Distribution of Arbuscular Mycorrhizal Fungi in Upland Field Soil of Japan

2. Spore Density of Arbuscular Mycorrhizal Fungi and Infection Ratio in Soybean and Maize Fields

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Abstract: In this study, soil samples were collected from upland fields where maize and soybeans had been cultivated and the density of AM (arbuscular mycorrhizal) fungal spores and the percentage of soybean roots infected with AM fungi (infection ratio) were assessed to determine the factors of the soil chemical properties affecting the mycorrhizal infection. The roots and rhizosphere soil were sampled from 9 soybean fields and 8 maize fields in the summer of 2004. The soil samples were examined for chemical properties (pH, electric conductivity, total phosphate, available phosphate, and phosphate absorption coefficient) and the density of AM fungal spores. Soybean roots were stained with trypan blue to determine the infection ratio. There was a significant difference in soil pH and available phosphorus content with the sampling site. The phosphorus absorption coefficient markedly varied with the sampling site and there was a significant difference in the phosphorus absorption coefficient with the site. The spore density in the soybean and maize fields markedly differed with the sampling site and there was a significant difference in spore density with the sampling site. The density of AM fungal spores in the soybean field was negatively correlated with the available phosphorus content, and showed a positive correlation with the phosphate adsorption. This means that an increase in the available soil phosphorus due to the application of phosphate fertilizers will lower the density of AM fungal spores in the soil and that the density of AM fungi spores is generally higher in soils with a higher phosphate absorption coefficient. It is considered that this tendency is marked in the soil with a low phosphate adsorption coefficient. The infection ratio was positively correlated with spore density, and negatively with the available phosphorus content. To increase mycorrhizal infection of soybeans, we need to decrease the amount of available soil phosphorus and simultaneously to increase the density of AM fungal spores. Excessive application of phosphate fertilizers should be avoided.

Key words: Arbuscular mycorrhizal fungi, Available phosphate, Infection ratio, Phosphate adsorption coefficient, Spore density.

The density of the fungi in soil must be increased to increase the level of crop infection with arbuscular mycorrhizal (AM) fungi (Hayman, 1970; Giovannetti, 1985). Although the density of AM fungal spores and propagules has been reported for particular regions and upland fields (Tawaraya et al., 1995; Isoi, 1997; Tsuchida and Nonaka, 2002; Saito et al., 2004), data is lacking on the density of AM fungal spores in various regions and soil types. Previously, we found a negative correlation between the density of AM fungal spores and the available phosphorus content of the soil (Isobe et al., 2007). On the other hand, an increase in the available phosphorus content by phosphate fertilizer application has been found to decrease the percentage of roots infected with AM fungi (infection ratio) and the density of fungal spores (Isobe and Tsuboki, 1997; Ryan and Ash, 1999; Mohammad et al., 2004; Lekberg and Koide, 2005), but the relationship between the density of AM fungal spores and infection ratio in different regions or soils has not been studied. We need to determine whether this negative relationship between available phosphorus content of soil and the density of AM fungal spores or the infection ratio holds true in various soil types and environmental conditions. In addition, because the density of AM fungal spores varies with the crop under cultivation (Isobe and Tsuboki, 1999; Troeh and Loyanchan, 2003; Usuki and Yamamoto, 2003), soil samples from upland fields of various crops need to be examined. The density of AM fungal spore varied with the kind of cultivated crop in the sampling field (Isobe et al., 2007). This fact indicated that the soil must be collected from the specific crop field in order to clarify the difference of AM fungal spore density of each region in Japan. In this study, we collected soil samples from upland fields of maize and soybeans and assessed the density of AM fungal spores and the infection ratio to determine which factors of the soil chemical properties caused the changes in these parameters.
Materials and Methods

The crop roots and rhizosphere soils were sampled from 9 soybean (*Glycine max* (L.) Merr.) fields and 8 maize (*Zea mays* L.) fields in the summer of 2004. In the maize field, the roots and field soil were sampled, and in the soybean field, only the roots were sampled. The size of the field was different with the sampling site, the smallest field was about 100 m$^2$ (Sampling site No. III, maize) and the biggest field was about 2000 m$^2$ (Sampling site No. I, soybean). The soil and roots were sampled at the center of each field. The roots were sampled from the neighboring 10 plants in one row and about 300 mL within 15 cm in depth was collected from the circumference of the sampled roots. Though the variety of the crop under cultivation was uncertain, the samples were obtained at the flowering stage. Table 1 shows the sampling site, sampling date and the type of sampled soil. Each sampling site had been used as a field for a long time. However, the cropping system of each sampling site was not always clear.

A portion of the soil sample was air-dried for the measurement of chemical properties and the remainder was used to determine the number of AM fungal spores. The pH (H$_2$O), electric conductivity, total phosphate, available phosphate, and phosphate absorption coefficient were measured by the glass electrode method, 1:5 water extraction method, wet ashing followed by molybdenum yellow colorimetry, Bray 2 method, and the ammonium phosphate method, respectively. Spores of AM fungi in the soil were sieved through a 53 μm mesh by a wet sieving method, recovered by sucrose density gradient centrifugation, and counted under a microscope while confirming their morphological characteristics (Gerdemann and Nicolson, 1963). Soybean roots were stained with trypan blue and measured for infection ratio by a grid crossing-point method while confirming the presence of hyphae of AM fungi, dendrophyses, and cystidia (Giovannetti and Mosse, 1980). The size

| Sampling site No. (sampling crops) | Location (Prefecture, City, Town or Village) | Soil type | Sampling date (Y/M/D) |
|-----------------------------------|---------------------------------------------|-----------|----------------------|
| I (Soybean Maize) | Kakudate farm (Hokkaido, Numata town) | Gray lowland soil | 2004/Aug./16 |
| II (Soybean) | Tohoku University (Miyagi, Sendai city) | Yellow soil | 2004/Jul./26 |
| III (Soybean Maize) | Nihon University (Kanagawa, Fujisawa city) | Ando soil | 2004/Aug./1 |
| IV (Soybean) | Yamanashi Prefecture Agriculture Center (Yamanashi, Kai city) | Gray lowland soil | 2004/Aug./11 |
| V (Maize) | Yamanashi Prefecture Agriculture Center (Yamanashi, Hokuto city) | Ando soil | 2004/Jun./30 |
| VI (Soybean Maize) | Wada farm (Nagano, Matsukawa village) | Gley soil | 2004/Jul./24 |
| VII (Soybean Maize) | Kyoto University (Kyoto, Kyoto city) | Gray lowland soil | 2004/Aug./3 |
| VIII (Soybean Maize) | Tottori University (Tottori, Tottori city) | Gray lowland soil | 2004/Aug./9 |
| IX (Soybean) | Yamaguchi University (Yamaguchi, Yamaguchi city) | Gray lowland soil | 2004/Aug./4 |
| X (Maize) | Yamaguchi Prefecture Agriculture Center (Yamaguchi, Yamaguchi city) | Gray lowland soil | 2004/Aug./4 |
| XI (Soybean Maize) | Kyusyu University (Fukuoka, Fukuoka city) | Yellow soil | 2004/Aug./5 |

Fig. 1. AM (arbuscular mycorrhizal) fungi spore density of each soil sample in soybean or maize field.

*: Means followed by the same letter in each field crop soil are not significantly different (p<0.05) according to Tukey’s multiple range test.
of grid was 5 mm quarters; the count number of the crossing of grid and root in one plant was lowest 200. The soil analysis and measurement of spore density, and infection ratio were repeated 5~10 times.

**Results**

The density of AM fungal spores in the soybean and maize fields markedly differed with the sampling site (Fig. 1). In the soybean field, the density of AM fungal spores was highest at site No.III, and low at sites No.II, VI, VII and VIII. In the maize field, the density of AM fungal spores was also highest at site No.III, and low at sites No.I, VI, VIII and X.

The infection ratio of soybean roots also varied greatly with the sampled root (Fig. 2). This tendency was almost equal to the spore density. The root samples at site No.III had the highest infection ratio followed by those from sites No. XI and IX. The infection ratio of the roots at sites No.II, VII and VIII was lower than that of the other samples.

The pH of the collected soil ranged from 6.8 to 4.6, and was slightly acidic at all soybean fields (Table 2). There was a significant difference in pH with the sampling site. The soil samples collected at sites No.VI, XI, VIII and VII had the highest pH, and the samples collected at No.IX, I and II had a lower pH than the other samples. The electric conductivity of the collected soil ranged from 0.472 to 0.188 dS m$^{-1}$, and significantly differed with the sampling site. It was highest at site No.III followed by No.VIII, IV and XI. The electric conductivity at sites No.I, II, VI, VII and IX was about 0.2 dS m$^{-1}$ being lower than that at other sampling sites. The total phosphoric acid content of soil in the soybean fields ranged from 5.07 g kg$^{-1}$ (site No.I) to 1.30 g kg$^{-1}$ (No.III) and there was a significant difference in total phosphoric acid content with the sampling site. Soil collected at sites No.III and No.VIII had the highest total phosphoric acid content, and the content at site No.I was lower than that at other sampling sites. The available phosphorus content ranged from 0.92 g kg$^{-1}$ (No.VI) to 0.03 g kg$^{-1}$ (No.III) and there was a significant difference in available phosphorus content with the sampling site. The available phosphorus content was highest at sites No.VI and No.VII followed by site No.VIII. The available phosphorus content at site No.III was lower than that at other sampling sites. There was a significant difference in phosphorus absorption coefficient with

![Fig. 2. AM (arbuscular mycorrhizal) fungi infection ratio of soybean root. *: Means followed by the same letter are not significantly different (p < 0.05) according to Tukey's multiple range test.](image)

| Sampling site No. | I   | II  | III  | IV  | VI  | VII | VIII | IX  | XI |
|-------------------|-----|-----|------|-----|-----|-----|------|-----|----|
| Soil pH           | 4.7c*| 5.0c| 5.6b | 6.8a| 5.8b| 6.0ab| 6.3a | 4.6c| 6.5a|
| Electric conductivity (dS m$^{-1}$) | 0.206c| 0.212c| 0.472a| 0.300b| 0.196c| 0.188c| 0.368b| 0.192c| 0.276b|
| Total phosphoric acid (g kg$^{-1}$) | 1.30d| 3.05bc| 5.07a| 2.00c| 4.23b| 3.99b| 4.64ab| 2.33c| 1.71cd|
| Available phosphorus (g kg$^{-1}$) | 0.20d| 0.43c| 0.05e| 0.20d| 0.92a| 0.82a| 0.63b| 0.36c| 0.16d|
| Phosphate absorption coefficient | 530c| 730b| 2150a| 680b| 440d| 280e| 780b| 220e| 470cd|

*: Means followed by the same letters are not significantly different (p < 0.05) according to Tukey’s multiple range test.
the sampling site. It was the highest at site No.III, and was low at sites No.VII and No.IX.

Table 3 shows the chemical property of the soil collected from the maize fields. The pH of the soil collected from the maize fields ranged from 6.4 to 4.6, and that from maize fields was also slightly acidic. There was a significant difference in pH with the sampling site. The soil samples collected at sites No.V, No. XI, No.X, No.VIII and No.VI had the highest pH, and that at site No.VII had a lower pH than other samples. The electric conductivity of the soil collected from the maize field ranged from 0.682 to 0.172 dS m$^{-1}$. There was a significant difference in electric conductivity with the sampling site. It was the highest in the soil collected at site No.III followed by that collected at site No.VI and was lower in the soil collected at site No. XI than that at other sites. The electric conductivity in soil collected from another maize field was approximately 0.2$\sim$0.3 dS m$^{-1}$. The total phosphoric acid content of soil in the maize fields ranged from 5.09 g kg$^{-1}$ (No. VI) to 1.72 g kg$^{-1}$ (No. XI), and there was a significant difference in total phosphoric acid content with the sampling site. The soil collected at sites No.VI and No.I had the highest total phosphoric acid content, and that at sites No. XI and No.X had the lowest. The available phosphorus content of maize field soil ranged from 1.81 g kg$^{-1}$ (No.VI) to 0.02 g kg$^{-1}$ (No. III) and there was a significant difference in available phosphorus content with the sampling site. It was the highest at site No.VI, followed by site No.VIII, and that at site No.III was lower than that at other sites. There was a significant difference in phosphorus absorption coefficient with the sampling site. It was the highest in the soil collected at site No.III, and lowest in the soil at sites No.VI, No.X and No. XI.

Table 4 shows the correlation coefficient between the density of AM fungal spores in field soil and soil chemical property. The density of AM fungal spores in the soil in the soybean fields was negatively correlated with the available phosphorus content, and positively correlated with the phosphate adsorption coefficient. The correlation coefficient was $r = -0.833$ and, $r = 0.699$, respectively and was significant at the 1% and 5% level, respectively. The density of AM fungal spores in the soil in the maize fields was positively correlated with the phosphate adsorption coefficient. The correlation coefficient was $r = 0.894$, and was significant at the 1% level.

Table 5. The correlation coefficients between infection ratio of soybean and spore density or soil chemical property.

| Soil pH | Electric conductivity | Total phosphoric acid | Available phosphorus | Phosphate absorption coefficient |
|---------|----------------------|----------------------|----------------------|-------------------------------|
| Soybean | Maize field soil     | Soybean field soil   | Maize field soil     |                               |
| 0.181ns | 0.560ns              | -0.028ns             | 0.699*               |                               |
| -0.145ns| 0.678ns              | -0.092ns             | 0.894**              |                               |
| ns, *, **: not significant and significant at 5%, and 1% level.

Table 3. Soil pH, electric conductivity, total phosphoric acid, available phosphorus and phosphate absorption coefficient in soil of maize fields.

| Sampling site No. | I | III | V | VI | VII | VIII | X | XI |
|------------------|---|-----|---|----|-----|------|---|----|
| Soil pH          | 5.6b*| 5.4b| 6.4a| 5.8ab| 4.6c| 6.0a| 6.2a| 6.4a|
| Electric conductivity (dS m$^{-1}$) | 0.540c| 0.682a| 0.340c| 0.480b| 0.286d| 0.320cd| 0.266d| 0.172e|
| Total phosphoric acid (g kg$^{-1}$) | 4.77a| 3.98c| 4.45b| 5.09a| 4.24bc| 4.47b| 2.02d| 1.72d|
| Available phosphorus (g kg$^{-1}$) | 0.55d| 0.02f| 0.13c| 1.81a| 1.03b| 0.74c| 0.40d| 0.17e|
| Phosphate absorption coefficient | 790c| 2260a| 1460b| 320e| 430f| 690c| 250ef| 300e|

* : Means followed by the same letters are not significantly different (p<0.05) according to Tukey's multiple range test.
level.

Table 5 shows the coefficient of correlation of the infection ratio with the density of AM fungal spores in the soybean fields and with the soil chemical properties. The infection ratio showed a positive correlation with the density of AM fungal spores, and negative correlation with the available phosphorus content. The correlation coefficient was \( r = 0.904 \) and, \( r = -0.724 \), respectively, and was significant at the 0.1% and 5% level, respectively. However, the correlation coefficients of the density of AM fungal spores with other soil chemical properties (soil pH, electric conductivity, total phosphoric acid and phosphate absorption coefficient) were not significant.

Discussion

Increasing the application amount of phosphorus fertilizer increases the amount of available phosphorus in the soil. This tendency is striking in the soil with a low phosphate adsorption coefficient (Takahashi et al., 1997). Isobe and Tsuboki (1998) found that the infection rate decreased with the increase in the amount of phosphorus fertilizer. In addition, it was reported that applying phosphorus fertilizer caused a decrease in the density of AM fungal spores in soil (Isobe et al., 1993; Tawaraya et al., 1995; Isobe and Tsuboki, 1997; Mohammad et al., 2004; Lekberg and Koide, 2005). In the present study, a significant, highly negative correlation was observed between the density of AM fungal spores and the content of available phosphorus in the soil from soybean fields (Table 4). Although the correlation was not statistically significant, a negative correlation coefficient of \(-0.606\) was observed between spore density and the amount of available phosphorus in the soil from maize fields. These findings indicate that an increase in the available soil phosphorus due to the application of phosphorus fertilizers lowers the density of AM fungi spores in the soil. There have been many reports that the more phosphorus fertilizer applied, the lower the spore density (Menge et al., 1978; Isobe et al., 1993; Kahiliuto et al., 2001). In other words, to increase the density of AM fungi spores in soil, it is important to avoid excessive application of phosphorus fertilizers.

Furthermore, in the present study, a positive correlation was observed between the density of AM fungi spores and the phosphate absorption coefficient in the soils from both soybean and maize fields (Table 4). This indicates that the densities of the AM fungi spores are generally higher in soils with higher phosphate absorption coefficients. Because volcanic ash soils generally have a higher phosphate absorption coefficient than non-volcanic ash soils, the densities of AM fungi spores tended to be higher in volcanic ash soils than in non-volcanic ash soils. In the present study, the soils sampled at sites No. III and V tended to have a higher density of AM fungi spores than the soils in other areas (Table 1, Fig. 1). This may be because the soil samples from these sampling sites were Andosol volcanic ash soils. Thus, the density of AM fungal spores in Japanese fields is greatly affected by the phosphorus content (available phosphorus content, phosphate absorption coefficient) of the soil. Moreover, the density of AM fungal spores generally varies significantly with the season and growth stage of the crop (Sutton and Barron, 1972; Tisdall and Oades, 1980; Giovannetti, 1985). The density of AM fungal spores increased from summer to autumn (Sutton and Barron, 1972). Therefore, it is necessary to sample the field soil as much as possible within a short period in order to investigate the relationship between the density of AM fungal spores and soil chemistry in detail. The spore germination and hyphal elongation of AM fungi, and infection of plant roots with AM fungi are all suppressed in acidic and alkaline soils and on acidic and alkaline agar media (Green et al., 1976; Hepper, 1984; van Aarle et al., 2002; Isobe et al., 2007). Therefore, the density of AM fungi spores was assumed to be lower in acidic and alkaline soils. However, no significant differences in soil pH were observed, which may be due to the rather narrow pH range (4.6 to 6.8, which are all weakly acidic) of the soil samples analyzed (Tables 2 and 3). The levels of spore density in the soils with a pH lower than 4 or higher than 7 remain uncertain. Hence, further analysis of the relationship between soil pH and spore density in soil samples with various pHs is needed.

For stimulation of growth and increased yield of the crop by AM fungal infection, it is important to lower the amount of available phosphorus and increase the infection ratio (Hayman, 1970; Giovannetti, 1985; Karasawa et al., 2001). In the present study, a high positive correlation was observed between the infection ratio and the density of AM fungi spores in the soil, while a negative correlation was observed between the infection ratio and the amount of available phosphorus in the soybean field (Table 5). These results are in general agreement with previous reports (Giovannetti, 1985; Tawaraya et al., 1995; Wuen et al., 2002; Mohammad et al., 2004). In addition, the presence of crop roots that can be infected by AM fungi is another important factor for increasing spore density because the AM fungi do not form spores unless the plant roots are infected by the fungi. One way to achieve an increased spore density would be to shorten the period of bare ground (Isoi, 1997; Isobe and Tsuboki, 1999; Usuki and Yamamoto, 2003). However, because the density of AM fungi spores greatly varies with the species and cultivar of the crop, the crop type and the crop system (Troeh and Loynachan, 2003; Usuki and Yamamoto, 2003), it is necessary to understand the correlation between spore production and the type of crop. It is anticipated that the composition of the species of the AM fungi differs with the sampling site. The
infection ratio of AM fungi differed with the species of AM fungi (Ezawa et al., 1995; Tawaraya et al., 1996; Isobe and Tsuboki, 1997). In this study, the difference in the AM fungi flora at the sampling site affected the infection ratio. Therefore, it is necessary to investigate the AM fungi flora at the sampling site to clarify the relationship between spore density and the infection ratio.

In this study, the density of AM fungal spores was higher in soils with a higher phosphate absorption coefficient. Furthermore, the infection ratio was positively with the density of AM fungal spores, and negatively correlated with the available phosphorus content. The density of AM fungal spores was lower in gray lowland soil and gley soil than in the other soils. However, the effects of other factors such as climate and vegetation on the spore density and infection ratio of AM fungi have not been clarified because only one type of soil was tested in each area. To elucidate these relationships, we need to analyze more types of soil in each area and compare the density of AM fungal spores with the infection ratio in various areas with different crops.

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