Distribution of Arbuscular Mycorrhizal Fungi in Upland Field Soil of Japan

1. Relationship between Spore Density and the Soil Environmental Factor

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Abstract: To quantify the effects of arbuscular mycorrhizal fungi (AM fungi) on the growth of upland field crops cultivated in Japan, we analyzed soil samples from 124 sites in 18 Japanese prefectures for available P content, pH and AM fungal spore density. The AM fungal spore density in the 124 soil samples was 1.7 per g DW on the average, and lower than 1.0 per g dry soil (DW) in about half of the soil samples. The maximum spore density was 20.6 spores per g DW. The density of AM fungal spore did not vary significantly with the sampling site and the kind of cultivated crop in the sampling field. The pH of the soil with a high spore density ranged from 6 to 8, and in the soil samples with a pH lower than 6 and higher than 8, the spore density was lower than 5 spores per g DW. Thus, in the acid or alkaline soil, the sporogenesis of AM fungi is suppressed. Because available P content was consistently low in the soil samples with a high spore density, P content was considered to correlate with the AM fungal spore density. Therefore, crop cultivation with limited P fertilizer application and reduced available P content may be important to increase AM fungal spore density in upland field soil.

Key words: Arbuscular mycorrhizal fungi, Available P, Spore density, Upland field.

Arbuscular mycorrhizal fungi (AM fungi) invade crop roots, absorb P from soil and supply it to the host crops (Sanders and Tinker, 1971). As a result, the yield of crops was increased by infection with AM fungi (Boswell et al., 1998; Mohammad et al., 2004). Thus, increased infection with AM fungi is important to enhance crop growth (Giovannetti, 1985; Isobe and Tsuboki, 1999a; Usuki and Yamamoto, 2003; Mohammad et al., 2004; Lekberg and Koide, 2005).

Although there are many reports demonstrating the crop growth enhanced by AM fungal infection, many of them are based on the experiments under highly controlled conditions in sterilized soil or in pots (Tawaraya et al., 1996; Isobe and Tsuboki, 1997). Moreover, although about 150 species of AM fungi have been identified, the growth enhancement of host crops varied with the combination of crop and fungal species (Ezawa et al., 1995; Isobe and Tsuboki, 1997; Isobe and Tsuboki, 1999a; Pelletier and Dionne, 2004). Therefore, to effectively utilize AM fungi for crop cultivation, it is extremely important to clarify the density and species of AM fungi in upland field soils.

Surveys on the distribution of soil-inhabiting AM fungi were limited to specific regions like grassland and forests (Tawaraya et al., 1995; Isoi, 1997; Tsuchida and Nonaka, 2002; Saito et al., 2004), and there have been no nationwide surveys on the AM fungi in upland field soils. The density of AM fungal spores in soil is known to vary with the soil environment, such as P content, presence of plant roots, and crop species (Hamel et al., 1994; Kurle and Pfleger, 1996; Boswell et al., 1998; Isobe and Tsuboki, 1999b; Troeh and Loynachan, 2003; Usuki and Yamamoto, 2003). However, it is unknown which environmental factors are associated with spore number in the soil. The objective of this study was to investigate the correlation between the density of AM fungal spore and some soil factors (pH and available P), and to determine if it is possible to predict the type of soil suitable for cultivating crops.

Materials and Methods

Soil was sampled at 124 sites in upland fields of 18 prefectures from May to September, 2003 (Table 1). The soil samples within 10-cm in depth were collected from each spot in each field. Table 1 shows the number of soil samples and the crops cultivated in each sampling field in each prefecture. The samples collected for this experiment consisted of various soil types, including volcanic ash soil and non-volcanic ash soil.

Aliquots of the soil samples were air-dried, sieved through a 2-mm mesh and pH and available P content. The glass electrode method was used for pH measurement and available P content was determined by the Bray 2 method (Bray and Kurtz, 1945). These measurements were repeated three times for each...
Ten-grams of fresh soil samples were wet-sieved through 53-µm and 500-µm meshes. Sample residues on the 53-µm mesh was subjected to sucrose density-gradient centrifugation (Hayman, 1984) to collect AM fungal spores. Spore number in each soil sample was counted under a microscope according to the manual by Schenck and Perez (1990). Another fresh soil sample was air-dried to determine the number of AM fungal spores per one-gram (g) dry soil (DW). This procedure was repeated five times for each sample.

**Results**

Soil pH of all samples ranged from 4.3 to 8.3, but it was predominately between 6.0 and 6.5 (Fig. 1). Available P content of the soils ranged from 0.0 to
260.0 mg per 100 g DW, but it was predominately less than 25.0 mg per 100 g DW (Fig. 2).

The AM fungal spore density in the 124 soil samples was 1.7 per g DW on the average. The spore number of about half of the soil samples was less than 1.0 per g DW. The maximum spore density was 20.6 per g DW (Fig. 3).

Fig. 4 shows the relationship between soil pH and spore density. The pH of soil sample with the highest spore density was 6.4. However, some soil samples with a pH lower than 6.0 and close to 8.0 had more than five spores per g DW and some samples with a pH between 6.0 and 7.0 had no spores.

Fig. 5 shows the relationship between available P content of soil sample and spore density. The available P content of the soil sample with the highest spore density was 8.0 mg per 100 g DW; and that of the samples with the second and third largest spore density was 1.2 mg and 10.9 mg per 100 g DW, respectively. Available P content of the collected soil samples was low, as a whole, and the soil samples with more than 5.0 spores per g DW (i.e. more than the mean) contained less than 50.0 mg available P per 100 g DW.

Fig. 6 shows the AM fungal spore density in the
soil sampled from the fields with different cultivated crops. In this experiment, six or more soil samples were collected from the field with each crop. The spore density was relatively low in the soil sampled from welsh onion and cabbage fields and high in the soil sampled from soybean and taro fields. However, no significant differences were found among the fields with different cultivated crops.

Fig. 7 shows the AM fungal spore density in the soils sampled from different regions. The spore density was lowest in the Chubu region and highest in the Okinawa region, but, there was no significant difference in spore density among regions.
Crops infected with AM fungi, receive various benefits, for example, increase in P content, improvement in plant growth and yield, and resistance to drought and disease (Niemira et al., 1996; Tawaraya et al., 1996; Puthur et al., 1998). The extent of such benefits varies with the environment, such as available P content of soil and soil moisture (Isobe and Tsuboki, 1997). However, one of the most important factors

**Discussion**

Crops infected with AM fungi, receive various benefits, for example, increase in P content, improvement in plant growth and yield, and resistance to drought and disease (Niemira et al., 1996; Tawaraya et al., 1996; Puthur et al., 1998). The extent of such benefits varies with the environment, such as available P content of soil and soil moisture (Isobe and Tsuboki, 1997). However, one of the most important factors
in promoting host crop growth is an increase in the rate of infection with AM fungi, which itself is strongly influenced by the amount of propagules (i.e. spore) 
(Giovannetti, 1985; Isobe and Tsuboki, 1999a, b; 
Usuki and Yamamoto, 2003; Mohammad et al., 2004; 
Lekberg and Koide, 2005). Therefore, to clarify how 
much field crops benefit from AM fungus infection, we 
investigated spore density in field soils. In this study, we 
collected soil samples from upland fields all over 
Japan to determine whether AM fungal spore density 
varied with the region, type of crop cultivated, and 
soil chemical properties. We found that AM fungal 
spore density did not vary with the region or the kind 
of cultivated crop in the field (Figs. 6, 7). However, 
none of the soil samples with a pH lower than 6 and 
higher than 8 had more than 5 spores per g DW (Fig. 
4). In general, spore germination, hyphal elongation 
and infection with AM fungi are suppressed on acidic 
or alkaline agar or soil (Green et al., 1976; Daniels 
and Trappe, 1980; van Aarle et al., 2002). This was 
also the case in this study. Sporogenesis is considered 
to be suppressed and spore density decreased in acid 
or alkaline soil. However, the optimum pH for spore 
germination of AM fungi has been reported to vary 
with the fungal species (Green et al., 1976). The 
species of AM fungi remains to be identified.

On the other hand, every soil sample with a high 
available P content had a small number of AM fungal 
spores (Fig. 5). This finding is consistent with the 
report that P application to soil and high available 
P content resulted in a decreased spore number, 
and suggests that available soil P content affects the 
production, survival and germination of AM fungal 
spores (Mohammad et al., 2004). Clearly, the amount 
of P fertilizer should not be in excess, to keep the 
available P in the field soils low, and to increase the 
AM fungal spore density. When the phosphate 
absorption coefficient of the soil is low, P applied as 
fertilizer is not fixed and accumulates in the soil as 
available P. Therefore, we consider that AM fungal 
spore density is low in the soil with a low phosphate 
absorption coefficient like volcanic ash soil.

Therefore, we consider that AM fungal 
spore density. AM fungal spore density in the 
field generally increases from summer to autumn, 
and decreases in winter (Hayman, 1970; Sutton and 
Barron, 1972; Giovannetti, 1985), and it is widely 
known that AM fungal spore density in the field soils 
shows marked seasonal variation (Clapperton and 
Reid, 1992; Sturmer and Bellei, 1994). Therefore, it 
is necessary to collect field soil samples within a shorter 
period than in this study to clarify the relationship 
among spore density, available soil P content and type 
of crop cultivated.

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