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Interaction of AMF and vegetable crops

Bachelor thesis

Bsc. Agriculture and food

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Declaration:

I declare that the topic "Interaction of AMF and vegetable crops" which I chose to work on is my own work and research. All the references which I used are listed as references.

Prague, Czech Republic

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Interaction of AMF and vegetable crops

Summary:

AMF-interaction has been proven very beneficial since decades in terms of higher yield, nutritional values, resilience against several biotic/abiotic stress factors, etc. Additionally, it is cost-effective so the AMF-plant interactions can lead to higher profitable business in commercial markets. The present study was conducted to examine the positive symbiotic development between selected strains of AMF (*Diversispora celata, Funneliformis mosseae,* and *Rhizophagus irregularis)* and vegetables (tomato, salad, and cabbage), along with the analysis of drought conditions in their symbiosis development. The plant height and leaves numbers were taken into the account as vegetative parameters of the crops. The combination of *Diversispora celata* and *Rhizophagus irregularis* appeared to be the best combination of strains and majority showed positivity. However, drought analysis also showed some positive results with specific cultivars such as, Albatros P.pozdani. In conclusion, *Diversispora celata* with *Rhizophagus irregularis* can be the most suitable combination for further studies, including drought stress conditions.

Key Words:

AMF; symbiosis; vegetable crops; drought stress; vegetative growth

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CHAPTER 01

Introduction

1.1 Introduction

Arbuscular mycorrhizal fungi (AMF) are a vastly spreaded species among the endotrophic mycorrhizal fungi which are found under the monophyletic phylum. AMF are using in agriculture as a bio fertilizer. AMF can proficiently increase the water supply and nutrient’s uptake for the plants such as phosphates and nitrates. However, in exchange that the fungi absorb around 20% of plant-fixed carbon (Parniske, 2008). For efficient plant growth and productivity AMF are usually applied mixed with substrates. It is conventionally practiced and claimed to be the most effective for the sustainability and the ecosystem of agriculture. The fundamental function of the symbiosis is the adequacy of AMF which is to evolve a network of mycelia which extends the proportion of surface area by 40% while producing the enzymes and discharging the organic substances. AMF are able to produce phosphatases to hydrolyze phosphate derived from organic phosphorus compounds in the soil. Therefore, it increases the quality of crops without using too many inputs (Koide and Kabir, 2000; Marschner, 2012). The AMF symbiosis also improves the uptake of insoluble and immobile phosphate ions because of interactivity between tri and bi cations from soil such as, Al^{3+}, Fe^{3+} and Ca^{3+}. The fundamental function of the symbiosis is the adequacy of AMF which is to evolve a network of mycelia which extends the proportion of surface area by 40% while producing the enzymes and discharging the organic substances. Moreover, other beneficial nutrients (ammonium), immobile micronutrients (Zn and Cu), and soil-acquired mineral cations (Ca2+, K+, Fe 3+, and Mg2+) are increased by the extraradical hyphae (Clark and Zeto, 2000; Smith and Read 2008). AMF is not only useful as a biofertilizer but also enhances phytohormonal balance in the crops. Therefore, it works as a bio protector and bioregulator. This is not only increasing the productivity, but also overall quality of the crops (Antunes et al., 2012).

The fungal strain which is used for the inoculation also plays a key role in the whole interaction process. For instance, Hayek et al. (2012) has claimed that three various fungal strains indicated positive effects while out of three only one was capable enough to preserve the crop from a pathogenic root fungus in Petunia. Thus, a proper combination of various genotypes and strains is really important, and the effect of that inoculation should be tested with different environmental conditions (Regvar et al., 2003). In many research, it has been found that the problem of consistency in the positive effectiveness of microbial inoculants and the behind this is the natural outcome of the symbiosis. It has been reported that dual or multiple inoculations consisting of two various microbial species are more advantageous because of synergetic tendencies and it comes with positive outcome rather than negative (Meena et al., 2018).

In many regions of the world, drought is negatively affecting crop production whereas AMF withstands under water drought conditions with higher tolerance (Al-karaki G, et al., 2004). The AMF symbiosis decreases water drought in plants which can be observed by the increased leaf elasticity, better network of roots in the soil, and maintains turgor pressure (Auge et al., 1987a; Auge et al., 1987b; Ellis et al., 1985; Davis et al., 1992).

Accordingly, the aim of this study was to analyze the possible symbiosis between the AMF and the different vegetable crops. As mentioned before, AMF leaves a greater impact on water drought stress crops. Thus, in this experiment, the symbiosis of three different AMF strains with three vegetable crops were also examined under drought stress conditions. The AMF has a vast range of host plants, and it is commonly believed to create a symbiosis with most terrestrial crops. However, cabbage is one of the crops which is recognized to be not highly AMF positive plants. Therefore, this research was focused on whether specific mixtures of AMF strains can vary in results.

1.2 Scientific Hypotheses and objective of the work

Hypothesis: All three AMF strains selected have significant symbiosis development with all three vegetable crops selected and improve the crop growth.

Objectives:

1. Analysis of the possible symbiosis development between crops and AMF and effect on crop growth.
2. Analysis of the effect of drought stress on symbiosis development between cabbage and AMF.

1.3 Literature overview

1.3.1 Arbuscular mycorrhizal fungi (AMF)

AMF are often known as soil-borne fungus which has been proved very essential in terms of plant nutrient uptake and to avoid any kind of biotic or abiotic stress factors (Sun et al., 2018). Arbuscular mycorrhizal (AM) is a particular group of fungi which pierce the cortical cells of the crop roots by creating arbuscules. AMF are categorized under the phylum of *Glomeromycota* unlike the true fungus (Schussler et al., 2001). More or less four orders of AMF have been recognized under this phylum namely, *Diversisporales*, *Archaeosporales*, *Paraglomerals*, and *Glomerals*.

According to some research study of Redecker et al. (2013) molecular phylogenetic and fossil data indicates that even the first ever land plant species was containing the AMF. Around 80-90% of AMF are produced by land crop species, and it is considered as the most ubiquitous crop symbiont (Newman and Reddell 1987). Land crop species such as *Alliaceae* (garlic, onion), some fruit trees (citrus), *Solanaceae* (petunia, tomato), herbs (thyme, basil), and ornamental plants (rose, cactus). Apart from some, almost each AMF can have mutual relationships with mycorrhizal crops (Smith and Read, 2008).

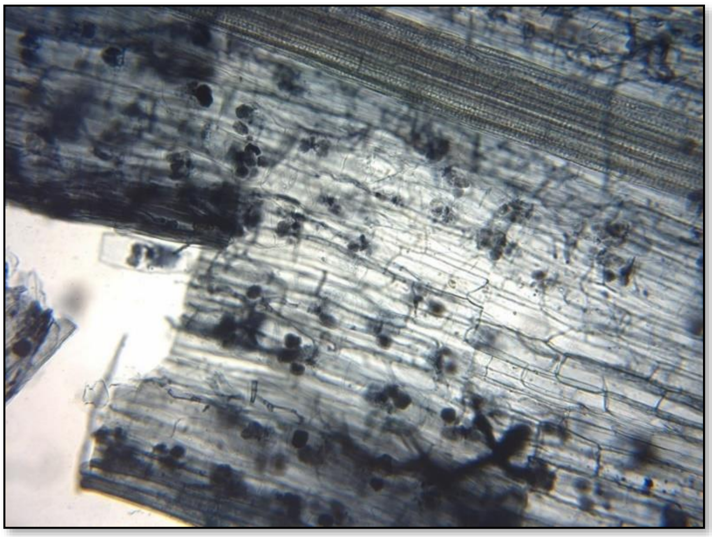

Figure 1: AM; flax root cortical cells containing paired arbuscules (MS Turmel 2006)

Thus, it is impractical to advise individual AMF strains with specific plants. Some of the species of genera are believed to be dangerous for the soil health such as *Scutelleospora* and *Gigaspora*, on that account most researchers focus on the species of the genera *Funelliformes* and *Rhizophagus*. These species can survive in various climate zones. Therefore, it can be found extensively in nearly all types of soils (Smith and Read, 2008). For the healthy crop life cycle, the AMF works as the mandatory lipids, biotrophs, and photosynthetic products (Jiang et al., 2017). AMF not only promotes nutrient and water uptake in the plants but also restricts soil fungal pathogens from damaging the crop's health and life cycle (Smith and Read, 2008; Jiang et al., 2017). Consequently, AMF stands for endosymbionts and is an important key factor in terms of increasing crop productivity and maintaining the habitat. They are also playing a fundamental role in impeding plant development (Gianinazzi et al., 2010).

1.3.2 AMF-Plant symbiosis

1.3.2.1 History of AMF

Looking back into history, the AMF- plant interaction was discovered 400 million years before (Selosse et al., 2015). The AMF was evolved in such a way that it can reproduce asexually which it has inherited from its ancestors. The discovery of the AMF has proved to be one of the successful biological processes which came with the greater impact in the natural ecosystem and agriculture system (Van der Heijden et al., 2015). For many decades, AMF has been used for translocation of nutrients in plants and for improving the plant and soil health.

1.3.2.2 AMF life cycle

AMF's life cycle initiates with chlamydospores in the soil which are produced asexually in the asymbiotic phase. To establish the life cycle, optimum temperature and humidity are essential factors.

AMF are considered as a biotrophs and they reverses the cytoplasm as they do not need the existence of plants and easily head back to the dormant stage. Further, this process leads to development of the primary germ tube, which takes place near the plant-roots and this operation is called a pre-symbolic stage (Giovannetti et al., 1993). The hyphophodia is produced on the root surface when the AMF meets the host. From the plant side, epidermal cells beneath hyphopodia and undergo the specific mycorrhiza process. This procedure constructs the transient intracellular structure which helps fungus to penetrate the roots of the plants (Genre et al., 2005). AMF begins to populate in the root cells and forms the fungal hyphae (Gianinazzi-pearson and Gianinazzi, 1988). After reaching to the inner cortex, the AMF changes the way of populating and expands making a complex-branched structure. This structure builds up in the apoplast of the root cells of the host and structure often known as arbuscules. Simultaneously, the fungus also searches for the nutrients from encircling soil and along with that it also initiates an interaction with surrounding microorganisms to populate themselves in the nearby roots of the plants whether they are same species or different. At this stage, both AMF and crops are interlinked with each other by the hyphae and the root web, and they are capable of surpassing messages and the nutrients (Read, 1998; Giovannetti et al., 2004; Mikkelsen et al., 2008; Song et al., 2010). The cycle terminates by creating new chlamydospores which can be located at extra radicular mycelium (Youssef Rouphael et al., 2015).

1.3.2.3 Characteristics of AMF symbiosis

AMF is the best instance to understand the mutual relationship in terms of seed germination and maturation of the plants. The AMF also able to create nexus within different plant species and ends up constructing a common mycorrhizal network (CMN). The CMN is very advantageous on terrestrial ecosystems, and it is showing greater impact on plant communities such as invasive crop species (Pringle et al., 2009). Invasive crop species rely on the AMF community to amplify their abilities and receive more benefits from them in comparison to the native crop species (Shah et al., 2009). CMN is also a considerable mediator when it comes to surpassing the nutrients such as phosphorus (P) and nitrogen (N) through fungi (Smith and Read, 2008). Besides, other beneficial nutrients as well travel to the plants via fungi in company with various effects. More or less this can be the reason why AMF associated plants are able to stand against biotic and abiotic circumstances (Plassard and Dell, 2010). Furthermore, it is capable of improving soil health along with it enhancing crop health under any stressful conditions (Navarro et al., 2014; Alqarawi et al., 2014a; Alqarawi et al., 2014b). Generally, AMF is considered to be a bio-fertilizer and it is applied in horticulture as a bio-inoculant (Barrow, 2012). In comparison with non-treated AMF soil, AMF-inoculated soil creates the number of continuous masses and is also very essential for forming over-comprehensive hyphae mycelium (Syamsiyah et al., 2018). There are certain factors which are affected by AMF inoculation such as, CO_2 assimilation, relative water content (RWC) in soil, PSII efficiency, leaf water potential, and stomatal conductance (He et al., 2017;

Chandrasekaram et al., 2019). AMF play a fundamental role for physiological alteration of some plant tissues and organs to work against water stress conditions (Barzana et al., 2012). Moreover, AMF inoculum gathers all the dry matter and also creates moist condition, in consequence it fights under stressful conditions such as, salinity and water drought. Overall, AMF is very helpful for increasing the yield, plant health and plant growth with the organic culturing (Figure 2).

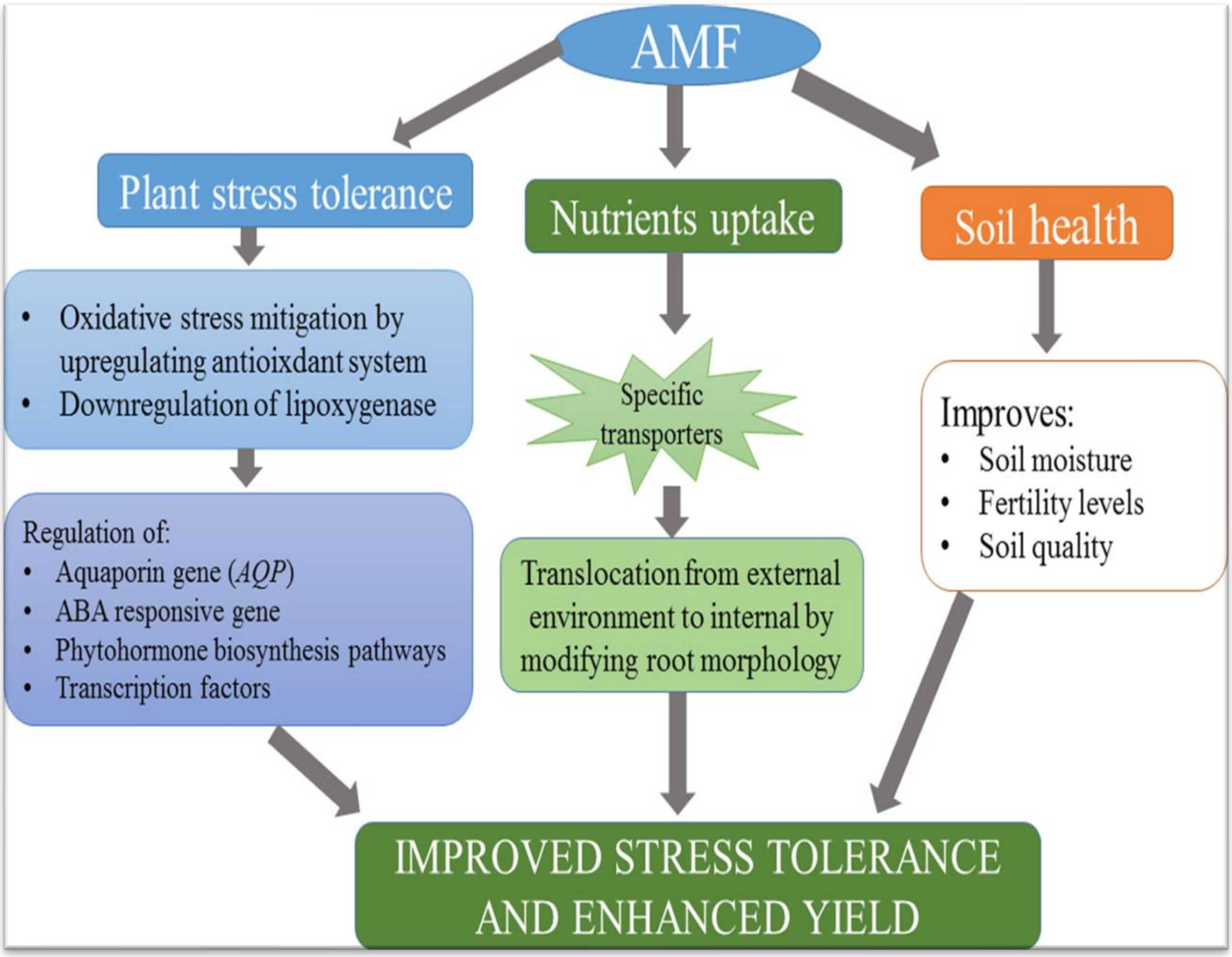

Figure 2: A diagrammatic of mycorrhizal functions showing certain processes in the environment and improving plant growth against stressful conditions (Begum et al., 2019).

1.3.3 Importance of AMF in agriculture/horticulture

AMF are used in agriculture from centuries due to their ability of constructing a symbiotic relationship with vast variety of crop species to enhance their capabilities and adapt in challenging environments. The complex communication between the host plant and AMF leads to amplify the rate of photosynthetic prosses and water uptake (Birhane et al., 2012).

AMF are also known to increase the quality of soil by enhancing the texture and structure of the soil, that improves plant growth (Zou et al., 2016; Thirkell et al., 2017).

1.3.4 Factors effect on AMF-plant symbiosis development

AMF-plant symbiosis functions synchronically in ideal conditions and benefits both AMF and the host plants. However, in some natural conditions there are some factors that affect the symbiosis in a negative way such as, drought, salinity, heavy metals, and temperature.

1.3.4.1 Drought

Drought stress disturbs the whole life cycle of plant growth such as, transpiration rhythm gets unbalanced because of drought and the plant roots are unable to have accessible amounts of water. Additionally, drought can also be the reason for the oxidative stress in plants (Impa et al., 2012; Hasanuzzaman et al., 2013). Drought stress is also responsible for the disruption of the activity of nutrient absorption, ion uptake, and enzyme endeavor (Ahanger and Agarwal, 2017; Ahanger at al., 2017a). Even so, some research studies have been claimed that AMF reduce the effect of drought stress condition on some crops such as soybean, maize, wheat, strawberry, onion, and barley (Mena-Violante et al., 2006; Ruiz-Lozano et al., 2015; Yooyongwech et al., 2016; Moradtalab et al., 2019). Several physiological and biochemical changes occur in plants with the AMF symbiosis including, stomal synchronizing by balancing abscisic acid (ABA) metabolism (Duan et al., 1996), enhancing osmotic adaptation (Kubikova et al., 2001), and improves the aggregation of proline or glutathione levels (Ruiz-Sanchez et al., 2010; Yooyoungwech et al., 2013; Rani, 2016). The symbiosis of plants and AMF give exclusive benefits under drought conditions such as it is enhancing the leaf area index, root size and its efficiency, and also biomass (Al-Karaki et al., 2004; Gholamhoseini et al., 2013). Along with that their mutual relation also supports the plant to stand against such harsh environmental conditions (Ruiz-Lozano, 2003). Other advantages of their symbiosis are escalated leaf-water relations, transpiration rate, gas exchange, and stomatal conductance (Morte et al., 2000; Mena-Violante et al., 2006).

1.3.4.2 Salinity

As the soil salinization has become a challenge ever since causing environmental issues. Salinity stress invades the growth of vegetation, and net assimilation which ends up decreasing harvest capacity (Hasanuzzaman et al., 2013; Ahanger et al., 2017a). There were several research held to find out the solution of increasing the yield productivity under the salinity condition. Here AMF matches the expectation in this stress factor as well. Some studies have proved that AMF helps to improve the growth of the plant and also enhances the outcome beneath salt affected soil (Talaat and Shawky, 2014; Abdel Latef and Chaoxing, 2014). El-Nashar (2017) has discovered that AMF - plant symbiosis drastically increases the water use capability, development ratio, and leaf water potential of *Antirrhinum majus*. Another successful study came out in the frame by Ait-El-Mokhtar et al. (2019) that the symbiosis also very helpful for some physiological parameters including, stomatal conductance, leaf water relations, and enhances the photosynthesis in the plants under salinity stress. Other than that, it also improves chlorophyll amount and gas exchange traits in some plant species like *Ocimum basillium* L. (Elhindi et al., 2017). The *Allium sativum* species has also revealed the enhancement in crop growth traits including some fresh and dry biomass and leaf area index beneath the salty environment (Borde et al., 2010). Wang et al. (2018) has proclaimed that AMF-inoculated plants are providing a considerable amount of nitrogen (N) to the roots under salt affected soil.

Moreover, the crops interacting with the AMF show more capability to synthesize salicylic acid, jasmonic acid and other essential inorganic compounds. As an example, in the *cucumis sativus* plant species, the ones which were inoculated with AMF resulted in more concentration of N, K+, Mg+2, Ca+2 and P, than the ones without interacted with AMF under the salt stressed environment (Hashem et al.,2018). AMF interaction with *Capsicum annuum* revealed amplified chlorophyll content with increased uptake of N and Mg+2 with decreased Na+ transport under the saline environment (Cekic et al., 2012). Furthermore, AMF inoculated lettuce plants have shown specifically decreased accumulation of Na+, increment in N uptake, enhanced synthesis of proline, and drastically higher capability of biomass production, rather than the lettuce non-inoculated with AMF under salt stressed conditions (Santander et al., 2019). On top of that, Aroca et al. (2013) noticed that AMF treated lettuce crops mediated increment in strigolactone. Also, AMF inoculated crops can reduce oxidative stress by subduing lipid membrane peroxidation under salt stress conditions (Abdel Latef and Chaoxing, 2014; Talaat and Shawky, 2014). In addition, the AMF colonized plants even showed gathering of many organic acids, developing an up-regulation of osmoregulation cycle in crop development under the salt stressed environment. Sheng et al., (2011) noticed increased concentration of some organic acids in maize crops developing in salt stressed conditions, in which AMF builds up the concentration of betnine. Betnine indicated the partial role of AMF in balancing the osmoregulation process in crops under saline conditions.

1.3.4.3 Heavy metals

AMF are strongly believed to enhance seed germination under the heavy metal concentrated soil, because of their ability to nourish the defense system of the AMF colonized crops to increase development and growth. Heavy metals can cause a variety of health risks in vegetables, fruits, and food plants (Lui et al., 2013; Yousaf et al., 2016). Plants that are grown in Cd and Zn concentrated soil showed noticeable decrease in root and shoot development, leaf chlorosis, and also death of the crop (Moghadam, 2016). Heavy metals can be put out of action in the fungal hyphae of the external and the internal origin (Ouziad et al., 2005) which has the capability to affix heavy metal in the cell wall and collect them in the vacuole or even chelate with other material in the cytoplasm (Punamiya et al., 2010) and effectively reduce toxicity in the crops. The noticeable effects of AMF on crop growth and development under various stressful environment is mostly because of the capability of AMF to enhance the physiological and morphological processes that escalate crop biomass and uptake of important anchored nutrients such as Zn, P, and Cu resulting in reduction of toxicity in the host crops (Kanwal et al., 2015; Miransari, 2017). It is also found that increased chelation and development can cause metal dilution in plant tissues in the rhizospheric soil (Kapoor et al., 2013 Audet, 2014). Reportedly, AMF is also known to tie Zn and Cd in the cortical cell and the cell wall of mantle hyphae, resulting the reduction of their uptake and enhancing crop development, nutrient status and yield growth (Andrade and Silveira, 2008; Garg and Chandel, 2012).

AMF can disarrange the uptake of various metals into crops from the rhizosphere and also their activity from the root area to the aerial parts (Dong et al., 2008; Li et al., 2015). The mycelia of different AMF have an increased absorption of metals and capacity of cation-exchange (Takacs and Voros, 2003). Furthermore, in perennial ryegrass (*Lolium perenne*) in customized polluted soil including different metals such as Zn, Cd and Ni, the AMF deducted the uptake and accumulating heavy metals and settled the polluted soil. As an example, in plants accumulated with AMF like *Glycine max* the increment of Si uptake has been observed by Yost and Fox, (1982) as similar for *Zea mays* (Clark and Zeto, 2000). In addition, Hammer et al.,(2011) as well noticed a significant uptake of Si in the endospore and mycelium of *Rhizophagus irregularis* and its transfer to the roots of the host. Also, low portability and toxicity of Cd could be conveyed with AMF by increasing the pH of the soil (Shen et al., 2006).

AMF has observed to be decreasing the amounts of Cd in cell walls and vacuoles, which is considered to be the detoxification of Cd (Li et al., 2016a). It has also been noticed by Wand et al. (2012) that in alfalfa (*Medicago sativa L.*), AMF increased tolerance against Cd which was most probably due to the tempering of chemical structures of Cd in various crop tissues. Different processes which take place through AMF are restricting the metal compounds, chelating heavy metals inside the fungus and precipitating polyphosphate substances in soil. (Figure 2).

1.3.4.4 Temperature

When there is an increment in the temperature, the crop community may also behave differently on AMF interconnections for production and sustainable yield (Bunn et al., 2009). The raise of temperature drastically affects crop development and growth in various ways such as, discouragement of seed germination and loss in plant's strength, saging and blazing of leaves and reproductive organs, injury and discolored fruits, reduction in biomass production, weakness and ablation of leaves, deceased cells and decrease in yield and inhibited growth rate (Wahid et al., 2007; Hasanuzzaman et al., 2013). Usually, AMF colonized crop species show finer growth under heat stress than non-AMF colonized crops (Gavito et al., 2005). Furthermore, Maya and Matsubara (2013) have discovered that AMF inoculated with *Glomus Fasciculatum* in plants showed constructive development and growth under heat stress environments.

AMF colonized crops are referred to as having more resistance against cold stress (Birhane et al., 2012; Chen et al., 2013; Liu et al., 2013). Several studies have even demonstrated that AMF colonized crops show better growth under cold temperature conditions rather than non-AMF inoculated crops (Zhu et al., 2010b; Abdel Latef and Chaoxing, 2011b; Chen et al., 2013; Liu et al., 2013). AMF encourages crops to build resistance against cold stress and ultimately improves crop development (Gamalero rt al., 2009; Birhane et al., 2012). Furthermore, AMF helps to maintain wetness in host plants (Zhu et al., 2010a). Also, AMF improves secondary biotransformation in crops resulting in increased strength in the immune system of the crop, and enhanced concentration of protein that helps crops to withstand cold stress environments (Abdel Latef and Chaoxing, 2011b). As an example, AMF colonized crops demonstrated enhanced water holding capacity and increased use proficiency (Zhu et al., 2010b). The colonization of AMF in crops raises the crop and water relationship and enhances osmotic adaptation and potential of gas exchange (Zhu et al., 2012). AMF increases the concoction of chlorophyll resulting in remarkable enhancement in the concentration of different biotransformations in crops to withstand cold stress environments (Zhu et at., 2010a; Adbel Latef and Chaoxing, 2011b). It is noticed that AMF modifies the protein concentration in tomato and other crops throughout low temperature conditions (Adbel Latef and Chaoxing, 2011b).

1.3.5 Importance of AMF in human nutritional value

For decades, the agriculture business was more focused on productivity rather than quality. However, the new and upcoming generation is more into the quality of products other than the quantity. Especially, agricultural products such as fruits and vegetables have become more nutritious in past years, and which will continue in further years as well (Gruda, 2009). Even in several stress conditions mycorrhiza is believed to increase the quality of the crops (Schnitzler and Gruda, 2002). Various studies established that AMF not only improves crop development but also it increases crop quality (Backhaus, 1983; Schnitzler, 1997; Feldmann et al., 1999). Many studies, which focused on horticulture products such as lettuce, tomato, pepper, and strawberries which significantly showed improved quality (Baslam et al., 2013a,b,c; Ulrichs et al., 2008; Mena-Violante et al., 2006; Castellanos-Morales et al., 2010). Baslam et al. (2013a,b,c) reviewed the effect of AMF on lettuce in terms of nutritional quality for humans. Some studies found several nutritional facts about few vegetable crops after inoculation with AMF such as, in sweet potato the β- carotene level improved (Tong et al., 2013), in chili the ascorbic acid level gets increased (Bagyaraj and Sreeramulu, 1982), accumulation of phenolics, carotenoids, minerals, and anthocyanins in lettuce leaves (Baslam et al., 2011, 2012, 2013 a,b,c). AMF colonized plants also showed secondary biotransformation synthesis which eventually improves antioxidants in lettuce crops that is proven to fill nutritional value in the human diet (Baslam and Goicoechea, 2012). Antioxidants such as anthocyanins and carotenoids at higher levels, whereas phenolics and chlorophyll at lower levels are found in lettuce leaves. Also, these studies found that the improvement of antioxidants were much higher when it had water drought stress rather than in a normal condition. Basically, these studies were indicating that the AMF symbiosis can upgrade the nutritional value of lettuce crop even under the stress conditions without even affecting its yield.

Besides improving plant chemical composition, AMF also develops chemical properties of the crops by diminishing insecticides and the pesticides implementation. For instance, phoxim is an extensively implied organophosphate pesticide in horticulture and its leftovers also frequently seen on some crops which indicates hazardous health issues (Baum et al., 2015). Wang et al. (2011) has stated that the AMF is increasing the yield of crops, decreasing pesticides residues and their growth medium. In tomatoes, AMF plays a key role not only in terms of improved fruit-production or nutritional values but also in the matter of fruit size and good sales standard (Schnitzler et al., 1996).

1.3.6 Future prospect of AMF in Vegetable cultivation

Many studies have claimed and proven that the AMF-plant symbiosis is very advantageous under any kind of biotic, abiotic stress conditions. Before few decades, the heated topic to research studies was benefits of AMF in nutrients absorption from soil but recently many scientists are studying more about several abiotic issues like, cold stress, nutrient stress, salinity, alkali stress, water drought, extreme temperature, etc. and, they have found out that AMF really resist all those stress factors and enlarge the production per hectare of many fruits and vegetables. Furthermore, to sustain the modern agriculture ecosystem, AMF should be uplifted very massively. AMF also encourages bio-healthy agriculture, it is constantly reducing the use of the synthetics, fertilizers, pesticides and develops horticulture market quality and quantity wise. Moreover, AMF-inoculated crops and the increased yield will also solve one of the big questions of the ecosystem which is world hunger (consumption requirement). Other than that, AMF is a very eco-friendly and cost-efficient approach due to international demand. Further research should be done on the gene and gene products which are affecting the growth of AMF under the biotic/abiotic stress factors. Also, the recognition of protein factors and the host which are linked in this whole symbiotic pathway under various surroundings and temperature. Grasping the sufferance mechanism under the AMF association can still boost the outcome in the horticulture market (Naheeda et al., 2019).

CHAPTER 02

Materials and method

2.1 Materials

2.1.1 Reagents

The reagents which were used in this research were 90% ethanol, 10% KOH (w/v), 5% HCL. trypan blue stain, 50% Lactic acid, and distilled water. All the reagents and chemicals were purchased from Sigma Aldrich (Prague, Czech Republic).

2.1.2 Plant Materials

All the seeds were bought from Moravo seed (Prague, Czech Republic).

Table 1: Vegetable types and cultivars used in this study.

Vegetable	Cultivar
Tomato	‘Palava’
	‘Mandat’
	‘Orkado’
Cabbage	‘Albatross’
	‘Libertos’
	Povrov pozdeni
Salad	‘Cassini’
	‘Amur’
	‘Apollo’

2.1.3 AMF Strains

Diversispora celata (BEG 231),
Funneliformis mosseae (BEG12), from Institute of Botany, Pruhonice.
Rhizophagus irregularis (DAOMI19798) (Symbiom s.r.o)

2.1.4 Sand Substrate for the plant cultivation

Soil (Agro, Czech Republic)
Agroperlite

2.1.5 Equipments

Scale (SCEOO001, Scaltec instruments, Germany)
Incubator (memmert, Germany)
Microscope (Olympus, Japan)
Autoclave (memmert, Germany)

The equipment which was used for the whole research was acquired from the Department of Horticulture, Czech University of Life Sciences in Prague, Czech Republic. All the equipment is listed above.

2.2 Methodology

2.2.1 Substrate preparation

The soil and agroperlit were mixed in 4:1 ratio and double sterilize it in an autoclave at 120° C for 3 hours. The 96 or 20 holes planting tray or open trays (Figure 3) were used and filled with the sterilized substrate. The tray with sterilized substrate and approximately 1 cm deep holes.

Figure 3: Planting tray preparation using open tray for single inoculation analysis.

2.2.2 Preparation of plant and inoculation

2.2.2.1 AMF culture preparation

Stock cultures of each AMF strain used were maintained separately in the same substrate mentioned above and *Plantago lanceolata* was used as the plant host in the stock cultures. Each culture was maintained in “Sun bags' ' individually at room temperature. Cultures were irrigated once a week or if necessary.

2.2.2.2 Inoculum preparation

Different AMF combinations were tested on different cultivars as mentioned in the table 2. Inoculum were prepared using the substrates from stock cultures maintained in “sun bags” with Plantago lanceolata. For the mixed inoculations, each inoculum was prepared mixing stock cultures in 1:1 ratio.

Inoculation of AMF into vegetables

Later, twenty parts were made in that tray and in each part around 1 cm of hole has been made. 1 g of AMF inoculums (single strain or mixed strains) was added in to that 1 cm hole along with particular seeds. The seeded trays were placed in the greenhouse for 8 weeks at 23° C. During culture maintain, the water was sprinkled twice per week. The control experiment was conducted in similar way as explained before but without the AMF inoculation. For the analysis of effect of drought stress on symbiosis development, inoculation of AB combination was done in the similar way and cultivated under same greenhouse conditions, but the amount of water added was radiused to ¼.

Table 2: AMF inoculation combinations

<table>
<tr><td>Vegetable</td><td>Cultivar</td><td colspan="6">AMF combinations</td></tr>
<tr><td rowspan="3">Tomato</td><td>‘Palava’</td><td rowspan="9">A</td><td rowspan="9">B</td><td rowspan="9">C</td><td rowspan="9">AB</td><td rowspan="9">AC</td><td rowspan="9">BC</td></tr>
<tr><td>‘Mandat’</td></tr>
<tr><td>‘Orkado’</td></tr>
<tr><td rowspan="3">Salad</td><td>‘Cassini’</td></tr>
<tr><td>‘Apollo’</td></tr>
<tr><td>‘Amur’</td></tr>
<tr><td rowspan="3">Cabbage</td><td>‘Liberto’</td></tr>
<tr><td>‘Albatros’</td></tr>
<tr><td>P.Pozdeni</td></tr>
</table>

ABBREVIATION

A	*Funneliformis mosseae* (BEG12)
B	*Diversispora celata* (BEG 231)
C	*Rhizophagus irregularis* (197198)

2.2.3 Collecting data and samples

After 8 weeks of successfully growing, plants were ready to be harvested. Initially all the plants were carefully removed from the soil. All the plant's height and the number of leaves were recorded. The roots were washed and cleaned by water and discreetly cut from 1 cm above the base. The cut roots were placed in microcentrifuge tubes and filled with 90% ethanol. Then samples were stored in the freezer below 20° C till staining will do.

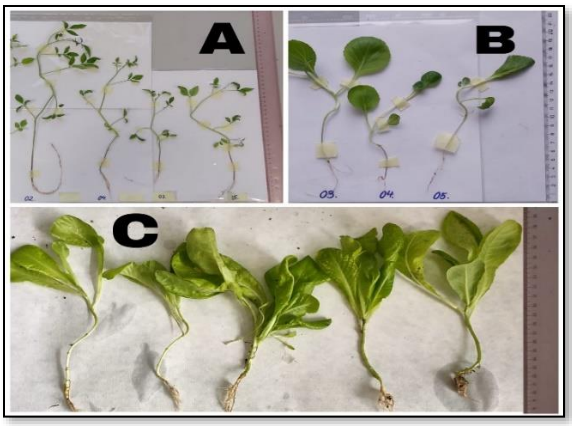

Figure 4: Measurements of plant height and leaf numbers of tested crop. A – Tomato; B – Salad; C – Cabbage.

2.2.4 Sample preparation for microscopic analysis

2.2.4.1 Root staining

Removed filled ethanol from the tubes and 10% KOH (w/v) was added. It was kept for 15-20 minutes at room temperature until the solution got yellowish color. Again, the solution was removed and 5% HCL was added to all the tubes. The solution was poured out after one minute. After that, the trypan blue stain was added and incubated at 90° C for 15 minutes. At last, the stain was removed, and all the tubes were filled with 50% lactic acid. The tubes were then stored in the freezer between 0-4° C until preparation of slides for microscopic observation.

2.2.5 Slides preparation

A thinner, clear, and small fraction of the stained root sample was taken and put on the slide. A small drop of 50% lactic acid was added on the root sample. Afterwards, the sample was covered with coverslip and slightly pressed to open the thinner issues. Prepared slides were stored in the fridge between 0-4° C.

2.2.6 Microscopic observations and analysis

The root samples from AMF inoculated and uninoculated were examined under the microscope to detect the presence of AMF colonization in the root issues. The samples were analyzed using software “Quickcam”.

All the data were statistically analyzed by the software “STATISTICA version 13”.

CHAPTER 03

Results

3.1 Results

In this study, various combinations of AMF strains were tested on three different vegetable types, tomato, salad, and cabbage including three cultivars for each. When paying attention on vegetative parameters of the treated plants such as general outer appearance, plants were in good vigor and healthy. However, when the height and the leaves numbers of the crops were comparatively lower than the control in most of the tested cultivars regardless the vegetable type.

In comparison with others, tomato has shown overall good results with mixed-inoculums and with the single strain inoculums. However, there is a significant difference in tomato cultivar 'Mandat', where there was a decreased height with *Funneliformes mosseae and Diversispora celata* mixed inoculums, but the height of cultivar 'Mandat' was significantly higher with both single inoculums *Funneliformes mosseae* and *Diversispora celata* (Figure 6). While Palava and Orkado did not show any significant difference. Their growth was not really declined but it was more or less similar in all mixed and single inoculums. When it takes cabbage into the account, with mixed inoculums the growth was similar or decreased. The inoculums *Diversispora celata* with *Rhizophagus irregularis* and *Funneliformes mosseae* with *Rhizophagus irregularis* did not prove successful in cabbage, especially with cultivar 'Albatros'. In both inoculums, height, and leaves of the cultivar 'Albatros' were seen to be reduced. Whereas, at some point, the positive interaction had been seen with strain *Diversispora celata* in 'Liberto' and 'Albatros'. Both mixed and single inoculums worked the same pattern in salad, there was none positive or negative differences were seen. Substantially, the growth was similar as in control with all the strains, except in cultivar 'Amur' which showed a positive interaction. Moreover, *Diversispora celata* with *Rhizophagus irregularis* strains, 'Amur' had shown optimistic response, but 'Cassini' and 'Apollo' were not (Figure 8). On the other hand, while 'Apollo' and 'Cassini' demonstrated upstanding host with *Funneliformes mosseae* and *Rhizophagus irregularis* mixed strains (Figure 8), 'Amur' showed the opposite. Overall, for the mixed inoculums *Funneliformes mosseae* with *Diversispora celata* did not show difference from the two others and *Funneliformes mosseae* with *Rhizophagus irregularis* which gave varied results such as, for tomato the results were similar, for cabbage it was decreased but for the salad some positive interactions had been seen. Among them, *Diversispora celata* with *Rhizophagus irregularis* strains had been proven very efficient for tomato and salad, except for cabbage. Quite the opposite, single inoculums presented significantly higher or similar growth to all the cultivars tested. The worst proven host was the cabbage among all mixed inoculums and tomato the best in single inoculums.

For the water drought, *Funneliformes mosseae* with *Diversispora celata* mixed inoculation had been used and the significant growth was distinguished affirmatively in ‘Albatros’. Subsequently, ‘P. pozdani’ had also improved its growth under drought conditions but a less than ‘Albatros’. However, there was no such difference was discerned in ‘Orkado’ and ‘Liberto’, which apparently appeared the same as control (Figure 9).

Figure 5: Growing crops under drought conditions at the 4th week of cultivation with AB AMF combination.

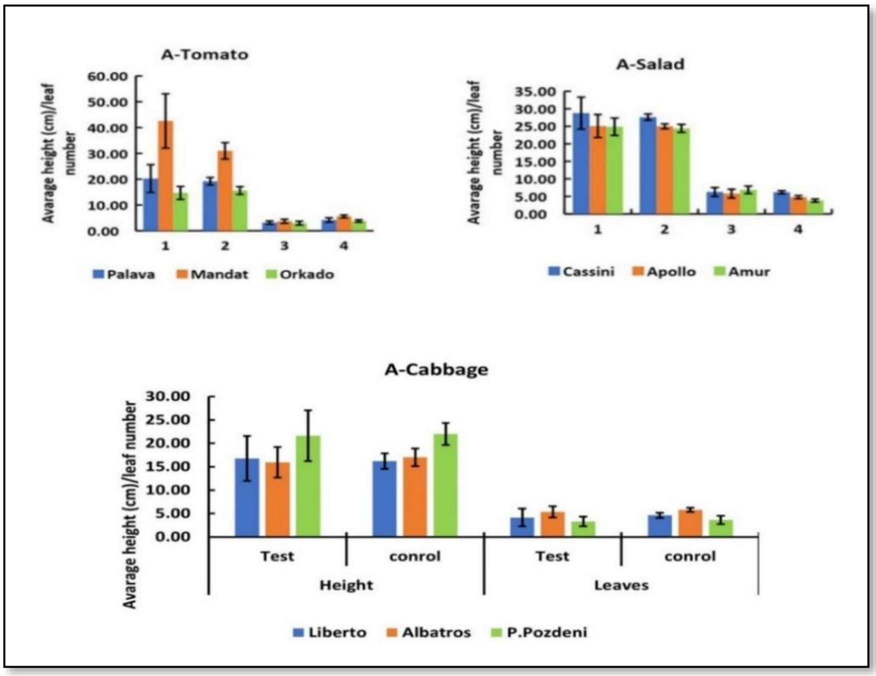

Figure 6: Average height and the leaf number of the crops with *Funneliformis mosseae* strain.

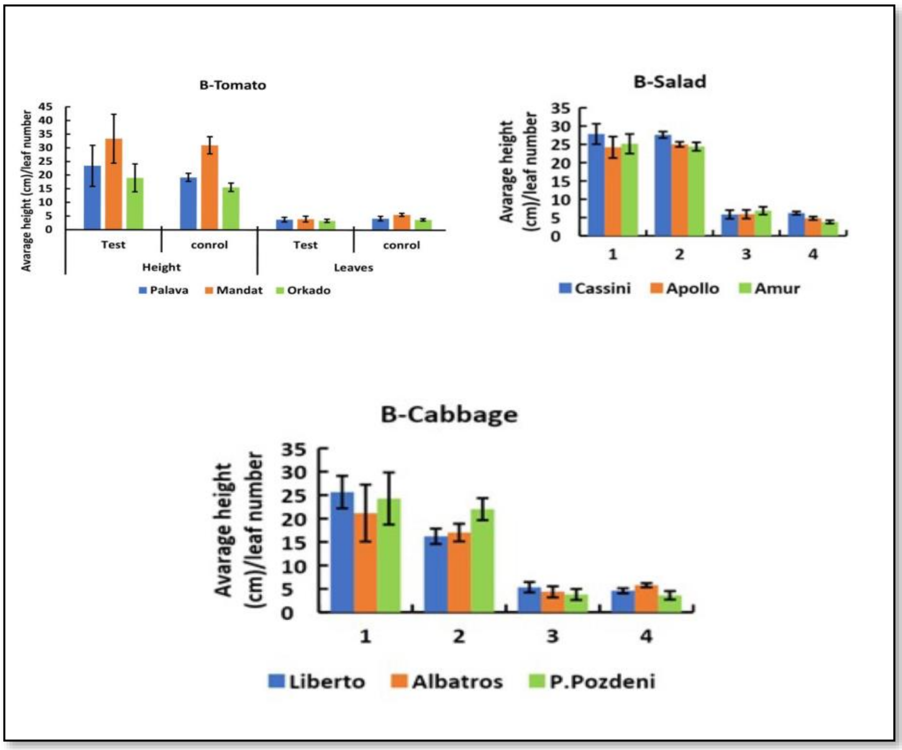

Figure 7: Average height and the leaf number of the crops with *Diversispora celata* strain.

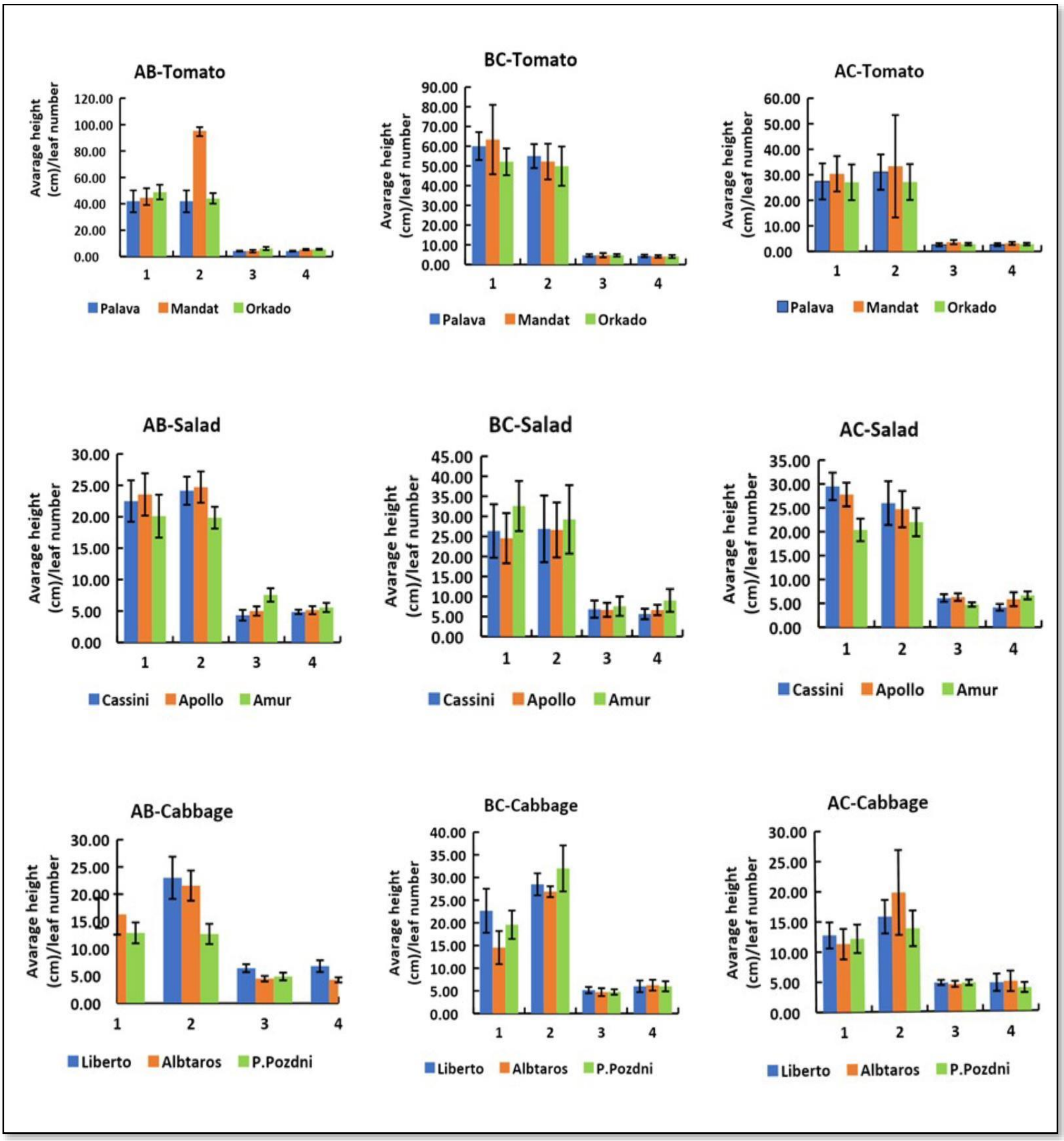

Figure 8: Average height and the leaf number of the crops under different combinations of AMF strains. A: *Funneliformes mosseae*, B: *Diversispora celata*, C: *Rhizophagus irregularis*.

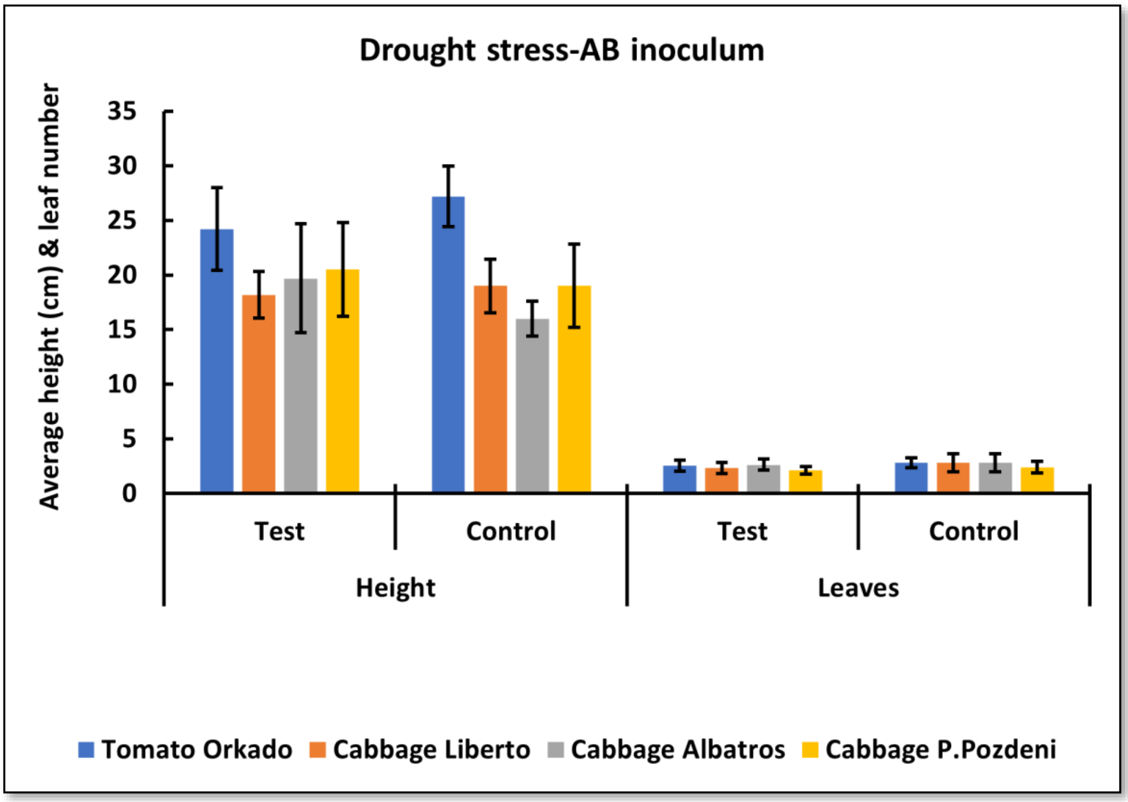

Figure 9: Average height and the leaf number of the crops under drought conditions

3.2 Statistical data

The statistical analysis was performed using ANOVA to determine the interaction between AMF treatments, vegetable types, and cultivars on their biomass. There is a significant effect on height and leaf number of almost all crops by AMF (Table 3). However, there is no significant effect on leaf number between single inoculations ($p=0.9$). Single interaction between AMF combinations, different vegetables and cultivars can be noticed based on the statistical data. Also, there is no significant interaction between single AMF inoculations and vegetable types in terms of crop height but there is a significant interaction between AMF and different cultivars (Figure 10).

Table 3: Results of ANOVA to detect the interaction between AMF treatments, vegetable types and cultivars on crop height and leaf number.

		Effect	MS	F	P
Height of the crop	Individual inoculations	AMF	285.00	5.71	0.004*
		Vegetable	578.60	11.60	0.000*
		Cultivar	833.70	32.54	0.000*
		AMF x Vegetable	86.60	1.74	0.142
		AMF x Cultivar	87.20	3.40	0.000*
	Mixed inoculations	AMF combinations	25.45	25.45	0.000*
		Vegetable	338.00	282.1	0.000*
		Cultivar	8139.00	72.70	0.000*
		AMF x Vegetable	1959.00	16.40	0.000*
		AMF x Cultivar	646.00	5.77	0.000*
Leaf number of the crop	Individual inoculations	AMF	0.12	0.11	0.900
		Vegetable	58.95	42.27	0.000*
		Cultivar	21.45	18.86	0.000*
		AMF x Vegetable	8.31	5.96	0.000*
		AMF x Cultivar	4.68	4.11	0.000*
	Mixed inoculations	AMF combinations	20.80	10.40	0.000*
		Vegetable	166.00	83.20	0.000*
		Cultivar	50.93	29.74	0.000*
		AMF x Vegetable	14.10	7.06	0.000*
		AMF x Cultivar	8.13	4074.00	0.000*

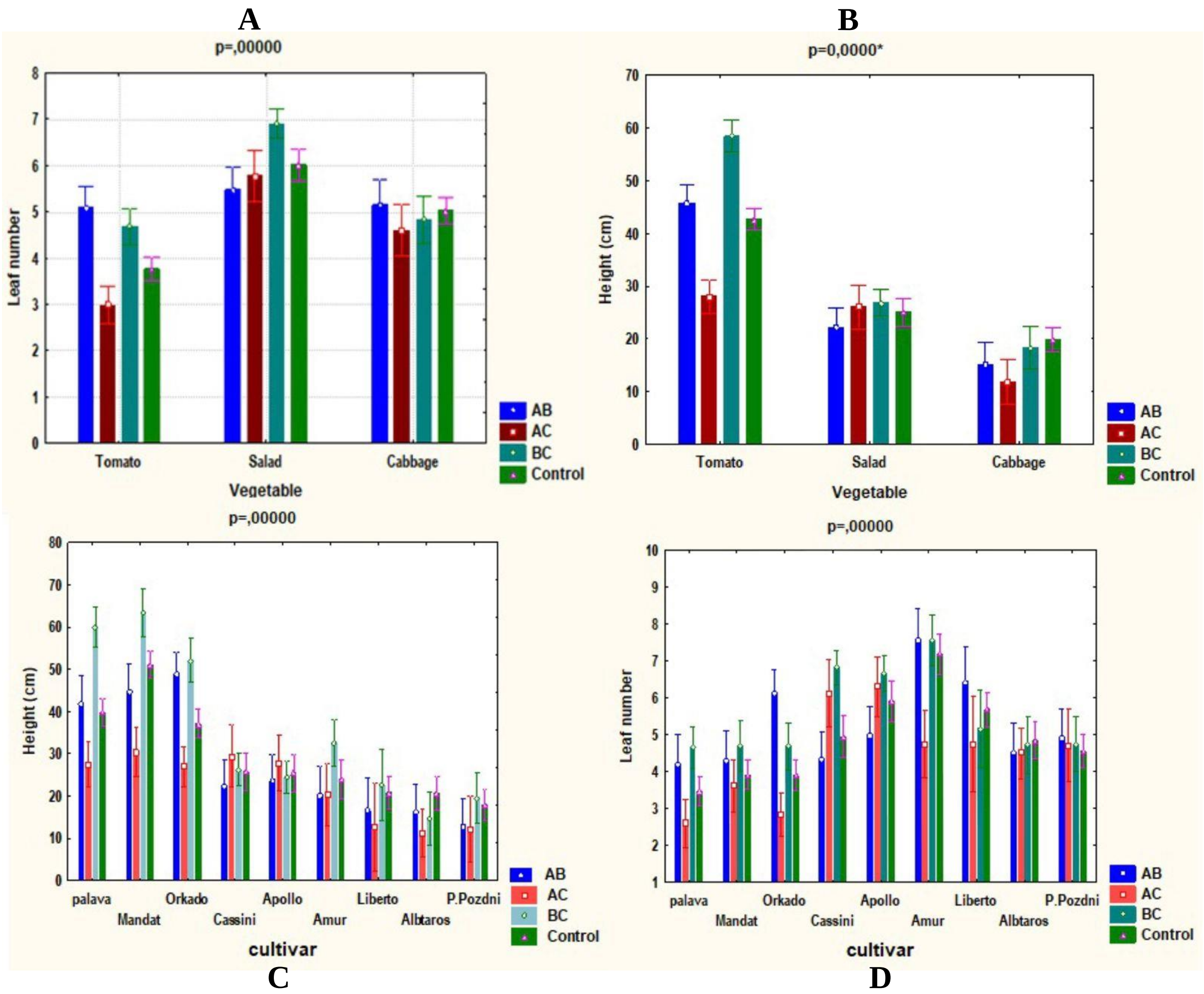

Figure 10: Variations of crop height and leaf number of crops under different AMF treatments.

3.3 Microscopic observations

The internal tissue observation of roots to detect the development of symbiosis was made under microscopy with 10x and 40x magnification. The capacity of development of symbiosis was varied among the vegetable types and among the cultivars (Table 4). Tomato plants are significantly presenting interaction with the AMF strains which is the highest observed (75-100%) in this study among all three vegetables tested. Tomatoes are vastly considered AMF positive plants.

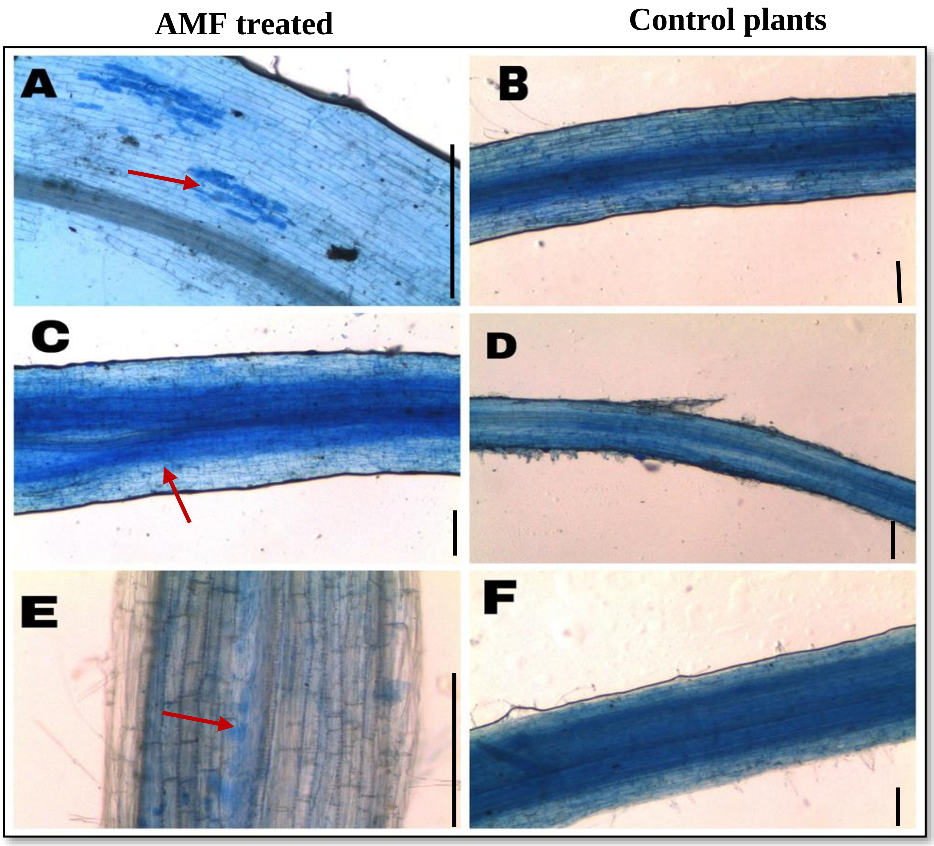

Figure 11: Microscopic observation of Crop-AMF interactions. A & B – Tomato (bar; A= 40×, B= 10×); C & D – Salad (bar; = 10×); E & F – Cabbage (bar; E= 40×, F= 10×). Arrows are showing the presence of AMF in the roots.

Along with tomatoes (Figure 11) all the root samples were shown positive AMF interactions and spores, and hyphae were clearly visible from 60-90% of root samples observed. Among all three cultivars of tomatoes, 'Mandat' showed the higher percentage, nearly 100% positivity while cultivar 'Albatros' had the lowest interaction with AMF by 40-65%.

Table 4: The intensity of AMF interaction with crops.

AMF combinations	Vegetable	Cultivar	Interaction
A	Tomato	‘Palava’	+++
		‘Mandat’	++++
		‘Orkado’	+++
	Salad	‘Cassini’	+++
		‘Apollo’	+++
		‘Amur’	+++
	Cabbage	‘Liberto’	+++
		‘Albatros’	+++
		‘P.Pozdeni’	++++
B	Tomato	‘Palava’	+++
		‘Mandat’	++++
		‘Orkado’	++++
	Salad	‘Cassini’	+++
		‘Apollo’	+++
		‘Amur’	+++
	Cabbage	‘Liberto’	+++
		‘Albatros’	+++
		P.Pozdeni	+++
C	Tomato	‘Palava’	+++
		‘Mandat’	++++
		‘Orkado’	+++
	Salad	‘Cassini’	+++
		‘Apollo’	+++
		‘Amur’	+++
	Cabbage	‘Liberto’	+++
		‘Albatros’	+++
		P.Pozdeni	++++
AB	Tomato	‘Palava’	++
		‘Mandat’	+++
		‘Orkado’	+++
	Salad	‘Cassini’	+++

++++ - Sample 100% positive for AMF

+++ - Sample 75% positive for AMF

++ - Sample 50% positive for AMF

+ - Sample 25% positive for AMF

+/- Sample <25% positive for AMF

Considerably, cabbage cultivars showed about 75% of crop - AMF symbiosis. However, in comparison to tomato and cabbage, the salad showed the lowest interaction with AMF resulting in only 60-75% positivity.

Apart from plant height and leaves number, when it takes the root zone, big soil clumps could be observed around the roots of most of AMF inoculated plants regardless the vegetables (Figure 12). The difference between control and the treated plant roots were noticeable as the most inoculated plants had soil clumps in bulk and roots spread deep inside the soil. Furthermore, in some individuals, though they had big soil clumps developed around the root zone, it was not detected any internal symbiosis under the microscopic analysis.

Figure 12: Root zone observations of cabbage. A & B: Root zone of control plants. C - F: AMF treated cabbage plants.

CHAPTER 04

Discussion and Conclusion

4.1 Discussion

The AMF interaction with plants had been a pattern for many decades that now had left its positive impression in horticulture (Rouphael et al., 2015). AMF has been implemented in agriculture for many reasons such as for photosynthesis, tolerance of abiotic and biotic stresses, amplifying crop productivity, increasing nutrient uptake, etc. (Liu et al., 2014, 2016; Cavagnaro et al., 2015). Although, AMF can be used individually or with combinations which continue in arguments as the mutual relationship with plants highly rely on the AMF strains. In this research, the used AMF inoculations were either single or multiple strains to observe the ability to develop symbiosis and to see whether it is affecting on plant growth, leaf numbers, height on three different vegetables tomatoes, cabbage, and salad. Many research studies had reported the negative effects of AMF on the selected crops. Some studies have claimed that single inoculums are far better than the multi or mixed inoculums as multi-inoculums have significantly decreased or similar effect on their biomass as control (Monicka et al., 2021).

In the study as well, it was observed varied effect on crop height and leaf number based on cultivar and type of vegetable. With single strain *Funneliformes mosseae,* the tomato 'Mandat' showed significant higher height than the control, however, with the same strain and cultivar the leaf number appeared to be decreased. Additionally, in tomato 'Palava' the height and leaf number were identical, with nixed inoculation (*Funneliformes mosseae* with *Diversispora celata*) and control. Whereas, in salad 'Cassini" with *Funneliformes mosseae* and *Rhizophagus irregularis* mixed strains, the height and the leaf numbers were significantly higher than the others. While comparing the cabbage, both cultivars 'Albatros' and 'P.pozdani' showed decreased vegetative parameters in symbiotically relationship with *Diversispora celata* and *Rhizophagus irregularis* mixed strains. The cultivar 'Mandat' showed 100% positive results with both mixed and single inoculums. In comparison between all three mixed inoculums, *Diversispora celata* with *Rhizophagus irregularis* was the most appropriate inoculum for the positive interaction with host and plant growth. Furthermore, *Funneliformes mosseae* with *Rhizophagus irregularis* combination has not proven the worst or the best as they showed the varied results so it might be depending on the cultivars. Moreover, the experiment with the single inoculums had been successful, unlike the mixed inoculums.

Chen et al. (2017) has declared that in his study he has found out that the dry weight, photosynthetic properties, height, nutrients, and chlorophyll content was remarkably higher in cucumbers with AMF combinations than the control. In that study, multiple strains were tested with different combinations such as VT which was one of the combinations with various AMF species from the unassociated genera (*Funneliformis sp*., *Glomus sp*., *Rhizophagus sp*., *Diversispora sp*., and *Claroideoglomus sp.*) and BF from the same genera with diverse species (*G. intradices, G. claroideum*, and *G. microageregatum*). Eventually both gave more positive effects than the single inoculum (*Funneliformis mosseae*). However, it is also important to use right combinations of the inoculums as Monicka et al. (2021) argued that disadvantageous results of mixed inoculums can occur due to AMF adversary interactions. In this study, some mixed combinations did not reach to the goal which does not mean that there is no interaction between AMF-host plants, but it can be said that there were few interactions as seen in the Figure 11. However, there are several reasons for the negative effect on symbiotic development such as, AMF might use the resources from plants and the soil which leads to decreases the biomass production. Additionally, AMF takes around 20% of photosynthetic product from the plants which can also be one of the reasons for negative effect.

In addition to the single and mixed inoculation analysis, drought conditions were applied to cabbage and tomato together with mixed inoculum of *Funneliformes mosseae* and *Diversispora celata*, to detect whether the stress conditions would stimulate the degree of symbiosis development. Previous studies have reported that cabbage as a low responsive crop towards AMF symbiosis due to having higher glucosinolate content. Nevertheless, during the study, cabbage showed significant symbiosis development with all tested AMF strains and combinations of inoculations. Among those, *Funneliformes mosseae* and *Diversispora celata* showed to be the combination with intermediate symbiosis development with cabbage. All together cabbage and this AMF combination could be ideal for such study of stress induced symbiosis development in non-responsive vegetable cultivars. The major noticeable difference of vegetative parameters was the height, and the leaves were comparatively less than the control plants in some cultivars. However, from the outer appearance the AMF-host plants looked healthier, unlike the control plants. The leaves of AMF-inoculated crops were not scrambled, and the stem of those crops looked in good physical conditions. In terms of root observations, the inoculated plants were ultimately holding the soil which can increase the water holding capacity while the control plant roots were apparently thin and not deeply escalated. Also, the crops did not have the soil clumps in mass which can lead to less soil holding strength and decreases the water adequacy. There was higher symbiosis development observed in single inoculated plants, contrasting from the mixed inoculums.

Overall, this research clearly represents that the combinations of AMF strains might be very effective for growth of some crop cultivars which can be negative or no effect on another cultivar. Along with right combinations of the strains, the cultivars are also equally important so that it does not adversely affect the biomass and growth of the crops. According to many studies, this study also gave a positive response for the single inoculums with each cultivar or crops.

4.2 Conclusion

The interaction between AMF and host plants can be improved the growth of the crops adding higher nutritional values, healthy crop life cycle, effective water supply, and tolerance against other biotic and abiotic stress factors. The findings of this study showed that, the appropriate combinations of the strains are important and the suitable host to perform the mutual interactions. As per this study, *Diversispora celata* with *Rhizophagus irregularis* strains proven the most suitable and successful strains where the positive AMF-host symbiosis development had been noticed. Additionally, single inoculums are also demonstrated as a prosperous achievement. Moreover, for specific cultivars such as, 'Albatros' and 'P.pozdani' in drought conditions had positive outcomes on growth with mixed AMF strains.

Therefore, for the future studies, some other mixed inoculums should be considered including more cultivars to get more detail information about their efficiency for developing the mutual relationship under any kind of biotic or abiotic stress factors. So far mixed inoculum *Diversispora celata* with *Rhizophagus irregularis* established positive response, these mixed strains should be used to find out the symbiosis development with other various crops or cultivars which are less studied for the prospects. Moreover, further studies also can be directed towards the acknowledgement of the gene and the proteins which are linked in this symbiotic pathway and accelerating both AMF and host plants to perform the symbiosis development.

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List of abbreviation

AMF	Arbuscular mycorrhizal fungi
AM	Arbuscular mycorrhizal
CMN	Common mycorrhizal network
P	Phosphorus
N	Nitrogen
RWC	Relative water content
KOH	Potassium hydroxide
HCL	Hydrochloric acid