

Article

Rootstock Effects on Water Relations of Young Almond Trees (cv. Soleta) When Subjected to Water Stress and Rehydration

Sara Álvarez ^{1,*}, Hugo Martín ¹, Enrique Barajas ¹, José Antonio Rubio ¹ and Gaetano Alessandro Vivaldi ²

¹ Instituto Tecnológico Agrario de Castilla y León, Ctra, Burgos km 119, 47071 Valladolid, Spain; marguthu@itacyl.es (H.M.); enrique.barajas@itacyl.es (E.B.); rubcanjo@itacyl.es (J.A.R.)

² Dipartimento di Scienze Agro Ambientali e Territoriali (DiSAAT), Università degli Studi di Bari Aldo Moro, Via Giovanni Amendola 165/A, 70126 Bari, Italy; gaetano.vivaldi@uniba.it

* Correspondence: alvmarsa@itacyl.es

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Abstract: Rootstocks with size controlling potential are being used in newly planted intensive almond orchards. Due to increased water scarcity, characterizing the response of these rootstocks to water deficit is required. The current work aims to assess whether the rootstock can improve their drought tolerance. We investigated the morphological and physiological response of *P. dulcis* “Soleta” either self-rooted or grafted on Rootpac-20 rootstock. Plant responses were evaluated during a water stress period (withholding irrigation for 20 days) and subsequent recovery in potted plants under greenhouse conditions. Self-rooted plants had a higher capacity to control vigour than plants grafted onto Rootpac-20, both under full irrigation and no irrigation conditions. Stressed plants exhibited severe dehydration, as indicated by lower leaf water potential and relative water content. Removing irrigation reduced stomatal conductance in grafted and self-rooted plants by a similar extent, suggesting an efficient stomatal control, while the reduction in the net photosynthesis rate was more marked in grafted plants compared to non-grafted plants. Self-rooted plants under water stress increased their root to shoot ratio and water use efficiency, which are positive aspects for growth and survival of these plants.

Keywords: *Prunus dulcis*; evapotranspiration; gas exchange; self-rooted; high density plantings; water use efficiency; grafting

1. Introduction

Almond is an important crop in the USA, Australia and Spain, which are the main world almond-producing countries. In the Mediterranean area, doubts about further financial support provided by the European Union to some traditional crops, such as vineyards and olive, and the low profitability of some crops, such as cereals, has led to new almond plantations. Almond is one of the major tree crops in Spain in terms of cultivated area, 822.878 ha according to Encuesta sobre Superficies y Rendimientos de Cultivos en España (ESYRCE) 2019 [1]. In Spain, the almond production is mainly located in the Mediterranean coastal regions, but it is becoming an interesting alternative to traditional crops in other regions of Spain, such as in the interior of the country [2]. Although in the world’s main almond-producing country (USA), most of orchards are irrigated, there are large areas in Mediterranean countries where rainfed conditions are not uncommon [3]. Almond (*Prunus amygdalus* Batsch, syn. *P. dulcis* (Mill.) D.A. Webb) is considered a drought-tolerant species, but its production increases enormously under full irrigation compared to rainfed conditions [4,5]. Thus, irrigation systems are

being installed in newly planted orchards. However, in the context of the scarcity of water resources, full irrigation might be an unrealistic option, and deficit irrigation strategies or rainfed conditions might be advised [6–8]. Even in irrigated orchards, some periods of water stress could occur due to the limited availability of irrigation water in some areas [5,9]. In such a situation, managing global water resources is needed as there is an important pressure in agriculture and fruit culture to cultivate crops more efficiently by increasing water savings [10]. Given the economic importance of almonds in Mediterranean countries, the almond response to water stress has been widely studied and various physiological and morphological mechanisms developed by this crop to confront water stress have been identified: osmotic adjust, elastic properties, control over stomatal regulation, the onset of leaf abscission and the presence of a deeply penetrating root system [3,11–14].

On the other hand, almond growing in the Mediterranean area has undergone significant changes in recent years. Current trends in almond orchards have focused on intensification and high-density plantings [2]. In this context, not only new cultivars but also new rootstocks are essential tools to achieve success in these new super-intensive or very high-density plantations. The use of rootstocks that reduce tree vigour is a common practice in modern olive orchards [15]. In contrast, knowledge about the influence of rootstock in the adaptation of this new high-density planting system of almond trees is very scarce. Selecting the right scion–rootstock combination is also important in the adaptation of the fruit tree to specific training system

Recently, rootstocks with low vigour were developed in Spain, such as the “Rootpac[®]” series, which opens the possibility to develop almonds under high-density planting system. The physiological and morphological responses of different scion–rootstock combinations of a commercial almond orchard under irrigation at high density planting were studied by BenYahmed et al. [16]. These authors conclude that “Rootpac-20”, the most dwarfing rootstock, resulted in bad adaptation to Mediterranean conditions. The information provided was important for selecting the right scion–rootstock association for the establishment of a new orchard under irrigation conditions. Considering the actual trend of increasing the almond acreage, especially in semi-arid conditions, it is of foremost importance to increase our knowledge on the behaviour of this crop and the mechanisms underlying the reduced vegetative growth of scions grafted on low-vigour rootstock, especially under water stress situations. Moreover, it is well known that the rootstocks may also confer tolerance to different biotic and abiotic stresses in the soil [17] and grafting is a potential approach that can mitigate negative drought effects [18]. In addition, farmers should select plant materials that have lower water requirements or are able to cope with water scarcity while maintaining yield and fruit quality [19]. In this sense, more recently, the production of self-rooted almond trees in hedges constitutes a technological innovation that opens the possibility of achieving greater profitability under non-irrigation conditions. The self-rooted plant offers the advantages of having the root system of the almond tree and its adaptation to drought, and also avoids grafting, thus lowering the cost of production in the nursery [20,21]. In woody crops, few and inconclusive studies have documented the response of these new low-vigour rootstocks [2,16,22–24]. The selection of the suitable plant material is very important, particularly in limited water conditions, however, only indirect information about the water relations and drought resistance is available. In this way, the knowledge of physiological processes that promote drought tolerance can improve our understanding of the mechanisms involved in scion/rootstock interactions and also on the selection of proper rootstocks to be used under different irrigation conditions. There are several rootstocks widely used around the world and their selection is, sometimes, more dependent on availability than on the actual information about their agronomic aptitude [9]. Knowledge about the relationship between drought tolerance and the rootstock, as well as information regarding the response of almond plants grafted on low-vigour rootstock and self-rooted plants during water stress recovery, is scarce, and the physiological mechanisms involved in the recovery process remain poorly understood [25–27].

In the context of almond production intensification under limited water supply, the objective of this research was to study the morphological and physiological response to water stress of one-year-old almond plants (cv. “Soleta”) grafted onto Rootpac-20 rootstock and self-rooted plants. The response

of the plant during drought recovery was also considered, which is important when selecting plant material to be used under different irrigation conditions.

2. Materials and Methods

2.1. Plant Material and Experimental Conditions

The experiment was conducted during the summer of 2018 at Itacyl Research Station, Valladolid, Spain (41°42' N, 4°42' W, 705 m a.s.l.). One-year-old almond plants (*Prunus dulcis* L. cv. Soleta from the breeding program of the Unidad de Fruticultura, Centro de Investigación y Tecnología Agroalimentaria de Aragón), either self-rooted or grafted on low-vigour rootstock, were used. One half of the plants was grafted on Rootpac-20 rootstock (*Prunus besseyi* × *Prunus cerasifera* from the breeding program of Agromillora), in order to assess the physiological responses to irrigation of the grafted combination (Rp) when compared to the self-rooted plants (Sr). Plants were grown in 5 L pots filled with an 8:7:1 mixture of coconut fibre:black peat:vermiculite and placed inside a plastic greenhouse equipped with a cooling system. The temperature and relative humidity were registered with a Hoboware Lite Data Logger (Escort Data Logger, Inc., Buchanan, VA, USA). The micro-climatic conditions registered to the total experiment were 26.8 °C (average) temperature, 59.68% (average) relative humidity, and 3.84 (mean maximum) and 1.66 (average) vapour pressure deficit (VPD). All the plants were watered daily for 3 weeks to field capacity prior to starting the treatments.

2.2. Treatments and Experimental Design

Almond plants were grown under greenhouse conditions and subjected to two irrigation treatments using a computer-controlled drip irrigation system from June to September 2018. The irrigation treatments were full irrigation (Control) and no irrigation (Stress). The irrigation treatments consisted of a control (C)—when substrate moisture was maintained close to container capacity, it was watered daily to 100% water holding capacity (leaching 15% (*v/v*) of the applied water), and a stress treatment—removing irrigation during 20 days (S). The different treatments will be named as follows: plants grafted under full irrigation (RpC); self-rooted plants under full irrigation (SrC); plants grafted submitted to water stress (RpS); self-rooted plants submitted to water stress (SrS). One drip nozzle, delivering 2 L h⁻¹ per pot, was connected to two spaghetti tubes (one on the side of every pot) and the duration of each irrigation episode was used to vary the amount of water applied, which depended on the season and on climatic conditions. The electrical conductivity of the water applied was 0.4 dS m⁻¹. In plants subjected to water stress, irrigation was withdrawn from day of the year (DOY) 198 until 218 (stress period). Once the stress period was completed, the plants were exposed to a recovery period of 35 days with the same irrigation regime applied to control plants, and pots were re-watered up to field capacity until the end of the experiment (recovery period, DOY 218–253).

2.3. Growth and Plant Water Measurements

At the end of the stress period (DOY 218) and at the end of the recovery period (DOY 253) eight plants per treatment were harvested and separated into leaves, stems and roots. They were then oven-dried at 80 °C until they reached a constant weight to measure the respective dry weights (DWs). Leaf number and leaf area (cm²), using a leaf area meter (Delta-T; Devices Ltd., Cambridge, UK), were determined in the same plants. In addition, the root to shoot ratio was determined in these plants and calculated by dividing root DW by leaf DW. Throughout the experiment, the plant height and trunk diameter were measured in 20 plants per treatment once a week.

To determine the maximum water holding capacity of the substrate, five samples were uniformly mixed and packed to a similar bulk density. The pot surfaces were covered with aluminium foil to prevent water evapotranspiration, and the lower parts were submerged to half the pot's height, in a water bath; then, the pots were removed and left to equilibrate overnight. The next day, the pots were removed and left to drain freely until drainage became negligible. The fresh weight was then recorded

for each individual pot and considered as the weight at field capacity. At the end of the experiment, the substrate was dried in an oven at 105 °C until constant weight in order to obtain the dry weight and calculate the volumetric water content. Later, the difference between the weight at field capacity and the oven-dry weight was measured and the volumetric water content was calculated (64%), which was considered as the substrate field capacity. Evapotranspiration (ET) was measured gravimetrically throughout the experimental period in five plants per treatment, using the difference in weights (weight after irrigation and weight before irrigating again), using a balance (Analytical Sartorius, Model 2501; capacity 5.2 g and accuracy of 0.01 g, SECURA Insurance, Fox Crossing, WI, USA). Then, the difference between the fresh weight and oven-dry weight was measured, giving the volumetric water content of these monitored pots. Moreover, at the end of the stress period (DOY 216), the weights of these pots were also recorded several times during the day, giving the hourly ET throughout the day.

Seasonal changes in stem water potential (Ψ_s), relative water content (RWC), stomatal conductance (g_s) and net photosynthetic rate (P_n) were determined in 6 plants per treatment during the central hours of illumination. In addition, at the end of the stress period, the diurnal patterns of ET, g_s , and P_n were measured at a 2 h interval (diurnal course). Stem water potential was estimated according to the method described by Scholander et al. [28], using a pressure chamber (Soil Moisture Equipment Co, Santa Barbara, CA, USA), for which leaves were placed in the chamber within 20 s of collection and pressurised at a rate 0.02 MPa s⁻¹ [29], while the RWC of leaves was calculated according to Barrs [30]. Stem water potentials were measured in non-transpiring leaves that had been bagged with both a plastic sheet and aluminium foil for at least 1 h before measurement in order to prevent leaf transpiration; in this way, the leaf water potential equalled stem water potential [31]. Gas exchange parameters (g_s and P_n) were determined in attached leaves using a gas exchange system (LI-COR, LI-COR Inc., Lincoln, NE, USA). Water use efficiency of production (WUE) was calculated at the end of the experiment by dividing the increment in dry weight by the water used.

2.4. Statistical Analyses of Data

In the experiment, 20 plants were randomly assigned to each treatment. The data were analysed by one-way ANOVA using SPSS 17.0 software (SPSS Inc., 2002, Chicago, IL, USA). Ratio and percentage data were subjected to an arcsine square-root transformation before statistical analysis to ensure homogeneity of variance. Treatment means were separated with Duncan's Multiple Range Test. Statistical comparisons were considered significant at $p \leq 0.05$.

3. Results

3.1. Substrate Water Content (SWC) and Evapotranspiration (ET)

The volumetric water content of the substrate before and after irrigation reflected the different irrigation treatments and the climatic conditions (Figure 1A). It was higher in the control plants and decreased during the water stress period in the stressed plants with respect to the controls. After irrigation, the substrate water content (SWC) in the full irrigated plants remained on average at about 0.60–0.64 m³ m⁻³, above and close to container capacity (Figure 1A). SWC in stressed plants was lower than in the controls and decreased from container capacity to 10% in RpS and to 8% in SrS, at the end of the stress period, coinciding with the time of maximum stress. The pots had an initial mean weight of 3.9 kg when the substrate was at field capacity and those of the stressed plants lost on average 2.6 and 2.7 kg (RpS and SrS, respectively) from the beginning of the experiment to the time of maximum stress (DOY 218). The recovery of the substrate water content was very fast. One day after the beginning of the rehydration (recovery period), SrS pots recovered 77.3% of their initial weight, followed by the RpS (75.9%). After two days, both plant materials (Rootpac-20 and self-rooted plants) had recovered almost their initial weight: 96.6% and 93.8% (SrS and RpS, respectively).

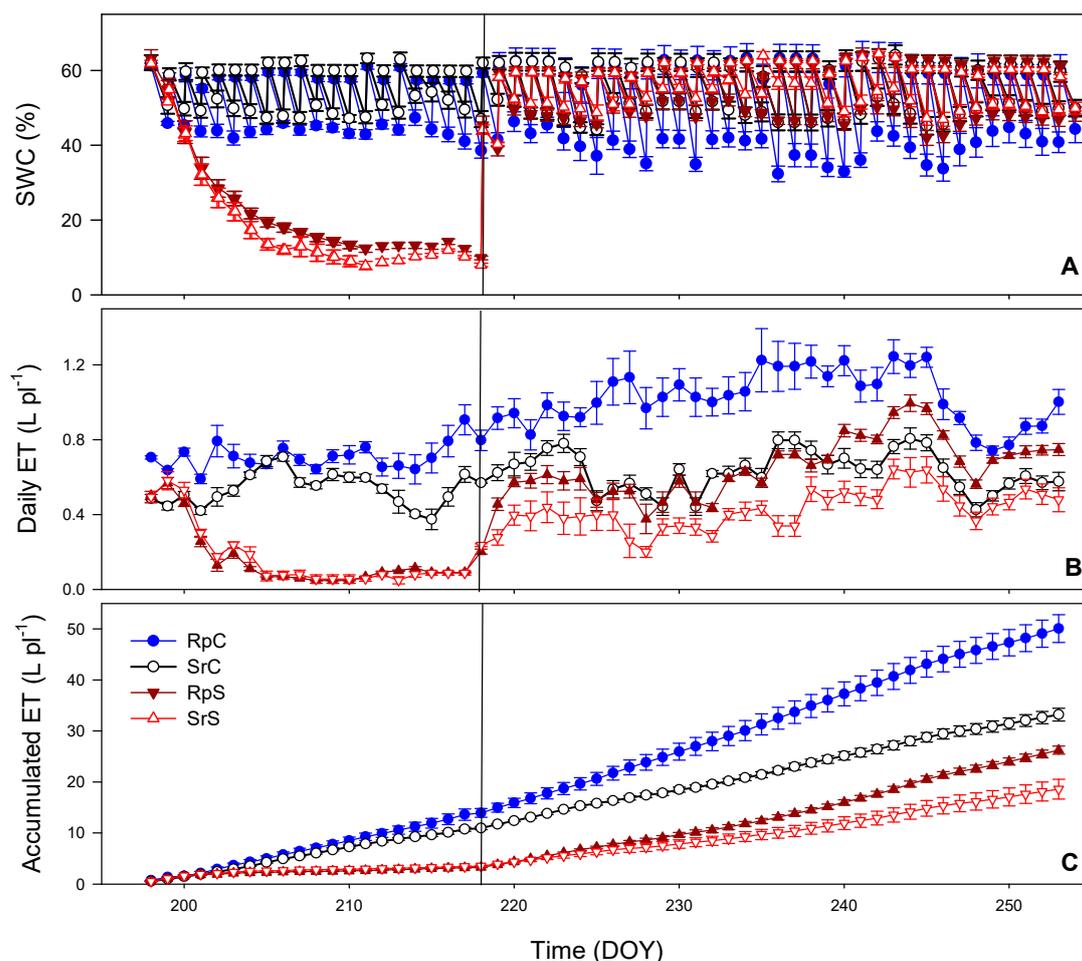


Figure 1. Evolution of the substrate volumetric water content (SWC, (A)), daily evapotranspiration (ET, (B)) and accumulated evapotranspiration (C) in *P. dulcis* plants submitted to different treatments. Values are means + s.e., $n = 5$. Symbols represent the different treatments: RpC (filled circles; plants grafted under full irrigation), SrC (open circles; self-rooted plants under full irrigation), RpS (filled triangles; plants grafted submitted to water stress) and SrS (open triangles; self-rooted plants submitted to water stress). Vertical line indicates irrigation change, the end of the water stress period and the beginning of recovery period.

The evolution of evapotranspiration (ET) along the study period is presented in Figure 1B. During the experimental period (DOY 198–253), daily evapotranspiration values ranged from 375 to 1250 mL d⁻¹ per pot in plants under full irrigation (Figure 1B), while ET values in water stressed plants were significantly lower. The rootstock regime also affected daily ET and differences between both well-irrigated treatments were evident throughout the experimental period in this respect. Evapotranspiration was higher in RpC plants than in plants of the SrC treatment, and these differences were greater as the time progressed. In the stressed plants, when the irrigation pattern was changed, the plants increased or decreased their water consumption (ET) and adjusted to the new conditions, but with some particular characteristics (Figure 1B). When plants were exposed to water stress conditions, SWC decreased progressively and plants of both stress treatments restricted their daily ET. During this phase, the ET of both stressed plants was similar, reaching very low ET values of below 70 mL per pot during most of the water stress period (DOY 205–217). Once well-watered conditions were restored (DOY 218), the humidity in the substrate immediately recovered. In contrast, ET values in the stressed plants increased more slowly and were still significantly lower than that in control plants during the 30 days following the beginning of the recovery period. Only at the end of the experiment, ET in the stressed plants matched that of plants that had been exposed to

full irrigation since the beginning of the experiment. The water consumption in each pot during the whole experimental period was 50.1 L for RpC plants and 33.2, 26.2 and 18.6 L for SrC, RpS and Sr-S plants, respectively (66.3%, 52.4% and 37.1% of the amount of water compared with RpC treatment) (Figure 1C). RpS had 52% of the amount of water supplied in the RpC and SrS had 55% of the amount of water supplied in the SrC.

The behaviour of the evapotranspiration rate on a representative day at the end of the stress period can be seen in Figure 2A. When plants were well irrigated, the highest ET values were reached between 13:00 and 18:00 h (11:00 and 16:00 solar time) especially in grafted plants (71 mL per 60 min in RpC and 52 mL in SrC), coinciding with the highest temperature and VPD, after which, evapotranspiration decreased (Figure 2B). In plants submitted to water stress, the transpiration curve was more stable throughout the day, independently of temperature and DPV changes. The ET of stressed plants (self-rooted and grafted) was similar and very low (coinciding with minimum water levels in the substrate, approximately of 10%). Only grafted plants (RpS) increased their ET at the end of the day. Although this did not occur in the case of SrS plants, in which ET remained low during all times of the day (from pre-dawn to afternoon).

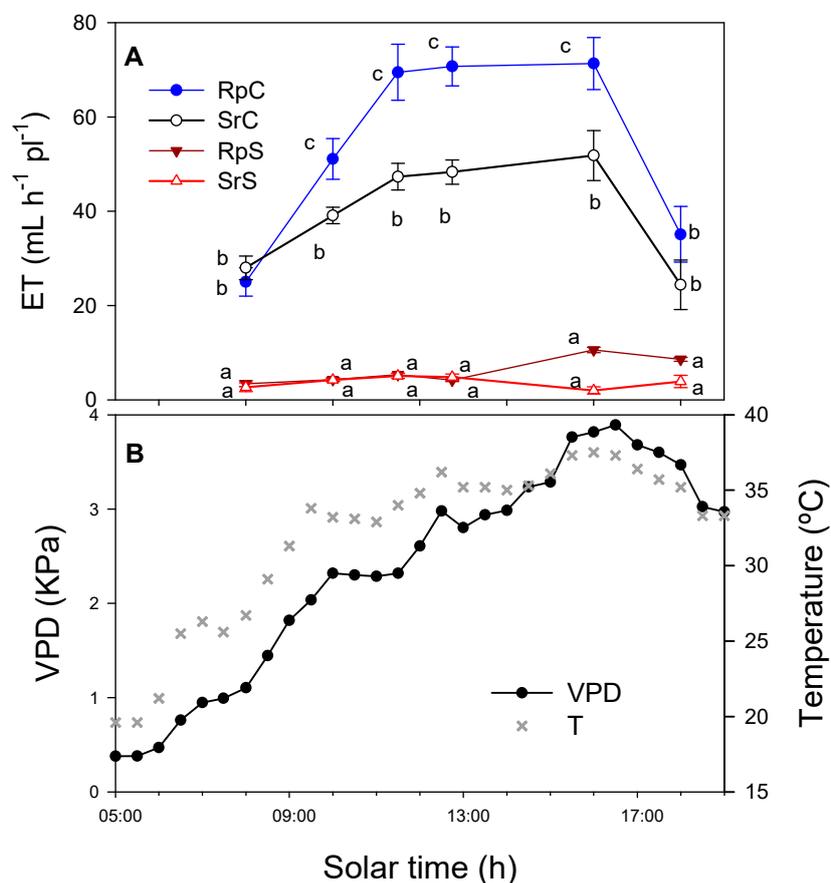


Figure 2. Evolution of hourly evapotranspiration (ET, (A)) and vapour deficit pressure (VPD and temperature (B)) throughout a representative day at the end of the water stress period in *P. dulcis* plants submitted to different treatments. Values are means + s.e., n = 5. Symbols represent the different treatments: RpC (filled circles; plants grafted under full irrigation), SrC (open circles; self-rooted plants under full irrigation), RpS (filled triangles; plants grafted submitted to water stress) and SrS (open triangles; self-rooted plants submitted to water stress). Different lowercase letters indicate significant differences between treatments according to Duncan 0.05 test.

3.2. Plant Growth

Water deficit had a significant effect on biomass accumulation of the almond plants at the end of the stress and recovery period (Table 1). Plants submitted to water stress for 20 days reduced leaf dry weight (DW) at the end of the stress period compared with the controls. When plants were full irrigated, the leaf area was higher in grafted (RpC) than in self-rooted plants (SrC). In contrast, in non-irrigated plants, the reduction in leaf area compared with controls was more marked in RpS than in SrS, 77% and 67% in RpS and SrS, respectively. The reduction in leaf area induced by water stress was due to a decrease in the number of leaves and in the individual leaf size.

Table 1. Growth parameters at the end of the stress and recovery period in *P. dulcis* subjected to different irrigation treatments: plants grafted under full irrigation (RpC), self-rooted plants under full irrigation (SrC), plants grafted submitted to water stress (RpS) and self-rooted plants submitted to water stress (SrS). Means within a row without a common letter are significantly different by the Duncan 0.05 test. Each value is the mean of eight plants per treatments.

Period	Parameters	RpC	SrC	RpS	SrS
Stress	Leaf DW (g pl ⁻¹)	14.9 ± 0.9 b	12.6 ± 0.9 b	3.8 ± 0.7 a	3.6 ± 0.7 a
	Stem DW (g pl ⁻¹)	48.6 ± 4.5 c	25.4 ± 2.9 b	28.5 ± 1.9 b	16.1 ± 0.8 a
	Root DW (g pl ⁻¹)	21.6 ± 2.5 bc	26.9 ± 1.2 c	10.6 ± 1.0 a	20.3 ± 0.7 b
	Root to shoot ratio	1.21 ± 0.07 a	2.36 ± 0.08 b	2.16 ± 0.05 ab	6.06 ± 0.39 c
	Total leaf area (cm ²)	1306 ± 66 c	970 ± 57 b	297 ± 55 a	316 ± 67 a
	Number of leaves	405.0 ± 14.0 c	336.7 ± 19.9 b	129.2 ± 22.5 a	129.3 ± 20.7 a
	Leaf blade area (cm ²)	3.2 ± 0.2 b	3.0 ± 0.2 b	1.9 ± 0.2 a	2.0 ± 0.3 a
Recovery	Leaf DW (g pl ⁻¹)	26.3 ± 1.6 c	14.3 ± 1.4 ab	16.0 ± 16.0 b	11.6 ± 0.8 a
	Stem DW (g pl ⁻¹)	83.9 ± 5.4 d	33.8 ± 2.5 b	33.8 ± 33.8 c	24.2 ± 1.4 a
	Root DW (g p ⁻¹)	32.9 ± 2.6 b	35.5 ± 1.9 b	18.7 ± 0.5 a	24.2 ± 0.3 a
	Root to shoot ratio	1.32 ± 0.05 a	2.42 ± 0.05 b	1.13 ± 0.05 a	2.22 ± 0.04 b
	Total leaf area (cm ²)	2278 ± 197 c	1120 ± 146.4 a	1594 ± 89 b	917 ± 55 a
	Number of leaves	491.3 ± 51.9 c	345.8 ± 48.3 b	403.8 ± 21.7 b	215.0 ± 26.4 a
	Leaf blade area (cm ²)	4.2 ± 0.3	3.6 ± 0.3	3.9 ± 0.3	4.4 ± 0.3

Means within a row without a common letter are significantly different by the Duncan 0.05 test.

Both self-rooted plants (Sr) had higher root to shoot ratios than grafted ones (Rp) at the end of stress period, being particularly marked in self-rooted plants submitted to water deficit (SrS). At the end of recovery period (DOY 253), root to shoot ratios in self-rooted plants were still higher than in grafted plants, but no differences were detected between RpS and SrS at that time. In addition, for each irrigation regime, grafted plants had higher aerial biomass production values (leaf DW, stem DW, leaf area and number of leaves) than those found for self-rooted at the end of the experimental period.

Trunk diameter and plant height increased with time in all irrigation treatments and material types. Figure 3A shows the values of the trunk diameter as a fraction of the diameter at the beginning (TD/TDi) of the experiment for each treatment. When water stress was induced, the trunk diameter accumulation decreased in the plants grafted on Rootpac-20 and at the end of the stress period, RpS had the lowest values for trunk diameter accumulation, while the well-irrigated plants grafted on Rp (RpC) had the highest values (Figure 3A). At this time, trunk diameter accumulation also decreased as a result of water stress in the self-rooted plants, but it was less affected than in Rp plants and no significant differences were observed between well-irrigated and water stressed in self-rooted plants. During the recovery period, trunk diameter growth slightly increased in SrS plants with respect to the values observed during the water stress period, but trunk diameter increased markedly in the RpS plants. At the end of recovery period, the plants with highest trunk diameter accumulation were those from RpC and the lowest from SrS, while the respective trunk diameter accumulations of RpS and SrS were similar.

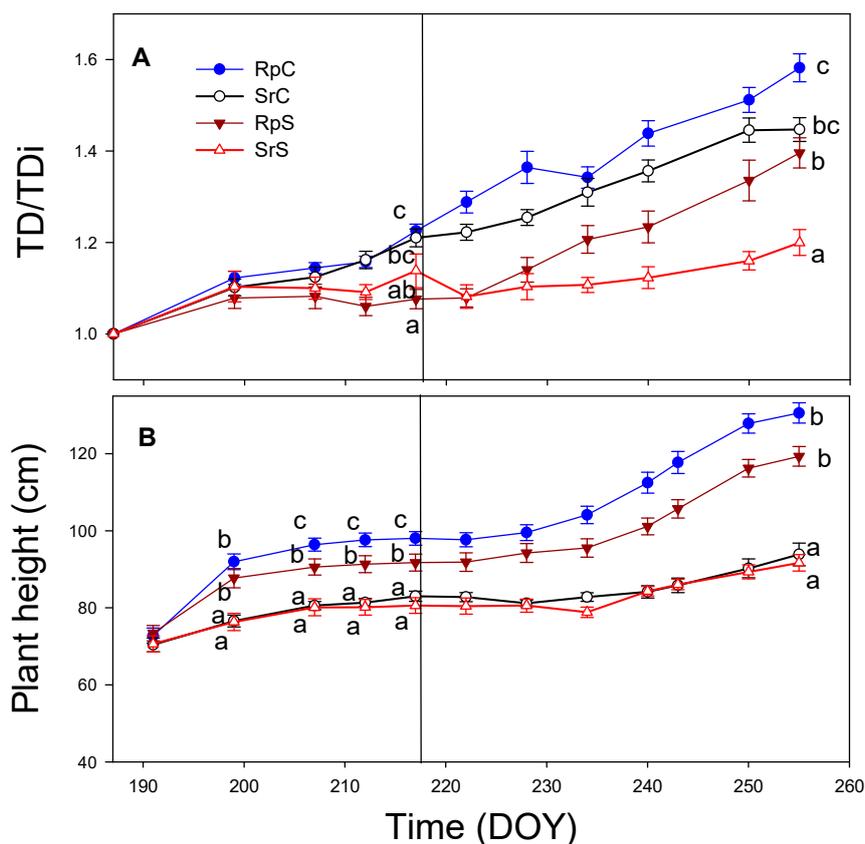


Figure 3. Evolution of trunk diameter as a fraction of the initial value (TD/TDi) (A) and plant height (B) in *P. dulcis* plants submitted to different treatments. Values are means + s.e., $n = 20$. Symbols represent the different treatments: RpC (filled circles; plants grafted under full irrigation), SrC (open circles; self-rooted plants under full irrigation), RpS (filled triangles; plants grafted submitted to water stress) and SrS (open triangles; self-rooted plants submitted to water stress). Different lowercase letters indicate significant differences between treatments according to Duncan 0.05 test. Vertical line indicates irrigation change, the end of the water stress period and the beginning of recovery period.

Plant height was less affected due to water stress than trunk diameter during the experimental period (Figure 3B). No significant changes were observed in the plant height of self-rooted plants by irrigation effect, but height decreased as a result of water stress in the grafted plants at the end of the water stress period. RpC plants reached the greatest height, while the self-rooted plants had a significant reduction from the beginning of the experiment, leading to the smallest plants. At the beginning of the experiment, plant height was similar in both the RpC and RpS treatment, but it was inhibited by the latter 2 weeks after application onwards (from 2 weeks after beginning of deficit irrigation treatments). At the end of the experiment, the reductions were around 9%, 28% and 30% for RpS, SrC and SrS, respectively, compared with RpC.

3.3. Plant Water Relations and Gas Exchange Parameters

In response to the different irrigation amounts and changes in soil water content, different seasonal trends in midday stem potential (Ψ_s) developed in each treatment (Figure 4A). Plants irrigated at full water requirements had Ψ_s values around -1.2 MPa throughout the experimental period, which was indicative that these plants were never short of water. By contrast, the non-irrigated plants had a decreasing Ψ_s as water stress developed with time, with minimum values at the end of stress period, when substrate water content values were lowest. The stem water potential at midday (Ψ_s) decreased in both stress treatments, especially in the RpS plants, in which values of -3.4 MPa were reached at

the end of the stress period (Figure 4A). This was followed by an increase in the Ψ_s values for water deficit treatments when the irrigation restriction ended, and all plants were fully irrigated. Ψ_s of SrS plants recovered rapidly to values similar to those of the fully irrigated plants (DOY 218, 1 day after rehydration), while in the most stressed plants (RpS) this recovery took more time (DOY 232, 14 days after rehydration).

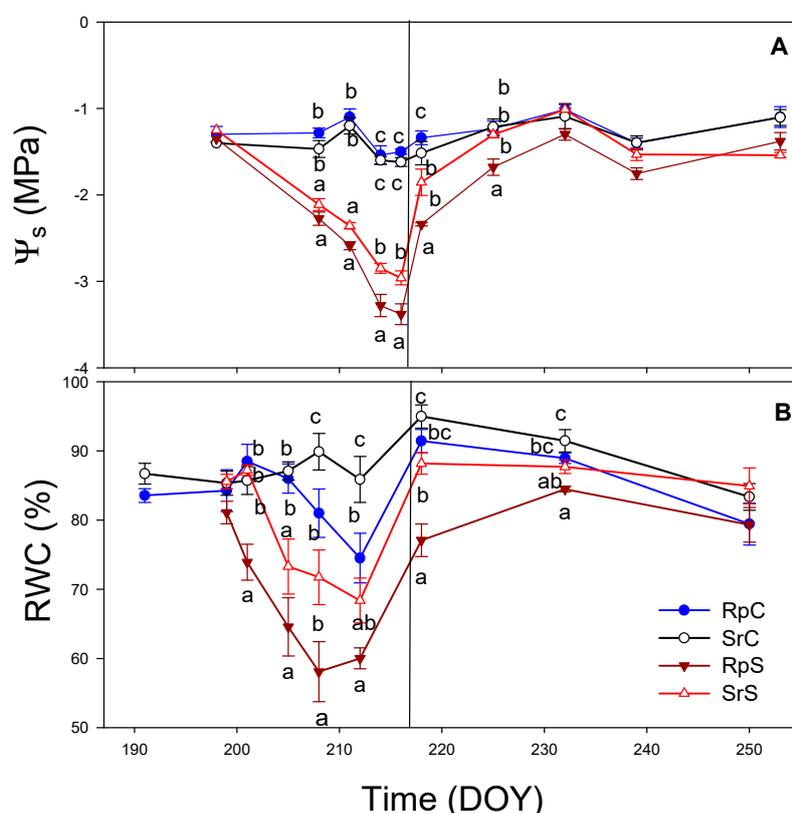


Figure 4. Evolution of the stem water potential (Ψ_s , (A)) and leaf relative water content (RWC, (B)) in *P. dulcis* plants submitted to different treatments. Values are means + s.e., $n = 6$. Symbols represent the different treatments: RpC (filled circles; plants grafted under full irrigation), SrC (open circles; self-rooted plants under full irrigation), RpS (filled triangles; plants grafted submitted to water stress) and SrS (open triangles; self-rooted plants submitted to water stress). Different lowercase letters indicate significant differences between treatments according to Duncan 0.05 test. Vertical line indicates irrigation change, the end of the water stress period and the beginning of recovery period.

The relative water content (RWC) showed a similar behaviour to that observed for Ψ_s , with plants subjected to water stress having the lowest values, especially in grafted (Rp) plants (Figure 4B). No pronounced differences in RWC were observed between self-rooted and grafted plants under full irrigation conditions (RpC and SrC plants) during most of the experimental period, although using Rootpac-20 as rootstock affected RWC on some days of the experiment (end of July), when lower values were observed in the RpC treatment compared with the SrC treatment. The lowest value for RWC was found in RpS plants, reaching a value of 58.0% at the end of the stress period, coinciding with the lowest values of Ψ_s (Figure 4A). The end of the irrigation restriction and the beginning of the rehydration period was followed by an increase in the values of RWC for stress treatments, and 1 day after the onset of rehydration period, differences with the controls were only observed in RpS plants. At the end of the experimental period, both the Ψ_s and RWC values of the plants that had been exposed to irrigation restriction were similar to those of the control treatments.

The values of the stomatal conductance (g_s) and the photosynthetic rate (P_n) at midday during the experimental period can be seen in Figure 5. In general, g_s values were highest in the control plants,

while the g_s values changed in the stressed treatments according to the irrigation applied in each phase (Figure 5A). When the change in irrigation involved a restriction of irrigation, g_s decreased in the plants of both stress treatments. During the stress period, these plants had lower g_s values than the fully irrigated plants, regardless of the rootstock. All the plants subjected to irrigation restriction had very low values of g_s at midday (below $50 \text{ mmol m}^{-2} \text{ s}^{-1}$) during most of the stress period (July, DOY 205–215). Such reductions with respect to the control plants were also observed in the photosynthesis levels at midday, although significant differences were observed between RpS and SrS (Figure 5B). The plants of SrS treatment had higher P_n values at midday than RpS plants throughout the stress period, despite having similar g_s values. When irrigation was restored, g_s and P_n increased in the plants of both stress treatments, although the plants did not reach the values of the control plants until 7 days after the onset of rehydration period. One day after the onset of the rehydration period, P_n and g_s values in the plants submitted to water stress (RpS and SrS) remained lower than control plants, despite having similar substrate water content values to the well-irrigated plants.

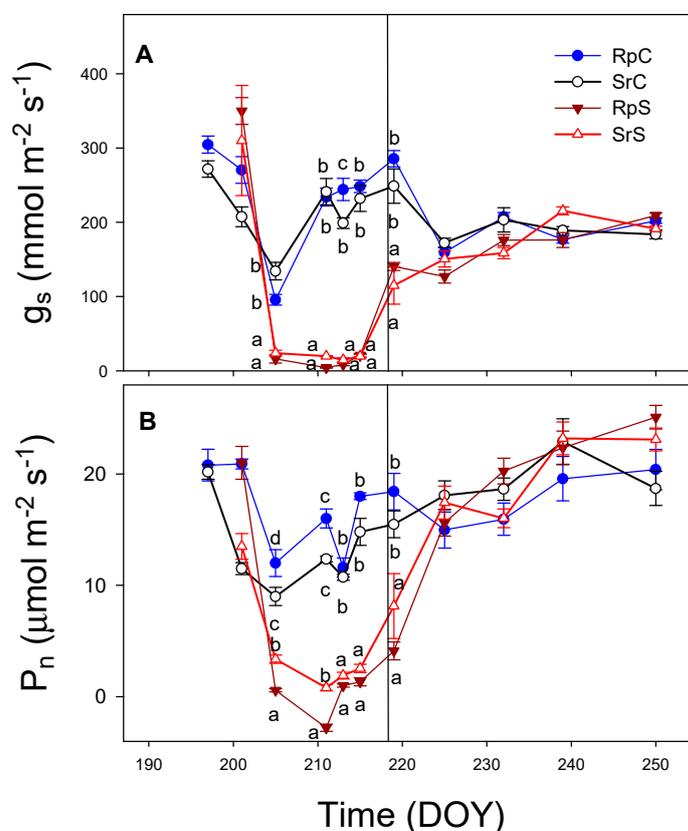


Figure 5. Evolution of stomatal conductance (g_s , (A)) and net photosynthesis rate (P_n , (B)) in *P. dulcis* plants submitted to different treatments. Values are means + s.e., $n = 6$. Symbols represent the different treatments: RpC (filled circles; plants grafted under full irrigation), SrC (open circles; self-rooted plants under full irrigation), RpS (filled triangles; plants grafted submitted to water stress) and SrS (open triangles; self-rooted plants submitted to water stress). Different lowercase letters indicate significant differences between treatments according to Duncan 0.05 test. Vertical line indicates irrigation change, the end of the water stress period and the beginning of recovery period.

The behaviour of g_s and P_n on a representative day of the end of the stress period can be seen in Figure 6. Maximum values of g_s were observed between 9:00 and 12:00 (solar time) in the fully irrigated treatments, especially in the case of RpC (Figure 6A). In both stress treatments, g_s was reduced to a similar extent compared with the controls, with plants of both stress treatments having very low values of below $50 \text{ mmol m}^{-2} \text{ s}^{-1}$ throughout the day, regardless of the rootstock. The diurnal pattern of P_n also consisted of a reduction in both stress treatments compared with controls (Figure 6B). RpC had,

in the early morning (7:00–9:00), significantly higher values of P_n compared to SrC. Later, at midday, these differences disappeared. Similarly, RpS plants had higher P_n than SrS plants in the early morning. In general, P_n increased in all treatments as the evaporative demand of the atmosphere increased during the day. However, RpS showed a gradual decrease through the day, having minimum P_n values at midday. At this time, Rp plants had even lower P_n values than the Sr, although these differences were not statistically significant.

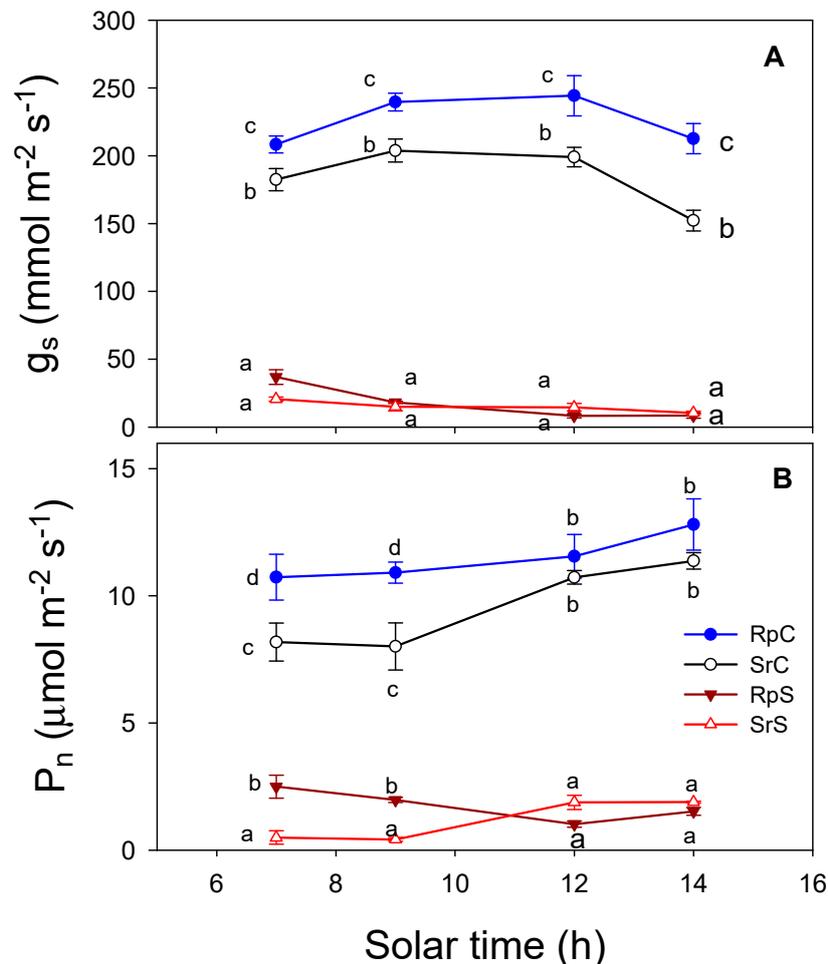


Figure 6. Evolution of hourly g_s (A) and P_n (B) throughout a representative day at the end of water stress in *P. dulcis* plants submitted to different treatments. Values are means + s.e., $n = 6$. Symbols represent the different treatments: RpC (filled circles; plants grafted under full irrigation), SrC (open circles; self-rooted plants under full irrigation), RpS (filled triangles; plants grafted submitted to water stress) and SrS (open triangles; self-rooted plants submitted to water stress). Different lowercase letters indicate significant differences between treatments according to Duncan 0.05 test.

Plants grown under well-watered conditions with the Rootpac-20 rootstock (RpC) had significantly higher values of water use efficiency (WUE) in leaf and stem than self-rooted plants under the same irrigation conditions (SrC), while no significant differences were observed in the case of root (Figure 7). Water deficit led to an increase in water use efficiency (WUE) in all parts of the plants (leaf, stem and root) in the SrS plants compared with SrC plants. While in Rootpac-20, these parameters did not change under water stress.

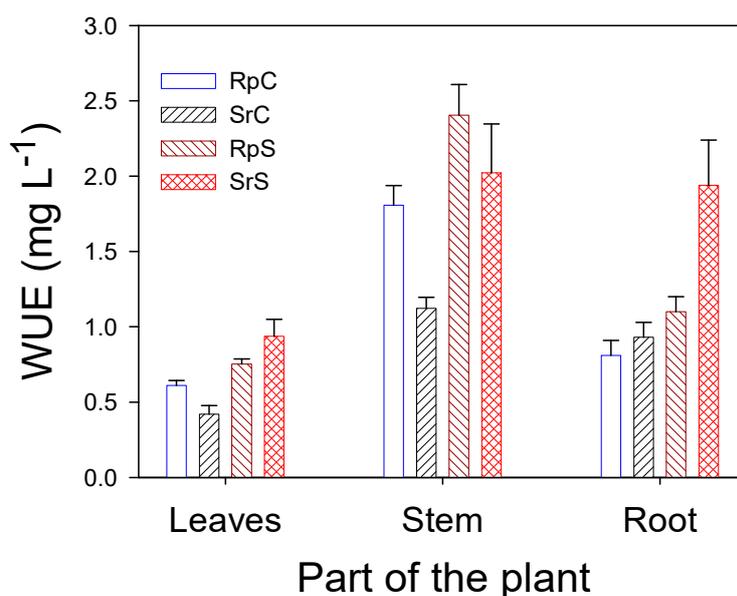


Figure 7. Water use efficiency of production (WUE) at the end of experimental period in *P. dulcis* plants. Means within a part of the plant without a common letter are significantly different by the Duncan 0.05 test.

4. Discussion

The different plant material studied in this work led to substantial differences in terms of growth and water relations in the cultivar Soleta, both under full irrigation and water stressed conditions. The rootstock used in our experiment influenced the growth responses of the almond plants to water stress, meaning that the plant material must be considered an important aspect when used under deficit irrigation strategies or rainfed conditions. In our study, the reduction in leaf area in response to water stress produced by withholding irrigation was much more evident in the variety grafted onto the Rootpac-20 rootstock than in self-rooted plants. The differences in the plant height and trunk diameter were also greater in grafted plants. According to these results, self-rooted plants would be more tolerant to water stress than Rp, because the vegetative growth was less affected by irrigation restriction. The reduction in leaf area due to water deficit is a common response, since expansive growth is the most sensitive process to water stress in plants and is affected even at relatively high leaf water potentials [32,33]. The inhibition of leaf growth under limited water availability is seen as an adaptive strategy, because it allows plants to reduce water consumption by restricting the evaporative surface and delaying the onset of more severe stress [34,35]. The differential provision of water to plant not only influences the aerial part but also the root system, which may be affected by drought [36]. In our conditions, exposure to irrigation water withdrawal caused a significant decrease in aerial dry mass, leaf area, number of leaves, plant height and trunk diameter, but had less effect on root mass, indicating that shoots and roots react differently to drought, influencing the dry matter partitioning between roots and shoots [37,38]. This was confirmed by the root to shoot ratio, which increased in plants under water deficit conditions, especially in self-rooted plants, which confer an advantage in water uptake. This aspect is an important factor for successful transplanting and establishment in the field, since root anatomy and structure may be decisive for plant survival [39]. Deficit irrigation has the potential to improve crop quality by increasing the root to shoot ratio, as previously reported for other woody crops by Moriana et al. [9] in pistachio, by Abrisqueta et al. [40] in peach, and by Yadollahi et al. [38] in several almond genotypes.

Under full irrigation conditions, the Rootpac-20 rootstock promoted higher vegetative growth than self-rooted plants. This greater growth during the experimental period resulted in different plant size. Figure 1 characterizes these differences very well, showing a much higher evapotranspiration during the experimental period for plants grafted under full irrigation compared to self-rooted

plants. Rootpac-20 is considered to be a rootstock of low vigour, with 40–50% vigour reduction in comparison to GF677 [24,41]. Similarly, Ben Yahmed et al. [16], reported that Rootpac-20 exhibited a high capacity to control tree vigour, based on the growth of the trunk. In our experimental conditions, self-rooted plants exhibited even higher capacity to control plant vigour than Rootpac-20. In fact, reduced vegetative growth is a positive aspect of fruit crops in the view of getting high density orchards with reduced cultural costs associated with harvesting and pruning [22,42,43]. However, this response may be an undesirable feature if it reduces crop yield through a lower assimilation capacity [32]. Despite the increasing commercial importance of dwarf orchard trees, the mechanisms related to reduced vegetative growth of scions grafted on low-vigour rootstocks have not been clearly identified [22,44,45]. These authors suggested that control growth is associated with hormonal relationships and hydraulic conductance of roots and stem. Lliso et al. [46] suggested that the dwarfing mechanisms are related to competition between reproductive and vegetative growth. Motisi et al. [47] reported lower stem water potential and lower hydraulic conductance on dwarfing rootstocks compared to vigorous rootstocks.

Water stress, characterized by stem water potential and leaf relative water content measurements, was more severe in plants grafted on Rp than in the self-rooted plants. The level of induced water stress was similar, as described in other studies in which Ψ_s values of -4 MPa have been reported for severe stress levels [11,12]. Ben Yahmed et al. [16] reported that leaf and stem water potential were lower for scions grafted on Rootpac-20 rootstocks than for scions grafted on more vigorous rootstocks. This behaviour is likely to be related to the lower water absorption capability of the root system of dwarfing rootstocks to fulfil the transpiration demand of canopy. In the present study, self-rooted plants had higher Ψ_s than plants grafted on Rp rootstocks (more vigorous). These variations in water status can be explained by the absence of grafting in self-rooted plants. The higher Ψ_s values in self-rooted plants were likely related to the smaller transpiring plant leaf area and biomass. Although the reduction in leaf area allowed a quicker recovery of some parameters after water stress was over, it could result in a reduction in the assimilation capacity of the plant, which could affect crop yield [32]. This hypothesis should be checked in field experiments. Almond is a very drought-resistant crop that tolerates high levels of tissue dehydration with a quick capacity of rehydration [48]. The rehydration capacity is also very important in this species and the stem water potential, relative water content and leaf conductance were quickly recovered. The above results indicate that self-rooted plants had more efficient mechanisms for tolerating water deficits than Rp did. Our data suggest that self-rooted plants were apparently the most drought resistant according to plant water status and this could be linked to a higher root to shoot ratio and the ability to control water loss via transpiration.

The water consumption of the plants varied during the experiment and was closely related with environmental factors, plant size and the irrigation regime [3]. Plants are able to adapt to a reduced moisture level and, as a result, transpiration is reduced [27]. In our conditions, daily evapotranspiration varied during the experiment and depended mainly on the available water content. This indicates that *P. dulcis* plants regulated their transpiration when subjected to water restrictions, which is a common response of plants grown in Mediterranean climates [49]. Several works have studied the evolution of water consumption in almond plants under different environmental conditions and it is well established that transpiration decreased in the deficit treatments as compared to full irrigated trees [12,50]. Knowledge of transpiration responses under different irrigation conditions is essential to formulate irrigation strategies [3,5,51]. Determining a precise estimation of crop water requirements is also an important aspect when selecting the right scion–rootstock combination. In this sense, the water consumption of the plants under full irrigation was reduced in non-grafted plants compared to grafted plants, despite the similar levels of water in the substrate. Nawaz et al. [18] also observed this same behaviour, in which plants grafted on rootstocks absorbed more water and ions than self-rooted plants and transported these water and ions to the aboveground scion. This was confirmed by the accumulated ET which increased by 50% in plants grafted onto Rootpac-20 under full irrigation conditions compared to self-rooted plants with the same irrigation regime. Surprisingly, relatively little

research has quantified irrigation requirements of woody crops with low vigorous rootstocks, although such knowledge would offer great possibility for water conservation [3,15].

Plant functioning and gas exchange parameters were affected by water deficit, as a marked decrease in P_n and g_s values were observed in stressed plants, especially at the end of stress period, when conditions were more stressful and SWC was lower. *P. dulcis* has been classified as a plant with very sensitive stomata that regulate stomata closure before reaching critical leaf water potential, which would cause cavitation events [3]. Li et al. [52] reported that water availability in the soil had a marked effect on stomatal morphology and movement that regulate plant water relations and plant growth. Shaker et al. [53] found that *P. dulcis* plants under water stress exhibited a stem water potential of about -1.4 to 1.8 MPa, which caused a reduction of 50% in g_s , suggesting that they had very sensitive stomata; this agrees with our results. As a result of the stomatal closure, net photosynthesis is unavoidably reduced due to decreased CO_2 availability at the chloroplast level [54], as seen in many other studies with different almond cultivars submitted to water deficit [3,38,55,56]. At the end of the water stress period, P_n was seen to be negatively affected in plants grafted than in self-rooted plants. The fact that such a reduction was less marked in Sr than in Rp plants confirms the higher drought tolerance compared to grafted plants.

As regards the behaviour of g_s along the day, it began to increase at dawn due to stomatal opening in full-irrigated plants, and was the highest at midday, when evaporative demand was also the highest. After midday, stomata closing began, producing a decrease in the g_s . The same observation was made for the evolution of evapotranspiration values. Maximum ET values were found at midday in full irrigation treatments, when hourly ET was also highest. By contrast, both stressed plants reduced g_s values throughout the day. Only, RpS plants showed slight increases in the gas exchange parameters in the early morning, when DPV was still low. Similar behaviour was found by Villalobos et al. [57], who reported a displacement to the pattern of gas exchange of the stressed trees towards the early morning hours. Some species under water stress are able to take advantage of the times of the day when the trade-off between water and CO_2 is more favourable, to perform then most of their daily gas exchange. Later, at midday, when VPD was high, stomata of stressed trees were fully closed, reaching very low g_s values.

The severe and prolonged inhibition of g_s has been interpreted as evidence of a gradual increase in the non-stomatal limitations of photosynthesis during the stressful conditions [58]. Álvarez and Sánchez-Blanco [59] reported that if plants showed g_s values below $100 \text{ mm m}^{-2} \text{ s}^{-1}$ for long periods, intrinsic water use efficiency is sharply reduced and non-stomatal limitation of P_n are predominant, which could delay plant recovery or even cause permanent damage. In this sense, the subsequent recovery in gas exchange parameters that occurred in almond plants suggest that withholding irrigation during a period of 20 days did not cause damage to leaf tissue, or, at least, it was not irreversible, indicating that plant functioning was not permanently affected by the stressful conditions experienced by plants [60].

Although self-rooted plants exposed to water deficit showed lower biomass accumulation, the water use efficiency (WUE) was higher in the water-stressed plants. The advantage in the case of these plants is that controlled drought may lead to an accumulation of carbohydrate reserves in the plants and, together with an increased root to shoot ratio, could promote a more rapid resumption of growth once irrigation is restored or rainfall events start [61,62].

5. Conclusions

Data of vegetative growth suggest that self-rooted plants had a higher capacity to control plant vigour than plants grafted onto Rootpac-20, both under full irrigation and no irrigation conditions. Under irrigation, the Rootpac-20 may be the best rootstock since it induces the biggest leaf stomatal conductance and vigour, which are likely to be accompanied by a more productive response. This greater growth was associated with higher photosynthetic rates but involved a 50% increase in water consumption compared to self-rooted plants.

The tolerance of almond plants to drought was related to an effective mechanism of stomatal control, together with a reduction in leaf area. This is also clear from the decline and subsequent recovery of gas exchange parameters. The results show that Soleta is highly resistant to drought stress, but the morphological and physiological responses differed between self-rooted plants and plants grafted onto Rootpac-20. In the case of dryland or deficit irrigation conditions, self-rooted plants might be a good choice for their drought tolerance, as these plants are able to maintain better plant water status, which resulted in smaller reductions in leaf area. It was accompanied by an increased root to shoot and water use efficiency, which are positive aspects that would allow plants a more rapid recovery at the onset of the autumn. Based on this behaviour, self-rooted can be regarded as an interesting plant material for high-density plantations under drought conditions.

The responses found in this work should be taken into account when selecting the rootstock for the establishment of a new orchard, knowing the irrigation management that will be used. In addition, these results suggest the need to evaluate the effect of these rootstocks on the productive response under field conditions.

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