African Journal of Microbiology Research Vol. 5(23), pp. 3946-3957, 23 October, 2011
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 ©2011 Academic Journals

Full Length Research Paper

Effect of bradyrhizobial inoculation on growth and seed yield of mungbean in Fluvisol and Humofluvisol

Dušica Delić[1]*, Olivera Stajković-Srbinović[1], Djordje Kuzmanović[1], Nataša Rasulić[1], Vesna Mrvić[1], Srdjan Andjelović[2] and Jelena Knežević-Vukčević[3]

[1]Institute for Soil Science, Teodora Drajzera 7, Belgrade, Serbia.
[2]Selsem d.o.o., Belgrade, Serbia.
[3]Faculty of Biology, University of Belgrade, Serbia.

Accepted 19 September, 2011

Nitrogen fixing effectiveness of selected *Bradyrhizobium* spp. strains to mungbean, *Vigna radiata* L. for better quantity and quality of grain and shoot dry matter was investigated in field conditions in two soil types with the aim of introducing of mungbean as a new crop in Republic of Serbia. Seed inoculation was done with four active strains both individually and in combination with fertilization of 20 kg N ha^{-1}. Quantity and quality of yield of grain and shoot dry matter was higher in Fluvisol than in Humofluvisol. On the both soil types, seed inoculation with and without 20 kg N ha^{-1} produced significantly higher grain yield (11-59%) and shoot dry weight (13-48%) as well as total N content and protein yield in respect to untreated control (Ø). The strains, soil types, mineral N and their interaction significantly influenced shoot dry matter and grain yield. According to high quantity and quality of grain and shoot dry matter yield, application of *B. japonicum* strain 542 in combination with 20 kg N ha^{-1} could replace fertilization of 40 kg N ha^{-1} and would be applied in production of mungbean.

Key words: *Bradyrhizobium* strains, *Vigna radiata* L., symbiotic N fixation, inoculation, yield.

INTRODUCTION

Mungbean, *Vigna radiata* (L.) Wilczek is an ancient and well-known pulse crop in Asia, particularly in the Indian subcontinent. It is now becoming popular in the other countries as a cheap, high quality and protein-rich food which can be considered as healthy nourishment. This annual legume is known to have high nutrient values with excellent source of vegetable protein (seeds and sprouts contain to 28% of proteins). Seeds contain 60-65% carbohydrates, low content of fat (1-1.5%) and 3.5-4.5% fibre. Sprout is rich in vitamins, minerals and amino acids (especially lizin). Mungbean is considered as a substitute of animal protein and forms a balanced diet when used with cereals (Khan and Malik, 2001; Anjum et al., 2006; Mansoor, 2007).

This legume species is used primarily as human food, but also it can be used as forage and green manure (Reedy et al., 1986). Predominantly, the grain that is consumed, but green matter and immature pods as well. Seed is more palatable, easy digestible and non-flatulent than other pulse allowing mungbean to consume in vegetarian and dietetic nourishment (Abbas et al., 2011). In addition, it contains isoflavonoids having estrogens and antioxidant activities that can be used in prevention of much disease such as cancer (Brouns, 2002). Further more, it exhibits antimicrobial and insecticidal activities (Sheteawi and Tawfik, 2007).

Although mungbean is grown mostly for grain production, early season forage production of mungbean can be followed by its grain production; it is dual-purpose of mungbean (El Karamany, 2006). Mungbean can produce a large amount of biomass (about 9 t ha^{-1}) and can be recovered after grazing to yield abundant grain. It has the potential of producing higher seed yield from 1295 to 2961 kg ha^{-1} depending on genotypes studied (El Karamany, 2006; Rafiei, 2009; Tien et al., 2002; Ullah et al., 2011).

High economic importance of mungbean, especially in the developing countries, is reflected in many ways as ability of cultivation in arid areas, growth on marginal

*Corresponding author. E-mail: vukmirdusica@yahoo.com. Tel:/Fax: +381 11 2667 123.

lands as well as the improvement of soil quality due to ability of symbiotic nitrogen (N) fixation (SNF). Thanks to the ability of rhizobial bacteria to fix N from the air (N_2) in symbiosis with legumes, huge natural source of N from the air can be taken up from this symbiotic association. This ability leads to decrease or absence of N mineral fertilizer application in the field (Rahman et al., 2002; Abbas et al., 2011).

According to some field trial, application of 30-50 kg mineral N ha^{-1} resulted in significant increases of mungbean productivity (Ashraf et al., 2003; Rahman et al., 2002). Amount of N mineral fertilizer applied depends on abiotic and biotic factors. One of the principle ways to avoid or decrease environmental pollution associated with really expensive mineral N fertilizer is to insist on maximal use of seed inoculation as microbiological N fertilizer. Mungbean in symbiosis with effective *Rhizobium* and *Bradyrhizobium* spp. can fix 30-60 kg N ha^{-1} depending on agro ecological conditions (Mansoor, 2007). Applying seed inoculation with effective rhizobial strains is important agronomic measure which improves intensity of SNF and satisfies plant need for N (Vlassak and Vanderleyden, 1997; Herridge et al., 2005).

It is known that indigenous rhizobial populations in symbiosis with host plant fix N_2 with different efficiency depending of their density and activity (Mpepereki et al., 2000; Musiyiwa et al., 2005).

Therefore, only highly effective rhizobial strains as plant inoculants in form of microbiological N fertilizer can increase production of legumes so therefore selection of highly effective strains is necessary process and important area of investigation. Seed inoculation with strains prior to sowing allows a reduction in N mineral fertilization, increases plant and seed quality and yield as well as decreases susceptibility to environmental stress (Tien et al., 2002; Herridge et al., 2005).

The characteristics of mungbean as healthy food as well as its grate similarity of growing way with soybean along with our intention for expansion of sustainable agriculture production (Myers, 2000) indicate that *V. radiata* should be introduced into agricultural production in temperate continental climate zone that Serbia belongs.

Nitrogen fixing effectiveness of selected bradyrhizobium strains to mungbean for better quantity and quality of grain and shoot dry matter was investigated in two different soil types in field conditions. The aim of this field research was to study the possibility of cultivation of mungbean (forage and seed) in temperate climate in Serbian soils, by applying seed inoculation with selected highly effective rhizobial strains.

MATERIALS AND METHODS

Bacterial strains and host plant

Bradyrhizobium japonicum strains 525, 526, 542 and 532 and *Bradyrhizobium* sp. isolate Knj from Collection of Institute of Soil Science, Belgrade were applied. The bradyrhizobial cultures were maintained on yeast mannitol agar (YMA) (Vincent, 1970). Mungbean, *V. radiata* was used as the host-plant. The seed of mungbean (domestic population) was obtained from the "Selsem", company for breeding and seed production (Belgrade, Serbia).

Inoculum preparation

Each of single bradyrhizobial strains was grown in 100 ml liquid Yeast Extract Mannitol medium (YMB) (Vincent, 1970) with shaking, for 72 h at 28°C up to approximately 10^9 cell ml^{-1} determined by colony-plating of dilutions. The culture of 40 ml of each single bradyrhizobial inoculum was mixed with 100 g sterile ground peat as inoculum carrier and packed in polyethylene bags. All operations were carried out aseptically in a laminar flow chamber. After period of incubation (15 days) single inoculums consisted of approximately 10^9 bradyrhizobia g^{-1} of peat (Somasegaran and Hoben, 2004).

Identification and characterization of soil types of field sites

The trial was set up at two field sites: Ratare (Y=7430288, X=4945438) and Grabovac (Y=7429637, X=4942333) locations. On the both field sites, soil profiles were done to determine type of soil and soil samples were taken from every generic horizon. The fallowing analysis were determined: granulometric composition (combined method of sieving and pipette method), pH in H_2O and pH in 1M KCl (electrometrically), $CaCO_3$ (volumetrically, using Scheibler calcimeter), humus (CNS Elemental Analyzer Vario EL III).

Using both field and laboratory data the soils have been classified according to national classification (Škorić et al., 1985) as Fluvisol soil type (Ratare) and Humofluvisol (meadow soils) (Grabovac) that corresponds to Fluvisol and Fluvisol Clayic, respectively (IUSS Working Group WRB, 2007). Fluvisol has been protected from flooding for a long time. Experimental field on the Fluvisol had clayed loam texture with the following characters in Amo horizon: sand 31.3%, silt 32.3%, clay 36.4%, pH 6.95, organic matter 4.23%, $CaCO_3$ 0.42%. The Humofluvisol had heavy clay texture with the following characters also, in Amo horizon: Sand 22.5%, silt 30.6%, clay 46.9%, pH 6.82, organic matter 4.21%, $CaCO_3$ 1.26%). Soil characteristics of the field sites are listed in Table 1.

Agro ecological conditions and field history

Field trial was set up in 2007 in the two field sites of the central part of Serbia with temperate continental climate. Meteorological data for experimental year were based on data from “Surcin” Geo-meteorological station, Republic Hydrometeorology Department of Republic of Serbia, Belgrade (www.hidmet.sr.gov.yu). In 2007 average annual temperature of air was 13.2°C and during the growing period (from March to October) average monthly temperature was 23.2°C with maximum in July 31.4°C. In Jun, July and August average maximal extreme temperature 29.0, 32.0 and 30.3°C, respectively. In this year total amount of rainfall was 770.9 mm and during the growing period was 532.3 mm with two long dry periods: the fist was in the middle of April and the second one was from the end of June to the beginning of August (Figure 1).

Mungbean has never been cultivated on Ratare and Grabovac locations. In addition, in the past 10 years no legumes have been grown in Ratare, while in Garbovac legumes have never been cultivated before. In the experimental sites previous crops were some vegetables. Before sowing and during vegetation mineral manure was not added as well as pesticides.

Table 1. Granulametric content and chemical properties of soil samples at field location.

Type of soil/field location	Horizontal	Depth cm	Granulometric content			pH (KCl)	$CaCO_3$ %	Humus %	N %	NH_4-N + NO_3-N mg/kg	P_2O_5 mg/100 g	K_2O mg/100 g
			Total send >0.02 mm	Silt 0.002-0.02 mm	Clay <0.002 mm							
Humofluvisol Grabovac	Amo	0-43	22.5	30.6	46.9	6.82	1.26	4.21	0.219	25	28.10	22.40
	AmoC	43-85	21.3	24.7	54.0	6.89	0.85	1.52	0.079	/	0.38	11.80
	C	85-105	21.4	25.5	53.1	7.21	1.27	1.06	0.055	/	0.32	9.20
	CG	105-125	25.6	25.9	48.5	7.32	3.39	1.09	0.057	/	0.33	9.20
Fluvisol Ratari	Amo	0-54	31.3	32.3	36.4	6.95	0.42	4.23	0.220	25	17.03	38.80
	C1	54-94	28.8	31.3	39.9	6.71	0.42	1.73	0.090	/	0.75	14.00
	C2	94-115	42.0	33.5	24.5	7.32	22.85	1.09	0.057	/	0.30	7.60
	C3	115-125	60.8	23.4		7.51	25.81	0.65	0.034	/	1.90	5.50

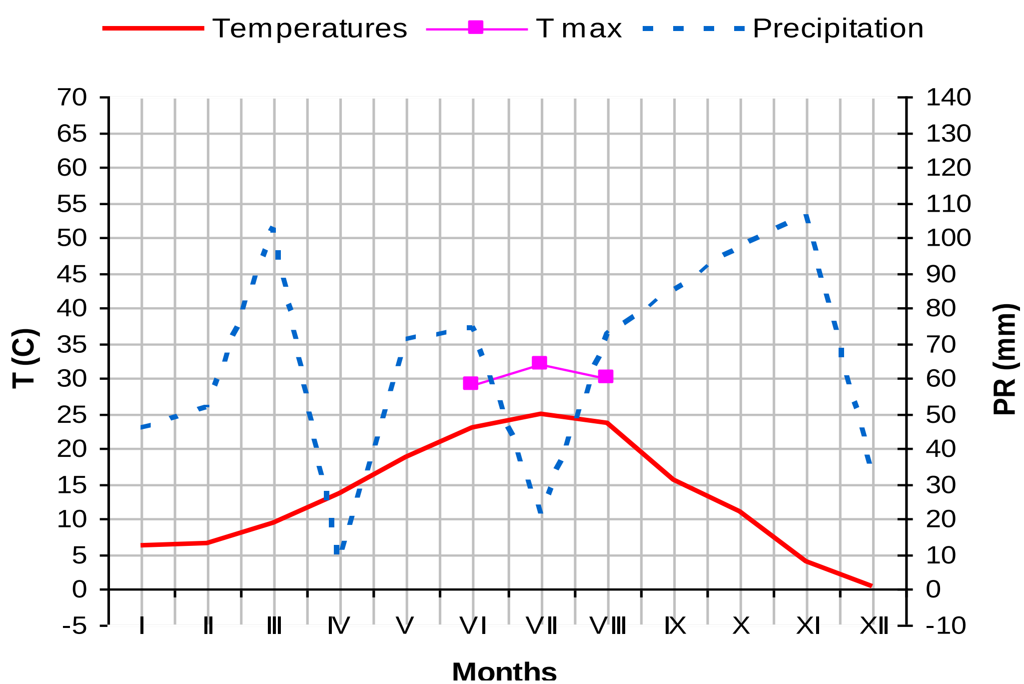

Figure 1. Climate diagram(Walter,1970) for 2007; average precipitations (PR), average day temperatures (T) and average maximum day temperatures (T max) in Republic of Serbia.

Experimental design of field trial

The trial was designed with eight inoculated and three uninoculated controlled treatments. Inoculated treatments consisted of 4 only inoculated treatments with single bradyrhizobial strains (542, 526, 525, and Knj) and 4 combined treatments of these single strains and mineral N rate of 20 kg N ha^{-1}. Inoculated treatments were compared with 3 controlled uninoculated treatments (Ø, N_{20}Ø and N_{40}Ø): untreated control-Ø (without any treating), fertilized control with 20 kg N ha^{-1}-N_{20}Ø and fertilized control with 40 kg N ha^{-1}-N_{40}Ø. Fertilization was applied as potassium ammonium nitrate-KAN (27% N).

The experiments were laid out in completely randomized system with three replicates. Experimental plot was 0.9 m x 2 m. Seeds were sown in the first week of May maintaining 30 cm row-to-row and 10 cm plant-to-plant spacing. Each plot was planted as three rows of 2 m length.

Measurement of plant parameters

Forty-two days after sowing (DAS) samples of 10 plants were randomly collected from each plot to evaluate root nodulation. Then, 67 DAS plants from the first row of experimental plots were harvested to determine quantity, shoot dry weight (SDW) as well as quality, %N in shoot dry matter (SDM). At the end of September, 120 DAS (at maturity stage) the crop was harvested to measure grain yield; the matured pods were collected by hand plucking from the plant of each treatments and air drying and threshing were done.

The percentage of shoot and grain nitrogen was determined by CNS Elemental Analyzer (Vario EL III) and it was used to calculate total and fixed N content as well as protein yield in kg ha^{-1}. Fixed N was calculated by subtracting total N content of control-Ø from total N content of each treatment inoculated (Vincent, 1970). Fixed N as percentage of N accumulated amount (total N content) was determined as the amount of fixed N x 100 / the amount of total N content (Remans et al., 2008).

Percentage of yield increase represented percentage of increase of a grain yield as well as a shoot dray weight yield in respect to control-Ø expressed as 100%.

Statistical analysis

All data were subject to ANOVA using the statistical analysis system (SPSS). Means of all treatments were calculated and the differences were tested for significance using the LSD test at the 0.5 probability level. Correlation coefficients were calculated to study the associative relations among the measured traits.

RESULTS

Grain yield

The effect of bradyrhizobial inoculation on quantity and quality of grain yield of mungbean in the two soil types, the Fluvisol and Humofluvisol, was estimated and results were presented in Table 2. Seed inoculation was done with four active bradyrhizobial strains both individually and in combination with 20 kg N ha^{-1} in the form of N mineral fertilizer.

In Fluvisol soil, among all inoculated treatments the greatest grain yield was recorded by seed inoculation with strain 542 in combination with (1.301 t ha^{-1}) and without 20 kg N ha^{-1} (1245 kg ha^{-1}). Results showed that there were no significant differences between these two treatments as well as fertilized control (N_{40}Ø) in which N mineral fertilizer of 40 kg N ha $^{-1}$ (1325 kg ha^{-1}) was applied. Significantly lower grain yield was obtained by inoculation with the remaining strains 526, Knj and 525 with and without application of N mineral fertilizer (1007-1167 kg ha^{-1}) as well as in fertilized control with 20 kg N ha $^{-1}$(N_{20}Ø) (1028 kg ha^{-1}).. Untreated control treatment-Ø gave the lowest grain yield (816 kg ha^{-1}), significantly lower from all the other treatments.

In Humofluvisol soil, the grain yield of all treatments was considerably lower by 15-43% than that in the Fluvisoli, it varied from 710 to 930 kg ha^{-1}. The greatest yield obtained by inoculation with strain 542 in combination with 20 kg N ha^{-1} (930 kg ha^{-1}) was not significantly higher than grain yield obtained with 542 strain (910 kg ha^{-1}) as well as in N_{40}Ø fertilized control (897 kg ha^{-1}). As in the Fluvisol, significantly lower grain yield was obtained by inoculation with the remaining strains without and with 20 kg N ha^{-1} (785-860 kg ha^{-1}) as well as in N_{20}Ø fertilized control (780 kg ha^{-1}). In addition, as in the Fluvisol, untreated control (Ø) gave the lowest grain yield (710 kg ha^{-1}).

The effect of bradyrhizobial inoculation on grain yield depended on treatments and soil types and it was expressed by percentage of grain yield increase in respect to untreated control-Ø (Table 2).

Inoculation with single strains increased the grain yield by 23-53% in the Fluvisol as well as by 11-28% in the Humofluvisol over control-Ø (Table 2). Combined treatments of inoculation and 20 kg N ha^{-1} increased the grain yield by 28-59% in the Fluvisol and by 11-31% in the Humofluvisol. In addition, fertilization with 40 kg N ha^{-1} increased grain yield by 62 and 26%, in the Fluvisol and Humofluvisol, respectively. Among inoculated treatments the highest percentage of grain yield increase was realized in treatments with strain 542 on the both soil types.

Data showed that quality of grain was also higher in the Fluvisol (by 28-70% according to total N content) than that in the Humofluvisol (Table 2). Percentage of N varied from 4.00 to 4.82% in the Fluvisol while in the Humofluvisol was lower and more variably; from 3.60 to 4.64%.

In Fluvisol location, total N content (TNC) and protein yields were in correlation with grain yield. Combined treatment of strain 542 and 20 kg N ha^{-1} as well as N_{40}Ø fertilized control recorded the same values of TNC, 60.13 and 60.15 kg ha^{-1}, respectively. The greatest values of protein yield were also in these treatments (about 370 kg ha^{-1}). Significantly lower TNC and protein yield had grain in treatments with 542 strain inoculation alone (56.46 and 353 kg ha^{-1}, respectively) and in combined treatment of 526 strain (53.30 and 333 kg ha^{-1}, respectively). In N_{20}Ø fertilized control and treatments with remaining strains

Table 2. Grain yield of mungbean (*Vigna radiata* (L.) Wilczek) and its quality in inoculated treatments with single bradyrhizobial strains and in combination with N mineral fertilizer on different soil types.

Soil type	Treatment*		Grain yield kg ha^{-1}	% grain yield increase over Ø	%N	Total N content kg ha^{-1}	Fixed N kg ha^{-1} (%)	Protein yield kg ha^{-1}
	Strain	N Fertilizer kg ha^{-1}						
Fluvisol	542	0	1245^{ab}	153	4.53	56.46^{b}	23.82^{a}	353^{b}
		20	1301^{a}	159	4.62	60.13^{a}	12.94^{b}	378^{a}
	526	0	1135^{c}	139	4.39	49.87^{cd}	17.23^{b}	312de
		20	1167^{bc}	143	4.57	53.30^{bc}	6.11^{c}	333^{bc}
	Knj	0	1058^{d}	130	4.59	48.55^{de}	15.91	303de
		20	1174^{bc}	144	4.48	52.63^{c}	5.44^{c}	329^{bcd}
	525	0	1007^{d}	123	4.56	45.95^{e}	13.31^{b}	287^{e}
		20	1042^{d}	128	4.82	50.18^{cd}	2.99^{c}	314^{cd}
	Ø	0	816^{e}	100	4.00	32.64^{f}	0	204^{f}
	N_{20}Ø	20	1028^{d}	126	4.59	47.19^{e}	0	295^{e}
	N_{40}Ø	40	1325^{a}	162	4.54	60.15^{a}	0	376^{a}
LSD 0.05			76			3.46	3.39	23
Humo fluvisol	542	0	910^{a}	128	3.73	33.92^{c}	8.36^{a}	290^{cd}
		20	930^{a}	131	3.80	35.31^{bc}	4.11^{bc}	309^{bc}
	526	0	790^{cd}	111	3.62	28.56^{e}	3.00^{c}	256^{e}
		20	860^{b}	121	3.75	32.27^{d}	1.07^{d}	273de
	Knj	0	785^{d}	111	4.41	34.60^{c}	9.04^{a}	292^{bcd}
		20	820^{c}	115	4.30	35.27^{bc}	4.07^{bc}	316^{b}
	525	0	805^{cd}	113	4.38	35.28^{bc}	9.72^{a}	276de
		20	790^{cd}	111	4.64	36.62^{b}	5.42^{b}	302^{bc}
	Ø	0	710^{e}	100	3.60	25.56^{f}	0	160^{f}
	N_{20}Ø	20	780^{d}	110	4.00	31.20^{cd}	0	195^{d}
	N_{40}Ø	40	897^{a}	126	4.36	39.11^{a}	0	361^{a}
LSD 0.05			34			1.40	1.51	23

*Ø -untreated control; $Ø_{20}$N and $Ø_{40}$N –uninoculated, N fertilizer controls with N content of 20 and 40 kg ha^{-1} respectively; *Bradyrhizobium* spp. strains with or without mineral N of 20 kg ha^{-1} N; Means with the same letter within a column do not differ significantly (P<0.05).

with and with out application of N mineral fertilizer, TNC and protein yield varied from 45.95 to 53.30 kg ha^{-1} and from 287 to 333 kg ha^{-1}. These data indicated that fertilization of 20 kg N ha^{-1} in combination with inoculation by effective strain 542 could be applied for quality grain in the Fluvisol and can replace fertilization of 40 kg N ha^{-1}.

In Humofluvisol location, grain yield increase was not followed by N% increase in grain as well as TNC and protein yield except in fertilized control; the highest TNC and protein yield in grain (39.1 and 361.01 kg ha^{-1}) were reached in fertilized control in the Humofluvisol. Despite of its greatest grain yield, combined treatment of strain 542 and 20 kg N ha^{-1} did not have the best quality due to lower value of N% (3.73%). There were no clear differences between treatments based on quality of grain. The similar quality of grain had all treatments with strain 525, 542 and Knj (with and without N fertilizer). However, considering the highest obtained grain yield, strain 542 in combination with 20 kg ha^{-1} can be recommended for application in the Humofluvisol. The lowest quality of grain (TNC of 25.56 kg ha^{-1}) was noted in the control treatment (Ø) on the booth soil types which was in line with grain yield.

These results indicated that in investigated soil types inoculation with the strain 542 gave high grain yield while high quality of grain obtained in its combination with

Table 3. Quantity and quality of shoot dry matter of mungbean (*Vigna radiata* (L.) Wilczek) in inoculated treatments with single bradyrhizobial strains and in combination with fertilization by mineral N on different soil types.

Soil type	Treatment*		Shoot dry weight (SDW) kg ha^{-1}	% SDW increase over Ø	%N	Total N content kg ha^{-1}	Fixed N Content %	Protein yield kg ha^{-1}
	Strain	Mineral N kg ha^{-1}						
Fluvisol	542	0	8786^{bc}	132	2.29	201.52^{cd}	72.53^{b}	1260^{cd}
		20	8666^{bc}	130	2.35	203.25^{cd}	41.06^{d}	1270^{cd}
	526	0	8453^{bc}	127	2.19	185.50^{e}	56.51^{c}	1156^{e}
		20	9807^{a}	148	2.27	222.25^{b}	60.06^{bc}	1389^{b}
	Knj	0	8231^{c}	124	2.43	199.72^{cd}	70.73^{bc}	1247^{d}
		20	7517^{d}	113	2.26	169.56^{f}	7.37^{e}	1060^{f}
	525	0	8408^{bc}	126	2.33	196.28^{d}	67.29^{bc}	1227^{d}
		20	8991^{b}	135	2.77	249.32^{a}	87.13^{a}	1557^{a}
	Ø	0	6649^{e}	100	1.94	128.99^{g}	0	806^{g}
	N_{20}Ø	20	7440^{d}	112	2.18	162.19^{f}	0	1040^{f}
	N_{40}Ø	40	8769^{bc}	132	2.40	210.37^{c}	0	1316 ^{c}
LSD 0.05			444			10.53	10.99	65
Humo fluvisol	542	0	7805^{cd}	133	3.00	234.15^{abc}	116.35^{a}	1463^{b}
		20	7570de	129	3.42	258.89^{a}	73.89^{b}	1617^{a}
	526	0	7734^{d}	131	2.96	228.93^{bc}	111.13^{a}	1431^{b}
		20	8342^{a}	142	2.05	254.01^{ab}	69.01^{bc}	1587^{a}
	Knj	0	7400^{ef}	126	2.44	180.56^{d}	62.76^{c}	1128^{e}
		20	7137^{f}	121	2.95	210.54^{c}	25.54^{e}	1316^{c}
	525	0	7600de	129	2.21	167.96^{d}	50.16^{d}	1047^{f}
		20	8020^{bc}	136	2.35	188.47^{d}	3.47^{f}	1178^{d}
	Ø	0	5890^{g}	100	2.00	117.80^{e}	0	736^{g}
	N_{20}Ø	20	7385^{ef}	125	2.50	185 d	0	1154^{d}
	N_{40}Ø	40	8110^{ab}	138	2.70	218.97^{bc}	0	1368^{c}
LSD 0.05			267			22.07	7.87	44

*Ø -untreated control; Ø$_{20}$N and Ø$_{40}$N –uninoculated, N fertilizer controls with N content of 20 and 40 kg ha^{-1} respectively; *Bradyrhizobium* spp. strains with or without mineral N of 20 kg ha^{-1} N; Means with the same letter within a column do not differ significantly (P<0.05).

20 kg N ha^{-1}. In both cases these treatments can replace fertilization of 40 kg N ha^{-1}.

Biomass production

The effect of bradyrhizobial inoculation on quantity and quality of SDM yield of mungbean on the both soil types was presented in Table 3. In Fluvisol, inoculation with strain 526 in combination with 20 kg N ha^{-1} gave significantly the greatest shoot dry weight (SDW), 9.807 t ha^{-1} (Table 3). There were no clear differences between the remaining treatments with exception of significantly the lowest SDW noted in combined treatment of strain Knj and 20 kg N ha^{-1} and untreated control (Ø) (6.649 t ha^{-1}). The second greatest yield was obtained in combined treatment of strain 525 and 20 kg N ha^{-1}. Based on shoot dry weight, all bradyrhizobial strains showed the same effectiveness with exception of strain Knj which showed lower SDW.

Similarly to the results of grain yield, in Humiofluvisol all treatments gave lower shoot dry yield than in Fluvisol. The highest shoot dry weight was as in treatment in Fluvisol: inoculation with strain 526 in combination with 20 kg N ha^{-1} (8.342 t ha^{-1}). There were no significant differences between this treatment and N_{40}Ø-fertilized control (8.11 t ha^{-1}). Significantly lower values of SDW were obtained by inoculation with single strains 542

Table 4. Correlation coefficient for plant characters in mungbean inoculated with bradyrhizobial strains with and without mineral fertilizer of 20 kg N ha-1.

	Grain yield	Shoot dry weight	Grain total N content	Shoot total N content	Grain fixed N	Shoot fixed N
Grain yield	1					
Shoot dry weight	0.697*	1				
Grain total N content	0.969***	0.718**	1			
Shoot total N content	0.208	0.616*	0.143	1		
Grain fixed N	0.446	0.399	0.460	0.044	1	
Shoot fixed N	0.052	0.383	-0.022	0.624*	0.459	1

*P < 0.05 (significant), ** P < 0.01 (highly significant), *** P < 0.001 (extremely significant).

(7.805 t ha^{-1}) and 525 (7.600 t ha^{-1}) without significant differences between them.

Inoculation influenced SDW increase by 24-32% in Fluvisol as well as by 26-33% in HumoFluvisol compared to untreated control-Ø while combined treatments increased SDW by 13-48% and 21-42% in Fluvisol and Humofluvisol, respectively (Table 3). N_{40}Ø fertilized control in the both soil types increased SDW by 32 and 38% in respect to untreated control (Ø). The highest percentage of shoot yield increase was in combined treatments of strain 526 and 20 kg N ha^{-1} on the both soil types.

Percentage of N in shoot dry matter of inoculated treatments varied from 2.19 to 2.77% and from 2.21 to 3.00% in Fluvisol and Humofluvisol, respectively. Slightly higher %N had the plants simultaneously inoculated and fertilized in respect to only inoculated plants.

The best quality in Fluvisol had combined treatment of strain 525 and 20 kg N ha^{-1} (249.32 kg ha^{-1} of TNC and 1.557 t ha^{-1} of protein yield) while significantly lower quality was detected also in combined treatment of strain 526 (222.25 kg ha^{-1} of TNC and 1.389 t ha^{-1} of protein yield). In Humofluvisol the best quality had combined treatments of strains 526 (254.01 kg ha^{-1} of TNC and 1587 kg ha^{-1} of protein yield) and strain 542 (258.89 kg ha^{-1} of TNC and 1617 kg ha^{-1} of protein yield).

These results indicated that in investigated soil types inoculation with the strain 526 in combination with 20 kg N ha^{-1} gave the highest yield and one of the highest quality of SDM and can replace fertilization of 40 kg N ha^{-1}, while strain 542 gave the highest quality in Humofluvisol. Based on quality and quantity of SDM obtained by inoculation with single strain, strains 526 and 542 showed high efficiency in the both soil types.

Fixed N in the grain and shoot dry matter

Amount of grain fixed N in the both soil types was low: in Fluvisol was up to 12.94 kg ha^{-1} in combined treatments and 23.82 kg ha^{-1} in treatments with inoculation alone while in Humofluvisol was less than 10 kg ha^{-1}. Also, the percentage of fixed nitrogen in grain total N content showed that N-fixing activity of strains was higher in the Fluvisol (29-42%) than in the Humofluvisol (11-26%) (Table 2).

Amount of fixed N was higher in shoot matter than that in the grain. In SDM single strains fixed 56.51-72.53 kg N ha^{-1} and 49.78-116.35 kg N ha^{-1} in Fluvisol and Humofluvisol, respectively which is 31-36% and 30-50% of total N content in SDM in Fluvisol and Humofluvisol, respectively (Table 3).

The strain 542 fixed N in SDM and the grain with the highest effectiveness on the both soil types Taking into account high efficiency of strain 542 and its high grain yield, we can conclude that strain 542 was more effective and has the greatest potential to be active agent of microbiological N fertilizer.

Correlation coefficient

Correlation coefficient between plant characters in mungbean - bradyrhizobium associations (Table 4) in the both soil types, indicated that grain yield was in positive significant correlation with SDW (r = 0.697) and grain total N content (r = 0.969). In addition, there is significant correlation between SDW and shoot (r = 0.616) and grain (r = 0.718) total N content. Shoot fixed N was in significant correlation with shoot total N content (r = 0.624).

ANOVA

In our investigation mungbean was evaluated for their symbiotic performances with four bradyrhizobial strains with and without N mineral fertilizer. The effects of the strain, mineral N and soil type on the variation of SNF were measured by three factorial analysis of variance for SDW and grain yield. Plant SDW is the best parameter to evaluate symbiotic activity of legume-Rhizobium associations while seed yield is an important trait as it measures the economic productivity in mungbean. Based on three

Table 5. Analysis of variance for shoot dry weight and grain yield of mungbean inoculated with four bradyrhizobial strains.

Source of variance	Shoot dry weight (kg ha^{-1})	Grain yield (kg ha^{-1})
Strain	^{s}126.37 ***	81.76 ***
Type of soil	214.40 ***	854.54 ***
N	50.72 ***	45.79 ***
Interactions		
Strain x type	5.65 **	12.69 ***
Strain x N	33.35 ***	4.12 **
Type x N	0.06 ^{ns}	8.74 **
Strain x type x N	5.33 **	3.13 *

^{s}F-values, *, **, *** Significant at P<0.05, 0.01 and 0.001, respectively.

factorial variance analysis, it was found that bradyrhizobial strains, mineral N application of 20 kg ha^{-1}, type of soil had highly significant (P<0.001) effects alone on SDW and grain yield on the both soil types (Table 5). Shoot dry weight was significantly affected by interaction between strain x type (P<0.01) and strain x type x N (P<0.01) as well as by strain x N (P<0.001) but not by type x N. Interactions of all factors in their combinations affected significantly grain yield.

DISCUSSION

Nitrogen fixing effectiveness of particular bradyrhizobial strains was the object of many investigations on mungbean with the aim of highly effective strain selection (Hafeez et al., 2001; Neeraj et al., 2008). The previous pot experiment in a semi-controlled greenhouse environment showed N fixing ability of specific rhizobial strains to mungbean (Delić et al., 2007). Highly effective strains from this pot experiment were chosen for checking their N fixing effectiveness in field condition. In the presented field trial, N fixing ability of these effective strains to mungbean for better quantity and quality of grain and SDM as well as reduced use of N mineral fertilizer was studied in two different soil types; Fluvisol and Humofluvisol.

Absence of nodules on plant roots of untreated control-Ø (data not shown) indicated that there were no indigenous rhizobial strains specific for mungbean in Fluvisol and Humofluvisol fields of presented study. This could be the consequence of a legume absence on investigated field locations because rhizobial soil count gradually decreases in parallel with the increase of the time elapsed from the presence of host plants in croprotation (Bottomley and Jenkins, 1983).

An adequate N supply for legumes is essential for normal plant growth and seed yield. The concentration of N containing solutes in the soil, especially that of nitrate may change quickly due to the processes such as uptake by plant roots and microorganisms, leaching and gentrification.

Therefore, it is recommended to apply appropriate amount of N mineral fertilizer directly before sowing even though a soil is well supplied with N (Mengel and Kirkby, 2001) as in presented trial. Application of N mineral fertilizers improves the plant productivity and enhances the grain yield (Rahman et al., 2002; Sarma and Sarma, 1999). Some authors have reported an increase in grain yield with increased level of N mineral fertilizer (Rahman et al., 2002; Sadeghipour et al., 2010; Ali et al., 1999; Nadeem et al., 2004). In mungbean production application of mineral N fertilizer varies from 15-90 kg N ha^{-1} depending on soil type and form of soil N available for the plant (Ahmad et al., 2001; Mahboob and Asghar, 2002). In native area (South and Southern Asia) mungbean is grown without application of mineral N fertilizer or with 10-20 kg N ha^{-1} (Ali, 2011; Ullah et al., 2011). In the presented trial the highest grain yield of mungbean was obtained in fertilized control treatment with 40 kg N ha^{-1}, in Fluvisol which is in agreement with results of some authors (Ayub et al., 1999; Mozumder et al., 2003). There is a level of N which represents an optimum supply for given crop on a particular soil type. Adding more mineral N fertilizer than an optimum amount does not increase yield and crop quality and will not be taken up by the crop usuallydue to the leaching to ground water. In addition, an important environmental factor which interferes with N_2 fixation is the presence of more mineral N in the soil, usually as nitrate (NO^-_3) (Heridge et al., 2005). Because of that, good management of N fertilization with particular reference to optimum amount of fertilizers is important for maximum growth of crop, enhancement of grain yield as well as N_2 fixation (Kaushal et al., 2006).

Many researches confirmed that inoculation of mungbean with effective rhizobial strains increase plant height and dry matter production as well as seed yield (Brar and Lal, 1991; Thakur and Panwar, 1995; Provorov et al., 1998; Sharma, 2001; Anjum et al., 2006; Mansoor, 2007). In this trial the inoculation of mungbean crop with bradyrhizobial strains significantly increased grain yield (from 11-53%) which is in concordance with findings of some authors (Mansoor, 2007). A significant effect of strains on grain yield was detected in our trail ($P<0.001$). Presented results showed that plant inoculation only with strain 542 enabled one of the highest grain yield that was not significantly different than grain yield obtained in fertilized control- N_{40}Ø which is in agreement with results of some authors (Mansoor et al., 2007). Based on grain quality, the second highly effective strain in Fluvisol was the strain 526 while in Humofluvisol it was 525.

However, some researches showed that the best grain yield was obtained by combined application of rhizobial inoculation and N mineral fertilizer (Provorov et al., 1998; Kashem et al., 2000; Ashraf et al., 2003; Nadeem et al., 2004; Anjum et al., 2006). Shivesh et al. (2000) reported that *Bradyrhizobium* inoculation and NPK fertilizer significantly increase the grain and straw yields of mungbean. Application of 20 kg N ha^{-1} by Tripathi et al. (1994) and Rahman et al. (2002) resulted in seed yield which was statistically similar with rhizobial inoculation alone. These results and data of mungbean N fertilization (Sadeghipour et al., 2010) as well as applied amount of 40 kg N ha^{-1} in N_{40}Ø-fertilized control directed our trial to research inoculation in combination with 20 kg N ha^{-1}. Presented results indicated that combined treatments of inoculation and 20 kg ha^{-1} enabled a slightly better quality and quantity of grain and SDW than treatments with inoculation alone.

According to our results in the both soil types, inoculation with the strain 542 gave one of the highest grain yield while the highest quality of grain was obtained also by N fixing ability of the strain 542 but in combination with 20 kg N ha. The nitrogen directly increases the plant protein content in the shoot and grain of *Vigna* (Shadi et al., 2002; Sadeghipour et al., 2010). Presented results indicated that the most effective strain proved to be *Bradyrhizobium* strain 542 which in combination with 20 kg N ha^{-1} could replace application of 40 kg N ha^{-1} on the both soil types.

Introduction of food legume into new areas faces the crop with new environmental conditions. Information of environmental factors effect on improvement of crop growth is important in development of appropriate management practices. One of the major causes of low crop yield is decline of soil fertility. Type of soil is one of the most important factor influencing the yield of mungbean which significant influence was confirmed in presented research by analysis of variance for shoot dry weight and grain yield of mungbean ($P<0.001$). In general, *Vigna radiata* has ability to grow vigorously under wide range of environmental condition and different soil types without addition supplement N (Rafiei, 2009). However, some studies suggested that plant growth in most temperate ecosystems is limited by combination of N and water (Reed et al., 2007). In this trial growing potential of mungbean in agro-ecological condition in Serbia which belongs to the temperate continental climate zone was investigated. Approximately about 360 000 ha of a Fluvisol and Humofluvisol soils are used as arable field in Serbia. It makes them the important soil types in Serbia. Fluvisols cover 5% of Europe (Soil Atlas of Europe, 2005). They have chemical characteristic that can be suitable for crop cultivation while physical composition of Humoflivisol is less suitable for plant growing. Humofluvisol contains more clay in respect to Fluvisol (at a depth of entire soil profile the clay content was above 45%). Prevalence of small pores in Humofluvisol makes infiltration and drainage slow and negatively influence yield of mungbean.

In addition, two dry periods in 2007 (in the middle of April and in the end of June to the beginning of August with 21.6 mm rainfall in July) as well as maximal temperature of 31.4˚C in July (Figure 1) influenced decrease of mungbean yield in Humofluvisol. In spite of grate amount of rainfall that was expressed in 2007, uneven rainfall distribution during the plant growing period had negative influence on yield attributes. In wet period at the beginning of vegetation Humofluvisol was more than wet with possible anaerobic processes but in dry period was tough and compacted with insufficient quantity of water accessible to the plants. These unfavourable environmental conditions could be one of the reasons for lower yield and plant quality of mungbean in Humofluvisol compared to Fluvisol. The process of N_2 fixation is sensitive to soil water decrease resulting in reduction of SDW as well as nodule number and weight (Ramos et al., 2003). In addition, high temperature influence persistence of rhizobial populations in the soils (Mansoor, 2007). However, thanks to the fact that mungbean is drought tolerant crop that can overcome adverse environmental condition (Anjum et al., 2006; Sheteawi and Tawfik, 2007; Abbas et al., 2011) in the presented trial mungbean has obtained the grain yield up to 930 kg ha^{-1} and SDW of about 8300 kg ha^{-1} in the Humofluvisol.

The highest grain yield of 1300 kg ha^{-1} (by 59% over control-Ø) obtained in Fluvisol pointed out possible good grain yield potential of mungbean in Serbian agro-ecological conditions which is in line with results of Tien et al. (2002). In addition, this value is in concordance with report of Mansoor (2007) that mungbean has the potential to produce seed yield higher than 1200 kg ha^{-1}. This was similar with results of FAO that the highest seed yields were recorded in growing countries like China (1286 kg ha^{-1}), Japan (1119 kg ha^{-1}) and Italy (1210 kg ha^{-1}) while the potential of mungbean to produce seed yield in Pakistan is 1200 kg (Mansoor, 2007). In the presented trial the grain yield from 710 to 1300 kg ha^{-1} was obtained. Untreated control (Ø) gave the lowest grain yield which is general findings of many scientific papers (Brar and Lal, 1991; Rajput et al., 1992; Rahman et al., 2002; Mansoor, 2007). In addition, values of grain yield in untreated controls in the presented trial indicated the big gap between potential and average yield of mungbean (Mansoor, 2007).

Good yield performance can be achieved by proper combination of agro technical measures (Sarkar et al 2004; Rajput et al., 1992; Nazir 1996; Boe et al., 1991; El Karamany, 2006; Mansoor, 2007; Sadeghipour, 2008). In this trial appropriate time of sowing, plant density and line to line spacing were applied to provide available sunlight and aeration as well as more space and nutrition in the root zone. They influenced effective nitrogen fixation (Talee et al., 1999; Rafiei, 2009). These agro technical measures resulted in satisfactory grain and shoot yield in Serbian agro-ecological condition without significant weed quantity and absence of plant protection during the period of vegetation. In addition, rhizobial inoculation positively influenced the grain yield ($P < 0.001$) which is in agreement with Provorov et al. (1998).

According to the results of some authors, *Rhizobium* inoculation in the field trial increased the grain yield by about 10% more than no inoculation (Adreshna et al., 1993; Sharma and Khurana, 1997). Similar results were obtained in our experiment in Humofluvisol (by 11-13% over untreated control-Ø) with exception for inoculation with strain 542 which increase grain yield much higher, by 28% over control-Ø. However, percentage of grain yield increase was higher in Fluvisol depending on strain and reached up to 53% with strain 542, compared to untreated control-Ø. Presented results of protein yield were in line with those reported by Rafiei (2009).

According to the presented results application of 20 kg N ha^{-1} in N_{20}Ø fertilizer treatment was statistically comparable with rhizobial inoculation which is in concordance with results of some authors (Tripathi et al., 1994; Rahman et al., 2002). Ayub et al. (1999) recorded 31% higher grain yield than control with 40 kg N ha^{-1} while in presented trial the increase of grain yield in the fertilized control depended on soil types; according to the presented results in Humofluvisol grain yield was lower (26%) but in Fluvisol doubled (62%) than untreated control-Ø indicating suitable soil characteristics for plant growing in Fluvisol.

Grain production of mungbean can follow its forage production. According to some authors mungbean can be used as dual-purpose crop; using its shoot matter and after its cuttings growing to the grain. However, its potential as dual-purpose crop is not clearly lighted. When seed production is the main object, mungbean can produce forage dry yield of about 4-5 t $ha^{-1}$and 2.62-2.76% of N depending on DAS (El Karamany, 2006). In this experiment quantity and quality of shoot dry matter of mungbean was investigated with the aim to use this plant as forage crop.

The nitrogen deficiency results in plant yield decrease (Sadeghipour et al., 2010). Presented results showed that inoculation with single rhizobial strains in Fluvisol and Humofluvisol increased the SDW in respect to untreated control-Ø which is in agreement with findings of Provorov et al. (1998) and Hayat et al. (2008). Amount of DSM of 9.8 t $ha^{-1}$was in line with results of Tien et al. (2002). Inoculation with single strain 542 and 526 increased the shoot dry yield by about 30% in the both soil types which was equal to application of 40 kg N ha in fertilized control. The combined treatment of inoculation and 20 kg N ha^{-1} increased the SDW for 13-48% and 21-42%, in Fluvisol and Humofluvisol, respectively. Mineral N and its interaction with strains highly influenced SDW ($P<0.001$) as well as grain yield ($P<0.01$). Our results indicated good quality of plant shoot dry matter in all inoculated treatments which is confirmed with results of Tien et al. (2002), El Kamarany (2006) and Rafiei (2009).

Our data of fixed N revealed that mungbean fixed substantial but variable quantities of N per hectare which is in agreement with data of Hayat et al. (2008). The most effective strain 542 fixed approximately 76 and 116 kg N ha^{-1} in Fluvisol and Humofluvisol, respectively which is more than fixation of effective rhizobial and bradyrhizobial strains in symbiosis with mungbean (about 55-60 kg N ha^{-1}) in some papers (Hayat and Ali, 2004; Mansoor, 2007). The total amount of fixed N is the resultant of proportion of plant fixed N and total N content accumulated in the crop (Hayat and Ali, 2004). According to the presented results coefficient of correlation showed that shoot fixed N was influenced by shoot total N content which is in agreement with the results of Hayat et al. (2008).

Genetic variation and environment were the object of investigation of some researchers (Atta and Shah, 2009; Ullah et al., 2011). Two tested soil types influenced the differences in mungbean growth in our trial. In addition, there were differences between inoculated treatments with and without N mineral fertilizer as in research of Atta and Shah (2009) and Ullah et al. (2011). In combined treatments on the both soil types, grain and shoot fixed N were mainly significantly lower in respect to inoculation alone since plants prefers mineral N in respect to N_2 from the air (Herridge et al., 1998).

Analysis of variance showed that strain, type of soil and mineral N and their interactions significantly effected shoot dry weight and grain yield. This suggested further investigation on different soil type, with high effective strains and different amount of mineral N for effective production of mungbean in symbiotic association with bradyrhizobial and rhizobial strains.

Presented results exhibited effectiveness of strains 542 and 526. According to the protein yield and total and fixed N content increase, strain 542 was highly effective without significant differences in comparison to its treatment in combination with mineral N.

Conclusion

All investigated characteristics of grain and SDM yield were significantly increased due to seed inoculation with particular rhizobial strains, with and with out 20 kg N ha^{-1}.

Shoot dry matter and grain yield were significantly influenced by bradyrhizobial strains, soil types, mineral N and their interaction. The combined application of seed inoculation with *B. japonicum* strain 542 and fertilization of 20 kg N ha^{-1} gave high quantity and quality of grain and SDM yield and can replace fertilization of 40 kg N ha^{-1}. This strain might be recommended as active agent of N microbiological fertilizer. Mungbean can be successfully grown as a food and forage crop in Fluvisol and Humofluvisol soils.

ACKNOWLEDGEMENT

This research was supported by the Ministry of Science and Environmental Protection of Republic of Serbia, Project, No TR-46007 and TR-20098.

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