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# Ecological Distribution of Endomycorrhizal Fungi in Pogil-do in Tadohae-haesang National Park

**SEO, HYUN CHANG[1], CHANG JIN KIM, SHIN DUK KIM, AND ICK DONG YOO***

*Genetic Engineering Research Institute, Korea Institute of Science and Technology, P.O. Box 17, Taedok Science Town, Taejon 305-606, Korea*
*[1]Department of Food and Nutrition, Shingu Junior College, Sungnam 462-743, Kyonggi-do, Korea*

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**The ecological distribution of endomycorrhizas in evergreen woody species native to the evergreen forest ecosystem of Tadohae-haesang National Park in southern Korea in February, 1989 was studied. The abundance and diversity of vesicular-arbuscular (VA) mycorrhizal fungi were also determined. The spore densities ranged from 14 to 326 per 100 g of soil. Most of the spores of mycorrhizal fungi collected from 25 soil samples belonged to the genera *Glomus* and *Gigaspora*. The frequency and number of spores in *Camellia japonica* varied with location. Spores belonging to the genus *Gigaspora* were not found in *Camellia japonica* in Yesongri evergreen forests adjacent to the sea. *Glomus* sp. was the major constituent of the spore assemblage at this site. The most abundant species in *Camellia japonica* in the Yesongri evergreen forests in Pogildo was *Glomus borealis*. In the soil of a mountain at Buwhangri, in the central location of the island at an elevation of 250 m, *Gigaspora* sp. was present and *Glomus* sp. was a major constituent of the spore assemblage. In the urban area of Haenam spore densities were much higher than in the Pogildo area. The most abundant species in *Camellia japonica* in the urban area of Haenam was *Gigaspora* sp..**

VA mycorrhizal associations in the rhizosphere are thought to be almost ubiquitous, having extremely wide host ranges, and are present in most existing species of vascular plants, including agricultural crops grown throughout the world (6). In addition to these wide host ranges there are many reports on improved plant growth due to the presence of mycorrhizal association (2, 5, 14, 16). These symbiotic associations with host plants have also been reported to promote plant health (7, 15, 19). Mycorrhizal plants are more resistant to plant stress induced by drought (15), high soil temperature (19) and root pathogens (7). These published reports of plant growth and health, and the real concern about pollution of the environment induced by chemical fertilizers and pesticides, have suggested the possible use of VA mycorrhizal fungi as "biofertilizer" (1, 13, 17).

However, for the effective use of VA mycorrhizal fungi as biofertilizer, we need to understand their ecology and select suitable fungi. Only about 120 species of VA mycorrhizal fungi have been identified and there are few studies dealing with the ecology of VA mycorrhizal fungi in native ecosystems (3, 4, 18).

In agroecosystems of Korea a lot of pesticides and chemical fertilizers have been used. This type of agriculture may have killed many useful soil microorganisms, including VA mycorrhizal fungi, and may have damaged the ecosystems. In this respect, as a first step for the selection of VA mycorrhizal fungi for agricultural use, we studied the ecological distribution of VA mycorrhizal fungi from an unpolluted ecosystem. In this environment diverse VA mycorrhizal fungal species might be present (20) and possible candidates for VA mycorrhizal fungal biofertilizers might to be abundant. Reports on the taxonomic distribution of ecto- and endomycorrhizal fungi from Korean soil were surveyed by Lee and Koo (11).

*Corresponding author
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The purpose of this study was to examine the ecological distribution of endomycorrhizal fungi in evergreen woody species native to the forest ecosystem of Tadohae-haesang National Park in southern Korea in February, 1989.

## MATERIALS AND METHODS

### Study Areas

Perennial plants were surveyed for colonization at 2 sites in the evergreen forests of Pogil-do and 1 site in the Haenam-up area at Chŏllanamdo (Fig. 1). A total of 25 soil samples were obtained from 19 woody species. Sampling site selection was based on general soil characteristics and floristic composition. The evergreen forests of Yesongri in Pogil-do were designated and preserved as a Natural Monument of Korea (No. 40). *Camellia japonica* was the major woody species at these sites. Site #1 was adjacent to a seashore of small pebbles and the soil was clay rich with abundant organic matter. Site #2 was in the Pogil-do forest ecosystem on the side of a mountain in Buwhangri,near the center of the island, at an elevation of 250 m. The sea is departed about 2.5 km from this site. In the urban area of Haenam, at site #3, samples were also collected for analysis of endomycorrhizal fungi. All the sites studied were characterized as temperate zone evergreen forests where evergreen woody species could survive perennially.

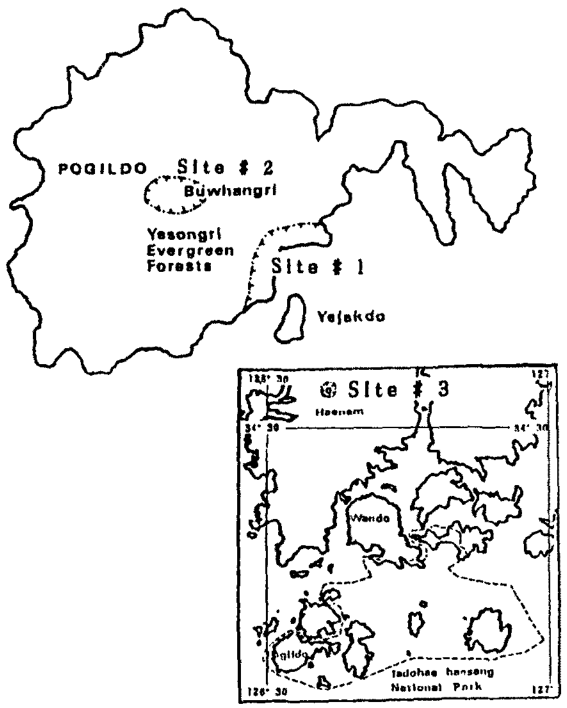


**Fig. 1. The Pogil-do area and its location in the Tadohae-haesang National Park in Chŏllanamdo, Korea, and the locations of sampling sites.**

### Isolation and Identification of Endomycorrhizal Fungi

Evergreen woody species were selected for sampling of mycorrhizal fungi. Soil and root samples were collected from the top 10 to 20 cm soil. A representative sample of the entire root system was obtained from five or six different portions of the root system, according to the plant species. These portions were combined rather than obtaining one large sample from a single portion. Samples of 2 to 3 Kg were collected of each plant species. To measure the density of spores subsamples of 200 ml of soil and root fragments were wet sieved and soil sievings were then centrifuged in 1 M sucrose solution to separate detritus from the spores (9). Spores were examined under a dissecting microscope (20~40x) and then grouped by color, shape and size. A compound microscope (100~1,000x) was used for further examination and identification of the spores. Spores were preliminarily identified and then compared for structural details against information in the original species description. Identification of endomycorrhizal fungi was based on the "Manual for the identification of VA mycorrhizal fungi" by INVAM and the "Compilation of the Endogonaceae" by Shannon M. Berch (3, 8, 18). Sudan grass (*Sorghum bicolor* L.) was grown for 5 months in pot culture in a sterile sand-vermiculite medium (1:1, v/v) with spores from each plant species as VA mycorrhizal inoculum.

### Observation of Infection Characteristics of VA Mycorrhiza

Root samples of fine terminal feeder roots attached to lower order roots, free of root systems of adjacent plants, were preserved in fixation solution (formalin-acetic acid-alcohol solution) before staining. Staining of the root samples was based on the method of Komanick (9).

## RESULTS AND DISCUSSION

### Distribution of Endomycorrhizal Fungi

Distribution of endomycorrhizal fungi was estimated as the number of spores per 100 g of soil collected in the rhizosphere of the evergreen forests. Most of the spores of mycorrhizal fungi collected from the 25 soil samples belonged to the genera *Glomus* and *Gigaspora*.

Among the sites surveyed, Sites #1 and #2 (Yesongri evergreen forests) have been well preserved as a Natural Monument. *Glomus* sp. was a major constituent of the spore assemblage at Site #1 (Fig. 2). The site was adjacent to the sea side and thought to be high in soil salinity. As VA mycorrhizal fungi have been shown to adapt to edaphic factors (10), salinity-resistant species or geno-

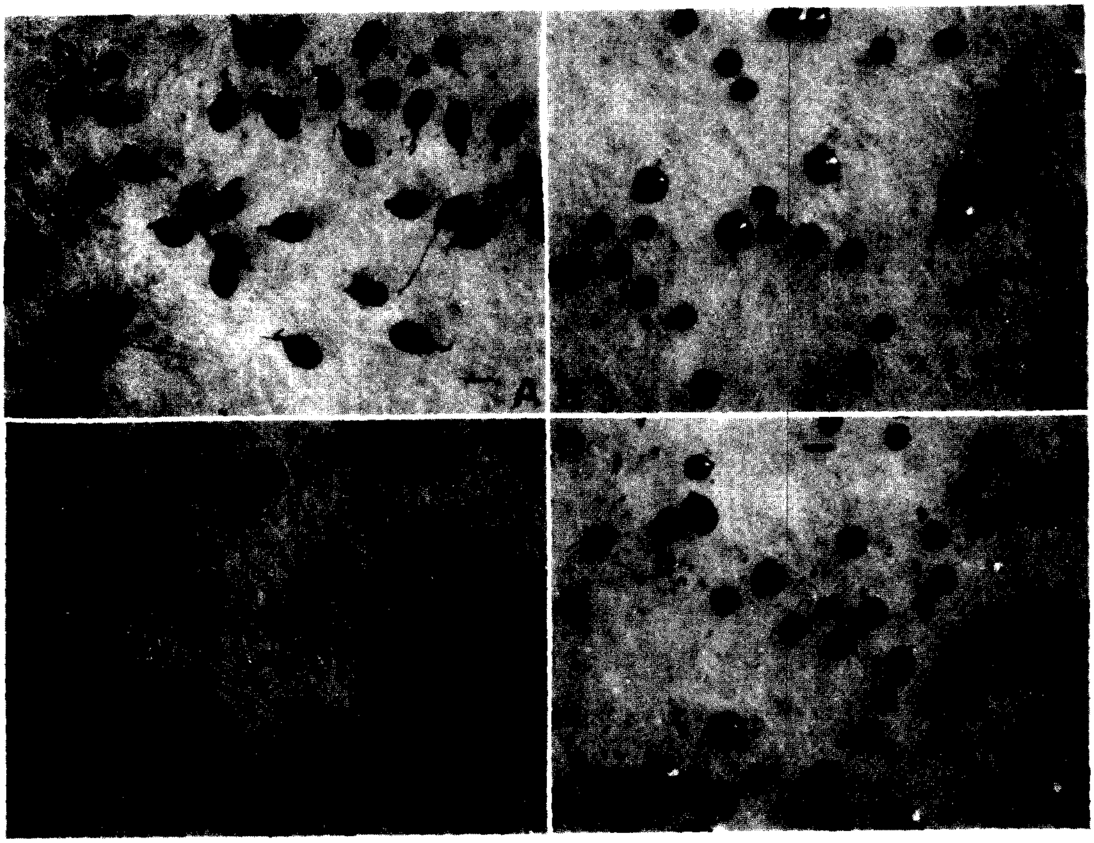


**Fig. 2. Diversities of spores of endomycorrhizal fungi isolated from *Camellia japonica* in the Yesongri evergreen forests of Pogil-do (Site #1). Scale line=100 μm.**

types may also exist in our isolates from coast areas. Spores belonging to the genus *Gigaspora* were not found at Site #1 and the wall layer of chramydospores found in *Camellia japonica* was generally thick. Site #2, a forest ecosystem in Pogil-do, was on a mountainside at an elevation of 250 m. The spore densities in evergreen woody species at the two sites in Pogil-do and one site in Haenam are compared in Table 1. The spore densities ranged from 14 to 326 per 100 g of soil at the 3 sites studied. Many environmental factors acting on the host plant may affect these differences in the production of VA mycorrhizal spores. However, the influence of host plants was thought to be minor among the factors, considering the spore densities in evergreen woody species common to the three sites (Table 1). The spore densities in the evergreen woody species *Camellia japonica*, common to the three sites were compared in Table 1. The spore densities were 58 in Site #1, 128 in Site #2, and 224 in Site #3. These differences are attributed to a difference in soil fertility, based upon the soil characteristics examined. The fact that the soil of Site #1 was clay rich with abundant organic matter, and the difference of spore density in *Cinnamomum*

**Table 1. Density of spores of endomycorrhizal fungi isolated in the Pogil-do and Haenam areas in February, 1989.**

| Sampling site | Plant species | Spores/100 g soil |
|---|---|---|
| Site #1 | | |
| | *Camellia japonica* | 58 |
| Yesong-ri | *Ilex integra* | 38 |
| Evergreen | *Cinnamomum japonicum* | 26 |
| Forests | *Kalopanax pictus* | 14 |
| in | *Cryptomeria japonica* | 60 |
| Pogil-do | *Neolitsea sericea* | 38 |
| | *Cinnamomum japonicum* | 16 |
| Site #2 | | |
| | *Chamaecyparis obtusa* | 28 |
| Buwhang-ri | *Camellia japonica* | 128 |
| in | *Machilus thunbergii* | 38 |
| Pogil-do | *Cinnamomum japonicum* | 48 |
| | *Elaeagnus umbellata* | 46 |
| Site #3 | | |
| Haenam | *Camellia japonica* | 224 |
| | *Ilex crenata* | 326 |

*japonicum* at Site #1 and Site #2 (Table 1) also support this suggestion. Changes in spore densities in agroecosystems have also been associated with changes in soil fertility (12). Schenck's result regarding the comparison of the density of spores between agroecosystems and native ecosystems (20) also supports our result that the differences are the result of soil fertility. Therefore, mycorrhizal fungal infestations of evergreen woody plants are thought to be dependent on the extent of organic matter in the soil. Thus, it can be said that *Camellia japonica* at Site #3 was more dependent on endomycorrhizal fungi than at Site #1.

**Comparison of the Types of Endomycorrhizal Fungi in *Camellia japonica* at Three Different Sites**

The types of spores in the evergreen woody species *Camellia japonica* common to the three sites were compared. The differences in spores, grouped by color, shape and size, are shown in Fig. 2 (Site #1) and Fig. 3 (Sites #2 and #3). The types of spores found in the two sites in Pogil-do were different from each other. As shown in Fig. 2, diverse mycorrhizal fungal species were found in *Camellia japonica* in the Yesongri evergreen forests at Site #1. *Glomus* sp. was a major spore type and *Gigaspora* sp. was not found at this site. The abundant species found in the site are shown in Fig. 2-(A) and spores of this type were not found in any site studied. In Site #2, *Gigaspora* sp. was found, but *Glomus* sp. was still the major spore type, while in Site #3 in the urban area of Haenam, *Gigasporas* sp. was the major spore type (Fig. 3-(B)). It is well known that the hyphae of VA mycorrhizal fungi serve as extensions of the root systems and are both physiologically and geometrically more effective organs of absorption than the roots themselves (2). Therefore, *Camellia japonica* in the urban area of Haenam studied is thought to be more dependent on VA mycorrhizal fungi than in the Pogil-do area.

**Identification of Endomycorrhizal Fungi**

Dominant spore types of endomycorrhizal fungi from *Camellia japonica* forests at Site #1 are shown in Fig. 2-(A). The spores have not been reported in Korea as yet so further analysis was performed. The spores were


**Fig. 3. Comparison of spores of endomycorrhizal fungi isolated from *Camellia japonica* in Buwhang-ri (Site #2, A) and the Haenam area (Site #3, B). Scale line=100 μm.**

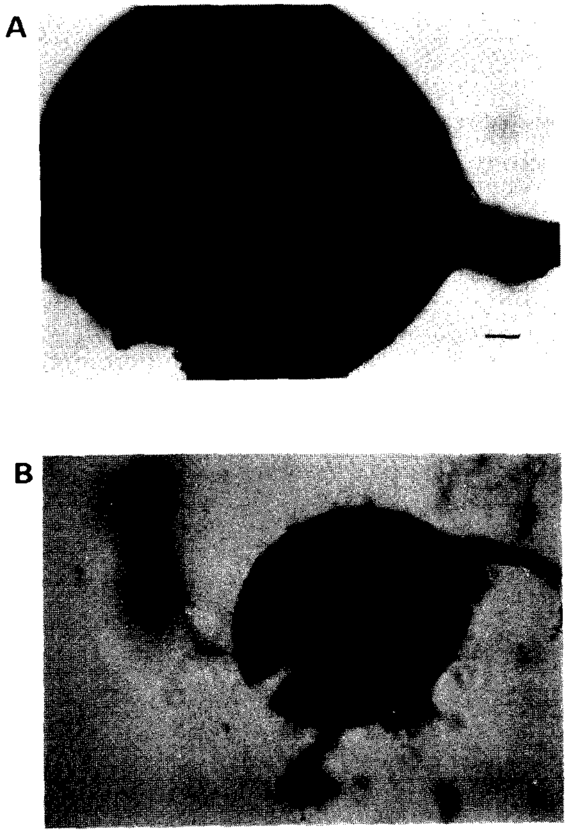


**Fig. 4. A spore of endomycorrhizal fungi, *Glomus borealis*, predominantly abundant in *Camellia japonica* in the Yesongri evergreen forests of Pogil-do . Scale line=10 μm.**

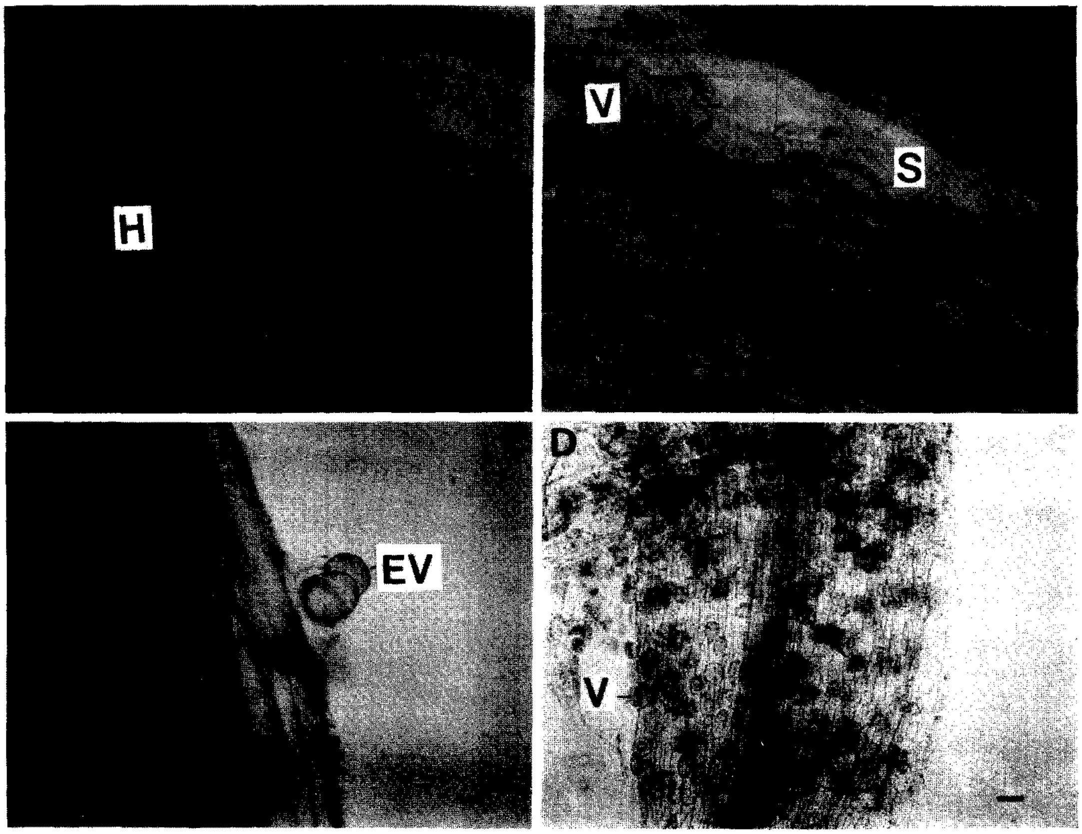


**Fig. 5. Infection characteristics of hyphae of *Glomus borealis* in *Camellia japonica*.**
(A, B) Intercellular hypha (H) and vesicles (V) found in *Camellia japonica*, (C) Intercellular hypha and extracellular vesicles (EV) found in *Camellia japonica*, (D) Abundant vesicles found intercellularly in *Camellia japonica* in the Yesongri evergreen forests of Pogil-do. Scale line = 10 μm.

reddish-brown about 100×140 μm in size, broadly and rather symmetrically ellipsoid, borne on a rather slender hyphae and frequently subtended by a septum (Fig. 4-(A)). The spores have a single layer spore wall. Spore contents were finely granular and more or less fibrous (Fig. 4-(B)). They were compared with published data and identified as *Glomus borealis* (3, 8, 18). The infection characteristics shown in Fig. 5 showed that the type of mycorrhiza was VA mycorrhiza. Fossil records of VA mycorrhiza-like structures have been dated at about 370 million years ago (6). Therefore, the widespread distribution of VA mycorrhizal fungi may have required millions of years for the dispersal and adaptation of mycorrhizal fungi. *Glomus borealis* is known only from Quebec, Canada and pot cultured mycorrhizal associations are not known (3). In this experiment sudan grass (*Sorghum bicolor* L.) was grown for 5 months in pot culture in a sterile sand-vermiculite medium (1:1, v/v) with spores of *Glomus borealis* as VA mycorrhizal inoculum. The result showed that sudan grass was a good pot cultured host plant for the mycorrhizal fungi. The importance of searching for endomycorrhizal fungi lies in the fact that most of the endomycorrhiza are a common feature of many woody and herbaceous plants, including agronomic crops. Further studies will be focused on the agricultural applications of these mycorrhizal fungi.

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