Increased Tolerance to Fusarium Wilt in Mycorrhizal Strawberry Plants Raised by Capillary Watering Methods

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Tolerance to fusarium wilt, caused by Fusarium oxysporum f. sp. fragariae (Fof), in strawberry (Fragaria × ananassa Duch., cv. Nohime) plants infected with arbuscular mycorrhizal (AM) fungi (Gigaspora margarita, Glomus fasciculatum, Gl. mosseae, Gl. sp. R10, Gl. aggregatum) was estimated under capillary watering conditions. Thirty days after Fof inoculation, the incidence of fusarium wilt ranged from a minimum of 22.2% in Gl. mosseae plot and a maximum of 100% in non-AM one; the incidence varied, depending on AM fungal species. Incidence and severity of browned vessels and roots became lower in AM plots than in non-AM one. Non-diseased and diseased AM plants had higher dry weight of shoots and roots than did diseased non-AM plants. No significant difference in phosphorus concentration in plants appeared between non-AM and AM plots 11 weeks after AM fungus inoculation (just before Fof inoculation) and 30 days after Fof inoculation. These findings suggest that tolerance to fusarium wilt occurred in AM fungus-infected strawberry plants, and the effect had less association with phosphorus concentration in plants.

Keywords: arbuscular mycorrhizal fungi, disease tolerance, fusarium wilt, strawberry, symbiosis

INTRODUCTION

In strawberry cultivation, fusarium wilt and anthracnose have been the serious diseases in the major strawberry producing regions in Japan, and the diseases caused great losses during nursery and fruit production period (Okayama, 1991; Tezuka and Makino, 1991; Mori and Kitamura, 2003). Recently, capillary watering as a cultural control method of these diseases has been rapidly introduced to strawberry cultivation, but the diseases are still difficult to control because no effective resistant variety or fungicide have been developed (Okayama, 1991; Akita, 2001). As for biological control of fusarium disease, non-pathogenic isolates of Fusarium oxysporum are tried to use for control in many vegetables such as asparagus (Blok et al., 1997), cucumber (Paulitz et al., 1987), celery (Schneider, 1984), and also attempted in strawberry (Tezuka and Makino, 1991). However, the method using non-pathogenic isolates is still not enough to control and the method has no growth promoting effect.

Arbuscular mycorrhizal (AM) fungi have the effect of promoting host plant growth mainly by enhancing phosphorus uptake through symbiosis (Gerdemann, 1964; Marschner and Dell, 1994). As for strawberry plants, growth enhancement through AM fungus inocula-
tion was reported in several combinations of AM fungal species and strawberry cultivars (Robertson et al., 1988; Chavez and Ferrera, 1990; Niemi and Vestberg, 1992; Williams et al., 1992; Varma and Schuepp, 1994). In addition, trials to inoculate *Phytophthora fragariae* to strawberry plants infected with AM fungus were reported and the effect of disease reduction differed with host cultivar and AM fungal species (Baath and Hayman, 1984; Mark and Cassells, 1996; Norman et al., 1996). On the other hand, Kobayashi (1988) and Singh et al. (2000) mentioned that AM fungus-infected plants showed tolerance to fusarium diseases in several crop plants except strawberry. However, it remains unclear whether strawberry plants infected with AM fungi have tolerance to fusarium wilt and the effect depends on AM fungal species.

In this study, tolerance to fusarium wilt in AM fungus-infected strawberry plants was investigated using five AM fungal species.

**MATERIALS AND METHODS**

_Inoculation of AM fungus._ Strawberry (*Fragaria×ananassa* Duch., cv. Nohime) plants were obtained by directly rooted runners from mother plants. Bedding soil, coconutshell medium (pH 5.5–6.0, autoclaved at 1.2 kg•cm⁻² and 121°C for 1 h), was packed in pot (10.5 cm in diameter) and strawberry runner plants were inoculated with *Gigaspora margarita* (GM), *Glomus fasciculatum* (Gf), *Gl. mosseae* (Gm), *Gl. sp. R10* (Gr), *Gl. aggregatum* (Ga), according to Matsubara et al. (1996), using commercial inocula; spore densities were 100 spores•g⁻¹ inoculum in GM and unknown in the others. Two weeks after AM fungus inoculation, the AM fungus-inoculated plants (AM plants) and non-inoculated control plants (NAM plants) were administered a mixed fertilizer (N : P : K= 13 : 11 : 13, 1 g per pot). Six plots, consisting of 30 plants per plot, were irrigated by capillary watering method as regularly in bench culture system and raised in a greenhouse.

_Inoculation with Fusarium oxysporum f. sp. fragariae._ *Fusarium oxysporum* f. sp. *fragariae* (Fof: strain 2S, derived from the diseased strawberry plants) were grown on potato-dextrose agar media. The conidia were harvested in potato-sucrose liquid media and incubated at 25°C in the dark for 7 days. The conidial suspension was sieved (45 µm) and its concentration adjusted to 10⁶ conidia•ml⁻¹. Each plant was then inoculated by pouring 50 ml of the conidial suspension on the soil 11 weeks after AM fungus inoculation. Eighteen plants per plot were raised in a growth chamber at 28±3°C, 60±5% relative humidity under natural light and photoperiod in September.

_Evaluation of AM fungal infection level._ Eleven weeks after AM fungus inoculation and 30 days after Fof inoculation, roots were sampled and stained according to Phillips and Hayman (1970) and the rate of AM fungal infections in 1-cm segments of lateral roots (RFISL) was calculated. Hence, RFISL expresses the percentage of 1-cm AM fungus-infected segments to the total 1-cm segments of all the lateral roots; the number of total segments was approx. 50 per a plant. The average was calculated from the values of 3 plants.

_Estimation of symptoms of fusarium wilt._ Thirty days after Fof inoculation, the symptoms of fusarium wilt in eighteen plants per plot were categorized into 6 degrees: 0, no symptom; frequency of diseased petiole in a plant: 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:

\[
\text{Disease index} = \frac{\sum(\text{number of plants} \times \text{degree of symptom})}{\text{Total number of plants}} \times 5
\]

_Determination of phosphorus in plants._ The P determination in plants was performed twice to investigate both the effect of AM fungus inoculation on P concentration in plants and
the persistence of the effect after Fof inoculation. Shoots and roots from 5 plants were sampled 11 weeks after AM fungus inoculation and 30 days after Fof inoculation to determine P. Dry matter was weighed after drying at 110°C for 2 days. The samples were ground, wet-ashed, and their P concentrations determined, according to Matsubara and Harada (1996).

RESULTS

Thirty days after Fof inoculation, symptoms of fusarium wilt were observed in all the plots (Fig. 1). The incidence of fusarium wilt ranged from a minimum of 22.2% in Gm plot and a maximum of 100% in NAM; the incidence varied with AM fungal species. As for the severity of symptoms, diseased plants of 4 and 5 degrees in symptoms appeared in NAM plot, however, the severity became lower in AM plots than in NAM one. In this case, the severity differed among the AM fungal species and it was significantly low in Gm and Ga plots. Thirty days after Fof inoculation, the disease index reached 91.1 in NAM plot, while it was

![Fig. 1](image1.png)

![Fig. 2](image2.png)

![Fig. 3](image3.png)

![Fig. 4](image4.png)

low as 6.6 in Gm plot (Fig. 2). Hence, the disease indices and incidence of fusarium wilt for the AM species followed a similar pattern. Incidence of browned vessels became 100% and the plants with maximum degree of symptom reached more than 50% in NAM plot (Fig. 3). In AM plots, incidence and extent of browned vessels were decreased, and Gm plot had the lowest incidence among the AM plots. On the other hand, incidence of browned roots became 100% in all the plots (Fig. 4). In this case, the plants with all-browned roots appeared more than 60% in NAM plot, while AM plots had lower severity of browning than did NAM.
one, especially in Gm and Ga plots. As for dry weight of plants, AM plots showed higher values both shoots and roots than did NAM ones 11 weeks after AM fungus inoculation; dry weight of GM plot was relatively lower than the other AM fungal ones (Fig. 5). Thirty days after Fof inoculation, both non-diseased and diseased AM plants showed higher dry weight of shoots and roots than did diseased NAM ones. RFISL became lowest in Gr among the AM fungal species both 11 weeks after AM fungus inoculation and 30 days after Fof inoculation in non-diseased and diseased plants. RFISL differed little between non-diseased and diseased plants except Gr 30 days after Fof inoculation (Fig. 6).

Eleven weeks after AM fungus inoculation, shoots of GM plot accumulated most phosphorus among all the plots, however, the P level in NAM plot was higher than in the other AM fungal plots except GM; P concentration in roots was highest in NAM plot (Fig. 7). Thirty days after Fof inoculation, P concentrations in shoots and roots were higher in NAM plot than in the most of the AM ones.

**DISCUSSION**

In the present study, growth promotion through AM fungal symbiosis appeared in strawberry plants, and both incidence and severity of symptoms of fusarium wilt were reduced by pre-infection with AM fungi. These findings indicate that tolerance to fusarium wilt was increased by AM fungi. The effect differed, however, with the AM fungal species with Gm being the most effective. Norman et al. (1996) reported that the incidence of the symptom caused by *Phytophthora fragariae* in strawberry plants was reduced by the inoculation of AM fungi though the effect differed with the AM fungal species. Our data supports their finding, but we could not clarify the difference in the tolerance to fusarium wilt among AM fungal species. Mark and Cassells (1996) mentioned that no relationship between AM fungal infection level in roots and the tolerance to *Phytophthora fragariae* occurred in strawberry plants. In our study, we could not investigate the competition in infection between AM fungi and Fof, further observations would be needed to clarify that point. As for the relationship between phosphorus concentration and disease tolerance, 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 characteristic difference in P concentration between AM and NAM plants, which indicate that P concentration in plants have little influence on the tolerance to fusarium wilt in AM fungus-infected plants. Thus, some other factor caused by AM fungal infection resulted in the tolerance to fusarium wilt.

Dehne and Schonbeck (1979) reported that the lignification in endodermis and stele enhanced by AM fungal infection suppressed fusarium wilt in tomato plants. 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*. On the other hand, Matsubara et al. (2003) reported that pectic substances in asparagus roots were increased by AM fungal infection, and supposing that
resulting rigidity of root tissue suppressed fusarium infection. Thus, some physiological and histological factors may be associated with the disease tolerance in AM fungus-infected plants.

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.

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REFERENCES


（和文抄録）

底面吸水育苗イチゴにおける菌根共生並びに萎黄病耐性

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イチゴ（Fragaria × ananassa, Duch., cv. 濃姫）のランナーにarbuscular菌根菌5菌種（Gigaspora margarita, Glomus fasciculatum, GL. mosseae, GL. sp. R10, GL. aggregatum）を接種し、菌根共生および萎黄病耐性を調査した。菌根菌接種11週間後に萎黄菌（Fusarium oxysporum f. sp. fragariae）を接種した。萎黄菌接種30日後、菌根菌無接種区における発病率は100％に達し、発病指数も90を上回った。一方、菌根菌接種区では菌種にかかわらず発病率および発病指数とも無接種区より低く、発病抑制効果には菌種間差がみられた。この場合、最も抑制効果が高かったのはGL. mosseae区の発病率22.2％、発病指数6.6であった。また、導管部および根の褐変程度についても特にGL. mosseae区で軽減され、植物体乾物重では、萎黄菌接種前後で菌根菌接種区が無接種区を上回った。菌根菌感染植物体の耐病性機構については不明な点が多く、耐性因子とされる植物体リン酸含量と本研究における耐病性との間に一貫した特徴はみられなかったことから、植物体リン酸含量の本耐性性への関与は低いことが示唆された。