



Effect of Arbuscular Mycorrhizal Fungi on Water Relations and Growth of Young Plum Trees under Severe Water Stress Conditions

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Authors' contributions

This work is a result of collaboration between the both authors. Author RR performed the experiment and wrote the first draft of the manuscript. Author AK designed the study and statistical analysis. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Arbuscular mycorrhizal fungi (AMF) can mitigate plant response to severe water stress. On this basis, an experiment was carried out under field conditions to evaluate the effect of arbuscular mycorrhization, realized by a mixture of two AMF species, *Rhizophagus intraradices* and *Funneliformis mosseae*, on drought tolerance of young plum trees.

Study Design: The experimental design was a criss cross with three variable factors: water regime, mycorrhization and plum variety.

Place and Duration of Study: the trial was conducted during one year (2013) under field condition in experimental station Ain Taoujdate of the Regional Agricultural Research Center of Meknes, in northern Morocco.

Methodology: The experiment was performed on four one year old mycorrhizal and non-mycorrhizal plum varieties submitted to two water regimes, 50% and 100% of crop evapotranspiration (ETc). Measurements have concerned vegetative growth parameters (shoot elongation, trunk growth and leaf area), water status (predawn and midday leaf water potential,

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stomatal conductance and leaf relative water content) and leaf phosphorus content).

Results: the young plum trees, even mycorrhized, did not tolerate water stress applied. However, plants were dependent on AMF under water stress, highly compensating its effects even at 50% of ETc. The compensatory effect of AMF was related to an increase of water potential and stomatal conductance without changing relative water content of plants. AMF also induced a significant increase of phosphorus uptake under water stress.

Conclusion: it was demonstrated that AMF significantly improve water and nutrient use efficiency of young plum trees submitted to water stress amounting to 50% of ETc. The observed improvements due to AMF were considerable, suggesting possibility of adoption of this water restriction to optimize deficit irrigation of mycorrhizal plants of this rosacea under low water availability conditions.

Keywords: *Prunus domestica*; water stress; arbuscular mycorrhizal fungi; water status; phosphorus; vegetative growth.

1. INTRODUCTION

Morocco's climate is mainly semi-arid. Rainfall is erratic during the year and varies from one year to another. Water resources preservation is very important for the development of Moroccan agriculture. The application of deficit irrigation associated with the use of arbuscular mycorrhizal fungi (AMF) is a promising way to save irrigation water. This study is particularly *justified* in semi-arid areas on plum trees that require a high quantity of irrigation water. This thematic is a new area of research in Morocco. Thus, the effect of mycorrhizal on fruit trees is little studied.

It is well known that mycorrhizal symbiosis have favorable effects on nutrients uptake and water relations of plants. Since its observation for the first time by Giuseppe Gibelli in 1879, many researchers have studied it on different plants and several studies have been published on this subject [1-4]. This symbiosis has attracted much interest for its use to mitigate stress effect on plants after obtaining synthetic strains since 1967 by Anna Fontana [5]. Since then and with development of molecular biology techniques and genetic analysis, several strains of mycorrhizal fungi were isolated, synthesized and tested on different plants. Especially for woody species, the emphasis was laid on symbiosis with AMF, belonging particularly to Glomeromycota family, for their encouraging results on implementation of these species in arid and semi-arid areas [6,7].

AMF Hyphae penetrate plant root cortex where they form intracellular arbuscles and vesicles. Arbuscles are the place of contact and exchange of elements between the two symbionts and vesicles constitute storage organs. Extra-root hyphae also grow over several centimeters

outside from the root and may bear a multitude of spores that are the reproductive structures of AMF [8]. In symbiosis, the two partners mutually exchange elements necessary for their proper development: mycorrhizal fungi convey water and nutrients to the plant in exchange for carbon molecules coming from photosynthesis. Inoculation with *Rhizophagus intraradices* significantly led to increase vegetative growth and the formation of lateral roots in rice plant [9]. In red tangerine seedlings, *Funnelliformis mosseae* notably improved shoots growth and parameters of root system architecture, including total root length, total root projected area, total root surface and total root volume and decreased root average diameter significantly, compared with the non-AMF control [10].

It is widely accepted that AMF play an important role in host plant adaptation to drought [11,12]. However, the underground nature and the fact that a part of the fungal biomass is included inside roots imply that some mechanisms of this symbiosis are unknown, although significant progress in the understanding of these mechanisms were made with development of ecophysiology and biotechnology techniques. Possible mechanisms of AMF positive effects could be related to increase of root hydraulic conductivity [13], improvement of stomatal regulation and transpiration rate [14], forcing of water absorption even under low soil moisture by the extra-radical mycorrhizal hyphae, osmotic adjustment which promotes maintenance of cell turgor even at low water potential of tissues [15] and increase of photosynthetic activity by improving nutrients absorption [16]. Furthermore, Fitter [17] claimed that the influence of AMF on plant water relations may be a secondary consequence of an increase of minerals absorption, especially phosphorus. But the

verification tests of this hypothesis have produced controversial results. Indeed, Nelson [3] found that water relations of onion were improved with increase of phosphorus concentration. Conversely, on rose and pepper plants, Augé et al. [15] found different levels of plant water stress resistance under the same phosphorus concentration.

Use of AMF seems to be a promising technique to improve water use efficiency of various plants subjected to water stress and save irrigation water. This technique would be particularly justified in semi-arid areas such as a high part of Morocco and on plants that require a high quantity of irrigation water such as plum trees. It is within that framework that this work was carried in order to quantify the effects of inoculation of young plum trees by arbuscular mycorrhizae, *Rhizophagus intraradices* and *Funnelliformis mosseae*, submitted to severe water stress under field conditions.

2. MATERIALS AND METHODS

2.1 Plant Material and Cultural Conditions

The trial was carried out under field conditions in experimental station Ain Taoujdate of the National Agronomic Research Institute, located in northern Morocco (33° 56 'E, 5° 13' N, 499 m). Meteorological data, collected from the experimental field station, during the year of study (2013) is presented in Fig. 1 showing that rainfall deficit was very pronounced from March to October. The soil is of calci-magnesian type, sandy clay containing an average of 7.7% CaCO₃, moderately rich in organic matter in surface (0-30 cm), with an average of 2% (0.92% in deep layer 30-60 cm). Soil pH is approximately neutral (7.3) and not saline with an electrical conductivity of 0.13 mS/cm in the first 60 centimeters.

The plant material used come from 48 one year old plants of four plum varieties (*Prunus domestica*) with similar size, which culture is widespread in Morocco: Angelino, Stanley, Fortuna and Black Amber grafted on Myrobalan rootstock, [18]. Before planting, the terminal roots were partially cut to stimulate plant growth and mycorrhizal colonization. Root inoculation was realized at plants plantation in field by 12 g/plant of an inoculum purchased on market from France containing 25 spores/g of *Rhizophagus intraradices* and 25 spores/g of *Funnelliformis mosseae* [19]. Indeed, the inoculum was put in contact with the roots to a depth of about 30 cm.

The choice of these AMF species is based on their high ability to colonize prunus rootstocks, demonstrated in previous research [20,21]. Plants plantation was realized in January with spacing of 5x4m in the experimental field where the soil was previously homogenized by cover crop. All plants, mycorrhizal and non-mycorrhizal, were pruned, fertilized (N-P₂O₅-K₂O = 60-40-80 kg/ha) and treated in the same way, except irrigation that varied to produce two water regimes during non-rainy days: 100% and 50% of crop evapotranspiration (ET_c) since trees plantation. ET_c values were estimated as the product of reference evapotranspiration (ET_o) obtained with the Hargreaves model [22] and the crop coefficients recommended by FAO adjusted to planting density and foliageu dimensions using a reduction coefficient (Kr) recommended for almond tree: $Kr = \pi D^2 N / 20000$ where "D" is the average of foliage diameters and "N" is planting density [23].

The experimental design was a criss cross with three variable factors: water regime (50% ET_c and 100% ET_c), mycorrhization (M + and M-) and plum variety. Indeed, the experimental orchard was divided into two equal and homogenous plots, one of which was fully irrigated (100% ET_c) and another was submitted to water stress of 50% ET_c. Both plots contain 24 young plum plants. Six of those were randomized per variety of which three were inoculated by AMF and another three were not.

2.2 Measurements

2.2.1 Vegetative growth measurements

The effect of water stress on vegetative growth of mycorrhizal and non-mycorrhizal plum trees was evaluated at the end of their growth cycle, at the end of october. Plant height, annual growth of trunk section, primary shoots length, secondary shoots length, number of secondary shoots per linear meter of primary shoot and leaf area were measured.

The plant height was measured from collar graft to the highest apex. The annual growth of trunk section was estimated by measuring trunk circumference at the beginning and the end of plants growth cycle at 10 cm above soil. The average of primary and secondary shoot elongation was determined by measuring the final length of all shoots per plant. The leaf area was evaluated on twenty fully developed leaves per plant, taken from medial portions of the primary shoots.

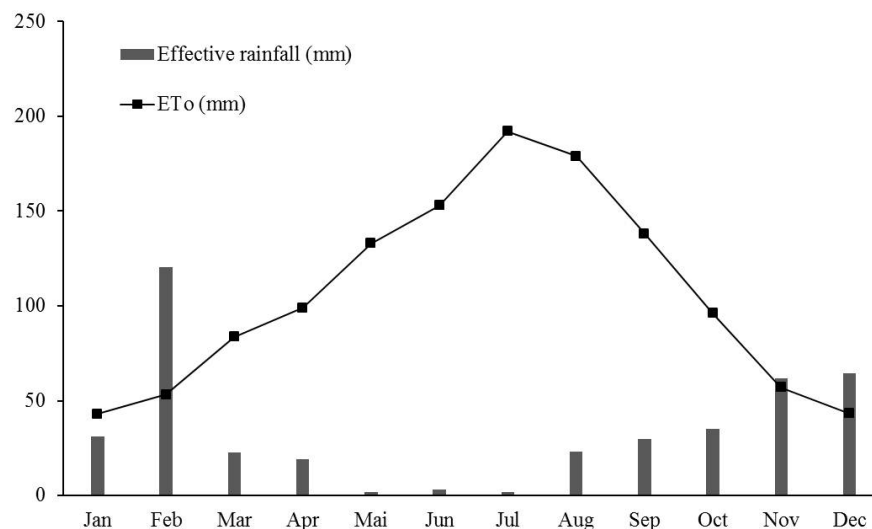


Fig. 1. Effective rainfall (80% of rainfall) and reference evapotranspiration (ETo) obtained from the Hargreaves model during the year of study in experimental field

2.2.2 Plants water status measurements

Plants water status was evaluated by monitoring predawn leaf water potential (Ψ_{pd}), midday leaf water potential (Ψ_{md}), midday stomatal conductance (gs) and midday leaf relative water content (RWC) for five different dates during plant growth cycle.

Ψ_{pd} was measured in the morning by a Scholander pressure chamber on two leaves per plant taken from shoot extremity (4th and 5th leaf), previously bagged by aluminum paper at sunset of the day preceding measurement. Ψ_{md} was measured on two leaves per plant taken from shaded shoot extremity.

Stomatal conductance and Ψ_{md} on selected leaves were measured. At the same time (13h GMT), five fully developed leaves per plant were taken from shaded shoot extremity to measure the relative water content. This parameter was determined following the formula of Turner (equation 1) [24]:

$$RWC = \frac{FW - DW}{SW - DW} \times 100 \quad (\text{Equation 1})$$

FW, DW and SW designate fresh, dry and saturation weights of leaf sample respectively. Leaves were saturated by placing their petioles in contact with water in boxes papered inside

with wet filter paper for 24 hours in a refrigerator set at 5°C and they were dried in an oven at 105°C for 48 hours.

2.2.3 Leaf phosphorus content

Leaf phosphorus content was determined in october on leaf samples taken from the middle portions of shoots toward the end of plant growth cycle. Phosphorus analysis was performed according to the method described by Rayan et al. [25]. Indeed, phosphorus was extracted on dried samples using a mixture of ammonium molybdate, ammonium vanadate and nitric acid and quantified by a spectrophotometer set at 410 nm.

2.2.4 Mycorrhizal colonization and sporulation

Mycorrhizal colonization on root collected from soil samples (approximately 250 g/plant) taken from root zone was determined at the end of plant growth at the end of November. The collected roots were washed thoroughly with distilled water and preserved in a lactoglycerol solution (63 ml glycerol, 62 ml distilled water, 875 ml of acetic acid). Staining of root was realized on fragments measuring approximately 1 cm following the method of Philips and Hayman [26]. Indeed, the roots fragments were placed in 10% KOH solution in a bain-marie set to a temperature of 90°C for 2 hours. They were

subsequently washed with distilled water and transferred into 2% HCl solution for 5 min before being placed in a staining solution (lactoglycerol with 0.05% trypan blue) in bain-marie at 90°C for 15 min. After staining, the Mycorrhizal colonization was estimated under an optical microscope (x 100) from the number of fragments showing arbuscles or vesicles on the total colored fragments.

We counted the number of mycorrhizal spores on soil samples collected from the root zone of mycorrhizal plum trees. Indeed, an amount of 200 g of soil of each sample was dried under the open air, softly stirred in 3 liters of distilled water and left to decant for 5 to 10 seconds, until the precipitation of the large particles of soil. The soil solution in suspension was passed through a series of piled sieves under tap water (250 µm, 106 µm and 63 µm). The fraction retained on the latest sieve (63 µm) was recovered and placed in a 50 ml conical tube which level was adjusted with distilled water to 25 ml. Using a syringe, 20 ml of a sucrose solution (50% w/w) was added in tube bottom. The tubes were then passed to the centrifuge (1000 rpm for 5 min). At the end of the centrifugation, the spores were concentrated to the sucrose-water interphase, that were collected using a pipette and sieved again (63 µm) in order to eliminate sugar residues. Finally, spores were isolated, placed in a petri dish containing 100 µl of distilled water and counted under a binocular microscope.

2.2.5 Statistical analysis

Data was used in order to analyze the variance (ANOVA) using the SPSS software (version 17.0) Mean comparisons were performed using student's test to compare the effect of AMF depending on water treatment.

3. RESULTS AND DISCUSSION

3.1 Vegetative Growth

Vegetative growth of all tested plum varieties, mycorrhizal and non-mycorrhizal, was affected by water stress of 50% of ETc. However, the affected parameters differed depending on varieties. For all varieties, water stress applied induced a significant decrease of shoots length and number of secondary shoots per linear meter of primary shoot, both in mycorrhizal and non-mycorrhizal plants (Table 1). This depressive effect of water stress was also significant for trunk growth of the Angelino variety. However, plant height and leaf area remained unchanged

in all varieties tested. These depressive effects of water stress on plant growth were observed in similar works even under moderate water stress of 75% of ETc [27,28].

Mitigation of water stress by AMF was partial, but statistically significant for all vegetative parameters affected. The mitigation rate due to AMF was 22% for primary shoot elongation and 57% for number of secondary shoots grown on linear meter of primary shoot. The decrease of trunk growth under water stress, observed only in *Angelino* variety was significantly alleviated by AMF by rate of 50%. Vegetative growth gain due to AMF was also observed for non-stressed plants, but with a relatively low magnitude. For all varieties, average of this gain was 12% and 22% respectively for primary shoot elongation and number of secondary shoots per linear meter of primary shoot. This mitigation of water stress due to AMF can be explained by their favorable effects on nutrient uptake and plant water relations under water stress conditions, as it has been demonstrated on several plants in previous studies [29-31].

Plum plants were therefore significantly dependent to mycorrhizal fungi under the level of water stress tested. However, it should be noted that it is often assumed that dependency of plants to arbuscular mycorrhizae decreases with water stress, even disappearing under severe stress [32,29]. This decline of AMF effect under severe water stress is mostly explained by ineffectiveness of mycorrhizal fungi at very low soil water potential, which would be attributed to low germination of spores and to AMF-soil-plant interactions [33]. Furthermore, mycorrhizal dependency of plants was relatively low under full irrigation, but statistically significant. The weakness of the mycorrhizal dependency under this latter water regime is explained by the low biomass gain observed in mycorrhizal plants, limited by the genetic growth potential of cultivars [34].

3.2 Leaf Phosphorus Content

The applied water stress causes decrease of phosphorus uptake for all non-mycorrhizal plants by different degrees depending on varieties, with an average of 35% (Table 2). This result is in line with many studies about the effects of phosphate nutrition of plants under water stress [35,36]. Reduction of leaf phosphorus content in stressed plants is certainly not related to a deficiency of this nutrient in soil solution, but rather to a decrease of number of rootlets

in response to water stress, which constitute the essential seat of mineral uptake [37].

This depressive effect of water stress on phosphorus uptake was significantly attenuated by AMF by 27% observed for *Stanley* variety to 100% for *Black Amber*, with an average for all varieties of 55%. The significant improvement of leaf phosphorus content in mycorrhizal plants comes from extra-root hyphae of AMF that operate as additional rootlets and also to their

ability to ramify the root system [38,39], thereby boosting nutrients uptake, including phosphorus. Mycorrhizal hyphae does not only explore the available phosphorus contained in soil solution, but they also have the ability to access to non-assimilable phosphorus and that integrated in organic matter by secreting phosphatase enzymes and various molecules that acidify the soil, making phosphorus more available [40].

Table 1. Vegetative growth parameters of mycorrhizal and non-mycorrhizal plants under different water treatments

| | | | Trunk growth (mm/year) | Plant height (cm) | Primary shoot (cm) | Secondary shoot (cm) | Number of secondary shoot (N/Lm) | Leaf area (cm ²) |
|----------------|------|----|---------------------------|-------------------------|--------------------------|-------------------------|--|------------------------------------|
| Angelino | R100 | M+ | 17.5 b | 2.5 b | 185.0c | 14.7 | 2.9 b | 19.7 |
| | | M- | 15.9 b | 2.1 ab | 159.2b | 11.6 | 1.9 a | 19.1 |
| | R50 | M+ | 13.8 ab | 2.3 ab | 150.0b | 21.7 | 2.4 b | 19.3 |
| | | M- | 11.7 a | 2 a | 138.3a | 8.2 | 1.5 a | 18.8 |
| Stanley | R100 | M+ | 17.5 | 1.5 | 106.7c | 11.4 | 10.0 c | 20.1 |
| | | M- | 15.9 | 1.5 | 98.3bc | 16.6 | 7.5 b | 19.6 |
| | R50 | M+ | 15.4 | 1.5 | 93.3b | 13.1 | 7.2 b | 19.7 |
| | | M- | 14.3 | 1.3 | 85.0a | 15.0 | 4.4 a | 19.3 |
| Fortuna | R100 | M+ | 15.9 | 2.3 | 163.3c | 11.6 | 8.8 b | 20.4 |
| | | M- | 15.4 | 2.3 | 130.7b | 21.2 | 7.7 b | 19.4 |
| | R50 | M+ | 14.3 | 2.0 | 80.0 a | 11.3 | 4.3 ab | 20.5 |
| | | M- | 12.7 | 1.9 | 78.3 a | 17.0 | 3.9 a | 19.9 |
| Black Amber | R100 | M+ | 20.7 | 2.2 ab | 165.0 b | 16.0 | 5.2 b | 19.1 b |
| | | M- | 15.9 | 2.0 a | 163.3 b | 8.5 | 4.9 ab | 19.0 b |
| | R50 | M+ | 22.3 | 2.4 b | 145.0 a | 7.9 | 4.6 ab | 17.6 a |
| | | M- | 20.2 | 2.1 ab | 143.3 a | 20.6 | 3.9 a | 17.5 a |
| Average | R100 | M+ | 17.9 | 2.1 | 155.0 c | 13.4 | 6.7 c | 19.8 |
| | | M- | 15.8 | 1.9 | 137.8 b | 14.5 | 5.5 bc | 19.3 |
| | R50 | M+ | 16.4 | 2.0 | 117.0 ab | 13.5 | 4.6 ab | 19.3 |
| | | M- | 14.7 | 1.8 | 111.2 a | 15.3 | 3.4 a | 18.9 |

The student test was applied for each variety separately; Values followed by different letters are statistically different at level of 95%; Values non-followed by letters are statistically equal at level of 95%; N/Lm: number secondary shoot per linear meter of primary shoot; R100: full irrigation regime; R50: water regime 50% of ETc; M+: mycorrhizal plant; M-: non-mycorrhizal plant)

Table 2. Leaf phosphorus content (mg/g dw) of mycorrhizal and non-mycorrhizal plants at the end of their growth cycle under different water regimes

| | | Angelino | Stanley | Fortuna | Black Amber | Average of all varieties |
|---------------|----|----------|---------|---------|-------------|-----------------------------|
| R100 | M+ | 2.7 b | 3.2 b | 2.4 b | 2.3 b | 2.7 b |
| | M- | 2.7 b | 3.0 b | 2.3 b | 2.2 b | 2.6 b |
| R50 | M+ | 2.2 ab | 2.2 ab | 2.2 b | 2.2 b | 2.2 ab |
| | M- | 1.7 a | 1.9 a | 1.5 a | 1.7 a | 1.7 a |
| Signification | | P=0.004 | P=0.004 | P=0.004 | P=0.004 | P=0.003 |

The student test was applied for each variety separately; Values followed by different letters are statistically different at level of 95%; Values non-followed by letters are statistically equal at level of 95%; R100: full irrigation regime; R50: water regime 50% of ETc; M+: mycorrhizal plant; M-: non-mycorrhizal plant

However, AMF has not had a significant effect on phosphorus uptake under full irrigation, although the obtained values generally show a tendency to a slight improvement of phosphorus uptake under this water regime. The non-significance of AMF effect under this water regime may be explained by the fact that the amount of rootlets developed by non-mycorrhizal plants under full irrigation was sufficient to uptake phosphorus at the similar level than mycorrhizal plants.

3.3 Plant Water Status

Plant water status was very influenced by water stress. Significant reductions of midday leaf water potential (Ψ_{md}), relative water content (RWC) and stomatal conductance (gs), measured at midday, were observed immediately on non-mycorrhizal plants upon application of water stress (Figs. 2,3,4), such as has been found in previous works on rosaceous fruit trees [41]. Over the monitored period, from May 07 to July 04, Ψ_{md} values were decreased by an average of -0.30 MPa for all tested varieties. RWC and gs values decreased by similar manner of Ψ_{md} values. Highly significant correlation was found between these parameters indicating this similarity (Fig. 5). Values of gs decreased by an average of 27%. However, the decrease in RWC was relatively low with an average of 4%, compared to non-mycorrhizal control.

Water stress applied does not induce immediate changes in leaf water potential (Ψ_{pd}), whose

values have remained unchanged for a period of three months after the application of stress, from March to May. However, with increase of crop evapotranspiration since June, Ψ_{pd} values began to decrease significantly in response to water stress, with an average of -0.08 MPa over the period from June 05 to July 04 (Fig. 6). According to Lampinen et al. [42], the decrease of Ψ_{pd} is explained by the fact that the root system of young trees is not sufficiently developed to explore all the wet parts of the rhizosphere and thus stabilize Ψ_{pd} values.

Moreover, water stress effect on plant water status was partially mitigated by AMF. Mitigation effect due to AMF was significant for Ψ_{md} and gs values. However, RWC values were not significantly affected by AMF although this parameter was significantly correlated with Ψ_{md} . According to works of Liu et al. [43], Duan et al. [44] and Davies et al. [45], this result indicates that under conditions of the present experiment, AMF induced an increase of Ψ_{md} , Ψ_{pd} and gs values in colonized plants by improvement of stomatal regulation and adjustment of osmotic potential through biochemical signals including essentially ABA and modification of hormonal balances, but without boosting water absorption, although this latter effect is known as a benefit of AMF [46]. A further explanation for this result is that AMF ensure maintenance of leaf cells turgor through accumulation of solutes, thereby stabilizing water potential and stomatal conductance values without changing leaf water content [47,44].

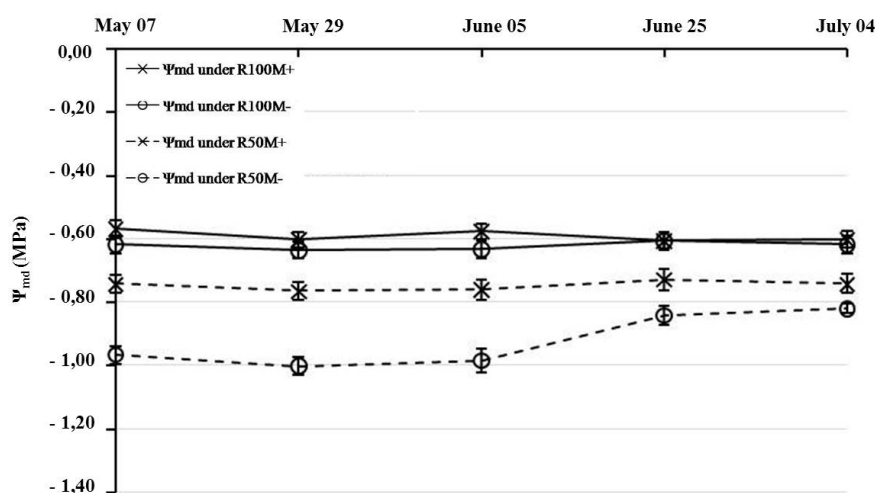


Fig. 2. Seasonal variation of midday leaf water potential (Ψ_{md}) of mycorrhizal (M+) and non-mycorrhizal (M-) young plum tree under full irrigation (R100) and water stress (R50) (average values for all tested varieties)

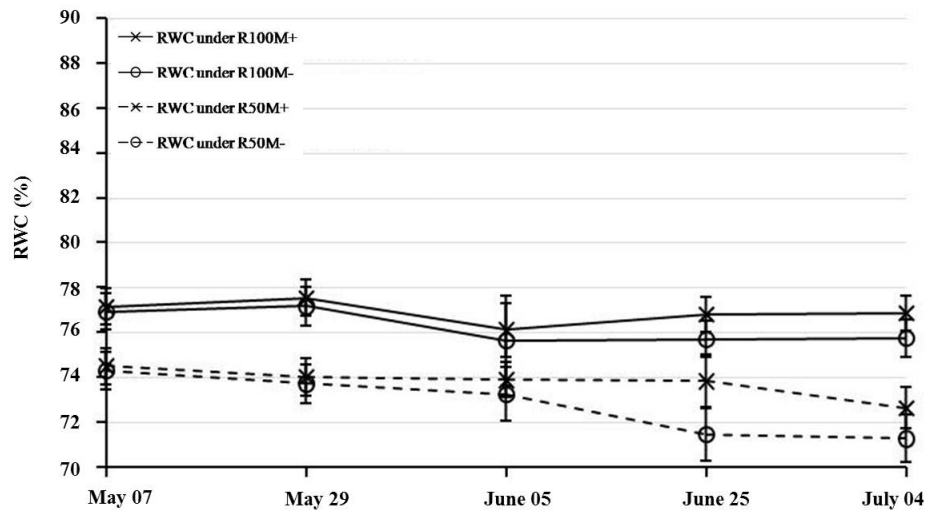


Fig. 3. Seasonal variation of midday leaf relative water content (RWC) of mycorrhizal (M+) and non-mycorrhizal (M-) young plum tree under full irrigation (R100) and water stress (R50) (average values for all tested varieties)

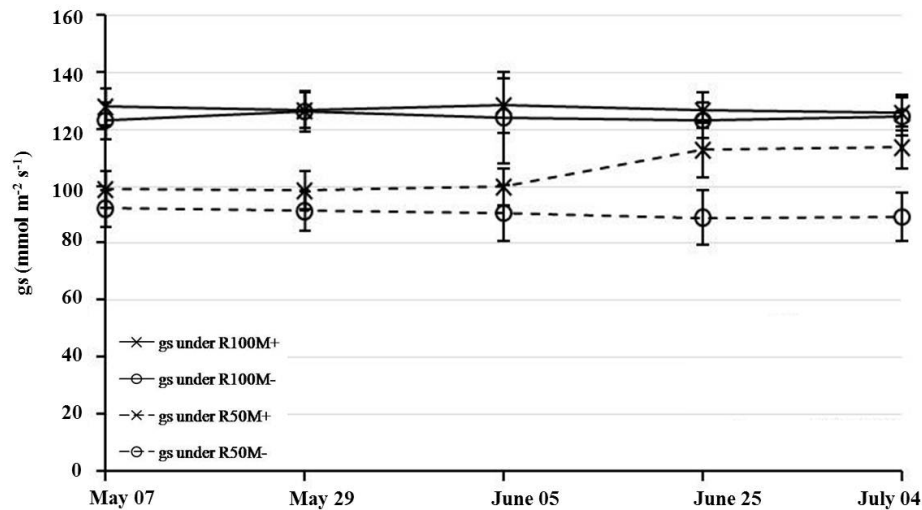


Fig. 4. Seasonal variation of midday stomatal conductance (gs), of mycorrhizal (M+) and non-mycorrhizal (M-) young plum tree under full irrigation (R100) and water stress (R50) (average values for all tested varieties)

These changes induced by water stress and AMF on plant water status were not statistically different between varieties. Indeed, variance analysis of Ψ_{pd} , Ψ_{md} , gs and RWC values at July 04, when the effects of water stress and AMF were more pronounced, revealed no significance differences between the tested varieties under all treatments (Table 3). This observation is explained probably by the fact that the tested varieties were grafted on the same rootstock.

3.4 Mycorrhizal Colonization and Sporulation

For non-inoculated plants, there is no root fragment colonized by eventual native AMF. However, all the inoculated plants were successfully colonized by AMF with varied colonization rates depending on water regime (Table 4). The differences between varieties were not significant probably because of the use of the same rootstock.

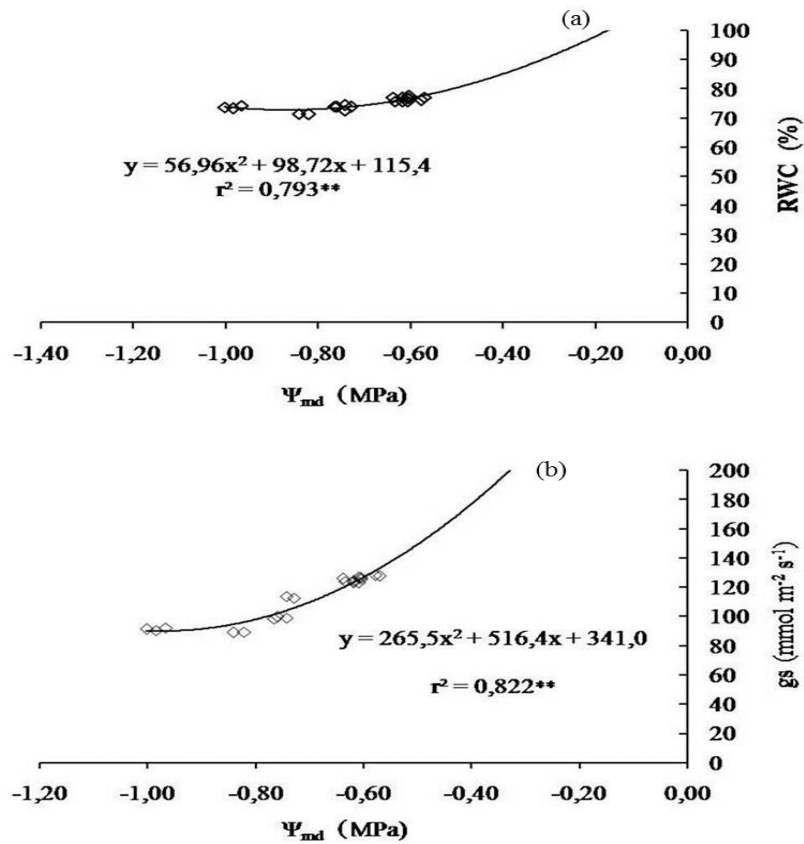


Fig. 5. Variation of water potential depending on relative water content (a) and stomatal conductance (b) measured at midday (all varieties and treatments combined)

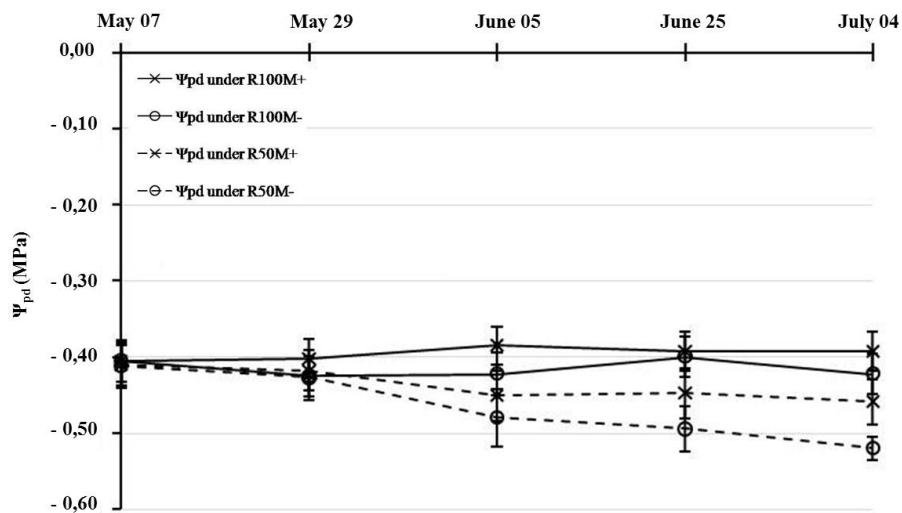


Fig. 6. Seasonal variation of predawn leaf water potential (Ψ_{pd}) of mycorrhizal (M+) and non-mycorrhizal (M-) young plum tree under full irrigation (R100) and water stress (R50) (average values for all tested varieties).

Table 3. P-values of variance analysis of water status parameters observed at July 04 depending on plum varieties

| | | Ψ_{pd} | Ψ_{md} | RWC | gs |
|------|----|-------------|-------------|-------------|-------------|
| R100 | M+ | $P = 0.632$ | $P = 0.792$ | $P = 0.56$ | $P = 0.662$ |
| | M- | $P = 0.845$ | $P = 0.810$ | $P = 0.782$ | $P = 0.851$ |
| R50 | M+ | $P = 0.657$ | $P = 0.795$ | $P = 0.759$ | $P = 0.854$ |
| | M- | $P = 0.865$ | $P = 0.815$ | $P = 0.874$ | $P = 0.697$ |

(Ψ_{pd} : predawn leaf water potential; Ψ_{md} : midday leaf water potential; RWC: leaf relative water content; R100: full irrigation regime; R50: water regime 50% of ETc; M+: mycorrhizal plant; M-: non-mycorrhizal plant).

Table 4. AMF colonization and sporulation under full irrigation (R100) and water stress of 50% ETc (R50)

| | Water regime | Plum varieties | | | | Average for all varieties |
|------------------------|--------------|----------------|---------|---------|-------------|---------------------------|
| | | Angelino | Stanley | Fortuna | Black Amber | |
| AMF colonization (%) | R100 | 42.5a | 52.2a | 46.3a | 50.8a | 47.9a |
| | R50 | 38.3a | 43.3b | 26.2b | 22.1b | 32.5b |
| AMF spores density | R100 | 60a | 75a | 96a | 88a | 80a |
| (spores/100 g dw soil) | R50 | 162b | 153b | 173b | 166b | 163b |

Even under full irrigation, mycorrhizal colonization was relatively low with an average of 48% for the four tested varieties. The low AMF colonization stems from the fact that under field conditions, colonization of new ramifications of root system by AMF is confronted to various constraints including essentially the remoteness of many rootlets from AMF spores and the development of weeds that competes the young plants as to mycorrhizae [48]. Under water stress, the AMF colonization was significantly reduced by an average of 32% for the four tested varieties. The mechanisms of this inhibition due to water stress are associated with a low rate of spore germination and disturbance of chemical transmission between fungus and roots [49].

As for AMF sporulation, it varied little following genotypes, but it was greatly affected by water regime. In response to water stress, AMF sporulation increased amply by an average of 103% to pass from 80 spores/100g of soil observed under full irrigation to 163 spores/100g of soil under water stress. This rise of AMF sporulation indicates that level of the applied stress (50% of ETc) was sufficient to induce the passage of mycorrhizal fungi to sporulation which constitutes their form of resistance to water stress [49].

4. CONCLUSION

In this experiment, we evaluated the capacity of AMF (*Rhizophagus intraradices* and *Funneliformis mosseae*) to alleviate severe water stress effects on young plum tree under field

conditions. It was found that without mycorrhizal fungi, the young plum tree does not tolerate water stress amounting to 50% of ETc. Plants response to water stress was marked by a significant deterioration of their water and nutritional status, thereby inducing considerable reductions of their vegetative growth. AMF have contributed to partially reduce the effects induced by water stress. The favorable influence of mycorrhizal fungi was not due to an improvement of plants relative water content, but rather to a partial stabilization of water potential and stomatal conductance. Arbuscular mycorrhization was therefore able to improve water and nutrient use efficiency of the young plum trees. However, it was unable to make them tolerant to water stress of 50% of ETc. Therefore, in order to optimize deficit irrigation of young plum in semi-arid areas, the obtained results suggest adoption of water regime of 50% of ETc associated to mycorrhizal symbiosis, under the condition that this water regime does not induce a consistent reduction of plants growth in the long term.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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