# Genotype × seed size interaction reveals contrasting early vigor strategies in durum wheat (Triticum durum Desf.): implications for trait-based selection in Mediterranean environments

**BELAGROUZ Abdenour**

belagrouz.abdenour@ensf.dz

Higher National School of Forests, KHENCHELA

**BENDADA Hocine**

University of Relizane

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# Genotype × seed size interaction reveals contrasting early vigor strategies in durum wheat (Triticum durum Desf.): implications for trait-based selection in Mediterranean environments

BELAGROUZ Abdenour [1, *], BENDADA Hocine[2]

[1] *Lab of Algerian Forests and Climate Change, Higher National School of Forests, KHENCHELA, Algeria*
belagrouz.abdenour@ensf.dz, https://orcid.org/0000-0003-2534-4945
[2]*Department of Agricultural Sciences, University of Relizane, Relizane (48000), Algeria*
agro.hocine@gmail.com, https://orcid.org/0000-0002-8997-5933

* Corresponding author: *belagrouz.abdenour@ensf.dz*

## Abstract

This study investigated genotype × seed size interactions on early vigor in 25 Algerian durum wheat (Triticum durum Desf.) genotypes under controlled conditions. Seeds were classified into two size fractions (small: < 3.5 mm; large: > 3.5 mm) and germinated using the paper roll method with eight replicates per genotype × seed size combination (400 experimental units). Ten morpho-physiological traits were measured after nine days, including coleoptile length (CL), leaf area (LA), specific leaf area (SLA), root parameters (PRL, LRL, TRL, RN, RA), and chlorophyll content (Chl a and Chl b). Agglomerative hierarchical clustering (Ward's method, 1,000 bootstrap replicates) grouped genotypes into six distinct clusters. Two-way ANOVA revealed highly significant varietal group effects ($P < 0.001$) for all traits, while seed size significantly influenced LRL, TRL, RA, RN, and SLA. Critically, significant genotype × seed size interactions were detected for eight of ten traits, with notable reversals in specific genotypic backgrounds. Principal component analysis showed that the first three axes explained 67.3% of total variance. All traits exhibited high broad-sense heritability (78.7–97.3%), with Chl a ($H^2$ = 97.3%) and Chl b ($H^2$ = 95.7%) showing the highest values. High genetic advance as percentage of the mean was observed for Chl b (108.5%), Chl a (69.1%), and LA (47.4%), identifying chlorophyll content as the most promising selection target—surpassing traditionally prioritized morphological traits. The demonstrated independence between root and shoot vigor dimensions offers a framework for multi-trait selection strategies applicable across Mediterranean breeding programs.

**Keywords:** Early vigor · Genotype × seed size interaction · Heritability · Chlorophyll content · Mediterranean agriculture

## 1. Introduction

Durum wheat (*Triticum durum* Desf.) is a major cereal crop in Mediterranean agricultural systems, playing a central role in food security and economic development across the region (Belagrouz 2021; Quagliata et al. 2025). In semi-arid environments, this crop faces significant challenges associated with intensifying climatic constraints, including recurrent drought, increasing soil salinity, and rising temperatures (Royo et al. 2021; Berkane et al. 2023). These environmental stresses are particularly detrimental during the early stages of the crop cycle—germination, emergence, and initial seedling development—which are critical for successful crop establishment (Abdel-Ghani et al. 2015).

Improved seedling vigor has long been recognized as a key target for enhancing crop resilience under water-limited conditions (Rebetzke and Richards 1999; Blum 2017). Vigorous early growth enables seedlings to optimize the use of limited resources, particularly water, while rapid canopy development limits soil evaporation and reduces weed competition (Zhao et al. 2017; Aharon et al. 2021). Simultaneously, a robust and deep root system ensures improved uptake of essential nutrients, particularly nitrogen (López-Castañeda and Richards 1994; Liu et al. 2022; Wang et al. 2023; Odone et al. 2025). These benefits have driven increasing interest in trait-based selection for early vigor in wheat breeding programs worldwide (Bourgault et al. 2020; Gracia-Romero et al. 2022; Alahmad et al. 2022; Fang et al. 2024; Spada et al. 2024).

Among the factors influencing seedling vigor, seed size has received considerable attention. Large seeds generally produce more vigorous seedlings with higher germination rates and enhanced initial development, owing to greater nutrient reserves (Ambika et al. 2014; Shahwani et al. 2014; Basu and Groot 2023). However, most studies have treated seed size as a fixed covariate or have examined its effects independently of genotypic background. Few investigations have systematically quantified genotype × seed size interactions on seedling vigor traits in durum wheat, despite evidence suggesting that the magnitude and even the direction of seed size effects may vary depending on genotype (Snider et al. 2020). This oversight is significant, because if seed size effects are genotype-dependent, selection protocols that ignore this interaction risk misclassifying genotypic potential.

A second gap concerns the traits used to assess early vigor. Traditional screening protocols have predominantly focused on morphological traits such as coleoptile length and leaf area (Rebetzke et al. 2007; Bourgault et al. 2020). The relative contribution of physiological traits—particularly chlorophyll content—to genetic variation in seedling vigor remains poorly quantified in durum wheat. Estimating genetic parameters such as heritability, genotypic coefficient of variation, and genetic advance for both morphological and physiological traits is essential for determining which indicators offer the greatest selection efficiency (Johnson et al. 1955; Paleo et al. 2024). If physiological traits prove to be more heritable and genetically variable than morphological ones, this would have direct implications for the design of vigor screening protocols in breeding programs.

Furthermore, understanding the correlation structure among seedling traits is critical for multi-trait selection. If root and shoot vigor are independently regulated, breeders could select for specific adaptation strategies—root-focused for drought avoidance or shoot-focused for rapid canopy closure—without negative trade-offs. Conversely, strong correlations between trait dimensions would constrain selection options. This information is currently lacking for durum wheat germplasm adapted to North African Mediterranean conditions.

This study addresses these three gaps by (i) systematically quantifying genotype × seed size interactions across a comprehensive panel of 25 Algerian durum wheat genotypes and ten morpho-physiological traits, (ii) comparing the selection potential of physiological versus morphological vigor indicators through genetic parameter estimation, and (iii) characterizing the functional coordination among seedling trait dimensions to inform multi-trait selection strategies. By focusing on the interaction between seed size and genotypic background—rather than describing either factor in isolation—this work aims to provide insights applicable to trait-based selection protocols across Mediterranean breeding programs.

## 2. Materials and Methods

### *2.1. Study site and plant material*

The experimental work was conducted at the National Center for Seed and Plant Control and Certification (CNCC), Sidi Bel Abbès, Algeria, with complementary analyses performed at the Higher School of Agronomy, Mostaganem, Algeria. A diverse panel of 25 durum wheat (*Triticum durum* Desf.) genotypes (Table 1) was selected to encompass both traditional landraces, valued for their resilience and long-standing adaptation to harsh environments, and improved varieties developed for enhanced productivity and stress tolerance. Seeds were obtained from the germplasm collections of both institutions.

**Table 1**. List of 25 durum wheat varieties used in the experiment, including their names, genotype types, pedigrees, and origins.

| Code | Genotypes | Type/Pedigree | Origin |
|---|---|---|---|
| V1 | Beliouni | Landrace | Algeria |
| V2 | Djenah Khotifa | Landrace | North Africa |
| V3 | Hedba 3 | Landrace ( hedba3/GDVZ619) | Algeria |
| V4 | Langlois | Landrace | Algeria |
| V5 | Mohammed Ben Bachir | Landrace | Algeria |
| V6 | Montpellier | Landrace | Algeria |
| V7 | Oued Znatie | Landrace | Algeria |
| V8 | ACSAD | Gerardo-vz-469/3/Jori-1//Nd-61- | ACSAD |
| V9 | Altar84 | Ruff/Flamingo,mex//Mexicali | CIMMYT |
| V10 | Ciccio | Appulo/Valnova(f6)//(f5)Valforte/Patr | Italy |
| V11 | Cirta | Hedba-3/Gerardo-vz-619 | Algeria |
| V12 | Core | Platani/Gianni | Italy |
| V13 | GTA Dur | Crane/4/Polonicum | CIMMYT |
| V14 | INRAT 69 | Mahmoudi/(bd-2777)Kyperounda | Tunisia |
| V15 | Korifla | Durum-dwarf-s-15/Crane//Geier | ICARDA |
| V16 | Mansourah | Bread wheat/MBB | Algeria |
| V17 | Massinissa | Ofanto/Bousselem | Algeria |
| V18 | Polinicum | Triticum polinicum/Zenati boulette | France |
| V19 | Sahell 77 | Cit"s"/4/Tace/4*tc//2*zb/wls/3/aa"s''/ | CIMMYT |
| V20 | Simito | Capeiti-8/Valnova | Italy |
| V21 | Sitifis | Bousselam/Ofanto | Algeria |
| V22 | Vitron | Turkey77/3/Jori/Anhinga//Flamingo | Spain |
| V23 | Waha | Plc/Ruff//Gta's/3/Rolette | ICARDA |
| V24 | ZB×FG | Zb/fg''s'' lk/3/ko 120/4/Ward cs | Algeria |
| V25 | Ofanto | Ademelio/Appulo | Italy |

### *2.2. Experimental procedure*

The filter paper roll method was employed to grow seedlings under controlled conditions (Figure 1), following established protocols for assessing root and shoot traits during the early seedling stage (Liu et al. 2017; Zhao et al. 2017; Royo et al. 2021).


Figure 1**.** Preparing seeds for germination in the laboratory of higher school of agronomy (A), Paper rolls containing the seeds were placed vertically inside the germination chamber (B, C), general view of the seedlings after 9 days of sowing (D).

Seeds were sorted into two size fractions based on diameter: small seeds (< 3.5 mm) and large seeds (> 3.5 mm). Prior to germination, seeds were surface-sterilized with 1% sodium hypochlorite for 5 minutes, followed by three rinses with distilled water. Twenty seeds of each genotype and size fraction were placed on moistened filter paper rolls (30 × 40 cm) with deionized water. The rolls were placed vertically in plastic containers containing 2 cm of water to maintain consistent moisture throughout the experiment.

Germination was carried out in a growth chamber set at 20 °C with 60% relative humidity and a 16/8 h light/dark photoperiod (light intensity: 200 µmol $m^{-2}$ $s^{-1}$), simulating Mediterranean spring conditions. Paper rolls were inspected daily and remoistened as needed. Each genotype × seed size combination was replicated eight times in a completely randomized design (CRD), yielding a total of 400 experimental units (25 genotypes × 2 seed sizes × 8 replicates).

### *2.3. Data collection*

After nine days of germination, seedlings were evaluated for ten vigor-related traits organized into three categories:

*Root parameters:* total root length (TRL, cm), root spread angle (RA, °), number of seminal roots (RN), and lengths of primary (PRL, cm) and lateral roots (LRL, cm).

*Shoot parameters:* coleoptile length (CL, cm), leaf area (LA, $cm^2$), and specific leaf area (SLA, $cm^2/g$).

*Chlorophyll content:* Chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) were quantified following the method of Arnon (1949). Fresh leaf samples (0.1 g) were ground in 10 mL of 80% acetone and centrifuged at 10,000 rpm for 10 minutes. Absorbance was measured at 663 nm and 645 nm using a UV-VIS spectrophotometer. Concentrations were calculated using the equations: Chl *a* (µg/g FW) = $[12.7 \times A_{663} - 2.69 \times A_{645}] \times V / (1000 \times W)$; Chl *b* (µg/g FW) = $[22.9 \times A_{645} - 4.68 \times A_{663}] \times V / (1000 \times W)$, where V is the volume of extract (mL) and W is the fresh weight of leaf tissue (g).

### *2.4. Statistical analysis*

Data were analyzed using a combination of multivariate and univariate statistical methods. *Agglomerative hierarchical clustering (AHC)* was performed on standardized genotype means using Ward's minimum variance method (Ward 1963) and Euclidean distance, implemented in R software version 4.0.2 (R Core Team 2020). Cluster robustness was assessed by bootstrap resampling (1,000 replicates). The resulting clusters were subsequently used as consolidated varietal groups (VG) in further analyses.

*Two-way analysis of variance (ANOVA)* was conducted using the linear model

$Y_{ijk} = \mu + VG_i + SS_j + (VG \times SS)_{ij} + \varepsilon_{ijk}$, where VG is the varietal group effect, SS is the seed size effect, and VG × SS is their interaction. Mean comparisons were performed using Fisher's LSD test at $P < 0.05$.

*Principal component analysis (PCA)* was performed on standardized genotype means to explore multivariate relationships among traits and visualize genotype differentiation. A biplot was constructed on the first two principal components. Pearson correlation analysis was conducted to examine pairwise relationships among traits using genotype means (n = 25). Correlations were therefore computed on averaged genotypic values, and within-genotype variation was not considered in this analysis.

*Genetic parameters* were estimated as follows:

broad-sense heritability

$H^2 = \sigma^2g / (\sigma^2g + \sigma^2e/r)$, where $\sigma^2g = (MSgenotype – MSerror) / r$, $\sigma^2e = MSerror$, and r =16 observations per genotype (8 replicates × 2 seed size fractions, pooled across seed sizes) Genotypic (GCV) and phenotypic (PCV) coefficients of variation, genetic advance (GA) at 5% selection intensity (k = 2.06), and GA as percentage of the mean (GAM) were calculated following Johnson et al. (1955). Phenotypic diversity was assessed using the coefficient of variation (CV), Shannon diversity index (H′), and evenness (E).

It is acknowledged that the varietal groups used as factors in the two-way ANOVA were derived from agglomerative hierarchical clustering performed on the same trait data. Consequently, the significance of the VG main effect is partly expected by construction and should be interpreted with caution. The primary analytical value of the two-way ANOVA resides in (i) the quantification of the seed size effect, which is entirely independent of the clustering procedure, and (ii) the estimation of the VG × SS interaction, which captures differential genotypic responses to seed size that are not predetermined by the clustering algorithm. This analytical framework, in which cluster-derived groups serve as consolidated factors in subsequent ANOVA, has been widely employed in germplasm characterization studies (Hennig et al. 2015; Vassileva et al. 2023).

# 3. Results

## *3.1. Agglomerative hierarchical clustering*

The agglomerative hierarchical clustering of the 25 durum wheat genotypes based on ten morpho-physiological traits identified six distinct clusters (Figure 2). Bootstrap support values from 1,000 replicates confirmed the robustness of the major branches. Cluster 1 (G1) comprised two genotypes (V20 and V24) characterized by low root development but high SLA and chlorophyll content. Cluster 2 (G2) included three genotypes (V6, V7, and V15) with intermediate trait profiles. Cluster 3 (G3) contained four genotypes (V5, V8, V13, and V21) distinguished by high coleoptile length. Cluster 4 (G4) was the largest group with six genotypes (V1, V2, V11, V17, V18, and V19), showing intermediate to high root and chlorophyll values. Cluster 5 (G5) included five genotypes (V4, V9, V22, V23, and V25) with high leaf area. Cluster 6 (G6) comprised five genotypes (V3, V10, V12, V14, and V16) exhibiting the strongest root development across all root parameters.

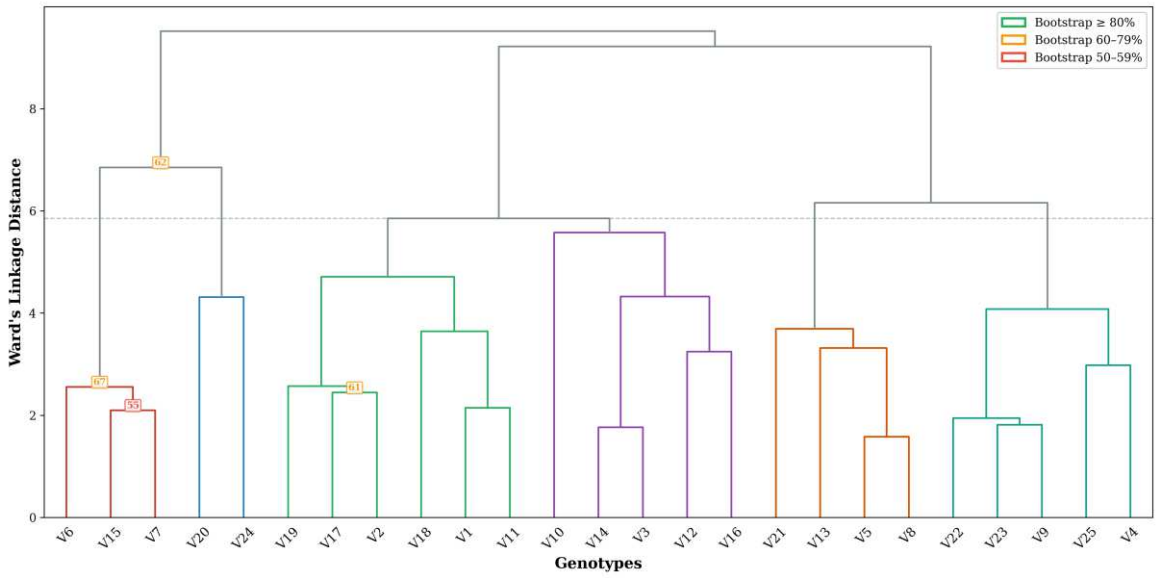


**Figure 2**. Agglomerative hierarchical clustering (AHC) of 25 durum wheat genotypes using Ward's method. Numbers at the nodes represent bootstrap support values based on 1,000 replicates.

## *3.2. Analysis of variance*

The two-way ANOVA revealed highly significant varietal group effects ($P < 0.001$) for all ten morpho-physiological traits (Table 2), confirming substantial genotypic differentiation for early vigor characteristics. The *F*-values for VG ranged from 6.11 (RN) to 51.99 (CL), with particularly strong effects observed for CL ($F = 52.0$), LA ($F = 51.8$), and TRL ($F = 31.4$).

The seed size (SS) effect was significant for five traits: LRL ($F = 18.50$, $P < 0.001$), TRL ($F = 24.50$, $P < 0.001$), RA ($F = 9.65$, $P < 0.01$), RN ($F = 8.11$, $P < 0.01$), and SLA ($F = 10.15$, $P < 0.01$). Conversely, seed size had no significant effect on CL, PRL, LA, Chl *a*, or Chl *b* ($P > 0.05$). The VG × SS interaction was significant for eight of the ten traits, with only CL and LA showing non-significant interactions.

**Table 2.** Two-way ANOVA F-values for ten early vigor traits in 25 durum wheat genotypes.

| Source | df | CL | PRL | LRL | RN | RA | LA | TRL | SLA | Chl a | Chl b |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **VG** | 5 | 52.0*** | 29.7*** | 23.6*** | 6.1*** | 11.4*** | 51.8*** | 31.4*** | 17.3*** | 24.3*** | 27.9*** |
| **SS** | 1 | 1.7 ns | 3.4 ns | 18.5*** | 8.1** | 9.7** | 0.3 ns | 24.5*** | 10.2** | 0.1 ns | 3.5 ns |
| **VG × SS** | 5 | 1.7 ns | 3.6** | 5.1*** | 3.6** | 5.3*** | 1.5 ns | 6.8*** | 3.2** | 3.7** | 7.0*** |
| **Error** | 388 | 0.786 | 8.875 | 6.253 | 0.229 | 288.2 | 0.736 | 212.8 | 5785 | 1.340 | 0.192 |

**VG: varietal group; SS: seed size. Last row shows MS Error. *** P < 0.001; ** P < 0.01; ns: not significant.**

### *3.3. Multivariate characterization of varietal groups*

The heatmap representation of standardized trait means across the six varietal groups (Figure 3) revealed distinct vigor profiles. Group G6 exhibited the highest values for root-related traits (PRL = 20.43 cm; LRL = 14.43 cm; TRL = 96.08 cm), indicating superior root system development. Group G5 showed the highest leaf area (LA = 4.35 cm²) and coleoptile length (CL = 4.62 cm), suggesting strong aerial vigor. Group G1, despite having the lowest root development (TRL = 67.33 cm), recorded the highest SLA (326.86 cm²/g), chlorophyll *a* (4.38 µg/g FW), and chlorophyll *b* (1.49 µg/g FW) concentrations, indicating potentially higher photosynthetic efficiency per unit leaf mass.

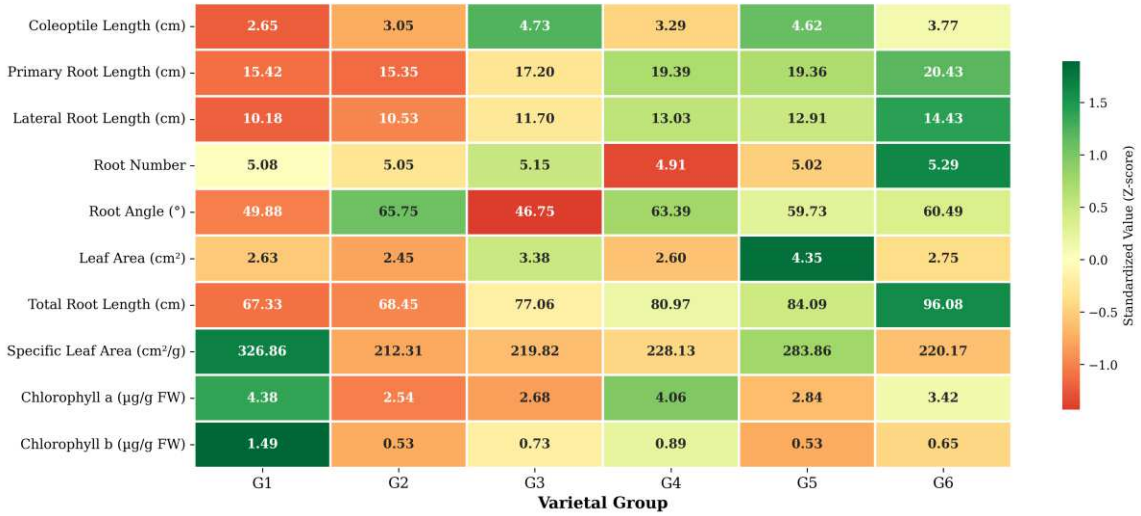


**Figure 3**. Heatmap illustrating variation in morpho-physiological traits among varietal groups. Cell values represent group means, while color intensity reflects standardized Z-score values for each trait.

### *3.4. Combined effects of seed size and varietal group*

The interaction between seed size and varietal group on each vigor parameter is illustrated in Figure 4. For shoot parameters, G5 exhibited the highest LA in both the large seed group (LSG: 4.45 cm²) and small seed group (SSG: 4.24 cm²), while G2 recorded the lowest values. Large seeds generally promoted greater root development, though the magnitude of this effect varied considerably across varietal groups. G6 exhibited a notable reversal, with higher TRL from small seeds (99.19 cm) compared to large seeds (92.97 cm). Group G3 showed the strongest positive response to large seed size for TRL (LSG: 85.85 cm vs. SSG: 68.28 cm, a 25.7% increase). Regarding chlorophyll content, G1 showed higher values from small seeds, indicating complex genotype-dependent responses.

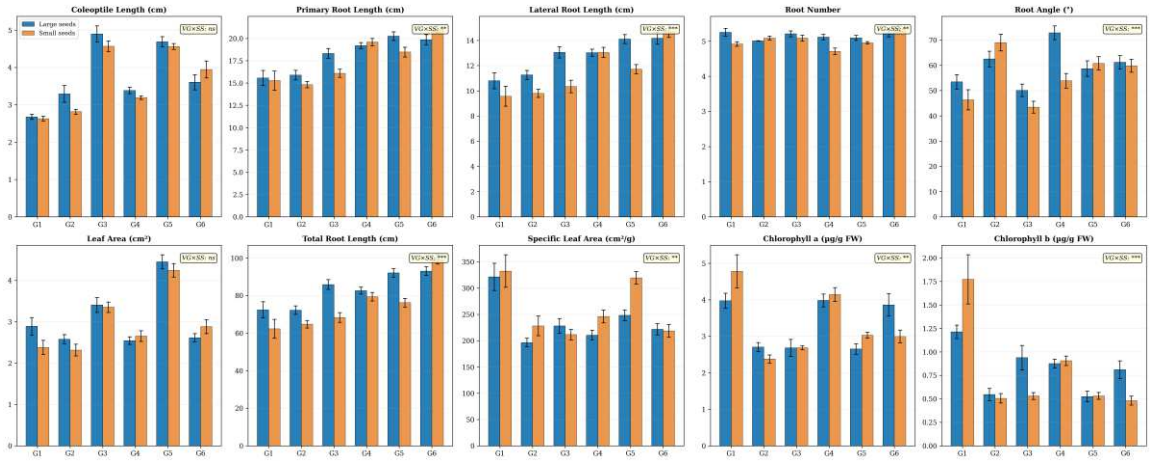


**Figure 4**. Effect of seed size on early vigor traits across varietal groups. Bars represent mean values ± standard error of the mean (SEM).

### *3.5. Principal component analysis*

Principal component analysis revealed that the first three principal components accounted for 67.3% of the total variance (Table 3). PC1 explained 29.2% of the variance and was primarily driven by root traits, with TRL (31.1% contribution), LRL (28.9%), and PRL (28.8%) as the dominant loadings. PC2 accounted for 21.2% of the variance and separated aerial development traits (CL: 24.9%; LA: 22.5%) from chlorophyll content (Chl *a*: 23.8%; Chl *b*: 20.2%), which loaded negatively. PC3 (16.8%) was associated with SLA (38.6%) and chlorophyll pigments with positive loadings.

**Table 3.** Principal component analysis of ten early vigor traits in 25 durum wheat genotypes.

| PC | Eigenvalue | Variance (%) | Cumulative (%) | Main contributing traits |
|---|---|---|---|---|
| **PC1** | 3.045 | 29.23 | 29.23 | TRL (31.1%), LRL (28.9%), PRL (28.8%) |
| **PC2** | 2.213 | 21.24 | 50.48 | CL (24.9%), Chl a (23.8%), LA (22.5%), Chl b (20.2%) |
| **PC3** | 1.755 | 16.85 | 67.32 | SLA (38.6%), Chl b (17.9%), Chl a (11.2%) |
| **PC4** | 1.115 | 10.70 | 78.03 | RN (74.9%), RA (11.7%) |

The PCA biplot (Figure 5) provided clear visualization of genotype differentiation and trait associations. Genotypes from G6 clustered in the positive region of PC1, confirming their superior root development, while G1 and G2 were positioned in the negative region. The spatial distribution of clusters on the biplot was largely consistent with the AHC groupings, providing independent validation of the clustering results.

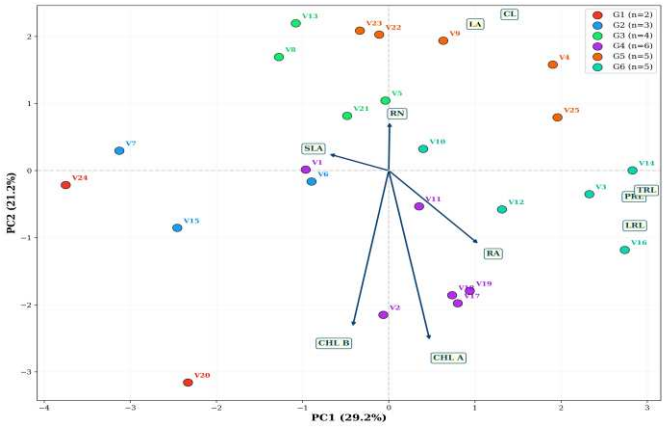


Figure **5**. PCA Biplot of 25 Durum Wheat Genotypes Based on Early Vigor Morpho-Physiological Traits

### *3.6. Correlation analysis*

Pearson correlation analysis revealed several significant associations (Figure 6). Very strong positive correlations were observed among root traits: LRL–TRL ($r = 0.92$, $P < 0.001$), PRL–TRL ($r = 0.83$, $P < 0.001$), and PRL–LRL ($r = 0.79$, $P < 0.001$), indicating tight functional coordination in root system development. A significant positive correlation was found between CL and LA ($r = 0.65$, $P < 0.001$). Additionally, LA was positively correlated with SLA ($r = 0.47$, $P < 0.05$), and Chl *a* was positively correlated with Chl *b* ($r = 0.60$, $P < 0.01$). No significant correlations were detected between root traits and chlorophyll content, suggesting independent regulation at the seedling stage.

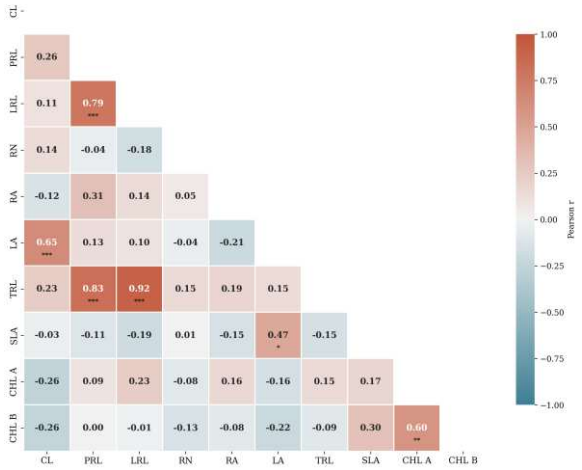


**Figure 6**. Pearson correlation matrix among early-vigor morphophysiological traits (n = 25 genotype means). Significance levels: ***P < 0.001, **P < 0.01, *P < 0.05.*

### *3.7. Genetic parameters*

All ten traits exhibited high broad-sense heritability ($H^2$), ranging from 78.7% (RA) to 97.3% (Chl *a*) (Table 4; Figure 7a). The consistently small differences between GCV and PCV across all traits indicated that observed phenotypic variation was predominantly attributable to genetic factors. GCV

ranged from 4.99% (RN) to 53.84% (Chl *b*). High GCV values (> 20%) were recorded for Chl *b* (53.84%), Chl *a* (34.00%), LA (23.87%), and CL (21.23%).

**Table 4.** Genetic parameters for ten early vigor traits in 25 durum wheat genotypes

| Trait | Mean | σ²g | σ²p | H² (%) | GCV (%) | PCV (%) | GA | GAM (%) | H² class |
|---|---|---|---|---|---|---|---|---|---|
| **CL** | 3.80 | 0.652 | 0.694 | 94.0 | 21.2 | 21.9 | 1.61 | 42.4 | High |
| **PRL** | 18.44 | 4.884 | 5.366 | 91.0 | 12.0 | 12.6 | 4.34 | 23.6 | High |
| **LRL** | 12.54 | 2.655 | 3.034 | 87.5 | 13.0 | 13.9 | 3.14 | 25.0 | High |
| **RN** | 5.08 | 0.064 | 0.076 | 84.1 | 5.0 | 5.4 | 0.48 | 9.4 | High |
| **RA** | 58.62 | 65.65 | 83.43 | 78.7 | 13.8 | 15.6 | 14.81 | 25.3 | High |
| **LA** | 3.09 | 0.543 | 0.586 | 92.7 | 23.9 | 24.8 | 1.46 | 47.4 | High |
| **TRL** | 81.40 | 95.51 | 109.9 | 86.9 | 12.0 | 12.9 | 18.77 | 23.1 | High |
| **SLA** | 242.4 | 1433 | 1800 | 79.6 | 15.6 | 17.5 | 69.56 | 28.7 | High |
| **Chl a** | 3.31 | 1.268 | 1.303 | 97.3 | 34.0 | 34.5 | 2.29 | 69.1 | High |
| **Chl b** | 0.75 | 0.162 | 0.170 | 95.7 | 53.8 | 55.0 | 0.81 | 108.5 | High |

. σ²g: genotypic variance; σ²p: phenotypic variance; H²: broad-sense heritability; GCV/PCV: genotypic/phenotypic coefficients of variation; GA: genetic advance at 5% selection intensity; GAM: genetic advance as % of mean.

Genetic advance as a percentage of the mean (GAM) ranged from 9.43% (RN) to 108.49% (Chl *b*). The combination of high H² with high GCV and high GAM for Chl *b*, Chl *a*, LA, and CL indicates that these traits are controlled predominantly by additive gene action and would respond favorably to direct phenotypic selection.

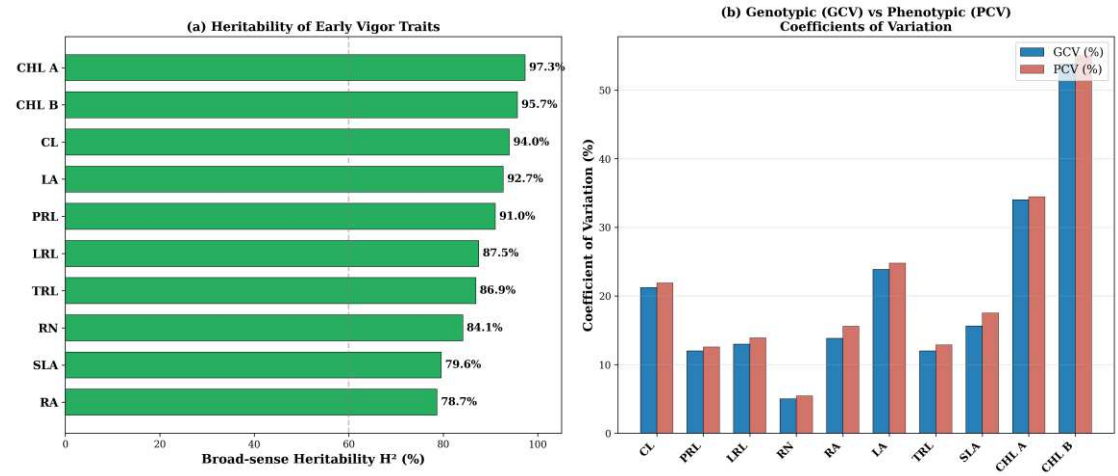


**Figure 7**. Genetic parameters of early-vigor morphophysiological traits in 25 durum wheat genotypes: broad-sense heritability (H²) and genotypic (GCV) versus phenotypic (PCV) coefficients of variation.

### *3.8. Phenotypic diversity*

Phenotypic diversity analysis revealed considerable variation among the 25 genotypes for most traits (Table 5). The highest coefficients of variation were observed for Chl *b* (55.0%), Chl *a* (34.5%), LA (24.8%), and CL (21.9%). Shannon diversity indices (H′) ranged from 1.11 (RN) to 1.54 (CL), with evenness values between 0.69 (RN) and 0.96 (CL), indicating generally well-distributed phenotypic variation. The relatively low diversity observed for RN (CV = 5.4%; H′ = 1.11) confirms the limited variation for this trait.

296 **Table 5.** Phenotypic diversity indices for ten early vigor traits across 25 durum wheat genotype means. H′:
297 Shannon diversity index (5 classes); Evenness: H′/H′max.

| Trait | Mean | SD | CV (%) | Min | Max | Range | H′ | Evenness |
|---|---|---|---|---|---|---|---|---|
| **CL** | 3.80 | 0.83 | 21.9 | 2.60 | 5.42 | 2.82 | 1.54 | 0.96 |
| **PRL** | 18.44 | 2.32 | 12.6 | 14.04 | 23.24 | 9.20 | 1.51 | 0.94 |
| **LRL** | 12.54 | 1.74 | 13.9 | 9.52 | 15.44 | 5.92 | 1.51 | 0.94 |
| **RN** | 5.08 | 0.28 | 5.4 | 4.35 | 5.99 | 1.65 | 1.11 | 0.69 |
| **RA** | 58.62 | 9.13 | 15.6 | 43.31 | 81.50 | 38.17 | 1.45 | 0.90 |
| **LA** | 3.09 | 0.77 | 24.8 | 2.25 | 4.80 | 2.55 | 1.31 | 0.82 |
| **TRL** | 81.40 | 10.48 | 12.9 | 62.52 | 101.6 | 39.09 | 1.46 | 0.91 |
| **SLA** | 242.4 | 42.43 | 17.5 | 180.6 | 335.6 | 158.5 | 1.46 | 0.91 |
| **Chl a** | 3.31 | 1.14 | 34.5 | 1.84 | 6.08 | 4.25 | 1.43 | 0.89 |
| **Chl b** | 0.75 | 0.41 | 55.0 | 0.23 | 2.14 | 1.90 | 1.19 | 0.74 |

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# 4. Discussion

## 4.1. Genotype × seed size interaction: a neglected dimension of early vigor

The most novel finding of this study is the pervasive genotype × seed size interaction, significant for eight of ten measured traits. This result contrasts sharply with the implicit assumption in most seedling vigor studies that seed size effects are consistent across genotypes. Ambika et al. (2014) and Shahwani et al. (2014) reported uniform positive effects of large seeds on seedling performance in bread wheat, leading to generalized recommendations for seed grading. However, the present results demonstrate that such generalizations do not hold across the genetic diversity of durum wheat: the magnitude, and in some cases the direction, of seed size effects is strongly genotype-dependent.

The most striking example is the reversal observed in G6, where small seeds produced higher total root length than large seeds (99.19 vs. 92.97 cm), contrary to the expected pattern. To our knowledge, this reversal has not been previously documented in durum wheat. It suggests that certain genotypic backgrounds possess compensatory mechanisms that offset the reduced nutrient reserves of smaller seeds—potentially through more efficient mobilization of seed storage compounds or earlier activation of autotrophic growth. Conversely, G3 showed the strongest positive response to large seed size (25.7% increase in TRL), indicating that some genotypes remain strongly seed-reserve-dependent during early development. These differential responses have direct practical implications: seed grading protocols should account for genotypic background rather than applying uniform size thresholds across varieties.

The non-significant VG × SS interaction for coleoptile length and leaf area is equally informative. It indicates that these two traits respond consistently to seed size across genotypic backgrounds, simplifying their use as selection criteria. Breeders can reliably screen for CL and LA without needing to control for seed size, which is a practical advantage in large-scale phenotyping programs.

It is acknowledged that the varietal groups used in the ANOVA were derived from clustering on the same trait data, and therefore the significance of the VG main effect is partly expected by construction. However, the seed size effect and the VG × SS interaction—which constitute the primary findings of this study—are entirely independent of the clustering procedure and represent genuinely novel information.

## 4.2. Chlorophyll content as a superior selection target: challenging the morphological paradigm

A second original contribution of this study is the identification of chlorophyll content as the trait with the greatest selection potential for early vigor in durum wheat. Chlorophyll b exhibited the highest GAM among all traits (108.5%), followed by chlorophyll a (69.1%), meaning that selection of the top 5% of genotypes would more than double the population mean for Chl b. These values substantially exceed the GAM observed for traditionally prioritized morphological traits such as coleoptile length (42.4%) and total root length (23.1%).

This finding challenges the longstanding emphasis on morphological indicators in vigor screening protocols. Rebetzke et al. (2007) established coleoptile length as the reference trait for early vigor selection in wheat, and subsequent studies have largely followed this framework (Bourgault et al. 2020; Rossi et al. 2024). While CL showed high heritability (94.0%) and

respectable GAM (42.4%) in the present study, the chlorophyll pigments consistently outperformed it across all three selection criteria ($H^2$, GCV, and GAM). The combination of very high heritability (Chl a: 97.3%; Chl b: 95.7%) with high genotypic coefficient of variation (Chl a: 34.0%; Chl b: 53.8%) suggests strong genetic control and high expected response to phenotypic selection. Such a combination of high heritability, GCV, and GAM is generally considered favorable for effective selection (Johnson et al. 1955).

The $H^2$ values reported here (78.7–97.3%) are comparable to or exceed those reported by Paleo et al. (2024) for similar traits in Argentine wheat (62–88%) and by Rebetzke et al. (2007) for coleoptile-related traits in Australian germplasm. The higher values observed in the present study likely reflect both the controlled experimental conditions, which minimize environmental variance, and the broad genetic diversity of the panel, which maximizes genotypic variance. The exceptionally high broad-sense heritability values observed under controlled conditions therefore reflect minimized environmental variance, allowing a more precise estimation of the underlying genetic signal. Such values should be interpreted as upper-bound estimates of selection efficiency under optimal phenotyping environments rather than direct predictors of field response. The consistently small gap between GCV and PCV across all traits further confirms that environmental influences were minimal relative to genetic effects.

From a practical standpoint, chlorophyll quantification through spectrophotometry is rapid, inexpensive, and scalable—making it suitable for high-throughput screening in breeding programs. The integration of chlorophyll-based vigor indicators alongside traditional morphological traits could substantially improve selection efficiency for early vigor in durum wheat.

### 4.3. Contrasting vigor strategies: evidence for independent root and shoot dimensions

The correlation analysis revealed a clear functional compartmentalization in seedling development. Root traits formed a tightly coordinated module (PRL–LRL–TRL: $r = 0.79–0.92$, $P < 0.001$), while aerial traits (CL–LA: $r = 0.65$, $P < 0.001$) formed a separate module. Critically, no significant correlations were detected between these two dimensions, indicating that root and shoot vigor are independently regulated at the seedling stage.

This independence has important implications for breeding. It means that selection for deep root systems—advantageous for drought avoidance in semi-arid environments (Manschadi et al. 2006; Lynch 2021; Bonfiglioli et al. 2024)—can be pursued without compromising aerial vigor, and vice versa. Breeders could therefore design ideotypes combining traits from different varietal groups: the root architecture of G6 (TRL = 96.08 cm) with the leaf development of G5 (LA = 4.35 $cm^2$) and the photosynthetic efficiency of G1 (Chl a = 4.38 µg/g FW; SLA = 326.86 $cm^2/g$). This modularity provides flexibility that would not be available if the trait dimensions were strongly correlated.

The independence between chlorophyll content and morphological traits is also noteworthy. Silva-Pérez et al. (2020) reported moderate correlations between photosynthetic capacity and leaf morphology in spring wheat, but those measurements were taken at later developmental stages. The present results suggest that at the seedling stage, photosynthetic pigment accumulation follows a developmental trajectory largely independent of structural growth—an encouraging finding for breeding multi-trait ideotypes that combine both high photosynthetic efficiency and robust structural vigor.

### 4.4. Genotypic diversity and clustering: a framework for targeted crossing

The agglomerative hierarchical clustering organized the 25 genotypes into six distinct varietal groups with differential vigor profiles, confirmed by bootstrap validation (1,000 replicates). The biological validity of these groups is independently supported by the PCA biplot, which was computed separately from the clustering: the spatial distribution of genotypes on PC1 (root vigor gradient) and PC2 (aerial vs. physiological traits) was largely consistent with the AHC groupings.

The six groups represent three distinct vigor strategies: root-dominant (G6), shoot-dominant (G5), and physiologically efficient (G1). This diversity is consistent with the heterogeneous origins of the studied panel, which includes both traditional landraces adapted to local conditions and improved varieties developed through modern breeding. The range of variation observed—TRL from 67.3 to 96.1 cm, CL from 2.65 to 4.73 cm, Chl b from 0.23 to 2.14 µg/g FW—provides substantial raw material for genetic improvement.

The observed diversity within Algerian germplasm mirrors patterns reported in Mediterranean collections from other regions (Vassileva et al. 2023; Singh et al. 2025), suggesting that North African landraces may harbor adaptive variation of interest to international breeding programs. The convergence of high heritability, significant genetic variation, and clearly differentiated vigor strategies positions this germplasm as a valuable resource for pre-breeding activities aimed at developing drought-resilient durum wheat cultivars.

### 4.5. Originality and broader implications

This study makes three original contributions to the international literature on wheat seedling vigor. First, it provides the first systematic quantification of genotype × seed size interactions across a comprehensive panel of durum wheat genotypes, revealing that seed size effects are not universal but strongly genotype-dependent—a finding that calls for revision of seed grading protocols in breeding programs. Second, it identifies chlorophyll content (Chl a and Chl b) as the traits with the highest selection potential for early vigor, challenging the traditional emphasis on morphological traits in vigor screening protocols. Third, the demonstrated independence between root and shoot vigor dimensions offers a practical framework for multi-trait selection strategies that can be applied across Mediterranean breeding programs regardless of local germplasm composition.

### 4.6. Limitations and future perspectives

While controlled conditions allow precise measurement of seedling traits and minimize environmental variance—thereby providing robust heritability estimates—field validation under actual Mediterranean stress conditions (drought, heat, salinity) is necessary to confirm the agronomic relevance of the observed vigor differences. In particular, it remains to be determined whether the high heritability values observed here are maintained under field conditions, where genotype × environment interactions may reduce selection efficiency. Additionally, molecular characterization of the identified varietal groups through marker-based analysis would strengthen the genetic basis of the observed phenotypic diversity and facilitate the development of marker-assisted selection tools. Future research should also investigate the physiological mechanisms underlying the compensatory responses observed in G6 and G1, where small seeds unexpectedly outperformed large seeds for specific traits.

## 5. Conclusion

This study addressed three underexplored aspects of early vigor in durum wheat: the genotype-dependence of seed size effects, the relative selection potential of physiological versus morphological vigor indicators, and the functional coordination among seedling trait dimensions.

The systematic quantification of genotype × seed size interactions across 25 genotypes and ten traits revealed that seed size effects are not universal: significant VG × SS interactions were detected for eight of ten traits, with notable reversals in specific genotypic backgrounds where small seeds outperformed large seeds. This finding challenges the widespread assumption that large seeds uniformly enhance seedling performance and calls for genotype-specific seed grading protocols in breeding programs.

The genetic parameter analysis identified chlorophyll content—particularly Chl b ($H^2$ = 95.7%; GAM = 108.5%) and Chl a ($H^2$ = 97.3%; GAM = 69.1%)—as the traits with the highest selection potential for early vigor, substantially exceeding the genetic gains achievable through traditionally prioritized morphological traits such as coleoptile length and total root length. This result suggests that integrating chlorophyll-based physiological indicators into vigor screening protocols could significantly improve selection efficiency.

The demonstrated independence between root and shoot vigor dimensions provides a practical framework for multi-trait selection: breeders can target root architecture for drought avoidance and aerial development for rapid canopy closure without negative trade-offs between these two strategies.

Collectively, these findings move beyond local germplasm characterization to offer methodological insights applicable to trait-based selection programs across Mediterranean durum wheat breeding systems. Field validation under representative stress conditions and molecular characterization of the identified vigor strategies constitute the necessary next steps toward translating these results into operational breeding tools.

## Declarations

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**Data availability** The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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