Diversity of Arbuscular Mycorrhizal Fungi and Their Roles in Ecosystems

Eun-Hwa Lee[1], **Ju-Kyeong Eo**[1], **Kang-Hyeon Ka**[2] and **Ahn-Heum Eom**[1,*]

[1]*Department of Biology Education, Korea National University of Education, Cheongwon 363-791, Korea*
[2]*Division of Wood Chemistry and Microbiology, Korea Forest Research Institute, Seoul 130-712, Korea*

Abstract Arbuscular mycorrhizal fungi (AMF) have mutualistic relationships with more than 80% of terrestrial plant species. This symbiotic relationship is ancient and would have had important roles in establishment of plants on land. Despite their abundance and wide range of relationship with plant species, AMF have shown low species diversity. However, molecular studies have suggested that diversity of these fungi may be much higher, and genetic variation of AMF is very high within a species and even within a single spore. Despite low diversity and lack of host specificity, various functions have been associated with plant growth responses to arbuscular mycorrhizal fungal colonization. In addition, different community composition of AMF affects plants differently, and plays a potential role in ecosystem variability and productivity. AMF have high functional diversity because different combinations of host plants and AMF have different effects on the various aspects of symbiosis. Consequently, recent studies have focused on the different functions of AMF according to their genetic resource and their roles in ecosystem functioning. This review summarizes taxonomic, genetic, and functional diversities of AMF and their roles in natural ecosystems.

Keywords Arbuscular mycorrhizas, Ecosystem, Functional diversity, Genetic diversity, Taxonomic diversity

Arbuscular mycorrhizal fungi (AMF) have mutualistic relationships with more than 80% of terrestrial plant species, from bryophytes to tracheophytes [1]. This symbiotic relationship is believed to have formed approximately 460 million years ago and would have had important roles in establishment of plants on land [2]. AMF produces highly branched fungal structures, arbuscules, within root cortical cells of their host plants, with which they exchange inorganic minerals, especially phosphorus and carbon compounds [1]. AMF are one of the most abundant organisms in the rhizosphere and the relationships can be found within a broad range of more than 200,000 species of host plants. Despite their abundance and wide range of relationship with plant species, AMF have been known to show low

species diversity and only approximately 240 species have been described within a fungal phylum, Glomeromycota, on the basis of morphology [3-5]. However, molecular studies have suggested that diversity of these fungi may be much greater [6]. In addition, high genetic variation of AMF has been reported within species, even within a single AMF spore [7, 8], and affects various important functions such as colonization rates, growth of extra-radical hyphae, and phosphorus uptake of AMF [9-12].

Most AMF successfully colonize a large number of host plant species, indicating lack of host specificity [1]. Due to the low species diversity and lack of host specificity of AMF, it has been assumed that AMF are functionally unnecessary [13] and the functional roles of AMF in ecosystems have been ignored. However, a variety of functions associated with plant growth responses to arbuscular mycorrhizal (AM) fungal colonization, not only phosphorus uptake, have been reported [10, 14, 15]. In addition, there is evidence indicating that differences in community composition of AMF may have different effects in plants, and play a potential role in determining plant diversity, ecosystem variability, and productivity. These studies have indicated high functional diversity of AMF because different combinations of host plant and AMF have different effects on various aspects of symbiosis. Consequently, recent studies have focused on the different functions of AMF according to their genetic resource and their roles in ecosystem functioning. This review provides a summary of recent studies of functional diversity of AMF and their roles in natural ecosystems.

TAXONOMIC DIVERSITY OF AMF

Fossil records and molecular data suggest that AM symbiosis is evolutionary ancient and that it occurred with the first terrestrial plants. However, species diversity of AMF is low, with only approximately 240 recognized species [3, 5], although molecular studies have suggested the existence of a significantly larger number of species [16]. It was suggested that low diversity in AMF is characteristic for mutualistic symbiosis because there is low selection pressure for speciation in the fungal partner [17].

Traditionally, the taxonomy of AMF has been based on the morphological characteristics of spores produced in soil features, including size, color, and various layers of the cell wall [18, 19]. However, AMF spores have simple structures with only a few morphological characteristics. In addition, spores collected from field soil are not in original condition, and several species of AMF may not produce spores under controlled conditions. These problems of morphological identification of AMF allow species identity to be determined only by experts and have obstructed ecological studies as well as systematics of AMF.

AMF have been placed within six genera of an order Glomales (Zygomycota) using phylogenetic analysis, using morphological characteristics [19]. However, sequence analysis of ribosomal RNA showed that all AMF species are included in a monophyletic clade and separated from all major fungal groups. In addition, it was so diverse within the group that all AMF species were placed into a new phylum, the Glomeromycota [4]. In a recent study, Krüger *et al.* [5] analyzed sequences of 136 species of AMF, including 27 undescribed species, and suggested that the number of described species was definitely underestimated. The region of small subunit (SSU) rDNA has been most widely used for phylogenetic sequence analysis for AMF, because intraspecific variation of the internal transcribed spacer region rDNA, which is generally used for identification and barcoding fungal species, is too high to be used in distinguishing species in AMF [20, 21]. However, use of SSU rDNA could also underestimate real diversity of Glomeromycota [22]. Thus, information from sequences, such as large subunit (LSU) and β-tubulin, would be required for better understanding of actual diversity of AMF [3, 23, 24].

Ecological studies of AMF have also relied on spores collected from soil. However, spore data collected from soil do not necessarily reflect the actual diversity and functionally active AMF colonizing plant roots. Therefore, identification of AMF species within plant roots is critical for their ecological study. However, identification of AMF hyphae to species level within roots using morphological features is not possible. In addition, due to the complex root systems, it is not possible to distinguish active symbionts of AMF in an individual plant from spore communities in soil. Development of suitable primers and protocols has enabled identification of AMF within plant roots. Specific PCR primers have been developed for all AMF lineages for use in PCR [25-27], and the LSU sequences were used for identification of AMF and for construction of a more accurate system [28-30].

GENETIC DIVERSITY OF AMF

Species diversity is an important factor affecting biodiversity and productivity of ecosystems [31]. Recently, molecular based methods in AMF studies have enabled direct identification of AM fungal species in plant roots or in soils, and it was revealed that actual AMF diversity in ecosystems could be higher than expected [22, 28]. In addition, DNA polymorphism within AM fungal isolates by different geographic origin, even within a single spore, was identified by use of molecular techniques [7, 20]. These findings incited an argument regarding the cause of genetic variation in AMF and their role in ecosystems.

Genetic diversity within species or among isolates originated from the genetic structure of AMF. Hundreds or thousands of nuclei exist together within a single spore or hypha of AMF, meaning that the genetic structure of AMF is 'multi genomic' [32]. Sanders *et al.* [20] identified intraspecific polymorphisms in the rDNA region within a single spore [20]. There has been debate regarding the genetic characteristics of nuclei in a spore or hypha. However, evidence supports that each nucleus within a single spore was not genetically identical and that genetic variation is inherited in an individual nucleus, and is not shared by nuclei [32, 33].

Despite recognition of the importance of genetic diversity of AMF, little is known about its role in ecosystems. Recent experimental evidence indicating that genetically different AM fungal isolates could differ in their ability of survival or functionality on their host plants [34] supports that genetic variation increases diversity of AM fungal communities more than expected. Although the roles of genetic variation in AM fungal communities and ecosystems are not yet clearly understood, the genetic variation offers the possibility of functional diversity of AMF in ecosystems. It is clear that genetically different AM fungal isolates, even from the same species, have different effects on their host plants [10, 35]. More studies on genetic variation within or among AMF species should be conducted for better understanding of the roles of functional diversity of AMF in ecosystems.

FUNCTIONAL DIVERSITY OF AMF

The function of mycorrhizal symbiosis could vary significantly between fungal species and even between isolates within a species [1]. Functional diversity of AMF can often refer to a variety of functions associated with plant growth responses to AM fungal colonization [36]. Although the mechanisms causing the functional diversity are still uncertain, involvement of the exchange of phosphorus and photosynthate between plants and AMF is evident [37].

The measure of symbiotic efficiency between host plants

and AMF is complex [38]. It can be defined as the amount of carbon gained by a growth response from the symbiotic relationship with AMF minus the amount of carbon lost by investment in maintaining the symbiotic relationship. In most cases, AMF inoculation promotes plant growth, but not always. On the contrary, some isolates of AMF can decrease the biomass of the host plant [1]. Recent studies have reported that the efficiency of AM symbiosis could differ according to the genotype of the two partners, host plants and AMF, as well as the combination of the partners [10, 11, 14]. Croll *et al.* [12] reported a strong preference for AM fungal genotype by host plants in his experiment. In addition, functional differences by genotype among AMF isolates for host plants were identified by Angelard *et al.* [9], who used genetically different AMF isolates, *G. intraradices*, to promote the growth of rice and found that specific AM fungal genotype could increase the biomass of rice up to five times compared with other isolates.

The specific AMF genotype affects host plants by different functionality, including spread of extraradical mycelia, efficiency of nutrient absorption, and mycorrhizal-specific gene expression, and causes different growth responses in host plants. The extraradical mycelium of AMF absorbs the mineral nutrients from soil and transports them to the host plant. However, the capacity of the mycelium to spread, and the viability, structure, and possibility of anastomosis might vary among AMF genotypes, even among those collected from the same origin [39-41]. In addition, it is known that there are differences in ability to take up and supply phosphorus and nitrogen to plants even within AMF species, caused by the expression levels of P-transporter and N-assimilation genes [42, 43].

Use of molecular method-based studies has enabled a clearer understanding of mycorrhiza-specific genes, expressed in the early stage after inoculation. In different AMF taxa, a similar gene expression pattern was identified in a set of nutrient-transport related genes in early stages of infection, and Liu *et al.* [44] reported a similar gene expression pattern in an AMF-related gene set in different plant taxa. However, Hohnjec *et al.* [45] reported that the gene expression pattern was similar in infections by two AMF species but that some genes were expressed more in specific host plants, meaning that mycorrhizae-specific gene expression was affected by the combination of host plant and AMF species [45].

Finally, the continuous signal exchange between AMF and host plant is important to completion of their life cycle from colonizing roots to sporulation in soils [46, 47]. Genetic variation caused by the composition of hyphal nuclei is important in mutual recognition of AM symbiosis. In addition, genetically different isolates of AMF could affect colonization strategy and mycorrhizal morphology of the plant.

Although AMF species diversity is lower than that of other fungal taxa, its effects on growth and structure of plant communities are very diverse. Thus, when considering the many causes that lead to the actual diversity of AMF in ecosystems, the functional differences among AMF species or isolates cannot be simply measured as shown.

ECOLOGICAL ROLES OF AMF DIVERSITY

AMF are distributed among a variety of environments as symbionts with plants from sub-polar to tropical latitudes and from swamps in low lands to mountainous areas of high elevation [48]. The host plants offer carbon compounds to AMF (approximately 20% of photosynthate) in exchange for inorganic minerals and protection against environmental stresses from AMF [1]. AMF play an important role in phosphorous absorption [49], circulation of nitrogen in ecosystems [43], tolerance to heavy metals and saline conditions [50], and increasing protection from nematodes and root diseases in the rhizosphere [51, 52].

Rhizospheres with AMF have very dense hyphal networks, akin to extended roots, which occupy an extensive biomass [53]. Although AMF have low host specificity in their symbioses with plants, they share hyphal networks with different host plants and affect not only a single plant species but also populations and plant species composition. Despite wide distribution and abundance of AMF in various environments as well as a broad range of relationship with most land plants, species diversity of AMF is low. However, molecular evidence has shown that the diversity would be much higher in ecosystems than expected. In addition, differences in functionality between AM fungal isolates could complement species diversity of AMF by changing the functional units interacting with plants from species to genotype. In such a point of view, we need a novel ecological concept of AMF from decomposer to producer or assistant producer of plants, increasing productivity and fitness of plants in ecosystems, not restricted in conventional view of fungi.

CONCLUSION

AMF are the most abundant symbionts in ecosystems. They are adapted to various environments and have symbiotic relationships with more than 200,000 plant species; however, only about 240 species have been described to date. This means that morphological diversity of AMF spores is not sufficient to reveal actual diversity of the fungi in ecosystems. Use of molecular techniques in studies of AMF has led to a significant increase in taxonomic diversity and studies have indicated high genetic diversity within a population and even within a single spore. Functional diversity by the combination of host plant and AMF results from the genetic structure of AMF, which is multi-genomic and composed of hundreds or thousands of nuclei with different genetic composition. The genetic variation of nuclei in a single spore affects genetic diversity at the population level and it could increase the functional diversity of AMF in ecosystems.

Experimental evidence supports that genetically different AMF isolates, even within species, could have different effects on host-plant growth. AMF are regarded as the most widespread microorganisms in soil, except in sterile or contaminated sites where plants cannot survive. They contribute to nutrient cycling (e.g., the N cycle) in ecosystems and improve soil conditions through soil aggregation and increasing the nutrient uptake of plants. However, the taxonomic species diversity of AMF reflects only a small part of AM fungal diversity in actual ecosystems compared with their functional diversity. The characteristics of AMF related to host-plant selection, rapid adaptation to various environments, and functional differences in growth response in host plants could be understood when the genetic diversity of AMF and their roles have been more clearly determined.

ACKNOWLEDGEMENTS

REFERENCES

1. Smith SE, Read D. Mycorrhizal symbiosis. 3rd ed. San Diego: Academic Press; 2008.

2. Redecker D, Kodner R, Graham LE. Glomalean fungi from the Ordovician. Science 2000;289:1920-1.

3. Schüßler A, Walker C. The Glomeromycota: a species list with new families and new genera. Edinburgh and Kew: The Royal Botanic Garden Kew, Botanische Staatssammlung Munich, and Oregon State University; 2010.

4. Schüßler A, Schwarzott D, Walker C. A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol Res 2001;105:1413-21.

5. Krüger M, Krüger C, Walker C, Stockinger H, Schüßler A. Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. New Phytol 2012;193:970-84.

6. Fitter AH. Darkness visible: reflections on underground ecology. J Ecol 2005;93:231-43.

7. Clapp JP, Rodriguez A, Dodd JC. Inter- and intra-isolate rRNA large subunit variation in *Glomus coronatum* spores. New Phytol 2001;149:539-54.

8. Vandenkoornhuyse P, Leyval C. SSU rDNA sequencing and PCR-fingerprinting reveal genetic variation within *Glomus mosseae*. Mycologia 1998;90:791-7.

9. Angelard C, Colard A, Niculita-Hirzel H, Croll D, Sanders IR. Segregation in a mycorrhizal fungus alters rice growth and symbiosis-specific gene transcription. Curr Biol 2010;20: 1216-21.

10. Munkvold L, Kjøller R, Vestberg M, Rosendahl S, Jakobsen I. High functional diversity within species of arbuscular mycorrhizal fungi. New Phytol 2004;164:357-64.

11. Avio L, Pellegrino E, Bonari E, Giovannetti M. Functional diversity of arbuscular mycorrhizal fungal isolates in relation to extraradical mycelial networks. New Phytol 2006;172:347-

57.

12. Croll D, Wille L, Gamper HA, Mathimaran N, Lammers PJ, Corradi N, Sanders IR. Genetic diversity and host plant preferences revealed by simple sequence repeat and mitochondrial markers in a population of the arbuscular mycorrhizal fungus *Glomus intraradices*. New Phytol 2008; 178:672-87.

13. Allen EB, Allen MF, Helm DJ, Trappe JM, Molina R, Rincon E. Patterns and regulation of mycorrhizal plant and fungal diversity. Plant Soil 1995;170:47-62.

14. Burleigh SH, Cavagnaro T, Jakobsen I. Functional diversity of arbuscular mycorrhizas extends to the expression of plant genes involved in P nutrition. J Exp Bot 2002;53:1593-601.

15. Smith SE, Smith FA, Jakobsen I. Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal reponses in grwoth and total P uptake. New Phytol 2004;162:511-24.

16. Redecker D. Molecular identification and phylogeny of arbuscular mycorrhizal fungi. Plant Soil 2002;244:67-73.

17. Law R. Evolution in a mutualistic environment. In: Boucher DH, editor. The biology of mutualism: ecology and evolution. London: Croons Helm; 1985. p. 145-70.

18. Morton JB. Taxonomy of VA mycorrhizal fungi: classification, nomenclature, and identification. Mycotaxon 1988;32:267-324.

19. Morton JB, Benny GL. Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order, Glomales, two new suborders, Glomineae and Gigasporineae, and two new families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomaceae. Mycotaxon 1990;37:471-91.

20. Sanders IR, Alt M, Groppe K, Boller T, Wiemken A. Identification of ribosomal DNA polymorphisms among and within spores of the Glomales: application to studies on the genetic diversity of arbuscular mycorrhizal fungal communities. New Phytol 1995;130:419-27.

21. Lloyd-Macgilp SA, Chambers SM, Dodd JC, Fitter AH, Walker C, Young JPW. Diversity of the ribosomal internal transcribed spacers within and among isolates of *Glomus mosseae* and related mycorrhizal fungi. New Phytol 1996; 133:103-11.

22. Husband R, Herre EA, Turner SL, Gallery R, Young JP. Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. Mol Ecol 2002;11:2669-78.

23. Msiska Z, Morton JB. Phylogenetic analysis of the Glomeromycota by partial β-tubulin gene sequences. Mycorrhiza 2009;19:247-54.

24. Morton JB, Msiska Z. Phylogenies from genetic and morphological characters do not support a revision of Gigasporaceae (Glomeromycota) into four families and five genera. Mycorrhiza 2010;20:483-96.

25. Helgason T, Fitter A, Young JP. Molecular diversity of arbuscular mycorrhizal fungi colonising *Hyacinthoides non-scripta* (bluebell) in a seminatural woodland. Mol Ecol 1999; 8:659-66.

26. Lee J, Lee S, Young JP. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi.

FEMS Microbiol Ecol 2008;65:339-49.

27. Wubet T, Weiss M, Kottke I, Teketay D, Oberwinkler F. Phylogenetic analysis of nuclear small subunit rDNA sequences suggests that the endangered African Pencil Cedar, *Juniperus procera*, is associated with distinct members of Glomeraceae. Mycol Res 2006;110(Pt 9):1059-69.

28. Gollotte A, Van Tuinen D, Atkinson D. Diversity of arbuscular mycorrhizal fungi colonising roots of the grass species *Agrostis capillaris* and *Lolium perenne* in a field experiment. Mycorrhiza 2004;14:111-7.

29. Pivato B, Mazurier S, Lemanceau P, Siblot S, Berta G, Mougel C, Van Tuinen D. *Medicago* species affect the community composition of arbuscular mycorrhizal fungi associated with roots. New Phytol 2007;176:197-210.

30. Rosendahl S, McGee P, Morton JB. Lack of global population genetic differentiation in the arbuscular mycorrhizal fungus *Glomus mosseae* suggests a recent range expansion which may have coincided with the spread of agriculture. Mol Ecol 2009;18:4316-29.

31. van der Heijden MG, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 1998;396:69-72.

32. Kuhn G, Hijri M, Sanders IR. Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi. Nature 2001;414:745-8.

33. Hijri M, Sanders IR. Low gene copy number shows that arbuscular mycorrhizal fungi inherit genetically different nuclei. Nature 2005;433:160-3.

34. Colard A, Angelard C, Sanders IR. Genetic exchange in an arbuscular mycorrhizal fungus results in increased rice growth and altered mycorrhiza-specific gene transcription. Appl Environ Microbiol 2011;77:6510-5.

35. Koch AM, Croll D, Sanders IR. Genetic variability in a population of arbuscular mycorrhizal fungi causes variation in plant growth. Ecol Lett 2006;9:103-10.

36. Johnson NC, Graham JH, Smith FA. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytol 1997;135:575-85.

37. van der Heijden MG, Boller T, Wiemken A, Sanders IR. Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. Ecology 1998; 79:2082-91.

38. Tinker PB, Durall DM, Jones MD. Carbon use efficiency in mycorrhizas theory and sample calculations. New Phytol 1994;128:115-22.

39. Sanders FE, Tinker PB, Black RL, Palmerley SM. The development of endomycorrhizal root systems: I. Spread of infection and growth-promoting effects with four species of vesicular-arbuscular endophyte. New Phytol 1977;78:257-68.

40. Abbott LK, Robson AD. Formation of external hyphae in soil by four species of vesicular-arbuscular mycorrhizal fungi. New Phytol 1985;99:245-55.

41. Giovannetti M, Fortuna P, Citernesi AS, Morini S, Nuti MP. The occurrence of anastomosis formation and nuclear exchange in intact arbuscular mycorrhizal networks. New Phytol 2001; 151:717-24.

42. Maldonado-Mendoza IE, Dewbre GR, Harrison MJ. A phosphate transporter gene from the extra-radical mycelium of an arbuscular mycorrhizal fungus *Glomus intraradices* is regulated in response to phosphate in the environment. Mol Plant Microbe Interact 2001;14:1140-8.

43. Govindarajulu M, Pfeffer PE, Jin H, Abubaker J, Douds DD, Allen JW, Bücking H, Lammers PJ, Shachar-Hill Y. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 2005;435:819-23.

44. Liu J, Blaylock LA, Endre G, Cho J, Town CD, VandenBosch KA, Harrison MJ. Transcript profiling coupled with spatial expression analyses reveals genes involved in distinct developmental stages of an arbuscular mycorrhizal symbiosis. Plant Cell 2003;15:2106-23.

45. Hohnjec N, Vieweg MF, Pühler A, Becker A, Küster H. Overlaps in the transcriptional profiles of *Medicago truncatula* roots inoculated with two different *Glomus* fungi provide insights into the genetic program activated during arbuscular mycorrhiza. Plant Physiol 2005;137:1283-301.

46. Genre A, Chabaud M, Faccio A, Barker DG, Bonfante P. Prepenetration apparatus assembly precedes and predicts the colonization patterns of arbuscular mycorrhizal fungi within the root cortex of both *Medicago truncatula* and *Daucus carota*. Plant Cell 2008;20:1407-20.

47. Parniske M. Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev Microbiol 2008;6:763-75.

48. Read DJ. Mycorrhizas in ecosystems. Experientia 1991;47: 376-91.

49. Lambert DH, Baker DE, Cole H Jr. The role of mycorrhizae in the interactions of phosphorus with zinc, copper, and other elements. Soil Sci Soc Am J 1979;43:976-80.

50. Feng G, Zhang FS, Li XL, Tian CY, Tang C, Rengel Z. Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. Mycorrhiza 2002;12:185-90.

51. Augé RM. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 2001;11:3-42.

52. Linderman RG. Effects of mycorrhizas on plant tolerance to diseases. In: Koltai H, Kapulnik Y, editors. Arbuscular mycorrhizas: physiology and function. Dordrecht: Springer; 2000. p. 345-65.

53. Leake J, Johnson D, Donnelly D, Muckle G, Boddy L, Read D. Networks of power and influence: the role of mycorrhizal mycelium in controlling plant communities and agroecosystem functioning. Can J Bot 2004;82:1016-45.