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Evaluation of water relation parameters in *vitis* rootstocks with different drought tolerance and their effects on growth of a grafted cultivar

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Abstract

Knowledge of root hydraulic resistance will allow us to better understand water relations arising in the soil-plant-atmosphere continuum. These are the basis for better control of plant behaviour in the current environmental context that is more and more affected by global warming and problems related to increased drought frequency and duration. The objectives of this study were to determine how the growth of a cultivar changes in response to the drought tolerance of the rootstock used in *Vitis* grown in a semi-arid area and how the root hydraulic resistivity and root hydraulic conductivity change with increased transpiration when adopting a rootstock with a different drought tolerance.

These experiments were carried out on intact plants of Gaglioppo grapevines grafted onto rootstocks of 779 P, a drought-tolerant American hybrid, and 420 A, a drought-susceptible American hybrid.

Root hydraulic conductivity was significantly higher in the roots of 779 P than in the roots of 420 A. Stomatal conductance, net assimilation of CO₂, leaf water potential, and relative water content were also higher in Gaglioppo grafted onto 779 P than that grafted onto 420 A. Leaf area, leaf dry weight, and specific leaf weight of Gaglioppo were also higher when grafted onto 779 P. Gaglioppo grapevine grafted onto 779 P showed superior growth and physiological performance.

Abbreviations: $\Delta\Psi$ = Water potential difference; T= leaf transpiration E_0 = Transpiration into gas exchange chamber; E = E_0 on root length basis; Ψ_{Soil} = Soil water potential; Ψ_{Leaf} = Leaf water potential; $\Psi_{\text{Root xylem}}$ = Root xylem water potential; gs = Stomatal conductance; Leaf Water Potential= Ψ_{LEAF} ; LDW = Leaf Dry Weight; L_p = Root hydraulic conductance; L_p' = Root hydraulic conductivity; Offset= non-zero y intercept; PPFD = Photosynthetic Photon Flux Density; PIF (Pressure Induced Flow); R = Root hydraulic resistance;; R' = Root hydraulic resistivity; RWC = Relative Water Content; Soil Water potential = Ψ_{SOIL} ; SLW = Specific Leaf Weight; Soil Water Content (SWC); SRL = Specific Root Length; THR = True Hydraulic Resistivity; TIF (Transpiration Induced Flow); VPD = Vapour Pressure Deficit; WUE = Water Use Efficiency.

Keywords: hydraulic resistivity, root, stomatal conductance, *Vitis*, 779 Paulsen, 420 A.

Introduction

In a grafted plant, metabolic functions are split between two genotypes. The root system of the rootstock ensures the provision of water and all minerals, whereas the shoot variety is responsible for carbohydrate synthesis. In *Vitis*, there are many rootstocks that are used in order to improve fertility

and development of plant and overcome vineyard problems related to soil characteristics, such as drought, excess water, or salinity.

The responses of grapevine rootstock to drought (Meggio *et al.*, 2014) are very important; an appropriate selection of rootstock for a given scion variety can improve transpiration efficiency and water use (Soar *et al.*, 2006). Shoot xylem architecture is an important factor in limiting water supply versus demand in grapevines (Lovisolò and Schubert, 1998; Lovisolò *et al.*, 2002), but studies have found that the root resistance to water flow through whole plants is greater when compared to other components of the plant (Kramer, 1983; Boyer, 1985; Tsuda and Tyree, 1997; Miyamoto *et al.*, 2001; Mu *et al.*, 2006). Indeed, root resistance can reach up to 90% of whole plant resistance (Hellkvist *et al.*, 1974; Roberts, 1977; Rieger, 1989). This is probably the principal reason for the absorption lag compared to transpiration, which is a characteristic of plants even when adequately supplied with water. Root hydraulic resistance can be estimated using the Ohm's law analogue (Elfving *et al.*, 1972). Root hydraulic resistivity (R' ; inverse of Root hydraulic conductivity = Lp') is obtained when root hydraulic resistance (R ; inverse of hydraulic conductance = Lp) is normalized to the adsorbent root length.

Water passes through the root cortical cells before entering the xylem. R strongly depends on root anatomy and morphology (Steudle and Peterson, 1998; Rieger and Litvin, 1999; Steudle, 2000). The radial resistance is composed of three factors: the apoplastic components (cell wall, middle lamella, and intercellular air space), symplastic component (cell to cell), and transcellular path.

The apoplastic flow is hampered by the existence of apoplastic barriers, such as the Casparian strip of the endodermis and the suberin lamella of the endodermis, exodermis, and periderm in woody species; (Esau, 1977; Peterson *et al.*, 1993; Steudle *et al.*, 1993; Peterson and Enstone, 1996; Rieger and Litvin, 1999; Schreiber *et al.*, 2005). The symplastic path is mediated by plasmodesmata which link adjacent cells so that a cytoplasmic continuum is formed. During the passage through the apoplast and symplast, no membranes are crossed, whereas on the transcellular path, two plasma membranes are crossed per cell layer. In the transcellular path, membrane permeability (cell hydraulic conductivity) plays an important role in water transport. The symplastic and transcellular flow components cannot be separated experimentally and are summarized as a cell-to-cell component of water flow.

In the cell-to-cell pathway, R depends on intrinsic plasma membrane properties, plasmodesmata (Oparka and Prior, 1992; Roberts and Oparka, 2003), and/or the abundance and activity of aquaporins (Javot *et al.*, 2003; Luu and Maurel, 2005; Gambetta *et al.*, 2012; Steudle and Peterson, 1998; Steudle, 2000; Maurel, 2007; Katsuhara *et al.*, 2008; Kjellbom *et al.*, 2008; Maurel *et al.*, 2008; Knipfer and Fricke, 2010; Postaire *et al.*, 2010).

How root hydraulic resistance changes in grapevine rootstocks is an important question. Recently, some interesting work was performed in *Vitis* rootstock, but no one has yet reported how these parameters change during transpiration in intact plants. In previous studies, the root hydraulic conductance values were determined using the pressure-induced flow (PIF): the instrumentation that use this method is the HPFM (de Herralde et al., 2006; Alsina et al., 2011; Romero et al., 2017). With this method, the measurement is performed on root systems after they are cut from whole plants, and the water flow in the root system is changed by application of external pressure. The less known system, transpiration-induced flow (TIF) (Rieger and Motisi, 1990; Motisi and Gullo; 2000) allows the use of intact plants. With this system, the flow in the plant is changed, thus altering the physiological transpiration of the canopy. However, in the literature, few studies have used this system, although it was more performing.

Furthermore, the current increase in drought in the environment implies that the characterization of rootstocks for drought tolerance is important for future crop success. Therefore, for better management of future plantings, the pattern of hydraulic resistivity or hydraulic conductivity of each main rootstock in the soil-plant-atmosphere continuum must be known.

In the present study, two related objectives have been pursued. The first experiment examined how the growth of a grapevine cultivar changes in response to rootstocks with different drought tolerance in a semi-arid area, such as the Mediterranean Basin. In the second experiment, we evaluated how root hydraulic resistivity and root hydraulic conductivity changed with increasing transpiration and how these two parameters changed between a drought-tolerant and drought-susceptible rootstock in intact plants.

Materials and Methods

Plant material

Both experiments were carried out on the same plants.

For each rootstock, 36 uniform cuttings (400 mm length, 6/8 mm diameter) taken from mother plants of 779 P (*Vitis berlandieri* × *Vitis rupestris*) and 420 A (*Vitis berlandieri* × *Vitis riparia*).were selected. The cuttings were obtained by pruning one-year-old branches. In general, 420 A rootstock confers a lower growth rate to the grafted grapevine cultivar and it is drought susceptible (Carbonneau, 1985). 779 P rootstock is more drought resistant (Ardenghi and Cauzzi, 2015) than 420 A and confers a higher growth rate to its scion.

The cuttings were grafted with Gaglioppo (*Vitis vinifera* L.) an important autochthonous grapevine cultivar of southern Italy. The surface of the cutting was sterilized by immersion in a 10% NaOCl solution for 10 min and the cuttings were rooted in 10-litre pots.

The pot was filled with a previously pasteurized soil. Pasteurization consisted of gradually raising the soil temperature up to 90 °C over a period of 60 min in an electric unit and then gradually cooling it. This method, which is not as aggressive as autoclave-based sterilization, effectively devitalized mycorrhizal fungal propagules in the soil (McGonigle and Miller, 1996). The soil was loamy-clay with low active lime, a good level of nitrogen (1.5-2‰), a good level of phosphorus (60 ppm), a medium level of potassium (150 ppm), and a pH of 6.8. The absence of mycorrhizae spores in the substrate, was evaluated using the technique proposed by Jenkins (1964) and modified by Walker *et al.* (1982). At the end of the experiment, a final check to visualize the absence of mycorrhizae was performed. Portions of lateral roots were sampled and submitted to an optimized staining technique by acid fuchsine. In particular, the Phillips and Haymann's technique (1970), modified by Torta *et al.* (2003), was used. The mycorrhization index (MI = % of stained tissue with respect to the hyaline portion on the length of the root) was established on three fragments of each sample, thus determining the average value. Finally, sectioned radical fragments (longitudinally and transversely) were mounted on a glass slide with a drop of lactophenol and observed with an optical microscope.

Experimental design

A randomized design with 3 blocks of twelve plants per graft combination (36 plants) was adopted for each rootstock.

Plant care

Each plant had two drippers installed with a flow rate of 2 L h⁻¹. The soil water potential was measured using Watermark® probes. The probe was installed at half of the height of the pot, 15 cm from the substrate surface. This is an inexpensive, fast, and easy-to-use method that provided the opportunity for automatic recording with a Watermark® Monitor Datalogger. Plants were irrigated every 72 hours to keep the soil water potential (ψ_{Soil}) at “field capacity”. Because the volume of the pot was small, the added water rapidly dispersed inside the pot and was consumed by the entire root system.

A tensiometric curve was generated according to the type of substrate used. Thus, it was possible to determine the water soil content by measuring the water potential.

Plants were trained to have a lateral shoot under the graft point. Each shoot had ten fully expanded leaves. For each plant, the shoots were selected within 10 cm above the surface of the root-containing substrate. The leaves on those shoots were used to estimate the xylem water potential at the point of attachment of the lateral shoot to the main stem as according to Powell (1974). The other shoots were periodically removed.

First experiment: Gas exchange measurements

Gas exchange measurements were performed under optimal weather conditions (average CO₂ partial pressure 38 Pa; saturated PPFD 1600 $\mu\text{mol m}^{-2} \text{s}^{-1}$) in mid-September 2 hours after irrigation when the

soil water potential was near 0, 72 hours after irrigation when the soil water potential was lower, and before water supply with irrigation. Leaf CO₂, stomatal conductance (gs), water use efficiency (WUE), and transpiration were measured on 36 mature leaves from the exterior canopy position for each rootstock (4 leaves × 3 plants × 3 blocks). Measurements were made using a portable photosynthesis system (Li-Cor 6400 XT; LI-COR, Lincoln, Nebraska, USA). The measurements were carried out during a clear sunny summer day (between 11:00 and 13:00).

The measurements of plant development used in this experiment (leaf area, leaf dry weight, fibrous root length, and fibrous root dry weight) were taken when the plants were destroyed at the end of the second experiment. Some measurements were also used for the second experiment (see below).

Second experiment: Transpiration induced flow

The root hydraulic resistance and resistivity were measured in September (at the end of first experiment) on three intact plants for rootstock using the TIF method: a semi-closed gas exchange system. This system was composed of a chamber (80 Liters), a control system of environmental parameters (VPD, CO₂, Temperature, and PPF), and a measurement system for water produced by transpiring leaves (Fig. 1).

The change in environmental conditions was created by varying the VPD, CO₂ concentration, and light intensity in the chamber. Light was provided by one 400-watt LED lamp. This lamp provided a max photosynthetic photon flux at the top of the canopy of at least 800 μmol s⁻¹ m⁻². Air temperature was kept at 25 °C (±0.2). Leaf temperature was lower than air temperature at a range of 2 to 3 °C. Chamber VPD was maintained within a range of -0.03 to +0.03 kPa of the set point depending on the desired level of transpiration (E₀). In the chamber, the CO₂ concentration can be changed within a range of 100 μL L⁻¹ to 1000 μL L⁻¹ by injecting air with 4000 μL CO₂/L. The setting of all these parameters in the chamber were kept stable by the control system.

The plants of each rootstock were brought to the laboratory and watered during the night. The measurements were performed on the day after the following procedure.

The canopy of each plant was enclosed in the gas exchange chamber. The pot and the lateral shoots (lead development at the base of the trunk) were left outside the chamber. Each pot was placed in a container with 10 mm of standing water in order to keep the water potential of the soil (ψ_{Soil}) close to zero. All fully developed leaves on the lateral shoots outside of the chamber were enclosed in parafilm and aluminium foil. This was done to stop transpiration and photosynthesis. As a consequence, the leaf xylem water potential came to equilibrium with the root xylem water potential (Ψ_{Root xylem}) as reported by Powell (1974), Rieger (1989), Rieger and Motisi (1990), and Motisi and Gullo (2000). The transpiration of the plants (E₀) in the gas exchange chamber was adjusted to the change in the environmental conditions. At the end of the experiment, the CO₂ was increased to 3000 ppm, the

chamber was covered with a black towel, and the relative humidity was increased to 90%. Under these conditions, the absence of transpiration was detected by the instrumentation.

For each new environmental condition, the measurements were performed when the plants reached a steady state; steady-state E_0 was assumed when the difference between consecutive measurements at 15- to 30-min intervals was $< 10\%$.

Some covered leaves on the shoot were detached and used to measure the water potential in a Schölander pressure chamber (Skye Instruments, Llandrindod Wells, UK).

Steady-state Ψ was assumed when sequential measurements at a 30-min interval differed by < 0.05 MPa. Both steady states (E_0 and Ψ) were attained within 1 h after changing the chamber to a new setting as reported by Rieger and Motisi (1990).

At the end of the measurements, the plants were destroyed. For each plant, the root system was gently cleaned of soil under water and cut at the interface between the root system and shoot. The leaves were also measured using a Li-Cor 3100.

Root and leaf dry mass was recorded after drying (Nobel and Huang 1992; Rieger, 1995) at constant mass at 70°C .

Root morphology and anatomy

Fine (or fibrous) roots were measured using the methodology adopted by Himmelbauer *et al.* (2004) and Gambetta *et al.* (2012). The fine root length and diameter were determined using Win-RHIZO version 2003a (Régent Instruments, Quebec, Canada).

The number of nodes (branching) per unit root length (m) was evaluated. Root surface area was calculated from diameter and assuming the root was a cylinder.

For anatomy parameters, for each rootstock 72 cross-section ($5\ \mu\text{m}$ thick and 10-30 mm behind the root tip, as effected by Rieger and Litvin, 1999) were used for measurements of root diameter, endodermis diameter, and exdermis diameter. The width of the cortex was calculated as the difference between the radii of the root epidermis and the endodermis.

Root hydraulic resistivity, root hydraulic conductivity

Root resistivity (R') was calculated as the slope of the plot of E vs $\Delta\Psi$. E is transpiration (E_0) expressed in $\text{mmol H}_2\text{O}$ per root length as reported by Rieger and Litvin (1999), measured after destroying the plant. $\Delta\Psi$ is the difference in the water potential between the soil and root. R' is in units of $\text{mmol}^{-1}\ \text{m}^{-1}\ \text{s MPa}$, and its inverse (hydraulic conductivity) is L_p' , which is in units of $\text{mmol m}^{-1}\ \text{s}^{-1}\ \text{MPa}^{-1}$. Because the water potential of the soil was maintained close to zero, $\Delta\Psi$ is equal to Ψ of the wrapped leaves and then to $\Psi_{\text{Root xylem}}$ (see above).

Root hydraulic resistance (R) and Root hydraulic conductance (L_p) were determined for each leaf's maximum transpiration rate for each rootstock.

From the ratios of the root hydraulic resistance to the root fibrous length and the root hydraulic conductance to the root fibrous length, we obtained the root hydraulic resistivity and root hydraulic conductivity, respectively.

For each plant, the slope of the linear regression between E and Ψ was used to statistically differentiate the two rootstocks. The slope of the tangent curve of the polynomial regression between L_p and E was used to statistically differentiate the two rootstocks.

Measure of transmembrane water movement and embolization

The HPFM was used exclusively to measure the transmembrane water movement and 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) and also adopted by Traontini et al. (2013). The root system of each rootstock was separated from the soil using a low-pressure water flow. Then a cut was inflicted at the interface between the root and shoot. It was then placed in a tension–pressure chamber. In the first phase, the tension–pressure chamber was filled with water only. A negative pressure of -80 kPa was applied for 5 min through the sleeve. After 5 min, the flow at the apical end of the trunk was constant; the conductance was measured at steady state. In the second phase, the root was treated with a solution of 0.05 mM $HgCl_2$ for 60 min (an additional 15-min wait allowed the system to stabilize) in order to inhibit mercury-sensitive aquaporins. The same pressure (-80 kPa) was applied for the same time (5 min). The difference between the two measures of conductance defines the portion of conductance sustained by transmembrane water movement.

The same pressure was applied for a further 5 min after a flushing of 100 kPa on the whole root in order to free the system from embolisms.

The difference between this value and the previous one defines the conductance achievable by the system through the transmembrane pathway in the absence of embolisms.

Statistical analysis

Data are expressed as averages with their corresponding standard errors. The results were submitted to a one-way ANOVA and Tukey's test using the statistical software package SPSS software (SPSS Inc, Cary; NC, USA)

Results and discussion

The TIF method adopted by Rieger and Motisi (1990) permitted the use of intact plants, thus avoiding problems associated with the commonly used HPFM, such as flow through unnatural paths (Koide, 1985) and lack of root-shoot communication (Markhart and, 1990).

It is known that rootstock plays an important role in grapevine tolerance to water stress (Carbonneau, 1985; Soar *et al.*, 2006; Alsina *et al.*, 2011; Marguerit *et al.*, 2012; Meggio *et al.*, 2014) and appropriate choice of rootstock for a given scion variety can improve transpiration efficiency and thus the water use (Soar *et al.*, 2006).

In the first experiment of the present study, *Vitis* rootstocks adopted have had an effect on vigour (Table 1). The Gaglioppo with the 779 P rootstock (G/779 P) had a larger leaf area ($1615.11 \text{ cm}^2 \pm 11.15$) compared to those with the 420 A rootstock (G/420 A; $1355.25 \text{ cm}^2 \pm 12.21$), which is 16% smaller. The leaf dry weight (LDW) of Gaglioppo was also different as function of rootstock (Table 1), and it was 32% higher in plants grafted onto 779 P compared to 420 A. In G/420 A plants, the value of the specific leaf weight (SLW; $4.02 \text{ mg.cm}^{-2} \pm 0.03$) was 21% lower compared to G/779 P rootstocks ($4.88 \text{ mg.cm}^{-2} \pm 0.03$; Table 1). Our results are consistent with those reported by Gambetta *et al.* (2012) for drought-tolerant and drought-susceptible rootstocks.

No difference in stomatal conductance and RWC was observed between plants when the soil water potential was close to 0 (Table 3). However, under water constraints, the stomatal conductance (g_s) and water status (leaf water potential, Ψ_{Leaf} , and Relative water content, RWC) of G/779 P were better than those of G/420 A. Indeed, 72 hours after watering, the g_s , the RWC and Ψ_{Leaf} were 70%, 17% and 0.19 MPa higher in G/779 P compared to G/420 A, respectively (Table 3). A stomatal closure is an early plant response to water deficit (Schroeder *et al.*, 2001). Consequently, CO_2 net assimilation was also higher in leaves of G/779 P compared to G/420 A (data not shown).

Marguerit *et al.*, (2012) reported that chemical signals sent by drying roots to the shoot regulate stomatal aperture; this remains a matter of debate, whereas several authors (Lovisolo *et al.*, 2002; De Souza *et al.*, 2003; Soar *et al.*, 2006; Alsina *et al.*, 2011; Serra *et al.*, 2014) reported that the ABA synthesized by roots and its concentration is responsible for stomatal closure as long as soil and root tissue are dehydrated. During the second experiment in the chamber, the change in CO_2 concentration (from 300 to 3000 $\mu\text{L L}^{-1}$), VPD (from 2.5 to 1 kPa), and light (from 800 to 0 $\mu\text{mol m}^{-2} \text{ s}^{-1}$) led to a change in transpiration from 0 to 0.30 $\text{mmol H}_2\text{O min}^{-1} \text{ m}^{-1}$. For each environmental setting, both (E_0) and $\Psi_{\text{Leaf xylem}}$ reached a steady state 90 min after the change in environmental conditions in the chamber. At the maximum value of E_0 , the 779 P rootstock exhibited a 64% higher root-hydraulic conductance ($84.25 \text{ mmol m}^{-1} \text{ s}^{-1}$) compared to 420 A ($51.5 \text{ mmol m}^{-1} \text{ s}^{-1}$) (Fig. 2). The higher vigour exerted on Gaglioppo by 779 P compared to 420 A in the first experiment, can be attributed to the higher hydraulic conductance of the root-system of this rootstock according to reports in the literature (Nardini *et al.*, 2006; Solari *et al.*, 2006; Clearwater *et al.*, 2004; Lovisolo *et al.*, 2007; Tramontini *et al.*, 2013).

The change in root-system hydraulic conductance between two rootstocks can depend on the biomass of root-system (Lovisolò *et al.*, 2007) and/or the root intrinsic hydraulic properties. For this reason, we have focused our attention on R' together with root biomass.

In both rootstocks, a different trend in transpiration was observed when the hydrostatic pressure gradient between the soil and xylem increased (tension in the xylem). Furthermore, in the relationship between Ψ and E (called the true hydraulic resistivity (THR) by Passioura and Munns; 1984), the slope was 6 times higher for 420 A (-1.17 rad) compared to 779 P (-0.187 rad); Fig. 3; Table 4.. For a transpiration value of $0.1 \text{ mmol H}_2\text{O min}^{-1} \text{ m}^{-1}$, the $\Psi_{\text{Root xylem}}$ was 27% lower in 779 P (-0.22 MPa) compared to the 420 A rootstock (-0.29 MPa).

The measurement of E was equal to zero when CO_2 was increased to 3000 ppm, the relative humidity was increased to 90%, and the chamber was covered with a towel. Under this condition, the relationship between Ψ (y axis) and E (x axis) showed that Ψ (y-intercept) was nonzero. This

For both rootstocks, the substrate volume (pot conditions) was limited and water availability was the same. However, after 72 hours of water administration, the ψ_{Soil} and SWC were lower in the pot with 779 P rootstock than the pot with 420 A rootstock (Table 3). Therefore, the higher drought tolerance of 779 P could not be explained by its capacity to explore larger and deeper soil volumes. Indeed, no differences were observed between the two rootstocks for length, dry weight (Table 1), diameter, and other anatomical structures of fibrous roots as stele diameter and cortex width (Table 2), but differences were observed in root morphology with 779 P showing greater root branching (Table 1).

The treatment with Hg significantly reduced the maximum hydraulic conductance (Fig. 5). Root hydraulic conductance was higher in 779 P than 420 A plants, both before and after Hg treatment. However, in 779 P rootstocks, the reduction was higher (-35%) whereas hydraulic conductance was not influenced by Hg treatment in 420 A rootstock (-12%) as observed by Tramontini *et al.*, 2013 in drought-tolerant and drought-susceptible rootstocks.

In the meristematic/elongation zone, it has been shown that L_p is higher and aquaporin activity reaches a peak in the root tip, whereas both are low in the secondary growth zone that coincides with the suberised epidermis (Zwieniecki *et al.*, 2003; Alsina *et al.*, 2011 Gambetta *et al.*, 2012).

Because of greater branching (Table 1) and a higher number of root tips in 779 P compared to 420 A, we hypothesize that the higher root hydraulic conductance in 779 P compared to 420 A can also be attributed to more root tips and aquaporins, as confirmed by observations on excised roots treated with Hg. Therefore, the higher aquaporin activity or greater abundance observed in 779 P facilitated xylem conduit refilling after drought conditions that produce embolisms in the xylem conduit. (Lovisolò *et al.*, 2008; Vandeleur *et al.*, 2009; Tramontini *et al.*, 2013).

After eliminating embolisms (with flushing of 100 kPa, for 5 min) by using the same pressure (-80 kPa) applied for the same length of time, the conductance increased 280% ($68 \text{ mmol m}^{-1} \text{ s}^{-1}$) in the 420 A rootstock and 190% in 779 P ($105 \text{ mmol m}^{-1} \text{ s}^{-1}$). Therefore, in 779 P rootstocks, the hydraulic conductance is less influenced by embolisms than in 420 A (Fig. 4).

Several authors found that higher conductance of the excised roots, higher cell-to-cell water transport, and higher tolerance to xylem cavitation processes (Tramontini *et al.*, 2013; Lovisolo *et al.*, 2008) were correlated with an intrinsic higher tolerance to water stress on rootstocks.

The pattern of L_p depends both on THR and offset; it approaches zero in the absence of transpiration according to Rieger and Motisi (1990) and Motisi and Gullo (2000). During an increase in transpiration, the gradient of the L_p curve was significantly higher in 779 P rootstocks compared to 420 A (Fig. 5). The slope of the tangent of the L_p curve for a transpiration rate of $0.1 \text{ mmol H}_2\text{O min}^{-1} \text{ m}^{-1}$, was significantly higher in 779 P rootstocks (1.09 rad) compared to 420 A (0.99 rad) (Fig. 5; Table 4). The L_p curve tends to flatten and to remain stable under higher transpiration rates, such as those approaching $0.2 \text{ mmol H}_2\text{O min}^{-1} \text{ m}^{-1}$ (Fig 5) according to Rieger and Motisi (1990) and Motisi and Gullo (2000). However, L_p was significantly higher in 779 P rootstock compared to 420 A.

Conclusion

In this study, we performed an analysis to improve the knowledge on water relation and behaviour of grapevines, using an autochthonous cultivar, Gaglioppo, grafted onto rootstocks adopted in some areas of the Mediterranean basin.

We monitored the change of water resistance and its inverse (L_p') in roots during transpiration using the TIF method. This method allowed for using intact plants and inducing flow in the plant from the roots to the leaves.

The results of this study help us to better understand the behaviour of water within the root system of the 779 P and 420 A in the grafted scions. The rootstock which induces the greatest vigour is also the one most tolerant to the lower water availability of the soil. This is attributable to two factors that influence the root hydraulic conductivity: the lower value of the offset and the lower true water resistance (slope of E vs ψ in a linear regression) of 779 P rootstocks compared to 420 A. Because no difference was observed between length and anatomical parameters of the two rootstocks, the causes are to be found in the greater branching of the fibrous roots and in the greater number of tips in particular. These are the main factors that increase the L_p' because the tips are rich in aquaporins. Furthermore, it is very important to define these parameters in grapevine rootstocks and to analyse the effects on the vigour of grafted scions.

Future research should take into account the important abiotic parameters of the soil, such as the soil texture.

These results make it possible to further characterize the two rootstocks by highlighting the strengths and weaknesses of each of them. Finally, these outcomes provide new information that is important for the optimal choice of rooting cuttings for new plantings and water management in the vineyard, especially when considering the actual climate. Climate change can be characterized by profound changes, such as an increase in temperature and drought as has happened in many areas of the Mediterranean basin.

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Table 1 – Main biometric parameters of scion and rootstock in the grapevine tree of CV Gaglioppo grafted onto 420 A and 779 P. Data are reported as a mean \pm standard error (n=72);

| Rootstock | Leaf Area cm ² | LDW g | SLW mg.cm ⁻² | Root nodes (Branching) N°m ⁻¹ | Root length m | Fibrous Root dry weight g |
|-----------|------------------------------|------------------|----------------------------|--|-----------------------|---------------------------------|
| 779 P | 1615.11 \pm 11.15b | 7.22 \pm 0.14b | 4.88 \pm 0.03b | 85.3 \pm 2.2b | 118.23 \pm 20.07n.s | 5.09 \pm 0.35n.s. |
| 420 A | 1355.25 \pm 12.21a | 5.45 \pm 0.21a | 4.02 \pm 0.03a | 48.3 \pm 1.9a | 128.44 \pm 26.11 | 5.12 \pm 0.31 |

For each column, different letters indicate significant differences per $p \leq 0.05$; n.s. not significant

Table 2 – Root anatomical characteristics in the two rootstocks of grapevine trees: 420 A and 779 P. Data are reported as a mean \pm standard error ($n=144$);

| Rootstock | Root diameter μm | Stele diameter μm | Cortex width μm^2 | SRL m.g^{-1} |
|-----------|--------------------------------|---------------------------------|---------------------------------|--------------------------|
| 779 P | 650.11 \pm 11.15ns | 260 \pm 0.14ns | 380 \pm 0.03ns | 21.37 \pm 2.2 ns |
| 420 A | 710 \pm 12.21 | 305 \pm 0.21 | 320 \pm 0.03 | 25.3 \pm 1.9 |

For each , different letters indicate significant differences per $p \leq 0.05$; n.s. not significant

Table 3 – Soil water potential (Ψ Soil), Soil water content (SWC), Stomatal conductance (gs), leaf water potential (Ψ Leaf) and relative water content (RWC) in Gaglioppo grapevine cultivar grafted onto 779 P and 420 A rootstocks. The measures were taken 2 hours and 72 hours after irrigation with exception of SWC that were taken only 72 hours water administration.. *Data are reported as means \pm standard error (n = 36).*

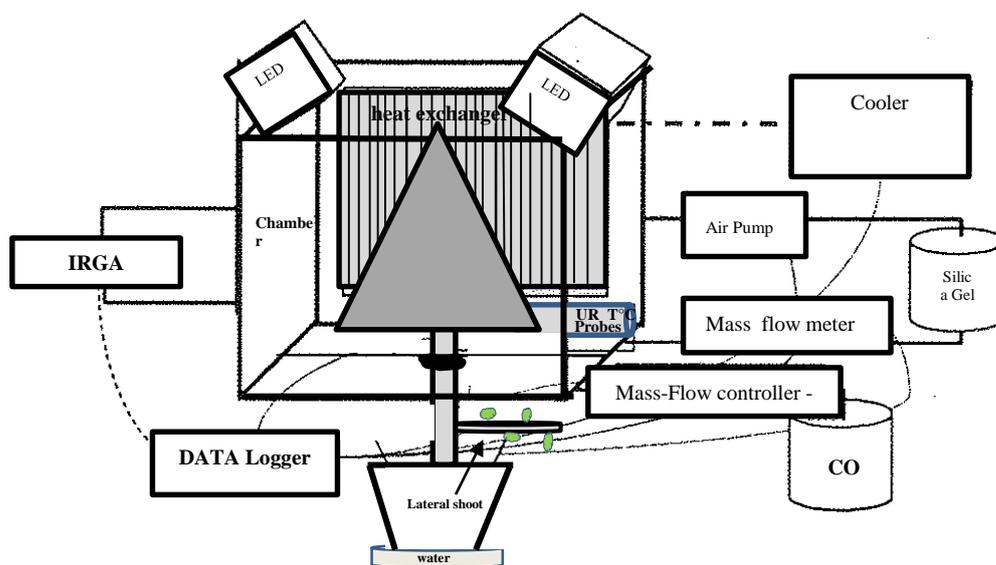
| Rootstock | Time after irrigation (hours) | Ψ Soil centibar | SWC $g \cdot g^{-1}$ | gs (mmol.m ⁻² .s ⁻¹) | Ψ Leaf (MPa) | RWC % |
|----------------|-------------------------------|----------------------|----------------------|---|-------------------|-------|
| Gaglioppo/420A | 2 | 1.1 \pm 0.09a | | 254.18 \pm 10.25a | -0.36a | 96.9c |
| Gaglioppo/779P | 2 | 1.2 \pm 0.08a | | 235.21 \pm 8.2a | -0.25a | 96.4c |
| Gaglioppo/420A | 72 | 15 \pm 1.1b | 0.31 \pm 0.4b | 88.12 \pm 07.1c | -0.88c | 71.4a |
| Gaglioppo/779P | 72 | 22 \pm 1.2c | 0.19 \pm 06a | 125.06 \pm 7.8b | -0.69b | 86.3b |

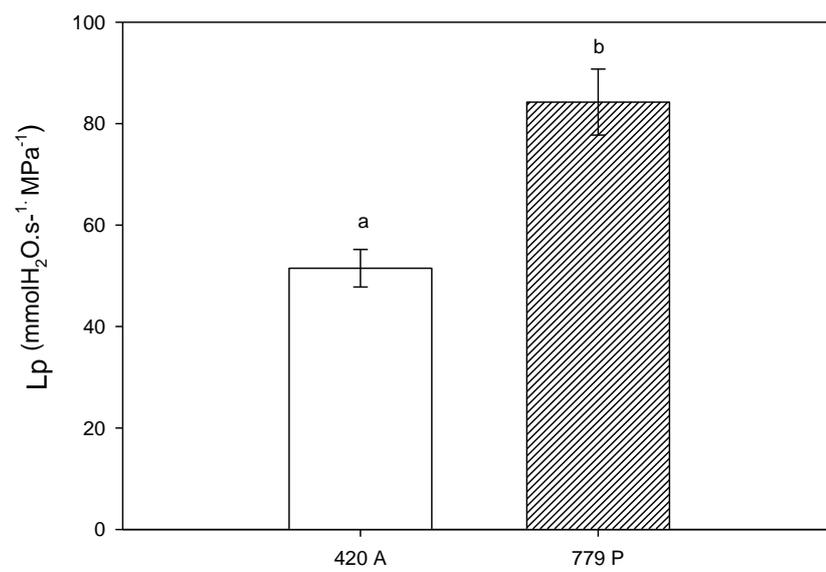
For each column, different letters indicate significant differences per $p \leq 0.05$; n.s. not significant

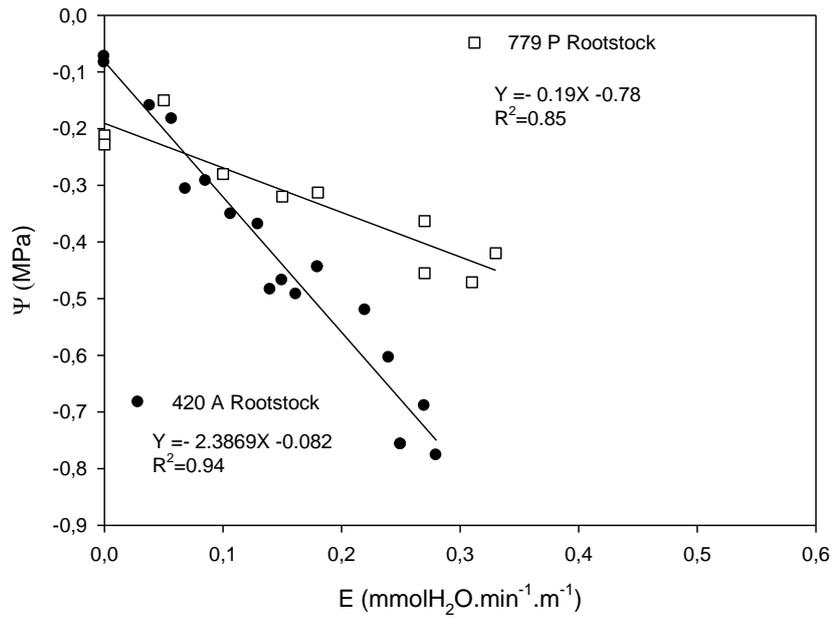
Table 4 –Slope of regression of the relationship between E and T and slope of the tangent to the LP curve, calculated for a transpiration of $0.1 \text{ mmolH}_2\text{O}\cdot\text{min}^{-1}\cdot\text{m}^{-1}$. Data are reported as means \pm standard error ($n = 36$).

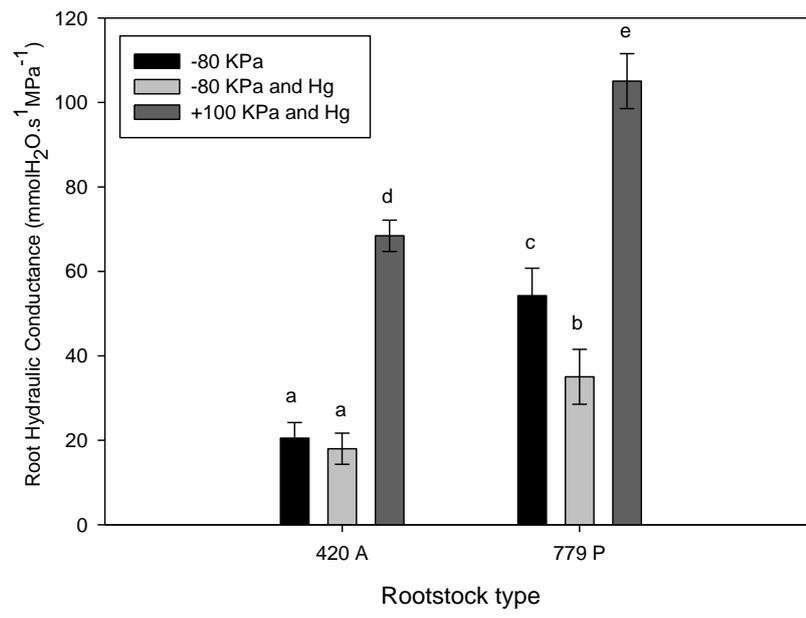
| Rootstocks | Slope of "E vs Ψ " linear regression (rad) | Slope of the tangent to LP (rad) |
|-----------------|---|----------------------------------|
| Gaglioppo/779 P | -0.187 ± 0.1 a | 1.09 ± 0.2 b |
| Gaglioppo/420 A | -1.17 ± 0.2 b | 0.99 ± 0.2 a |

*Different letters indicate significant differences per $p \leq 0.05$; n.s. not significant.









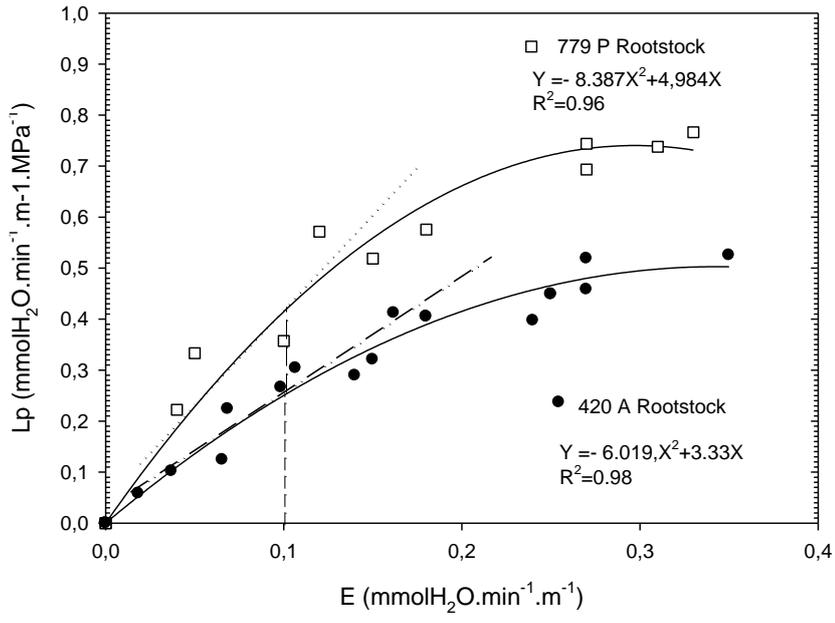


Figure Legends

Fig. 1 TIF: semi-closed gas exchange system

Fig 2 Root hydraulic conductance (E_0 = max transpiration) in 779 P and 420 A grapevine rootstock; (n= 12)

Fig 3 Relationship between the root water potential (Ψ) and transpiration (E) in 779 P and 420 grapevine rootstock (each point is the mean of three measurements)
n= 12)

Fig.4. Hydraulic conductance measured on excised root of 779 P and 420 A during 3 phases: depressurizing at -80 kPa, treating with Hg and depressurizing at -80 kPa, pressure flushing of +100 kPa on the Hg-treated roots and depressurizing again at -80 kPa (n=12)

Fig 5 Relationship between the root hydraulic conductivity (L_p) and transpiration (E) in 779 P and 420 A grapevine rootstock; (each point is the mean of three measurements)
n= 12)

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