Original research article

Changes in SOD isozyme in mycorrhizal asparagus inoculated with *Fusarium oxysporum*

Jia Liu[1] and Yoh-ichi Matsubara[2]

[1]The United Graduate School of Agricultural Science, Gifu University, Gifu 501-1193, Japan
[2]Faculty of Applied Biological Sciences, Gifu University, Gifu 501-1193, Japan

Corresponding author: Yoh-ichi Matsubara, E-mail: ymatsu@gifu-u.ac.jp, Phone: +81-58-293-2887, Fax: +81-58-293-2887

Received on May 10, 2016; Accepted on September 26, 2016

Abstract: Symbiosis-specific changes in antioxidative ability and superoxide dismutase (SOD) isozyme in mycorrhizal asparagus plants through the inoculation with *Fusarium oxysporum* f. sp. *asparagi* (Foa, MAFF305556) were investigated. Dry weight of shoots and roots increased more in AMF (Gi, *Glomus intraradices*; GM, *Gigaspora margarita*)-inoculated plants than the control. Eight weeks after Foa inoculation, the incidence and severity of Fusarium root rot were alleviated in both AMF-inoculated plants, compared to the control, which were not alleviated. Superoxide dismutase (SOD) activity, ascorbate peroxidase (APX) activity, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity increased in both AMF-inoculated plants more than in the control before and after Foa inoculation, and increased in all asparagus roots due to Foa inoculation. As for SOD isozymes in roots before Foa inoculation, the Rf = 0.37 (Cu/Zn-SOD) band had a higher intensity in both Gi and GM compared to the control. Eight weeks after Foa inoculation, Rf = 0.33 and 0.37 (Cu/Zn-SODs) showed a higher intensity in both Gi and GM compared to the control. Though AM fungal difference appeared in the intensity of Rf = 0.25 (Mn/Fe-SOD); GM had a higher intensity than Gi, Cu/Zn bands had a clearly higher intensity than Mn/Fe band. These findings supposed that tolerance to Fusarium root rot in mycorrhizal asparagus plants might be closely associated with the changes in SOD activity and isozymes, especially in Cu/Zn-SODs.

Keywords: Arbuscular mycorrhizal fungi, Biocontrol, *Fusarium oxysporum* f. sp. *asparagi*, SOD isozyme

Introduction

Fusarium crown and root rot, caused mainly by *Fusarium oxysporum* f. sp. *asparagi* (Foa), results in early decline of yields in cultivated asparagus (He et al. 2002, Elmer 2015). It is difficult to control this disease successfully with cultural and chemical methods due to the perennial nature of the crop, and highly resistant cultivars have not been developed (Pontaroli et al. 2000, He et al. 2002, Elmer 2015).

Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil inhabitants, and form a symbiotic relationship with the roots of most of the terrestrial plants. AMF promotes host plant growth by enhancing phosphorus uptake through symbiosis (Marschner and Dell 1994), and hence is an alternative to high inputs of chemical fertilizers in sustainable crop production systems. Additionally, several investigators earlier reported a higher resistance of mycorrhizal plants to biotic and abiotic stresses (Garmendia et al. 2006, Wu et al. 2006). As for asparagus, Wacker et al. (1990) reported that application of AMF in greenhouse trials was directly associated with the reduced damage from crown and root rot, and Liu et al. (2016) demonstrated the tolerance to Fusarium root rot in mycorrhizal asparagus plants in decline fields. However, many points remain unclear about the mechanisms of disease tolerance in mycorrhizal plants.

Liu J and Matsubara Y 2016 Changes in SOD isozyme in mycorrhizal asparagus inoculated with *Fusarium oxysporum*. Plant Root 10:26-33. doi:10.3117/plantroot.10.26

When plants suffer pathogenesis infection, production of a higher concentration of reactive oxygen species (ROS) such as H_2O_2, superoxide anion (O_2^-), and hydroxyl radical has been shown to create cytotoxic conditions (Sahoo et al. 2007, Wahid and Close 2007). To overcome this negative consequence of ROS, plants have evolved various protective mechanisms either to reduce or completely eliminate antioxidative abilities of producing antioxidant enzymes and substances (Moghaddam et al. 2006, Sahoo et al. 2007). The superoxide dismutase (SOD) plays a primary role in defensive reactions and detoxifies superoxide (O_2^-) among the antioxidant enzymes; thus, SOD activity is considered the most important key enzyme in a plant's antioxidant ability (Fridovich 1986). In addition, SODs are classified into three different types in plants, depending on their site and metal ion: mitochondrial manganese (Mn-SODs), cytosolic and chloroplasts copper/zinc (CuZn-SODs), and chloroplastic iron forms (Fe-SOD); these isoenzymes differ in their sensitivity to H_2O_2 and KCN and can be easily distinguished by gel electrophoresis (Fridovich 1986).

In mycorrhial plants, in *Pisum sativum-G. mosseae* symbiosis, no qualitative differences among the SOD isozyme patterns in both mycorrhizal and non-mycorrhizal roots were observed, although a higher SOD activity was detected in mycorrhizal pea roots (Arines et al. 1994). Pozo et al. (2002) reported that Mn-SOD as a new isozyme was detected in mycorrhizal (*G. mosseae*) tomato plants before and after *Phytophthora parasitica* inoculation without SOD activity analysis. However, studies on the relationship between SOD activity and SOD isozyme changes through AMF symbiosis and pathogen-inoculated mycorrhizal plants are still rarely reported. In addition, there are no reports on the changes in SOD isozymes in mycorrhizal plants inoculated with *Fusarium oxysporum* as a major pathogen, including AM fungal difference.

We aimed to clarify symbiosis-specific changes in SOD activity and isozymes in mycorrhizal asparagus plants (*Asparagus officinalis* L., cv. Welcome, susceptible to Fusarium root rot) by *Fusarium oxysporum* f. sp. *asparagi* (Foa) inoculation. Firstly, changes in antioxidative enzymes [activity of SOD and ascorbate peroxidase (APX) activity], 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (frequently estimated as total antioxidative substance) by AMF and Foa inoculation were determined. Then, SOD isozymes in mycorrhizal asparagus plants through the inoculation with Foa were investigated.

Materials and Methods

Inoculation of AMF

Seeds of asparagus (*Asparagus officinalis* L., cv. Welcome, susceptible to Fusarium root rot) were sown in commercial soil (autoclaved at 1.2 kg cm^{-2} and 121°C for 1 hour) in plastic container (19.0W × 33.5L × 15.5H cm). During the time of seed sowing, plant holes were made, and each hole contains 3 g $plant^{-1}$ commercial AMF of *Glomus intraradices* (Gi, supplied by Idemitsukosan Co. Ltd. Tokyo, Japan.), *Gigaspora margarita* (GM, supplied by Centralgrass Co. Ltd. Tokyo, Japan.). After sowing, plant holes were covered with soil and administered by a mixed fertilizer (N: P: K = 13: 11: 13, 0.5 g $plant^{-1}$). Twenty plants per plot with three replications were irrigated regularly and grown in a greenhouse. Eight weeks after AMF inoculation, the dry weight of the shoots and roots were investigated. The average was calculated from the values of 10 plants in each replication.

Inoculation of Fusarium oxysporum f. sp. asparagi

Fusarium oxysporum f. sp. *asparagi* (Foa; MAFF305556) were grown on potato dextrose agar media. The conidia were harvested in potato sucrose liquid media and incubated at 25°C in the dark condition for 7 days. The conidial suspension was sieved and the concentrations adjusted to 10^6 conidia mL^{-1}. Eight weeks after AMF inoculation, each plant was inoculated by pouring 50 mL of the conidial suspension onto the soil. Eight weeks after Foa inoculation, the symptoms were categorized into five degrees: of percentage of storage roots with root lesions in a plant: 1, less than 20%; 2, 20-40%; 3, 40-60%; 4, 60-80%; 5, 80-100%. In addition, the disease index was calculated by the following formula:

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

Evaluation of AMF colonization level

Eight weeks after AMF-inoculation and 8 weeks after Foa inoculation, the roots of the asparagus were preserved with 70% ethanol and stained according to Phillips and Hayman (1970). The percentage of AM fungal colonization in 1-cm segments of the lateral roots (abbreviated PFCSL) was calculated from approximately 30 samples of 1-cm segments per plant, for five plants per plot and time point.

Analysis of antioxidative ability

Eight weeks after AMF inoculation and 8 weeks after Foa inoculation, plants were sampled and partitioned into shoots and roots. Then, the samples were frozen in liquid nitrogen. Antioxidative abilities were analyzed by the following methods.

As for SOD activity, aliquots (0.1 g) of the roots and shoots were homogenized in 4 mL of 50 mM phosphate-borate buffer (pH 7.8). The filtrate was centrifuged (EF-1300, Tomy Co., Ltd., Tokyo, Japan) at 13,000 rpm for 10 min. The supernatant was used for crude enzyme extract. The activity was determined using the Nitro Blue Tetrazolium (NBT) reduction method (Beauchamp and Fridovich, 1971).

APX was extracted with 3 mL of an ice-cold 50 mM phosphate buffer (pH 7.0), and the filtrate was centrifuged at 13,000 rpm for 15 min and determined using the method of Wu et al. (2006) by measuring the oxidation of ascorbate at 290 nm using a spectrophotometer (U-1900, HITACHI).

DPPH radical scavenging activity was measured according to the method of Burits and Bucar (2000). A one gram sample was ground in 40 mL of 90% methanol. The 10 µL of the extract was introduced into test tubes, and 2.7 mL of DPPH solution was added. The tubes were mixed and left to stand for 30 min in the dark. Absorbance was read against a blank at 520 nm using a spectrophotometer (U-1900, HITACHI).

SOD isozyme was measured according to the method of Davis (1964) and Sohoo et al. (2007). Roots (0.5 g, fresh weight) were frozen in liquid nitrogen and pulverized in a mortar with 3 ml extraction buffer containing 75 mg L^{-1} polyoinylpolypyrrolidone (PVPP), 12.1 g/L Tris, 68 g L^{-1} sucrose, 170 mg L^{-1} EDTA, 0.031 mL L^{-1} Tween 80 and 800 mg L^{-1} sodium-thioglycolate. The filtrate was centrifuged at 13,000 rpm for 15 min in 5 °C and the supernatant (18 µL) was mixed with 2 µL 40% sucrose and 1 µL BPB. All extracts were analyzed by a 12.5% polyacrylamide gel (e-PAGEL E-T 12.5L, ATTO Co., Ltd., Tokyo, Japan). Electrophoresis (PAGE) under native conditions (100 V, 20 mA, 130 min) using an electrophoresis apparatus (AE-6500, ATTO Co., Ltd., Tokyo, Japan) was carried out. SOD isoforms were detected directly on the gel after electrophoresis by the method described by Beauchamp and Fridovich (1971) based on the inhibition to the staining buffer. The relative distance (Rf value) of the bands was derived from the mean value of many ˜runs″, calculated after assigning Rf = 1 to the fastest band and Rf = 0 to the start point of the ˜runing″gel (Mangaris and Alston 1992).

Statistical analysis

Mean values were separated by t test or Tukey's multiple range test at $P < 0.05$. All analyses were performed using XLSTAT pro statistical analysis software (Addinsoft, New York, NY).

Result

Eight weeks after AMF inoculation, the dry weight of the shoots and roots of AMF plants significantly increased compared to the control, especially in Gi (Fig. 1). AMF colonization occurred in all the inoculated plants successfully. RFCSL reached more than 60% in both AMF plants, and no significant difference occurred among the plots throughout the experimental period (Fig. 2). In the analysis of

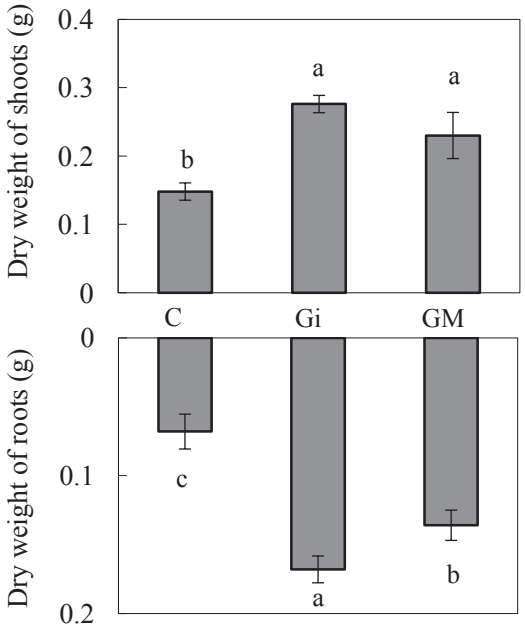

Fig. 1. Dry weight of shoots and roots in asparagus plants 8 weeks after AMF inoculation. C, control; Gi, *Glomus intraradices*; GM, *Gigaspora margarita*. Bars represent standard errors (n = 10). Columns denoted by different letters indicate significant difference according to Tukey's test ($P < 0.05$).

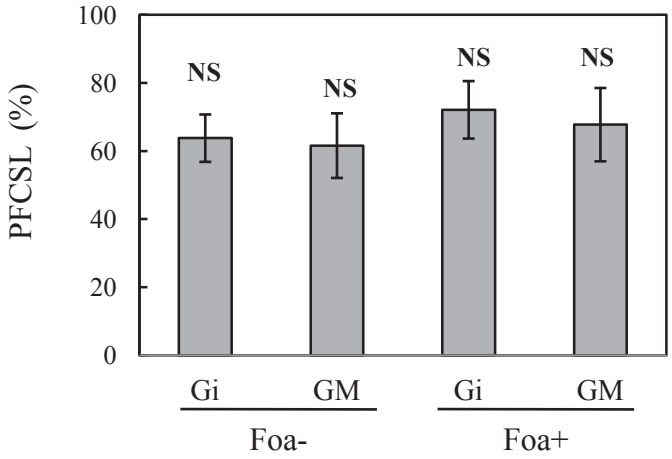

Fig. 2. AMF colonization level (PFCSL) in asparagus plants before and after *Fusarium oxysporum* f. sp. *asparagi* (Foa) inoculation. Gi, *Glomus intraradices*; GM, *Gigaspora margarita*. Foa-, before *Fusarium oxysporum* f. sp. *asparagi* inoculation; Foa+, after *Fusarium oxysporum* f. sp. *asparagi* inoculation. Bars represent standard errors (n = 5). NS, indicate no significant difference among the treatments according to Tukey's multiple range test ($P < 0.05$).

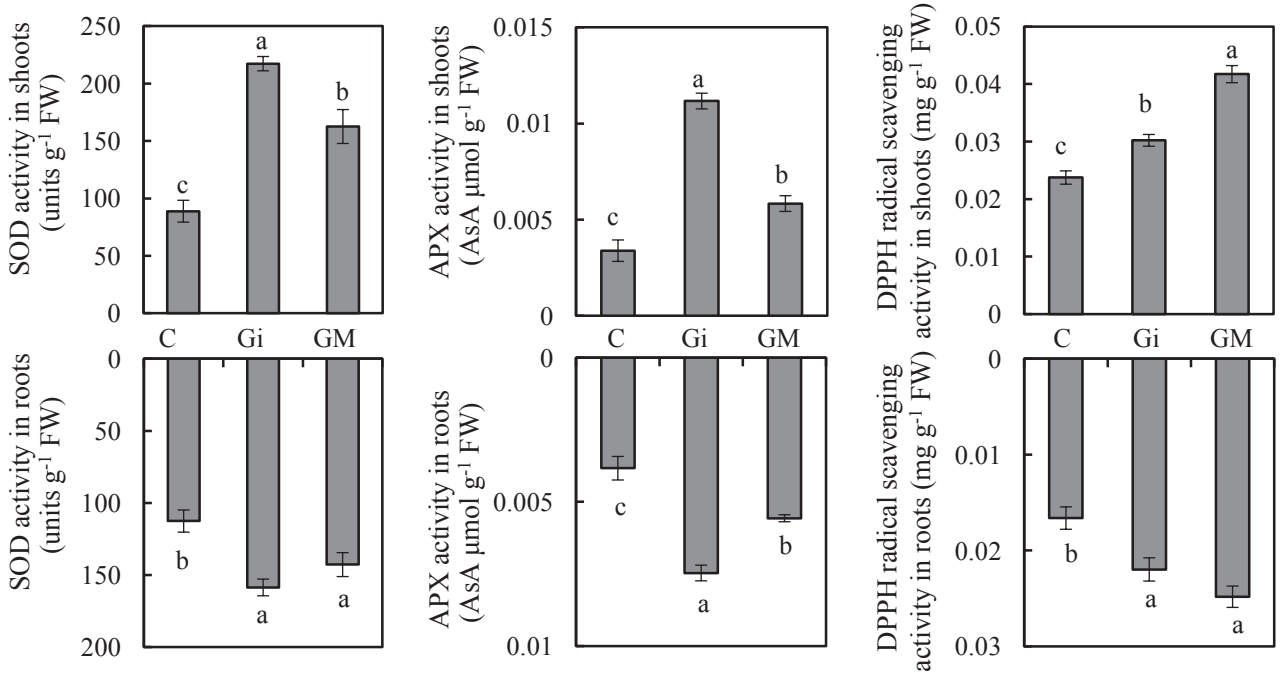

Fig. 3. Superoxide dismutase (SOD) activity, ascorbate peroxidase (APX) activity and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity in shoots and roots 8 weeks after AMF inoculation. C, control; Gi, *Glomus intraradices*; GM, *Gigaspora margarita*. Bars represent standard errors (n = 10). Columns denoted by different letters indicate significant difference according to Tukey's test ($P < 0.05$).

antioxidative ability eight weeks after AMF inoculation, SOD and APX activity were significantly higher in the AMF plants than the control in both the shoots and roots, especially in Gi (Fig. 3). On the other hand, DPPH radical scavenging activity increased with significance in shoots and roots of the AMF plants, especially in GM.

Eight weeks after Foa inoculation, incidence of Fusarium root rot in roots reached up to 80% in the

control plants (Fig. 4), but was lower in Gi plants (30%) and GM plants (50%). The severity of the symptoms in the roots became lower in both Gi and GM compared to control. The disease indices of Fusarium root rot reached more than 40 in control plants, whereas it was as low as 8 in Gi and 14 in GM (Fig. 4). Hence, the disease indices and incidence of Fusarium root rot for the AMF and control plants followed a similar pattern. As for antioxidative ability 8 weeks after Foa inoculation, SOD and APX activity were significantly higher in AMF roots, especially in Gi (Fig. 5). DPPH radical scavenging activity increased significantly in AMF roots compared to the control; GM was higher than Gi. In addition, SOD, APX and DPPH radical scavenging activity in roots increased in all the asparagus roots due to Foa inoculation (Figs. 3 and 5).

As for SOD isoenzymes in roots, Cu/Zn- (Rf = 0.33 and 0.37) and Mn/Fe-SOD (Rf = 0.25) were detected in both AMF and control roots before and after Foa inoculation (Fig. 6). In this case, Cu/Zn bands had higher intensity than Mn/Fe in all the plots, and no difference appeared in the band positions among the plots. Before Foa inoculation (Foa-), SOD-1 (Rf = 0.37, Cu/Zn-SOD) bands became higher in intensity in both Gi and GM compared to the control. Eight weeks after Foa inoculation (Foa+), SOD-1 and -2 (Rf = 0.37, 0.33, Cu/Zn-SODs) showed higher intensity in both Gi and GM than the control. The intensity of those Cu/Zn bands decreased after Foa inoculation in control. In addition, SOD-3 (Rf = 0.25, Mn/Fe-SOD) in GM increased in intensity compared to control after Foa inoculation. Comparing states before and after Foa inoculation, SOD-1,2 (Cu/Zn-SODs) decreased in the control and GM, but increased in Gi; SOD-3 (Mn/Fe-SOD) increased in GM due to Foa inoculation.

Discussion

In this study, the dry weight of shoots and roots increased in AMF plants compared to the control, suggesting that growth-promoting effect through AMF symbiosis appeared in asparagus plants. As for tolerance to Fusarium root rot, the incidence and severity of symptoms were alleviated by AMF, with Gi having better results than GM, though no significant difference appeared in AMF colonization level. These results indicated that tolerance to Fusarium root rot occurred in asparagus plants inoculated with AMF. In addition, the antioxidative ability of mycorrhizal plants increased before and after Foa inoculation compared to control. In this case, the enhancement of SOD antioxidant capacity in both Gi and GM plants is considered as a result of increased isozymes. From these facts, it is suggested that tolerance to Fusarium root rot in mycorrhizal plants is closely associated with the increase in antioxidative ability.

In the *Pisum sativum-G. mosseae* symbiosis, no qualitative differences among the isozyme patterns in both mycorrhizal and non-mycorrhizal roots were observed, although higher SOD activity was detected in mycorrhizal pea roots (Arines et al. 1994). In a pathogen stress condition, Pozo (2002) reported that Mn-SOD as a new isozyme was detected in mycorrhizal (*G. mosseae*) tomato plants without SOD activity analysis. In the report, no changes appeared in the band pattern before and after *Phytophthora* inoculation, so that only AMF colonization affected Mn-SOD isozymes. In this study, the intensity of the Cu/Zn isozyme changed through AMF colonization, and Fusarium inoculation induced additional changes in the intensity of Cu/Zn- and Fe/Zn-SOD isozymes including AM fungal difference. These are new findings compared to those former reports.

Some reports described that AMF colonization itself induced a temporary increase in antioxidative abilities such as SOD, guaiacol peroxidase (G-POD), catalase (CAT), APX, DPPH radical scavenging activity and flavonoid content, suggesting that colonization might be a temporary stress for host plants (Wu et al. 2006, Zhu et al. 2010). Antioxidant levels in plants and activities of ROS scavenging enzymes have been reported to be correlated with tolerance to different environmental stresses (Chaitanya et al. 2002) including pathogen attack in plants (Miyazawa et al. 1998). Our results showed similar patterns to those findings as the increase in SOD, APX and DPPH radical scavenging activity by AMF colonization before and after Foa inoculation.

On the other hand, Moghaddam et al. (2006) mentioned that higher SOD activity and newly formed SOD isozyme (Rf = 0.53) was confirmed in a resistant strawberry cultivar than susceptible one with *Mycosphaerella fragariae* infection; the SOD isozyme was supposed to be concerned with the disease resistance. In this experiment, Gi plants showed higher dry weight and lower disease index than control. In addition, suppression of the Cu/Zn-SODs (Rf = 0.33, 0.37) appeared in control roots, and increased intensity of those Cu/Zn-SODs appeared in Gi after Foa inoculation. From these findings, especially, Cu/Zn isozymes might be closely associated with resistance to Fusarium root rot in mycorrhizal asparagus.

Antioxidative substances, frequently estimated totally as DPPH radical scavenging activity, have

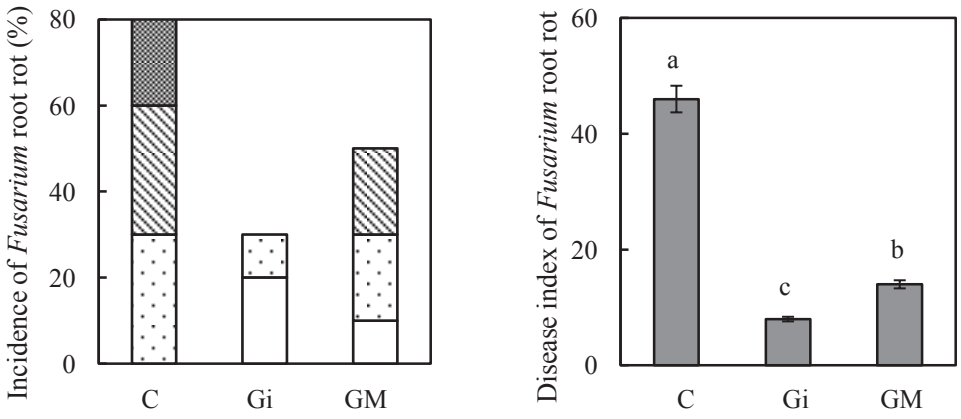

Fig. 4. Incidence and disease indices of Fusarium root rot in asparagus plants 8 weeks after *Fusarium oxysporum* f. sp. *asparagi* inoculation. C, control; Gi, *Glomus intraradices*; GM, *Gigaspora margarita*. Ratio of diseased storage roots; □, 0-20 ; ⁘, 20-40 ; ▨, 40-60; ▩ 60-80; ■ 80-100 (%). Bars represent standard errors (n = 20). Columns denoted by different letters indicate significant difference according to Tukey's test ($P < 0.05$).

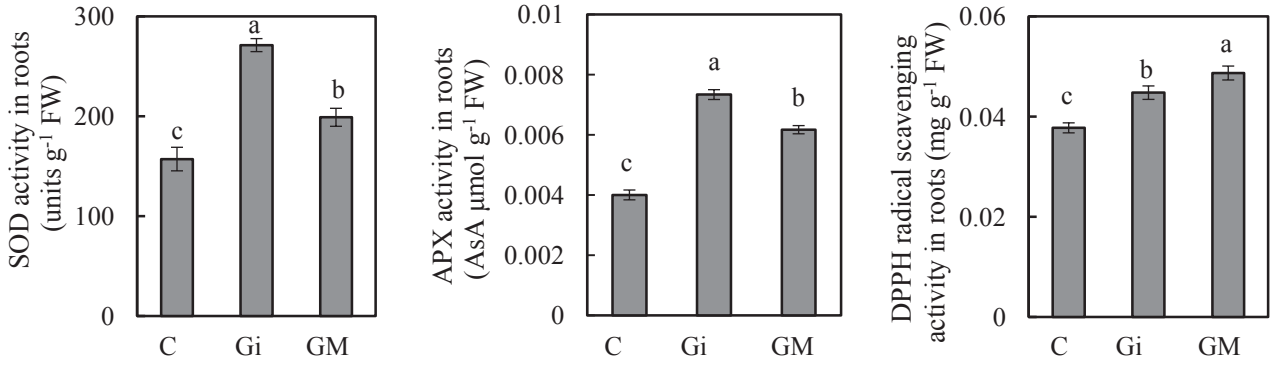

Fig. 5. Superoxide dismutase (SOD) activity, ascorbate peroxidase (APX) activity and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity in roots 8 weeks after *Fusarium oxysporum* f. sp. *asparagi* inoculation. C, control; Gi, *Glomus intraradices*; GM, *Gigaspora margarita*. Bars represent standard errors (n = 10). Columns denoted by different letters indicate significant difference according to Tukey's test ($P < 0.05$).

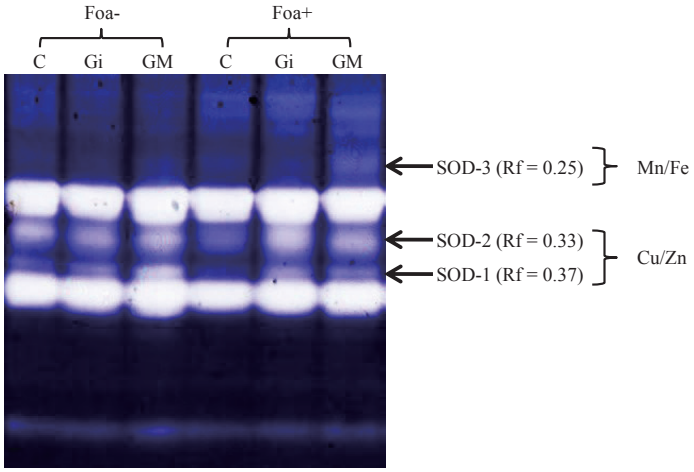

Fig. 6. Changes in superoxide dismutase (SOD) isozyme of mycorrhizal asparagus roots before and after *Fusarium oxysporum* f. sp. *asparagi* (Foa) inoculation. Gi, *Glomus intraradices*; GM, *Gigaspora margarita*; Foa-, before *Fusarium oxysporum* f. sp. *asparagi* inoculation; Foa+, after *Fusarium oxysporum* f. sp. *asparagi* inoculation. Rf = relative mobility (i.e., relative electrophoretic mobility with respect to the bromophenol blue band).

lower electron reduction potential than the electron reduction potential of oxygen radicals; as a result, they directly scavenge reactive oxygen intermediates without promoting further oxidative reactions (Ainsworth et al. 2007). Vanitha et al. (2009) mentioned that total phenol content increased bacterial wilt in tomato upon *Ralstonia solanacearum* inoculation. Hichem et al. (2009) reported that salt stress induced DPPH free radical scavenging activity and polyphenolic compounds in maize. In mycorrhizal St. John's wort plants inoculated with *Colletotrichum gloeosporioides*, ascorbic acid content and disease tolerance increased (Richter et al. 2011). In this experiment, disease index became lower in both Gi and GM than control, and the increase in DPPH radical scavenging activity also occurred in both AMF plants. Hence, antioxidative substances also have an association with tolerance to Fusarium root rot in mycorrhizal asparagus plants, the same as antioxidative enzymes.

Norman et al. (1996) reported that the incidence of the symptom caused by *Phytophthora fragariae* in strawberry plants was reduced by the inoculation of AMF, though the effect differed with AM fungal species. Ozgonen and Erkilic (2007) reported that growth promotion and tolerance to *Phytophthora capsici* had no correlation with the mycorrhizal colonization levels in pepper. In this study, the AMF colonization level was only checked twice, so it is difficult to estimate when AMF reached maximum colonization level during the experimental period, and how the colonization level affects SOD isozymes in host plants. Further study would be needed to clarify the relationship between AMF colonization level and SOD isozyme including the relation with other host and pathogenic fungi.

Conclusion

AMF (Gi and GM) plants showed an increase in the dry weight of shoots and roots. Eight weeks after Foa inoculation, the incidence and severity of Fusarium root rot were both alleviated in AMF-inoculated plants to a greater extent than control. SOD and APX activity and DPPH radical scavenging activity increased in both AMF-inoculated plants compared with the control before and after inoculation. Eight weeks after Foa inoculation, especially, Rf = 0.33 and 0.37 bands (Cu/Zn-SODs) showed higher intensity in both Gi and GM compared to the control, and AM fungal difference appeared in the Rf = 0.25 (Mn/Fe-SOD) intensity. These findings suggest that tolerance to Fusarium root rot in mycorrhizal asparagus plants might be closely associated with the changes in SOD activity and isozymes, especially in Cu/Zn-SODs.

Acknowledgements

This study was supported by Grants-in-Aid for Scientific Research (No. 21580029), Japan Society for the Promotion of Science (JSPS).

References

Ainsworth EA, Gillespie KM 2007 Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat. Prot. 2: 875-877.

Arines J, Quintela M, Vilariño A, Palma JM 1994 Protein patterns and superoxide dismutase activity in non-mycorrhizal and arbuscular mycorrhizal *Pisum sativum* L. plants. Plant Soil 166: 37-45.

Beauchamp C, Fridovich I 1971 Superoxide Dismutase: Improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44: 276-287.

Burits M, Bucar F 2000 Antioxidant activity of Nigella sativa essential oil. Phytother. Res. 14: 323-328.

Chaitanya KV, Sundar D, Masilamani S, Reddy R 2002 Variation in heat stress-induced antioxidant enzyme activities among three mulberry cultivars. Plant Growth Reg. 36: 175-180.

Davis BJ 1964 Disc electrophoresis. II. Method and application to human serum proteins. Ann. N. Y. Acad. Sci. 121: 404-427.

Elmer WH 2015 Management of Fusarium crown and root rot of asparagus. Crop Prot. 73: 2-6.

Fridovich I 1986 Biological effects of the superoxide radicals. Arch. Biochem. Biophys. 247: 1-11.

Garmendia I, Aguirreolea J, Goicoechea N 2006 Defence-ralated enzymes in pepper roots during interactions with arbuscular mycorrhizal fungi and/or *Verticillium dahliae*. Biocontr. 51: 293-310.

He CY, Hsiang T, Wolyn DJ 2002 Induction of systemic disease resistance and pathogen defence responses in *Asparagus officinalis* inoculated with nonpathogenic strains of *Fusarium oxysporum*. Plant Pathol. 51: 225-230.

Hichem H, Mounir D, Naceur EA 2009 Differential responses of two maize (*Zea mays* L.) varieties to salt stress: changes on polyphenols composition of foliage and oxidative damages. Ind. Crops Prod. 30: 144-151.

Liu J, Taniguchi A, Matsubara Y, Kojima T, Takahashi K 2016 PCR-SSCP analysis and disease control by the application of biological and chemical agents in asparagus decline. Acta Hort. (In press)

Manganaris AG, Alston FH 1992 Inheritance and linkage relationships of peroxidase isoenzymes in apple. Theor. Appl. Genet. 83: 392-399.

Marschner H, Dell B 1994 Nutrient uptake in mycorrhizal symbiosis. Plant Soil 159: 89-102.

Miyazawa J, Kawabata T, Ogasawara N 1998 Induction of an acidic isozyme of peroxidase and acquired resistance to

wilt disease in response to treatment of tomato roots with 2-furoic acid, 4-hydroxybenzoic hydrazide or salicylic hydrazide. Physiol. Mol. Plant Pathol. 52: 115-126.

Moghaddam B, Charles MT, Carisse O, Khanizadeh S 2006 Superoxide dismutase responses of strawberry cultivars to infection by *Mycosphaerella fragariae*. J. Plant Physiol. 163: 147-153.

Norman JR, Atkinson D, Hooker JE 1996. Arbuscular mycorrhizal fungal-induced alteration to root architecture in strawberry and induced resistance to the root pathogen *Phytophthora fragariae*. Plant Soil 185: 191-198.

Ozgonen H, Erkilic A 2007 Growth enhancement and *Phytophthora* blight (*Phytophthora capsici* Leonian) control by arbuscular mycorrhizal fungal inoculation in pepper. Crop Prot. 26: 1682-1688.

Phillips JM, Hayman DS 1970 Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection.Trans. Br. Mycol. Soc. 55: 158-163.

Pontaroli AC, Camadro EL, Babinec FJ, Ridao A 2000 Responese of *Asparagus officinalis* pollen to the culture filtrate of *Fusarium oxysporum* f. sp. asparagi. Sci. Hort. 84: 349-356.

Pozo MJ, Cordier C, Dumas-Gaudot E, Gianinazzi S, Barea JM, Azcón-Aguilar C 2002 Localized vs systemic effect of arbuscular mycorrhizal fungi on defence response to *Phytophthora* infection in tomato plants. J. Exp. Bot. 53: 525-534.

Richter J, Baltruschat H, Kabrodt K, Schellenberg I 2011 Impact of arbuscular mycorrhiza on the St. John's wort (*Hypericum perforatum*) wilt disease induced *Colletotricuhum* cf. *gloeosporioides*. J. Plant Dis. Prot. 118: 109-118.

Sahoo MR, Dasaupta M, Kole PC, Bhat JS, Mukherjee A 2007 Antioxidative enzymes and isozymes analysis of taro genotypes and their implications in *Phytophthora* blight disease resistance. Mycopathol. 163: 241-243.

Vanitha SC, Niranjana SR, Umesha S 2009. Role of phenylalanine ammonia lyase and polyphenol oxidase in host resistance to bacterial wilt of tomato. J. Phytopathol. 157: 552-557.

Wacker TL, Safir GR, Stephens CT 1990 Effect of *Glomus fasciculatum* growth of asparagus and the incidence of Fusarium root rot. J. Amer. Soc. Hort. Sci. 115: 550-554.

Wahid A, Close TJ 2007 Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves. Biol. Plant. 51: 104-109.

Wu Q, Zou Y, Xia R 2006. Effect of water stress and arbuscular mycorrhizal fungi on reactive oxygen metabolism and antioxidant production by citrus (*Citrus tangerine*) roots. Eur. J. Soil Biol. 42: 166-172.

Zhu X, Song F, Xu H 2010 Influence of arbuscular mycorrhiza on lipid peroxidation and antioxidative enzyme activity of maize plants under temperature stress. Mycorrhiza 20: 325-332.