Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

STUDY REGARDING THE INFLUENCE OF NITRIC, AMMONIACAL NITROGEN FERTILIZATION AND VARIETY ON WINTER WHEAT (*Triticum aestivum* L.) PROTEIN CONTENT

Denisa Cristiana HETEA[1], Simona NIȚĂ[1], Ștefan-Laurențiu BĂTRÎNA[1], Ioana-Alina HÎNDA[1], Piotr PRUS[2], Florin IMBREA[1]

[1]University of Life Sciences "King Mihai I" from Timișoara, 119 Calea Aradului Street, Timisoara, Romania
[2]Bydgoszcz University of Science and Technology, Al. prof. S. Kaliskiego, 7, Bydgoszcz, Poland

Corresponding author email: denisa.hetea@yahoo.com

Abstract

The aim of this study was to analyse e the effect of variety, nitrogen rate and type and their interaction on protein content and to determine the economic feasibility of varieties and fertilizer application for the highest values. The subject of the experiment consisted in testing during two wheat growing seasons 2021-2023, twenty-seven winter wheat varieties fertilized with nitric and ammoniacal nitrogen in three different rates - 120, 150 and 170 kg N ha^{-1}. The layout of the experimental plan was done using stratified randomization block method. Cultivars were factorially combined and arranged in completely randomized blocks. We concluded that compared with the mean of the experience of 14.75%, variety and nitrogen fertilization systems interaction had a significant effect on wheat protein content. The highest protein content was obtained by the Tika Taka variety - 15.78%. Regarding the fertilization system, with very significant differences the values above the average of the experience, 15.32%, 15.27% and 15.36% were obtained the levels of fertilization with nitric nitrogen and those with ammoniacal nitrogen had negative values of 14.04%, 14.20% and 14.32%.

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

INTRODUCTION

Wheat is the second most widespread cultivated plant in the world, it is estimated that this crop covers 200 million hectares, able to grow in temperate, mediterranean and subtropical regions of the two hemispheres, thanks to its enormous genetic diversity with more than 25,000 varieties of *Triticum aestivum* L. adapted to different climate and soil conditions (Frankin et al., 2021; de Sousa et al., 2021). 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 small 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 (Harari, 2015).

Nitrogen is an important factor limiting plant growth and consequently wheat pro-duction and quality 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 wheat plant growth and development (Rossini et al., 2018; Noor et al., 2023).

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 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 (Ali et al., 2011; Barraclough et al., 2014).

Nitrogen is indispensable for both production yields and wheat quality. The benefits of nitrogen fertilizer on yield and quality depend on the rate and timing of application (Grzebisz et al., 2023; Bogard et al., 2010).

The use of mineral fertilizers in agricultural systems for growing wheat is essential to support the growing world population. Wheat is demanding in terms of fertilization, be-cause the root system is poorly developed, given the architecture of the root, therefore it explores a small volume of soil and has a low power to solubilize and absorb nutrients from the soil reserve (Ma et al., 2021).

Nitrogen is the essential element for increasing production yields and economic profitability. Nitrogen availability for wheat production is almost as important as soil water availability. Without an adequate N supply, all growth stages are severely stunted (Zhong et al., 2019; Song et al., 2020). N applied at sowing stimulates tillering and vegetative growth, while N applied in the generative phases has a greater influence on the protein concentration of the caryopses (Habash et al., 2006). Studies suggest that N use efficiency is higher when N is applied later (at tillering) than when applied at sowing (Zhong et al., 2018). Doses, types of nitrogen fertilizers and the time of N application influence nitrogen uptake and its translocation within the plant (Khan et al., 2022). Thus, the timing of fertilizer application and doses have a significant role in determining the amount of absorption and its efficient use by the plant. N from the above-ground parts of plants is actively recycled and transported into plants as they grow. This redistribution of nitrogen from aboveground parts of wheat has been studied extensively. The flow and amount of nitrogen redistributed to developing caryopses varies according to the availability-absorption ratio, which is influenced by climatic conditions (temperature, precipitation) and inherent properties of plant organs (Ahmed et al., 2020; Guo et al., 2019). It has also been suggested that N remobilization from roots may play an important role in whole plant N utilization (Daba et al., 2021).

Increased leaf mass improvement in wheat plants when nitrate nitrogen fertilizers are applied compared to ammoniacal nitrogen fertilizers (Aluko et al., 2023). Growth factors consisting of tiller number, root volume, specific leaf mass, standard leaf area, and total leaf area per-formed better with NO_3^- compared to NH_4^+ fertilizers. Along with the above, the use of nitrogen fertilizers based on ammoniacal nitrogen decreases the nitrate content of plants, while nitrogen fertilizers based on nitric nitrogen increase it (He et al., 2022). Furthermore, a significant decrease in the soluble sugar content of wheat caryopsis exposed to NH_4^+ compared to NO_3^- was observed (Sinha et al., 2020). In addition, investigations 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 (Ukalska-Iaruga et al., 2020; Yadav et al., 2023).

Today we share in the success of agriculture's evolution - a two-sided coin, given the ever-growing population and the climate crisis. From 1.6 billion in 1900, the world's population has grown to more than 7 billion today and will reach 9 billion by 2050. In this context, it will be impossible to feed the ever-growing population without a significant increase in wheat quality. Data from the United Nations show us that in the next 30 years the global demand for food will increase by 70% as a result of population growth, and the battle of the future will be over food and water. Food security is systematically analyzed by the Food and Agriculture Organization of the United Nations, which draws attention to the anticipated food crisis. Thus, international forums explain why population growth is likely to outpace production yields and call for greater investment to keep pace with future demand. According to a 2022 Food and Agriculture Organization (FAO) report, global wheat production was 777 million tons, production that needs to be doubled by 2050 and tripled by 2100 to ensure food security, as a result of population growth. How this will be achieved is unknown, as the extent of cultivated arable land is not expected to increase, but rather to decrease as a result of urbanization, increased soil erosion, and areas affected by drought and salinization (UNESCO; FAO 2024; European Commission; From Farm to Fork; Mălinaș et al., 2022).

MATERIALS AND METHODS

The study was conducted in 2021-2023 in the Dudeștii Noi experimental fields (45°50′51″N 21°06′30″E, 87 m altitude, Garmin device GPSMAP 64 st), located in the Western Plain of Romania, which is a part of one of the largest agricultural regions of the country. The territory of the area where the research was carried out is located in the forest-steppe area, with a high hydrostatic level of the water table and differentiated parental rocks, which determined a varied pedological background. The formation of sedimentary rocks in this area is closely related to the existence of the Pannonian Lake, which, by retreating, created an area of accumulation of coarse materials brought to the unstable bed of the Mureș River. The parent rocks, on the basis of which the soils around the commune of Dudeștii Noi were formed, consist of quartz sands with a varied content of calcium carbonate, as well as medium-textured löessoid materials. In some places, in micro-depression zones, the water table has salted the mentioned rocks, thus forming areas with salinized or alkalized soils. The experimental field was located on a cambic chernozem soil, wet phreatic, slightly levigated, medium clay loam, developed on fluvial, carbonate, medium fine materials with a well-defined profile and with insignificant differences regarding the physical, hydric and chemical properties. The chernozems in general and the cambium equally represent soils with a bioenergetic potential and a good production capacity. Cambic chernozem is a very deep soil with loamy texture, generated by transported and redeposited materials, constituted by aeolian deposits (loess and carbonate loessoid deposits) (Crista, 2017). The soil profile is characterized by the sequence of the following horizons: Ap, Am, Bv, Ck. Ap horizon: 0-15 cm, gradual transition, dark gray brown clayey texture, small glomerular structure, plastic, weak adhesive, small pores, frequent roots, dry. Am horizon: 15-35 cm, gradual transition, dark brown loamy texture, medium glomerular structure, well developed, plastic, adhesive, small pores, weak compact, thin roots frequent. Bv horizon: 35-60 cm, gradual transition, dark gray-brown loamy texture, medium subangular polyhedral structure, plastic, adhesive, fine porous, moderately compact, sparse thin roots, dry. Ck horizon: 60-100 cm, gradual transition, yellowish brown light gray loamy texture, subangular and medium angular polyhedral structure well developed, plastic, adhesive, dry (Munteanu, 2012).

Physical and hydraulic properties: The loamy-clay texture - dusty in the first 70 cm and loamy-dusty in the rest, makes the apparent density (AD) medium throughout the profile of the soil, with 1.46 g/cm^3 in the Ap horizon and 1.58 g/cm^3 in Am. Total porosity (TP) is medium in Ap (48.8%) and high in Am (58.2%). Air porosity (AP) is medium in Ap (15.2%), high in Am (23.8%), medium in Bv (21.7%) and low in Ck (12.2%). The ranges of variation for clay are between 33.4-40.3%. The processed Ap horizon has a clay content of 36% compared to the Am horizon with a content of 40.3%. In horizons Bv and Ck, the amount of clay shows lower values (33.8%) and 33.4%, respectively. The soil is weakly to moderately compacted on the horizons below the tilled horizon. The values of the hydrophysical indices show different values, correlated with the physical properties. A medium value of the withering coefficient is noted in the Ap and Am horizons (11.7%), high in Bv (13.2%) and medium in Ck (12%) (Obrejanu, 1964).

The field capacity (FC) is medium over the entire soil profile, with values of 23.3% in Ap, Am, Bv and 22.4% in Ck. Chemical properties were determined using the Kjeldahl and Olsen et al. methods (Dumitru et al., 2011). The soil has a humus content of 3.04% in the Ap horizon and 1.78% in the Am horizon, decreasing by 1.24% in the Bv horizon. It is medium supplied in total nitrogen, with 0.172% in Ap, very well supplied in Am with mobile phosphorus (168 ppm) and well supplied with mobile potassium (248 ppm). The pH was tested using the distilled glass electrode method, 1:2.5 soil and water. The reaction of the soil on the profile is weakly acid in Ap horizon 5.58, weakly alkaline 7.66. The amount of exchangeable bases is medium (23.2 in Ap, 24.8 in Am), and the degree of saturation in bases shows values of 92.62% which gradually increases on the Bv horizon. Trace element content is high for zinc (176 ppm) in the Ap and extremely low in the Bv horizon (0.40 ppm). Climatic data during the period of the experiment were collected from the meteorological station of Dudeștii Noi and are represented in Figure 1. The climate of this area is temperate - continental with slight Mediterranean influences. The average annual temperature is 10.8°C and the warmest month was July. Temperatures were also extremely high for September and October. As can be seen, during the entire experimental period and especially during the vegetation months, the average monthly temperatures exceeded the multiannual averages, with the highest values during the vegetation period being recorded between June and September in all two experimental years.

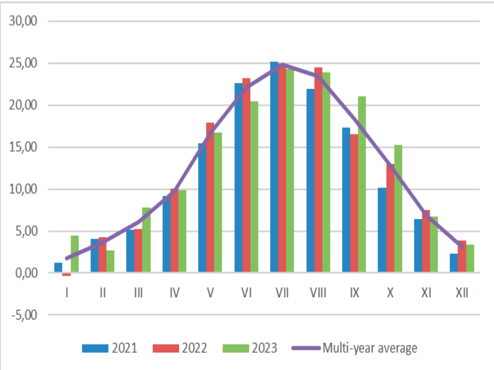

Figure 1. The average monthly temperatures (°C) recorded in Dudeștii Noi (2021-2023)

The average annual precipitation was frequently around 540 mm. In May and July, as a rule, the maximum pluviometric occurs (Figure 2). Sowing was carried out late, after successful application of a watering to help the suitable preparation of the field. The months from August until October were dry. These were registered against the back-ground of acute lack of precipitation. The last three years, especially, have positioned the western area of Romania in an unprecedented position. The Western Plain of Romania, an important region of the country for wheat cultivation, has recently had to deal with long periods of very hot and dry weather and very wet and rainy periods with heavy rainfall in a short period of time. These climate events often exceed the relevant meteorological observations, some of which vary strongly from the multi-year mean values recorded to date.

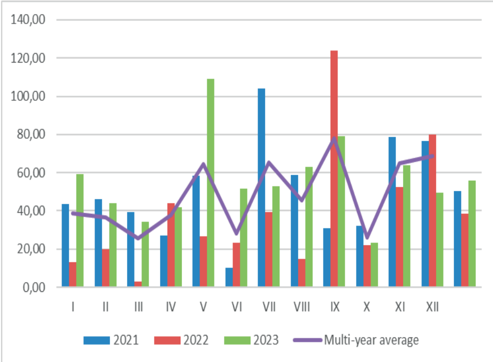

Figure 2. The average monthly rainfall (mm) recorded in Dudeștii Noi (2021-2023)

The biological material is represented by 27 Romanian, German and French 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, high tolerance to disease and pests attack and specific qualities for milling and baking indices.

The layout of the experimental plan was made using the stratified randomization block method. Cultivars were factorially combined and arranged in completely randomized blocks. This experimental method was chosen in order to avoid the interfering effects of various environmental factors and to adequately and accurately estimate nitrogen utilization. The size of each plot was 1.2 m × 7 m, the distance between plots and adjacent blocks was kept at 0.5 m and 1.5 m, respectively, and the buffer strip was 2.5 m. Each block 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 - cultivated wheat variety:

a1 - Dacic, a2 - Miranda, a3 - Alex, a4 - Litera, a5 - Ciprian, a6 - Crișana, a7 - Biharia, a8 - Glosa, a9 - Bohemia, a10 - Sothys, a11 - Sacramento, a12 - Rubisko, a13 - Certiva, a14 -

Aurelius, a15 - Aspect, a16 - Papillon, a17 - Activus, a18 - Centurion, a19 - Tika Taka, a20 - Chevignon, a21 - Sosthene, a22 - Vivendo, a23 - Sophie, a24 - Solindo, a25 - Tiberius, a26 - Arrezo and a27 - Apexus.

FACTOR B - fertilization system:

b1 - Ammoniacal nitrogen; b2 - Nitric nitrogen.

FACTOR C - fertilization level:

c1 - 120 kg ha^{-1} N a.s.; c2 - 150 kg ha^{-1} N a.s.; c3 - 170 kg ha^{-1} N a.s.

Nitrogen doses were established starting from the maximum ceiling accepted by The Agency for Intervention and Payments in Agriculture in Romania, 170 kg N ha^{-1} (APIA, 2021). The lowest dose of 120 ha^{-1} was chosen according to the use of nitrogen in wheat crop, the humus content and the available nitrogen in the soil. The fertilizer consisted of two types of nitrogen (nitric and ammoniacal) that were applied in the form of ammonium sulfate $(NH_4)_2SO_4$ and calcium nitrate $Ca(NO_3)_2$, in three contrasting fertilization doses and administered to the plants in - a single dose, unfractionated, at the stage of growth BBCH 30-31. In both years of study, the sowing rate for each variety was established considering aspects related to the sowing rate such as purity, germination and the 1,000 kernel weight, and the density at sowing was 550 germinating grains/m^2. Wheat was sown at a depth of 4 centimeters with a row spacing of 12.5 cm. The treatments applied were V1 - nitric N dose of 120 kg ha^{-1} N a.s., V2 - nitric N dose of 150 kg ha^{-1} N a.s, V3 - nitric N dose of 170 kg ha^{-1} N a.s, V4 - ammoniacal N dose of 120 kg ha^{-1} N a.s., V5 - ammoniacal N dose of 150 kg ha^{-1} N a.s, V6 - ammoniacal N dose of 170 kg ha^{-1} N a.s. and the Mt - control variant was the average of the experimental field.

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 (ASRO, 2022), 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 this parameter. 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. In parallel, two determinations were performed for each analyzed sample.

Based on the results of two years field experiment, the wheat protein content parameter was calculated using the Analysis of variance (ANOVA) and Student t tests.

RESULTS AND DISCUSSIONS

Wheat quality is a complex trait resulting from interactions between numerous protein components. Protein substances usually represent 10-16% of the mass of the grain (with limits between 8 and 24%) and are mostly located towards the peripheral parts of the grain (coats, aleurone layer), in the embryo and scutellum. The amount and composition of proteins give the nutritive quality of the grain. Proteins form, mainly, gluten, a mixture of protein substances that occupy the space between the starch granules in the endosperm and which, after grinding, in the flour, embeds the starch granules. The accumulation of proteins in the grain depends on a number of factors, such as: the wheat species, the variety, the climatic conditions, the natural fertility of the soil and the doses of nitrogen fertilizers used. Wheat grains contain on average 8-30% proteins, which are divided into two major categories: prolamins which include gliadins and glutenins, and nonprolamins consisting of water-soluble albumins and saline-soluble globulins. The albumin/globulin fraction accounts for 20-25%, while gliadins and glutenins account for 75-90% of wheat proteins (Sulek et al., 2018; Liu et al., 2022). Wheat storage proteins, namely gliadins and glutenins, are the main components of gluten, which determine the nutritional values and baking properties of wheat grains due to their impact on the water absorption capacity of the dough, its elasticity and extensibility. Gliadins give the dough extensibility and viscosity and glutenins are responsible for elasticity and resistance (Lafiandra et al., 2022; Veraverbeke et al., 2002).

Table 1. Variation analysis

Variation source	SSP (SP)	Degree of Freedom	Weighed Least of Squares - WSL (s^2)	F test for s^2 error		
				Value	P	Signification
A (variety)	137.63	26	5.29	6.5	0.000000	***
B (agrofund)	159.26	5	31.85	39.3	0.000000	***
A×B	102.98	130	0.79	1.0	0.551313	ns
Error	262.39	324	0.81			
Total	662.26					

*, **, or *** indicate statistically significant differences between sample means and Mt based on t-test, at p ≤ 0.05, p ≤ 0.01, or p ≤ 0.001, respectively. NS (not significant) indicates the t-test difference between sample means was p > 0.05.
0, 00 or 000 also indicate significant differences between sample means and Mt based on t-test at p ≤ 0.05, p ≤ 0.01, or p ≤ 0.001, respectively, but with negative values.

The F test (Table 1, column p) shows that: factor A - variety and factor B - agrofund had a very significant effect, that is: between the varieties followed in the experiment there are very significant differences; between the 6 agrofunds there are very significant differences; the interaction A × B, insignificant effect, the 27 varieties did not react differently within the 6 agrofunds. In conclusion, the null hypothesis H0 is rejected for factor A (variety) and factor B (agrofund), it is accepted for the interaction A × B.

Table 2. Student test for factor A (variety) – witness (Mt), average of the field

Variety	Protein content (%)	Difference (%)	Signification
a1 - Dacic	14.98	0.22	ns
a2 - Miranda	14.41	-0.34	ns
a3 - Alex	14.84	0.09	ns
a4 - Litera	14.87	0.12	ns
a5 - Ciprian	15.68	0.92	**
a6 - Crișana	15.05	0.30	ns
a7 - Biharia	15.03	0.28	ns
a8 - Glossa	14.35	-0.40	ns
a9 - Boema	14.79	0.04	ns
a10 - Sothys	14.18	-0.57	ns
a11 - Sacramento	13.95	-0.80	00
a12 - Rubisko	14.08	-0.68	0
a13 - Certiva	14.23	-0.52	ns
a14 - Aurelius	15.32	0.57	ns
a15 - Aspekt	14.68	-0.08	ns
a16 - Papillon	13.99	-0.76	*
a17 - Activus	14.85	0.10	ns
a18 - Centurion	13.75	-1.00	000
a19 - Tika Taka	15.78	1.03	***
a20 - Chevignon	15.34	0.59	*
a21 - Sosthene	14.30	-0.45	ns
a22 - Vivendo	14.89	0.14	ns
a23 - Sophie	15.02	0.27	ns
a24 - Solindo	14.57	-0.18	ns
a25 - Tiberius	15.69	0.94	**
a26 - Arrezo	14.95	0.20	ns
a27 - Apexus	14.71	-0.04	ns
Mean	14.75	Mt	
DL 5% = 0.584; DL 1% = 0.769; DL 0.1% = 0.981			

*, **, or *** indicate statistically significant differences between sample means and Mt based on t-test, at p ≤ 0.05, p ≤ 0.01, or p ≤ 0.001, respectively. NS (not significant) indicates the t-test difference between sample means was p > 0.05.
0, 00 or 000 also indicate significant differences between sample means and Mt based on t-test at p ≤ 0.05, p ≤ 0.01, or p ≤ 0.001, respectively, but with negative values.

The protein content achieved using the 27 winter wheat varieties is shown in Table 2. Compared to the control - the average of the experience of the two years of study, significant differences were recorded by the Papillon and Chevignon varieties; distinctly significant differences were recorded by the Ciprian and Tiberius varieties, and very significant values were recorded by the Tika Taka variety. Below the experience average were the Rubisko varieties with significant differences, Sacramento with distinctly significant differences and Centurion with very significant differences. The other varieties did not register differences. The variety with the highest value of this index is Tika Taka - 15.78%, Tiberius - 15.69% and Ciprian - 15.68%. The varieties Sacramento - 13.95% and Centurion - 13.75% ranked below the experience average. The other varieties did not register differences.

Table 3. Student test for factor B (level of fertilizer) – witness (Mt), average of the field

Variant	Protein content (%)	Difference (%)	Signification
V1 - 120 kg ha^{-1} N a.s nitric N	15.32	0.57	***
V2 - 150 kg ha^{-1} N a.s nitric N	15.27	0.52	***
V3 - 170 kg ha^{-1} N a.s nitric N	15.36	0.61	***
V4 - 120 kg ha^{-1} N a.s ammoniacal N	14.04	-0.72	000
V5 - 150 kg ha^{-1} N a.s ammoniacal N	14.20	-0.55	000
V6 - 170 kg ha^{-1} N a.s ammoniacal N	14.32	-0.43	00
Mean	14.75	Mt	
DL 5% = 0.275; DL 1% = 0.363; DL 0.1% = 0.462			

*, **, or *** indicate statistically significant differences between sample means and Mt based on t-test, at p ≤ 0.05, p ≤ 0.01, or p ≤ 0.001, respectively. NS (not significant) indicates the t-test difference between sample means was p > 0.05.
0, 00 or 000 also indicate significant differences between sample means and Mt based on t-test at p ≤ 0.05, p ≤ 0.01, or p ≤ 0.001, respectively, but with negative values.

The protein content achieved using the nitric and ammoniacal nitrogen fertilization is shown in Table 3. Compared to the control group - the mean of the experience of the years of study, very significant differences were recorded, regardless of the level or type of N fertilizer.

Table 4. Student test for A × B interaction (variety × level of fertilization) – witness (Mt), average of the field

Variety	V 1		V 2		V 3		V 4		V 5		V 6	
	P	Diff	P	Diff	P	Diff	P	Diff	P	Diff	P	Diff
a1 - Dacic	15.40	0.62	15.70	0.60	15.75	0.25	14.00	0.17	14.20	0.61	15.05	0.62
a2 - Miranda	15.05	0.62	14.45	0.60	14.85	0.25	13.85	0.17	14.15	0.61	14.10	0.61
a3 - Alex	15.60	0.62	15.65	0.60	16.00	0.25	12.85	0.17	14.40	0.61	14.55	0.61
a4 - Litera	15.30	0.62	14.25	0.60	14.90	0.25	13.25	0.17	15.65	0.61	15.85	0.61
a5 - Ciprian	16.70	0.62	15.8	0.60	16.60	0.25	14.50	0.17	15.35	0.61	15.10	0.61
a6 - Crișana	16.20	0.62	15.00	0.60	15.10	0.25	15.40	0.17	14.25	0.61	14.35	0.61
a7 - Biharia	16.05	0.62	16.50	0.60	15.65	0.25	13.90	0.17	13.75	0.61	14.35	0.61
a8 - Glossa	15.20	0.62	14.80	0.60	15.05	0.25	13.35	0.17	13.75	0.61	13.95	0.61
a9 - Boema	15.15	0.62	15.25	0.60	15.40	0.25	14.20	0.17	14.40	0.61	14.35	0.61
a10 - Sothys	15.00	0.62	14.50	0.60	14.60	0.25	13.70	0.17	13.40	0.61	13.90	0.61
a11 - Sacramento	14.75	0.62	14.20	0.60	14.40	0.25	13.80	0.17	13.05	0.61	13.50	0.61
a12 - Rubisko	14.50	0.62	14.85	0.60	15.00	0.25	13.15	0.17	13.60	0.61	13.35	0.61
a13 - Certiva	14.70	0.62	14.85	0.60	14.75	0.25	13.65	0.17	13.50	0.61	13.95	0.61
a14 - Aurelius	16.40	0.62	15.90	0.60	15.70	0.25	14.65	0.17	14.65	0.61	14.60	0.61
a15 - Aspekt	14.50	0.62	16.10	0.60	15.60	0.25	14.45	0.17	13.85	0.61	13.55	0.61
a16 - Papillon	14.50	0.62	14.35	0.60	14.45	0.25	13.75	0.17	13.35	0.61	13.55	0.61
a17 - Activus	15.40	0.62	15.90	0.60	15.95	0.25	14.40	0.17	13.60	0.61	13.85	0.61
a18 - Centurion	14.55	0.62	14.00	0.60	14.15	0.25	12.85	0.17	13.60	0.61	13.35	0.61
a19 - Tika Taka	16.90	0.62	16.40	0.60	16.10	0.25	15.20	0.17	14.90	0.61	15.20	0.61
a20 - Chevignon	16.30	0.62	15.80	0.60	15.70	0.25	15.10	0.17	14.60	0.61	14.55	0.61
a21 - Sosthene	14.70	0.62	14.75	0.60	15.70	0.25	13.30	0.17	13.55	0.61	13.80	0.61
a22 - Vivendo	14.85	0.62	14.90	0.60	14.85	0.25	14.50	0.17	14.90	0.61	15.35	0.61
a23 - Sophie	13.20	0.62	16.10	0.60	16.10	0.25	15.10	0.17	15.15	0.61	14.45	0.61
a24 - Solindo	15.45	0.62	15.40	0.60	15.05	0.25	13.70	0.17	13.85	0.61	13.95	0.61
a25 - Tiberius	16.55	0.62	16.25	0.60	16.05	0.25	15.15	0.17	14.95	0.61	15.20	0.61
a26 - Arrezo	15.60	0.62	15.90	0.60	16.15	0.25	13.30	0.17	14.40	0.61	14.35	0.61
a27 - Apexus	15.05	0.62	14.85	0.60	15.10	0.25	13..90	0.17	14.65	0.61	14.70	0.61
Mean	14.75											
DL 5% = 0.486; DL 1% = 0.528; DL 0.1% =0.667												

*, **, or *** indicate statistically significant differences between sample means and Mt based on t-test, at p ≤ 0.05, p ≤ 0.01, or p ≤ 0.001, respectively. NS (not significant) indicates the t-test difference between sample means was p > 0.05.
0, 00 or 000 also indicate significant differences between sample means and Mt based on t-test at p ≤ 0.05, p ≤ 0.01, or p ≤ 0.001, respectively, but with negative values.

However, it should be noted that: at the V1, V2 and V3 treatments the protein content values obtained are above the experience average.
The highest value of 15.36% was obtained at the V3 treatment, followed by V2 - 15.27% and the minimum level of nitrogen fertilization, V1 - 15.32%. Below the experience mean are the protein content values from the ammoniacal nitrogen fertilization levels, the values being very significant for the first two nitrogen rates, V4 and V5 and distinctly significant for the maximum level of fertilization with this type of nitrogen.
There are no significant differences in Table 4, the same is confirmed by the F test from the analysis of variance.
Influence of variety on protein content - the protein content values range between 13.7% (Centurion variety) and 15.7% (Tika Taka variety). All other 25 varieties have values between 13.8 and 15.5%. The differences between varieties are highly significant ($p<0.001$), according to the F-test value.
Influence of fertilization system on protein content - for the first three fertilization levels, the protein content values are approximately identical. From the fertilization level of 120 kg ha^{-1} a.s. ammoniacal nitrogen to that of 170 kg ha^{-1} a.s. ammoniacal nitrogen, the trend is slightly upward, with protein content values ranging between 14 and 14.4%. The differences between agrofunds are very significant ($p<0.001$), according to the F-test value.
Influence of the A × B interaction on protein content: the lowest value of this index was recorded for the Alex variety - 12.9% at the fertilization level of 120 kg ha^{-1} a.s. ammoniacal nitrogen; and the highest value of this index was obtained by the Tika Taka variety - 16.9% at the fertilization level of 120 kg ha^{-1} a.s. nitric nitrogen.
Factor A - variety contributes to the formation of the protein content index by 20.78%, factor B - fertilization system by 24.05%, and the interaction of the two mentioned factors A × B by 15.55% (Figure 3). However, it is very important to mention that the greatest contribution to the formation of protein content is made by other factors that were not taken into account in this study, being followed by factor B - fertilization system, then with a very small difference factor A - variety, in last place being the interaction between the two factors, variety - fertilization system.

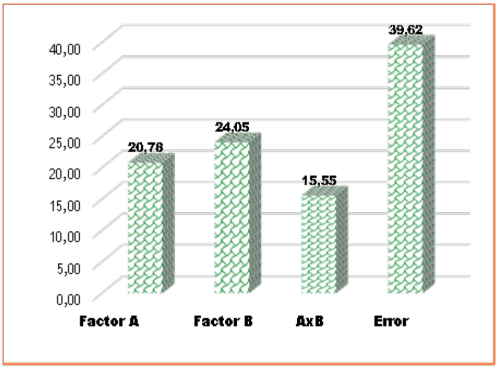

Figure 3. The contribution of factors and the interaction of factors on protein content

CONCLUSIONS

The average of the two years study for the protein content is 14.75%. The highest values of this index were obtained by the varieties Tika Taka - 15.78%, Tiberius - 15.69%, Ciprian - 15.68% and Chevignon - 15.34%. Below the average of the experience, with the lowest values, Rubisko - 14.08%, Sacramento - 13.95% and Centurion - 13.75% varieties were ranked. Regarding the fertilization levels and types of nitrogen, the highest value of 15.36% was obtained at V3 (170 kg ha^{-1} a.s. nitric nitrogen, followed by V1 (120 kg ha^{-1} a.s. nitric nitrogen) - 15.32% and V2 (150 kg ha^{-1} a.s. nitric nitrogen) - 15.27%.
The variants in which fertilization was carried out with ammoniacal nitrogen were ranked below the average of the experience with values of 14.04 - V4 (120 kg ha^{-1} a.s. ammoniacal nitrogen), 14.20 - V5 (150 kg ha^{-1} a.s. ammoniacal nitrogen and 14.32% - V6 (170 kg ha^{-1} a.s. ammoniacal nitrogen). Influence of the A × B interaction on the protein content: no significant differences were recorded.
Wheat mainly uses nitric nitrogen, under the influence of microorganisms, being immediately available and easily absorbed by plants, much more 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 (Barraclough et al., 2014). Absorption of ammoniacal nitrogen occurs specifically through the root plasma membrane, which serves as an ammonium ion transporter. These uptake transporters have a high affinity for ammonium ions, allowing wheat plants to achieve this even in the presence of low external ammonium concentrations. And once used, ammonium can be directly assimilated into organic compounds through the process of glutamate dehydrogenase (GDH). This process catalyzes the formation of glutamate from ammonium and 2-oxoglutarate. However, the efficient uptake mechanism may not have positive effects on wheat plants that are exposed to excessive soil ammonium levels, causing toxicity such as stunted root growth and reduced nutrient uptake efficiency (Ijato et al., 2021). Several studies have tested the molecular mechanisms behind the physiological responses of wheat plants to ammonia nitrogen fertilization. It has been found that NH_4^+ application can decrease proton efflux lipid peroxidation and protein carbonylation in root tissues, consequently causing plant growth suppression (Du et al., 2021). This is associated with the rate of ammonium absorption exceeding the rate of assimilation in the plant tissues, which leads to the disturbance of ion homeostasis, especially in potassium ions (Xiao et al., 2023). This disturbance of potassium ion balance can appear as leaf chlorosis, leaf scorch and lower biomass production. In addition, ammonium toxicity leads to damage to the chloroplast ultrastructure, which in turn affects photosynthetic activity and plant growth (Castro et al., 2022).

ACKNOWLEDGEMENTS

This work was carried out with the support of University of Life Sciences "King Mihai I" from Timișoara.

REFERENCES

Ahmed, M., Rauf, M., Akhtar, M., Mukhtar, Z., & Saeed, N. A. (2020). Hazards of nitrogen fertilizers and ways to reduce nitrate accumulation in crop plants. *Environmental Science and Pollution Research*; *27*(15), 17661–17670.

Ali, A. Ahmad, A., Syed, W.H., Khaliq, T., Asif, M., Aziz, M., Mubeen, M. (2011). Effects of nitrogen on growth and yield components of wheat (report). *Sci. Int.*, *23*(4), 331-332. Aluko, O. O., Kant, S., Adedire, O. M., Li, C., Yuan, G., Liu, H., & Wang, Q. (2023) Unlocking the potentials of nitrate transporters at improving plant nitrogen use efficiency. *Frontiers in Plant Science;* 14.

Barraclough P.B., Lopez-Bellido R., Hawkesford M.J. (2014). Genotypic variation in the uptake, partitioning and remobilization of nitrogen during grain-filling in wheat. *Field Crops Research*; 156: 242–248.

Barraclough, P.B., Lopez-Bellido, R., Hawkesford, M.J. (2014). Genotypic variation in the uptake, partitioning and remobilisation of nitrogen during grain-filling in wheat. *Field Crops Research* 156: 242-248.

Bogard, M., Allard, V., Brancourt-Hulmel, M., Heumez, E., Machet, J.M., Jeuffroy, M.H., Gate, P., Martre, P., Le Gouis, J. (2010). Deviation from the grain protein concentration-grain yield negative relationship is highly correlated to post-anthesis N uptake in winter wheat. *J Exp Bot.*; *61*(15): 4303-12.

Castro, S.A.Q.d., Kichey, T., Persson, D.P., Schjoerring, J.K. (2022). Leaf Scorching following Foliar Fertilization of Wheat with Urea or Urea–Ammonium Nitrate Is Caused by Ammonium Toxicity. *Agronomy*, 12, 1405.

Crista, F. (2017). *Conservarea Fertilității Solului si Managementul Nutrientilor*, 2nd ed.; Eurobit Publishing House: Timisoara, Romania, 2017; pp. 66–71.

Daba, N.A., Li, D., Huang, J., Han, T., Zhang, L., Ali, S., Khan, M.N., Du, J., Liu, S., Legesse, T.G., et al. (2021). Long-Term Fertilization and Lime-Induced Soil pH Changes Affect Nitrogen Use Efficiency and Grain Yields in Acidic Soil under Wheat-Maize Rotation. *Agronomy*, 11, 2069.

de Sousa, T., Ribeiro, M., Sabença, C., Igrejas, G. (2021). The 10,000-Year Success Story of Wheat! *Foods* 2021, 10, 2124

Du, W., Zhang, Y., Si, J., Zhang, Y., Fan, S., Xia, H., & Kong, L. (2021). Nitrate alleviates ammonium toxicity in wheat (*Triticum aestivum* L.) by regulating the tricarboxylic acid cycle and reducing rhizospheric acidification and oxidative damage. *Plant Signaling & Behavior, 16*(12), 1991687.

Dumitru, M., Manea, A., et al. (2011). *Chemical and microbiological analysis methods used in the soil monitoring system*, 2nd ed.; Sitech Publishing House, Craiova, Romania, pp. 54-89.

Frankin, S., Roychowdhury, R., Nashef, K., Abbo, S., Bonfil, D.J., Ben-David, R. (2021). In-Field Comparative Study of Landraces vs. Modern Wheat Genotypes under a Mediterranean Climate. *Plants* 10, 2612.

Grzebisz, W., Zielewicz, W., & Przygocka-Cyna, K. (2023). Deficiencies of Secondary Nutrients in Crop Plants - A Real Challenge to Improve Nitrogen Management. *Agronomy*, *13*(1), 66.

Guo, J., Jia, Y., Chen, H., Zhang, L., Yang, J., Zhang, J., Hu, X., Ye, X., Li, Y., & Zhou, Y. (2019). Growth, photosynthesis, and nutrient uptake in wheat are affected by differences in nitrogen levels and forms and potassium supply. *Scientific Reports, 9*(1), 1248.

Habash, D.Z., Bernard, S., Schondelmaier, J., Weyen, J., Quarrie S.A. (2006), The genetics of nitrogen use in hexaploid wheat: N utili-sation, development and yield. *Theor. Appl. Genet.*, 114: 403–419.

Harari, Y. N. (2015). *Sapiens: A Brief History of Humankind*, 1st ed.; Harper Publishing House Hbrew, Israel, 2015; pp: 75-84.

He, X., Chi, Q., Meng, L., Zhao, C., He, M., Dan, X., Huang, X., Zhao, J., Cai, Z., Zhang, J., & Müller, C. (2022). Plants with nitrate preference can regulate nitrification to meet their nitrate demand. *Soil Biology and Biochemistry*, 165, 108516.

Ijato, T., Porras-Murillo, R., Ganz, P., Ludewig, U., & Neuhäuser, B. (2021). Concentration-dependent physiological and transcriptional adaptations of wheat seedlings to ammonium. *Physiologia Plantarum*, *171*(3), 328–342.

Khan, G.R., Alkharabsheh, H.M., Akmal, M., AL-Huqail, A.A., Ali, N., Alhammad, B.A., Anjum, M.M., Goher, R., Wahid, F., Seleiman, M.F., et al. (2022). Split Nitrogen Application Rates for Wheat (*Triticum aestivum* L.) Yield and Grain N Using the CSM-CERES-Wheat Model. *Agronomy,* 12, 1766.

Lafiandra, D., Shewry, P.R. (2022). Wheat Glutenin Polymers 2. The Role of Wheat Glutenin Subunits in Polymer Formation and Dough Quality. *Journal of Cereal Science*, 106, 103487.

Liu J., Zhang J., Zhu G., Zhu D., Yan Y. (2022). Effects of water deficit and high N fertilization on wheat storage protein synthesis, gluten secondary structure, and breadmaking quality. *The Crop Journal, 10*, 216–223.

Ma Q., Wang M., Zheng G., Yao Y., Tao R., Zhu M., Ding J., Li C., Guo W., Zhu X. (2021). Twice-split application of controlled-release nitrogen fertilizer met the nitrogen demand of winter wheat. *Field Crops Research,* 267: 108163.

Mălinaş, A., Vidican, R., Rotar, I., Mălinaş, C., Moldovan, C.M., Proorocu, M. (2022), Current Status and Future Prospective for Nitrogen Use Efficiency in Wheat (*Triticum aestivum* L.). *Plants*, 11, 217.

Mooshammer, M., Wanek, W., Hämmerle, I., Fuchslueger, L., Hofhansl, F., Knoltsch, A., Schnecker, J., Takriti, M., Watzka, M., Wild, B., et al. (2014). Adjustment of microbial nitrogen use efficiency to carbon: Nitrogen imbalances regulates soil nitrogen cycling. *Nature Communications*; 5: 1–7.

Munteanu, I., Florea, N. (2012). *Romanian System of Soil Taxonomy*. 2nd ed.; Sitech Publishing House, Craiova, Romania, pp. 84-129.

Noor, H., Yan, Z., Sun, P., Zhang, L., Ding, P., Li, L., Ren, A., Sun, M., Gao, Z. (2023). Effects of Nitrogen on Photosynthetic Productivity and Yield Quality of Wheat (*Triticum aestivum* L.). *Agronomy*, 13, 1448.

Obrejanu, G. (1964). *Soil research methods, 1st ed*.; Academy of RPC Publishing House, Bucharest, Romania, pp. 221-246.

Rossini, F., Provenzano, M.E., Sestili, F., Ruggeri, R. (2018). Synergistic Effect of Sulfur and Nitrogen in the Organic and Mineral Fertilization of Durum Wheat: Grain Yield and Quality Traits in the Mediterranean Environment. *Agronomy*, 8, 189.

Sinha, S. K., Kumar, A., Tyagi, A., Venkatesh, K., Paul, D., Singh, N. K., & Mandal, P. K. (2020). Root architecture traits variation and nitrate-influx responses in diverse wheat genotypes under different external nitrogen concentrations. *Plant Physiology and Biochemistry*; 148, 246–259.

Song, L., Li, L., Zhao, L., Liu, Z., Li, X. (2019). Effects of Nitrogen Application in the Wheat Booting Stage on Glutenin Polymerization and Structural-Thermal Properties of Gluten with Variations in HMW-GS at the Glu-D1 Locus. *Foods*, 18; 9(3): 353.

Sułek, A., Cacak-Pietrzak, G. (2018). The influence of production technology on yield selected quality parameters of spring wheat cultivars. Agric. *Sci. Crop Sci. Animal Sci. Res. Rural Develop,* 2, 42–48.

Ukalska-Jaruga, A., Siebielec, G., Siebielec, S., Pecio, M. (2020). The Impact of Exogenous Organic Matter on Wheat Growth and Mineral Nitrogen Availability in Soil. *Agronomy*, 10, 1314.

Veraverbeke, W.S., Delcour, J.A. (2002). Wheat Protein Composition and Properties of Wheat Glutenin in Relation to Breadmaking Functionality. Crit. Rev. *Food Science and Nutrition*, 42, 179–208.

Xiao, C., Fang, Y., Wang, S., & He, K. (2023). The alleviation of ammonium toxicity in plants. *Journal of Integrative Plant Biology*; *65*(6), 1362–1368.

Yadav, M.R., Kumar, S., Lal, M.K., Kumar, D., Kumar, R., Yadav, R.K., Kumar, S., Nanda, G., Singh, J., Udawat, P. et al. (2023). Mechanistic Understanding of Leakage and Consequences and Recent Technological Advances in Improving Nitrogen Use Efficiency in Cereals. *Agronomy*, 13, 527.

Zheng B., Zhang X., Wang Q., Li W., Huang M., Zhou Q., Cai J., Wang X., Cao W., Dai T., et al. (2021). Increasing plant density improves grain yield, protein quality and nitrogen agronomic efficiency of soft wheat cultivars with reduced nitrogen rate. *Field Crops Research*; 267:108145.

Zhong Y., Yang M., Cai J., Wang X., Zhou Q., Cao W., Dai T., Jiang D. (2018). Nitrogen topdressing timing influences the spatial distribution patterns of protein components and quality traits of flours from different pearling fractions of wheat (*Triticum aestivum* L.) grains. *Field Crops Research*, 216: 120–128.

Zhong, Y., Wang, W., Huang, X., Liu, M., Hebelstrup, K.H., Yang, D., Cai, J., Wang, X., Zhou, Q., Cao, W., Dai, T., Jiang, D. (2019). Nitrogen topdressing timing modifies the gluten quality and grain hardness related protein levels as revealed by iTRAQ, *Food Chemistr*y, 277, 135-144.

***https://food.ec.europa.eu/horizontal-topics/farm-fork-strategy_en

***https://unesdoc.unesco.org/ark:/48223/pf0000261424

***https://www.fao.org/faostat/en/#compare

***https://magazin.asro.ro/ro/standard/186772

***https://www.apia.org.ro/files/pages_files/Brosura_Cod__C3.pdf