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SHORT COMMUNICATION

Inoculation of arbuscular mycorrhizal fungi can substantially reduce phosphate fertilizer application to *Allium fistulosum* L. and achieve marketable yield under field condition

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Abstract The effects of inoculating arbuscular mycorrhizal (AM) fungi on the growth, phosphorus (P) uptake, and yield of Welsh onion (*Allium fistulosum* L.) were examined under the non-sterile field condition. Welsh onion was inoculated with the AM fungus, *Glomus* R-10, and grown in a glasshouse for 58 days. Non-inoculated plants were grown as control. Inoculated and non-inoculated seedlings were transplanted to a field with four available soil P levels (300, 600, 1,000, and 1,500 mg $P_2O_5 kg^{-1}$ soil) and grown for 109 days. AM fungus colonization, shoot P concentration, shoot dry weight, shoot length, and leaf sheath diameter were measured. Percentage AM fungus colonization of inoculated plants was 94% at transplant and ranged from 60% to 77% at harvest. Meanwhile, non-inoculated plants were colonized by indigenous AM fungi. Shoot length and leaf sheath diameter of inoculated plants were larger than those of non-inoculated plants grown in soil containing 300 and 600 mg $P_2O_5 kg^{-1}$ soil. Shoot P content of inoculated plants was higher than that of non-inoculated plants grown in soil containing 300 and 600 mg $P_2O_5 kg^{-1}$ soil. Yield (shoot dry weight) was higher for non-inoculated plants grown in soil containing 1,000 and 1,500 mg $P_2O_5 kg^{-1}$ soil than for those grown in soil containing 300 and 600 mg P_2O_5 kg^{-1} soil. Meanwhile, the yields of inoculated plants (200 g $plant^{-1}$) grown in soils containing the four P levels were not significantly different. Yield of inoculated plants grown in soil containing 300 mg P_2O_5 kg^{-1} soil was similar to that of non-inoculated plants grown in soil containing 1,000 mg $P_2O_5 kg^{-1}$ soil. The cost of AM fungal inoculum for inoculated plants was US$ 2,285 ha^{-1} and lower than the cost of superphosphate (US$ 5,659 ha^{-1}) added to soil containing 1,000 mg P_2O_5 kg^{-1} soil for non-inoculated plants. These results indicate that the inoculation of AM fungi can achieve marketable yield of *A. fistulosum* under the field condition with reduced application of P fertilizer.

Keywords Arbuscular mycorrhiza · Field · Inoculation · Phosphate · Welsh onion · Yield

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Introduction

Phosphorus (P) is one of the essential elements for plant growth and often the first limiting nutrient in soils due to its low availability. The heavy application of P fertilizer has been carried out for crop production to avoid growth decline due to P deficiency. Rock phosphate is the raw material of P fertilizer, and it is estimated that the world's reserve would last for only 90 years (Stewart et al. 2005). The expected global peak of P production is predicted to occur around 2030 (Cordell et al. 2009). The heavy application of P fertilizer accelerates P enrichment of water, and this, in turn, leads to the eutrophication of rivers, lakes, and marshes. In this regard, it is necessary to develop sustainable and environmentally safe technologies for the use of P resources.

Arbuscular mycorrhizal (AM) fungi promote the growth of many plants by enhancing P uptake (Smith and Read 2008). The elongation of the extraradical hyphae of AM fungi into soil increases the surface area for the uptake of P, which is often depleted in rhizosphere soil solution. Generally, P acquisition by crop plants is accomplished by extending roots into soil. Crop plants of Alliaceae, such as Welsh onion, onion, Chinese chive, garlic, and leek, have a less well-developed root system than most other species (Greenwood et al. 1982). Studies have shown that the inoculation of AM

fungi improved P uptake and growth of *Allium cepa* (Mosse and Hayman 1971; Smith et al. 1986), *Allium fistulosum* (Tawaraya et al. 1999, 2001), and *Allium porrum* (Hepper et al. 1988; Stribley et al. 1980) under the pot culture condition. Thus, the utilization of AM fungi may reduce the amount of P fertilizer to be applied to these plant species, thereby conserving P resources. The purpose of the present study was to clarify the effect of inoculating *A. fistulosum* with AM fungi under the field condition to reduce application of P fertilizer and achieve marketable yield.

Materials and methods

Inoculation

Andosol was collected from Tsuruoka, Yamagata Prefecture, Japan. The soil was air-dried, sieved to <5 mm particle, and steam-sterilized twice at 80°C for 45 min. Ammonium sulfate, superphosphate, and potassium sulfate were added at the rate of 1.00 gN, 1.00 g P_2O_5, and 0.83 gK kg^{-1} soil, respectively. Soil pH (H_2O) was adjusted to 5.8 by the addition of lime. AM fungal inoculum (Idemitsu Kosan, Co. Ltd., Tokyo, Japan), which consisted of spores, extraradical hyphae, and infected roots of *Glomus* R-10 (14 propagules g^{-1}), was mixed with soil at the rate of 75 g kg^{-1} soil. Control plants were not inoculated. Two hundred twenty paperpots (26×26×38 mm, Nippon Beet Sugar Mfg. Co., Ltd., Tokyo) were placed in rearing trays (280×580× 30 mm each), and each tray was filled with 4 kg of inoculated soil or non-inoculated soil. Seeds of Welsh onion (*A. fistulosum* L. cv. Motokura) were sown in the paperpots on 2 April 2008. One thousand seven hundred sixty inoculated or non-inoculated paperpots were prepared. The seedlings were grown in a glasshouse at Yamagata University, Tsuruoka, Japan (38°44′N, 139°50′E) for 58 days and irrigated with tap water every other day.

Transplanting

The field was located in Tozawa Village, Yamagata Prefecture, Japan (38°46′N, 140°9′E). Properties of the soil were as follows: pH (H_2O), 5.23; texture, loamy sand; organic C, 0.91%; total N, 0.47%; available P, 94.38 mg P_2O_5 kg^{-1}. Ammonium sulfate and potassium sulfate were applied at the rate of 200 kg N ha^{-1} and 200 kg K ha^{-1}, respectively. Superphosphate was applied at the rate of 0; 4,135 (703 P_2O_5); 9,847 (1,674 P_2O_5); and 12,711 (2,161 P_2O_5) kg ha^{-1}. The available soil P concentrations of these soils 2 weeks after application were 300, 600, 1,000, and 1,500 mg P_2O_5 kg $soil^{-1}$. The fertilizers were broadcasted and incorporated manually with the top 10 cm of the soil surface. Each plot measured 2×3 m and had two 2-m rows. The paperpots were planted in a plot spacing of 5 cm. In total, there were 80 paperpots per plot. The plots were arranged in a randomized complete block design with two AM fungal inoculations (inoculated and non-inoculated) and four soil P levels (300, 600, 1,000, and 1,500 mg P_2O_5 kg $soil^{-1}$) with four replications. Thirty-two plots were employed.

Harvest and sample analysis

Shoots and roots were harvested on 8 October 2008 (167 days after sowing). Twenty plants growing in a 1-m row were harvested. In total, 40 plants were harvested from one plot. Shoot length and leaf sheath diameters were measured. Shoot dry mass was determined after drying at 70°C. Ground shoots were digested with HNO_3–$HClO_4$–H_2SO_4 solution. P concentration in the digested solution was determined colorimetrically with the vanadomolybdate-yellow assay (Olsen and Sommers 1982). Roots were cleared and stained with 500 mg L^{-1} aniline blue solution (Tawaraya et al. 1998). Percentage AM fungus colonization was examined using the gridline intersect method (Giovannetti and Mosse 1980). Data were subjected to the two-way analysis of variance, and means were compared by the least significant difference method (P=0.05) using the statistical software StatView 4.5 (Abacus Concepts).

Results

Arbuscular mycorrhizal colonization

The AM fungus *Glomus* R-10 colonized well in the roots of seedlings in the paperpots. Percentage AM fungus colonization was 94±3% (n=4) 58 days after sowing. No colonization was noted in the roots of non-inoculated seedlings. AM fungus colonization was observed in both inoculated and non-inoculated seedlings at harvest (Table 1). AM fungus colonization of inoculated plants ranged from 60% to 77% and was not different among the four soil P levels. Non-inoculated plants were colonized by indigenous AM fungi, but the AM fungus colonization of non-inoculated plants was less prominent than that of inoculated plants and decreased with increasing soil P level.

Shoot P uptake

Shoot P content of non-inoculated plants increased with increasing soil P level (Table 1). In contrast, shoot P contents of inoculated plants grown in soil containing the four soil P levels were not significantly different. Shoot P content of inoculated plants grown in soil containing 300 and 600 mg P_2O_5 was higher than that of non-inoculated plants grown under the same conditions.

Table 1 Mycorrhizal colonization, shoot height, diameter of leaf sheath, and shoot P content grown at four soil P levels with or without inoculation

Inoculation	Soil P level (mg P_2O_5 kg^{-1})	Mycorrhizal colonization (%)	Shoot height (cm)	Diameter of leaf sheath (cm)	Shoot P content (mg P $plant^{-1}$)
Control	300	52±4 abc[a]	52.8±5.1 b	1.7±0.3 b	18±8 b
Control	600	47±5 abc	60.0±2.8 ab	2.0±0.2 ab	30±8 b
Control	1,000	27±7 bc	64.5±3.6 a	2.4±0.1 ab	40±7 ab
Control	1,500	18±9 c	70.5±7.6 a	2.5±0.1 a	41±4 ab
Glomus R-10	300	77±8 a	76.6±2.2 a	2.4±0.1 a	54±3 a
Glomus R-10	600	65±10 ab	77.1±2.9 a	2.5±0.1 a	63±8 a
Glomus R-10	1,000	66±5 ab	74.2±1.8 a	2.6±0.1 a	59±4 a
Glomus R-10	1,500	60±14 ab	78.2±2.5 a	2.7±0.0 a	67±4 a

[a] Means (±standard error, n=4) followed by different letters in the column are significantly (P=0.05) different as determined by the Tukey's HSD test

Shoot length and leaf sheath diameter

Shoot length and leaf sheath diameter of non-inoculated plants increased with increasing soil P levels (Table 1, Fig. 1). In contrast, shoot length and leaf sheath diameter of inoculated plants grown in soil containing the four soil P levels were not significantly different. Shoot length and leaf sheath diameter of inoculated plants grown in soil containing 300 mg P_2O_5 were higher than those of non-inoculated plants.

Shoot dry weight

Shoot dry weight of non-inoculated plants increased with increasing soil P levels (Fig. 2). In contrast, shoot dry weight of inoculated plants was higher than 15 g/plant regardless of the soil P level and was not significantly different from those of non-inoculated plants grown in soil containing 600, 1,000, and 1,500 mg P_2O_5.

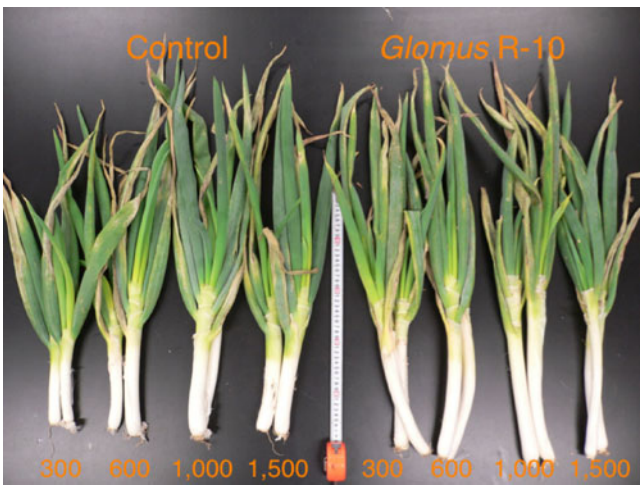

Fig. 1 Shoot growth of *A. fistulosum* inoculated with AM fungus, *Glomus* R-10, and grown in soil containing four levels (300, 600, 1,000 and 1,500 mg P_2O_5 kg^{-1}) of soil P, measured 167 days after sowing. Non-inoculated plants were used as control

Discussion

It is established that inoculation of AM fungi increased shoot P uptake and shoot growth under pot culture conditions. However, there have been few papers showing reduction of P fertilizer application and satisfactory yield with inoculation of AM fungi under field conditions. Inoculation of AM fungi did not increase yield of garlic under field condition (Sari et al. 2002). Pre-inoculation of transplants with arbuscular mycorrhizal fungi increased growth of leek under semi-field condition (Sorensen et al. 2008). Our data shows that inoculation of AM fungi increase shoot P uptake and growth of *A. fistulosum* and achieve marketable yield under filed condition. Moreover, shoot dry weight of inoculated plants grown in soil containing 300 mg P_2O_5 was 207 g $plant^{-1}$ and was not different from that of non-inoculated plants grown in soil containing 1,000 mg P_2O_5

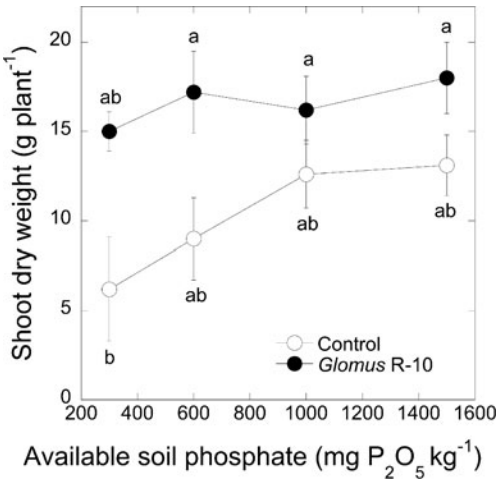

Fig. 2 Yield (shoot dry weight) of *A. fistulosum* inoculated with AM fungus, *Glomus* R-10, and grown in soil containing four levels of soil P, measured 167 days after sowing. Non-inoculated plants were used as control. Means (±standard error, n=4) followed by *different letters* are significantly (P<0.05) different by Tukey's HSD test

(Fig. 2). This indicates that the exposure to 300 mg P_2O_5 plus the inoculation of AM fungi at the nursery stage increased shoot dry weight to a value that was similar to that of the non-inoculated plant grown in soil containing 1,000 mg P_2O_5. The inoculation of AM fungi reduced the amount of superphosphate applied from 9,850 kg ha^{-1} to 0 kg P_2O_5 ha^{-1}. Shoot dry weight of inoculated Welsh onions (>15 g $plant^{-1}$) was the same as that of conventionally grown non-inoculated Welsh onions in Japan. The potential savings in P fertilizer application for soybean due to inoculation with AM fungi were estimated under glasshouse condition (Kelly et al. 2001; Plenchette and Morel 1996). To our knowledge, this is the first report showing that the inoculation of AM fungi reduced the amount of P fertilizer to be applied and achieved economically valuable yields under the field condition.

The costs of AM fungal inoculum and P fertilizer were calculated. Price of superphosphate and AM fungal inoculum in 2008 was 0.57 and 8.40 US$ kg^{-1}, respectively. Nine thousand eight hundred fifty kilograms of superphosphate per hectare was applied to non-inoculated plants, which was equivalent to 1,000 mg P_2O_5. In contrast, no superphosphate was applied to the inoculated plants exposed to 300 mg P_2O_5, and only 272 kg of AM fungal inoculum per hectare was applied. Total cost of P fertilizer was 5,629 US$ ha^{-1}. On the other hand, total cost of AM fungal inoculum was 2,285 US$ ha^{-1}. The cost difference was US$ 3,374. This means that the use of AM fungal inoculum is valuable for farmers. Heavy application of phosphate fertilizer has been carried out on horticultural crops (Mishima et al. 2010; Reijneveld et al. 2010). Rock phosphate has been used to produce phosphate fertilizers, and availability of rock phosphate may peak in about 2030 (Cordell et al. 2009). It is necessary for responses of this problem (1) to reduce application of P fertilizer to agricultural crops, (2) to make crop plants more efficient at acquiring soil P and using it, and (3) finally, to recycle organic P for agriculture. Inoculation of AM fungi is one of useful techniques to achieve the reduction of phosphate fertilizer application.

Colonization by indigenous AM fungi was noted in the roots of non-inoculated plants grown in soil containing the four P levels, 167 days after sowing. However, shoot growth of non-inoculated plants was slower than that of inoculated plants. This indicates that the introduced *Glomus* R-10 was more efficient in growth improvement than the indigenous fungi. AM fungus colonization of non-inoculated plants tended to decrease with increasing soil P levels. On the other hand, AM fungus colonization of inoculated plants did not change significantly even with an increase of soil P levels. It is well known that the heavy application of P fertilizer to plant inhibits AM fungus colonization in roots (Smith and Read 2008). The effect of P operates on spore germination, growth of germ tube, initiation of entry points, development of intraradical mycelium, and formation of arbuscules. Percentage AM fungus colonization of inoculated plants was 94% at transplant. Steps from the spore germination to the initiation of entry points of inoculated plants were not affected by the high soil P concentration. Therefore, the inoculation of AM fungi at the nursery stage avoids the decrease in colonization caused by the application of P fertilizer.

The AM fungus *Glomus* R-10 was not indigenous to the soil of this field experiment. Introduction of this fungus can affect composition and activity of soil microbial community. There has been little known about how long inoculated AM fungi survive in the soil and how they affect a community of indigenous soil microbes including AM fungi. Research on impact of exotic AM fungi on local soil microbial species and community is required to elucidate best management practices for mycorrhizal treatment (Schwartz et al. 2006).

Conclusions

The inoculation of AM fungi at the nursery stage promoted the growth of *A. fistulosum* under the non-sterile field condition. The yield of *A. fistulosum* grown in soil containing 300 mg P_2O_5 kg^{-1} plus AM fungal inoculum was equal to that of plants grown in soil containing 1,000 mg P_2O_5 kg^{-1} but no AM fungal inoculum. The inoculation of AM fungi reduced the amount of P fertilizer to be applied and the production cost of *A. fistulosum* under the field condition.

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