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Multivariate analysis of spike traits to identify optimal phenotypic profiles of productive tiller number in novel hybrid soft winter wheat breeding lines

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Abstract

Background. The ability to comprehend how spike traits contribute to total phenotypic variation and genetic divergence among genotypes is essential for breeding programs to be effective. Specifically, in wheat, genetic improvement of yield centers on manipulating component traits like productive tiller number and spike architecture. Therefore, identifying the optimal multivariate phenotypic profile of spikes for maximizing productive tiller number in hybrid backgrounds is crucial.

Purpose. Therefore, this study aimed to identify superior genotypes with optimal productive tiller numbers from novel soft winter wheat hybrids by analyzing multivariate spike trait profiles, in order to provide actionable selection criteria for enhancing breeding programs.

Materials and methods. The research was conducted at the Kaluga Agricultural Experimental Station during 2022-2023 period. Data were collected from 1,056 individual sets of spikes (plant stand) representing productive tiller groups across multiple lines and analyzed key spike yield-related traits such as number of productive tillers spikes per plant, spikes weight per plant, and grain weight per plant stand, along with productivity efficiency ratios and analyzed by principal component and cluster analyses using Python 3.11.7 in the Jupiter notebook environment.

Results. Principal component analysis identified two major components that together explained 84.78% of the observed phenotypic variation in the soft winter wheat hybrids. The first principal component (PC1) was primarily associated with grain weight, exhibiting a loading of 0.386, indicating its dominant contribution to variation along this dimension. The second principal component (PC2) was strongly influenced by number of

spikes, with a loading of 0.749, and also reflected yield efficiency traits, including grain weight (loading 0.591) and grain weight per spike number (loading 0.604), capturing variation related to spike productivity and grain filling efficiency. Cluster analysis distinguished three genotype groups with contrasting yield strategies: Cluster 1 had low number of spikes and grain weight with high variability, Cluster 2 combined high number of spikes and grain weight with moderate variability, and Cluster 3 exhibited fewer spikes but high grain weight per spike with low variability, indicating efficient grain filling. The top 5% of genotypes showed a high average number of spikes (productive tillers) (12.75) and grain weight (30.25 g), with excellent grain filling ratio (80.15%) and low variability, confirming the importance of balancing spike quantity and quality in breeding for enhanced yield.

Keywords: cluster analysis; grain yield; hybrid genotype; phenotypic trait; principal component analysis

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Introduction

The global food security mainly relies on agricultural productivity, and this is highly cereal crop-specific [5]. To sustain the ever-increasing needs of an expansionary global population at an accelerated growth rate, it is imperative to maximize crop yield and quality [26]. However, achieving superior agricultural productivity is elusive due to the medley of abiotic and biotic pressures hindering crop development and growth [15; 22]. It is thus imperative to identify high yielding genotypes and understand the traits directly influencing crop productivity and devise effective breeding measures for enhancement. The tiller number, which is one of the vital indicators in agricultural science, plays an integral role in crop productivity analysis [27]. This indicator reveals the potential of plants to branch and demonstrates their competency in producing additional stems or shoots potentially resulting in the formation of extra grain bearing spikes [8; 23]. To maximize crop yield and enhance agricultural productivity in general, there is essential importance in the analysis between productivity and the tiller number [8].

Estimation of tillering capacity is critical in soft winter wheat breeding, as it directly determines the number of functional spikes per plant and significantly impacts grain yield. Tillering capacity varies among genotypes [19], influencing spike number and, consequently, grain production. Yield is also influenced by

components like grains per spike, single spike yield, fertile tiller number, and tiller output [4; 9]. Studies report considerable genotype-dependent variation in viable tiller numbers, ranging from 6.78 to 28.06 and averaging 13.49 with observed limits between 8.60 and 16.06 [23]. The interaction of genotype and environment governs tillering stability, with some varieties consistently producing tillers across conditions, demonstrating genetic control of this trait [19].

Following earlier research that emphasizes the efficacy of cluster and principal component analyses (PCA) in evaluating genetic diversity and relationships among wheat genotypes [7], numerous scholars have employed these multivariate techniques to gain insights into the complex interrelations between tillering potential and spike characteristics in wheat breeding lines. Cluster analysis categorizes varieties based on a multitude of intricate traits rather than a singular attribute, providing a more holistic perspective of genetic variability which is essential considering the multifaceted nature of winter wheat yield [1; 16]. PCA minimizes dimensionality by converting correlated variables into a reduced number of principal components, thereby aiding in the identification and interpretation of traits [3; 20]. [18] suggested conducting PCA before cluster analysis to enhance data clarity and support the selection of prospective parents for hybridization initiatives. For instance, Mustafa et al. utilized PCA and cluster analysis on maize genotypes subjected to drought stress, determining four principal components that accounted for 86.7% of the total phenotypic variation. [12] identified four components that explained 84% of the variability in traits among spring wheat genotypes. [6] reported that in their study of rice landraces, the first three principal components, each with eigenvalues greater than one, collectively explained 72.9% of the total variability among the genotypes analyzed. In a similar vein, [2] implemented factor and cluster analyses to categorize bread wheat genotypes into three distinct clusters, uncovering notable correlations between yield and its constituent components. Moreover, multivariate analyses facilitate the concurrent investigation of various spike-related traits, assisting in genotype classification and the assessment of superior tillering capacity [17]. This methodology enables breeders to group lines based on patterns of spike traits, thereby streamlining the selection of desirable parents with improved tillering potential and advancing breeding strategies focused on optimizing crop architecture and yield stability [3].

This study aimed to integrate fine-scale spike trait phenotypes with multivariate statistical methods to form an effective framework for analyzing genotypes with optimum tillering capacity in novel soft winter wheat breeding lines. The combined approach enhances the analysis of complex relationships among

the traits and genetic diversity necessary in the improvement of wheat cultivars with the potential to yield highly.

Materials and methods

Plant material and experimental Site

The research was carried out in the Kaluga Agricultural Experimental Station, Peremyshlsky District of Kaluga Region of Russia (54°25'10" N, 36°05'11" E) where light-gray forest soils provide good conditions for winter wheat growth. The research object was hybrid new breeding lines (L) derived from a batch of winter soft wheat genotypes (H5174-17, H5154-17, H5171-17) that were produced by cross-pollinating the leading breeding line Kasar with a set of tester lines (gA7, gA42, gB23). The research was conducted for the whole duration of the 2022–2023 growing season.

Experimental design, Sampling and data collection

The seeds of the hybrid genotypes were sown in a field plot measuring 10 meters in width and 50 meters in length, arranged in four distinct rows. The field experiment involved sowing seeds on September 25, 2022, at 500 seeds/m² followed by harvesting on July 29, 2023. The sowing density was adjusted to ensure optimal plant spacing, minimizing intra-line competition and allowing for accurate phenotypic assessment.

At physiological maturity, through mass selection a total of 1,056 individual sets of spikes representing productive tiller groups across multiple genotypes were sampled across the experimental plot to ensure a representative assessment of the population. Sets of spikes from productive tiller group of each sampled genotype were meticulously harvested to prevent physical damage and subsequently transported to crop production laboratory for comprehensive phenotypic characterization. The number of productive tiller spikes per plant (NS), the weight of all productive tiller spikes per plant (WS) measured in grams(g), and the grains obtained from productive tiller spikes per plant (WG), also weighed in grams(g), were recorded. Additionally, productivity-indicating ratios (%WG/WS, WG/NS, WS/NS) were calculated. These parameters provided critical data for evaluating tiller numbers and grain yield potential at the individual plant level.

Data Analysis

All analyses were conducted using Python version 3.11.7 within the Jupyter Notebook environment. Before performing multivariate analysis, all variables underwent Z-score normalization to standardize each trait to a mean of zero and scale to unit variance.

Principal component analysis (PCA)

Principal Component Analysis (PCA) was applied to standardized variables to reduce dimensionality and reveal the main patterns of variation within the multivariate spike trait dataset. An initial PCA was performed without limiting the number of components to identify eigenvalues and the proportion of variance explained.

Components with eigenvalues exceeding one were retained for further analysis, following the Kaiser criterion [14]. Subsequently, a final PCA was conducted specifying the selected number of components, producing principal component scores that represent each sample's position in a reduced-dimensional space, alongside loadings that indicate the contribution of each variable to the components. Scree plots were used to visually evaluate the explained variance and confirm the adequacy of component selection.

Clustering Analysis

Hierarchical clustering methods were applied to the PCA-transformed data to identify groups of genotypes with similar spike trait profiles. The appropriate cluster count was determined by balancing compactness (by inertia in the elbow approach) with cluster separation quality (as measured by silhouette scores). Ward's linkage [25] was used resulting in a dendrogram that visualises genotype associations. The optimal number of clusters was selected by maximizing the average silhouette score computed over cluster numbers ranging from 2 to 10. Each genotype was awarded cluster membership by dividing the dendrogram into the optimal number of clusters. Cluster characterization entailed calculating mean trait values for each cluster, which aided in the understanding of agronomic profiles associated with each group. A Spike Model was generated by first ranking all genotypes using a weighted index across spike traits. The top 5% of performers were then selected, and their trait means, standard deviations (SD) and coefficient of variation (%CV) were calculated to define the desired phenotypic ranges and acceptable variation for breeding purposes.

Selection Index Calculation

To combine numerous qualities into a composite selection criterion, each genotype's selection index was determined as a weighted linear combination of the standardized trait values. The index was calculated using the dot product of trait values and weights. This index allowed for the ranking of genotypes based on their overall attractiveness across different agronomic parameters.

The selection index for each cluster is determined as the weighted sum (linear combination) of the trait means, following the method described by [9].

$$I_i = \sum_{j=1}^n w_j x_{ij}$$

Where:

I_i is the selection index score for the i -th spike/genotype,

n is the total number of traits included in the index,

w_j is the normalized economic or breeding weight assigned to the j -th trait,

x_{ij} is the phenotypic value of the j -th trait for the i -th spike.

Results and discussion

Dimensional reduction of spike trait variability using principal component analysis

The PCA output results (Table 1) indicate that the first two principal components (PC1 and PC2) effectively summarize the multivariate phenotypic structure of the dataset, capturing 84.70% of the total variance. PC1 alone, with an eigenvalue of 2.491, explained 49.78% of the variance, highlighting a primary source of structural variation among the measured traits. PC2, with an eigenvalue of 1.747, contributed an additional 34.91% of the explained variance. The substantial variance explained by these first two components suggests the presence of strong underlying patterns and interrelationships within the trait set, providing a concise, simplified representation of a more complex multivariate structure. These findings align with established applications of PCA in plant phenotyping. Previous studies on wheat genotypes have similarly reported that a limited number of principal components often between two and four, each with eigenvalues greater than one account for the majority (72.9% to 86.8%) of total phenotypic variability [6, 12, 18]. This consistency across independent studies underscores the robustness of PCA as a tool for reducing dimensionality and capturing the core axes of variation in complex phenotypic datasets.

Table 1.

Eigen values, Percentage of variance explained and cumulative percentage by principal components

Component	Eigenvalue	% Variance Explained	Cumulative %
1	2.491	49.78	49.78
2	1.747	34.91	84.70
3	0.735	14.69	99.39
4	0.028	0.56	99.95
5	0.002	0.05	100.00

Trait Loadings on the first two principal components with eigenvalues greater than one

The first two principal components (PC1 and PC2, Table 2), both with eigenvalues greater than 1, represent the most significant sources of variation in the data. PC1 is primarily associated with yield per spike, as indicated by strong positive loadings for grain weight per spike (Wg/Ns, 0.604) and spike weight per spike (Ws/Ns, 0.568). It also shows moderate positive loadings for grain weight (Wg, 0.387) and grain-to-spike weight percentage (Wg/Ws%, 0.401). In contrast, the number of spikes (Ns) contributes minimally to PC1, given its low loading of 0.058. This suggests PC1 captures a dimension of spike size and filling efficiency, independent of spike number.

PC2 reflects variation strongly linked to spike number (Ns), with a high positive loading of 0.749, and also shows a substantial positive loading for total grain weight (Wg, 0.591). However, the per-spike efficiency traits (Wg/Ns, Ws/Ns, and Wg/Ws%) all exhibit slight negative loadings on PC2. This indicates that PC2 represents a spike number and total yield potential axis, which is distinct from (and slightly inversely related to) the per-spike yield components that define PC1.

Together, these two orthogonal components summarize key, independent biological dimensions influencing yield architecture. PC1 explains variation in spike size and grain-filling intensity, while PC2 emphasizes variation in spike number and total grain weight. This clear separation provides a robust framework for interpreting trait variation relevant to wheat breeding objectives.

Table 2.

Trait loadings on principal components

Trait	PC1	PC2
Ns	0.058	0.749
Wg	0.387	0.591
Wg/Ws%	0.401	-0.087
Wg/Ns	0.604	-0.204
Ws/Ns	0.568	-0.201

Determination of optimal cluster number in wheat spike trait grouping using the elbow method and silhouette score

Figure 1 displays two diagnostic plots used to determine the optimal cluster number. The Elbow Method plot (left) shows the inertia (within-cluster sum of squares) decreasing as the number of clusters (k) increases. The curve exhibits a visible bend, or “elbow,” at $k = 3$, indicating that further increases in k yield

diminishing returns in reducing inertia. This suggests that three clusters may provide an optimal balance between cluster compactness and interpretability. The Silhouette Analysis plot (right) shows the average silhouette score for each k , a metric that assesses how similar samples are to their own cluster compared to other clusters. The highest silhouette score occurs at $k = 3$, indicating the most cohesive and well-separated clustering at this value. A study by [21] supports this interpretation, noting that higher silhouette scores are associated with tighter, more distinct groups, while lower scores indicate over-segmentation or poor separation. Therefore, the diagnostic evidence in Figure 1 strongly supports the selection of three clusters as best capturing the underlying structure of the data, which is consistent with conventional clustering evaluation practice.

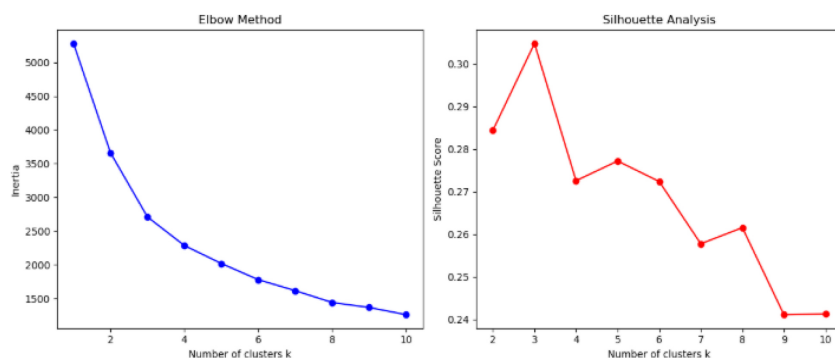


Fig. 1. Elbow Method and Silhouette scores indicating clustering performance for various k values, with optimal cluster count $K=3$

Principal component and cluster analysis reveal key trait drivers and population structure in soft winter wheat spikes

The application of PCA facilitated the reduction of multidimensional trait data and captured the key underlying structure, simplifying the interpretation of complex phenotypic relationships [13]. The biplot (Figure 2) visualizes the relationships among genotypes within the principal component space. The orientation and length of the trait vectors reinforce the component interpretation: vectors for yield-per-spike traits (Wg/Ns , Ws/Ns) project strongly along the positive PC1 axis, confirming their dominance in defining this spike-size dimension. Notably, the grain-to-spike weight percentage ($Wg/Ws\%$) vector aligns closely with these per-spike yield vectors indicating a strong positive correlation among these efficiency traits. This suggests that heavier, better-filled spikes also tend to allocate a greater proportion of their biomass to grain, highlighting a synergistic component

of spike efficiency. In contrast, the spike number (Ns) vector projects almost vertically along the positive PC2 axis, and the grain weight (Wg) vector points into the upper-right quadrant, indicating its positive correlation with both components. The near-orthogonal angles between the Ns vector and the cluster of per-spike efficiency vectors (Wg/Ns, Ws/Ns, Wg/Ws%) graphically illustrate the statistical independence of the spike-number and spike-size trade-offs. Consequently, genotypes are distributed according to these trait associations. Those positioned in the direction of the Wg/Ns, Ws/Ns, and Wg/Ws% vectors (lower-right quadrant: high PC1, low PC2) exhibit the profile of few but heavy, well-filled spikes with high grain partitioning efficiency. Genotypes projected in the direction of the Ns vector (upper-left quadrant: low PC1, high PC2) achieve yield primarily through high spike count. Genotypes in the upper-right quadrant (high PC1, high PC2), aligned with the Wg vector, combine both large spike size and high spike number, representing a high-yielding ideotype.

This spatial representation confirms the orthogonal nature of the primary yield components and provides a direct visual tool for selecting parents based on specific targets enhancing spike filling and partitioning efficiency (following the cluster of per-spike vectors), increasing tillering (following the Ns vector), or combining both.

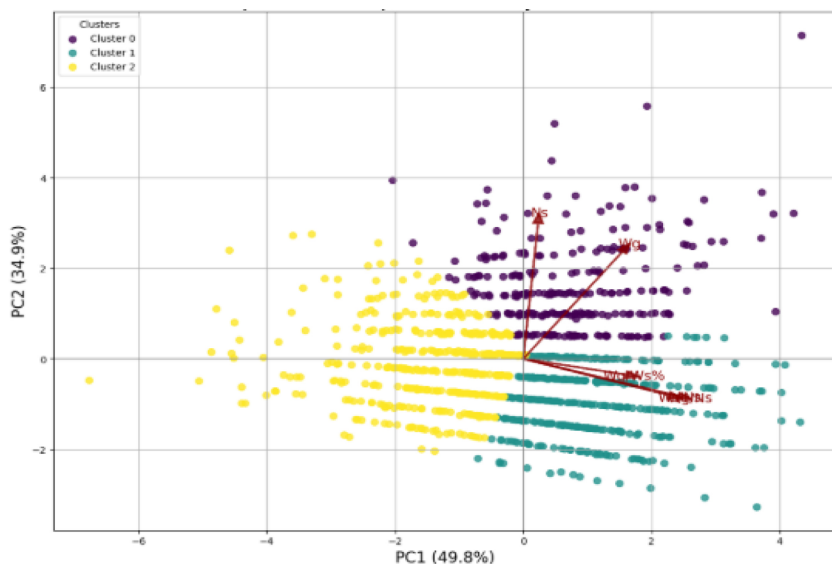


Fig. 2. PCA biplot showing the first two principal components (PC1 and PC2) of a dataset colored by clusters identified from k-means clustering.

Hierarchical clustering of wheat spike traits of tiller group

Figure 3 presents a dendrogram generated from wheat spike trait data using Ward's linkage method. This hierarchical approach begins with each sample as a distinct cluster and iteratively merges pairs to minimize the increase in within-cluster variance. Branches merging at lower heights indicate greater phenotypic similarity, while joins at higher levels represent more distinct groups.

The dendrogram is color-coded to delineate three primary clusters, a result consistent with the optimal cluster number ($k = 3$) indicated by prior elbow and silhouette analyses. The orange, green, and red branches represent groups of samples with distinct spike phenotypes. This clustering likely reflects biologically meaningful variation in yield components and spike morphology among the breeding lines.

Overall, the dendrogram provides a hierarchical visualization of phenotypic similarities, revealing inherent structure within the population. The coherent separation into three robust clusters, corroborated by independent validation metrics, underscores the reliability of this grouping. These distinct phenotypic categories offer a valuable framework for targeted parental selection and further investigation in wheat breeding

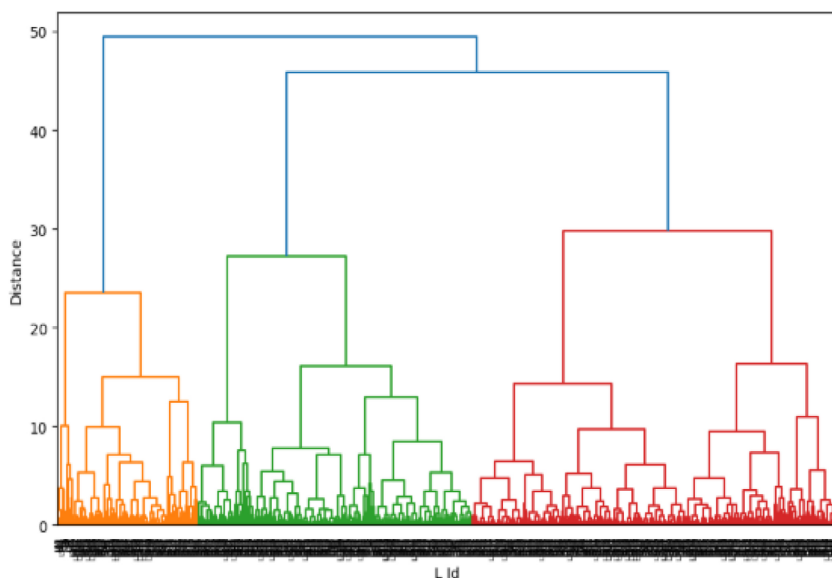


Fig. 3. Hierarchical clustering dendrogram (Ward Method) of wheat spike samples of tiller groups

Hierarchical cluster analysis of characteristics of productive tiller spikes

The hierarchical clustering analysis results (Table 4) describe three distinct clusters of productive tillers, each characterized by specific attributes for spike number, grain weight, and associated efficiency ratios.

Cluster 1 exhibited the lowest mean spike number (6.07) and grain weight (8.16 g), coupled with the highest coefficients of variation (>40% for both traits). This indicates a highly variable and heterogeneous group where individual plants display widely divergent productivity. It also recorded the poorest grain-to-spike weight ratio (71.88%) with substantial variability, reflecting reduced and inconsistent grain-filling efficiency. This combination defines Cluster 1 as a dynamic but unreliable phenotypic group, warranting further investigation into the genetic and environmental drivers of its high variability.

Cluster 2 presented a contrasting profile, with the highest mean spike number (9.63) and grain weight (19.37 g), alongside moderate and more uniform variation. Its grain-to-spike weight ratio improved to 77.40%, accompanied by one of the lowest variability measures. This cluster represents a consistently high-performing group where productive tillers combine high spike number with efficient and predictable grain packing. Its balanced trait expression and stability identify Cluster 2 as a steady performer, making it a prime target for breeding programs focused on reliable, high-yielding phenotypes.

Table 4.

Hierarchical cluster-wise variation in spike number, grain weight, and grain-to-spike weight ratios in productive tillers per plant

Cluster ID	Cluster 1			Cluster 2			Cluster 3		
Size	191			371			494		
Trait	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV
Ns	6.07	2.57	42.27	9.63	2.20	22.84	5.11	1.40	27.46
Wg	8.16	3.29	40.36	19.37	5.95	30.71	12.16	4.06	33.40
%Wg/Ws	71.88	6.61	9.20	77.40	3.27	4.22	79.35	2.67	3.36
Ws/Ns	1.95	0.57	28.99	2.59	0.41	15.94	3.00	0.52	17.27
Wg/Ns	1.39	0.34	24.76	2.01	0.35	17.30	2.39	0.43	18.15

Cluster 3 displayed a distinct quality-oriented strategy. Despite having the fewest spikes (5.11), it achieved a moderate grain weight (12.16 g) and the highest grain-to-spike weight ratio (79.35%) with minimal variability. The high ratios for spike weight and grain weight per spike suggest that tillers in this cluster prioritize quality over quantity, producing fewer but larger, heavier spikes with highly efficient and stable grain filling. This profile exemplifies an optimized

phenotype characterized by superior partitioning efficiency and consistency, making it particularly valuable for breeding programs aimed at maximizing yield components in a predictable and controlled manner.

Optimal phenotypic traits in elite wheat spikes: Wheat tiller group traits statistics for breeding selection

The results (Table 5) summarizes key statistics for the top 5% of wheat spikes in terms of genotype tillering productivity traits. These genotypes exhibit a high average spike number (12.75) with moderate variability (%CV ~19.8%), indicating consistently large spike counts. This implies that a sufficient number of reproductive units is required for better grain output, which is consistent with previous research on the effect of spike number on yield [28]. Grain weight of the productive tiller spikes is also notably high (30.25 g) with relatively low variation (18.12%), reflecting strong and stable grain development. The grain-to-spike weight ratio is very efficient at 80.15% with minimal variation (2.79%), suggesting excellent grain filling. The ratios of grain weight to spike weight per spike numbers (2.41 and 3.01, respectively) illustrate the balance between spike quantity and quality, reinforcing the superior productivity and consistency of this elite spike group. This supports the concept that higher spike biomass corresponds to higher-yielding lines [11]. Moreover, these ratios emphasize the importance of both the number of spikes and their individual productivity, as a greater grain weight per spike often equates to increased total yield potential. Overall, these results confirm the significance of combined spike-related traits and their ratios in selecting superior wheat lines, thereby supporting breeding strategies aimed at optimising both spike numbers and grain filling efficiency to maximise yield.

Table 5.

Summary Statistics of optimal yield traits in the top 5% productive tiller spikes per plant

Trait	Mean	SD	%CV
NS	12.75	2.53	19.82
WG, g	30.25	5.48	18.12
WG/WS%	80.15	2.24	2.79
WG/NS	2.41	0.37	15.47
WS/NS	3.01	0.44	14.47

Selection of superior wheat lines of tiller group using spike trait index

The results (Table 6) outlines a group of leading wheat lines selected on the basis of high spike productivity traits and a selection index (I). They have high spike counts (Ns) between 12 and 21 and high grain weights (Wg) between 29.9 and 55.6

grams. Grain weight-to-spike weight ratios (%Wg/Ws) always remain high, well above 80%, implying efficient grain filling operations. Grain weight-to-spike number (Wg/Ns) and weight of spikes-to-spike number (Ws/Ns) ratios indicate fluctuation among the lines, but they indicate vigorous spike and grain growth. Selection index (I) consistently prioritizes the lines on the basis of their integrated spike productivity, and lines like L799, L633, and L301 are indicated as high-quality breeding material.

Table 6.

Key spike yield traits and selection index of 10 top performing wheat lines

Line Id	Spike traits					
	Ns	Wg	% Wg/Ws	Wg/Ns	Ws/Ns	I _i
L799	21	55.6	80.93	2.65	3.27	32.69
L633	13	39.6	83.54	3.05	3.65	28.57
L301	19	39.6	78.73	2.08	2.65	28.41
L336	14	40.1	80.52	2.86	3.56	28.21
L291	13	38.8	80.50	2.99	3.71	27.80
L350	14	35.8	81.55	2.56	3.14	27.41
L881	12	35.9	80.31	2.99	3.73	26.99
L317	13	29.9	86.42	2.30	2.66	26.86
L163	12	30.8	84.38	2.57	3.04	26.56
L858	15	32.8	80.00	2.19	2.73	26.54

I_i-Selection index

Conclusion

Principal component analysis identified two main components that together explained 84.78% of the phenotypic variance. These components highlight critical trait combinations that underlie phenotypic diversity and yield potential, offering valuable insights for soft winter wheat breeding programs.

Cluster analysis revealed three distinct groups with unique spike and grain trait profiles. Cluster 1 was characterized by low spike number and grain weight with high variability, indicating an unpredictable phenotype. Cluster 2 exhibited the highest spike number and grain weight with low variability, representing a stable, high-performing group. Cluster 3 had fewer spikes but showed higher grain weight of productive tiller spikes and the highest grain-to-spike weight ratio with low variability, indicating efficient grain filling and consistency. These distinct genetic profiles present valuable resources for breeding, where crossing divergent groups may enhance heterosis and accelerate genetic gains.

The elite top 5% of genotypes showed consistently high tillering productivity traits, with an average spike number of 12.75 and moderate variability. Grain

weight of productive tillers was high and stable, and an efficient grain-to-spike weight ratio indicated excellent grain filling. Together, these findings confirm the significance of combining spike quantity and grain quality traits to optimize wheat yield, guiding breeding strategies towards enhanced productivity and stability.

Conflict of interest information. The authors declare that there is no conflict of interest.

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