ISSN 1392-3196
Zemdirbyste / Agriculture, vol. 93, No. 4 (2006), p. 210-220
UDK 631.461.5:631.415.1

THE INFLUENCE OF SOIL ACIDITY ON SYMBIOTIC AND NON - SYMBIOTIC NITROGEN FIXATION

Edmundas LAPINSKAS, Loreta PIAULOKAITĖ-MOTUZIENĖ

Lithuanian Institute of Agriculture
Vėžaičiai, Klaipėda district
E-mail: edmundas@vezaiciai.lzi.lt

Abstract

The data of laboratory and field experiments on biological nitrogen fixation by *Sinorhizobium meliloti*, *Rhizobium leguminosarum* bv. *trifolii*, *Rhizobium galegae* and *Rhizobium leguminosarum* bv.. *viciae* in different acidity soils was generalized in the paper. Depending on the species of rhizobia and soil pH_{KCl} legume symbiosis fixed from 160 to 264 kg N ha^{-1}. Investigating symbiotic nitrogen fixation, the minimal (5.0 pH_{KCl}) and optimal (6.5-7.0 pH_{KCl}) soil acidity was established for all species studied. The liming ($CaCO_3$ rate 6.2 t ha^{-1}) in combination with inoculation of red clover increased the fixed nitrogen from 169 to 275 kg N ha^{-1}. Ecologically adapted rhizobia strains formed more effective symbiosis with goat's rue and field bean than non-adapted. Associative nitrogen fixing bacteria in rhizoplane of barley and timothy accumulated on average 18.0-20.4 kg N ha^{-1} from the atmosphere.

In acid soils free nitrogen fixing microorganisms accumulated 0.6-2.3 μM N g^{-1} soil h^{-1} depending on soil pH_{KCl} and duration of plant vegetation.

Key word: nitrogen fixation, rhizobia, species, symbiosis, nitrogenase, soil acidity.

Introduction

The process of biological nitrogen fixation has a great importance in sustainable and organic farming systems. From atmosphere biological nitrogen is accumulated by symbiotic and non-symbiotic microorganisms which are named diazotrophs /Hamdi, 1982/. Mostly nitrogen is fixed by the symbiosis between rhizobia and legumes. The total value of biological nitrogen depended on genetic and physiological properties of symbiotic system. Perennial legumes fixed 40-470 kg N ha^{-1} /Kelner et al., 1997; Elgersma, Schlepers, 2001; Koc', 2001/ and annual legumes fixed 30-200 kg N ha^{-1} /Carranca et al., 1999/. Significantly less (14-50 kg N ha^{-1}) of biological nitrogen was accumulated by the associative and free nitrogen fixing microorganisms /Umarov, 1987/.

The scale of fixed nitrogen is defined by many factors. One of the most influencing ones are the form and rate of this process is soil acidity. The minimal soil pH for different species of rhizobia is unequal. *Sinorhizobium meliloti,* for example, is attached to mostly sensitive to soil acidity bacteria. The critical pH for nitrogen fixation ranges from 5.0 to 6.0. Considerable tolerance (pH 4.0-4.7) to soil acidity has *Rhizobium leguminosarum* bv. *trifolii* /Lindstrom, Myllyniemi, 1987; Ricghardson et al., 1989/ and *Rhizobium leguminosarum* bv. *viciae* – pH 4.5 /Ozawa et al., 1999/. Finally, the opinion that *Bradyrhizobium lupini* is most tolerant to acidic soil reaction exists. They can fix the biological nitrogen even when soil pH_{KCl} is 3.8-4.0 /Kaminskij, Golodna, 2000/. The optimal soil reaction for most of rhizobia species is close to neutral. However, the significance of present soil pH means can change and it depends on soil conditions especially on Al toxicity /Shilnikov et al., 1996/.

One of the central topics in increase on nitrogen fixation is legume inoculation with effective and ecological adapted rhizobia strains, which have become more tolerant to soil acidic pH and especially to mobile aluminium on 400 mM concentration /Ozawa et al., 1999/. In the presence of low pH means and deficit of Ca amount in the soil, rhizobia cannot form the efficient symbiosis /Lindstrom, Myllyniemi, 1987; Čiuberkis et al., 2005/. Even the small rates of Ca fertilizers (3.0 t ha^{-1}) decrease the toxicity of mobile Al and negative impacts of heavy metals more than twice, improve the metabolism of nitrogen and phosphorus in the soil, because soil microorganism activity and nitrogen fixation increases /Shilnikov et al., 1996/. From average Ca rates (7 t ha^{-1}) the nitrogen fixation increases to 30 % /Paraxin, Petrova, 2001/. The high Ca fertilizer rate (20 t ha^{-1}) eliminates the Al toxicity entirely. The first indication of their efficiency was visible on the 5th day after introducing the Ca fertilizers /Andrejuk et al., 2001/. Liming of acidic soil improved both inoculation process of legumes and nitrogen fixation /Delavechia et al., 2003/.

Our objective was to determine and estimate the symbiotic and non-symbiotic nitrogen fixation depending on soil acidity and liming.

Materials and methods

Laboratory, field and lysimetric experiments were carried out, in which different species of rhizobia were investigated. In this work we summarized symbiotic efficiency of *Sinorhizobium meliloti* – 40 strains, *Rhizobium leguminosarum bv. trifolii* – 108, *Rhizobium galegae* – 19 and *Rhizobium leguminosarum* bv. *viciae* – 90 strains.

The colony size measured in nutrient yeast agar medium (pH from 4.2 to 7.0) after 6 days' growing at 25 ^{o}C. Tolerance (percentage) of rhizobia species to acidity of medium was calculated by measuring the colony size in acid medium and dividing it by size in neutral medium and multipling by 100.

Field trials were carried out in both *Dystri – Endohypogleyic Albeluvisol* and *Orthi-Haplic Luvisol* where pH_{KCl} was 4.7-6.4, organic C – 1.17-3.14 %, available phosphorus – 64-139 and potassium – 92-118 mg kg^{-1}. The legumes were sown in 28 m^2 (2.0 x 14.0 m) plots. Their inoculation was carried out with ecologically adapted and non-adapted strains of rhizobia in the different acidity soils /Lapinskas, 2002/. For establishing the biological nitrogen fixation ryegrass (*Lolium perenne* L.) was grown in parallel plots. Experimental area was fertilized with $P_{60}K_{60}$. Just before legume sowing the seeds were inoculated with rhizobia bacterial suspension at $600x10^9$ cfu (colony forming units) ha^{-1}. After legumes and cereal plant cuts (2-3 times in every year) 0.5 kg herbage samples from all plots were taken for dry matter content analysis. All samples were dried and weighed to a constant weight in an oven controlled at 105^0 C and the amount of dry matter harvested was determined. The root samples were taken from soil monoliths (20x 20x 20 cm size). The amount of fixed nitrogen was calculated according to this equation:

$$F_n = (U_n + R_n) – (U_s + R_s)$$

F_n – fixed nitrogen, U_n – total nitrogen in over ground part of legume, R_n – total nitrogen in legume roots, U_s – total nitrogen in over ground part of cereal plants, R_s – total nitrogen of cereal roots. In all cases the dimension was kg N ha^{-1}.

Efficiency of associative nitrogen fixing bacteria was examined under field conditions. Soil was Dystri-Endohypogleyic Albeluvisol (pH$_{KCl}$ 5.6-5.9). Fertilizing with $P_{60}K_{60}$ the background was formed. The seed of barley and timothy were inoculated by preparations from sequential bacteria: *Azospirillum lipofera* (strain 137), *Agrobacterium radiobacter* 10, *Arthrobacter mycorens* 7 and *Rhizobium leguminosarum* bv. trifolii 348a. The biological nitrogen was calculated from total nitrogen differences of inoculated and uninoculated plants /Umarov, 1987/.

The enzyme nitrogenase (classification No 1.18.6.1) of free living nitrogen fixing bacteria was determined in field experiments, whose soil was *Dystric Albeluvisol*. Different soil pH$_{KCl}$ (from <4.7 to >6.7) and fertilization (three levels of mineral fertilizers: non-fertilized, $N_{45}P_{39}K_{57}$ and $N_{135}P_{117}K_{171}$ on average per crop rotation) were formed in the trials. The activity of nitrogen fixing enzyme -nitrogenase by free living of nitrogen fixing bacteria was measured by gas chromatograph CHROM – 5 using acetylene. The activity of nitrogenase is expressed in μM N g^{-1}soil h^{-1} /Andrejuk et al., 1988; Lapinskas et al., 2005/.

The efficiency of soil liming, red clover inoculation and leaching of Ca and nitrates from soil were measured in lysimetric experiments. Before liming soil pH$_{KCl}$ was 5.2 and after liming (6.2 t ha^{-1} $CaCO_3$) – pH$_{KCl}$ 6.2. In lysimeter trial the soil was *Dystri Albeluvisol*. The seed of red clover was inoculated by *Rhizobium leguminosarum* bv. *trifolii* strain 348a. The plot of this trial was 1.0 m^2 and depth –1.1 m. Experiments had three replicates.

Pattern analysis was processed using programs ANOVA and STAT /Tarakanovas, Raudonius, 2003/.

Results and discussion

The data of laboratory analyses showed that most sensitive to acid soil reaction of all rhizobia species was *Sinorhizobium meliloti*. Index of rhizobia tolerance to acid soil reaction was 67 %, while that of remaining species 82-87 %. In acid medium (pH 4.2) no strains of this bacteria grew, while other bacteria species grew upon the average and formed the colonies in size from 2.1 to 5.9 mm (Table 1). The other species of rhizobia were more tolerant to soil acidity compared with *Sinorhizobium meliloti*. In neutral medium (pH 7.0) all species of rhizobia grew very well and formed colonies from 6.9 to 10.3 mm in diameter.

The results of field experiments established that different species of rhizobia had unequal response to soil pH (Fig. 1). For nitrogen fixation by *Rhizobium leguminosarum* bv. *trifolii* the minimal soil acidity was pH$_{KCl}$ 4.5. At that time other species of this bacterium have not fixed the atmospheric nitrogen. When soil pH$_{KCl}$ was 5.0 all rhizobia species fixed nitrogen from 123 to 205 kg N ha^{-1}. The inhibitory effect of low soil pH has been indicated in references /Kaminskij, Golodna, 2000; Lapinskas, 2002/. Optimal

Table 1. The impact of nutrient medium pH on growth of rhizobia species biomass

1 lentelė. *Mitybinės terpės pH įtaka skirtingų rūšių gumbelinių bakterijų biomasės augimui*

Rhizobia species *Gumbelinių bakterijų rūšys*	Nutrient medium pH and colonies size on the 6th day mm *Terpės pH ir kolonijų dydis 6 parą mm*					LSD$_{05}$ *R$_{05}$*	Index of rhizobia tolerance to acid reaction % *Gumbelinių bakterijų tolerantiškumo rūgščiai reakcijai indeksas %*
	4.2	5.4	6.0	6.3	7.0		
S. *meliloti*	0	4.6	6.4	6.5	6.9	0.9	67
R. leguminosarum bv. *trifolii*	2.1	8.1	10.2	10.6	10.3	1.3	82
R. galegae	5.5	7.5	8.5	8.4	8.4	1.1	89
R. leguminosarum bv. *viciae*	5.9	8.0	8.8	8.9	9.2	1.3	87

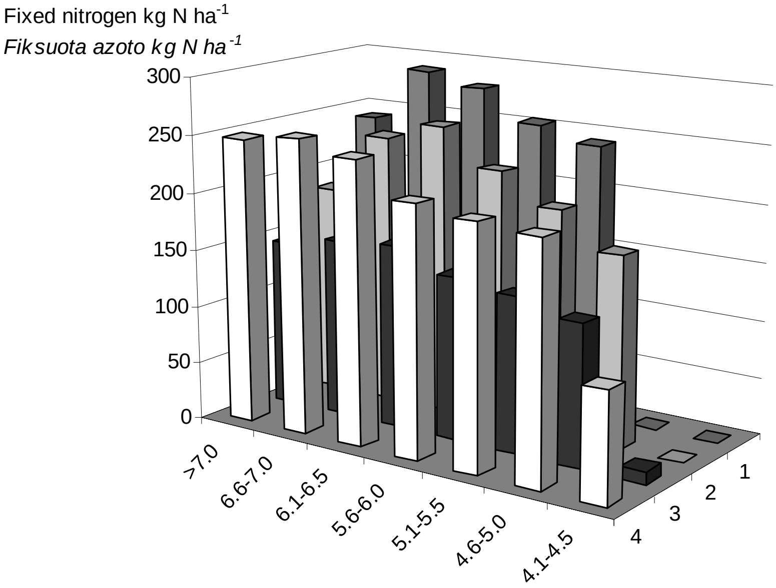

Figure 1. The impact of soil pH$_{\mathrm{KCl}}$ on biological nitrogen fixation by different species of rhizobia:

1 – *Rhizobium galegae,* 2 – *Sinorhizobium meliloti*, 3 – *Rhizobium leguminosarum* bv. *viciae*, 4 – *Rhizobium leguminosarum* bv. *trifolii*

1 paveikslas. *Dirvožemio pH$_{KCl}$ įtaka skirtingų rūšių gumbelinių bakterijų biologinio azoto fiksacijai:*

1 – Rhizobium galegae, 2 – Sinorhizobium meliloti, 3 – Rhizobium leguminosarum bv. viciae, 4 – Rhizobium leguminosarum bv. trifolii reaction for various species was different. *Rhizobium leguminosarum* bv. *viciae, Sinorhizobium meliloti* and *Rhizobium leguminosarum* bv. *trifolii* mostly fixed biological nitrogen when soil pH_{KCl} was 6.5, as *Rhizobium galegae* formed efficient symbiosis at pH_{KCl} 7.0. Up to now it has been known that *Sinorhizobium meliloti* have higher requirements for soil pH, but not *Rhizobium galegae* /Lindstrom, Myllyniemi, 1987/.

Therefore soil liming was an effective measure for improving the symbiosis and nitrogen fixation. Acid soil liming (lime rate 6.2 t ha^{-1} of $CaCO_3$) enriched the soil with biological nitrogen by 57 kg N ha^{-1} (Table 2). Liming in combination with inoculation of red clover fixed the highest amount of nitrogen – 275 kg N ha^{-1}. Agricultural means effectively increased DM yield by 2.4 t ha^{-1} and crude protein content by 365 kg ha^{-1}.

Table 2. The effect of inoculation and liming on red clover symbiosis and leaching of nutrients

2 lentelė. *Inokuliavimo ir kalkinimo reikšmė raudonųjų dobilų simbiozei bei maisto medžiagų išsiplovimui*

Treatment *Variantas*	Fixed nitrogen kg N ha^{-1} *Fiksuota azoto kg N ha^{-1}*	DM yield t ha^{-1} *SM derlius t ha^{-1}*	Crude protein *Žali baltymai*		Leached nutrients kg ha^{-1} *Išplauta maisto medžiagų kg ha^{-1}*	
			%	kg ha^{-1}		
Uninoculated, unlimed *Neinikuliuota, nekalkinta*	169	4.60	13.63	627	134	18
Inoculated, unlimed *Inokuliuota, nekalkinta*	202	5.41	13.49	730	126	13
Uninoculated, limed *Neinokuliuota, kalkinta*	226	5.92	14.17	839	138	11
Inoculated, limed *Inokuliuota, kalkinta*	275	7.00	14.17	992	128	15
LSD_{05} / R_{05}	43	0.60	1.67	86	14.2	2.54

The data of nutrients leaching analysis showed that combination of soil liming with legume seed inoculation had impact on leaching of Ca. However, somewhat more nitrates (18 kg ha^{-1}) leached in the plots where soil was unlimed and uninoculated clover grew. The explanation can be that the same measures intensified clover growth because there was less leached nutrients from area under clover crop /Hamdi, 1982/.

In other series of field experiments we estimated the efficiency of different species and strains of *Rhizobium* depending on their ecological adaptation. The results showed that uninoculated legume formed less number of nodules in comparison with inoculated plants (Table 3). Adapted strains of *Rhizobium leguminosarum* bv. *trifolii, Rhizobium galegae* and *Rhizobium leguminosarum* bv. *viciae* were more virulent and formed nodules more markedly than non-adapted. Conversely, among *Sinorhizobium meliloti* strains the same differences were not found. Depending on species of rhizobia and soil pH legumes symbiosis have fixed 160-264 kg N ha^{-1} on average. The highest amount of nitrogen was accumulated by *Rhizobium leguminosarum* bv. *trifolii* (222-264 kg N ha^{-1}) and the lowest by *Rhizobium leguminosarum* bv. *viciae* (160-183 kg N ha^{-1}).

Table 3. Symbiotic efficiency of different species of rhizobia
3 lentelė. *Įvairių rūšių gumbelinių bakterijų simbiotinis efektyvumas*

Strains / *Štamai*	Number of nodules per plant *Augalo gumbelių skaičius*	Fixed nitrogen kg N ha^{-1} *Fiksuota azoto kg N ha^{-1}*	Crude protein *Žali baltymai*		DM yield t ha^{-1} SM *derlius t ha^{-1}*
			%	kg ha^{-1}	
Sinorhizobium meliloti					
Uninoculated *Neinokuliuota*	23	184	15.78	887	5.62
Not adapted *Neadaptuota*	38	205	15.80	989	6.26
Adapted *Adaptuota*	41	213	16.12	1024	6.35
LSD$_{05}$ /R_{05}	4.2	12.8		57	0.30
Rhizobium leguminosarum bv. *trifolii*					
Uninoculated *Neinokuliuota*	139	222	16.74	1070	6.39
Not adapted *Neadaptuota*	184	264	18.33	1272	6.94
Adapted *Adaptuota*	202	261	17.00	1256	7.39
LSD$_{05}$ / R_{05}	16.6	9.3		42	0.2
Rhizobium galegae					
Uninoculated *Neinokuliuota*	48.2	43.1	14.65	375	2.56
Not adapted *Neadaptuota*	89.8	167.1	15.60	763	4.89
Adapted *Adaptuota*	129.0	213.3	16.47	944	5.73
LSD$_{05}$ /R_{05}	19.3	22.5		24	0.30
Rhizobium leguminosarum bv. *viciae*					
Uninoculated *Neinokuliuota*	62	160	29.81	772	3.05
Not adapted *Neadaptuota*	78	171	29.86	824	3.25
Adapted *Adaptuota*	119	183	29.46	878	3.51
LSD$_{05}$ /R_{05}	17.7	8.6		29	0.29

From adapted strains only *Rhizobium galegae* and *Rhizobium leguminosarum* bv. *viciae* had an advantage as compared to non-adapted. At that time *Sinorhizobium meliloti* and *Rhizobium leguminosarum* bv. *trifolii* strains from the standpoint of nitrogen fixation were of the same efficiency.

The content of crude protein depended on symbiosis. In all cases legume inoculation had a positive effect on accumulation of crude protein in legume yield. The inoculation of goat's rue and field bean with adapted rhizobia strains increased efficiency of their symbiosis and had a positive influence on legume yield and biological nitrogen fixation.

Over study on sphere of application of associative nitrogen fixing bacteria it was indicated that different species of diazotrophs fixed unequal amounts of biological nitrogen in rhizoplane of barley and timothy (Fig. 2). The highest amount of nitrogen was accumulated by *Agrobacterium radiobacter, Rhizobium leguminosarum* bv. *trifolii and Arthrobacter mycorens.* They fixed 11.0-20.4 kg N ha^{-1} from atmosphere on average. It is known that in non-acid and rich in phosphorus and potassium soils the associative nitrogen fixers accumulate to 50 kg N ha^{-1} and more /Elgersma, Schlepers, 2001/.

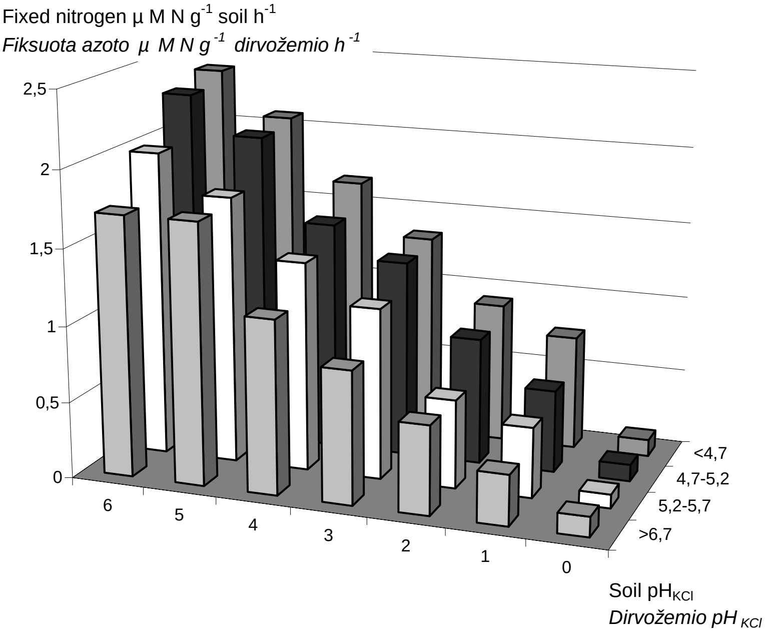

Figure 2. The impact of non–symbiotic nitrogen fixing microorganisms on biological nitrogen fixation in rhizoplane by barley and timothy:
1 – *Flavobacterium spp.,* 2 – *Arthrobacter mycorens,* 3 – *Agrobacterium radiobacter,* 4 – *Azospirillum lipoferum,* 5 – *Rhizobium leguminosarum* bv. *Trifolii*

2 paveikslas. *Nesimbiotinio azotą fiksuojančių mikroorganizmų įtaka biologinio azoto fiksacijai miežių ir motiejukų rizoplanoje:*
1 – Flavobacterium spp., 2 – Arthrobacter mycorens, 3 – Agrobacterium radiobacter, 4 – Azospirillum lipoferum, 5 – Rhizobium leguminosarum bv. trifolii

The highest activity of soil nitrogenase was in non – fertilized soil in all pH levels (Fig. 3). The amount of fixed nitrogen was 0.6-2.3 µM N g^{-1} soil h^{-1} on average respectively at the beginning of plant vegetation and after 25 weeks after vegetation resumed in very acid soil ($pH_{KCl} < 4.7$). At that time in close to neutral soil ($pH_{KCl} > 6.7$) 0.2 and 1.1 µM N g^{-1} soil h^{-1} was fixed. In earlier investigations it was established that free living nitrogen fixers accumulated nitrogen from atmosphere in more acid soil /Čiuberkis et al., 2005/. It can be explained, that in the same soils *Azotobacter chroococcum* and other nitrogen fixing bacteria which are sensitive to low soil pH were not found. In that case biological nitrogen is fixed by tolerant to acid soil reaction bacteria such as *Clostridium pasteurianum* and other /Arlauskienė, 2000/.

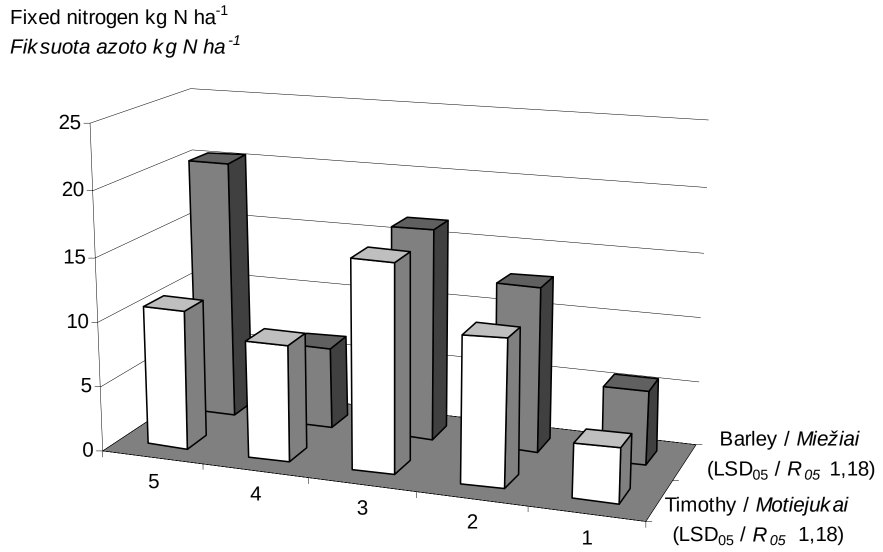

Figure 3. The impact of soil pH and period of crop vegetation on the activity of nitrogenase by non-symbiotic nitrogen fixing microorganisms:
1 – Beginning of vegetation, 2 – After 5 weeks, 3 – After 10 weeks, 4 – After 15 weeks, 5 – After 20 weeks, 6 – After 25 weeks
3 paveikslas. *Dirvožemio pH ir augalų vegetacijos trukmės įtaka nesimbiotinių azotą fiksuojančių mikroorganizmų nitrogenazės aktyvumui:*
1 – Vegetacijos pradžia, 2 – Po 5 sav., 3 – Po 10 sav., 4 – Po 15 sav., 5 – Po 20 sav., 6 – 25 sav.

The mathematical analysis of data showed conformity between soil acidity and symbiotic nitrogen fixation by both uninoculated and inoculated legumes (Table 4).

In all cases the symbiotic nitrogen fixation by uninoculated alfalfa, goat's rue and field bean was more depended on soil acidity (R = 0.65, 0.61 and 0.82, respectively) than inoculated legumes. There was identified a weak relationship between nitrogen fixing efficiency by red clover and soil pH_{KCl}.

Table 4. The inoculation efficiency on symbiotic nitrogen fixation (y) in relation to soil pH_{KCl} (x)

4 lentelė. *Ankštinių augalų inokuliavimo efektyvumo simbiotinio azoto fiksacijai (y) ir dirvožemio pH_{KCl} (x) ryšys*

Inoculation *Inokuliavimas*	Equation of regression *Regresijos lygtis*	R probability % *R tikimybė %*	Fisher's test % *Fišerio kriterijus %*
	Alfalfa / *Liucernos*		
Uninoculated *Neinokuliuota*	$y = 7665.9-2617.86x+228.28x^2$	0.65	5.53^*
Inoculated *Inokuliuota*	$y = 8111.7-2758.91x+240.1x^2$	0.61	4.48
	Red clover / *Raudonieji dobilai*		
Uniniculated *Neinokuliuota*	$y = -3330.6+1287.07x-115.837x^2$	0.40	3.00
Inoculated *Inokuliuota*	$y = -3585.0+1379.11x-123.027x^2$	0.38	2.78
	Goat's rue / *Rytiniai ožiarūčiai*		
Uninoculated *Neinokuliuota*	$y = 0.8497 \cdot e^{1.0610\,x}$	0.61	3.56
Inoculated *Inokuliuota*	$y = 4277.5-1376.75x+114.319x^2$	0.34	0.62
	Field bean / *Pašarinės pupos*		
Uninoculated *Neinokuliuota*	$y = 3203.5-1014.51x+82.654x^2$	0.82	12.54^{**}
Inoculated *Inokuliuota*	$y = 2968.4-934.19x+76.411x^2$	0.81	11.04^{**}

Conclusions

The results of this study indicated that different *Rhizobium* species were unequally tolerant to soil acidity. Index of tolerance of *Sinorhizobium meliloti* was 67 %, as more tolerant *Rhizobium galegae* and *Rhizobium leguminosarum bv. viciae* was 89 and 87 % respectively comparing their growth in acid (pH 5.0) and neutral (pH 7.0) mediums.

Under field conditions rhizobia, depending on their species and soil pH_{KCl}, fixed biological nitrogen from 0 to 282 kg N ha^{-1}. The most active fixer of atmospheric nitrogen was *Rhizobium galegae* and the worst – *Rhizobium leguminosarum* bv. *viciae.* It was established that minimal soil acidity for all species studied was pH_{KCl} 5.0 and optimal - pH_{KCl} 6.5-7.0.

The most important factors to activate nitrogen fixation were acid soil liming and legume seed inoculation with effective and in some cases – ecologically adapted rhizobia strains. Liming ($CaCO_3$ rate 6.2 t ha^{-1}) in combination with inoculation of red clover increased the amount of fixed biological nitrogen from 169 to 275 kg N ha^{-1}. Effective and ecologically adapted strains had an advantage if comparing to non-adapted only in forming symbiosis with *Rhizobium galegae* and *Rhizobium legumi-*

nosarum bv. *viciae*. However, adapted strains formed markedly more nodules in legume roots than non-adapted.

The application of associative nitrogen fixing bacteria on non – legumes has great prospects. Our experimental data showed that barley and timothy fixed 18.2-20.4 kg ha^{-1} of biological nitrogen, when their seeds were inoculated with diazotrophs (*Agrobacterium radiobacter, Rhizobium leguminosarum* bv. *trifolii* and *Arthrobacter mycorens*). The determined amount of nitrogen fixed by free nitrogen fixing bacteria was 0.6-2.0 µM N g^{-1} h^{-1} on average depending on soil acidity and duration of crop vegetation.

Received 05 09 2006
Accepted 09 11 2006

REFERENCES

1. Ambrazaitienė D. Activity of symbiotic nitrogen fixation in the *Dystric Albeluvisol* differing in acidity and fertilization (summary) / Simbiotinio azoto fiksavimo aktyvumas skirtingo rūgštumo įvairiai tręštame nepasotintame balkšvažemyje // Agriculture: scientific articles / Žemdirbystė: mokslo darbai / LŽI, LŽUŪ. - Akademija, 2003, t. 83, Nr. 3, p. 173-186. - In Lithuanian

2. Andrejuk K. I., Iutinska G. A., Antipchuk A. F. Funkcionuvanne mikrobnych cenoziv gruntu v umovach antropogennogo navantazhenija. - Kiiv, 2001, ch. 1-2. - 240 c.

3. Andrejuk K. I., Iutinskaja G. A., Dul'gerov A. N. Soil microorganisms and intensive agriculture. - Kiiv, 1988. - 190 p.

4. Arlauskienė E. A. Effect of soil acidity on the intensity of microbiological process // Soil Acidification and Liming. - Vėžaičiai, 2000, p. 15-20

5. Carranca A., Varennes de A., Rolston D. Biological nitrogen fixation by faba been, pea and chickpea under field condition, estimated by the N - 15 isotope dilution technique // Europian Journal of Agronomy. - 1999, vol. 10, iss. 1, p. 49-56

6. Čiuberkis S., Čiuberkienė D., Končius D. ir kt. Effect of liming and fertilizing systems on soil properties and productivity of agrocenoses (summary) / Kalkinimo bei tręšimo sistemų poveikis dirvožemio savybėms ir agrocenozės produktyvumui // Agricultural sciences / Žemės ūkio mokslai. - 2005, Nr. 2, p. 1-12. - In Lihuanian

7. Delavechia C., Hampp E., Fabia A., Castro S. Influence of pH and calcium on growth, polysacharide production and symbiotic association of *Sinorhizobium meliloti* SEMIA 116 with alfalfa roots // Biology and Fertility of Soils. - 2003, vol. 38, iss. 2, p. 110-114

8. Elgersma A., Schlepers H. N – use efficiency in grass – clover mixtures // Grassland Science in Europe. - 2001, vol. 6, p. 73-75

9. Hamdi Y. A. Application of nitrogen – fixing systems in soil improvement and management // Soil's Bulletin. - Rome, 1982, vol. 49. - 188 p.

10. Kaminskij V. F., Golodna A. V. Zernobobovi kul'tury dzerelo biologichnogo azotu // Visnik agrarnoi nauky. - 2000, spec. vyp., s. 45-47

11. Kelner D. J., Vessey J. K., Antz M. H. The nitrogen of 1,-2-and 3-years stands of alfalfa in a cropping system // Agriculture Ecosystems and Environment. - 1997, vol. 64, iss. 1, p. 1-10

12. Koc' S. J. Fiziologichi osoblivosti formuvannia ta funkcionuvannia symbiotychnyh system bobi roslyny-bul'buchkovi bakterii // Ontogenez roslyn, biologichna fiksacija molekuliarnogo azotu ta azotnyj metabolizm. - Ternopil, 2001, s. 86-90

13. Lapinskas E. Effectiveness of *Sinorhizobium meliloti* strains adapted to soil acidity // Advances of Agricultural Sciences Problem Issues. - Warsaw, 2002, iss. 482, p. 323-328

14. Lapinskas E., Ambrazaitienė D., Piaulokaitė-Motuzienė L. Estimation of soil microbiological properties in relation to soil acidity and fertilization // Agronomijas Vestis. - Jelgava, 2005, No. 8, p. 32-36

15. Lindstrom K., Myllyniemi H. Sensitivity of red clover rhizobia to soil acidity factors in pure culture and in symbiosis // Plant and Soil. - 1987, vol. 98, No 3, p. 353-362

16. Ozawa T., Imai Y., Sukiman H. I. et al. Low pH and aluminium tolerance of *Bradyrhizobium* strains isolated from acid soils in Indonesia // Soil Science and Plant Nutrition. - 1999, vol. 45, iss. 4, p. 987-992

17. Парахин Н.В., Ретрова С.Н. Сравнительная симбиотическая активность, урожайность и белковая продуктивность многолетних бобовых трав в условиях Орловской области // Известия ТСХА. - 2001, вып. 3, с. 18-33. - Rus.

18. Шильников И.А., Кирпичников Н.А., Удалова Л.П. и др. Проблемы известкования почв // Химия в сельском хозяйстве. - 1996, No. 5, с. 18-21. - Rus.

19. Tarakanovas P., Raudonius S. Agronominių tyrimų duomenų statistinė analizė taikant kompiuterines programas ANOVA, STAT, SPLIT, PLOT iš paketo „Selekcija” ir Irristat. - Akademija, 2003. - 56 p.

20. Umarov M. M. Nitrogen in the modern biological farming // Proceedings of 13 Congress International Society of Soil Science, 1986. - Hamburg, 1987, vol. 6, p. 950-953

ISSN 1392-3196
Žemdirbystė, t. 93, Nr.4 (2006), p. 210-220
UDK 631.461.5:631.415.1

DIRVOŽEMIO RŪGŠTUMO REIKŠMĖ SIMBIOTINIO IR NESIMBIOTINIO AZOTO FIKSACIJAI

E. Lapinskas, L. Piaulokaitė-Motuzienė

Santrauka

Apibendrinti laboratorinių ir lauko bandymų duomenys biologinio azoto fiksacijos klausimais. Įvertintas labiausiai paplitusių keturių rūšių gumbelinių bakterijų: *Sinorhizobium meliloti, Rhizobium leguminosarum* bv. *trifolii, Rhizobium galegae ir Rhizobium leguminosarum* bv. *viciae* simbiotinis efektyvumas įvairaus rūgštumo (pH nuo 4,7 iki 7,2) dirvožemiuose. Priklausomai nuo gumbelinių bakterijų rūšies ir dirvožemio pH_{KCl}, ankštinių simbiozė sukaupė biologinio azoto nuo 160 iki 264 kg N ha^{-1}. Simbiotinio azoto fiksacijai visų tirtų gumbelinių bakterijų kritiškas pH_{KCl} 5,0, optimalus pH_{KCl} 6,5-7,0. Derinant kalkinimo ($CaCO_3$ norma 6,2 t ha^{-1}) ir raudonųjų dobilų inokuliavimą, padidėjo simbiotinio azoto fiksacija nuo 169 iki 275 kg N ha^{-1}. Ekologiškai adaptuoti gumbelinių bakterijų štamai formavo efektyvesnę simbiozę su rytiniais ožiarūčiais ir pašarinėmis pupomis, negu neadaptuoti. Asociatyvios azotą fiksuojančios bakterijos miežių ir motiejukų rizoplanoje fiksuoja vidutiniškai 18,2-20,4 kg N ha^{-1}. Rūgščiuose dirvožemiuose laisvieji azotą fiksuojantieji mikroorganizmai sukaupia 0,6-2,3 µMN g^{-1} dirvožemio h^{-1}, priklausomai nuo dirvožemio pH ir augalų vegetacijos trukmės.

Reikšminiai žodžiai: azoto fiksacija, gumbelinės bakterijos, rūšys, simbiozė, nitrogenazė, dirvožemio rūgštumas.