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EFFECT OF INOCULATION WITH NITROGEN FIXING BACTERIA ON CHICKPEA (*CICER ARIETINUM* L.) YIELD AND RHIZOSPHERE MICROFLORA

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Summary. The effect of either a single or mixed inoculation of chickpea with *Bradyrhizobium sp. (*cicer*)* and *Azospirillum brasilense* was studied in a pot experiment on leached smolnitza (Bojurishte, Sofia district). Three *Bradyrhizobium* strains and A.brasilense Sp.107 were used for presowing treatment of seeds. The virulence of nodule bacteria and the main physiological groups of rhizospheric microorganisms were studied at the blossoming phase. Grain yield was determined as well. It was established that the mixed inoculation of chickpea was more effective than the single one. The inoculation effect depended on the *Bradyrhizobium* strain. The greatest effect was observed after a mixed inoculation with *Bradyrhizobium sp.* (cicer) № 7 and *A.brasilense* Sp.107. In most cases inoculation increased the amount of the studied rhizosphere microorganisms connected with plant nutrition.

Key words**:** *Azospirillum brasilense, Bradyrhizobium,* Cicer arietinum, effectiveness, inoculation, virulence.

INTRODUCTION

In the last years the interest on chickpea, an old traditional crop for Bulgarian agriculture, is higher. Based on data of the Ministry of Agriculture for 2005, areas sown with chickpea are 35% from the total area occupied with leguminous crops (58102 da), followed by bean and peas for grain. In worldwide scale chickpea is the third significant leguminous crop. Chickpea, having high nutritional qualities of seed rich in protein, fats, amino acids and vitamins is characterized also by lower demands for soil conditions and high sustainability to drought. It possesses high nitrogen fixing ability as well – about 80% of the necessary nitrogen for this crop is provided by nitrogen fixation.

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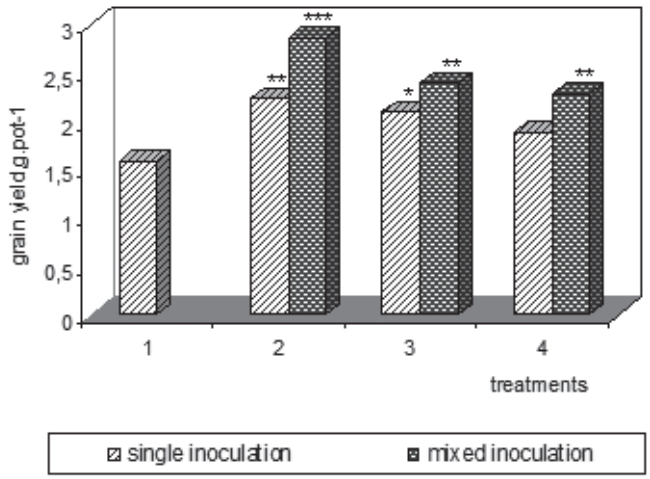
All this makes chickpea a valuable crop in technical, ecological and economic aspects. Its importance grows especially in conditions of the global heating. In our country, the investigations with chickpea are in agronomic aspect (Mihov et al., 2002; Atanassova and Mihova, 2005). Studies on the symbiotic system "chickpea – *Bradyrhizobium* sp. (cicer)" have not been conducted. The effectiveness of this system can be enhanced by using bioinoculants containing nitroge-fixing microorganisms. The aim of the present study was to examine the effect of inoculation with different *Bradyrhizobium* sp. (cicer) strains and *Azospirillum brasilense* on grain yield and the microflora connected with transformation of nutrient substances in chickpea rhizosphere.

MATERIAL AND METHODS

The effect of a single or mixed inoculation of chickpea (hybrid line N 27) was studied in a pot experiment on leached smolnitza (Bojurishte, Sofia district). The soil had the following characteristics: $pH_{(KCl)}$ – 6.4; humus (by Tjurin) – 5.3 %; hydrolysable N (by Bremner) – 27.8 mg 1000 g $^{-1}$; mobile P (by Ivanov) – 13.1 mg 100g $^{-1}$; mobile K (by Ivanov) – 23.5 mg 100g $^{-1}$. Two plants were grown in pots with 1 kg air dry soil in four replications. 70 mg kg^{-1} N and 160 mg kg^{-1} P as NH_4NO_3 and KH_2PO_4, respectively were applied as a background in conditions of soil humidity 60% of WHC. *Bradyrhizobium* sp. (cicer) strains №№ 7 and 10, isolated from chickpea, grown on leached smolnitza, Sadovo (Plovdiv district), strain №3352 (USA) and *A.brasilense* Sp.107 (CA-TEC) were used for presowing treatment of seeds at a final concentration of 10^8 CFU cm^3. The virulence of the *Bradyrhizobium sp.* (cicer) strains and the main physiological groups of rhizosphere microorganisms (Goushterov et al., 1970) were studied at the blossoming phase. Grain yield was determined as well. Data were processed by the method of dispersion. Comparison of means was performed by the Fisher LSD test.

RESULTS AND DISCUSSION

The results obtained showed that native rhizobia were absent in the leached smolnitza from Bojurishte – there were no nodules on the roots of the uninoculated control. In case of chickpea inoculation with *Bradyrhizobium sp.*(cicer) strains nodules were formed as follows: 13, 12 and 8 with strains № 7, № 10 and № 3352, respectively. These results showed that the strains had good virulence, better expressed with strain № 7, where big double and triple nodules were observed. The mixed inoculation with *Bradyrhizobium* sp. (cicer) and *Azospirillum brasilense* Sp. 107 stimulated nodule formation. The nodule number was increased as compared to the single inoculation. The best virulence was found in the case of mixed inoculation with strain № 7 *Bradyrhizobium* sp. (cicer) and *Azospirillum brasilense* Sp. 107 where the nodule number was increased by 30% as compared to the single inoculation with the same strain. The positive effect of the mixed inoculation can be explained by the action of growth promoting substances produced by associative bacteria of *Azospirillum* genus, which stimulated



1 – control ; 2 – strain № 7 ; 3 – strain № 10; 4 – strain № 3352
LSD $p \leq 5\% - 0.3892^{*}$; $p \leq 1\% - 0.5457^{**}$; $p \leq 0.1\% - 0.7714^{***}$

Fig. 1. Effect of chickpea inoculation on grain yield.

the penetration of nodule bacteria into root hairs (German et al., 2000). The effect of chickpea inoculation on grain yield is presented in Fig.1. The results showed that *Bradyrhizobium* sp. (cicer) strains isolated from leached smolnitza, Sadovo, were effective. The increase of grain yield was 40% and 31% with strains № 7 and № 10, respectively as compared to uninoculated control. Strain № 3352 showed significantly lower effectiveness (19%). The effectiveness of mixed inoculation with *Azospirillum brasilense* Sp. 107 depended on the *Bradyrhizobium sp.* (cicer) strain. The mixed inoculation was more effective than the single one with strains №№ 7 and 3352. The differences in yield increase were statistically significant at $p \leq 0{,}1\%$. The greatest increase of grain yield (28%) was found with strain № 7. Obviously, in case of this combination, the best favourable relationships between leguminous plant and the studied bacteria were created. In our previous works with other legumes we found also that the mixed inoculation was more effective than the single one (Altimirska and Markova, 2008; Chanova et al., 1997; Markova et al., 2005). Similar data have been reported by other authors (Hamaoui et al., 2001; Remans et al., 2008). Our results showed changes in the major groups of rhizosphere microorganisms due to chickpea inoculation (Table 1). In most cases inoculation increased the amount of the studied microorganisms, especially bacteria transforming nitrogen in soil and connected with plant nutrition . The increase was greater in case of bacteria utilizing mineral nitrogen, thus indicating more advanced mineralization processes. This tendency was stronger in the case of chickpea inoculation with strain № 7 – cellulose decomposing microorganisms and actinomycetes were increased as well. So, more favourable conditions for plant nutrition in their

Table 1. Effect of chickpea inoculation on rhizosphere microorganisms [CFU g^{-1} soil].

Variants	Bacteria x 10^6			Cellulose decomposing microorganisms x 10^4	Actino-mycetes x 10^6	Microscopic fungi x 10^4
	Ammonifying	Utilizing mineral nitrogen	Total			
1. Control	42.2	6.8	49.0	16.0	1.0	28.0
2. Strain 7	78.0	8.6	86.6	20.0	4.0	27.2
3. Strain 7 + *A. brasilense*	15.6	14.0	29.6	16.0	12.0	8.0
4. Strain 10	28.0	11.4	39.4	10.0	8.0	14.0
5. Strain 10 + *A. brasilense*	13.0	8.6	21.6	10.0	10.0	6.0
6. Strain 3352	21.8	13.8	35.6	10.0	16.0	14.0
7. Strain 3352 + *A. brasilense*	42.4	8.8	51.2	8.0	12.0	20.0
LSD ($P \leq 0.05$)	12.6	4.6	20.1	3.8	4.6	5.2

rhizosphere were created. These data correlated positively with changes in yield, better expressed with strain № 7 which was the most effective strain (Fig. 1). Inoculation caused a decrease in microscopic fungi amount (Table1). This tendency could be considered as a positive one, because there were many phytopatogenes among the fungi population. The results obtained give us the reason to continue our investigations for searching effective pairs of nitrogen-fixing microorganisms for chickpea inoculation as an element of the breeding technology of this crop.

CONCLUSIONS

The effectiveness of a single chickpea inoculation depended on the *Bradyrhizobium* sp. (cicer) strain. The highest effectiveness was found with strain № 7, the increase of grain yield being higher by 40% as compared to uninoculated control. The mixed inoculation with *Azospirillum brasilense* Sp. 107 was more effective than the single one. The best effect was established with strain №7 *Bradyrhizobium* sp.(cicer) and *Azospirillum brasilense* Sp. 107. The virulence was enhanced and the grain

yield was increased by 28% as compared to the single inoculation. In most cases, inoculation increased the amount of the rhizosphere microorganisms connected with chickpea nutrition.

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