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The Activity of Glutamine Synthetase Enzyme, Content of Ammonia and Phytometrical Indexes of Spring Wheat Planting Depending on Different Time Periods and Doses of Nitrogen Fertilizers

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Abstract

The research studied the influence of time periods and doses of nitrogen fertilizers on the activity of glutamine synthetase enzyme (GS), content of ammonia in the leaves, photosynthetic activity of planting and yield capacity of spring wheat grain. It was found out that GS was mostly active in the leaves in the blooming period. Nitrogen fertilizers in the dose of 2/3 of the calculated norm in the tillering period increased yield capacity by 1.32 t/ha in comparison with the control variant and by 0.31 t/ha in comparison with the variant where the whole norm of nitrogen had been introduced before planting. Fertilizers in the dose of 1/3 of the calculated norm of nitrogen improved the quality of spring wheat grain in the booting and blooming periods.

Keywords: Spring wheat; Nitrogen fertilizers; Glutamine synthetase enzyme; Yield capacity; Photosynthetic potential; Photosynthesis net productivity

Introduction

At present cereal crops provide almost a half of all protein reserves for humanity. Intensification of synthesis of a large percentage of protein with increased nutrient value by the plants is one of the most important tasks of modern times. The study of biochemical processes, which are in the basis of taking inorganic nitrogen by the plants, plays a great role in the solution of this problem [1].

The study of the level of enzyme activity, which defines taking inorganic nitrogen, is acute both from the theoretical and practical points of view. This base can solve the issues of diagnostics of nitrogen supply to the plants, necessity and periods of nitrogen fertilizers introduction, and ways of their use with the aim to achieve maximum plant productivity [2,3].

Cereal crops need nitrogenous nutrition from the beginning of plant development. This demand is determined by the necessity to form a well-developed assimilating system. However, nitrogen doses in the early periods shall be moderate [4,5].

The question now arises of how to determine the correct time periods and doses of introducing nitrogen fertilizers. The solution may be found in the study of the activity of enzymes participating in nitrogen assimilation. One of the main enzymes of this process is glutamine synthetase (GS) catalyzing the reaction of glutamine synthesis from the glutamic acid [6,7].

The aim of the research is to study the activity of glutamine synthetase enzyme, the formation of leaf surface of planting, and the yield capacity of spring wheat grain depending on the time periods and doses of nitrogen fertilizers in conditions of the eastern part of the Volga-Vyatka region.

Materials and Methods

The research was conducted in 1998-1999 in the test field of the Mari State University. The soil of the test area was sod-low-podzolic midclay, humus content was 1.8%, active forms of phosphorus and exchange potassium were middle and high, soil acidity (pH) was 6.0. Cultivation technology of spring wheat of Priokskaya grade was common for the region. Its predecessor had been fall rye. Norms of mineral fertilizers were calculated using the balance method to provide 3 t wheat grain from one hectare and were introduced according to the experiment scheme:

1. Control (without fertilizers);
2. NPK before planting;
3. PK,1/3N before planting + 2/3N in the tillering period;
4. PK,1/3N before planting + 1/ in the tillering period + 1/3N in the heading period;
5. PK,1/3N before planting + 1/3N in the booting period + 1/3N in the blooming period.

Change of activity of glutamine synthetase enzyme was determined in the plants of spring wheat in the laboratory conditions. The selected samples were frozen, and then ground in the cold with quartz sand (1:1). After that homogenate was extracted during 1 h by 0.05 M tris-HCl buffer with pH 7.4 in the presence of additives stabilizing enzyme activity. It was centrifuged at 3,000 rpm during 25 min. Ammonia content was determined in the supernatant fluid by the microdiffusion method [5]. Protein quantity was defined in the extract after dialysis using Lowry's method and the activity of GS enzyme using the transferase method. Enzyme quantity which catalyzed the formation of 1 μmol—γ-GGL during 15 min was taken as an activity unit of glutamine synthetase. Phytometrical indexes of spring wheat planting were determined using A.A. Nichiporovich's method [8].

Results and Discussion

The research showed that the wheat leaves of the variants under research had the least activity of GS enzyme in the tillering period—0.1-0.2 unit/mg of protein with high ammonia content of 73.0-93.0 mg/g of wet weight (Table 1). At the same time ammonia content in the plant tissue was almost twice and more as high as in the following periods. High ammonia content might have had a negative effect on enzyme synthesis.

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Indexes	Phenological period	Variant				
		Control (without fertilizers)	NPK before planting	PK+1/3N before planting +2/3N in the tillering period	PK+1/3N before planting +1/3N in the tillering period +1/3N in the heading period	PK+1/3N before planting +1/3N in the booting period +1/3N in the blooming period
GS activity, unit/mg of protein	Tillering	0.2	0.1	0.1	0.1	0.1
	Booting	0.2	0.4	0.5	0.8	0.2
	Heading	1.8	2.0	2.3	2.0	2.7
	Blooming	2.7	3.7	4.2	3.9	3.4
	Average during the period of tillering—blooming	1.2	1.5	1.8	1.7	1.6
Ammonia content, mg/g of wet weight	Tillering	73.0	93.0	86.0	86.0	86.0
	Booting	49.0	54.0	46.0	42.0	37.0
	Heading	27.0	63.0	57.0	24.5	41.0
	Blooming	26.0	21.0	25.0	38.0	38.0
	Average during the period of tillering—blooming	43.8	57.8	53.5	47.6	50.5
r		−0.86	−0.84	−0.78	−0.63	−0.56

Table 1: GS activity and ammonia content in the spring wheat leaves depending on the time periods and doses of nitrogen fertilizers

Further GS activity increased against the decrease and stabilizing of ammonia content in the wheat leaves. It is necessary to note that the highest enzyme activity was in the blooming period. In this period spring wheat had the increased capability for ammonia assimilation. Therefore, spring wheat shall be fed with nitrogen fertilizers in the heading period.

The results of our research showed that the increase of nitrogen fertilizers dose did not lead to the increase of activity of glutamine synthetase. Thus, the introduction of 2/3 of the calculated nitrogen norm in the tillering period suppressed enzyme's activity a little—0.5 unit/mg of protein, whereas 1/3 of nitrogen increased the activity of glutamine synthetase up to 0.8 unit/mg of protein. In the heading period the activity level of glutamine synthetase soared and was in variants of 2.3 and 2.0 unit/mg of protein correspondingly. At the same time late nitrogen fertilizing of spring wheat increased the activity of glutamine synthetase.

All agrotechnical measures, which are carried out with the purpose to increase economic yield, may be efficient just in the case they have a positive effect on photosynthetic activity of planting [9]. Together with the increase of activity of glutamine synthetase enzyme, nitrogen fertilizers influenced the formation of leaf-area duration of spring wheat planting, their functioning duration and biomass growth (Table 2).

The research showed that the use of mineral fertilizers increased photosynthetic potential of spring wheat planting for the whole period of observation: by 46.4-87.5%—tillering-booting and 68.4-113.9%—booting-heading.

Photosynthesis net productivity characterizes planting photosynthetic rate and may vary during vegetation. Thus, in the period from tillering to booting of spring wheat it was from 3.38 to 7.40 g/m^2 a day in the experimental variants. With split nitrogen dosing PNP was significantly high against single use and control variant. In the period from booting to heading of spring wheat the accumulation of dry substances synthesized by the leaf surface increased by 6.1-63.9% depending on the variant.

Variant	Periods of growth and development			
	tillering-booting		booting-heading	
	PP, thous. m^2 / ha × day	PNP, g/m^2 × day	PP, thous. m^2 / ha × day.	PNP, /m^2 × day
1. Control (without fertilizers)	56	3.38	165	5.54
2. NPK before planting	105	6.20	306	6.87
3. PK, 1/3N before planting +2/3N in the tillering period	100	7.21	353	7.89
4. PK, 1/3N before planting +1/3N in the tillering period +1/3N in the heading period	88	7.40	315	7.85
5. PK, 1/3N before planting +1/3N in the booting period +1/3N in the blooming period	82	7.00	278	7.79
HCP_{05}	18.1	0.2	32.3	0.21

Table 2: Photosynthetic potential (PP) and photosynthesis net productivity (PNP) of spring wheat planting depending on the time periods and doses of nitrogen fertilizers

Variant	Years		Average	+, − to control
	1998	1999		
1. Control (without fertilizers)	1.16	1.00	1.08	−
2. NPK before planting	2.38	1.79	2.09	+1.01
3. PK, 1/3N before planting +2/3N in the tillering period	2.86	1.94	2.40	+1.32
4. PK, 1/3N before planting +1/3N in the tillering period +1/3N in the heading period	2.54	1.92	2.23	+1.15
5. PK, 1/3N before planting +1/3N in the booting period +1/3N in the blooming period	2.43	1.87	2.15	+1.07
HCP_{05}	0.04	0.02		

Table 3: Yield capacity of spring wheat depending on the time periods and doses of nitrogen fertilizers, t/ha

The formation of the higher yield of spring wheat grain was encouraged by the introduction of 1/3 of nitrogen, phosphorous, and potassium fertilizers before planting together with nitrogen fertilizers at the early stage of plant growth and development (Table 3).

Maximum yield capacity of spring wheat grain was gotten after the introduction of 1/3 of calculated nitrogen dose before planting together with fertilizing with the dose of 2/3 of the norm in the tillering period. Thus, increase in grain in comparison with control was 1.32 t/ha. Other variants had significantly lower yield capacity.

Mineral fertilizers truly improved the quality of spring wheat grain (Table 4).

Variant	Protein content, %	Crude gluten content, %	Hardness, %	Quality group	Natural weight, g/l	Mass of 1,000 grains, g
1	11.0	21.0	48.3	I	720.0	24.5
2	12.8	25.6	50.5	I	732.0	26.2
3	12.4	25.9	47.5	I	726.0	26.6
4	12.8	29.8	53.0	I	726.0	27.4
5	14.4	31.6	50.0	I	728.0	27.1
HCP_{05}	0.7	1.2	2.1		3.7	1.4

Table 4: The quality of spring wheat grain depending on the time periods and doses of nitrogen fertilizers

Thus, depending on the variant, protein content increased by 1.4-3.4%, crude gluten content—by 4.6-10.6%; grain natural weight was 726-732 g/l, and the mass of 1,000 grains was 26.2-27.4 g. At the same time the most efficient was nitrogen fertilizing of spring wheat planting in the booting and blooming periods.

Conclusion

The results of the research make it possible to deduce that spring wheat cultivation with split dosing of nitrogen fertilizers against full phosphorous and potassium feeding promotes activation of glutamine synthetase enzyme in the plants. The highest activity of glutamine synthetase in the leaves was observed in the blooming period. It is necessary to note that nitrogen fertilizers in the dose of 2/3 of the calculated norm in the tillering period increased yield capacity by 1.32 t/ha in comparison with control and by 0.31 t/ha—with the variant where the whole nitrogen norm was introduced before planting. The processing properties of spring wheat grain were improved by nitrogen fertilizers in the dose of 1/3 of the calculated norm in the booting and blooming periods. In comparison with the control variant protein content increased by 3.4%, crude gluten content—by 10.6%, the mass of 1,000 grains—by 2.6 g.

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