




MULTIVARIATE IDENTIFICATION OF KEY YIELD DETERMINANTS IN BREAD WHEAT USING PATH ANALYSIS AND PCA TECHNIQUES

Zekiye BUDAK BAŞÇİFTÇİ^{1a} - Nazife Gözde AYTER ARPACIOĞLU^{1b*} - Murat OLGUN^{1c}

¹Field Crop Department, Agricultural Faculty, Eskişehir Osmangazi University, Eskişehir, Türkiye

*Corresponding Author E-mail: gaytergu.edu.tr

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a:  ORCID 0000-0002-4034-2537, b:  ORCID 0000-0002-5121-4303, c:  ORCID 0000-0001-6981-4545

ABSTRACT. Understanding the multivariate structure of yield components is fundamental for improving grain yield in bread wheat (*Triticum aestivum* L.). This study evaluated twelve commercial wheat varieties using a comprehensive set of agronomic traits to identify the key determinants of seed yield and to characterize varietal differences through integrated statistical approaches. Descriptive statistics revealed substantial phenotypic variability among genotypes, indicating strong potential for selection and genetic improvement. Path analysis demonstrated that seed weight per spike and seed number per spike were the most influential traits, exhibiting the highest direct and indirect effects on yield, respectively. Thousand-seed weight, harvest index, and plant height contributed mainly through indirect pathways, highlighting their secondary yet supportive roles in the yield formation process. Principal Component Analysis (PCA) further confirmed that the first two components explained over half of the total variation, with seed weight, seed number, spikelet number, thousand-seed weight, and plant height clustering along the primary productivity axis. Overall, the integrated analytical framework emphasizes that grain yield in bread wheat is governed primarily by the balance between grain number and grain weight, with morphological and physiological traits providing additional support. These findings offer valuable guidance for breeding programs aiming to enhance yield potential through targeted trait selection and ideotype development.

Keywords: Bread wheat; *Triticum aestivum* L.; seed yield; yield components; path analysis; seed number; seed weight; thousand-seed weight; harvest index; plant height.

INTRODUCTION

Grain yield in wheat is the result of a complex interaction between genotype and environment, and is determined by numerous yield-related traits such as spike architecture, grain number, grain weight, plant height, and various physiological and phenological characteristics. Growing global population demands and uncertainties associated with climate change have made it strategically essential to quantitatively identify the key components that govern wheat yield. Modern breeding programs increasingly require not only information on average yield performance, but also a deeper understanding of the relationships among yield components—including grain number, grain weight, spike structure, and biomass partitioning—that collectively shape final yield outcomes. Previous studies highlight that wheat yield is largely determined by the interaction between “grain number \times grain weight,” with grain number per spike and thousand-grain weight frequently identified as critical determinants [1-2]. However, classical correlation analysis is often insufficient for distinguishing whether these traits influence yield directly or indirectly. At this point, path analysis provides a powerful statistical tool for examining yield formation more comprehensively. By partitioning correlation coefficients into direct and indirect effects, path analysis enables researchers

to quantify the extent to which traits such as grain number per spike exert direct effects on yield, or whether their influence is mediated indirectly through factors such as thousand-grain weight or spike length. Numerous studies have reported that traits such as grain number per spike, grain weight per spike, thousand-grain weight, and test weight exhibit strong direct effects on yield, whereas plant height and heading date tend to influence yield predominantly through indirect pathways [3-4]. Thus, path analysis plays a crucial role in identifying which traits can serve as effective direct selection criteria to enhance genetic gain in wheat breeding. Because yield components often exhibit high levels of intercorrelation (multicollinearity), classical regression approaches may be inadequate for evaluating them simultaneously. Therefore, Principal Component Analysis (PCA) has increasingly been used to assess the multivariate structure of yield-related traits in wheat. PCA reduces large sets of correlated variables into a smaller number of principal components that explain most of the total variance, enabling breeders to classify genotypes and identify ideotypes based on trait combinations associated with high yield. Genotypes may cluster according to grain number and grain weight traits, spike and plant architecture traits, or quality-related characteristics, providing valuable insight into yield stability and physiological efficiency [5-6]. Studies integrating both path analysis and PCA show that the two methods complement each other effectively. While PCA simplifies multidimensional trait structures, path analysis quantifies their causal pathways toward yield. Thus, traits that load heavily on the first principal component—such as grain number per spike and thousand-grain weight—can be evaluated for whether they also possess strong direct effects on yield. This integrative approach has been shown to be particularly successful in explaining yield stability among wheat genotypes grown under dryland or semi-arid conditions [7-8]. The objective of this study is to elucidate the effects of major yield components on grain yield in bread wheat by jointly applying path analysis and PCA. By examining both the direct–indirect causal effects and the multivariate relationships among spike and grain morphology traits, this work aims to identify the primary selection criteria essential for improving grain yield. The findings are expected to contribute significantly to understanding the complex interactions among yield components and support the development of high-yielding, environment-adapted wheat genotypes.

MATERIALS AND METHODS

This study was conducted using twelve commercially cultivated bread wheat (*Triticum aestivum* L.) varieties widely grown in Türkiye in 2020-21 and 2021-22. The experiment was established under field conditions at the Research and Application Farm of Eskişehir Osmangazi University, Agricultural Faculty. A randomized complete block design (RCBD) with three replications was employed. Each plot consisted of six rows, 6 m in length, with 20 cm row spacing. Standard agronomic practices for fertilization, irrigation, and weed control were applied to ensure optimal crop growth. No major biotic or abiotic stresses were observed during the growing season. A comprehensive set of agronomic and yield-related traits was recorded following standard protocols. Traits included spike length (SpikeL), plant height (PlantHe), spikelet number per spike (SpkltN/Sp), seed weight per spike (SeedW/Sp), seed number per spike (SeedN/Sp), thousand seed weight (ThouSeedW), harvest index (Har.Ind), and seed yield per plot (SeedY). Measurements were taken from ten representative plants in each plot and averaged prior to statistical analysis. Minimum, maximum, and mean values with standard deviations were calculated for all traits to assess phenotypic variation among genotypes. Prior to multivariate

analyses, all variables were standardized (mean = 0, SD = 1) to eliminate scale effects and ensure comparability across traits.

Path analysis was performed to partition the observed correlations into **direct** and **indirect** effects of each yield component on seed yield. The analysis followed the causal modeling approach proposed by Wright (1934), using seed yield as the dependent variable. Direct effect coefficients (P_iY), total correlations (r_iY), and the sum of indirect effects ($\sum r_{ij}P_jY$) were calculated. This method enabled the identification of primary determinants of yield and the evaluation of compensation mechanisms among traits. Statistical computations were carried out using the *lavaan* and *plspm* packages in R and SPSS AMOS. PCA was conducted to evaluate the multivariate structure of the dataset and identify the most influential yield components. Eigenvalues, explained variance ratios, and cumulative variance ratios were derived for each principal component. Trait loadings on PC1 and PC2 were used to interpret trait clustering and to characterize underlying biological processes governing yield variation. Biplots were generated to visualize trait relationships and genotype distribution across principal axes. All PCA computations were performed in R using the *FactoMineR* and *factoextra* packages. All statistical analyses were conducted using RStudio (version 4.2), IBM SPSS (version 26), and Python (scikit-learn environment). Graphical outputs (path diagrams, PCA biplots, clustering visualizations) were produced using *ggplot2*, *matplotlib*, and *factoextra*.

RESULTS AND DISCUSSION

Understanding the contribution of yield components to final seed yield is essential for guiding effective wheat breeding strategies. Seed yield in wheat is shaped by a complex interaction of traits such as spike morphology, seed number, seed weight, and physiological attributes, making it necessary to apply advanced statistical tools to dissect these relationships. Path analysis enables the separation of direct and indirect effects of these traits on yield, identifying those with the strongest causal influence, while Principal Component Analysis (PCA) provides a multivariate view of how yield components collectively structure variation among genotypes. Together, these methods offer a powerful framework for identifying key selection criteria that can accelerate yield improvement in wheat breeding programs. Understanding the contribution of yield components to final seed yield is essential for guiding effective wheat breeding strategies. Seed yield in wheat is shaped by a complex interaction of traits such as spike morphology, seed number, seed weight, and physiological attributes, making it necessary to apply advanced statistical tools to dissect these relationships. Path analysis enables the separation of direct and indirect effects of these traits on yield, identifying those with the strongest causal influence, while Principal Component Analysis (PCA) provides a multivariate view of how yield components collectively structure variation among genotypes. Together, these methods offer a powerful framework for identifying key selection criteria that can accelerate yield improvement in wheat breeding programs. Maximum, minimum and average values of yield and yield components in wheat are given in Table 1.

Table 1. Maximum, minimum and average values of yield and yield components in wheat

Trait	Minimum	Maximum	Mean
SpikeL.	34.000 ± 2.9637	48.000 ± 2.9637	40.9688 ± 2.9637
PlantHe.	46.600 ± 7.7404	82.600 ± 7.7404	65.8438 ± 7.7404
SpkltN/Sp.	107.000 ± 12.6516	159.000 ± 12.6516	135.0000 ± 12.6516
SeedW/Ppk	3.500 ± 2.2623	13.200 ± 2.2623	8.0563 ± 2.2623
SEedN/Sp.	103.000 ± 49.7130	362.000 ± 49.7130	225.2396 ± 49.7130
Thou.SeedN.	26.506 ± 4.0505	46.127 ± 4.0505	35.4644 ± 4.0505
Har.nd.	0.250 ± 0.0871	0.659 ± 0.0871	0.4673 ± 0.0871
SeedY.	100.000 ± 76.3511	443.333 ± 76.3511	239.0620 ± 76.3511

The descriptive statistics of the evaluated yield-related traits in wheat reveal substantial phenotypic variation within the dataset. Spike length ranged from 34.000 ± 2.96 to 48.000 ± 2.96 cm, with a mean of 40.97 cm, indicating moderate variability in spike architecture. Plant height also exhibited broad variation (46.600 ± 7.74 to 82.600 ± 7.74 cm), reflecting differences in vegetative growth potential among genotypes. Spikelet number per spike showed a considerable spread (107.000 ± 12.65 to 159.000 ± 12.65), while seed weight per spike varied between 3.500 ± 2.26 and 13.200 ± 2.26 g, suggesting strong divergence in resource allocation to reproductive structures. Seed number per spike displayed the widest range (103.000 ± 49.71 to 362.000 ± 49.71), highlighting the high genetic and physiological variability in grain set capacity. Thousand-seed weight (26.506 ± 4.05 to 46.127 ± 4.05 g) and harvest index (0.250 ± 0.087 to 0.659 ± 0.087) also showed meaningful differences, reflecting variation in grain filling efficiency and biomass partitioning. Ultimately, seed yield ranged from 100.000 ± 76.35 to 443.333 ± 76.35 g, with a mean of 239.06 g, demonstrating substantial yield diversity likely driven by the combined effects of these yield components.

Investigation of the effects of yield factors on grain yield in wheat using statistical methods

Statistical methods play a vital role in agricultural research by enabling the accurate analysis of complex biological data and the identification of meaningful relationships among traits. These tools help researchers evaluate treatment effects, understand variability, optimize experimental designs, and make reliable decisions based on quantitative evidence. Through techniques such as ANOVA, regression, correlation, PCA, and path analysis, statistics provides a scientific foundation for improving crop productivity, resource efficiency, and overall agricultural sustainability.

Path Analysis

Path analysis is widely used in agricultural research to separate the direct and indirect effects of yield-related traits, allowing researchers to identify which characteristics have the most meaningful causal influence on crop performance. By decomposing correlations into specific pathways, this method helps breeders determine the most effective selection criteria for improving yield, quality, and stress tolerance in crop improvement programs. Explaining the effect of yield factors on grain yield in wheat by path analysis was given in Table 2 and Figure 1.

Table 2. Explaining the effect of yield factors on grain yield in wheat by path analysis.

Trait	Total Correlation with Seed Yield (r_{iY})	Direct Effect on Seed Yield (P_iY)	Sum of Indirect Effects ($\sum r_{ij} P_jY$)
SeedW_Ppk (Seed Weight per Spike)	0.132	1.879	-1.747
SeedN_Sp (Seed Number per Spike)	0.119	-1.191	1.310
PlantHe (Plant Height)	0.141	0.083	0.058
Har_nd (Harvest Index)	-0.107	-0.252	0.145
ThouSeedN (Thousand Seed Weight)	0.066	-0.754	0.820
SpikeL (Spike Length)	0.082	-0.006	0.088

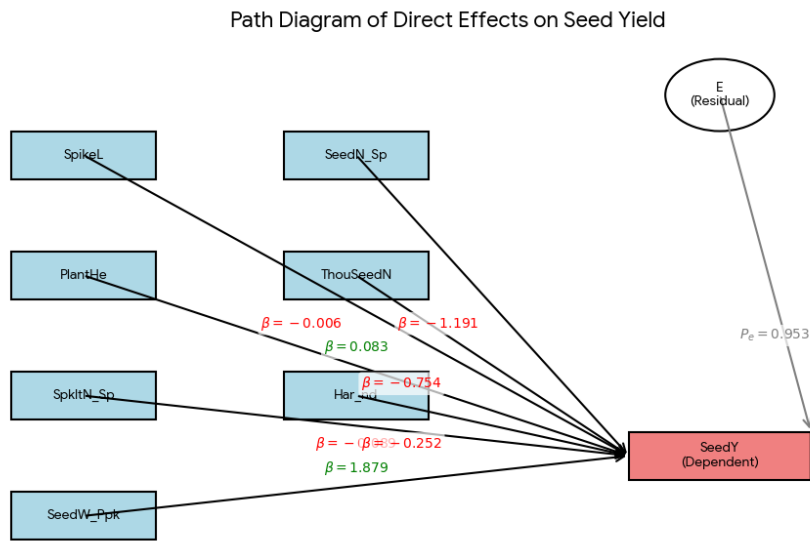


Figure 1. Explaining the effect of yield factors on grain yield in wheat by path analysis

The path analysis table provides a detailed quantitative assessment of how wheat yield components contribute to final grain yield by separating their total correlations into direct and indirect effects. Among all traits examined, seed weight per spike shows the strongest positive direct effect on grain yield, indicating that spikes capable of accumulating more assimilates into grain mass play a dominant role in determining yield. This finding agrees with several physiological studies reporting that improved assimilate partitioning toward the spike and enhanced grain filling capacity are critical drivers of high yield potential [1, 2]. Although seed weight per spike also exhibits negative indirect effects reflecting interactions and trade-offs with grain number and spike morphology the magnitude of its direct effect clearly identifies it as the most influential yield component under the studied conditions. In contrast, seed number per spike displays a negative direct effect but a positive and sizable indirect effect. This pattern illustrates a well-known biological compensation mechanism: genotypes that produce many grains may suffer reductions in individual grain weight due to limited assimilate availability, thereby creating a negative direct pathway. However, seed number remains a central yield determinant because of its strong indirect contributions through other interconnected traits. This trade-off between grain number and grain weight is widely documented, showing that final yield is often

maximized when a balanced contribution of both components is achieved [9, 10]. Thus, although seed number does not directly elevate yield in this dataset, its indirect influence reflects its integral role in the overall yield formation system.

Secondary traits such as plant height, thousand-seed weight, and harvest index demonstrate more moderate influences. Plant height contributes a small positive direct effect, consistent with reports that moderately taller plants may possess stronger biomass production or enhanced spike fertility while avoiding excessive lodging [11]. Thousand-seed weight exhibits a negative direct effect but strong positive indirect effects, indicating that heavier grains alone do not ensure higher yield unless they occur alongside favorable spike and fertility traits. Similar trends have been observed in earlier path analyses where thousand-seed weight often influences yield indirectly by interacting with grain number and grain filling traits [4]. Harvest index shows a slight negative direct effect, suggesting variability in biomass partitioning efficiency among genotypes, although its indirect effects partially offset this reduction. The traits spike length and spikelet number per spike exhibit very small direct effects, implying that their influence on final grain yield is mainly indirect and mediated through their relationships with seed number and spike fertility rather than through independent contributions. Previous studies have similarly noted that spike morphological traits contribute only weakly to yield unless integrated with strong reproductive efficiency and grain set capacity [3].

The path diagram visually reinforces these interpretations by illustrating the magnitude and direction of the relationships among traits. The thick and prominent arrow from seed weight per spike to grain yield highlights its dominant role. The negative arrows from seed number per spike and thousand-seed weight depict trade-offs and biological constraints, while thinner arrows from spike length and spikelet number confirm their limited direct importance. This visual representation aligns with the conceptual understanding that wheat grain yield is determined primarily by the interplay between grain number and grain weight, complemented by secondary influences from plant architecture and biomass allocation patterns [1, 9]. Collectively, the table and figure indicate that improving wheat yield requires a targeted emphasis on enhancing spike grain mass while maintaining an optimal balance with grain number. These insights are consistent with previous literature and provide a robust framework for trait prioritization in wheat breeding programs.

Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is widely used in agricultural research to reduce the complexity of large, multivariate datasets and to identify the most influential traits contributing to variation among genotypes or treatments. By transforming correlated variables into a smaller number of independent principal components, PCA helps researchers detect patterns, classify cultivars, and reveal trait combinations associated with high yield, stress tolerance, or superior quality. This method provides a powerful exploratory tool for plant breeding and agronomy, enabling more efficient selection decisions and a deeper understanding of trait interrelationships under varying environmental conditions. PCA analysis of yield components in wheat was presented in Table 3 and Figure 2.

Table 3. PCA analysis of yield components in wheat

Component	Eigenvalue	Explained Variance Ratio	Cumulative Variance Ratio	Component	Eigenvalue	Explained Variance Ratio	Cumulative Variance Ratio
PC ₁	3.566	0.441	0.441	PC ₄	0.799	0.099	0.825
PC ₂	1.223	0.151	0.592	PC ₅	0.704	0.087	0.912
PC ₃	1.082	0.134	0.726	PC ₆	0.408	0.050	0.963

Variable	PC ₁	PC ₂	Variable	PC ₁	PC ₂
Seed W Sp	0.510	-0.104	Spike L	0.275	0.610
Seed N Sp	0.474	-0.128	Thou Seed N	0.340	-0.042
Spklt N Sp	0.360	0.353	Har Ind	0.297	-0.577
Plant He	0.316	-0.071	Seed Y	0.075	0.368

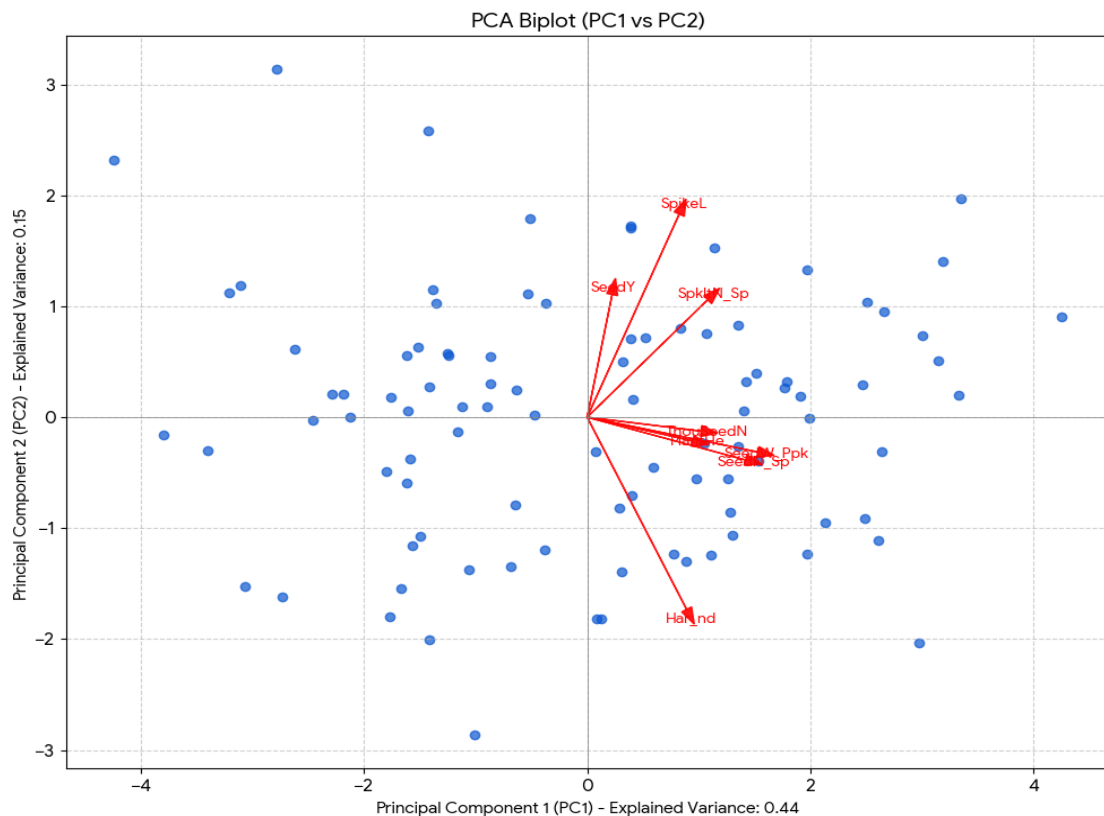


Figure 2. PCA analysis of yield components in wheat was presented in

The PCA table shows that the first principal component (PC1) is the dominant source of variation among the wheat yield traits, explaining 44.1% of the total variance. High positive loadings for seed weight per spike, seed number per spike, spikelet number, thousand-seed weight, and plant height indicate that PC1 represents a general productivity axis that integrates spike fertility, grain formation, and vegetative growth. This result aligns with previous multivariate studies in wheat, which consistently report that grain number, grain weight, and spike fertility traits cluster together in the primary component because they share strong physiological and genetic connections [5-6]. The second principal component (PC2), accounting for 15.1% of variance, is characterized by strong positive loadings for spike length and seed yield, contrasted by a strong negative loading for harvest index. This pattern suggests a trade-off between structural spike traits and biomass allocation efficiency, a relationship frequently highlighted in wheat physiology

research, where harvest index and spike morphology often vary independently depending on genotype and environmental conditions [9-11]. Together, PC1 and PC2 explain 59.2% of the total variation, indicating that these two axes sufficiently summarize most of the multivariate structure of the dataset.

The PCA figure visually reinforces this interpretation by illustrating how each trait contributes to the first two components. The long arrows corresponding to seed weight per spike, seed number per spike, spikelet number, thousand-seed weight, and plant height show their strong influence on PC1 and confirm that these traits collectively define the primary axis of variation. Similar clustering of yield-related traits has been observed in several wheat PCA studies, where yield potential is closely associated with grain number and grain weight components [2-7]. In contrast, spike length and seed yield point strongly toward PC2, suggesting that spike structural attributes play an important role in differentiating genotypes along this secondary dimension. Meanwhile, the negative positioning of harvest index along PC2 illustrates its distinct behavior relative to spike morphology traits, consistent with earlier findings that biomass partitioning efficiency does not always parallel changes in spike structure [1]. Overall, the PCA figure shows that wheat yield is positioned closer to spike-related traits, indicating that spike morphology contributes meaningfully to yield variation in this dataset. Both the table and the figure together demonstrate that wheat yield variability arises from two major biological dimensions: one associated with grain production capacity and fertility traits, and another linked to spike architecture and biomass allocation patterns. These findings align well with established literature emphasizing the combined influence of grain number, grain weight, and spike morphology in shaping wheat yield.

The results of the PCA clearly demonstrate that wheat grain yield is mainly governed by a core group of interrelated traits that define the crop's productivity potential. Among these, seed weight per spike, seed number per spike, spikelet number, thousand-seed weight, and plant height emerged as the most influential components, strongly shaping the primary axis of variation (PC1). These traits collectively represent the fundamental physiological processes of spike fertility, grain formation, and biomass production, which are widely recognized as the main drivers of yield in wheat. The secondary axis (PC2) highlighted the contrasting roles of spike length and harvest index, indicating that spike structural attributes and biomass allocation efficiency differentiate genotypes beyond basic productivity traits. Overall, the multivariate analysis confirms that wheat yield variation is largely determined by the combined influence of grain number, grain weight, and spike morphology, with seed weight per spike and seed number per spike appearing as the most critical traits for yield improvement. These findings are consistent with previous studies emphasizing that yield potential is maximized when both grain number and grain weight are optimized through effective spike development and assimilate partitioning.

CONCLUSION

The integrated evaluation of wheat yield components through path analysis and PCA revealed that grain yield is primarily governed by two critical physiological determinants: seed weight per spike and seed number per spike. Path analysis clearly showed that seed weight per spike exerts the strongest positive direct effect on grain yield, demonstrating that enhanced assimilate accumulation in the spike is a decisive factor in achieving high productivity. In contrast, seed number per spike displayed a negative direct but a strong positive indirect effect, reflecting the well-known trade-off between grain number and

grain weight. This compensation mechanism highlights that increasing grain number alone is insufficient unless supported by adequate resource allocation and grain filling capacity. Secondary traits, including plant height, thousand-seed weight, and harvest index, contributed to yield mainly through indirect pathways, indicating that their influence is dependent on their interactions with primary yield components. Spike morphological traits such as spike length and spikelet number per spike had minimal direct impacts, confirming that structural characteristics alone do not drive yield unless accompanied by strong fertility and assimilate distribution traits. PCA supported these findings by demonstrating that the major axis of multivariate variation (PC1)—representing 44.1% of total variance—is dominated by seed weight per spike, seed number per spike, spikelet number, thousand-seed weight, and plant height. These traits collectively describe the core yield-forming processes related to spike fertility and grain development. The second component (PC2) emphasized the contrasting contributions of spike length and harvest index, indicating an additional dimension of variability driven by spike structure and biomass allocation efficiency.

Overall, the combined analyses confirm that seed weight per spike and seed number per spike are the most influential and biologically meaningful determinants of grain yield in wheat, while traits such as thousand-seed weight, plant height, and harvest index provide complementary support through their indirect effects. These findings highlight the necessity of breeding strategies that optimize both grain number and grain weight simultaneously, ensuring efficient assimilate partitioning and robust spike fertility. By identifying and prioritizing these key components, wheat breeding programs can design superior ideotypes with enhanced and stable yield performance across environments.

This is the standard font (Times New Roman, 12-pt) and layout (single spacing) for the individual paragraphs. The complete paper has to be written preferably in Rich Text Format or in an MS-Word 2003 compatible file, with a maximum of 30 pages including text, figures, and tables. Page size: A4, margins: 3 cm on each side, line spacing: single, font type: Times New Roman.

In the text, references should be cited in by number as [1]. If you need more than one citation, you should place the references as [2, 3, 4]. If you need to cite without parenthesis, use this example: As previously reported by Alexandrova et al. [5].

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