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RESPONSE OF BEANS TO INOCULATION AND FERTILIZERS

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ABSTRACT
Aiming at a more effective production of beans, the paper displays the result of inoculation of two beans varieties with different strains of *Rhizobium leguminosarum bv. phaseoli* with and without the application of calcium ammonium nitrate.
The experiment was conducted on vertisol . The size of experimental plots was 250m^2.Before sowing, the seed of beans varieties Palanacka rana and Biser was inoculated with two strains of *Rhizobium leguminosarum bv. phaseoli* (strains B_2 and P_4) and NS Nitragin .The contol variant was not inoculated. Besides inoculation, at the beginning of the flowering phase 150 kg ha^{-1} of calcium ammonium nitrate (40 kg N) was introduced into the half of the experimental plots.
The parameters that were determined included: height of plant (cm), dry matter mass of plant (g), number of pods per plant, number of grains per plant and dry matter mass of the grains per plant (g). At the end of vegetation period, the total number of micro-organisms, the number of fungi and the number of azotobacter were determined in rhizospheric soil.
Mineral fertilizer had a negative effect but inoculation had a positive effect on the majority of the investigated parameters. There was no negative effect of fertilizer when both ammonium nitrate and inoculation were applied.
Microbiological activity was high in all variants. The fertilizers and inoculation increased the total number microorganisms.

KEY WORDS:
rhizobia, beans, fertilizer, microorganisms

1. INTRODUCTION

Beans live in symbiotic relationship with *Rhizobium leguminosarum bv. phaseoli*, *R. tropici*, *R. mongolense*, *R. gallicum* i *R. etli.* The most numerous bacterium in most types of soil is *Rhizobium leguminosarum bv. phaseoli.* The number and activity of autochthonous population of these bacteria depend on the characteristics of soil, beans variety, fertilization etc. (Nutman, 1976).

The amount of fixed nitrogen in such a symbiotic relationship is 25-120 kg Nha^{-1} a year. Therefore, it is recommended that effective strains should be used in the production of beans. The effective strains of *Rhizobium leguminosarum bv. phaseoli* increase the number of these bacteria in soil and change the balance of micro-organisms in the rhizospheric soil of beans (Martins et al., 2003., Jarak et al., 2002).

Most commonly, the number of micro-organisms increases, however, there can occur antagonism between rhizobia and other micro-organisms (Pugashetti et al., 1982). For a better growth of the beans plant it is recommended that up to 30 kg N ha^{-1} should be introduced into soil. After effective nodules have been formed, the necessary amount of nitrogen is obtained in the process of nitrogen fixation.

In different types of soil in Serbia, the number of *Rhizobium leguminosarum bv. phaseoli* in one gram of soil amounts to 10^{1} in pseudogley, 10^{3} in humogley, 10^{2} in vertisol i 10^{5} u chernozem soil (Jarak et al., 1994).Beans are grown in all these types of soil, but the yield is not always satisfactory. A reason for this is the small number and the low activity of autochthonous rhizobia strains.

Aiming at a more effective production of beans, the paper displays the result of inoculation of two beans varieties with different strains of *Rhizobium leguminosarum bv. phaseoli* with and without the application of calcium ammonium nitrate.

2. MATERIAL AND METHODS

The experiment was conducted on vertisol (pH in KCl 6,5., humus 3,9%, $N\text{-}NH_4$ 6,3 mg kg^{-1}, $N\text{-}NO_3$ 7,5 mg kg^{-1}, total nitrogen 0,19%, P_2O_5 48,7 mg $100g^{-1}$, K2O 27,8 mg $100g^{-1}$) during 2001 and 2002.The size of experimental plots was $250m^2$.

Before sowing, the seed of beans varieties Palanacka rana and Biser (selection by Centre for vegetable crops, Smederevska Palanka, SCG) was inoculated with two strains of *Rhizobium leguminosarum bv. phaseoli* (strains B_2 and P_4, of the Faculty of Agriculture, Novi Sad, SCG) and NS Nitragin (microbiological fertilizer, Science Institute of Field and Vegetable Crops, Novi Sad, SCG). The contol variant was not inoculated. The inoculant was made from pure cultures which multiplied in yeast manitol broth (Vincent, 1970). 50 ml of culture (10^{9} cells ml^{-1}) was introduced into 100 g of sterile peat. This amount of inoculants was mixed with 5 kg of seed. Besides inoculation, at the beginning of the flowering phase 150 kg ha^{-1} of calcium ammonium nitrate (40 kg N) was introduced into the half of the experimental plots.

The parameters that were determined included: height of plant (cm), dry matter mass of plant (g), number of pods per plant, number of grains per plant and dry matter mass of the grains per plant (g). At the end of vegetation period, the total number of micro-organisms (Benson, 2000), the number of fungi in Czapek-Dox medium (Sharlau, 2000) and the number of azotobacter in Fjodorov medium (Anderson, 1965) were determined in rhizospheric soil. The most probable number method (MPN) was used for determination of the number of micro-organisms.

Statistical differences between treatments (with and without inoculation, with and without fertilization and interaction between inoculation and fertilization) were determined using analysis of variance (ANOVA) and were grouped by LSD procedure (* $P< 0,05$ and ** $P< 0,01$).

3. RESULTS AND DISCUSSION

The result of beans inoculation and application of calcium ammonium nitrate were various (tables 1 and 2).In the variants where only mineral fertilizer was applied-Palanacka rana, the following parameters decreased: length of the plant (**$P< 0,01$), dry matter mass of plant (**$P< 0,01$), number of pods (*$P< 0,05$) and grains mass (**$P< 0,01$).With Biser variety , fertilization negatively affected the length of the plant. Other parameters also decreased in fertilized variants, but not so significantly.

The results of many investigations stress the need for smaller amount of mineral fertilizers at the beginning of the vegetation period of beans (Henson i Bliss,

1991) because autochthonous populations of rhizobia strains often do not provide the plant with enough nitrogen (Tsai et al., 1993).

On the other hand, investigations point out to the negative effect of mineral nitrogen fertilizers on symbiotic nitrogen fixation (Rennie and Kemp, 1983) especially if the soil contains a sufficient amount of mineral nitrogen. Viera at al.(1998) claims that even 20 kg N ha^{-1} decreases the amount of fixed nitrogen. This investigation included the application of 40 kg N ha^{-1}.

Inoculation had a positive effect on the majority of the investigated parameters. With Palanacka rana dry matter mass of plant significantly increased (strain B_2, **P<0,01) as well as the number of grains per plant (NS Nitragin and strain B_2,** P< 0,01).

With Biser variety, there was significant increase in dry matter mass. Although the amount of fixed nitrogen in beans is smaller than in other legumes (Piha i Munns, 1987), by isolating and idetifying those rhizobia strains which can provide the plant with enough nitrogen in the process of nitrogen fixation, the yield and the total amount of nitrogen in the grain can be increased (Martins et al., 2003).

The effect of inoculation depends also on rhizobia strain and beans variety (Pacowsky et al.,1984, Milic et al., 1999). This investigation has shown that the strains had similar activity but the results were better with Palanacka rana variety.

Table 1. The effect of inoculation (i) and fertilization (f) on the length of plant, dry matter mass of plant, number of pods per plant , number of grains per plant and dry matter mass of grains per plant, Palanacka rana variety

Parameters	NH_4NO_3	without inoculation	NS Nitragin	strain B_2	strain P_4
length of plant (cm)	-	33.80	32.33	33.87	33.40
	+	30.60	35.00	32.73	33.93
dry matter mass (g)	-	16.09	17.37	19.17	17.03
	+	12.37	16.60	16.17	20.83
number of pots	-	10.93	12.67	13.00	11.67
	+	9.27	12.40	12.40	13.67
number of grains	-	29.00	37.93	37.73	32.33
	+	29.00	36.20	34.53	42.33
mass of grains (g)	-	8.67	9.23	9.17	7.80
	+	7.10	9.10	9.33	11.23

Statistical analyses (least statistical differences, LSD ,**P<0,01, *P<0,05):

LSD	length of plant		dry matter mass of plant		number of pots		number of grains		dry mass grains	
	<0.01	<0,05	<0.01	<0,05	<0.01	<0,05	<0.01	<0,05	<0.01	<0,05
f	2.60	1.86	2.10	1,5	2.13	1,52	3.84	2.75	1.35	0.97
i	3.68	2.64	2,97	2,13	3,01	2,15	5.43	3.88	1.92	1.37
f x i	5,21	3,73	4,20	3,01	4,26	3,05	7.67	5.49	2.71	1.94

There was no negative effect of fertilizer when both ammonium nitrate and inoculation were applied. With Palanacka rana, dry matter mass increased (f x P_4, **P< 0,01), but also the number of grains per plant (f x N, f x B_2, f x P_4) and dry matter mass of the grain. With Biser variety, dry matter mass of the plant increased (f x P_4).This is also confirmed by the results of Hungria et al.(2003), Vargas et al.(2000) which show that the synergistic effect of mineral fertilizers and highly effective rhizobia strains increases the yield of beans.

Table 2. The effect of inoculation (i) and fertilization (f) on the length of plant, dry matter mass of plant, number of pots per plant, number of grains per plant and dry matter mass of grains per plant, Biser variety

Parameters	NH_4NO_3	without inoculation	NS Nitragin	strain B_2	strain P_4
length of plant (cm)	-	53.00	48.43	49.90	48.40
	+	44.87	52.33	50.33	44.10
dry matter mass (g)	-	23.67	24.53	25.23	26.53
	+	23.63	25.70	25.93	26.63
number of pots	-	16.20	15.20	15.73	16.33
	+	14.13	15.67	14.80	16.40
number of grains	-	59.47	55.87	59.73	61.53
	+	53.30	57.40	57.40	62.47
mass of grains (g)	-	12.93	11.47	13.00	13.30
	+	11.43	12.43	12.57	13.10

Statistical analyses (least statistical differences , LSD, **P<0,01, *P<0,05):

LSD	*length*		*dry matter mass*		*number of pots*		*number of grains*		*dry mass grains*	
	<0.01	<0,05	<0.01	<0,05	<0.01	<0,05	<0.01	<0,05	<0.01	<0,05
f	11.12	4.82	19.73	8.55	9.07	3.93	36.28	15.73	9.11	3.95
i	7.36	5.25	5.53	3.95	2.67	1.91	13.14	9.37	2.39	1.70
f x i	10.41	7.42	7.83	5.58	3.78	2.69	18.58	13.25	3.38	2.41

Microbiological activity of soil is also important when providing optimal conditions for plant growth. In this investigation, ammonium nitrate had a positive effect on the total number of micro-organisms and on the number of azotobacter with Palanacka rana variety, as well as on the number of azotobacter with Biser variety (*P<0.05) (table 3).

With both varieties, inoculation with NS Nitragin had a positive effect on the total number of micro-organisms (*P<0.05) and the number of azotobacter (*P<0.05). P_4 strain had a positive effect on the total number of micro-organisms (*P<0.05) with Palanacka rana variety whereas it had a negative effect with Biser variety (*P<0.05). With Biser, B_2 strain had a negative effect on the total number of micro-organisms (**P<0.01), whereas it had a positive effect on the number of azotobacter (*P<0.05).

Table 3. Total number microorganisms (TN, 10^6 g^{-1}), number of fungi (F, 10^4 g^{-1}) and number of azotobacter (azb,10^2 g^{-1})

Variants	NH_4NO_3	Palanacka rana			Biser		
		TN	F	azb	TN	F	azb
without inoculation	-	202.53	8.27	8.43	117.00	14.67	26.33
	+	266.53	11.47	24.87	118.00	18.00	39.33
NS Nitragin	-	244.03	16,50	18.67	151.33	10.00	40.00
	+	313.93	19.77	53.47	77.00	19.33	47.33
strain B_2	-	222.57	8.74	11.20	56.67	14.67	31.67
	+	253.23	27.47	57.90	23.33	23.00	43.33
strain P_4	-	226.67	13.50	11.77	82.67	17.67	27.67
	+	347.23	13.10	19.93	56.00	28.33	43.67

Statistical analyses (least statistical differences, LSD):

	LSD	*Palanacka rana*			*Biser*		
		f	i	f x i	f	i	f x i
TN	**P<0,01	22.04	31.17	44.08	130,31	48.21	68.19
	*P<0,05	15.77	22.31	31.55	56.50	34.39	48.63
F	**P<0,01	5.91	8.36	11.83	26.58	5,95	8,42
	*P<0,05	4.23	5.99	8.47	11.52	4,25	6,00
azb	**P<0,01	8.41	11.89	16.82	14,96	6,46	16,82
	*P<0,05	6.02	8.51	12.03	6,48	4,60	12,03

In the experimental fields where ammonium nitrate and inoculation were applied, the number of micro-organisms increased (*P<0.05) with Palanacka rana whereas with Biser the number of azotobacter increased (**P<0.01).

The number of micro-organisms in rhizospheric soil depends on the conditions in the soil, agro-technical measures and ecological factors (Alexander, 1977). This investigation shows that the number of micro-organisms depends on inoculation, beans variety and fertilization which was proved by other numerous results (Kent and Triplet, 2002, Innes et al .2004).

4. CONCLUSION

The result of beans inoculation and application of calcium ammonium nitrate were various.

Mineral fertilizer decreased length of the plant, dry matter mass of plant, number of pods and grains mass with Palanacka rana variety. With Biser variety, fertilization negatively affected the length of the plant.

There was no negative effect of fertilizer when both ammonium nitrate and inoculation were applied.

Inoculation had a positive effect on the majority of the investigated parameters.

Microbiological activity was high in all variants.The fertilizers and inoculation increased the total number microorganisms.

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