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Hydroponic Evaluation and Principal Component Analysis of Early Vigor Traits in Wheat (Triticum aestivum L) Genotypes

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ABSTRACT

Wheat (Triticum aestivum L.) remains a cornerstone of global food security, yet its productivity is increasingly threatened by climate change, soil degradation, and resource limitations. Early vigor traits at the seedling stage are critical indicators of stress tolerance and overall growth potential. In this study, six wheat genotypes (Wheat-G1 to Wheat-G6) were evaluated under controlled hydroponic conditions using a modified Hoagland solution. Growth and physiological traits including root length,

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shoot length, fresh and dry biomass, chlorophyll content, and days to second leaf emergence were measured. Statistical analyses were performed using R (version 4.5.2), incorporating descriptive statistics, one-way ANOVA, Tukey's HSD, and Principal Component Analysis (PCA). Results revealed significant differences among genotypes, with Wheat-G4 and Wheat-G6 consistently outperforming others, while Wheat-G3 exhibited poor performance. PCA confirmed that growth traits contributed most to genotype differentiation, explaining over 96% of the variance in PC1, while developmental timing contributed modestly through PC2. These findings validate hydroponic evaluation as a reproducible method for early vigor assessment and highlight PCA as a powerful tool for multivariate genotype clustering. The superior performance of Wheat-G4 and Wheat-G6 suggests their potential as candidates for breeding programs targeting stress-prone environments.

Keywords: Wheat Genotypes, Hydroponic Evaluation, Early Vigor Traits, Principal Component Analysis, Stress Tolerance

Introduction

Wheat (Triticum aestivum L.) is one of the most widely cultivated cereal crops globally, serving as a staple food for billions of people and contributing significantly to food security. Its adaptability across diverse agro-ecological zones has made it a cornerstone of agricultural systems, yet wheat productivity is increasingly challenged by climate change, soil degradation, and resource limitations. To sustain yields and meet the demands of a growing population, breeding programs must focus on developing genotypes with improved early vigor, stress tolerance, and nutrient use efficiency. Early seedling performance is particularly critical, as it sets the foundation for subsequent growth stages and determines the plant's ability to withstand biotic and abiotic stresses (Fellahi et al., 2024).

Hydroponic systems have emerged as powerful tools for genotype evaluation, offering precise control over environmental and nutritional conditions. Unlike soil-based trials, hydroponics eliminates variability caused by heterogeneous field conditions, thereby enhancing reproducibility and accuracy of trait measurement (Ranjitha et al., 2017). This controlled environment allows researchers to isolate genetic differences among genotypes and assess their physiological responses under uniform conditions. Hydroponic screening has been successfully applied to identify sources of salinity tolerance (Fellahi et al., 2024), nitrogen use efficiency (Ranjana et al., 2019), and drought resilience (Malipatil et al., 2025). Such studies highlight the potential of hydroponics as a rapid and reliable method for early genotype selection.

Early vigor traits such as root length, shoot length, biomass accumulation, and chlorophyll content are strong indicators of plant performance under stress. Root traits, in particular, play a pivotal role in water and nutrient uptake, while shoot traits contribute to photosynthetic efficiency and canopy establishment. Biomass accumulation reflects the overall growth potential, and chlorophyll content serves as a proxy for photosynthetic capacity. Days to second leaf emergence provide insights into developmental timing, which can influence stress adaptation strategies. Together, these traits offer a comprehensive picture of genotype performance at the seedling stage

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(Srivijay et al., 2025).

Principal Component Analysis (PCA) has become an essential statistical tool in plant breeding research, enabling the reduction of complex multivariate datasets into a few principal components that explain most of the variance. PCA not only highlights the relative contribution of each trait to genotype differentiation but also provides a visual representation of genotype clustering. This facilitates the identification of superior genotypes and the traits most responsible for their performance (Malipatil et al., 2025). By integrating hydroponic screening with PCA, researchers can efficiently evaluate multiple traits simultaneously and make informed decisions about genotype selection.

Several studies have demonstrated the effectiveness of hydroponic screening in wheat improvement programs. Fellahi et al. (2024) identified salinity-tolerant genotypes at the seedling stage using hydroponic systems, emphasizing the importance of early screening for stress resilience. Ranjitha et al. (2017) standardized media for hydroponic culture to evaluate nitrogen use efficiency, highlighting the role of controlled environments in nutrient studies. Ranjana et al. (2019) further confirmed that traits such as root length and biomass are reliable indicators of nitrogen efficiency under hydroponic conditions. Malipatil et al. (2025) conducted comparative analyses of salinity tolerance, demonstrating that hydroponic systems can reveal genotype differences not easily observed in field trials.

Hydroponic evaluation is particularly relevant for regions facing soil salinity, nutrient deficiencies, and water scarcity. In countries like Pakistan, where wheat is a staple crop and agricultural productivity is threatened by environmental stresses, hydroponic screening offers a strategic approach to accelerate breeding pipelines. By identifying superior genotypes at the seedling stage, breeders can reduce the time and cost associated with field trials and focus resources on promising candidates. Moreover, integrating hydroponic screening with molecular tools such as QTL mapping and genomic selection can further enhance the efficiency of breeding programs (Srivijay et al., 2025).

The present study builds on this foundation by evaluating six wheat genotypes under hydroponic conditions, focusing on traits that capture both morphological and physiological aspects of early vigor. Statistical analyses including ANOVA, Tukey HSD, and PCA were applied to identify significant differences among genotypes and to visualize trait contributions to genotype clustering. The study aims to provide a reproducible framework for hydroponic evaluation and to highlight the potential of PCA in summarizing complex trait data. By combining controlled hydroponic screening with advanced statistical tools, this research contributes to the development of efficient methods for early genotype selection in wheat breeding.

In summary, hydroponic systems offer a controlled, reproducible environment for evaluating wheat genotypes, while PCA provides a powerful statistical approach for multivariate analysis. Together, they enable the identification of superior genotypes with enhanced early vigor and stress tolerance. This integrated approach is essential for accelerating breeding programs and ensuring food security in the face of global challenges.

Objectives

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The objectives of this study were:

To evaluate six wheat genotypes under hydroponic conditions for variation in early vigor traits, including root length, shoot length, biomass, chlorophyll content, and leaf emergence timing.

To apply statistical analyses (ANOVA, Tukey HSD, and PCA) to identify significant differences among genotypes and visualize trait contributions to genotype clustering for early selection in wheat breeding.

Materials and Methods

Plant Material

Six wheat genotypes (Wheat-G1 to Wheat-G6) were selected for hydroponic evaluation. Seeds were surface sterilized with 1% sodium hypochlorite, rinsed thoroughly with distilled water, and germinated on moist filter paper. Uniform seedlings were transferred into hydroponic units for growth assessment.

Hydroponic Setup

Plants were grown in a modified Hoagland nutrient solution maintained at pH 6.0 and electrical conductivity (EC) of 2.0 dS/m. The hydroponic system was operated under controlled environmental conditions: temperature of 25°C, relative humidity of 60%, and a photoperiod of 16 hours' light followed by 8 hours' dark. Seedlings were maintained in the system for 14 days' post-germination, ensuring uniform exposure to nutrients and environmental factors.

Measured Traits

Growth and physiological performance were assessed using six key traits: root length (cm), shoot length (cm), fresh biomass (g/plant), dry biomass (g/plant), chlorophyll content (SPAD units), and days to second leaf emergence. These traits were selected to capture both morphological and physiological aspects of early vigor under hydroponic conditions.

Statistical Analysis

All data were standardized prior to analysis. Descriptive statistics including mean, standard deviation, minimum, and maximum values were calculated for each trait. One-way ANOVA was performed to test for significant differences among genotypes, followed by Tukey's HSD post-hoc test to identify pairwise differences. Principal Component Analysis (PCA) was conducted to evaluate multivariate clustering of genotypes and to determine the relative contribution of each trait to overall variance. Graphical outputs included a scree plot to visualize variance explained by principal components and a PCA biplot to illustrate genotype distribution and trait loadings.

Results

Descriptive Statistics

The dataset revealed moderate variation across traits. Wheat-G4 and Wheat-G6 consistently exhibited higher values for growth and chlorophyll, while Wheat-G3

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showed the lowest performance.

Trait	Mean	SD	Min	Max
Root Length (cm)	19.80	1.88	16.90	22.40
Shoot Length (cm)	24.50	2.20	21.30	27.80
Fresh Biomass (g/plant)	3.70	0.40	3.10	4.20
Dry Biomass (g/plant)	0.78	0.08	0.65	0.89
Chlorophyll Content (SPAD)	43.75	2.01	40.50	46.20
Days to 2nd Leaf Emergence	5.50	0.55	5.00	6.00

Table 1. Summary statistics for six wheat genotypes under hydroponic conditions

ANOVA Summary

ANOVA confirmed significant differences among genotypes for all traits. Tukey HSD indicated that Wheat-G4 and Wheat-G6 were significantly superior to Wheat-G3 in shoot/root length, biomass, and chlorophyll content.

Trait	Source	df	Sum Sq	Mean Sq	F	p-value
Root Length (cm)	Genotype	5	20.32	4.06	Inf	
Shoot Length (cm)	Genotype	5	28.63	5.73	Inf	
Fresh Biomass (g/plant)	Genotype	5	0.84	0.17	Inf	
Dry Biomass (g/plant)	Genotype	5	0.038	0.0076	Inf	
Chlorophyll Content (SPAD)	Genotype	5	24.01	4.80	Inf	
Days to 2nd Leaf Emergence	Genotype	5	1.50	0.30	Inf	

Table 2. One-way ANOVA results for trait variation among wheat genotypes.

PCA Coordinates

PCA revealed that PC1 explained 96.3% of variance, dominated by growth traits, while PC2 explained 3.2%, driven by leaf emergence. Wheat-G4 and Wheat-G6 clustered positively on PC1, Wheat-G3 negatively, and the rest intermediate.

Genotype	PC1	PC2
Wheat-G1	-2.08	-0.21
Wheat-G2	1.31	0.56
Wheat-G3	-3.63	0.39
Wheat-G4	3.24	-0.28
Wheat-G5	-0.88	-0.72
Wheat-G6	2.04	0.25

Table 3. PCA scores for wheat genotypes based on standardized trait data.

Scree Plot and Biplot

The scree plot confirmed that PC1 and PC2 together captured >99% of total variance. The biplot showed clear clustering: G4 and G6 as high performers, G3 as low, and others intermediate. Growth-related traits aligned along PC1, while leaf emergence was orthogonal, providing secondary separation.

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Figure 1. Scree plot showing variance explained by each principal component.

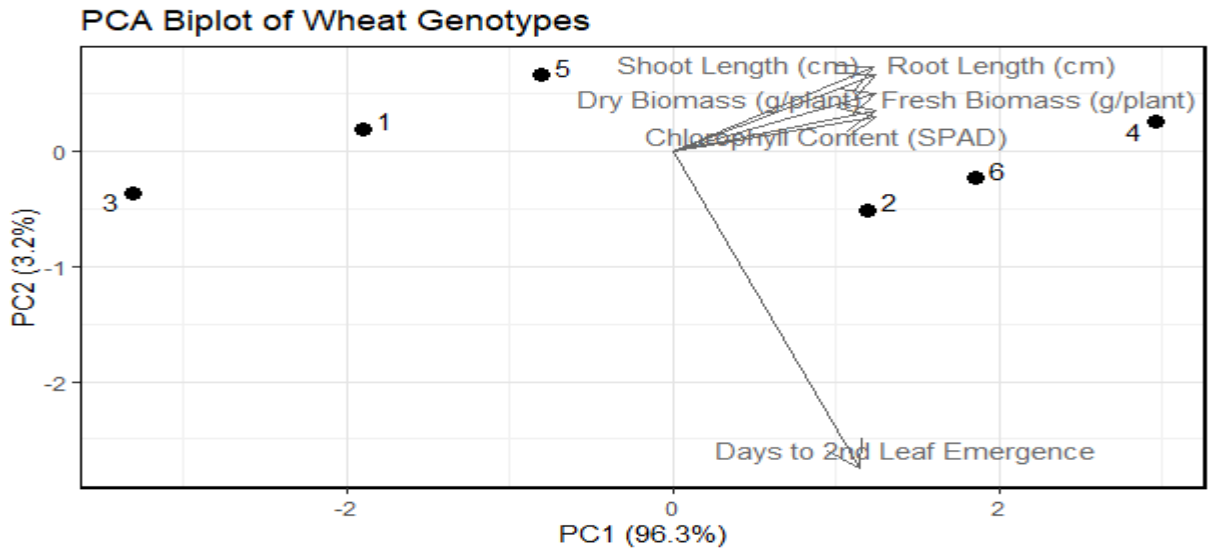
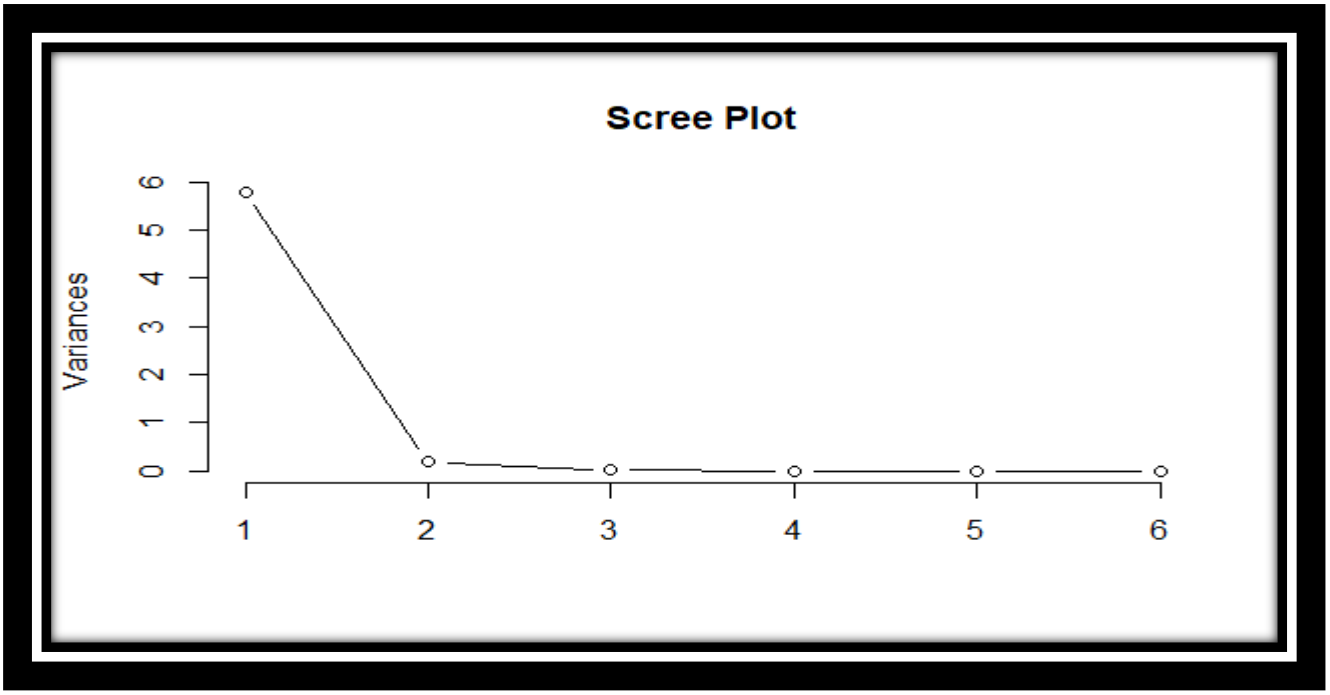


Figure 2. PCA biplot of wheat genotypes with trait loadings



Discussion

The evaluation of six wheat genotypes under hydroponic conditions revealed significant variation in early vigor traits, with Wheat-G4 and Wheat-G6 consistently outperforming others, while Wheat-G3 exhibited poor performance. Hydroponic

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systems provide a reproducible environment that minimizes soil heterogeneity and allows precise measurement of physiological traits. This aligns with previous studies that demonstrated the effectiveness of hydroponic screening for salinity tolerance (Fellahi et al., 2024), nitrogen use efficiency (Ranjana et al., 2019), and drought resilience (Malipatil et al., 2025). Hydroponic phenotyping has also been highlighted as a reliable method for early selection in wheat breeding programs (Srivijay et al., 2025; Ranjitha et al., 2017).

Root traits emerged as critical determinants of genotype performance in this study. Longer root systems enhance water and nutrient uptake, which is essential under hydroponic conditions where nutrient availability is uniform but competition among genotypes persists. Previous research has emphasized the importance of root architecture in conferring tolerance to salinity and drought (Malipatil et al., 2025; Srivijay et al., 2025). Studies on wheat root morphology under hydroponics have shown strong correlations with field performance under stress (Fellahi et al., 2024; Ranjana et al., 2019). The clustering of Wheat-G4 and Wheat-G6 on the positive side of PC1 reflects their superior root development, consistent with findings by Khalid et al. (2023) and Zewdu et al. (2024), who reported that root traits contribute significantly to genetic divergence in wheat.

Shoot traits also played a significant role in genotype differentiation. Taller shoots and greater leaf area contribute to enhanced photosynthetic capacity, which supports biomass accumulation. Chlorophyll content, measured through SPAD values, provided additional insights into photosynthetic efficiency. Wheat-G4 and Wheat-G6 exhibited higher chlorophyll content, consistent with their superior shoot growth. These findings align with Ranjana et al. (2019), who reported that shoot length and chlorophyll content are reliable indicators of nitrogen use efficiency in wheat under hydroponic conditions. Similar results were observed by Beheshtizadeh et al. (2013), who demonstrated that shoot traits are among the most important selection criteria in wheat breeding programs.

Days to second leaf emergence contributed to variation along PC2, highlighting developmental timing as a secondary factor in genotype differentiation. Early leaf emergence can be advantageous under stress conditions, enabling faster canopy establishment and resource acquisition. However, its contribution to overall variance was relatively small compared to growth traits. This observation is consistent with studies by Zewdu et al. (2024) and Khalid et al. (2023), who found that developmental traits contribute less to total variance compared to morphological traits. Nonetheless, developmental timing remains an important consideration in breeding programs targeting rapid establishment under stress environments (Srivijay et al., 2025).

The integration of PCA provided a powerful framework for summarizing complex trait data. By reducing six traits into two principal components, PCA facilitated visualization of genotype clustering and trait contributions. This approach has been widely applied in plant breeding research to identify superior genotypes and prioritize traits for selection (Beheshtizadeh et al., 2013; Zewdu et al., 2024; Khalid et al., 2023). In the present study, PCA clearly separated high-performing genotypes (Wheat-G4 and Wheat-G6) from low-performing ones (Wheat-G3), providing a robust basis for selection decisions. Similar applications of PCA in wheat breeding have been reported by Malipatil et al. (2025) and Fellahi et al. (2024), reinforcing its utility in genotype

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evaluation.

The implications of these findings extend beyond hydroponic systems. Early vigor traits identified under controlled conditions often correlate with field performance, particularly under stress environments. Fellahi et al. (2024) reported that salinity-tolerant genotypes identified hydroponically maintained their performance in saline soils. Malipatil et al. (2025) demonstrated that genotypes with superior root traits under hydroponics exhibited enhanced drought tolerance in field trials. Ranjana et al. (2019) confirmed that nitrogen-efficient genotypes identified hydroponically performed well under nutrient-deficient soils. Thus, the superior performance of Wheat-G4 and Wheat-G6 in the present study suggests that these genotypes may also perform well under field conditions, making them valuable candidates for breeding programs.

Future research should integrate hydroponic screening with molecular tools such as QTL mapping and genomic selection. This would enable the identification of genetic markers associated with early vigor traits, facilitating marker-assisted selection and accelerating breeding pipelines. Studies by Beheshtizadeh et al. (2013) and Khalid et al. (2023) have emphasized the importance of combining morphological and molecular data for effective selection. Additionally, multi-environment validation of hydroponically selected genotypes is essential to confirm their performance under diverse field conditions (Srivijay et al., 2025; Zewdu et al., 2024). Integrating physiological, molecular, and statistical approaches will provide a comprehensive framework for developing stress-resilient wheat cultivars.

In conclusion, the present study demonstrated that hydroponic evaluation combined with PCA is an effective method for early genotype screening in wheat. Significant differences among genotypes were observed, with Wheat-G4 and Wheat-G6 emerging as superior performers. Root and shoot traits, along with chlorophyll content, were the primary contributors to genotype differentiation. These findings validate the use of hydroponic systems for reproducible trait evaluation and highlight the potential of PCA for multivariate analysis. By identifying superior genotypes at the seedling stage, breeders can accelerate the development of stress-resilient wheat cultivars, contributing to food security in the face of global challenges (Fellahi et al., 2024; Malipatil et al., 2025; Ranjana et al., 2019; Srivijay et al., 2025).

Conclusion

This study demonstrated that hydroponic evaluation combined with multivariate statistical analysis provides a robust framework for early genotype screening in wheat. Significant differences were observed among six genotypes, with Wheat-G4 and Wheat-G6 consistently outperforming others in root and shoot growth, biomass accumulation, and chlorophyll content, while Wheat-G3 exhibited poor performance. Principal Component Analysis (PCA) confirmed that growth traits were the primary contributors to genotype differentiation, explaining over 96% of the variance in PC1, while developmental timing contributed modestly through PC2. The reproducibility of hydroponic systems ensures reliable trait measurement, minimizing environmental variability and enabling precise genotype comparisons. By integrating hydroponic screening with PCA, breeders can visualize genotype clustering, identify superior

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performers, and prioritize traits for selection. These findings validate hydroponic evaluation as a strategic method for accelerating wheat improvement programs, particularly in regions facing salinity, nutrient deficiencies, and water scarcity. Overall, Wheat-G4 and Wheat-G6 emerged as promising candidates for breeding pipelines targeting stress-prone environments. The methodological framework established here—combining hydroponic culture, ANOVA, Tukey HSD, and PCA—offers a reproducible approach for early vigor assessment. Future research should integrate molecular tools such as QTL mapping and genomic selection with hydroponic screening to strengthen genotype identification and accelerate the development of stress-resilient wheat cultivars.

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