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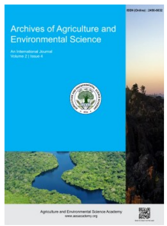

ORIGINAL RESEARCH ARTICLE

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Genetic diversity and morphological characterization of wheat (*Triticum aestivum* L.) landraces in Nepal

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ABSTRACT

This study examined the genetic diversity and phenotypic characteristics of 60 wheat landraces from 24 districts across Nepal. The study was conducted at the National Agriculture Genetic Resources Centre (NAGRC) in Khumaltar, where it employed a non-replicated augmented block design to assess eight qualitative and sixteen quantitative traits based on Bioversity International descriptors. The Shannon-Weaver diversity index (H') for wheat landraces varies from 0.457 to 0.979 across qualitative traits. Tillering capacity shows the highest diversity at 0.979, and glume hairiness has the lowest diversity at 0.457. The coefficient of variation (CV) for the quantitative traits of wheat landraces varied widely; percentages ranged from 7.58% for days of heading, suggesting relatively low variability, to 36.56% for spike exertion, indicating high variability among the samples. Principal Component Analysis (PCA) with an eigenvalue greater than 1 revealed that five principal components accounted for 70.95% of the variability, with traits like plant height and spike exertion playing pivotal roles in genotype differentiation. A dendrogram generated using a UPGMA clustering approach organized the landraces into two groups. Cluster-I consists of 56 accessions (93.33%), and Cluster–II consist of 4 Accessions (6.67%) separated by 2374.99, indicating phenotypic differentiation between the groups. These findings underscore the importance of targeted breeding programs based on specific trait performance, supported by further correlation analysis to identify optimal characteristics for breeding. This research highlights the need to continue evaluating these landraces through environmental trials and biotechnological approaches to fully capitalize on their genetic potential for improving wheat cultivation.

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is a vital cereal crop cultivated worldwide, serving as a significant source of daily caloric intake for over one-fifth of the global population and accounts for approximately 28% of global cereal production (Bai *et al.*, 2024). This crop is crucial to global food security, especially in developing countries (Grote *et al.*, 2021). Approximately 9000 years ago, an accidental hybridization between domesticated emmer wheat (*T. turgidum* conv. turgidum, 2n = 4x = 28) and goat grass (Ae. tauschii spp. strangulate, 2n = 2x = 14) with chromosome doubling naturally resulted in the generation of a free-threshing hexaploid common wheat (*T. aestivum*, 2n = 6x = 42) (Wan *et al.*, 2023). In 2022, global wheat production reached 946.16 million tons and over 242.67 million hectares were cultivated, highlighting its importance in global agriculture (FAOSTAT, 2022). In Nepal, wheat is a critical crop, contributing significantly to national food security with a production of 3,106,397 metric tons and a productivity of 3.15 mt/ha in 2022/23. It is nutritious, easy to store and transport and can be processed into various types of food. Wheat is a good source of protein, minerals, B-group vitamins and dietary fiber (Shewry, 2007). Although environmental conditions can affect the nutritional composition of wheat grains with their essential coating of bran, vitamins and minerals, it is an excellent health-building food.

Wheat is a mesophytic plant, so for the cultivation of wheat, the temperature range is relatively narrow, ranging from 10°C-15° C during sowing to 21°C-26°C during the ripening period. However, some wheat varieties can grow at 35°C (Khan *et al.*, 2020). Nepal's unique geographic and climatic diversity has fostered a rich array of wheat landraces, each adapted to specific local conditions and possessing valuable traits such as disease resistance and drought tolerance (Kaduwal *et al.*, 2019; Kumar *et al.*, 2023). These landraces represent a valuable genetic resource for enhancing wheat production and resilience, especially under changing climatic conditions (Broccanello *et al.*, 2023). Preserving and utilizing genetic diversity is essential for creating new varieties to tackle emerging challenges such as climate change and pest attacks and enhancing food security (Vincent *et al.*, 2016; Hoban *et al.*, 2021). Plant genetic diversity allows researchers to develop new, improved varieties with desirable traits, which accommodate both farmer's and breeder's preferred traits (Temesgen, 2021). Integrating landraces into breeding programs can ensure sustainable wheat production by leveraging their adaptability and resilience (Cheng *et al.*, 2024). This becomes especially important as climate variability increases, necessitating the development of wheat varieties suited to diverse environmental conditions (Zhang *et al.*, 2022). Nepal can maintain and enhance its wheat productivity by preserving these genetic resources while protecting the environment (Joshi *et al.*, 2013)

The advent of high-yielding wheat varieties and modern agricultural technologies has undoubtedly boosted wheat productivity worldwide, including in Nepal. However, this progress has often come at the cost of neglecting traditional landraces with unique traits crucial for long-term sustainability (Prasai, 2017). Although there are many genetic resources available on a national and international level, breeders tend to focus only on adapted and improved cultivars, often overlooking landraces and wild and weedy relatives in their breeding program (Upadhyaya *et al.*, 2014). Integrating these local varieties into breeding programs is now more critical than ever. While the genetic diversity and morphological characterization of wheat landraces are essential, a comprehensive assessment of Nepalese landraces using qualitative and quantitative traits has been lacking. This research addresses this gap by assessing the genetic diversity and morphological characteristics of 60 wheat landraces collected from different districts of Nepal and conserved in the National Agriculture Genetic Resources Centre (Genebank). This approach allows for developing resilient genotypes that thrive in Nepal's diverse agroecological conditions. It represents a commitment to a more sustainable and secure future for Nepal's wheat production (Joshi *et al.*, 2013).

Existing literature lacks a comprehensive assessment of Nepalese wheat landraces that combines morphological and genetic diversity analyses to identify superior genotypes for specific traits (Karkee *et al.*, 2023). There is a need for studies that go beyond simple characterization and provide actionable information for breeders and conservationists to utilize these genetic resources better. This research assesses Nepalese wheat landraces' genetic diversity and morphological characteristics, highlighting their potential to improve breeding programs. By understanding genetics and morphological variations, the study aims to identify superior genotypes for developing improved varieties that can adapt to local conditions and withstand biotic and abiotic stresses. The specific objectives of this study was to assessing the extent of genetic diversity among these landraces using qualitative and quantitative traits, identifying key morphological traits that contribute significantly to this diversity, and identifying superior genotypes with desirable traits for potential use in wheat breeding programs.

MATERIALS AND METHODS

Experimental site

An on-station experiment was conducted at a research field of the National Agriculture Genetic Resources Centre (NAGRC), NARC, Khumaltar, Lalitpur, during the primary cropping season (Figure 1). The research station is located at 27.6471°N latitude, 85.3233°E longitude, and an altitude of 1368 masl. The location represents the mid-hill region with a subtropical climate, which receives annual rainfall of around 1250 mm. The dominant soil in research is loamy clay, with a pH of 5-7.

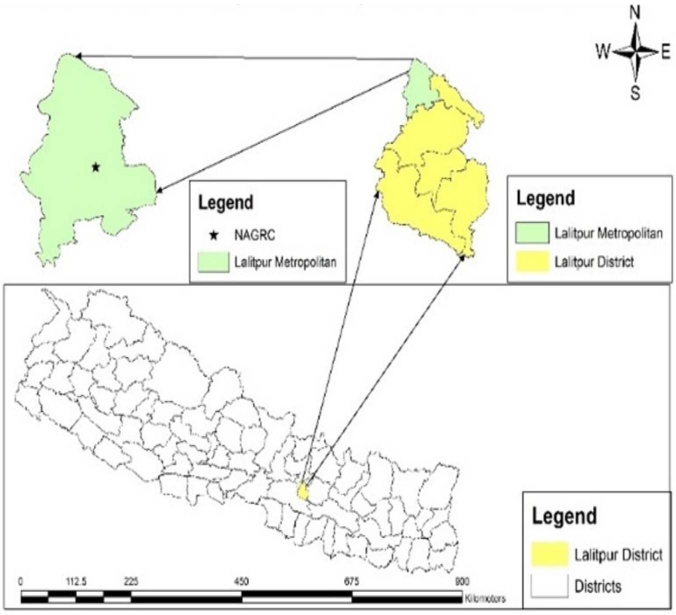

Figure 1. *Study area showing Gene Bank, Khumaltar, Lalitpur, Nepal.*

Experimental design and materials

Sixty landraces of wheat collected from 24 districts of Nepal are grown in the field of NAGRC for regeneration and agro-morphological characterization. An experiment was conducted using a non-replicated augmented block design. The distance between rows was maintained at 25cm, while the space between plants was set at 15cm. Each plot had a width of 3 meters and was separated by 1 meter. Before the land was prepared, Farmyard manure (FYM) was applied as the main fertilizer at 25 Mt/ha, 10 days in advance. This was followed by carefully applying chemical fertilizer at the row, with a dose of 60:40:60 NPK/ha. This systematic approach, which included the meticulous preparation of the land, ensured that the plants received the necessary nutrients and water during their critical growth periods.

Data collection

Data was taken from the description of vegetative and phenological characters. The agro-morphological traits were measured at various growth stages according to the Wheat descriptor developed by (International Board for Plant Genetic, 1985), now known as Bioversity International. We used a descriptor list to record qualitative and quantitative characters. A descriptor list was made based on field observation during the ethnobotanical survey and characterization. For each accession, six central plants were tagged for data recording. The eight qualitative traits were observed at different growth stages: Growth habit, Plant height, Seed colour, Seed size, Glume colour, Glume hairiness, Tillering capacity and Spike density. Similarly, sixteen quantitative traits were recorded following the IGPRI descriptor for wheat through visual observation: days of heading, flag leaf length, flag leaf width, spike density, plant height, spike exertion, spike length, number of seeds per spikelet, Number of spikelet per spike, Number of seeds per spike, 1000 grain weight, seed length, seed width, days to 80% maturity, days to harvest, yield per hectare.

Data analysis

Descriptive statistics such as minimum mean, maximum standard deviation, standard error, the mean coefficient of variation, Principal component analysis, and correlation were calculated with the R-studio. Principal Component Analysis (PCA) was conducted to identify the major components contributing to the variability among the genotypes. The principal components with an Eigenvalue of more than 1 were chosen for further study. The average method was used for cluster analysis, and the data matrix employed was identical to the one used in PCA. A circular dendrogram was constructed using Originpro to visualize the hierarchical clustering of the genotypes.

The diversity index was calculated using MS Excel 2016. (Thapa *et al.*, 2021) also used this tools to analyze diversity. To analyze diversity, the Standardized Shannon-Weaver diversity index (H') (Shannon CE & W Weaver, 1949) was calculated using the formula:

$$H' = [\sum(n/N) * \{Log2(n/N) * (-1)\}]/log2k$$

Where H'= standardized Shannon–Weaver diversity index, K = number of phenotypic classes for a character, n = frequency of a phenotypic class of that character, and N= Number of observations/landraces for that character. Accessions were divided into ten phenotypic classes to measure H' for quantitative traits. For this; x-2.5sd, x-2sd, x-1.5sd, x-1sd, x-0.5sd, x, x+0.5sd, x-1sd, x-1.5sd, x-2sd, & gt; x+2sd are as the margin of the classes, where x is average and sd is standard deviation. H'≥0.60, 0.40≤H'≤0.60, 0.10≤H'≤0.40 are considered as traits of high, intermediate and low diversity index, respectively.

RESULTS AND DISCUSSION

Diversity based on qualitative traits

The Shannon-Weaver diversity index (H') is an index capable of measuring diversity in a population, with higher values representing the higher diversity in that population. The diversity index computed for eight qualitative characters exhibited notable variation, with glume colour showing a relatively high diversity index (H'=0.651), as shown in Table 1. Among the eight qualitative traits, only two traits had a diversity index (H') > 0.60, which are considered to have a high diversity index: seed colour (H'=0.63) and glume colour (H'=0.651). This aligns with the findings by (Ullah Ajmal *et al.*, 2013), who noted the relevance of qualitative traits in differentiating genotypes in wheat diversity studies. In contrast, glume hairiness had the lowest diversity index (H'=0.457), suggesting limited variation and possible dominance of specific phenotypes (Fellahi *et al.*, 2024). Other traits such as plant height (H'=0.579), growth habit (H'=0.502), spike density (H'=0.6), and seed size (H'=0.469) displayed low to intermediate diversity. The high diversity in traits such as seed, and glume colors indicates genetic variability and potential adaptability. In contrast, the lower diversity in traits like glume hairiness highlights a strong selection pressure or genetic uniformity within the population. These findings collectively highlight the significant genetic diversity among the wheat landraces, which is crucial for breeding programs and conservation efforts.

Diversity based on quantitative traits

The mean, standard deviation, maximum, minimum and coefficient of variation (CV%) for 16 quantitative traits of the 60 landraces are depicted in Table 2. The days of the heading varied between 108 and 143 days, with an average of 117.3 days, a standard error (SE) of 1.15, and a coefficient of variation (CV) of 7.58%. The flag leaf length ranged from 10.64 cm to 24.68 cm, averaging 16.27 cm, and showed moderate variability with a CV of 16.9%. The width of the flag leaf exhibited less variation, with an average width of 1.4 cm and a range from 1.06 cm to 1.9 cm. Spike density per square meter displayed considerable variability, varying from 86 to 483 spikes, with an average density of 282.23 and a higher CV of 27.46%. Plant height varied widely, from 64.92 cm to 139.8 cm, with an average height of 108.64 cm, while spike exertion ranged significantly from 4.16 cm to 35.92 cm, with an average of 18.68 cm and the highest CV

Table 1. Shannon-weaver diversity index (H'), descriptor's state, and frequency distribution for qualitative traits among wheat landraces.

S. No.	Qualitative characters	H'	Descriptor's state	Frequency	Proportion
1	Growth habit	0.502	3- Semi Erect	6	10
			5- Intermediate	12	20
			7- Prostrate	42	70
2	Plant height	0.579	0-Dwarf	8	13.33
			1-Semi-dwarf	22	36.67
			2-Tall	30	50
3	Seed color	0.63	1-White	30	50
			2-Red	30	50
			3-Purple	0	0
4	Seed size	0.469	3- small	3	5
			5- Intermediate	24	40
			7- large	33	55
			9- Very Large	0	0
5	Glume color	0.651	1-White	29	48.33
			2-Red to Brown	19	31.67
			3-Purple to Black	12	20
6	Glume Hairiness	0.457	0-Absent	47	78.33
			3-low	11	18.33
			7- High	2	3.33
7	Tillering capacity	0.979	3-Low	35	58.33
			7-High	25	41.67
8	Spike Density	0.6	1-Very lax	0	0
			3-Lax	13	21.67
			5-Intermediate	33	55
			7-Dense	14	23.33
			9-Very dense	0	0

Table 2. Coefficient of variation of 16 quantitative traits of wheat landraces.

Traits	Minimum	Maximum	Mean	SE mean	SD	CV
Days of Heading	108	143	117.3	1.15	8.89	0.07580
Flag leaf length (cm)	10.64	24.68	16.27	0.35	2.74	0.16904
Flag leaf width (cm)	1.06	1.9	1.4	0.03	0.21	0.15067
Spike density (per sq.m)	86	483	282.23	10.007	77.51	0.27464
Plant height (cm)	64.92	139.8	108.64	2.29	17.80	0.16384
Spike exertion (cm)	4.16	35.92	18.68	0.88	6.83	0.36556
Spike length (cm)	6.64	14.98	9.55	0.24	1.84	0.19288
Number of seeds per spikelet	2	4	3.1	0.051	0.39	0.12875
Number of spikelets per spike	15	55	20.23	0.64	4.99	0.24675
Number of seeds per spike	35	70	49.78	1.105	8.56	0.17196
1000 grains (g)	26	64.2	44.58	1.15	8.93	0.20038
Seed length (mm)	5.406	8.304	6.77	0.091	0.71	0.10514
Seed width (mm)	2.664	4.112	3.55	0.041	0.32	0.09064
Days to 80% maturity	147	174	158.18	0.50	3.93	0.02486
Days to harvest	173	178	175.08	0.32	2.48	0.01419
Yield kg per hectare	1.06	5.45	3046.21	113.51	879.24	0.28863

among the traits at 36.56%. Spike length and the number of seeds per spikelet were relatively consistent, with mean values of 9.55 cm and 3.1, respectively. The number of spikelet's and seeds per spike exhibited considerable variability, with mean values of 20.23 and 49.78, respectively. Grain characteristics, including 1000 grain weight, seed length, and seed width, displayed significant diversity, with the weight of 1000 grains ranging from 26 gm to 64.2 gm and averaging 44.58 gm. The measurements of seeds varied, with lengths spanning from 5.406 mm to 8.304 mm and widths from 2.664 mm to 4.112 mm. Days to 80% maturity and days to harvest were less variable, characterized by narrower ranges and lower CV values. Yield per hectare demonstrated the widest range among all traits, varying from 1.06 kg to 5.45 kg per hectare, with an average yield of 3046.21 kg per hectare, emphasizing the potential for significant yield improvements through focused breeding and agricultural practices. This detailed analysis highlights the variability and opportunities for genetic enhancement in wheat landraces. Similar variations between the quantitative characteristics were found in the study conducted for the wheat landraces by (Mohammadi *et al.*, 2022). Breeders can develop varieties that offer better productivity and economic returns by focusing on landraces with higher yields.

Table 3. Showcasing the description of the 60 wheat landraces based on collected location and hardiness level.

S. No.	Genotype label	Accession number	Local name	Collected site	Latitude	Longitude	Altitude (masl)
1	N1	NGRC00004	Local gahun	Baglung, Bhimpokhara	28.29	83.5309	1963
2	N2	NGRC00017	Local gahun	Jumla, Patarashi	29.34	82.5	2697
3	N3	NGRC00062	Local gahun	Kanchanpur, Suda	28.9862	80.2323	251
4	N4	NGRC00073	Local gahun	Kanchanpur, Suda	28.9862	80.2323	251
5	N5	NGRC00105	Local gahun	Kanchanpur, Suda	28.9862	80.2323	251
6	N6	NGRC00107	Local gahun	Kanchanpur, Suda	28.9862	80.2323	251
7	N7	NGRC00108	Local gahun	Kanchanpur, Suda	28.9862	80.2323	251
8	N8	NGRC00132	Local gahun	Kanchanpur, Suda	28.9862	80.2323	251
9	N9	NGRC00134	Local gahun	Kanchanpur, Suda	28.9862	80.2323	251
10	N10	NGRC00136	Local gahun	Kanchanpur, Suda	28.9862	80.2323	251
11	N11	NGRC00138	Local gahun	Kanchanpur, Suda	28.9862	80.2323	251
12	N12	NGRC00149	Local gahun	Kanchanpur, Suda	28.9862	80.2323	251
13	N13	NGRC00153	Local gahun	Kanchanpur, Suda	28.9862	80.2323	251
14	N14	NGRC00157	Local gahun	Kanchanpur, Suda	28.9862	80.2323	251
15	N15	NGRC00158	Local gahun	Kanchanpur, Suda	28.9862	80.2323	251
16	N16	NGRC00159	Local gahun	Kanchanpur, Suda	28.9862	80.2323	251
17	N17	NGRC00162	Local gahun	Kanchanpur, Suda	28.9862	80.2323	251
18	N18	NGRC00172	Local gahun	Kanchanpur, Suda	28.9862	80.2323	251
19	N19	NGRC00184	Dho gahun	Mustang, Khinga	28.8238	83.8235	3216
20	N20	NGRC00188	Local gahun	Myagdi, Raulthar	28.3728	83.5668	1006
21	N21	NGRC00195	Dudhe murilo	Rukum, Pyaugha	28.5826	82.3829	823
22	N22	NGRC02464	Local gahun	Jumla, Bumrimadichaur	29.4082	82.13	2896
23	N23	NGRC02466	Geru gahun	Mugu, Pina	29.4768	82.1605	2035
24	N24	NGRC02471	Dabde gahun	Jumla , Patarasi	29.34	82.5	2713
25	N25	NGRC02544	Muraala gahun	Rolpa, Khumel	28.2725	82.6843	1500
26	N26	NGRC02548	Hanse gahun	Salyan, Tharmare	28.466	82.2707	1040
27	N27	NGRC02556	Local gahun	Mustang, Jharkot	28.8219	83.8435	3353
28	N28	NGRC02584	Sete gahun	Taplejung, Nankholyang	27.32	87.71	1402
29	N29	NGRC02618	Mudule gahun	Dolakha, Mirge	27.62	86.15	1899
30	N30	NGRC04415	Local gahun	Salyan, Dhanbang	28.2004	82.3561	1418
31	N31	NGRC04423	Local gahun	Surkhet, Gadi	28.66	81.62	1455
32	N32	NGRC04424	Local gahun	Ramechhap	27.5963	86.2648	1752
33	N33	NGRC04450	Local gahun	Surkhet, Kunathari	28.69	81.51	526
34	N34	NGRC04464	Thulo gahun	Dailekh, Badalamji	28.9065	81.609	1358
35	N35	NGRC04466	Local sano gahun	Dailekh, Dullu	28.8571	81.5917	1434
36	N36	NGRC05103	Local gahun	Jumla	29.225	82.2583	2514
37	N37	NGRC06600	Local gahun	Dadeldhura, Katal	29.1364	80.34554	830
38	N38	NGRC06601	Local gahun	Rupendehi, Bhairahawa	27.5309	83.4579	82
39	N39	NGRC06265	Local gahun	Dang, Namai	27.8667	82.5167	605
40	N40	NGRC06285	Local gahun	Rupendehi,Bhairahawa	27.5309	83.4579	82
41	N41	NGRC06314	Local gahun	Rupendehi, Bhairahawa	27.5309	83.4579	82
42	N42	NGRC06356	Local gahun	Rupendehi, Bhairahawa	27.5309	83.4579	82
43	N43	NGRC06386	Local gahun	Rupendehi, Bhairahawa	27.5309	83.4579	82
44	N44	NGRC06527	Local gahun	Kailali, Durgauli	28.5646	81.1472	170
45	N45	NGRC06522	Local gahun	Doti, Dipayal	29.2605	80.9318	1310
46	N46	NGRC06534	Local gahun	Rupendehi, Bhairahawa	27.5309	83.4579	82
47	N47	NGRC06560	Local gahun	Rupendehi, Bhairahawa	27.5309	83.4579	82
48	N48	NGRC06567	Local gahun	Rupendehi, Bhairahawa	27.5309	83.4579	82
49	N49	NGRC07590	Local gahun	Rupendehi, Bhairahawa	27.5309	83.4579	82
50	N50	NGRC07594	Local gahun	Rupendehi, Bhairahawa	27.5309	83.4579	82
51	N51	NGRC07610	Local gahun	Rupendehi, Bhairahawa	27.5309	83.4579	82
52	N52	NGRC07662	Local gahun	Rupendehi, Bhairahawa	27.5309	83.4579	82
53	N53	NGRC07999	Local gahun	Mustang	29.1347	83.815	3606
54	C1	Co13668	Local gahun	Rupandehi, Gaidahawa	27.4495	83.37299	132
55	C2	Co13789	Local gahun	Sankhuwasabha, Chyamtang	27.7656	87.43334	2200
56	C3	Co14157	Mudule gahun	Jajarkot	28.655	81.926	1155
57	C4	Co14747	Local gahun	Nuwakot, Pahare thok	27.7231	85.23568	1314
58	C5	Co14820	Local gahun	Dhading, Dharapani	27.9036	84.77161	1170
59	C6	Co14936	Mudula gahun	Dhading, Hagetar	27.799	84.87926	431
60	C7	Co15013	Local gahun	Rasuwa, Bhalayodanda	28.0119	85.19676	1286

Principal Component Analysis (PCA)

Principle component analysis (PCA) reduces many related variables into a smaller set of key variables called principal components (Mujaju & Chakauya, 2008). Several researchers (Kandel *et al.*, 2018; Karkee *et al.*, 2023; Siddquie & Hoque, 2023) Suggest using principal component analysis to study the differences and relationships among wheat genotypes, and these methods help to identify the best parent plants for breeding programs (Mustafa *et al.*, 2015). PCA (Table 4) shows that the first five principal components have an eigenvalue of more than one, accounting for 70.95% of the total variation. Suggesting these principal component scores might be used to summarize the original 16 variables in further data analysis. The first principal component (PC1) explains 26.36% of the variance, with positive loading from traits such as plant height (0.315) and spike exertion (0.35), while negative loading from flag leaf width (-0.352) and 1000-grain weight (-0.381). PC2 contributed about 17.01% of the total variation, and traits such as flag leaf length (0.48), Days of Heading (0.43) and spike length (0.32) have significant positive loadings. In contrast, traits such as seed width (-0.25) and 1000 grain weight (-0.24) have substantial negative loadings. The third principal component (PC3) accounted for 11.25% of the total variation, with traits like spike density per square meter (0.315) and number of seeds per spike (0.321) showing notable positive loadings. In contrast, plant height (-0.414) and spike exertion (-0.35) have significant negative loadings. PC4 represented 9.12% of the overall variance, with significant positive loadings for the number of seeds per spikelet (0.62), Number of seeds per spike (0.42) and days to harvest (0.339). In contrast, traits like days of heading (-0.33) have significant negative loadings. The fifth principal component (PC5) explained 7.198% of the total variation, with attributes like yield (-0.66) and Spike density per sq.m (-0.47) showing significant negative loadings. These findings highlight the key traits contributing to the genetic diversity among the wheat landraces, which are crucial for breeding programs and conservation efforts.

The Principal Component Analysis (PCA) biplot (Figures 2 and 3) of wheat genotypes helps visualize how agro-morphological traits vary among samples, explaining about 43.4% of the data's variation. The top right quadrant, populated by Accessions like NGRC07662 (N52) and Co14820 (C5) near vectors such as Average flag leaf width (AFLW) and Average Seed Length (ASL), indicates high values for these variables, showing strong positive correlations with both principal components. Conversely, the top left quadrant features observations such as NGRC02556 (N27) near the Days to Heading (DOHe) vector, suggesting unique traits with negative scores on the PC1 but positive on the PC2. The bottom left quadrant, with points like NGRC00004 (N1), NGRC00105 (N5), Co14157 (C3), and NGRC02548 (N26), exhibit traits that are inversely related to those in the top right, indicating lower variable values. The accessions like NGRC06386 (N43) and NGRC06285 (N40), at the bottom right quadrants, show positive and negative influences, illustrating the complex interactions of traits within the dataset. The key traits contributing to the variance in the PCA include days of heading, flag leaf length and width, spike density, plant height, and yield per hectare (Table 4). These traits are critical for understanding genetic diversity and the potential for improvement in wheat landraces (Karkee *et al.*, 2023). Yield per hectare is a direct measure of productivity, making it a crucial trait for breeding programs. This comprehensive analysis underscores the genetic diversity in the wheat landraces, essential for developing resilient and high-yielding wheat varieties. By focusing on the key traits identified through PCA and the coefficient of variation, breeding programs can effectively enhance wheat performance and adaptability (Kandel *et al.*, 2018, Verma *et al.*, 2024).

Table 4. Principal Component Analysis (PCA) table of 16 quantitative traits of wheat landraces.

Dimensions	Dim-1	Dim-2	Dim-3	Dim-4	Dim-5
Eigenvalue	4.21	2.72	1.8	1.46	1.15
Variance Percent	26.36	17.01	11.25	9.12	7.198
Cumulative variance per cent	26.36	43.37	54.63	63.75	70.95
Traits	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Days of Heading	0.087255	0.43867	0.189455	-0.33164	0.010892
Flag leaf length (cm)	0.08455	0.480056	-0.22547	0.059185	-0.19688
Flag leaf width (cm)	-0.35294	0.271009	0.078285	-0.01308	-0.19105
Spike density per sq.m	0.206955	-0.16522	0.315144	-0.11632	-0.47903
Plant height (cm)	0.315931	0.08418	-0.41452	0.169535	-0.2924
Spike exertion (cm)	0.350542	0.022078	-0.35045	0.263625	-0.0906
Spike length (cm)	-0.22331	0.327602	-0.18829	0.064904	0.040089
Number of seeds per spikelet	-0.11437	0.100106	0.259015	0.629771	-0.11635
Number of spikelets per spike	-0.02023	0.179396	0.299948	-0.10219	0.221389
Number of seeds per spike	-0.09105	0.32354	0.321696	0.42114	0.001239
1000 grains weight (gm)	-0.38197	-0.24068	-0.22377	0.086275	-0.02657
Seed length (mm)	-0.37178	0.047566	-0.2242	0.058682	-0.18207
Seed width (mm)	-0.3747	-0.25143	-0.11844	0.123263	0.114546
Days to 80% maturity	-0.11341	0.194859	-0.20711	-0.20355	-0.15088
Days to harvest	0.269785	-0.12701	0.11173	0.339521	0.159198
Yield (kg per hectare)	-0.11549	-0.19258	0.21781	-0.0562	-0.66499

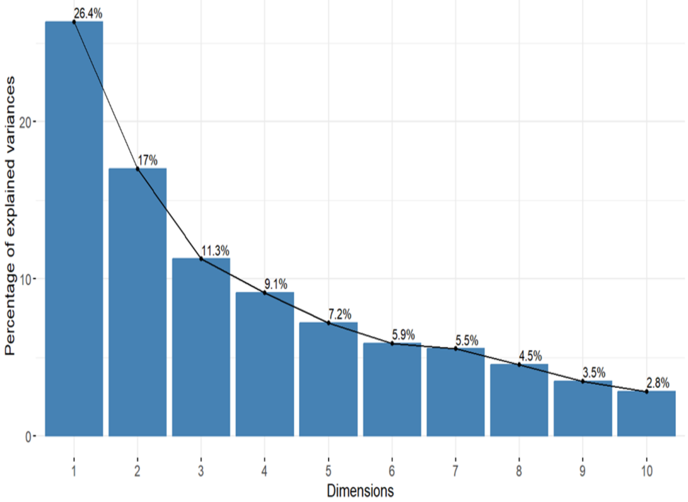

Figure 2. *Scree-plot between dimension and percentage of variance.*

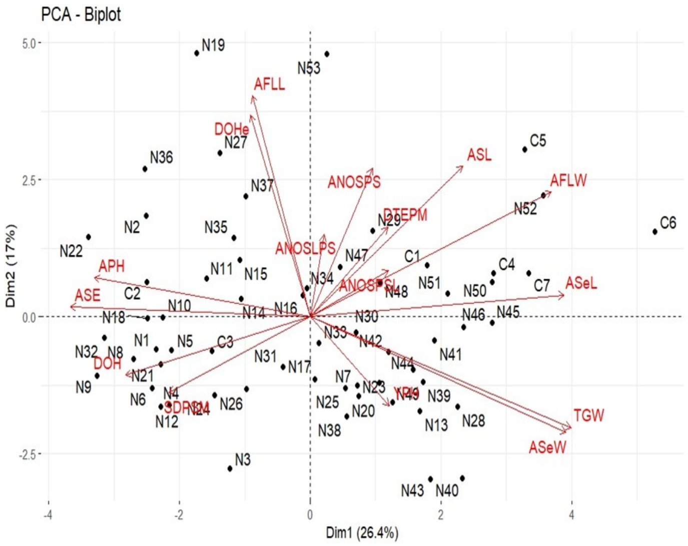

Figure 3. *PCA-Biplot showing quantitative traits of wheat landraces.*

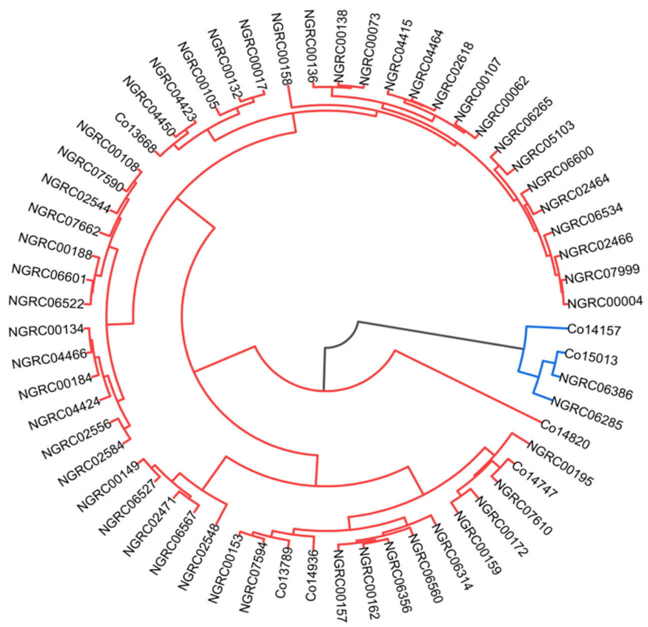

Figure 4. *Circular cluster dendrogram of different accession of wheat.*

Cluster analysis

A dendrogram (Figure 4) was created for 60 wheat landraces using a UPGMA clustering method, which employs an average linkage and Euclidean distance to evaluate 16 quantitative traits. The clustering divided the landraces into two clear groups: Cluster I and Cluster II. Cluster I contains 56 accessions (93.33%), while Cluster II comprises four accessions (6.67%), with a notable separation of 2374.99, indicating a significant phenotypic distinction between the clusters. The descriptive statistics for each distinct cluster are presented in Table 5. Regarding leaf characteristics, Cluster I has longer (16.32 cm) but slightly narrower flag leaves (1.41 cm) than Cluster II, which may influence photosynthesis, biomass production, and overall yield. Cluster II shows a higher spike density at 321.25 per square meter, compared to 279.446 in Cluster I, suggesting a potential for greater seed production. Plants in Cluster I are taller (108.836 cm) and exhibit more spike exertion (18.93 cm), factors that could complicate the harvesting process and heighten the risk of lodging. The seeds in Cluster II are larger, with a greater seed length (7.12 mm), width, and 1000-grain weight (50.22 g), which may improve yield and market appeal. The time taken to reach 80% maturity and the days until harvest is less in Cluster II, indicating a faster development cycle that could be favorable in certain agricultural conditions. Lastly, the yield in Cluster II is significantly greater at 5262.5 kg per hectare compared to 2887.91 kg per hectare in Cluster I, reflecting a considerable disparity in productivity between the two clusters. The earlier maturity of Cluster II could be advantageous for areas with shorter growing seasons. Similar studies, such as those conducted by (Karkee *et al.*, 2023; Khadka *et al.*, 2020; Poudel *et al.*, 2017; Thapa *et al.*, 2024) have also categorized germplasm using multivariate analysis techniques.

Correlation analysis

The Pearson correlation method was implemented to estimate the correlation among the 16 quantitative traits (Figure 5). Strong and significant correlations were found between the APH and ASE and days to heading (0.82^{***}), TGW and ASeW (0.82^{***}), ASel and TGW (0.74^{***}), ASeL and AFLW (0.56^{**}), which is considered as a preferable trait. In contrast, a significant negative correlation was observed among ASew with DOHe, TGW DOHe, AFLW, and ASE. Days to heading were also negatively correlated with 1000-grain weight and grain weight, which suggests that early maturing entries could be selected for higher grain yield; similar results were also reported. The study of variability among accession in different quantitative and qualitative traits could be used to select advantageous adaptive traits for crosses in the breeding program, which farmers can directly use. It can be used as an important tool for the management of crop germplasm collection.

Table 5. Mean value of quantitative traits in each cluster.

Traits	Cluster-1	Cluster- 2
Number of Landraces	56 (NGRC00004, NGRC00017 ,NGRC00062 ,NGRC00073 ,NGRC00105, NGRC00107, NGRC00108, NGRC00132, NGRC00134, NGRC00136, NGRC00138, NGRC00149, NGRC00153, NGRC00157, NGRC00158, NGRC00159, NGRC00162, NGRC00172, NGRC00184, NGRC00188, NGRC00195, NGRC02464, NGRC02466, NGRC02471, NGRC02544, NGRC02548, NGRC02556, NGRC02584, NGRC02618, NGRC04415, NGRC04423, NGRC04424, NGRC04450, NGRC04464, NGRC04466, NGRC05103, NGRC06600, NGRC06601, NGRC06265, NGRC06314, NGRC06356, NGRC06527, NGRC06522, NGRC06534, NGRC06560, NGRC06567, NGRC07590, NGRC07594, NGRC07610, NGRC07662, NGRC07999, Co13668 ,Co13789, Co14747, Co14820 ,Co14936)	4 (CO14157, CO15013, NGRC06386, NGRC06285)
Days of Heading	117.71	111.5
Flag leaf length (cm)	16.32	14.86
Flag leaf width (cm)	1.41	1.5
Spike density per sq.m	279.446	321.25
Plant height (cm)	108.836	105.95
Spike exertion (cm)	18.93	15.23
Spike length (cm)	9.53	9.92
Number of seeds per spikelet	3.08	3.25
Number of spikelets per spike	20.42	17.5
Number of seeds per spike	50.17	44.25
1000 grain weight (gm)	44.18	50.22
Seed length (mm)	6.74	7.12
Seed width (mm)	3.54	3.72
Days to 80% maturity	158.25	157.25
Days to harvest	175.23	173
Yield (kg per hectare)	2887.91	5262.5

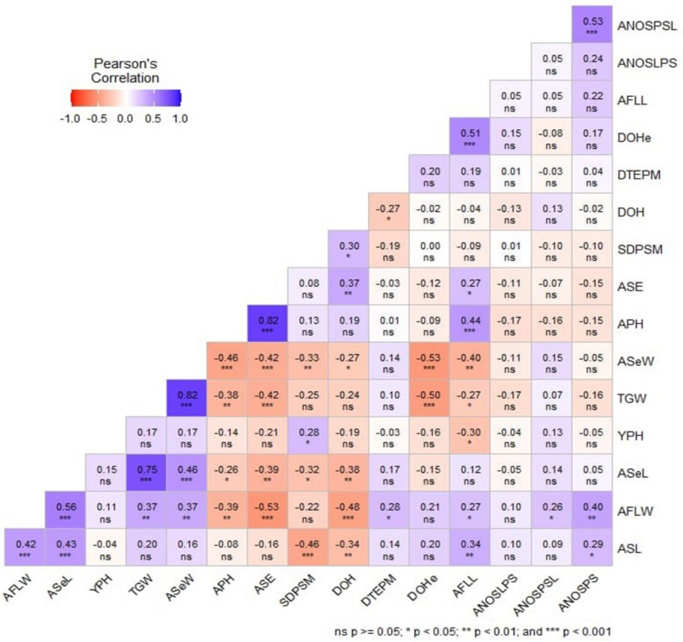

Figure 5. *Correlation among 16 quantitative traits observed over 60 wheat accessions. DOHe=Days to heading, AFLL= Average flag leaf length, DTEPM= Days to 80% Maturity, SDPSM= Spike density per square meter, ASE= Average spike exertion, APH= Average Plant height, ASeW= Average seed width, ANOSLPS= Average number of spikelet per spike, ANOSPSL= Average number of seed per spikelet, ANOSPS= Average number of seed per spike, TGW= Thousand grain weight, YPH = Yield per hectare, ASeL=Average spike length, AFLW= Average flag leaf width, ASL = Average Seed Length.*

Conclusion

In this research, we investigated the genetic variability of 60 wheat landraces from Nepal, using various statistical methods to reveal the differences and connections among qualitative and quantitative traits. The Shannon-Weaver diversity index indicated that traits such as seed color and glume color exhibit high levels of diversity, signifying a wealth of genetic variation within these landraces. This genetic diversity is crucial for breeding initiatives as it increases the likelihood of developing resilient and adaptable wheat varieties. Utilizing principal component analysis (PCA), we found that the top five principal components account for around 70.95% of the overall variation, having an eigenvalue of more than one, with significant input from traits like plant height and spike exertion. These traits are essential as they affect the adaptability and productivity of wheat, making them vital targets for genetic enhancement. Additionally, cluster analysis displayed two clusters among the landraces, each defined by traits that could be leveraged in breeding efforts. This segregation implies that specific landraces within each cluster have advantageous agronomic traits, positioning them as ideal candidates for breeding programs to improve yield and adaptability. The correlation analysis offered further understanding, revealing strong positive relationships among key traits such as days to heading and grain weight, which reflect the landraces' potential for early maturity and high yield.

DECLARATIONS

Author contribution statement

Conceptualization: M.B. and J.C.; Methodology: M.B.; Software and validation: J.C., P.S. and J.J.LC.; Formal analysis and investigation: J.C.; Resources: M.B.; Data curation: P.S.; Writing—original draft preparation: P.S.; Writing—review and editing: J.C.; Visualisation: J.J.LC.; Supervision: M.B.; Project administration: M.B.; Funding acquisition: M.B. All authors have read and agreed to the published version of the manuscript.

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