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Effects of Arbuscular Mycorrhizal Inoculation by Indigenous Fungal Complexes on the Morpho-Physiological Behavior of *Argania spinosa* Subjected to Water Deficit Stress

Imane Ouallal [1], Younes Abbas [2], Houda ElYacoubi [1], Hamada Imtara [3,*], Mashail N. Al Zain [4], Mohemed Ouajdi [5], Younes El Goumi [6], Nurah M. Alzamel [7], Omar Mohammed Noman [8] and Atmane Rochdi [1,*]

1 Natural Resources & Sustainable Development Laboratory, "AgroPhysiology, Biotechnology & Environment" Research Unit, Faculty of Science of Kenitra, Ibn Tofail University, Kenitra 14000, Morocco; imaneouallal@gmail.com (I.O.); houda.elyacoubi@uit.ac.ma (H.E.)
2 Polyvalent Team in R&D Laboratory, Polydisciplinary Faculty of Beni-Mellal, Sultan Moulay Slimane University, Beni Mellal 23000, Morocco; abbasfbpm@gmail.com
3 Faculty of Arts and Sciences, Arab American University Palestine, P.O. Box 240, Jenin 44862, Palestine
4 Department of Biology, College of Sciences, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia; mnalzain@pnu.edu.sa
5 Forestry Research Centre, Rabat 10080, Morocco; ouajdim@gmail.com
6 Polyvalent Team in R&D, Higher School of Technology of Fkih Ben-Salah, Sultan Moulay Slimane University, Beni Mellal 23000, Morocco; elgoumiyounes@hotmail.com
7 Department of Biology, College of Science and Humanities, Shaqra University, Shaqra 11961, Saudi Arabia; nalzamel@su.edu.sa
8 Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia; onoman20@gmail.com
* Correspondence: hamada.tarayrah@gmail.com (H.I.); atmane.rochdi@uit.ac.ma (A.R.)

Abstract: Our objective is to test selected mycorrhizal complexes to verify the contribution of mycorrhizal symbiosis as a biological tool promoting the development of the argan tree under hostile conditions. In addition, this study aims to assess the impact of soil drought caused by stopping watering of young argan plants inoculated with strains of fungal complexes indigenous to the species in comparison to non-inoculated plants. Under conditions of water deficit stress, the most marked reductions in fresh and dry biomass were recorded in non-mycorrhizal plants. The most negative values of leaf water potential Ψf and Ψb were also noted in non-mycorrhizal plants. On the other hand, plants inoculated with mycorrhizal Bouyzakarne inoculum were relatively less affected by watering discontinuation compared to those inoculated with mycorrhizal Argana inoculum. Water stress caused a reduction in potassium and phosphorus content in the leaves and roots of all plants. However, mycorrhizal plants exhibited the highest P and K values compared to non-mycorrhizal ones. Therefore, mycorrhization compensates for the deficit in absorption of inorganic nutrients during drought. Sodium gradually decreased in the leaves but increased in the roots, and this delocalization of Na^+ ions under water deficit stress resulted in higher concentrations in the roots than in the leaves of all plants. However, the mycorrhizal plants exhibited relatively lower values of root Na^+ compared to the non-mycorrhizal controls. The water deficit reduced the content of chlorophyll a and b in the leaves and the chlorophyll a/b ratio in stressed plants. The lowest chlorophyll values were recorded in non-mycorrhizal plants. The levels of proline and soluble sugars in the leaves and roots of argan plants increased in all plants, especially with the extension of the duration of stress. However, proline accumulation was higher in mycorrhizal plants, with superiority in plants inoculated with the Bouyzakarne complex in comparison with that of Argana. In contrast, the accumulation of soluble sugars was higher in non-mycorrhizal plants than in mycorrhizal plants. We concluded that with a correct choice of the symbiotic fungi complexes, AMF inoculation biotechnology can benefit argan cultivation, especially under stressful conditions in arid regions with structural drought, where native Arbuscular mycorrhizal fungi levels are low.

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Keywords: *Argania spinosa*; autochthonous arbuscular mycorrhizal fungi; water deficit; tolerance

1. Introduction

The argan tree (*Argania spinosa*), endemic to Morocco, is one of the most remarkable species in the Moroccan forest landscape, ecologically, environmentally, and from a socio-economic point of view. However, argan ecosystems are experiencing a very worrying regressive dynamic due to the joint effects of the aridity of the climate, the scarcity and irregularity of precipitation, and human pressure [1]. On the other hand, the natural regeneration of the argan tree is practically absent in the main distribution area of the species [2]. Intervention's backup and fast rescue of arganeraies are required. However, water deficit and soil infertility considerably reduce the success of plantations [3].

Drought is considered one of the most serious abiotic stressors that limit plant growth and reduce crop production in numerous regions worldwide [4]. Under the context of climate change, drought has been and is becoming an acute problem constraining plant growth and terrestrial ecosystem productivity in many regions all over the world, particularly in arid and semi-arid regions [5]. The main effect of water stress is the reduction of plant growth, which depends on cell division, cell enlargement, and differentiation and involves genetic, morphological, physiological, and biochemical events [6,7]. Therefore, it is imperative to improve the drought tolerance of crops under changing circumstances. Currently, there are no economically viable technological means to facilitate agricultural production in times of drought. However, the development of crops tolerant to water stress could be a promising approach to restoring or re-establishing degraded ecosystems [8,9]. Asmelash et al. [10] noted that any program to restore a degraded ecosystem must integrate components from different ecosystem levels, especially soil microorganisms that are closely related to plants. Indeed, arbuscular mycorrhizal fungi (AMF) are ubiquitous elements in most ecosystems around the world. They are considered one of the key factors in controlling major nutrient cycles and maintaining plant cover [11–13]. AMF symbiosis not only stimulates plant growth but also helps improve the tolerance of plants to abiotic stress, such as drought [14,15], salinity [16,17], and heat stress [18]. Studies have indicated that AMF symbiosis is able to enhance the drought tolerance of plants [19,20], but the exact mechanisms are not fully known.

Some studies carried out on the mycorrhization of argan trees have shown that the use of an allochthonous (commercial) inoculum based on *Glomus intraradices* improved the nutrition and growth of plants [21,22]. However, El Mrabet et al. [23] showed that controlled mycorrhization of young argan plants with native inoculum from a defended area (Admine region) improved growth by 51% compared to control plants. However, the effect of inoculation by AMF on the resistance of the argan tree to water stress has not yet been studied.

In this context, the present work aimed to evaluate the potential of arbuscular mycorrhizal inoculation by native AMF complexes—previously selected according to their mycorrhizogenic potential [24,25]—on the morphophysiological behavior of young argan plants subjected to the stress of soil water deficit.

2. Materials and Methods

2.1. Soil/Root Sampling

Artificial inoculation was carried out with two complexes ("Bouyzakarne" and "Argana") of mycorrhizal fungi that were previously selected from among several complexes of AMF native to the soil rhizosphere of argan trees. These complexes of fungal symbionts were chosen according to the mycorrhizogenic power of the soils and the natural state of conservation of Moroccan argan groves [24]. These two forest stands represent non-degraded (Argana) and degraded (Bouyzakarne) sites. The climate of these regions is Mediterranean arid. Table 1 shows the ecological characteristics of these sites.

Table 1. Ecological Characteristics of Inoculum Source Sites.

Station	Coordination	Province	Altitude	Average Annual Rainfall (mm)	Annual Mean Temperature (°C)	Tree Density/Hectare	Use
Bouyzakarne	N: 29.1879 W: 009.7421	Goulmim Es-smara	690	120	21	5	Pasture
Argana	N: 30.7544 W: 009.1545	Taroudante	735	500	20.9	30	Pasture

2.2. Preparation of the Inoculum

Samples of soil/roots were collected from the two argan groves (Bouyzakarne and Argana), and the propagules (spores, hyphae, and mycorrhizal root fragments) were multiplied on a mycotrophic annual plant (barley: *Hordeum vulgare*) that has a dense root system and is therefore well suited to AMF propagation. The barley seeds were washed several times with tap water, soaked in a 20% sodium hypochlorite solution for 30 min, rinsed 3 times with distilled water, and then sown for three months in pots containing the soil samples (either Argana or Bouyzakarne) collected from the two argan groves. Watering the pots was done according to the plant's needs. The substrates and roots from these three-month-old cultures served as the inoculum for our experiment.

2.3. Plant Material

Argan seeds from the Amskroud forest in west-central Morocco were provided by the Marrakech Forestry Research Center. The seeds were rinsed several times with tap water, then disinfected with 20% sodium hypochlorite for 30 min, and well rinsed with distilled water. Seeds were then soaked for 48 h in tap water and pre-germinated between two thin layers of damp black peat. Subsequently, the pre-germinated seeds with a 1–2 cm radicle were transplanted and sunk under a thin layer of substrate in "WM"-shaped pots containing a mixture (1V/1V) of the moist black peat and sterile sand. For the mycorrhizal pots, we added a thin layer of the inoculum (~10 g) near the radicle and covered with a layer of substrate used. These cultures were initiated in April in a greenhouse under semi-controlled conditions. The average maximum / minimum temperatures were 28 ± 1 °C and 15 ± 1 °C, respectively. Plants were watered as needed for up to 16 months.

2.4. Experimental Setup

A total of 90 sixteen-month-old argan plants were used: 30 plants inoculated with the mycorrhizal complex of Argana (a preserved forest), 30 plants inoculated with the mycorrhizal complex of Bouyzakarne (a very degraded forest), and 30 control plants (non-inoculated plants). The water deficit stress consisted of completely stopping the watering of all plants from August 1 to 28 when the maximum / minimum average temperatures were 32/18 °C, respectively.

2.5. Analysis of the Effects of Controlled Mycorrhization on the Morphophysiological Responses of the Argan Tree Tolerance to Water Stress

The roots and mature leaves of the same vegetative rank (taken from the upper third of the plants) were analyzed each week during the month of August.

2.5.1. Leaf Water Potential

Plant water stress was characterized directly by measuring leaf water potential.

Basic Leaf Water Potential (Ψb)

The measurements were taken at the end of the night (near 6 a.m. (GMT + 1)) in the absence of transpiration (closed stomata), where the leaf water potential is balanced over the entire soil–plant–atmosphere continuum [24]. In these conditions of no transpiration,

the measurement of the basic leaf potential makes it possible to approach the average water potential of the soil in the root area.

It is measured using a pressure chamber (PMS model 1000 pressure chamber instrument) on the upper third of the plants in the part of the stem with the last developed leaves. We opted for 5 repetitions (one plant per repetition) per treatment.

Leaf Water Potential at Noon (Ψf)

The minimum leaf water potential was measured at midday to determine the state of minimal hydration reached by the plant. It responds to a combination of factors, such as local climatic demand, plant water availability, stomatal conductance, and hydraulic conductivity of the xylem vascular system. The measurements were performed on 5 plants (repetitions) per treatment.

2.5.2. Biomass

The fresh and dry masses of the aerial and root parts were measured on 3 plants per treatment. The different parts were dried using an oven at 75 °C for 48 h.

2.5.3. Mineral Content

The content of mineral ions (Na^+ and K^+) was determined using a flame photometer after dry mineralization of the aerial and root parts of argan plants. The dosages were carried out on 3 plants (replicates) per treatment.

The phosphorus content was determined by colorimetric assay; after nitric oxidation and incineration, the phosphoric acid was colorimetrically assayed on the hydrochloric solution of the ashes using the yellow color it gives with the vanadomolybdic reagent.

2.5.4. Chlorophyll Pigment Content

Photosynthetic pigments were quantified according to the Arnon method [26] with 3 repeats per treatment (one plant per repetition). The OD was measured at 645 and 663 nm using a spectrophotometer. The chlorophyll content was calculated using the following equations [27]:

$$\text{Chlorophyll a (mg/L)} = 12.7 \times \text{OD } 663 - 2.63 \times \text{OD } 645$$

$$\text{Chlorophyll b (mg/L)} = 22.9 \times \text{OD } 645 - 4.68 \times \text{OD } 663$$

$$\text{Chlorophyll b (mg/L)} = 22.9 \times \text{OD } 645 - 4.68 \times \text{OD } 663$$

$$\text{Total chlorophyll (mg/L)} = (20.2 \times \text{OD } 645) + (8.02 \times \text{OD } 663)$$

2.5.5. Free Proline Content

The concentration of free proline was determined according to the method described by Bates et al. on 3 repeats per treatment (one plant per repetition) [28]. A quantity of 100 mg of fresh tissue (obtained from root or the young fully developed leaves) was mixed with 4 mL of a 3% sulfosalicylic acid solution and then stirred for 1 h before being filtered through filter paper (Whatman No. 1). The residue was centrifuged at 1200 rpm for 10 min. A volume of 1 mL of plant extract was placed in a tube to which 1 mL of ninhydrin reagent (2.5%) and 1 mL of glacial acetic acid were added, and the tube was placed in a water bath and boiled at 100 °C for 60 min. The reaction mixture was vigorously mixed with 5 mL toluene with the addition of a pinch of anhydrous sodium sulfate. The absorbance was determined at 528 nm (L-proline was used as a standard and 100% toluene as a blank).

2.5.6. Total Soluble Sugar Content

The sugar content was determined by the phenol–sulfuric acid method described by Dubois et al. on 3 repetitions per treatment (one plant per repetition) [29]. The extraction of carbohydrates from the roots or the young fully developed leaves of each plant sample was made by grinding 100 mg of tissue in 5 mL of 80% ethanol. To evaporate the alcohol,

the tubes were placed at 80 °C in a ventilated oven. Then, the test solution was obtained by adding 10 mL distilled water to the extract in each tube. To a 1 mL aliquot of each solution tested, 1 mL of 5% phenol solution and 5 mL of concentrated sulfuric acid (96%) were added. The resulting yellow to orange solution was vortexed to homogenize it, and the tubes were then left to stand for 10 min before being placed in a water bath for 15–20 min at a temperature of 30 °C. The absorbance was taken at a 485 nm wavelength.

2.6. Statistical Analyses

The normality of the data distribution was analyzed by the Shapiro–Wilk test, and it was performed by transformation when necessary. A two-way and three-way analysis of variance (ANOVA) was used to assess the effect of the inoculum, stress duration, plant part, and their interactions on the measured variables. The averages were compared by Tukey's multiple comparison tests (honestly significant difference) ($p \leq 0.05$). A canonical discriminant analysis (CDA) was performed for the aerial (a) part using 11 variables and for the root (r) part using 7 variables. Then, another CDA was established for all parameters (18 variables) of the whole plant (p) as predictors of the diagnostic group. All statistical analyses were performed using JMP SAS Pro software database software (JMP®, Version <14>. SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Analysis of Variance of the Parameters Measured for the Aerial and Root Parts

The analysis of variance (Table 2) shows significant to highly or very highly significant differences in stress duration (D), mycorrhizal treatment (inoculum), and plant part (PP). Regarding the interaction between the different factors (inoculum X D; inoculum X PP; D X PP), the degree of significance differs from one combination to another.

Table 2. Result of the Analysis of the Variance of the Means of the Measured Parameters, Depending on the Inoculum, Duration, and Plant Part.

Source of Variation	df	Proline (µg/g)	Sugar (µmol/g)	K^+ (mg/g)	Na^+ (mg/g)	Ratio K/Na	Phosphorus (mg/g)	Fresh Matter (g)	Dry Matter (g)
Inoculum	2	1094.11 ***	4854.55 ***	24.29 ***	4.63 *	0.97 **	97.52 ***	315.09 ***	58.29 ***
Duration (D)	3	26,295.61 ***	15,808.79 ***	514.83 ***	814.34 ***	11.54 ***	60.00 ***	269.06 ***	49.83 ***
Plant Part (PP)	1	4232.00 ***	6610.39 ***	3493.50 ***	1328.96 ***	7.45 ***	38.75 ***	353.82 ***	6.28 ***
Inoculum*Duration	6	2437.89 ***	15.24 ns	1.61 ns	11.23 *	0.41 ns	0.77 ns	4.60 ***	1.81 ***
Inoculum*PP	2	2413.00 ***	1825.16 ***	1.81 *	18.60 ***	0.35 ns	0.27 ns	45.31 ***	8.44 ***
D*PP	3	9422.11 ***	3271.16 ***	73.04 ***	2123.73 ***	110.55 ***	6.01 ***	8.34 ***	2.95 ***
Model	17	45,894.72 ***	32,385.28 ***	4109.09 ***	4301.49 ***	131.26 ***	203.32 ***	996.22 ***	127.60 ***
Error	54	2371.89	1225.20	14.97	35.10	3.62	4.06	7.26	2.77
C. Total	71	48,266.61	33,610.48	4124.05	4336.59	134.87	207.37	1003.48	130.37

Source of Variation	df	Ratio FM (A/R)	Ratio DM (A/R)	Chlorophyll a (mg/L)	Chlorophyll b (mg/L)	Ratio Chlorophyll a/b	Ψb (bar)	Ψf (bar)
Inoculum	2	0.1014 ***	0.5024 ***	13.72 **	16.06 ***	0.0524 ns	33.50 ***	50.89 ***
Duration (D)	3	0.1899 ***	0.2587 ***	302.83 ***	195.33 ***	1.4910 ***	746.33 ***	715.67 ***
Inoculum*D	6	0.0208 ***	0.0349 *	12.54 **	14.83 ***	0.0515 ns	9.83 *	3.33 ns
Model	11	0.3122 ***	0.7960 ***	329.09 ***	226.22 ***	1.5949 ***	789.67 ***	769.89 ***
Error	24	0.0073	0.0426	17.84	10.67	0.7603	13.33	6.00
C. Total	35	0.3195	0.8386	346.93	236.89	2.3552	803.00	775.89

*, **, ***: significant at the 5%, 1%, and 0.1% thresholds, respectively; ns: not significant. df: degrees of freedom.

3.2. Morphophysiological Characters

The first physiological effects recorded during the application of water stress concerned leaf water status. Indeed, our results showed a decrease in the leaf water potential (Ψf) and the basic leaf water potential (Ψb) in parallel with the lengthening of the stress duration (Figure 1). The plants most affected by water stress showed the most negative values for these two parameters. The most negative values were noted in the non-mycorrhizal plants. In addition, the effect of mycorrhization was recorded for these parameters. Ψf went from −11 to −23 bar for mycorrhizal plants with Argana inoculum and from −10 to −22 bar for mycorrhizal plants with Bouyzakarne inoculum. However, Ψb decreased from

−8 bar during the 1st week to −20 bar during the 4th week of stress for both mycorrhizal complexes (Argana and Bouyzakarne).

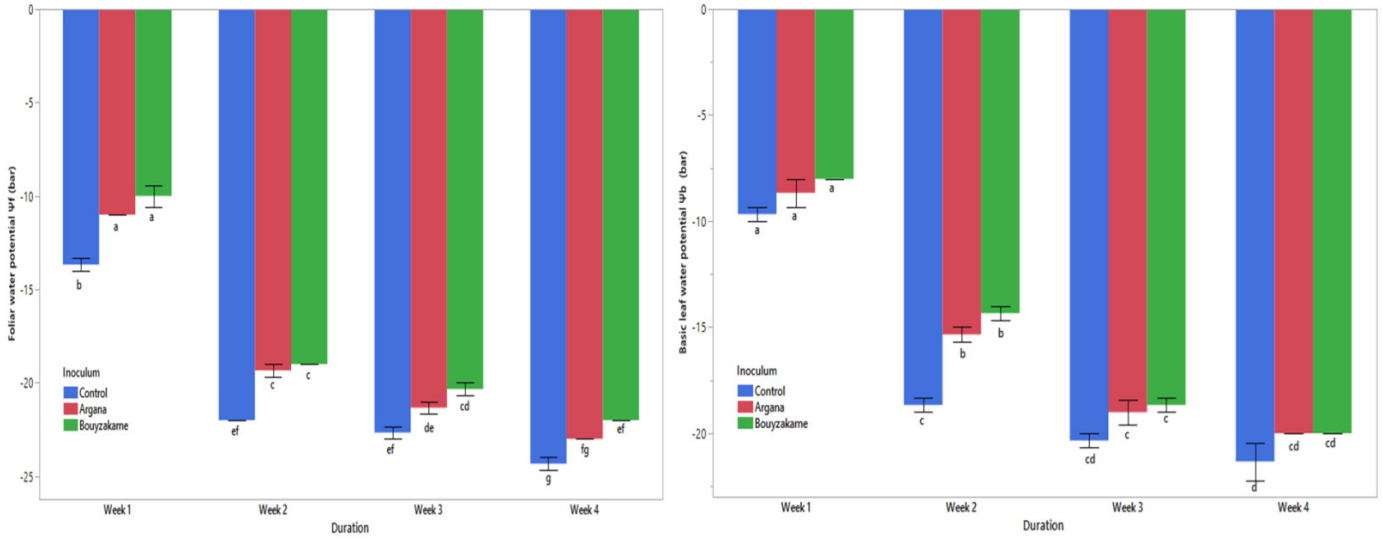


Figure 1. Foliar water potential (Ψf) and basic leaf water potential (Ψb) of mycorrhizal and non-mycorrhizal argan plants subjected to the stress of soil water deficit for one month (each error bar is constructed using 1 standard error from the mean). Values with different letters are significantly different (Tukey's test $p \leq 0.05$).

Differences in fresh and dry biomass production were also recorded between the control and mycorrhization treatments (Argana and Bouyzakarne) under water stress conditions. Indeed, by stopping watering, the argan plant had a reduced weight of aerial and root parts of the plant, along with the lengthening of the duration of stress. The amplitude of loss of the total weight of the plants at the end of the stress ([(W1 − W4)/W1] × 100) was 38.94%, as well as 36.05% and 33.86%, in the control and mycorrhizal 'Argana' and 'Bouyzakarne', respectively. This reduction is greater in the aerial part than in the root part (Figure 2), and the most marked reductions were recorded in non-mycorrhizal plants. In addition, plants mycorrhizal with Bouyzakarne inoculum were generally less affected by watering discontinuation compared to those mycorrhizal with Argana inoculum. Towards the end of the experiment (week 4), the amplitude of variation of the total weight of the mycorrhizal plants compared to the controls ([(Treatment − control)/control] × 100) was 28.49% and 45.72% in the mycorrhizal complex 'Argana' and mycorrhizal complex 'Bouyzakarne', respectively.

Figure 2 shows that the more the duration of stress increases, the more the ratio of the root part to the aerial part (fresh and dry matter) increases. However, the minimum values of the ratio were noted in the non-mycorrhizal plants. On the other hand, the highest values are noted in plants mycorrhizal by Bouyzakarne inoculum, followed by those mycorrhizal by Argana inoculum.

In the leaves and roots, the potassium ion concentration systematically decreases with the lengthening of the stress duration. In addition, potassium content remained higher in the leaves than in the roots. However, mycorrhizal plants had the highest levels of leaf and root K^+ compared to non-mycorrhizal plants, with a slight difference between mycorrhizal plants with Bouyzakarne complex, as well as with the Argana complex, toward the end of the experiment (week 4) (Figure 3).

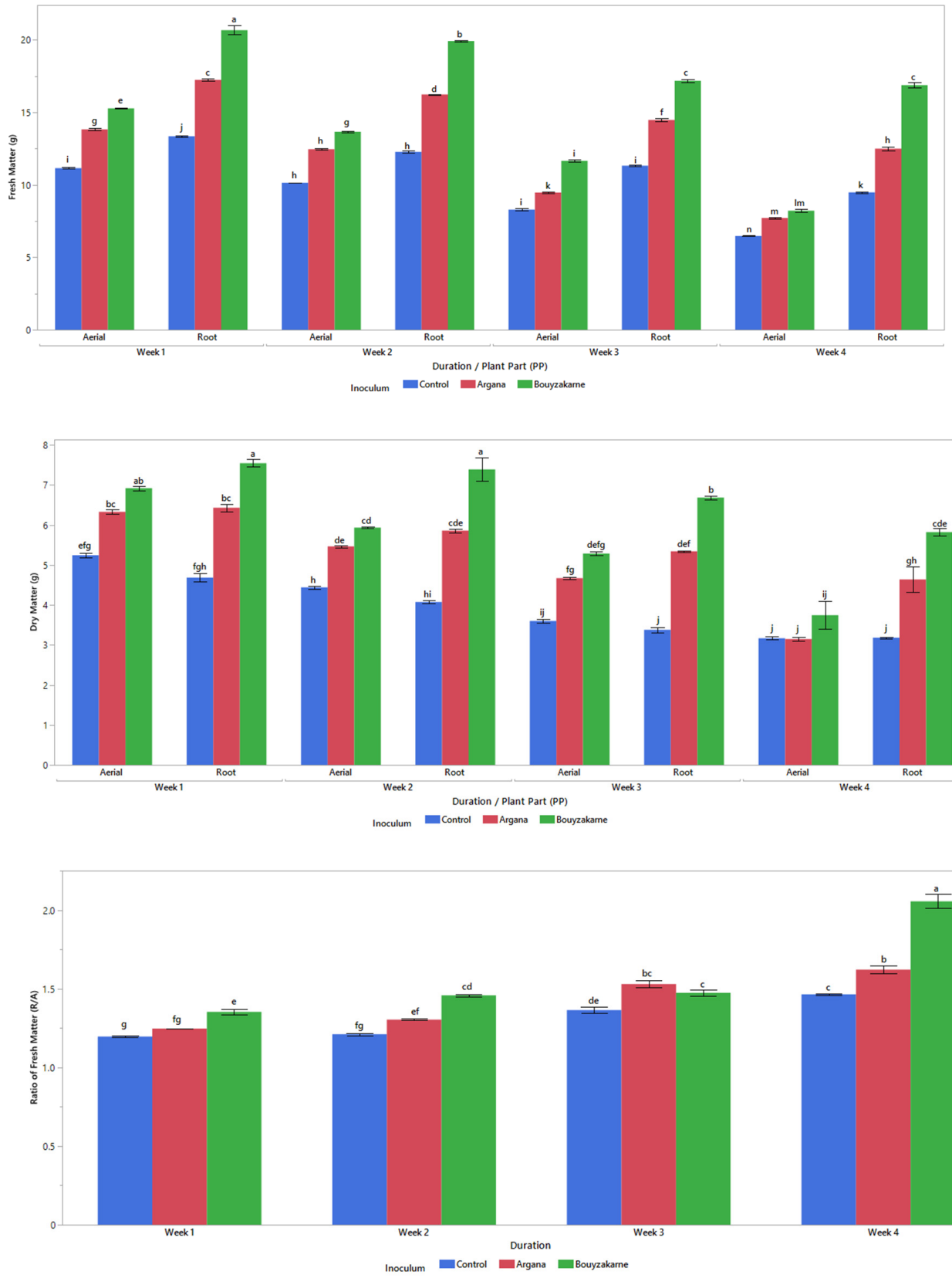


Figure 2. *Cont.*

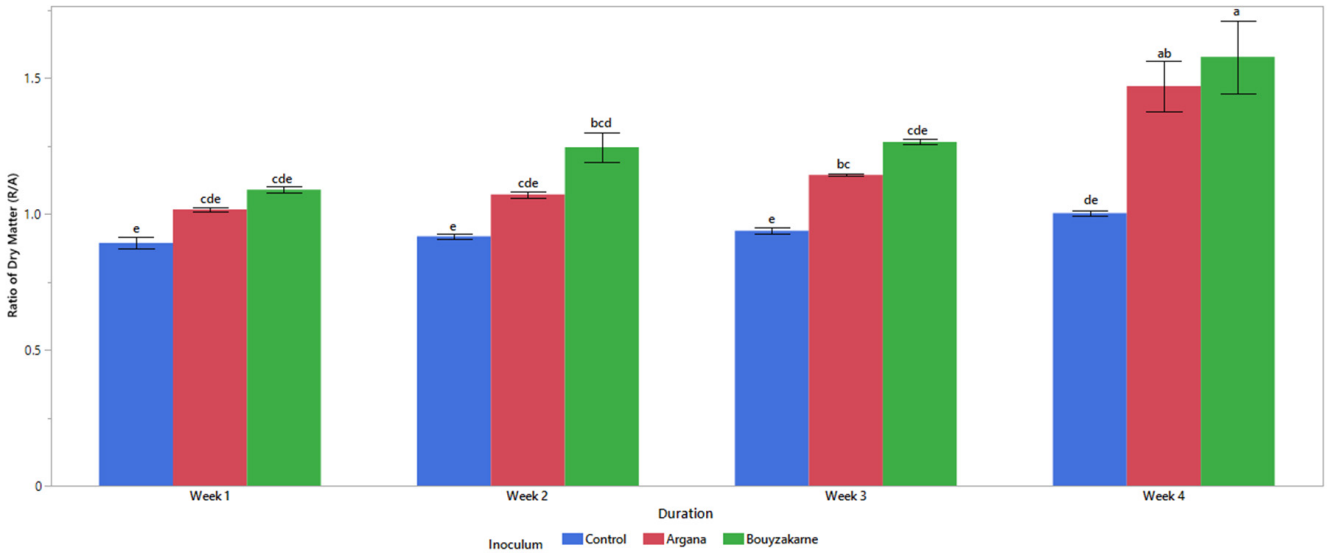
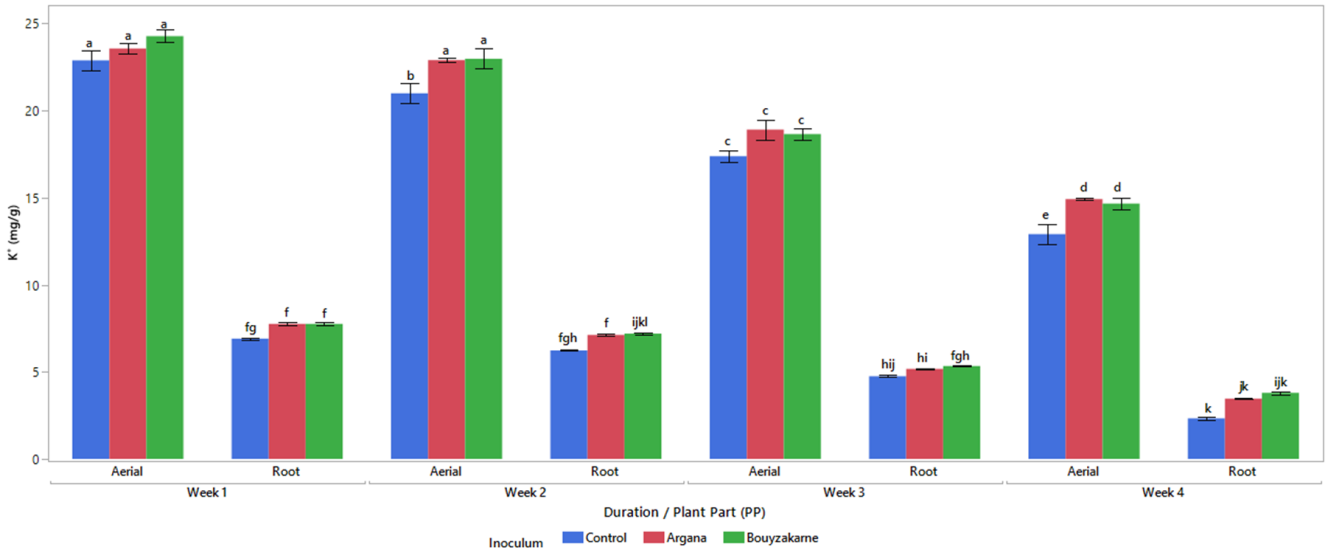


Figure 2. Fresh (FM) and dry (DM) matter of the aerial (A) and root (R) parts and the ratio of the material mass of the root part to the aerial part of mycorrhizal or non-mycorrhizal argan plants subjected to the stress of soil water deficit for a month (each error bar is constructed using 1 standard error from the mean). Values with different letters are significantly different (Tukey's test $p \leq 0.05$).



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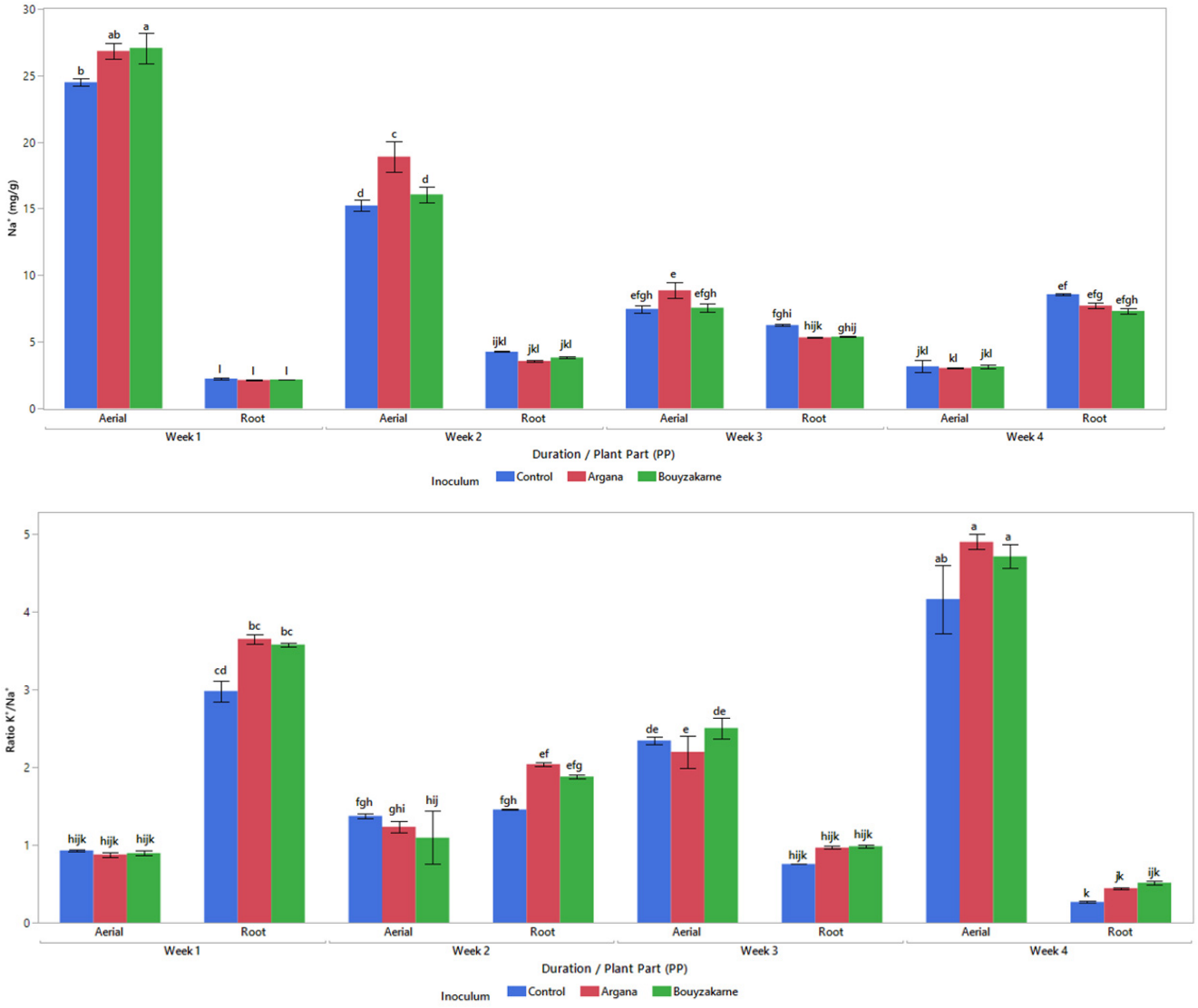


Figure 3. Potassium and sodium ion content and K^+/Na^+ ratio in the leaves and roots of mycorrhizal or non-mycorrhizal Argan plants subjected to soil water deficit stress for one month (each error bar is constructed using 1 standard error from the mean). Values with different letters are significantly different (Tukey's test $p \leq 0.05$).

Water stress reduced the sodium tissue concentration at the leaf level and, on the other hand, increased it at the root level of argan plants. The decrease in leaf level and the accumulation at the root level of Na^+ become more marked as the duration of stress increases. On the other hand, in the leaves, the highest values of the Na^+ concentration values were observed in the mycorrhizal plants. In the roots, the highest values of the Na^+ concentration was observed in the non-mycorrhizal plants (Figure 3).

Under conditions of water stress, the foliar K^+/Na^+ ratio increases in parallel with the lengthening of the duration of stress for the control (non-mycorrhizal) and the "Argana" and "Bouyzakarne" treatments. Moreover, in the roots, K^+/Na^+ ratio decreases in parallel with the lengthening of the duration of stress (Figure 3). For the first two weeks of stress, the K^+/Na^+ ratio was higher in the roots than in the leaves. In contrast, for the third and fourth weeks of stress application, the K^+/Na^+ ratio was much higher in the leaves than in the roots. In addition, the root K^+/Na^+ ratio drops below unity, especially in the 3rd and 4th weeks of water deficit stress.

Furthermore, the roots of mycorrhizal plants always maintained a higher K^+/Na^+ ratio than in the roots of the controls (non-mycorrhizal). Also in the leaves, in the 4th

week of stress, the leaf K^+/Na^+ ratio of mycorrhizal plants was higher than that of non-mycorrhizal plants.

The phosphorus content was higher in the leaves than in the roots for the mycorrhizal and non-mycorrhizal treatments. However, the phosphorus content in both organs systematically decreased with increasing duration of stress. In addition, mycorrhizal plants had the highest phosphorus concentrations compared to non-mycorrhizal plants, with superiority for the Bouyzakarne complex compared to the Argana complex (Figure 4).

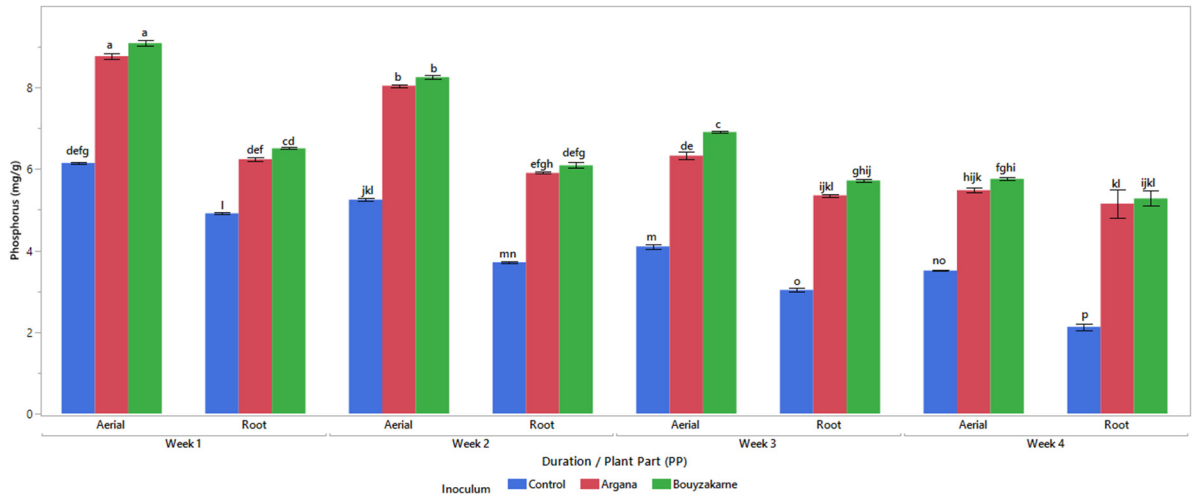
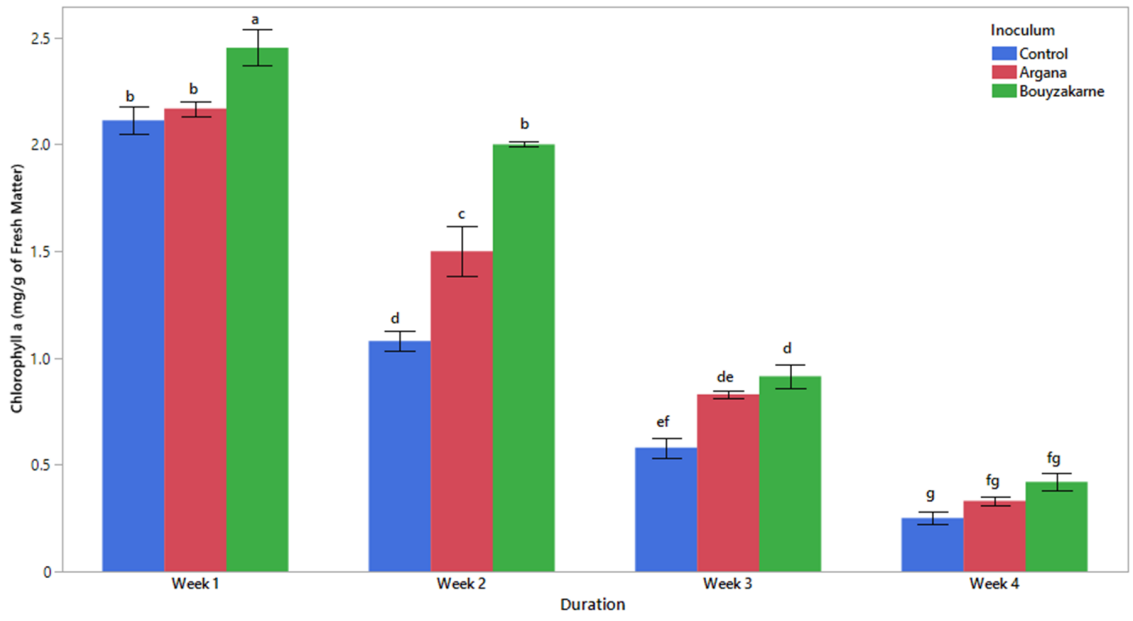


Figure 4. Phosphorus content in the leaves and roots of mycorrhizal and non-mycorrhizal Argan plants subjected to soil water deficit stress for one month (each error bar is constructed using 1 standard error from the mean). Values with different letters are significantly different (Tukey's test $p \leq 0.05$).

Stopping watering dramatically altered the chlorophyll content in the leaves. Chlorophyll a and b content and a/b ratio decreased in stressed plants from the mycorrhizal and non-mycorrhizal treatments. The lowest chlorophyll content values were recorded in non-mycorrhizal plants. Furthermore, during the last weeks, the highest values for chlorophyll content were recorded in plants inoculated with the Bouyzakarne complex, followed by those inoculated with the Argana complex (Figure 5).



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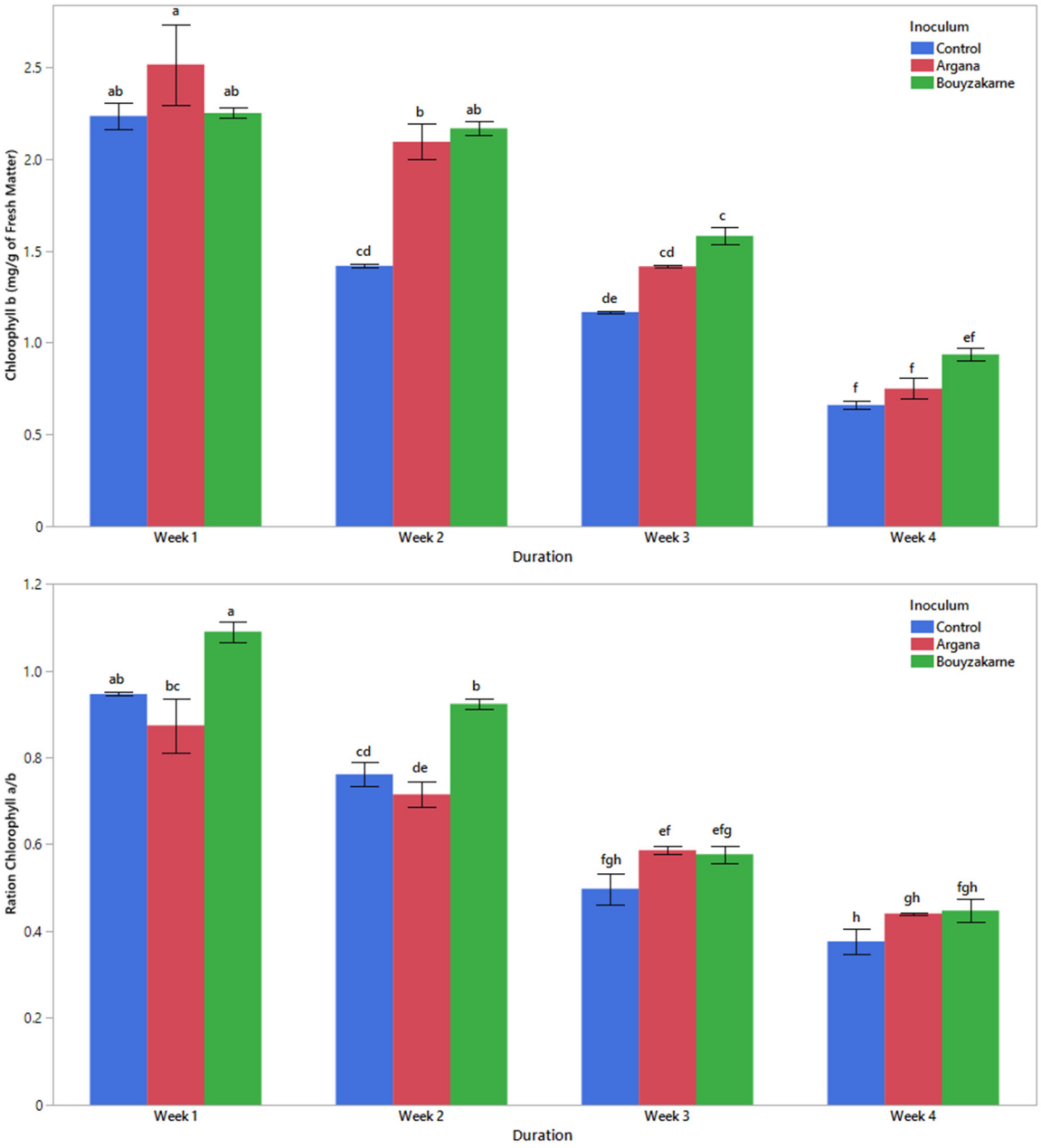

Figure 5. Chlorophyll a, b content and chlorophyll a/b (Chl a/b) ratio of mycorrhizal or non-mycorrhizal Argan plants, subjected to soil water deficit stress for one month (each error bar is constructed using 1 standard error from the mean). Values with different letters are significantly different (Tukey's test $p \leq 0.05$).

Proline dosage results showed a significant accumulation of this amino acid in the leaves and roots of argan plants, along with increased stress duration. However, the proline content in the leaves was higher than in the roots, especially during the 3rd and 4th weeks (Figure 6).

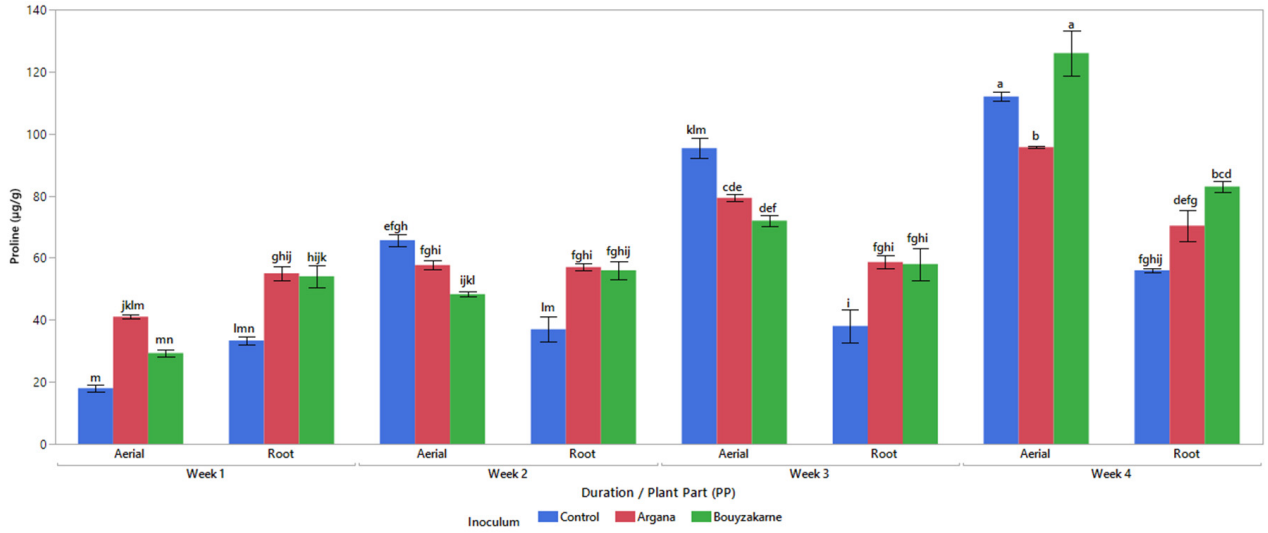

Figure 6. Proline content in the leaves and roots of mycorrhizal or non-mycorrhizal Argan plants subjected to soil water deficit stress for one month (each error bar is constructed using 1 standard error from the mean). Values with different letters are significantly different (Tukey's test $p \leq 0.05$).

On the other hand, during the 2nd, 3rd, and 4th weeks of stress, the leaves of non-mycorrhizal plants showed a very significant accumulation of proline (Figure 6). In contrast, in roots, a higher accumulation of proline was recorded in mycorrhizal plants compared to non-mycorrhizal ones (Figure 6). Moreover, around the 4th week of the watering stoppage, the argan tree plants inoculated with Bouyzakarne inoculum recorded the highest proline content values in the leaves and roots.

In the leaves and roots of argan plants, the content of soluble sugars increased systematically as the duration of stress increased. In addition, the leaves and roots of non-mycorrhizal plants showed higher carbohydrate contents than in mycorrhizal plants (Figure 7). Moreover, for the mycorrhizal plants, the leaves of the plants inoculated with the Argana complex had the highest soluble sugar content compared to those inoculated with the Bouyzakarne inoculum. The roots of plants mycorrhizal with the Bouyzakarne complex accumulated more sugar than those inoculated with Argana inoculum (Figure 7).

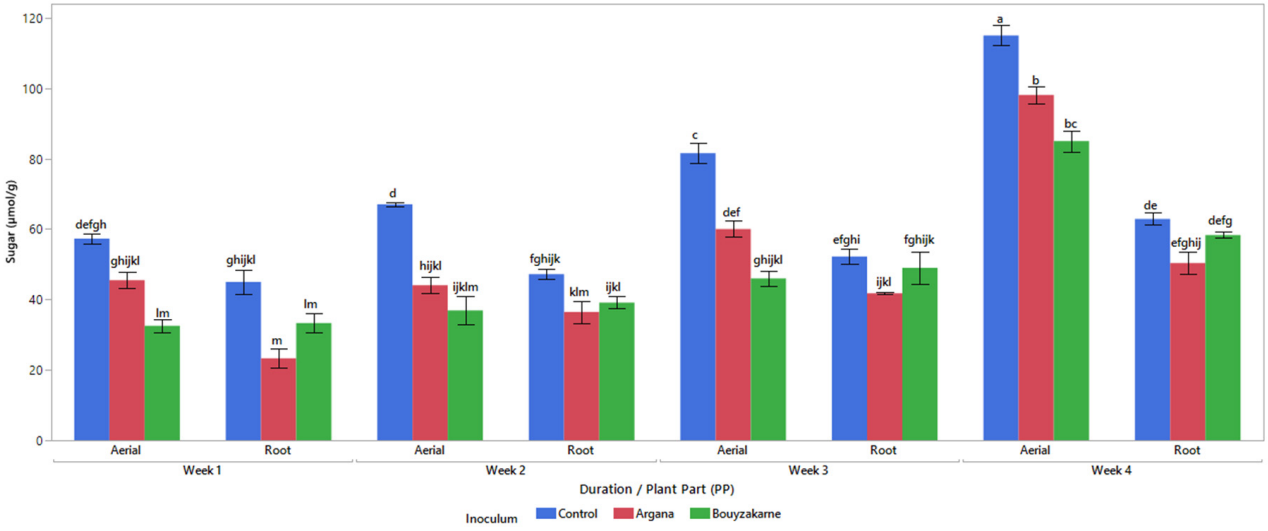

Figure 7. Soluble sugar content of the leaves and roots of mycorrhizal and non-mycorrhizal Argan plants subjected to the stress of soil water deficit for one month (each error bar is constructed using 1 standard error from the mean). Values with different letters are significantly different (Tukey's test $p \leq 0.05$).

3.3. Canonical Discriminant Analysis of Results

The two-dimensional (2D) scatterplots of the discriminant space (canonical plot) compared to the two discriminant functions (CF1 and CF2) relating to the aerial and root parts (Figures 8–10) showed a good separation between the control and the two fungal treatments 'Argana' and 'Bouyzakarne'.

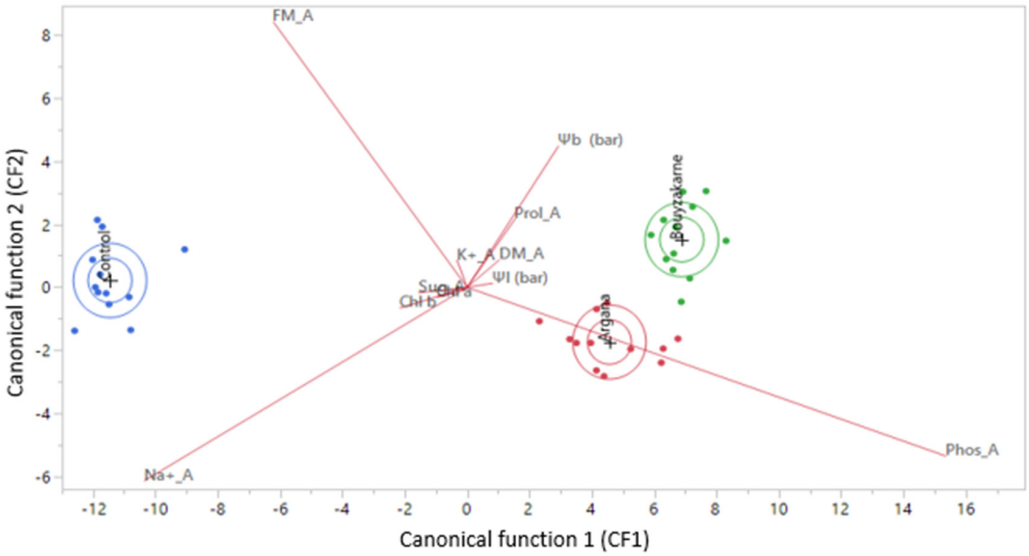

Figure 8. Two-dimensional point cloud showing the separation of the inoculum studied according to the two gradients of the discriminant functions obtained by CDA for the parameters recorded in the aerial part (FM_A: aerial fresh matter, DM_A: aerial dry matter, Sug_A: aerial sugar, Prol_A: aerial proline, Phos_A: aerial phosphorus).

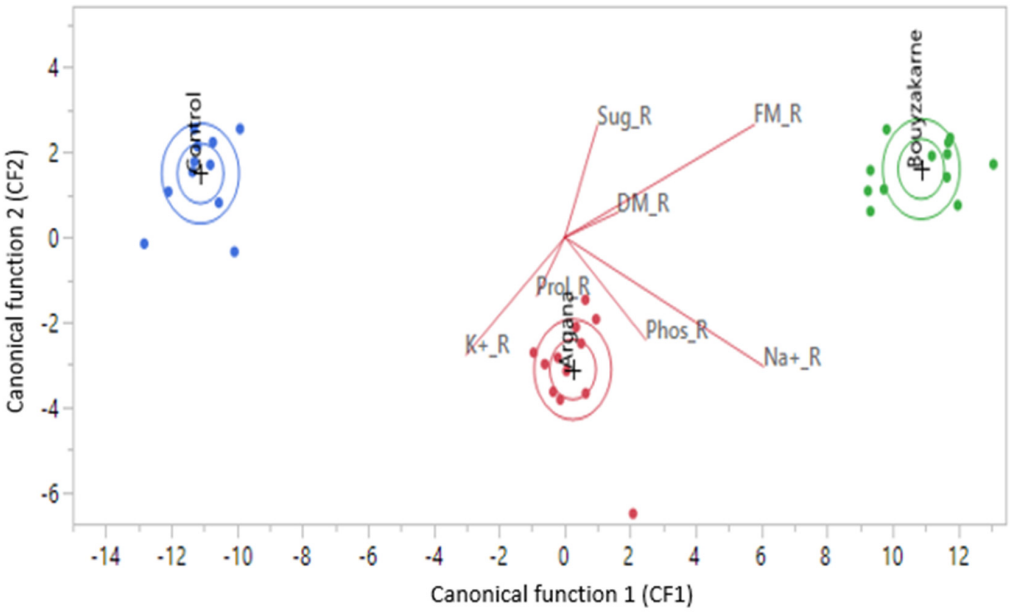

Figure 9. Two-dimensional point cloud showing the separation of the inoculum studied according to the two gradients of the discriminating functions obtained by CDA for the parameters recorded in the root part (FM_R: root fresh matter, DM_R: root dry matter, Sug_R: root sugar, Prol_R: root proline, Phos_R: root phosphorus).

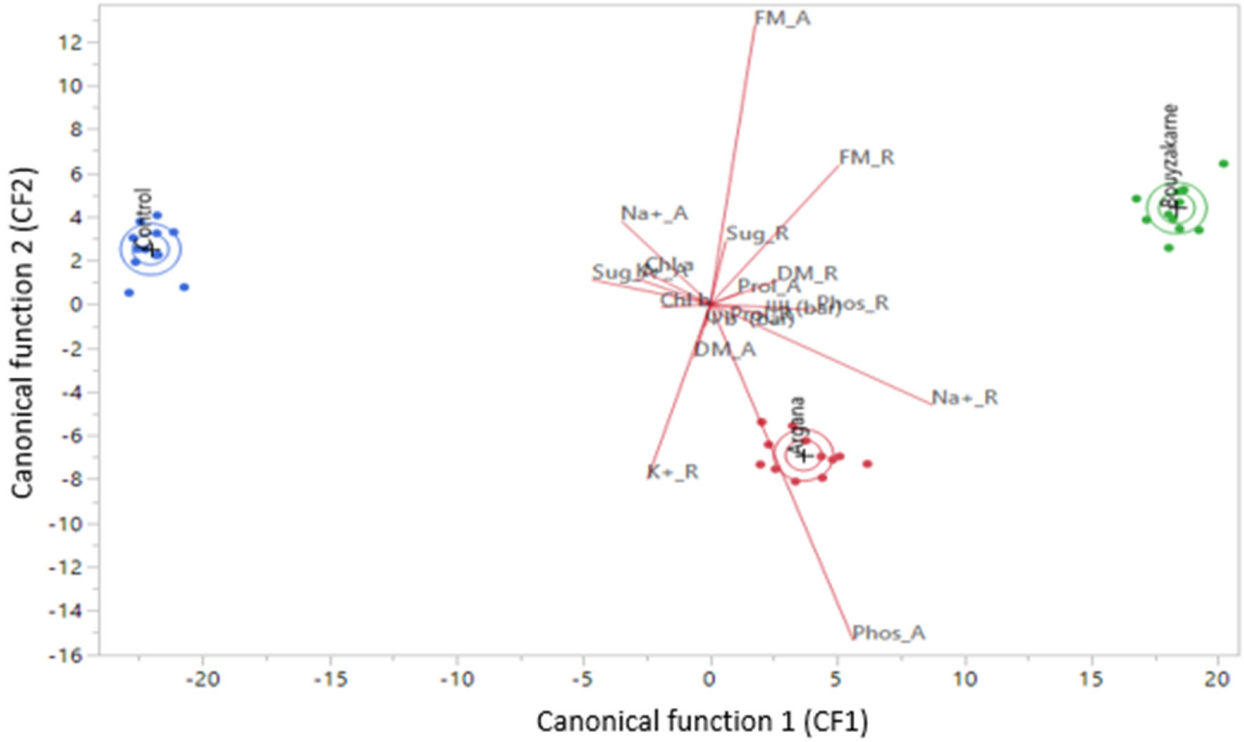

Figure 10. Two-dimensional point cloud showing the separation of the inoculates studied according to the two gradients of the discriminating functions obtained by ACD for the parameters recorded in the aerial and root parts (FM_A: aerial fresh matter, DM_A: aerial dry matter, Sug_A: aerial sugar, Prol_A: aerial proline, Phos_A: aerial phosphorus, FM_R: root fresh matter, DM_R: root dry matter, Sug_R: root sugar, Prol_R: root proline, Phos_R: root phosphorus).

Regarding the canonical discriminant analysis (CDA) performed for the aerial (a) part [CDAa] (Figure 8), horizontal separation was provided by the first canonical function (CF). This one quantifies the degree to which the mycorrhization treatments differ in their parameters related to the aerial part. The control was clearly separated from the two treatments by a low accumulation of phosphorus and a high accumulation of sodium. Due to the second CF, "Argana" treatment was slightly distinguished from "Bouyzakarne" treatment and the "control" by a higher Na+ concentration in the aerial part of plants.

Regarding the canonical discriminant analysis (CDA) performed for the root (r) part [CDAr] (Figure 9), the first CF revealed that the control was clearly separated from the two mycorrhization treatments by a sharp decrease in K^+ concomitant with a strong accumulation of Na^+. This suggests that the control suffers the repercussion of soil drought much more compared to the mycorrhizal plants. The second CF showed, on the one hand, that the Argana treatment was essentially distinguished by a strong accumulation of sugars in the roots, and on the other hand, that the Bouyzakarne treatment was essentially distinguished by a low accumulation of Na^+.

The CDAp point cloud illustrated the separation of the three treatments (Control, "Argana", and "Bouyzakarne") taking into account all parameters studied (Figure 10). The CDAp revealed a strong discrimination of the treatments, and it perfectly and positively separated the Bouyzakarne treatment from the other two treatments (Control, Argana). The first CF revealed that the control was clearly separated from the two inocula by a low accumulation of phosphorus and low production of fresh root and aerial matter. The second CF showed that the Argana treatment was separated from the other treatments (Control and Bouyzakarne) by a low accumulation of root Na+. The Bouyzakarne inoculum was positively distinguished from the two other treatments (Control, Argana) by the two functions (CF1 and CF2) by a higher production of fresh root matter and higher accumulation of phosphorus than the other two treatments (Control and Argana).

4. Discussion

Water stress is a determining factor for plants, especially in arid and semi-arid regions, where it induces a significant reduction in the production of plant biomass [30]. It has a negative impact on total yield and plant distribution [31,32]. In the present study, we simulated the effect of soil water deficit stress by completely stopping irrigation to study certain morphological and physiological mechanisms of water stress tolerance in mycorrhizal and non-mycorrhizal argan seedlings.

The measurement of water potential and water contents in plant tissue is the most direct way to measure the impact of drought on tissue hydration. When watering was stopped, the argan plants inoculated with the arbuscular mycorrhizal fungi complex 'Bouyzakarne' or 'Argana' were able to maintain their leaf water potential (Ψf and Ψb) at less negative values than the "non-inoculated" (control) plants. The positive contribution of AM symbiosis in plant resistance to water deficit stress has been demonstrated in several other studies [9,33,34]. In fact, Marulanda et al. showed that inoculation with drought-tolerant AMF can reduce the water requirements of plants by up to 42% for *Retama sphaerocarpa* [35]. An increase in the efficiency of water use by plants induced by mycorrhizae has also been reported [36,37]. The improvement in plant water nutrition by AMF may be due to the increased surface area for water absorption provided by AMF hyphae, increased access to small soil pores, or improved apoplastic water flow [20].

AM fungi release various types of glycoproteins defined as "glomalins" or "glomalin-related soil proteins" in soils [38]. These proteins play an important role in soil aggregation, together with the tridimensional hyphal network. Both features increase soil water holding capacity, which results in an increased availability of water for plants [19]. The water deficit hampered the growth of the argan plants in terms of the production of fresh and dry matter. This reduction was more marked in the aerial part than in the root part. However, the greatest biomass reductions were recorded in the non-inoculated argan plants. Similarly, in other works, an attenuation of the adverse effect of water deficit stress on biomass production has also been recorded in mycorrhizal plants compared to non-mycorrhizal plants [19,39,40]. This stimulation of the production of fresh and dry matter by mycorrhizae is thought to be the result of an improvement in the nutritional and especially phosphate status of plants [41,42] as well as of an improvement in the photosynthesis of plants [43,44]. By extending the root absorption surface, AMF increase the total absorption surface of the inoculated plants and thus improves the access of the plants to nutrients, in particular those whose ionic forms have a low rate of mobility or those that are present in low concentrations in the soil solution and consequently they increase in the dry biomass of the plants [45,46]. The importance of AM fungi under difficult conditions is also evidenced by the increase of the potential of mycorrhization in response to water deficit [47]. Similar results were observed in corn inoculated with *Rhizophagus irregularis* [47], Coriander inoculated with *Glomus hoi* [48], in lettuce inoculated with *G. deserticola* [12], and in soybeans inoculated with *G. mosseae*, but especially at advanced stages of water stress [2].

The mineral analysis of our argan plants showed a diversity of behavior between inoculated/non-inoculated plants, tested for water deficit stress, in terms of inorganic ions accumulation, as well as differences in accumulation between the organs of absorption and photosynthesis. Indeed, although the stress of water deficit did decrease the tissue concentrations of potassium and phosphorus, the leaves retained higher P and K^+ contents than the roots. In addition, the mycorrhizal argan plants maintained higher levels of P and K^+ compared to the non-inoculated controls. Several studies have shown that AMF contributes to plant growth through the assimilation of immobile nutrients from the soil [16,48]. This is because mycorrhizal plants have been shown to absorb more metallic nutrients via extra-radical hyphae, which explore a larger volume of soil than roots, reduce the diffusion distance, and improve the uptake of immobile metallic nutrients [49–51].

The improvement in the phosphate nutrition of plants is due, first of all, to the increase in the root absorption surface of the plant via the extra-matrix network [13,52], but it could also be explained by the secretion of phosphatases, enzymes that catalyze the hydrolysis

of organic bonds to release orthophosphate, by the hyphae of the fungus, which favors the release of immobile phosphorus into the soil and allows the mineralization of organic phosphate sources [53].

The contribution of mycorrhizal symbiosis to improving the potassium nutrition of plants is still little studied, and the bibliography reports only a few studies concerning the mycorrhizal contribution to the acquisition of K^+ [54]. However, in some studies, similar responses have been reported for other plants [55,56]. Moreover, in view of our results in mycorrhizal argan plants, and in particular the parallelism observed between the evolution of the tissue contents of K^+ and P, in addition to the maintenance of their higher concentrations in the roots and the leaves compared to the controls non-mycorrhizal, we hypothesize that in addition to the proven functions of K^+ in plant nutrition and metabolism, potassium may also be positively involved in the translocation of phosphorus from the fungal symbiont to the host plant. Regarding the interaction between potassium and phosphorus plant nutrition, it should be noted that a strong correlation between potassium and phosphorus during AM symbiosis has also sometimes been reported [57,58]. According to Garcia and Zimmermann [59], data demonstrating the strong link between these two elements suggests that K^+ is a more important component of mycorrhizal symbiosis than previously suspected.

Regarding the effect of mycorrhization on tissue sodium concentration, the relatively lowest values of Na^+ content in roots were noted in mycorrhizal argan plants. This result can be explained by the fact that arbuscular mycorrhizal fungi improve the tolerance of plants under saline stress by excluding salt from plant cells by accumulating salt ions in fungal hyphae [60,61].

In the aerial part of the argan tree, the relatively highest values of leaf sodium concentration were noted in mycorrhizal plants. In fact, under saline stress conditions, Slama [62] showed that plants that have the capacity to accumulate high levels of Na+ in their leaves are clearly more tolerant, and this capacity would be a reflection of the efficiency of the system cell compartmentalization [63]. In addition, the maintenance of a higher K^+/Na^+ ratio in the mycorrhizal plants of the argan tree than in the controls (non-mycorrhizal) also shows that the AM fungal symbionts improve the tolerance of the argan tree by avoiding the loss of homeostasis and therefore reducing the deleterious effects of stresses that can be caused by drought stress.

Mycorrhization has an important compensatory effect on chlorophyll under water stress conditions. Indeed, the highest values of chlorophyll were maintained in the mycorrhizal plants of the argan tree. Zhu et al. [64] also noted that the chlorophyll concentrations in *Zea mays* mycorrhizal and cultured under water stress conditions are much higher than in controls. Zaouchi et al. [65] suggested that symbiosis reduces the negative influence of water deficit on the PSII reaction center and increases the photosynthetic efficiency of stressed plants, resulting in a relative increase in the concentration of chlorophyll pigments.

A plant strategy for tolerating abiotic stress due to drought and salinity is to accumulate a high concentration of low molecular weight organic solutes, such as soluble sugars, proline, and other amino acids, to regulate the osmotic potential of cells subjected to the stress [66,67]. Our results show that the tissue concentrations of proline and soluble sugars increased during water stress in all inoculated and non-inoculated argan plants. These results are in agreement with previous work [68–70].

The proline content in the leaves of the argan tree was significantly lower in the mycorrhized plants than in the non-mycorrhized plants subjected to water deficit stress. This result is in agreement with Wu and Xia and Manoharan et al. [71,72], who showed that mycorrhized plants accumulate relatively less proline in their leaves than non-mycorrhized plants under conditions of severe water stress. On the other hand, in the roots, we recorded a higher accumulation of proline in the inoculated argan plants compared to those not inoculated. Both an increase and a decrease in proline can indicate a higher tolerance to drought induced by mycorrhiza. Indeed, the accumulation of proline could reflect a higher

osmoregulation in the plant, while a decrease could indirectly result from a greater water status in AM-colonized plants.

These results can be attributed to less injury or greater drought tolerance in mycorrhizal plants grown under drought stress conditions [73,74]. Some plants produce a large amount of proline to improve osmosis and prevent dehydration [75]. Thus, the accumulation of organic solutes in the roots could provide the plant with an osmotic mechanism to maintain a favorable gradient for water entry into the roots [76], leading to less stress injury in plants [77].

AMF provide water and nutrition to their host plants, and in return host plants transfer their carbohydrates to AMF for the energy source. Our results showed that under water stress conditions, the accumulated proportions of soluble sugar by non-inoculated plants were higher than those of inoculated plants. This suggests that AM symbiosis mainly demands soluble sugar provided by the host plant. However, the transferring processes of carbohydrates from host plants to AMF are unclear. Moreover, a relatively lower accumulation of soluble sugars in mycorrhizal plants could be due to the involvement of these organic compounds in the functioning and growth of arbuscular mycorrhizal fungi [78].

It seems likely that a high-level soluble sugar accumulation may play a role in drought tolerance and make plants survive short droughts and recover from stress [79,80]. Our study indicated that AM colonization decreased the soluble sugar accumulation of AM seedlings' leaves and roots. The results suggested that AM colonization enhanced host plant drought tolerance, which did not correlate with soluble sugar but with proline, K^+, and phosphorus.

5. Conclusions

In summary, under water deficit stress conditions, the mycorrhizal symbiosis achieved by the selected AMF complexes improved the growth and chlorophyll content of argan trees, their water, and mineral nutrition, in addition to the accumulation of osmoregulators, in particular proline. Canonical discriminant analysis showed that argan trees inoculated with the "Bouyzakarne" complex were distinguished by a high accumulation of phosphorus and a better production of root and aerial fresh matter; this shows that this mycorrhizal inoculum possesses and confers a relatively more efficient tolerance system to the effects of drought. These indigenous drought-tolerant AMF complexes, which are much more adapted to aridity, could be an effective biological means of assisting vegetation restoration in arid and semi-arid areas where plants are under increasing stress from water deficits.

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