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ORIENTATION OF PROCESSES OF BIOLOGICAL NITROGEN TRANSFORMATION IN WINTER RYE AGROECOSYSTEMS UNDER DIFFERENT LEVELS OF FERTILIZATION BACKGROUND

V. V. Volkogon, I. G. Korotka

Institute of Agricultural Microbiology and Agro-Industrial Production, NAAS of Ukraine
97, Shevchenka Str., Chernihiv, Ukraine, 14027

e-mail: chychvaga_i@i.ua

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Aim. To determine physiologically expedient rates of mineral nitrogen in winter rye production on sod-podzolic soils based on the orientation of the processes of biological nitrogen transformation in the plants rhizosphere. **Methods**. Field studies, gas chromatography determination of potential nitrogen fixation activity and potential emissions of N_2O. **Results.** The results obtained have demonstrated that the rates of mineral nitrogen, not exceeding 60 kg/ha, can be considered physiologically expedient for winter rye production on sod-podzolic soils. Under the application of microbial preparation Diazobakteryn, there is a higher physiological need of plants for nitrogen, which allows increasing the rates of nitrogen fertilizers up to 90 kg/ha. **Conclusions**. The orientation of the processes of biological nitrogen transformation in the root zone of plants is a reliable indicator of determining the appropriateness of nitrogen fertilization of crops.

Keywords: nitrogen fixation, emission of N_2O, biological nitrogen transformation, physiological need for nitrogen.

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INTRODUCTION

Nitrogen fertilizers are known as one of the most powerful pollutants of environment. The degree of their consumption by crops is in the range of 35–50 % [1], which is taken into consideration while calculating the fertilization rates, required for the harvest (calculation formulas envisage the corresponding increase in the amount of fertilizers). Non-consumed nitrogen fertilizers pollute ground waters and cause the emission of N_2O. The problem could be avoided if nitrogen fertilizers were used in the range of physiological needs of plants. However, the standard of truth – what is the need of plants for nitrogen? – is unknown, thus, it is frequently deemed that this indicator reflects the content of nitrogen compounds in the yield of the corresponding quality.

It has recently become possible to determine the physiological need of plants for nitrogen due to numerous works, proving a high degree of association between some kinds of microorganisms and plants [2–4]. Here nitrogen-fixation associative bacteria are unique microorganisms. Being in close spatial and trophic interaction with plant roots, they form an integral biological system, thus tracing the changes in the functional activity of the bacterial component as a response to some degree of nitrogen fertilization would allow estimating the similar response of the very plant to a specific concentration of mineral nitrogen. In 1985 Umarov *et al*. [5] demonstrated the increase in the activity of the associative nitrogen-fixation due to the application of specific doses of nitrogen fertilizers, and formulated the notion of "physiological optimum of nitrogen for plants" as a rate of fertilizers, ensuring the highest level of activity of associative nitrogen-fixation. The researchers from the Philippines came to the same conclusion [6]. The mechanism of stimulating the activity of associative nitrogen-fixation by physiologically optimal rates of mineral nitrogen comes down to the increase in the amount of root exudates which are the source of carbon and energy for rhizosphere nitrogen-fixers. For instance, there are data about the 9.5-fold increase in the root exudation compared to the volume of root exudates in the control (without nitrogen fertilizers) plants [7]. Root exudates may have a larger or smaller amount of nitrogen compounds depending on the amount of the introduced fertilizers [8], which has an immediate impact on the functional activity of rhizosphere microorganisms.

While working at the mentioned issue, microbiologists tried to substantiate the rate of fertilizers, the application of which would result in the highest amount of "biological" nitrogen (bound to air nitrogen-fixating bacteria) coming into the agroecosystem. We made an effort of estimating the situation from another point of view. Based on our understanding that the scale of associative nitrogen-fixation process in conditions of moderate climate is not high, our aim was to determine the threshold of the rates of nitrogen fertilizers, exceeding which would result in the decrease in the activity of associative nitrogen-fixation compared to the control (variant without any fertilizers). In other words, the rates of nitrogen fertilizers, not exceeding the mentioned rate, may be considered to be physiologically expedient.

An additional test (besides determining nitrogen-fixation) of the appropriateness of nitrogen fixation may be the determination of the activity of biological dinitrification, as diazotrophs are capable of fulfilling the functions of dinitrofixators at the excess of the bound nitrogen [9, 10].

Therefore, taking into account the capability of nitrogen-fixers to have functional changes depending on the content of nitrogen in the system, it is possible to substantiate the rates of nitrogen fertilizers, neither decreasing the activity of nitrogen fixation in the root zone of plants nor leading to the excessive activity of dinitrification, which is an indirect evidence of optimal fertilization of the crop.

The aim of this work was to experimentally check the abovementioned assumptions during winter rye production with the consideration of the nitrogen fertilization impact on the yield capacity, and to determine the impact of the pre-sowing inoculation on the degrees of physiological appropriateness of nitrogen fertilizers for plants.

MATERIALS AND METHODS

Field experiments were conducted on sod-podzolic silt-sandy soil (pH_{salt} − 6.2; humus content − 1.2 %; content of easily hydrolyzed nitrogen according to Cornfield − 54.9 mg/kg; P_2O_5 − 330 mg/kg; K_2O – 148 mg/kg) of the Institute of Agricultural Microbiology and Agro-Industrial Production, NAAS of Ukraine in 2011−2015. The experiments were conducted in two variants – without inoculation and with the application of a microbial preparation Diazobakteryn (TC U 24.1-00497360-002:2005). Winter rye, Syntetyk-38 cultivar, in both variants was cultivated on the background of increasing rates of mineral fertilizers:

1. no fertilizers;
2. $N_{30}K_{20}$ (N_{10} in autumn + N_{20} early spring);
3. $N_{60}K_{40}$ (N_{30} in autumn + N_{30} early spring);
4. $N_{90}K_{60}$ (N_{30} in autumn + N_{30} early spring + N_{30} in the phase of stem elongation);
5. $N_{120}K_{80}$ (N_{30} in autumn + N_{45} early spring + N_{45} in the phase of stem elongation).

The phosphoric fertilizers were not applied due to high content of mobile phosphates in the soil.

The potential activity of nitrogen fixation in the plant rhizosphere was determined in dynamics [11]. The nitrogenase activity was determined by the acetylene reduction method on gas chromatograph Chrom-5 with flame-ionization detector; the potential activity of dinitrification in the rhizosphere soil – with the addition of the solution of glucose and potassium nitrate [12]. The reduction of nitrogen oxide was determined at the chromatograph Tsvet-560M with the thermal conductivity detector.

The yield was registered. The results were statistically processed by the method of two-factor dispersion analysis [13] using Microsoft Office Excel 2007.

RESULTS AND DISCUSSION

The highest indicators for potential activity of nitrogen fixation in the plant rhizosphere were observed in the control variant in the tillering phase in spring. Specific introduction of fertilizers in the doses of $N_{30}K_{20}$, $N_{60}K_{40}$, $N_{90}K_{60}$, $N_{120}K_{80}$ demonstrates the decrease in the process activity with the increase in the dose. The analyses in the blossoming phase demonstrate high indices of nitrogenase activity in the variants with the introduction of $N_{30}K_{20}$, the tendency to increase − with the application of $N_{60}K_{40}$ and the prolongation of the process inhibition − in the variants with $N_{90}K_{60}$

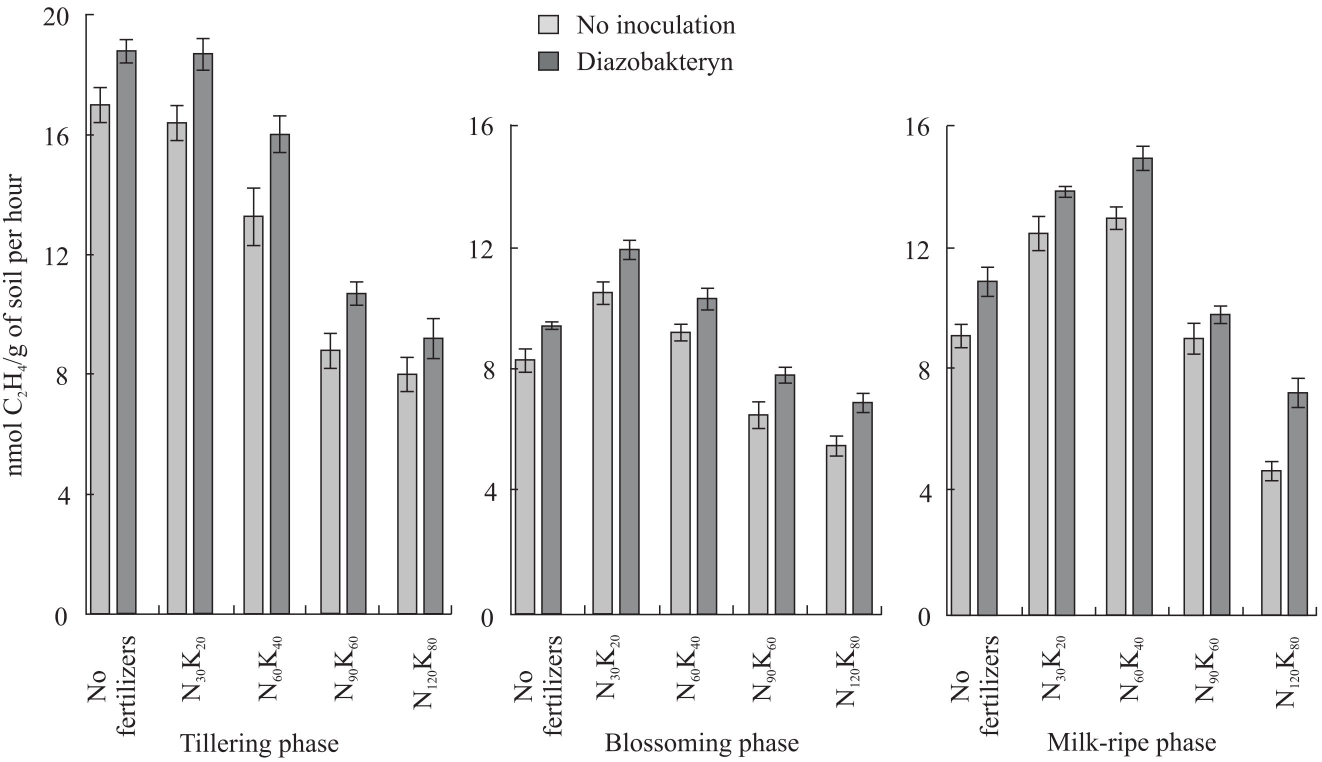

Fig. 1. Potential nitrogenase activity of winter rye plant rhizosphere under the impact of inoculation and fertilizers, field experiment 2013

and $N_{120}K_{80}$. The subsequent studies demonstrated the highest indices in the variants with the introduction of mineral nitrogen in the rates, not exceeding 60 kg/ha. It should be noted that even at the end of the vegetative period of winter rye the rate of mineral nitrogen of 120 kg/ha inhibits the nitrogenase activity in the root zone of plants, which testifies to its inappropriateness while cultivating rye on sod-podzolic soil (Fig. 1).

Noteworthy is the stimulation of nitrogen fixation process with not high rates of mineral nitrogen at first, followed by the use of higher rates. The rate of mineral nitrogen of 60 kg/ha, being compromise regarding the provision of constructive metabolism of plants and also not excessive for environment, is capable of stimulating the activity of nitrogen fixation process, and the increase in the nitrogenase activity in the plant root zone compared to the control (without the introduction of nitrogen fertilizers) is a reliable criterion of physiological and ecologic appropriateness of nitrogen fertilization of the crop.

The application of Diazobakteryn mitigates the negative impact of mineral fertilizers considerably and restores the activity of the nitrogen-fixation activity significantly. This biopreparation stimulates the activity of the nitrogen fixation process at the introduction of N_{60} in the highest degree, but the process activity, higher than control indices, with inoculation is also observed with the application of N_{90}. This fact may be explained by the nitrogen consumption from fertilizers by inoculation-initiated plants, and thus by the decrease in the concentration of nitrogen compounds in the root zone. Therefore, the threshold of physiological appropriateness of nitrogen fertilizers increases in these conditions. The application of Diazobakteryn does not allow restoring the nitrogen fixation activity in the variants with the introduction of high rates of fertilizers (even at the end of vegetation the indices are lower than the control ones).

During the studies the absolute indices of nitrogen fixation activity changed, but the specificities of the process depending on the rates of nitrogen fertilization were stable.

The study of potential emission of nitrogen oxide revealed the increase in the mentioned indices regarding the increase in the rates of mineral nitrogen fertilizers (Fig. 2). Here the loss of nitrogen is in proportion to the introduced rates of fertilizers. Diazobakteryn enhances the emission of nitrogen oxide at the beginning of vegetation, especially in the variants with the introduction of mineral fertilizers. This is explained by the fact that

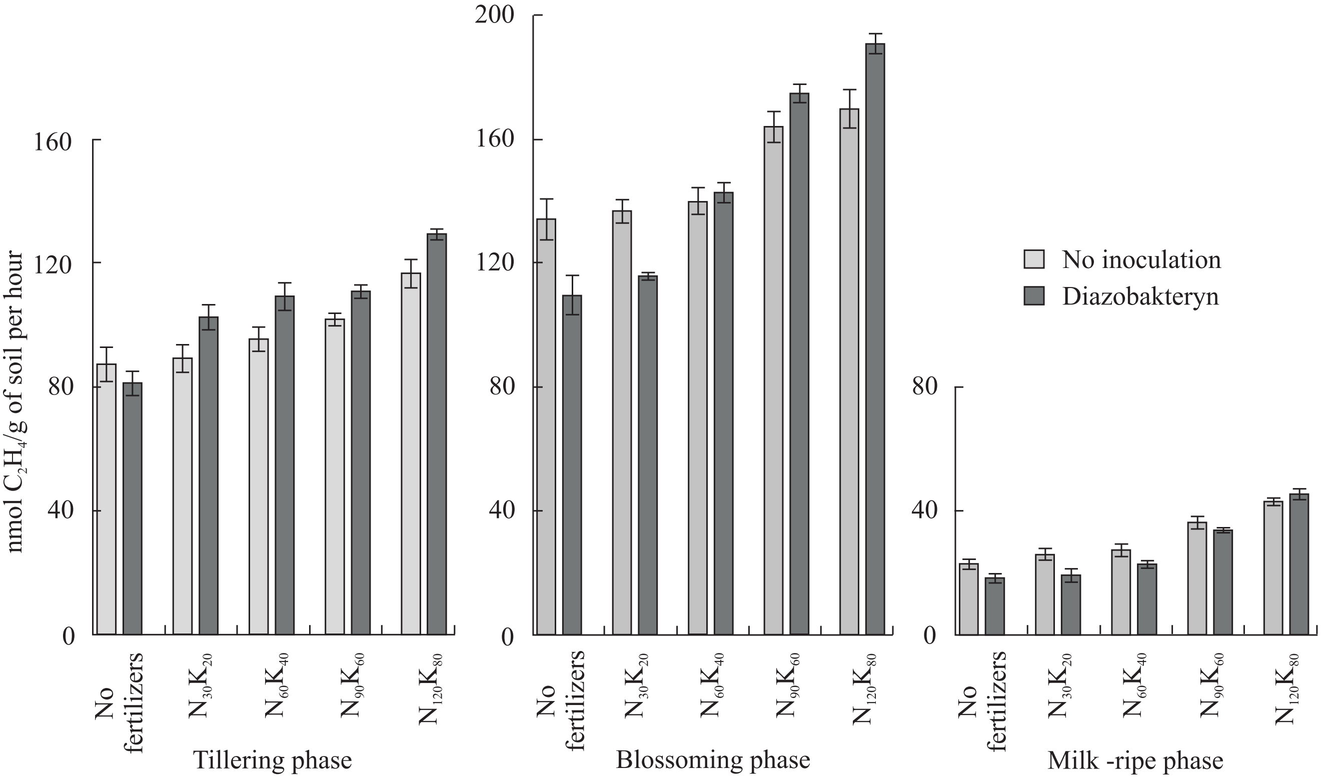

Fig. 2. Potential emission of nitrogen oxide from winter rye plant rhizosphere under the impact of inoculation and fertilizers, field experiment 2013

even small rates of fertilizers are excessive for juvenile plants. The enzymatic level faces changes, conditioned by the balance of natural resources: the synthesis of nitrogen fixation enzymatic nitrogenase complex is inhibited, nitrate reductase is synthesized instead. Here the bacteria, introduced in the agroecosystem, are temporarily involved in the dinitrification process, which reflects on the increase in its activity.

During the subsequent phases of plant development, the application of Diazobakteryn in physiologically appropriate agrobackgrounds promotes the decrease in gasiform losses of nitrogen, which is explained by exhausting the excessive amount of nitrogen compounds and the transition of the bacteria to the nitrogen fixation function.

The comparison of the biological dinitrification intensity in the experimental variants demonstrates that with the dosed introduction of fertilizers in the norm of 60 kg/ha of mineral nitrogen, although the emission of nitrogen oxide has a tendency towards increasing, it is not statistically different from the control variant indices, starting with the blossoming phase. At the same time the application of N_{90} and N_{120} conditions reliable losses in gasiform nitrogen compounds during the vegetation period.

Therefore, based on the results of studying the orientation of the processes of biological nitrogen transformation in the root zone of winter rye plant production on sod-podzolic soil, it is possible to consider the rates of mineral nitrogen, not exceeding 60 kg/ha, to be physiologically expedient in these conditions (with the dosed application), as their long-term introduction results in the increase in the nitrogen fixation activity as a criterion of physiological optimization and ecologic well-being along with the insignificant increase in the loss of gasiform nitrogen compounds. The nitrogen norm of 60 kg/ha should be considered physiologically threshold due to short-time stimulation of nitrogen fixation activity and promoting the loss in gasiform nitrogen compounds during the whole vegetation period.

The pre-sowing inoculation using Diazobakteryn leads to a considerable increase in the physiological optimum in bound forms of nitrogen for inoculation-initiated winter rye plants and the mentioned rate becomes physiologically expedient. It is not reasonable from the physiological point of view to introduce higher rates, as this leads to the long-term decrease in nitrogenase activity and the excessive loss of gasiform nitrogen compounds, which testifies to the excessive dose of nitrogen nutrition of plants.

It is worth mentioning while analyzing the yield data that the increase in the rate of mineral nutrition leads to the decrease in the yield return (Table).

Thus, the application of the smallest rate of fertilization in the experiment ensures the yield surplus by 37.5 %, and that of the highest one – only by 5.2 % from the previous one. The application of Diazobakteryn corrects the situation considerably. The return of fertilizers is much higher. In particular, the crop yield with the introduction of $N_{30}K_{20}$ and inoculation ensures the formation of almost the same yield, achieved with the introduction of fertilizers at the rate of $N_{60}K_{40}$. The combination of the biopreparation and the rate of fertilizers $N_{60}K_{40}$ promotes obtaining the same yield, observed for the introduction of $N_{90}K_{60}$ (without inoculation) − 4.81 and 4.84 t/ha respectively. Taking into consideration the data of the experimental yield, we may state that the impact of Diazobakteryn and the effect of mineral fertilizers on the crop productivity at the rate of $N_{30}K_{20}$ are equivalent. The positive effect of the pre-sowing inoculation of the seeds on the formation of crop productivity is explained by a number of reasons. These are the increase in the nitrogen fixation activity, the enhanced immune status of plants, and the direct limitation of the development of harmful microorganisms in the root spheres. However, it may find its best explanation in the effect on the development of the root zone, its consumption ability and the increase in the coefficients of plants, consuming the active substance from the fertilizers, which is confirmed by the literature data [14, 15].

The studies demonstrate the possibility of determining the rates of mineral nitrogen, appropriate from the physiological and ecological points of view, as the nitrogen fixation process is not possible in the presence of excessive amount of nitrogen fertilizers.

The results obtained may be used to highlight that the ecologic situation in agroecosystems is optimized using the presence of specific (physiologically appropriate) rates of fertilizers, not their absence, as it could have been expected. This does not testify in favor of the concept of complete refusal from the application of mineral fertilizers in the technologies of cultivating crops. On the contrary, fertilizers should be applied, but only on the grounds of their physiological appropriateness for the crops. The consideration of the abovementioned methodological approaches to the ecologic estimation

The yield of winter rye at the impact of mineral fertilizers and microbial preparation Diazobakteryn

Experiment variant	Yield, t/ha (average for 2011–2015)	Surplus after each subsequent dose of fertilizers*		Surplus due to inoculation	
		tons/ha	%	tons/ha	%
No inoculation					
No fertilizers, control	2.75	–	–	–	–
$N_{30}K_{20}$	3.78	1.03	37.5	–	–
$N_{60}K_{40}$	4.33	0.55	14.6	–	–
$N_{90}K_{60}$	4.84	0.51	11.8	–	–
$N_{120}K_{80}$	5.09	0.25	5.2	–	–
Inoculation with Diazobakteryn					
No fertilizers, control	2.95	0.20	7.3	0.20	7.3
$N_{30}K_{20}$	4.15	1.20	40.7	0.37	9.8
$N_{60}K_{40}$	4.81	1.03	27.2	0.48	11.1
$N_{90}K_{60}$	5.24	0.91	21.0	0.40	8.3
$N_{120}K_{80}$	5.43	0.59	12.2	0.34	6.7
HIP_{05}					
for experiment	0.67				
for fertilizers	0.42				
for inoculation and interaction	0.31				

*Including the combination with inoculation.

of the rates of mineral nitrogen could approximate the positions of such sciences as agrochemistry, microbiology, and plant physiology in terms of selecting rational strategies of fertilization for crops. It is known that the optimization of mineral nutrition for crops should be aimed at solving rather various tasks, including stable yields with optimal indices of biological quality and hygienic purity, decrease in relative loss of nutrients during the production process of crops, minimization of loading chemization means on soils to preserve their fertility and ecologic functions [16]. Therefore, the efficiency of applying fertilizers should be estimated not only from the position of maximal provision of crops with the compounds of biogenic elements and economic indices of a specific vegetation period, but also from the point of view of their impact on environment.

The focus of many researchers was concentrated on the ecologic side of the problem of applying mineral fertilizers in agricultural production. However, the estimates of the required amount of fertilizers, including nitrogen ones, are traditionally made for the anticipated yield with the consideration of possible coefficients of plants, consuming the active substance from fertilizers, agrochemical indices of soil, the after-effect of fertilization of previous crops in the crop rotation, nitrogen content in after-harvest remains, etc. There are several dozens of modifications of this method, the essence of which being the estimation of the return of the elements with the anticipated yield [17]. Possible ecologic problems are ignored here. Therefore, we suggest determining the rates of nitrogen fertilization of crops by physiological needs. The rates of phosphate and potassium fertilizers may be estimated by the known principle of balance for the rate of nitrogen fertilizers, taking into account the results of previous diagnostics of soils.

The materials, presented in the article, demonstrate that the physiological optimum of plants regarding nitrogen may change. In particular, the inoculation-initiated plants demand a larger amount of nitrogen for constructive metabolism. Here is the corresponding possibility of introducing a larger amount of fertilizers to ensure the additional yield without any risk of environmental pollution. Indeed, the indicators of the increase in the physiological need of plants for nitrogen may be other agents, besides microbial preparations, for instance, stimulators of growth and development, microelements, *etc*.

CONCLUSIONS

The studies have demonstrated that the rates of mineral nitrogen, not exceeding 60 kg/ha, can be considered to be physiologically expedient for winter rye production on sod-podzolic soils. Under the application of microbial preparation Diazobakteryn, there is a higher physiological need of plants for nitrogen, which allows applying the rates of mineral nitrogen at the rate of 90 kg/ha. The impact of the pre-sowing inoculation of seeds on rye productivity while cultivating the crop on physiologically expedient agrobackgrounds is equivalent to the effect of mineral fertilizers at the rate of $N_{30}K_{20}$. The orientation of the processes of biological nitrogen transformation in the root zone of plants is a reliable indicator of determining the appropriateness of nitrogen fertilization of crops.

Спрямованість процесів біологічної трансформації азоту в агроценозах з житом озимим за різних рівнів удобрення культури

В. В. Волкогон, І. Г. Коротка

e-mail: chychvaga_i@i.ua

Інститут сільськогосподарської мікробіології та агропромислового виробництва НААН
Вул. Шевченка, 97, Чернігів, Україна, 14027

Мета. За спрямованістю процесів біологічної трансформації азоту в ризосфері рослин жита озимого визначити фізіологічно доцільні норми мінерального азоту при вирощуванні культури на дерново-підзолистому ґрунті. **Методи.** Польові дослідження, газохроматографічне визначення потенційної активності азотфіксації та потенційної емісії N_2O. **Результати.** Фізіологічно доцільними нормами мінерального азоту при вирощуванні жита озимого на дерново-підзолистому ґрунті є такі, що не перевищують 60 кг/га. За використання мікробного препарату Діазобактерину фізіологічні потреби рослин в азоті зростають, що дозволяє підвищити норми азотних добрив до рівня 90 кг/га. **Висновки.** Спрямованість процесів біологічної трансформації азоту в кореневій зоні рослин є надійним індикатором визначення доцільності азотного живлення сільськогосподарських культур.

Ключові слова: азотфіксація, емісія N_2O, біологічна трансформація азоту, фізіологічні потреби в азоті.

Направленность процессов биологической трансформации азота в агроценозах с рожью озимой при разных уровнях удобрения культуры

В. В. Волкогон, И. Г. Короткая

e-mail: chychvaga_i@i.ua

Институт сельскохозяйственной микробиологии и агропромышленного производства НААН
Ул. Шевченко, 97, Чернигов, Украина, 14027

Цель. По направленности процессов биологической трансформации азота в ризосфере растений ржи озимой определить физиологически целесообразные нормы минерального азота при выращивании культуры на дерново-подзолистой почве. **Методы.** Полевые исследования, газохроматографическое определение потенциальной активности азотфиксации и потенциальной эмиссии N_2O. **Результаты.** Физиологически целесообразными нормами минерального азота при выращивании ржи озимой на дерново-подзолистой почве являются такие, которые не превышают 60 кг/га. При использовании микробного препарата Диазобактерина физиологические потребности растений в азоте возрастают, что позволяет повысить нормы азотных удобрений до уровня 90 кг/га. **Выводы.** Направленность процессов биологической трансформации азота в корневой зоне растений является надежным индикатором определения целесообразности азотного удобрения сельскохозяйственных культур.

Ключевые слова: азотфиксация, эмиссия N_2O, биологическая трансформация азота, физиологические потребности в азоте.

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