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Research article

Salicylic acid increases drought adaptability of young olive trees by changes on redox status and ionome

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ABSTRACT

Different SA concentrations (10, 100 and 1000 μM) were applied in young olive trees (*Olea europaea* L.) subjected to drought and rewatering. Plants treated with 10 μM exhibited a close behavior to SA-starved plants. Although both 100 and 1000 μM improved the balance between ROS production and scavenging, 100 μM was more efficient. During drought, 100 μM improved ROS detoxification and scavenging by the maintenance or overaccumulation of soluble proteins. During recovery, soluble proteins return to well-watered values and increased the investment in non-enzymatic antioxidants. 100 μM was also the most effective in plant ionome regulation, improving macro and micronutrients uptake, namely P, Fe, Mn and Zn, and changing mineral allocation patterns. Therefore, 100 μM also countered the drought-induced decline in total plant biomass accumulation. The application of suitable SA concentrations is an efficient tool to improve cellular homeostasis and growth of plants subjected to recurrent drought episodes.

1. Introduction

Olive tree (*Olea europaea* L.) is one of the oldest cultivated plants native of the Mediterranean region, where is produced most of the world's olive oil. Olive oil is related with several beneficial effects in human health (Ghanbari et al., 2012), what have been contributed to the increase in its consumption and demand all over the world (IOC, 2018). The major factors affecting crop production in Mediterranean agro-ecosystems are water and minerals (Porras-Soriano et al., 2009). Olive tree is traditionally grown under poor soils and water limited environments (Therios, 2009). Concomitant with a rainfall decrease during summer, climate models predict a stronger inter- and intra-annual weather variability (IPCC, 2013). These scenarios give even more prominence to the concept of drought adaptability, that integrates much more than drought resistance, playing recovery capacity also a fundamental role in plants growth and survival (Chen et al., 2016).

Drought stress results in increased generation of reactive oxygen species (ROS) due to energy accumulation, which increases the photooxidative effect (Waraich et al., 2011). Prevention of cellular oxidative damage has been suggested as one of the mechanisms of stress tolerance (Yildirim et al., 2008). To fight against the resulting oxidative stress, olive trees invest in several enzymatic and non-enzymatic antioxidant mechanisms (Sofo et al., 2004, 2008; Bacelar et al., 2006, 2007; Petridis et al., 2012). However, high stress levels can create an imbalance between ROS production and scavenging (Farooq et al., 2012), damaging lipids, proteins, carbohydrates, pigments and DNA (Sofo et al., 2004; Bacelar et al., 2006, 2007; Farooq et al., 2009; Petridis et al., 2012). Those limitations may also influence the biochemical metabolism and the signal cascade in recovery by rewatering events (Xu et al., 2010). In addition, drought stress affects uptake, transport, and subsequent distribution of nutrients within the plant (Farooq et al., 2009), causing an imbalance in plant nutrition. The disequilibrium in plant nutrition is associated with several secondary effects, since mineral nutrients serve numerous functions in plants, as structural components in macromolecules, co-factors in enzymatic reactions, osmotic solutes and maintenance of charge balance in cellular compartments (Grusak, 2001). Therefore, the occurrence of oxidative damage in droughted plants can be even more notorious when plants also suffer nutrient deficiencies (Cakmak, 2008). Consequently, biomass accumulation, allocation patterns and productivity are eventually affected (Farooq et al., 2009, 2012).

Salicylic acid (SA) is a phytohormone increasingly recognized as abiotic stress-tolerance enhancer, via SA-mediated control of major plant-metabolic processes (Khan et al., 2015). Nevertheless, the basic

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mechanisms supporting this tolerance remain less discussed (Khan et al., 2015). SA regulates several proteins associated with signal transduction, stress defense and protein metabolism (Kang et al., 2012), and modulates mineral nutrients uptake and metabolism, affecting growth and development under stressful conditions (El-Tayeb, 2005; Gunes et al., 2007; Yildirim et al., 2008; Khan et al., 2015). However, the effect of SA on ROS concentration, cell membrane stability, osmoprotectants and proteins accumulation and antioxidant defense system is highly dependent on several factors, as applied concentration, kind and level of stress and the species in study (El-Tayeb, 2005; Chen et al., 2007; Harfouche et al., 2008; Hayat et al., 2008; Yildirim et al., 2008; Kang et al., 2012; Fayez and Bazaid, 2014; Kabiri et al., 2014). The information about SA role in abiotic stress mitigation in olive tree is reduced. To illustrate, SA in a suitable concentration (1 mM) improved the biochemical responses under freeze conditions (Hashempour et al., 2014), at 0.25 mM SA promoted growth under salt stress conditions (Aliniaeifard et al., 2016) and at 0.1 mM improved physiological processes and growth under drought conditions (Brito et al., 2018a).

We hypothesized that a suitable SA concentration can improve olive trees drought adaptability. Accordingly, we aimed: 1) to appraise the SA influence on oxidative damage and antioxidant defense system during drought and recovery events; 2) to assess the SA influence on plant mineral status and growth after drought and rewatering events; 3) and, to achieve a most profit SA concentration to improve olive tree drought tolerance and recovery capacity.

2. Material and methods

2.1. Growth conditions, plant material and experimental set-up

The experiment was carried out between June and September 2014 at the University of Trás-os-Montes and Alto Douro, Vila Real, Northeast Portugal (41°17′17.83″N, 7°44′12.81″W, 448 m a.s.l.). Climate is Mediterranean-like, warm-temperate with dry and hot summers, classified as Csb according to Köppen-Geiger's classification. Mean annual rainfall is 1023 mm, most of which in the autumn-winter and negligible during the summer. However, 2014 had an atypical summer with some rainfall events (13.7, 11.8 and 13.0 mm during the 1st, 2nd and 3rd recovery periods, respectively). The warmest months are July/August and the coldest months are December/January, with mean daily temperatures of 21.3/21.7 °C and 6.8/6.3 °C, respectively (IPMA, 2017).

Own-rooted 3 years-old olive trees (*Olea europaea* cv. Cobrançosa), were grown outdoors in 16 L pots containing a mix of sandy-loam soil and horticultural substrate Siro Oliva (Siro-Leal & Soares SA, Mira, Portugal) (2:1). Pots surface was covered with a thin layer of perlite and then were sealed with plastic film and aluminum foil to avoid temperature raise, evaporation and rain water entering. Pots were randomly arranged and periodically rotated to the neighboring position to minimize the effects of environmental heterogeneity. When applicable, plants were watered to field capacity, determined gravimetrically. Care was taken to ensure negligible leaching through the pots bottoms during irrigation.

Prior to the experiment, eighty-eight uniform plants, selected based on height, leaf number and leaf area were left for 30 days in the study site for acclimatization, being watered every other day to field capacity, determined gravimetrically. Then, at the beginning of the experiment, 6th July, eight plants randomly chosen were harvested to assess the initial biomass of the different plant organs. The remaining eighty plants were divided in five groups, each one comprising sixteen plants. One group was sprayed with distilled water and kept under well-watered conditions (WW, control plants) throughout the entire experimental period, in which plants were watered every day. The four other groups were subjected to three "drought-re-watering cycles" (D). Three groups were sprayed with 10 μM (D10), 100 μM (D100) or 1000 μM (D1000) salicylic acid (SA) concentrations. SA firstly dissolved in absolute ethanol, and then added to water (ethanol: water; 1:1000, v/v). The fourth group (D0) was sprayed with distilled water plus ethanol (0,1%, v/v). Each plant was treated with a mean volume of 150 mL of spraying solution. All spray applications were supplemented with 0.1% (v/v) Tween 20 and conducted according to good efficacy practice standard operating procedures adjusted for agricultural experiments. Care was taken during the application of foliar sprays to avoid overspraying non-target trees, covering them with a plastic sheet.

Due to environmental conditions limitation, in the 1st and 2nd cycles drought was imposed by withholding water until the occurrence of natural precipitation. In the 3rd cycle drought was imposed until the stomatal conductance for water vapour during mid-morning (peak of photosynthetic activity) dropped around 50 mmol m^{-2} s^{-1} a threshold value indicating a situation of severe drought stress experienced by the plants, a value where photosynthetic activity becomes predominantly inhibited by metabolic processes, besides stomatal limitations (Flexas and Medrano, 2002). When occurred precipitation (1st and 2nd cycle), or when olive trees reached the desired drought intensity (3rd cycle), the trees were rewatered to field capacity in the evening and during the following days until net photosynthesis was almost restored to control values (recovery). The 1st, 2nd and 3rd "drought-re-watering cycles" had the duration of 12-6 days, 9-3 days and 21-16 days, respectively.

Each group of sixteen plants was divided in two subgroups, each one with eight plants. Plants from one subgroup were used for biochemical destructive measurements, and plants from the other subgroup were used for final biomass and plant minerals assessment. All the plants were manually defruited immediately after fruit set to avoid yield influences on the measured variables. A schematic representation of the experiment is presented in Fig. 1. All the measurements detailed bellow were performed 8 times per treatment (n = 8), one per plant. Biochemical measurements at leaf level were measured in healthy, full expanded mature leaves 21 days after starting the 3rd drought period (DP), at the peak of stress and 8 days after starting the respective recovery period (RP). Final biomass accumulation and plant organs ionome was evaluated at the end of the experiment, 16 days after starting the 3rd RP.

2.2. Foliar metabolic assays

Total reactive oxygen species (ROS) were determined with 2′,7′-dichlorofluorescein diacetate (DCFH-DA) (Sigma–Aldrich,Germany) (Kong et al., 2013). A 25 mM solution was prepared in dimethyl sulphoxide for pending use. Twenty microliters of each sample were loaded into a small well ELISA plate containing 0.2 ml of PBS buffer (pH 7.4) and 12 μM of DCFH-DA and incubated for 20 min at 25 °C. Fluorescence was measured at 485 nm and 530 nm (excitation and emission wavelength, respectively), in a CARY 50 Bio (Eclipse, Australia) every 15 min until 60 min after the incubation. 2′,7′ -dichlorofluorescein was used to obtain a calibration curve. Results were expressed as nM DCF g^{-1} DW. Hydrogen peroxide (H_2O_2) concentration were determined using a method described by Junglee et al. (2014), with some modifications. The absorbance was measured at 350 nm and H_2O_2 was used to obtain a calibration curve.

β-carotene was extracted with acetone–hexane mixture (4:6) and determined according to Barros et al. (2011). Total soluble proteins (TSP) were quantified using the method of Bradford (1976), using bovine serum albumin as a standard. Total thiols (–SH) in TSP extract were assessed according to Ellman (1959), using an extinction coefficient of 13,600 M^{-1} cm^{-1}. Total phenolic compounds (TPC) in leaf methanolic extracts were quantified following the Folin–Ciocalteu procedure (Singleton and Rossi, 1965), using gallic acid as a standard. Flavonoids were determined according to Zhishen et al. (1999) in the

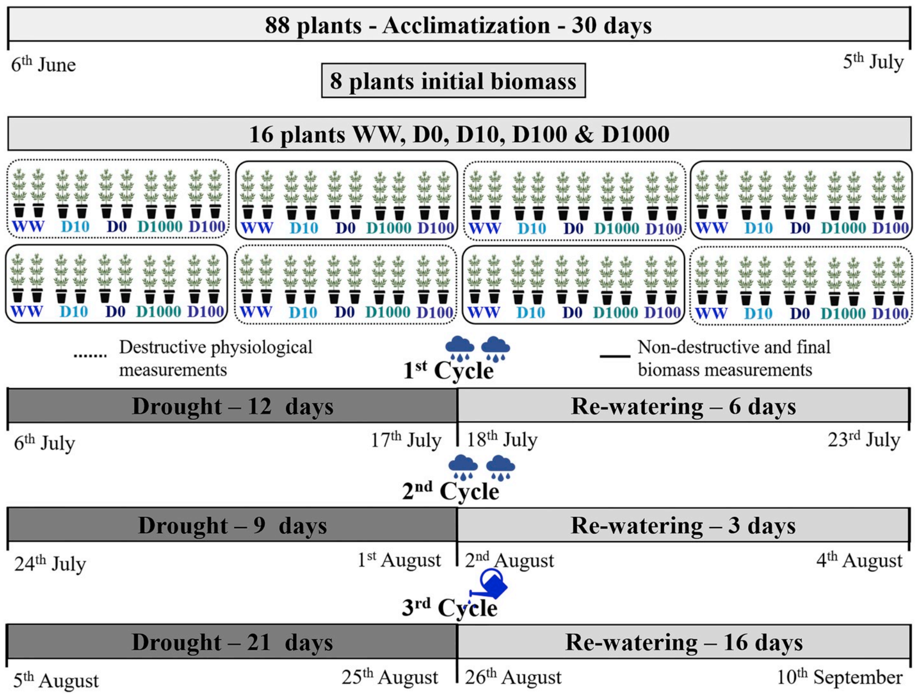

Fig. 1. Schematization of the experiment. Well-watered controls (WW) and droughted plus salicylic acid plants, 0 μM (D0), 10 μM (D10), 100 μM (D100) and 1000 μM (D1000).

same leaf extracts of TPC, using (+)-catechin as a standard. Ascorbate was quantified using a method adapted from Klein and Perry (1982), using L-ascorbic acid as a standard.

Total antioxidant capacity (TAC), based on DPPH-free radical scavenging, was evaluated according to a method adapted from Xu and Chang (2007). Leaf methanolic extracts, and methanol for negative control, were mixed with DPPH methanolic solution (0.1 mM) and left to stand for 30 min in dark at room temperature. The absorbance for the sample (A_{sample}) and negative control ($A_{control}$) was measured at 517 nm against methanol blank. The percent of DPPH radical reduction was calculated as follows = 100 x ($A_{control}$ − A_{sample})/$A_{control}$. The free radical scavenging activity was expressed as μM of Trolox equivalents, TE = (% DPPH radical reduction/a), where a is the slope of the standard curve ($y = ax$).

2.3. Plant biomass accumulation and mineral analysis

Dry weight of plant samples (leaves, stems and roots) were oven-dried at 70 °C to a constant weight. Based on these data, the total biomass increase (TBI) and the fraction of biomass allocation among plant organs were calculated. Following ground, N concentration was determined by Dumas method in an elemental analyser (Primac, Skalar, The Netherlands). The concentrations of other elements (P, K, Ca, Mg, S, B, Fe, Cu, Zn, and Mn) were determined by ICP-OES (Quantima, GBC, Australia), after dry digestion and ash dissolution with HNO_3 (Mills and Jones, 1996).

The quantity of nutrients accumulated per plant was obtained by multiplying the concentration (dry weight basis) in each plant organ by the respective biomass. Physiological nutrient-use efficiency (UE) has been calculated as total plant biomass per unit of mineral element acquired, while minerals root uptake efficiency has been calculated as the total amount of minerals in each plant per unit of root biomass.

2.4. Statistical analysis

The statistical analysis was performed using the statistical software program SPSS for Windows (v. 22). All data sets satisfied the assumptions of ANOVA based on homogeneity of variances and normality. In all parameters, data were analyzed one-way factorial ANOVA and the post hoc Tukey's test. Significant differences were considered for ($P < 0.05$). For statistical analysis of TBI, arcsine transformation was performed in percentage data.

3. Results

3.1. Oxidative stress and defense systems

Oxidative stress, at leaf level, can be accessed based on total ROS and H_2O_2 concentrations (Table 1). The results showed that water regime and SA concentrations modulate total ROS and H_2O_2 accumulation in both sampling dates. Treatments influenced total ROS concentration in the following order: D10 ≥ D0 ≥ D1000 ≥ D100 = WW and D0 ≥ D10 ≥ D1000 ≥ D100 = WW during the drought and recovery periods, respectively. Meanwhile, H_2O_2 concentrations varied in the following way: WW = D1000 > D100 > D10 > D0 and D100 > WW = D10 = D0 > D1000 during drought and after recovery, respectively.

Water regime and SA also modulated leaf non-enzymatic antioxidant defenses, which usually change upon rewatering (Table 1, Fig. 2). Total soluble proteins concentration was higher in D10 and D100 than in D0 plants during drought, whereas WW and D100 leaves showed higher TSP levels than in D0 plants after rewatering. Drought induced decline in total thiols concentration was annulated by the application of SA during the drought period, whereas their concentration was lower in all stressed plants upon rewatering (Table 1). β-carotene was significantly affected by the applied treatments, presenting control plants higher values during the drought phase, whereas D0 leaves had higher values than WW and D10 treatments, upon rewatering (Table 1). Ascorbate concentration was modulated by water regime and SA treatments. During drought, D0 and D100 plants presented the highest and lowest values, respectively, whereas upon rewatering D0 continued to present the highest concentrations and D100 and control plants showed the lowest levels (Table 1). The concentrations of TPC and flavonoids were affected during the drought period following the order

Table 1
Oxidative stress indicators and foliar metabolites concentrations of well-watered (WW) and droughted plus salicylic acid (D) plants during drought (DP) and recovery (RP) periods. Total reactive oxygen species (ROS, nmol.g^{-1} DW), H_2O_2 (μmol.g^{-1} DW), total soluble proteins (TSP, mg.g^{-1} DW), total thiols (-SH, nmol.mg^{-1} DW), β-Carotene (mg.g^{-1} DW), total phenolic compounds (TPC, mg.g^{-1} DW), flavonoids (mg.g^{-1} DW) and ascorbate (mg.g^{-1} DW).

		WW	D0	D10	D100	D1000	Sig.
ROS	DP	0.276 ± 0.020^{c}	0.422 ± 0.019^{ab}	0.470 ± 0.040^{a}	0.297 ± 0.048^{c}	0.356 ± 0.024^{bc}	**
	RP	0.391 ± 0.026^{c}	0.560 ± 0.039^{a}	0.490 ± 0.022^{ab}	0.380 ± 0.029^{c}	0.437 ± 0.012^{bc}	**
H_2O_2	DP	22.7 ± 0.1^{a}	11.9 ± 0.1^{d}	19.9 ± 0.2^{c}	21.1 ± 0.2^{b}	22.6 ± 0.1^{a}	***
	RP	18.0 ± 0.5^{b}	17.8 ± 0.6^{b}	17.8 ± 0.9^{b}	25.6 ± 0.5^{a}	14.4 ± 0.6^{c}	***
TSP	DP	7.04 ± 0.36^{b}	2.65 ± 0.13^{c}	9.92 ± 0.60^{a}	8.78 ± 0.38^{ab}	7.57 ± 0.85^{b}	***
	RP	7.87 ± 0.48^{a}	3.54 ± 0.12^{c}	6.02 ± 0.26^{b}	6.78 ± 0.38^{ab}	7.37 ± 0.88^{ab}	***
-SH	DP	1.96 ± 0.07^{a}	0.99 ± 0.07^{b}	1.77 ± 0.16^{a}	1.95 ± 0.18^{a}	1.60 ± 0.11^{a}	***
	RP	3.28 ± 0.51^{a}	1.10 ± 0.04^{b}	1.06 ± 0.04^{b}	1.36 ± 0.13^{b}	1.20 ± 0.02^{b}	***
β-Carotene	DP	0.562 ± 0.010^{a}	0.528 ± 0.011^{b}	0.519 ± 0.004^{b}	0.512 ± 0.004^{bc}	0.492 ± 0.005^{c}	***
	RP	0.536 ± 0.010^{bc}	0.607 ± 0.012^{a}	0.527 ± 0.014^{c}	0.582 ± 0.013^{ab}	0.572 ± 0.021^{abc}	**
TPC	DP	38.6 ± 0.5^{b}	42.4 ± 0.3^{a}	39.2 ± 0.3^{b}	35.5 ± 0.3^{c}	34.2 ± 0.4^{d}	***
	RP	34.2 ± 0.5^{c}	41.9 ± 0.4^{b}	42.9 ± 0.5^{b}	46.8 ± 0.4^{a}	47.3 ± 0.3^{a}	***
Flavonoids	DP	12.0 ± 0.6^{d}	22.6 ± 0.2^{a}	15.9 ± 0.4^{b}	14.5 ± 0.1^{c}	16.6 ± 0.1^{b}	***
	RP	15.4 ± 0.3^{e}	17.6 ± 0.4^{d}	21.9 ± 0.5^{b}	20.1 ± 0.3^{c}	24.6 ± 0.4^{a}	***
Ascorbate	DP	1.36 ± 0.44^{b}	1.60 ± 0.05^{a}	0.485 ± 0.01^{c}	0.227 ± 0.009^{d}	1.41 ± 0.02^{b}	***
	RP	1.15 ± 0.02^{d}	2.16 ± 0.04^{a}	1.56 ± 0.05^{b}	1.28 ± 0.04^{cd}	1.38 ± 0.06^{c}	***

Values are means ± SE. Different letters indicate significant differences among treatments within each date (**P < 0.01, ***P < 0.001).

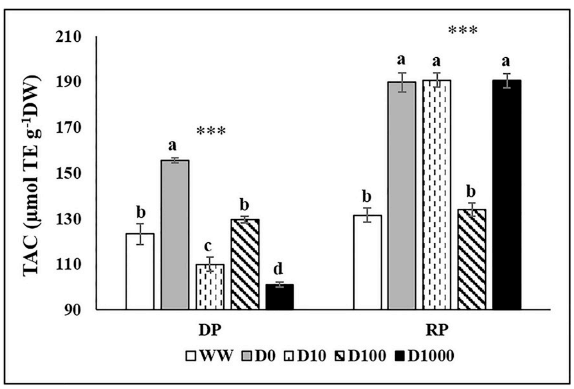

Fig. 2. Leaf total antioxidant capacity (TAC) of well-watered (WW) and droughted plus salicylic acid (D) plants during drought (DP) and recovery (RP) periods. Each column is average and vertical bars represent the S.E. (n = 8). Different letters indicate significant differences among treatments within each date (***P < 0.001).

D0 > WW = D10 > D100 > D1000 and D0 > D10 = D1000 > D100 > WW, respectively, while after rewatering the orders were D1000 = D100 > D10 = D0 > WW and D1000 > D10 > D100 > D0 > WW, respectively (Table 1).

The influence of treatments on antioxidant system is somehow reflected in the TAC estimated by the DPPH assay, being TAC higher in D0 under drought, namely relatively to D1000 plants, whereas upon rewatering D100 and WW plants presented lower values than the other treatments (Fig. 2).

3.2. Plant biomass accumulation and mineral dynamics

Total biomass increase through the experiment was reduced by drought imposition, being this decline attenuated by SA in a dose dependent manner, as D100 plants presented higher TBI than D0 plants (Fig. 3A). The fraction of biomass allocation among plant organs was also distinctly affected by water regime and SA concentration. All stressed plants increased the investment in roots, highlighting D100 plants, that conversely reduced the investment in stems and leaves fractions (Fig. 3B).

The content of minerals, except for nitrogen and calcium, was affected by water availability and exogenous SA supply, although dependent on the plant organ (Table 2). The application of SA decreased leaf P, while drought reduced stem P, mainly in the absence of SA. On the other hand, drought dropped K content on leaves and roots, mainly in SA-treated and D0 plants, respectively. Furthermore, substantial changes were observed in magnesium contents. Leaf Mg was higher in D10 than in D0 plants, stem Mg was superior in D0, whereas root Mg was higher in D100 than in D0 and D10 plants. Meanwhile, the leaf sulfur content was higher in D0 plants, at the expenses of stem S content. In addition, interesting changes were observed in micronutrients contents, namely under the application of SA. The content of boron increased in roots of droughted plants, namely in D100 trees, whereas the contents of iron and manganese increased in leaves and roots with SA application, highlighting D10 on leaves and D100 on both organs for Fe, and D10 and D100 on leaves and D1000 on roots for Mn. On the other hand, the content of Zn only changed on roots, being higher in D100 than in D0 and D10 plants, while the content of Cu on leaves was higher in D10 than on SA-starved plants, while the content on roots decreased under drought.

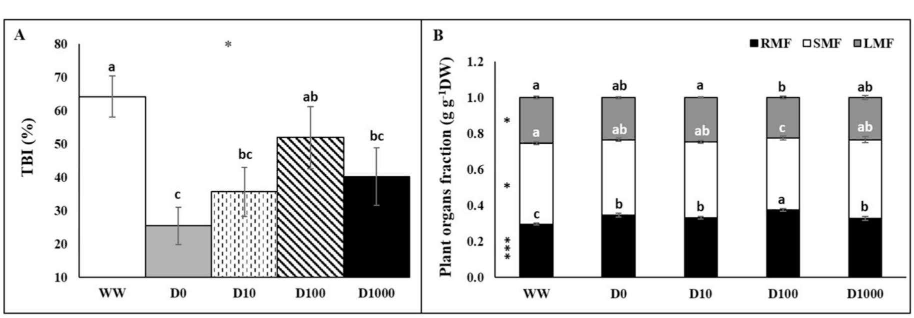

Fig. 3. Total biomass increase (TBI) (A) and plant organs fraction (RMF, root mass fraction; SMF, stem mass fraction; LMF, leaf mass fraction) (B) of well-watered (WW) and droughted plus salicylic acid (D) plants at the end of the experiment. Each column is average and vertical bars represent the S.E. (n = 8). Different letters indicate significant differences among treatments (*P < 0.05, ***P < 0.001).

Table 2
Nutrients content ($g.kg^{-1}DW$ for macronutrients, and $mg.kg^{-1}DW$ for micronutrients) in the different plant organs of well-watered (WW) and droughted (D) plus salicylic acid plants at the end of the experiment.

	Nutrients content					Sig.
	WW	D0	D10	D100	D1000	
N_{Leaf}	20.0 ± 0.8	20.0 ± 0.9	19.6 ± 0.5	19.6 ± 0.8	19.8 ± 0.8	n.s.
N_{Stem}	5.02 ± 0.23	5.80 ± 0.21	5.54 ± 0.20	5.54 ± 0.14	5.79 ± 0.33	n.s.
N_{Root}	13.8 ± 0.4	15.0 ± 0.6	13.2 ± 0.8	14.3 ± 0.5	15.5 ± 0.5	n.s.
P_{Leaf}	3.52 ± 0.21^{a}	2.93 ± 0.11^{a}	2.41 ± 0.15^{b}	2.66 ± 0.11^{b}	2.62 ± 0.12^{b}	***
P_{Stem}	2.93 ± 0.20^{a}	1.57 ± 0.34^{c}	2.22 ± 0.05^{b}	2.59 ± 0.13^{ab}	2.42 ± 0.26^{ab}	**
P_{Root}	1.97 ± 0.29	2.11 ± 0.40	1.72 ± 0.58	1.28 ± 0.15	1.71 ± 0.31	n.s.
K_{Leaf}	12.6 ± 0.7^{a}	10.4 ± 0.2^{b}	8.30 ± 0.98^{c}	7.16 ± 0.31^{c}	8.59 ± 0.56^{c}	***
K_{Stem}	6.61 ± 1.33	5.14 ± 0.17	2.67 ± 0.97	5.21 ± 0.94	5.63 ± 1.02	n.s.
K_{Root}	7.87 ± 0.44^{a}	3.40 ± 0.77^{c}	3.44 ± 1.03^{c}	5.98 ± 0.33^{ab}	5.56 ± 0.45^{b}	***
Ca_{Leaf}	3.37 ± 0.26	3.80 ± 0.96	4.93 ± 0.39	4.62 ± 0.65	3.84 ± 0.97	n.s.
Ca_{Stem}	1.74 ± 0.40	2.345 ± 0.43	0.93 ± 0.42	1.80 ± 0.60	1.32 ± 0.23	n.s.
Ca_{Root}	1.99 ± 0.523	2.51 ± 0.93	2.75 ± 0.57	2.23 ± 0.17	3.25 ± 0.38	n.s.
Mg_{Leaf}	0.462 ± 0.094^{b}	0.402 ± 0.086^{b}	0.808 ± 0.034^{a}	0.694 ± 0.099^{ab}	0.586 ± 0.12^{ab}	*
Mg_{Stem}	0.217 ± 0.076^{b}	0.492 ± 0.080^{a}	0.288 ± 0.067^{b}	0.267 ± 0.060^{b}	0.189 ± 0.01^{b}	*
Mg_{Root}	1.78 ± 0.13^{ab}	1.27 ± 0.21^{b}	1.34 ± 0.26^{b}	1.59 ± 0.08^{ab}	1.97 ± 0.12^{a}	*
S_{Leaf}	2.95 ± 0.17^{ab}	3.29 ± 0.26^{a}	2.25 ± 0.19^{b}	2.35 ± 0.06^{b}	2.35 ± 0.38^{b}	*
S_{Stem}	0.328 ± 0.047^{a}	0.135 ± 0.042^{b}	0.152 ± 0.04^{b}	0.324 ± 0.006^{a}	0.284 ± 0.03^{a}	**
S_{Root}	0.667 ± 0.095	0.759 ± 0.264	0.627 ± 0.302	0.683 ± 0.079	0.646 ± 0.110	n.s.
B_{Leaf}	28.4 ± 0.8	25.8 ± 1.8	26.6 ± 1.9	27.8 ± 2.2	27.9 ± 2.0	n.s.
B_{Stem}	22.5 ± 1.1	29.0 ± 2.7	28.6 ± 3.0	33.3 ± 3.4	32.8 ± 4.4	n.s.
B_{Root}	29.4 ± 1.7^{c}	39.2 ± 3.3^{b}	38.1 ± 2.7^{b}	50.1 ± 2.7^{a}	40.9 ± 2.9^{b}	**
Fe_{Leaf}	14.4 ± 1.9^{bc}	9.9 ± 0.9^{c}	30.8 ± 3.9^{a}	25.2 ± 4.5^{ab}	17.8 ± 5.0^{bc}	**
Fe_{Stem}	10.6 ± 2.1	11.3 ± 2.7	5.5 ± 1.5	12.7 ± 3.9	9.78 ± 1.17	n.s.
Fe_{Root}	246.1 ± 22.7^{bc}	262.3 ± 49.8^{bc}	179.2 ± 33.3^{c}	499.4 ± 107.0^{a}	377.5 ± 38.1^{ab}	**
Zn_{Leaf}	17.4 ± 2.2	17.0 ± 2.0	21.7 ± 1.0	18.7 ± 2.3	18.4 ± 4.9	n.s.
Zn_{Stem}	9.8 ± 1.7	14.9 ± 1.6	7.6 ± 2.9	14.6 ± 2.8	16.7 ± 2.6	n.s.
Zn_{Root}	54.0 ± 3.9^{ab}	34.0 ± 9.7^{bc}	30.1 ± 6.3^{c}	64.6 ± 5.3^{a}	54.7 ± 2.3^{ab}	**
Mn_{Leaf}	27.3 ± 5.6^{c}	21.8 ± 6.7^{c}	59.2 ± 2.6^{ab}	62.1 ± 11.0^{a}	40.2 ± 4.9^{bc}	**
Mn_{Stem}	4.94 ± 0.15	9.65 ± 1.90	6.64 ± 1.73	13.09 ± 5.89	5.96 ± 1.60	n.s.
Mn_{Root}	35.9 ± 9.9^{b}	54.1 ± 17.4^{b}	50.3 ± 11.4^{b}	71.7 ± 12.3^{ab}	92.9 ± 7.7^{a}	*
Cu_{Leaf}	14.0 ± 1.0^{b}	12.9 ± 1.6^{b}	22.8 ± 1.3^{a}	18.1 ± 2.5^{ab}	17.3 ± 3.9^{ab}	*
Cu_{Stem}	9.17 ± 1.37	10.6 ± 2.5	7.95 ± 1.35	10.6 ± 0.9	10.5 ± 0.9	n.s.
Cu_{Root}	25.5 ± 2.6^{a}	10.1 ± 2.4^{bc}	9.74 ± 1.49^{bc}	7.14 ± 1.88^{c}	14.3 ± 2.3^{b}	***

Values are means ± SE. Different letters indicate significant differences among treatments (n.s.- not significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

The minerals allocation patterns, with the exception of calcium, were affected by the applied treatments, although differently among SA concentrations and water regime, being leaves and roots more influenced than stems (Table 3). D10 plants maintained the allocation of N into leaves and roots, as in WW plants, whereas the other stressed plants decreased their allocation of N into leaves, in order to invest more N in roots. SA-treated plants presented similar patterns of P allocation, as well-irrigated plants, while drought-starved SA increased allocation into leaves, at the expenses of stems. Meanwhile, minor changes were observed in K allocation, as only D10 plants presented more investment of K on leaves than D100 and D1000 counterparts. More evident were the effects of the application of SA and drought on the allocation of magnesium, as D10 plants presented higher distribution of Mg to leaves, D0 exhibited superior Mg allocation to stems than WW, D100 and D1000 trees, and D1000 had larger Mg investment in roots than D0 and D10 plants. Relatively to sulfur allocation, D0 plants showed higher allocation to leaves than D100 and D1000, while these treatments, joining with well-watered, had higher S allocation to stems than drought-starved SA plants. On the other hand, the allocation of B to leaves decreased in plants submitted to drought, whereas the opposite trend was observed in roots, namely in D100 treatment. Iron, zinc and copper allocation patterns were almost similar, being registered higher allocations to leaves of D10 plants. Nonetheless, WW plants presented higher Cu allocation to roots than droughted plants. Relatively to Mn, D10 plants presented again the highest distribution to leaves, whereas D1000 trees stand out in allocation of Mn to roots.

The total quantity of minerals acquired by plants showed in Table 4 reflects treatment effects on both tissue concentrations and biomass production and are, in general, strictly associated with the root uptake efficiency (RUE). Droughted plants presented lower P, K, S and Cu root uptake efficiency and, thus, inferior minerals yield than well-watered trees, whereas Ca, Mg and B were not significantly affected. In addition, WW plants had larger N_{RUE} than D0 and D100, while D100 presented superior uptake of P than D0 plants. Moreover, in general, D100 and D1000 plants absorbed more Fe, Zn and Mn than the other treatments.

In terms of physiological nutrient use efficiency (UE), our data demonstrated that P_{UE} increased in water-stressed trees, K_{UE} was highest in D10, WW had larger B_{UE} than D100 and D1000 and higher Mn_{UE} than all exogenous-SA treatments, and D10 presented superior Fe_{UE} and Zn_{UE} than D100 and D1000 plants. Meanwhile, no significant effects of treatments were reported on nitrogen, calcium, magnesium, sulfur and copper physiological use efficiency (Table 5).

4. Discussion

4.1. Oxidative stress and defense systems

Whether ROS would act as signaling molecules or might cause oxidative stress to the tissues, it depends on the refined balance between ROS production and scavenging (Mattos and Moretti, 2015). In this study, the sharp increase in total ROS observed in D10 and D0 plants, both under drought and after rewatering, suggest that D100 and D1000 treatments contributed to maintain reduced values of ROS, just as in WW plants. ROS react with structural and functional proteins, lipids and nucleic acids, causing oxidative damage and impairing cellular functioning (Farooq et al., 2009). Yet, each type of ROS molecule has its own distinct reactivity, being singlet oxygen ($^1O_2$) and hydroxyl radical ($OH^{\bullet}$) the most reactive, while H_2O_2 is less reactive (Gill and

Table 3
Nutrients allocation (%) by the different plant organs of well-watered (WW) and droughted (D) plus salicylic acid plants at the end of the experiment.

	Nutrients allocation					Sig.
	WW	D0	D10	D100	D1000	
N_{Leaf}	48.1 ± 0.4^{a}	40.2 ± 1.6^{b}	45.1 ± 0.4^{a}	39.5 ± 0.5^{b}	40.3 ± 1.9^{b}	***
N_{Stem}	26.2 ± 0.7	24.7 ± 1.31	26.8 ± 0.8	25.1 ± 0.8	26.2 ± 2.1	n.s.
N_{Root}	25.7 ± 1.0^{b}	35.1 ± 2.9^{a}	28.1 ± 0.7^{b}	35.4 ± 0.9^{a}	33.5 ± 2.3^{a}	**
P_{Leaf}	31.1 ± 1.7^{b}	38.6 ± 1.5^{a}	28.0 ± 2.5^{b}	26.9 ± 2.2^{b}	26.8 ± 3.0^{b}	**
P_{Stem}	55.6 ± 0.5^{a}	34.7 ± 5.5^{b}	54.1 ± 3.6^{a}	57.6 ± 2.0^{a}	54.1 ± 6.4^{a}	**
P_{Root}	13.3 ± 1.8	26.7 ± 4.6	17.9 ± 5.6	15.5 ± 1.7	19.1 ± 4.6	n.s.
K_{Leaf}	40.2 ± 6.4^{ab}	41.1 ± 1.8^{ab}	51.5 ± 5.8^{a}	28.3 ± 1.8^{b}	33.9 ± 5.6^{b}	*
K_{Stem}	41.2 ± 7.2	42.8 ± 2.6	31.1 ± 6.9	42.3 ± 4.7	43.7 ± 5.6	n.s.
K_{Root}	18.6 ± 1.3	16.1 ± 4.3	17.4 ± 4.37	29.4 ± 3.2	22.4 ± 2.2	n.s.
Ca_{Leaf}	39.9 ± 3.0	34.9 ± 8.1	52.8 ± 3.8	41.9 ± 7.0	36.8 ± 5.1	n.s.
Ca_{Stem}	40.5 ± 7.5	42.1 ± 4.1	18.7 ± 7.2	33.0 ± 10.5	28.0 ± 4.1	n.s.
Ca_{Root}	19.6 ± 5.3	23.0 ± 7.7	28.5 ± 6.6	25.1 ± 3.6	35.2 ± 5.0	n.s.
Mg_{Leaf}	20.6 ± 4.9^{b}	13.2 ± 1.8^{b}	31.4 ± 3.1^{a}	21.2 ± 2.6^{b}	18.6 ± 2.9^{b}	*
Mg_{Stem}	19.6 ± 6.0^{b}	37.3 ± 6.6^{a}	23.1 ± 5.9^{ab}	18.5 ± 4.6^{b}	13.6 ± 1.3^{b}	*
Mg_{Root}	59.8 ± 3.6^{ab}	49.5 ± 6.0^{b}	45.5 ± 7.0^{b}	60.3 ± 2.0^{ab}	67.8 ± 3.7^{a}	*
S_{Leaf}	70.9 ± 2.1^{ab}	76.2 ± 3.91^{a}	72.3 ± 6.6^{ab}	60.1 ± 1.80^{b}	62.8 ± 3.9^{b}	*
S_{Stem}	16.7 ± 1.9^{a}	6.3 ± 1.63 ^{b}	11.4 ± 4.5^{ab}	18.6 ± 1.21^{a}	18.5 ± 4.4^{a}	*
S_{Root}	12.4 ± 2.0	17.5 ± 4.66	16.3 ± 7.4	21.3 ± 2.30	18.7 ± 2.5	n.s.
B_{Leaf}	28.6 ± 0.4^{a}	19.6 ± 0.55^{c}	21.9 ± 0.8^{b}	17.0 ± 0.7^{d}	19.6 ± 0.9^{c}	***
B_{Stem}	48.7 ± 0.5	46.1 ± 1.85	48.8 ± 1.7	45.1 ± 1.5	49.8 ± 2.7	n.s.
B_{Root}	22.7 ± 0.5^{c}	34.3 ± 1.97^{ab}	29.3 ± 1.5^{b}	37.9 ± 1.6^{a}	30.6 ± 2.8^{b}	***
Fe_{Leaf}	6.6 ± 0.9^{b}	3.3 ± 0.7^{b}	16.7 ± 4.7^{a}	4.2 ± 0.8^{b}	4.3 ± 1.3^{b}	**
Fe_{Stem}	9.9 ± 1.3	10.3 ± 4.4	6.8 ± 2.7	5.5 ± 2.4	5.2 ± 1.0	n.s.
Fe_{Root}	83.5 ± 1.0	86.4 ± 2.0	76.5 ± 7.3	90.3 ± 3.0	90.5 ± 2.2	n.s.
Zn_{Leaf}	21.6 ± 1.8^{b}	19.9 ± 3.4^{b}	35.3 ± 3.8^{a}	14.5 ± 1.9^{b}	16.0 ± 3.2^{b}	***
Zn_{Stem}	25.9 ± 3.8	36.9 ± 6.0	23.1 ± 6.1	24.6 ± 3.9	31.8 ± 3.1	n.s.
Zn_{Root}	52.5 ± 4.4	43.2 ± 8.5	41.6 ± 6.6	60.9 ± 2.6	52.2 ± 3.6	n.s.
Mn_{Leaf}	41.3 ± 4.7^{ab}	23.3 ± 6.3^{c}	52.2 ± 6.4^{a}	34.8 ± 5.3^{bc}	26.8 ± 3.7^{bc}	**
Mn_{Stem}	18.3 ± 2.8	20.8 ± 3.2	11.0 ± 2.3	16.9 ± 7.6	8.4 ± 1.9	n.s.
Mn_{Root}	40.4 ± 5.0^{bc}	55.9 ± 6.3^{ab}	36.8 ± 4.5^{c}	48.3 ± 5.1^{bc}	64.8 ± 4.2^{a}	**
Cu_{Leaf}	26.7 ± 2.1^{b}	30.5 ± 5.2^{b}	47.4 ± 3.2^{a}	35.5 ± 3.46^{b}	30.1 ± 4.3^{b}	**
Cu_{Stem}	36.2 ± 4.0	45.1 ± 3.5	33.9 ± 4.6	47.7 ± 5.01	42.0 ± 2.7	n.s.
Cu_{Root}	37.1 ± 2.6^{a}	24.4 ± 3.9^{b}	18.7 ± 2.5^{b}	16.8 ± 3.26^{b}	27.9 ± 4.8^{ab}	**

Values are means ± SE. Different letters indicate significant differences among treatments (n.s.- not significant, *P < 0.05, **P < 0.01, ***P < 0.001).

Tuteja, 2010; Pinto-Marijuan and Munne-Bosch, 2014). Nonetheless, when accumulated at high level, H_2O_2 becomes toxic because it can be converted to OH˙ (Belkadhi et al., 2014). Still, none of the droughted plants overaccumulate H_2O_2 in relation to the WW controls. Furthermore, H_2O_2 being the product of other ROS detoxification (Gill and Tuteja, 2010; Pinto-Marijuan and Munne-Bosch, 2014), it is also a potent signaling molecule, due to its long half-life and the ability to cross cellular membranes (Petrov and Van Breusegem, 2012).

Table 4
Nutrients acquisition (A, g.plant^{-1}for macronutrients and mg.plant^{-1} for micronutrients) and root uptake efficiency (RUE) (macronutrients, mg.g^{-1} root, and micronutrients, μg.g^{-1} root) of well-watered (WW) and droughted (D) plus salicylic acid plants at the end of the experiment.

	WW	D0	D10	D100	D1000	Sig.
N_{A}	1.43 ± 0.08	1.20 ± 0.06	1.21 ± 0.10	1.38 ± 0.07	1.33 ± 0.12	n.s.
P_{A}	0.393 ± 0.031^{a}	0.216 ± 0.004^{c}	0.240 ± 0.011^{bc}	0.284 ± 0.028^{b}	0.267 ± 0.011^{bc}	***
K_{A}	1.142 ± 0.111^{a}	0.612 ± 0.017^{b}	0.478 ± 0.078^{b}	0.743 ± 0.111^{b}	0.728 ± 0.093^{b}	***
Ca_{A}	0.301 ± 0.037	0.285 ± 0.059	0.259 ± 0.019	0.318 ± 0.029	0.273 ± 0.035	n.s.
Mg_{A}	0.079 ± 0.003	0.071 ± 0.010	0.074 ± 0.009	0.091 ± 0.005	0.083 ± 0.006	n.s.
S_{A}	0.14 ± 0.01^{a}	0.105 ± 0.008^{b}	0.087 ± 0.007^{b}	0.110 ± 0.008^{b}	0.098 ± 0.011^{b}	**
B_{A}	3.43 ± 0.22	3.18 ± 0.26	3.42 ± 0.40	4.55 ± 0.29	3.89 ± 0.44	n.s.
Fe_{A}	7.78 ± 0.76^{b}	8.35 ± 1.51^{b}	5.83 ± 0.87^{b}	18.3 ± 3.1^{a}	11.8 ± 1.3^{ab}	***
Zn_{A}	2.78 ± 0.33^{ab}	2.22 ± 0.37^{bc}	1.82 ± 0.29^{c}	3.60 ± 0.18^{a}	3.04 ± 0.30^{ab}	**
Mn_{A}	2.25 ± 0.40^{c}	2.56 ± 0.76^{c}	3.32 ± 0.40^{bc}	4.96 ± 0.39^{a}	4.09 ± 0.34^{ab}	**
Cu_{A}	1.86 ± 0.21^{a}	1.12 ± 0.16^{b}	1.33 ± 0.02^{b}	1.42 ± 0.16^{ab}	1.49 ± 0.13^{ab}	*
N_{RUE}	54 ± 1.5^{a}	43.9 ± 3.9^{b}	46.8 ± 2.7^{ab}	40.3 ± 0.6^{b}	46.9 ± 3.0^{ab}	*
P_{RUE}	14.8 ± 0.8^{a}	7.83 ± 0.36^{b}	9.32 ± 0.33^{b}	8.21 ± 0.48^{b}	9.47 ± 0.57^{b}	***
K_{RUE}	43.1 ± 3.6^{a}	22.3 ± 1.5^{b}	18.8 ± 3.7^{b}	21.3 ± 2.5^{b}	25.4 ± 2.6^{b}	***
Ca_{RUE}	11.3 ± 1.3	10.1 ± 1.8	10.3 ± 1.4	9.25 ± 0.72	9.61 ± 1.20	n.s.
Mg_{RUE}	2.99 ± 0.17	2.54 ± 0.31	2.86 ± 0.32	2.64 ± 0.11	2.93 ± 0.20	n.s.
S_{RUE}	5.43 ± 0.29^{a}	3.86 ± 0.50^{b}	3.38 ± 0.30^{b}	3.20 ± 0.12^{b}	3.45 ± 0.40^{b}	***
B_{RUE}	129.6 ± 4.6	114.7 ± 8.6	130.1 ± 6.6	133.0 ± 8.1	137.8 ± 15.7	n.s.
Fe_{RUE}	294.4 ± 26.0^{b}	295.1 ± 44.8^{b}	225.2 ± 32.4^{b}	541.7 ± 103.1^{a}	415.2 ± 34.7^{ab}	**
Zn_{RUE}	104.5 ± 8.6^{a}	78.7 ± 10.0^{ab}	72.1 ± 14.4^{b}	106.0 ± 7.8^{a}	106.58 ± 7.9^{a}	*
Mn_{RUE}	85.0 ± 15.1^{b}	90.4 ± 24.9^{b}	129.0 ± 16.9^{ab}	146.2 ± 14.2^{a}	144.4 ± 10.3^{a}	*
Cu_{RUE}	69.7 ± 5.6^{a}	39.4 ± 4.1^{b}	52.4 ± 4.5^{b}	40.9 ± 2.7^{b}	52.9 ± 5.0^{b}	***

Values are means ± SE. Different letters indicate significant differences among treatments (n.s.- not significant, *P < 0.05, **P < 0.01, ***P < 0.001).

Table 5
Physiological nutrient use efficiency (UE) (g biomass g $^{-1}$ mineral for macronutrients and mg biomass g^{-1} mineral for micronutrients) of well-watered (WW) and droughted (D) plus salicylic acid plants at the end of the experiment.

	Nutrient Use Efficiency					Sig.
	WW	D0	D10	D100	D1000	
N_{UE}	95.3 ± 2.7	86.4 ± 3.4	93.2 ± 3.3	90.4 ± 3.0	87.6 ± 3.7	n.s.
P_{UE}	351.3 ± 23.4^{b}	480.0 ± 26.6^{a}	467.3 ± 22.2^{a}	448.4 ± 23.8^{a}	432.2 ± 11.5^{a}	**
K_{UE}	123.2 ± 12.8^{b}	168.7 ± 5.8^{b}	257.1 ± 36.2^{a}	182.8 ± 27.4^{b}	167.8 ± 21.9^{b}	*
Ca_{UE}	484.7 ± 72.8	422.4 ± 82.6	440.2 ± 38.3	402.6 ± 30.8	444.7 ± 49.7	n.s.
Mg_{UE}	1729.6 ± 66.0	1581.4 ± 246.1	1572.7 ± 136.2	1388.9 ± 66.0	1404.5 ± 67.8	n.s.
S_{UE}	956.3 ± 60.7	1010.8 ± 87.9	1332.6 ± 147.0	1142.1 ± 40.1	1265.7 ± 192.7	n.s.
B_{UE}	39.8 ± 1.7^{a}	33.2 ± 2.7^{ab}	33.8 ± 2.7^{ab}	27.8 ± 1.9^{b}	30.9 ± 3.0^{b}	*
Fe_{UE}	19.2 ± 1.6^{ab}	15.5 ± 3.9^{ab}	26.5 ± 6.5^{a}	8.02 ± 1.39^{b}	10.7 ± 1.3^{b}	*
Zn_{UE}	50.5 ± 4.5^{ab}	51.6 ± 8.3^{ab}	67.0 ± 9.0^{a}	35.0 ± 2.5^{b}	38.9 ± 3.0^{b}	*
Mn_{UE}	67.7 ± 10.8^{a}	57.1 ± 17.5^{ab}	35.7 ± 4.4^{bc}	25.8 ± 2.2^{c}	28.4 ± 0.6^{bc}	*
Cu_{UE}	75.9 ± 7.4	102.6 ± 17.8	84.7 ± 6.0	90.3 ± 5.3	79.7 ± 8.3	n.s.

Values are means ± SE. Different letters indicate significant differences among treatments (n.s.- not significant, *P < 0.05, **P < 0.01).

Interestingly, the distinct pattern of H_2O_2 and total ROS accumulation during drought, with higher H_2O_2 accumulation in WW, D100 and D1000 treatments might be a reflex of the balance between each type of ROS. Thus, D0 and D10 plants seems to display a reduced capacity to scavenge and detoxify highly reactive species. In agreement, there are evidences that SA pretreatment induces H_2O_2 production (Chen et al., 2007; Harfouche et al., 2008; Belkadhi et al., 2014), which in turns might induce antioxidant enzymes activity, decreasing cellular ROS levels (Arfan, 2009).

General responses to stress involve the signaling stress detection and, consequently, the increase in antioxidant responses (Farooq et al., 2009). The synthesis of stress proteins plays crucial role in drought tolerance development (Farooq et al., 2009). However, although in some studies total soluble proteins increased in response to drought (Bacelar et al., 2006, 2007), we found a markedly decline of TSP in D0 plants, in agreement with other experiments (El-Tayeb, 2005; Jalal et al., 2012; Kabiri et al., 2014). In fact, depending on drought severity (Jalal et al., 2012), the generation of ROS causes the oxidation of amino acids and burst proteins structure (Kabiri et al., 2014). Notably, SA prevented the TSP decline and/or induced its overaccumulation in droughted leaves, starting to stabilize during the recovery period. The positive effect of SA on proteins accumulation is commonly observed in stressed plants, either through developing mechanisms that avoids their degradation or by inducing specific stress related proteins (Jalal et al., 2012; Kang et al., 2012; Belkadhi et al., 2014; Hashempour et al., 2014; Kabiri et al., 2014). In fact, the application of SA changed the protein patterns of drought stressed plants (Jalal et al., 2012; Kang et al., 2012), inducing the expression of proteins associated with signal transduction, stress defense, photosynthesis, carbohydrate metabolism, protein metabolism, and energy (Kang et al., 2012). Again, the lower -SH concentrations of D0 plants during drought stress must be the result of the oxidation by ROS (Zagorchev et al., 2013). In agreement, similar results were reported in droughted olive trees by Bacelar et al. (2006, 2007). The maintenance of high -SH levels during water scarcity is an advantage to SA-treated plants, since -SH groups are involved in the antioxidant defense system and, thus, they are crucial to plants stress tolerance (Zagorchev et al., 2013).

The restauration and/or overaccumulation of drought-declined β-carotene concentration after stress relief suggests an important function of this carotenoid in recovery processes. β-carotene allows the physical quenching of ^{3}Chl* and $^1O_2$ (Pinto-Marijuan and Munne-Bosch, 2014), helping in the detoxification processes. Many abiotic stresses, and the resulted oxidative stress, induce the phenylpropanoid metabolism in plants (Grace, 2007), leading to an accumulation of phenolic compounds in olive leaves in response to drought (Bacelar et al., 2006; Petridis et al., 2012), as observed in our study. Noteworthy, the increase of TPC in D0 plants during drought was largely associated with the increase in flavonoids, important bioactives plant secondary metabolites (Gill and Tuteja, 2010). This association suggests an increased necessity of protection against photooxidative damage, as flavonoids protects photosynthetic apparatus against photoinhibition under excessive light (Zhou et al., 2016) and absorbs the damaging UV-B radiation (Grace, 2007). The lower accumulation of TPC in SA-treated plants during drought, mainly in D100 and D1000, is in conformity with a previous study in salt and drought stressed barley plants (Fayez and Bazaid, 2014). Such data suggests a reduced oxidative stress in D100 and D1000 trees, which was confirmed by the lower accumulation of ROS in these plants, and/or the investment in other defense mechanisms, such as the maintenance of high TSP concentrations. Meanwhile, ascorbate, besides to directly scavenge ROS, is also substrate to ascorbate peroxidase, that use it as specific electron donor to reduce H_2O_2 to H_2O (Pinto-Marijuan and Munné-Bosch, 2014; Mattos and Moretti, 2015). Thus, the overaccumulation of ascorbate and the lowest H_2O_2 concentration in D0 suggest an improvement of ascorbate peroxidase activity. Moreover, in chloroplasts ascorbate acts as a cofactor of violaxantin de-epoxidase, sustaining the dissipation of excess excitation energy (Mattos and Moretti, 2015), suggesting again higher necessity of photoprotection.

The higher TAC exhibited by D0 plants at the peak of drought claim the role of phenolic compounds as potent free radical scavengers, since the measured TAC depends on the reduction of DPPH radical, where phenolics are known to overcome other antioxidants (Xu and Chang, 2007; Mattos and Moretti, 2015). In agreement, the lower accumulation of phenolic compounds in SA-treated plants than in D0 plants was also evident on TAC data. Still, the higher TAC of D100 plants, relatively to D10 and D1000 plants suggests that other antioxidant substances, rather phenolic compounds, were involved in this response, that were further repressed during rewatering.

In short, D10 plants although invested largely in TSP accumulation, exhibited a close behavior to D0 plants. D0 plants, exhibited higher accumulation of phenolic compounds and ROS, and invested in β-carotene and ascorbate pool restauration upon rewatering. The responses induced by D100 and D1000 treatments were more similar. D100 plants had a slightly higher and lower accumulation of TSP and ROS, respectively, and increased the investment in TPC and β-carotene concentrations, suggesting that 100 μM SA induced drought tolerance by reducing the oxidative damages caused by ROS levels in association with higher concentration of soluble proteins and antioxidant responses. Upon rewatering, both D100 and D1000 plants increased largely the investment in phenolic compounds. However, although D100 plants reduced TSP to D1000 and WW levels, continued to display the lower and higher concentrations of total ROS and H_2O_2, respectively. This response might be the result of a higher detoxification of stronger ROS. By this, the activation or restoration of other mechanisms and/or metabolites, like ascorbate, may be involved in this response.

4.2. Plant ionome dynamics

Olive trees in many areas of the world are among the least fertilized trees, and because of such inadequate nutrition, among other factors, biennial bearing is quite frequent (Therios, 2009). Moreover, limited nutrient uptake and reduced minerals concentrations is a regular response to lower water availability (Farooq et al., 2009), although in our study we only observed relevant decreases in some elements (P, K, S and Cu), in a closely association with root uptake efficiency. Several reasons can be given for the reduction of nutrient uptake under drought stress, including the lower transpiration rate, due to inferior stomatal conductance and total leaf area, as demonstrated previously (Brito et al., 2018b), the inhibition of ATP synthesis, the reduction of nutrient supply through mineralization (Sanaullah et al., 2012), the reducing nutrient diffusion and mass flow in the soil (Chapin, 1991) and the decrease in the concentration of root nutrient-uptake proteins (Bista et al., 2018). It is noteworthy that the decline in the contents of those minerals in some plant organs was evident even with the concentration effect due to the lower production of biomass. In opposition, drought-starved SA plants presented higher contents of Mg and B on stems and roots, respectively, in association with higher allocation of minerals to these organs. In the same way, these plants showed higher allocation of N to roots, at the expenses of leaves, and of P to leaves, at the expenses of stems, suggesting that the change on the allocation of minerals is an adaptive mechanism for growth under water limitation, confirming that the 'functional equilibrium' or 'resource balancing' theory might apply for the allocation of mineral nutrients within plants, as proposed by Weih et al. (2011). Meanwhile, as drought stress did not affect the uptake and transport of all minerals to the same extent, changes in the element stoichiometry may cause nutrient imbalances, conducting to perturbations of physiological functions, as reported earlier (Brito et al., 2018a,b), and to a reduction of biomass accumulation. In addition, nutrient imbalances can also lead to ROS formation, as demonstrated above, which can result in oxidative stress. Thus, for all these reasons, we believe that the optimization of fertilizer practices in a drought environment would offer a considerable challenge.

Notably, this study clearly demonstrated that plant ionome of droughted plants was modulated by the application of SA. Although some reductions in element contents were observed, as P, K and S in leaves, and Mg in stems, mainly associated to changes in allocation patterns among organs, in general organs of SA-treated plants presented higher contents of mineral elements, as P and S on stems, Fe and Mn on leaves, and K, Mg, B, Fe, Mn and Zn on roots, highlighting D1000 and, mainly, D100 plants. The contents on roots are particularly interesting, as the higher values were obtained in spite of the higher root biomass accumulation in D100 plants, meaning that the responses were more related with enhanced nutrient root uptake efficiency, namely for Fe, Zn and Mn. Overall, the drought-induced decline in minerals uptake and contents were attenuated, and in some cases even overcompensated by SA application in relation to WW controls. Despite the fact that few studies have been done, particularly that they include all plant organs, these results were consistent with other works who reported nutrient status improvement by SA application in drought and salt stressed plants (El-Tayeb, 2005; Gunes et al., 2007; Yildirim et al., 2008; Nazar et al., 2015). In the present study it is also notorious that exogenous SA affected micronutrients at a higher extent than macronutrients. Although micronutrients are presented in plant tissues in much lower concentration than macronutrients, they are largely required to activate several physiological, biochemical and metabolic processes, being essential to help macronutrients in growth and drought alleviation (Waraich et al. 2011; Duman, 2012).

The higher content of K on roots of D100 and D1000, relatively to D0 plants, has a significant relevance as K is a principal cation in vacuoles, contributing to osmotic adjustment and thus to increased expansion of cells via high cell turgor pressure, while the superior root Mg, more evident in D1000 plants, is important due to the involvement in protein synthesis and phosphorylation reactions, and as enzymatic cofactor (Grusak, 2001; Waraich et al., 2011). Meanwhile, B has major functions on the structure of cell walls and on regulation of carbohydrates metabolism, promoting root cell elongation and, thus, root growth (Li et al., 2016), whereas zinc is a micronutrient required in many plant processes, as various enzymatic and oxidation-reduction reactions, membrane integrity, energy transfer and protein synthesis (Hafeez et al. 2013). Zn is also involved in tryptophan synthesis, a precursor of an essential growth hormone, IAA (Waraich et al. 2011; Hafeez et al. 2013), that enhance root growth, which in turn improves drought tolerance. Furthermore, the general higher Fe and Mn contents, both on leaves and roots of superior exogenous SA treatments, have major effects on drought adaptation as Fe is required for chlorophyll synthesis, respiration, photosynthesis and is an enzymatic cofactor (Rout and Sahoo, 2015). In addition, iron promote cell division in the meristematic cells of adventitious root primordial, as well the lateral root elongation, mediated by auxins signals (Hilo et al., 2017). On the other hand, Mn assists iron in chlorophyll formation, is fundamental on the splitting of water and on electron transport to the chlorophyll reaction centers, being also and enzymatic cofactor, including in an important antioxidant enzyme, superoxide dismutase (Ciríaco da Silva et al., 2011). Thus, for all these reasons we may assume that SA regulates the responses of olive tree to drought stress and could be used as plant growth regulator to rebalancing mineral nutrients stoichiometry in plant tissues and to increase nutrient reserves that can promote plant's capacity to recover from drought stress events.

Nutrient allocation reflects the balance between the capacity to obtain, transport and store nutrients (He et al., 2015), being also dependent on where and how nutrients are used by the plant, and whether this pattern is changed under atypical conditions (McGrath and Lobell, 2013). In this way, the different allocation patterns verified in SA-treated plants suggest a selective behavior according to the plant needs, for instance of elements that acts as antioxidant-enzyme cofactors (Ciríaco da Silva et al., 2011; Waraich et al. 2011), which is also supported by the higher concentration of TSP in these plants. Likewise, the influence of SA in plant minerals allocation was also confirmed by other studies (El-Tayeb, 2005; Yildirim et al., 2008). Meanwhile, minor changes in physiological nutrients use efficiency were induced by water availability. In fact, only PUE was affected, being higher under drought stress, which suggests a better distribution of phosphorus resources among the different metabolic processes involved in biomass production and, thus, we may conclude that P use efficiency is an important trait to improve growth under drought conditions. On the contrary, SA provoked higher changes on nutrients use efficiency, depending from dosage, as D10 plants presented enhanced K_{UE}, Fe_{UE} and Zn_{UE} values, mainly relatively to the D100 plants, in a strictly association with the higher allocation of these minerals into leaves and to a lesser investment of minerals in protection mechanisms.

4.3. Biomass accumulation and allocation and implications in ionome

The reduced growth and dry matter accumulation under drought are well established (Bacelar et al., 2007; Farooq et al., 2012), primarily due to lower leaf area dimension and to stomatal closure and then due to the inhibition of C assimilation by photosynthetic impairment (Medrano et al. 2002; Farooq et al. 2009). In fact, none of the stressed plants reached the TBI exhibited by WW controls, although SA attenuated this decline in a dose dependent manner, highlighting D100 treatment. To illustrate, while WW plants showed a mean accumulation of 135.4 g of dry mass per plant, D0, D10, D100 and D1000 showed values of 103.4, 118.8, 125.2 and 115.6 g per plant, respectively (Brito et al., 2018a). Likewise, this positive effect of SA has been described on the literature (Umebese et al., 2009; Habibi, 2012; Kang et al., 2012; Fayez and Bazaid, 2014; Nazar et al., 2015). This response was found to be related with the higher capacity to maintain and recover the photosynthetic performance, during and after drought relief (Brito et al., 2018a). Moreover, the maintenance of a better balance between oxidants and antioxidants, as discussed above, certainly contributed to the improved photosynthetic capacity and consequent biomass accumulation. In the same way, Gunes et al. (2007) argued that the dry matter increases in SA-treated salt stressed plants might been related to the induction of antioxidant responses. In line with the higher biomass accumulation D100 plants increased the acquisition of some minerals, mainly micronutrients (Table 4), reflecting selective acquisition induced by SA.

A differential partitioning of dry matter between root and shoot is commonly observed in Mediterranean species to improve tolerance to repeated cycles of drought (Toscano et al., 2014; Brito et al., 2018a). As observed in our study, with special emphasis in D100 plants, instead of investing in photosynthetic tissues, olive tree enhances the allocation of dry matter into roots, as in other studies (Umebese et al., 2009; Kang et al., 2012; Aliniaeifard et al., 2016), in order to improve water and minerals uptake from soil, as confirmed by the higher root uptake efficiency of some micronutrients (Table 4) and the improved water status of those plants (Brito et al., 2018a). Moreover, roots also give mechanical support to plants and supply hormones that affect many physiological and biochemical processes associated with growth and development (Fageria and Moreira, 2011).

5. Conclusions

Overall, the results successfully confirmed our previous hypothesis, as a suitable concentration of SA (100 μM SA) improves olive trees drought adaptability. This effectiveness is achieved by the root biomass accumulation, the higher uptake of essential minerals, mainly micronutrients, and the improvement of the equilibrium between ROS production and scavenging. Notably, our findings give new insights about how SA regulate drought and recovery responses in olive tree. This understanding is of great importance as olive tree growing areas are typically subjected to oscillating drought-rewatering events. Moreover, in this study SA-treated plants were exposed to a realistic combination of abiotic stresses, making the present results very promising for established olive groves. Hence, the use of an appropriate SA concentration could be a cost-effective tool under the current and predicted extreme and variable environmental conditions, being a procedure of major interest in the commercial production of olive trees and others fruit crops.

6. Authors contribution

Cátia Brito was responsible for establish and maintain the experiment, collected data on the field, performed the laboratory analyses and was responsible for data analysis and manuscript writing. Lia-Tânia Dinis and José Moutinho-Pereira collaborated in data collection on the field and in the critical review of the article. Helena Ferreira collaborated in data collection on the field and assisted in the laboratory analyses. João Coutinho carried out the mineral's analysis. Carlos Correia was responsible for design the experiments, data collection on the field, critical review and final approval of the manuscript. All the authors reviewed and approved the final manuscript.

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