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Progress and Headaches in Endomycorrhiza Biotechnology

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Abstract

Endomycorrhiza biotechnology is still very much in its infancy because of the paucity, until very recent years, of sufficient knowledge about how mycorrhizae develop and function, and consequently a justified reluctance for widescale applications. Although commercially viable techniques for bulk inoculum production and widescale endomycorrhizal inoculation of field-grown crop plants are not yet available, the use of endomycorrhizal fungi to improve plant productivity in certain important agricultural and horticultural areas, including orchard crops and container ornamentals, is already feasible. The culture of endomycorrhizal fungi on synthetic media, will lead to more widescale applications, with the possibility of genetic manipulation and selection of improved strains.

Keywords: endomycorrhiza, biotechnology, inoculum production

Abbreviations: VA, vesicular-arbuscular

Introduction

Endomycorrhizae are the most widespread root symbiosis and are formed by the large majority of plant species. The presence of endomycorrhizal fungi within roots can greatly improve plant growth (Fig. 1) and stress resistance by their beneficial effect on plant mineral nutrition (see Gianinazzi et al., 1982; Harley and Smith, 1983; Gianinazzi-Pearson and Gianinazzi, 1986).

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Figure 1. Inoculation trials under field conditions.
(A. Trouvelot, S. Gianinazzi and V. Gianinazzi-Pearson, unpublished data)
(a) The influence of soil disinfection and controlled VA endomycorrhizal inoculation on the growth of five different plant species. ND, non disinfected plots; D, disinfected plots; M1, disinfected plots inoculated with *Glomus mosseae*; M2, disinfected plots inoculated with *G. intraradices*. Endomycorrhiza formation is essential for good growth of asparagus (2), ash (3), catalpa (4) and leek (5) while sugar beet (1), a non-mycorrhizal plant, shows no growth response to inoculation. Ash plants infected by indigenous mycorrhizal fungi in nondisinfected soil (6) grow less well than those inoculated with selected fungi in disinfected plots (3).
(b) Asparagus production in disinfected soil with (M) and without (NM) inoculation of *G. monosporum*. (see opposite page)

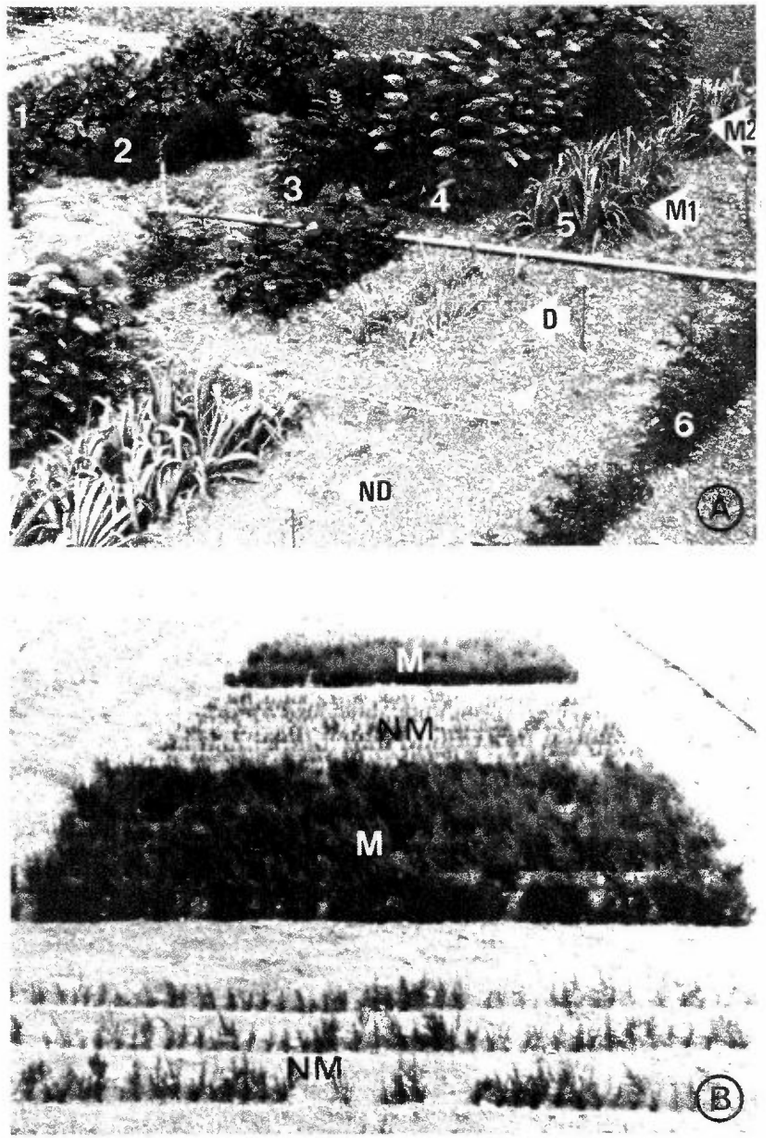

Figure 1

Their use in cultivated crops could therefore be an important factor in increasing or ensuring good productivity, even under adverse conditions, as well as enabling a more rational use of fertilizers (Menge, 1983; Gianinazzi-Pearson, 1986). In spite of this, mycorrhiza biotechnology is still very much in its infancy, probably owing to the lack until very recent years, of sufficient knowledge about how endomycorrhizae develop and function and to the fact that the fungi forming the more economically interesting type of endomycorrhiza, the vesicular-arbuscular (VA) mycorrhiza, cannot be grown in pure culture. However, for certain crops (citrus, *Vaccinium myrtillus* or bell-pepper, for example) the use of endomycorrhizal fungi is already commercially and economically feasible (Menge et al., 1977; Powell, 1981; Johnson and Menge, 1982; Haas et al., 1986).

Types of Endomycorrhizae

Three distinct types of endomycorrhiza are formed by various plant and fungal taxa: ericoid, orchid and VA endomycorrhizae.

Ericoid endomycorrhizae are found exclusively in genera of the Ericaceae, like *Calluna* sp., *Vaccinium* sp., *Erica* sp., *Rhododendron* sp. and *Azalea* sp. The fungi involved are Ascomycetes, and for the moment only one species, *Hymenoscyphus (Pezizella) ericae* (Read) Korf and Kernan, has been taxonomically defined (Read, 1974; Kernan and Finocchio, 1983).

Orchid endomycorrhizae are also limited to one plant family, the Orchidaceae, but the mycorrhizal fungi come from several genera in the Basidiomycetes, the most common of which are *Tulasnella, Thanatephorus, Marasmius, Armillaria, Sebacina* and *Ceratobasidium.* Orchid mycorrhizal fungi can also be either normal soil saprophytes or parasites on other plants; *Thanatephorus cucumeris* (Syn. *Rhizoctonia solani*) for example, has received considerable attention as a root pathogen (see Harley and Smith, 1983).

VA endomycorrhizae are not restricted to any one plant family. It is estimated that this type of endomycorrhiza is formed by about 80% of plant species and it concerns most agricultural and horticultural crops, fruit trees and a large number of forest tree species. VA endomycorrhizae are formed by fungi from a fairly limited taxonomic range; these are restricted to four genera (*Glomus, Gigaspora, Acaulospora, Sclerocystis*) belonging to one family (Endogonaceae) in the Zygomycetes (Trappe, 1982).

Isolation, Culture and Storage of Endomycorrhizal Fungi

The fungi responsible for endomycorrhizae of ericaceous and orchidaceous species can be easily isolated from surface sterilized fine roots of wild plants

and grown on a sterilized culture media. The fungal isolates can be obtained by subculturing the mycelium growing out of the root and then maintained on a simple agar media (Pearson, 1970; Pearson and Read, 1973; Hadley and Ong, 1978).

One of the major problems with VA endomycorrhizal fungi is that attempts to grow them in pure culture have so far failed and fungal collections have to be maintained on living host plants under non sterile conditions. These fungi, however, form characteristic resting spores or sporocarps (Trappe, 1982; Hall, 1984) that can be collected by passing a suspension of soil through a series of graded sieves. Individual clean spores can then be isolated under a binocular microscope or in a density gradient, identified, surface sterilized and used as inoculum to establish pure starter cultures on young host plants (e.g. onion or clover) growing in sterilized soil or rooting media (see Schenck, 1982). Since the fungal species forming VA endomycorrhizae can have very different ecological preferences, it is advisable to start cultures in the soil from which they have been isolated or in a soil or rooting medium having very similar characteristics. Furthermore, although fertilizer needs for starter cultures will depend on the soil type and host plant requirements, it is preferable to supply phosphate at relatively low levels, as high concentrations of this nutrient can inhibit the development of the fungal infection within host roots (see Gianinazzi-Pearson, 1986). Slow release fertilizers, for example, are apparently not inhibitory at normal fertilizer rates (Kormanik et al., 1977; Maronek et al., 1981). Collections of different fungal isolates can be established with such starter cultures and maintained by successively inoculating young host plants with infected roots or spores. Mycorrhizal plants of culture collections must evidently be grown under controlled conditions, in order to avoid contamination by pathogens, and isolate purity should be checked regularly. Although fungi forming VA endomycorrhiza cannot be grown in pure culture, surface sterilized spores can be used to establish *in vitro* infections in whole plants or excised roots growing on synthetic media (Pearson and Tinker, 1975; Pons et al., 1983; Hepper, 1984). However, this has been done with very few fungal species and there is still need for further research in order to develop a method for long-term maintenance of VA endomycorrhizal fungi in axenic culture. Such a method would greatly facilitate the maintenance of large fungal collections and make controlled, high-quality, pathogen-free inoculum always available for starter cultures in large-scale production.

Selection and Commercial Production of Endomycorrhizal Fungi

Production of endomycorrhizal inoculum for commercial use requires practically viable techniques that will ensure a high inoculation potential at economically acceptable costs, and assumes the selection of efficient fungal strains adapted to different agricultural situations.

Little is known of the variability that exists between orchidaceous endomycorrhizal fungi in their effects on plant growth, but it has been observed that fungal isolates forming endomycorrhizae with ericaceous plants can vary in their ability to infect and stimulate growth of host plants (Gianinazzi-Pearson, unpublished data). Furthermore, since these fungi can be easily cultured on both solid and liquid medium, production of large quantities of inoculum should pose no real technical problems. In spite of this, there has been no program for the selection or commercial production of inoculum for ericaceous species, even though commercial micropropagation techniques are being developed for such plants.

VA endomycorrhizal fungi have received more attention because of their potential economic impact. Isolates of VA fungi showing different abilities to stimulate plant growth are available in several laboratories around the world and an international endomycorrhizal collection is being set up at Florida University (U.S.A.) by N.C. Schenk. As isolates can be characterized for their ecological requirements vis-à-vis certain soil factors like soil phosphate levels (see Table 1) or pH, it is now possible to select appropriate fungal inocula to ensure the establishment of an effective mycorrhizal association under given edaphic conditions.

VA endomycorrhizal inoculum, however, is not yet produced on a real commercial scale although certain research institutions and nurseries produce sufficient amounts for local use. Large scale multiplication of selected fungi has been achieved by inoculating appropriate host plants like clover, ray grass, sudan grass, strawberry, lettuce, etc. growing in sterilized soil or a variety of rooting media containing vermiculite, sawdust, bark, perlite, pumice, peat, sand, gravel, expanded clay or a mixture of these (see for example Gianinazzi, 1982; Menge, 1984). Spores, hyphae, infected roots and infested soil or rooting medium obtained from this type of culture can, separately or combined, constitute a source of crude inoculum.

Although attempts have been made to produce pure inoculum by concentrating spore suspensions or infected roots, this risks being costly on a large scale and currently VA mycorrhizal inoculum can only be produced economically and in large quantities in a bulk form consisting of a mixture

Table 1. Screening of different VAM fungi for their ability to infect and improve plant yield (10-week old leeks) in a field soil with high available phosphate (155 ppm Olsen P.).

	Yield (g/plant)	Infection (% length mycorrhizal root)
Disinfected soil	64.3	0
Nondisinfected soil (indigenous fungi)	98.9**	18.8
Disinfected soil + *Glomus*:		
– *monosporum*	132.2**	38.9
– *intraradices*	128.9**	30.5
– *epigaeum* (1)	89.3	6.9
– *epigaeum* (2)	75.0	0.2
– *mosseae*	131.9**	32.9
– *fasciculatum*	105.0**	69.7
– *caledonium*	79.7	31.7
– E_3	84.4	29.8
– spp. 1	76.5	3.3
– spp. 2	110.2**	41.8
Gigaspora spp.	64.9	0.2

**Different from disinfected soil at $P \leq 0.01$.

of inoculum sources. Such inoculum can be of high quality and, for certain rooting media, also light and easy to transport, but the bulk of inoculum required for the introduction of selected VA fungi into many agricultural situations remains an important limiting factor to their widescale inoculation. This underlines once more the necessity for much more research directed towards obtaining axenic cultures of VA mycorhizal fungi in order to be able to economically produce large amounts of high quality inoculum that is easy to transport, store and introduce into usual agricultural practices.

Inoculation, Inoculum Survival and Competition

If inoculation is to be successful, it should guarantee survival of fungi once introduced into the rooting medium and ensure that mycorrhizal infection by the introduced inoculum is rapidly established early in plant development.

For nursery produced ericaceous plants and orchids, this can be achieved by mixing suspensions from mycelial cultures into the appropriate growing medium (Powell, 1981; Berjaud and Minier, 1982) and where these plants are micropropagated, it is possible to envisage inoculation directly *in vitro* (Pons et al., 1982), so facilitating subsequent plant manipulation.

The situation is more complex for plants forming VA endomycorrhizae and it is necessary to distinguish between plants that are grown in disinfected field or nursery soils, or rooting media devoid of mycorrhiza fungi, from those that are produced in untreated soils or soil mixtures and containing an indigenous mycorrhizal population. It is already possible to realistically envisage controlled inoculation of endomycorrhizal fungi under nursery conditions because of the frequent lack of indigenous fungi and the relatively limited quantity of inoculum required. Crude inoculum is already being successfully used, for example, in nurseries for the production of mycorrhiza dependent plants like citrus and bell-pepper that are outplanted into disinfected field soils (Menge et al., 1977; Menge, 1983; Haas et al., 1986).

An increasing number of nursery plants forming endomycorrhiza are being produced by micropropagation; micropropagated plantlets can be mycorrhizally infected *in vitro* (Pons et al., 1983; Gianinazzi et al., 1986) and, if developed, this technique could provide a convenient method for producing high quality, mycorrhizal plants (Morandi et al., 1979). Most endomycorrhizal crops, however, are produced in non disinfected field soils which poses not only the problem of producing sufficient quantities of inoculum, but also that of successful competition and effectiveness of introduced selected fungi vis-à-vis indigenous mycorrhizal populations. Techniques are now available for evaluating the infectivity and effectiveness of mycorrhizal soil populations in order to assess whether inoculation will be beneficial (Gianinazzi-Pearson et al., 1985), but virtually nothing is known about the competitive abilities of VA mycorrhizal fungi (Abbott et al., 1983). However, since ineffective indigenous populations of VA fungi can exist in agricultural soils (Dodd et al., 1983; Gianinazzi-Pearson et al., 1985), the possibility of improving or ensuring yields by field inoculations of mycorrhiza-dependent high value crops deserves particular attention.

Conclusion

Developments in endomycorrhizal biotechnology are recent but important advances are now being made towards applications in plant production. Nevertheless, a number of difficulties still have to be overcome before commercially viable techniques will be available for widescale inoculation of endomycorrhizal fungi, especially for field-grown crops. Research efforts need to be particularly directed towards:

- developing economically and practically feasible methods for massive inoculum production and inoculation,

- the selection of fungal inocula adapted to specific edaphic conditions, highly effective for plant growth and able to compete with eventually less efficient indigenous mycorrhizal populations.

The results obtained with citrus in the U.S.A. (Menge et al., 1977; Menge, 1983) and bell-pepper in Israel (Haas et al., 1986) should, however, encourage practitioners to consider endomycorrhizal inoculation as a potential alternative for improving plant production especially in disinfected soils and inert rooting media. Furthermore, research into growing VA fungi on synthetic media, together with a better understanding of how they function at the molecular level, will open the possibility of genetic manipualtion to produce improved strains.

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