Interaction of VAM Fungi and Rhizobium Bacteria on *Pisum sativum* in Visonta Mine Spoils

I. VÖRÖS, K. KÖVES-PÉCHY and J. SZEGI

Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences, Budapest

The vesicular-arbuscular endomycorrhizal fungi (VAM) are obligate symbionts with the roots of most legumes, and promote the water and nutrient uptake of the plants (MOSSE, 1957; GERDEMANN, 1964, 1972; NICOLSON, 1967). Over 100 species belonging to six genera (*Acaulospora, Entrophosphora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora*) of the *Zygomycetes* family are known to occur in symbiotic association (MORTON & BENNY, 1990).

The hyphae elongated from the roots perform certain root hair functions, e.g. by increasing the absorptive surface, sometimes by as much as 80- to 100-fold, and by penetrating the soil far beyond the zone depleted of nutrients by the plant roots. VA mycorrhizae may also utilize the nutrients of larger soil volumes. This effect is usually registered mostly as an increase in the phosphorus and amino acid contents of the plants, or as an increase in crop yield (MOSSE, 1957; GERDEMANN, 1964; SIEVERDING, 1991).

In the developed countries, research on VA mycorrhizal fungi is well established and wide-ranging, while in Hungary, it has only been underway for 2-3 years.

The Rhizobium - legume symbiosis effect is already well-known. Rhizobium bacteria are able to take up atmospheric nitrogen and transfer it to the plant; therefore, as the result of Rhizobium inoculation, a yield increase and N surplus can be achieved. According to literary data, VAM fungi have a favourable effect on plants, not only individually, but also by promoting the success of each other (BAREA et al., 1988; TORREGROZA et al., 1991).

The present examinations were conducted with three different objectives:

1. To study the role played by endomycorrhizal fungi in successfully promoting the recultivation of industrially damaged areas.

2. To study how phosphorus and nitrogen fertilizers can be replaced by environmentally sound technologies.

3. To study the VAM - Rhizobium-legume tripartite symbiosis and its interactions.

Materials and Methods

Rocks originating from the upper levels of the Visonta (Hungary) lignite layer, exploited by open-cut mining, are being brought to the surface where they occupy the place of the chernozem brown forest soil originally found there. These spoils do not contain any toxic substances, but they need recultivation in both the environmental and agricultural sense.

After 8 years of recultivation on two types of Visonta dump soils (Pannonian yellow clay and yellow sand) investigations were made on the frequency (F%) of mycorrhizal fungi occurring on the roots of *Pisum sativum*, the inoculation efficiency with *Rhizobium leguminosarum*, and the interaction of the two types of symbiosis on the dry matter production.

The experiments were set up in the spring of 1983 in a field trial. Samples were put into standard concrete well tubings of 80 cm diameter, sunk into the spoil banks to a depth of 100 cm. Both types of dump soils, alone and covered to a thickness of 60 cm with the original soil, were included. The recultivation treatments were as follows:

1. Control;
2. NPK fertilizer;
3. NPK + lignite;
4. Sewage sludge, 10 t/ha;
5. Sewage sludge, 40 t/ha.

In the fertilized variants, NPK was applied at rates of 400 kg N/ha, 200 kg K_2O/ha and 200 kg P_2O_5/ha, while 1.6 tons/ha lignite grist was added as well.

The test plant was sweet sorghum and the crop (plant) was worked into the spoils as green manure, except in the cases of the untreated control and the sewage sludge-treated variants. The NPK fertilization was repeated every autumn, and 240 kg N/ha, 120 kg P_2O_5/ha and 120 kg K_2O/ha fertilizers were applied in variants 2. and 3. Lignite grist was also added to variant 3 at a rate of 1.2 t/ha/year. The communal sewage sludge treatments were carried out in 1983 and 1985. Subsequently, wheat was cultivated with maize as a second crop in certain years. Later on, barley and maize were sown.

In the autumn of 1990, NPK was applied to the fertilized variants at a rate of 120 kg P_2O_5/ha and 120 kg K_2O/ha, with only 46 kg N. Then, in the spring of 1991, peas were sown. In every variant both Rhizobium-inoculated and non-inoculated treatments were applied.

After harvesting the peas, the plant production (dry matter weight of the total aboveground plant parts) was measured, and the roots of 5 plants per treatment were assessed using the aniline blue staining technique (KRJUEGER et al., 1968). The F% value was determined microscopically according to the method of PHYLLYPS and HAYMAN (1970).

Results

The air-dried weights of peas cultivated on the spoils are demonstrated in Table 1, while the endomycorrhizal infection (F%) on the roots can be seen in Table 2.

Table 1
Air-dried weight of *Pisum sativum* in mine spoils, g/pot

Treatment	Thickness of soil cover, cm	Type of mine spoils			
		Yellow Clay		Yellow Sand	
		uninocu-lated	inocu-lated	uninocu-lated	inocu-lated
Control	-	120	240	320	520
	60	230	380	350	480
NPK	-	220	360	380	620
	60	180	380	340	600
NPK+ lignite	-	190	500	170	440
	60	180	220	160	520
Sewage sludge					
10 t/ha	-	960	960	800	1020
40 t/ha	-	1140	1220	790	1000

Table 2
VAM fungal infection frequency (F%) on the roots of *Pisum sativum* in mine spoils

Treatment	Thickness of soil cover, cm	Type of mine spoils			
		Yellow Clay		Yellow Sand	
		uninocu-lated	inocu-lated*	uninocu-lated	inocu-lated*
Control	-	100	100	98.1	93.3
	60	90.5	100	96.7	100
NPK	-	83.3	100	50	79.1
	60	90.4	100	85.1	68.9
NPK + lignite	-	96.7	58	23.3	96.6
	60	45.5	95.3	30	20
Sewage sludge					
10 t/ha	-	100	100	100	96.7
40 t/ha	-	100	93.3	100	100

$LSD_{5\%}$ = 16; * inoculated with *Rhizobium leguminosarum*

The following conclusions can be drawn from the results:

Rhizobial inoculation enhanced the plant production in all cases, both in the control and fertilized variants.

For pea, crop yields were higher in yellow sand than in the yellow clay. The dry weight was not increased in any of the recultivated variants by topsoil covering, compared to natural spoils treated in the same manner.

For yellow clay, the mycorrhizal infection frequency (F%) was fairly high; the endomycorrhizal frequency was generally improved by rhizobial inoculation. The F% value in the spoil control was also high, though this was significantly decreased by fertilization and especially by the lignite treatment.

The dry matter production of pea was primarily affected by rhizobial inoculation and only secondarily by the treatment and the spoil type. The influence of spontaneously occurring VAM infection on the yield production was pushed into the background in this experiment.

The positive effect of sewage sludge treatments on the yield was so strong that it masked the effect of both VA mycorrhizal fungi and Rhizobium inoculation. On the other hand, the highest values of F% could be observed in these treatments.

Summary

The types of mine spoils (yellow sand and yellow clay from Visonta, Hungary were examined after eight years of recultivation for the dry matter production of *Pisum sativum* L. The effect of Rhizobium inoculation, the infection frequency in the VA mycorrhizal fungi (F%) and their interaction were considered in the following treatments: control, NPK fertilizer, NPK + lignite, sewage sludge (10 t/ha and 40 t/ha), the spoils were either uncovered or covered with the original topsoil (chernozem brown forest soil).

It was established that Rhizobium inoculation increased the plant dry weight in all cases both in the controls and in treatments with various fertilizers. The infection frequency with VA mycorrhizal fungi was relatively high in clay mine spoils, becoming higher after Rhizobium inoculation.

The F% data for sandy mine spoils were the same in the control and less in the case of fertilizer and lignite treatments, compared to the clay spoils.

The dry matter production of peas was affected mostly by Rhizobium inoculation and less influenced by the treatments and the type of mine spoils. The role of spontaneous VA mycorrhizal infection (F%) in the yield was not significant in this experiment.

The sewage sludge treatment enhanced the yield to such an extent that the effect of spontaneous VAM fungi and Rhizobium inoculation was marked. On the other hand, the highest values of F% could be observed in these treatments.

References

BAREA, J. M., AZCON-AQUILAR, C. & AZOON, R., 1988. The role of mycorrhiza in improving the establishment and function of the Rhizobium-legume system under field condition. In: Nitrogen Fixation By Legumes in Mediterranean Agriculture. (Eds.: BECK, D. P. & MATERON, L. A.). 153-162. Martinus Nijhoff Publ., Dordrecht, Boston, Lancaster.

GERDEMANN, J. W., 1964. The effects of mycorrhiza on the growth of maize. Mycologia. **56.** 342.

GERDEMANN, J. W., 1972. The significance of VA mycorrhizae in plant nutrition. In: Root Diseases and Soilborne Pathogens. (Eds.: TOUSSOUN, T. A., BEGA, R. V. & NELSON, P. E.). Univ. California Press. Berkeley.

KRJUEGER, L. et al., 1968. Uchenie zapiski Permskogo pedagogicheskogo instituta. Perm. 69-80.

MORTON, J. B. & BENNY, G. L., 1990. Revised classification of arbuscular mycorrhizal fungi (Zigomycetes): A new order, Glomales, two new suborders, Glomineae and Gigasporineae, and two new families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomaceae. Mycotaxon. **37.** 471-491.

MOSSE, B., 1957. Growth and chemical composition of mycorrhizal and non-mycorrhizal apples. Nature. London. **179.** 922.

NICOLSON, T. H., 1967. VA mycorrhiza - a universal plant symbiosis. Sci. Prog. Oxford. **53.** 561.

PHYLLYPS, J. M. & HAYMAN, D. S., 1970. Improved procedure for declearing and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. **55.** 158-161.

SIEVERDING, E., 1991. Vesicular-Arbuscular Mycorrhiza Management in Tropical Agrosystems. Eschborn.

TORREGROZA, E. et al., 1991. Double symbiosis in woody legumes (Water stress-Proline analyses) In: Third European Symp. on Mycorrhizas. Mycorrhizas in Ecosystems - Structure and Function. Sheffield, August 19-23. Abstracts.