Histological Observations of Arbuscular Mycorrhizal Fungus Infection to Root Tissue of *Asparagus officinalis* L. Seedlings

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Summary

The infection process of arbuscular mycorrhizal (AM) fungus, *Gigaspora margarita*, in asparagus (*Asparagus officinalis* L.) seedling roots was histologically investigated to clarify the mechanism as to how the organism enhances asparagus plant growth. Such information may lead to a better understanding of growth through symbiosis. The sequential infection process was classified into four main stages.

Stage I: a hypha penetrated into the host roots via the intercellular spaces of the epidermal cells to the cortex. Stage II: the hypha initially elongated longitudinally in the outer cortex and then in the inner cortex. Stage III: the hypha invaginated to the surface layer of the cortical cell; at the invaginating point, the primary cell wall became thin and indistinguishable; the secondary cell wall also became thin but remained visible. After invaginating, the hypha formed a ramified arbuscule which pushed the host cell plasmalemma inwardly but did not crush it. No hypha penetrated the endodermis. Stage IV: the arbuscule collapsed into a mass.

This study clarified the histological characteristics of host cell wall and middle lamella during an infection by the AM fungus (*Gigaspora margarita*) in asparagus seedling roots.

Introduction

Infection of arbuscular mycorrhizal (AM) fungus promotes host plant growth mainly by enhancing mineral uptake through symbiosis which is based on the formation of symbiotic organs in the roots. We previously reported that growth enhancement by host-fungus symbiosis appeared mostly in liliaceous vegetables including asparagus, and that, especially in asparagus, this growth enhancement might be useful for developing vigorous seedlings and for shortening the seedling-raising period (Matsubara et al., 1994).

To understand the mechanism of growth enhancement through host-fungus symbiosis, it is important to clarify the infection process of AM fun-

gus in the host plant. There are some studies on the infection process of AM fungus, which have been conducted mycologically, but rarely histologically.

We previously reported some aspects of AM fungus infection process observed under a light microscope (Matsubara et al., 1994). However, the infection process of AM fungus needs to be clarified more sufficiently at the ultrastructure level; e.g., it is essential to examine the infection-induced changes in both walls and organelles of the host plant cells.

In the present study, to clarify the mechanism of enhancing asparagus plant growth through AM fungus (*Gigaspora margarita*) infection and establish a further effective method for the growth enhancement, the infection process of AM fungus in asparagus seedling roots was histologically investigated with electron microscope.

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Materials and Methods

1. Preparation of plant materials, inoculation of AM fungus, and growth of infected plants

Seeds of asparagus (*Asparagus officinalis* L., cv. MW500W) were germinated on moistened filter paper in a Petri dish (11 cm in diameter). Short primary roots of ten-day-old seedlings were inoculated with spores of *Gigaspora margarita* at a density of 100 spores per gram of inoculum.

Sieved soil with an available-P content (61.3 mg/100 g dry soil, Truog) was autoclaved at 121 °C and 1.2 kg·cm⁻² for one hour and then packed in a plastic vat [46 cm×30 cm×16 cm (H)]. The AM fungus-inoculated plants were transplanted to the vat, kept under natural light conditions in a greenhouse, and watered adequately. The plants were not fertilized throughout the experiments.

2. Light microscope observation

Root samples were collected every 2~3 days after inoculation and cleared in 10% KOH for 10 min at 115°C and 1 kg·cm⁻². The samples were then stained with 0.05% trypan blue by the method of Phillips and Hayman (1970).

3. Scanning electron microscope (SEM) observation

Root samples were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer solution (pH 7.2) for 2 hours at 5°C and postfixed with 1% osmium oxide in 0.1 M phosphate buffer solution for 2 hours at 0°C. The samples were dehydrated through a graded buthanol series and embedded in

paraffin. Twenty-micrometer-thickness sections were prepared and dried up to a critical point with the critical point dryer (Hitachi, HCP-2), and then coated with Pt-Pd with ion sputter (Hitachi, E-1030). The sections were examined with SEM (Hitachi, S-800) at 10 kV.

4. Transmission electron microscope (TEM) observation

Root samples were collected weekly after inoculation, fixed with 1% glutaraldehyde-3.7% formalin in 0.1 M phosphate buffer solution (pH 7.2) for a week at 5°C, and postfixed with 1% osmium oxide in 0.1 M phosphate buffer for 2 hours at 0 °C. The samples were dehydrated through graded ethanol series followed by propylene oxide, infiltrated in Spurr's resin and polymerized at 70°C. Ultrathin sections were cut and poststained with 2 % uranyl acetate and lead citrate. Stained sections were examined under TEM (JEM, 100s) at 80 kV.

Results

From sequential observation, the authors classified the infection process into four main stages (see Fig. 1).

1. Hyphal penetration into the epidermis of host roots (stage I)

A hypha formed an appressorium on the surface of the feeder root, and penetrated into the cortex of the host root through the intercellular space of the epidermal cells (Fig. 2-1).

Fig. 1. Classification of AM fungus (*Gigaspora margarita*) infection stage.

Fig. 2. Hyphal penetration into the epidermis of the host root (stage I) and hyphal elongation in the cortex of the host root (stage II). (1) ($\times 200$), (2-4) ($\times 100$).
ep: epidermis, st: stele, h: hypha, ap: appressorium.

2. Hyphal elongation in the cortex of host roots (stage II)

The hypha elongated longitudinally in the outer cortex through the intercellular spaces. The intercellular hyphae initially predominated longitudinally in the outer cortex (Fig. 2-2), and the frequency of hyphal elongation increased in the inner cortex closer to the stele (Fig. 2-3, 2-4). Hyphae elongated were not observed at the root-branched portions (Fig. 3).

3. Hyphal invagination to the host cortical cell (stage III-1)

Before the formation of an arbuscule (a symbiotic organ playing a role in nutrient exchange between the host and fungus), the intercellular hyphae invaginated to the host cortical cells (Fig. 4). At the invaginating point, hypha formed a peg like projection which pushed the walls of the cortical cell inward. At that point, the primary cell wall became thin and invisible, whereas the secondary cell wall which also became thin and remained visible. Host cell plasmalemmas were separated from the secondary cell wall and became wavy.

Fig. 3. Branching portion of the roots ($\times 40$).
fr: feeder root, lr: lateral root of feeder root, st: stele, h: hypha.

Fig. 4. Hyphal invagination to the host cortical cell (stage III-1, $\times 6,000$). hpw: host primary wall, hsw: host secondary wall, hpl: host cell plasmalemma, hmi: host mitochondria, fv: fungal vacuole, fw: fungal wall, lg: lipid globule.

4. Branching of hyphae (stage III-2)

After invagination, the hypha began to branch repeatedly and formed an arbuscule (Fig. 5-1, 5-2), the top of which became globular (Fig. 5-3). Arbuscules at various stages of development were concentrated in the three inner cortical layers immediately adjacent to the endodermis where infection had not occurred (Fig. 6). Hyphae elongated intercellularly in the host cortex and caused transformation of the cells. The intercellular hyphae seemed to exist closer to the portion where the host organelles were concentrated. There was no infection of cortical cells containing crystals of oxalic acid, a substance usually found in asparagus

Fig. 5. Photomicrograph of arbuscule (symbiotic organ) formation in the asparagus cortical cell (stage III).

(1) Cross section of the feeder root infected with AM fungus ($\times 600$).

(2) Arbuscule ($\times 3,000$), (3) Arbuscule ($\times 20,000$).

(4) A crystal of oxalic acid ($\times 3,500$).

ar: arbuscule, st: stele, oa: a crystal of oxalic acid, hew: host cell wall.

Fig. 6. Cross section of a feeder root infected with AM fungus ($\times 1,000$).

en: endodermis, ar: arbuscule, ih: intercellular hypha.

roots (Fig. 5-4).

5. Completion of an arbuscule (Stage III-3)

The arbuscule occupied most of the cytoplasmic space in the infected host cell, and the arbuscule forming cells enlarged more than did the noninfected cells (Fig. 7). The arbuscule did not penetrate the host cell plasmalemma; an interfacial matrix filled the space between the host cell plasmalemma and the fungal arbuscular wall. Organelles in the infected host cell were located between the fine arbuscular branches and did not fuse with them. In infected host cells, a vacuole divided into small masses; the number of mitochondria increased; the nuclei became enlarged (data not shown).

6. Degeneration of an arbuscule (Stage IV)

The old arbuscule collapsed into a mass near the invaginating point (Fig. 8-1). The host cell plasmalemma surrounded the mass and, at the invaginating point, a thin layer (like the secondary cell wall of the host cell) surrounded the fungal arbuscular wall (Fig. 8-2). The cell volume of the arbuscule-forming cells matched the size of noninfected cells when the arbuscule degenerated.

Fig. 7. Photomicrograph of an arbuscule in the asparagus cortical cell (stage III-3, $\times 4,000$).

ar: arbuscule, hmi: host mitochondrion, hew: host cell wall, ih: intercellular hypha, im: interfacial matrix, hv: host vacuole.

Fig. 8. Photomicrograph of an arbuscule in the asparagus cortical cell (stage IV).

(1) ($\times 8,000$), (2) ($\times 6,000$). ca: collapsed arbuscule, hcw: host cell wall, hpl: host cell plasmalemma, fw: fungal wall.

Discussion

Holley and Peterson (1979) reported that, at the fungus-invaginating point in the infected host cell, the hypha was constricted and the host cell wall became invisible around the invaginating hypha. They did not mention the changes in either host primary or secondary cell walls at the invaginating point. In our observation, the hyphae invaginated without constriction, and the host secondary cell wall was clearly visible around the invaginating hypha, though the host primary cell wall became unclear. The different conditions of primary and secondary cell walls at the invaginating point of the infected host cells might be associated with the qualitative characteristics, i.e., contents of pectic substance, lignin and suberin, etc. of the host cell wall.

The process of arbuscule formation in *Gigaspora margarita* was similar to that in *Glomus* spp. (Carling and Brown, 1982; Cox and Sanders, 1974; Holley and Peterson, 1979). We observed arbuscules in the three inner layers of the cortex immediately adjacent to the endodermis. This fact suggests that these areas might be favorable for nutrient exchange between host and fungus. Cox and Sanders (1974) and Carling and Brown (1982) stated that in the arbuscule-forming cell, an increase in host cell volume and an enlargement of the host nucleus occurred. We observed those same phenomena; furthermore, the number of host mitochondria increased in the arbuscule forming cell. Hence, in asparagus those changes in the host organelles are closely related to growth enhance-

ment because symbiotically-induced growth appeared after arbuscule formation (data not shown). In the infected host cell, the mitochondria were uniformly distributed among the fine arbuscular branches without crossing each other, so that the mitochondria seem to play a role in the nutrient exchange between the host and fungus.

Interfacial matrix filled the space between the host cell plasmalemma and the branching arbuscular wall. This feature of the matrix was also reported in *Glomus* spp. (Cox and Sanders, 1974; Holley and Peterson, 1979; Scannerini and Bonfante, 1979), the composition of the matrix varied depending on the host-fungus combination (Scannerini and Bonfante, 1983). However, the membrane-like structure between the infected host cell wall and the fungal arbuscular wall remained indistinguishable. Scannerini and Bonfante (1979) reported that the interfacial matrix contained polysaccharides and proteins, i.e. the same materials as that found in the host cell wall. The interfacial matrix (the matrix between asparagus plant and *Gigaspora margarita*) is possibly made by the host cell wall because the primary cell wall became thin and indistinguishable at the invaginating point; the secondary cell wall either disappears or becomes extremely thin around the fine arbuscular branches.

The degeneration process of an arbuscule of *Gigaspora margarita* was similar to that of *Glomus* spp. (Craling and Brown, 1982; Cox and Sanders, 1974; Holley and Peterson, 1979). Generally, in AM fungus infection, the membrane at the invaginating point loses its structural integrity when the arbuscule degenerated. Likewise, we observed that although a thin layer resembling the host secondary cell wall surrounded the fungal arbuscular wall, it could not be determined whether the secondary cell wall persisted, disappeared or had reproduced around the fine arbuscular branches.

Thus, we found that phenomena concerning the infection process of AM fungus (*Gigaspora margarita*) in asparagus seedlings were specific to the cortical cells of asparagus roots, and did not occur in the endodermis, the vascular bundle sheath cells and the branching portion of the roots. Our results suggest that the infection-induced structural changes in both primary and secondary cell walls at the invaginating point of the infected host cells play an important role in the infection pro-

cess of AM fungus.

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アスパラガス実生における arbuscular 菌根菌感染過程の組織学的観察

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摘 要

arbuscular 菌根 (AM) 菌感染によってアスパラガスの生育が促進される機構を明らかにし、さらに効果的な生育促進技術確立するため、アスパラガス (*Asparagus officinalis* L.) 実生の根における AM 菌 (*Gigaspora margarita*) 感染過程について組織学的手法による観察・調査を行った。感染過程を4つの主要なステージに類別した。(1)宿主根表皮における菌糸の侵入 (Stage I) : 菌糸は表皮細胞間隙から根組織内へ侵入した。(2)皮層組織における菌糸の伸長 (Stage II) : 皮層組織に侵入した菌糸は、皮層の外層を中心柱と平行に伸長し、やがて皮層内層部へ侵入した。(3)樹枝状体 (菌と宿主の物質交換器官) の形成 (Stage III)

: 菌糸が細胞表層へ陥入し始めた部位では、宿主1次細胞壁は陥入菌糸周辺部で薄化し明瞭となり、2次細胞壁は薄化した明瞭な状態で存在した。菌糸は、陥入後に、宿主細胞の原形質膜を押し延ばしながら細かく分岐し続け、樹枝状体を形成した。その場合、樹枝状体は宿主細胞の原形質膜を貫通して細胞質中へ侵入することはなかった。菌糸は内皮組織には侵入しなかった。(4)樹枝状体の退化 (Stage IV) : 老化した樹枝状体は集合体を形成した。

このように、宿主の根の組織学的特性 (細胞の表層構造および組織の中層の状態) が AM 菌の感染に関与していることが明らかになった。