

EFFECTS OF NITRIC AND AMMONIACAL NITROGEN FERTILIZATION ON THE ZELENY SEDIMENTATION INDEX IN COMMON WHEAT (*Triticum aestivum* L.)

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Abstract

The present study evaluated the effects of nitrogen form and fertilization level on the Zeleny sedimentation quality index of bread wheat (Triticum aestivum L.). A bifactorial experiment was conducted including 27 wheat cultivars and six fertilization regimes combining three nitrogen rates (120, 150, and 170 kg ha⁻¹ N) applied either as nitric or ammoniacal nitrogen, arranged in three replications. Analysis of variance revealed that cultivar, fertilization regime, and their interaction had a highly significant effect ($p < 0.001$) on the Zeleny index. Mean sedimentation values ranged from 44.12 to 60.94 mL among cultivars, while fertilization treatments varied between 48.31 mL under ammoniacal nitrogen and 55.93 mL under nitric nitrogen. Nitric nitrogen consistently produced significantly higher Zeleny index values compared to ammoniacal nitrogen at all fertilization levels. The fertilization regime accounted from the proportion of total variance (2.97%), followed by the cultivar $\times$ fertilization interaction (32.21%) and cultivar effect (28.46%). These results highlight the dominant role of nitrogen form in determining wheat technological quality and emphasize genotype-specific responses to fertilization strategies.

Key words: bread wheat, grain quality, nitrogen form, protein quality, wheat quality, Zeleny sedimentation index.

INTRODUCTION

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 catalyses 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 (Castro et al., 2023). In addition, ammonium toxicity leads to

damage to the chloroplast ultrastructure, which in turn affects photosynthetic activity and plant growth. Nitrogen, which is essential in the formation of chlorophyll, which is stressed when the plant is exposed to excess ammonium (Kong et al., 2022). However, ammonium toxicity can be ameliorated to some extent by preventive strategies. Studies have revealed how ammonium uptake could be mitigated when nitric nitrogen is included in the fertilizer formulation. Wheat plants innately have the mechanism to store unused nitrate in vacuoles, where it is used for plant development, providing a way to mitigate the effect of ammonium toxicity (Wang et al., 2022). High levels of ammoniacal nitrogen in plants have the effect of significantly altering the oxidative metabolism of wheat roots, as well as most of their enzymatic activities. Research has shown that wheat root activity is negatively affected by ammonia nitrogen through reduced activity of key enzymes such as ascorbic acid, phosphoenolpyruvate carboxylase and mannose pyro phosphorylase (Lyu et al., 2022). In addition, NH_4^+ , through its means of disrupting the electron transport chain, has been shown to negatively influence membrane-bound oxidase and thereby increase cellular production of reactive oxygen, damaging photosynthesis, and organs that enhance uptake electrons inhibits the photosynthesis process (Wang et al., 2020). Nitrogen fertilizers used in agriculture include nitrates (salts of nitric acid), ammonium salts, and various organic nitrogen compounds. Nitric nitrogen and ammoniacal nitrogen fertilizers are mostly found on the market in the form of ammonium sulphate and calcium nitrate (Roman et al., 2011). Ammonium sulphate $(\text{NH}_4)_2\text{SO}_4$ is a nitrogen fertilizer containing 21.21% N, characterized by high water solubility and an acidic physiological reaction. Its long-term application may contribute to the acidification of neutral soils. Calcium nitrate $\text{Ca}(\text{NO}_3)_2$ is a readily soluble nitrogen fertilizer with an alkaline physiological reaction, making it suitable for use on acidic soils. It is typically available in hydrated crystalline forms with varying stability and hygroscopic properties. These contrasting properties influence soil reaction and nutrient availability, which are relevant for the present

study (Sala, 2008; Imbrea, 2014; Marschner, 2012).

Moreover, the maximum required amount of plant nutrients occurs in a short period of time from stem elongation to maturity, during which about 80% N, <80% P and <85% K are taken up by plants. Wheat quality indices depend on the variety, climate, and applied agronomic practices. One of the most important factors influencing the baking quality of wheat is nitrogen fertilization, particularly through its effect on protein content and the Zeleny sedimentation index. Zeleny sedimentation values are strongly influenced by the amount of nitrogen applied and its efficient uptake by plants. Numerous studies have shown that bread-making quality indices tend to improve with higher rates of nitrogen fertilization, mainly due to their positive effect on grain protein content and sedimentation values (Ma et al., 2021; Zhong et al., 2018; Kadar et al., 2018; Balla et al., 2019).

Wheat's Zeleny sedimentation index value is directly dependent to a complex of many natural and agrotechnical factors and their mutual connections (cultivated wheat variety, soil type, crop management and weather conditions). Abiotic stress, such as extreme temperatures and water shortages due to climate change, can affect wheat production and wheat baking quality indices worldwide (UNESCO, 2018; FAO, 2024; European Commission, 2024; Mălinaş et al., 2022).

In the context of increasing climatic variability and the occurrence of atypical weather conditions during the wheat growing season, the evaluation of agronomic factors affecting grain technological quality becomes particularly important. Irregular precipitation patterns and temperature fluctuations can influence nitrogen uptake and assimilation, which in turn affects protein accumulation and gluten quality. Under such environmental conditions, the efficiency of nitrogen fertilization and the form in which nitrogen is supplied to the crop may play a crucial role in determining wheat technological quality. However, the response of different wheat cultivars to various nitrogen forms and fertilization levels under atypical climatic conditions remains insufficiently documented. Therefore, the aim of the present study was to evaluate the effect of nitrogen fertilization on

the technological quality of bread wheat, assessed through the Zeleny sedimentation index. Specifically, the study aimed to (i) analyze the influence of different nitrogen forms (nitric and ammoniacal) on sedimentation values, (ii) assess the effect of increasing fertilization levels on gluten quality, and (iii) examine the interaction between wheat cultivars and fertilization regimes under the particular climatic conditions of the experimental year. The results of this research are expected to contribute to a better understanding of the relationship between nitrogen management, genotype response, and environmental conditions, providing useful information for improving wheat quality in modern agricultural systems.

MATERIALS AND METHODS

Site, Soil and Climatic Characteristics

The field experiment was conducted during the 2025–2026 growing seasons at the experimental station in Dudeștii Noi, located in the Western Plain of Romania (45°50'51" N, 21°06'30" E; altitude 87 m). This region represents one of the country's most important agricultural areas and belongs to the forest–steppe climatic zone. The area is characterized by relatively shallow groundwater levels and heterogeneous parent materials, which contribute to a diverse pedological background.

The geological evolution of the region is associated with the retreat of the Pannonian Lake, which resulted in extensive sediment accumulation. These deposits include coarse materials transported by the unstable bed of the Mureș River. The parent materials underlying the soils in the Dudeștii Noi area consist mainly of quartz sands with variable calcium carbonate content and medium-textured loess-like deposits. In certain micro depression areas, the influence of shallow groundwater has promoted salinization and alkalization processes.

The experimental plots were established on a wet phreatic cambic chernozem, slightly leached and characterized by a clay-loam texture, developed on fluvial carbonate deposits of medium to fine granulometry. The soil profile is well developed and shows relatively uniform physical, hydro physical, and chemical properties. Cambic chernozems are generally

characterized by high biological activity and good agricultural productivity. According to Crista (2017), these soils are typically deep and formed from transported and redeposited aeolian materials, such as loess and carbonate loess-like sediments.

The soil profile includes the following horizons: Ap (0–15 cm), Am (15–35 cm), Bv (35–60 cm), and Ck (60–100 cm) (Munteanu, 2012). The upper horizons are dark brown to dark gray brown in colour, with clay-loam to loam textures and well-developed granular structures that favour root penetration and aeration. The Bv horizon exhibits a subangular blocky structure and moderate compaction, whereas the Ck horizon consists of carbonate-rich parent material with a well-developed blocky structure.

Physical and Hydro physical Properties

The soil has a clay-loam texture, with silty-clay characteristics in the upper 70 cm and silty-loam features in the deeper layers. Bulk density values indicate moderate compaction throughout the soil profile, with 1.46 g cm⁻³ in the Ap horizon and 1.58 g cm⁻³ in the Am horizon. Total porosity is moderate in the Ap horizon (48.8%) and higher in the Am horizon (58.2%). Air-filled porosity shows moderate values in the Ap horizon (15.2%), higher values in the Am (23.8%) and Bv horizons (21.7%), and lower values in the Ck horizon (12.2%).

Clay content ranges from 33.4% to 40.3%, with the highest proportion recorded in the Am horizon. Slightly lower values were observed in the deeper Bv (33.8%) and Ck (33.4%) horizons. Hydro physical indices reflect these textural characteristics. The wilting coefficient ranged from 11.7% in the Ap and Am horizons to 13.2% in the Bv horizon, while the field capacity remained relatively uniform across the profile, with values of 23.3% in the Ap, Am, and Bv horizons and 22.4% in the Ck horizon (Obreja, 1964).

Chemical Properties

Soil chemical properties were determined using the Kjeldahl method for total nitrogen and the Olsen method for available phosphorus (Dumitru et al., 2011). Humus content was 3.04% in the Ap horizon, decreasing to 1.78% in the Am horizon and 1.24% in the Bv horizon. Total nitrogen content in the Ap horizon was

0.172%, indicating a moderate nitrogen supply. The soil was very well supplied with available phosphorus (168 ppm in the Am horizon) and well supplied with available potassium (248 ppm).

Soil reaction was measured potentiometrically in a 1:2.5 soil-to-water suspension, indicating a slightly acidic reaction in the Ap horizon (pH 5.58) and a slightly alkaline reaction in deeper horizons (up to pH 7.66). Exchangeable bases showed moderate values (23.2 cmol kg⁻¹ in Ap and 24.8 cmol kg⁻¹ in Am), while the base saturation degree reached approximately 92.6%, increasing slightly toward the Bv horizon. Regarding micronutrients, zinc concentration was relatively high in the Ap horizon (176 ppm) but extremely low in the Bv horizon (0.40 ppm).

Weather conditions during the experimental period

Climatic conditions recorded during the experimental period (October 2025-July 2026) were compared with the long-term climatic averages for the Timișoara meteorological station, located near the experimental field. (Figure 1). The regional climate is characterized as temperate continental with sub-Mediterranean influences, typical for the western part of Romania.

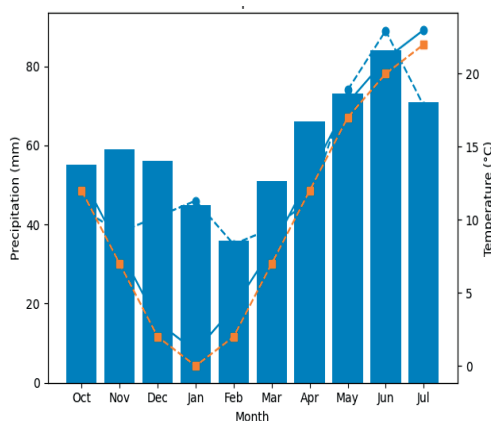


Figure 1. The average monthly climograph temperatures (°C) and rainfall (mm) recorded in Dudeștii Noi, (2025-2026)

The mean air temperature during the study period followed the typical seasonal pattern of the region, with a gradual decrease from autumn toward winter and a subsequent increase during

spring and summer. The lowest temperatures were recorded during January, while the highest values occurred in July. Compared with the multiannual average, temperatures during the experimental period showed slightly higher values during late spring and summer, which are favourable for crop growth and physiological activity.

Precipitation distribution during the experimental period showed moderate seasonal variability. Rainfall amounts were relatively moderate during autumn and winter months, while a significant increase occurred during late spring and early summer. The highest precipitation levels were recorded in June, consistent with the long-term climatic pattern of the region, when convective rainfall events are most frequent.

Overall, the climatic conditions recorded during the study period were close to the multiannual averages for the region, providing favourable moisture and thermal regimes for crop development.

Experimental Design and Plant Material

The biological material consisted of 27 winter wheat (*Triticum aestivum* L.) cultivars originating from breeding programs in Romania, Germany, and France. These cultivars are among the most widely cultivated in the Western Plain of Romania and were selected based on several agronomic criteria, including high yield potential, tolerance to major diseases and pest pressure, and favourable technological characteristics related to milling and baking quality.

The field experiment was arranged using a stratified randomized block design in order to minimize the influence of environmental heterogeneity and to ensure a reliable estimation of nitrogen utilization efficiency. Cultivars and fertilization treatments were factorially combined and distributed in randomized blocks with three replications.

Each experimental plot measured 1.2 m × 7 m, while the distance between neighbouring plots and adjacent blocks was 0.5 m and 1.5 m, respectively. A 2.5 m buffer strip was maintained between experimental units to reduce border effects. Each block contained 27 experimental plots, corresponding to the tested cultivars.

The study was based on a three-factor experiment arranged in subdivided plots, including 27 wheat cultivars and three nitrogen fertilization levels. The experimental factors were defined as follows:

Factor A - Wheat cultivar

Dacic, Miranda, Alex, Litera, Ciprian, Crișana, Biharia, Glosa, Bohemia, Sothys, Sacramento, Rubisko, Certiva, Aurelius, Aspect, Papillon, Activus, Centurion, Tika Taka, Cheignon, Sosthene, Vivendo, Sophie, Solindo, Tiberius, Arrezo, and Apexus.

Factor B - Nitrogen fertilizer form

b1 - ammoniacal nitrogen

b2 - nitric nitrogen

Factor C - Nitrogen fertilization rate

c1 - 120 kg N ha⁻¹

c2 - 150 kg N ha⁻¹

c3 - 170 kg N ha⁻¹

The nitrogen application rates were established considering the maximum allowable nitrogen input of 170 kg N ha⁻¹, according to the regulations of the Romanian Agency for Payments and Intervention in Agriculture (APIA, 2021). The lowest fertilization rate (120 kg N ha⁻¹) was selected based on soil fertility indicators, including humus content and available soil nitrogen, as well as typical nitrogen requirements for winter wheat cultivation.

Nitrogen was supplied in two chemical forms: ammoniacal nitrogen applied as ammonium sulphate (NH₄)₂SO₄ and nitric nitrogen applied as calcium nitrate Ca(NO₃)₂. Fertilizers were applied as a single, non-fractionated dose at the beginning of stem elongation (BBCH growth stage 30-31).

During both years of the study, the seeding rate for each cultivar was calculated based on seed purity, germination capacity, and thousand kernel weight, resulting in a sowing density of 550 viable seeds m⁻². Wheat was sown at a depth of 4 cm, with a row spacing of 12.5 cm.

The fertilization treatments were defined as follows:

V1 - nitric nitrogen, 120 kg N ha⁻¹

V2 - nitric nitrogen, 150 kg N ha⁻¹

V3 - nitric nitrogen, 170 kg N ha⁻¹

V4 - ammoniacal nitrogen, 120 kg N ha⁻¹

V5 - ammoniacal nitrogen, 150 kg N ha⁻¹

V6 - ammoniacal nitrogen, 170 kg N ha⁻¹

The control treatment (Mt) corresponded to the mean value of the experimental field.

Determination of the Zeleny Sedimentation Index

✓ Principle of the Method

The method is based on the formation of a suspension of wheat flour in a lactic acid solution in the presence of bromophenol blue solution. In this acidic environment, the gluten proteins hydrate and swell, forming aggregates that settle during the sedimentation period. The resulting sediment volume is measured after a fixed time interval and is used to estimate the gluten strength and protein quality of the flour. The Zeleny sedimentation value is influenced by several factors, including the total protein content of the flour, the qualitative characteristics of gluten proteins, and the hardness of the wheat kernels. As a result, the test is commonly used in cereal quality evaluation and wheat breeding programs to estimate the technological quality of wheat.

Apparatus

The determination of the sedimentation index was performed using equipment compliant with ISO 5529:2007, including:

- a special four-roll mill designed for preparing wheat samples for the Zeleny sedimentation test (LM 3310 PerkinElmer);
- a metal sieve with a mesh size of 150 μm used for flour standardization;
- a four-position tube shaker equipped with a timer for controlled agitation;
- a sedimentation unit provided with a timer to ensure standardized sedimentation conditions;
- glass sedimentation tubes (flat-bottom cylinders with plastic stoppers), graduated in cm³, with a capacity of 100 cm³;
- an analytical balance with a precision of 0.01 g.

✓ Reagents

The following reagents were used during the analysis:

- bromophenol blue solution, used as a dye indicator to facilitate the visualization of sediment formation;
- lactic acid solution 2% (v/v), which provides the acidic medium necessary for gluten swelling and sediment formation.

All reagents were prepared according to the specifications indicated in the reference standards.

✓ Methodology

Prior to analysis, the wheat sample was cleaned to remove large mechanical impurities and broken kernels. This was carried out by manual

sieving using a sieve with openings of 20 mm × 1 mm. The cleaned sample was subsequently milled using the LM 3310 PerkinElmer mill, ensuring a uniform particle size suitable for the sedimentation test.

The resulting flour was then sieved for 1.5 minutes through a 150 µm metal sieve, in accordance with the requirements of ISO 5529:2007, to obtain a standardized flour fraction for analysis. A sample of 3 g flour was weighed with an analytical balance to an accuracy of 0.01 g and transferred into sedimentation test tubes. Subsequently, 50 mL of bromophenol blue solution were added to each tube. The tubes were sealed with plastic stoppers, and the contents were mixed by manually shaking the tubes horizontally for 5 seconds to ensure homogeneous dispersion of the flour particles.

The tubes were then placed in a mechanical shaker and agitated for 5 minutes. After this initial agitation period, 25 mL of lactic acid solution (2% (v/v)) were added to each tube. The tubes were returned to the shaker and agitated for an additional 5 minutes, ensuring proper interaction between the flour suspension and the acidic medium.

Following agitation, the tubes were placed in the sedimentation unit and allowed to stand undisturbed to permit sediment formation. After a sedimentation period of 15 minutes, the sediment volume was read directly from the graduated scale of the tube and expressed in millilitres (mL). This value represents the Zeleny sedimentation index.

✓ Calculation of Results

For each sample, two parallel determinations were performed under identical experimental conditions in order to ensure the reliability and reproducibility of the results. The final sedimentation index was calculated as the arithmetic mean of the two measurements and expressed as a whole number.

RESULTS AND DISCUSSIONS

The Zeleny sedimentation index is a widely used parameter for assessing the quantity and quality of gluten in wheat flour. It is expressed as the volume (mL) of sediment obtained from a suspension of flour in lactic acid solution after a standardized sedimentation period. The sedimentation value reflects the swelling

capacity of gluten proteins in an acidic medium and is therefore considered an indirect indicator of the baking quality of wheat flour (Hrušková et al., 2003; Kibkalo, 2022).

Higher sedimentation values are generally associated with increased protein content and stronger gluten network formation, leading to improved dough strength and baking performance. Conversely, lower values indicate weaker gluten and reduced baking quality. The Zeleny sedimentation value is positively correlated with grain protein content and gluten composition, particularly glutenin fractions, which are key determinants of bread volume and technological quality (Guerrini et al., 2020).

Thus, the sedimentation test provides an integrated assessment of gluten quantity and quality, including gluten resistance, beyond indices that evaluate these attributes separately (Hrušková et al., 2003; Kibkalo, 2022). From a practical perspective, sedimentation values between 20-34 mL indicate satisfactory flour quality, 35-50 mL indicate good quality, while values above 50 mL are characteristic of excellent baking performance (Jankowski et al., 2015).

The experimental data were statistically analysed using analysis of variance (ANOVA) to evaluate the effects of the studied factors and their interactions. When significant differences among treatments were detected, Student's t-test was applied to compare the means. Statistical significance was considered at $p \leq 0.05$.

F test (Table 1, p column), shows that: A factor (variety), B factor (level of fertilization) and their interaction $A \times B$ had very significant action, meaning: wet gluten content differences between varieties are very significant; there are very significant differences between the 6 fertilization systems; $A \times B$ interaction has very significant action. Hence, the 27 winter wheat varieties reacted differently; within the 6 agrofunds, in terms of wet gluten content, the values obtained differ significantly between them. In conclusion, the null hypothesis H_0 is rejected for factor A (variety), factor B (level of fertilization) and the $A \times B$ interaction. To examine the differences between the different varieties, the differences between the agrofunds, as well as the interaction $A \times B$ (variety $\times$ agrofund) we apply the t-test and we will use as the error variant: $s^2 = 3.6$, degrees of freedom = 324.

Table 1. Analysis of variance

Variation source	SSP (SP)	Degree of Freedom	Weighted Least of Squares WSL (s ²)	F test for s ² error		
				Value	P	Significance
A (variety)	7248	26	279	63,6	0,0000	***
B (agrofund)	18511	5	3702	844,7	0,0000	***
A×B	10867	130	84	19,1	0,0000	***
Error	1420	324	4			
Total	38045					

*, **, or *** indicate statistically significant differences between sample means and Mt based on t-test, at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, 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 $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively, but with negative values.

The Zeleny sedimentation value achieved using the 27 winter wheat varieties is shown in Table 2. Compared to the control - the average of the experience, the following values of the sedimentation index were obtained: the highest Zeleny sedimentation values were recorded in the varieties Tika Taka (60.94 mL), Tiberius and Ciprian (approximately 58 mL), Aurelius, Cheignon, and Sophie, followed by Crișana,

Biharia, and Arrezo (around 54-55 mL), all exceeding the threshold of 50 mL associated with very good baking quality. In contrast, the lowest values were observed in Papillon (44.12 mL), Centurion (45.57 mL), Rubisko, and Sothys (approximately 46 mL), indicating comparatively weaker gluten strength. The remaining varieties showed intermediate values without significant differences.

Table 2. Influence of variety on winter wheat Zeleny sedimentation value

Measured Parameter	Zeleny sedimentation value / Significance		Difference
Variety	mL		%
a1 – Dacic	54.26/**		1.7
a2 – Miranda	51.18/0		-1.4
a3 – Alex	54.39/**		1.8
a4 – Litera	51.05/0		-1.5
a5 – Ciprian	57.78/**		5.2
a6 – Crișana	55.44/**		2.9
a7 – Biharia	55.29/**		2.7
a8 – Glossa	50.93/00		-1.7
a9 – Boema	52.80/NS		0.2
a10 – Sothys	46.38/000		-6.2
a11 – Sacramento	48.58/000		-4.0
a12 – Rubisko	45.77/000		-6.8
a13 – Certiva	48.97/000		-3.6
a14 – Aurelius	57.18/**		4.6
a15 – Aspekt	53.38/ns		0.8
a16 – Papillon	44.12/000		-8.5
a17 – Activus	53.28/NS		0.7
a18 – Centurion	45.57/000		-7.0
a19 – Tika Taka	60.94/**		8.4
a20 – Cheignon	56.94/**		4.4
a21 – Sosthene	49.26/000		-3.3
a22 – Vivendo	52.95/NS		0.4
a23 – Sophie	56.87/**		4.3
a24 – Solindo	52.84/NS		0.3
a25 – Tiberius	57.85/**		5.3
a26 – Arrezo	54.59/**		2.0
a27 – Apexus	51.38/0		-1.2
Average	52.59	Cv	
LSD 5% = 1.17; LSD 1% = 1.54; LSD 0.1% = 1.96			

*, **, or *** indicate statistically significant differences between sample means and Mt based on t-test, at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, 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 $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively, but with negative values.

The Zeleny sedimentation value achieved using the nitric and ammoniacal nitrogen fertilization is shown in Table 3. Compared to the control group - the average of the experience, very significant increases were obtained at the all of the 6 treatments. The highest value of this index was obtained at V1 of 55.93 mL, followed by V3 - 55.88 mL and V2 - 55.66 mL. Ammoniacal nitrogen fertilization levels ranked below the experience average.

Relative to the experimental mean (control), the Zeleny sedimentation index showed both positive and negative statistically significant deviations (Table 4), indicating that certain cultivars recorded values either above or below the trial average. This variability highlights the differential response of wheat genotypes to nitrogen fertilization regimes.

Table 3. Influence of nitrogen fertilizers on winter wheat Zeleny sedimentation value

Measured Parameter	Zeleny sedimentation value / Significance	Difference
Variant	mL	%
V1	55.93/***	3.34
V2	55.66/***	3.07
V3	55.88/***	3.29
V4	48.31/000	-4.28
V5	49.18/000	-3.41
V6	50.57/000	-2.02
Average	52.59	Cv
LSD 5% = 0.584; LSD 1% = 0.769; LSD 0.1% = 0.981		

*, **, or *** indicate statistically significant differences between sample means and Mt based on t-test, at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, 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 $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively, but with negative values.

Table 4. Influence of variety $\times$ nitrogen fertilizers interaction on winter wheat Zeleny sedimentation value

Variety	Zeleny sedimentation value and significations											
	V 1		V 2		V 3		V 4		V 5		V 6	
	SV	Diff.	SV	Diff.	SV	Diff.	SV	Diff.	SV	Diff.	SV	Diff.
a1- Dacic	57.05	4.5**	58.80	6.2***	57.80	5.2***	47.60	-5.0 ⁰⁰⁰	49.25	-3.3 ⁰	55.05	2.5
a2- Miranda	53.55	1.0	52.65	0.1	55.35	2.8	47.90	-4.7 ⁰⁰	51.85	-0.7	45.75	-6.8 ⁰⁰⁰
a3- Alex	57.05	4.5**	59.15	6.6***	61.55	9.0***	41.25	-11.3 ⁰⁰⁰	50.95	-1.6	56.40	3.8
a4- Litera	56.80	4.2**	49.50	-3.1 ⁰	51.85	-0.7	42.25	-10.3 ⁰⁰⁰	58.55	6.0***	47.35	-5.2 ⁰⁰⁰
a5- Ciprian	64.35	11.8***	58.05	5.5***	60.95	8.4***	50.55	-2.0	56.70	4.1**	56.05	3.5*
a6- Crišana	60.70	8.1***	54.50	1.9	56.80	4.2**	56.00	3.4*	48.70	-3.9 ⁰⁰	55.95	3.4*
a7- Biharia	59.55	7.0***	65.00	12.4***	56.40	3.8**	47.15	-5.4 ⁰⁰⁰	47.90	-4.7 ⁰⁰	55.75	3.2*
a8- Glossa	55.80	3.2*	53.65	1.1	55.25	2.7	45.20	-7.4 ⁰⁰⁰	47.20	-5.4 ⁰⁰⁰	48.45	-4.1 ⁰⁰
a9- Boema	55.70	3.1*	56.15	3.6*	56.50	3.9**	49.00	-3.6 ⁰	49.65	-2.9 ⁰	49.80	-2.8
a10- Sothys	51.55	-1.0	48.50	-4.1 ⁰⁰	49.15	-3.4 ⁰	44.75	-7.8 ⁰⁰⁰	41.85	-10.7 ⁰⁰⁰	42.45	-10.1 ⁰⁰⁰
a11- Sacramento	53.45	0.9	49.00	-3.6 ⁰	52.05	-0.5	47.50	-5.1 ⁰⁰⁰	44.00	-8.6 ⁰⁰⁰	45.50	-7.1 ⁰⁰⁰
a12- Rubisko	49.10	-3.5 ⁰	50.95	-1.6	49.40	-3.2 ⁰	40.45	-12.1 ⁰⁰⁰	43.90	-8.7 ⁰⁰⁰	40.80	-11.8 ⁰⁰⁰
a13- Certiva	51.75	-0.8	52.50	-0.1	51.10	-1.5	45.55	-7.0 ⁰⁰⁰	44.15	-8.4 ⁰⁰⁰	48.75	-3.8 ⁰⁰
a14- Aurelius	62.00	9.4***	58.35	5.8***	60.30	7.7***	55.75	3.2*	51.20	-1.4	55.45	2.9*
a15- Aspekt	58.55	6.0***	59.00	6.4***	57.05	4.5**	48.65	-3.9 ⁰⁰	46.85	-5.7 ⁰⁰⁰	50.20	-2.4
a16- Papillon	48.65	-3.9 ⁰⁰	47.10	-5.5 ⁰⁰⁰	41.30	-11.3 ⁰⁰	43.30	-9.3 ⁰⁰⁰	41.30	-11.3 ⁰⁰⁰	43.05	-9.5 ⁰⁰⁰
a17- Activus	56.00	3.4*	58.30	5.7***	56.30	3.7*	51.45	-1.1	45.35	-7.2 ⁰⁰⁰	52.25	-0.3
a18- Centurion	49.15	-3.4 ⁰	45.65	-6.9 ⁰⁰⁰	51.25	-1.3	39.30	-13.3 ⁰⁰⁰	44.20	-8.4 ⁰⁰⁰	43.85	-8.7 ⁰⁰⁰
a19- Tika Taka	66.30	13.7***	65.70	13.1***	60.60	8.0***	57.80	5.2***	56.10	3.5*	59.15	6.6***
a20- Chevignon	61.05	8.5***	59.90	7.3***	61.35	8.8***	56.55	4.0**	50.80	-1.8	52.00	-0.6
a21- Sosthene	51.95	-0.6	51.25	-1.3	56.35	3.8**	42.50	-10.1 ⁰⁰⁰	43.60	-9.0 ⁰⁰⁰	49.90	-2.7
a22- Vivendo	52.80	0.2	54.80	2.2	52.30	-0.3	51.75	-0.8	52.45	-0.1	53.60	1.0
a23- Sophie	44.10	-8.5 ⁰⁰⁰	61.05	8.5***	66.95	14.4***	56.55	4.0**	57.60	5.0***	54.95	2.4
a24- Solindo	58.35	5.8***	58.35	5.8***	57.55	5.0***	47.05	-5.5 ⁰⁰⁰	47.25	-5.3 ⁰⁰⁰	48.50	-4.1 ⁰⁰
a25- Tiberius	62.80	10.2***	62.60	10.0***	59.80	7.2***	54.30	1.7	54.20	1.6	53.40	0.8
a26- Arrezo	57.70	5.1***	58.85	6.3***	61.05	8.5***	46.50	-6.1 ⁰⁰⁰	50.65	-1.9	52.80	0.2
a27- Apexus	54.40	1.8	53.45	0.9	52.40	-0.2	47.90	-4.7 ⁰⁰	51.75	-0.8	48.35	-4.2 ⁰⁰
Average	52,59											
DL 5% = 2.86; DL 1% = 3.76; DL 0.1% = 4.80												

*, **, or *** indicate statistically significant differences between sample means and Mt based on t-test, at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, 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 $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively, but with negative values.

Under nitric nitrogen fertilization, several cultivars consistently exhibited sedimentation index values exceeding the experimental mean. At the fertilization level of 120 kg N ha⁻¹, the cultivars Dacic, Alex, Litera, Ciprian, Crișana, Biharia, Glossa, Boema, Aurelius, Aspect, Activus, Tika Taka, Chevignon, Solindo, Tiberius, and Arrezo recorded values above the experimental average. Increasing the nitrogen rate to 150 kg N ha⁻¹ resulted in above-average sedimentation index values for Dacic, Litera, Ciprian, Biharia, Boema, Aurelius, Aspect, Activus, Tika Taka, Chevignon, Sophie, Solindo, Tiberius, and Arrezo. At the highest fertilization level (170 kg N ha⁻¹), the cultivars Dacic, Alex, Crișana, Biharia, Boema, Aurelius, Aspect, Activus, Tika Taka, Chevignon, Sosthene, Sophie, Solindo, Tiberius, and Arrezo maintained sedimentation index values above the experimental mean. In contrast, under ammoniacal nitrogen fertilization, a smaller number of cultivars exceeded the experimental average. At 120 kg N ha⁻¹, the cultivars Crișana, Aurelius, Tika Taka, Chevignon, and Sophie showed higher sedimentation index values than the trial mean. At 150 kg ha⁻¹, only Litera, Ciprian, and Sophie recorded above-average values, while at 170 kg N ha⁻¹ the cultivars Ciprian, Crișana, Biharia, Aurelius, and Tika Taka exceeded the experimental mean. Overall, the results indicate that nitric nitrogen fertilization generally promoted higher Zeleny sedimentation index values compared with ammoniacal nitrogen, suggesting a stronger positive effect on gluten quality and wheat technological performance.

The analysis of variance indicates that the variability of the Zeleny sedimentation index is primarily determined by the combined influence of genetic factors and their interaction with fertilization practices. The relatively high proportion of variance attributed to the genotype × fertilization interaction (32.21%) suggests that the response of wheat cultivars to nitrogen supply is strongly genotype-specific. This highlights the importance of considering cultivar-dependent responses when designing fertilization strategies aimed at improving technological grain quality.

The genotype effect itself accounted for 28.46% of the total variance, confirming the major role of genetic background in controlling gluten

strength and protein quality, which are key determinants of sedimentation capacity.

Although the direct contribution of the fertilization regime was comparatively small (2.97%), its interaction with genotype demonstrates that nitrogen fertilization remains an important agronomic factor capable of enhancing grain quality when applied in combination with well-adapted cultivars. The relatively high proportion of unexplained variance (36.37%) may be associated with environmental variability during the experimental period, particularly under atypical climatic conditions that can influence nitrogen uptake, protein synthesis, and gluten formation (Figure 2).

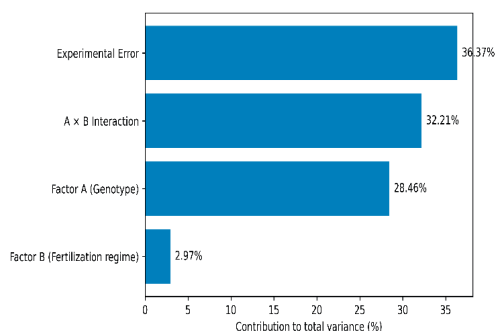


Figure 2. Contribution of experimental factors to total variance of the Zeleny sedimentation index

Overall, these findings emphasize that the technological quality of wheat, expressed through the Zeleny sedimentation index, results from a complex interaction between genetic potential, nitrogen management, and environmental conditions. Consequently, improving wheat quality requires an integrated approach that combines cultivar selection with optimized fertilization practices adapted to specific agroecological conditions.

CONCLUSIONS

The results of the present study highlight the complex interaction between nitrogen fertilization, genetic background, and environmental conditions in determining the technological quality of bread wheat, as expressed through the Zeleny sedimentation index. The experimental data obtained under the agro-climatic conditions of the Western Plain of

Romania revealed considerable variability among cultivars and fertilization treatments.

The analysis of variance showed that the genotype $\times$ fertilization interaction was the main source of variability for the sedimentation index, accounting for 32.21% of the total variance, followed by the genotype factor, which contributed 28.46%. In contrast, the direct effect of fertilization regime was relatively limited, accounting for only 2.97% of the total variance. The relatively high experimental error, 36.37%, suggests that environmental conditions during the experimental year also had a substantial influence on wheat quality expression.

Nitrogen form clearly affected wheat technological quality. Across fertilization levels, nitric nitrogen produced more favorable sedimentation responses than ammoniacal nitrogen, as reflected by the higher number of cultivars exceeding the experimental mean. This effect was particularly evident at the rate of 120 kg ha⁻¹, where sixteen cultivars exceeded the mean under nitric nitrogen, compared with only five under ammoniacal nitrogen. These results indicate that nitric nitrogen had a stronger positive influence on gluten quality under the conditions of this study.

Although increasing nitrogen fertilization generally improved sedimentation values, the magnitude of response differed among cultivars. Several genotypes, including Dacic, Boema, Aurelius, Activus, Tika Taka, Chevignon, Solindo, and Tiberius, consistently recorded values above the experimental mean under multiple fertilization treatments. This behavior indicates stable technological performance and a strong genetic capacity for protein and gluten formation.

These findings are consistent with previous research showing that nitrogen fertilization influences grain protein content and gluten quality, both of which are closely associated with the Zeleny sedimentation index (Zeleny, 1947; Triboi & Triboi-Blondel, 2002; Gooding et al., 2007). The Zeleny test remains a reliable indicator of gluten strength and baking quality and is widely used in wheat quality assessment and breeding programs (Wrigley et al., 2006; Wieser, 2007). The genotype-dependent response to nitrogen fertilization observed in this study has also been reported by other authors (Slafer et al., 2014; Zörb et al., 2018).

Overall, the improvement of wheat technological quality requires an integrated approach that combines appropriate nitrogen management with the selection of cultivars capable of efficiently using nitrogen for protein and gluten synthesis. Under the conditions of this study, nitric nitrogen fertilization proved more effective than ammoniacal nitrogen in enhancing the Zeleny sedimentation index.

The main findings can be summarized as follows:

- The genotype $\times$ fertilization interaction was the strongest determinant of variation in the Zeleny sedimentation index, confirming the importance of genotype-specific responses to nitrogen management.
- Nitric nitrogen was more effective than ammoniacal nitrogen in improving sedimentation values, especially at higher fertilization levels.
- Cultivars such as Dacic, Boema, Aurelius, Activus, Tika Taka, Chevignon, Solindo, and Tiberius showed stable above-average technological performance.
- Environmental conditions contributed substantially to the observed variability, indicating that wheat quality expression is strongly influenced by year-specific agro-climatic factors.
- Optimizing wheat grain quality requires combining cultivar selection with suitable nitrogen fertilization strategies rather than relying on fertilization level alone.

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