Arbuscular Mycorrhizal Fungal Colonization. Factors Involved in Host Recognition

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The arbuscular mycorrhizal (AM) symbiosis is the association between fungi of the order Glomales (Zygomycetes) and the roots of terrestrial plants (Harley and Smith, 1983). Conservative estimates suggest that this ancient symbiosis, dating back to the early Devonian age (398 million years ago), affects approximately 90% of the Earth's land plant species (Remy et al., 1994). This symbiosis is increasingly being recognized as an important and integral part of natural ecosystems throughout the world. The AM fungus-plant association is a mutually beneficial event: The plant supplies the fungus with carbon (from its fixed photosynthates) while the fungus assists the plant in its uptake of phosphate and other mineral nutrients from the soil (Smith and Gianinazzi-Pearson, 1988; Smith and Read, 1997). This bidirectional exchange of nutrients takes place through extensively branched haustoria, termed arbuscules. In addition to increased nutrition, mycorrhizal plants also show increased resistance to root pathogens and tolerance to drought stress, and their hormonal balance is altered (Smith and Gianinazzi-Pearson, 1988; Hwang et al., 1992).

A major challenge for the mycorrhizologist is to understand the extremely harmonious AM fungus-host signaling mechanisms and the colonization process. This harmonious symbiotic relationship is reflected in the obligate biotrophic nature of the fungi, which cannot be cultured in the absence of a host (Williams, 1992). The most accepted reason for the obligate biotrophy is that the fungus, during the long evolution of its symbiotic relationship with the host plant, lost some of its carbon-fixing capabilities or the genetic machinery that supports them, and became completely dependent on the host plant for fixed carbon supply. The empirical evidence for this hypothesis is still lacking, but several indirect approaches to the study of this relationship have been developed.

In all of the current methods of cultivating AM fungi, the presence of the host plant is indispensable. Many variants of these methods have been developed, including the classical soil-based system, aeroponic and hydroponic systems, and the recent in vitro root organ culture system. The root organ culture system is the most attractive cultivation methodology for research; it uses root-inducing transfer-DNA-transformed roots of the host plant to develop the symbiosis on a specific medium in vitro (Bècard and Fortin, 1988). These techniques, though challenging, have proven useful in adding to our understanding of various aspects of the AM fungal-host symbiosis (Douds, 1997).

The observation that approximately 150 species of AM fungi (Morton and Bentivenga, 1994) colonize an estimated 225,000 species of plants (Law and Lewis, 1983) has led to the conclusion that AM fungi have wide host ranges. This situation indicates a high degree of adaptability and integration of the symbiotic process across a wide range of plant species (Smith and Read, 1997); but does it mean that the fungi have no preferences among plants? Do all host plants emit signals that indicate their availability for colonization by all AM fungi? The very fact that plants respond to colonization by other soil biota, e.g. by initiating diverse biochemical and physiological changes, but do not do so when "invaded" by AM fungi supports the hypothesis that a specific signal(s) emitted by the AM fungi trigger(s) a cascade of events that culminate in colonization without eliciting any adverse defense reaction from the host.

Here, we discuss the question of host specificity in this unique category of symbiotic interactions and update the reader on the existing evidence for mutual recognition mechanisms between the host and the fungus. Emphasis will be placed on how the host responds to colonization by the AM fungus during the early stages of the interaction and on the basic mechanism of recognition by the host. Current exciting developments in the field have set the stage for revealing the roles played by the factors involved in recognition and colonization of the host plant.

EVIDENCE FOR SIGNALING IN PRE-INFECTION STAGES

The signaling events between host root and AM fungi before and after colonization are not yet fully understood; however, distinct morphological stages for AM fungal development have been defined (Smith and Read, 1997), and can be classified as "host-dependent" and "host-independent." Of the

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www.plantphysiol.org/cgi/doi/10.1104/pp.010783.

host-dependent factors, plant root exudates have been shown to enhance spore germination but are not a prerequisite for this process. Experimental evidence indicates that the quality and source of the exudates play an important role in triggering germination. For example, the exudates from non-host plants such as *Brassica* spp. or *Lupinus* spp. do not stimulate germination (Giovannetti et al., 1993). After germination, the spores must find a host root in their vicinity, to trigger the subsequent colonization stages. Over the years, evidence has accumulated that roots emit a volatile signal that stimulates the directional growth of the AM fungus toward them (Koske, 1982). One of the prime candidates for this volatile signal is CO_2, which can stimulate extensive hyphal growth of some AM fungi in vitro (Bècard and Piché, 1989).

Soluble chemical compounds, primarily plant exudates, elicit a positive growth response from AM fungi, and this effect is increased when combined with high CO_2 (Bècard and Piché, 1989). It has been suggested that CO_2 is an essential carbon source for hyphal growth, and it may be involved in the catabolism of lipids in the growing hypha. Evidence to support this suggestion lies in the observation that carbon from ^{14}C-labeled CO_2 is fixed by *Gigaspora rosea* in vitro, suggesting that an anaploretic pathway(s) in the fungus fixes this carbon source (Bècard and Piché, 1989). Thus, the possibility of a dual role for CO_2 in AM mycorrhizal biology as both a trigger for germination and a carbon source cannot be ruled out. Whether this possibility is relevant to all AM fungi is an interesting question for which evidence needs to be sought.

Other, as-yet-unknown, signal compounds could also exist; these may be analogous to the oligosaccharins that act as signal molecules in both plant-pathogen and legume-*Rhizobium* spp. interactions. Mycorrhizal fungi have been demonstrated to have weak cellulase and endopolygalacturonase activities, and both of these enzymes have the ability to catalyze the release of oligosaccharides or oligosaccharins from the plant cell wall (Fry et al., 1993). The latter could trigger the colonization and spread of the fungus by a cascade of events that are autoregulated and controlled by the host. A detailed discussion of this possibility has been presented in a review by Salzer and Boller (2000).

HOW DOES THE FUNGUS RESPOND TO THE PRESENCE OF A HOST?

Hyphae elongate 20 times more slowly in the absence of host roots than in their presence (Bècard and Piché, 1989). AM fungi respond to host exudates with extensive hyphal growth and branching (Giovannetti et al., 1993, and refs. therein). Despite the high mycelial growth in the presence of the roots, hyphae do not always appear to exhibit "directional growth" toward the roots until they are very close to the host (Mosse and Hepper, 1975). Once contact occurs, branching on the root surface takes place. The directional attraction may not be a general phenomenon, but may be more characteristic of the specific host tested (Vierheilig et al., 1998).

Giovannetti et al. (1993) used the "membrane sandwich" technique to study hyphal branching in the presence of host roots. Development of a densely branched hyphal network was evident on the surface of a membrane placed immediately over the roots of host plants but not over those of non-host plants. Preliminary evidence suggests that a factor(s) that elicits branching of *Glomus mosseae* is a compound of $<$500 D (Giovannetti et al., 1996). Such a factor(s) is exuded from the roots of many host species but not at the same level in all cases. Using a different experimental system, Nagahashi and Douds (2000) showed that, in response to a soluble host factor derived from the roots, the branching pattern of *Gigaspora gigantea* changed from dense to scattered. This phenomenon was concentration dependent and temporal in nature. It is interesting that the factor derived from the non-host was inhibitory. Buee et al. (2000), using the same system, showed that all mycotrophic plants produce a soluble factor(s), which induced hyphal branching in *Gigaspora margarita*. This branching factor was found to be strikingly absent from non-mycotrophic plants, e.g. *Brassica* spp. The challenge now lies in characterizing this signaling compound.

Close observations have revealed that as the main hypha (diameter 20–30 μm) approaches a root, it puts out a characteristic fan-shaped complex of lateral branches (Giovannetti et al., 1993). These fan-like structures were also observed by Karandashov et al. (2000) under in vitro conditions. Prevention of actual contact with the roots by means of the membrane-sandwich technique did not, however, prevent the development of these fan-like structures. It can be concluded that in the presence of the host, specific morphogenesis of the fungus take place, a process that the non-host plant is unable to elicit.

A very recent study supports the suggestion that hyphal growth and branching are controlled by the same or a distinct regulatory signal(s) specific to the pre-infection stages (David-Schwartz et al., 2001). This observation implies that not only can the root exudates stimulate the branching capacity of the fungi but at the same time, inhibitor molecules may also be involved in regulating the symbiotic event. Interestingly and analogously, legume plants produce isoflavonoid compounds that induce *nod* gene expression in some rhizobial cells, but act as antagonists of the same process in others (Vance, 1996). Whether branching inhibitors are exuded from the host or are produced in the rhizosphere following exudation of other substances remains to be demonstrated, but the fact that this phenomenon can be related to the genetic makeup of the host opens new possibilities for the discovery of new complementary

AM fungus-host interactions. Whether the same chemical acts at each checkpoint or whether there are different signals remains to be determined. Above all, it is clear that the host plant can stimulate hyphal growth by means of different categories of signal molecule (diffusible and volatile) at several major checkpoints during fungal colonization. Whether the signal(s) also plays a role in regulating fungal morphogenesis is not yet known.

THE PROGRESS OF FUNGAL COLONIZATION IN THE ROOTS

Recent studies have indicated that topographical or biochemical signals on the root surface may be necessary for appressorium formation. Nagahashi and Douds (1997) showed that appressoria formed on isolated epidermal cell walls derived from carrot roots, but not on isolated cortical or vascular cell walls. Apart from attachment to isolated cell walls, formation of penetration hyphae was not observed, indicating the absence of a physiochemical signal intrinsic to the living epidermal cells. Following the successful recognition events, the formation of appressoria takes place on the root epidermal cells (Fig. 1). Penetration is characterized by localized production of wall-degrading hydrolytic enzymes by the fungus and by the exertion of hydrostatic pressure by the hyphal tip (Bonfante and Perotto, 1995). So far, no plant signal has been implicated in appressorium formation, but there is evidence that a shoot factor(s) may be involved in the inhibition of appressorium formation in *Lupinus* spp. (Gianinazzi-Pearson and Gianinazzi, 1992).

As penetration and colonization of the root tissues proceed, the host responds in a number of ways (see below), which probably vary in different plant-fungus interactions (Smith and Read, 1997). Internal infection of the root involves the formation of intercellular hyphae, coils, and arbuscules. The arbuscules, which branch from the longitudinally spreading hyphae and provide a considerable increase in the area of contact between fungus and cortical cells are likely to be involved in nutrient and carbohydrate transfer (Saito, 2000). In arbuscule-containing cells, the plant nuclei migrate from the periphery of the cell to the center, with the increases in size and condensation of the chromatin. These cytological modifications, associated with alterations in H^+-ATPase and phosphatase localization, clearly indicate a remarkable degree of coordinated development. Nevertheless, arbuscules are short-lived: In most host-fungus interactions, they degenerate within 7 to 12 d. Thus progression of colonization requires ongoing arbuscule formation as the fungus spreads in the host roots.

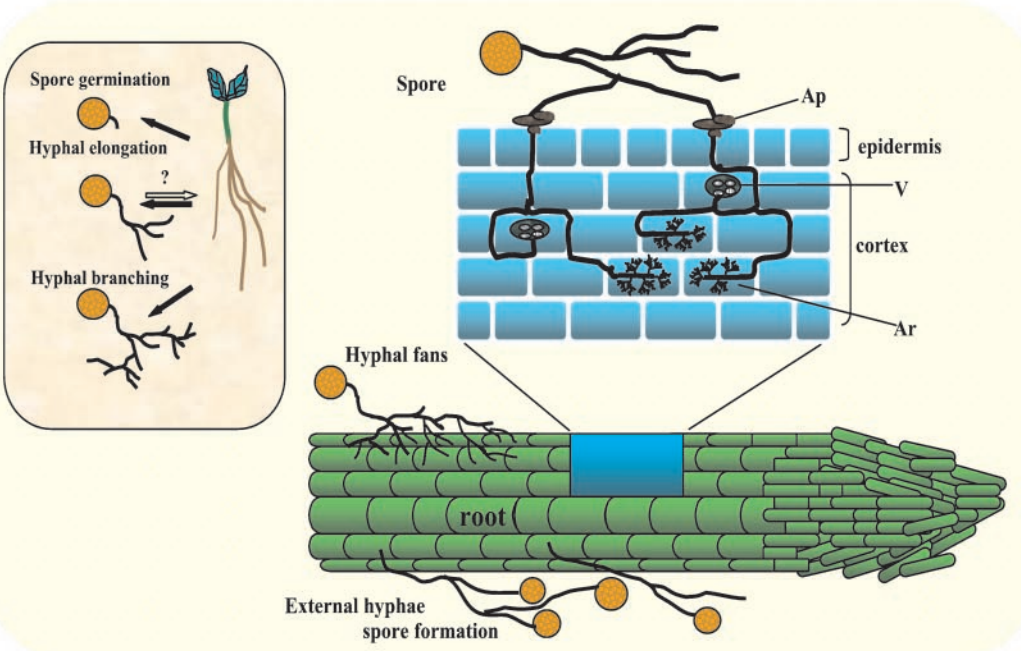
AFTER PENETRATION, DOES THE PLANT RECOGNIZE AM FUNGI?

In plant-pathogen interactions, plants respond to fungal attack by eliciting various mechanisms, some of which are well characterized; the main one among them is the plant defense response. Such resistance responses, i.e. incompatible interactions, occur when plants recognize elicitor compounds in the presence of an invading pathogenic fungus. Biochemical and physiological responses of the plant, such as production of antifungal metabolites, deposition of lignin, production of low-M_r antimicrobial phytoalexins, etc., are triggered to limit the progress of the fungal invasion.

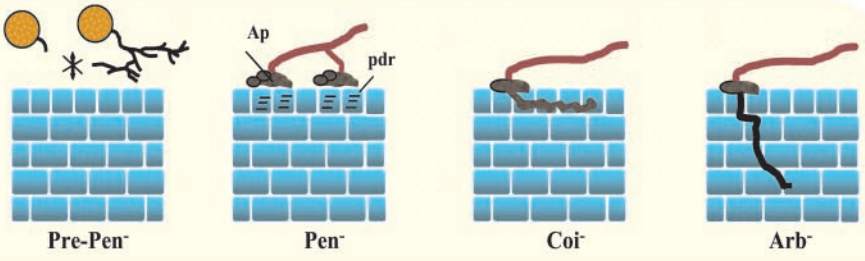
Although there are indications of elicitor involvement in the early stages of mycorrhizal formation, the elicited defense response is generally less vigorous than that observed in plant-pathogen interactions (Salzer and Boller, 2000) and is often completely suppressed (David et al., 1998). In contrast to the plant's interactions with pathogens, plant-mycorrhizal associations are exceptional in being compatible. Infection by AM fungi appears to initiate some plant defense responses in the host tissue, but these do not seem to reach levels that would prevent colonization. Furthermore, expression of defense genes is localized to arbuscule-containing parenchyma cells, and the elicitation of the defense reaction in other cells of the roots is not overcome (Gianinazzi-Pearson, 1996). Whether the plant recognizes the fungus as a mutualistic organism or whether the fungus suppresses plant defense responses remains an open question.

The evidence for the activation of defense-related processes during AM fungus-plant interactions has been considered in detail in a recent review (Gianinazzi-Pearson, 1996). Nevertheless, it is important to note that typical structural defense barriers and cell wall modifications are not found. Pathogenesis-related (PR) proteins, which have antimicrobial properties and are induced when plants are exposed to physical damage or to certain chemicals, have also been extensively studied in various mycorrhizal symbioses. In general, only weak, transient, and in most cases, localized and uncoordinated host defense responses are elicited (Dumas-Gaudot et al., 2000). In addition, Shaul et al. (2000) discussed the possible involvement of a suppression mechanism in the *Glomus intraradices*-tobacco interaction. Finally, Vierheilig et al. (1995) demonstrated that transgenic tobacco plants that over-express some of the PR genes do not restrain AM fungal colonization. These inconsistencies in the expression of PR genes and of protein activities in relation to the AM fungal symbiosis have revealed that PR defense reactions do not necessarily respond to the AM fungal invasion via a typical defense-related pathway (Dumas-Gaudot et al., 2000). It might be concluded that signal reception or recognition of AM fungi by a plant does

A. Compatible interactions between host root and arbuscular mycorrhizal (AM) fungi



B. Plant mutants that are defective in different stages of AM development



L. japonicus B-129 Gifu		***mcbep***	282-287, 282-288, 2557-1, 282-227, EMS-1749, ***mcbex***, ***mcbee***	
M. sativa		MN NN-1008		MN IN-3811
M. truncatula cv. jemalong		C71, B129, Y6, P1, TR25, TR26, TRV25		
M. alba cv. U389		BT35, BT58, BT61, BT69, BT70, BT71		
P. vulgaris cv. OAC Rico				R69
P. sativum cv. Final				RisNod24
cv. Frisson		P1, P2, P3, P4, P6, F4-1, (P53), F4-141, (P55)		
cv. Rondo		K24		
cv. Sparkle		N/A, R72, NMU1, NEU5, R19, E140, R25		
V. faba		Indian 778		
L. esculentum cv. 76R		***rmc***		
cv. Micro-Tom	***pmi***			
Z. mays	***nope1***			

Figure 1. (*Legend appears on opposite page.*)

not elicit a typical plant defense response or, alternatively, that such a response is rapidly suppressed by parallel mechanism(s) induced by the AM fungus.

Secondary metabolism, including the phenylpropanoid pathway, may be involved in signaling host restrictions in plant-fungus interactions. This biochemical pathway induces the production of a number of critical secondary metabolites including lignin, phytoalexins, isoflavonoids, and anthocyanins. Legumes act in response to pathogen invasion by elevating certain enzyme activities, leading to the production of defense-related compounds such as medicarpin in alfalfa, a compound that exhibits antimicrobial activity (Lawton and Lamb, 1987). Medicarpin inhibits *G. intraradices* spore germination (Guenoune et al., 2001), but enzymes for its biosynthesis have been found to be induced in cells containing arbuscules, indicating that its induction may have a regulatory role in AM fungal colonization of the root. Harrison (1999) suggested that recognition of compatibility between the plant and the fungus terminates the elicitation of the plant defense response. The brief mobilization of defense responses may result in the production of suppressors by the mycorrhizal fungi, which prevent recognition of elicitors (Lambias and Mehdy, 1993). To date, no suppressor has been identified, and two alternative hypotheses have recently been presented by Salzer and Boller (2000) and Shaul et al. (2000).

The existence of plants that exhibit defense responses and plants that do not, as well as plants that suppress their defense responses during mycorrhizal formation, suggests the involvement of coevolutionary processes in the development of this symbiosis.

MUTANTS AS A TOOL TO DEFINE CONTROL STEPS IN AM FUNGAL COLONIZATION

The life cycle of AM fungi is a plant-dependent, multiple-step process that involves recognition, signaling, and communication between the host root and the fungus. Spore germination and initial hyphal growth do not necessarily depend on the presence of the host plant (Giovannetti et al., 1993), but all of the subsequent processes require it. The genetically determined events that control communication between the host root and the fungus, thereby enabling a successful symbiosis, remain unknown. Analysis of host plants defective in the mycorrhizal phenotype offers an exciting possibility for obtaining information about the genetic mechanism involved in normal mycorrhizal development and about the key control steps involved. Several groups have published reports describing host mutants that are stage-defective in mycorrhizal symbiosis (Peterson and Guinel, 2000; Marsh and Schultze, 2001). These mutants can be classified according to the defects observed during the developmental stages following fungal infection in the root. These stages of colonization can be broadly classified into 1) Pre-Pen (spore germination, hyphal elongation and branching), 2) Pen, 3) intracellular development and spread in the cortical region, and 4) Arb (Fig. 1A). Although the categories defined in Figure 1B may be appropriate for some of the legume species described to date, they are not to be interpreted as an absolute nomenclature defining all stages thus far reported. The stages presented in Figure 1B are a broad indicator of the categories of mutants currently available. For example, the mutants *mcbex* (mycorrhizal colonization blocked in the cortex) and *mcbee* (mycorrhizal colonization blocked between epidermis and exodermis) in *Lotus japonicus* are blocked somewhere between Pen and Arb stages of development. In these mutants, there is a overproduction of deformed appressoria, inner cortical invasion does not occur, and abnormal arbuscules are occasionally formed (Marsh and Schultze, 2001). The existence of a mutation in the Pre-Pen stage has recently been described in maize and tomato, two non-leguminous plants (David-Schwartz et al., 2001; Paszkowski et al., 2001). The fact that these phenotypes were observed in non-legumes and not so far in legumes stresses the usefulness of exploring the mutation phenomena in other plants, due to their potential for uncovering more control steps in mycorrhizal formation.

The observation that many of the mycorrhizal-defective legume mutants are impaired in nodule formation suggests that there is some overlap between rhizobial and mycorrhizal establishment and function (Hirsch and Kapulnik, 1998). Accordingly, many of the mycorrhizal mutants available today share the common origin of having been isolated

Figure 1. A, The complete life cycle of the AM fungi involving recognition, communication and establishment of symbiosis between the fungi and the host. Ap, Appressoria; V, vesicle; and Ar, arbuscules. The pregermination stages may be stimulated by the plant root exudates, but may also occur in its absence. B, Illustration of the different stages in plant mutants that are defective in AM colonization. Table describes the different mutants based on table presented by Marsh and Shultze (2001). Prepenetration (Pre-Pen) stage, Includes all steps that may be involved in the early recognition event(s) leading to the formation of appressoria. Penetration (*Pen*), Swollen appressoria (*Ap*) are formed, but the epidermis is not penetrated, evidence of the involvement of plant defense response (pdr) signals. Cortex invasion phenotypes (*Coi*), Successful penetration but intracellular spread leading to cortical infection aborted at various stages. Arbuscules (*Arb*), Mutants do not form either fully functional arbuscules or could be reduced in number or be altogether absent. References for *pmi* tomato mutants and *nope* 1 maize mutant are David-Schwartz et al. (2001) and Paszkowski et al. (2001), respectively, and for the *Melilotus alba* mutants, Lum et al. (2002).

from legumes defective in nodule formation (Nod^- phenotype). These mutants probably represent only a partial spectrum of the potential control steps induced by the host. Nevertheless, it has been proposed that rhizobial and AM fungi have evolved functionally similar recognition systems for plant colonization (van Rhijn et al., 1997). Thus, the gene products of the host may have a common function in an early step(s) of both symbiotic interactions, but the perception mechanisms for the two microsymbionts probably differ (Peterson and Guinel, 2000). Alternatively, legume hosts share features at the molecular level, which both *Rhizobium* spp. and AM fungi have exploited to enable the development of specific plant-host interfaces for the benefit of both partners. A more detailed characterization of the interactions between a symbiont and each legume mutant could potentially reveal the ultimate control of each step in the colonization process.

Mutations that are unique to AM fungal symbiosis might be expected to have particular impact at two key stages: precolonization and arbuscular development. For example, mutations in key stages leading to fungal colonization, which trigger pre-infection hyphal branching and appressorium formation, have yet to be discovered. Initial findings suggest that such stages exist. To meet this challenge, considerable research needs to be directed to obtain non-legume host mutants that exhibit the necessary attribute of lack of colonization at a particular stage of AM fungal infection. Such efforts are currently being pursued with tomato and maize as the non-leguminous hosts (Barker et al., 1998; David-Schwartz et al., 2001).

The study of stage-defective mutants should advance our understanding of the control steps of AM symbiosis and aid in the better molecular dissection of the complex genetic association that controls harmonious symbiosis.

ACKNOWLEDGMENT

Our sincere apologies to the colleagues we have not cited due to space limitations.

Received August 27, 2001; returned for revision September 12, 2001; accepted September 17, 2001.

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