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Relation between Fiber and Pectic Substances in Root Tissue and Tolerance to Fusarium Root Rot in Asparagus Plants Infected with Arbuscular Mycorrhizal Fungus

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Summary

Tolerance to fusarium root rot, caused by *Fusarium oxysporum* f. sp. *asparagi* (FOA; MAFF305556, SUF844, SUF1226, SUF1229), in asparagus (*Asparagus officinalis* L., cv. Mary Washington 500W) plants infected with arbuscular mycorrhizal (AM) fungus (*Glomus* sp. R10) was estimated, and the relationships between fiber (cellulose, hemicellulose and lignin) and pectic substances (water-soluble, hexametaphosphate-soluble and HCl-soluble pectin) in root tissue and the tolerance to fusarium root rot were investigated.

Twelve weeks after FOA inoculation, the incidence and symptoms were significantly lower in AM plants than in non-AM ones, regardless of the FOA strains. Among non-AM plants, disease index reached 70-92, it ranged between 8-16 in the AM plants. Non-diseased and diseased AM plants produced more dry weight of feeder and storage roots than did diseased non-AM ones. Phosphorus concentration in feeder and storage roots differed little between non-AM and AM plots 10 weeks after AM fungus inoculation (just before FOA inoculation) and 12 weeks after FOA inoculation. Total fiber and each of its constituents in the feeder and storage roots did not differ between AM and non-AM plots before and after FOA treatment. In storage roots, total content of pectic substances was higher in AM plots than it was in non-AM ones at the end of the periods; especially, the increase in water-soluble pectin content in the AM plots. In feeder roots, the individual pectic substances in AM and non-AM plots were similar. These findings suggest that root rot tolerance in storage roots of AM fungus-infected plants is closely associated with the pectic substances, such as water-soluble pectin, rather than with the fiber content in root tissue.

Key Words: arbuscular mycorrhizal fungi, asparagus, fusarium root rot, fiber, pectic substances.

Introduction

Fusarium root rot caused by *Fusarium oxysporum* f. sp. *asparagi* (FOA) remains a serious disease in asparagus (*Asparagus officinalis* L.) 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 *F. oxysporum* was attempted by inoculation with non-pathogenic isolates of the species (Blok et al., 1997). Matsubara et al. (2001) previously reported the occurrence of fusarium root tolerance in asparagus plants that were infected with arbuscular mycorrhizal (AM) fungus, which promoted host plant growth mainly by enhancing phosphorus uptake. Furthermore, tolerance to fusarium root rot in AM fungus-infected asparagus plants was increased by adding coconut charcoal or manure of coffee residue to bed soil (Matsubara et al., 2002). However, it remains unclear how fusarium tolerance develops in AM fungus-infected asparagus plants and whether the tolerance differs among FOA strains.

Biological control of soil-borne disease by AM fungal infection was reported in citrus (Davis and Menge, 1980), cucumber (Kobayashi, 1992), tomato (Dehne and Schonbeck, 1979a; Caron et al., 1986), eggplant (Matsubara et al., 1995) and mung bean (Kasiamdari et al., 2002). Davis and Menge (1980) indicated that phytophthora root rot was decreased by an increase in phosphorus concentration through AM fungal infection in citrus. Dehne and Schonbeck (1979b) reported that the lignification in endodermis and stele enhanced by AM fungal infection suppressed fusarium-wilt in tomato plants. Matsubara and Harada (1998) associated the presence of pectic substances with rigidity of root tissue and the rigidity was closely related to AM fungal infection. They supposed that pectic substances in roots might also inhibit infection of pathogenic fungi such as fusarium.

In this study, the relationship between fiber and pectic substances in root tissue and tolerance to fusarium root rot in asparagus plants infected with AM fungus were investigated.

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Materials and Methods

Inoculation of AM fungus

Ten day-old seedlings of asparagus cv. Mary Washington 500 W were inoculated with *Glomus* sp. R10, according to Matsubara et al. (1996), using commercial inocula supplied by Idemitsukosan Co., Ltd.. Bedding soil, a mixture of soil : vermiculite (1:1, v/v) was autoclaved at 1.2 kg·cm^{-2} and 121 ℃ for 1 hr. The mixture (pH 6.0) with an available-P content of 30.5 mg/100 g dry soil was packed in plastic containers[36 cm × 30 cm × 18 cm (H)]to which AM fungus-inoculated plants (AM plants) and noninoculated control plants (NAM plants) were transplanted. Each plot was administered a mixed fertilizer (N:P:K=4:1:3, 0.5 g·liter^{-1} soil). Eight plots, consisting of 40 seedlings with no replication, were irrigated as needed and raised in a greenhouse.

Inoculation with FOA

Four strains of FOA (MAFF305556, SUF844, SUF1226 and SUF1229) were grown on potato-dextrose agar media. The conidia were harvested in potato-sucrose liquid media and incubated at 25 ℃ in the dark for 5 days. The conidial suspension was sieved (45 μm) and its concentration adjusted to 10^6 conidia·ml^{-1}. Each plant was then inoculated by pouring 50 ml of the conidial suspension on the soil 10 weeks after AM fungus inoculation. Twenty plants per plot were raised in a growth chamber at 25 ℃ under natural light and photoperiod.

Evaluation of AM fungal infection level

Ten weeks after AM fungus inoculation and 12 weeks after FOA inoculation, roots were sampled and preserved with 50% ethanol. The specimen were stained according to Phillips and Hayman (1970) and the rate of AM fungal infections in 1-cm segments of lateral roots (RFISL) calculated. Hence, RFISL expresses the percentage of 1-cm AM fungus-infected segments to the total 1-cm segments of all the lateral roots (feeder roots in asparagus) that branched from the main roots of a plant; the number of total segments was approx. 50 per a plant. The average was calculated from the values of three plants.

Estimation of symptoms of fusarium root rot

Twelve weeks after FOA inoculation, the symptoms of fusarium root rot in 20 plants per plot were categorized into 6 degrees: 0, no symptom; frequency of diseased storage roots in a root system: 1, less than 20%; 2, 20-40%; 3, 40-60%; 4, 60-80%; 5, 80-100%. The disease index was calculated by the following formula:

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

Determination of phosphorus in roots

The P determination on root samples was performed twice to investigate both the effect of AM fungus inoculation on P concentration in plants and the persistence of the effect after FOA inoculation. Roots from 5 plants were sampled 10 weeks after AM fungus inoculation and from 3 plants 12 weeks after growing in SUF1226-inoculated plot to determine P. Root dry matter was weighed after drying at 110 ℃ for 2 days. The samples were ground, wet-ashed, and their P concentrations determined, according to Matsubara and Harada (1996).

Determination of fiber substances in root tissue

Roots were sampled as the same method in P determination and divided into feeder and storage roots, and dried at 110 ℃ for 2 days. The samples were combined respectively and extracted with 70% ethanol. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin were then extracted from the alcohol insoluble solid (AIS) by the detergent fiber method (Fig. 1) of Soest and Wine (1967). Cellulose and hemicellulose contents were calculated by the following formula: cellulose= ADF - lignin, hemicellulose= NDF - ADF.

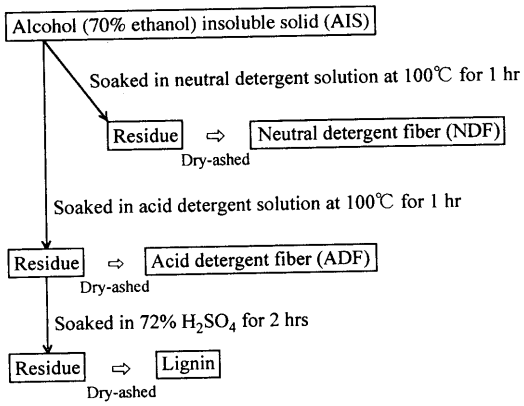

Fig. 1. Flow chart for extraction of fiber substances.

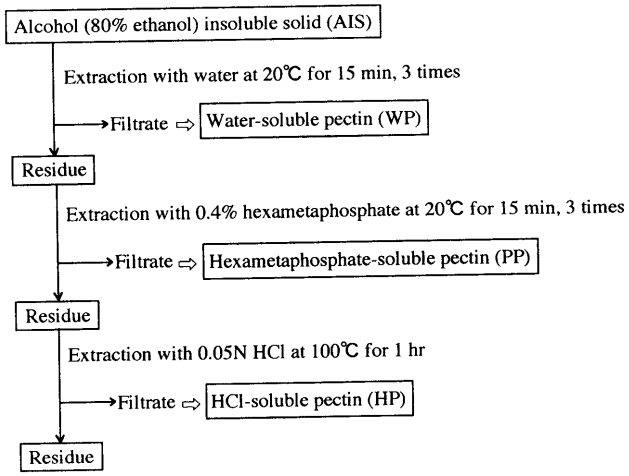

Fig. 2. Flow chart for extraction of pectic substances.

Determination of pectic substances in root tissue

Root samples collected as above were separated as feeder root (approx. 5 cm in length, excluding the tip) and storage root (approx. 10 cm, excluding root tip) at 5 ℃ to minimize pectinase activity. The root samples were extracted separately with 80% ethanol. The alcohol insoluble solid (AIS) was then extracted successively with water (water-soluble pectin, WP), hexametaphosphate (hexametaphosphate-soluble pectin, PP) and hydrochloric acid (HCl-soluble pectin, HP) (Fig. 2). The pectic substances in the three fractions were determined by the carbazole-sulfuric acid method .

Results

Twelve weeks after FOA inoculation, the incidence of fusarium root rot reached 100% in all the NAM plants; the severity of symptoms varied, depending on FOA strains. The pathogenicity was higher in MAFF 305556 and SUF 1226 than in the others (Fig. 3). However, the incidence and the extent of symptoms were significantly lower in AM plants than in NAM ones, regardless of the FOA strains. The incidence ranged 30-60% and the symptom showed only 1 and 2 degrees in AM plots. Among NAM plants, the disease index reached 70-92, while it was low as 8-16 in the AM plants (Fig. 4). Hence, the disease index and incidence of fusarium root rot for the NAM and AM plants followed a similar pattern. Feeder and storage roots of non-diseased and diseased AM plants were equally heavier (DW) than those of the diseased NAM ones, regardless of the FOA strains (Fig. 5). RFISL reached approx. 40-50% in AM plants, but no differences were noted between non-diseased and diseased AM plants, independent of the FOA strains (Fig. 6).

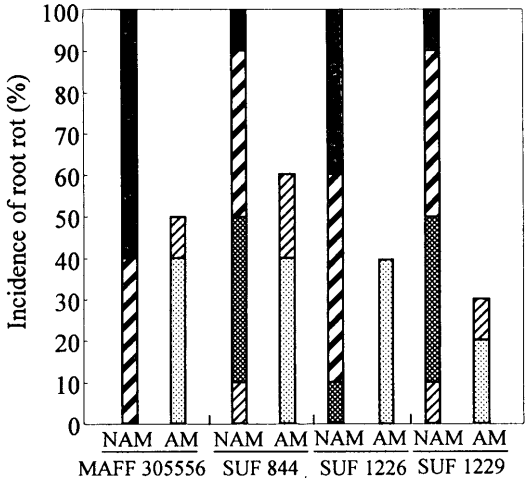

Fig. 3. Incidence and symptoms of root rot in asparagus 12 weeks after FOA inoculation. NAM, AM fungus-noninoculated; AM, AM fungus-inoculated. Ratio of diseased storage roots in a root system was; ▨, less than 20% (1: degree in symptom); ▨, 20-40% (2); ■, 40-60% (3); ◪, 60-80% (4); ■, 80-100% (5).

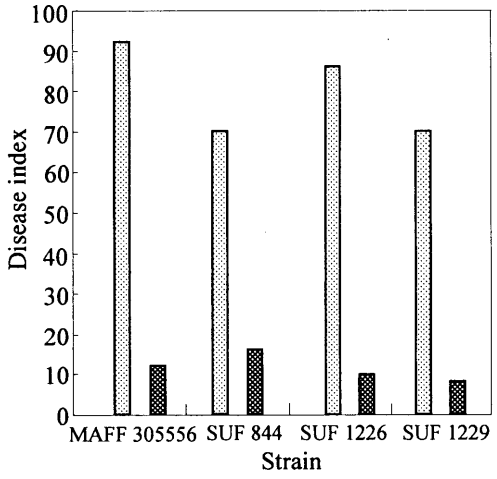

Fig. 4. Disease index of root rot in asparagus 12 weeks after FOA inoculation. ▨, AM fungus-noninoculated; ■, AM fungus-inoculated.

As for P concentration, storage roots accumulated more P than did feeder roots, irrespective of AM inoculation. No significant difference existed in P levels between feeder and storage roots from NAM and AM plants before or after FOA inoculation (Fig. 7).

Total fiber content in storage roots became relatively higher in AM plants, compared with NAM ones at 10 weeks after fungal inoculation; however, the situation was reversed 12 weeks after FOA inoculation. In this case, lignin content differed little between AM and NAM plots, the hemicellulose content was relatively lower in AM plot than in NAM one 12 weeks after FOA inoculation (Fig. 8). No major difference existed in total fiber content in feeder roots between AM and NAM plots.

Stotage roots from AM plots had higher total pectic substances than did the NAM ones, especially the WP content. HP content was relatively lower in AM than

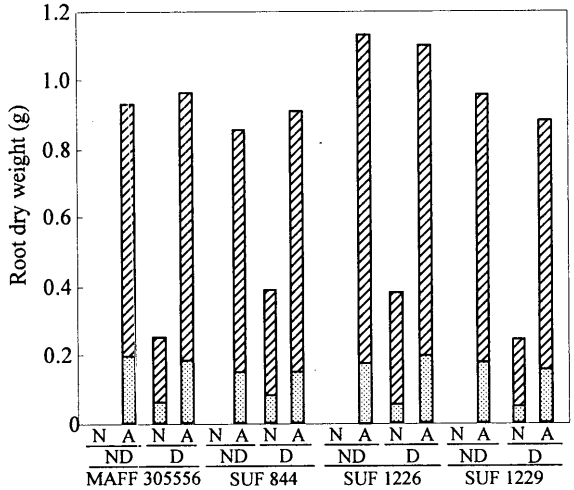

Fig. 5. Dry weight of roots in asparagus 12 weeks after FOA inoculation. N, AM fungus-noninoculated; A, AM fungus-inoculated. ND, non-diseased plants; D, diseased plants. ▨, feeder roots ; ▨, storage roots.

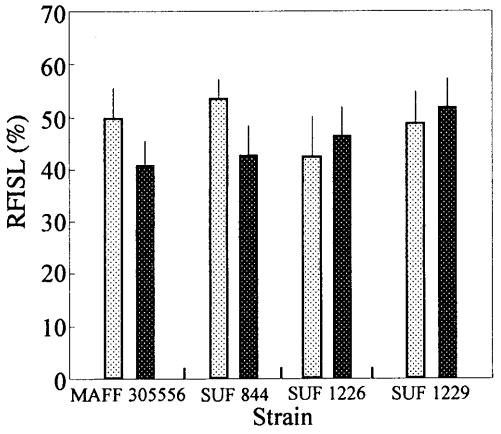

Fig. 6. Rate of AM fungus-infected segments in lateral roots (RFISL) of asparagus 12 weeks after FOA inoculation. ▒, non-diseased plants; ▓, diseased plants. Vertical bars represent SE (n=3).

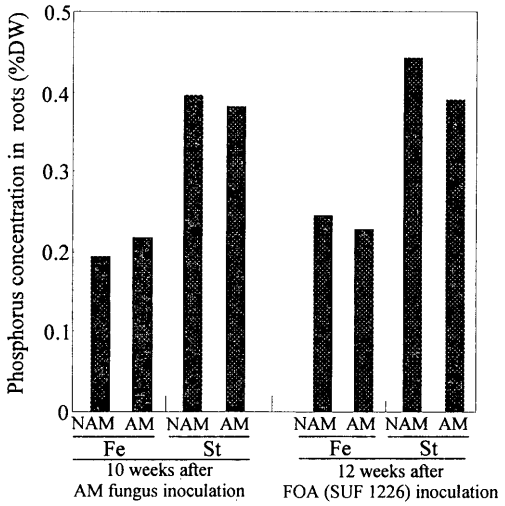

Fig. 7. Phosphorus concentration in asparagus roots 10 weeks after AM fungus inoculation and 12 weeks after FOA (SUF 1226) inoculation. NAM, AM fungus-noninoculated; AM, AM fungus-inoculated. Fe, feeder roots; St, storage roots.

NAM plots, but the PP content was prominently lower than WP and HP in all plots (Fig. 9). Total content of pectic substances in feeder roots from NAM plots resembled that in AM ones throughout the experiment. No major difference among individual pectic substances existed between those plots.

Discussion

In the previous study, asparagus plants infected with 3 AM fungal species showed tolerance to fusarium root rot caused by SUF1226; the AM species, *Glomus* sp. R10, especially, was effective (Matsubara et al., 2001). In this study, tolerance to fusarium root rot caused by 4 FOA strains was exhibited by *Glomus* sp. R10-infected asparagus plants, but the effect differed among FOA

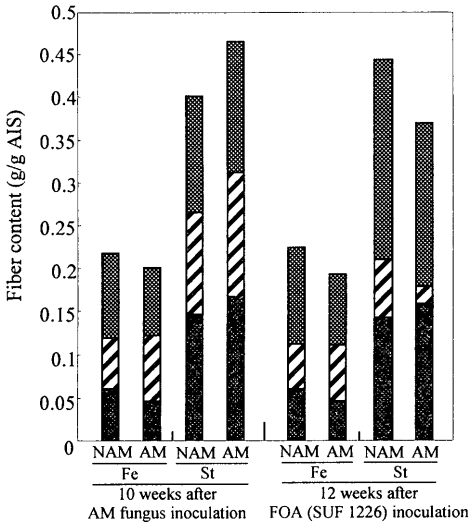

Fig. 8. Fiber content in asparagus roots 10 weeks after AM fungus inoculation and 12 weeks after FOA (SUF 1226) inoculation. NAM, AM fungus-noninoculatedj AM, AM fungus-inoculated. Fe, feeder roots; St, storage roots. ▒, cellulose; ▨, hemicellulose; ▓, lignin.

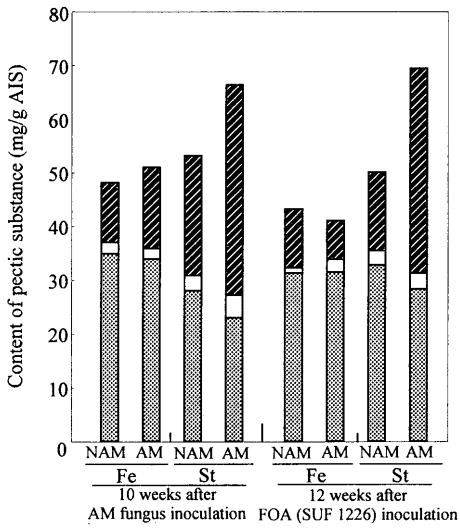

Fig. 9. Content of pectic substance in asparagus roots 10 weeks after AM fungus inoculation and 12 weeks after FOA (SUF 1226) inoculation. NAM, AM fungus-noninoculated; AM, AM fungus-inoculated. Fe, feeder roots; St, storage roots. ▨, water-soluble pectin (WP); □, hexametaphosphate-soluble pectin (PP); ▒, HCl-soluble pectin (HP).

strains. These results indicate that the root rot tolerance in asparagus depends on the combination of AM fungal species and FOA strains. Blok et al. (1997) reported that the incidence of fusarium root rot in asparagus plants was reduced by the inoculation of non-pathogenic isolates of *F. oxysporum*, though the effect differed with the strains. Our data supports their finding.

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), however, found no relationship between increased P concentration and the tolerance to *Fusarium* disease in AM fungus-infected tomato plants. Our data show that there is no difference in P concentration between AM and NAM plants in feeder and storage roots 10 weeks after AM fungus inoculation and 12 weeks after FOA inoculation, which indicate that P concentration in plants have little influence on the tolerance to fusarium root rot in AM fungus-infected plants.

Our results reveal that 1) fiber and pectic substances in feeder roots differed little between AM and NAM plots; 2) hyphae of AM fungus and FOA preferentially elongated into short cells in the dimorphic exodermis of feeder roots in asparagus, and 3) pre-infection with AM fungus in short cells reduced FOA infection (Matsubara et al., 2001). These findings suggest that suppression of FOA infection in short cells by pre-infection with AM fungus is closely associated with disease tolerance rather than the changes in fiber and pectic substances in feeder roots. However, a decrease of root rot occurred in AM plants but no mycorrhizal infection was found in the storage roots (Matsubara et al., 2001). Matsubara (1999) previously described that the structure of the exodermis of storage roots differed from that in feeder roots in asparagus. Thus, other factor(s), associated with AM fungal infection, resulted in the tolerance to fusarium in storage roots. Dehne and Schonbeck (1979b) reported that the lignification in endodermis and stele enhanced by AM fungal infection suppressed fusarium-wilt in tomato plants, but lignin content differed little between our AM and NAM asparagus plots.

However, WP that consists mainly of pectic acid and pectin, which are located in middle lamellae of root tissue, increased in storage roots of AM plots before and after FOA inoculation. Hence, AM fungal infection may indirectly induce an increase in WP in storage roots before FOA inoculation. Perhaps, the resulting rigidity of storage root tissue suppressed FOA infection in storage roots of AM plants. These questions on the possible relationship between pectic substances and root rot tolerance require additional study.

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Arbuscular菌根菌が感染したアスパラガスの立枯病耐性と根組織における繊維成分およびペクチン質含量との関連

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摘　要

Arbuscular菌根(AM)菌(*Glomus* sp. R10)が感染したアスパラガス(*Asparagus officinalis* L.)‘メリーワシントン500W’の立枯病耐性と根組織内における繊維成分およびペクチン質含量との関連について調査した．AM菌接種10週間後に立枯病菌(*Fusarium oxysporum* f. sp. *asparagi*; MAFF-305556, SUF844, SUF1226, SUF1229)を接種した．立枯病菌接種12週間後，発病個体率は立枯病菌の系統に関わらずAM菌接種区で無接種区より低くなり，発病指数は無接種区では70~90を示したのに対し，接種区では8~16と顕著に低下した．また，AM菌接種区の非罹病株および罹病株とも，無接種区の罹病株より吸収根および貯蔵根乾物重が増大した．一方，植物体中のリン酸含量および根組織内の繊維成分含量には，調査したAM菌接種10週間後(立枯病菌接種直前)と立枯病菌接種12週間後において処理区間に大きな差はみられなかった．しかし，ペクチン質含量については，吸収根では両処理区ほぼ同様であったが，貯蔵根中の総ペクチン質含量は両調査日ともAM菌接種区が無接種区より高かった．この場合，接種区の水溶性ペクチン含量の増大が顕著であった．これらのことから，AM菌が感染したアスパラガスの立枯病耐性は，貯蔵根組織中の水溶性ペクチンを主体とするペクチン質含量と関連があることが示唆された．