Investigation of Natural Rhizobial Populations in Hungarian Soils, Demonstrated on Four Leguminous Plants

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The biological fixation of atmospheric nitrogen is influenced by numerous biotic and abiotic factors. Among the abiotic factors, the activity of microorganisms is greatly influenced by the physical and chemical properties of the soil, the temperature, pH, moisture content, oxidation-reduction potential, quantity and quality of macro- and microelements and the toxicity. For this reason, the soil, as an environment, is very important in the rhizobial inoculation of legumes, because the Rhizobium is closely connected with it /HAMDI, 1982/. The inoculated population - which is only able to multiply within a definite interval - comes into antagonism with the indigenous microbe community, which has already been adapted to the environmental conditions for some time. So, among the valuable microbe communities artificially introduced into the soil, there is permanent competition in the continuously changing environment around them. The capability for competition is defined by the biochemical potential, ecological tolerance, and geno- or phenotypic plasticity of the species /SZENDE, 1987; BEATTIE, 1988/. As symbiotic nitrogen fixation is influenced to a large extent by different soil types, it would seem reasonable to characterize the original Rhizobium populations of the major soil types in a national survey and to investigate the possibility of combining rhizobial inoculation with a simple agrochemical technique /SZEGI et al., 1984; BAKONDI--ZÁMORY et al., 1986/. Within the framework of a five-year work programme, investigations have been carried out on spontaneous infection. In addition, the efficiency of the BAKTOLEG inoculum preparated at the Research Institute for Soil Science and Agricultural Chemistry has been tested on non-fertilized soils and on soils with optimum contents of different macro- and microelements /KÖVES-PÉCHY et al., 1988/. The results of the first 3 years of this programme are reported here.

Materials and methods

Soil samples were collected from different sites in Hungary, from areas which have not been fertilized, or only to a slight extent, in recent years.

The sites and soil types investigated were as follows: 1. Calcareous sandy soil /Őrbottyán/; 2. Ramann's brown forest soil /Keszthely/; 3. Brown forest soil with clay illuviation /Kenyeri/; 4. Brown forest soil with clay

illuviation /Putnok/; 5. Brown forest soil with clay illuviation /Zalaegerszeg/; 6. Brown forest soil /Bicsérd/; 7. Brown forest soil /Eszterág/; 8. Chernozem brown forest soil /Kompolt/; 9. Calcareous chernozem soil /Nagyhörcsök/; 10. Lowland chernozem soil /Nagyszénás/; 11. Chernozem with forest residuals /Martonvásár/; 12. Chernozem soil /Debrecen/; 13. Meadow chernozem soil /Békéscsaba/; 14. Meadow solonetz soil /Karcag/; 15. Calcareous meadow soil /Agyagosszergény/; 16. Alluvial meadow soil /Magyaróvár/.
The soils were utilized after physical and chemical analyses.

From the 16 soil samples collected, pot trials were set up in a plant growth chamber. The experiment was carried out using Vincent's method /VINCENT, 1970/. The soils were put into 2 kg plastic pots with holes in the bottom and a lower layer of 200 g of gravel for aeration and water drainage. Before use, the soils were screened through a 5 mm ∅ mesh under air-dried conditions. The test plants used were lucerne /species Nagyszénás/, peas /Rhenish Dwarf/, horse-beans /Lippó/ and soybeans /Ewans/. Based on the physical and chemical analyses of the soil samples and on literary data /BUZÁS and FEKETE, 1979/, a soil enriched with nutrients was selected. In each case, a nitrogen-free fertilizer was mixed into 50% of the soil samples.

The peat-based BAKTOLEG inoculum was used, containing $5x10^8$/g of Rhizobium bacterium. 50% of the seed was treated with the inoculum, so inoculated and non-inoculated variants were obtained. This meant that the experiment involved testing about 350 pots per year, because for each soil there were inoculated and non-inoculated variants, treated or non-treated with mineral nutrients, with 4 different legumes, in four replications:
1. Control; 2. Rhizobium inoculation /with peat-based BAKTOLEG inoculum/;
3. Additional fertilization /with N-free macro- and microelements/ and
4. Additional fertilization + Rhizobium inoculation.

The pot soil was moistened first after additional fertilization and then, one day before sowing to 65% water capacity. 21 lucerne seeds were planted per pot at a depth of 0.5 cm, while 7 peas, horse-beans and soybeans were planted per pot at a depth of 1-1.5 cm. After moistening with distilled water, the seeds of non-inoculated variants were treated with sterile peat, whereas in the inoculated variants lucerne was inoculated with R. meliloti, peas and horse-beans with R. leguminosarum and soybeans with Bradyrhizobium japonicum. The plants were watered as required using distilled water.

Results

After an incubation period of 8-9 weeks, the following parameters were investigated:
1. Dry matter production /g per 100 plants/;
2. N content as a % of dry matter;
3. Nodule number /per 100 plants/;
4. Acetylene reduction activity /ARA/ nmol C_2H_4/24 hours/100 plants.
The present paper contains the results obtained for points 1 and 2.

The plants were selected randomly for evaluation: 15 plants per pot for lucerne, and 5 plants per pot for peas, horse-beans and soybeans. The shoots were dried at 40 °C and measured using an analytical balance. The nitrogen content of the plants was determined by the Kjeldahl method.

In the case of lucerne /Table 1/ without fertilization, a rhizobial inoculation effect was observed, between the limits of 2-68%, in most of the 16 soils tested. A significantly higher dry matter production was obtained after inoculation on two chernozem soils /Békéscsaba: 68.5% and Kompolt: 30.2%/ and a calcareous sandy soil /Őrbottyán: 29.2%/. A brown forest soil with clay illuviation /Putnok/, a calcareous chernozem /Nagy-

Table 1

Changes in dry matter weight and N % of lucerne as the result of rhizobial inoculation and PK fertilization, in different soil types

Soil site	Dry matter /g/ per 100 plants				N %			
	non-fertilized		fertilized		non-fertilized		fertilized	
	non-in-oculated	inoc-ulated	non-in-oculated	inoc-ulated	non-in-oculated	inoc-ulated	non-in-oculated	inoc-ulated
1. ŐRBOTTYÁN	32.04	41.39^{x}	42.41	37.46	2.82	2.61	2.84	2.89
2. KESZTHELY	31.94	27.25	32.99	33.54	3.33	3.55	2.30	3.62^{x}
3. KENYERI	17.77	12.15	38.42	36.00	2.66	2.74	3.55	3.83
4. PUTNOK	14.86	18.72	21.05	24.81	3.39	4.85^{x}	4.68	4.68
5. ZALAEGERSZEG	4.82	4.45	20.43	20.37	5.48	6.68^{x}	5.18	4.70
6. BICSÉRD	26.65	27.47	36.45	30.22	3.82	2.98	3.81	4.00
7. ESZTERÁG	22.56	23.02	27.15	28.07	3.72	3.02	3.30	3.98
8. KOMPOLT	19.36	25.20^{x}	24.85	35.73^{x}	4.05	4.35	4.60	4.25
9. NAGYHÖRCSÖK	28.39	29.83	30.94	38.39^{x}	4.25	3.35	4.57	3.53
10. NAGYSZÉNÁS	6.99	10.53	11.75	14.93	3.94	4.07	3.92	3.49
11. MARTONVÁSÁR	23.65	26.78	24.12	36.91^{x}	4.97	5.20	4.93	4.88
12. DEBRECEN	25.79	21.72	21.06	16.10	5.63	6.10	5.40	5.78
13. BÉKÉSCSABA	9.85	16.60^{x}	14.80	17.54	3.70	4.19	4.21	4.52
14. KARCAG	22.68	26.12	25.41	23.65	6.29	6.20	5.83	6.48
15. AGYAGOSSZERGÉNY	16.82	15.51	17.43	18.55	2.11	2.83	3.14	3.05
16. MAGYARÓVÁR	6.65	8.43	15.91	19.88	2.18	2.45	3.40	3.55
$P < 0.5$		4.91				0.8		

x = a significant difference originated from rhizobial inoculation

/-/ = a significant difference originated from fertilization

Table 2

Changes in dry matter weight and N % of peas as result of rhizobial inoculation and PK fertilization, in different soil types

Soil site	Dry matter /g/ per 100 plants				N %			
	non-fertilized		fertilized		non-fertilized		fertilized	
	non-in-oculated	inoc-ulated	non-in-oculated	inoc-ulated	non-in-oculated	inoc-ulated	non-in-oculated	inoc-ulated
1. ŐRBOTTYÁN	117.63	122.51	129.84	144.63	3.04	4.45x	3.27	4.66x
2. KESZTHELY	118.10	113.66	136.51	124.95	3.61	3.72	3.54	3.28
3. KENYERI	54.99	75.52x	68.22	108.25x	2.45	3.02	2.69	1.88
4. PUTNOK	32.55	34.85	34.05	38.85	4.68	4.80	4.85	4.70
5. ZALAEGERSZEG	35.35	28.10	41.45	54.60	5.78	4.20	5.88	5.88
6. BICSÉRD	86.27	92.15	141.22	96.46	3.47	2.86	3.14	3.72
7. ESZTERÁG	75.67	104.32x	82.20	64.84	3.08	2.95	2.38	2.98
8. KOMPOLT	73.96	63.28	75.95	139.38x	5.17	4.75	5.48	4.70
9. NAGYHÖRCSÖK	91.56	102.74	87.92	98.03	2.65	1.74	2.09	2.44
10. NAGYSZÉNÁS	63.40	113.75x	80.80	124.70x	5.16	4.14	4.93	4.32
11. MARTONVÁSÁR	78.75	80.65	91.65	97.57	5.28	4.23	5.20	5.08
12. DEBRECEN	40.75	43.05	39.15	35.95	5.48	5.85	5.68	6.03
13. BÉKÉSCSABA	89.35	102.75	49.15	91.44x	4.19	4.51	4.81	3.54
14. KARCAG	41.35	37.15	36.95	36.70	6.25	6.50	6.28	5.53
15. AGYAGOSSZERGÉNY	72.20	74.86	41.59	94.89x	2.20	2.90	1.66	2.18
16. MAGYARÓVÁR	63.90	53.52	92.52	91.27	2.66	2.36	3.29	3.59
P < 0.5			19.5			1.0		

x = a significant difference originated from rhizobial inoculation
/-/ = a significant difference originated from fertilization

Table 3

Changes in dry matter weight and N % of horse-beans as result of rhizobial inoculation and PK fertilization, in different soil types

Soil site	Dry matter /g/ per 100 plants				N %			
	non-fertilized		fertilized		non-fertilized		fertilized	
	non-in-oculated	inoc-ulated	non-in-oculated	inoc-ulated	non-in-oculated	inoc-ulated	non-in-oculated	inoc-ulated
1. ŐRBOTTYÁN	428.01	345.83	405.54	444.61	2.92	3.20	2.95	3.00
2. KESZTHELY	322.26	318.34	380.77	392.90	3.15	3.53	4.18	3.46
3. KENYERI	210.15	209.12	417.22	354.12	1.90	2.71^x	2.70	2.73
4. PUTNOK	195.75	175.25	239.10	251.10	4.41	4.33	4.63	4.75
5. ZALAEGERSZEG	84.45	106.60	184.70	178.00	3.72	4.23	4.08	4.35
6. BICSÉRD	354.05	360.45	340.30	428.40^x	2.73	3.22	3.14	2.80
7. ESZTERÁG	334.10	332.80	363.72	418.27^x	3.19	3.15	3.62	3.27
8. KOMPOLT	346.75	376.35	457.25	526.50^x	4.42	4.24	4.72	4.70
9. NAGYHÖRCSÖK	282.06	258.25	432.23	407.20	2.88	2.68	2.59	3.07
10. NAGYSZÉNÁS	182.85	245.60^x	168.60	246.65^x	2.89	2.44	2.97	2.54
11. MARTONVÁSÁR	323.10	326.00	414.85	444.50	4.08	4.18	4.05	4.06
12. DEBRECEN	341.25	307.15	299.00	345.00	4.67	4.90	4.80	4.98
13. BÉKÉSCSABA	227.10	234.10	198.70	261.60^x	2.53	2.82	3.00	3.24
14. KARCAG	354.35	350.85	366.00	391.80	4.73	4.80	5.05	5.48
15. AGYAGOSSZERGÉNY	216.55	219.70	321.26	290.45	2.07	2.35	2.13	2.58
16. MAGYARÓVÁR	247.15	216.60	328.37	317.77	1.91	2.22	2.16	2.06
$P < 0.5$		46.6				0.7		

x = a significant difference originated from rhizobial inoculation
/-/ = a significant difference originated from fertilization

Table 4

Changes in dry matter weight and N % of soybeans as result of rhizobial inoculation and PK fertilization, in different soil types

Soil site	Dry matter /g/ per 100 plants				N %			
	non-fertilized		fertilized		non-fertilized		fertilized	
	non-in-oculated	inoc-ulated	non-in-oculated	inoc-ulated	non-in-oculated	inoc-ulated	non-in-oculated	inoc-ulated
1. ŐRBOTTYÁN	210.00	332.70^{x}	255.19	362.79^{x}	2.80	3.24	3.49	3.13
2. KESZTHELY	185.39	302.68^{x}	191.14	297.87^{x}	2.13	3.21^{x}	2.43	3.47^{x}
3. KENYERI	201.27	179.47	114.39	240.77^{x}	1.68	2.68^{x}	1.94	2.38
4. PUTNOK	189.55	289.75^{x}	296.45	431.30^{x}	2.00	2.90^{x}	1.93	4.33^{x}
5. ZALAEGERSZEG	144.00	139.10	296.35	262.90	3.00	3.70^{x}	2.65	4.28^{x}
6. BICSÉRD	231.94	253.59	298.70	256.60	2.66	2.89	2.69	2.67
7. ESZTERÁG	310.52	276.60	359.00	349.92	3.16	3.24	3.68	2.86
8. KOMPOLT	348.95	469.30^{x}	245.50	727.35^{x}	3.15	4.21^{x}	1.70	4.48^{x}
9. NAGYHÖRCSÖK	275.66	281.31	171.32	328.30^{x}	3.50	3.06	3.04	3.57
10. NAGYSZÉNÁS	110.81	122.61	121.66	147.08	1.75	4.02^{x}	1.76	4.42^{x}
11. MARTONVÁSÁR	493.40	527.50	603.80	700.60^{x}	3.33	3.83	4.28	4.63
12. DEBRECEN	261.40	305.45	225.10	267.60	2.05	4.33^{x}	2.05	4.03^{x}
13. BÉKÉSCSABA	132.33	148.66	122.91	136.98	3.71	4.17	3.85	3.76
14. KARCAG	180.35	298.55^{x}	168.15	330.35^{x}	1.98	5.65^{x}	2.10	5.38^{x}
15. AGYAGOSSZERGÉNY	176.00	141.85	158.50	276.55^{x}	1.37	2.27^{x}	2.23	2.14
16. MAGYARÓVÁR	160.25	147.95	145.85	206.63	1.87	2.48^{x}	2.44	3.26^{x}
$P < 0.5$	70.9				0.6			

x = a significant difference originated from rhizobial inoculation
/–/ = a significant difference originated from fertilization

szénás/ and a meadow solonetz soil /Karcag/ also gave good results. In 10 cases, the yield of lucerne was significantly increased by fertilization. When this was combined with rhizobial inoculation, a significantly higher dry matter production was obtained in 9 soils. The effectiveness of rhizobial inoculation could not be observed in the following cases: in a brown forest soil with clay illuviation /Kenyeri/, in a chernozem /Debrecen/ where the original N content of the soil was high, and in another brown forest soil with clay illuviation /Zalaegerszeg/ where, on the other hand, the pH value of the soil was very low /3.5/, possibly preventing inoculation from being effective. In the case of lucerne the nitrogen content was significantly increased by rhizobial inoculation in two soils; when combined with fertilization, the effect was only significant in one case.

Experiments with peas /Table 2/ showed a significant effect of rhizobial inoculation in three soils: to the greatest extent in a calcareous chernozem soil /Nagyszénás, 79.3%/ and also in two brown forest soils /Kenyeri, Eszterág 37%/. In the case of 5 soils, rhizobial inoculation combined with fertilization showed a positive significant difference: e. g. in a calcareous chernozem /Nagyszénás 96.0%/, in a brown forest soil /Kompolt 88.3%/, in a meadow chernozem /Békéscsaba 86.6%/. In a meadow chernozem soil /Agyagosszergény/ the dry matter production was increased by 31.4%. Rhizobial inoculation caused a significant increase in the % nitrogen content of pea plants both with and without fertilization in a calcareous sandy soil /Őrbottyán, 46.3% and 53.2% respectively/.

When horse-beans were the test plant /Table 3/ the yield was significantly increased by rhizobial inoculation without fertilization in a calcareous chernozem soil /Nagyszénás, 33.9%/. With fertilization, the dry matter value after inoculation was significantly higher in 5 soils. These are as follows: chernozem brown forest soil /Kompolt 51.9%/, calcareous chernozem /Nagyszénás 34.9%/, brown forest soil /Bicsérd 25.2%/, loess brown forest soil /Eszterág 25.2%/ and meadow chernozem /Békéscsaba 15.2%/. The effect of additional fertilization was the most promising for horse bean plants. After rhizobial inoculation, the yield was significantly increased in 14 out of 16 soil samples compared to the similarly inoculated, but non-fertilized variants. The nitrogen content values were not increased either by rhizobial inoculation or by fertilization in the majority of cases.

For soybeans /Table 4/ rhizobial inoculation led to a significant increase in yield without fertilization /meadow solonetz soil Karcag 65.1%, Ramann's brown forest soil Keszthely 63.3%, calcareous sandy soil, Őrbottyán 58.4%; chernozem brown forest soil, Putnok 52.7%, and chernozem brown forest soil Kompolt 34.5%/ and in 9 soils after fertilization.

Examples of the significantly higher yield obtained when fertilization was combined with rhizobial inoculation, compared to the non-fertilized, rhizobium-inoculated variant, are a calcareous meadow soil, Agyagosszergény 57.1%; chernozem brown forest soil, Kenyeri 19.6%; and calcareous chernozem, Nagyhörcsök 19.1%.

In non-fertilized variants, the nitrogen content showed a significant increase /73.7% on average/ compared to the control due to the effect of rhizobial inoculation. When combined with fertilization, this value showed an average increase of 97.8%.

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