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Morphology of Arbuscular Mycorrhizal Roots and Effects of Root Age and Soil Texture on the Mycorrhizal Infection in *Panax ginseng* C.A. Meyer

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Abstract : The objectives of this study were to investigate the morphology of mycorrhizal roots, and the effects of root age and soil texture on the mycorrhizal infection in ginseng (*Panax ginseng* C. A. Meyer) growing in Korea. Ginseng roots at ages of two to six years were collected from fields in late June. Their infection by arbuscular mycorrhizal fungi (AMF) was studied by clearing the roots and staining fungal hyphae with trypan blue. Root infection varied greatly depending on the developmental stages of young roots. Young tertiary roots, in diameter of smaller than 0.8 mm, formed during the current growing season had root hairs and were frequently and in some cases heavily infected by AMF. Hyphal coils and arbuscules were abundant, while vesicles were rarely observed. Older secondary or tertiary roots in diameter of bigger than 1.0 mm with fully differentiated primary xylem formed during the previous growing season had no root hairs, and were not infected at all. The rates of mycorrhizal infection in the young tertiary roots were not affected by the age of the ginseng plants, suggesting that fungal populations might have not much changed during the aging of the cultivated fields up to six years. The differences in the infection rates among the different ages of ginseng were caused by differences in the amount of young tertiary roots in the samples. Soil texture, either sandy loam or clay loam, did not affect the rate of root infection. There were large variations in the infection rates among the different farms and locations within a farm. It strongly suggested that infection rates of the ginseng roots by AMF would be influenced by the practice of the farmers, possibly by avoiding consecutive planting, introduction of new topsoil, and the ways of handling the soil before transplanting the ginseng, such as fumigation or sterilization that might have affected indigenous inoculum sources of the AMF.

Key words : Young root, root hair, primary xylem, tertiary root, mycorrhizal infection, vesicles, endomycorrhiza

INTRODUCTION

Ginseng (*Panax ginseng* C. A. Meyer) has been cultivated by farmers for a long period of time in Korea. One of the major problems in cultivation of ginseng is root diseases encountered during six years of ginseng cultivation for a single harvest. In Korea Damping-off is caused by *Fusarium* sp., *Rhizoctonia solani*, and *Pythium* sp., root rots by *Cylindrocarpon destructans*, and root blight by *Phytophthora cactorum*.[1)] In Japan *Alternaria panax* was one of the common pathogens in *Panax ginseng*.[2)] In Canada *Pythium* sp. and *Rhizoctonia solani* caused damping-off, and *Cylindrocarpon destructans* was responsible for root rot in Ontario.[3)] In the U. S. *Phytophthora cactorum* was reported in Wisconsin.[4)] Consecutive planting of ginseng in the same bed may bring in serious root diseases[5)] and root rot caused by *Fusarium* sp.[6)]

Farmers in Korea have paid a special attention to the bed soil to avoid introduction of pathogens of root diseases. They might completely exchange the topsoil of the beds with a new soil that has not been used for any type of agriculture. Or they might sterilize or fumigate the soil to kill any pathogenic organisms in the beds.

Higher plants in terrestrial ecosystems have been reported to form either ectomycorrhizae or endomycorrhizae. It has been known that mycorrhizae promote nutrient absorption, enhance resistance to harsh environments, such as extreme pH, soil, and temperature, and suppress root diseases in some agricultural systems.[7)] Recently mycorrhizae have been applied to environmentally friendly agriculture to minimize the use of pesticides and fertilizers. In ginseng cultivation, uses of pesticides are

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strongly discouraged because of their slow breakdown and their possible accumulation in the soil. Ginseng is normally grown for two to six years in the same bed, during which residual pesticides would be absorbed by the ginseng roots.

Researches on ginseng mycorrhizae have received little attention in Korea. Park[8] confirmed the presence of arbuscular mycorrhizae in the cultivated ginseng field. Han *et al.*[9] showed a potential to use arbuscular mycorrhizae in suppression of root rot pathogens. However, natural abundance of endomycorrhizae in different ginseng farms and the rate of root infection by mycorrhizal fungi have not been studied in relation to the aging of the roots.

The objectives of this study were to understand the morphology of mycorrhizal roots, and the effects of root age and soil texture on the mycorrhizal infection in ginseng (*Panax ginseng* C. A. Meyer).

MATERIALS AND METHODS

Sampling and survey of infected roots with AMF

Ginseng (*Panax ginseng* C. A. Meyer) roots used in this experiment were obtained from farms located in Icheon and Hwaseong, Gyonggi Province. Three farms in Icheon area were established in sandy loam and had a tradition of making 40cm-deep ditches to promote fast drainage in the beds. Other three farms in Hwaseong area were established in clay loam and maintained 20cm-deep ditches, which might have delayed drainage. All the ginsengs in these farms were grown from transplanted one-year-old seedlings.

Ginseng has been known not to form fibrous root systems like grasses. Instead, the main or primary roots, also called taproots, become thick as a storage organ and are harvested for medicinal purposes at least four and in maximum six years after seed sowing. Thus, in the present study the ginsengs at the age from two to six years were used. For mycorrhizal study, secondary lateral roots deriving from the taproots and tertiary roots branching from the secondary roots were collected in late June. Therefore, actual targets of the mycorrhizal study were secondary and tertiary lateral roots at small diameter before their secondary thickening[10] regardless of the ginseng age, because mycorrhizae are normally formed in the fine roots only. The diameter of the collected lateral roots ranged from about 0.4 to 1.2 mm.

The collected roots were put into a FAA (formalin-acetic acid-alcohol) solution in the field and stored in the same solution until further treatment. The cellular contents in the roots were cleared with KOH, bleached with H_2O_2, and stained with trypan blue[11]. The squashed root samples were put on the slide glasses and observed under a light microscope for presence of fungal structures. The percentage of mycorrhizal infection was counted by a grid-intersection method[12].

RESULTS

Morphology of young roots and mycorrhizae

Young lateral ginseng roots might be classified into three groups based on their stages of differentiation. The first group is young current-year lateral roots at the early stage of primary xylem differentiation (Fig. 1). This figure shows a young tertiary lateral root in 2-year-old ginseng. All the root samples used in this study were collected in late June, and this young root might have been formed very recently in the current year. The average diameter of young tertiary roots in the first group was 0.6 mm and they had partially differentiated primary xylem in the stele as shown in Fig. 1. It had abundant root hairs, suggesting its active function in absorbing water and nutrients. However, it was not infected by mycorrhizal fungi.

The second group is current-year lateral roots at mid stage of primary xylem differentiation (Fig. 2). This picture was taken from a young tertiary lateral root in a 2-year-old ginseng. This young root might have been formed in the current year, but earlier than the younger root in Fig. 1. This second group of young roots had an average diameter about 0.8 mm, and had more differentiated primary xylem than the first group in Fig. 1. It had

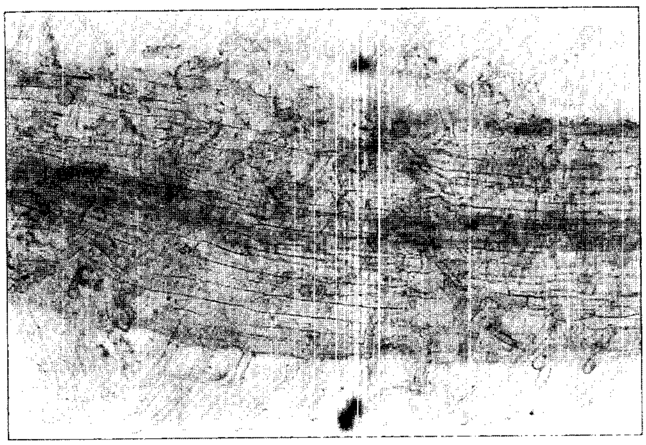

Fig. 1. A current-year young tertiary root (average diameter of 0.6 mm with partially differentiated primary xylem) of 2-year-old ginseng with abundant root hairs, but without infection caused by AMF.

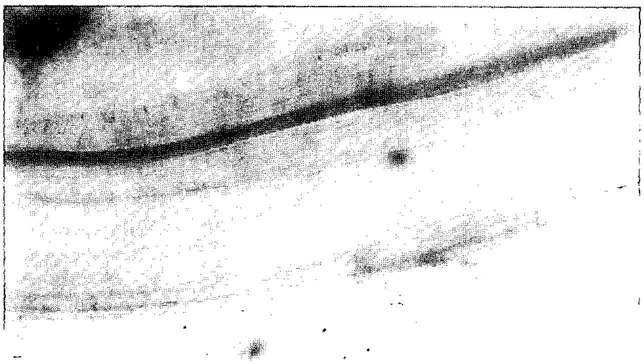

Fig. 2. A current-year young tertiary root (average diameter of 0.8 mm with more differentiated primary xylem) of 2-year-old ginseng with root hairs, but without infection caused by AMF.

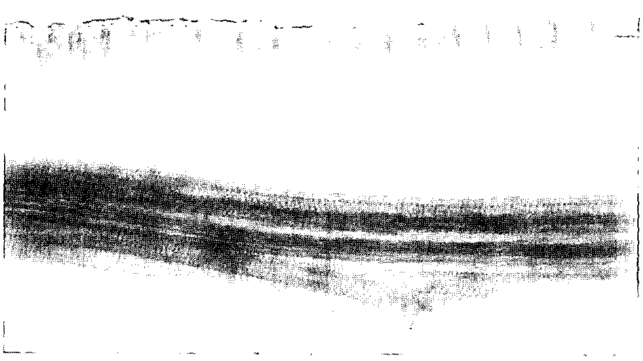

Fig. 3. An one-year-old secondary root (average diameter of 1.0 mm with fully differentiated primary xylem, but without secondary thickening) of 4-year-old ginseng with no root hairs, and with no infection by AMF.

abundant root hairs, but was not infected by mycorrhizal fungi.

The third group is one-year-old young roots at more advanced stage of primary xylem differentiation (Fig. 3). The young roots in this third group were formed during the previous growing season and would be secondary roots that had an average diameter of about 1.0 mm or more. This root had fully differentiated primary xylem, but showed no secondary thickening. This particular picture (Fig. 3) was taken from a 4-year-old ginseng. This third group had no root hairs and was not infected by mycorrhizal fungi. In Fig. 4, the bigger diameter of a one-year-old young root without infection (left side) could be easily distinguished from smaller diameter of a current-

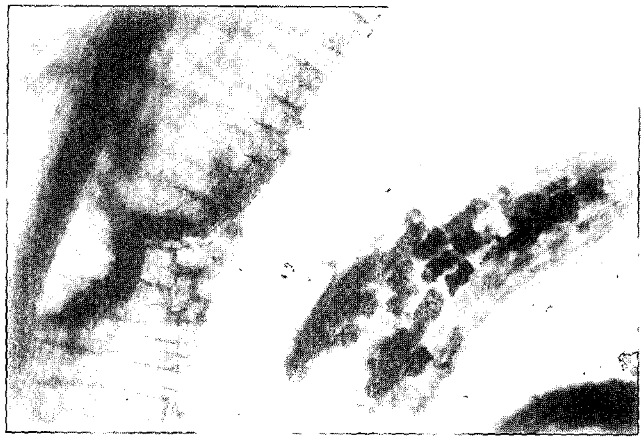

Fig. 4. A difference in the size of root diameter between an one-year-old root in bigger diameter without infection (left) and a current-year root in smaller diameter with infection (right) caused by AMF in 5-year-old ginseng.

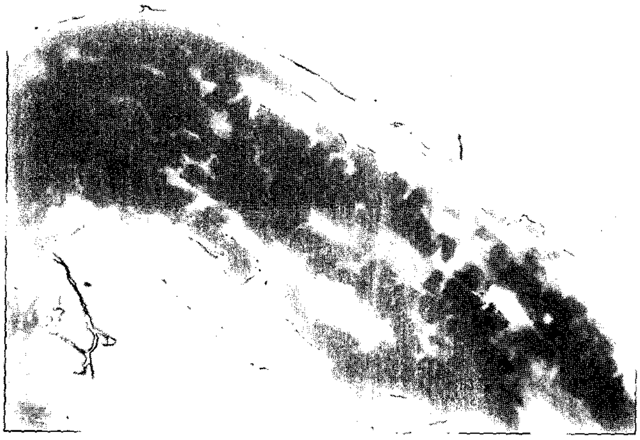

Fig. 5. A current-year young root of 3-year-old ginseng infected heavily by AMF and with abundant arbuscules.

year young root with mycorrhizal infection (right side).

Fig. 5 shows a current-year young root of 3-year-old ginseng infected heavily by arbuscular mycorrhizal fungi (AMF). It had many root hairs and abundant arbuscules in the cortex. Infection of the roots was progressed toward the root apex and the arbuscules was arranged in the longitudinal axis as shown in Fig. 6. Arbuscules were formed in a relatively short period of time in *Panax quinquefolius*,[13] with rapid turn over of arbuscules at different stages of development as shown in Fig. 7. Arbuscules were reported to be formed continuously until August to reach a maximum and decrease thereafter in *Panax quinquefolius*.[14] In the present study the rate of infection observed in late June may not reflect the maximum level of infection possible in August. However, authors had more interest in the rate of root infection at different ages, rather than different sea-

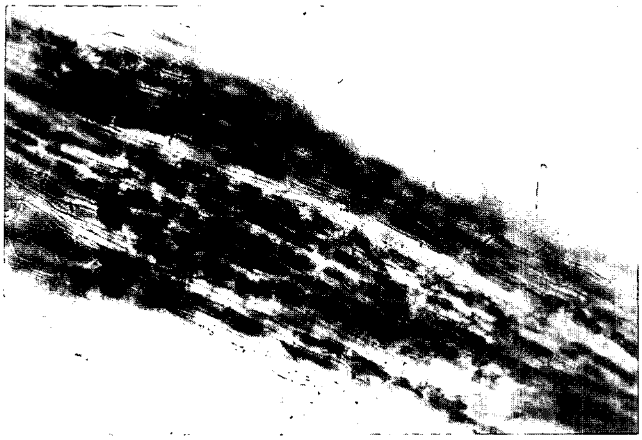

Fig. 6. Abundant arbuscular formations along the longitudinal axis in a young root with infection caused by AMF in 2-year-old ginseng.

Fig. 7. Arbuscules at different stages of development in a young root of 2-year-old ginseng.

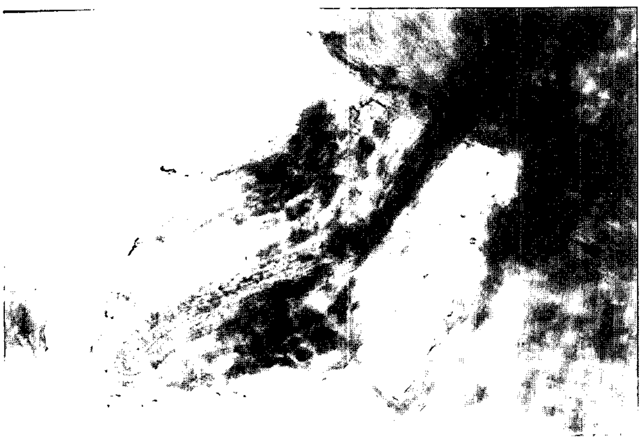

Fig. 8. Mycorrhizal formation near the root tip of a young root of 4-year-old ginseng. The root tip is free of mycorrhizal infection.

Fig. 9. Hyphal coils of AMF in a young root of 2-year-old ginseng, with hyphae being confined in individual cells to form a rectangular shape.

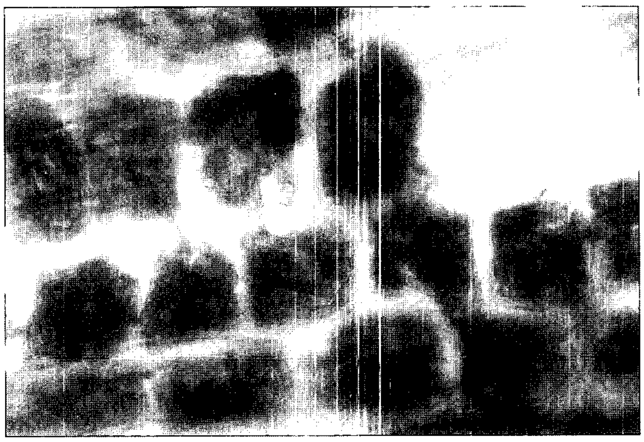

Fig. 10. Detailed arrangement of hyphal coils of AMF in a young root of 2-year-old ginseng.

sons, during the six years of cultivation.

Roots do not form mycorrhizae near the root tip where active cell divisions are under way. Fig. 8 shows a root apex of a young root of 4-year-old ginseng. The root tip is free of mycorrhizal infection. Hyphal coils in AMF were abundant in this study and sometimes confined in individual cells to form a cube shape as shown in Fig. 9 and Fig. 10. Arbuscules showed different shapes depending on the root samples. Fig. 11 shows internal hyphae and arbuscules developed from these hyphae in a young root of 3-year-old ginseng. They were at different stages of development.

Vesicles were rarely found in this study. Absence or rare occurrence of vesicles in ginseng roots was also reported previously[13] and could be characteristics of ginseng. Fig. 12 shows an exceptional case of abundant vesicles in a

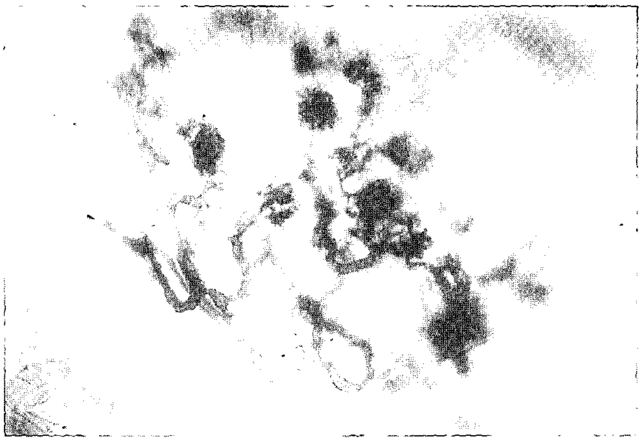

Fig. 11. Internal hyphae and arbuscules of AMF at different stages of development in a young root of 3-year-old ginseng.

Fig. 12. Abundant vesicles in a young root of 3-year-old ginseng infected by AMF. Vesicles were rarely observed in this study.

Fig. 13. Internal hyphae of AMF running along the longitudinal axis by passing intracellularly through cortical cells in a young root of 2-year-old ginseng.

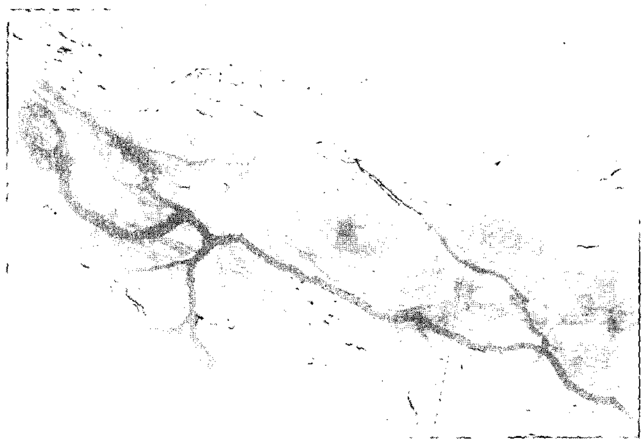

Fig. 14. External hyphae of AMF on the surface of a young root in 4-year-old ginseng.

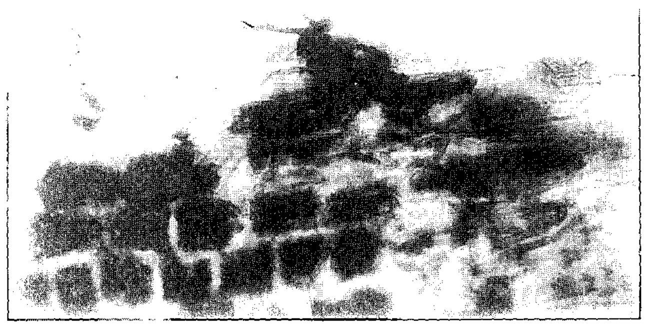

Fig. 15. Appresoria formation on the surface of a young root as an entry point in 2-yer-old ginseng.

young root of 3-year-old ginseng. Vesicles were observed in two cases only in this study.

Internal hyphae of AMF were sometimes abundant, but had different shapes depending on the samples. Fig. 13 shows the internal hyphae in a young root of 2-year-old ginseng. These internal hyphae ran along the longitudinal axis by passing intracellularly through cortical cells without being confined in individual cells. Other hyphae of AMF formed intensive coils that were confined in individual cells as shown in Fig. 9. These external hyphae were abundant in this study as shown in Fig. 14. They were stained more heavily by trypan blue, and had bigger diameter than internal hyphae.

Mycorrhizal fungi may enter the host roots through root hairs or epidermis. In the present study, entry point of a fungus was not frequently observed, but fungus formed appressoria on the surface of epidermal cells as shown in Fig. 15.

Table 1. Mycorrhizal infection rates (%) of field-grown *Panax ginseng* at different root ages and soil texture

Root ages	Sandy loam[1]						Clay loam[2]					
	Bed[3] 1	Bed 2	Bed 3	Bed 4	Bed 5	Mean	Bed[4] 1	Bed 2	Bed 3	Bed 4	Bed 5	Mean
2 years	10	50	33	40	58	38	0	0	0	–	–	0
3 years	0	3	0	0	5	2	35	13	18	60	–	32
4 years	1	0	55	3	2	12	30	2	2	–	–	13
5 years	5	23	28	23	2	16	–	–	–	–	–	–
6 years	0	2	0	3	0	1	30	28	28	8	15	22
Mean						14						17

[1]The soil was collected from a ginseng farm located in Icheon, Gyonggi Province.
[2]The soil was collected from a ginseng farm located in Hwaseong, Gyonggi Province.
[3]The bed was 1m wide and had 40cm-deep ditches to promote fast drainage.
[4]The bed was 1m wide and had 20cm-deep ditches which might have delayed drainage.

Infection rate at different root ages and soil texture

Table 1. shows mycorrhizal infection rates of ginseng at ages from two to six years and in different soil texture. Overall average of infection rates in this study was 15%. This low infection rate may partly come from the root characteristics of cultivated ginseng. Through fertilization, weeding, and adequate spacing, farmers control the quality of ginseng roots. Thus cultivated ginseng develops a thickened primary (tap) root and very few secondary and tertiary lateral roots. Mycorrhizae are formed in fine roots only in both ecto- and endomycorrhizae. In case of cultivated ginseng, mycorrhizae would be formed in the secondary roots at the early stage of their development while they do not undergo secondary thickening. Instead tertiary roots may form mycorrhizae while they have small diameter with root hairs. Eventually cultivated ginseng roots have very few sites for mycorrhizal symbiosis.

There were large variations in mycorrhizal infection among the different ages of ginseng roots. In addition, large variations were also noticed among the different beds or locations. For example, some roots had no mycorrhizae at all, while one of the samples showed 60% infection rate. Age of the roots did not influence the infection rates. For example, average infection rate in sandy loam fluctuated widely with increasing age of the roots, from 38% in the two-year-old roots to 1% in the six-year-old roots. The same large variations in the infection rate were also observed in the clay loam, from 32% in three-year-old roots to 0% on the two-year-old roots.

The differences in the infection rates among the different age of ginseng were caused by differences in the amount of young roots in the samples. It was found later during the courses of investigating roots under a microscope that all the young roots with mycorrhizal infection had root hairs and had diameter of 0.8 mm or less with partially differentiated primary xylem. During the sampling of roots in the field, authors tried to collect as many roots in smaller diameter as possible. But due to limited number of young roots, and to large variations in the amount and size of young roots, root samples contained different amount of young roots at varying diameter. Therefore, the root infection rates were affected by the amount of young roots at smaller diameter.

Soil texture seemed to have no influence on the mycorrhizal infection. Infection rates in the overall average showed no differences between the two kinds of soil texture. Average infection rate in sandy loam was 14%, while that of clay loam was 17%. On the other hand, large variations in the infection rates within a farm were noticed in Table 1. For example, four-year-old roots in sandy loam showed no infection in Bed 2, while neighboring Bed 3 in the same farm had 55% infection.

DISCUSSION

Present study confirmed mycorrhizal infection in *Panax ginseng* in the cultivated fields in Korea. Arbuscular mycorrhizae (AM) were the major type in this symbiosis. Among the two types of AM, known as Arum type and Paris type, the latter was found in *Panax quinquefolius*.[15] Based on the present study it may be said that Korean ginseng might have Paris type AM that typically has extensive hyphal coils and arbusculate coils. Further study may be needed to clarify the classification.

In the present study, the 15% average mycorrhizal infection rate in two- to six-year-old roots seemed to be low in the cultivated ginseng. The low infection rate may be partly explained by the partial loss of fine roots during the transplanting of one-year-old seedlings. Cultivated ginseng first develops primary root, also called taproot, after germination from seed, and later develop extensive secondary fine roots during the first year of growth. However, many fine roots would be lost during the process of transplanting the one-year-old seedlings to new beds. Therefore, during the second growing season, the number of secondary and tertiary young roots where mycorrhizae would normally be formed were smaller than that in the first growing season.

The low mycorrhizal infection rates in the present study are comparable to the same trend in the wild ginsengs. Wild ginsengs found in the forests also had low infection rates. Lee and Park[16] who observed 176 samples of wild ginseng roots throughout the country found that 42% samples had no mycorrhizae at all, 53% showed a trace of mycorrhizal infection, and 5% only were extensively infected, indicating low infection of ginseng roots in natural conditions. It has been reported that wild ginsengs grow under very diverse environments,[17] but it is not clear how much ginseng relies on mycorrhizal formation for its survival under natural environments. Further study may be needed to understand the infectivity and effectivity of mycorrhizal fungi in ginseng.

Large variations in the infection rates were noticed among different root ages, farms, and locations within a farm as shown in Table 1. The infection rates in the Table 1 were determined with a duplicate in each bed and with a duplicate again in microscopic observations from a single bed. The infection rates within a duplicate also showed large variations. These large variations indicated that mycorrhizal infection of ginseng roots in cultivated fields in Korea might be quite variable or unpredictable, which was not desirable in sustainable agriculture.

The mycorrhizal infection was variable in ginseng roots at different ages. There was no relationship between root ages and mycorrhizal infection. Above observations suggested that fungal populations in the ginseng beds might have not increased during the period of six years of cultivation for a single harvest. Accordingly, there would be no fungal succession during the period. Further study is needed to monitor AM fungal diversity and population dynamics in relation to the aging of the ginseng beds.

It is quite reasonable to infer that Korean farmer's practices in ginseng cultivation might be quite unsuitable for natural introduction of mycorrhizal symbiosis to the system. The possible biological and edaphic factors that might discourage mycorrhizal symbiosis in agriculture would be lack of fungal inoculum, low host preference for mycorrhizal formation, and toxic root exudates[18] as biological factors, and high or extremely low nutrient levels, toxic materials in the soil, and extreme soil pH as edaphic factors.[7] The present study did not determine the reasons of low mycorrhizal infection in ginseng. However, it may be assumed that loss of fine roots during the process of transplanting and lack of fungal inoculum in the new beds would be the major reasons of low infection. Korean farmers are very concerned about the root diseases during the six years for a single harvest. It has been a common practice that farmers avoid consecutive cultivation of ginseng in the same field and for preparation of new beds brings in new topsoil that has not been previously used for agriculture to discourage the root diseases. An alternative would be soil fumigation or sterilization of the field soil that might kill fungal inoculums as well as pathogens in the soil.

The present study focused on the morphology of mycorrhizal infection in ginseng in relation to the development of young roots and to the age of the roots. Further study is needed to investigate the mycorrhizal infection of current-year ginseng growing in the seedbeds, and to investigate reasons for variable and low infection rates in the aged ginseng roots. In addition, the effects of mycorrhizal infection on the growth stimulation of hosts and deterrence of root pathogens should be further studied to promote environmentally friendly agriculture that saves fertilizers and pesticides, even though there is a report that mycorrhizal ginseng may grow slower than non-mycorrhizal plants.[19]

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