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MULTIVARIATE ANALYSIS OF GRAIN QUALITY TRAITS IN OAT (*Avena sativa* L.) CULTIVARS UNDER DIFFERENT NITROGEN FERTILIZATION LEVELS

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Abstract

Understanding relationships among grain quality traits is essential for improving oat performance under varying agronomic conditions. This study used principal component analysis (PCA) to assess the multivariate structure of key physical and chemical grain quality traits in eleven oat cultivars grown under different nitrogen fertilization levels during 2023-2025. A total of 55 cultivar × fertilization combinations were evaluated for hectoliter weight, protein content, fat content, and nitrogen-related traits. The first two principal components explained 93.4% of the total variance. The first component (72.9%) was mainly associated with protein and nitrogen content, indicating the strong influence of nitrogen nutrition on grain composition. The second component (20.5%) was related to hectoliter weight and fat content, reflecting variation in physical grain quality among cultivars. PCA scores clearly differentiated cultivar–fertilization combinations, highlighting distinct cultivar responses to nitrogen supply. These findings confirm that PCA is an effective approach for identifying major determinants of oat grain quality and supports cultivar selection and nitrogen management strategies in oat production systems.

Key words*: hectoliter weight, multivariate analysis, nitrogen fertilization, oat cultivars.*

INTRODUCTION

Oat (*Avena sativa* L.) is a cereal crop of increasing importance due to its high nutritional value, adaptability to diverse agroecological conditions, and growing demand in human nutrition and functional foods. Oat grain is distinguished from other cereals by its relatively high protein content, favorable lipid composition, and the presence of bioactive compounds such as β-glucans, which contribute to health benefits including cholesterol reduction and glycemic control (Peterson, 1992; Wood, 2007; Ibrahim et al., 2020). As a result, improving oat grain quality has become a major objective in both breeding programs and agronomic management strategies. Grain quality in oat is a complex trait influenced by genetic background, environmental conditions, and crop management practices. Key physical and chemical parameters, such as hectoliter weight, protein content, fat content, and nitrogen concentration, are commonly used to evaluate oat quality for food, feed, and industrial purposes (Mut et al., 2018; Capouchová et al., 2021; Sitnicki et al., 2024). Understanding the relationships among these traits is essential for identifying superior cultivars and optimizing production systems. Substantial genetic variability exists among oat cultivars with respect to grain composition and physical characteristics. Several studies have demonstrated significant differences in protein, lipid, and mineral content among oat genotypes grown under similar environmental conditions (Sykut-Domańska et al., 2013; Özcan et al., 2017). This variability provides opportunities for breeding programs aimed at improving grain nutritional quality while maintaining agronomic performance. Recent research has applied multivariate and genomic approaches to better understand the genetic basis of quality traits in oat. Carlson et al. (2019) showed that multivariate genome-wide association analyses can effectively identify loci controlling seed fatty acid composition, highlighting the complexity of lipid-related traits. Similarly, Ihsan et al. (2022) reported considerable diversity in nutritional composition among oat accessions, emphasizing the usefulness of

principal component analysis (PCA) and cluster analysis for classifying genotypes based on multiple quality attributes. Nitrogen availability is one of the most important agronomic factors affecting oat grain quality, particularly protein and nitrogen content (Smuleac et al., 2020). Numerous studies have reported strong positive relationships between nitrogen fertilization and grain protein concentration, reflecting the central role of nitrogen in amino acid and protein synthesis (Allwood et al., 2023; Zhang et al., 2025). However, increased nitrogen supply may also influence other quality traits, including lipid content and physical grain characteristics. The response of oat cultivars to nitrogen fertilization is often genotype-dependent, leading to significant cultivar × nitrogen interactions (Kebede et al., 2023). Excessive nitrogen application can result in diminishing returns or undesirable trade-offs between yield and quality, underscoring the need for integrated evaluations of multiple traits (Köse, 2022). Consequently, assessing how nitrogen-related traits interact with physical grain parameters such as hectoliter weight is critical for developing efficient and sustainable fertilization strategies (Chițu et al., 2024). Hectoliter weight is a widely used physical indicator of grain density and milling quality, while protein and fat content represent major chemical attributes determining nutritional and processing value. Studies have shown that these traits are not always positively correlated, indicating complex physiological relationships during grain development (Mut et al., 2018; Capouchová et al., 2021). Protein and nitrogen content are typically strongly correlated, as nitrogen concentration is a direct determinant of protein synthesis (Ihsan et al., 2021). In contrast, fat content often shows weaker or variable relationships with protein, reflecting differences in carbon and nitrogen allocation within the grain (Ahmet et al., 2019; Banaś & Harasym, 2021). Understanding these interrelationships is essential for identifying cultivar profiles suited to specific end uses, such as food processing or feed formulation. Given the multidimensional nature of grain quality, multivariate statistical techniques have become increasingly important in oat research. Principal component analysis (PCA) is widely used to reduce data dimensionality, identify major sources of variation, and reveal relationships among traits that may not be evident through univariate analyses (Bose et al., 2014; Ihsan et al., 2022). PCA has been successfully applied to evaluate genotype performance across environments, assess quality trait associations, and discriminate cultivars based on integrated quality profiles (Köse, 2022; Yan et al., 2025). By grouping correlated variables into principal components, PCA facilitates the interpretation of complex datasets and supports decision-making in breeding and agronomic management. In oat studies, PCA has revealed that protein and nitrogen-related traits often dominate the first principal component, while physical traits such as hectoliter weight and lipid content contribute to subsequent components (Ihsan et al., 2022; Yan et al., 2025). These findings highlight the value of PCA for identifying key drivers of grain quality and for selecting cultivars with balanced trait combinations.

MATERIALS AND METHODS

Field experiments were conducted over three consecutive growing seasons (2023-2025) to evaluate the effect of nitrogen fertilization on grain quality traits of oat (*Avena sativa* L.).

The experiment was arranged in a factorial design with two experimental factors.

Factor A - Cultivar included eleven oat cultivars: a1 - Jeremy; a2 - Ovidiu; a3 - Mureșana; a4 - GK; a5 - Pillango; a6 - Prokop; a7 - Effectiv; a8 - Overdrive; a9 - Venafor; a10 - Earl; a11 - Genziana.

Factor B - Nitrogen fertilization level was applied on a uniform agrochemical background of P60K60 and consisted of five levels:

b1 - N0; b2 - N30; b3 - N60; b4 - N90; b5 - N120. The combination of the two factors resulted in a total of 55 treatment variants (11 cultivars × 5 nitrogen levels).

For the purpose of multivariate analysis, mean values across the three experimental years were calculated to reduce the influence of annual climatic variability. Grain samples were collected at harvest maturity and analyzed for key physical and chemical quality parameters. Hectoliter weight (HW) was determined and expressed in kg hL^{-1} as an indicator of grain physical quality. Protein content and fat content

were expressed as percentages of dry matter. Nitrogen fertilization level was included as a quantitative explanatory variable reflecting nitrogen input intensity. These parameters were selected due to their agronomic, nutritional, and technological importance in oat production.

Prior to multivariate analysis, descriptive statistics were calculated for all measured variables. Principal component analysis (PCA) was performed using the correlation matrix to standardize the data and eliminate scale effects among variables.

Eigenvalues, explained variance, and cumulative variance were used to determine the number of significant principal components. The interpretation of PCA results was based on factor loadings, eigenvectors, variable contributions, and factor scores. Biplots were generated to illustrate the relationships among variables and to visualize the distribution of cultivar × nitrogen fertilization combinations within the space defined by the first two principal components. All statistical analyses were carried out using STATISTICA software**.**

RESULTS AND DISCUSSIONS

Explained variance and eigenvalue structure of the PCA

Principal component analysis (PCA) applied to the grain quality dataset revealed a strong dimensionality reduction. As shown in Table 1, the first principal component (PC1) had an eigenvalue of 2.916 and explained 72.90% of the total variance, while the second principal component (PC2) had an eigenvalue of 0.820 and explained an additional 20.50%. Together, PC1 and PC2 accounted for 93.41% of the cumulative variance, indicating that these two components captured nearly all the information contained in the original variables.

Table 1. Eigenvalues of correlation matrix, and related statistics

Component	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	2.916188	72.90471	2.916188	72.9047
2	0.820120	20.50301	3.736309	93.4077
3	0.215058	5.37645	3.951367	98.7842
4	0.048633	1.21584	4.000000	100.0000

The scree plot of eigenvalues (Figure 1) displayed a sharp decline after PC1 and a clear leveling-off after PC2, confirming that the retention of the first two components is statistically justified. The third and fourth components explained only 5.38% and 1.22% of the variance, respectively, and therefore had limited biological and agronomic relevance.

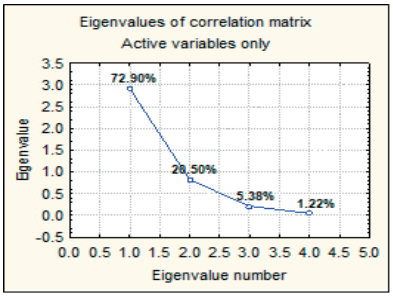

Figure 1. The scree plot of eigenvalues

Factor loadings and variable correlations with principal components

The correlations between the original grain quality variables and the principal components are presented in Table 2.

PC1 showed strong negative correlations with nitrogen (−0.983), protein content (−0.939), fat content (−0.823), and hectoliter weight (−0.625). The magnitude of these loadings indicates that PC1 represents a general grain composition axis, dominated primarily by nitrogen and protein accumulation.

Table 2. Factor-variable correlations, based on correlations

Variable [parameter]	Factor 1 [CP1]	Factor 2 [CP2]	Factor 3 [CP3]	Factor 4 [CP4]
HW	-0.625212	0.762461	0.161254	-0.041954
Fat	-0.822974	-0.485186	0.287421	-0.068534
Protein	-0.939381	-0.047444	-0.324583	-0.099793
Nitrogen	-0.982637	-0.033416	-0.033025	0.179493

In contrast, PC2 was strongly and positively correlated with hectoliter weight (0.762) and negatively correlated with fat content (−0.485), while protein and nitrogen exhibited negligible correlations with this component.

This statistical structure suggests that PC2 primarily reflects physical grain quality, largely independent of nitrogen-related chemical traits.

Contribution of variables to principal components

The relative contribution of each variable to the principal components is detailed in Table 3 and

graphically illustrated in Figures 2 and 3. Nitrogen (33.11%) and protein content (30.26%) were the dominant contributors to PC1, followed by fat content (23.23%) and hectoliter weight (13.40%).
These results confirm that PC1 is mainly driven by nitrogen nutrition and its effect on grain protein synthesis.

Table 3.Variable contributions, based on correlations

Variable [parameter]	Factor 1 [CP1]	Factor 2 [CP2]	Factor 3 [CP3]	Factor 4 [CP4]
HW	0.134041	0.708856	0.120910	0.036192
Fat	0.232251	0.287038	0.384133	0.096579
Protein	0.302599	0.002745	0.489886	0.204771
Nitrogen	0.331109	0.001362	0.005071	0.662458

For PC2, hectoliter weight accounted for 70.89% of the total contribution, while fat content contributed 28.70%. Protein and nitrogen contributed less than 1% each, reinforcing the interpretation of PC2 as a physical quality component.
The contrasting contribution patterns between PC1 and PC2 highlight the multidimensional nature of oat grain quality.

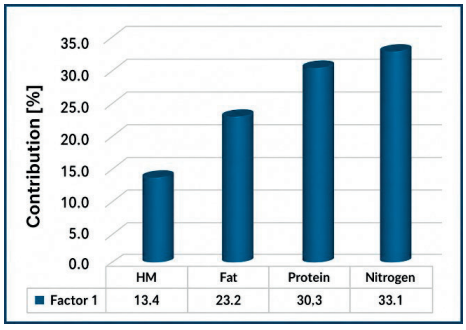

Figure 2. Contribution of variables to CP1

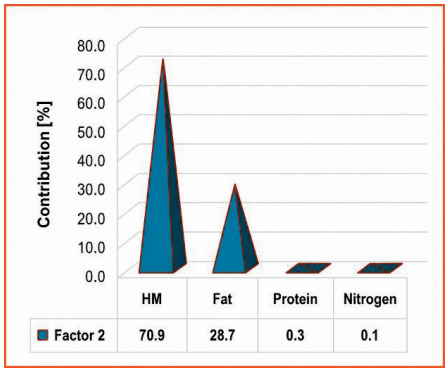

Figure 3. Contribution of variables to CP2

Projection of variables in the factorial plane (PC1 × PC2)

The projection of variables in the factorial plane defined by PC1 and PC2 (Figure 4) provides a visual representation of the relationships among grain quality traits. Nitrogen and protein variables were closely grouped and oriented along the negative direction of PC1, confirming their strong positive association. Fat content was positioned between PC1 and PC2, indicating partial dependence on both chemical composition and physical grain attributes. Hectoliter weight was oriented almost exclusively along the positive direction of PC2, demonstrating its independence from nitrogen and protein accumulation. This spatial separation suggests that increasing nitrogen fertilization enhances protein content but does not necessarily improve physical grain quality, emphasizing a potential trade-off between chemical and physical traits.

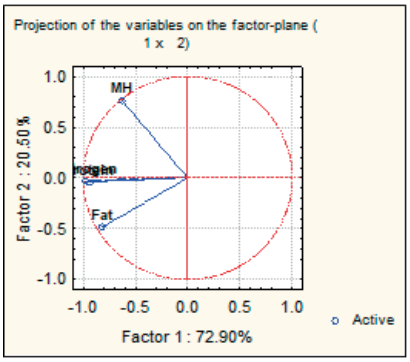

Figure 4. Projection of variables in the plane of the factorial axes of the two main components [Factor 1/CP1 and Factor 2/CP2]

Distribution and contribution of cultivar × nitrogen fertilization combinations

The projection of the 55 cultivar × nitrogen fertilization combinations in the PC1–PC2 plane is shown in Figure 5. A wide dispersion of treatments along both principal components was observed, indicating substantial variability in grain quality responses among cultivars and fertilization levels.
The contribution of individual combinations to PC1-PC4 is reported in Table 4 and illustrated in Figures 6 and 7. Treatments with high contributions to PC1 were mainly associated with higher nitrogen fertilization levels,

reflecting increased nitrogen and protein content. In contrast, combinations contributing strongly to PC2 were characterized by higher hectoliter weight, suggesting superior physical grain quality.

This clear separation demonstrates that PCA effectively discriminates cultivar × fertilization combinations based on integrated quality profiles rather than individual traits.

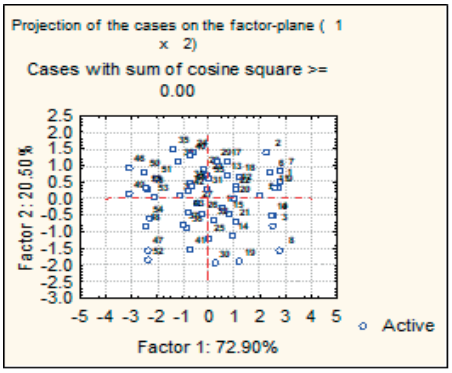

Figure 5. Case projection [combinations of variety x fertilization levels] in the plane of the factorial axes of the two main components [Factor 1/CP1 and Factor 2/CP2]

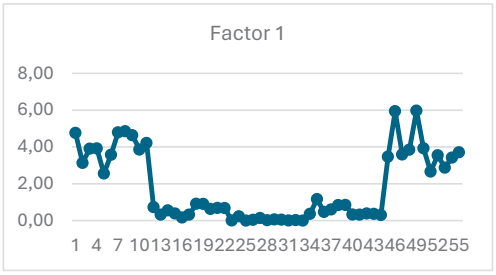

Figure 6. Contribution of combinations [variety x fertilization levels] to CP1

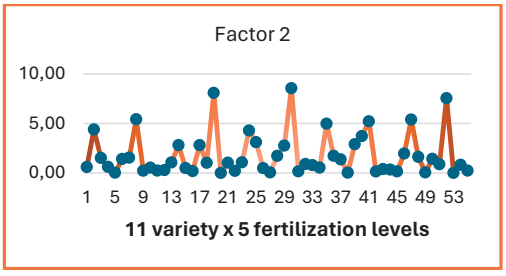

Figure 7. Contribution of combinations [variety x fertilisation levels] to CP2

Table 4. Case contributions, based on correlations

Combination [variety x fertilization levels]	Factor 1 [CP1]	Factor 2 [CP2]	Factor 3 [CP3]	Factor 4 [CP4]
1	4.7657	0.6028	0.5149	2.5720
2	3.1327	4.3932	0.0016	0.2882
3	3.9086	1.5172	0.1861	0.0120
4	3.9203	0.6108	2.6286	0.1356
5	2.5568	0.0236	4.4303	2.1152
6	3.5778	1.4085	0.1159	0.0122
7	4.7934	1.5444	0.0105	2.4787
8	4.8561	5.4233	0.4197	0.7872
9	4.6398	0.2277	1.3592	2.1656
10	3.8598	0.5384	2.6964	0.0944
11	4.2135	0.2377	5.3510	0.0777
12	0.7315	0.2896	0.0891	0.2252
13	0.3232	1.0605	0.3031	4.4485
14	0.5535	2.8116	0.5045	2.8291
15	0.3866	0.4937	2.2930	3.0988
16	0.1676	0.1895	6.0281	7.2920
17	0.3202	2.8188	0.0635	3.7951
18	0.9090	1.0250	0.0027	0.0075
19	0.9020	8.1030	0.1312	0.5028
20	0.6270	0.0010	2.2766	0.4807
21	0.6866	1.0391	2.5679	1.5885
22	0.6769	0.2125	8.5869	1.5902
23	0.0020	1.0718	1.2090	0.0217
24	0.2320	4.2959	0.0077	4.9943
25	0.0003	3.1109	0.2974	0.1126
26	0.0378	0.4875	4.1048	0.6749
27	0.1354	0.0542	4.3107	2.8093
28	0.0187	1.7345	0.2423	0.3540
29	0.0595	2.7592	0.1420	2.9386
30	0.0433	8.5657	0.0063	0.3358
31	0.0010	0.1608	0.9709	0.0075
32	0.0237	0.9035	3.1568	0.1154
33	0.0001	0.7807	5.3478	0.0444
34	0.3598	0.5332	0.9697	5.5009
35	1.1606	4.9785	0.0855	0.0502
36	0.4669	1.7284	0.2265	1.9996
37	0.6021	1.3772	1.0724	1.0738
38	0.8396	0.0232	3.9048	0.4727
39	0.8501	2.9071	0.1010	0.2740
40	0.3271	3.7329	1.1575	7.5009
41	0.3212	5.2270	0.0073	3.1257
42	0.3869	0.1461	1.1347	4.7258
43	0.3630	0.3884	4.0005	2.3392
44	0.2974	0.3353	3.9321	4.2817
45	3.4688	0.1527	0.3596	0.3733
46	5.9422	1.9695	5.8200	8.4315
47	3.5861	5.3974	0.8594	0.1198
48	3.8477	1.6190	1.9166	0.0094
49	5.9722	0.0517	5.2200	5.2536
50	3.9295	1.4214	0.0224	0.0011
51	2.6603	0.8799	0.2681	3.1478
52	3.5505	7.5675	0.0277	0.0300
53	2.8716	0.0079	0.4830	2.2243
54	3.4221	0.8245	3.1163	0.0000
55	3.711880	0.234755	4.958246	0.058982

Eigenvectors, component equations, and factor scores

The eigenvectors of the correlation matrix are presented in Table 5, providing further insight into the structure of the principal components. The eigenvector coefficients confirm the dominant negative weights of nitrogen and protein in PC1 and the strong positive weight of hectoliter weight in PC2.

Table 5. Eigenvectors of correlation matrix

Variable [parameter]	Factor 1 [CP1]	Factor 2 [CP2]	Factor 3 [CP3]	Factor 4 [CP4]
HW	-0.366117	0.841936	0.347722	-0.190243
Fat	-0.481924	-0.535759	0.619785	-0.310771
Protein	-0.550090	-0.052389	-0.699918	-0.452516
Nitrogen	-0.575420	-0.036900	-0.071213	0.813916

Based on these eigenvectors, the component equations (Z1 and Z2) describe the multivariate relationships among traits and quantify their relative importance. The factor score coefficients for variables (Table 6) and for treatment combinations (Table 7) allowed the precise positioning of cultivars and fertilization levels within the multivariate space.

$\mathbf{Z_1} = -0.366117*x_1 - 0.481924*x_2 - 0.550090*x_3 - 0.575420*x_4$
$\mathbf{Z_2} = 0.841936*x_1 - 0.535759*x_2 - 0.052389*x_3 - 0.036900*x_4$
where: x1-HW; x2-Grasimi; x3-protein; x4-fertilize N

Table 6. Factor score coefficients, based on correlations for variables

Variable [parameter]	Factor 1 [CP1]	Factor 2 [CP2]	Factor 3 [CP3]	Factor 4 [CP4]
HW	-0.214394	0.929694	0.74981	-0.86266
Fat	-0.282209	-0.591604	1.33648	-1.40920
Protein	-0.322126	-0.057850	-1.50928	-2.05195
Nitrogen	-0.336959	-0.040746	-0.15356	3.69073

Table 7. Factor score coefficients, based on correlations for cases [variant]

Variable [parameter]	Factor 1 [CP1]	Factor 2 [CP2]	Factor 3 [CP3]	Factor 4 [CP4]
1	1.60421	0.57051	0.52731	1.17850
2	1.30064	1.54023	0.02921	-0.39450
3	1.45280	-0.90516	-0.31704	-0.08044
4	1.45497	-0.57429	1.19141	0.27059
5	1.17502	0.11278	1.54673	-1.06874
6	1.38996	0.87213	-0.25016	-0.08128
7	1.60887	0.91324	-0.07545	1.15693
8	1.61935	-1.71132	-0.47606	0.65197
9	1.58288	0.35063	0.85673	1.08141
10	1.44370	-0.53921	-1.20667	-0.22583
11	1.50841	0.35824	-1.69986	0.20479
12	0.62852	0.39543	0.21933	-0.34869
13	0.41778	0.75676	-0.40454	-1.54990
14	0.54670	-1.23218	-0.52197	-1.23600
15	0.45690	-0.51633	1.11276	-1.29358
16	0.30080	-0.31987	1.80421	-1.98436
17	0.41580	1.23375	-0.18511	-1.43155
18	0.70061	0.74398	-0.03823	0.06348
19	0.69793	-2.09180	-0.26613	-0.52106
20	0.58189	0.02380	1.10876	-0.50950
21	0.60890	-0.74906	-1.17757	-0.92617
22	0.60461	0.33878	-2.15336	-0.92667
23	-0.03324	0.76078	0.80801	0.10834
24	-0.35394	1.52309	0.06465	-1.64222
25	0.01251	-1.29611	-0.40074	-0.24656
26	-0.14286	-0.51309	1.48882	-0.60368
27	-0.27044	-0.17103	1.52570	-1.23167
28	-0.10062	0.96780	-0.36175	-0.43724
29	0.17929	1.22065	0.27687	1.25971
30	0.15289	-2.15070	-0.05837	0.42584
31	-0.02274	0.29468	0.72406	0.06359
32	0.11323	-0.69848	-1.30563	0.24964
33	0.00673	0.64929	-1.69936	-0.15491
34	-0.44077	0.53657	0.72363	1.72352
35	-0.79165	1.63963	-0.21493	-0.16470
36	-0.50212	-0.96608	0.34976	1.03913
37	-0.57018	-0.86237	0.76100	0.76147
38	-0.67335	0.11188	1.45209	0.50523
39	-0.67752	1.25294	-0.23357	0.38466
40	-0.42026	1.41979	0.79059	2.01258
41	-0.41647	-1.68005	-0.06271	1.29919
42	-0.45709	0.28085	0.78279	1.59748
43	-0.44276	-0.45795	-1.46978	1.12392
44	-0.40077	0.42554	-1.45716	1.52057
45	-1.36862	0.28714	0.44067	0.44897
46	-1.79131	1.03127	-1.77279	-2.13378
47	-1.39157	-1.70722	-0.68122	-0.25437
48	-1.44144	-0.93501	1.01735	-0.07109
49	-1.79583	0.16704	1.67892	-1.68433
50	-1.45669	0.87609	-0.11009	-0.02486
51	-1.19856	0.68930	-0.38052	1.30377
52	-1.38466	-2.02150	0.12224	-0.12720
53	-1.24526	0.06544	0.51072	1.09595
54	-1.35938	-0.66727	-1.29724	0.00214
55	-1.41577	0.35604	-1.63629	-0.17847

The factor scores revealed that nitrogen fertilization intensity exerted a stronger influence on PC1, while cultivar-specific characteristics were more influential along PC2. This distinction emphasizes the combined role of agronomic management and genetic background in determining oat grain quality.

The integrated interpretation demonstrates that nitrogen fertilization is the main driver of chemical grain quality traits, particularly protein and nitrogen content, while physical quality traits such as hectoliter weight are more strongly influenced by cultivar characteristics. The clear separation of these trait groups suggests that optimizing oat grain quality requires balanced strategies combining appropriate nitrogen inputs with cultivar selection.

Overall, the PCA approach proved to be a powerful tool for identifying key sources of variability in oat grain quality and for supporting decision-making in breeding programs and nitrogen management strategies.

CONCLUSIONS

The multivariate analysis of oat grain quality traits demonstrated that principal component analysis (PCA) is an effective tool for integrating physical and chemical parameters and for discriminating cultivar responses to nitrogen fertilization. The first two principal components explained over 93% of the total variability, confirming that a limited number of

components can adequately describe the complex structure of oat grain quality.

Nitrogen and protein content were the dominant contributors to the first principal component, highlighting the central role of nitrogen fertilization in shaping grain chemical composition. The strong association between these traits confirms that increased nitrogen supply primarily enhances protein accumulation in oat grain. In contrast, hectoliter weight contributed mainly to the second principal component, indicating that physical grain quality is largely independent of nitrogen-driven chemical traits and is more strongly influenced by cultivar characteristics.

The clear separation of grain quality traits into chemical and physical dimensions revealed potential trade-offs between maximizing protein content and maintaining favorable physical grain properties. The dispersion of cultivar × nitrogen fertilization combinations in the factorial plane demonstrated differential cultivar responses to nitrogen input, emphasizing the importance of genotype-specific nitrogen management.

Overall, the results underline the necessity of integrated agronomic strategies that combine appropriate nitrogen fertilization with cultivar selection to achieve balanced oat grain quality. The PCA-based approach provides valuable support for breeding programs and sustainable nitrogen management in oat production systems.

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