Effect of Arbuscular Mycorrhizal Fungus Infection on the Incidence of Fusarium Root Rot in Asparagus Seedlings

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Summary

The incidence of fusarium root rot, caused by *Fusarium oxysporum* f. sp. *asparagi* (Foa), was investigated in seedlings of asparagus (*Asparagus officinalis* L., cv. Mary Washington 500W) by inoculation with three species of arbuscular mycorrhizal (AM) fungi; *Gigaspora margarita* (GM), *Glomus fasciculatum* (GF), and *Glomus* sp. R10 (GR).

Ten weeks after AM fungal inoculation, the inoculated plants were taller; they produced more shoots and roots and accumulated more dry matter in the shoots and roots than noninoculated ones. The infection levels in a root system differed with fungal species.

Six weeks after Foa inoculation, 90% of the noninoculated plants exhibited symptoms of fusarium root rot, whereas 20-50% of the inoculated plants did. The effect was more pronounced in GR, GM, and GF, in that order. As for the disease indices, it was lower in the inoculated plants than in the noninoculated ones. The indices differed among the AM fungal species; it was significantly low in GR. The number of Foa hyphae invading feeder roots decreased in the inoculated plants, compared with the noninoculated ones. In addition, AM fungal hyphae preferentially elongated into short cells in the exodermis of feeder roots, while the Foa hyphae also elongated into short cells as well as AM fungus. However, no short cells became infected with both AM fungus and Foa.

These results reveal that tolerance to fusarium root rot was conditioned by AM fungal infection in asparagus seedlings, although the effect differed with the AM fungal species. It seems that the tolerance to fusarium root rot was partially caused by AM fungal pre-infection in short cells which suppressed invasion by Foa in feeder roots.

Key Words: arbuscular mycorrhizal fungi, asparagus, exodermis, fusarium root rot.

Introduction

Fusarium root rot caused by *Fusarium oxysporum* f. sp. *asparagi* (Foa) remains a serious disease in asparagus cultivation in Japan and abroad (Tsuchiya, 1989; Minagawa, 1993; Blok et al., 1997). The disease is difficult to control because no resistant cultivar or effective fungicides have been developed. Recently, biological control of *Fusarium oxysporum* was attempted by inoculation with non-pathogenic isolates of the species (Blok et al., 1997).

Arbuscular mycorrhizal (AM) fungus has the effect of promoting host plant growth mainly by enhancing phosphorus uptake through symbiosis with symbiotic organ formation in the roots (Marschner and Dell, 1994). Biological control of soil-borne disease by AM fungal infection was reported in citrus (Davis and Menge, 1980), asparagus (Wacker et al., 1990), cucumber (Kobayashi, 1992), tomato (Dehne and Schonbeck, 1979a; Caron et al., 1986) and eggplant (Matsubara et al., 1995), etc. Wacker et al. (1990) reported that incidence of fusarium root rot caused by *Fusarium oxysporum* was reduced by inoculation of AM fungus (*Glomus fasciculatum*). However, the mechanism of the disease tolerance remains unknown. It is unclear how tolerance to fusarium root rot caused by Foa develops in AM fungus-infected asparagus plants, and whether the tolerance differs among AM fungal species.

Matsubara et al. (1994) reported that asparagus has a high affinity for AM fungus and the symbiotic relationship produced vigorous asparagus plants. Therefore, there is a possibility that the AM fungal symbiosis may offer an effective method for biological control of fusarium root rot caused by Foa in asparagus.

In this study, the effect of pre-inoculation of asparagus seedlings with three AM fungal species on incidence of fusarium root rot caused by Foa was investigated.

Materials and Methods

Inoculation of AM fungus

Seeds of asparagus (*Asparagus officinalis* L., cv. Mary Washington 500W) were germinated on a moist filter paper in a Petri dish (11 cm in diameter). Ten-day
old seedlings with 2 cm long radicles were inoculated with Gigaspora margarita (GM), Glomus fasciculatum (GF) and Glomus sp. R10 (GR), using commercial inoculums; spore densities were 20 spores $\cdot$ $g^{-1}$ inoculum in GM, and 100 spores $\cdot$ $g^{-1}$ inoculum in GF and GR. Inoculation was carried out according to Matsubara et al. (1994).

Bed soil [mixture of soil : vermiculite (1:1, v/v); autoclaved at 1.2 kg $\cdot$ cm$^{-2}$ and 121°C for one hour; pH 6.0 (H$_2$O); available-P content, 21.9 mg/100 g dry soil] were packed in plastic containers (13.5 cm $\times$ 27.0 cm $\times$ 15.5 cm (H)). AM fungus-inoculated plants (AM plants) and noninoculated control plants (NAM plants) were then transplanted to the bed soil and administered a mixed fertilizer (N:P:K=8:3:10, 0.5 g $\cdot$ liter$^{-1}$ soil). The 15 seedlings per plot were irrigated as needed and raised in a greenhouse.

Inoculation with Foa

Foa (SUF1226) was grown on potato–dextrose agar medium. The conidia were harvested in potato–sucrose liquid medium and incubated at 25°C in the dark for 5 days. The conidial suspension was sieved (45 $\mu$m) and the concentration of inoculum was adjusted to $10^{6}$ cfu $\cdot$ ml$^{-1}$. Each plant was then inoculated by spreading 50 ml of the conidial suspension into the bed soil ten weeks after AM fungal inoculation. The plants were raised in a growth chamber at 25°C under natural light and day-length. Ten plants per treatment were used.

Observation of AM fungal infection in roots, and evaluation of AM fungal infection level

Ten and 16 weeks after AM fungal inoculation, roots were fixed with 50% ethanol and stained according to Phillips and Hayman (1970) to observe the rate of AM fungal infection in segments of lateral roots (RFISL). RFISL expresses the percentage of 1 cm AM fungus–infected segments to the total 1 cm segments of the lateral roots of a plant. The average is calculated from the values of four plants.

Estimation of symptoms of fusarium root rot

Six weeks after Foa inoculation, the symptoms of fusarium root rot were categorized into 6 degrees: 0, no symptom; ratio of diseased storage roots in a root system was; 1, less than 10%; 2, 10–20%; 3, 20–30%; 4, 30–40%; 5, more than 40%. In addition, the disease index was calculated by the following formula:

$$\text{Disease index} = \frac{1}{5} \left( \frac{\text{number of plants} \times \text{number of degree in symptom}}{\text{Total number of plants} \times 5} \right) \times 100$$

Results

Ten weeks after AM fungal inoculation, AM plants showed higher values for plant height, no. of shoots, and dry weight of shoots and roots than those of NAM plants. The effect differed little among the species (Table 1). AM fungal infection occurred in all the treatments. RFISL reached 50.8% (highest) in GR and 36.3% (lowest) in GF.

Six weeks after Foa inoculation, RFISL in healthy plants differed among the fungal species, with a maximum of 60.3% in GR (Fig. 1). However, symptoms of fusarium root rot were observed in all the treatments. The incidence of root rot ranged from a minimum of 20% in GR and a maximum of 90% in NAM (Fig. 2). As for the severity of symptoms, plants in which more than 40% of storage roots was diseased occurred only in NAM plants. The severity differed among the AM fungal species; it was significantly low in GR. The disease indices were lower for AM plants as compared to NAM plants; NAM reached 56.0, but only 4.0 for GR (Fig. 3). Hence, the disease indices and incidence of fusarium root rot for the AM species followed a similar pattern.

The AM fungal infection occurred only in feeder roots; the hyphae preferentially elongating into the short cells in exodermis located in outer cortex but not to long cells (Fig. 4–1). In the infected roots, many vesicles (storage organs of AM fungi) were formed in the cortex; hyphal density was higher in the inner cortex than in the outer cortex (Fig. 4–2). Foa infection occurred in both

<table>
<thead>
<tr>
<th>AM fungal inoculation'</th>
<th>Plant height (cm)</th>
<th>No. of shoots</th>
<th>Diameter of shoots (mm)</th>
<th>No. of storage roots</th>
<th>Diameter of storage roots (mm)</th>
<th>Dry weight of shoots (g)</th>
<th>Dry weight of roots (g)</th>
<th>RFISL'</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAM</td>
<td>30.0b$^3$</td>
<td>4.3b</td>
<td>0.54b</td>
<td>6.2b</td>
<td>1.84b</td>
<td>0.14</td>
<td>0.15</td>
<td>0</td>
</tr>
<tr>
<td>GM</td>
<td>44.5a</td>
<td>7.4a</td>
<td>0.72a</td>
<td>13.5a</td>
<td>1.79c</td>
<td>0.95</td>
<td>0.90</td>
<td>42.6b</td>
</tr>
<tr>
<td>GF</td>
<td>40.5a</td>
<td>8.2a</td>
<td>0.59ab</td>
<td>10.5ab</td>
<td>1.88b</td>
<td>0.97</td>
<td>0.98</td>
<td>36.3c</td>
</tr>
<tr>
<td>GR</td>
<td>44.2a</td>
<td>8.0a</td>
<td>0.66ab</td>
<td>11.7ab</td>
<td>2.03a</td>
<td>0.91</td>
<td>0.87</td>
<td>50.8a</td>
</tr>
</tbody>
</table>

$^3$ NAM, AM fungus – noninoculated; GM, inoculated with Gigaspora margarita; GF, inoculated with Glomus fasciculatum; GR, inoculated with Glomus sp. R10.

$^\gamma$ Rate of AM fungus – infected segments in whole lateral roots.

$^x$ Mean separation within columns by Duncan’s multiple range test, 5% level.
feeder roots and storage roots. In feeder roots, hyphae of Foa preferentially elongated into the short cells as did the AM fungus (Fig. 4-3, 4).

In NAM plants, the no. of Foa hyphae invading feeder roots reached 119.0 per plant. No hypha penetrated the long cells of exodermis, whereas the rate of hyphal invasion into short cells reached 67.8% (Table 2). In AM plants, the no. of Foa hyphae invading into feeder roots was small, compared with NAM plants. The percentage of AM fungus hypha infection in short cells was higher than that in long cells, irrespective of the fungal species. However, no short cells were infected with both AM fungus and Foa.

**Discussion**

In this study, both incidence and severity of symptoms of fusarium root rot were reduced by a pre-infection with AM fungi, which indicates that tolerance to fusarium root rot was increased by AM fungi. The effect differed, however, with the AM fungal species with GR being the most effective. Blok et al. (1997) reported that the incidence of fusarium root rot in asparagus plants...
Table 2. Infection rate of feeder roots 16 weeks after AM fungus inoculation and 6 weeks after FoA inoculation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fungus</th>
<th>Total no. of invading hyphae (plant)</th>
<th>Rate of hyphal invasion into long cells (%)</th>
<th>Rate of hyphal invasion into short cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FoA only</td>
<td>FoA</td>
<td>119.0 ± 27.5</td>
<td>0</td>
<td>67.8</td>
</tr>
<tr>
<td>GM+Foa</td>
<td>GM</td>
<td>132.0 ± 19.9</td>
<td>0</td>
<td>74.6</td>
</tr>
<tr>
<td>GM+Foa</td>
<td>FoA</td>
<td>53.8 ± 7.1</td>
<td>0</td>
<td>60.2</td>
</tr>
<tr>
<td>GF+Foa</td>
<td>GF</td>
<td>166.6 ± 28.7</td>
<td>0</td>
<td>60.5</td>
</tr>
<tr>
<td>GF+Foa</td>
<td>FoA</td>
<td>71.8 ± 18.7</td>
<td>0</td>
<td>56.2</td>
</tr>
<tr>
<td>GR+Foa</td>
<td>GR</td>
<td>122.8 ± 12.5</td>
<td>0</td>
<td>80.3</td>
</tr>
<tr>
<td>FoA only</td>
<td>FoA</td>
<td>62.6 ± 9.4</td>
<td>0</td>
<td>72.2</td>
</tr>
</tbody>
</table>

* FoA, inoculated with *Fusarium oxysporum* l.sp. asparagusi, GM, inoculated with *Gigaspora margarita*, GF, inoculated with *Glomus fasciculatum*; GR, inoculated with *Glomus* sp. R10.

* Mean ± S.E.

* Ratio of total no. of hyphae invading long cells to total no. of hyphae invading feeder roots.

* Ratio of total no. of hyphae invading short cells to total no. of hyphae invading feeder roots.

was reduced by the inoculation of non-pathogenic isolates of *Fusarium oxysporum*, though the effect differed with the strains of the inoculum. Our data supports their finding, in that more asparagus roots were infected with GR than with GM and GF. Matsubara et al. (1995) reported that in eggplant, the effect of verticillium wilt control by AM fungus inoculation differed with the AM fungal species; i.e., plants with a high level of AM fungal infection were less susceptible to the wilt disease. Our results are similar to the findings in eggplant.

Davis and Menge (1980) indicated that phytophthora root rot was decreased by an increase in P concentration through AM fungal infection in citrus. Caron et al. (1986) and Wacker et al. (1990), however, found no relationship between increased P concentration and the tolerance to *Fusarium* disease in AM fungus-infected plants of tomato and asparagus, respectively. In our study, no significant difference in P concentration occurred among the treatments six weeks after FoA inoculation (data not shown), so that P concentration in plants may have little relationship with the tolerance to fusarium root rot in AM fungus-infected plants. However, Wacker et al. (1990) reported that populations of *Fusarium oxysporum* in the soil decreased with P increase in the soil.

We found that the total number of invading hyphae of FoA decreased in AM plants, compared with NAM ones, that no infection occurred in long cells of exodermis, and that hyphal invasion into short cells was frequently recognized for both fungi. Matsubara (1999) reported that AM fungal hyphae preferentially elongated into short cells and no infection took place in long cells. In addition, the frequency of hyphal invasion into short cells differed with AM fungal species; GR showed higher frequency than GM and GF. Kamura et al. (1994) noted that hyphae of *Fusarium culmorum* preferentially elongated into short cells in asparagus roots, and that no infection occurred in long cells. These findings indicate that short cells might provide preferential entry points into the cortex for AM fungi and FoA. Thus, we propose that pre-infection with AM fungus resulting in infection of only short cells may reduce FoA infection in asparagus roots and that high disease tolerance in GR-infected plants is partially associated with the high infectivity of short cells by GR. However, the decrease in root rot occurred in AM plants but no infection of AM fungi was found in the storage roots. Matsubara (1999) previously described that the structure of exodermis in storage roots differed with that in feeder roots in asparagus. Thus, some factor caused by AM fungal infection resulted in the tolerance to root rot in storage roots. Baltruschat and Schonbeck (1975) demonstrated that in tobacco plants, an increase in both arginine and citrulline occurred in AM fungus-infected plants, which inhibited the propagation of *Thielaviopsis basicola*, whereas Dehne and Schonbeck (1979b) reported that the lignification in endodermis and stele enhanced by AM fungal infection suppressed fusarium-wilt in tomato plants. Thus, some physiological and histological factors may be associated with the tolerance to root rot in storage roots in this study.

Biologically controlling soil-borne disease by AM fungus infection may have advantages over the use of resistant cultivars, agrochemicals, or soil sterilization because the symbiotic relationship enhances plant growth, while suppressing several soil-borne diseases. The results of our experiment may lead to such a control method.

Acknowledgement

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Literature Cited


Arbuscular菌根菌が感染したアスパラガス実生への立枯病菌接種の影響

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摘要

Arbuscular菌根（AM）菌 [Gigaspora margarita (GM), *Glomus fasciculatum* (GF) および *Glomus* sp. R10 (GR)] が感染したアスパラガス (Asparagus officinalis L., cv. Mary Washington 500W) 実生へ立枯病菌 (Fusarium oxysporum f. sp. asparagi) を接種し、罹病状態について調査した。

AM菌接種10週間後において、AM菌接種区では菌種に関わらず、草丈、葉数、地上部および地下部乾物重の増加といった生長促進効果が見られた。しかし、AM菌感染率 (1個体の根系における感染率) は菌種により異なった。

AM菌接種10週間後に立枯病菌を接種し、立枯病菌接種6週間後、根棚は全ての処理区で現れた。しかし、罹病個体率はAM菌無接種区で最高の90%であり、AM菌接種区ではGF接種区で50%、GM接種区で40%、GR接種区で最低の20%であった。また、根系における罹病程度については、AM菌接種区で無接種区より著しく小さく、その効果はGR接種区において顕著であった。吸収根におけるAM菌および立枯病菌の感染状態を観察したところ、AM菌接種区では無接種区より立枯病菌の侵入菌糸数が少なかった。また、AM菌および立枯病菌とも複数外皮の短細胞で高頻度の感染がみられたが、両菌が共に感染している短細胞はみられなかった。

これらのことを考えると、AM菌が感染したアスパラガス実生において立枯病の罹病性がみられ、その効果にはAM菌の菌種間差があることが示唆された。また、この罹病性は、吸収根の複数外皮の短細胞に感染したAM菌による立枯病菌の感染抑制が一つとなっていることが示唆された。