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Nitrogen-Driven Genotypic Diversity of Wheat (*Triticum aestivum* L.) Genotypes

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Abstract: Imbalanced use (form, quantity, and ratio) of nitrogen fertilization can result in decreased grain yields and increased nitrogen loss, leading to adverse effects on overall environmental quality. Globally, limited empirical research has been conducted on the comprehensive effects of different levels of N that can significantly influence wheat agronomic and genotypic traits. Therefore, this study aimed to evaluate wheat genotypes for two consecutive years (2020–2021 and 2021–2022) under different N fertilization treatments: N0 (native N, without external application of N), N75 (1/2 of the recommended dose of N), and N150 (recommended dose of N). The study findings revealed that 'HD 3249' and 'HD 3117' were the top-performing genotypes in terms of grain yield (5.3 t ha^{-1}; 5.0 t ha^{-1}), straw yield (6.9 t ha^{-1}; 6.7 t ha^{-1}), biological yield (12.2 t ha^{-1}; 11.8 t ha^{-1}), and harvest index (42.9%; 42.4%). In particular, the application of N75 and N150 increased grain yields by 142.6% and 61.3%, respectively; straw yields by 72.3%; and by 110.6% over N0. Furthermore, N levels (N75 and 150) significantly increased the higher concentration of N in grain (23.1% and 33%) and straw (21.1% and 29.8%); N uptake in grain (70.2 and 104.2) and straw (64.8 and 41.5); and total N uptake (68.8% and 101.4%) than N0, respectively. Additionally, correlation analysis revealed that there were positive correlations between yields, harvest index as well as N concentration and uptake. This study identified the two elite genotypes, 'HD 3249' and 'HD 3117', with N150 splits giving a better response, which can be used as selection criteria for developing wheat varieties that are more efficient in using nitrogen, leading to high yields and N uptake.

Keywords: nitrogen levels; nitrogen uptake; wheat genotypes; yields

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1. Introduction

The application of nitrogen (N) fertilizers involves significant expenditures for cultivators and is accompanied by potential environmental repercussions, such as nitrate leaching

and N_2O emissions resulting from bacteria-facilitated soil denitrification processes. These impacts deserve close attention given their potential to exacerbate the issue of greenhouse gas emissions. Seeing this problem, nitrogen-efficient genotypes (N) present a viable approach to mitigate the need for excessive N fertilizer applications while still maintaining acceptable yields. This strategy involves selecting and breeding plant varieties with the inherent ability to use N more efficiently, thus minimizing the need for the external input of N. By adopting this approach, growers can significantly reduce their dependence on N fertilizers, contributing to sustainable agricultural practices.

Numerous studies have documented the existence of genetic variability in N uptake and utilization in various winter wheat genotypes [1–3]. These findings highlight the potential to develop crop varieties that exhibit superior N uptake, which can greatly enhance their ability to absorb and use N from the soil while minimizing the need for excessive fertilization with N. These results underscore the importance of leveraging genetic diversity in crop improvement programs that aim to improve yields and N uptake in wheat genotypes. The variation between wheat genotypes offers an agronomist the opportunity to select wheat genotypes. The adoption of such selection practices is believed to have played a pivotal role in promoting the development of wheat genotypes that exhibit enhanced N uptake and utilization traits. Using this approach, it becomes possible to identify and propagate plant varieties that exhibit robust characteristics of nutrient uptake, such as increased N uptake and utilization while maintaining high crop yields. Implementing this strategy has allowed significant advances in wheat breeding efforts, resulting in the development of varieties that require a reduced N input.

A critical challenge plant breeders are facing is to identify and select genotypes that show a consistent response to N and possess desirable traits related to N utilization [4]. To address this challenge, it is necessary to gain a comprehensive understanding of the existing genetic variation in response to N, which can be achieved using a combination of field-based and controlled environment approaches. When these methods are used, the responsiveness of different genotypes to N supply can be effectively evaluated, allowing the identification of higher N uptake traits that are amenable to use in breeding programs. Furthermore, a detailed dissection of N metabolic pathways is needed to obtain information on the molecular mechanisms that underlie N utilization in plants. This information is vital in identifying the crucial genes and enzymes involved in nitrogen metabolism, which can be targeted in breeding programs to enhance N response traits.

The expression of N uptake, utilization, and N response traits has been found to exhibit variability, which is subject to influence by environmental factors such as location and year [5–7]. The integration of multi-omics data, encompassing agronomic, physiological, and molecular information [8–10], shows significant potential for the identification and selection of exceptional genotypes exhibiting elevated yields and enhanced nutrient uptake, tailored to specific environmental circumstances. By harnessing innovative and high-throughput technologies, a comprehensive understanding of the genetic and metabolic determinants that underlie the yield and nutrient uptake traits can be obtained. This knowledge can facilitate the development of more precise and effective breeding programs. The proposed strategy exhibits promising potential to accelerate the rate of genetic advancement toward optimal N uptake. This could ultimately lead to the development of genotypes that demonstrate efficient utilization of N while mitigating negative environmental impacts.

The current global situation, characterized by increased economic costs and growing environmental concerns, has led to a greater need to manage the application of fertilizers that contain N in a more sustainable and efficient manner. Furthermore, these pressures are projected to escalate due to the increasing demand for food and energy with the continuing expansion of the global human population. Therefore, it is crucial to have a comprehensive understanding of the traits of crops that influence yields at varying levels of N to improve nitrogen uptake, and utilization; reduce the usage of N fertilizers; and simultaneously increase or maintain crop yield. However, this matter has received

relatively little attention, with only a limited number of investigations conducted so far. In this study, we conducted field experiments in New Delhi, India, using ten wheat genotypes grown at different levels of N to examine their morphological characteristics and yields and to identify traits that contribute to the variation in yield among genotypes under different N conditions. The study aimed to evaluate the genetic diversity of winter wheat genotypes concerning their uptake of nitrogen and their corresponding yield performance at different levels of fertilization with N. Specific objectives are to (i) evaluate the responsiveness of wheat genotypes to low, half, and recommended N supply; (ii) compare the differences in economic, straw, and biological yields as well as harvest index; and (iii) investigate genetic variation in nitrogen uptake and related traits at three N levels.

2. Materials and Methods

2.1. Experimental Site and Soil Characteristics

The current investigation was carried out under open-field conditions during the crop growing seasons from November to April for the years 2020–2021 and 2021–2022 at the ICAR-Indian Agricultural Research Institute in New Delhi (located at 28°38′ N 77°10′ E), India (Figure 1). The experimental site was characterized by maximum and minimum temperature ranges of 27.1 °C and 10.6 °C in the first season (2020–2021) and 26.9 °C and 11.5 °C in the second season, respectively, with an annual average rainfall of 74.3 mm in the first season and 181.5 mm in the second season (Figures 2 and 3). Before the sowing of wheat genotypes began, unmodified soil samples were obtained from the upper 0.02 m of the soil profile, demonstrating sandy loam soil with a pH of 7.8 (1:2.5, soil:water), 0.4% organic carbon [11], 221 kg ha^{-1} of available nitrogen [12], 15.23 kg ha^{-1} of Olsen P [13], and 252.2 kg ha^{-1} [14] of available potassium as preliminary soil properties.

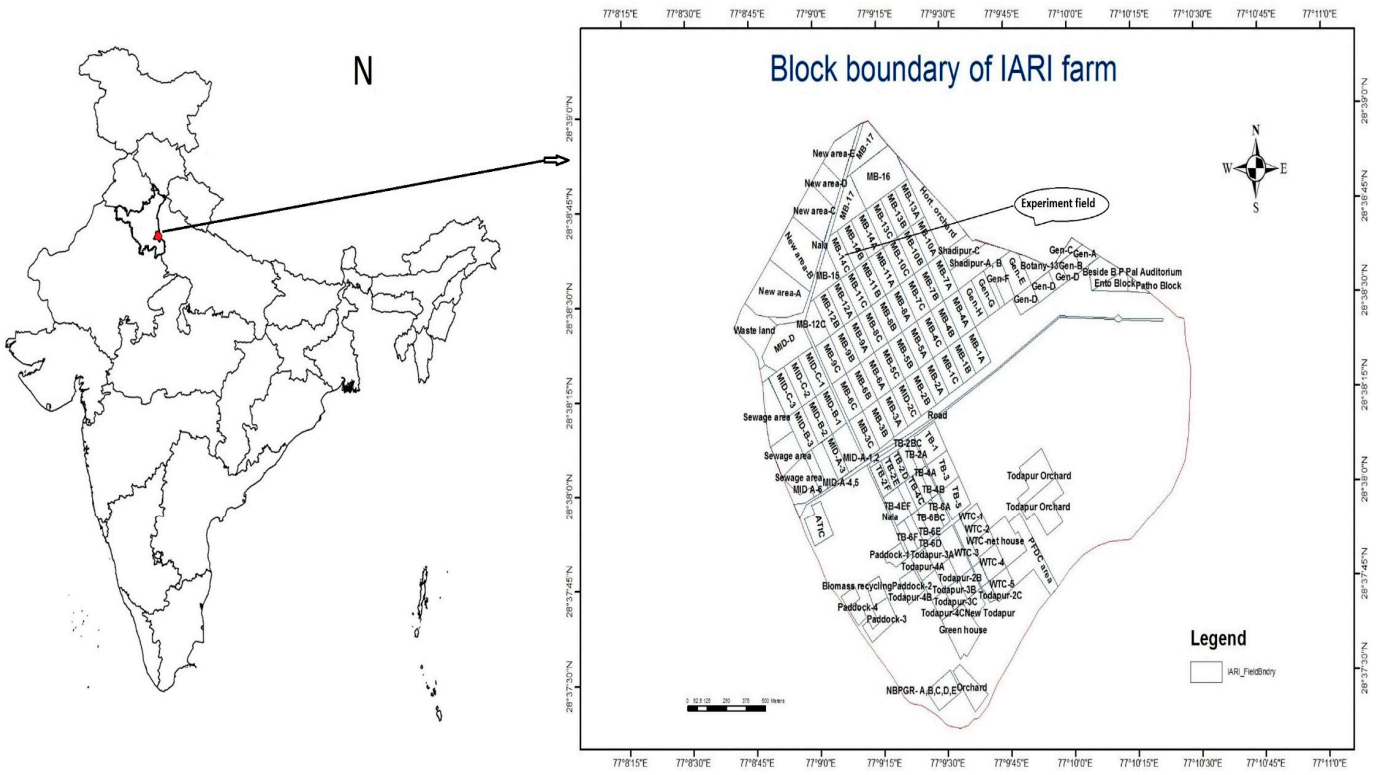


Figure 1. Geographical landscape of the study area.

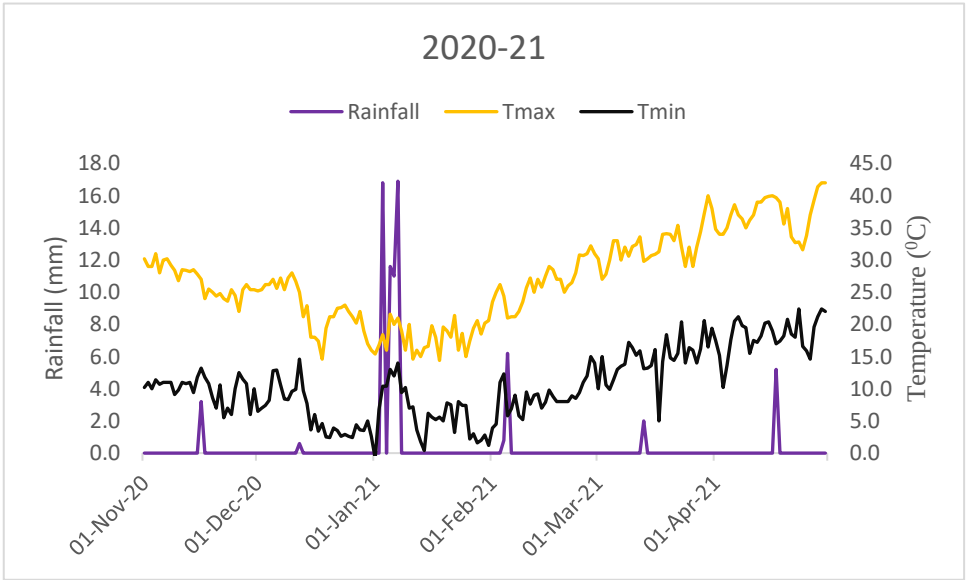


Figure 2. Variations in monthly mean temperatures and total rainfall at ICAR-IARI in New Delhi throughout the crop growing period (2020–2021), (Nov: November; Dec: December; Jan: January; Feb: February; Mar: March; and Apr: April).

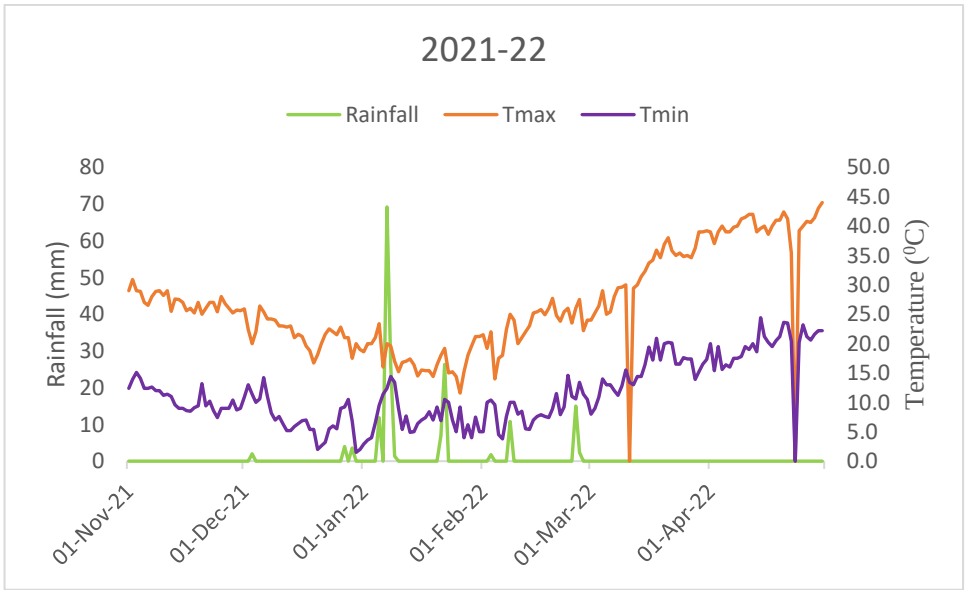


Figure 3. Variations in monthly mean temperatures and total rainfall at ICAR-IARI in New Delhi throughout the crop growing period (2021–2022), (Nov: November; Dec: December; Jan: January; Feb: February; Mar: March; and Apr: April).

2.2. Experimental Design and Treatment Details

The experimental field was organized in a split plot design. The primary plot was composed of three levels of nitrogen fertilization: N0 (native N, without any external nitrogen application), N75–75 kg N ha^{-1} (half of the recommended nitrogen dosage), and N150–150 kg N ha^{-1} (recommended nitrogen dosage). Neem-oil-coated urea (organic in nature, with a 46.6% N content; one ton of neem-coated urea contained 0.5 kg of neem oil) was used as a nitrogen source. The sub-plot consisted of ten distinct wheat genotypes, namely V1: HD 3226, V2: HDCSW 18, V3: HD 2967, V4: HD 3086, V5: HD 3249, V6: HD 2733, V7: PBW 550, V8: PBW 343, V9: HD 3117, and V10: HD 3298, each with their unique genetic makeup and phenotypic characteristics. A supplementary table (Table S1) has been provided for the characteristics of these wheat genotypes. Each plot was 5 m × 2.5 m with

a spacing of 22.5 cm between seeds. Before sowing, phosphorus (60 kg P_2O_5 ha^{-1}) and potash (60 kg K_2O ha^{-1}) were applied as basal fertilizers, while nitrogen was applied in three separate splits: half as basal, 1/4th during the crown root initiation stage (20–25 DAS), and 1/4th during the tillering stage (40–45 DAS). Standardized measures for pest and disease control were implemented in all wheat genotypes in accordance with established wheat cultivation protocols.

2.3. Sampling and Measurements

During the harvesting stage, the four middle rows of the ground were selected to measure grain and straw yield, with two border rows on each side of plot not used for measurements. The harvested product was then subjected to sun drying for a period of 20–25 days to reduce the moisture content to 12–14%. After this, the yields were measured separately at each plot level. For straw yield measurement, plants are cut at ground levels. After that, the data on the above-ground biomass yield were used in the computation of the harvest index for the various wheat genotypes.

2.4. Chemical Analysis of N Content in Plant Samples

In the harvesting stage, samples were collected from different parts of wheat genotypes to perform nitrogen analysis. These samples were first washed with tap water and then treated with diluted HCL (0.05 N). The plant samples were comminuted to a fine particle size using a 1 mm mesh screen and subjected to Kjeldahl digestion in adherence to the established protocol as explained by Prasad et al. [15].

2.5. Computation of Nitrogen Uptake in Genotypes

To determine total N uptake (kg ha^{-1}), the N uptake in both grain and straw was summed at the harvesting stage.

Straw N uptake (kg/ha^{-1}) was calculated as shown in the following equation:

Straw N uptake = N concentration in straw × straw yield in each plot

The uptake of N in grains (kg/ha^{-1}) was calculated as shown in the following equation.

Grain N uptake = N concentration in grain × grain yield in each plot

Total N uptake = Grain N uptake + Straw N uptake

Partial N balance (kg/kg) = Nitrogen removed (kg)/Nitrogen applied (kg)

Internal nitrogen use efficiency (kg/kg) = Grain yield (kg)/Total nitrogen uptake (kg)

2.6. Statistical Analysis

The replicated data in years were subjected to a statistical analysis using R Studio (version of the agricolae package), following the method described by de Mendiburu [16]. A significance level of $p < 0.05$ was adopted for intertreatment comparisons, with the least significant difference (LSD) determined. The mean data of the recorded crop traits from the two years were used to construct a Pearson's correlation coefficient matrix/diagram using the performance analytics package in R Studio. This analysis aimed to explore the interrelationships between crop traits and gain insight into their dependencies.

3. Results

3.1. Yields and Harvest Index

The pooled data analysis revealed a significant interaction effect of years, N levels, and genotypes on grain yield (GY), straw yield (SY), biological yield (BY), and harvest index (HI) (Table 1; Figures 4 and 5). Although no significant interaction was observed between years and N levels for GY and HI, a significant interaction effect was found for SY and BY. GY and HI exhibited successive increases with increasing levels of N up to N150, while SY and BY increased significantly up to N75 in the first year and up to N150 in the second year.

Yield and HI data showed a substantial year–variety interaction. Only HD 3236, HDCSW 18, and HD 2967 exhibited similarities, but all other genotypes had statistically

different GY, with 2020 having a greater GY than 2021. At N150, 'HD 3249' had the greatest grain yield (GY), but in the second year, it was at par with 'HD 3117'.

Table 1. Effect of nitrogen × genotype interaction on straw and biological yield of wheat.

Nitrogen × Variety		HD 3226	HDCSW 18	HD 2967	HD 3086	HD 3249	HD 2733	PBW 550	PBW 343	HD 3117	HD 3298	Mean
Straw yield—SY (t/ha)												
2020–2021	N0	3.03 ^{k}	3.36 ^{ijk}	4.10 ^{gh}	4.12 ^{fgh}	4.57 ^{fg}	3.59 ^{hij}	4.12 ^{fgh}	3.19 ^{jk}	4.66 ^{f}	3.75 ^{hi}	3.85 ^{b}
	N75	4.40 ^{fg}	5.87 ^{e}	6.39 ^{de}	6.44 ^{d}	7.28 ^{c}	6.11 ^{de}	6.50 ^{d}	5.86 ^{e}	6.58 ^{d}	6.14 ^{de}	6.16 ^{a}
	N150	6.57 ^{d}	8.04 ^{b}	8.55 ^{ab}	8.78 ^{a}	8.87 ^{a}	8.03 ^{b}	8.33 ^{ab}	6.44 ^{d}	8.39 ^{ab}	8.41 ^{ab}	8.04 ^{a}
	Mean	4.67 ^{g}	5.76 ^{e}	6.34 ^{bc}	6.45 ^{b}	6.91 ^{a}	5.91 ^{de}	6.31 ^{bc}	5.16 ^{f}	6.54 ^{b}	6.10 ^{cd}	
	* N × V = 0.6/* V × N = 2											
2021–2022	N0	3.18 ^{lm}	3.13 ^{m}	3.91 ^{ijklm}	4.08 ^{hijk}	4.68 ^{hi}	3.62 ^{jklm}	4.28 ^{hij}	3.30 ^{klm}	4.75 ^{h}	3.75 ^{jklm}	3.87 ^{c}
	N75	3.76 ^{jklm}	3.84 ^{jklm}	8.07 ^{bcd}	6.96 ^{fg}	7.13 ^{efg}	7.41 ^{def}	6.72 ^{fg}	4.00 ^{hijkl}	7.09 ^{efg}	7.91 ^{cde}	6.29 ^{b}
	N150	6.44 ^{g}	8.05 ^{bcd}	8.80 ^{ab}	8.78 ^{ab}	8.97 ^{a}	8.26 ^{abc}	8.82 ^{ab}	6.44 ^{g}	8.86 ^{ab}	8.76 ^{ab}	8.22 ^{a}
	Mean	4.46 ^{d}	5.01 ^{c}	6.93 ^{a}	6.61 ^{ab}	6.93 ^{a}	6.43 ^{b}	6.61 ^{ab}	4.58 ^{cd}	6.90 ^{ab}	6.81 ^{ab}	
	* N × V = 0.8/* V × N = 1.8											
	Pooled	4.6 ^{f}	5.4 ^{d}	6.6 ^{b}	6.5 ^{b}	6.9 ^{a}	6.2 ^{c}	6.5 ^{b}	4.9 ^{e}	6.7 ^{ab}	6.5 ^{b}	
	Year-1 = 6/Year-2 = 6.1/*SY × Year = 0.72/*SY × V = 0.28											
Biological yield—BY (t/ha)												
2020–2021	N0	4.67 ^{q}	5.33 ^{opq}	6.64 ^{mn}	6.82 ^{mn}	7.77 ^{kl}	5.72 ^{op}	6.91 ^{lmn}	4.99 ^{pq}	7.86 ^{k}	6.05 ^{no}	6.28 ^{b}
	N75	7.01 ^{klm}	9.64 ^{j}	10.80 ^{ghi}	10.94 ^{ghi}	12.59 ^{f}	10.16 ^{ij}	11.09 ^{gh}	9.55 ^{j}	11.35 ^{g}	10.26 ^{hij}	10.34 ^{a}
	N150	10.57 ^{ghi}	13.36 ^{ef}	14.89 ^{bc}	15.44 ^{ab}	16.26 ^{a}	13.58 ^{de}	15.12 ^{bc}	10.60 ^{ghi}	15.28 ^{bc}	14.41 ^{cd}	13.95 ^{a}
	Mean	7.42 ^{g}	9.45 ^{e}	10.78 ^{c}	11.07 ^{bc}	12.21 ^{a}	9.82 ^{de}	11.04 ^{bc}	8.38 ^{f}	11.50 ^{b}	10.24 ^{d}	
	* N × V = 0.9/* V × N = 3.7											
2021–2022	N0	4.90 ^{m}	5.02 ^{lm}	6.37 ^{jk}	6.71 ^{ij}	7.96 ^{i}	5.83 ^{jklm}	7.15 ^{ij}	5.18 ^{klm}	8.03 ^{i}	6.05 ^{jklm}	6.32 ^{c}
	N75	5.96 ^{jklm}	6.24 ^{jkl}	13.42 ^{de}	11.76 ^{fgh}	12.48 ^{efg}	12.11 ^{efg}	11.52 ^{gh}	6.45 ^{jk}	12.19 ^{efg}	13.01 ^{def}	10.51 ^{b}
	N150	10.56 ^{h}	13.37 ^{de}	15.14 ^{abc}	15.32 ^{ab}	16.37 ^{a}	13.82 ^{cd}	15.60 ^{ab}	10.60 ^{h}	15.87 ^{ab}	14.88 ^{bc}	14.15 ^{a}
	Mean	7.14 ^{e}	8.21 ^{d}	11.64 ^{ab}	11.26 ^{bc}	12.2^{7} ^{a}	10.59 ^{c}	11.42 ^{b}	7.41 ^{e}	12.03 ^{ab}	11.31 ^{bc}	
	* N × V = 1.3/* V × N = 3.4											
	Pooled	7.3 ^{h}	8.8 ^{f}	11.2 ^{cd}	11.2 ^{cd}	12.2 ^{a}	10.2 ^{e}	11.2 ^{c}	7.9 ^{g}	11.8 ^{b}	10.8 ^{d}	
	Year-1 = 10.2/Year-2 = 10.3/*BY × Year = 2.2/* BY × V = 0.45											

* LSD (p = 0.05) for nitrogen means at same or different level of varieties; * LSD (p = 0.05) for varieties means at same or different level of nitrogen. Values in a column followed by the different letters were significantly different at $p < 0.05$ as determined with LSD; letters indicate the comparison among genotypes under different N levels. V (variety).

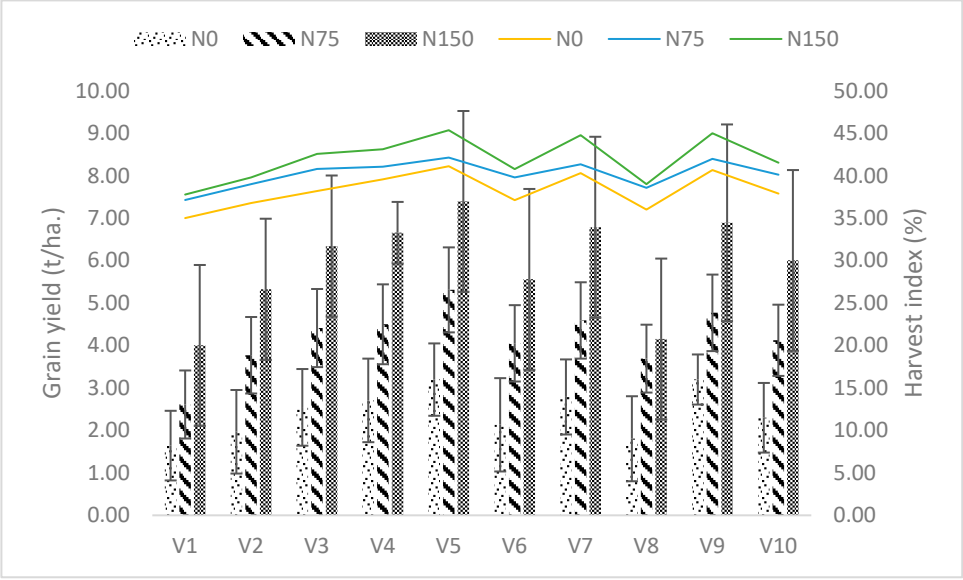


Figure 4. Wheat genotypes grain yield and harvest index under different N fertilization treatments during 2020–2021 [(vertical lines indicate the standard error, N_0 (control), N_{75} ($\frac{1}{2}$ recommended N), and N_{150} (recommended N)), (V1: HD 3226, V2: HDCSW 18, V3: HD 2967, V4: HD 3086, V5: HD 3249, V6: HD 2733, V7: PBW 550, V8: PBW 343, V9: HD 3117, and V10: HD 3298)].

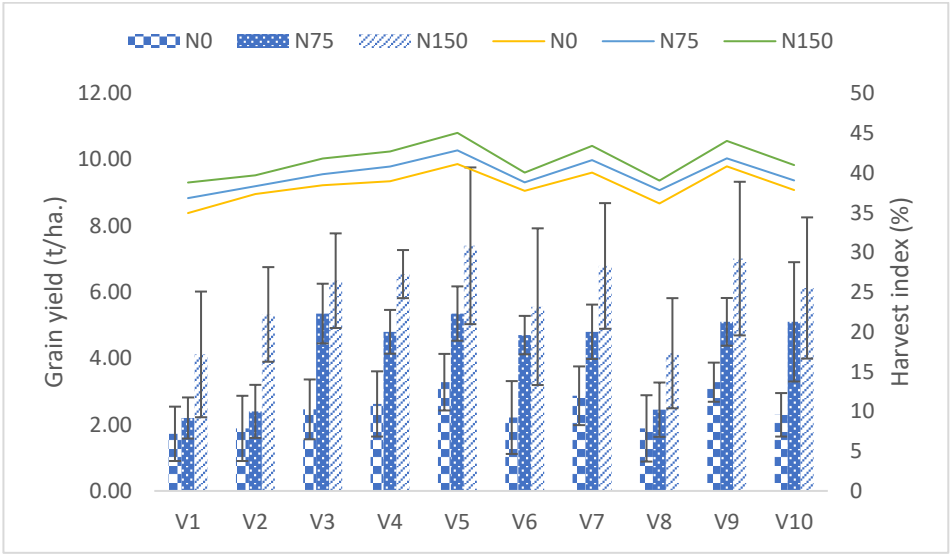


Figure 5. Wheat genotypes grain yield and harvest index under different N fertilization treatments during 2021–2022 [(vertical lines indicate the standard error, N_0 (control), N_{75} (1/2 recommended N), and N_{150} (recommended N)), (V1: HD 3226, V2: HDCSW 18, V3: HD 2967, V4: HD 3086, V5: HD 3249, V6: HD 2733, V7: PBW 550, V8: PBW 343, V9: HD 3117, and V10: HD 3298)].

The pooled data over two years indicated that 'HD 3249' had the greatest GY of all genotypes, whereas 'HD 3117' had the second highest and was statistically different. 'HD 3226' had the lowest GY and was substantially lower than all other genotypes. At low, medium, and high N levels, 'HD 3249' and 'HD 3117' produced the best yields, demonstrating their grain production efficiency. The analysis revealed that the order of genotypes, from highest to lowest grain yield, was HD 3249 > HD 3117 > PBW 550 ≥ HD 3086 ≥ HD 2967 > HD 3298 > HD 2733 > HDCSW 18 > PBW 343 > HD 3226. Only four genotypes—'HD 3086', 'HD 3249', 'PBW 550', and 'HD 3117'—had statistically equivalent grain yields. 'HD 3249' had the greatest grain yield at N150 and was at par with 'HD 2967', 'HD 3086', 'HD 2733', and 'HD 3117'. The pooled results of two years showed that 'HD 3249' had the highest SY, statistically at par with 'HD 2967', 'HD 3086', and 'HD 3117'.

Genotypes were ranked in decreasing order of SY as HD 3249 ≥ HD 3117 ≥ HD 2967 = HD 3086 = HD 3298 = PBW 550 > HD 2733 > HDCSW 18 > PBW 343 > HD 3226. Only 'HD 3086', 'HD 3249', 'PBW 550', and 'HD 3117' had BY at par in both years. At N level N150, 'HD 3249' had the highest BY and was at par with HD 2967, HD 3086, PBW 550, and HD 3117. In decreasing order of BY, genotypes were listed as HD 3249 > HD 3117 > HD 3086 ≥ PBW 550 ≥ HD 2967 ≥ HD 3298 > HD 2733 > HDCSW 18 > PBW 343 > HD 3226. The only genotype with a statistically significant HI difference was 'HD 3249', and it shows the highest HI. In the decreasing order of HI, genotypes were HD 3249 > HD 3117 > PBW 550 > HD 3086 > HD 2967 > HD 3298 > HD 2733 > HDCSW 18 > PBW 343 > HD 3226. These results suggest that 'HD 3249' may have potential for high yield and HI under similar environmental conditions, while 'HD 3226' may have lower yield and HI.

3.2. N Concentration and Uptake

Pooled data analysis showed significant interactions between years, N levels, and genotypes for N uptake in grain (NUG), straw (NUS), and total N uptake (TNU). The NCG and NCS had no significant interactions (Tables 2–4). The mean NCG was stable across all treatments, although NCS, NUG, NUS, and TNU varied greatly over the two years. The NCG, NCS, and NUS showed substantial year-by-N interaction, but NUG and TNU did not. In the first season, NCG increased to N150, while NCS increased to N75 in the second. Year and variety interacted significantly for NUG, NUS, and TNU. All genotypes except 'HD3249' have statistically distinct NUG and TNU. Except for 'HD 3226', 'PBW 343', and 'HD 3298', all genotypes exhibited

statistically identical NUS, However, 2020 had a considerably higher NUG, NUS, and TNU than 2021. 'HD 3249' consistently had the greatest NUG, NUS, and TNU values in both years.

Table 2. Effect of nitrogen fertilization on N concentration (%) in grain and straw of wheat at harvesting stage.

Nitrogen × Variety	N Concentration in Grain (NCG-%)			N Concentration in Straw (NCS-%)		
	2020–2021	2021–2022	Pooled	2020–2021	2021–2022	Pooled
N0	1.60 ^{c}	1.61 ^{b}	1.60 ^{c}	0.392 ^{b}	0.393 ^{c}	0.392 ^{c}
N75	1.97 ^{b}	1.98 ^{a}	1.98 ^{b}	0.474 ^{a}	0.477 ^{b}	0.476 ^{b}
N150	2.13 ^{a}	2.14 ^{a}	2.13 ^{a}	0.509 ^{a}	0.510 ^{a}	0.509 ^{a}
LSD (p = 0.05)	0.10	0.15	0.06	0.062	0.015	0.02
HD 3226	1.60 ^{f}	1.62 ^{f}	1.61 ^{f}	0.443 ^{def}	0.446 ^{ef}	0.445 ^{fg}
HDCSW 18	1.99 ^{b}	2.00 ^{b}	2.00 ^{b}	0.443 ^{def}	0.437 ^{fg}	0.44 ^{gh}
HD 2967	1.65 ^{ef}	1.66 ^{ef}	1.66 ^{f}	0.453 ^{cde}	0.458 ^{d}	0.455 ^{ef}
HD 3086	2.01 ^{b}	2.03 ^{b}	2.02 ^{b}	0.433 ^{ef}	0.436 ^{fg}	0.435 ^{gh}
HD 3249	2.20 ^{a}	2.20 ^{a}	2.20 ^{a}	0.487 ^{ab}	0.487 ^{ab}	0.487 ^{ab}
HD 2733	1.97 ^{b}	1.98 ^{bc}	1.97 ^{b}	0.460 ^{cd}	0.456 ^{de}	0.458 ^{de}
PBW 550	1.71 ^{e}	1.72 ^{e}	1.71 ^{e}	0.427 ^{f}	0.433 ^{g}	0.430 ^{h}
PBW 343	1.81 ^{d}	1.82 ^{d}	1.81 ^{d}	0.467 ^{bc}	0.471 ^{c}	0.469 ^{cd}
HD 3117	2.16 ^{a}	2.16 ^{a}	2.16 ^{a}	0.497 ^{a}	0.498 ^{a}	0.498 ^{a}
HD 3298	1.90 ^{c}	1.91 ^{c}	1.90 ^{c}	0.473 ^{bc}	0.477 ^{bc}	0.475 ^{bc}
LSD (p = 0.05)	0.07	0.07	0.05	0.022	0.011	0.012
Interaction	ns	ns	ns	ns	ns	ns
Year-1			1.91 ^{a}			0.458 ^{a}
Year-2			1.90 ^{a}			0.460 ^{a}
NCG/NCS × Year			0.022			0.33
NCG/NCS × V			0.05			0.012

LSD (p = 0.05) = least significant difference; letters indicate the comparison among genotypes under different N levels; ns = non-significant, NCG/NCS = nitrogen concentration in grain/straw, and V (variety).

Table 3. Effect of nitrogen × genotype interaction on N uptake in grain and straw of wheat.

Nitrogen × Variety		HD 3226	HDCSW 18	HD 2967	HD 3086	HD 3249	HD 2733	PBW 550	PBW 343	HD 3117	HD 3298	Mean
N uptake of grain (NUG—kg ha^{-1})												
2020	N0	20.0 ^{p}	36.8 ^{mno}	32.6 ^{no}	47.7 ^{m}	64.8 ^{l}	35.4 ^{no}	37.8 ^{mn}	26.0 ^{op}	63.7 ^{l}	35.4 ^{no}	40.03 ^{b}
	N75	43.7 ^{mn}	81.6 ^{hijk}	75.1 ^{jkl}	92.9 ^{gh}	118.5 ^{f}	82.8 ^{hij}	81.0 ^{ijk}	70.7 ^{kl}	104.4 ^{g}	82.6 ^{hij}	83.35 ^{ab}
	N150	77.2 ^{ijk}	104.6 ^{g}	126.7 ^{ef}	149.2 ^{bc}	175.0 ^{a}	124.8 ^{ef}	139.8 ^{cd}	87.7 ^{hi}	160.7 ^{b}	130.6 ^{de}	127.62 ^{a}
	Mean	47.0 ^{h}	74.3 ^{f}	78.1 ^{ef}	96.6 ^{c}	119.4 ^{a}	81.0 ^{def}	86.2 ^{d}	61.5 ^{g}	109.6 ^{b}	82.9 ^{de}	
	* N × V = 11.70/* V × N = 49.97											
2021	N0	21.5 ^{q}	35.1 ^{opq}	31.5 ^{pq}	46.3 ^{no}	66.6 ^{kl}	36.9 ^{op}	38.9 ^{nop}	27.3 ^{pq}	65.3 ^{lm}	35.6 ^{opq}	40.5 ^{b}
	N75	37.1 ^{op}	52.2 ^{mn}	91.8 ^{hij}	99.2 ^{ghi}	120.3 ^{ef}	96.6 ^{hi}	85.1 ^{ij}	47.4 ^{no}	112.0 ^{fg}	102.8 ^{gh}	84.4 ^{ab}
	N150	79.8 ^{jk}	104.9 ^{gh}	126.8 ^{de}	149.4 ^{bc}	174.8 ^{a}	125.1 ^{ef}	139.9 ^{cd}	87.5 ^{ij}	163.2 ^{ab}	133.5 ^{de}	128.5 ^{a}
	Mean	46.1 ^{e}	64.1 ^{d}	83.4 ^{c}	98.3 ^{b}	120.6 ^{a}	86.2 ^{c}	88.0 ^{c}	54.1 ^{e}	113.5 ^{a}	90.6 ^{bc}	
	* N × V = 52.5/* V × N = 8.2											
	Pooled	46.6 ^{h}	69.2 ^{f}	80.8 ^{e}	97.5 ^{c}	120.0 ^{a}	83.6 ^{de}	87.1 ^{d}	57.8 ^{g}	111.6 ^{b}	86.8 ^{d}	
	Year-1 = 83.7 ^{a} /Year-2 = 84.5 ^{a} /* NUG × Year = 3.1/*NUG × V = 5.2											
N uptake of straw (NUS—kg ha^{-1})												
2020	N0	11.6 ^{m}	12.9 ^{lm}	16.1 ^{kl}	15.3 ^{l}	19.3 ^{jk}	14.1 ^{lm}	14.9 ^{lm}	12.8 ^{lm}	20.2 ^{j}	15.1 ^{lm}	15.2 ^{b}
	N75	20.3 ^{j}	27.3 ^{i}	30.1 ^{ghi}	29.1 ^{hi}	36.6 ^{ef}	29.6 ^{hi}	28.7 ^{i}	28.2 ^{i}	33.8 ^{fg}	30.2 ^{ghi}	29.4 ^{a}
	N150	32.5 ^{gh}	39.6 ^{de}	43.0 ^{bcd}	42.2 ^{cd}	48.1 ^{a}	41.1 ^{d}	40.1 ^{de}	33.8 ^{fg}	46.4 ^{ab}	44.8 ^{abc}	41.1 ^{a}
	Mean	21.5 ^{f}	26.6 ^{de}	29.7 ^{bc}	28.9 ^{bc}	34.6 ^{a}	28.3 ^{bcd}	27.9 ^{cd}	24.9 ^{e}	33.4 ^{a}	30.0 ^{b}	
	* N × V = 1.27/* V × N = 2.28											
2021	N0	12.0 ^{l}	11.8 ^{l}	15.3 ^{jkl}	14.9 ^{jkl}	19.7 ^{hi}	13.9 ^{jkl}	15.6 ^{ijkl}	13.1 ^{kl}	20.8 ^{h}	15.1 ^{jkl}	15.2 ^{c}
	N75	17.7 ^{hij}	17.1 ^{hijk}	38.8 ^{de}	31.9 ^{fg}	35.9 ^{ef}	34.5 ^{fg}	30.3 ^{g}	19.6 ^{hi}	36.0 ^{ef}	39.4 ^{de}	30.1 ^{b}
	N150	31.5 ^{g}	39.4 ^{de}	43.9 ^{bc}	42.5 ^{cd}	48.1 ^{ab}	42.6 ^{cd}	42.6 ^{cd}	33.6 ^{fg}	48.6 ^{a}	46.2 ^{abc}	41.9 ^{a}
	Mean	20.4 ^{d}	22.8 ^{d}	32.7 ^{ab}	29.8 ^{c}	34.6 ^{a}	30.3 ^{bc}	29.5 ^{c}	22.1 ^{d}	35.1 ^{a}	33.6 ^{a}	
	* N × V = 9.9/* V × N = 2.5											
	Pooled	20.9 ^{e}	24.7 ^{d}	31.2 ^{b}	29.3 ^{c}	34.6 ^{a}	29.3 ^{c}	28.7 ^{c}	23.5 ^{d}	34.3 ^{a}	31.8 ^{b}	
	Year-1 = 28.6 ^{a} /Year-2 = 29.1 ^{a} /*NUS × Year = 28.9/* NUS × V = 1.6											

* LSD (p = 0.05) for nitrogen means at same or different level of varieties; * LSD (p = 0.05) for varieties means at same or different level of nitrogen. Values in a column followed by the different letters were significantly different at $p < 0.05$ as determined with LSD; letters indicate the comparison among genotypes under different N levels. V (variety).

Table 4. Effect of nitrogen × genotype interaction on total N uptake of wheat.

Nitrogen × Variety		HD 3226	HDCSW 18	HD 2967	HD 3086	HD 3249	HD 2733	PBW 550	PBW 343	HD 3117	HD 3298	Mean
Total N uptake (TNU kg ha^{-1})												
2020	N0	31.6 ^{q}	49.7 ^{nop}	48.7 ^{op}	63.0 ^{mn}	84.1 ^{l}	49.5 ^{nop}	52.7 ^{mno}	38.8 ^{pq}	83.8 ^{l}	50.6 ^{mnop}	55.3 ^{b}
	N75	64.1 ^{m}	109.0 ^{ijk}	105.3 ^{jk}	122.0 ^{i}	155.1 ^{fg}	112.4 ^{ijk}	109.6 ^{ijk}	98.9 ^{k}	138.2 ^{h}	112.8 ^{ij}	112.7 ^{ab}
	N150	109.7 ^{ijk}	144.2 ^{gh}	169.7 ^{de}	191.4 ^{c}	223.1 ^{a}	165.8 ^{ef}	179.9 ^{cd}	121.4 ^{i}	207.1 ^{b}	175.4 ^{de}	168.8 ^{a}
	Mean	68.5 ^{g}	100.9 ^{e}	107.9 ^{de}	125.5 ^{c}	154.1 ^{a}	109.2 ^{d}	114.1 ^{d}	86.4 ^{f}	143.1 ^{b}	112.9 ^{d}	
	* N × V = 13.80/* V × N = 61.55											
2021	N0	33.4 ^{o}	46.9 ^{mno}	46.9 ^{mno}	61.2 ^{klm}	86.3 ^{j}	50.8 ^{lmn}	54.5 ^{klmn}	40.4 ^{no}	86.1 ^{j}	50.7 ^{lmn}	55.7 ^{b}
	N75	54.8 ^{klmn}	69.3 ^{jk}	130.6 ^{gh}	131.1 ^{fgh}	156.2 ^{de}	131.0 ^{fgh}	115.3 ^{hi}	67.0 ^{kl}	148.0 ^{ef}	142.1 ^{efg}	114.6 ^{ab}
	N150	111.3 ^{i}	144.3 ^{efg}	170.7 ^{cd}	191.8 ^{b}	222.9 ^{a}	167.7 ^{cd}	182.5 ^{bc}	121.2 ^{hi}	211.8 ^{a}	179.7 ^{bc}	170.4 ^{a}
	Mean	66.5 ^{e}	86.8 ^{d}	116.0 ^{c}	128.1 ^{b}	155.1 ^{a}	116.5 ^{c}	117.4 ^{c}	76.2 ^{e}	148.6 ^{a}	124.2 ^{bc}	
	* N × V = 62.2/* V × N = 9.8											
	Pooled	67.5 ^{h}	93.9 ^{f}	112.0 ^{e}	126.8 ^{c}	154.6 ^{a}	112.9 ^{de}	115.8 ^{de}	81.3 ^{g}	145.8 ^{b}	118.5 ^{d}	
	Year-1 = 112.3 $^{a/}$ Year-2 = 113.6 $^{a/}$ *TNU × Year = 32.1/*TNU × V = 6.2											

* LSD (p = 0.05) for nitrogen means at same or different level of varieties; * LSD (p = 0.05) for varieties means at same or different level of nitrogen. Values in a column followed by the different letters were significantly different at $p < 0.05$ as determined with LSD; letters indicate the comparison among genotypes under different N levels. V(variety).

The genotype order of decreasing NUG and NUS was as follows: HD 3249 > HD 3117 > HD 3086 > PBW 550 ≥ HD 3298 ≥ HD 2733 ≥ HD 2967 > HDCSW 18 > PBW 343 > HD 3226 and HD 3249 ≥ HD 3117 ≥ HD 3298 ≥ HD 2967 > HD 3086 ≥ HD 2733 ≥ PBW 550 > HDCSW 18 ≥ PBW 343 > HD 3226. Similarly, the order of genotypes with significantly decreasing TNU was as follows: HD 3249 > HD 3117 > HD 3086 > HD 3298 > PBW 550 ≥ HD 2733 ≥ HD 2967 > HDCSW 18 > PBW 343 > HD 3226.

3.3. Partial N Balance

Pooled analysis data showed that mean nitrogen (N) input conditions for partial N balance were the same in both years (Table 5). Among all genotypes, the highest and lowest partial N balance was observed in genotype 'HD 3249' and 'HD 3226'.

Table 5. Effect of nitrogen × genotype interaction on partial nitrogen balance of wheat.

Nitrogen × Variety		HD 3226	HDCSW 18	HD 2967	HD 3086	HD 3249	HD 2733	PBW 550	PBW 343	HD 3117	HD 3298	Mean
Partial N balance—PNB (Kg N removed per kg N applied)												
2020–2021 (N)	N75	1.07 ^{jk}	1.82 ^{de}	1.75 ^{def}	2.03 ^{c}	2.59 ^{a}	1.87 ^{cd}	1.83 ^{d}	1.65 ^{efg}	2.30 ^{b}	1.88 ^{cd}	1.88 ^{a}
	N150	0.91 ^{k}	1.20 ^{j}	1.41 ^{i}	1.59 ^{fgh}	1.86 ^{cd}	1.38 ^{i}	1.50 ^{ghi}	1.01 ^{k}	1.73 ^{def}	1.46 ^{hi}	1.41 ^{b}
	Mean	0.99 ^{g}	1.51 ^{e}	1.58 ^{de}	1.81 ^{c}	2.22 ^{a}	1.63 ^{de}	1.66 ^{d}	1.33 ^{f}	2.01 ^{b}	1.67 ^{d}	
	* N × V = 0.179/* V × N = 0.176											
2021–2022	N75	0.93 ^{i}	1.20 ^{gh}	2.18 ^{c}	2.19 ^{c}	2.6^{0} ^{a}	2.18 ^{c}	1.92 ^{d}	1.12 ^{hi}	2.47 ^{ab}	2.37 ^{bc}	1.91 ^{a}
	N150	0.91 ^{i}	1.16 ^{h}	1.42 ^{fg}	1.60 ^{ef}	1.86 ^{d}	1.40 ^{fg}	1.52 ^{f}	1.01 ^{hi}	1.77 ^{de}	1.50 ^{f}	1.42 ^{a}
	Mean	0.92 ^{e}	1.18 ^{d}	1.80 ^{bc}	1.89 ^{b}	2.23 ^{a}	1.79 ^{bc}	1.72 ^{c}	1.06 ^{de}	2.12 ^{a}	1.93 ^{b}	
	* N × V = 0.22/* V × N = 0.48											
	Pooled	0.96 ^{d}	1.34 ^{c}	1.69 ^{b}	1.85 ^{b}	2.23 ^{a}	1.71 ^{b}	1.69 ^{b}	1.20 ^{c}	2.07 ^{a}	1.80 ^{b}	
	Year-1 = 1.64 ^{a} /Year-2 = 1.66 ^{a} /*PNB × Year = 0.52/*PNB × V = 0.10											

* LSD (p = 0.05) for nitrogen means at same or different level of varieties; * LSD (p = 0.05) for varieties means at same or different level of nitrogen. Values in a column followed by the different letters were significantly different at $p < 0.05$ as determined with LSD; letters indicate the comparison among genotypes under different N levels. V = variety.

The order of genotypes, ranked according to the significantly decreasing partial N balance, was as follows: HD 3249 ≥ HD 3117 > HD 3086 ≥ HD 3298 ≥ HD 2733 ≥ HD 2967 ≥ PBW 550 > HDCSW 18 ≥ PBW 343 > HD 3226.

3.4. Internal Nitrogen Use Efficiency

The internal nitrogen use efficiency (IE_N) decreased with increasing N inputs (Table 6). Pooled analysis data showed that mean nitrogen input conditions for IE_N were the same in

both years. Among all genotypes, the highest IE_N was observed in genotype 'PBW 550', which is at par with 'HD 2967' genotypes, and the lowest IE_N was observed in genotypes 'HD 3117' and 'HD 3249'. The order of genotypes, ranked according to the significantly decreasing partial IE_N, was as follows: PBW 550 ≥ HD 2967 ≥ HD 3226 > HDCSW 18 ≥ HD 3086 ≥ PBW 343 ≥ HD 3298 ≥ HD 2733 > HD 3117 ≥ HD 3249.

Table 6. Effect of nitrogen × genotype interaction on internal nitrogen use efficiency of wheat.

Nitrogen × Variety		HD 3226	HDCSW 18	HD 2967	HD 3086	HD 3249	HD 2733	PBW 550	PBW 343	HD 3117	HD 3298	Mean
2020–2021 (N)	N75	40.8 ^{a}	37.0 ^{b}	42.0 ^{a}	36.9 ^{b}	34.3 ^{e}	36.5 ^{bcd}	41.9 ^{a}	37.4 ^{b}	34.7 ^{de}	36.6 ^{bc}	37.6 ^{a}
	N150	36.6 ^{bc}	35.0 ^{cde}	37.5 ^{b}	34.9 ^{cde}	33.3 ^{e}	33.8 ^{e}	38.0 ^{b}	34.5 ^{e}	33.5 ^{e}	34.3 ^{e}	35.3 ^{b}
	Mean	38.7 ^{a}	36.0 ^{b}	39.8 ^{a}	35.9 ^{b}	33.8 ^{d}	35.1 ^{bc}	39.9 ^{a}	36.0 ^{b}	34.1 ^{cd}	35.5 ^{b}	
	* N × V = 1.7/* V × N = 1.8											
2021–2022	N75	40.1 ^{a}	36.8 ^{b}	40.9 ^{a}	36.6 ^{bc}	34.2 ^{def}	36.0 ^{bcd}	41.6 ^{a}	36.5 ^{bc}	34.5 ^{def}	35.8 ^{bcde}	37.1 ^{a}
	N150	37.0 ^{b}	34.8 ^{cdef}	37.2 ^{b}	34.2 ^{def}	33.3 ^{f}	33.4 ^{f}	37.3 ^{b}	34.5 ^{def}	33.2 ^{f}	34.1 ^{ef}	35.1 ^{a}
	Mean	38.5 ^{a}	35.8 ^{b}	39.1 ^{a}	35.4 ^{b}	33.7 ^{c}	34.7 ^{bc}	39.5 ^{a}	35.5 ^{b}	33.9 ^{c}	34.9 ^{bc}	
	* N × V = 1.86/* V × N = 1.91											
	Pooled	38.6 ^{b}	35.9 ^{c}	39.4 ^{ab}	35.7 ^{cd}	33.8 ^{e}	34.9 ^{d}	39.7 ^{a}	35.7 ^{cd}	34.0 ^{e}	35.2 ^{cd}	
	Year-1 = 36.5 ^{a} /Year-2 = 36.1 ^{a} /* INUE × Year = 2/* INUE × V = 0.9											

* LSD (p = 0.05) for nitrogen means at same or different level of varieties; * LSD (p = 0.05) for varieties means at same or different level of nitrogen. Values in a column followed by the different letters were significantly different at $p < 0.05$ as determined with LSD; letters indicate the comparison among genotypes under different N levels; N (nitrogen), * INUE (internal nitrogen use efficiency), and V (variety).

3.5. Correlation Analysis among Crop Traits

The crop traits, including GY (grain yield), SY (straw yield), BY (biological yield), HI (harvest index), PNCG (percentage N content in grain), PNCS (percentage N content in straw), NUG (N uptake in grain), NUS (N uptake in straw), and TNU (total N uptake), were analyzed for their relationships using the performance analytics package. A correlation coefficient matrix (Figure 6) was generated, indicating a significant positive correlation ($p < 0.01$) among all traits of the crop at mean levels of N.

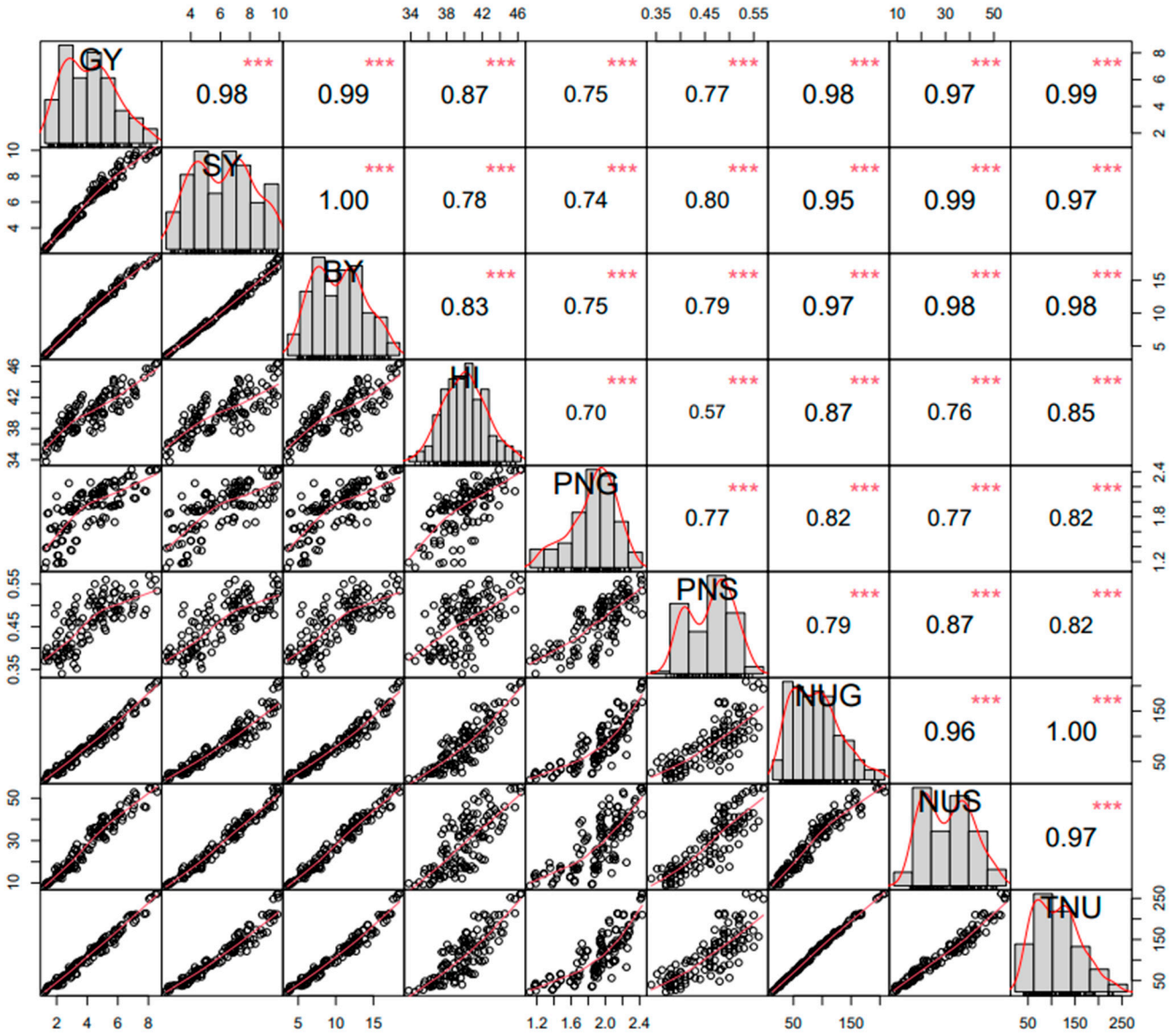


Figure 6. Pearson's correlation among crop traits/parameters of ten wheat genotypes under nitrogen fertilization. Grain yield (GY), straw yield (SY), biological yield (BY), harvest index (HI), percentage N concentration in grain (PNG) and straw (PNS), N uptake in grain (NUG) and straw (NUS), and total N uptake (TNU). The correlation coefficient (r values) was calculated from mean of two years of data from 2020–2021 to 2021–2022. ***: highly significant ($p < 0.001$). Diagonals indicate how each parameter is distributed. At the bottom of diagonal scatter plots, lines are available. At the top of the diagonal, values of correlations and significance level are available. Size of correlation values and intensity of color show correlation coefficients.

4. Discussion

In this study, we performed a statistical analysis over two years, N levels, and genotypes to investigate the potential interaction between them for various crop parameters. Our analysis revealed the presence of significant interaction of years on crop parameters such as, straw yield (SY), biological yield (BY), N concentration in grain (NCG/NCS) and straw, N uptake in straw (NUS), and total N uptake (TNU) of genotypes. This suggests that the performance of different genotypes under varying N levels may be influenced by the specific year or years in which the crops are grown. In addition, this interaction can also be influenced by the specific traits of each genotype. The interaction between N levels and genotypes was common across most of the crop parameters studied.

Overall, annual variations in the data were observed, primarily due to the high rainfall in the second year. While grain yields (GY) remained statistically similar over the two years of the study, the interaction of years, nitrogen levels, and genotypes had a significant impact on most of the crop traits.

In spite of variations in N inputs and their timing of application, it has been observed that using a N rate of N_{150} leads to higher crop yields compared to N rates of N_{75} and N_0. This may be attributed to the fact that neem-oil-coated urea (NOCU) is a slow-release N fertilizer that releases nutrients slowly and undergoes gradual conversion to ammonia and nitrate in the soil. This characteristic allows for an extended period of available N compared to N inputs N75 and N0. In a study conducted by Singh et al. [17], it was observed that the use of NOCU in wheat resulted in a significant enhancement of approximately 5–6% in GY compared to the use of urea at equivalent levels.

This study aimed to assess how various wheat genotypes respond to N fertilization. Genotypes that give the highest response to the application of N would automatically have higher nitrogen uptake. These findings highlight the need for a more comprehensive understanding of the complex interactions between different factors in crop growth and development, which could lead to more effective crop production management strategies. Furthermore, our results suggest that future research should aim to investigate the underlying mechanisms that contribute to these interactions in order to better understand how to optimize crop performance under varying environmental conditions. The study provides valuable information on the potential impact of genotype, N levels, and year-to-year variability on crop performance, which can inform future research efforts and agricultural practices.

Studies have shown that applying N fertilizers based on N uptake and utilization to wheat genotypes with varying levels of nutrient uptake patterns (low, moderate, and high) can improve wheat yield. Wheat genotypes from the same genetic lineage have been observed to exhibit differential responses to varying levels of N at different times of the year [18,19]. Our results show that traits of the winter wheat genotype traits such as GY, SY, BY, HI, and nutrient uptake had a high level of genetic diversity. Therefore, selecting genotypes with high yields and N uptake would be beneficial for breeding programs. To improve N utilization in wheat, two potential strategies can be used, namely genotype screening and genotype improvement. The former approach involves the identification of genotypes that possess a high capacity for nitrogen (N) uptake and utilization based on their capacity to react to diverse levels of N application rates, influenced by climate and soil conditions. The latter approach focuses on developing wheat genotypes with enhanced N uptake and utilization efficiency through selective breeding techniques [20].

Several previous studies in wheat cultivars have also reported significant nitrogen × genotype interactions (N × G) for GY and other agronomic traits [1,20–26]. The interaction of N × G is a crucial factor that affects the performance of various genotypes under different ecologies. According to Hitz et al. [1], the interaction between N × G was a significant factor that offers breeders a means to differentiate genotype performance between two different environments of N.

4.1. Effects of N Fertilization on Wheat Yields, Harvest Index, and Nitrogen Uptake

This study revealed that N rates significantly affect wheat genotypes GY, SY, and BY. All genotypes showed substantial yield and HI variation. The highest yields and HI yields were with 'HD 3249' and with 'HD 3117' as the second. These genotypes had the best yields because N150 splits increased spikes per unit area and spike grain, leading to a high GY. The N levels from 75 to 150 kg N ha^{-1} reliably boost yields, harvest index, and N absorption. Some genotypes performed well at all N rates; Kubar et al. [27] found similar findings.

The research also revealed that genotype yield and HI were positively correlated with N levels. Thus, identifying an appropriate N application rate was an excellent way to balance crop economic advantages and ecological concerns. Wheat genotypes with N75

and N150 treatments yielded 72.3% and 142.6% more than N0. Compared to N75, N150 has a 41% greater GY. The two genotypes ('HD 3249' and 'HD 3117') with the greatest straw yield had N150 splits, which increased photosynthesis and dry matter output. The top dressing of wheat genotypes with the right dosage and timing of N may boost yields and HI [27–30]. The huge production discrepancies between the three N levels show that crop yields may be increased, particularly in plots with low N fertilization. Studies have shown that an adequate N fertilizer improves GY [31,32]. N nutrition exerts a significant effect on economic yield, with an increased N application rate showing remarkable effects on winter wheat yields and N uptake of up to N level N150. Other investigations have shown that N is essential for grain and straw production. Therefore, it can be considered as a pivotal factor in this process [33,34].

Studies have reported moderate heritability for above-ground biomass in wheat [24,35], indicating that cultivating wheat varieties with greater biomass and a high HI may improve grain production more specifically. Field trials in low, medium, and high N environments help assess potential genotypes [36]. This method compares genotype genetic progress across N levels to determine performance similarities and differences [37]. This technique, as previously studied by Cormier et al. [36], is essential to an effective genotype selection framework, giving vital information on genotype production and yield stability.

Wheat genotypes, weather conditions, and growing years influenced N buildup and absorption throughout maturity. The total N uptake from wheat genotypes ranged from 66.5 to 155.1 kg ha^{-1}, with an average of approximately 112.3 kg ha^{-1}. The highest N uptake was observed in the cultivar 'HD 3249' and the lowest in the cultivar 'HD 3226' in both years. Every genotype displayed a variable response to N levels owing to their diverse N absorption patterns and physicochemical, agronomic, and genetic composition [20,38]. Brasier et al. [39] found genotypic variance in wheat germplasm nitrogen utilization. Hitz et al. [1] studied wheat N absorption with different N fertilization rates and severe N stress. Their findings indicate that screening genotypes in low-N environments alongside environments with high N is crucial to identifying genotypes of high-N uptake and utilization. Brasier et al. [40] recommend testing wheat genotypes for high-N-use genetic materials in three N rates and diverse settings. Our use of three N fertilizer rates suggests that increasing the number of rates could improve the significance of our results.

Thus, it is crucial to identify and choose genotypes that possess a good genetic potential for the efficiency of N to optimize crop production while minimizing the use of N fertilizers. Sylvester-Bradley and Kindred [8] proposed an alternative objective for N uptake and utilization. They defined the economic N optimum as the amount of N needed to maximize earnings by maximizing yields and minimizing input expenses. It is clear from the economic N optimal that N responsiveness is needed to improve N uptake and utilization, although it cannot be used as a breeding target [41]. Sylvester-Bradley et al. [42] reported that the responsiveness of genotypes to varying levels of N might facilitate the screening of cultivars with high responsiveness to both high and low levels of N. In a study by De Oliveira Silva et al. [43], it was found that genotypes with high mean response and high variability in the response to intensified management (IM) across years could offer greater opportunities for producers to maximize yield as those genotypes showed greater yield gain from IM when conditions favored their response.

Furthermore, the intensive use of N fertilizers in agricultural regions has led to serious environmental problems associated with the enrichment of the atmosphere, soil, and water with various forms of N [44]. Overuse of N fertilization can lead to environmental issues such as eutrophication, greenhouse gas emissions, and nitrate pollution.

Therefore, the implementation of optimal N fertilization management is crucial to achieve a more favorable N balance and reduce environmental hazards. To maximize wheat yield while minimizing environmental problems associated with N use, it is recommended to use a suitable N rate for each genotype that considers regional weather and soil conditions.

Partial N balance (PNB) analysis provides valuable insights into N dynamics concerning their uptake in relation to the applied N within the system. Regarding nitrogen (N), PNB values were consistently above one in various wheat genotypes, except for 'HD 3236' with a value of 0.96. This data revealed that N removal was greater than the applied N inputs. However, N was supplied from diverse sources, such as irrigation water, atmospheric N, etc.

Internal Nitrogen Use Efficiency (IE_N) refers to the quantitative assessment of a crop's inherent ability to transform nitrogen derived from various sources into the ultimate economic yield [45]. High IE_N indicates N deficiency, and low IE_N implies poor internal nitrogen conversion due to various stresses (nutrient deficiency and toxicity, drought, heat stress, pests, etc.) [46]. In this experiment, IE_N spanned from 33.2 to 42 kg kg^{-1} (mean: 36.3 kg kg^{-1}), which was below the IE_N of 62.3 kg kg^{-1}, reported by [47]. This value lies between the IE_N range (18.30–65.90 kg kg^{-1}) observed in cereal-based systems [48].

4.2. Relationship between Crop Traits/Parameters of Wheat

Our study revealed a positive correlation between yields, harvest index (HI), nitrogen (N) concentrations, and their uptake in different parts of the plant during various growth stages. The results are in accordance with earlier studies conducted by Ramanjaneyulu et al. [49], Huang et al. [50], Wang et al. [51], Singh et al. [52], and Samonte et al. [53]. Wheat genotypes exhibited significant variability in grain yield, amplified by N rate–environment interactions. Key determinants of wheat grain yield include genetic factors, adaptability, and stability to varying environmental conditions as well as optimal water and nutrient uptake [39,54–57]. Therefore, it is recommended to prioritize the investigation of these N nutrition traits in wheat breeding programs, as they have the potential to improve the productivity and efficiency of N utilization in genotypes. The 'HD 3249' and 'HD 3117' genotypes exhibited better N uptake and grain yield at three levels of N, highlighting their potential for inclusion in regional breeding programs. Nitrogen uptake is influenced by root biomass, morphology that allows exploration of regions with high N levels, and the physiological ability to absorb nutrients [58].

Thus, comprehending N metabolism in a particular genotype and identifying wheat genotypes with enhanced nitrogen uptake, combined with optimizing nitrogen (N) input strategies, could lead to improved N uptake, grain yield (GY), and mitigation of detrimental effects resulting from excessive N fertilizer application.

5. Conclusions

In conclusion, N levels considerably affect each genotype's agronomic attributes and nitrogen (N) uptake. All examined genotypes showed a different directional response to three N levels, suggesting direct selection and optimization of N application for particular genotypes. The selection of genotypes with high nitrogen absorption and excellent yields enables breeding progress and yield improvements. Genotype-specific N application increased yields, HI, N uptake, and partial N balance. 'HD 3249' and 'HD 3117' had greater yields, HI, and N absorption at the same N application. Indian wheat breeding efforts may use these genotypes to boost production and minimize N losses. Wheat yields, HI, and N uptake are positively correlated. Thus, breeding programs may study these N nutrition features to create novel wheat genotypes that are highly productive and effectively use N. Future selection and breeding attempts to minimize N losses in Indian wheat will benefit from these findings. Our work supports the importance of selecting optimal N levels to boost wheat yields, HI, N absorption, and utilization.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13102447/s1, Table S1: Some of the basic characteristics of the wheat cultivars used in the current research experiment. References [59–64] are cited in the supplementary materials.

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