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Differences in Growth and Water Use Efficiency in Four Almond Varieties Grafted onto Rootpac-20

Sara Álvarez [1,*], Lidia Núñez [1], Hugo Martín [1], Enrique Barajas [1] and José Manuel Mirás-Avalos [2]

[1] Instituto Tecnológico Agrario de Castilla y León, Ctra. Burgos, 119, 47071 Valladolid, Spain
[2] Departamento de Sistemas Agrícolas, Forestales y Medio Ambiente, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Avda. Montañana 930, 50059 Zaragoza, Spain
* Correspondence: alvmarsa@itacyl.es

Abstract: Almond cultivation in the Mediterranean area has undergone important changes leading to the current trend of intensification. In this scenario, low-vigor rootstocks have recently been developed, such as the 'Rootpac®' series, but knowledge about the rootstock's influence on adaptation to high-density planting systems is very scarce. The objective of this work was to assess the morphological and physiological response of four almond cultivars grafted on 'Rootpac-20'. To this end, one-year-old almond plants (*Prunus dulcis* (Mill) D. A. Webb.) cv 'Penta' (P), 'Guara' (G), 'Vialfas' (V) and 'Soleta' (S) were grown in pots, irrigated at field capacity (from June to September). Plant height and trunk diameter were measured periodically, and the water use efficiency of production was determined at the end of the trial. Evapotranspiration was measured throughout the experiment. The trunk diameter was greater in S and G at the end of the trial. Similarly, the tallest plants were G while the shortest ones were P. In general, G and S had a higher water use efficiency than V and P. 'Soleta' grafted on 'Rootpac-20' had higher evapotranspiration values (+25%), which is an important issue when selecting plant material, especially in the case of limited water availability for irrigation.

Keywords: biomass; cultivars; evapotranspiration; *Prunus dulcis*; rootstock; super high-density system; water relations

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1. Introduction

Almond (*Prunus dulcis* (Mill) D. A. Webb) is considered one of the most important fruit nuts in the world. Almond is commercially cultivated worldwide, with the USA, Australia, and Spain being the main producing countries [1,2]. In the Mediterranean area, almond trees have been traditionally cultivated under rainfed conditions [3,4]. However, the increase in yield as a response to irrigation has promoted the installation of irrigation systems in newly planted orchards, which has increased the demand for water supply in these areas [5,6]. Due to the limited availability of water in some areas, especially in the Mediterranean Basin, assessing crop water needs and water use efficiency is becoming very important [7]. This has led to the use of sustainable irrigation strategies in some Mediterranean regions [8]. Among the management strategies available to cope with scarce water resources, the selection of plant material (both rootstock and scion genotypes) with lower crop water requirements or with improved water use efficiency (WUE) is crucial for the sustainability of the agricultural activities in the long term [9]. Knowledge of the water needs of each cultivar is necessary to select the most appropriate plant material and avoid inadequate irrigation estimations, which increase costs, waste irrigation water, and cause a negative impact on the environment, especially in areas characterized by water scarcity, such as the Mediterranean Basin, where sufficient water amounts for supplying the full requirements of some crops are not available [10].

Additionally, agricultural intensification and the establishment of high-density plantations have increased in recent years [11]. In such a situation, not only new cultivars but also new rootstocks are essential tools to achieve the sustainability of these super-intensive plantations [12,13]. Low-vigor rootstocks have recently been developed, such as the 'Rootpac®' series, opening the possibility of growing almond trees in high-density systems, with the potential benefits of reducing labor costs, especially for pruning and harvesting operations [14,15]. Knowledge about the influence of rootstock on adaptation to this new high-density planting system is very scarce [14,16,17]. In this context of intensification of almond cultivation, the choice of suitable plant material is crucial. Very few studies have quantified the growth response and water relations of each cultivar grafted onto a dwarfing rootstock, 'Rootpac-20', although this knowledge would contribute to better water management in the Mediterranean area. In this sense, Álvarez et al. [18] have identified the varying water needs of *P. dulcis* cv. 'Soleta', either self-rooted or grafted on 'Rootpac-20' under different watering regimes, showing that the morphological and physiological responses of this almond cultivar differed between self-rooted plants and those grafted.

To advance in this knowledge, the current study aimed at evaluating morphological and physiological responses of four almond cultivars ('Penta', 'Guara', 'Vialfas' and 'Soleta') grafted on 'Rootpac-20' without limitations in water supply, by studying water relations, vegetative growth, and gas exchange. Isolating the effects of the cultivar will provide valuable knowledge for selecting plant material when planning new almond plantations.

2. Materials and Methods

2.1. Plant Material and Experimental Conditions

Four almond cultivars grafted onto 'Rootpac-20' (*Prunus besseyi* x *Prunus cerasifera*), a low-vigor rootstock, were used in this study. The scion cultivars selected were 'Vialfas' (V), 'Guara' (G), 'Penta' (P), and 'Soleta' (S). All of them are important commercial cultivars of agronomic interest in Spain and include two late-blooming genotypes (G and S) and two extra late-blooming genotypes (V and P). Plants of V, G, and S cultivars were obtained at the Agrifood Research and Technology Centre (CITA), Aragon (Spain) and P was obtained in the Almond Breeding Program at CEBAS-CSIC, in Murcia (Spain).

The experiment was carried out during the summer of 2018 using a total of 120 plants (30 plants per cultivar). One-year-old almond plants were transplanted into 25 × 20 cm pots (5L) filled with a mixture of coconut fibre, black peat, and vermiculite (8:7:1) amended with 10 g per pot of Osmocote plus (14:13:13 N, P, K plus microelements) and placed inside a plastic greenhouse equipped with a cooling system at Itacyl Research Station, Valladolid, Spain (41°42′ N 4°42′ W, 705 m above sea level). The conditions registered in the greenhouse over the study period were 26.8 °C (average temperature) and 59.7% (average relative humidity).

The experimental period lasted 12 weeks, from 21 June to 13 September. During this period, all the plants were watered daily to field capacity using a computer-controlled drip system. Substrate moisture was maintained close to container capacity by daily irrigating to 100% water holding capacity (leaching 15% (*v/v*) of the applied water), using good-quality water with very low electrical conductivity (EC) = 0.4 dS m^{-1}.

The volumetric water content at field capacity was calculated to determine the maximum water holding capacity of the substrate (64%) as described in Álvarez et al. [18].

2.2. Growth and Plant Water Measurements

At the end of the experimental period, eight plants per cultivar were taken out and their aboveground parts were divided into leaves and stem. These were dried in an oven at 105 °C until they reached a constant weight to obtain their corresponding dry weights (DW). In addition, leaf area and leaf number were determined on the same plants before drying, using a leaf area meter (DeltaT Devices Ltd., Cambridge, UK).

Plant height and trunk diameter were weekly measured in 20 plants per cultivar throughout the experimental period using a measuring tape and a digital calliper, respectively. Plant height is the measurement of the plant from the base (ground level) to its highest point. The trunk diameter was measured at 15 cm above the ground level (above the graft union). At that level, the trunk was marked on its perimeter at the beginning of the experiment.

Volumetric water content was periodically calculated in five pots per cultivar throughout the experimental period. This was made by weighing the pots before and after irrigation, using a balance (Analytical Sartorius, Model 5201; capacity 5.2 kg and accuracy of 0.01 g). Then, the difference between the fresh weight and oven-dry weight was measured, giving the volumetric water content of these monitored pots. Moreover, daily evapotranspiration (ET) was calculated using the difference in weights (weight after irrigation and weight before irrigating again) in five pots of each cultivar.

During the experiment, stem water potential (Ψ_s), relative water content (RWC), stomatal conductance (g_s), and net photosynthesis rate (P_n) were measured weekly, approximately, in six plants per cultivar in mature leaves at midday. The Ψ_s was determined according to the method described by Scholander et al. [19], 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 of 0.02 MPa s^{-1} [20]. Ψ_s was measured in non-transpiring leaves that had been bagged with both a plastic sheet and aluminium foil for at least 1 h before measurement to prevent leaf transpiration [21], while leaf RWC was calculated according to Barrs [22]. Leaf stomatal conductance (g_s) and net photosynthetic rate (P_n) were determined using a gas exchange system (LI-6400, Li-cor Inc., Lincoln, NE, USA). Leaf gas exchange was measured on six young, fully expanded leaves per cultivar, placed in a 2 cm^2 leaf cuvette. The CO_2 concentration in the cuvette was maintained at 400 µmol mol^{-1} (≈ambient CO_2 concentration). Measurements were performed at a saturating light intensity of 1200 µmol m^{-2} s^{-1} and at ambient temperature and relative humidity. In addition, the water use efficiency of production (WUE) was calculated in eight plants at the end of the experimental period by dividing the increment in dry weight by the water used.

2.3. Statistical Analysis

The obtained data were submitted to one-way ANOVA using SPSS Statistics software (version 23.0 for Windows, SPSS Inc.; Chicago, IL, USA), after checking the normality and homoscedasticity assumptions. The variation factor was the almond cultivar. When significant differences were detected, treatment means were separated with Duncan's Multiple Range Test.

3. Results

3.1. Plant Growth

Trunk diameter increased during the experiment in all almond cultivars, with S and G being those plants with the highest trunk diameter at the end of the trial (Figure 1A), while V plants showed the lowest trunk diameter values.

Periodic measurements of plant height were also made throughout the experimental period (Figure 1B). Two growth periods were observed, one at the start of the experiment (1 July) and another one at the end of August (Figure 1B). From the first weeks of the experiment, differences in plant height were observed among varieties, with V and P plants being smaller. At the end of the second growth period, G plants were the tallest ones, while P plants were the shortest ones. At the end of the experiment, plant heights were 5, 12, and 17% smaller for S, V, and P, respectively, compared to the G plants.

In addition, the biomass accumulation was significantly different among the evaluated cultivars (Table 1). At the end of the experimental period, the highest aboveground DW was observed in the S cultivar due to a higher stem DW, since the leaf DW was similar in all varieties (Table 1). Moreover, S had the highest number of leaves per plant, although the leaves in this variety were slightly smaller, so the four cultivars had a similar total leaf area by the end of the experiment.

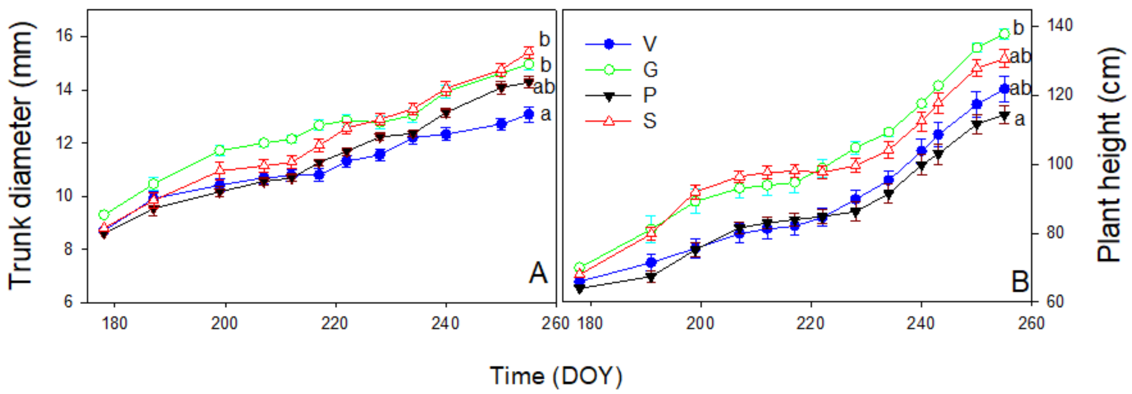

Figure 1. Evolution of (**A**) trunk diameter, and (**B**) plant height in four almond cultivars grafted in Rootpac-20 over the course of 12 weeks (DOY = Day of the year). Values are means + s.e., n = 20. Symbols represent the different cultivars: 'Vialfas' = V; 'Guara' = G; 'Penta' = P; and 'Soleta' = S. Different lowercase letters indicate significant differences among cultivars according to the Duncan test ($p < 0.05$).

Table 1. Vegetative growth parameters of four cultivars of almond plants grafted on 'Rootpac-20' at the end of the experiment. Each value is the mean of 8 plants per treatment ± standard error.

Parameters	'Vialfas'	'Guara'	'Penta'	'Soleta'	Significance
Aboveground DW (g plant^{-1})	83.4 ± 4.6 a	92.0 ± 5.3 a	85.2 ± 4.8 a	110.2 ± 6.7 b	**
Leaf DW (g plant^{-1})	23.8 ± 1.3	23.8 ± 4.7	23.1 ± 1.5	26.3 ± 1.6	ns
Stem DW (g plant^{-1})	59.6 ± 3.8 a	68.2 ± 4.7 a	62.0 ± 3.5 a	83.9 ± 5.4 b	**
Total leaf area (cm^2)	2292 ± 160	1999 ± 107	2178 ± 142	2279 ± 197	ns
Number of leaves	378 ± 15 a	373 ± 27 a	353 ± 28 a	491 ± 5 b	*
Leaf blade area (cm^2)	6.1 ± 0.2 b	5.4 ± 0.4 b	6.2 ± 0.3 b	4.6 ± 0.2 a	**

Mean values in each row followed by different letters are significantly different according to Duncan's Multiple Range Test (ns = not significant; * $p < 0.05$; ** $p < 0.01$). DW = Dry weight.

3.2. Water Consumption

The substrate in all the pots remained close to field capacity after each irrigation event, as indicated by periodic measurements of the VWC of the substrate over the experimental period (Figure 2A). The VWC decreased as the day progressed, until the next day when irrigation restored the substrate to field capacity. The minimum values of VWC were observed for the S plants (Figure 2A).

The evolution of evapotranspiration (ET) during the experimental period is shown in Figure 2B. Evapotranspiration increased over the duration of the experiment in all cultivars due to plant growth. In addition, climatic conditions also influenced ET, which decreased at the end of the experiment (September), when temperature and VPD were lower than on previous days (July–August).

Throughout the experimental period, evapotranspiration was higher in S plants than in the rest of the cultivars, especially from day of the year (DOY) 228 to DOY 244 (Figure 2B). Water consumption was lower in P and G plants compared with S plants, from the beginning of the experiment, despite having similar levels of water in the substrate.

The S plants had higher accumulated evapotranspiration throughout the experimental period than the rest of the cultivars (Figure 2C). The accumulated water consumption per plant during the whole experimental period was 49.2 L for S plants and 40.5, 39.1, and 38.2 L for V, P, and G plants, respectively (82, 79, and 78% of the amount of water consumed by S).

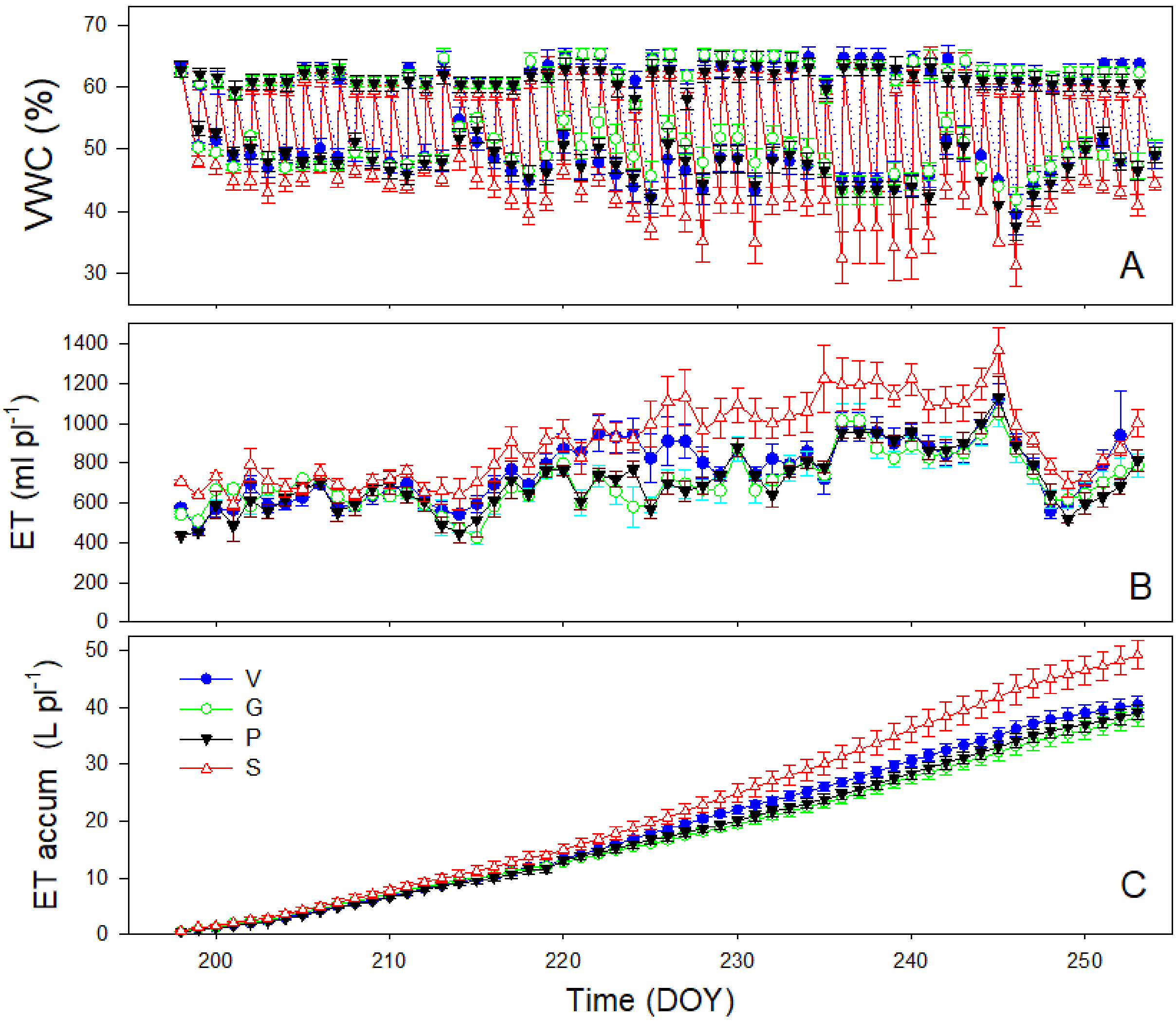

Figure 2. Evolution of soil volumetric water content (VWC; (**A**)), daily evapotranspiration (ET; (**B**)), and accumulated evapotranspiration (ET accum; (**C**)) over the course of 12 weeks in four almond cultivars grafted in Rootpac-20 (DOY = Day of the year). Values are means + s.e., n = 5. Symbols represent the different almond cultivars: 'Vialfas' = V, 'Guara' = G, 'Penta' = P, and 'Soleta' = S.

3.3. Water Relations

Regarding plant water relations, P and S plants had more negative Ψ_s values than V and G plants (Figure 3A). In S, the Ψ_s values reached −1.4 MPa at the end of August, coinciding with the period in which substrate VWC was lower. The relative water content (RWC) presented a similar behaviour to that of the stem water potential, from the DOY 220 onwards, with S plants having the lowest values, although RWC oscillated over the experimental period (Figure 3B). However, at the beginning of the measurement period, especially on the first two dates, RWC behaved differently to Ψ_s, as there were significantly higher RWC in V and G when compared to P and S (Figure 3B).

On average, especially from DOY 220 onwards, the P and S plants presented higher stomatal conductance values than V and G plants (Figure 4A), coinciding with less negative Ψ_s values. However, there were no significant differences among varieties in the net photosynthesis rates over the experimental period, ranging between 9 and 23 μmol m^{-2} s^{-1} (Figure 4B). These higher values of stomatal opening coincided with the lower values of stem water potential registered at midday in these plants.

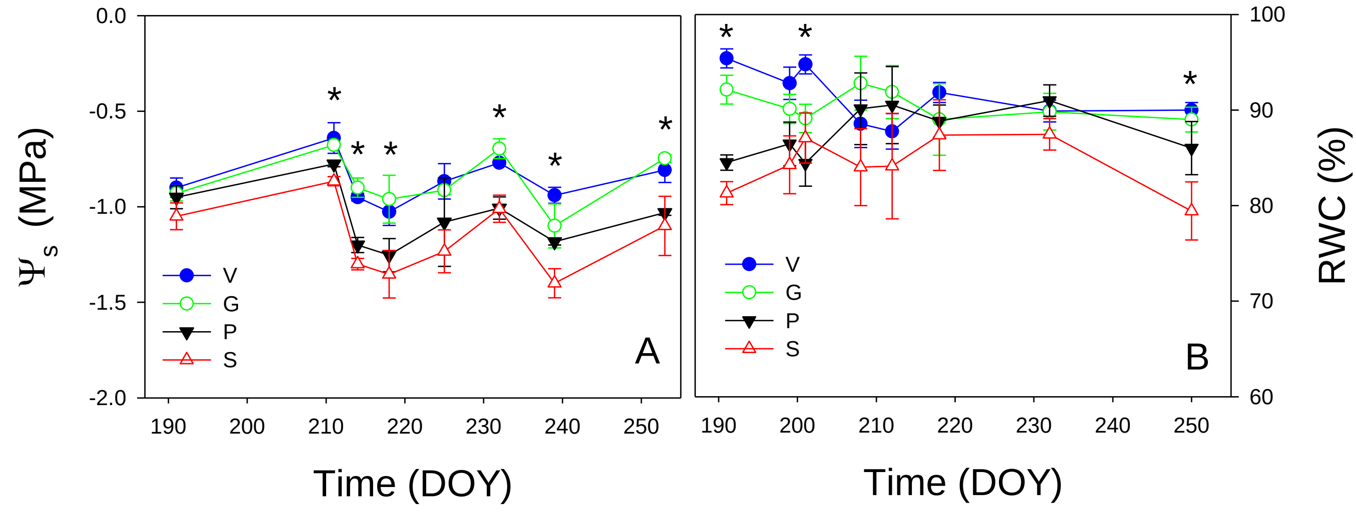

Figure 3. Evolution of stem water potential (Ψ_s; (**A**)) and leaf relative water content (RWC; (**B**)) at midday in four almond cultivars grafted in Rootpac-20 over the course of 12 weeks (DOY = Day of the year). Values are means + s.e., n = 6. Symbols represent the different cultivars: 'Vialfas' = V; 'Guara' = G; 'Penta' = P; and 'Soleta' = S. Asterisks indicate significant differences among cultivars on a given date (Duncan's test at $p < 0.05$).

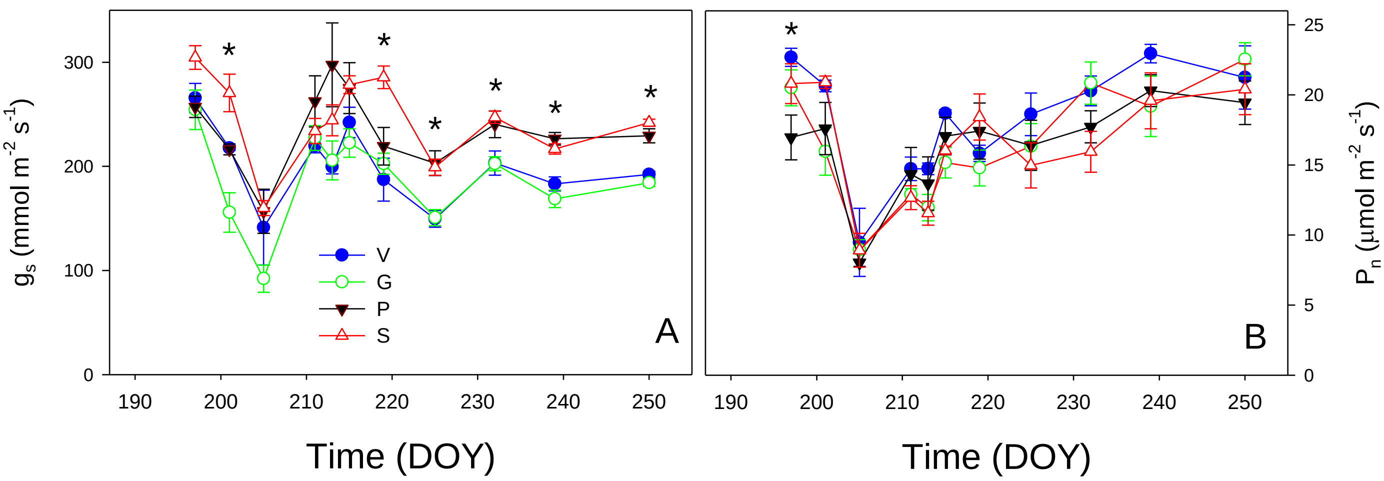

Figure 4. Evolution of stomatal conductance (g_s; (**A**)) and net photosynthesis rate (P_n; (**B**)) at midday in four almond cultivars grafted in Rootpac-20 over the course of 12 weeks (DOY = day of the year). Values are means + s.e., n = 6. Symbols represent the different cultivars: 'Vialfas' = V; 'Guara' = G; 'Penta' = P; and 'Soleta' = S. Asterisks indicate significant differences among cultivars on a given date (Duncan's test at $p < 0.05$).

Regarding the increase in biomass in relation to the water applied (Figure 5), the G and S plants had a higher water use efficiency of production (WUE) than the V and P plants under the same irrigation conditions. Aboveground biomass was higher in G plants, which partitioned more dry weight to the stems for each litre of water applied, although not significantly different from S plants (Figure 5).

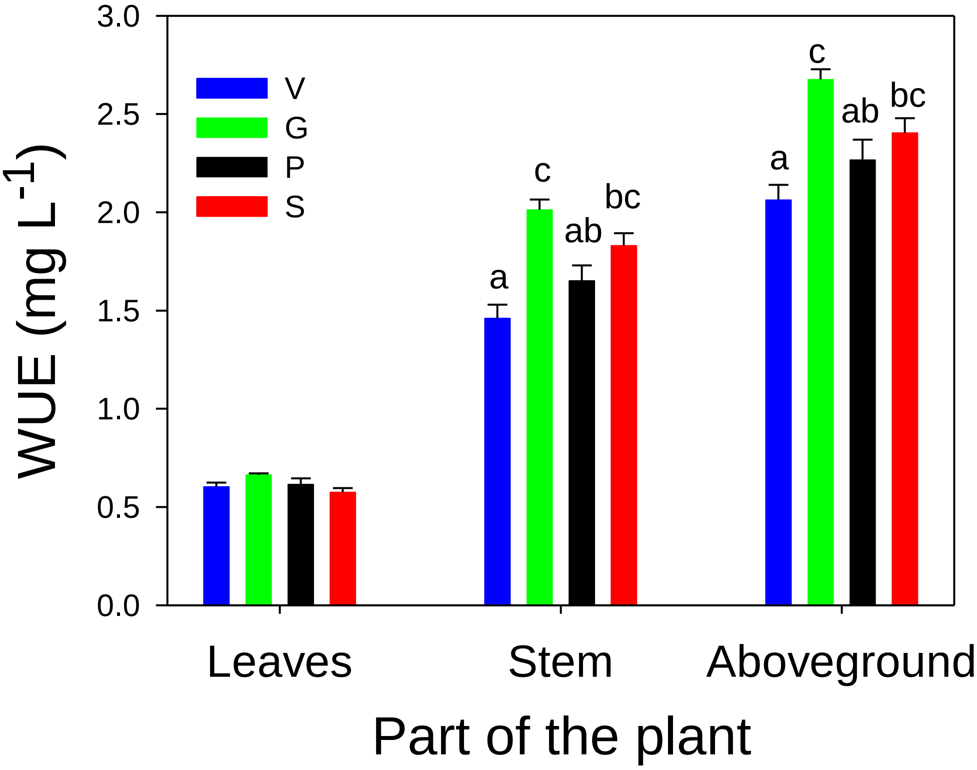

Figure 5. Water use efficiency of production (WUE) at the end of the experimental period in four almond cultivars grafted in Rootpac-20. Values are means + s.e., n = 8. Symbols represent the different cultivars: 'Vialfas' = V; 'Guara' = G; 'Penta' = P; and 'Soleta' = S. Different lowercase letters indicate significant differences among cultivars according to the Duncan test ($p < 0.05$).

4. Discussion

Our study showed the effects of a newly bred rootstock ('Rootpac-20') on the vegetative growth and water relations of four almond cultivars. Since all cultivars were grafted on the same rootstock and received the same watering regime, our study isolated the effect of cultivar genotype on almond water consumption. Therefore, the knowledge obtained can help growers when planning the establishment of a high-density almond orchard in a region suffering from water restrictions for irrigation. The responses of these scions to the same irrigation scheduling (involving the absence of water stress) differed considerably, as 'Soleta' plants were taller and consumed more water than the other three almond cultivars, under the conditions of this study. Assessing these traits is of great relevance in the current scenario where high-density orchards are being established all over Spain and other Mediterranean countries, and scarce information for managing this new type of plantation is available [18].

In the current study, the 'Soleta' plants were taller, while the smallest plants were those of 'Vialfas' and 'Penta'. However, these results must be interpreted with caution because our study was performed in a greenhouse, with potted plants (which limits root growth) and only for a single season, so further research is needed under field conditions and for longer experimental periods to reach solid conclusions. In almond trees, as in other woody crops, vegetative growth is a key factor that influences canopy volume, which in turn affects tree water requirements, but could have an additional impact on the potential crop load [23]. In addition, plant growth and architectural traits are of great relevance for the management of the plantation, especially pruning operations. For instance, the rootstock used in the current study usually reduces the branch length of scions [24] and other vegetative growth traits such as the number of branches [16]. Reduced leaf area and a low number of leaves are desirable traits when facing water stress conditions, as well as for facilitating management operations in super high-density orchards [11,25], so 'Vialfas', 'Guara, and 'Penta' could be preferred against 'Soleta', according to the results of the current study, but these findings must be confirmed with field studies over longer time spans. Indeed, high-density almond orchards imply short distances between trees (both in the row and the interrow), thus requiring smaller trees with low vegetative vigor to facilitate the passing of machinery and reduce operational costs, mainly in pruning and harvesting [11,26]. In this scenario, low-vigor rootstocks that fulfil the requirements for high-density plantations have been bred in recent years [17]. The rootstock employed in the current study, 'Rootpac-20', provides several characteristics appropriate for high-density plantations, including low vigor (around 40–50% less than GF-677), high productivity, adaptability to warm and colder climates, etc. [17].

As the effect of rootstock on tree architecture is still unclear [16], the magnitude of plant growth traits in the current study depended on the scion–rootstock combination. In our study, 'Guara' plants were significantly taller than 'Penta' plants, while trunk diameter was greater in 'Guara' and 'Soleta' plants than in 'Vialfas' plants. This is in accordance with previous research in which the magnitude of rootstock effect on tree vigor depended on the specific scion–rootstock combination [16,27,28]. Further research is required to discern the effects of 'Rootpac-20' on vegetative growth traits of almond trees under field conditions, but the results from the current study proved the relevance that this rootstock has on the growth of four well-irrigated almond cultivars.

In this scenario of well-watered plants, evapotranspiration values varied throughout the period and were closely related to climatic conditions, although they were also influenced by active growth periods (Figure 2B) [3]. This information should be considered when selecting almond cultivars before planting [29]. Surprisingly, very few works have quantified the irrigation requirements of woody crops grafted on low-vigor rootstocks, even though this would offer great possibilities for the conservation of water resources [18]. In this sense, our study showed that 'Soleta' cultivar, when grafted onto 'Rootpac-20', consumed more water than the other three cultivars considered. This finding is of relevance for horticulture in Mediterranean countries, where water resources are limited and will likely become scarcer due to the negative effects of climate change [30,31]. In this context, planting scion/rootstock combinations that produce profitable yields, while consuming less water is desirable. Our study provides preliminary insights into the water consumption of four almond cultivars that are increasingly being planted in Spain and other Mediterranean countries. In general, G and S plants had a significantly higher water use efficiency (aboveground and stem WUE) than V and P plants under the same irrigation conditions, while no differences were observed in the case of leaves.

In addition, water stress indicators and gas exchange parameters showed significant differences among cultivars on certain dates (Figures 3 and 4), suggesting that the studied cultivars have a different responses to water restrictions. The values of RWC differed among cultivars, being greater for V and G than for P and S, on the first date of measurement, when the Ψ_s values were similar in the four almond cultivars. However, from DOY 220 onwards, RWC did not differ among cultivars, but Ψ_s values were less negative for V and G than for P and S. Despite this inconsistency, both RWC and Ψ_s values indicated that the plants were not subjected to water stress conditions in the current study, as RWC was never lower than 80% until the end of the experiment and Ψ_s values were always more positive than -1.5 MPa over the whole experiment, as reported for fully irrigated trees [32]. Moreover, the apparent discrepancy between Ψ_s values and g_s rates could be indicating that the almond cultivars studied present a different behaviour with respect to water relations, likely being V and G near isohydric and P and S near anisohydric [33]. The absence of differences in photosynthesis rates among cultivars could be explained by the fact that the plants were well irrigated in the current study, so they did not suffer from water stress and could perform photosynthesis without restrictions.

Vegetative growth and stem WUE were reduced in V and P compared with S, which may be of interest for growers since lower costs for pruning operations (reduced worktime) are expected [34], although further studies under field conditions are needed to confirm this hypothesis. The major effects of variety in this study were related to a decrease in vegetative growth (stem), whereas leaf growth was unaffected. From the perspective of the sustainability of water resources, V, G, and P allowed for an average of 20% reduction in water consumption compared to S plants. This reduction in vegetative growth does not seem to be directly related to the amount of water and is desirable for high-density orchards to optimize light interception by creating a compact canopy over the tree row [35]. Therefore, under conditions of water scarcity, and according to the results from the current study, the 'Soleta' cultivar would not be recommended due to its higher water consumption, whereas the 'Guara' cultivar has also high vegetative vigor, which may increase management costs in high-density orchards. Therefore, 'Vialfas' and 'Penta' could be preferred over 'Soleta' and 'Guara'; however, these results must be confirmed with further research on adult trees, involving field experiments and yield assessments.

Even though our study was performed in pots with young almond plants, our results could be seen as a first step for providing growers with information to make decisions when planning a new high-density almond plantation, as the growers will have the information derived from this study to help them choose the more appropriate cultivar for the water resources available in each area. However, other factors should be considered, such as yield performance and pest and disease tolerance, which require further research under field conditions.

5. Conclusions

Despite being carried out under controlled conditions and using young plants, the current study provides key information for supporting decisions when planning a new almond plantation. The four almond cultivars studied performed differently despite being grafted on the same rootstock ('Rootpac-20'). Under the conditions of this study, 'Soleta' plants showed significantly higher aboveground and stem dry weights, and the number of leaves when compared to the other three cultivars. In addition, this study revealed that 'Guara' plants had the highest water use efficiency among the four almond cultivars considered. Furthermore, the current study showed that 'Soleta' plants consumed more water than the other three cultivars (25% higher water consumption, approximately), which is essential information for disregarding a cultivar for planting when water availability is scarce. Therefore, under conditions of water scarcity, the 'Soleta' cultivar is not recommended due to its higher water consumption, whereas the 'Guara' cultivar has also high vegetative vigor, which may compromise management operations in high-density orchards. Therefore, 'Vialfas' and 'Penta' could be preferred over 'Soleta' and 'Guara'. Nevertheless, the results of this study must be confirmed with research under field conditions for assessing the yield performance of these cultivars.

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