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**Symbiotic interactions between a newly identified native mycorrhizal fungi complex and the endemic tree *Argania spinosa* mediate growth, photosynthesis, and enzymatic responses under drought stress conditions**

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**The significant symbiotic interactions between a native mycorrhizal fungi complex newly identified and the endemic tree *Argania spinosa* Skeels mediate growth, photosynthesis and enzymatic responses under drought stress conditions.**

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2 **identified and the endemic tree *Argania spinosa* Skeels mediate growth, photosynthesis**  
3 **and enzymatic responses under drought stress conditions.**

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**Abstract**

Water deficit or drought is the most important abiotic stress limiting plant growth performance and plant community development, which is the case in the Mediterranean area where plants are often both severely and permanently water limited. This is the case of the Argan tree (*Argania spinosa* Skeels) being one of the most affected species by desertification and global warming. To advance knowledge on how this tree can withstand drought stress, Arbuscular mycorrhizal fungi (AMF) inoculation with a native complex, mainly formed of *Glomus* genus, was studied on a set of growth and physiological parameters. Under controlled conditions, inoculated and non-inoculated Argan seedlings were grown for three months under three water regimes (25%, 50%, 75% relatively to the field capacity of used soil substrate). Results showed that the Argan tree had different growth abilities to develop and withstand the various applied water limitations. The AMF complex stimulates growth and mineral nutrition of Argan seedlings under the different imposed levels of water deficiency). The Relative water content (RWC) in leaves, the hydric potential and the stomatal conductance in Argan leaves had shown a general improvement in inoculated seedlings compared to non-inoculated ones. Soluble sugar and proline contents significantly increased in non-inoculated compared with inoculated seedlings under water-limiting conditions (25%). This was similar to oxidative enzyme (Catalase, peroxidase, superoxide dismutase) whose activity increased significantly in drought stressed seedlings. Non-inoculated seedlings had shown the highest level in accumulation of these enzymes. Moreover, mycorrhizal symbiosis establishment positively correlated with Argan tree seedlings in terms of growth, mineral nutrition, soluble sugar, proline contents and enzymes activities. The main ensued results from the current study suggest that AMF improve the ability of *Argania spinosa* to tolerate drought via the enhancement of mineral nutrition and the carriage of a high water level by enhancing the relative water content and the hydric potential in leaves. Finally, the alleviation of the destructive effects of reactive oxygen species (ROS) was modulated by enzymatic scavenging activity. Hence the use of AMF in the technical itinerary of production of Argan seedlings is highly recommended in different ecofriendly restoration strategies based on Argan tree to produce high quality seedlings able to tolerate drought stress.

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**Key words:** *Mycorrhizae*. *Drought*. *Plant growth*. *Argania spinosa*

## 64 1. Introduction

65 Drought stress is one of the most threatful factors trammeling the plant production worldwide,  
66 especially in the Mediterranean being one of the most vulnerable regions of the world due to  
67 drought episodes and irregular precipitations (Diffenbaugh and Giorgi, 2012). The negative  
68 effects are marked with climate changes such as increasing of global temperature and soil  
69 drought (Rasmussen et al., 2020). This global phenomenon leads to irreversible desertification  
70 process, particularly when human intervention is lucking. Both drought and fertility loss of  
71 soils are idiosyncratic of these Mediterranean areas (Blondel et al. 2010; Jiao et al., 2016).  
72 The belowground microbial richness and diversity are drastically affected; particularly the  
73 community of Arbuscular mycorrhizal fungi (AMF) is stricken ( Allen, 1986; Albaladejo et  
74 al., 1998). This fungal community is considered as a key factor in biogeochemical cycles of  
75 the major nutrients including phosphorus (Walder et al., 2015), which is a limiting factor for  
76 plant growth and production ( Requena et al.2001; Armada et al., 2015). Furthermore AMF  
77 allow the plant to maintain a high level of tissue water content ( Ruiz-Lozano and Aroca,  
78 2010; Augé et al., 2014). This crucial role of AMF in water nutrition is provided by the extra-  
79 radical fungal hyphae (Liu et al., 2015). Additionally the extension of the root system by the  
80 fungal hyphae makes a better water nutrition possible while exploiting significantly more soil  
81 volume surrounding roots ( Johnson et al., 1997; Ouahmane et al., 2007b; Püschel et al.,  
82 2020; Remke et al., 2021)

83 The Argan tree (*Argania spinosa* Skeels) is one of the most emblematic species of Moroccan  
84 forest covering approximately 900.000 ha (NFI 2005) mainly in the south west Morocco. This  
85 multipurpose tree is used for edible and cosmetic oil, firewood, timber, forage, and for cereal  
86 crops (Alados and Aich, 2008). Grazing activity and intensive agriculture under the arid  
87 climate occurring in a large part of Argan cultivated areas affect drastically the sustainability  
88 of the Argan ecosystem (Mcgregor et al., 2009). A huge degradation of the physico- chemical  
89 and biological properties of soil were recorded (Lybbert et al., 2010; El Mrabet et al., 2014).  
90 This degradation is usually manifested by a reduction in the diversity and the activity of  
91 rhizosphere microbes (Kennedy and Smith, 1995). The reduction or loss of the microbial  
92 activity, especially the one associated to AMF establishment in the rhizosphere soil can  
93 influence the growth and the nutritional status of plants and limit the success of plantations (

94 Sylvia, 1990; Van Der Heijden et al., 1998; Kyriazopoulos et al., 2014).  
95 Important findings showed that inoculation of plants with AMF not only facilitates  
96 establishment of plants (Herrera et al., 1993; Sally E. Smith, 2008, Alguacil et al., 2011,

97 Remke et al. 2021), but also improves the physico-chemical and biological properties of the  
98 soil ( Schmid et al.,2008; Rillig et al., 2015). In general, beneficial effects of inoculation with  
99 these mycosymbiotes have been widely demonstrated in terms of the water retention  
100 improvement capacity, infiltration rate (Augé 2004) soil aggregation and stability (Morris et  
101 al.2019). Furthermore, the improvement of the metabolic activity is a regular consequence of  
102 inoculation of plants with AMF. Biochemical activities, biosynthesis and oxidative enzymes  
103 activity usually follow the establishment of mycorrhizae in roots of targeted plants (Zebarth et  
104 al., 1999; Caravaca et al., 2003; Fuentes et al., 2010; Wu et al., 2013, 2006).

105 Reforestation with Argan tree has been initiated in Morocco without achieving the main goals  
106 expected due to the harsh soil and climatic conditions prevailing in the arid and semi-arid  
107 south-west of Morocco. In addition, nutrients limitation such as phosphorus, nitrogen and  
108 water contributes these miscarriages in yearly plantation program with Argan tree seedlings  
109 (El Mrabet et al. 2014). Furthermore, in these planting programs the community of native  
110 AMF associated with Argan tree in the targeted ecosystems was not glamorized. It is also  
111 worth mentioning that neither inoculation of seedlings produced in forest nurseries, nor the  
112 assessment of mycorrhizal soil infectivity were practiced. This, should be adopted within a  
113 holistic approach to boost the planted seedling's colonization in the field ( Requena et al.,  
114 2001; Caravaca et al., 2003; Duponnois et al., 2011;). In this context, the importance of  
115 inoculation of plants with AMF under similar soil and climatic conditions significantly  
116 contributed to plants growth and cope with nutrients deficiency, drought, salinity, and  
117 metallic pollution (Martínez and Pugnaire, 2009; Martínez-García et al., 2012). Indeed,  
118 previous studies have investigated the effects of a mycorrhizal inoculation of *Argania spinosa*  
119 seedlings with *Glomus intraradices* (*Rhizophagus intraradices*) on growth and nutrition of  
120 seedlings (Nouaim and Chaussod, 1994; Bousselmame et al., 2003; Echairi et al., 2008).  
121 Hence in the current study, the main objective is to highlight the beneficial role of inoculation  
122 with a selected native mycorrhizal complex on the Argan seedlings growth and tolerance to  
123 drought stress. The AMF complex was isolated from Argan tree roots in Agadir zone  
124 (Morocco), and its composition was analysed after DNA Sequencing. The height, the fresh  
125 and dry biomass, the membrane stability, the stomatal conductance, the relative water content,  
126 the chlorophyll a and b, the total soluble sugars, the protein, the proline contents and the  
127 activities of three well reported oxidative stress enzymes: Catalase, Peroxidase and  
128 Superoxide Dismutase were under study.

129 Hence, the identification and the production of a native arbuscular mycorrhizal consortium  
130 could be efficient in the improvement of the growth of Argan plantations and in the

131 alleviation of the drought treats in these specific harsh conditions mediating consequently the  
132 sustainability of Argan tree ecosystems. In fact, the most important loss of seedlings in  
133 reforestation programs occurs in early stage of plantation. Thus the use of Mycorrhizal fungi  
134 inoculation in the technical itinerary is proposed as an ecofriendly technic contemplated to  
135 produce high quality seedlings able to tolerate drought stress and the consequences of the  
136 global warming.

## 137 **2. Materials and Methods**

### 138 *2.1. Mycorrhizal fungi propagules and Inoculum preparation*

139 The targeted soil for the mycorrhizal fungi propagules was collected in the region of Agadir  
140 ( 9 ° 36 '22' 'W and 30 ° 55' 39 " N, the elevation above sea level is : 279 m), Morocco. The  
141 Bioclimate is arid with average annual rainfall of about 224.1 mm. Soil samples were collected  
142 around the Argan tree roots at depths varying between 10 and 30 cm and stored immediately  
143 at 4°C. The mean soil characteristics are clay% (20), Silt%(39.9), Sand%(40.1), pH(8.54), C%(2.01),  
144 N%(0.19), P mg/Kg(18).

145 The inoculum preparation first begun with setting up the mycorrhizal trap culture using Maize  
146 (*Zea mays L.*) as an endophyte host plant. Maize seeds were surface disinfected and  
147 germinated in pots containing the rhizospheric soil sampled under the Argan tree to 1m from  
148 the trunk. Maize culture was maintained for three months before that AMF spores were  
149 isolated using the wet sieving method (Sieverding, 1991). Extracted spores were surface  
150 disinfected with a solution of chloramine T and streptomycine (both at 0.2 g l<sup>-1</sup>) (Mosse,  
151 1973) and used to inoculate Maize seedling planted in an autoclaved (140 °C for 3 h)  
152 substratum sandy soil. Three days after their germination, Maize seeds were then inoculated  
153 with a 10 ml suspension of the surface sterilized mycorrhizal spores mixture formerly  
154 extracted using the wet sieving method and kept up growing for a period of 3 months. The  
155 roots colonized by the AMF complex were rinsed three times with sterile distilled water and  
156 cut into 1 cm fragments before being used as a fresh mycorrhizal inoculum for Argan  
157 seedlings ( Wang et al., 2008; Douds et al., 2010; Trejo-Aguilar et al., 2013; Selvakumar et  
158 al., 2016) .

### 159 *2.2. Molecular identification of the mycorrhizal complex*

160 Fungal DNA was extracted from a sub-sample of 40 mg of surface sterilized Argan roots  
161 (ground using FastPrep-24 homogenizer (MP biomedical Europe, Illkirch, France)) from  
162 nine months old Argan seedlings inoculated with disinfected Arbuscular Mycorrhizal fungi

163 spore mixture. DNA was extracted using FastDNA® SPIN kit (MP biomedical Europe)  
164 according to the manufacturer's instructions. Guanidine thiocyanate is added to improve the  
165 extraction process. DNA extracts were purified by adding 20-30 mg polyvinyl polypyrrolidone  
166 (PVPP) to limit the presence of PCR inhibitors. Fungal DNA amplification was performed  
167 targeting 18S rRNA gene that was amplified using the primers NS31 and AML2 (Lee et al.,  
168 2008; Simon et al., 1992). The PCR Products were then freeze-dried before being sent for  
169 sequencing according to the provider's instruction. Bioinformatic data processing was  
170 conducted by Frederic mahe (<https://github.com/fredericmahe/stampa>). Molecular operational  
171 taxonomic unit (OTU) representative sequences were then searched and, sequences received  
172 taxonomical assignments using the stampa pipeline.

### 173 *2.3. Argan seeds germination*

174 Seeds of Argan obtained from a single tree in Admine forest in Agadir region were immersed  
175 in hydrogen peroxide for 30 min, thoroughly rinsed with sterile water where they were kept  
176 for four days. Seeds were transferred for germination in Petri dishes containing wet sterile  
177 filter papers. Seeds were then germinated at 28°C for one week before seedlings were  
178 individually transplanted into pots filled with 2 kg of disinfected soil at 140 °C for 3 h.

### 179 *2.4. Inoculation and experimental design*

180 The experimental design consisted of two treatments with or without AMF inoculation. The  
181 inoculation of germinated seeds was carried out by mixing an individual germinated Argan  
182 seed with 2g of fresh mycorrhizal root fragments in a hole in the middle of the pot containing  
183 2 kg of sterilized soil collected under Argan tree. The experiment was conducted under  
184 greenhouse conditions at the Cadi Ayyad University of Marrakesh. The average day/night  
185 temperature was 36/25 °C; the relative humidity (RH) was 55/86 % and a photoperiod of  
186 about 16 hours light / 8 hours dark. After six months of plant growth under daily irrigation to  
187 saturation, Argan seedlings were subjected to three water regimes for three months (25%,  
188 50% and 75% of field capacity). The saturation level (100% field capacity) of the culture  
189 substrate was first defined. Pot cultures (2L) were then watered and weighed regularly to  
190 maintain the following water regimes: 25%, 50%, and 75% of field capacity with six  
191 treatments encompassing inoculated and non-inoculated plants under 25%, 50% and 75% of  
192 field capacity water regimes with forty repetitions for each treatment. Five repetitions were  
193 used for all the analyzed parameters.



194           2.5. *Mycorrhizal parameters and Plant growth performance*

195                 2.5.1. *Mycorrhizal parameters*

196   The mycorrhizal colonization parameters of roots were determined through microscopic  
197   observation after staining methods. Root fragments were washed in 10% KOH at 90 °C for 2  
198   h, and 5 min soak in 5% HCl. Roots were then stained in a solution of 0.05% trypan blue  
199   (1:1:1 water, glycerol and lactic acid) at 90 °C for 15 min. The mycorrhizal Frequency and  
200   colonization rates were determined according to the method of Trouvelot et al. (1986). The  
201   mycorrhizal frequency (%) was defined as the percentage of mycorrhizal root fragments  
202   related to the total number of fragments observed. The mycorrhizal intensity (%)  
203   corresponded to the proportion of observed root fragments colonized by the AMF. Each  
204   analyzed fragment is then placed in a mycorrhizal identity class according to five classes of  
205   colonization 0 (non mycorrhized at all), 1 (trace of mycorrhization), 2 (less than 10%  
206   colonization), 3 (between 11% and 50% colonization), 4 (between 51% and 90%), 5 (more than  
207   91% colonization) ( Brundrett et al. 1996; McGonigle et al., 1990)

208                 2.5.2. *Morpho-metric parameters*

209   After three months under drought stress, the nine month aged Argan seedlings were harvested  
210   and put under measurement. Shoots height (cm) and collar diameter (mm) were measured.  
211   The shoots and roots fresh weights (g) were weighted and their respective dry weights were  
212   determined after one week drying at 62°C. Leaf surface area was determined using image  
213   analysis software imagej (NIH).

214                 2.5.3. *Mineral contents analysis*

215   Plant samples were oven-dried at 62 °C for one week, ground and passed through a 1 mm  
216   sieve. The total nitrogen was measured by the Kjeldhal method. The total phosphorus (P), the  
217   potassium (K<sup>+</sup>), the calcium (Ca<sup>2+</sup>) the magnesium (Mg<sup>2+</sup>) and the sodium (Na<sup>+</sup>) contents  
218   were measured using the “ICP: inductively coupled plasma spectrophotometer” (National  
219   Center for Scientific and Technical Research, Rabat, Morocco).

220           2.6. *Plant Physiological and biochemical changes*

221                 2.6.1. *Relative water content*

222   The relative water content (RWC) was determined using the formula developed by Talaat and  
223   Shawky (2014).  $RWC = 100 \times [(FW-DW) / (TW-DW)]$  in which FW, DW and TW represent

224 Fresh weight, dry weight and turgid weight respectively. The turgid weight (TW) was  
225 determined after placing the leaves, fully submerged, in water in the dark for 24 h at 4 °C.

#### 226 2.6.2. *Hydric potential*

227 The leave water potential in petiole level was measured using the pressure chamber. It  
228 consists on pressing the petiole under lens observation. The water potential measured at 12  
229 O'clock is the pressure allowing the emergence of a water droplet from the pressed tissues.  
230 The leave water potential was measured at the final stage of the experiment just before  
231 stopping the growth of the Argan seedlings (Barrs and Kozlowski, 1968; Scholander et al.,  
232 1965).

#### 233 2.6.3. *Stomatal conductance*

234 The stomatal conductance was measured at a temperature of 25°C using a leaf porometer  
235 (Model SC-1, Decagon devices) at very specific time of the day, generally at 12 O'clock.

#### 236 2.6.4. *Membrane stability*

237 The membrane stability was determined according to the method developed by Shanahan et  
238 al. (1990), a conducto-metric technique which assesses membrane damage by measuring  
239 electrolyte leakage, 100 mm<sup>2</sup> leaf fragments were rinsed then placed in test tubes containing  
240 10 mL of distilled water and placed in test tube shaker racks for 24 hours, the initial  
241 conductivity C1 is then measured. Final conductivity C2 was measured after autoclaving the  
242 samples for 10 min at 0.1 MPa and cooling them down to room temperature (25°C). The  
243 membrane stability index was then calculated based on the formula:  $MSI = [1 - (C1/C2)] * 100$ .

#### 244 2.6.5. *Total Chlorophyll*

245 The fresh leaf material (50 mg) was ground in 3 ml of 90% acetone solution then centrifuged  
246 at 100 rpm for 10 min. After three hours incubation in the dark, optical density (OD) was read  
247 at 663 and 645 nm and chlorophyll a, chlorophyll b and total chlorophyll contents were  
248 calculated according to (Raimbault et al., 2004).

249 Chlorophyll a (µg/ml) =  $11,93DO_{664} - 1,93DO_{647}$

250 Chlorophyll b (µg/ml) =  $20,36DO_{647} - 5,5 DO_{664}$

251

#### 252           2.6.6. *Total Soluble sugars*

253   The total soluble sugar content was determined according to (Dubois et al., 1956). 100 mg of  
254   fresh plant matter were ground in 4 ml of 80% ethanol then centrifuged at 4000 rpm for 10  
255   min. 2.5 mL of 5% phenol and 2.5 mL of 97% sulfuric acid are added to 0.5 mL of the  
256   supernatant, the mixture is homogenized then allowed to rest for 5min. Optical density was  
257   measured at 485 nm and a glucose standard curve was used to determine TSS content.

#### 258           2.6.7. *Protein content*

259   The protein extract was obtained by grinding and homogenizing 100 mg of leaves sample in  
260   0.1 mL of 50mM potassium phosphate buffer (7.5pH), 1% pvpp (polyvinylpolypyrrolidone)  
261   and 0.1 mM EDTA. The resulting mixture was centrifuged for 20 min at 4° C (12500xg) and  
262   the supernatant was used for protein content enzymatic activity determination. Total proteins  
263   were determined using the method of Bradford (Bradford, 1976). 100 µl of diH<sub>2</sub>O were added  
264   to 100 µl of the protein extract and 2 mL of Bradford's reagent. The samples are then  
265   incubated for 5min and the optical density (OD) was read at 595 nm. Protein content was  
266   determined using a serum bovine albumin standard curve.

#### 267           2.6.8. *Proline content*

268   The leaf material (400 mg) was homogenized in 5ml of 95% Ethanol and rinsed three times  
269   using 70% Ethanol. For each sample, 5 mL of the combined supernatant are recovered and 2  
270   mL of chloroform are added along with 3 mL of water. The samples are then allowed to  
271   incubate for 12 hours (Nguyen and Paquin, 1971). A 0.2 to 1 mL aliquot of the superior phase  
272   is then added to a ninhydrin solution and glacial acetic acid and placed in a 100°C water bath  
273   for 45 min. After cooling, 2 mL of toluene are added and the samples are allowed to rest for  
274   30 min. Optical density (OD) of the superior phase was then read at 520 nm and a standard  
275   curve is used to determine proline concentration (Bates L, Waldren RP, 1973; Singh et al.,  
276   1973).

#### 277           2.6.9. *Oxidative enzyme activity*

278   The resistance to the effects of oxidative stress is evaluated by the determination of, catalase ,  
279   peroxidase and superoxide dismutase in the leaves of inoculated and non-inoculated Argan  
280   seedlings after three months under drought stress conditions (Patterson et al., 1984). Catalase  
281   (CAT) activity was determined in the protein extract by determining the rate of disappearance

282 of the 15mM hydrogen peroxide. The reaction mixture contained 940µl of 50 mM phosphate  
283 buffer (7.0 pH), 40 µl of hydrogen peroxide and 40 µl of the protein extract. The change in  
284 OD was determined by spectrophotometry at 240nm for 3min ( $\epsilon = 39.4 \text{ mM cm}^{-1}$ ) (Aebi,  
285 1984).

286 Superoxide dismutase (SOD) activity was determined by measuring the reduction of  
287 Nitroblue tetrazolium according to the method of (Beyer Jr and Fridovich, 1987). The  
288 reaction mixture contained 2550 µl of 100 mM phosphate buffer (pH 7.8), 75 µL 55 mM  
289 methionine, 300 µl 0.75 mM nitro blue tetrazolium (NBT) and 50 µL of the enzyme extract,  
290 60 µL of 0.1 mM riboflavin are added and the mixture is subsequently incubated under 2  
291 fluorescent lamps (20W) for 15 min at 25°C. The OD was read at 560nm. An enzymatic unit  
292 is defined as the amount necessary to inhibit the reduction of the NBT by 50% (Patterson et  
293 al., 1984).

294 Peroxidase (POD) activity was measured by following the change in absorption at 470 nm  
295 due to guaiacol oxidation. The activity was assayed for 1 min in a reaction solution (3 mL  
296 final volume) containing 20 mM of guaiacol, 10 mM of H<sub>2</sub>O<sub>2</sub>, and 0.35 mL of an enzyme  
297 extract in a 100 mM potassium phosphate buffer (pH 6.8) (Polle et al., 1994), POD was  
298 analyzed according to (Barceló, 1998).

## 299 2.7. Statistical analysis

300 Statistical analysis was conducted using two-way ANOVA using SPSS 20 (IBM) software  
301 with AMF inoculation (AMF) and field capacity (FC) as first and second factors, respectively.  
302 The significance of the differences between treatments and factor interactions was calculated  
303 at 5% and mean comparisons were determined using Tukey's HSD test ( $p \leq 0.05$ ).

## 304 3. Results

### 305 3.1. Molecular identification of the AMF complex associated with *Argania spinosa*

306 The AMF complex belongs to, Glomeromycota phylum, Glomeromycetes class, where the  
307 Glomerales order forms 99.07% (Family of Glomeraceae (98.64%) and Family  
308 Claroideoglomeraceae (0.43%). Only 0.25% is formed from paraglomerales order (family of  
309 paraglomeraceae). At the Genus level, the complex is composed of *Claroideoglomus* (0.43%)  
310 *Glomus* (83.82%), *Rhizophagus* (14.74%), *Sclerocystis* (0.08%), *Paraglomus* (0.008%) and  
311 unidentified Genus (0.67%). In terms of species richness, the mycorrhizal complex  
312 composition encompasses *Glomus sp.*, *Rhizophagus intraradices*, *Rhizophagus clarus*,

313 *Sclerocystis sinuosa*, *Paraglomus majewskii* and other unidentified *Glomus*, and *Paraglomus*  
314 *species*.

### 315 3.2. Mycorrhizal colonization and plant growth parameters

#### 316 3.2.1. Mycorrhization parameters

317 Analysis of the Mycorrhizal colonization parameters in roots of Argan seedlings after nine  
318 months culture and three months under water deficit had shown that the plants were infected  
319 by the mycorrhizal fungi complex, the frequency is 100%. The mycorrhizal colonization rate  
320 showed that at least half the root system is colonized by the fungi structures (Hyphae,  
321 vesicles, arbuscules and spores). The rates ranged between 55.34% for (25% FC) and 70.34%  
322 for ( 50% FC). Whereas, the seedlings under 75% FC were medium with 60.76%. This result  
323 showed a successful establishment of the mycorrhizal symbiosis between roots and the used  
324 fungal consortium (Tab.1).

#### 325 3.2.2. Morpho-metric parameters

326 The inoculation with the native mycorrhizal consortium had shown a tremendous increase of  
327 all the growth parameters considered in this study in the entire water regimen, in comparison  
328 with the plants without mycorrhizal inoculation (Tab.1). The growth parameters under study  
329 were the height, the diameter to the collar, the areal dry biomass, the root dry biomass and the  
330 leaf surface area. By and large, the 25% field capacity treatment had represented the lowest  
331 growth parameters in both inoculated and non-inoculated seedlings. On the other hand the  
332 75% field capacity treatment had shown a significant increase in the height, the collar  
333 diameter, the aerial and root dry biomasses and the leaf surface area either in inoculated or in  
334 non-inoculated nine month aged Argan seedlings subjected to three months drought stress  
335 (Tab.1). The water shortage had led to a significant curtailment of the shoots height, the collar  
336 diameter, the aerial and root biomass and the leaf surface area. Inoculated seedlings were  
337 slightly affected by drought stress compared to non- inoculated ones. The 50% Field capacity  
338 treatment had largely described the medial state between the two extremes (25% and 75%  
339 field capacity) either in inoculated or non-inoculated seedlings for all the analysed morpho-  
340 metric parameters (Tab.1). The inoculation of the Argan seedlings with the AMF complex  
341 garnered from roots of Argan tree had led to a significant upgrading of the morpho-metric  
342 parameters of the growth in the entire applied water deficiency regimen.

343           3.2.3. *Effects of the AMF complex inoculation on the plant mineral nutrition statut*

344   The analysis of different mineral contents in the shoots of Argan seedling after three months  
345   under drought stress had shown that the most important accumulation of the targeted mineral  
346   elements (N, P, K<sup>+</sup>, Ca<sup>++</sup>, Mg<sup>++</sup>, Na<sup>+</sup>) was recorded in inoculated more than in no-inoculated  
347   seedlings and that these contents decreased drastically with increasing the water drought  
348   (Tab.2). The highest contents were encountered in inoculated plants subjected to 75% field  
349   capacity; and the lowest contents were performed in non-inoculated seedlings subjected to  
350   25% field capacity. Whereas the inoculated seedlings subjected to 50% field capacity, the  
351   inoculated plants subjected to 25% field capacity and finally the non-inoculated plants  
352   subjected to 50% field capacity were classified between the two extremes previously defined.  
353   Consequently it seems that the addition of the mycorrhizal complex as an input in the culture  
354   substrate could improve significantly the mineral nutrition of Argan seedlings. The majority  
355   of analyzed elements had shown that their contents in no-inoculated seedlings were multiplied  
356   by at least 2 factors in inoculated plants (Tab.2).

357           3.3. *Physiological and biochemical changes in Agran seedlings after inoculation with the*  
358           *AMF complex*

359           3.3.1. *Relative water content*

360   The relative water content (RWC) had shown lower levels in the non- mycorrhized seedlings  
361   of *Argania spinosa*, whereas inoculated seedlings had witnessed a high level of it. The (RWC)  
362   was drastically reduced in leaves of Argan seedlings under high drought stress conditions  
363   (25% FC). (Fig.1)

364           3.3.2. *Hydric potential*

365   The water potential measured in the level of the petioles of the Argan leaves had proven  
366   higher values in the inoculated seedlings comparatively to non-inoculated seedlings. The most  
367   noteworthy extension of the water potential was recorded under the most drastic water regime  
368   (25% field capacity). This improvement of the water potential was significant in the moderate  
369   water regime (50% field capacity) and no-significant in the light drought treatment (75% field  
370   capacity). (Fig.2)

371           3.3.3. *Stomatal conductance*

372   The calculated stomatal conductance had displayed a large divergence between the non-  
373   inoculated seedlings which presented the lowest levels of conductance and the inoculated  
374   Argan seedlings with the higher levels. Inoculated seedlings, even under the severe treatment  
375   (25% field capacity), had exhibited high stomatal conductance comparing to all the non-  
376   inoculated seedlings (25%, 50%, 75% field capacity). (Tab.3).

377           3.3.4. *Membrane stability*

378   The membrane stability had shown a significant difference between the inoculated and non-  
379   inoculated Argan seedlings, except for the 75% field capacity treatment. The mycorrhizal  
380   input had shown a valuable contribution of the mycorrhizal fungi complex to the  
381   improvement of the membrane stability particularly under harsh conditions (25% and 50%)  
382   (Tab.3).

383           3.3.5. *Total Chlorophyll*

384   The total chlorophyll contents in leaves of Argan seedlings after culture under drought  
385   stressful conditions was drastically reduced with the increase of the drought constraint. The  
386   lowest total chlorophyll content was recorded in no-inoculated seedlings subjected to 25%  
387   field capacity forces. The use of the arbuscular mycorrhizal complex had led to a common  
388   enhancement of the total chlorophyll contents in leaves of Argan seedlings. Some specific  
389   responses were recorded for the chlorophyll (b) where the difference between inoculated and  
390   no-inoculated seedlings was not significant (75% and 50%) (Tab.4).

391           3.3.6. *Total Soluble Sugars*

392   About the accumulation of the soluble sugars in leaves of Argan seedlings, it seems that the  
393   sugar heap is increased with the raise of the drought constraint either in non-inoculated or  
394   inoculated seedlings. The highest soluble sugars accumulation was recorded in non-inoculated  
395   seedlings under the hardest drought stress (25% field capacity). A general alleviation of the  
396   effects of the drought stress was recorded *via* a significant reduction of accumulated sugars in  
397   inoculated seedlings (Tab.5).

398           3.3.7. *Total Protein content*

399   Total protein content in leaves of Argan seedlings subjected to three months drought stress  
400   had shown a large decrease with the raise of the water constraint. The lowest contents were  
401   recorded in the drastic conditions (25% field capacity) both in non-inoculated and inoculated  
402   seedlings. Whereas, the highest contents were encountered in the slight drought stress  
403   treatment (75% field capacity). In addition, in each water regime the inoculated seedlings had  
404   shown significant high protein contents comparing with the non-inoculated seedlings, which  
405   support the beneficial effects of the mycorrhizal fungi complex alleviating the drought stress  
406   treats to the Argan plants (Tab.5).

407           3.3.8. *Proline content*

408   The proline was abundantly accumulated in leaves of Argan seedlings when the water  
409   deficiency became marked. The highest proline content was recorded in non-inoculated plants  
410   under 25% of field capacity treatment. Whereas the lowest proline content was recorded in  
411   inoculated seedlings subjected to the lightest drought stress regime (75% field capacity). Thus  
412   the accumulation of proline in leaves of Argan seedlings was significantly mitigated after  
413   inoculation with the used AMF complex (Tab.5).

414           3.3.9. *Oxidative enzyme activity*

415   The catalase activity had indicated high values when the seedlings were subjected to  
416   aggressive water deficit, particularly the non-inoculated seedlings had recorded the highest  
417   catalase activity under 25% field capacity regime. The lowest catalase activity was recorded  
418   in inoculated seedlings subjected to light drought stress (75% and 50%) (Tab.6). Superoxide  
419   dismutase activity had shown a similar model of appearance in catalase activity. This activity  
420   is strengthened when the water regime is more drastic from (75% to 25%) in both inoculated  
421   and non-inoculated Argan seedlings. On the other hand the non-inoculated seedlings had  
422   performed the highest Superoxide dismutase activity specifically under 25% regime where  
423   this enzyme recorded the pick activity (Tab.6). Peroxidase activity had shown a significant  
424   increase with the raise of the imposed water deficiency in both non-inoculated and inoculated  
425   Argan seedlings. Additionally, the peroxidase activity is marked in non-inoculated Argan  
426   seedlings such as for catalase and superoxide dismutase (Tab.6).



#### 427 4. Discussion

428 From this study, it's obvious that the application of drought stress had a negative effect on the  
429 growth, on the mineral nutrition, on the water status, on the physiological traits and on the  
430 biochemical activity of the Argan tree. Additionally, responses are more market when the  
431 hydric constraint becomes aggressive. These results are in accordance with those of many  
432 previous studies in Mediterranean area on different tree plants like *Cupressus atlantica*,  
433 *Tetraclinis articulata*, *Ceratonia siliqua*, *Phoenix dactylifera* (Zarik et al.2016; Jadrane et  
434 al.2021). On the other hand the enrichment of the culture substrate by mycorrhizal fungi input  
435 had deeply improved morpho-metric, hydric, physiological and biochemical traits of Argan  
436 seedlings in early age under greenhouse conditions. In the current case native mycorrhizal  
437 fungi complex trapped under Argan tree was used. This complex was subjected to advanced  
438 process for characterization by massive sequencing. The result had shown that the native  
439 mycorrhizal complex associated with roots of *Argania spinosa* is mainly formed of the Genus  
440 *Glomus*. This major Genus is accompanied by, *Rhizophagus*, *Sclerocystis* and *Paraglomus*.  
441 It's the first time that the mycorrhizal complex associated with *Argania spinosa* in Morocco is  
442 revealed using molecular tools. Definitely the most presented endomycorrhizal species in  
443 association with Argan roots as revealed by massive sequencing are *Glomus sp*, *Rhizophagus*  
444 *intraradices*, *Rhizophagus clarus*, *Sclerocystis sinuosa*, *Paraglomus majewskii* and other  
445 unidentified *Glomus*, and *Paraglomus species*. This mycorrhizal complex had shown high  
446 effectiveness colonizing roots of Argan seedlings, particularly when the imposed drought  
447 stress becomes harsh (25% and 50% of field capacity). Similarly, the most important  
448 responses of Argan seedlings to water deficit were recorded in the stark conditions described  
449 above. Consequently the roles of the inoculation of Argan seedlings with the autochthonous  
450 mycorrhizal fungi complex mitigating the harmful effects of drought stress were marked in  
451 the case of drastic conditions (25% and 50% field capacity). Differences were statistically  
452 significant comparing inoculated and non-inoculated Argan seedlings subjected to drought  
453 stress. Indeed, the roles of mycorrhizal symbioses alleviating the drought stress effects on  
454 plants is now well demonstrated (Abbaspour et al., 2012). The mean response of inoculated  
455 seedlings to drought stress is the maintenance of a valid level of growth, mineral nutrition,  
456 water status, photosynthetic activity, metabolites accumulation and antioxidative enzyme  
457 activity. In fact the roots of Argan seedlings have given evidence of a high colonization rate  
458 by the mycorrhial complex used in these experiments. The differences in terms of growth  
459 between inoculated and non-inoculated seedlings which traduce the increment rate induced by

460 the mycorrhizal input, express the mycorrhizal dependence of Argan seedlings from  
461 mycorrhizal symbiosis. Thus the Argan seedlings are highly dependent from mycorrhizal  
462 symbiosis especially in the nine early months investigated in this study. Previous studies had  
463 shown that Argan seedlings are highly dependent from mycorrhizal symbiosis (Mrabet et al.,  
464 2014). Furthermore, the complex isolated under Argan tree, in Agadir region had shown  
465 satisfaction in terms of infectivity and establishment in young seedling roots. Thus the  
466 application of drought stress of 25% and 50% field capacity had significantly reduced the  
467 growth parameters of the Argan tree (Kyriazopoulos et al., 2014). The seedlings height, the  
468 diameter to the collar, the aerial and root dry Biomass and the leaf surface were largely  
469 higher in inoculated seedlings than in non-inoculated ones. It's admitted that the mycorrhizal  
470 symbioses safeguard their host plants from deleterious effects of water scarcity (Jumrani and  
471 Bhatia, 2018). These beneficial effects of the establishment of a mycorrhizal symbiosis are  
472 generally conferred to the important uptake of water and mineral nutrients from the solution  
473 in soil, basically by absorbing hairs and additionally by the extraradical mycorrhizal hyphae (  
474 Remke et al. 2021). Water and mineral nutrients are directly reachable by the host plant *via*  
475 the mycorrhizal hyphae, particularly water and phosphorus are privileged (Doubková et al.,  
476 2013; Sfairi et al., 2018). In deed all the analyzed mineral contents in leaves of *Argania*  
477 *spinosa* (N, P, K<sup>+</sup>, Ca<sup>++</sup>, Mg<sup>++</sup>, Na<sup>+</sup>) were increased in inoculated seedlings comparing to  
478 non-inoculated seedlings under all the tested water regimes (Kim et al., 2008; Tamayo et al.,  
479 2014). The improvement of the mineral nutrition of mycorrhized plants is generally attributed  
480 to the increase of the absorption and release from non-labile sources of the most important  
481 nutrients like phosphorus (Ouahmane et al., 2007a; Li et al., 2014). Similarly the mycorrhizal  
482 inoculation had boosted the water balance in Argan seedlings under drought stress conditions.  
483 Divers hydric parameters were investigated in this study as the relative water content, the  
484 hydric potential, the stomatal conductance and the membrane stability. The relative water  
485 content (RWC) reflects the water balance in the tissues of the targeted plant and the  
486 availability of water for metabolic reactions and osmotic regulation. (Shaw et al., 2002;  
487 Anjum et al., 2011; Gholami et al., 2012; Rostami and Rahemi., 2013). The (RWC) in the  
488 level of leaves of inoculated Argan seedlings was significantly higher than in non-inoculated  
489 plants. The mycorrhizal fungal extraradical Hyphae is widely implicated in the water supply  
490 for the plants (Zhang et al., 2010). In the same way, the water potential had shown a very  
491 important effect of the inoculation improving the retention and consequently the availability  
492 of water in seedlings tissues. The hydric potential is another reliable parameter to measure  
493 and assess the water balance in the seedlings (Rapparini and Peñuelas, 2014). Furthermore,

494 the stomatal conductance is in accordance with the hydric potential and the relative water  
495 content (Subramanian et al., 2006; Shao et al., 2008). The water flow among the plant tissues  
496 is mediated by the hydric potential in different compartments of the plant and by the stomatal  
497 conductance which ensure the water perdition by transpiration or the water thrift after stoma  
498 closure (Augé et al., 2014). Likewise, the membrane stability had shown a resistance to the  
499 drought stress particularly when Argan seedlings were inoculated (Nadeem et al., 2014). The  
500 analysis of the chlorophyll a and the chlorophyll b in foliar tissues of Argan seedlings had  
501 shown the negative effects of drought on chlorophyll contents which informs about how the  
502 photosynthetic activity is reduced in non-inoculated seedlings subjected to drought stress.  
503 Inversely, the presence of AMF in the neighborhood of Argan seedlings had heavily promoted  
504 the chlorophyll contents in the leaves which report the high level of photosynthesis activity in  
505 mycorrhized seedlings (Rivero et al., 2007). Furthermore, in the current study, osmotic  
506 regulation assessment was engaged by evaluation of the mean metabolic components  
507 concerned like total soluble sugars, total protein and proline contents. This latest amino- acid  
508 is considered by divers studies as the stress amino-acid since its accumulation behavior in the  
509 tissues of stressed plants. These osmolytes mediate the water flow and translocation in the  
510 plants (Ruiz-lozano, 2003; Farooq et al., 2009; Ruiz-Lozano and Aroca, 2010; Khoyerdi et  
511 al., 2016). The accumulation of proline and soluble sugars in cells diminishes the osmotic  
512 potential and consequently increases the cell turgor. This mechanism enables the cells to  
513 refurbish the water contents and ovoid deleterious effects of drought stress. The osmo-  
514 protection of proline and soluble sugar was underlined in diverse studies dealing with drought  
515 stress (Yamada et al., 2005). The highest accumulation was recorded in non-mycorrhized  
516 plants at 25% of field capacity regime. Whereas, in presence of mycorrhizal fungi a decrease  
517 in accumulation of proline and soluble sugars in Argan seedlings was noted comparing to no-  
518 inoculated seedlings. This result had shown that the AMF complex had played a role of a  
519 substantial warrantor against the imposed drought stress (Porcel and Ruiz-lozano, 2004; Tang  
520 et al., 2009; Rahimzadeh and Pirzad, 2017; Wu et al., 2017; Zunzunegui et al., 2017). Plants  
521 under drought stress often experience oxidative damages after accumulation of Reactive  
522 Oxygen Species (ROS) following the cascade of electron transfer among different oxygen  
523 forms. The products (peroxide, superoxide...) are deeply harmful for cell membranes.  
524 Scavenging these accumulated oxygen molecules is necessary to save plant cells from  
525 degradation and death. Various processes are involved to alleviate the toxic effects of ROS.  
526 Enzymatic and non-enzymatic ways are known such respectively the polyphenol action and  
527 the antioxidative enzyme activity, particularly the Catalase, the Superoxide dismutase and the

528 Peroxidase are closely involved ( Wu et al., 2006; Chang et al., 2012). Enzymes activity  
529 assessment in leaves of Argan seedlings had shown a substantive accumulation in stressed  
530 plants and that this accumulation is exacerbated in no-inoculated seedlings (Wu and Xia,  
531 2006). In this study the three enzyme activities had shown a similar tendency which  
532 demonstrates the activation of the antioxidative stress against all the forms mediated by these  
533 targeted enzymes. The decrease and stabilization of the antioxydative activity in presence of  
534 the fungal symbionts summarizes the important role played by the mycorrhizal fungi complex  
535 associated with roots of *Argania spinosa* in the alleviation and tolerance to drought stress  
536 (Mcmichael et al.,2004; Baslam and Goicoechea, 2012). The mycorrhizal effect evidence  
537 against the drought stress is the avoidance behavior manifested by massive water uptake by  
538 extraradical fungal hyphae (Fouad et al., 2014). Similarly phosphorus uptake *via* extraradical  
539 fungal hyphae contributes the alleviation of the destroying effect of ROS. In deed the  
540 photosynthetic activity of inoculated seedlings as revealed by the Chlorophyll contents in  
541 leaves is maintained even in drastic conditions (Beltrano and Ronco, 2008; Birhane et al.,  
542 2012).

## 543 5. Conclusion

544 In the current study it is well demonstrated that the AMF complex clearly contributed to  
545 alleviation of drought stress in Argan tree "*Argania spinosa* Skills" which can be explained  
546 by the active functioning of the mutualistic interaction between the fungi and the host plant.  
547 Mainly the extra-radical fungi hyphae played a pivotal role in water and mineral nutrition. In  
548 addition the mycorrhizal symbiosis establishment mediated the boosting of physiological,  
549 biochemical parameters and antioxydative stress enzymes production. These drought stress  
550 responses are probably dues to a stress avoidance strategy mainly induced by the AMF  
551 symbionts. This research supports that *Argania spinosa* is a highly mycorrhizal dependent  
552 species. Thus the use of the native mycorrhizal complex formed essentially of *Glomus*,  
553 *Rhizophagus* and *Claroideoglomus* would be an efficient ecological engineering method  
554 handling Argan tree seedlings in an early stage in nurseries before their transplantation into  
555 areas affected by drought and climate changes.

## 556 Conflict of interest statement

557 The authors have *no* conflict of interest to declare.

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Draft

Draft

**Table 1:** Effects of water deficit on morphological and mycorrhizal parameters of *Argania spinosa* seedlings nine month aged and subjected to a 3-month period of drought. Mean values  $\pm$  SE in the same column followed by the same lower case letters are not significantly different at  $P \leq 0.05$  by Tukey HSD test.

	Drought stress treatment	Roots mycorrhizal frequency (%)	Roots mycorrhizal colonization (%)	Height (cm)	Diameter to collar (mm)	Aerial dry weight (g)	Root dry weight (g)	Leaf surface area. (cm <sup>2</sup> )
<b>Mycorrhizal seedlings</b>	25%	100 <sup>a</sup>	54.33 <sup>a</sup> $\pm$ 1.53	17.80 <sup>d</sup> $\pm$ 0.46	25.2 <sup>d</sup> $\pm$ 0.6	8.0 <sup>d</sup> $\pm$ 0.07	5.83 <sup>d</sup> $\pm$ 0.07	66 <sup>d</sup> $\pm$ 0.04
	50%	100 <sup>a</sup>	66.33 <sup>a</sup> $\pm$ 3.78	26.63 <sup>b</sup> $\pm$ 0.41	34.6 <sup>b</sup> $\pm$ 0.2	11.3 <sup>b</sup> $\pm$ 0.03	8.16 <sup>b</sup> $\pm$ 0.04	85 <sup>b</sup> $\pm$ 0.05
	75%	100 <sup>a</sup>	60.66 <sup>a</sup> $\pm$ 2.08	29.55 <sup>a</sup> $\pm$ 0.37	36.6 <sup>a</sup> $\pm$ 0.4	14.7 <sup>a</sup> $\pm$ 0.08	9.45 <sup>a</sup> $\pm$ 0.01	100 <sup>a</sup> $\pm$ 0.02
<b>Non Mycorrhizal seedlings</b>	25%	-	-	14.75 <sup>e</sup> $\pm$ 0.30	17.2 <sup>f</sup> $\pm$ 0.7	4.6 <sup>f</sup> $\pm$ 0.03	4.6 <sup>e</sup> $\pm$ 0.05	52 <sup>e</sup> $\pm$ 0.04
	50%	-	-	17.33 <sup>d</sup> $\pm$ 0.73	24.3 <sup>e</sup> $\pm$ 0.3	7.7 <sup>e</sup> $\pm$ 0.07	6.3 <sup>c</sup> $\pm$ 0.01	72 <sup>c</sup> $\pm$ 0.07
	75%	-	-	19.00 <sup>c</sup> $\pm$ 0.55	27. <sup>c</sup> $\pm$ 0.03	09.2 <sup>c</sup> $\pm$ 0.03	06.6 <sup>c</sup> $\pm$ 0.02	82 <sup>b</sup> $\pm$ 0.07

**Table 2 :** Effects of water deficit on mineral contents of nine month aged *Argania spinosa* seedlings subjected to a 3-month period of drought. Mean values ± SE in the same column followed by the same lower case letters are not significantly different at P ≤ 005 by Tukey HSD test.

	<b>Drought treatment</b>	<b>Total N%</b>	<b>P (mg .plant<sup>-1</sup>)</b>	<b>K (mg.plant<sup>-1</sup>)</b>	<b>Ca (mg.plant<sup>-1</sup>)</b>	<b>Mg (mg.plant<sup>-1</sup>)</b>	<b>Na (mg.plant<sup>-1</sup>)</b>
<b>Mycorrhizal Seedlings</b>	25%	51.23 <sup>c</sup> ±0.81	26.4 <sup>d</sup> ±003	9.24 <sup>c</sup> ±0.33	3.65 <sup>d</sup> ±0.10	2.64 <sup>c</sup> ±0.06	8.63 <sup>b</sup> ±0.48
	50%	71.7 <sup>b</sup> ±0.59	53.2 <sup>b</sup> ±0 15	9.68 <sup>b</sup> ±0.36	6.26 <sup>b</sup> ±025	3.61 <sup>b</sup> ±0.09	4.72 <sup>d</sup> ±0.68
	75%	103.1 <sup>a</sup> ±049	107.8 <sup>a</sup> ±064	11.69 <sup>a</sup> ±0.40	7.44 <sup>a</sup> ±015	7.37 <sup>a</sup> ±0.023	3.31 <sup>f</sup> ±0.68
<b>Non-Mycorrhizal seedlings</b>	25%	27.6 <sup>f</sup> ±0.40	12.5 <sup>f</sup> ±014	3.3 <sup>f</sup> ±0.13	1.62 <sup>f</sup> ±0.05	1.74 <sup>e</sup> ±0.06	14.41 <sup>a</sup> ±0.53
	50%	25.5 <sup>e</sup> ±0.19	15.7 <sup>e</sup> ±0.13	3.67 <sup>e</sup> ±032	2.46 <sup>e</sup> ±0.25	2.20 <sup>d</sup> ±0.17	6.50 <sup>c</sup> ±056
	75%	28.6 <sup>d</sup> ±0.06	41.6 <sup>c</sup> ±0.85	5.49 <sup>d</sup> ±0.80	3.48 <sup>c</sup> ±0.24	2.56 <sup>c</sup> ±0.07	5.19 <sup>e</sup> ±0.70

**Table 3:** Stomatal conductance (SC), and Membrane stability (MS) in non-inoculated (NM) and inoculated (MY) Argan seedling. Mean values  $\pm$  SE in the same column followed by the same lower case letters are not significantly different at  $P \leq 0.05$  by Tukey HSD test.

Water regime (% FC)	AMF Inoculation	SC (mmol.m <sup>2</sup> S <sup>-1</sup> )	MS (%)
75%	NM	160.04 <sup>c</sup> $\pm$ 0.42	84.4 <sup>a</sup> $\pm$ 0.22
	MY	204.28 <sup>a</sup> $\pm$ 0.45	86.67 <sup>a</sup> $\pm$ 0.66
50%	NM	140.24 <sup>e</sup> $\pm$ 0.82	74.8 <sup>b</sup> $\pm$ 0.36
	MY	176.44 <sup>b</sup> $\pm$ 0.28	86.03 <sup>a</sup> $\pm$ 0.36
25%	NM	112.72 <sup>f</sup> $\pm$ 0.14	66.1 <sup>c</sup> $\pm$ 0.42
	MY	152.12 <sup>d</sup> $\pm$ 0.62	76.3 <sup>b</sup> $\pm$ 0.18



**Table 4:** Leaf chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (Chl a + b) content in non-inoculated (NM) and inoculated (MY) *Argania spinosa* seedlings. Mean values  $\pm$  SE in the same column followed by the same lower case letters are not significantly different at  $P \leq 0.05$  by Tukey HSD test.

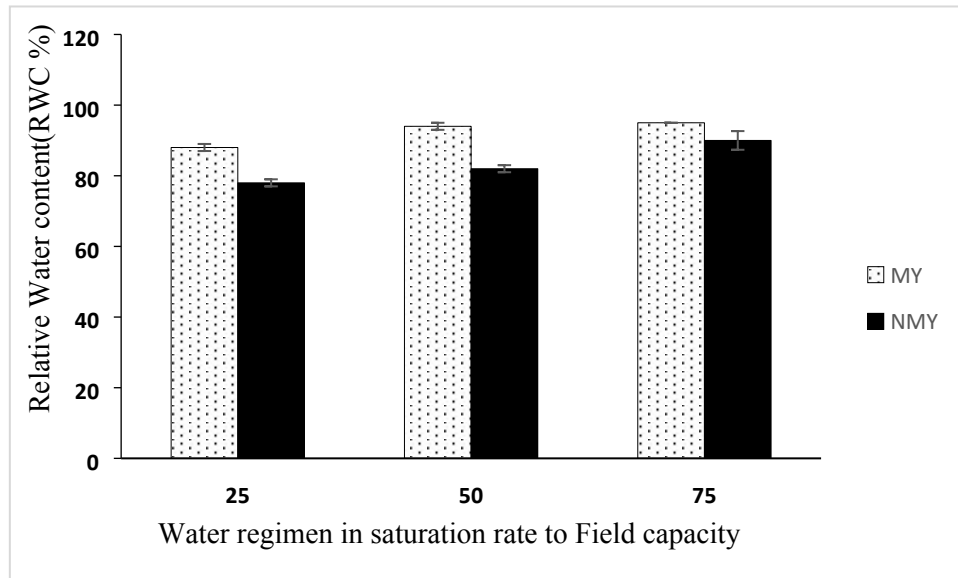
Water regimen (% FC)	AMF Inoculation	Chl a (mg.g FW <sup>-1</sup> )	Chl b (mg.g FW <sup>-1</sup> )	Chl a+b (mg.g FW <sup>-1</sup> )
75%	NM	0.26 <sup>b</sup> $\pm$ 0.07	1.11 <sup>a</sup> $\pm$ 0.25	1.37 <sup>b</sup> $\pm$ 0.20
	AMF	0.33 <sup>a</sup> $\pm$ 0.04	1.3 <sup>a</sup> $\pm$ 0.44	1.66 <sup>a</sup> $\pm$ 0.50
50%	NM	0.22 <sup>b</sup> $\pm$ 0.06	0.81 <sup>b</sup> $\pm$ 0.1	1.03 <sup>d</sup> $\pm$ 0.34
	AMF	0.30 <sup>a</sup> $\pm$ 0.03	0.96 <sup>b</sup> $\pm$ 0.04	1.26 <sup>b</sup> $\pm$ 0.61
25%	NM	0.12 <sup>c</sup> $\pm$ 0.06	0.43 <sup>c</sup> $\pm$ 0.03	0.55 <sup>e</sup> $\pm$ 0.02
	AMF	0.30 <sup>a</sup> $\pm$ 0.05	0.86 <sup>b</sup> $\pm$ 0.05	1.16 <sup>c</sup> $\pm$ 0.11

**Table 5:** Effects of water deficit on biochemical parameters in nine month aged *Argania spinosa* seedlings subjected to a 3-month period of drought. Mean values  $\pm$  SE in the same column followed by the same lower case letters are not significantly different at  $P \leq 0.05$  by TukeyHSD test.

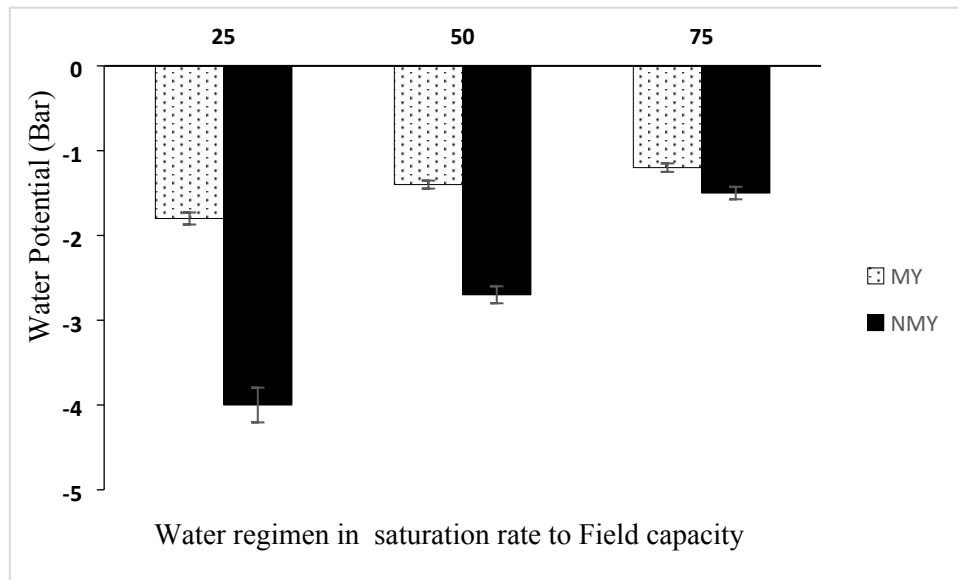
	<b>Drought stress treatments</b>	<b>Soluble sugars (mg.g fresh material<sup>-1</sup>)</b>	<b>Total protein (mg.g fresh material<sup>-1</sup>)</b>	<b>Proline (mg.g fresh material<sup>-1</sup>)</b>
<b>Inoculated seedlings</b>	25%	8.63 <sup>b</sup> $\pm$ 0.48	2.2 <sup>c</sup> $\pm$ 0.44	11.24 <sup>b</sup> $\pm$ 0.66
	50%	4.72 <sup>c</sup> $\pm$ 0.68	2.8 <sup>b</sup> $\pm$ 0.41	5.44 <sup>d</sup> $\pm$ 0.53
	75%	3.31 <sup>f</sup> $\pm$ 0.68	4.4 <sup>a</sup> $\pm$ 0.66	2.96 <sup>f</sup> $\pm$ 0.27
<b>Non –Inoculated seedlings</b>	25%	14.41 <sup>a</sup> $\pm$ 0.53	1.6 <sup>d</sup> $\pm$ 0.32	23.4 <sup>a</sup> $\pm$ 0.49
	50%	6.50 <sup>e</sup> $\pm$ 0.56	2.7 <sup>b</sup> $\pm$ 0.44	10.51 <sup>c</sup> $\pm$ 0.61
	75%	5.19 <sup>d</sup> $\pm$ 0.70	3.6 <sup>a</sup> $\pm$ 0.55	4.41 <sup>e</sup> $\pm$ 0.57

**Table 6:** Catalase (CAT), Superoxide dismutase (SOD) and Peroxidase (POD) activities in fresh leaves of nine month aged *Argania spinosa* seedlings subjected to a 3-month period of drought. Mean values  $\pm$  SE in the same column followed by the same lower case letters are not significantly different at  $P \leq 0.05$  by Tukey HSD test.

	<b>Water regimes (%FC)</b>	<b>CAT (mg.g fresh material<sup>-1</sup>)</b>	<b>SOD (mg.g fresh material<sup>-1</sup>)</b>	<b>POD (mg.g fresh material<sup>-1</sup>)</b>
<b>Mycorrhizal seedlings</b>	25%	3.48 <sup>d</sup> $\pm$ 0.54	7.70 <sup>c</sup> $\pm$ 0.59	26.39 <sup>c</sup> $\pm$ 0.32
	50%	2.35 <sup>e</sup> $\pm$ 0.55	6.55 <sup>d</sup> $\pm$ 0.61	26.39 <sup>c</sup> $\pm$ 0.44
	75%	1.87 <sup>f</sup> $\pm$ 0.22	3.27 <sup>e</sup> $\pm$ 0.48	15.38 <sup>e</sup> $\pm$ 0.52
<b>Non Mycorrhizal seedlings</b>	25%	9.31 <sup>a</sup> $\pm$ 0.75	21.19 <sup>a</sup> $\pm$ 0.78	69.92 <sup>a</sup> $\pm$ 0.54
	50%	8.26 <sup>b</sup> $\pm$ 0.60	14.64 <sup>b</sup> $\pm$ 0.33	60.46 <sup>b</sup> $\pm$ 0.62
	75%	4.54 <sup>c</sup> $\pm$ 0.95	3.47 <sup>e</sup> $\pm$ 0.59	20.42 <sup>d</sup> $\pm$ 0.68



**Figure 1:** Relative water content of shoots of nine month aged *Argania spinosa* seedlings subjected to a 3-month period of drought.



**Figure 2:** Water Potential(Bar) in leaves petiole level of nine month aged *Argania spinosa* seedlings subjected to a 3-month period of drought.