ASSESSMENT OF PROTEIN CONTENT BASED ON THE INTERACTION BETWEEN NITROGEN LEVEL OF FERTILIZATION AND WINTER WHEAT VARIETY CULTIVATED AT DUDEȘTII NOI, AN IMPORTANT AGRICULTURAL AREA OF ROMANIA

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Abstract. *The aim of this study was to analyse the effect of wheat variety, nitrogen fertilizer rate and type and their interaction on protein content and to determine the economic feasibility of application the fertilizer and varieties, two key drivers that contribute guiding the future with efficient agronomic practices to guarantee wheat quality in the advent of the most significant changes for agriculture. The subject of the experiment consisted in testing during one wheat growing season, twenty-seven modern winter wheat varieties fertilized with nitric and ammoniacal nitrogen in three different rates - 120, 150 and 170 kg N ha*$^{-1}$ *active substance. The biological material is represented by 27 romanian and foreign autumn wheat varieties, some of the most cultivated wheat varieties in the Western Plain of Romania, and the criteria that were the basis of their choice are the high production potential, the high tolerance to disease attack and pests and specific qualities for milling and baking. This study reflects the results obtained in the research Laboratory for seed quality control at the University of Life Sciences "King Mihai I" from Timișoara, using the standard method with a whole seed NIR multiparameter analyzer using near-infrared transmission (transmittance) technology. the experience average for protein content was 12.94%. In the case of cultivars, there were no differences from the field average. As for agricultural funds, the highest value was obtained at the fertilization level of 170 kg ha*$^{-1}$ *a.s. ammoniacal nitrogen of 13.79%, and the lowest at the fertilization level of 120 kg ha*$^{-1}$ *a.s. nitric nitrogen of 12.11. The other agricultural funds did not show differences. Even in the case of the variety-agricultural interaction, there were no statistically guaranteed differences.*

Keywords: *ammoniacal N, fertilization rate, nitric N, protein content, wheat genotype.*

INTRODUCTION

Wheat is one of the most important cultivated plants with a high alimentary value, grown in over one hundred countries. The plant has a high ecological plasticity, being cultivated in areas with different climates and soils. The history of wheat cultivation is as long as the history of civilization. Historical data tells us that wheat is the oldest cultivated plant. It is believed that its domestication took place in the Fertile Crescent about 10,000 years ago and was part of the Neolithic Revolution, when man acquired enough knowledge about the surrounding world and discovered this plant in the spontaneous flora that could serve as food, fact which represented "*a radical change*", as archaeologist and researcher Vere Gordon Childe put it, "*full of revolutionary consequences for whole species*", a plant that then spread to all parts of the world through the first farmers, who adapted local populations to different climates. (VENSKE ET AL., 2019; WILLIAM ET AL, 2011; DUBCOVSKY ET AL., 2007).

The success of this plant is inextricably linked to the ability of the gluten protein fraction that allows flour to be processed to produce bread, other pastries, noodles and pasta. (PENG ET AL., 2011; DUBCOVSKY ET AL., 2007; SHIFERAW ET AL., 2013) This, too, being an important source of carbohydrates, proteins, dietary fiber and fats, as well as minerals (including P, K, Ca and Mg), the B vitamin complex and other bioactive substances. Wheat alone provides a fifth of the calories and protein in food globally. Currently, wheat is one of the most economically important crops worldwide (GREWAL ET AL., 2015; SHEWRY ET AL., 2015; RANUM ET AL., 2014), one of the three cereals (along with rice and maize) that are the most important sources of food for humanity and whose total global consumption represents over 90% of total grain consumption. (KHOKHAR ET AL. 2017; ASSENG ET AL., 2019)

In northern European countries, such as Germany and Great Britain, wheat varieties are rated according to their protein concentration. This protein concentration requires high rates of post-emergence fertilizers for high storage protein accumulation. Despite the great efforts of breeders, the negative relationship of yield with grain protein concentration is difficult to break (MOSLETH, 2015), although grain quality in modern cultivars has increased by increasing the concentration of storage proteins in the caryopsis. Due to increased nitrogen fertilizer rates and improved genotypes, the average protein concentration of currently cultivated wheat genotypes has increased from about 7–8% crude protein in the 1960s to 12–16% in modern genotypes (LAIDIG, 2017). There is a limitation of N growth in cereals due to the inverse relationship between yield and protein concentration, leading to high yield genotypes on the one hand and high quality genotypes on the other. Although some authors have suggested that grain protein concentration may not be limited by yield (HELLEMANS, 2018; JAN ET AL., 2011), there is no clear indication that this is universally true for wheat. However, some new cultivars with high crude protein content (13–16%) performed at par with production levels (CARVAHLO ET AL., 2016; SCHULZ ET AL., 2015).

Nitrogen is an important factor limiting plant growth and consequently wheat production worldwide. Plants consume nitrogen present in both the atmospheric air and soil minerals. The ability of plants to take up nitrogen naturally or applied as fertilizers is one of the critical factors limiting the efficient use of N by plants. Despite the fact that N is one of the most abundant elements on earth, nitrogen deficiency is one of the most common problems affecting plant growth and development (VENSKE ET AL., 2019; WILLIAM ET AL., 2011).

Wheat generally contains 3–5% nitrogen in its tissue biomass, which is by far the most important soil-derived nutrient outside of oxygen, hydrogen, and carbon (ALI ET AL., 2011).

Nitrogen has a complex role, it is responsible for plant growth and development, involved in a wide range of physiological processes such as photosynthesis, protein synthesis and enzyme activity, it is a basic constituent of nucleic acids (DNA and RNA), proteins, enzymes, cell wall and pigment system, amino acids, vitamins (biotin, thiamin, niacin and riboflavin), all proteins and a wide range of nitrogen-containing organic molecules. Nitrogen assimilation occurs throughout the growing season, but with different intensities depending on the growth and development phenophase and critical periods of nutrition (NOOR ET AL., 2023; BARRACLOUGH ET AL., 2014).

N applied at sowing stimulates twining and vegetative growth, while N applied in the generative phases has a greater influence on the protein concentration of the caryopses (ZHENG ET AL., 2021).

MATERIALS AND METHODS

The biological material used in the research is represented by twenty-seven varieties of wheat: Dacic, Miranda, Alex, Litera, Ciprian, Crişana, Biharia, Glosa, Boema, Sothys, Sacramento, Rubisko, Certiva, Aurelius, Aspekt, Papilon, Activus, Centurion, Tika Taka, Chevignon, Sosthene, Vivendo, Sophie, Solindo, Tiberius, Arrezo and Apexus and the criteria that were the basis of their choice are the high production potential, the high tolerance to the attack of diseases and pests and the specific qualities for milling and baking. The experiment was carried out at the Seed quality control laboratory of Faculty of Agriculture.

The experimental plots were located in a location in Timiș County, famous for the large areas on which wheat is grown in the Western Plain, on the territory of Dudeștii Noi locality.

The layout of the experimental plan was done using the stratified randomized block method. Cultivars were factorially combined and arranged in completely randomized blocks. This experimental method was chosen to avoid the interfering effects of various environmental factors and to adequately and accurately estimate nitrogen utilization. Each agricultural plot consisted of 27 plots with three replicates. The study is based on a trifactorial experiment, in subdivided plots, on the 27 × 3 type, with the following grading of the experimental factors: factor A – wheat variety, factor B – level of nitrogen fertilization 120, 150 and 170 kg N ha^{-1} and factor C - type of N fertilizer, nitric and ammoniacal nitrogen.

The wheat samples were cleaned in a fully automated process using the MLN Sample Cleaner - Pfeuffer equipped with a cyclone for light bodies, a ball screening system for cleaning, additional screening screens for sorting coarse and small grains and then homogenized with an automatic grain sampler Vario 1G – Pfeuffer equipped with an integrated electric actuator (adjustment cylinder) according to ISO 24333:2010, then the tests for bakery quality indices were carried out. Protein content was measured using a whole seed NIR multiparameter analyzer using near-infrared transmission technology (transmittance). A volume of 600 milliliters of sample was used to analyze these parameters. The measurement process was automated, the transport rotor ensured constant density, and the built-in infrared spectrometer with a range of 950 to 1,540 nanometers scanned each sample 1,500 times. Thanks to this technology, each sample was completed by printing an analysis report in less than a minute.

RESULTS AND DISCUSSIONS

Based on the results of one year field experiment, the production parameter was calculated for optimal nitrogen dose and maximum yield of winter grain for that dose. First of all, we used the Analysis of variance (ANOVA) represented in Table 1 which is an analysis tool used in statistics that splits an observed aggregate variability found inside a data set into two parts: systematic factors and random factors.

Table 1

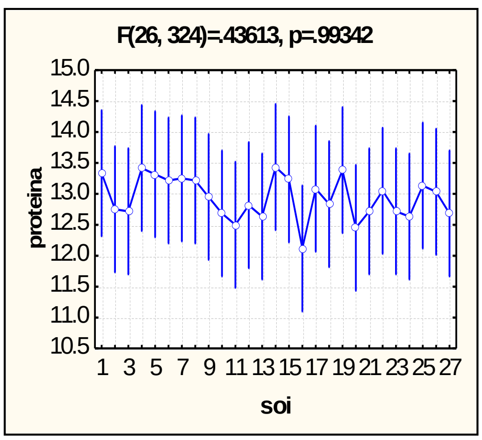
Variation analysis

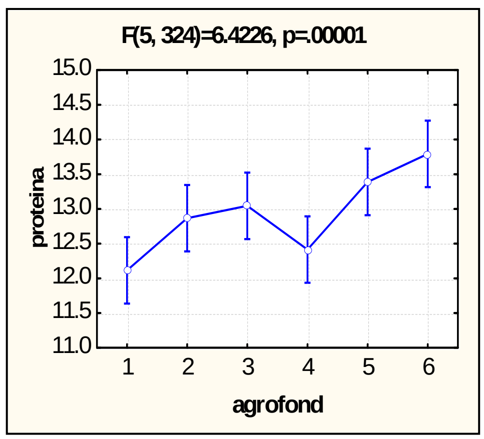
Variation source	SSP (SP)	Degree of freedom	Weighed Least of Squarez WSL (s^2)	F test for s^2 error		
				Value	P	Signification
A (variety)	54,38	26	2,09	0,44	0,993417	ins
B (level of fertilization)	154,01	5	30,80	6,42	0,000010	***
A×B	74,80	130	0,58	0,12	1,000000	ins
Error	1553,89	324	4,80			
Total	1837,08					

ins p>0,05; * p≤0,05; ** p≤0,01; *** p≤0,001

o Factor A (variety): p<0.001
o Factor B (level of fertilization): p<0.001
o A×B interaction: p<0.001

The F test (the table above, column p), shows that: the factor A - the variety, had an insignificant action, the factor B - the agricultural fund had a very significant action and the interaction A × B had an insignificant action, that is: between the varieties followed in the experience there are no significant differences; between the 6 agricultural funds there are very significant differences; the 27 varieties did not react differently within the 6 agrofunds. In conclusion: the null hypothesis H_0 is rejected for the factor B (farm background), the null hypothesis H_0 is accepted for the factor A (variety) and for the interaction A × B.





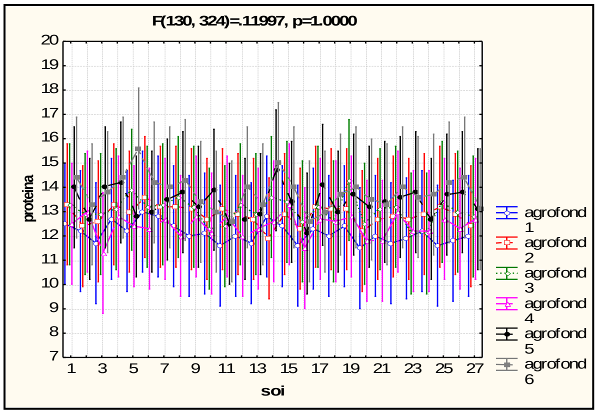


Figure 1 The influence of the variety and the influence of the agrofund on the protein content

Influence of variety on protein content – Protein content values range from 12.1% (Papillon variety) to 13.4% (Aurelius variety). All other 25 varieties have values of 12.4 and 13.3%. The differences between the varieties are insignificant (p>0.05), according to the F test value.

The influence of the agricultural fund on the protein content – From the first level of fertilization of 120 kg/ha s.a. nitric nitrogen at 170 kg/ha s.a. nitric nitrogen, the trend is upward, and from the previously mentioned fertilization level to that of 120 kg/ha s.a. ammoniacal nitrogen the trend is downward, after which the trend is again upward at the last level of ammonium nitrogen fertilization. The differences between the agricultural funds are insignificant (p>0.05), according to the value of the F test.

The influence of the A × B interaction on the protein content: The highest values of this index were obtained by the varieties Ciprian – 15.6% and Aspekt – 15.1% at the fertilization level of 170 kg/ha s.a. ammoniacal nitrogen. The lowest values were recorded in the varieties Alex – 11.3% and Chevignon – 11.6% at the fertilization level of 170 kg/ha s.a. nitric nitrogen.

Table 2

Student test for A factor (variety) – witness (W), average of the field

Variety	Protein content (%)	Difference (%)	Significance
a1 – Dacic	13,33	0,40	
a2 – Miranda	12,75	-0,19	
a3 – Alex	12,72	-0,22	
a4 - Litera	13,42	0,48	
a5 – Ciprian	13,32	0,38	
a6 – Crișana	13,22	0,28	
a7 – Biharia	13,25	0,31	
a8 – Glossa	13,22	0,28	
a9 – Boema	12,95	0,01	
a10 – Sothys	12,68	-0,25	
a11 – Sacramento	12,50	-0,44	
a12 – Rubisko	12,82	-0,12	
a13 – Certiva	12,63	-0,30	

a14 – Aurelius	13,43	0,50	
a15 – Aspekt	13,23	0,30	
a16 – Papillon	12,12	-0,82	
a17 – Activus	13,08	0,15	
a18 – Centurion	12,83	-0,10	
a19 - Tika Taka	13,38	0,45	
a20 – Chevignon	12,45	-0,49	
a21 – Sosthene	12,72	-0,22	
a22 – Vivendo	13,05	0,11	
a23 – Sophie	12,72	-0,22	
a24 – Solindo	12,63	-0,30	
a25 – Tiberius	13,13	0,20	
a26 – Arrezo	13,03	0,10	
a27 – Apexus	12,68	-0,25	
Average	12,94	Wt	
DL 5%= 1,431; DL 1% = 1,884; DL 0,1% = 2,403			

No difference is significant.

Table 3

Student test for B factor (level of fertilizer) – witness (W), average of the field

Factor B (level of fertilization)	Protein content (%)	Difference (%)	Significance
b1 – 120 kg nitric N a.s. ha^{-1}	12,11	-0,82	0
b2 – 150 kg nitric N a.s. ha^{-1}	12,87	-0,07	
b3 – 170 kg nitric N a.s. ha^{-1}	13,04	0,11	
b4 - 120 kg ammoniacal N a.s. ha^{-1}	12,41	-0,52	
b5 - 150 kg ammoniacal N a.s. ha^{-1}	13,39	0,45	
b6 - 170 kg ammoniacal N a.s. ha^{-1}	13,79	0,86	*
Average	12,94	mt	
DL 5% = 0,675; DL 1% = 0,888; DL 0,1% = 1,133			

No difference is significant except for fertilization levels of 120 kg nitric N ha^{-1} and 170 ammoniacal N ha^{-1}, where the differences compared to the average of the experience are significant, the value for the first mentioned agricultural fund is negative, and for the second one positive.

Table 4

Student test for A×B factors (variety × level of fertilizer) – witness, average of the field

Protein content and significances (to the witness, the average of the experience)												
Variety	Agrofund 1		Agrofund 2		Agrofund 3		Agrofund 4		Agrofund 5		Agrofund 6	
	P	Diff.	P	Diff.	P	Diff.	P	Diff.	P	Diff.	P	Diff.
a1- Dacic	12.50	-0.44	13.30	0.36	13.30	0.36	12.50	-0.44	14.00	1.06	14.40	1.46
a2- Miranda	12.20	-0.74	12.40	-0.54	12.90	-0.04	13.00	0.06	12.70	-0.24	13.30	0.36
a3- Alex	11.70	-1.24	12.60	-0.34	12.90	-0.04	11.30	-1.64	14.00	1.06	13.80	0.86
a4- Litera	12.70	-0.24	13.30	0.36	13.10	0.16	12.80	-0.14	14.20	1.26	14.40	1.46
a5- Ciprian	12.20	-0.74	13.00	0.06	13.90	0.96	12.40	-0.54	12.80	-0.14	15.60	2.66
a6- Crișana	13.00	0.06	13.60	0.66	13.20	0.26	12.30	-0.64	13.00	0.06	14.20	1.26
a7- Biharia	12.80	-0.14	13.20	0.26	13.30	0.36	12.70	-0.24	13.50	0.56	14.00	1.06
a8- Glossa	12.40	-0.54	13.20	0.26	13.60	0.66	12.00	-0.94	13.80	0.86	14.30	1.36
a9- Boema	12.00	-0.94	13.10	0.16	13.20	0.26	12.80	-0.14	13.20	0.26	13.40	0.46

a10- Sothys	12.10	-0.84	12.70	-0.24	12.30	-0.64	12.10	-0.84	13.90	0.96	13.00	0.06
a11- Sacramento	11.60	-1.34	13.10	0.16	12.40	-0.54	12.80	-0.14	12.50	-0.44	12.60	-0.34
a12- Rubisko	12.00	-0.94	12.90	-0.04	13.30	0.36	12.00	-0.94	12.70	-0.24	14.00	1.06
a13- Certiva	11.70	-1.24	12.70	-0.24	12.90	-0.04	12.30	-0.64	12.90	-0.04	13.30	0.36
a14- Aurelius	12.80	-0.14	11.90	-1.04	13.60	0.66	12.60	-0.34	14.70	1.76	15.00	2.06
a15- Aspekt	12.40	-0.54	12.90	-0.04	13.40	0.46	13.30	0.36	13.40	0.46	14.00	1.06
a16- Papilon	11.60	-1.34	12.30	-0.64	12.60	-0.34	11.50	-1.44	12.10	-0.84	12.60	-0.34
a17- Activus	12.30	-0.64	13.20	0.26	13.20	0.26	12.70	-0.24	14.10	1.16	13.00	0.06
a18- Centurion	12.00	-0.94	13.10	0.16	12.60	-0.34	12.60	-0.34	13.00	0.06	13.70	0.76
a19- Tika Taka	12.40	-0.54	13.10	0.16	14.30	1.36	12.80	-0.14	13.70	0.76	14.00	1.06
a20- Chevignon	11.50	-1.44	12.20	-0.74	12.50	-0.44	11.80	-1.14	13.20	0.26	13.50	0.56
a21- Sosthene	12.00	-0.94	12.70	-0.24	13.10	0.16	11.80	-1.14	13.40	0.46	13.30	0.36
a22- Vivendo	11.70	-1.24	12.80	-0.14	13.20	0.26	13.00	0.06	13.60	0.66	14.00	1.06
a23- Sophie	11.90	-1.04	12.70	-0.24	12.10	-0.84	12.20	-0.74	13.80	0.86	13.60	0.66
a24- Solindo	12.20	-0.74	12.90	-0.04	12.10	-0.84	12.20	-0.74	12.70	-0.24	13.70	0.76
a25- Tiberius	11.60	-1.34	13.20	0.26	13.40	0.46	12.70	-0.24	13.70	0.76	14.20	1.26
a26- Arrezo	11.80	-1.14	12.90	-0.04	13.00	0.06	12.30	-0.64	13.80	0.86	14.40	1.46
a27- Apexus	12.00	-0.94	12.40	-0.54	12.80	-0.14	12.70	-0.24	13.10	0.16	13.10	0.16
Average	12,94											
DL 5% = 3,506 DL 1% = 4,615 DL 0,1% = 5,885												

No difference is significant.

CONCLUSIONS

In the agricultural year 2022 - 2023, the experience average for protein content was 12.94%. In the case of cultivars, there were no differences from the field average. As for agricultural funds, the highest value was obtained at the fertilization level of 170 kg ha^{-1} a.s. ammoniacal nitrogen of 13.79%, and the lowest at the fertilization level of 120 kg ha^{-1} a.s. nitric nitrogen of 12.11. The other agricultural funds did not show differences. Even in the case of the variety-agricultural interaction, there were no statistically guaranteed differences.

Factor A - the variety contributes to humidity by 2.96%, factor B - the agrofund contributes by 8.38%, the interaction A×B by 4.07%. The biggest contribution is made by other factors that were not taken into account, followed by the factor B - the agrofund, the interaction A×B, followed by the variety, and in the last place is the factor A - the variety.

Reduction of N fertilizer use (up to 20–40%) without loss of quality and quantity could be achievable. The quality of wheat is not only determined by the cultivated variety and the applied technology, but is also influenced by the growing and climatic conditions The most effective approach will be the next strategies: using wheat varieties without unnecessary storage protein genes and the use of an optimal nitrogen fertilizer application strategy. Improvements associated with wheat protein content may include: selection and breeding of new genotypes that can be grown using lower amounts of fertilizers; the use of modern agricultural techniques that ensure sustainability and improve the efficiency of the use of water and nutrients necessary for the growth and development of wheat plants; development of germplasm with increased resistance to biotic and abiotic stress.

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