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ORIGINAL RESEARCH ARTICLE



Evaluation of elite spring wheat genotypes for grain yield and other agronomic attributes in hills of Sudurpaschim Province, Nepal

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ABSTRACT

Selection and release of high yielding and disease resistant varieties is the cost effective and ecologically sound approach for increasing the production and productivity of agricultural crop in Nepal. Twenty-two advanced bread wheat (*Triticum aestivum* L.) genotypes including commercial check variety "Sorgadwari", newly released check variety "Khumal Shakti" and Local Check variety "Jhadde" were evaluated under irrigated conditions at Gokuleshwor Agriculture and Animal Science College (GAASC), Baitadi, Nepal during 2022/2023. This study was carried out for the identification of high yielding genotypes under irrigated condition in western hills. The experiment was conducted in Alpha lattice design with two replications. The highly significant difference ($p < 0.01$) among the genotypes was found for most of the traits viz., days to heading, days to anthesis, days to maturity, plant height, spikes per square meter, number of grains per spike, grain weight per spike, flag leaf area, thousand kernel weight, biomass yield and grain yield and non-significant difference for spike length. The mean grain yield ranged from 1908 to 4146 kg/ha with grand mean of 2766 kg/ha. The highest grain yield was produced by genotype NL 1474 (4146 kg/ha) which was followed by NL 1475 (3994 kg/ha), NL1597 (3536 kg/ha) and NL 1590 (3070 kg/ha). The check variety Sorgadwari and Khumal Shakti produced 3480 and 3070 kg/ha respectively while the local check variety Jhadde produced 2655 kg/ha. Similarly, highest TKW was produced by NL 1487 (68.5 g) followed by BL 5148 (67.2 g) and WK 3730 (66.3 g). The correlation analysis revealed that grain yield showed highly significant positive correlation with biomass yield (0.90**), and number of grains per spike (0.6**), spikes per square meter (0.7**), plant height (0.5**) and non-significant positive correlation with spike length (0.21) and grain weight per spike (0.1) and non-significant negative correlation with days to heading (-0.2) and days to maturity (-0.2). Cluster analysis revealed that Cluster III consists of 4 genotypes namely NL 1474, NL 1475, NL 1597 and Sorgadwari. This cluster represent with highest grain yield, number of spikes per meter square, number of grains per spike and grain weight per spike. Among the tested genotypes, NL 1474, NL 1475, NL 1597 and NL 1590 were found superior for grain yield and yield-related traits in comparison to three checks and could be recommended for hills of Sudurpaschim province after further testing in multi-environment and in farmer's field.

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is the third most important staple

cereal crop grown in Nepal after rice and maize (Farnworth *et al.*, 2019; Garapaty *et al.*, 2021). It occupies about 24%

(7,16,978 hectares) of the area under cereal cultivation and contributes 23.5% to the total cereal production in the country (MoALD, 2022). The mid and high hills, spanning the central to the far western regions, represent a sizable 43% (3,17,458 hectares) of the national wheat acreage. This region contributes about 34.57% (6,49,801 metric tons) of the total wheat output in Nepal (Pant et al., 2023). Wheat farming thus plays a vital socioeconomic role in the predominantly subsistence-based rural livelihoods in these hilly areas by providing food security, income and employment for a majority of households (Aryal et al., 2021). While genetic improvements have enhanced productivity from 1 metric ton/hectare in 1960s to about 3 metric tons/hectare presently, yields remain below yield potential across all wheat growing regions (Devkota & Upadhyay, 2013; Subedi et al., 2020). Rainfed wheat cultivation predominates in the mid-hills, with limited irrigation infrastructure (Subedi et al., 2020). Nonetheless, rich landrace diversity exists, making it crucial to harness genetic resources for developing resilient cultivars (Castagnetti et al., 2021). The far western mid-hills offer favorable agroclimatic conditions for wheat farming. However, persistent challenges related to low external input use, lack of assured irrigation, soil nutrient mining and emerging climate risks hamper productivity in this region (Adhikari et al., 1999; CIAT et al., 2017). There have been limited research efforts comprehensively evaluating the genetic potential and adaptability of diverse wheat genotypes under the unique far western mid-hill environments. This knowledge gap restricts strategic breeding endeavors to develop improved cultivars tailored to the specific climate risks, soil limitations, and socioeconomic contexts of the

region (Khan et al., 2022; Pour-Aboughadareh et al., 2021). This research therefore aimed to: evaluate advanced breeding lines under far western mid-hill conditions, determine grain yield relationships with other traits, and identify high yielding genotypes adapted to regional climate risks. The findings can equip local breeding programs to develop resilient and high-yielding wheat varieties that enhance livelihood security for smallholder farmers across the far western mid-hills of Nepal.

MATERIALS AND METHODS

Experimental site

The field experiment was conducted at the research farm of Gokuleshwor Agriculture and Animal Science College, Nepal (27°45'N, 80°50'E; 700 masl), during the winter season of 2022-2023. The site has a sandy loam soil texture under a rice-wheat-maize rotation.

Plant material and experimental design

The study evaluated 25 bread wheat genotypes as shown in Table 1, including 22 advanced lines from the National Wheat Research Program and 3 released varieties i.e., Khumal Shakti, Sorgadwari & Jhadde (*local*) as checks. Field experiment was designed following the Alpha Lattice design with two replications. In each replication, there were 5 blocks consisting of 5 plots. Each experimental unit (plot) was 2 × 2 m² in size. In each plot, there were 8 rows with row-to-row distance of 25 cm and continuous seeding was done. There was a gap of 0.5 m between the blocks and plots were continuous within the block.

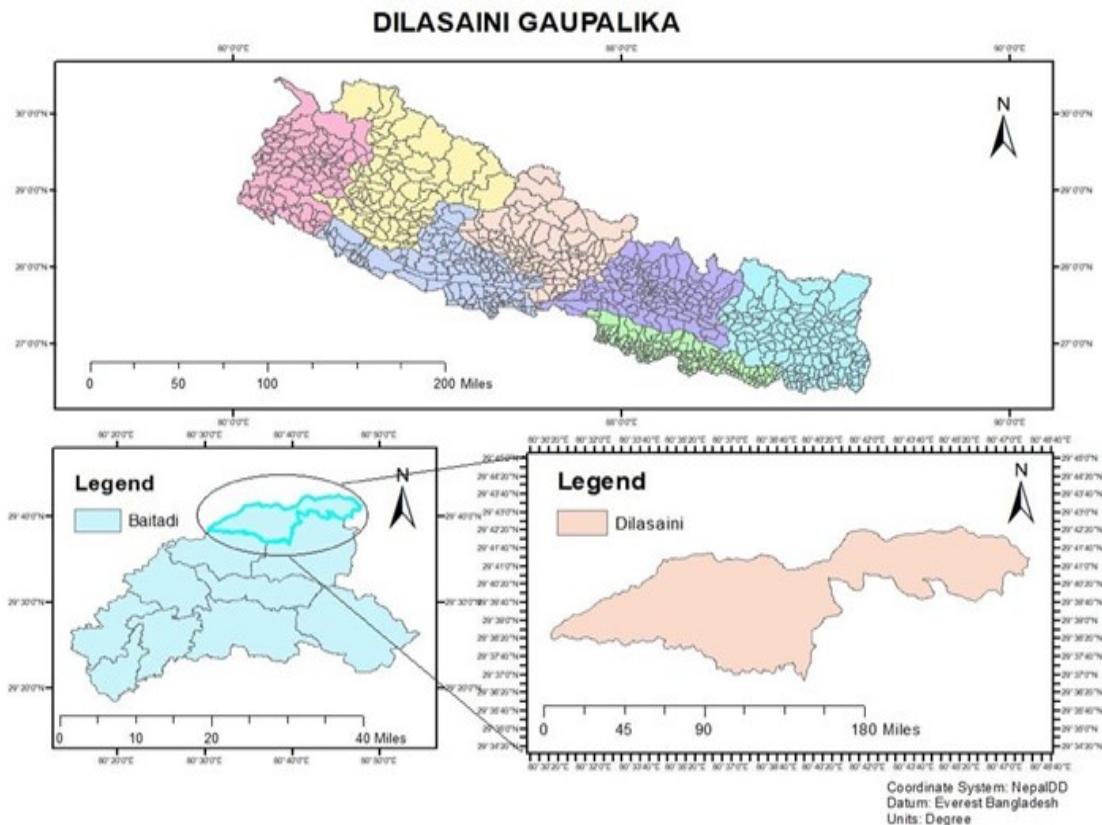


Figure 1. Map of Nepal Showing experimental site.

Seed rate, fertilizer and sowing

Seeding was done on November, 2022. Seed rate was applied at the rate of 120 kg per hectare. The standard agronomic cultivation practice recommended for the area was followed to raise the crop. Fertilizers were applied at the rate of 120:60:60 NPK Kg/ ha with two split applications. Half dose of nitrogen and full dose of phosphorus and potassium (60:60:60 NPK Kg/ha) was applied at a basal dose and remaining half dose of nitrogenous fertilizer was top dressed. First top dress was applied after 25 days of sowing at crown root initiation (CRI) stage after first irrigation and second top dress was applied 60 days after sowing at booting stage. Irrigation was given at two important stages: - crown root initiation (CRI) stage and flowering stage.

Sampling and observation

Observation was taken for following parameters:

Days to heading: Days to heading refers to the emergence of ear from the flag leaf sheath. The number of days from planting to the days when 50% of the plants in the plot had emerged head was recorded as heading days.

Days to flowering/anthesis: The number of days from seeding to the days when 50% of the plants show flowering was recorded as days to anthesis.

Days to maturity: This corresponds to the number of days from seeding to the date when 90% of the spikes in the plot turn yellow (glumes lose chlorophyll and turn yellow).

Flag leaf area: Flag leaves were collected from five randomly selected plants to determine flag leaf area using formula: Flag leaf Area = Length × Breadth × 0.7 attributed to Agarawal & Sinha, 1987 (Liu et al., 2018). Flag leaf length was measured from the base to the tip of the flag leaf with the help of ruler in cm. The average of width taken from base, middle and tip of the flag leaf was taken as flag leaf width.

Spike length: Length from the basal spikelet to the apical spikelet, excluding awns was measured as spike length and was taken from five sampled plants.

Plant height: The plant height was measured from soil surface in cm up to tip of apical spikelet excluding awn of the main tiller using a meter scale at the time of harvesting.

Thousand kernel weight (TKW): After harvesting and drying, 500 grains from each plot were counted and weighed (in gm) using electronic balance and multiplied by 2 to calculate the 1000 grain weight.

Number of grain per spike: Five spikes from randomly selected plants were hand threshed and number of grains was counted and averaged to obtain number of grains per spike.

Grain yield per spike: The average of weight of grains from five sampled plants was taken as grain weight per spike.

Grain yield (kg/ha): Harvested crop from net plot was threshed using a plot thresher. The grain yield was recorded in kg/ha with adjustment for 12% grain moisture content.

Straw yield (t ha⁻¹): The biomass harvested from the whole plot 'Bundle' was weighted (kg) before threshing and straw weight was calculated after deducting grain weight (kg) from the bundle weight.

Statistical analysis

Data entry and processing was carried out using Microsoft Office Excel 2007. Analysis of variance (ANOVA) and mean estimation were done with the software- R Studio version 4.3.1. Correlation analysis, UPGMA clustering and PCA was done using R studio. The statistical significance (alpha) was declared at 5% level of probability.

RESULTS AND DISCUSSION

The present study was carried out for evaluating the 25 wheat genotypes for yield potential (Table 1), character association, cluster analysis and principal component analysis and the results obtained were presented as below:

Table 1. Genotypes used in the experiment at Gokuleshwor (2022-23).

S.N.	Genotype	S.N.	Genotype
1.	BL 5082	14.	NL 1596
2.	NL 1472	15.	NL 1597
3.	NL 1474	16.	NL 1598
4.	NL 1475	17.	NL 1605
5.	NL 1487	18.	NL 1607
6.	WK 3385	19.	WK 3669
7.	WK 3387	20.	WK 3715
8.	WK 3391	21.	WK 3722
9.	BL 5148	22.	WK 3730
10.	BL 5157	23.	Khupal Shakti (Newly released check)
11.	BL 5168	24.	Sorgadwari (Commercial check)
12.	NL 1590	25.	Jhadde (Local Check)
13.	NL 1593		

Table 2. Performance of genotypes for different observed traits in an experiment at Gokuleshwar (2022-2023).

S. No.	Genotypes	DTH (days)	DTA (days)	DTM (days)	PH (cm)	NSPM	SL (cm)	NGPS	GWPS	FLA	TGW (g)	GY (kg/ha)	BY (kg/ha)
1	BL 5082	107	117	146	85	239	11.2	31	1.9	15.2	59.4	2351	4871
2	BL 5148	103	115	144	107	254	12	41	2.7	21.9	67.2	2840	6434
3	BL 5157	110	118	148	89	209	10.3	48	2.3	19.4	60.9	2960	7177
4	BL 5168	106	116	144	93	209	10	50	3.2	14.9	63.9	2778	6235
5	NL 1472	107	115	142	84	228	10.5	40	2.5	15.6	57.4	2408	6030
6	NL 1474	106	112	143	107	314	11.3	57	2.6	17.6	45	4146	8134
7	NL 1475	110	120	151	98	275	9.3	52	2.3	14.5	43.3	3994	8074
8	NL 1487	105	111	142	93	189	8.9	40	2.7	17.4	68	2810	6439
9	NL 1590	104	111	141	89	271	9.5	46	3.2	14.7	63.2	3193	6296
10	NL 1593	105	113	142	91	214	10.5	47	2.6	19.5	55.1	2642	5855
11	NL 1596	109	118	148	88	168	8.7	42	3	19.4	62.4	1962	4176
12	NL 1597	107	116	142	90	213	9.5	51	3.4	16	57.9	3536	7793
13	NL 1598	106	112	143	88	166	10.1	40	3.2	18.7	63.8	2506	5284
14	NL 1605	113	121	152	83	201	10.2	44	2.4	16.4	56.4	2240	6035
15	NL 1607	108	112	146	85	166	9.6	37	2.8	20.9	64.1	1908	4959
16	WK 3385	108	116	148	90	246	9.7	43	2.9	18.2	58.5	2808	5756
17	WK 3387	111	119	148	92	177	9.8	47	2.6	16.2	63.2	2044	4552
18	WK 3391	112	124	154	92	235	9.4	45	2.3	21.7	42.8	2555	5610
19	WK 3669	104	111	141	101	279	9.7	48	2.8	16.1	58.9	2931	5964
20	WK 3715	98	110	144	91	176	9.4	41	2.9	15.4	61.4	2303	5049
21	WK 3722	110	115	152	87	188	11.2	52	2.8	22.7	53.2	2449	5561
22	WK 3730	104	111	143	80	198	9.5	49	3.7	16	66.3	2577	4757
23	Jhadde (Local check)	98	108	144	89	222	8.2	41	1.9	17.2	42.4	2655	6092
24	Sorgadwari (Check)	99	106	147	87	204	10.9	41	3.6	25.9	62	3480	6545
25	KhumalShakti (Check)	110	118	146	86	302	9.9	45	2.7	20.8	58.4	3070	7151
	Grand Mean	106.1	114.7	145.6	90.6	221.8	10	44.7	2.7	18.1	58.2	2765.8	6033.1
	LSD value	3.5	2.4	1.8	8.1	65.2	1.9	8.2	0.5	5.3	8.3	657.4	1630.8
	CV (%)	1.59	0.86	0.55	4.03	13.15	9.13	8.1	8.75	12.57	6.83	10.35	11.57
	P value	<0.00	<0.00	<0.00	<0.00	0.004	0.096	0.002	<0.001	0.01148	<0.001	<0.001	0.008

Table 3. Mean value of traits for 4 clusters obtained from hierarchical cluster analysis.

Clusters	Cluster I	Cluster II	Cluster III	Cluster IV	Grand Centroid
DTH (days)	106	106	105	109	106
DTA (days)	115	114	114	116	115
DTM (days)	146	144	146	147	146
PH (cm)	87.0	93.5	95.3	88.5	91.1
NSPM	207	245	252	170	218
SL (cm)	10.0	10.0	10.2	9.4	9.9
NGPS	43	45	50	42	45
GWPS	2.6	2.8	3.0	2.8	2.8
FLA	17.8	17.9	18.5	18.8	18.3
TGW (g)	55.8	62.4	52.1	63.2	58.4
GY (kg/ha)	2469	2924	3789	1971	2788

Table 4. Vector loadings and proportion of variance explained by the first five principal components.

Traits	PC1	PC2	PC3	PC4	PC5
DTH	-0.347	-0.365	0.344	0.059	0.272
DTA	-0.393	-0.360	0.186	0.083	0.336
DTM	-0.319	-0.383	0.078	-0.053	-0.489
PH	-0.243	0.377	-0.053	0.235	0.328
NSPM	-0.348	0.351	-0.126	0.132	0.236
SL	-0.102	0.125	0.227	0.789	-0.469
NGPS	-0.305	0.248	0.461	-0.356	-0.061
GWPS	0.306	0.175	0.633	-0.201	-0.115
TGW	0.423	-0.050	0.381	0.327	0.396
GY	-0.259	0.464	0.114	-0.134	-0.129
Loadings					
Eigen value	3.005	2.752	1.269	1.130	0.609
Standard deviation	1.734	1.659	1.127	1.063	0.781
Proportion of Variance	0.301	0.275	0.127	0.113	0.061
Cumulative Proportion	0.301	0.576	0.703	0.816	0.877

Analysis of variance

The analysis of variance indicated highly significant differences ($p \leq 0.001$) among the 25 genotypes for several metric traits including days to heading (range 98-113 days), anthesis (106-124 days) and maturity (141-154 days), plant height (80-107cm), grains/spike (31-57), grain weight/spike (1.9-3.7g) and ultimately grain yield (1908-4146 kg/ha). Significant variation ($p \leq 0.01$) was also observed for biological yield (4176-8134 kg/ha), spikes/m² (166-314) and flag leaf area (14.45-25.9 cm²). Non-significant difference was found only for spike length (8.2-12cm). The wide variability observed for yield attributes, phenology, and agro-morphological traits aligns with findings from previous diversity studies in wheat (Ikram-ul-Haq et al., 2022; Khyber et al., 2019). This could be attributed to the diverse genetic backgrounds and ancestral relationships among the advanced breeding lines evaluated, which likely contribute to capturing favorable allelic variations controlling these quantitative traits (Khadka et al., 2020). The earliness for heading and maturity was recorded in WK3715 (98 days) and local check variety Jhadde (98 days). The highest plant stature was exhibited by BL5148 and NL1474 (107cm) while NL1474 also showed maximum spikes/m² (314), grains/spike (57) and grain yield (4146 kg/ha) coupled with highest biological yield (8134 kg/ha). In contrast, local check Jhadde despite early heading had the lowest grain weight/spike (1.9g) and 1000-grain weight (42.4g). The genotypes thus showed wide variability for yield attributes giving scope for genetic improvement through selection.

The superior performance of NL1474 for grain yield combined with its excellent expression of yield components like spikes/m², grains/spike, and plant height is consistent with previous reports highlighting the importance of improving spike fertility traits along with optimizing plant architecture to maximize grain yield potential (Guo et al., 2018; Molero & Reynolds, 2020). The observed positive correlations between grain yield and these synergistic determinants reinforce their combined influence, as documented in other studies on wheat (Hussain et al., 2022; Lin et al., 2019).

However, the negative correlations between grain weight and number components like grains/spike and spikes/m² suggest trade-offs that can constrain simultaneous improvement in grain size and number. This compensation effect has been frequently reported, with larger grains often associated with reduced grain numbers (Sheoran et al., 2019). Breeding efforts must, therefore, carefully balance selection for these inversely related traits to optimize grain yield. Based on mean performance, genotype NL1474 was identified as best for plant height, spikes/m², grains/spike, grain yield and biological yield. For earliness, WK3715 matured in 144 days similar to Jhadde. The highest 1000-grain weight was shown by NL1487 (68g) while maximum grain weight/spike by WK3730 (3.7g). Among poor performers, NL1607 gave the lowest grain yield (1908 kg/ha) and NL1596 minimum biological yield (4176 kg/ha). BL5082 showed the least grains/spike (31) and grain weight/spike (1.9g). Local checks Jhadde and Sorgadwari exhibited contrast features with both desirable and poor traits.

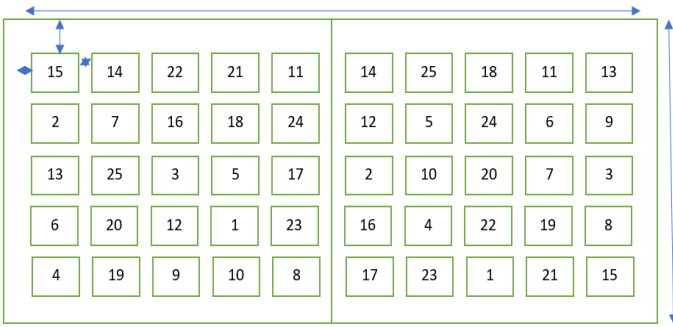


Figure 2: Experimental layout used in an experiment at Gokuleshwar (2022/23).



Figure 3. Correlation matrix among different traits for 25 wheat genotypes, evaluated at Gokuleshwar, Sudurpaschim Pradesh, Nepal.

(Note: GY: Grain Yield; BY: Biomass Yield; NGPS: Number of Grains per Spike; TGW: Thousand Grains Weight; PH: Plant Height; NSPM: Number of spikes per square meter; DTH: Days to Heading and DTM: Days to Maturity and SL: Spike length.)

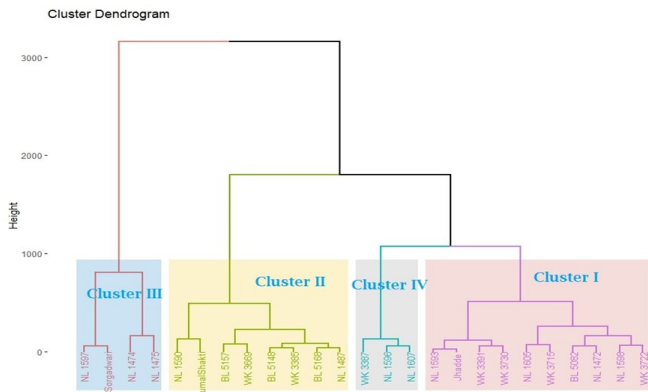


Figure 4. Hierarchical clustering of 25 wheat genotypes evaluated at Gokuleshwar.

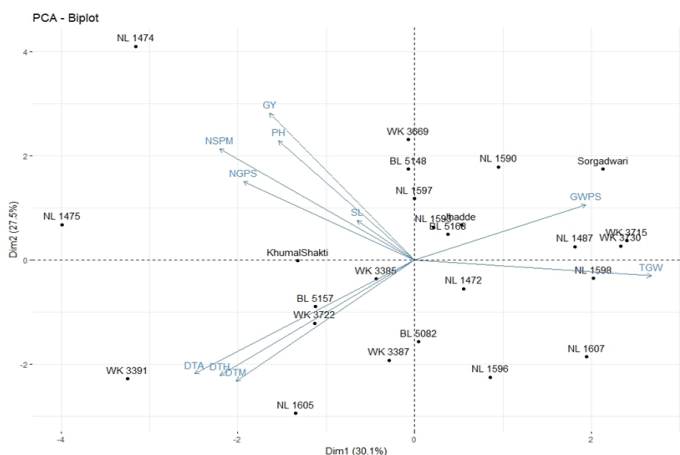


Figure 5. Biplot between first and second principal components.

Correlation study

The correlation analysis helps to understand the connections between important factors that determine wheat growth and yield, which can be useful for breeding and variety development to improve yield and quality. Aligning with previous studies (Mdluli et al., 2020; Mwadzingeni et al., 2016; Rezzouk et al., 2022), days to heading (DTH) exhibited strong positive correlations with days to anthesis (DTA) (0.90^{***}) and maturity (0.6^{**}), indicating that breeding for earlier DTH can accelerate developmental phases. However, unlike some prior reports (Dukamo et al., 2023; Mwadzingeni et al., 2016), DTH and DTA showed non-significant associations with grain yield (-0.2) and thousand grain weight (-0.2). This demonstrates the potential to reduce pre-anthesis period duration without penalizing productivity in the studied germplasm. As expected, thousand grain weight (TGW) positively correlated with grains per spike (Hussain et al., 2022; Mwadzingeni et al., 2016; Sheoran et al., 2019). However, TGW exhibited trade-offs with grain number components like spikes per m² (-0.4^{*}) and grains per spike (-0.4^{*}), as well as with grain yield (-0.4^{*}). Simultaneously improving grain size and number remains challenging, reflecting the frequently observed compensation effect where larger grains are often associated with reduced grain numbers (Sheoran et al., 2019). Reinforcing previous studies (Hussain et al., 2022; Lin et al., 2019; Mwadzingeni et al., 2016), grain yield expressed highly significant positive correlations with biomass accumulation (0.90^{**}), spikes per m² (0.7^{**}), grains per spike (0.6^{**}), and plant height (0.5^{**}). Jointly improving these synergistic yield determinants may provide the greatest potential to maximize genetic gains in productivity. In contrast, some traits like spike length exhibited weaker associations with grain yield.

Cluster analysis and principal component analysis

The 25 bread wheat genotypes were grouped into 4 clusters based on phenotypic traits using hierarchical cluster analysis (Table 2). Cluster analysis effectively discriminated genotypes into distinct groups based on phenotypic similarity, providing a convenient means to explore diversity and identify potential heterotic groups and parental lines for hybridization. This aligns with the utility of cluster analysis for such purposes, as highlighted in previous wheat studies (Khan et al., 2022; Pour-Aboughadareh et al., 2021). Cluster III, represented by genotypes NL1474, NL1475, NL1597, and the check Sorgadwari, appears the best source of favorable alleles for improving grain and biomass yield. This cluster displayed maximum expression for traits like grain yield, spikes/m², spike length, grains/spike, and plant height. In contrast, Cluster IV genotypes exemplified by NL1596, NL1607, and WK3387 should be discarded owing to their overall poor performance for yield attributes. Identifying such superior and inferior genotype groups through clustering can guide parental selection and population development for further enhancement, as observed in earlier studies (Ali et al., 2021). The principal component analysis (PCA) revealed that the first five principal components (PC1 to PC5) explained a cumulative variance of 87.7%, capturing important axes of

genetic variation among the genotypes. PC1 accounted for 30.1% of the variance, predominantly influenced by thousand grain weight, phenology traits (days to heading, anthesis, and maturity), plant height, spikes/m², and grain weight per spike. PC2 explained an additional 27.5% variation, mainly contributed by phenology, plant height, spikes/m², grains/spike, and grain yield. Similarly, thousand grain weight, grains/spike, spikes/m², and spike length were the principal discriminating traits for PC3, while plant height and spike length played major roles in PC4 and PC5, respectively.

These findings align with previous studies that have identified similar key traits contributing to phenotypic diversity in wheat through multivariate analyses (Ikram-ul-Haq et al., 2022; Pour-Aboughadareh et al., 2021). The PCA enabled identifying major traits that could be targeted through simultaneous selection to improve overall crop yield. Emphasis should be placed on traits with high weightages across principal components while selecting candidate genotypes for use in crossing and genetic enhancement programs. The biplot based on the first two principal components (explaining 57.6% variance) provided a visual representation of the relationships among genotypes and agro-morphological traits. It distinctly separated genotypes into high-performing and low-performing groups based on traits like grain yield, spikes/m², and grains/spike. Genotypes NL1474, NL1475, NL1597, and the check Sorgadwari clustered together on the positive side of PC1, exhibiting the highest expression for yield attributes, including grain yield. In contrast, genotypes NL1596, NL1607, and WK3387, positioned on the negative PC1, showed the least performance for these traits. The vectors for thousand grain weight, days to maturity, plant height, and grains/spike were perpendicular, indicating their maximum influence on PC1 differentiation, while PC2 was predominantly determined by grain yield, days to heading, and spikes/m². The biplot complemented the cluster analysis in identifying promising (NL1474, NL1475) and poor-yielding genotypes (NL1596, NL1607), which could be exploited for wheat improvement through strategic crossing and progeny selection. This integration of clustering and biplots to discriminate genotypes based on target traits has been successfully employed in previous wheat breeding studies (Ali et al., 2021; Khan et al., 2022).

Conclusion

This study evaluated 25 advanced bread wheat genotypes for performance under irrigated conditions in the far western mid-hills of Nepal. The results demonstrated substantial genetic variability among the tested lines for critical yield-related traits like days to maturity, plant height, spikes per unit area, grains per spike, 1000-grain weight, and ultimately grain yield. The genotype NL1474 emerged as the top performer, exhibiting maximum values for grain yield (4146 kg/ha), biomass yield, spikes/m², grains/spike, and plant height. In contrast, local checks like Jhadde showed lower productivity despite early maturity. Correlation analysis revealed that while grain yield exhibited strong positive associations with yield components

such as biomass, fertile spikes, spike grains, and plant height, it displayed trade-offs with grain size (1000-grain weight). Simultaneously improving grain number and grain weight remains challenging due to inherent compensatory mechanisms. Multivariate techniques like cluster analysis and principal component analysis effectively discriminated the genotypes into distinct high-yielding and low-yielding groups. Promising genotypes like NL1474, NL1475, and NL1597 clustered together based on maximum expression of favorable traits, while inferior lines like NL1596 and NL1607 showed the opposite trend. The substantial variability observed for yield determinants, along with the identification of superior genotypes like NL1474, underscores the potential of the studied germplasm for genetic enhancement of productivity in Nepal's far western mid-hills. However, multi-location field testing across representative environments is crucial to validate the adaptability and stability of outstanding genotypes before considering them for large-scale cultivation. This study provides a foundation for local breeding programs to harness available diversity and develop high-yielding, climate-resilient wheat varieties tailored to the agro-ecological conditions and farmers' needs in the far western mid-hill regions of Nepal.

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AUTHORS CONTRIBUTION

Conceptualization, AN and KRP; Methodology, BS, DST, MKS, KBB, AN, and KRP; Software, KRP and AN; Validation, KRP; Data curation, KRP and AN; Writing—original draft preparation, AN, BS, DST, MKS, and KBB; Writing—review and editing, KRP and AN; Investigation, BS, DST, MKS, KBB, and AN; Resources, KRP; Visualization, KRP and AN; Supervision, KRP; Funding acquisition, no funding was received from any source. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest: The authors declare no conflict of interest.

Ethical approval: Not applicable.

Data availability: The data that support the findings of this study are available on request from the corresponding author.

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