EFFECTS OF DIAZOTROP ON QUALITY OF WHEAT AND LAND GRAIN IN SUSTAINABLE WHEAT PRODUCTION

Gorica Cvijanović

Megatrend University Belgrade, Faculty of Biofarming BackaTopola, Serbia cvijagor@yahoo.com Vojin Cvijanović

Institute of soil science, Belgrade Serbia, Serbia cvija91@yahoo.com

Nenad Đurić

Megatrend University Belgrade, Faculty of Biofarming Bačka Topola, Serbia cvijagor@yahoo.com Ljubiša Živanović

Faculty of Agriculture, University of Belgrade Serbia ljuba@agrif.bg.ac.rs **Svetlana Roljević**

Institute of Agricultural Economics, Belgrade, Serbia svetlana_r@iep.bg.ac.rs

Abstract: Wheat is the important crop in the diet of the population. The production of wheat for flour is represented on all continents, and in undeveloped areas of the world it takes up 85% of the total production. In the program for the creation of new varieties of wheat, it was constantly working on increased yields, then on introducing the genes for resistance to pathogenic organisms. Today, it works to create varieties that are tolerant to abiotic factors and to increase the resistance to low temperatures, and resistance to drought. This is very topical in recent years when climate change has become more pronounced. Considering the importance of wheat as bread grain in human nutrition, it is necessary to develop methods in primary wheat production to maintain / improve the quality and quantity of wheat, while reducing the possibility of degradation of production characteristics of the soil. In addition to quantity, the quality of wheat is defined by proteins found in grains at an average of 8-16%. The synthesis of proteins is significantly influenced by genetic and agroecological factors as well as their interactions. Proteins deposited in the endosperm of seeds consist of different fractions, where 80 % constitute a fraction called gluten. Gluten plays a crucial role in forming most of the features that define the quality of wheat. Generally, the higher the protein content, the wheat quality, as the raw material for the preparation of bread, is better. In order to protect the geobiosphere from the point of view of food production, the United Nations Food and Development Organization (FAO) has adopted and implemented various programs of financial and pragmatics to stimulate agricultural production within the framework of sustainable development. Therefore, the goal of the work was to determine the protein content of wheat grains, as well as the nitrogen content (easily accessible forms and total nitrogen) in the soil under conditions of application of different groups of microorganisms-diazotrophs. The research was carried out on the experimental plot in Bačka Topola in the vegetation period 2014/2015 and 2015/2016 (factor A). The aim of this study was to determine the optimal protein content of the wheat grain in the different amounts of mineral nitrogen 60, 90, 120 and 150 kgN/ha (factor B). As a factor (C) used is a microbial inoculum liquid with different types diazotrophes Azotobacter chroococcum, Azospirillum lipoferum, Klebsiela planticola which inoculating seed prior to the sowing, and foliar treatment performed in the phase of tillering wheat. On the content of protein in wheat grains significant influence had both factors. On average, the use of diazotrophs influenced the increase in protein in wheat grains by 6.93 %. In the year with a lower amount of precipitation for the period IX-VI (162,8 mm), the highest increase in the protein content of 10,89-9,70 % was at the lowest doses of mineral nitrogen, 60 and 90 kgN/ha. In the year of the precipitation of 432,2 mm (IX-VI) the greatest increase from 12,31 to 10,34 % of the protein was in the variants of 60 and 120 kgN/ha. Entered diazotrophs into the soil affected the amount of easily accessible forms of nitrogen in the soil. In the year of the precipitation of 432,2 mm (IX-VI) the greatest increase from 12,31 to 10,34 % of the protein was in the variants of 60 and 120 kgN/ha. Entered diazotrophs into the soil affected the amount of easily accessible forms of nitrogen in the soil. The increase in the content (NO₃ + NH₄)-N in the average with the use of diazotroph was from 17,40-33,16 %. Based on the results obtained, it can be concluded that diazotrophs can increase the amount of protein in grains especially in years with unfavorable agrometeorological factors. Also under the same conditions the content (NO₃ + NH₄)-N is increased, which is very significant if the same parcel is used for the use of crops.

Keywords: wheat, proteins, fertilizers, diazotrophs

UTICAJ DIAZOTROFA NA KVALITET ZRNA PŠENICE I ZEMLJIŠTA U ODRŽIVOJ PROIZVODNJI PŠENICE

Gorica Cvijanović

Univerzitet Megatrend, Fakultet za bioframing, Bačka Topola, Srbija cvijagor@yahoo.com Vojin Cvijanović

Institut za zemljište, Beograd, Srbija, Serbia cvija91@yahoo.com

Nenad Đurić

Univerzitet Megatrend, Fakultet za biofarming, Bačka Topola, Srbija cvijagor@yahoo.com Ljubiša Živanović

Univerzitet u Beogradu, Poljoprivredni fakultet, Srbija ljuba@agrif.bg.ac.rs Svetlana Roljević

Institut za ekonomiku poljoprivrede, Beograd, Srbija svetlana_r@iep.bg.ac.rs

Rezime: Pšenica je osnovna ratarska kultura značajna u ishrani stanovništva. Proizvodnja pšenice za brašno je zastupljena na svim kontinetima, a u nerazvijenim delovima sveta zauzima 85 % ukupne preoizvodnje. U programu stvaranja novih sorti pšenice konstantno se radilo na povećanu prinosa, zatim na unošenju gena za otpornost prema patogenim organizmima. Danas se radi na stvaranju sorti koje su otporne prema abiotičkim faktorima i to povećanje otpornosti na niske temperature, i otpornost prema suši. Ovo je veoma aktuelno poslednjih godina kada su klimatske promene sve izraženije. S obzirom na značaj pšenice kao hlebnog žita u ishrani ljudi neophodno je razvijati metode u primarnoj proizvodnji pšenice kojima bi se održao/poboljšao kvalitet i kvantitet pšenice uz smanjenje mogućnosti degradcije proizvodnih osobina zemljišta. Pored kvantiteta kvalitet pšenice je definisan proteinima koji se nalaze u zrnu u proseku 8-16 %. Na sintezu proteina značajno utiču genetički i agroekološki faktori kao i njihove interakcije. Proteini deponovani u endospermu semena sastoje se od različitih frakcija, gde 80 % čine frakcije pod imenom gluten. Gluten ima presudnu ulogu u formiranju većine osobina koja definišu kvalitet pšenice. Generalno, što je veći sadržaj proteina to je i kvalitet pšenice, kao sirovine za spravljanje hleba, bolji. U cilju zaštite geobiosfere sa aspekta proizvodnje hrane Organizacija Ujedninjenih nacija za ishranu i razvoj (FAO) donela i realizovala različite programe finansijske i nučno-tehnološke za podsticaj poljoprivredne proizvodnje u okviru održivog razvoja. Zato je za cili rada postavljeno da se utvrdi sadržaj proteina u zrnu pšenice, kao i sadržaj azota (lakopristupačnih formi i ukupnog azota) u zemljištu u uslovima primene različitih grupa mikroorganizama-diazotrofa. U tom cilju su sprovedena istraživanja na oglednoj parceli u Bačkoj Topoli u vegetacionom periodu 2014/2015 i 2015/2016 (faktor A). Cili istraživanja bio je da se utvrdi optimalni sadržaj proteina u zrnu pšenice pri različitim količinama mineralnog azota 60, 90, 120, 150 kgN/ha (faktor B). Kao faktor (C) korišćen je mikrobiološki tečni inokulum sa različitim vrstama diazotrofa Azotobacter chroococcum, Azospirillum lipoferum, Klebsiela planticola kojim se inokulisalo seme pred setvu i obavio folijarni tretman u fazi bokorenja pšenice. Na sadržaj proteina u zrnu pšenice značajan uticaj imala su oba faktora. U proseku primena diazotrofa uticala je na povećanje proteina u zrnu pšenice za 6,93%. U godini sa manjom sumom padavina za period IX-VI (162,8 mm) najveće povećanje sadržaja proteina 10,89-9,70 % bilo je pri najmanjim dozama mineralnog azota, 60 i 90 kgN/ha. U godini sa količinom padavina od 432,2 mm (IX-VI) najveće povećanje proteina 12,31-10,34 % bilo je pri đubrenju od 60 i 120 kgN/ha. Uneti diazotrofi u zemljište uticali su na količinu lakopristupačnih formi azota u zemljištu. Povećanje sadržaja (NO₃+NH₄)-N u proseku sa primenom diazotrofa iznosilo je od 17,40-33,16%. Na osnovu dobijenih istraživanja može se zaključiti da se diazotrofima može povećati količina proteina u zrnu pogotovu u godinama sa nepovoljnim agrometeoročoškim faktorima. Takođe u istim uslovima povećava se sadržaj (NO₃+NH₄)-N što je veoma značajno ukoliko se ista parcela koristi za postrbi usev.

Ključne reči: pšenica, proteini, đubrenje, diazotrofi

1. INTRODUCTION

Wheat is the most cultivated plant species. It began to grow at the time of the original farming another 8-10000 years before the new era. Wheat spread on all continents of the world, although it mostly corresponds to regions

with moderate climate Zohary and Hopf ¹ Gustafson i sar ². It is the main food in almost all parts of the world. Global demand for wheat grows by about 2% annually, twice as fast as the rate of increase in the genetic potential for yield Skovmand and Reynolds³. The great needs of the population for bread cereals in food, especially in the underdeveloped part of the world, are the development of programs in breeding. These programs concerned the creation of varieties that would have a high fertility potential to yield yields of 8 t / ha. However, wheat has a limiting architecture and the geometry of the plant Denčić⁴, so changes in the tree and the leaf to increase yields would not lead to positive changes. The planned yield of wheat depends on the proper selection of the variety according to the agroecological conditions of the region on which it is grown, as well as the sowing density of different genotypes Đurić⁵. In addition to high yield, grain quality is also very important, which is determined by nutritional content. Mature wheat grains contain 8-16% and up to 20%, which can be divided into soluble solvents (soluble in water), globulins (soluble in salt solutions), gliads (soluble in dilute alcohol), and glutenins (soluble in diluted bases or acids). Achieving quality standards is complex because it is under the influence of genotype, environment, nutrition and their interactions. The protein content of the gluten is increased when eating more nitrogen. Also, the gluten content increases and under high temperature conditions because they break the starch synthesis Alternbach⁶. Today the current question is how agro-technical measures in wheat production can increase the yields and quality of wheat grain, while preserving the environment. The basic measure for a stable and high yield is the nutrition of plants with nitrogen, which must be balanced with phosphorus and potassium Đekić. Based on many studies, depending on the state of the soil and the genotypes of plants, high yield of wheat grains should be applied from 120 to 160 kg / ha of nitrogen. Considering the environmental protection measures, the research should focus on reducing the amount of mineral fertilizers in wheat production. One of the measures is certainly to apply knowledge about the role of microorganisms in the circulation of matter and the biological fixation of nitrogen. The natural phenomenon of these processes by a special group of microorganisms, it gets practical application in sustainable production in order to protect the land as a natural and national resource of each country. Associated microorganisms play an important role in the circulation of nitrogen forms in nature. These groups of microorganisms produce stimulating substances for plant growth, activate the autochthonous microbial population, whereby faster mineralization of organic matter is carried out and the nutrients are faster available to the plants. By increasing microbial biomass, the amount of organic nitrogen in the soil increases, which is significant for feeding the next crop.

Therefore, the aim of the work was to determine the role of the application of associative diazotrophs in which amounts of mineral nitrogen to protein grain synthesis in the grain as well as to the amount of alkaline nitrogen form in the soil after the harvest of wheat.

2. MATERIALS AND METHODS

Design of experimental research - Experimental research was conducted on the experimental plot in Vojvodina in Bačka Topola in the vegetation period 2014/2015 and 2015/2016. years. The variety of wheat Pobeda is planted in the density of 500 plants/m^2 . The surface of the experimental plot was 138 m^2 , and the elementary plot was 5 m^2 (1,0 x 5,0 m). The parcels were placed according to the plan of the divided plots in three repetitions. The predus was corn.

¹ Zohary D., Hopf M. Domestication of plants in the old world. Oxford University Press, Oxford (2000)

² Gustafson P., Raskina O., Ma X., Nevo E. *Wheat evolution, domestication, and improvement.* In: Carver B.F. (ed) Wheat: science and trade. Wiley, Danvers (2009)

³ Skovmand B., Reynolds M.P. (2000): Increasing yield potential for marginal areas by exploring genetic resources collections. The Eleventh Regional Wheat Workshop for Eastern, Central and Southern Africa. Ethiopia, (2000)

⁴ Denčić S., *Genetics and Breeding of Grain Cereals, Proceedings*, Institute for Field and Vegetable Crops Novi Sad (2006)

⁵ Đurić N., Cvijanović G., Dozet G., Trkulja V., Rajičić V., Cvijanović V. Correlation analysis of more significant production traits of certain winter wheat pkb varieties XXXI Agronomic counseling Proceedings book (2017)

⁶ Alternbach, S. B. New insigths into the effects of high temperature, drought and post-anthwsis fertilizer on wheat grain development J. Cereal Science (2012)

Dekić V., Milivojević J., Staletić M., Jelić M., Popović V. Branković S., Terzić D. *The influence of mineral nutrition on winter wheat yield (Triticum aestivum l.)* XXII Biotechnology counseling Proceedings book, (2017)

Factor A: Basic fertilization was carried out in autumn with complex NPK fertilizers in relation to 8:24:16, and nitrogen Urea 46% N in the spring. The quantities and types of nitrogen fertilizer introduced expressed the values of pure nutrients: N_0 - bez đubrenja, N_{80} - 80 kgN/ha, 60 kg P_2O_5 , 40 kg K_2O /ha, N_{120} -120 kgN/ ha, 90 kg P_2O_5 /ha, 60 kg K_2O /ha; N_{150} - 150 kgN/ha,120 kg P_2O_5 /ha, 80 kg K_2O /ha.

Factor B: Bacterization of wheat seed prior to sowing and foliar treatments in phosphor and piercing phases was carried out using a liquid inoculum in which the cell mixture *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Klebsiela planticola* and *Bacillus sibtilis*.

Meteorological and soil conditions of plant growth - The temperature of the air during the vegetation period in 2015/2016 was higher by 1.5 °C, while the amount of rainfall was 287 mm higher, which are good prerequisites for achieving high yields and wheat quality.

Tab. 1. Average monthly temperatures (°C) and precipitation (mm) for the growing period of wheat

	Average monthly air temperature (⁰ C)										
Year	X	XI	XII	I	II	III	IV	V	VI	VII	Average
2014/15	13.0	7.5	2.7	1.8	1.9	6.8	11.3	17.4	20.6	24.1	10.7
2015/16	10.6	6.7	2.6	0.6	6.7	7.8	14.1	17.1	22.2	23.9	11.2
	Sums of monthly precipitation anount (mm)										
2014/15	60,8	9,2	53,6	72.0	35,6	36.0	13.0	128,4	20,4	15.0	136.0
2015/16	86.6	40.0	7.4	55.0	67.8	24.8	17.2	31.2	66.4	26.6	423.0

Source: Measuring Station Agricultural Expert Service Bačka Topola

The effect of the applied measures was examined on the content of total proteins (%) in wheat grain according to the micro-Kjeldalh method (Jones, 2001), as well as the light-chain forms of nitrogen in soil (kg/ha).

Data were processed using the two-factorial view (parcel allocation) in the DSAASTAT program Statistics 2011. The significance of the differences between the mean values of the treatment was tested with the LSD test

3. RESEARCH RESULTS

1. Wheat protein content - Based on the results obtained in the research, the wheat plant bacterization with a mixture of different types of diazotrophs was observed to increase protein in wheat grain. In both years of research, bacteria and fertilization significantly increased protein content, while their mutual relationship did not affect the level of statistical significance on the protein content of the grains.

In 2014/2015, the average increase in protein content in bacteria was 6.46%, which was statistically significant at p<0.01 (Table 2). The highest protein content of 15.51% was found in fertilization with 150 kgN/ha, which was at the level of statistical significance p<0.01 only in relation to control. However, with this amount of nitrogen, the minimum percentage of protein increase was only 0.32%. The greatest influence of bacterization was in the fertilizer-free variant because the highest percentage of 10.89% of the protein was increased. Fertilizers of 80 and 120 kgN/ha also had a high percentage of protein increase from 9.70% to 7.52% compared to the fertilizer variant.

Tab. 2. The effect of bacterization and ways fertilization on the protein content in grain wheat 2014/2015

		yeai				
Pasterization (P)	Proteins	Level of r	Arrama ma (D)			
Bacterization (B)	Froteins	N_0	N_{80}	N ₁₂₀	N ₁₅₀	Average(B)
ish harstaningsian	%	14.15	15.03	15.28	15.51	14.99
with bacterization	Index level	110.89	109.70	107.52	100.32	106.46
Maria de la construction	%	12.76	13.70	14.21	15.46	14.08
without bacterization	Index level			100,00		AB
Average (A)	%	13.45	14.36	14.74	15.48	14.51

	Index level	100.00	106.76	109.59	115.09	107.86
	F test		LSD 5%		LSD 1%	
Fertilization (A) 31.207			-	-	-	
Bacterization (B) 24.213**			0.6	506	0.9	918
Interaction(A x B) 3.343			n	ıs	ns	

In 2015/2016, higher protein content in both variants was determined than in 2014/2015 (Table 3). Bacterization was statistically significant at p<0.01, increasing protein content by 7.38%. Also, the amount of nitrogen statistically significantly influenced the increase in protein content. In the fertilizer-free variant, the lowest protein content was found at 14.60%, but the highest increase was found for 12.31%. The highest protein content was found in fertilization with 150 kgN/ha with the lowest percentage increase of 5.05%. In fertilizing with 120 kgN/ha, 16.12% of proteins were found, which was less by 0.52% than at 150 kgN/ha. The protein content of 15.03% in fertilization with 80 kgN/ha was only 0.25% lower than at 150 kg N/ha. These differences were not at the level of statistical significance.

Tab. 3. The effect of bacterization and ways fertilization on the protein content in grain wheat 2015/2016

year								
Bacterization (B)	Proteins	Level	Prosek (B)					
Dacterization (B)	1 Totellis	N_0	N_{80}	N_{120}	N ₁₅₀	_ 110sck (b)		
with bacterization	%	14.60	15.35	16.12	16.64	15.55		
with bacterization	Index level	112,31	105,97	110,34	105,05	107,38		
without bacterization	%	13.00	14.44	14.65	15.84	14.48		
without bacterization	Index level			AB				
Prosek (A)	%	13.80	14.87	15.38	16.24	15.07		
Flosek (A)	Index level	100.00	107.75	111.45	117.68	109.22		
	F test		LSD 5% LS			SD 1%		
Fertilization (A)	81.308**		-			-		
Bacterization (B)	24.094**		0.724		1	1.098		
Interaction (A x B)	3.343		ns			ns		

In both years of research it was noted that the protein content of bacteria and 80 kgN/a (15.03% -15.35%) was the same / higher than in the variant without bacterization and 120 kgN/ha (14.74% -15, 38%). Bacterization and 120 kgN/ha influenced the protein content of 15.28%-16.12%, which was negligible less than in the variant without bacterization and 150 kgN/ha. This can be explained that the microorganisms introduced into the soil significantly increased the number and enzymatic activity of the microbial soil population. Cvijanović et al. 8 seed inoculation with highly effective strains of different genera of associative diazotrophs can increase the number of azotobacter in

⁸ Cvijanović, G., Milošević, N., Đalovic, I., Cvijović, M., Paunović, A. *Nitrogenization and N fertilization effects on protein contents in wheat grain* Cereal Research Communications, (2008)

wheat rice by 27.30%. In addition to physiological groups of microorganisms Roljević et al.⁹ have found an increase in the total number of microorganisms of 3.7% and 28% for combinations of organic and microbiological fertilizers in different wheat genotypes.

Based on the obtained results, significant differences were found in the years of research. In 2015/2016 the protein content in both variants (14.48%-15.55%) was significantly higher than in 2014/2015 (14.08%-14.49%). These differences were created under the influence of favorable agro-weather conditions in 2015/2016. Pan¹⁰ have also found that the average daily temperatures, total sunshine and rainfall are the most significant meteorological parameters involved in the determination of protein content.

Tab. 4. Interaction of bacterization x fertilization on content (NO3+NH4)-N (kg/ha)

Year	Bakterization/fertilization	N_0	N ₈₀	N ₁₂₀	N ₁₅₀	level	Index level
2014/2015	with bacterization	36.18	38.99	40.98	40.66	39.20	132,61
2014/2015	without bacterization	28.18	21.12	30.67	38.00	29.56	
2015/2016	with bacterization	110.54	117.07	107.83	122.85	114.57	117,39
	without bacterization	81.90	107.83	103.73	96.91	97.59	
A	with bacterization	73.36	78.03	81.91	81.75	78.76	106.54
Average	without bacterization	55.04	49.57	67.21	67.45	73.92	
Index level		133,28	157.41	121.87	142,29	106,54	

By analyzing the results in tab.4, it can be concluded that there are significant differences in the amount of mineral nitrogen after harvesting wheat. On average, in 2014/2015, the increase in nitrogen content was 32.61%, while in 2015/2016 this increase was less 17.39%. These differences can be explained that a higher amount of precipitation influenced the life activity of microorganisms in soil and in variant without bacterization. On average, the largest quantities of nitrogen were found in bacterization and 120 kgN/ha (81.91 kgN/ha). On average for the two years of bacterial research, nitrogen content increased by 6.54% at all levels of fertilization. This is very important from the aspect of preserving the soil, as well as determining the amount of nitrogen in the feed of the crop.

4. CONCLUSION

Based on the results obtained, it can be concluded that the protein content was different by the research factors. Agrometeorological conditions influenced the parameters tested. In 2014/2015. In the year that had a water deficit and lower average air temperature, an increase of 7.86% of wheat protein was found, in 2015/2016 this increase was 9.22%. Bacterization and the amount of nitrogen statistically significantly influenced the protein content. The highest protein content (15.51% -16.64%) was found in fertilization with 150 kgN/ha, but with the lowest percentage increase (0.32% -5.05%). It can be said that a large amount of nitrogen has a depressing effect on the activity of the microbes in the soil, and the plants were more likely to adopt nitrogen from the fertilizer. The protein content under bacterial conditions and 80 kgN/h and 120 kgN/ha was statistically significantly higher than the protein content in the non-bacterial variant. Protein content in these variants is at the level of protein content at 150

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⁹ Roljević Nikolić, S., Kovačević, D., Cvijanović G., Dolijanović Ž., Marinković J., *Grain yield and rhizosphere microflora of alternative types of wheat in organic production* Romanian Biotechnological Letters, (2018)

¹⁰ Pan, J., Zhu, Y., Cao, W., Dai, T., Jiang, D. Predicting the protein content of grain in winter wheat with meteorological and genotypic factors. Plant Prod. Sci. 9(3) (2006)

kgN/ha without bacterization, and it can be said that the optimal amounts of nitrogen in wheat feeding are 80 and 120 kgN/ha with seed cropping and foliar treatment of plants with compatible groups of microorganisms.

Bacterization significantly influenced the amount of mineral nitrogen left in the soil after harvest. On average, this increase was 6.54%. The largest amounts of mineral nitrogen in soil were determined during bacterization and 120 kgN/ha. In order to maintain steady green fields, the amount of mineral nitrogen left in the soil is important from the economic and ecological aspect of the sowing of posterior or subsequent crops.

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