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# Genetic Diversity and Dissection of Agronomic Traits in Durum Wheat Grown Under Contrasting Environments in Algeria

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## Abstract

Durum wheat productivity in Mediterranean regions faces growing challenges from drought and heat stress. Understanding the genetic architecture of diverse germplasm is therefore essential to support pre-breeding efforts and enhance stress adaptation. In this context, 125 durum wheat genotypes were evaluated for agro-morphological traits across two contrasting Algerian locations over two growing seasons. A subset of 94 genotypes, selected on the basis of phenotypic characterization, was genotyped using the Illumina 7K SNP array. Population structure analysis revealed two to four subgroups, with linkage disequilibrium decaying at 4.09 Mb. Genome-wide association analysis identified 27 distinct significant SNPs associated with eight traits, with most associations detected for spike length, thousand-kernel weight, and plant height. The marker TGWA25K-TG0010 on chromosome 4A showed pleiotropic effects on plant height and peduncle length and co-localized with the *Dwarf8* and *gibberellic-acid-insensitive* genes. Additionally, wsnp_Ex_c2033_3814035 on chromosome 2A was associated with heading earliness and the number of fertile spikelets per spike, and wsnp_Ku_c51039_56457361 on chromosome 5A with plant height and peduncle length in a single site and season. Several other environment-specific associations were also identified. These results support future studies in which the identified markers may be deployed in breeding strategies aimed at improving yield stability and stress adaptability in durum wheat under Algerian conditions.

**Keywords:** durum wheat; resilience; candidate genes; GWAS; SNPs



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## 1. Introduction

Durum wheat is a major crop in the Mediterranean region, mainly grown in rainfed conditions [1]. In Algeria, durum wheat is consumed in a variety of forms, including semolina and pasta. It is grown on 1.49 million hectares, producing 2.58 million tons with

an average yield of 1.8 t/ha [2], and holds significant agricultural importance, as most crops are cultivated in semi-arid regions characterized by low and irregular precipitation. Despite its resilience to difficult conditions, durum wheat is endangered by climate change, particularly by drought stress [3]. Recent studies by Zaïm et al. [4] show that breeding efforts have been directed toward generating varieties with enhanced resilience, enabling them to avoid or tolerate such stresses. The exploitation of genetic diversity present in local germplasm collections, including landraces, traditional cultivars, and breeding lines [5] is a crucial step toward discovering alleles associated with key agronomic traits, including yield potential, stress resistance, and overall adaptability, which are essential for sustainable crop improvement [6,7], and improved nutritional content [8].

Agro-morphological characterization represents a fundamental first step in the exploration and identification of plant genetic resources, as it reveals observable variation in key agronomic traits [9]. This approach is also a valuable management tool for validating the identity of specific accessions [10] and provides a basis for crop improvement targeting desirable traits [11]. The main objective of this characterization is to identify predictive traits that can be used to optimize grain yield, a complex polygenic trait influenced by multiple factors [12]. In addition, the study of traits with high heritability allows the prediction of phenotypic performance, which is an indicator of breeding value and provides insight into the transmission of traits from parents to offspring [13]. Understanding the interrelationships among traits may further assist breeders in direct and indirect selection, particularly for traits that are difficult to measure or exhibit low heritability [14]. Phenotypic traits are highly affected by the environment; thus, conducting multi-environment and multi-year trials allows a more reliable evaluation of genotype × environment interactions and facilitates the selection of genotypes with stable and broad adaptation [15]. Molecular markers therefore offer a complementary and more reliable approach to assess genetic variation and understand complex traits [16].

The genotyping technique known as the "genotyping array" plays a key role in wheat genotyping. It allows researchers to examine different wheat varieties, detect genetic variants associated with important traits, and develop markers to incorporate into breeding strategies [17]. Building on this approach, advances in high-throughput SNP genotyping have considerably enhanced the assessment of genetic diversity and population structure among various wheat types and genetic backgrounds [18]. Given their high resolution, these platforms are particularly well suited for genome-wide association studies (GWAS) [19], enabling the identification of genomic loci involved in traits of agronomic interest [20]. GWAS relies on linkage disequilibrium (LD), which offers the possibility of accurately exploring the genetic basis of complex traits [21]. This knowledge facilitates the introgression of useful alleles from wild relatives or traditional germplasm into improved varieties through marker-assisted selection or genomic selection [20,22].

In recent years, numerous studies have successfully identified genomic regions and quantitative trait loci (QTLs) associated with several traits in wheat [23], such as grain yield [24,25], drought tolerance [26], and disease resistance [27,28]. Also, flowering time is mainly governed by several QTLs, including a major locus on chromosome 2B linked to copy number variation at *Ppd-B1*, which explains around 26% of the observed variation [29]. Plant height, another fundamental agronomic trait, has been associated with five QTLs on chromosomes 1A, 2B, 6B, 7A, and 7B in Ethiopian durum wheat accessions [30]. Spike fertility, which directly determines grain number, has been linked to 25 stable QTLs detected across two doubled haploid populations [31]. In terms of spike length and yield components, eight QTLs were identified on chromosomes 2B, 4A, 5A, 5B, 7A, and 7B,

including a novel QTL on chromosome 2B under drought stress [30], while Mangini et al. [32] reported 39 QTLs for thousand-kernel weight, grain dimensions, and grain shape across environments. Physiological traits such as flag leaf chlorophyll content have been dissected; Yang et al. [33] identified 29 QTLs across multiple environments, including the stable *Qchl.saw-5A.3* explaining up to 23.25% of variation under drought. Nevertheless, most agronomic traits in wheat are complex and polygenic, influenced by numerous genes and their interactions with environment [34]. Identifying QTLs that consistently express their effects under varying environmental conditions represents an essential step toward enhancing yield stability and broad adaptability [24]. Although GWAS is effective in identifying marker-trait associations for yield traits, studies across different regions highlight the strong impact of environmental factors and the significant role of genotype × environment interactions [35]. According to Eltaher et al. [36], the integration of environment-oriented breeding approaches with molecular marker-assisted selection can significantly enhance the efficiency of wheat genetic improvement. These studies highlight the importance of integrating phenotypic characterization, high-density genotyping, and GWAS to identify loci associated with key agronomic traits, thereby supporting breeding efforts aimed at stress adaptation and yield stability.

In this context, the objective of this study was to evaluate the genetic diversity and population structure of a panel of 94 durum wheat genotypes with diverse origins using the 7K Infinium SNP array. In addition, a genome-wide association study was then conducted to detect genomic regions linked to major agro-morphological traits across two contrasting locations and cropping seasons.

## 2. Materials and Methods

### *2.1. Plant Material*

The initial plant material consisted of a germplasm collection of 125 durum wheat genotypes originating from diverse agro-ecological zones, and includes both landraces, old, and modern varieties. This germplasm collection was assembled and is maintained by the Plant Breeding and Biotechnology Division of the National Agronomic Research Institute of Algeria. The majority of genotypes (32%) originate from the seed banks of CIMMYT and ICARDA, while the most represented countries of collection are Mexico (22.4%) and Algeria (17.6%). Detailed information for code, name, and pedigree is provided in Table S1.

### *2.2. Description of Sites*

All genotypes were tested under rainfed conditions at two contrasting agro ecological sites representative of Mediterranean cereal-growing environments in Algeria. The first site was the experimental station of the National Agronomic Research Institute of Algeria (36°68′ N, 3°11′ E, 18.5 m a.s.l.) in Algiers (Central zone) during the 2020–2021 and 2021–2022 cropping seasons. This site is located at lower altitude in the fertile Mitidja plain and belongs to a dry sub-humid climatic zone, with milder temperatures and moderate rainfall [37]. The second site was the El-Khroub experimental farm, located 14 km southeast of Constantine (36°28′ N, 6°67′ E, 640 m a.s.l.), where trials were conducted in 2018–2019 and 2020–2021. This site is characterized by a semi-arid bioclimatic zone, at higher altitude characterized by cooler temperatures, lower rainfall, and continental conditions with a wide thermal amplitude [38]. At both sites, soils are predominantly clay-textured, as determined according to the texture triangle of Hénin et al. [39].

### *2.3. Climatic Conditions*

Climatic data were recorded from September to July for each growing season at both sites. In Algiers, total rainfall reached 474.2 mm in 2020–2021, slightly higher than the 461.4 mm recorded in 2021–2022. In the first season, peak precipitation occurred in December (146.5 mm), enhancing soil water storage and favoring good crop establishment, while in the second season, two peaks were recorded in November (180.2 mm) and March (121.6 mm), with the latter coinciding with the stem elongation–heading stage. In Constantine, rainfall was 467.6 mm in 2018–2019 and 326.4 mm in 2020–2021, representing a deficit of 142.2 mm. The highest monthly totals were observed in October (142 mm) and January (97.2 mm) for the first season, and in December (55 mm) for the second.

At both sites, the lowest temperatures were recorded from December to February, often with frost events in Constantine, followed by a steady increase from March, with relatively high temperatures in Constantine during May coinciding with the stem elongation and the heading. Over the two seasons, average monthly temperatures ranged from 6.75 °C to 33.65 °C in Algiers and from 0.95 °C to 35.75 °C in Constantine. The thermal amplitude was higher in Constantine (21.8 °C and 19.6 °C, respectively) compared with Algiers (13.9 °C and 15.7 °C, respectively). Detailed climatic data are presented in Figure S1.

### *2.4. Experimental Design and Phenotypic Data Measurements*

The field experiment was carried out using an augmented randomized complete block design (ARCBD) with 4 blocks, each containing 30 test entries (genotypes) and 5 checks (C1: Waha; C2: Cirta; C3: Sigus; C4: Beni Mestina; and C5: Gta/dur), which were repeated twice in each block, for a total of 160 experimental plots. This design is suitable for evaluating numerous genotypes while allowing reliable adjustment through replicated checks. Each genotype was sown in 2 rows of 1-m length and 0.25 m apart. The sowing at both sites was carried out at the end of December, while harvesting took place between late June and early July. All recommended crop management practices were implemented to ensure a healthy crop stand.

Several phenotypic traits were measured, including days to 50% heading (DH, days), counted from the date of sowing to the stage when 50% of the spikes were halfway out from the flag leaf. At the heading stage, the distance from the base to the tip of the leaf was calculated using the formula for flag leaf area FLA = 0.606 (L × l), where L represents the total length of the leaf, l is the average width of the leaf, and 0.606 is the regression coefficient described by Spagnoletti Zeuli et al. [40]. The chlorophyll content (CC) was measured using an SPAD-502 chlorophyll meter, with readings taken at the midpoint of the fully expanded flag leaf. At maturity, plant height (PH, cm) was measured from the soil surface to the tip of the spike (excluding awns), peduncle length (PL, cm) as the internodal distance between the base of the flag leaf blade and the base of the spike, and spike length (SL, cm) as the distance from the base of the spike to its tip (excluding awns).

Grain yield-related components were determined including thousand-kernel weight (TKW, g), where samples of 1000 grains were counted using a grain counter and then weighed with a precision balance, and number of fertile spikelets per spike (NFSS), determined by counting the spikelets containing at least one grain per spike. A mean value for each genotype's agro-morphological parameters was calculated based on the sampling of three plants from each plot.

### *2.5. DNA Extraction and Genotyping*

Four seeds of each genotype were sown in alveolar trays under controlled greenhouse conditions at the molecular biology laboratory of the University of Modena and Reggio

Emilia in Italy. Fresh leaf tissues were collected from 10- to 12-day-old seedlings for DNA extraction using modified CTAB method [41]. To reduce redundancy and ensure a representative panel capturing the overall phenotypic diversity, a principal component analysis (PCA) based on the measured traits was performed and used to guide genotype selection (Figure S2). A total of 93 genotypes were selected from the initial collection based on pedigree information and phenotypic similarity across morpho-agronomic traits. Additionally, the cultivar SVEVO was included as a reference, bringing the total number of genotypes to 94 (Table S2). The extracted DNA samples (50 ng/µL per sample), arranged in a 96-well plate format, were genotyped at TraitGenetics GmbH (Gaterslegen, Germany) using the optimized Illumina Infinium 7K wheat array. 7K array is a subset (6707) aimed at genomic selection studies of the 25K array developed by SGS Institut Fresenius TraitGenetics section (SGS IF TG). The physical position of each SNP was assigned based on the physical map of the Svevo durum wheat, available at https://www.interomics.eu/durum-wheat-genome.

### *2.6. Statistical Analysis of Phenotypic Data*

Each site × year combination was considered as a distinct environment. For each environment, descriptive statistics (minimum, maximum, and mean) and separate analyses of variance (ANOVA) were carried out in R version 4.3.2 using the 'augmentedRCBD' package. The adjusted means for each environment were then used for the combined analysis following a simple linear model with checks, genotypes, environments, and blocks, and the genotype x environment interactions were treated as fixed effects, allowing direct comparison of genotype performance across environments. The model is described as follows: lm (formula = Trait ~ Genotypes + Checks + Block + Check × Genotype + Environment + Genotypes × Environment).

Broad-sense heritability ($H^2_{bs}$) was calculated for individual environments using the ANOVA results obtained with the augmentedRCBD package and for the combined environments using the formula: $H^2_{bs} = \frac{\sigma^2_G}{\sigma^2_p} = \frac{\sigma^2_G}{(\sigma^2_G + \frac{\sigma^2_{GE}}{e} + \frac{\sigma^2_e}{er})}$, according to Aquaah [42]; where $\sigma^2_p$ is the phenotypic variance, $\sigma^2_G$ is the genotypic variance, $\sigma^2_{GE}$ is the genotype × environment interaction variance, $\sigma^2_e$ is the residual error variance, e is the number of environments, and r is the number of replications. The variance components were estimated as follows:

$$\sigma^2_p = \sigma^2_G + \sigma^2_{GE} + \sigma^2_E,\ \sigma^2_G = (MS_G - MS_e)/r,\ \sigma^2_{GE} = (MS_{GE} - MS_e)/r,\ \sigma^2\ e = MS_e$$

$MS_G$ is the mean square due to genotypes, $MS_{GE}$ is the mean square due to genotype × environment interaction, and $MS_e$ is the mean square of the residual error. Pearson correlation analysis was executed using the R package 'metan'.

### *2.7. SNP Filtering, Linkage Disequilibrium, and Population Structure*

Genotypic data from the set of 7000 SNPs were filtered using TASSEL software (version 5.2.92, Institute for Genomic Diversity, Ithaca, NY, USA) to exclude markers with more than 10% missing data, a minor allele frequency (MAF) of less than 5%, a heterozygosity rate greater than 20%, and a call rate per site of 85%. Linkage disequilibrium (LD) between SNP marker pairs ($r^2$) was estimated using TASSEL version 5.2.92. The linkage disequilibrium decay graph was produced using R software version 4.3.2 with the LOESS model [43]. A threshold of $r^2 = 0.2$, commonly applied in cereal genomics studies, was used to determine the point at which linkage disequilibrium was considered to have decayed sufficiently [44].

Linkage disequilibrium (LD) pruning was performed with an $r^2$ threshold of 0.5 to eliminate markers exhibiting strong linkage disequilibrium. This resulted in a dataset of 1433 SNPs, which were used only for the analysis of population structure.

The population structure was assessed using two complementary approaches. First, STRUCTURE software version 2.3.4, a model-based Bayesian approach, was run with 25,000 burn-in iterations and 25,000 Markov Chain Monte Carlo (MCMC) repetitions. The admixture model with correlated allele frequencies was applied to evaluate the number of clusters (K) ranging from 1 to 10, with five independent runs for each K value. The results were visualized using the online software STRUCTURE Selector (available at http://lmme.qdio.ac.cn/StructureSelector/, accessed on 6 May 2024), and the optimal number of subpopulations (K) was determined based on the delta K method described by Evanno et al. [45]. Second, discriminant analysis of principal components (DAPCs), a non-parametric multivariate method, was conducted using the R package 'adegenet' implemented in R version 4.3.2. In this case, the optimal K value was identified based on the lowest Bayesian Information Criterion (BIC).

### *2.8. Identification of Genomic Loci and Candidate Genes Associated with Traits*

Genome-wide association analysis was conducted separately for each environment (site × year) and also combined across environments using the Multi-Locus Mixed Model (MLMM) implemented in the GAPIT package in R (version 4.3.2). This model was selected to mitigate the high level of genetic stratification observed in the diversity analysis. By incorporating principal component analysis (PCA) as a fixed effect and a kinship matrix as a random effect, the MLMM effectively accounts for both population structure and cryptic relatedness, thereby minimizing Type I errors (false positives) [46]. To minimize false-positive associations, a Bonferroni correction was applied at a significance level of $\alpha = 0.05$. A significance threshold of $-\log_{10}(p) = 4.67$ was used to identify markers significantly associated with the traits of interest shown in a Manhattan plot and QQ plot.

In order to define candidate genes, high confidence gene models in the confidence interval (CI) of the QTLs were extracted from Svevo version 1 annotation data, retrieved from GrainGenes cereal database (https://graingenes.org/GG3/, accessed on 1 April 2025).

### *2.9. Artificial Intelligence*

During the preparation of this manuscript the authors used ChatGTP 5.1 for the purposes of generating a scheme for the Graphical Abstract that was then implemented with icons from BioRender (www.biorender.com). Grammar and spelling editing were also performed.

## 3. Results

### *3.1. Phenotypic Variability of Measured Traits*

The combined analysis of variance for the eight studied traits indicated highly significant differences ($p < 0.001$) among genotypes, environments, and genotype × environment interactions (Table 1), highlighting the strong influence of environmental conditions and differential genotype responses across environments. Both checks and test genotypes also exhibited significant variation ($p < 0.001$) for most traits, with some exceptions such as chlorophyll content (CC) and flag leaf area (FLA) which were not significant for the genotype × check comparison.

**Table 1.** Mean squares and significance levels for the traits studied in durum wheat genotypes using combined ANOVA analysis.

| Source | Environments | Genotype vs. Check | Checks | Genotypes | Genotypes | Environment:Genotypes | Error |
|---|---|---|---|---|---|---|---|
| | | | | Tests | | | |
| Df | 3 | 1 | 4 | 119 | 124 | 372 | 57 |
| DH | 28,673.2 *** | 188.2 *** | 73.3 *** | 99.9 *** | 101 *** | 14.8 *** | 3.2 |
| CC | 8032.1 *** | 9 $^{ns}$ | 16.6 ** | 26.5 *** | 27 *** | 11.5 *** | 3.7 |
| PH | 5544.8 *** | 2593.9 *** | 124.9 *** | 897.6 *** | 892.2 *** | 40.6 *** | 7 |
| PL | 1213.62 *** | 224.67 *** | 24.14 *** | 57.50 *** | 59.02 *** | 9.67 *** | 1.36 |
| SL | 50.052 *** | 14.369 *** | 5.483 *** | 2.069 *** | 2.282 *** | 0.354 *** | 0.133 |
| NFSS | 56.647 *** | 31.027 *** | 33.918 *** | 11.948 *** | 12.896 *** | 2.989 *** | 0.854 |
| TKW | 2894.61 *** | 25.66 ** | 50.67 *** | 92.56 *** | 93.45 *** | 21.76 *** | 2.48 |
| FLA | 1158.24 *** | 11.66 $^{ns}$ | 194.94 *** | 58.22 *** | 62.58 *** | 24.21 *** | 7.82 |

Df—degree of freedom; DH—days to heading(days); PH—plant height (cm); FLA—flag leaf area ($cm^2$); SL—spike length (cm); PL—peduncle length (cm); TKW—thousand kernels weight (g); NFSS—number of fertile spikelets per spike; CC—chlorophyll content (SPAD). ns: not significant, ** $p < 0.01$, *** $p < 0.001$

Mean days to heading (DH) ranged from 95.66 days in Algiers during the 2021–2022 season to 124.84 days in Constantine in 2018–2019 (Table S3). Average plant height (PH) varied from 80.35 cm in the former to 94.68 cm in the latter during the 2020–2021 growing season. Across environments, the mean values of fertile spikelets per spike (NFSS), spike length (SL), and peduncle length (PL) were 18 fertile spikelets/spike, 7.03 cm, and 18.19 cm, respectively. Flag leaf area (FLA) ranged from 24.52 $cm^2$ in Constantine in 2020–2021 to 30.58 $cm^2$ in Algiers in 2021–2022. The highest CC was recorded in Constantine during 2020–2021 (55.12 SPAD units), whereas the maximum thousand kernel weight (TKW) was obtained in Algiers in 2021–2022 (49.71 g)

In Constantine, a decrease in PH, DH, TKW, and PL, was observed during the second year, while CC increased, possibly reflecting a stress response. In Algiers, the highest broad-sense heritability value was recorded for PH in 2020–2021 ($H^2bs = 0.99$), whereas the lowest value was observed for CC in 2021–2022 ($H^2bs = 0.24$) (Table S3).

### *3.2. Phenotypic Correlation Between Traits Analyzed*

Correlation analysis among all parameters measured was carried out using Pearson's correlation matrix (Figure 1). PH showed a significant positive correlation with PL (r = 0.18, * $p < 0.05$), SL (r = 0.75, *** $p < 0.001$), and FLA (r = 0.40, *** $p < 0.001$). DH was positively correlated with PH (r = 0.60, *** $p < 0.001$), FLA (r = 0.52, *** $p < 0.001$), and NFSS (r = 0.50, *** $p < 0.001$). NFSS also showed a significant positive correlation with SL (r = 0.19, * $p < 0.05$). FLA was positively associated with most traits, particularly with PH, PL, DH, and NFSS, but negatively correlated with chlorophyll content (CC) (r = −0.18, * $p < 0.05$). CC exhibited weak but negative correlations with almost all traits, except TKW, where the correlation was weakly positive (r = 0.05) but not significant. Finally, TKW showed a slight negative correlation with NFSS (r = −0.17) and DH (r = 0.11), both non-significant, possibly suggesting a weak compensatory effect among yield-related components.

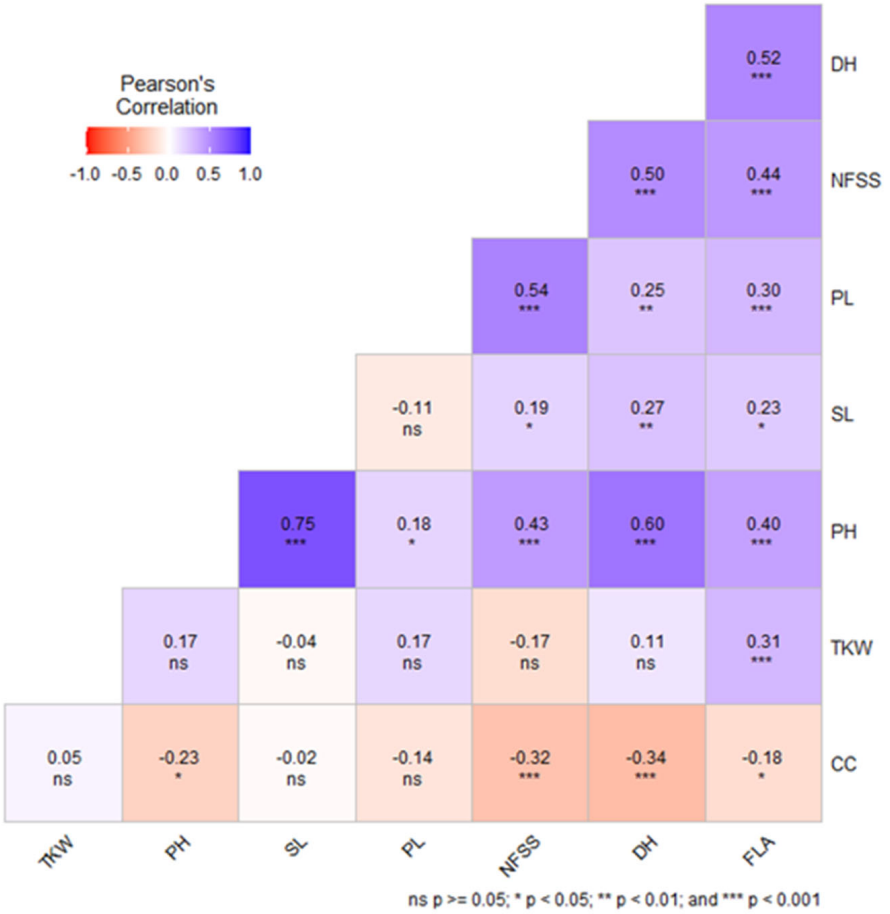


**Figure 1.** Pearson's correlation matrix among the measured traits across combined environments. DH—days to heading(days); PH—plant height (cm); FLA—flag leaf area ($cm^2$); SL—spike length (cm); PL—peduncle length (cm); TKW—thousand kernels weight (g); NFSS—number of fertile spikelets per spike; CC—chlorophyll content (SPAD).

### *3.3. Genotyping Distribution and Genomic Size of SNPs and Linkage Disequilibrium*

After filtering the initial set of 7000 SNPs, a total of 2353 high-quality SNPs was retained and used for further analyses. Among these, 1058 SNPs (46%) were distributed on the A genome and 1295 SNPs (54%) on the B genome. The number of SNPs per chromosome ranged from 90 SNPs for chromosome 4A to 227 for chromosome 1B. In terms of genomic coverage, chromosome 3B showed the largest span of SNPs, covering approximately 835 Mbp, while chromosome 1A exhibited the smallest coverage, at around 584 Mbp (Table S4). This variation in genomic coverage was found to be proportional to the physical length of the chromosomes, consistent with results obtained by Maccaferri et al. [44]. Furthermore, the marker density aligned with the distribution patterns of the original 7K SNP set, specifically reflecting the lower abundance of SNPs on chromosome 4A and the higher concentration observed on chromosomes 1B and 3B.

The maximum observed linkage disequilibrium decay value was 0.4592, with the half-decay point ($r^2$ = 0.2296) at an average physical distance of 4.09 Mb across both genomes A and B, using a threshold of $r^2$ = 0.2. Accordingly, a ±4.09 Mb interval around each trait-associated SNP was considered as the confidence interval for the corresponding candidate genes (Figure 2).

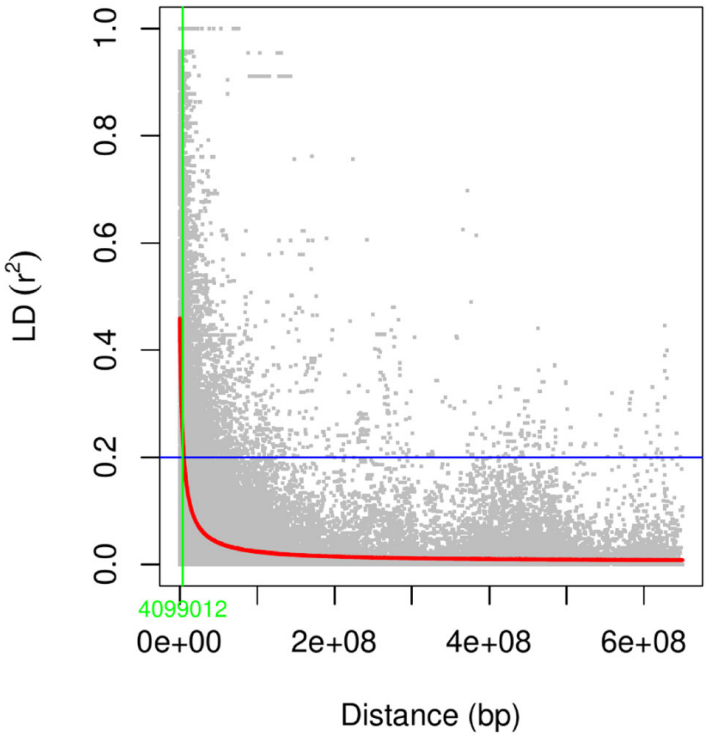


**Figure 2.** Scatter diagram showing the LD decay of the panel of durum wheat genotypes using a 2353 SNPs at an $r^2$ value of 0.2. The red line shows the smoothed LD decay, the blue horizontal line represents the $r^2$ threshold (0.2), and the vertical green line indicates the LD decay distance defined by the intersection of the red decay curve with the blue threshold line.

### *3.4. Population Structure*

Population structure analysis using STRUCTURE version 2.3.4 indicated an optimal number of two clusters (K = 2), based on the highest ΔK value (Figure 3a), allowing the discrimination of two main populations, with genotypes assigned to a given population according to the Q probability ($Q \geq 0.6$ indicating a pure line, $Q < 0.6$ indicating admixture) (Figure 3b, Table S5). The first population (q1), included 58 genotypes of durum wheat, distributed as follows: 10 from Algeria, 1 from Australia, 2 from France, 4 from Italy, 4 from Syria, 1 from Spain, 1 from the United States, 1 from Tunisia, 8 from CIMMYT, 12 from Mexico and 14 from ICARDA. The second population (q2) consisted of 28 genotypes, including 11 from Algeria, 1 from Cyprus, 1 from Iran, 2 from France, 4 from Italy, 3 from ICARDA, and one genotype from each of the following countries: Spain, Jordan, Tunisia, Mexico, the United States, and Syria (Table S5). Moreover, eight genotypes not assigned to either population 1 or 2, were identified as admixed.

The discriminant analysis of principal components (DAPC) method indicated that the optimal number of groups is four, based on the lowest value of the Bayesian information criterion (BIC) (Figure 3c). The scatter plot of the DAPC analysis revealed group sizes of 19, 48, 12, and 15 durum wheat genotypes, respectively, for groups 1, 2, 3, and 4 (Figure 3d, Table S5). The results of the DAPC analysis revealed an overlap between groups 2 and 3—consistent with the STRUCTURE method—which classified them in population 1; meanwhile a clear separation was observed between groups 1 and 4. Group 1 included nine accessions from Algeria, one from Spain, one from the USA., two from France, three from ICARDA, one from Italy, one from Tunisia, and one from Iran. Group 4 comprised three accessions from Italy, one from Jordan, two from Mexico, one from Syria, one from Cyprus, three from Algeria, one from Tunisia, two from ICARDA, and one from France.

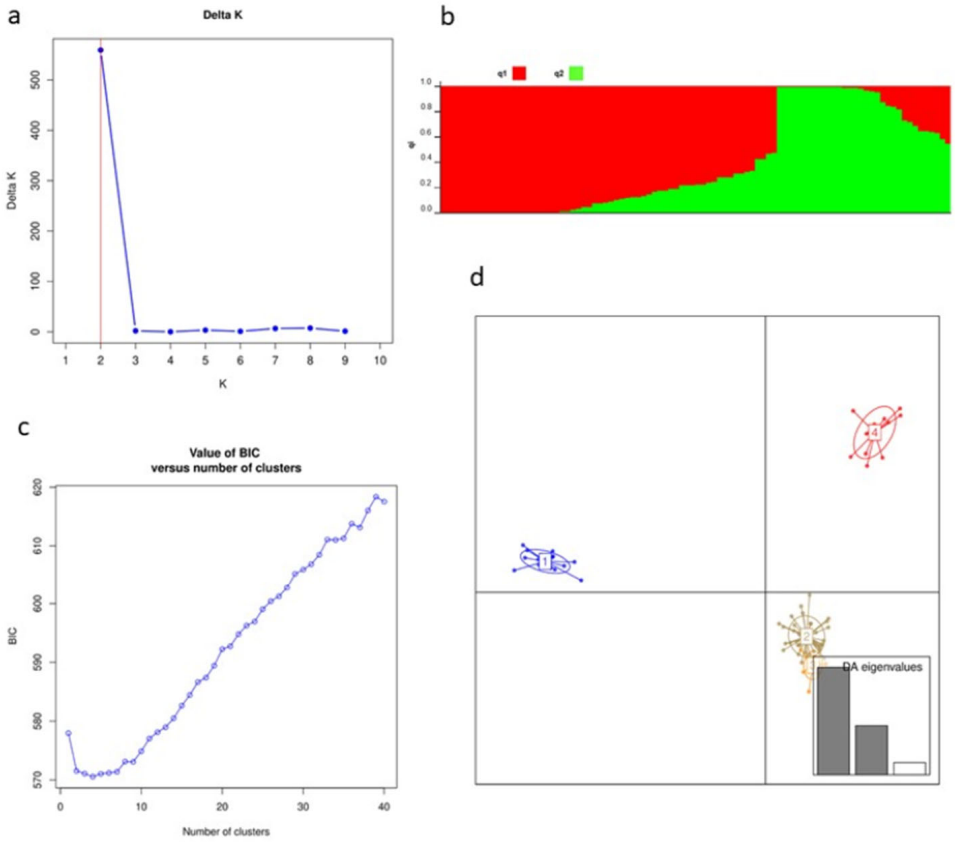


**Figure 3.** Population structure of the durum wheat panel, the vertical line in red indicates the optimal K value, corresponding to the highest delta K, and the blue curve represents the variation of ΔK values for K ranging from 1 to 10. (**a**). Determination of the value of K based on the Evanno delta K. (**b**). Population grouping of genotypes according to STRUCTURE results; q1 (red), q2 (green). (**c**). Number of optimal groups based on the BIC values. (**d**). Scatter plot of the DAPC analysis showing the different populations.

### *3.5. Marker-Trait Association Study*

Genome wide association analysis was performed using the set of 2353 filtered SNPs. The analysis identified a total of 27 distinct significant SNPs and 30 SNP—trait associations across eight measured traits (TKW, PH, PL, SL, NFSS, DH, CC, and FLA) (Table 2).

At the Algiers site, six traits (DH, TKW, PH, PL, SL, and NFSS) showed associations during the 2020–2021 season, whereas in 2021–2022, associations were observed for four traits (DH, PH, PL, and FLA) (Figure S3, Table 2). TKW exhibited the highest number of MTAs, identified in genomic regions 2A, 2B, 4B, 5B, and 7A. A pleiotropic effect was observed for the marker wsnp_Ex_c2033_3814035 on chromosome 2A, associated with DH and NFSS. Another pleiotropic marker TGWA25K-TG0010 on chromosome 4A was associated with PH and PL. The minor alleles of these markers were associated with reductions in the respective traits (Table 2).

At the Constantine site, three traits (PH, SL, and PL) were associated during the 2018–2019 season, while in 2020–2021, associations were observed for PH and PL (Figure S3, Table 2). SL exhibited the highest number of markers, detected in genomic regions 2B, 3A, 3B, and 6A. The marker wsnp_Ku_c51039_56457361 on chromosome 5A showed a pleiotropic effect on PH and PL.

Across all environments, the SNPs for DH, PH, and PL matched those detected at the Algiers site, including the marker TGWA25K-TG0010. Two additional SNPs were identified: one located on chromosome 5B, and another on chromosome 7A, associated

with CC (Figure S3, Table 2). These markers explained a substantial proportion of the phenotypic variance.

**Table 2.** List of SNP markers significantly associated with individuals and across environments.

| Environment | Traits | SNPs | Chr | Pos (bp) | −log10 (*p*) | Effect | PVE (%) |
|---|---|---|---|---|---|---|---|
| Alg 20–21 | SL | AX-158554307 | 7B | 126402042 | 8.66 | −0.75 | 46.25 |
| | | BS00068050_51 | 2A | 7171578 | 7.10 | 0.49 | 17.05 |
| | NFSS | BobWhite_c7454_492 | 3B | 9977207 | 6.80 | −1.81 | 30.54 |
| | | wsnp_Ex_c2033_3814035 | 2A | 36293464 | 5.40 | −1.48 | 28.52 |
| | DH | BS00078506_51 | 2B | 205979500 | 5.43 | −5.17 | 55.90 |
| | | wsnp_Ex_c2033_3814035 | 2A | 36293464 | 8.63 | −4.33 | 25.14 |
| | TKW | AX-158555676 | 2A | 84493443 | 6.80 | 4.14 | 21.37 |
| | | AX-158561628 | 2A | 685543691 | 6.14 | −3.87 | 14.92 |
| | | AX-158547515 | 2B | 1056812 | 9.77 | 4.85 | 16.44 |
| | | BS00108020_51 | 5B | 432748323 | 5.84 | 4.8 | 19.50 |
| | | TGWA25K-TG0269 | 7A | 571700366 | 5.76 | 3.01 | 11.32 |
| | | AX-94707905 | 4B | 34852445 | 9.54 | −3.23 | 7.54 |
| | PH | TGWA25K-TG0010 | 4A | 575089903 | 9.64 | −14.53 | 65.06 |
| | | AX-111656814 | 5A | 465829618 | 5.94 | 8.99 | 23.31 |
| | PL | TGWA25K-TG0010 | 4A | 575089903 | 5.25 | −4.3 | 70.87 |
| Alg 21–22 | PH | TGWA25K-TG0010 | 4A | 575089903 | 11.4 | −17.81 | 86.48 |
| | PL | TGWA25K-TG0010 | 4A | 575089903 | 8.49 | −5.08 | 80.09 |
| | DH | AX-158523625 | 3A | 720338696 | 5.37 | −12.03 | 70.32 |
| | FLA | BS00070991_51 | 1A | 578641384 | 5.97 | −5.65 | 34.31 |
| | | AX-158523970 | 3A | 34213247 | 4.78 | −4.47 | 28.75 |
| Const 18–19 | PH | TGWA25K-TG0010 | 4A | 575089903 | 12.6 | −14.24 | 74.19 |
| | | AX-111656814 | 5A | 465829618 | 5.53 | 6.94 | 15.60 |
| | PL | TGWA25K-TG0010 | 4A | 575089903 | 6.30 | −4.02 | 73.52 |
| | SL | AX-158575250 | 2B | 206985531 | 18.6 | −0.72 | 24.39 |
| | | Kukri_c4294_371 | 2B | 637407331 | 5.29 | 0.26 | 6.42 |
| | | AX-158538087 | 2B | 769288005 | 11.7 | −0.34 | 4.66 |
| | | AX-158523255 | 3A | 690801141 | 5.19 | 0.25 | 4.16 |
| | | AX-109276379 | 3B | 423388447 | 13.5 | 0.37 | 5.71 |
| | | Excalibur_c8197_381 | 6A | 16755164 | 13 | 0.43 | 17.15 |
| | | RFL_Contig2605_672 | 6A | 85672533 | 14.4 | 0.66 | 33.36 |
| Const 20–21 | PH | wsnp_Ex_c1255_2411550 | 1A | 581428253 | 4.73 | 8.09 | 39.86 |
| | | wsnp_Ku_c51039_56457361 | 5A | 491036134 | 6.84 | −8.23 | 41.99 |
| | PL | wsnp_Ku_c51039_56457361 | 5A | 491036134 | 4.84 | −3.24 | 59.19 |
| Across environments | DH | wsnp_Ex_c2033_3814035 | 2A | 36293464 | 5.05 | −1.97 | 14.94 |
| | | AX-158523625 | 3A | 720338696 | 9.11 | −8.63 | 61.31 |
| | CC | AX-95197108 | 7A | 87965802 | 6.17 | 3.00 | 68.11 |
| | PH | TGWA25K-TG0010 | 4A | 575089903 | 12 | −14.52 | 74.43 |
| | | AX-111656814 | 5A | 465829618 | 5.26 | 7.11 | 15.93 |
| | PL | TGWA25K-TG0010 | 4A | 575089903 | 7.93 | −3.88 | 79.48 |
| | SL | Excalibur_c97201_294 | 5B | 234455169 | 4.78 | 0.62 | 72.04 |

Pos—position; Chr—chromosome; PVE—phenotypic variance explained; DH—days to heading (days); PH—plant height (cm); FLA–flag leaf area (cm2); SL—spike length (cm); PL—peduncle length (cm); TKW—thousand kernels weight (g); NFSS—number of fertile spikelets per spike; CC—chlorophyll content. Localities: Alg = Algiers; Const = Constantine. ENV = Environment. Years18–19; 20–21; 21–22.

### *3.6. Functional Prediction and Annotation of Genes Located in Trait-Associated Loci*

Consistent with reports in durum germplasm, LD decay in this study reached the background threshold ($r^2 < 0.2$) at approximately 4 Mb [44,47]. This relatively slow decay is characteristic of the species' self-pollinating nature and the intensive selection pressure applied during modern breeding programs, which has resulted in large conserved genomic blocks [48]. Similar decay distances have been documented in Mediterranean durum wheat collections, suggesting that the current marker density is enough to capture most of the functional variation across the A and B genomes. Following the approach suggested by Gaur et al. [49], we initially searched for candidate genes within a 1-Mbp window flanking the significant SNPs; notably, all identified candidates were located within a narrow 3-kb range of the associated markers, indicating high mapping precision. To account for the extended linkage disequilibrium in durum wheat, an initial ±4.09 Mb window was also considered for functional annotation. Genes located within these intervals were retrieved based on high-confidence annotations and analyzed according to their functional annotation and homology with known genes in model plants and related cereals. The identified genes encoded diverse functional proteins, including kinases and transcription regulators involved in signal transduction, resistance-related proteins such as NBS-LRR and germins, biosynthetic enzymes including transferases and hydrolases, and structural or transport-related proteins such as kinesins, actin-related proteins, and ribosomal components (Table S6).

Several genes were identified as potential candidates based on previous studies. The genes *TRITD4Av1G194130.6* and *TRITD4Av1G194130.1*, both located on chromosome 4A and encoding the *dwarf8* and *Gibberellic-Acid Insensitive* (GAI) proteins, respectively, were associated with PH and PL. The same locus was also reported by Taranto et al. [50] as encoding the *Rht-A1* gene. The gene *TRITD2Av1G253030.2*, located on chromosome 2A and encoding a cytochrome P450-type protein, was associated with thousand-kernel weight, consistent with previous findings [51–54]. Another gene, *TRITD2Av1G039600.2* on chromosome 2A, encodes a glucosyltransferase also associated with TKW [55]. The gene *TRITD1Av1G227700.11* on chromosome 1A encodes a disease resistance protein (NBS-LRR class) was associated with FLA, in agreement with results reported by Bhatta et al. [56], while association of *TRITD2Av1G019050.3* on chromosome 2A, which encodes a NETWORKED 2D G protein, was detected for days to heading, consistent with findings from a study on Foxtail millet by Li et al. [57].

## 4. Discussion

### *4.1. Phenotypic Variability and Relation Among Traits Data*

This study revealed significant variability among genotypes for the evaluated traits, with performance differing across sites and cropping seasons. Beyond the main environmental effects, these differences were mainly explained by highly significant genotype × environment interactions, reflecting both the adaptive capacity of individual genotypes to specific climatic conditions and the genetic diversity of the material, which included landraces, old, and modern varieties.

According to Fellahi et al. [8], local landraces, although tall, late-maturing, and low-yielding, are still cultivated for their resilience to stress, high straw yield, and weed competitiveness, with plant height being advantageous under drought conditions. Similarly, Bapela et al. [5] reported that landraces remain underutilized in breeding programs due to limited characterization and the presence of undesirable alleles. Nevertheless, they might represent valuable sources of traits, such as drought tolerance.

The influence of site- specific climatic conditions on genotype responses was evident for DH, PH, PL, and TKW, which showed a mean reduction during the second cropping

season at the Constantine site (Const 20–21). This season was marked by prolonged drought from January to July, leading to a shortened vegetative cycle, reduced plant height, and lower grain weight. In contrast, CC increased on average at Const 20–21 but decreased at Alg 20–21. Atar et al. [58], highlighted that seasonal variations in chlorophyll content differ among species because of their genetic characteristics and are strongly affected by environmental conditions, particularly those specific to each site. Similarly, Anjum et al. [59] reported that higher chlorophyll concentrations indicate a greater potential for dry matter accumulation under oxidative stress induced by water and heat constraints. The contrasting rainfall and temperature regimes between Algiers and Constantine strongly influenced the expression of agronomic traits. In Algiers, milder temperatures and higher, more evenly distributed rainfall—particularly during the autumn period—favored rapid and uniform crop establishment at early developmental stages. These conditions promoted faster vegetative growth and development, which were reflected in improved growth- and yield-related traits such as plant height, flag leaf area, and thousand-kernel weight. Moreover, the accelerated crop development likely enabled a partial strategy of escape from terminal drought during grain filling and maturity. In contrast, the lower and more variable rainfall, greater thermal amplitude, and occurrence of frost events in Constantine, especially during the early growing season, likely hindered timely crop establishment, constrained plant development, delayed phenology, and reduced biomass accumulation. Overall, these results indicate that differences in temperature and rainfall patterns strongly influenced genotype performance, particularly for phenological, morphological, and physiological traits.

To further interpret the phenotypic responses observed across environments, relationships among the studied traits were examined. A positive correlation was observed between DH and PH, indicating that tall cultivars showed delayed heading but produced more straw [60]. A positive correlation was also observed between PH and PL, which have been suggested as key criteria for selecting genotypes under drought stress, given their role in nutrient accumulation and translocation to the grain [61]. In addition, SL showed a positive relationship with NFSS, consistent with the findings of Jamal et al. [62], who reported that increased spike length, along with a greater number of grains and spikelets per spike, contributes substantially to yield enhancement.

In contrast CC, showed a negative association with the measured parameters. According to Mansouri et al. [63], who investigated part of the same genetic panel, the absence of correlation between CC and other agro-morphological traits may reflect the breeding history of these genotypes, as chlorophyll content was not used as a direct selection criterion.

### *4.2. Linkage Disequilibrium and Population Structure*

In the present study, the average linkage disequilibrium decay distance (LD) was estimated to be 4.09 Mb. This finding is consistent with values reported in other studies [28,64]. All markers falling within an LD interval are assumed to represent the same quantitative trait locus (QTLs), and the genes located in this region are potentially involved in the regulation of the analyzed trait [28].

In this context, analyzing the genetic structure of durum wheat from diverse origins is essential for exploiting its diversity, identifying promising genotypes, and broadening the genetic base of modern varieties [65]. In this study, population structure analysis using the STRUCTURE software (version 2.3.4) identified two distinct populations. The first population mainly included cultivars originating from Algeria, CIMMYT, ICARDA, and Mexico, suggesting a shared genetic composition among these materials. The second population was largely composed of Algerian genotypes, most of which corresponded to landraces according to their passport data, along with a few accessions from other regions, reflecting a valuable source of allelic diversity Some cultivars with pedigrees involving

traditional landraces were also included. Similar patterns of genetic admixture between local and improved germplasm have been reported in previous studies on durum wheat, including Kabbaj et al. [66].

Using the DAPC method four distinct groups were identified, consistent with findings by Degu et al. [67] in barley. Tehseen et al. [68] noted that DAPC and PCA analyses are generally considered more reliable than STRUCTURE, especially since the value K = 2 in some cases may not accurately reflect the true genetic structure. Groups 2 and 3 overlapped, corresponding to population 1 in STRUCTURE, whereas groups 1 and 4 were clearly separated, although grouped in population 2 by STRUCTURE. The eight genotypes classified as admixed by STRUCTURE were distributed across the DAPC groups, reflecting their mixed ancestry and genetic divergence.

### *4.3. Associations Mapping (GWAS) and Candidate Genes*

Association mapping is a powerful approach for identifying genes associated with phenotypic traits. It complements QTL analysis by enabling the identification of shared genomic regions that involve multiple traits and major regulatory genes [69]. In this study, GWAS revealed multiple marker–trait associations across most chromosomes of the durum wheat genome, highlighting key loci controlling important agro-morphological traits. Accordingly, the SNPs identified in Algiers appeared to be associated with traits related to production and adaptation, whereas those detected in Constantine were linked to traits involved in adaptive mechanisms. These findings highlight the role of environment-specific conditions in determining locus expression. Candidate gene search strategies were guided by previously reported approaches [49,70], ensuring that only genes within a biologically meaningful distance from the SNP peak were considered. Although the limited size of our collection presents a potential risk for the identification of robust and significant MTAs, the SNPs associated with the target traits can be considered stable. This is evidenced by the fact that some of these associations have been previously identified and reported in the literature. This consistency supports the reliability and validity of our GWAS analysis, as previously demonstrated [71–74].

Starting with the phenological trait, days to heading, which reflects flowering time in wheat, is mainly regulated by genes controlling vernalization (*Vrn* genes), photoperiod sensitivity (*Ppd* genes), and earliness per se (*Eps* genes) [75]. In this study, three genomic regions associated with DH were detected on chromosomes 2A, 2B, and 3A, with similar associations previously reported on 2A [76] and 2B [77]. These loci therefore confirm previously reported genomic regions, whereas the association detected on chromosome 3A may represent a novel locus. The presence of the minor allele reduced the number of days to heading by 5, 3, and 12 days, respectively, suggesting a potential role in adaptation through flowering earliness. Three genes were identified for DH. On chromosome 2A, *TRITD2Av1G019050*, encoding the protein NETWORKED 2D G, matched the QTL *qHD2-2* and corresponds to the gene *Seita.2G44460* in foxtail millet, which was previously identified in a population of 313 RILs as a novel gene involved in response to photoperiod sensitivity [57]; its association with DH in wheat is reported here for the first time. On chromosome 2B, *TRITD2Bv1G078470* encodes an Alpha/Beta fold. The Alpha/Beta fold hydrolase constitutes a core structural framework for various phytohormone and ligand receptors, including those participating in gibberellin, strigolactone, and karrikin signaling [78]. Given that gibberellins regulate critical developmental processes such as seed germination, stem elongation, floral initiation, and the timing of flowering [79], it is possible to hypothesize that *TRITD2Bv1G078470* may influence heading earliness through GA-mediated flowering regulation, representing a novel association with DH in wheat. On chromosome 3A, *TRITD3Av1G273510.4* encodes a signal recognition particle protein,

previously defined as a protein for drought tolerance [80]. To our knowledge, this gene has not previously been linked to heading date in cereals, and thus this locus represents a potentially novel association with DH in wheat.

The NETWORKED 2D G protein—annotated for both days to heading (DH) and number of fertile spikelets per spike (NFSS)—showed in our study an association on chromosome 2A with DH and NFSS, suggesting a potential pleiotropic role of *TRITD2Av1G019050* in regulating these traits. This is consistent with Campana [81], who identified this gene in expression analysis of spikelet fertility, using the functional network KnetMiner to reveal shared functions with genes involved in flowering, heading date, and grain number, indicating a common genetic basis likely due to pleiotropy. Pleiotropic markers such as *TRITD2Av1G019050* are particularly valuable for breeding programs, as they enable the simultaneous selection of multiple favorable traits from a single locus, accelerating genetic improvement and enhancing crop performance and stability under challenging environmental conditions.

Plant height (PH) represents a fundamental component of wheat architecture and crop performance [82]. In our study, significant MTAs for PH were detected on chromosomes 1A, 4A, and 5A. The most prominent locus on chromosome 4A was significantly associated with both plant height and peduncle length, indicating a pleiotropic effect. This association was consistently detected across three individual environments and in the combined analysis, highlighting its stability and explaining the higher percentage of the phenotypic variance among all MTAs detected, representing a major effect locus and reducing plant height by approximately 14 cm, an effect advantageous for reducing lodging under high nitrogen fertilization [83] and improving adaptation to semi-arid environments by lowering evapotranspiration and facilitating assimilate translocation. Several detected loci overlapped with genomic regions previously reported for plant height, including Chr 1A [84], Chr 4A [50,85], and Chr 5A [76]. On chromosome 4A, the candidate genes *TRITD4Av1G194130.6* and *TRITD4Av1G194130.1*, corresponding to the *Dwarf8* and *GAI* genes, respectively, are orthologous to *GAI* in Arabidopsis and *d8* in maize [86]. The proximity of this region to the well-known *Rht-A1* locus, together with previous reports [50] linking a similar candidate gene to reduced height alleles *Rht-A1*. *Rht* genes, including *Rht-B1*, *Rht-D1*, and *Rht-A1*, were pivotal in the Green Revolution, as they confer semi-dwarf stature, reduce lodging, and improve harvest index [87] by reducing the response to gibberellin (gibberellin insensitive dwarfing genes) [84]. On chromosome 5A, two MTAs were identified: *TRITD5Av1G170950.3* encoding a basic helix–loop–helix (bHLH) transcription factor for PH, known for roles in plant development and stress adaptation [88], and *TRITD5Av1G180320.4* encoding a kinesin-like protein for both PH and PL. Kinesin, a molecular motor protein, is crucial for plant development, particularly in early growth stages, where it affects growth dynamics, yield, and quality [89]. Chen et al. [90] identified a total of 155 *Kinesin TaKIN* genes in wheat, carrying specific functional domains and hormone-responsive cis-elements (GA, Aux, SA, MeJa), their expression profiles across tissues and stages indicate a broad role in wheat growth and development. On chromosome 1A, *TRITD1Av1G229310.2* encodes a Phosphatase 2 protein, previously identified for coleoptile length [91] and involved in phytohormone signaling, growth regulation, and stress responses [92]. Interestingly, Sesiz et al. [91] reported a strong linkage disequilibrium (LD, $r^2$= 0.71) between *QCol.su.4BS* and *QPh.su.4BS*, loci associated with coleoptile length and plant height, respectively. The association detected here between *TRITD1Av1G229310.2* and plant height therefore supports and extends these previous findings in durum wheat. This relationship highlights opportunities for breeding durum wheat with long coleoptiles and reduced plant height.

Grain size, a major determinant of TKW, is a crucial yield trait in durum wheat, with numerous QTLs identified and functional markers providing useful tools for marker-assisted selection [32,93]. In the present study, some of these loci were also detected, notably on Chr 4B, 7A, and 2B [94], on Chr 2A [51,95], and on Chr 5B [96]. The two MTAs identified on Chr 2A correspond to the genes *TRITD2Av1G253030.2* and *TRITD2Av1G039600.2*, encoding cytochrome P450 and glucosyltransferase proteins, respectively. This finding is consistent with Sesiz [51], who reported MTAs QAS.su.2A1, implicated in area grain size and encoding a glucosyltransferase, and QTGW.su.2A2, encoding a cytochrome P450. Moreover, Ma et al. [97] demonstrated that a cytochrome P450 gene, *TaCYP78A3*, increases seed size and weight in wheat, suggesting that members of the P450 family may contribute to the phenotypic variation observed in our panel. In addition, the gene *TRITD5Bv1G144190.1*, encodes a protein kinase. According to Khan et al. [98], the kinase family is involved in regulating both grain number and size. Similarly, the gene *TRITD4Bv1G014180.1* on Chr 4B encodes a serine/threonine protein kinase. As reported by Ur Rehman et al. [99], these proteins play an essential role in increasing TKW and grain number per spike. GWAS in bread wheat linked serine/threonine-protein kinases and protein kinase-like genes to TKW, confirming their role in yield regulation [100]. On chromosome 2B, *TRITD2Bv1G000750.3* encodes for an AT-rich interactive domain-containing protein 2 (ARID). Family members of ARID play vital roles in the regulation of development and/or tissue-specific gene expression [101]. Gutiérrez et al. [102] identified *MTR_8g022980*, an ortholog in *Vicia faba* that encodes the same protein associated with the number of pods per plant, suggesting that this family may be involved in regulating factors related to productivity. This study represents the first report of a potential association between ARID domain-containing genes and yield component traits in durum wheat.

Flag leaves have morphological characteristics such as shape and area that contribute to wheat productivity, by affecting photosynthetic capacity and plant architecture [103]. In this study, flag leaf area (FLA) was found to be associated with chromosomes 1A and 3A [94]. Two genes were identified; *TRITD1Av1G227800.1*, located on chromosome 1A, encodes a leucine-rich repeat (LRR) family protein that is involved in various fundamental metabolic and signaling processes in plants, including growth, defense responses such as disease resistance signaling, and abiotic stress responses [104]. On chromosome 3A, *TRITD3Av1G017430.2* encodes for an F-box family protein. Baute et al. [105] reported that the *AtFBX92* gene, a member of the F-box protein family, suppresses leaf growth in Arabidopsis by regulating cell division during the early stages of leaf development.

Chlorophyll content (CC) of the flag leaf is an important key element for the photosynthesis trait for drought resistance in wheat under drought stress [33]. In this study, CC was associated with chromosome 7A, consistent with Huang et al. [106]. The identified MTA explained approximately 68% of the phenotypic variation, which is relatively high and represents a potentially novel association. This locus and corresponds to *TRITD7Av1G041160.1*, encoding strictosidine synthase (STR). STR is involved in the production of terpenoid indole alkaloids (TIAs) and β-carboline alkaloids (BCAs) [107]. These alkaloids belong to one of the most biologically active groups of natural metabolites, generally linked to plant defense responses and various ecological interactions [108]. Similarly, the *SSL6* (*strictosidine synthase-like*) gene in Arabidopsis appears to be involved in the regulation of chlorophyll under stress conditions, likely through its contribution to defense signaling and oxidative balance [109]. Previous studies have reported more than 80 QTLs associated with chlorophyll content, distributed across all wheat chromosomes, and explaining variable proportions of phenotypic variation [110,111]. However, little consistency was observed among them, mainly due to differences in measurement methods, developmental stages considered, and genetic backgrounds used [33].

Spike length (SL) is a key trait influencing lodging resistance and yield, making its genetic understanding essential for wheat improvement [112]. In this study, seven genomic regions associated with SL were identified on chromosomes 2A, 2B, 3A, 3B, 5B, 6A, and 7B, consistent with previous findings on 2A, 3A, 7B, 5B [17], and 2B [76].

Within these regions, two genes, *TRITD2Bv1G257970.2* and *TRITD3Av1G259110.1*, belong to the F-box family. According to Hong et al. [113], F-box genes participate in targeted protein degradation and are involved in flowering, seed development, leaf senescence, and hormone signaling, suggesting their possible influence on growth-related signaling pathways. Another gene, *TRITD3Bv1G135990.1*, corresponds to a zinc finger protein ZPR1. The zinc finger protein (ZFP) family is large and diverse, contributing to plant growth and development while also regulating stress responses under abiotic conditions [114]. *TRITD2Bv1G078930.1* was annotated as a small ubiquitin-related modifier (SUMO), which modulates cellular protein activity and contributes to stress tolerance [115]. On chromosome 6A, *TRITD6Av1G007220.1* corresponds to a kelch repeat-containing protein, a subclass of F-box proteins [116]. Similarly, Chen et al. [117] described an F-box protein with a Kelch repeat domain (OsFBK12) involved in regulating leaf senescence, seed size, and grain number in rice.

Another locus, *TRITD6Av1G037000.1*, was identified as a 26S proteasome non-ATPase regulatory subunit-like protein. Fatima et al. [118] reported in their study on seedling biomass and root traits under contrasting water regimes the presence of the gene *TraesCS4B02G049700* on chromosome 4B, which encodes a non-ATPase regulatory subunit (subunit 8) of the 26S proteasome, highlighting its potential role in wheat growth and development under different water regimes.

On chromosome 5B, the identification of a highly effective SNP explaining over 72% of the phenotypic variation represents a major novel finding for durum wheat breeding, highlighting a potentially unexploited regulatory mechanism for yield component stability. This SNP is associated with the gene *TRITD5Bv1G079720.1* (Xyloglucan 6-xylosyltransferase 1). Xyloglucan is one of the major hemicellulosic components of the primary cell wall in flowering plants [119]. Because it contributes to wall loosening during cell expansion, the genes involved in its biosynthesis are of particular interest [120]. In this context, Han et al. [121] demonstrated the important role of xyloglucan in primary cell wall remodeling during spike development in wheat.

## 5. Conclusions

In this study, a germplasm collection of 125 durum wheat genotypes was initially characterized for a set of morpho-agronomic traits related to the adaptation of the crop to semi-arid and sub-humid growing conditions. Subsequently, 94 of the most diverse accessions were genotyped using the Illumina Infinium 7K wheat array. GWAS identified 27 significant SNPs distributed across 13 chromosomes associated with key traits such as thousand-kernel weight, plant height, and days to heading under two contrasting Mediterranean sites. Candidate genes associated with these loci provide novel insights into the genetic architecture of these traits, offering potential targets for breeding programs in Algeria, where yield stability and adaptation to water-limited conditions are major priorities. Overall, the findings demonstrate the potential of marker-assisted selection to accelerate the development of improved durum wheat cultivars for Mediterranean environments.

Future studies would benefit from increasing both the population size and SNP density, along with extending evaluations across multiple environments and years. This would help capture greater genetic diversity and maximize the number of detected marker-trait associations. Such efforts would help us better understand the genetic basis of complex

traits evaluated in variable environments and support the development of durum wheat cultivars better adapted to future changes in climate conditions.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture16030370/s1, Figure S1: Total rainfall and the mean temperature across all studied environments. Localities: Alg = Algiers; Const = Constantine. Years = 18–19; 20–21; 21–22; Figure S2: Principal Component Analysis (PCA) of the 125 durum wheat genotypes based on morpho-agronomic traits; Figure S3: Genome-wide association (GWA) results obtained using the Multi-Locus Mixed Model (MLMM) across all studied environments (a) Manhattan plots showing the genomic associations identified for each trait, location, and year. The X-axis represents the chromosomes, while the Y-axis shows the $-\log_{10}(p)$ values. A significance threshold of $-\log_{10}(p) = 4.67$ was used to identify markers significantly associated with the traits. (b) Quantile–quantile (QQ) plots comparing the observed $-\log_{10}(p)$ values (Y-axis) with the expected distribution under the null hypothesis (X-axis, red line). Localities: Alg = Algiers; Const. = Constantine.; Traits: DH—days to heading (days); PH—plant height (cm); FLA—flag leaf area (cm2); SL—spike length (cm); PL—peduncle length (cm); TKW—thousand kernels weight (g); NFSS—number of fertile spikelets per spike; CC—chlorophyll content.; Table S1: List of 125 genotypes of durum wheat used for phenotyping; Table S2: List of 94 genotypes used for genotyping by 7K SNP Infinium array; Table S3: Descriptive analysis and heritability for the eight traits studied; Table S4: Distribution of SNPs and chromosome sizes across the 14 chromosomes of the durum wheat genome; Table S5: Genetic structure of the durum wheat panel based on STRUCTURE and DAPC analyses; Table S6: Putative genes and functional annotations associated with traits detected in the GWAS analysis.

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