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Research article

Root verbascoside and oleuropein are potential indicators of drought resistance in olive trees (*Olea europaea* L.)

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ABSTRACT

Polyphenols are constituents of all higher plants. However, their biosynthesis is often induced when plants are exposed to abiotic stresses, such as drought. The aim of the present work was to determine the phenolic status in the roots of olive trees grown under water deficit conditions. The results revealed that roots of water-stressed plants had a higher content of total phenols. The main compound detected in well-watered olive tree roots was verbascoside. Oleuropein was established as the predominant phenolic compound of water-stressed plants. The oleuropein/verbascoside ratio varied between 0.31 and 6.02 in well-watered and water-stressed plants respectively, which could be a useful indicator of drought tolerance in olive trees. Furthermore, this study is the first to provide experimental evidence showing that luteolin-7-rutinoside, luteolin-7-glucoside and apigenin-7-glucoside were the dominant flavonoid glucosides in olive tree roots and showed the most significant variations under water stress.

1. Introduction

Olives (*Olea europaea* L.) are grown mostly in arid and semi-arid regions, where plants are frequently subjected to low availability of water in soil (Dichio et al., 2009), high root-zone salinity (Melgar et al., 2009), cold stress (Ortega-García and Peragón, 2009) and high irradiance levels (Remorini et al., 2009). All of which contribute to make the habitat harsh and unfavorable for plant development. Among all factors, severe water deficit is considered to be one of the most important abiotic factors limiting olive trees growth and survival in such areas (Fernández, 2014). It has been shown that water deficit alters the olive tree vegetative growth, xylem hydraulic properties and photosynthetic metabolism (Bacelar et al., 2007; Ben Abdallah et al., 2018), leads to the reduction in leaf and fruit turgor pressure and a decrease in the fruit growth (Girón et al., 2015), affects the quality and volatile composition of olive oils (Servili et al., 2007; Caruso et al., 2014) and generates reactive oxygen species (ROS) which oxidize photosynthetic pigments, proteins, membrane lipids, RNA and DNA (Bacelar et al., 2006; Ahmed et al., 2009a,b)

Most studies focusing on olive tree response to water deficit stress have emphasized growth aspects, photosynthetic and the intracellular accumulation of osmolytes functioning as osmoprotectants, such as sugars and proline (Bacelar et al., 2007; Ahmed et al., 2009a,b; Dichio et al., 2009; Ben Abdallah et al., 2018). More recently, it has been shown that the accumulation of phenolic compounds is a well-known adaptive mechanism in the olive tree against drought conditions (Cetinkaya et al., 2016; Petridis et al., 2012a) and salinity (Petridis et al., 2012b). Phenolic compounds accumulation, induced by water deficit stress in plants, has been shown to play a key role in cellular protection (Agati et al., 2012; Falahi et al., 2018). Phenolics have a great potential to scavenge ROS once they are formed during stress conditions (Nakabayashi and Saito, 2015).Generally the synthesis of phenolic compounds is stimulated in response to abiotic/biotic stresses (Gaquerel et al., 2014; Nakabayashi and Saito, 2015).

Studies on phenolic compounds in *Olea europaea* L. have made progress (Quirantes-Piné et al., 2013; Talhaoui et al., 2014). However, most of the studies focused on comparison of metabolite variation in different *Olea europaea* organs such as olive fruits, leaves, buds and flowers (Cabrera-Bañegil et al., 2017; Laguerre et al., 2009; Taamalli et al., 2013). The prevalent classes of phenolic compounds found in olive fruits, leaves, buds and open flowers are phenolic alcohols (hydroxytyrosol and tyrosol), flavonoids (luteolin-7-rutinoside, apigenin-7-glucoside luteolin-7-glucoside, quercetin, apigenin, catechin), secoiridoids (oleuropein), hydroxycinnamic acid derivatives (verbascoside) and lignans (pinoresinol) (Cabrera-Bañegil et al., 2017; Laguerre et al., 2009; Taamalli et al., 2013). Other polar compounds were also

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identified in olive fruits and leaves such as quinic acid, gallic acid, vanillin and p-hydroxybezoic acid (Quirantes-Piné et al., 2013; Cabrera-Bañegil et al., 2017). However, other *Olea europaea* organs, such as roots, have received little attention leading to a limited knowledge about their phytochemical composition. The interest in the phenolic compounds in the plant roots derives from the fact that they are considered to have an ecological role (Mandal et al., 2010). Some studies suggested that they are related to plant defense mechanisms against pathogenic attack (Khan et al., 2010). A range of conjugated and soluble phenolic compounds involved in nodule morphogenesis and rhizobial defense have been detected in root nodules and roots of *Arachishypogaea* L. (Chakraborty and Mandal, 2008). Phenolic compounds are also known to play multifunctional roles in rhizospheric plant-microbe interactions (Martens, 2002). It has been shown that phenolic compounds play a key role in the establishment of plant root endosymbioses with arbuscular mycorrhiza fungi, rhizobia and Frankia bacteria (Abdel-Lateif et al., 2012). Mandal et al. (2010) reported that the accumulation of flavonoids and phenolic compounds in the host, as a result of arbuscular mycorrhizal fungi inoculation, acts as signaling molecules in the initiation of the establishment of arbuscular mycorrhizal symbioses and can act as agents in plant defense. In our previous study, we have indicated that olive tree roots contain significant amounts of phenolic compounds, important factors for antioxidant capacity, which can be substantially modified by colonization of olive trees with arbuscular mycorrhizal fungi (Mechri et al., 2015). All together, these data suggest that the change of the phenolic compounds pattern plays a regulatory role in plant roots.

According to the literature, the main components of olive roots are hydroxytyrosol, tyrosol, catechin and oleuropein (Del Río et al., 2003; Ortega-García and Peragón, 2010; Petridis et al., 2012a). Changes in hydroxytyrosol and oleuropein levels under water deficit stress (Petridis et al., 2012a), salt stress (Petridis et al., 2012b) and cold stress (Ortega-García and Peragón, 2009) have been reported adequately. However, rather limited data are available concerning other constituents such as flavonoids (apigenin-7-glucoside, luteolin-7-rutinoside and luteolin-7-glucoside), lignans (pinoresinol) and cinnamic acid derivatives (verbacoside). Therefore, the aim of the present work was to identify and quantify different kinds of phenolic compounds with significant physiological and biological functions in olive tree roots, and to establish similarities and differences in terms of identification and quantification of the phenolic compound variation in olive trees subjected or not to water deficit stress (well-watered vs water-stressed plants).

2. Material and methods

2.1. Experiment description

A greenhouse experiment was performed to determine the phenolic status in the roots of olive trees (*Olea europaea* L. cv. Meski) grown under well-watered and water deficit conditions. Olive plantlets (20 cm long and three pairs of leaves) were grown in a glasshouse in 10 L pots filled with a sandy-loam soil (55% sand, 30% silt, 15% clay) collected directly from an olive tree field. Olive trees were grown for 9 months under natural daylight under greenhouse conditions. The mean temperature, relative humidity and photosynthetic photon flux density inside the glasshouse were respectively 50–65%, 25°-32 °C and about 1200 μmmol m^{-2} s^{-1} at midday, on a clear day. After nine months of plant growth, uniform plants (based on leaf number, height and total leaf area) were selected for the experiment. One group of plants was used as a control in which plants were watered every two days to maintain their soil water content near to field capacity (ψ soil = 0.01 MPa). The other group of plants were stressed by withholding irrigation. Experiment was stopped when soil moisture in stressed plants decreased less than soil wilting point after 30 days (−2.5 MPa). Well-watered and water-stressed trees were placed randomly with three replicates per treatment (30 plants were used corresponding to five plants for each treatment). At the end of the experiment, well-watered and water-stressed olive plants were harvested and fine root samples were collected from each treatment.

2.2. Extraction of phenolic compounds

1 g from well-watered and drought-stressed fresh olive tree roots was extracted in 10 ml of methanol (HPLC gradient grade) on a shaker at 200 rpm for 24 h. The obtained extracts were centrifuged for 10 min at 5000 rpm, filtered (nylon filters of 0.45 μm) and stored in opaque vials at −20 °C until analyses (Taamalli et al., 2012).

2.3. Determination of total phenols in roots of well-watered and water-stressed plants

Total phenolic contents were determined using Folin & Ciocalteu reagent according to the method of Montedoro et al.(1992) with slight modifications. 0.4 ml of root methanolic extracts was mixed with 10 ml of diluted Folin–Ciocalteu reagent and 8 ml of sodium carbonate solution (75 g/L). The mixtures were allowed to stand for 1 h in obscurity and then the absorbance was measured at 765 nm.

2.4. HPLC analysis of phenolic compounds

In order to identify and quantify the main polyphenols, olive root extracts were analyzed using an HPLC (High-performance liquid chromatography) instrument (Agilent LC 1100 series; Agilent Technologies, Inc., Palo Alto, CA, USA) controlled by the Chemstation software. HPLC separation of root extracts was carried out on a Hewlett-Packard system comprising a Rheodyne model 7725 injector (Cotati, CA, USA, loop volume 20 μl), an UV detector and a HP-1100 pump equipped with a C18 Eclipse XDB column (4.6 × 250mm, 5 μm) from Agilent Technologies. The flow rate was 0.8 ml/min, and the absorbance was detected at 280 nm. Separation was carried out through a linear gradient method using 0.2% sulfuric acid (A) and acetonitrile (B), starting the sequence with 15% B and programming the gradient to obtain 40% B at 12 min, 60% B at 14 min, 80% B at 18 min, 90% B at 20 min, 100% B at 24 min. Phenolic compounds in olive root extracts were then identified by comparison of their retention times with the corresponding standards. Cinnamic acid was used as internal standard for the quantification of polyphenols.

2.5. Statistical analysis

All of the experiments were repeated three times and the results were expressed as the mean value ± standard deviation. Statistical comparisons were made using one-way analysis of variance (ANOVA) followed by Duncan post hoc test for multiple comparisons (SPSS, version 16). Differences were considered to be significant at $p < 0.05$.

3. Results and discussion

3.1. Chromatographic separation

Fig. 1A shows the typical HPLC–UV chromatogram of the standard mixture solution. All investigated phenolic compounds had responses at 280 nm, where they were successfully separated. Fig. 1B and Fig. 1C show the peak chromatograms of well-watered and water-stressed plants respectively. The compounds were identified by comparing their retention times with those of authentic standards. As can be seen from Fig. 1B and C, the identified phenolic compounds, under well-watered and water-stressed conditions, belong to different classes; namely, secoiridoid (oleuropein), hydroxycinnamic acid derivatives (verbascoside), phenolic alcohols (hydroxytyrosol and tyrosol), flavonoids (catechin hydrate, quercetin, apigenin, luteolin-7-rutinoside, apigenin-7-glucoside and luteolin-7-glucoside) and lignan (pinoresinol).

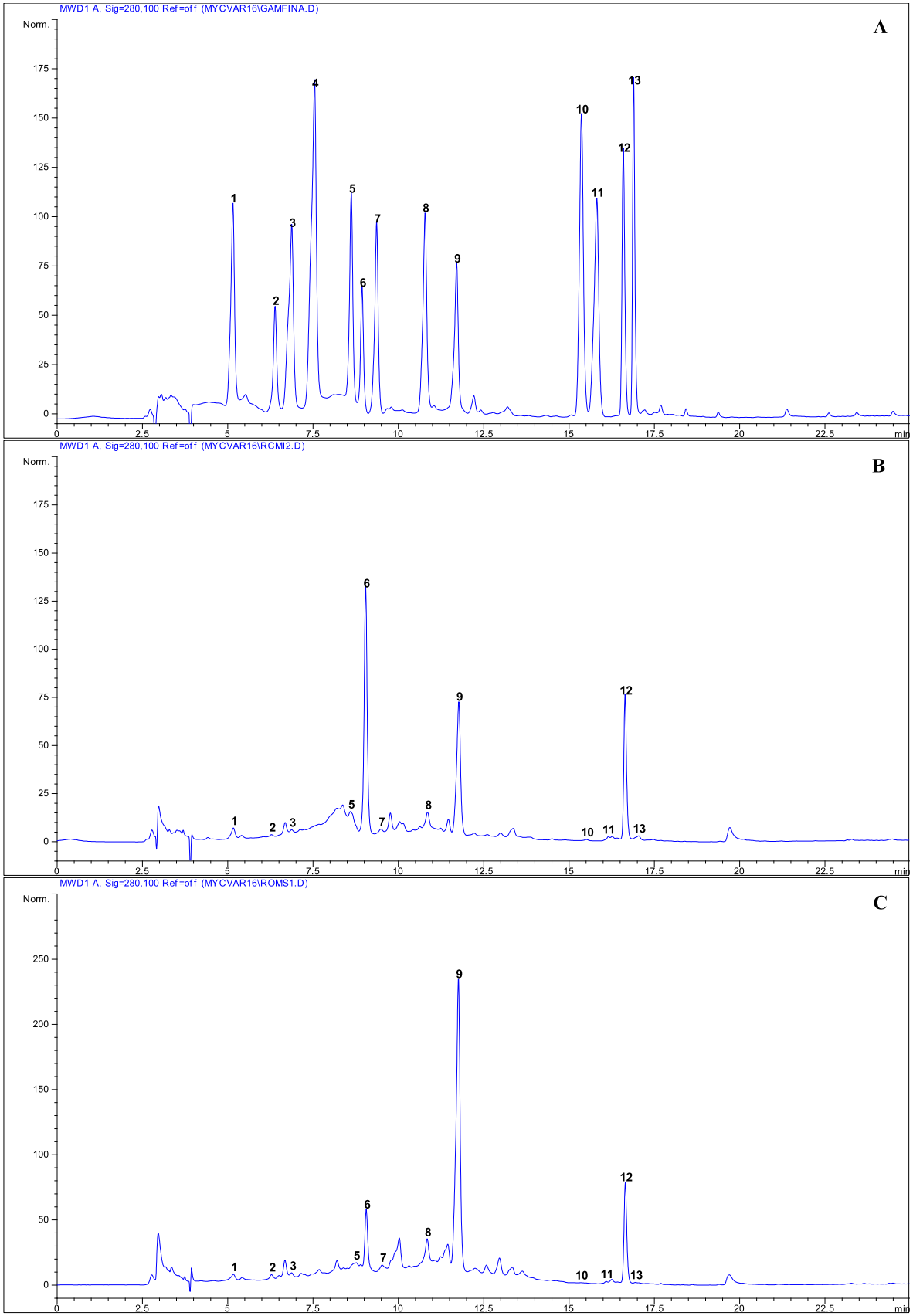

Fig. 1. Typical HPLC–UV chromatograms of standard mixture solution (A), roots of well-watered (B) and roots of water-stressed plants (C). 1. hydroxytyrosol; 2. catechin hydrate; 3. tyrosol; 4. p-Hydroxybenzoic acid; 5. luteolin-7-rutinoside; 6. verbascoside; 7. luteolin-7-glucoside; 8. apigenin-7-glucoside; 9. oleuropein; 10. quercetin; 11. pinoresinol; 12. cinnamic acid; 13. apigenin.

To compare the relative contents of the eleven phenolic compounds under well-watered and water-stressed treatments, data were subjected to one-way analysis of variance (ANOVA) followed by Duncan post hoc test. The results showed that the concentrations of oleuropein ($P < 0.001$), tyrosol ($P = 0.005$), hydroxytyrosol ($P < 0.001$), luteolin-7-rutinoside ($P = 0.025$), luteolin-7-glucoside ($P < 0.001$), apigenin -7-glucoside ($P < 0.001$), quercetin ($P = 0.003$) and verbascoside ($P < 0.001$) were all significantly affected under water-stressed conditions. However, the level of apigenin ($P = 0.236$), catechin ($P = 0.354$) and pinoresinol ($P = 0.611$) did not appear to be significantly influenced by water deficit. Moreover, the relative contents of oleuropein, luteolin-7-glucoside and apigenin -7-glucoside were higher under water-stressed treatment than those under well-watered treatment. The increased contents of these phenolic compounds indicated resistance to the water deficit stress and an adaptation to the abiotic stress.

3.2. Concentration of phenolic compounds in the roots of well-watered and water-stressed plants

Most of woody plants have different mechanisms when responding to drought stress. Accumulation of osmotically active compounds such as sugars, sugar alcohols, amino acids, numerous terpenes and simple or complex phenols is one of the compatibility mechanisms (Dichio et al., 2009; Selmar and Kleinwächter, 2013). In our study, water deficit caused a significant increase in total phenolic content in the root system. After 30 days of water deficit, the total phenol content in roots of water-stressed plants was 60% higher than in well-watered plants (Fig. 2). The induction of phenylpropanoid metabolism in olive trees have been observed in various environmental stresses and it was claimed that phenolics acted as antioxidants (Ahmed et al., 2009a,b; Ortega-García and Peragón, 2009; Remorini et al., 2009). Petridis et al. (2012a) determined the changes in total phenol content, malondialdehyde content and antioxidant activity in the leaves of four greek olive cultivars ('*Gaidourelia*', '*Kalamon*', '*Koroneiki*' and '*Megaritiki*') grown under water deficit conditions. These authors showed that the cultivar '*Gaidourelia*' possessed the highest phenolic concentration and antioxidant activity and the lowest lipid peroxidation. These results clearly demonstrate the protective effect of phenolic compounds against water deficit stress. Another mechanism underlying the antioxidant properties of phenolics is their ability to alter peroxidation kinetics by modification of the lipid packing order and to reduce membrane fluidity (Arora et al., 2000; Wu et al., 2013).

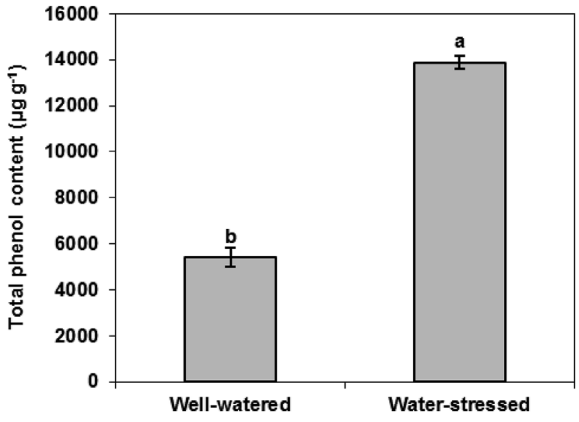

Fig. 2. Total phenol content in roots of well-watered and water-stressed olive trees (cv. Meski). Bars represent the mean of each treatment, and error bars indicate standard deviation (n = 3). Means with different letters are significantly different at $p < 0.05$ (Duncan test).

Table 1
Content of phenolic alcohols, flavonoids, lignan, secoiridoids and hydroxycinnamic acid derivatives, expressed in µg/g (mean ± SD%, n = 3), in roots of well-watered and water-stressed olive trees.

Compounds	well-watered	water-stressed
Phenolic alcohols		
Hydroxytyrosol	110.88 ± 5.89[a]	54.01 ± 5.96[b]
Tyrosol	88.99 ± 5.95[a]	67.41 ± 2.91[b]
Flavonoids		
Catechin hydrate	66.27 ± 5.05[a]	68.14 ± 5.39[a]
Luteolin-7-rutinoside	157.82 ± 2.54[a]	150.66 ± 3.19[b]
Luteolin-7-glucoside	87.65 ± 14.05[b]	198.21 ± 10.99[a]
Apigenin-7-glucoside	212.71 ± 17.98[b]	366.25 ± 8.43[a]
Quercetin	32.55 ± 3.05[a]	15.82 ± 3.33[b]
Apigenin	34.08 ± 2.04[a]	35.78 ± 0.55[a]
Lignan		
Pinoresinol	17.06 ± 3.31[a]	18.89 ± 4.73[a]
Secoiridoids		
Oleuropein	271.98 ± 23.13[b]	1795.41 ± 62.19[a]
Hydroxycinnamic acid derivatives		
Verbascoside	866.19 ± 34.45[a]	298.05 ± 17.43[b]
Specific ratio		
Oleuropein/Verbascoside	0.31 ± 0.01[b]	6.02 ± 0.14[a]

The effect of water deficit treatment was tested with one-way ANOVA (mean value ± SE, n = 3), and mean values in individual line followed by the same letter are not significantly different at $p < 0.05$ (Duncan test).

3.3. Verbascoside was the main phenolic compound found in the roots of well-watered plants

Verbascoside is a phenylethanoid glycoside present in several plant families that possess beneficial activities for human health (Alipieva et al., 2014). Generally, verbascoside can be considered as a phenolic compound of four chemical moieties: hydroxytyrosol (HT, phenylethanoidaglycone), caffeic acid (CA), rhamnose (Rha) and glucose (Glu, central saccharide) group (Qi et al., 2013).Verbascoside is a potent antioxidant extracted from olive oil mill wastewater (Cardinali et al., 2012). It was also detected in olive leaves (Fu et al., 2010; Quirantes-Piné et al., 2013), olive fruits (Cabrera-Bañegil et al., 2017), buds and flowers (Taamalli et al., 2013). Our results showed that verbascoside was the main phenolic compound detected in olive roots of well-watered plants (Table 1). Ortega-García and Peragón (2010) investigated the role of oleuropein, hydroxytyrosol and tyrosol in the metabolism of stems and roots of *Olea europaea* L. cv. *Picual* during olive ripening. The authors observed that in roots, the concentrations of the three phenolic compounds were significantly lower than in stems, and oleuropein was not the predominant phenolic compound found in the roots. The HPLC chromatogram obtained in this study revealed that the main phenolic compound found in the roots of well-watered plants was verbascoside (Fig. 1B). To our knowledge, this is the first report indicating that verbascoside is the major phenolic compound in the olive tree roots. Several biological properties of verbascoside are extensively reported in the literature and include antifungal (Oyourou et al., 2013), Antioxidant (Gonçalves et al., 2015), anti-inflammatory (Akdemir et al., 2011), antimicrobial (Pendota et al., 2013), as well as protection from UV irradiation (Alipieva et al., 2014). Cardinali et al. (2012) considered that verbascoside recuperated from olive mill wastewater, as a promising nutraceutical compound for the treatment of oxidative stress-related diseases, which might have interesting applications in nutraceuticals, cosmetics or functional foods. The results presented in this study suggested that roots of olive trees not subjected to drought stress condition could be considered as a potential source of verbascoside that are suitable for applications involving food and dietary supplements (Cardinali et al., 2012).

Application of water deficit stress caused a significant decrease in the level of verbascoside (by 65% comparing to well-watered plants) (Table 1). Recently, an opposite behaviour, regarding verbascoside

concentration, was recorded in roots of *Scrophularia striata*. Falahi et al. (2018) observed a high accumulation of verbascoside and echinacoside in roots of *Scrophularia striata* as a response to water stress. These data revealed that the improvement of stress tolerance due to verbascoside accumulation is species-dependent.

3.4. Oleuropein was the main phenolic compound found in the roots of water-stressed plants

Oleuropein has been described previously as being the main component of olive leaves (Fu et al., 2010; Quirantes-Piné et al., 2013). It was also detected in olive fruits (Cabrera-Bañegil et al., 2017; Vinha et al., 2005), olive roots (Ortega-García and Peragón, 2010), buds and open flowers (Taamalli et al., 2013). The results presented in this study showed that oleuropein was the predominant phenolic compound found in the chromatographic profile of root methanolic extracts of water-stressed plants (Fig. 1C). The application of water deficit stress caused a significant increase in the level of oleuropein (by 85% comparing to well-watered plants). Accumulation in oleuropein in other *olea europaea* organs such as fruits and leaves has been observed in many olive tree cultivars subjected to various abiotic stresses (Ahmed et al., 2009a,b; Ortega-García and Peragón, 2009; Petridis et al., 2012a). The latter authors, along with our findings, confirmed the effect of various stresses on the phenolic compound metabolism in olive trees, and revealed that the accumulation of oleuropein is a common response to abiotic stresses. According to Smirnoff (1993), water deficit is often associated with increased levels of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{-\bullet}$), singlet oxygen ($^1O_2$) and hydroxyl radical ($HO^{\bullet}$). ROS are highly reactive species, cause extensive damage to DNA, protein, lipids, and thereby affects the normal metabolism of plants (Kaushik and Roychoudhury, 2014). To ensure survival, plants are endowed with a developed efficient antioxidant machinery to cope with ROS. (i) enzymatic components like ascorbate peroxidase, catalase, superoxide dismutase, dehydroascorbate reductase, monodehydroascorbate reductase, glutathione reductase and guaiacol peroxidase (Apel and Hirt, 2004; Kaushik and Roychoudhury, 2014); (ii) non-enzymaticlow molecular weight antioxidantssuch as carotenoids, α-tocopherol, reduced glutathione, ascorbate, proline and phenolic compounds (Gill and Tuteja, 2010; Kaushik and Roychoudhury, 2014). The production of ROS in plants is mainly localized in the chloroplast, mitochondria and peroxisomes. There are secondary sites as well like the cell membrane, endoplasmic reticulum, cell wall and the apoplast (Kaushik and Roychoudhury, 2014). As a result, the production of ROS in the leaves is several magnitudes higher than in the roots. Thus, the increment of oleuropein content under water deficit stress could be restricted for the essential process of osmoregulation in the root zone. Osmotic adjustment through enhancing of a variety of primary and secondary metabolites such as soluble sugars, polyols, free amino acids, alkaloids and simple or complex phenols is a common mechanism for plants to protect cellular integrity against osmotic stress (Fang and Xiong, 2015).

According to Selmar and Kleinwächter (2013), when plants are exposed to various stress situations, their natural products such as terpenes, alkaloids, simple or complex phenols are enhanced. This well-known phenomenon is presumably due to a passively enhanced rate of biosynthesis, caused by greatly elevated concentrations of NADPH in stressed plants. However, any stress-related increase in the biosynthesis of specialized plant products might also be caused by a corresponding up-regulation of the genes encoding the involved enzymes. Nasrollahi et al. (2014) reported that drought stress enhanced the transcriptome of the gene encoding key enzymes involved in the biosynthesis of triterpenoid saponins in liquorice (*Glycyrrhiza glabra* L.). Recently, Yahyazadeh et al. (2018) showed that drought stress-enhanced biosynthesis of alkaloids was correlated with increasing gene expression of stylopine synthase. Considering the high level of oleuropein in water-stressed plants, further research is needed to determine whether or not the stress related increase of oleuropein biosynthesis in general is – at least in part – due to an enhancement of the transcriptome of the genes encoding the key enzyme in oleuropein biosynthesis, the iridoid synthase. It is known that iridoid synthase is involved in oleuropein biosynthesis in olive (*Olea europaea* L.) (Alagna et al., 2012).

3.5. Oleuropein/verbascoside ratio as a potential indicator for water deficit stress

Verbascoside and oleuropein were the main phenolic compounds detected in roots of well-watered and water-stressed plants respectively. The roots of well-watered plants contained 44,5% of verbascoside and 14% of oleuropein. However, the roots of water-stressed plants contained 58.5% of oleuropein and 10% of verbascoside. It seems that there is a reverse relationship between verbascoside and oleuropein. Both verbascoside and oleuropein share the same hydroxytyrosol moiety and it's possible that a part of verbascoside degradation, caused by water deficit stress, might contribute to the rise in the level of oleuropein. This observation corroborates the correlation already reported by Amiot et al.(1986) and later noticed by Esti et al..(1998), Servili et al.(1999) and Vinha et al.(2005) suggesting that there is a metbolic relationship between oleuropein and verbascoside.

Application of water deficit stress revealed shifts in the oleuropein/verbascoside ratio. The oleuropein/verbascoside ratio increased significantly from 0.31 in roots of well-watered plants to 6.02 in roots of water-stressed plants, indicating that the biosynthesis of oleuropein is augmented (Table 1). These results suggest that the oleuropein/verbascoside ratio could be considered as a useful indicator of drought tolerance in olive trees. Thus, we suggest that there is a potential to use the oleuropein/verbascoside ratio for the selection of drought-tolerant olive tree cultivars. To our knowledge, the elevated values of the oleuropein/verbascoside ratio can help controlling the water status of olive plants and avoiding serious oxidative damage induced by water deficit stress.

3.6. Water-deficit stress reduced the accumulation of hydroxytyrosol and tyrosol

Hydroxytyrosol and tyrosol are natural phenolic antioxidants present in olives (Cabrera-Bañegil et al., 2017), leaves (Benavente-García et al., 2000), stems and roots (Ortega-García and Peragón, 2010), olive mill wastewater (De Marco et al., 2007) and olive oil (Ben Brahim et al., 2017). They have shown a various biological activity such as antiviral (Yamada et al., 2009), antibacterial (Capasso et al., 1995), fungicidal (Yangui et al., 2010), but especially are considered as powerful antioxidant agents (De Marco et al., 2007; Obied et al., 2005; Pérez-Bonilla et al., 2014). The results presented in this study showed that the trend of root tyrosol and hydroxytyrosol concentrations was different from that of oleuropein. The concentrations of hydroxytyrosol and tyrosol decreased significantly after water deficit stress (Table 1). Hydroxytyrosol and tyrosol concentrations in roots of water-stressed plants were 51 and 24% lower than in roots of well-watered plants respectively. Reduction in the levels of hydroxytyrosol and tyrosol was previously reported under cold stress (Ortega-García and Peragón, 2010). Many kinds of environmental stresses have also been shown to cause the decrease of hydroxytyrosol level in olive plant cells, including salinity (Petridis et al., 2012b) and water deficit stress (Petridis et al., 2012a). These results suggest that hydroxytyrosol reduction is a response of olive tree to abiotic stresses. A possible explanation for the decrease of root hydroxytyrosol is that this compound is involved in the synthesis of oleuropein. It is known that hydroxytyrosol can be derived from oleuropein degradation under the action of different factors such as light, air, base, acid, metal ions, high temperatures and enzymatic process (Briante et al., 2002; Yuan et al., 2015). Ryan et al. (2002) reported that the levels of simple phenols such as hydroxytyrosol and tyrosol in the olive fruit differed during growth and ripening of the

drupe, the decrease in their levels is consistently correlating with the synthesis of the component of higher mass.

3.7. Water-deficit stress induced the accumulation of luteolin-7-glucoside and apigenin-7-glucoside

Flavonoids are plant secondary metabolites derived from the phenylpropanoid pathway (Ferrer et al., 2008). They are responsive to almost all abiotic stresses (Nakabayashi and Saito, 2015). Different families of flavonoids are found in different parts of olive, including flavones, flavonols, flavanonols and flavonols (Ghanbari et al., 2012; Talhaoui et al., 2015). In this study, several flavonoids were detected in olive-root extracts and all of them were identified by comparison of their retention time with those of the standards. With HPLC-ultraviolet detector it was possible to establish the presence of the flavone apigenin, the flavanol catechin and the flavonols quercetin. These compounds were commonly present in all the products derived from the olive trees (Cabrera-Bañegil et al., 2017; Ben Mohamed et al., 2018; Taamalli et al., 2013). Among the principle naturally occurring flavonoid derivatives, we found the glycosidic forms that are situated primarily in cell vacuoles throughout the plant (Stalikas, 2007). Among these, luteolin-7-rutinoside, luteolin-7-glucoside and apigenin-7-glucoside are the most cited in the literature (Cabrera-Bañegil et al., 2017; Quirantes-Piné et al., 2013; Taamalli et al., 2013). Apigenin-7-glucoside was the main flavonoid detected in roots of well-watered and water-stressed plants (Table 1). Water deficit did not affect all the flavonoids in the same way. The levels of luteolin-7-rutinoside and quercetin decreased significantly under water stress conditions. However, the levels of luteolin-7-glucoside and apigenin-7-glucoside increased. Water deficit also increased the level of catechin and apigenin, but this increase was not significant (Table 1). This is the first report to show the boost in the concentration of luteolin-7-glucoside and apigenin-7-glucoside in the roots of olive trees after water deficit stress. According to previous studies, luteolin-7-glucoside and apigenin-7-glucoside possess a wide range of biological activities, such as antioxidant, anti-inflammatory and antimutagenic activities (McKay and Blumberg, 2006; Seelinger et al., 2008). We hypothesized that luteolin-7-glucoside and apigenin-7-glucoside functions in olive plants were to reduce the damage caused by water deficit stress. Thus, luteolin-7-glucoside and apigenin-7-glucoside play a key role in olive tree resistance to abiotic stresses. There are few studies about the influence of water deficit on luteolin-7-glucoside and apigenin-7-glucoside and the contribution of these compounds in plant resistance against abiotic stresses. In one of these studies, for example, Hojati et al. (2011) reported that the tolerance to water deficit stress in chamomile (*Matricaria chamomilla* L.) was related to the changes in growth variables, antioxidants and the apigenin-7-glucoside content. These authors observed that drought stress caused a higher reduction in morphological and productive parameters and a higher increase in the apigenin-7-glucoside content and antioxidant enzyme activity.

4. Conclusions

The results presented here demonstrated that, when *Olea europaea* L. cv. Meski is exposed to water deficit stress, luteolin-7-glucoside, apigenin-7-glucoside and oleuropein are three important elements involving in the response of olive tree to such stress. We hypothesize that a coordinate control system between secoiridoid and flavonoid pathways operated in roots of olive trees can help to avoid serious oxidative damage induced by water deficit stress, decrease the osmotic potential and allow the root cell to absorb more water from soil.

Author contributions

Beligh Mechri did the laboratory job and drafted the manuscript. Hechmi Chehab and Meriem Tekaya did the experimental work. Mohamed Hammami and Meriem Tekaya participated in the statistical analysis and the correction of the manuscript. All authors contributed to manuscript revision, read and approved the final manuscript.

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