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VARIATION OF SOME WHEAT QUALITY INDICES IN RELATION TO THE NITROGEN FERTILIZATION RATE AND FOLIAR BIOFERTILIZER BASED ON ALGAE EXTRACT

SUMMARY

The study analyzed the influence of nitrogen and the Super Fifty algae-based biofertilizer, at variable application rates, on some wheat quality indices. The study was conducted in the specific conditions of the Western Plain of Romania, within the Didactic and Experimental Resort, University of Life Sciences "King Mihai I" from Timisoara, between 2018 and 2022. The 'Alex' wheat cultivar was cultivated on a chernozem soil, non-irrigated system. Nitrogen (N) was applied in spring in five doses (0, 50, 100, 150 and 200 kg ha⁻¹ active substance; ammonium nitrate fertilizer). The algae-based biofertilizer Super Fifty (SF) was applied foliarly, at each N level, in six doses (0, 1, 2, 3, 4, and 5 L ha⁻¹). Thirty experimental variants (T1 – T30) resulted, each with three repetitions. The two factors (N, SF) differentially influenced the starch (ST), gluten (GLT), protein (PRO), Zeleny index (ZEL), hectoliter weight (HW) and moisture (Moist) content. Convergent action of the factors was recorded in the variation of the GLT, PRO, and ZEL indices, and divergent action in the formation of the ST, and HW indices. Based on the multivariate analysis, in PC1 the N factor loaded, with very strong positive action, associated with the PRO, GLT, ZEL, and ST indices. In PC2 the SF loaded with strong positive action, associated with HW. Cluster analysis grouped the variants based on similarity (Coph.corr.=0.791), and Ranking-scaling analysis generated the hierarchy of the variants in relation to the values of the quality indices. The results of the study have scientific and practical relevance for optimizing wheat crop technology through fertilization models, in which foliar biofertilizers are of interest in the context of climate change. The fertilization model, based on the two fertilizer resources (nitrogen and algae-based biofertilizer), can be adjusted for different wheat genotypes and environmental conditions. Also, the model can be developed with multiple fertilizer elements (e.g., NPK) for specific environmental conditions, and the algae-based biofertilizer Super Fifty can

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enhance the effect of these elements on crop productivity, yield and quality indices.

Keywords: algae-based biofertilizer, mathematical models, multivariate analysis, variable nitrogen rate

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) has been studied in relation to various ecological, economic and social factors, for sustainable agricultural practices, with yields and appropriate quality indices in relation to the finished products categories (Šeremešić *et al.*, 2021; Gao and Liu, 2023; Djikic *et al.*, 2024; Nozdrina *et al.*, 2024). In response to environmental changes, local varieties and genotype collections of wheat has been studied under different crop conditions (e.g. intercropping), aiming to evaluate ecological efficiency, yields, quality, consumer preferences, and environmental health (Luo *et al.*, 2021; Radchenko *et al.*, 2023; Velimirović *et al.*, 2023).

Morphological variability in wheat, along with variations in certain quality indices and biochemical parameters, has been investigated in connection with technological practices and abiotic environmental factors (Xhulaj and Koto, 2022; Nazarenko and Horshchar, 2024).

Nitrogen is the nutrient with the major influence on yield formation, and the interaction between nitrogen, soil, environmental conditions and wheat crop has been analyzed from multiple perspectives (Wang *et al.*, 2023). The relationship between nitrogen and wheat has been analyzed at different levels and physiological processes, starting from seed germination (Kolupaev *et al.*, 2025).

Although nitrogen is a key mineral element for grain yield, nitrogen use efficiency has remained low under many crop conditions, or in various studies and experiments (Walsh *et al.*, 2022). The authors reported the variation in yield-effective nitrogen rates for agricultural crops, according to crop locations.

The efficiency of nitrogen fertilization was studied in wheat crops under different climatic conditions, in relation to watering methods, water application frequency, yield, and some productivity and seed quality parameters (Hao *et al.*, 2023; Chadda *et al.*, 2025). Variable nitrogen rates were analyzed in relation to yield and water factor in wheat crop, and different nitrogen levels with ecologically advantageous impact on yield were identified (Noor *et al.*, 2023). A large study evaluated the influence of variable nitrogen application rates on wheat crops, particularly in terms of nitrogen use efficiency, soil and environmental impacts (Feyisa *et al.*, 2024).

The influence of nitrogen at different application rates was analyzed in relation to quality indicators, such as starch content and starch fractions in the endosperm of wheat grains (Xiong *et al.*, 2014). Improvement in grain quality was recorded in relation to nitrogen fertilization rates, even in conditions where there were low effects on yield in different study locations (Walsh *et al.*, 2022). Experiments with variable nitrogen rates applied at different physiological stages of wheat crop facilitated the identification of the dose and application times that

led to the physiological performance of wheat plants, with a positive impact on grain yield and quality (Kubar *et al.*, 2022). Nitrogen rates associated with other agronomic treatments have been studied in wheat crops in relation to yield, quality indices and grain milling parameters (Božek *et al.*, 2022).

Variable nitrogen rates have been studied in relation to technological quality indices in wheat, and increasing variations in protein content, gluten and sedimentation index (Zeleny) associated with increasing nitrogen rates have been recorded (El Chami *et al.*, 2023). The interaction between sowing date, seed density and nitrogen fertilization rate was analyzed in relation to winter wheat quality indices, and the authors were able to select the effective combinations for the study area (Lachutta *et al.*, 2024).

The protein content of wheat grains was analyzed in relation to nitrogen application rate, in relation to different specific soil conditions, climate and crop location (An *et al.*, 2024). Mineral fertilization with nitrogen, at different rates, associated with organic fertilization generated a positive response in yield and quality indices in wheat (Jin *et al.*, 2024). Nitrogen fertilization rates and differentiated light conditions were analyzed in relation to wheat yield and starch content, and the authors recorded the differential influence of the two factors on wheat yield and quality elements (Yang *et al.*, 2025).

In the context of climate change, and variations in the balance of the "plant-soil" relationship, foliar fertilization and foliar biostimulatory products are increasingly promoted within crop technologies for nutrient supplementation, yield control and production quality (Szczepanek *et al.*, 2018; Ferrari *et al.*, 2025). Favorable effects of treatments with foliar biostimulants based on algae extracts were recorded in wheat crops, on agronomic parameters of plants, yield and quality parameters of seeds (Szczepanek *et al.*, 2018). Foliar fertilization with products based on algae extracts in relation to different doses of nitrogen generated a significant increase in some agronomic parameters of plants and more efficient use of water in wheat cultivation (Bakhoun *et al.*, 2023).

The aim of the study was to evaluate the direct and interaction influence of nitrogen and foliar biofertilizer Super Fifty in different rates on wheat quality indices, to describe the interdependence of quality indices, the loading of variables in the principal components, and to group based on similarity and to rank the experimental variants in relation to the performance given by the index values.

MATERIAL AND METHODS

Location and experimental conditions. The study was conducted at the University of Life Sciences "King Mihai I" from Timisoara, Romania, between 2018 and 2022. The field experiments were located in the Didactic and Experimental Resort (DER), in flat land conditions, on a chernozem soil type, Figure 1. The climatic conditions recorded during the experimental period are presented in Figure 2.



Figure 1. Experimental field, Didactic and Experimental Resort, ULS “King Mihai I” from Timisoara

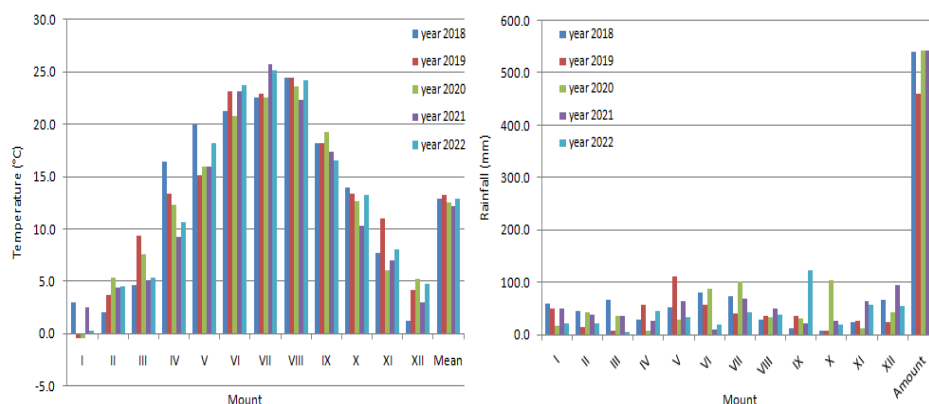


Figure 2. Climatic conditions during the study period, according to the Timisoara Meteorological Station (2018-2022)

The wheat crop was in a non-irrigated system. The soil was prepared for sowing by plowing, followed by disc and combinatory works. Sowing was done in the first decade of October, in each experimental year.

Experimental factors. The biological material used was 'Alex' wheat cultivar. The wheat cultivar was created in ARDS Lovrin, and is a cultivar with high yield potential (over 7000 kg ha⁻¹) and good quality indicators. This wheat cultivar is well adapted to the specific climatic conditions of the Western Plain of Romania, where the experimental field was located.

Nitrogen was applied at variable rates, in the form of granulated

ammonium nitrate (33.5 % N, $\text{N}-\text{NO}_3^-$, $\text{N}-\text{NO}_4^+$). Five levels of nitrogen fertilization were applied: 0, 50, 100, 150 and 200 kg ha⁻¹ N a.s. (a.s. – active substance). Nitrogen was applied in spring, at the end of tillering – beginning of stem elongation, growth stage 29-30 BBCH Code (Meier, 2001).

The Super Fifty (SF) foliar algae-based biofertilizer was applied in six concentrations, at each nitrogen fertilization level. Six levels of the foliar biostimulator were provided: 0, 1, 2, 3, 4, and 5 L ha⁻¹. The application of the Super Fifty product was made foliarly, with a manually operated atomizer, at the end of stem elongation stage, growth stage 37-39 BBCH code (Meier, 2001).

The combination of the two factors (N, SF) resulted in 30 experimental variants, denoted T1 to T30. The surface area of an experimental plot was 18 m². The experimental variants were placed in three repetitions.

Wheat quality indicator. At harvest maturity, 99 BBCH Code, grain samples were collected to determine quality indices. Starch content (ST, %), gluten content (GLT, %), protein content (PRO, %), Zeleny index (ZEL, %), hectoliter weight (HW, kg hl⁻¹) and moisture (Moist, %) were determined. The determination of quality indices was done with the AgriCheck device.

Statistical data analysis. Based on the experimental data recorded in each experimental year, the mean value was calculated for each index and experimental variant. The mean values were analyzed to find out the reliability of the data and the presence of the variance in the set of results (ANOVA Test). To evaluate the interdependence of the independent variables (N, SF) and the dependent ones (quality indices) the correlation analysis (Pearson's) was applied. The variation of quality indices in relation to N and SF was evaluated by regression analysis.

The position of the variables in relation to the principal components, mode and intensity of action, were evaluated by multivariate analysis (PCA). The grouping of experimental variants based on similarity, in relation to the recorded quality indices, was evaluated by cluster analysis (CA). The value hierarchy of the experimental variants was done by the Ranking-scaling method. In relation to the mathematical and statistical analysis tests of the experimental data, the calculation module in EXCEL, the PAST software v.4.17c (Hammer *et al.*, 2001), the JASP software v.0.16.4 (2022), and the Mathematica software v.14.2 (2024) were used.

RESULTS AND DISCUSSION

Nitrogen fertilization at variable rates ranging from 0–200 kg ha⁻¹ (N, a.s.) and foliar treatment with the Super Fifty (SF) biofertilizer at doses ranging from 0–5 L ha⁻¹, determined differentiated values of quality indices in wheat, 'Alex' cultivar, Table 1. ANOVA Test (Two-Factor) confirmed the reliability of the data, and the presence of variance in the experimental data set, according to Table 2 (Alpha = 0.05).

Table 1. The quality indices values for wheat, 'Alex' cultivar on the experimental variants

Trial	N; SF	ST	GLT	PRO	ZEL	HW	Moist
T1	0; 0	68.99±0.35	26.78±1.08	12.16±0.46	48.45±2.96	74.54±0.28	12.42±0.06
T2	0; 1	69.37±0.32	27.87±1.02	12.67±0.45	52.51±2.68	76.13±0.27	12.39±0.03
T3	0; 2	69.39±0.29	28.30±1.06	12.53±0.52	53.11±2.65	77.49±0.31	12.33±0.01
T4	0; 3	70.08±0.35	29.32±0.98	13.22±0.42	56.74±2.61	77.97±0.19	12.61±0.07
T5	0; 4	69.56±0.30	29.17±0.99	13.16±0.41	55.39±2.25	76.51±0.20	12.43±0.05
T6	0; 5	69.36±0.26	28.33±1.04	12.81±0.44	52.65±2.61	75.87±0.24	12.32±0.01
T7	50; 0	68.90±0.35	30.08±1.08	13.47±0.46	52.70±2.96	74.67±0.28	12.48±0.06
T8	50; 1	68.86±0.32	30.20±1.02	13.53±0.45	53.21±2.68	75.43±0.27	12.28±0.03
T9	50; 2	68.80±0.29	30.18±1.06	13.04±0.52	54.34±2.65	75.76±0.31	12.31±0.01
T10	50; 3	69.02±0.35	31.32±0.98	14.04±0.42	55.58±2.61	77.23±0.19	12.31±0.07
T11	50; 4	69.09±0.30	31.84±0.99	14.26±0.41	59.13±2.25	77.30±0.20	12.13±0.05
T12	50; 5	68.40±0.26	32.30±1.04	14.47±0.44	57.90±2.61	77.13±0.24	12.37±0.01
T13	100; 0	68.03±0.35	30.42±1.08	13.74±0.46	56.51±2.96	74.70±0.28	12.16±0.06
T14	100; 1	68.26±0.32	31.24±1.02	14.30±0.45	58.90±2.68	74.76±0.27	12.39±0.03
T15	100; 2	68.55±0.29	31.93±1.06	14.58±0.52	60.84±2.65	76.30±0.31	12.39±0.01
T16	100; 3	68.92±0.35	33.20±0.98	14.81±0.42	64.66±2.61	77.00±0.19	12.40±0.07
T17	100; 4	68.34±0.30	33.37±0.99	14.57±0.41	64.60±2.25	77.01±0.20	12.16±0.05
T18	100; 5	68.09±0.26	33.06±1.04	14.67±0.44	64.04±2.61	76.63±0.24	12.32±0.01
T19	150; 0	67.60±0.35	32.00±1.08	14.40±0.46	61.08±2.96	76.06±0.28	12.46±0.06
T20	150; 1	67.99±0.32	32.92±1.02	14.78±0.45	63.43±2.68	76.23±0.27	12.48±0.03
T21	150; 2	68.14±0.29	33.51±1.06	15.01±0.52	64.18±2.65	76.80±0.31	12.33±0.01
T22	150; 3	68.45±0.35	34.23±0.98	15.34±0.42	66.82±2.61	76.91±0.19	12.31±0.07
T23	150; 4	68.10±0.30	33.86±0.99	15.14±0.41	65.65±2.25	76.30±0.20	12.29±0.05
T24	150; 5	68.21±0.26	33.92±1.04	15.19±0.44	65.38±2.61	76.39±0.24	12.36±0.01
T25	200; 0	67.25±0.35	33.14±1.08	14.88±0.46	65.21±2.96	75.13±0.28	12.51±0.06
T26	200; 1	67.57±0.32	33.61±1.02	15.22±0.45	65.96±2.68	75.78±0.27	12.36±0.03
T27	200; 2	67.71±0.29	34.04±1.06	15.02±0.52	66.56±2.65	75.98±0.31	12.31±0.01
T28	200; 3	68.01±0.35	34.56±0.98	15.49±0.42	68.13±2.61	77.04±0.19	12.22±0.07
T29	200; 4	68.03±0.30	34.83±0.99	15.58±0.41	67.50±2.25	76.36±0.20	12.30±0.05
T30	200; 5	67.81±0.26	33.99±1.04	15.27±0.44	66.40±2.61	75.89±0.24	12.40±0.01

Table 2. ANOVA Test results

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	119159.5	29	4108.948	4587.780	3E-200	1.546
Columns	78.19178	5	15.63836	17.461	1.64E-13	2.277
Error	129.8662	145	0.895629			
Total	119367.5	179				

Correlation analysis (Pearson's r) was applied to evaluate the level of interdependence between quality indices as well as in relation to influencing factors. According to the values of the correlation coefficient, positive or negative correlation was identified, with different levels of intensity and statistical safety, Table 3 ($p < 0.05$, *; $p < 0.01$, **; $p < 0.001$, ***).

Table 3. The correlation level at the level of study elements, 'Alex' wheat cultivar

Variables		N	SF	ST	GLT	PRO	ZEL	HW	Moist
N	Pearson's r	—							
	p-value	—							
SF	Pearson's r	0	—						
	p-value	1	—						
ST	Pearson's r	-0.898***	0.156	—					
	p-value	< .001	0.411	—					
GLT	Pearson's r	0.892***	0.320	-0.739***	—				
	p-value	< .001	0.084	< .001	—				
PRO	Pearson's r	0.903***	0.285	-0.748***	0.987***	—			
	p-value	< .001	0.126	< .001	< .001	—			
ZEL	Pearson's r	0.899***	0.311	-0.714***	0.959***	0.955***	—		
	p-value	< .001	0.095	< .001	< .001	< .001	—		
HW	Pearson's r	-0.094	0.595***	0.325	0.230	0.188	0.261	—	
	p-value	0.621	< .001	0.079	0.222	0.319	0.164	—	
Moist	Pearson's r	-0.103	-0.274	0.131	-0.229	-0.177	-0.147	-0.109	—
	p-value	0.588	0.143	0.490	0.223	0.350	0.439	0.567	—

Notes: $p < 0.05$, *; $p < 0.01$, **; $p < 0.001$, ***

Very strong, positive correlation was recorded between protein (PRO) and fertilizer nitrogen (N), $r = 0.903^{***}$, between GLT and PRO indices, $r = 0.987^{***}$, between GLT and ZEL indices, $r = 0.959^{***}$, and between PRO and ZEL indices, $r = 0.955^{***}$. Strong negative correlation was recorded between starch (ST) and nitrogen (N), $r = -0.898^{***}$, and strong positive correlation between GLT and N, $r = 0.892^{***}$, respectively between ZEL and N, $r = 0.899^{***}$. Moderate, negative correlation, was recorded between ST and GLT, $r = -0.739^{***}$, between ST and PRO, $r = -0.748^{***}$, respectively between ST and ZEL, $r = -0.714^{***}$. At the level of the other indices, or with the factors N and SF, a low value correlation was recorded, and without statistical certainty, Table 3.

The variation of the quality indices in relation to the experimental influence factors (N, SF) in the doses used was evaluated by quadratic regression analysis. The general equation (1) resulted, with the values of the coefficients and statistical safety parameters presented in Table 4.

$$QI = ax^2 + by^2 + cx + dy + exy + f \quad (1)$$

where: QI – quality indices in wheat grains (explained in Table 4);

x – Nitrogen doses (N, kg ha⁻¹); y – Super Fifty (SF, L ha⁻¹); a, b, c, d, e, f – the coefficients of equation (1), with the values corresponding to each quality index in Table 4.

Table 4. Regression analysis results

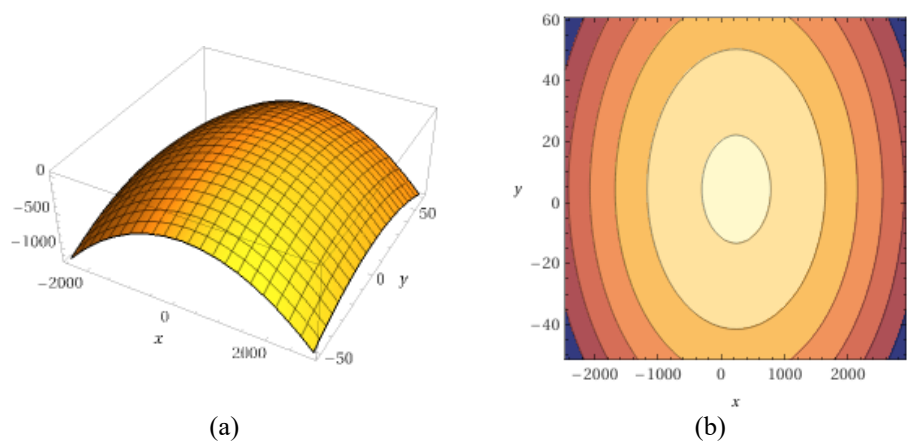
Regression analysis elements		Quality Indices (QI)				
		ST	GLT	PRO	ZEL	HW
Equation (1) coefficients	a	0.00002	-0.00012	-0.00005	-0.00010	0.00000
	b	-0.07221	-0.11143	-0.03706	-0.35186	-0.18095
	c	-0.01372	0.05332	0.02279	0.10015	0.00144
	d	0.37642	1.04216	0.38421	3.10966	1.32787
	e	0.00046	-0.00072	-0.00038	-0.00323	-0.00118
	f	69.16345	26.82894	12.15621	48.01428	74.70778
Statistical parameters	R ²	0.928	0.966	0.950	0.927	0.656
	F	62.3077	139.0395	92.0350	74.7023	9.1770
	p	<0.001	<0.001	<0.001	<0.001	<0.001
	RMSE	0.1777	0.4023	0.2144	1.3879	0.5123

From the analysis of the coefficient values of Equation (1), and Table 4, it was found that the two factors (N, SF) presented convergent action in the formation of gluten content (GLT), protein content (PRO) and Zeleny index (ZEL). The graphical expression of variation of the three indices in relation to N and SF is presented in Figure 3.

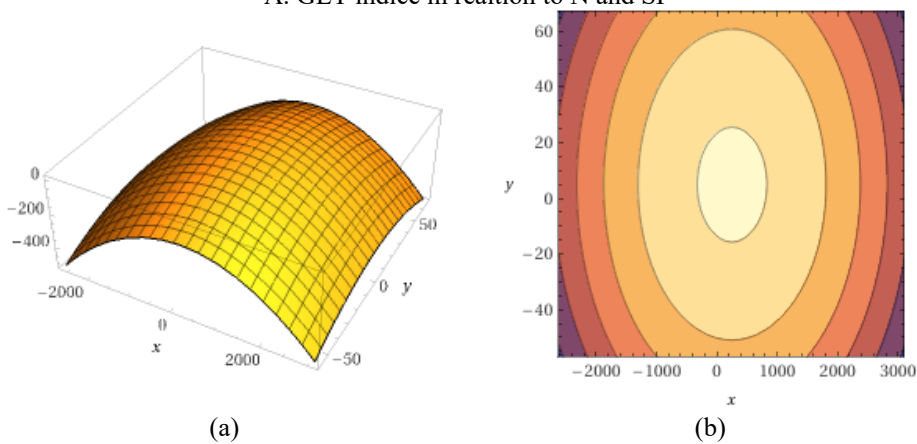
Under the conditions of the convergent action of the two factors (N, SF) it is possible to optimize them simultaneously in relation to the quality indices. Thus, for example, the optimal values of nitrogen (N) and Super Fifty (SF) were calculated in relation to the GLT index, where $N_{opt}=212.52$ kg ha⁻¹ N a.s., respectively $SF_{opt}=3.99$ L ha⁻¹, and in relation to the PRO index, where $N_{opt}=222.75$ kg ha⁻¹ N a.s., respectively $SF_{opt}=4.03$ L ha⁻¹.

From the analysis of the graphic distribution of the GLT, PRO and ZEL indices, a wider variation of the index values was found in relation to nitrogen (N), than in relation to Super Fifty (SF).

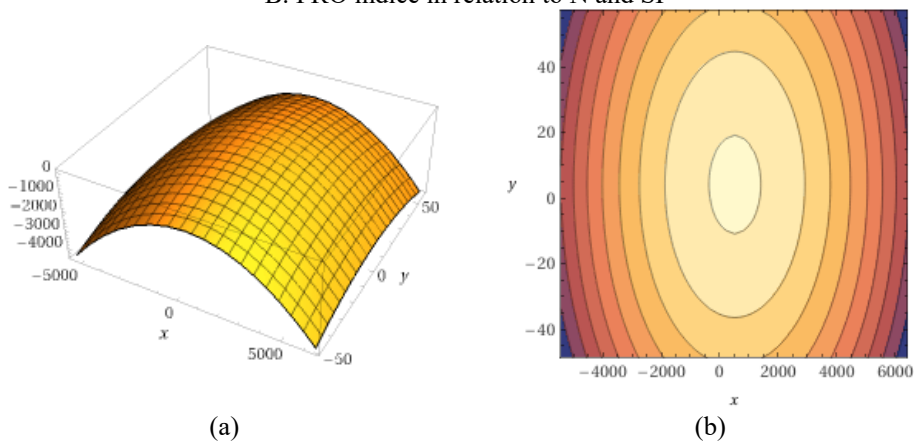
From the analysis of the coefficient values of Equation (1), and Table 4, it was found that the two factors (N, SF) presented divergent action in the formation of the values of starch content (ST), and hectoliter weight (HW). The graphic expression of variation of the two indices in relation to N and SF is presented in Figure 4.



A. GLT indice in relation to N and SF



B. PRO indice in relation to N and SF



C. ZEL indice in relation to N and SF

Figure 3. Graphical expression of GLT, PRO and ZEL indices in relation to N (x – axis) and SF (y – axis); (a) – 3D model, (b) model in isoquants format

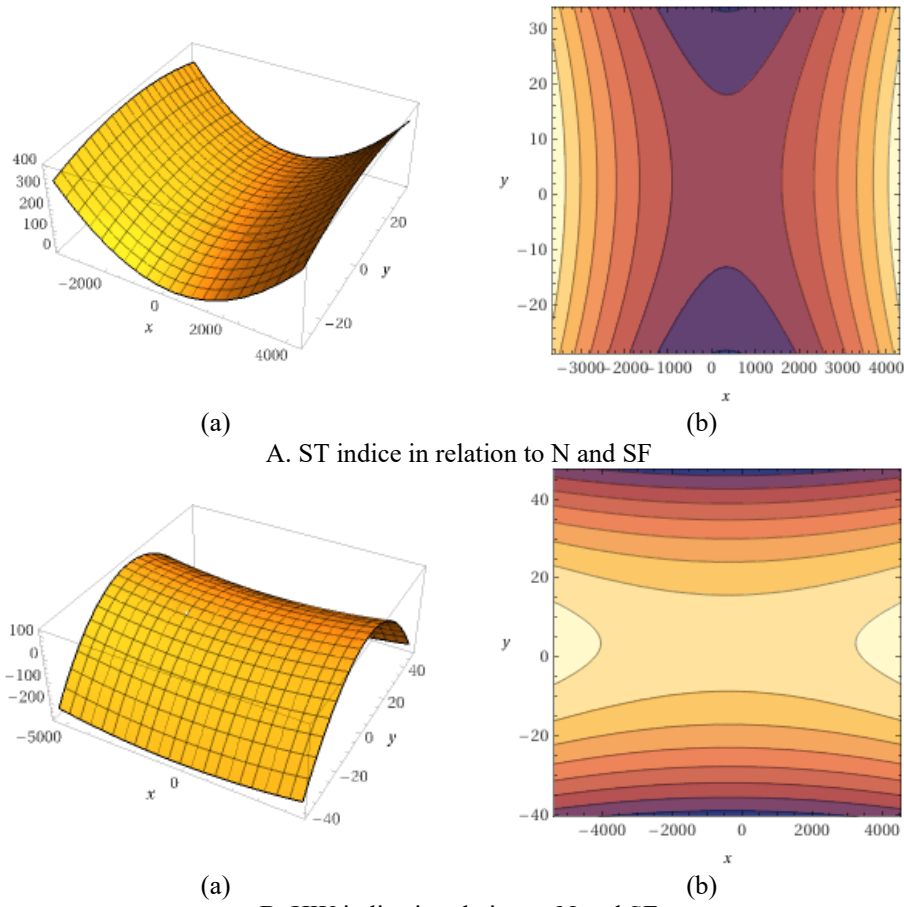


Figure 4. Graphical expression of ST and HW indices in relation to N (x – axis) and SF (y – axis); (a) – 3D model, (b) model in isoquant format

Table 4. Factor loadings in the principal components

Factors and indices	PC1	PC2	Uniqueness
N	0.984		0.028
PRO	0.943		0.037
GLT	0.935		0.024
ZEL	0.921		0.050
ST	-0.911		0.089
SF		0.880	0.222
HW		0.877	0.227
Moist			0.847

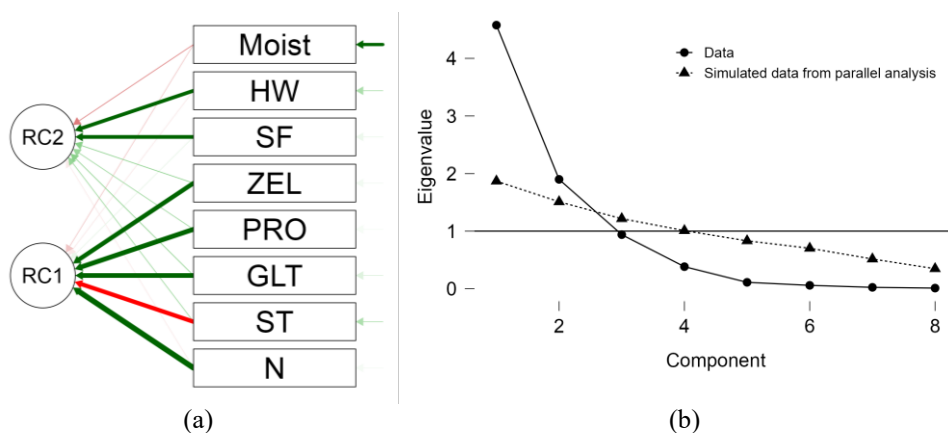


Figure 5. PCA diagrams; (a) path diagram – factor loadings on the principal components, (b) scree plot – graphical distribution of the Eigenvalue – Component interaction

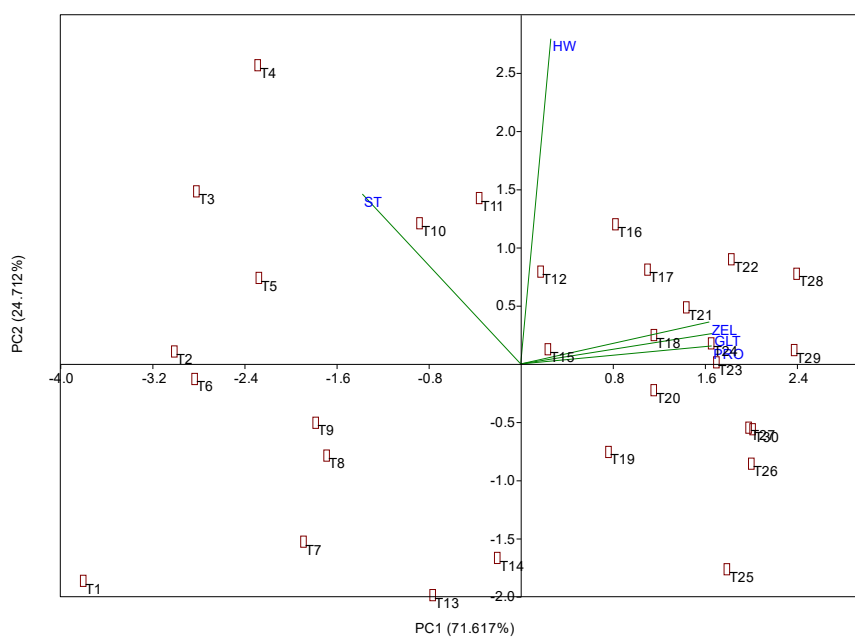


Figure 6. PCA diagram with the variants distribution in relation to quality indices

From the analysis of the graphic distribution, it was observed that the ST index had higher amplitude of variation in relation to nitrogen, compared to the Super Fifty biofertilizer. In the case of the HW index, higher amplitude of variation was observed in relation to SF than to the nitrogen. The results showed that a differentiated approach to nitrogen fertilization and foliar fertilization with Super Fifty is necessary to optimize the starch content (ST) and hectoliter weight

(HW) in wheat, under the study conditions.

Multivariate analysis was applied to explain the positioning of the influencing factors (N, SF) and the quality indices (ST, GLT, PRO, ZEL, HW, Moist) in relation to the principal components, Table 4, and Figure 5. In PC1, the N factor was loaded, with a very strong positive effect ($r=0.984$), associated with the PRO ($r=0.943$), GLT ($r=0.935$), ZEL ($r=0.921$), and ST ($r=-0.911$) indices. In PC2, SF was loaded with a strong effect ($r=0.880$) associated with HW ($r=0.877$). Humidity (Moist) was positioned independently, with $r=0.847$. The distribution of variants in relation to quality indices is presented in Figure 6, in which the main two components explained 96.339% of total variance. Three directions of orientation of quality indices were observed, with independent positions of ST and HW indices, and grouped PRO, GLT and ZEL indices.

Cluster analysis generated the grouping of variants based on similarity in relation to the overall values of the quality indices within each variant (Coph.corr. = 0.791), Figure 7.

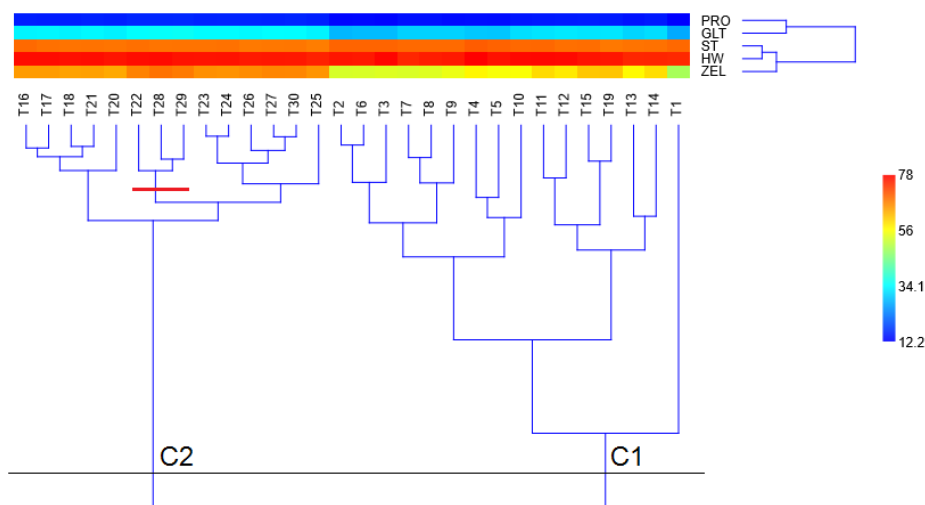
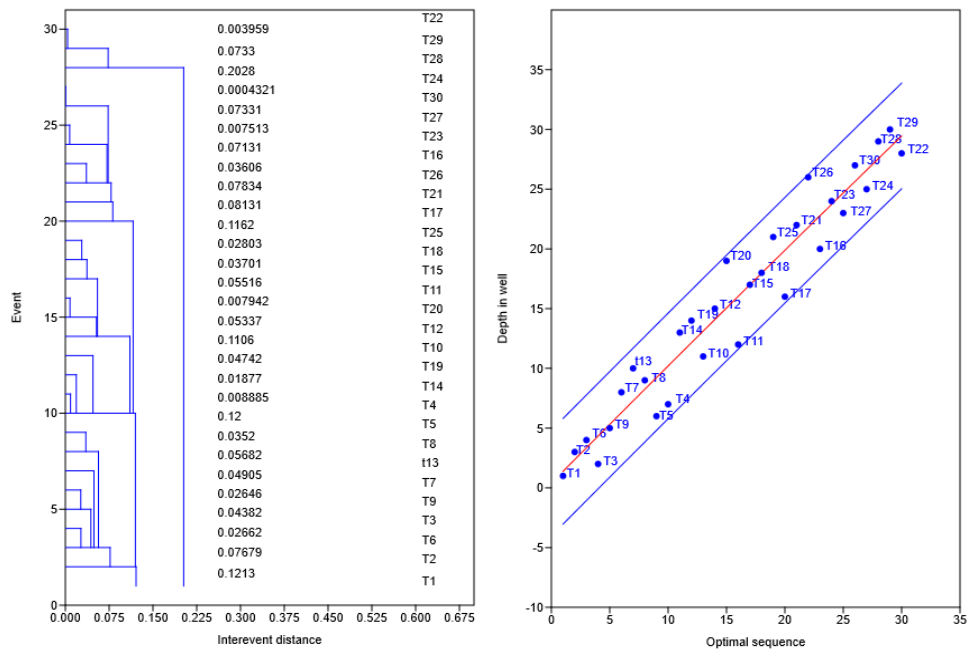


Figure 7. Cluster dendrogram based on Euclidean distances, in relation to quality indices, 'Alex' wheat cultivar

Two distinct clusters resulted (C1, and C2), and each cluster contained several subclusters. The independent position was occupied by variant T1, in cluster C1. The variants with the best and balanced values for the quality indices considered, occupied the median position in cluster C2, subcluster (T22,(T28,T29)), Figure 7 (marked with a red line). The highest level of similarity was recorded between variants T23 and T24 with $SDI=0.3150$, followed by variants T27 with T30, $SDI=0.3297$, cluster C2 (SDI – Similarity and Distance Indices).

Based on the values of the quality indices, the ranking of the experimental

variants was evaluated. The result was a ranking diagram of the variants, Figure 8(a), with the scattergram distribution of the variants in relation to the protein content, Figure 8(b). In the upper positions of the ranking diagram (upper quartile) the variants T22 were positioned, followed by T29, T28, T24, T30, T27, and T23.



(a) Ranking of experimental variants based on quality performance (b) Scattergram distribution of experimental variants

Figure 8. Ranking of experimental variants based on quality indices (a), and distribution of variants in scattergram format with confidence interval (b)

Knowledge of technological factors, especially nutritional ones, with a decisive influence on wheat plants and wheat quality indices was of interest in relation to the optimization of crop technology, wheat processing and the quality of finished products (Sheng *et al.*, 2022; Khalid *et al.*, 2023; An *et al.*, 2024).

Luo *et al.* (2021) recorded variations in physiological processes (e.g. photosynthetic rate) and agronomic parameters in wheat plants in relation to nitrogen rate. The level of 120 kg ha⁻¹ N positively influenced the light interception rate, photosynthetic rate, biomass and yield in wheat (Luo *et al.*, 2021). An increase in wheat grain quality, especially protein, associated with nitrogen rate (range of N rate variation between 0 and 360 kg ha⁻¹) has been reported, even if no yield variations were recorded (Walsh *et al.*, 2022). The rate of 225 kg ha⁻¹ N was reported as favorable for photosynthetic processes and physiological parameters in wheat (Kubar *et al.*, 2022). Nitrogen rates of 100–200 kg ha⁻¹ have been reported to be optimal in relation to yield, grain protein content and water utilization in wheat (Wang *et al.*, 2023). In relation to the

thermal threshold of 14°C, yield utilized N rates better at lower rates, and protein utilized N rates better at higher rates (Wang *et al.*, 2023).

Noor *et al.* (2023) recorded differential effect of nitrogen rate on water accumulation (favorable N rate 210 kg ha⁻¹), and dry matter accumulation (favorable N rate 150 kg ha⁻¹) in wheat at different growth stages. The rate of 210 kg ha⁻¹ N reduced gliadin content in grains compared to the rate of 180 kg ha⁻¹ N, which presented yield and ecological advantages (Noor *et al.*, 2023). Significant increases in protein and starch content were recorded in wheat in relation to nitrogen fertilization and organic fertilization (Jin *et al.*, 2024). An *et al.* (2024) reported optimal nitrogen rate between 240 and 300 kg ha⁻¹, in relation to wheat grain protein, in relation to the study conditions. The authors showed that the nitrogen fertilization rate depended greatly on the specific crop conditions. Three fertilization ranges were identified, with rates of 180–240 kg ha⁻¹ nitrogen rate, under climate and soil conditions above 100 m altitude (N-W China area), rates of 240–300 kg ha⁻¹ nitrogen rate under soil conditions with moderate nutrient content (S-E China area), and over 300 kg ha⁻¹ nitrogen rate under soil conditions with low nutrient content, on lands at altitudes below 100 m (An *et al.*, 2024). Chadda *et al.* (2025) reported nitrogen fertilization options that generated high protein content in grains (e.g. 15.3%), respectively with optimal values for specific grains gravity (80.7 kg hl⁻¹), under semi-arid cultivation conditions.

The applied statistical analyses explained in this study the interdependence between the dependent variables (quality indices) and the independent variables (N, SF). Based on the values of the correlation coefficient, the ST, GLT, PRO and ZEL indices showed close interdependence with the N rate, and the HW indicator showed close interdependence with SF. Quadratic regression analysis explained that for the GLT, PRO and ZEL indices, the independent variables N, SF showed convergent action and can be optimized concomitantly, and for the ST and HW indicators they showed divergent action and require an independent approach (e.g. optimizing SF on each N rate).

The positioning of the two independent variables (N, and SF) in relation to the principal components (N in PC1, very strong positive action; SF in PC2, strong positive action) confirmed their mode of action. The positioning of the ST indicator in PC1, with negative action ($r = -0.911$), and of the HW indicator in PC2, with positive action ($r = 0.877$), explained their differential association with the two independent variables. Under the conditions of the present study, nitrogen fertilization rates of 212.52 kg ha⁻¹ and 222.75 kg ha⁻¹, associated with algae-based biofertilizer Super Fifty, at a level of 3.00 – 4.03 L ha⁻¹, resulted to be optimal for gluten content and protein content of the 'Alex' wheat cultivar.

CONCLUSIONS

Quality indices of wheat grains, 'Alex' wheat cultivar recorded differentiated values in relation to the nitrogen (N) rate and the foliar biostimulator Super Fifty (SF) applied. Nitrogen showed a very strong, positive correlation with protein ($r=0.903^{***}$), and a strong positive correlation with

Zeleny ($r=0.899^{***}$), starch ($r=0.998^{***}$) and gluten ($r=0.992^{***}$). The Super Fifty biofertilizer showed a weak correlation with hectoliter weight ($r=0.595^{***}$).

Quadratic regression analysis showed that the N and SF factors presented convergent action in the variation of gluten content, protein, and Zeleny index and divergent action in the variation of starch content and hectoliter weight. In relation to gluten content, $N_{opt}=212.52 \text{ kg ha}^{-1} \text{ N a.s.}$, respectively $SF_{opt}=3.99 \text{ L ha}^{-1}$ resulted, and in relation to protein content, $N_{opt}=222.75 \text{ kg ha}^{-1} \text{ N a.s.}$, respectively $SF_{opt}=4.03 \text{ L ha}^{-1}$ resulted.

In relation to the principal components, the classification of factors and quality indices was obtained, so that in PC1 the N factor was loaded, with very strong positive action ($r=0.984$), associated with the PRO ($r=0.943$), GLT ($r=0.935$), ZEL ($r=0.921$), and ST ($r=-0.911$) indices. In PC2 the SF was loaded with strong action ($r=0.880$) associated with HW ($r=0.877$). The moisture (Moist) was positioned independently, with $r=0.847$. The results of the multivariate analysis were consistent with the results of the correlation analysis and the results of the quadratic regression analysis and, overall, explained much more clearly the interactions between the experimental variables (factors and quality indices).

Cluster analysis and ranking scaling facilitated the grouping of variants based on similarity and their value hierarchy, in relation to the recorded quality indices, and differentiated the variants positioned in the upper quartile.

Based on the results recorded in the wheat crop, the Alex variety, for protein and gluten, nitrogen doses between 212.52 and 222.75 kg ha^{-1} active substance, associated with algae-based biofertilizer Super Fifty in concentrations of 3.99 to 4.03 L ha^{-1} can be recommended.

Considering the "genotype x environment" interaction, these values are approximate, and optimization for other wheat genotypes and environmental factors, their adjustment through field experiments would be appropriate. Also, the fertilization model based on these fertilizer resources can be extended to other grassy cereal crops, but also to other crop plants.

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