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

Denisa Cristiana HETEA, F.CRISTA, F. IMBREA

University of Life Sciences "King Mihai I" from Timișoara
Faculty of Agriculture, 300645, Aradului Street 119, Timişoara
Corresponding author: denisa.hetea@yahoo.com

Abstract. *The gluten content is directly correlated with the level of fertilization of nitrogen which is strongly influenced by pedoclimatic conditions and wheat variety. The aim of this study was to show the effect of wheat variety, nitrogen rate and type and their interaction on wet gluten content and to determine the most effective N dose application 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 contrasting rates - 120, 150 and 170 kg N ha^{-1} active substance, applied in a single dose at BBCH 30-31. The biological material is represented by 27 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 is the 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, Perten Glutomatic System according to ISO 21415-2:201.*

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

INTRODUCTION

Wheat is the second most widely cultivated plant in the world, it is estimated that this crop covers 200 million hectares (FAO, 2022). Due to relatively modest cultivation requirements, high adaptability to climate and soil, and high nutritional value, wheat spread rapidly from its center of origin, a narrow area in the Middle East (Jordan, Palestine, and Lebanon) to Syria, Turkey, Iraq and Iran and then around the world, propelling itself into the orbit of eternity thanks to its importance. Therefore, it has transformed from a simple wild herbaceous plant, apparently insignificant, to a ubiquitous one and continues to be the staple food, like a *Panis caelestis* for humanity. The first ancient forms of wheat, einkorn, emmer and spelt, not only played an important role as a food source, but became the ancestors of the modern species currently cultivated around the world, with *Triticum aestivum* L. now accounting for about 95% of world production. 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; DUBOVSKY ET AL., 2013; SHIFERAW ET AL., 2013)

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

Research has reported increased leaf mass improvement in wheat plants when nitrate nitrogen fertilizers are applied compared to ammoniacal nitrogen fertilizers. (MICKAN ET AL., 2022)

Other studies found that growth factors consisting of sibling number, root volume, specific leaf mass, standard leaf area, and total leaf area performed better when applied with NO_3^- compared to NH_4^+. Along with the above, the use of nitrogen fertilizers based on ammonia nitrogen lowers the nitrate content of plants, while nitrogen fertilizers based on nitric nitrogen increase it (AHMED ET AL., 2020) Furthermore, a significant decrease in the soluble sugar content of wheat caryopses exposed to NH_4^+ compared to NO_3^- was observed (GUO ET AL., 2019) In addition, investigations in China indicated higher plant nitrogen use efficiency in wheat plants fertilized with NO_3^-, as well as reduced photosynthesis and carbon assimilation rates in the case of NH_4^+ fertilization (DABA ET AL., 2021; ALUKO ET AL., 2023). There is an absolute nitrogen requirement for wheat cultivation, and the yield and quality of productions depend on substantial nitrogen inputs. Initially, this leads to the formation of the leaf mass necessary for photosynthesis which, in turn, leads to the formation of the ear. The availability of fertilizers and the growing demand for wheat encourages overuse, and environmental problems in some agricultural ecosystems have become a major problem. (YADAV ET AL., 2023; BARRACLOUGH ET AL., 2010)

Nitric nitrogen is absorbed by plants and then becomes mobile in the xylem vessels. Ammoniacal nitrogen bypasses the process of conversion into amino acids and enters directly into the plant's metabolism. Wheat mainly uses nitric nitrogen, under the influence of microorganisms, being immediately available and easily absorbed by plants, being mobile in the soil, which leads to its solubilization in the root zone, especially on sandy soils or in conditions of abundant precipitation. In soil, nitric nitrogen moves mainly by mass flow with water movement and partly by diffusion, while ammonia nitrogen moves mainly by diffusion and less by mass flow (MOOSHAMMER ET AL., 2014). The rate of movement of soil solution through mass flow depends on plant transpiration rate, soil water content, and soil texture (BARBER, 1984).

Gluten consists of 90% protein, 8% fat, and 2% carbohydrate (PRONIN ET AL., 2011; PERTEN ET AL., 1992) Gluten can be defined as the "cohesive, visco-elastic protein material" resulting as a by-product obtained by isolating starch from wheat flour. The minimum gluten content of wheat flour should be about 24% (wet) and 8% (dry) (SINGH, 2006) Gluten is formed from the proteins gliadin and glutenin, when combined with water, it forms a network capable of it retains the carbon dioxide released during fermentation. This quality is determined by rheological tests that allow the prediction of the behavior of the flour during the baking process and the characteristics that the final products will have (XUE ET AL., 2019).

MATERIALS AND METHODS

The biological material used in the research is represented by twenty-seven varieties of wheat: Dacic, Miranda, Alex, Litera, Ciprian, Crișana, Biharia, Glosa, Boema, Sothys, Sacramento, Rubisko, Certiva, Aurelius, Aspekt, Papilon, Activus, Centurion, Tika Taka, Chevignon, Sosthene, Vivendo, Sophie, Solindo, Tiberius, Arrezo and Apexus. 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. Wet gluten content was measured using the Perten Glutomatic System according to ISO 21415-2:2015 An apparatus system consisting of the Perten Glutomatic 2000 gluten washer, the Gluten Index 2010 centrifuge and the Glutork 2020 gluten dryer. The samples were ground with a laboratory steel hammer mill fitted with a 200 µm sieve to produce a flour of the appropriate particle size for testing, and the flour was allowed to cool for one hour before being analyzed .
Principle of the method for obtaining wet gluten: for each sample, 10 g of flour were ground with a laboratory mill with a precision of 0.01 g. Then a dough was formed from the flour mass and 4.8ml of NaCl solution which was mixed with the help of the aluminum paddles of the device for 20 seconds in the washing vessel equipped with a sieve with 88µ holes. The separation of protein substances in the form of gluten consists in washing with sodium chloride solution 20g/l (2%), at a temperature of 22°C, used as a reagent of the formed dough. For the preparation of NaCl, distilled water was used so that the results would not suffer changes due to minerals or other substances with which the tap water could have been contaminated. The formed dough is placed in the washing vessel equipped with a fine sieve with holes of 88 microns and washed for 5 minutes with sodium chloride solution with a flow rate of 50-56 ml/minute. The gluten obtained in this way is placed in specially designed boxes in the centrifuge for whipping and measuring the gluten index. The samples are processed for one minute, the centrifuge operating at 6,000 revolutions/minute. The gluten that passed through the sieve is considered weak gluten, it is removed with the help of a spatula and weighed with an accuracy of 0.01g, it is left on the scale, then the gluten that passed through the sieve, considered to be gluten, is added hard and weigh again. The entire amount of gluten is introduced into the dryer, the dry gluten being determined by drying the sample at a constant temperature of 150°C for 5 minutes, then weighing and obtaining the dry gluten content. In parallel, two determinations were performed for each analyzed sample.

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

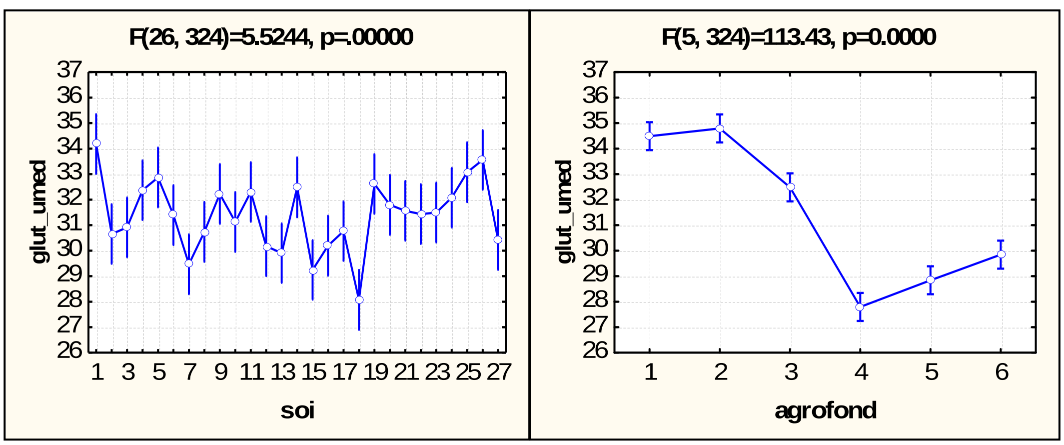
Variation analysis

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)	907,1	26	34,9	5,52	0,000000	***
B (level of fertilization)	3581,6	5	716,3	113,43	0,000000	***
A×B	2374,2	130	18,3	2,89	0,000000	***
Error	2046,2	324	6,3			
Total	8909,08					

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 (above table, column p), shows that: factor A (variety), factor B (agrofund) and the interaction A × B had a very significant action, that is: the differences between varieties are very significant; between the 6 agricultural funds there are very significant differences; A × B interaction, highly significant action. So the 27 varieties reacted differently within the 6 agrofunds, as far as the wet gluten content is concerned, the values obtained differ very significantly between them. In conclusion, the null hypothesis H0 is rejected for the factor A - the variety, the factor B - the agrofund and the interaction A × B.



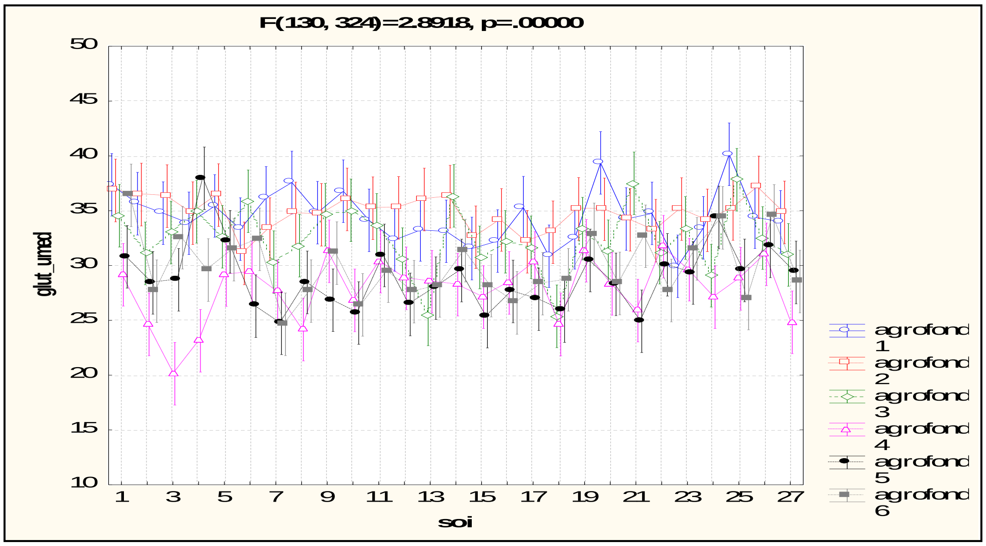


Figure 1. The influence of the variety and the influence of the agrofund on the wet gluten content

The influence of the variety on the wet gluten content: The values of the wet gluten content vary between 28 % (Centurion variety) and 34.1% (Dacic variety). The wet gluten content values for all other 25 cultivars range between 29.1 and 33.5%. The differences between the varieties are very significant ($p<0.001$), according to the F test value.

The influence of the agrofund on the content of wet gluten: The values of the content of wet gluten vary between 35.9% (120 kg nitric N a.s. ha^{-1}) and 28.8% (120 kg ammoniacal N a.s. ha^{-1}). The differences between the agrofunds are very significant ($p<0.001$), according to the value of the F test. The value of the wet gluten content has an upward trend from the first fertilization level of 120 kg nitric N a.s. ha^{-1}, at the second of 150 kg nitric N a.s. ha^{-1}, after which the trend is downward until the fertilization level of 150 kg ammoniacal N a.s. ha^{-1}, and from this level of fertilization the trend is again upward until the last level of fertilization of 170 kg ammoniacal N a.s. ha^{-1}.

The influence of the A × B interaction on the wet gluten content: The lowest values of this index were recorded in the varieties Alex (20.1%), Litera (24%) and Miranda (24.9%) at the fertilization level of 120 kg/ha s.a. nitric nitrogen. The highest values of the wet gluten content were recorded at the same level and type of fertilizer in the varieties Chevignon (39.9%) and Tiberius (40%).

Table 2

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

Variety	Wet gluten content (%)	Difference (%)	Significance
a1 – Dacic	34,18	2,81	***
a2 – Miranda	30,65	-0,72	
a3 – Alex	30,91	-0,46	
a4 - Litera	32,37	1,00	
a5 – Ciprian	32,87	1,50	
a6 – Crișana	31,39	0,02	
a7 – Biharia	29,47	-1,91	0
a8 – Glossa	30,74	-0,64	
a9 – Boema	32,22	0,85	
a10 – Sothys	31,12	-0,25	
a11 – Sacramento	32,30	0,93	
a12 – Rubisko	30,18	-1,20	
a13 – Certiva	29,90	-1,47	
a14 – Aurelius	32,48	1,11	
a15 – Aspekt	29,25	-2,13	0
a16 – Papillon	30,19	-1,18	
a17 – Activus	30,77	-0,61	
a18 – Centurion	28,07	-3,30	000
a19 - Tika Taka	32,61	1,24	
a20 – Chevignon	31,79	0,42	
a21 – Sosthene	31,56	0,19	
a22 – Vivendo	31,43	0,06	
a23 – Sophie	31,49	0,12	
a24 – Solindo	32,08	0,70	
a25 – Tiberius	33,08	1,70	*
a26 – Arrezo	33,56	2,18	**
a27 – Apexus	30,42	-0,95	
Average	31,37	Wt	
DL 5%= 1,640; DL 1% = 2,159; DL 0,1% = 2,753			

Compared to the control - the average of the experience, the following values of the wet gluten content were obtained: significant positives in the variety Tiberius and negatives in Biharia and Aspekt; significant differences in the Arrezo variety. The variety with a superior value of wet gluten content was obtained by the Dacic variety, and the variety with a very significant but negative value is Centurion. For the other 21 varieties 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}	34,49	3,12	***
b2 – 150 kg nitric N a.s. ha^{-1}	34,79	3,42	***
b3 – 170 kg nitric N a.s. ha^{-1}	32,48	1,11	**
b4 - 120 kg ammoniacal N a.s. ha^{-1}	27,79	-3,58	000
b5 - 150 kg ammoniacal N a.s. ha^{-1}	28,84	-2,53	000
b6 - 170 kg ammoniacal N a.s. ha^{-1}	29,84	-1,53	000
Average	31,37	wt	
DL 5% = 0,675; DL 1% = 0,888; DL 0,1% = 1,133			

Compared to the control - the average of the experience, very significant values of the wet gluten content were obtained at the 6 agricultural holdings, regardless of the level of fertilization or the type of nitrogen applied, with the exception of the fertilization level of 120 kg/ha s.a. ammoniacal nitrogen where the difference is distinctly significant. It should be noted that at fertilization levels of 120, 150 and 170 kg nitric N a.s. ha^{-1} the values obtained are higher than the experience average; and at the fertilization levels of 120, 150, 170 kg ammoniacal N a.s. ha^{-1}, the values of this index are negative, below the experience average.

Table 4

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

Wet gluten content and significances (to the witness, the average of the experience)												
Variety	Agrofund 1		Agrofund 2		Agrofund 3		Agrofund 4		Agrofund 5		Agrofund 6	
	WG	Diff.	WG	Diff.	WG	Diff.	WG	Diff.	WG	Diff.	WG	Diff.
a1- Dacic	37.36	5.99**	36.84	5.47**	34.54	3.17	29.15	-2.22	30.79	-0.58	36.40	5.03*
a2- Miranda	35.64	4.27*	36.47	5.10*	31.10	-0.27	24.63	-6.74^{00}	28.42	-2.95	27.66	-3.71
a3- Alex	34.78	3.41	36.33	4.96*	33.00	1.63	20.13	-11.24^{000}	28.69	-2.68	32.54	1.17
a4- Litera	33.85	2.48	34.78	3.41	34.91	3.53	23.13	-8.24^{000}	37.96	6.59**	29.59	-1.78
a5- Ciprian	35.43	4.06*	36.42	5.05*	32.61	1.24	29.14	-2.23	32.16	0.79	31.45	0.08
a6- Crișana	33.32	1.95	31.11	-0.26	35.86	4.49*	29.38	-1.99	26.30	-5.07^{0}	32.39	1.02
a7- Biharia	36.16	4.79*	33.32	1.95	30.31	-1.06	27.64	-3.73	24.73	-6.64^{00}	24.64	-6.73^{00}
a8- Glossa	37.57	6.20**	34.75	3.38	31.81	0.44	24.16	-7.21^{000}	28.46	-2.92	27.67	-3.70
a9- Boema	34.82	3.45	34.63	3.26	34.64	3.26	31.29	-0.08	26.84	-4.53^{0}	31.13	-0.24
a10- Sothys	36.78	5.41**	36.03	4.66*	35.04	3.66	26.82	-4.55^{0}	25.67	-5.70^{00}	26.41	-4.96^{0}
a11- Sacramento	34.12	2.75	35.23	3.86	33.70	2.33	30.35	-1.02	30.92	-0.45	29.48	-1.89
a12- Rubisko	32.33	0.96	35.25	3.88	30.59	-0.78	28.83	-2.54	26.45	-4.92^{0}	27.61	-3.76
a13- Certiva	33.24	1.87	36.02	4.65*	25.55	-5.83^{00}	28.52	-2.85	27.94	-3.43	28.14	-3.23
a14- Aurelius	33.12	1.75	36.28	4.91*	36.35	4.98*	28.25	-3.12	29.54	-1.83	31.36	-0.01
a15- Aspekt	31.55	0.18	32.58	1.21	30.73	-0.64	27.12	-4.25^{0}	25.33	-6.04^{00}	28.16	-3.21
a16- Papilon	32.23	0.86	34.15	2.78	32.16	0.78	28.41	-2.96	27.63	-3.74	26.59	-4.78^{0}
a17- Activus	35.28	3.91	32.16	0.79	31.66	0.29	30.23	-1.14	26.92	-4.45^{0}	28.34	-3.03
a18- Centurion	30.85	-0.52	33.04	1.67	25.37	-6.01^{00}	24.62	-6.75^{000}	25.85	-5.52^{00}	28.69	-2.68
a19- Tika Taka	32.52	1.15	35.16	3.79	33.37	1.99	31.34	-0.03	30.46	-0.91	32.84	1.47
a20- Chevignon	39.36	7.99***	35.13	3.76	31.31	-0.06	28.32	-3.05	28.28	-3.09	28.36	-3.01
a21- Sosthene	34.24	2.87	34.17	2.80	37.50	6.12**	25.89	-5.48^{00}	24.91	-6.46^{00}	32.67	1.30
a22- Vivendo	34.75	3.38	33.18	1.81	31.17	-0.20	31.72	0.35	30.06	-1.31	27.72	-3.65
a23- Sophie	29.93	-1.44	35.15	3.78	33.42	2.05	29.62	-1.75	29.32	-2.05	31.52	0.15
a24- Solindo	33.43	2.06	34.12	2.75	29.07	-2.30	27.11	-4.26^{0}	34.38	3.01	34.34	2.97
a25- Tiberius	40.15	8.78***	35.16	3.79	37.82	6.45**	28.78	-2.59	29.55	-1.82	26.99	-4.38^{0}
a26- Arrezo	34.41	3.04	37.13	5.76**	32.51	1.14	31.02	-0.35	31.75	0.38	34.52	3.15
a27- Apexus	33.98	2.61	34.84	3.47	30.97	-0.41	24.82	-6.55^{00}	29.37	-2.00	28.54	-2.83
Average	31,37											
DL 5% = 4,017 DL 1% = 5,287 DL 0,1% = 6,742												

The significance of the differences in terms of wet gluten content compared to the average of the experience, are reproduced in table 4 from where it can be seen that the varieties had values from significant to very significant regardless of the agro-fund, with the exception of the following varieties which have insignificant values:

• 120 kg nitric N a.s. ha^{-1}: Alex, Litera Crișana, Sacramento, Rubisko, Certiva, Aurelius, Aspekt, Papillon, Activus and Tika Taka;

• 150 kg nitric N a.s. ha^{-1}: Litera, Crișana Biharia, Glossa, Boema, Sacramento, Rubisko, Aspekt, Papillon, Activus, Centurion, Tika Taka, Chevignon, Sosthene, Vivedno, Sophie, Solindo, Tiberius and Apexus;
• 170 kg nitric N a.s. ha^{-1}: Dacic, Alex, Litera, Ciprian, Glossa, Boema, Sothys, Sacramento, Rubisko, Aspekt, Papillon, Activus, Tika Taka, Chevignon, Sosthene, Vivendo, Sophie, Solindo, Arrezo and Apexus;
• 120 kg ammoniacal N a.s. ha^{-1}: Dacic, Ciprian, Crișana, Biharia, Boema, Sacramento, Rubisko, Certiva, Aurelius, Papillon, Activus, Tika Taka, Chevignon, Vivedno, Sophie, Tiberius and Arrezo;
• 150 170 kg ammoniacal N a.s. ha^{-1}: Dacic, Miranda, Alex, Ciprian, Glossa, Sacramento, Certiva, Aurelius, Papillon, Tika Taka, Chevignon.
• 170 kg ammoniacal N a.s. ha^{-1}: Miranda, Alex, Litera, Ciprian, Crișana, Glossa, Boema, Sacramento, Rubisko, Certiva, Aurelius, Aspekt, Activus, Centurion, Tika Taka, Chevignon, Sosthene, Vivendo, Sophie, Solindo, Arrezo and Apexus.

Statistically assured wet gluten content values were both positive and negative, i.e. values were both below and above the experimental mean. Varieties with a wet gluten content clearly above the experience average are classified as follows:
• 120 kg nitric N a.s. ha^{-1}: Dacic, Miranda, Cyprian, Biharia, Glossa, Sothys, Chevignon and Tiberius;
• 150 kg nitric N a.s. ha^{-1}: Dacic, Miranda, Alex, Ciprian, Sothys, Certiva, Aurelius and Arrezo;
• 170 kg nitric N a.s. ha^{-1}: Crisana, Aurelius, Sosthene and Tiberius;
• 150 kg ammoniacal N a.s. ha^{-1}: Litera
• 170 kg ammoniacal N a.s. ha^{-1}: Dacic

CONCLUSIONS

The gluten content is directly correlated with the level of fertilization of nitrogen which is strongly influenced by pedoclimatic conditions and wheat variety. However, wheat genotype is considered the most important factor influencing gluten quality characteristics. Increasing total nitrogen content of wheat correlates positively with gluten content.

In the agricultural year 2021-2022, the average of the experiment for the content of wet gluten is 31.37%. The varieties with the highest values of this index are Arrezo - 33.56% and Dacic - 34.18%, and the lowest value of 28.07% was obtained by the Centurion variety.

In the case of fertilization levels, the highest value of 34.79% was obtained at agrofund 2, followed by the fertilization level of120 kg nitric N a.s. ha^{-1} – 34.49% and the maximum of 170 kg nitric N a.s. ha^{-1} where the wet gluten value was 32.48%.

In the case of ammoniacal nitrogen fertilization levels, the averages of these agrofunds ranked below the experience average. The influence of the A × B interaction on the wet gluten content: The lowest values of this index were recorded in the varieties Alex (20.1%), Litera (24%) and Miranda (24.9%) at the fertilization level of 120 kg nitric N a.s. ha^{-1}. The highest values of the wet gluten content were recorded at the same level and type of fertilizer in the varieties Chevignon (39.9%) and Tiberius (40%).

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