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Elmostapha Outamamat, Said El Mrabet, Hanane Dounas, Adnane Bargaz, Robin Duponnois, Lahcen Ouahmane

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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.

Outamamat Elmostapha[1], El Mrabet Said[2], Dounas Hanane[1], Bargaz Adnane[3], Duponnois Robin[4] , Ouahmane Lahcen[1*].

1- Laboratory of Microbial biotechnologies, Agrosciences and Environment, Faculty of sciences-Semlalia , Cadi Ayyad University, Marrakesh, Morocco.

2 - Regional Department of Forestry and Combating Desertification, Agadir, Morocco.

3- Agrobiosciences, Mohammed VI Polytechnic University, Benguerir, Morocco

4-LSTM, University of Montpellier, CIRAD, INRA, IRD, Montpellier SupAgro, Montpellier, France

[*]**Corresponding author**: *Laboratory of Microbial biotechnology, Agrosciences and Environment, Faculty of Sciences-Semlalia, Cadi Ayyad University, Marrakesh, 40000, Morocco.*

E-mail: l.ouahmane@uca.ac ma, Tel : 0021268729336. https://orcid.org/0000-0002-7378-549X.

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, peoxydase, 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.

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

1. Introduction

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

The Argan tree (*Argania spinosa* Skeels) is one of the most emblematic species of Moroccan forest covering approximately 900.000 ha (NFI 2005) mainly in the south west Morocco. This multipurpose tree is used for edible and cosmetic oil, firewood, timber, forage, and for cereal crops (Alados and Aich, 2008). Grazing activity and intensive agriculture under the arid climate occurring in a large part of Argan cultivated areas affect drastically the sustainability of the Argan ecosystem (Mcgregor et al., 2009). A huge degradation of the physico- chemical and biological properties of soil were recorded (Lybbert et al., 2010; El Mrabet et al., 2014). This degradation is usually manifested by a reduction in the diversity and the activity of rhizosphere microbes (Kennedy and Smith, 1995). The reduction or loss of the microbial activity, especially the one associated to AMF establishment in the rhizosphere soil can influence the growth and the nutritional status of plants and limit the success of plantations (Sylvia, 1990; Van Der Heijden et al., 1998; Kyriazopoulos et al., 2014).

Important findings showed that inoculation of plants with AMF not only facilitates establishment of plants (Herrera et al., 1993; Sally E. Smith, 2008, Alguacil et al., 2011,

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

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

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

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

2. Materials and Methods

2.1. Mycorrhizal fungi propagules and Inoculum preparation

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

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

2.2. Molecular identification of the mycorrhizal complex

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

spore mixture. DNA was extracted using FastDNA® SPIN kit (MP biomedicals Europe) according to the manufacturer's instructions. Guanidine thiocyanate is added to improve the extraction process. DNA extracts were purified by adding 20-30 mg polyvinyl polypyrrolidon (PVPP) to limit the presence of PCR inhibitors. Fungal DNA amplification was performed targetting18S rRNA gene that was amplified using the primers NS31 and AML2 (Lee et al., 2008; Simon et al., 1992). The PCR Products were then freeze-dried before being sent for sequencing according to the provider's instruction. Bioinformatic data processing was conducted by Frediric mahe (https://github.com/fredericmahe/stampa). Molecular operational taxonomic unit (OTU) representative sequences were then searched and, sequences received taxonomical assignments using the stampa pipeline.

2.3. Argan seeds germination

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

2.4. Inoculation and experimental design

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

2.5. Mycorrhizal parameters and Plant growth performance

2.5.1. Mycorrhizal parameters

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

2.5.2. Morpho-metric parameters

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

2.5.3. Mineral contents analysis

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

2.6. Plant Physiological and biochemical changes

2.6.1. Relative water content

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

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

2.6.2. Hydric potential

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

2.6.3. Stomatal conductance

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

2.6.4. Membrane stability

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

2.6.5. Total Chlorophyll

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

Chlorophyll a (µg/ml) = 11,93DO664 –1,93DO647

Chlorophyll b (µg/ml) = 20,36DO647 –5,5 DO664

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_2O 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

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

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

Peroxidase (POD) activity was measured by following the change in absorption at 470 nm due to guaiacol oxidation. The activity was assayed for 1 min in a reaction solution (3 mL final volume) containing 20 mM of guaiacol, 10 mM of H2O2, and 0.35 mL of an enzyme extract in a 100 mM potassium phosphate buffer (pH 6.8) (Polle et al., 1994), POD was analyzed according to (Barceló, 1998).

2.7. Statistical analysis

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

3. Results

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

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

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

3.2. Mycorrhizal colonization and plant growth parameters

3.2.1. Mycorrhization parameters

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

3.2.2. Morpho-metric parameters

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

3.3.3. Stomatal conductance

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

3.3.4. Membrane stability

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

3.3.5. Total Chlorophyll

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

3.3.6. Total Soluble Sugars

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

3.3.7. Total Protein content

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

3.3.8. Proline content

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

3.3.9. Oxidative enzyme activity

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

4. Discussion

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

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

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

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

5. Conclusion

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

Conflict of interest statement

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

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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 ± SE in the same column followed by the same lower case letters are not significantly different at P ≤ 005 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}±1.53	17.80^{d}±0.46	25.2^{d}±0.6	8.0^{d} ±0.07	5.83^{d} ± 0.07	66^{d} ±0.04
	50%	100^{a}	66.33^{a}±3.78	26.63^{b}±0.41	34.6^{b}±0.2	11.3^{b}±0.03	8.16^{b}±0.04	85^{b} ±0.05
	75%	100^{a}	60.66^{a}±2.08	29.55^{a}±0.37	36.6^{a}±0.4	14.7^{a}±008	9.45^{a}±0.01	100^{a}±0.02
Non Mycorrhizal seedlings	25%	-	-	14.75^{e} ±0.30	17.2^{f}±0.7	4.6^{f}±0.03	4.6^{e}±0.05	52^{e}±0.04
	50%	-	-	17.33^{d}±0.73	24.3^{e}±0.3	7.7^{e}±0.07	6.3^{c}±0.01	72^{c} ±0.07
	75%	-	-	$19.00\pm^{c}0.55$	$27.^{c}$±003	09.2^{c}±0.03	06.6^{c}±0.02	82^{b} ±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**.**

	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}±0.81	26.4 ^{d}±003	9.24 ^{c} ±0.33	3.65 ^{d}±0.10	2.64^{c} ±0.06	8.63 ^{b} ±0.48
	50%	71.7 ^{b}±0.59	53.2 ^{b}±0 15	9.68 ^{b} ±0.36	6.26 ^{b}±025	3.61^{b} ±0.09	4.72^{d} ±0.68
	75%	103.1 ^{a}±049	107.8 ^{a} ±064	11.69 ^{a} ±0.40	7.44 ^{a}±015	7.37^{a} ±0.023	3.31^{f} ±0.68
Non-Mycorrhizal seedlings	25%	27.6 ^{f}±0.40	12.5 ^{f}±014	3.3 ^{f} ±0.13	1.62 ^{f}±0.05	1.74 ^{e} ±0.06	14.41^{a} ±0.53
	50%	25.5 ^{e}±0.19	15.7 ^{e}±0.13	3.67 ^{e} ±032	2.46 ^{e}±0.25	2.20^{d} ±0.17	6.50^{c} ±056
	75%	28.6 ^{d}±0.06	41.6 ^{c}±0.85	5.49 ^{d} ±0.80	3.48 ^{c}±0.24	2.56^{c} ±0.07	5.19^{e} ±0.70

Table 3: Stomatal conductance (SC), and Membrane stability (MS) in non-inoculated (NM) and inoculated (MY) Argan seedling. 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 regime (% FC)	**AMF Inoculation**	**SC ($mmol.m^2S^{-1}$)**	**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	$0.26^{b} \pm 0.07$	$1.11^{a} \pm 0.25$	$1.37^{b} \pm 0.20$
	AMF	$0.33^{a} \pm 0.04$	$1.3^{a} \pm 0.44$	$1.66^{a} \pm 0.50$
50%	NM	$0.22^{b} \pm 0.06$	$0.81^{b} \pm 0.1$	$1.03^{d} \pm 034$
	AMF	$0.30^{a} \pm 0.03$	$0.96^{b} \pm 0.04$	$1.26^{b} \pm 0.61$
25%	NM	$0.12^{c} \pm 0.06$	$0.43^{c} \pm 0.03$	$0.55^{e} \pm 0.02$
	AMF	$0.30^{a} \pm 0.05$	$0.86^{b} \pm 0.05$	$1.16^{c} \pm 0.11$

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 ± SE in the same column followed by the same lower case letters are not significantly different at P ≤ 005 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} ±048	2.2^{c}±0.44	11.24^{b} ±0.66
	50%	4.72^{e} ±068	2.8^{b} ±0.41	5.44^{d} ±0.53
	75%	3.31^{f} ±068	4.4^{a} ±0.66	2.96^{f} ±0.27
Non –Inoculated seedlings	25%	14.41^{a} ±053	1.6^{d}±0.32	23.4^{a} 6±0.49
	50%	6.50^{c} ±056	2.7^{b} ±0.44	10.51^{c} ±0.61
	75%	5.19^{d} ±070	3.6^{a} ±0.55	4.41^{e} ±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

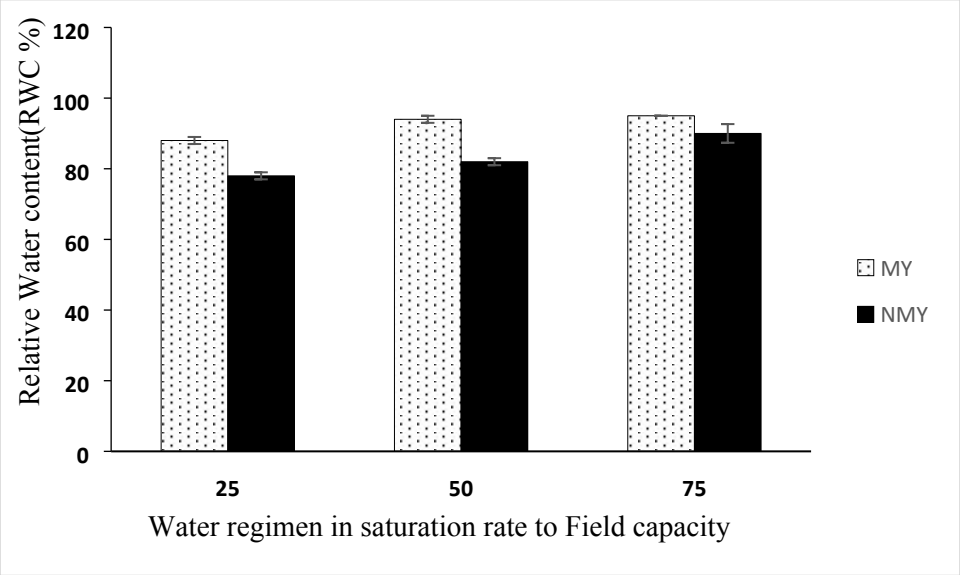

Figure 1: Relative water content of shoots of nine month aged *Argania spinosa* seedlings subjected to a 3-month period of drought**.**

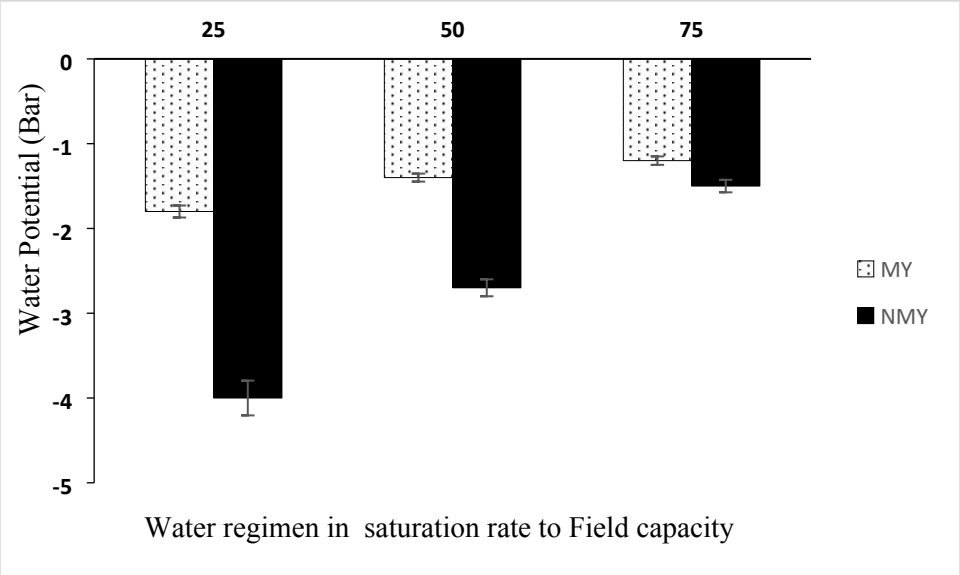

Figure 2: Water Potential(Bar) in leaves petiole level of nine month aged *Argania spinosa* seedlings subjected to a 3-month period of drought.