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Research Paper

Genotypic variation of physiological and morphological traits of seven olive cultivars under sustained and cyclic drought in Mendoza, Argentina

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The effects of two deficit irrigation regimes on the physiological, morphological, vegetative, and reproductive traits of seven olive cultivars were studied in a pot experiment. Specific leaf area (SLA) and SPAD chlorophyll meter reading (SCMR) were measured over the experimental period. Their relationships with physiological traits and with each other were also tested. Three-year-old plants in pots were subjected to control regime (CI replaced daily water use), sustained deficit regime (SDI was applied daily at 35% of CI), and five successive drought cycles of 30 days followed by rewatering (CDI). In all cultivars, SDI decreased stem water potential compared to CI. Under CDI, stem water potential reached the minimum, ∼−8 MPa, but all cultivars recovered to potentials similar to the rewatering period. Stomatal conductance was significantly reduced in both deficit irrigation regimes compared to the control. Rewatering caused a slower recovery of stomatal conductance in 'Selección Mendoza', 'Villalonga' and 'Arbequina' than in the rest of cultivars. Across cultivars, specific leaf area decreased with water deficit, while SCMR increased. Specific leaf area was positively related with stem water potential ($R^2 = 0.60$), stomatal conductance ($R^2 = 0.44$) and trunk growth ($R^2 = 0.31$). In contrast, SCMR values were negatively related with stem water potential ($R^2 = 0.59$), stomatal conductance ($R^2 = 0.52$) and trunk growth ($R^2 = 0.62$). 'Changlot', 'Arauco' and 'Nevadillo Blanco' cultivars maintained lower SLA and higher SCMR under both deficit irrigation regimes and higher stomatal conductance recovery after rewatering. These cultivars also appear to be better adapted to drought prone environments.

1. Introduction

In Argentina, olive cultivation has expanded considerably since the 1990s to reach approximately 100.000 ha (SAGPyA, 2010). Currently, this is the second most widely planted fruit crop in Argentina after vineyards, and it is key to the economy of the provinces of Mendoza, San Juan, La Rioja and Catamarca. More than 95% of olive plantations are located in arid and semi-arid environments between latitudes 28°S and 40°S having annual rainfall ranging from 100 to 500 mm concentrated during the summer months (Gómez-del-Campo et al., 2010). Consequently, irrigation throughout the growing season, mainly spring and autumn, is essential for profitable yield and quality (Rousseaux et al., 2009; Trentacoste et al., 2015). Water is actually the most limiting factor to both olive production and area expansion. In the coming years, lower water availability and higher costs are expected due to competition among different uses (industrial, urbanization, recreation, etc.) (Fereres and Evans, 2006). At present, many agronomic interventions are available in Argentinian olive orchards to increase irrigation technology and water use efficiency; however, the evaluation of olive cultivars under water deficit conditions (commonly known as drought) could be the best strategy to cope with water availability uncertainty (Rousseaux et al., 2009; Ruane et al., 2008).

The olive is widely considered to be well adapted to drought (Connor, 2005; Lo Gullo and Salleo, 1988). A large number of morphological and physiological traits have been found to be related to drought adaptation, such as restriction of water loss through modulation of stomatal closure (Sofo et al., 2008), development of osmotic adjustment (Dichio et al., 2003), higher water potential gradient between canopy and root system (Chartzoulakis et al., 1999; Xiloyannis et al., 1997) and increase of stomatal density and decrease of leaf area (Bosabalidis and Kofidis, 2002). Olive tree response to water deficit varies among cultivars (e.g. Ennajeh et al., 2008). Olive cultivars better adapted to drought conditions reveal

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smaller leaf area, lower specific leaf area and leaf water potential, lower stomatal conductance and stem growth under drought compared to well-irrigated conditions (Bacelar et al., 2006; Bosabalidis and Kofidis, 2002; Ennajeh et al., 2010; Tugendhaft et al., 2016). Most studies on olive trees focus on leaf traits because (i) leaves are the first organs to show visible morphological and physiological signs of drought and (ii) there is a tendency for studies to be conducted on young, non-producing plants. However, an integrated view of olive cultivar response to water deficit should include reproductive traits, such as return bloom (Gucci et al., 2007).

Among leaf traits, specific leaf area (SLA- ratio between leaf area and leaf dry weight) and SPAD chlorophyll meter reading (SCMR) have been investigated in a wide range of annual species such as peanut (Puangbut et al., 2009; Songsri et al., 2009), cowpea (Anyia, 2004), wheat (Fotovat et al., 2007) and trees (Aspelmeier and Leuschner, 2006) as a useful selection criteria of genotypes to drought conditions. Leaf water content is the most important factor to determine SLA, which is related to both leaf thickness and tissue density (Meziane and Shipley, 1999). SLA is generally lower in genotypes adapted to more drought prone environments (Rieger et al., 2003) because leaf expansion is generally more sensitive than photosynthesis when plant water potential decreases (Tardieu et al., 1999). In olive cultivars, lower SLA in leaves developed under water deficit has been related to an increase in leaf tissue density (Bacelar et al., 2006; Boughalleb and Hajlaoui, 2011). SCMR is an indicator of the light-transmittance characteristics of the leaf which is dependent on the leaf chlorophyll content (Richardson et al., 2002). A reduction of the relative water content of leaves causes an increase in SCMR (Martínez and Guiamet, 2004). SLA is negatively and closely correlated to water use efficiency, while SCMR and water use efficiency are positively related (Songsri et al., 2009). In this context, in annual species, it has been hypothesized that genotypes with high SCMR and low SLA have more photosynthetic capacity per unit leaf area, and hence, potential for greater assimilation under water stress (Nigam and Aruna, 2008; Puangbut et al., 2009).

Olive cultivar response to drought can vary in relation to the severity and duration of water deficit (Boussadia et al., 2008). In Mendoza, the target region for the present work, two future drought patterns are probable in relation to the irrigation technology used by farmers: (i) sustained deficit irrigation applied by modern farmers utilizing reservoirs and drip irrigation, and (ii) cycles of drought and posterior rewatering by traditional farmers using surface irrigation and without reservoirs. In sustained deficit irrigation, water deficit develops slowly allowing more plausible plant adaptation (Fereres and Soriano, 2006), in contrast to cyclic drought and re-watering, where the plants should be enabled to recover the main physiological processes after severe water deficit (Angelopoulos et al., 1996).

The aims of this work were: (i) to evaluate the response of five foreign and two local olive cultivars under sustained deficit and cyclic drought-alleviated conditions on morphological and physiological traits at leaf level, and vegetative growth and inflorescence density at plant level, and (ii) to investigate the relationships between specific leaf area and SCMR with physiological parameters (stem water potential, stomatal conductance and plant growth) in a wide range of olive cultivars and water conditions.

2. Materials and methods

2.1. Plant material and watering regimes

The study was carried out from September 2015 to November 2016 at the experimental farm of INTA Junín (33°06′S, 68°29′W, 653 masl). One-year old olive trees were transplanted in 40 L plastic pots (1 per pot) in the spring of 2013, two years before initiating the experiment. The pots were filled with a mixture of sandy soil and peat (4:1, v/v). Pots were made from black plastic covered with aluminium foil in order to avoid soil evaporation, minimize container temperature and exclude the entrance of rain. Plants were well irrigated and fertilized before starting the experiment. The pots were kept on the field under plastic netting of high transmittance (75%) to avoid hail damage and to prevent changes in microclimatic conditions.

Seven olive cultivars were evaluated, including five foreign cultivars: 'Arbequina', 'Changlot', 'Morchiaio', 'Nevadillo Blanco' and 'Villalonga' and two local cultivars: 'Arauco' and 'Selección Mendoza' (Trentacoste and Puertas, 2011).

Three different watering regimes were assigned to four plants per cultivar. In the control irrigation (CI) regime, plants were irrigated to replace evapotranspiration loss. The amount of water evapotranspired was calculated once per week, weighing one pot per cultivar for two consecutive days. Next, the average daily weight difference across cultivars was applied during the rest of the week. Sustained deficit irrigation (SDI) was restricted to 35% of the control amount. In both, CI and SDI, irrigation was performed daily. Cyclic deficit irrigation (CDI) was restricted to 35% of the control amount, but irrigation was applied on one day, each event approximately 30 days apart, exposing plants to five cycles of water deficit – rewatering during the experiment. The plants under CDI and SDI received the same amount of water but with a different frequency. The contrasting watering regimes were maintained from September 2015 to April 2016, after which all treatments were irrigated with the same amount of water as the control treatment until November 2016. Average, maximum absolute, and minimum absolute temperatures from September 2015 to April 2016 were 23.7 °C, 41.5 °C, and 2.0 °C, respectively.

Before the experiment, the water retention characteristics of the soil substrate were determined for two additional pots under the same experimental conditions. These pots were irrigated to excess and allowed to drain until they achieved constant weight. In CDI treatment, the end of irrigation withholding cycles was determined when water applied and accumulated in SDI treatment by pots achieved the maximum storable water. During the experiment, soil water content was monitored by a 10 cm long capacitance sensor (EC-5 Decagon Pullman WA) inserted vertically into the middle of one pot per treatment only in 'Arbequina' (Fig. 1) since there were only three sensors available.

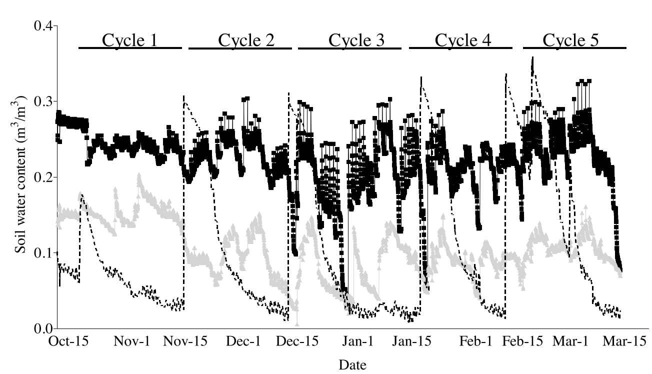

Fig. 1. Seasonal pattern of soil water content for control (solid black lines), sustained deficit irrigation (gray lines) and cyclic deficit irrigation (dotted black lines) treatments during part of the experimental period (October to mid-March). Control treatment was irrigated to replace evapotranspiration loss, determined by weighing. For sustained deficit irrigation treatment, irrigation was restricted to 35% of control. For cyclic deficit irrigation, plants were irrigated monthly with the same water accumulated in sustained deficit irrigation during this period and applied on one day. During the experimental period, cyclic deficit irrigation plants were exposed to 5 cycles of drought and rewatering.

2.2. Plant water status and stomatal conductance

Midday stem water potential (SWP) was measured in all irrigation treatments and cultivars, one day before and after irrigation in the CDI treatment. Midday SWP was measured on sunny days between 11:30 h and 12:30 h solar time using a Scholander-type pressure chamber (BioControl, Buenos Aires, Argentina). One leaf per plant was sampled following the procedure outlined by McCutchan and Shackel (1992). Mature leaves were enclosed in a small plastic bag covered with aluminium foil at least 90 min before measurements. As olive leaf petioles are too short to allow measurements with a pressure chamber, approximately 1 cm of leaf lamina on each side of the petiole was cut with a sharp blade and then immediately placed inside the chamber.

Stomatal conductance (gs) was measured the same days as SWP measurements. Measurements of abaxial stomatal conductance were taken from two fully-expanded leaves per plant during mid-morning (10:00-11:30 h) using a steady state porometer (Decagon device SC-1, USA).

2.3. Vegetative growth and morphological leaf traits

In winter 2015, before budburst, four one-year-old shoots were selected at random from each pot. Shoot length was measured in September 2015 and April 2016, and total shoot growth was then estimated as the difference between the two measurements. Trunk circumference was measured at 20 cm from the ground together with shoot length.

Fifteen mature leaves from each pot were gathered on current-year shoots from each pot in April 2016. The leaf area (LA) of those leaves was estimated taking photographs and subsequently using the program imageJ ® (National Institute of Health, USA, http://imagej.nih.gov/ij). Then the leaves were oven-dried at 60 °C for 48 h and their dry weight (LDW) determined. Specific leaf area (SLA = LA/LDW in $m^2\ kg^{-1}$) was estimated. Plant leaf area was estimated in April 2016 as the product between total leaves per plant and average leaf area.

SPAD chlorophyll meter reading (SCMR) was measured in four leaves from each pot on November 18th, 2015 (beginning of cycle 2 when the leaves of the current-season were well developed) and March 21st, 2016 (at the end of cycle 5). In recording SCMR, care was taken to ensure that the SPAD (Hansatech Model CL-01, UK) meter sensor fully covered the leaf lamina. Average SCMR was calculated as the mean of two evaluation times.

2.4. Inflorescence density

The effect of water regime on the following season was evaluated on return bloom. During the winter of 2016, length and node number was recorded on four shoots selected in the previous season for each pot. The number of inflorescences in the four selected shoots was counted in October 2016. Afterwards, inflorescence density was determined by dividing the number of inflorescences on each shoot by their length.

2.5. Data analysis

The factorial combination of three water regimes and seven cultivars was arranged in a completely randomized design with four replicates (i.e. 84 pots). ANOVA was used to test the effect of cultivar, water regime, and their interaction on response variables, and means were separated using the DGC- test (Di Rienzo et al., 2002). Statistical analyses were performed using the InfoStat 1.5 program. Regression analysis was applied to study the relationships between SLA and SCMR and stem water potential, stomatal conductance and increase in trunk circumference. These analyses and the resulting plots were made using GraphPad Prism version 5.01 software (GraphPad Prism Software, California, USA).

3. Results

3.1. Soil water content and stem water potential

Fig. 1 shows the seasonal pattern of soil water content (SWC) in three irrigation regime treatments of 'Arbequina'. In control irrigation (CI), the seasonal pattern of SCW tended to be maintained around 0.22 $m^3\ m^{-3}$ over the experimental period. During the experiment, irrigation for all treatments was occasionally interrupted due to power cuts. On these days, the control treatment SWC dropped near to 0.1 $m^3\ m^{-3}$, but it increased rapidly when irrigation was restored. In sustained deficit irrigation (SDI), SWC values were almost constant around 0.14 $m^3\ m^{-3}$ in October and November, after which they declined, fluctuating around 0.12 and 0.08 $m^3 m^{-3}$ until mid-March. In cyclic deficit irrigation (CDI), irrigation increased SWC up to values even higher than the control treatment, but then dropped sharply the first seven days after irrigation, remaining low at around 0.01 $m^3\ m^{-3}$ until a new irrigation event occurred. In CDI, the first, second, and fourth cycle lasted four weeks during which irrigation was completely stopped, the third cycle lasted five weeks, while the fifth cycle was shorter and lasted three weeks.

Average midday SWP through the experiment revealed a significant interaction between cultivars and irrigation regime ($P = 0.01$, Fig. 2A). Stem water potential under well irrigated conditions remained almost constant around −1.3 MPa, which was similar for the seven cultivars (Fig. 2). Under the SDI treatment, the SWP pattern exhibited a similar trend in all cultivars. SWP decreased during the first two months reaching minimum values in December (SWP ranged from −2.8 to −3.7 MPa) then SWP increased fluctuating between values of −1.1 and −2.3 MPa. Among cultivars, 'Arbequina' and 'Nevadillo Blanco' showed a significantly lower average SWP over the season than 'Arauco', 'Morchiaio' and 'Villalonga', while 'Selección' and 'Changlot' showed intermediate values. In all cultivars, average midday SWP under SDI was significantly lower than under CI (Fig. 2A).

Under CDI, SWP dropped sharply during drought periods and tended to recover to the level of the control well-irrigated plants, and this was similar in all cultivars. During the drought period, SWP varied from −2.0 to −3.0 MPa in the two first drought cycles and from −4.0 to −6.0 MPa in the last three cycles, with minimum values of ∼−8 MPa in 'Arauco' and 'Changlot'. Cultivars 'Changlot' and 'Arbequina' showed a significantly lower average SWP over the season than 'Villalonga', while the other cultivars showed intermediate values. In all cultivars, the average midday SWP under CDI decreased significantly compared to SDI and CI (Fig. 2A).

3.2. Stomatal conductance

In Fig. 3 stomatal conductance patterns under SDI and CDI are shown as proportions of values reached in CI, which was assigned a value of 1 in each cultivar and measurement date. Under SDI, relative gs pattern was similar to control irrigation at the beginning of the experiment; afterwards, relative gs declined and remained 40% to 50% lower than CI until February-March when stomatal conductance increased to the level of CI, similar in all cultivars. Under CDI, differential recovery levels of relative gs with intermittent cycles of drought-rewatering among cultivars were observed: (1) relative gs increased to the level of CI in the five cycles of drought re-watering for 'Morchiaio'; (2) relative gs increased to the level of CI, or occasionally higher than CI, when pots were re-watered in four cycles for 'Arauco', 'Nevadillo Blanco' and 'Changlot'; (3)

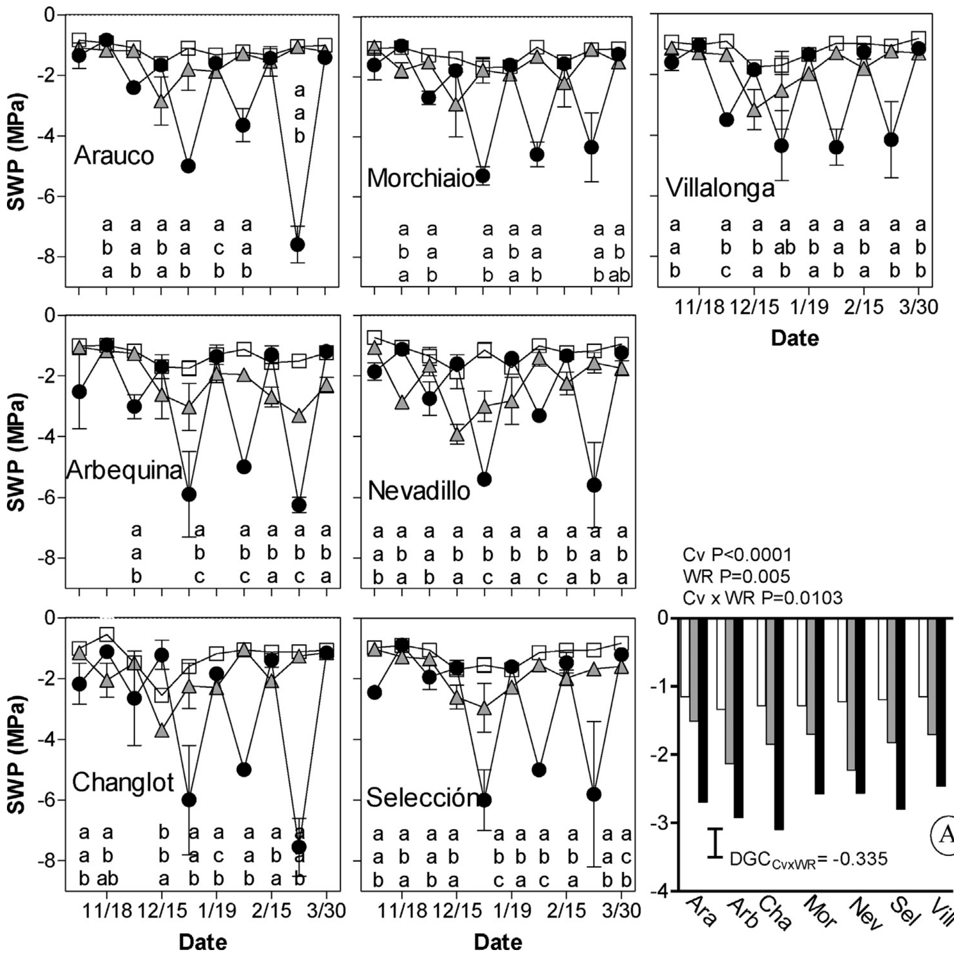

Fig. 2. Midday stem water potential (SWP) of seven olive cultivars subjected to three irrigation regimes (control: empty symbols, sustained deficit irrigation: gray symbols, and cyclic deficit irrigation: black symbols). Each symbol represents the mean of four measurements. Error bars are two standard errors of the mean. Values with the same letter are not significantly different among water regimes for each measurement data by DGC's test at $P \leq 0.05$, only presented when ANOVA indicated a significant effect. For comparative purposes, low panel (A) shows average SWP during the experimental period for each cultivar (Cv) and water regime (WR), where the vertical bar indicates DGC ($P < 0.05$) for comparison between cultivars and water regime combinations.

relative gs increased to the level of CI in three cycles for 'Selección Mendoza' and 'Villalonga'; and (4) relative gs was similar to CI after rewatering in two cycles for 'Arbequina'.

Average gs in the experiment revealed a significant interaction between cultivars and irrigation regime ($P = 0.001$, Fig. 3A). Average gs under well-irrigated conditions was highest in 'Arbequina' (451 mmol m^{-2} s^{-1}), significantly higher than for the rest of cultivars evaluated (average 362 mmol m^{-2} s^{-1}). Under SDI, average gs decreased significantly than CI in all cultivars, except in 'Arauco'. In this water regime, average gs was highest in 'Arbequina', 'Nevadillo Blanco', 'Arauco' and 'Villalonga' cultivars (362 mmol m^{-2} s^{-1}), significantly higher than 'Selección Mendoza' and 'Morchiaio' (230 mmol m^{-2} s^{-1}), while 'Changlot' had the lowest average gs (129 mmol m^{-2} s^{-1}). Under CDI, average gs was significantly lower than CI in all cultivars and SDI in all cultivars, except 'Morchiaio'. Under CDI, average gs was highest in 'Nevadillo Blanco' and 'Morchiaio' (224 mmol m^{-2} s^{-1}) followed by 'Arbequina' and 'Selección Mendoza' (154 mmol m^{-2} s^{-1}), significantly higher than 'Villalonga' and 'Arauco' (average gs 139 mmol m^{-2} s^{-1}), while 'Changlot' (46 mmol m^{-2} s^{-1}) showed a lower average gs value than the rest of the cultivars (Fig. 3A).

3.3. *Vegetative growth and leaf morphological characteristics*

Shoot growth and increase in trunk circumference did not respond to the interaction between cultivars and water regime; hence, the effects of each factor were studied individually (Table 1). 'Villalonga', 'Nevadillo Blanco', 'Morchiaio', 'Changlot', 'Arauco' and 'Selección Mendoza' showed significantly higher shoot growth over the experimental period than 'Arbequina'. Total increment of trunk circumference showed a similar pattern among cultivars, although the differences were more evident. 'Villalonga', 'Changlot', 'Morchiaio' and 'Arauco' cultivars had a significantly higher increase in trunk circumference; 'Nevadillo Blanco' and 'Selección' had an intermediate trunk increase, and 'Arbequina' had the smallest increase in trunk circumference of all cultivars. Plants under SDI and CDI showed similar shoot length, but significantly lower than CI. Shoot length under SDI and CDI was reduced by 45% and 55% respectively, compared to CI. The increase in trunk circumference was significantly reduced under SDI (28%) and CDI (50%) than CI, while plants under CDI showed remarkably lower trunk circumference increase than SDI by 31%.

The interaction between cultivars and water regimes was not statistically significant for plant leaf area, leaf area, SLA and SCMR (Table 1); therefore, the effects of each factor are discussed separately. Across water regimes, leaf area and plant leaf area varied among cultivars, while SLA did not vary. Leaf area was significantly higher in 'Villalonga' than in the rest of cultivars. 'Arauco' and 'Selección Mendoza' showed higher leaf area than 'Changlot', 'Nevadillo Blanco', 'Morchiaio' and 'Arbequina' cultivars. Plant leaf area was highest in 'Selección Mendoza', 'Villalonga', 'Nevadillo Blanco', 'Arauco', 'Changlot' and 'Morchiaio' cultivars, while 'Arbe-

Table 1
Shoot elongation, plant leaf area and total increment of trunk circumference (ITC) from September 2015 to April 2016 and inflorescence density on return bloom (October 2016) as affected by olive cultivar and water regimes (control irrigation CI, sustained deficit irrigation, SDI and cyclic deficit irrigation, CDI). [a]SPAD chlorophyll meter reading (SCMR).

Source of variation		Shoot growth (cm)	ITC (cm)	Leaf area (cm^2)	Plant leaf area (cm^2 $plant^{-1}$)	Specific leaf area (m^2 kg^{-1})	SCMR[a]	Inflorescence density (#cm^{-1})
Cultivars	'Arauco'	45.25 a	4.25 a	5.18 b	1239.2 a	3.95	74.79 a	0.16 b
	'Arbequina'	18.84 b	2.29 c	3.34 c	722.3 b	4.00	58.03 b	0.48 a
	'Changlot'	47.17 a	4.65 a	4.12 c	1201.3 a	3.61	74.86 a	0.01 c
	'Morchiaio'	51.40 a	4.35 a	3.66 c	1082.2 a	3.75	65.15 b	0.24 b
	'Nevadillo Blanco'	55.90 a	3.61 b	3.63 c	1316.4 a	3.70	78.46 a	0.31 b
	'Selección Mendoza'	36.26 a	3.35 b	4.88 b	1492.6 a	4.05	61.74 b	0.47 a
	'Villalonga'	59.07 a	4.69 a	5.97 a	1421.1 a	3.65	71.59 a	0.02 c
Water regime	CI	65.90 a	5.27 a	4.68 a	1530.6 a	4.29 a	54.20 c	0.13 b
	SDI	36.32 b	3.77 b	4.72 a	1118.2 b	3.73 b	67.49 b	0.30 a
	CDI	32.30 b	2.61 c	3.79 b	983.5 b	3.43 b	85.16 a	0.30 a
P-value	Cultivar	<0.0001	<0.0001	<0.0001	0.0001	0.598	<0.0001	<0.0001
	Water Regime	0.008	<0.0001	<0.0001	0.0045	0.0001	<0.0001	0.0003
	Cv x WR	0.323	0.091	0.847	0.1582	0.628	0.1256	0.193

Values with the same letter within each column are not significantly different by DGC's test at $P \leq 0.05$, only presented when ANOVA indicated significant effect.

quina' showed the lowest values. Across cultivars, plant leaf area was reduced by both SDI and CDI treatments by 27% and 36% in comparison to CI. Leaf area expansion was reduced by 20% in plants under CDI with respect to CI, while SDI regime did not affect leaf area in comparison with CI. Similarly, specific leaf area (SLA) was reduced by 25% in CDI over CI. SLA was similar between plants under SDI and CI treatments. Average SCMR values were highest in 'Changlot', 'Arauco', 'Villalonga' and 'Nevadillo Blanco' cultivars, while 'Morchiaio', 'Arbequina' and 'Selección Mendoza' showed the lowest values. SCMR was 1.3 and 1.6 times greater under SDI and CDI than under CI, respectively, while plants under CDI showed 1.3 times greater SCMR than under SDI.

Amongst the well-irrigated and water-deficit regime plants, the increase in specific leaf area was positive and linearly related to average stem water potential ($R^2 = 0.60$; $P < 0.001$, Fig. 4A), stomatal conductance ($R^2 = 0.44$; $P < 0.01$, Fig. 4B), and seasonal increase in trunk circumference ($R^2 = 0.31$; $P < 0.05$, Fig. 4C). In contrast, stem water potential ($R^2 = 0.59$; $P < 0.001$, Fig. 4D), stomatal conductance ($R^2 = 0.52$; $P < 0.001$, Fig. 4E), and increase in trunk circumference ($R^2 = 0.25$; $P = 0.02$, Fig. 4F) decreased linearly with the increase in SCMR. SLA and SCMR were closely and negatively related ($R^2 = 0.62$; $P < 0.001$) (Fig. 4G). 'Arbequina' showed a particular response, i.e. a narrow variation in SLA and SCMR and a marked variation in SWP (Fig. 4A and D), gs (Fig. 4B and E) and trunk circumference increase (Fig. 4C and F) as a result of contrasting water regimes.

3.4. Inflorescence density

The number of inflorescences per centimeter of shoot chosen during the experimental season water regime and measured during the following season did not respond to the interaction between cultivars and water regime (Table 1). Inflorescence density was significantly different among cultivars, 'Arbequina' and 'Selección Mendoza' showed the highest inflorescence density (0.47 inflorescences on average per cm of shoot), followed by 'Morchiaio', 'Nevadillo Blanco' and 'Arauco' (0.24 inflorescences per cm of shoot), while the lowest inflorescence density was observed in 'Villalonga' and 'Changlot' (0.01 inflorescences on average per cm of shoot). Both water deficit regimes, SDI and CDI, had 2.3 times greater inflorescence density than well-irrigated plants. Inflorescence density was negative and slightly related to the node number ($R^2 = 0.44$; $P = 0.006$) formed in the previous season when water treatments were applied (Fig. 5).

4. Discussion

4.1. Stem water potential and stomatal conductance

The pattern and average values of midday SWP, an indicator of plant water status widely used in olive plants (Trentacoste et al., 2015), were similar for the seven well-irrigated cultivars, confirming the low genetic variability of SWP under well-watered conditions previously observed in olive cultivars by Tugendhaft et al. (2016). The exposure to water deficit regimes, however, led to a differential response of midday SWP among cultivars, similar to those reported in previous studies (Bacelar et al., 2006; Boughalleb and Hajlaoui, 2011; Lo Bianco and Scalisi, 2017). In these studies, olive cultivars were mainly compared under various levels of sustained deficit irrigation, but not under multiples cycles of drought with intermittent recovery during the growth season (Abdallah et al., 2017). Here, the amount of water applied in both SDI and CDI regimes was similar; however, in terms of average SWP, the cultivars under CDI regimes were exposed to higher deficit conditions than those under SDI. All cultivars reaching average minimum values of SWP within a range of −4 to −6 MPa and a rapid recovery

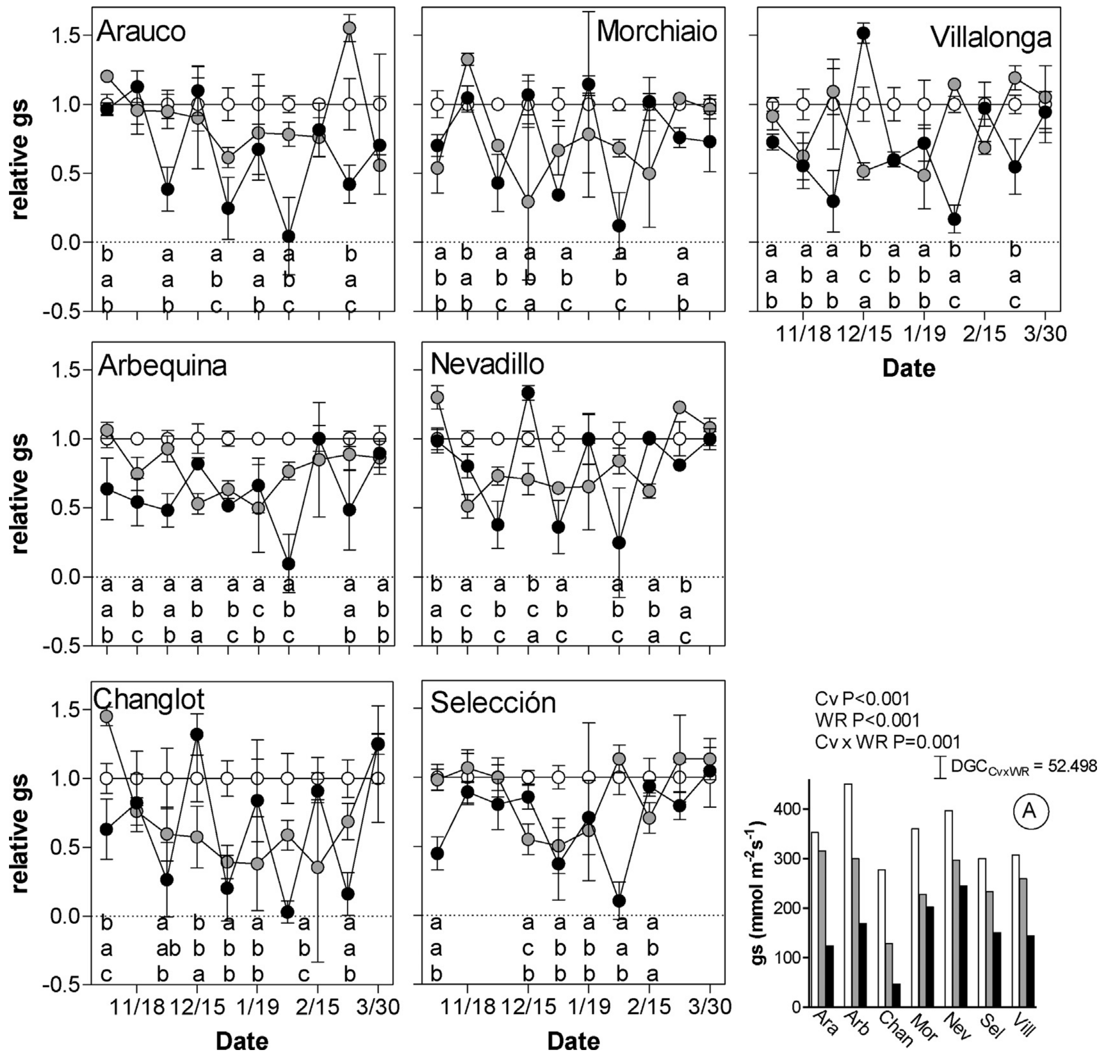

Fig. 3. Abaxial stomatal conductance (gs) of seven olive cultivars under sustained deficit irrigation (gray symbols) and cyclic deficit irrigation (black symbols) relative to well-irrigated plants (empty symbols). Each symbol represents the mean of four measurements. Error bars are two standard errors of the mean. Values with the same letter are not significantly different among water regimes for each measurement data by DGC's test at $P \leq 0.05$, only presented when ANOVA indicated a significant effect. For comparative purposes, low panel (A) shows average gs during the experimental period for each cultivar (Cv) and water regime (WR), where the vertical bar indicates DGC ($P < 0.05$) for comparison between cultivars and water regime combinations.

of water status after resumption of full irrigation at values similar to control plants. In olive trees have been reported water potentials as low as −8 MPa, keeping a rapid recovery capacity after rehydration (Boughalleb and Hajlaoui, 2011; Giorio et al., 1999; Guerfel et al., 2009). Similarly, Ennajeh et al. (2008) observed that a water potential of around −6 MPa was not enough to generate a significant reduction of hydraulic conductivity in olive cultivars.

Among cultivars, during SDI and CDI regimes, 'Villalonga' maintained the highest average SWP, and 'Arbequina' and 'Nevadillo Blanco' cultivars showed the lowest average SWP under SDI, while 'Arbequina' and 'Changlot' showed the lowest SWP under CD Bacelar et al. (2009) compared five adult olive cultivars, including 'Arbequina' under field experimental conditions with low water availability. The authors found that 'Arbequina' had the lowest predawn and midday SWP compared to the rest of cultivars, associated with the low cell wall elasticity of 'Arbequina' leaves.

Average abaxial stomatal conductance ranged between 50 and 400 mmol m^2 s^{-1}, higher than those previously reported in an experiment conducted on 'Arbequina' in Mendoza field conditions (Trentacoste et al., 2011). This may be due to the fact that the current experiment was conducted under plastic netting inside a tunnel structure, where plants were exposed to higher relative humidity and lower wind speed (i.e. low deficit pressure vapor) which is associated to an increase in stomatal conductance, similar to that observed by Tugendhaft et al. (2016).

Cultivars differences in stomatal conductance were observed under well-irrigated conditions, where 'Arbequina' tended to have higher gs than the rest of cultivars. The reduction of the plant water status under SDI induced a non-significant decrease in stomatal conductance in 'Arauco', in contrast to a significant reduction of gs in the rest of cultivars. Under CDI, regardless the cultivar, the full recovery of stem water potential was probably due to the partial recovery of stomatal conductance, previously descripted in olive trees by Xiloyannis et al. (1997). The slow recovery of gs after the restoration of plant water status is not yet clear in olive trees (Torres-Ruiz et al., 2015). In this study, some level of genetic variation in gas exchange recovery to water deficit suggests an intraspecific variability of stomatal response to drought (Chartzoulakis et al., 1999; Tognetti et al., 2002). 'Arauco' and 'Villalonga' under SDI, and 'Nevadillo Blanco' under CDI, had a lower grade of gs variation compared to the control regime, and a larger capacity for recovering stomatal conductance. 'Changlot' showed reduced SWP and gs under CDI, with a marked ability to recover plant water status rapidly. In comparison, 'Arbequina' maintained

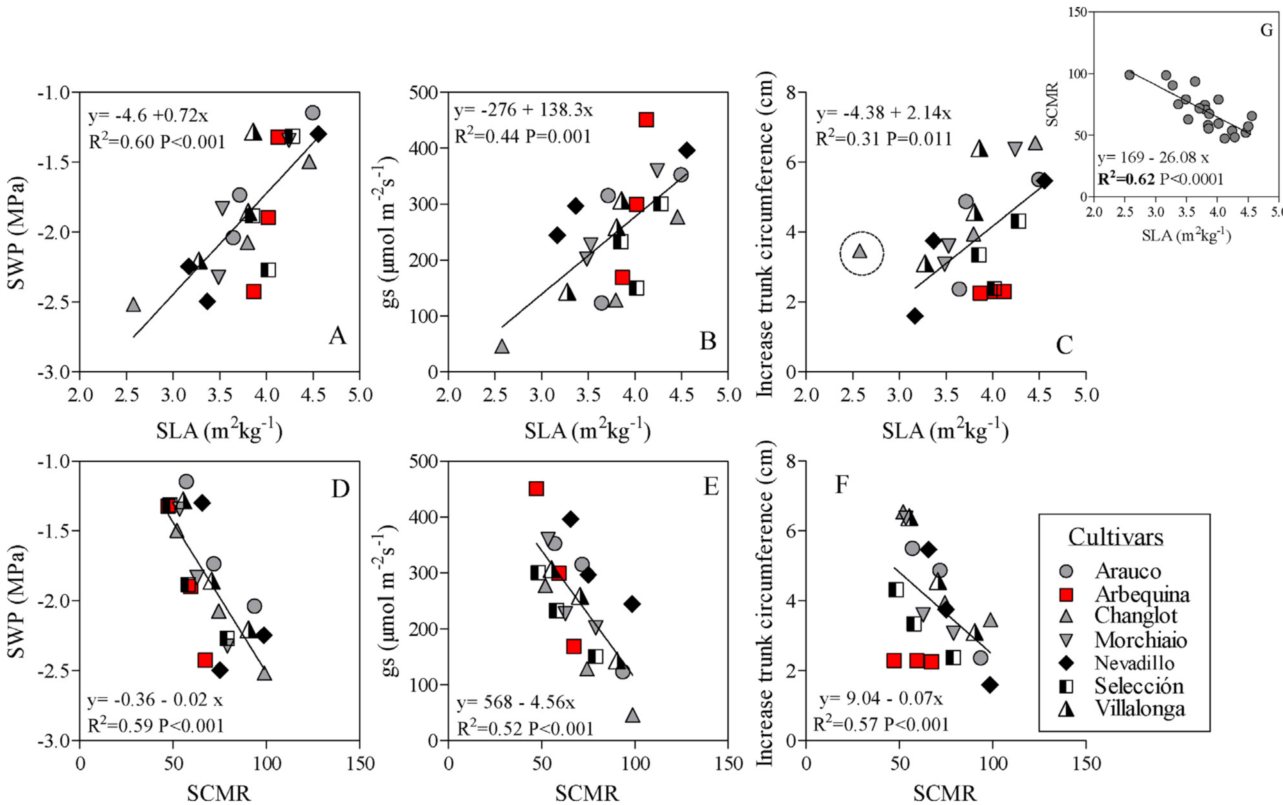

Fig. 4. Relationships between specific leaf area (SLA) in upper panels and SPAD chlorophyll meter reading (SCMR) in lower panels with average midday stem water potential (SWP) (A and D); average abaxial stomata conductance (gs) (B and E) and increase in trunk circumference (C and F) of seven olive cultivars subjected to control irrigation, sustained deficit irrigation, and cyclic deficit irrigation. The inset panel (G) shows the relationship between SCMR and SLA, pooling data from seven cultivars and three water regimes.

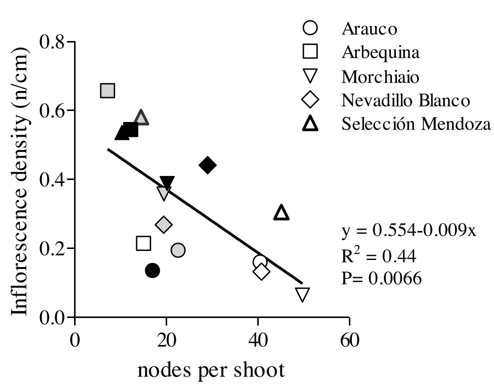

Fig. 5. Relationship between number of nodes per shoot developed during the 2015–2016 season and inflorescence density on return bloom (2016–2017 season) in five olive cultivars under well irrigation (empty circles), sustained deficit irrigation (gray circles) and cyclic deficit irrigation (black circles). 'Changlot' and 'Villalonga' cultivars were not considered because of very low inflorescence number during the experimental period.

higher gs under both well-watered and water deficit regimes, leading to the lowest values of SWP, a high grade of gs depressed in SDI and CDI, and a lower ability to gs recovery after irrigation under CDI. Thus, 'Arauco', 'Villalonga', 'Nevadillo Blanco' and 'Changlot' cultivars appear to employ a conservative strategy in water use, in contrast to 'Arbequina' which uses a non-conservative strategy. This coincides with the findings of Torres-Ruiz et al. (2015) in 'Arbequina' who reported a complete and rapid recovery of water potential but not of stomatal conductance in plants exposed to cycles of water deficit followed by water recovery.

4.2. Vegetative growth and leaf morphology

It is well established in olive plants that vegetative growth depends on the amount of water used. All cultivars reduced vegetative growth when irrigation was restricted. Trunk growth was more sensitive than shoot growth, as found in previous works (Livellara et al., 2011); thus, trunk growth has been proposed as a good estimator of olive plant water status (Fernández et al., 2011). Across water regimes, 'Villalonga', 'Changlot', 'Arauco', and 'Morchiaio' cultivars showed both higher trunk and shoot growth, in contrast with the lower vigor of 'Arbequina'.

Total leaf area was sharply reduced in all cultivars in both restrictive water regimes with respect to well-watered plants. Lower plant leaf area in SDI was mainly explained by a combination of lower leaf production over the growing season and/or the shedding of older leaves. Under CDI, reduced leaf plant area was a result of the loss of leaves, lower leaf production, and additionally, a reduced expansion of younger leaves (Table 1). The decrease in total leaf area and consequently of whole plant transpiration has been described in olive trees as common drought response (Connor 2005). Comparing cultivars, 'Arbequina' presented the smallest area per leaf and whole-plant, which could prevent even more marked plant water status decrease in water deficit conditions, similar to those observed by Bacelar et al. (2004). In contrast, 'Villalonga' showed both greatest area per leaf and plant also had higher vegetative growth (Table 1). These traits could confer greater susceptibility to drought (Bacelar et al., 2004).

4.3. Inflorescence density

A single relationship between inflorescence density and node number per shoot (Fig. 5) showed that low vigor cultivars or water deficit promoted early-bearing (Grattan et al., 2006). 'Arbequina' was the most precocious cultivar while 'Changlot' and 'Villalonga' were the most delayed. In addition, our results suggest that cultivar with non-reproductive development changes such as 'Changlot' provided fitness benefits in dry environments. Possible explanations for this apparent behavior need further research in adult trees, but they could be related to lower stomatal conductance, with the subsequent improvement in efficient water use observed in 'Changlot' during reproductive bud formation, in contrast to 'Arbequina'.

4.4. Relationships between SLA and SCMR with physiological plant traits

Analysis of variance confirmed that (1) specific leaf area was the most stable trait among cultivars growing under broad water regime conditions (Ennajeh et al., 2010; Guerfel et al., 2009) and that (2) there was a significant decrease of SLA in response to the shortage of water applied. Previously, it had been widely observed in olive plants that SLA is reduced under drought conditions, which provides fitness benefits in dry environments (Bacelar et al., 2006; Ennajeh et al., 2010; Guerfel et al., 2009). SCMR was influenced by genotype and proved to be more responsive to plant water status than SLA. Across cultivars, SCMR increased significantly from CI to SDI and CDI, consistent with previous studies on peanuts (Nigam and Aruna, 2008).

This is the first quantitative report in olive trees to demonstrate a single negative and close correlation between SLA and SCMR using a contrasting range of cultivars and water regimes (Fig. 4G). In previous studies in grain crops, this close relationship between SLA and SCMR was reported under different water regimes (Nigam and Aruna, 2008; Upadhyaya, 2005), where both parameters were used in selecting genotypes for more drought prone environments. SCMR had a closer association than SLA with plant water status and vegetative growth traits such as midday SWP, stomatal conductance and trunk growth, whereas SCMR had higher genotype variability compared with SLA. Thus, SCMR could be a valuable tool for screening of large number of olive genotypes under contrasting environments.

'Changlot', 'Arauco' and 'Nevadillo Blanco' cultivars maintained lower SLA and higher SCMR under drought conditions, and consequently, should have greater water use efficiency in drought prone environments (Puangbut et al., 2009). 'Arbequina' exhibited more stability in SLA, SCMR, and vegetative growth traits to tackle reduction in water availability. This could be evidence of a low plastic response (Caruso et al., 2006; Nicotra and Davidson, 2010), similar to Bacelar et al. (2009) who found that 'Arbequina' showed a low capacity to acclimate to drought conditions.

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