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Host genotype and the formation and function of VA mycorrhizae[1]

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Abstract

VA mycorrhizae, the most ancient type of mycorrhizal symbiosis, are present in the most phytogenetically advanced groups. Few plants have evolved mechanisms to completely prevent infection by VAM fungi. Yet, plant species that are less dependent on VA mycorrhizae for nutrient acquisition (e.g., grasses) generally have less root colonization in the field than more dependent species (e.g., *Citrus*). Among closely related Citrus genotypes, there is a greater tendency for less dependent species to limit the rate but not the extent of colonization, even in high-P soils. We hypothesize that colonization represents a significant carbon cost that may be regulated by the host genotype. Carbon expenditure on the fungus at high P may result in mycorrhizal-induced growth depression. The potential value of breeding plants for greater susceptibility to colonization will depend on the cost/benefit of VA mycorrhizae for the specific crop, soil and environmental conditions. Although the genetics and physiology of host control over VAM colonization are barely known, recently discovered mycorrhizal colonization mutants (myc^-) of pea offer great promise for the study of host–fungus compatibility.

Introduction

VA mycorrhizae are the most common of plant–fungal infections. Perhaps as much as 80% of all plant species form VA mycorrhizae with a comparatively small number of zygomycetous species in the order Glomales (Gianinazzi, 1991). The early origin of the zygomycetous symbiosis is supported by the fossil record and patterns of mycorrhizal habit in present-day plants (Trappe, 1987). The VA mycorrhizal association is ancient, yet has been maintained in even the most advanced plants. Complete resistance to infection by mycorrhizal fungi has evolved in few plant orders. However, phytogenetically advanced groups with fine roots that are less dependent on VA mycorrhizae for nutrient acquisition (e.g., grasses) generally have lower levels of root colonization in the field (Crush, 1973; Sparling and Tinker, 1978) than mycorrhizal dependent plants with coarse roots (e.g., *Citrus*) (Graham et al., 1991).

Given the lack of host specificity of VA mycorrhizal fungi, the genes for host–fungus compatibility would presumably be homologous among plant and fungus genotypes. The genes involve plant/fungus recognition, fungal infection/plant reaction, and the ultimate morphology and function of the mycorrhiza in carbon/nutrient exchange. The genetics of host–fungus compatibility are just now coming under study (Duc et al., 1989; Gianinazzi, 1991); thus, the precise physiological mechanisms underlying the different processes leading to mycorrhizal function are barely understood.

The purpose of this review is to appraise the costs of mycorrhizal colonization and the potential for crop benefit from plant breeding for

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increased fungal colonization in agricultural situations. The prospects for study of the genetics of mycorrhizal formation and function will also be briefly discussed.

Functional relationship between P uptake and carbon expenditure

VA mycorrhizal colonization is most notably affected by P nutrition of the host (Stribley et al., 1980). Plants growing in low-P environments and deficient in tissue P are more readily colonized by mycorrhizal fungi than plants of high-P status. Host carbon must cross the plasma membrane at the host–fungus interface before becoming available to the fungus. Functionally speaking, phospholipid content of root cells regulates the symbiosis via membrane leakage of carbon to support the activities of the fungus (Graham et al., 1981). However, passive leakage alone may not completely explain the means of regulation since even when membrane permeability is reduced at high root-P content, sufficient carbon for fungal growth in the roots is available for colonization in high-P environments although at much lower rates (Schwab et al., 1991).

In low-P soils, rate of colonization is often correlated with growth responses (Abbott and Robson, 1984). However, the increase in leaf area and carbon assimilation may feed back carbon to the fungus even though tissue P is increased and membrane permeability reduced. In plant species with a greater growth response, more carbon will feed back from the shoot to the fungus than in less responsive plant species. Thus, a correlation between mycorrhizal dependency (VAM plant dry weight / non-mycorrhizal plant dry weight) and colonization at low P may represent a phenotypic response of the fungus to greater carbon availability. The correlation does not reveal the inherent ability of a host genotype to regulate carbon expenditure for the symbiosis (Graham et al., 1991).

Host genotype relationship

In addition to various environmental factors influencing colonization, host genotype is another important factor controlling the rate and extent of VAM formation. In high-P soil, colonization varies substantially among plant families, genera and even among closely related genotypes of the same species (Graham et al., 1991; Krishna et al., 1985; Manske, 1989; Toth et al., 1990). Where there is no growth benefit in high-P soils, colonization of plants cannot be promoted by carbon feedback. We hypothesize (Graham et al., 1991) that plants may be more fit (i.e., able to produce offspring) if the rate of carbon expenditure on the mycorrhizal fungus is limited during periods of little or no P benefit. When young roots of citrus genotypes were examined in an orchard high in P fertility (> 160 µg/g Mehlich I extractable), species of low mycorrhizal dependency had lower rates of VAM colonization than those of high mycorrhizal dependency (Graham et al., 1991). The rate rather than the extent of colonization tended to be limited in these genotypes. Presumably, the plant's expenditure of forming mycorrhizae was prolonged so that carbon could be expended for other competing needs that were more likely to provide a more immediate return on the investment, such as leaf area for carbon assimilation. Among citrus genotypes, this observation suggests that rate of colonization can represent a significant carbon cost and that there has been some evolutionary selection for this trait. The cost vs benefit of the host plant by restricting carbon expenditure on mycorrhizal formation would be a function of the amount of carbon saved and the likelihood that heavily colonized roots would be needed for P uptake in the near future. During their evolutionary history, plant genotypes that are highly mycorrhizal dependent would not exhibit reduced rates of colonization because they rarely grew in environments where they were not benefited by mycorrhizae.

In order for plants to be under selection pressure to limit the rate of carbon expenditure for mycorrhizal fungal growth during periods of no P benefit (i.e., high P fertility), carbon expenditure must be significant. In citrus species, which have substantial colonization even in high-P soil, growth is depressed by the mycorrhizal fungus. For instance, incidence of

colonization of Volkamer lemon by *Glomus intraradices* Schenck and Smith is about 60% at 52 days after inoculation in high-P soil (Peng et al., 1993). This level of colonization is associated with a 37% higher rate of root plus soil respiration and a 10 to 20% lower specific daily carbon gain compared to non-mycorrhizal plants. Differences in soil/root respiration are linked to 19% higher root dry weight and 10% higher root growth rate. There is also a two-fold increase in total-fatty acid content of roots due to the presence of 16:0 and 16:1 fatty acids in the intraradical vesicles of *G. intraradices*. Quantities of fungal lipids in citrus roots are correlated with root construction cost, i.e., the amount of simple carbohydrate (e.g., glucose) required to build all the organic molecules present in the root (Williams et al., 1987). The cost of mycorrhizal formation at high P includes a 50% greater root allocation in mycorrhizal plants, 11% expenditure for building of lipid rich roots and the remaining 39% for maintenance of fungal tissue in the root and growth and maintenance of extramatrical hyphae. Therefore, the growth depression of plants at high P is attributed to carbon expenditure on construction of mycorrhizal roots and their maintenance (Peng et al., 1993). Similar mycorrhizal-induced growth reductions in high-P soils have been demonstrated for ryegrass, ferns, forage legumes, apple, tobacco and onions (Buwalda and Goh, 1982; Cooper, 1975; Crush, 1976; Miller et al., 1985; Modjo and Hendrix, 1986; Mosse, 1973). Thus, the potential among plant species for mycorrhizal fungi to cause growth depressions is quite widespread.

Evaluation of carbon expenditure by citrus genotypes

We sought to link mycorrhizal dependency at low P with the tendency of the plant genotype to limit colonization and, therefore, carbon expenditure at high P.

To evaluate genotypic variation in carbon expenditure associated with mycorrhizal dependency, five citrus genotypes (Fig. 1) were grown at low- and high-P supply with and without *G. intraradices*. In the first experiment,

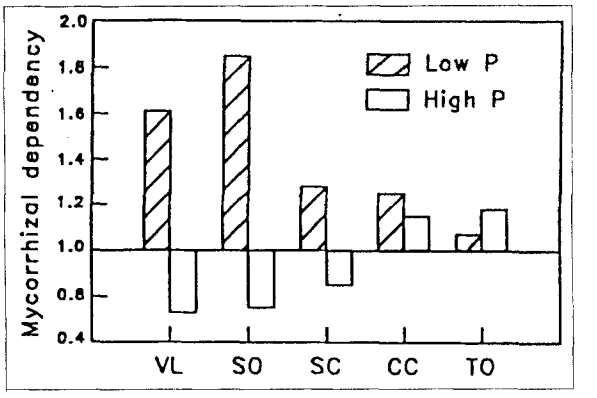

Fig. 1. Mycorrhizal dependency (ratio of VAM plant dry wt and nonmycorrhizal plant dry wt) for seedlings of five citrus genotypes grown at low- and high-P supply. VL = Volkamer (*Citrus volkameriana* Tan. and Pasq.), SO = Sour orange (*C. aurantium* L.), TO = trifoliate orange (*Poncirus trifoliata* (L.) Raf.), SC = Swingle citrumelo (*C. paradisi* Macf. X *P. trifoliata*), and CC = Carrizo citrange (*C. sinensis* (L.) Osb. X *P. trifoliata*).

total biomass was assessed after 155 days of growth in 125 cm^3 containers and mycorrhizal dependency of each cultivar expressed as the ratio of total dry weight of the mycorrhizal plant to that of the non-mycorrhizal plant (n = 5). Thus, values of less than one at high-P supply indicate growth depression. In the second experiment, the five genotypes were grown in 1,100 cm^3 pots for 125 days. At harvest, colonization was assessed as incidence of vesicles, arbuscules and hyphae in 20 root segments per plant (Graham et al., 1991) and intensity of vesicle formation was rated on a scale of 0–5 (0 = no vesicles present) (n = 5). Dried roots were ground and the fatty-acids extracted with methanol, saponified and derivatized to methyl esters. The Microbial Identification System (MIS) (Sasser, 1990) was used to quantify fatty-acid content of roots which was used as an index of fungal cost of mycorrhizal formation. Concentrations of individual fatty acids were expressed as relative amounts based on their integrated areas of the fatty-acid profile. Root construction costs were determined using the heat of combustion method (Williams et al., 1987).

In the first experiment, growth enhancement by *G. intraradices* was greater for the more

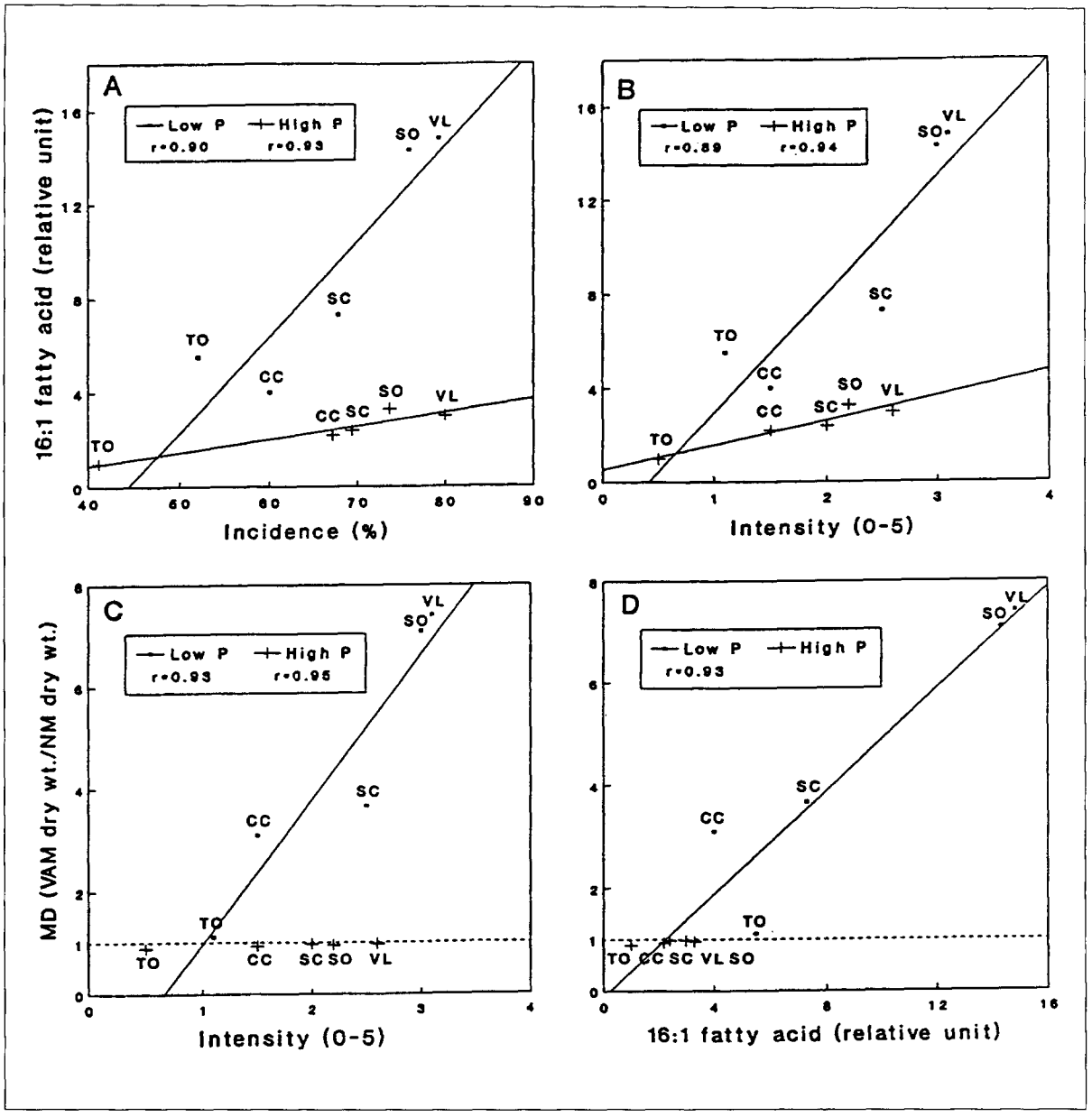

Fig. 2. Relationship among 16:1 fatty-acid content of roots, colonization by *Glomus intraradices* and mycorrhizal dependency of seedlings of five citrus genotypes growing at low- and high-P supply. See Fig. 1 for identification of genotypes. Correlation coefficients are significant at $P \leq 0.05$.

dependent genotypes, Volkamer lemon and sour orange, at low-P soil supply (Fig. 1). At high-P supply, growth of the dependent genotypes by *G. intraradices* was depressed, whereas growth of the least dependent genotypes was increased slightly. Thus, greater mycorrhizal dependency at low P appeared to be linked with the levels of growth depression at high P.

In the second experiment, incidence of colonization and intensity of vesicle formation by *G. intraradices* for the five citrus genotypes were highly correlated with the content of 16:1

fatty acid (Fig. 2a, b). This fatty acid was associated principally with infected roots and was in low quantities in non-mycorrhizal citrus roots. Furthermore, mycorrhizal dependency of the citrus genotypes was correlated with intensity of vesicle development at both low- and high-P supply (Fig. 2c) and was related to 16:1 fatty-acid content at low P (Fig. 2d). At high P, fatty-acid content of mycorrhizal roots did not vary substantially among the five citrus genotypes (Fig. 2d). This small variation in fatty-acid content suggests that there were only small differences in fungal carbon expenditure among citrus genotypes at high P in this experiment. This is consistent with the narrow range of mycorrhizal increases in root construction costs (-4 to 2%; data not shown) and of growth depression (2 to 6%; Fig. 2c) found among the genotypes. Consequently, growth depression at high P was not linked to mycorrhizal dependency at low P as in the first experiment.

In the second experiment, the failure to establish a relationship between mycorrhizal dependency and carbon expenditure on the fungus at high P may have occurred for several reasons. The range in colonization among citrus genotypes at high P was not great enough to detect clear differences in expenditure and a relationship to growth depression. The citrus genotypes may have varied in their root allocation in response to extrinsic factors other than the mycorrhizal fungus. The cost of resisting colonization by the different citrus genotypes may vary independently from the root allocation responses. In this study, plant growth, colonization levels and fatty-acids in roots were only determined at one point in the colonization process. These comparisons need to be made at different stages of root colonization and plant development.

Host genotype interactions and plant breeding

Several researchers have advocated the use of plant breeding to increase host 'susceptibility' to mycorrhizal fungi and, therefore, enhance the efficiency of mycorrhizae (Krishna et al., 1985; Lackie et al., 1988; Manske, 1989; Toth et al., 1984). This assumes that rapid and high levels of colonization are beneficial but ignores the potential costs particularly in high-P agricultural soils. Under P-limiting conditions, greater colonization within a group of plant genotypes has been correlated with increased tissue P concentrations and mycorrhizal dependency (Krishna et al., 1985; Manske, 1989). However, in some cases, a relationship between extent of mycorrhizal root length, P uptake, and greater growth response has not been demonstrated (Azcon and Ocampo, 1981; Crush and Caradus, 1980). A more complex interaction between colonization and plant growth response may exist than one related only to P or carbon economy of the genotype. Toth et al. (1990) recently reported that inbred lines of corn selected for a range of resistance to a variety of fungal leaf pathogens had lower mycorrhizal colonization, slower maturation and larger root systems.

Whether plant breeding programs that select for resistance to fungal pathogens are influencing mycorrhizal dependency of the host may partly depend on whether the relationship between cost and benefit of VA mycorrhizae is affected. If rapidly colonizing indigenous fungi cause growth depressions in high-P soils, as has been reported for field grown tobacco (Modjo and Hendrix, 1986), then selection of traits that reduce the rate or extent of colonization may be beneficial. By contrast, if the plant genotype is dependent on mycorrhizae for nutrient acquisition, then a delay in colonization may have adverse effects on yield. Cost vs benefit cannot be addressed unless the crop genotype, fungal genotype/populations, soil fertility and the environmental conditions are specified. For example, growth depression of wheat cultivars in a high-P soil occurred in a year of low light intensity in the greenhouse but not in a year of high light intensity (Manske, 1989). Likewise, we have demonstrated growth depression of young citrus trees in high-P soil resulting from colonization by indigenous mycorrhizal fungi in one orchard location but not in an adjacent field under similar soil conditions in another year.

The interaction of host and different fungus genotypes may also influence the cost/benefit of enhanced colonization. Fungi, like *G. intraradices*, that depress host growth, may be more opportunistic in their ability to colonize

and consume carbon in plants susceptible to colonization than fungi that are less opportunistic and/or more efficient in their carbon utilization. Johnson et al. (1992) have hypothesized that yield decline with continuous corn and soybean monoculture is in part due to proliferation of less beneficial mycorrhizal fungi. Ultimately, the equation of cost and benefit will depend on the soil nutrient supply since this will have the most direct bearing on dependency of the host under a given cultural condition (Fig. 1). Thus, the merits of breeding for greater mycorrhizal formation will depend largely on the objectives of the crop management program (e.g., level of phosphate fertilizer inputs).

Genetics of host–fungus compatibility

The study of colonization in non-mycorrhizal plant species has provided some insight into the stages at which VAM fungus–host incompatibility may occur, but the basis for the incompatibility remains obscure (Glenn et al., 1988). Recently, chemically induced mutants of pea (*Pisum sativum*) were discovered that are characterized by aborted infections (Duc et al., 1989). Analysis of F1 crosses of the 'myc$^-$' mutants and the wild type indicate the involvement of at least three genes (Gianinazzi, 1991). The myc$^-$ character is constantly associated with the nod$^-$ genotype controlling nodulation by *Rhizobium leguminosarum*. This is intriguing because it suggests that there is a genetic linkage and presumably tight control of the two carbon consuming and potentially competing symbioses. The genetics of myc$^-$ in peas may be unique to legumes. Nevertheless, isogenic lines, except for the myc$^-$, nod$^-$ genes, offer unprecedented possibilities for study of the genes and gene products which control different stages of the VAM colonization process.

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