

Root Age-Dependent Changes in Arbuscular Mycorrhizal Fungal Communities Colonizing Roots of *Panax ginseng*

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Abstract In this study, we examined arbuscular mycorrhizal fungal (AMF) community structure colonizing field-cultivated ginseng roots according of different ages, such as 1- to 5-year-old plant, collected from Geumsan-gun, Korea. A total of seven AMF species namely, *Funnelliiformis caledonium*, *F. moseae*, *Gigaspora margarita*, *Paraglomus laccatum*, *P. occultum*, *Rhizophagus irregularis*, and *Scutellospora heterogama* were identified from the roots using cloning, PCR-restriction fragment length polymorphism and sequence analysis of the large subunit region in rDNA. AMF species diversity in the ginseng roots decreased with the increase in root age because of the decreased species evenness. In addition, the community structures of AMF in the roots became more uniform. These results suggest that the age of ginseng affects mycorrhizal colonization and its community structure.

Keywords AMF, Korean ginseng, LSU, Species diversity

Arbuscular mycorrhizal fungi (AMF) belong to phylum Glomeromycota and symbiosis with the roots of most terrestrial plants [1]. AMF improve the nutrient uptake efficiency of the host plant and also enhance drought resistance, improving resistance to leaf herbivory by insects, increasing heavy metal and salt tolerance, and increasing soil pathogen resistance [2-5]. Thus, we thought AMF play important roles in plant physiology, plant health and nutrient cycling of the ecosystem with basis of commensalism [6].

Korean ginseng (*Panax ginseng* C. A. Mey.) is a slow growing perennial herb with thick roots and palmate leaves. The herb belongs to the genus *Panax* of family Araliaceae. Ginseng roots have been used as a medicine for

thousands of years in Asian countries, especially in Korea, China, and Japan. Medicinal effects of ginseng are attributed to ginsenosides found mainly in their roots [7]. The biochemical properties of the ginsenosides and their abundance are influenced by the genetic composition of ginseng as well as by the environmental conditions, including chemical composition of the soil, climate and interaction with other organisms including soil microorganisms [8].

AMF can increase the survival of American ginseng, *Panax quinquefolius* and inoculation with AMF promote growth and increases root ginsenoside content [9]. Each AMF species affects plant growth and nutrient uptake differently [10] as has been described previously for different species of AMF that colonize ginseng roots [11]. Thus, understanding AMF diversity in ecosystems is an important factor for developing sustainable agriculture.

There are previous reports on the detection of AMF in the rhizosphere and roots of ginseng [12, 13]. Recently, Kil *et al.* [14] reported that AMF spore community structures in the soil of field-cultivated ginseng changed and AMF species diversity decreased with the increase in the age of ginseng roots. However, it is not clear whether AMF species found in the soils colonized the ginseng roots. Previous studies showed that the positive relationship between AMF spore communities in the soil and active AMF colonizing within roots was low [15, 16] and there is no reports on AMF community in the ginseng roots with different ages. In this study, we examined community compositions of AMF colonizing the interior of the ginseng roots collected

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from Geumsan-gun, Korea and investigated the relationship of AMF colonization rate and community structure according to the ginseng root age.

Root samples and staining. Field-cultivated ginseng roots were collected 20 different plots growing ginsengs from 1 to 5 year-old plants in Geumsan-gun, Korea (N 36°03'~05', E 127°32'~33'), with 4 replicate plots for each age. A root sample was used for each plot in this study, making a total of 20 samples. To examine AMF colonization rates, ginseng roots were washed, and stained with trypan blue [15]. The roots were observed under the microscope (AXIO Imager; Carl Zeiss, Jena, Germany) and root colonization rates were measured by the previously described magnified intersects technique [16].

Cloning, restriction fragment length polymorphism (RFLP), and sequencing.

DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Venlo, Netherlands) from 20 field-cultivated ginseng root samples. The nested PCR was performed to amplify the 28S rDNA using primers LR1 and FLR2 for first PCR, and LR1 and FLR4 for second PCR as previously described [17-19]. The PCR product was purified by using the QIAquick Gel Extraction kit (Qiagen) and cloned using the pGEM-T Easy Vector System following the manufacturer's instructions (Promega Co., Madison, WI, USA). Typically, twenty clones from each root sample were selected randomly for RFLP in order to group for sequencing. Restriction enzymes *Hinf*I and *Hsp*92II (both from Promega Co.) were used for PCR-RFLP analysis. The nucleotide sequence of one clone from each RFLP type was determined using an automated sequencer (SolGent Co., Daejeon, Korea), and submitted to the GenBank at NCBI (accession Nos. JX975296~JX975326).

Data analysis. Using MEGA 5, rDNA sequences were aligned with other Glomeromycota sequences as reference sequences from GenBank to test the topological appropriation. From this analysis, a phylogenetic tree was constructed by the neighbor-joining method, with a bootstrap value of 1,000 based on Tamura-Nei model using MEGA 5 [20]; *Geosiphon pyriformis* was used as an outgroup.

Statistical analysis. Relative abundances of each fungal phlotypes were estimated based on clone counts for each RFLP type. Shannon diversity index were calculated for AMF community analysis [21]. Univariate analysis of variance (ANOVA) was used to evaluate the fixed effect of root ages on percent mycorrhizal root colonization rates and species diversity of AMF communities, and principle component analysis was performed to compare the compositions of AMF communities using a statistical package SPSS-WIN ver. 12 (SPSS Inc., Chicago, IL, USA).

Mycorrhizal colonization rates significantly increased with the age of the root ($p < 0.05$) (Fig. 1). The mean colonization rate increased gradually from 5% in the 1-

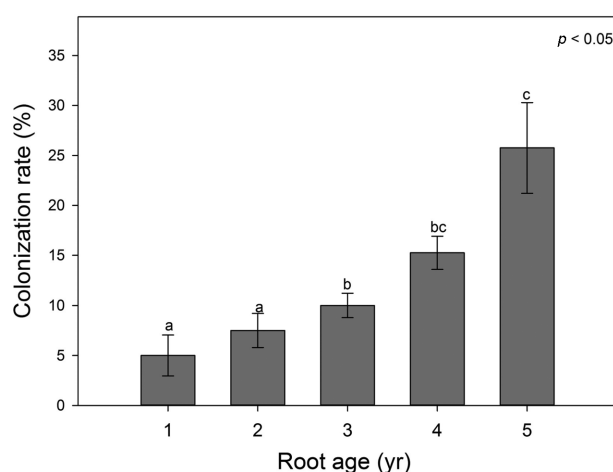


Fig. 1. Mycorrhizal root colonization rates of *Panax ginseng* with cultivation periods. Means and standard errors are shown. Different letters on each bar indicate significantly different mean at $p < 0.05$ according to least significant difference of one-way ANOVA.

year-old ginseng roots to over 25% in the 5-year-old roots. Root colonization rate is influenced by numerous biotic or abiotic factors in the field. Moreover, the overuse of soil fertilizer containing phosphorus and limited light availability has been shown to reduce the rate of colonization by AMF [22, 23]. In addition, root growth at a faster rate than that of the AM fungal hyphae, can also decrease the colonization rate [24]. Even if some of the possible causes described above or other complex environmental factors result in low AMF colonization rates, this neither implies a low AMF-dependence of the host plant nor is it a reflection of the effects of AMF on the host plant [1]. Here, we found that the AMF colonization rate in ginseng roots increased while the species diversity simultaneously decreased with age. Therefore, we found no positive relationship between the colonization rate and species diversity of AMF.

In total, 31 RFLP types were obtained from the 200 clones and one clone representing each RFLP type was sequenced from each group. These sequences were used to construct a phylogenetic tree based on the large subunit (LSU) regions of rDNA (Fig. 2). Based on the phylogeny, all AMF clones were divided into 7 AMF clades: *Funnelliformis caledonium*, *F. mosseae*, *Gigaspora margarita*, *Paraglomus laccatum*, *P. occultum*, *Rhizophagus irregularis*, and *Scutellospora heterogama* (Table 1, Fig. 2).

AMF community composition significantly changed with age of the roots (Table 1). *R. irregularis* was the most dominant species in ginseng roots of all ages used in this study. On the other hand, *G. margarita* was observed only in the 1-year-old roots. The relative abundance of *R. irregularis* increased from 71.3% in 1-year-old ginseng roots to 92.5% in 5-year-old roots. However, abundance of other species, including *F. mosseae*, *P. laccatum* decreased despite widespread presence in cultivated soil; *F. mosseae*, which seems to have

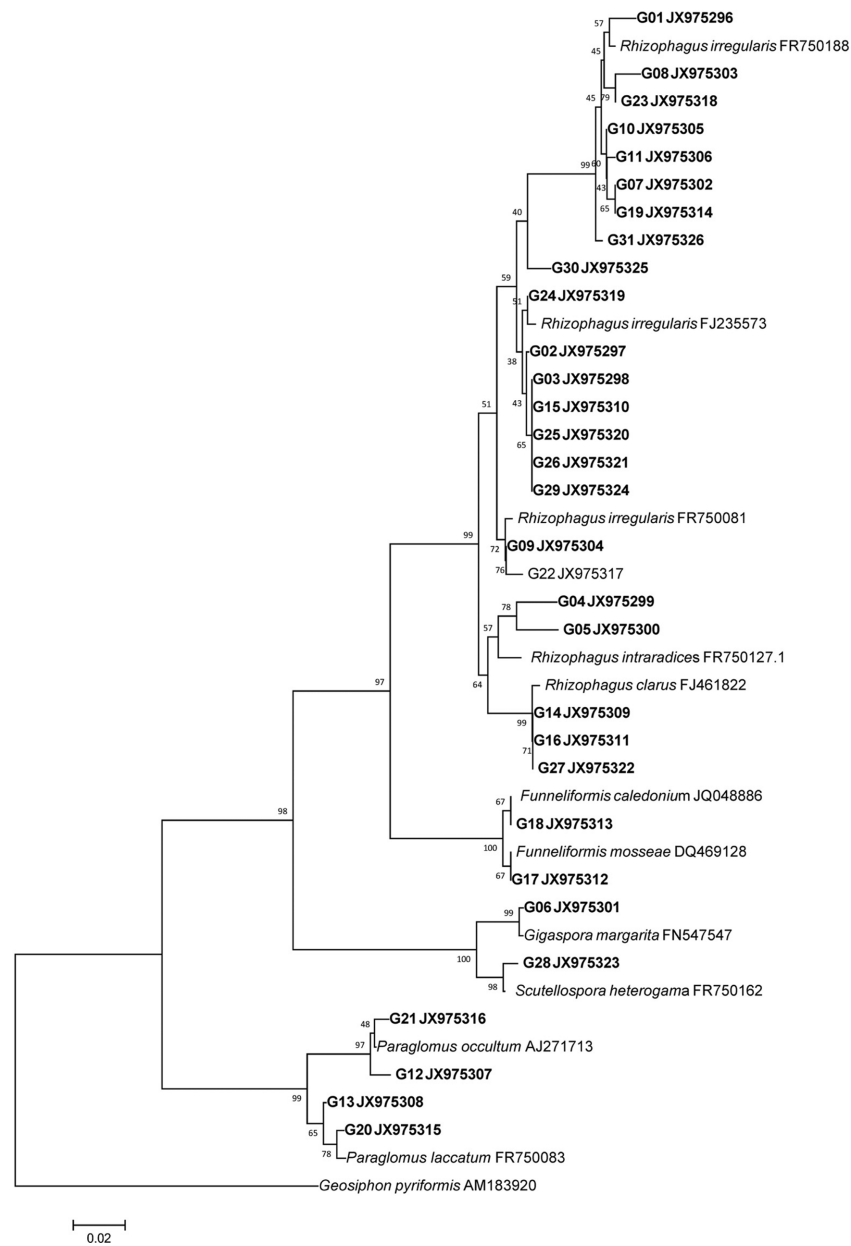


Fig. 2. Neighbor joining phylogenetic tree of arbuscular mycorrhizal fungi colonizing on field-cultivated *Panax ginseng*. Large subunit of rDNA region was sequenced. *Geosiphon pyriformis* was used as an outgroup and bootstrap values are shown at the branches (1,000 replicates). Bold letters indicates clone sequences from this study.

Table 1. Relative abundance (%) of phylotypes of arbuscular mycorrhizal fungi colonizing in the roots of *Panax ginseng* with different ages

Phylotypes	Root age (yr)				
	1	2	3	4	5
<i>Funnelliformis caledonium</i>	2.5 ± 0.5	2.5 ± 0.5	2.5 ± 0.5	5.0 ± 1.0	2.5 ± 0.5
<i>Funnelliformis mosseae</i>	2.5 ± 1.4	1.3 ± 0.3	1.3 ± 0.3		
<i>Gigaspora margarita</i>	1.3 ± 0.3				
<i>Paraglomus occulturn</i>	10.0 ± 2.0	8.8 ± 1.5	6.3 ± 1.0	1.3 ± 0.3	1.3 ± 0.3
<i>Paraglomus laccatum</i>	12.5 ± 2.0	15.0 ± 3.0	12.5 ± 2.0	5.0 ± 1.0	2.5 ± 0.5
<i>Rhizophagus irregularis</i>	71.3 ± 8.3	72.5 ± 6.5	77.5 ± 10.3	88.8 ± 4.3	92.5 ± 6.0
<i>Scutellospora heterogama</i>					1.3 ± 0.2

Values are presented as mean ± standard errors.

adapted successfully to agricultural soil conditions did not colonize substantially with the roots of *P. ginseng* in this study [25-27]. The field management for ginseng cultivation differs from other crops. It is therefore likely that the AMF fungal community composition changed as result of this difference in the agricultural system. In our study, *S. heterogama* was observed to colonize the roots of only 5-year-old ginseng at a relatively lower rate ($1.3 \pm 0.2\%$). Overall, the mean colonization rate increased with an increase in the age of ginseng roots. We observed a higher rate of colonization for *R. irregularis*. This species has not been detected in cultivated soil and ginseng roots previously [28]. In our future work, we will study how symbiosis between *R. irregularis* and *P. ginseng* affects ginsenoside content of perennial herbaceous plants.

Eom *et al.* [13] reported two species of AMF, *P. brasilianum* and *Glomus spurgum* from the roots of 2-year-old field-cultivated ginseng using primers AML1/AML2. Other AMF species were not observed to colonize ginseng in this study. Several other studies have reported that *G. intraradices* dominates the AMF community in other plant roots, while fungi from the Acaulosporaceae or Gigasporaceae family are lower in abundance. Although several studies have identified *G. intraradices* or other species that belong to the Acaulosporaceae family, these species were not detected in our study. However, species diversity of AMF colonizing the roots significantly decreased with the root age (Fig. 3). The diversity of AMF in 1- to 3-year-old ginseng roots was higher than in 4- to 5-year-old ginseng roots. This was reflected primarily through the decreased species evenness

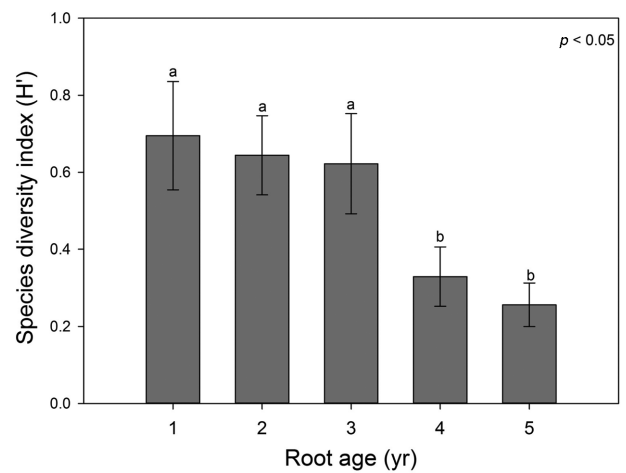


Fig. 3. Shannon's diversity index of arbuscular mycorrhizal fungi colonizing different ages of ginseng roots. Means and standard error are shown. Different letter on each bar indicates significantly different mean at $p < 0.05$ according to least significant difference test of one-way ANOVA.

because the richness of AMF was not significantly affected by the age of the ginseng roots. Our results are consistent with those of previous studies because the observed increase in the colonization by *R. irregularis* with the age of the roots and the absence of other species from more mature roots.

PCR analysis used to determine the influence of the age of ginseng roots on AMF communities and revealed that

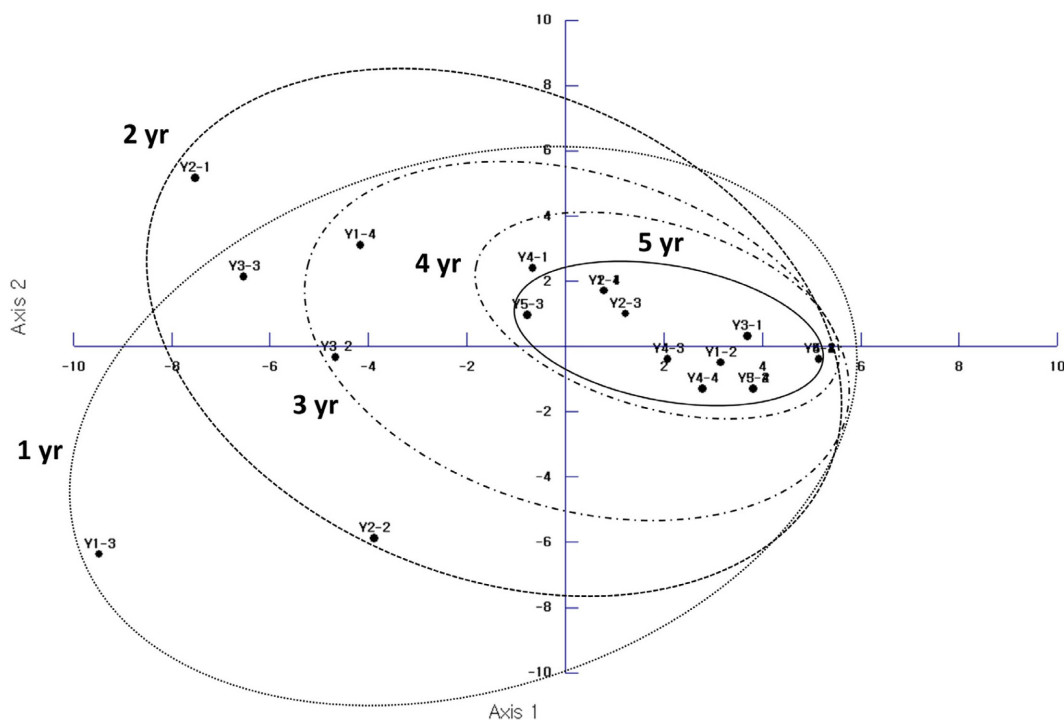


Fig. 4. Principle component analysis ordination of arbuscular mycorrhizal fungal communities colonizing different ages of field-cultivated *Panax ginseng*.

the AMF communities were able to discern among them (Fig. 4). This is similar to a previous study based on the data of AMF spores in soil from the field of cultivated ginseng [11]. In our study, AMF community composition changed with the age of ginseng roots. Based on the results of AMF spore study, the diversity of AMF species was found to be greater in the soil of field-cultivated ginseng than in the ginseng roots. However, not all the species identified from the soil were isolated from the roots. Six AMF species: *Glomus* sp., *G. intraradices*, *G. etunicatum*, *Acaulospora longula*, *Archaeospora trappei*, *P. brasilianum* were isolated only from the soil of cultivated ginseng and four AMF species: *F. caledonium*, *F. mosseae*, *P. occultum*, *S. heterogama* were observed both in the soil and the roots. Of these, *P. brasilianum* has also been detected in another study on ginseng and AMF symbiosis [13]. Hijri et al. [29] observed that there was a good agreement between the spores isolated from soil and the phylotypes amplified from roots in a survey of five agricultural fields. However, others have reported a poor species match between AMF spores in the soil and AMF in the roots of grassland plants [30].

In this study, root colonization rates of AMF increased with the age of ginseng roots. However, with an increase in the root age, species diversity and evenness of AMF colonizing the roots decreased, whereas the dominance of *R. irregularis* increased. The AMF community colonizing ginseng roots became more uniform with an increase in root age indicating that changes in the preferences of AMF for ginseng roots were age-dependent.

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