



## Improvement of Continuous Deficit Irrigation Efficiency on Young Plum Tree Using Arbuscular Mycorrhizal Fungi

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### Authors' contributions

This work was carried out in collaboration between all authors. Author RR designed the study, performed the experiment and statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AK supervised the study and managed the literature searches. Authors MA, EHB and CDK realized the measurements. All authors read and approved the final manuscript.

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### ABSTRACT

This work aimed to improve the efficiency of continuous deficit irrigation (CDI) on plum tree through using symbiosis with arbuscular mycorrhizal fungi (AMF). Thus, an experiment was conducted in pots to evaluate the effects of arbuscular mycorrhization on the growth of young plum trees, in two cases of CDI (50% and 75% of full crop evapotranspiration - ET<sub>c</sub>) compared to full irrigation (100% ET<sub>c</sub>). We used a mixture of two mycorrhizal fungi species, *Rhizoglomus intraradices* and *Funneliformis mosseae*. The measurements concerned: 1) morphological parameters of the root system (total fresh weight, total dry weight, total volume and hairy root dry weight); 2) morphological parameters of aerial parts (primary shoot elongation, number of secondary shoots, trunk growth, leaf area, total fresh weight and total dry weight); and 3) nutritional status parameters (leaf phosphorus content and chlorophyll pigments content). Compared to full irrigation, the two CDI levels induced a significant decrease of hairy root percentage without significantly affecting

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total root weight and volume. The use of AMF enabled to limit this depressive effect because it stimulates root ramification, but this is effective only under moderate water stress (75% of ETc). Under this CDI regime, hairy root percentage has been enhanced by 87% in mycorrhizal plants comparatively to non-mycorrhizal plants. Water stress effects on vegetative growth were partially alleviated using AMF even under severe decrease of irrigation (50% of ETc): shoot elongation was higher for mycorrhizal plants exceeding non-mycorrhizal ones by an average of 13%. AMF induced also a significant increase of phosphorus, nitrogen and chlorophyll pigments concentration in mycorrhizal plants. Thus, AMF significantly improves CDI efficiency on young plum tree, even at level of 50% of ETc. The observed improvements due to AMF were considerable under 75% of ETc, suggesting the possibility to adopt this CDI level associated with AMF to optimize deficit irrigation on young plants of this rosaceous under low water availability conditions.

**Keywords:** *Prunus domestica*; continuous deficit irrigation; arbuscular mycorrhizal fungi.

## 1. INTRODUCTION

The advantages of mycorrhizal symbiosis have been demonstrated for different species, especially under arid conditions [1]. This symbiosis is established naturally for all fruit species and may be controlled in nursery and during transplantation of young plants to the field by using specific mycorrhizal fungi. However, several studies have shown the importance of early inoculations in nursery on sterilized substrate to avoid the natural mycorrhizal infection of plants when they are transplanted [2,3].

Particular attention was given to arbuscular mycorrhizal fungi (AMF) because of their polyvalence and benefits for the implantation of fruit and forest species in arid areas [4,5]. However, each fruit tree develops symbiosis more easily with specific AMF species. According to a study conducted by Clavet et al. [6] on eighteen *Prunus* rootstocks, this symbiosis was more easily achieved with *Rhizogloium intraradices*, followed by *Funneliformis mosseae*. However on apple tree, symbiosis succeeded better with *Funneliformis mosseae* than with *Rhizogloium intraradices* [7]. Other AMF genus tested on fruit trees have also yielded encouraging results such as *Gigaspora* on olive tree [8] and *Diversispora* on citrus [9].

Mycorrhizal dependency of fruit trees may remain significant even under severe water stress conditions. Indeed, Bagheri et al. [10] found a significant mycorrhizal dependency on pistachio tree inoculated by *Funneliformis mosseae* and *Rhizogloium intraradices* under water regime amounting to 25% of crop evapotranspiration. The mycorrhizal dependency improves water and nutritional status of trees thereby impacting shoot and root growth and fruit

yield levels. On young peach plants, Rutto and Mizutani [11] found that mycorrhizal symbiosis with *Funneliformis mosseae* improved shoot growth by an average of 30% compared to non-mycorrhizal plants. In terms of mineral nutrition, this symbiosis has induced spectacular increases of leaf nutrient concentrations with averages ranging from 28% for magnesium to 450% for zinc. On this same fruit specie, Wu et al. [12] found that the most significant effect of AMF on root system resides in boosting its ramification thereby changing its architecture. On young plum and apple plants inoculated by *Funneliformis mosseae* and *Rhizogloium intraradices*, Fortuna et al. [7] observed an improvement of shoot and root system growth of more than 130% for both fruit species, accompanied by a significant increase of phosphorus uptake estimated at 220% compared to non-inoculated plants.

Certainly, AMF improve fruit trees performance, whose effects are notable since the first year of the inoculation. However, the magnitude of the effects varies depending on fruit species, AMF species, soil types and climatic conditions. This technique would be particularly relevant in orchards where deficit irrigation is imposed, which constitute the bulk of Moroccan tree orchards. This study is particularly relevant on plants that require higher amount of irrigation water such as plum trees that has an economic importance in Morocco. This work is intended to quantify the effects of a mixture of two AMF species, *Rhizogloium intraradices* and *Funneliformis mosseae*, on efficiency of moderate and severe continuous deficit irrigation of young plum trees on the basis of root growth, nutritional status and vegetative growth measurements. Studies undertaken in some countries have indicated that these AMF species are particularly efficient for *Prunus* plants [13,14], but the present work provides data to use them

in mixture at different levels of water stress under climatic conditions of Morocco.

$$ET_o = 0.16 * T_{max} + 0.14 \quad (r^2 = 0.93; \text{see} = 0.02) \quad (1)$$

## 2. MATERIALS AND METHODS

### 2.1 Cultural Conditions

The trial was carried out on twenty four young plum trees; cv. *Stanley* grafted onto the rootstock *Myrobalan* which is widely used in Morocco [15]. The seedlings were one year old and their size was approximately similar. The plants were planted in January in 60 liter pots containing a mixture of sand and soil (2:1 v/v) previously sieved (mesh size of 5 mm). The soil was taken from surface layer of uncultivated land. Resulting soil texture was sandy clay containing an average of 7% CaCO<sub>3</sub>, poor in organic matter with an average of 0.9%. Soil was approximately neutral (pH<sub>water</sub> 7.3) and not saline with an electrical conductivity of 0.13 mS/cm. The plants were then placed under glasshouse conditions and were cultivated in the same way.

Before planting, the terminal roots were partially cut to stimulate plant growth and promote mycorrhizal inoculation. Root inoculation was achieved with 12 g/plant of an inoculum purchased on the market containing 25 spores/g of *Rhizoglyphus intraradices* and 25 spores/g of *Funnelformis mosseae*, whose viability has been checked before implementation of the experiment. The choice of these AMF species was based on their high ability to colonize *prunus* rootstocks as demonstrated in previous research [16]. In addition, it was chosen to use a mixture of these AMF species instead to test them separately because it has been shown in several studies that the mixtures of AMF are generally more efficient [17-19].

During the first month after planting (February), the plants were fully irrigated to ensure satisfactory plant growth start. Then, from March to October, three water-application treatments were applied: 100%, 75% and 50% of crop evapotranspiration (ET<sub>c</sub>), considering the radius of pots as spacing planting. The ET<sub>c</sub> values were estimated as the product of reference evapotranspiration (ET<sub>o</sub>) obtained through equation 1 provided below, linking ET<sub>o</sub> to maximal temperature (T<sub>max</sub>), established for Meknes region based on 11 years of climate data (1996-2006) [20]. This estimation method of ET<sub>o</sub> has been proven effective for controlling irrigation in previous trials [21,22].

Irrigation was applied twice a week without exceeding the easily usable water reserve of the substrate, equivalent to 2/3 of its usable water reserve. The experimental design was a split plot with two factors: water regime (50% ET<sub>c</sub>, 75% ET<sub>c</sub> and 100% ET<sub>c</sub>) and mycorrhization (M + and M-). Each water treatment has been applied to eight plum trees of which four are inoculated by AMF.

### 2.2 Measurements

#### 2.2.1 Mycorrhizal colonization and root growth measurements

Mycorrhizal colonization was determined on 1 cm root segments sampled at harvest from all plants (nine months after inoculation). This measurement was also realized on non-inoculated plants in order to verify that there was no root colonization by native AMF. The root segments were washed thoroughly with distilled water and preserved in a lactoglycerol solution (63 ml glycerol, 62 ml distilled water, 875 ml of ethanoic acid). Staining of root was realized following the method used by Philips and Hayman [23]: the root segments were placed in 10% KOH solution in a water bath set at a temperature of 90°C for 2 hours. Then they were washed with distilled water and transferred to 2% HCl solution for 5 min before being placed in a staining solution (lactoglycerol with 0.05% trypan blue) in water bath at 90°C for 15 min. After staining, the mycorrhizal colonization was estimated under an optical microscope (x100) from number of root segments showing arbuscules, vesicles and hyphae on total colored root segments.

Root growth parameters were measured in October on the entire root system of each plant carefully removed from pot. Measurements concerned maximal root length, root system volume, total fresh weight, total dry weight and hairy root dry weight. The root system volume was measured by dipping it in a water-filled tube.

#### 2.2.2 Vegetative growth measurements

The effect of water stress on vegetative growth of mycorrhizal and non-mycorrhizal plum trees was evaluated at the end of the shoot growth cycle, in October. The measurements concerned plant height, trunk diameter, primary shoot length,

secondary shoots length, number of secondary shoots per linear meter of primary shoot, leaf area, total fresh weight and total dry weight of plant.

The plant height was measured from collar graft to the highest apex. The annual growth of trunk diameter was measured 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 average leaf area was measured using a leaf area meter (type ADC Bioscientific A350) on twenty fully developed leaves per plant, taken from medial portions of the primary shoots. The total fresh weight was determined after plants grubbing before being dried at 105°C for 48 hours to determine their dry weight. The difference between dry weights of mycorrhizal and non-mycorrhizal plants were calculated to determine mycorrhizal dependency (MD) according to the formula: MD (%) = [100 x (mycorrhizal plant dry weight – non-mycorrhizal plant dry weight) / mycorrhizal plant dry weight].

### **2.2.3 Leaf phosphorus content**

Leaf phosphorus content was measured on leaf samples taken from the middle portions of shoots at the end of plant growth cycle, in October. Phosphorus analysis was performed according to the method described by Rayan et al. [24]. Indeed, phosphorus was extracted on samples dried using a mixture of ammonium molybdate, ammonium vanadate and nitric acid and quantified by spectrophotometer set at 410 nm.

### **2.2.4 Chlorophyll content**

Chlorophyll content was determined following the method used by Singh and Billore [25] on young leaves collected from the apex of shoots in October. After lyophilization and grinding of leaf samples, 5 mg of the ground product was agitated in 1 ml of 80% acetone in Eppendorf tubes for 1 h 30 min to extract all chlorophyll pigments. The extract was centrifuged at 14000 rpm for 15 min under 4°C. The optical density (OD) of supernatant was measured at 645 nm and 663 nm. The concentration of chlorophyll a ( $Ch_a$ ) and chlorophyll b ( $Ch_b$ ) are given by the following formulas:

$$Ch_a = [12.7 (OD_{663}) - 2.69 (OD_{645})]$$

$$Ch_b = [22.9 (OD_{645}) - 4.86 (OD_{663})]$$

## **2.3 Statistical Analysis**

Significant differences between treatments were determined using one-way ANOVA carried out on the SPSS 17.0 for Windows. Normality assumption of the distributions was checked prior using kurtosis, skewness and W-value significance of Shapiro-Wilk test ( $p < 0.05$ ). For homogeneity assumption of the variances, it was checked following Levene test. Indeed, all variables had a normal distribution except maximal length of roots and ratio of  $Ch_a/Ch_b$  for which was applied a log-normal transformation before proceeding to ANOVA test. Means comparison was performed for all normal variables using student's test to compare the effect of water treatments on mycorrhizal and non-mycorrhizal plants.

## **3. RESULTS AND DISCUSSION**

### **3.1 Mycorrhizal Colonization**

Root mycorrhizal colonization was similar under all water treatments with an average of 85% (Table 1) that is high compared to results obtained by Calvet et al. [16] on *Myrobolan* rootstock. This result is related to differences in environmental conditions and also to the variety used. Indeed, the variations in hydromineral requirements between varieties generate variations in root growth of rootstock and in root exudates composition that induce spore germination, growth and ramification of mycorrhizal hyphae [26].

The non-significance of water stress conditions on mycorrhizal colonization is linked to the fact that substrate moisture level induced by all water treatments was sufficient to ensure good conditions for spore germination and contact between AMF hyphae and roots, especially since under pot conditions, the produced spores and roots are not distant. In contrast, Buee et al. and Logi et al. [26,27] report that water stress exerts a depressive effect on mycorrhizal colonization under field conditions. The mechanisms of mycorrhizal inhibition under water stress is linked to low levels spore germination and to disruption of chemical transmission between fungus and roots [27,28].

### **3.2 Root System Growth**

Among parameters measured on root system, only the percentage of hairy root was reduced by water stress (Table 1). For non-mycorrhizal

plants, reduction of this parameter was higher under moderate water stress (75% of ETc) with an average of 26% compared to the control treatment (100% of ETc). However, under severe water stress (50% ETc), the decrease of this parameter was lower with an average of 13%. The water deficit seemed induce a preferential allocation of biomass to roots, especially when stress intensity was more accentuated. This is expressed by values of aerial biomass reduction on severely stressed plants significantly higher than that observed on moderately stressed plants. Similar results were reported on *Pinus radiata* [29], *Cedrus atlantica* [30] and *Fagus sylvatica* [31].

This result partially corroborates those of Abrisqueta et al. [32] who found that water stress on peach trees induces significant reductions of the majority of biometric parameters related to root growth. However, our result contradicts the finding of Romero et al. [33] who observed on almond tree that root growth was stimulated by water deficit. According to Burkart et al. [34], these differences in water stress effects on root growth are largely related to soil depth. They argue that in a situation of deep soil, plants adapt to water stress conditions by significantly increasing the volume of root system. In contrast, on shallower soils such as the case of pots, the root system is limited by soil depth making it more exposed to water stress effect.

Compensation of water stress effect on hairy root percentage through AMF was significant only under moderate water stress of 75% ETc by an average of 51% compared to non-mycorrhizal plants used as control. Under severe stress of 50% ETc, mycorrhization did not compensate the depressive effects of water stress on hairy root. This result suggests that under severe stress,

mycorrhizal root ramification stimuli are ineffective or insufficient to compensate the depressive effect of water stress. Moreover, under full irrigation, mycorrhization had a positive effect, but not statistically significant, on hairy root. This may imply that AMF promotes the action of naturally existing root ramification stimulus under full irrigation, which becomes inhibited under water stress conditions.

Under moderate water stress, AMF induced a significant change in root system architecture, induced by stimulating root ramification thereby increasing the hairy root percentage. Gutjahr et al. [35] observed the same effect on rice inoculated by *Rhizoglyphus intraradices*. However, several trials on different plants indicated that AMF effect on root system architecture varies depending on plant species and mycorrhizal strain. On tomato inoculated by *Funneliformis mosseae*, Trotta et al. [36] indicated that AMF did not induce significant effect on root system architecture. However, on mandarin tree inoculated by *Funneliformis mosseae*, Wu et al. [37] reported a significant improvement of root system architecture via an increase of root length and root volume associated with a significant decrease in roots diameter. A similar finding was also obtained on peach tree inoculated by *Funneliformis mosseae* [38]. Gahoonia et al. [39] reported that root system architecture changes are governed by internal and external parameters of mycorrhizal symbiosis. Until now, these parameters are not known. Allen et al. [40] reported an increase of phytohormone production on roots of mycorrhizal plants and estimate that these substances stimulate rhizogenesis. Furthermore, Tolsma et al. [41] indicated that AMF stimulates ramification of root system by inducing an accumulation of phosphorus and carbohydrates in roots.

**Table 1. Root growth parameters of mycorrhizal and non-mycorrhizal of young plum trees under different water treatments**

	Non-mycorrhizal trees			Mycorrhizal trees			p-value
	R100	R75	R50	R100	R75	R50	
Mycorrhizal colonization (%)	-	-	-	85.2	88.6	81.1	0.221
Log. maximal length (cm)	1.96	2.03	1.92	1.94	2.00	1.93	0.112
Volume (ml)	292.5	252.5	315.0	300.0	307.5	342.5	0.900
Fresh weight (g)	273.7	208.9	245.4	283.4	249.6	232.9	0.968
Dry weight (g)	97.2	73.0	83.0	101.1	101.5	62.0	0.875
Dry hairy root weight (g)	28.0	15.0	19.4	27.1	24.6	13.9	0.672
Percentage of hairy root (% DM)	27.4b	20.2a	23.9ab	28.3b	23.9ab	22.6ab	0.014
Aerial part dry weight / Root dry weight	0.59	0.50	0.51	0.60	0.61	0.43	0.865

R100: Control treatment 100% of ETc, R75: water treatment 75% of ETc, R50: water treatment 50% of ETc  
Values followed by the same letters or unmarked by letters are significantly equal

### 3.3 Vegetative Growth

The water restrictions significantly affected vegetative growth of plum trees both mycorrhizal and non-mycorrhizal, particularly primary shoots elongation, number of secondary shoots and trunk growth (Table 2). On non-mycorrhizal plants, the water treatment of 50% ETc induced a decrease of trunk diameter by 0.25 cm and shoot elongation by 15 cm associated with a reduction of the number of secondary shoots grown on one linear meter of primary shoot by an average of 4 shoots, in comparison with non water-stressed plants. Under the treatment of 75% ETc, these decreases were lower but remained significant with a decrease in trunk diameter of 0.1 cm and a decrease of 9 cm with regards to primary shoot elongation. These results corroborate those of Barradas et al. [42] and Remorini et al. [43] who found that the young rosaceous fruit trees do not tolerate water stress even at moderate level as 75% of ETc.

The mitigation effect of AMF on the way water stress affects vegetative growth was limited, but statistically significant under all water treatments. In comparison to non-mycorrhizal plants fully irrigated, the mitigation rate obtained varied from 43 to 90% for all vegetative parameters under the water treatment of 75% ETc and from 16 to 56% under the treatment of 50% ETc. Vegetative growth gain due to AMF was also observed for non-stressed plants, but only with regards to trunk growth and the number of secondary shoots. This gain was important with an average of 34% for trunk growth and 47% for secondary shoots number. The improvements observed on mycorrhizal plants come mainly from the favorable effects induced by AMF on nutrient

uptake and plant-water relations even under water stress conditions, as has been demonstrated on several plants in previous studies [44-46].

Vegetative growth of plum trees is therefore significantly dependent on mycorrhizal fungi under the two tested water stress intensities. However, it is often assumed that dependency of plants to arbuscular mycorrhizae decreases with water stress intensity to the point that no dependency remains at severe water stress levels [47]. This decrease of AMF effect under severe water stress is essentially explained by ineffectiveness of mycorrhizal fungi at very low soil moisture, which has been attributed to the limited germination of spores and to the inhibition of chemical transmissions between AMF and plants [48]. Furthermore, mycorrhizal dependency of plants was relatively low under full irrigation, but statistically significant. The low value of the mycorrhizal dependency under the treatment 100% of ETc can be explained by the low biomass gain observed in mycorrhizal plants, limited by the genetic growth potential of the used cultivar [49].

### 3.4 Leaf Phosphorus Content

Moderate water stress of 75% ETc did not affect phosphorus uptake. However, this uptake significantly decreased for all plants both mycorrhizal and non-mycorrhizal under a severe water stress of 50% ETc (Table 3). For non-mycorrhizal plants, leaf phosphorus content decreased in response to this water stress level by an average of 0.6 mg/g. The decrease was only 0.3 mg/g for mycorrhizal plants, indicating a considerable compensatory effect of AMF of 50%.

**Table 2. Vegetative growth parameters of mycorrhizal and non-mycorrhizal young plum trees under different water treatments**

	Non-mycorrhizal trees			Mycorrhizal trees			p-value
	R100	R75	R50	R100	R75	R50	
Trunk diameter (mm year <sup>-1</sup> )	7.2b	6.3ab	4.7a	9.7c	6.3ab	6.1ab	0.001
Plant height (cm)	130.5b	124.0b	88.2a	136.5b	127.5b	111.2b	0.001
Total fresh weight (g)	407.3cd	327.5ab	303.1a	420.6d	361.9b	319.4ab	0.007
Total dry weight (g)	173.0cd	143.2ab	130.5a	183.1d	158.8b	139.7ab	0.002
Primary shoot length (cm)	38.1b	29.0a	23.1a	38.9b	29.4a	26.1a	0.001
Number of secondary shoot (N Lm <sup>-1</sup> )	8.1bc	5.0ab	4.4a	11.9c	6.9b	4.6a	0.001
Leaf area (cm <sup>2</sup> )	24.7b	21.7ab	18.6a	25.7b	24.4b	18.9a	0.003
Mycorrhizal dependency (%)	-	-	-	5.5a	9.8b	6.6ab	0.021

R100: Control treatment 100% of ETc, R75: Water treatment 75% of ETc, R50: Water treatment 50% of ETc.

Values followed by the same letters are significantly equal.

N Lm<sup>-1</sup>: Number of secondary shoots per linear meter of primary shoot

**Table 3. Leaf phosphorus and chlorophyll pigments content of mycorrhizal and non-mycorrhizal young plum trees under different water treatments**

		<b>P</b> <b>(mg g<sup>-1</sup>)</b>	<b>Ch<sub>a</sub></b> <b>(mg g<sup>-1</sup>)</b>	<b>Ch<sub>b</sub></b> <b>(mg g<sup>-1</sup>)</b>	<b>Ch<sub>a+b</sub></b> <b>(mg g<sup>-1</sup>)</b>	<b>Log. Ch<sub>a</sub> /</b> <b>Ch<sub>b</sub></b>
R100	M+	2.4b	2.54b	1.54c	4.08c	0.23a
	M-	2.3b	2.46ab	0.87b	3.33b	0.46ab
R75	M+	2.3b	2.15ab	0.32a	2.42a	0.85b
	M-	2.4b	2.10ab	0.36a	2.51a	0.80ab
R50	M+	2.0ab	2.10ab	0.17a	2.28a	1.13c
	M-	1.7a	2.01a	0.19a	2.20a	1.04c
p-value		0.004	0.004	0.001	0.004	0.001

*R100: Control treatment 100% ETc, R75: Water treatment 75% ETc, R50: Water treatment 50% ETc,*

*M+: Mycorrhizal plant; M-: Non-mycorrhizal plant.*

*Values followed by the same letters are significantly equal*

This depressive effect of water stress on phosphorus uptake is in agreement with many studies on plant phosphate nutrition under water stress conditions [50,51]. 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 rootlets number in response to water stress, which constitute the essential seat of mineral uptake [52]. The significant improvement of phosphorus uptake on mycorrhizal plants comes from extra-root hyphae of AMF that operate as additional rootlets and also to their ability to ramify the root system [35,38], thereby boosting nutrients uptake, including phosphorus. Mycorrhizal hyphae does not only explore the available phosphorus contained in soil solution, they have also the ability to access non-assimilable phosphorus and organic phosphorus by secreting phosphatase enzymes and various molecules that acidify the soil, making phosphorus more available [53].

Under full irrigation, there was no effect of AMF on phosphorus uptake. Indeed, the amount of rootlets developed by non-mycorrhizal plants under full irrigation was sufficient to uptake phosphorus at the same level as mycorrhizal plants.

### 3.5 Chlorophyll Content

In non-mycorrhizal plants, the two applied levels of water stress induced a similar decrease of total chlorophyll content by an average of 29% (Table 3). This effect was mainly linked to degradation of chlorophyll b (Ch<sub>b</sub>) which was reduced by 58% under water stress of 75% ETc and by 78% under water stress of 50% ETc. However, concentration of chlorophyll a (Ch<sub>a</sub>) did not change under water stress of 75% ETc

whereas it decreased by 18% under water stress of 50% ETc. This water stress effect significantly increased the concentration ratio of the two chlorophyll pigments (Ch<sub>a</sub>/Ch<sub>b</sub>). The average value of this ratio under full irrigation (2.88) was doubled under water stress of 75% ETc and was quadrupled in response to water stress of 50% ETc. This high sensitivity of Ch<sub>b</sub> to water stress was reported by Chutia and Borah [54] for rice. However, Mafakheri et al. [55] found that Ch<sub>a</sub> was more sensitive to water stress in chickpea. In fact, the sensitivity of chlorophyll pigments to water stress varies according to plant species as well as the duration and severity of water stress [56].

Although Ch<sub>b</sub> was more affected by water stress than Ch<sub>a</sub>, the compensatory effect due to AMF was significant for Ch<sub>a</sub> whose values increased with AMF under water stress of 50% ETc and were significantly equal to those observed under full irrigation. Ch<sub>b</sub> content tends to decrease by AMF under water stress conditions. This effect may indicate that AMF induces an increase of total chlorophyll content under water stress by promoting synthesis of Ch<sub>a</sub> to the detriment of Ch<sub>b</sub>. However, under full irrigation, the positive effect of AMF had the same amplitude for both Ch<sub>a</sub> and Ch<sub>b</sub> as shown by the significant increases of chlorophyll pigments concentration in mycorrhizal plants under this water regime. Indeed, under this water regime, AMF induced an increase of Ch<sub>b</sub> content by an average of 77% against 3% for Ch<sub>a</sub>, thereby increasing total chlorophyll content by 22%. These different changes identified on chlorophyll pigments content in response to water stress and AMF effects are closely linked to absorption and translocation of nitrogen involved on synthesis of glutamate which is the main precursor of chlorophyll [57,58]. These changes are also

explained by the variation of leaf tissue turgor which determines the status of chloroplast membranes [59].

#### 4. CONCLUSION

In this experiment, we evaluated the ability of AMF to improve continuous deficit irrigation efficiency on young plum trees. Without mycorrhizal fungi, the young plum trees were significantly affected by water restriction even at moderate level of 75% ETc. Plants response to water stress was marked by a significant reduction on root ramification and deterioration of their nutritional status, thereby inducing considerable reductions of their vegetative growth. The effect of AMF was partial under the two tested levels of water restrictions, but considerably alleviated water stress tolerance of plants by an improvement of root ramification and nutrients uptake. Given irrigation at 75% of ETc, AMF induces a significant increase of shoot elongation and ramification that is sufficient for suitable tree architecture. Thus, AMF makes possible the adoption of this CDI regime on young plum trees under low water availability conditions.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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