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ORIGINAL ARTICLE

Water relations and carbohydrate partitioning of four greenhouse-grown olive genotypes under long-term drought

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Abstract
Key message **The four olive genotypes exhibited different responses to drought involving leaf drop and g_s reductions (broad-leaved genotypes, good leaf hydration); or growth reductions (narrow-leaved genotypes, dehydration tolerance). There was minor effect on carbohydrate levels.**
Abstract Olive plants of broad-leaved 'Minuta' (MN) and 'Nocellara del Belice' (NB) and narrow-leaved 'Passulunara' (PA) and 'Biancolilla Siracusana' (BS) were studied to evaluate their responses to drought. In a greenhouse, 2-year-old rooted cuttings were irrigated to field capacity (WW) or with 20% of WW evapotranspiration (DS) for over 3 months. Subsequently, all pots were rewatered to field capacity for 20 days. Gravimetric soil water content (SWC), leaf relative water content (RWC), stomatal conductance (g_s), leaf carbohydrates, percentage of leaf drop and shoot elongation were determined throughout the trial. In WW, SWC fluctuated between 80 and 100% of field capacity, whereas in DS, SWC decreased sharply reaching a minimum level around 30–35% of field capacity after 2 months of drought. At this time, drought induced a significant reduction of: (a) RWC in PA and BS, (b) g_s in MN, NB, and PA, and (c) shoot elongation (−23%) in PA. Conversely, drought increased leaf drop in all genotypes, especially in MN and NB. RWC and g_s levels were mostly restored after rewatering. Initially, drought induced an increase of mannitol and total carbohydrates in MN and a decrease in NB. At more advanced drought stages, mannitol and total carbohydrates decreased in PA and BS. NB exhibited a general increase of the (mannitol + glucose)/sucrose ratio in response to drought. The two broad-leaved genotypes (MN and NB) maintained similar leaf hydration levels in DS and WW plants proving to be generally intolerant to dehydration, whereas the two narrow-leaved genotypes (PA and BS) tolerated a fair degree of dehydration.

Keywords Leaf abscission · Mannitol · Glucose · Relative water content · Stomatal conductance · Water deficit

Introduction

Olive (*Olea europaea* L.) is a species that grows in Mediterranean and semi-arid regions where it commonly faces high temperatures and irradiation along with long periods of water deficit. In those regions, most of the olive oil is produced from trees typically grown under rainfed conditions, and their growth and yields largely depend on the resistance to environmental stress (Connor and Fereres 2005; Moriana et al. 2003).

During stress periods, olive is able to reduce water content and potential in leaves and roots, stopping growth but maintaining some photosynthesis and carbohydrate accumulation (Dichio et al. 2003; Xiloyannis et al. 1999). This has been mainly attributed to the accumulation of compatible solutes (osmotic adjustment). In particular, mannitol (along with glucose and malic acid) seems to contribute to osmotic adjustment under water deficit (Xiloyannis et al. 1999) and salt stress (Gucci et al. 1998), and it increases in response to low temperatures in vitro

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(Rejšková et al. 2007), whereas sorbitol plays a similar role in apple (Wang and Stutte 1992), cherry (Ranney et al. 1991), and peach (Lo Bianco et al. 2000).

Sorbitol and mannitol are polyols, or sugar alcohols, and are widely distributed in the plant kingdom (Bieleski 1982). Specifically, mannitol comprises a significant portion of the soluble carbohydrate in species of the Oleaceae, Apiaceae, and Rubiaceae families (Barker 1955; Bieleski 1982; Zimmermann and Ziegler 1975). It is synthesized in mature leaves after reduction of mannose-6-phosphate by an NADPH-dependent mannose-6-phosphate reductase followed by dephosphorylation by a mannitol-6-phosphate phosphatase. Once synthesized, mannitol may be accumulated in source tissues or transported to sink organs (Conde et al. 2007) where it is oxidized to mannose by an NAD-dependent mannitol dehydrogenase (Noiraud et al. 2001; Stoop et al. 1996).

It has also been suggested that mannitol and other polyols are strong water-structure formers acting as effective stabilizing/protecting agents at both molecular and whole-cell level (Galinski 1993). Furthermore, polyols may function as scavengers of reactive oxygen species and represent a non-enzymatic mechanism to protect cells from oxidative stress (Smirnoff and Cumbes 1989). In transgenic plants exposed to salt or water stress, mannitol induces better survival and/or performance compared to wild types (Abebe et al. 2003; Macaluso et al. 2007; Tarczynski et al. 1993), and this does not seem to be attributable to osmotic protection by mannitol (Abebe et al. 2003; Karakas et al. 1997) but rather to a more specific radical scavenging mechanism (Shen et al. 1997). A similar mechanism has been proposed in olive under oxidative stress induced by paraquat applications (Lo Bianco et al. 2011).

The identification and use of drought-tolerant olive genotypes would allow for minimization of yield reductions due to environmental stress along with maximization of profits and health benefits. Despite the amount of work classifying olive as a drought-tolerant species, a relatively high number of cultivars and genotypes have been identified worldwide (Bartolini et al. 1998) and even in Sicily (La Mantia et al. 2005), which may be different for their degree of stress tolerance. In particular, a number of Sicilian olive genotypes have been identified for their ability to accumulate high quantities of mannitol and other carbohydrates along with leaf traits indicative of drought tolerance (Lo Bianco et al. 2013). More detailed work is now needed to test the physiological behavior of those genotypes indicated as potentially stress tolerant. In this study, we used four Sicilian olive genotypes, two with leaf traits indicative of drought tolerance and two with leaf traits indicative of drought sensitivity, to evaluate their water relations, growth and carbohydrate partitioning in response to drought.

Materials and methods

Plant material and greenhouse conditions

The experiment was conducted in a greenhouse located at the Department of Agricultural and Forest Sciences in Palermo, Sicily, and yielding approximately 60% integrated daily solar radiation transmission and temperatures ranging from 16 to 24 °C during the trial period. A total of 48 uniform olive rooted cuttings at the second leaf were grown in 2-L plastic pots. The potting mix included peat moss, compost, vermiculite, and perlite, and had 50% water content at field capacity (FC) and a pH of 7.0. Four different olive genotypes (12 plants each) were included in the trial, namely ‘Minuta’ (MN), ‘Nocellara del Belice’ (NB), ‘Passulunara’ (PA), and ‘Biancolilla Siracusana’ (BS). In a previous characterization, MN and NB were found to have relatively broad leaves and low leaf mannitol and glucose (traits indicative of low drought tolerance), whereas PA and BS were found to have relatively narrow leaves and high leaf mannitol and glucose (traits indicative of high drought tolerance) (Lo Bianco et al. 2013).

Drought treatments and recovery

The experiment was carried out from November 5, 2010 to April 22, 2011 because under the climate of Sicily, cooler months provide a good opportunity to impose gradual drought stress inside a greenhouse and trigger plant metabolic responses. Two water regimes were imposed. One half of the plants were watered to FC (WW) two or three times per week depending on their evapotranspiration (ET). Before and after irrigations, plants from the WW group were weighed to the nearest gram to determine the average daily ET rate. On the same days, water was supplied to the other half of the plants (DS) at rates of 20% of WW ET. In the DS group, water was supplied to plastic saucers beneath the pots to assure that water reached growing roots. All plants were supplied with soluble fertilizer in the irrigation water during and following the drought period. Plants were arranged in a completely randomized design with six replicate plants for each genotype and irrigation treatment.

Starting on February 27, 2011, drought was suspended and all plants (WW and DS) were regularly watered to FC to study the ability of DS plants to recover. Soil water content (SWC) was estimated gravimetrically throughout the trial period and expressed as percentage of FC.

Plant water status

Measurements of leaf relative water content (RWC) were carried out to estimate plant water status following the

method of Barrs and Weatherley (1962). On November 22, January 13, and March 1, one leaf per plant was wrapped in parafilm and aluminum foil, collected, and transported to a nearby laboratory for determination of fresh weight (FW) to the nearest milligram. Leaf samples were placed in glass tubes with deionized water and, after 24 h at 10 °C, their weight at full turgor (TW) was recorded. The rehydration procedure (standing leaf) was chosen according to Arndt et al. (2015); rehydration time (24 h) was selected based on preliminary tests. Finally, the leaf samples were oven-dried at 60 °C until constant weight (DW). RWC was calculated as (FW − DW)/(TW − DW) × 100. To establish a relationship between RWC and stem water potential (WP_{stem}), 24 MN leaves and 24 BS leaves from plants outside the trial were collected during the experiment, and RWC and WP_{stem} were measured simultaneously in each leaf. On each date, leaves were covered with parafilm and aluminum foil, equilibrated with the rest of the plant for about 1 h, WP_{stem} was measured with a pressure chamber, and FW, TW, and DW were determined as described above to calculate RWC. On four leaves of each genotype, WP_{stem} was also measured after full rehydration.

On November 10 and 25, January 13, March 1, and April 22, stomatal conductance (g_s) was measured in one leaf per plant with an AP4 Delta-T leaf dynamic porometer (Delta-T Devices, Cambridge, UK). Air temperature and solar radiation were also recorded at the time of measurements. Shoot growth was estimated by measuring elongation of three newly formed shoots per plant during the trial period. At the end of the drought period, the percentage of leaf drop was estimated on three shoots per plant by counting dropped leaves and leaves still present in each sampled shoot.

Soluble carbohydrates

Leaves from the same shoots and next to the ones used for RWC measurements were sampled, transferred to the laboratory, and stored at −40 °C for subsequent determination of soluble carbohydrates. Extraction was carried out using about 0.3 g of leaf blade, cut into small pieces, transferred into a 1.5-mL Eppendorf tubes, and finely ground with a V-shaped pestle in the presence of liquid nitrogen. Ground dry tissues were weighed and extracted with 1 mL of 80% (v:v) methanol solution. The homogenate was vortexed for 1 min and centrifuged for 5 min at 3000*g*. The supernatant was stored at −40 °C for subsequent quantification of glucose, fructose, sucrose, mannitol, and galactinol by liquid chromatography/mass spectrometry (LC/MS) using Thermo TSQ Quantum Access equipment and a Hypercarb PGC (100 × 2.1 mm, 5 µm) column (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). Sample preparation and LC/MS quantification were performed by the Centro Grandi Apparecchiature (UniNetLab), University of Palermo. Calibration curves were constructed with separate standards using reagents from Sigma Aldrich (St. Louis, Missouri, USA). Carbohydrate contents were expressed in milligrams per gram of DW.

All measurements and leaf samples were taken between 10:00 and 12:00 HR.

Statistical data analysis

Data were analyzed by analysis of variance using Systat procedures (Systat Software Inc., Richmond, CA, USA). When appropriate, means were compared using Fisher's least significant difference. Linear regression analysis was used to establish relationships between RWC and WP_{stem}. The slopes of the two regressions were compared running a *t* test on coefficients and standard errors from the regression analysis.

Results and discussion

During the 6 months of trial, the climatic conditions were typical of a non-heated greenhouse in Sicily during late fall and winter. At mid to late morning, vapor pressure deficit (VPD) ranged between 0.7 and 1.8 kPa, whereas photosynthetic photon flux density (PPFD) was between 300 and 850 µmol m^{-2} s^{-1} (Fig. 1).

During the drought period, SWC exhibited very similar trends in all four genotypes, while some differences were found after rewatering (Fig. 2). As expected, SWC of DS pots decreased sharply in the first two months of trial reaching a constant minimum level around 35% of FC in January and February; only PA pots reached a slightly lower minimum SWC (around 30% of FC) than pots of other genotypes (Fig. 2). On the contrary, fluctuations of SWC in WW pots were generally contained between 80

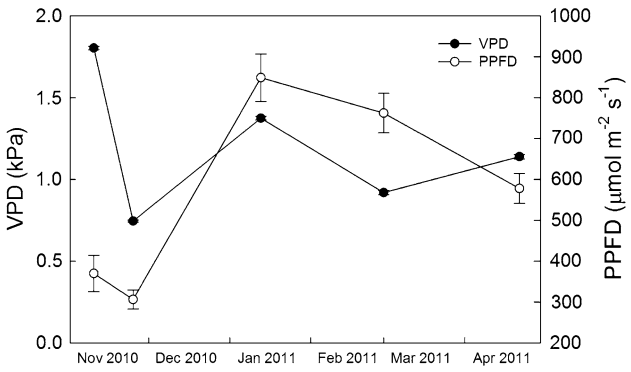

Fig. 1 Vapor pressure deficit (VPD) and photosynthetic photon flux density (PPFD) measured throughout the experimental trial inside the greenhouse. *Error bars* indicate standard errors of means

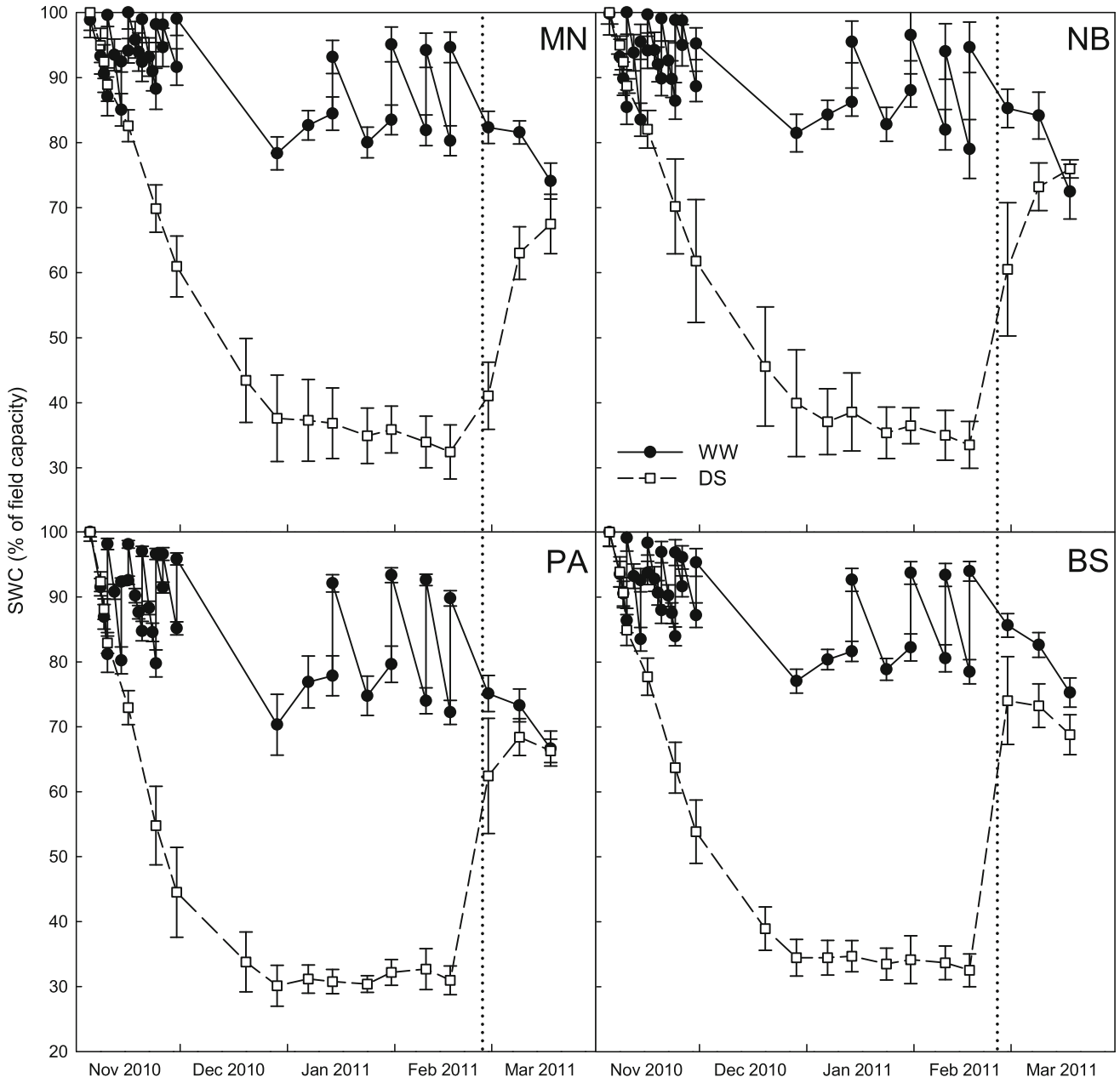

Fig. 2 Soil water content (SWC) in well-watered (WW) and drought-stressed (DS) pots of 2-year-old 'Minuta' (MN), 'Nocellara del Belice' (NB), 'Passulunara' (PA), and 'Biancolilla Siracusana' (BS) olive plants during drought and after rewatering. The *dotted vertical lines* indicate rewatering. *Error bars* indicate standard errors of means

and 100% of FC; also in this case, PA pots exhibited a lower minimum SWC (around 70% of FC) than pots of other genotypes. During the 20 days of rewatering, SWC of DS pots increased to the level of WW pots in all genotypes, but with different speeds and trends. In particular, DS pots of MN, and partly of NB, did not respond immediately to rewatering, and the SWC level of WW pots was reached only after 20 days; DS pots of PA responded promptly to rewatering and soon reached SWC levels of WW pots (Fig. 2). On the other hand, DS pots of BS responded quickly to rewatering, but never reached the exact level of WW pots. Those differences among genotypes suggest a more prompt recovery of transpiration and/or water uptake after rewatering in MN and NB than in PA and BS plants.

A direct linear relationship between RWC and WP_{stem} was found in leaves of both MN and BS (Fig. 3), indicating that RWC is indeed a good estimate of plant water status in olive. Similar linear relationships were found in young potted olive plants across a wide range of RWC and water potential (Dichio et al. 2006; Guerfel et al. 2009).

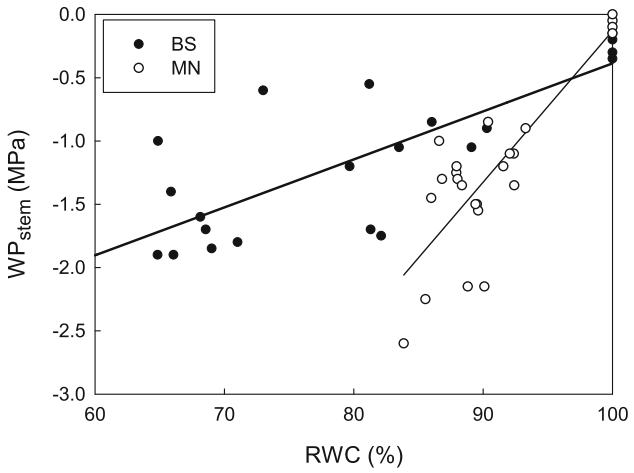

Fig. 3 Relationship between leaf relative water content (RWC) and stem water potential (WP_{stem}) in 2-year-old 'Minuta' (MN) and 'Biancolilla Siracusana' (BS) olive plants. In MN, $WP_{stem} = -12.1 + 0.120$ RWC, $R^2 = 0.703$, $P < 0.001$; in BS, $WP_{stem} = -4.18 + 0.038$ RWC, $R^2 = 0.646$, $P < 0.001$

Nevertheless, the slope of the regression line for MN was significantly greater ($P < 0.001$) than the slope for BS, suggesting that similar variations of RWC in different genotypes may correspond to different changes of WP_{stem}. This is easily explained by the fact that RWC measures leaf water deficit and takes into account any possible solute accumulation and osmotic adjustment in response to drought (Barrs 1968; Jones 2007). In our specific case, over the same WP_{stem} range, MN leaves were less dehydrated than BS leaves, suggesting that the former genotype may be able to counteract dehydration by some sort of solute accumulation (Fig. 3).

In mid-January, when SWC of DS pots reached the minimum levels, RWC of DS plants was lower than RWC of WW plants in PA and BS (Fig. 4). No significant difference of RWC between WW and DS plants was observed in MN and NB. Only in BS, RWC of DS did not recover immediately to the levels of WW after rewatering. In terms of water status, two types of responses were observed: a homeostatic response to drought, or tendency to maintain similar RWC, in broad-leaved MN and NB, and the ability to tolerate significant dehydration in the narrow-leaved PA and BS. Also, the effect of drought on RWC was observed very early in PA where plants tended to slowly rehydrate with time, and strongly delayed in BS where leaves were not able to recover quickly after rewatering (Fig. 4). In WW plants, RWC fluctuations over time were generally due to changes in light and VPD (Fig. 1). Also, leaves with RWC above 85% generally showed no sign of dehydration.

By mid-January (advanced drought), with increasing PPFD and VPD (Fig. 1), g_s was significantly lower in DS than in WW plants of all genotypes but BS, for which no drought effect was observed throughout the entire trial (Fig. 5). After rewatering, g_s of all DS plants generally tended to recover to the level of WW plants. Similar reductions of g_s in response to soil drying have been reported in field-grown olives (Giorio et al. 1999), where g_s seems to be controlled primarily by soil water content. The latter is also in agreement with our observations, where at least in three genotypes (except PA) there is no relationship between RWC and g_s, and SWC alone may be the main factor driving g_s changes. This suggests that a feed-forward mechanism could be invoked in the response of stomata to soil drying. A similar behavior was observed during late summer in field-grown olive trees (Giorio et al. 1999). If we consider leaf water status (Fig. 4), we can also assume that g_s reductions were at least in part responsible for avoiding dehydration in MN and NB leaves, whereas g_s reductions were not able to avoid dehydration in PA leaves.

The percentage of leaf drop was generally greater in MN and NB than in PA and BS (Fig. 6). Regardless of the genotype, drought stress also increased leaf drop by about 7%. Leaf drop is a typical response to long-term drought already documented in walnut (Tyree et al. 1993) and other species (Bargali and Tewari 2004; Chaves et al. 2009). In olive, it has been reported mainly in response to salinity (Bongi and Loreto 1989). Our results suggest that olive leaf drop under saline conditions may be, at least in part, a direct response to tissue dehydration rather than to ion toxicity, and a strategy of the species to limit dehydration by reducing the total transpiring surface. Leaf drop can be considered an effective strategy, in addition to g_s reduction, to avoid dehydration in MN and NB, but not in PA or BS.

A significant interaction between genotype and drought ($P = 0.047$) indicated that shoot growth responses to drought were genotype dependent. Under WW conditions, PA was the most vigorous and MN the least vigorous, while NB and BS showed similar intermediate vigor (Table 1). Drought did not induce any significant shoot length reduction in MN and BS, whereas it reduced the length of PA shoots by about 23% and that of NB shoots by 18% (non-significant). Similar shoot growth reductions were observed in field-grown olive trees under deficit irrigation (Iniesta et al. 2009) and in container-grown olive trees under water stress (Ben-Gal et al. 2010). Compared to leaf drop, reductions of shoot extension are of less adaptive value for drought stress tolerance. In other words, shoot growth reductions are generally regarded as passive responses to drought with negative impact on yields, especially in less vigorous genotypes. On the contrary, shoot growth reductions in vigorous olive genotypes, such as PA, may be beneficial for reducing pruning costs and could be used to attenuate alternate bearing.

As for carbohydrates, there was a significant interaction between genotype, date, and irrigation. Regardless of genotype, date or irrigation, mannitol was by far the most

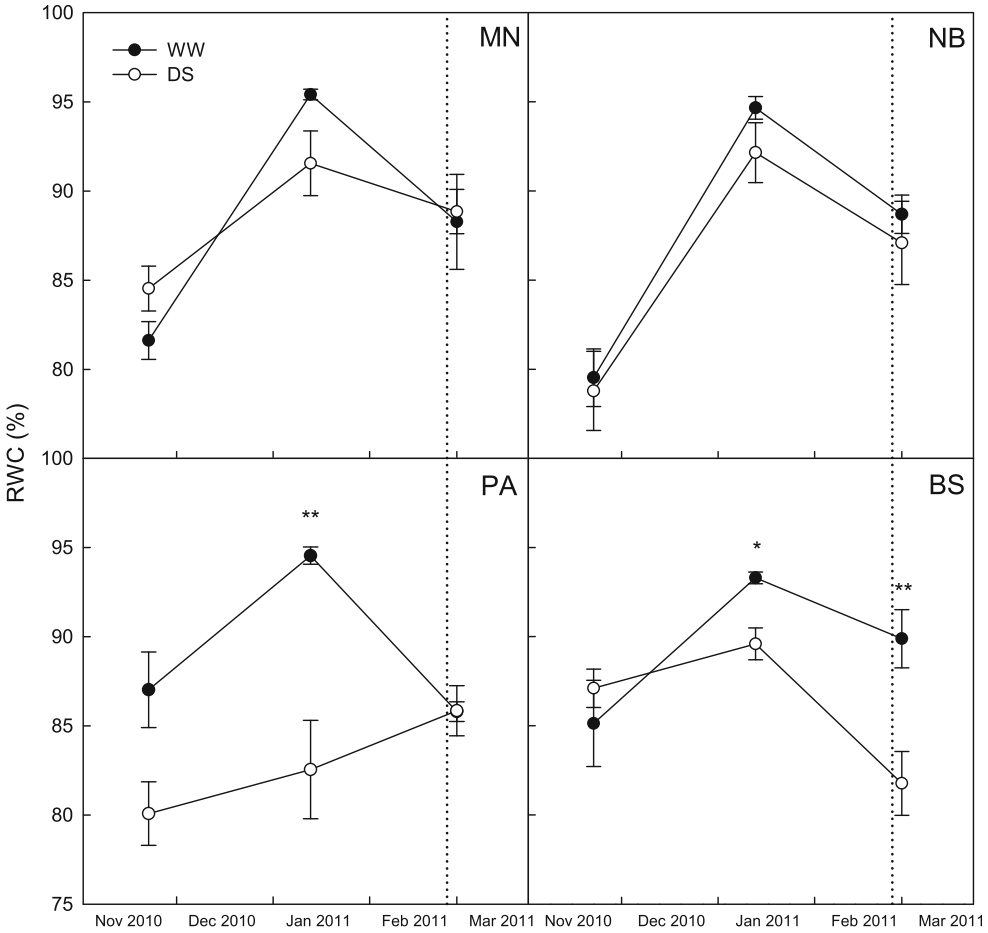

Fig. 4 Leaf relative water content (RWC) in well-watered (WW) and drought-stressed (DS) 2-year-old plants of 'Minuta' (MN), 'Nocellara del Belice' (NB), 'Passulunara' (PA), and 'Biancolilla Siracusana' (BS) olive during drought and after rewatering. The *vertical dotted lines* indicate rewatering. *Error bars* indicate standard errors of means. *Significantly different for $P < 0.05$; **significantly different for $P < 0.01$

abundant carbohydrate in olive leaves followed by glucose (Table 2). This is consistent with carbohydrate levels reported in previous studies conducted on Sicilian olive genotypes (Lo Bianco et al. 2013; Lo Bianco and Avellone 2014). Fructose was always present in relatively low amounts, while galactinol and sucrose reached glucose levels only in March, after rewatering. Generally, glucose, fructose, and mannitol showed a tendency to decrease from November to March, whereas galactinol and sucrose were relatively constant during drought and showed a marked increase after rewatering. As a result, total leaf carbohydrates decreased with time in MN and NB, while no evident change was observed between January and March in PA and BS.

The effect of drought on carbohydrate levels was sporadic and inconsistent among genotypes and dates (Table 2). In particular, drought induced a significant decrease of glucose, fructose, and sucrose in NB at the beginning of drought (November); a significant increase of glucose and sucrose in PA after rewatering (March); an increase of mannitol in MN at the beginning of drought, while a decrease of mannitol in PA and BS in January; a decrease of galactinol during advanced drought in NB and PA. In November, drought led to an increase of total carbohydrates in MN and a decrease in NB, whereas in January, it caused a decrease of total carbohydrates in PA and BS. Carbohydrate depletion under severe drought conditions (especially after several weeks of drought like in January) has often been reported in the literature and is consistent with a significant decrease in photosynthesis and assimilation rates (Chaves et al. 2009; Pinheiro et al. 2001). In this regard, PA and NB appear to be very sensitive genotypes followed by BS. Indeed, the reductions in g_s observed in DS plants of NB and PA (Fig. 5) suggest that carbohydrate decreases may be due to a substantial drop in assimilation rates. On the contrary, temporary increases (e.g., in November) in total carbohydrates, especially those that have been shown to play an osmotic role (e.g., mannitol and glucose), indicate a clear tendency of plants to accumulate carbohydrates to lower osmotic potential and maintain cell turgor, for as long as plant water status allows for fair carbon assimilation rates. A similar accumulation of carbohydrates to maintain cell turgor has been reported in olive under short-term (i.e. 15 days) water deficit (Dichio et al. 2009). In this regard, MN appears to be the only genotype that must have maintained good assimilation

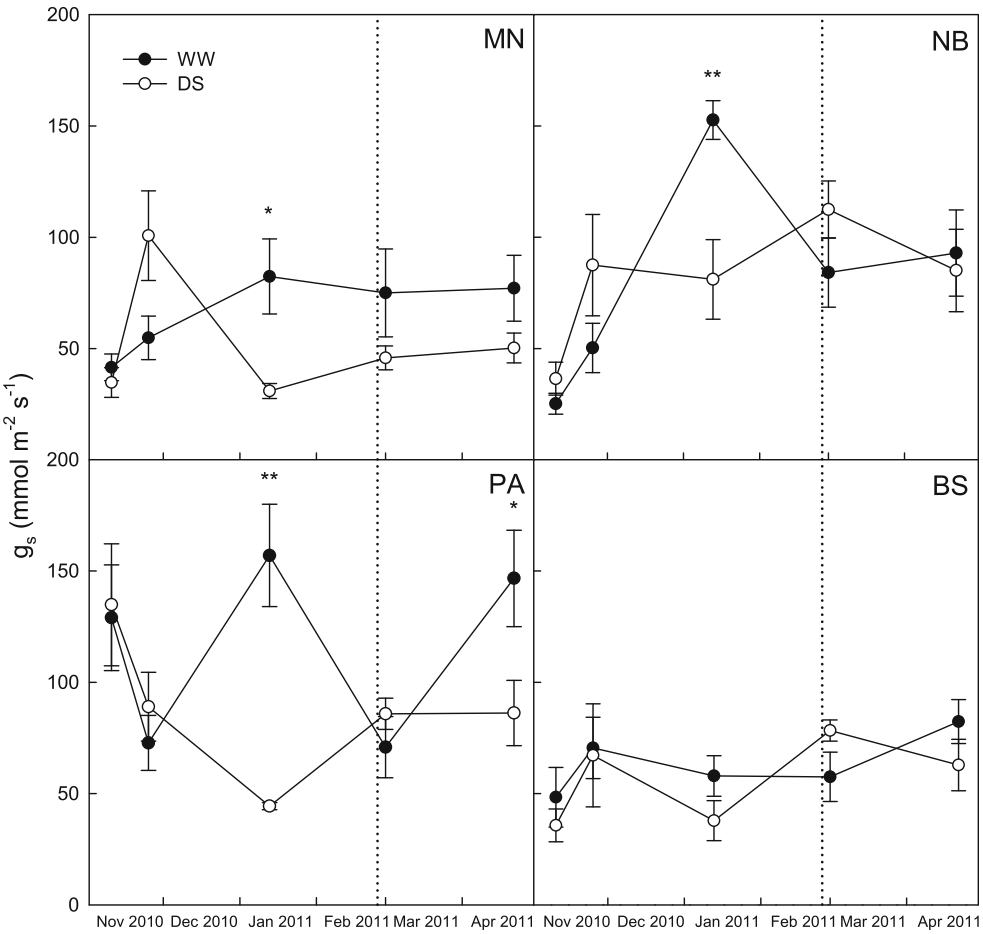

Fig. 5 Leaf stomatal conductance (g_s) in well-watered (WW) and drought-stressed (DS) 2-year-old plants of 'Minuta' (MN), 'Nocellara del Belice' (NB), 'Passulunara' (PA), and 'Biancolilla Siracusana' (BS) olive during drought and after rewatering. The *vertical dotted lines* indicate rewatering. *Error bars* indicate standard errors of means. *Significantly different for $P < 0.05$; **significantly different for $P < 0.01$

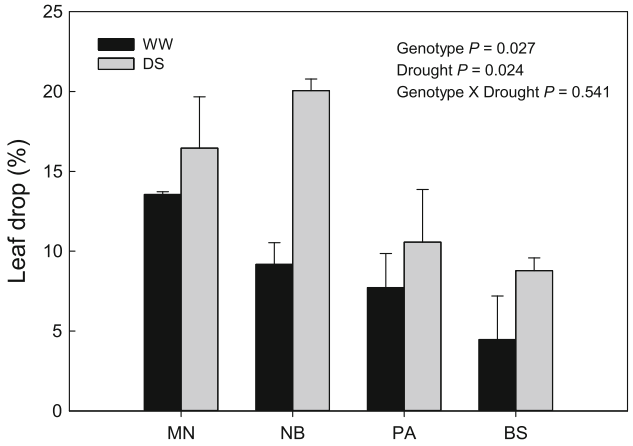

Fig. 6 Percentage of leaf drop in well-watered (WW) and drought-stressed (DS) 2-year-old plants of 'Minuta' (MN), 'Nocellara del Belice' (NB), 'Passulunara' (PA), and 'Biancolilla Siracusana' (BS) olive after 3 months of drought. *P* values indicate significance levels of factors tested in the analysis of variance. *Error bars* indicate standard errors of means

Table 1 Shoot elongation (mm) in 2-year-old plants of 'Minuta' (MN), 'Nocellara del Belice' (NB), 'Passulunara' (PA), and 'Biancolilla Siracusana' (BS) olive under well-watered (WW) and drought (DS) conditions

Genotype	WW	DS
MN	22.2	22.4
NB	26.9	22.1
PA	34.5	26.7
BS	28.8	31.5
LSD	6.38	

Means compared by Fisher's least significant difference (LSD) at $P \leq 0.05$

rates under initial drought and used carbohydrates to maintain some turgor. This is also consistent with the ability of DS MN plants to maintain RWC to the level of WW plants (Fig. 4) and with contained reductions of g_s (Fig. 5).

As for carbohydrate partitioning, the hexose/sucrose ratio showed a clear and significant decrease in all genotypes after rewatering (Table 3). Drought effects were

Table 2 Carbohydrate content (mg g^{-1} DW) in leaves of 'Minuta' (MN), 'Nocellara del Belice' (NB), 'Passulunara' (PA), and 'Biancolilla Siracusana' (BS) olive plants under well-watered (WW) and drought (DS) conditions

Genotype	Date	Irrigation	Glucose	Fructose	Mannitol	Galactinol	Sucrose	Total
MN	22 Nov	WW	57.4	4.94	204	5.5	6.8	278
		DS	58.4	7.13	263	7.1	7.8	343
	13 Jan	WW	31.8	4.49	214	6.1	7.2	264
		DS	34.9	4.69	223	5.7	7.2	276
	1 Mar	WW	7.4	1.10	147	10.1	7.8	174
		DS	6.3	1.12	148	10.9	7.8	174
NB	22 Nov	WW	69.0	7.04	262	12.4	12.0	363
		DS	44.8	4.53	222	14.3	7.1	292
	13 Jan	WW	39.0	3.73	206	16.2	12.4	278
		DS	30.9	3.45	195	9.4	7.7	246
	1 Mar	WW	12.5	1.01	149	17.6	13.3	193
		DS	18.7	1.18	157	14.3	12.1	203
PA	22 Nov	WW	51.5	4.68	233	11.6	7.6	309
		DS	41.1	3.38	220	15.5	8.8	288
	13 Jan	WW	33.9	4.12	205	12.7	11.2	267
		DS	27.0	2.77	147	5.5	8.3	190
	1 Mar	WW	8.1	1.21	206	14.3	13.4	243
		DS	24.7	1.66	199	19.3	20.4	265
BS	22 Nov	WW	43.0	5.50	247	7.8	13.0	316
		DS	45.0	4.93	254	5.4	10.7	320
	13 Jan	WW	46.6	6.59	250	3.4	13.0	320
		DS	56.0	6.89	167	2.2	8.2	240
	1 Mar	WW	16.6	3.17	210	15.3	19.1	264
		DS	29.5	3.26	189	16.7	18.4	257
		LSD	16.9	2.25	57.5	5.29	4.85	63.7

Two-year-old plants during early drought (22 November), advanced drought (13 January) and after rewatering to field capacity (1 March). Means compared by Fisher's least significant difference (LSD) at $P \leq 0.05$

minor and inconsistent with a decrease in November for PA and an increase in January for BS. For those reasons, changes in hexose/sucrose ratio (due to both a decrease of hexoses and an increase of sucrose) should be mainly associated with lower metabolic rates of senescent leaves. A similar association between leaf age/metabolism and carbohydrate partitioning has been already documented in citrus (Iglesias et al. 2002) and common bean (Morris and Arthur 1984).

Similarly, the ratio between osmotically active compounds (mannitol and glucose) and sucrose decreased after rewatering (Table 3). In this case, however, drought induced a significant increase of the (Glu + Man)/Suc ratio throughout the entire drought period in NB; similar trends were observed also in BS and MN (non-significant). These changes may be indicative of internal metabolic adjustments toward the accumulation of more osmotically active carbohydrates, even when carbohydrate accumulation per se is impaired by unfavorable conditions for carbon assimilation, i.e., drought limits photosynthesis. Thus, NB, which was very sensitive to drought in terms of g_s, and possibly carbon assimilation, was able to adjust carbohydrate partitioning toward more osmotically active compounds in an attempt to limit turgor loss.

Conclusions

The four genotypes under trial exhibited different responses to drought. Indeed, this study provides evidence of different degrees of dehydration tolerance in olive, which seem to be genotype related and not just associated with water management. As a matter of fact, the two broad-leaved genotypes (MN and NB) were able to maintain similar leaf hydration levels in DS and WW plants via different strategies, proving to be relatively intolerant to dehydration. Specifically, MN dropped a considerable amount of leaves and partly reduced gas exchange, using a portion of the assimilated carbon to accumulate primarily mannitol, lower osmotic potential, and maintain turgor. On the other hand, NB markedly reduced gas exchange and leaf carbohydrates, shifted the accumulation of

Table 3 Carbohydrate ratios in leaves of 'Minuta' (MN), 'Nocellara del Belice' (NB), 'Passulunara' (PA), and 'Biancolilla Siracusana' (BS) olive plants under well-watered (WW) and drought (DS) conditions

Genotype	Date	Irrigation	(Glucose + fructose)/sucrose	(Glucose + mannitol)/sucrose
MN	22 Nov	WW	5.61	37.7
		DS	5.52	43.9
	13 Jan	WW	3.22	37.1
		DS	3.81	35.5
	1 Mar	WW	0.61	19.7
		DS	0.58	20.0
NB	22 Nov	WW	4.24	32.2
		DS	3.79	45.9
	13 Jan	WW	2.10	22.1
		DS	3.01	37.0
	1 Mar	WW	0.57	11.6
		DS	0.84	15.0
PA	22 Nov	WW	4.22	35.8
		DS	2.64	29.2
	13 Jan	WW	2.83	27.9
		DS	2.12	21.5
	1 Mar	WW	0.43	16.9
		DS	0.70	10.7
BS	22 Nov	WW	2.30	21.3
		DS	2.41	27.3
	13 Jan	WW	2.43	21.7
		DS	3.99	24.7
	1 Mar	WW	0.61	11.5
		DS	0.94	11.5
		LSD	1.27	8.63

Two-year-old plants during early drought (22 November), advanced drought (13 Jan) and after rewatering to field capacity (1 Mar). Means compared by Fisher's least significant difference (LSD) at $P \leq 0.05$

carbohydrates toward more osmotically active forms, and dropped the largest amount of leaves to limit dehydration.

On the contrary, the two narrow-leaved genotypes (PA and BS) tolerated a fair degree of dehydration using only to some extent similar mechanisms. In particular, PA reduced drastically g_s, but dropped a small amount of leaves and consumed a significant amount of carbohydrates to maintain the basic metabolism (respiration); the result was a pronounced dehydration level and reduction of shoot growth. Similarly, BS dropped few leaves and consumed significant amounts of carbohydrates for respiration, but maintained similar low levels of g_s in DS and WW plants; in this case, the resulting dehydration level was more moderate than in PA and typical of a slower growing genotype.

Author contribution statement RLB designed and supervised the experiment, conducted statistical analysis and interpretation of all data and contributed for the most part to manuscript writing. AS conducted most of the measurements in the greenhouse and the laboratory, and contributed to manuscript revision and formatting.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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