South African Journal of Botany 121 (2019) 152–158

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Influence of arbuscular mycorrhizal fungi inoculation on the control of stomata functioning by abscisic acid (ABA) in drought-stressed olive plants

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ARTICLE INFO

Article history:
Received 28 June 2018
Received in revised form 6 September 2018
Accepted 29 October 2018
Available online xxxx

Edited by R Baraldi

Keywords:
AM-symbiosis
Hormone
Olea europaea
Rewatering
Stomata
Water deficit

ABSTRACT

Abscisic acid (ABA) is the key phytohormone modeling the stomata behavior under drought conditions. However, stomata closure is not correlated with leaf ABA content in mycorrhizal olive plant. The aim of the present study is to evaluate the impact of arbuscular mycorrhizal (AM) symbiosis on the control of stomata functioning by ABA in two olive tree (*Olea europaea* L.) cultivars 'Zarrazi' and 'Meski' subjected to dehydration–rehydration treatment. AM-inoculated (Myc+) and non-inoculated (Myc-) olive plants were subjected to water stress and then rewatered (recovery). Leaf ABA content, stomatal conductance and transpiration rate were measured in: (1) irrigated control, (2) moderately and severely stressed and (3) recovered plants. In both Zarrazi and Meski Myc– plants, ABA content increased in parallel with drought intensity and stomatal closure. However, an intraspecific variability appeared in inoculated plants; in Meski Myc+ plants ABA content was not influenced by drought and recovery treatments, whereas in Zarrazi Myc+ plants the ABA amount increased under moderate water stress and even further after water relief, but decreased under severe water stress. However, in Myc+ plants of both cultivars, stomatal closure was not correlated with leaf ABA content. The results reveal that the ABA is not the key factor controlling the stomatal closure in AM-inoculated olive plants under drought conditions. In fact, other AM-related factors are involved in the control of stomata regulation in mycorrhizal olive plants exposed to severe drought. These factors act specifically in the drought-resistant cultivar 'Zarrazi' permitting a suitable stomata behavior.

1. Introduction

In most Mediterranean countries, like Tunisia, olive orchards rely mainly on rainfall rather than irrigation. The region is characterized by large oscillations in total annual rainfall, and episodes of acute drought are common. Fortunately, olive trees are a drought-tolerant species, well adapted to Mediterranean climate, thanks to morpho-anatomic adaptations and physiological and biochemical defense mechanisms (Ennajeh et al. 2006; Mekahlia et al., 2013). Nevertheless, intra-species differences in drought tolerance do exist and can be exploited to further improve the performance of elite cultivars under drought (Ennajeh et al. 2006, 2009).

Like most fruit plants, the olive tree forms beneficial associations with AM fungal (Calvente et al. 2004; Ouledali et al. 2018). It is well established that mycorrhizae can help plants to overcome stressful conditions by acting as bio-fertilizers, bio-protectors and also as bio-regulators of plant development (Gianinazzi et al. 2010). The interaction between AM fungal and plants is extremely ancient, it dates back to about 460 million years when plants started to conquer terrestrial ecosystems (Santander et al. 2017). Approximately 80% of terrestrial plants, including most cultivated and forest species, develop mutualistic mycorrhizal associations (Smith and Read 2008; Smith and Smith 2011; Wu et al. 2013; Azcón-Aguilar and Barea 2015). Both associated partners acquired benefits; as obligate biotrophs, AM fungal receive photosynthetic products (Smith and Read 2008) and lipids (Bravo et al. 2017; Jiang et al. 2017) from root cells, while the plant obtains better water and mineral uptake (Smith and Read 2008). Furthermore, AM fungal reinforces plant's resistance to abiotic stresses (Sun et al. 2018), such as drought stress (Smith and Read 2008; Ouledali et al. 2018; Wu 2017; Xu et al. 2018) and high levels of heavy metals (Andres and Andrea

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https://doi.org/10.1016/j.sajb.2018.10.024
0254-6299/

2002; Rozpadek et al. 2014), but also to biotic aggression (Mechri et al. 2008).

Regarding mycorrhizae utility, researchers focused mainly on their potential as bio-fertilizers, and on the mycorrhizae benefits in plant nutrition, particularly in the improvement of phosphorus uptake (Vosátka et al. 2012). However, the AM fungal–plant association can also induce the activation of several signals especially phytohormones such as cytokinins, gibberellins, ethylene, abscisic acid, auxine, and jasmonic acid (Ludwig-Müller 2000).

Drought is the main abiotic factor responsible for reduction of plant growth and yield in various regions of the world (Kramer and Boyer 1997). The ABA is considered as the stress hormone (Ludwig-Müller 2010), as it can regulate plant development and survival under environmental stress conditions (Schroeder and Nambara 2006). ABA is the hormone most involved in coordinating the plant's response to drought (Zhang et al. 2006; Christmann et al. 2006; Ton et al. 2009). The main known role of the ABA is the regulation of plant water status through the control of stomata aperture by regulating guard cell turgidity when the soil dries out. Fine roots synthesize ABA which is transported upward, through the transpiration stream, to the stomata guard-cells provoking stomata closure. Furthermore, ABA induces the activation of genes encoding enzymes and other proteins involved in cellular dehydration tolerance (Bray 2002; Xiong and Zhu 2003). The impact of the colonization of roots by AM fungal on ABA balance has been documented in many plant species like tomato (*Solanum lycopersicum*), corn (*Zea mays*), lettuce (*Lactuca sativa)*, chile ancho pepper (*Capsicum annuum*), and cowpea (*Vigna unguiculata*), (Herrera-Medina et al. 2007; Kandowangko et al. 2009; Aroca et al. 2008; Estrada-Luna and Davies Jr 2003; Ruiz-Lozano et al. 2009; Duan et al. 1996).

The aims of the present study were to investigate the impact of AM-supply on ABA balance, and to determine if AM-inoculation of roots influences the ABA-control of stomata functioning in two olive tree cultivars subjected to drought followed by rehydration period. Like in several plant species, we hypothesize that mycorrhizal symbiosis affects the ABA content in leaves of olive tree under drought conditions. So the stomatal regulation, which is the principal instantaneous response to water deficit, could be accomplished by an AM-depending factor.

2. Materials and methods

2.1. Plant culture and treatments

We used two contrasting olive (*Olea europaea*) cultivars: 'Zarrazi' and 'Meski'; the first one is a reputed drought-tolerant cultivar widely cultivated in arid and semi-arid Tunisian areas, while the drought-sensitive Meski cultivar grows in the more humid Tunisian North. One-year-old olive plants, of homogenous size, were obtained from the nursery of the National Office of Oil (ONH, Tunisia). For each cultivar, thirty plants of homogeneous size were individually transplanted into 17-L pots filled with a sterilized soil (10% clay, 1% silt, 39% coarse sand, 42% fine sand and 8% very fine sand), having a pH of 8.17 and with known mineral composition (0.133% N, 2.07% Ca, 0.127% K, 0.26% Mg, 0.06% Fe, 0.066% Na, 0.000065% available P and 1.39% organic matter). The soil was steam-sterilized with two 12 h cycles at 120 °C. The pots were top-covered with plastic film and aluminum foil to reduce evaporation from the soil surface and to minimize solar heating.

At transplantation (end January 2015), half of the plants were randomly chosen and were inoculated with AM fungal (Myc+ plants) by mixing 80 g per pot of the commercial AM mix 'Symbivit' (INOCULUMplus, Dijon, France), into the potting soil. 'Symbivit' contains propagules of six different AM fungal species: *Glomus etunicatum, Glomus microaggregatum, Glomus intraradices, Glomus claroideum, Funneliformis mosseae, Glomus geosporum*. The remaining plants were not inoculated (Myc– plants). All plants were watered weekly to field capacity with tap water for four months. At the end of this period the mycorrhization status was checked (see following section) on six plants by cultivar (three *Symbivit*-added and three not treated), and the stress treatments were applied. The stress experiment was conducted outdoor in the experimental field of the campus of the Faculty of Science of Gabes (southern Tunisia 33°50′ N, 10°5′ E) during the dry season (June–August 2015). No rainfall occurred during this experimental period, and monthly mean of minimum and maximum temperatures fluctuated between 18 and 21 °C and 32–47 °C, respectively.

In the stress experiment, six plants of each cultivar (three Myc+ and three Myc–) were used as irrigated controls. The 18 remaining plants (nine Myc+ and nine Myc–) were drought-stressed by stopping watering until two levels of water stress (moderate and severe) were imposed. These two levels were assessed on the basis of predawn leaf water potential (Ψ_{pd}) measurements. Based on previous results on the same plant material (Ennajeh et al. 2008; Ouledali et al. 2018), severe stress was set for Zarrazi at a Ψ_{pd} between −7 and −8.5 MPa, whereas, for Meski cultivar, a severe water stress level corresponds to values of Ψ_{pd} between −5 and −7 MPa. The moderate stress level was set at mid-value Ψ_{pd} of the severe stress level. For each inoculation treatment, three plants were subjected to moderate stress and six to severe stress; at the end of the stress period, three among the six severely stressed plants were fully rewatered for one month to induce recovery from stress.

Predawn leaf water potential (Ψ_{pd}) was assessed at regular intervals in order to assess water stress level. Stomatal conductance (g_s) and leaf transpiration rate (E) were measured in unstressed, water-stressed (moderately and severely) and water-stress recovering plants. Thereafter, fully expanded mature leaves were collected, dipped in liquid nitrogen and then stored in a freezer (−30 °C) for subsequent ABA analysis.

2.2. Microscopic observations and estimation of mycorrhizal colonization

Mycorrhizal colonization was assessed at start June 2015 (after four months of inoculation) on fresh roots from three, randomly selected, *Symbivit*-inoculated and three non-inoculated plants for each cultivar. Roots were stained with trypan blue (0.05%) (Philips and Hayman, 1970). The mycorrhization parameters were evaluated by the overall assessment of 30 root fragments (1 cm length) per plant as described by Trouvelot et al. (1986). Mycorrhizal frequency (F%) and intensity of colonization (M%) were determined with the 'Mycocalc' program (www.dijon.inra.fr/mychintec/). Microphotographs of AM fungal colonized roots were taken with a digital camera (CMEX 5.0) coupled with photonic microscope (Euromex-Holland) interfaced to a computer using image manager Zeiss software.

2.3. Measurements of predawn leaf water potential and leaf gas exchange

The predawn leaf water potential (Ψ_{pd}) was measured early in the morning before sunrise to ensure that no transpiration occurred. It was measured on small terminal brindle using the Scholander pressure chamber (PMS Instrument Company, Albany, Oregon, USA) according to Scholander et al. (1965).

Stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}) and transpiration rate (E, mmol H_2O m^{-2} s^{-1}) were measured on fully expanded mature leaves using the LCi portable gas exchange system (ADC BioScientific Ltd., Hoddesdon, UK). Measurements were done between 09:30 and 10:30 h under saturating light conditions at temperatures between 20 and 30 °C. The measurements were repeated three times per leaf, three leaves per plant were used, and the three replicate plants for each drought stress treatment were considered.

2.4. ABA analysis

The ABA content was quantified on fully expanded mature leaves collected separately from the replicate plants of each water treatment: (1) unstressed (irrigated), (2) moderately stressed, (3) severely

stressed and (4) recovered. Five leaves by plant were used, freeze-powdered by liquid nitrogen and then lyophilized.

ABA was extracted from the leaves of olive trees according to the method described by Materán et al. (2009). Briefly, lyophilized leaf material (0.2 g) was homogenized in 5 ml of 80% methanol and kept overnight at 4 °C (16–18 h). Samples were then centrifuged at 7500 rpm for 5 min, after that the supernatant was moved to a flask and the methanol was evaporated in a Rotavap at 30–33 °C. Two ml of phosphate buffer (pH 8) were added to each sample, and the pH was adjusted in the range 8–9 with NaOH 1 N. After that, 1 ml of ethyl acetate was added for each sample, and a second centrifugation was performed at 7500 rpm for 5 min. The resulting supernatant was discarded and the remaining of tubes was transferred to another set of tubes, the pH was adjusted in the range of 2–3 with HCl 1 N. Two ml of ethyl acetate were added to each sample, after centrifugation, the supernatant was transferred into a flask and ethyl acetate was evaporated in a Rotavap at 30–33 °C. Finally, the dried samples were re-suspended in 1 ml of methanol and filtrated using 1 ml syringe equipped with 0.2 μl filters. The filtrated drops were recovered into dark glass vials and injected into a 1260 Infinity HPLC-DAD System (Agilent Technologies, Cernusco sul Naviglio, Milano, Italy).

The concentration of ABA was determined by reference to a standard curve prepared using ABA standard solutions ranging in concentration from 0.039 to 100 mg L^{-1}.

2.5. Statistical analysis

The experimental set up was arranged as a Completely Randomized Design with three replicates. All statistical analysis was performed. Analysis of variance with the GLM procedure, and the Duncan *post-hoc* test ($P = .05$) were applied using SAS software (SAS Institute 1999).

3. Results

3.1. Mycorrhizal colonization

The microscopic observations showed that, four months after transplantation, the roots of inoculated olive plants (Myc+) of both cultivars were colonized by endomycorrhizal fungus (Fig. 1), while Myc– plants were not infected. Colonization exhibited a frequency (F%) of 42.94 and 43.33 and an intensity (M%) of 5.39 and 3.63 in Zarrazi and Meski cultivars, respectively.

3.2. Leaf ABA content

In the Zarrazi cultivar, leaf ABA content was influenced by water stress intensity and AM fungal treatments (Fig. 2A). In leaves of non-inoculated plants, ABA content was significantly correlated with water stress intensity ($P = .004$), increasing 2.5-folds higher under moderate

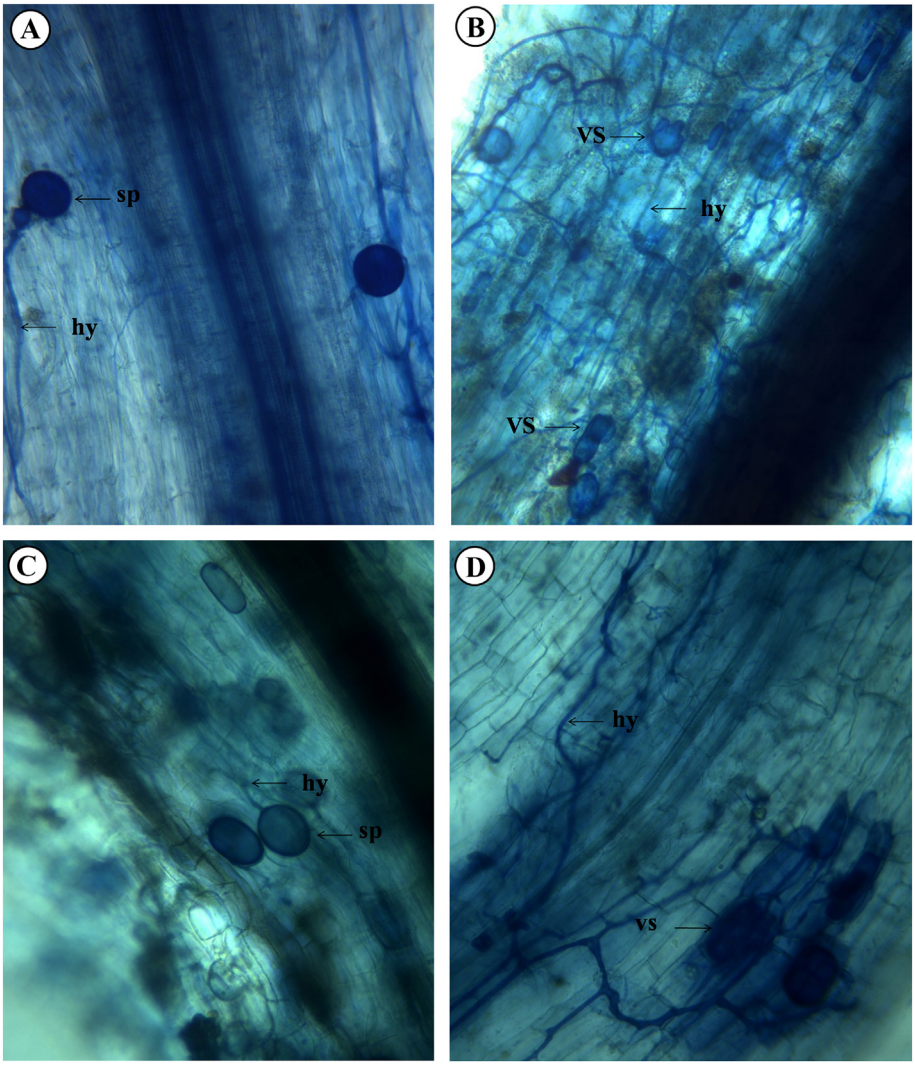

Fig. 1. Mycorrhizal colonization in roots of Zarrazi (A, B) and Meski (C, D) olive plants: spore (sp) attached to intercellular hyphae (hy); vesicles (vs) formed between cells in root cortex of olive plants (G:400×).

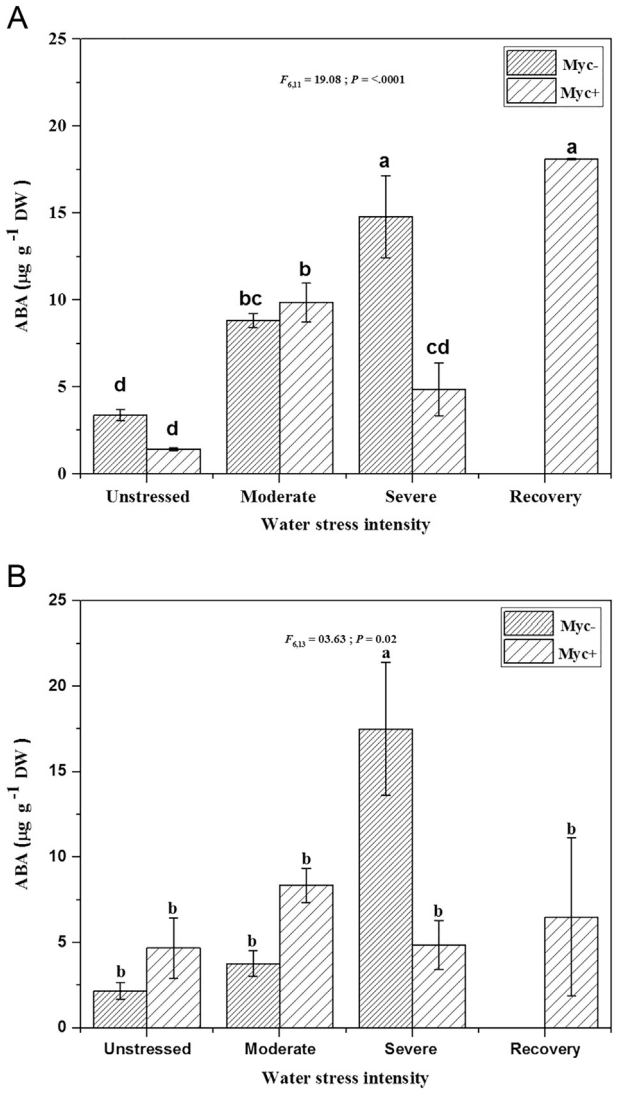

Fig. 2. ABA content in leaves of well-watered (unstressed), water stressed (moderate and severe) and water-recovered plants of two olive cultivars: (A) Zarrazi and (B) Meski. Myc + indicates the inoculated AM fungal plants, while Myc- represents the non-inoculated ones. Statistical analysis based on the Duncan post-hoc test were presented by letters. For each cultivar, the different letters on bars indicates significant difference between treatments ($P \leq .05$). Bars are mean values, and error bars represent SE (n = 3).

stress and more than 4-folds under severe water stress compared to unstressed plants. In leaves of well-irrigated mycorrhizal plants, ABA content was low and similar to the level found in the leaves of control Myc– plants. Moderate water stress induced an increase in leaf ABA content of both Myc– and Myc+ plants (more than 6-folds compared to unstressed controls in the latter case). Contrary to Myc– plants, severe water deficit caused a decrease in ABA content in Myc+ plants by 46% compared to moderate water stress level.

In the cultivar Meski, ABA content was less influenced by water stress intensity and mycorrhization (Fig. 2B). Only the severely stressed Myc– plants showed a significant increase of ABA concentration, however the hormone level did not change significantly among the other water treatments and with or without fungal inoculation.

During the recovering stage, ABA content was not quantified in leaves of Myc– plants of both Zarrazi and Meski cultivars because trees did not recover from drought, after water stress they dried and dead (Fig. 2). However, Myc+ plants (of both cultivars) survived the stress and recovered. They behaved differently concerning the accumulation of ABA in their leaves. In Zarrazi, the ABA amount increased sharply in Myc+ plants exceeding the concentration measured in all water stressed plants. However, in Myc+ plants of Meski, the ABA content during the recovering phase was not significantly different to other water treatments.

3.3. Physiological changes in response to water stress

Stomatal conductance (g_s) varied between the two cultivars and was affected by water stress severity and mycorrhization (Fig. 3). Under well-watered conditions, in Zarrazi cultivar stomatal conductance was higher in Myc+ plants compared to Myc– plants, whereas in Meski cultivar g_s was similar in Myc+ and Myc– plants.

Under moderate water stress, g_s decreased in both olive tree cultivars, but to a greater extent in Myc– plants than Myc+ plants. This decrease was gradual for Zarrazi plants, but was rapid for Meski plants especially in Myc– ones. Under severe water stress, g_s was very low, and Zarrazi plants showed full stomatal closure at leaf water potential below −8 MPa. In contrast, Meski plants (Myc+ and Myc–) did not fully close their stomata despite the decrease of Ψ_{pd} below −7 MPa. In inoculated plants after resuming watering, g_s was not recuperated in Meski but it was partially recuperated in Zarrazi the drought-resistant cultivar.

Similarly to stomatal conductance, also transpiration rate (E) was significantly lower in droughted than in irrigated control plants, (Fig. 4). Under moderate water stress, Myc– plants of both cultivars showed lower E compared to Myc+ plants, however Meski plants were more susceptible to stress. An increase of stress (severe level) resulted in additional decrease of E, even if transpiration resulted higher in Meski compared to Zarrazi. After rehydration period, Zarrazi-Myc+ plants recuperate partially their transpiration process but not Meski-Myc+ plants.

In order to highlight the impact of mycorrhization on ABA-controlled stomata functioning, we examined the correlation between leaf ABA content and stomatal conductance in Myc+ and Myc– plants under the different water stress intensities (Fig. 5). In both cultivars, the correlation between leaf ABA content and g_s was influenced by mycorrhization. Indeed, this correlation was highly negative in Myc– plants, especially in Zarrazi cultivar ($R^2 = 0.927$) revealing that ABA was the principal stomata aperture controller in Myc– plants. However, Myc+ plant did not show a correlation between ABA and g_s ($R^2 = 0.002$ for Zarrazi-Myc+ and 0.129 for Meski-Myc+). This result suggested

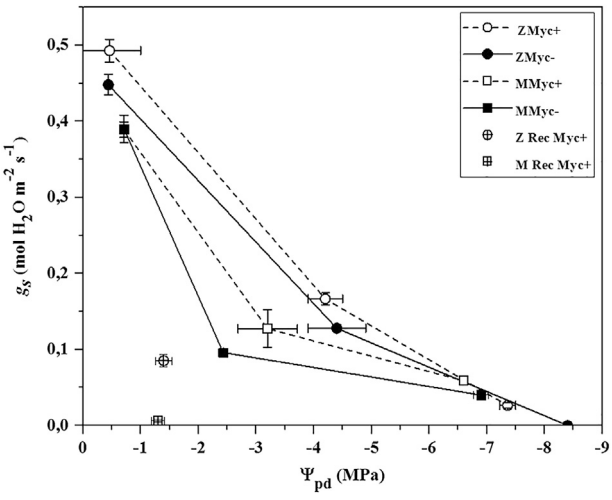

Fig. 3. Stomatal conductance (g_s) in relation to predawn leaf water potential (Ψ_{pd}) in plants of two olive cultivars Zarrazi (Z) and Meski (M). Myc + indicates the inoculated, mycorrhizal plants, while Myc- denotes the non-inoculated ones. Left: unstressed (well-irrigated); center: moderate stress; right: severe stress. Single symbols indicate recuperated mycorrhizal plants of Zarrazi and Meski (Z Rec Myc + and M Rec Myc+). Circle and square symbols represent average values and error bars are SE (n = 3).

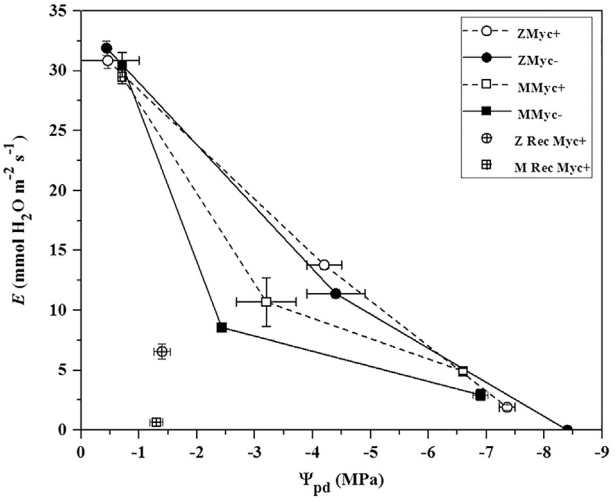

Fig. 4. Leaf transpiration rate (E) in relation to predawn leaf water potential (Ψ_{pd}) in plants of two olive cultivars Zarrazi (Z) and Meski (M). Myc + indicates the inoculated, mycorrhizal plants, while Myc- denotes the non-inoculated ones. Left: unstressed (well-irrigated); center: moderate stress; right: severe stress. Single symbols indicate recuperated mycorrhizal plants of Zarrazi and Meski (Z Rec Myc + and M Rec Myc+). Circle and square symbols represent average values and error bars are SE (n = 3).

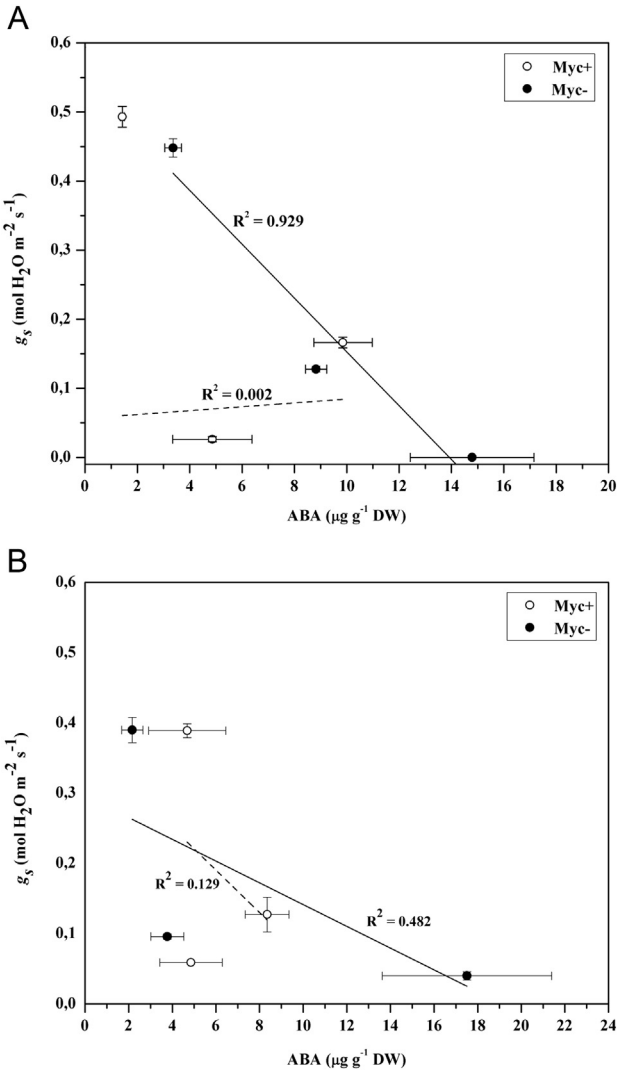

Fig. 5. Correlation between stomatal conductance (g_s) and leaf ABA content in plants of two olive cultivars: (A) Zarrazi and (B) Meski subjected to water-stress. Myc + indicates the inoculated, mycorrhizal plants, while Myc- denotes the non-inoculated ones. Circles represent average values and horizontal and vertical bars indicate SE (n = 3). R^2 is the correlation coefficient between the two parameters.

that in Myc + plants, stomatal functioning was not principally mediated by ABA, but another AM fungal factor was involved.

4. Discussion

Plants adapt to their environment by modulating metabolic and physiological processes. To accomplish this adaptation, they may interact with some organisms through symbiotic associations. Under drought conditions, the AM fungal symbiosis is beneficial for the plant and offers several advantages (Rapparini and Peñuelas 2014). In addition to its role in the improvement of the hydro-mineral uptake, it may influence the hormonal balance of the plant to mount an integrated response to drought. ABA is the key hormone modeling the plant responses to water deficit and it is also involved in the regulation of stomatal functions. ABA reduces leaf transpiration rate (Holbrook et al. 2002; Zhang et al. 2006) by limiting stomatal conductance (Bray 2002; Zhang et al. 2006). It also increases the susceptibility of plants to AM colonization and contributes to the development of complete arbuscules and their functionality (Herrera-Medina et al. 2007; Martín-Rodríguez et al. 2016).

In the present work, we analyze the changes in ABA concentration in the leaves of two olive cultivars associated or not with AM fungal and under drought conditions. Our results show that the colonization with AM fungal influences the accumulation of ABA in host plants compared to non-mycorrhizal ones. Indeed, in the leaves of Myc– plants, higher ABA content was observed with an increase in water stress. However, in leaves of Myc + plants, ABA concentration increased under moderate water stress but decreased when the stress became severe. Our results agree with previous reports showing that, under severe drought stress, the concentration of ABA is higher in non-inoculated than inoculated plants (Estrada-Luna and Davies Jr 2003; Ludwig-Müller 2000). Furthermore, Allen et al. (1980) found that in AM-infected *Bouteloua gracilis*, ABA decreased in the leaves but was unchanged in the roots. Estrada-Luna and Davies Jr (2003) indicated that colonized plants had lower leaf ABA than non-colonized ones under severe dehydration; Kandowangko et al. (2009) showed that the inoculation of corn plants with AM fungal reduced ABA content under drought conditions, and Chitarra et al. (2016) detected lower ABA levels in droughted mycorrhizal tomato plants under drought stress.

In both cultivars and in both Myc + and Myc– plants, water stress caused a decrease in g_s and E. However, Myc + plants maintained slightly higher g_s and E than Myc– plants. Guard cells respond to various stimuli including plant hormones and elicitors (Munemasa et al. 2007). ABA is the principal plant hormone inducing stomatal closure (Kandowangko et al. 2009). In Myc– plants of both cultivars, stomata closure was highly correlated with ABA concentration in the leaves as water stress intensified (Fig. 5). However, stomatal regulation is not correlated to the ABA accumulation in Myc + plants. It has been shown that higher g_s rates in mycorrhizal plants are associated with low xylem-sap ABA concentrations and thus lower ABA content in the leaves (Ebel et al. 1997). Goicoechea et al. (1997) reported that high g_s and E in mycorrhizal *Medicago sativa* plants is associated with altered ABA/cytokinins ratios in their leaves. Thus, the mycorrhizal symbiosis can influence the production of ABA and cytokinins in roots exposed to drying soil in order to regulate stomatal opening and limit water loss. Ruiz-Lozano et al. (2009) showed that AM-inoculated plants regulate better their ABA level than no-inoculated ones, resulting in a suitable balance between leaf transpiration and root water absorption during drought stress and recovery. As well, a recent study conducted by Xu et al. (2018) proved that AM symbiosis could modulate the stomatal behavior through the regulation of the 14-3-3 genes in the ABA signaling pathway in order to improve drought stress tolerance. Thus our results

suggest that AM-inoculation of the two studied olive cultivars helped to some extent to maintain transpiration and stomatal conductance in Myc+ plants under water deficit conditions. This is crucial for growth and development of the plant (Ba et al. 2000; Estaun et al. 2003; Meddad-Hamza et al. 2010).

Our results on Myc– plants concerning ABA-g_s correlation corroborate with previous study of Ruiz-Lozano et al. (2016) on tomato and lettuce plants. However, the behavior of Myc+ plants was different and warrants a deeper discussion. In the present study, stomata closure was well correlated with ABA accumulation in the leaves of Myc+ plants of both cultivars when water deficit was moderate. However, under severe water stress, Myc+ plants of both cultivars closed their stomata despite the lower concentration of ABA in their leaves. This suggests the possible presence in olive of an AM-dependent signal replacing ABA when dehydration is severe. The lower ABA concentration may be caused by a negative feed-back exerted by the AM-induced signal on the ABA-biosynthesis pathway, or also competition for a shared common precursor between ABA and the signal. ABA signaling is interconnected to other pathways involving nitric oxide, ethylene, salicylic acid and jasmonic acid acting either antagonistically or synergistically (Manthe et al. 1992; Leung and Giraudat 1998; Evans 2003; Anderson et al. 2004; Suhita et al. 2004; Desikan et al. 2006; Mosher et al. 2010). In addition to ABA, stomatal closure is also induced by methyl jasmonate and by ethylene (Desikan et al. 2006; Munemasa et al. 2007).

At the end of the recovering period, leaf ABA concentration was different between the two cultivars. The Zarrazi Myc+ plants, reputed drought-tolerant, accumulated more ABA than Meski Myc+ plants, the drought-sensitive cultivar. Moreover, Zarrazi rehydrated Myc+ plants had higher leaf ABA content than Myc+ plants severely dehydrated. However, in Meski, rehydrated Myc+ plants had leaf ABA content similar to that of Myc+ but lower than Myc– under severe drought stress conditions. The higher concentration of ABA in leaves of rehydrated Myc+ Zarrazi plants could be a specific mechanism of resilience to drought.

When dehydration became more severe, E and g_s became very low in Zarrazi cultivar and reached zero in Myc– plants. Thus, stomatal regulation was efficient in this drought-tolerant cultivar (Ouledali et al. 2018). In contrast, in drought sensitive (Ennajeh et al. 2006) Meski, E and gs decreased gradually, but both Myc– and Myc+ plants did not fully close stomata. The stomatal regulation in Meski was not efficient enough to protect the plant from dehydration under severe water stress.

Ruiz-Lozano et al. (2016) reported that AM fungal symbiosis induces strigolactone (SL) biosynthesis under drought and improves drought tolerance in lettuce and tomato. SLs share their biosynthetic precursors with ABA, both being derived from carotenoids (Matusova et al. 2005; Stauder et al. 2018). ABA functions at multiple levels to regulate AM-symbiosis (Martín-Rodríguez et al. 2016). A regulatory role of ABA in strigolactone biosynthesis was proposed since a correlation between ABA and strigolactone content was observed (Aroca et al. 2013; López-Ráez et al. 2010). On the other hand, recent evidence shows that genetic SL depletion may affect ABA concentration and stomatal sensitivity to ABA in *Lotus japonicus* (Liu et al. 2015) and in tomato (Visentin et al. 2016). Thus an AM-dependent interplay between ABA and SL could contribute to modulate stomatal regulation under drought in olive.

5. Conclusion

In olive plant species, AM-inoculation influenced leaf ABA balance and stomatal functions under drought conditions. In Myc– plants, the stomata closure was well correlated with ABA concentration in the leaves and ABA content increased as water stress intensified. On the contrary, in Myc+ plants, stomatal closure was not correlated with leaf ABA concentration when drought became severe. This leads us to suggest that stomata closure in AM-inoculated olive plants may be mediated by a putative mycorrhiza-dependent metabolite replacing ABA. This metabolite may be produced by the fungus itself or by the plant but its accumulation is a result of the symbiosis with the AM fungal.

In the recovering phase, Myc+ plants of drought-resistant Zarrazi, showed higher leaf ABA content than Myc+ plants of Meski, the drought-sensitive cultivar. This behavior may reflect the better resilience to drought of Zarrazi cultivar. To shed more light on the cause (s) of the decrease in ABA concentration in the leaves of Myc+ olive plants, and the influence of the AM-symbiosis on the modulation of the stomatal function under drought and rewatering conditions, the levels of strigolactones, ethylene, jasmonic acid and gibberellins should be monitored.

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