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Responses of Soybean Plants to Vesicular-Arbuscular Mycorrhizal Inoculation

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The responses of growth and seed yield of soybean to the inoculation of indigenous micro-organisms were compared in three Kyushu soils, each differing in soil phosphate status. The inoculation to irradiated Kasuya soil (high in both total and available phosphate) and Osumi soil (high in total phosphate but low in available phosphate) resulted in increased dry matter and seed yields. However, seed yields decreased after the inoculation to irradiated Kashii soil, which was low in both total and available phosphate.

The effect of inoculation with spores of vesicular-arbuscular mycorrhizal fungi, *Glomus mosseae* and *G. etunicatus* on growth and seed yield of soybean was tested in Aso soil, which was high in total phosphate but low in available phosphate. Inoculation greatly increased the dry matter and seed yields in irradiated soil with or without added phosphate fertilizer. In unsterilized soil the effect of inoculation was much clearer when phosphate fertilizer had been applied than when its addition was omitted.

INTRODUCTION

In many countries during the last decade, much attention has been paid to research on vesicular-arbuscular (VA) mycorrhizas (Smith, 1980 ; Barea and Azcbn-Aguilar, 1983). Until recently, however, this subject has been ignored in Japan although Asai (1943), in his pioneering work, stressed the roles of VA mycorrhizas in plant nutrition. Tawaraya *et al.* (1985) investigated VA mycorrhizal infection of several crop plants in Hokkaido. Their results showed that the percentage of infection did not correlate with the quantity of available phosphate or the pH value. Kawai and Yamamoto (1986) reported that VA mycorrhizal inoculation increased the formation and nitrogen fixation of soybean nodules. The present paper describes the effect of VA mycorrhizal inoculation on the growth and yields of soybean plants cultured in some Kyushu soils, including andosols which are low in available phosphate.

MATERIALS AND METHODS

Experiment I.

Soil samples were collected from the following three areas: Kasuya, Fukuoka Prefecture ; Osumi (Kushira) Kagoshima Prefecture ; and Kashii, Fukuoka city. Two-thirds of each soil sample was irradiated with a 1 Mrad gamma dose from ^{60}Co in the Co-60 radiation Laboratory, Kyushu University. This eliminated the indigenous VA mycorrhizal fungi. The soil pH was then adjusted to 6.6-7.0 using a dose of 5 g calcium carbonate per 1 kg of soil. Two kg samples of soil were next placed into pots and 0.1 g nitrogen and 0.5 g potassium were applied to each. For the plus phosphate soil specimens (i. e. those with added phosphate), each received 0.13 g phosphorus.

Mycorrhizal inoculation was carried out by placing a mixture of 0.2 kg irradiated soil and 0.2 kg unsterilized soil on top of 1.6 kg irradiated soil in a sample pot. For each soil sample four replicates were made for minus phosphate treatment (i.e. no added phosphate) and six replicates for plus phosphate treatment.

Sterilized soybean (Glycine *max* L. AGS-2) seeds were inoculated with *Rhizobium japanicum* and germinated in a petri dish. When germination had taken place, the seeds were transferred to sterilized vermiculite contained in a paper cup. Thirteen days later, the plants were transplanted into pots containing the prepared soil. The pots were kept inside an area enclosed by netting and watered with domestic tap water between June 22 and November 20, 1983. At harvest time the dry weight of plant parts was measured. These were then ground for later analysis.

Experiment II.

Half of the andosol (Aso soil) collected in Namino (Kumamoto Prefecture) was irradiated with a gamma dose of 1 Mrad. Two kg of either irradiated or unsterilized soil (with the pH adjusted to 6.8) was applied with 0.5 g potassium and 0.26 g phospho rus (plus phosphate) or with 0.5 g potassium alone (minus phosphate). The treated soil was then placed in a pot. Eight-day-old nodulated soybean *(Glycine max* L. AGS-2) seedlings were then planted in the soil. The soil in the pot was then inoculated with a mixture containing 1,000 spores of each of *Glomus mosseae* and G. *etunicatus*. The duplicate pots were maintained below a maximum temperature of 30°C inside a greenhouse between June 26 and November 15, 1984. Water was supplied uniformly to the pots by a capillary feeding system, consisting of a polyvinylformyl sponge sheet. At harvest, the plants were separated into seed, pod shell, and petiole plus stem. The dry weight of each part was then measured.

Ground samples were digested by the Kjeldahl method to determine nitrogen content by distillation-titration and phosphorus content colorimetrically.

‘RESULTS

Experiment I.

Some properties of the soils used are shown in Table 1. The Kasuya soil is high in both total and available phosphate, while the Osumi soil is high in total phosphate but low in available phosphate, and the Kashii soil is low in both total and available phosphate. The Osumi soil has the highest phosphate fixing capacity.

Plant height was compared 50 days after germination. In soils to which phosphate fertilizer had not been applied, the growth was best in the Kasuya soil, followed by that of the Osumi soil and lastly that of the Kashii soil. Growth was greatest in the Kasuya soil and there was no difference in growth between the Osumi and Kashii soils where phosphate fertilizer had been added. Although growth was slightly superior in the

Table 1. Some properties of the soils used in the experiments

Soil	Vegetation	Soil type	Total phosphate	Available* phosphate	Phosphate absorption	Total nitrogen
			mg P_2O_5／100g soil			%
Kasuya	Arable	Gray lowland soil	106	11.6	542	0.17
Kashii	Pine	Red-yellow soil	34	0.17	642	0.03
Osumi	Arable	Andosol	151	0.81	2330	0.42
Aso	Arable	Andosol	203	1.23	3190	0.74

* Available phosphate was determined by Bray II method (Bray and Kurtz, 1945).

Table 2. Dry weight of inoculated or uninoculated soybean plants grown in three different soils.

Soil	Treatment	Dry weight −P total	Dry weight −P seed	Dry weight +P total	Dry weight +P seed
		g／pot			
Kasuya	unsterilized	60	22	66	26
	irradiated uninoculated	43	11	56	16
	irradiated inoculated	68	20	77	27
Osumi	unsterilized	6.9	1.8	17	4.9
	irradiated uninoculated	–	–	15	4.0
	irradiated inoculated	14	3.4	21	6.5
Kashii	unsterilized			11	4.1
	irradiated uninoculated			19	4.8
	irradiated inoculated			11	2.9

irradiated Kasuya soil as compared to the unsterilized sample, the inoculation of micro-organisms and the application of phosphate fertilizer did not affect plant height in Kasuya soil. In the irradiated Osumi soil, the absence of both micro-organisms inoculation and phosphate fertilizer resulted in the poorest soybean growth, and ultimate plant death. Application of phosphate fertilizer to Kashii soil increased plant height. In this soil where no phosphate fertilizer had been added, plants ceased growth 50 days after germination.

Seed yields were almost identically reflected by the plant growth (Table 2), i. e. seed yields were greater in the Kasuya soil, then in the Osumi and Kashii soils, respectively. Seed yields were also greater in soils with added phosphate fertilizer than in soils without added phosphate fertilitzer. In the irradiated Kasuya and Osumi soils, seed yields were increased by inoculation of micro-organisms in both the presence and absence of phosphate fertilizer. When the Kashii soil was not amended by phosphate fertilizer, all the plant died. In the Kashii soil, with added phosphate fertilizer, inoculation of micro-organisms did not increase seed yields.

Accumulation of phosphorus and nitrogen in the shoot is shown in Table 3. Trends in phosphorus and nitrogen accumulation were almost the same as dry matter

Table 3. Phosphorus and nitrogen accumulation in inoculated or uninoculated soybean plants grown in three different soils.

Soil	Treatment	P content		N content	
		−P	+P	−P	+P
		mg/pot		g/pot	
Kasuya	unsterilized	248	301	2.10	2.48
	irradiated uninoculated	158	208	1.11	1.59
	irradiated inoculated	262	300	2.30	2.70
Osumi	unsterilized	13	42	0.16	0.47
	irradiated uninoculated	_	34	_	0.35
	irradiated inoculated	32	60	0.34	0.65
Xashii	unsterilized	_	30		0.35
	irradiated uninoculated	_	36	_	0.45
	irradiated inoculated	_	27		0.30

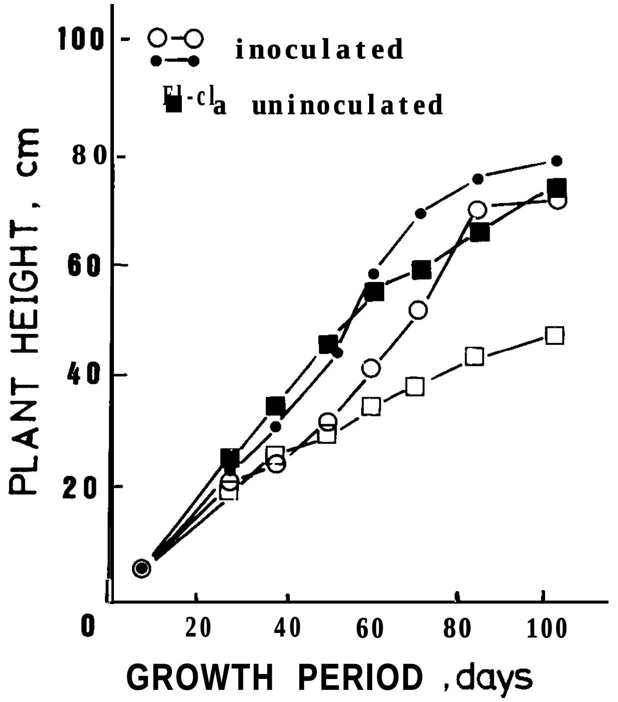

Fig. 1. Effect of vesicular-arbuscular mycorrhizal inoculation on the height of soybean plants grown in irradiated Aso soil with (closed symbol) or without (open symbol) added phosphate fertilizer.

production or seed yields. Phosphorus accumulation by shoots in the Kasuya soil was remarkably large compared to that by shoots in other soils. This coincides with the

Table 4. Effects of inoculation of VA mycorrhizal fungus spores and application of phosphate fertilizer on the dry weight of soybean plants grown in irradiated or unsterilized Aso soil.

Treatment			Dry weight			
			leaf	stem	pod shell	seed
			g／pot			
Irradiated	Inoculated	- P	10.3	6.3	3.1	5.2
		+ P	31.7	21.1	14.8	21.5
	Uninoculated	- P	2.0	0.4	0.1	0.2
		+ P	11.8	8.1	6.1	8.9
Unsterilized	Inoculated	- P	19.7	13.6	7.9	11.1
		+ P	35.1	25.0	15.4	25.6
	Uninoculated	- P	22.1	12.9	8.5	12.3
		+ P	32.5	21.2	11.5	19.6

fact that Kasuya soil has the heighest available phosphate (Table 1). Phosphorus concentration in stem tissue was slightly higher in inoculated plants than that in uninoculated plants. However, phosphate fertilization did not always cause an increase in phosphorus concentration of tissue.

Experiment II.

The Aso soil is high in total phosphate but low in available phosphate, and hence very similar to the Osumi soil used in Experiment I (Table 1).

When phosphate fertilizer was not applied to unsterilized soil, only uninoculated plants showed symptoms of mild phosphorus deficiency. The height of those plants was similar to that of other plants up to 40 days after sowing. Phosphorus deficiency symptoms appeared 20 days after sowing when irradiated soil did not receive phosphate fertilizer. In this soil, inoculated plants recovered growth 50 days after sowing, while growth remained repressed in uninoculated plants (Fig. 1). Dry matter and seed yields are shown in Table 4. Application of phosphate fertilizer increased dry matter and seed yields in irradiated and unsterilized soils. The inoculation of VA mycorrhizal fungus spores caused slight increases in seed yields in irradiated soil without added phosphate fertilizer, and greater increases in irradiated soil given phosphate fertilizer. When phosphate fertilizer was not applied in irradiated soil, seed yields of uninoculated soybean were almost nil. Seed yields of inoculated plants grown in irradiated soil with added phosphate fertilizer were at the same level as the control plants (unsterilized and uninoculated soil with added phosphate fertilizer). In unsterilized soil the inoculation of VA mycorrhizal fungus spores increased the seed yields in phosphate-fertilized soil, but had no effect in the phosphate-unfertilized soil.

Accumulation of phosphorus and nitrogen was reflected by dry matter production (Fig. 2). Accumulation of phosphorus and nitrogen was greater in unsterilized soil than in irradiated soil. The application of phosphate fertilizer and the inoculation of VA mycorrhizal fungus spores increased the accumulation of phosphorus and nitrogen. In soils without added phosphate fertilizer, inoculated VA mycorrhizas contributed to a stimulated uptake of 23 mg of phosphorus per pot, indigenous VA mycorrhizas did

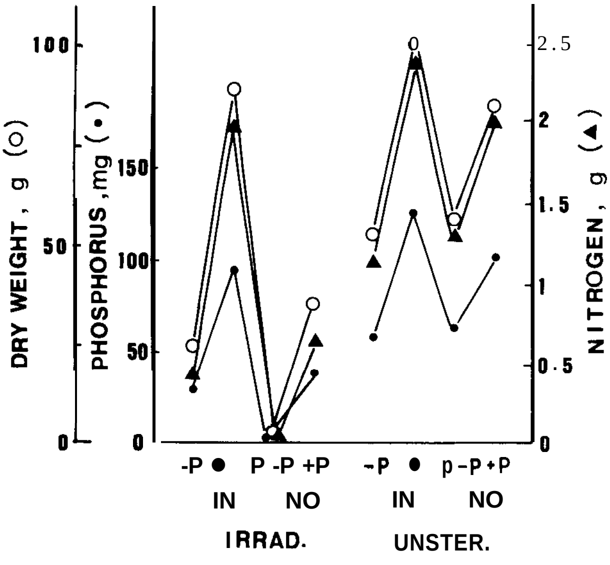

Fig. 2. Effects of vesicular-arbuscular mycorrhizas and phosphate fertilizer on dry weight and phosphorus and nitrogen accumulation in soybean plants in irradiated (IRRAD) or unsterilized (LJNSTER) Aso soil. IN : inoculated plant, NO : uninoculated plant.

to that of 48 mg phosphorus per pot, and inoculated plus indigenous VA mycorrhizas did to that of 43 mg phosphorus per pot. On the other hand, when soils received phosphate fertilizer (260 mg), phosphorus accumulation was increased by 46, 50 and 71 mg phosphorus per pot for inoculated VA mycorrhizas, indigenous VA mycorrhizas and inoculated plus indigenous VA mycorrhizas, respectively. This was because soybean roots alone absorbed 30 mg phosphorus per pot from irradiated and uninoculated soil with added phosphate fertilizer (Fig. 2).

After harvest, the roots were cleaned with 10 % potassium hydroxide and stained with lactophenol and trypan blue after Phillips and Hayman (1977), and then examined microscopically. Vesicules and spores were found in the samples of roots inoculated with VA mycorrhizal fungi or grown on unsterilized soil.

DISCUSSION

The addition of unsterilized soil as an inoculum increased dry matter and seed yields of soybean plants in Kasuya soil and Osumi soil. This was not so in Kashii soil. It is thought that in Kasuya and Osumi soil the indigenous micro-organisms, including VA mycorrhizal fungi improved the nutritional status of plants which were inoculated. In Kashii soil the lack of indigenous VA mycorrhizal fungi might be responsible for the failure of growth stimulation by addition of unsterilized soil inoculum because this soil was not arable.

When phosphate fertilizer was not applied to the irradiated Osumi soil (low in available phosphate), inoculation was indispensable for continuous (from germination to harvest) growth. This suggests that inoculation could promote the plant uptake of phosphate from the soil. Addition of large amounts of phosphate to soils increases the available phosphate, and is known to decrease mycorrhizal infection (Mosse, 1973). In the present experiment the effects of inoculation were found even when phosphate fertilizer was applied to Kasuya soil (high in available phosphate) and Osumi soil (low in available phosphate). Therefore, it is considered that the low rate of phosphate fertilizer application did not affect mycorrhizal infection in these soils. Even when phosphate fertilizer was applied, plant growth was poorer in Osumi and Kashii soils than in the Kasuya soil. Phosphorus concentrations in the stem and pod shell of plants grown in Osumi and Kashii soils were considerably lower than those of plants in Kasuya soil. It was supposed that the limited sources of phosphorus were not adequate to provide sufficient nitrogen-fixing activity by nodules in order to maintain vigorous plant growth in these soils. The greatest nitrogen accumulation of plants occurred in Kasuya soil. This shows that nitrogen-fixing activity was maintained in this soil during the growth period, due to relatively high supply of phosphorus.

In Experiment I, a possible contribution of micro-organisms other than VA mycorrhizal fungi cannot be excluded because unsterilized soil was used as propagules for VA mycorrhizal fungi. Inoculation of VA mycorrhizal fungus spores, however, clearly stimulated growth and increased seed yields in the Aso soil, which is similar to Osumi soil in its chemical properties. This increment was larger with the application of phosphate fertilizer. Hence we think that the function of VA mycorrhizas was apparent in Aso soil due to enhanced uptake of phosphate from the soil. The Aso soil was low in available phosphate in spite of a high total phosphorus content, and did receive a low rate of phosphate fertilizer in this experiment. These conditions might have had a favorable effect on VA mycorrhizal colonization and may have caused increased seed yields in the Aso soil.

Inoculation of VA mycorrhizal fungus spores increased the seed yields in unsterilized Aso soil which received phosphate fertilizer. This was not so in unsterilized soil which did not receive phosphate fertilizer. This suggests that inoculated fungi may be inactive or less active than indigenous fungi when the soil does not receive phosphate fertilizer, and that inoculated fungi may become more effective than indigenous fungi when phosphate fertilizer is applied.

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