THE EFFICIENCY OF LEGUMINOUS SEED TREATMENT WITH NODULE BACTERIA, DIFFERENT SELECTED STRAINS, COMPARATIVELY TO NATURAL SYMBIOTIC ONES

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ABSTRACT

The paper presents the results of a study carried out in 1995 in a 11 experimental fields of different pedo-climatic conditions, concerning the influence of the natural symbiotic bacteria on the bacterization treatment of leguminous seeds. The experiments were conducted on soybean, pea and bean crops. "Infection plant" technics were used for counting the most probable number of specific rhizobia from organized competition trials using 6 strains for each of *Rhizobium leguminosarum* biovar. *viciae, Rh. leg.* biovar. *phaseoli* and *Bradyrhizobium japonicum.* The quality and the quantity of biopreparations for seed bacterization may be better established for a certain leguminous crop, taking into account the abundance and effectiveness of natural specific rhizobial population.

Key words: leguminous seed bacterization; soil native rhizobia.

INTRODUCTION

The action of leguminous seed bacterization is an exercise in applied bacterial ecology and its prime objective is the nodulation of the host plant (Brockwell, 1977).

For obtaining an efficient biopreparation, a number of qualitative and quantitative conditions should be accomplished:

the selected *Rhizobium* strains must be more competitive than the naturalized rhizobia for nodulating root hairs;

to survive under different environmental conditions;

to prove genetic stability and a high level of effectiveness of the atmospheric nitrogen fixation.

Rhizobium number for a good treatment with Nitragin must be at least 100-1000 bacteria per seed (Date *et al*, 1965). A severe competition by rhizobia already existing in soil may demand 100,000-1 million selected bacteria per seed (Ireland and Vincent, 1968). For this purpose, Weaver and Frederick (1974) demonstrated that the number of bacteria given on the seeds has to be 1000 fold superior to the number of natural specific bacteria existing in 1 g soil, to form only 50% of nodules. The interest for the presence and effectiveness of natural rhizobia was shown by several researches: Keyser et al.,1982; Balatti and Pueppre, 1992; Cattelan and Hungria, 1995. The aim of the present study consists in the appreciation of the efficiency of some selected *Rhizobium* and *Bradyrhizobium* strains comparatively to natural specific symbiont bacteria in soil.

MATERIALS AND METHODS

The trials were conducted in 1995, in the laboratory and green-house on nutritive media and sterilized sand and in the fields of Agricultural Experimental Stations, on different soil types: Fundulea - Cãlãraºi, Vãceni - Teleorman, Caracal - Olt, Secuieni - Neamþ and Podu-Iloaiei - Iaºi (on cambic chernozem); Livada - Satu Mare and Oradea - Bihor (on brown luvisol), Valu lui Traian - Constanþa (vermi-calcaro-calcic chernozem); Albota - Argeº (albic luvisol).

Six bacterial strains, for each leguminous crop, were prepared in laboratory, on YEMA medium, as follows: *Rhizobium leguminosarum* biovar. *viciae* for *Pisum sativum, Rh. leg.* biovar. *phaseoli,* for *Phaseo-*

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lus vulgaris and *Bradyrhizobium japonicum*, for *Glycine max*.

The method used for counting natural rhizobia was "plant dilution" technics, initiated by Wilson (1926), Berezova et al. (1955) and detailed by Vincent (1970). The "plant dilution" technique consists in treating with 1 ml of different soil dilutions the desinfected seeds of leguminous plants, germinated on filter paper under aseptic conditions. Each soil sample was diluted from 10^{-1} to 10^{-6} and two replicates for each dilution and two replicates for each leguminous plant were performed. After three weeks, the presence of nodules on the roots was noted. The number of specific *Rhizobium* sp. was calculated by the method of "the most probable number" in one gramme of desiccated soil.

RESULTS AND DISCUSSIONS

The estimation made by using the "plant infections" technique are given in table 1 and can be easely characterized using the standard appreciations of Vincent and Humphrey (1971) given in table 2.

Table 1. Number of *Rhizobium leguminosarum* biovar. *viciae* and *Bradyrhizobium japonicum*, native in different soils, determined by "plant infections" technique

Agricultural Research Station	*Rhizobium leguminosarum* biovar.		*Bradyrhizobium*
	viciae	*phaseoli*	*japonicum*
	Number of bacteria/g soil		
Teleorman	16.250	1,600	34
Caracal	136.400	3,310	112
Podu Iloaiei	8.284	0	0
Valu lui Traian	118	0	356
Oradea	70	0	0
Livada	0	0	0
Turda	373	0	0

Table 2. Appreciation of *Rhizobium* sp. abundance in soil native microflora (by Vincent and Humphrey, 1971)

Approximate number of bacteria	Estimation
< 25	absent or rare
25-1000	few
1000-100,000	abundant
> 100,000	very abundant

Generally, *Rhizobium leguminosarum* biovar. *viciae* bacteria are widely distributed, ranging between 70 per g of soil in Oradea field and 136,400 per g of soil in Caracal field. *Rhizobium leguminosarum* biovar. *phaseoli* bacteria are abundant in Oradea, Caracal and Teleorman fields and *Bradyrhizobium japonicum* bacteria are only few in Teleorman and Caracal fields and absent in the other experimental fields (Podu Iloaiei, Valu lui Traian, Oradea, Livada and Turda).

The counting data obtained by using the "plant infection" technique were sustained by nodule formation control in the plots where the seeds were not treated with *Rhizobium* and *Bradyrhizobium* bacteria.

Considering the minimum standard number of rhizobia per gramme of soil, needed for a good nodulation, ranging between 100 and 1000 (Date et al.,1995) and the optimal level for a good nodulation ranging between 100,000 and 1 million (Ireland and Vincent,1968), the position of the estimates for different soil conditions can be easely observed.

Rhizobium leguminosarum biovar. *viciae* natural populations, detected in the fields of Caracal, Podu Iloaiei, Teleorman and Turda and *Rhizobium leguminosarum* biovar. *phaseoli* natural populations in the fields of Teleorman, Caracal and Oradea overcome the minimum standards for a good nodulation. *Bradyrhizobium japonicum* population is absent or markedly deficient for all the studied fields. Bacterization of soybean seeds is a necessity.

The competition between different rhizobial strains and the success or failure of bacterization reported to uninoculated plants (control plots), expressed in q/ha seed yield, are given in tables 3, 4 and 5. In several cases, the seed yield obtained after bacterization of soybean, pea and bean seeds has overcame significantly the uninoculated variant (control).

For soybean crop, the strains SO 3407 (the best in 5 from 6 fields), SO 7and SO 618 (the best in 4 from 6 fields), were the most efficient.

For pea crop, the strains MZ 809 (the first in 6 from 7 fields), MZ 217 and MZ 804 (the first in 5 from 7 fields) were the most efficient. For Caracal field, pea bacterization was not efficient, because the natural *Rhizobium leguminosarum* biovar. *viciae* population was very abundant (over 100,000 rhizobia /g soil). *Rhizobium leguminosarum* biovar. *phaseoli* FL 400 strain proved to be the most efficient, contributing to obtaining the highest yields in 5 from 7 studied fields.

One can observe, comparing the estimates of natural abundance of specific rhizobia and the bacterization effect in different pedoclimatic regions, that the yield increase percents were lower in the fields where natural specific bacteria were in a high number. In these cases, others methods to increase bacterization effectiveness should be tried as selection of more infections symbiotic lines and utilization of new cultural techniques.

Table 3. Influence of some selected strains of *Bradyrhizobium japonicum* on the level of soybean seed yield

SO-strains	Yield (q/ha) and ranking categories					
	Valu lui Traian	Teleorman	Secuieni	Livada	Oradea	Fundulea
Untreated seeds	26.8 c	33.0 c	14.89 c	1.87 b	10.8 c	18.32 b
7	33.8 a	35.3 a	17.33 b	24.6 a	13.4 a	19.27 b
3407	33.0 a	35.2 a	19.09 a	22.o a	12.5 b	20.36 a
618	30.6 b	35.1 a	18.14 a	21.5 b	13.0 a	20.54 a
FR10	30.0 b	36.8 a	16.99 b	25.4 a	13.8 a	20.00 b
3154	29.5 b	34.6 b	16.30 c	22.1 a	12.1 b	22.03 a
USDA122	28.4 c	34.6 b	17.52 b	24.6 a	12.2 b	20.50 a
Limit differences:						
P 5%	1.74*	1.37	0.86	3.6*	1.11*	1.99*
P 1%	2.45	1.87*	1.18*	5.0	1.55	2.73
P 0.1%	3.46	2.55	1.61	6.8	2.11	3.71

Table 4. Influence of some selected strains of *Rhizobium leguminosarum* biovar. *viciae* on the level of pea seed yield

MZ-strains	Yields (q/ha) and ranking categories						
	Teleorman	Turda	Podu Iloaiei	Lovrin	Albota	Caracal	Fundulea
Untreated seeds	31.1 b	39.04 b	16.50 c	16.8 b	8.8 b	26.69	15.20 c
12	31.5 b	45.98 a	17.90 b	18.3 a	15.2 a	27.87	20.03 b
144	33.5 a	42.87 a	15.80 b	18.3 a	14.8 a	26.97	19.85 b
217	33.0 a	43.69 a	18.60 a	18.1 a	14.7 a	25.41	20.45 b
264	33.0 a	39.22b	18.90 a	17.7 a	13.5 a	27.90	19.67 b
804	33.5 a	44.14 a	17.00 c	17.9 a	14.3 a	27.17	21.10 a
809	33.4 a	42.32 a	17.40 a	18.4 a	13.7 a	27.7	23.06 a
Limit differences:							
P 5%	1.32	6.29*	0.90*	1.5*	3.01*	2.074	2.099*
P 1%	1.81*	8.73	1.20	2.0	4.23	2.844*	2.871
P 0.1%	2.47	12.32	1.70	2.7	5.97	3.871	3.919

Table 5. Influence of some selected strains of *Rhizobium leguminosarum* biovar. *phaseoli* on the level of bean seed yield

FL-straits	Yields (q/ha) and ranking categories						
	Teleorman	Turda	Podu Iloaiei	Oradea	Albota	Caracal	Secuieni
Untreated seeds	20.6 b	14.59 b	9.50 c	15.8 d	7.6 b	19.46 b	12.23 c
8	22.3 a	19.77 a	10.70 c	19.4 a	8.8 a	20.33 a	14.83 b
171	22.6 a	19.23 a	14.30 a	16.2 c	8.9 a	20.11 a	15.04 b
247	23.1 a	20.65 a	11.50 b	17.0 b	9.1 a	22.27 a	15.68 a
256	22.4 a	20.33 a	13.00 a	17.2 b	9.5 a	19.35 b	16.20 a
400	22.2 a	17.45 a	14.30 a	17.1 b	9.2 a	21.26 a	15.79 a
Rhph	21.8 a	18.67 a	12.70 a	16.0 c	9.0 a	19.80 b	15.42 a
Limit differences:							
P 5%	1.30	2.76*	1.60*	0.3	1.01	2.332	0.78*
P 1%	1.79*		2.20	0.8	1.64*	3.198*	1.08
P 0.1%	2.43		3.10	1.1*	2.50	4.353	1.47

In Podu Iloaiei and Turda (for bean crop) and Valu lui Traian and Livada (for soybean crop), in the case of high grain yield the increase percent corresponds to a rare or absent population of specific rhizobia. Bacterization in these fields is a necessity.

The determinations of the natural rhizobial abundance offer precious information about the symbiotic bacteria quality and about the number of bacteria destinated for leguminous seeds bacterization. These informations are of a real help in bacterization efficiency improvement.

CONCLUSIONS

Leguminous seed bacterization with selected *Rhizobium* strains contributed in obtaining high level of grain yield increase, in the experimental fields characterized by scarce or absent natural rhizobial populations.

The appreciation of the presence or absence of soil specific *Rhizobium* sp.for certain leguminous crops contributes to the determination of the bacterial culture quantity required for an efficient treatment.

The experiences carried out in different pedoclimatic regions pointed out the most efficient *Rhizobium* strains, specific for pea, bean and soybean crops.

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Table 1. Number of *Rhizobium leguminosarum boivar. viciae* and *Bradyrhizobium japonicus*, native in different soils, determined by "plant infections" technique

Agricultural Research from:	*Rhizobium leguminosarum biovar.*		*Bradyrhizobium japonicum*
	viciae	*phaseoli*	
	Number of bacteria/g soil		
Teleorman	16,250	1,600	34
Caracal	136,400	3,310	112
Podu Iloaiei	8,284	0	0
Valu lui Traian	118	0	356
Oradea	70	0	0
Livada	0	0	0
Turda	373	0	0

Table 2. Appreciation of *Rhizobium sp.*abundance in soil native microflora (by Vincent and Humphrey, 1971)

Approximate number of bacteria	Estimation
< 25	absent or rare
25-1000	few
1000-100,000	abundant
> 100,000	very abundant

Table 3.Influence of some selected strains of *Bradyrhizobium japonicum* on the level of soybean seed yield

SO-strains	Yield (q/ha) and ranking categories					
	Valu lui Traian	Teleoman	Secuieni	Livada	Oradea	Fundulea
Untreated seeds	26,8c	33,0c	14,89c	1,87b	10,8c	18,32b
7	33,8 a	35,3 a	17,33 b	24,6 a	13,4 a	19,27 b
3407	33,0 a	35,2 a	19,09 a	22,o a	12,5 b	20,36 a
618	30,6 b	35,1 a	18,14 a	21,5 b	13,0 a	20,54 a
FR10	30,0 b	36,8 a	16,99 b	25,4 a	13,8 a	20,0 b
3154	29,5 b	34,6 b	16,30 c	22,1 a	12,1 b	22,03 a
USDA122	28,4 c	34,6 b	17,52 b	24,6 a	12,2 b	20,50 a
Limit differences						
P 5%	1.74*	1.37	0.86	3.6*	1.11*	1.99*
P 1%	2.45	1.87*	1.18*	5.0	1.55	2.73
P o,1%	3.46	2.55	1.61	6.8	2.11	3.71

Table 4. Influence of some selected strains of *Rhizobium leguminosarum biovar. viciae* on the level of pea seed yield

MZ-strains	Yields (q/ha) and ranking categories						
	Teleorman	Turda	Podu Iloaiei	Lovrin	Albota	Caracal	Fundulea
Untreated seeds	31.1 b	39.04 b	16.50 c	16.8 b	8.8 b	26.69	15.20 c
12	31.5 b	45.98 a	17.90 b	18.3 a	15.2 a	27.87	20.03 b
144	33.5 a	42.87 a	15.80 b	18.3 a	14.8 a	26.97	19.85 b
217	33.0 a	43.69 a	18.60 a	18.1 a	14.7 a	25.41	20.45 b
264	33.0 a	39.22b	18.90 a	17.7 a	13.5 a	27.90	19.67 b
804	33.5 a	44.14 a	17.00 c	17.9 a	14.3 a	27.17	21.10 a
809	33.4 a	42.32 a	17.40 a	18.4 a	13.7 a	27.7	23.06 a

Limit differences

P 5%	1.32	6.29*	0.90*	1.5*	3.01*	2.074	2.099*
P 1%	1.81*	8.73	1.20	2.0	4.23	2.844*	2.871
P 0.1%	2.47	12.32	1.70	2.7	5.97	3.871	3.919

Table 5. Influence of some selected strains of *Rhizobium leguminosarum biovar. phaseoli* on the level of bean seed yield

FL-straits	Yields (q/ha) and ranking categories						
	Teleorman	Turda	Podu Iloaiei	Oradea	Albota	Caracal	Secuieni
Untreated seeds	20.6 b	14.59 b	9.50 c	15.8 d	7.6 b	19.46 b	12.23 c
8	22.3 a	19.77 a	10.70 c	19.4 a	8.8 a	20.33 a	14.83 b
171	22.6 a	19.23 a	14.30 a	16.2 c	8.9 a	20.11 a	15.04 b
247	23.1 a	20.65 a	11.50 b	17.0 b	9.1 a	22.27 a	15.68 a
256	22.4 a	20.33 a	13.00 a	17.2 b	9.5 a	19.35 b	16.20 a
400	22.2 a	17.45 a	14.30 a	17.1 b	9.2 a	21.26 a	15.79 a
Rhph	21.8 a	18.67 a	12.70 a	16.0 c	9.0 a	19.80 b	15.42 a
Limit differences							
P 5%	1.30	2.76*	1.60*	0.3	1.01	2.332	0.78*
P 1%	1.79*		2.20	0.8	1.64*	3.198*	1.08
P 0.1%	2.43		3.10	1.1*	2.50	4.353	1.47