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STUDIES ON APPLICATION OF VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI TO ASPARAGUS CULTIVATION.

1. Effects of vesicular-arbuscular mycorrhizal fungi inoculation on growth of asparagus (*Asparagus officinalis* L.) seedling.

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Introduction

Asparagus (*Asparagus officinalis* L.), a perennial crop, requires at least three years from sowing time to first harvest period. It is already well-known that growing good seedlings relate strongly to high yield of spears of mature plants[1]. Many investigations have been carried out to establish the method of raising unfavorable environment-resistant and vigorous seedlings[2,3,4,5,6].

Vesicular-arbuscular mycorrhizal (VAM) fungi has an effect to promote plant growth by establishing symbiosis from mycorrhizae formation in roots. It is reported that asparagus (*Asparagus officinalis* L.) is infected with *Glomus spp.* fungi[7,8,9,10]. Powell et al. and Burrows et al. clarified VAM had an effect on the seedling growth in low P soil[11,12]. In these studies, we have expected VAM fungi would be available for developing good asparagus seedlings with high yielding ability induced by microorganism.

This study aimed at investigating VAM fungus inoculation method for more active mycorrhizae formation and asparagus seedling growth, and at clarifying morphogenesis of seedling roots infected with VAM fungi.

Materials and methods

Mycorrhizal fungus inoculation. Seeds of asparagus (*Asparagus officinalis* L. cv. Franklim, Bohnlim, Welcome, Mary Washington 500W and Hokkai 100) were prepared for mycorrhizal fungus inoculation. All seeds were germinated on a moistened filter paper in a Petri dish (11 cm in diameter), Ten-day-old seedlings were used in the present experiments.

Two lines of *Glomus spp.* '301' and '401', which were supplied by Kyowa Hakko Kogyo Co. Ltd., were inoculated mainly at a density of 1000 spores per g

of inoculum which had consisted of uniformly powdered vermiculite with adequate moisture. Only in a spore density test, the spore density was adjusted to 0, 100, 500 and 1000 spores/g inoculum by diluting the original inoculum at 1000 spores/g inoculum.

Sieved soil, as a medium, was obtained from Experimental Farm of Hokkaido University (Sapporo, Japan), and autoclaved at 121°C and 1.2kg/cm^2 for one hour to avoid the influences of other indigenous microbes on the seedlings or the vesicular-arbuscular mycorrhizal fungi. The soil was packed at 10 cm in depth in a vat (38×60×14 cm) and paperpots which are a hexagonal tube (13 cm in length)-glued cluster, and made by Nippon Beet Sugar MFG Co. Ltd.

In *Glomus spp.* '301', one-gram inoculum at prescribed spore densities was inoculated onto roots of seedlings, and only a density of 1000 spores/g inoculum was used especially in 'Mary Washington 500W' and 'Bohnlim'. The seedlings inoculated were transplanted to the vat or to the paperpot. In *Glomus spp.* '401' one-gram inoculum (1000 spores/g inoculum) was inoculated onto roots of seedlings in 'Franklim', 'Bohnlim' and 'Welcome', and the seedlings inoculated transferred to the vats. All mycorrhizal fungus-inoculated seedlings were kept in a greenhouse under natural day light conditions, watered adequately, but not fertilized.

Measurement of the growth of mycorrhizal fungus-inoculated seedlings. To observe microscopically the VAM fungus activities in roots, asparagus roots were collected and stained with cotton blue modified by the method of Phillips and Hayman[13]. The mycorrhizal infection level i.e. percentage of VAM fungi forming internal hyphae were evaluated by Gridline Intersect Method of Giovannatti and Mosse[14]. One hundred and eighty days after the inoculation with *Glomus spp.* '301', number of storage roots, number of feeder roots (per storage root segment 1 cm in length), length of a storage root and a feeder root, and the diameter of a storage root were recorded and calculated to determine the differences in these between the inoculated and noninoculated.

Results

Sixty days after VAM fungus inoculation, differences in shoot length were scarcely recognized according to spore densities, and began to appear among five asparagus cultivars tested. On the 180th day from the inoculation, the mycorrhizal infection levels had gradually increased until then with the increase of plant growth duration, and in all five cultivars, showed 23.8-41.8% uniformly in the inoculation at 1000 spores/g inoculum (Table 1). In the present experiment, shoot length also increased more rapidly at the highest spore density (1000 spores/g inoculum) (Fig.1).

In the paperpot, shoot length of the VAM fungus-inoculated seedling, of which mycorrhizal infection level was about 2-5 times higher than that of the

Table 1. Effect of spore densities in inoculum on growth of asparagus seedlings inoculated with VAM fungus.

Asparagus cultiver	Spore density in inoculum[z] (spores/g inoculum)	60 days after inoculation		180 days after inoculation	
		Mycorrhizal infection level in root (%)[y]	Shoot length (cm)	Mycorrhizal infection level in root (%)[y]	Shoot length (cm)
Welcome	0	0	14.8±0.25	0	26.3±4.07
Welcome	100	8.9	14.1±0.39	25.8	30.7±4.87
Welcome	500	8.4	16.5±0.40	15.0	33.0±9.60
Welcome	1000	14.2	16.1±0.45	37.1	40.1±14.10
Franklim	0	0	13.0±0.22	0	27.4±3.28
Franklim	100	6.2	13.5±0.26	28.5	27.0±3.27
Franklim	500	11.0	13.7±0.22	37.9	31.4±2.92
Franklim	1000	25.6	13.5±0.36	40.4	34.1±4.85
Hokkai 100	0	0	11.9±0.62	0	24.0±6.33
Hokkai 100	100	10.9	11.9±0.66	21.9	28.1±5.81
Hokkai 100	500	14.6	16.4±0.76	46.0	35.0±5.55
Hokkai 100	1000	11.0	14.6±0.57	23.8	37.6±7.62
Bohnlim	0	0	12.4±0.36	0	24.6±2.89
Bohnlim	1000	29.5	19.9±0.72	41.8	32.2±6.00
Mary Washington 500W	0	0	11.2±0.35	0	16.3±1.96
Mary Washington 500W	1000	21.1	13.6±0.47	34.4	24.3±2.92

[z] *Glomus spp.* '301'.

[y] Evaluated by Gridline Intersect Method: root segments 1 cm in length are uniformly and randomly excised from all roots of each seedling; criteria in a portion of the segment surface layer that is selected by the method are microscopicaly observed; percentages of root segments with a cystidium are calculated. Confidence limits (t*s.e. at P=0.05).

Fig. 1. Asparagus seedlings 120 days after inoculation of *Glomus spp.*
Left : noninoculated seedlings.
Right : inoculated (100 spores/g inoculum) seedling.

seedlings planted in the vat, was as long as of the noninoculated (Table 2).

Table 2. Type of container plays an important role in some cases of the tests on VAM-infected seedling growth.

Asparagus cultivar	Container	Inoculation of VAM fungus[z]	120 days after inoculation	
			Mycorrhizal infection level in root (%)[y]	Shoot length (cm)
Franklim	vat	No	0	20.9
Franklim	vat	Yes	25.6	35.2
Franklim	paperpot[x]	No	0	14.6
Franklim	paperpot	Yes	59.8	15.0
Welcome	vat	No	0	24.6
Welcome	vat	Yes	14.2	32.5
Welcome	paperpot	No	0	14.3
Welcome	paperpot	Yes	71.0	13.5
Hokkai 100	vat	No	0	19.7
Hokkai 100	vat	Yes	11.0	24.2
Hokkai 100	paperpot	No	0	15.0
Hokkai 100	paperpot	Yes	39.0	15.0
Bohnlim	vat	No	0	19.5
Bohnlim	vat	Yes	29.5	33.0
Bohnlim	paperpot	No	0	17.9
Bohnlim	paperpot	Yes	56.0	17.0
Mary Washington 500W	vat	No	0	14.1
Mary Washington 500W	vat	Yes	21.1	31.5
Mary Washington 500W	paperpot	No	0	13.4
Mary Washington 500W	paperpot	Yes	46.5	10.3

[z] *Glomus spp.* '301', 1000-1500 spores/g inoculum.

[y] Evaluated by Gridline Intersect Method: root segments 1 cm in length are uniformly and randomly excised from all roots of each seedlings; criteria in a portion of the segment surface layer that is selected by the method are microscopicaly observed; percentages of root segments with a cystidium are calculated.

[x] A hexagonal tube (1.9 cm in diameter)-glued cluster.

Both *Glomus spp.* '301' and 401' steadily promoted the growth of seedlings. Average length of the longest shoot of the seedlings inoculated with *Glomus spp.* '301' or '401' reached 36.1-48.9 cm ('301') and 43.0-52.8 cm ('401') that were approximately two times more than those of the noninoculated shoot. In this study, average length of the shoot of the seedlings inoculated with *Glomus spp.* '401' was a little more than that with *Glomus spp.* '301' (Table 3).

Table 3. Effects of inoculation of mycorrhizal fungi *Glomus spp.* on growth of asparagus seedlings.

Asparagus cultivar	Inoculation of Glomus spp.[z]	Average shoot length (cm)[y] Days after inoculation 100	180	250
Franklim	Noninoculated	8.9	12.7	20.8
Franklim	301-inoculated	13.2	19.3	36.1
Franklim	401-inoculated	17.0	22.9	43.0
Bohnlim	Noninoculated	10.8	13.1	25.3
Bohnlim	301-inoculated	23.2	26.4	48.9
Bohnlim	401-inoculated	21.9	30.8	51.7
Welcome	Noninoculated	10.9	11.9	19.2
Welcome	301-inoculated	16.4	22.7	39.4
Welcome	401-inoculated	18.5	33.4	52.5

[z] *Glomus spp.* 1000 spores/g inoculum.

[y] Average length of the longest shoots.

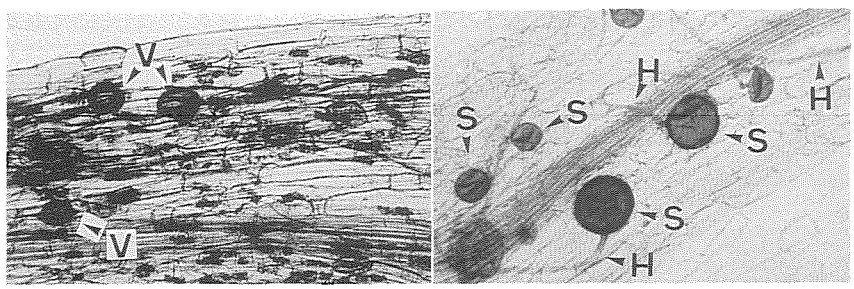

Fig. 2. Vesicular-Arbuscular Mycorrhizal fungi in infected asparagus seedling roots. H : hyphae. S : Spore. V : Vesicular.

Table 4. Morphological characteristics of asparagus seedling roots inoculated with *Glomus spp.*

Asparagus cultiver	Inoculation of VAM fungus[z]	Shoot length (cm)	Storage root No./seedling	Length (cm)	Diameter (mm)	Feeder root No./storage root segment	Length (mm)
MW 500W	No	16.3±1.96	4.2	22.0±2.38	1.97±0.074	3.6	–
MW 500W	Yes	24.3±2.92	6.7	22.8±2.53	2.24±0.073	3.2	–
Bohnlim	No	24.6±2.89	4.6	32.3±2.94	2.26±0.081	2.8	–
Bohnlim	Yes	32.3±6.00	7.0	25.9±2.50	2.60±0.063	3.4	–
Franklim	No	27.4±3.28	–	–	–	3.0	51.2±6.60
Franklim	Yes	34.1±4.85	–	–	–	4.1	24.0±3.22

[z] *Glomus spp.* '301', 1000 spores/g inoculum.

Confidence limits (t*s.e. at P=0.05).

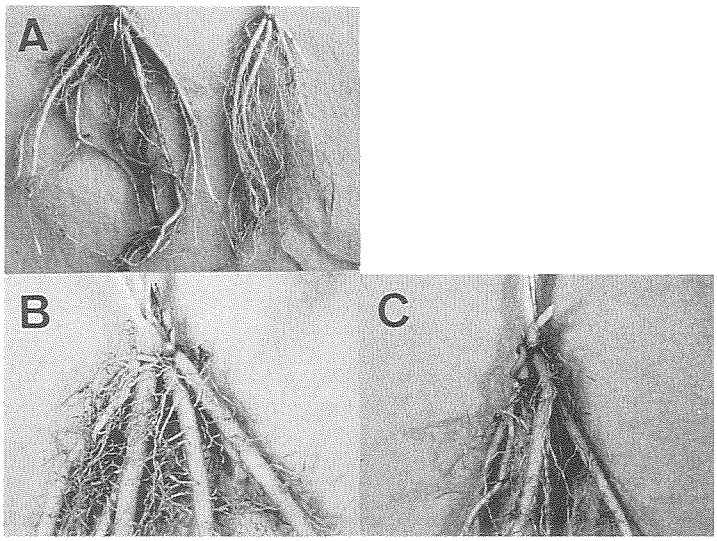

Fig. 3. Asparagus seedling roots inoculated with *Glomus spp.*
A : Seedling roots inoculated with *Glomus spp.* Inoculated (Left). Noninoculated (Right)
B : Crown infected
C : Crown noninfected

VAM infection in the seedling roots was observed in a surface layer of feeder roots (Fig. 2) but not in that of storage roots. A little significant differences in the average length of the storage roots existed between VAM fungus-inoculated and noninoculated seedlings. Average number and diameter of VAM fungus-inoculated storage roots were about 1.5 times (number) and 1.1 times (diameter) more than those of the noninoculated. In contrast, average length of VAM fungus-inoculated feeder roots was shorter than that of the noninoculated (Table 4, Fig. 3).

Discussion

In all experiments, among five cultivars used, there was little significant difference in the effect of VAM fungi inoculation on the seedling growth. This shows that VAM fungi has no peculiarity to infect the seedling roots of various asparagus cultivars. Increasing the VAM spore density resulted in raising the mycorrhizal infection level, and subsequently, led to the formation of long shoots 60 to 180 days after the inoculation. These observations suggests that the symbiotic relationship between VAM fungi and asparagus seedlings is established, and that subsequent VAM performance effectively promote growth of the seedling. The fact that 1000 spores/g inoculum is most effective on seedling growth will require a test concerning the effect of more than 1000 spores/g inoculum on seedling growth.

Although seedlings grown in a paperpot showed high mycorrhizal infection

levels in roots, their shoot elongations were less rapid than that of seedlings grown in a vat. This may be caused by various, complicated environmental conditions such as soil volume per seedling, difference in space where roots elongate.

Both *Glomus spp.* '301' and '401' had an effect to promote considerably the seedling growth. Average shoot length of the seedlings inoculated with *Glomus spp.* '401' was a little more than that with *Glomus spp.* '301'. Chang and Plenchette et al. reported that the growth of seedlings slightly differed according to the kinds of *Glomus spp.*[7,8]. The effect of both *Glomus spp.* '301' and '401', should definitely be examined by further investigations.

It was found the morphogenesis of root was changed by the VAM infection. VAM infection in seedling roots increased number and diameter of the storage roots. Such changes might be related to the vigorous growth in epigeous parts of a seedling. Length of feeder root of VAM fungus-inoculated seedlings was shorter than that of the noninoculated. The result may be caused by active phosphorus-and water-absorption enhancement of VAM external hyphae. This indicates that asparagus is a highly mycorrhizae-dependent crop[7] such as certain kinds of citrus[15].

From these results, it was clarified the inoculation of both *Glomus spp.* '301' and '401' (at 1000 spores/g inoculum) in asparagus young seedlings is noticeably effective to grow good seedlings.

Summary

Influences of inoculation with Vesicular-Arbuscular mycorrhizal fungi on the growth of asparagus (*Asparagus officinalis* L.) seedlings was examined using *Glomus spp.* '301' and '401' which were breeded by Kyowa Hakko Kogyo Co. Ltd.

Differences in shoot length began to appear clearly between mycorrhizal fungus-inoculated and noninoculated seedlings 60 days after the inoculation, and higher density (1000 spores/g inoculum) of *Glomus spp.* '301' spores gave a higher mycorrhizal infection level, and made shoots longer.

It was revealed that both *Glomus spp.* '301' and '401' had an effect on the growth of asparagus seedlings, and the *Glomus spp.* '401' promoted the seedling growth more intensively than '301'.

VAM infection was observed only in the feeder roots. In a VAM-infected seedling, the number and thickness of storage roots increased, whereas feeder roots were shortened.

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