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THE EFFECTS OF RHIZOBIAL INOCULATION ON GROWTH AND YIELD OF *Vigna mungo* L. IN SERBIAN SOILS

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Abstract: *Vigna mungo* (L.) Hepper is an important annual leguminous species in tropical and sub-tropical regions. The aim of this research was to investigate the possibility of *V. mungo* cultivation as a newly introduced crop in Serbia, by applying seed inoculation with highly effective rhizobial strains in the form of microbial nitrogen (N) fertilizer. The effects of inoculation with rhizobial strain were estimated in field conditions on alluvium soil types. Inoculated plants produced significantly higher shoot dry weight (SDW) yield, total N content as well as protein yield in respect to untreated control (Ø). According to plant shoot yield and yield attributes strain 542 was highly effective without significant differences in comparison to its treatment in combination with mineral N as well as uninoculated control with full rate of mineral N, 80 kg ha^{-1} N (NØ). Taking into account these results and aims of sustainable agriculture 542 strain might be recommended as active agent of N microbiological fertilizer.

Key words: *Vigna mungo*, *Bradyrhizobium* spp., symbiotic N fixation, biological yield, fixed N

Introduction

Blackgram, *Vigna mungo* (L.) Hepper, a member of the Asian Vigna crop group, is annual pulse crop native to central Asia. It is staple crop in central and south east Asia. However, it is extensively used only in India and is now grown in the Southern United States, the West Indies, Japan and other tropics and subtropics. It is summer pulse crop with short duration (90-120 days) and high nutritive value *(El Karamany, 2006)*. It valued for its high digestibility and freedom from flatulence effect *(Fary, 2002)*. Blackgram is used for human food (vegetable diets), green manure, a cover crop, forage, silage, hay and chicken pasture *(Munro and Small, 1997)*. Although it is grown mostly for food seed production, it can be cultivating in the form of double cropping after barley, wheat, oat in Serbia. Beside that blackgram potential as a dual-purpose crop for early season forage production follows by seed production should be examined *(Imrie, 2005; El Karamany, 2006)*. Blackgram is sown on most soil but it can grow on heavier soils (pH 5.5-7.5). Fore legume, nitrogen (N) is more useful because it is the main component of amino acid as well as protein. As an excellent source of plant protein blackgram is highly responsive to nitrogen *(Imrie, 2005; Kulsum et al., 2007)*. Mineral N fertilizers are costly and detrimental to the environment. Otherwise blackgram can obtain nitrogen (N) by atmosphere by fixation in their root nodules in symbiosis with soil rhizobia, and thus have the potential to yield well in N deficit soils *(Ahmad et al., 2001)*. This characteristic is particularly important in developing countries due to the relatively high cost and restricted availability of chemical N fertilizer. To reduce the production cost with application mineral N fertilization and provide environment protection, more pulse production could be achieve through seed inoculation with *Bradyrhizobium* biofertilizer.

They are known to influence nodulation, symbiotic N fixation and growth and yield of pulses. Seed inoculation with *Bradyrhizobium* bacteria prior to sowing allows a reduction in nitrogen mineral fertilization and decreases susceptibility to environmental stress. The characteristics of these leguminous plants indicate a need to introduce them into agricultural production in Serbia.

The goal of this research is to obtain profitable and healthy food by introducing new type of legume *Vigna mungo* applying effective strains of *Bradyrhizobium* in the form of microbiological nitrogen fertilizes, which would enable a reduction or avoidance of nitrogen mineral fertilizers in their agricultural production.

Materials and Methods

Field experiments were conducted during 2007 in village Ratare, near Obrenovac, Serbia. The chemical properties of the soil were shown in Table 1. The experiment was designed with 8 inoculated treatments; 4 treatments with single rhizobial strains and 4 treatments with single strains in combination with mineral N rate of 40 kg N ha^{-1} and 2 controlled uninoculated treatments: Ø -untreated control and ØN –uninoculated, N fertilizer control with full N content of 80 kg N ha^{-1}. Mineral N was added in the form of KAN (27% N). The experimental plots (2.4 m^2) were arranged in a randomized complete block system with three replicates. Seeds were sown in the first week of May maintaining 30 cm line to line and 10 cm plant to plant spacing. A sowing dose of blackgram of 200 000 ha^{-1} was employed. Blackgram seeds were inoculated with peat-based inoculants of single rhizobial strains (*Bradyrhizobium* spp. KNj and *Bradyrhizobium japonicum* 525, 526 and 542) that were selected from preliminary experiment as a high efficiency strains in

N fixation before sowing. A samples of 10 plants were collected from each plot at early pod filling to evaluate root nodulation and the plant high was measured. In 11th week after sowing the plants were harvested to determine quantity and quality of plant shoot yield (t ha^{-1}). The percentage of shoot N was determined (CNS Elemental Analyzer vario EL III) and it was used to calculate shoot total and fixed N content. All data were subject to ANOVA using the statistical analysis system (SPSS). Means of all treatments were calculated and the differences tested for significance using the LSD test at the 0.5 probability level.

Table 1. Chemical properties of the soil in 2007 year

Depth (cm)	pH KCl	Humus (%)	$CaCO_3$ (%)	N mg 1000 g^{-1}			P_2O_5 mg $100g^{-1}$	K_2O mg $100g^{-1}$
				Total	NH_4^+	NO_3^-		
0-20	6.60	4.00	0.33	25.00	18.20	7.00	21.68	29.0

Results

There were no significant differences in plant high between all the treatments during the pod-fill (27.1-33.8 cm) except untreated control (Ø) (Table 2). The highest plant high in absolute value was found at 542N treatment in which 40 kg ha^{-1} of N with 542 strain was applied.

The highest shoot dry weight (SDW) of the blackgram was obtained in treatments 542N (6.54 t ha^{-1}) and 525N (6.62 t ha^{-1}) and lower, without significant differences, in 526N and KNjN (Table 2). There were no significant differences between nitrogen fertilizer control- ∅N and these treatments. The lowest SDW was in untreated control-∅ (4.73 t ha^{-1}). Plants inoculated with single strains showed lower SDW than in combination with 40 kg ha^{-1} of mineral N.

Crude protein percentage of SDW varied from 12.6 (∅) to 26.25% (542N). The protein yield (PY) in plant shoot weight was wide-ranging from 592 kg $ha^{-}1$ in ∅ to 1717 kg $ha^{-}1$ in 542N and was in correlation with SDW (r=0.691).

The effects of inoculation on SDW and PY depended on treatments. Interaction effect of rizobial strains in combination with mineral N increased SDW and total N content by 28-38% and 37-189%, respectively compared to Ø while the increase with single strain inoculation were 21% and 75.87% (526) and 28% and 164.99% (542) respectively. Effect of inoculation on SDW and total N content with single another two strains was marginal 10% and 23% (KNj) and 13% and 25% (525). Inoculation with 542 strain alone and in combination with 40 kg ha^{-1} mineral nitrogen (N) had the similar effect on SDW as nitrogen fertilizer control- ∅N (80 kg ha^{-1} the mineral N) while the same treatments showed higher protein yield than ∅N.

Table 2. Influence of *Bradyrhizobium* strains on shoot dry weight (SDW) and its attributes of *Vigna mungo* under field conditions

Soil types	Treatments *	Plant high (cm)	SDW (t ha^{-1})	Crude protein in SDW %	Yield protein in SDW (t ha^{-1})	Total N in SDW (kg ha^{-1})	Fixed N kg ha^{-1}	% SDW increase over Ø	% N increase over Ø
Alluvium	525	27.6 b	5.36 c	13.94	0.747 de	119.53 de	25	113.32	125.82
	525N	32.5 a	6.52 a	12.50	0.815 d	130.34 d	35	137.78	137.20
	KNJ	30.1 ab	5.21 c	14.06	0.732 de	117.14 de	22	110.07	123.30
	KNjN	33.5 a	6.09 ab	15.50	0.944 cd	151.05 cd	56	128.76	159.00
	526	30.4 ab	5.76 b	18.12	1.044 bc	167.07 bc	72	121.80	175.87
	526N	29.1 ab	6.23 ab	19.37	1.207 b	193.19 b	98	131.76	203.36
	542	32.5 a	6.07 ab	25.94	1.573 a	251.75 a	157	128.25	264.99
	542N	33.8 a	6.54 a	26.25	1.717 a	274.69 a	180	138.27	289.14
	Ø	27.1 b	4.73 d	12.50	0.592 e	94.67 e	-	100.00	100.00
	ØN	32.3 a	6.29 ab	19.56	1.231 b	196.93 b	-	133.03	207.29
	LSD 0.05	0.22	0.38		0.150	0.190			

*Ø -untreated control; ØN –uninoculated, N fertilizer control with full N content; *Bradyrhizobium* strains with or without 40 kg ha^{-1} N mineral fertilizer; Means with the same letter within a column do not differ significantly (p<0.05

Discussion

Application of *Bradyrhizobium* strains showed significant effect on plant high, SDW and PY in plant shoot weight. In our investigation SDW (18.8% of shoot fresh weight) is satisfactory (Table 1). The highest fixed N was obtained in inoculation with and without 542 strain. For legumes adequate supply of nitrogen is essential for normal growth and yield. In native areas (South and Southeast Asia, poor countries) blackgram is grown without application of mineral N fertilizer or with 10-20 kg ha^{-1} of N. According to some authors, 30-60 kg N ha^{-1} was applied *(Ahmad et al., 2001; Mahboob and Asghar, 2002)*. In Serbia this crop cultivation is on the experimental level for scientific purposes and small production of sprout in healthy food market. In concordance with available date from agricultural practice in Serbia ("Selsem" company applies soybean production technology with or without application of N mineral fertilizer and rizobial inoculation), amount of 80 kg N ha^{-1} was applied in this work. The largest leaf area that influences to shoot mass was obtained with 80 kg N ha^{-1} *(Kulsum et al., 2007)*. Application of this rate of N mineral fertilizer under field conditions (∅N treatment) in our experiment increased SDW for 33% over control. This amount of mineral fertilizer affected positively yield and its attributes (Table 2). Most of the treatments realized the same or higher values of yield and yield attributes than ∅N.

In our experiment plant were grown in alluvium soil (Table 1) with good physical and chemical characteristics and good provided with mineral N. However, the concentration of N containing solutes, especially that of nitrate may change quickly due to processes such as uptake by plant roots and microorganisms, leaching and gentrification. Inoculated legume might be N-limited because suppressive effect of mineral N or ineffective rhizobial strains *(Herridge et al., 2005)*. Bearing in mind this, we chose half of applied amount of N mineral fertilizer under field conditions in Serbia (40 kg ha^{-1}) for combination with rhizobial strain inoculation. Our results indicated effectiveness of strains 542 and 526 and unsuppressive effect of 40 kg ha^{-1} in all inoculated treatments in combinations with N mineral fertilizer. According to yield protein, fixed N and % total N increase over ∅, strain 542 was highly effective without significant differences in comparison to its treatment in combination with mineral N. Taking in to account these results and aims of sustainable agriculture 542 strain might be recommended as active agent of N microbiological fertilizer. According *Kulsum et al. (2007)* blackgram is very poor as compare to many other legumes crop due to slow rate of dry weight accumulation during pre-flowering phase, on-set of leaf senescence during the period of pod development and low partitioning efficiency of assimilates to grain identified as the main physiological constraints for increasing yield. In contrast to that our results indicate good quality of plant shoot dry mass in some treatments (542, 542N and 526N) on the bases crude protein percentage (around 19-26%), yield protein and total N content in shoot weight.

Conclusion

Inoculation with high effective strain *B. japonicum* 542 and in combination with 40 kg N ha^{-1} provided good blackgram yield and yield attributes in Serbian soil and might be recommended as active agent of microbiological N fertilizer for black gram production.

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Efekat rizobijalne inokulacije na rast i prinos *Vigna mungo* L. u zemljištu Srbije

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Rezime

Cilj ovog istraživanja je bio da ispita mogućnost gajenja *V. mungo* u Srbiji uz primenu inokulacije visoko efektivnim sojevima rizobija u obliku mikrobiološkog azotnog đubriva. Eksperiment je urađen pod poljskim uslovima na aluvijalnom tipu zemljišta uz inokulaciju pojedinačnim sojevima rizobija i u kombinaciji sa mineralnim azotnim đubrivom. Biljke inokulisanih tretmana su dale značajno veću suvu nadzemnu masu, ukupni azot kao i prinos proteina u odnosu na netretiranu kontrolu (Ø). Najefektivniji soj, 542 koji je ostvario najveće vrednosti parametara prinosa biljne mase, primenjen pojedinačno ili u kombinaciji sa mineralnim azotom mogao bi da se preporuči kao aktivni agens mikrobiološkog azotnog đubriva.

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