Trade-off between hydraulic sensitivity, root hydraulic conductivity and water use efficiency in grafted *Prunus* under water deficit

Guillermo Toro [a], Claudio Pastenes [b], Ariel Salvatierra [a], Paula Pimientel [a,*]

[a] *Centro de Estudios Avanzados en Fruticultura (CEAF), Las Parcelas 882, Rancagua, Chile*
[b] *Departamento de Producción Agrícola, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago, Chile*

ARTICLE INFO

ABSTRACT

Sweet cherry is mainly cultivated in arid and semi-arid areas. In the last decade, these areas have experienced a dramatic reduction in rainfall, which has resulted in water shortage for sweet cherry. The use of specific rootstock and scion combinations could help improve the tolerance of plants to water shortage events. This study reports on the influence of rootstocks on whole-plant performance under water deficit as detected by hydraulic sensitivity, root hydraulic conductivity (L_p), water use efficiency and sugar content. Four *Prunus* rootstocks/scion combinations - 'Bing/Colt', 'Lapins/Colt', 'Bing/Mx60', 'Lapins/Mx60', and two self-rooted rootstocks Colt and Maxma 60 - were acclimated for 30 days and then exposed to well-watered (WW) and water deficit (WD) conditions for 36 days. Whole-plant transpiration and growth were both influenced by WD, and two groups were identified based on responses: 'Bing/Colt', 'Bing/Mx60' and 'Mx60' had an early reduction (conservative strategy), whereas 'Lapins/Mx60', 'Lapins/Colt' and 'Colt' had late reduction (productive strategy) in transpiration as WD increased. Among the combinations, 'Lapins/Colt' and 'Colt' showed a remarkable growth response to the WD being less affected in shoot and root biomass. The 'Colt' rootstock maintained a higher Ψ_{gs50} (near-isohydric behavior) than combinations using the 'Mx60' rootstock (near anisohydric behavior). The relationship between L_p and the variation of $\Psi_{pre-dawn}$-Ψ_{midday} showed differences among rootstock/scion combinations, and under WD condition the reduction in L_p induced by WD affected the whole-plant WUE of combinations differently. Sucrose and sorbitol content in leaves and roots of WD-tolerant combinations such as 'Colt' and 'Lapins/Colt', showed a remarkable increase under WD condition. Our finding highlights the importance of the specific interaction between rootstock and scion, suggesting that combinations characterized by a higher water uptake capacity under conditions of lower water availability would be sustainable under minimal to moderate water deficit.

1. Introduction

Water deficit is an adverse component of climate change that threatens the productivity of different ecosystems (Pareek et al., 2020). With the reduction of rainfall and the increase in temperature over the past few decades, the magnitude and severity of the drought events have increasingly affected irrigated crops (Hussain et al., 2019), representing one of the major causes of agricultural production loss worldwide (Campbell et al., 2016; Lesk et al., 2016). In the last 10 years, the annual precipitation in central Chile has decreased by 55–75% resulting in a period of megadrought (Garreaud et al., 2017), while in arid and semi-arid regions an increased water shortage is expected as intensity and frequency of rainfall decrease (Edenhofer, 2015; Núñez et al.,

2011).

Sweet cherry (*Prunus avium* L.) has become one of the most appealing and economically prolific fruit crops around the world (Gonçalves et al., 2021). In general, sweet cherry is grown under controlled and scheduled irrigation systems (Blanco et al., 2018), and water limitation is therefore expected to negatively affect the productivity and yield (Blanco et al., 2020). The use of rootstocks has been widely adopted around the world due to benefits related to resistance to soil diseases and abiotic stresses such as water limitations (Pedroso et al., 2014). The ability to cope with water stress in grafted sweet cherry plants depends on the integration of the communication mechanisms between the scion and the rootstock (Serra et al., 2014). Species within the *Prunus* genus have been widely distributed throughout the world (Gonçalves et al., 2021) and exhibit a

variety of physiological strategies when confronting water deficit, making it possible to identify and select genotypes with high tolerance to water limitation (Jiménez et al., 2013; Opazo et al., 2020).

Water deficit leads to a decrease in plant water content and turgor, and consequently causes a decrease in cellular expansion and alters various vital physiological, biochemical and molecular processes (Shakeel et al., 2011). Under water deficit, the most critical response is the decline in stomata conductance because of its negative effect on photosynthesis; stomatal closure decrease the availability of CO_2 at the carboxylation sites, reducing both shoot and root growth and ultimately affecting productivity (Lovisolo et al., 2010; Mashilo et al., 2017). A study previously reported a strong reduction in gas exchange in grafted *Prunus* induced by water shortage, but also found a high water-use efficiency (WUE) associated with the most vigorous rootstocks (Jiménez et al., 2013). The importance of the rootstock how grafted plants tolerate a water deficit has been reported for several species (Opazo et al., 2020; Silva et al., 2021; Wright et al., 2019), and the influence of rootstocks on the hydraulic strategies are key focal points in the response to water limitations.

Contrasting information has been reported on hydraulic strategies that would define water deficit tolerance among species (Gambetta et al., 2020). Classically, plants have been classified on the iso- to anisohydric trait continuum according to their stomatal behavior as the water deficit increases (Martínez-Vilalta et al., 2014). There are at least three metrics to evaluate hydraulic strategies according the stomatal control of water loss based on the plant water potential during water deficit: i) the slope (σ) of the relationship between pre-dawn (Ψpre-dawn) and Ψmidday (Martínez-Vilalta et al., 2014), ii) the region bounded by Ψpre-dawn-Ψmidday regression and 1:1 line, namely the hydroscape area (Li et al., 2019), and iii) the relationship between stomatal conductance (gs) and Ψmidday (Carminati and Javaux, 2020). The use of different metrics could provide a wide perspective of the response of different species, cultivars, and even between rootstock and scion to water deficit conditions. For instance, species with stringent stomatal control and a small hydroscape area tend to be more vulnerable to embolism, and less likely to protect stem hydraulic integrity (Li et al., 2019). Recently, this metric has been used to distinguish stomatal behavior among almond cultivars (Álvarez-Maldini et al., 2021).

From the agronomic point of view (Sade et al., 2012), under minimal or moderate water deficit, a near anisohydric behavior may have a productive advantage over the more isohydric behavior by keeping the stomata open at lower water potentials (Pou et al., 2012). In trees, it is often observed that isohydric species are not necessarily limited by carbon starvation compared to anisohydric species during extreme water deficit because of higher carbon reserves (Garcia-Forner et al., 2017). Plant productivity under water limitations depends on the sensitivity of the plant and its stomatal responsiveness to water deficit. Early stomatal closure caused by water depletion is useful in conserving water (conservative strategy), but inconvenient for CO_2 assimilation, forcing the plant to rely on stored carbohydrates (mainly in woody species) (Hartmann et al., 2021; Moshelion, 2020). On the other hand, in late stomatal closure plants, the sugar production is maintained by keeping stomata open longer during water deficit (productive strategy) with the risk of hydraulic failure (Hartmann et al., 2021; Moshelion, 2020; Sade et al., 2012; Zacarias Rafael et al., 2020). Delayed or early stomatal closure (productive or conservative strategies, respectively) can be considered one of the most important traits in maintaining the productivity of plants under water limitation. There is evidence that under water deficit, the tolerant *Prunus* rootstock induces a late decline in transpiration and an increased WUE, affecting the biomass accumulation less than in water deficit-sensitive rootstocks (Opazo et al., 2020). Recently, it has been reported that the first cause of stomatal closure is associated with hydraulic restrictions in roots rather than other factors related to cavitation (Carminati and Javaux, 2020; Rodriguez-Dominguez and Brodribb, 2020).

Root hydraulic conductance is sensitive to water limitations, likely controlling the whole-plant hydraulic conductance (Rodriguez-Dominguez and Brodribb, 2020) as well as the plant vigor (Gonçalves et al., 2007; Jiménez et al., 2013). Evidence suggests that the differences in number, size or frequency of the xylem vessels between *Prunus* rootstocks and scion could strongly impact on the root hydraulic conductance (Olmstead et al., 2006b; Peschiutta et al., 2013). Furthermore, root hydraulic conductance can substantially affect the gas exchange (Rodríguez-Gamir et al., 2016), although root hydraulic conductance is poorly correlated with a direct effect on the WUE under water deficit.

WUE refers to the relationship between carbon gain and its associated cost in water (Medrano et al., 2018). In *Prunus*, the carbon gain is mainly related to the formation of two types of sugars, sorbitol and sucrose (Lo Bianco et al., 2000) The dynamic accumulation and role of sorbitol and sucrose in leaves and roots is considerably affected by water deficit (Noiraud et al., 2001). Sorbitol is one of the main sugar alcohols that has a key role in osmotic adjustment (Jiménez et al., 2013; Lo Bianco et al., 2000; Ranney et al., 1991). In fact, most vigorous *Prunus* rootstock under water deficit had a high WUE, which was explained by the osmotic adjustment due to high accumulation of sorbitol in the leaves (Jiménez et al., 2013). A similar result was reported for grapevine, where osmotic adjustment promoted the maintenance of open stomata at lower water potential (Pou et al., 2012). On the other hand, the role of sucrose in plant responses to water deficit is related to maintaining the energy level in growing tissues, for instance, preserving root respiration to sustain root growth, and then water uptake capacity (Silva et al., 2021).

Chile ranks fourth among the countries with the largest production and second with highest exportation of sweet cherries around the world (Gonçalves et al., 2021). The main sweet cherry producing regions are located in central Chile areas, which is currently suffering severe water scarcity, which is predicted to worsen in the near future (Garreaud et al., 2020). Therefore, the development and assessment of new sweet cherry rootstocks adapted to local climate conditions is urgent, as is the physiological characterization of different rootstock/scion combinations to better understand the effects of water stress in grafted plants. Here, we hypothesized that in grafted sweet cherry plants, the stomatal regulation is controlled by the rootstock through root hydraulic conductivity, in turn affecting water uptake capacity and WUE. We aimed to determine the influence of rootstocks on whole plant performance under water deficit through hydraulic sensitivity, root hydraulic conductivity and water use efficiency.

2. Materials and methods

2.1. Plant material and growth conditions

The experiment was conducted in the summer season using 2-years-old rootstocks/scion combinations of sweet cherry in vegetative stage. Two genotypes of *Prunus avium* L. were used as scion: 'Bing' (Black Republican x OP) and 'Lapins' (Van x Stella) (Fernandez i Marti et al., 2012), selected on the based on the commercial relevance in Chile. The scions were grafted onto two vigorous commercial genotypes of *Prunus* rootstocks: Colt (*Prunus avium* (L.) L. × *P. pseudocerasus* Lindl.) and Maxma 60 ('Mx60') (*Prunus mahaleb* x *P. avium*), generating the following combinations: 'Bing/Colt', 'Lapins/Colt', 'Bing/Mx60', 'Lapins/Mx60', and self-rooted 'Colt' and 'Mx60' (for descriptive convenience, we will refer also self-rooted as "plants or rootstock/scion combination"). The rootstock/scion combinations generated in this study correspond to those most used in sweet cherry fields in central zone of Chile (AGV, 2022). Plants were potted in 20-L container filled with a substrate mixture of 1:1 perlite:peat supplemented with Basacote® Plus 9 M 16–8–12 (BASF, Limburgerhof, Germany) at 6 g L^{-1} (slow release fertilizer). Before the experiment began, all plants were maintained during 30 days for acclimation under greenhouse conditions (with polycarbonate panels) and watered daily. During the experiment, the night/day temperature was 17/30 ºC, and the relative humidity

fluctuated between 40% and 50% and with a photoperiod of 16/8 day/night and a maximum light intensity of 1000 ± 55 µmol PAR m^{-2} s^{-1} on the top of the plants at midday.

2.2. Treatments of water availability

After acclimation, the rootstock/scion combinations were grown under two water availability conditions: well-watered (WW) and water deficit (WD). One day before starting the substrate dry-down, all pots (both WW and WD conditions) were saturated with water and left to drain overnight to constant weight., In the next morning, the pots were covered with a black plastic film to avoid the water evaporation from the substrate. After the weight of each pot at maximum substrate water holding capacity, the field capacity (FC) was determined. Each pot was weighted daily (PWD) at the same time each morning to calculate the water to be added.

To setting WW condition, five plants of each rootstock/scion combination were daily weighted and manually irrigated when the water content of the substrate is less than 80% of the FC, keeping the water content in each pot as constant as possible close to 75–85% of the FC. The amount of water to add under WW condition was calculated as follows: FC−PWD− (FC−[0.8 ×FC]). To setting the WD condition, to five plants of each combination, the substrate was allowed to gradually dry-down hand watering an 80% of the water transpired on the previous day, this allows to avoid a drastic physiological impact due to water deficit. The progressive water deficit was maintained for 36 days until the plants reached the permanent wilting point.

2.3. Normalized transpiration rate and fraction of transpirable soil water threshold

All pots were weighed daily in the evening and the daily transpiration rate (DTR) was calculated as the difference in weight of each pot on successive days. At the end of the experiment, the total transpirable substrate water (TTSW) to each pot was calculated as the difference between the initial weight (IW) and final weight (FW). The FW of pots was established near to permanent wilting point of each plant.

The fraction of transpirable soil water (FTSW) was calculated by equation proposed by Sinclair and Ludlow (1986) as follows:

$$FTSW = \frac{DW - FW}{TTSW}$$

where, DW correspond to daily weight of each pot.

The plant transpiration under WD and WW conditions were used to calculate the transpiration rate (TR) by the equation as follows:

$$TR = \frac{DTR}{DTR_a}$$

where, DTR_a is the mean of DTR of the five pots under WW conditions to each plant combination. To normalize the TR (NTR) and centred around 1.0, since plants under water deficit may vary the size, the DTR of each plant was divided by the mean DTR of four days while the plants were under WW condition (Sinclair and Ludlow, 1986). The fraction of transpirable soil water threshold (FTSW$_{threshold}$) was calculated at the point when the NTR began to decline in an 80%.

2.4. Plant growth

At the beginning of the experiment, one plant per each rootstock/scion combination under each water availability condition were taken from an extra batch of plants. At the end of the experiment, one plant per each rootstock/scion combination under each water availability condition from the same batch of plants of the experiment were carefully extracted from substrate mixture, and roots were gently cleaned and separated from shoot. Plant, root and shoot dry biomass was determined

after drying in an oven at 70 °C for 72 h.

2.5. Stomatal conductance and leaf water potential

Stomatal conductance (g_s) was measured in one fully expanded and photosynthetically active leaf per each rootstock/scion combination under each water availability condition by using a CIRAS-2 portable IRGA photosynthesis system (PPSystem, Hitchin, UK) with a controlled environment CIRAS PLC cuvette (broad windows 2.5 cm^2). The CO_2 concentration inside the cuvette was adjusted to 400 µmol mol^{-1}. Air temperature and relative humidity inside the chamber were recorder, ranging 30–35 °C and 35–45%, respectively. g_s was measured from 10:00–13:30 h on clear days.

Leaf water potential was measured in pre-dawn ($\Psi_{pre-dawn}$) before sunrise (4:30–6:30 h) and midday (Ψ_{midday}) nearby to solar noon (12:30–14:30 h) on fully sun-exposed leaves (different of stomatal condition) per each rootstock/scion combination under each water availability condition using a Schölander pressure chamber (PMS Instrument Company, Corvallis, OR, USA). Each leaf was carefully cut and quickly placed inside the Schölander chamber with the petiole protruding from the lid, and the xylem sap emerging from the cut end was observed with an amplifier glass. The daily variation of leaf water potential was calculated by $\Psi_{pre-dawn}$ - Ψ_{midday} (Klein, 2014).

To know the stomatal sensitivity of the different rootstock/scion combinations to water deficit, the relationship between $\Psi_{pre-dawn}$ and Ψ_{midday} was performed according to Fu and Meinzer (2019). The slope of the entire trajectory of the linear regression (σ) between $\Psi_{pre-dawn}$ and Ψ_{midday} was used to know the relative sensitivity of the transpiration rate and hydraulic conductance. Then, the plant behavior to water deficit was determined as follow: σ < 1 as partial isohydric, σ = 1 as strict anisohydric, and σ > 1 as extreme anisohydric. To calculate the hydroscape area (HA), the stomatal regulation of Ψ_{midday} prior to complete stomatal closure ($\Psi_{pre-dawn} = \Psi_{midday}$) was considered. Then, the HA was calculated according to Fu and Meinzer (2019), as follows:

$$HA = \frac{\alpha^2}{2(1 - \beta)}$$

where α is the estimate Ψ_{midday} when $\Psi_{pre-dawn} = 0$ and β correspond to the slope of the linear regression between α and the point where $\Psi_{pre-dawn} = \Psi_{midday}$.

In order to analyze the relationship between g_s and Ψ_{midday} we performed a logistic function ($g_s(\Psi_{midday})$) according to Klein (2014) and Guyot et al. (2012), as follow:

$$g_s = \frac{g_{s\,max}}{1 + \left(\frac{\Psi_{midday}}{\Psi_{gs50}}\right)^s}$$

where, $g_{s\,max}$ is the maximum g_s reached, Ψ_{midday} is the midday leaf water potential, Ψ_{gs50} is the midday leaf water potential when g_s decline in a 50% respect to $g_{s\,max}$ and s correspond to the rate of g_s declining as a function to Ψ_{midday}.

2.6. Root hydraulic conductivity

The root hydraulic conductance in intact root system was determined at the end of the experiment using a high-pressure flow meter (HPFM, Dynamax, USA), as described by (Tyree et al., 1995). Briefly, the method consists of measuring the water flow of whole root system applying different pressure levels. Before measurements, five whole and intact root systems of each rootstock/scion combination per water availability condition were separated from the aerial part by cutting the stem about 10 cm above the substrate surface. The HPFM was connected to each whole-root system by pressure couplings, and setting as transient mode (Tyree et al., 1995). The pressure applied into the root system was increased 5 kPa s^{-1}, and both pressure and flow were recorded every 2 s.

The root hydraulic conductance was calculated as the slope of the curve between pressure and flow applied. Root hydraulic conductivity (L_p) was determined dividing root hydraulic conductance by total root dry biomass and expressed as mmol H_2O s^{-1} MPa^{-1} g^{-1} DW.

2.7. Whole-plant water-use efficiency

Five whole-plants of each combination and treatment were harvested at the start and the end of the experiment to quantify the initial (DW_i) and final (DW_f) dry biomass of the whole-plant. The total water consumed during the experiment was calculated as the sum of the daily water consumption. The whole-plant water-use efficiency (WUE) was determined according to Tomás et al. (2012) as follows:

$$\text{WUE} = \frac{(DW_f - DW_i)}{\text{total water consumed}}$$

2.8. Determination of sorbitol and sucrose concentration

At the end of the experiment, one gram of leaf and root tissue were collected from per rootstock/scion combination under each water availability condition after gas exchange measurement. The complete procedure to take samples and sugar analysis were performed according to Pastenes et al. (2014). Briefly, samples were carefully cut from the petiole and immediately frozen in liquid nitrogen and stored at − 80 ºC until the analysis. Both tissues were ground with liquid nitrogen to a fine powder. For soluble solids 0.5–1 g were transferred to tubes containing 10 mL of Milli-Q water and incubated at 60 ºC for 30 min. The extracts were clarified by centrifugation for at $4000 \times g$ and 4 ºC by 10 min 600 μL of supernatant were mixed with 600 μL of acetonitrile, centrifuged for at $12000 \times g$ and 4 ºC by 10 min and filtered through a 0.22 mm membrane.

The sugars (sorbitol and sucrose) were analyzed using an Agilent 1200 series HPLC system. Finally, 20 μL of each sample were injected. The sugars were detected by a refractive index detector with the reference cell maintained at 45 ºC. A Zorbax carbohydrate column (4.6 mm × 150 mm, 5 μm) was used with a Zorbax NH_2 guard column cartridge (4.6 mm × 12.5 mm, 5 μm). The column was kept at 35 °C. The samples were separated with acetonitrile:water (75:25) and a flow rate of 1.5 mL min^{-1}. Sorbitol and sucrose were detected for their retention time and quantifications were performed using the external standard method with commercial standards (Sigma Chemical Co.). Sorbitol and sucrose concentration were expressed in mg g^{-1} FW.

2.9. Experimental design and statistical analysis

The experiment was performed using a completely randomized design with five replications. Six levels of rootstock-scion combinations were used: 'Bing/Colt', 'Lapins/Colt', 'Bing/Mx60', 'Lapins/Mx60', 'Colt' and 'Mx60' and two levels of water availability: well-watered (WW) and water deficit (WD). A two-way analysis of variance (ANOVA) was conducted on the influence of two independent factors (rootstock/scion combinations, water availability) on all parameters and Tukey's range (multiple comparison) test was performed when differences were identified. The R software was used for statistical analysis and graphics (Team, 2013). All data from linear or non-linear regression were analyzed and performed using R software (Team, 2013).

3. Results

3.1. Plant transpiration and growth responses to water deficit

Fig. 1 shows the response of the transpiration rate of each rootstock/scion combination to water reductions at the root level. The NTR of the
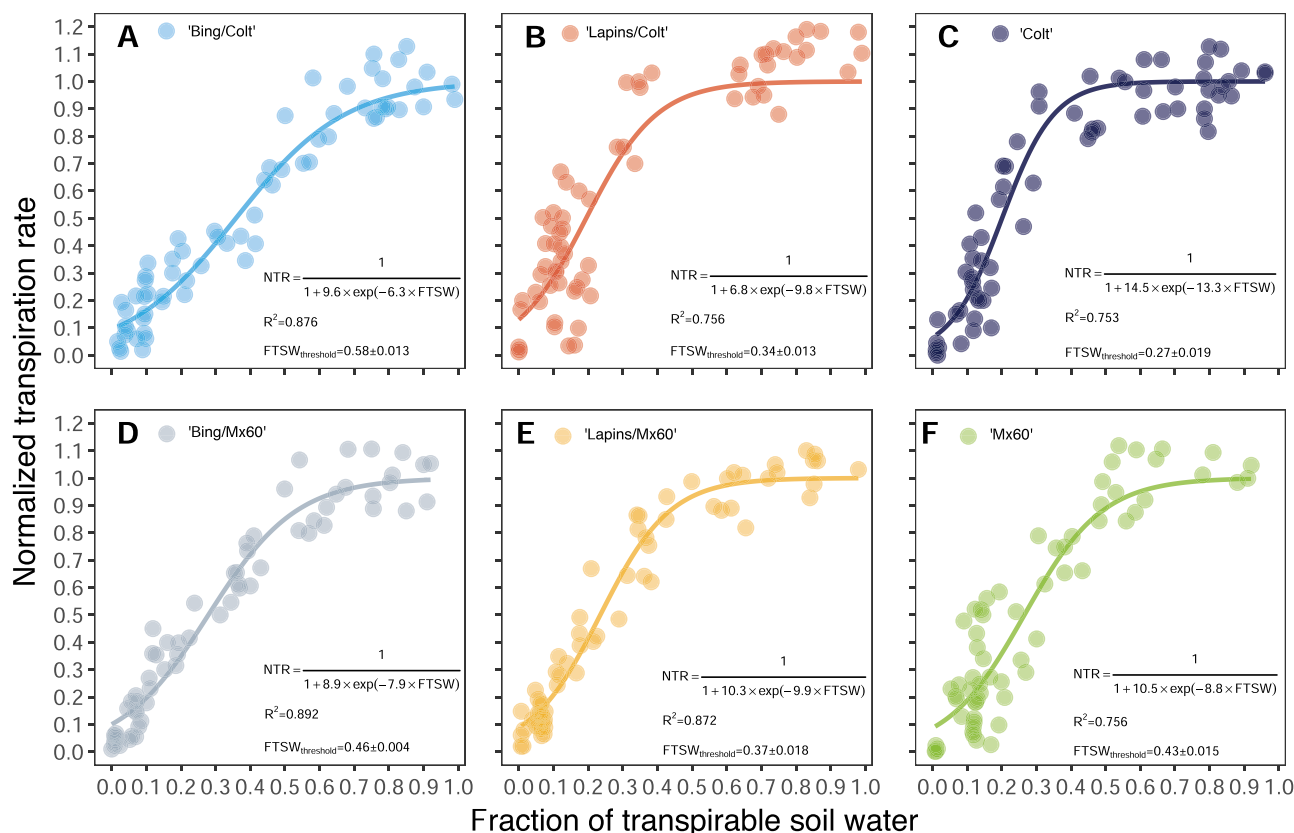

Fig. 1. Variability of the relationship between normalized transpiration rate (NTR) and fraction of transferable soil water (FTSW) of four rootstock/scion combinations and two self-rooted *Prunus* plants under water deficit condition. Each data point represents the average of five replicate of each rootstock/scion combinations of the NTR related to FTSW to each day during the experiment.

rootstock/scion combinations started declining at FTSW values with FTSW$_{threshold}$ ranging between 0.58 and 0.27 (Fig. 1). Two groups of behaviors were identified in the different rootstock/scion combinations, which exhibit a contrasting NTR response to FTSW. The first group is represented by combinations with early reduction of NTR as the FTSW decreases, thus with higher values of FTSW$_{threshold}$ such as 'Bing/Colt', 'Bing/Mx60' and 'Mx60' (0.58, 0.46 and 0.43, respectively) (Fig. 1A, D and F). By contrast, the second group is represented by combinations with a late reduction of NTR as the FTSW decreases, i.e., low FTSW$_{threshhold}$ values, as in 'Lapins/Colt', 'Colt' and 'Lapins/Mx60' (0.37, 0.34 and 0.27, respectively) (Fig. 1B, C and E).

The impact of WD on shoot, root and plant biomass on the rootstock/scion combinations is shown in Fig. 2. Under WW condition, the shoot biomass weight was similar in all rootstock/scion combinations, but WD negatively affected the shoot biomass of 'Bing/Colt', decreasing between 50% and 75% and 'Bing/Mx60', 'Lapins/Mx60' and 'Mx60' with reductions of nearly 50% (Fig. 2A). By contrast, both 'Lapins/Colt' and 'Colt' were less affected by WD reducing the shoot biomass in around 25% (Fig. 2A). Under WW condition, there were no difference between the rootstocks/scion combinations (Fig. 2B). However, the WD negatively affected the root biomass accumulation of 'Bing/Mx60', 'Lapins/Mx60' and 'Mx60' in 25%, and to 'Bing/Colt' in a 50%, while that of 'Lapins/Colt' and 'Colt' were not affected by WD (Fig. 2B). Under WD condition, the most affected combinations in plant biomass were those using 'Mx60' as rootstock, and the 'Bing/Colt' combination, while the best performance was observed in 'Lapins/Colt' and 'Colt', which maintained the plant biomass productivity similar to the WW condition (Fig. 2C).

3.2. Response of stomatal conductance to changes in leaf water potential

To determine the different behaviors to water deficit, the response of g_s to the changes in the Ψ_{midday} was studied in the different rootstock-scion combinations. Fig. 3 shows a strong logistic relationship between Ψ_{midday} and g_s of the rootstock/scion combinations. The $g_{s\ max}$ values (Fig. 3, Table 1) ranged between 0.521 and 0.369, where 'Lapins/Colt' and 'Bing/Mx60' reached the highest $g_{s\ max}$ and 'Colt' the lowest. 'Colt' rootstock induced higher Ψ_{gs50} values, nearly − 1.28 MPa on average (Fig. 3A, Table 1), while scions using 'Mx60' reached lower Ψ_{gs50} values, − 1.70 MPa on average (Fig. 3B, Table 1). The rate of g_s reduction to Ψ_{midday} decline is shown as the slope of the linear portion of the logistic regression (s), and no differences were found among the rootstock/scion combinations (Table 1).

3.3. Sensitivity to water deficit of rootstock/scion combinations

The Ψ_{midday} vs. $\Psi_{pre-dawn}$ relationship of the different rootstock/scion

combinations showed a clear variability in plant sensitivity to water deficit (Fig. 4). 'Bing/Colt' and 'Lapins/Mx60' had the highest sensitivity of the transpiration rate and hydraulic conductance (σ) to the decrease in water availability, behaving as partially isohydric with σ values of 0.73 and 0.75, respectively (Fig. 4A, Table 2). 'Lapins/Colt', 'Colt' and 'Bing/Mx60' showed a behavior close to strict anisohydric with σ of 0.99, 0.98 and 0.93, respectively (Fig. 4A, Table 2), while 'Mx60' showed a lower plant sensitivity to WD indicating an extreme anisohydric behavior with a σ values of 1.23 (Fig. 4A, Table 2). The hydroscape area (HA) showed a wide variability ranging from 0.9 to 5.2 MPa^2 among rootstock/scion combinations (Fig. 4B). 'Colt' and 'Mx60' rootstocks showed no significant differences in the HA, averaging 3.4 MPa2 (Fig. 4B and Table 2). 'Mx60' rootstock induced similar HA for 'Bing/Mx60' and 'Lapins/Mx60' with 2.5 and 2.2 MPa^2 of HA, respectively (Fig. 4B and Table 2). On the other hand, 'Colt' rootstock induced a higher HA in 'Lapins/Colt' but not in 'Bing/Colt' (Fig. 4B and Table 2). 'Lapins/Colt' showed the largest (p < 0.001) HA of the rootstock/scion combinations, which was 108% and 130% higher than 'Bing/Mx60' and 'Lapins/Mx60', respectively, and 5-fold higher than 'Bing/Colt' (p < 0.05) (Fig. 4B and Table 2).

3.4. Relationship between root hydraulic conductivity on the daily variation of leaf water potential and whole-plant water use efficiency

The positive exponential relationship between L_p and the variation of $\Psi_{pre-dawn}$-Ψ_{midday} showed differences among rootstock/scion combinations (Fig. 5A). After 36 days of experiment, sensitivity to WD condition that determined a slight variation in $\Psi_{pre-dawn}$-Ψ_{midday} could be due to the reduction in L_p (Fig. 5A and Table 3). 'Lapins/Colt', 'Bing/Mx60' and 'Mx60' slightly reduced the variation of $\Psi_{pre-dawn}$-Ψ_{midday} compared to the reduction in the L_p, while 'Lapins/Mx60' and 'Colt' experienced a reduction of $\Psi_{pre-dawn}$-Ψ_{midday} induced by a drastic reduction in L_p; moreover, 'Bing/Colt' showed an extreme reduction in $\Psi_{pre-dawn}$-Ψ_{midday} compared to L_p (Fig. 5A and Table 3). In the same vein, the reduction in L_p induced by water deficit affected the whole-plant WUE (Fig. 5B and Table 3). Under WW condition, high whole-plant WUE was found in 'Colt', with high L_p, but the variability in L_p had no effect on whole-plant WUE in the other rootstock/scion combinations (Fig. 5B and Table 3). However, we found that the WD condition generated changes in the whole-plant WUE associated with changes in the L_p (Fig. 5B and Table 3). We observed that under WD condition, 'Colt', 'Lapins/Colt', 'Mx60' and 'Bing/Mx60' reduced, on average, 35% of L_p compared to the WW condition, while 'Lapins/Mx60' and 'Bing/Colt' reduced L_p by 60% and 80% compared to the WW condition (Fig. 5B and Table 3).
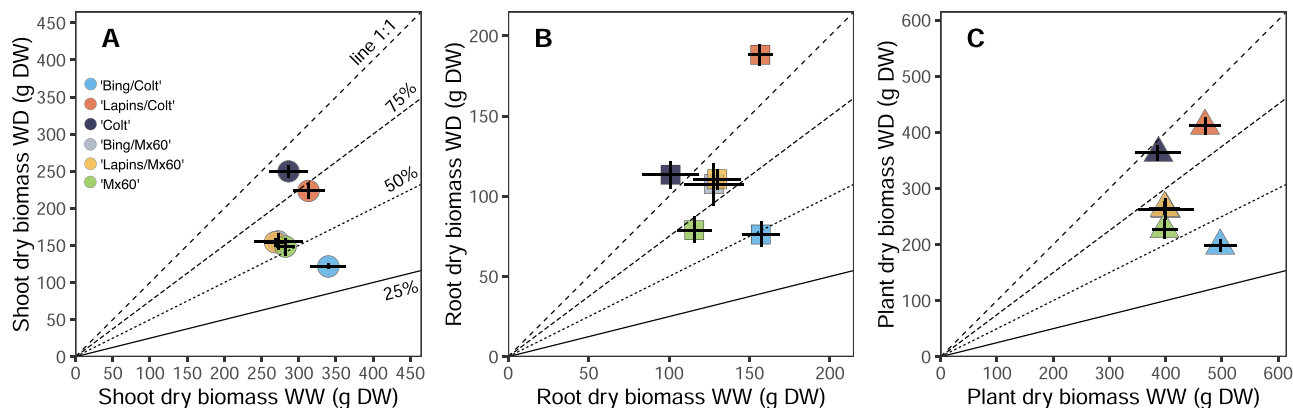

Fig. 2. Relationship of dry biomass of four rootstock/scion combinations and two self-rooted *Prunus* plants under water deficit and well-watered conditions. A) shoot (circle symbol), B) root (square symbol) and C) plant (triangle). n = 5.
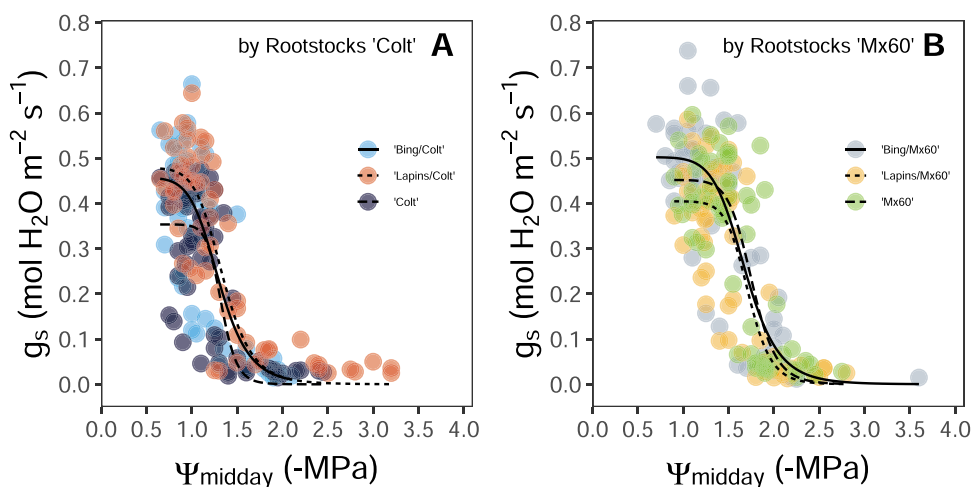
Fig. 3. Relationship between midday leaf water potential (Ψ_{midday}) and stomatal conductance (g_s) of different rootstock/scion combinations grafted onto A) 'Colt' and B) 'Mx60' rootstocks.

Table 1

Average of the parameters derived from the stomatal conductance (g_s) vs. midday leaf water potential (Ψ_{midday}) relationship of different rootstocks/scion combinations under WW and WD conditions. The parameters showed correspond to maximum stomatal conductance ($g_{s\ max}$, mol H_2O m^{-2} s^{-1}), leaf water potential at 50% of $g_{s\ max}$ (Ψ_{gs50}, MPa) and the slope of the lineal part of the curve (s, mol MPa m^{-2} s^{-1}). Data are means $\pm$ standard error, n = 5.

Combinations	$g_{s\ max}$		Ψ_{gs50}		s		R^2
'Bing/Colt'	0.440 ± 0.01	a b	-1.28 ± 0.10	a	7.8 ± 2	a	0.720
'Lapins/Colt'	0.484 ± 0.03	a	-1.26 ± 0.06	a	6.5 ± 2	a	0.671
'Colt'	0.369 ± 0.02	b	-1.31 ± 0.04	a	15.7 ± 7	a	0.520
'Bing/Mx60'	0.521 ± 0.02	a	-1.63 ± 0.10	b	9.7 ± 2	a	0.699
'Lapins/Mx60'	0.417 ± 0.03	a b	-1.70 ± 0.08	b	13.6 ± 4	a	0.550
'Mx60'	0.448 ± 0.02	a b	-1.78 ± 0.04	b	13.2 ± 4	a	0.668

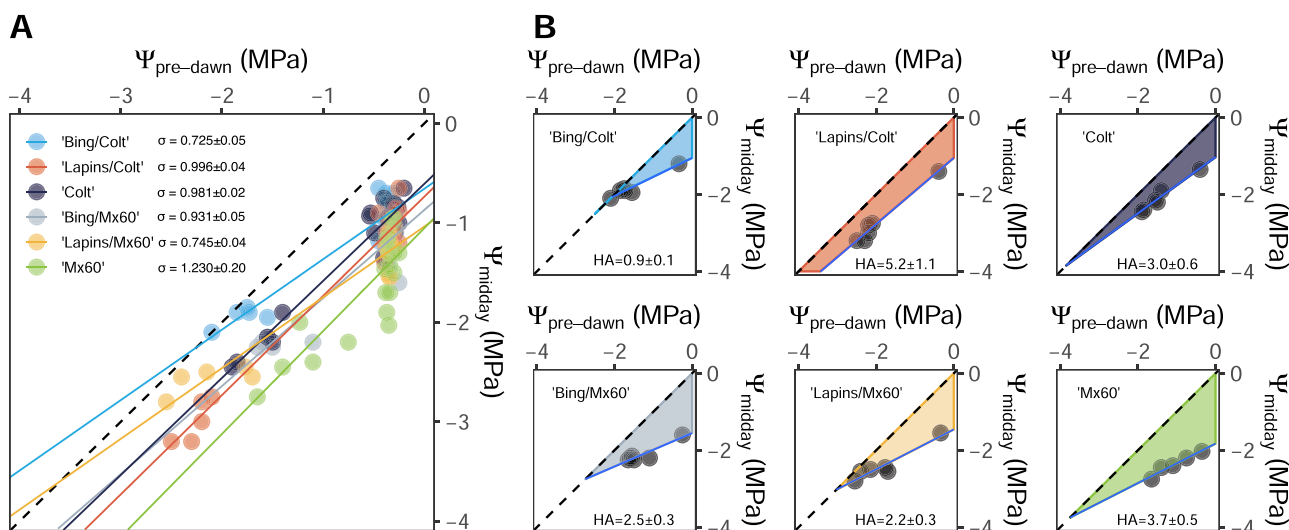Different letters indicate significant differences (p < 0.05) among rootstock/scion combinations.

Fig. 4. A) Relationship between pre-dawn ($\Psi_{pre-dawn}$) and midday (Ψ_{midday}) leaf water potential and B) hydroscape area of four rootstock/scion combinations and two self-rooted *Prunus* plants under water deficit condition. The black dashed represent 1:1 line. Linear regression lines are also depicted for each plant. The intercept (λ) and the slope (σ) of the relationship are shown in the insert.

3.5. Sucrose and sorbitol and content in roots and leaves

There was a significant effect of water deficit on the sucrose and sorbitol contents in both the leaves and roots of the different rootstock/scion combinations. Fig. 6 shows the relationship between the WW and WD conditions with the sucrose and sorbitol content in leaves and roots. At the end of the experiment, the WD reduced the sucrose content of the

leaves of 'Bing/Colt', 'Bing/Mx60' and 'Lapins/Mx60' by 25% compared to the WW condition (Fig. 6A). In roots, the sucrose content of 'Colt' was significantly lower (between 50% and 75%), followed by 'Lapins/Colt' and 'Bing/Mx60' (almost 50%), while 'Lapins/Mx60', 'Bing/Colt' and 'Mx60' tended to increase compared to the WW condition (Fig. 6B). To the leaf sorbitol content, a significant increase was found in 'Lapins/Colt', 'Colt' and 'Mx60' induced by the WD condition,

Table 2

Average of the parameters derived from the relationship between pre-dawn ($\Psi_{\text{pre-dawn}}$) and midday (Ψ_{midday}) leaf water potential and plant sensitivity of the different rootstock/scion combinations under well-watered and water deficit conditions. The parameters showed correspond to the slope of the linear regression between $\Psi_{\text{pre-dawn}}$ and Ψ_{midday} (σ, the relative sensitivity of the transpiration rate and hydraulic conductance) and the hydroscape area (HA). Data are means ± standard error, n = 5.

Combinations	σ		HA		Plant sensitivity
'Bing/Colt'	0.725 ± 0.05	b	0.962 ± 0.12	c	Partial isohydric
'Lapins/Colt'	0.996 ± 0.04	a b	5.169 ± 1.15	a	Strict anisohydric
'Colt'	0.981 ± 0.02	a b	3.019 ± 0.64	a b c	Strict anisohydric
'Bing/Mx60'	0.931 ± 0.05	a b	2.528 ± 0.26	b c	Strict anisohydric
'Lapins/Mx60'	0.745 ± 0.04	b	2.175 ± 0.33	b c	Partial isohydric
'Mx60'	1.230 ± 0.20	a	3.732 ± 0.48	a b	Extreme anisohydric

Different letters indicate significant differences (p < 0.05) among rootstock/scion combinations.

while 'Bing/Colt' and 'Lapins/Mx60' decreased the sorbitol content by 25% and 'Bing/Mx60' showed no change (Fig. 6C). The sorbitol content in the roots showed significant differences in most combinations; however, the WD induced a remarkable increase in the sorbitol content of 'Colt' and 'Lapins/Mx60' (Fig. 6D).

4. Discussion

In this study we investigated whether the relationship between rootstock and scion plays a role in the response to water deficit, associated with an improved performance of water uptake capacity, variability of stomatal conductance and leaf water potential as well as root hydraulic conductivity and whole-plant water use efficiency. We found that *Prunus* rootstocks with a higher water uptake capacity increased the productivity (biomass) and water use efficiency (g DW L^{-1} H$_2$O) of a specific rootstock/scion combination under water deficit. Then, in grafted *Prunus* under water deficit, the rootstock greatly affects the scion stomatal regulation controlling the transpiration through hydraulic mechanisms at the root.

The water movement across the plant depends on the sum of resistances from root to shoot (Venturas et al., 2017). The transpiratory

stream may be negatively affected by several environmental conditions, but water deficit is one of the most detrimental, affecting plant growth and crop yield (Lovisolo et al., 2010; Mashilo et al., 2017). In grafted crops such as *Prunus*, rootstock is key to the regulation of the water uptake capacity as the water deficit increases (Opazo et al., 2020). In general, there is a wide variability in the hydric balance of crops in response to water deficit, which are in a trade-off between carbon gain and the risk of deleterious soil water depletion (Sade et al., 2012; Tardieu et al., 2018). In *Prunus*, some species can take advantage of low soil water content, even below a threshold where transpiration is reduced by more than 80%, and thus maximize carbon gain (Opazo et al., 2020, 2019). In our study, we found that rootstocks regulate the plant transpiratory stream in response to a decrease in the FTSW (Fig. 1). Our results highlight two contrasting responses, in which 'Colt' rootstock maintains transpiration as the water deficit increases, while 'Mx60' reacts early to low water availability, reducing transpiration when FTSW is still close to maximum (Fig. 1). The maintenance of high g$_s$ under water deficit, sustaining plant growth, may be related to a "productive" strategy by the crops, which could result in a better agronomic performance (Sade et al., 2012). By contrast, more "conservative" stomatal response, convenient for maintaining the soil water availability (Adiredjo et al., 2018; Casadebaig et al., 2008; Sinclair and Muchow, 2001). These opposite behaviors found in 'Colt' and 'Mx60' rootstocks are transferred partially to scion, because 'Colt' affects 'Lapins', but did not on 'Bing', while 'Mx60' transfers the "conservative" trait both to the 'Lapins' and 'Bing' scions. In grafted citrus, Santana-Vieira et al. (2016) found that the transfer of behavior from rootstock to variety was linked mainly to hormonal a mechanism, determining either the productive or conservative strategies in response to dehydration.

The strategy for transpiratory regulation that a crop may adopt will directly affect both crop growth and productivity (Opazo et al., 2020; Sade et al., 2012). Our results show that the productivity of

Table 3

Two-ways ANOVA performed between water availability conditions (Treatments) and rootstock/scion combinations (Combinations) on root hydraulic conductance (L$_p$, mmol H$_2$O s^{-1} MPa^{-1} g^{-1} DW), the differences between $\Psi_{\text{pre-dawn}}$ and Ψ_{midday}, and whole-plant water-use efficiency (WUE, g DW L^{-1} H$_2$O).

Factors	L$_p$	$\Psi_{\text{pre-dawn}}$-Ψ_{midday}	Whole-plant WUE
Treatment	* *	*	ns
Combinations	*	* *	* *
Treatment x Combinations	* **	* *	* **

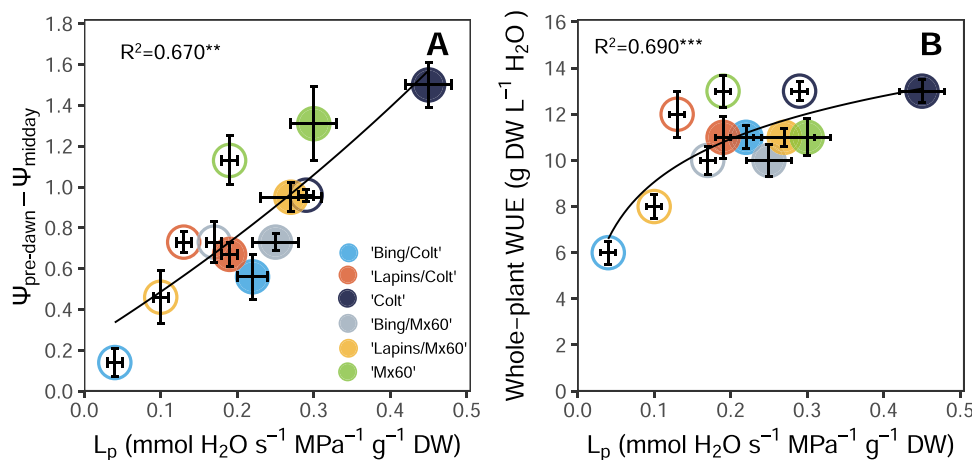Asterisk indicates significant level, p < 0.001 * ** ; p < 0.01 * *; p < 0.05 * . ns: not significant.

Fig. 5. Relationship between root hydraulic conductivity (L$_p$) and the difference in pre-dawn ($\Psi_{\text{pre-dawn}}$) and midday (Ψ_{midday}) leaf water potential A) and whole-plant water use efficiency (WUE) B) of four rootstock/scion combinations and two self-rooted *Prunus* plants under well-watered (filled circles) and water deficit (open circles) for 36 days. Data on graph are represented by means of each rootstock/scion combination under well-watered and water deficit conditions, n = 5.
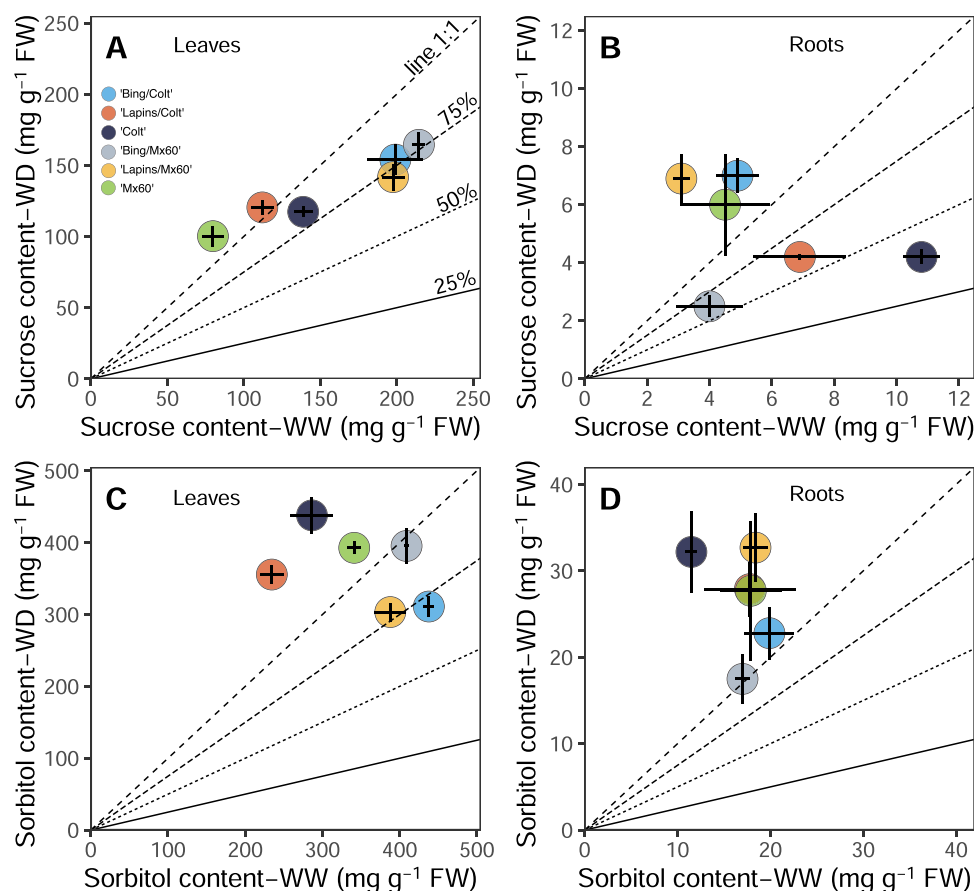
Fig. 6. Relationship of sucrose (A and B) and sorbitol (C and D) contents in both leaves and roots of four rootstock/scion combinations and two self-rooted *Prunus* plant between well-watered and water deficit conditions after 36 days. Data on graph are represented by means of each rootstock/scion combinations, n = 5.

rootstock/scion combinations under water deficit, as whole-plant biomass accumulation, was partially less reduced with 'Colt' as root-stock ('Bing/Colt'), but the rootstock/scion combinations using 'Mx60' rootstock exhibited a higher reduction of plant growth under water deficit (Fig. 2C). In addition, the strategy observed in 'Colt' was asso-ciated with root growth by favoring carbon allocation to this organ (Fig. 2B). This could suggest a possible link between the "productive" strategy and the allocation of resources to the specific respiratory components of growth and ion uptake over the maintenance component. Although this has not been previously reported for water deficit, it has been reported for stresses such as saline and hypoxia (Del-Saz et al., 2017; Toro et al., 2018). Since the success of sweet cherry crops depends on an optimum water supply, orchards are equipped with controlled drip irrigation systems. However, it is possible to reduce irrigation in sweet cherry orchards without a negative effect on productivity, with significant potential water savings. In this context, the use of root-stock/scion combinations with a "productive" strategy could be more convenient than combinations with "conservative" responses (Sade et al., 2012).

Stomatal regulation is the most important mechanism for the control of water losses from leaf to atmosphere (Buckley, 2019). Typically, two kinds of plant sensitivity have been described to explain the stomata behavior under water deficit. Near-isohydric behavior indicates a nar-row range of experienced leaf water potential due to a high g_s sensitivity to water deficit, while near-anisohydric behavior indicates a wider range of leaf water potential resulting from a lower stomatal sensitivity (Carminati and Javaux, 2020; Hochberg et al., 2018; Martínez-Vilalta et al., 2014; Tardieu et al., 2018). Stomatal regulation may be affected by environmental conditions, leaf or root hormone signaling and hy-draulic response or both, and even in grafted plants, the rootstock could

be directly involved in stomatal control (Hochberg et al., 2013; Lavoie-Lamoureux et al., 2017; Peccoux et al., 2018). We found that 'Colt' rootstock maintained a higher Ψ_{gs50} (near-isohydric behavior) than combinations using the 'Mx60' rootstock (near-anisohydric behavior), even with similar g_s of 0.230 µmol m^{-2} s^{-1} (Fig. 3). This result supports the idea that rootstock could control the stomatal response mainly through hydraulic responses rather than hormonal signaling (mainly ABA). According to Peccoux et al. (2018), the grape-vine rootstock controls the transpiration rate, g_s and Ψ_{midday} of shoot, mainly through hydraulic responses rather than through ABA signaling, and the Ψ_{midday} could be purely related to a hydraulic response of the rootstock, closely related to the root-soil interface (Carminati and Jav-aux, 2020). In fact, recent findings introduce the parameter Ψ_{gs50} as a support to synthesize belowground interactions between soil drying and stomata regulation because Ψ_{gs50} is not only a function of leaf traits, but also dependent on the soil-root hydraulic condition (Carminati and Javaux, 2020). Then, this would suggest that 'Colt' rootstock exhibit mechanisms associated with the root hydraulics which maintain hy-draulic safety (higher Ψ_{midday}) despite a reduction in g_s down to a 50% of g_{smax}.

It should be noted that in order to have a broader perspective and knowledge about plant behavior under water deficit, it could be desir-able to use several metrics for an accurate analysis (Martínez-Vilalta et al., 2014) and use caution in comparing the response mechanisms for stomatal control and the concomitant leaf water potential (Garcia--Forner et al., 2017). Plant response to water deficit has commonly been determined as follows: σ < 1 as partial isohydric, σ = 1 as strict aniso-hydric, and σ > 1 as extreme anisohydric (Fu and Meinzer, 2019). We found differences between rootstocks in stomatal behavior through g_s-Ψ_{midday} curves (Fig. 3) but no differences were found in the

relationship between $\Psi_{pre\text{-}dawn}$ and Ψ_{midday} (Fig. 4). In this regard, our results on the stomatal sensitivity deducted from the Ψ_{midday} to $\Psi_{pre\text{-}dawn}$ relationship, showed three categories of stomatal regulation: partial isohydric ($\sigma < 1$) in 'Bing/Colt' and 'Lapins/Mx60', strict anisohydric ($\sigma = 1$) in 'Lapins/Colt', 'Colt' and 'Bing/Mx60', and extreme aniso-hydric ($\sigma > 1$) in 'Mx60' (Fig. 4 and Table 2). The differences between may be due, in part, to the complexity between the stomatal response and leaf water potential, since the water potential gradient under low water availability would not be determined directly by stomatal sensitivity but by the sensitivity of the plant hydraulic system as a whole, as reported by Martínez-Vilalta et al. (2014). These authors also argue that highly stomatal sensitive plants could still show a strict anisohydric behavior provided that their hydraulic transport system is even more sensitive than the stomata to reductions in water availability. Thus, 'Lapins/Colt' and 'Colt' (and pertinent to 'Lapins/Mx60') will show a critically high leaf water potential (isohydric) and low sensitivity of the transpiration rate and plant hydraulic conductance, running the risk of hydraulic failure (strict anisohydric) as the water availability declines. By contrast, 'Bing/Colt' consistently showed an isohydric behavior ($\sigma < 1$) and was highly and negatively affected by water deficit. These criteria for stomatal behavior are consistent with the hydroscape area, which according to Meinzer et al. (2016) integrates more accurate information on the degree of iso- or anisohydrism than other metrics. In *Prunus*, Álvarez-Maldini et al. (2021) found that cultivars more tolerant to water deficit showed a large hydroscape area, which was related to anisohydric behavior and maintenance of photosynthetic capacity compared to the cultivars with lower hydroscape area. Our results showed that 'Bing/Colt' has the most stringent stomatal control and small hydroscape area, which could explain its lower biomass accumulation due to carbon limitations. Carbon starvation due to early stomatal closure could explain the low biomass accumulation exhibited by the 'Bing/Colt' combination (Fig. 2); however, it has been previously reported that isohydric plants will not necessarily be carbon starved by making use of the reserves present in stems and roots (Garcia-Forner et al., 2017).

Root hydraulic conductance is a physiological trait sensitive to water deficit (Rodriguez-Dominguez and Brodribb, 2020), and in most cases, it has been reported that hydraulic conductivity loss is closely associated with gain in hydraulic safety (Gonçalves et al., 2007). In addition, it has recently been demonstrated that the decrease in root hydraulic conductance and even in the hydraulic of the root-soil interface is the primary cause of hydraulic limitation leading to stomatal closure prior to cavitation (Carminati and Javaux, 2020; Rodriguez-Dominguez and Brodribb, 2020). In this regard, we found at the end of the experiment that the most conservative combination in water use, 'Bing/Colt', loses functionality of the root hydraulic conductivity at lower water potential gradients ($\Psi_{pre\text{-}dawn}$-Ψ_{midday}), while more productive combinations do not reduce the root hydraulic conductivity (as 'Colt'), favoring the dynamics of the soil-to-leaf water potential gradient (Fig. 5A). Recently, Rodriguez-Dominguez and Brodribb (2020) reported that in olive plants under moderate water stress (Ψ_{stem}=−2.5 MPa) at least 95% of the whole-plant hydraulic resistance is controlled by root hydraulic resistance (i.e., a radial pathway from the xylem to the soil-root interface). In this regard, one limitation of our study was the lack of continuous measurements of root hydraulic conductance and its correlation with data obtained from root cross-sections. However, our results suggest that the differences found in root hydraulic conductance are associated with specific resistances resulting from differences in the diameter of xylem vessels between rootstock and scion from each combination.

In our study, we found that despite 'Lapins' and 'Bing' scions being grafted onto the same rootstocks ('Colt' or 'Mx60'), both expressed differences arguably associated with genetic background; for instance, the remarkable differences in hydraulic conductivity were exhibited between 'Lapins/Colt' and 'Bing/Colt' under water deficit (Fig. 5). In this regard, a study by Peschiutta et al. (2013) anatomically compared the 'Lapins' and 'Bing' scions grafted onto *P. domestica* rootstock under

well-watered condition found genetic differences associated with stem hydraulic traits (xylem vessel frequency and diameter). The authors showed that 'Lapins' scion had lower density and smaller xylem vessels (120 vessels mm^{-2} and 18 µm, respectively) compared to the 'Bing' scion (190 vessels mm^{-2} and 21 µm, respectively), concluding that 'Lapins' could be less vulnerable to stem cavitation than 'Bing'. The higher stem vulnerability to cavitation reported in 'Bing' suggests that is mainly affected by the high tension in the xylem caused by the water deficit in the soil, while 'Lapins' could avoid the embolism in vessels. Nevertheless, it is possible that the differences observed between combinations may also be due to the variations in the anatomical configuration of rootstock xylem vessels (Olmstead et al., 2006a). Regarding the xylem vessels of rootstocks, in general, 'Colt' has been reported with 35 vessels mm^{-2} and 40 µm (Olmstead, 2006; Olmstead et al., 2006a), whereas for 'Mx60' we found no information about xylem vessels. However, if we use MaxMa14 (*P. mahaleb* x *P. avium*) as a reference showing similar vigor (Gonçalves et al., 2007), we may assume that 'Mx60' could have around 300 vessels mm^{-2} and 25 µm (Gonçalves et al., 2007; Hajagos and Végvári, 2013). For a close hydraulic connection between the scion and the rootstock, the scion should have smaller xylem vessels than the rootstock (Olmstead et al., 2006a). Therefore, we can hypothesize that the losses in hydraulic functionality of 'Bing/Colt' in water deficit could be associated with large vessels of both 'Bing' and 'Colt', which could increase the vulnerability of the plant xylem to cavitation (Peschiutta et al., 2013), while the other combinations have a better hydraulic connection either for having fewer and/or smaller xylem vessels (Olmstead et al., 2006b).

The effect of the rootstock on scion vigor through the water relationships related to root hydraulic conductance is well known (Jones, 2012; Tombesi et al., 2010). Despite both 'Colt' and 'Mx60' having been classified as vigorous rootstocks (Csihon et al., 2018; Olmstead et al., 2010; Sotirov, 2020), we found a slight difference in water use strategy between 'Colt' (productive) and 'Mx60' (intermediate), which could redefine the classification of vigor according to the strategy: 'Colt' maintains its vigorous trait due to its high capacity to extract water (Fig. 1) and to maintain ('Lapins/Colt') or increase biomass (as in root) (Fig. 2), but 'Mx60' maintains a semi-vigorous trait through the intermediate capacity of water uptake (Fig. 1) and intermediate growth (Fig. 2). We believe that the vigor of the rootstocks should be rigorously reviewed under the new climate scenarios. Interestingly, we found evidence of a correlation between root hydraulic conductance and whole-plant WUE, revealing that rootstock/scion combinations with low root hydraulic conductance exhibited low whole-plant WUE (Fig. 5B). The finding related to whole-plant WUE suggests that root hydraulic conductivity may not only be controlling the water flow in roots and the plant, but also that it can maintain the vigor of the whole plant.

The whole-plant WUE referes to the analysis of the balance between two components: the biomass accumulation given by new growth and the water consumption during a period of time. Jiménez et al. (2013) found that the most vigorous rootstock induced higher WUE concomitant to the accumulation of osmoregulators, mainly leaf sorbitol. Our results show that rootstocks with high whole-plant WUE under water deficit increased sorbitol content in leaf (Fig. 6C). Sorbitol is considered the major end-product of photosynthesis and the main form of translocated and storage carbon in many fruit tree species of the *Prunus* genus (Centritto, 2005; Loescher, 1987). It has also been found that sorbitol transport (both via xylem and phloem) increases under water deficit condition (Noiraud et al., 2001) to perform osmotic adjustment functions in both roots and leaves (Jiménez et al., 2013; Lo Bianco et al., 2000). An early work showed a high capacity to adjust osmolarity of 'Colt' rootstock under water deficit, mainly associated with accumulation of sorbitol in roots and leaves (Ranney et al., 1991). In 'Colt' and 'Lapins/Colt', osmotic adjustments induced by sorbitol accumulation in leaves may contribute to the maintenance of open stomata at lower water potentials by sustaining turgor in response to an imposed water depletion (Pou et al., 2012). Thus, our results would suggest that the

relationship between rootstock and scion should consider an interaction that maximizes water uptake capacity, even as water deficit increases, since this will favor the mobility of sugars, either to increase osmolarity or as a source of energy for growing organs. Therefore, maintaining relatively high non-structural sugar levels during water deficit stress and keeping them available, i.e., during the recovery phase, may be crucial to tree vigor and survival (Tomasella et al., 2019). As for sucrose, Lo Bianco et al. (2000) found no influence in osmotic adjustment in peach plants. This could be due to sucrose having energy rather than osmotic functions. In our study, the increase in sucrose in roots under water deficit suggests that this sugar may support energy mainly for root respiration and the maintenance of root growth, as observed in the 'Colt' and 'Lapins/Colt' combinations (Fig. 2). Although it has been reported that tolerance to water deficit may be associated with low root respiration (Durand et al., 2016), in citrus, Silva et al. (2021) revealed that rootstock with tolerance to water deficit was able to use photo-assimilates as an energy source to increase root respiration under water deficit, and then to maintain root growth and improve drought tolerance.

5. Conclusion

It Is important to consider that rootstocks with a great influence of productive strategy on whole-plant performance should be considered an "opportunistic risk-taker" because this behavior, characterized by a water uptake capacity under conditions of low water availability, is only sustainable under minimal to moderate water stress and in the short term (Sade et al., 2012). In our study, we report that the water deficit-sensitive combination ('Bing/Colt') exhibited an early reduction in its water uptake capacity (higher FTSW values), a concomitant lower hydroscape area and a remarkable reduction in its whole-plant WUE. Moreover, this was the combination most negatively affected in its biomass accumulation. Conversely, combinations with late reductions in their water uptake capacities (lower FTSW values) or risk-taker combinations ('Lapins/Colt' and 'Colt') presented a greater hydroscape area, with barely affected shoot biomass and even with an increase in biomass at root level under water deficit. Our study shows that there should not be a strict rule on whether it is the rootstock or scion that controls the behavior of the whole plant; rather, an integrative view of the physiology of both grafted components should be taken. According to the above considerations, the choice of which rootstock to take the risk in order to maintain a sustained water uptake capacity from the soil will be fundamental to maintaining high productivity. This research highlights the importance of studying the physiological response of grafted plants in order to select better rootstock genotypes supporting the scion and generating a productive plant with an improved performance to cope with dry environments.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agwat.2023.108284.

References

Adiredjo, A.L., Casadebaig, P., Langlade, N., Lamaze, T., Grieu, P., 2018. Genetic analysis of the transpiration control in sunflower (*Helianthus annuus* L) subjected to drought. Vegetos - Int. J. Plant Res. 31, 1–6. https://doi.org/10.4172/2229-4473.1000368.

AGV, 2022. Plantas Frutales y Vides Comercializadas en Chile 2017–2021. https://viverosdechile.cl/anuario-agv/wp-content/themes/Divi-child/anuario/anuario2agvfinal-oct-2022.pdf. (accessed 09 March 2023).

Álvarez-Maldini, C., Acevedo, M., Pinto, M., 2021. Hydroscapes: a useful metric for distinguishing iso-/anisohydric behavior in almond cultivars. Plants 10. https://doi.org/10.3390/plants10061249.

Blanco, V., Domingo, R., Pérez-Pastor, A., Blaya-Ros, P.J., Torres-Sánchez, R., 2018. Soil and plant water indicators for deficit irrigation management of field-grown sweet cherry trees. Agric. Water Manag. 208, 83–94. https://doi.org/10.1016/j.agwat.2018.05.021.

Blanco, V., Blaya-Ros, P.J., Torres-Sánchez, R., Domingo, R., 2020. Influence of regulated deficit irrigation and environmental conditions on reproductive response of sweet cherry trees. Plants 9. https://doi.org/10.3390/plants9010094.

Buckley, T.N., 2019. How do stomata respond to water status. N. Phytol. 224, 21–36. https://doi.org/10.1111/nph.15899.

Campbell, B.M., Vermeulen, S.J., Aggarwal, P.K., Corner-Dolloff, C., Girvetz, E., Loboguerrero, A.M., Ramirez-Villegas, J., Rosenstock, T., Sebastian, L., Thornton, P. K., Wollenberg, E., 2016. Reducing risks to food security from climate change. Glob. Food Sec 11, 34–43. https://doi.org/10.1016/j.gfs.2016.06.002.

Carminati, A., Javaux, M., 2020. Soil rather than xylem vulnerability controls stomatal response to drought. Trends Plant Sci. 25, 868–880. https://doi.org/10.1016/j.tplants.2020.04.003.

Casadebaig, P., Debaeke, P., Lecoeur, J., 2008. Thresholds for leaf expansion and transpiration response to soil water deficit in a range of sunflower genotypes. Eur. J. Agron. 28, 646–654. https://doi.org/10.1016/j.eja.2008.02.001.

Centritto, M., 2005. Photosynthetic limitations and carbon partitioning in cherry in response to water deficit and elevated [CO$_2$. Agric. Ecosyst. Environ. 106, 233–242. https://doi.org/10.1016/j.agee.2004.10.011.

Csihon, Á., Bicskei, D.K., Dremák, P., Gonda, I., 2018. Performance of sweet cherry cultivars grafted on Colt rootstock. Int. J. Hortic. Sci. 24, 7–10. https://doi.org/10.31421/IJHS/24/1-2./1540.

Del-Saz, N.F., Romero-Munar, A., Alonso, D., Aroca, R., Baraza, E., Flexas, J., Ribas-Carbo, M., 2017. Respiratory ATP cost and benefit of arbuscular mycorrhizal symbiosis with Nicotiana tabacum at different growth stages and under salinity. J. Plant Physiol. 218, 243–248. https://doi.org/10.1016/j.jplph.2017.08.012.

Durand, M., Porcheron, B., Hennion, N., Maurousset, L., Lemoine, R., Pourtau, N., 2016. Water deficit enhances C export to the roots in arabidopsis thaliana plants with contribution of sucrose transporters in both shoot and roots. Plant Physiol. 170, 1460–1479. https://doi.org/10.1104/pp.15.01926.

Edenhofer, O., 2015. Climate Change 2014: Mitigation of Climate Change (Intergovernmental Panel on Climate Change). Cambridge University Press, Cambridge.

Fernandez i Marti, A., Athanson, B., Koepke, T., Font, I., Forcada, C., Dhingra, A., Oraguzie, N., 2012. Genetic diversity and relatedness of sweet cherry (*Prunus avium* L.) cultivars based on single nucleotide polymorphic markers, 116-116 Front. Plant Sci. 3. https://doi.org/10.3389/fpls.2012.00116.

Fu, X., Meinzer, F., 2019. Metrics and proxies for stringency of regulation of plant water status (iso/anisohydry): a global data set reveals coordination and trade-offs among water transport traits. Tree Physiol. 39, 122–134. https://doi.org/10.1093/treephys/tpy087.

Gambetta, G.A., Herrera, J.C., Dayer, S., Feng, Q., Hochberg, U., Castellarin, S.D., 2020. The physiology of drought stress in grapevine: towards an integrative definition of drought tolerance. J. Exp. Bot. 71, 4658–4676. https://doi.org/10.1093/jxb/eraa245.

Garcia-Forner, N., Biel, C., Savé, R., Martínez-Vilalta, J., 2017. Isohydric species are not necessarily more carbon limited than anisohydric species during drought. Tree Physiol. 37, 441–455. https://doi.org/10.1093/treephys/tpw109.

Garreaud, R.D., Alvarez-Garreton, C., Barichivich, J., Boisier, J.P., Christie, D., Galleguillos, M., LeQuesne, C., McPhee, J., Zambrano-Bigiarini, M., 2017. The 2010-2015 megadrought in central Chile: impacts on regional hydroclimate and vegetation. HESS 21, 6307–6327. https://doi.org/10.5194/hess-21-6307-2017.

Garreaud, R.D., Boisier, J.P., Rondanelli, R., Montecinos, A., Sepúlveda, H.H., Veloso-Aguila, D., 2020. The Central Chile Mega Drought (2010–2018): A climate dynamics perspective. Int. J. Clim. 40, 421–439. https://doi.org/10.1002/joc.6219.

Gonçalves, B., Correia, C., Silva, A., Bacelar, E., Santos, A., Ferreira, H., Moutinho Pereira, J., 2007. Variation in xylem structure and function in roots and stems of scion–rootstock combinations of sweet cherry tree (*Prunus avium* L.). In: Trees, 21, pp. 121–130. https://doi.org/10.1007/s00468-006-0102-2.

Gonçalves, B., Aires, A., Oliveira, I., Afonso, S., Morais, M.C., Correia, S., Martins, S., Silva, A.P., 2021. Sweet Cherry, in: Mandal, D., Wermund, U., Phavaphutanon, L.,

Cronje, R. (Eds.), Temperate Fruits: Production, Processing, and Marketing. Apple Academic Press, pp. 333–415.

Guyot, G., Scoffoni, C., Sack, L., 2012. Combined impacts of irradiance and dehydration on leaf hydraulic conductance: insights into vulnerability and stomatal control. Plant Cell Environ. 35, 857–871. https://doi.org/10.1111/j.1365-3040.2011.02458.x.

Hajagos, A., Végvári, G., 2013. Investigation of tissue structure and xylem anatomy of eight rootstocks of sweet cherry (*Prunus avium* L.). Trees 27, 53–60. https://doi.org/10.1007/s00468-012-0766-8.

Hartmann, H., Link, R.M., Schuldt, B., 2021. A whole-plant perspective of isohydry: stem-level support for leaf-level plant water regulation. Tree Physiol. https://doi.org/10.1093/treephys/tpab011.

Hochberg, U., Degu, A., Fait, A., Rachmilevitch, S., 2013. Near isohydric grapevine cultivar displays higher photosynthetic efficiency and photorespiration rates under drought stress as compared with near anisohydric grapevine cultivar. Physiol. Plant. 147, 443–452. https://doi.org/10.1111/j.1399-3054.2012.01671.x.

Hochberg, U., Rockwell, F.E., Holbrook, N.M., Cochard, H., 2018. Iso/Anisohydry: A plant–environment interaction rather than a simple hydraulic trait. Trends Plant Sci. 23, 112–120. https://doi.org/10.1016/j.tplants.2017.11.002.

Hussain, H.A., Men, S., Hussain, S., Chen, Y., Ali, S., Zhang, S., Zhang, K., Li, Y., Xu, Q., Liao, C., Wang, L., 2019. Interactive effects of drought and heat stresses on morpho-physiological attributes, yield, nutrient uptake and oxidative status in maize hybrids. Sci. Rep. 9, 3890. https://doi.org/10.1038/s41598-019-40362-7.

Jiménez, S., Dridi, J., Gutiérrez, D., Moret, D., Irigoyen, J.J., Moreno, M.A., Gogorcena, Y., 2013. Physiological, biochemical and molecular responses in four Prunus rootstocks submitted to drought stress. Tree Physiol. 33, 1061–1075. https://doi.org/10.1093/treephys/tpt074.

Jones, H.G., 2012. How do rootstocks control shoot water relations. N. Phytol. 194, 301–303. https://doi.org/10.1111/j.1469-8137.2012.04110.x.

Klein, T., 2014. The variability of stomatal sensitivity to leaf water potential across tree species indicates a continuum between isohydric and anisohydric behaviours. Funct. Ecol. 28, 1313–1320. https://doi.org/10.1111/1365-2435.12289.

Lavoie-Lamoureux, A., Sacco, D., Risse, P.-A., Lovisolo, C., 2017. Factors influencing stomatal conductance in response to water availability in grapevine: a meta-analysis. Physiol. Plant. 159, 468–482. https://doi.org/10.1111/ppl.12530.

Lesk, C., Rowhani, P., Ramankutty, N., 2016. Influence of extreme weather disasters on global crop production. Nature 529, 84–87. https://doi.org/10.1038/nature16467.

Li, X., Blackman, C.J., Peters, J.M.R., Choat, B., Rymer, P.D., Medlyn, B.E., Tissue, D.T., 2019. More than iso/anisohydry: hydroscapes integrate plant water use and drought tolerance traits in 10 eucalypt species from contrasting climates. Funct. Ecol. 33, 1035–1049. https://doi.org/10.1111/1365-2435.13320.

Lo Bianco, R., Rieger, M., Sung, S.-J.S., 2000. Effect of drought on sorbitol and sucrose metabolism in sinks and sources of peach. Physiol. Plant. 108, 71–78. https://doi.org/10.1034/j.1399-3054.2000.108001071.x.

Loescher, W.H., 1987. Physiology and metabolism of sugar alcohols in higher plants. Physiol. Plant. 70, 553–557. https://doi.org/10.1111/j.1399-3054.1987.tb02857.x.

Lovisolo, C., Perrone, I., Carra, A., Ferrandino, A., Flexas, J., Medrano, H., Schubert, A., 2010. Drought-induced changes in development and function of grapevine (*Vitis* spp.) organs and in their hydraulic and non-hydraulic interactions at the whole-plant level: a physiological and molecular update. Funct. Plant Biol. 37, 98–116. https://doi.org/10.1071/FP09191.

Martínez-Vilalta, J., Poyatos, R., Aguadé, D., Retana, J., Mencuccini, M., 2014. A new look at water transport regulation in plants. N. Phytol. 204, 105–115. https://doi.org/10.1111/nph.12912.

Mashilo, J., Odindo, A.O., Shimelis, H.A., Musenge, P., Tesfay, S.Z., Magwaza, L.S., 2017. Drought tolerance of selected bottle gourd [*Lagenaria siceraria* (Molina) Standl.] landraces assessed by leaf gas exchange and photosynthetic efficiency. Plant Physiol. Biochem. 120, 75–87. https://doi.org/10.1016/j.plaphy.2017.09.022.

Medrano, H., Tortosa, I., Montes, E., Pou, A., Balda, P., Bota, J., Escalona, J.M., 2018. Genetic Improvement of Grapevine (*Vitis vinifera* L.) Water Use Efficiency: Variability Among Varieties and Clones. In: García Tejero, I.F., Durán Zuazo, V.H. (Eds.), Water Scarcity and Sustainable Agriculture in Semiarid Environment. Academic Press, pp. 377–401.

Meinzer, F.C., Woodruff, D.R., Marias, D.E., Smith, D.D., McCulloh, K.A., Howard, A.R., Magedman, A.L., 2016. Mapping 'hydroscapes' along the iso- to anisohydric continuum of stomatal regulation of plant water status. Ecol. Lett. 19, 1343–1352. https://doi.org/10.1111/ele.12670.

Moshelion, M., 2020. The dichotomy of yield and drought resistance. EMBO Rep. 21, e51598 https://doi.org/10.15252/embr.202051598.

Noiraud, N., Maurousset, L., Lemoine, R., 2001. Transport of polyols in higher plants. Plant Physiol. Biochem. 39, 717–728. https://doi.org/10.1016/S0981-9428(01)01292-X.

Núñez, J.H., Verbist, K., Wallis, J.R., Schaefer, M.G., Morales, L., Cornelis, W., 2011. Regional frequency analysis for mapping drought events in north-central Chile. J. Hydrol. 405, 352–366. https://doi.org/10.1016/j.jhydrol.2011.05.035.

Olmstead, M., 2006. Xylem Vessel Anatomy of Sweet Cherries Grafted Onto Dwarfing and Non-Dwarfing Rootstocks. Journal of the American Society for Horticultural Science. American Society for Horticultural Science 131, 577–585. https://doi.org/10.21273/JASHS.131.5.577.

Olmstead, M., Lang, N., Lang, G., Ewers, F., Owens, S., 2006a. Examining the vascular pathway of sweet cherries grafted onto dwarfing rootstocks. HortScience 41, 674–679. https://doi.org/10.21273/HORTSCI.41.3.674.

Olmstead, M., Lang, N., Lang, G., 2010. Carbohydrate profiles in the graft union of young sweet cherry trees grown on dwarfing and vigorous rootstocks. Sci. Hortic. 124, 78–82. https://doi.org/10.1016/j.scienta.2009.12.022.

Olmstead, M.A., Lang, N.S., Ewers, F.W., Owens, S.A., 2006b. Xylem vessel anatomy of sweet cherries grafted onto dwarfing and nondwarfing rootstocks. J. Am. Soc. Hortic. Sci. jashs 131, 577–585. https://doi.org/10.21273/JASHS.131.5.577.

Opazo, I., Toro, G., Solis, S., Salvatierra, A., Franck, N., Albornoz, F., Pimentel, P., 2019. Late reduction on transpiration is an important trait for water deficit tolerance in interspecific *Prunus* rootstock hybrids. Theor. Exp. Plant Physiol. 31, 493–506. https://doi.org/10.1007/s40626-019-00162-w.

Opazo, I., Toro, G., Salvatierra, A., Pastenes, C., Pimentel, P., 2020. Rootstocks modulate the physiology and growth responses to water deficit and long-term recovery in grafted stone fruit trees. Agric. Water Manag. 228, 105897 https://doi.org/10.1016/j.agwat.2019.105897.

Pareek, A., Dhankher, O.P., Foyer, C.H., 2020. Mitigating the impact of climate change on plant productivity and ecosystem sustainability. J. Exp. Bot. 71, 451–456. https://doi.org/10.1093/jxb/erz518.

Pastenes, C., Villalobos, L., Ríos, N., Reyes, F., Turgeon, R., Franck, N., 2014. Carbon partitioning to berries in water stressed grapevines: The role of active transport in leaves and fruits. Environ. Exp. Bot. 107, 154–166. https://doi.org/10.1016/j.envexpbot.2014.06.009.

Peccoux, A., Loveys, B., Zhu, J., Gambetta, G.A., Delrot, S., Vivin, P., Schultz, H.R., Ollat, N., Dai, Z., 2018. Dissecting the rootstock control of scion transpiration using model-assisted analyses in grapevine. Tree Physiol. 38, 1026–1040. https://doi.org/10.1093/treephys/tpx153.

Pedroso, F.K.J.V., Prudente, D.A., Bueno, A.C.R., Machado, E.C., Ribeiro, R.V., 2014. Drought tolerance in citrus trees is enhanced by rootstock-dependent changes in root growth and carbohydrate availability. Environ. Exp. Bot. 101, 26–35. https://doi.org/10.1016/j.envexpbot.2013.12.024.

Peschiutta, M.L., Bucci, S.J., Scholz, F.G., Kowal, R.F., Goldstein, G., 2013. Leaf and stem hydraulic traits in relation to growth, water use and fruit yield in *Prunus avium* L. cultivars. Trees 27, 1559–1569. https://doi.org/10.1007/s00468-013-0904-y.

Pou, A., Medrano, H., Tomàs, M., Martorell, S., Ribas-Carbó, M., Flexas, J., 2012. Anisohydric behaviour in grapevines results in better performance under moderate water stress and recovery than isohydric behaviour. Plant Soil 359, 335–349. https://doi.org/10.1007/s11104-012-1206-7.

Ranney, T.G., Bassuk, N.L., Whitlow, T.H., 1991. Osmotic adjustment and solute constituents in leaves and roots of water-stressed cherry (*Prunus*) trees. J. Am. Soc. Hort. Sci. 116, 684–688. https://doi.org/10.21273/JASHS.116.4.684.

Rodriguez-Dominguez, C.M., Brodribb, T.J., 2020. Declining root water transport drives stomatal closure in olive under moderate water stress. N. Phytol. 225, 126–134. https://doi.org/10.1111/nph.16177.

Rodríguez-Gamir, J., Primo-Millo, E., Forner-Giner, M.Á., 2016. An integrated view of whole-tree hydraulic architecture. does stomatal or hydraulic conductance determine whole tree transpiration? PLoS One 11, e0155246. https://doi.org/10.1371/journal.pone.0155246.

Sade, N., Gebremedhin, A., Moshelion, M., 2012. Risk-taking plants: anisohydric behavior as a stress-resistance trait. Plant Signal. Behav. 7, 767–770. https://doi.org/10.4161/psb.20505.

Santana-Vieira, D.D.S., Freschi, L., Almeida, L.Ad.H., Moraes, D.H.Sd, Neves, D.M., Santos, L.M.D., Bertolde, F.Z., Soares Filho, W.D.S., Coelho Filho, M.A., Gesteira, Ad. S., 2016. Survival strategies of citrus rootstocks subjected to drought, 38775-38775 Sci. Rep. 6. https://doi.org/10.1038/srep38775.

Serra, I., Strever, A., Myburgh, P.A., Deloire, A., 2014. Review: the interaction between rootstocks and cultivars (*Vitis vinifera* L.) to enhance drought tolerance in grapevine. Aust. J. Grape Wine Res 20, 1–14. https://doi.org/10.1111/ajgw.12054.

Shakeel, A.A., Xiao-yu, X., Long-chang, W., Muhammad, F.S., Chen, M., Wang, L., 2011. Morphol., Physiol. Biochem. Responses Plants Drought Stress. Afr. J. Agric. Res 6, 2026–2032. https://doi.org/10.5897/AJAR10.027.

Silva, S.F., Miranda, M.T., Costa, V.E., Machado, E.C., Ribeiro, R.V., 2021. Sink strength of citrus rootstocks under water deficit. Tree Physiol. S. https://doi.org/10.1093/treephys/tpab008.

Sinclair, T., Ludlow, M., 1986. Influence of soil water supply on the plant water balance of four tropical grain legumes. Funct. Plant Biol. 13, 329–341. https://doi.org/10.1071/PP9860329.

Sinclair, T.R., Muchow, R.C., 2001. System analysis of plant traits to increase grain yield on limited water supplies. Agron. J. 93, 263–270. https://doi.org/10.2134/agronj2001.932263x.

Sotirov, D., 2020. Evaluation of different rootstocks with 'Van' sweet cherry cultivar, 1281 ed. International Society for Horticultural Science (ISHS), Leuven, Belgium, pp. 179–184.

Tardieu, F., Simonneau, T., Muller, B., 2018. The physiological basis of drought tolerance in crop plants: a scenario-dependent probabilistic approach. Annu. Rev. Plant Biol. 69, 733–759. https://doi.org/10.1146/annurev-arplant-042817-040218.

Team, R.D.C., 2013. R: A language and environment for statistical computing. R Fundation for Statistical Computing, Vienna, Austria.

Tomás, M., Medrano, H., Pou, A., Escalona, J.M., Martorell, S., Ribas-Carbó, M., Flexas, J., 2012. Water-use efficiency in grapevine cultivars grown under controlled conditions: effects of water stress at the leaf and whole-plant level. Aust. J. Grape Wine Res 18, 164–172. https://doi.org/10.1111/j.1755-0238.2012.00184.x.

Tomasella, M., Petrussa, E., Petruzzellis, F., Nardini, A., Casolo, V., 2019. The possible role of non-structural carbohydrates in the regulation of tree hydraulics. Int. J. Mol. Sci. 21. https://doi.org/10.3390/ijms21010144.

Tombesi, S., Johnson, R.S., Day, K.R., DeJong, T.M., 2010. Relationships between xylem vessel characteristics, calculated axial hydraulic conductance and size-controlling capacity of peach rootstocks. Ann. Bot. 105, 327–331. https://doi.org/10.1093/aob/mcp281.

Toro, G., Pinto, M., Pimentel, P., 2018. Root respiratory components of *Prunus* spp. rootstocks under low oxygen: regulation of growth, maintenance, and ion uptake

respiration. Sci. Hortic. 239, 259–268. https://doi.org/10.1016/j.scienta.2018.05.040.

Tyree, M.T., Patiño, S., Bennink, J., Alexander, J., 1995. Dynamic measurements of roots hydraulic conductance using a high-pressure flowmeter in the laboratory and field. J. Exp. Bot. 46, 83–94. https://doi.org/10.1093/jxb/46.1.83.

Venturas, M.D., Sperry, J.S., Hacke, U.G., 2017. Plant xylem hydraulics: what we understand, current research, and future challenges. J. Integr. Plant Biol. 59, 356–389. https://doi.org/10.1111/jipb.12534.

Wright, D.E.J., Cline, J.A., Earl, H.J., 2019. Physiological responses of four apple (*Malus* × *domestica* Borkh.) rootstock genotypes to soil water deficits. Can. J. Plant Sci. 99, 510–524. https://doi.org/10.1139/cjps-2018-0276.

Zacarias Rafael, D., Arriagada, O., Toro, G., Mashilo, J., Mora-Poblete, F., Contreras-Soto, R.I., 2020. Plasticity of the root system architecture and leaf gas exchange parameters are important for maintaining bottle gourd responses under water deficit. Plants 9. https://doi.org/10.3390/plants9121697.