Volume 5 No.9, September 2015 ISSN 2224-3577
International Journal of Science and Technology

http://www.ejournalofsciences.org

Response of legumes to co-inoculation with nodule bacteria and plant growth promoting rhizobacteria

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ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) of the genus *Pseudomonas* have been reported to increase the yield of non-leguminous plants. Inoculation with *Pseudomonas* has been found by some authors to improve nodulation of legumes. This work examined the response of various legumes to a combined inoculation with *Pseudomonas* and nodule bacteria in pot experiments under field conditions in gray forest soil of Russia's southern Moscow Region. Co-inoculation increased the weight of pea and soybean grain, alfalfa and clover shoots in comparison with nodule bacteria alone. Addition of mixed bacterial cultures promoted macro- and micronutrient uptake by legumes from soil. *Pseudomonas* improved N nutrition of pea and soybean by increasing amounts of N derived from air, soil and ^{15}N-labelled starter fertilizer. Nodulation and N_2 fixation of soybean inoculated with *Pseudomonas* and nodule bacteria were promoted by addition of P fertilizer in a greenhouse pot experiment. The beneficial effect of combined inoculation resulted from the promotion of symbiotic N_2 fixation and nutrient uptake by leguminous plants without any considerable change in the chemical composition of produce. PGPR-based inoculants can be used to increase the yield of leguminous crops without microfertilizers added.

Keywords *Pseudomonas, Legumes, Nutrient uptake, N_2 fixation*.

I. INTRODUCTION

Specific strains of pseudomonads have been shown, when incorporated into soil or inoculated into the rhizosphere, to increase the growth and yield of some crops [1, 2]. Our previous experiments have shown selective strains of *Pseudomonas* bacteria to increase the yield of such non-leguminous crops as radish [3], red beet and fodder beet [4, 5], winter wheat [6], oat and barley [7]. Mixed cultures of nodule and *Pseudomonas* bacteria when used as inoculants of various leguminous species improve nodulation compared with rhizobia alone [8-12]. Inoculant microbes can stimulate the performance of legumes by affecting symbiotic N_2 fixation. There are little data on the influence of inoculation with *Pseudomonas* on the yield and mineral nutrition of leguminous plants. Addition of *P. putida* M17 in greenhouse and field experiments did not considerably alter the yield of common bean despite stimulated nodulation [8]. None of *P. putida* strains tested had any effect on the growth of pea in the field and some strains promoted nodulation, N_2 fixation and growth of lentil in laboratory and field experiments [11]. Conversely, inoculation of alfalfa with a combination of *Rhizobium meliloti* and *P. fluorescens* PSIA12 enhanced shoot dry production in greenhouse and field experiments [12]. Some combined N is known to be required for the maximum growth of and nodule formation in legumes [13].

The aim of this work was to study the effects of *Pseudomonas* PGPR in combination with nodule bacteria on the yield of pea, soybean, alfalfa and clover; nutrient uptake by plants and symbiotic N_2 fixation against the background of NPK fertilizers added to gray forest soil under field conditions.

II. MATERIALS AND METHODS

Pot experiments under field conditions

Pea (*Pisum sativum* L. cv. Smaragd), soybean (*Glycine max* L. cv. Mageva), alfalfa (*Medicago sativa* L. cv. Slavyanskaya mestnaya) and clover (*Trifolium pratense* L. cv. VIK1) were grown in southern Moscow Region. Plants were grown in separate experiments in bottomless plastic pots (0.33×0.33×0.33 m, 0.1 m^{-2}) containing 30 kg of gray forest soil and sunk into the upper layer of soil. Each pot contained 11 plants. Soils were fertilized with 0.4 g N pot^{-1} (4 g N m^{-2}) as $(NH_4)_2SO_4$ against the background of 0.8 g P and 0.8 g K pot^{-1} (8 g P and 8 g K m^{-2}) as KH_2PO_4 and K_2SO_4 before sowing seeds. In experiments with pea and soybean, N fertilizer labelled with ^{15}N was used. Seeds were inoculated with water suspensions of pure and mixed bacterial cultures.

Pea was inoculated with a pure culture of *R. leguminosarum* bv. *viceae* 250a nodule bacteria in the first treatment and a mixture of *R. leguminosarum* bv. *viceae* 250a and the bacterium *P. fluorescens* 20 in the second treatment. In the experiment with pea, 11 oat plants (*Avena sativa* L. cv. Gambo) were grown in separate pots under the same conditions as the non-N_2-fixing control. In the first treatment, oat was treated with an autoclaved

culture of *P. fluorescens* 20; in the second treatment, with a living culture of the bacterium.

Soybean was amended with *Bradyrhizobium japonicum* USDA 110 nodule bacteria and combinations of *B. japonicum* USDA 110 and *P. fluorescens* 20 or *P. fluorescens* 21. In the treatment without inoculation, autoclaved suspensions of bacteria were used as the non-N_2-fixing control.

Alfalfa was treated with *Sinorhizonium meliloti* VKMB117 nodule bacteria and a mixture of *S. meliloti* VKMB117 and *P. fluorescens* 20.

Clover was inoculated with *R. trifolii* 348a nodule bacteria and a combination of *R. trifolii* 348a and *P. fluorescens* 20.

Nodule bacteria were added at a rate of 10^6 cells per plant. Nodule bacteria and *Pseudomonas* were applied at a ratio of 5:1. Treatments were done in four repeats. Pots remained fully exposed to atmospheric conditions and were irrigated with water up to 60% of water holding capacity (WHC) of soil when necessary.

Pea and soybean were harvested at maturity, alfalfa and clover were grown for two vegetation periods. Green mass of leguminous grasses was cut at flowering. In the first year, three cuttings of alfalfa and one cutting of clover were done; in the second year, two cuttings of both grasses were performed. Plants were dried at 70°C and weighed. Dry samples were thoroughly ground to uniform consistency; total N and ash elements in plant samples as well as atom% ^{15}N excess in pea grain and soybean plants were determined.

Greenhouse pot experiment

Three soybean plants were grown in a plastic pot (9 cm dia, 30 cm height) containing 3 kg of a soil–sand mixture (1:1 v/v). Phosphorus was added at 0 and 5 mg P 100 g^{-1} against the background of 4 mg N and 20 mg K 100 g^{-1} substrate. Seeds were inoculated with *B. japonicum* USDA 110 alone in the first set of treatments and with a combination of *B. japonicum* USDA 110 and *P. fluorescens* 21 in the second set. The same procedures as described above were carried out for inoculation with bacteria, fertilization and shoot dry-weight estimation. Treatments were done in five repeats. Substrate was watered up to 60% WHC. At the beginning of the pod setting stage (85 days after coming-up), shoots were harvested, roots and nodules were separated from the soil–sand mixture by washing with water. Weight, number and N_2ase activity of fresh nodules were established.

Plant analysis

Total N in plants was measured using the indophenol reaction after digestion of a 100-mg plant sample with 20-ml diluted H_2SO_4 (H_2SO_4:H_2O, 2:1 v/v) and 1-g catalyst (K_2SO_4 : Zn : Se: $CuSO_4 \cdot 5H_2O$, 100 : 24 : 2 : 0.2) as described earlier [14]. The atom% ^{15}N excess of the plant sample was determined by a NOI-5 emission spectrometer (Statron, Germany). A 100-mg plant sample was digested with a 5-ml mixture of concentrated H_2SO_4 and $HClO_4$ (10:1 v/v) to analyze for P with ascorbic acid as a reductant [15] and for K by a FlaPho 4 flame photometer (CarlZeiss, Germany). A 1-g plant sample was also digested with a 20-ml mixture of concentrated HNO_3 and $HClO_4$ (2:1 v/v) and analyzed for Ca, Mg, Fe, Zn and for Cu, Co, Mo by atomic absorption spectrophotometers (Perkin Elmer 303 (USA) and Perkin Elmer 503 (USA), respectively).

Quantification of N_2 fixation, ^{15}N-labelled fertilizer and soil N in plants

The percentage of N derived from ^{15}N-labelled fertilizer (%NdfFert) in pea grain, in grain and straw of soybean plants in pot experiments under field conditions was estimated as follows:

$$\%\text{NdfFert} = \frac{\text{Atom \% } ^{15}\text{N excess in plant sample}}{\text{Atom \% } ^{15}\text{N excess in fertilizer}} \times 100.$$

The percentage of N derived from the atmosphere (%Ndfa) or fixed N in pea grain and soybean plants in pot experiments under field conditions was found by the ^{15}N isotope dilution technique using, respectively, oat plants and non-inoculated soybean plants as a non-N_2-fixing control [16] as follows:

$$\%\text{Ndfa} = 1 - \left\{ \frac{\text{Atom \% } ^{15}\text{N excess (fs)}}{\text{Atom \% } ^{15}\text{N excess (nfs)}} \right\} \times 100,$$

where fs is the fixing system – plants inoculated with a mixed culture of nodule bacteria and *Pseudomonas*;
nfs is the non-fixing system – oat plants in the experiment with pea, or soybean plants inoculated with autoclaved bacterial cultures.
Soil N uptake by pea grain and above-ground mass of soybean plants was estimated as the difference between the total N and fixed N plus the uptake of ^{15}N-labelled fertilizer N.

The N_2ase activity of fresh nodules detached from roots in the greenhouse pot experiment was determined by the acetylene reduction assay in a C_2H_2 atmosphere (10% v/v) immediately after harvesting as described previously [17].

Statistical analysis

The results of the studies were processed statistically by the analysis of variance, in which the treatment effects were evaluated. Significant differences between treatments were established by the least significant differences (LSD). Calculations were performed using the Statgraphics software package. All tests were considered

significant at a 5% level ($P < 0.05$). Data for some parameters were also expressed as the mean ± standard deviation for each treatment. Significant differences between inoculated treatments and uninoculated control were determined using Student's *t*-test ($\alpha = 0.05$).

III. RESULTS

Crop yield in the field

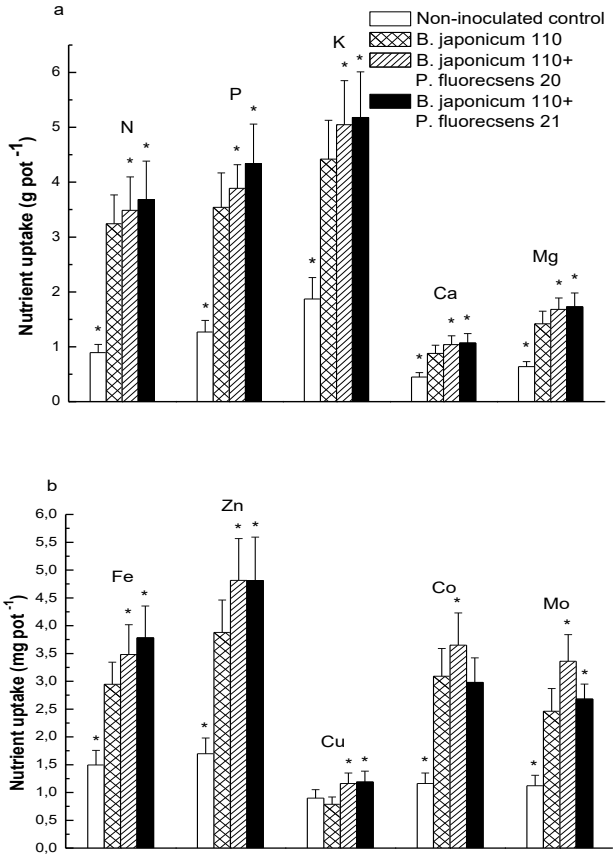
Table 1 shows the dry weights of leguminous plants grown in pot experiments under field conditions. Inoculation with a mixture of nodule bacteria and bacteria of the genus *Pseudomonas* had a favourable influence on plant growth resulting in a greater weight of pea and soybean grain, alfalfa and clover shoots in comparison with nodule bacteria alone. A mixed culture of *R. leguminosarum* bv *viceae* 250a and *P. fluorescens* 20 increased the grain weight of pea by nearly 30% over a single inoculation with nodule bacteria. Despite a more than twofold increase of the yield of soybean inoculated with nodule bacteria alone compared with non-treated plants, which was associated with the absence of *Bradyrhizobium* in gray forest soil, application of *P. fluorescens* 20 or *P. fluorescens* 21 had a significant additional effect. Mixed bacterial cultures increased the soybean grain weight over a single inoculation with nodule bacteria by 20% on average. Addition of *P. fluorescens* 20 in combination with *S. meliloti* VKMB117 increased the shoot dry weight of alfalfa by 33% for two vegetative seasons over the treatment with *S. meliloti* VKMB117. *P. fluorescens* 20 bacteria in combination with *R. trifolii* 348a increased the shoot dry yield of clover by 16% for two growing seasons unlike nodule bacteria alone.

Table 1. Yield of leguminous plants inoculated with nodule and *Pseudomonas* bacteria in pot experiments under field conditions. Different letters for each crop indicate statistically significant differences (P < 0.05).

Crop	Treatment	Dry weight (g pot^{-1})
Pea (grain)	*R. leguminosarum* bv. *viceae* 250a	39.1b
	R. leguminosarum bv. *viceae* 250a + *P. fluorescens* 20	49.7a
Soybean (grain)	no inoculation	18.7c
	B. japonicum 110	49.1b
	B. japonicum 110 + *P. fluorescens* 20	58.0a
	B. japonicum 110 + *P. fluorescens* 21	59.5a
Alfalfa (green mass of two growing seasons)	*S. meliloti* VKMB117	171.9b
	S. meliloti VKMB117 + *P. fluorescens* 20	227.9a
Clover (green mass of two growing seasons)	*R. trifolii* 348a	164.6b
	R. trifolii 348a + *P. fluorescens* 20	191.2a

Nutrient uptake by plants in the field

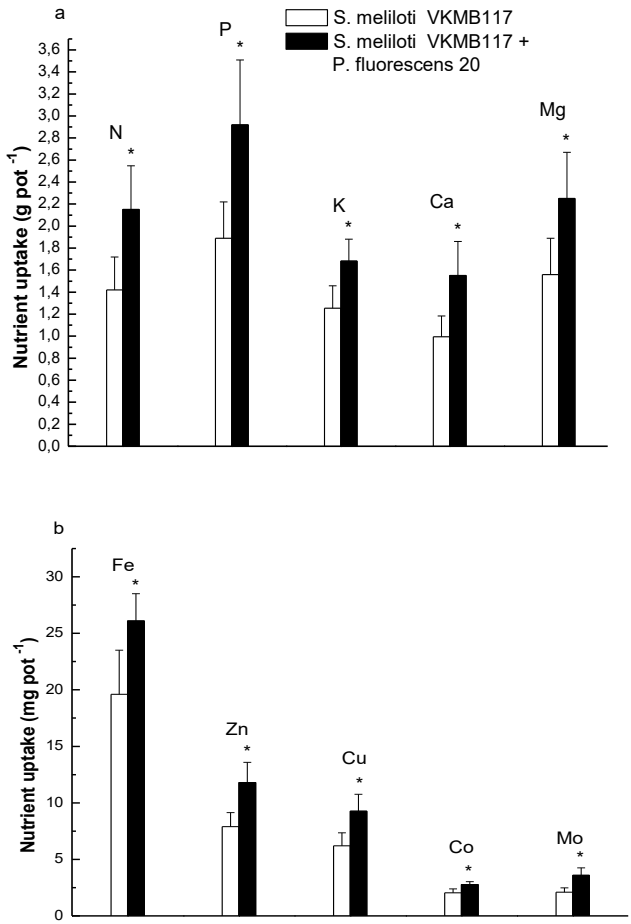
Addition of *Pseudomonas* in pot experiments under field conditions promoted mineral nutrition of soybean, alfalfa and clover (Figures 1-3). Despite a 3.6-fold increase of N uptake by grain of soybean inoculated with nodule bacteria *B. japonicum* 110 over the treatment without any bacteria, additional application of *P. fluorescens* 20 or *P. fluorescens* 21 increased this index by another 9–14% as compared with the treatment by nodule bacteria. The difference between the effects of the *Pseudomonas* strains was not statistically significant. Shoot N of clover and alfalfa treated with, respectively, *R. trifolii* 348a and *S. meliloti* VKMB117 in combination with *P. fluorescens* 20 was 26% and 52% higher than that of single inoculations with nodule bacteria. Application of mixed bacterial cultures considerably promoted ash element uptake by soybean, alfalfa and clover from soil. Highly significant increases in ash element uptake by soybean grain were recorded as the result of combined inoculation with nodule bacteria and pseudomonads. Accumulation of P, K, Ca



Asterisked data denote the values that differ significantly from those for the treatment with *B. japonicum* 110 ($P < 0.05$). P × 10, K × 10, Ca × 10, Mg × 10, Co × 10, Mo × 10.

Figure 1. Nutrient uptake by soybean grain in pot experiment under field conditions.

Mg, Fe and Zn in the grain of soybean treated with both mixed bacterial cultures were 10 to 28% higher than that in the treatment by *B. japonicum* 110. Addition of the bacterium *P. fluorescens* 20 increased the amounts of Co and Mo in soybean grain by, respectively, 18 and 37%, whereas in the treatment with *P. fluorescens* 21 a tendency for an increase was observed only for Mo. Cu uptake by soybean grain in the presence of both mixed bacterial cultures was 1.5 times greater than that in the treatment by nodule bacteria alone. Similar trends were obtained for alfalfa and clover plants. Combined inoculation with *P. fluorescens* 20 and *S. meliloti* VKMB117 promoted ash element accumulation in alfalfa shoots by 33–71% for two vegetative periods as compared with *S. meliloti* VKMB117. The highest Mo uptake by dry alfalfa shoots was observed for plants inoculated with the bacterial mixture. The bacterium *P. fluorescens* 20 in a mixed culture with *R. trifolii* 348a enhanced accumulation of ash elements except Cu in dry clover shoots by 14–30% for two growing seasons compared with rhizobia alone.



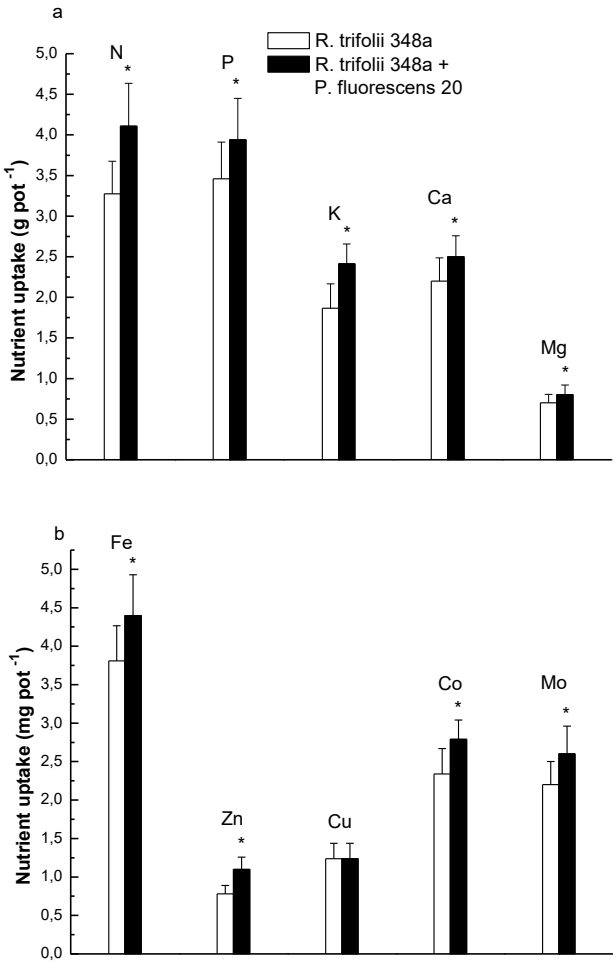
Asterisked data denote the values that differ significantly from those for the treatment with *S. meliloti* VKMB117 ($P < 0.05$). P × 10, Mg × 10, Cu × 10, Co × 10, Mo × 10^2.

Figure 2. Nutrient uptake by alfalfa green mass of two growing seasons in pot experiment under field conditions.

Application of *Pseudomonas* had a weak influence on the contents of most nutrients in leguminous plants (Tables 2, 3). The content of N increased only in alfalfa shoots of the

Table 2. Macronutrient concentrations in leguminous plants inoculated with nodule and *Pseudomonas* bacteria in pot experiments under field conditions. Soybean – grain, alfalfa and clover – green masses of second cuttings. Different letters for each crop indicate statistically significant differences ($P < 0.05$).

Crop	Treatment	N	P	K	Ca	Mg
		%				
Soybean	*B. japonicum* 110	6.61a	0.72	0.90	0.18	0.29
	B. japonicum 110 + *P. fluorescens* 20	6.01b	0.67	0.87	0.18	0.29
	B. japonicum 110 + *P. fluorescens* 21	6.19b	0.73	0.87	0.18	0.29
Alfalfa	*S. meliloti* VKMB117	2.09b	0.26	1.73	1.53	0.27
	S. meliloti KMB117 + *P. fluorescens* 20	2.45a	0.28	1.75	1.80	0.29
Clover	*R. trifolii* 348a	1.85	0.20	1.08	1.36	0.43
	R. trifolii 348a + *P. fluorescens* 20	2.00	0.20	1.22	1.37	0.42



Asterisked data denote the values that differ significantly from those for the treatment with *R. trifolii* 348a ($P < 0.05$). P × 10, Co × 10, Mo × 10^2.

Figure 3. Nutrient uptake by clover green mass of two growing seasons in pot experiment under field conditions

Table 3. Micronutrient concentrations in leguminous plants inoculated with nodule and *Pseudomonas* bacteria in pot experiments under field conditions. Soybean – grain, alfalfa and clover – green masses of second cuttings.

Crop	Treatment	Fe	Zn	Cu	Co	Mo
		(mg kg^{-1})				
Soybean	*B. japonicum* 110	60	79	15	6	5
	B. japonicum 110 + *P. fluorescens* 20	60	83	20	6	6
	B. japonicum 110 + *P. fluorescens* 21	60	78	20	5	5
Alfalfa	*R. meliloti* VKMB117	240	118	10	7	0.4
	R. meliloti VKMB117 + *P. fluorescens* 20	215	130	11	8	0.6
Clover	*R. trifolii* 348a	211	450	6	14	0.8
	R. meliloti VKMB117 + *P. fluorescens* 20	232	550	5	14	1.1

[a] *Acetylene reduction activity, μM C_2H_2 pot^{-1} h^{-1}*
[b] *Means ± SE of 5 replications*

second cutting affected by a mixed bacterial culture; conversely, in grain of double-inoculated soybean plants this index tended to decrease. Addition of the bacterium *P. fluorescens* 20 enhanced only the concentration of Mo in alfalfa and clover shoots of the second cuttings. Concentrations of other nutrients in each leguminous plant were the same both for the single and double inoculation.

Amounts of N fixed from the atmosphere, soil and ^{15}N-labelled fertilizer in pea grain and soybean plants in pot experiments under field conditions are given in Table 4.

Table 4. N derived from atmosphere, soil and ^{15}N fertilizer in pea and soybean plants inoculated with nodule and *Pseudomonas* bacteria in pot experiments under field conditions

Crop	Treatment	Ndfa	Soil N	NdfFert
		(mg pot^{-1})		
Pea (grain)	*R. leguminosarum* pv. *viceae* 250a	381	952	146
	R. leguminosarum pv. *viceae* 250a + *P. fluorescens* 20	588	1126	166
	LSD (P < 0.05)	95	119	14
Soybean (grain+ straw)	no inoculation	ND	845	156
	B. japonicum 110	2195	967	183
	B. japonicum 110 + *P. fluorescens* 20	2473	1106	210
	B. japonicum 110 + *P. fluorescens* 21	2399	1247	198
	LSD (*P* < 0.05)	120	100	15

ND – not dtermined.

Using the ^{15}N dilution method, combined inoculation with *Bradyrhizobium* and *Pseudomonas* was found to increase the amount of N derived from air in pea grain and soybean above-ground mass relative to nodule bacteria alone. Uptake of soil and ^{15}N-labelled fertilizer N by these leguminous plants inoculated with mixed bacterial cultures was also higher than that in the treatment by a single inoculation with nodule bacteria.

Soybean growth and N_2 fixation in greenhouse

P fertilization had a significant impact on the growth of and N_2 fixation by soybean in the greenhouse pot experiment (Table 5). Without P fertilization there was no

Table 5. Shoot dry weight, nodulation and nodule N_2ase activity of soybean inoculated with nodule and *Pseudomonas* bacteria in greenhouse pot experiment

Treatment	P (mg 100 g^{-1})	Shoot dry weight (g pot^{-1})	Nodule number (n pot^{-1})	Nodule raw weight (g pot^{-1})	Nodule ARA[ab]
B. japonicum 110	0	11.6	45	3.1	0.01 ± 0.01
B. japonicum 110 +*P.fluorescens* 21	0	10.1	36	3.1	0.01 ± 0.00
B. japonicum 110	5	11.9	56	3.2	0.16 ± 0.03
B. japonicum 110 +*P.fluorescens* 21	5	12.6	56	4.0	1.60 ± 0.44
LSD (P < 0.05)		1.8	10	0.4	

difference in shoot dry weight and nodulation of soybean between inoculations with *B. japonicum* 110 and a mixture of nodule bacteria (*B. japonicum* 110) with the bacterium *P. fluorescens* 21. The nodule N_2ase activity (μM C_2H_2 pot^{-1} h^{-1}) of non-fertilized soybean was very low in both treatments. Addition of 5 mg P 100 g^{-1} substrate substantially promoted the N_2ase activity of nodules especially in the presence of a mixed bacterial culture as compared with *B. japonicum* 110. Mixed bacterial culture caused an increased nodule weight, a 10-fold increase in nodule N_2ase activity and only a slight increase in shoot dry weight.

IV. DISCUSSION

The results of these studies indicate that a combined inoculation of pea, soybean, alfalfa and clover with *Pseudomonas* PGPR and nodule bacteria promotes the growth and mineral nutrition of leguminous plants in gray forest soil under field conditions of Russia's southern Moscow Region. Enhanced accumulation of major and minor elements (mg tube^{-1}) in clover plants inoculated with rhizospheric microorganisms including the bacterium *P. putida* was also observed in a laboratory experiment [9]. Addition of the bacterium *P. fluorescens* PsIA12 in combination with nodule bacteria increased P and K uptake by alfalfa shoots in pot and field experiments [12].

Promotion of nutrient uptake by legumes amended with *Pseudomonas* is undoubtedly associated with improved bioavailability of the nutrients in rhizospheric soil. Using the split-root technique, inoculation with *Pseudomonas* PGPR was shown to increase accumulation of nutrients in maize (*Zea mays* L.) mainly as a result of a direct influence on their availability in soil. Moreover, the

bacteria promoted the absorbing capacity of inoculated roots, which is due to an enhanced secretion of phenolic compounds by them [18]. Probably, the increase in the yield of legumes by inoculation with *Pseudomonas* is also due to production of growth factors by bacteria [19, 20] and inhibition of plant pathogenic fungi [1]. The culture media of *Pseudomonas* PGPR including the bacterium *P. fluorescens* 20 were found to accumulate indole-3-acetic acid [19]. Some PGPR may influence plant growth by synthesizing plant hormones or facilitating nutrient uptake from soil through solubilization of phosphorus and synthesis of siderophores for iron sequestration, making nutrients more available to plants [20].

The presented results on improved nodulation of soybean induced by *Pseudomonas* confirm the findings by other authors for faba beans [8], clover [9], soybean [10], lentil [11] and alfalfa [12]. Using the ^{15}N dilution technique, addition of *Pseudomonas* was proved to enhance the amount of N derived from air in pea and soybean plants as well as from soil and ^{15}N-labelled fertilizer. Stimulation of symbiotic N_2 fixation by combined inoculation can also be explained by an increased availability of Mo in rhizospheric soil; Mo is a constituent of the cofactor of N_2ase – an N_2-fixing enzyme – and other elements involved in microbial fixation of molecular N. Mineral nutrient deficiencies are a major constraint limiting legume nitrogen fixation and yield [21]. Chemical elements exert both a direct effect on plant growth by entering enzymes' active centres and an indirect effect by altering the metabolic processes in the plant. A possible explanation of the increased benefit for growth and N_2 fixation of legumes in the presence of *Pseudomonas* might include an improvement of physiological and biochemical processes in plants. The leaf area and CO_2 exchange of soybean and red beet plants [17, 22] and the activity of nitrate reductase enzyme in red beet leaves [22] were established to increase at an addition of *Pseudomonas*.

The results of a greenhouse pot experiment indicate that the deficit of P also limits symbiotic N_2 fixation and growth of soybean amended with a mixture of *Bradyrhizobium* and *Pseudomonas*. It was reported that the growth, nodulation and symbiotic N_2 fixation of soybean inoculated with various strains of *R. japonicum* in the pot experiment depended considerably on the P fertilization level [23]. Without P fertilization, N_2 fixation was observed to be low, and the presence of a mixed bacterial culture of *B. japonicum* 110 and *P. fluorescens* 21 was found to have no effect on soybean growth and nodulation. P fertilization improved nodulation and N_2 fixation of soybean inoculated with a mixed bacterial culture. Addition of P after the treatment of soybean with *Bradyrhizobium* and *Pseudomonas* promoted in the first place nodulation and most of all N_2 fixation.

Pseudomonas bacteria promoted macro- and micronutrient uptake from soil by legumes as the result of plant weight increase, but the concentrations of most elements in plants including grain did not change significantly; consequently, the quality of produce did not change. Efficient strains of *Pseudomonas* PGPR enhanced nutrient uptake from gray forest soil by various non-leguminous crops without significant changes in the contents of most elements in plants in pot experiments under field conditions [5, 6]. Moreover, *Pseudomonas* promotes accumulation of micronutrients in leguminous plants from soil without any microfertilizers added. It is known that application of mineral fertilizers including microfertilizers can change the concentrations of chemical elements, especially micronutrients, and the ratios of elements in plants [24, 25]; consumption of such a produce causes various human and animal diseases [24]. Probably, enhanced nutrient uptake by plants inoculated with *Pseudomonas* PGPR without altering the chemical composition of the yield is, unlike mineral fertilizers, due to biological mechanisms of plant–microbe interaction.

V. CONCLUSIONS

These results demonstrate that, in Russia's temperate climate, inoculation with selective *Pseudomonas* PGPR strains in combination with nodule bacteria increases the yield of pea and soybean grain, green mass of alfalfa and clover in gray forest soil under field conditions in comparison with nodule bacteria alone. Combined inoculation enhances the uptake of macro- and micronutrients by leguminous plants from soil slightly altering their chemical composition. Bacteria of the genus *Pseudomonas* improve N nutrition of legumes increasing the amounts of N derived from air, soil and ^{15}N-labelled fertilizer in pea and soybean. P fertilizer promotes nodulation and symbiotic N_2 fixation of soybean amended with a mixed bacterial culture of *Bradyrhizobium* and *Pseudomonas*. *Pseudomonas* promotes micronutrient uptake by plants from soil and legume growth without any microfertilizers added.

Acknowledgements

The author would like to express his appreciation to Dr. V. Yu. Smolin for technical support and helpful discussion throughout these studies.

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