

Elements of nitrogen metabolism based on the nitrogen uptake of common wheat (*Triticum aestivum* L.) cultivars grown in a four-field crop rotation

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Abstract

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In the period 2018–2020, characteristics of nitrogen metabolism in five cultivars of common winter wheat were studied. The experiment was conducted in a four-field crop rotation after the predecessors rapeseed, peas, sunflower and corn. Mineral fertilization was carried out with increasing nitrogen rates of 0, 60, 120 and 180 kg N/ha on a background of P₂O₅ 60 kg/ha and K₂O 60 kg/ha. Nitrogen uptake in wheat organs is mainly controlled by mineral fertilization. The share of influence reaches 36.15 percent for total biomass and 31.47 percent for nitrogen absorbed in grain. Increasing nitrogen rates leads to a decrease in the efficiency of nitrogen utilization from 45.07 to 34.85 kg grain per kg of nitrogen absorbed from total biomass. The type of predecessor also affects the nitrogen conversion capacity. The strongest negative effect is reported after the predecessor rapeseed.

Keywords: wheat cultivars; elements of nitrogen metabolism; predecessor; fertilization

Introduction

The series of challenges facing agriculture (growing food demands, unreasonably applied practices, abrupt climate changes, soil degradation and desertification, reduction of arable land, etc.) requires solutions to several problems related to the use of specific agricultural production systems. Stabilization and sustainable intensification are imperative, mainly connected with the way agricultural production is managed. This is possible only based on a thorough understanding of the potential of soil fertility, the characteristics of the agro-ecological region, and their complex interaction with the agricultural practices applied to the cultivated crops (Reddy and Reddy, 2019; Ghafoor et al., 2021; Ahmed et al., 2022).

Building and maintaining a sustainable agricultural production system inevitably requires reduced intensification

or even a complete transition to organic production. Global climate change requires that crop cultivation methods be thoroughly studied and optimized. Regardless of the production system – organic or conventional – all agronomic approaches must be adapted to the specific soil and climatic conditions of the region (Campiglia et al., 2015).

Wheat yields and the quality of the final product depend on the overall management of the production process and on the genotype, as part of complex agronomy practices and their interaction. Low soil fertility, particularly nitrogen (N) deficiency, is one of the main factors limiting wheat production. High levels of N supply lead to increased protein content, but improved efficiency of its utilization is achieved only when protein concentration rises, and grain yield remains stable (Ortiz-Monasterio et al., 1997; Peng et al., 2022).

According to Iqtidar et al. (2006), wheat provides more protein than any other cereal crop in the world. However,

the global challenge facing crop nutrition is to increase grain yield while maintaining the same level of its protein (Tilman et al., 2002).

As early as 1991 Papakosta and Gagianas (1991) reported that 50 to 95% of the nitrogen present in the grain at harvest is remobilized from the leaves, stem, and the non-grain part of the spike. Studies by Gaju et al. (2014) demonstrated increased nitrogen remobilization under nitrogen limitation or water deficiency during grain filling, as stress limits nitrogen uptake after flowering, forcing the plant to remobilize stored nitrogen to the grains.

The use of nitrogen fertilizers over the past few decades has far exceeded the amount of nitrogen that wheat can absorb. This, in turn, caused a significant reduction in the nitrogen efficiency of wheat in terms of productivity and quality (de Silva et al., 2020). In modern wheat varieties grown under favorable conditions, more than 50% of the aboveground biomass is allocated to the grain, i.e. the harvest index (HI) is more than 0.50 (Maeoka et al., 2020).

In this regard, nitrogen use efficiency (NUE) is a complex characteristic, depending on its uptake (NUp) and its efficiency (NUpE) and use/utilization (NUt) and utilization efficiency (NUtE) (Moll et al., 1982; Fiez et al., 1995; Ortiz-Monasterio et al., 1997; Hitz et al., 2017; Rawal et al., 2022). Essentially, these indices, on the one hand, reflect the ability of the plants to absorb and accumulate nitrogen in their organs, and on the other hand, the efficiency of their utilization, i.e. the ability of the plant to utilize the absorbed nitrogen to the grain (Delogu et al., 1998). It was found that NUtE in wheat varies depending on the level of soil nitrogen supply, including cases of foliar application. This result suggests that the efficiency of N utilization and distribution from the vegetative parts of the plant to the grain is influenced by the contribution of each crop organ during different stages of development to the parameters characterising its metabolism.

Tran and Tremblay (2000), using ^{15}N , found that the timing of nitrogen application plays a significant role in the amount of NUpE in wheat. It is lower when applied early compared to when applied in the later stages of crop growth and development. Studies have shown that the amount of economic yield can increase with effective N application, which leads to a decrease in N losses from the soil-plant system, by increasing nitrogen uptake efficiency (NUpE) and its translocation efficiency (NUtE) (Muurinen et al., 2007).

Fundamental research shows that cultivars differ in nitrogen use efficiency (NUE) as a result of differences in nitrate absorption (Rodgers and Barneix, 1988) and the remobilization of N (van Sanford and Mac Kown, 1986; Jariwala et al., 2022). However, modern breeding often develops without considering the cultivars' ability to grow and produce yield

in soils with a lower level of fertility, and they are developed for high productivity when grown under high agronomic conditions (Wuebbles, 2009; Govindasamy et al., 2023).

The interaction of environmental factors with genetic factors is a complex characteristic regarding nitrogen use efficiency (Lupini et al., 2021). This interaction, when grown in different conditions, is capable of provoking unstable and therefore unsatisfactory results (Getahun et al., 2022).

Nitrogen use efficiency is a key trait associated with nitrogen metabolism and a major objective in crop research. Current studies address this topic through two main approaches: optimization of agronomic practices in conventional production systems and breeding of cultivars with improved nutrient uptake and utilization, particularly of nitrogen (Fixen et al., 2014; Fixen et al., 2015; Cormier, 2015; Cormier et al., 2016; Ren et al., 2017; Ali et al., 2025).

Even in 1992, Feil indicated that varieties producing large amounts of biomass have more NUpE, which may reduce NUtE (Feil, 1992). In turn, the overall level of NUE is thus decreased in modern varieties.

The varieties differed also by their ability to transport nitrogen to the grain (Singh and Arora, 2001), which depends on the grain harvest index (GHI) and the ability to produce more biomass from 1 kg of nitrogen input. However, breeding for a higher GHI may reduce grain N content, as protein reduction is associated with high NUtE (Ortiz-Monasterio et al., 1997; Feil et al., 1997). At the same time, the authors pointed out that the efficiency of production from nitrogen biomass (NBPE) was slightly changed in the long selection process.

High-yielding varieties are desired for both agricultural systems, briefly characterized as low and high levels of agronomy practices. Nitrogen utilization is important in both cases, as higher yields are obtained with the same amount of N absorbed by the plant, which is affected by the weather conditions (Ferreira et al., 2021). This means that in both cases, more nitrogen uptake is important, but for different reasons. In intensive, high-cost farming, high nitrogen uptake efficiency (NUpE) avoids the leaching of nitrogen absorbed, but not transported to the grain, which is beneficial for sustainable agriculture. In low-cost farming, high nitrogen uptake efficiency (NUpE) is also desirable (van Ginkel et al., 2001). In their research, the authors found that the breeding of materials with increased ability to absorb nitrogen is more successful in soils with low nitrogen availability, while for increased translocation of nitrogen to the grain, high soil nitrogen availability is desirable.

The main objective of the study is to determine changes in selected characteristics of nitrogen metabolism related to nitrogen uptake and accumulation in the grain of mod-

ern Bulgarian cultivars of common wheat (*Triticum aestivum* L.), grown in a four-field crop rotation, under clearly defined experimental factors including predecessor, rate of fertilization treatment under intensive conventional production system.

Materials and Methods

Description of the study site

The experiment covers the period 2018 – 2020, and was conducted in a stationary long-term field experiment in the Experimental Field of the Dobrudzha Agricultural Institute – General Toshevo. The Institute is located in Northeastern Bulgaria, in the center of the South Dobrudzha geographical region. The main zonal soils in the region are different subtypes of Chernozem soils. The experiment according to World Base of Soil Resources is located on Haplic Chernozems (Schad, 2023). A conventional production system was tested in a four-field crop rotation.

Treatment and experimental design

Several cultivars of common winter wheat (Dragana, Rada, Pchelina, Kosara and Kalina) were tested in a four-field crop rotation, which were grown after the predecessors rapeseed, peas, sunflower and corn (Table 1) (Tsenov et al., 2026).

In mineral fertilization, four increasing nitrogen rates (0, 60, 120 and 180 kg N/ha) were applied against the background of P_2O_5 – 60 kg/ha and K_2O – 60 kg/ha. The experiment was conducted in four replications. The field experiment occupied a total area of 5 hectares, including the predecessors of wheat. Classical mineral fertilizers were used for fertilization: ammonium nitrate (33–34% N), triple superphosphate (46% P_2O_5) and potassium chloride (60% K_2O).

Phosphorus and potassium fertilizers were applied before the main soil cultivation. Nitrogen fertilization was performed manually at the beginning of the perennial spring vegetation within one day for all fertilization options and replications.

Data collection and measurement

At technical maturity of wheat (stage 91–92) by Zadoks et al. (1974) above-ground parts of plants from each replication of an area of 50 × 50 cm were taken to determine the biological yield and its components. Nitrogen was determined by the classical Kjeldahl method in the following organs: leaves, stems, non-grain part of the spikes (NGPS) and grain. Based on the studies conducted on the productivity of individual organs and the nitrogen content in them, two of the key parameters of nitrogen metabolism were determined: nitrogen uptake (NUp) and the amount and efficiency of nitrogen uptake in the grain (NUtE). The same was done according to Fiez et al. (1995), Gaju et al. (2011) and Cormier (2015). The authors' approach to determining NUtE is to divide the grain dry matter yield (GDM) by the total nitrogen accumulated in the plant (NTA) at technical maturity:

$$\text{NUtE} = \text{GDM} / \text{NTA}$$

where: NUtE is expressed as kg GDM kg⁻¹ N in the plant.

Statistical analysis was performed according to the analysis of variance (ANOVA) procedure. Data were analyzed with statistical package SPSS version 13.0, which allows us to determine the share of each of the factors in relation to the total variation. Treatment means were compared by the F test, and when significant differences were established, means were separated using the Waller-Duncan (1955) multiple range test at $P \leq 0.05$. Pearson correlation coefficients (R) were also calculated and tested for significance.

Meteorological conditions

Southern Dobrudzha belongs to the Eastern climatic region of the Danubian Hilly Plain of the temperate continental climatic subregion of the European continental climatic region.

The most unfavorable conditions for crop development were established in 2020 (Fig. 1). In terms of the amount of autumn-winter precipitation, it was lower than both the previous two years and the climatic norm. The long spring vegetation began with a serious lack of moisture, and the

Table 1. Short principal information of the cultivars studied

Cultivars	Pedigree	Group of quality (French classification)	Year of development
Dragana	Miryana/Nadya	B (Superior)	2009
Rada	Enola/Preslav	B (Superior)	2012
Pchelina	Eryth.2582/89-3/969-69	A (premium type)	2012
Kosara	Enola/Karina	C (Medium)	2012
Kalina	Space 2*/Enola	B (Superior)	2010

Source: Authors' own elaboration

large amount of precipitation in June (192.2 mm) delayed the harvest. The most favorable conditions for crop development were established in the 2018 harvest. It was characterized by 357.6 mm of precipitation, which is 113.1 mm above the climatic norm. The formed autumn-winter moisture reserve helps to more easily overcome the low amounts of precipitation in April and June. Conditions were relatively favorable in 2019, especially in terms of vegetation precipitation.

In terms of temperature, all three years were characterized by higher temperatures than the climate norm. This fact was especially pronounced during the autumn-winter period of 2020, as well as during grain filling.

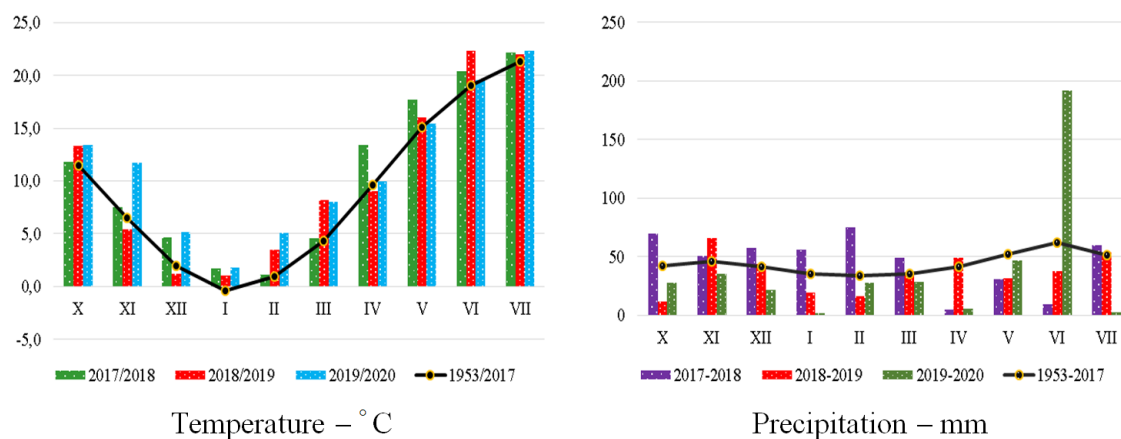


Fig. 1. Meteorological conditions during the study period

Source: Authors' own elaboration

Table 2. Analysis of variance of interaction of factors on nitrogen uptake by the non-economic part of common wheat

Source	df	Leaves/ $P \leq 0.001$			Stems/ $P \leq 0.001$			NGPS/ $P \leq 0.001$		
		Mean Square	F	%	Mean Square	F	%	Mean Square	F	%
Year (1)	2	11.39	4172.39	14.96	25.31	2773.4	15.03	15.31	1526.8	29.63
Fertilization (2)	3	17.3	6340.31	34.10	48.26	5289.12	43.01	9.48	945.43	27.53
Cultivars (3)	4	3.676	1347.06	9.66	0.321	35.194	0.38	0.326	32.466	1.26
Predecessor (4)	3	0.821	300.778	1.62	1.541	168.915	1.37	0.174	17.328	0.50
1×2	6	2.154	789.195	8.49	4.311	472.438	7.68	1.637	163.26	9.51
1×3	8	0.411	150.57	2.16	1.007	110.367	2.39	0.223	22.274	1.73
2×3	12	0.521	190.881	4.11	0.486	53.215	1.73	0.254	25.282	2.94
1×2×3	24	0.444	162.743	7.00	1.41	154.554	10.05	0.341	34.002	7.92
1×4	6	0.65	238.157	2.56	1.514	165.956	2.70	0.214	21.293	1.24
2×4	9	0.299	109.636	1.77	0.529	57.992	1.41	0.172	17.149	1.50
1×2×4	18	0.305	111.834	3.61	0.639	70.058	3.42	0.175	17.436	3.05
3×4	12	0.083	30.313	0.65	0.228	25.021	0.81	0.099	9.909	1.15
1×3×4	24	0.094	34.596	1.49	0.232	25.473	1.66	0.123	12.283	2.86
2×3×4	36	0.123	44.93	2.90	0.222	24.38	2.38	0.068	6.766	2.36
1×2×3×4	72	0.104	38.107	4.92	0.279	30.573	5.97	0.098	9.75	6.81

% – Share of total factors variation

Source: Authors' own elaboration

fects show statistical significance at $P \leq 0.001$. Fertilization forms the largest share of the variation in leaves and stems. It explains 34.10% of the variability in leaves and 43.01% in stems. Year also has a strong influence. Its share reaches 14.96% in leaves, 15.03% in stems and 29.63% in glumes. Varietal differences have a more limited contribution. They explain 9.66% of the variation in leaves and less than 2% in the other organs. The predecessor also shows a weak effect. Its share remains less than 2% in all studied organs. The interaction between year and fertilization shows a significant contribution to the variability. It reaches 8.49% in leaves, 7.68% in stems and 9.51% in glumes. The triple interaction *year x fertilization x cultivars* also has a noticeable effect. Its share reaches 7.00% in leaves, 10.05% in stems and 7.92% in glumes. The remaining interactions form a limited part of the variation. Their share in most cases remains below 5%.

The analysis of variance on the nitrogen absorbed by the grain and the total biomass of common wheat is also of high statistical reliability $P \leq 0.001$. Fertilization occupies a leading place among the main factors (Table 3). It explains 49.10% of the variation in nitrogen in the grain and 51.63% in the total biomass. The year also has a significant influence. Its share reaches 21.41% in the grain and 19.99% in the total biomass. These results show a strong dependence of nitrogen accumulation on environmental conditions and the level of mineral nutrition. The variety and the predecessor form a limited part of the total variability. Varietal differences explain 0.62% of the variation in the grain and 0.32% in the total biomass. The predecessor occupies a similar share. It

reaches 0.56% in the grain and 0.78% in the total biomass. Among the interactions, the strongest effect is shown by the combination *year x fertilization*. It explains 3.49% of the variation in grain and 4.02% in total biomass. The triple interaction *year x fertilization x cultivars* also has a significant contribution with a share of 7.06% in grain and 7.21% in total biomass. The remaining interactions form a smaller part of the total variability. Their share usually remains below 3%.

Nitrogen uptake is largely regulated by the amount of water available to plants in the soil, so during times of water deficit, crops can become both water and nitrogen limited (Hawkesford and Riche, 2020; Jariwala et al., 2022).

The differences in the amounts of NUp in the grain and the total aboveground biomass follow those of NUp in the non-economic part of production, since they are largely its function (Fig. 2). For the conditions of 2019, the highest values are reported for all cultivars and organs. In the leaves, the amount reaches 13.92 kg/ha for the Kosara variety and 11.84 kg/ha for Dragana. In the stems, the most intensive accumulation of nitrogen is established. The Rada variety reaches 21.53 kg/ha, and Dragana and Kosara, respectively, 19.30 and 19.22 kg/ha. A similar trend is observed in the glumes, where the value reaches 13.46 kg/ha for Dragana. In 2020, a decrease in the absorbed nitrogen in the leaves is established for all cultivars. The values range from 4.19 kg/ha for Kalina to 6.56 kg/ha for Kosara. In the stems, the decrease is less pronounced. Some cultivars have higher accumulation. The Kalina variety reaches 15.06 kg/ha, and the Pchelina variety reaches 14.64 kg/ha.

Table 3. Analysis of variance of interaction of factors on nitrogen uptake by grain and total biomass of common wheat

Source	df	Grain/ $P \leq 0.001$			Total biomass/ $P \leq 0.001$		
		Mean Square	F	%	Mean Square	F	%
Year (1)	2	904.023	5536.21	21.41	1520.54	6298.762	19.99
Fertilization (2)	3	1382.52	8466.51	49.10	2618.05	10845.14	51.63
Cultivars (3)	4	12.995	79.583	0.62	12.243	50.715	0.32
Predecessor (4)	3	15.751	96.457	0.56	39.49	163.584	0.78
1×2	6	49.122	300.82	3.49	102.012	422.582	4.02
1×3	8	15.158	92.828	1.44	21.841	90.474	1.15
2×3	12	3.643	22.31	0.52	7.965	32.994	0.63
1×2×3	24	24.84	152.117	7.06	45.687	189.257	7.21
1×4	6	27.1	165.957	1.93	51.402	212.929	2.03
2×4	9	14.667	89.823	1.56	25.308	104.836	1.50
1×2×4	18	11.66	71.402	2.48	23.624	97.86	2.80
3×4	12	8.88	54.382	1.26	11.496	47.62	0.91
1×3×4	24	6.63	40.6	1.88	9.025	37.385	1.42
2×3×4	36	6.462	39.572	2.75	8.489	35.165	2.01
1×2×3×4	72	4.624	28.316	3.94	7.644	31.666	3.62

% – Share of total factors variation

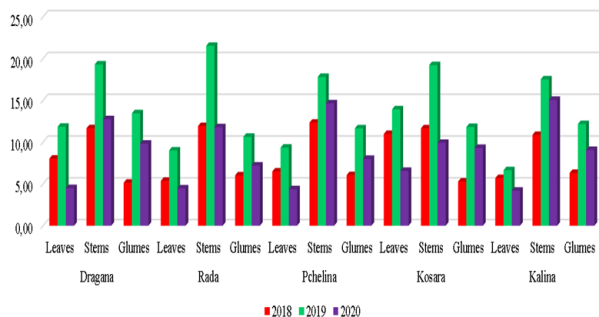
Source: Authors' own elaboration

In grain, the highest nitrogen uptake was recorded in 2019. The Rada cv. reached 130.26 kg/ha, and Dragana 120.51 kg/ha. In 2018, the Pchelina cv. showed the highest value of 113.92 kg/ha. In 2020, all cultivars recorded lower values between 68.98 and 76.68 kg/ha. Similar dynamics were observed in total biomass. The highest values were recorded in 2019. The Rada cv. reached 171.43 kg/ha, and Dragana 165.10 kg/ha. In 2020, the values decreased and varied between 96.04 and 100.33 kg/ha.

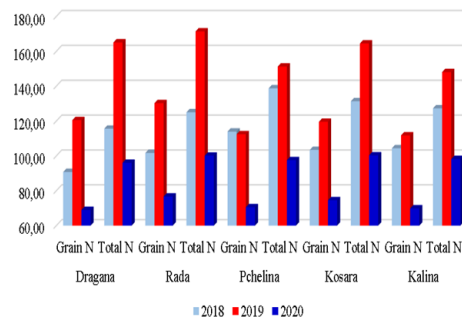
In leaves, the highest values are found after corn. The Kosara variety reaches 12.68 kg/ha, and Dragana 9.26 kg/ha. After rapeseed and peas, the values remain lower (Fig. 3). The Kalina variety shows the lowest nitrogen accumulation in the leaves of all predecessors with a range from 5.06 to 5.95 kg/ha. In stems, a higher absorption is again found after corn. The Rada variety reports the highest value with 17.08 kg/ha. The Dragana and Pchelina cv. reach 16.36 kg/ha. After sunflower and rapeseed, the values range mainly between

13 and 15 kg/ha. In glumes, the highest nitrogen accumulation is observed in the Dragana variety after corn with 10.87 kg/ha. For the other cultivars, the values vary between 7.58 and 9.91 kg/ha. Kalina variety shows relatively higher values after sunflower with 9.91 kg/ha.

In grain, the highest nitrogen uptake is also found after corn. The Rada cv. reaches 114.45 kg/ha, and Kosara 107.86 kg/ha. After rapeseed and sunflower, the values remain slightly lower. The Pchelina variety shows a high value after rapeseed with 107.23 kg/ha. A similar trend is observed in total biomass. The highest values are recorded after corn. The Rada cv. reaches 147.80 kg/ha, and Kosara 144.56 kg/ha. After rapeseed and peas, the values range mainly between 118 and 130 kg/ha. The results show higher nitrogen uptake in wheat after corn. Cultivar differences are mainly manifested in the leaves and grain, with the Kosara, Rada and Pchelina showing a higher potential for nitrogen accumulation.



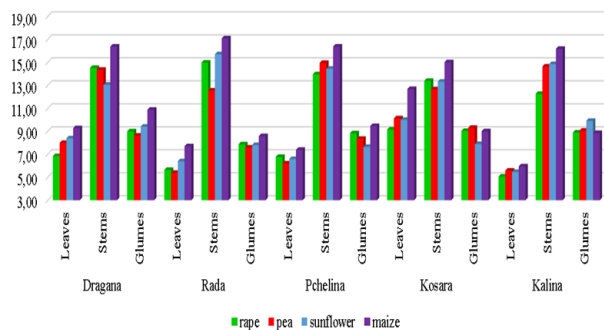
Non-economic part



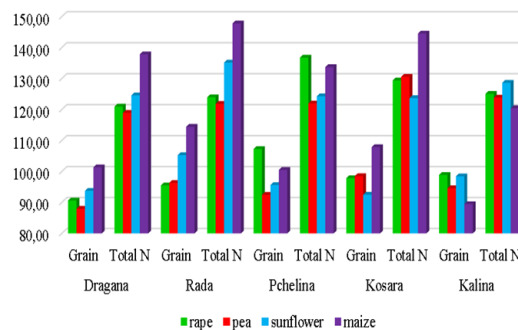
Grain and total aboveground biomass

Fig. 2. Nitrogen uptake (NUP) by organs of the non-economic part, grain and total aboveground biomass of wheat (kg/ha), depending on the methods of its cultivation, by years of study

Source: Authors' own elaboration



Non-economic part



Grain and total aboveground biomass

Fig. 3. Nitrogen uptake (NUP) by non-economic organs, grain and total aboveground biomass of wheat (kg/ha), depending on the type of predecessor

Source: Authors' own elaboration

Increasing the fertilization dose from T0 to T3 leads to a significant increase in accumulated nitrogen in all cultivars and organs (Fig. 4). The leaves show the strongest relative increase in nitrogen uptake. In the Kosara variety, the value increases from 2.94 kg/ha at T0 to 14.94 kg/ha at T3, which represents an increase of 408.16%. In the Dragana cv., the value increases from 3.15 to 13.61 kg/ha or 332.06%. In the Pchelina cv., the increase is from 2.46 to 10.89 kg/ha, which represents 342.68%. In the Rada cv., the value increases from 2.47 to 9.63 kg/ha or 289.88%, while in the Kalina cv., an increase is reported from 2.36 to 8.78 kg/ha or 272.03%.

In the stems, a significant increase in nitrogen uptake was also observed with increasing fertilization levels. In the Rada cv., the value increased from 7.39 kg/ha at T0 to 23.47 kg/ha at T3, which represents an increase of 217.73%. In the Pchelina cv., the increase was from 7.44 to 23.08 kg/ha or 210.22%. In the Kalina cv., the value increased from 7.95 to 23.61 kg/ha, which corresponds to 196.98%. In the Kosara cv., the increase was from 5.96 to 22.26 kg/ha or 273.49%, and in the Dragana cv., from 8.02 to 20.91 kg/ha or 160.72%.

A clear increase in nitrogen uptake is also reported for glumes. For the Rada cv., the value increases from 4.69 kg/ha at T0 to 10.15 kg/ha at T3, which represents an increase of 116.42%. For the Pchelina cv., the value increases from 4.74 to 10.72 kg/ha or by 126.16%. For the Kosara cv., the increase is from 5.27 to 11.64 kg/ha or by 120.87%. For the Kalina cv., the value increases from 5.20 to 12.64 kg/ha, which represents an increase of 143.08%. For the Dragana cv., an increase is reported from 5.46 to 12.09 kg/ha or by 121.43%.

In grain, the increase in nitrogen uptake with increasing fertilization dose is also distinct. In the Kosara cv., the

value increases from 50.82 kg/ha at T0 to 141.39 kg/ha at T3, which represents an increase of 178.22%. In the Pchelina variety, the value increases from 56.15 to 139.05 kg/ha or by 147.64%. In the Rada cv., an increase is reported from 57.71 to 137.67 kg/ha or by 138.55%. In the Dragana cv., the increase is from 54.44 to 123.83 kg/ha or by 127.46%, while in the Kalina cv., the value increases from 52.84 to 129.74 kg/ha or by 145.55%.

A similar relationship is observed for total biomass. For the Kosara cv., the value increases from 64.99 kg/ha at T0 to 190.24 kg/ha at T3, which represents an increase of 192.72%. For the Pchelina cv., an increase is reported from 70.80 to 183.73 kg/ha or 159.51%. For the Rada cv., the value increases from 72.27 to 180.92 kg/ha, which corresponds to an increase of 150.35%. For the Dragana cv., an increase is observed from 71.06 to 170.44 kg/ha or 139.87%, and for the Kalina cv. from 68.34 to 174.77 kg/ha or 155.73%. The largest relative increase was found in leaf mass, while the increase in grain and total biomass remained more moderate. The cultivars Kosara, Pchelina and Rada showed a higher potential for nitrogen uptake and accumulation at higher fertilization levels.

There are a number of reasons that lead to the inefficient use of imported mineral nitrogen. On the one hand, this is the one-time import of the aggressively high nitrogen rate for the region of 180 kg N/ha, which does not contribute to the effective use of nitrogen. On the other hand, the extremely unfavorable conditions in 2020 led to a sharp decrease in the values of a number of parameters characterizing nitrogen metabolism in wheat (Atanasov, 2024).

Nitrogen use efficiency is one of these indicators that allows us to compare the two agricultural production systems.

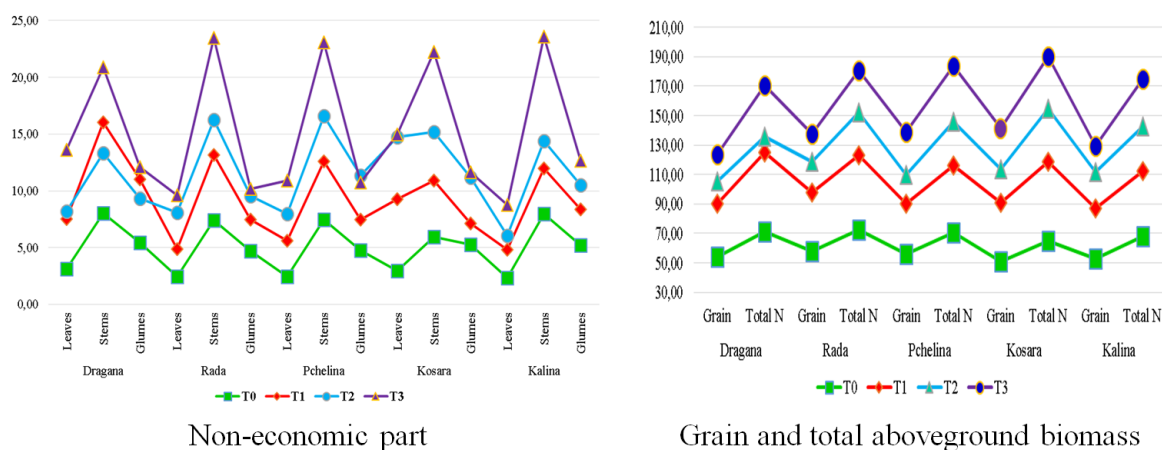


Fig. 4. Influence of cultivars and fertilization on nitrogen uptake in the non-economic part, grain and total biomass, kg/ha

Source: Authors' own elaboration

It is also known as biomass production efficiency (BPE). Essentially, this indicator applies the idea of quantifying the efficiency of nitrogen uptake from total biomass, i.e. how much grain is produced from 1 kg of nitrogen used from total aboveground biomass. According to Congreves et al. (2021), this indicator is also one of the important characteristics of nitrogen metabolism in plants, built on a plant basis. Of strategic importance for yield and quality are the processes leading to the accumulation of nitrogen in grain. Research in this area dates back decades.

At the dawn of intensive research on the specifics of nitrogen metabolism, Cox et al. (1985) found significant differences in the amount of nitrogen remobilized after husk removal. According to Knowles and Watkins (1993), most of the nitrogen absorbed by plants enters the grain directly or through mobilization from other parts of the plant. Several studies have shown that about two-thirds of the nitrogen in the wheat grain is absorbed before flowering, and the rest is absorbed during grain filling (Barbottin et al., 2005). However, this can vary significantly depending on soil moisture and nitrogen availability during the grain filling period.

Several studies have shown that nitrogen in wheat grain is largely the result of its translocation from vegetative parts after flowering (Hötensteiner and Feller, 2002; Haberer et al., 2008). This translocation is under genetic control (Anbessa et al., 2009), but also depends on environmental conditions (Asseng and Milroy, 2006) and the applied mineral fertilization (Perez et al., 1983; Congreves et al., 2021; Ren et al., 2022; Langridge et al., 2022; Zhang et al., 2023). Since the remobilization of nitrogen from vegetative tissues is associated with the senescence process and its translocation to the grain, this may reduce the total amount of nitrogen absorbed by the plant. This is mainly due to reduced leaf activity (Kichey et al., 2007), which in turn reduces the total amount of nitrogen available to the grain. Ercoli et al. (2008) found that under conditions without mineral nutrition, dry matter accumulation and nitrogen uptake increased until maturity. They concluded that under such conditions, nitrogen enters the grain primarily by translocation from the leaves and stems, rather than by uptake from the soil during grain filling.

Descriptive statistics show the variability of nitrogen use efficiency NUtE during the period 2018 – 2020 (Table 4). The average value of the indicator increased from 36.39 in 2018 to 36.95 in 2019 and reached 46.03 in 2020. The average value for the entire period is 39.79. The minimum values range from 8.02 in 2018 to 24.98 in 2020, and the maximum values range between 73.06 and 78.15. The range of variation is highest in 2018 with a value of 67.51, while in 2019 and 2020 a lower variability of 54.82 and 53.17 was report-

ed, respectively. The standard deviation ranges from 11.63 to 13.07. The coefficient of variation decreased over the years from 35.92% in 2018 to 26.64% in 2020. On average for the period, the coefficient of variation was 32.87%, indicating moderate variability in nitrogen use efficiency.

Table 4. Descriptive statistics parameters of NUtE by year and average for the study period, depending on the wheat farming system, by years and average for 2018–2020

Year	df	2018	2019	2020	2018–2020	
					df	Value
Mean	160	36.39	36.95	46.03	480	39.79
Minimum	160	8.02	18.24	24.98	480	8.02
Maximum	160	75.53	73.06	78.15	480	78.15
Range	160	67.51	54.82	53.17	480	70.13
SD	160	13.07	11.63	12.26	480	13.08
CV%	160	35.92	31.47	26.64	480	32.87

Source: Authors' own elaboration

The analysis of statistical reliability shows the statistical significance of the influence of the studied factors on nitrogen uptake efficiency (NUtE) in individual years (Table 5). The main factors fertilization, predecessor and variety show very high statistical significance in all years of the study $P \leq 0.001$. Differences between individual years are found in the interactions. The interaction *fertilization* \times *predecessor* does not show statistical significance in 2018, while in 2019 and 2020 the influence is highly significant $P \leq 0.001$. The same trend is observed in the interaction *predecessor* \times *variety*, which is insignificant in 2018, but shows very high statistical significance in the following two years. The interaction *fertilization* \times *variety* is statistically significant in all years $P \leq 0.001$. The triple interaction *fertilization* \times *predecessor* \times *cultivar* shows weaker significance in 2018 $P \leq 0.05$, while in 2019 and 2020 the influence is very pronounced $P \leq 0.001$.

The statistical analysis shows a high reliability of the influence of all studied factors on the efficiency of nitrogen use

Table 5. Statistical reliability (F probability) in ANOVA, the influence of factors in agricultural production systems on NUtE by years of study

Factors	2018	2019	2020
Fertilization (1)	***	***	***
Predecessor (2)	***	***	***
Cultivars (3)	***	***	***
1 \times 2	n.s.	***	***
1 \times 3	***	***	***
2 \times 3	n.s.	***	***
1 \times 2 \times 3	*	***	***

Significance levels: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Source: Authors' own elaboration

Table 6. Statistical reliability of the influence of factors in conventional production on NUtE on average for the study period 2018–2020

Factors	Sig	Combination	Sig	Combination	Sig	Combination	Sig
Year (1)	***	1×2	***	2×4	***	2×3×4	***
Fertilization (2)	***	1×3	w***	3×4	***	1×2×3×4	***
Predecessor (3)	***	1×4	***	1×2×3	***		
Cultivars (4)	***	2×3	***	1×2×4	***		

Significance levels: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Source: Authors' own elaboration

NUtE on average for the period (2018 – 2020). The main factors year, fertilization, predecessor and variety show very high statistical significance $P \leq 0.001$ (Table 6).

The same degree of significance is found for all studied interactions between factors. The two-way interactions *year* × *fertilization*, *year* × *predecessor*, *year* × *cultivar*, *fertilization* × *predecessor*, *fertilization* × *cultivar* and *predecessor* × *cultivar* show a reliable influence. The three-way interactions *year* × *fertilization* × *predecessor*, *year* × *fertilization* × *cultivar* and *fertilization* × *predecessor* × *cultivar* are also statistically significant, as well as the four-way interaction between all factors.

Gaju et al. (2011) reported that the average NUtE, expressed as kg dry matter of grain per kg aboveground nitrogen at harvest, increased from 34.0 under high nitrogen fertilization to 52.1 kg dry matter/kg N under low nitrogen conditions.

A number of authors in their studies conducted in different time periods indicate that NUtE values decrease with increasing nitrogen fertilization rates (Delogu et al., 1998; Lopez-Bellido and Lopez-Bellido, 2001; Ortiz-Monasterio et al., 2002; Hussain et al., 2002; Hirel et al., 2007).

The leaves, and especially the flag leaf, are more efficient than the stem and non-grain part of the ear at remobilizing nitrogen, regardless of the presence of moisture. Application of nitrogen fertilization usually reduces the efficiency of remobilizing the whole plant when nitrogen is available during grain filling (Bahrani et al., 2011). In their studies, Shangguan et al. (2000), Gonzalez-Dugo et al., (2010), Li et al., (2020) found that under water deficit conditions, the efficiency of nitrogen remobilization increased up to a fertilization rate of 80 kg/ha, but further increase leads to a decrease in reuse. Li et al., (2022) in their research “suggest that long-term soil health and nutrient improvement should be more conducive to increasing yield and resource use efficiency in wheat production.”

It has long been known that crop rotation has significant advantages in agricultural production compared to monocultures or biennial crop rotations. In his review of this topic, Bullock (1992) points out that the expectations that mineral

fertilizers, with their appearance in 1950–1960, would replace the role of crop rotation were not justified.

The average data on the crop's ability to assimilate nitrogen in relation to the tested factors are also summarized (Fig. 5). The average nitrogen use efficiency NUtE for the period 2018 – 2020 varies between 36.39 and 46.03 kg DM/kg N by year, for 2018 and 2020, respectively. Depending on the predecessor, NUtE is highest after peas – 48.52 kg DM/kg N, followed by rapeseed – 42.92 kg DM/kg N and sunflower – 42.77 kg DM/kg N. It is lowest after corn – 38.61 kg DM/kg N.

The results obtained show that cultivation methods have a significant impact on the ability of wheat to use nitrogen for the economic part of production. Crop rotation, with the variety of plant residues they leave behind, tillage and the overall soil nutrition regime, in combination with additional nutrient input, contribute to this fact. All agronomic practices affect the availability of available nitrogen to plants and its uptake by plants (Ortiz-Monasterio et al., 1997; Reddy, 2011; Lehman et al., 2012; Salim and Raza, 2020; Mirzaee and Nafchi, 2025).

One explanation for the differences in remobilization efficiency is that during grain filling, the plant retains part of the absorbed nitrogen until maturity, which it needs for survival and various biological functions, while the rest is released for remobilization. This nitrogen retention appears to depend on growth and developmental conditions, although genetic variability in nitrogen remobilization has also been reported (Kirda et al., 2001; de Silva, 2020).

In our study, the contribution of the genotypic factor to obtaining larger amounts of nitrogen uptake at the highest temperature is also evident. By variety, Kalina shows the highest efficiency – 47.56 kg DM/kg N, followed by Dragana – 45.39 kg DM/kg N and Kosara – 43.59 kg DM/kg N. It is the lowest for Rada – 39.06 kg DM/kg N.

The data show that in terms of fertilization rates, the highest efficiency is recorded at T_1 – 45.07 kg DM/kg N, followed by T_2 – 39.71 kg DM/kg N and T_0 – 39.53 kg DM/kg N. It is lowest at T_3 – 34.85 kg DM/kg N. In this regard, Gamage et al. (2023) point out that a fundamental reassessment and

transformational redesign of agricultural systems based on agroecological principles is needed to reduce environmental pressures, especially in the areas of nitrogen fertilization and tillage systems. According to Zhang et al. (2023), achieving smart manufacturing is impossible without interdisciplinary research.

Janssen et al. (1990) also found negative correlations between NUtE and nitrogen/protein content at certain levels of nitrogen uptake. The authors explained this fact by the processes of nitrogen concentration dilution (as a result of growth), which leads to a limitation of the relationship between yield and nitrogen uptake in the final phase.

The increase in nitrogen uptake with increasing mineral fertilization rates outpaces the increase in yield, because we have less nitrogen transfer to the grain with increasing nitrogen rates. In addition, mineral fertilization in combination with other agronomic factors leads to significant changes in the efficiency of nitrogen use in the Mediterranean climate, which inevitably determines the interrelationships in the elements of this efficiency (Bellido and Pedregal, 2001).

The correlation analysis shows negative relationships between nitrogen use efficiency NUtE and most of the studied indicators (Table 7). The strongest relationship is found with

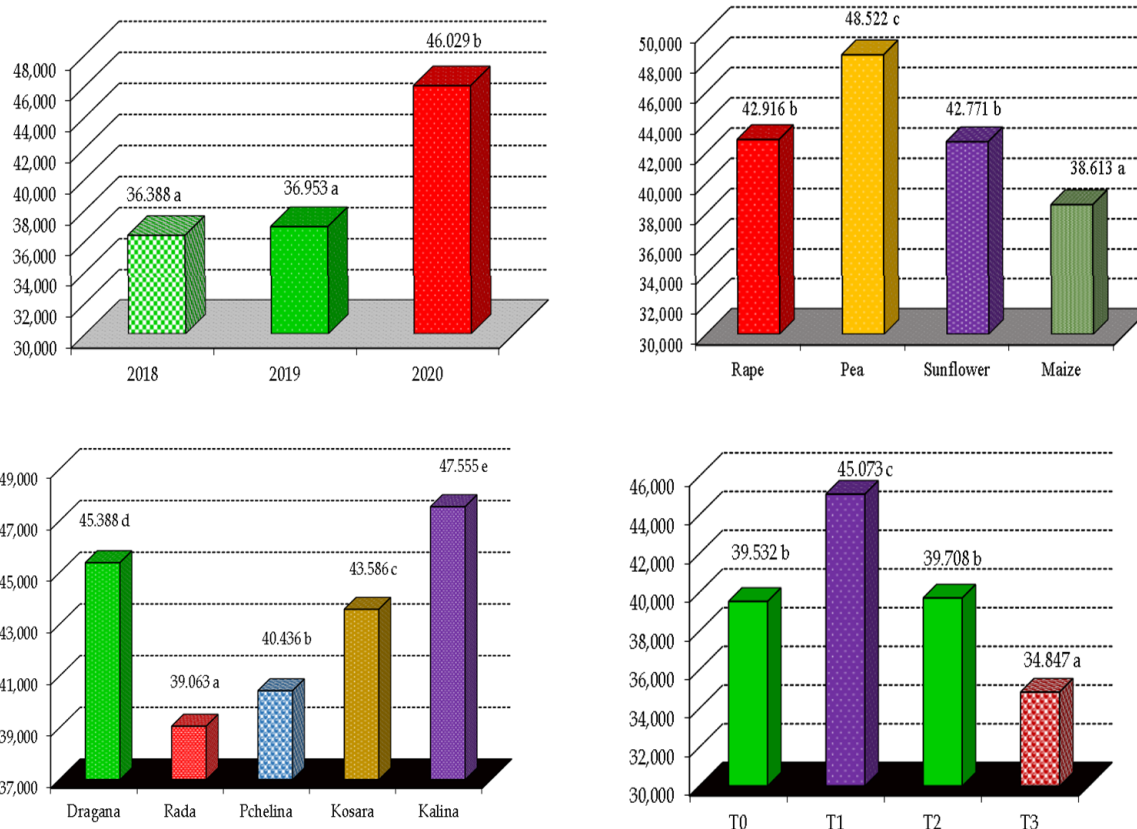


Fig. 5. Nitrogen utilization efficiency on average for the experiment, regardless of the production method, for the period 2018–2020 (kg DM/kg N)

Different letters indicate statistically significant differences among means according to the Waller–Duncan multiple range test at $P \leq 0.05$.

Source: Authors' own elaboration

Table 7. Correlations between nitrogen utilization efficiency with protein, yield and absorbed nitrogen

Production systems	Grain yield		Protein		Nitrogen uptake		
	Yield	GHI	Protein %	Yield	Total biomass	Grain	NUp
NUtE	-0.006	-0.190(**)	-0.527(**)	-0.265(**)	-0.588(**)	-0.575(**)	-0.323(**)

Significance levels: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Source: Authors' own elaboration

total biomass $r = -0.588$ and nitrogen uptake in grain $r = -0.575$. Protein content also shows a relatively strong negative correlation $r = -0.527$. A weaker, but statistically significant relationship is found with protein yield $r = -0.265$, the harvest index GHI $r = -0.190$ and total nitrogen uptake NUpE $r = -0.323$. No relationship is found between NUtE and grain yield $r = -0.006$.

Conclusions

Nitrogen uptake in the non-economic part, grain and total biomass increases with increasing fertilization dose. For example, in the leaves of the Kosara cultivar, the NUP value increases from 2.94 kg/ha at T0 to 14.94 kg/ha at T3 (an increase of 408%), in the stems – from 5.96 to 22.26 kg/ha (273%), in the grain – from 50.82 to 141.39 kg/ha (178%), and in the total biomass – from 64.99 to 190.24 kg/ha (193%).

The highest nitrogen uptake efficiency among the cultivars was observed in Kalina and Dragana, 47.56 kg DM/kg N and 45.39 kg DM/kg N, respectively, and the lowest in Rada – 39.06 kg DM/kg N.

Among the predecessors, peas provide the highest nitrogen uptake efficiency – 48.52 kg DM/kg N, followed by rapeseed – 42.92 kg DM/kg N and sunflower – 42.77 kg DM/kg N. The lowest efficiency is observed after corn – 38.61 kg DM/kg N.

In terms of fertilization rates, the highest nitrogen uptake efficiency was recorded at T1 – 45.07 kg DM/kg N, followed by T2 – 39.71 kg DM/kg N and T0 – 39.53 kg DM/kg N. The lowest value was observed at T3 – 34.85 kg DM/kg N.

The interactions between the factors year, fertilization, predecessor and variety are statistically significant ($p \leq 0.001$) and affect nitrogen uptake, with the triple interaction fertilization x predecessor x cultivar being weakly significant in 2018 ($p \leq 0.05$) and highly significant in 2019 – 2020 ($p \leq 0.001$).

Correlation analysis showed negative relationships between nitrogen assimilation efficiency NUtE and protein content ($r = -0.527$), total biomass ($r = -0.588$) and nitrogen assimilated in grain ($r = -0.575$), while the relationship with grain yield was practically absent ($r = -0.006$).

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