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Running title: Response of almond submitted to reclaimed water and deficit irrigation

Physiological responses of almond trees under regulated deficit irrigation using saline and desalinated reclaimed water

Gaetano Alessandro Vivaldi[a], Salvatore Camposeo[a], Cristina Romero-Trigueros[a,b], Francisco Pedrero[b], Gabriele Caponio[a], Giuseppe Lopriore[c], Sara Álvarez[d,*],

[a] Dipartimento di Scienze Agro Ambientali e Territoriali (DiSAAT), Università degli Studi di Bari Aldo Moro, Via Giovanni Amendola 165/A, 70126, Bari. Italy

[b] Departamento de Riego. Centro de Edafología y Biología Aplicada del Segura (CSIC). P.O. Box 164, E-30100 Murcia, Spain

[c] Department of Science of Agriculture, Food and Environment, University of Foggia, Foggia, Italy

[d] Unit of Woody and Horticultural Crops. Instituto Tecnológico Agrario de Castilla y León (ITACyL). Ctra. Burgos km 119. 47071 Valladolid, Spain

[*] Corresponding author

Abstract

Regulated deficit irrigation (RDI) strategy using reclaimed water (RW) is becoming a common procedure in some Mediterranean regions. Full and regulated deficit irrigation were combined with desalinated (EC_w 1 dS m^{-1}) and saline (EC_w 3 dS m^{-1}) reclaimed water to irrigate young potted almond trees over a 3-year period. The full irrigation treatments received 130% of the crop evapotranspiration (ET_c) and the RDI treatments received 80% of ET_c during the kernel filling. Trunk diameter decreased in both RDI treatments at the end of the experimental period, although this response was more marked in the trees irrigated with saline RW. There were negative relationships between shoot growth and leaf Na^+ and Cl^+ contents in the saline treated trees, in which the accumulation of salts in leaves was associated with osmotic adjustment, which was responsible for maintaining midday leaf turgor. Plant water status, measured by the leaf and water potential, decreased in almond exposed to water deficit or irrigated with saline RW, indicating a slight dehydration in these plants due to the difficulty in water uptake from the substrate. Trees subjected to both deficit irrigation treatments showed lower stomatal conductance values than full irrigated treatments during the RDI period. However, at the end of experimental period, the lowest P_n values were found in plants irrigated with saline RW, especially in trees irrigated with saline RW combined with RDI strategy, verifying the relevance of duration of exposure to the stress. Saline and desalinated RW can be successfully used for irrigating almond trees, which might be of great economic and competitive significance for agriculture, but further research focused on a longer term should be carried out since detrimental effects might appear. Deficit irrigation combined with saline RW in *P. dulcis* is not recommended since it intensifies the negative effects of water and salt stress applied individually.

Keywords:

Ion uptake; osmotic adjustment; *Prunus dulcis*; salinity; treated wastewater; water relations

Abbreviations

EC_w: water electrical conductivity; SAR_w: water sodium adsorption ratio; Ψ_s: stem water potential; Ψ_l: leaf water potential; Ψ_o: osmotic water potential; Ψ_t: turgor water potential ; Ψ_{100s}: leaf osmotic potential at full turgor; P_n: net photosynthesis rate; g_s: stomatal conductance; E: leaf transpiration rate; RGR: relative growth rate; VPD: vapour pressure deficit; ET_0:reference evapotranspiration; FI; full irrigation, RDI; regulated deficit irrigation; S; saline reclaimed water; D; desalinated reclaimed water; RW; reclaimed water; ET_c: crop evapotranspiration;

1. Introduction.

Water shortages are very frequent in many countries, and, together with the rising demand for industry, growth of human population, climate change and specifically the trend towards irrigated agriculture, have led to widespread problems of water scarcity, especially in Mediterranean countries (Romero-Trigueros et al., 2020). This situation imposes the need to optimize its use in all human activities (Fulcher et al., 2016, Álvarez et al., 2019). Among the different productive uses of water, agriculture is by far the main water user in most water scarce regions and, consequently, any potential improvement in the use of the available water resources may play a role toward achieving a more sustainable use of water (Fereres and Soriano, 2007; Alcon et al., 2013). Stakeholders involved in water resource management are looking for knowledge necessary to successfully irrigation management. To achieve this objective, several water conservation strategies have been recommended, for example by using precise tools for assessing crop water requirements (Mirás-Avalos et al., 2016), reclaimed water (Grant et al., 2012; Nicolás et al., 2018; Erel et al., 2019), drought and salt tolerant genotypes or rootstocks (Yadollahi et al., 2011; Jiménez et al., 2013; Álvarez et al., 2020), applying deficit irrigation strategies (Ruiz-Sánchez et al., 2010; Romero-Trigueros et al., 2019a; Sánchez-Blanco et al., 2019) and providing information about the water use requirements of the trees depending on the phenological periods varying (Goldhamer and Beede, 2004).

Almond (*Prunus dulcis* (Mill.) D.A. Webb) is a major tree nut species whose production and profitability are highly dependent on irrigation supply (Egea et al., 2010), especially in regions such as the Mediterranean basin characterized by low rainfall and high evaporative demand during the almond growing season. The physiological and morphological response of almond to different irrigation levels has been extensively investigated (Torrecillas et al., 1988, Shackel, 2007; Egea et al., 2010; Espadafor et al., 2017). One of the most promising techniques to maximize the water use in tree crops with little or no impact on crop yield and quality is regulated deficit irrigation (RDI), which is defined as an irrigation strategy that provides irrigation water below the full needs for specific development stages. Past research has revealed the interest of this irrigation technique, with special attention paid to fruit crops, including almonds, where it has been successfully employed (Goldhamer and Viveros, 2000; Girona et al., 2005; Romero and Botía, 2006; Stewart et al., 2011).

Due to the increasing pressure on fresh water resources, the use of saline waters and reclaimed water (RW) has become a reliable alternative for irrigation in agriculture (Acosta-Motos et al., 2016; Romero-Trigueros et al., 2019b). This situation imposes the need to use non-conventional water resources (desalinated or reclaimed water) for irrigation. Saline RW has been successfully used in several fruit crops like citrus (Nicolás et al., 2016), olive (Erel et al., 2019) and other species (Pedrero et al., 2018; Perulli et al., 2019). The main conclusions from these studies are that treated wastewater can be used as an additional water resource for tree irrigation in water-scarce Mediterranean environments. Little information is available on the effect of using saline or reclaimed water to irrigate almond trees, although they represent an important section of fruit trees production and such information be of great interest for designing and promoting water conservation strategies (Phogat et al., 2018). Notably, the studies related to salinity in almonds are specifically focused on the evaluation of salt tolerance in genotypes (Rouhi et al., 2007; Dejampour et al., 2012; Rajabpoor et al., 2014; Bahrami et al., 2015) and rootstocks (Doll et al., 2014; Momenpour et al., 2018). Research on the physiological performance of *P. dulcis* irrigated with RW is still lacking, as the previous studies about salinity tolerance of almonds have been conducted just on irrigation NaCl solutions and it is well known that the chemical properties of the water applied also affect the response of plant, being the kind of water a relevant aspect (Gómez-Bellot et al., 2013). In addition, increasing water resource problems in arid regions are even leading to growers to use the RW combined with deficit strategies (Mounzer et al., 2013). Many works have focused on water and salt stress applied individually, but very few have evaluated the physiological changes that take place when both stresses applied simultaneously, despite the fact of being known that the response by plants to combination of these two stresses may differ if water and salt stress applied individually or simultaneously (Brown et al., 2006; Sucre and Suárez, 2011; Glenn et al., 2012).

In general, under saline or drought conditions, plants reduce their water uptake capacity, but using irrigation waters with high salt concentrations can also cause ion toxicities and nutritional imbalance, depending on the kind of salts in the irrigation water (Acosta-Motos et al., 2014). In this sense, RW may contain high concentrations of salts and, in consequence, their use in irrigation for long term may have negative effects on soils and plants (Ayers and Westcot, 1985), particularly for those crops relatively sensitive to salinity, such as almonds (Phogat et al., 2018). It is for these reasons that reducing salt concentration in these water resources, leading to desalinated RW, could be an interesting option to reduce problems associated with salinity, providing different solutions to agriculture of arid and semi-arid environments.

However, studies regarding the physiological effects of the irrigation with RW on almond trees are lacking. The short-term response in term of growth and yield of almond trees to irrigation with desalinated and saline RW combined with RDI strategy during 1 year has been well described in a previous study (Vivaldi et al., 2019), but no information is available on its physiological response of several years of irrigation with these non-conventional water resources. Due to that the response of plants to salinity depends not only on the water composition, but also on the time of exposure to salt stress (Nicolás et al., 2016), the present work was carried out over a three-year period in *P. dulcis*, using the same trees tested by Vivaldi et al. (2019) but after two additional growing seasons (three seasons in total from 2017 to 2019).

The main objective of this research was to study the mid-term effects on young almond potted trees exposed to RW and different irrigation strategies, with special interest in plant growth, ion accumulation and tree water status, in order to identify the mechanisms that the plants develop to cope with these stresses. For this, a three-year experiment was designed to evaluate single and interaction effects of different levels of irrigation and salinity, in an attempt to check the sustainability of these irrigation strategies and to identify the most adequate in each context. The results can also be important contributions to scheduling irrigation strategies in water scarce regions, where low quality waters are often combined with deficit irrigation strategies, as well as making more sustainable almond crop production in regions with limited water resources. Our working hypothesis were: responses of almonds irrigated with reclaimed water would be different from those of trees irrigated with saline water with the same level of salinity; degree of salt stress tolerance would be correlated with the ability to control ion accumulation in leaves; and reclaimed water would confer a protective effect on water stress tolerance, as observed for other species.

2. Material and methods

2.1. Plant material and experimental conditions

The study was performed during three consecutive seasons (2017-2019) in an experimental orchard cultivated with 3-years old (in 2017) almond trees (*Prunus dulcis* (Mill.) D.A. Webb, cv. “Guara”) grafted on a hybrid Rootpak 20® of *Prunus besey* x *Prunus cerasifera* L-H. Bailey and Ehrh. Plants were transplanted in January 2017 into 100L polyethylene pots (diameter 50 cm, height 65 cm) filled with soil. The soil texture was classified as loam (44.78% sand, 12.32% clay and 42.90%silt) (USDA textural soil classification). Plants were placed outdoors in a plot in the University of Bari experimental station located in the southeast of Italy (Bari, Apulia Region) (41º06’41’’N, 16º52’57’’E, 5 m above sea level). Pots were on the ground with a 1.85 x 2.10 m planting system in rows oriented N-NE to S-SW.

The climate data were recorded by an automatic weather station located about 100 m from the experimental site. Air temperature, solar radiation, air relatively humidity, rainfall and wind speed 2.5 m above the soil surface were collected every 15 min. These data were used for calculating ET_0 and crop water requirements. All treatments received the same amounts of fertilizer (N-P_2O_5-K_2O), applied through the drip irrigation system. Pest control practices and pruning were those commonly used by growers in the area, and no weeds were allowed to develop in the pots.

2.2. Treatments

Two irrigation water sources were used. The first was desalinated RW (D), produced on the DESERT prototype and obtained by treating secondary wastewater from the Bari (Italy) secondary wastewater treatment plant, using ultrafiltration, active carbon, and reverse osmosis to reach an ECw of 1.0 dS $m^{-1.}$ DESERT (Low-cost water DEsalination and SEnsoR Technology) is an innovative water desalination compact module that enhances the energy savings by using solar energy developed in the framework of the DESERT European project (Water JPI, 2016). The second source was saline RW (S), obtained by mixing the secondary wastewater from Bari SWTP with the brine produced in the DESERT prototype, reaching a final ECw of 3 dS m^{-1}. For each water source, two irrigation treatments were established. The full irrigation (FI) treatments involved irrigation with D or S during the whole season at 130% ET_c (D-FI and S-FI, respectively). The RDI treatments consisted of irrigation at 130% ET_c, except during the kernel filling, when received 80% of the ETc (from late June to early August, 35-40 days) (D-RDI and S-RDI). Irrigation was applied each year from March to October. Therefore, four irrigation treatments based on the water quality of the irrigation source and water quantity were performed. The irrigation treatments consisted of a desalinated full irrigation treatment (D-FI) irrigated through the growing season to fully satisfy crop water requirements using desalinated reclaimed water, a saline full irrigation treatment (S-FI) using saline reclaimed water, and two regulated deficit irrigation treatments: desalinated regulated deficit irrigation (D-RDI) plants were irrigated using desalinated RW, and saline regulated deficit irrigation (S-RDI) plants were irrigated using saline RW.

The irrigation doses were scheduled on the basis of the daily crop evapotranspiration (ET_c), calculated by the water balance method. Et_c was calculated using the following equation recommended by FAO:

Et_c=Kr. Kc. ET_0

where Kr is the reduction coefficient (Kr=0.75), Kc is the crop coefficient as described by Vivaldi et al. (2019) and ET_0 is reference evapotranspiration. ETo was calculated by Penman-Montheith equation using climate data provided by the climate station located 100 m from the experiment. The water was supplied by drip irrigation with three pressure compensated drippers per tree, each with a flow rate of 2 L h^{-1}. Trees were irrigated daily during the three-year experiment. The irrigation was controlled automatically by a head unit programmer and the amount of water applied for each irrigation treatment were measured with in-line flowmeters placed in each treatment. A total of 40 trees made up this assay (10 per treatment). Each irrigation treatment had five replicates, distributed in a completely randomized design. Each replicate consisted of two trees.

2.3. Water quality

The inorganic solute content, pH and EC_w of each irrigation water source were assessed monthly during the irrigation season in 2017, 2018 and 2019. The samples were collected in glass bottles, transported in an ice chest to the laboratory, and stored at 5ºC before processed for chemical and physical analyses. The concentrations of Na^+, K^+, Ca^{+2}, B^{+3} and Mg^+ were determined by inductively coupled plasma optical emission spectrometer (ICP-ICAP 6500 DUO Thermo, England). Anions (Cl^-, NO^{3-}, PO_4^{3-}, SO_4^{2-}) were analysed by ion chromatography with a liquid chromatograph (Metrohm, Switzerland). EC_w was determined using a PC-2700 meter (Eutech Instruments, Singapore), and pH was measured with a pH-meter Crison-507 (Crison Instruments S.A., Barcelona, Spain).

2.4. Plant growth and water status measurements

At the beginning and at the end of each growing season, trunk diameter was measured in five trees per treatment with a sliding caliper, 0.20 m above the soil surface. Shoot length values were collected during growing period in 2018, by measuring the length of two shoots for each tree and four trees per treatment, and the relative growth rate (RGR) was calculated as the rate of increase of length per unit of initial shoot length. At the end of growing period in 2018, twenty leaves per tree in four trees per treatment were washed with distilled water and dried at 80ºC, before stored at room temperature for inorganic solute analyses. The concentration of Cl^- was analyzed by chloride analyzer (Chloride Analyser Model 926, Sherwood Scientific Ltd.) in the aqueous extracts obtained when mixing 100 mg of dry vegetable powder with 40 mL of water before shaking for 30 min and filtering. The concentrations of Na^+ were determined in a digestion extract with HNO_3:$HCl0_4$ (2:1, v/v) by inductively coupled plasma mass spectrometry (ICP-ICAP 6500 DUO Thermo, England).

Seasonal changes in leaf water potential (Ψ_l), stem water potential (Ψ_s), leaf osmotic potential (Ψ_o), leaf turgor potential (Ψ_t), leaf osmotic potential at full turgor (Ψ_{100s}), stomatal conductance (g_s), net photosynthesis rate (P_n) and transpiration (E) were determined in five trees per treatment in mature leaves at midday. Ψ_l was estimated according to the method described by Scholander et al. (1965), using a pressure chamber (Model 3000; Soil Moisture Equipment Co, Santa Barbara, CA, USA), for which leaves were placed in the chamber within 20 s of collection and pressurized at a rate of 0.02 MPa s^{-1} (Turner, 1988). Ψ_s was measured in non-transpiring leaves that had been bagged with both a plastic sheet and aluminum foil for at least 2 h before measurement in order to prevent leaf transpiration: in this way leaf water potential equaled stem water potential (Begg and Turner, 1970). In six representatives dates during the experimental period, leaves from the Ψ_l measurements were frozen in liquid nitrogen (-196ºC) and stored at -30ºC. After thawing, the osmotic potential (Ψ_o) was measured in the extracted sap using a WESCOR 5520 vapour pressure osmometer (Wescor Inc., Logan, UT, USA), according to Gucci et al. (1991). Ψ_t was estimated as the difference between leaf water potential (Ψ_l) and leaf osmotic potential (Ψ_o). Throughout the experimental period, leaf osmotic potential at full turgor (Ψ_{100s}) was estimated as indicated above for Ψ_o, using excised leaves with their petioles placed in distilled water overnight to reach full saturation. Ψ_l and Ψ_{100s} were only measured during 2018 and 2019. Leaf stomatal conductance (g_s), net photosynthesis rate (P_n) and leaf transpiration rate (E) were determined in attached leaves using a gas exchange system (LI-6400, LI-COR Inc., Lincoln, NE, USA), while the P_n/g_s ratio was used as an estimation of the intrinsic water use efficiency.

2.5. Statistical analyses of data

The data were analyses by one-way ANOVA using Statgraphics Plus for Windows 5.1 software. Ratio and percentage data were subjected to an arcsine square-transformation before statistical analysis to ensure homogeneity of variance. Treatments means were separated by Duncan´s Multiple Range Test (P<0.05). Pearson´s correlation analysis was used to test for relationship between leaf ion concentrations and RGR of shoot length.

3. Results

3.1. Irrigation, water quality and volume applied

Significant differences on the water quality were found between the two irrigation sources during the whole experiment (Table 1). Saline reclaimed water (S) had high salinity, with EC_w values (≈3 dS m^{-1}) higher than those measured in the desalinated reclaimed water (D) (≈1-1.1 dS m^{-1}). S also increased the concentrations of some nutrients such as NO_3^-, PO_4^{3-}, SO_4^{2-}, K^+, Mg^+ and Ca^{+2} with respect to D, whereas the concentration of B^{+3} remained invariable. The high Na^+ concentration observed in S, also increased the water sodium adsorption ratio (SAR_w) level from a mean value close to 4.6 $[meq/L]^{0.5}$ observed in the D source to about 6.7 $[meq/L]^{0.5}$ in the S source. It is also noteworthy that concentrations of phytotoxic elements such as Na^+ and Cl^- concentrations were clearly different in each irrigation water source. D had lower Na^+ and Cl^- concentration as a result of desalination process, reaching on the average, values of Na^+ (165 mg L^{-1}) and Cl^- (211 mg L^{-1}), as compared to the S (328 for Na^+ and 416 mg L^{-1} for Cl^-).

The mean annual ET_0 and rainfall for the three experimental seasons were 1291 and 586 mm (Fig. 1) The amounts of irrigation water applied in 2017, 2018 and 2019 in the full irrigation treatments were 368, 246 and 221 mm, while in the RDI treatments were 305, 201 and 184 mm respectively, which meant reductions of about 20% each year in the RDI treatments (Fig. 1).

3.2 Plant growth and leaf mineral concentrations

Regulated deficit irrigation and reclaimed water affected the growth and size of the almond trees and a significant decrease in trunk diameter and shoot length compared with D-FI trees was measured during the experimental period. However, the specific response depended on the treatment and parameter in question. Throughout the experiment, trunk diameter was similar in all treatments, but began to be inhibited three years after application of the RDI (Table 2). At the end of the experimental period, trunk diameter was significantly inhibited by both RDI compared to full irrigation trees, the smallest plants (4.2 cm), being those subjected to RDI combined with saline RW. As regard RGR as a function of shoot length, no differences between the D-FI and D-RDI treatments were observed, but lower rates were found in the trees irrigated with saline RW (Fig. 2; F= 17.91, P= 0.00003). While no accumulation of Na^+ and Cl^- was found in the leaves of trees submitted to D-RDI treatment compared with D-FI treatment, the contents of both ions increased in the leaves of the trees irrigated with saline RW (Fig. 2). In all treatments, the Cl^- content in the leaves was markedly higher than the corresponding of Na^+ content (between 12 and 30 fold higher), despite that the concentrations of both ions in the irrigation water were similar (7.0 and 5.5 mmol L^{-1} in D; 11.7 and 10.6 mmol L^{-1} in S, for Na^+ and Cl^- respectively). This means that *P. dulcis* plants are able to restrict Na^+ accumulation in leaves to a greater extent than Cl^-. Thus, the trees increased their leaf Cl^- content sharply, reaching a value of 2.4% in S-RDI plants. The highest Cl^- value was measured in leaves of S-RDI plants, while the highest Na^+ value was in S-FI, which means that the retention and transport of both ions was different. Significant relationships between RGR and leaf Na^+ and Cl^- content were observed in the almond trees in 2018 at the end of the deficit irrigation period (Fig. 2).

3.3. Plant water relations and osmotic adjustment

The seasonal evolution of stem water potential (Ψ_s) and leaf water potential (Ψ_l) during the experimental period for both water sources (D and S) and irrigation treatments (FI and RDI) is shown in Fig. 3A, B. Plants irrigated at full water requirements using desalinated RW maintained the Ψ_s close to -1.0 MPa during the three-year experimental period (Fig. 3A). By contrast, under drought conditions, the Ψ_s values progressively decreased in both RDI treatments compared with full irrigation trees, reaching values at midday of -2.5 and -2.6 MPa for the S-RDI and D-RDI treatments, respectively at the end of deficit irrigation period in 2018. However, these values were never reached in 2019 which was a year characterized by a larger amount of rainfall over the deficit irrigation period and lower evaporative demand compared with 2017 and 2018 (Fig. 1). Once full irrigation conditions were restored, Ψ_s in the RDI treatments immediately recovered and matched that of the full irrigation trees during the first two growing seasons. While in 2019, this recovery took more time, especially in S-RDI, and differences among treatments only disappeared at the end of the experimental period.

No pronounced differences in Ψ_s were found between trees of both full irrigation treatments (D-FI and S-FI) during most of the experimental period, although lower Ψ_s values were observed in plants irrigated with saline RW compared with trees irrigated with desalinated RW in 2019. Similarly, in plants subjected to both RDI, 3 years after the beginning of the irrigation with reclaimed water S-RDI plants had the lowest Ψ_s. As expected, Ψ_s and Ψ_l were higher in general in the trees of both full irrigation treatments than in both RDI treatments, although Ψ_s measured at midday showed lower variability than Ψ_l (Fig. 3A, B). Thus, Ψ_s was able to detect differences among treatments to a greater extent than Ψ_l.

The application of the RDI strategy, with desalinated or saline RW, reduced the leaf osmotic potential (Ψ_o) values compared with D-FI during the water stress periods, which induced similar values of leaf turgor potential (Ψ_t) in the D-FI and S-RDI treatments, and even higher in D-RDI at the end of the deficit period in 2019 (Fig. 3C). The lowest values for Ψ_t were found in D-RDI trees, reaching a value of 0.5MPa during the RDI period in 2018, coinciding with the lowest value of Ψ_s (Fig. 3D).

At the end of the deficit irrigation periods in 2018 and 2019, leaf osmotic potential values at full turgor (Ψ_{100s}) decreased in trees irrigated with saline RW (S-FI and S-RDI), especially under the combination of saline reclaimed water and regulated deficit irrigation (S-RDI) (Table 3). This reduction was indicative of the osmotic adjustment that took place in these trees as a consequence of the irrigation (0.37 MPa and 0.79 MPa for S-FI and S-RDI treatments, respectively).

3.4. Leaf gas exchange

Stomatal conductance (g_s) and the photosynthetic rate (P_n) are shown in Fig. 4. The trees subjected to both deficit irrigation treatments showed lower g_s values than the full irrigated trees during the RDI period, especially in the case of trees irrigated with desalinated RW in 2018 (Fig. 4A). Such reductions with respect to the full irrigation trees were also observed in photosynthesis levels, although the differences were less marked (Fig. 4B).

Once well-watered conditions were restored, both the P_n and g_s values of the plants that had been exposed to deficit irrigation showed recovery with respect to the full irrigation treatments and similar values of P_n and g_s were obtained in all treatments at the end of each growing season. Trees irrigated at full water requirements using saline water reduced P_n with respect to D-FI in 2019, 3 years after the beginning of the saline irrigation. At that time, the lowest P_n values were found in S-RDI plants, this is when the two constraints were combined.

In general, trees subjected to RDI treatments showed higher P_n/g_s ratios (intrinsic water use efficiency) than full irrigated trees during the deficit irrigation period, but these differences between treatments disappeared when irrigation was restored (Fig. 4C). All treatments showed a decline in leaf transpiration rate (E) as the evaporative demand of the atmosphere increased, whereas more pronounced E reductions were found in D-RDI and S-RDI treatments in response to a decrease in the irrigation amount (Fig. 4D). This parameter changed in the RDI treatments according to the irrigation applied in each phase. In the RDI treatments, when irrigation pattern was changed, the trees increased or decreased their leaf transpiration (E) and adjusted to the new conditions, but with some particular characteristics. When trees were exposed to deficit irrigation after full irrigation conditions, plants of both RDI treatments restricted their E in relation to the full irrigation trees. This reduction was earlier and more marked in plants irrigated with desalinated RW, while the leaf rate readjustment in S-RDI took more time and during the first deficit irrigation period the leaf transpiration rate of S-RDI was similar to that of full irrigation plants, despite the lower levels of water applied (Fig. 4D). Once well-watered conditions were restored, the E in D-RDI plants quickly recovered and their E matched that of trees that had been well irrigated since the beginning of the experiment. In contrast, E values in the S-RDI plants increased more slowly and even were significantly lower than that in full irrigated trees at the end of growing season in 2017.

The net photosynthetic rates (P_n) decreased as stomatal conductance decreased, particularly when g_s was below 150 mmol m^{-2} s^{-1}, (Fig. 5A). In addition, plants showed higher P_n/g_s ratios when stomatal conductance decreased from maximum to around 100 mmol m^{-2} s^{-1}, but when g<100 mmol m^{-2} s^{-1}, the decline in P_n/g_s was more pronounced (Fig. 5B). Only trees of the D-RDI treatment reached g_s values below 100 mmol m^{-2} s^{-1} during the deficit irrigation period. Fig. 6 presents the response of g_s for all treatments to the decline of Ψ_s. Stomatal conductance was maintained relatively high until Ψ_s reached at about -1.2 MPa. Afterwards, it declined gradually, and by the time Ψ_s was below -2 MPa, g_s was lower than 100 mmol m^{-2} s^{-1}.

4. Discussion

Fruit trees in general have demonstrated wide variability in their reaction to water stress and salinity. Variations in plant growth have been previously used to identify water or salt tolerant plants (Tattini and Traversi, 2008; Sidari et al., 2008). In our experiment, shoot growth in *Prunus dulcis* plants was more influenced by the irrigation with saline reclaimed water than by water deficit. However, Pedrero et al. (2015) irrigated young grapefruit trees for 3 consecutive years with saline RW with the same level of salinity used in our essay (EC=3 dS m^{-1}) reporting no reductions of the canopy volume, which confirms the differences between species, and points to the higher relative salt sensitivity of almond compared with grapefruit when using reclaimed water.

The effects of salinity and water stress on plant growth were additive, as trees submitted to RDI combined with saline RW had the lowest values of shoot length and trunk diameter, as previously reported by Glenn et al. (2012). Each of the different stresses tested in our study caused differences in growth responses of *P. dulcis*, indicating that the kind of stress and their interaction are key factors to success when using reclaimed water and /or regulated deficit irrigation strategies. In contrast to shoot length, trunk diameter was not reduced by saline RW irrigation under full irrigation, while trees submitted to both deficit irrigation treatments showed the lowest values of trunk diameter. Therefore, in almond trees, trunk growth was more sensitive to water deficit than to salinity. The reported differential response between shoot elongation and trunk diameter to salinity and deficit irrigation might be attributed to the variation in the time required by salts to affect each parameter. In fact, not all growth parameters are similarly affected by ionic and osmotic stress. This result is in agreement with Munns and Tester (2008), who reported that the reduction in growth parameters like plant size or trunk cross sectional area is evident much later than the reduction in cell production in young leaves.

P. dulcis plants can cope with water shortage during kernel filling or irrigation with saline RW with no important reduction in growth. However, growth was markedly reduced by the combination of saline reclaimed water and regulated deficit irrigation, which is a negative aspect, as the reduction of the tree canopy could affect the crop fruit load /potential yield (Nicolás et al., 2016; Romero-Trigueros et al., 2017).

In general, an increase in Na^+ and Cl^- concentration in the irrigation water led to an accumulation of Na^+ and Cl^- in the plant tissues of numerous species (Munns and Tester, 2008; Zrig et at., 2015; Álvarez et al., 2018; Momenpour et al., 2018). In our study, higher Na^+ and Cl^- contents were observed in the leaves of *P dulcis* trees irrigated with S, correlating with their lower shoot growth (Acosta-Motos et al., 2017). Under salt stress conditions, the ability to control the Na and/or Cl contents of the leaves, minimizing entry through the roots, retaining ions in the roots and lower stem and/or limiting transport to the aerial parts, is a crucial mechanism that can result in improved plant growth and survival under saline conditions (Pérez-Alfocea et al., 2000; Colmer et al., 2005).

In the present study, *P. dulcis* were not able to retain Na^+ and Cl^- in the woody parts of the tree, especially for Cl^- ions, although the retention and transport of each ion was different, as previously reported by Tattini and Traversi (2008). Trees irrigated with saline RW of 3 dS m^{-1} EC increased their Cl^- content in leaves, especially in S-RDI, while the increase in the Na^- content was similarly for both levels of irrigation, regardless the amount of water. The greater decline in plant growth observed in the almond trees submitted to RDI combined with saline RW could be attributed to excessive Cl^- in leaves. Indeed, in certain species Cl^- toxicity seems to be more severe than Na^+ (Fornes et al., 2007).

In plants exposed to water deficit, turgor maintenance is usually obtained by means of increasing concentrations of solutes as a consequence of cell volume reduction, which has been described as more efficient that turgor maintenance achieved through the production of organic solutes (Navarro et al., 2009; Turner, 2018). In our assay, the almond trees exposed to RDI reduced leaf osmotic potential as a tolerance mechanism to drought, which allow trees to maintain the high cell turgor pressure values. In addition, the irrigation with saline RW pointed to lower values of leaf osmotic potential at full turgor in almond trees, which is indicative of the osmotic adjustment process that occurs in these trees, as previously found in almond trees subjected to similar salinity levels (Shibli et al., 2003; Zrig et al., 2015). However, osmotic adjustment was not observed in almond trees exposed to water deficit when irrigated with desalinated RW.

Similar observations are described in other species grown under salinity and water stress, where lower osmotic adjustment was found in water stress than in salt stress (Sucre and Suarez, 2011; Álvarez et al., 2012; 2018). The osmotic adjustment can be achieved by uptake the inorganic ion from the soil solution or by synthesis of organic solutes, the latter being the more cost-effective strategy (Slama et al., 2008). This could indicate that these solutes (Na and Cl) were responsible for the osmotic adjustment observed in almond plants when irrigated with saline RW. Zrig et al. (2015) found that Cl^- and Na^+ were the main osmolytes involved in osmotic adjustment in almonds subjected to salinity. However, this mechanism of leaf turgor maintenance by the accumulation of inorganic solutes, especially Cl^-, can have deleterious effects on the plant (Pérez-Pérez et al., 2007). In the present investigation, it appeared that the high accumulation of Cl^- in leaves of both S, especially S-RDI may have been responsible for the reduction in growth and death of older leaves observed in these plants.

Plant water status, measured by the leaf and water potential, decreased in almond exposed to water deficit or irrigated with saline RW, indicating a slight dehydration in these plants (Zrig et al., 2015; Espadafor et al., 2017). During the first two years, the lowest values of the Ψ_s and Ψ_l were observed in D-RDI, producing the lowest values of stomatal conductance and leaf transpiration rate. However, in the last year of the experiment (2019) the lowest values were observed for plants irrigated with S, especially when combined with RDI, due to the accumulation of salts together with passive dehydration (Slama et al., 2008). Álvarez and Sánchez-Blanco (2015) reported that stem water potential measured at midday can be used as an indicator of the stress resulting from water and salt stress in *C. laevis* plants, not only with salinity and water deficit separately but also when combined. A similar response was also noted in our essay with *P dulcis* when saline and desalinated reclaimed water was used for irrigation combined with RDI, as Ψ_s was the most discriminating indicator for these stresses (Choné et al., 2001).

P. dulcis is considered a species with high stomatal sensitivity to water deficit, that prevents xylem cavitation by controlling stomatal function (Espadafor et al., 2017). Studies aimed at developing regulated deficit irrigation strategies in almonds have reported that plant water status had a dominant role in controlling stomatal closure and the reduction in stomatal conductance was attributed to a decrease in leaf and stem water potential. The results of this study are consistent with the finding of Shakel et al. (2007), who reported a reduction of 50% in g_s when Ψ_s was -1.4 to -1.8 MPa, a level of water stress that may be moderate for almond, as values as low as -4.0 MPa have been reported for severe stress levels. The decline in g_s observed in the present work in response to the lowering of Ψ_s suggests a high sensitivity to almond to water deficit. In this sense, almond trees are able to adapt to a reduced moisture level in the soil and, as a result, leaf transpiration rate (E) is reduced (Espadafor et al., 2017). In our experiment, environmental conditions and level of irrigation applied clearly affected transpiration, as pointed out by Phogat el al. (2013), Álvarez et al. (2013) and Fereres et al. (2014).

Although the amount of water applied was the same in both RDI treatments, the lowest values for both E and g_s were found in the trees submitted to RDI combined with desalinated RW (for the first two years) and in trees submitted to RDI combined with saline RW for the third year. This indicates that these parameters do not only depend on the amount of water applied but also on the EC of the water applied.

The inhibition of transpiration under deficit irrigation or salinity is seen as an adaptation and it is one of the key mechanisms allowing plans to reduce water losses, delay the onset of more severe stress under drought conditions and limit the accumulation of toxic ions in the shoots in plants exposed to salinity. Evapotranspiration reductions have been attributed to lower stomatal conductance in the short term and to the reduction in leaf area in the long term as stress increases (Ali et al., 2012; Espadafor et al., 2017). In this sense, wilting and a great leaf loss was observed during the last growing season in S-RDI treatment, when salt injury became evident in the old leaves.

A decline in P_n due to irrigation with saline reclaimed water has also been described in a variety of species, such as Carrizo citrange, a plant sensitive to salinity (Pérez-Pérez et al., 2007) or in *M. communis,* an ornamental plant tolerant to salinity (Acosta-Motos et al., 2014). Nevertheless, this parameter is not always decreased by the irrigation with reclaimed water. Nicolás et al. (2016) reported that the rates of photosynthesis were not reduced in mandarin trees after six years irrigating with reclaimed water with the same salt level (3 dS m^{-1}) and Hassena et al. (2018) mentioned that irrigation with treated wastewater significantly increased the P_n in young olive trees in similar experimental conditions.

As indicated in the results, no pronounced differences in photosynthesis were observed during the first two years of the experiment between plants irrigated under full irrigation, regardless the quality of the water source (D-FI and S-FI). However, the cumulative effect of irrigation with saline RW (3 dS m^{-1}) for a longer period (3 years) was a reduction of P_n. In our study, trees submitted to the combination of water deficit and saline RW showed a small degree of P_n reduction during the first two years. However, the third year the reduction in P_n and g_s was more pronounced in S-RDI than in D-RDI plants. Several works have verified that the morphological and physiological responses of plants to the combination of water and salt stress are more complex than a simple additive effect of water and salt stress applied individually (Mittler 2006, Pérez-Pérez et al., 2007). Some studies have shown that drought may magnify the adverse effects of salinity, reporting more negative impact on plant growth than their individual effects (Álvarez and Sánchez-Blanco, 2015). However, numerous studies have demonstrated that the addition of salt to plants submitted to water deficit actually has a positive effect on biomass accumulation in several species and found that salinity mitigates the deleterious effects of water stress and enhances plant survival (Glenn and Brown, 1998; Martínez et al., 2005; Alla et al., 2011, Sucre and Suárez, 2011; Glenn et al. 2012). Álvarez and Sánchez-Blanco (2014; 2015) in *C. citrinus* and *C. laevis* reported that if plants show g_s values below 100 mmol m^{-2} s^{-1} for long periods, reductions in P_n are mainly caused by non-stomatal factors and intrinsic water use efficiency is sharply reduced, which could delay plant relief or cause irreversibly effects. As indicated in the results the values of g_s observed in our study were maintained relatively high, being above 200 mmol m^{-2} s^{-1} during most of the experiment, while g_s values lower than 100 mmol m^{-2} s^{-1}, corresponding to Ψ_s <-2 MPa were only observed occasionally. In this sense, the subsequent recovery in P_n that occurred in these plants when irrigation was restored suggest that water stress did no cause irreversible damage to leaf tissue, indicating that the maximal PSII primary photochemistry was not permanently affected-by the stressful conditions experienced by plants and leaves can recover photosynthetic capacity after stress (Álvarez et al., 2018).

5. Conclusion

Our results showed that although both regulated deficit irrigation and saline reclaimed water slightly decrease tree vegetative growth in *Prunus dulcis,* plants displayed different morphological and physiological responses to each stress, being different between water and salt stress and the combination of both stresses. The use of desalinated reclaimed water of low conductivity (1 dS m^{-1}) combined or not with RDI treatment is a viable irrigation management strategy for saving water without compromising the overall performance of the almond trees. The tolerance of *P. dulcis* to drought was related to an effective mechanism of stomatal control and its ability to recover water status and photosynthesis capacity, accompanied by an increased water use efficiency (P_n/g_s ratio), which are aspect positives of deficit irrigation. *Prunus dulcis* responded to irrigation with saline reclaimed water of moderate conductivity (3 dS m^{-1}) combined or not with RDI treatment by reducing vegetative growth, restricting Na^+ accumulation in leaves in a great extent than Cl^- and showing osmotic adjustment. Thought *Prunus dulcis* appears to develop mechanisms to confront drought and salt stress separately, saline reclaimed water combined with deficit irrigation is not recommended, due to it reduced photosynthesis, affected the capacity of plants to recover water and carbon balance after water stress and induced a great reduction in growth due to leaf tissue dehydration and the high content of Cl and Na accumulated in leaves. Based on the results of this study, desalinated and saline reclaimed water can be used as additional water resources for almond tree irrigation. The use of desalinated RW could be successfully applied combined or not with RDI strategies, while the use of saline RW could be only recommended if full irrigation is carried out so to ensure the sustainability of almond trees. This finding should be borne in mind when deciding irrigation strategies for use in this kind of crops in water-scarce Mediterranean environments.

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Author contributions

GAV, SC and FP: design of the research; CRT, FP, GC and GL: performance of the research; CRT and GC: data analysis; GAV, CRT and SA: data Interpretation; SA: writing the manuscript; GAV, CRT and SA: critical revision of the manuscript; GAV and SC: funding acquisition. All authors reviewed and commented on the manuscript.

Declaration of Competing Interest

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

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Figure captions

Fig. 1. Monthly values of reference evapotranspiration (ET_0, mm month^{-1}), rainfall (R, mm month^{-1}), vapour pressure deficit (VPD, kPa), and irrigation in the full irrigation (FI) and regulated deficit irrigation (RDI) treatments (mm month^{-1}), during 2017-2019.

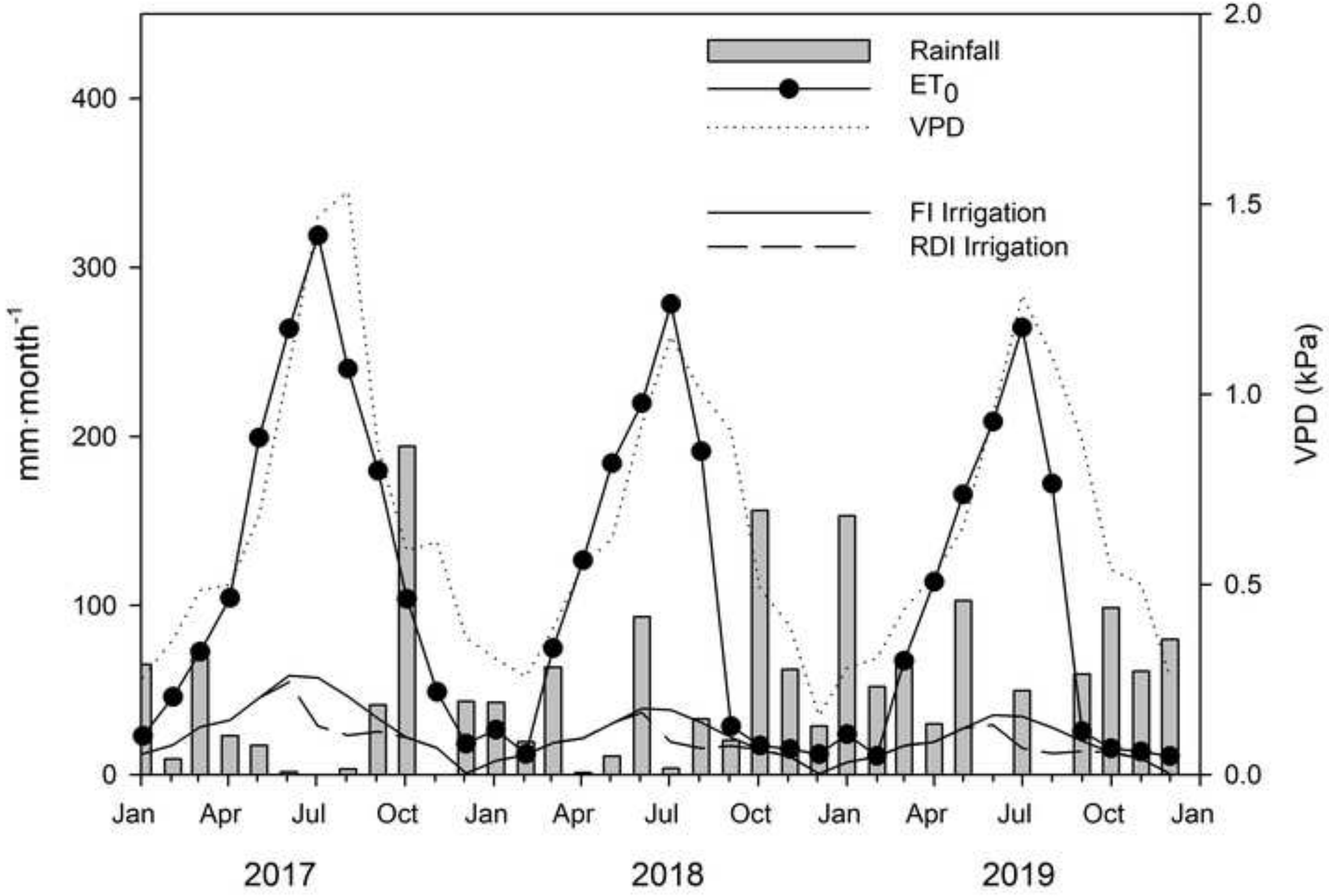
Fig 2. Relationship between RGR as a function of shoot length and Cl^- (A) and Na^+ (B) leaf content at the end of the growing season in 2018 in *P. dulcis* plants subjected to different irrigation treatments. Each point represents a single plant. Symbols represent the different treatments: Desalinated full irrigated (filled circles), desalinated regulated deficit irrigation (open circles), saline full irrigation (filled triangles) and saline regulated deficit irrigation (open triangles).

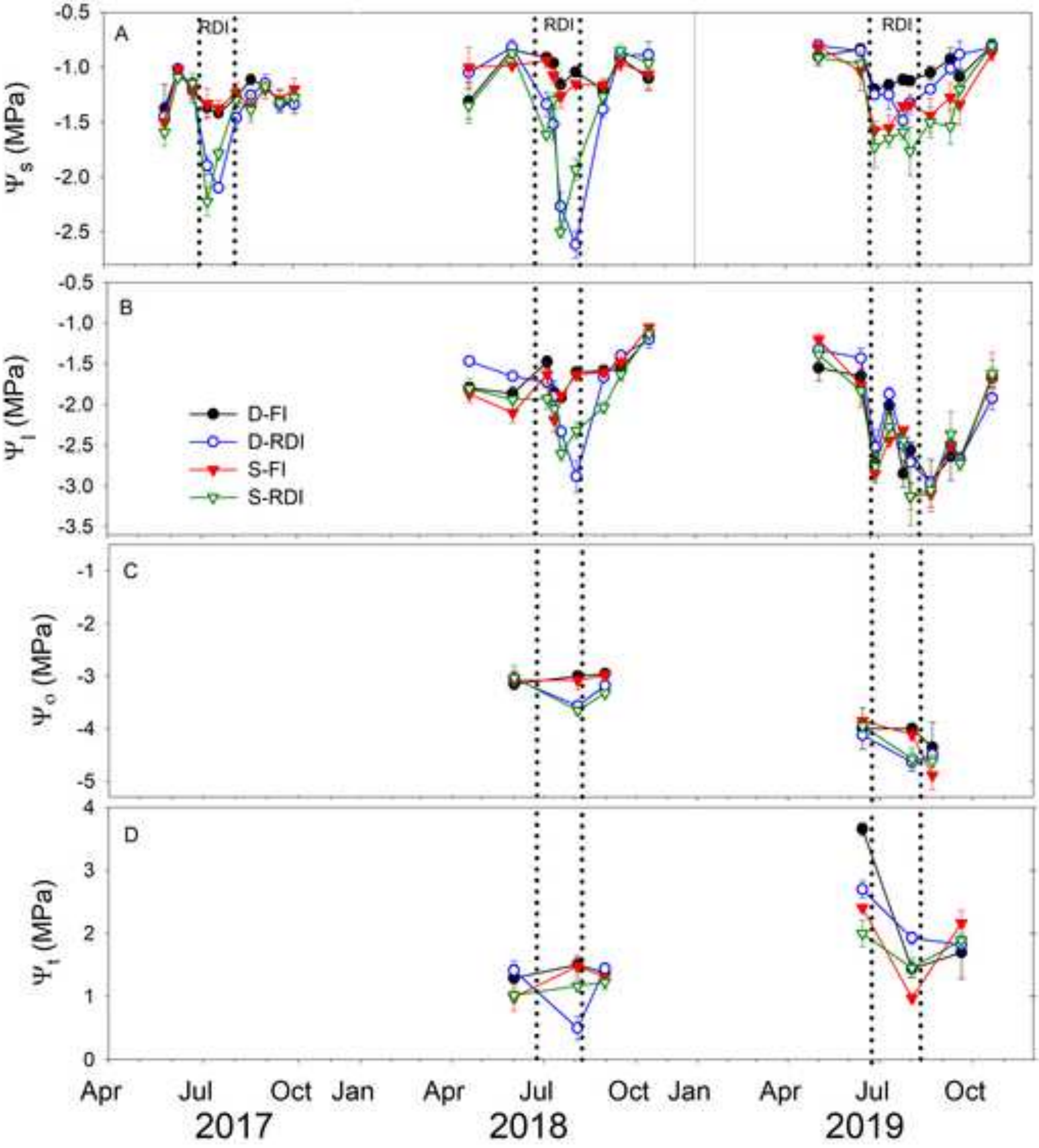
Fig. 3 Evolution of the stem water potential (Ψ_s, A), leaf water potential (Ψ_l, B), leaf osmotic potential (Ψ_o, C) and leaf turgor potential (Ψ_t, D) in *P. dulcis* plants submitted to different irrigation treatments. Values are means ± s.e., n = 5. Symbols represent the different treatments: Desalinated full irrigated (filled circles), desalinated regulated deficit irrigation (open circles), saline full irrigation (filled triangles) and saline regulated deficit irrigation (open triangles). Dashed lines represent the beginning and end of the regulated deficit irrigation periods

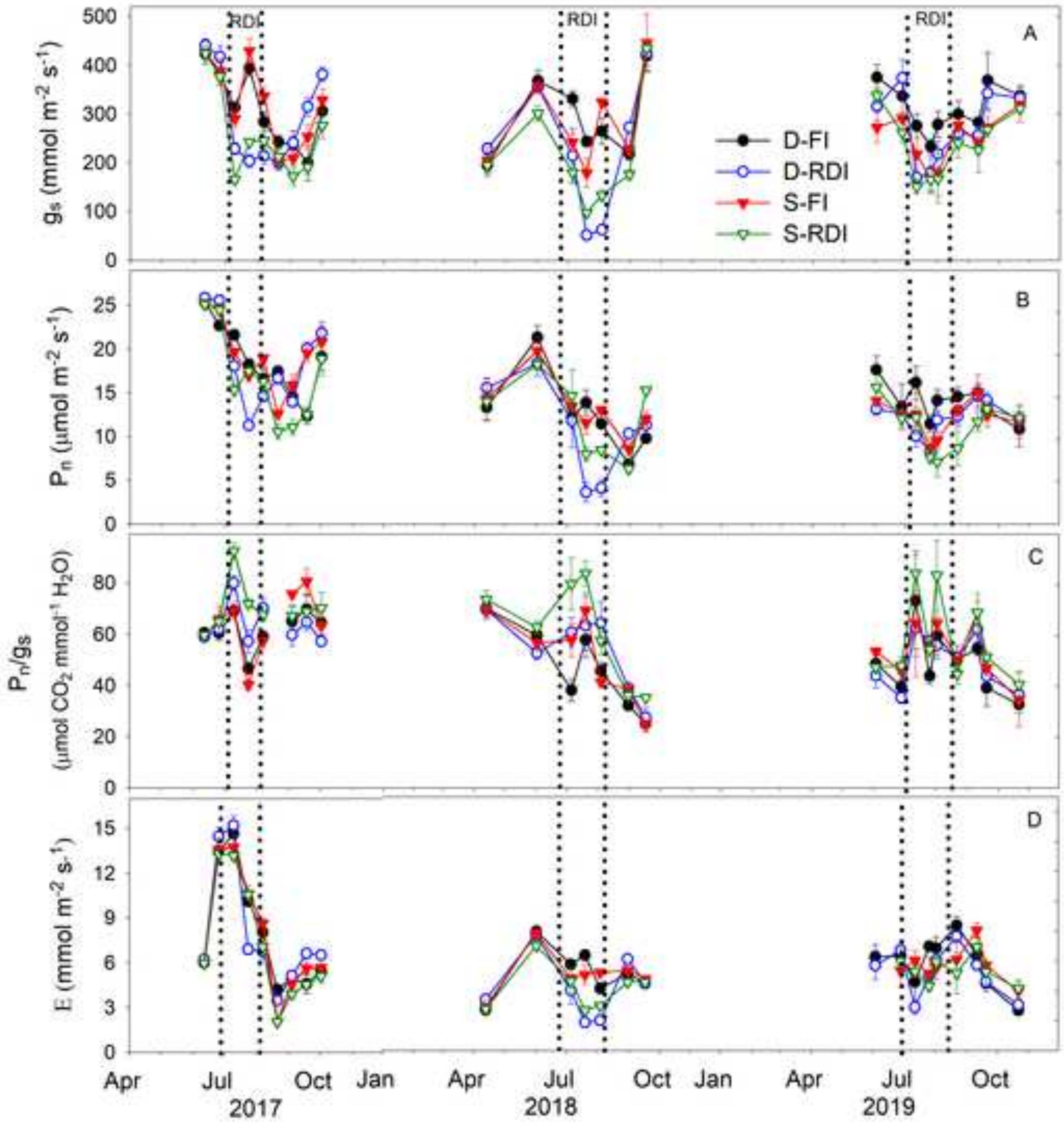
Fig. 4. Evolution of stomatal conductance (g_s, A), net photosynthesis rate (P_n; B), intrinsic water use efficiency (P_n/g_s, C) and leaf transpiration rate (E, D) in *P. dulcis* plants submitted to different irrigation treatments. Values are means ± s.e., n = 5. Symbols represent the different treatments: Desalinated full irrigated (filled circles), desalinated regulated deficit irrigation (open circles), saline full irrigation (filled triangles) and saline regulated deficit irrigation (open triangles). Dashed lines represent the beginning and end of the regulated deficit irrigation periods.

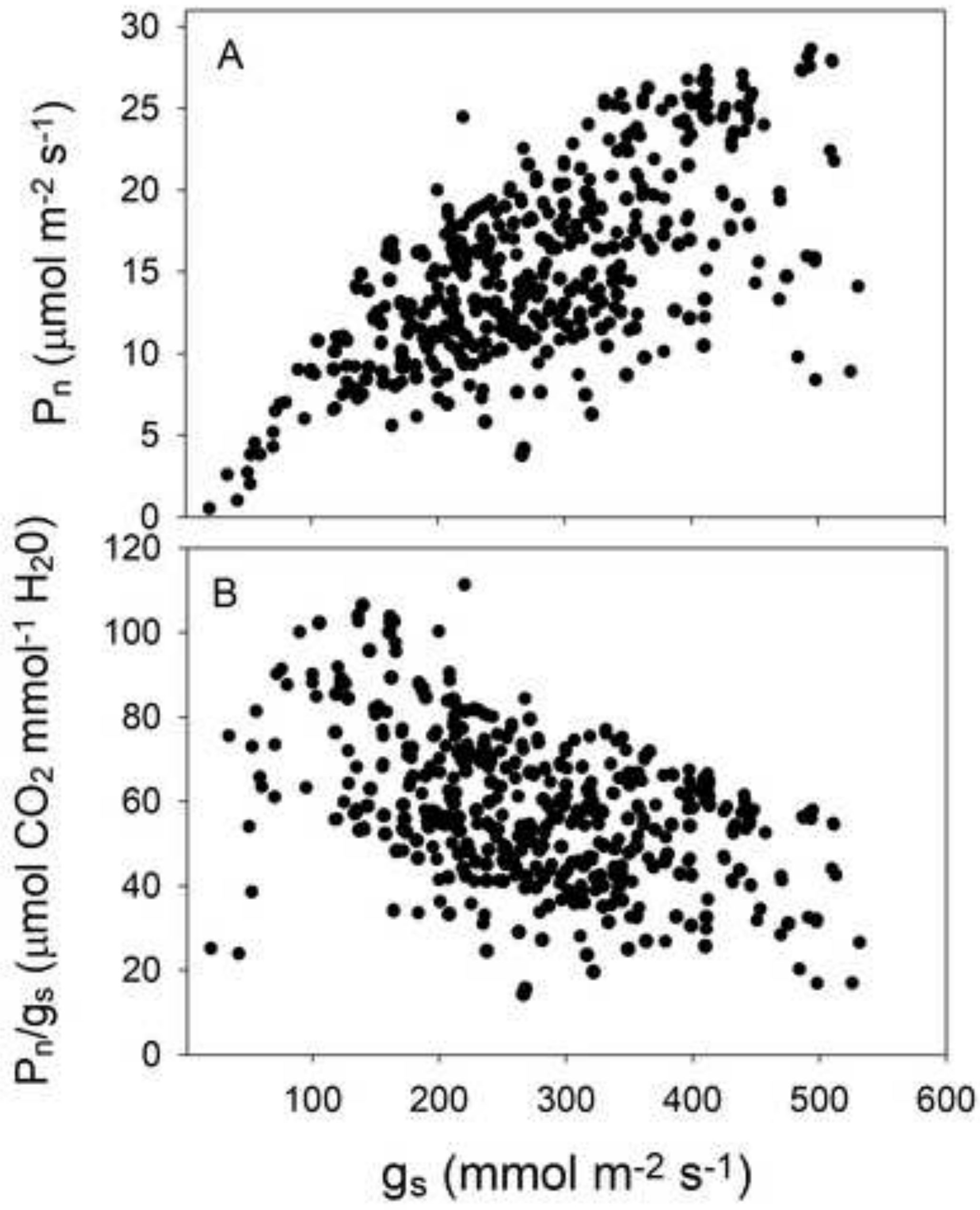
Fig 5. Relationship between net photosynthetic rate (P_n) and stomatal conductance (g_s) (A) and between intrinsic water use efficiency (P_n/g_s) and g_s (B) in *P. dulcis* plants submitted to different irrigation treatments. Each point represents a single plant.

Fig 6. Relation between stem water potential (Ψ_s) and stomatal conductance (g_s) in *P. dulcis* plants submitted to different irrigation treatments. Each point represents a single plant.


Rainfall
ET$_0$
VPD
FI Irrigation
RDI Irrigation
mm·month^{-1}
VPD (kPa)
0
100
200
300
400
0.0
0.5
1.0
1.5
2.0
Jan
Apr
Jul
Oct
Jan
Apr
Jul
Oct
Jan
Apr
Jul
Oct
Jan
2017
2018
2019


A
B
C
D
RDI
RDI
RDI
Ψs (MPa)
Ψl (MPa)
Ψo (MPa)
Ψt (MPa)
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
-1
-2
-3
-4
-5
0
1
2
3
4
D-FI
D-RDI
S-FI
S-RDI
Apr
Jul
Oct
Jan
2017
2018
2019


RDI
RDI
RDI
A
B
C
D
D-FI
D-RDI
S-FI
S-RDI
g_s (mmol m^{-2} s^{-1})
P_n (μmol m^{-2} s^{-1})
P_n/g_s
(μmol CO_2 mmol^{-1} H_2O)
E (mmol m^{-2} s^{-1})
500
400
300
200
100
0
25
20
15
10
5
0
80
60
40
20
0
15
12
9
6
3
0
Apr
Jul
Oct
Jan
Apr
Jul
Oct
Jan
Apr
Jul
Oct
2017
2018
2019


A
B
P_n (µmol m^{-2} s^{-1})
P_n/g_s (µmol CO$_2$ mmol^{-1} H$_2$0)
g_s (mmol m^{-2} s^{-1})
0
5
10
15
20
25
30
0
20
40
60
80
100
120
100
200
300
400
500
600

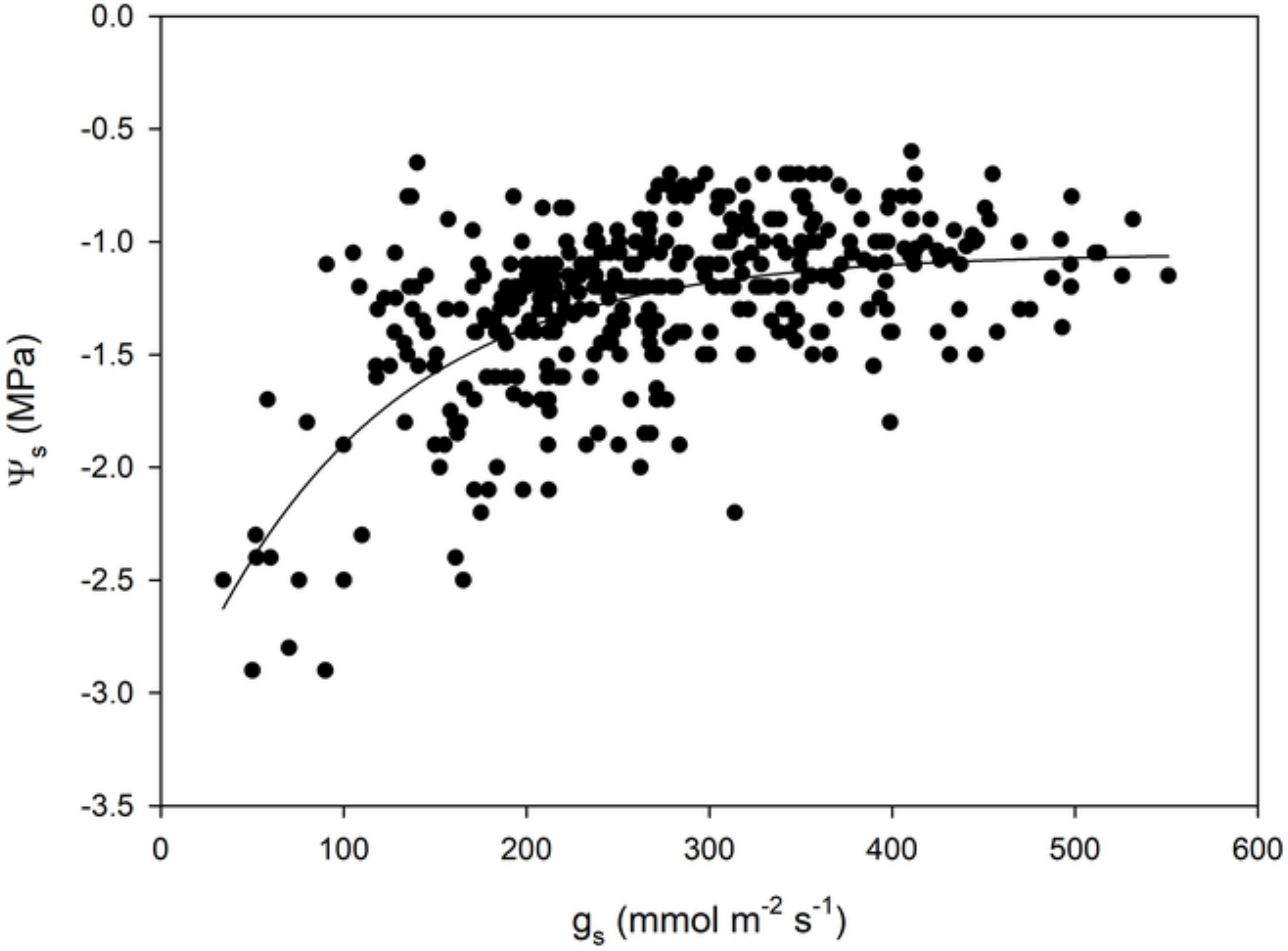

0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
Ψ_s (MPa)
0
100
200
300
400
500
600
g_s (mmol m^{-2} s^{-1})

Table 1 Physical and chemical properties for desalinated reclaimed water (D) and saline reclaimed water (S) in 2017, 2018 and 2019

		2017				**2018**				**2019**			
Property	**Units**	**D**		**S**		**D**		**S**		**D**		**S**	
pH		7.53 ± 0.31	b	8.15 ± 0.20	a	8.11 ±0.32	b	8.44 ±0.34	a	7.70±0.13	b	7.89±0.11	a
EC_w	dS m^{-1}	1.00 ± 0.15	b	3.00 ± 0.45	a	1.13 ±0.61	b	3.00 ±0.89	a	1.17±0.04	b	2.56±0.30	a
SAR_w	(meq/L)$^{0.5}$	3.70 ±0.42	b	7.20 ±1.52	a	4.79±1.94	b	5.69 ±1.62	a	5.52±0.75	b	7.09±1.54	a
Ca^{+2}	mg L^{-1}	56.28 ±11.30	b	121.3 ± 22.1	a	50.76±21.52	b	108.05±57.15	a	65.18±8.07	b	140.80±8.12	a
Mg^{+}	mg L^{-1}	20.9 ± 5.40	b	35.5 ± 6.10	a	18.31±8.12	b	35.96±16.82	a	13.56±1.25	b	31.81±2.28	a
K^{+}	mg L^{-1}	20.67 ± 8.81	b	42.76 ± 6.30	a	20.37±9.77	b	33.54±12.60	a	14.49±1.03	b	30.02±3.76	a
Na^{+}	mg L^{-1}	148.4 ± 53.2	b	353.2 ± 48.7	a	160.1±85.7	b	270.7±126.4	a	186.2±25.0	b	359.2±79.3	a
B^{+3}	mg L^{-1}	0.14 ± 0.06		0.15 ± 0.07		0.13±0.05		0.14±0.04		0.14±0.01		0.15±0.01	
NO_3^{-}	mg L^{-1}	15.83 ± 2.53	b	36.16 ± 9.28	a	28.39±25.08	b	42.70±19.93	a	25.6±3.1	b	11.52±1.14	a
PO_4^{3-}	mg L^{-1}	1.3 ± 0.61	b	3.1 ± 0.52	a	2.01±0.52	b	2.51±1.45	a	2.09±0.43	b	2.30±0.29	a
SO_4^{-2}	mg L^{-1}	98.0 ± 16.2	b	227.4 ± 37.5	a	92.4±66.1	b	144.9±92.1	a	49.3±1.3	b	95.5±0.3	a
Cl^{-}	mg L^{-1}	198.1 ± 54.1	b	379.5 ± 72.3	a	199.8±184.7	b	380.2±181.3	a	236.0±28.7	b	487.4±112.8	a

Values are the mean±SE of 12 individual samples taken throughout the crop cycle. Letters denote statistically significant differences at $P < 0.05$ level between treatments for each element and year.

Table 2. Trunk diameter at the end of each growing season in *P. dulcis* subjected to different irrigation treatments. Values are the mean±SE of five trees.

Year	**Treatments**				**P**
	D-FI	**D-RDI**	**S-FI**	**S-RDI**	
2017	3.73 ± 0.09 a	3.69 ± 0.05 a	3.64 ± 0.14 a	3.49 ± 0.17 a	ns
2018	4.63 ± 0.19 a	4.36 ± 0.10 a	4.54 ± 0.06 a	4.24 ± 0.16 a	ns
2019	4.86 ± 0.13 a	4.54 ± 0.07 b	4.76 ± 0.05 a	4.20 ± 0.09 c	*

Means within a row without a common letter are significantly different by Duncan $_{0.05}$ test.
(P; probalility level, ns; non significance, *P<0.05)

Table 3. Leaf osmotic potential at full turgor (Ψ_{100s}) at the end of each growing season in *P. dulcis* subjected to different irrigation treatments. Values are the mean±SE of five trees.

Year	**Treatments**				P
	D-FI	**D-RDI**	**S-FI**	**S-RDI**	
2018	-1.89 ± 0.08 a	-2.04 ± 0.05 a	-2.12 ± 0.03 bc	-2.25 ± 0.06 c	**
2019	-1.79 ± 0.05 a	-1.83 ± 0.18 a	-2.20 ± 0.14 b	-2.59 ± 0.10 c	**

Means within a row without a common letter are significantly different by Duncan $_{0.05}$ test
(P; probalility level, **P<0.01)