Université catholique de Louvain
Faculté des bioingénieurs
Earth and Life Institute
Applied Microbiology
Laboratory of Mycology

Impact of arbuscular mycorrhizal fungi on growth and physiology of argan tree and maize under water stress conditions

Thèse de doctorat présentée par

Ganoudi Matike

en vue de l'obtention du grade de Docteur en Sciences Agronomiques et Ingénierie Biologique

Promoteur:

Prof. Stéphane Declerck (UCLouvain, Belgium)

Membres du jury

Prof. Vanclooster Marnik	UCLouvain, Belgium
Prof. Muriel Quinet	UCLouvain, Belgium
Dr. Maryline Calonne-Salmon	UCLouvain, Belgium
Prof. Anissa Lounes-Hadj Sahraoui	Université du Littoral Côte d'Opale, France
Prof. Mohammed Ibriz	Université Ibn Tofail, Maroc

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Acknowledgments

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List of abbreviations

A	CO_2 Assimilation Rate
ABA	Abscisic Acid
AMF	Arbuscular Mycorrhizal Fungi
ANOVA	Analysis Of Variance
ANDZOA	Agence Nationale pour le Développement des Zones Oasiennes et de l'Arganier
ARES	Académie de Recherche et d'Enseignement Supérieur
BTC	Belgian Technical Cooperation
CESAMM	Center of Study on Arbuscular Mycorrhizae Monoxenics
CIPF	Centre Indépendant de Promotion Fourragère
DS	Drought Stress
DW	Dry Weight
E	Transpiration Rate
ERM	Extraradical Mycelium
FAO	Food and Agriculture Organization
GINCO	Glomeromycota *In vitro* Collection
gs	Stomatal Conductance
H_2O_2	Hydrogen Peroxide
Hoagland ^{lowPi}	Pi-impoverished Modified Hoagland Solution
IAV	Agronomic and Veterinary Institute
ICP- AES	Inductive Coupled Plasma-Atomic Emission Spectrometer
INRA	Institut National de la Recherche Agronomique
IRGA	Infrared Gaz Analyzer
IRM	Intraradical Mycelium
MDA	Malondialdehyde
MDP	Myclium Donor Plant
MS	Murashige and Skoog
MUCL	Mycothèque de l'UCLouvain
MW	Moderately Watered
NAA	1-Naphthaleneacetic Acid
ONSA	Office National de la Sécurité Alimentaire
PW	Poorly Watered
PPF	Photosynthetic Photon Flux
REML	Restricted Maximum Likelihood
ROS	Reactive Oxygen Species
RWCL	Leaf Relative Water Content
SA	Salicilic Acid
SAS	Statistical Analysis Software
SARDI	South Australian Research and Development Institute
SW	Saturated Weight
TSS	Total Soluble Sugars
TW	Turgid Weight
UNESCO	United Nations Educational, Scientific and Cultural Organization
UPOV	International Union for the Protection of New Varieties of Plants
USDA	United States Department of Agriculture
WS	Water Stressed
WR	Water Regime
WUEi	Instantaneous Water Use Efficiency
WW	Well Watered
ww	Wet Weight
RWC	Relative Water Content

Glossary

Accession: *A distinct and identifiable sample of a species collected in a given region, representing a population, variety, or genotype. It retains its genetic identity (origin, geographic location, ecotype, cultivars, etc.). For example, an argan accession collected in one region will be different from one collected in another, even if they are the same species* (FAO, 2010)

Arbuscular mycorrhizal fungi (AMF): *Fungi that belong to the phylum Glomeromycota, forming mutualistic symbioses with approximately 72% of vascular plant species* (Brundrett and Tedersoo, 2018). *These fungi evolved over 500 million years ago, during the Ordovician period, and are characterized by the formation of highly branched, tree-like structures called "arbuscules" within the root cortex cells of host plants* (Pirozynski and Dalpe, 1989).

Abiotic stress: *Is defined as the negative impact of abiotic factors on living organisms in each environment. Stresses include drought, salinity, low or high temperatures, and other environmental extremes. Abiotic stresses, particularly over-salinity and drought, are the major causes of crop loss worldwide. Abiotic stress can limit growth, development, and productivity, particularly in plants where it affects physiological and molecular processes. Strategies to mitigate abiotic stress often involve genetic, agronomic, and biotechnological approaches or inoculation of plants by symbiotic microorganisms* (Powell, 2006).

Biotization: *Metabolic response of plant material grown in vitro to (a) microbial inoculant(s), resulting in developmental and physiological changes that enhance biotic and abiotic stress resistance of the derived plant propagules. The establishment of artificial symbiotic associations could also be included in this category* (Nowak, 1998).

Cuttings: *The most common method of vegetative propagation of ornamental trees and shrubs and some herbaceous perennials. There are winter (hard) and summer (green) cuttings. Poplar, gooseberry, sea buckthorn, currant, tamarisk, willow, etc., are propagated by hardwood cuttings. The material for cuttings should be prepared in autumn, after the leaves have fallen and the sap has stopped flowing* (Herman et al., 2019).

Drought tolerance: *The ability of a plant to withstand low soil moisture for long periods of time without significant signs of disturbance to their physiological condition and viability. It is an important indicator in assessing the reclamation qualities of cultivars* (Herman et al., 2019).

Drought recovery (plant): *The ability of a plant to resume growth and increase yield after exposure to severe drought stress that causes a complete loss of turgor pressure and leaves* (Luo, 2010). *It has been proposed that drought recovery plays an important role in plant adaptation to drought and involves specific physiological mechanisms* (Chen et al., 2016).

Extraradical mycelium: *Hyphal network that develops in the rhizosphere where it absorbs inorganic nutrients that are transferred to the host plant via intraradical hyphae* (Bonfante and Genre, 2010).

Grafting: *Operation in which a vegetative part - a graft - is transferred to another plant- the rootstock - with which it grows, forming the whole organism* (Herman et al., 2019).

Hydroponic systems: *Include all systems that deliver the nutrients in a liquid form, with or without an aggregate medium to anchor the plant roots* (Hayden, 2006).

Intraradical mycelium: *Component of arbuscular mycorrhizal fungi composed of the hyphae that extend within the intercellular root space of the root epidermis and cortex, branching and anastomosing*

to form specific structures such as arbuscules, coils, and, in some species, vesicles and spores (Dalpé et al., 2005).

Micropropagation: *Practical rapid propagation of plant material to produce large numbers of progeny using tissue culture micropropagation: In vitro vegetative propagation of plants* (Funt et al., 2017).

Resistance of plants: *The ability of the plant to withstand the action of extreme environmental factors such as soil salinity, drought, and low or high temperatures, is largely determined by its morphological, physiological, and biochemical features. These features are developed and genetically fixed during the evolutionary process. The level of resistance to various factors often varies between species and varieties* (Herman et al., 2019)

Vegetative propagation: *The method of propagation by division of shrubs or individual organs or parts thereof. It is of great importance in ornamental horticulture because it is a way of passing on to the progeny, with certain variations or distortions, the decorative characteristics of the original forms, which are passed on by seed propagation, especially in the case of hybrids. There are the following types of vegetative propagation: by cuttings, layers, grafting (budding), and by tubers, bulbs, and rhizomes* (Herman et al., 2019).

Summary

Argania spinosa (L) Skeels is a tree endemic to Morocco. It is essential to the livelihoods of rural populations thanks to its oil production, and also plays a crucial ecological role by preventing soil erosion. However, it is under threat from human pressure and recent climate changes, with reduced rainfall, are further jeopardizing its survival and oil production. Maize is also an important crop in Morocco, with an area of 25 635 ha. It is the third most irrigated crop after alfalfa (*Medicago sativa L.*) and berseem (*Trifolium alexandrinum L.*) in the country. However, maize often suffers greater grain yield losses in the event of water stress and is more susceptible to drought.

Both plants form symbiotic associations with arbuscular mycorrhizal fungi (AMF). These obligate root symbionts play an important role in improving plant mineral nutrition, water uptake, and above all, drought tolerance. They are therefore seen as an innovative strategy to help plants better resist and recover from drought.

Using argan seeds, we first succeeded in the *in vitro* association of three accessions (City Hanchan, Mejji and Tidzi) with *Rhizophagus irregularis* MUCL 41833. The roots of the three accessions were well colonized after 11 weeks of growth in the extraradical mycelial network of the fungus. Five months after transfer to the greenhouse, the biomass of the mycorrhizal plants was significantly higher than that of non-mycorrhizal plants. These results suggest that the *in vitro* association of argan plants with *R. irregularis* is a robust method for producing plants with stronger growth after their transfer to the hardening phase.

This material was then tested for root colonization by *R. irregularis*, biomass, mineral nutrient content, physiological, and biochemical parameters under two water regimes: well-watered (WW) (100% of field capacity (FC)) and water stressed (WS) (15% FC). Root colonization by the AMF improved the performance of argan trees under water stress for all three accessions, but with different responses depending on the accession, with Tidji and Mejii responding better than City Hanchan.

Using maize seeds, we tested the impact of AMF on plant growth and physiological parameters in a semi-hydroponic cultivation system under three water regimes: well-watered (WW, 100% FC), moderately watered (MW, 50% FC) and poorly watered (PW, 25% FC).

After the stress period, the plants were re-watered to FC. Whatever the water regime applied before recovery, AMF-colonized plants used inorganic phosphorus (Pi) more efficiently during recovery than the non-mycorrhizal plants. They had lower stomatal conductance and transpiration rates but higher CO_2 assimilation and instantaneous water use efficiency (WUEi)

than the non-mycorrhizal plants. These results suggest that AMF can improve the drought tolerance of maize by increasing Pi uptake and water use efficiency during recovery from drought stress.

In summary, our results show that early colonization of plants (i.e., argan or maize) by AMF is a promising strategy to help these plants better resist and recover from drought stress. These results must now be demonstrated in the field as a promising strategy in drought stress situations.

Author's Contribution

The work presented here was strictly realized during the time course of my PhD. The sections**: Introduction, Outline of the thesis, State of the Art, Material and Methods, General Discussion, Conclusion and Perspectives** were written by myself and have not been published.

Chapter 1 is a research paper published in Symbiosis (2021). My contribution to this chapter is estimated at 90%. All the experiments were carried out by myself, with the exception of the evaluation of root colonization, which was carried out by François Ferrais. The manuscript was written by myself under the supervision of Pr. Stéphane Declerck and Dr. Maryline Calonne-Salmon. All authors have read and approved the manuscript.

Chapter 2 is a manuscript in preparation. My contribution to this chapter is estimated at 90%. All experiments were carried out by myself and Nouhaila Manan (a master's student at the Mohammed V University, Faculty of Sciences, Rabat, supervised by myself). Data collection and analysis were done by myself, Nouhaila Manan, and Soumia El Malahi. The manuscript was written by myself under the supervision of Pr. Stéphane Declerck and Dr. Maryline Calonne-Salmon. All authors have read and approved the manuscript.

Chapter 3 is a research paper published in Frontiers in Plant Science (2019). In this paper, we had an equal contribution, myself and Olivia Le Pioufle, another PhD student from the group of Pr. Declerck. The experiments and data collection and analysis were carried out by myself, Olivia Le Pioufle and Fatma Ben Dhaou (a master's student from Ecole Polytechnique de Sousse, Tunisia, supervised by me). The manuscript was written under the supervision of Prof. Stéphane Declerck and Dr. Maryline Calonne-Salmon. All authors have read and approved the manuscript.

Introduction

Argan (*Argania spinosa* (L.) Skeels) is a tree species endemic to Morocco. It is the only member of the family Sapotaceae and one of the most important forest species (Achour et al., 2015), with significant socio-economic potential for the arid and semi-arid regions of the country, particularly in the areas of Souss-Massa and Essaouira (Alados and El Aich 2008). Its oil is highly prized for its nutritional and cosmetic properties (Mechqoq et al., 2021, El Oumari et al., 2022), providing an important source of income for local communities. In addition, its wood provides excellent charcoal and its leaves, fruit pulp, and oilcake (oil extraction residue) are very attractive and provide excellent fodder for livestock (M'Hirit et al., 1998; El Aich et al., 2007; Sinsin et al., 2020). The tree also plays an important role in carbon storage, helping to reduce the amount of carbon dioxide in the atmosphere (Bayssi et al., 2024).

Despite its importance, the argan tree faces a number of challenges that threaten its cultivation. Among these, the climate change with more frequent and intense drought periods as well as the anthropogenic pressure on land are the most significant, reducing the area of cultivation by 50 % in the last decades (Abourouh, 2007; De Waroux and Lambin, 2012). To tackle this problematic, the conservation of the argan tree in its ecosystem, as well as the restoration of degraded areas, have become a national priority.

Propagation via different methods have been assayed but often showed repeated failures, generally attributed to the poor quality of the plants produced, their weak and poorly branched root systems and the low resistance of young plants to environmental stress during transplanting (Bouselemen et al., 2001; Berrichi et al., 2007; Justamante et al., 2017; Aït Hammou et al., 2018). Therefore, research focused on optimizing rooting, and acclimatization is urgently needed.

Maize is also an important crop in Morocco, widely cultivated for its dual function as a staple food for human consumption and an important feed for livestock. However, its production faces significant challenges due to water penury, as Morocco is characterized by arid and semi-arid climates with irregular and limited rainfall. Water stress is the main factor limiting maize yield, as it directly affects physiological processes such as photosynthesis, transpiration, and biomass accumulation (Chen et al., 2016). In addition, the intensification of drought events exacerbated by climate change can lead to yield losses of 15 and 20% per year under severe water deficit conditions (McMillen et al., 2022).

Drought has become a major challenge for agriculture as well as forests due to the accelerated effects of climate change. Water stress is one of the most important factors limiting plant growth and reducing agricultural productivity and yields worldwide especially in arid and semi-arid regions (Wu and Zou, 2017). Moreover, results from various regions globally indicate that no forest type or climate zone is immune to climate change, even in areas typically not considered water- limited (Laftouhi et al., 2022). In Morocco, droughts have become more extreme, longer, and more frequent and the country has been classified as highly vulnerable (Cherif et al., 2017). Droughts, rising average temperatures, heat waves, and changes in rainfall patterns continue to affect crops and forests. For instance, in Morocco, it has been predicted that warming combined with a slight decrease or increase in rainfall will lead to a decline in maize yields (Cakir, 2004). In addition, the region where argan trees grow is prone to arid climatic conditions and the additional decrease in rainfall has a direct impact on the health of the argan trees, leading to a decrease in their productivity and an overall weakening of the trees.

The intensification of drought highlights the urgent need for effective management strategies to mitigate its adverse effects and ensure increased crop productivity, food security and forest resilience. Among these strategies are the use of specific microorganisms such as arbuscular mycorrhizal fungi (AMF). These obligate root symbionts play an important role in plant nutrition and resistance to biotic stresses as well as abiotic stresses such as drought (Diagne et., 2020). The *in vitro* co-culture of plants (e.g., banana - Koffi and Declerck 2015; rubber - Sosa-Rodriguez et al. 2013) with AMF improves their survival, maintenance of a functional root system, and rapid recovery after transfer to hardening conditions. AMF have been shown to alleviate drought stress in a wide range of plant species. For example, they have been reported to maintain higher water potential, stomatal conductance, and relative water content in clover (Meddich et al., 2000), date palm (Baslam et al., 2014), plum (Razouk and Kajji, 2015), and carob (Essahibi et al., 2018). Increased biomass and productivity have also been reported in crops such as rubber (Tienebo et al., 2019), olive (Fouad et al., 2014), and date palm (Benhiba et al., 2015) under drought conditions. In addition, AMF can enhance plant P nutrition by accessing distant inorganic P (Pi) locations via their extraradical hyphae (ERM - Smith and Read, 2008), and it has been shown that this enhanced Pi nutrition can maintain optimal growth and water relations and therefore increase plant resistance to drought (Augé, 2001; Balestrini et al., 2020, Keller-Pearson et al., 2023).

Until today, no study has reported the *in vitro* mycorrhization of argan trees and the resistance of this plant to drought stress. Therefore, *in vitro* mycorrhization of argan trees with AMF could

provide a novel strategy to improve mass production of this plant species and accelerate its transplantation in the field, in addition to understanding the role of these root symbionts in argan adaptation to drought. A similar observation can be made for maize, although in this case, the plants are not produced *in vitro*, but can potentially benefit from their association with AMF.
Although the role of AMF in enhancing drought resistance is well documented, their contribution to stress recovery is less well understood, particularly in maize and argan. Recovery refers to the ability of a plant to resume physiological functions and growth after a period of water deficit. AMF may play a crucial role in this process by facilitating faster water uptake, improving nutrient (especially P) assimilation, and maintaining cellular integrity during rehydration. In addition, AMF may modulate stomatal behavior and metabolic adjustments during rehydration. Investigation of the mechanisms underlying AMF-mediated recovery could provide valuable insights into enhancing plant resilience in arid and semi-arid environments where drought events are intermittent.

The thesis is divided into three main objectives,

- ⇒ The first objective aims to investigate the *in vitro* association between the argan tree and *Rhizophagus irregularis* MUCL 41833 and to assess the impact of AMF on growth parameters during the post-*vitro* growth in the greenhouse.

- ⇒ The second objective aims to evaluate the influence of the AMF *Rhizophagus irregularis* MUCL 41833 on morphological, physiological, and biochemical factors associated with water stress tolerance in argan tree

- ⇒ The third objective aims to explore if the adaptive responses of argan tree to water stress in the presence of AMF can also be applied to maize.

The thesis was developed in three research chapters, which are summarized in **Figure 1**.

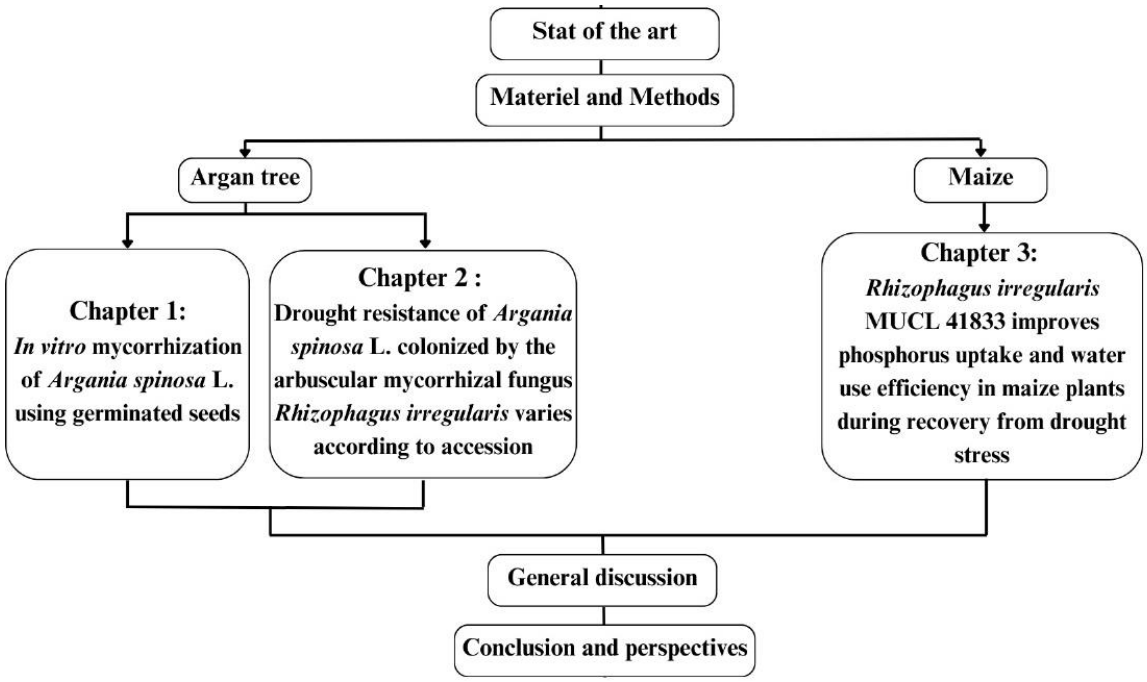


Figure 1. Outline of the thesis

In the **State of the Art section**, we first focused our attention on the argan tree, its taxonomy and ecological requirements. We reviewed its importance for the economy and environment of Morocco and the challenges posed by its production via several cultivation methods with their advantages and limitations. We also summarized what is currently known on their association with AMF. We then focused our attention on maize, its taxonomy and ecology. We reviewed its economic importance for Morocco and vulnerability to drought stress. We also focused on its symbiotic association with AMF and the role of these microorganisms in enhancing plants' tolerance to drought.

In the **Materials and Methods section**, we described the biological materials used and provided detailed explanations of the main methods and techniques applied in the different experiments of the thesis.

In Chapter 1, we developed an *in vitro* cultivation system to associate argan plants to AMF and evaluated the effects of this association on the post-*vitro* growth of the plants. We considered three argan accessions (TD, MJ, and CH) and the AM fungus ***R. irregularis*** MUCL 41833. We adapted the mycelium donor plant (MDP) *in vitro* culture system developed by Voets et al. (2009) to colonize surface-disinfected seeds of argan. The different structures of colonization were observed in the roots of the accessions. Roots of plants that survived transfer from *in vitro* to greenhouse conditions remained heavily colonized after five months, and a significant increase in growth and chlorophyll content of mycorrhizal plants was observed as compared to the non-mycorrhized controls.

The results of chapter 1 are published in Symbiosis (2021) 85, 57-68.

In Chapter 2, we investigated the role of *R. irregularis* MUCL 41833 on the growth and physiology of argan accessions (TD, MJ CH) under two water regimes: well-watered (WW) (100% of field capacity) and water stressed (WS) (15% of FC). Measurements included growth parameters, mineral nutrient content, physiological and biochemical parameters. Under WS conditions, the AMF-colonized plants had a significantly higher number of leaves, shoot and root length, shoot and root biomass in CH and MJ accessions, and leaf number, shoot length, shoot, and root biomass in TD accession. AMF also increased phosphorus and potassium in the accessions. Physiologically, AMF improved chlorophyll content and stomatal conductance under both water regimes. AMF also alleviated oxidative and osmotic stress by stabilizing total soluble sugars and reducing MDA and H_2O_2 levels, particularly in MJ and TD.

The results of chapter 2 are accepted in Frontiers in Plant Science.

In Chapter 3, we used a semi-hydroponic cultivation system to investigate the effect of *R. irregularis* MUCL 41833 on plant Pi uptake and leaf gas exchange parameters during drought recovery of maize plants. Maize plants were associated or not with the AMF and grown for three weeks under three different water regimes well-watered (WW) (100% of FC), moderately watered (MW) (50% of FC), and poorly watered (PW) (25% FC): a recovery period was induced by circulating a Hoagland solution for 42 h. The dynamics of Pi uptake in the plant roots was assessed, and physiological parameters were measured. During recovery, plants associated with AMF used Pi more efficiently than their non-mycorrhizal counterparts, regardless of the water regime (WR) applied before recovery. Their stomatal conductance and transpiration were lower than those of non-mycorrhizal plants, but they assimilated CO_2 and had higher instantaneous water use efficiency (WUEi).

The results of chapter 3 are published in in Frontiers in Plant Science (2019) Vol 10 Article 897.

In the General Discussion section, we summarized and discussed the main results obtained in the three different chapters of the thesis.

In the Conclusions section, we provided an overview of the results and the limitations of the present study, while in the **Perspectives section** we proposed hypotheses and strategies for improving the robustness of argan rooting and increasing the drought tolerance of maize and argan plants.

State of the Art

Importance of the species: Argan tree and Maize

Argan and maize are key crops in Morocco's national agricultural strategy, including the Green Morocco Plan “Plan Maroc Vert” (PMV) and the current Green Generation (GG) initiative. Their ecological and agronomic importance justifies their inclusion in this study. Argan (*Argania spinosa*) has exceptional ecological, socio-economic, and cultural value, particularly in the production of argan oil, which is recognized worldwide for its cosmetic and medicinal properties (Gebrai et al., 2025). However, pressures related to climate and land-use changes threaten its conservation, making research into its sustainable management and resilience particularly urgent. Maize (*Zea mays*), on the other hand, is one of Morocco's most important food crops, contributing directly to food security and serving as a major source of animal feed (Achli et al., 2025). Its production is a national priority, particularly within the framework of the Green Morocco Plan, which aims to improve food self-sufficiency of the country and crop resilience to environmental stress. Together, these crops represent the two pillars of Moroccan agriculture: the argan tree symbolizes long-term ecological sustainability and cultural heritage, while maize is essential for national food production and rural economies. Studying the association of these two crops with AMF allows us to investigate ways to enhance their growth and improve their resilience under water stress.

I. *Argania spinosa* (L) Skeels

1. Argan reserve.

The argan tree (*Argania spinosa* (L) Skeels), formerly known as *Sideroxylon spinosum* (Mechqoq et al., 2021), is a species endemic to Morocco. It is one of the most important forest species in this country, along with holm oak and Cedar, forming a unique landscape of hills and steep slopes, designed a biosphere reserve by UNESCO in 1998 (Guillaume et al., 2019). They form vast natural areas known as argan groves, estimated to cover more than 871,000 ha for Moukrim et al. (2018) and 800,000 ha for Msanda et al. (2021). These figures are probably overestimated and outdated according to Benabdellah and Ibnezzyn (2018), who estimate the area occupied by the argan tree at around 550,000 ha. This decrease in the area is attributed to the pressure of human activity and climate change.

2. Geographical distribution

The Moroccan argan forest is mainly located in central and western Morocco, along the Atlantic coast and in the foothills of the Atlas Mountains, in the provinces of Essaouira, Agadir Ida Outanan, Taroudant, Chtouka Ait Baha, and Tiznit **(Figure 2**). This area is known as the Argan Biosphere Reserve (ABR). The ABR corresponds to the southern slope of the High Atlas Mountains and the coastal lowlands, with an average altitude of 200 m (Santoro et al., 2023). The dispersal of seeds by animal and human vectors has given rise to two distinct lines of argan trees: one located southeast of Rabat (Grou Valley), the other in northwest Morocco (Beni-Snassen, Oujda) (Bellefontaine et al., 2010). The great genetic variability of the species is reflected in the great phenotypic variability observed, which enables it to survive in extremely difficult ecological conditions, particularly in terms of soil aridity (Ferradous et al., 1995).

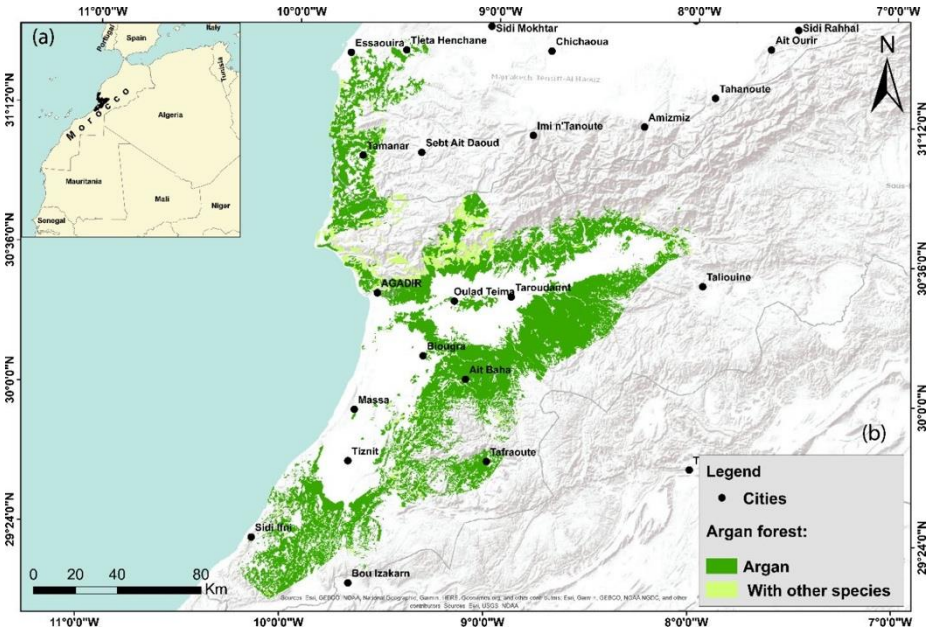


Figure 2. Spatial distribution of the argan forest in Morocco (Santoro et al., 2023)

3. Taxonomy and botanical description

According to the phylogenetic classification (APG IV, 2016), *A. spinosa* belongs to the tropical family Sapotaceae (**Figure 3**). It is the only representative of this family in North Africa and is the only species of the monospecific genus *Argania* (Achour et al., 2015). This unique and remarkable species is a multipurpose forest tree (forest, fruit, fodder) of great importance to Morocco in terms of biology, ecology, and biodiversity, as well as in economic and social terms. There are two types of argan trees, one weeping and the other erect, suggesting that there are two variants or biological races within the species (Buemor et., 2021).

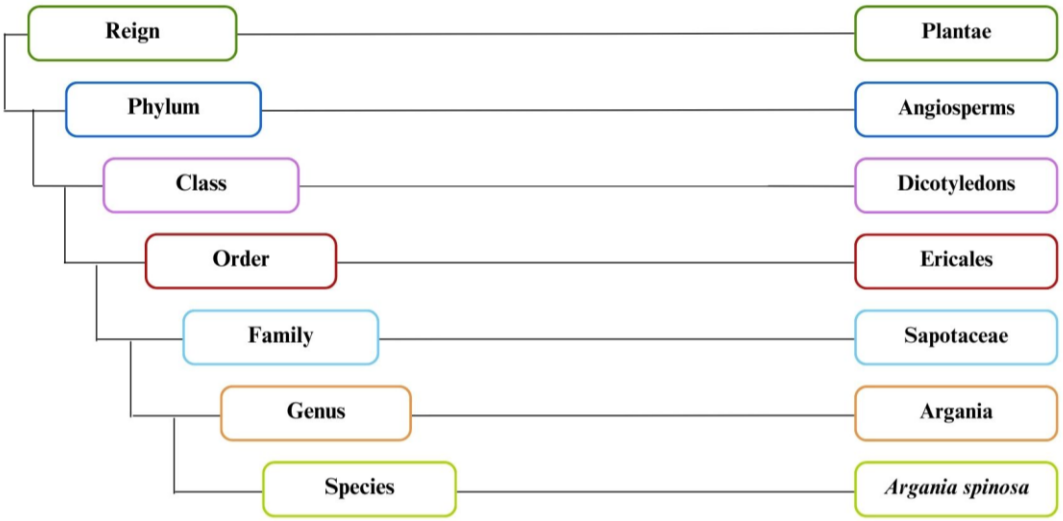


Figure 3. Classification of the argan tree according to (Radi, 2003)

The argan tree resembles an olive tree. Its height varies between 8 and 10 m (Naima et al., 2010) and can reach a height of 11 m under certain ecological conditions (**Figure 4a**). Its crown is broad and dense, with rounded edges. According to Bani-Ameur (2004) and Berka et al. (2019), the trunk is short and robust, often formed by several intertwined stems that originate from the same base or from closely spaced shoots.

Argan leaves come out in Spring, mainly between mid-March and mid-April. They are evergreen, alternate, dark green on top and light green underneath, and are between 1.5 and 5 cm in length (Zahidi et al., 2013). The leaves are glabrous, with a pale midrib and fine branched lateral veins. The argan tree has two types of leaves: simple leaves on young shoots or branches with indeterminate growth and grouped leaves on older, woody branches (**Figure 4b**)

Argan flowers are hermaphroditic, pentamerous, complete, and rather inconspicuous. The greenish, then yellowish, bell-shaped corolla with five fused petals has five stamens alternating with five staminodes with short filaments, inserted at the base of the corolla, usually on two whorls (**Figure 4b**). The style is short and conical. The ovary is superior and has two to three cells, each containing an ovule. Flowering takes place in May-June, giving rise to fruits that ripen around September (Bani-Aameur, 2000; Benlahbil, 2003).

The fruit of the argan tree is a berry with a wide variety of shapes: round oval, fusiform, apical oval, oval drop-shaped, rounded, and spherical (**Figure 4c**). Its size varies from 1 to 5 cm. The fruit is green to pale yellow in color, turning yellow or red when ripe (Bani-Aameur et al., 1999). It consists of an extremely hard central core, which accounts for about 25% of the weight of the fresh fruit, and a soft pericarp (Berka et al., 2019). Inside the hard shell of the argan nut

are usually one to three almond-shaped seeds. These seeds are used in the production of argan oil (**Figure 5**)

The argan tree has a short and tortuous trunk with rough and cracked bark and thorny branches, hence the species name spinosa (**Figure 4d**). The taproot system has a dense network of surface roots that have a strong ability to regenerate themseves after every rainy season (Benismail et al., 2002). The roots of the argan tree penetrate very deeply (up to 30 m) into the soil, allowing the plant to withstand prolonged drought periods and thrive in poor soils (Mokhtari, 2002; Kenny, 2007).

Figure 4. **(a)** Argan tree, **(b)** Leaves and flowers, **(c)** Fruits **(d)** Short and tortuous trunk.

Figure 5. Dried fruit of the argan tree to obtain seeds. Seeds broken to recover the kernels.

4. Ecological requirement

The argan tree is a thermoxerophilous species that can grow under semi-arid and desertic conditions (**Figure 6**). Its ability to defoliate makes it resistant to high temperatures and tolerant to prolonged droughts (Peltier, 1983). According to Msanda et al. (2005), the tree can survive temperatures of up to 50°C with a minimum temperature of 3°C and is satisfied with rainfall of between 120 and 300 mm per year. This explains its absence from the High Atlas, where the heavy rainfall and colder conditions do not provide an ideal environment for its growth. It grows on all types of soil, including saline soils (Nouaim et al., 1991). It is found on shale, limestone, and alluvial soils. It also appears to tolerate a range of pH between 4.6 and 7.5 (Nouaim et al., 1991).

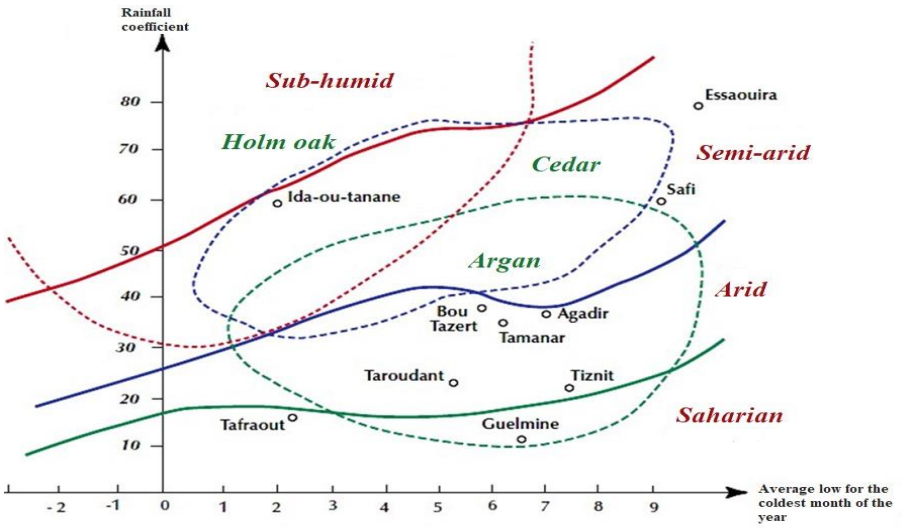


Figure 6. The bioclimatic region of the argan tree on the pluviothermal climate diagram of Emberger (Msanda et al., 2005).

5. *Argania spinosa*: a tree with multifaceted functions

The argan tree is a fruit, fodder, and forest tree (Prendergast, 1992). Every part of the tree can be used to earn money or sustain the farmers' livelihood. The wood of the tree is used as firewood, the leaves and fruits are used to feed goats and camels, and the oil extracted from the kernel is used for human consumption, traditional and modern medicine, and cosmetics. These characteristics allow this tree to play important socio-economic, ecologic, and nutritional roles (Sinsin et al., 2020).

5.1. Socio-economic values

The population of the regions where the argan tree grows is about 3 million, 2.2 millions of whom live in rural areas and depend directly on the exploitation of the argan forest (Laaribya et al., 2017). The exploitation of the argan tree products provide more than 20 million working days, including 7.5 million working days for women, just to extract the argan oil (Charrouf, 2007). The creation of women's cooperatives for the extraction and production of argan oil (**Figures 7a and 7b**) aims to reduce rural poverty. These cooperatives have contributed to local economic development, women's empowerment, and environmental protection (Msanda et al., 2021). Argan oil is now considered to be the most expensive food oil in the world, and even when it is sold as a cosmetic product (**Figure 7c**), its price remains astronomical (Romagny and Guyon, 2009). The export demand for oil for food, cosmetic, and medicinal purposes continues to grow. Argan oil exports recorded an annual growth rate of 12% between 2008 and 2020. Morocco exports argan oil to more than 100 countries with the main importing countries being

France (37%), United states (16%), Germany (13%) and South Korea (8%) (https://www.agrimaroc.ma/huile-argan-maroc-arganier).

Figure 7. **(a)** Manuel crushing of seeds to obtain the nuts, **(b)** Pressing of the argan nuts with a traditional mill which allows to obtain a paste. The obtained paste is kneaded, added with warm water, and then pressed to extract the argan oil. **(c)** Various cosmetic products based on argan oil.

5.2. Traditional uses, chemical composition, and biological activities

Argan oil has been used in human nutrition for centuries (Figure 8). The local population uses argan oil to prepare several original dishes, such as amlou, a spread made from argan oil, ground almonds, and honey. Amlou is often served on special occasions or at receptions. There is also labsis, a snack served on religious occasions or at the end of argan oil extraction process, and tagoulla, a traditional meal prepared with barley and argan oil (Barkaoui et al., 2022).

The chemical analysis of argan oil (Figure 8) shows that it is mainly composed of triglycerides, polyphenols, and saponins. It is also rich in polyunsaturated fatty acids such as oleic acid and linoleic acid (Mechqoq et al., 2021; Barkaoui et al., 2022). The antioxidant activity of argan seed oil and pulp has been reported by El Monfalouti et al. (2012. Recently, Azizi et al. (2021) described the phenolic content and antioxidant activity of argan flowers and leaves (**Figure 8**). Argan oil is also recognized for its cosmetic properties. In fact, it is used externally to moisturize the skin and to treat various skin problems such as burns, scars, and marks, as well as adolescent acne. Dried argan kernels (seeds) are used externally in powder to treat eczema, sprains, wounds, skin burns, and for hair care (Mechqoq et al., 2021).

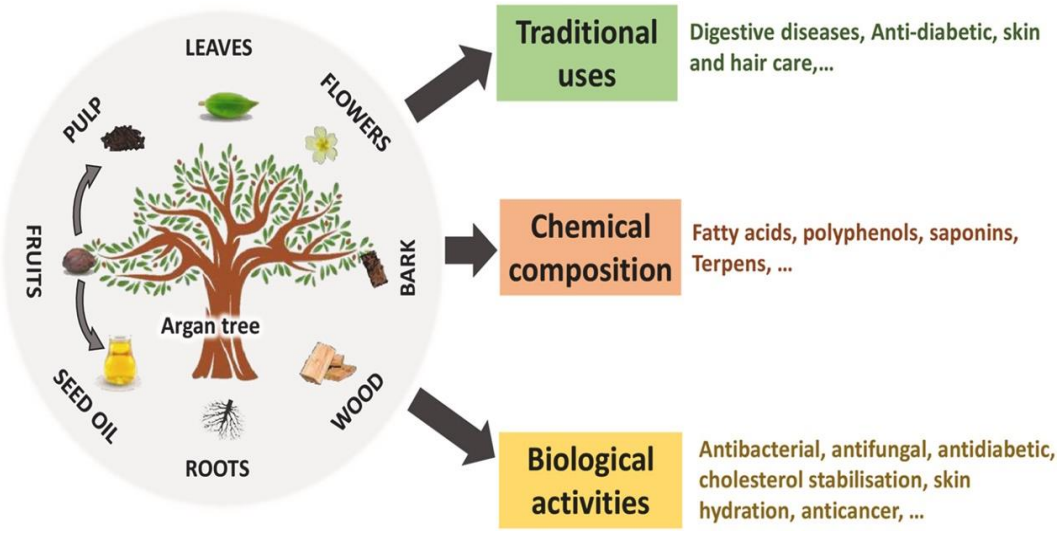


Figure 8. Traditional use, chemical composition, and biological activities of each part of the argan tree (Mechqoq et al., 2021)

5.3 Ecological values

Argan tree is essential for preserving the soils on which it grows. It slows down the desertification that is increasingly threatening southern Morocco and helps prevent soil erosion caused by strong winds or heavy rains by fixing the soil in place (Charrouf, 2007). It is considered a "green belt" against desertification in the far south. Under its shade live fauna and flora whose presence is crucial to the ecological balance of the region. The tree also helps regulate the local microclimate by providing shade (Karmaoui, 2019). Finally, it also plays an important role in carbon storage, helping to reduce the amount of carbon dioxide in the atmosphere (Bayssi et al., 2024).

6. Constraints on argan tree development

As we have just seen, the argan tree has great ecological, cultural, and economic value, and has historically supported a significant proportion of the Moroccan population. Despite its many benefits, the argan tree is in decline in terms of area and density (Bellefontaine et al., 2010). In less than 50 years, the average density of argan trees has decreased from 100 trees per hectare to 30 trees per hectare (Abourouh, 2007). A study carried out by De Waroux and Lambin (2012) in the Taroudante region between 1970 et 2007 showed that the average density of trees has decreased from 27.4 to 15.2 trees per hectare (**Figure 9**). This decline is due to the cumulative effects of overgrazing (**Figures 10a, 10b**), as almost all parts of the tree as well as young plants are grazed by goats (Cordier and Genin, 2008), and to anthropogenic activities, including those

related to urbanization (**Figure 10c**), illegal collection of firewoods (**Figure 10d**), and extensive agricultural development (Cordier and Genin, 2008; De Waroux and Lambin, 2012)

In addition to the above challenges, propagating the argan tree remains a complex task. Many researchers have explored both traditional propagation methods and advanced tissue culture techniques to improve its reproduction. Despite these efforts, significant obstacles remain in almost all approaches (Koufan et al., 2020; Mazri et al., 2022).

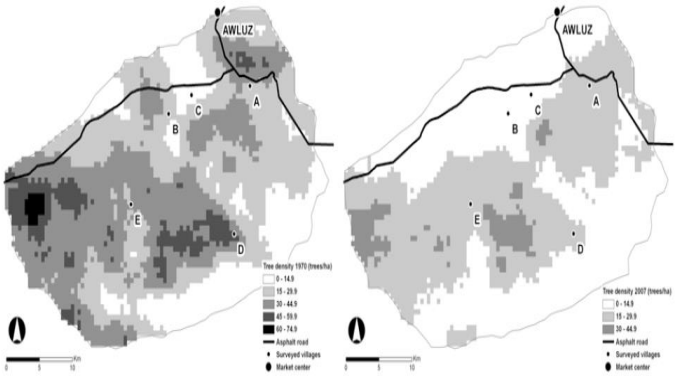


Figure 9. Density changes in the Arganeraie. left: density in 1970, right: density in 2007. (De Waroux and Lambin, 2012)

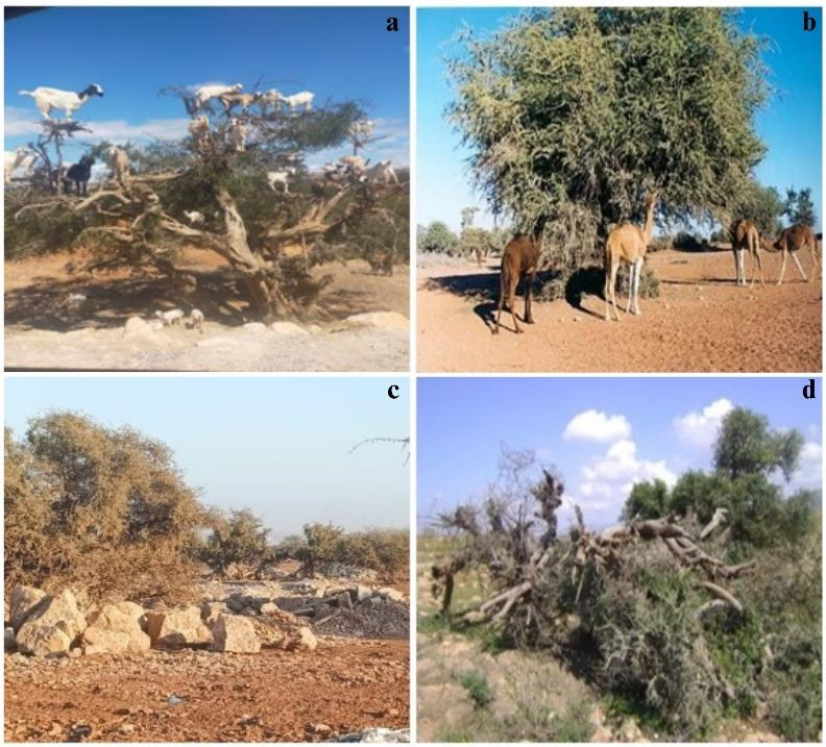


Figure 10. Factors in the degradation of argan trees. **(a)** and **(b**) argan tree grazed by goats and camels **(c)** destruction of argan trees for urban expansion and housing construction **(d)** exploitation of argan tree for firewood.

7. Cultivation methods.

The argan tree is considered a wild tree and not a cultivated tree (Michon et al., 2017). To protect and conserve the argan tree from overexploitation, the zone covered with argan trees was designated a UNESCO biosphere reserve in 1998, and the Moroccan government has made many efforts to rehabilitate it. In 2010, the government created the National Agency for the Development of Oasis Zones and the Argan Tree (ANDZOA). To combat deforestation and the risk of desertification, the Moroccan government has developed a ten-year program (2015-2024) and the contract has been extended until 2030, through the High Commission for Water and Forests (Msanda et al., 2021). Argan grove managers must ensure the sustainable management of this endemic agroforestry system to solve the problem of gradual loss of stands. To ensure better reproduction and domestication, it is necessary to intensify efforts to protect this species. Knowledge of the many techniques for regenerating the argan tree has improved considerably in recent years. Traditionally, argan has been propagated through seeds. However, significant progress has been achieved in its vegetative propagation using techniques such as stem cuttings, grafting, and tissue culture (Metougui et al., 2017; Benbya et al., 2019; Lamaoui et al., 2019).

7.1. Argan propagation by seeds

Propagation by seed is a classic method of sexual reproduction. More than 25,000 articles have been published on the subject (Mazri et al., 2022). The first research on seed propagation began in the 20th century with the work of Perrot (1907). Seed propagation is characterized by high genetic variability, which ensures the maintenance of diversity but does not allow the conservation of the characteristics of the mother tree (Msanda, 1993). Several publications have noted that seed-derived plants have a long juvenile period (Alouani and Bani-Aameur, 1998, 1999; Alouani, 2003; Ferradous, 2010; Metougui et al., 2017) and those young plants have poor recovery after transplantation (Harrouni et al., 1995; El Mrabet et al., 2014). However, the propagation of argan by seed is hampered by the lack of suitable conditions and the excessive use of seeds in the food, cosmetic, and pharmaceutical industries (Mazri et al., 2022).

7.2. Vegetative propagation of argan

Vegetative propagation is a highly effective method of maintaining the desired characteristics of the argan tree, such as oil content, nutritional value, and tolerance to environmental stress (Harrouni, 2002). The preferred methods of vegetative propagation are cutting and grafting. *In*

vitro cultivation is also a growing alternative for accelerating the mass production of selected trees.

7.2.1. Argan propagation by grafting.

Grafting is a technique that involves combining two parts of the plant: a rootstock, which provides the nutrients needed for the new plant to grow, and a scion, which provides the genetic characteristics of the plant to be propagated. The first attempts to graft an argan tree were made in the 1990s at the Hassan II Agronomic and Veterinary Institute (IAV) in Agadir. However, the results obtained were difficult to reproduce due to the limited number of grafted plants and the lack of conclusive statistical studies. Research has shown that division grafting requires high humidity (above 70%) and an average temperature of 25°C, while increasing the temperature to 29°C can lead to complete failure (Nasiri, 2007). Grafting techniques using simple apical division and apical or lateral perforation give the best results (Nouaim et al., 2002).

Studies carried out in 2007 and 2009 at the Regional Forestry Research Center in Marrakech showed that the argan tree remains a difficult species to graft when grafts are taken from old argan trees, especially in dry climates (Bellefontaine et al., 2011). Mokhtari et al. (2011) investigate the effect of rootstock age on grafting success. A low success rate (<30%) was obtained using rootstocks aged 6 to 8 months, while the most suitable rootstocks were those aged 18 days to 6 weeks. Mokhtari et al. (2011) investigated the effect of rootstock age on grafting success and found that young rootstocks (18 days to 6 weeks) had a higher grafting success rate (65-93%) than 6–8-month-old rootstocks (<30%). Successful union between rootstock and scion was observed after 15-20 days under the following conditions: temperature, 18-25°C; light intensity, 2000-4000 lux; and humidity >90%. The grafting and hardening of the grafted plants were carried out in two steps: The first step was carried out within 3-4 days after nebulization at a humidity of 70-100% and a temperature of 20-32°C. The second step took place in a greenhouse with shading nets and frequent irrigation by sprinklers. Other authors (e.g., Metougui et al., 2017) have studied the effect of rootstock and scion genotype on argan grafting. The results showed that grafting success mainly depends on scion/rootstock compatibility, but little on scion genotype and not at all on rootstock genotype. The same authors compared the propagation of argan by stem cutting and grafting and found that grafting was the best method of argan propagation. The highest rooting percentage of stem cuttings was 66.7%, while the percentage of successfully grafted plants was 95.8% (Metougui et al., 2017).

7.2.2. Argan propagation by stem cuttings.

Propagation of the argan tree by cuttings is the best way to establish productive orchards and preserve endangered clones, as it allows mass production of plants identical to the mother plant (Bellefontaine et al., 2010). The first attempt on the cutting of argan tree showed that cuttings from immature plant material (young branches) could be used for propagation (Platteborze, 1976). In a study by Kaaya (1998), argan cuttings from different genotypes were taken from young branches 8–10 cm long in December (1994), May (1995), and March (1996). The results showed that only 44 out of 260 cuttings were able to from root, especially those taken in March. This author further demonstrated that there were differences in the percentage of rooting depending on the genotype propagated and the substrate used. The highest rooting rate was obtained with Terragreen. Vegetative propagation of argan by cuttings was attempted by Nouaim (2002) using lignified cuttings collected from adult trees or young stems from three-years old mother plants. The rate of rooting seemed to depend on the genotype, the geographical origin of the mother plants and the substrate used. They found that cuttings planted in Terragreen had a higher percentage of rooting (33%) than cuttings planted in vermiculite (17%) or siliceous substrate (no rooting). Between 2007 and 2009, a study was carried out by Bellefontaine et al. (2010) on the propagation of argan trees by cuttings. Almost 2490 cuttings were tested. The results showed that 1467 could be rooted and 1020 weaned, giving average rooting, and weaning rates of 58.9% and 69.5% respectively, and an overall success rate of 41%. Cuttings are relatively easy if appropriate techniques are used: choice of mother plant, type of cuttings, substrate used, auxin treatment, cutting environments. Another study was conducted on argan herbaceous cuttings collected according to three factors: the age of the mother plant, the position of the branches on the tree, and the hormonal treatment of the roots (Ait Hammou et al., 2018). The results showed that root formation varied depending on the age of the mother plant. The rooting percentage was 33.3% in cuttings taken from adult trees (> 40 years) under controlled conditions (95% relative humidity, 30°C) and was much higher (76.2%) in those taken from young trees (8 years). These results highlight that the ability of argan genotypes to propagate from cuttings is strongly dependent on genetic and environmental factors and the relationships between these factors. In the study of Benbya et al. (2019), it was noted that the success of cuttings from mature material in argan reached 63.8% survival and 96.25% rooting with the use of indole-3-butyric acid, auxin and Hoagland nutrient solution.

In conclusion, the success of cuttings depends on several parameters, such as the age of the mother plant, the type of cutting, and the type and concentration of auxins used. In general, all

these factors have a strong influence on the rooting rate and root quality, and special attention should be paid to their architecture and growth (Ait Hammou et al., 2022; Metougui et al., 2017).

7.2.3. Tissue culture propagation of argan

Because of limitations and slowness of conventional methods of vegetative propagation, *in vitro* culture has been proposed as an alternative for producing argan seedlings. This is a rapid and controlled method of propagating plant tissues. It is based on the culture of micro-cuttings from selected mother trees in synthetic media that promote the initiation, multiplication, and rooting of explants. The effects of the growth regulators 1-naphthaleneacetic acid and IBA and activated charcoal, and of coconut fiber on the induction and elongation of roots of argan seedlings produced *in vitro* were studied by Bousselmam et al. (2001). They used Murashige and Skoog (MS) medium (1962) modified with tyrosine, sucrose, 6-benzylaminopurine, kenitine and indole-3-acetic acid for the establishment phase. For the induction phase, MS/2 medium was tested with the effect of indole-3-acetic acid and 1-naphthaleneacetic acid. MS/2 medium without growth regulators was used for the elongation phase. The results showed that the combination of indole-3-butyric acid and 1-naphthaleneacetic acid resulted in a rooting rate of 85% with an average of 16 roots per plant after 14 days of incubation in the dark. Moreover, the development of the elongation phase on a substrate composed of coconut fibers improved the growth of primary and secondary roots. For vegetative propagation of argan by cuttings, Nouaim et al. (2002) used woody cuttings taken from adult trees or young stems from three-year-old mother plants. They found that the number of rooted cuttings varied depending on the genotype and geographical origin of the mother plants. Another study by Justamante et al. (2017) has focused on the *in vitro* germination of argan seeds in MS medium. The newly produced *in vitro* plantlets were then transferred to MS medium. The *in vitro* micro-cuttings were rooted using MS medium supplemented with 1-naphthaleneacetic acid and indole-3-acetic acid. The rooted micro-cuttings were then transplanted into soil and acclimatized in a greenhouse. A significant percentage of rooting was obtained, reaching 100%. All the transplanted, rooted micro-cuttings survived. For *in vitro* multiplication of argan tree, 1 to 2 cm of nodal segments with one to two buds were used as explants (Lamaoui et al., 2019). The explants were transferred to MS medium containing different combinations of hormones. The maximum frequency of *in vitro* shoot development (93%) was induced on MS medium containing 2.5 mg/L 6-benzyladenine in combination with 1 mg/L indole-3-acetic acid.

Repeated subcultures of node segments were also performed on medium supplemented with lower concentrations of 6-benzyladenine (1.5 mg/L). The ability to induce *in vitro* rooting of *A. spinosa* was obtained on MS basal medium containing 5 mg/L indole-3-acetic acid and 1 mg/L 1-naphthaleneacetic acid. Well-rooted shoots were successfully acclimatized and transferred to field conditions with 100% survival.

An *in vitro* organogenesis regeneration system has recently been described by Amghar et al. (2021a) for two argan genotypes (Mejji and R'zwa). Epicotyl segments derived from seedlings were cultured on MS medium supplemented with 2 mg/L 6-benzylaminopurine under dark conditions. After 4 weeks, organogenesis rates reached 79.1% for Mejji and 62.5% for R'zwa genotypes. For shoot bud multiplication, explants were transferred to MS medium supplemented with 1 mg/L 6-benzylaminopurine and 2 mg/L gibberellic acid. The multiplication rate varied between 3.8 and 4.0 shoots per explant depending on the genotype (Amghar et al., 2021b). Successful *in vitro* root induction was achieved on a modified MS medium with half the normal concentration of ammonium nitrate supplemented with 1.5 mg/L indole-3-acetic acid, 0.5 mg/L 1-naphthaleneacetic acid, 2 mg/L silver nitrate, and 160 mg/L putrescine. Root formation rates were 86.6% for Mejji and 84.4% for R'zwa, with a remarkable survival rate of 100% for both genotypes (Amghar et al., 2021a).

Whatever propagation method is used, particular attention must be paid to the architecture and growth of the root system (Nouaim et al., 2002; Mazri et al., 2022).

8. Advantages and limitations of the different argan propagation methods.

Many researchers have attempted to develop effective propagation methods using both conventional and tissue culture techniques. Conventional propagation has been carried out using seeds, cuttings, and grafting. *In vitro* propagation has been carried out by seed germination, nodal segment culture, and organogenesis from explants. However, advantages and limitations have been encountered in almost all cases (Koufan et al., 2020). The following **Table 1** summarizes the advantages and limitations of the different methods of argan propagation.

Table 1. Advantages, and limitations of the different argan propagation methods (Mazri et al., 2022)

Propagation method		Advantages	Limitations
Conventional methods	Propagation by seed germination and seedling establishment	1. Preserves genetic variability 2. Relatively easy compared to other propagation methods 3. Can be used for genetic improvement 4. Low production cost	1. Cannot be used to produce true-to-type plants 2. Long juvenile period 3. Depends on climatic and edaphic conditions 4. The germination rate varies depending on the genotype
	Propagation by stem cuttings	1. Production of genetically uniform plants. 2. Easier than *in vivo* grafting.	1. Rooting recalcitrance 2. Successful rooting depends on the genotype, age, and type (lignified, herbaceous, or semi-herbaceous) of cuttings, planting season and substrate
	Propagation by *in vivo* grafting	1. Allows to preserve the desirable characteristics of rootstock (e.g., adaptation to soil, stress tolerance) 2. Allows to preserve the genetic characteristics of mother plants 3. Allows to overcome the rooting recalcitrance of stem cuttings	1. Incompatibility reactions between rootstock and scion 2. Requires specific conditions 3. Requires skilled workers
Tissue cultures techniques	*In vitro* germination and seedling development	1. Easy and fast 2. Preserves genetic variability 3. Can be used to produce rootstocks for micrografting	1. Cannot be used to produce true-to-type plants 2. Long juvenile period 3. The frequency of germination varies depending on the genotype
	In vitro propagation by nodal segmentation of elongated shoots	1. Mass production of superior genotypes 2. Production of disease- and pathogen-free plants 3. Production of genetically uniform plants	1. Rooting recalcitrance 2. High production cost
	Micrografting	1. Allows to preserve the desirable characteristics of rootstock 2. Allows to preserve the genetic characteristics of mother plants 3. Allows to overcome the rooting recalcitrance of microshoots	1. Plant acclimatization should be improved 2. High production cost 3. Relatively laborious for routine use
	Adventitious organogenesis	1. Can be used for rapid and large- scale propagation of argan 2. High survival rate during acclimatization 3. Can be used for genetic improvement	1. Achieved from zygotic explants 2. Cannot be used to produce true-to-type plants

The use of beneficial microorganisms, as arbuscular mycorrhizal fungi, known to increase plant nutrition and stress resilience, could represent a complementary technique to promote the argan survival and growth, in arid and semi-arid environmental conditions. This aspect is currently understudied and deserves larger considerations. The chapter 1 thus focused on whether different argan accessions (TD, MJ, and CH) can be associated with the ERM network of the AMF *R. irregularis* MUCL 41833 and (2) whether this association is beneficial to the growth

and development of the argan accessions following their transfer from *in vitro* to greenhouse conditions.

II. Maize (*Zea mays* L.)

1. Historical

Maize (*Zea mays* L.) is an important crop in Morocco with historical roots going back several centuries. It was introduced to the country during the period of European colonization, by the Portuguese and Spanish (Shoryer et al., 1990). This introduction marked the beginning of maize cultivation in the Gharb region. Later, maize cultivation spread inland to other regions, as Moroccan farmers adopted the crop and integrated it into their traditional farming practices. The expansion of maize cultivation in Morocco gained momentum during the 20th century with advances in agricultural technology and infrastructure development. Government initiatives to promote agricultural productivity have further facilitated the spread of maize in the various regions of the country.

2. Geographical distribution in Morocco

Today, maize is grown in a variety of agro-ecological zones in Morocco, including the fertile plains of the Gharb and Haouz regions, the mountainous regions of the Atlas Mountains, and the arid plains of the Souss valley (MAPM, 2016).

Maize is cultivated in several regions of Morocco, each with its own unique environmental characteristics and farming practices **(Figure 11**). One of Morocco's main maize-growing regions is Gharb, in the north-west of the country. This region benefits from fertile alluvial soils deposited by rivers such as the Sebou. The availability of irrigation water also favors maize production in this region. Another important maize-growing region is Haouz, in the central plains of Morocco. This region is characterized by clay soils and benefits from the proximity of the High Atlas Mountains, which provide water for irrigation through extensive canal systems. The Souss valley, in south-west Morocco, is also an excellent maize-growing region. Despite its arid climate and sandy soils, the Souss Valley uses drip irrigation systems to grow maize. Other maize-growing regions in Morocco include the Rif mountains in the north and the Middle Atlas region in the center of the country. These regions have different soil types and climatic conditions, and maize is grown using traditional methods or modern agricultural techniques, depending on local conditions. In the northern provinces (Al Hoceima, Tetouan, Tangier, Fez and Benslimane) and in some areas of Loukkous, maize is grown in bour (rainwater).

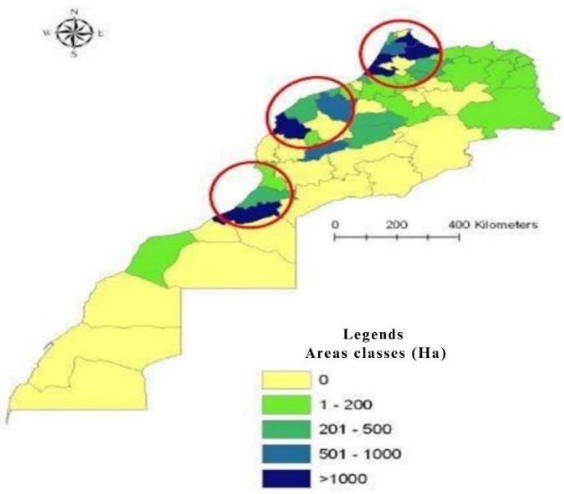


Figure 11. Maize production areas in Morocco (MAPM, 2016)

3. Taxonomy and botanical description.

Zea mays belongs to the Kingdom Plantae, Phylum Angiospermae, Class Monocotyledonae, and Order Poales. It is a member of the family Poaceae, commonly known as the grass family (Doebley and Iltis, 1980). Within the Poaceae family, *Z. mays* belongs to the genus Zea. It comprises several subspecies and cultivars, each with distinct morphological and genetic characteristics (Matsuoka et al., 2002) (**Figure 12**).

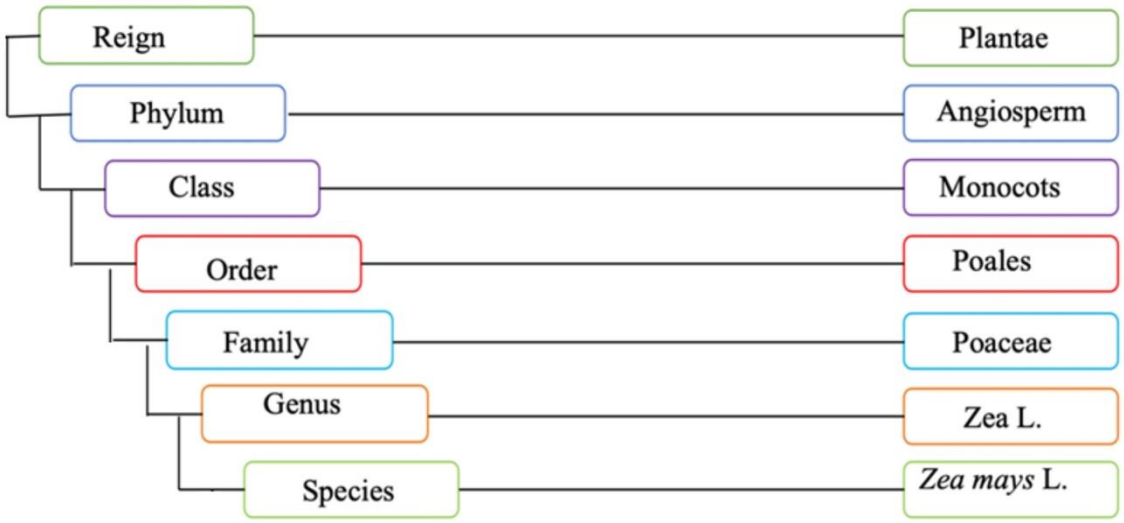


Figure 12. Botanical Classification of Maize (Awata et al., 2019)

Zea mays, commonly known as maize or corn, is an annual grass characterized by its tall stature, generally reaching 2 to 3 m in height. It has a robust stem with distinct nodes and internodes, and its leaves are long, narrow, and arranged alternately along the stem. The reproductive structures of maize are contained within the inflorescences, which consist of the tassel and the

ear (**Figure 13a**). The tassel, located at the top of the plant, contains numerous spikelets arranged in a panicle-like structure, each of which bears male flowers, or staminate florets, that produce pollen. The ear develops from the axils of the leaves and bears the female flowers, or pistillate florets, arranged in rows along a central axis called the cob. Each pistillate floret is enclosed in a modified leaf structure known as a husk or sheath, which protects the developing kernels, the seeds of the maize plant. The kernels vary in color, size, and shape depending on the maize variety, ranging from white, yellow, and red to blue and purple (**Figure 13b**) (www.gnis-pedagogique.org). The root system of maize is fibrous and shallow, extending laterally into the soil to anchor the plant and absorb water and nutrients (Doebley and Iltis, 1980).

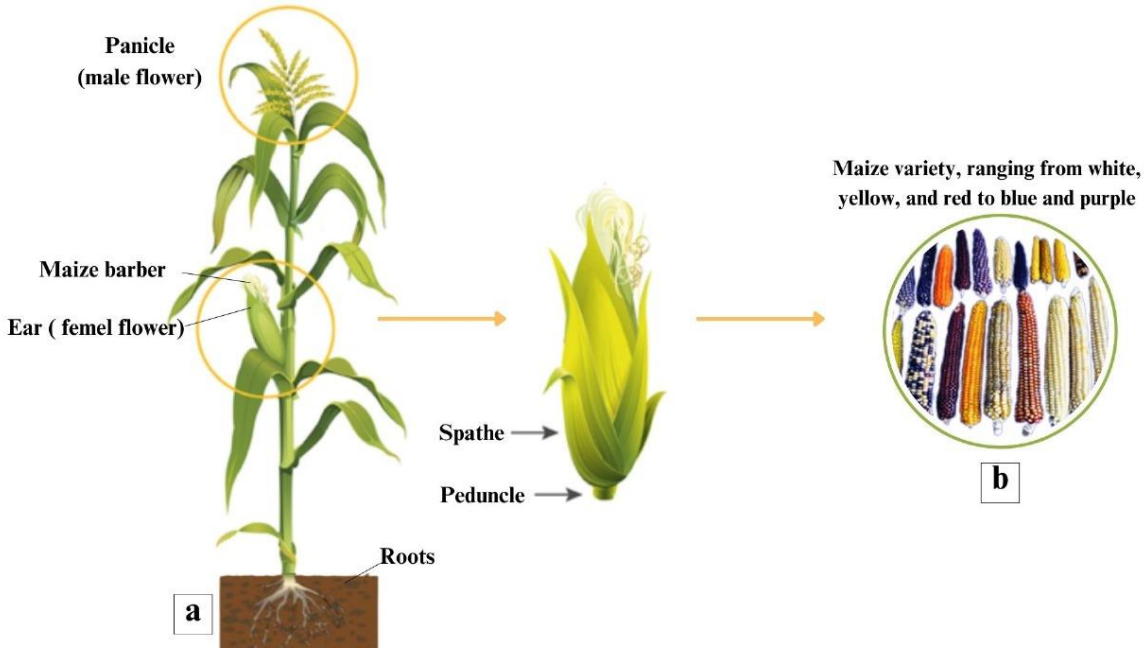


Figure 13. (a) Structure of a maize plant, **(b)** maize variety, ranging from white, yellow, and red to blue and purple (https://www.semae-pedagogie.org/sujet/mais-origine-caracteristiques)

4. Maize ecology

Maize is a C4 plant that grows best in warm, sub-humid climates with high rainfall (between 600 and 1,500 mm) and long photoperiods (Norman et al., 1995). It is a demanding plant in terms of temperature, with a minimum threshold between 8 and 10°C and an optimal growth temperature between 20 and 30°C (FAO, 2006). Water requirements vary according to precocity, variety, and harvest stage (Allen et al., 1998). During the growing season, maize requires a minimum annual rainfall of about 375 mm, but the best yields are obtained in areas where rainfall exceeds 1,000 mm (Allen et al., 1998). Maize grows on soils that vary greatly in fertility, texture, and depth. However, the best yields are obtained on deep, sandy-loamy, or clay-loamy soils that are well drained, rich in organic matter, and have a pH between 5 and 7 (USDA, 2008). Maize's ability to adapt to different soils has enabled it to be planted in different production areas from the north to the south of Morocco, the limit to production being the supply of irrigation water. The genetic progress made over the years to improve maize production has implicitly led to an increase in the crop's water requirements, as its intrinsic water efficiency has not changed from one variety to another. In general, the average irrigation requirement for maize is between 130 and 180 mm and can reach 300 mm under very unfavorable soil conditions (FAO, 1992).

5. Socio-economic importance

Maize is a major cereal crop and a staple food for over 900 million people in the developing world. It has earned the esteemed title of "Queen of Cereals" due to its worldwide demand and impressive adaptability. Maize is the most widely produced cereal in the world (FAO, 2022), with a staggering production of 1,148 million metric tons. In 2010, maize production in industrialized or intensive agricultural countries, such as Belgium, was 9828,9 kg/ha. In contrast, in countries with less intensive agriculture, such as Morocco, production did not exceed 714 kg/ha. This means that Belgium's maize production was almost 14 times that of Morocco (https://www.fao.org/faostat/en/data/QCL, date of consultation 01/01/2025).

Maize is a crop of nutritional, industrial, and agronomic importance. It is processed by populations for food consumption in various forms: whole grain (boiled or roasted maize), flour, and semolina. It is also used as silage for animal feed. Industrially, maize is used to produce oil, proteins, alcoholic beverages, biofuels, and starch (FAO, 1993). This versatility is based on its ability to thrive and adapt to a wide range of climatic conditions throughout the world (Gezahegn et al., 2021). In regions with favorable climatic conditions and appropriate

management practices, farmers can exploit the potential of double cropping to maximize maize yields and income (Li-Juan et al., 2011).

Maize production in Morocco declined sharply between 1965 and 2010 (source FAO-UN, date of consultation 18/12/2024) due to several years of drought recorded in the country during this period. Extreme droughts were recorded in 1986-1987, 1991-1992, 1994-1995 and 1998-2000, and floods in 2014, 2011 and 2009 (Benabdelouahab et al., 2019). Since 2010, Morocco has started to import maize (**Figure 14**). This importation is due to the low production of maize, which cannot keep up with the continuous and sustainable growth of the agricultural sector. According to the United States Department of Agriculture (USDA), imports of Argentinean and Brazilian maize account for 90% of Morocco's maize purchases. According to the USDA, Argentina, the world's third largest maize producer, is expected to face a 4 billion tons drop in annual production due to drought. As a result, Morocco's maize imports from Argentina are expected to fall from 2.5 million tons in 2021 to 2.3 million tons in 2023. https://www.agrimaroc.ma.

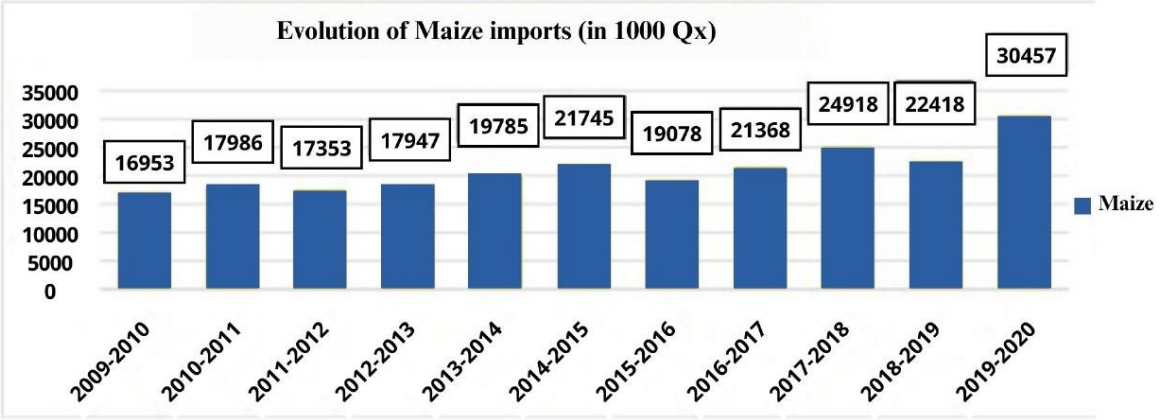


Figure 14. Evolution of maize imports (in 1000 Qx) between 2009-2010 and 2019-2020 (Source: National Interprofessional Office for Cereals and Legumes (ONICL), 2020).

6. Vulnerability of maize yields to rainfall variability.

Climate change is a major challenge for the world in general and Africa in particular. It can adversely affect agricultural yields and thus food security. In Morocco, drought, rising average temperatures, heat waves, and changes in rainfall patterns have a major impact on crops such as wheat, barley, and maize. Future climate projections for Morocco (1.3°C increase in mean annual temperature and 11% decrease in mean annual precipitation by 2050) (Achli et al., 2022), mean that the country will face economic and food security problems, as agriculture in Morocco is highly dependent on the climate (Ouatik et al., 2019; Salhi et al., 2019). The effects of climate change have a negative impact on the agricultural sector, each degree of warming

reduces corn yields by 7% (Achli et al., 2022). A study conducted by Achli et al. (2022) on the vulnerability of maize yields to growing season precipitation variability in Morocco showed that maize is highly vulnerable and has low adaptive capacity. To reduce the impact of climate change on climate-vulnerable agriculture in Morocco, Kamaoui (2019) noted that biofertilization could be a potential technique to increase soil fertility, improve plant resilience to abiotic stresses, and promote sustainable agricultural practices.

III Drought stress and their mechanisms in plants.

Abiotic stresses are considered as the major cause of crop yield loss worldwide, as they severely inhibit plant growth (Mittler, 2002). These stresses include drought, salinity, extreme temperature, and exposure to pollutants such as lead or cadmium or soil contamination by persistent organic pollutants.

Water stress is one of the most worrying abiotic factors threatening crop growth and productivity worldwide (Farooq et al., 2009). It causes several negative effects on plants, such as osmotic imbalance, growth decrease, restricted diffusion of CO_2 into chloroplasts due to stomatal closure, reduction of photosynthetic rates, disturbances in cellular metabolism, abnormal distribution of CO_2 and water, and accelerated leaf senescence (Benhiba et al., 2015; Ruiz Lozano et al., 2016; Quiroga et al., 2017; Essahibi et al., 2018; Dai et al., 2022).

Plants respond to drought stress by adopting different strategies that allow them to avoid stress and/or improve their drought tolerance (**Figure 15**). These adaptation strategies allow plants to withstand water-limiting conditions by maintaining a higher water status (Bahadur et al., 2019). Arbuscular mycorrhizal fungi may help in this scenario by improving water uptake and/or minimize water loss.

In response to drought stress, plants activate sophisticated defense mechanisms (**Figure 15**). When stress is perceived, signaling cascades involving secondary messengers such as calcium ions (Ca^{2+}), abscisic acid, reactive oxygen species (ROS) and protein kinases, are triggered (Wani et al., 2023; Zhang et al., 2023). These signals lead to morphological and physiological adjustments, either directly or indirectly via signal transduction. Water stress indirectly induces the expression of genes whose functional products, such as proline, glycine betaine, soluble sugars, late embryogenesis abundant proteins and aquaporins (AQP), contribute to metabolic regulation and plant stress tolerance (Cheng et al., 2020).

At the morphological level, adaptation mechanisms are reflected by a reduction in leaf area. To cope with drought, plants generally develop smaller leaves, greater leaf thickness and higher tissue density (Werner et al., 1999). These modifications directly impact photosynthesis and crop yield, making them some of the most observable responses to water stress. Another visible adaptation is leaf rolling, which occurs when a water deficit reduces turgor pressure in the upper epidermis of leaves (Willick et al., 2018). Root characteristics are also critical for adapting to stress, as roots are the primary organs responsible for water uptake (Lobet et al., 2014). The configuration of the root system, including root hairs, branching patterns and root density, plays a major role in improving water acquisition under arid conditions. Studies have emphasized the

importance of observable morphological characteristics. For instance, leaf curling in maize during severe drought helps to preserve photosynthetic capacity (Saglam et al., 2014; Chandarak et al., 2025). Root system architecture plasticity also plays a pivotal role. For instance, in maize significant genetic variation in the architecture responses to hydroponically simulated drought and salinity, has been reported, highlighting its adaptive significance (Keerthi et al., 2025).

At the physiological level, plants employ various strategies to cope with stress, such as closing their stomata to reduce transpiration, accumulating compatible solutes to maintain turgor, and adjusting their root architecture to enhance water uptake. Furthermore, the interaction between plant hormones, particularly abscisic acid, jasmonic acid and salicylic acid, regulates gene expression and metabolic pathways, thereby orchestrating stress responses (You et al., 2023). For example, abscisic acid pre-treatment in barley speeds up transcriptional reprogramming and encourages effective stomatal closure, improving drought recovery (Collin et al., 2025).

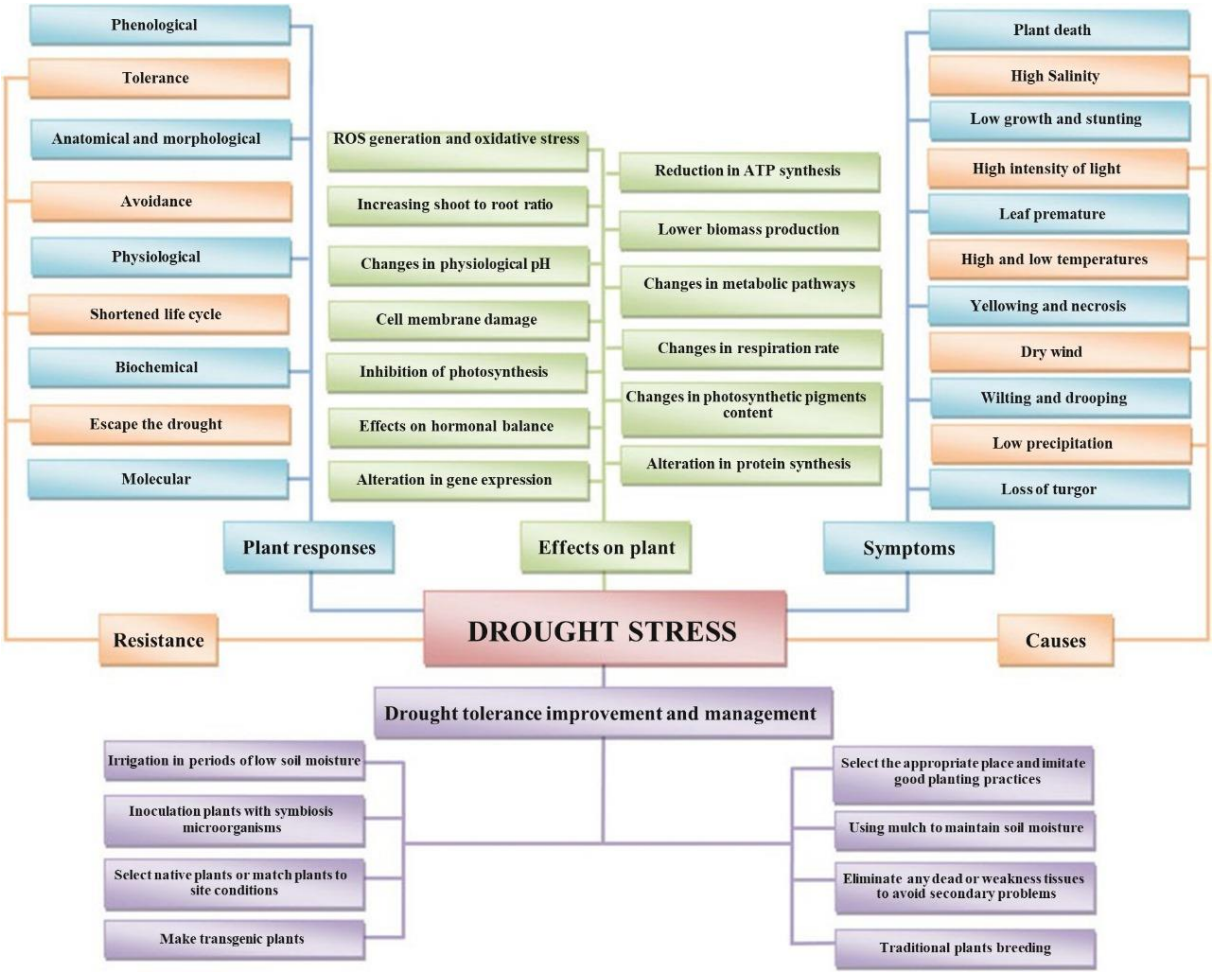


Figure 15. Causes of drought stress and its effects on plants, symptoms of water stress, plant responses to drought and mechanisms involved in resistance, and some useful strategies for drought management (from Lisar and Agdam, 2016).

IV. Arbuscular mycorrhizal fungi (AMF)

1. Phylogeny of AMF

According to Stürmer (2012), who conducted an extensive study of the taxonomy and systematics of Glomeromycota, the evolution of the taxonomy and phylogeny of AMF can be divided into four phases. The first phase was descriptive (1845–1974), followed by a morphology-based phase (1975–1989). The third phase was cladistic (1990–2000) and was characterized by the integration of molecular tools with morphological methods. The fourth and current phase is phylogenetic (2001–present) and relies predominantly on molecular and computational approaches. Until 2001, AMF were not considered as a monophyletic group (Morton, 2000). However, phylogenetic analyses based on the small subunit (SSU) rRNA gene confirmed that AMF constitute a distinct monophyletic lineage, separate from other fungal phyla (Schüßler et al., 2001). Consequently, AMF were assigned to their own fungal phylum, Glomeromycota (Schüßler et al., 2001). Subsequent phylogenetic trees of the Glomeromycetes have combined morphological traits (e.g. spore wall structure and germination) with molecular markers including internal transcribed spacer (ITS) regions, small and large subunit rRNA genes, and partial β-tubulin genes (Oehl et al., 2011). Currently, the Glomeromycota thus has been divided in four orders (Archaeosporales, Diversisporales, Glomerales and Paraglomerales) with eleven families (Acaulosporaceae, Ambisporaceae, Archaeosporaceae, Claroideoglomeraceae, Diversisporaceae, Geosiphonaceae, Gigasporaceae, Glomeraceae, Pacisporaceae, Paraglomeraceae and Sacculosporaceae), twenty-five genera (*Acaulospora, Ambispora, Archaeospora, Cetraspora, Claroideoglomus, Corymbiglomus, Dentiscutata, Diversispora , Funneliformis, Geosiphon, Gigaspora, Glomus, Intraornatospora, Otospora, Pacispora, Paradentiscutata, Paraglomus, Racocetra, Redeckera, Rhizophagus, Sacculospora, Sclerocystis, Scutellospora, Septoglomus,* and *Tricispora*) **(Figure 16),** and more than 350 species (Kokkoris et al., 2024; Tedersso et al., 2024, amf-phylogeny.com).

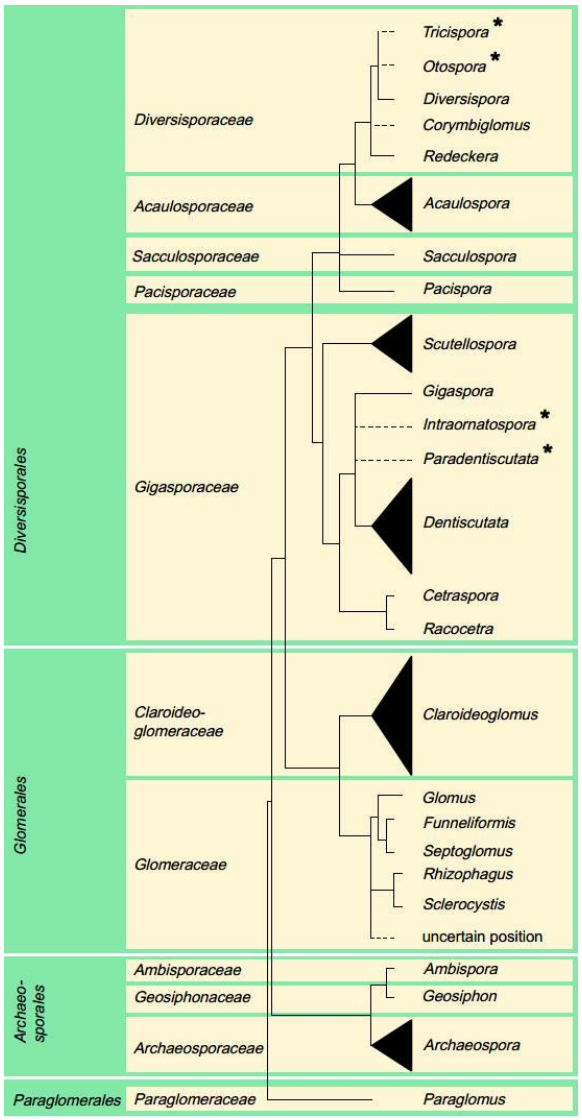


Figure 16. Classification of Glomeromycota as reviewed and formalized by Redecker et al. (2013). Genera marked by asterisks are questionable with respect to data used for description and/or with respect to phylogenetic position.

2. Definition and characteristics

AMF are soil microorganisms whose origin dates back almost 450 million years (Lanfranco et al., 2016). They form mutualistic symbioses with an approximate of 72% of terrestrial plants (Smith and Read, 2008, Brundrett and Tedersoo, 2018), colonizing roots via propagules such as spores, sporocarps and vesicles. Within the roots, they form highly branched, tree-like structures known as arbuscules, for nutrients exchange, and vesicles (for some genera) for storage and reproduction (Declerk et al., 2004). In this symbiotic relationship, plants provide carbohydrates as a source of carbon, while AMF improves the acquisition by plants of phosphorus (P) and nitrogen (N), nutrients that often limit plant growth (**Figure 17**). A key

characteristic of AMF is their extensive network of hyphae, which significantly extends the surface area for nutrient uptake by the plant beyond the root zone. This network allows access to areas of the soil that cannot be reached by roots alone (Miller et al., 2002).

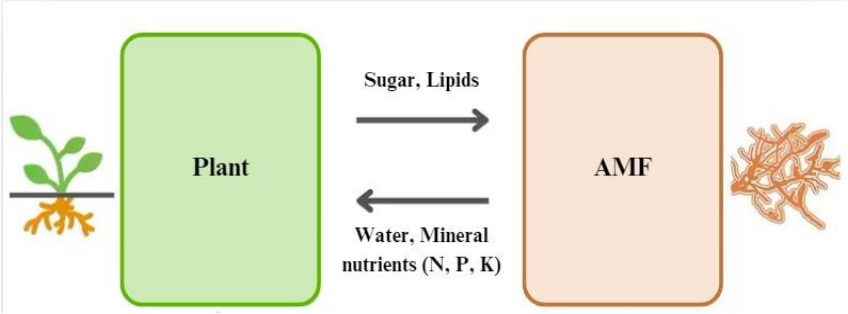


Figure 17. The process of exchange between the plant and the AMF. The plant gives the fungus some of the products of photosynthesis. In return, the fungus provides the plant with water and nutrients from the soil.

3. Life cycle of AMF

The life cycle of AMF can be divided in three phases: (1) asymbiotic, (2) pre-symbiotic and (3) symbiotic **(Figure 18**).

(1) During the asymbiotic phase, AMF propagules (e.g., spores, sporocarps, vesicles) germinate in the absence of any signal from a host. The fungal growth during this phase is limited. In the case of spores, it has been shown that if no host root is found, the cytoplasm retracts into the spore, which then becomes dormant and can germinate again when conditions become favorable (e.g., presence of a suitable host root) (Giovannetti et al., 2010).

(2) The pre-symbiotic phase begins with the recognition between the two symbionts. Roots release strigolactones (e.g., 5-deoxy-strigol) and volatiles compounds that can promote or suppress spore germination, suggesting the existence of fungal receptors sensitive to changes in the chemical composition of the environment (Akiyama et al., 2005). In response, the fungus release lipochitooligosaccharides, known as Myc factors (Maillet et al., 2011), inducing variations in calcium concentration in the cytosol and nucleus of plant cells, transcriptional regulation of genes, and root branching. Consequently, host/non-host discrimination occurs to some extent at this early stage of the interaction.

(3) The symbiotic phase starts with the development of a hyphopodium on the surface of the root epidermis. This point of attachment of the fungus on the root epidermis is the future point of penetration in the root. Plant cells beneath the hyphopodium develop a pre-penetration apparatus in response to chemical and mechanical cues, facilitating the

penetration of the fungus into the root interior (Filho et al., 2016). Root epidermal cells undergo remodeling of their cytoskeleton, nucleus, and membranes (Genre et al., 2005). Once the fungus has penetrated the root, it is able to grow in the inter- and intracellular spaces until it reaches the cortical cells. In these cortical cells, the fungus develops highly branched structures called arbuscules. Within a number of genera (i.e., *Glomus*, *Rhizophagus*), the AMF also develop thick-walled intercellular or intracellular structures called vesicles, which are thought to serve as storage structures but are also capable of colonizing new roots (Declerck et al., 2004). AMF then develop external hyphae and produce spores, either inside or outside the root, able of colonizing new hosts.

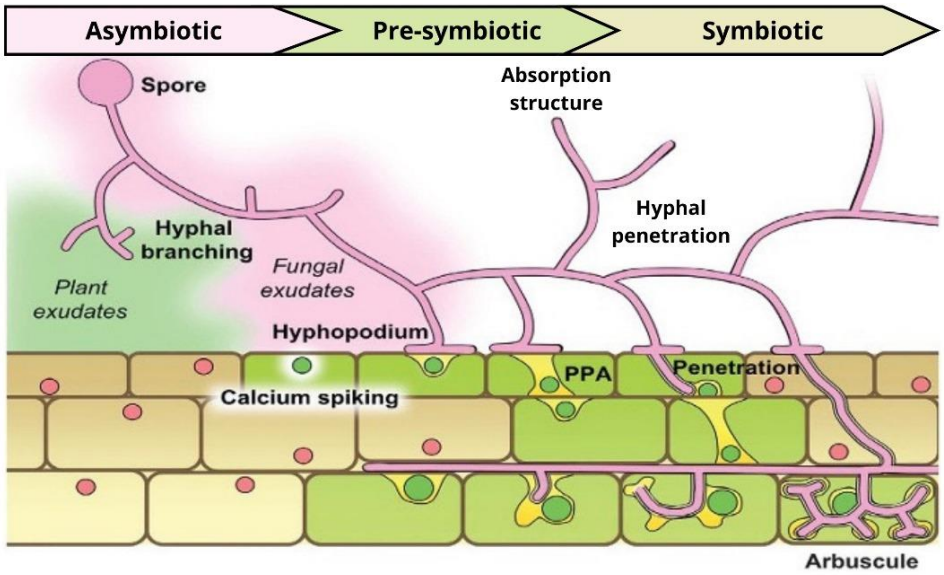


Figure 18. *The different stages of AMF colonization. The asymbiotic phase: the AMF germinates and forms some branches without the help or presence of the plant partner. The pre-symbiotic phase: exchange of diffusible signals without direct contact between the two partners. The plant secretes strigolactones that are perceived by the AMF. The AMF also produces signals (i.e., lipochitooligosaccharides) that are perceived by the root cells, inducing variations in calcium levels in the cytoplasm and nuclei and the activation of plant genes. The symbiotic phase: the AMF forms a hyphopodium on the surface of the epidermis and the plant sets up a pre-penetration apparatus (PPA) to guide the development of the AMF through the different cell layers to the cells of the inner cortex, where arbuscules are formed* (adapted from Bonfante and Genre, 2010)

4. Roles of AMF

Arbuscular mycorrhizal fungi play key roles in the ecosystem. They provide nutrients and water to their host plants and increase their resistance to biotic and abiotic stresses (Bhantana et al., 2021). They also improve soil quality by producing glycoproteins involved in micro-aggregates (Bhantana et al., 2021).

4.1. Plant nutrition

Numerous studies have demonstrated the beneficial effect of AMF on the uptake of minerals (Bhupenchandra et al., 2024), especially P and N, in nutrient-poor environments (Smith and Smith, 2011; Garcés-Rioz et al., 2017; Calonne-Salmon et al., 2018; Le Pioufle et al., 2019).
Phosphorus is by far the most studied mineral in the symbiosis between plants and AMF. There are basically two pathways for P transport to the plant (**Figure 19**): the direct pathway (soil/plant), where root cells extract Pi from the rhizosphere (Smith et al., 2011), and the indirect pathway (or mycorrhizal pathway) corresponding to the uptake and transport of Pi by the ERM (Ferrol et al., 2019).
AMF substantially enhances plant nutrition compared to non-mycorrhizal plants, particularly P. For example, mycorrhizal plants often demonstrate increased uptake of P and N uptake (Wang et al., 2017), improved acquisition of micronutriments such as Zn and Cu (Liu et al., 2000). According to Smith et al. (2004), up to 80% of plant P requirements can be supplied through AMF pathways, highlighting their central role in plant nutrition.
AMF play a crucial role in increasing the availability of P to plants by various biological and chemical processes (Mitra et al., 2020). Their extensive extraradical mycelium (ERM) allows them to explore a large volume of soil that is inaccessible to roots and root hairs. The mycelium is composed of small-diameter hyphae allowing the AMF to penetrate small soil pores, inaccessible to root hairs, resulting in higher P influx rates per unit area (Bücking et al., 2012). These hyphae actively take up Pi from the soil using high-affinity phosphate transporters (PT1, PT6 and PT3 - Walder et al., 2016, Mercy et al., 2017, Giovannini et al., 2022) coupled to proton pumps, H^+-ATPases and Na^+-ATPases (Ezawa and Saito, 2018).

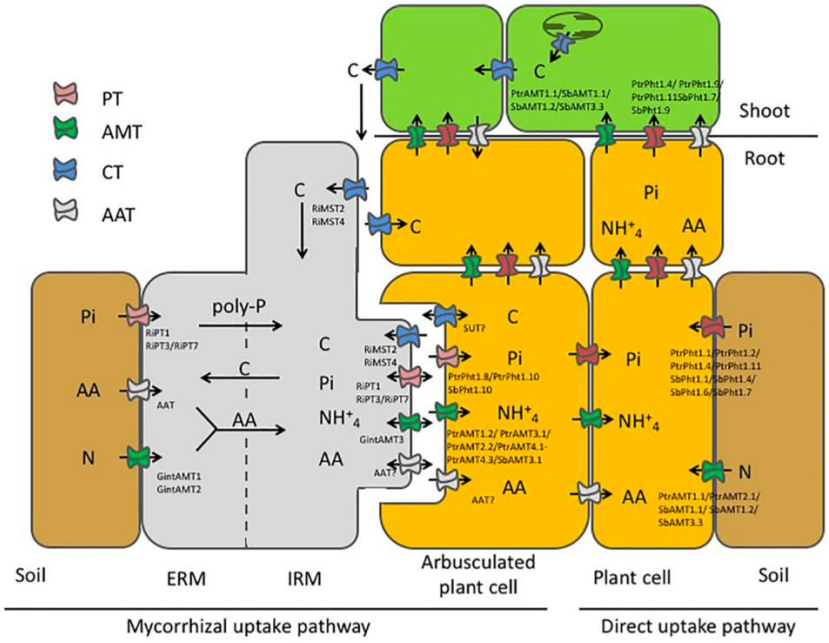


Figure 19. *Schematic representation of the mycorrhizal nutrient uptake pathway and the direct nutrient uptake pathway in the model systems poplar and sorghum when colonized by R. irregularis. In the direct uptake pathway (right hand side) nutrients, i.e. inorganic phosphate (Pi) and nitrogen (N) are taken up from the rhizosphere by phosphate transporters (PT) and ammonium transporters (AMT) and are transported to the shoot. In symbiotic interaction, the AMF partially takes over nutrition of the plant. Nutrients are taken up by specialized transporters in the extraradical mycelium (ERM) and are further transported to the intraradical mycelium (IRM) where they are transferred to the peri arbuscular space, to be taken up by plant transporters. N has been suggested to be additionally taken up from the soil in form of amino acids (AA) by predicted amino acid transporters (AAT). In exchange for the transfer of the mineral nutrients the mycorrhizal fungus is rewarded with essential carbohydrates from the plant. As some transporters were specifically induced by mycorrhization a possible localization at the peri arbuscular membrane was assumed for the plant transporters. High induction of mycorrhizal transporters in the IRM compared to the extraradical mycelium ERM suggest that these transporters are mainly involved in nutrient exchange at the symbiotic interface. The reality is probably much more complex, with reuptake of nutrients (double-headed arrow) at the biotrophic interface, allowing both partners to, at least partially, control the exchanges* (from Calabrese et al., 2019)

According to Miyauchi et al. (2020), AMF are unable to fully exploit organic nutrient sources in soil because they lack most genes encoding the carbohydrate-active enzyme (CAZyme). As such, they cannot use organic P because they lack phosphatase enzymes (Tisserant et al., 2013; Zhang et al., 2016, Zhang L. et al., 2018). To compensate for this deficiency, AMF recruit and interact with phosphate-solubilizing bacteria (PSB) such as *Rahnella aquatilis* (Zhang L. et al., 2018). For instance, in the cooperation between the AMF *R. irregularis* and *R. aquatilis*, the AMF provide fructose to the bacterium, stimulating the expression of phosphatase genes by the bacterium as well as the rate of phosphatase release into the growth medium by regulating its protein secretory system. The phosphatase activity is subsequently increased, promoting the mineralization of organic phosphorus (i.e., phytate) into inorganic phosphorus **(Figure 20),**

stimulating simultaneously the processes involved in Pi uptake by the AMF (Zhang L. et al., 2018).

AMF cooperate specifically with bacteria harboring many genes encoding CAZyme in their genome (Kakouridis et al., 2024) rather than cooperating randomly with soil bacteria (Rozmoš et al., 2022). These bacteria produce a variety of organic acids, including oxalic, citric, malic, and fumaric acids, as well as acid phosphatases, ketogluconic acids, gluconic acid, protons, and siderophores, which are crucial for mineralizing organic P (Bhupenchandra et al., 2024).

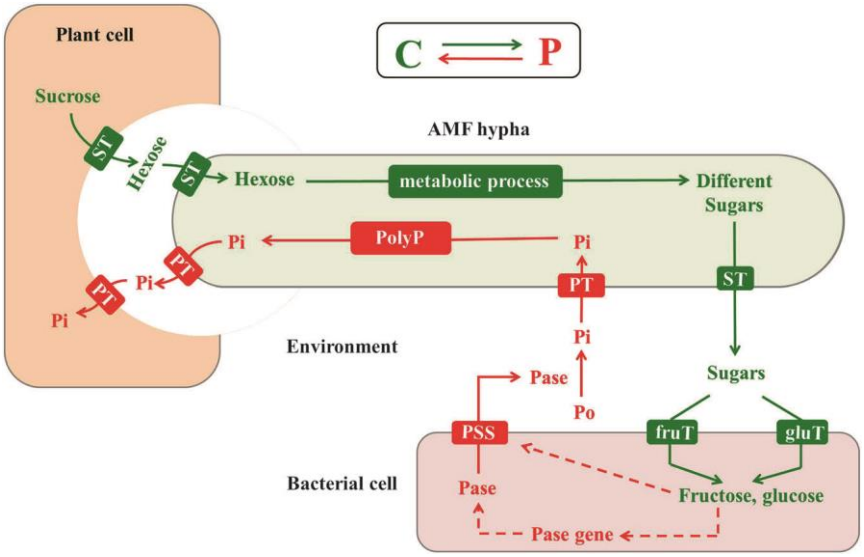


Figure 20. *Schematic representation of the reciprocal rewards of carbon (C) and phosphorus (P) between the arbuscular mycorrhizal fungus Rhizophagus irregularis and the phosphate-solubilizing bacterium Rahnella aquatilis. ST: sugar transporter, fruT: fructose transporter, glut: glucose transporter, PT: phosphate transporter, PSS: protein secretory system, Pase: phosphatase, Pi: inorganic P, Po: organic P* (from Zhang L. et al., 2018).

Once in the hyphae, the Pi is rapidly stored in vacuoles as polyphosphates (Poly-P) by the vacuolar transporter chaperone (VTC1/2/4) complex (Kikuchi et al., 2016). The vacuoles are translocated from the extraradical to the intraradical hyphae by cytosolic flux (Bücking et al., 2012, Kikushi et al., 2016). In the apoplast, Poly-P granules are hydrolyzed to inorganic orthophosphates and released in the cytoplasm through a vacuolar Pi efflux transporter (VPE), currently unidentified (Xu et al., 2019). Subsequently, the PT7 transporter exports free Pi into the peri-arbuscular space, making it available for plant absorption through their PT4, the phosphate transporter specifically located in root cells containing arbuscules (Javot et al., 2007). Meanwhile, PT1 localized on the peri-arbuscular membrane can reabsorb Pi from the peri-

arbuscular space to meet the demand during fungal growth when Pi in arbuscules becomes very limiting (Benedetto et al., 2005; Fiorilli et al., 2022; Xie et al., 2021).

Regarding N, it has been shown that the ERM of AMF absorbs inorganic N from the soil and metabolizes it to amino acids through the glutamine synthetase/glutamine oxoglutarate aminotransferase (GS/GOGAT) pathway, ultimately converting it to arginine (**Figure 20**). This arginine is transported from the ERM to the intraradical mycelium (IRM), where it is converted to urea and ornithine. During this process, ammonia is released as a byproduct of urea hydrolysis and is subsequently absorbed by plants at the symbiotic interface. However, the exact mechanism by which arginine is translocated from the ERM to the IRM remains to be elucidated (Bhupenchandra et al., 2024).

The presence of ammonium transporters (AMTs) such as GintAMT1, GintAMT2, and GintAMT3 has been observed in *Rhizophagus irregularis* during the symbiosis (Figure 18). These AMTs are activated under low ammonium concentrations and increase the ability of the fungus to take up NH_4^+ from the soil. Researchers suggest that arbuscular branches are the primary locations for ammonium transporter activity, as shown in *Glycine max* and *Medicago sativa*, where GmAMT4.1 and ATM2.3 are specifically localized in arbuscules (Breuillin-Sessoms et al., 2015).

In addition, recent studies demonstrated the recognition and preferential migration of rhizobia along ERM to their specific host via chemoattraction by specific signals transported and released by the AMF connected to one or diverse legume plant (He et al., 2024). This resulted in the improvement of nodulation on hosts plants, and possibly larger nitrogen fixation.

4.2. Biotic and abiotic stress alleviation

4.2.1. Biotic stress alleviation

It is well known that plants in nature are often attacked by microorganisms such as bacteria, fungi, nematodes, as well as insects, and that mycorrhizal plants are generally more resistant to these aggressors than non-mycorrhizal plants. Indeed, several studies have reported the beneficial effects of root colonization by AMF on plant resistance to biotic stresses (Gallou et al., 2011; Vos et al., 2012a, 2012b; Cameron et al., 2013; Anene and Declerck, 2016; Comby et al., 2017; Spagnoletti et al., 2020; Dowarah et al., 2022; Sharma et al., 2023).

The increased resistance of mycorrhizal plants has been demonstrated against both belowground and aboveground pathogens (Whipps, 2004). Several mechanisms have been

suggested, such as improved nutritional status of the host plant, changes in root growth and morphology, competition for host colonization sites and photosynthesis, microbial changes in the mycorrhizosphere and elicitation of defense genes in plants via a mechanism named mycorrhiza induced resistance (MIR) (Diagne et al., 2020). However, the effectiveness of these mechanisms varies with the host plant, AM fungus, pathogen and cropping system (Diagne et al., 2020).

In the rhizosphere, AMF provide effective protection against pathogens through a combination of biochemical, structural, and ecological mechanisms. They improve the uptake of essential nutrients, notably P, thereby enhancing root health and resistance to pathogen attack. This improved nutrition also limits the resources available to pathogens in the rhizosphere, creating spatial and nutrient competition unfavorable to their proliferation (Kumari and Prabina, 2019; Dowarah et al., 2022). Furthermore, by colonizing the roots, AMF modify the composition of root exudates, releasing compounds that favor beneficial microorganisms (such as plant growth-promoting bacteria) while inhibiting pathogens through antimicrobial effects (Pozo et al., 2013; Kumari and Prabina, 2019).

AMF also reinforce root structural barriers through callose deposition and increased lignification, making tissues more resistant to pathogen penetration. For example, in tomato, mycorrhization with *Funneliformis mosseae* reduces root necrosis caused by *Phytophthora nicotianae* (Cordier et al., 1996). This is associated with the accumulation of non-esterified pectins and pathogenesis- related (PR1) proteins in the cell walls (Cordier et al., 1996). The ERM network of AMF also contributes to the physical occupation of the rhizosphere, preventing pathogens from accessing the roots (Pozo et al., 2013; Kumari and Prabina, 2019). Finally, AMF induce local immunity by recognizing molecules associated with microorganisms' patterns (MAMP); this activates a MAMP-triggered immunity (MTI) characterized by the production of antimicrobial compounds and the stimulation of defense genes in the roots (Pozo et al., 2013; Dowarah et al., 2022). Via this mechanism, AMF induce priming, preparing plants for rapid responses to pathogen attacks by enhancing the expression of defense-related genes and boosting the production of antimicrobial compounds. This local priming ensures that the plant can quickly and effectively counteract stress when exposed to pathogens (Pozo et al., 2010; Dowarah et al., 2022).

Although localized in the roots, AMF also induce protective effects in the aerial parts of plants via systemic mechanisms. This phenomenon, MIR relies on hormonal signaling, mainly salicylic acid, jasmonic acid and ethylene pathways. Salicylic acid is involved in activating

defenses against biotrophic pathogens, while jasmonic acid and ethylene stimulate defenses against necrotrophs; hemibiotrophs and herbivores (Pozo et al., 2010). These hormones trigger the production of PR proteins, the synthesis of callose, and the accumulation of phenolic compounds in leaves, reinforcing the physical and chemical barriers of aerial tissues (Kumari and Prabina, 2019).

Another key mechanism is the role of common mycelial networks (CMN). These networks allow mycorrhizal plants to transmit warning signals to their neighbors when attacked by pathogens or herbivores. These signals induce a "priming" of defenses in neighboring plants, increasing their ability to respond quickly and effectively to a future attack (Pozo et al., 2013). Finally, AMF indirectly affects aerial parts by enhancing overall plant health through improved nutrition, thereby reducing their susceptibility to biotic stresses.

4.2.2. Abiotic stress alleviation

In dry conditions, mycorrhizal plants absorb more water due to the higher volume of soil they explore compared to non-mycorrhizal plants (Augé, 2001; Khalvati et al., 2005; Ruth, 2011; Zhang F. et al., 2018).

Several authors have reported the beneficial effects of AMF in different plant species subjected to drought stress. For example, Rani et al. (2024) evaluated the potential of the AMF *Funneliformis mosseae* on a drought tolerant (WH 1025) and a susceptible (WH 1105) wheat variety. They showed that the colonization of the plants increased the mean WUE by 3.6% in WH 1025 and 0.11% in WH 1105. In another study, Bolandnazar et al. (2007) evaluated the effects of AMF inoculation (*Glomus versiforme*, *Glom*us *intraradices*, *Glomus etunicatum*) on onion under water deficit conditions and found that AMF significantly improved WUE, yield, and growth parameters. Finally, Ouledali et al. (2018) highlighted the cultivar-specific benefits of AMF inoculation (Commercial product 'Symbivit' (INOCULUM plus, Dijon, France) in olive trees (cultivars Meski and Zarrari). They showed that AMF improve survival rates, water status, and photosynthetic assimilation differently in 'Meski' and 'Zarrazi' cultivars. Arbuscular mycorrhizal fungi improve host plant tolerance to drought (**Figure 21**) through several biochemical, physiological, and molecular mechanisms:

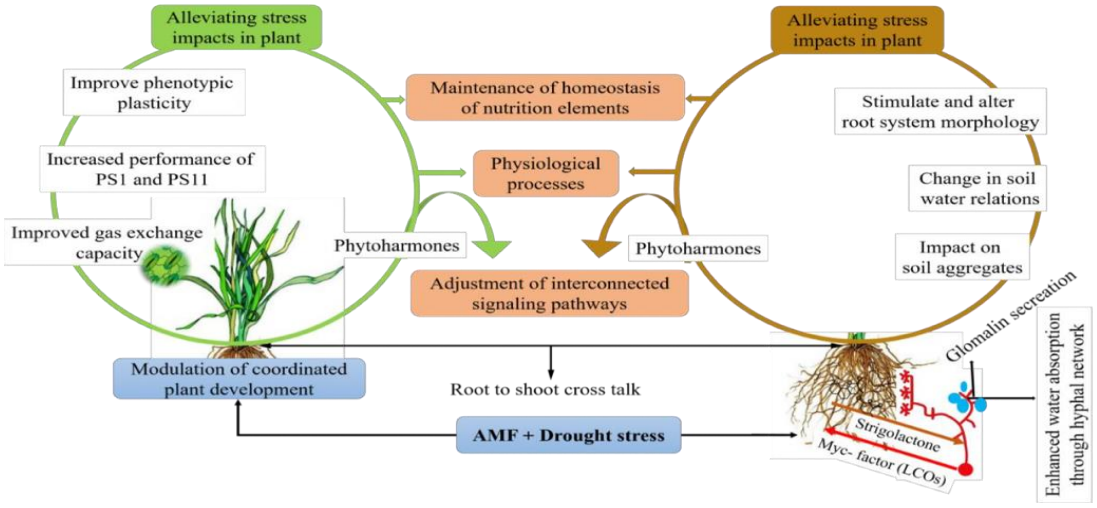


Figure 21. *This illustration shows how AMF help plants cope with drought stress. AMF forms a symbiotic relationship with plant roots, initiating a network of mechanisms that improve plant resistance to drought. This symbiosis leads to changes in root physiology and growth that improve water and nutrients uptake through both biochemical and molecular processes. The presence of AMF can improve communication between roots and shoots, helping the plant to maintain water, hormonal, and ionic balance. Specifically, AMF promotes water uptake by producing external mycelium that increases the effective root surface area. They also stimulate the formation of lateral roots, increasing the plant's access to soil resources. Water and nutrient transfer occur through specialized structures called arbuscules, which form inside plant root cells and allow direct exchange between the fungi and the plant. This AMF-mediated adaptation allows plants to alter their growth and gene expression in response to drought, helping them to better withstand stress conditions* (from Bahadur et al., 2019).

- Improvement of the soil structure and the water retention

AMF produce and secrete glomalin via their ERM contributing to the soil particles aggregation and improving the soil moisture (Zou et al., 2014; Querejeta et al., 2017; Chi et al., 2018).
The air gaps in the soil-root interface can be reduced in soils containing AMF hyphae, improving the soil-root contact, and promoting the soil-root hydraulic conductivity (Augé, 2003). Hydraulic conductivity is improved in soil containing AMF mycelium in case of water stress (Bitterlich et al., 2018).

- Plant morphological adaptations

Biomass, length, volume, and surface area, as well as root-hair density and length, and root branching in mycorrhizal trifoliate orange is increased under water stress conditions in comparison with non-mycorrhizal control plants (Moradi et al., 2013; Zou et al., 2017; Liu et al., 2018; Zhang F. et al., 2018). These adaptations suggest a larger area for water absorption, resulting in a better water and nutrient uptake (Cheng et al., 2021; Basyal, 2024).

- Improved nutrition of the plants

Drought stress often limits nutrient availability in the soil, affecting plant growth and development (Basyal, 2024). Literature often reported a better nutrients acquisition by mycorrhizal plants subjected to drought stress (Wu and Zou, 2009, Farias et al., 2018; Balestrini et al., 2020; Nouri et al., 2020).

For instance, in field, Subramanian et al. (2006) demonstrated that colonization of tomato roots by the AMF *R. intraradices* allowed the plants to grow well under water stress conditions due to improved nutrient levels and WUE. AMF improved nutrient uptake by expressing the genes of specific transporters, including ammonium transporters, phosphate transporters, and transporters for carbon, nitrate, and sulfur in plants (Nguyen et al., 2019; Balestrini et al., 2020). In a recent study, transcriptomic analysis revealed an increase of genes involved in symbiotic transport in the intraradical mycelium of *R. irregularis* in carrot roots submitted to water stress (Keller-Pearson et al., 2023). The expression of Pi transporters genes and K channels were upregulated during drought in *R. irregularis*.

- Improve water absorption by plants

The association with AMF improves plant water uptake by expressing specific transporters, as aquaporin. In carrot, bean, and sorghum, one to three AM-specific aquaporin transcripts were upregulated in mycorrhizal roots in comparison with non-mycorrhizal plants (Recchia et al., 2018; Symanczik et al., 2020; Keller-Pearson et al., 2023). Nonetheless, different expression patterns could be observed between fungal species under water stress conditions and could also depend on irrigation conditions and severity of soil drought (Bárzana et al., 2014; Symanczik et al. 2020). This suggests a complex interaction between plants and their AMF associates to regulate the water uptake of the host plant (Keller-Pearson et al., 2023).

- Fungal water absorption

In a study with *Poncirus trifoliata* inoculated in pots with *F. mosseae* and *Paraglomus occultum,* exposed to two water regimes (i.e., well-watered (WW) and water stress (WS), Zhang F. et al. (2018) demonstrated that water consumption by hyphae was more important in plants under WS stress regime than in plants under WW regime. According to Rhut (2011), the direct and indirect contribution of hyphae to total water uptake is estimated to be about 20%.

Furthermore, according to Khalvati et al. (2005), hyphae move about 4% of the water from a hyphal compartment to the root compartment. The up-regulation of genes coding for AQP, as AQPF1, AQPF2 and AQP3 was demonstrated (Li et al., 2013, Keller-Pearson et al., 2023; Wang et al., 2023). This suggests a facilitated water uptake and an increased water exchange potential to the host roots to maintain sufficient hydration (Cheng et al., 2021).

- Hormonal regulation

Other mechanisms include the production of signaling molecules that have the potential to act as phytohormones under certain conditions, such as ethylene, abscissic acid, jasmonic acid, strigolactones, cytokinins and indole-3-acetic acid, which collectively help maintain higher relative water content (RWC) and WUE under drought conditions (Bahadur et al., 2019). AMF colonization can influence the synthesis and regulation of these hormones (Ludwig-Müller et al., 2010). For example, the effects of drought on lettuce and tomato plant performance and hormone levels were studied in mycorrhizal and non-mycorrhizal plants. The results showed that AM symbiosis alleviates drought stress by altering hormonal profiles and affecting host plant physiology. Furthermore, a correlation between AMF root colonization, strigolactones levels, and drought severity was demonstrated, suggesting that under these adverse conditions, plants may increase strigolactones production to promote symbiosis establishment to cope with stress (Ruiz-Lozano et al., 2016).
The phytohormone abscissic acid, considered an 'abiotic stress hormone', increased during drought in mycorrhizal plants to cope with stress. It is essential for the successful establishment of AMF colonization in plant roots, with evidence of its impact on arbuscules formation and function. The continuous adjustment of plant abscissic acid levels in response to AM symbiosis elucidates its crucial importance in communicating drought tolerance. During drought stress, AMF induce abscissic acid biosynthesis which ultimately increase the abscissic acid levels in plants and promotes the stomatal closure to minimize water loss by transpiration (Bahadur et al., 2019). This hormone also promotes the accumulation of osmoprotectants, such as sugars and proline, further enhancing drought tolerance (Basyal, 2024).

- Osmotic adjustment and osmolytes production

Symbiosis with AMF improves growth performance and osmotic adjustment through the accumulation of various compounds, such as soluble sugars, proline, and free amino acids,

under drought conditions. Osmotic adjustment helps plants maintain the water potential gradient for soil-root water flow (Bahadur et al., 2019).

Soluble sugars can function as a signal molecule that activates regulatory pathways controlling the transport of photosynthetic products (Poór et al., 2019). Sucrose, fructose, and glucose can protect and stabilize macromolecule structures (Cheng et al., 2021).

Proline is an amino acid produced by plants in response to drought stress. It helps maintain cellular osmotic balance, thereby reducing the effects of drought stress. Proline also plays a key role in osmoregulation and free radical control. In addition, it acts as a molecular chaperone, stabilizing cellular structures and protecting plant cells from the harmful effects of drought (Bahadur et al., 2019). Numerous studies have shown that in mycorrhizal plants, proline accumulation leads to better stress resistance than in non-mycorrhizal plants (Zhu et al., 2011; Zhang F. et al., 2018; Zhen et al., 2020).

The increase in polyamines in mycorrhizal plants subjected to WS could reduce oxidative damage by maintaining cell pH and ion homeostasis and enhancing antioxidant defense systems (Zhang et al., 2020).

In an experiment with *Zenia insignis* seedlings inoculated separately with three AMF (*F. mosseae, R. intraradices,* and *Diversispora versiformis*) or inoculated with a mixture of the three species under two water regimes (i.e., WW and WS), Bagheri et al. (2019) showed that AMF had a positive effect on osmolytes of plants under WS. A modulation of gene expression coding for enzymes involved in the synthesis of osmo-protectants and osmo-regulators, such as *P5CS* and *MeP5CS* in proline biosynthesis, *GmLEA8* and *GmLEA10* in Late Embryogenesis Abundant (LEA) Proteins, and *PtADC* and *PtPAO* in polyamine regulation was demonstrated in mycorrhizal plants, enhancing their ability to cope with water deficit (Wang et al., 2023; Basyal, 2024).

- ROS metabolism

In plants, drought stress triggers the generation of toxic reactive oxygen species (ROS), because of the overflow of electrons from chloroplasts, mitochondria, peroxisomes and plasma membranes. Accumulation of ROS induces an oxidative burst in plants subjected to drought stress, and thus causes oxidative damage, including lipid peroxidation, nucleic acid damage, protein oxidation and programmed cell death. Therefore, the adverse effects of drought on plant growth are partly attributed to ROS accumulation (Zou et al. 2021). Plants also develop

enzymatic and non-enzymatic antioxidant defense systems to reduce their oxidative stress (Choudhary et al. 2018; Li et al., 2022).

Most research in recent years has focused on the symbiotic mechanisms of AMF to protect plants against drought stress. The first mechanism involves the direct uptake of water by the hyphae and its transfer to the host plant, increasing the water content and decreasing the generation of ROS such as hydroxyl radicals (OH^-) singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and a superoxide radical anion (O_2^-) (Huang et al., 2020). The second mechanism involves an increase in the production of enzymatic (as superoxyde dismutase, catalase, ascorbate peroxidase, glutathione peroxidase) and non-enzymatic antioxidants induced by the symbiotic association (Bahmani et al., 2018; Diagne, 2020). These enzymatic and non-enzymatic antioxidants can control and eliminate ROS in plants, along with a reduction of malondialdehyde (biomarker of the lipid peroxydation) concentration (Rani et al., 2018).

- Photosynthesis improvement

Photosynthesis is often hampered by drought-induced stomatal closure, chloroplast structural damage, including the structure and function of the PSII reaction center, and the inhibition of electron transport (Zhang et al., 2016), thus limiting the availability of carbon dioxide for the photosynthetic process (Basyal, 2024). AMF positively impact the photosynthesis and carbon assimilation in host plants under WS (Birhane et al., 2012, Abd-Allah et al., 2019), via an increase of the chlorophyll content, the integrity and stability of PSI and PSII and enhanced photosynthetic and transpiration rates (Abd-Allah et al., 2019; Mathur et al., 2019).

4. What do we know about AMF and argan tree?

4.1. Diversity of AMF in natural argan forest soils

Like many forest species, the argan tree forms symbiotic associations with AMF (Achouri, 1989). A predominance and wide distribution of the genus *Glomus* (now renamed *Rhizophagus* for most species) was observed in the Souss Massa region, which is characterized by low rainfall and alkaline soils with reduced mineral content (Achouri, 1989).

The diversity of AMF communities associated with argan trees in different edaphoclimatic situations has been studied by several authors. Maazouzi et al. (2023) conducted a study on two argan sites, Beni Snassene (Berkane) and Aklim, in northwestern Morocco. The identification of isolated spores revealed the presence of 28 species belonging to 7 genera: *Acaulospora,*

Dentiscutata, Claroideoglomus, *Funneliformis*, *Glomus*, *Rhizophagus*, and *Pacispora*, and 5 families: *Glomeraceae* (7 species), *Acaulosporaceae* (10 species), *Pacisporaceae* (2 species), *Claroideoglomeraceae* (2 species), and *Gigasporaceae* (1 species). Other authors have studied the diversity of fungi associated with the rhizosphere of 9 argan sites in south-west Morocco (Essaouira, Admine, Amskroud plaine, Amskroud montagne, Imouzzer, Argana, Lekhssas, and Bouizakarne). The analysis of the morphological characteristics revealed the presence of two genera (*Glomus/Rhizophagus* and *Scutellospora*). The genus *Glomus* was represented by five species (*R. aggregatus*, *F. constrictum*, *Sclerocystis sinuosa*, *R. intraradices*, *R. irregularis*) and the genus *Scutellospora* by two species *(Scutellospora sp1* and *Scutellospora sp2*) (Ouallal et al., 2018). Identification of isolated spores from seven sites (Essaouira, Taghazout, Ait melloul, Tamanar, Toufalazte, Taroudant, Tiznit, Lakhsas) in southwestern Morocco revealed the presence of 26 species of mycorrhizal fungi, divided into five genera (*Glomus/Rhizophagus*, *Scutellospora*, *Entrophospora*, *Pacispora, Gigaspora*) (Sellal et al., 2016). El Maati et al. (2015) demonstrated the dominance of the genera *Rhizophagus* and *Glomus* in five argan sites (Alma, Amskroud, Ait Baha, TizinTarkatin, and Tirhmi).

4.2. Effects of AMF on plant growth parameters and nutrient content of the argan tree

The beneficial effects of AMF on the growth and nutrition of argan plants have been reported in several studies conducted under greenhouse or field conditions. For example, inoculation of young argan plants with a strain of *R. irregularis* showed a positive and significant effect on plant size and biomass, compared to non-mycorrhizal plants (Nouaim and Chaussod, 1994; 2002; Bouselmane et al., 2002; Echairi et al., 2008). El Mrabet et al. (2014) evaluated the influence of mycorrhizal inoculation with a native mycorrhizal complex of species dominated by the genus *Glomus/Rhizophagus* and containing species of *Scutellospora* and *Acaulospora* and the addition of biocompost on the establishment of *A. spinosa*. They observed a positive effect of the fungi on the growth of the seedlings and an increased survival rate and N and P content in the plant tissues. Sellal et al. (2017) tested the effect of a combination of six genera: *Acaulospora, Glomus, Scutellospora, Entrophospora, Pascispora, and Gigaspora,* on the growth of argan plants in the nursery. They observed a positive and significant effect on the growth of inoculated plants (shoot and root biomass) compared to the controls. Furthermore, the strong dependence of argan trees on mycorrhization for optimal morphological and physiological development was confirmed by Ouallal et al. (2019). Soufiani et al. (2022) studied the effect of a multi-species consortium of indigenous mycorrhizal fungi, known as

Rhizargan (complex of seven genera from 15 Moroccan argan soils) and a commercialized strain of *R. irregularis* on the growth and mineral nutrition of argan trees on two contrasting argan ecotypes, Admine and Lakhsas. The results showed that inoculation with the 'Rhizargan consortium' improved plant growth and mineral nutrition more than *R. irregularis*.

4.3. Application of AMF for the protection of argan plants against drought stress

It is well known from the literature that AMF increase nutrient uptake and improve plant water relations (Wu et al., 2013; Bahadur et al., 2019; Begum et al., 2019; Effa, 2020; Chandrasekaran et al., 2022). On the contrary, little is known about the role of AMF in argan tolerance to drought stress. Ouallal et al. (2022) studied the effects of water deficit stress on argan plants inoculated with two complexes ("Bouyzakarne" and "Argana") of mycorrhizal fungi previously selected from several AMF complexes native to the rhizosphere of the argan tree soil. The results showed that mycorrhizal plants had the highest levels of P and K and a higher accumulation of proline compared to non-mycorrhizal plants. Inoculation with a native AMF complex consisting mainly of the genus *Glomus* was studied for growth and physiological parameters. Inoculated and non-inoculated argan seedlings were grown for three months under three water regimes (25%, 50%, and 75% of field capacity) (Outamamat et al., 2022). The AMF complex stimulated the growth and mineral nutrition of argan seedlings under the different levels of imposed water deficit. Leaf relative water content (RWC), water potential, and stomatal conductance of argan leaves showed a general improvement in the inoculated seedlings compared to the non-inoculated ones. Soluble sugar and proline contents increased significantly in non-inoculated seedlings compared to inoculated seedlings under water deficit conditions (25%). Soufiani et al. (2023) evaluated the effect of a multi-species association of the indigenous mycorrhizal fungus `Rhizargan' and the strain *R. irregularis* on argan growth, mineral nutrition, and phosphatase activities under water stress conditions for two argan ecotypes, Admine and Lakhsas. Inoculation with `Rhizargan` significantly improved P, K, Ca, Mg, and Na concentrations in the rhizosphere, roots, and leaves, as well as plant growth, especially in the Lakhsas argan ecotype, compared to inoculation with the *R. irregularis* strain. Under severe water stress, inoculation with `Rhizargan` significantly increased the P content in leaves, roots, and rhizosphere in the Lakhsas argan ecotype compared to inoculation with the *R. irregularis* strain.

These studies, generally led on one genotype, were complemented by the study on various genotypes and compared their tolerance to drought in the work presented in Chapter 2 of this

thesis. This chapter highlighted the variability in drought tolerance among different argan accessions when colonized by AMF, thereby extending, and reinforcing previous findings on the role of mycorrhization in improving plant performance. In addition, photosynthesis, and oxidative stress biomarkers, not yet studied in argan subjected to drought, were analyzed.

5. What do we know about AMF and maize?

5.1 Effects of AMF on plant growth parameters and nutrient content of maize.

AMF are widely recognized as key symbiotic partners that enhance nutrient uptake, growth, and productivity in maize. These benefits extend to morphological, physiological, and nutritional characteristics, highlighting the potential of AMF as sustainable biofertilizers that can reduce the need for chemical inputs while maintaining or even improving maize yields. Field experiments have demonstrated that applying commercial AMF inoculum to soil, with or without P fertilization, significantly increases maize yields by improving soil P availability in both NPK and NK treatments (Cozzolino et al., 2013). Further greenhouse and field studies using native AMF inoculum combined with NPK fertilization have confirmed additional advantages, including increased root colonization, higher leaf chlorophyll content, and elevated N, P, and K concentrations in maize tissues (Fall et al., 2023). Field trials have also reported significantly higher maize yields when AMF were applied alongside 50% NPK, highlighting their potential to maintain productivity while reducing fertilizer use (Fall et al., 2023). Additional experiments have shown that AMF improve the availability of soil and plant micronutrients, resulting in maize grains with higher concentrations of micronutrients and improved nutritional quality (Subramanian et al., 2013). AMF also regulate zinc uptake, optimizing absorption and supporting maize growth (Saboor et al., 2021). Furthermore, Kazadi et al. (2022) reported that combining AMF inoculation with low doses of P fertilizer shortened the flowering period, promoted vigorous growth, and produced longer, thicker maize ears. Additionally, symbiosis with AMF enhanced osmotic regulation in maize by increasing soluble sugar and protein levels while reducing proline accumulation compared to non-mycorrhizal controls. These findings suggest more efficient metabolic homeostasis (Wu et al., 2017).

5. 2 Application of AMF for the protection of maize plants against drought stress

Numerous studies have examined the impact of AMF on maize performance under drought conditions. Here, we summarize selected research highlighting the beneficial role of AMF. For

instance, inoculation of maize with *R. intraradices* significantly increased silage yield across five irrigation regimes. This led to higher fresh and dry matter production, as well as altered biomass partitioning, with increased leaf/stem and reduced ear/stem ratios compared to non-inoculated plants (Celebi et al., 2010). Similarly, inoculation with *R. intraradices* improved the growth, P content, and WUE of maize grown in coal-mining spoils, while decreasing its C/P and N/P ratios. This indicates improved nutrient balance and enhanced tolerance to water limitation (Zhao et al., 2015; Ge et al., 2012). As earlier described, AMF help maintain maize plant performance by modulating photosynthesis, antioxidant activity, and osmotic balance in maize. Inoculation with *Glomus versiforme* improved drought tolerance by up-regulating the antioxidant system, protecting membrane integrity, maintaining photosynthetic performance, and enhancing osmolyte accumulation, which promoted tissue hydration and overall plant stability (Begum et al., 2019). Similar findings were reported by Quiroga et al. (2017), who observed that AMF-inoculated maize, particularly drought-sensitive cultivars, accumulated more biomass, exhibited higher PSII efficiency, showed improved membrane stability, and reduced lipid peroxidation. Gene expression analysis also revealed that AMF regulated several drought-responsive genes in sensitive cultivars, whereas only a few genes were regulated in tolerant cultivars (Quiroga et al., 2017). Furthermore, inoculation of maize with *F. mosseae* greatly reduced leaf ABA content, and postponed the decline in photosynthetic rate, stomatal conductance, and osmotic adjustment. Malondialdehyde (MDA) level was significantly lower in mycorrhizal plants than in non-inoculation plants. However, inoculation with *F. mosseae* increased antioxidant enzyme activities including peroxidase (POD) and superoxide dismutase (SOD) (Ren et al., 2019). Consistently, AMF-inoculated maize plants maintained higher PSII efficiency and chlorophyll content than non-mycorrhizal plants under both well-watered and drought conditions (Hu et al., 2020). Finally, inoculation of maize plants with a consortium composed of *Rhizophagus clarus* and *Bacillus* sp. improved P uptake, regardless of the water regime, demonstrating a synergistic effect on nutrient acquisition (Silva et al., 2023).

Whereas numerous studies have reported the better resistance/tolerance of AMF-colonized maize plants to drought, the beneficial effects of these fungi during recovery from drought is much less investigated, especially in maize. To our knowledge, before the start of the thesis, only Subramanian and Charest (1997) demonstrated an accelerated recovery of leaf-turgidity in AMF-colonized plants and an improved Pi nutrition. That is why we examined how AMF may help plants to better recover from drought via the study of the short-term dynamics of Pi uptake and leaf gas exchanges in presence/absence of these root symbionts in chapter 3.

Material and Methods

I. Biological materiel

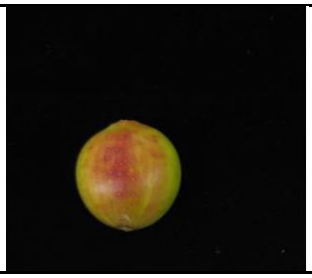
1. Plant material

1.1. Argan tree (*Argania spinosa* L.)

Three argan (*Argania spinosa* (L) Skeel) accessions from three different sites in Essaouira (Tidzi (TD), Mejii (Mj), and Cité el Hanchan (CH)) were selected for our study (TD, Mj and CH argan accessions, thereafter). The TD, MJ, and CH argan accessions originate from different regions and environments of Morocco, reflecting diverse ecological backgrounds and considerable phenotypic variability. In addition, they are among the most productive accessions in terms of yield and oil quality. These characteristics make them particularly valuable for assessing intraspecific differences in physiological performance and response to AMF colonization under water stress conditions. These stations are located in a semi-arid Mediterranean climate with an average annual rainfall of 343 mm. The Tidzi site is located approximately 26 km south of Essaouira, the Mejji site 45 km east of the city, and the Cité el Hanchan site 35 km east of the city. The argan fruits harvested from the trees (**Table 2**) of these sites were significantly different being oval in TD, elongated in MJ, and rounded in CH (**Table 2**). The pulp was then removed from the fruits, and the seeds were stored at room temperature in the laboratory until use.

Table 2. Phenotypic characteristics of different *Argania spinosa* accessions from Essaouira: Mejji, Tidzi and City Hanchan

Characteristic	**Tidzi** (altitude of 151 m, longitude of 09°43)	**City Hanchan** (altitude 298 m, longitude 09°26')	**Mejji** (altitude 306 m, longitude 09°27')
Tree			
Tree shape	spherical	erected	Other
Vigor	medium	strong	Strong
Growth habit	spreading	droping	
Shoot apical dominance	absent	absent	absent

Shoot : density of spines	large	small	medium
Shoot :type of branching	drooping	erected	erected
Shoot : internode	medium	long	short
leaf			
density	medium	strong	medium
leaf blade : size	medium	medium	large
Color of upper side	Light green	Green	Dark green
Leaf shape	Spatula	Lanceolate	Lanceolate
Leaf blade: shape of apex	Rounded	Rounded	Rounded
Leaf blade: shape of base	Cuneiform	Cuneiform	Cuneiform
Leaf blade : length	long	Medium	Medium
Leaf blade: width	Narrow	Medium	Vast
Flowers			
Flower time of flowering	late	early	phased
Flower insertion	On the nodes of branches	On the nodes of branches	On the nodes of branches
Fruit			

Fruit: shape	oval	rounded	elongated
Fruit : width	short	medium	short
Fruit: weight	low	Medium	Medium

1.2. Maize (Zea mays L)

Maize (*Zea mays* L.) seeds (Inra 258) were provided by the National Office for Health and Food Security of Morocco. They were used to produce the AMF inoculum in the greenhouse for the experiment in Chapter 2. For the experiment in Chapter 3, maize seeds (cv. ES Ballade) were provided by the Centre independent de la promotion forager (CIPF, Belgium) and used to study the dynamics of Pi uptake by maize/AMF associations under drought stress conditions in a semi-hydroponic cultivation system.

1.3. *Medicago truncatula*

Medicago truncatula Gaertn. cv. Jemalong A 17 seeds were provided by SARDI (Australia). The seeds were surface-disinfected (**see protocol in section 2.2**), placed in Petri dishes (90 mm diameter) filled with 35 ml of MSR medium without sucrose or vitamins, solidified with 3 g L^{-1} of Phytagel (Sigma-Aldrich, St. Louis, USA), and incubated in the dark at 27°C for 3 days and then placed in the light in a growth chamber (24°C/20°C (day/night), 70% relative humidity, a photoperiod of 16 hours per day, and a photosynthetic photon flux of 215 µmol m^{-2} s^{-1}). Germinated seeds were associated with *R. irregularis* MUCL 41833 to set up the mycorrhizal donor plant (MDP) *in vitro* culture system (**Section 2.1**).

2. Arbuscular mycorrhizal fungus (AMF)

The AMF *Rhizophagus irregularis* is a well-studied specie that is widely available in international collections. Due to its strong root colonization capacity and its ability to enhance P uptake and capacity to improve plant tolerance to water stress (Garcés-Ruiz et al., 2017; Le Pioufle et al., 2019), this species was selected as the model AMF for this study. In addition, *R. irregularis* is one of the most widespread AMF species, being found across the globe in diverse environments and pedoclimatic conditions, including both natural ecosystems and arable soils (Alaux et al., 2018; Savary et al., 2018). This broad distribution further reinforces its relevance as a model species in this study.

The AMF *Rhizophagus irregularis* MUCL 41833 was provided by the Glomeromycota *In vitro* COllection (GINCO). The fungus was associated with transformed carrot (*Daucus carota* L.) roots in bi-compartmented Petri dishes (St-Arnaud et al., 1996; Cranenbrouck et al., 2005) on the modified Strullu-Romand (MSR) medium (Declerck et al., 2005) to produce inoculum. The Petri dishes consisted of a root compartment (RC) containing the carrot root and the AMF and a hyphal compartment (HC) in which only the fungus was allowed to proliferate. The Petri dishes were inverted and incubated at 27°C in the dark. Within three months, thousands of spores had formed in the HC of the bi-compartment Petri dish.

II. *In vitro* mycorrhization of argan tree and greenhouse acclimatization

1. Preparation of the mycelium donor plant (MDP) *in vitro* culture system

The MDP *in vitro* culture system developed by Voet et al. (2009) was used for the *in vitro* mycorrhization of argan accessions. The system consisted of a small Petri dish (55mm diam, the root compartment - RC) placed in a larger Petri dish (145 mm diam, the hyphal compartment – HC). Both compartments were filled with MSR medium without sugar and vitamins and 3 g L^{-1} of Phytagel (20 ml in the RC and 100 ml in the HC). The roots of *M. truncatula* were positioned on the surface of the MSR medium in the RC while the shoot protruded out of the Petri dish through a 2-3 mm diam hole drilled in the bottom and lid of the 145 mm diam Petri dish (**Figure 22**). The opening was carefully sealed with silicone grease and the Petri dishes with parafilm (VWR Chemicals, Prolabo, Belgium). One hundred spores of *R. irregularis* were placed near the roots of *M. truncatula* (hereafter the donor plant). After a few weeks the hyphae of the AMF started to proliferate in the HC. The MDP *in vitro* culture systems were then wrapped in aluminum foil and kept in a growth chamber under controlled temperatures (22 °C/18 °C, day/night, 70% relative humidity, 16 h d^{-1} photoperiod, and 215 µmol m^{-2} s^{-1}).

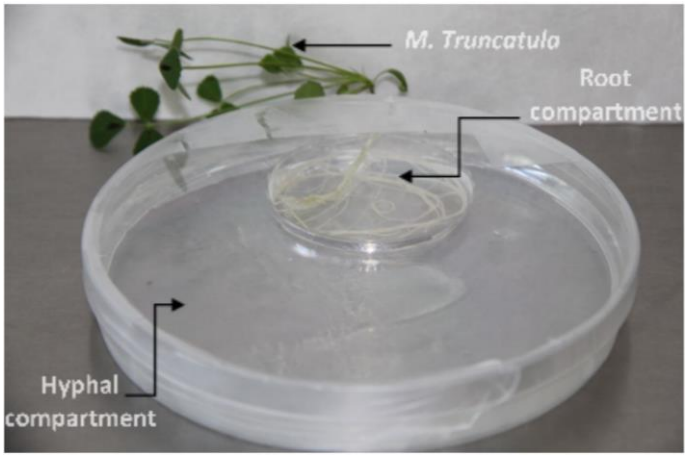


Figure 22 The mycelium donor plant *in vitro* culture system with *Medicago truncatula* (the donor plant) associated to the AMF (*Rhizophagus irregularis* MUCL 41833) in the root compartment and hyphae extending in the hyphal compartment. The roots are plated on the MSR medium, and shoot is protruding outside the Petri dish via a hole.

2. Disinfection, germination, and growth conditions of the argan seeds

The argan nuts (**Figure 23A**) were separated from the pulp and endocarp and then manually cracked to extract the almonds (**Figure 23B and C**). The almonds were then surface- sterilized by immersion in 8% sodium hypochlorite for 15 min, followed by three rinses (15 min each) in sterilized (121°C for 15 min) deionized water to ensure complete removal of any residual chemicals. These steps were performed under a horizontal laminar flow hood. The almonds were then placed in Petri dishes (90 mm diam) containing 35 ml of MSR medium without sucrose and vitamins, solidified with 3 g L^{-1} Phytagel (Sigma-Aldrich, St. Louis, USA), and adjusted to pH 5.5 before sterilization (**Figure 23D**). Petri dishes were incubated at 27°C in the dark, and germination occurred within 8 to 12 days (**Figure 23E**). After 12 days, plantlets measuring 2 to 10 cm with unbranched roots 1 to 10 cm long were obtained (**Figure 23F**).

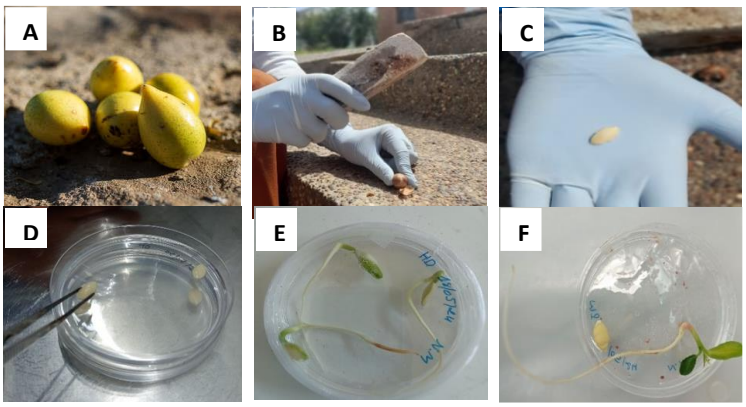


Figure 23. Argan almond isolation and *in vitro* germination, A: Mature argan fruit, B: Crushing of the seed, C: Argan almond, D: *In vitro* culture of almonds, E: Germination of almonds, F: Germination after 12 days of acclimation to light.

3. Transfer of the germinated seeds in the MDP systems

After 8 weeks of incubation of *M. truncatula* roots with AMF, the hyphae of AMF crossed the partition wall separating the RC from the HC, forming a dense extraradical mycelium (ERM) network in the HC. At this stage, two *in vitro* argan plants of 12 days old with a 2-10 cm taproot were placed in the HC. The roots were placed in contact with the dense mycelium network of *R. irregularis.* The shoots extended out of the Petri dish through a hole (2-3 mm diam) made at the base of the 145 mm diam Petri dish (**Figure 24**). The *in vitro* culture systems were then sealed with Parafilm, and the holes carefully plastered with silicone grease (**Figure 24**). The systems were then covered with aluminum foil and placed in a growth chamber under the same conditions as above.

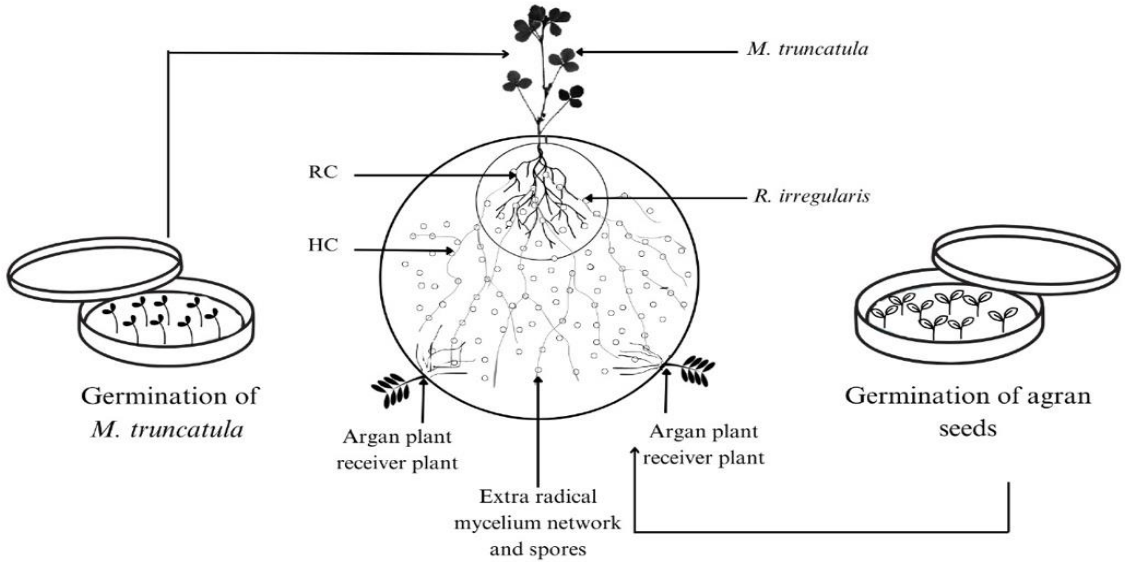


Figure 24 Schematic representation of different steps of the mycelium donor plant (MDP) *in vitro* culture system associating *M. truncatula* (donor plant) with *R. irregularis* in the root compartment (RC). Eight weeks after inoculation, the extraradical mycelium (ERM) densely covered the hyphal compartment (HC). At that time, two *in vitro* argan plants (received plants) from each accession were placed in the ERM for root colonization.

3.1. System maintenance

Plants transpiration leads to rapid depletion of the MSR medium, requiring the addition of medium every week to the RC and HC. Therefore, the systems were refilled with MSR at regular intervals under the laminar hood to avoid contamination.

4. Acclimatization of mycorrhizal plant accessions

Following their mycorrhization under *in vitro* conditions during 11 weeks, the argan plants were transferred in the greenhouse for evaluating their growth. Pots of 400 ml were filled with a sterilized substrate consisting of sand (quartz No. 4, Euroquartz, Belgium) and peat in a 1:1 (v:v) ratio. The substrate was autoclaved twice (2 X 1 h at 121 °C). The *in vitro* mycorrhizal plants were carefully removed from the MDP systems and transferred to the pots. The pots were randomly placed in a greenhouse and maintained under controlled conditions for 5 months, with a temperature of 25°C/18°C (day/night), a relative humidity of 70%, a photoperiod of 16 h, and a photosynthetic photon flux of 170 µmol m^{-2} s^{-1}. The plants were watered as needed with deionized water. In addition, 500 ml of Hoagland's nutrient solution (Hoagland and Arnon, 1950) was added after 2 and 4 months to support plant growth (**Figures 25 and 26**).

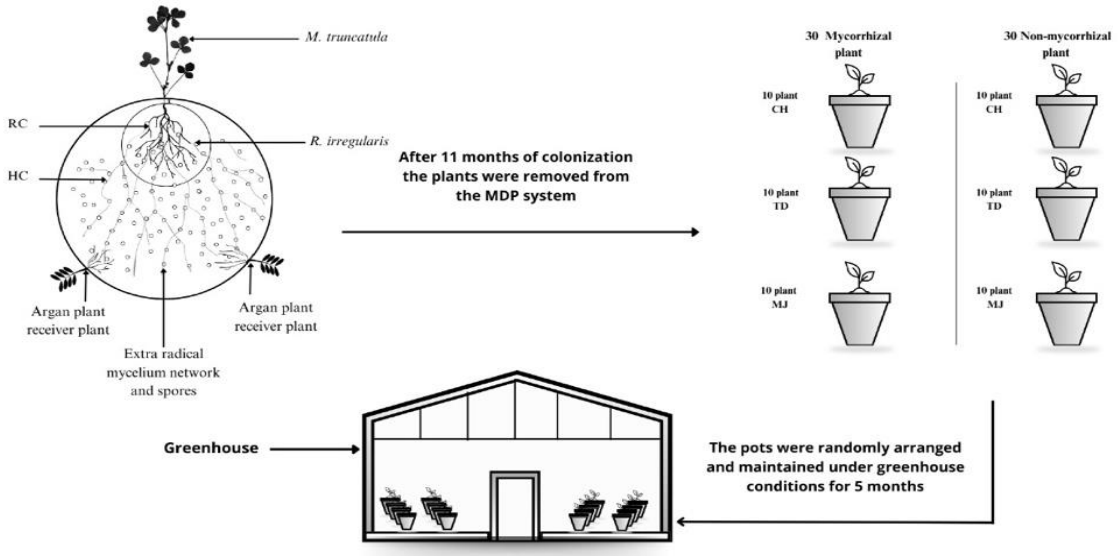


Figure 25. Schematic representation of the transfer of plants of each accession from the mycelium donor plant (MDP) *in vitro* culture system to the individual pots; ten mycorrhizal plants and ten non-mycorrhizal plants of each accession were maintained in the greenhouse for five months.

Figure 26. Overview of the plants of the different accessions (TD, MJ, and CH) after 5 months of acclimatization in the greenhouse.

5. Measurement

5.1. Evaluation of root colonization by AMF

Roots were first cleared and stained using the method of Phillips and Hayman (1970) before evaluation of colonization. Roots were cleared in 10% KOH and incubated in a water bath at 90°C for one hour. After thorough rinsing with deionized water, they were neutralized and bleached with 1% HCl for a few minutes. The roots were then stained with 2% Parker blue ink (USA) in 1% HCl at 70°C for 30 minutes. A bleaching step with a hydrogen peroxide solution (H_2O_2) for 10 min at 90°C in a water bath was included for the samples of chapter 2 (Figure 27). The roots were then cut into 1-cm segments and mounted on slides for microscopic observation under an Olympus BH2-RFCA microscope (Olympus Optical GmbH, Hamburg, Germany) at X125 magnification. To quantify colonization, four slides per plant were analyzed using the magnified intersect method (McGonigle et al., 1990) (**Figure 27**). The total colonization percentage, as well as the percentages of arbuscules, vesicles/spores, and hyphae, were recorded and calculated according to the formulas below on a minimum of 150 root intersections per plant.

$$\% \text{ TC} = \frac{\text{total number of intersections examined} - \text{negative intersect}}{\text{total number of intersections examined}} * 100$$

$$\% \text{ AC} = \frac{\text{number of intersections with the presence of arbuscules}}{\text{total number of intersections examined}} * 100$$

$$\%\text{VC} = \frac{\text{number of intersections with the presence of spores/vesicles}}{\text{total number of intersections examined}} * 100$$

$$\%HC = \frac{\text{number of intersections with the presence of hyphae}}{\text{total number of intersections examined}} * 100$$

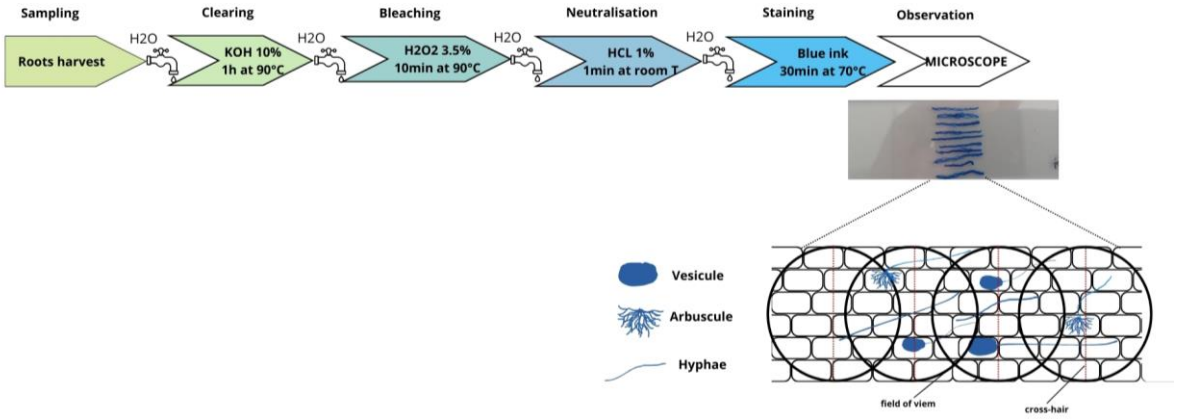


Figure 27. Schematic representation of the staining procedure and AMF colonization assessment in argan roots.

5.2. Chlorophyll dosage

For the analysis of photosynthetic pigments, fresh leaves were collected. Hundred mg were ground in a mortar with 4 ml of 80% acetone. The ground material was poured in a 15 ml Falcon tubes. The volume of the Falcon tubes was adjusted to 10 ml with acetone. The Falcon tubes were placed on ice in the dark and centrifuged at 3000 rpm for 10 minutes at 4 °C. After centrifugation, they were immediately transferred to ice before measurement using a spectrophotometer.

The absorbance of the solution was read at 2 wavelengths: 663.2; 646.8 nm; blank = 80% acetone.

Chlorophyll concentrations were expressed in mg L^{-1} of acetone according to the formula:

Chl a (mg L^{-1}) = (12,25 * D663,2) – (2,79 * D646,8)

Chl b (mg L^{-1}) = (21,50 * D646,8) – (5,10 *D663,2)

The following formulas were used to calculate chlorophyll concentrations in mg g^{-1} MF:

Chl a (mg g^{-1} MF) = [Ca (mg L^{-1}) / poids (g)] * [10 (ml)/1000]

Chl b (mg g^{-1} MF) = [Cb (mg L^{-1}) / poids (g)] * [10 (ml)/1000]

III. Greenhouse assay for studying the impact of drought stress on argan plants

1. Experimental design

The experiment was designed to evaluate the effects of AMF colonization on the growth and development of three argan accessions (CH, MJ, and TD) under drought stress conditions.
In brief, 15-days old argan seeds of CH, MJ, and TD produced *in vitro*, with a primary root of 2 to 10 cm, were transplanted into trays with or without AMF for a period of three months. The plants were then transferred to individual 5 L pots for an acclimatization period of 50 days. Two water regimes (well-watered – WW: 100% FC; Water-stressed – WS: 15% FC) were subsequently applied. For the WS plants, watering was stopped until reaching 15% FC (i.e., after 45 days) and kept at this FC for an additional 7 days. The WW plants were kept non-stop at 100% FC. The total duration of water stress was 52 days (**Figure 28**).

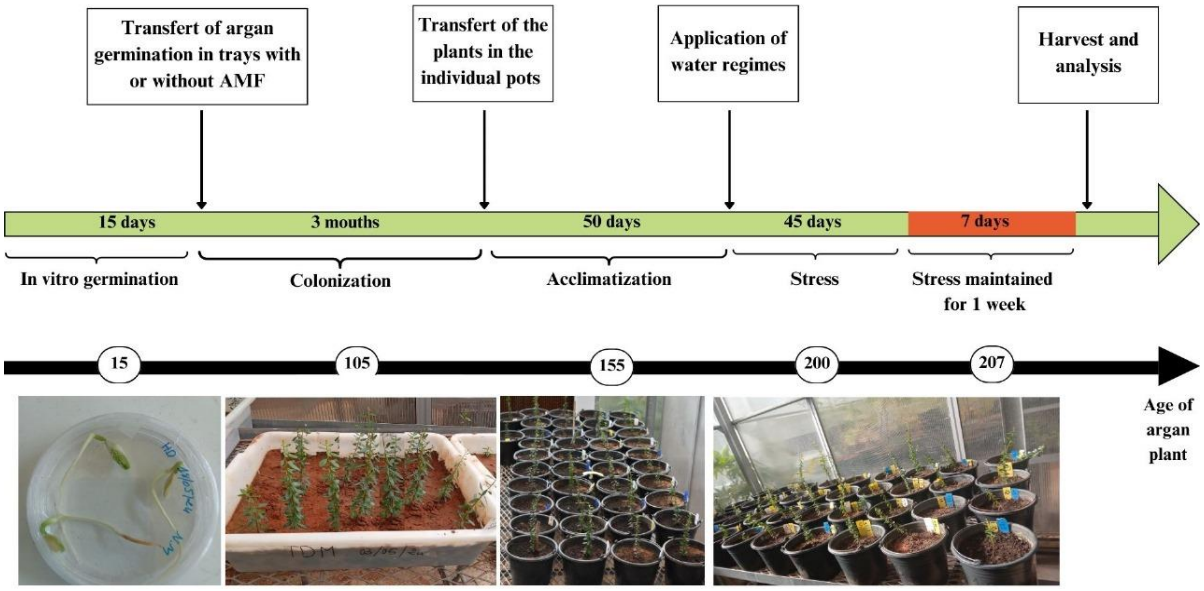


Figure 28. Timeline of the experiment in the greenhouse with argan plants. The main events of the experiment (green arrows and brackets) and the duration of the different growth phases of argan plants under different growth conditions (black brackets) are shown. The 7 days of maintained stress are indicated in red. The main thick black line indicates the age of the argan plants during the experiment and is illustrated with pictures of argan plants at ages 15, 105, 155, 200 and 207 days.

Plants were arranged in the greenhouse as follows: For each treatment, four plants were arranged in triplicate. These replicates were randomly arranged on two tables in the greenhouse (**Figure 29**).

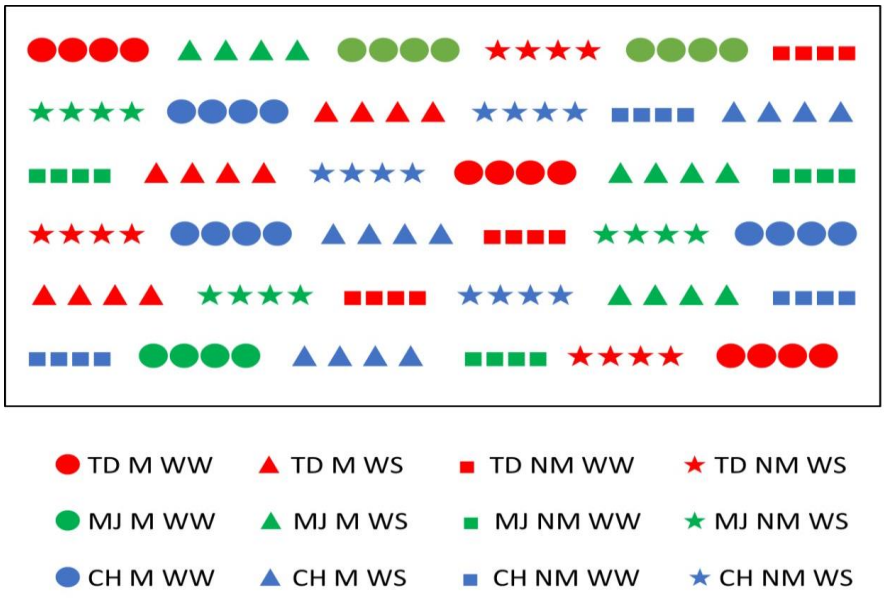


Figure 29. Diagram of the dispositive experimental in the greenhouse

2. Measurement

2.1. Mineral nutrients

Plants were dried in an oven at 60-70°C for 48 hours and subsequently finely ground in a mill or analytical grinder to obtain a uniform powder. Hundred mg of the powder was placed in a beaker and calcined at 450-550°C. A few drops of hydrochloric acid (HCl) was finally added to the ash for 15 minutes and the mixture filtered into a flask.

The measurement of potassium (K^+) and sodium (Na^+) concentration was done as follows: Firstly, the flame photometer (BWB-XP Technologies UK LTD) was calibrated using standard solutions of K^+ and N Na^+ (e.g., 1, 5 and 10 ppm). Once the instrument was correctly calibrated, the prepared sample solution was injected into the instrument, and the concentrations of. K^+ and Na^+ measured. The results were given in mg L^{-1}. To obtain the final concentration in mg g^{-1} dry matter, the values were adjusted according to the initial dilution factor used during sample preparation.

The measurement of P concentration was done as follows: A coloring reagent was first prepared by mixing ammonium molybdate and vanadate in dilute sulfuric acid. This reagent reacts with P to form a colored complex. Next, 5 mL of the dye reagent was added to 5 mL of the digested solution, and the mixture was allowed to react for 15 minutes at room temperature or in a water bath. After the reaction time, the absorbance was measured using a spectrophotometer (METASH 5100 model UV/VIS) at 410 nm. The P concentration was then quantified by

comparing the absorbance values with a calibration curve, which was prepared using phosphate standard solutions. The results of the spectrophotometric analysis were expressed in mg L^{-1}. To determine the actual concentration of P in the plant sample, the measured values were multiplied by the dilution factor used during sample preparation. This conversion allowed the P content to be expressed in mg g^{-1} of dry matter.

2.2 Biochemical analysis

2.2.1. Total soluble sugar dosage

Five hundred mg of fresh plant material was dried at 60°C for 48 hours and then crushed with a mortar and pestle. Four ml of 70% ethanol was added to the crushed sample, followed by centrifugation at 4000 rpm for 10 minutes. After centrifugation the supernatant was carefully collected and transferred in a new tube. To ensure maximum extraction, a further 4 ml of 70% ethanol was added to the remaining pellet, followed by a second centrifugation at 4000 rpm for 10 minutes. The resulting supernatant was collected and combined with the first extract, and the final extract was stored at -20°C for future use.

Half a ml of 5% phenol and 2.5 ml of concentrated sulfuric acid (90%) were added to 0.5 ml of the extract. The mixture was then incubated for 30 minutes on ice in the dark to ensure the stability of the reaction. After incubation, the optical density (OD) at 490 nm was read using a spectrophotometer (GENESYS 10S UV. VIS).

A calibration curve was prepared as follow: stock solution was prepared by dissolving 1 g of glucose in 100 mL of distilled water. From this stock solution, a series of dilutions were prepared to establish the calibration (**Table 3**).

Table 3. Represents the different dilutions of the calibration curve used to determine total soluble sugar of the samples.

Cc (mL/L)	0	5	10	15	20	30	40
D-glu	0	0.5	0.1	0.15	0.2	0.3	0.4
DW (mL)	10	9.95	9.9	9.85	9.8	9.7	9.6

The total soluble sugar was then calculated as follows:

$$\text{Total soluble sugar (mg/g)} = \frac{\text{Sample O.D.} \times \text{Concentration du standard (mg/mL)} \times \text{Dilu}}{\text{Standard O.D.} \times \text{Poids de l'chantillon (g)}}$$

2.2.2. Malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) dosage

Two hundred mg of fresh plant material was ground using liquid nitrogen. The resulting homogenate was then suspended in 5 ml of 5% (w/v) trichloroacetic acid. The homogenate was centrifuged at 12,000 g for 10 min at 4°C. The supernatants were used for determination of MDA and hydrogen peroxide (H_2O_2)

The measurement of MDA was done as follows: Two ml of the supernatant was collected and mixed with 2 ml of 0.5% thiobarbituric acid. This mixture was then heated in a heating block at 95°C for 30 min. After heating, the solution was rapidly cooled on ice to stop any further reaction. To remove any remaining particles, the mixture was centrifuged again at 12,000 g for 1-2 min at 4°C. The absorbance of the supernatant was then measured at 532 nm and 600 nm. The MDA content was estimated using an extinction coefficient of 155 mM^{-1} cm^{-1}, and the concentration was expressed as nanomoles per gram of fresh weight (nmol g^{-1} FW).

The measurement of H_2O_2 was done as follow: Half a mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide were added to 0.5 mL of the supernatant. The reaction mixture was then incubated in the dark for 20 min. After incubation, the absorbance of the reaction mixture was measured at 390 nm using a spectrophotometer (GENESYS 10S UV. VIS). To ensure accuracy, a blank was also prepared using 0.5 mL of 5% TCA, 0.5 mL of phosphate buffer, and 2 mL of potassium iodine reagent. To maintain accuracy, a new blank was prepared after every 10 samples. Absorbance of the reaction mixture was read at 390 nm, and H_2O_2 content was determined using a calibration curve obtained with different concentrations of H_2O_2 and expressed as µmole per gram of fresh weight (µmol g^{-1} FW).

The protocols necessitate the preparation of a number of solutions detailed below:

- ***Trichloroacetic acid solution***

A solution of 5% trichloroacetic acid was prepared. To do so, 20 g of trichloroacetic acid were dissolved in 395 mL of deionized water. Then 5 mL of glycerol was added to the solution and mixed until the trichloroacetic acid was completely dissolved. The solution was finally stored on ice to maintain its stability.

- ***Thiobarbituric acidsolution***

A solution of 0.67% thiobarbituric acid was prepared. To do so, 0.67 g of thiobarbituric acid was dissolved in 100 mL of 5% trichloroacetic acid. The solution was then warmed at 50°C until the thiobarbituric acid was completely dissolved.

- ***Potassium iodide solution***

A solution of 1M potassium iodide was prepared. To do so, 16.6 g of potassium iodine was added in 100 mL demineralized water and stirred until complete dissolution. The solution was subsequently stored in a tightly sealed container away from light to prevent degradation.

- ***Potassium phosphate solution (K_2HPO_4)***

A solution of 1M of potassium phosphate was prepared. To do so, 17.42g of K_2HPO_4 was dissolved in 100 mL of demineralized water.

IV. Greenhouse assay for studying the impact of drought stress on maize

1. Experimental design.

The experiment was designed to evaluate the effect of AMF colonization on the growth and development of maize plants under drought stress conditions.

In brief, mycorrhizal (M) and non-mycorrhizal (NM) maize plants were transplanted into 500 ml wash bottles, containing each 25 g of dried perlite. Unplanted control (NV) bottles were prepared similarly. The plants were then acclimated for 24 days and subsequently divided in three homogeneous groups and subjected to three water regimes: well-watered (WW), moderately watered (MW), and poorly watered (PW) (**Figure 30**). The WW plants received water until the substrate reached saturation, which was determined when the first drops of water began to flow from the base of the pot. For the MW and PW regimes, maize plants were irrigated with 60 ml and 30 ml of deionized water, respectively. These volumes corresponded to approximately 50% and 25% of the FC of perlite. The mycorrhizal and non-mycorrhizal plants were grown for 3 weeks in a circulatory semi-hydroponic cultivation system.

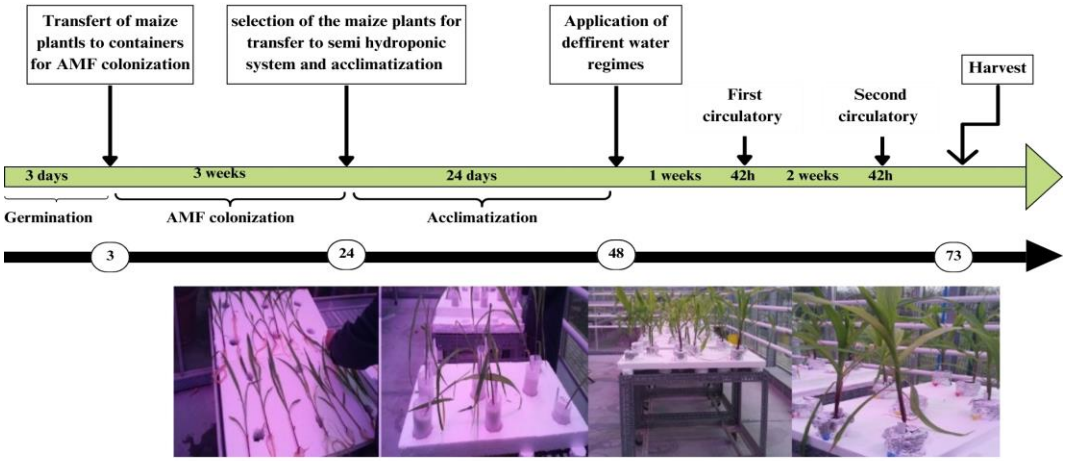


Figure 30. Timeline of the experiment in the greenhouse with maize plants. The main events of the experiment (green arrows and brackets) and the duration of the different growth phases of maize plants under different growth conditions. The main thick black line indicates the age of the maize plants during the experiment and is illustrated with pictures of maize plants at ages 03, 24, 48, and 73 days.

Plants were arranged in the greenhouse as follows: Nine pots were systematically arranged in a single row on each table. A minimum of three mycorrhizal and three non-mycorrhizal plants were assigned to each table, with nine non-vegetated control pots placed in the remaining positions to fill the empty spaces. The positioning of the pots on each table was randomized to minimize positional bias (**Figure 31**)

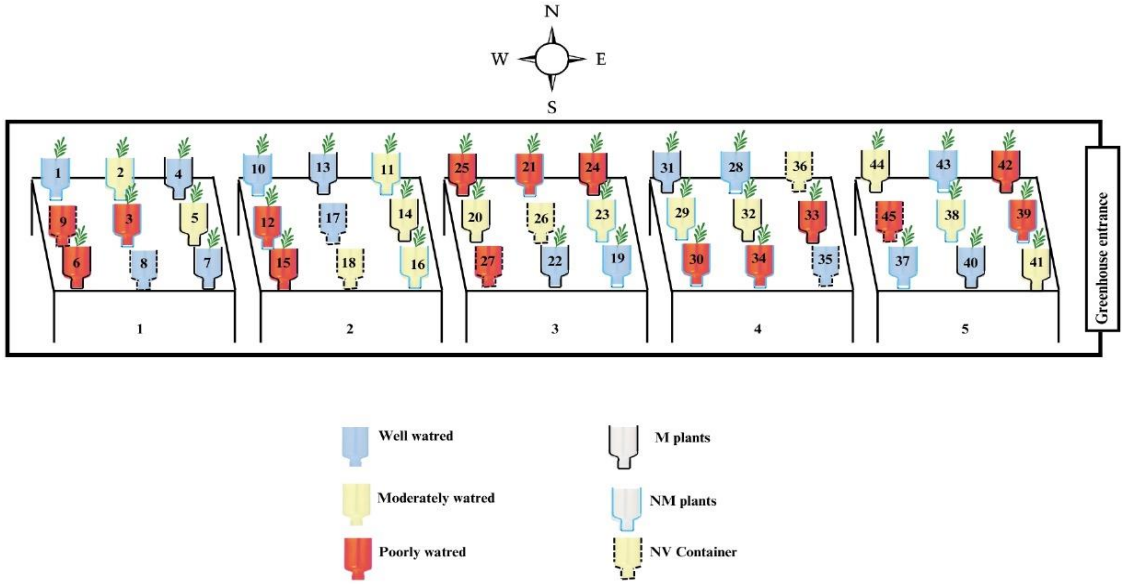


Figure 31. Experimental design of the greenhouse experiment with mycorrhizal (M) and non-mycorrhizal (NM) maize plants grown in a semi-hydroponic cultivation system. Each treatment is represented at least one time per table. NV= non-vegetated containers.

The semi-hydroponic circulatory system (**Figure 32**) was operated as follows: First, a flush was performed to obtain an equal concentration of nutrients in the containers. Two hundred mL of Hoagland$^{low\text{-}Pi}$ (Garcés-Ruiz et al., 2017)) solution was added to each container. The solution was drained from the containers using a multi-channel peristaltic pump set at 44 mL min^{-1} and discarded. After the acclimatization and flushing step, the circulatory system was set up as follows: Each plant container (bottle) was connected to a peristaltic pump via silicone tubing, which was also connected to a 1L glass bottle filled with Hoagland$^{low\ Pi}$ solution. Before activating the pump, a 15 mL sample of the solution was taken from the glass bottle to measure the Pi concentration at the initial time. Once the pump was started, it operated at a flow rate of 7.4 mL min^{-1}. The nutrient solution was pumped from the glass bottle into the plant container, and the liquid flowed back into the bottle by gravity.

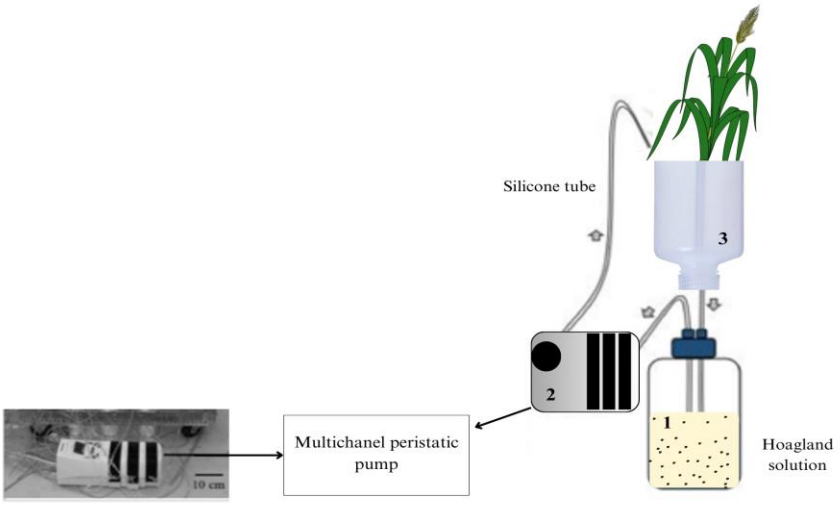


Figure 32. Schematic representation of the semi-hydroponic circulatory system. The Hoagland solution circulated through the containers supporting the maize plants. The nutrient solution in the glass bottle (1) is pumped using a peristaltic pump (2) to the top of the plant container (3) via silicone tubes. The solution flows through the plant container back into the glass bottle. The arrows indicate the direction of flow of the nutrient solution in the tube (adapted from Garcés-Ruiz et al., 2017).

2. Measurement

2.1. Root colonization of the maize plants

Three plants from the M and NM treatments were randomly selected and root colonization was assessed to ensure that colonization was sufficiently high (usually above 70% − see protocol for root staining and colonization rate - **section 2.6.1**).

2.2. Phosphorus in solution and plants

The measurement of P in the nutrient solution was done as follows: samples collected in Falcon tubes were directly diluted 5 times with ultrapure water and digested with 20 µl of nitric acid (65%, Merck, Germany) before analysis. Phosphorus was analyzed by ICP-AES (ICAP 6500, Thermo-Scientific, USA).

The measurement of P in plant was done as follows: shoots and roots were oven dried and ground separately in a grinder, 500 mg of the obtained samples were transferred to a 25 mL Erlenmeyer flask (Pyrex, USA), and 4 mL of 65% nitric acid was added. The Erlenmeyer flasks were covered with a watch glass and heated at 60°C overnight. After an evaporation step at 120 °C, the samples were dissolved in 2 mL of aqua regia solution (3/4 38% HCl to 1/4 65% HNO_3). The solutions were neutralized with approximately 15 mL of purified MilliQ (MilliporeTM). The solutions were filtered into a 50 mL graduated flask and diluted to the mark with purified MilliQ water. Solutions were homogenized before analysis by ICP-AES (ICAP 6500, Thermo-Scientific, USA).

2.3. Physiological analysis

Photosynthetic activity, transpiration rate, and stomatal conductance of maize leaves were measured using an infrared gas analyzer (IRGA), a portable model (IRGA-LCi photosynthesis system, ADC BioScientific Ltd.) with the narrow chamber (5.8 cm^2 area) in the 4^{th} or 5^{th} fully expanded leaf (of maize plants with 7–9 expanded leaves). The measurements were done under day mode with a photosynthetic photon flux of 120 µmol m^{-2} s^{-1}.

RESEARCH RESULTS

Chapter 1

In vitro mycorrhization of Argania spinosa L. using germinated seeds

Matike Ganoudi, Maryline Calonne-Salmon, Mohamed Ibriz, Stéphane Declerck

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Preface

Argan, a tree endemic to Morocco, plays a vital role in supporting local ecosystems and rural livelihoods. However, its production by methods such as stem cuttings, grafting and tissue culture, is hampered by problems such as low survival rates after transplantation and slow growth in harsh environments. Interestingly, AMF have been reported to associate with various plants *in vitro* such as hevea (Sosa-Rodriguez et al. 2013) and banana (Koffi and Declerck 2015) resulting in a higher survival and better growth following transfer to the greenhouse. Therefore, the application of AMF to argan plants *in vitro* could represent an innovative approach to increase survival, growth and development of the plant following transfer from *in vitro* to *in vivo* conditions.

In chapter 1, we used the mycelium donor plant (MDP) *in vitro* culture system developed by Voets et al. (2009) to investigate (1) whether different argan accessions (TD, MJ and CH) can be associated with the extraradical mycelium (ERM) network of the AMF *Rhizophagus irregularis* MUCL 41833 and (2) whether this association is beneficial to the growth and development of the argan accessions following their transfer from *in vitro* to greenhouse conditions.

Abstract

The beneficial effects of arbuscular mycorrhizal fungi (AMF) on the growth and nutrition of argan plants have been reported in several studies conducted under greenhouse or field conditions. However, none of these studies considered the *in vitro* mycorrhization of those plants, despite the benefits of this technology on the enhanced survival and fast recovery at transfer to hardening conditions reported in several plants of economic importance (e.g. banana and hevea). In our study, we succeeded for the first time the association of argan plants with an AMF (*Rhizophagus irregularis* MUCL 41833) under *in vitro* culture conditions and their successful transfer from *in vitro* to greenhouse conditions. We tested three argan accessions, Tidzi (TD), Mejji (MJ) and City Hanchan (CH) and grew surface-sterilized seeds in the mycelium donor plant *in vitro* culture system. Whatever the accession, root colonization, with the presence of arbuscules, vesicles/spores and hyphae, was observed after 8 weeks of growth in the extraradical mycelium network of the fungus and reached values between circa 30 and 50% of total root colonization after 11 weeks, with significant differences between the accessions. Plants survived transfer from *in vitro* to greenhouse conditions (peat-sand mixture substrate), and roots remained highly colonized after five months. Whatever the accession, a significant increase was noticed in the growth of AMF-colonized plants. Differences were also observed between accessions, with TD showing the highest relative mycorrhizal dependency and CH the lowest. The relative mycorrhizal dependency was correlated with the % of total colonization, % of arbuscules colonization and % of hyphae colonization within roots. The concentration of chlorophyll a and b was also increased in the leaves of the AMF-colonized plants, whatever the accession. In conclusion, the *in vitro* association of argan seedlings with *R. irregularis* MUCL 41833 allowed good root colonization and improved the growth of the plants after transfer to the greenhouse. This technology thus represents a promising approach to produce biotized argan plants with faster recovery and growth at transfer to the greenhouse and possibly to the field.

Keywords: *Argania spinosa*; *Rhizophagus irregularis;* Mycelium Donor Plant; Acclimatization

Introduction

Argan tree (*Argania spinosa* (L.) Skeels) is an endemic forest essence of the southwest of Morocco covering an area of 800000 ha (Msanda et al., 2005; Charrouf and Guillaume, 2009). This tree is widely exploited for the production of oil whose nutritional, medicinal as well as cosmetic properties are largely recognized (Echairi et al., 2008; Nouaïm et al., 2007). Therefore, argan oil is a socio-economic and cultural pillar of the indigenous populations of Morocco (Alados and Aich, 2008). This tree also plays an important agro-environmental role (Le Houérou, 1989), feeding animals, sheltering agricultural crops and protecting soils against erosion and desertification (Msanda et al., 2005; Achour et al., 2011). However, despite their considerable economic and agro-environmental importance, the area and density of argan trees have been reduced by half in the recent 50 years, mainly because of their over-exploitation by the local populations (Lybbert et al., 2011; Waroux et al., 2012), the clearing of forests for agricultural crops, the extension of towns (El Yousfi and Benchekron, 1992) and the arid climate.

The natural regeneration of the argan tree is very low or even absent (M'Hirit et al., 1998; Tarrier and Benzyane, 2003; Nouaïm et al., 2007; Bellefontaine, 2010). Therefore, the artificial regeneration by tree planting has become part of an ambitious program of reforestation for the Water and Forest Department of Morocco (http://www.eauxetforets.gov.ma/). However, the program has not achieved the expected results mostly because the plants produced in nurseries have a root system that is poorly vigorous and weakly branched and, therefore that, cannot withstand post-transplantation stress. Indeed, despite the fact that the vegetative propagation by cuttings and grafting has yielded some encouraging results, the starting material and multiplication conditions need further standardization (Mokhtari, 2002). For instance, Metougui et al. (2017) noticed that the rate of multiplication via cuttings differed significantly between genotypes. Their study also indicated that the success of grafting was dependent on graft/rootstock compatibility. Finally, Ait Hammou et al. (2018) revealed that the success of cuttings was mainly depending on the genotype, the age of the mother plant, the nature of cuttings and the environmental conditions.

Vegetative propagation is another promising avenue for the mass production of argan trees and their transfer to the field. Micro-cuttings from juvenile material were used by Bousselmame et al. (2001) and Aaouine and Bazagra (1990), but no satisfactory growth was obtained. With micro-cuttings from adult trees, the rate of success increased for the induction and proliferation phase, but rooting and survival remained low, never exceeding 30% (Bakkali-Kacimi, 1997).

(The rooting of lignified cuttings collected from adult trees was shown to be strongly dependent on genotype (Nouaim et al., 2002; Ferradous et al., 2011), while better results were obtained from young stems (Nouaim et al., 2002). A rooting percentage varying from 9 to 46% was obtained from two-year-old shoots (Ferradous et al., 2011). In a recent study by Justamante et al. (2017), the rooting rate by lignified hardwood shoots was better when a combination of indole-3-butyric acid and 1-naphthaleneacetic acid was used.

In vitro culture is another important approach for mass multiplication and, moreover, would contribute to the restoration and rapid regeneration of the argan forest ecosystem. However, controlling the multiplication and production of argan *in vitro* produced plants is not sufficient to ensure their survival. Root initiation and the quality of the root system *in vitro* also have important consequences on the quality of plants after transplantation under *ex vitro* conditions. Therefore, research focusing on fine-tuning and optimizing rooting and acclimatization are strongly needed.

Biotization, that is to say the *in vitro* co-culture of plant tissue explants with beneficial microorganisms (Nowak, 1998), is another option that proved promising to improve plant production and to ensure the maintenance of a functional root system after transplantation into the field. Indeed, the *in vitro* co-cultivation of plants with beneficial microorganisms may enhance their survival and fast recovery at transfer to hardening conditions (Koffi and Declerck 2015). Numerous experimental evidences have been obtained with bacteria (bacterization) and arbuscular mycorrhizal fungi (AMF) (mycorrhization), suggesting their potential application in commercial micropropagation. Using the mycelium donor plant *in vitro* culture system developed by Voets et al. (2009), plants such as banana (Koffi and Declerck 2015) and hevea (Sosa-Rodriguez et al., 2013) were successfully colonized *in vitro* by AMF and showed marked growth increase at transfer to greenhouse when compared to non-colonized controls. The *in vitro* multiplication of the argan tree in association with AMF may thus represent an innovative approach to improve rooting and acclimatization of the plants to nursery conditions and possibly accelerate their transplantation to the field.

Interestingly, Achouri (1989) noticed that argan trees, like numerous forest species, form symbiotic associations with AMF. A predominance and wide distribution of the genus *Glomus* (nowadays renamed *Rhizophagus* for most of the species) was observed in the Souss Massa region characterized by low rainfall and alkaline soils with reduced content of minerals (Achouri, 1989). Numerous studies have been conducted under controlled conditions and reported the beneficial effects of AMF on the growth and nutrition of argan trees seedlings

(Nouaim and Chaussod, 1994; Bousselmane et al., 2002; Echairi et al., 2008). These experiments confirmed the strong relative mycorrhizal dependency (Plenchette et al., 1983) of the argan tree. Indeed, Nouaim and Chaussod (1994) reported a relative mycorrhizal dependency of 78% after six months of growth, while it was 48% after nine months of growth in the study of Echairi et al. (2008). Both studies were conducted with *Glomus intraradices* (renamed *R. intraradices*) under controlled conditions in the greenhouse. Using a native mycorrhizal complex of species dominated by the genus *Glomus* and containing *Scutellospora* and *Acaulospora* species, El Mrabet et al. (2014) obtained a relative mycorrhizal dependency of 39% after six months of growth in the greenhouse. To the best of our knowledge, the association of the argan tree with AMF has never been tested *in vitro*, while this procedure could represent an option to increase the percentage of recovery and to speed-up the growth of argan trees after transplantation into the nursery and the field thus improving their resilience to environmental stresses (e.g. drought and salinity). The objective of the present study was to investigate whether argan seedlings could be colonized *in vitro* with AMF and whether this colonization results in a growth promotion at transfer to the greenhouse as compared to non-colonized plants. Three argan tree accessions from Tidzi (TD), Mejji (MJ) and City Hanchan (CH) were associated with the AMF *Rhizophagus irregularis* MUCL 41833, and the dynamics of root colonization during *in vitro* cultivation and subsequent plant growth following transfer to the greenhouse were evaluated.

Materials and Methods

Biological material

Argania spinosa

Fruits of *Argania spinosa* (L.) Skeels were collected at Essaouira (Morocco) in three forest accessions located in Tidzi (TD), Mejji (MJ) and City Hanchan (CH) (hereafter named accession) at an altitude of 151, 306 and 298 m and longitude of 09°43', 09°27' and 09°26', respectively. The climate is Mediterranean semi-arid with an average annual rainfall of 343 mm. The accessions were chosen because of their adequate rooting, high productivity, and quite different phenotypic characteristics (see annexes). The fruits highly differed between the three collecting sites. They are oval, elongated and rounded in TD, MJ, and CH, respectively and their average weight varies from 4.7 ± 0.6 g in TD to 6 ± 0.5 g in the two other sites. The fruits are yellow, formed of a fleshy pericarp (the pulp) which represents 55 to 75% of the weight of the fresh fruit. The pulp covers a very hard wood core called argan nut that represents 25 to 45% of the weight of the fresh fruit and that contains one to three almonds.

The argan nuts were used for the *in vitro* cultivation of the plants as follows: they were first separated from the pulp and endocarp and manually broken to recover the almonds. The almonds were then surface disinfected for 15 min by immersion in sodium hypochlorite (8% active chloride) and rinsed three times for 15 min in sterilized (121°C for 15 min) deionized water. The surface-disinfection steps were conducted under a horizontal laminar hood. The almonds were then placed in 90 mm Petri plates filled with 35 ml of Modified Strullu-Romand medium (Declerck et al., 1998) solidified with 3 g L^{-1} Phytagel (Sigma-Aldrich, St. Louis, USA) and with pH adjusted to 5.5 before sterilization. The Petri plates were finally incubated at 27°C in the dark. The almonds germinated within 8 to 10 days. Plantlets of 3 to 9 cm in size with non-ramified roots ranging from 1 to 10 cm in length were obtained after 10 days.

Medicago truncatula

Seeds of *Medicago truncatula* Gaertn. cv. Jemalong A17 (SARDI, Australia) were disinfected following the same protocol as the one used for the disinfection of argan seeds. The seeds were then placed on Petri plates as above and incubated at 27°C in the dark. Seeds germinated within 1 to 2 days, producing plantlets that were ready to use after 4 days.

Arbuscular mycorrhizal fungus

A strain of *Rhizophagus irregularis* (Błaszk. Wubet, Renker and Buscot) C. Walker and A. Schüßler comb. nov. MUCL 41833 was purchased from the Glomeromycota *in vitro* collection (GINCO). The fungus was associated with Ri T-DNA transformed carrot (*Daucus carota* L.) roots in Petri plates (90-mm diameter) containing 35 mL Modified Strullu-Romand medium solidified with 3 g L^{-1} Phytagel. The Petri plates were incubated at 27°C in the dark in an inverted position. The inoculum was then cultivated in bi-compartmented Petri plates as explained by St-Arnaud et al. (1996) and Cranenbrouck et al. (2005) to produce a stock culture. Thousands of spores were produced within a period of three months in the hyphal compartment (free of hairy roots of carrot) of the bi-compartmented Petri plates and were used to inoculate plantlets of *M. truncatula*.

Experiment 1: *In vitro* mycorrhizal interactions between argan accessions (TD, MJ, CH) and *Rhizophagus irregularis* MUCL 41833

The *in vitro*-produced argan plants were associated with the AMF in the mycelium donor plant *in vitro* culture system (Figure 33) as detailed in Voets et al. (2009) and Koffi and Declerck (2015), with some modifications. Briefly, the mycelium donor plant *in vitro* culture system consisted of a bi-compartmented Petri plate allowing the separation of a root compartment (a 55-mm diameter Petri plate) containing a *M. truncatula* seedling associated with an approximate of 100 spores of *R. irregularis* from a hyphal compartment (145-mm diameter Petri plate). The partition wall thus consisted in the plastic wall of the 55-mm Petri dish. Another modification consisted of a skirted Falcon tube, which after being cut to three quarters, was sealed around a hole (30-mm diameter using a heated punch) on the lid of the Petri dishes, above the boundary between the two compartments. This modification was made to facilitate the addition of medium in both the root and hyphal compartments. The entire system was then sterilized with gamma rays at 25 kGy (Sterigenics, Belgium) before use. In both compartments, Modified Strullu-Romand medium without sucrose and vitamins and solidified with 3 g l^{-1} Phytagel was added (20 ml in the root compartment and 100 ml in the hyphal compartment). The roots of *M. truncatula* were placed on the surface of the medium and the shoot extended outside the Petri plate via a hole (2–3 mm in diameter) made in the cover and base of the 145-mm diameter Petri plate. Plugs of medium containing hundreds of spores from the hyphal compartment of the bi-compartmented Petri plates from the stock culture were dissolved with sterilized sodium citrate buffer (10 mM, pH 6) passed through an Acrodisc® Syringe Filter (0.2 µm Supor Membrane; Pall Corp., New York, NY, USA). Around 100 spores were then

pipetted, rinsed in sterile water, and placed in the vicinity of *M. truncatula* (donor plant) roots. The mycelium donor plant *in vitro* culture system was sealed with Parafilm, and the hole carefully plastered with silicon grease (VWR Chemicals, Prolabo, Belgium). The mycelium donor plant *in vitro* culture system was then covered with aluminum foil and incubated in a growth chamber under controlled conditions (°T of 22°C/18°C Day/night, 70% relative humidity, a photoperiod of 16 h d^{-1} and a photosynthetic photon flux of 215 µmol m^{-2} s^{-1}).

After 8 weeks of incubation, the AMF started to cross the partition wall separating the root compartment from the hyphal compartment and developed a dense extraradical mycelium (ERM) network in the hyphal compartment. At that time, two single *in vitro* rooted plants of argan (12-days-old, with pivotal root ranging from 2 to 10 cm) were introduced in the hyphal compartment with their roots plated on the medium and shoots extending outside the system, each via a hole (2-3 mm in diameter) made in the cover and base of the 145-mm diameter Petri plate. The mycelium donor plant *in vitro* culture system was sealed with Parafilm and the holes on both sides of the system carefully plastered with silicon grease (Figure 33). The system was then covered with aluminum foil and placed in a growth chamber under the same conditions as above. To maintain the volume of the medium and the nutrient content in the root and hyphal compartments at optimal level, sterilized Modified Strullu- Romand medium without sucrose and vitamins and solidified with 3 g L^{-1} Phytagel was added every week for 8 weeks (10 to 20 ml in the root compartment and 50 mL in the hyphal compartment) via the Falcon tube glued on the top of the lid (**Figure 33**). Twenty mycelium donor plant *in vitro* culture systems were set up for each argan accession.

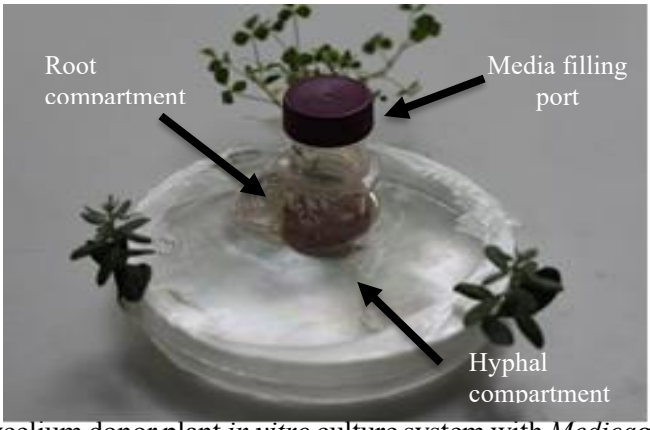

Figure 33. The mycelium donor plant *in vitro* culture system with *Medicago truncatula* (the donor plant) associated to the AMF (*Rhizophagus irregularis* MUCL41833) in the root compartment. The roots are plated on the medium and shoot is extending outside the plate via a hole. The hyphae cross the plastic barrier separating the root compartment from a hyphal compartment within 7 weeks. At that time, two receiver argan plants are planted with their roots on the medium and shoots extending outside the plate via two holes. The system is closed with Parafilm and cellophane, and holes are plastered with silicon grease. The plants are nourished via the Falcon tube glued on the top of the lid (i.e. the media filling port)

Plant growth parameters

At the end of the experiment, i.e., 11 weeks after the transfer of the *in vitro* propagated plants of argan in the mycelium network of the mycelium donor plant *in vitro* culture systems, the length of the shoots and roots of the accessions was estimated. The root length of the mycorrhizal (M) and non-mycorrhizal (NM) plants was estimated using the modified line intersect method (Newman, 1966) and shoot height was measured from the collar to the apex.

Root colonization

Root colonization was estimated after 8, 9 and 11 weeks of contact of the argan plants with the mycelium network of *R. irregularis*. Six plants (i.e. replicates) were sampled randomly at each time, and colonization was estimated following staining of the roots by the method of Phillips and Hayman (1970), with some modifications. Roots were first cleared in 10% KOH and placed in a water bath at 90°C for 1 h. They were then rinsed with deionized water, neutralized, and bleached with 1% HCl for a few minutes and finally stained with ink 2% (Parker blue ink, USA) in 1% HCl at 70°C for 30 minutes. Roots were subsequently cut in 1-cm length and mounted on slides for microscopic observations (Olympus BH2-RFCA, Olympus Optical GmbH, Hamburg, Germany) at ×125 magnification. Four slides were used to estimate total (% total colonization), arbuscular (% arbuscules colonization), vesicular/spores (% vesicles colonization) and hyphae (% hyphae colonization) colonization, according to the magnified intersects methods (McGonigle et al., 1990). For each plant, a minimum of 150 root intersections was evaluated.

Experiment 2: Acclimatization of the *in vitro* plants in open pots

Ten plants of each argan accession were inoculated with *R. irregularis* (i.e. the M plants) as described above. The same number of plants was placed in similar systems without the AMF (NM plants). All the culture systems were subsequently incubated in a growth chamber under the same growth conditions as described in experiment 1. Sterilized Modified Strullu-Romand medium (± 50 ml) without vitamins and sucrose (pH 5.5) was added weekly to the root compartment and hyphal compartment of the systems.

The plants were removed from the mycelium donor plant *in vitro* culture systems after 11 weeks and transferred into plastic pots (400 ml) filled with a sterilized (2 times 1h at 121°C) substrate of sand (quartz N°4, Euroquartz, Belgium) and peat (1:1, v:v). The pots were arranged randomly and maintained under greenhouse conditions for five months at 25°C/18°C (day/night), a

relative humidity of 70%, a photoperiod of 16 h day^{-1} and a photosynthetic photon flux of 170 µmol m^{-2} s^{-1}. Each plant was watered when appropriate with a similar quantity of deionized water. Five hundred ml of Hoagland (Hoagland and Arnon, 1950) nutrient solution was added after two and four months.

Root colonization

Root colonization was estimated at harvest, i.e. after 5 months in the sand-peat mixture substrate, on three randomly selected M and NM replicates for each accession following the same protocol as in experiment 1. The bleaching of the roots was done in a solution of H_2O_2 for 10 min at 90°C in the water bath.

Plant growth parameters

At harvest, the height of the plants was estimated from the collar to the apex. The twigs on the vegetative part were numbered and length measured. The collar diameter at the base was measured with caliper scales. The fresh and dry weights of the shoots and roots were measured for both treatments. The dry weights of the shoots and roots were determined after drying in an oven at 65°C for 72 hours. The relative mycorrhizal dependency of the argan accessions was calculated from the dry weight of the M shoots and the dry weight of the NM shoots as a percentage of the dry weight of the M shoots (Declerck et al., 1995).

Chlorophyll content

Chlorophyll content was determined in 80% acetone extract. One hundred mg of fresh leaves was homogenized in 4 ml of ice-cold acetone 80% and then centrifuged at 3000 g for 10 min at 4°C. The supernatant was used for the determination of chlorophyll pigments with a spectrophotometer (DU 800 spectrophotometer) at 663 nm for chlorophyll a (Chl*a*) and 646 nm for chlorophyll b (Chl*b*). The concentration of Chl*a* and Chl*b* was quantified as mg g^{-1} of fresh weight according to Arnon. (1949)**.**

Statistical analyses

Data (shoot and root lengths, plant height, stem diameter, number of twigs and plant biomass) were analyzed by a standard least squares test (two-way ANOVA) with a restricted maximum likelihood estimation with the AMF inoculation and the accessions regarded as fixed factors. The Tukey's honestly significant difference post-hoc test ($p \leq 0.05$) was used to discriminate among means between all treatments. The normal distribution of residuals was checked before

analyzes. Data that did not follow the normal distribution (stem diameter, root fresh and dry weights) were log10-transformed before analyzes. The factors 'time' and 'accession' were regarded as fixed factors for the arcsin-transformed percentages of argan root colonization analysis by a standard least-squares test (two-way ANOVA) with a restricted maximum likelihood estimation followed by a Tukey honestly significant difference post-hoc test ($p \leq 0.05$) to discriminate among all treatments. The normal distribution of residuals was checked before analyses. A one-way ANOVA followed by a Tukey honestly significant difference post-hoc test ($p \leq 0.05$) was used to test the arcsin-transformed percentages of argan root colonization after 5 months of acclimatization in pot cultures. The normal distribution of residuals was checked before analyses. A Pearson correlation was conducted at $P \leq 0.05$ to assess the relationships between the relative mycorrhizal dependency and % total colonization, % arbuscules colonization, % vesicles colonization or % hyphal colonization. Data analyzes were performed with the JMP Pro 15 statistical software (SAS Institute Inc., USA).

Results

Experiment 1: *In vitro* mycorhizal interactions between argan accessions (TD, MJ, CH) and *Rhizophagus irregularis* MUCL 41833

Plant growth parameters

At the start of the experiment (i.e. before transfer of the argan plants in the mycelium donor plant *in vitro* culture systems), the plants of the three accessions had primary roots, cotyledon unfolding and leaf initiation. Six plants of each accession were harvested after 11 weeks of growth in the mycelium donor plant *in vitro* culture system, and the length of the shoot and roots was measured (**Table 4**). No significant effect of the factors AMF, accession or interaction AMF x accession was noticed for shoot length. Conversely, the factors AMF and accession had a significant effect on the root length. Indeed, the root lengths of the M plants were longer as compared to the NM ones and this was significant for the plants from the TD accession, for which root length was more than doubled as compared to the NM plants. In addition, the root lengths of the NM plants for the CH and MJ accessions were significantly longer as compared to the root length of the NM plants for the TD accession

Table 4. Shoot and root lengths of argan plants (accessions TD (Tidzi), MJ (Mejji) and CH (City Hanchan)) grown 11 weeks in presence (M) or absence (NM) of *Rhizophagus irregularis* MUCL 41833 in the mycelium donor plant *in vitro* culture system

Accession	Shoot length (cm)	Root length (cm)
TD/M	12.9 ± 3.2 a	110.6 ± 31.1 a
TD/NM	12.2 ± 1.6 a	50.4 ± 6.1 b
MJ/M	11.9 ± 2 a	146.2 ± 18.4 a
MJ/NM	12.2 ± 2.1a	115.2 ± 16.1 a
CH/M	1.2 ± 2.5 a	148.6 ± 18.8 a
CH/NM	10.5 ± 2 a	121 ± 34.9 a
Effect (p-value)		
AMF	ns	***
Accession	ns	***
AMF x accession	ns	ns

For each parameter, the data represent means ± SD of 8 and 6 replicates for the shoot length and root length, respectively. Data were analyzed by a two-way ANOVA (ns = no significant difference, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$). Data in a column with different lower-case letters indicate significant differences between treatments, according to the Tukey's honestly significant difference post-hoc test ($p \leq 0.05$

Root colonization

Six plants of each accession (TD, MJ, and CH) were harvested 8, 9 and 11 weeks after plating in the extraradical mycelium of the AMF developing in the hyphal compartment, and the dynamics of root colonization were evaluated (**Table 5**). Root colonization was observed at each harvest time, and arbuscules, vesicles/spores and hyphae were detected in the root cortex of each accession (**Figure 34**). A significant effect of the factors accession, time and interaction accession x time was noticed for % total colonization, % hyphae colonization and % vesicles colonization, while for % arbuscules colonization, only the factor accession had a significant effect. The significant interaction time x accession suggested that the influence of the accession on colonization differed with time of analysis. This is exemplified for % total colonization which significantly increased with time only for the TD accession (from 3.6% at week 8 to 52.3% at week 11).

No difference in % total colonization was noticed with harvesting time for MJ and CH, while it was significantly higher at week 11, for TD as compared to week 8 and week 9. Whatever the time of observation, no difference in % total colonization was observed between the MJ and CH accessions, while this percentage was significantly higher at week 8 and week 9 as compared to the TD accession. To the contrary, the TD accession presented a higher % total colonization as compared to the CH accession 11 weeks after plating in the hyphal compartment.

The % hyphae colonization was significantly higher at week 11 as compared to week 8 and 9 for accession TD. No significant difference was noticed between all weeks for accession CH, while it was significantly higher at week 9 versus week 8 for accession MJ. No difference in % hyphae colonization was noticed at week 11 for the three accessions, while at week 9, it was significantly higher for MJ accession, as compared to the TD accession. Finally, at week 8, no significant difference was noticed between CH and MJ, while both accessions had significantly higher % hyphae colonization as compared to accession TD.

The % vesicles colonization significantly increased between week 8 and week 11 for accessions TD, while it did not differ between week 8 and week 9. Whatever the time of observation, no significant difference was noticed for accession MJ and CH. At week 8, the % vesicles colonization was significantly higher for accession CH versus accession MJ. At week 9, no significant difference in % vesicles colonization was noticed between the three accessions. Finally, at week 11, the % vesicles colonization was significantly lower for accessions MJ and CH versus TD, while it did not differ between these two accessions.

The % arbuscules colonization was not affected by the factor time or interaction time x accession. Indeed, whatever the accession, no difference in % arbuscules colonization was observed with harvest time. Conversely, a significant effect of the factor accession was observed. At week 8, the % arbuscules colonization was significantly lower for accession TD versus CH and MJ, while it did not differ at weeks 9 and 11. No significant difference was noticed in % arbuscules colonization between accessions MJ and CH.

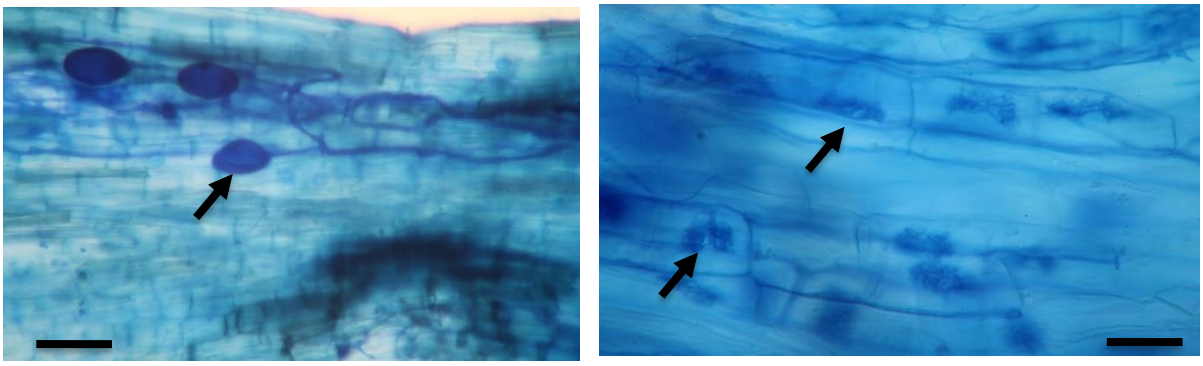

Figure 34. Intraradical structures of *Rhizophagus irregularis* MUCL 41833 in roots of argan plants after 11 weeks of associations under *in vitro* culture conditions. a Vesicles and intraradical hyphae. b Arbuscules. Bar = 40 µm

Table 5. Root colonization of argan plants (accessions TD (Tidzi), MJ (Mejji) and CH (City Hanchan)) associated with *Rhizophagus irregularis* MUCL 41833 after 8, 9 and 11 weeks of growth in the mycelium donor plant *in vitro* culture system.

Accession	%total colonization	%hyphae colonization	%vesicles colonization	%arbuscules colonization
TD/week 8	3.6 ± 3.8 d	1.5 ± 1.4 e	1 ± 1.4 bc	1.2 ± 1.5 b
TD/week9	12.1 ± 6.3cd	7 ± 1.4 d	1.2 ± 1.1 bc	3.9 ± 4.8 ab
TD/week11	52.3 ± 26.7 a	29.7 ± 10.2 a	13.8 ± 10.5 a	8.7 ± 8.4 ab
MJ/week 8	30.7 ± 10.7 abc	15.7 ± 5.3 bcd	0.2 ± 0.4 c	14.8 ± 7.9 a
MJ/week9	41.8 ± 5.7 ab	30.4 ± 4.1 a	1.2 ± 1.6 bc	10.2 ± 3.1 a
MJ/week11	39.2 ± 9.6 ab	26.2 ± 8.2 ab	2.2 ± 2.4 bc	10.7 ± 3.7 a
CH/week 8	27.2 ± 11.4 bc	10.6 ± 3.6 cd	5.2 ± 4.2 ab	11.4 ± 10.7 a
CH/week9	32.2 ± 8.5 abc	17 ± 6.2 bcd	3.2 ± 2.3 bc	11.9 ± 5.3 a
CH/week11	27.2 ± 8.3 bc	19.3 ± 7.2 abc	0.8 ± 0.7 bc	7.1 ± 2.7 ab
Effect (p-value)				
Accession	***	***	*	***
Time	***	***	*	ns
Time x accession	***	***	***	ns

For each parameter, the data represent means ± SD of 6 replicates. Data were analyzed with a two-way ANOVA (ns = no significant difference, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$). Data in a column with different lower-case letters indicate significant differences between treatments, according to the Tukey's honestly significant difference post-hoc test ($p \leq 0.05$)

Experiment 2: Acclimatization of *in vitro* plants in open pot

Root colonization

Root colonization of M and NM *in vitro*-produced argan plants was evaluated five months after acclimatization in the greenhouse. Vesicles/spores and intraradical hyphae were observed in each accession. Contrariwise, arbuscules were observed only in the TD and MJ accessions. The % total colonization significantly differed between the three accessions. The highest value was recorded in TD (77% ± 6%) then MJ (50% ± 3%) and the lowest in CH (35% ± 5.8%). The % hyphae colonization was also significantly higher in the accession TD as compared to the accession CH, while no difference was noticed between accession MJ and the two others accessions (result not showed). The % arbuscules colonization did not differ between accession TD and MJ. No significant difference was noticed in % vesicles colonization between the three accessions. No root colonization was observed in the NM plants.

Plant growth parameters

After five months of acclimatization in the greenhouse, a significant effect of the factor AMF was observed on all the plant growth parameters, while no effect of the factors accession, or interaction AMF x accession were noticed (**Figure 35 and Table 6**). More in details, with the exceptions of plant height and the number of twigs, each growth parameter for the TD accession was significantly higher for the M plants as compared to the NM plants. For the MJ accession,

the shoot dry weight was significantly increased in the M plants, while for the CH accession only the stem diameter was significantly larger as compared to the NM plants. Whatever the accession, no significant difference was noticed in the height, stem diameter, number of twigs, shoot and root fresh and dry weights of both the NM and M plants. The relative mycorrhizal dependency was the highest for the MJ accession (53%) then TD (50%) and the lowest for the CH accession (33%). The relative mycorrhizal dependency was correlated with % total colonization ($r^2 = 0.70$, $p \leq 0.05$), % hyphae colonization ($r^2 = 0.70$, $p \leq 0.05$) and strongly correlated with % arbuscules colonization ($r^2 = 0.83$, $p \leq 0.01$). On the other hand, it was negatively, but non-significantly, correlated with % vesicles colonization ($r^2 = -0.61$).

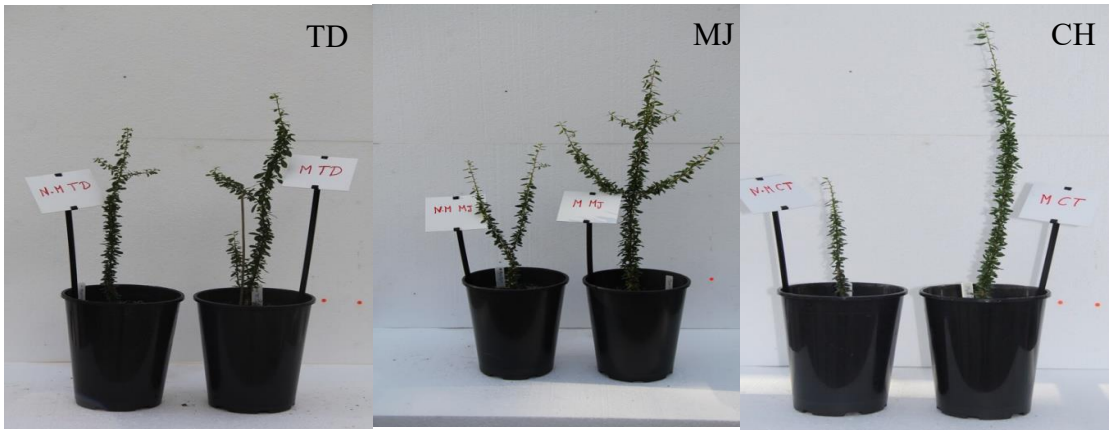

Figure 35. Impact of *Rhizophagus irregularis* MUCL 41833 on growth of argan plants (accessions TD, MJ, and CH) 5 months after of acclimatization in the greenhouse. N-M = non-my corrhizal and M = mycorrhizal. Bar = 5

Table 6. Height, stem diameter, number of twigs, shoot and root fresh and dry weights of argan plants (accessions TD (Tidzi), MJ (Mejji) and CH (City Hanchan)) grown during five months in the greenhouse, after 11 weeks of growth in presence (M) or absence (NM) of *Rhizophagus irregularis* MUCL 41833 in the mycelium donor plant in *vitro* culture system

	Height (cm)	Stem diam (cm)	Number of twigs	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
TD/M	76.8 ± 25.5 ab	6.8 ± 0.7 a	3.8 ± 0.8 a	14.7 ± 4.6 a	14.0 ± 7.2 a	5.9 ± 1.7 a	3.9 ± 2.1 a
TD/NM	63.7 ± 19.1 ab	4.9 ± 1.1 c	1.7 ± 0.8 ab	7.6 ± 2.3 b	5.9 ± 2.0 b	2.9 ± 0.7 bc	1.5 ± 0.5 b
MJ/M	89.2 ± 35.7 a	6.3 ± 0.4 ab	3.0 ± 1.6 ab	11.4 ± 5.3 ab	8.8 ± 2.3 ab	5 ± 1.8 ab	2.7 ± 1 ab
MJ/NM	46.2 ± 10 ab	5.3 ± 0.6 bc	0.8 ± 0.8 b	7.6 ± 2.3 b	6.9 ± 2.4 ab	2.3 ± 1.3 c	1.8 ± 0.7 ab
CH/M	60.6 ± 23.8 ab	6.7 ± 0.3 a	2.7 ± 2.7 ab	9.8 ± 2.5 ab	10.3 ± 4.1 ab	4.0 ± 1 abc	2.6 ± 1 ab
CH/NM	47.6 ± 11.7 b	4.5 ± 0.5 c	1.0 ± 0.6 b	6.8 ± 1.8 b	6.9 ± 2.4 b	2.7 ± 0.8 bc	1.7 ± 0.7 b
Effect (p value)							
AMF	**	***	***	***	***	***	***
Accession	ns	ns	ns	ns	ns	ns	ns
AMF x accession	ns	ns	ns	ns	ns	ns	ns

For each parameter, the results represent means ± SD of 6 replicates for the M and NM plants of the CH accession and the NM plants of the TD accession and 5 replicates for the M and NM plants of the MJ accession and the M plants of the TD accession. Data were analyzed by a two-way ANOVA (ns = no significant difference, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$). Data in a column with different lower-case letters indicate significant differences between the NM treatments of the accessions. Data in a column with different upper-case letters indicate significant differences between the M treatments of the accessions. The presence of § indicates a significant difference between the M and NM treatments within each accession, according to the contrasts test (Tukey) $p \leq 0.05$).

Chlorophyll content

Chlorophyll concentration of M and NM *in vitro*-produced argan plants was evaluated five months after acclimatization in the greenhouse (**Table 7**). A significant effect of the factor AMF was noticed on Chl*a* and Chl*b*, but not on the ratio between Chl*a* and Chl*b*, while no effect of the factors accession, and interaction AMF x accession were noticed. The concentration of Chl*a* and Chl*b* was generally higher in M plants as compared to NM ones. This was particularly marked for the Chl*b* concentration of the CH accession. No significant difference was noticed in Chl*a* and Chl*b* concentration of both NM and M plants between accessions.

Table 7. Concentration of chlorophyll a (Chl a – mg/g of fresh matter), chlorophyll b (Chl b – mg/ g of fresh matter) and Chla / Chlb ratio of argan leaves (accessions TD (Tidzi), MJ (Mejji) and CH (City Hanchan)) grown during five months in greenhouse, after 11 weeks of growth in presence (M) or absence (NM) of *Rhizophagus irregularis* MUCL 41833 in the mycelium donor plant *in vitro* culture system

Accession	Chl a (mg/gfm)	Chl b (mg /gfm)	Chl a/ Chl b
TD/M	1.4 ± 0.4 ab	0.5 ± 0.1 ab	2.9 ± 0.3 a
TD/NM	1.1 ± 0.3 ab	0.3 ± 0.1 ab	3.1 ± 0.2 a
MJ/M	1.4 ± 0.3 ab	0.4 ± 0.1 ab	3.1 ± 0.1 a
MJ/NM	0.9 ± 0.1 b	0.3 ± 0.04 ab	2.9 ± 0.3 a
CH/M	1.6 ± 0.4 a	0.6 ± 0.2 a	3.0 ± 0.2 a
CH/NM	1.1 ± 0.2 ab	0.3 ± 0.06 b	3.3 ± 0.1 a
Effet (p-value)			
AMF	***	***	ns
Accession	ns	ns	ns
AMF x accession	ns	ns	ns

For each parameter, the data represent mean ± SD of 6 replicates for the M and NM plants of the CH accession and the M plants of the TD accession, five replicates for the NM plants of the TD accession and the M plants of the MJ accession and 3 replicates for the NM plants of the MJ accession. Data were analyzed by a two-way ANOVA (ns = no significant, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$). Data in a column with different lower-case letters indicate significant differences between treatments, according to the Tukey's honestly significant difference post-hoc test ($p \leq 0.05$)

Discussion

In the present study, we reported for the first time the *in vitro* mycorrhization of three argan accessions (TD, MJ, and CH) by the AMF *Rhizophagus irregularis* MUCL 41833. The plants were successfully colonized using the mycelium donor plant *in vitro* culture system. Following their transfer to the acclimatization phase, a significant increase in plant biomass was noticed as compared to the non-colonized controls, thus opening the door to the production of *in vitro* biotized argan plants.

Whatever the accession, root colonization was observed after 8 weeks of growth in the ERM network of the AMF, with arbuscules, vesicles/spores and hyphae observed in the root system of each plant. The % colonization was high and remained almost similar between week 8 and 11 for

the MJ and CH accessions while it was low at week 8 and increased substantially at week 9 and 11 for the TD accession. The high values of % total colonization (between 30 and 50 % according to the accession at week 11) and increase over time (for the TD accession) was also observed with other woody plants such as pear (Lotfi et al., 2019) and hevea (Sosa-Rodriguez et al., 2013) or herbaceous plants such as potato (Fernández Suárez et al., 2017), banana (Koffi and Declerck 2015) and medic (Voets et al., 2009) grown in similar *in vitro* culture systems. In parallel, root length of the M plants significantly increased after 11 weeks of association for the three accessions in comparison with their respective NM plants, while shoot length did not differ between M and NM plants. The marked increase in root length of the M versus NM plants is probably due to the genetic control of the host, the fungus, or more likely a complex interaction between both partners of the symbiosis and the environmental conditions, as suggested by Sylvia et al. (2003). In our experiment, it is difficult to attribute the higher root length to an improved nutrient uptake of the M plants since the minerals were in soluble form and readily accessible to all roots, whether the plants were colonized or not. However, it is well know from the literature that lipochitooligosaccharides (myc factors) are released by the AMF in response to the strigolactones exuded by the roots and that these signals stimulate root growth and branching by the symbiotic DMI (does not make infection) signaling pathway in *M. truncatula* (Maillet et al., 2011). Therefore, it is not excluded that the dense hyphal network in the hyphal compartment stimulated root development, resulting in longer and more branched roots. This observation contrasted with the study of Koffi and Declerck (2015) on bananas. In this study no differences in root length were noticed with the controls, but the plants were in contact with the ERM for only 5 weeks, which strongly differs from the 11 weeks in our *in vitro* culture conditions. Enhanced root branching and root lengths were reported by Elmeskaoui et al. (1995) with strawberry grown *in vitro* from 10 to 20 days, but the system was a tripartite with mycorrhizal carrot roots growing in close contact with the strawberry roots, a system that strongly differed from the bi-compartmented mycelium donor plant *in vitro* culture system used by Koffi and Declerck (2015) and in the present study. The significant increase in the root/shoot length ratio in the present experiment (results not shown) during *in vitro* culture could represent an interesting factor of pre-adaptation of micropropagated plants to acclimatization as suggested by Elmeskaoui et al. (1995). The mycelium donor plant *in vitro* culture system used for the early mycorrhization of argan tree may thus represent an effective way to select the best combinations of host-symbiont associations. This technique clearly demonstrated the potential benefits of early mycorrhization (i.e. during the pre-emergence of roots *in vitro*) under autotrophic conditions on the acclimatization of argan plants during transplantation.

Five months after transfer from *in vitro* to pot culture in the greenhouse, the root colonization of plants grown in pots remained high, with values increasing as compared to those obtained *in vitro*. The highest value for total root colonization was recorded for the TD accession, followed by the MJ and CH accessions. Interestingly, under *in vitro* culture conditions (i.e. before transfer to the pots), the same tendency was noted, with the highest values of total root colonization noticed for TD, then MJ and finally CH, although the differences were only significant between TD and CH. The parallel increase in root colonization for the three accessions at transfer to the pots suggested a link between genotype and colonization values and demonstrated that the fungus was adapted to the substrate and developed concomitantly to the plant root system.

Whatever the accession, the M plants generally developed better than the NM ones. The height of shoots, the stem diameter, and the number of twigs as well as the shoot and root dry weights were generally significantly greater than those of the respective NM plants. This was also noticed for pears (Lotfi et al., 2019), bananas (Koffi and Declerck 2015) and strawberry (Cassells et al., 1996), suggesting that colonized plants may adapt more quickly to the *ex vitro* conditions in pot cultures, which presents a useful pre-adaptation for plants during transfer to the acclimatization stage (Elmeskaoui et al., 1995). The increase in plant growth parameters was further confirmed by the relative mycorrhizal dependency with values ranking from 35% to 50% and 53% for accessions CH, TD and MJ, respectively. The MJ and TD accessions showed a higher colonization rate than the CH accession, thus corroborating a correlation between the % total colonization and % hyphae colonization and the relative mycorrhizal dependency. This result confirms those obtained by Nouaim et al. (2002) who reported that clones of the argan tree - which have a high relative mycorrhizal dependency - present a higher number of arbuscules. The values of relative mycorrhizal dependency observed in our experiment further confirm the results obtained by Nouaim and Chaussod (1994) and Echairi et al. (2008) in pot cultures with values of 82% after 6 months and 48% after 9 months, respectively. Both studies were conducted with *Glomus intraradices*. Another study conducted with a native mycorrhizal complex of species reported a relative mycorrhizal dependency value of 39% after six months of growth under greenhouse culture conditions (Mrabet et al., 2014). These studies thus corroborate our work, confirming that the argan tree is a strong mycotrophic plant. Interestingly, a significant effect of AMF was noticed on the concentration of Chl*a* and Chl*b* suggesting a more efficient photosynthetic activity which depends on two factors: first, a redistribution of carbon between plant and fungus, secondly an improvement of plant nutrition, which concomitantly could have increased plant biomass. Plants increase their photosynthetic activity to cover their needs and those of the fungus (Smith and Read 2008); in return, the AMF improve the water and mineral acquisition of plants for their growth.

Conclusion

This study is the first to report on the *in vitro* mycorrhization of argan plants. Three contrasting accessions were successfully associated to *R. irregularis* MUCL41833 using the mycelium donor plant *in vitro* culture system. Plants were readily colonized within 11 weeks, and growth was strongly increased after transfer to *ex vitro* conditions. This study widens the perspectives for application; it offers not only the possibility of a large-scale production of M argan plants that perform well during acclimatization but possibly also that of an early selection of the best accessions. *In vitro* culture (by organogenesis or somatic embryogenesis) constitutes a promising route for mass multiplication of argan plants and, moreover, would contribute to the restoration and rapid regeneration of the forest ecosystem. However, controlling the multiplication and production of argan *in vitro*-produced plants is not sufficient to ensure the survival of the plants. Research should be focused on fine-tuning and optimizing rooting and acclimatization. The multiplication of argan tree by organogenesis or somatic embryogenesis in association with AMF can thus represent an innovative approach to improve rooting and their acclimatization and possibly to accelerate their transplantation in the field. Studies are now needed for the adaptation of this technology to the *in vitro* mass production of argan trees colonized by AMF.

Chapter 2

Drought resistance of *Argania spinosa* L. colonized by the arbuscular mycorrhizal fungus *Rhizophagus irregularis* varies according to accession.

Adapted from the research article accepted in: Frontiers in Plant Science

Matike Ganoudi[1,2], Soumia El Malahi[3], Nouhaila Manan[4], Mohammed Ibriz[5], Maryline Calonne-Salmon[2], Stéphane Declerck[2,*]

Preface

In the first chapter we demonstrated that argan seedlings could be associated with an AMF *in vitro* and that this association was accompanied by improved growth when the mycorrhizal plants were transferred from *in vitro* culture to the greenhouse. This improved growth was associated with better mineral nutrition and higher chlorophyl concentration and can therefore potentially be accompanied by increased resistance to certain abiotic stresses, such as drought.

Drought stress is, indeed, a critical global challenge for agriculture and forestry. In particular, the argan tree grows in the arid and semi-arid regions of Morocco, where water shortages are a constant threat to its survival, thus requiring innovative solutions to guarantee its productivity and resistance under these harsh conditions. Among these solutions, the use of symbiotic microorganisms, such as AMF, has attracted particular attention due to the ability of these obligate root symbionts to improve plant tolerance to environmental stresses such as drought.

In chapter 2, we investigated the effects of the AMF *R. irregularis* MUCL 41833 on the growth and water relations of argan accessions under water stress. Argan plants were grown in trays with or without AMF for three months in the greenhouse. The plants were then transferred to individual pots for an acclimatization period of 50 days. After acclimatization, two water regimes were applied: well-watered (WW, 100% FC) and water stressed (WS, 25% FC). For the WS plants, watering was stopped for 45 days and maintained at this level for 7 days. At the end of the experiment, the plants were harvested, and root colonization, plant growth parameters (leaf number, root and shoot length, fresh and dry shoot, and root weights), mineral nutrient content (P, K, and Na), physiological parameters (chlorophyll content, stomatal conductance, and relative water content), and biochemical parameters (total soluble sugar, proline, hydrogen peroxide, and malondialdehyde) were determined.

Abstract

Rhizophagus irregularis MUCL 41833 on three argan (*Argania spinosa* L. Skeels) accessions (Tidzi, Mejji, and City Hanchan) under well-watered (100% field capacity) and water-stressed (15% field capacity) conditions. Whatever the water regime, AMF colonization was observed in all accessions, but Tidzi showed significantly higher total root colonization than City Hanchan, while Mejji showed intermediate levels. Under well-watered conditions, the colonized plants exhibited higher biomass, root length, chlorophyll content, stomatal conductance, and root potassium (K) concentration in all accession. In the Mejji accession, colonized plants also had significantly higher shoot concentrations of phosphorus (P) and K. Under water-stressed conditions, plant response varied with accession. Compared to their respective controls, Mejii had significantly higher biomass, shoot K concentration, chlorophyll content, stomatal conductance, and reduced oxidative stress, Tidzi had also significantly higher biomass, root P and K concentration, and chlorophyll content with lower oxidative stress, while City Hanchan had significantly higher biomass and root P concentration but had higher H_2O_2 concentration. We can conclude that mycorrhization benefits all three accessions under stress conditions, with the Mejji and Tidzi accessions responding more favorably than the City Hanchan accession. These results highlight the role of AMF in improving argan tree performance under water-limiting conditions and demonstrate the variability in response between accessions.

Keywords: *Argania spinosa*, arbuscular mycorrhizal fungi, water stress, phosphorus uptake, antioxidant metabolism, accession variability

Introduction

Argania spinosa (L.) Skeels is an endemic tree species native to Morocco which is of immense economic and social importance, particularly in the arid and semi-arid regions of the country. It plays a vital role in the local economy, providing valuable products such as argan oil (Ganoudi et al.2025), which is renowned for its many nutritional, cosmetic, and medicinal properties and uses. Roasted kernels produce culinary oils, while unroasted kernels produce cosmetic oils (Mechqoq et al., 2021; El Oumari et al., 2022; Mouahid et al., 2022).

In Morocco, historical data show a clear trend of increasing temperatures and decreasing rainfall (Ohamdouch and Bhir, 2017), contributing to more frequent and severe droughts (Abdelmajid et al., 2021; Cherfi et al., 2023; Ongoma et al., 2024). These changes particularly affect argan groves, which are sensitive to rainfall variability and rising temperatures, threatening the long-term sustainability of both the ecosystem and local communities (El Ghazali et al., 2021; Afi et al., 2024)

A number of studies have identified several physiological and biochemical mechanisms by which argan trees respond to drough stress: leaf water potential and stomatal conductance have been shown to decrease significantly under drought stress, limiting CO2 fixation and thus reducing photosynthetic efficiency (Chakhchar et al., 2016; Mouafik et al., 2022). Drought stress has been reported to affect nutrient uptake leading to nutritional imbalances in plants (Chakhchar et al., 2018a). A significant decrease in the hydraulic conductivity of argan roots has also been observed under conditions of increasing drought stress (Chakhchar et al., 2019). This phenomenon can significantly impact plant survival, especially under prolonged drought conditions. Finally, an increase in the production of reactive oxygen species (ROS) has been reported under conditions of drought stress, causing oxidative damage to carbohydrates, protein synthesis, and lipid metabolism, as well as to membranes, resulting in plant cell death (Meslem et al., 2015; Chakhchar et al., 2018 b).

The TD, MJ, and CH argan accessions originate from different regions and environments, reflecting diverse ecological backgrounds and considerable phenotypic variability. In addition, they are among the most productive accessions in terms of yield and oil quality. These characteristics make them particularly valuable for assessing intraspecific differences in physiological performance and response to AMF colonization under water stress conditions.

To combat drought stress and support argan tree production, traditional irrigation methods are used. These include, among others, localized irrigation using ramps equipped with drippers (Wifaya and Mimouni, 2011). In addition, genetic diversity within the species means that the trees

best adapted to drought conditions can be identified and used in plantations (Msanda et al., 2005; Majourhat et al., 2008; Ait Aabd et al., 2020). Other approaches, such as the use of beneficial soil microorganisms, can also help to improve plant resistance to drought (Yang et al., 2021; Dai et al., 2022). For instance, arbuscular mycorrhizal fungi (AMF) have been reported to help plants resist drought stress through various mechanisms. A study synthesizing data from 187 research papers found that AMF inoculation significantly enhanced plant biomass and nutrient uptake, with greater effects on phosphorus than nitrogen (Wu and Wang, 2024). Another meta-analysis showed that AMF improved plant photosynthesis and water status under drought stress by enhancing gas exchange and water use efficiency, although the effects varied depending on drought severity (Chandrasekaran et al., 2024). In wheat, AMF inoculation increased chlorophyll content, the maximum quantum efficiency of PSII, and relative water content (RWC%), while regulating antioxidant enzyme activity, proline levels, and reducing hydrogen peroxide (H_2O_2), superoxide (O_2^-), electrolyte leakage (EL%), and lipid peroxidation (MDA) under drought stress (Abdelaal et al., 2024). Meta-analyses also highlighted the role of AMF in promoting soil functions, such as aggregation and microbial biomass, enhancing soil multifunctionality and drought resistance (Tang et al., 2024). Overall, AMF improved nutrient uptake, strengthened soil structure, increased water use efficiency, and boosted antioxidant defenses mechanisms, particularly in drought-stressed environments (Das and Sarkar, 2024; Nie et al., 2024). As a result, inoculating argan trees with AMF to increase their resistance to drought is an option that should be considered, although it is not given much thought at present. Only a few studies have shown that AMF inoculation can improve the growth, mineral nutrition, and physiological parameters of argan plants under drought stress (El Mrabet et al., 2021; Ouallal et al., 2022; Soufiani et al., 2023).

Given the importance of argan tree in arid ecosystems and its vulnerability to water scarcity, it is crucial to evaluate whether AMF can improve its drought tolerance. Therefore, in the present study, we investigated the impact of the AMF *Rhizophagus irregularis* MUCL 41833 on the morphological, physiological, and biochemical parameters, nutrient status, osmolyte accumulation, and antioxidant metabolism responses of three argan accessions grown under two distinct water regimes: well-watered and water- stressed. The aim of the study was to gain a better understanding of some of the mechanisms by which the AMF can increase the resistance of argan trees to drought stress and to explore whether there are differences between accessions, ultimately contributing to the selection of the best accessions for cultivation in water-scarce regions.

Materials and methods

Biological material

Argania spinosa (L.) Skeels

Argan fruits were collected from three different sites in the Essaouira region of Morocco, namely City Hanchan (CH), Mejji (MJ) and Tidzi (TD) (hereafter referred to as argan accessions). The fruits of the TD accession are oval-shaped, those of the CH accession are rounded, while the fruits of the MJ accession are elongated. These accessions were selected for their strong rooting, high productivity, and distinct phenotypic characteristics (see for more details in Ganoudi et al., 2021).

Arbuscular mycorrhizal fungus

Rhizophagus irregularis (Błaszk., Wubet, Renker and Buscot) C. Walker and A. Schüßler comb. nov., strain MUCL 41833, was obtained from the Glomeromycota IN vitro COllection (GINCO[1]). It was cultured in bi-compartmented Petri plates with Ri-T DNA-transformed roots of carrot (*Daucus carota* L.) strain DC2, as described by St-Arnaud et al. (1996) and Cranenbrouck et al. (2005). After three months, thousands of spores were produced in the hyphal compartment (devoid of carrot hairy roots) of the bi-compartmented Petri plates. The spores were used to inoculate maize plants in 1 L pots containing sterilized (twice at 121°C for 15 min) vermiculite and lava stone (w:w, 1:1) to produce the AMF inoculum. After 3 months, root colonization reached 98.5% and tens of thousands of spores were produced.

Experimental design

The experimental design aimed at evaluating the effects of AMF colonization (M treatment) on the drought resistance of three argan accessions (TD, MJ, and CH) grown under two water regimes: well-watered (WW = 100% Field Capacity, FC) and water- stressed (WS = 15% FC). Non-mycorrhizal plants (NM treatment) were produced and grown in a strict similar way.

The plants were first grown in trays (46 x 24 cm) in the greenhouse at a temperature of 25 ± 3°C, 330 µmol m^{-2} s^{-1} light intensity, and a relative humidity (RH) of 60% for 3 months in presence (M) or absence (NM) of inoculum of the AM fungus. They were then transferred to 1 L pots for acclimatization during 50 days. Finally, half of the plants were subjected to a decrease in water content (during 45 days) until reaching 15% of FC and then kept for an additional 7 days under

1 Glomeromycota IN vitro Collection, BCCM-MUCL, http://www.mycorrhiza.be/ginco-bel, https://bccm.belspo.be/about-MUCL

this WS condition, while the other half was kept at 100% FC (WW) during the 52 days. This resulted in four treatments: (1) M/WW, (2) M/WS, (3) NM/WW (4) NM/WS for each accession. (see below for details for the whole process). Each treatment consisted of 12 plants per accession. Plants were randomly placed on two tables in the greenhouse and pots were rotated every two weeks to minimize positional bias.

Seed sterilization, germination, and growth conditions

The experimental process began with the preparation of the argan seeds. The fruits were peeled and shelled to release the almonds, which were surface disinfected with a solution of 8% sodium hypochlorite for 15 minutes. The almonds were then rinsed three times (15 min each) with sterilized (121°C for 15 min) water to ensure the complete removal of the sodium hypochlorite. The almonds were finally placed per two in Petri plates on the Modified Strullu-Romand (MSR) medium (Declerck et al., 1998) for germination. The Petri plates were incubated in the dark at 27°C for 12 days, achieving a germination rate of 81.5%. The Petri plates with germinated almonds were then transferred to a growth chamber with a photoperiod of 16 h day^{1} (24°C/20°C, day/night), a RH of 50%, and a light intensity of 170 µmol m^{-2} s^{-1} for four days.

Mycorrhizal inoculation and greenhouse growth

Nine trays (three trays per argan accession) were prepared, containing 1200 g of sterilized soil (twice at 121°C for 1 hour). The soil sterilization was done to avoid root colonization by the AMF present in the collected soil. The sterilized soil was then left two weeks to allow for escape of volatile phytotoxic/ mycotoxic substances produced during the process of heating (Koide and Li, 1989; Mwangi et al., 2011). The sterile soil was mixed with 500 g of substrate containing *R. irregularis* inoculum. The chemical characteristic of the soil is presented in **Table 8.** Nine trays containing the same soil substrate without AMF inoculum were considered for the NM treatments.

Table 8: Chemical properties of soil samples from each site and from the greenhouse

Sites	pH	Organic matter (%)	P_2O_5(ppm)	K_2O(ppm)	CE (mS/Cm)
TD	8.26	4.9	0.8	295.2	1.6
MJ	8.03	1.8	2.4	1000.2	4.4
CH	8.17	8.7	145.0	102.4	1.5
Greenhouse soil	8.10	1.2	18.6	168.7	0.6

Sixteen-days-old argan plants, with a primary root of 2 to 10 cm, were transferred into the trays (i.e., 16 plants per tray 3 trays per accession) and were maintained under controlled greenhouse conditions (temperature of 25 ± 3°C, light intensity of 330 µmol m^{-2} s^{-1} and a RH of 60%) for three months. Plants were initially watered with distilled water and then every three days with 1 L of Hoagland's solution (Hoagland and Arnon, 1950). At the end of this phase, root colonization was assessed on three randomly selected M and NM plants for each accession. The total root colonization reached 86.2% ± 7.3%, 79% ± 2.1%, and 75.7% ± 8% for TD, MJ, and CH, respectively, while no root colonization was detected in the plants of the NM treatments.

Plants acclimatization

Among the 48 M and NM argan plants per accession in total, 36 were selected based on uniformity of size and number of leaves and transferred into 1 L pots (one plant per pot) containing a sterilized (twice at 120°C for 1 h) mixture of soil collected from the field site of each accession and compost (2/3 soil, 1/3 potting soil (Plantaflor, European Union), v/v). The pot cultivation ensured uniform growth conditions, but their limited size imposed physical confinement to the root system, potentially limiting root/AMF exploration, thus potentially influencing nutrient acquisition and the magnitude of AMF benefits. The chemical characteristics of the soils from each site are presented in **Table 8.** The plants were acclimatized in the greenhouse for 50 days at a temperature of 25 ± 3°C, a light intensity of 330 µmol m^{-2} s^{-1}, and a RH of 60%. All plants were irrigated to maintain 100% FC to ensure uniform development prior to stress application.

The calculation of FC was as follows: The different soil/compost substrates were saturated by adding distilled water until puddling occurred. After 24-48 hours of free drainage in a controlled environment at 24°C, the wet weight (ww) of the substrates were determined. They were then oven dried at 105°C for 24 hours to obtain the dry weight (dw). The FC was calculated using the formula: FC (%) = ww-dw*100/dw.

Soil moisture management using weight-based soil drying method

After the acclimatization phase, 24 plants of uniform size were selected from the 36 acclimated plants in each treatment (M or NM) and accession to apply the water regimes. The selected plants were then divided into WW and WS conditions, constituting twelve conditions as follows: $TD^{M/WW}$, $TD^{M/WS}$, $TD^{NM/WW}$, $TD^{NM/WS}$, $MJ^{M/WW}$, $MJ^{M/WS}$, $MJ^{NM/WW}$, $MJ^{NM/WS}$, $CH^{M/WW}$, $CH^{M/WS}$, $CH^{NM/WW}$, $CH^{NM/WS}$. Twelve plants were considered per treatment and randomly distributed in the greenhouse. Pot rotation was performed every two weeks to minimize positional bias.

Temperature, RH, and photoperiod were kept constant throughout the experiment, as described earlier.

Soil moisture levels were controlled using a precise weight-based soil drying method, using a high precision balance (Bionlock, BS 3000). This approach involved monitoring the combined weight of the pot, soil, and plant daily to maintain target moisture levels corresponding to specific FC. For the plants of the WW treatment, pots were initially weighed at full FC, and water was added as required to maintain this level. For the plants of the WS treatment, plants were not watered until pots reached 15% of their FC (after 45 days) This FC was kept for an additional 7 days by controlled irrigation.

Plant harvest

At the end of the experiment (i.e., 52 days after initiating the two water regimes), plants were harvested and root colonization, plant height and number of leaves, mineral nutrient content (phosphorus (P) potassium (K) and sodium (Na)), physiological (relative water content (RWC), chlorophyll content, and stomatal conductance (g_s)), and biochemical (total soluble sugars (TSS**),** proline content, malondialdehyde (MDA) and hydrogen peroxide content (H_2O_2)) parameters were measured (see sections 2.4, 2.5, 2.6, 2.7 and 2.8). In each treatment, twelve plants were used to assess height and number of leaves. From these 12 plants, three were further used to determine root colonization, three for fresh and dry weights and mineral nutrient content, three for physiological parameters and three for biochemical parameters.

Assessment of the root colonization

The percentage of root length colonized (%CRL) by the AMF was estimated following the method of Phillips and Hayman (1970). Roots were carefully harvested, thoroughly washed-free from debris, and cleared with 10% KOH. After neutralization with 1% HCl for three min, the roots were bleached in a solution of hydrogen peroxide (H_2O_2) 3.5% at 90°C for 10 min in a water bath. The roots were then stained with 2% CROWN Blue Ink (PRC) in 1% HCl (Walker, 2005) by placing the tubes in a water bath at 70°C for 30 min. Root colonization was quantified using optical microscopy (Olympus CX33RTFS2, Hamburg, Germany, version 2024) at 40X magnification (McGonigle et al., 1990). Three slides with 30 1-cm-long root fragments were made per plant to estimate the percent colonization using the line-intercept method, noting hyphae, arbuscules and spores/vesicles. For each plant, between 100 and 150 intercepts were observed (McGonigle et al. 1990).

Evaluation of plant height, number of leaves and weights

Shoot height and number of leaves were measured. Shoot height was measured from the base of the stem to the crossing point of the last leaf. Shoot and root fresh weights (SFW and RFW, respectively) were determined. Shoots and roots dry weights (SDW and RDW, respectively) were determined after drying in an oven at 65°C for 72h, until a constant weight was reached.

Determination of mineral nutrient

Phosphorus, potassium, and sodium concentrations were determined in fresh leaves, oven-dried at 60-70 °C for 48 hours, and then incinerated at 500°C for 5 hours. The P concentration was registered using a METASH 5100 model UV/VIS spectrophotometer at a wavelength of 825 nm. Potassium and Na concentrations were measured using a flame photometer (BWB-XP Technologies UK LTD), according to the method described by Gustav and Havre (1961). Available P (P_2O_5) was determined using the Olsen method (Olsen and Sommers, 1982), with sodium bicarbonate extraction performed at pH 8.5. Available P (P_2O_5) was recorded using a JENWAY 6405 UV-visible spectrophotometer.

Physiological Parameter Assessment

Relative Water Content (RWC)

Relative water content (RWC) was measured on five whole leaves per plant. The RWC was calculated as RWC = (FW−DW)/(TW−DW) × 100, where FW and DW are the fresh and dry (drying at 70°C for 48h) weights, respectively, and TW is the turgid weight after leaves were soaked in distilled water for 24h at 4°C in the dark.

Stomatal Conductance (gs)

Stomatal conductance (gs) was measured using a leaf porometer (SC-1 leaf porometer, version 2014, Decagon Devices, USA) between 10:00 and 12:00 AM. Data were collected from the same leaf located at the center of the plant.

Chlorophyll content

Chlorophyll content was measured using a SPAD-502 portable chlorophyll analyzer developed by Soil and Plant Analysis Developments (KONICA MINOLTA, Japan). The SPAD-502 is a widely used, handheld instrument designed for the rapid and precise assessment of leaf

chlorophyll content. It functions by measuring the light transmitted through the leaf in the red and near-infrared wavelengths (700 nm–780 nm) (Shibaeva et al. 2020). Prior to taking measurements, the SPAD meter was calibrated using the manufacturer's reference checker. The SPAD value for each leaf was determined as the average of 6 readings. The meter provides a digital reading between 0.0 and 99.9 SPAD units, which corresponds to chlorophyll content.

Biochemical parameter evaluation

Total soluble sugars (TSS)

Fresh leaves (500 mg) were dried at 60° for 48h, extracted with 5 mL of 70% ethanol and centrifuged at 4000 rpm for 10 min. Extraction was repeated 2 times with 70% ethanol and supernatants were collected into 25 mL volumetric flasks. The extract (0.5 mL) was pipetted from each treatment into separate test tubes containing 0.5 mL of 5% phenol, followed by 2.5 mL of concentrated sulfuric acid (90%). The mixture was then incubated for 30 minutes on ice in the dark to ensure reaction stability. The intensity of color was read at 490 nm using a spectrophotometer (GENESYS 10S UV. VIS). A standard curve was prepared using 1g of glucose per 100 mL of distilled water. Total soluble sugar (mg/g) = Sample O.D x standard concentration (mg/mL) x dilution factor/standard O.D x sample weight (g)

Proline concentration

The proline concentration was assessed according to the procedure of Carillo and Gibon (2011). Fifty mg of leaf sample was weighed and then extracted using ethanol (500 µL of 80% (v/v) heated at 80°C for 20 min). One hundred µL of a solution consisting of 1% (w/v) ninhydrin, 20% absolute ethanol (v/v), 60% glacial acetic acid (v/v), and 20% ultrapure water was added to 75 µL supernatant in the 96 wells microplate. The microplate was boiled at 80°C for 30 min. After cooling at room temperature, the absorbance was recorded at 520 nm using a microplate reader. Proline concentration was calculated from a calibration curve using proline as standard. (Carillo and Gibon, 2011).

Malondialdehyde and hydrogen peroxide concentrations

The extraction was performed by 5 mL of 5 % (w/v) trichloroacetic acid (TCA) per 0.20 g of tissue powder. After extraction in the ice bath, homogenates were centrifuged for 10 min at 12,000× g at 4°C and supernatants were used for determination of lipid peroxidation and hydrogen peroxide.

Lipid peroxidation was measured as concentration of malondialdehyde (MDA) in the leaves by the method of Verma and Dubey (2003). A total of 2 mL of the supernatant was mixed with 2 mL of 0.67% thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA) and was thereafter heated for 30 min at 95°C in a heating block and then cooled on ice. After centrifugation for 1 min at 12,000× g at 4°C, the absorbance of the supernatant was read at 532 and 600 nm. The concentration of MDA was estimated by using the extinction coefficient of 155 mM^{-1} cm^{-1}, and concentration was expressed as nanomole per gram of fresh weight (nmol g^{-1} FW).

Hydrogen peroxide (H_2O_2) concentration was quantified according to Velikova et al. (2000). After the addition of 0.5 mL of supernatant into 10 mM potassium phosphate buffer (pH 7.0) (0.5 mL) and 1 M potassium iodide (1 mL), reaction mixture was stored in the dark for 20 min. Absorbance of the reaction mixture was read at 390 nm, and H_2O_2 concentration was determined using a calibration curve obtained with different concentrations of H_2O_2 and expressed as µmole per gram of fresh weight (µmol g^{-1} FW).

Statistical analysis

Treatment effects were analyzed using analysis of variance (ANOVA) at a 95% confidence level. All analyses were conducted in Minitab® version 20 (Minitab, Inc., State College, PA, USA). Data were first checked for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. For datasets meeting both assumptions, one-way ANOVA was applied, followed by Tukey's post hoc (HSD) test for pairwise comparisons. Tukey's HSD inherently adjusts for the number of comparisons, thereby controlling the family-wise error rate and minimizing Type I error inflation.

When normality or variance assumptions were not satisfied, the Kruskal–Wallis test was employed as a non-parametric alternative. In cases of significant Kruskal–Wallis results, pairwise comparisons between treatments were performed using Dunn's test with Bonferroni correction to account for multiple testing and control Type I error. The selection of parametric versus non-parametric procedures was based strictly on the distributional properties of each parameter. Specifically, ANOVA was used to analyze root length, shoot height, number of leaves, fresh and dry weight of roots and shoots, and concentrations of P, K, and Na, as well as %CRL. Conversely, chlorophyll content, gs, RWC, and biochemical parameters were evaluated with the Kruskal–Wallis test.

Results

Root colonization

Root colonization was measured at harvest, i.e., after the application of the two WR (WW and WS) during 52 days. Whatever the WR, a significant higher %CRL was noticed for the TD accession (74.1 ± 7.0% and 62.4 ± 7.8%, for WW and WS, respectively) compared to the CH accession (63.0 ± 3.4% and 53.8 ± 4.5%, for WW and WS, respectively), while no significant difference was measured between these two accessions and the MJ accession (72.7 ± 4.8% and 60.7 ± 7.3%, for WW and WS, respectively). The %CRL decreased significantly by 14.6% between the WW and WS treatments only for the CH accession, while no significant differences were observed between these two WR for TD and MJ accessions (**Figure 36**).

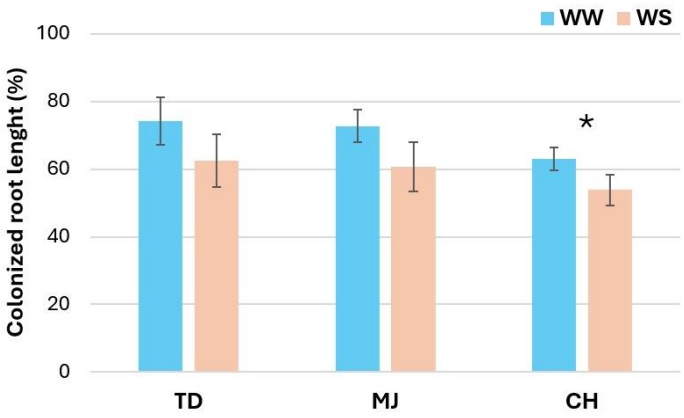

Figure 36. Effect of water regime (WR) (i.e., 100% of field capacity= Well-Watered (WW) and 15% of field capacity = Water-Stressed (WS)) on the percentage of colonized root length (%CRL) by *Rhizophagus irregularis* MUCL 41833 of three argan accessions (i.e., TD, MJ, and CH). Data are presented as means ± standard deviation. The presence of * indicates a significant difference among the means between WW and WS treatments for each accession, according to a one-way ANOVA followed by a Tukey's HSD post-hoc test ($P \leq 0.05$

Plants growth parameters

At harvest, a significant effect of the interaction "accession x AMF x WR" ($p \leq 0.001$) was measured on the number of leaves. Whatever the accession and WR, a significant higher number of leaves was observed in the M plants compared to the NM plants (Figure 1A). Similarly, whatever the accession and presence/absence of AMF, a significant higher number of leaves was noticed for plants grown in the WW condition compared to the WS condition (**Figure 37A**).

A significant effect of the interaction "AMF x WR" was observed on shoot length ($p \leq 0.01$) (results not shown). Averaged over the accessions, the shoot length of the NM plants was significantly lower in the WS condition (13.0 ± 2.8 cm) compared to the WW condition (19.7 ±

4.2 cm), while no significant difference was observed between M plants grown under WW (23.6 ± 3.0 cm) and WS (23.4 ± 2.7 cm) conditions (results not shown). Under WW conditions, no significant difference in shoot length was observed between M and NM plants, whatever the accession. However, under WS conditions, M plants had significantly longer shoots than NM plants.

A significant effect of the interaction "accession x AMF" and "AMF x WR" ($p \leq 0.01$ and $p \leq 0.001$, respectively) was observed on root length. Averaged over the two WR, the root length was significantly higher in M plants of the CH and MJ accessions compared to their respective NM plants, while no significant difference was observed for TD accession (**Figure 37B**). Averaged over the accessions, no significant difference was observed in root length between M plants grown under WW (36.8 ± 2.3 cm) and those grown under WS (37.8 ± 7.0 cm) conditions. In contrast, for the NM plants, the root length was significantly lower under WS conditions (16.8 ± 4.8 cm) compared to WW conditions (30.3 ± 7.4 cm) (results not shown). Similar to shoot length, no significant difference in root length was observed between M and NM plants under WW conditions, regardless of accession, while under WS conditions, M plants had significantly longer roots than NM plants.

Plant biomasses (i.e., SFW, SDW, RFW and RDW) were significantly impacted by the factor "AMF" ($p \leq 0.001$, $p \leq 0.001$, $p \leq 0.01$ and $p \leq 0.001$ respectively). Averaged over both the accession and the WR, the SFW, SDW, RFW and RDW were significantly higher for the M plants compared to the NM plants (**Figure 37C**). Except for SDW ($p = 0.06$), the factor "WR" also influenced the SFW, RFW and RDW ($p = 0.02$, $p = 0.02$ and $p = 0.03$, respectively). Averaged over the accessions and the presence/absence of AMF, no significant difference was observed in SFW and SDW between plants grown under WW and WS conditions, while RFW and RDW were significantly lower under WS conditions (**Figure 37D**). No significant effect of the interaction "AMF x WR" was observed on SFW, SDW, RFW and RDW ($p = 0.5$, $p = 0.2$, $p = 0.3$ and $p = 0.3$, respectively).

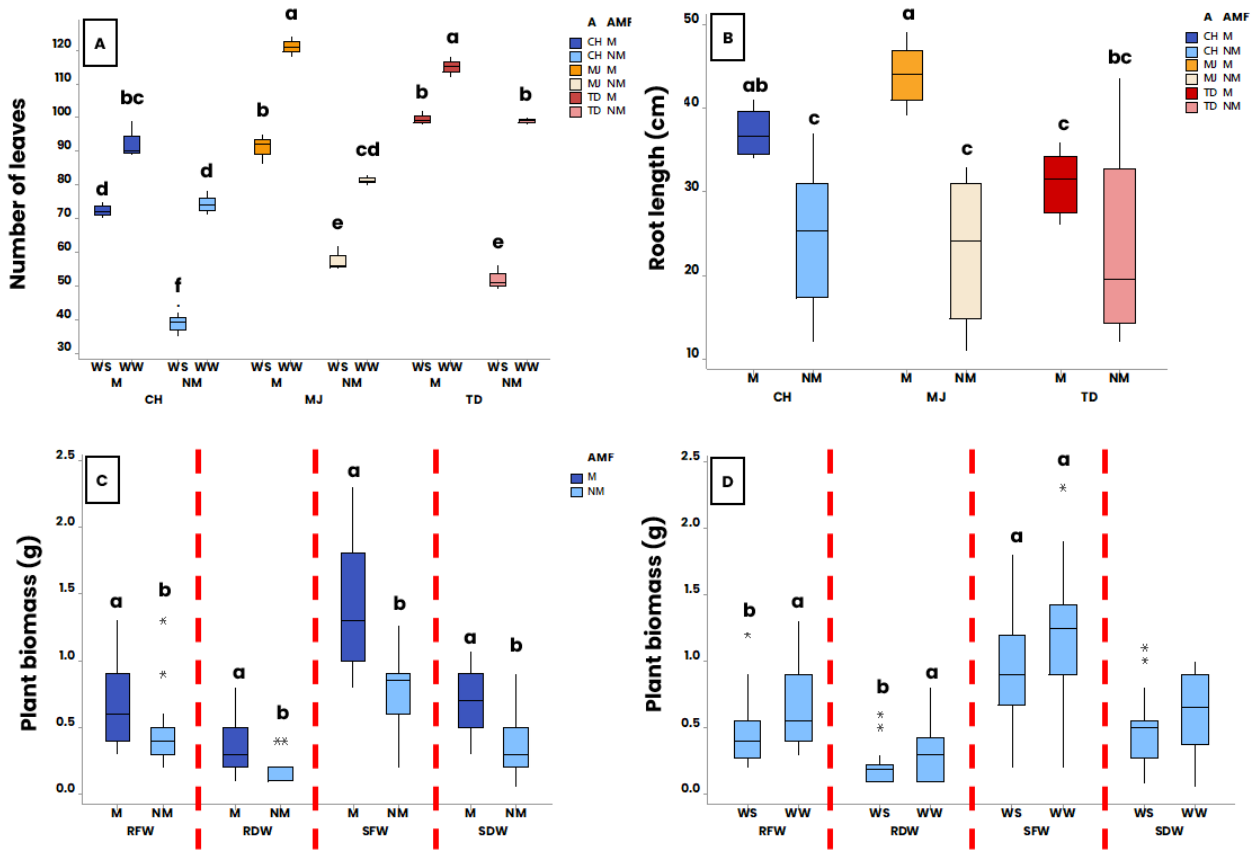

Figure 37. Effect of water regime (WR) (i.e., 100% of field capacity = Well-Watered (WW) and 15% of field capacity = Water-Stressed (WS)), AMF colonization by *Rhizophagus irregularis* MUCL 41833 (i.e. mycorrhizal (M) or non-mycorrhizal (NM)) and accession (i.e., TD (Tidzi), MJ (Mejji), and CH (City El Hanchan)) on plant growth parameters at harvest (52 days after the start of the WR application). Significance of (A) accession, AMF and WR interaction on the number of leaves, (B) accession and AMF interaction on root length, (C) AMF on total biomass (root and shoot fresh and dry weights (RFW, RDW, SFW, SDW)) and (D) WR on total biomass. The box plots display the maximum, upper quartile, and lower quartile minimum values. Data were analyzed by a three-way ANOVA followed by a Tukey post-hoc test ($P \leq 0.05$). For one parameter, data sharing similar lower-case letters are not significantly different. (*) indicates an extreme point (> 1.5 times the interquartile range beyond the quartiles), while (**) denote a very extreme point (> 3 times the interquartile range).

Plant nutrient concentrations

At harvest, P, K and Na concentrations were measured in roots and shoots (**Figure 38**).

Phosphorus dynamic under water conditions.

Phosphorus concentration was impacted by the interaction "accession x AMF x WR" in both roots ($p \leq 0.001$) and shoots ($p \leq 0.01$). In roots, no significant difference in P concentration was measured between plants grown under WW conditions and those grown under WS conditions for M and NM plants in the CH and MJ accessions, as well as for NM plants in the TD accession **(Figure 38A left)**. In contrast, a significant increase in P concentration was observed in M plants of the TD accession grown under WS conditions in comparison with those grown under WW

conditions. Under WS conditions, a significant difference in P concentration was observed between M and NM plants of the CH and TD accessions, whereas no significant difference in P concentration was observed between M and NM plants of these accessions under WW conditions. No significant difference in P concentration was observed between M and NM plants of the MJ accession under WS conditions, whereas NM plants presented a lower P concentration than the M plants under WW conditions. `

In shoots, no significant difference in P concentration was measured between M and NM plants grown under WW and WS conditions in the CH accession (**Figure 38A right**). In contrast, a significant difference in P concentration was observed between plants grown under WW and WS conditions in the MJ accession. For the TD accession, no significant difference in P concentration was found between M plants grown under WW and those grown under WS conditions, whereas NM plants grown under WS conditions had a lower P concentration than NM plants grown under WW conditions. Whatever the WR, no significant difference was observed between M and NM plants in the TD and CH accessions. In the MJ accession, the P concentration in the shoots of M plants grown under WW conditions was significantly lower than in those grown under WS conditions, whereas in the NM plants, the P concentration under WW conditions was significantly higher than in those grown under WS conditions.

Potassium dynamic under water conditions `

The K concentration in roots was influenced by the factor "AMF" ($p \leq 0.01$). Averaged over both the accession and WR, K concentration was higher in M plants (1.7 ± 0.6 mg/g) than in NM plants (1.3 ± 0.5 mg/g) (results not shown). In shoots, K concentration was impacted by the interactions "accession × AMF" ($p \leq 0.001$), "accession x WR" ($p \leq 0.05$), and "AMF x WR" ($p \leq 0.05$). Averaged over the two WR, no significant difference in K concentration was observed between M and NM plants in the CH accession (**Figure 38B**). Conversely, whereas the M plants had significantly higher K concentrations than NM plants in the MJ accession, the opposite was observed in the TD accession. Averaged over the accessions, no significant difference in K concentration in shoot was observed between WW (2.2 ± 1.1 mg/g) and WS (1.8 ± 0.5 mg/g) conditions for the M plants. Similarly, no significant difference in K concentration was observed between WW (1.6 ± 0.5 mg/g) and WS (2.4 ± 0.7 mg/g) conditions for the NM plants. On the other hand, whereas shoot of M plants presented a lower K concentration than the NM plants, the shoot K concentration remained similar between M and NM plants grown under WS conditions. Averaged over the M and NM plants, no significant difference in shoot K concentration was observed between plants grown under WW and WS conditions, irrespective of the accession

(**Figure 38C**). On the other hand, whereas plants grown under WW conditions presented similar K concentration, a significantly higher K concentration was measured in the TD accession in comparison with plants of the MJ accession, under WS conditions.

Sodium dynamic under water conditions

In drought conditions, measuring Na^+ content is relevant since water deficit often increases salt concentration in the rhizosphere, and the ability of plants to regulate Na^+ uptake and maintain a favorable K^+/Na^+ ratio is a key determinant of drought tolerance (Fayyaz et al., 2013).

The shoot Na concentration was impacted by the interaction "accession x AMF x WR" ($p \leq 0.001$). No significant difference in Na concentration was observed between M plants grown under WW and WS conditions in the CH and MJ accessions (**Figure 38D**). Conversely, a significant higher Na concentration was observed in the shoots of M plants grown under WS conditions in the TD accession. In addition, a significant difference in Na concentration was observed between NM plants grown under WW and WS conditions in the CH and MJ accessions, while it remained similar for the TD accession. In the CH accession, Na concentration was higher in NM plants grown under WS conditions than in those grown under WW conditions, while the opposite was observed in the MJ accession.

In the roots, Na concentration was influenced by the factor "WR" ($p \leq 0.001$) and the interaction "accession x AMF" ($p \leq 0.001$). Averaged over both the accessions and the presence/absence of AMF, the Na concentration was significantly higher in roots of plants grown under WS conditions (6.2 ± 0.9 mg/g) than in those grown under WW conditions (4.8 ± 1.0 mg/g) (results not shown). Averaged over the WR, no significant difference was observed in Na concentration between M and NM plants for all accessions.

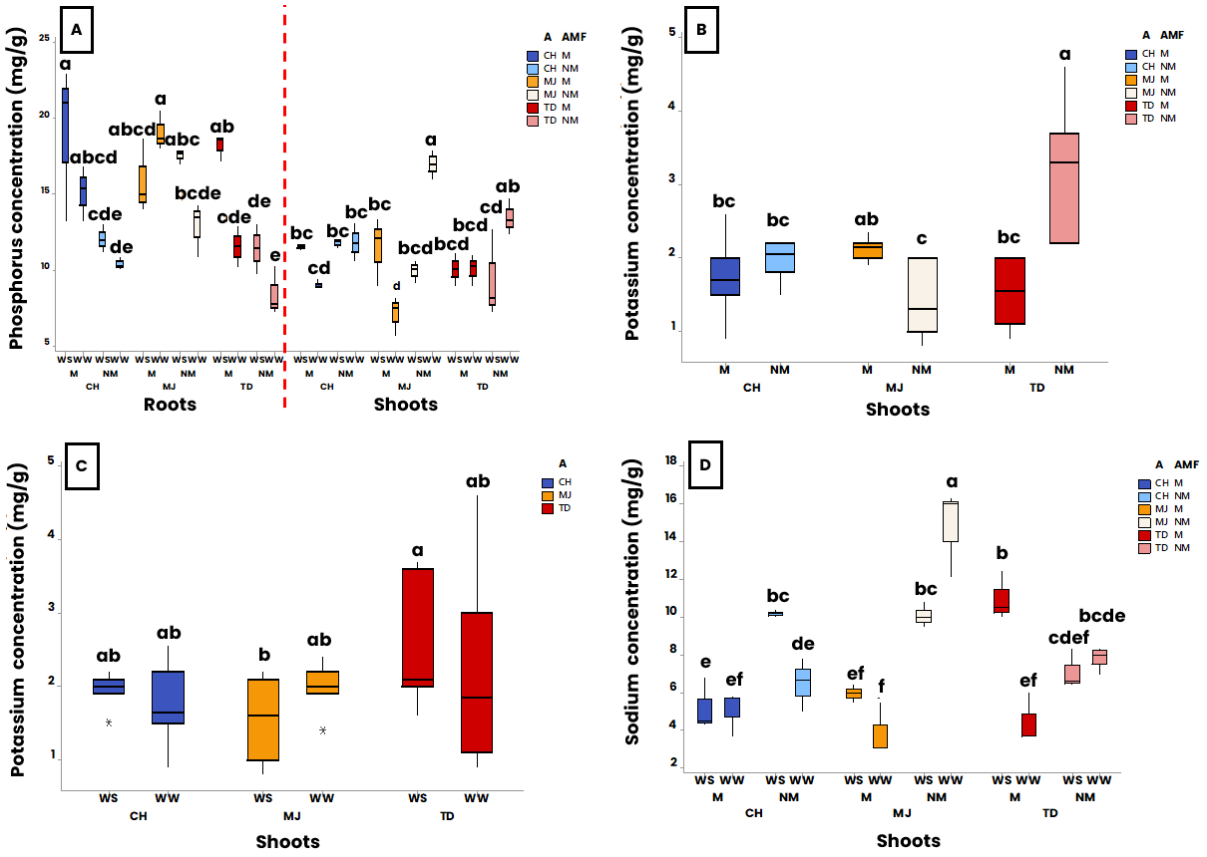

Figure 38. Effect of water regime (WR) (i.e., 100% of field capacity = Well-Watered (WW) and 15% of field capacity = Water-Stressed (WS)), AMF colonization by *Rhizophagus irregularis* MUCL 41833 (i.e. mycorrhizal (M) or non-mycorrhizal (NM)) and accession (i.e., TD (Tidzi), MJ (Mejji), and CH (City El Hanchan)) on nutrients concentrations at harvest (52 days after the start of the WR application). Significance of (A) accession, AMF and WR interaction on phosphorus concentration (B) accession and AMF interaction on potassium concentrations in shoots, (C) accession and WR interactions on potassium concentrations in shoots and (D) accession, AMF and WR interaction on sodium concentrations in shoots. The box plots display the maximum, upper quartile, and lower quartile minimum values. Data were analyzed by a three-way ANOVA followed by a Tukey post-hoc test ($P \leq 0.05$). For one parameter, data sharing similar lower-case letters are not significantly different.

Physiological parameters

At harvest, plant physiological parameters (i.e., Chl, g_s, and RWC) were measured in plants (**Figure 39**).

A significant effect of the interaction "accession x WR" ($p \leq 0.001$) and "AMF x WR" ($p \leq 0.01$) was observed for Chl content. Averaged over the presence/absence of AMF, Chl content was significantly higher in plants grown under WW conditions compared to those grown under WS conditions for all the accessions (**Figure 39A**). In addition, whatever the WR, the Chl content in accession TD was significantly higher than in MJ and CH accessions. Averaged over the accessions, the Chl content was significantly higher in the M plants than in NM plants, in both WW and WS conditions (**Figure 39B**). In both the M and NM plants, the Chl content was also

significantly higher in the plants grown under WW conditions compared to those grown under WS conditions.

The parameter g_s was impacted by the factor "AMF" and the factor "WR" ($p \leq 0.01$ for each). Averaged over both the accession and the WR, g_s was significantly higher in the M plants (262.5 ± 25.5 mmol m^{-2} s^{-1}) compared to the NM plants (150.4 ± 38.7 mmol m^{-2} s^{-1}) (results not shown) and averaged over both the accession and the presence/absence of AMF, plants grown under WW conditions had significantly higher g_s (235.5 ± 50.7 mmol m^{-2} s^{-1}) than those grown under WS conditions (177.4 ± 66.6 mmol m^{-2} s^{-1}) (results not shown).

The RWC was significantly influenced by the factor "WR" ($p \leq 0.01$). Averaged over both the accession and the presence/absence of AMF, plants grown under WW conditions had the highest RWC (90.2 ± 3.0 %) compared to those grown under WS conditions (78.3 ± 9.7 %) (results not shown)

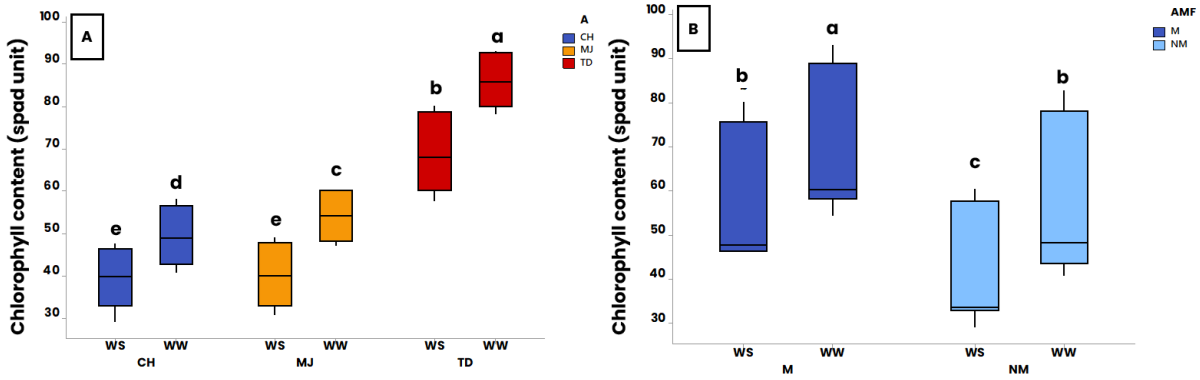

Figure 39. Effect of water regime (WR) (i.e., 100% of field capacity = Well-Watered (WW) and 15% of field capacity = Water-Stressed (WS)), AMF colonization by *Rhizophagus irregularis* MUCL 41833 (i.e. mycorrhizal (M) or non-mycorrhizal (NM)) and accession (i.e., TD (Tidzi), MJ (Mejji), and CH (City El Hanchan)) on chlorophyll content at harvest (52 days after the start of the WR application). Significance of (A) accession and WR interaction on chlorophyll content, (B) AMF and WR interaction on chlorophyll content. The box plots display the maximum, upper quartile, and lower quartile minimum values. Data were analyzed by a three-way ANOVA followed by a Tukey post-hoc test ($P \leq 0.05$). For one parameter, data sharing similar lower-case letters are not significantly different.

Biochemical parameters

At harvest, TSS, MDA, proline concentration and H_2O_2 concentrations were measured in the shoots of plants (**Figure 40**).

The TSS was impacted by the interaction "accession x AMF x WR" ($p \leq 0.01$). No significant difference in TSS concentration was measured in the M plants grown under WW and WS

condition, while in the NM plants, the TSS concentration was significantly higher under WS conditions compared to the WW conditions, irrespective of the accession (**Figure 40A**).

The MDA concentration was also impacted by the interaction "accession x AMF x WR" ($p \leq 0.001$). A significantly higher MDA concentration was measured under the WS condition compared to the WW condition, irrespective of the accession and the presence/absence of AMF (**Figure 40B**). In addition, under WS conditions, MDA concentration in shoots was significantly lower in M plants than in NM plants.

Proline concentration was impacted by both the factor "WR" and the interaction "accession x AMF" ($p \leq 0.001$ and $p \leq 0.05$, respectively). Averaged over both the accession and the presence/absence of AMF, plants grown under WS conditions had a significantly higher proline concentration (4.2 ± 1.6 µmol/g) than those grown under WW conditions (2.4 ± 1.6 µmol/g) (results not shown). Averaged over the two WR, no significant difference was observed in proline concentration between the M and NM plants of the CH and TD accessions, while in MJ, a significant higher proline concentration was observed in the NM plants compared to the M plants (**Figure 40C**). The proline concentration was significantly higher in the M and NM plants of the CH accession in comparison with the accessions MJ and TD.

The hydrogen peroxide concentration in shoot was impacted by the factor "accession", "AMF" and "WR" ($p \leq 0.001$ for each). Averaged over both the presence/absence of AMF and the WR, the CH accession presented significantly higher H_2O_2 concentration (2.7 ± 0.5 µmol/g) than MJ (1.9 ± 0.9 µmol/g) which also presented a significantly higher H_2O_2 concentration than TD (1.6 ± 0.8 µmol/g) (result not shown). Averaged over both the accession and the WR, NM plants had significantly higher H_2O_2 concentration (2.9 ± 0.3 µmol/g) than M plants (1.7 ± 0.9 µmol/g) (result not shown). Finally, averaged over both the accession and the presence/absence of AMF, plants grown under WS conditions had significantly higher H_2O_2 concentration (3.0 ± 1.0 µmol/g) than those grown under WW (1.1 ± 0.3 µmol/g) conditions.

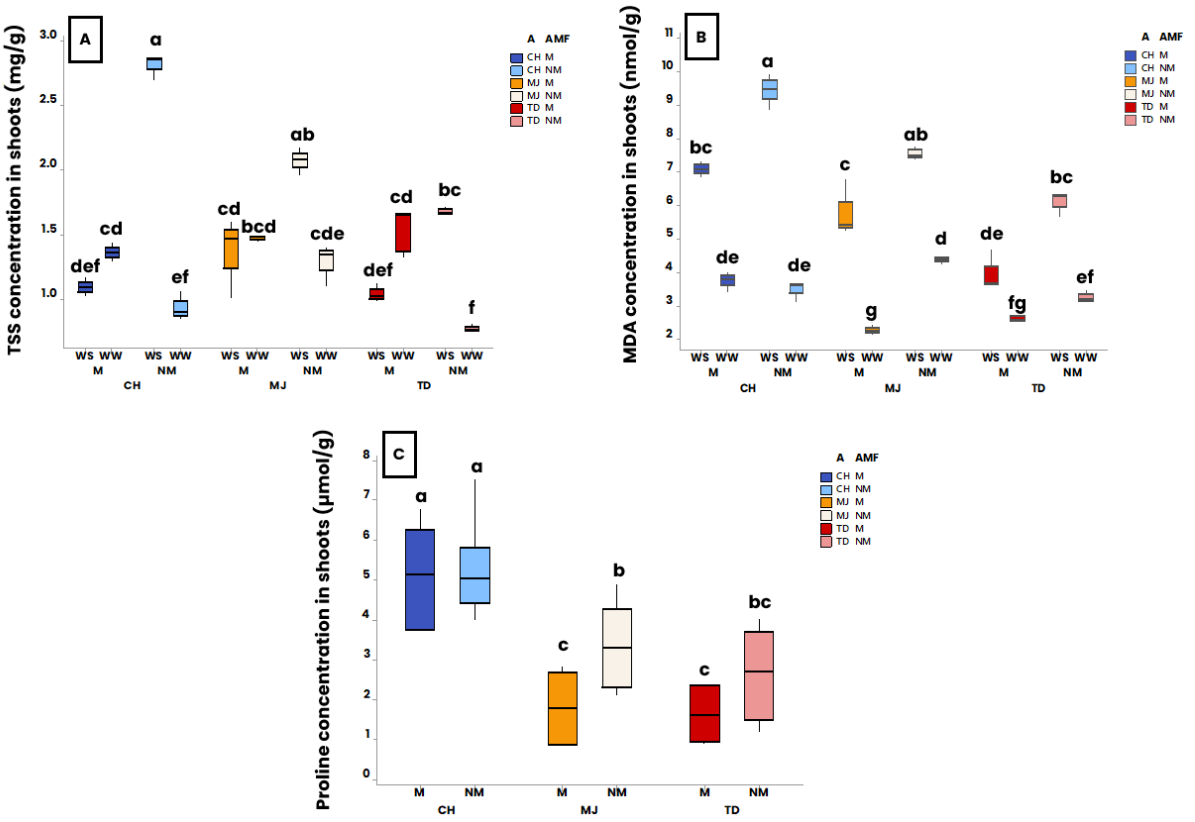

Figure 40. Effect of water regime (WR) (i.e., 100% of field capacity= Well-Watered (WW) and 15% of field capacity = Water-Stressed (WS)), AMF colonization by *Rhizophagus irregularis* MUCL 41833 (i.e. mycorrhizal (M) or non-mycorrhizal (NM)) and accession (i.e., TD (Tidzi), MJ (Mejji), and CH (City El Hanchan)) on biochemical parameters at harvest (52 days after the start of the water regime application). Significance of (A) accession, AMF and WR interaction on TSS concentration, (B) accession, AMF and WR interaction on malondialdehyde (MDA), and (C) accession and AMF interaction on proline concentration. The box plots display the maximum, upper quartile, and lower quartile minimum values. Data were analyzed by a three-way ANOVA followed by a Tukey post-hoc test ($P \leq 0.05$). For one parameter, data sharing similar lower-case letters are not significantly different.

Discussion

Drought is one of the main environmental threats affecting plant growth and productivity. The role of AMF in mitigating the effects of this factor on plants is well established (Begum et al., 2019; Bahadur et al., 2019; Diane et al., 2020; Al Karaki and Williams, 2021) and has been reported on argan (Outmamat et al., 2021). However, it is not known whether the response of this tree to AMF under drought conditions varies according to accession. Here, we investigated the effects of the AMF *Rhizophagus irregularis* MUCL 41833 on growth, nutrient concentrations, physiological parameters, and biochemical responses of three argan accessions (Tidzi, Mejji, and City Hanchan), grown under two water regimes (WR): well-watered (WW) and water- stressed (WS).

AMF positively impact argan accessions under well-watered conditions

All accessions were colonized, with TD and MJ showing significantly higher root colonization than CH, which correlated with a significant increase in leaf number, root length, and biomasses compared to non-colonized plants. Root length was also significantly increased in the accessions CH and MJ, confirming the often-observed role of AMF in promoting root system development (Ruiz-Lozano, 2003). However, this was not accompanied by a significant difference in shoots or roots P concentration between AMF-colonized and non-colonized plants in the CH and TD accessions, whereas in the MJ accession, P concentration was significantly lower in the shoots and higher in the roots of AMF-colonized plants. The absence of effect on shoot P concentration could reflect dilution, as suggested by Smith et al. (2011), given that colonized plants from all accessions had significantly higher biomass. Though, this was not confirmed in our study; the total P content in shoots did not differ between the AMF-colonized plants and the non-colonized ones (see supplementary results). This lack of differences may be linked to the form of P and the size of the pots. In fact, P was applied in the form of KH_2PO_4 in Hoagland's solution (1 L every three days) in small pots (1 L), so in a form that was easily available to the roots and in a reduced exploration volume. This probably reduced the added value of hyphae that are able to recover P from less accessible forms or beyond the root depletion zone. Other factors have also been suggested to explain differences between plants, such as AMF species/strain, with some plant/AMF combinations being more efficient than others (Klironomos, 2003; Smith et al., 2011), but these were not explored in the present study.

The AMF-colonized plants had a significantly higher K concertation in roots, irrespective of accession. This suggests that *R. irregularis* MUCL 41833 can improve the uptake of this nutrient via its extraradical hyphae and store it in the intraradical fungal structures and/or root cells. This observation is in agreement with the study by Scheloske et al. (2004), showing a higher concentration of K in *Aster tripolium* roots colonized by *R. irregularis* compared with non-colonized plants. Regarding the shoot K concentration, the plant response differed with accessions. In accession MJ, the AMF-colonized plants had a significantly higher K concentration than the non-colonized plants as previously shown for *Pelargonium peltatum* (Perner et al., 2007) and *Lactuca sativa* (Baslam et al., 2014), while in accession CH, there was no significant difference and in accession TD, it was significantly lower than in the non-colonized plants. This demonstrates, once again, that the effects of AMF may vary depending on plant genotype and host compatibility (Klironomos, 2003; Smith and Smith, 2011). Regardless of the accession, the concentration of Na in the shoots and roots did not differ between AMF-colonized and non-

colonized plants. The presence of AMF probably did not influence Na concentration, as plants are probably able to maintain ion homeostasis through their own regulatory mechanisms when water is not a limiting factor (Smith and Read, 2008).

Irrespective of the accession, the chlorophyll content and stomatal conductance of AMF-colonized plants were significantly higher than those of their respective controls. Similar results were obtained by Outamamat et al. (2022). This suggests a greater photosynthetic efficiency and improved gas exchange regulation in AMF-colonized plants, as previously documented in other crops such as *Catalpa bungei* (Chen et al., 2020), wheat (Mathur et al., 2019) and hybrid poplar (Liu et al., 2015).

Oxidative stress was reduced in plants colonized by AMF in all accessions in absence of stress. A significantly lower concentration of MDA and H_2O_2 was observed in AMF-colonized plants compared with the non-colonized plants. This suggests that the symbiotic relationship enhances the plant's ability to mitigate oxidative damage, probably by activating antioxidant defense systems and stabilizing cellular structures. It has been shown in the literature that lower MDA concentrations reflect reduced lipid peroxidation, while lower H_2O_2 concentrations suggest increased scavenging of reactive oxygen species (Gill and Tuteja, 2010). These results demonstrate that AMF support plant growth and play a role in optimizing oxidative metabolism under normal growth conditions. Furthermore, the accumulation of proline is generally a physiological response in plants exposed to various abiotic stresses. In absence of stress, AMF may have little effect on proline concentration, or may even reduce concentration slightly, as the plant does not need to accumulate osmoprotectants such as proline. This was confirmed in CH and TD accessions, for which proline concentrations did not differ between treatments. In contrast, colonized plants from the MJ accession had significantly lower proline concentrations than their non-colonized counterparts. A significant increase in TSS concentration was observed in AMF-colonized plants compared to non-colonized plants for both CH and TD accessions under WW conditions. This suggests that AMF may enhance carbohydrate metabolism even in the absence of drought stress, likely by improving nutrient uptake and photosynthesis activity (Augé, 2001; Ruiz-Lozano, 2003). The increase in TSS concentration could reflect improved carbon allocation or increased transport of sugars from source to sink organs, both have been associated with AMF symbiosis (Smith and Read, 2008). However, no significant differences were observed for the MJ accession, which suggested a possible genotypic response to AMF colonization. Variability in mycorrhizal responsiveness between genotypes has been widely documented (Wang et al., 2021) and could be attributed to differences in root architecture, mycorrhizal dependency, or

physiological characteristics. The lack of response in the MJ accession suggests that its carbon metabolism under WW conditions may be less influenced by AMF.

AMF mitigates the impact of drought on argan tree accessions

Growth responses of argan accessions under drought stress conditions and role of AMF colonization

Whatever the argan accession, plant growth (i.e., number of leaves, shoot and root length and biomass production) was markedly affected under WS conditions, in the presence as well as absence of AMF colonization. This is consistent with previous studies reporting the detrimental effects of drought stress on the growth of argan trees (Bezzalla et al., 2018). The decrease in biomass can be attributed to a reduction in nutrients uptake. When soil water content is reduced, stomata close, transpiration decreases and, as a result, the flow of water and nutrients slows down due to the reduced rate of diffusion of nutrients through the soil to the root surface (Yoo et al., 2009). The more severe the drought, the lower the flow of water and nutrients and the more limited the availability of nutrients for uptake by the root system. This was emphasized in our study, in which shoot P concentration was significantly lower under WS than WW treatment in non-colonized plants of TD and MJ accessions. Conversely, no significant differences were observed in root P concentration of non-colonized plants across all accessions under WW and WS conditions. This suggests that P accumulates in the roots and is not transferred to the shoots, due to reduced transpiration. Similarly, a significant increase in Na was noticed in the roots of the non-colonized plants under WS compared to WW conditions, likely due to reduced transpiration limiting Na transport to shoots (da Silva et al., 2011). Regarding K, no significant difference was noticed in shoot concentration between non-colonized plants grown under WW and WS conditions, suggesting that K homeostasis was maintained under different water regimes (Wang et al., 2013).

AMF-colonized plants had a significantly higher number of leaves, shoots and roots length, as well as biomass production compared to the non-colonized plants, regardless of accession. Similar observation has been made in argan by Outamamat et al. (2022), Ouallal et al. (2022) and Soufiani et al. (2022) as well as in other plants such as date palm (Baslam et al., 2014; Benhiba et al., 2015), olive tree (Fouad et al., 2014), and carob (Essahibi et al., 2018). This suggest that AMF play a critical role in mitigating the effects of drought stress, possibly by increasing root hydraulic conductivity and nutrient uptake (Sánchez-Romera et al., 2016; Begum et al., 2019).

In roots, particularly in the CH and TD accessions, a significantly higher P concentration was observed in the AMF-colonized plants compared to the respective non-colonized plants. This higher P concentration could be attributed to an increased uptake by the extraradical mycelium of the fungus and possible accumulation in the intraradical structures. AMF improves the acquisition of P by facilitating water transport through its extraradical hyphae, which promotes the mobilization and translocation of inorganic P to the root cortical cells. This process is regulated by the water potential gradient generated by host plant transpiration and the activity of fungal aquaporins. Together, these factors play a critical role in P transport (Kikuchi et al., 2016). In shoot, no significant difference in P concentration was observed between AMF-colonized and non-colonized plants in all accessions, while in the MJ accession, shoot P concentration in AMF-colonized plants was significantly higher in plants under WS than plants under WW conditions. However, shoot P content was significantly higher in AMF-colonized plants compared to non-colonized plants under WS conditions, regardless of the accession (see Supplementary Results). This effect could be explained by the increased acquisition of P by the hyphal network, under WS conditions, even in confined spaces such as 1 L pots.

The higher P concentration in AMF-colonized plants versus non-colonized plants under WS conditions can be explained by the unique physiological and structural characteristics of AMF. Their extraradical hyphae can grow and remain active at water potentials much lower than those of roots, allowing them to continue to take up and transport water and nutrients, including P, when root function is slowed down (Augé, 2001; Allen, 2007). Root hairs are more susceptible to desiccation and rapidly lose their efficiency in dry soils (North and Nobel, 1997). In addition, the diameter of hyphae is much smaller than root hairs, enabling them to penetrate tiny soil pores and access water and nutrients that are physically inaccessible to root hairs, particularly during drought when diffusion is limited. This small-scale exploration of the soil increases the effective volume of soil available to the plant for water and P uptake (Jakobsen et al., 1992; Smith and Read, 2008). Under WS conditions, the efficiency of the direct P uptake pathway by roots is often reduced. In contrast, the mycorrhizal pathway is increasingly important, as AMF contribute to maintain nutrient acquisition. This increased contribution of the mycorrhizal pathway promotes P uptake, thus compensating for the reduced functionality of the direct root pathway (Smith and Smith, 2011; Ruiz-Lozano et al., 2016).

The AMF-colonized plants had a significantly higher concentration of K and higher content of K (see supplementary results) in roots than non-colonized plants, regardless of the accession but also water regime, suggesting that AMF played a beneficial role in enhancing K uptake. This could be

attributed to improved transport capacities and soil exploration facilitated by the mycorrhizal network (Garcia and Zimmermann, 2014; Al Karaki and Wiliams., 2021). In shoots, no significant difference in K concentration was observed between WS and WW conditions in both AMF-colonized and non-colonized plants. This suggests that K homeostasis was maintained regardless of water availability or mycorrhizal status. As K plays a crucial role in osmoregulation, stomatal opening and enzyme activation, plants may prioritize maintaining its levels during drought stress to preserve essential physiological functions. In some species, K is efficiently remobilized, or its uptake is tightly regulated under stress, resulting in stable tissue concentrations (Wang et al., 2013).

Physiological responses of argan accessions to drought stress and role of AMF colonization

Whatever the accession, the non-colonized plants grown under WS conditions had significantly lower chlorophyll content than those under WW conditions. This reduction is a typical physiological response to drought, as drought stress disrupts chlorophyll metabolism by increasing chlorophyll degradation and inhibiting the biosynthesis of chlorophyll pigments (Anjum et al., 2011). These disturbances in pigment metabolism reduce the net photosynthetic rates, thereby compromising plant physiological performance under drought conditions. (Augé, 2001; Ruiz-Lozano et al., 2016; Bhardwaj et al., 2023; Cui et al., 2024).

Similarly, under WS conditions, the g_s of non-colonized plants significantly decreased compared to those under WW conditions. This is a well-known strategy of plants to reduce transpiration and keep water. However, this reduction also limits CO_2 uptake, leading to decreased photosynthesis and growth (Flexas et al., 2004; Chaves et al., 2003). These results corroborate those of Chakhchar et al. (2016), which reported a decrease of g_s in argan trees under water-stressed conditions. Comparable response has been documented in olive trees (El Yamani et al., 2020; Boussadia et al., 2023) and wheat (Ahmed et al., 2018). Finally, the RWC of the non-colonized plants grown under WS conditions was significantly lower than under WW conditions, confirming the findings of Keyvan (2010) and Patané et al. (2022), which demonstrated that a decrease in RWC under drought stress is associated with reduced water uptake and increased transpiration.

Plant colonization by AMF positively influenced the physiological parameters of argan accessions under WS conditions. Regardless of the accession, AMF-colonized plants had significantly higher RWC, chlorophyll content, and gs than non-inoculated plants. A significant increase in leaf RWC was noticed, indicating improved plant water status under WS conditions. This increase is likely due to the ability of AMF to promote water uptake through, among others, their extensive hyphal network and stimulation of root physiological activity (Wu et al., 2013). By maintaining better

hydration, symbiosis with AMF could indirectly promote higher gs and improve photosynthetic performance under WS conditions (Augé, 2001).

The AMF-colonized plants had significantly higher chlorophyll content than non-colonized plants. This increase can be related to several physiological mechanisms. Firstly, AMF increase the uptake of water and nutrients, particularly P, which are vital for the synthesis of chlorophyll and photosynthesis. This was corroborated in our study by a significant increase in P concentration and content in both roots and shoots of AMF-colonized plants, regardless of accession. This increased nutrient availability helps to maintain and synthesize chlorophyll pigments under WS conditions. Similar observations were made in gas exchange parameters, such as gs, between colonized and non-colonized plants. This suggests that AMF colonization could increase photosynthesis by improving the gas exchange capacity of plants under WS conditions. AMF are considered a metabolic sink for the mobilization of photosynthates to plant roots, thereby providing signals for increased photosynthetic activity (Metra et al., 2021). Numerous studies on the argan tree have shown that AMF-colonization can improve RWC, chlorophyll content and gas exchange (Outamamat et al., 2022; Ouallal et al., 2022).

Biochemical responses of argan accessions to drought stress and role of AMF colonization

Whatever the presence/absence of AMF, WS conditions significantly increased the concentration of H_2O_2 and MDA in all accessions compared to the WW conditions. Nonetheless, the impact was significantly more marked in the non-colonized plants, indicating a less pronounced oxidative stress when plants are associated with the AMF. Similar observations have been reported by Fouad et al. (2014) in olive plants and Chitarra et al. (2016) in tomato, which demonstrated that drought stress enhances the production of ROS, including H_2O_2. A significantly higher H_2O_2 concentration was observed in the CH accession, compared to the MJ and TD accessions, regardless the presence/absence of AMF and water regime, further suggesting a larger oxidative stress and a possible weaker antioxidant defense. This variation in H_2O_2 accumulation between accessions may be related to genetic differences in antioxidant defense mechanisms and stress tolerance (Gill and Tuteja, 2010). The colonization of plants with AMF resulted in a significantly lower concentrations of H_2O_2 and MDA across all accessions compared to non-colonized plants. The lower MDA concentrations in AMF-colonized plants versus non-colonized plants was already reported by Wu et al. (2006) in *Poncirus trifoliata citrus tangerine* and Hau et al. (2018) in *Triticum aestivum.* A reduced accumulation of ROS was also reported by Baslam et al. (2014) in *Phoenix dactylifera*, by Fouad et al. (2014) in olive plants and by Benhiba et al. (2015) in date palm, indicating reduced oxidative stress in AMF-colonized plants. Indeed, under drought stress,

excessive ROS production can cause cellular damage, especially lipid peroxidation. These observations highlight the protective role of AMF in helping plants maintain cellular balance under drought stress conditions. Among accessions, TD showed the most efficient antioxidant response following AMF colonization (lowest H_2O_2), while CH showed the highest oxidative stress indicators. This highlights the importance of genotype in modulating the effectiveness of AMF associations under drought. In addition to this role in mitigating oxidative stress, AMF colonization influences other biochemical responses to drought stress, including the regulation of osmolyte and osmoprotectant accumulation. As such, both TSS and proline concentration increased significantly in the non-colonized plants under WS conditions across all accessions, while AMF-colonized plants maintained stable TSS concentration. This suggests that AMF helps to regulate osmolyte accumulation, by improving water and nutrient uptake such as P and K, particularly observed in MJ and TD accessions, maintaining photosynthetic activity, and minimizing oxidative stress (Ruiz-Sánchez et al., 2010; Barros et al., 2018). These results support the protective role of AMF under drought conditions (Benhiba et al., 2015; Essahibi et al., 2018; Outamamat et al., 2022).

The accumulation of proline is a well-known adaptive response to drought stress and is often considered a reliable biochemical marker of stress resistance in plants (Szabados and Savouré, 2010; Hayat et al., 2012), highlighting its multifaceted role as a key osmoprotectant. Proline contributes to cellular osmotic adjustment by balancing the intracellular osmotic potential, thereby helping cells retain water during dehydration. Proline also plays a critical role in stabilizing proteins, enzymes, and cell membranes, thereby protecting structural integrity under stressful conditions (Kavi Kishor and Sreenivasulu, 2014; Szabados and Savouré, 2010). Moreover, proline acts as a molecular chaperone and an efficient scavenger of ROS, thereby mitigating oxidative damage in stressed tissues (Szabados and Savouré, 2010). The higher proline concentration regardless the accession and the AMF colonization under WS than plant under WW conditions, reflects a well-known plant response to drought stress. This accumulation is widely documented across plant species and is recognized as a reliable biochemical marker of water deficit (Kishor et al., 2005). The consistency of this response, independent of genotype or symbiotic status, suggests that proline biosynthesis is a fundamental and conserved mechanism of plant stress resistance (Szabados and Savouré, 2010).

Conclusion

In this study, we demonstrated that AMF significantly improve morphological, physiological, and biochemical traits of argan plants under drought stress conditions. The benefits of AMF colonization were particularly evidenced in the TD and MJ accessions. AMF-colonized plants showed increased shoot and root length, shoot, and root biomass, and enhance uptake of both immobile (e.g., P) and mobile (e.g., K) nutrients compared to their respective non-colonized plants. AMF also increased chlorophyll content and stomatal conductance under drought stress conditions. In terms of biochemical traits, AMF colonization reduced oxidative stress by lowering the accumulation of MDA and H_2O_2 and modulating the accumulation of proline and TSS.

The three argan accessions responded differently to colonization by *R. irregularis* under drought stress conditions. Mejii accession showed a positive response to drought stress, demonstrating significant improvements in growth, nutrient uptake, and stomatal conductance, as well as reduced oxidative stress. Tidzi accession also performed well, showing increased biomass and nutrient uptake, as well as reduced oxidative damage. In contrast, City Hanchan accession showed some growth improvements, but also showed higher H_2O_2 concentrations, indicating higher sensitivity to drought stress. Overall, MJ and TD accessions exhibited a significantly enhanced responses to mycorrhization under drought stress compared to CH accession. Performing transcriptome analyses would strengthen our understanding on the variability of responses of the argan accessions, revealing some of the mechanisms involved in the increased resistance of AMF-colonized argan trees to drought stress.

This study highlights the genotypic variability of argan accessions in their response to AMF colonization, which has important implications for breeding programs aimed at improving drought resistance. This variability suggests that the selection and propagation of AMF-compatible genotypes could be a promising strategy to improve the adaptability of argan trees to climate change. However, the results are based on controlled pot experiments that do not fully consider the complexity of natural soil and field conditions. Pot experiments may exaggerate root confinement, alter soil microbial interactions, and limit long-term plant-fungus dynamics. Therefore, future research should prioritize field trials in diverse agroecological zones to validate these findings and better understand the stability of AMF benefits under varying environmental conditions. Integrating AMF inoculation strategies into argan breeding programs could represent a sustainable approach to improve the resilience and productivity of this species in regions subject to drought.

Chapter 3

Rhizophagus irregularis MUCL 41833 improves phosphorus uptake and water use efficiency in maize plants during recovery from drought stress.

Olivia Le Pioufle, Matike Ganoudi, Maryline Calonne-Salmon, Fatma Ben Dhaou and Stéphane Declerck

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Olivia Le Pioufle and Ganoudi Matike had the equal contribution in this work

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Preface

In the second chapter, we demonstrated that under water stress conditions, the AMF-colonized TD and MJ accessions had a significantly higher number of leaves, shoot and root length, shoot and root biomass compared to their respective non-colonized controls. Nutrient uptake, including P and K, was also significantly increased in the AMF-colonized TD and MJ accessions compared to the controls. Whatever the accession, the AMF-colonized plants showed a better water-use efficiency (WUE), showed by a significantly higher chlorophyll content and stomatal conductance compared to the controls. Finally, AMF contributed to stress alleviation, with the colonized plants having a significantly reduced MDA and H_2O_2 accumulation highlighting a stronger protective effect in MJ and TD accessions.

Given the benefits of AMF in improving drought tolerance of argan tree, we extended our research to maize, a staple crop of great economic importance and food security in Morocco. Numerous studies have reported a better resistance/tolerance of mycorrhizal maize to drought, whereas the beneficial effects of AMF (in term of P acquisition and WUE) during drought recovery have been less investigated. The main objective of this third chapter was to investigate the potential of AMF in helping maize to recover after a drought stress period.

Maize plants were grown in pots with or without AMF for three weeks. The plants were then transferred to individual containers consisting of 500 ml wash bottles for an acclimatization period of 24 days in a semi-hydroponic cultivation system. Three water regimes were applied: well-watered (WW, 100% FC), moderately watered (MW, 50% FC) and poorly watered (PW, 25% FC). The water regimes were applied for 1 week, after which the circulatory system was started for the first time with Hoagland$^{low\text{-}Pi}$ solution without any analysis. The same water regimes were then applied for 2 weeks before the circulatory system was restarted for the second time. Each period was followed by a recovery period of 42 h. At the end of the experiment, the plants were harvested, and root colonization, plant growth parameters, Pi uptake/immobilization, and physiological parameters linked to the WUE, such asCO_2 assimilation rate, stomatal conductance, transpiration, instantaneous WUE, and relative water content, were determined.

Abstract

Irregular precipitations are likely to affect maize production in the future. Arbuscular mycorrhizal fungi (AMF) have been reported to increase maize resistance to drought, but their role on the short-term inorganic phosphorus (Pi) uptake, leaf gas exchange parameters and water content during recovery after drought remains poorly understood. Here, we investigated these parameters in maize plants colonized or not by *Rhizophagus irregularis* MUCL 41833. The mycorrhizal (M) and non-mycorrhizal (NM) plants were grown for a 3-weeks period in a circulatory semi-hydroponic cultivation system and were submitted to well-, moderately- or poorly- watered conditions (WW, MW and PW, respectively), the two latter conditions corresponding to moderate and severe droughts. The plants were then watered at field capacity for 42 h with a Pi impoverished Hoagland nutrient solution and the dynamics of Pi depletion in the nutrient solution, corresponding to Pi uptake/immobilization by the maize-AMF associates, was evaluated at 0, 9, 21 and 42 h. The CO_2 assimilation rate (A), stomatal conductance (gs), transpiration (E) and instantaneous water use efficiency (WUEi) were also assessed at 0 and 42 h of circulation. Plant biomass, plant water content, phosphorus concentration and content, and leaf relative water content were evaluated at harvest. During recovery, Pi uptake was significantly higher in M versus NM plants whatever the water regime (WR) applied before recovery. AMF colonization did not affect leaf gas exchange parameters before recovery but modulated gs and E, and improved WUEi after 42 h of recovery. At harvest, no significant difference in dry biomass was found between M and NM plants but shoot fresh weight was significantly higher in M plants. This resulted in an increased shoot water content in M plants grown in the MW and PW treatments. Surprisingly, leaf relative water content was significantly lower in M plants when compared with NM plants. Finally, P content and concentration were significantly higher in roots but not in shoots of M plants. Our results suggested that AMF can play a role in drought resistance of maize plants by increasing the Pi uptake and WUEi during recovery after drought stress.

Keywords: maize, arbuscular mycorrhizal fungi, drought recovery, phosphorus uptake, leaf gas exchange, water use efficiency.

Introduction

Maize (*Zea mays* L.) is the third most consumed cereal worldwide, after rice and wheat (FAO, 2013). It is considered a main staple food in Southern and Eastern Africa, Central America and Mexico, playing an important role in food security (Ranum et al., 2014). The trend for more erratic rainfall in the next 50 years poses, however, a severe threat to maize production (Li et al., 2009; Challinor et al., 2014; Rosenzweig et al., 2014) Indeed, as a C4 plant, maize is known to have a higher water use efficiency than C3 plants such as wheat and barley (Akita and Moss 1972). Yet, maize tends to have higher grain yield reduction under water shortage conditions and a higher drought sensitivity when compared to wheat (Daryanto et al., 2016). This could be explained partly by the drought avoidance strategy of maize plants that show decreased stomatal conductance, transpiration and eventually lower photosynthesis rate under lower soil water content (Sinclair et al., 1975).

The mechanisms involved in plant resistance to drought have been widely documented (see review by Fang and Xiong (2015), while those involved during drought recovery have been less explored. A recent study conducted on maize indicated, however, that plant physiological responses differed during drought and recovery and that the recovery phase could play a more important role than previously believed (Chen et al., 2016). This corroborated the study of Hayano-Kanashiro et al. (2009) who observed that gene expression patterns between drought tolerant and drought sensitive maize genotype differed more during recovery from drought than during drought. Similar findings were reported in drought-tolerant cowpea genotype (Anyia and Herzog 2004; Rivas et al., 2016) pointing at the importance of studying plant recovery to improve adaptability of plants to water paucity.

A number of studies have demonstrated the role of soil microorganisms in increasing drought resistance to drought stresses (Kim et al., 2012; Coleman-Derr and Tringe 2014). Among these are the arbuscular mycorrhizal fungi (AMF). These soil microorganisms form symbiotic associations with an estimate of 72% of land plant species (Brundrett and Tedersoo 2018) and have been suggested as promising root-associates for drought mitigation in plants (Boomsma and Vyn 2008) via several mechanisms. For instance, improved cell turgor via osmotic adjustment in shoots (Wu and Xia 2006) neutralization of reactive oxygen species in tissues via the synthesis of enzymes (e.g., superoxide dismutase) (Ruiz-Lozano et al., 1996), the increased access to soil pores inaccessible to roots or root hairs (Smith and Read 2008), and transport of water via the extraradical mycelium to the root cells (Sánchez-Díaz and Honrubia 1994; Ahmad Khan et al., 2003) the enhanced water uptake in plants by stimulating the growth of root hairs (Zou et al.,

2017) and by increasing root hydraulic conductivity through modulation of root aquaporin expression (Bárzana et al., 2012 ; Quiroga et al., 2018) have been reported (for details see reviews by Augé 2001; Plouznikoff et al., 2016). Remarkably, in inorganic P (Pi) limiting soils, AMF can ameliorate P nutrition of plants by accessing to remote locations of Pi through their extraradical hyphae (Smith and Read 2008) and it has been demonstrated that this improved Pi nutrition can maintain optimal growth and water relations and therefore increase plant resistance to drought (Nelsen and Safir 1982; Fitter 1988; Augé, 2001).

In contrast to the many studies conducted on the role of AMF in plant drought resistance (see above), the beneficial effects of these microorganisms during recovery from drought is much less investigated, especially in maize. An improved Pi nutrition was reported in AMF-colonized maize (Subramanian et al., 1997) and wheat (Bryla and Duniway 1997) plants following drought recovery. However, it was not clear which from the Pi uptake during recovery or during drought contributed the most to the improved P nutrition. Studies conducted by Levy and Krikun (1980) in citrus plants and by Goicoechea et al. (2004) in *Anthyllis cytisoides* revealed an improved water status in AMF-colonized plants together with an improved photosynthesis, leaf stomatal conductance and transpiration, during a five- and 20-days recovery period, respectively. In maize, Subramanian and (Subramanian et al., 1997) indicated an accelerated recovery of leaf-turgidity in AMF-colonized plants. To understand how AMF may help plants to better recover from drought it could be important to examine and compare the short-term dynamics of Pi uptake and to analyze leaf gas exchanges in presence/absence of these root symbionts. Recently, a semi- hydroponic cultivation system developed by Colpaert et al. (1999) for pine associated to ectomycorrhizal fungi, was adapted by Garcés-Ruiz et al. (2017) to study the short-term dynamics of Pi uptake/mobilization by maize plants associated with AMF. The mycorrhizal maize plants were grown in containers on perlite and irrigated with a Hoagland Pi-impoverished nutrient solution circulating in a closed loop. The Pi uptake dynamics by the plant/AMF associates were deduced from the Pi depletion in the circulating nutrient solution.

In the present study, the semi-hydroponic cultivation system was used to assess the effect of AMF on the dynamics of Pi uptake and on leaf gas exchanges of maize plants during the first 42 h of recovery from drought. The plants inoculated with *Rhizophagus irregularis* MUCL 41833 and their non-colonized counterparts were grown under well-, moderately- or poorly- watered conditions for three weeks and then watered at field capacity for 42 h with a Pi-impoverished nutrient solution circulating in a close loop. The dynamics of Pi uptake was monitored at 0, 9, 21 and 42 h, and leaf gas exchanges measured at times 0 and 42 h after setup of the circulatory

system. Finally, the plant biomass, P content and concentration, water content and leaf relative water content were assessed at the end of the experiment.

Materials and Methods

Biological Material

The arbuscular mycorrhizal fungus (AMF) *R. irregularis* (Baszk., Wubet, Renker, and Buscot) C. Walker and A. Schüßler as ["irregulare"] MUCL 41833 was supplied by the Glomeromycota in vitro collection (GINCO)1 and grown *in vitro* on excised Ri T-DNA transformed roots of carrot (*Daucus carota* L.) as described in Cranenbrouck et al. (2005).

Seeds of maize (*Z. mays* cv. ES Ballade) were supplied by the Centre Indépendant de Promotion Fourragère (CIPF)2. They were surface-disinfected as described in Garcés-Ruiz et al. (2017). and placed on wet paper (N1, Whatman, United Kingdom) in closed plastic containers (Aarts Plastics, Netherlands) in the dark at room temperature (20°C) for germination for 4 days

Preparation of the maize plantlets

Three 5 L pots containing a sterilized (121C for 15 min) substrate composed of vermiculite (PullRhenen, Netherlands), sand (quartz N4, Euroquartz, Belg) and lava stone (DCM, Belgium) (w:w:w, 1:1:1) were prepared to produce mycorrhizal (M) plants. In each pot, six maize seedlings were placed with their roots in contact with an approximate of 100 spores of an in vitro culture of *R. irregularis*. Three other pots containing a similar substrate but without AMF inoculum were used to produce non-mycorrhizal (NM) plants. All the pots were maintained 4 months under greenhouse conditions (21 C/18 C (day/night), a relative humidity (RH) varying from 40 to 70%, a photoperiod of 16 h day^{-1} and a photosynthetic photon flux (PPF) of 120 µmol m^{-2} s^{-1}) to produce maize plants with strong root systems either colonized or not with the AMF. The shoots of the plants were then cut, and six new 3-days-old, disinfected maize seedlings were planted in the pots containing or not the AMF extending from the roots of the cut maize plants. The plants were watered twice a week with 1400 mL of deionized water and received additionally 500 mL of Pi-impoverished modified Hoagland solution ($Hoagland^{lowPi}$ – see Garcés-Ruiz et al. (2017) for chemical composition). The plants were kept in a growth chamber set at 21/21°C (day/night), a RH of 75%, a photoperiod of 16 h day^{-1} and a PPF of 300 µmol m^{-2} s^{-1}.

Experimental Set Up

After 3 weeks, the maize plants were gently removed from the pots and roots carefully rinsed from the substrate with deionized water. Three plants from the M and NM treatments were randomly selected and root colonization evaluated (see below). The other plants were transferred to the greenhouse into individual containers that consisted of 500 mL wash bottles filled with 25 g of dry perlite [see Garcés-Ruiz et al. (2017) for details]. The bottles were inverted [i.e., with neck below closed by a nylon mesh of 100 µm size pore (Prosep B.V.B.A., Belgium) to avoid substrate loss] and disposed in holes made in flex foam supports disposed on tables. Nine containers without plants were also included as non-vegetated (NV) controls. Every 2 days, all the plants and the NV control containers received the same volume (i.e., between 60 and 100 mL) of Hoagland^{lowPi} solution. Deionized water was further added until saturation of the substrate (estimated until the first drops of water were released from the opening at the bottom of the containers). After 24 days of acclimatization in the perlite, the M and NM maize plants had the same height ($P = 0.923$). They were separated in three homogenous groups and grown under three WR (water regime): well-watered (WW^M and WW^{NM}), moderately watered (MW^M and MW^{NM}) or poorly watered (PW^M and PW^{NM}), the two later corresponding to moderate and severe drought conditions, respectively (see below for details). Six replicates were considered per treatment and three NV control containers were assigned to each WR. Treatments and controls were randomly distributed among five tables, with each treatment represented at least once on each table. Position of the containers were finally randomized on each table. The plants were subsequently grown under the three WR for a 3 week-period. Every 2 days, the plants in the WW treatments were supplied with deionized water to maintain saturation of the perlite (see details above), while in the MW and PW treatments, the plants received 60 and 30 mL of deionized water corresponding to 50 and 25% of the volume of water necessary to saturate the substrate (estimated to 120 mL deionized water, data not shown). After 1 week, the circulatory system was started a first time with Hoagland^{lowPi} solution for 42 h without any analysis. In the two following weeks, plants were watered as described above before setting the circulatory system a second time. During this second period, the MW and PW treatments induced drought stresses in the maize plants as noticed by the severe decrease in CO_2 assimilation rate and the wilting of the leaves (data not shown). To summarize, the maize seeds were germinated for 3 days, then associated or not with the AMF in 5 L pots for 3 weeks (21 days). The M and NM plants were then transferred in the semi-hydroponic cultivation system and acclimatized for 24 days. Three water regimes (WW, MW, and PW) were then applied for 1 week and an additional 2-week period, each period being followed by 42 h of

recovery. At harvest, the plants were grown in the semi- hydroponic cultivation system for 49 days and were 73 days old.

Measurement of short-term Pi depletion in the Hoagland^{lowPi} solution circulating through the containers containing the M and NM maize plants and non-vegetated controls

The short-term depletion of Pi in the Hoagland^{lowPi} solution was monitored using a closed semi-hydroponic cultivation system as described by Garcés-Ruiz et al. (2017). Briefly, 1 L of Hoagland^{lowPi} solution contained in glass bottles circulated via peristaltic pumps through the containers with M or NM maize plants and returned to the bottles in a closed loop **(see Figure 41)**. After a flushing step to equally saturate perlite in Hoagland^{lowPi} solution (Garcés-Ruiz et al., 2017), the Pi uptake by the plants and AMF was deduced from the Pi depletion in the circulating solution during 42 h. Initial Pi concentration in the nutrient solution was determined by sampling 15 mL of the solution from each bottle [considered as Time 0 h (T0)] before the start of the circulatory system. The circulatory system was then initiated at a rate of 7.4 mL min^{-1}, and 15 ml of nutrient solution was collected at 9, 21, and 42 h. The samples were then stored at 4C in the dark and Pi concentration subsequently measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES) as described in Garcés-Ruiz et al. (2017). Data were then normalized according to the NV controls (Garcés-Ruiz et al., 2017). Finally, the data expressed in mg kg^{-1} were converted into % of Pi concentrations remaining in the nutrient solution relative to the initial concentration at T0 using the following formula:

$$\%\ \text{of Px} = ([\text{Px}]\text{T} + [\text{PNV}]\text{T0} - [\text{PNV}]\text{T}) / [\text{Px}]\text{T0} \times 100$$

where:

[P] = Pi concentration in the solution

x = sample

NV = non-vegetated containers (i.e., perlite Control) respective to the sample analyzed

[PNV] = mean Pi concentration (3 replicates) for the non-vegetated containers

T= time considered (9, 21, or 42 h after the start of the circulatory system)

T0 = 0 h before the start of the circulatory system

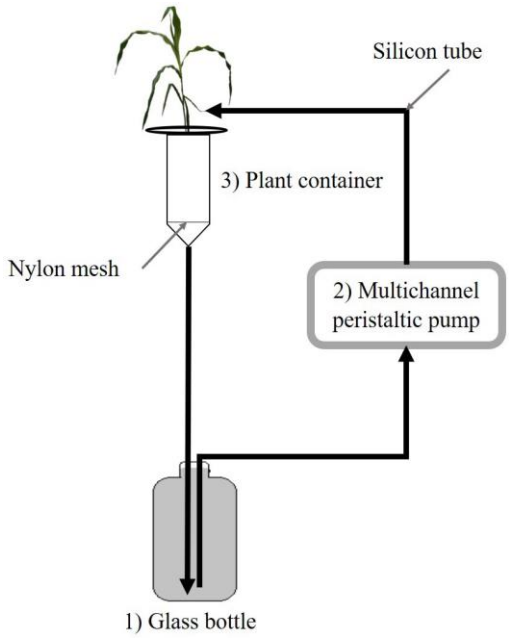

Figure 41. Schematic representation of the semi-hydroponic circulatory system [adapted from Garcés-Ruiz et al. (2017)]. Pi depletion is monitored during 42 h in the Hoagland^{lowPi} solution circulating through containers with mycorrhizal (M) or non-mycorrhizal (NM) maize plants. The nutrient solution in the glass bottle (1) is pumped with a peristaltic pump (2) to the upper part of the container (3) via silicon tubes. The solution percolates then through the plant container back into the glass bottle. Dark arrows indicate the direction of flow of the nutrient solution in the tubing.

Leaf gas exchange parameters in maize leave

Net CO_2 assimilation (A), stomatal conductance (g_S), and transpiration rates (E) were measured in the morning (from 8 to 12 AM) before starting the circulatory system (considered as time = 0 h), and at the end of the circulation (at time = 42 h), using an InfraRed Gaz Analyzer (IRGA-LCi Photosynthesis system, ADC BioScientific Ltd.). For each plant, an average of three measures recorded from a 5.8 cm² area on the 4 or 5th leaf was considered for the analysis. Instantaneous water use efficiency was calculated using the following equation (Bacelar et al., 2012) : WUEi = A/E.

Plant Harvest

At the end of the experiment, i.e., 49 days after transfer into the systems, the plants were harvested. The shoot and roots fresh weights (SFW and RFW, respectively) of each plant were evaluated and a leaf sample (approx. 1 x 7 cm) was cut from the 5th leaf to evaluate leaf relative water contents (see below). Shoots and roots were oven-dried at 50C for 7 days for evaluation of shoot and roots dry weights (SDW and RDW, respectively) and leaf samples were further used for the determination of the leaf relative water contents (RWCL-see below).

The root systems were subsequently separated in two parts to evaluate AMF root colonization and P concentration in tissues. After evaluation of dry weight, 500 mg of shoot and roots were ground separately for P analysis. Samples were digested following the procedure described in (Garcés-Ruiz et al. (2017). Phosphorus concentration was quantified using ICP-AES.

RWC_L was determined at harvest from a sample of maize leaf (see above). After determination of the FW, the leaf sample was placed in a Petri dish containing water at 4C in the dark for one night and turgid weight (TW) was estimated. Leaf samples were then oven-dried at 50C for 4 h and dry weight (DW) was estimated. The RWC_L was calculated using the equation of Barrs and Weatherley (1962): RWC_L (%) = (FW DW)/(TW DW) x 100.

AMF root colonization

Root colonization was estimated on three replicates from the M and NM plants at the time of transfer to the systems (i.e., 24 days old plants) and on each replicate of the six treatments at harvest (i.e., 49 days after transfer into the systems – 73 days old plants). Roots were sampled, washed from the substrate, cut into small pieces, and placed into 50 mL Falcon tubes (Sarstedt, Germany). Twenty-five ml of KOH 10% was added to the roots before incubation at 70°C in a water bath for 1 h. The KOH solution was removed, and roots were washed with HCl 1%. The roots were further stained with ink 2% (Parker Blue Ink, United States) in HCl 1% (Walker, 2005) by placing the tubes at 70°C in a water bath for 1 h. The roots were rinsed and stored in deionized water before observation (Walker, 2005). Percentages of total (%TC), arbuscular (%AC), and vesicular (%VC) root colonization were estimated under a dissecting microscope (Olympus BH2–RFCA, Japan) at 10 x magnification (McGonigle et al., 1990). At each intersection, the presence or absence of fungal structures was noted. Around 100 intersections were observed per plant.

Statistical Analysis

Data analysis was performed with JMP 13.2 and SAS 9.4 statistical software (SAS Institute Inc., Cary, NC, United States) with a 5% a-threshold. Levene and Shapiro–Wilk tests were run prior to the statistical analysis to confirm homogeneity of variance and normality in the distribution, respectively. Phosphorus depletion in the Hoagland^{lowPi} nutrient solution was analyzed with a mixed model for repeated measurements, where “time” of sampling (i.e., 9, 21, and 42 h), “plant number” (from 1 to 36) and “table” (five different tables) were considered as random factors while “AMF” and “WR” (i.e., six conditions) were regarded as fixed factors. Plant biomass, tissue P content and concentration, and RWC_L were analyzed using a mixed model analysis with REML

estimation where “AMF” and “WR” were regarded as fixed factors and “table” as random factor. Following significance of the factors, contrast analysis ($p < 0.05$) was conducted to determine differences among groups. Data of g_s at time 0 h and data of g_s and E at time 42 h were submitted to a square root transformation to meet the assumption of the homogeneity of variance. Before and after circulation, A, g_s and WUE_i were analyzed using a mixed model with REML estimation followed by contrast analysis as described above. When a significant interaction between factors was found, a one-way ANOVA was run for each level of each factor “AMF” and “WR,” separately. Water contents was analyzed using Wilcoxon’s non-parametric comparison for each level of each factor “AMF” and “WR,” separately. Results of multiple pairwise comparisons were taking in account Bonferroni’s correction of *p*-values. Finally, root mycorrhizal colonization percentages were arcsine transformed and analyzed using a one-way ANOVA followed by a pairwise comparison using Tukey-HSD test.

Results

Root colonization

The root colonization parameters were measured at transfer of the maize plants into the containers and at harvest in the WW, MW and PW treatments (**Table 9**). The %TC in the plants at transfer did not differ from the %TC in the plants of the WW treatment at harvest, while it was significantly higher as compared to the plants in the MW^M and PW^M treatments. Whatever the water regime, no significant differences were observed in %AC between the plants at transfer and those at harvest. The %VC was significantly higher in the plants of the WW^M treatment as compared to the plants in the PW^M treatment but did not change significantly from the plants in the MW^M treatment and from the plants at transfer. No significant difference in %VC was noted between the plants in the MW^M and PW^M treatments as compared to the plants at transfer.

Table 9. Percentages of arbuscular- (%AC), vesicular- (%VC) and total colonization (%TC) of maize roots associated with the arbuscular mycorrhizal fungus *Rhizophagus irregularis* MUCL 41833 at transfer into the semi- hydroponic container system (after 21 days of pre-mycorrhization) and at harvest (in 73-days-old plants that were grown for a three-weeks period under well- (WW), moderately (MW) or poorly watered (PW) condition).

	AC (%)	VC (%)	TC (%)
At plant transfer	68 ± 6.4a	42 ± 6.3a	97 ± 1.8a
Harvest time			
HW	69 ± 13.5a	47 ± 12ab	77 ± 15ab
MW	51 ± 8.7a	37 ± 15ab	69 ± 18b
PW	54 ± 13a	25 ± 11b	63 ± 19b

Data were analyzed with a one-way ANOVA followed by pairwise comparison using Tukey-HSD test. Data in a column followed by similar letter do not differ significantly ($p \leq 0.05$). Three replicates were considered at plant transfer into the containers and six per water regime (WW, MW, PW) at harvest. Data are presented as mean ± SD.

Phosphorus depletion in the nutrient solution

The short-term dynamics of Pi depletion in the nutrient solution was assessed on maize plants that were grown for a 3-week- period under three contrasting WR (WW, MW, and PW) before watering them at field capacity for 42 h. Pi depletion (throughout the text expressed as relative percentage of Pi concentration in the medium or %Pi) was measured 0, 9, 21, and 42 h after setup of the circulatory system. Whatever the WR preceding circulation of the nutrient solution, a decrease was noticed in the %Pi in the nutrient solution between time 0 and 42 h (**Figure 42**), suggesting a concomitant Pi-uptake by the plant and AMF associates. Using the mixed model for repeated measures, the %Pi was affected by the factors AMF, WR, Time, and the interaction WR x Time ($P < 0.0001$), this later indicating that the impact of the WR significantly differed with time. Indeed, the %Pi was lower for the plants in the WW treatments when compared with those in the MW or PW treatments at any time but this difference was less significant at time 42 h than at times 9 and 21 h (at times 9 and 21 h, $P < 0.0001$ for plants in both MW and PW treatments when compared with plants in the WW treatments while at time 42 h, $P = 0.007$ and $P = 0.004$ for the plants in the MW and PW treatments, respectively, when compared with those in the WW treatments). No difference was found between the plants in the MW or PW treatments at times 9, 21 and 42 h ($P = 0.425$, $P = 0.883$, and $P = 0.967$, respectively). Conversely, the %Pi depletion was not affected by AMF x WR, AMF x Time, and AMF x WR x Time interactions ($P > 0.05$). This suggested that the effect of AMF on the %Pi depletion in the nutrient solution was effective whatever the WR or time considered. Interestingly, the plants in the MW^M and PW^M treatments took up proportionally more Pi (as compared to their respective NM controls) as compared to the

plants in the WW^{M} treatment. Indeed, for the plants in the MW^{M} treatment, the decrease in %Pi was two times higher than for those in the MW^{NM} treatment at 9 and 21 h (i.e., 18 and 9% at 9 h and 43 and 23% at 21 h for the M and NM plants, respectively). For the plants in the PW^{M} treatment the decrease in %Pi as compared to the plants in the PW^{NM} treatment was 50 and 18% at times 9 and 21 h, respectively, while for the plants in the WW^{M} treatment, it was only 10 and 13% at times 9 and 21 h, respectively, as compared to those in the WWNM treatment.

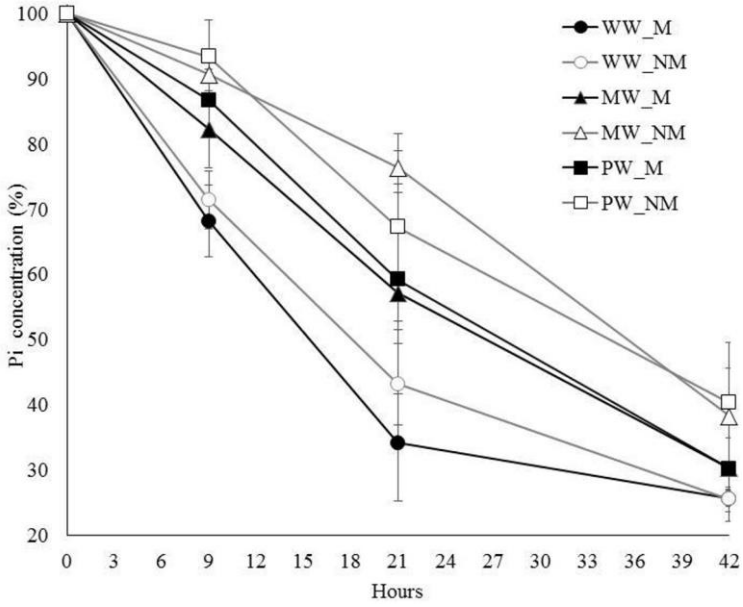

Figure 42. Dynamics of Pi depletion in the Hoagland^{lowPi} nutrient solution circulating in containers with mycorrhizal (M) and non-mycorrhizal (NM) maize plants grown under well- (WW), moderately- (MW), or poorly watered (PW) conditions for a 3-week period before the start of the circulatory system. Percentages of Pi concentration remaining in the nutrient solution were expressed from Pi concentrations measured at times 9, 21, and 42 h relative to Pi concentrations measured at time 0 h. The AMF was *Rhizophagus irregularis* MUCL 41833. Data are presented as mean ± SD of six replicates per treatment.

Physiological Parameters

The values for A, g, E, and WUE were analyzed in leaves before (i.e., Time 0) and after circulation of the nutrient solution (i.e., Time 42 h) (Table 9). At time 0 h, no significant effects of the factor AMF or the interaction AMF x WR were noticed for any of the parameters evaluated in the leaves. A significant effect of the factor WR was noticed on A, g_S, and WUE_i, but not on E. When compared with the plants in the WW treatments, the A, g_S, and WUE_i significantly decreased in leaves by 58, 83, and 48%, respectively, in the plants of the MW treatments, and decreased by 25, 60, and 34%, respectively, in the plants of the PW treatments.

At 42 h following circulation, the WUE_i measured in leaves was significantly higher in the M plants as compared to the NM ones (**Table 10**). The WR had an impact on A. Indeed, A

significantly increased for the plants on the MW and PW treatments when compared to those in the WW treatments. The interaction AMF x WR significantly influenced g_S and E, suggesting that the impact of WR was dependent of the root colonization by the AMF and vice-versa. Indeed, whereas the parameters E and g_S remained similar in leaves of NM plants whatever the WR, their value increased in the leaves of the plants in the PW^M treatment as compared to those in the WW^M treatment. In addition, the plants in the WW^M treatment had a significant lower g_S than those in the WW^{NM} treatment, while under MW and PW conditions, no significant differences were noticed between M and NM plants (Table 9). Indeed, the g_S measured in the leaves of the plants in the WW^M treatment was 72.3% lower as compared to those in the WW^{NM} treatment. Similarly, E significantly decreased by 57 and 42.9% in the leaves of the M plants as compared to NM leaves, under WW and MW WR, respectively.

Table 10 Impact of different water regimes (WW, MW, and PW) and AMF colonization by R. irregularis MUCL 41833 on the net CO2 assimilation (A), stomatal conductance (gs), transpiration rate (E), and instantaneous water use efficiency (WUEi) measured in maize leaves at the start (0 h) and after 42 h of circulation of the nutrient solution. Mycorrhizal (M) and non-mycorrhizal (NM) maize plants were grown under well- (WW), moderately- (MW) or poorly-watered (PW) conditions for three weeks.

	Time = 0h				Time = 24h			
	A ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	g_s ($mmol\ m^{-2}\ s^{-1}$)	E ($mol\ H_2O\ m^{-2}\ s^{-1}$)	WUE_i ($\mu mol\ CO_2\ mol^{-1}\ H_2O$)	A ($\mu mol\ CO_2 m^{-2}\ s^{-1}$)	g_s ($mmol\ m^{-2}\ s^{-1}$)	E ($mol\ H_2O\ m^{-2}\ s^{-1}$)	WUE_i ($\mu mol\ CO_2\ mol^{-1}\ H_2O$)
Groups								
M	0.8 ± 0.5	2.8 ± 4	0.2 ± 0.1	4.1 ± 2.4	1.4 ± 1.1	17 ± 22	0.5 ± 0.5^{b}	3.0 ± 1.6^{a}
NM	0.9 ± 0.4	2.2 ± 3.4	0.2 ± 0.1	5.4 ± 3.4	1.1 ± 1	23 ± 15	0.7 ± 0.4^{a}	1.8 ± 1.2^{b}
WW	1.2 ± 0.3α	4.7 ± 4.4α	0.2 ± 0.1	6.5 ± 3.7α	0.8 ± 0.6γ	14 ± 15	0.5 ± 0.4	2.4 ± 1.9
MW	0.5 ± 0.4γ	0.8 ± 2.1β	0.2 ± 0.1	3.4 ± 2.6β	1.3 ± 0.6β	17 ± 13	0.5 ± 0.3	2.7 ± 1.4
PW	0.9 ± 0.2β	1.9 ± 3.3β	0.2 ± 0.1	4.3 ± 1.5β	1.6 ± 1.5α	28 ± 25	0.8 ± 0.5	2.1 ±1.3
WW ^{M}	1.2 ± 0.5	5 ± 4.6	0.2 ± 0.1	6.1 ± 2.6	0.8 ± 0.6	6.1 ± 8* ^{B}	0.3 ± 0.2* ^{B}	3.5 ± 1.9
WW ^{NM}	1.2 ± 0.2	4.4 ± 4.6	0.2 ± 0.1	7 ± 4.7	0.8 ± 0.8	22 ± 16* ^{a}	0.7 ± 0.4* ^{a}	1.2 ± 0.8
MW ^{M}	0.4 ± 0.3	1.1 ± 2.7	0.2 ± 0.1	2.1 ± 0.9	1.4 ± 0.8	12 ± 6.9 ^{AB}	0.4 ± 0.2* ^{AB}	3.1 ± 1.4
MW ^{NM}	0.7 ± 0.5	0.6 ± 1.4	0.2 ± 0	4.7 ± 3.2	1.3 ± 0.5	22 ± 15 ^{a}	0.7 ± 0.4* ^{a}	2.4 ± 1.4
PW ^{M}	0.8 ± 0.2	2.2 ± 4.0	0.2 ± 0.1	4.1 ± 1.8	1.9 ± 1.6	20 ± 12 ^{A}	0.6 ± 0.2 ^{A}	2.5 ± 1.4
PW ^{NM}	0.9 ±0.3	1.7 ± 2.8	0.2 ± 0.1	4.5 ± 1.3	1.3 ± 1.5	24 ± 11 ^{a}	0.7 ± 0.4 ^{a}	1.8 ± 1.2
Effects (P-value)								
AMF	ns	Ns	ns	ns	ns	ns	*	**
WR	***	*	ns	*	**	ns	ns	ns
AMF × WR	ns	Ns	ns	ns	ns	*	*	ns
Table (% of the total effect)	0	18.2	4.45	16.6	60.62	32.96	45.3	21.16

The effect of the fixed factors AMF colonization (AMF) and water regime (WR) as well as their interactions on plant physiological parameters were tested using the mixed model with REML analysis ($p \leq 0.05$). Percentages of the effect due to the random factor Table are reported. When no significant interactions between fixed factors were found, significant differences among WR and between M and NM plants are indicated using contrast analysis. In the case of a significant interaction, the significant differences between the different levels of each treatment were tested separately using a one-way ANOVA followed by Tukey-HSD test ($p \leq 0.05$). – Different lower-case letters indicate significant differences between M and NM plants according to the contrast analysis. – Different Greek letters indicate significant differences between WR according to the contrast analysis. – Different upper-case letters and different upper-case letters in italics indicate significant differences between WR inside each M and NM level, respectively. – Asterisks indicate significant differences between M and NM plants inside each WR level. Data are represented as mean ± SD of six replicates per treatment. For the P-values, ns = no significant difference, *p≤ 0.05, **≤ p 0.01, ***p ≤ 0.001

Plant growth parameters

At the end of the experiment, plants were harvested and the FW and DW, the P concentration and content, as well as the WC and RWC_L were evaluated (Table 10). Whatever the WR, the SDW, P concentration and total P content in shoots did not differ significantly between the M and NM plants, while the SFW was significantly higher in the M plants as compared to the NM ones. Conversely, the RWC_L was significantly higher in the NM plants as compared to the M plants. No significant difference was noticed in the shoot WC between the plants in the WW^M and WW^{NM} treatments, while under MW and PW conditions, a significant increase of shoot WC (by 2.9 and 2.6%, respectively) was noticed in the M plants as compared to the NM ones. Whatever the presence or absence of AMF within roots, the WR significantly impacted the SFW, SDW, and P concentration in shoots, but not the total P contents and RWC_L. Indeed, the SFW and SDW significantly decreased in the plants of the MW and PW treatments. A significant lower P concentration was measured in the shoots of the plants in the WW treatments as compared to those in the MW or PW treatments. Nonetheless, when reported to P contents in shoots, no significant impact of WR was noticed. No significant interaction of WR x AMF was observed for any of the shoot parameters, demonstrating the absence of interactions between WR and presence/absence of AMF (results not presented).

In roots, no significant effect of the factor AMF was observed on the RFW and RDW whatever the WR, while P concentration and content were significantly higher in the M plants as compared to their respective NM controls (**Table 10**). These parameters were also significantly affected by the factor WR but not by the interaction AMF x WR. Indeed, the RFW and RDW as well as the P contents in roots decreased in the plants of the MW and PW treatments whatever the presence/absence of AMF. To the contrary, the P concentration in roots increased in the plants of the MW and PW treatments as compared to those in the WW treatments. No significant impact of AMF or WR could be observed on root WC.

Table 10 Impact of different water regimes (WW, MW, and PW) and AMF colonization by R. irregularis MUCL 41833 on fresh weight (FW), dry weight (DW), water contents (WC), P concentration ([P]), and P contents of shoots and roots and on leaf relative water contents (RWCL) of maize at harvest time, after 73 days of growth. Mycorrhizal (M) and non-mycorrhizal (NM) maize plants were grown under well- (WW), moderately- (MW) or poorly-watered (PW) conditions for three weeks.

	Shoots						**Roots**				
	FW (g)	DW (g)	WC (%)	Leaf RWC (%)	[P] (mg P g^{-1} of DW)	P content (mg $plant^{-1}$)	FW (g)	DW (g)	WC (%)	[P] (mg P g^{-1} of DW)	P content (mg $plant^{-1}$)
Groups											
M	52 ± 7.7^{a}	8.2 ± 2.7	85 ± 3.4^{a}	75 ± 18^{a}	1.5 ± 0.6	11 ± 2	23 ± 8.6	3.6 ± 1.4	84 ± 23^{b}	1.3 ± 0.2^{a}	4.5 ± 1.3^{a}
NM	46 ± 12^{b}	8.2 ± 2.7	82 ± 2.6^{b}	90 ± 25^{b}	1.5 ± 0.5	11 ± 5.5	23.4 ± 7.1	3.4 ± 1.1	86 ± 14^{a}	1 ± 0.2^{b}	3.2 ± 0.9^{b}
WW	59 ± 7.3α	11 ± 1.4α	80 ± 2.8β	78 ± 23	1 ± 0.5γ	12 ± 6.6	30 ± 6.6α	4.7 ± 1α	84 ± 27	1 ± 0.6γ	4.9 ± 1.1α
MW	49 ± 7.3β	7.3 ± 1.2β	85 ± 1.8α	84 ± 20	1.5 ± 0.3β	11 ± 2.7	23 ± 5.4β	3.3 ± 0.7β	86 ± 9.6	1.1 ± 0.3β	3.7 ± 1.2β
PW	40 ± 6.4γ	5.7 ± 0.7γ	85 ± 2.4α	88 ± 26	2 ± 0.4α	11 ± 2.1	17 ± 4.5γ	2.4 ± 0.6β	85 ± 19	1.3 ± 0.3α	3 ± 0.9γ
HW ^{M}	60 ± 5.3	11 ± 1.6	81 ± 3 ^{B}	71 ± 21	0.9 ± 0.2	9.7 ± 2.4	30 ± 8.5	5 ± 1.3	83 ± 3.4 ^{A}	1.1 ± 0.1	5.7 ± 1
HW ^{NM}	59 ± 9.4	11 ± 1.3	80 ± 2.8 ^{a}	83 ± 24	1.2 ± 0.6	14 ± 9	30 ± 4.8	4.5 ± 0.5	85 ± 1.5 ^{a}	0.9 ± 0.1	4.1 ± 0.4
MW ^{M}	52 ± 4	7.3 ± 0.8	86 ± 0.7* ^{A}	77 ± 14	1.7 ± 0.2	12 ±1.7	22 ± 6.3	3.2 ± 0.7	85 ± 0.9 ^{A}	1.4 ± 0.3	4.3 ± 1.3
MW ^{NM}	46 ± 8.7	7.4 ± 1.6	83 ± 1.4* ^{a}	92 ± 22	1.4 ± 0.3	10 ± 3.3	23 ± 4.8	3.3 ± 0.6	86 ± 1.1 ^{a}	0.9 ± 0	3 ± 0.5
PW ^{M}	44 ± 4.5	5.7 ± 0.7	87 ± 0.6* ^{A}	79 ± 21	2.1 ± 0.5	12 ± 2.6	16 ± 4.4	2.6 ± 0.6	84 ± 1.5* ^{A}	1.4 ± 0.1	3.6 ± 0.7
PW ^{NM}	35 ± 3.7	5.6 ± 0.7	83 ± 1.9* ^{a}	96 ± 30	1.8 ± 0.3	10 ± 1.3	17 ± 5.1	2.3 ± 0.6	86 ± 1.4* ^{a}	1.1 ± 0.3	2.4 ± 0.6
Effects (P-value)											
AMF	*	ns	*	*	ns	ns	ns	Ns	*	***	***
WR	***	***	***	ns	***	ns	***	***	ns	***	***
AMF × WR	ns	ns	-	ns	ns	ns	ns	Ns	-	ns	ns
Table (% of the total effect)	0	0.8	-	8.7	7.6	0	3	7.8	-	6.5	30

The effect of the fixed factors AMF colonization (AMF) and water regime (WR) as well as their interactions (result not presented) on plant growth parameters was tested using mixed model with REML analysis (p 0.05). Percentages of the effect due to the random factor Table are reported. Water contents (WC) were analyzed by running Wilcoxon's test on each factor separately and using Bonferroni's correction for pairwise comparisons, without interaction analysis. For the [P], data in ppm were converted in mg g

- ✓ Different lower-case letters indicate significant differences between M and NM plants according to the contrast analyses
- ✓ Different Greek letters indicate significant differences between WR according to the contrast analysis –
- ✓ Different upper-case letters and lower-case letters in italic indicate significant differences between WR inside each M and NM level, respectively. – Asterisks indicate significant differences between M and NM treatments inside each WR level.

Discussion

Drought is a major threat to maize production and many strategies (e.g. irrigation, breeding) have been put in place to limit its impact. In maize, recovery after drought is poorly studied and the role played by AMF in this process is almost unknown. Here, we reported on the impact of *R. irregularis* MUCL 41833 on the dynamics of Pi uptake and on the modifications in leaf gas exchanges parameters (CO_2 assimilation rate (A), stomatal conductance (g_s), transpiration (E) and instantaneous water use efficiency (WUE_i) in maize plants that were grown under three contrasting water regimes (i.e. well-, moderately- and poorly-watered, WW, MW and PW, respectively) and subsequently watered at field capacity for 42 h. Plants were grown in a semi-hydroponic cultivation system to follow-up the depletion of Pi in the circulating nutrient solution. During the circulation, Pi uptake was increased in the mycorrhizal plants, whatever the WR. At initiation of circulation (time 0 h), A, g_s, and WUE_i were impacted by the WR but not by AMF, except for E which stayed unchanged whatever the condition. A, g_s, and WUE_i were indeed lower in plants grown in the MW and PW treatment when compared with those in the WW treatment. After 42 h of recovery, A recorded in the plants in the MW and PW treatments exceeded those in the WW treatment, a significant interaction between AMF and WR was found for g_s and E, and WUE_i was improved in M plants when compared with NM plants whatever the WR. At harvest, the plants in the MW^M and PW^M treatments had higher shoot WC but also lower leaf RWCL when compared to their non- mycorrhizal counterparts.

Total and arbuscular root colonization parameters evaluated at harvest were not impacted by the WR. This result is consistent with previous studies conducted on maize (Bárzana et al., 2014; Quiroga et al., 2017) and wheat under moderate and severe drought stress (Beltrano and Ronco 2008) and suggested that the fungus was able to develop in the root system of plants under different WRs, even the most severe, as in our study. However, a significant decrease in %VC was found in the plants grown in the PW^M treatment when compared to those grown in the WW^M treatment. This corroborated the study of Martínez-García et al. (2012) that observed a decrease in vesicles but not in arbuscules in shrubs during seasonal drought. They suggested that under drought conditions, AMF would rather allocate energy to the production of arbuscules than to storage structures (i.e., vesicles) to maintain transfer of water and nutrients to the plant. Our results on Pi uptake during recovery (see below) tend to support this hypothesis with a proportionally higher uptake of Pi in the plants grown in the PW^M treatment versus those grown in the PW^{NM}

treatment as compared to the plants grown in the WW^{M} treatment versus those grown in the WW^{NM} treatment, even if the data are non-significantly different.

The dynamics of Pi uptake/immobilization by the maize-AMF associates were measured indirectly by monitoring the % of Pi concentration remaining in the nutrient solution circulating through the plants. Whatever the presence/absence of AMF, the decrease in %Pi in the circulating solution in the plants of the WW treatments was fast from 0 to 21 h and slowed down onward 21 until 42 h. This dynamic is similar to the one encountered in studies using the same semi-hydroponic cultivation system with maize (Garcés-Ruiz et al., 2017) and Medicago (Calonne-Salmon et al., 2018) plants. A comparable, but less pronounced, decrease in %Pi was noticed in the M and NM plants grown under MW and PW treatments, although no slowdown was noticed after 21 h of circulation. Indeed, the Pi uptake in the plants of the MW and PW treatments was significantly lower as compared to those in the WW treatments whatever the time considered even if this difference was less significant at time 42 h. The lower Pi uptake could be explained by the significant lower root biomass of the plants in the MW and PW treatments as compared to those in the WW treatments, probably combined with a negative effect of drought on the plant and/or AMF physiology.

Whatever the WR, the Pi uptake by the plant/AMF associates during the 42-h circulation, increased significantly in the M versus NM plants. The absence of differences in root biomass between M and NM plants under similar WR, combined with the high levels of root colonization, suggested that the increased rate of Pi uptake in the M plants could be attributed to an increased acquisition of Pi by the extraradical mycelium extending in the substrate. However, the relatively small volume of the container but also the continuous percolation of the nutrient solution through the substrate, might prevent the formation of Pi-depletion zones around roots and hyphae. Therefore, the increased Pi uptake in the M plants could probably result from an increased expression of fungal Pi transporters (Colpaert et al., 1999) rather than to an increased foraging by the extraradical hyphae. This result was also correlated with an improved Pi content in maize roots. However, shoot and root biomass as well as shoot P content and concentration at harvest were not improved in the M plants as compared to the NM plants under similar WR, as already reported in the study of Subramanian and Charest (1997) and Zhu et al. (2012) with maize. As suggested by Garcés-Ruiz et al. (2017), it is not excluded that Pi accumulated in the intraradical structures of the AMF (i.e., hyphae and vesicles) in the form of polyphosphate granules. Interestingly, similar shoot P contents under all WR were associated with significantly lower SDW in the plants of the MW and PW treatments as compared with those in the WW treatments.

This suggested that water was the principal factor limiting plant growth. Pi accumulation in the intraradical structures of the fungus could thus been explained by the lower demand of Pi by the plant.

Leaf gas exchange parameters measured at the initiation of circulation (time 0 h) drastically differed after 42 h of circulation. A high increase of gs and E was noticed between time 0 and 42 h of circulation. As environmental conditions did not differ between both times (i.e., vapor pressure deficit, temperature, photosynthetically active radiation, not presented data), we hypothesized that these differences were related to the 42-h circulation. Interestingly, an increase of gs and E values in plants of the WW treatments was also noted between both times suggesting that perlite moisture at time 0 h was not optimal. It is indeed not excluded that a decrease in perlite moisture in the WW treatments had happened within 2 days, impacting gas exchange parameters event if no leaf rolling was observed in the plants in contrast to the MW and PW treatments.

At time 0 h, i.e., before plants received the nutrient solution, A, g_S, and WUE_i in maize leaves were decreased in the plants of the MW and PW treatments as compared to those in the WW treatments. Conversely, after 42 h of circulation, g_S and WUE_i were not impacted anymore by the WR indicating that rehydration occurring through the circulation of the nutrient solution was sufficient to eliminate the differences between plants in the WW treatments and drought-stressed plants as already observed (Grzesiak et al., 2006; Hayano-Kanashiro et al., 2009 ; de Carvalho et al., 2011). In addition, the plants in the MW and PW treatments had significantly higher A values than those in the WW treatments at time 42 h and significantly increased shoot water contents at harvest. Increased CO_2 assimilation has been considered a plant strategy to alleviate drought stress (Gale and Zeroni 1985) This increased metabolism and water demand compared to the plants in the WW treatments suggest that plants in the MW and PW treatments tried to compensate the lack of growth and water supply during recovery.

At time 0 h, AMF did not improve leaf gas exchange parameters in plants that were grown under WW, MW, or PW conditions. Although various studies showed a beneficial effect of AMF on these parameters in well-watered or drought- stressed maize plants (Subramanian et al., 1995; Zhu et al., 2012; Augé et al., 2015), a few others reported no impact of AMF on g_S in drought-stressed maize plants (Quiroga et al., 2017) and on E parameters in well-watered maize plants (Subramanian and Charest 1999), As suggested by (Quiroga et al., 2017), the absence of effect could be due to the variability of response in different maize genotype. After 42 h of circulation, the AMF did not improve A but modulated E and g_S in function of the WR. Indeed, E and g_S were significantly lower in the plants of the WW^M treatment when compared to those in the MW^M

and PW^M treatments. As photosynthesis was also significantly lower in the plants of the WW treatment when compared with those in the MW and PW treatments, it mechanically resulted in an improved WUE_i in the M plants comparatively to the NM plants.

An increased WC was observed in the plants of the MW and PW^M treatments during drought recovery which could be partly explained by the improvement of the WUE_i as observed in the plants at harvest. Other mechanisms such as improved root hydraulic properties during drought (Bárzana et al., 2012) or following recovery (Levey and Krikun 1980) have also been proposed as possible reasons for increased WC in M plants under drought stressed conditions. Curiously, the higher WC in the plants of the MW^M and PW^M treatments was not associated with higher RWC_L measured from leaves. Indeed, RWC_L was significantly decreased by 15% in M plants when compared to NM plants. However, WC and RWC_L cannot strictly be compared as WC measurement is based on whole plant and takes in account water in leaves, stems, grains, and flowers (if there are any), whereas RWC_L is measured from a small sample taken from the 4^{th} to 5^{th} leaf. This result seems also contradictory with previous studies which observed a higher RWC_L (Subramanian et al., 1997) and leaf moisture percentages (Zhao et al., 2015) in M maize plants during recovery. It has been shown that structure and physiology of maize leaves differed between mature and juvenile leaves (Bongard-Pierce et al., 1996). This difference in RWC_L could thus potentially be explained by the different maturity of the leaf measured in our study.

Meanwhile, the values of RWC_L measured in the plants of the WW treatments tended also to be lower than those in the plants in the MW and PW treatments. Thus, it seems that lower RWC_L values measured in plants of WW treatments as well as in M plants could reflect higher water binding properties of the plant tissues rather than a difference in the turgidity of the leaves. Plants tissues can indeed display different water binding properties depending on their structural and macromolecular composition. In wheat for example, it has been shown that leaves of drought tolerant cultivars had a higher affinity for strongly bound water when compared with drought sensitive ones (Rascio et al., 1999). This hypothesis is reinforced as the leave water contents (calculated without the contribution of the turgid weight) shows no significant differences among the treatments (not presented data).

Conclusion

In this study, we demonstrated a beneficial effect of AMF on the recovery of maize plants after a drought stress. An improved Pi uptake as well as an increased WUE_i and WC in shoots was noticed in the plant/AMF associates during the 42 h of recovery. Under a drought environment, where Pi is less available (Gahoonia et al., 1994) and precipitations are erratic, a more efficient Pi uptake by the fungus can help plants to mitigate the impact of drought stress and maintain growth (Singh et al., 1999) by improving net photosynthesis and water contents for instance (Garg et al., 2004). The ability of AMF to increase plant WUE_i during recovery from drought is of high agronomical importance as limited transpiration in maize plants is a major driver of yield performance in drought-susceptible environments (Messina et al., 2015). Therefore, future challenge could be to develop drought-adapted inbred lines highly responsive to AMF for faster recovery after drought stress.

GENERAL DISCUSSION

Morocco is a predominantly agricultural country, with rural populations heavily dependent on agriculture for their livelihood. The climate of the country is generally classified as arid to semi-arid. Since the 1960s, average temperatures have risen by around 0.2°C per decade (Cherif et al., 2023) and frequency and intensity of droughts, exacerbating desertification, have become regular in the last decennia. These changes are expected to intensify in the coming decades, potentially disrupting the water cycle and posing significant challenges to the agricultural sector and economic activities (Cherif et al., 2023).

Argan and maize are two key cultures in Moroccan agriculture. Argan trees cover approximately 830,000 hectares (Santoro et al., 2023), while maize is cultivated on about 158,000 hectares (MAPM, 2025). Both cultures play an essential role in rural development. The argan tree, endemic to southwestern Morocco, supports thousands of rural families through the production of argan oil, a high-value product with strong global demand (FAO, 2022). Maize, on the other hand, is a staple crop grown throughout the country and is an essential source of food for people and fodder for animals. Together, argan and maize contribute significantly to food security, income generation, and the sustainable development of rural communities.

Today, both cultures face numerous challenges, the most significant being climate change. Rising temperatures, erratic rainfall and prolonged droughts threaten their productivity and resilience. In addition to climatic stress, argan cultivation is also impacted by overgrazing, land degradation, and limited natural regeneration. These challenges underline the urgent need for sustainable agricultural practices and adaptation strategies to ensure the long-term use of these vital resources.

One promising strategy is the use of arbuscular mycorrhizal fungi (AMF), which have been shown to significantly improve plant tolerance to environmental stresses, including drought. These fungi are among the most important underground symbionts, forming associations with the roots of approximately 72% of terrestrial plant species (Brundrett and Tedersoo, 2018). They play an essential role in the survival of plants and the restoration of ecosystems, by reinforcing various defense mechanisms and helping plants to cope with drought and other abiotic stresses (Meddich et al., 2015; Begum et al., 2019; Diagne et al., 2020). Their successful *in vitro* association to plants such as banana (Koffi and Declerck, 2015) or hevea (Sosa-Rodríguez et al., 2013) has improved plant survival and fast recovery after transfer from *in vitro* to greenhouse conditions, opening the door to a novel biotechnological approach for argan trees, for which mass production via classical methods is below expectations.

In the present study, our objective was to explore the potential of AMF to increase argan and maize resilience to drought stress conditions. Three main objectives were pursued.

- First, develop an *in vitro* mycorrhization system for argan tree and evaluate the effects of AMF on key growth parameters such as rooting and biomass production during the transfer from *in vitro* to greenhouse conditions.
- Second, study whether AMF colonization can improve the morphological, physiological, and biochemical responses of argan accessions to drought stress and assess whether potential differences are accession dependent.
- Third, assess whether the mechanisms by which AMF improve drought resistance of argan tree are also effective in maize.

In vitro **colonization of argan tree by** ***R. irregularis*** **increases plant grow after transfer to the greenhouse.**

We have demonstrated the feasibility and effectiveness of *in vitro* mycorrhization of argan accessions using *R. irregularis*. Plant colonization as well as growth were highly increased after transfer from *in vitro* to the greenhouse, representing a major advance in the propagation of this ecologically and economically important species.

Traditional propagation techniques of argan are done by seeds. However, this approach is hindered by high seed dormancy, genetic variability, long juvenile stage, and poor survival after transfer (Ferradous et al., 2011; El Mrabet et al., 2014; Metougui et al., 2017). Vegetative propagation via cuttings and grafting ensures genetic uniformity but have low success rates due to poor rooting and slow growth (Ait Hammou et al., 2022; Metougui et al., 2017). Tissue cultures are promising, but their low rooting efficiency (Ait Hammou et al., 2022; Mazri et al., 2022) and difficulties in scaling up (Mazri et al., 2022) have also limited their use.

It is well-known from the literature that AMF can stimulate root development and improve seedling establishment (Giovannetti and Avio, 2002; Smith and Read, 2008). Moreover, the mycelium donor plant (MDP) *in vitro* culture system (see details in Lalaymia and Declerck, 2020) was used with success by Koffi and Declerck (2015) and Sosa-Rodriguez et al. (2013) for the *in vitro* propagation of banana and hevea, respectively. This technique has therefore been extended to *in vitro* propagation of the argan tree.

Seeds from three argan accessions Tidzi (TD), Mejji (MJ), and City Hanchan (CH) were surface-disinfected and germinated in the MDP *in vitro* culture system. Most seeds germinated within a

period of 10 days and root colonization, with the presence of arbuscules, vesicles/spores and hyphae, was observed within 8 to 11 weeks, although differences were observed between accessions, with the highest value for accession TD (total root colonization of 52.7 ± 26.7) and lowest for accession CH (total root colonization of 27.2 ± 8.3). During *in vitro* growth, there was no difference in shoot length between the colonized and non-colonized plants, while the roots of colonized plants were longer than those of non-colonized plants, with for instance a root length doubled for accession TD. This suggests that symbiosis with AMF can play an important role in improving root development during the *in vitro* culture, particularly in certain genotypes.

At transfer to the greenhouse, no plant death was noticed, and after five months, the AMF-colonized plants had significantly greater vegetative growth compared to non-colonized controls. For accession TD, all growth parameters were significantly higher in colonized plants compared to non-colonized ones. In accession MJ, shoot dry weight was significantly higher in colonized plants, while in accession CH, only stem diameter was significantly larger in colonized plants. This suggest that the response to AMF colonization is strongly genotype- dependent (Ronga et al., 2019). A significant effect of AMF inoculation was also observed on chlorophyll (*Chl a* and *Chl b*) concentration, which was generally higher in colonized plants than in non-colonized ones. These results confirm the beneficial effects of AMF on plant establishment during hardening phase. The elevated chlorophyll concentration suggests an improvement in photosynthetic capacity, which is often correlated with better nutrient uptake, particularly of phosphorus (P), facilitated by mycorrhizal symbiosis (Smith and Read, 2008). Increased vegetative growth indicates that AMFs not only improve nutrient and water uptake, but also actively contribute to overall plant vigor as has been reported for hevea (Sosa-Rodríguez et al., 2013), banana (Koffi and Declerck, 2015) and *Prunus domestica* (Lotfi et al., 2019), in which improved survival and recovery were observed after transfer from *in vitro* to *ex vitro* conditions following *in vitro* mycorrhization.

The successful *in vitro* mycorrhization and further acclimatization of argan plants is a crucial step towards integrating the AMF biotechnology into large-scale argan propagation programs. Indeed, argan micropropagation using tissue culture techniques such as organogenesis and somatic embryogenesis are often limited by low rooting efficiency (Mazri et al., 2022). Therefore, synchronizing the root development stage of both regeneration techniques with root colonization by AMF would be an important advance for efficient large-scale propagation of argan tree.

The process of organogenesis, particularly via shoot regeneration followed by auxin-induced rooting, often takes 8 to 12 weeks. This is perfectly compatible with the mycorrhization period of 8 to 11 weeks observed in our study. This chronological compatibility makes organogenesis an ideal candidate for integration into the MDP *in vitro* culture system.

Although somatic embryogenesis is theoretically capable of producing complete and genetically uniform plantlets, a reliable and reproducible system for somatic embryogenesis in argan plants has yet to be established (Mdarhri Alaoui et al., 2011; Achnin et al., 2017; Lamnouni et al., 2019; Oukmi et al., 2019). The complete somatic embryogenesis process can take 10 to 16 weeks, which may exceed the optimal mycorrhization period observed in our study. Nevertheless, the high regeneration potential and fidelity offered by somatic embryogenesis make it a promising approach for large-scale production if the timing of root emergence can be adjusted and mycorrhization targeted during germination.

In conclusion, achieving *in vitro* root colonization within a timeframe compatible with the root emergence phase of both regeneration techniques is essential for the efficient mass production of mycorrhizal argan plantlets. Integrating AMF into these tissue culture approaches could help to overcome some of their current limitations by enhancing root formation and improving plantlet vigor during the acclimatization phase

On the more fundamental perspective, these results provide a solid basis for (i) investigating the potential of argan/AMF couples to mitigate the effects of drought and (ii) selecting the most responsive argan accessions to drought stress, before (iii) selecting AMF species/strains from arid/semi-arid regions that may be better adapted to drought conditions (not done in the present thesis).

Inoculation with *R. irregularis* MUCL 41833 improves the resistance of argan plants to drought and stimulates their growth and physiological characteristics, with benefits varying according to accessions.

We have demonstrated that root colonization by the AMF *R. irregularis* MUCL 41833 increases the drought resistance of argan tree. All accessions benefited from AMF colonization (i.e., increased growth and physiological performance), but the magnitude of response varied between accessions.

Several studies have shown that, under normal growing conditions (i.e., without stress), AMF colonization and plant growth promotion vary from one plant species to another and, within the same plant species, from one variety or cultivar to another (Declerck et al., 1995; Sensoy et al., 2007). Under water-stress (WS) conditions, it is widely reported that AMF improve plant resistance through various physiological and biochemical mechanisms (Augé et al., 2015; Quiroga et al., 2018; El Yamani et al., 2020; Cheng et al., 2021), but whether this effect varies with variety/cultivar (or accession in our case) is less-well studied.

Under WS conditions, AMF colonization improved the nutritional performance of argan, although differences were noticed between accessions. AMF-colonized plants from MJ accession had significantly higher shoot P and K concentrations than non-colonized plants. Similarly, AMF-colonized plants from TD accession had significantly higher root P and K concentrations than non-colonized plants. In contrast, AMF-colonized plants from CH accession only had significantly higher root P concentrations than uncolonized plants.

It is often admitted that AMF-colonized plants absorb more mineral nutrients than non-mycorrhizal plants under non-stressed conditions but also under stressed (i.e., drought) conditions. This is due to the greater efficiency of the extraradical hyphae, compared with the root hairs, to absorb water and nutrients when water is limited. The fine extraradical hyphae (sometimes < 1 µm for the branched absorbing structures) extend beyond the root zone, accessing soil pores that are inaccessible to roots and root hairs. This allows the hyphae to absorb water at lower water potentials than the roots. The fine hyphae also maintain hydraulic continuity by bypassing soil air voids, thereby facilitating the transport of water from dry areas to the roots (Kikuchi et al., 2016). Despite their smaller diameter, calculations indicate that hyphae transport water efficiently at more negative soil matric potentials than those tolerated by roots, particularly in coarse-textured soils (Carminati et al., 2023). This ability enables AMF hyphae to absorb water in drier soils, where water extraction by roots is limited. In addition, AMF stimulate aquaporin expression in fungal and plant cells, thereby improving water transport from the soil to the plant (Kikuchi et al., 2016). Together, these mechanisms increase the water uptake of mycorrhizal plants, providing benefits beyond P nutrition alone (Safir et al., 1972; Harris et al., 1985). Higher P uptake promotes energy transfer and photosynthesis (Ahmed et al., 2017), while K contributes to regulating water balance and stomatal function (Wang et al., 2013; Hasanuzzaman et al., 2018). As a result, mycorrhizal plants show higher biomass, better leaf growth and greater photosynthetic efficiency compared to non-colonized plants under water stressed conditions.

These observations may be of interest under the harsh environmental conditions, characterized by poor, shallow and rocky soils, in which argan trees grow. These soils often have low fertility and limited availability of essential nutrients such as P and K. This is particularly pronounced in regions such as Essaouira, where soil analyses have revealed reduced nutrient availability impacting plant growth and vitality (Afi et al., 2024). In this context, the use of AMF as inoculants for argan trees grown under stressful conditions due to lack of nutrients and drought could be a strategy to establish argan trees in the field and make the plants more drought resistant.

Physiological mechanisms, including osmotic adjustment and antioxidant systems, also play a key role in increasing the resistance of AMF-colonized plants to water stress (Wu and Zou, 2017). This can be attributed to the role of AMF in enhancing antioxidant defenses by increasing the activity of enzymes such as catalase and superoxide dismutase, which help to detoxify ROS (Baslam et al., 2014; Benhiba et al., 2015; Essahibi et al., 2018). Importantly, a reduction in oxidative stress markers (H_2O_2 and MDA), was observed in TD and MJ accessions, suggesting that both accessions are more responsive to AMF-colonization due to their capacity to mitigate drought-induced oxidative damage. These physiological responses suggest that these accessions developed more effective protective mechanisms in response to drought stress when colonized by AMF. In contrast, the CH accession exhibited relatively higher levels of oxidative markers despite mycorrhizal colonization, suggesting lower symbiotic efficiency. This may be due to a less compatible host-fungus interaction or slower activation of antioxidant pathways, resulting in greater oxidative sensitivity. The contrasting responses observed between the different accessions are consistent with the hypothesis that the interaction between genotype and AMF plays a key role in plant performance (Ronga et al., 2019). However, as our study included only one AMF species (*R. irregularis*) and three argan accessions, further research exploring a larger AMF strains diversity and combinations with different argan genotypes is necessary to identify the most effective AMF-argan accession combinations improving drought resistance and promoting the development of more resilient agroecosystems.

Inoculation with *R. irregularis* MUCL 41833 improves drought resistance and drought recovery in maize by enhancing phosphorus uptake and water use efficiency

We have demonstrated that *R. irregularis* MUCL 41833 promotes drought resistance and recovery in maize by improving phosphorus uptake and water use efficiency. The mycorrhizal plants showed greater Pi uptake and improved WUEi during rehydration, irrespective of water regime.

In the context of climate change, where rainfall events are becoming less frequent but more intense, understanding how plants recover from drought stress is of increasing importance. These conditions expose plants to prolonged periods of water deficiency, often followed by heavy rainfall. This requires not only drought resistance but also efficient recovery mechanisms. AMF have been shown to facilitate plant recovery from drought as reported by Aroca et al. (2008) with *Lactuca sativa* and Boutasknit et al. (2020) with *Ceratonia siliqua.* They improve root system functionality, maintain membrane stability, and enhance water and nutrient uptake upon rehydration. This allows for the faster restoration of physiological processes such as photosynthesis and transpiration (Aroca et al., 2008; Ruiz-Lozano et al., 2016). Therefore, focusing on plant recovery from drought provides a better understanding of plant resilience in the face of increasingly erratic episodes of water availability. Although the beneficial effects of AMF on drought recovery have been demonstrated in various plant species, such recovery mechanisms have not yet been documented in maize. Maize is a major crop in Morocco and is frequently exposed to drought due to increasingly erratic water availability. It is therefore important to evaluate how AMF can improve drought resistance of maize and help the plant to recover after drought stress.

In our study, the decrease in transpiration rate (E) and stomatal conductance (gs) observed in mycorrhizal maize leaves can be explained by the improved drought prevention mechanisms associated with AMF, such as improved water uptake and stomatal control (Augé, 2001). This is consistent with the recognized role of AMF in regulating stomatal behavior, optimizing water conservation, and facilitating gas exchange during the transition from drought stress to recovery (Duan et al., 2024). The regulation of stomatal behavior is an adaptive response that improves plant WUE under drought conditions (Augé, 2001). Mycorrhizal maize plants also showed a substantial increase in shoot water content, particularly under moderate to severe drought conditions. This finding supports the hypothesis that AMF enhance water transport and retention by increasing root hydraulic conductivity and stimulating aquaporin activity (Cheng et al., 2020). Together, these responses represent key adaptive mechanisms that strengthen strategies to cope with drought stress. Several studies have demonstrated that AMF improves plant drought resistance, mainly through increased P uptake. For example, AMF combined with P supplementation improves growth and drought tolerance in barley, increases P content and antioxidant responses in *Populus cathayana*, and regulates phosphate transporter activity in tomato to mitigate drought stress. Additionally, foliar phosphate application improves physiological traits such as chlorophyll content, gas exchange efficiency, and antioxidant activity

under drought (dos Santos et al., 2004; Porcel et al., 2004; Ahmad et al., 2018; Meddich et al., 2021; Han et al., 2022; Szentpéteri et al., 2023).

During rehydration following drought, plants face high metabolic demands to repair tissues, resume growth, and re-establish physiological functions (Bruce et al., 2007). Phosphorus is essential during this phase, as it plays a central role in energy metabolism (ATP), membrane repair (phospholipids), and cellular signaling (Schachtman et al., 1998; Bruce et al., 2007). AMF can enhance P acquisition by increasing the effective root surface area through extraradical hyphae, supporting the plant's nutritional needs during recovery. In our study, Pi uptake was significantly higher in M plants than in NM plants during the recovery phase, regardless of the water regime. At harvest, P content and concentration were also significantly higher in the roots, but not the shoots, of M plants. This result is consistent with previous research. For example, Xiao et al. (2023), reported that AMF colonization increased P content in *Cinnamomum migao*, while Subramanian et al. (1997) observed that colonized maize plants maintained higher P content at the end of the drought and recovery stages.

CONCLUSION AND PERSPECTIVES

This work provides comprehensive evidence for the positive role of the AMF *R. irregularis* MUCL 41833 in improving plant growth, physiological performance, and stress resilience of two economically important species grown in Morocco: *Argania spinosa* and *Zea mays.* Within this thesis we tried to answer three main questions and delineated some further perspectives:

1- Is it possible to associate AMF and argan plants under *in vitro* culture conditions? Does *in vitro* mycorrhization of argan plants impact their subsequent growth and development under *ex vitro* conditions?

In our study, we have successfully associated argan seedlings with *R. irregularis* MUCL 41833 *in vitro* and have demonstrated their better growth following transfer to the greenhouse. This is a significant advance for this species, particularly given the historical challenges associated with its propagation. The association with the AMF promoted early and robust colonization, increased rooting in the *in vitro* stages, increased chlorophyll content and significantly improved plant growth following transfer to the greenhouse. Differences in response to AMF were observed between the three argan accessions tested (Tidzi, Mejji, and City Hanchan), with Tidzi exhibiting the highest responsiveness.

Perspectives:

We have shown that *in vitro* culture is a promising approach for mass propagation of AMF-colonized argan plants which can contribute to the reintroduction of this plant in difficult ecosystem. The chronological compatibility observed in our study between organogenesis or somatic embryogenesis and optimal mycorrhization is promising for developing regeneration and mass propagation protocols of robust plants. Further research should focus on developing and optimizing rooting processes *in vitro* and, following acclimatization under controlled conditions, evaluate the survival and regrowth of AMF-colonized plants after transfer to the field. Therefore, evaluating the impact of AMF on the root system architecture, including root length, branching, density, and overall biomass, needs to be investigated more deeply. Particular attention should therefore be paid to hormones such as auxins and cytokinins, which play a key role in root initiation and growth. AMF have been shown to influence these hormonal pathways, thereby promoting root and shoot development in various plant species (Ludwig-Müller, 2010). However, it is unclear whether the same phenomenon occurs in argan. Future research should therefore explore whether AMF can affect auxin and cytokinin levels in argan seedlings. This could

improve our understanding of how AMF impact root architecture, which is essential for successful *in vitro* regeneration and plant survival during acclimatization.
To investigate whether AMF influence auxin and cytokinin levels in argan seedlings, inoculated and non-inoculated plants can be compared, and hormone levels in roots and shoots quantified using methods such as HPLC-MS/MS

2- Does *Rhizophagus irregularis* MUCL 41833 protect argan accessions against drought stress?

In our study, we have demonstrated that *R. irregularis* MUCL 41833 has a protective effect on drought stress alleviation in three argan accessions (TD, MJ, and CH). This was evidenced by improved growth and physiology (e.g., increased biomass, chlorophyll content and stomatal conductance). Furthermore, reduced H_2O_2 and MDA concentrations were observed in TD and MJ, suggesting that root colonization can mitigate oxidative stress. While all three accessions benefited from AMF inoculation under drought conditions, TD and MJ demonstrated superior responses in terms of nutrient uptake (particularly P and K), water retention and reduced oxidative damage. This highlights the importance of genetic variability in AMF responsiveness.

Perspectives:

Due to the different responses of the TD, MJ, and CH accessions to *R. irregularis* inoculation under drought stress condition, further research should focus on identifying the genetic factors that determine AMF responsiveness. Identifying molecular markers responsible for increased AMF compatibility would allow for the targeted selection of argan genotypes that are potentially better adapted to arid conditions.
To study the genetic factors that control argan response to AMF, argan genotypes can be inoculated with AMF. Techniques such as qPCR, RNA sequencing, and molecular marker analysis can be used to identify genes and markers associated with larger colonization and growth. (Given that the genome of argan tree is still largely unknown and unannotated, these techniques will only be possible once the genome will be available).

As argan trees are naturally adapted to arid and semi-arid environments, focusing on the selection and use of AMF strains that have been isolated from natural argan environments appears another important perspective. These AMF, whether native or adapted to arid conditions, may possess specific traits that enhance plant tolerance to drought stress. Evaluating the performance of these AMF in symbiosis with argan trees could help to identify the most effective combinations for improving drought resistance.

Selection and isolation of AMF from natural argan environments involve collecting rhizosphere soil around healthy argan roots, extraction, and molecular identification of the species, and a final selection of strains that efficiently colonize argan and enhance drought tolerance and nutrient uptake.

In addition to measuring oxidative damage markers such as H_2O_2 and MDA under drought conditions, drought resistance is also considered an indicator of a plant's ability to detoxify ROS through the upregulation of various genes involved in defense mechanisms (Fahad et al., 2017; Abideen et al., 2020; Rady et al., 2020). Detoxification of excess ROS in plants is maintained by enzymatic and non-enzymatic antioxidants during drought stress (Choudhary et al., 2018). In plant cells, ROS scavenging is carried out by enzymatic antioxidants such as SOD, CAT, and APX, as well as non-enzymatic antioxidants, including glutathione and ascorbic acid. Although it is known that AMF can improve drought resistance by modulating ROS detoxification (Li et al., 2022), the specific roles of these enzymatic and non-enzymatic antioxidants in *Argania spinosa* remain unclear. Future research should, therefore, focus on elucidating these mechanisms to better understand how AMF contribute to the regulation of oxidative stress in *A. spinosa* under drought conditions.

To elucidate these mechanisms, the measurement of the activity and expression of both enzymatic (e.g., SOD, CAT, APX) and non-enzymatic (e.g., glutathione, ascorbic acid) antioxidants in AMF-colonized and non-colonized argan trees under drought could be processed. Molecular approaches such as gene expression coupled to proteomics can help identifying the specific genes and pathways involved in ROS detoxification mediated by AMF.

3- Does *Rhizophagus irregularis* MUCL 41833 induce similar adaptive responses to water stress in maize as those observed in the argan tree?

AMF significantly affected foliar gas exchange parameters of maize plants during recovery from drought. Mycorrhizal plants had stable CO_2 assimilation rates but exhibited reduced transpiration (E) and stomatal conductance (gs), resulting in increased instantaneous water use efficiency (WUEi), compared to their non-mycorrhizal counterparts. The reduction in stomatal conductance and transpiration contributed to an increase in shoot water content. Furthermore, AMF colonization enhanced P uptake during the early recovery phase and at harvest, thereby improving the P nutritional status of mycorrhizal plants. This improved nutritional status likely promoted better physiological functioning under stress. These results highlight the functional role of AMF not only in promoting recovery from drought but also in improving the drought resistance of

maize. They also demonstrate the potential of AMF as a sustainable biotechnological tool for mitigating the adverse effects of water deficits in maize cultivation, particularly in the context of climate change.

Perspectives:

The accumulated P uptake capacity of the AMF-colonized roots during the recovery phase, highlighted the crucial role of these symbioses in nutrient acquisition under adverse conditions. Further research is needed to examine whether these results are correlated with a change in root architecture and the overexpression of phosphate transporters in mycorrhizal maize plants. Understanding the mechanisms by which *R. irregularis* improves plant resilience to drought stress, particularly via the modulation of phosphate transporters or root remodeling, could offer interesting perspectives for crop improvement strategies. A relevant approach to further exploring this hypothesis would be to combine transcriptomic analyses and root imaging.

As this study used only *R. irregularis* MUCL 41833, it is important to evaluate the ability of other AMF strains particularly indigenous ones to improve our understanding of the genetic and physiological determinants of drought resistance and higher recovery potential. This could facilitate the selection of effective combinations of AMF and maize varieties, thereby enhancing crop yields under drought conditions.

Future research could involve isolating and propagating indigenous AMF strains, followed by controlled inoculation of maize varieties under well-watered and drought-stress conditions. Subsequent assessment of root colonization, physiological responses, and recovery after re-watering would allow identification of the most effective AMF–maize combinations for drought resilience.

Development of mycorrhizal inoculum the argan tree (*Argania spinosa*) and maize (*Zea mays*) and limitation of use.

From a Moroccan perspective, argan and maize are both essential for ecological sustainability and socioeconomic development. Argan tree, which is endemic to south-west Morocco, support biodiversity and sustain rural livelihoods through the production of argan oil, while maize is a key crop for food security and animal feed. However, both species face major challenges due to climate stress, with the argan tree struggling in harsh soils and experiencing the effects of climate change, and the maize crop facing water scarcity. These problems threaten the productivity and ecological integrity of both species. Incorporating AMF into their cultivation can improve nutrient uptake and optimize water use, reducing reliance on chemical fertilizers and providing a sustainable approach to building resilience. This strategy aligns with Moroccan national initiatives such as the Green Morocco Plan (Plan Maroc Vert (PMV)) and the Green Generation (GG) 2020–2030 program, which promote environmental preservation, sustainable agriculture, and economic development.

To develop an effective AMF inoculum for argan and maize, it is essential to carefully select fungal strains that are compatible with each species and capable of enhancing drought tolerance. Argan requires long-term colonization to enhance nutrient uptake in poor soils, whereas maize benefits from rapid root colonization to promote immediate growth and efficient water use.

For argan tree several studies have demonstrated the beneficial effects of specific inocula. Soufiani et al. (2022) tested a consortium of seven native mycorrhizal fungal species called 'Rhizargan', comprising *Glomus* (15 species), *Acaulospora* (10 species), *Entrophospora* (4 species), *Gigaspora* (4 species), *Scutellospora* (3 species), *Pacispora* (2 species) and *Ambispora* (1 specie). This consortium was found to significantly improve seedling growth and resilience to stress. Under severe drought stress, Rhizargan also markedly increased the phosphorus content of argan plants' leaves and roots. Similarly, Ouallal et al. (2022) tested two complex inocula: one originating from Bouyzakarne, and the other from Argana. Their study showed that the Bouyzakarne inoculum was particularly effective in promoting higher P accumulation and greater fresh biomass production in both roots and shoots. This suggests that the Bouyzakarne inoculum provides relatively more efficient tolerance to drought stress. Additionally, our study in Chapter 2 revealed that inoculation with *R. irregularis* significantly enhanced drought resistance in two *A. spinosa* accessions.

Taken together, these findings suggest that an effective inoculum for argan trees should ideally combine native, multispecies AMF consortia, such as the Rhizargan or Bouyzakarne complexes,

with well-characterized, widely used strains, such as *R. irregularis*. This would exploit the adaptive traits of the native fungi, which are already adapted to the local arid pedoclimatic conditions, while also benefiting from the strong colonizing and stress-mitigating properties of *R. irregularis*. This integrated approach could maximize P uptake, enhance biomass production, and improve drought tolerance, providing a more reliable strategy for argan tree cultivation under water-limited conditions.

For Maize plants, several studies have highlighted the beneficial role of AMF in improving the tolerance of maize plants to water stress. For instance, inoculation with *R. intraradices* has been shown to optimize the nutrient balance and enhance tolerance to water limitation (Zhao et al., 2015). Similarly, inoculation with *Glomus versiforme* was found to significantly improve the drought tolerance of maize plants (Begum et al., 2019). Another study investigated the use of *F. mosseae* inoculation to mitigate the adverse effects of water stress in maize. Additionally, a consortium of *R. clarus* and *Bacillus sp*. promoted P uptake by plants under heavy watering and water-limiting conditions (Silva et al., 2023). In our own research, presented in Chapter 3, inoculation with *R. irregularis* was found to enhance P uptake and water use efficiency in maize plants during recovery from water stress.

Based on these results, the development of an effective maize inoculum should consider the selection of highly effective AMF species, as well as the potential benefits of microbial consortia. Examples of such strains include *R. intraradices*, *G. versiforme, F. mosseae* and *R. irregularis*. Furthermore, the observed synergistic effect of the *R. clarus* and *Bacillus* sp. consortium (Silva et al., 2023) underlines the importance of combining AMF with complementary plant growth-promoting microorganisms to enhance P uptake and resilience in response to varying water conditions. Therefore, producing an effective maize inoculum would involve formulating a multi-strain consortium incorporating native, well-characterized AMF species and beneficial bacteria to ensure broad-spectrum benefits under different environmental conditions.

However, optimizing inoculum production does not solve the significant challenge of adapting laboratory protocols to field conditions. Soil variability, microbial interactions, and environmental stressors all strongly influence the success of inoculation, thus limiting its potential for large-scale application. Furthermore, introducing exotic strains of inoculants into natural ecosystems can disrupt local microbial diversity (Schwartz et al., 2006). Therefore, careful attention to strain selection, production methods, ecological compatibility and long-term sustainability is essential to optimize the use of AMF in agriculture.

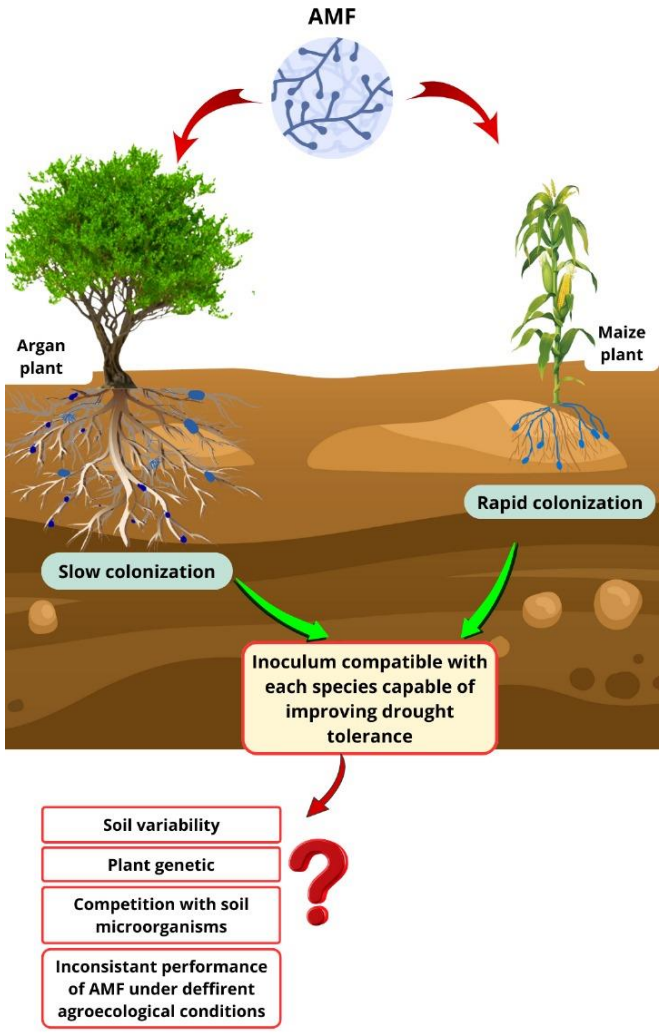

Figure 43: Illustration of the development of a dual-purpose mycorrhizal inoculum for the argan tree (*Argania spinosa*) and maize (*Zea mays*). The figure highlights the potential of this inoculum as a key strategy for sustainable agricultural systems in the face of climate change, emphasizing the complex interactions between AMF, soil variability, plant variability, and competition with other microorganisms (Adapted from Canvas.)

Limitations of results obtained under controlled conditions.

The results of drought resistance observed in argan (*Argania spinosa* L.) and maize (*Zea mays* L.) under controlled conditions must be confirmed by field trials. For maize, this verification is relatively simple, as its growth cycle is short and can be assessed in a single growing season. In contrast, the argan tree has a long development cycle, which means that assessing its resilience to drought in natural conditions requires several years of observation. Nevertheless, field validation remains essential to ensure that the positive effects observed under controlled conditions are consistent and applicable in real agricultural and ecological conditions.

Socio-economic impact of AMF inoculation of argan tree and maize on Moroccan agriculture

Inoculating argan and maize with AMF to improve their drought resistance has major socioeconomic implications for Morocco. Argan contributes to rural livelihoods through the production and marketing of argan oil, which has high global economic value. This oil is an important source of income for local populations, particularly through women's cooperatives (Gebrai et al., 2025). In 2022, the global argan oil market was estimated to be worth USD 299.45 million, and it is expected to grow at a compound annual rate of 11.4% between 2023 and 2030 (Report of Gran View Research, 2024 https://www.grandviewresearch.com/industry-analysis/argan-oil-market). This reflects growing demand from industry. Similarly, maize is one of Morocco's main food crops, essential for food security and rural economies. By mitigating the negative effects of drought through symbiosis with AMF, maize productivity can be stabilized, thereby reducing dependence on imports. According to the USDA, 90% of Morocco's maize is imported from Argentina. In conclusion, applying AMF to improve the water stress tolerance of argan and maize could promote sustainable socio-economic development in Morocco.

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OVERVIEW OF SCIENTIFIC ACHIEVEMENT

Scientific publication

Matike Ganoudi, Olivia Le Pioufle, Maryline Calonne -Salmon, Fatma Ben Dhaou and Stéphane Declerck., 2019. ***Rhizophagus irregularis*** **MUCL 41833 improves phosphorus uptake and water use efficiency in maize plants during recovery from drought stress**. Frontiers in Plant Cciences. https://doi.org/ 10.3389/fpls.2019.00897

Matike Ganoudi, Maryline Calonne-Salmon, Mohamed Ibriz, Stéphane Declerck., 2021. ***In vitro*** **mycorrhization of *Argania spinosa* L. using germinated seeds,** Springer Nature**.**

Matike Ganoudi, Imane Ouallal**,** Mohamed Ibriz, Driss Iraqi., 2023. **Diversity of endomycorrhizal fungi in argan forest stands implications for the success of reforestation programs**, **Forest**. htpps://doi.org/10.3390/f1. 2023. 4081649

Matike Ganoudi, Soumia El Malahi, Nouhaila Manan, Mohammed Ibriz, Maryline Calonne-Salmon, Stéphane Declerck, 2025. Drought resistance of *Argania spinosa* L. colonized by the arbuscular mycorrhizal fungus *Rhizophagus irregularis* varies according to accession. Article accepted in Frontiers: plant sciences

Conference participation.

Ganoudi M, Ibriz M, Iraqi D, Declerck S. ***In vitro*** **mycorrhization of argan plantlets with the *rhizophagus irregularis* MUCL41833 improves their growth during the weaning phase**. International Argan Congress, 10- 11 December 2019.

Ganoudi M, Ibriz M, Iraqi D, Declerck S**. Impact of arbuscular mycorrhizal fungi on phosphorus uptake by argan trees under drought stress conditions.** International Argan Congress, 20- 22 November 2017.

Ganoudi M, Ibriz M, Crozilhac P, Lepioufle O, Diria G, abdelwahd R, Mentag R, Declerck S. ***In vitro*** **mycorrhization of Argan tree: impact on development and growth**. 21 National Symposium on applied Biological Sciences, February 2016.

Ganoudi M, Ibriz M, Crozilhac P, Lepioufle O, Diria G, Declerck S. **Caractérisation et identification morphologique des souches fongiques associées aux rhizosphères de l'arganier,** International Argan Congress, 17- 19 Decembre 2015.

Ganoudi Matike, Amghar Ilham, Abdelwahd rabha, Diria ghgizalne, Ibriz mohamed, Stepahne declerck**. Caractérisation et identification des souches fongiques associées aux rhizosphères de l'arganier**, International Congress on Mycorrhizae. Mycorrhizal Symbiosis a key factor for improving plant productivity and ecosystems restoration, Marrakesh, Morocco, 15-17 October 2014.

Training and students' supervision

- International Training on *in vitro* Culture of Arbuscular Mycorrhizal Fungi, from 15/05/2015 to 23/05/2015, plant system sessions. UCLouvain, Belgium.
- International Training on *in vitro* Culture of Arbuscular Mycorrhizal Fungi from 09/05/2016 to 15/05/2016, plant system sessions. UCLouvain, Belgium
- International Training on *in vitro* Culture of Arbuscular Mycorrhizal Fungi from 23/10/2016 to 28/10/2016, root disinfection sessions. UCLouvain, Belgium
- Student supervision of Fatma Ben Dhaou (October to June 2018-2019), a master's student from the Ecole Polytechnique de Sousse, Tunisia. Title of her master thesis: « Impact of arbuscular mycorrhizal fungi on phosphorus absorption dynamics by Maize under hydric stress».
- Student supervision of Fahd Chabli (March to Novembre 2021), a master's student from Institut Agronomique et Veterinaire Hassan II, Maroc Title of his master thesis «Effect of arbuscular mycorrhizal fungi on morphological and physiological traits of drought tolerance in *Argania spinosa* »
- Student supervision of Nouhaila Manan (19 February to 24 July), a master's student from Mohammed V University of Rabat, Faculty of Sciences, Maroc. Title of her master thesis: «Effect

of arbuscular mycorrhizal fungi on morphological and physiological traits of drought tolerance in *Argania spinosa* ».

- Student supervision of Chaymae Lamchichi (15 January to 30 June 24), a master's student from Ibn Tofail University of Kenitra, Faculty of Sciences, Maroc. Title of her master thesis: «Evaluation of the mycorrhizal potential of argan rhizosphere soils in the Essaouira region».
- Student supervision of Nouhaila Ouahib (01May to 30 September), a master's student from Ibn Tofail University of Kenitra, Faculty of Sciences, Maroc. Title of her master thesis: «Evaluation of the mycorrhizal potential of argan rhizosphere soils in the Essaouira region ».

ANNEXES

Annex I

In vitro mycorrhization of *Argania spinosa* L. using germinated seeds

Supplementary table 1. The composition of the various stock solutions involved in developing the MSR medium.

Macroelement stock solution (g/l)		**Microelement stock solution (g/l)**		**Calcium nitrate solution (g/l)**		**NaFeEDTA (g/l)**	
KNO_3	7,6	$MnSO_4.7H_2O$	2,45	$Ca(NO_3)_2.4H_2O$	35,9	NaFeEDTA	1,6
KCL	6,5	$ZnSO_4.7H_2O$	0,28				
KH_2PO_4	0,41	H_3BO_3	1,85				
$MgSO_4.7H_2O$	73,9	$CuSO_4.5H_2O$	0,22				
		$(NH_4)6Mo_7O_{24}.4H_2O$	0,034				
		$Na_2MoO_4.2H_2O$	0,0024				

Methods

In a 1l volumetric flask, mix:

10 ml of macroelement solution
10 ml of calcium nitrate solution
5 ml of NaFeEDTA solution
1 ml of microelement solution

Make up the volume to the mark on the graduated flask.

Homogenize with a magnetic stirrer

Adjust PH to **5.50** ± 0.05

Add **3 g L^{-1}** Phytagel or Gelrite

Dissolve the gelling agent by boiling the solution on a heated magnetic stirrer.

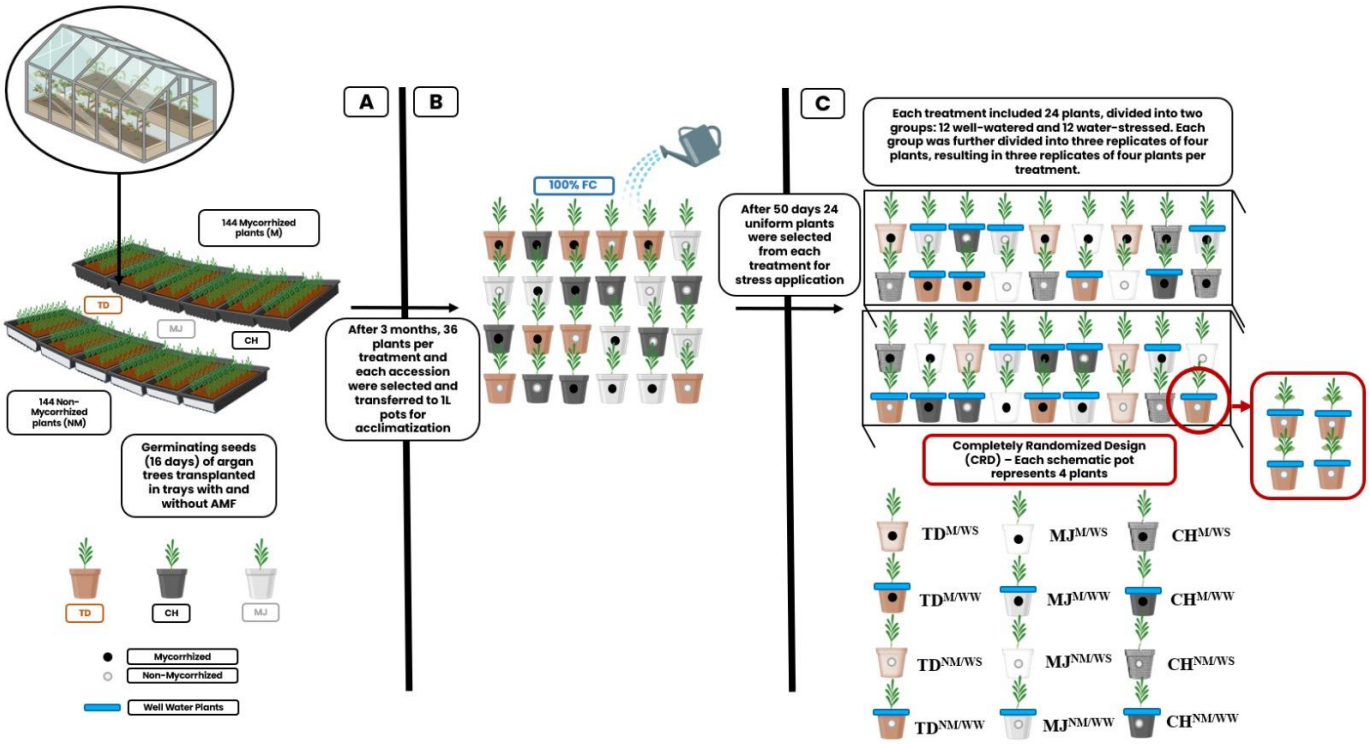
Autoclave the solution for 15 minutes at 121°C and 1bar pressure.

Use solution directly or store in oven (40°C - 60°C).

NB: This solution can be stored in the oven for up to 4 days.

Annex II

Drought resistance of *Argania spinosa* L. colonized by the arbuscular mycorrhizal fungus *Rhizophagus irregularis* varies according to accession.



Supplementary figure 1. Experimental set up to evaluate the effects of mycorrhization on three argan accession grown under two water regimes.

Supplementary table 1. Effects of two water regimes (i.e., 100% of field capacity = well-watered (WW) and 15% of the field capacity = water stress (WS)) on the mean total percentage of colonized root length (%CRL), the mean %CRL by hyphae (%CRLH), by arbuscules CRLA), and vesicles (%CRLV) of argan plants accessions (TD (Tidzi), MJ (Mejji), and CH (City Hanchan)) colonized by *Rhizophagus irregularis* MUCL 41833 at the transfer into individual pots and at harvest (52 days after the start of the water regime application).

		At plant transfer	At harvest	
			WW	WS
TD	**%CRL**	86.20 ± 7.31^{a}	74.13 ± 6.96^{ab}	62.42 ± 7.84^{b}
	%CRLH	43.13 ± 4.87^{a}	40.49 ± 3.89^{ab}	32.01 ± 2.58^{b}
	CRLA	57.64 ± 3.43^{a}	28.02 ± 1.98^{b}	21.97 ± 3.40^{b}
	CRLV	40.96 ± 4.11^{a}	36.24 ± 3.97^{a}	24.22 ± 2.94^{b}
MJ	**CRL**	79.00 ± 2.12^{a}	72.72 ± 4.84^{ab}	60.73 ± 7.35^{b}
	%CRLH	37.02 ± 6.42	44.29 ± 6.82	31.33 ± 3.05
	%CRLA	20.92 ± 2.01	18.63 ± 3.21	15.35 ± 2.59
	%CRLV	56.22 ± 1.79^{a}	38.60 ± 3.18^{b}	30.95 ± 2.28^{c}
CH	**CRL**	75.70 ± 7.99^{a}	63.01 ± 3.37^{ab}	53.85 ± 4.49^{b}
	%CRLH	37.13 ± 6.13^{a}	34.39 ± 3.69^{a}	20.92 ± 2.01^{b}
	%CRLA	28.56 ± 7.43^{a}	22.06 ± 3.44^{ab}	10.97 ± 1.68^{b}
	%CRLV	47.60 ± 19.90	32.17 ± 2.26	26.89 ± 3.15

Data were analyzed using a one-way analysis of variance (ANOVA) to assess significant differences among treatments. Post hoc comparisons were conducted using the Tukey-HSD test at a significance level of P≤ 05. Means sharing the same lower-case letter within a row do not differ significantly. Results are expressed as mean ± SD (n = 3)

Supplementary table 2. Effects of accessions (CH, MJ, TD), water regimes (WW and WS) and AMF colonization (NM and M) by *R. irregularis* MUCL 41833 on the proline and H_2O_2 concentrations of argan plants after 52 weeks of stress conditions.

	Proline (µmol/g)	**H_2O_2 (µmol/g)**
Effect A		
CH	5.23 ± 1.28	2.67 ± 1.51[a]
MJ	2.58 ± 1.33	1.94 ± 0.94[b]
TD	2.15 ± 1.14	1.56 ± 0.75[c]
Effect AMF		
NM	3.78 ± 1.66	2.39 ± 1.34 [β]
M	2.86 ± 1.94	1.73 ± 0.91[α]
Effect WR		
WW	2.40 ± 1.57[b]	1.13 ± 0.27
WS	4.24 ± 1.64[a]	2.98 ± 0.99
Effect A * AMF		
CH/NM	5.32 ± 1.31	3.13 ± 1.79
CH/M	5.13 ± 1.37	2.21 ± 1.15
MJ/NM	3.37 ± 1.18	2.24 ± 1.06
MJ/M	1.80 ± 1.00	1.65 ± 0.78
TD/NM	2.65 ± 1.29	1.79 ± 0.83
TD/M	1.64 ± 0.76	1.32 ± 0.64
Effect A * WR		
CH/WW	4.36 ± 0.79	1.34 ± 0.29
CH/WS	6.09 ± 1.09	4.00 ± 0.85
MJ/WW	1.61 ± 0.81	1.12 ± 0.20
MJ/WS	3.55 ± 0.98	2.77 ± 0.53
TD/WW	1.23 ± 0.36	0.93 ± 0.15
TD/WS	3.07 ± 0.82	2.19 ± 0.51
Effect AMF * WR		
NM/WW	2.88 ± 1.57	1.29 ± 0.23
M/WW	4.68 ± 1.24	3.49 ± 1.03
NM/WS	1.92 ± 1.50	0.98 ± 0.23
M/WS	3.79 ± 1.93	2.48 ± 0.66
Effect A * AMF * WR		
CH/NM/WW	4.81 ± 0.93	1.50 ± 0.23
CH/NM/WS	5.83 ± 1.62	4.76 ± 0.12
CH/M/WW	3.91 ± 0.30	1.19 ± 0.29
CH/M/WS	6.35 ± 0.70	3.24 ± 0.23
MJ/NM/WW	2.33 ± 0.25	1.30 ± 0.06
MJ/NM/WS	4.40 ± 0.45	3.17 ± 0.43

MJ/M/WW	0.89 ± 0.05	0.95 ± 0.04
MJ/M/WS	2.70 ± 0.11	2.36 ± 0.16
TD/NM/WW	1.50 ± 0.30	1.06 ± 0.06
TD/NM/WS	3.80 ± 0.26	2.53 ± 0.35
TD/M/WW	0.95 ± 0.02	0.79 ± 0.02
TD/M/WS	2.33 ± 0.08	1.85 ± 0.42
	p-value	
Effect A	0.000	**0.000**
Effect AMF	0.000	**0.000**
Effect WR	**0.000**	**0.000**
Effect A * AMF	**0.028**	0.997
Effect A * WR	0.907	0.067
Effect AMF * WR	0.847	0.548
Effect A * AMF * WR	0.063	0.707

Data are presented as means +/- SD. Data were analyzed by a three-way ANOVA followed by contrasts post-hoc test ($p<0.05$) for normalized data. For non-normalized data, a Kruskall-Wallis test was applied. For each effect in a column, different lower-case letters indicate significant difference among the treatments. The absence of any sign indicates no difference among the treatments.

Supplementary table 3. Effects of accessions (CH, MJ, TD), water regimes (WW and WS) and AMF colonization (NM and M) by *R. irregularis* MUCL 41833 on the shoot and root lengths of argan plants after 52 weeks of stress conditions.

	Shoot length (cm)	**Root length (cm)**
Effect A		
CH	18.38 ± 4.84	30.75 ± 8.83
MJ	21.42 ± 6.33	33.50 ± 12.61
TD	19.96 ± 4.55	27.08 ± 9.23
Effect AMF		
NM	16.33 ± 4.81	23.58 ± 9.20
M	23.50 ± 2.71	37.31 ± 6.22
Effect WR		
WW	21.64 ± 3.99	33.58 ± 7.23
WS	18.19 ± 5.95	27.31 ± 12.24
Effect A * AMF		
CH/NM	14.83 ± 3.87	24.58 ± 8.55^{C}
CH/M	21.92 ± 2.54	36.92 ± 2.69^{AB}
MJ/NM	17.17 ± 6.34	23.08 ± 8.79^{C}
MJ/M	25.67 ± 2.16	43.92 ± 3.47^{A}
TD/NM	17.00 ± 4.43	23.08 ± 11.65^{C}
TD/M	22.92 ± 2.20	31.08 ± 3.64^{BC}

Effect A * WR		
CH/WW	19.58 ± 2.87	33.50 ± 5.01
CH/WS	17.17 ± 6.31	28.00 ± 11.33
MJ/WW	24.00 ± 4.24	36.75 ± 7.43
MJ/WS	18.83 ± 7.36	30.25 ± 16.41
TD/WW	21.33 ± 4.03	30.50 ± 8.61
TD/WS	18.58 ± 4.96	23.67 ± 9.22
Effect AMF * WR		
NM/WW	19.67 ± 4.06[a]	30.33 ± 7.36[b]
M/WW	13.00 ± 2.78[a]	16.83 ± 4.81[ab]
NM/WS	23.61 ± 2.96[b]	36.83 ± 5.77[c]
M/WS	23.39 ± 2.62[a]	37.78 ± 6.96[a]
Effect A * AMF * WR		
CH/NM/WW	17.67 ± 2.52	30.67 ± 5.69
CH/NM/WS	12.00 ± 2.65	18.50 ± 6.26
CH/M/WW	21.50 ± 1.80	36.33 ± 2.52
CH/M/WS	22.33 ± 3.51	37.50 ± 3.28
MJ/NM/WW	21.67 ± 4.73	30.50 ± 2.78
MJ/NM/WS	12.67 ± 4.16	15.67 ± 4.51
MJ/M/WW	26.33 ± 2.52	43.00 ± 3.61
MJ/M/WS	25.00 ± 2.00	40.83 ± 3.82
TD/NM/WW	19.67 ± 5.03	29.83 ± 13.27
TD/NM/WS	14.33 ± 1.53	16.33 ± 5.13
TD/M/WW	23.00 ± 2.65	31.17 ± 2.84
TD/M/WS	22.83 ± 2.25	31.00 ± 5.00
	p-value	
Effect A	0.080	**0.033**
Effect AMF	**0.000**	**0.000**
Effect WR	**0.003**	**0.003**
Effect A * AMF	0.608	**0.031**
Effect A * WR	0.515	0.955
Effect AMF * WR	**0.005**	**0.001**
Effect A * AMF * WR	0.889	0.916

Data are presented as means +/- SD. Data were analyzed by a three-way ANOVA followed by contrasts post-hoc test ($p<0.05$) for normalized data. For non-normalized data, a Kruskall-Wallis test was applied. For each effect in a column, different lower-case letters indicate significant difference among the treatments. The absence of any sign indicates no difference among the treatments.

Supplementary table 4. Effects of accessions (CH, MJ, TD), water regimes (WW and WS) and AMF colonization (NM and M) by *R. irregularis* MUCL 41833 on the K and Na concentrations on roots and on the k concentrations on shoots of argan plants after 52 weeks of stress conditions.

	Roots		Shoots
	K (mg/g)	**Na (mg/g)**	**K (mg/g)**
Effect A			
CH	1.42 ± 0.42	6.742 ± 2.38	1.11 ± 0.13
MJ	1.64 ± 0.28	8.69 ± 4.52	1.14 ± 0.39
TD	1.24 ± 0.39	7.6 ± 2.62	1.08 ± 0.22
Effect AMF			
NM	1.22 ± 0.31^{B}	9.42 ± 3.05	1.22 ± 0.29
M	1.65 ± 0.35^{A}	5.93 ± 2.63	1.22 ± 0.29
Effect WR			
WW	1.29 ± 0.37	7.09 ± 3.99$^{\alpha}$	1.14 ± 0.34
WS	1.57 ± 0.36	8.26 ± 2.46$^{\beta}$	1.07 ± 0.16
Effect A * AMF			
CH/NM	1.13 ± 0.11	8.35 ± 2.22	1.18 ± 0.08
CH/M	1.71 ± 0.41	5.13 ± 1.16	1.03 ± 0.14
MJ/NM	1.52 ± 0.28	12.45 ± 3.00	1.35 ± 0.39
MJ/M	1.75 ± 0. 25	4.93 ± 1.46	0.93 ± 0.29
TD/NM	0.99 ± 0.22	7.47 ± 0.91	1.14 ± 0.30
TD/M	1.49 ± 0.38	7.73 ± 3.77	1.01 ± 0.09
Effect A * WR			
CH/WW	1.28 ± 0.28	5.78 ± 1.40	1.05 ± 0.17
CH/WS	1.56 ± 0.50	7.70 ± 2.88	1.17 ± 0.03
MJ/WW	1.60 ± 0.37	9.35 ± 6.21	1.21 ± 0.55
MJ/WS	1.67 ± 0.18	8.03 ± 2.32	1.07 ± 0.17
TD/WW	1.00 ± 0.22	6.15 ± 2.08	1.18 ± 0.21
TD/WS	1.48 ± 0.38	9.05 ± 2.39	0.98 ± 0.20
Effect AMF * WR			
NM/WW	1.06 ± 0.23	9.71 ± 4.11	1.41 ± 0.24^{a}
M/WW	1.37 ± 0.31	9.13 ± 1.65	1.04 ± 0.19^{b}
NM/WS	1.52 ± 0.35	4.48 ± 1.24	0.90 ± 0.16^{ab}
M/WS	1.77 ± 0.32	7.39 ± 2.90	1.10 ± 0.1^{ab}
Effect A * AMF * WR			
CH/NM/WW	1.04 ± 0.42	6.50 ± 1.41	1.18 ± 0.13
CH/NM/WS	1.21 ± 0.09	10.20 ± 0.20	1.18 ± 0.26
CH/M/WW	1.51 ± 0.18	5.07 ± 1.18	0.91 ± 0.29
CH/M/WS	1.90 ± 0.51	5.20 ± 1.39	1.15 ± 0.21
MJ/NM/WW	1.29 ± 0.18	14.80 ± 2.34	1.70 ± 0.09
MJ/NM/WS	1.75 ± 0.05	10.10 ± 0.66	0.99 ± 0.07
MJ/M/WW	1.91 ± 0.13	3.90 ± 1.39	0.71 ± 0.13

MJ/M/WS	1.59 ± 0.25	5.97 ± 0.45	1.15 ± 0.23
TD/NM/WW	0.85 ± 0.16	7.83 ± 0.76	1.35 ± 0.15
TD/NM/WS	1.15 ± 0.17	7.10 ± 1.04	0.94 ± 0.29
TD/M/WW	1.16 ± 0.14	4.47 ± 1.33	1.01 ± 0. 10
TD/M/WS	1.82 ± 0.08	11.00 ± 1.32	1.00 ± 0.1.05
	p-value		
Effect A	0.527	**0.021**	0.078
Effect AMF	**0.007**	0.255	0.084
Effect WR	0.074	**0.000**	0.739
Effect A * AMF	0.075	**0.001**	**0.000**
Effect A * WR	0.260	0.177	**0.028**
Effect AMF * WR	0.977	0.690	**0.042**
Effect A * AMF * WR	0.540	0.770	0.357

Data are presented as means +/- SD. Data were analyzed by a three-way ANOVA followed by contrasts post-hoc test ($p<0.05$) for normalized data. For non-normalized data, a Kruskall-Wallis test was applied. For each effect in a column, different lower-case letters indicate significant difference among the treatments. The absence of any sign indicates no difference among the treatments.

Supplementary table 5. Effects of accessions (CH, MJ, TD), water regimes (WW and WS) and AMF colonization (NM and M) by *R. irregularis* MUCL 41833 on the gs and RWC of argan plants after 52 weeks of stress conditions.

	gs (mmol m^{-2} s^{-1})	**RWC (%)**
Effect A		
CH	208.90 ± 73.60	82.61 ± 8.55
MJ	199.30 ± 57.50	84.11 ± 9.49
TD	211.30 ± 69.20	86.03 ± 10.56
Effect AMF		
NM	150.42 ± 38.73	80.16 ± 11.67
M	262.52 ± 25.47	88.34 ± 3.21
Effect WR		
WW	235.50 ± 50.80	89.60 ± 3.70
WS	177.40 ± 66.60	78.90 ± 10.38
Effect A * AMF		
CH/NM	145.90 ± 44.30	78.11 ± 10.29
CH/M	271.82 ± 21.29	87.11 ± 2.54
MJ/NM	151.20 ± 39.20	80.49 ± 12.54
MJ/M	247.37 ± 13.74	87.73 ± 3.07
TD/NM	154.10 ± 39.50	81.87 ± 13.83
TD/M	268.40 ± 33.80	90.19 ± 3.59
Effect A * WR		

CH/WW	238.70 ± 57.50	92.06 ± 3.13
CH/WS	179.10 ± 80.50	80.00 ± 12.18
MJ/WW	223.20 ± 39.90	88.72 ± 4.41
MJ/WS	175.30 ± 65.60	79.50 ± 11.31
TD/WW	244.70 ± 59.70	88.03 ± 2.48
TD/WS	177.80 ± 65.40	77.20 ± 9.19
Effect AMF * WR		
NM/WW	187.78 ± 2.88	90.72 ± 4.24
M/WW	113.06 ± 6.27	69.59 ± 4.50
NM/WS	283.28 ± 18.38	88.48 ± 2.87
M/WS	241.76 ± 8.40	88.21 ± 3.70
Effect A * AMF * WR		
CH/NM/WW	187.78 ± 5.36	88.97 ± 2.25
CH/NM/WS	103.98 ± 4.93	85.25 ± 0.83
CH/M/WW	185.13 ± 4.43	87.08 ± 2.75
CH/M/WS	124.88 ± 2.68	69.14 ± 3.97
MJ/NM/WW	184.92 ± 3.29	86.16 ± 3.80
MJ/NM/WS	114.93 ± 2.95	89.31 ± 1.32
MJ/M/WW	191.48 ± 4.32	91.28 ± 3.80
MJ/M/WS	132.58 ± 3.30	69.70 ± 5.42
TD/NM/WW	191.22 ± 4.26	93.81 ± 3.91
TD/NM/WS	120.17 ± 2.60	90.06 ± 5.67
TD/M/WW	202.62 ± 4.35	90.31 ± 0.12
TD/M/WS	144.80 ± 3.49	69.94 ± 5.94
	z-value	
Effect A	0.318	0.258
Effect AMF	**0.004**	0.066
Effect WR	**0.000**	**0.002**
Effect A * AMF		
Effect A * WR		
Effect AMF * WR		
Effect A * AMF * WR		

Data are presented as means +/- SD. Data were analyzed by a three-way ANOVA followed by contrasts post-hoc test ($p<0.05$) for normalized data. For non-normalized data, a Kruskall-Wallis test was applied. For each effect in a column, different lower-case letters indicate significant difference among the treatments. The absence of any sign indicates no difference among the treatments.

Supplementary results 1.

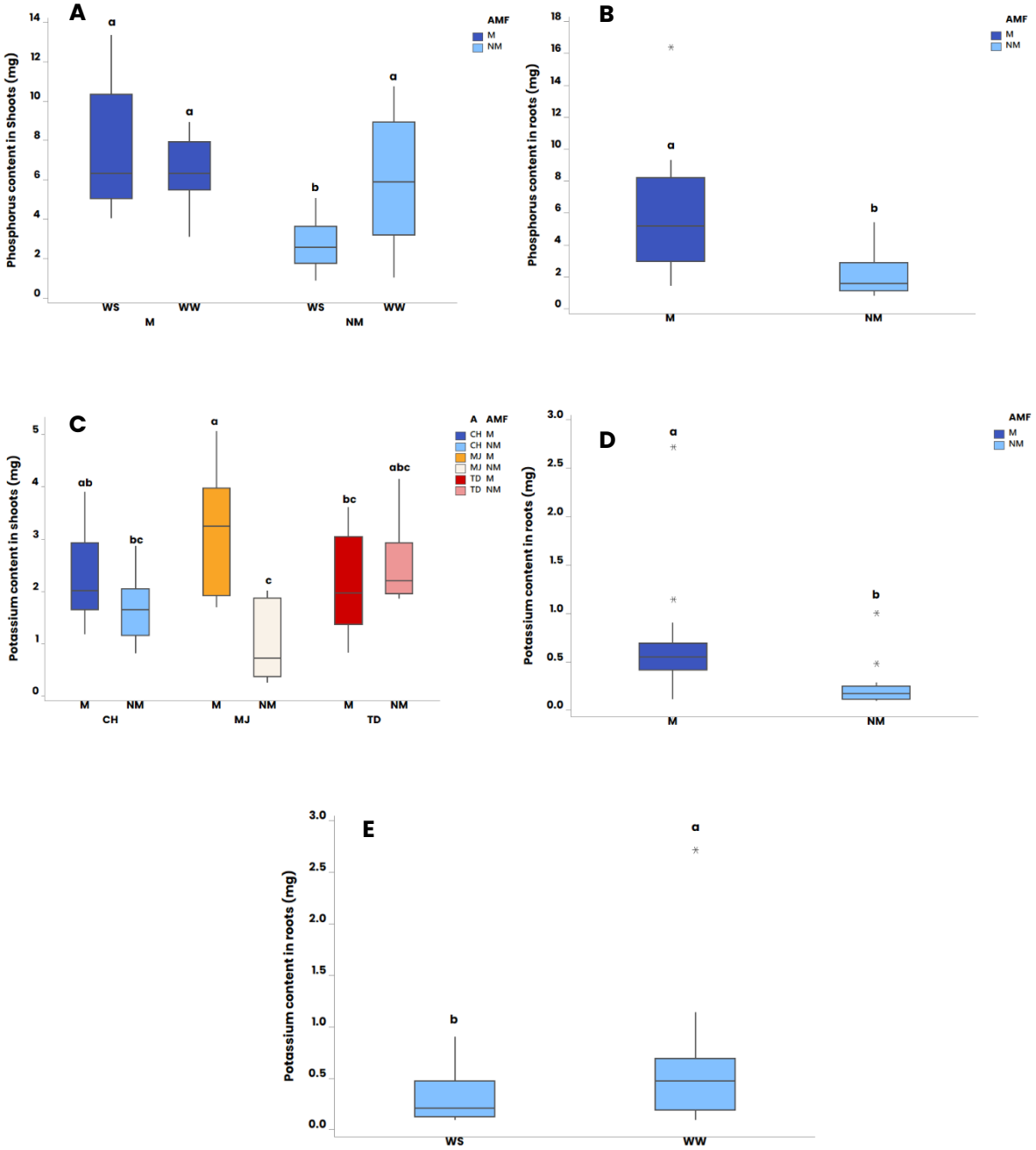


Figure 2. Effect of water regime (WR) (i.e., 100% of field capacity = Well-Watered (WW) and 15% of field capacity = Water-Stressed (WS)), AMF colonization by *Rhizophagus irregularis* MUCL 41833 (i.e. mycorrhizal (M) or non-mycorrhizal (NM)) and accession (i.e., TD (Tidzi), MJ (Mejji), and CH (City El Hanchan)) on nutrients content at harvest (52 days after the start of the WR application). Significance of (A) AMF and WR interaction on phosphorus content in shoots, (B) AMF on phosphorus content in roots, and AMF and accession interaction, (C) accession and AMF interactions on potassium content in shoots and (D) AMF on potassium content in roots, (E) WR on potassium content in roots. The box plots display

the maximum, upper quartile, and lower quartile minimum values. Data were analyzed by a three-way ANOVA followed by a Tukey post-hoc test ($P \leq 0.05$). For one parameter, data sharing similar lower-case letters are not significantly different.

Annex III

Rhizophagus irregularis MUCL 41833 improves phosphorus uptake and water use efficiency in maize plants during recovery from drought stress.

⇒ Preparation of a 90% Pi-impoverished Hoagland (Hoagland ^{lowPi}) solution

Table 1 Composition of the stock solutions (**x100)** for preparation of Hoagland ^{lowPi}

Stock solutions	Elements	Concentration of stock solution ($g\ L^{-1}$)	Concentration of stock solution ($mol\ L^{-1}$)	Final concentration ($g\ L^{-1}$)
Solution A pH 5.3	NH_4NO_3	8.00	0.1	0.0777
	$Ca(NO_3)_2 \cdot 4H_2O$	82.60	0.35	0.8019
	KNO_3	35.70	0.353	0.3466
	KCl	4.51	0.061	0.0438
	K2SO4	10.54	0.061	0.1023
Solution B pH 4.4	KNO_3	5.00	5.00	0.049
	KH_2PO_4	2.74	2.74	0.02
	$MgSO_4$	12.04	12.04	0.1
	$MnSO_4\ H_2O$	0.05	0.05	0.00031
	H_3BO_3	0.14	0.14	0.0023
	$CuSO_4 \cdot 5H_2O$	0.02	0.02	0.00006
	$(NH4)_6Mo_7O_2 \cdot 4H_2O$	0.01	0.01	0.000007
	$ZnSO_4 \cdot 7H_2O$	0.06	0.06	0.00021
Solution C	Fe-EDTA	1.90	0.005200	0.0184

Hoagland ^{lowPi} solution is prepared right before use

To prepare 1 liter volume of solution
10 ml of solution A
10 ml of solution B
10 ml of solution C

Dissolve completely in 800 ml of H_2O

Adjust pH at 5.6