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Water stress tolerance is coordinated with water use capacity and growth under water deficit across six fruit tree species

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Abstract

Compare water stress tolerance traits between different fruit tree species under the same experimental conditions can provide valuable information for understanding the mechanisms underlying water stress tolerance. This work aimed to determine and compare the water stress tolerance of six fruit tree species typically cultivated in Mediterranean regions and evaluate its association with water use and growth under water deficit. Six fruit tree species were used in this study: pomegranate, fig, mandarin, avocado, and two Prunus species ('R40' and 'R20'). Iso-anisohydric behavior (low to high water stress tolerance) was assessed through a multi-trait approach and associated with growth and water use under wellwatered and water deficit conditions. Avocado and mandarin were classified as species with more strict stomatal control over water potential, while pomegranate, fig, and *Prunus* spp. showed less stomatal control. This classification was supported by the multi-traits analysis, which showed that avocado and mandarin, in contrast to the rest of the species, were characterized by higher gas-exchange thresholds (more sensitive). A more isohydric behavior was associated with lower water, but higher root hydraulic conductivity, and a lower growth capacity. Some traits, such as the FTSW_{threshold} to the drop of relative transpiration, root hydraulic conductivity, and residual soil water content, provide valuable information to discriminate between species or genotypes that are better adapted to water deficits. These traits explain the position of the species in the iso-anisohydric spectrum and allow us to understand and develop better strategies for water management in agricultural systems.

Introduction

The impact of global warming alongside a growing human population of over 7.7 billion, strongly challenges agricultural systems and increases the need for a better understanding of how crops and fruit trees can survive and yield under hotter and dryer environments (Venkatramanan et al., 2020). In this context, the understanding of water stress tolerance of agricultural species is a key issue, especially in fruit tree species because of their lower flexibility in comparison to crops, and because increased irrigation water use is not necessarily a sustainable solution. While many studies have addressed the topic of water stress tolerance in forest systems (Manzoni et al., 2013a, Klein, 2014, Skelton et al., 2015, Johnson et al., 2018, Väänänen et al., 2019), fewer studies have been conducted on fruit tree species, particularly comparing different fruit trees (Garrido and Vergara 2022; Sorek et al. 2022), with *Vitis vinifera* being one of the main species studied (Charrier et al., 2018, Gambetta et al., 2020).

To assess water stress tolerance, a common approach is the stomatal behavior assessment, commonly named iso-anisohydric behavior (Klein, 2014, Martínez-Vilalta et al., 2014). Those categories have been incorporated into the "hydraulic-carbon framework" of the physiological mechanism of tree mortality (McDowell et al., 2008). It is expected that, at lower water availability, species of relatively isohydric behavior escape from stress by closing their stomata and by maintaining a constant minimum water potential over days of water deficit. Alternatively, species with a relatively anisohydric behavior would tolerate water stress maintaining a relatively higher stomatal conductance and experiencing a decline in minimum water potential over time (Meinzer et al., 2016). Thus, during prolonged drought, isohydric

species would experience carbon starvation due to photosynthesis limitation imposed by stomatal closure, while anisohydric species would experience hydraulic failure by cavitation of the water column at low water potentials (McDowell, 2011). Since the stomatal behavior is a continuum, its determination is complex because often there is a species x environment interaction (Martínez-Vilalta and Garcia-Forner, 2017) and a lack of accurate drought resistance indicators and standardized water stress methodology (Lawlor, 2013).

Stomatal closure acts as a mechanism that controls the rate of cavitation (Trifilò et al., 2015), which injures the hydraulic system of the plant, i.e. hydraulic design that drives the water movement from roots to leaves, and therefore defines the canopy volume that can be supplied with water (Cruiziat et al., 2002, Cosme et al., 2017). This strong relationship between hydraulics and gas exchange makes the hydraulic traits key variables for understanding the functional response of plants during drought and recovery periods (Skelton et al., 2017).

The existence of trade-offs between stomatal behavior and hydraulics (Trifilò et al., 2015), as the advantages of adopting a more iso-anisohydric strategy based on the duration and intensity of the water deficit (Vadez et al., 2013), made very difficult define the degree of water stress tolerance of a species. This could be more challenging for agricultural species, where the productivity under water deficit is as important as their survival capacity. It is therefore relevant to establish the relationship between resistance to water deficit and the water use and growth capacity of agricultural species. Additionally, a further definition of water stress tolerance is necessary (Gambeta et al. 2020). For example, Dayer et al. (2020) demonstrated through hydraulic traits such as water potential at leaf turgor loss point and vulnerability to embolism that *Vitis vinifera* cv. 'Granache' was less tolerant to drought than cv. 'Semillion'. Nevertheless, a more conservative water use (less maximum transpiration rate) allows 'Granache' to delay reaching critical water potential, being more resistant to drought. This study demonstrated the importance of integrating multiple traits in characterizing drought resistance.

This work aimed to determine and compare the water stress tolerance of six fruit tree species typically cultivated in Mediterranean regions and evaluate its association with water use and growth under water deficit. We hypothesize that a multiple-traits approach can accurately determine the water stress tolerance, and that a more tolerant strategy (e.g. more anisohydric behavior) will be associated with higher water consumption and greater growth capacity under water deficit. The species were pomegranate and fig (tolerant; Ammar et al., 2020, Volschenk, 2021), mandarin and avocado (sensitive; Panigrahi et al., 2014, Kourgialas and Dokou, 2021), and two genotypes of *Prunus* sp. usually used as rootstock, Rootpac®40 rootstock (*Prunus dulcis* [Mill.] DA Webb x *P. persica* Batsch) and Rootpac®20 rootstock (*Prunus besseyi* Bailey × *P. cerasifera* Ehrh) selected as tolerant and sensitive, respectively (Opazo et al., 2019). All plants were evaluated in the entire range of their fraction of transpirable soil water (FTSW; Sinclair and Ludlow, 1986), a standardized supply variable that allows determining the sequence in which various physiological processes are affected and it has been mentioned as a good approach to determine a more or less conservative use of water by a crop, in a genotype background-dependent manner (Vadez et al., 2013).

Materials and methods

Plant material and experimental conditions

This study was conducted at the Centro de Estudios Avanzados en Fruticultura (CEAF), Rengo, Chile (34°19'S 70°50'W). The climate corresponds to a warm temperate climate with an annual precipitation of 350 mm, an average maximum temperature in January of 28.5°C, and an average minimum temperature in June of 2°C. The reference evapotranspiration for the summer period (December to February) is 465 mm (CIREN 2023).

Fruit trees included in the trial were genetically distant, have a wide range of drought tolerance, and are economically important in different edaphoclimatic locations. One-year-old plants of avocado (*Persea americana* Mill.), mandarin (*Citrus reticulata* Blanco), pomegranate, fig, Rootpac®40 rootstock (*Prunus dulcis* [Mill.] DA Webb x *P. persica* Batsch), and Rootpac®20 rootstock (*Prunus besseyi* Bailey × *P. cerasifera* Ehrh) were obtained from a commercial nursery (Table 1). We worked with grafted mandarin and avocado plants to represent the way these crops are traditionally grown.

Scion	Rootstock	Specie or genera origin and climate	Reference
Pomegranate cv. 'Wonderfull' (<i>Punica granatum</i> L.)	Own-rooted	Transcaucasia and Central Asia – Subtropical to desertic	(Chandra et al., 2010)
Fig cv. 'Black Mission' (<i>Ficus carica</i> L.)	Own-rooted	Middle East – Arid to mild-temperate	(Mars, 2003)
		Central and southwest Asia – Desertic to subtropical (almond or peach respectively)	
Rootpac®40 (<i>Prunus dulcis</i> [Mill.] DA Webb x <i>P. persica</i> Batsch)	Own-rooted		(Kester et al., 1991, Ladizinsky, 1999)
Rootpac®20 (<i>Prunus besseyi</i> Bailey × <i>P. cerasifera</i> Ehrh)	Own-rooted	From the Balkans to the Caucasian mountains in southwest Asia – Temperate to semiarid (<i>P. cerasifera</i>)	(Horvath et al., 2008)
Mandarin cv. 'Orogrande' (<i>Citrus</i> <i>reticulata</i> Blanco)	'Carrizo' (<i>Citrus sinensis</i> L. Osb.× <i>Poncirus trifoliata</i> L. Raf.)	Southeastern Asia and Australia – Subtropical (<i>Citrus</i> spp)	(Scora, 1975)
		Mesoamerica – Tropical to subtropical	
Avocado cv. 'Hass' (<i>Persea americana</i> Mill.)	'Mexícola' (<i>Persea americana</i> Mill.)		(Galindo- Tovar et al., 2008)

Table 1 List of the studied cultivars and rootstocks, tree species origin, and climate of the center of origin.

Plants were transferred to 20 L pots filled with a mixture of 1:1 peat/perlite supplemented with Basacote Plus 9M at a 6 g L⁻¹ as a controlled-release fertilizer (BASF, Limburgerhof, Germany). After the transplant, plants were pruned to activate multiple growth points. Plants were grown for 30 days in a shade house (50% sunlight) and were then transferred to field conditions (full sunlight). An acclimation period of two weeks was allowed before the experiment started. The irrigation system consisted of two drippers per plant with a flow rate of 2 L h⁻¹. Pots were covered with black plastic bags to avoid evaporation. Sixteen uniform and healthy plants of each species were selected for all evaluations. Of the sixteen plants, four plants were used to determine the initial biomass, and the remaining 12 plants were used for final biomass and physiological measurements during the water deficit period.

Irrigation treatments

Plants were subjected to two irrigation conditions: well-watered (WW) and water deficit (WD), in a completely randomized experimental design. Each pot was saturated with tap water, allowed to drain, and covered with plastic bags to avoid evaporation for 24 h. After, a similar initial weight of each container was reached (ca. 11 kg) and recorded. This was established as 100% of soil water content (SWC) and considered as field capacity. WW plants were irrigated three times per week adding tap water until 100% of the SWC for each pot. For WD the irrigation was withheld for 45 days, weighing each container three times per week to determine the water consumption of every single plant. During this period all the containers were filled with the volume of water necessary to reach the same water content of the plant with the lowest transpiration rate which, in turn, was not irrigated. Therefore, after the irrigation, all the WD containers had the same SWC (Opazo et al., 2019, Opazo et al., 2020) and the progression of the water deficit was similar between species. The minimum water content in the pot (0% SWC) was determined by drying the substrate in an oven at 100°C until constant weight. The SWC was calculated for each container as Eq. 1 (Supp. Figure 1):

$$\mathrm{SWC}\left(\%
ight) = rac{\mathrm{DSW} - \mathrm{DW}}{\mathrm{MSW} - \mathrm{DW}}; \mathrm{[Eq.1]}$$

where DSW is the daily substrate weight, DW is the substrate dry weight reached in the oven and MSW is the maximum substrate weight at field capacity.

Relative transpiration (RT) rate and residual soil water content (RSWC)

The point of reduction in transpiration under WD was calculated by the relationship between the relative transpiration rate (RT) and the fraction of transpirable substrate water (FTSW) (Sinclair and Ludlow, 1986). The transpiration rate corresponds to the daily amount of transpired water under WD, divided by the average daily transpiration of the WW plants for each species. The fraction of transpirable substrate water corresponds to the fraction of water inside the container that plants can use to transpire. The RT of each plant was divided by the respective mean RT of each plant during the period when the soil was still well-watered to normalize the initial values (Sinclair and Ludlow, 1986). According to Bindi et al. (2005),

the initial point for stress (FTSW_{threshold}) is around a RT value of 0.9. Then, RT was adjusted to a logistic equation as in Eq. 2:

$$\mathrm{RT} = rac{1}{1+lpha^{*}e^{-eta^{*}\mathrm{FTSW}}}; \mathrm{[Eq.2]}$$

where α and β are constants to be determined for each plant related to the curvature of logistic regression.

At the end of the experiment, containers were weighed before the plant harvest. After, the fresh weight of leaves, stems, and roots, plus the weight of an empty container, were discounted to the initial weight to obtain the substrate weight at this moment on every WD plant. That substrate weight was compared with the dry weight (70°C) to determine the residual water content in WD plants.

Stomatal conductance, net photosynthesis, stem water potential, and osmotic potential

Stomatal conductance (g_s) and net photosynthesis (A) were measured weekly on fully active and expanded leaves between 9:30 and 11:00 a.m. on the day before irrigation. One leaf per experimental unit was measured using a portable photosynthesis system (model CIRAS-2, PPSystem, Hitchin, UK) equipped with a 2.5 cm² LED lighting cuvette (model CIRAS PLC, PPSystem). Measurements were made at 25°C, with photosynthetic active radiation of 1000 µmol PAR m⁻² s⁻¹, CO₂ concentration of 400 µmol mol⁻¹, and relative humidity of 50%. Midday stem water potential (Ψ_{stem}) was measured on similar leaves and the same-day gas exchange measurements. One leaf per experimental unit was covered with plastic bags coated with aluminum foil to stop transpiration and allow them to balance with the stem water potential for at least 2 hours before measuring. The measurement was made at solar noon (between 13:00 and 15:00 local time) with a Schölander pressure chamber (Schölander et al., 1965).

Relative stomatal conductance (gs_{rel}) and relative net photosynthesis (A_{rel}) were estimated by the ratio between measurements made on WD plants and average WW. Negative values of A were assumed equal to 0. These relative traits were related to FTSW in the same manner as described in Eq. 2 to estimate gas-exchange thresholds. With the same purpose a relative stem water potential ($\Psi_{stem rel}$) was calculated as in Eq. 3:

$$\Psi_{ ext{stemrel}} = rac{ ext{WD}\Psi_{ ext{stem}} - ext{minimum}\Psi_{ ext{stem}}}{ ext{averageWW}\Psi_{ ext{stem}} - ext{minimum}\Psi_{ ext{stem}}}; ext{[Eq.3]}$$

where minimum Ψ_{stem} corresponds to the more negative Ψ_{stem} value detected during the experimental period in WD plants for each species.

Osmotic water potential (Ψ_0) was measured after 45 days of treatment. Fully active and expanded leaves were collected and kept in distilled water for 24 h and then frozen at -80°C until measurement. Leaves

were thawed and put in a syringe to press them and extract their sap. The osmolality was evaluated with an osmometer (Osmomat 3000, Gonotec GmbH, Germany). The osmotic potential was obtained using the Van't hoff equation, where osmotic potential = C*T*R, where *C* is the osmolality (mOs mol kg⁻¹ H₂O), *T* the absolute temperature and *R* is the gas constant (0.00831 kg MPa mol⁻¹ K⁻¹).

Whole plant hydraulic conductance

Whole plant hydraulic conductance (K_{pl} ; Kg [H_2O] MPa⁻¹ m⁻² s⁻¹) was determined at day 38 of the water deficit period (plants severely stressed) on four plants per treatment. The whole plant transpiration (E; Kg [H_2O] m⁻² s⁻¹) were calculated from Eq. 4:

$$\mathrm{E} = \frac{\Delta \mathrm{W}}{\mathrm{LA}^* \Delta \mathrm{t}}; [\mathrm{Eq.4}]$$

where Δt (seconds) was the interval time between dawn and midday, ΔW was the weight container change (kg) between pre-dawn and midday, and LA was leaf area (m²), which was determined at the end of the experiment (7 days later). Pre-dawn and midday leaf water potential were measured with a pressure chamber. K_{pl} was estimated as in Eq. 5 (Tsuda and Tyree, 2000):

$$\mathrm{K_{pl}} = rac{\mathrm{E}}{\mathrm{\Psi_{soil}} - \mathrm{\Psi_{leaf}}}; \mathrm{[Eq.5]}$$

Root hydraulic conductivity

The root hydraulic conductivity (L_p) was determined by a High-Pressure Flow Meter (HPFM, Dynamax, Houston, TX, USA) at the end of the experiment, according to Tyree et al. (1995) on six plants per treatment. The night before measurements, WD plants were fully irrigated to recover the water columns, reducing artifacts due to cavitation (Alsina et al., 2011). At the end of the water deficit period, L_p measurements were carried out in the whole root system below the rootstock/scion junction in grafted plants, and at 10 cm over the substrate's surface in non-grafted plants, twice per plant. Subsequently, this value was normalized by the dry weight of the whole root system (Vandeleur et al., 2014).

Iso-anisohydric behavior

On days 24, 31, and 38 of water deficit, leaf water potential was measured at pre-dawn (Ψ_{pd}) and midday (Ψ_{md} ; between 13:00 and 15:00 h) on four plants per treatment. A modified hydroscape area (Meinzer et al., 2016) was estimated by the method proposed by Johnson et al. (2018). In this method, the hydroscape area (HA: MPa²) is calculated as the area of a polygon formed by the 1:1 lines ($\Psi_{md} = \Psi_{pd}$) and the leaf midday water potential. A greater HA will be associated with a more anisohydric stomatal regulation on leaf water potential. The polygon areas were obtained through digital image analysis with the ImageJ software (Schindelin et al., 2015).

Isotopic composition of ¹³C

To determine the leaf isotopic composition of ¹³C (δ^{13} C; ‰) fully expanded and sun-exposed leaves were sampled on day 45 of water deficit. Samples were dried at 60°C in a forced-air oven until constant weight and then ground in a mill to a homogeneous fine powder. Two subsamples from each sample were weighed with an analytical balance (Precisa 125A) in tin capsules for δ^{13} C measurements. Each leaf sample isotopic composition was determined using standard procedures at the Stable Isotope Laboratory at the Faculty of Agronomic Sciences (University of Chile), with an INTEGRA2 isotopic ratio mass spectrometer (IRMS) (Sercon Ltd. Cheshire, UK), with a precision of 0.3‰ for δ^{13} C.

Wood density and stomatal density

Wood density and stomatal density were measured after 45 days of treatment. Wood density was estimated as the ratio between the dry mass of a trunk segment and its maximum fresh volume. Segments were placed in distilled water for 24 hours, then the bark was removed, and their volume was determined through a dimensional method by measuring their length and diameter (average between the beginning, middle, and end of the segment). Subsequently, the segments were dried in a forced-air oven at 80°C until constant weight for dry mass measurement. Stomatal imprints were made by applying a nail varnish on the abaxial surface of the leaves, avoiding the midrib and the leaf margin. After drying, the nail varnish film was gently peeled off using transparent adhesive tape and was fixed on a clean labeled microscope slide (Kardel et al., 2010). The stomatal imprints were analyzed with a light microscope (Olympus BX43, Olympus, Hamburg, Germany). For each imprint, two images were taken in different zones. Stomatal density (number of stomata per mm2) was assessed by counting all the stomata of the image (known area) and extrapolating to 1 mm2.

Whole-plant water-use efficiency and growth

At the beginning and end of the experiment, plants were harvested and divided into leaves, stems, and roots. The dry weight of each plant part was determined after placing the samples in an oven at 70°C until to reach a constant weight. Growth for each plant part was calculated as the means of the difference between the final biomass of leaves, stems, and roots and the average of the initial biomass of each species. The specific leaf area (cm² g⁻¹) was measured by scanning leaves and then analyzed with ImageJ software (version 1.51j8 NIH) with a reference area (O'Neal et al., 2002) and then dried in an oven at 70°C until reaching a constant weight. The total leaf area per plant was estimated by multiplying the specific leaf area and the total dry weight of the leaves.

Whole-plant water-use efficiency (WUE_{wp}) was calculated as the difference between the total biomass at the end of the water deficit period minus the average biomass at the beginning of the experiment, divided by water consumption of the respective period, as in Eq. 6.

$$WUE_{wp} = \frac{Finaldrybiomass - Initialdrybiomass}{Totalwater consumption}; [Eq.6]$$

Statistical analysis

Differences in FTSW_{threshold}, gas exchange threshold, SWC to FTSW = 0, L_p, K_{pl}, biomass, WUE_{wp}, and δ^{13} C were tested using irrigation, species, and their interactions as fixed factors. Heteroscedastic models were used when necessary, followed by a Ficher-LSD *post hoc* analysis when appropriate. For analysis, a significance level of 0.05 was set. A principal component analysis (represented by a biplot) was carried out as an exploratory analysis of the associations between traits, and the similarity between species under well-watered and water deficit conditions. All the statistical analyses were made using InfoStat (version 2016e, Universidad de Córdoba, Córdoba, Argentina) statistical software (Di Rienzo et al., 2011)d v 4.1.1. (R-Core-Team, 2020).

Results

Water use, gas exchange, and stem water potential in response to soil water depletion

From the relationship between relative transpiration (RT) and fraction of transpirable soil water (Supp. Figure 2) were calculated the soil water content in which RT drop to 0.9 and 0. Avocado and mandarin were the more conservative species in terms of water use. These species started a reduction in transpiration at SWC of 90 and 80%, respectively. An intermediate response was observed in pomegranate and 'R20' with a SWC of 57 and 61%, respectively. Late reduction of RT was found in fig and 'R40' with a SWC of 51% in both species (Fig. 1a).

There were differences among species in the residual soil water content (RSWC), i.e. FTSW = 0 which represents the point at which plants could not extract more water from the substrate. Avocado plants reached this point at 67% of the SWC. Mandarin and 'R20' had 56 and 39% of the SWC, respectively. 'R40', pomegranate, and fig exhibited the lower SWC, 33% in pomegranate and 'R40', and 28% in fig (Fig. 1a), showing a higher water use capacity. Species with more water use capacity such as pomegranate, fig and 'R40' had more negative Ψ_{stem} when FTSW = 0 (Fig. 1b).

Figure 2 shows the relationship between physiological traits and the fraction of transpirable soil water (FTSW). Avocado, mandarin, pomegranate, and 'R20' had the earliest drop of relative *A* values with FTSW_{threshold} of 0.41, 0.33, 0.35, and 0.28, respectively (Fig. 2a, b, c, and d), while fig and 'R40' had the more belated reduction of *A* with FTSW_{threshold} of 0.24 and 0.27, respectively (Fig. 2e and f). Avocado and mandarin evidenced the earliest reduction of relative g_s values with FTSW_{threshold} of 0.73 and 0.65, respectively (Fig. 2a and b). Pomegranate, 'R20', fig, and 'R40' had a late reduction of relative g_s values with FTSW_{threshold} of 0.39, 0.39, 0.34, and 0.27, respectively (Fig. 2c, d, e, and f). Avocado, mandarin, and 'R20' showed an earlier reduction of relative Ψ_{stem} values with FTSW_{threshold} of 0.54, 0.51, and 0.49, respectively (Fig. 2a, b, and d). Instead, pomegranate, fig, and 'R40' evidenced a late drop of relative Ψ_{stem} values with FTSW_{threshold} of 0.25, 0.34, and 0.26, respectively (Fig. 2c, e, and f).

Root and whole plant hydraulic conductance

There was a significant interaction between water availability treatments and species for root hydraulic conductivity (L_p ; Fig. 3a) and plant hydraulic conductance (K_{pl} ; Fig. 3b). After 45 days of the experiment, all species except for pomegranate and R20, reduced their L_p under WD. Under WW, avocado and mandarin had the highest L_p , followed by R20. Fig and R20 had lower L_p , and pomegranate had the lowest. Under WD there were two groups. Avocado, mandarin, and R20 with Lp higher than R40, fig, and pomegranate (Fig. 3a). After 38 days of the experiment, under WW condition the highest values of K_{pl} were recorded in pomegranate, fig and 'R40', followed by 'R20' (Fig. 3b). These four species reduced strongly the K_{pl} values under WD condition to 19, 24, 30, and 22% respect to WW condition, respectively (Fig. 3b) and there was no difference in K_{pl} between mandarin and avocado in both irrigation conditions (Fig. 3b).

Hydroscape area as a proxy of iso-anisohydric behavior

Two groups of behavioral groups were identified across the hydroscape area (HA), the near anisohydric species, as pomegranate, fig, 'R40' and 'R20' with HA over 1 MPa², and near isohydric species, as mandarin and avocado with HA below 1 MPa² (Fig. 4).

Biomass and water-use efficiency

Water deficit caused a severe restriction in leaves biomass of pomegranate, fig, mandarin, and avocado, with 32, 47, 45, and 78% less leaf dry matter, respectively, while in 'R40' and 'R20' were no statistical differences (Fig. 5a). In fig, 'R20' and avocado, the stem biomass accumulation was significantly lower under WD than WW conditions falling by 45, 32, and 94%, respectively (Fig. 5b). Pomegranate, fig, 'R40' and 'R20' had the highest root biomass under WW condition, and, oppositely, 'R20' and avocado to WD condition reduced their root biomass up to 30 and 77%, respectively (Fig. 5c). Total leaf area was lower under WD condition in pomegranate, fig, 'R20', mandarin and avocado in a 32, 47, 28, 47 and 78% respectively, while 'R40' rootstock was not affected by WD condition in this parameter (Fig. 5d).

There was no interaction between the factors species and water availability for wood density and stomatal traits, but the factor species was significant (Supp. Figures 7 and 8). Fig and avocado were the species with the lesser wood density, while mandarin and avocado had the higher stomatal density.

Pomegranate and mandarin plants exhibited higher WUE_{wp} values during the water deficit period (Fig. 6a). Under WD, 'R40' increased the WUE_{wp} by 22% for WW condition (Fig. 6a). By contrast, fig, 'R20' and mandarin in control conditions did not modify the WUE_{wp} compared to WD plants. Avocado was the only species that reduced their WUE_{wp} values under WD conditions, and these WD plants showed the lowest values among all species and irrigation treatments, with a reduction of 54% (Fig. 6a). Pomegranate plants had the highest δ^{13} C level regardless of the irrigation treatment (Fig. 6b). Compared with pomegranate, fig, 'R40' and avocado WD plants reached similar values (Fig. 6b). Only fig and 'R40'

increased δ^{13} C levels under WD condition compared to WW plants (Fig. 6b). Mandarin plants had the lowest δ^{13} C values in both irrigation treatments (Fig. 6b).

Traits association

Species displayed a wide variation in all measured traits. The principal component analysis explained 70.5% of the observed variability. Figure 7 shows the biplot with two groups through Principal Component 1, being the species more important than the water regime as a discriminant factor (significances in Supp. Figure 9). The first one was composed of avocado and mandarin, which were characterized by having high values of traits associated with sensitivity to water deficit (A_{thr} , g_{sthr} , FTSW_{thr}, $\Psi_{stem thr}$, and RSWC) and a high L_p value. On the other hand, fig, pomegranate, and *Prunus* spp. ('R40' and 'R20') had high values of HA, biomass accumulation (DM_t), and LA. Principal Component 2 was determined by wood density (W\delta) and water-use efficiency of the whole plant (WUE_{wp}), which showed high values in pomegranate, and full turgor osmotic potential (Ψ_o), which had higher values in fig trees under WW and WD conditions, and in avocado under WD. Regarding the associated with HA but negatively associated with A_{thr}, $\Psi_{stem thr}$, g_{sthr} , FTSW_{thr}, and RSWC. The conductance variables, L_p and K_{pl} were negatively associated. In particular, L_p was positively associated with A_{thr}, $\Psi_{stem thr}$, g_{sthr} , FTSW_{thr}, and RSWC.

Discussion

Multi-traits approach is a good proxy to determine water stress tolerance of different species and it was related to the origin of each species

Through the hydroscape area (HA), avocado and mandarin were classified as species with more strict stomatal control over water potential, suggesting a more isohydric behavior. Alternatively, pomegranate, fig, and *Prunus* spp. showed a less conservative or anisohydric behavior (Delzon, 2015). This classification was supported by the multivariate analysis (Fig. 7), where avocado and mandarin, in contrast to the rest of the species, were characterized by higher gas-exchange thresholds (more sensible), lower water use, but higher root hydraulic conductivity, and a lower growth capacity. As suggested by Martínez-Vilalta et al. (2014) and Fu and Meinzer (2018), our results indicate more isohydric behavior in species from mesic environments than species from dry climates. Species such as avocado and mandarin from tropical and subtropical climates were more sensitive to water deficit than fig, pomegranate, and *Prunus* spp. from desertic to temperate climates (Table 1).

An iso-anisohydric behavior has consequences on growth and water use efficiency

A mild and moderate water deficit, like in this study, favors anisohydric behavior avoiding carbon starvation (Vadez et al., 2013) defined as a lack of carbon because of stomatal closure that has a consequent reduction in dry matter accumulation (McDowell et al., 2008, McDowell, 2011). Anisohydric species like pomegranate, fig, and *Prunus* sp. were less affected in their growth under water deficit, especially in shoots and roots (Fig. 5). These species were able to extract about twice the volume of water in the same volume of substrate compared with avocado plants before reaching severe stress (Fig. 1a). Avocado and mandarin responses to water deficit through an early stomatal closure that limit its growth, a response observed in isohydric plants (McDowell et al., 2008, McDowell, 2011, Manzoni et al., 2013b, Martínez-Vilalta et al., 2014).

At the plant level, near isohydric species such as avocado and mandarin did not show differences in K_{pl} (Fig. 3b), which may be due to the earlier and strong stomatal control (Fig. 2). Also, K_{pl} was positively correlated with dry matter production (Fig. 7). In general, drought-tolerant species have efficient hydraulic systems to extract water quickly in a competitive environment (Rieger et al., 2003, Manzoni et al., 2013b). Tolerant species had higher K_{pl} in WW plants, which could be helpful for quick water consumption, but it was strongly reduced under WD condition (Fig. 3b).

Gas-exchange parameters like g_s *A*, and WUE_i have been widely used in plant physiology studies to determine the point of onset of water stress (Ingram and Bartels, 1996, Yordanov et al., 2000, Reddy et al., 2004, Valliyodan and Nguyen, 2006). Nevertheless, there is not always a good correlation among WUE on different scales (Tomás et al., 2012). Indeed, we observed a low correlation of WUE_{wp} and δ^{13} C with other parameters (Fig. 7). WUE_{wp} and δ^{13} C consider a long period and that's why these parameters are integrative (Glenn, 2010, Belko et al., 2012, Tomás et al., 2012). Anyway, a clear correlation is not frequent (Ma et al., 2010, Liu et al., 2012, Tomás et al., 2012, Wei et al., 2016, Zheng et al., 2020). Due to the variable nature of δ^{13} C throughout the plant, it is crucial to obtain representative samples (Bchir et al., 2016). Thus, it is probable that WUE_{wp} is more accurate and useful to evaluate the phenotypic variability associated with water deficit tolerance, since it is related to the plant growth, while leaves can suffer different degrees of compensation that could not reflect the total plant WUE (Belko et al., 2012, Tomás et al., 2012). Notably, WUE_{wp} was strongly correlated with wood density and oppositely related with leaf osmotic potential (Fig. 7), all expected responses in a tolerant species.

Water deficit response is linked to water use capacity and residual soil water content

We observed that avocado and mandarin plants were severely stressed when the soil water content was 67 and 53% of the field capacity, respectively. This high residual water means a narrow margin between full transpiration and stomatal closure (FTSW = 0), and could be related to a lower water extraction capacity (Martínez-Vilalta et al., 2014). Avocado and mandarin had the highest stomatal control and less root dry matter in comparison with more anisohydric species (Fig. 5c), which may be associated with a

lower root density and a concomitant higher soil-root hydraulic resistance (Passioura, 1983). This wasn't compensated by the less leaf area in both species (Fig. 5d).

The relationship between the fraction of transpirable soil water and relative transpiration (FTSW ~ RT) has been proposed as a good trait to characterize WD tolerance (Vadez et al., 2013, Gambetta et al., 2020). However, based on the results reported in this study, we propose that it is also important to consider the RSWC, since pomegranate and 'R20' had equal FTSW_{threshold} at RT drop (Suppl. Figure 1) but at different RSWC, which would evidence different water uptake capacities (Fig. 1a). The FTSW value does not consider the real SWC reached by each species, it is a relative value intrinsic of the plant, and we observed remarkable differences among species in RSWC (Fig. 1a), so could be a good complement to the FTSW ~ RT relationship.

Drought-tolerant plants can deal with more negative plant water potential and drier soils (Martínez-Vilalta et al., 2014), and this more negative tension inside the plant could explain a bigger water uptake capacity from the soil (Fig. 1b), taking into account of the cohesion tension theory (Manzoni et al., 2013b). It is known that water deficit generates a reduction in hydraulic conductivity, caused by xylem embolism under low water potential, dehydration, and death of roots, being small roots more sensitive than shoots to xylem cavitation (Maherali et al., 2004, McDowell et al., 2008, Manzoni et al., 2013b). It has been postulated that the efficiency traits, i.e. water transport capacity, are compensated by the tolerance to cavitation (Meinzer et al. 2010). In our study root hydraulic conductivity correlates positively with gas-exchange thresholds (Fig. 7), suggesting a more conservative water use under water restriction in species with higher root hydraulic efficiency. Additionally, it is postulated that the mechanical requirements to tolerate high stresses within the xylem conducts, require a higher density of the tissues, which would translate into greater mechanical resistance. Thus, the sensitivity of the xylem to cavitation depends on the diameter of vascular bundles, which can be estimated from the wood density (Hacke et al., 2001). In our study, avocado, the less water stress tolerance species, had the lowest wood density, followed by fig (Suppl. Figure 4).

Finally, it is important to consider in the definition of water stress tolerance a temporal dimension. For example, the slow reduction in the SWC in mandarin plants can be the factor responsible for a 'delayed stress onset' (Lawlor, 2013) or a bigger 'stress distance' (Gambetta et al., 2020), which would represent the amount of time that a plant could stay without water until it reaches the critical water potential threshold (Gambetta et al., 2020). A bigger stress distance could mean a better performance under water deficit conditions if this condition does not last for a long time. In this context, has been observed that the lower water loss evidenced by drought-tolerant tetraploid citrus trees (*Citrus volkameriana* Tan. And Pasq.) meant a delay in the onset of water deficit in this species compared to the drought-sensitive ones (Khalid et al., 2021).

Conclusions

The effect of water deficit on different crops has been studied and discussed for a long time. However, in woody fruit tree species, few comparative studies have been conducted and could be a useful approach for selecting and improving fruit orchards in temperate and sub-tropical zones. Our results reveal that some traits such as the FTSW_{threshold} to the drop of relative transpiration, root hydraulic conductivity, and residual soil water content provide valuable information that allows discriminating species or genotypes better adapted to water deficits even among closer species. These traits explain the position of the studied species in the iso-anisohydric spectrum approached by hydroscape area. Additionally, in this study, a multi-trait approach was capable to discriminate between contrasting species like pomegranate and avocado, and between R40 and R20. More anisohydric species were capable of extracting water in a more drier soil than isohydric species, and in consequence, grew more in a water-deficit period. These mechanistic observations allow us to understand that if avocados induce early stress as the soil dries out and the residual soil water content is relatively high, there is a need for a higher watering frequency with less amount of water to prevent both, water stress and loss of efficiency by drainage.

Declarations

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Competing of interest

The authors have no relevant financial or non-financial interests to disclose."

Author Contributions

"All authors contributed to the study conception, design and analysis. Material preparation and data collection were performed by Ismael Opazo, Paula Pimentel, Ariel Salvatierra, Mauricio Ortiz and Guillermo Toro. The first draft of the manuscript was written by Ismael Opazo and Marco Garrido, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript."

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures



Substrate water content (SWC) when relative transpiration (RT) reaches 0.9 in water deficit (WD) plants (a), and the relationship between residual soil water content (RSWC) and stem water potential (Ψ_{stem}) when the FTSW was equal to 0 (b). White bars indicate the SWC at the start of the reduction in relative transpiration. Black bars indicate the minimum SWC reached by a plant (RSWC). Mean ± SE (n=6). *** indicates statistical significance for linear regression (p < 0.001).



Relationship between the fraction of transpirable soil water (FTSW) and relative photosynthesis (A_{rel}), relative stomatal conductance ($g_{s rel}$), and relative stem water potential ($\Psi_{stem rel}$) of (a) avocado, (b) mandarin, (c) pomegranate, (d) 'R20', (e) fig, and (f) 'R40'. The dotted lines indicate RT is equal to 1. All logistic regressions were significant (p < 0.001). On top of each graph, the value of the initial point for stress (FTSW_{threshold}), which corresponds to a RT value of 0.90, is indicated using the logistic curves

derived from all the replicates within a species. Mean \pm SE (n=6). Different letters denote significant differences (p < 0.05) among all species and variables according to Fisher's LSD test.



Figure 3

Root hydraulic conductivity (L_p) after 45 days of water deficit (a) and whole-plant hydraulic conductivity (K_{pl}) after 38 days of water deficit (b). Black and white bars represent well-watered (WW) and water deficit (WD) treatments, respectively. Different letters denote significant differences (p < 0.05) within the interaction according to Fisher's LSD test. The top tables indicate the statistical significance of each factor from the analysis of variance. *** indicates p < 0.001. Mean ± SE (n=6 and 4 for L_p or K_{pl} , respectively). The top arrow indicates the interaction plotted.



Hydroscape area (HA; MPa2) of (a) pomegranate, (b) fig, (c) 'R40', (d) 'R20', (e) mandarin, and (f) avocado. The HA was estimated following the method proposed by Johnson *et al.* (2018), as the area of the polygon that surrounds the ordered pairs (Ψ pd, Ψ md), having as upper limit the line 1:1. Black and white circles represent well-watered (WW) and water deficit (WD) conditions, respectively (each point is a mean of n=4).



Accumulation of dry leaves biomass (a), stem biomass (b), root biomass (c), and total leaf area (d) after 45 days of water deficit. Black and white bars represent well-watered (WW) and water deficit (WD) treatments, respectively. Different letters denote significant differences (p < 0.05) within the same interaction according to Fisher's LSD test. The top tables indicate the statistical significance of each factor from the analysis of variance. *, **, *** indicates p < 0.05, 0.01, or 0.001, respectively. Mean ± SE (n=6). The top arrows indicate the interaction plotted.



Whole-plant water-use efficiency (a) and leaf isotopic composition of ¹³C (δ^{13} C; b) after 45 days of water deficit. Black and white bars represent well-watered (WW) and water deficit (WD) treatments, respectively. Different letters denote significant differences (p < 0.05) within the interaction according to Fisher's LSD test. The top tables indicate the statistical significance of each factor from the analysis of variance. ns = non-significant factor. *, **, *** indicates p < 0.05, 0.01, or 0.001, respectively. Mean ± SE (n=6). The top arrows indicate the factor or interaction plotted.



Principal component analysis (PCA) of 15 traits associated with water deficit tolerance, hydraulic architecture, and growth of avocado (A), pomegranate (P), mandarin (M), fig (F), Rootpac®40 rootstock ('R40') and Rootpac®20 rootstock ('R20') under well-watered (WW) and water deficit (WD) conditions. The species were grouped according to the water regime, with blue points for WW and red points for WD. The variables used in this analysis were the fraction of transpirable substrate water where relative net photosynthesis, relative stomatal conductance, relative transpiration, and relative stem water potential (Arel, gsrel, Trel, and Ψ (stem)rel, respectively) reach 90% of their maximum value, leaf isotopic ¹³C composition (d13C), plant hydraulic conductance (K_{pl}), root hydraulic conductivity (L_p), wood density (W\delta), hydroscape area (HA), full turgor osmotic potential (Ψ_o), leaf area (LA), total dry matter (DMt), whole plant water-use efficiency (WUE_{wp}), residual water content (RSWC) and the difference between minimum leaf water potential ($\Delta\Psi$) of species under WW and WD conditions.

Supplementary Files

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