Arbuscular mycorrhizal fungi enhance growth of tomato under normal and drought conditions, *via* different water regulation mechanisms

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A R T I C L E   I N F O

A B S T R A C T

Arbuscular mycorrhizal fungi (AMF) are soilborne microorganisms that establish a mutualistic symbiotic association with most of land plants. To investigate the effects of AMF symbiosis under different water status conditions, we grew AMF-inoculated and non-inoculated tomato plants in the greenhouse under two irrigation regimes, 70% and 30% of growth-substrate water holding capacity. Two different AMF inoculation strains, *Funneliformis mosseae* and *Rhizophagus irregularis*, were applied as single inocula. AMF colonization significantly enhanced plant vegetative growth by 40% and 50–60%, under normal and reduced irrigation respectively. In the presence of the AMF, phosphorous concentrations in the leaves were increased under both watering regimes while K, Ca, Mg, Zn, and Mn were also increased under limited watering to levels similar to those of non-stressed plants. Transpiration and stomatal conductance increased by an average 80% and 65% respectively in the presence of the AMF under full watering, but were kept stable and coupled to reduced leaf area-to-leaf biomass ratios and to increased metabolic water use efficiency under limited irrigation. This indicates a different mode of action induced by AMF colonizers, prioritizing water conservation in tomato plants under drought stress.

1. Introduction

Scarcity of water is a major environmental constraint to plant survival, growth and productivity (Farooq et al., 2009). Crops experience drought stress due to imbalances between water supply to the roots and transpiration rate needs. Generally, drought stress conditions elicit a cascade of morphological, physiological, biochemical and molecular responses that affect plant metabolism (Osakabe et al., 2014). Among others, stomatal closure is induced, causing a decrease in carbon dioxide uptake followed by a reduction in photosynthetic activity (Dubey et al., 2001). Moreover, drought causes nutrition disturbances as roots are unable to take up a range of nutrients from the soil due to reduced root activity, slow ion diffusion and water movement rates, and disruption of water continuity in soil pores (Gómez et al., 2012).

To overcome the consequences of water deficit, plants evolved different kinds of adaptive strategies, that include stress avoidance and stress tolerance mechanisms (Fang and Xiong, 2015). Apart from direct protective physiological and morphological adaptations, plant roots and rhizosphere soil accommodate various microorganisms that may promote plant fitness and abiotic stress amelioration under unfavorable conditions. One of the most studied and widespread mutualistic plant–microorganism associations, developed during millions of years of co-evolution, is the symbiosis established with arbuscular mycorrhizal fungi (AMF) (Kiers et al., 2011). AMF represent a monophyletic fungal lineage (*Glomeromycota*) that establishes an intimate association with the roots of most land plants (Bonfante and Genre, 2010). The fungus benefits from the host plant by obtaining photosynthetic carbon in the form of carbohydrates and lipids (Keymer et al., 2017), and in reverse, the fungal mycelium exploits the rhizosphere soil and the fungus helps the plant to acquire water and mineral nutrients (Smith and Smith, 2011).

AMF contribute to the alleviation of various biotic and abiotic plant stresses (Begum et al., 2019). In particular, the symbiotic relationship between AMF and the roots of higher plants aids plants to cope with drought stress. This has been shown for a number of host plants and fungal species (Chitarra et al., 2016; Moradtalab et al., 2019;

Ruiz-Lozano and Aroca, 2010). The ability of AMF-colonized plants to tolerate drought effectively is based on both direct and indirect mechanisms. Water uptake and transport by fungal mycelium, as well as nutritional and physiological benefits induced by mycorrhization have been shown (Smith and Smith, 2011; Wu et al., 2007). Due to their small diameter (2–20 μm), their high surface-to-volume ratio and their capacity for rapid exponential growth, fungal hyphae have access to soil pores inaccessible to plant roots and root hairs, resulting in more efficient water extraction by mycorrhizal than by non-mycorrhizal plants (Allen, 2007). Moreover AMF alter root morphology, mainly by promoting lateral root proliferation (Gutjahr and Paszkowski, 2013). Physiological effects include modifications of foliar water relationship characteristics (Wu et al., 2007), adjustments in root-to-shoot signaling and hormone production (Duan et al., 1996), biosynthesis of secondary metabolites, as strigolactones that act as cues in establishing and maintaining AMF symbiosis with the host plant (Stassen et al., 2021) and regulation of antioxidative enzymes (Caravaca et al., 2005; Rivero et al., 2018).

In the present study we investigate the effects of the colonization of tomato plants by AMF under different water availability, focusing on plant growth, physiology characteristics, plant water status and nutrition. To address that, we evaluated the role of two AMF strains of *Funneliformis mosseae* and *Rhizophagus irregularis,* applied to tomato seedlings grown in the greenhouse under two water regimes (full and reduced irrigation). *F. mosseae* and *R. irregularis* are phylogenetically distinct species that have been widely applied in tomato plants (Chitarra et al., 2016; Rivero et al., 2018; Volpe et al., 2018) and furthermore they were chosen to examine potential plant-AMF specificity effects regarding responses to abiotic stress. Our study shows that under reduced irrigation, the plant response to colonization by the AMF is differentiated, by apparently prioritizing water use and metabolic efficiency.

2. Materials and methods

2.1. Biological material

Tomato (*Lycopersicon esculicon* cv. EVIA F$_1$) was used as experimental plant material. EVIA F$_1$ is a modern high-yield tomato hybrid, with a determinate growth habit, which is mainly cultivated in the field. Seeds were surface sterilized in 1.25% sodium hypochlorite for 20 min, rinsed three times in sterile distilled water, sown in sterile Klasmann-TS2 soil (Klasmann-Deilmann, Geeste, Germany) distributed in 50 ml plastic pot trays and allowed to germinate in the dark. Plantlets were grown in the glasshouse (latitude 37.98° N, longitude 23.70° E) under controlled conditions of 25–30 °C and 60–80% relative humidity during the summer season.

Two AMF species were used, the model arbuscular mycorrhizal fungus *Rhizophagus irregularis* DAOM 197198 (Tisserant et al., 2013) and a *Funneliformis mosseae* strain, which belongs to the Agricultural University of Athens (AUA) Soils-Lab AMF collection and was isolated from a certified organic farm. *F. mosseae* was propagated on *Zea mays* L. trap plants grown for 3 months in pots filled with sterilized 2 : 1 v/v sand: vermiculite substrate. The final inoculum consisted of the potting substrate containing colonized *Z. mays* roots, hyphae, and spores. *R. irregularis* DAOM 197198 was purchased from Agronutrition (Labège, France) and consisted of a liquid preservation solution containing colonized roots, hyphae, and spores. To ensure homogeneity of AMF application, the *R. irregularis* DAOM suspension was mixed with sterilized 2:1 v/v sand: vermiculite (the substrate of the *F. mosseae* formulation), prior to plant inoculation.

2.2. Experimental set-up

The experiment was set up as a 2 × 3 factorial randomized blocks design, with two levels of water regime, full irrigation (70% of water

holding capacity, WHC) and limited irrigation (30% of WHC, determined by preliminary trials), and three inoculation treatments (non-AMF, *R. irregularis* and *F. mosseae*). At 27 days after sowing (DAS), and at the stage of 3 true leaves, tomato seedlings were transplanted to pots (1.4 L) filled with one kg of sterilized 2 : 1 v/v sand: vermiculite substrate. During transplantation, 10 g of the respective AMF inoculum (approximately 60 spores plus colonized roots and hyphae) were added per plant, while non-inoculated plants were supplied with the same amount of an autoclaved form of the same inoculum. Plants were irrigated to pot water capacity until the stage of 4 true leaves on a daily basis, and fertilized with modified Hoagland nutrient (Supplementary Table 1). Afterwards, water stress treatment was applied for a time-period of 1 month. The experimental design and all the treatments are summarized in Supplementary Fig. 1. All treatments consisted of five plants, each of which constitutes a single biological replicate, and all measurements were performed individually on every plant of each treatment.

2.3. Morpho-anatomical measurements

At harvest, 30 days after the drought stress application, plant growth was determined by measuring total length of shoot, total number of leaves and shoot and root fresh weights (destructive sampling). Plant leaves were scanned for the determination of leaf area (LA) with the use of Gimp (ver. 2.10.20, GIMP Development Team). Afterwards, the plant material was oven dried at 60 °C for three days to determine dry shoot and root weight.

2.4. Determination of root colonization by AMF

At the end of the experiment, roots of all plants were collected. They were washed free of soil, and a subsample (2 g fresh weight) was used to estimate AMF colonization with trypan blue stain (Sylvia, 2018). Mycorrhizal colonization was estimated on slides according to McGonigle et al. (1990).

2.5. Leaf gas exchange

Net photosynthetic rate (P$_N$), stomatal conductance (g$_s$), transpiration rate (E) of tomato plant leaves were recorded with a Li-6400, portable photosynthesis system (LiCor Bioscience Inc., Lincoln, NE, USA) in the morning (between 09:30 and 11:00 a.m.) at a light-saturated photosynthetic photon flux (PPF, greater than 1000 μmol m^{-2} s^{-1}) and a reference CO$_2$ concentration of 380 μmol mol^{-1}. In each plant, the measurement was carried out on one healthy, non-senescing and fully expanded leaf, at the same physiological age (in the middle part of the plant, counting the third-fourth leaf from the shoot apex). Photosynthetic water use efficiency (pWUE) was also calculated as the ratio between P$_N$ and E of each measurement.

2.6. Leaf relative water content measurements

Leaf relative water content (RWC) was measured as described by Sade et al. (2015), after 30 days of water scarcity. Leaf samples were collected in the morning and weighed immediately (fresh weight). To obtain turgid weight, the samples were dipped into 5 μM CaCl$_2$ and kept overnight in the dark (turgid weight). Full turgor was reached the next morning (after 10–11 h). Dry weight was obtained after placing the samples in a drying oven set at 60 °C for 48 h (dry weight). RWC was calculated based on the following equation and expressed as a percentage:

RWC (%) = [(fresh weight − dry weight)/(turgid weight − dry weight)] x 100.

2.7. Tissue analysis for mineral nutrients

At the end of the experiment, samples of the dried leaves of all plants were finely ground in a stainless-steel Wiley mill. A subsample of 0.5 g was heated to ash at 550 °C, solubilized with 5 ml of 65% HNO_3, and diluted to 50 ml with dH_2O. Total concentration of P was determined following the Murphy and Riley color rection method with a PG T60 UV/VIS Spectrophotometer, at 880 nm wavelength. Concentration of Mg, Fe, Zn, Mn, Cu were determined by flame atomic absorption spectrophotometry (Varian, A–300; Varian Techtron Pty. Limited, Australia), using an air–acetylene flame, while Ca concentration was determined using an acetylene–N_2O flame. K and Na were measured by flame photometry (PG 2000 Instruments).

2.8. Statistical analysis

Statistical analysis was performed in R v3.6 (R Core Team, 2013). All data were subjected to a two-way analysis of variance (ANOVA) for the determination of the main and interaction effects of drought-stress and inoculation treatments. For the comparisons between means the Duncan's multiple range test was employed ($p < 0.05$), using the R package *agricolae*. All plots were generated with the R packages *ggplot2* and *ggrpub*.

3. Results

3.1. Plant growth

Overall, limited irrigation led to drought stress symptoms and reduced growth (Fig. 1, Supplementary Fig. 2). Limited irrigation led to reduced shoot and root dry weights by 39 & 28% respectively, in control non-inoculated plants (Fig. 1A and B). Leaf dry weight was also reduced by 48% (Fig. 1C), while the 19% reduction trend in leaf area was not significant (Fig. 1D). AMF inoculation of the two strains induced a similar positive effect on shoot growth of the tomato plants that resulted in 40% increase under full irrigation and 60% increase under limited irrigation (Fig. 1A). Leaf weight increased similarly by 40% in the presence of both AMF strains under full irrigation, while it was doubled under drought (Fig. 1C). Leaf surface area was doubled under full irrigation as a result of AMF inoculation, while it increased by 50% under limited irrigation (Fig. 1D). The two AMF strains led to increased root growth by 30% under limited irrigation only (Fig. 1B). Overall, inoculation with the AMF strains, clearly reinstated plant growth of drought stressed plants to the levels of fully irrigated non-inoculated plants, while inoculation boosted plant growth of fully irrigated plants even further (Fig. 1).

3.2. Physiological variables

Physiological variables were estimated after one month of continuous water deprivation, to examine possible mechanisms related to the alleviation of prolonged drought stress in AMF inoculated plants. A clear 50% decrease trend in net CO_2 assimilation rates (P_N) was observed for the control non-inoculated plants under limited irrigation (Fig. 2A), while stomatal conductance (g_s) and transpiration rates (E) were not affected (Fig. 2C). The two AMF inoculations induced similar changes, which were however, more pronounced for *R. irregularis* compared to *F. mosseae*: Under full irrigation P_N, g_s and E increased by 27%, 56% and 45% in plants inoculated with *F. mosseae* and by 55%, 100% and 91% in plants inoculated with *R. irregularis* compared to non-inoculated plants. Under prolonged drought, however, only P_N showed a clear increase trend of about 85%, for both inoculation treatments (Fig. 2A), while g_s and E were not affected by inoculation (Fig. 2B and C).

3.3. Plant water status

The photosynthetic water use efficiency (pWUE), the ratio of P_N-to-E, is an indicator of the metabolic use efficiency of water, i.e. the cost of
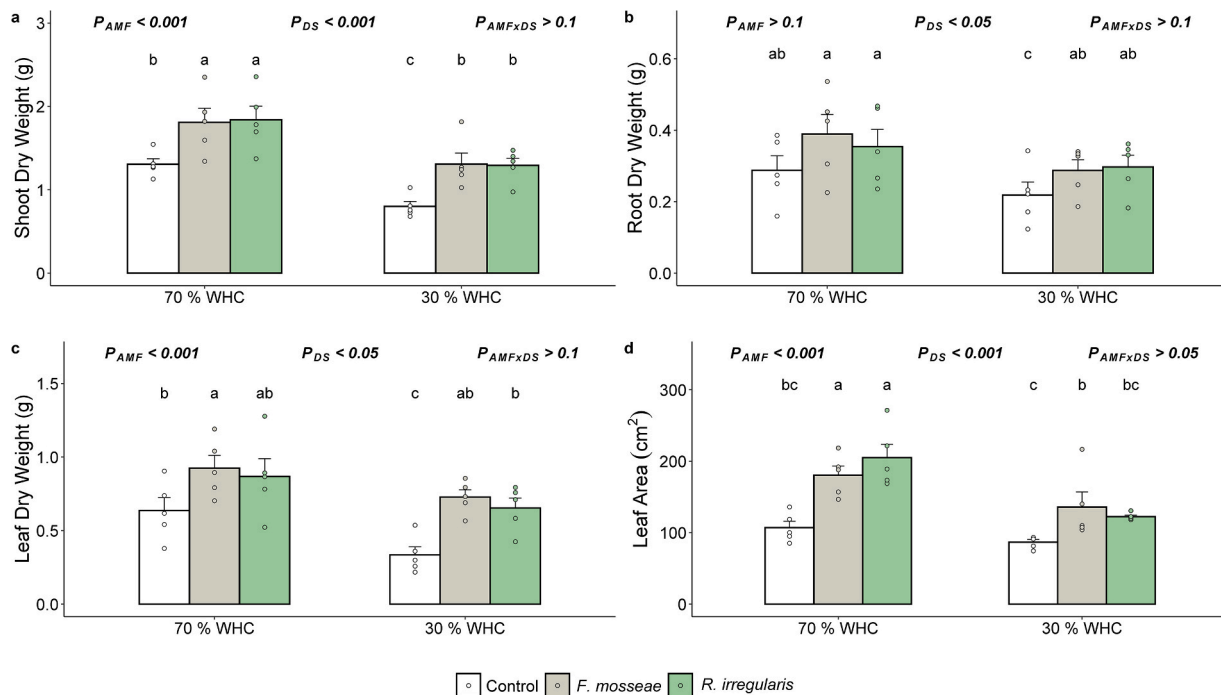

Fig. 1. The effects of irrigation regime and inoculation with two AMF species on (a) Shoot Dry Weight, (b) Root dry Weight, (c) Leaf Dry Weight and (d) Leaf Area of tomato plants after one month under drought stress conditions. Non-inoculated and inoculated plants were grown under normal (70%) or limited (30%) irrigation schemes. Data represent average values (n = 5) and error bars are standard errors of the means. P_{AMF}, probability value for the inoculation with the *F. mosseae* and *R. irregularis* species; P_{DS}, probability value for the moisture treatment; P_{AMFxDS}, probability value for the AMF × moisture interaction. P values higher than 0.05 indicate lack of significant effect. Different letters between columns indicate significant differences at p < 0.05 (Duncan's multiple range test).
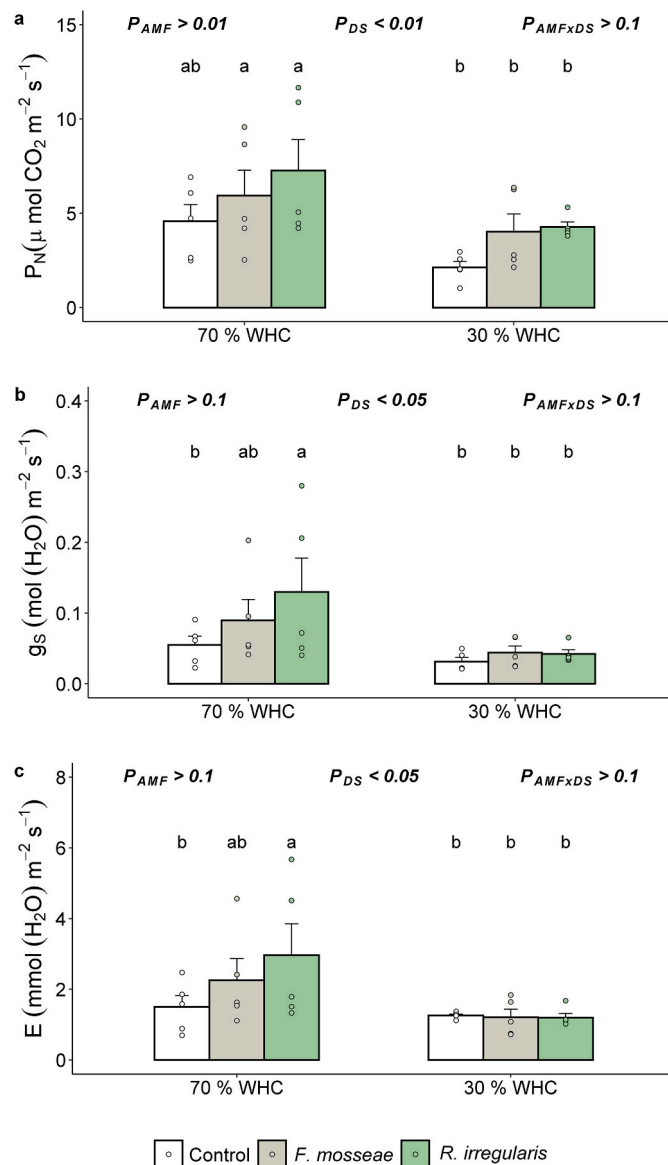
Fig. 2. The effects of irrigation regime and inoculation with two AMF species on (a) Net photosynthetic rate (P_N), (b) stomatal conductance (gs) and (c) transpiration rate (E) of tomato plants after one month under drought stress conditions. Non-inoculated and inoculated plants were grown under normal (70%) or limited (30%) irrigation schemes. Data represent average values (n = 5) and error bars are standard errors of the means. P_{AMF}, probability value for the inoculation with the *F. mosseae* and *R. irregularis* species; P_{DS}, probability value for the moisture treatment; P_{AMFxDS}, probability value for the AMF × moisture interaction. P values higher than 0.05 indicate lack of significant effect. Different letters between columns indicate significant differences at p < 0.05 (Duncan's multiple range test).

Fig. 3. The effects of irrigation regime and inoculation with two AMF species on (a) photosynthetic Water Use Efficiency (pWUE) and (b) Relative Water Content (RWC) of tomato plants after one month and 21 days respectively under drought stress conditions. Non-inoculated and inoculated plants were grown under normal (70%) or limited (30%) irrigation schemes. Data represent average values (n = 5) and error bars are standard errors of the means. P_{AMF}, probability value for the inoculation with the *F. mosseae* and *R. irregularis* species; P_{DS}, probability value for the moisture treatment; P_{AMFxDS}, probability value for the AMF × moisture interaction. P values higher than 0.05 indicate lack of significant effect. Different letters between columns indicate significant differences at p < 0.05 (Duncan's multiple range test).

photosynthetic carbon assimilation in terms of water loss *via* leaf stomata. The photosynthetic water use efficiency (pWUE) dropped to 50% in the control non-inoculated plants under limited irrigation compared to that of the fully irrigated plants (Fig. 3A). The relative water content of the leaves (%RWC) was also reduced significantly by 20% (Fig. 3B).

AMF inoculation of plants grown under full irrigation did not result in improved pWUE rather a trend for wasteful plant-water utilization was observed; however, when plants were grown under limited irrigation a doubling of pWUE was observed for plants inoculated with *F. mosseae* and an over-doubling for plants inoculated with *R. irregularis* compared to the non-inoculated control plants (Fig. 3A). Inoculation with AMF, also, resulted in a significant increase in the %RWC compared
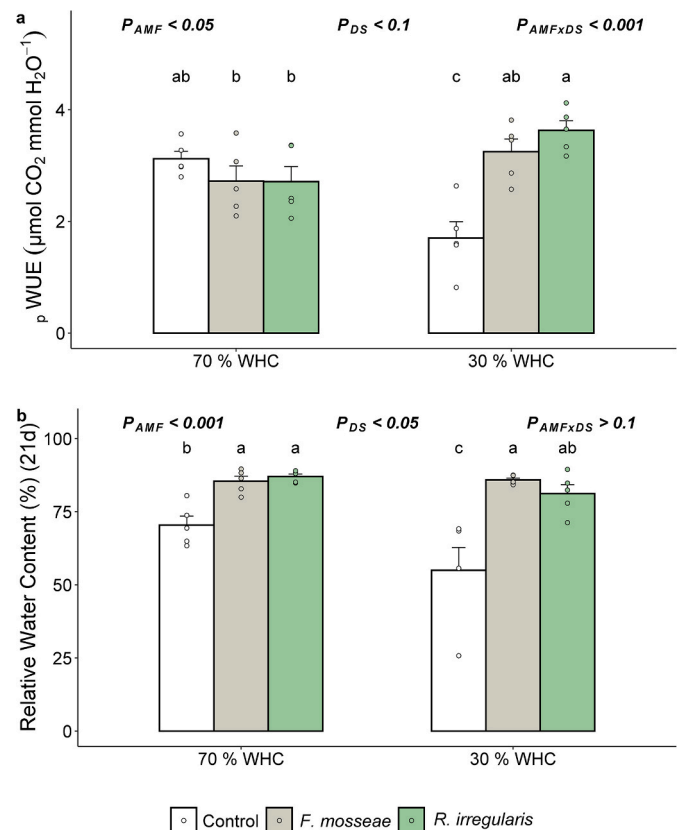
to control non-inoculated plants. The increase was greater for the plants grown under limited irrigation, around 50% for both AMF strains, and all inoculated plants reached the same level of %RWC independent of irrigation scheme (Fig. 3B). No differences were observed between the two AMF strains on the way they affect pWUE and %RWC.

3.4. Leaf macro- and micro-nutrient concentrations

Concentrations of P, K, Mg & Ca in the leaves were significantly reduced, by 47%, 37%, 35% and 57% respectively, when the non-inoculated tomato plants were grown under limited irrigation (Fig. 4).

Concentrations of micronutrients were not significantly affected by irrigation alone, however Fe tended also to be reduced (Supplementary Fig. 3). A positive effect was observed in the nutrient profile of the AMF-inoculated plants grown under limited irrigation regarding P, K, Mg and Ca (Fig. 4) as well as Zn and Mn (Supplementary Fig. 3), where nutrient concentrations were maintained within the levels of non-stressed plants. However, under full irrigation regime, positive effects were observed for P only (Fig. 4 & Supplementary Fig. 3) which increased approximately by 13% for *F. mosseae* and by 35% for *R. irregularis* compared to non-inoculated plants.
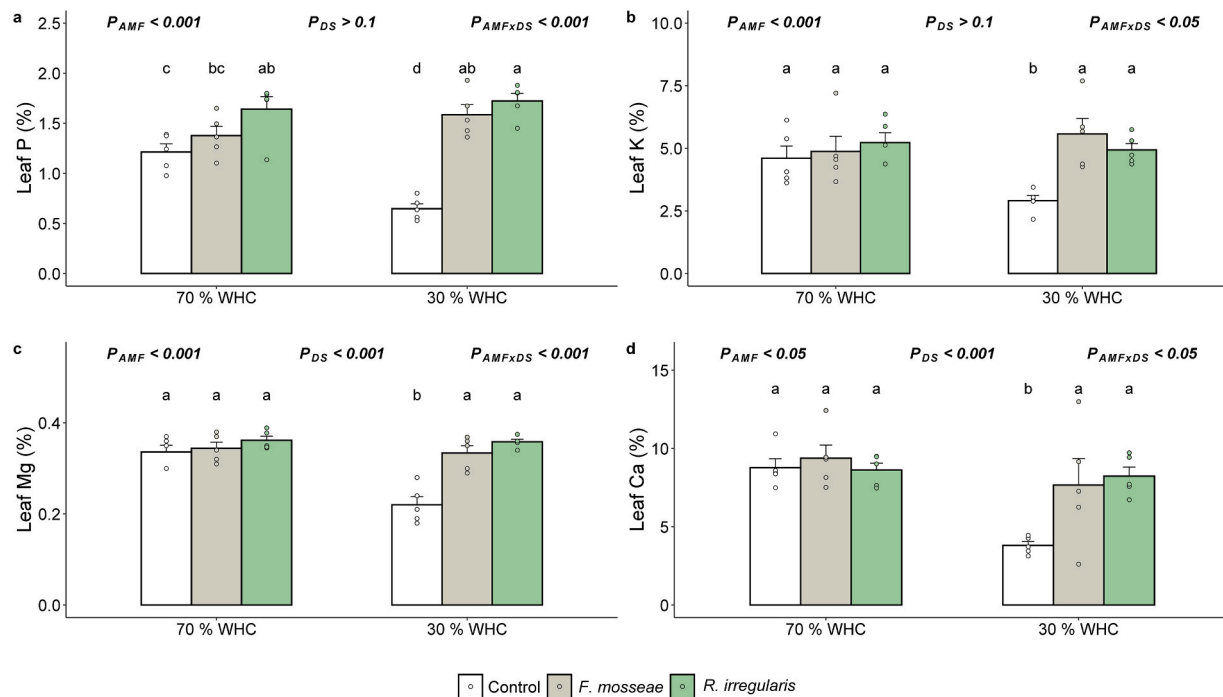
Fig. 4. The effects of irrigation regime and inoculation with two AMF species on leaf concentration of (a) Phosphorus (P), (b) Potassium (K) (c) Magnesium (Mg) and (d) Calcium (Ca) of tomato plants after one month and 21 days respectively under drought stress conditions. Non-inoculated and inoculated plants were grown under normal (70%) or limited (30%) irrigation schemes. Data represent average values (n = 5) and error bars are standard errors of the means. P_{AMF}, probability value for the inoculation with the F. *mosseae* and *R. irregularis* species; P_{DS}, probability value for the moisture treatment; P_{AMFxDS}, probability value for the AMF × moisture interaction. P values higher than 0.05 indicate lack of significant effect. Different letters between columns indicate significant differences at p < 0.05 (Duncan's multiple range test).

3.5. Root length colonization

No mycorrhizal structures were observed in the roots of control plants. Generally, tomato plants showed about 25% relative root length colonization with AMF, which was doubled under drought stress (Fig. 5). Effects were similar for both AMF species (Fig. 5).
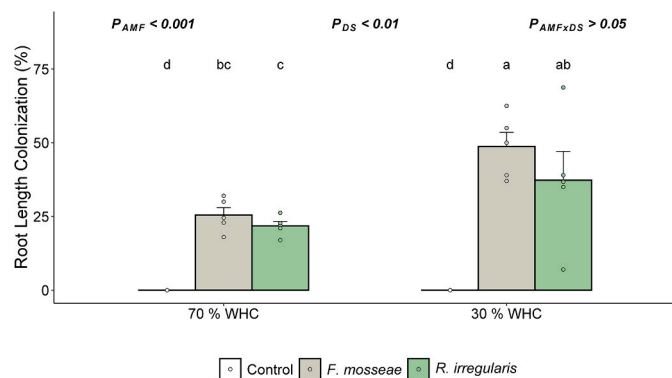

Fig. 5. The effects of irrigation regime and inoculation with two AMF species on % root length colonization of tomato plants by the AMF, after one month under drought stress conditions. Non-inoculated and inoculated plants were grown under normal (70%) or limited (30%) irrigation schemes. Data represent average values (n = 5) and error bars are standard errors of the means. P_{AMF}, probability value for the inoculation with the F. *mosseae* and *R. irregularis* species; P_{DS}, probability value for the moisture treatment; P_{AMFxDS}, probability value for the AMF × moisture interaction. P values higher than 0.05 indicate lack of significant effect. Different letters between columns indicate significant differences at *p* < 0.05 (Duncan's multiple range test).

4. Discussion

4.1. Tomato plants under full irrigation

Under full irrigation mycorrhizal plants grew better than their non-inoculated counterparts and showed increased relative water content in the leaves. The latter occurred in spite of increased transpiration and gas exchange rates and of increased area -to- biomass ratios in the leaves (plants inoculated with *F. mosseae* increased leaf area -to- biomass ratio by 17% compared to non-inoculated plants, while plants inoculated with *R. irregularis* showed an even more prominent increased by 53%). These observations clearly indicate that facilitated water transport and reduced hydraulic resistance throughout the soil-plant-air continuum was a key mechanism for advancing plant performance characteristics under full irrigation. Extraradical hyphal growth has been linked to enhanced exploitation of available water in the root zone and to enhanced water uptake (Hardie, 1985; Ruiz-Lozano and Azcón, 1995) a mechanism that may, however, be of secondary importance under full watering. Apoplastic water transport in the roots, a major process of radial transfer, was also shown to be increased in AMF-colonized tomato plant roots under both full watering and water-stress conditions (Bárzana et al., 2012). Cell-to-cell water flow was also shown to be facilitated following colonization, an effect related to the expression of several aquaporin genes (Bárzana et al., 2014) and observed even under flooding conditions (Calvo-Polanco et al., 2014). However, AMF also confer a metabolic cost to the plant host, as AM colonizers impose a strong carbon sink strength to the host plant, using up to 20% of photoassimilates (Wright et al., 1998). The increased stomatal conductance, the doubled leaf area and the clear trend for increased photosynthesis rates (especially for the plants inoculated with *R. irregularis*) indicate that a carbon sink stimulation mechanism operated in the colonized tomato plants, compensating for increased photoassimilate drainage. This mechanism was convincingly suggested by Kaschuk et al. (2009),

and was shown to be unrelated to foliar P content increases (Schweiger et al., 2014). Direct evidence for its operation in cucumber plants was also recently provided (Gavito et al., 2019).

4.2. Tomato plants under water stress

Limited irrigation led to reduced growth of control non-inoculated tomato plants. Leaves grew thinner and a clear trend for reduced photosynthesis rates was observed. Water stress resulted in reduced water content in the leaves, and a dramatic reduction in metabolic water use efficiency. Inoculation with the AMF led to major amelioration of these effects. Plants grew better than their non-inoculated counterparts reaching the growth characteristics of non-inoculated plants under full irrigation. However, the AMF appear to operate under prolonged water limitation *via* a different mechanism, prioritizing water conservation: Leaf transpiration rates were not increased in inoculated plants under limited irrigation, and stomatal conductivity remained low. Considering the smaller leaf area of the mycorrhizal plants under limited irrigation, this results to low transpiration at the plant level as well. Mycorrhizal plants have been shown to respond to drought and salt stress and to keep low transpiration rates by down-regulating the expression of plasma membrane aquaporins (Ouziad et al., 2006). Down-regulation of certain (but not all) aquaporin genes was also shown in mycorrhizal tomato plants under prolonged water stress (Chitarra et al., 2016). However, aquaporin gene expression under water stress is highly complex and depends on stress longevity (Barzana et al., 2014). Although the extra-radical and intraradical hyphae may improve water uptake of mycorrhizal plants, (Khalvati et al., 2005), the direct water uptake *via* fungal hyphae appears to be minor compared to plant transpiration needs (Graham and Syvertsen, 1984), even under drought conditions (Khalvati et al., 2005; Püschel et al., 2020). Apoplastic water flow, however, was shown to be highly increased following inoculation by AMF in the roots of tomato plants under both full watering and water stress conditions (Barzana et al., 2012). A composite model, reviewed by (Barzana et al., 2012) likely operates where colonization by AMF aids plant response to water stress by regulating (a) apoplastic and (b) cell-to-cell water transport balance. Enhanced K^+ uptake, as observed in this study, may indeed lead to increased root hydraulic conductivity under drought (El-Mesbahi et al., 2012) and robust stomata operation, while control of the cell-to-cell high resistance path, by osmoregulation and regulation of cell membrane aquaporin expression, may lead to reduced water losses and maintenance of high metabolic activity in mycorrhizal plants under drought. This is in line with the high leaf relative water content and nutrient uptake maintained by mycorrhizal plants under water stress, which was even higher than the fully watered non-mycorrhizal controls.

We also showed greatly decreased leaf area-to-biomass ratios for both AMF inoculants compared to non-inoculated tomato plants under water stress (contrary to the increased ratios observed following inoculation under full irrigation). Growing thicker leaves with reduced area-to-biomass ratios in the mycorrhizal tomato plants apparently preserved the function of the photosynthetic apparatus. This is also in line with the elevated Mg concentrations in the leaves, indicating higher chlorophyll contents. In total, this led to the doubled metabolic water use efficiency, which, contrary to controls, was retained tended to exceed even that of fully watered plants. It has been shown that CO_2 drawdown from sub-stomatal cavities to chloroplasts increases in leaves with higher biomass to surface area ratios (Niinemets et al., 2009) and that this CO_2 drawdown to chloroplasts as well as mesophyll diffusion conductance (g_m) are reduced under water stress in tomato plants (Warren, 2008). The potential role of mycorrhizal colonization in improving plant metabolic performance by allowing for alleviations of photosynthesis limitations in the mesophyll needs to be further investigated.

4.3. Tomato plant nutrition

Improved plant nutrition following inoculation with AMF has been

well established (Smith and Smith, 2011). Our results showed that P, K, Mg & Ca concentrations in the leaves of the tomato plants were highly increased by both inoculations under water stress (resulting in remarkably multifold increases of total uptake, Supplementary Fig. 4). However, only P concentration showed a significant though smaller increase, under full watering. The latter is in line with recent results presented by Püschel et al. (2021) who show improved P acquisition under a wide range of soil moisture contents but a greater mycorrhizal effect towards lower water regimes. Greater mycorrhizal effect on P acquisition of tomato plants under deficit irrigation was also shown by Bowles et al. (2016), who also observed three-fold higher rates of root sap exudation in these plants compared to the respective non-mycorrhizal plants, indicating amelioration of water stress. Volpe et al. (2018) showed that inoculation of tomato plants with *F. mosseae* and *R. irregularis* led to dissimilar ecophysiological plant performances under water stress and related these dissimilarities to different phosphate uptake efficiencies presented by the two fungi. The similar P acquisition response by both inocula in our study, coupled to their similar functional role on tomato plant physiological adaptation are consistent with this, supporting a key role for P uptake efficiency on plant physiological adaptation to water scarcity. Moreover, they indicate that differences at the subspecies level may be critical for the functional profile of AMF inocula. The improved K absorption in mycorrhizal plants under drought has been related to increased root hydraulic conductivity in inoculated plants (El-Mesbahi et al., 2012), so overall, our results indicate an indirect role of inoculation on plant ecophysiological adaptation under water stress *via* the doubling of P and K uptake. Among micronutrients, the increased concentration and total uptake of Zn under water stress is consistent with its slow diffusion characteristics and with relevant reports summarized by Lehmann et al. (2014). The increased uptake of Mn under drought, has been previously reported (Bagheri et al., 2012) and is in line with a metadata analysis by Lehmann and Rillig (2015) who showed that Mn uptake is usually negatively affected by AMF inoculation, but perennial herbs are a notable exception.

5. Conclusions

Overall, increased photosynthesis rates and transpiration rates by an average 40% and 70% respectively under both AMF strains, doubled leaf area, and increased relative water content of the plant leaves by 25% indicate that improved growth effects by AMF colonizers derived from facilitated water transport coupled to carbon sink stimulation in the fully irrigated tomato plants.

On the contrary the over-doubled vegetative plant growth and nutrient concentrations in the plant leaves under drought, were coupled to conserved transpiration and gas exchange rates, indicating an efficient drought stress amelioration mechanism in AMF-colonized plants, *via* prioritization of water status conservation *in planta* and protection of the photoassimilation apparatus.

Induction of increased leaf area-to-leaf biomass ratios under full irrigation, contrary to reduced ratios under water stress appear to be a key differentiation in phenotypic adaptation of mycorrhizal tomato plants to soil water potential, while the role of mycorrhization in aquaporin regulation and function appears as a promising target for future research.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rhisph.2021.100394.

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