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Bibliographic review

An approaching to Ectomycorrhizae world

Un acercamiento al mundo de las Ectomicorrizas

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ABSTRACT: Ectomycorrhizae are generally considered the most advanced symbiotic association between higher plants and fungi, because although they involve only 3 % of seed plants, all plants species are woody species; trees and shrubs, including most fruit trees. Therefore the ectomycorrizal association is important worldwide due to the large area covered by plants and due to its economic value as source of wood. In this review, references are made to the characteristics of the association, taking into account the morphology of the symbiosis, the mechanisms used to establish the association and how they influence plant nutrition.

Key words: functioning, nitrogen, phosphorus transport.

RESUMEN: Las ectomicorrizas generalmente se consideran la asociación simbiótica más avanzada entre plantas superiores y hongos porque, aunque involucran solo alrededor del 3% de las plantas con semillas, todas estas son especies leñosas, árboles y arbustos, incluida la mayoría de los árboles forestales. Por lo tanto, la asociación ectomicorrízica es importante a nivel mundial debido a la gran área cubierta por estas plantas y debido a su valor económico como fuente de madera. En la presente revisión se hace referencia a las características de la asociación, teniendo en cuenta la morfología de la simbiosis, los mecanismos empleados para establecer la asociación y como influencia en la nutrición de las plantas.

Palabras clave: transporte de nutrientes, fósforo, nitrógeno.

INTRODUCTION

Mycorrhizal fungi are widespread in most terrestrial habitats and form a mutualistic symbiotic association with more than 80 % of plant species (1). There are two types of mycorrhizae depending on the relationship that the hyphae establish with plant root cells. In this sense, endomycorrhizae form their associations within the root cells of the host plant, whereas ectomycorrhizae associate externally (2).

Arbuscular mycorrhizas are associated with 80 % of plants, whereas ectomycorrhizae (EcM) occur between certain fungal species and the roots of about 2 % of vascular plant species. Generally in woody plants, including species of the genera *Betula*, *Dipterocarpus*, *Myrtus*, *Fagus*, *Salix*, *Pinus* and also species of the genus *Quercus*, such as oaks and *Rosa* (2). However, due to the processes of anthropic degradation suffered by the forest planet systems (3), which leads to the decrease of biodiversity and therefore to the decrease of the services they provide (4, 5), these associations have become vitally important. In tropical and temperate forests, most timber plant species are obligate symbionts of ectomycorrhizae, which provide nutrients and water in exchange for photosynthetically fixed carbon (6).

DEVELOPMENT

Deforestation is one of the greatest challenges facing the earth with impacts that can range from destroying the habitat of different animals and plants, limiting genetic diversity by isolating populations and making it difficult for some species to survive and reproduce. Deforestation releases amounts of carbon into the atmosphere that contribute to global warming, changing weather patterns, which directly impacts agriculture and water sources. Forests play an important role in the planet's water and temperature regulation cycle, so maintaining forest health would allow for improved living conditions. Reforestation projects are essential to combat climate change and preserve biodiversity. In these projects Ectomycorrhizae use is from vital importance since these microorganisms play an important role in alleviating biotic and abiotic stresses on plants. In this review we propose to approach to the ectomycorrhizae world and learn about advantages they provide to their host plants.

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Evolution

In the Devonian, about 380 million years ago, lignin appeared, which allowed some plant species to reach large dimensions (7) when decomposing, the tissues of these plants provided large amounts of wood waste. At about the same time, basidiomycetes and ascomycetes fungi, which could decompose lignin, differed from glomeromycetes, which already formed arbuscular mycorrhizae, according to taxonomic analysis of EcM lines. However, on at least five occasions EcM evolved from non-mycorrhizal or facultative mycorrhizal ancestors (*Coccoloba*, *Persicaria vivipara*, *Gymnopodium* and *Pisonieae* within the *Caryophyllales* and *Kobresia* within the *Poales*) (8). This approach is in contradiction with some authors (9) who suggest that only AM can be the ancestors of EcM; however, the latter author excludes the groups mentioned in parentheses (8).

In any case, the plant species that evolved to form symbiosis with these fungi had the ability to colonize substrates unfavorable to arbuscular mycorrhizae, i.e., substrates rich in phenols and tannins, which allowed them to accumulate organic matter and preserve this association (10).

Characteristics of EcM

As its name indicates, ectomycorrhizal symbiosis does not involve the mycelium entering the plant root cells. The fungal hyphae envelop the host plant root in a pattern of intercellular penetration, where the hyphae form a network between cortical cells called Hartig's network (11, 12). This network constitutes the site of exchange between the plant, which provides organic carbon, and the fungus, which provides various nutrients and water (6). Unlike the hyphae of mycorrhizal fungi, which are coenocytic (cytoplasm common to many nuclei, without septa), the hyphae of ectomycorrhizal fungi are septate.

Ectomycorrhizal roots are morphologically different from non-ectomycorrhizal roots. First, the production of hairs is inhibited (13). The effectiveness of these structures is far exceeded by that of mycelium. Second, the cortex is hypertrophic, which increases the space available for the Hartig network. Third, the roots are more branched and have less longitudinal growth (14).

In EcM, hyphae attack the epidermal cells of the emerging lateral roots. This hyphae proliferate and differentiate into a series of hyphal layers to form a psudoparenchymatous tissue known as Harting' sheath or mantle. This structure contains water and air channels that transport nutrients to symbiotic cells and develops around cortical cells in angiosperms and both cortical and epidermal cells in gymnosperms (12).

Hartig's mantle (resulting in a complex maze of hyphal branching and thus a large surface area) forms an efficient interface for bidirectional transport of nutrients across the cells forming the host surface (15, 16).

Fungal species involved

Although arbuscular mycorrhizae are the most common form of mycorrhizal symbiosis, the number of fungal species involved remains limited (approximately 200) compared with the number of ectomycorrhizal fungal species, which amount, according to some authors, to tens of thousands, distributed in a few hundred genera (17-19).

Most of the fungal species in ectomycorrhizal associations are part of the basidiomycetes, ascomycetes, and some of the genus Endogone (*Mucoromycotina*) (20).

In contrast to arbuscular fungi, which have not yet been successful in axenic culture, some species of ectomycorrhizal fungi are easy to cultivate. This is particularly the case for *Boletus*, *Amanita* and *Lacaria*. Other genera, such as *Tuber* (truffles) and *Lactarius*, are more difficult to cultivate, while others remain elusive (2).

Mechanism of infection by EcM

A model has been described by which EcM fungi colonize host plants. This model contains several stages.

First: the host plant has a restricted set of genes that are induced during the preinfection phase and during colonization of the apoplastic space (21, 22).

Second: EcM fungi can alter root metabolism so that hyphae are accommodated in the root, similar to what has been observed for MA symbiosis (23, 24).

Prior to physical contact, the colonizing hyphae alter endogenous auxin metabolism, root signal molecules, and cellular responses of the colonized plant through the use of mechanisms that include a variety of diffusible molecules (such as plant auxins and fungal sesquiterpenes) such that more roots are produced, providing the fungus with a wide colonization area (25, 26, 27).

Third: gene function associated with defense responses during the early stages of EcM invasion is attenuated by the secretion of a fungal effector called MiSSP. In the nucleus, MiSSP interacts with the transcriptional repressor JAZ 6 (JASMONATE ZIM DOMAIN) which is the key regulator of the jasmonate signaling pathway. In the rest of the cell, when jasmonate levels are low or absent, JAZ6 inhibits transcriptional activation of genes responsible for jasmonic acid synthesis, similar to what occurs in MA symbiosis with the effector protein Sp7, which allows the EcM fungus to colonize the root while “escaping” or subverting plant defense (25, 28).

Molecular studies have shown that these effector proteins are secreted into host plant cells and translocate to the nucleus where they suppress the expression of defense genes by physically interacting with their target proteins (29). These mechanisms of weakening defense responses are crucial to achieve hyphal penetration into the apoplastic spaces (28, 30). On the other hand, the host plant responds to develop its ectomycorrhizal interaction by secreting its own effector protein and chemical signals in response to fungal effectors (30).

Fourth: the fungal effectors, by regulating wall-degrading enzymes as part of the plant defense system, modify cell-cell contact to allow the fungal hyphae to become lodged in the root (31, 32).

Finally, once in the apoplastic space and followed by the establishment of bidirectional nutrient transport with the host plant, the hypha continuously shields itself from detection by plant defense through masking proteins (such as hydrophobins (33)) or decoys (MiSSPs) as entertainment tactics.

Functioning of EcMs. Nutrient uptake and transport

N transport

To supplement plant tissues with N, EcM must first take it from their surrounding environment. EcM fungi encode transporters for the acquisition of nitrate and ammonium from the soil, as well as a set of enzymes and transporters for the utilization of organic N sources (34-36).

Ammonium is the preferred source of inorganic nitrogen taken up by EcMs, since, as nitrate, it is immediately reduced to ammonium and requires more energy (37). AMT1 and AMT2 are ammonium transporters that have been well characterized in several EcM species, including *Hebeloma cylindrosporum* (38, 39), *Tuber borchii* (40) and *Amanita muscaria* (41). Homologs of these genes have been identified in other EcM fungi from transcriptomic studies (42, 43). These transporters have been characterized as high affinity and their expression is up-regulated at low ammonium concentrations (39, 41).

Nitrate transporters such as LbNRT2 have also been found in *Laccaria bicolor* (44) and HcNRT2 in *H. cylindrosporum* (45), and their regulation is usually governed by the enzyme nitrate reductase which is required for their assimilation (46, 44). Nitrate uptake is depressed in the presence of ammonium, but not by the presence of other organic N sources, allowing simultaneous uptake from organic and inorganic sources (45, 44).

These fungi also secrete peptidases to utilize soil proteins and present transporters for amino acids, oligopeptides, and dipeptides (34, 47). The expression of these organic N transporters, as well as the secretion of peptidases, is also reduced in the presence of ammonium, indicating fungal preference for this source (48, 47).

Once soil nitrogen is taken up, it is metabolized, stored, and transported to other fungal cells (47). Part of this N is exchanged with the plant. This requires high coordination between the expression and activity of fungal and plant transporters.

Most studies have shown that the exchange between symbionts is not reciprocal (49-52). The plant allocates C to the fungus when it is produced in excess, however, it continues to transport it to the fungus even when N transport from the fungus to the plant is affected (49). In this regard, some authors found that under N-limited conditions in a boreal forest, plants could increase C transfer, but were not rewarded with increased N transport by the fungus (53).

Phosphorus transport

Most of the P in forests is found as part of complexes. Most of it is in the form of phosphate esters (54). It is considered that EcMs can acquire this P as a whole molecule (55). The identification of three genes encoding glycerophosphoinositol transporters supports this theory; however, the activity of these transporters has not been demonstrated (56), for which phosphate must be released from this bond by phosphatase enzymes (57). Phytate is the form in which P is found in most ecosystems including forests (58). It is the form in which P is stored in seeds (59) and is hydrolyzed during germination by intracellular phytases to supply P to seedlings; however, if this phytate is not hydrolyzed it becomes part of the P content of the soil. The efficiency of organisms in mobilizing phytate from the soil solution depends on their ability to produce phytases (56).

The ability of EcM fungi to release phytases is still a subject of research and there is contradiction in the literature; some studies have reported EcM with a high ability for this (60, 61), without the ability (62), or very low (62, 63) in axenic media. Most studies have made it clear that these fungi enhance phosphorus nutrition using sources other than phytates (64, 65).

P acquisition by EcMs takes place via plasma membrane transporters. The first P transporter of EcMs was identified based on the homology of this transporter with yeast P transporters (66). Most EcMs have three to five transporters belonging to the *Pht1* subfamily (67; P/H+-type transporters). However, the transporters encoded by the *TmPT3* gene are classified as P/Na+ transporters (*Pht2*). The latter type of transporter has been identified in the yeast *Saccharomyces cerevisiae* (68). This suggests that the efficiency of phosphorus uptake by the external hyphae of these fungi is influenced by the pH of the surrounding medium (56). From all the P transporters identified in EcM, *HcPT1.1*, *HcPT2*, and BePT have been characterized by their heterologous expression to those of yeast (69, 70).

These transporters respond differently to different P concentrations. *HcPT1.1* is strongly expressed when P concentrations are very low or only trace amounts of the element are present (66, 70), however, *HcPT2* transcript levels are independent of solution P concentration (69).

The interaction with mycorrhizal fungi is based on bidirectional nutrient transfer. Because there is no symplastic continuity between EcM fungal hyphae and the root, P must move into the apoplastic interfacial space before being taken up (71). This transport involves passive movement of P and C across the fungal and plant membranes, respectively, into the interfacial space and active nutrient uptake by both symbionts employing a proton pump (H+/ATP loop) (72). P moves within hyphae to supply plant demands (72) and it has been suggested that this passive movement of P across fungal membranes is ensured by maintaining low cytosolic P concentrations, as suggested for AM associations, maintaining a P gradient at the expense of poly-P degradation in the cytosol and efficient uptake through plasma membrane transporters (73, 74). In addition, it has been found that this P efflux from the hypha can be affected by the presence in the exchange zone of K+, Na+, and carbohydrates (75). Alternatively, P efflux from the fungus to the apoplast may employ an active mechanism involving P transporters whose presence and activity is regulated by host demand (76).

In contrast to AM symbiosis, little is known about the transporters responsible for P acquisition by the host plant (56). However, the expression and activation of *Pht1* genes involved in P acquisition by the plant have been documented (77, 78). As in AM symbiosis, it has been observed that as the EcM fungus supplies the P needs of the plant, active uptake transporters from the root soil solution begin to be turned off as a cellular economy mechanism (79).

Prospects for EcM use

There is evidence that the use of these fungi as tools for reforestation could be effective (6). Anthropogenic activities negatively affect the abundance and richness of EcM communities due to erosion, changes in land use, introduction of chemicals, fire, and invasion of non-native plants (80, 81, 6). Inoculation of these microorganisms could facilitate the establishment and growth of species of interest in degraded ecosystems while improving soil quality (82, 83). Reforestation and ecosystem restoration projects are not always imminently successful, not to mention that in most cases they are context dependent (6). There are a low number of studies using EcM to restore boreal and tropical forests, however, there are numerous reports in the literature of evidence that restoring microbiomes often recovers plants that were considered lost to these communities (84), which opens a door for the potential use of these fungi.

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