Review: the interaction between rootstocks and cultivars (Vitis vinifera L.) to enhance drought tolerance in grapevine

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Abstract

Water scarcity is a key limiting factor in agriculture. Grapevines react at the physiological, biochemical and genetic level to tolerate water constraints. Even though grapevines are considered relatively tolerant to water deficits, grapevine growth and yield can be seriously reduced under water deficit. Drought-tolerant rootstocks are expected to enable the scion to grow and yield when water supply is limited. Genetic machinery allows rootstocks to control water extraction capacity and scion transpiration. Numerous works have demonstrated the positive role of drought-tolerant rootstocks on the control of cultivar’s leaf stomatal conductance and therefore on canopy transpiration. The mechanisms, in terms of signalisation and gene functioning, need further study. Furthermore, there is no standardised methodology to rank rootstocks in terms of their tolerance to drought. A potential effect of rootstocks on stomatal development is also discussed. This review will critically discuss the current knowledge of the mechanisms of drought tolerance afforded by rootstocks, taking into account the scion/rootstock interaction, and will present some of the challenges for future investigations.

Keywords: climate change, drought tolerance, rootstock, water deficit, water use

Introduction

The use of rootstocks is common in most viticultural areas, and most rootstocks currently used around the world were developed before 1930 from American Vitis species in an effort to avoid the damage caused by phylloxera, which devastated the European vineyards in the last half of the 19th century (Granett et al. 2001). Currently, scion cultivars are grafted onto rootstocks that are either North American species or inter-specific hybrids (Mullins et al. 1992) (Figure 1) that have a limited genetic background due to the fact that 90% of all rootstocks used around the world originated from less than ten different rootstock cultivars (Keller 2010). Rootstocks are selected for their resistance to phylloxera; however, several other characteristics are also required, such as suitability for grafting, rooting and propagation, and resistance to nematodes and Pierce’s disease; tolerance to lime, drought, salinity and vigour conferred are also considered (Granett et al. 2001). Possible water scarcity in the near future (Intergovernmental Panel on Climate Change 2008) increases the interest in drought tolerance afforded by rootstocks.

Drought induces senescence of older leaves (Jackson 1997), a decrease in growth, a decrease in plant water potential, stomatal closure, lower transpiration and photosynthetic rates (Yordanov et al. 2000). The drought responses of a plant involve a series of physiological and biochemical changes. Stomata are pores that control the gas exchange between leaves and the atmosphere (Hetherington and Woodward 2003), which is necessary for photosynthesis. In C₃ plants, during a mild water constraint, a reduction in photosynthesis is mainly due to stomatal closure, with a transition phase with stomatal and non-stomatal limitations, while during severe water deficit the non-stomatal limitation to photosynthesis is dominant (Lovisolo et al. 2010). This may include a decline in Rubisco activity (Dias and Brüggemann 2010). Many studies have shown that grapevine response to water deficit involves a reduction in stomatal conductance and photosynthesis (Iacono et al. 1998, de Souza et al. 2003, Koundouras et al. 2008); a decrease in leaf expansion and internode elongation (Schultz and Mathews 1988, Cramer et al. 2007, Lovisolo et al. 2010); and a reduction in yield (dos Santos et al. 2003, Chaves and Oliveira 2004). Drought can cause cellular water loss, which induces osmotic stress that affects cell division and elongation and which, in turn, affects the growth of different organs (Bartels and Sunkar 2005). The degree of growth limitation can vary depending on the nature of the tissue, e.g. shoots, leaves or roots (Wu and Cosgrove 2000). The rate at which water constraints develop, i.e. gradually or abruptly, could also determine the extent of growth limitation (Christmann et al. 2007). Furthermore, the cell will have to deal with the production of reactive oxygen species that negatively affect cell metabolism and cell wall structure (Bartels and Sunkar 2005). Therefore, the sensitivity of growth to drought will depend on regulation at the physiological, biochemical and genetic level that can control changes in the cell wall (tightening and loosening) (Moore et al. 2008). Turner (1986) suggested three mechanisms of plant adaptation to water deficit, namely drought escape, drought tolerance with...
low plant water potential and drought tolerance with high plant water potential. In terms of drought tolerance, rootstocks are expected to enable the scion to grow and function normally when water supply is limited. The mechanisms of tolerance to drought by rootstocks are not yet fully understood. In tomato, a higher scion fruit yield under salinity was related to a greater capacity of the rootstock to improve water flow to the scion, probably due to an enhancing vascular cylinder area and xylem cell lignification, compared with that of a non-grafted cultivar (Asins et al. 2010). In apple, peach and cherry, the effect of rootstock genotype on scion vigour has been related to the influence on the hydraulic conductance capacity (Atkinson et al. 2003, Tombesi et al. 2010, Zorić et al. 2012). Furthermore, in kiwi, it was found that differences in phenology between scion and rootstock combinations appear to be responsible for the rootstock influence on shoot growth (Clearwater et al. 2007). In grapevine, high vigour rootstocks have higher fine-root hydraulic conductivity due in part to higher aquaporin expression and activity (Gambetta et al. 2012). Furthermore, rootstocks with higher inherent vigour perform better than low vigour rootstocks under water deficit conditions (Williams 2010). Nevertheless, the effect of vigour on the drought tolerance of the plant is still not clear (Jones 2012). It has been postulated that using drought-tolerant rootstocks in the grapevine can help to minimise the effect of water constraints via improved water uptake and transport (Carbonneau 1985, Soar et al. 2006) and by controlling the plant’s transpiration through chemical signalling (Lovely and Kriedemann 1974, Stoll et al. 2000, Soar et al. 2006) and hydraulic signalling (Vandeleur et al. 2009). The aim of this review is to identify and discuss the main advances in the understanding of the rootstock/scion interaction in the regulation of water use by grapevines.

**Root anatomy**

In general, grapevines are considered relatively tolerant to water deficits, due in part to their relatively large xylem vessels in comparison with those of other plants (Comas et al. 2010), allowing a quick recovery from water constraints (Lovisolo et al. 2008a). Furthermore, grapevine roots have larger xylem vessels (Figure 2) in comparison with those in their stems, causing them to be more prone to xylem cavitation (Lovisolo et al. 2008a). Cavitation and embolism can affect whole-plant hydraulic conductance at different levels: leaves, stem and roots. It has been suggested that the sensitivity to cavitation and embolism might be related to plant mechanisms to adapt to water deficit conditions involving regulation of stomatal conductance (Domec and Johnson 2012). In peach and cherry, it was found that rootstocks that induce more vigour have larger xylem vessels and lower vessel density in comparison with those considered dwarfing rootstocks, resulting in a different hydraulic conductance capacity (Tombesi et al. 2010, Zorić et al. 2012). In the same way, citrus rootstocks that have higher hydraulic conductance appear to have larger xylem vessels (Tombesi et al. 2010, Zorić et al. 2012). In grafted grapevines, the anatomical characteristics of the xylem of the rootstocks might influence the water uptake and transport/conductance capacity. Besides differences in hydraulic architecture due to genetic origin, soil type can affect plant adaptation to drought in terms of changes in whole-plant hydraulic conductance by affecting xylem tissue development (Tramontini et al. 2012).
Root growth and development

Having a well-developed root system may improve water uptake by exploiting more efficiently the resources available in the soil. Most of the roots are found in the top 1 m of soil, although they can be found at a depth of up to 6 m (Seguin 1972) or more. The root system consists of the main framework roots (6–100 mm in diameter) and smaller, permanent roots (2–6 mm in diameter) (Mullins et al. 1992). Root density can be affected by soil water availability and type of irrigation (Soar and Loveys 2007), canopy manipulation (Hunter and Le Roux 1992, McLean et al. 1992, Serra-Stepke 2010), trellis system (Archer et al. 1988) and rootstock genotype (Southey and Archer 1988, Morano and Kliewer 1994). The pattern of new lateral root growth will depend on the climatic conditions where the vineyard is located. Grapevines in temperate and Mediterranean climates show root growth activity mainly between flowering and veraison, followed by some root growth during summer if the soil water content is favourable (Van Zyl 1984). In addition, a smaller postharvest growth of roots can occur in temperate climates. In subtropical climates, root growth occurs primarily postharvest, with no spring flush (Comas et al. 2010). Escalona et al. (2012) found that under irrigation, the estimated carbon losses due to respiration amounted to 47–65 g per plant. This equated to 30–50% of the total estimated gains due to photosynthesis. Furthermore, respiration by the root system represented 70–80% of the total carbon losses, illustrating the large requirements of this organ. The remaining proportion consisted of both leaf and stem respiration.

Early studies proposed that a genetic variability exists regarding rooting depth (Pongrác 1983, Pouget 1987), e.g. V. riparia is described with a root system that is well branched and shallow growing, in contrast with 140 Ruggeri (Van Huyssteen 1983). A survey showed that this critical penetration into soil if the penetration resistance exceeds about 2 MPa (Van Huyssteen 1983). Grapevine roots cannot grow readily in deep layers (Van Huyssteen 1983). Root systems that differ in their ability to confer vigour and resistance limited root system development in a wide range of Australian vineyard soils (Myburgh et al. 1996). In young, grafted grapevines, scion genotype can determine root development (Tandonnet et al. 2010). Limited soil nitrogen (N) content could enhance root growth in order to improve the acquisition of this particular nutrient (Grechi et al. 2007). Lateral root formation can be initiated by the presence of a high soil nitrate concentration, even when root N concentration is adequate (Dodd 2005). This suggests that nitrate could be considered as an N resource, as well as a signal that influences root system development. The grapevine root system responds to available N in soil with production of new roots that have a high capacity for N uptake (Volder et al. 2005).

Drivers of root system development

Due to the heterogeneity of the soil structure, water and nutrients will be located irregularly. It has been shown, however, that during periods of minimal transpiration, water movement within a single plant can occur from roots located in wet soil to roots in dry soil patches (Smart et al. 2005, Bauerle et al. 2008a). Despite the general belief that the rooting pattern is mainly due to the genetics of the rootstock (Pouget 1987), experiments have shown that the main driver for root development is soil water content (Morlat and Jacquet 1993, Conradie et al. 2002, Comas et al. 2005), which explains why it is possible to modify the rooting pattern through irrigation (Myburgh 1996, 2007, 2011, Soar and Loveys 2007). Soil structure and texture, which influence the nutrient retention and water-holding capacity of the soil and the air-to-water ratio (Figure 3), can affect root growth (Nagarajah 1987). Soil physical limitations, e.g. layers with a bulk density in excess of 1.4 kg/m³, can also limit root penetration and development in deeper layers (Van Huyssteen 1983). Grapevine roots cannot grow readily into soil if the penetration resistance exceeds about 2 MPa (Van Huyssteen 1988). A survey showed that this critical penetration resistance limited root system development in a wide range of Australian vineyard soils (Myburgh et al. 1996). In young, grafted grapevines, scion genotype can determine root development (Tandonnet et al. 2010). Limited soil nitrogen (N) content could enhance root growth in order to improve the acquisition of this particular nutrient (Grechi et al. 2007). Lateral root formation can be initiated by the presence of a high soil nitrate concentration, even when root N concentration is adequate (Dodd 2005). This suggests that nitrate could be considered as an N resource, as well as a signal that influences root system development. The grapevine root system responds to available N in soil with production of new roots that have a high capacity for N uptake (Volder et al. 2005).

In general, soil properties have a greater influence on root distribution than rootstock genotype (Southey and Archer 1988, Smart et al. 2006). Nevertheless, under similar soil conditions, rootstocks that differ in their ability to confer vigour and drought tolerance to the scion can give rise to differences in root origin of the rootstock on the root system development cannot be discarded, it is not possible to understand the role of the rootstock on the plant adaptation to drought without considering the exogenous factors and the genotype-environment interaction.
development, which could be related to different strategies to tolerate a water deficit. Under periods of water constraint, rootstocks that tend to induce more vigour and drought tolerance may exhibit more rapid root growth later in the season in wetter soil conditions (Bauerle et al. 2008b). In contrast, rootstocks that induce lower vigour and less drought tolerance could form more roots in deeper soil layers early in the growing season, no matter what soil moisture conditions prevail (Bauerle et al. 2008b). Such grapevines with deep root systems will be better buffered against drought conditions, particularly during the latter part of the season. Furthermore, it was found that roots located deeper in the soil have a longer lifespan in comparison with shallow roots (Anderson et al. 2003). In a similar way, it was found that drought-tolerant grapevine rootstocks formed more new roots in the soil profile during a dry, hot season, thereby increasing the uptake of water, compared with that of drought-sensitive rootstocks (Alśina et al. 2011). Since root tips are highly active in absorbing water (Zwieniecki et al. 2003), the formation of new roots could improve water supply to the plant (Alśina et al. 2011).

Various studies have been carried out to understand how grapevine canopy size and irrigation can affect root growth and lifespan dynamics (Anderson et al. 2003, Comas et al. 2005), as well as root metabolic activity (Comas et al. 2000). It is still not clear, however, whether different rootstock genotypes have a better tolerance for soil water deficits due to a longer root lifespan and/or different root metabolic activities, which allow improved water uptake and/or soil water deficit sensing via the roots. Several studies have found that rootstock genotypes vary in their nutrient acquisition capacity (Ruhl 1989, Grant and Matthews 1996, Keller et al. 2001, Mpelasoka et al. 2003) and that root physiology and age influence the rate of nutrient uptake (Volder et al. 2005). In a similar manner, rootstock genotypes have different mechanisms that involve root functioning and root tissue differentiation in response to a soil water deficit. Differences in root life span between balance pruned grapevines, i.e. 44 buds left per kilogram of cane prunings from the previous winter, and minimally pruned ones, i.e. only cutting the hanging stems to 1 m above the ground where canopy pruning decreased root life span, suggest that it might be due to differences in root composition related to carbon concentration (Comas et al. 2000).

Root functioning

Water uptake and transport

It has been proposed that water moves passively into roots as a result of a water potential controlled by transpiration (Steudle and Peterson 1998). Initially, water flows radially through the different tissues into the xylem vessels. This is followed by axial conductance, which depends on the size and number of xylem vessels (Tyrman et al. 2009). The composite transport model explains how the water flows through individual cells and various tissues (Steudle and Frensch 1996), involving apoplastic as well as cell-to-cell, i.e. symplastic and transcellular, pathways operating in parallel (Tyrman et al. 2009).

More drought-tolerant rootstocks have higher hydraulic conductance, which could be related to improved xylem development and lower vessel embolisation (Lovisolo et al. 2008b). One aspect that could explain these differences is the presence in plants of aquaporins (Maurel et al. 1993), which are special proteins that act as water conduits (Tyrman et al. 2009). Aquaporins are involved in the regulation of water movement across plasma membranes in the cell-to-cell pathway (Tyrman et al. 1999), and in the recovery from xylem embolism (Lovisolo and Schubert 2006). Eight putative aquaporins were identified that enabled a series of studies at the molecular level in 110 Richter (Baiges et al. 2001), which is considered to be a drought-tolerant rootstock (Keller 2010). Furthermore, it was found that the expression of the aquaporin genes in 110 Richter varied between the leaves and the roots (Galmés et al. 2007). In this study, it appeared that the expression of the aquaporin genes in the leaves decreased to limit water loss via transpiration, whereas the expression of the same aquaporin genes increased in the roots to enhance water uptake to avoid plant water constraints when water deficits occurred. This particular study also showed a negative correlation between stomatal conductance and abscisic acid (ABA) but not with leaf water potential and hydraulic conductivity in the plant. The latter is attributed in part to the expression of the aquaporins, which means that 110 Richter on own roots is able to maintain the same leaf water status, irrespective of the soil water deficit. During drought conditions, the intensity of aquaporin regulation in the roots of different *V. vinifera* cultivars determines their ability to tolerate soil water deficits (Vandeleur et al. 2009). Differences in aquaporin expression and activity between rootstocks have been detected mainly in the root tip (apical 2 cm of the fine root) in comparison with the mature root zone (10–20 cm behind the tip) (Gambetta et al. 2012).

The graft union, which can play a key role in water transport, is also an important aspect. A successful graft union has to differentiate functional phloem and xylem connections across the graft surface (Keller 2010) in order to allow the transport of water, nutrients and photosynthates. It has been shown that grafting can have a negative effect on the hydraulic conductivity (Bavaresco and Lovisolo 2000) and therefore on the development and lifespan of the scion. In general, the most important requirement for grafting is the formation of a normal vascular connection across the grafting area and, secondly, the maintenance of rootstock-to-scion communication (Aloni et al. 2010).

Nutrient uptake

In addition to water uptake, the adsorption of nutrients can have a significant impact on the vigour of grapevine vegetative growth. The apical regions of the root exhibit the greatest rate of nutrient uptake and a rapid decline in this capacity with age (Wells and Eissenstat 2003). A similar trend is shown with phosphate uptake in apple and citrus trees (Bouma et al. 2001). In the grapevine, the rate of nitrate uptake declines to 50% of the starting rate in fine lateral roots after a single day (Volder et al. 2005). Differences in nutrient uptake among grapevine rootstocks have been described mainly in relation to N (Keller et al. 2001), phosphorus (Grant and Matthews 1996) and potassium (Ruhl 1989, Mpelasoka et al. 2003). Therefore, the capacity of the rootstock to generate new roots will have a positive impact on the capacity of nutrient uptake.

Assessment of drought tolerance of different rootstocks

Drought tolerance varies among *Vitis* species and is related to the vine’s adaptation to their natural habitats (Whiting 2004). Several rankings of drought tolerance for grapevine rootstocks have been proposed (Pongrácz 1983, Padgett-Johnson et al. 2003, Dry 2007, Keller 2010), but there is no standardised methodology for the classification of rootstocks based on their drought tolerance. Different rankings for the same rootstock can be due to differences in the soil properties and climate where the trial was carried out, as well as the intensity and duration of water deficits imposed on the plants and the choice of drought-
related parameters that were studied. For example, early evaluations of drought tolerance induced by rootstocks were based primarily on vegetative vigour (trunk circumference), fruit attributes (berry size, berry colour estimate, total soluble solids and total acids) and yield (Lider 1957), but the latter has been the more important measure of rootstock adaptation in the past (May 1994). More recent studies have incorporated physiological indicators, such as stomatal conductance (Carbonneau 1985), leaf water potential (Ezzahouani and Williams 1995, Choné et al. 2001, Deloire et al. 2004, Williams 2010), ABA in the xylem, stomatal conductance (Iacono and Peterlunger 2000) and the chlorophyll content index (ratio of transmission at 931 nm to 653 nm through a leaf) in rootstocks (Pavlosek 2011). Nevertheless, a classification has been proposed by several authors based on field observations (Samson and Castéran 1971, Fregoni 1977) and evaluations in pots involving different levels of water deficit (Carbonneau 1985) (Table 1). It is important to note that the assessment of drought tolerance should consider the ability of a specific scion/rootstock combination to produce an acceptable yield under conditions of water deficit. The early detection of drought tolerance using parameters that correlate with yield is desirable. Nevertheless, some parameters measured, such as leaf water potential and instantaneous leaf water-use efficiency, are not always reflected in yield results (Whiting 2004).

Table 1. Rootstock classification based on adaptation to drought, as proposed by Samson and Castéran (1971), Fregoni (1977) and Carbonneau (1985) (adapted from Dillot, INRA Bordeaux, France, personal communication).

<table>
<thead>
<tr>
<th>Name</th>
<th>Crossing</th>
<th>Samson and Castéran</th>
<th>Fregoni</th>
<th>Carbonneau</th>
</tr>
</thead>
<tbody>
<tr>
<td>110R</td>
<td>V. rupestris × V. cinerea var. helleri ‘Resseguier#2’</td>
<td>Good</td>
<td>High resistance</td>
<td>High resistance</td>
</tr>
<tr>
<td>140Ru</td>
<td>V. rupestris × V. cinerea var. helleri ‘Resseguier#2’</td>
<td>Average</td>
<td>High resistance</td>
<td>High resistance</td>
</tr>
<tr>
<td>44–53M</td>
<td>V. riparia × V. cordifolia-V. rupestris</td>
<td>Good</td>
<td>High resistance</td>
<td>High resistance</td>
</tr>
<tr>
<td>1103P</td>
<td>V. rupestris × V. cinerea var. helleri ‘Resseguier#2’</td>
<td>Good</td>
<td>High resistance</td>
<td>Resistance</td>
</tr>
<tr>
<td>504</td>
<td>V. riparia × V. cinerea var. helleri ‘Resseguier#2’</td>
<td>Weak</td>
<td>Weak resistance</td>
<td>Resistance</td>
</tr>
<tr>
<td>99R</td>
<td>V. rupestris × V. cinerea var. helleri ‘Resseguier#2’</td>
<td>Average</td>
<td>Average resistance</td>
<td>Resistance</td>
</tr>
<tr>
<td>3309C</td>
<td>V. riparia × V. rupestris</td>
<td>Good</td>
<td>Weak resistance</td>
<td>Sensitive</td>
</tr>
<tr>
<td>240A MGt</td>
<td>V. riparia × V. cinerea var. helleri ‘Resseguier#2’</td>
<td>Weak</td>
<td>Weak resistance</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Feral</td>
<td>V. cinerea var. helleri ‘Resseguier#2’ × V. vinifera</td>
<td>Average</td>
<td></td>
<td>Sensitive</td>
</tr>
<tr>
<td>5BB</td>
<td>V. riparia × V. cinerea var. helleri ‘Resseguier#2’</td>
<td>Bad</td>
<td>Weak resistance</td>
<td>Sensitive</td>
</tr>
<tr>
<td>161–49C</td>
<td>V. riparia × V. cinerea var. helleri ‘Resseguier#2’</td>
<td>Weak</td>
<td>Mid resistance</td>
<td>Sensitive</td>
</tr>
<tr>
<td>41B MGt</td>
<td>V. cinerea var. helleri ‘Resseguier#2’ × V. vinifera</td>
<td>Average</td>
<td>High resistance</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Rupestris du Lot</td>
<td>V. rupestris</td>
<td>Bad</td>
<td>Weak resistance</td>
<td>Sensitive</td>
</tr>
<tr>
<td>101–14 Mt</td>
<td>V. riparia × V. rupestris</td>
<td>Bad</td>
<td>Weak resistance</td>
<td>Very sensitive</td>
</tr>
<tr>
<td>Riparia Gloire de Montpellier</td>
<td>V. riparia</td>
<td>Bad</td>
<td>Weak resistance</td>
<td>Very sensitive</td>
</tr>
<tr>
<td>313EM</td>
<td>V. cinerea var. helleri ‘Resseguier#2’ × V. vinifera</td>
<td>Good</td>
<td>Mid resistance</td>
<td>Very sensitive</td>
</tr>
</tbody>
</table>

Mechanisms of drought tolerance in rootstocks

Drought escape involves the ability of the plant to complete the whole life cycle before severe water constraint occurs. Drought tolerance with low plant water potential involves desiccation tolerance and the maintenance of turgor, mainly by osmotic adjustment. Drought tolerance with high plant water potential involves a reduction of water loss and an increase in water uptake, which is a way to avoid drought (Chaves and Oliveira 2004). Grapevines do not fall under the drought escape mechanism. Most of the grapevines cultivated around the world are located in a Mediterranean type of climate, meaning that most of the vegetative and reproductive growth occurs under moderate to severe water constraints if irrigation is not applied. Grapevine roots and rootstocks present drought tolerance mechanisms related to low and high plant water potential (Figure 4, Tables 2–4) involving drought responses, such as stomatal closure, decrease of cell growth and photosynthesis, activation of respiration, and accumulation of osmolytes and proteins (Shinozaki and Yamaguchi-Shinozaki 2007). In addition, grapevine rootstocks can affect leaf area and root development depending on the vigour inducing capacity (Gambetta et al. 2012) affecting the canopy water demand and supply. During dry hot seasons, higher vigour rootstocks can explore root zones to an extent greater than low vigour rootstocks (Bauerle et al. 2008b) and as a consequence can access water from deeper soil layers (a drought avoidance strategy). Gambetta et al. (2012) found that the higher canopy water demand due to the effect of rootstocks that promote scion vigour appears to be balanced by adjustments in root hydraulic conductivity through fine root hydraulic conductivity and higher root surface area. The mechanisms involved can develop in different time scales, from minutes to months. For example, an adjustment to stomatal conductance can occur within minutes or less, whereas osmotic adjustment and the response to ABA can occur in hours and adaptations in terms of root system development can take several days or weeks (Passioura 1996).

Although many genes related to drought response have been identified, their physiological relevance is not always known (Chaves et al. 2003). Drought-tolerance characteristics are controlled by many genes, known as quantitative traits (Bartels and Sunkar 2005), which will complicate the understanding of the plant response to water deficits at a molecular level. QTLs are regions within genomes that contain genes associated with a particular quantitative trait (Jones et al. 1997). Recently, a study carried out on QTLs identified one genomic region of the grapevine rootstock that was related to water extraction capacity and scion transpiration and acclimation (Marguerit et al. 2012). This finding supports previous hypotheses that rootstocks differ in their ability to provide water to the
scion and that chemical signalling, primarily ABA, and hydraulic signalling via aquaporins regulate stomatal conductance.

Osmotic adjustment in roots
Osmotic adjustment, i.e. the active accumulation of solutes involving inorganic solutes taken up from the substrate and organic solutes synthesised by the plant, is a response to drought that enables maintained water absorption and cell turgor pressure (Cattivelli et al. 2008). Evidence for a decrease in osmotic potential in grapevine roots in response to drought was reported by Düring (1984). Furthermore, osmotic adjustment in roots and the maintenance of a positive root water status in grapevines subjected to soil water deficits were shown to have a positive influence on leaf gas exchange (Düring and Dry 1995). It was also speculated that osmotic adjustment may reduce the sensitivity of roots as sensors, and therefore restrict the production of root signals such as ABA (Düring and Dry 1995).

Control of water loss
Many studies have shown that rootstocks can modify their leaf gas exchange capability in response to water deficit conditions (Candolfi-Vasconcelos et al. 1994, Düring 1994, Bica et al. 2000, Padgett-Johnson et al. 2000). Such responses, however, could vary according to different rootstock/scion combinations (Keller et al. 2012), as well as the level of water deficit experienced (Soar et al. 2006). The effect of rootstock on the photosynthetic capacity of the scion appears to increase under higher water constraint conditions (Soar et al. 2006). Under well-watered conditions, it has been reported that the scion genotype predominates the determination of transpiration efficiency, i.e. the CO₂ assimilation to H₂O transpiration ratio compared with the rootstock (Gibberd et al. 2001, Virgona et al. 2003). In the absence of root-to-shoot signals, differences in the leaf anatomy of the scion might play a more relevant role in the regulation of photosynthesis, as they can present different mesophyll conductance to CO₂ (gₘ), i.e. the capacity for CO₂ diffusion inside leaves (Flexas et al. 2008). It has been shown that differences in leaf anatomical properties associated with differences in gₘ explained the differences in photosynthesis between two pine species (Peguero-Pina et al. 2012). In relation to grapevines, it has been suggested that the level of gₘ could be related to the carboxylation efficiency of the specific genotype (Düring 2003). Furthermore, it has been shown that grapevine shoots have some ability to regulate ABA concentration under conditions of low water constraints, independent of root-to-shoot signalling (Soar et al. 2004).

Water losses could also be reduced by limiting transpiration through the regulation of stomatal conductance. Under conditions of water constraint, drought-sensitive rootstocks induce a lower stomatal conductance of the scion, leading to a higher reduction in photosynthetic carbon assimilation rates compared with that of drought-tolerant rootstocks (Alsina et al. 2011). Stomatal density and stomatal size determine the possible maximum stomatal conductance (Franks and Beerling 2009).
The control of stomatal movement is mediated by changes in guard cell turgor, cytoskeleton organisation, membrane transport and gene expression (Hetherington 2001). Many mechanisms for stomatal regulation have been postulated, such as changes in hydraulic conductivity (Schultz 2003, Christmann et al. 2007), abscisic acid synthesis (Davies et al. 2005, Dodd 2005, Jiang and Hartung 2008) and alkalinisation of the xylem pH (Davies et al. 2002, 2005). Grapevine roots are responsible for sensing the soil water deficit and sending a signal to the shoots, thereby primarily regulating shoot growth and water use (Lovisolo et al. 2010).

Chemical signalling is based on evidence that stomatal closure is well correlated with soil water deficits, whereas it only correlates weakly with leaf water potential (Comstock 2002). Abscisic acid is one of the most studied hormones and is considered to be the most important in root-to-shoot water deficit signalling (Davies et al. 2005, Schachtman and Goodger 2008). This does not, however, rule out the possibility that other compounds are involved (Schachtman and Goodger 2008). It has been confirmed that ABA is synthesised in the roots in response to drought (Lovisolo et al. 2002). Following this, ABA is transported via the xylem to the aerial parts of the plant, where it

Table 2. Proposed drought-tolerance mechanism via stomatal regulation based on chemical signalling originating either from scions or rootstocks from field or pots experiments.

<table>
<thead>
<tr>
<th>Criteria used to measure stomatal regulation by chemical signalling</th>
<th>Scion/rootstock</th>
<th>Genetic origin of the root system</th>
<th>Set up</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root, stem and leaf ABA</td>
<td>Pinot Noir grafted onto 5BB</td>
<td>V. cinerea var. helleri ‘Resseguier#2’ × V. riparia</td>
<td>P</td>
<td>Lovisolo et al. (2002)</td>
</tr>
<tr>
<td>Leaf xylem ABA</td>
<td>Monastrell grafted onto 1103 P SO4</td>
<td>V. cinerea var. helleri ‘Resseguier#2’ × V. rupestris</td>
<td>F</td>
<td>Romero et al. (2012)</td>
</tr>
<tr>
<td>Leaf xylem ABA</td>
<td>Shiraz grafted onto 5C SO4 140 Ru, Ramsey, K51-40, 420A MGt, Schwarzmann, Shiraz own roots</td>
<td>V. cinerea var. helleri ‘Resseguier#2’ × V. rupestris</td>
<td>F</td>
<td>Soar et al. (2006)</td>
</tr>
<tr>
<td>Leaf xylem ABA, xylem pH and exogenous ABA with different pH buffers</td>
<td>V. riparia × V. labrusca</td>
<td>V. riparia × V. labrusca</td>
<td>P</td>
<td>Li et al. (2011)</td>
</tr>
<tr>
<td>Foliar ABA and phaseic acid</td>
<td>Cabernet Sauvignon own roots</td>
<td>V. vinifera</td>
<td>P</td>
<td>Loveys and Kriedemann (1974)</td>
</tr>
<tr>
<td>Endogenous ABA, exogenous ABA and benzyladenine</td>
<td>Bacchus own roots Forta own roots Müller-Thurgau own roots Riesling own roots</td>
<td>V. vinifera</td>
<td>P</td>
<td>Düring and Broquedis (1980)</td>
</tr>
<tr>
<td>Exogenous benzyladenine, leaf xylem sap ABA, xylem sap pH, ABA and cytokinins (zeatin + zeatin riboside) from roots</td>
<td>Cabernet Sauvignon own roots Chardonnay own roots Sultana own roots</td>
<td>V. vinifera</td>
<td>F and P</td>
<td>Stoll et al. (2000)</td>
</tr>
<tr>
<td>Transcript abundance of genes (ABA and cytokinin) of plants under water and salinity constraints in comparison with plants with no constraint state</td>
<td>Cabernet Sauvignon own roots</td>
<td>V. vinifera</td>
<td>P</td>
<td>Cramer et al. (2007)</td>
</tr>
<tr>
<td>Bulk leaf ABA, leaf xylem ABA, root ABA and xylem pH</td>
<td>Mavrodafni own roots Sabatiano own roots</td>
<td>V. vinifera</td>
<td>P</td>
<td>Beis and Patakas (2010)</td>
</tr>
</tbody>
</table>

ABA, abscisic acid; F, field; P, pots.
Table 3. Proposed drought tolerance mechanism via stomatal regulation in combination with various other regulating or signalling mechanisms from field or pots experiments.

<table>
<thead>
<tr>
<th>Stomatal regulation mechanism</th>
<th>Criteria used to measure stomatal regulation by non-chemical signalling</th>
<th>Criteria used to measure stomatal regulation by chemical signalling</th>
<th>Scion/rootstock</th>
<th>Genetic origin of the root system</th>
<th>Set up</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical signalling and regulation of homeostasis by aquaporins</td>
<td>Expression of aquaporins genes in roots and leaves</td>
<td>Leaf xylem ABA</td>
<td>110 R on own roots</td>
<td>V. cinerea var. helleri ‘Resseguier#2’ × V. rupestris</td>
<td>P</td>
<td>Galmés et al. (2007)</td>
</tr>
<tr>
<td>Chemical signalling and embolism repair by aquaporins</td>
<td>Hydraulic conductivity recovery of root, shoot and leaf petiole</td>
<td>Foliar ABA</td>
<td>Grenache grafted onto 420A MG</td>
<td>V. riparia × V. cinerea var. helleri ‘Resseguier#2’</td>
<td>P</td>
<td>Lovisolo et al. (2008a)</td>
</tr>
<tr>
<td>Hydraulic signalling</td>
<td>Leaf-specific hydraulic conductance</td>
<td>None</td>
<td>Grenache and Syrah grafted onto V. rupestris × V. cinerea var. helleri ‘Resseguier#2’</td>
<td>V. rupestris × V. cinerea var. helleri ‘Resseguier#2’</td>
<td>F</td>
<td>Schultz (2003)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical and hydraulic signalling</td>
<td>Root hydraulic conductance</td>
<td>None</td>
<td>Chardonnay own roots</td>
<td>V. vinifera</td>
<td>P</td>
<td>Vandeleur et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>Plant water status</td>
<td>Foliar ABA</td>
<td>Concord own roots</td>
<td>V. labruscana</td>
<td>P</td>
<td>Liu et al. (1978)</td>
</tr>
<tr>
<td></td>
<td>Plant hydraulic conductivity</td>
<td>Leaf xylem ABA</td>
<td>110 R on own roots</td>
<td>V. cinerea var. helleri ‘Resseguier#2’ × V. rupestris</td>
<td>P</td>
<td>Pou et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>Plant water status</td>
<td>Leaf xylem ABA and xylem sap pH</td>
<td>Castelão and Muscat of Alexandria grafted onto 1103 P</td>
<td>V. cinerea var. helleri ‘Resseguier#2’ × V. rupestris</td>
<td>F</td>
<td>Rodrigues et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>QTLs identification with genes associated to hydraulic regulation</td>
<td>QTLs identification with genes associated to ABA regulation</td>
<td>Cabernet Sauvignon grafted onto V. vinifera cv. Cabernet Sauvignon × V. riparia cv. Gloire de Montpellier</td>
<td>V. vinifera × V. riparia</td>
<td>P</td>
<td>Marguerit et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>Leaf water potential</td>
<td>Leaf xylem ABA and exogenous ABA application to roots</td>
<td>Semillon own roots</td>
<td>V. vinifera</td>
<td>F</td>
<td>Rogiers et al. (2012)</td>
</tr>
</tbody>
</table>

ABA, abscisic acid; F, field; P, pots; QTL, quantitative traits loci.
regulates stomatal functioning and the activity of shoot meristems (Jiang and Hartung 2008). In *V. vinifera*, there are two genes, *VvNCED* and *VvZEP*, that have been described putatively to be involved in the ABA biosynthetic pathway (Soar et al. 2004) in response to soil water deficit in the roots (Seo and Koshiba 2002). Soar et al. (2006) have suggested that a difference in concentration in xylem ABA among rootstocks is not due to their ability to synthesise ABA but primarily due to a difference in water content constraints experienced by the rootstock genotypes caused by variable water uptake capacity. The intensity of the root-to-shoot ABA signal is regulated at four anatomical levels: (i) the rhizosphere; (ii) the root cortex; (iii) the stem; and (iv) the leaves (Jiang and Hartung 2008). In *V. riparia* × *V. labrusca*, the intensity of the root-sourced ABA signal is intensified along its way, due in part to a higher xylem pH at higher node positions, resulting in a lower stomatal conductance of leaves at higher nodes compared with that of lower nodes on the stem (Li et al. 2011). Consequently, the stomatal conductance of leaves at higher nodes along the stem is lower compared with that of leaves at lower nodes. Cytokinins (CKs), which are synthesised mainly in the roots (Aloni et al. 2005), have been described as an antagonist to ABA in stomatal closure (Dodd 2005). In *V. vinifera*, zeatin and zeatin riboside have been found to be reduced by partial root zone drying (Stoll et al. 2008). Nevertheless, the mechanisms involved are still under debate (Buckley 2005). Using *Arabidopsis* mutants that are deficient in ABA biosynthesis and defective in ABA signalling, it was demonstrated that water constraint-induced stomatal closure requires hydraulic as well as ABA signals (Christmann et al. 2007). It was concluded that the generation of the hydraulic signal is not dependent on ABA biosynthesis and/or ABA signalling, which proves that the hydraulic signal precedes the ABA signal. It was found that own-rooted grapevine cultivars that differ in their response to soil water deficits via differences in the regulation of the leaf water potential also vary in their root response to water soil deficits in terms of aquaporin expression (Vandeleur et al. 2009). This finding suggests a close relationship between root water transport and shoot transpiration. Domec and Johnson (2012) suggested that whole-plant hydraulic conductance is driven by leaf hydraulic conductance under no water deficit and by root hydraulic conductance under water deficit.

The relative importance of chemical and hydraulic signalling in the control of stomatal functioning is debatable (Chaves et al. 2010). Some grapevine studies have concluded that hydraulic signals play a dominant role when water deficits occur (Rodrigues et al. 2008), whereas others have shown that the control is primarily due to ABA signalling and that hydraulic signalling plays a secondary role (Pou et al. 2008). Only hydraulic signalling, however, is involved during recovery from water deficits (Pou et al. 2008). Hydraulic and chemical signalling are considered to be the most important mechanisms in the regulation of stomatal conductance, and these signals probably function in an integrated way (Comstock 2002, Rodrigues et al. 2008).

Our results showed that stomatal density and size, i.e. number of stomata per unit area, are affected by water constraints and light and that the same scion grafted onto different rootstock cultivars can have different stomatal densities and sizes (Figures 5 and 6). Soil water deficit induced a response in stomatal size (Figures 5 and 6). Soil water deficit induced a response in stomatal density but bigger pore diameter than leaves growing under full light and that the same scion grafted onto different rootstock cultivars can have different stomatal densities and sizes (Figures 5 and 6). Soil water deficit induced a response in stomatal size (Figures 5 and 6). Soil water deficit induced a response in stomatal density but bigger pore diameter than leaves growing under full

### Table 4. Proposed grapevine tolerance to drought via osmotic adjustment, aquaporins and root foraging on its own or in combination with different levels of plant water status regulation from field or pots experiments.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Criteria used to measure plant water status regulation</th>
<th>Scion/rootstock</th>
<th>Genetic origin of the root system</th>
<th>Set up</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmotic adjustment in roots</td>
<td>Osmotic potential of roots</td>
<td>Silvano own roots</td>
<td><em>V. vinifera</em></td>
<td>P</td>
<td>Düring (1984)</td>
</tr>
<tr>
<td></td>
<td>Osmotic potential of roots</td>
<td>Riesling own roots</td>
<td><em>V. vinifera</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Presence of aquaporins</td>
<td>5BB on own roots</td>
<td><em>V. cinerea var. helleri</em> × <em>V. riparia</em></td>
<td>P</td>
<td>Düring and Dry (1995)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>110 R on own roots</td>
<td><em>V. cinerea var. helleri</em> × <em>V. rupestris</em></td>
<td>P</td>
<td>Baiges et al. (2001)</td>
</tr>
<tr>
<td>Root foraging</td>
<td>Root growth dynamics in response to soil moisture availability</td>
<td>1103 P and 101-14 MGt</td>
<td><em>V. cinerea var. helleri</em> × <em>V. rupestris</em></td>
<td>F</td>
<td>Bauerle et al. (2008b)</td>
</tr>
<tr>
<td>Root foraging and different degree of stomatal conductance control</td>
<td>Root growth dynamics and hydraulic conductance</td>
<td>Merlot grafted onto 1103 P and 101-14 MGt</td>
<td><em>V. cinerea var. helleri</em> × <em>V. rupestris</em></td>
<td>F</td>
<td>Alsina et al. (2011)</td>
</tr>
</tbody>
</table>

F. field; P. pots.
These results might have implications in the interaction of vigour induced by the rootstock (canopy microclimate) and canopy water demand. Significant differences in stomatal density and size were observed on Pinotage leaves grafted onto different rootstocks, where plants grafted onto 140 Ruggeri presented lower stomatal density but bigger pore diameter than those grafted onto 110 Richter and 1103 Paulsen (Figure 6). Scienza and Boselli (1981) found that rootstocks considered drought tolerant have lower stomatal density in their leaves in comparison with that of rootstocks considered drought sensitive. The mechanisms involved in stomatal development, as affected by rootstock, cannot be explained at this stage. It is hypothesised, however, that differences in hydraulic conductance between rootstocks affect the plant water status, thereby affecting leaf growth, and that they consequently cause variability in stomatal density and size that is closely related to leaf gas exchange and water use efficiency (Xu and Zhou 2008).

Scion and rootstock interaction

There is a differential response of roots and shoots to water deficits. Under drought conditions, vegetative growth, e.g. internode elongation, leaf expansion and tendril extension, as well as transpiration will be reduced (Lovisolo et al. 2010). Nevertheless, the root system is less sensitive to drought. Grapevines can rehydrate ‘dry’ roots with water moved through the root system at night (Bauerle et al. 2008a). It has been shown that grapevine root growth is enhanced under moderate water constraints but decreased under severe water constraints (Van Zyl 1984). It has been postulated that a higher root-to-shoot ratio...
Rootstocks to enhance drought tolerance in grapevine

Choosing a rootstock is an important decision not only because of the potential benefit that viticulturists expect, such as resistance to pests (phylloxera and nematodes), pathogens in the soil and drought tolerance, but also because establishing a vineyard is a long-term investment. The rootstock influence on vigour and the effect on drought tolerance need to be clarified; aspects, such as differences in root growth, root hydraulic capacity and stomatal development, should be taken into consideration. Most of the previous studies have focused on root system development and structure but little is known about the genetic regulation of root branching and root mineral uptake. Molecular studies of grapevine drought tolerance are limited, and even more so studies considering rootstocks, compared with research carried out on cereals, for example. Understanding the mechanisms of drought tolerance induced and regulated by rootstocks might be helpful for breeding programmes in order to develop more drought tolerant rootstocks.

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