JARQ 31, 179-183 (1997)

Regulation of Arbuscular Mycorrhiza Symbiosis: Hyphal Growth in Host Roots and Nutrient Exchange

Masanori SAITO

Department of Ecology, National Grassland Research Institute
(Nishinasuno, Nasu, Tochigi, 329-27 Japan)

Abstract

Arbuscular mycorrhizal (AM) fungi, which have often been designated as vesicular-arbuscular mycorrhizal (VAM) fungi, symbiotically inhabit plant roots. The promotion of phosphorus uptake and plant growth of host by AM fungi is now well recognized. Therefore, it is anticipated that AM symbiosis could be applied for sustainable agriculture. The author investigated the regulation of the symbiosis with emphasis placed on nutrient exchange, namely phosphorus transfer from AM fungi to host plant and reverse transfer of carbon. The following results were obtained: 1) The growth of AM fungi in host roots was regulated by the supply of photosynthates and the growth of both partners in the symbiosis was well harmonized. 2) A new method for the isolation of intraradical hyphae from host root tissues was developed. The characteristics of enzyme activities in the isolated intaradical hyphae suggest that alkaline phosphatase activity is localized in arbuscular hyphae and that glucose is one of the carbon sources from host plant to AM fungi. 3) Electrophoresis analysis showed that symbiosis-specific may originate from AM fungi. Based on these findings, the regulation of the symbiosis was discussed.

Discipline: Soils, fertilizer and plant nutrition/Biotechnology
Additional key words: vesicular-arbuscular mycorrhizal fungi, VAM, phosphatase, enzyme

Introduction

It is generally recognized that symbiotic association between terrestrial plant roots and the fungi belonging to the order Glomales (designated as arbuscular mycorrhizal (AM) or vesicular-arbuscular mycorrhizal (VAM) symbiosis) improves not only the P nutrition of the host plant but also its growth, which may result in the increase of the resistance against drought stress and some diseases. Therefore, AM offers a great potential for sustainable agriculture. In Japan, the interest about the AM fungi (AMF) has increased since the pioneer work by Ogawa[9]. However, research had been mainly focused on the ecology and the practical application of AM fungi in agriculture or horticulture[5,10,11,20]. Some companies have already launched the commercialization of inoculum production of AMF. For application of AMF to rather intensive agriculture in Japan, evaluation of the function of AM under field conditions is needed. However, since the basic aspects of the symbiosis have not been well investigated, the methodology for such evaluation has not been developed. Various practical problems tend to be addressed on an empirical basis rather than on basic understanding of the function of AM. We have investigated the mechanism controlling the regulation of the growth of symbiotic fungi in host roots in relation to nutrient exchange between the symbionts. In this paper the current progress of our research on the topic is reviewed and future prospects are also outlined.

Growth interrelationship between host plant and mycorrhizal fungi

AMF depend for their carbon and energy sources upon the photosynthates of host plants[4]. Although the mycobiont (fungal partner of the symbiosis) contributes to host growth by supplying P, there is a competitive relationship between the mycobiont and host for photosynthates. To analyze the host-symbiont relationships from the view point of carbon

allocation, the growth response of the mycobiont can be examined when the host plant growth is depressed due to environmental stresses such as low temperature.

Therefore, the effects of low temperature and reduced light on a *Glycine-Bradyrhizobium-Glomus* spp. symbiosis were examined in pot experiments[12]. Soybean plants (*Glycine max*) were grown with N fertilization or inoculation with *Bradyrhizobium japonicum* plus P fertilization or inoculation with an AMF, *Glomus mosseae*, in a glasshouse. After the flowering stage, half of the pots with soybean plants were exposed to low temperature with light reduced by shading. At 0, 7, 16 and 28 days after the application of the treatments, the growth, nodulation, mycorrhizal infection and the N and P contents of the soybean plants were examined. In all the symbiont-fertilization combinations, the low-temperature treatment reduced the production of dry matter by the soybeans. Both the nodule weight and the infected root length were linearly related to shoot dry weight regardless of the treatment and of the symbiont-fertilization combination used (Fig. 1). Since the infected root length has been recognized to be an index of mycobiont biomass, the results suggest that the growth of the symbionts on the root was in harmony with the shoot growth of the host, irrespective of climatic conditions. Shoot growth may be recognized as an index of the supply of photosynthates to symbionts. Therefore, these observations indicate that there is a considerable degree of host control for symbiont growth through the supply of photosynthates and furthermore, that there is a balance between host and symbiont irrespective of competition for photosynthates.

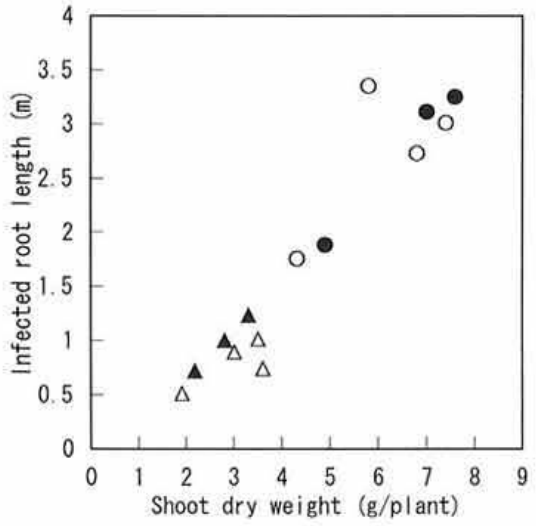

Fig. 1. Relationship between shoot dry weight and root length of soybean infected with *Glomus mosseae*
○: Control plants grown with *Glomus mosseae* + nitrogen fertilizer.
●: Low temperature-shading treatment with *Glomus mosseae* + nitrogen fertilizer.
△: Control plants with *Glomus mosseae* + *Bradyrhizobium* sp.
▲: Low temperature-shading treatment with *Glomus mosseae* + *Bradyrhizobium* sp.

Characteristics of mycorrhizal infection in relation to enzyme activities of roots

The nutrient exchange between host and mycobiont involves both phosphorus transfer from mycobiont to host and the reverse transfer of carbon[4]. Host regulation of the mycobiont growth as observed in the above experiments may be ascribed to carbon transfer from host to mycobiont. The biochemical mechanisms of this nutrient exchange have been often reviewed, but are still largely speculative[3,6,14,16,17]. Therefore, we attempted to clarify the mechanism of the nutrient exchange by comparing the differences in the enzymes expressed in mycorrhizal and non-mycorrhizal roots. For this objective, we examined various enzymes which were considered to be related to the C and P exchange and the energy metabolism[14].

Soluble protein, mitochondria- and plasma membrane-enriched fractions were prepared from both arbuscular mycorrhizal onion (*Allium cepa*) roots with *Gigaspora margarita* and non-mycorrhizal roots. The activities of 11 enzymes involved in glycolysis, TCA cycle, carbohydrate and phosphate metabolism were measured for the soluble protein fraction. The activity of succinate dehydrogenase (SDH) for the mitochondria-enriched fraction and H^+-ATPase for the plasma membrane-enriched fraction was also measured, respectively. Only 4 enzymes (trehalase, hexokinase, phosphofructokinase, SDH) showed consistently higher specific activities (protein basis) in mycorrhizal roots (Table 1). Most of the other enzymes did not show higher specific activities in mycorrhizal roots but showed higher activities on a root weight basis because soluble protein contents were higher in mycorrhizal roots.

Previously, we did not observe any changes in the composition and the amount of symbiotic-specific amino acid, organic acids and sugars between mycorrhizal onion roots with *Glomus mosseae* and non-mycorrhizal roots[19], suggesting that nutrient exchange did not involve metabolic processes specific

Table 1. Comparison of specific activities (protein basis) of enzymes in mycorrhizal and non-mycorrhizal onion roots[a)]

Tendency	Phosphate metabolism	Carbohydrate metabolism	Glycolysis, TCA cycle
Myco. > Non-myco.		Trehalase	HK, PFK, SDH[c)]
Nyco. ≦ Non-myco.	ACP, ALP, H^+-ATPase[b)]	Sucrose synthase, Invertase	G6PDH, MDH, ICDH, Malic enzyme

a): Acid phosphatase (pH 4.0)(ACP), Alkaline phosphatase (pH 8.5)(ALP), Hexokinase (HK), Glucose-6-phosphate dehydrogenase (G6PDH), Phosphofructokinase (PFK), Malate dehydrogenase (MDH), Isocitrate dehydrogenase (ICDH), Succinate dehydrogenase (SDH).
b): Plasma membrane-enriched fraction.
c): Mitochondria-enriched fraction. Others: Soluble protein fraction.

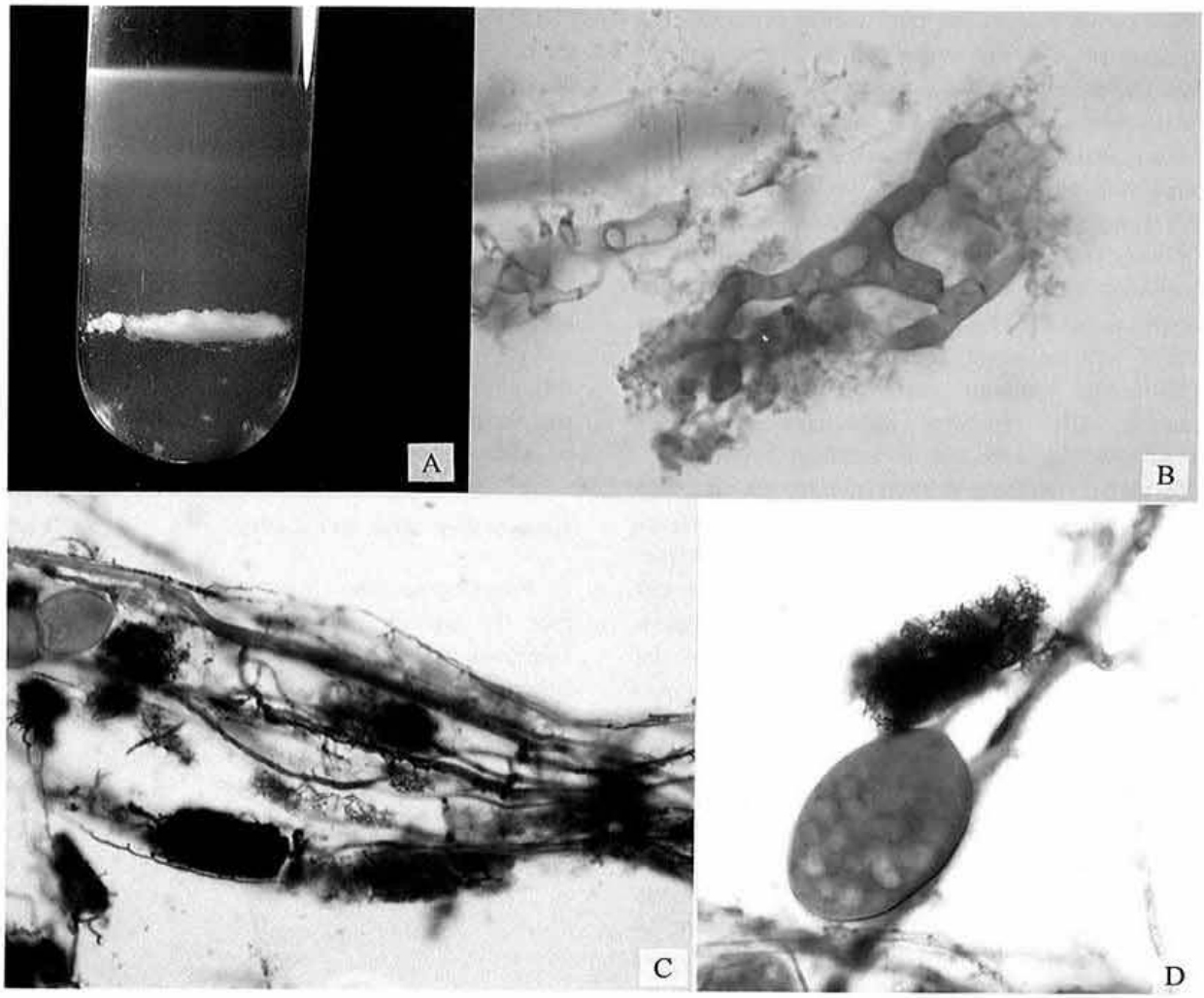

Plate 1. Isolation of intraradical hyphae from mycorrhizal roots and their phosphatase activities
A: Intraradical hyphal fraction of arbuscular mycorrhizal onion roots with *Gigaspora margarita*, separated by discontinuous gradient centrifugation of Percoll.
B: Arbuscular hyphae isolated from mycorrhizal onion roots (acid fuchsin staining).
C: Alkaline phosphatase activity (pH 8.5) of the intraradical hyphae isolated from mycorrhizal onion roots with *Glomus* sp. E3. Phosphatase activity was stained black and the hyphae without the activity were counterstained in red with acid fuchsin.
D: Alkaline phosphatase activity in arbuscular hyphae of *Glomus* sp. E3 stained black. Vesicle not showing phosphatase acitivity was stained red only with acid fuchsin.

to the symbiosis. Although the results did not enable to solve the problems related to nutrient exchange, it was suggested that the root metabolic activities were enhanced by the increase of the protein content of cells, which was in agreement with the report of McArthur and Knowles[8]. The higher activities of HK, PFK and trehalase suggest that the glucose and trehalose metabolism play a significant role in arbuscular mycorrhizae.

Enzyme activities of the intraradical hyphae isolated from arbuscular mycorrhizal roots

In order to analyze the biochemical processes occurring in the nutrient exchange, it is necessary to isolate the mycobiont from host tissues and to examine its biochemical activities. Therefore, a method for the isolation of metabolically active intraradical hyphae was newly developed[13]. In this method, onion roots infected with arbuscular mycorrhizal fungi were treated with a digestion solution containing cellulase and pectinase, followed by homogenization with a blender at a low speed. Then, the hyphal fraction was collected from the homogenate by discontinuous gradient centrifugation of Percoll (Plate 1A, 1B). Enzyme histochemical staining showed that the collected intraradical hyphae contained active succinate dehydrogenase, alkaline and acid phosphatases. Especially, alkaline phosphatase activity was characteristic in fine arbuscular hyphae, suggesting that this phosphatase is involved in nutrient exchange (Plate 1C, 1D). Specific activities (protein basis) of several enzymes in a crude extract of the isolated intraradical hyphae of *Gigaspora margarita* were examined. The hexokinase activity was much higher in the intraradical hyphae than in the root tissues, irrespective of the mycorrhizal infection. These findings indicate that glucose is actively metabolized in the intraradical hyphae, and further suggest that glucose might be one of the energy sources which are supplied by the host plant to intraradical hyphae of the fungi. NMR study showed that glucose supplied exogenously was utilized by mycorrhizal roots but not by non-mycorrhizal ones[15], which supports our assumption. Recently we have examined the use of sugars by the intraradical hyphae by radiorespirometry and confirmed that the hyphae mainly used glucose as a substrate for respiration[18].

Symbiosis-specific phosphatase

Transfer of phosphorus from AM fungi to host plant may take place as follows: inorganic phosphate absorbed with the extraradical hyphae from the soil solution is transformed into polyphosphate, which is driven by cytoplasmic streaming, and hydrolyzed in the arbuscule for its supply to host cell[4,17]. The phosphatase which was found to be localized in arbuscular hyphae (Plate 1C, 1D) may play a key role in the above phosphate transfer[2,13]. Electrophoresis of soluble protein from mycorrhizal roots and subsequent enzyme staining of the gel showed the presence of a symbiosis-specific phosphatase in *Gigaspora margarita-Allium cepa* (onion) symbiosis[7]. This specific band of phosphatase on the gel was only detected when the assay was conducted under alkaline conditions (pH 8.5). The isolated intraradical hyphae also showed the presence of the phosphatase with the same relative mobility, indicating that the symbiosis-specific phosphatase found on the gels is of fungal origin. The phosphatase is now being purified and characterized. Ezawa and Yoshida[1] also reported the presence of a phosphatase specific to mycorrhizal infection in *Glomus etunicatum-Tagetes patula* (marigold) and it was partially purified for characterization. The origin of this phosphatase is still unclear. Further studies on the phosphatases may enable to elucidate the process of nutrient exchange.

Conclusion and prospects

Fragmentary knowledge obtained so far indicates that the growth of both partners involved in AM symbiosis is interrelated and in harmony with each other, and that the glucose metabolism is essential to elucidate the mechanisms of the nutrient exchange. Assuming that the balanced growth may be ascribed

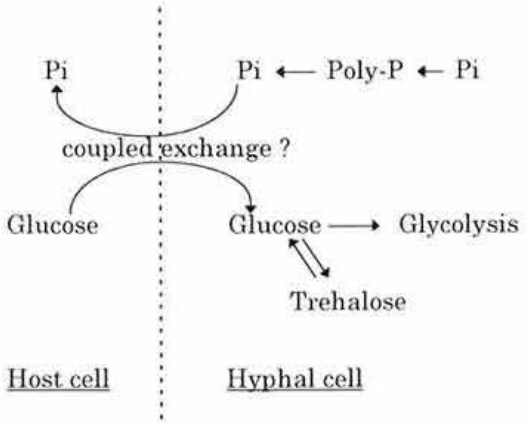

Fig. 2. Hypothetical scheme of nutrient exchange at arbuscule between AM fungi and host plant

to the nutrient exchange in the symbiosis, it was proposed that phosphorus transfer from AM fungi to host is biochemically coupled with carbon transfer (Fig. 2). Characterization of the specific enzymes such as the phosphatases described above may enable to validate this hypothesis. Further studies may enable to develop gene or protein probes for the symbiosis-specific enzymes. These probes would be powerful tools for tracing the introduced AM fungi and for the evaluation of their function in a soil–plant system.

This study was partly supported by a Grant-in-Aid (Bio-Media Program, BMP-V-1-④) from the Ministry of Agriculture, Forestry and Fisheries, Japan.

References

1) Ezawa, T. & Yoshida, T. (1994): Acid phosphatase specific to arbuscular mycorrhizal infection in marigold and possible role in symbiosis. *Soil Sci. Plant Nutr.*, **40**, 255–264.
2) Ezawa, T., Saito, M. & Yoshida, T. (1995): Comparison of phosphatase localization in the intraradical hyphae of arbuscular mycorrhizal fungi, *Glomus* spp. and *Gigaspora* spp. *Plant Soil*, **176**, 57–63.
3) Gianinazzi-Pearson, V. et al. (1991): Enzymatic studies on the metabolism of vesicular-arbuscular mycorrhizas. V. Is H^+-ATPase a component of ATP-hydrolyzing enzyme activities in plant-fungus interfaces? *New Phytol.*, **117**, 61–74.
4) Harley, J. L. & Smith, S. E. (1983): Mycorrhizal symbiosis. Academic Press, London, 64–103.
5) Kobayashi, N. (1989): Suppression of *Rhizotonia, Pythium* damping-off cucumber by microorganisms in charcoal and vesicular-arbuscular mycorrhizal fungi. *In* Recent advances in microbial ecology. ed. Hattori, T. et al., Japan Scientific Press, Tokyo, 242–246.
6) Koide, R. T. & Schreiner, R. P. (1992): Regulation of the vesicular-arbuscular mycorrhizal symbiosis. *Ann. Rev. Plant Physiol. Mol. Biol.*, **43**, 557–581.
7) Kojima, T., Hayatsu, M. & Saito, M. (1996): Phosphatase originated from symbiotic fungus, *Gigaspora margarita*, in arbuscular mycorrhizal onion roots. *Abstr. Annu. Meeting Jpn. Soc. Soil Sci. Plant Nutr.*, **42**, 60 [In Japanese].
8) McArthur, D. A. & Knowles, N. R. (1993): Influence of vesicular-arbuscular mycorrrhizal fungi on the response of potato to phosphorus deficiency. *Plant Physiol.*, **101**, 147–160.
9) Ogawa, M. (1987): Symbiotic microorganisms joining plants and soil. Nobunkyo, Tokyo [In Japanese].
10) Ogawa, M. (1989): Inoculation methods of VAM fungi: charcoal ball method and rice hull method. *In* Recent advances in microbial ecology. ed. Hattori, T. et al., Japan Scientific Press, Tokyo, 247–254.
11) Saito, M. (1990): Charcoal as a micro-habitat for VA mycorrhizal fungi, and its practical implication. *Agric. Ecosystems Environ.*, **29**, 341–344.
12) Saito, M. & Kato, T. (1994): Effects of low temperature and shading on relationships between nodulation, vesicular-arbuscular mycorrhizal infection and shoot growth of soybean. *Biol. Fertil. Soils*, **17**, 206–211.
13) Saito, M. (1995): Enzyme activities of the internal hyphae and germinated spores of an arbuscular mycorrhizal fungus, *Gigaspora margarita* Becker & Hall, *New Phytol.*, **129**, 425–431.
14) Saito, M. & Yasuda, M. (1995): Enzymological characteristics of arbuscular mycorrhizal onion roots. *In* Abstr. 7th Int. Symp. Microb. Ecol., 140.
15) Shachar-Hill, Y. et al. (1995): Partitioning of intermediary carbon metabolism in vesicular-arbuscular mycorrhizal leek. *Plant Physiol.*, **108**, 7–15.
16) Schwab, S. M., Menge, J. A. & Tinker, P. B. (1991): Regulation of nutrient transfer between host and fungus in vesicular-arbuscular mycorrhizas. *New Phytol.*, **117**, 387–398.
17) Smith, S. E. & Smith, F. A. (1990): Structure and function of the interfaces in biotrophic symbioses as they relate to nutrient transport. *New Phytol.*, **114**, 1–38.
18) Solaiman, M. Z. & Saito, M. (1997): Use of sugars by intraradical hyphae of arbuscular mycorrhizal fungi revealed by radiorespirometry. *New Phytol.*, **136** (In press).
19) Tawaraya, K. & Saito, M. (1994): Effect of vesicular-arbuscular mycorrhizal infection on amino acid composition in roots of onion and white clover. *Soil Sci. Plant Nutr.*, **40**, 339–343.
20) Yorifuji, T. & Suzuki, M. (1995): How to use mycorrhizal fungi. (Kinkonkin no ikashikata). Nobunkyo, Tokyo [In Japanese].

(Received for publication, June 3, 1996)