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Biochemical and Physiological basis of AMF-Host Association in Horticultural Crops

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Microbial populations are key component of soil plant system where they are immense in a network of interactions affecting plant development. Several symbiotic groups, phosphorus solubilizers, plant growth promoters and other such beneficial important micro- organisms are reported from different soils. Balanced microbial system contributes to the sustainability in agriculture, horticulture, forestry and range management. In this regard, an excellent example of microbe- plant mutualism is 'Mycorrhiza'. The mycorrhizal symbiotic association appears to have evolved with plants since the colonization of dry land by plant began as a survival mechanism for fungi and higher plants, thus allowing each to survive in the existing environment of low temperature, low soil fertility, periodic drought, diseases, extreme environments and other stress situations. Among the different types of mycorrhiza, arbuscular mycorrhizae (AM) are the important beneficial micro- organisms of the soil edaphon in most agro-ecosystems. AM, the mother of plant root endosymbiosis, is a wide spread mutualistic symbiosis between land plants and fungi of the phylum Glomeromycota. AM plant symbiosis to be established, molecular signaling events must occur that lead to various physiological and anatomical changes in both symbionts. Horticultural crop and flowers have been used as the host plants in several experimental tests for application of arbuscular mycorrhizal fungi. Mycorrhizal fungi can stimulate horticultural plants and vegetable crop's growth especially in the soils with lower fertility and the positive effect of mycorrhiza on plants mainly due to improved phosphorus uptake has been documented. AMF enable their host plant to tolerate environmental extremes such as nitrogen and phosphorus deficiency, drought, low pH, soil pollution, negative effects of some root pathogens etc.

Key words: AMF-Host, Horticulture crops, microbial population.

Mutually beneficial associations between different organisms play an important role in the ecology of natural ecosystems, but also in terms of sustainable agriculture (Hause and Schaarschmidt 2009; Sesan and Tanase, 2009). Arbuscular mycorrhizal associations occur in a wide spectrum of agricultural crops, most shrubs, tropical tree species and some temperate tree species. Many horticultural plants form AM. Arbuscular mycorrhizal (AM) fungi comprise the most common mycorrhizal association and form mutualistic relationships with over 80% of all vascular plants (Brundrett and Abbott 2002).

AM fungi are obligate mutualists belonging to the phylum Glomeromycota and have a ubiquitous distribution in global ecosystems. Chemical mutagenesis has further revealed that AM symbiosis is established through a multistep

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process consisting of a "cascade of recognition events" leading to a complete morphological and physiological interaction of two partners (Bohra *et al*., 2007). Arbuscular mycorrhizae act as biofertilizers and bioprotectants. 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 (Trotta et al. 2006) or anthropogenic soils rehabilitation (Cardinale *et al*. 2010) etc. Promoting sustainable agriculture in developing contries is a key to achieving food security. It is necessary to increase investment in agriculture, broaden acces to food, improve governance to global agricultural trade and increase productivity, while conserving the natural resources base (Diouf 2011). The efforts were made to study the effects of Arbuscular Mycorrhizal fungi (AM) on the morphological and biochemical changes of different vegetable seedlings grown under nursery conditions. The symbiotic association between AM fungi and plant roots provides a significant contribution to plant nutrition and growth (M. Lenin *et al.,* 2010). They are also described to improve the absorption of several plant nutrients like N, P, K Mg, Cu, Ca and Fe by the roots of plants (Liu *et al.,* 2002). The mycorrhizal infection enhances plant growth by increasing nutrient uptake through increasing the absorbing area of root and by mobilizing sparingly available nutrient sources; or by excretion of chelating compounds or ecto-enzymes (Marscher and Dell, 1994). The AM fungi can increase plant uptake of nutrients and consequently increase root and shoot biomass and improve plant growth and yield (Ryan and Angus, 2003). The inoculation of AM fungi improves the physico-chemical and biochemical properties of amended soil (Caravaca *et al*., 2004). Phosphorus (P) solubilizing microorganisms increase the mycorrhizal colonization by production of specific metabolites like vitamins, amino acids and hormones (Barea et al.,1997). Lettuce can establish a mutualistic association with arbuscular mycorrhizal fungi (AMF). The establishment of the symbiosis involves a continuous cellular and molecular dialogue between both symbionts, which includes the activation of antioxidant, phenylpropanoid or carotenoid metabolic pathways (Marouane e*t al.,* 2013).

Taxonomic diversity of am fungi

In the present scenario, the major thrust area of research is the scientific classification of AM fungi. Due to the absence of fossil records, it is difficult to develop a non controversial scientific classification system of AM fungi. Taxonomy is entering a new phase and many researchers have attempted to propose a suitable classification for AM fungi on the basis of morphological, biochemical and molecular genetics techniques.

The vesicular arbuscular mycorrhiza forming fungi were classified as the member of Zygomycota and were placed under the order Glomales (Morton et al., 1990). But, the gene encoding analysis of the small subunit (18S) ribosomal RNA show the AM fungi are not related to Zygomycota and probably share common ancestry with Ascomycota and Basidiomycota. So, they have been assigned to a new monophyletic group, Glomeromycota (Schussler *et al.,* 2001). Based on data from molecular, morphological and biochemical investigations, Mortan and Redecker (2001) erected two new families in the order Glomales i.e. Archaeosporaceae and Paraglomaceae with two new genera *Archaeospora* and *Paraglomus* respectively. The ordinal name 'Glomales' has now been changed to Glomerales and phylum Glomeromycota has been divided into four orders i.e. Glomerales, Paraglomerales, Diversisporales and Archaeosporales (Schussler *et al.,* 2001). Several taxonomic and phylogenetic relationships of AM fungi based on molecular

characterization have also been reviewed by Reddy *et al.* (2005). As the number of AM species is increasing day by day, it is quite pertinent to revise the classification of these fungi. The genera, which form AM fungal association are *Acaulospora*, *Ambispora*, *Archaeospora*, *Diversispora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Intraspora*, *Kuklospora*, *Otospora*, *Pacispora*, *Paraglomus*, *Sclerocystis* and *Scutellospora* (Oehl *et al*,.2004, Sieverding *et al.*, 2006, Walker *et al*., 2007, Palenzuela *et al* 2008).

Establishment of symbiosis and cytological features of am plant roots

Fungal development starts with the germination of hyphae from resting spores. In the absence of a host plant, AM fungi show only limited hyphal growth whereas in the presence of root exudates growth and branching of hyphae is strongly increased (Tamasloukht et al. 2003). This presymbiotic fungal reaction is characterized by the activation of specific genes followed by subsequent physiological and morphological changes. In return, germinating spores produce diffusible factors which are perceived by plant roots leading to the expression of specific genes even in the absence of direct physical contact (Kosuta et al. 2003). The chemical nature of both plant and fungal diffusible factors is not yet known. After the first physical contact between hyphae and plant roots, the fungus forms appressoria and subsequently penetrates the root surface colonizing the intercellular space of the root cortex. The plant actively mediates at least two steps allowing the fungus to penetrate the rhizodermis (Demchenko et al. 2004): (1) anticlinal cell walls of two adjacent epidermal cells separate from each other in the vicinity of fungal hyphae allowing the intercellular passage of the hyphae; and (2) fungal hyphae are allowed to pass intracellularly through an exodermal cell and an adjacent cell from the outermost cortical layer. After the subsequent penetration of the innermost cortical layers, tree-like fungal structures (arbuscules) are formed within individual root cortical cells by repeated dichotomous branching of fungal hyphae. Except for species from the genera Scutellospora and Gigaspora, all AM fungi form intra- or intercellular storage organs, lipid-rich vesicles, to varying degrees in late phases of the symbiosis (Smith and Read 1997). The arbuscules are the key features of AM and are responsible for nutrient exchange (Fig.1). They represent a dead end in the growth of AM fungi (Bonfante and Perotto 1995), because they finally senesce and collapse after 4–10 days of symbiosis (Sanders et al. 1977). The fungal structures are then degraded completely by the plant cell and the plant cell recovers its original morphology. This way, cortical cells are able to allow a second fungal penetration and arbuscule formation. The life cycle of AM fungi is completed by the formation of extraradical spores, which may enter another colonization process.

During colonization, the fungal arbuscule occupies a major portion of the plant cortex cell, but is separated from the cell protoplast by a part of the host plasma membrane, the periarbuscular membrane. This membrane completely surrounds the arbuscule, leading to up to a fourfold increase of the surface of the plasma membrane. Although it originates from the plant plasma membrane, the periarbuscular membrane exhibits different properties. In particular, phosphate transporters were shown to be located specifically in the periarbuscular membrane (Rausch et al. 2001). Moreover, a high amount of H+-ATPase activity (Gianinazzi-Pearson et al. 1996) accompanied by the highly acidic nature of the space separating plant and fungal plasma membranes has been found. These findings are consistent with the involvement of the periarbuscular membrane in the active transport of nutrients between the symbiotic partners. The space separating plant and fungal plasma membranes corresponds to a new apoplastic compartment and represents the symbioticinterface. It is continuous with the peripheral plant cell wall, but its structure differs from it (Peterson and Bonfante 1994). Its components reflect the composition of the wall of the host cell that is being invaded. Pectins, xyloglucans, nonesterified polygalacturonans, arabinogalactans, and hydroxyproline-rich glycoproteins have been localized within this interface (Balestrini et al. 1994; Bonfante and Perotto 1995). The mixture of primary plant cell wall components indicates that the arbusculated cells maintain their ability to synthesize and secrete cell wall material. Colonization by an AM fungus induces dramatic changes in the shape and number of organelles of root cortical cells. Differentiated cells of the root cortex are extensively reorganized

after penetration by an AM fungus (Bonfante and Perotto 2010). The central vacuole is fragmented, the volume of cytoplasm and the number of cell organelles increase significantly, and the nucleus moves into a central position. The nucleus of arbusculated cells undergoes hypertrophy (Balestrini et al. 1994) and is characterized by enhanced fluorochrome accessibility, increased nuclease sensitivity, and chromatin dispersion (Gianinazzi-Pearson 1996). These features reflect a higher transcriptional activity of the plant genome in colonized cells in comparison to non-colonized cells. The increase in the amount of host cytoplasm and the number of organelles surrounding the branching hyphae was shown first by electron microscopy (Bonfante and Perotto 1995). The analysis of organelles labelled by the green fluorescent protein using confocal laser scanning microscopy provided new details and led to the discovery of network-like organelle structures in colonized cells. Such structures covering the developing arbuscule can be observed for plastids (Fester *et al.* 2001), mitochondria (unpublished data), and the ER indicating a strong activation of the metabolism in the colonized root cortical cell. In the case of plastids, the networks are formed by

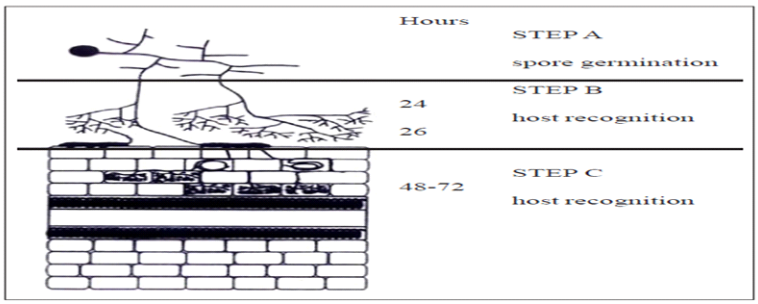

Fig. 1. The spatio-temporal development of arbuscular mycorrhizal symbiosis (Bonfante and Perotto, 1995)

tubular extensions, which have been referred to as stromules (Kohler and Hanson 2000).

Step A

A germinated spore show a linear growth pattern, consisting of branches extending in all directions, functional for soil exploration and for an efficient exploitation of resources. Step B: As early as 24h after the perception of host derived signals, a different hyphal growth pattern is expressed, functional to the location of infection sites. 36 h after the beginning of symbionts interaction, dramatic morphogenetical changes occur in hyphal tips, leading to the formation of appressoria. Step C: After root penetration,

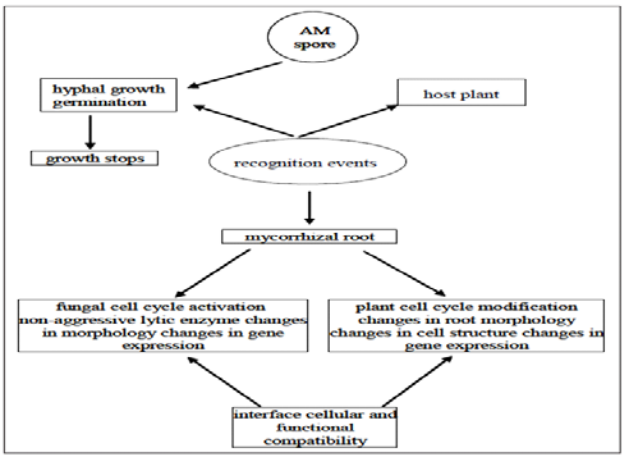

Fig. 2. The flow chart summarizes some of the check points which control the establishment of AM symbiosis (Bonfante and Perotto, 1990)

intercellular hyphae colonize the root, producing intracellular branched structures, the arbuscules, as early as 42-72 h after the beginning of the interaction.

Modification in fungus and host cell architecture

Modifications in the fungus

Besides formation of the regular appressoria and arbuscules by the fungus in a compatible interaction, major modification also occurs in fungal cell wall compartments, such as the cell wall, which becomes progressively thinner as infection develops in the roots and the cytoplasm changes its organization. Changes also occur in the storage components from lipid to glycogen, and the nuclear reorganization can change the physico-chemical properties of the fungal wall, resulting in alternation of its permeability and resistance to turgor pressure, thus, influencing molecular exchanges between the two symbionts (Bonfante and Scannerini 1992). Specific fungal enzyme activities are also known to change during plant tissue infection, such as the expression of a vacuolar alkaline phosphatase (Tisserant et al.1993). The mechanisms, controlling differentiation of AM fungal structure, in particular, the arbuscular, are so far not adequately understood.

Modifications in the plant

Formation of arbuscules inside the cortical cells of root by the AM fungi also induced several morphological modifications in the cell's architecture. Dramatics modification of host cell architecture such as invagination of the plant plasmalema, fragmentation of the vacuoles, disappearance of amyloplast and increase in the number of organelles, such as golgi bodies (Bonfante and Perotto 1992). The presence of fungus radically and effects the morphology of the plant nucleus, which maintains its ploidy, but increases in size owing to unfolding of its chromatin (Berta et al. 1990). Position of the nucleus is also affected, which moves from a peripheral position, typical of uninfected cell, to a central position in infected cells. This nuclear movement as well as many of the responses of the plant to fungal penetration probably results from modification in the organization of the plant cytoskeleton (Kobayashi and Kunoh, 1992).

One of the most important events that mark successful colonization of plant cells of plant cells by AM fungi is the formation of an interface compartment at the contact area between plant and fungal cell surfaces. It is composed of the membrane of both partners, separated by an apoplastic region and assumed to have a role in allowing a two-way exchange of nutrients (Bonfante and Scannerini, 1992; Smith *et al*.1994). When root cells are colonized by AM fungi, the host plasmalemma invaginates and proliferates around the developing fungus around the arbuscules. Apoplastic material is laid down between the invaginated plasma membrane and fungal cell surface, creating a new compartment. This compartment is structurally complex since it is composed of the host membrane, the interfacial material, the fungal wall and the membrane (Gollotte *et al.* 1996a). The material surrounding fungal branches topologically continues with the host wall, but its texture undergoes modification during arbuscules development. The material appears electron dense at the penetration point; it thins around arbuscular branches and thickens again around collapsed branches. It has a zone of high molecular complexity, molecules common to the plant primary wall, such as b-1, 4 glucans, nonesterfied polygalactouronase, hemicellulose such as xyloglucans, protein rich in hydroxyproline (HRGPs) and arabinogalactan proteins have been found in many different plant AM fungi combinations (Bonfante-Fasolo *et al*. 1991; Gianinazzi-Pearson *et al*. 1992; Gollotte *et al.* 1996b) . Chitin and b-1, 3 glucans were not detected in the interfacial material, whereas they are detected in the wall of many AM fungi (Bonfante and Perotto 1990). Current model assumes that plant cell wall consist of three interwoven domains: a network of cellulose and hemicellulose, another of heterogeneous pectin's and a third of proteins. The presence of these molecules typical of the primary plant cell wall indicates that the newly synthesized membrane, termed the perifungal membrane, retains the enzymatic machinery involved in the synthesis (cellulose) and secretion (pectins, hemicellulose, HRGPs) of cell wall material. In mycorrhizal association, because of its position around the fungus, the term perifungal membrane is suggested, which has a wider meaning than peri-arbuscular membrane limited to the membrane surrounding the arbuscular branches. The ATPase activity, revealed in the perifungal membrane might

be very important in terms of nutrient transport. Part of this activity is attributable to an H+/ATPase present in the perifungal membrane invaginated around the arbuscules, but cytochemically undetectable along other plant membranes. But its activity is absent around aborted arbuscules formed by the late pea mutants (Gianinazzi-Pearson, 1995). The two-way transfer between the plant and AM fungi also involves fungal membrane and have consistent H+-ATPase activity and exchange occurs across both the arbuscule interface, and the interface produced by cortical cell walls and intercellular hyphae. The presence of H+/ATPase seem to be typical of mutualistic symbiosis as it is also found on the plant membrane surrounding bacteria inside the nodul(Brewin, 1990). By contrast, no active membrane-associated ATPase was found on the membrane surrounding haustoria in plant-pathogen interaction, which might explain the unidirectional nutrient influx towards the

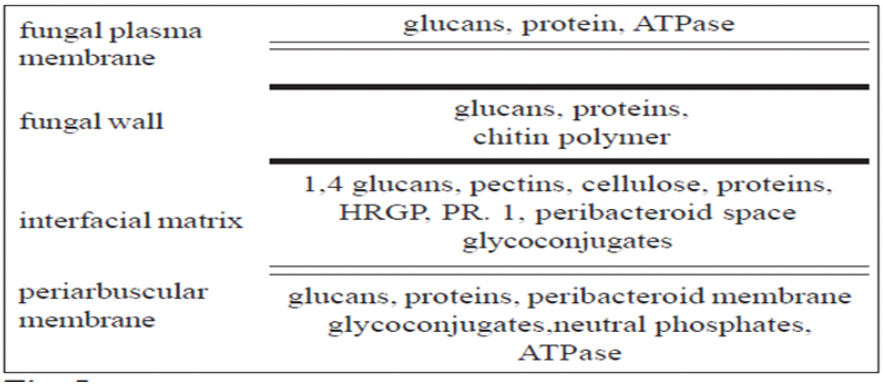

Fig. 3. Diagrammatic representation of the distribution of molecules characterizing the arbuscular interface (Gollotte *et al.* 1996)

fungus observed in pathogenic interactions (Smith and Smith, 1990).Formation of the interfacial compartment involves de novo synthesis of cell wall and membrane molecules.

Arbuscular mycorrhizal effects on host plant physiology

Enhanced plant efficiency in absorbing water and nutrients from the soil

Arbuscular mycorrhizae improved plant physiological performance (growth and development, productivity, crop quality) under normally growing condition, with producer and consumer benefits. Morever, 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.

Fungal H+-ATPases involved in symbiotic nutrient transfer have been characterized by Ferrol *et al.* (2002) and Requena *et al*. (2003). Regarding the transport of individual nutrients, the transfer of carbohydrates is thought to be the main benefit for the fungal symbiotic partner.

Enzymes and transporters described to be specifically induced in AM roots are indicated. Membrane transport of most metabolites can be expected to be pH-dependent and to be powered by the activity of plant (1) and fungal (2) H+ -ATPases. Fungal H+ -ATPases have been described not to be restricted to arbuscules,

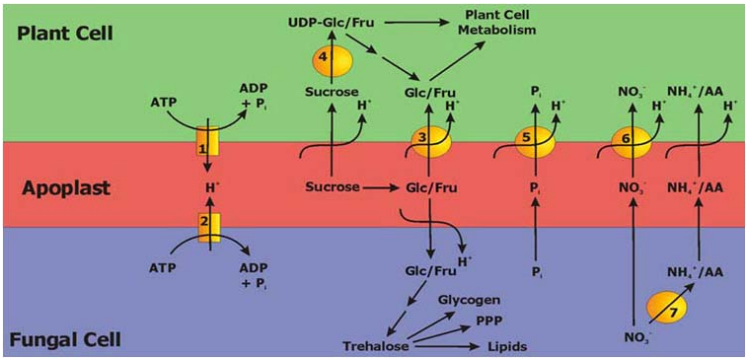

Fig. 4. Nutrient transfer in AM roots (Ferrol *et al*. 2002)

suggesting active transport at intercellular hyphae as well. Sucrose from the phloem is either cleaved by apoplastic invertases and taken up by the plant (3) or fungal hexose transporters or imported into root cortical cells and cleaved there by a cytoplasmic sucrose synthase (4). The fungus transforms hexoses rapidly into trehalose, which is either metabolized by the pentose phosphate pathway, or used for the biosynthesis of glycogen and lipids. These compounds are then exported to fungal vesicles or to the external mycelium. The plant cell takes up phosphate from the periarbuscular space using specific, H+ -dependent plant phosphate transporters (5). Regarding nitrogen supply, AM-induced plant nitrate transporters (6) have been found, suggesting a similar transport mechanism as referring to phosphate. On the other hand, the observation of increased transcript levels of a fungal nitrate reductase (7) suggests the transfer of nitrogen in a reduced form (as ammonium or in an organic form). 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.

Enhancing plant health and vigor, and minimizing stress

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 and 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.

Increased pathogen resistance/protection

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 *Verticilium 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 mychorrizal plants, shorthly 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 facsiculatum*, *Glomus etunicatum*, *Glomus intraradices* and *Scutellospora* sp. applied to green pepper (*Capsicum annum*), parsley (*Petroselinum crispum*), carrot (*Daucus carrota*) 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

Table 1. Some Plant Resistance Marker Molecules Investigated in Fungus-Root Interaction I Arbuscular Mycorrhiza (Gianinazzi- Pearson *et al*. 1996a)

Molecules	Modification	References
Phytoalexins	Late or transient increase in some flavonoids PAL, CHS, and CHI transcripts during root colonization. Localization of PAL and CHS transcripts in arbuscular containing cells. No increase in IFR transcripts	Morandi et al.1984; Harrison and Dixon 1993, 1994.
Callose	β-1, 3 glucans in host wall at the base of arbuscule trunks	Gollotte et al.1995; Gianinazzi-Pearson 1995.
Peroxidase	Increase in total and wall bound activity in early stage of colonization. No localization in arbuscular containing cells	Spanu and Bonfante- Fasolo 1988; Gianninazzi and Gianninazzi-Pearson 1992; Mc Arthur and Knowles 1992.
Chitinase	Early increase in transcripts and activity, generally followed by suppression in later stage of colonization. New isoforms	Spanu et al. 1989; Volpin et al.1994; Lambais and Mehdy 1993; Dumas Gaudot et al. 1992ab, 1994a.
β-1,3 glucanase	No detectable quantitative changes in protein and decrease in transcripts in later stage of colonization	Dumas et al.1989; Lambais and Mehdy 1993.
PR-1 protein	Slight increase in transcripts. Localization around living arbuscules	Gianinazzi-Pearson et al. 1992.

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*. Morewer, 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.

Refering 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.

Benefits of mycorrhizal fungus inoculation for woody plant growth

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).

Enhance the survival percentage of micropropagated seedlings

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 and Teixeira de Silva, 2010). 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 perfomed 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.

Enhanced plant transplant Establishment

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 (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 biopriming 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.

Enhanced rooting of cuttings

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 basidimiomycete *Piriformospora indica* inoculation was noticed as a new tool to improve vegetative propagation by cuttings (Druege *et al.* 2007).

Accumulation of secondary metabolites

The presence of AMF colonizing roots of greenhouse-grown lettuces can induce an accumulation of secondary metabolites, vitamins and minerals in leaves that overcome the dilution effect due to the increased size of mycorrhizal plants (Marouane *et al.,* 2013). Therefore, AMF would allow the intake of minerals and compounds with antioxidant properties to be enhanced without increasing the consumption of lettuce in the diet. In addition, increased quantities of secondary metabolites may help lettuce plants to withstand biotic and abiotic stresses.

CONCLUSION

In conclusion, the first transplant resulted in an adaptative condition to the growing stage and to the mycorrhizal colonization causing an additional energetic expense in plants. However, in the second transplant the co - inoculation improved plant growth and protected against oxidative stress. 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). The different ways to communicate with their hosts and to establish compatibility in divergent AM and root endophytic fungal lineages, reflected in the different amount and expression patterns of genes encoding for example, SSPs, hydrolytic enzymes, lectins and genes involved in signal transduction, suggest that similar func-tional properties and outputs of interactions (e.g. phos- phate transfer, growth promotion and establishment of biotrophy) have evolved independently through convergent evolution.

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