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QUANTITATIVE ABILITIES OF BIOLOGICAL NITROGEN REDUCTION FOR *Rhizobium galegae* CULTURES BY GOAT'S RUE

ILOŚCIOWE MOŻLIWOŚCI BIOLOGICZNEJ REDUKCJI AZOTU PRZEZ BAKTERIE *Rhizobium galegae* WSPÓŁŻYJĄCE Z RUTWICĄ WSCHODNIĄ

Abstract: The field experiment (microplots) was carried out in 2005–2007 in the experimental site belonging to the University of Podlasie in Siedlce. Nitrogen ^{15}N at 10.3 at. % enrichment was applied in a form of $(^{15}NH_4)_2SO_4$ at the amount of 1.66g per 1 m^2 in early spring. In parallel to goat's rue (*Galega orientalis* Lam.) cultivation, also plant that did not show the ability of biological N_2 reduction (spring barley (*Hordeum sativum*) was grown, and it was also fertilized with ^{15}N in the form of $(^{15}NH_4)_2SO_4$ at 10.3 at. % enrichment.

The quantitative abilities of biological nitrogen reduction for *Rhizobium galegae* cultures living together by goat's rue (*Galega orientalis* Lam.) was determined by means of isotope dilution method. The abundance of at. % ^{15}N was determined by spectroscopic method on the spectrophotometer NOI – 6E, then the amount of nitrogen transferred from the air due to biological N_2 reduction process, was calculated.

The yield of as a sum from three cuts of dry matter of tested plant in subsequent study years was [kg · m^{-2}]: 2005 – 1.092; 2006 – 0.831; 2007 – 0.509. The quantity of biologically reduced nitrogen reached up the mean value for three experimental years at the level of 28.863 g N · m^{-2} but in each year as follows [g N · m^{-2}]: 2005 – 37.603; 2006 – 26.080; 2007 – 22.906 during the whole vegetation period.

Keywords: *Rhizobium galegae* cultures, goat's rue, yield, spring barley

High prices of nitrogen fertilizers considerably increase the costs of animal fodder production. However, there is an opportunity to decrease them due by cultivating the fodder plant species that have the ability of biological N_2 reduction [1]. The goat's rue (*Galega orientalis* Lam.) can be the example.

Process of biological reduction of gaseous nitrogen from atmospheric air consists its incorporating into the biological system [2, 3]. *Rhizobium* bacteria living in a symbiosis with papilionaceous plants, as well as freely living bacteria (*Azotobacter, Clostridium*), fungi (*Rhizopus*), and actinomycetes (*Streptomyces*), show such ability [4, 5]. Their

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common feature is that they contain nitrogenase – a principle enzyme responsible for elemental nitrogen reduction. Nitrogenase is composed of two protein complexes. Protein containing Mo-Fe is the enzyme that reduces N_2, while protein containing only Fe provides with electrons necessary for reduction process.

Recently, more and more intensive studies are performed upon the goat's rue (perennial papilionaceous plant species) originating from Caucasus or Estonia [6, 7, 8, 9]. The plant can be grown for green forage, dried fodder, protein concentrate, and as energetic plant [10]; it can also be used for conserving potential wastelands.

The present study aimed at evaluating the amount of nitrogen from air biologically reduced by goat's rue biomass (*Galega orientalis* Lam.) during three subsequent vegetation periods.

Material and methods

The field experiment (microplots) was conducted in 2005–2007 on goat's rue plantation (9th, 10th, and 11th years of cultivation). Studies were carried out on a soil developed from strong loamy sand at pH value 6.9 in 1 mol KCl dm^{-3}. The experiment was performed on soil which contained 31.2 $g \cdot kg^{-1}$ of total carbon and 3.6 $g \cdot kg^{-1}$ of total nitrogen. Its abundance in available phosphorus and potassium was considered as moderate, while magnesium – poor. Nitrogen ^{15}N at 10.3 at. % enrichment was applied in a form of $(^{15}NH_4)_2SO_4$ at the amount of 1.66 g per 1 m^2 in early spring. Along with the goat's rue (*Galega orientalis* Lam.), also other plant that had not the feature of gaseous nitrogen reduction, was cultivated (spring barley – *Hordeum sativum*), and it was also fertilized with ^{15}N in form of $(^{15}NH_4)_2SO_4$ at 10.3 at. % enrichment.

Each year of study, three cuts of the test plant were harvested at the budding stage. The fresh and dry matter yields were determined. During the harvest of subsequent cuts of goat's rue (*Galega orientalis* Lam.), whole plant samples were collected, and they were dried and ground. Total nitrogen content was determined by means of Kjeldahl's method after wet digestion [4]. Analogous procedure was applied to spring barley as a control plant.

The quantitative abilities of biological nitrogen reduction by goat's rue were evaluated be means of isotope dilution method using spectrophotometer NOI – 6E for determination of (at. % ^{15}N), then the amount of nitrogen from the air due to biological reduction of gaseous nitrogen, was calculated [4]. Percentage of nitrogen plant from air calculated according to model [5]:

$$\% \text{ N} = [1 - (\text{at. } \% \ ^{15}\text{N enrichment fx : at. } \% \ ^{15}\text{N enrichment nfx})] \cdot 100$$

fx – papilionaceous plant, nfx – non-papilionaceous plant.

Achieved results were statistically processed applying variance of analysis, whereas significant differences were calculated using Tukey's test at the significance level of $p = 0.05$.

Results and discussion

Data on rainfalls and air temperatures during vegetation seasons 2005–2007 are presented in Table 1.

Table 1

Air temperatures and rainfall in the vegetation in the years 2005–2007.
Reported by the measurement centre in Siedlce

Means air temperature [^{o}C]								
Months	Years	IV	V	VI	VII	VIII	IX	Mean
Mean monthly	2005	8.6	13.0	15.9	20.2	17.5	15.0	15.0
	2006	8.4	13.6	17.2	22.3	18.0	15.4	15.8
	2007	8.3	14.5	18.2	18.5	18.6	13.1	15.2
Multiyear mean		7.7	10.0	16.1	19.3	18.0	13.0	11.4
Total monthly rainfall [mm]								Sum
Sum monthly	2005	12.3	64.7	44.1	86.5	45.4	15.8	268.8
	2006	29.8	39.6	24.0	16.2	227.6	22.0	359.2
	2007	21.2	59.1	59.9	70.2	31.1	67.6	309.1
Multiyear sum		52.3	50.0	68.2	45.7	66.8	60.7	343.7

Mean monthly temperature in subsequent vegetation periods was similar (15.0 to 15.8 ^{o}C), which was much higher as compared to many-year average. Temperatures recorded during vegetation favored the process of biological reduction of N_2 [11]. Mean sum of rainfalls during vegetation was lower than the many-year value. Only in 2006, it was slightly higher (by 15.5 mm), which resulted from high precipitation sum in August 3-fold exceeding the many-year average level.

Mean yield of dry matter biomass of goat's rue (*Galega orientalis* Lam.) harvested at budding stage amounted to 0.878 kg $\cdot$ m^{-2}, which was significantly differentiated for studied factors, as well as their interaction (Table 2). Significantly the highest yields were achieved for the I cut. For subsequent cuts of goat's rue, a considerable decrease of yields was recorded. When considering subsequent study years, it can be univocally concluded that the highest yield of the test plant was harvested in 2005 (1.092 kg $\cdot$ m^{-2}), whereas it decreased by about 100% in the third year. Mean nitrogen content in goat's rue biomass (*Galega orientalis* Lam.) was 37.4 g $\cdot$ kg^{-1} dm (Table 2). Here achieved results upon nitrogen contents are similar to those recorded by Ignaczak [6] for the first and second cuts. Symanowicz and Kalembasa [8], in the field experiment involving goat's rue seeds infected by *Rhizobium galegae*, determined slightly higher total nitrogen amounts (39.0–43 g $\cdot$ kg^{-1} dm). In other study with goat's rue, in which nitrogen contents in the plant biomass were compared in the third and seventh cultivation years [12], mean nitrogen content at budding stage ranged around 29.3 g $\cdot$ kg^{-1} dm in the third and 48.1 g $\cdot$ kg^{-1} dm in the seventh year. However, in the pot experiment, Andrzejewska and Ignaczak [7] observed lower nitrogen concentrations in aboveground parts of goat's rue (0.81–2.33 %). Probably, the field conditions are more favorable for biological reduction of N_2. Significantly the highest nitrogen content was determined in the first cut. Subsequent study years affected the total nitrogen content in the biomass at the level of about 39.2–37.8 $g \cdot kg^{-1}$ dm).

Table 2

Yield of dry matter [$kg \cdot m^{-2}$] and the content of total nitrogen [$g \cdot kg^{-1}$] of got's rue (*Galega orientalis* Lam.) of fertilization ^{15}N

Cuts (A)	Dry matter yield [$kg \cdot m^{-2}$]				Nitrogen content [$g \cdot kg^{-1}$]			
	Research years (B)							
	2005	2006	2007	Mean	2005	2006	2007	Mean
I	0.772	0.557	0.270	0.533	47.2	41.6	41.6	43.5
II	0.276	0.140	0.273	0.230	33.6	30.4	33.6	32.5
III	0.044	0.134	0.166	0.115	36.7	33.9	38.1	36.2
Sum (Mean)	1.092	0.831	0.509	0.878	39.5	35.3	37.8	37.4
LSD $_{0.05}$ for:								
cuts (A)	0.005				1.4			
years (B)	0.005				1.4			
interaction (A×B)	0.009				2.5			

A summarized nitrogen uptake along with the three cuts of goat's rue dry matter was 35.343 $g N \cdot m^{-2}$ (Table 3). Significant differences in nitrogen uptake were present between subsequent cuts and study years as well as interaction of these factors. Significantly the highest nitrogen uptake with goat's rue biomass yield (23.614 $g N \cdot m^{-2}$) was recorded for the first cut, and it reached up to about 3-fold higher level in relation to that for the second cut.

Table 3

Uptake of nitrogen in the yield of dry matter of goat's rue [$g N \cdot m^{-2}$] and at. % ^{15}N enrichment

Cuts (A)	Uptake [$g N \cdot m^{-2}$]				At. % ^{15}N enrichment			
	Research years (B)							
	2005	2006	2007	Mean	2005	2006	2007	Mean
I	36.438	23.171	11.232	23.614	0.037	0.035	0.039	0.037
II	9.274	4.256	9.173	7.568	0.017	0.019	0.018	0.018
III	1.615	4.543	6.325	4.161	0.009	0.011	0.011	0.010
Sum (Mean)	47.327	31.970	26.730	35.343	0.021	0.022	0.023	0.023
LSD $_{0.05}$ for:								
cuts (A)	0.262				0.01			
years (B)	0.262				ns			
interaction (A×B)	0.453				ns			

A considerable decrease of nitrogen uptake with goat's rue dry matter occurred in subsequent years. Particular cuts and subsequent study years differentiated the nitrogen uptake. Significantly the lowest nitrogen uptake was calculated for the third cut. Table 3 also presents the atomic enrichment percentage (at. % ^{15}N enrichment) for particular cuts and years. These values were calculated from the difference between the nitrogen amount expressed as at. % ^{15}N of examined sample and a standard (nitrogen contained in the air – 0.3663 % ^{15}N). Statistical processing revealed significant differentiation of the enrichment with ^{15}N isotope at goat's rue only for cuts.

The highest isotope ^{15}N enrichment was recorded for the first cut in subsequent study years (0.037, on average). In the second and third cut, the enrichment value decreased reaching its minimum levels in the last cut. It can be supposed that lower ^{15}N enrichment was associated with its dissolution in goat's rue yields. The ^{15}N isotope enrichment was also determined at spring barley (as a control plant) that has not the ability of biological gaseous nitrogen reduction. The value was – 0.214.

The percentage of total nitrogen share at goat's rue (*Galega orientalis* Lam.) due to biological reduction of atmospheric nitrogen was significantly differentiated for cuts and experimental years (Table 4). The highest values were achieved for the third cut (90.9%).

Table 4

Percentage share of total nitrogen from the air as a result of biological reduction of N_2 and amount of nitrogen biologically reduced for *Rhizobium galegae* cultures living together by goat's rue [g N · m^{-2}]

Cuts (A)	% total N from biological reduction of N_2				Amount of nitrogen biologically reduced from air [g N · m^{-2}]			
	Research years (B)							
	2005	2006	2007	Mean	2005	2006	2007	Mean
I	82.7	79.3	81.6	812	28.034	18.375	9.165	18.525
II	92.0	87.1	88.5	89.2	8.133	3.707	8.118	6.653
III	95.8	88.0	88.9	90.9	1.436	3.998	5.623	3.686
Sum (Mean)	90.2	84.5	86.3	87.1	37.603	26.080	22.906	28.863
$LSD_{0.05}$ for:								
cuts (A)	3.01				0.072			
years (B)	3.01				0.072			
interaction (A×B)	ns				0.125			

A high percentage of total nitrogen due to biological reduction was confirmed in studies by Andrzejewska and Ignaczak [7].

During its whole vegetation season of the goat's rue the biological reduction of nitrogen from air reached 28.863 g N · m^{-2} from the air (Table 4). Studied factors as well as their interaction significantly differentiated the amount of nitrogen uptaken from the air. The highest quantities of biologically reduced nitrogen were determined in the biomass of the first cut of goat's rue harvested at budding stage. The highest summarized amounts of nitrogen for the three cuts were recorded in the first study year – 2005 (37.603 g N $\cdot$ m^{-2}). Achieved results were similar to those reported by Symanowicz et al [13], in which the amounts of biologically reduced nitrogen by goat's rue harvested at budding stage were 379.7 kg N $\cdot$ ha^{-1}. Calculated correlation coefficient (r = 0.99) indicated the dependence between the quantity of biologically reduced nitrogen in subsequent cuts and experimental years.

Conclusions

1. The harvest of the first cut of goat's rue (*Galega orientalis* Lam.), significantly pointed out the highest values for biomass yield, total nitrogen content, nitrogen uptake, at. % ^{15}N enrichment, and biologically reduced nitrogen amounts were recorded.
2. Significant decrease of studied parameters occurred in subsequent experimental years.
3. Mean amount of biologically reduced nitrogen during the whole vegetation period of goat's rue (*Galega orientalis* Lam.) was 28.863 g N $\cdot$ m^{-2}.

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ILOŚCIOWE MOŻLIWOŚCI BIOLOGICZNEJ REDUKCJI AZOTU PRZEZ BAKTERIE *Rhizobium galegae* WSPÓŁŻYJĄCE Z RUTWICĄ WSCHODNIĄ

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Abstrakt: Doświadczenie polowe mikropoletkowe przeprowadzono w latach 2005–2007 na polu należącym do Akademii Podlaskiej w Siedlcach. Azot ^{15}N o wzbogaceniu 10,3 at. % stosowano w formie $^{15}(NH_4)_2SO_4$ w ilości 1,66 g na 1 m^2 wczesną wiosną. Równolegle z uprawą rutwicy wschodniej (*Galega orientalis* Lam.) uprawiano roślinę niemającą zdolności biologicznej redukcji N_2 (jęczmień jary – *Hordeum sativum*), którą również nawożono ^{15}N w formie $^{15}(NH_4)_2SO_4$ o wzbogaceniu 10,3 at. %.

Ilościowe możliwości biologicznej redukcji azotu przez kultury bakterii *Rhizobium galegae* współżyjące z rutwicą wschodnią (*Galega orientalis* Lam.) określono po zastosowaniu metody izotopowego rozcieńczenia.

Na spektrofotometrze NOI – 6E oznaczono at. % ^{15}N, a następnie obliczono ilość azotu pochodzącego z powietrza w wyniku biologicznej redukcji N_2.

Sumaryczny plon suchej masy rośliny testowej w kolejnych latach badań wynosił [kg · m^{-2}]: 2005 – 1,092; 2006 – 0,831; 2007 – 0,509. Ilość biologicznie zredukowanego azotu osiągnęła średnią wartość z trzech lat badań na poziomie 28,863 g N · m^{-2}, a w kolejnych latach kształtowała się następująco [g N · m^{-2}]: 2005 – 37,603; 2006 – 26,080; 2007 – 22,906 w ciągu okresu wegetacyjnego.

Słowa kluczowe: bakterie *Rhizobium galegae*, rutwica wschodnia, plon, jęczmień jary