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Temperature-Stress Tolerance of Asparagus Seedlings through Symbiosis with Arbuscular Mycorrhizal Fungus

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Summary

Temperature-stress tolerance through symbiosis with arbuscular mycorrhizal (AM) fungi [*Gigaspora margarita* (GM) and *Glomus* sp. R10 (GR)] in seedlings of asparagus (*Asparagus officinalis* L., cv. Mary Washington 500W) was investigated.

Seven weeks after inoculation under a bed soil kept at 25 ℃/20 ℃ (day/night) under a 16-hr photoperiod, AM fungus-infection levels in a root system reached 63.0% in GM and 20.0% in GR. AM fungus-infected plants were taller, produced more shoots, accumulated more dry matter and attained higher P concentration in both shoots and roots than the noninoculated plants.

Under a constant 15 ℃ bed soil for 4 weeks followed by an elevation to 25 ℃/20 ℃, shoot elongation was promoted in AM fungus-infected plants, especially after the third emergence; the effect was more pronounced in GR than in GM plots. Eleven weeks after inoculation, AM fungus infection levels reached 48.9% in GM and 58.9% in GR. Plant height, no. of shoots, no. of crowns, dry weight, and phosphorus concentration in shoots and roots became greater in AM fungus-infected plants than in noninoculated ones. When bed soil was heated to 30 ℃, shoot growth after the fourth emergence became restricted in noninoculated plants, whereas shoot emergence and elongation were promoted, especially after the fifth and fourth emergences in GM and in GR plots, respectively. Eleven weeks after inoculation, AM fungus infection levels reached 66.3% in GM and 36.7% in GR. All measured parameters in AM fungus-infected plants were larger than in the noninoculated plants; the effect appeared significantly greater in GM than in GR plots.

These results reveal that the asparagus seedlings infected with AM fungus tolerated greater temperature stress through symbiosis and that the degree of tolerance differed with the fungal species.

Key Words: arbuscular mycorrhizal fungus, asparagus seedlings, symbiosis, temperature-stress tolerance.

Introduction

Infection of arbuscular mycorrhizal (AM) fungus promotes the growth of host plants mainly by enhancing phosphorus uptake through host-fungus symbiosis in plant roots (Raju et al., 1990; Marschner and Dell, 1994).

In asparagus production, it is important to raise vigorous seedlings with high yields for short period (Tadagi, 1992). Kim and Sakiyama (1989a, b) and Kim et al. (1989) reported that in asparagus, shoot growth is mainly influenced by temperature, and shoot growth was restricted at 30 ℃ and 15 ℃. These findings encouraged us to establish techniques in obtaining good growth under temperature-stressed condition, such as 15 ℃ and 30 ℃, thus, resulting in vigorous seedlings during a short period in asparagus production.

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Matsubara et al.(1994) found growth enhancement through symbiosis in asparagus seedlings, and that such enhancement lead to vigorous plants and shortened the nursery stage in asparagus cultivation. To use AM fungus in asparagus cultivation, environmental conditions, especially temperature, are important factors in inducing AM fungus infection in roots (Hayman, 1974; Raju, et al., 1990). Matsubara and Harada (1996b) reported that, in asparagus, the optimum temperature for AM fungus infection differed with AM fungal species. However, temperature-stress tolerance in AM fungus-infected asparagus plants is poorly understood.

In this study, the limits of temperature stress on the growth of asparagus seedlings infected with AM fungus were estimated.

Materials and Methods

AM fungus inoculation and growing plants

Seeds of asparagus (*Asparagus officinalis* L., cv. Mary Washington 500W) were germinated on moistened filter

paper in a Petri dish (11 cm diameter). Ten-day-old seedlings with radicles, approx. 20-mm long, were inoculated with *Gigaspora margarita* (GM) and *Glomus* sp. R10 (GR).

Bed soil [mixture of soil and vermiculite (1:1, v/v); autoclaved at 1.2 kg·cm^{-2} and 121 ℃ for one hour; pH 6.0-7.0 (H_2O); available-P content, 21.9 mg/100 g dry soil] was packed in plastic containers [13.5 cm × 27.0 cm × 15.5 cm (H)]. AM fungus-inoculated plants were then transplanted to the bed soil and fertilized with a mixed fertilizer (N:P:K=8:3:10, 0.5 g·l^{-1} soil). The 12 seedlings per plot were irrigated as needed. The GM- and GR-inoculated plants were raised for 7 weeks in a growth chamber with the bed soil kept at 25 ± 1 ℃ (day)/20 ± 1 ℃ (night) under a 16-hr photoperiod supplimented with fluorescent light source (approxi. 40 μmol·m^{-2}·s^{-1}) and 60%RH; the air temperature approximated that of the bed soil. Seven weeks after inoculation, AM fungus-inoculated seedlings were raised for 4 weeks in growth chambers in which the bed soil was maintained at 15 ± 1 ℃ and 30 ± 1 ℃; all other conditions were unchanged.

Observation of AM fungus infection in roots, and evaluation of AM fungus infection level

Seven and eleven weeks after inoculation, plants were dug and their roots were stained according to Phillips and Hayman (1970) to observe the rate of fungal infection in segments of lateral roots (RFISL). RFISL expresses the percentage of total 1-cm AM fungus-infected segments to 1-cm segments consisting of whole lateral roots and is calculated by averaging the values of five plants.

Determination of phosphorus in plants

The above samples were analyzed for P concentration according to Matsubara and Harada (1996a).

Results

Effect of AM fungus inoculation on growth and P concentration of seedlings before temperature-stressed conditions

Plant height increased most rapidly between 6 and 7 weeks after inoculation in AM fungus-inoculated plants, regardless of the species (Fig. 1). Seven weeks after inoculation, AM fungus infection level in a root system reached 63.0% in GM, and 20.0% in GR (Table 1). AM fungus-infected plants gave greater values in the following parameters: plant height, no.of shoots, dry weight of shoots, and P concentration in the shoots and roots than did those in noninoculated plants. The effect was more pronounced in GM than in GR.

Growth and P concentration of AM fungus-infected seedlings during temperature-stressed conditions

During the 15 ℃ treatment, plant height in AM fungus-infected plants exceeded that in noninoculated plants, regardless of the fungal species (Fig. 1). The emergence and elongation of shoots were hastened in AM fungus-infected plants, especially after the second emergence but prior to being stressed (Fig. 2). During the stressed condition, the shoots of AM fungus-infected plants

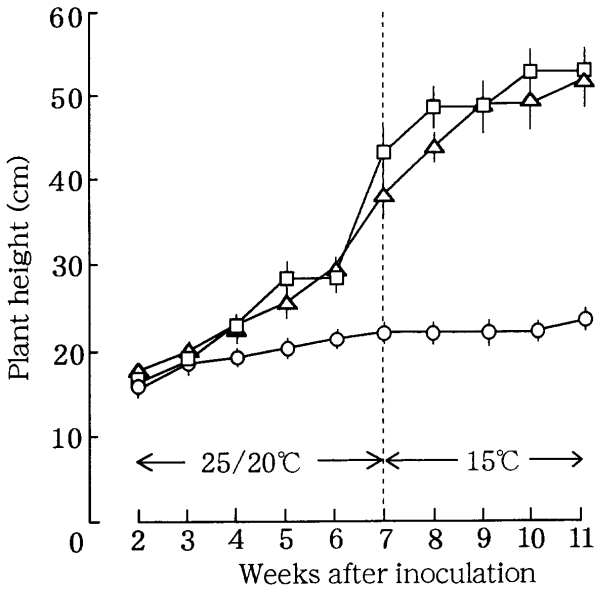

Fig. 1. Growth curves showing the effect of low temperature stress on growth of AM fungus-infected and noninfected asparagus seedlings.
○—○, noninoculated; □—□, inoculated with *Gigaspora margarita*; △—△, inoculated with *Glomus* sp. R10. Bars represent standard error.

Table 1. Effect of AM fungus inoculation and noninoculation on growth of asparagus seedlings[z].

AM fungus inoculation[y]	Plant height (cm)	No. of shoots	No. of crowns	Dry weight		P concentration		RFISL[x] (%)
				shoots (g)	roots (g)	shoots (%DW)	roots (%DW)	
None	22.2b[w]	2.4b	1.7a	0.2	0.3	1.8	0.7	0
GM	43.3a	4.0a	1.6a	0.9	0.3	7.0	4.7	63.0
GR	38.3a	4.3a	2.6a	0.6	0.3	4.1	2.4	20.0

[z] Date were collected 7 weeks after inoculation from 10 plants.
[y] None, noninoculated; GM, inoculated with *Gigaspora margarita*; GR, inoculated with *Glomus* sp. R10.
[x] Rate of AM fungus-infected segments in segments of lateral roots.
[w] Mean separation within columns by Duncan's multiple range test, 5% level.

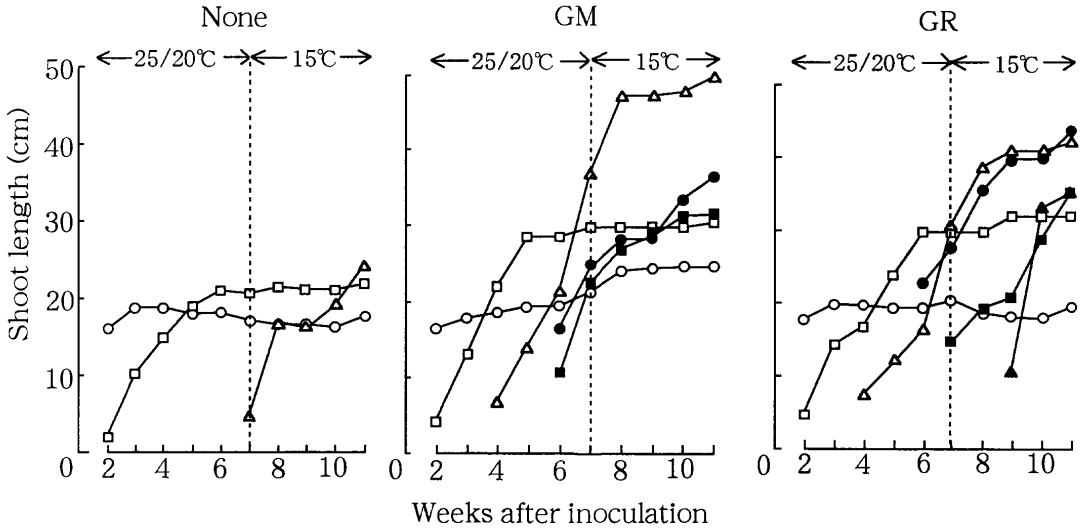

Fig. 2. Growth curves of low-temperature stressed AM fungus-infected and noninfected asparagus seedlings.
○—○, 1st order shoot; □—□, 2nd; △—△, 3rd; ●—●, 4th; ■—■, 5th; ▲—▲, 6th.
None, noninoculated; GM, inoculated with *Gigaspora margarita*; GR, inoculated with *Glomus* sp. R10.

Table 2. Effect of low temperature stress (25/20 ℃ → 15 ℃) on growth of AM fungus-infected and noninfected asparagus seedlings[z].

AM fungus inoculation[y]	Plant height (cm)	No. of shoots	No. of crowns	Dry weight		P concentration		RFISL[x] (%)
				shoots (g)	roots (g)	shoots (%DW)	roots (%DW)	
None	23.5b[w]	2.7b	1.6b	0.3	0.6	0.8	0.5	0
GM	52.7a	4.4a	3.3a	2.2	1.5	2.2	2.2	48.9
GR	51.8a	4.6a	3.4a	2.2	2.4	3.0	2.1	58.9

[z] Date were collected 11 weeks after inoculation from 10 plants.
[y] None, noninoculated; GM, inoculated with *Gigaspora margarita*; GR, inoculated with *Glomus* sp. R10.
[x] Rate of AM fungus-infected segments in segments of lateral roots.
[w] Mean separation within columns by Duncan's multiple range test, 5% level.

elongated faster, especially after the third emergence; the effect was more pronounced in GR than in GM. In GR-infected plants under stress, the 6-ordered shoots emerged and elongated rapidly, whereas those of GM plants did not even emerge. Eleven weeks after inoculation, AM fungus infection level reached 48.9% in GM, 58.9% in GR (Table 2). These fungus-infected plants exhibited higher values in all parameters measured compared with the noninoculated ones; the fungal species made no difference in the results.

During the 30 ℃, the AM fungus-infected plants became taller than the noninoculated ones, irrespective of the fungal species (Fig. 3). Shoot emergence after the fourth ceased in noninoculated plants (Fig. 4). The emergence of shoots and their elongation were promoted in both AM fungus-infected plants, especially after the fifth emergence in GM and fourth in GR during the stressed condition. In GM-infected plants, the 7th-ordered shoots emerged and rapidly elongated while for GR, no 7th-ordered shoot emerged. Eleven weeks after inoculation, RFISL were 66.3% in GM and 36.7% in GR (Table 3). Our data reveal that AM fungus-infected plants grew taller and were heavier and accumulated

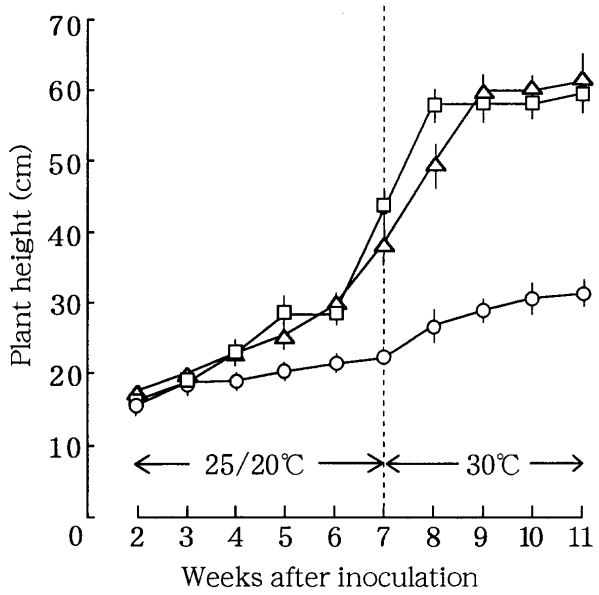

Fig. 3. Growth curves showing the effect of high temperature stress on the growth of AM fungus-infected and noninfected asparagus seedlings.
○—○, noninoculated; □—□, inoculated with *Gigaspora margarita*; △—△, inoculated with *Glomus* sp. R10. Bars represent standard error.

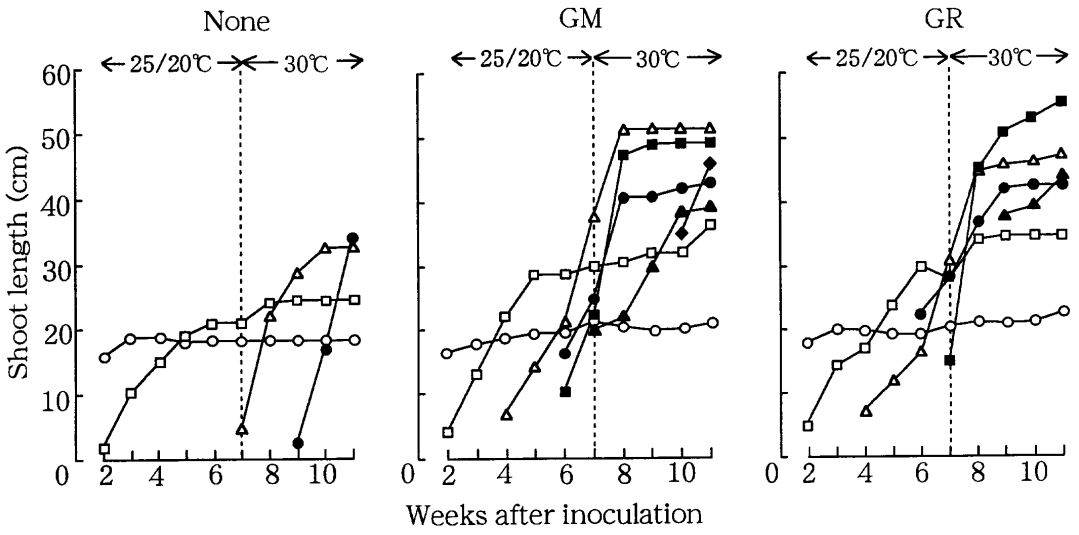

Fig. 4. Growth curves of high-temperature stressed AM fungus-infected and noninfected asparagus seedlings.
○—○, 1st order shoot; □—□, 2nd; △—△, 3rd; ●—●, 4th; ■—■, 5th; ▲—▲, 6th; ◆—◆, 7th.
None, noninoculated; GM, inoculated with *Gigaspora margarita*; GR, inoculated with *Glomus* sp. R10.

Table 3. Effect of high temperature stress (25/20 ℃ → 30 ℃) on growth of AM fungus-infected and noninfected asparagus seedlings[z].

AM fungus inoculation[y]	Plant height (cm)	No. of shoots	No. of crowns	Dry weight		P concentration		RFISL[x] (%)
				shoots (g)	roots (g)	shoots (%DW)	roots (%DW)	
None	31.2b[w]	2.8b	1.6b	0.3	0.6	0.9	0.3	0
GM	59.6a	6.0a	3.0a	2.6	1.4	3.0	3.6	66.3
GR	61.3a	4.6a	2.6a	1.4	1.3	2.1	2.4	36.7

[z] Date were collected 11 weeks after inoculation from 10 plants.
[y] None, noninoculated; GM, inoculated with *Gigaspora margarita*; GR, inoculated with *Glomus* sp. R10.
[x] Rate of AM fungus-infected segments in segments of lateral roots.
[w] Mean separation within columns by Duncan's multiple range test, 5% level.

more P in the shoots and roots than the noninoculated plants during the entire experiment; the effect was more pronounced in GM than in GR.

Discussion

Our results demonstrate that AM fungus infection induced temperature-stress tolerance in asparagus plants. As to the uncertainty about temperature-stress tolerance in AM fungus-infected plants, our study suggested that the tolerance in AM fungus-infected plants differed with the AM fungal species; GM-infected plants possessed greater tolerance than GR at 30 ℃, but the reverse was true at 15 ℃. In addition, it is supposed that the tolerance might be partially caused by P increase in plants through AM fungus infection.

Matsubara and Harada (1996b) reported that the optimum temperatures for both spore germination and hyphal growth of AM fungus differred between *Gigaspora margarita* and *Glomus etunicatum*, whereas Schenck et al. (1975) reported that the optimum temperatures for spore germination are 20 ℃-25 ℃ for *Glomus mosseae*, and 34 ℃ for both *Gigaspora coralloidea* and *Gigaspora heterogama*. Thus, the optimum temperature for fungal growth differs among the species, so that the difference in temperature-stress tolerance between the two fungi in this study might be associated with similar characteristics for fungal growth.

Matsubara and Harada (1996b) also reported that, in asparagus, growth enhancement and promotion of P accumulation through symbiosis did not occur under a constant bed soil temperature of 20 ℃, despite *Gigaspora margarita* infection. However, in the present experiment, plant growth was promoted and P concentrations increased in GM-infected plants at 15 ℃ after being pre-conditioned at 25/20 ℃. These results indicate that once symbiosis between host and fungus is established under a favorable temperature condition, such as 25/20 ℃ , the temperature range for inducing plant growth promotion through symbiosis might be extended, compared with those raised under unfavorable temperature condition, such as 20 ℃ (constant). The reasons for the different responses to low temperature followed by favorable or unfavorable temperature conditions for symbiosis-established plants are unclear. We suppose that hyphal viability, mainly in absorbing phosphorus, might account for the difference.

In this experiment, AM fungus infection level reached 63.0% in GM 7 weeks after inoculation, but decreased to 48.9% after the 15 ℃-stressed period. Hayman (1974), and Daniels and Bloom (1984) found that at a constant 10℃ and 15℃, the number of arbuscules decreased. We attribute the decrease in the AM fungus infection level in GM in our trial to a possible decrease in arbuscules on account of lower temperature. However, in GR, the AM fungus infection increased more at 15 ℃ than at 30℃, which indicates that the optimum temperature for arbuscule formation may be lower in GR than in GM.

Kim and Sakiyama (1989b) reported that the restriction of shoot growth in asparagus plants occurred at 30 ℃, whereas Krug (1998) demonstrated that a high temperature during July-August reduced or even inhibited the sprouting of asparagus crowns. In our study noninoculated plants at 30℃ responded similarly. However, in GR-infected plants, shoot emergence and elongation continued. Kim et al. (1989) mentioned that sugar translocation from storage roots to tops is associated with the difference in the elongation rate of asparagus shoots under different temperature treatments. Hence, the inhibition of sugar translocation from the storage roots to the top under high temperature might be eased by AM fungus infection, thus, allowing continued emergence and elongation even under 30℃.

Tadagi (1992) mentioned that to raise vigorous asparagus seedlings quickly in the nursery is important. Kim and Sakiyama (1989a) reported that asparagus shoot growth is influenced more by temperature than by light. Our results reveal that the symbiotic relationship provides vigorous shoot growth under uncertainly fluctuating temperatures, resulting in fast growing, vigorous seedlings with a high yield potential, and that the selection of appropriate combinations with a high affinity between host and fungus species which tolerate temperature stress is important.

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Arbuscular菌根菌が共生したアスパラガス実生の温度ストレス耐性

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摘 要

Arbuscular 菌根菌 (AM 菌)[*Gigaspora margarita* (GM), *Glomus* sp. R10 (GR)] が共生したアスパラガス (*Asparagus officinalis* L., cv.Mary Washington 500W) 実生の生長に及ぼす温度ストレスの影響について調査した.

変温条件 [床土温度 : 25℃ (16時間)/20℃ (8時間)] 下で7週間育苗後, AM菌感染率 (1個体の根系における感染率) はGM接種区で63.0%, GR接種区で20.0%に達し, これらの植物体では菌種に関わらず共生関係成立による植物体生長促進効果が発現された.

接種7週間後から低温ストレス条件 (床土温度 : 15℃, 恒温条件) 下で4週間育苗したが, この間, 無接種区では植物体生長が緩慢となった. AM菌が共生した植物体では, 特に4次茎以降の萌芽・伸長が促進され, その効果は特にGR接種区で大きく現れた. 接種11週間後では, 感染率はGM接種区で48.9%, GR接種区で58.9%となり, 接種区の生長は無接種区よりも良好で, 植物体中のリン含有率も接種区で高かった.

接種7週間後から高温ストレス条件 (床土温度 : 30℃, 恒温条件) 下で4週間育苗したが, この間, 無接種区では5次茎以降の萌芽が抑制された. 接種区では特に5次茎または6次茎以降の萌芽・伸長が促進され, その効果はGM接種区で大きく現れた. また, 接種11週間後では, 感染率はGM接種区で66.3%, GR接種区で36.7%を示し, 両菌種で無接種区より旺盛な生育がみられた.

これらのことから, AM菌が共生したアスパラガス実生において低温および高温に対する温度ストレス耐性がみられることが明らかとなり, 耐性がみられる温度域には菌種間差があることも示唆された.