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Clustering Wheat (*Triticum aestivum* L.) Varieties Based on Agronomic Traits: A Key Approach for Breeding High-Yielding Cultivars under Rainfed Conditions

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ABSTRACT

Wheat is a vital crop for global food security, particularly in regions with unpredictable rainfall, such as Iran. Understanding the factors that influence wheat yield under adverse conditions is essential for developing effective management strategies to ensure sustainable productivity. Clustering analysis is a valuable tool for identifying distinct groups of wheat genotypes with similar agronomic traits, enabling targeted breeding for improved yield and adaptability, especially under rainfed conditions. This study evaluated seven wheat varieties (Nafis, Dehghan, Sadra, Kamal, Hashtrood, Baran, and Varan) to identify key agronomic traits influencing grain yield under such conditions. Traits measured included Shoot length (LSHOOT), the number of seeds (Nseed), thousand-grain weight (Wseed), number of spikes (Nspike), harvest index (HI), leaf area (LA), photosynthesis (Ps), vegetative stage (Veg), booting stage (BOOT), flowering stage (FLOW), grain filling period (Gfill), biological yield (Ybio), and grain yield (Ygrain). The clustering analysis revealed four distinct clusters based on these agronomic traits. Cluster 1 included Kamal and Varan, which shared strong similarities in harvest index and growth period. Cluster 2 consisted of Hashtrood and Sadra, exhibiting similar phenological traits such as stem elongation and booting dates. Cluster 3 was comprised solely of Baran, which demonstrated unique yield components distinguishing it from the other varieties. Finally, Cluster 4 grouped Dehghan and Nafis, which showed greater variability in their yield-related and phenological traits, indicating the need for more customized management practices. The results of the clustering analysis also highlighted two primary trait groups. The first group, including number of spikes (Nspike), thousand-grain weight (Wseed), photosynthesis (Ps), tillering stage (Till), and flowering stage (Flow), is associated with early growth and development stages. The second group, comprising grain yield (Ygrain), biological yield (Ybio), and leaf area (LA), is more closely related to the final yield outcome. These findings emphasize the importance of selecting wheat cultivars based on specific agronomic traits to enhance yield potential under rainfed conditions and underscore the potential for improving wheat production through targeted breeding strategies.

Keywords: Wheat, Rainfed conditions, Agronomic traits, Grain yield, biological yield, clustering

Introduction

Wheat (Triticum aestivum L.), a vital member of the Poaceae family, is one of the world's most important cereal crops, playing a central role in global food security. It provides approximately 36% of the global population's food and contributes around 20% of total caloric intake [1]. In arid and semi-arid regions such as

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Iran, wheat yield is highly variable due to unpredictable and low rainfall. This variability poses a significant challenge to breeders, as improving yield under such fluctuating environmental conditions requires the selection of genotypes with stable and high performance across years [2] Wheat production in dryland areas is not only vital for ensuring national food security in Iran but also holds significant potential for increasing agricultural productivity. Given the prevalence of drought and the expansive area under rainfed wheat cultivation, the development and introduction of drought-tolerant, high-yielding varieties is a priority. Field evaluation of these new cultivars under farmers' conditions is essential to assess their adaptability and yield stability. This participatory approach helps farmers recognize the potential of improved lines and enables them to choose cultivars that outperform traditional varieties, thereby facilitating rapid dissemination of improved genotypes and increasing adoption of research outputs [3]. Grain yield in wheat is a complex trait affected by numerous morphological and physiological characteristics. Among these, spikes per square meter, 1000-grain weight, and plant height are the most influential, explaining up to 75% of the variation in grain yield [4]. Furthermore, thousand-grain weight, number of grains per spike, and effective tiller number are considered the primary yield components [1]. Other agronomic traits, including flowering date, spike length, and plant height, also contribute to yield indirectly through their interaction with these key components. According to Marino and Alvino 2021[5], yield in wheat is determined through a sequence of developmental stages, with early traits such as tiller formation and spike development influencing final yield components like grain number and grain weight. Therefore, understanding the variation and relationship among these traits is critical for designing effective breeding strategies, especially under water-limited conditions. Cluster analysis is a powerful statistical tool widely used in plant breeding to classify genotypes based on multiple traits simultaneously. It facilitates the identification of genetically distinct groups, allowing breeders to select promising genotypes that exhibit desirable combinations of traits. Tavana and Saba (2017) [2] reported significant genetic diversity among wheat lines in terms of both phenological and agronomic traits. Their cluster analysis revealed that genotypes in the first phenological cluster and second agronomic cluster—characterized by early flowering, greater height, longer awns, and higher biomass—were associated with superior yield-related traits, such as more spikes per plant, increased grain number per spike, and higher thousand-grain weight. These traits, due to their ease of evaluation and relatively high heritability, were recommended for indirect selection under dryland conditions. Similarly, Ghasemi et al. (2023) [6]., in their study on 42 rainfed wheat genotypes, found significant variation in grain yield and related traits. Cluster analysis divided the genotypes into four distinct groups and identified superior lines that could be used to improve grain yield and spike characteristics. In their study, genotypes in cluster II were recommended for grain yield improvement, while those in cluster IV were suitable for enhancing spike-related traits. Given the need to breed for both high yield and early maturity in drought-prone environments, identifying genotypes that combine these traits is essential. Early-maturing genotypes are valuable in dryland areas, as they escape terminal drought, though they may sometimes have lower yield potential compared to longer-duration genotypes [2]. Thus, balancing earliness with yield potential remains a key objective in breeding programs.

Considering the importance of identifying drought-tolerant and high-yielding wheat genotypes for rainfed regions, the present study aims to classify a set of wheat lines using cluster analysis based on key phenological and agronomic traits. The goal is to identify superior groups of genotypes that can serve as promising candidates for cultivation under dryland conditions or as parental lines in future breeding programs

Materials and Methods

Study Location and Conditions

The experiment was conducted at the Shirvan Dryland Agricultural Research Station in North Khorasan Province, Iran (37°24'N, 57°55'E; 1100 m above sea level). The region has a semi-arid climate, with an average annual temperature of 14°C and annual precipitation of 300 mm, primarily occurring in winter and spring. Rainfall during the growing season ranged from 4 mm to 80 mm, peaking in May. Frost was common in winter but ceased after April. The soil at the experimental site is classified as clay loam, with a slightly alkaline pH of 7.8, organic carbon content of 0.85%, and available nitrogen of 0.08%. These environmental and soil conditions underscore the need for effective water and nutrient management in wheat production under rainfed conditions.

Experimental Design

Seven wheat cultivars were selected based on their adaptability to rainfed conditions and specific agronomic traits. The spring wheat cultivars, Nafis and Dehghan, are early-maturing and drought-tolerant, with thousand-grain weights of 37 g and 31 g, respectively. Nafis exhibits resistance to yellow and brown rust, while Dehghan demonstrates resistance to terminal heat stress. The winter wheat cultivars—Sadra, Kamal, Hashtrood, Baran, and Varan—display diverse traits. Sadra is adaptable and stable in yield but moderately susceptible to yellow rust. Kamal is early-maturing and resistant to drought and cold but moderately prone to lodging.

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Hashtrood is well-suited to cold and semi-arid regions, with moderate resistance to yellow rust. Baran, recognized for its excellent baking quality, is highly resistant to cold, drought, lodging, and grain shattering. Varan is early-maturing and resistant to both cold and drought. The thousand-grain weights of the winter cultivars ranged from 35 g to 40 g.

Traits Measured

Growth and development were monitored at key stages—emergence, tillering, stem elongation, flowering, and grain filling—using the Zadoks growth scale. Important traits were measured at specific stages. Chlorophyll content, indicating photosynthetic activity, was recorded at the flag leaf stage with a SPAD-502 meter. Leaf area (LA), reflecting canopy size and light use efficiency, was measured at flowering using a LI-COR 3100C. Shoot length was measured to determine plant height. Grain yield was calculated from the central rows of each plot and adjusted to 12% moisture. Other traits measured included number of grains per spike, thousand-grain weight, biological yield, and harvest index. These findings help identify wheat cultivars suitable for rainfed and stress-affected environments.

Statistical Analyses

Hierarchical clustering analysis was conducted in R using the cluster and pvclust packages to classify wheat cultivars based on their measured traits. The Euclidean distance metric was used to calculate pairwise dissimilarities between cultivars, and clusters were formed using Ward's minimum variance method to minimize within-cluster variation. The pvclust package was employed to assess the robustness of the clustering results through multiscale bootstrap resampling, providing both approximately unbiased (AU) p-values and bootstrap probabilities (BP) for each cluster. These values were used to determine the statistical support for the identified groupings, with AU values above 95% indicating highly reliable clusters.

Results and Discussion

Clustering Results of Agronomic Traits of Seven Varieties

Hierarchical clustering analysis was conducted to identify relationships among the agronomic traits of seven wheat varieties. The analysis grouped the traits into two primary clusters based on their similarity and dissimilarity, as determined by Euclidean distances and using Ward's method. The clustering results were further validated by Approximately Unbiased (AU) and Bootstrap Probability (BP) p-values.

The first cluster comprised traits such as Number of Spikes (Nspike), Thousand-Grain Weight (Wseed), Photosynthesis (Ps), Tillering Stage (Till), and Flowering Stage (Flow). These traits displayed strong internal similarity, with sub-clusters supported by AU p-values of 100%, underscoring their robust associations. For instance, Wseed and Nspike formed a highly stable sub-cluster, while Ps, Till, and Flow grouped together to reflect their roles in physiological and phenological processes contributing to yield. These traits primarily represent intermediate physiological and developmental factors influencing productivity.

The second cluster included Grain Yield (Ygrain), Biological Yield (Ybio), and Leaf Area (LA). Traits in this cluster showed high interdependence, with AU p-values of 97% and 95%. This strong association emphasizes the critical roles of photosynthetic capacity and canopy size in determining yield potential.

These traits serve as direct indicators of productivity and are fundamental to yield improvement.The distinction between the two clusters is evidenced by the greater height at which they merge in the dendrogram, reflecting differences in their functional roles. Traits in the second cluster, such as Ygrain and Ybio, are direct targets for breeding programs due to their immediate impact on yield. In contrast, traits in the first cluster, such as Ps and Nspike, indirectly influence productivity and are considered supporting traits.

These results provide actionable insights for breeding strategies. Optimizing traits in the first cluster can enhance the performance of primary productivity traits in the second cluster. The stability of sub-clusters, such as the pairing of Wseed and Nspike, indicates their potential for simultaneous selection to achieve yield improvement. This study highlights the importance of integrating physiological, developmental, and output traits to improve wheat productivity under semi-arid, rainfed conditions.

Clustering Results of Wheat Varieties Based on Agronomic Traits

Clustering analysis of wheat varieties revealed significant relationships based on their agronomic traits, identifying several key clusters with varying levels of statistical support.

Cluster 1: Kamal (K) and Varan (V) Kamal and Varan formed a highly stable cluster, with an AU p-value of 99%. These varieties exhibited strong similarities in traits such as harvest index and growth period. The consistency of these traits across bootstrap samples highlights the robustness of this cluster.

Cluster 2: Hashtrood (H) and Sadra (S) Hashtrood and Sadra were grouped with an AU p-value of 99%, reflecting a stable relationship. Their similarities in phenological traits, such as stem elongation and booting dates, suggest shared developmental cycles, making them suitable for similar growing conditions and management practices.

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Cluster 3: Baran (B) Baran formed a distinct cluster with an AU p-value of 82%, indicating moderate support. While Baran exhibited some similarities to Kamal and Varan in growth traits, its unique yield components distinguished it from other varieties. These traits provide opportunities for specific breeding objectives.

Cluster 4: Dehghan (D) and Nafis (N) Dehghan and Nafis were grouped with a relatively lower AU p-value of 73%, indicating less stability in this cluster. This variability suggests greater differences in their yield-related and phenological traits, requiring tailored management practices to optimize performance.

The dendrogram's y-axis, representing Euclidean distances, illustrates the degree of dissimilarity among varieties. Smaller merging heights, as seen with Kamal and Varan, indicate greater similarity, while larger merging heights, such as for Dehghan and Nafis, reflect greater dissimilarity.

Implications for Breeding and Management These findings provide insights into the relationships among wheat varieties. Kamal and Varan, as well as Hashtrood and Sadra, exhibit strong similarities in growth and phenological traits, making them suitable candidates for breeding programs targeting specific environmental conditions, such as drought or temperature stress. Baran's distinct traits add diversity to breeding objectives, while the variability of Dehghan and Nafis highlights the need for customized agronomic practices.

In conclusion, hierarchical clustering analysis offers valuable insights into varietal relationships. Strongly supported clusters identify varieties with consistent traits, which are advantageous for breeding and management. Weaker clusters highlight areas for further investigation. This analysis aids breeders and agronomists in making informed decisions to enhance wheat production under diverse.

The cluster analysis of seven wheat cultivars revealed meaningful groupings based on agronomic traits, providing insight into their performance under rainfed conditions and their potential utility in breeding programs. These groupings, supported by statistical measures, also align with biological and agronomic interpretations, illustrating how specific traits influence yield and adaptation to environmental stress.

Cluster I, which included Kamal and Varan, demonstrated high similarity (AU = 99%) in adaptive traits such as early maturity, high harvest index (HI), and strong drought resilience. Both cultivars also produced high biological yield, indicating efficient biomass partitioning under limited water availability. Their strong performance under drought is likely the result of a drought escape mechanism, where early phenological development allows them to complete their life cycle before the onset of terminal drought stress. (Tavana and Saba ,2017[2] noted that early-maturing genotypes tend to perform better under dry conditions due to their ability to avoid stress during sensitive growth stages. This explains why Kamal and Varan are particularly well-suited to water-limited environments, and how their combination of earliness and biomass efficiency can be used in breeding programs to stabilize grain yield in unpredictable climates.

Cluster II, consisting of Hashtrood and Sadra (AU = 99%), showed close similarity in phenological traits such as stem elongation and booting dates. These cultivars had a longer growth period and balanced grain and biomass yield, suggesting a different strategy from Cluster I. Their yield potential may be attributed to extended grain filling duration and greater assimilate accumulation, which enhances both grain weight and straw output. In semi-arid environments where both grain and straw are important, these cultivars offer dual benefits. This illustrates how phenological plasticity and moderate maturity can support yield stability and adaptability across a range of conditions.

Cluster III was represented by Baran alone (AU = 82%), with distinct characteristics including superior cold tolerance, good grain quality, and resistance to lodging. While Baran shares some growth traits with Cluster I cultivars, its unique qualities set it apart. Its adaptability to cold and drought conditions makes it a strong candidate for environments with multiple abiotic stresses. The separation of Baran from other cultivars suggests that different physiological or genetic mechanisms, such as stronger stem structure or better stress signaling pathways, may be involved. This highlights how such distinct genotypes can serve as specialized parents in breeding programs aiming for quality improvement and stress resilience.

Cluster IV included Dehghan and Nafis, with lower statistical support (AU = 73%), indicating greater trait variability. These cultivars exhibited broad adaptability but inconsistent yield performance. Their variability suggests a strong genotype-by-environment interaction, meaning their performance may depend more on specific management practices. Adjusting planting dates, nutrient management, or irrigation schedules could help optimize their yield potential. This highlights the importance of site-specific agronomy in maximizing the productivity of cultivars with broader but less predictable adaptation.

Earliness remains a key trait in dryland breeding because it helps crops escape late-season drought. However, as Tavana and Saba (2017) [2] pointed out, early-maturing cultivars may sacrifice yield potential due to shorter grain filling periods. This trade-off between drought escape and maximum yield potential must be considered in breeding programs, especially when targeting environments with variable rainfall patterns. In contrast, longer-duration cultivars may yield more in favorable seasons but are more vulnerable to terminal drought.

Rahmati et al. (2022) [3] emphasized that selecting early-maturing genotypes with high yield under rainfed conditions is essential.

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The cultivars in Cluster II, which combine reasonable maturity with yield stability, may serve as promising options for planting in dryland areas or for use as parents in breeding programs.
Additionally, key yield components such as thousand-grain weight, number of grains per spike, and effective tillering—identified as critical traits by Soleymanifard et al. (2012) and Zhuang et al. 2024[1]—were found to be closely associated with high-yielding cultivars in this study. These traits help explain the grouping patterns and performance outcomes of the analyzed cultivars.

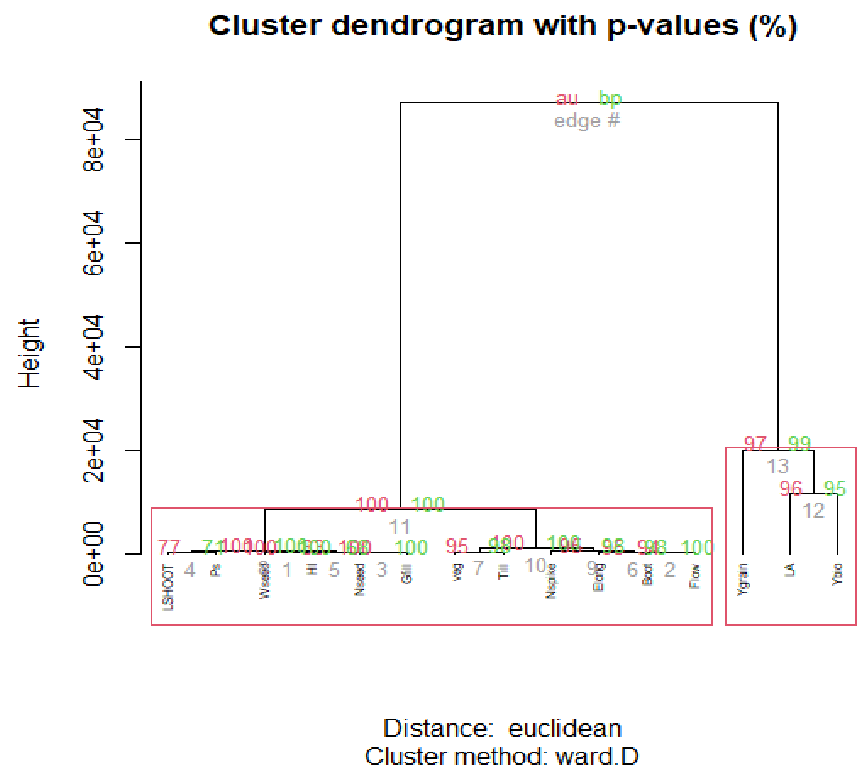

Figure 1. Cluster Analysis of Agronomic Traits in Seven Wheat Varieties

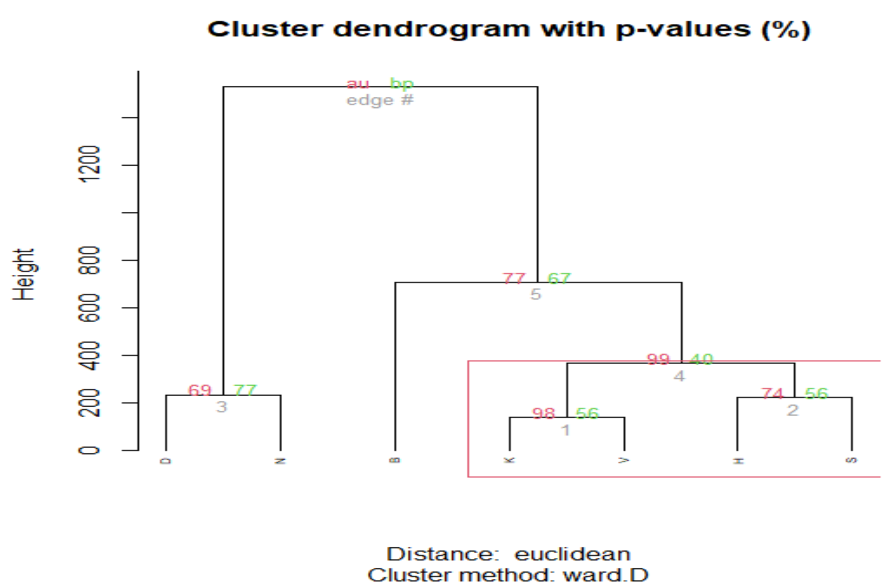

Figure 2. Cluster Analysis of of Wheat Varieties Based on Agronomic Traits (Dehghan(D), N(nafis), Baran(B),K (Kamal) , V(Varan), H(hashtrood), S(sadra))

Conclusion

The results emphasize that evaluating wheat cultivars based on measurable agronomic traits, such as grain yield, biomass, and thousand-grain weight, can guide the selection of varieties most suited to specific growing environments. Kamal and Varan are recommended for environments with limited water availability, while Hashtrood and Sadra are better suited for multi-output systems where both grain and biomass are important. Baran's unique traits make it a good candidate for grain quality improvement. The study also highlights the importance of using agronomic traits like leaf area and photosynthesis to optimize wheat yield potential across diverse growing conditions

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