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MULTIVARIATE EVALUATION OF MORPHOLOGICAL TRAITS IN WHEAT UNDER CONTRASTING NITROGEN REGIMES FOR IDENTIFYING NITROGEN-EFFICIENT GENOTYPES

Divya Sharma[1], B.K Sinha[1], Gurdev Chand[1], Swati[1], Marvi Sharma[1], Mandeep Kour[1], Monika Kumari[1], Jyotsana Kalsi[1], Muneeba Banoo[1], Farzana Kouser[1], Sapalika Dogra[2] and Nikhil Thakur[3]*
[1]Division of Plant Physiology, Faculty of Basic Sciences, *SKUAST-J,* Chatha, Jammu- 180009, UT of J&K, India
[2]Division of Plant Physiology, Faculty of Basic Sciences, SKUAST-Kashmir, India.
[3]Division of Fruit Science, Faculty of Horticulture and Forestry, SKUAST-J, Chatha, Jammu- 180009, UT of J&K, India
*Corresponding author E-mail: nikhilthakur1024@gmail.com
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ABSTRACT

Nitrogen is a critical nutrient influencing the morphological performance and yield potential of wheat. This study aimed to assess the variability in key morphological traits among 26 wheat genotypes under contrasting nitrogen regimes using multivariate analysis. The experiment was conducted in a randomized block design with three replications and two nitrogen treatments: control (no nitrogen) and treated (120 kg N/ha). Morphological traits including plant height, number of productive tillers per plant, leaf area, and total biomass at booting were recorded and analysed. Significant genotypic and treatment effects were observed across all traits, with superior performance under nitrogen-fertilized conditions. Multivariate analysis through pair plot visualization revealed strong interrelationships among traits, particularly between plant height and biomass accumulation. Leaf area and tiller number also exhibited positive associations under both nitrogen levels, although treatment effects were distinctly visible in their distribution patterns. The study highlights the utility of pair plot analysis for trait-based selection and genotype differentiation under nutrient stress. These findings contribute to identifying nitrogen-responsive genotypes for sustainable wheat improvement.

***Keywords* :** Plant height, tiller number, leaf area, total biomass, multivariate analysis, genotypic variability.

Introduction

Wheat (*Triticum aestivum* L.) is a major cereal crop cultivated globally, serving as a staple food for a large portion of the world’s population. With increasing global food demands and shrinking agricultural resources, enhancing wheat productivity under nutrient-limited conditions has become a major objective in crop improvement programs. Among essential nutrients, nitrogen (N) plays a crucial role in the structural and functional development of plants, influencing key processes such as chlorophyll biosynthesis, cell division, and protein formation (Fageria *et al.,* 2010; Xu *et al.,* 2012). However, nitrogen use efficiency (NUE) in wheat remains suboptimal, primarily due to unbalanced fertilizer applications, poor uptake efficiency, and environmental losses (Hirel *et al.,* 2007).

Morphological characteristics such as plant height, leaf area, number of productive tillers, and total biomass are directly linked to nitrogen availability and reflect the physiological performance of a genotype under varying nutrient conditions. These traits are not only critical for canopy development and photosynthetic efficiency but also influence spike emergence, sink capacity, and ultimately grain yield (Rafii and Shamsuzzaman, 2017). Under nitrogen-deficient conditions, a considerable reduction in tiller number, biomass accumulation, and leaf area has been reported, which restricts the plant’s potential to fully exploit its genetic yield capability (Mahajan and Gupta, 2009).

Given the complexity of plant responses to nitrogen, multivariate statistical approaches offer robust tools for dissecting the interactions among multiple traits and treatments. Visual methods like pair plot analysis allow for simultaneous observation of inter-trait relationships, treatment effects, and distribution patterns, making them especially useful in genotype-by-environment interaction studies. These plots integrate scatterplots and kernel density curves to identify positive or negative trait associations while differentiating treatment responses (Takahashi and Takahashi, 2023; Yan & Rajcan, 2002).

In this context, the present investigation was carried out to evaluate the morphological performance of 26 wheat genotypes under two contrasting nitrogen regimes (control and 120 kg N/ha). The objective was to explore the multivariate relationships among four key morphological traits plant height, leaf area, number of productive tillers per plant, and total biomass at booting using pair plot analysis. The results aim to identify nitrogen-responsive genotypes and trait associations that can be exploited in future breeding programs for improving nitrogen use efficiency and yield stability.

Material and Methods

Experimental Site and Genotypic Material

The field investigation was carried out during the rabi season of 2021–22 and 2022-23 at the research farm of Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu (SKUAST-Jammu), located in the subtropical region of the Jammu division (India). The experimental site is characterized by loamy soil, moderate organic carbon content, and low to medium nitrogen availability. The climate of the region is semi-arid, with cool winters and moderate rainfall during the growing season.

A set of twenty-six genetically diverse wheat (*Triticum aestivum* L.) genotypes was selected for evaluation. The genotypes comprised both released cultivars and advanced breeding lines, representing a wide range of morphological variability and potential nitrogen responsiveness. These genotypes were chosen to assess differential responses to nitrogen regimes based on their prior agronomic performance and breeding background.

Experimental Design and Treatments

The experiment was laid out in a two-factorial Randomized Block Design (RBD) with three replications. The two treatment factors were:

T1 – Nitrogen-stressed (Control): Plots received single super phosphate (SSP) and muriate of potash (MOP) only, without any nitrogen supplementation.

T2 – Nitrogen-fertilized (Treated): Plots were supplied with 120 kg N/ha using urea (in three equal splits), along with basal application of DAP, MOP, and SSP.

Nitrogen in the treated plots was applied in **three equal splits**: at sowing, crown root initiation (CRI), and booting stage. Standard agronomic practices, including seed rate, spacing, irrigation, and pest management, were uniformly followed across all treatments to minimize non-nutritional variability.

Morphological traits recorded

The following morphological attributes were assessed during the crop growth cycle:

1. **Plant Height (cm):** Measured at physiological maturity from the soil surface to the tip of the terminal spike (excluding awns) using a meter scale.
2. **Number of Productive Tillers per Plant:** Counted manually at the booting stage from five randomly selected plants per plot.
3. **Leaf Area per Plant (cm^2):** Estimated using the graph paper tracing method, where the area of the leaf lamina was outlined and measured to obtain total leaf area per plant.
4. **Total Biomass at Booting (g/m^2):** Recorded as the fresh weight of the aboveground plant parts sampled from a 1 m^2 area within each plot at the booting stage.

All observations were replicated and averaged to obtain reliable trait values for subsequent analysis.

Statistical and Multivariate Analysis

Statistical analyses were conducted to evaluate genotypic responses to contrasting nitrogen regimes. Two-way ANOVA revealed highly significant effects ($p < 0.01$) of genotype, treatment, and their interaction for all key morphological traits plant height, number of productive tillers, leaf area, and total biomass at booting indicating substantial genetic variability and nitrogen sensitivity.

To explore multivariate trait relationships, Principal Component Analysis (PCA) was applied. The first two principal components explained >98% of the total variation in all traits. Scree plots confirmed the sufficiency of PC1 and PC2 for interpretation. PCA biplots and correlation circles showed clear separation of genotypes under nitrogen-stressed and control conditions. Feature loading heatmaps indicated strong positive loadings for control treatments on PC1, while treated plants contributed more to PC2. Density overlays and pair plots highlighted clusters of genotypes with contrasting nitrogen responses (Sakin *et al.,* 2011; Karkaji *et al.,* 2023).

PCA effectively identified genotypic patterns and major contributing traits under nitrogen variability, supporting selection of nitrogen-efficient wheat lines for sustainable production.

Results and Discussion

Plant Height

Plant height was significantly influenced by both genotype and nitrogen regime, with a marked reduction observed under nitrogen stress (Ali *et al*., 2005). Genotypic means ranged from 73.00 cm (Ajanta) to 141.33 cm (PxW273-10). The mean plant height under control (120 kg N/ha) was 106.54 cm, compared to 92.82 cm under the nitrogen-stressed treatment (0 kg N/ha). These results indicate that nitrogen availability plays a crucial role in internodal elongation and overall vegetative growth, consistent with earlier findings. (Ali *et al.,* 2018). PCA for plant height revealed that PC1 explained 91.3% of the variation, capturing the bulk of genotype and treatment-induced variability (Figure 1). The scree plot sharply plateaued after PC1, validating dimensional reduction. The PCA biplot (Figure 2) clearly separated high-performing genotypes (e.g., PxW273-10, PxCim49-265, BWL 4433) on the positive side of PC1, corresponding to higher values under control conditions (Shi *et al.,* 2022). Genotypes like PxBaj-62 and Ajanta clustered on the negative axis, indicating low performance under stress. The correlation circle (Figure 3) further illustrated directional treatment effects. Control vector aligned positively with PC1, while the treated vector opposed it reinforcing the conclusion that nitrogen availability was the primary driver of variation. The feature loading heatmap (Figure 4) showed nearly equal PC1 loadings from both control and treated values (~0.56), while PC2 showed inverse relationships (Control: –0.69, Treated: +0.73), underlining the contribution of treatment contrast to total variance. The PC1–PC3 pair plot (Figure 5) and the density scatter plot (Figure 6) reinforced these findings, with high-density genotype clusters forming around central PC1 values, and nitrogen-efficient genotypes distinctly separated.

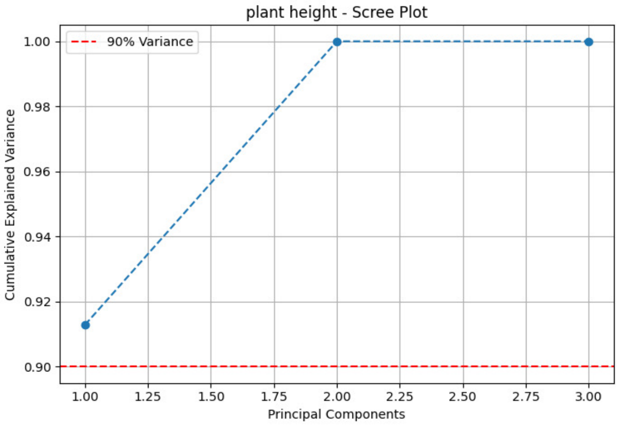

Fig. 1: Scree plot showing cumulative variance explained by the top principal components for plant height.

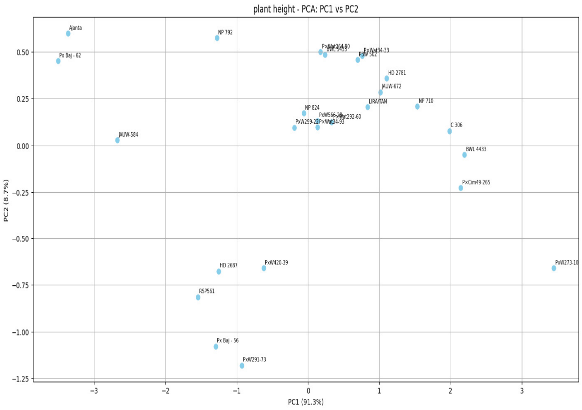

Fig. 2: PCA scatter plot (PC1 vs PC2) for plant height in wheat genotypes under control and nitrogen-stressed conditions

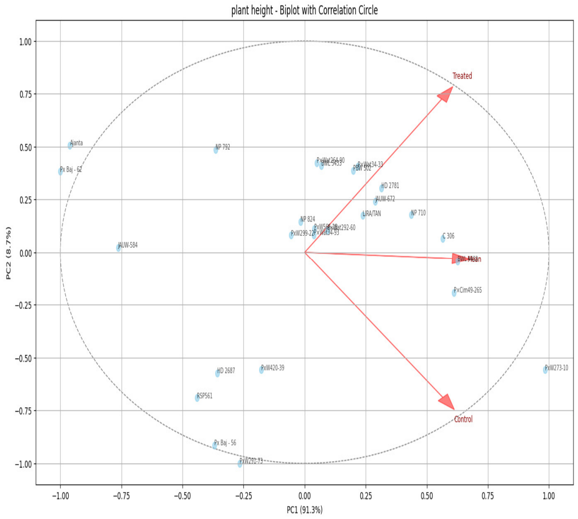

Fig. 3: Correlation circle biplot showing the relationship between plant height under different nitrogen treatments and principal components.

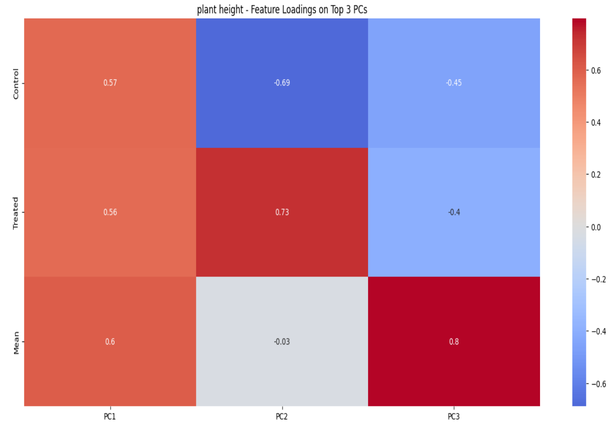

Fig. 4: Feature loadings heatmap depicting contribution of plant height (control, treated, and mean) to the top three PCs.

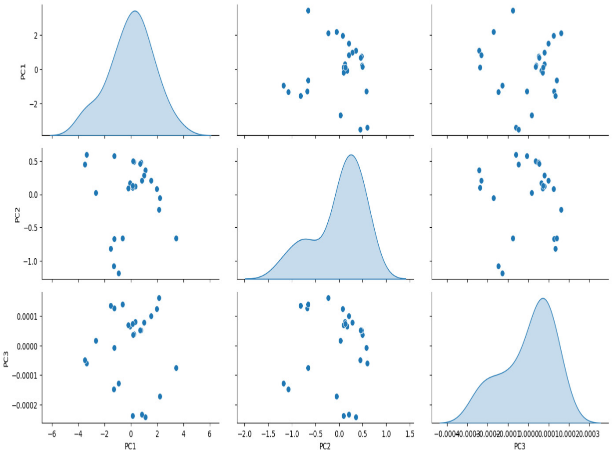

Fig. 5: Pair plot showing distribution and pairwise relationships among top three PCs for plant height

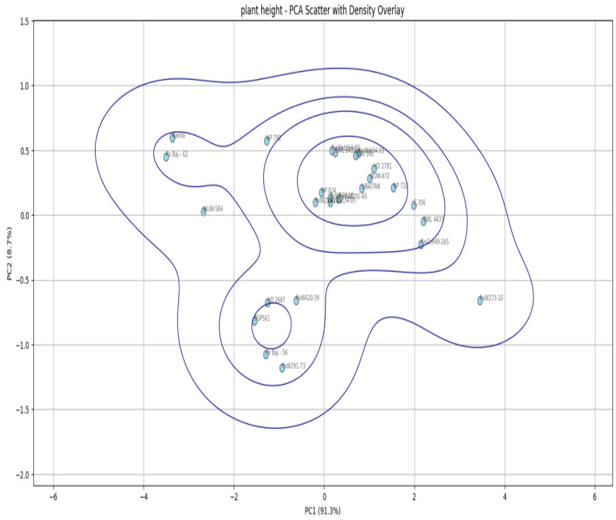

Fig. 6: PCA scatter plot with density overlay for plant height, showing clustering of wheat genotypes based on principal components

Number of productive tillers per plant

Nitrogen treatment significantly impacted tiller production. The mean number of productive tillers per plant increased from 3.28 under nitrogen stress to 4.16 under control conditions (Osman *et al.,* 2021). Genotypes such as BWL 4433, PxCim49-265, and PxW420-39 maintained higher tiller counts under both conditions, indicating strong genetic potential for tillering even in low nitrogen environments. The scree plot (Figure 7) confirmed that PC1 accounted for 94.3% of the variability, indicating strong dimensional structure. The PCA biplot (Figure 8) showed clear separation between genotypes that were nitrogen-responsive and those that were not. In the correlation circle (Figure 9), nitrogen treatments projected in nearly opposite directions, with the control vector strongly influencing genotypes with high tillering ability. Genotypes such as P×Baj-62 and P×Baj-56 projected in the negative quadrant, confirming nitrogen sensitivity. The feature loading heatmap (Figure 10) emphasized contrasting PC2 signs for control (+0.72) and treated (–0.70) tiller values, further validating PCA separation of nitrogen response. Supporting this, the PC pair plot (Figure 11) and density overlay (Figure 12) showed more diffuse genotype clustering compared to plant height, reflecting the polygenic and environmentally sensitive nature of tiller number.

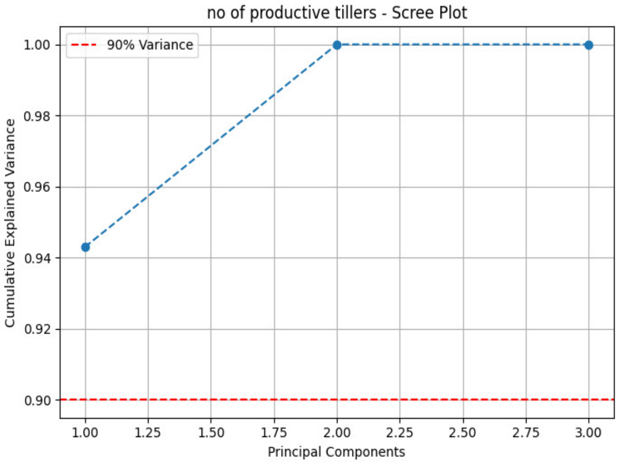

Fig. 7: Scree plot illustrating the cumulative explained variance by principal components (PCs)for number of productive tillers.

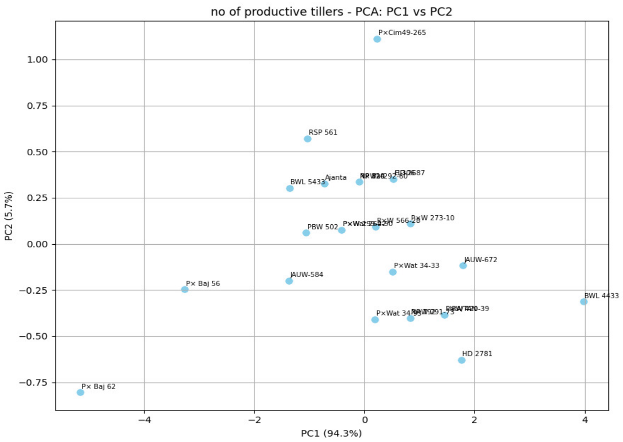

Fig. 8: Principal Component Analysis (PCA) scatter plot showing genotype distribution based on PC1 and PC2

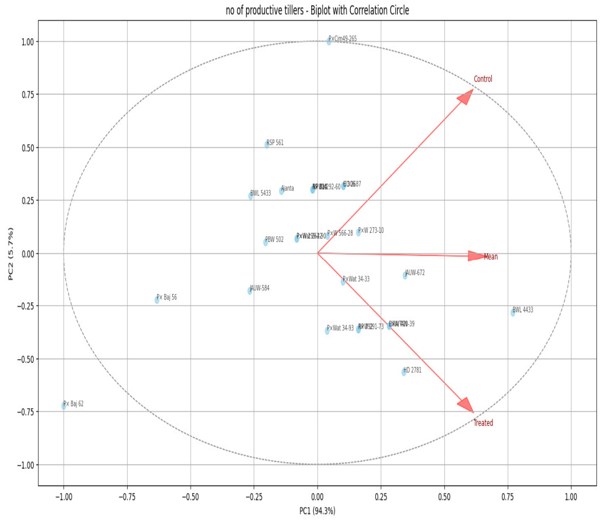

Fig. 9: PCA biplot with correlation circle depicting separation of control, treated, and mean vectors based on number of productive tillers.

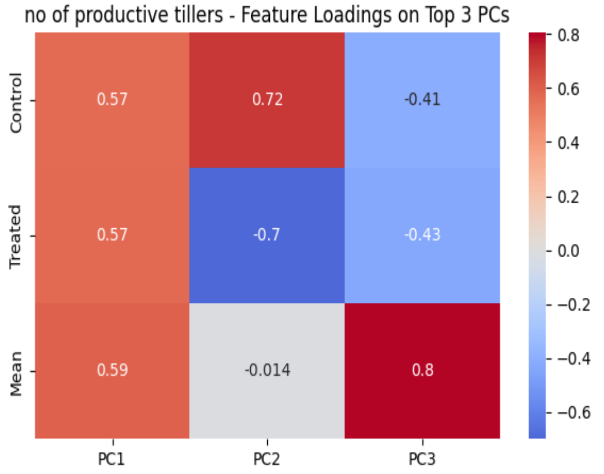

Fig. 10: Heatmap of feature loadings on top 3 principal components (PC1, PC2, and PC3) for number of productive tillers under control and treated conditions

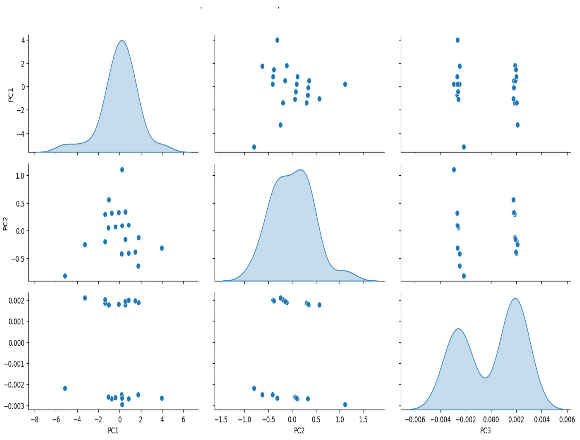

Fig. 11: Pairplot matrix showing scatter distribution and density plots of principal components (PC1, PC2, PC3) for number of productive tillers.

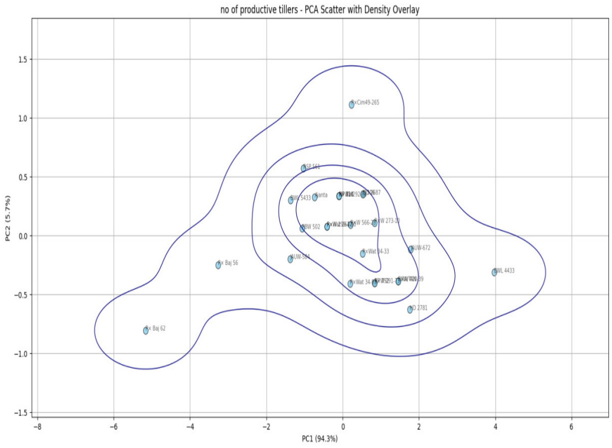

Fig. 12: PCA density contour plot for number of productive tillers showing clustering intensity of wheat genotypes across PC1 and PC2.

Leaf Area

Leaf area per plant is a direct indicator of canopy expansion and photosynthetic potential. The control treatment resulted in a significantly larger mean leaf area (23.1 cm²) than nitrogen-stressed plants (17.48 cm²) (Hitz *et al,* 2017). Genotypes such as PxCim49-265, PBW 502, and PxW273-10 exhibited high leaf area under both regimes.PCA results showed that PC1 explained 90.1% of the total variance (Figure 13). The PCA biplot (Figure 14) confirmed that genotypes with higher leaf area under nitrogen sufficiency clustered along the positive axis of PC1. Those with low canopy expansion under stress (e.g., NP 792, P×Baj-62) were projected negatively.

The correlation circle (Figure 15) and feature loading heatmap (Figure 16) supported this, with PC2 again showing clear divergence between control and treated samples (Control: +0.73, Treated: –0.69), validating the nitrogen-specific contrast in leaf expansion.

The PC1–PC3 plot (Figure 17) and density scatter overlay (Figure 18) demonstrated moderate genotype concentration, with PCA clearly differentiating nitrogen-responsive entries.

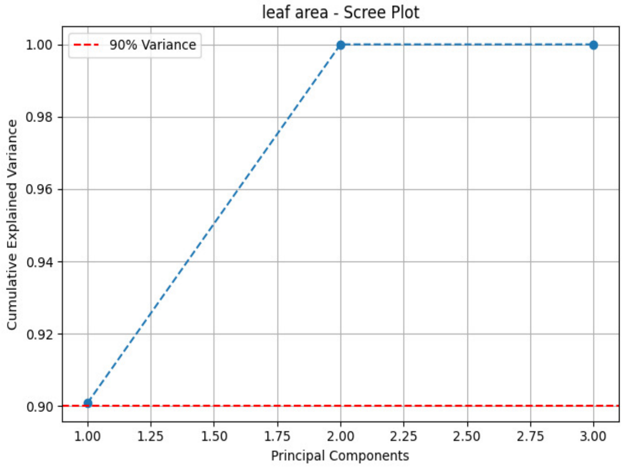

Fig. 13: Scree plot showing cumulative explained variance by principal components for leaf area

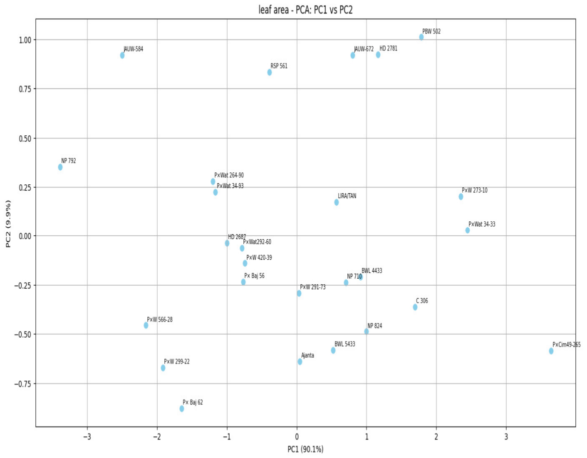

Fig. 14: PCA score plot of genotypes for leaf area based on PC1 and PC2

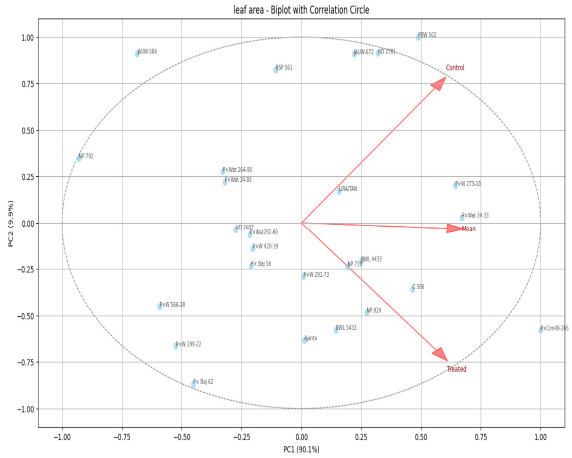

Fig. 15: Biplot with correlation circle for leaf area showing treatment vectors

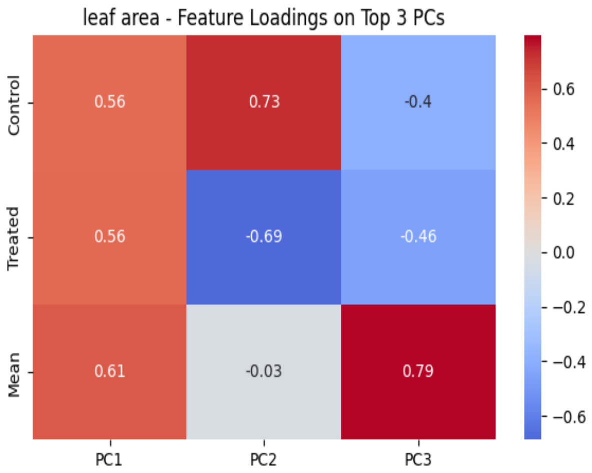

Fig. 16: Feature loadings heatmap of leaf area across top 3 principal components

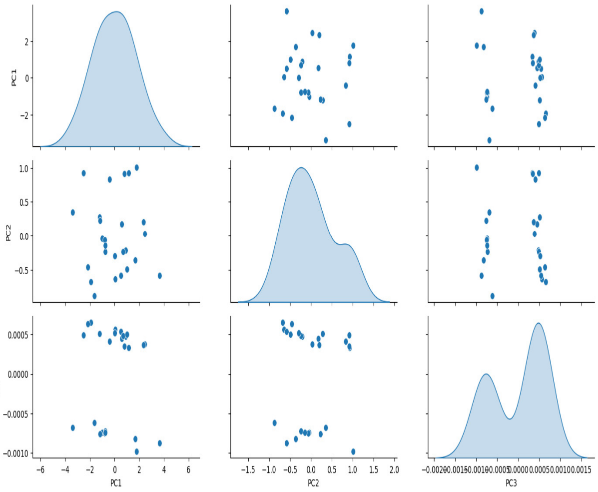

Fig. 17: Pairwise scatter matrix of top 3 principal components for leaf area

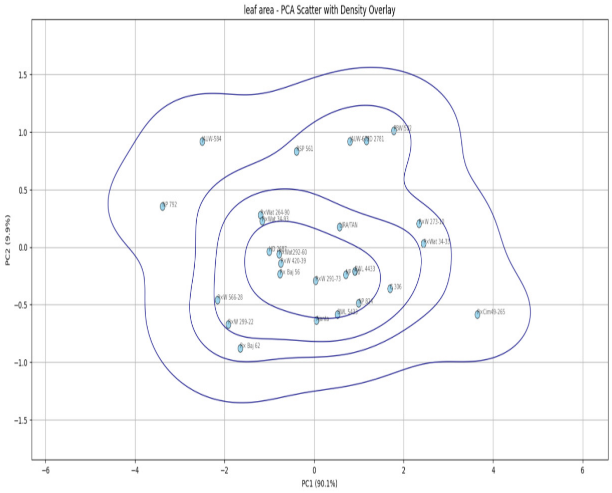

Fig. 18: PCA scatter plot with density contour overlay for leaf area

Total Biomass at Booting

Total above-ground biomass is a cumulative outcome of tillering, leaf area, and stem elongation. Nitrogen fertilization significantly enhanced biomass accumulation, with control plots averaging 9031.23 g/m², compared to 6127.52 g/m² under nitrogen stress (Ali *et al.*, 2005). The scree plot (Figure 19) showed that PC1 accounted for 91.35% of total variation. The PCA biplot (Figure 20) clustered high biomass genotypes such as PxCim49-265, HD 2781, and PxW34-33 on the right-hand side, aligning with the control vector. In the correlation circle (Figure 21), control and treated vectors projected in opposite directions, indicating distinct nitrogen-induced expression. This was mirrored in the heatmap (Figure 22), where PC2 differentiated between the two treatment types with high clarity.

The PC pair plot (Figure 23) and density-enhanced scatter plot (Figure 24) showed high separation of nitrogen-efficient genotypes, confirming the robustness of PCA in trait discrimination.

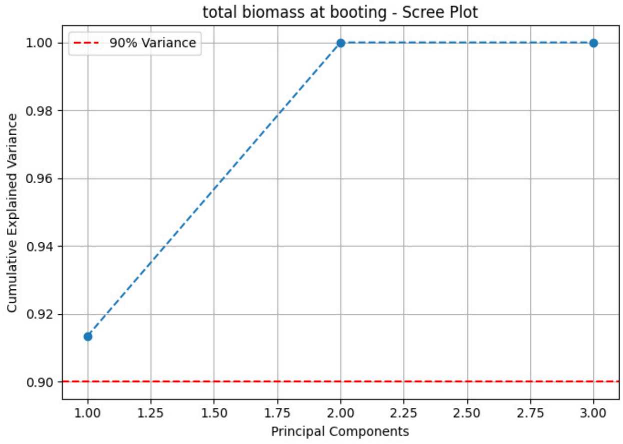

Fig. 19: Scree Plot for Total Biomass at Booting

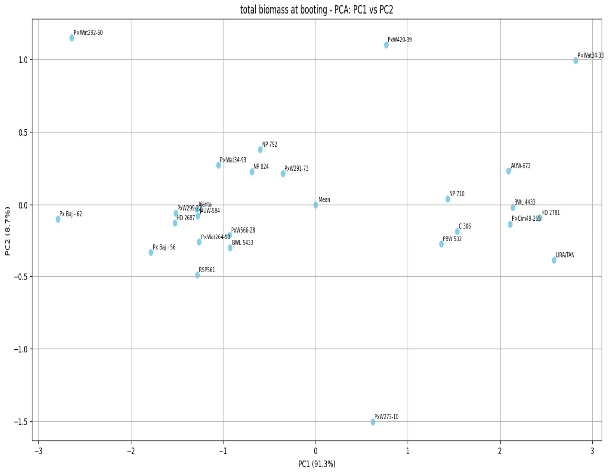

Fig. 20: PCA Scatter Plot of Genotypes Based on Total Biomass at Booting (PC1 vs PC2)

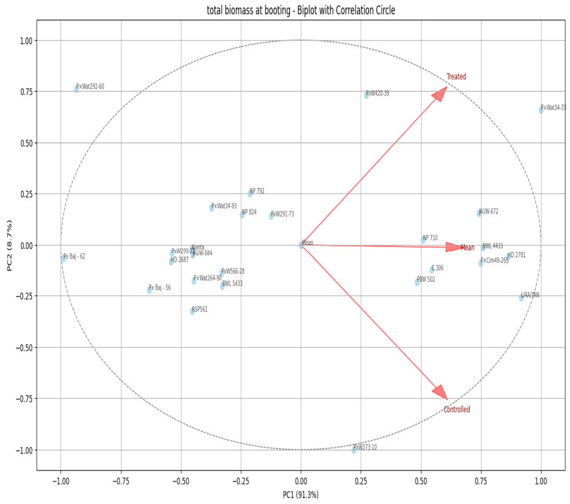

Fig. 21: PCA Biplot with Correlation Circle for Total Biomass at Booting

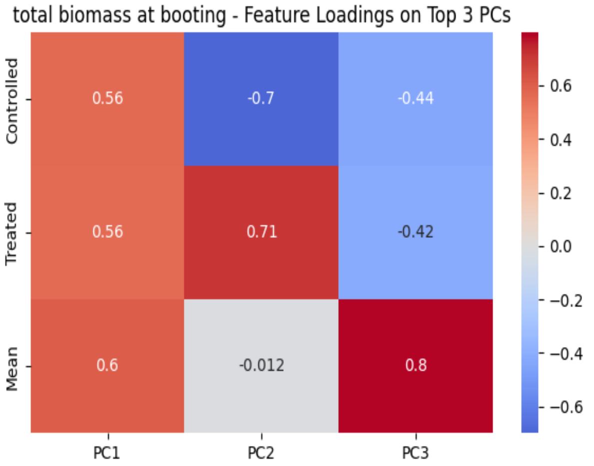

Fig. 22: Heatmap of Feature Loadings for Total Biomass at Booting on Top 3 Principal Component

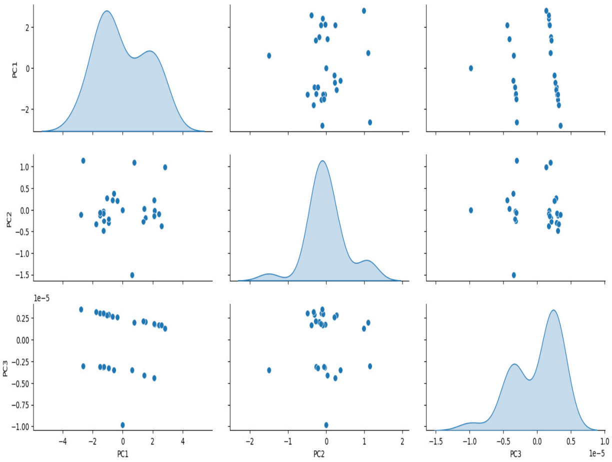

Fig. 23: Pairplot Matrix for Total Biomass at Booting Based on PCA Scores

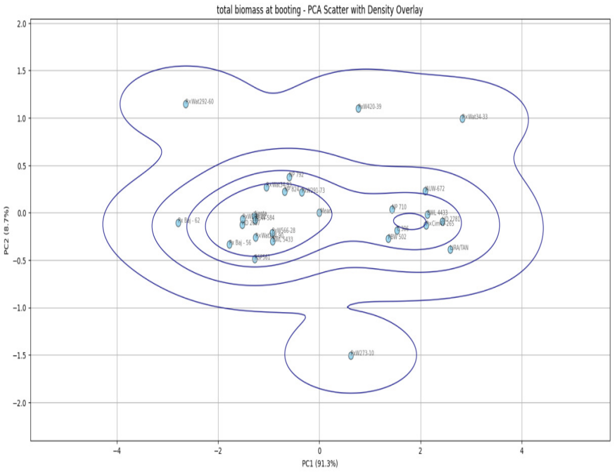

Fig. 24: PCA Scatter Plot with Density Contours for Total Biomass at Booting

Conclusion

The present investigation highlighted considerable genotypic variability in key morphological traits such as plant height, number of productive tillers, leaf area, and total biomass across control and nitrogen-stressed conditions, emphasizing the potential for selecting nitrogen-efficient wheat genotypes. Statistical analysis using two-way ANOVA revealed that both genotype and nitrogen level significantly influenced trait expression, indicating strong genotype × environment interactions. Principal Component Analysis (PCA) effectively differentiated the genotypes based on their morphological responses, with genotypes like C 306, NP 710, PxCIM49-265, and HD 2781 showing consistent clustering in favorable PCA quadrants under nitrogen stress, suggesting superior adaptability and nitrogen use efficiency (Gupta *et al.,* 2022). Feature loadings and biplots underscored trait contributions to variability and helped identify those most responsive to nitrogen availability. Such multivariate approaches are crucial for screening stress-resilient genotypes and are supported by earlier studies demonstrating the utility of PCA and clustering in wheat under abiotic stress conditions (Khalid *et al.,* 2023; Hirel *et al*., 2007; Marinho *et al.,* 2022). These findings offer valuable insights for breeding programs targeting improved nitrogen use efficiency and sustainable yield under low-input systems.

References

Ali, H., Ahmad, S. H., Ali, H. I., & Hassan, F. S. (2005). Impact of nitrogen application on growth and productivity of wheat (*Triticum aestivum* L.). *Journal of Agriculture and Social Sciences*, **1**(3), 216–218.

Akhter, M. M., Hossain, A., Timsina, J., Teixeira, D. S. J. A., & Islam, M. S. (2016). Chlorophyll meter A decision-making tool for nitrogen application in wheat under light soils. *Not specified journal/source*.

de Lucena Marinho, J., Silva, S. R., de Batista Fonseca, I. C., & Zucareli, C. (2022). Nitrogen use efficiency and yield of wheat genotypes affected by nitrogen fertilizing and environmental conditions in Southern Brazil. *International Journal of Plant Production*, **16**(3), 495–510. https://doi.org/10.1007/s42106-021-00155-w

Fageria, N. K., Baligar, V. C., & Jones, C. A. (2010). *Growth and mineral nutrition of field crops* (3rd ed.). CRC Press.

Gupta, V., Kumar, M., Singh, V., Chaudhary, L., Yashveer, S., Sheoran, R., & Nagpal, S. (2022). Genotype by environment interaction analysis for grain yield of wheat (*Triticum aestivum* L.) genotypes. *Agriculture*, **12**(7), 1002. https://doi.org/10.3390/agriculture12071002

Hirel, B., Le Gouis, J., Ney, B., & Gallais, A. (2007). The challenge of improving nitrogen use efficiency in crop plants: Towards a more central role for genetic variability and quantitative genetics within integrated approaches. *Journal of Experimental Botany*, **58**(9), 2369–2387. https://doi.org/10.1093/jxb/erm097

Hitz, K., Clark, A. J., & Van Sanford, D. A. (2017). Identifying nitrogen-use efficient soft red winter wheat lines in high and low nitrogen environments. *Field Crops Research*, **200**, 1–9. https://doi.org/10.1016/j.fcr.2016.09.010

Karkaji, F. A., Hervan, E. M., Roustaii, M., Bihamta, M., & Mohammadi, S. (2023). Comprehensive stability analysis of wheat genotypes through multi-environmental trials. *Journal of Agricultural Sciences*, **29**(1), 317–334.

Khalid, A., Hameed, A., & Tahir, M. F. (2023). Estimation of genetic divergence in wheat genotypes based on agro-morphological traits through agglomerative hierarchical clustering and principal component analysis. *Cereal Research Communications*, **51**(1), 217–224. https://doi.org/10.1556/0806.50.2022.015

Mahajan, A., & Gupta, R. D. (2009). *Integrated nutrient management (INM) in a sustainable rice–wheat cropping system*. Springer. https://doi.org/10.1007/978-1-4020-9875-8

Osman, R., Tahir, M. N., Ata-Ul-Karim, S. T., Ishaque, W., & Xu, M. (2021). Exploring the impacts of genotype-management-environment interactions on wheat productivity, water use efficiency, and nitrogen use efficiency under rainfed conditions. *Plants*, **10**(11), 2310. https://doi.org/10.3390/plants10112310

RAFII, M., & Shamsuzzaman, S. (2017). Response of yield, nitrogen use efficiency and grain protein content of wheat (*Triticum aestivum* L.) varieties to different nitrogen levels. *Bangladesh Journal of Botany*, **46**, 389–396.

Reynolds, S. G. (1970). The gravimetric method of soil moisture determination Part I: A study of equipment and methodological problems. *Journal of Hydrology*, **11**(3), 258–273. https://doi.org/10.1016/0022-1694(70)90066-2

Sakin, M. A., Akinci, C., Duzdemir, O., & Donmez, E. (2011). Assessment of genotype × environment interaction on yield and yield components of durum wheat genotypes by multivariate analyses. *African Journal of Biotechnology*, **10**(15), 2875–2885.

Shi, H., Chen, M., Gao, L., Wang, Y., Bai, Y., Yan, H., & Ma, Y. (2022). Genome-wide association study of agronomic traits related to nitrogen use efficiency in wheat. *Theoretical and Applied Genetics*, **135**(12), 4289–4302. https://doi.org/10.1007/s00122-022-04201-y

Takahashi, K., & Takahashi, L. (2023). Data visualization. In *Materials Informatics and Catalysts Informatics: An*

Introduction (pp. 143–170). Springer Nature Singapore. https://doi.org/10.1007/978-981-99-2402-7_6

Xu, G., Fan, X., & Miller, A. J. (2012). Plant nitrogen assimilation and use efficiency. *Annual Review of Plant Biology*, **63**(1), 153–182. https://doi.org/10.1146/annurev-arplant-042811-105532

Yan, W., & Rajcan, I. (2002). Biplot analysis of test sites and trait relations of soybean in Ontario. *Crop Science*, **42**(1), 11–20. https://doi.org/10.2135/cropsci2002.1100

Fageria, N. K., Baligar, V. C., & Jones, C. A. (2010). *Growth and mineral nutrition of field crops*. CRC press.

Xu, G., Fan, X., & Miller, A. J. (2012). Plant nitrogen assimilation and use efficiency. *Annual review of plant biology*, **63**(1), 153-182.