Influence of Arbuscular Mycorrhizal Fungi and Sodium Chloride on Fusarium Root Rot and Antioxidative Abilities in Asparagus Plants

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Influence of arbuscular mycorrhizal fungi [AMF, \textit{Glomus} sp. R10 (Gr) and \textit{Gigaspora margarita} (GM)] and sodium chloride (NaCl) on \textit{Fusarium} root rot and antioxidative abilities in asparagus (\textit{Asparagus officinalis} L., ‘Welcome’) plants was investigated. AMF plants accumulated higher dry weight of shoots than non-AMF plants with or without NaCl treatment during 16 weeks after AMF inoculation. Ten weeks after \textit{Fusarium oxysporum} \textit{f. sp. asparagi} (Foa, MAFF305556, N9-31, SUF1226, and SUF844) inoculation, the incidence and severity of \textit{Fusarium} root rot were eased in AMF plants; most of the Gr plants showed lower severity than GM. In this case, a synergetic effect in disease suppression occurred in some of the AMF plants treated with NaCl. As for antioxidative ability, SOD activity of shoots and roots increased in most of the Gr plants in both NaCl- and non-treated plants 10 weeks after Foa inoculation. Gr plants had higher DPPH radical scavenging activity of shoots and roots than non-mycorrhizal plants with or without NaCl, especially 16 weeks after AMF inoculation. Ascorbic acid contents of shoots and roots increased in most of the Gr plants without NaCl treatment both 16 weeks after AMF and 10 weeks after Foa inoculation. From these findings, AMF induced a growth promotion effect and alleviation of \textit{Fusarium} root rot in asparagus plants, and the synergetic effect of disease suppression could be expected by the combination use of AMF and NaCl. In addition, it supposed that changes in antioxidative ability might be associated with disease reduction in mycorrhizal asparagus plants.

Key Words: asparagus decline, \textit{Fusarium oxysporum} \textit{f. sp. asparagi}, growth promotion, symbiosis.

Introduction

Asparagus decline is a serious and increasing threat in asparagus producing regions throughout the world (Hamel et al., 2005; Knaflowski et al., 2008; Reid, et al., 2001; Wong and Jeffries, 2006). Asparagus decline is supposed to be caused by the contribution of both biotic (disease) (Knaflowski et al., 2008; Wong and Jeffries, 2006) and abiotic (allelopathy etc.) factors (Lake et al., 1993; Miller et al., 1991; Yong, 1984). As biotic factors, the most common phenomenon is \textit{Fusarium} crown and root rot, caused by \textit{Fusarium oxysporum} \textit{f. sp. asparagi} (Foa), \textit{Fusarium proliferatum} (Fp), and \textit{Fusarium redolens} etc. (Knaflowski et al., 2008; Reid et al, 2002; Wong and Jeffries, 2006). In Japan, Nahiyani et al. (2011) demonstrated that Foa and Fp are dominant \textit{Fusarium} species in asparagus decline fields by PCR-SSCP analysis. The diseases are still difficult to control because no resistant cultivar or disinfecting method has been developed. On the other hand, biological control of \textit{Fusarium} disease was tried by inoculation with non-pathogenic isolates of the \textit{Fusarium} species (Blok et al., 1997; Elmer, 2004); however, the method is not enough for control and has no growth promoting effect.

Arbuscular mycorrhizal fungus (AMF) has the effect of promoting host plant growth, mainly by enhancing phosphorus uptake through symbiosis (Marschner and Dell, 1994). Previously, we reported disease reduction of \textit{Fusarium} root rot in mycorrhizal asparagus (‘MW500W’) plants (Matsubara et al., 2003). On the other hand, chemical control of \textit{Fusarium} disease was also tried by treatment of sodium chloride (Elmer, 1992; Reid et al., 2001), however, many points remain unclear about the mechanisms of disease reduction in sodium chloride (NaCl) and AMF-treated asparagus plants. Generally, promotion of active oxygen synthesis in plants occurs under environmental stresses, such as plant disease, salinity, high temperature, and drought, and the antioxidative abilities are related with the resolution (Li...
Materials and Methods

Inoculation of AMF

Seeds of asparagus (Asparagus officinalis L., ‘Welcome’) were inoculated with 2 AMF species [Glomus sp. R10 (Gr), Gigaspora margarita (GM), supplied by Idemitsukosan Co., Ltd., Japan for Gr, and Centralgrass Co., Ltd., Japan for GM] according to Matsubara et al. (2003). The inoculated plants (AMF+) and the uninoculated control plants (AMF−) were raised in autoclaved commercial soil and administered mixed fertilizer (N : P : K = 13 : 11 : 13, 0.5 g per plant). Forty plants per plot with three replications were irrigated regularly and grown in a greenhouse.

Treatment with sodium chloride

Treatment with sodium chloride (NaCl) was carried out according to the method of Reid et al. (2001). From 8 weeks after AMF inoculation, NaCl (50, 100 mM) was added (10 mL/plant, NaCl+) to bed soil once a week until Foa inoculation (16 weeks after AMF inoculation). Non-NaCl-added (NaCl−) plants were treated with distilled water.

Inoculation of Fusarium oxysporum f. sp. asparagi

Four isolates of Foa (N9-31, MAFF305556, SUF1226, and SUF844) 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 and the concentrations adjusted to 10^6 conidia per mL. Sixteen weeks after AMF inoculation, each plant was inoculated with 50 mL of the conidial suspension onto the roots.

Estimation of symptoms of Fusarium root rot

Ten weeks after inoculation of Foa, the symptoms of Fusarium root rot were rated as follows: 0, no symptoms; frequency of diseased storage roots in a root system: 1, <20%; 2, 20–40%; 3, 40–60%; 4, 60–80%; 5, 80–100%.

Evaluation of AMF colonization level

Sixteen weeks after AMF inoculation and 10 weeks after inoculation of Foa, roots of asparagus were preserved with 70% ethanol and stained according to Phillips and Hayman (1970). The rate of AMF colonization in 1-cm segments of lateral roots (RFCSL) was calculated. Hence, RFCSL expresses the percentage of 1-cm AMF-colonized segments to the total 1-cm segments of all lateral roots; the number of total segments was approx. 30 per plant. Average colonization was calculated from the values of 5 plants.

Determination of antioxidative abilities

Sixteen weeks after AMF (Gr) inoculation and 10 weeks after inoculation of Foa (N9-31), plants were sampled and partitioned into shoots and roots from 10 plants, and all samples were frozen in liquid nitrogen until use. Antioxidative enzyme activities and antioxidative substances were analyzed by the following methods.

1. SOD activity

SOD activity was determined using the Nitro Blue Tetrazolium (NBT) reduction method (Beauchamp and Fridovich, 1971). One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of NBT reduction measured at 560 nm using a spectrophotometer (U-1900, HITACHI, Tokyo, Japan).

2. APX activity

APX activity was determined according to Wu et al. (2006). One unit of activity was defined as the amount of oxidized L-ascorbic acid 1 min after the addition of H_2O_2 solution.

3. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity

DPPH radical-scavenging activity was measured according to the method of Brurits and Bucar (2000). The activity was calculated as the percent of inhibition relative to the control.

4. Polyphenol content

Polyphenol content was determined as quercetin equivalent using an equation obtained from a standard curve against quercetin according to the Folin-denis method (1915).

5. Ascorbic acid content

The assay was performed by the 2,4-dinitrophenyl hydrazine method (Roe et al., 1948). Ascorbic acid content was determined using an equation obtained from a standard curve against L-ascorbic acid.

Results

Sixteen weeks after AMF inoculation, AMF+ (Gr and GM) plants had higher dry weight of shoots than AMF− plants in NaCl− plots, regardless of the fungal spices (Fig. 1). In NaCl+ plots, the dry weight of shoots in AMF+NaCl+ and roots in Gr+NaCl 50 increased compared to AMF−NaCl+; no significant difference occurred in the dry weight of shoots and roots by NaCl treatment in control plants (AMF−NaCl−). AMF colonization was confirmed in all the inoculated plants, and no colonization occurred in AMF− plants.
Colonization levels reached more than 60% in all the plots 16 weeks after AMF inoculation; no difference appeared between Gr and GM (Fig. 2). As for disease incidence, AMF−NaCl− plants showed 100% incidence and the most severe symptoms in each Foa isolates (Fig. 3); no disease symptoms appeared in plants without Foa inoculation (data not shown). However, Gr+ and GM+ plants with or without NaCl showed lower incidence and severity than AMF− plants in all the Foa isolates, except GM+NaCl 100 in SUF1226. In AMF− plants, severity was eased in NaCl+ than NaCl− plots; in addition, some of the AMF+NaCl+ plots showed lower severity than AMF+NaCl−, especially NaCl 50 plots.

In the analysis of antioxidative abilities, SOD activity in roots had decreased by NaCl treatment in AMF− plots 16 weeks after AMF inoculation; however, Gr+NaCl 100 showed higher SOD activity in both shoots and roots than Gr+NaCl− (Fig. 4). APX activity in shoots of Gr+NaCl 50 became higher than AMF−NaCl 50, but no difference appeared in roots among the plots. All the Gr+ plants showed higher DPPH radical-scavenging activity in shoots and roots than AMF− plants in each plot. No difference occurred in polyphenol contents in most of the plots. In AMF+ plots, ascorbic acid contents were higher in shoots of Gr+NaCl 50 and 100, and roots in Gr+NaCl 0 than AMF− plants. On the other hand, 10 weeks after Foa inoculation, SOD activity of both shoots and roots increased in Gr+ plants compared to AMF− plants with or without NaCl, except roots in NaCl 100 (Fig. 5). In APX activity, no increase occurred in Gr+ plants compared to AMF− among the treatments. In Gr plots with NaCl 0 and 100, DPPH radical-scavenging activity became higher in roots of Gr+NaCl 0, 100 plants than AMF−. Polyphenol contents showed no difference most of the plots. Ascorbic acid contents in shoots of AMF−NaCl 50, 100 were higher than AMF−NaCl−, and Gr+NaCl− plants had higher contents in both shoots and roots than AMF−NaCl−.

**Discussion**

In this study, the dry weight of shoots increased in AMF+NaCl− plants compared to AMF−NaCl− plants. In addition, AMF+NaCl+ plants showed higher dry weight of shoots than AMF−NaCl+ plants. From these findings, growth promotion effect through symbiosis appeared in mycorrhizal asparagus plants in both NaCl+ and NaCl− conditions. Porras-Soriano et al. (2009) reported that the dry weight of shoots and roots increased in mycorrhizal olive plants compared to control plants under NaCl treatment. They also mentioned that no significant difference occurred in AMF colonization levels by NaCl treatment, which supposed that reduction of salt stress appeared in mychorrhizal plants. Our results partially agreed with the findings and suggested that AMF could induce growth promoting effect in host plants under NaCl treatment. In addition, it is expected that AMF might induce the reduction of salt stress on horticultural plants. Recently, salt stress has been used...
to increase functional constituents, such as sugar and amino acids; however, salt stress resulted in growth reduction and a decrease in yield and fruits size in tomato (Kitano et al., 2008). In our results, growth promoting effect under NaCl treatment appeared in some of the mycorrhizal asparagus plants, and ascorbic acid contents increased in the shoots of mycorrhizal plots. From these findings, AMF might have the potential to enhance plant growth and increase functional constituents in host plants under NaCl treatment.

Matsubara et al. (2003) reported that mycorrhizal asparagus (‘MW500W’) plants showed lower incidence and severity of Fusarium root rot than control. In addition, NaCl-treated asparagus plants had lower severity of Fusarium root rot symptoms than non-NaCl plants (Elmer, 2004; Reid et al., 2001). Most of our results in ‘Welcome’ agreed with those findings, and additionally, synergetic effects on the alleviation of Fusarium root rot symptoms using AMF and NaCl were confirmed. In this study, NaCl treatment was carried out according to Reid et al. (2001), and NaCl 50 showed better results than NaCl 100. However, it is necessary to investigate sustainable method of NaCl treatment including the chemical properties of soil to induce growth enhancement and disease suppression under field conditions. In our results, AMF promoted the growth of asparagus plants during 16 weeks after AMF and 10 weeks after Foa (data not shown) inoculation. In addition, both the incidence and severity of symptoms in Foa were alleviated by pre-colonization with Gr and GM. Ozgonen and Erkilic (2007) reported that growth promotion and reduction of Phytophthora capsici had no correlation with the mycorrhizal colonization level in peppers. Lozano et al. (1996) reported that alleviation of drought showed no correlation with the mycorrhizal colonization level in lettuce. In our results, most of the Gr plants showed lower symptoms of Fusarium root rot than GM among the 4 Foa isolates, with no significant difference in colonization level between the 2 species. Thus, the colonization level might have less association with the reduction of Fusarium root rot in this study.

On the other hand, some reports described that the AMF colonization level induced antioxidative abilities, such as SOD, guaiacol peroxidase (G-POD), catalase (CAT), APX, and flavonoid content, suggesting that colonization might be temporary stress for host plants
Our results showed that SOD activity of shoots increased in some of the Gr+ plants, and DPPH radical-scavenging activity had increased in both shoots and roots in all Gr+ plants 16 weeks after AMF inoculation. However, other antioxidative abilities, such as APX activity and polyphenol content, were not stimulated, so is difficult to assess whether AMF colonization is a stress factor for asparagus plants.

SOD plays a primary role in defensive reactions and detoxifies superoxide (O$_2^-$) among the antioxidative enzymes; thus, SOD activity is considered the most important key enzyme in antioxidative abilities in plants (Fridovich, 1986). Garmendia et al. (2006) reported that alleviation of *Verticillium dahliae* and an increase in SOD activity occurred in mycorrhizal pepper plants. Moghaddam et al. (2006) mentioned that SOD activity was higher in a resistant strawberry cultivar than susceptible cultivars with *Mycosphaerella fragariae* infection. In the present study, alleviation of *Fusarium* root rot appeared in mycorrhizal asparagus plants; in addition, SOD activity and several antioxidative abilities were stimulated in some parts of mycorrhizal plants without NaCl. From these results, changes in antioxidative ability might be associated with the suppression of *Fusarium* root rot in mycorrhizal asparagus plants; however, in this experiment, antioxidant analysis was carried out only twice, a long time after Foa inoculation, so that it is difficult to clarify detailed relationship between reduction of disease symptoms and antioxidative ability. Further investigation is needed on this point.

In our results, suppression of *Fusarium* root rot appeared in AMF–NaCl+ plants; however, most of the antioxidative abilities, including SOD activity, were not stimulated by NaCl treatment. These findings supposed that the mechanisms of disease reduction in NaCl-treated asparagus plants might differ from those in mycorrhizal asparagus plants. On the other hand, Pozo et al. (2002) reported that in tomato plants with a split root system, suppression of *Phytophthora parasitica* appeared in both non-AMF-inoculated roots and inoculated roots in AMF plants, so that induced systemic disease suppression was recognized. In this study, some of the antioxidative abilities increased in shoots, where no colonization occurred. From these findings, we will assess the induced
systemic disease suppression in mycorrhizal asparagus plants with split root system, and the relationship between antioxidative ability and induced disease suppression.

Our results suggest that AMF could inhibit symptoms of *Fusarium* root rot in asparagus plants, and synergetic effect of disease suppression could be expected by the combination use of AMF and NaCl. This proposal seeks to develop a sustainable practice to manage the disease and improve plant health, thus contributing to improve asparagus decline.

**Literature Cited**


