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Review

Applying mycorrhiza biotechnology to horticulture: significance and potentials

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Abstract

Mycorrhiza are symbiotic associations between plant roots and certain soil fungi which play a key role in nutrient cycling in the ecosystem and also protect plants against environmental and cultural stress. Most of the major plant families are able to form mycorrhiza, the arbuscular mycorrhizal (AM) association being the commonest mycorrhizal type involved in agricultural systems. AM biotechnology is feasible for crops using a transplant stage, as is the case with horticultural systems. Recent developments and insights regarding the potentials of AM symbiosis in horticultural practices are discussed. Given the effects of AM inoculation on plant growth and health, as biofertilizers and bioprotectors, it is accepted that an appropriate management of this symbiosis would permit a satisfactory reduction of chemical fertilizer and pesticide inputs, key aspects for sustainable horticultural plant production approaches. Maximum benefits will only be obtained from inoculation with efficient AM fungi and a careful selection of compatible host/fungus/substrate combinations. The performance of micropropagated plants or artificial seeds may be greatly improved by ensuring a suitable mycorrhizal establishment at outplanting. In particular, woody horticultural plants which are difficult to root in vitro have been shown to improve their survival rate and quality when inoculated with AM fungi. Interactions between AM fungi and rhizobacteria have the potential to be a useful biotechnological tool for benefiting plant development and health throughout an integrated management approach. Mycorrhizal inoculum production techniques need to be improved for the proper application of AM biotechnology in commercial horticultural plant production systems. © Elsevier Science B.V.

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1. Introduction

The roots of most plant species live in symbiosis with certain soil fungi by establishing what are known as mycorrhiza. The fungus biotrophically colonizes the root cortex and develops an extra-matrical mycelium that helps the plant to acquire mineral nutrients from soil (Harley and Smith, 1983). It has been recognized that mycorrhizal symbioses play a key role in nutrient cycling in the ecosystem and also protect plants against environmental and cultural stress (Barea and Jeffries, 1995). Most of the major plant families are able to form mycorrhiza naturally, the arbuscular mycorrhizal (AM) associations being the commonest mycorrhizal type involved in agricultural systems (Barea et al., 1993). Since AM symbiosis can benefit plant growth and health there is an increasing interest in ascertaining their effectiveness in particular plant production situations and, consequently, in manipulating them, when feasible, so that they can be incorporated into production practices. Evidence is accumulating to show that indigenous and/or introduced AM fungi (AMF) are involved in the development of different plant production systems including either field sown and plantation crops, or transplantable horticultural crops. Although usually ignored, a number of diverse plant types of economic interest are related to AM activity. These include, firstly, annual crops, such as cereals and herbaceous legumes, in which the natural endophytes normally present in arable soils can operate. In these cases, however, the biotechnological approaches required for the appropriate management of AM potential are rather complex. Secondly, they include other plant production systems, for which the application of mycorrhizal biotechnology is also feasible. These include vegetable crops, temperate fruit trees or shrubs, tropical plantation crops, ornamentals, spices, etc., in summary, horticultural plant crops.

In fact, many high-value ornamental and edible horticultural crops form arbuscular mycorrhiza. The plant species involved usually display a considerable degree of mycotrophy and their optimal development is, therefore, dependent on early AM symbiosis establishment. In particular, AM inoculation can be carried out when, as in many instances, the seedlings are produced in potting mixtures or in disinfected nursery beds, where AM propagules are present in low number, or even absent, such as in micropropagated plant material (Gianinazzi et al., 1990a).

The aim of this review is to discuss recent developments and insights regarding the potentials of AMF in horticulture practices. Given the effects of AM symbiosis on plant growth and health, it is expected that an appropriate management of this symbiosis would permit a satisfactory reduction of chemical fertilizer and pesticide inputs, key aspects for sustainable horticultural plant production approaches. In fact, the potential of AMF as biofertilizers and bioprotectors which enhance crop productivity is well recognized, although it has not yet been well exploited (Lovato et al., 1995). In order to give a conceptual background to nonspecialist readers and to facilitate a better understanding of the mycorrhizal effects some aspects of the biology and ecology of the AM symbiosis and its overall significance for soil–plant ecosystems will first be briefly reviewed. A comprehensive review of the most relevant case studies and the current information concerning the significance of mycorrhizas in horticultural plants will then be given. Finally, the possibilities of a rational management of AM activity with respect

to how it might improve horticultural plant production will be analyzed. Afterwards, the future perspectives for basic, strategic and applied research on the topic will be outlined.

2. Arbuscular mycorrhizas

2.1. General concepts on the mycorrhizal status

The term 'mycorrhiza' refers to the association between fungi and roots. This association is usually considered a mutualistic symbiosis because of the highly interdependent relationship established between both partners, where the host plant receives mineral nutrients via fungal mycelium (mycotrophism), while the heterotrophic fungus obtains carbon compounds from the host's photosynthesis (Harley and Smith, 1983; Azcón-Aguilar and Bago, 1994).

During the process of mycorrhiza formation, in which the plant 'accepts' the fungal colonization without any significant rejection reaction, a series of root–fungus interactions give way to the integration of both organisms. This in turn, leads to the development of a well adapted 'unity' within the context of the soil–plant ecosystem. Despite the scarcity of experimental information, it has been accepted that the establishment of the symbiosis must be the result of a continuous molecular 'dialogue' between plant and fungus, as exerted through the exchange of both recognition and acceptance signals. The result of this dialogue will finally depend on the genome expression of both partners (Smith and Gianinazzi-Pearson, 1988; Gianinazzi-Pearson et al., 1994).

The fungus, in fact, becomes an integral part of the root system. The symbiosis is considered the most metabolically active component of the absorbing organs of the autotrophic host plant which, in addition to furnishing the heterotrophic fungal associate with organic nutrients, is an ecologically protected habitat for the fungus.

Mycorrhiza can be found in almost any kind of soil. All but a few vascular plant species (those belonging mainly to the families Cruciferae, Chenopodiaceae, Cyperaceae, Caryophyllaceae and Juncaceae) are able to form mycorrhiza. The physiology of the plant is highly affected by the presence of the fungal symbionts (Harley and Smith, 1983; Smith and Gianinazzi-Pearson, 1988; Azcón-Aguilar and Bago, 1994).

It is obvious that the universality of this symbiosis implies a great diversity in the taxonomic features of the fungi and the plants involved. There are, in fact, great differences in the morphology of the mycorrhizal type and this is reflected in the resulting physiological relationships (Gianinazzi-Pearson, 1984; Azcón-Aguilar and Bago, 1994; Smith et al., 1994). Five types of mycorrhiza can be recognized whose structural and functional features have been detailed in several publications (Smith, 1980; Harley and Smith, 1983), thus only a brief consideration will be given here to differentiate these groups.

About 3% of the higher plants, mainly forest trees in the Fagaceae, Betulaceae, Pinaceae, *Eucalyptus*, and some woody legumes, form ectomycorrhiza. The fungi involved are mostly higher Basidiomycetes and Ascomycetes which colonize the cortical root tissues. A lack of intracellular penetrations is characteristic. In general, the fungus develops a sheath or mantle around the feeder roots.

Three other types of mycorrhiza can be grouped as endomycorrhiza, in which the fungus can colonize the root cortex intracellularly. One of these is restricted to some species in the Ericaceae ('ericoid' mycorrhiza), the second to the Orchidaceae ('orchid' mycorrhiza), and the third, the arbuscular mycorrhizas, which is by far the most widespread type.

There is a fifth group, the ectendomycorrhiza, composed of plant species in families other than Ericaceae but in the Ericales, and in the Monotropaceae. They have sheaths and produce intracellular penetrations ('arbutoid' and 'monotropoid' mycorrhiza).

The ecological and economical value of arbuscular mycorrhiza can be directly inferred from the fact that about four-fifths of all land plants, including the agronomically important crops, form this type of mycorrhiza. As stated before, the arbuscular mycorrhiza are by far the commonest in horticultural plants. Therefore from here on this study will be concerned only with the 'arbuscular' type which will be referred to in this paper as 'AM symbiosis' or, simply 'mycorrhiza' (Walker, 1995).

Both the fungus and the AM symbiosis are distributed worldwide. The fungi belong to the class Zigomicotina, order Glomales (Walker, 1992; Rosendahl et al., 1994; Morton et al., 1995). About 150 species in the only six genera which are able to form AM (*Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora*) have been systematized; none of these fungi have yet been successfully cultured axenically (Azcón-Aguilar and Barea, 1995). Molecular biology approaches are now being developed to explore the biodiversity and characterization of AMF (van Tuinen et al., 1994).

Several of the properties inherent in all symbiotic systems are particularly relevant in AM associations, namely dependency, compatibility, specificity, etc.

It seems that certain fungi played a critical role in the evolution of 'plants' as they colonized the land in the late Silurian early Devonian period (400×10^6 years ago) in that they appear to have become associated with such 'plants', thus facilitating the nutrient uptake processes (Nicolson, 1975). These associations may be considered mycorrhiza, and the plant/mycorrhiza coevolution may account for the worldwide spread of AM symbiosis and its symbiotic properties. These properties are determined by: (i) the ability of a plant to acquire nutrients through a fungus (mycotrophy), (ii) the difficulties of the fungus to complete its life-cycle independently of the host plant, since it is a physiologically obligate symbiont (fungal dependency), and (iii) the characteristics of the plant as expressed by the degree to which it is dependent on mycorrhizas for its proper development (mycorrhizal dependency of a plant). This dependency on mycorrhizas varies from one plant species to another, since some need the AM symbiosis to survive, others, to improve their growth, and others to achieve their maximum development (Hayman, 1983; Barea et al., 1993), this being determined by the genetic characteristics of the plant.

On the other hand, there is a lack of 'specificity', strictly speaking, in AM associations. Any AMF can colonize any suitable plant species, a single root system can support different AMF species, and roots of various plant species can be linked by the mycelium of a single AMF (Mosse et al., 1981; Harley and Smith, 1983; Gianinazzi-Pearson, 1984). Nevertheless, different plant species, and even cultivars within the same species (Azcón and Ocampo, 1981), vary greatly in their level of 'susceptibility' to

AMF. This indicates that the plant genotype controls the amount of root tissue which would became mycorrhizal (Gianinazzi-Pearson, 1984; Smith, 1995). Since the fungi also differ in the extent to which they colonize the root system of a given host plant, it follows that a certain type of 'specificity' can be recognized in the symbiosis in which they participate. It is here that the concept of 'compatibility' arises, which must be associated to that of 'symbiotic effectiveness' to establish that of 'functional compatibility' (Gianinazzi-Pearson, 1984). The latter refers to the phenotipic expression of the AM status as a result of the environmental influences on the expression of the genotypic equipment of both the plant and the fungus involved (Smith and Gianinazzi-Pearson, 1988). According to Gianinazzi-Pearson (1984) and Azcón-Aguilar and Bago (1994), there is evidence of fungus–plant 'recognition' processes, at several stages. Such evidence includes: (i) a cell to cell contact, which gives way to appressorium formation; (ii) certain morphological and structural changes within the fungus colonizing root tissues, mainly concerning fungal cell wall organization; (iii) integration of the physiology of both symbionts, and (iv) redistribution of enzymatic activities, especially those involved in nutrient exchange between symbionts.

2.2. *Formation of AM symbiosis*

AM colonization begins with hyphae that arise from soil-borne propagules (large resting spores of the AMF or mycorrhizal root fragments), or from an AM plant growing nearby.

Upon the arrival of the fungal hyphae at the root surface, an appressorium is usually formed on the epidermal cells. The colonizing hyphae originating from appressoria pass through the intercellular spaces and then enter root tissues spreading between and through cells of the cortical root layers. Once the hyphae reach the inner cortex, they will grow into the cells and, by means of repeated dichotomous branching, form tree-like structures called 'arbuscules'. The life-span of individual arbuscules is about 4–14 days. As internal colonization spreads, the extra-radical hyphae ramify, and grow along the root surface forming more penetration points. They also grow outwards, into the surrounding soil, thus developing an extensive tri-dimensional network of mycelium which interfaces with soil particles. The fungus in some genera may form 'vesicles', oval-to-spherical structures with a storage (mainly lipids) function. Most AMF form large resting spores on the external mycelium.

The fungus in the intracellular colonizations, as in the case of arbuscules, is always surrounded by the intact host-cell plasmalemma. Arbuscule formation therefore, represents a large surface of cellular contact between both symbionts. This facilitates the exchange of metabolites between host and fungus. In fact, the arbuscule is probably the main transfer site of mineral nutrients from the fungus to the plant and C compounds to the fungus (Smith and Gianinazzi-Pearson, 1988; Smith and Smith, 1990; Bonfante and Bianciotto, 1995). Data recorded by Smith and Gianinazzi-Pearson (1988) indicate that the length of the external hyphae growing in soil associated with mycorrhizal roots reaches an average of $1\,m\,cm^{-1}$ root, but values of up to $10–14\,m\,cm^{-1}$ root have also been recorded. This mycelial network can extend several centimetres outwards from the root surface, bridging over the zone of nutrient depletion around roots to absorb

low-mobile ions (mineral plant nutrients) from the bulk soil. The extra-radical hyphae also interact with components of the rhizosphere microbiota, and together they contribute to the formation of water-stable aggregates which are critical for a good soil structure. In summary, AMF act as a major interface between plants and the biotic and abiotic components of the complex soil ecosystem (Bethlenfalvay and Linderman, 1992).

Several features of the host plant, depending mainly (but not exclusively) on the morphological and physiological characteristics of the root system, condition its ability to acquire nutrients and, consequently, the benefit the host obtains from its mycorrhizal status. Certain factors which affect mycorrhiza formation can also indirectly affect the functioning of the symbiosis.

In general, those plants which are able to absorb relatively immobile soil resources, like phosphorus, are less dependent on mycorrhiza formation (Brundrett, 1991). It is known that the ability of the root system to absorb low mobility nutrients from the soil solution is positively correlated to root surface area, which is in turn a consequence of root system architecture (branching pattern, length and diameter of the roots, length and number of root hairs, etc.). Thus, plants with low branching frequency, low number of lateral roots and few (and/or short) root hairs appear to be more dependent on mycorrhiza for mineral nutrient uptake (Brundrett, 1991).

However, the geometry of the root system is not the only characteristic relevant to nutrient uptake and mycorrhizal dependency. The activity of the root system, its growth rate and plasticity (the ability to respond quickly to localized or temporal changes in soil conditions, or in nutrient levels in soil profiles) also affect the ability of the plant to cope with unfavourable soil conditions. Consequently, and depending on these characteristics, the plant will need, to a greater or lesser extent, to rely on mycorrhiza establishment in order to overcome these problems.

2.3. Effects of AM on the plant–soil ecosystem

The key effects of AM symbiosis can be summarized as follows: (i) improved rooting and plant establishment; (ii) improved uptake of low mobile ions; (iii) improved nutrient cycling; (iv) enhanced plant tolerance to (biotic and abiotic) stress; (v) improved quality of soil structure; (vi) enhanced plant community diversity.

AM symbiosis, therefore, influences several aspects of plant physiology, such as mineral nutrition, plant development and plant protection (Gianinazzi et al., 1990b). AMF also have an indirect influence on plant growth because of their effects on soil structure stabilization, i.e. soil aggregate formation and humic substances accumulation (Bethlenfalvay and Linderman, 1992; Bethlenfalvay and Schüepp, 1994).

The primary effect of AM symbiosis is to increase the supply of mineral nutrients to the plant, particularly those whose ionic forms have a poor mobility rate, or those which are present in low concentration in the soil solution. This mainly concerns phosphate, ammonium, zinc and copper (Barea, 1991).

The well-known effect of AM symbiosis increasing P concentration and/or content in plant tissues, and on the acquisition of other nutrients, is usually concomitant with considerable increases in phytomass production. Nutrient supply by the mycorrhizal

mycelium activity exerts, in the aerial part of the plant, a feed-back regulation on the photosynthesis itself and on the translocation of the photosynthate. Hence, fewer photosynthesis products are allocated to the root; the shoot/root ratio is, therefore, usually higher in AM plants than in their corresponding controls (Smith, 1980). It has also been recently recognized that AM colonization affects a wide range of morphological parameters in developing root systems (Schellenbaum et al., 1991; Atkinson et al., 1994; Berta et al., 1995), a greater root branching being the most commonly described effect. It has been argued that changes in the hormonal balance and in the meristematic activity may explain these effects on plant development.

Another activity of arbuscular mycorrhizas concerns plant protection against biotic stress. As is well known, many types of microbe–microbe interactions, either beneficial or antagonistic, take place in rhizosphere microhabitats (Stoztky, 1972). Any microorganism which is antagonistic to harmful, 'soil-borne plant pathogens' will be beneficial to its host plant, concerning health protection (Linderman, 1994). Therefore, the appropriate management of such antagonistic organisms in order to protect the plant against pathogens (bacteria, fungi, nematodes, etc.) is a form of biological control. AM associations have been suggested as biocontrol agents, and the general conclusion is that they can reduce or even suppress, damage caused by soil-borne plant pathogens (Linderman, 1994; Hooker et al., 1994b). However, it is important to point out that the enhancement of root resistance/tolerance to pathogen attack in AM plants is not exerted with the same effectivity by all AM fungi, is not applicable to all pathogens, and is not expressed in all substrates or in all environmental conditions. Nevertheless, there are examples demonstrating that prior colonization of selected AM fungi protect plants against pathogenic fungi, such as *Phytophthora*, *Gaeumanomyces*, *Fusarium*, *Thielaviopsis*, *Pythium*, *Rhizoctonia*, *Sclerotium*, *Verticillium*, *Aphanomyces*, etc., or nematodes such as *Rotylenchus*, *Pratylenchus*, *Meloidogyne*, etc. The review papers by Linderman (1994) and Hooker et al. (1994b) summarize experiments which support such statements.

The mechanisms that have been suggested to explain the protective action of AM symbiosis include the improvement of plant nutrition, damage compensation, competition for photosynthates or for colonization/infection sites, induction of changes in the morphology/anatomy of the root system, induction of changes in mycorrhizosphere populations and activation of plant defence mechanisms (Linderman, 1994; Hooker et al., 1994b).

Mycorrhizal fungi also enable plants to cope with abiotic stress by means of alleviating nutrient deficiencies, improving drought tolerance, overcoming the detrimental effects of salinity, enhancing tolerance to pollution and improving the adaptation of sterile micropropagated plants to unsterile substrates and to field conditions (Barea et al., 1993).

2.4. Interaction of AMF with components of soil microbiota

Microbial interactions involving AMF appear to play a key role in ecological and biotechnological approaches to improving sustainability of soil–plant systems. Rhizosphere microorganisms can affect AM formation by acting on AM propagule germina-

tion, and/or the establishment of entry points of the AM fungi in the roots. Conversely, by altering the quality and quantity of root exudates, AM symbiosis can modulate the activity and/or number of the microbial components in the soil surrounding the mycorrhizal root system, giving way to the so-called mycorrhizosphere (Linderman, 1992; Azcón-Aguilar and Barea, 1992). Specific types of organisms are able to establish relationships with mycorrhizal fungi which affect plant growth and health, and also soil quality. These interactions are summarized from Barea et al. (1996) and recorded in Table 1.

2.5. *Modulation of AM activity by environmental and cultural factors*

In addition to the intrinsic characteristics of both partners (the fungus and the plant) a wide range of environmental influences and cultural practices modulate the efficiency of AM associations in terms of improving plant performance.

Some characteristics of the rooting media can influence the formation and/or effect of mycorrhiza on plant crop development. In general, low to moderate soil fertility favours AM formation and function although adaptation of certain AM fungi to higher levels of P and N in soil has been reported (Hayman, 1983).

A special case of a substrate effect is that exerted by the potting mixes used in horticulture (Nemec, 1986; Calvet et al., 1992b). Two main problems arising from the use of these substrates are currently receiving attention: (i) the selection of those potting mix materials which favour AM formation and functioning, and (ii) the interaction between AMF and other components of the microbiota of the potting mixture, mainly concerning the biological control of root diseases.

There is much published information to show that increasing soluble phosphate fertilizers usually decrease the overall level of AM colonization. The amount of external mycelium, the density of arbuscular development and the number of mycorrhizal entry points, can be greatly reduced by added soluble P fertilizers (Barea, 1991).

From the agricultural point of view, some important conclusions can be drawn: (i) P fertilizers may have a positive effect on AM activity when the soil fertility level is low; (ii) mycorrhizal plants reach an acceptable level of productivity at a dose of P lower

Table 1
Interactions of arbuscular mycorrhizas with beneficial soil microorganisms

Type of microorganism	Results of the interaction
N_2-fixing bacteria (biofertilizers)	N_2 fixation, N-cycling, N 'transfer'
Phosphate solubilizers (biofertilizers)	P-cycling, use of rock and organic phosphates as an alternative P source
Plant hormone producers (phytostimulators)	Rooting and establishment of seedlings
Agents for biological control of plant diseases ('biopesticides', bioprotectors)	Increased resistance/tolerance to root diseases
Bacteria and fungi related to formation of stable aggregates (ecosystem bioremediators)	Improvement of soil quality

than those needed for non-mycorrhizal controls to reach the same productivity; (iii) there is a level of P application at which mycorrhizal crops do not derive additional benefits, in term of plant growth, over the non-mycorrhizal counterparts (Barea et al., 1993). The use of slow-release fertilizers and the selection of high P-tolerant fungal ecotypes can be appropriate alternatives (Nemec, 1986).

Nitrogen fertilizers have also been described to decrease AM formation and activity, but as in the case with P, the field responses are unpredictables because they depend on the initial level of the nutrient in soil and on the endophyte adaptation properties. The effects of compound NPK fertilizers are obviously complex and depend on many variables.

It is likely that agricultural practices greatly modify the diversity and distribution of mycorrhizal fungi. Both spore number and species composition can be changed. Besides, the host can select particularly efficient endophytes (Mosse et al., 1981; Sieverding, 1991). Apart from the effects of fertilizer applications on the AM response, other agricultural practices are known to affect AM activity. Among these can be considered crop management intensity, transplant practices, organic matter applications, cover cropping, crop rotation (especially when it involves non-host plants or fallow periods), irrigation practices, weed control, minimal cultivation, pesticide applications, treatments which raise the soil heavy metal content, etc. These factors will be considered in Section 4.2 regarding horticulture systems.

3. Mycorrhizas in horticultural systems

The effects of AMF on the growth and development of horticultural plants have been studied and described in many research papers (Lovato et al., 1995). In general, fruit crops have received more attention than vegetable and ornamental crops.

Obviously, the interest of horticulturists in AM technology is due to the ability of AMF to increase the uptake of phosphorus and other nutrients, and to increase resistance to biotic and abiotic stress.

Basically, plant propagation in horticultural systems usually starts from seedlings, cuttings or graftings produced or developed in soil or in substrates that have been treated to lower the level of pathogenic organisms. In the case of micropropagation, sterility is obviously a key component. Therefore, the most common propagation techniques include treatments whose aim is to eliminate or reduce the microbial population in the rooting medium, although this obviously affects also the beneficial ones, such as the AMF. However, these practices provide the most rewarding situation for the application of mycorrhizal biotechnology by reintroducing appropriately selected AMF at suitable stages of the plant production schedules.

It is accepted that the first evidence of the positive influence of the AM symbiosis on horticultural production was provided by Menge et al. (1977). This paper demonstrated that AM propagule inoculation was a prerequisite to get the establishment of citrus plants in biocide-treated nursery beds. Since that publication a number of experiments have been carried out, as reviewed by Miller et al. (1986), Nemec (1986), Gianinazzi et al. (1990a,b), Barea et al. (1993), Chang (1994), Lovato et al. (1995), Varma and

Schüepp (1995) and Lovato et al. (1996). These reviews show the potentiality for the application of AM inoculation in horticulture. In summary, the information published, and recorded in the above review papers refers to: (i) vegetable crops and spices, such as lettuce, onion, leek, celery, asparagus, pepper, cucumber, beans, tomato, strawberry, potato, muskmelon, watermelon, etc.; (ii) temperate fruit crops, including citrus, apple, almond, peach, peach–almond hydrid, olive, grapevine, blackberry, pear, kiwifruit, raspberry, cherry, plum, etc.; (iii) tropical plantation crops, among them coffee, rubber, cacao, papaya, annanas, oil palm, cocoa, avocado, pineapple, passion fruit, cherimoya, etc.; (iv) floricultural crops (pot plants, foliage plants, and cut flowers), such as rose, lilac, verbena, primula, cyclamen, chrysanthemum, geranium, begonia, hortensia, gerbera, *Liquidambar*, *Ampelopsis*, marigold, etc.

The main effects of AM inoculation in horticultural crops include: (i) enhanced seedling growth; (ii) reduced phosphate requirements; (iii) increased survival rate and development of micropropagated plantlets; (iv) increased resistance to fungal root pathogens; (v) increased resistance to abiotic stresses; (vi) earlier flowering and fruiting; (vii) increased crop uniformity; (viii) improved rooting of cuttings and (ix) increased fruit production (Barea et al., 1993; Chang, 1994).

In conclusion, it can be stated that: (i) a careful selection of functionally compatible host/fungus/substrate combination is critical for success and (ii) an early establishment of the AM status, after sowing or at outplanting, is a key factor to improve plant performance in horticultural practices (Gianinazzi et al., 1990a,b).

4. Management of mycorrhizal biotechnology to improve horticultural plant production

4.1. Mycorrhizal inoculation management

Since most horticultural crops exhibit a considerable mycotrophic habit, their productivity is expected to be increased if functionally compatible AMF are available to colonize the developing plant root system. Furthermore, biotechnological approaches to AMF management for improving crop productivity appear to be conceivable in horticultural production systems.

In a general context, there are two types of particular situations where inoculation can be feasible and rewarding: (i) when a population of low-effective propagules is present and (ii) when indigenous fungi are absent or in low levels in the rooting medium. It is obvious that this second alternative is the most promising and that the corresponding biotechnology can be applied. Target plant production systems are those related to horticulture, including fruticulture and floriculture, and forestry. As already stated, in these systems there is a plantlet production stage where the seedlings develop on artificial substrates, potting mixtures or disinfected nursery beds. A special case is that of micropropagated plant material, as produced by tissue culture, where AMF are completely absent (Vidal et al., 1992; Gianinazzi et al., 1990a,b; Vestberg and Estaún, 1994).

The majority of the nursery-produced horticultural plants must have a subsequent growth stage in the field. Therefore, biotests to determine the natural mycorrhizal

potential and the field situations where AM inoculation is advisable must first be carried out.

One critical step in the AM inoculation process is the appropriate fungal selection. With this aim, a number of methodological approaches and parameters to be considered have been proposed (Lovato et al., 1995). Among them the effectiveness in promoting plant growth, and in protecting against plant pathogens and/or abiotic stress such as salinity or drought, are the most common aspects to be taken into account. In this respect, it is important to consider environmental conditions at which the mycorrhizal plants will be introduced, and to include in the AMF selection studies native ecotypes, supposedly better adapted to the prevailing conditions of the experimental site. Once fungal selection has been made, the next step is the production of large quantities of inoculum, which must be based on well-defined fungal cultures. The INVAM (International Culture Collection of Arbuscular Mycorrhizal Fungi) and the BEG (La Banque Européene de Glomales) represent useful germplasm stores of voucher specimens which allow accurate taxonomic positioning and preservation (Dodd et al., 1994).

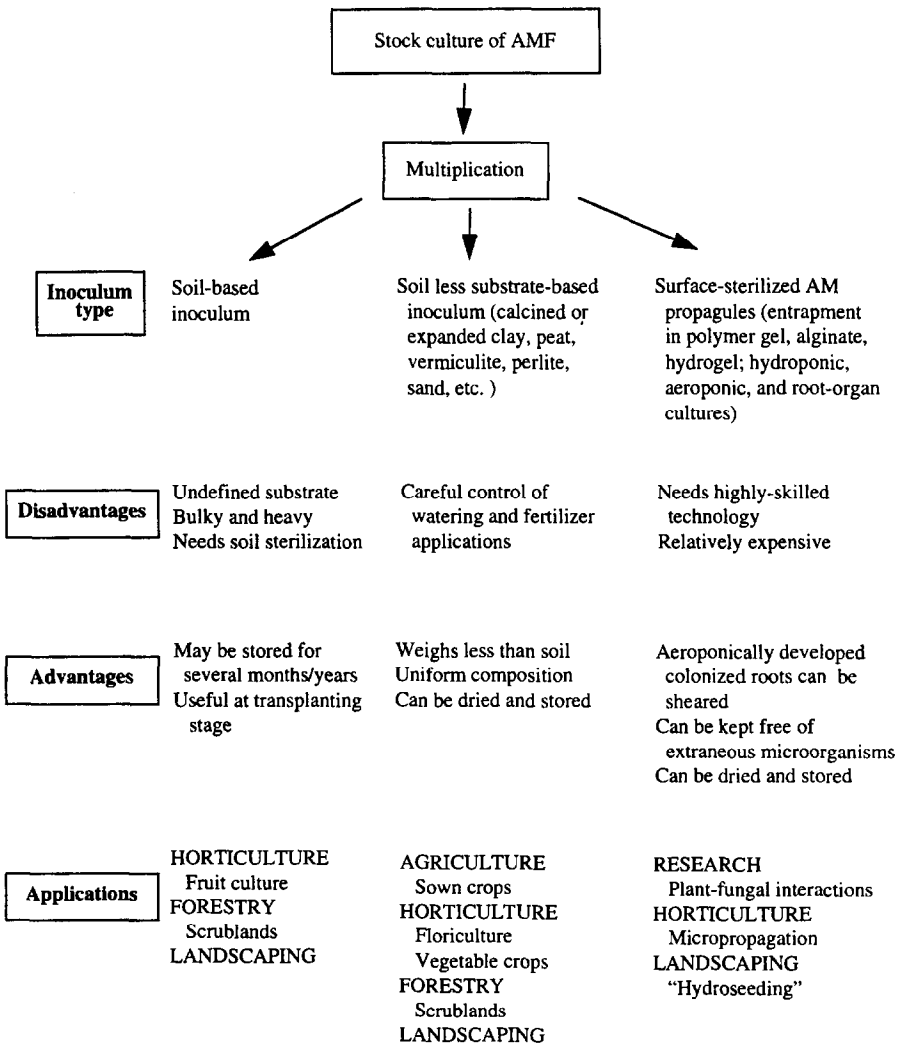
The materials to be inoculated are seeds, seedlings, cuttings or micropropagated plants. Considering the genetic variability for both the plant and the fungal partners, biotests which assess the functional compatibility between the symbionts must be performed. These will enable the selection of the suitable fungus for the cultivar (genotype) or plant species involved, always considering the prevalent edophoclimatic conditions. Inocula possesing more than one AM fungal species can also be tried (Lovato et al., 1995).

Since AMF are obligate symbionts, they must be produced on living roots. This is problematic when mass production is needed, and also because of the risk of plant pathogen contamination. However, as stated by Gianinazzi et al. (1990a), the fact that it is compulsory to reproduce the fungi as a plant-symbiont diminishes the chances of the fungi losing their symbiotic properties.

The procedures for inoculum production, type of inoculant and potential usages are outlined in Scheme 1.

Individual fungal isolates must be multiplied and their colonization of the roots of the stock culture plants must be maintained. These roots (in fact, mycorrhizas), or the AMF spores formed, are used to produce larger amounts of inoculum on soil-based media or artificial substrates (Sieverding and Barea, 1991). The soil-based inocula are quite easy to obtain and, after 6–12 months, highly infective inoculants can be yielded. However, this kind of inoculum is too heavy for extensive use in agriculture. Instead, it may be appropriate for plant production systems which involve a transplant stage, as is common in horticulture or forestry (Gianinazzi et al., 1990a)

Some AMF inoculum production systems have been proposed, and several patented (Gianinazzi et al., 1990b; Sieverding and Barea, 1991; Mulongoy et al., 1992; Jarstfer and Sylvia, 1992; Sylvia and Jarstfer, 1994; Thompson, 1994; Lovato et al., 1995). One inoculant form, which is produced in containers using light expanded clay aggregates (LECA) as substrate (Dehne and Backhaus, 1986), appears to be of interest. This porous material can be easily separated from the host roots and contains infective mycelium and spores (Feldmann and Idczak, 1992). The aggregates can be surface-sterilized and applied to field grown crops in small quantities (50–150 $kg\,ha^{-1}$) (Baltruschat, 1987).



Scheme 1. Inoculum production and potential usages.

Another inorganic carrier for AM inoculum production could be vermiculite, which behaved as an appropriate substrate for AMF spore formation (Barea et al., 1993). Sheared-root inocula (Sylvia and Jarstfer, 1992), obtained in aeroponic cultures (Hung and Sylvia, 1988; Jarstfer and Sylvia, 1995), have also been proposed. Recently, some other clay-derived substrates, often applied as soil-conditioners, are being successfully used for AMF inoculum production. Surface-disinfected AMF propagules can also be used, especially for micropropagated plants.

As regards inoculum management, the critical factors are the dosages and time of inoculation. Theoretically, a single propagule is enough to produce AM colonization. The process of colonization in such a case, however, is too slow to be of agronomic

interest. According to Lovato et al. (1995), about 1–2 kg of bulk soil inoculum (which means approximately 5000–10 000 propagules) per m^2 of seedbed could be an appropriate application rate.

The time of inoculation is also critical and, as a general rule, is can be said that the earlier the inoculation is performed, the greater the benefit to the plant (Barea et al., 1993).

The most common bulk inoculum composition is a mixture of spores, colonized roots, hyphae, and the supporting substrate from pot cultures, usually grown in pasteurized soil or soil-less media (Sylvia and Jarstfer, 1994). Some attention has been given to long-term AMF preservation and to the storing of the inocula.

Studies have also been carried out on a number of other aspects, such as: (i) the use of pesticides for inoculum production; (ii) inoculum quantities and richness; (iii) inoculum placement and spread, etc., (Jeffries and Dodd, 1991; Lovato et al., 1995).

There have been several attempts to produce AM inoculum on a commercial scale. Jeffries and Dodd (1991), Mulongoy et al. (1992), Sylvia and Jarstfer (1994) and Lovato et al. (1995), give examples in their review papers.

Wood (1991), in reviewing the potential markets and constraints for AM inoculum commercialization, concluded that the failure of large-scale commercial production could be attributed to: (i) a poor understanding of fungal biology; (ii) inappropriate information regarding the advantages of mycorrhiza, resulting in low demand for the product; (iii) fragmented markets due to the fact that many potential consumers are currently applying cultural practices which interfere with AM formation and function (e.g. large quantities of fertilizer and fungicides) and (iv) unreasonable expectations.

4.2. Horticultural practices influencing mycorrhizal biotechnology

It is noteworthy that soil disinfection, a current practice in horticultural nurseries leads to better opportunities for the application of mycorrhizal biotechnology. Chemical treatments (Chloropicrin, Methyl bromide, Vapam, and a number of fungicidal soil drenches) are currently being applied to kill harmful organisms present in propagation substrates (Hartmann et al., 1990). Such treatments, which also eliminate beneficial microorganisms, do not seem to seriously disrupt the physical and chemical characteristics of the substrates. After some time for aeration of the substrate, planting is possible, and the residues of the fumigant/fungicide do not appear to interfere with the activity of reintroduced AMF (Gianinazzi et al., 1990b; Barea et al., 1993).

Artificial potting mixes and other substrates are commonly used in nurseries where soil is rarely incorporated. Thus, AMF are not normally present in these rooting media. Because the receptivity of such substrates to AMF is variable, tests are needed to define appropriate substrate–crop–AMF combinations. Successful AM colonization has been reported to take place in substrates containing sand, gravel, peat, expanded clay, pumice, perlite, bark, sawdust, vermiculite or a mixture of these (Gianinazzi et al., 1990b).

In horticultural production fertilization should satisfy the maximum plant yield as well as the maintenance of the symbiosis and its properties as biofertilizer and bioprotector against biotic and abiotic stress. Therefore, appropriate fertilization regimes must be carefully established with regard to quantities, nutrient composition and application schedules (Lovato et al., 1995).

It has been demonstrated that certain pesticide treatments are compatible with AMF. Furthermore, mycorrhizal inoculation and these compatible pesticides cooperate to control pathogens. This would lead to a decrease in pesticide use and, consequently, to a reduction in their impact on the environment (Lovato et al., 1995). Captan appears to stimulate the development of AMF mycelium in horticultural substrates (Schüepp and Bodmer, 1991; Guillemin and Gianinazzi, 1992). In some cases, it has been shown that fosetyl-Al appears to have no significant effect on AM formation (Aziz et al., 1990). Metalaxyl has been also commended for AM inoculum production (Seymour, 1994).

4.3. The case of micropropagation

Micropropagation is a technique which uses aseptic tissue culture for the multiplication of important cultivars. It has become a fundamental practice for the commercial nursery propagation of many kinds of plants. The uses and advantages of micropropagation for commercial nurseries can be summarized as follows (Hartmann et al., 1990; Hooker et al., 1994a; Vestberg and Estaún, 1994): (i) mass propagation of specific clones (end point of breeding programmes); (ii) production of high quality, uniform, pathogen-free plants (for germplasm maintenance and transnational commercial distribution); (iii) clonal propagation of parental stocks for hybrid seed production and (iv) supply to year-round nursery production.

Despite many successful applications of this technique, there are still several problems limiting its widespread use. The transfer of in vitro plantlets to ex vitro conditions is one of the most critical steps of the micropropagation process. High mortality rates are recorded for certain micropropagated plant species upon transfer to ex vitro conditions due to the plantlet limitations in resisting the transplant stress. These limitations include a poorly developed cuticle, non-functional stomata, a heterotrophic habit, and a weak root system (Vestberg and Estaún, 1994; Elmeskaoui et al., 1995). In order to increase growth and survival rates during the weaning stage, recent research has focused on the control of the environmental conditions in the propagation system.

As stated before, horticultural plants commonly multiplied by micropropagation techniques, frequently develop AM relationships and exhibit a high degree of dependence on this symbiosis for normal development (Nemec, 1986). However, the media used, not only for the in vitro stages (obviously sterile), but also for the subsequent plantlet growth in potting substrates or in fumigated nursery beds, usually lack AM propagules. Therefore, plantlets should be inoculated early in the micropropagation process so that they can achieve a suitable growth (Gianinazzi et al., 1990a). Thus, research is being conducted world-wide to integrate both biotechnological approaches: mycorrhizal inoculation and micropropagation, regarding plant species of interest (Lovato et al., 1995, 1996; Varma and Schüepp, 1995).

There are three suitable seedling stages for AM inoculation: (i) in vitro during the rooting phase; (ii) ex vitro, immediately after the rooting phase, at the beginning of the acclimatization period and (iii) ex vitro, after the acclimatization phase, but before starting the post-acclimatization period under greenhouse conditions (Vestberg and Estaún, 1994).

As regards in vitro mycorrhizal inoculation and considering that the rooting phase normally takes place in agar-based media, it is important to remember that spore

germination and early stages of mycelial growth of AMF in axenic conditions have been intensively studied (see Azcón-Aguilar and Barea, 1995 for references). It has been shown that: (i) most AMF spores readily germinate on media with low nutrient content, and (ii) high nutrient levels, however, can inhibit spore germination and growth.

The AM symbiosis has been synthesized in vitro by using seedlings and root organ cultures (Mosse and Hepper, 1975; Bécard and Fortin, 1988; Elmeskaoui et al., 1995). In fact, surface sterilized spores and other AM propagules are able to develop mycorrhizal relationships when inoculated during the in vitro propagation of various horticultural species (Chávez and Ferrera-Cerrato, 1990), particularly woody plants (Pons et al., 1983; Ravolanirina et al., 1987). However, this technique presents a number of difficulties, which arise as a result of (i) differences in the requirements of the media for both root initiation and AM formation; (ii) loss of the roots produced during the in vitro stages after outplanting (Ravolanirina et al., 1987; Schubert et al., 1990) and (iii) more complex and time-consuming manipulation of the mycorrhizal propagules. In spite of this, Elmeskaoui et al. (1995) have proposed a method for developing AM associations in micropropagated plantlets in vitro. This method is based on a system for culturing the AMF *Glomus intraradices* with Ri T-DNA-transformed carrot roots, or nontransformed tomato roots. After root induction, micropropagated plantlets were grown on cellulose plugs, in contact with the mycorrhizal root organ culture. They were then placed in growth chambers under an atmosphere enriched with 5000 p.p.m. CO_2 and fed with a minimal medium. After 20 days of tripartite culture, all the plantlets were mycorrhizal.

In contrast to the in vitro inoculation, inoculation at the stage in which the axenically propagated plantlets are transplanted into the potting media, appears to be both more feasible and promising (Ravolanirina et al., 1989a,b; Schubert et al., 1990).

Mycorrhizal inoculation ex vitro can be performed either after the rooting phase in vitro, at the very beginning of the acclimatization stage, or after the acclimatization, before the beginning of the hardening phase under greenhouse conditions. Several studies have been published describing experiments of this kind (see Vestberg and Estaún, 1994). The micropropagated crop plants include grapevines (Ravolanirina et al., 1989b; Schubert et al., 1990; Schellenbaum et al., 1991), oil palm (Blal et al., 1990), raspberries (Varma and Schüepp, 1994); banana (Jaizme-Vega et al., 1991; Jaizme-Vega and Azcón, 1995), pineapple (Jaizme-Vega and Azcón, 1991, 1995; Guillemin et al., 1992, 1994; Varma and Schüepp, 1994), strawberry (Niemi and Vestberg, 1992; Williams et al., 1992; Vestberg et al., 1994), pear and peach clonal rootstocks (Rapparini et al., 1994), *Pistacia* sp. (Schubert and Martinelli, 1988), kiwifruit (Schubert et al., 1992), apple, peach and plum rootstocks (Sbrana et al., 1994), *Prunus* rootstocks (Estaún et al., 1994; Berta et al., 1995), avocado (Vidal et al., 1992; Azcón-Aguilar et al., 1992), cherimoya (Azcón-Aguilar et al., 1994), quince rootstocks (Calvet et al., 1995), apple microcuttings (Branzanti et al., 1992; Uosukainen and Vestberg, 1994); hortensia (Varma and Schüepp, 1994), etc.

In comparing the time of inoculation, either at the onset of the acclimatization stage or at the beginning of the hardening phase, it was found in some cases that, although the symbiosis could be established at both stages, a better plant response was produced when inoculation was carried out at the beginning of the hardening phase (Vidal et al., 1992; Azcón-Aguilar et al., 1992). In this context, a key aspect is that mycorrhizal

colonization is known to take place only on young secondary roots, before suberization (Barea, 1991). Since these fine young roots are sometimes produced later during the ex vitro development, inoculation would be meaningless during the early acclimatization stages, but advisable when transferred to open pots. This is probably the case above mentioned for avocado and cherimoya.

Inoculation ex vitro is obviously easier than in vitro. Therefore, it is probably the most appropriate method for commercial nurseries. Differences in root growth and in the development rate of the target plants are important for the choice of the inoculation time. At an early ex vitro stage, plantlets still possess a certain degree of heterotrophy, and the effectiveness of AM inoculation is dependent on the development of autotrophy. Thus, and as stated by Vestberg and Estaún (1994), an inoculation protocol should be designed for each case. For such protocol the following variables might be considered: (i) root development rate; (ii) length of the weaning and hardening periods (hetero- to autotrophy change) and (iii) objectives of the AMF inoculation (enhanced growth, increased survival, lower fertilization inputs, increased resistance to biotic and abiotic stress).

The selection of the right fungal partner is essential for an efficient AM development in micropropagated plants (Guillemin et al., 1992; Vestberg, 1992; Lovato et al., 1995). The influence of the fungal strain on symbiosis efficiency also varies with the growing media and with the fertilization regime. In this context, the substrates for the micropropagated plants must ensure that both the development of the symbiosis and plant performance must be improved. The substrates commonly used are peat, perlite and vermiculite (Vestberg and Estaún, 1994). Calvet et al. (1992a), Calvet et al., 1992b) found that certain types of peat and composted substrates had a negative effect on the establishment of the AM symbiosis, although neither spore germination nor the early mycelial growth were affected. Furthermore, potting media may include sterilized soil, a substrate component that usually improves AM formation in micropropagated plants (Gianinazzi et al., 1990a). Vidal et al. (1992) recorded that the symbiosis could be established in peat–sand mixes although soil–sand mixes were more conductive to AM colonization of micropropagated avocado plants. Schubert et al. (1990) also found good AM establishment in micropropagated grapevine growing in a peat-based medium.

The controlled-release fertilizers, such as Osmocote, are of special interest for the mycorrhization of micropropagated plants because the AMF seem to cooperate well with this type of controlled P-supplying fertilizers (Williams et al., 1992).

4.4. Management of mycorrhizosphere interactions in horticulture

As stated in Section 4.2, and recorded in Table 1, certain rhizosphere/mycorrhizosphere microorganisms are able to interact with AMF to develop biological activities of great relevance to plant growth and health. Examples of microorganisms, which have been described to interact with AMF, when introduced as mixed inoculants, to benefit horticultural crops, are the following: (i) *Rhizobium* spp.; (ii) Plant growth promoting rhizobacteria and (iii) Biocontrol agents.

As regards *Rhizobium*, there is much information to support the beneficial effects of its co-inoculation with selected AMF on leguminous horticultural crops (Barea et al., 1992) and no further comments will be made here.

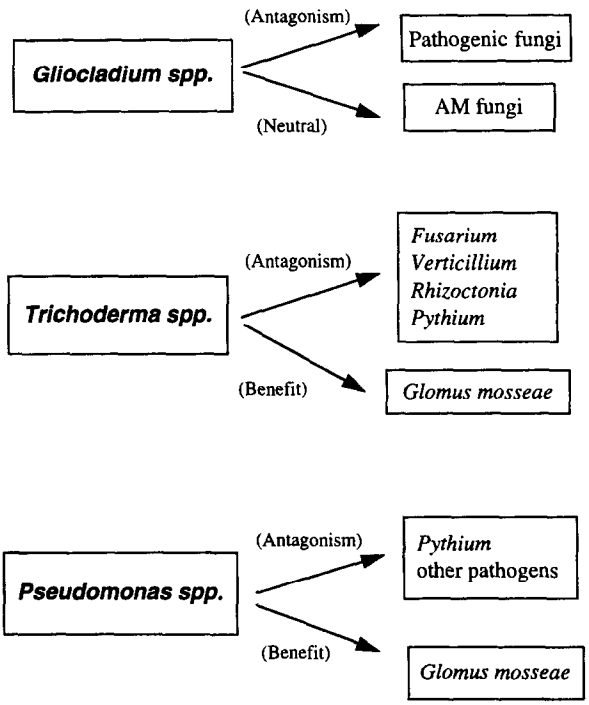
The attribute 'rhizobacteria' refers to the ability of some soil bacteria to colonize 'aggressively' the root–soil interface, where they establish and maintain, for a time, a considerable number of cells. These bacteria, which can promote plant growth by means of several mechanisms, are now commonly designated by the acronym PGPR (Kloepper et al., 1991; Linderman, 1992; Glick, 1995). The growth promoting activity of PGPR is related to effects on seedling emergence, root formation, nutrient cycling and nodulation promotion (Kloepper et al., 1991). However, one of the most remarkable effects of PGPR on plant growth derives from their ability as biological control agents for plant pathogens (Kloepper, 1992). Commercial formulations based on PGPR have already entered the market (Schippers et al., 1995).

There is experimental evidence that some PGPR, mainly *Pseudomonas* spp. and *Bacillus* spp., interact with AM fungi developing synergistic activities which lead to improved plant growth and nutrition. Besides, both groups of microorganisms (the saprophytes PGPR and the symbionts AMF) are able to interact with each other, improving their establishment in the rhizosphere (Azcón-Aguilar and Barea, 1992; Linderman, 1992).

The prophylactic ability displayed by some AM fungi could be exploited in cooperation with other rhizosphere microorganisms which have demonstrated antagonistic abilities against pathogens, and that are being used as biological control agents (Linderman, 1994). Among the microorganisms catalogued as antagonists to fungal pathogens, there are both fungi, such as *Trichoderma* spp. and *Gliocadium* spp., and bacteria, which are actually PGPR, like *Pseudomonas* spp. and *Bacillus* spp. (Kloepper et al., 1991; Linderman, 1994). It has been shown that microbial antagonists towards fungal pathogens do not necessarily have the same detrimental effect against AM fungi. Some examples are available concerning horticultural plants: *Glomus* + *Trichoderma* + wilt pathogenic fungi (Calvet et al., 1990); *Glomus* + *Trichoderma* + *Fusarium*, in tomato (Datnoff et al., 1995); *Glomus* + *Trichoderma* + *Pythium*, in marigold (Calvet et al., 1993). The information related to the interactions between biological control agents and soil fungi, either pathogenic or mycorrhizal, is summarized in Scheme 2. It is noteworthy that microbial antagonists of fungal pathogens can improve AM formation (Calvet et al., 1992a,b; Linderman, 1994; Vidal et al., 1996). It can, therefore, be concluded that the accurate management of these interactions, by tailoring appropriate mycorrhizosphere systems relevant to plant growth and health in an integrated approach, is among the main objectives of a sustainable horticulture (Azcón-Aguilar and Barea, 1992; Linderman, 1992; Barea and Jeffries, 1995).

4.5. Potential for the use of mycorrhizas in commercial horticultural production

As stated before, horticulture is one of the target systems in which AM biotechnology can express its potential. This is due to the characteristics of the components and the plant production strategies in horticultural practices, which make it necessary and easy to inoculate plants with AMF. Considering the high production costs of horticultural and ornamental crops, it seems that the application of AM inocula may represent only a small contribution to the input costs, particularly if AM management is associated with other biotechnologies such as micropropagation. Thus, the production of orchard and



Scheme 2. Differential effects of biological control agents on mycorrhizal or pathogenic fungi.

ornamental crops is probably the area in which the application of AMF as bioprotectors, biostimulators or biofetilizers will most readily become a reality (Lovato et al., 1995).

It has become clear that AMF can be introduced within commercial propagation systems to reduce chemical inputs. To this end, two kinds of requirements must be met. Firstly, there needs to be a readily available supply of inoculants and, secondly, research is needed to assure more predictable and consistent responses of plantlets (Hooker et al., 1994a). Commercial inoculants are now available (Mulongoy et al., 1992; Sylvia and Jarstfer, 1994), and the technical feasibility to apply these commercial AMF inocula for horticultural crops has been demonstrated (Hooker et al., 1994a; Lovato et al., 1995). However, a more in-depth evaluation of the available commercial inocula is clearly necessary. It is likely that inoculation with AMF will become an integral part of most horticultural systems in the future. Their application will result in savings in time, energy and chemical inputs, and will make horticultural production systems not only more economically competitive, but also more sustainable (Hooker et al., 1994a).

Micropropagation, which is an effective tool for multiplying any kind of horticultural plant, is now recognized as a viable technique for commercial plant production (Varma and Schüepp, 1995). In Europe alone, 175×10^6 plants were produced by micropropagation during 1990 (O'Riordain, 1992). In some cases, these plants have problems at critical stages of the micropropagation process. Such problems could be overcome by AMF inoculation as has been demonstrated in several experiments (Varma and Schüepp,

1994; Vestberg and Estaún, 1994). However, there is still a gap in the research and development framework in this area which concerns technology transfer. Undoubtly, as soon as the potential of AM inoculation has been clearly demonstrated to the growers, commercial nurseries will have to include the AMF inoculation as a standard procedure in their production protocols (Vestberg and Estaún, 1994).

5. Conclusions

Arbuscular mycorrhizal symbiosis must be considered an essential factor for promoting plant health and productivity. AM biotechnology is feasible and rewarding mainly for crops which involve a transplant stage, as in horticultural systems where plants are produced in nursery beds, containers or by tissue culture. Maximum benefits will only be obtained from inoculation with efficient AMF and a careful selection of compatible host/fungus/substrate combinations. In general, the earlier the mycorrhizal symbiosis is established at the appropriate stage of seedling production the greater the benefit. The performance of micropropagated plants or artificial seeds may be greatly enhanced by ensuring a suitable mycorrhizal establishment at outplanting. In particular, woody plants which are difficult to root have been shown to improve their survival rate when inoculated with AMF. The interactions between AMF and rhizobacteria could be a biotechnological tool essential for benefiting plant growth and health in horticultural practices. All in all, it is important to improve inoculum production techniques for the proper application of AM biotechnology in commercial horticultural plant production systems.

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