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IMPORTANCE OF "AZOTOFIKSIN" IN PRODUCTION OF LEGUME FOODER CROPS IN SERBIA[1]

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Contents: Experiment was set up in greenhoused vegetative pots containing soil with Azotofiksin- a microbial nitrogen fertilizer whose active agent is a live nitrogen-fixing microorganism (Rhizobium spp.) with aim reducing the application of mineral nitrogen fertilizers or completely avoided in the production of major fodder legumes, i.e. alfalfa, white and red clover and Galegae orientalis. Standard microbiological methods were employed. The application of Azotofiksin with specifically effective strains compatible with those legumes clearly showed an increase in the yield of shoot dry weight and the quality of hay proteins and in some cases equalling application of the mineral N fertilizer . It fortified soil with nitrogen without causing any adverse effects on human health or the environment. Azotofiksin is a realistic alternative to mineral nitrogen fertilizers in the production of fodder legumes.

Key words: Azotofiksin, alfalfa, clover, Goat's rue, nitrogen fixation, rhizobia

Introduction

Alfalfa, red and white clover and goat's rue are fodder plants characterised by high production potentials, quality and quantity of yield and nitrogen fixation ability (*Radenović, 2000*). These fodder crops share a capacity of fixing atmospheric nitrogen in symbiosis with their own specific bacteria named rhizobia (fam. *Rhizobiaceae*) (*Stougard, 2000*). They fix considerable amounts of nitrogen, and this fact enables alfalfa to provide by nitrogen fixation 70-80% of total nitrogen, red clover 86% and white clover 75% (*Boller and Nösberger, 1987; Wivstad et al., 1987*). According to this process, these legumes were naturally supplied with nitrogen, making them selves healthy and cheaper forage crops. Many Serbian soils have indigenous rhizobia populations for alfalfa and red clover, but that is not so with *G. orientalis* as the proportion and abundance of its rhizobia differ depending on soil type and presence of host-plant (*Vojinović et al, 1989; Vukmir, 1993*). But, in some cases spontaneous simbiotic N fixation can be reduced or absent, because of apsence of rizobia effective strains in soil (*Petrović i Vojinović,* 1963, *Bottomley i Jenkins,* 1983, *Vojinović i sar.,* 1989.; *Vukmir-Delić i sar.,* 1995). Application of crop-specific active rhizobia strains in inoculum provides most of plant need in atmospheric nitrogen, which considerably reduces or fully eliminates application of mineral nitrogen fertilizers. Thanks to own laboratory's rizobia collection, Microbiological Laboratory of Institute for soil science, Belgrade produced »AZOTOFIKSIN« tj. microbial nitrogen fertilizer whose active agent is *Rhizobium spp*. which supplies the plant with nitrogen from air in a symbiotic process with the host- plant (legume), thus making the plant less dependent on soil N.

Our research focused on the application of Azotofiksin, and on its effect on the yield and nitrogen content in dry weight of alfalfa, red clover, white clover and goat's rue with aim to reduce applying mineral N fertilizer.

Materials and methods

Investigation was conducted in semicontrolled laboratory conditions in greenhouse. Alfalfa, *Medicago sativa* L. (cultivar K-22), red clover, *Trifolium pratense* L. (cultivar K-17), white clover, *Trifolium repens* L. (cultivar K-33) and goat's rue, *Galega orientalis* Lam. in simbiosis with specific bacteria rhizobia were used. Standard microbiological methods were employed. A trial with alfalfa and clovers was set up in vegetative pots on a smonitza soil and with goat's rue on chernozem soil with four replications and without an autochthonous population of their specific rizobia *S. meliloti*, *R. trifolii* and *R. galegae*, respectively. Besides control without N (θ), and a nitrogen-added control (θN-full N content), strains of *S. meliloti, R. trifolii* and *R.*

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galega ("single strain inoculum"), originating from the Institute for soil science collection, were applied in the form of Azotofiksin by seed inoculation immediately before sowing.

The plants was grown for 45 days. After that, the shoot dry weight yield of the tested crops was measured, the percentage and total content of N in dry weight was determined by Kjeldahl method and effectiveness of nitrogenfixation was calculated. LSD test was done.

Results and discussion

Our investigation show the effect of *S. meliloti, R. leguminosarum* bv. *trifolii and R. galegae* strains in the form of Azotofiksin on shoot dry weight and nitrogen content in alfalfa, red clover, white clover and goat's rue. The results clearly indicate the influence of the applied Azotofiksin was significant because of very high quantity and quality yield expresed with the average values of these parameters. Effectiveness of nitrogen fixation in the tested plants expressed as the average values of all investigated parameters, is shown in tables 1, 2 and 3. Simbiotic effectivity or nitrogen fixation efficiency can be expresed as ratio of shoot dry matter/total N content of inoculated plants and control N fertilizered. If the yield of inoculated plants is expressed as a percentage, so that the yield of control plants with a full nitrogen rate (θN) equals 100%, the yield achieved in the process of nitrogen fixation was the some value in percent (%). On the some way, a values of the total N content were expressed.

Table 1. Effect of Sinorhizobium meliloti strains in the form of Azotofiksin on shoot dry weight and nitrogen content in alfalfa, Medicago sativa L., cultivar K-22

Treatments	*Shoot dry weight		%N	*Total nitrogen content		*Fixed nitrogen content
	g/pot	%		g/pot.	%	g/pot.
203	2,145	63	2,44	0,052	41	0,006
204	3,334	98	3,69	0,123	96	0,077
215	2,694	79	3,50	0,094	73	0,048
219	2,772	82	3,54	0,098	77	0,052
224	3,257	96	3,65	0,119	93	0,073
236	3,296	97	3,70	0,122	95	0,076
239	2,933	87	3,76	0,110	86	0,064
241	2,985	88	3,47	0,104	81	0,057
244	2,927	86	3,68	0,108	84	0,062
249	3,044	90	3,82	0,116	91	0,070
**θ	1,939	57	2,38	0,046	36	-
θN	3,392	100	3,78	0,128	100	-
LSD 005	0,364					
001	0,493					

* Each value is the mean of results for 30 plants ** θ-uninoculated control plants without N ferilizing; Nθ - uninoculated control plants on soil containing full mineral nitrogen content

The data in table 1 indicates that all investigated symbiotic associations achieved in the process of nitrogen fixation yields and total nitrogen contents lower than values of the corresponding parameters in control plant growing on a full nitrogen (θN) rate soil. The eight of ten applying strains were achieved shoot dry weight and total N content 82-97% in ratio to the control plants with full nitrogen content (θN) (100%). Strains 204, 224, 236 and 249 were the best, especially the strain 204 which was the most effective (98%). No statistically significant differences were found regarding yield dry weight and total nitrogen contents between inoculated plants with these strains and control plants with full nitrogen (N) rate. These findings agree with results reported by other researchers (*Petrović, 1962; Petrović and Vojinović, 1963; Boller and Nösberger, 1987; Vojinović et al., 1989*). According to various reports, alfalfa normally utilizes mineral nitrogen from its medium better than it does so in a process of symbiotic nitrogen fixation (*Gibson, 1976; Vojinović et al., 1989*).

In table 2, we show that about 2/3 of symbiotic assotiations red clover-*R. leguminosarum bv. trifolii* was achieve quantitative and qualitative yield 81-97% in ratio to the control plants with full nitrogen content (θN) (100%). The results indicates that mostly investigated symbiotic associations achieved in the process of

nitrogen fixation yields and total nitrogen contents equal or lower than values of the the corresponding parameters in control plants growing on a full nitrogen rate soil(θN), except the symbiotic association with strains 461 and 468, 104% and 106% respectively. No statistically significant differences were found regarding yield dry weight and total nitrogen contents between plants inoculated with strain 461 and 468 and θN control plants, which shows that there are compatible symbiotic assotiations to meet the plant's regular nitrogen requirement in a process of nitrogen fixation.

The same *R. leguminosarum bv. trifolii* strains were inoculated white clover. The similar average values were in this assotiation. These strains supplied host plant with 81-103 % N of full N content in control treatment (θN) (Tabela 2). Based on the quality of plant weight,strain 409 utilized atmospheric molecular nitrogen with similar effectiveness as mineral nitrogen from the soil. Strain 461 was the most effective strain which in the N fixation process achieved higher yield and total N content than some in θN (103%). This strain was equaly effective with both clover (tabela 2).

Our findings shown in Table 3 clearly indicate that *R. galegae* strain 802 was the most effective strain in spite of high effectiveness of two others strains, 801 and 804. There were no statistical signification between dry weight yields of assotiations *G. orientali/-R. galegae strains* 801 and 802 and velue of the some parameters of control treatment with N fertilizer(θN). Our findings agree with results reported by other researchers (Lindstrom et al., 1989) and in our earlier investigations (*Miličić et al., 1996, 1997).*

Table 2. Effect of R. leguminosarum bv. trifolii strains in the form of the Azotofiksin on the yield of the shoot dry weight and the nitrogen content in red clover, Trifolium pratense L and white clover, Trifolium repens L.

Treatments	Red clover, *Trifolium pratense* L.						White clover, *Trifolium repens* L.					
	*Shoot dry weight		%N	*Total N content		*Fixed N content	*Shoot dry weight		%N	*Total N Content		*Fixed N content
	g/pot	%		g/pot	%	g/pot	g/pot	%		g/pot	%	g/pot
402	2,120	95	3,68	0,078	100	0,056	1,186	90	3,26	0,039	87	0,026
405	2,003	79	3,72	0,075	96	0,052	1,256	95	3,48	0,044	98	0,031
407	2,094	94	3,52	0,074	95	0,051	1,241	94	3,57	0,043	96	0,030
408	2,016	91	3,40	0,069	89	0,046	1,024	78	3,44	0,035	78	0,022
409	2,158	97	3,47	0 ,075	96	0,053	1,318	100	3,60	0,047	104	0,034
410	1,486	67	1,80	0,027	35	0,005	0,853	65	1,70	0,015	33	0,002
413	1,964	88	3,12	0,061	78	0,039	1,039	79	2,84	0,030	67	0,017
417	2,068	93	3,54	0,073	94	0,051	1,124	85	3,28	0,037	82	0,024
421	1,861	84	3,22	0,060	77	0,038	1,024	78	3,05	0,031	69	0,018
424	1,835	83	3,15	0,058	74	0,036	0,985	75	3,24	0,032	71	0,019
433	2,029	91	3,30	0,067	86	0,045	1,124	85	3,16	0,036	80	0,023
437	1,771	80	3,12	0,055	70	0,033	1,024	78	3,20	0,033	73	0,020
445	1,796	81	3,18	0,057	73	0,035	1,070	81	3,25	0,035	78	0,022
447	2,068	93	3,50	0,073	93	0,051	1,303	99	3,40	0,044	98	0,031
461	2,378	107	3,40	0,081	104	0,059	1,365	103	3,48	0,048	107	0,035
462	1,422	64	1,74	0,025	32	0,003	0,814	62	1,76	0,014	31	0,001
464	2,145	97	3,37	0,072	92	0,050	1,303	99	3,46	0,045	100	0,032
465	1,887	85	3,46	0,065	83	0,043	1,101	84	2,98	0,033	73	0,020
468	2,352	106	3,54	0,083	106	0,061	1,287	98	3,36	0,043	96	0,030
469	1,990	90	3,18	0,063	81	0,041	1,163	88	3,24	0,038	84	0,025
**θ	1,292	58	1,72	0,022	28	-	0,775	59	1,68	0,013	29	-
θN	2,223	100	3,52	0,078	100	-	1,318	100	3,42	0,045	100	-
LSD 005	0,289						0,189					
001	0,387						0,237					

* Each value is the mean of results for 50 plants ** θ-uninoculated control plants without N ferilizing; Nθ - uninoculated control plants on soil containing full mineral nitrogen content

Table 3. Effect of Rhizobium galegae strains in the form of the Azotofiksin on the yield and nitrogen content in shoot dry Goat's rue (Galega orientalis Lam.)

Treatments	Shoot dry weight*		%N	Total nitrogen content*		Fixed nitrogen content*
	g/pot	%		g/pot.	%	g/pot.
801	2,144	89	3,55	0,076	84	0,041
802	2,293	95	3,80	0,087	97	0,052
804	1,873	78	3,24	0,061	68	0,026
θ**	1,357	56	2,56	0,035	39	
θN	2,415	100	3,73	0,090	100	
LSD 005	0,231					
001	0,314					

* Each value is the mean of results for 15 plants ** θ-uninoculated control plants without N ferilizing /; Nθ - uninoculated control plants on soil containing full mineral nitrogen content/

Results indicate that plant nitrogen derived from the nitrogen fixation process is to be supplemented with mineral nutrition (fertilization or natural nitrogen source in the soil) in order to fulfill the plants' normal needs for nitrogen (*Gibson, 1976*).

Conclusions

The acquired results clearly indicate that a successful production of alfalfa, red and white clover and *G. orientalis* requires treatments with Azotofiksin, i.e. that crop-specific, highly active and competitive rhizobia that are not part of the indigenous populations in domestic soils should be incorporated with seeds at the time of sowing. Azotofiksin thus represents a realistic alternative for reducing or fully substituting mineral nitrogen fertilizers in the production of these crops.

ZNAČAJ "AZOTOFIKSINA" U PROIZVODNJI LEGUMINOZNE STOČNE HRANE U SRBIJI

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R e z i m e

Eksperiment je izveden u stakleniku gde je na određenom tipu zemljišta u vegetacionom sudovima ispitivano dejstvo Azotofiksina na kvantitet i kvalitet krmnih leguminoza: lucerke, *Medicago sativa* L., crvene, *Trifolium pratense* L. i bele dateline, *Trifolium repens* i stočne galege, *Galega orientalis* Lam.. Biljke su bile inokulisane Azotofiksinom, mikrobiološkim azotnim đubrivom koje kao aktivan agens sadrži efikasne sojeve rizobia, simbioznih bakteria azotofiksatora. Inokulisane biljke su poređene sa kontrolnim tretmanima sa (biljke đubrene azotom) i bez azota. Cilj rada je bio da se pokaže mogućnost smanjenja primene N mineralnog đubriva kod lucerke, crvene i bele dateline, i galege na račun simbiozne azotofiksacije. Korišćene su standarder mikrobiološke metode. Rezultati ukazuju da primena Azotofiksina kod ispitivanih leguminoza jasno utiče na povećanje prinosa suve nadzemne mase i kvaliteta proteina u njoj koji se zahvaljujući određenim sojevima specifičnim za sve ispitivane leguminoze, nisu statistički značajno razlikovali od prinosa ostvarenog đubrenjem kontrolnih biljaka punom normom azota. Azotofiksin pretstavlja zdrav način snabdevanja biljke azotom što ima pozitivan efekat i na zdravlje životinja koje se hrane pomenutim lrguminozama a time i na čoveka i spoljašnju sredinu. On je alternativa mineralnom N đubrivu u proizvodnji krmnog bilja.

Ključne reči: Azotofiksin, lucerka, crven detelina, bela detelina, galega, azotofiksacija, rizobia

References

11. BOLLER B.C. AND I. NÖSBERGER (1987): Symbiotically fixed nitrogen from field grown white and red clover mixed with rye grasses at low levels of ^{15}N fertilization. Plant and Soil, vol. 104, No. 2, 219-226.
12. BOTTOMLEY, P.J. AND M.B. JENKINS (1983): Some characteristics of *Rhizobium meliloti* isolates from alfalfa fields in Oregon. Soil Science Society of America Journal, 47: 1153-1157
13. GIBSON, H.A. (1976.): Symbiotic nitrogen fixation in plant. International biological program. Cambridge university press. Edited by P.S. Nutman
14. LINDSTROM K. (1989): Rhizobium galegae a new species of legume voot nodula bacteria. J. of Sistem. Bacteriology, 39, 365-367.
15. MILIČIĆ B., Đ. KUZMANOVIĆ, DRAGOSLAVA RADIN I DRAGANA JOŠIĆ (1997): Azotofiksaciona aktivnost sojeva *Rhizobium galegae* specifičnih za *G. orientalis* i *G. officinalis*. Deveti kongres I.D.P.Z. Uređenje, korišćenje i očuvanje zemljišta, Novi Sad, 457-464.
16. MILIČIĆ B., KUZMANOVIĆ Đ., RADIN DRAGOSLAVA, RADENOVIĆ B. (1996): Gala Azotofiksin novo mikrobiološko đubrivo u proizvodnji *G. orientalis* Lam. u našoj zemlji. Zbornik radova sa VIII Jugoslovenskog simpozijuma o krmnom bilju, Novi Sad, 26, 449-445.
17. PETROVIĆ V. (1962): Uporedna ispitivanja aktivnosti sojeva *R. meliloti* i *R. trifolii*. Arhiv za poljoprivredne nauke God. XV, sv. 47, 92-98.
18. PETROVIĆ V. I VOJINOVIĆ Ž. (1963): O dejstvu inokulacije lucerke (*Medicago sativa*) i crevene deteline (*Trifolium pratense*) u poljskim ogledima. Zemljište i biljka god. XII, No. 1-3, 295-300
19. WIVSTAD M., A.M. MARTENSSON AND H.D. LJUNGGREN (1987): Field measurement of symbiotic nitrogen fixation in an established lucerne ley using ^{15}N and an acetylene reduction method. Plant and Soil, vol. 97, No. 1, 93-104.
20. RADENOVIĆ B. (2000): Semenarstvo krmnog bilja. Monografija. Ed Velarta, Beograd, Srbija i Crna Gora.
21. STOUGARD J. (2000): Regulators and regulation of legume root nodule development. Plant Physiol. 124, 531-540.
22. VUKMIR DUŠICA (1993): Određivanje prisustva i aktivnosti bakterije *Rhizobium meliloti* u različitim tipovima zemljišta Kruševačkog regiona. Magistarski rad, Biološki Fakultet, PMF, Univerzitet u Beogradu.
23. VOJINOVIĆ, B. MILIČIĆ, DRAGOSLAVA RAJIN I Đ. KUZMANOVIĆ (1989): Prisustvo i aktivnost sojeva *R. meliloti* i *R. trifolii* u nekim zemljištima Srbije. Mikrobiologija, vol. 26, No. 1, 69-81.
24. WIVSTAD M., A.M. MARTENSSON AND H.D. LJUNGGREN (1987): Field measurement of symbiotic nitrogen fixation in an established lucerne ley using ^{15}N and an acetylene reduction method. Plant and Soil, vol. 97, No. 1, 93-104.