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Research Article

Characterization of Nepalese Bread Wheat Landraces Based on Morpho-Phenological and Agronomic Traits

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Abstract: Due to the presence of valuable genes that contribute to a variety of functional traits, landraces kept in Genebank can be extremely important in wheat breeding. A study was conducted based on agro-morphological traits of Nepalese bread wheat landraces to explore genetic diversity among them. Using a replicated rod row design, 200 landraces were evaluated during the winter season of 2018 and 2019 at Khumaltar, Lalitpur, Nepal. The degree of variations among landraces was determined using univariate and multivariate statistical tools. The Shannon-Weaver diversity index (H') showed a wide range of variations among the studied landraces, ranging from 0.55 to 0.91 in quantitative traits and 0.63 to 0.85 in qualitative traits.Principal component (PC) analysis with an eigenvalue greater than 1 reveals that 68% of the variability for quantitative traits is contributed by the first five principal components whereas 67% of the variability of qualitative traits is governed by the first four principal components. UPGMA (Unweighted pair-groups methods through arithmetic average) clustered 202 landraces into five groups according to quantitative charterers.Identified advantageous adaptive traits through the analysis of variability within the accessions, will be used by breeders for crosses in the breeding or used directly by farmers.

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1. Introduction

The annual plant *Triticum aestivum* L., commonly known as bread wheat, has 21 chromosome pairs arranged into three sub-genomes, A, B, and D. It is a hexaploidy species (2n=6x=42) (Genome BBAADD). It belongs to the grass family Poaceae's Triticeae subtribe (Sears, 1952). Around 8 500 years ago, it was created by Aegilopstauschii, the domesticated tetraploid progenitor (genome BBAA) and the diploid donor of the D sub-genome (Levy and Feldman, 2022). The most significant cereal crop in the world is wheat, with production and productivity estimates of 760.93 million tons and 3.47 mt ha^{-1}, respectively. It is cultivated on 219 million ha of surface area (FAOSTAT, 2022). In terms of production and area, wheat is Nepal's third-largest cereal crop after rice and maize (Karkee et al., 2019). It is grown on 711 000 ha, with a productivity of 2.99 mt ha^{-1} and a total production of 2.127 million tons (MoALD, 2021).

Primitive cultivars, landraces, and wild relatives of crop plants make up a source of valuable genetic diversity needed for successful breeding programs (Routray et al., 2007). Landraces are essential for overcoming the effects of climate change on agriculture because they are locally adapted to the environmental conditions and provide valuable genetic resources for breeding programs (Mainali et al., 2020). Due to their genetic diversity, unique adaptation to local environmental conditions, and presence of genes that confer resistance to biotic and abiotic stresses, wheat landraces are important geneticresources (Lopes et al., 2015; Robbana et al., 2019). These are a mixture of different genotypes that were selected and evolved over time using both natural and artificial selection techniques (Gharib et al., 2020).

Despite the fact that there are many genetic resources available on a national and international level, breeders tend to focus only on adapted and improved materials while avoiding landraces and wild and weedy relatives in their breeding program (Upadhyaya et al., 2014). Traditional varieties, also known as landraces, have highyield stabilityunder low-input agricultural systems and have the capacity to tolerate biotic and abiotic stresses despite having a lower yield than improved and hybrid varieties (Manohara et al., 2018). One of the primary causes of the low utilization of landraces is a lack of knowledge about particular characteristics of them (Thapa et al., 2021). In order to improve the use of specific traits in breeding programs, it is crucial to evaluate the diversity of wheat that is currently available. Economically significant traits require evaluation, characterization, and tagging (Joshi et al., 2020). Agro-morphological characterization (Ghimire and Magar, 2017) and genotyping-by-sequencing (GBS) results in both showed that there is a high genetic diversity among Nepalese wheat landraces (Khadka et al., 2020). A crucial step in managing and utilizing the genetic diversity of any crop and for the development of better varieties is agro-morphological characterization (Manzano et al., 2001; Ali et al., 2022; Yüce et al., 2022). Smith and colleagues (1991) stated that significant traits of local landraces need to be assessed, characterized, and labeled. In the current study, agro-morphological traits were used to describe the diversity among landraces and explored the wheat intra-varietal diversity collected from different parts of Nepal.

2. Materials and Methods

2.1. Plant materials and site description

At the National Agriculture Genetic Resources Centre (NAGRC), Khumaltar, Lalitpur, Nepal. The genetic material consists of 200 landraces of bread wheat that have been preserved. As checks, two varieties, WK1204 and Morocco, were used. The landraces came from 42 different Nepalese districts, ranging in altitude from 148 to 3 353 meters above sea level (masl) (Figure 1). The study area is situated in the sub-tropical mid-hill region at latitude 27°40'N, longitude 085°20'E, and elevation 1 368 m (Genebank, 2018; Karkee et al., 2021). The research site's soil is of the black loamy variety (Ghimire and Magar, 2017). Figures 2 and 3 display the meteorological data for the wheat growing seasons of 2019 and 2020.

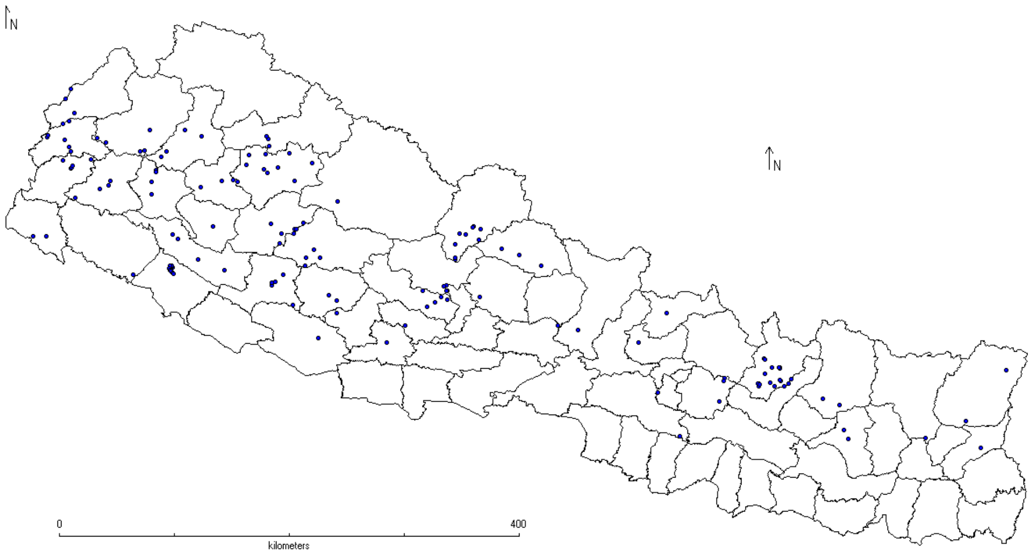

Figure 1. Collection sites of wheat landraces.

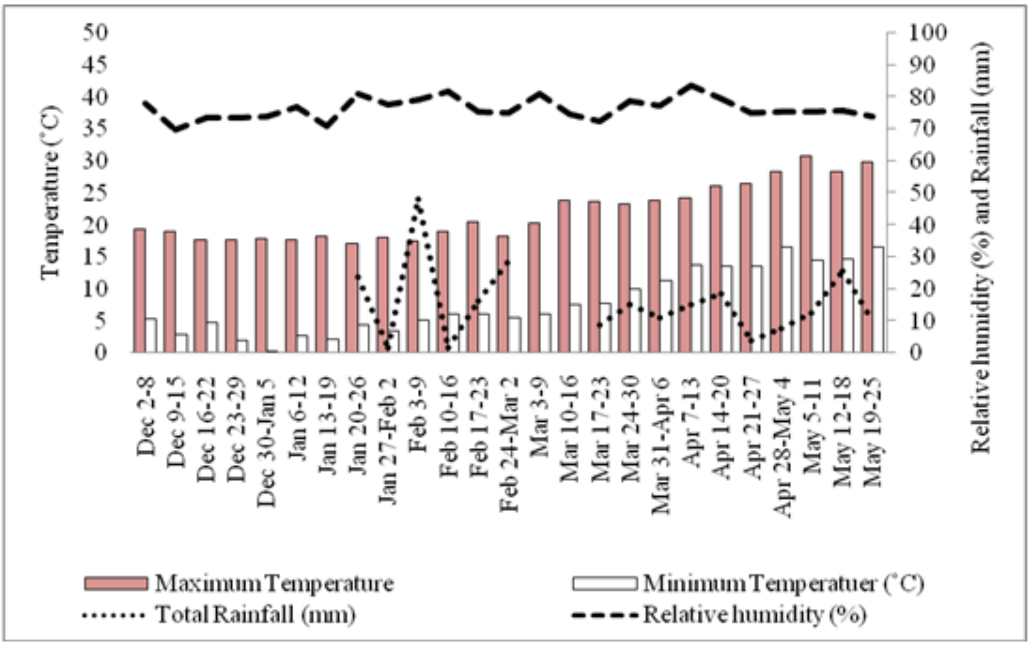

Figure 2.Average maximum and minimum temperatures (°C), total precipitation (mm), and average relative humidity (%) measured in Khumaltar, Lalitpur, Nepal, between December 2018 and May 2019 (Source: National Agronomy Research Centre, Khumaltar, 2019).

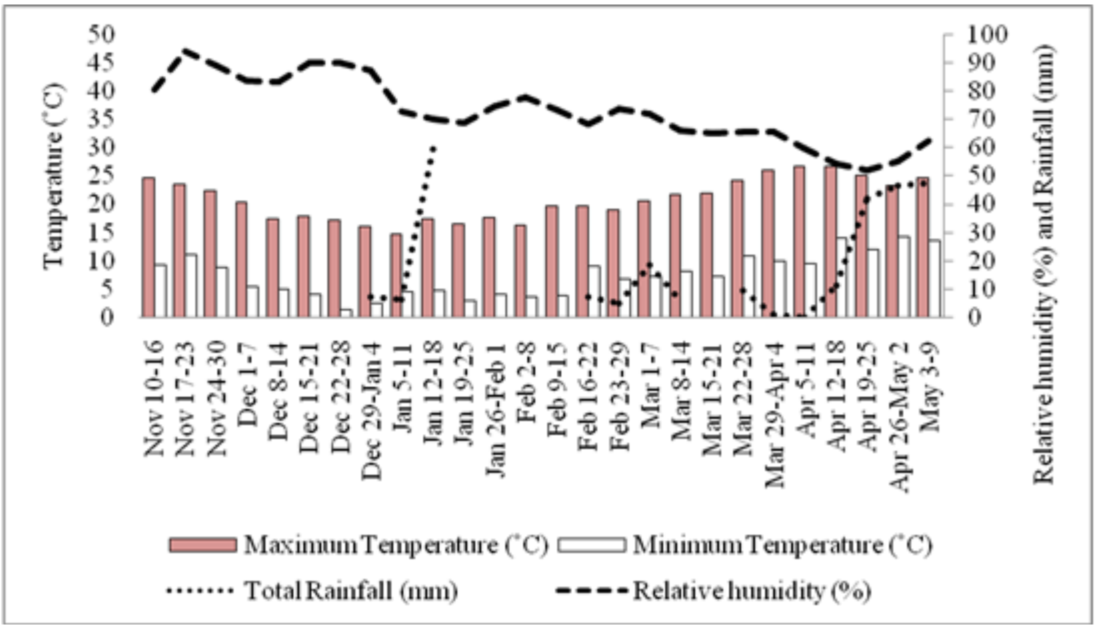

Figure 3. Average maximum and minimum temperatures (°C), total precipitation (mm) and average relative humidity (%) measured at Khumaltar in Lalitpur, Nepal, between November 2019 and May 2020 (Source: National Agronomy Research Centre, Khumaltar, 2020).

2.2. Field experiment and cultural practices

The tests were carried out at NAGRC Khumaltar in Lalitpur, Nepal, during the 2018–19 and 2019–20 crop seasons. They were arranged in a rod row design with two replications. The sowing was finished on 6th December 2018 and 15th November 2019. Two rows of each landrace, each measuring two meters in length, were continuously sown with 25 cm row spacing. The fertilizer was applied during the land preparation with 6 mt ha^{-1} FYM and with N:P_2O_5:K_2O (100:50:0 kg ha^{-1}) provided by di-ammonium phosphate (DAP), urea, and murate of potash. A full dose of P_2O_5 and K_2O and half a dose of N were applied during the land preparation process. The remaining N was distributed in two split doses during the tillering and flowering phases. Intercultural operations, such as irrigation, weeding, and other agronomic procedures, were carried out in accordance with the National Wheat Research Program, Bhairahawa, and the National Plant Breeding and Genetics Research Centre, Khumaltar, Lalitpur, Nepal (NWRP, 2017; Yadav et al., 2020).

2.3. Data collection and data analysis

Eight qualitative and sixteen quantitative morphological data were recorded according to IPGRI (1985) wheat descriptor. The diversity of the landraces was calculated and examined using the Shannon-Weaver diversity index (H'), frequency distribution, and some descriptive statistics.

Multivariate principal component analysis (PCA) was carried out in MINITAB version 17 for landrace classifications. 2-D plots were created based on the first and second principal components (PCs). Hierarchical clustering was carried out using the Average Euclidean distance method. The dendrogram was produced utilizing the unweighted pair group average method (UPGMA). R version 4.2.1 was applied to create the dendrogram.

3. Results and Discussion

3.1. Diversity based on quantitative characters

A higher coefficient of variations with more than 20% CV for four parameters among 16 quantitative characters was found, which suggests that the variability exists in a wider range in the studied landraces. The parameters with lower CV (20%) were found on days to emergence, heading, flowering, and maturity; plant height; flag leaf length and width; spike length; the number of seeds per spikelet and spike, and seed length and seed width (Table 1). This indicates that the estimate of this identified parameter disperses minimally and estimates accurately. Together, genetic and environmental factors may have an impact on the variability of a crop's quantitative traits (Karkee et al., 2020). The diversity of quantitative characters in wheat is an important factor in practical wheat breeding, as it provides breeders with a wide range of traits to select from and can help to improve the overall performance and adaptability of wheat varieties. Typically, the yield of wheat grains is analyzed in terms of several yield components, including the number of spikes per square meter, the number of grains per spike, the number of grains per square meter, and the thousand kernel weight. These components are interrelated and compensate for each other to contribute to overall yield (Beral et al., 2020) and the diversity of these components will help to increase the choices available for breeders to select from.

Table 1. Descriptive statistics and Shannon-Weaver diversity indices (H') for 16 quantitative traits

Traits	Minimum	Maximum	Mean	CV (%)	SD	H'
Days to emergence	4	13	12	6.9	0.79	0.77
Days to heading	104	123.00	112	3.7	4.17	0.90
Days to flowering	109	129	119	3.3	3.96	0.91
Days to maturity	139	169	154	4.5	6.93	0.86
Plant height (cm)	98	173	122	7.5	9.15	0.85
Flag leaf length (cm)	10.8	45.5	17.2	18.9	3.24	0.78
Flag leaf width (cm)	0.81	2.53	1.26	19.2	0.24	0.83
Spike length (cm)	6.9	14.8	9.9	11.1	2.16	0.89
Spike per square meter	223	450	367	21.9	40.88	0.87
Number of seed per spikelets	7	12	9	8.2	0.69	0.84
Number of seed per spike	25	49	33	13.7	4.51	0.91
Awn length (cm)	0.48	5.98	3.82	39.6	1.51	0.55
Thousand grain weight (g)	15	56	36	22.8	8.18	0.86
Seed length (mm)	4.41	7.23	5.90	8.0	0.47	0.86
Seed width (mm)	1.95	3.29	2.71	8.7	0.23	0.88
Yield (mt ha^{-1})	1.43	5.81	3.51	25.0	0.88	0.90

SD = Standard Deviation, CV = Coefficient of Variation, H' = Notation for Shannon–Weaver diversity index.

3.2. Diversity based on qualitative characters

Shannon-Weaver diversity indices, frequencies, and proportions are shown in Table 2 for each qualitative trait. The studied genotypes of wheat have higher diversity for the qualitative traits, as evidenced by the diversity index (H') from 0.63 to 0.85 with a 0.76 average value. In terms of qualitative characteristics, 75% of landrace plants had higher tillering capacity, 54% had red seeds, 59% had intermediate seeds, 48% had lax-type spike density, and 59% had awns. They also had white glumes, 67% had hairy glumes, 54% had white glumes, and 54% had red seeds. Qualitative characters are traits that are controlled by one or a few genes and exhibit clear-cut phenotypic differences. These traits are often important for determining important agronomic traits such as end-use quality and consumer preferences. Therefore, the diversity of qualitative traits in wheat can be useful in a practical wheat breeding program, as these traits can be manipulated through controlled crosses and selections.

The awning characteristic in wheat, which contributes to spike photosynthesis, is crucial for increasing yield potential as the amount of assimilate produced and primarily derived from the spike, flag leaf, and leaf sheath, also determines the grain yield of crops (Borner et al., 2005). According to Rebetzke et al. (2016) awns participate in photosynthesis when the canopy assimilation is limited due to water scarcity and Bruening (2019) suggests that awned-type genotypes offer protection benefits, recommending them to farmers who face wildlife problems such as deer damage when choosing a wheat variety. Some wheat characteristics can serve as field markers to identify desirable traits, making it more practical to select Glu-B3 alleles that contain elite lines by observing their ‘glume color’ in the breeding field instead of using DNA-MAS, which require laboratory procedures (Kiyoshi et al., 2011). The Glu-B3b and Glu-B3g alleles are becoming increasingly prevalent due to their association with bread-making quality (Si et al., 2013). Moreover, the breeding program can utilize the findings of the current study on the glume color of the relevant genotypes.

The function of glume hairiness in wheat is a topic of debate, but some researchers suggest that it plays a role in enhancing crop resilience and disease resistance. Warham (1988) suggests that hairy glumes may confer resistance to Karnal bunt disease. Studies by Reynolds et al. (1999), Skovmand et al. (2003), and Trethowan et al. (1998) have demonstrated that leaf pubescence can improve drought tolerance and cold tolerance in wheat.

Grain traits such as shape and size play a crucial role in determining wheat quality and yield, as they are linked to the crop's initial vigor (Kehel et al., 2020). Aparicio et al. (2002) reported that seed size has a direct impact on the growth of the first two leaves, which in turn affects how emergence and development of seedlings. The color of wheat seeds may also varies depending on consumer habits and preferences for the final product. Pre-harvest sprouting is less likely to occur in colored grains; this may be due to the pleiotropic effect within these traits or the genetic linkage between them (Groos et al., 2002). The majority of our landraces have colored grains due to the beneficial effects of color genes.

In our study, young plants with an upright growth habit were more prevalent (149 out of 202) than those with a prostrate habit. The same situation is described by Laino et al. (2015), who discovered that landraces with prostrate growth habits were less common than those with erect habits in their study. Overall, the diversity of qualitative characteristics in wheat is an important resource for wheat breeding, as it can help to improve the performance, quality, and adaptation of wheat varieties, making it a valuable tool for breeders to achieve their breeding objectives.

Table 2. S-W diversity index (H′), phenotypic class and their frequency and proportion for eight qualitative traits

Characters	H'	Observed phenotypic class	Frequency	Proportion %
Awnedness	0.85	Awnless	120	59
		Awnletted (short awns)	26	13
		Awned (Conspicuous awns)	56	28
Glumes colour	0.80	White	109	54
		Red to brown	80	40
		Purple to black	13	6
Glumes hairiness	0.78	Absent	136	67
		Low	31	15
		High	35	17
Tillering capacity	0.82	Low	52	26
		High	150	74
Seed colour	0.63	White	93	46
		Red	109	54
Seed size	0.76	Small	32	16
		Intermediate	119	59
		Large	44	22
		Very large	7	3
Spike Density	0.65	Very lax	12	6
		Lax	96	48
		Intermediate	83	41
		Dense	11	5
Growth habit of young plant	0.83	Upright	149	74
		Prostrate	53	26

3.3. Principal component analysis (PCA)

The genetic diversity of the studied genotypes was demonstrated by using PCA. The PCA can be used to quantify the independent influence of specific characteristics on overall variance, and each proper vector's coefficient illustrates the involvement of individual variables that every principal component is connected to each other (Nachimuthu et al., 2014). The better they are at separating the landraces, irrespective of sign, the higher the value. The distribution of landraces along the axes was quantified using quantitative characters' principal components 1 and 2, and the level of phenotypic variation in the collection was shown (Figure 4).

The total variance (68%) of the quantitative traits comes under the initial five Principal components with eigenvalues >1 (Table 3), demonstrating that the identified traits within the axes had a significant impact on the quantitative parameters of the genotype under study. Thousand-grain weight, seed length, spike length, yield, and seed width are the traits responsible for this variation in the first PC which covered 29% total variance. The second PC in which days to heading and flowering, the number of seed per spike, and flag leaf width were the primary determinants that cover 15 variances. Likewise, 9% total variance accounted for the third PC, which is affected by both the number of spikes/m^2 and the number of seed/spikes.

Among the qualitative traits, the first four PCs explained 67% of the total variance with less than 1 eigenvalue, indicating a significant impact of the identified traits on the genotypes under study (Table 4). The first principal component, which comprises 25% of the overall variation, was made up specifically of awareness and seed size. Important traits like spike density, glume color, and glume hairiness made up the second component, which had a 15% variance. The third variable, which covered 14% overall variation, was composed of seed color as well as tillering capacity. Growth habit, the last variable, accounted for 13% of the variance.

Table 3. Principal component analysis for sixteen quantitative characters of wheat landraces

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	4.60	2.43	1.42	1.37	1.17
Proportion	0.29	0.15	0.09	0.09	0.07
Cumulative variance (%)	0.29	0.44	0.53	0.61	0.68
Days to emergence	0.07	0.07	-0.29	-0.31	0.18
Days to heading	-0.13	0.51	-0.32	0.08	0.12
Days to flowering	-0.17	0.48	-0.36	0.12	0.12
Days to maturity	-0.16	0.02	-0.22	-0.34	-0.03
Plant height	-0.27	-0.12	-0.09	0.27	0.16
Flag leaf length	0.13	0.05	-0.04	0.09	0.60
Flag leaf width	0.13	0.25	0.03	0.20	0.14
Number of spike m^{-2}	0.01	-0.05	0.40	-0.20	0.66
Spike length	0.33	0.19	-0.09	-0.39	-0.02
Number of seed/ spikelets	-0.22	0.38	0.28	0.02	-0.13
Number of seed/ spikes	-0.08	0.41	0.50	-0.03	-0.23
Awn length	-0.30	0.14	0.00	-0.49	-0.11
Thousand-grain weight	0.41	0.12	0.10	0.18	0.03
Seed length	0.39	0.09	0.03	0.11	-0.02
Seed width	0.33	0.13	0.13	0.29	0.00
Yield	-0.37	0.11	0.31	-0.28	0.15

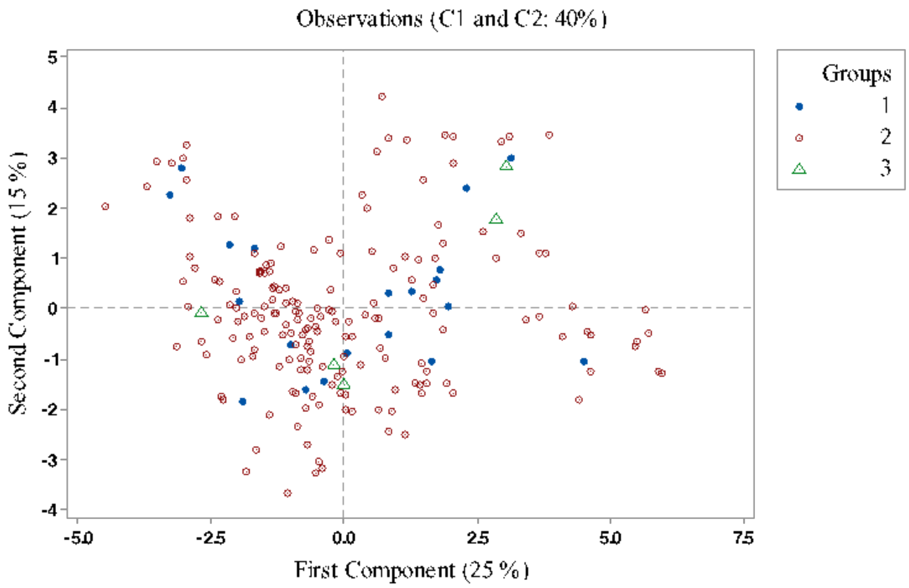

Figure 4. First two Principal Component Analysis (PCA) plots of the wheat landraces based on the 16 quantitative characters.

Table 4. Principal component for eight qualitative characters of wheat landraces

Qualitative traits	PC1	PC2	PC3	PC4
Eigenvalue	2.03	1.17	1.15	1.04
Proportion	0.25	0.15	0.14	0.13
Cumulative	0.25	0.40	0.54	0.67
Growth habit	0.03	0.22	0.23	0.88
Tillering capacity	0.25	0.14	0.61	0.08
Spike density	0.39	0.44	0.29	0.01
Awnedness	0.47	0.01	0.00	0.07
Glume colour	0.07	0.61	0.48	0.10
Glume hairiness	0.37	0.45	0.04	0.42
Seed colour	0.43	0.22	0.44	0.10
Seed size	0.49	0.35	0.27	0.17

3.4. Cluster analysis

Based on the estimated relatedness or kinship, a dendrogram was produced using UPGMA. The dendrogram is shown in Figure 5. The wheat landraces could be divided into five clusters. According to cluster analysis, there are differences in how different landraces are grouped based on agro-morphological traits. The reresulting dendrogram revealed five distinct groups: Groups I, IV, and V are comprised of 19 accessions (9%) in each, group II of 55 accessions (27%) and largest group III consists of 90 (45%) accessions. The descriptive statistics of the distinct cluster are presented in Figure 5. Based on theresults of the cluster analysis, cluster-I was determined to be superior in quantitative characteristics when compared to the other cluster. On the basis of cluster analysis, Cluster-I was found superior in terms of quantitative character as compared to the other clusters. Cluster-I includes landraces having higher mean values for the number of seeds per spikelets, number of seeds per spike, and yield but lower mean values for flag leaf length and width, number of spikes per square meter, spike length, and seed length. On the other hand, Cluster II landraces have higher mean values for flag leaf length and width, but lower mean values for the number of seeds pers pikelet and total yield but moderate mean values for other traits. Likewise, Cluster-III has a higher mean value for the number of spikes per square meter and a moderate mean value for all remaining traits. Cluster-IV has higher values for days to maturity and plant height, but lower values for the number of seeds per spike, Awn length, thousand-grain weight and seed width while Cluster-V includes landraces with higher values for spike length, awn length, thousand-grain weight and seed width, but lower values for days to maturity and plant height (Table 5). Using cluster analysis, Singh and Dwivedi (2002) also reported the genetic divergence among the tested wheat genotypes. These materials that have been identified can serve as candidates for selective breeding programs aimed at developing traits to interest in the future. It is likely that the crosses between members of clusters separated by inter-cluster distances would be advantageous for further improvement (Yatung et al., 2014).

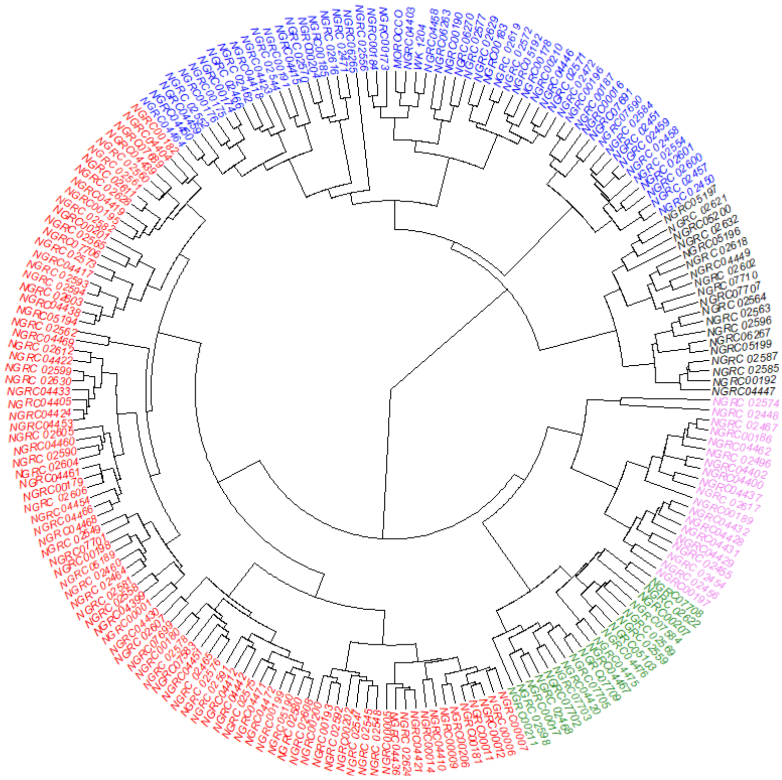

Figure 5. Dendrogram of 202 bread wheat landraces by UPGMA cluster analysis.

Table 5. Descriptive statistics of quantitative traits within clusters of 202 wheat genotypes

Parameter	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
No of landraces	19	55	90	19	19
Days to Emergence	11±1.86 (4-13)	12±0.5 (11-13)	11±0.5 (10-13)	12±0.4 (11-13)	12±0.5 (11-13)
Days to Heading	115±4.04 (110-121)	110±3.8 (104-119)	111±3.3 (104-118)	116±3.7 (111-123)	116±2.1 (112-121)
Days to Flowering	122±3.15 (117-128)	117±4.0 (110-124)	118±3.1 (109-125)	123±3.0 (120-129)	122±1.9 (117-125)
Days to Maturity	157±5.02 (148-166)	153±7.4 (139-169)	153±6.3 (139-169)	161±5.4 (151-168)	152±6.9 (141-165)
Plant height (cm)	126.0±8.1 (110.5-143.9)	119.6±7.4 (100.8-136.5)	124.4±8.1 (109.9-173.2)	127.3±6.3 (115.1-135.4)	107.7±5.3 (97.6-117.0)
Flag leaf length (cm)	16.1±2.01 (13.2-20.7)	18.4±4.5 (13.4-45.5)	16.4±2.4 (12.1-25.4)	17.8±2.5 (14.2-23.1)	17.3±3.1 (10.8-22.7)
Flaf leaf width (cm)	1.2±0.13 (1.0-1.4)	1.4±0.3 (0.8-2.5)	1.2±0.2 (0.8-1.6)	1.2±0.1 (1.0-1.4)	1.4±0.3 (1.0-1.9)
No of Spikesm^{-2}	349±33.65 (280-415)	367±41.5 (223-440)	374±38.0 (223-441)	360±40.8 (250-424)	369±53.9 (223-450)
Spike Length (cm)	8.4±1.27 (6.9-12.5)	11.2±2.0 (7.1-14.7)	8.5±0.8 (6.9-11.3)	11±1.9 (8-14)	13.3±0.8 (11.8-14.8)
Number of seed per spikelets	9.6±0.87 (8.5-12.3)	8±0.5 (7-9)	8.4±0.4 (6.7-9.3)	8±0.7 (7-10)	9±0.5 (8-10)
Number of seed per spike	40±4.21 (33-49)	31±2.5 (27-40)	32.4±2.8 (25.8-38.4)	28±2.9 (25-33)	38±4.7 (31-45)
Awn Length (cm)	3.1±0.98 (2.1-4.1)	4.7±0.8 (2.8-6.0)	1.9±1.3 (0.5-5.3)	2.4±1.2 (0.5-4.8)	4.7±0.7(3.0-5.6)
Thousand Grain Weight	30.2±3.80 (22.0-36.0)	43.6±7.7 (29.7-56.1)	32.8±3.5 (24.8-39.9)	25.7±5.4 (15.4-34.7)	44.3±4.5 (35.5-51.7)
Seed length (mm)	5.6±0.21 (5.2-6.0)	6.3±0.5 (5.6-7.2)	5.7±0.3 (5.0-6.5)	5.6±0.4 (4.4-6.3)	6.3±0.4 (5.5-7.2)
Seed width (mm)	2.6±0.14 (2.2-2.8)	2.9±0.2 (2.4-3.3)	2.7±0.2 (2.2-3.1)	2.4±0.2 (1.9-2.7)	2.9±0.2 (2.5-3.2)
Yield (mtha^{-1})	4.7±0.62 (3.7-5.8)	2.7±2.7 (1.4-3.8)	3.7±0.6 (2.3-5.4)	4.0±0.7 (2.6-5.3)	3.2±0.9 (1.9-4.8)

Conclusion

The cluster analysis as well as principal component analysis(PCA) showed the presence of genetic diversity in the evaluated landraces. Overall, this study offers a novel insight into the genetic diversity of wheat landraces in Nepal, which may support efforts to improve wheat breeding programs.

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Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' contribution statement

AjayaKarkee: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper. Ram Prasad Mainali, Krishna Hari Ghimire, Pradip Thapa, Bal Krishna Joshi, SudeepSubedi, and Jiban Shrestha: Contributed data analysis, Reviewed the initial draft of the manuscript.

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