Efficiency of combination of phytohormones and *R. leguminosarum bv. trifolii* and *S. meliloti* strains for clover and lucerne

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Laboratory and field experiments were carried out over the period 1991–1999. The soil of the experimental site was sod podzolic gleyic light loam (pH_{KCl} 5.2–6.0).

It was determined that the phytohormones β-indolil-acetic acid (IAA, heteroauxin) and kinetin stimulated biomass growth (size of colony) of some *Rhizobium leguminosarum bv. trifolii* and *Sinorhizobium meliloti* strains.

In field experiments heteroauxin tended to increase the symbiotic efficiency of all the investigated *R. leguminosarum bv. trifolii* strains; the dry matter yield increase made up 0.70–1.05 t/ha. However, heteroxin had no effect on the efficiency of lucerne legume bacteria.

The data of correlation–regression analysis show that reduction of soil pH_{KCl} from 5.0 to 6.0 resulted in a gradual reduction of inoculation efficiency of all strains ($\eta = 0.653$).

The highest net income (113.05–171.05 Lt/ha) was obtained when inoculating clover with effective strains in combination with heteroauxin.

Key words: phytohormones, *Rhizobium*, strains, efficiency, clover, lucerne

INTRODUCTION

In the conditions favourable for symbiosis, legume bacteria not only efficiently fix atmospheric nitrogen, but also synthesise many biologically active compounds stimulating the growth of legume crops [1–4]. However, in acid soils with a deficiency of plant nutrients, legume plants are unable to fully meet vitamin and phytohormone needs of plants.

It is known that indolylacetic acid, cytokinin and other phytohormones increase the activity of nitrogenase, malate-succinatedehydrogenases in legume bacteria, which in turn activate the processes of symbiotic nitrogen fixation [5–7].

The objective of this study was to ascertain the effect of phytohormones β-indolylacetic acid and kinetin on the symbiotic efficiency of various strains of red clover *(Rhizobium leguminosarum bv. trifolii)* and lucerne (*Sinorhizobium meliloti)* bacteria.

MATERIALS AND METHODS

In 1991–1999, laboratory and field experiments were conducted to study the following strains of *R. leguminosarum bv. trifolii* and *S. meliloti*:

Rhizobium strains		Institutions and locations where strains of legume bacteria were isolated
clover	lucerne	
348a	425a	Russian Institute of Agricultural Microbiology, St. Petersburg
D2	AL3	Crop Production Institute, New Southern Wales, Australia
R165	M41	University of Havaii, the USA
R91	M1	LIA Vėžaičiai Branch, Lithuanian soils
R99	M9	"
3RM5*	M37	"
5RM3*	2M24	"
8RM1*	2M32	"

* *R. leguminosarium bv. trifolii* mutants were produced by treatment of initial strain R99 with UV rays [1].

Biomass growth intensity of strains was investigated by growing bacteria on Norris agar medium [1], selecting optimum concentration (10^{-6}M) of IAA and kinetin.

Field experiments were conducted on a sod podzolic gleyic light loamy soil with a pH_{KCl} level of 5.2–6.0, humus 2.24–2.68%, mobile P_2O_5 and K_2O 110–179 and 143–210 mg/kg of soil, respectively.

In the trials we cultivated the blue-flowering lucerne variety 'Žydrūnė' and the red clover variety 'Liepsna' on a $P_{60}K_{60}$ fertilisation background. The size of a record plot was 18 m^2, with 4 replications.

Shortly before legume herbage sowing the seed was inoculated with *Rhizobium* suspension, calculating $100 \cdot 10^9$ bacteria cells per one hectare seed rate.

RESULTS AND DISCUSSION

S. meliloti strains formed colonies 6.9 mm in diameter, when grown for 6 days in the control medium without phytohormones. The largest colonies 9.7 mm in diameter were produced by the local strain M37, which exceeded in size colonies of the imported strain 425a by 2.6 mm or by 27%. The local strain M1 produced the largest colonies.

Heteroauxin and kinetin stimulated the growth of lucerne legume bacteria. In most of the strains the effect of both phytohormones was similar.

Clover legume bacteria without phytohormones grew in colonies 8.8 mm in diameter and were by 1.9 mm (28%) larger than those of lucerne. Individual strains demonstrated a positive response to this phytohormone. An especially marked increase through the application of heteroauxin occurred in the colonies of strains 348a, R165 and mutants 3RM5 and 8RM1. Kinetin in most cases stimulated the growth of clover legume bacteria.

Legume bacteria symbiotic efficiency with legume plants is best revealed by the changes in the dry matter yield of biologically pure legume plants resulting from inoculation or other means of effect [7–9].

While assessing the efficiency of clover legume bacteria strains or phytohormones in the field trials, it was determined that inoculation of plants gave a statistically significant yield increases through the application of strain 348a (0.72 t/ha) and strain R99 (0.70 t/ha). The other strains were not efficient for clover (Table).

Heteroauxin stimulated the efficiency of all strains of clover on average by 0.70–1.05 t/ha. Strain R99 was most susceptible to this phytohormone. It should be also noted that when red clover sward was sufficiently dense (over 90% of clover), inoculation did not have any effect on the botanical composition. Heteroauxin stimulated persistence of inoculated clover in the sward and did not have any effect on non-inoculated plants.

Protein content in clover yield due to inoculation did not increase in most cases. Heteroauxin stimulated protein content in non-inoculated clover plants and in the plants inoculated by strain D2. When clover was grown without heteroauxin, plants inoculated by strain 348a accumulated the highest content (961 kg/ha) of crude protein. Heteroauxin distinctly increased the efficiency of clover legume bacteria strains R165 and D2, which resulted in crude protein yield increase by 151 and 229 kg/ha, respectively. Heteroauxin application resulted in an inc-

Table. **Efficiency of phytohormones and *Rhizobium* strains on clover and lucerne**

Rhizobium strains	Yield of DM of bot. pure plants		Sown legume grasses % in the sward	Crude protein		Profit through seed treatment Lt/ha
	t/ha	yield increase t/ha		%	kg/ha	
			R. leguminosarum* bv. *trifolii			
			Without phytohormones			
Not inoculated	5.90	–	90.1	13.80	814	–
348a	6.62	0.72	91.6	14.32	961	92.10
R165	6.14	0.24	92.2	13.88	852	13.85
D2	6.32	0.42	90.2	12.90	815	42.20
R99	6.60	0.70	92.2	13.67	902	88.10
			IAR			
Not inoculated	6.70	0.80	90.4	14.66	982	129.20
348a	7.32	1.42	95.9	13.33	976	205.15
R165	7.14	1.24	93.9	14.05	1003	175.45
D2	7.32	1.42	94.2	14.26	1044	205.15
R99	7.65	1.75	94.0	13.12	1004	259.15
LSD_{05}	0.66		4.0	1.34	117	13.11
			S. meliloti			
			Without phytohormones			
Not inoculated	5.76	–	93.2	14.67	845	–
425a	6.44	0.68	89.4	13.60	876	89.5
M41	6.36	0.60	86.4	15.31	974	75.5
AL3	6.46	0.70	91.5	15.63	1010	92.3
2M24	6.50	0.74	92.0	14.98	966	99.3
			IAR			
Not inoculated	6.00	0.24	90.1	15.70	942	40.2
425a	6.59	0.83	89.8	14.78	974	44.3
M41	6.48	0.72	91.9	14.32	928	96.1
AL3	6.18	0.42	88.8	14.21	878	44.3
2M24	6.31	0.55	88.1	14.28	901	66.7
LSD_{05}	0.71		3.6	0.78	88	8.98

rease in crude protein mass (by 168 kg/ha) of non-inoculated clover, too.

Of all strains, 2M24 was most efficient for lucerne. It gave a 0.74 t/ha dry matter yield increase of botanically pure herbage. The other strains, 425a and AL3, were less effective. It is noteworthy that heteroauxin did not give an expected result for lucerne. Apparently spontaneous legume bacteria and the used strains of legume bacteria adequately synthesised this phytohormone in plants.

Heteroauxin stimulated crude protein synthesis only in the yield of non-inoculated plants. Heteroauxin application resulted in an increase in crude protein yield of only non-inocucalated plants and of the plants inoculated with strain 425.

According to L. Materon and C. Hagedorn, there is no clear connection between the efficiency of legume bacteria strains and crude protein content in legume plants [10].

All inoculation and heteroauxin combinations of red clover were effective from the viewpoint of economy and net profit made up from 113.05 to 171.05 Lt/ha. From the viewpoint of economy heteroauxin was efficient when sowing non-inoculated lucerne.

The highest inoculation efficiency of clover and lucerne legume bacteria strains was determined at a pH_{KCl} level of 5.0. A decline in soil acidity to pH_{KCl} 6.0 resulted in a consistent reduction of the efficiency of strains (η = 0.653).

Similar changes in the efficiency of legume bacteria strains were also determined at increasing soil humus content from 2.2 to 2.7% (η = 0.647) and increasing the content of phosphorus and potassium from 100 to 180 mg/kg of soil (η = 0.522 and 0.571).

References

1. Lapinskas E. Biological nitrogen fixation and inoculums. Akademija, 1998: 218 (in Lithuanian).
2. Parmar N, Dadarwall KR. J Appl Microbiol 1999; 86 (1): 36–44.
3. Волобуева ОГ, Шильникова ВК. Биологический азот в растениеводстве. 1996: 1262–81.
4. Груодене ЯП. Микробиологические процессы в почвах и урожайность сельскохозяйственных культур. Вильнюс, 1986: 92–4.
5. Hungria M, Starey G. Soil Biol Biochem 1997; 29 (5–6): 819–30.
6. Shukla RS, Singh SB, Dubey JN. Teor Aēpl Geneticū 1989; 78 (3): 433–5.
7. Мишустин ЕН. Биологический азот в сельском хозяйстве. Москва, 1989: 207.
8. Наумов ГФ, Кисель МС. Доклады II Всесоюзной научной конференции СОИСАФ. Калуга.1991: 76–9.
9. Шабаев ВП, Смолин ВЮ, Проворов НА. и др. Известия Российской АН. Сер. биол. 1996; 3: 351–8.
10. Materon LA, Hagedorn C. Communications in Soil Science and Plant Analysis 1984; 15 (1): 33–45.

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FITOHORMONŲ IR R. *LEGUMINOSARUM BV. TRIFOLII* BEI S. *MELILOTI* KAMIENŲ DERINIMO VEIKSMINGUMAS DOBILAMS IR LIUCERNOMS

S a n t r a u k a

1991–1999 m. daryti laboratoriniai ir lauko bandymai. Dirvožemis – velėninis jaurinis glėjiškas lengvas priemolis (pH_{KCl} 5,2–6,0).

Nustatyta, kad fitohormonai β indolilacto rūgštis (heteroauksinas) ir kinetinas stimuliavo *Rhizobium leguminosarum bv. trifolii* ir *Sinorhizobium meliloti* kai kurių kamienų biomasės augimą (kolonijos dydį).

Lauko bandymuose heteroauksinas didino visų tirtų *R. leguminosarum bv. trifolii* kamienų simbiotinį efektyvumą; raudonųjų dobilų sausųjų medžiagų derliaus priedas sudarė 0,70–1,05 t/ha. Liucernų gumbelinių bakterijų efektyvumui heteroauksinas neturėjo įtakos.

Koreliacinės–regresinės analizės duomenimis, mažėjant dirvožemio rūgštumui pH_{KCl} nuo 5,0 iki 6,0, palaipsniui mažėjo visų kamienų inokuliavimo veiksmingumas (η = 0,653).

Daugiausia grynųjų pajamų (113,05–171,05 Lt/ha) gauta inokuliuojant raudonuosius dobilus efektyviais kamienais kartu su heteroauksinu.

Raktažodžiai: fitohormonai, Rhizobium, kamienai, efektyvumas, dobilai, liucernos