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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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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 an 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 plantating 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⁻¹) (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⁺), the calcium (Ca²⁺) the magnesium (Mg²⁺) and the sodium (Na⁺) 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 mm² 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 diH₂O 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 H₂O₂, 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⁺, Ca⁺⁺, Mg⁺⁺, Na⁺) 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⁺, Ca⁺⁺, Mg⁺⁺, Na⁺) 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 ²)
Mycorrhizal seedlings	25%	100 ^a	54.33 ^a \pm 1.53	17.80 ^d \pm 0.46	25.2 ^d \pm 0.6	8.0 ^d \pm 0.07	5.83 ^d \pm 0.07	66 ^d \pm 0.04
	50%	100 ^a	66.33 ^a \pm 3.78	26.63 ^b \pm 0.41	34.6 ^b \pm 0.2	11.3 ^b \pm 0.03	8.16 ^b \pm 0.04	85 ^b \pm 0.05
	75%	100 ^a	60.66 ^a \pm 2.08	29.55 ^a \pm 0.37	36.6 ^a \pm 0.4	14.7 ^a \pm 0.08	9.45 ^a \pm 0.01	100 ^a \pm 0.02
Non Mycorrhizal seedlings	25%	-	-	14.75 ^e \pm 0.30	17.2 ^f \pm 0.7	4.6 ^f \pm 0.03	4.6 ^e \pm 0.05	52 ^e \pm 0.04
	50%	-	-	17.33 ^d \pm 0.73	24.3 ^e \pm 0.3	7.7 ^e \pm 0.07	6.3 ^c \pm 0.01	72 ^c \pm 0.07
	75%	-	-	19.00 ^c \pm 0.55	27. ^c \pm 0.03	09.2 ^c \pm 0.03	06.6 ^c \pm 0.02	82 ^b \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 \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 treatment	Total N%	P (mg .plant⁻¹)	K (mg.plant⁻¹)	Ca (mg.plant⁻¹)	Mg (mg.plant⁻¹)	Na (mg.plant⁻¹)
Mycorrhizal Seedlings	25%	51.23 ^c \pm 0.81	26.4 ^d \pm 0.03	9.24 ^c \pm 0.33	3.65 ^d \pm 0.10	2.64 ^c \pm 0.06	8.63 ^b \pm 0.48
	50%	71.7 ^b \pm 0.59	53.2 ^b \pm 0.15	9.68 ^b \pm 0.36	6.26 ^b \pm 0.25	3.61 ^b \pm 0.09	4.72 ^d \pm 0.68
	75%	103.1 ^a \pm 0.49	107.8 ^a \pm 0.64	11.69 ^a \pm 0.40	7.44 ^a \pm 0.15	7.37 ^a \pm 0.023	3.31 ^f \pm 0.68
Non-Mycorrhizal seedlings	25%	27.6 ^f \pm 0.40	12.5 ^f \pm 0.14	3.3 ^f \pm 0.13	1.62 ^f \pm 0.05	1.74 ^e \pm 0.06	14.41 ^a \pm 0.53
	50%	25.5 ^e \pm 0.19	15.7 ^e \pm 0.13	3.67 ^e \pm 0.32	2.46 ^e \pm 0.25	2.20 ^d \pm 0.17	6.50 ^c \pm 0.56
	75%	28.6 ^d \pm 0.06	41.6 ^c \pm 0.85	5.49 ^d \pm 0.80	3.48 ^c \pm 0.24	2.56 ^c \pm 0.07	5.19 ^e \pm 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 ² S ⁻¹)	MS (%)
75%	NM	160.04 ^c \pm 0.42	84.4 ^a \pm 0.22
	MY	204.28 ^a \pm 0.45	86.67 ^a \pm 0.66
50%	NM	140.24 ^e \pm 0.82	74.8 ^b \pm 0.36
	MY	176.44 ^b \pm 0.28	86.03 ^a \pm 0.36
25%	NM	112.72 ^f \pm 0.14	66.1 ^c \pm 0.42
	MY	152.12 ^d \pm 0.62	76.3 ^b \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 ± SE in the same column followed by the same lower case letters are not significantly different at P ≤ 005 by Tukey HSD test.

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

	Drought stress treatments	Soluble sugars (mg.g fresh material⁻¹)	Total protein (mg.g fresh material⁻¹)	Proline (mg.g fresh material⁻¹)
Inoculated seedlings	25%	8.63 ^b \pm 0.48	2.2 ^c \pm 0.44	11.24 ^b \pm 0.66
	50%	4.72 ^c \pm 0.68	2.8 ^b \pm 0.41	5.44 ^d \pm 0.53
	75%	3.31 ^f \pm 0.68	4.4 ^a \pm 0.66	2.96 ^f \pm 0.27
Non –Inoculated seedlings	25%	14.41 ^a \pm 0.53	1.6 ^d \pm 0.32	23.4 ^a \pm 0.49
	50%	6.50 ^e \pm 0.56	2.7 ^b \pm 0.44	10.51 ^c \pm 0.61
	75%	5.19 ^d \pm 0.70	3.6 ^a \pm 0.55	4.41 ^e \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 ± SE in the same column followed by the same lower case letters are not significantly different at P ≤ 005 by Tukey HSD test.

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

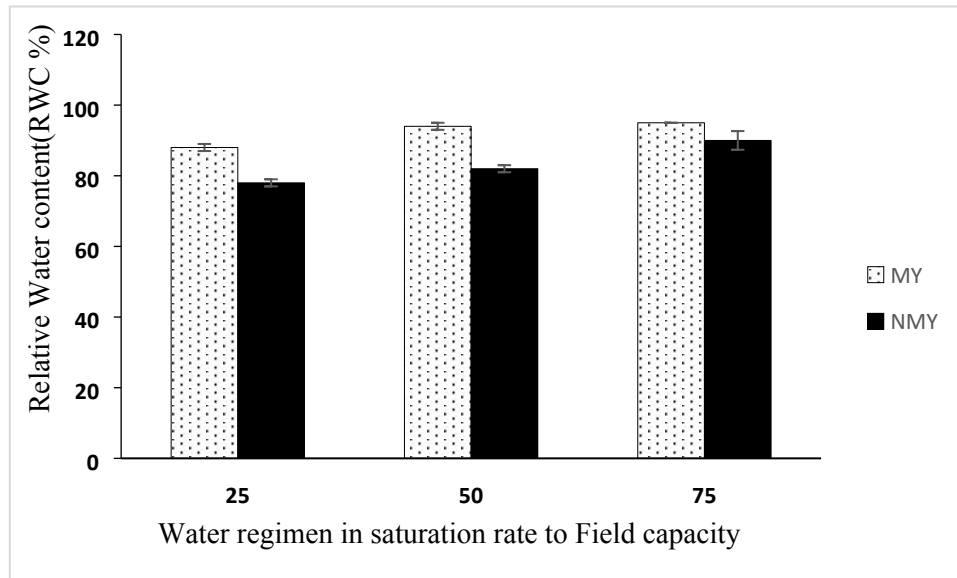


Figure 1: Relative water content of shoots of nine month aged *Argania spinosa* seedlings subjected to a 3-month period of drought.

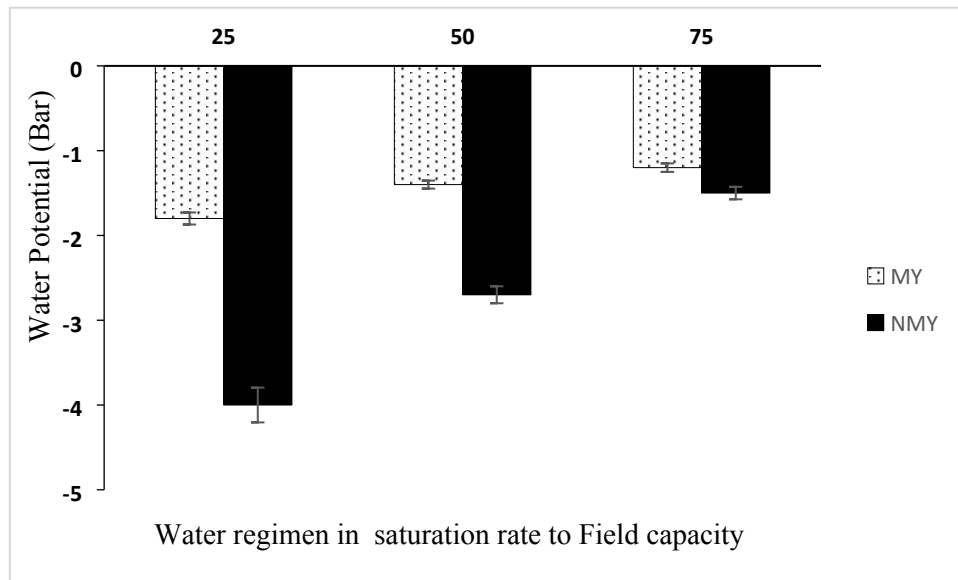


Figure 2: Water Potential(Bar) in leaves petiole level of nine month aged *Argania spinosa* seedlings subjected to a 3-month period of drought.