

ARBUSCULAR MYCORRHIZAE: AN OVERVIEW

Elena DELIAN*, Adrian CHIRA, Lenuța CHIRA, Elena SĂVULESCU

Univeristy of Agronomical Sciences and Veterinary Medicine Bucharest,
Faculty of Horticulture, Mărăști Blvd., No.59, Bucharest, Romania
*Corresponding author: E. Delian, E-mail: delianelena@yahoo.com

Abstract. *Physiological performance of plants in general, particularly cultured plant productivity and crops quality depend on the rhizosphere characteristic feature, an area of great interest to plants, producers, consumers and environmental health. Among the rhizosphere components, arbuscular mycorrhizae are one of the most common types of symbiotic associations between some rhizosphere microorganisms and plants roots. Understanding these beneficial mutualistic associations thanks to advanced research developed techniques and performed studies carried out in recent years to come open the way for wider implementation of biotechnology techniques, based on mycorrhizal inoculants, with maximum efficiency in the context of promoting benefits for plants, producers, consumers and ecosystems. The purposes of this paper are to summarize the available literature regarding: molecular signaling at the rhizosphere level, in the context of promoting mycorrhiza symbiosis; an overview concerning arbuscular mycorrhiza, one of the most widespread mycorrhiza type; arbuscular mycorrhizae beneficial effects in the context of sustainable agroecosystems. Research questions that address issues of fungal colonization and functionality, as well as the potential of managed inoculation could provide practical information for those working in rural, suburban and urban horticulture. In the future, by creating specific channels of interdisciplinary communication, can be achieved more efficient exploration and exploitation of soil resources in agroecosystems, the fastest progress to maximize crop yield, to increase its quality, to increase economic profitability, to conserve biodiversity and to assure environmental protection.*

Key words: *arbuscular mycorrhizae, plant physiology,, crops quality.*

INTRODUCTION

The world is on the brink of a new agriculture, one that involves the marriage of plant biology and agroecology under the umbrella of biotechnology and germplasm improvement (Vance et al. 2003). One in six people in developing countries suffer from chronic hunger, so, food security at local, regional and global levels will need to be realized in the face of

emerging challenges. First, we assist to the rapidly changes socio-economic environment. It is estimated that the world population will increase from about 7 to 9 billion people by 2050. The proportion living in urban areas will increase from about 50% to 70% by 2050. People's diets will change, shifting to increased proportions of vegetables, fruits and livestock products. The second challenge is climate change (Diouf 2011). To maintain or increase agricultural productivity and more efficient exploration and exploitation of soil resources in agroecosystems should be practicing a sustainable agriculture, both economically and environmental protection view point.

Therefore, we need a better knowledge of the processes and factors that govern the bioavailability of soil nutrients to plants, thus including the root-soil interactions understanding of microorganisms in the rhizosphere (Hinsinger et al. 2008, Martinez & Jonson 2010). As Samuil (2007) mentioned, organic farming promotes sustainable production systems, diversified and balanced crop, to prevent pollution and the environment damages. Moreover, the importance of the mycorrhizal arbuscular fungi in organic farming and farmers' potential to increase the benefits of arbuscular mycorrhizae (AM) associations in such systems represented interesting subjects as it was synthesized by Gosling et al. (2006). Research accumulated over the years on the chemistry, microbiology and soil pedoenzymology allowed to shape their own ideas about soil fertility and possibility to assess its level (Ghinea et al. 2007). Combination of conventional breeding techniques, molecular biology, genetic engineering and natural variation will improve the ability of plants to nutrients uptake and fixation, and improve roots system performance (Den Herder et al. 2010).

In this context, mutually beneficial associations between different organisms play an important role in the ecology of natural ecosystems, but also in terms of sustainable agriculture (Hause & Schaarschmidt 2009; Sesan & Tanase, 2009). Many horticultural plants form AM. However, other types of mycorrhiza are important in specific situations: ecto-mycorrhiza, for reforestation programs; ericoid mycorrhiza for fruit production, orchid's mycorrhiza, to improve propagation. As Toma & Toma (2004) noticed in the first bibliographic synthesis on mycorrhiza in Romania, this type of symbiosis large extended in nature is a common evolutionary strategy in the plant kingdom, an alternative to root morphological changes, as response to the soil quality where plants grown.

Understanding the mechanisms underlying mycorrhizae associations is one of the biologists priority, knowing that this kind of symbiotic relationship can be considered and used as a biological control agent in

stabilizing ecosystems and ecological restoration of contaminated soils in terms of assisting plant survival (Ike-Izundu 2007; Dong et al. 2008; Popa et al. 2008), assuring horticultural products quality and reducing postharvest losses (Paliyath et al. 2008), old-field restoration (Standish et al. 2007), low cost and sustainable phytoremediation techniques (Khan 2005; Trotta et al. 2006) or anthropogenic soils rehabilitation (Cardinale et al. 2010) etc. Promoting sustainable agriculture in developing countries is a key to achieving food security. It is necessary to increase investment in agriculture, broaden access to food, improve governance to global agricultural trade and increase productivity, while conserving the natural resources base (Diouf 2011).

Taking into consideration that one of the main goals of sustainable horticulture is to benefit farmers and consumers (Shalhevet et al. 2001) and arbuscular mycorrhizae act as biofertilizers and bioprotectants, the purpose of this paper is to review some of the recent research results on this topic. There are reviewed: rhizosphere characteristic feature related to AM symbiosis promoting; AM benefits related to plants performance (in terms of physiological importance for the producer, in terms of plants productivity, plants resistance to stress factors, satisfy consumer needs by high crop quality etc.; the role of AM in terms of soil quality as a support and nutritive media supply for cultivated plants, but also as soil attributes in the agroecosystems (structure, compaction, diversity of microorganisms, rehabilitation etc.)

1. Rhizosphere: Concept and molecular signaling in the context of promoting mycorrhizal symbiosis

Plants growth and development is under the control of internal signals that depend on assuring adequate amounts of mineral elements from soil, to roots. After Hiltner (1904) (cited by Hartman et al. 2008), *rhizosphere* is the volume of soil under the influence of roots and roots itself. This area is by far a more complex environment from the physical, chemical and biological view point, than the aboveground area, around the aerial part. Rhizosphere is under the influence of root and microflora (Aragno 2005). Living together is a common situation of the biological world, particularly in the case of plants and underground environmental conditions (Bonfante-Fasolo & Silvia Perotto 1992).

Below-ground biological systems need the same care as above ground systems, as is the case of AM which are essential in sustainable management practices (Robinson-Boyer et al. 2009). Extremes changes of the chemical composition within minutes and spaces of millimeters, and the density and diversity of soil organisms play a major role (Rost & Bloom

2006). Anthropogenic action on it, namely the introduction of fertilizers and pesticides, including adding organic amendments should be also considered in terms of its influence on microbial activity (Celik et al. 2004), on the agricultural soils biological indicators (Onet, 2010), considering that the soil biodiversity determine economic benefits for society, as part of a broader strategy of conservation and use of the agro biodiversity (Brussaard et al. 2007).

Roots are plants organs which very easily adapt their growth to the most profitable areas, thanks to a chemical communication between plant roots and between these and other rhizosphere organisms (Badri et al. 2009; Badri & Vivanco 2009; Hause & Schaarschmidt 2009; Hodge 2009). Products of the living roots into soil (rhizodeposits) are not only a source of carbon substrate for microorganisms growth, but also signal molecules that promote chemotaxis of microorganisms in the soil, to rhizosphere (Dakora & Phillips 2002; Lesuffleur et al. 2007; Ludovic 2008; Dennis et al. 2010). Rhizodeposition is a carbon and energy flow which gives to the rhizosphere the characteristics of a rich habitat, as regard as nutrients supply to the heterotrophic micro-organisms. As Jones et al. (2004) noticed (cited by Ludovic 2008), rhizodeposition is under the influence of many factors. For instance, amino acids concentration is a result of flows between roots, soil and plants, with physiological and ecological effects on plant-soil-microorganisms interactions (Lesuffleur et al. 2007). Schiliemann et al. (2008) showed for the first time that there are clear differences in the primary and secondary metabolism during *Medicago truncatula* plant development, in relation to root colonization by the *Glomus intraradices* fungus. During the most active stages of root colonization there were registered high levels of amino acids (glutamine, asparagine) and of some fatty acids (palmitic and oleic), corresponding to the plastids metabolism activation. Also, there was noticed an accumulation of fungus-specific fatty acids (palmitvaccenic and vaccenic acids) that can be used as markers for fungal root colonization. At the final stages of colonization it was observed a stimulation of apocarotenoids accumulation (cyclohexenone and mycorradicin), without registering a change of the typical cell wall bound specific phenols.

Thus, understanding the molecular signaling processes and functions that are regulated in the rhizosphere will play a central role in promoting coexistence of plant-beneficial microorganisms to overcome existing limits and setting new strategies to ensure inoculants, with applications in biotechnology with a view to promote agroecosystem sustainability.

2. General aspects as regard as arbuscular mycorrhizae symbiosis

Mycorrhizae are a classic examples of mutualistic symbiosis, primarily characterised by carbon gain by the fungus from the plant, also a reciprocal nutrient transfer from fungus to plant, next to other effects relating to improving water relation and pathogens tolerance (Smith & Read 1997). Arbuscular mycorrhizae symbiosis between roots of 80% of all terrestrial plant species and member of phylum *Glomeromycota* (Wang et al. 2008) are diffuse and often nonspecific (Selosse et al. 2006). When mycelium fungus colonizes roots and link together two or more plants, sometimes belonging to several species, common mycorrhizal network is formed (CMN), a major component of terrestrial ecosystems, with important effects on plant community (Selosse et al. 2006), on invasive plants trajectory (Pringle et al. 2009), as well as on the mycorrhizal community structure and functionality in invaded habitats (Shah et al. 2009).

Depending on their morphology and the involved plants and fungus species, it can be distinguish several types of mycorrhizae associations. The first *mycorrhizae classification* was made by Frank (1887) and subsequently there have been various classifications (see reviewed by Imhof 2009). For instance, based on hyphal structures, mycorrhizal interactions have been classified into ectomycorrhiza and endomycorrhiza (Bonfante 2000). Brundrett (2004) recommended to define and classify mycorrhizal association by anatomical criteria, regulated by the host plant. He also provided a new classification scheme for categories, subcategories and morphotypes.

Today, in the literature are recognized *seven types of mycorrhiza*: arbuscular mycorrhizae (AM), ecto-mycorrhizae (ECM), ect-endo-mycorrhizae, arbutoid mycorrhizae, monotropoid mycorrhizae, ericoid mycorrhizae and orchideoidal mycorrhizae. The most widespread is AM (Smith & Read 2008). After recent data the used terminology is - arbuscular-mycorrhizal associations, to describe the associations of phycomicetes septate fungus, belonging to *Glomeromycota*, corresponding to four genera: *Glomus*, *Gigaspora*, *Acaulospora* and *Sclerocystis*. Subsequently another type added: *Scutellospora*. Although these associations have been previously called vesicular - arbuscular mycorrhiza (as some were characterized by the presence of vesicles, also arbuscules), now, it is known that not all associations with *Glomeromycota* form vesicles (as in fact not all forms arbuscules). The use of arbuscular mycorrhiza terminology means maintaining a known name and the argument is stability and simplification in the future. Based on spore morphology, 214 species have been described (Schüssler, 2009) and studies carried out by Schüssler et al. (2011) highlights that experimental works published during

the last 25 years on an AMF named '*Glomus. versiforme*' or 'BEG47' refer to *Diversispora epigaea*, a species that is actually evolutionarily separated by hundreds of millions of years from all members of the genera in the *Glomerales* and thus from most other commonly used AMF 'laboratory strains'. Detailed redescriptions substantiate the renaming of *Glomus. epigaeum* (BEG47) as *D. epigaea*, positioning it systematically in the order *Diversisporales*, thus enabling an evolutionary understanding of genetical, physiological, and ecological traits, relative to those of other AMF. *D. epigaea* is widely cultured as a laboratory strain of AMF, whereas *G. versiforme* appears not to have been cultured nor found in the field since its original description.

Concerning plant species which perform or not such associations, representative are data presented by Wang & Qiu (2006), (cited by Smith & Read 2008).

Plants compatibility with mycorrhizal fungus is a widespread and an ancient phenomenon. Fossil samples suggest that this kind of symbiosis have been with more than 400 million years ago, in the tissues of the first terrestrial plants (Remy et al. 1994). Plant capacity to form AM must be under the control mechanisms that were preserved in the new plant species that occurred during evolution, involving selective recognition processes and certain specific plants to discriminate between beneficial microorganisms and harmful ones. As Requena et al. (2007) noticed, the molecular basis of this process started to be understood and point to common signaling pathways shared with other microbe plant associations and to AM specific signaling pathways.

Symbiotic relationships involve the development of specific adaptations for both partners and coordination of their development, with specific signals (Hause & Schaarschmidt, 2009). Plants produce an early signal, to initiate interaction, and fungus produces at least three signals: one diffusible, the second locally, allowing the plant to detect the appressorium position and last one, an autonomously cell signal, in the colonized cells which induces gene expression. As regard as the molecular bases of this interaction, there are already published a number of research results and of course researches continue. As literature have been mentioned, flavonoids have been proposed as the active molecule (Phillips & Tsai 1992), but they are not absolutely essential in host-fungus interactions (Bécard et al. 1995). First defined recognition phenomenon by the host fungus is the hyphae ramification induction, followed by appressorium formation on the root surface. Epidermal and hypodermic cells are in contact with these first infection structures and do not express significant cytological changes or typical defense responses (Gianinazzi-Pearson et al. 1996). The fungus

invades the root, both intercellular and intracellular, determines extensive cytological changes, as well as in terms of pathogenesis related proteins (PR proteins) (Collinge et al. 1994; Bonfante et al. 1996, Gianinazzi-Pearson et al. 1996). Plastids appear to be key cell organites to establishing symbiotic interface in the case of arbuscular mycorrhiza (Fester et al. 2007).

At the plant level, recently it was identified the initial signal, respectively strigolactones. Strigolactones are a group of sesquiterpenes lactones (reviewed by Steinkellner et al. 2007), signal molecules that play an important role in host recognition by parasitic plants such as *Striga*, *Orobancha* and *Phelipanche* (among the most harmful weeds in agriculture). Ana Höniges (2009) studied the root exudates of *Carduus* (plant host *Orobancha reticulata*) and noticed that strigolactones are active even in very low concentrations (10^{-10} to 10^{-14}) and different types of strigolactones varied for different amounts of root exudates. There were also addressed issues related to changes in their structure and possible function in the host recognition by AM fungi (reviewed by Cardoso et al. 2011). There were characterized symbiotic program components, in terms of genetic and anatomical view point, too (Reinhart 2007).

In recent years there have been multiple studies on AM and there are numerous reviews on this topic published everywhere. There were registered important steps to elucidate plants mechanisms that govern arbuscular symbiosis using "mycorrhiza-deficient" mutant plants, cytology and molecular biology techniques and comparing the interactions of plants with microorganisms in other systems. However, there were numerous gaps in the physiology and genetics understanding, possible due in part by the biological systems complexity and on the other hand by the arbuscular mycorrhizal fungus status, as obligated symbionts. Using the literature data, Ramos et al. (2008) proposed a hypothetical mechanism based on ion signaling between plant roots and AM fungi. Communication between symbionts starts before any physical contact between partners. Factors released by host plant roots (strigolactones, proteins, carbon dioxide and extra cellular protein fluxes) are recognized by specific fungal plasma membrane receptors, and these factors trigger a signal transduction cascade.

Scientific results obtained by Hildebrandt et al. (2007) have open the way for a better understanding of the required stimuli for fungus growth and sporulation. Essential genetic determinants to establish the AM are common in the wider part of the plant kingdom, and differential expression of symbiosis associated genes among different AM associations is a phenotypic response to the different fungal and plant genotypes and the

environment they inhabit. Functional diversity is therefore the rule rather than the exception (Feddermann et al. 2010). *Medicago truncatula* is a widely used model system to elucidate mutualism interactions of roots with some AM from the genetically view point and more of the results are synthesised by Krajinski & Frenkel (2007).

Despite increasing interest and performed studies, thanks to the developing molecular techniques there is still much to be learned (Satyanarayana & Johri 2005). Advances in molecular biology have revolutioned the analysis of fungal population colonising roots and forming mycorrhiza. Initially these studies were qualitative and more recently the methodology has developed for direct quantification of AMF within roots as quantitative PCR: the means to study spatial distribution and individual quantification of AMF in mixed communities over time (Robinson-Boyer et al. 2009). The identification of the fungal symbiosis signal (Myc factor) and of e-corresponding Myc factor receptor and other new insights into AM genetics, have opened new avenues to address early communication and functional aspects of AM symbiosis (Ercolin & Reinhardt 2011).

3. Arbuscular mycorrhizae benefits in the context of agroecosystems sustainability

Mycorrhizae have a particularly importance for plants and ecosystem. From the biological view point, mycorrhizal fungus affects two aspects of sustainable agriculture: plant production and soil quality (Bethlenfalvay & Barea 1994). The beneficial effects of mycorrhizae fungus on plants performances and soil health are essential for sustainable management of agricultural ecosystems (Celik et al. 2004; Currie et al. 2011; Diouf et al. 2003; Dong et al. 2008; Finlay 2004; Franco-Correa et al. 2010; Gianinazzi et al. 2010; Hause & Schaarschmidt 2009; Hildebrandt et al. 2007; Idoia et al. 2004; Kaya et al. 2009; Lingua et al. 2001; Martin & Stutz 2004; Martino & Perotto 2010; Navarro et al. 2009; Nicoara et al. 2010; Singh 2011; Smith et al. 2010; Trotta et al. 2006; Wu & Zou 2010 etc), being also one of the low cost and sustainable phytoremediation techniques.

Arbuscular mycorrhizae have multiple functions (reviewed by Turk et al. 2006) in the effective exploitation of soil mineral resources and because of their bioprotective role against many soil pathogens are suitable tools for survival of many species of plants in different ecosystems, including many species of cultivated plants. Besides these function it can be remember: assure nutrients cycle and prevent the loss of ecosystem; assure carbon transport from the plants roots to other soil organisms involved in the processes of soil nutrient cycle (Rooney et al. 2009); soil hyphae may intervene in the nutrients cycle, by taking substances from saprophytic

fungi; mycorrhizal fungus fructifications and root with mycorrhiza represent food sources and habitat for invertebrates; influence microbial populations in soil and exudates from the micorhizosphere and hyphosphere areas (Milleret et al. 2009); VA contribute to soil structure, aggregation of soil particles, respectively (Kahiluoto et al. 2011).

Arbuscular mycorrhizae improved plant physiological performance (growth and development, productivity, crop quality) under normally growing condition, with producer and consumer benefits. Moreover, AM alleviate abiotic and biotic stress factors action.

Many studies regarding AM influence have been carried out on vegetables. Evelin et al. (2009) presented an overview about the role of AM to alleviate saline stress. Besides AM practical importance there were mentioned: improving mineral elements supply (P, N, Mg and Ca), maintaining the ratio of K^+ : Na^+ , biochemical changes (accumulation of proline, betaines, polyamines, carbohydrates and antioxidants); physiological changes (photosynthesis efficiency, relative permeability, the water status, abscisic acid accumulation, nodulation and molecular nitrogen biological fixation); molecular changes (genes expression: PIP, Na^+ / H^+ antiport pumps) and ultrastructural changes. Hammer et al. (2011) indicated that AMF can selectively uptake minerals such as K and Ca, which act as osmotic equivalents, while avoiding toxic Na absorption, in the case of salt stress.

On the other hand, Kaya et al. (2009) showed that at the mycorrhized pepper plants have operated special mechanisms which have protected the cell membrane. Possible that mycorrhizal plant have accumulated some potential antioxidants that blocked the activities of reactive oxygen species, which could have caused damage to membranes and therefore would be promoted electrolyte leakage. Pepper plants not only require mycorrhizae for acclimatization, but also for continuity of nutrients absorption during the progress of the growth and development stages.

Tomato (*Lycopersicon esculentum*) as a major vegetable plant and moderately sensitive to salinity have been a biological material exposed to many research and has been selected as a model crop for such studies due to its commercial importance as a horticulture cash crop in many areas. Cuperman et al. (1996) showed that although AM originally from saline soils do not promoted tomato plant growth, reducing the chlorine concentration in the leaf of AM plants mediated by fungus, at moderate levels of salinity, may have beneficial implications for survival plants on saline soils. Also, pre-inoculation of tomato transplants demonstrated to be economically feasible in agrosystems affected by salts (Al-Karaki 2006). For instance, recently Huang et al. (2010) noticed that colonization

enhance the ability to plant growth and antioxidant defense enzymes: SOD (superoxide dismutase), POD (peroxidase), APX

(ascorbate peroxidase), CAT (catalase) in tomato leaves and roots, under different salt stress condition. *Vinca* plant was determined to tolerate the alkalinity of irrigation water and to increase the phosphorous absorption, associated with increased alkaline phosphatase activity (Cartmill et al. 2008). In the same context, Tarafdar & Rao (1997) highlighted the beneficial effect of inoculation with VAM on vegetables grown in arid conditions. Recent results confirm the potential application of mycorrhizae as biotechnology tools in sustainable horticulture for arid and semi-arid areas (Fan et al. 2010).

Strawberry plants AM treatments associated with phosphorus application increased the stems dry and fresh weight, leaves area and leaves number also, compared with only phosphorus application. It has been shown that practically is possible to increase strawberry runners, by VAM inoculation, a technique which was recommended to producers, with a view to produce strawberry certified planting material (Khanizadeh et al. 1995). AM also favored growth and absorption of nutrients by strawberry plants (Kawai & Hotta 1994). Salts reduce strawberry plant growth, while adding AMF can avoid damage on growth. Salinity significantly reduced plant dry mass, while adding AMF significantly increased this indicator value.

As regard as *plants resistance to biotic stress factors*, following studies conducted under controlled conditions, by infecting the vine roots, cv. Pinot Blanc with *Pseudomonas fluorescens* and *Glomus mosseae* the most significant result was that both treatments reduced the symptoms of chlorosis made from vines grafted on sensitive rootstock (101-14) (Bavaresco & Fogher 1996). VAM pre-inoculated potato plants resisted more effectively to infection by pathogens *Rhizoctonia solani*, compared with non-mycorrhizal plants (Nasim et al. 1996) and mycorrhizal populations alone and together with a plant growth promoting rhizobacterium reduces damage caused by *R. solani* and enhances plant vigour (Hanna Furugård 2000). Next to the AM protection against soil-born pathogens as *Rhizoctonia solani*, *Fusarium oxysporum* or *Verticillium Wilt* and by fungal-like *Oomycetes* such as *Phytophthora sp.*, *Phythium sp.* or *Aphanomyces euteiches*. Gallou et al. (2011) demonstrated AM role in the control of above-ground hemibiotrophic pathogens (for instance *Phytophthora infestans* in potato plants). There were also induced two pathogenesis related genes (PR1 and PR2) in leaves of mycorrhizal plants, shortly after infection with *P. infestans*. Thus, a systemic resistance was induced related to the priming of the two PR genes.

In the case of bean plants, symbiosis efficiency depended on the particular combination of the fungus that causes VAM, *Rhizobium* strain involved and plant cultivar. In all combinations, VAM and rhizobia significantly increased pods production (Daniels-Hylton et al. 1994). A mixture of AMF inoculum containing *Glomus mosseae*, *Glomus fasciculatum*, *Glomus etunicatum*, *Glomus intraradices* and *Scutellospora* sp. applied to green pepper (*Capsicum annum*), parsley (*Petroselinum crispum*), carrot (*Daucus carota*) and tomato (*Lycopersicon esculentum*) significantly increased pepper and parsley plants biomass and carrot roots biomass. There was also registered an increase in chlorophyll content in mycorrhizal parsley and a significant increase in carotenoid pigments content in mycorrhizal plants of parsley, carrots and tomato fruits. Moreover, there was noticed increased the mycorrhizal potential of soil and thus the growth of non-inoculated plants in the second season (Regvar et al. 2003). AMF consortium rather than a single AMF species significantly restrained *Fusarium* wilt of cucumber, therefore, an inoculum rich in AMF diversity is more ecologically beneficial (Hu et al. 2010).

Co-inoculation of melon plants with AMF and *Trichoderma harzianum* did not result in an additive effect on plant growth and nutritional status. As regard as the nutrient uptake, co-inoculation with *T. harzianum* and *G. mosseae* results were more effective than *G. intraradices*, *G. claroideum* and *G. constrictum*. Moreover, the effect of the AMF was influenced by the fertilizer dose. Combination of the AMF and *T. harzianum* were able to control *Fusarium* wilt more effectively than each AMF applied alone, but their effectiveness was similar to that of *T. harzianum* applied alone (Martínez-Medina et al. 2011). Krishna et al. (2010) emphasized AM beneficial effects in the case of apple affected by cancer incidence due to *Botryosphaeria* pathogen.

Referring to the herbivore insects effect, experiments carried out by Currie et al. (2011) with white clover (*Trifolium repens*) inoculated with the AM fungi *Glomus fasciculatum* and *Glomus mosseae* individually and in combination, and larvae of the clover root weevil (*Sitona lepidus*) on mycorrhizal and non-mycorrhizal plants demonstrated that a specialist feeder is less affected by the presence of AMF, than a generalist species.

There were also demonstrated the benefits of mycorrhizal fungus inoculation for woody plant growth and confirmed that woody plants and different cultivated plants share the same mycorrhizal fungi. Woody plants act as reservoirs for inoculated or indigenous mycorrhizal fungi, for surrounding crops or other annual vegetation (Ingleby et al. 2007). In fruit trees growing practices, micropropagation techniques have been expanded with a view to increase production speed and production of healthy plants

for fruit trees propagation, including apple trees (Dobránski & Teixeira de Silva 2010). Apple is one of the most important fruit of temperate areas and the third most important fruit (64.3 million tones per ha) in the world after bananas (81.3 million tones per year) and grapes (66, 3 million tons per year) (FAO, 2009). But, the main commercial problem for plants obtained by micropropagation is poor survival and increasing after their passage on field conditions. Associated losses for micro-propagated plants are due by less functional microorganism into the rhizosphere, as is the mycorrhiza case. Studied performed by Pathak and Dhawan (2010) using inorganic fertilizer, mycorrhiza (AM, *Glomus intraradices*) and farmyard manure on growth of micropropagated apple rootstock M.7 emphasized that none of the used treatments had a significant effect on survival percentage, root length and emergence of new leaves. Considering that 100% of plants treated with these two treatments attained graftable thickness in 6 months as against 8% plants in the control, it can be successfully applied for the production of grafted apple trees in lesser time.

Inoculation of AMF to the roots micropropagated plantlets play a beneficial role on their post-transplanting performance: development of a superior root system; increased photosynthetic efficiency; enhanced nutrient uptake; alleviate environmental stress; averts attack by harmful soil borne pathogens (reviewed by Kapoor et al. 2008). The cooperation between micropropagation and mycorrhizal inoculation is an important tool in more sustainable horticultural production (Fortunato et al. 2005). *In vitro* raised plantlets of *Terminalia bellerica* were biotized using an endosymbiotic root fungus *Piriformospora indica* during their hardening and acclimatization. Improved overall growth and higher rate of survival were observed with colonized plantlets. The fungus colonized in more than 80% of inoculated plantlets and about 90% of such plantlets showed survival in the greenhouse and subsequent under nursery shed. Colonization of fungus also promoted root growth, increased biomass and total chlorophyll content in inoculated plantlets. The study demonstrated the potential of *P. indica* as a bioprimer agent for achieving better growth and survival of tissue culture raised plantlets (Chittora et al. 2010). Susek et al. (2010) after the experiment based on inoculation with AMF or/and *Agrobacterium radiobacter* of micropropagated and vegetative (by rhizome cuttings) plants of Christmas rose (*Helleborus niger* L.) concluded that biotization can be beneficial in *in vitro* plant production systems, but inoculum have to be carefully selected.

Data regarding successful commercial utilisation of the AM symbiosis and its introduction into the *ornamental floriculture practices* are reviewed by Koltai (2010), with a view to promote cost-effective use of AMF in floriculture. Strong promotion of root formation in the moderately rooting pelargonium and

poinsettia cuttings in response to the basidiomycete *Piriformospora indica* inoculation was noticed as a new tool to improve vegetative propagation by cuttings (Druege et al. 2007).

Knowing that AM are obligated endo-symbionts fungus and can not be grown on laboratory culture media, producing a sufficient quantity of inoculums, respectively soil *inoculation under field conditions are difficult and expensive*. In the case of horticultural plants, in most cases planting material production is done under greenhouse and inoculation before seedlings transplanting can help plants to survive to this procedure and to further improve their performance. For example, pepper seedlings inoculated with different species (*G. mosseae*, *G. clarum*, *G. caledonium*, *G. intraradices* and *G. etunicatum* - propagated on corn roots) had positive effects on seedlings growth and quality. Inoculated plants were characterized by higher values of dry mass of aerial part, root dry mass as well as higher amounts of P and Zn in the aerial organs. Also, inoculated seedlings had an earlier flowering time, compared to those no inoculated. Differentiated behavior in relation to the used fungus species must to be further studied in order to sustain inoculum production protocol and to implement this kind of technology (Ortas et al. 2011).

Artificial mycorrhizae production by efficient fungi is an alternative to soil chemical treatments, by increasing the efficiency of nutrients uptake and reducing the fertilizer administration in the context of soil fertility management. It can ensure the development of plants, which otherwise difficult tolerate soils with low fertility or different improper systems (Zamfirache & Toma, 2000; Kahiluoto et al. 2009). Miransari et al. (2009) noticed that different species of AM may be able to adapt themselves with different ecological and environmental conditions. Moreover, resource availability generates evolved geographic structure in symbiosis among plant and soil organisms. So, edaphic origin of AM fungi should be considered when managing for their benefits in agriculture, ecosystem restoration and soil-carbon sequestration (Johnson et al. 2010). It is important to know which type of mycorrhiza corresponds to special interest and is more efficient *to inoculate very young plants*, because larger plants require more inoculums quantity and costs are higher. The next decision is that regarding the inoculum type to be bought and which is the best application way. The cheapest method is seed inoculation by powder applying. Successful are also incorporation into the soil or growing medium. Mycorrhizal inoculum can be as powder, liquid or granules (Amaranthus et al. 2009). Performance differences between isolates indicate that finding the best AM fungus inoculum for inoculation in the field conditions is crucial for the success of such practices. Pellegrino (2011) showed that native

inoculum is at least as effective as exotic isolates selected according to their efficiency. Therefore, use of the farm produced inoculum with native plant species can be a convenient alternative for produce VA-commercial inoculum and also provides economic and environmental benefits of sustainable or organic production systems.

In non-agricultural conditions, the natural role of plant growth promoting bacteria,

P-solubilizing bacteria, mycorrhizal helping bacteria and arbuscular mycorrhizal fungi in maintaining soil fertility is more important than in conventional agriculture, horticulture and forestry (reviewed by Khan, 2005). As the results obtained by Bainard et al. (2011) demonstrated, *in urban conditions* (with a weaker presence and diversity of mycorrhizal fungi, compared to rural or natural ecosystems) inoculation would increase levels of colonization and growth of trees. As fungi play several key sociological roles, their establishment may be essential for the integrity and sustainability of restoration projects. Research question that address issues of fungal colonization and functionality, as well as the potential of managed inoculation could provide practical information for those working in the field of urban ecological restoration, as reviewed by Newbound et al. (2010).

The biotechnology of microsymbiont inoculation on wild legumes that was applied for revegetation strategies of desertified ecosystems in southern Europe was also successful for semi-arid soils of anthropogenic origin, as Cardinale et al. (2010) showed. The addition of the microbial inoculants on the Mediterranean legume shrubs (the genisteae *Spartium junceum* L.) and the thermopsidea *Anagyris foetida* L. significantly affected plant growth and survival in the greenhouse and in the field and soil bacterial community. The drastic effect of the tripartite symbiosis on the structure of soil bacterial community largely overcomes plant species effect and suggest synergism of arbuscular mycorrhizal fungi and mycorrhizosphere to affect directly and indirectly the physical-chemical and biological soil properties that contributing to the pedogenesis of anthropogenic soils.

In terms of *qualitative indicators*, arbuscular mycorrhizae improve horticultural product quality as well as postharvest behavior (Paliyath et al. 2008). Recent research conducted by Binet et al. (2011) have highlighted the impact of microbial inoculation on the production of aromatic volatile oil at aromatic species such as *Artemisia umbelliformis* Lam. and also influence the supply of phosphorus. Research carried out by Zubek et al. (2010), by inoculate under laboratory conditions the species *Inula ensifolia* L. (*Asteraceae*), a species with therapeutic value, using different strains of AM fungi concluded that: there was an increase of roots thymol derivatives after inoculation with *G. clarum* and a decrease in production of these

metabolites for *G. intraradices* treatments. In this paper there were addressed the practical consequences of these mechanisms by AMF control the phytochemical substances concentration changes. As demonstrated Baslam & Goicoechea (2011) mycorrhizal symbiosis improved the accumulation of antioxidant compounds, mainly carotenoids and anthocyanins, and to a lesser extent chlorophylls and phenolics, in leaves of lettuce. These enhancements were higher, under water deficit than under optimal irrigation. Therefore, results suggest that mycorrhizal symbiosis can improve quality of lettuce and may allow restrict irrigation without reducing production.

Arbuscular mycorrhizal are key factors to soil quality characteristic feature, heavy metal control and agroecosystems sustainability. Plant production is directly related to soil quality which is based on its physical, chemical and biological properties. Some specific known soil functions essential for food production, also some functions of wider societal or ecosystem significances were listed by Powlson et al. (2011). Earlier, Bethlenfalvai & Barea (1994) have highlighted mycorrhizae role in sustainable agriculture. As we previously already mentioned, it is known that AM colonize plant roots and soil around them. They transfer mineral elements in plant and carbon from plant to the soil, thus having a dual role, both on plant and soil microflora composition. Experiments performed using *G. mosseae* fungus do not significantly alter the seed production (8%), but aggregation of soil particles has improved by 400%, in a soil rich in organic matter and phosphorus. In a soil containing little organic matter and phosphorus, seed production increased significantly (57%), but were only small changes in the aggregation of soil particles (50%). So, plant carbon allocation (measured as seed production) and soil (measured as formation of water stable aggregates) was influenced by VAM.

AM beneficially influence the *soil structure* by: growth of external hyphae into the soil to create a skeletal structure that holds soil particle together; creation by external hyphae of conditions of micro-aggregates; enmeshment of microaggregates by external hyphae and roots to form macroaggregates, and directly tapping carbon resources of the plants to the soils (Miller & Jastrow 1990). Soil quality also results by a combined action of extracellular hyphae and glomalin (Rilling et al. 2002; Bedini et al. 2008). Glomalin is an insoluble, hydrophobic protein (Bedini et al. 2009), whose levels range from 1.6 to 2.3 mg / g soil (Bedini et al. 2010) and has a cementing capacity to maintain soil particle together (Borie et al. 2008). Rilling (2004) reviewed studies as regard as glomalin related soil protein (GRSP). GRSP has been shown to be correlated with soil aggregate water stability, also at that period he mentioned that research of GRSP holds may

be a foundation for novel biotechnological application. Effects of arbuscular mycorrhiza to alleviate the stressful impact on soil compaction in the case of corn culture growth and nutrient uptake is mainly dependent on the competition with other soil microorganisms. AM significantly increased root fresh (maximum of 94% increase) and dry (maximum of 100 % increase) weights in the compacted soil (Miransari et al. 2007).

Mycorrhizae associations are useful to rehabilitate the disturbed and polluted soils.

Few plants named metallophytes are able to cope the adverse conditions on heavy metal soils due to their various physiological adaptation. Also, AMF considerably reduce the uptake of heavy metals into plant cell (reviewed by Hildebrandt et al. 2007) and contribute to a successful rehabilitation of degraded and polluted substrata (Straker et al. 2007). As Trotta et al. (2006) noticed, use of arbuscular mycorrhiza inoculation can be a low cost and sustainable phytoremediation techniques. These characteristics also apply to many plants species, including horticultural plants, which naturally have specific capacities or may be genetically transformed for beneficial purposes. Experiments conducted on pepper (*Capsicum annuum* L.) by inoculation with AM (*G. mosseae*) and the addition of Cu in the soil have concluded that mycorrhizal fungus was able to maintain an effective symbiosis in Cu contaminated soils, improving plant growth due reducing tissue accumulation of copper in the plant, reducing oxidative stress and lipid damage, or increase the antioxidant capacity (Latef 2011). Under an elevated Pb conditions mycorrhizae could promote plant growth by increasing P uptake and mitigate Pb toxicity by sequestering more Pb in roots (Chen et al. 2005). AM symbiosis influences the growth and Cd uptake of transgenic tobacco designed for high heavy metal tolerance and uptake. Mycorrhiza decreased Cd accumulation by the transgenic tobacco relatively to the non-transgenic tobacco (Janoušková et al. 2005).

Ker & Charest (2010) showed through experiments conducted in greenhouse conditions that on sunflower, colonization of AM (*G. intraradices*) resulted in increased absorption of the Ni element, applied in different doses and its transfer from roots to the stem, and at the roots level glutamine synthase activity significantly increased, as an indicator of tolerance to nickel increases. The obtained data support the hypothesis that symbiosis enhances Ni acquisition, plants tolerance to Ni and may be considered as part of phytoremediation strategies.

As regard as mercury, *G. mosseae* inoculation in artificial soil contamination conditions led to the conclusion that Hg accumulation was lower in mycorrhizal roots as against with non-mycorrhizal, while at the stem level showed no differences. Hg absorption was lower for mycorrhizal

roots, which highlights the role of inoculation in increasing Hg arresting into the soil and reduce its absorption by roots (Yu et al. 2010). Bona et al. (2011) using proteomic techniques showed that AM colonization of fern root (*Pteris vittata*) alleviated arsenic stress both up-regulating many arsenic-induced enzymes and modulating the process of protein degradation/turnover.

In Romania, research carried out in connection with the analysis of biotic elements of anthropogenic ecosystems through fungi capacity in reconstruction of degraded soils by mining activities led to the identification of several fungi species (belonging to *Basidiomycota*) in Călimani Massif on waste dumps, which may represent inoculum source from species of fungi adapted to extreme environmental conditions, for obtaining mycorrhizae on the seedlings roots (Grudnicki & Cenușă 2007).

CONCLUSIONS

In the context of current challenges related to population growth, food production need and environment changes, it is clear that the recent arbuscular mycorrhiza field research progress and those in the future should be focus on crops maximize, increasing its quality, increase economic profitability, biodiversity conservation and environmental protection. All of these challenges can be achieved at the disciplines interface by specific ways to create interdisciplinary communication and collaboration of all researchers in the field. Petre & Teodorescu (2010) noticed that biotechnology integrates multidisciplinary knowledge of microbiology, biochemistry, molecular biology, cell biology, immunology, enzymology, genetics, biophysics, bioengineering, chemical engineering, mathematics, in order to use microorganisms and their components to enhance their metabolism with maximum efficiency and usefulness in favor of the human factor.

Land use management will affect AM fungal population in soils. The use of AM fungi as inoculants in agriculture and environmental rehabilitation is becoming more widely accepted as being the key to maintaining soil health and vitality, leading to enhancement of nutrient cycling processes and development of sustainable ecosystems (Ike-Izundu 2007). Research question that address issues of fungal colonization and functionality, as well as the potential of managed inoculation could provide practical information for those working in the field of urban ecological restoration too, as reviewed by Newbound et al. (2010).

As Mattoo and Teasdale (2010) mentioned, sustainable agriculture systems tend to reach the general requirements in terms of productivity, profitability and conservation by local application of the principles according to climate, soil and available markets. Besides these approaches it can be mention organic farming to conventional farming, with the latest genetic and technological inputs. Sound ecological principles as well as physiological and genetic principles hold the most promise for addressing the challenges of the 21st century.

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