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Mapping Agronomic and Quality Traits in Elite Durum Wheat Lines under Differing Water Regimes

Rosa Mérida-García 1, Alison R. Bentley 2, Sergio Gálvez 3, Gabriel Dorado 4, Ignacio Solís 5, Karim Ammar 6 and Pilar Hernandez 1,*

1 Institute for Sustainable Agriculture (IAS-CSIC), Consejo Superior de Investigaciones Científicas (CSIC), Alameda del Obispo s/n, 14004 Córdoba, Spain; rmerida@ias.csic.es
2 The John Bingham Laboratory, NIAB, Huntingdon Road, Cambridge CB3 0LE, UK; alison.bentley@niab.com
3 Departamento de Lenguajes y Ciencias de la Computación, Campus de Teatinos s/n, Universidad de Málaga, Andalucía Tech, ETSI Informática, 29071 Málaga, Spain; galvez@uma.es
4 Departamento de Bioquímica y Biología Molecular, Campus Rabanales C6-1-E17, Campus de Excelencia Internacional Agroalimentario (ceiA3), Universidad de Córdoba, 14071 Córdoba, Spain; bb1dopeg@uco.es
5 ETSIA (University of Seville), Ctra de Utrera km1, 41013 Seville, Spain; isolis@agrovegetal.es
6 International Maize and Wheat Improvement Center (CIMMYT), C.A.P. Plaza Galerías, Col. Verónica Anzures, Ciudad de Mexico 11305, Mexico; k.ammar@cgiar.org

* Correspondence: phernandez@ias.csic.es

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Abstract: Final grain production and quality in durum wheat are affected by biotic and abiotic stresses. The association mapping (AM) approach is useful for dissecting the genetic control of quantitative traits, with the aim of increasing final wheat production under stress conditions. In this study, we used AM analyses to detect quantitative trait loci (QTL) underlying agronomic and quality traits in a collection of 294 elite durum wheat lines from CIMMYT (International Maize and Wheat Improvement Center), grown under different water regimes over four growing seasons. Thirty-seven significant marker-trait associations (MTAs) were detected for sedimentation volume (SV) and thousand kernel weight (TKW), located on chromosomes 1B and 2A, respectively. The QTL loci found were then confirmed with several AM analyses, which revealed 12 sedimentation index (SDS) MTAs and two additional loci for SV (4A) and yellow rust (1B). A candidate gene analysis of the identified genomic regions detected a cluster of 25 genes encoding blue copper proteins in chromosome 1B, with homoeologs in the two durum wheat subgenomes, and an ubiquinone biosynthesis O-methyltransferase gene. On chromosome 2A, several genes related to photosynthetic processes and metabolic pathways were found in proximity to the markers associated with TKW. These results are of potential use for subsequent application in marker-assisted durum wheat-breeding programs.

Keywords: durum wheat; genome wide association study; GWAS water use; agronomic traits; MTAs; candidate genes; TKW; sedimentation volume; SDS; YR

1. Introduction

Wheat is one of the most widely grown crops worldwide (FAO, 2015), and is essential for the human diet [1]. Its importance and worldwide dominance are due, in part, to its agronomic adaptability. Durum wheat (Triticum durum) is a tetraploid wheat species (AABB genomes) mainly grown in the Mediterranean basin, in the Northern Plains (between the USA and Canada), in the arid areas of South Western USA and in Northern Mexico [2]. Durum wheat is well-adapted to a broad range of climatic conditions (including dry environments) and marginal soils, and has low water requirements [3,4]. Climatic conditions, as temperature and water availability, together with biotic stresses, can strongly affect durum wheat development and production [3–6]. Crop adaptation
is a central objective for breeding progress, driving improvement in final production, quantity and quality [7,8]. For over two decades, CIMMYT (International Maize and Wheat Improvement Center) has had an intensive breeding and improvement programme focused on the acceleration of durum productivity in developing countries.

Grain quality is an important breeding aim determining product end-use linked to financial returns. It is influenced by both genetic and environmental conditions [9], and biotic and abiotic stresses during growth and at key development stages [10]. Temperature, water availability and soil properties, especially nitrogen content, influence the final quality and protein content of wheat and its end-products [11–13].

There is a growing need to increase wheat yield without losing grain quality [14,15]. Key end-use grain quality traits include grain protein content (GPC), gluten strength, kernel size and vitreousness [7,16] and are all influenced by climatic conditions [17]. A number of agronomic components influence final productivity, including phenology (maturity) and plant architecture (plant height and lodging resistance). The majority of important agronomic traits, including yield, are controlled or influenced by multiple genes and are quantitatively inherited [18]. In addition, most are influenced by the environment and interactions between environmental and genetic (GxE) effects [19–23]. One of the most common methods currently used for dissection of quantitative agronomic and quality traits is the association mapping (AM) approach [24].

AM, originating in human genetics, was initially combined with linkage disequilibrium (LD) to identify the role of genes and linked markers for the determination of disease loci [25]. It is now widely used in plant and crop genetics. Some of the first studies based on LD mapping applied in plants were done in maize [26], rice [27] and oat [28]. AM has the main objective of determining, based on LD, correlations between genotypes and phenotypes in a panel of selected individuals [29]. It can support the development of new genetic markers for use in marker-assisted plant breeding [30]. It also facilitates the analysis of genetic variation underlying traits for further characterisation of the loci of interest [31].

Single nucleotide polymorphism (SNP) markers are commonly used in quantitative trait loci (QTL) mapping experiments [32,33] and genome-wide association studies (GWAS) for the detection of marker-traits associations (MTAs) in wheat [34–38]. DArTseq, a variant of the microarray-based DArT technology, has also been widely used in QTL mapping [39–41]. It reduces the complexity of the genome, using combinations of restriction enzymes [42] and next-generation sequencing. Several studies have assessed MTAs in durum wheat. The analyzed traits include grain yield, yield and yield components [6,37,43,44], heading date [6], and grain quality traits (thousand kernel weight, vitreousness, protein content, sedimentation index [17,45–47], yellow colour [48,49]).

In this study, three panels of elite durum wheat lines from CIMMYT were assessed in field trials conducted over multiple seasons and with differing water regimes. The AM approach was used to detect SNP and DArT markers associated with heterogeneous agronomic and quality trait data in order to test the approach as a tool for marker discovery within a live and ongoing breeding programme.

2. Material and Methods

2.1. Plant Material, Phenotyping and Genotyping

Panels of elite durum wheat lines from CIMMYT were assessed in field trials conducted over multiple seasons and with differing water regimes. The AM approach was used to detect SNP and DArT markers associated with heterogeneous agronomic and quality trait data in order to test the approach as a tool for marker discovery within a live and ongoing breeding programme.
field seasons is included in Supplementary Materials Figure S1. The agronomic and quality assessment of the panels over seasons is summarised in Table 1.

Table 1. Agronomic and quality assessment of wheat field trials. The number of lines, year, location and water regime applied is shown.

| Wheat Panel | No. of Assessed Lines | Field Season   |
|-------------|-----------------------|----------------|
| 1           | 98                    | YAQ-FI         |
| 2           | 97                    | YAQ-FI YAQ-RI  |
| 3           | 98                    | YAQ-FI YAQ-RI  |

YAQ: Yaqui (Mexico); FI: Full irrigation; RI: Reduced irrigation.

Field experiments were conducted at CIMMYT’s experimental station in the Yaqui Valley, Mexico (27.282848° N; 109.923878° W) over four field seasons (2012 to 2015 harvest years, inclusive). Wheat panels 2 and 3 were phenotypically assessed across years, while panel 1 was only grown in 2012. The experimental plots (1.6 × 3 m) were sown in November/December of each year and harvested in May of the following year. Data for yellow rust were assessed in semi-controlled conditions at CIMMYT’s experimental station in Toluca (Mexico).

Plant material for genetic analysis was harvested for each line at the 4th leaf stage (growth stage 14 on the Zadoks scale [51]) and immediately frozen in dry-ice. Samples were stored at −80 °C until DNA extraction. Approximately 100 mg of frozen tissue was used for DNA isolation with a DNeasy Plant Mini Kit from Qiagen, following the manufacturer’s protocol. DNA sample quality and concentration were assessed using electrophoresis on a 0.8% agarose gel and the restriction enzyme TruII (ThermoFisher) was used to check for the absence of nucleases in DNA prior to genotyping.

Samples were genotyped by Diversity Arrays Technology Pty Ltd. (Montana St, University of Canberra, Bruce ACT 2617, Australia) (DArT) using DartSeq™. A total of 35,509 polymorphic dominant DArT markers and 9142 biallelic SNP markers were generated. Both datasets were thinned by removing one marker from each pair with a correlation coefficient of >0.95. The final dataset consisted on 14,588 DArT markers (of which 8411 were mapped) and 5716 SNP markers (4142 mapped markers). DartSeq™ genotyping and mapping of the corresponding markers to the wheat reference genome sequence ReSeq v1 from the International Wheat Genome Sequencing Consortium (IWGSC, http://www.wheatgenome.org/) was performed by DArT (diversityarrays.com), as described by Sukumaran et al. [43]. The distribution of markers across the A and B subgenomes is given in Table 2.

Table 2. Molecular markers distribution across the wheat genome. The distribution of DArT (Diversity Arrays Technology, left) and single nucleotide polymorphism (SNP, right) markers across the durum wheat A and B subgenomes and the number of unmapped markers are shown.

| Chr | A   | B   | Un  | Total 1 | A   | B   | Un  | Total 1 |
|-----|-----|-----|-----|---------|-----|-----|-----|---------|
| 1   | 577 | 866 | 37  | 1280    | 193 | 320 | 375 | 16      |
| 2   | 563 | 791 | 69  | 1423    | 320 | 375 | 19  | 714     |
| 3   | 496 | 818 | 33  | 1347    | 250 | 352 | 11  | 613     |
| 4   | 585 | 325 | 10  | 920     | 283 | 171 | 4   | 458     |
| 5   | 312 | 725 | 13  | 1050    | 162 | 376 | 2   | 540     |
| 6   | 449 | 690 | 21  | 1160    | 262 | 308 | 5   | 575     |
| 7   | 623 | 573 | 35  | 1231    | 293 | 287 | 9   | 589     |
| Total 2 | 3405 | 4788 | 218 | 8411    | 1763 | 2313 | 66  | 4142    |
| Un  | 6177 | 1574 |     |         |     |     |     |         |

Total 14,588 5716

Chr: chromosome; A: wheat A subgenome; B: wheat B subgenome; Un: unmapped; 1: total markers by group; 2: total markers by genome.
2.2. Phenotypic Data

Ten agronomic and quality traits were assessed for three durum wheat elite line panels: days to heading (days, DTH); plant height (cm, PH); lodging (%, LOD); yellow rust (% YR); yellow colour (YC); sedimentation index (cm$^3$, SDS); sedimentation volume (cm$^3$, SV); grain protein content (% GPC); thousand kernel weight (g, TKW); and grain yield (Kg/ha, GY). Agronomic traits (DTH, PH, LOD, YR, TKW and GY) were assessed under both water treatments (FI and RI), and quality traits (YC, SV, SDS and GPC) were only evaluated under FI conditions. Visual disease evaluation and phenology assessments were made in the field, while quality parameters were evaluated on grain samples post-harvest. DTH, PH and LOD and YR were visually assessed at the field trials in Yaqui, while YR was assessed at Toluca. To assess DTH, heading date was recorded as the time when 50% of the spikes have emerged from the flag leaf sheath (stage 59 in Zadoks scale [51]); PH was recorded by measuring the distance between the stem’s base and the top of the spike (awns not included); LOD was assessed as the percentage of lodging plot; and YR was assessed as the percentage of leaves with rust pustules. YC was assessed by a rapid colorimetric method with a Minolta color meter following CEN/TS 15,465:2008 [52–54]; SDS was evaluated following UNE 34,903:2014 [55,56]; SV and GPC were assessed by Near-infrared spectroscopy (NIRs) [57]; TKW was measured by weighing 2 samples of 100 entire kernels randomly chosen previously dried at 70 °C for 48 h.

The correlation between the assessed traits was analysed using the ‘cor’ function in R [58–60]. Then, an analysis of variance (ANOVA) was undertaken, using the ‘aov’ function in R [61], to obtain the descriptive statistics for each trait.

Traits were analysed using a Q + K linear mixed-model [62,63] which follows the model equation:

$$y = X\beta + S\alpha + Qv + Z\mu + \varepsilon$$

(1)

where $y$ is a vector of observed phenotypes; $X$, $S$ and $Z$ are matrices related to $\beta$, $\alpha$ and $\mu$, respectively; $\beta$ is a vector of fixed effects; $\alpha$ is a vector of marker effects; $Qv$ is a vector of population effect; $\mu$ is a vector of polygenic effects (with covariance proportional to a kinship or relationship matrix); and $\varepsilon$ is a vector of residuals.

These analyses were carried out using GenStat (14th Edition) to generate the best linear unbiased estimates (BLUEs) of variety performance in different ways: (i) across years and blocks; (ii) across years for each block (FI and RI); (iii) across a reduced dataset (years 2013 and 2014) and blocks; and (iv) across the reduced dataset for each block. The resulting datasets (available in Supplementary Material Table S2) were then used in different association mapping analyses.

2.3. Population Structure and Linkage Disequilibrium

Population structure was assessed using principal component analysis (PCA) based on the combined DAriT and SNP genotyping datasets. Euclidean distances were calculated using the R package ‘ggfortify’ [64] and the PCA was visualised with ‘ggplot2’ [65].

The pattern of linkage disequilibrium (LD) was assessed between each pair of SNP markers on the same chromosome across the two constitutive genomes with the allele frequency correlation ($r^2$) using the ‘popgen’ package in R [66]. A heatmap was obtained with the $D'$ and $r^2$ values for each chromosome and a scatterplot to determine LD decay (genetic distance in cM).

2.4. Association Mapping (AM)

The AM analyses were performed on the BLUEs obtained above using an additive model with ‘rrBLUP’ [67] and ‘GWASpoly’ [68] packages in R in different ways. Two marker-based kinship matrices (k-matrix), created from a subset of 14,588 DAriT and 5716 SNP markers, respectively, were used for the adjustment based on relatedness of individuals (Supplementary Materials Tables S3 and S4). A minor allele frequency (maf) threshold of 0.05 was used. To establish a $p$-value detection threshold
for statistical significance of associations, the Bonferroni correction, which employs a threshold of $\alpha/m$ to ensure the genome-wide type error I of 0.05, was applied with a total of 1000 permutations.

Associated DartSeq$^{\text{TM}}$ and SNP markers were blasted against the wheat reference assembly RefSeqv1 [69] with no indels or mismatches allowed, using an ad hoc Java program, to confirm their physical mapping location on the A or B genomes. The molecular markers were also mapped against the durum wheat genome (https://www.interomics.eu/durum-wheat-genome) to confirm their physical positions. In addition, to identify candidate genes, results were filtered, selecting the hits with best $e$-value for each molecular marker, and candidate genes were manually selected based on their annotations.

3. Results

3.1. Phenotypic Assessment

Results from the ANOVA for all the traits across years and water treatments are shown in Table 3. The mean phenotypic values across years were calculated for each block and panel to evaluate the influence of water conditions on the assessed traits (Table 3). Days to heading during the field seasons assessed ranged from 63 to 94 days. In plots with lower water availability (RI block), the spike emergence from the flag leaf took place approximately 11 days earlier than in FI plots. Plant height ranged from 39 to 110 cm showing differences between water regimes, with a decrease of 25–30 cm under RI conditions. Likewise, and as result of the RI treatment, GY (ranging from 1.35 to 10.63 ton/ha) and TKW (from 29.6 to 63.2 g) also varied, being reduced by 4–5 tons/ha and 7–10 g, respectively, in the low water availability RI treatment. This strong RI treatment resulted in very low heritability values for DTH, PH and LOD.

Several significant phenotypic correlations were observed between the analysed traits (Figure 1 and Supplementary Materials Table S5). The most correlated traits were PH and GY ($r = 0.90$, $p$-value $< 2.2 \times 10^{-16}$), followed by DTH and GY ($r = 0.87$, $p$-value $< 2.2 \times 10^{-16}$), SDS and SV ($r = 0.85$, $p$-value $< 2.2 \times 10^{-16}$) and also DTH and PH ($r = 0.82$, $p$-value $< 2.2 \times 10^{-16}$). Other traits showed important positive correlations too, including YC and DTH ($r = 0.69$, $p$-value $= 2.82 \times 10^{-09}$), GY and TKW ($r = 0.66$, $p$-value $< 2.2 \times 10^{-16}$), PH and TKW ($r = 0.62$, $p$-value $< 2.2 \times 10^{-16}$), GY and YC ($r = 0.53$, $p$-value $= 0.039$) and DTH and TKW ($r = 0.49$, $p$-value $< 2.2 \times 10^{-16}$). Negative correlations were also observed for SDS and GPC ($r = −0.38$, $p$-value $< 2.2 \times 10^{-16}$), and for GPC and YC ($r = −0.08$, $p$-value $< 2.2 \times 10^{-16}$) (Figure 1).

![Figure 1. Phenotypic correlations. YR: yellow rust; DTH: days to heading; PH: plant height; GY: grain yield; TKW: thousand kernel weight; YC: yellow colour; SV: sedimentation volume; SDS: sedimentation index; and GPC: grain protein content.](image-url)
Table 3. Summary of overall phenotypic data including analysis of variance (ANOVA) results and broad sense heritability ($h^2$) for agronomic and quality traits.

| Panel          | Min 1 | Mean 2 | Max 3 | Df  | Sum Sq | Mean Sq | F Value | Pr (>F) | Significance | $h^2$  | 1  | 2  | 2  | 3  | 3  |
|----------------|-------|--------|-------|-----|--------|---------|---------|---------|--------------|-------|----|----|----|----|----|
| Water regimes  |       |        |       |     |        |         |         |         |              |       |    |    |    |    |    |
| DTH (days)     | 63    | 79.38  | 94    | 1   | 1.8    | 1.768   | 0.24    | 0.626   | ***          | 0.14  | 87.55 | 83.73 | 72.54 | 81.95 | 70.51 |
| PH (cm)        | 39    | 81.97  | 110   | 1   | 167.5  | 167.51  | 8.025   | 6.59 x 10^-03 | **          | 0.16  | 92.98 | 93.31 | 62.58 | 89.32 | 63.74 |
| GY (ton/ha)    | 1.35  | 5.68   | 10.63 | 1   | 24.52  | 24.516  | 121.6   | 4.16 x 10^-15 | ***         | 0.44  | 10   | 7   | 2.03 | 6   | 2.33 |
| TKW (g)        | 29.6  | 44.69  | 63.2  | 1   | 38.6   | 38.65   | 2.064   | 1.57 x 10^-03 | -            | 0.41  | 49.25 | 47.58 | 37.29 | 47.06 | 40.22 |
| YR             | 0     | 2.286  | 40    | 1   | 358.5  | 358.5   | 6.702   | 1.25 x 10^-02 | *            | 0.00  | 4.77  | 3.98  | 0    | 0    | 0    |
| LOD (%)        | 0     | 2.142  | 90    | 1   | 0      | 0       | -       | -       | -            | 0.01  | 0.1   | 0    | 9.25 | 0    | -    |
| YC             | 14.6  | 16.59  | 20    | 1   | 0.008  | 0.0085  | 0.02    | 8.89 x 10^-03 | -            | 0.59  | 17.21 | 17.09 | -    | 16.35 | -    |
| SV (ml)        | 7     | 10.51  | 14.5  | 1   | 2.54   | 2.536   | 2.388   | 0.128   | -            | 0.57  | 10.87 | 10.14 | -    | 10.36 | -    |
| SDS            | 0.54  | 0.854  | 1.19  | 1   | 0.0927 | 0.09268 | 14.86   | 3.25 x 10^-04 | ***          | 0.55  | 1     | 1     | -    | 1    | -    |
| GPC (%)        | 10.4  | 12.33  | 14.9  | 1   | 5.965  | 5.965   | 16.41   | 1.74 x 10^-04 | ***          | 0.31  | 11.67 | 12.38 | -    | 12.12 | -    |

DTH: days to heading; PH: plant height; LOD: lodging; GY: grain yield; TKW: thousand kernel weight; YR: yellow rust; LOD: lodging; YC: yellow color; SV: sedimentation volume; SDS: sedimentation index; GPC: grain protein content; FI: full irrigation block; RI: reduced irrigation block; 1: minimum value across years and water regimes; 2: mean across years and water regimes; 3: maximum value across years and water regimes; Df: degrees of freedom; Sum Sq: sum square; F value: measure of significance in the F-test; Pr (>F): p-value associated with the F statistic; Significance levels: 0 = ‘***’; 0.001 = ‘**’; 0.01 = ‘*’; 0.1 = ‘’; Df = degrees of freedom.
3.2. Population Structure and Linkage Disequilibrium

The PCA used a total of 14,588 DArT and 5716 SNP markers. The first and second principal components explained 3.91% of the genetic variation (Figure 2). No underlying genetic structure was detected within or between the panels assessed. LD was estimated using the mapped SNP markers dataset. LD decay was determined within 20–30 cM for all the chromosomes (Figure 3). Using the classification defined by Maccaferri et al. [70], the markers presented loose linkage (Class 2), showing a distance value between 21 to 50 cM.

Figure 2. Principal components analysis (PCA) of the genotypic data.
Figure 3. Linkage disequilibrium (LD) decay analysis using SNP data. Estimates for $r^2$ versus linkage distance on chromosome in centimorgan (cM) was represented. The LD decay was established between 20–30 cM.
3.3. AM Analysis

Thirty-seven significant marker-trait associations (MTAs) were detected for TKW and SV across all years and water treatments with most of the significant markers located on chromosome 2A (Table 4). Twenty DArT and seven SNP markers were found in association with TKW on chromosome 2A (with additive effects ranging from $-3.41$ to $3.46$). In addition, eight unmapped DArT and one SNP marker were also associated with TKW (additive effects ranged from $-3.39$ to $3.46$ g). Most of these MTAs showed a negative additive effect, reducing the final weight value (ranging from $-2.84$ to $-3.19$ g), and only two MTAs were found to increase TKW (values of $2.97$ and $3.09$ g). Finally, a single SNP associated with SV was located on chromosome 1B (showing a positive effect increasing the final value by $1.26$ mL). The resulting Manhattan and QQ-plots from this AM analysis are included in Supplementary Materials Figures S2–S5.

The AM analyses on partitioned subsets of the data consistently detected the QTLs for TKW and SV. Nevertheless, the individual assessment of the water treatments significantly reduced the number of MTAs found, due in part to less available data for the RI block (Supplementary Material Table S6). The initial dataset of 294 durum wheat elite lines was reduced to 200 lines (assessed during the 2013 and 2014 seasons) to give a dataset balanced across assessment years. Using this reduced dataset for AM analysis, the results confirmed the QTLs previously found for the full dataset (Supplementary Materials Table S6). The analysis also detected an additional locus for SV on chromosome 4A, and a locus for YR on chromosome 1B (with additive effects of $-0.84$ and $2.79$, respectively).
Table 4. Significant marker-trait associations found in durum wheat elite lines across years and water treatments.

| Trait | Threshold | Marker | Chromosome | Pos (cM) | \(-\log_{10}(p\text{-Value})\) | Marker Effect | Mapping in Pseudomolecule | Physical Pos (bp) | Mapping in Durum | Physical Pos (bp) |
|-------|-----------|--------|------------|----------|----------------------------|----------------|---------------------------|------------------|------------------|------------------|
| SV    | 5.09      | SNP620 | 1B         | 139.21   | 5.5                         | 2.6            | 1B                        | 555,056,387      | 1B               | 547,593,323      |
| TKW   | 5.71      | DArT3154 | 2A       | 60.5     | 6.42                        | 3.21           | 2A                       | 533,610,520      | 2A               | 527,494,277      |
| TKW   | 5.71      | DArT3155 | 2A       | 60.5     | 6.11                        | 3.1            | 2A                       | 174,036,184      | 2A               | 171,903,830      |
| TKW   | 5.71      | DArT3156 | 2A       | 60.5     | 7.28                        | 3.41           | 1B                       | 134,638,820      | 2A               | 127,479,665      |
| TKW   | 5.09      | SNP1153 | 2A         | 68.47    | 5.38                        | 3.37           | 2A                       | 532,153,681      | 2A               | 526,046,873      |
| TKW   | 5.71      | DArT3146 | 2A       | 69.27    | 7.1                         | 3.41           | 2A                       | 566,208,089      | 2A               | 559,043,176      |
| TKW   | 5.71      | DArT3150 | 2A       | 69.42    | 7.23                        | 3.4            | 2A                       | 541,302,108      | 2A               | 535,046,952      |
| TKW   | 5.71      | DArT3162 | 2A       | 70.06    | 6.71                        | 3.31           | 2A                       | 535,235,854      | 2A               | 529,032,623      |
| TKW   | 5.09      | SNP1183 | 2A         | 70.31    | 6.45                        | 3.15           | 2A                       | 541,200,911      | 2A               | 534,959,463      |
| TKW   | 5.09      | SNP1184 | 2A         | 70.31    | 5.45                        | 2.86           | 2A                       | 541,391,854      | 2A               | 535,114,521      |
| TKW   | 5.09      | SNP1185 | 2A         | 70.31    | 5.45                        | 2.86           | 2A                       | 541,391,854      | 2A               | 535,114,521      |
| TKW   | 5.71      | DArT3165 | 2A       | 70.31    | 7.17                        | 3.46           | 2A                       | 541,391,851      | 2A               | 534,959,463      |
| TKW   | 5.71      | DArT3169 | 2A       | 70.53    | 7                           | 3.42           | 2B                       | 477,405,138      | 2A               | 534,564,999      |
| TKW   | 5.71      | DArT3172 | 2A       | 70.84    | 6.46                        | 3.16           | 2A                       | 542,687,204      | 2A               | 536,453,217      |
| TKW   | 5.71      | DArT3174 | 2A       | 70.96    | 5.91                        | 3.13           | 2A                       | 567,734,347      | 2A               | 557,562,938      |
| TKW   | 5.71      | DArT3175 | 2A       | 71.04    | 6.7                         | 3.37           | 2A                       | 566,457,122      | 2A               | 558,803,456      |
| TKW   | 5.71      | DArT3176 | 2A       | 71.14    | 6.13                        | 3.2            | 2A                       | 544,391,768      | 2A               | 538,104,252      |
| TKW   | 5.71      | DArT3180 | 2A       | 71.38    | 7.03                        | 3.43           | 2A                       | 567,736,123      | 2A               | 557,501,162      |
| TKW   | 5.71      | DArT3181 | 2A       | 71.38    | 6.2                         | 3.2            | 2A                       | 582,287,689      | 2A               | 551,184,512      |
| TKW   | 5.71      | DArT3182 | 2A       | 71.38    | 6.41                        | 3.27           | 2A                       | 569,404,524      | 2A               | 555,838,382      |
| TKW   | 5.09      | SNP1198 | 2A         | 71.64    | 6.39                        | 3.09           | 2A                       | 572,356,489      | 2A               | 552,087,972      |
| TKW   | 5.09      | SNP1199 | 2A         | 71.75    | 5.75                        | 2.97           | 2A                       | 567,737,911      | 2A               | 557,449,430      |
| TKW   | 5.71      | DArT3187 | 2A       | 71.94    | 7.07                        | 3.35           | 2A                       | 541,302,102      | 2A               | 535,046,946      |
| TKW   | 5.71      | DArT3198 | 2A       | 72.36    | 6.47                        | 3.24           | 2A                       | 535,235,860      | 2A               | 529,032,620      |
| TKW   | 5.71      | DArT3201 | 2A       | 72.56    | 6.19                        | 3.12           | 2A                       | 569,404,462      | 2A               | 555,838,444      |
| TKW   | 5.71      | DArT3206 | -        | -        | 6.16                        | 3.16           | 2A                       | 566,964,200      | 2A               | 556,292,372      |
| TKW   | 5.09      | SNP3905 | -        | -        | 6.41                        | 2.85           | 2A                       | 532,853,960      | 2A               | 526,751,856      |
| TKW   | 5.71      | DArT20759 | -     | -        | 7.23                        | 3.43           | 2A                       | 541,302,217      | 2A               | 535,047,061      |
| TKW   | 5.71      | DArT20961 | -     | -        | 7.17                        | 3.46           | 2A                       | 532,080,607      | 2A               | 525,972,283      |
| TKW   | 5.71      | DArT21317 | -     | -        | 6.06                        | 3.18           | 2A                       | 568,431,288      | 2A               | 556,806,491      |
| TKW   | 5.71      | DArT21609 | -     | -        | 7.04                        | 3.36           | 2A                       | -                | -                | -                |
| TKW   | 5.71      | DArT21773 | -     | -        | 6.34                        | 3.17           | 2A                       | -                | -                | -                |
| TKW   | 5.71      | DArT21834 | -     | -        | 6.35                        | 3.28           | 2A                       | 546,445,800      | 2A               | 540,140,602      |
| TKW   | 5.71      | DArT22064 | -     | -        | 6.75                        | 3.39           | 2A                       | -                | -                | 549,657,924      |

Pos (cM): position in chromosome in centimorgan; bp: base pairs; SV: sedimentation volume; TKW: thousand kernel weight; “-”: unmapped marker; 1: physical position based on the wheat reference assembly RefSeqv1 [69]; 2: physical position based on the durum wheat assembly [71].
3.4. Candidate Genes Analysis

The marker SNP620, located on chromosome 1B and detected in association with SV, was found included into a cluster of 12 genes encoding blue copper proteins (BCP), with homeologs in the two durum wheat subgenomes (Figure 4, Table 5 and Supplementary Material Figure S6). In the hexaploid wheat genome, this set of genes form a cluster of homeolog triads [72] with a total of 31 genes (Supplementary Materials Table S7 and Figure S7). Additionally, another interesting gene (TraesCS1B01G568400LC.1) was found closer this marker, coding for the ubiquinone biosynthesis O-methyltransferase.

There were several markers located in chromosome 2A, in close proximity to some interesting genes. Markers SNP1183, SNP1184 and DArT3165 were found next to several genes encoding reductase-1 (Figure 4 and Table 5). In addition, the marker SNP8395, also located on the same chromosome, was found in proximity to the gene TraesCS2A01G309700.1, which encodes a type A response regulator 1 (Figure 4 and Table 5).

Significant MTAs from the partitioned analysis also allowed the identification of potentially interesting genes. On chromosome 1B, marker DArT1744, previously described by Mérida-García et al. [73] related to high molecular-weight glutenin subunits, was found in proximity to genes encoding isocitrate dehydrogenase kinase/phosphatase G and leucine-rich repeat receptor-like protein kinase family protein. These genes participate in the carbohydrate metabolism during the Krebs cycle and play a crucial role in plant development and stress responses, respectively [74,75]. On this chromosome, another marker (SNP809) was found in proximity to some interesting genes encoding sugar transporter proteins. Additionally, some markers located on chromosome 2A were found in proximity to Acyl-CoA N-acyltransferase genes (SNP1206, SNP8395 and DArT3180) and chloroplastic zeaxanthin epoxidase (SNP1189) (Supplementary Materials Table S8).

![Figure 4](image-url). Location of candidate genes on chromosomes 1B and 2A. Markers are indicated with the symbol ‘-‘; blue copper proteins are shown in blue colour; ubiquinone biosynthesis O-methyltransferase in purple colour; the regulator response gene in brown colour; and for reductase 1 genes in green colour.
Table 5. Candidate genes for markers with significant marker-traits associations. Genes located in proximity of markers found in association with TKW and SV across years and water treatments (within a ±50 kb window). Values for physical position and distance are indicated in base pairs (bp). Chr: chromosome position. Blue copper proteins are shown in blue colour; ubiquinone biosynthesis O-methyltransferase in purple colour; the regulator response gene in brown colour; and for reductase 1 genes in green colour. Physical positions and gene annotations are based on the wheat reference assembly RefSeqv1 [69].

| Marker Identity | Transcript Chr | Physical Position | Distance | Description |
|-----------------|----------------|-------------------|----------|-------------|
| DArT3156        | 88.525         | 1B 134,604,832    | −33,988  | LINE-1 reverse transcriptase-like protein |
|                 | 1B 134,605,786  | −33,034           |          | Retrotransposon protein. putative. unclassified |
|                 | 1B 134,607,645  | −31,175           |          | Retrotransposon protein. putative. unclassified |
|                 | 1B 134,621,105  | −17,715           |          | Retrotransposon protein. putative. unclassified |
|                 | 1B 134,622,150  | −16,207           |          | Retrotransposon protein. putative. unclassified |
|                 | 1B 134,622,613  | −16,070           |          | Retrotransposon protein. putative. unclassified |
|                 | 1B 134,632,362  | −458              |          | RNA-directed RNA polymerase (reverse transcriptase)-related family protein |
|                 | 1B 134,633,505  | −5315             |          | LINE-1 reverse transcriptase-like |
|                 | 1B 134,639,730  | 910               |          | Transposon Ty3-G Gag-Pol polyprotein |
|                 | 1B 176,756,965  | −3171             |          | Retroposition protein. putative. LINE subclass |
|                 | 1B 176,768,886  | 23,969            |          | Phototrophic-responsive NPH3 family protein |
|                 | 1B 176,779,104  | 29,972            |          | Disease resistance protein (TIR-NBS-LRR class) family |
|                 | 1B 176,790,698  | 30,562            |          | Transposon protein. putative. Mutator sub-class |
|                 | 1B 176,791,607  | 31,471            |          | Transposon protein. putative. Mutator sub-class |
|                 | 1B 176,793,792  | 33,656            |          | GRF zinc finger family protein. expressed |
|                 | 1B 176,803,822  | 43,686            |          | BH/POZ domain containing protein. expressed |
| SNP620          | 100            | 1B 555,010,255     | −46,132  | Blue copper protein |
|                 | 1B 555,018,059  | −38,328           |          | Blue copper protein |
|                 | 1B 555,029,816  | −26,571           |          | Blue copper protein |
|                 | 1B 555,056,445  | 58                |          | Ubiquinone biosynthesis O-methyltransferase |
|                 | 1B 555,057,255  | 938               |          | Blue copper protein |
|                 | 1B 555,060,627  | 4240              |          | Blue copper protein |
|                 | 1B 555,063,537  | 7150              |          | Blue copper protein |
|                 | 1B 555,065,967  | 9600              |          | Blue copper protein |
|                 | 1B 555,068,889  | 12,302            |          | Blue copper protein |
|                 | 1B 555,071,301  | 15,043            |          | Blue copper protein |
|                 | 1B 555,076,734  | 20,347            |          | Blue copper protein |
|                 | 1B 555,088,083  | 31,696            |          | Blue copper protein |
|                 | 1B 555,090,695  | 34,308            |          | Blue copper protein |
|                 | 1B 555,095,491  | 39,104            |          | Blue copper protein |
|                 | 1B 555,096,179  | 39,792            |          | Disease resistance protein (TIR-NBS-LRR class) family |
|                 | 1B 555,097,247  | 58                |          | Blue copper protein |
|                 | 1B 555,098,272  | 41,885            |          | Blue copper protein |
|                 | 1B 555,101,471  | 45,084            |          | Blue copper protein |
|                 | 1B 555,103,053  | 46,666            |          | Blue copper protein |
Table 5. Cont.

| Marker | Identity | Transcript Chr | Physical Position | Distance | Description |
|--------|----------|----------------|-------------------|----------|-------------|
| DArT3155 | 93.939 | TraesCS2A01G213400LC.1 2A | 174,011,203 | −24,981 | Retrotransposon protein. putative. unclassified. expressed |
|        |         | TraesCS2A01G213500LC.1 2A | 174,024,497 | −11,687 | APOLO |
|        |         | TraesCS2A01G201000.1 2A | 174,026,194 | −9990 | Cytochrome P450-like |
|        |         | TraesCS2A01G213600LC.1 2A | 174,034,039 | −2145 | Retrotransposon protein. putative. unclassified. expressed |
|        |         | TraesCS2A01G213700LC.1 2A | 174,039,127 | 2943 | Cytochrome P450 |
|        |         | TraesCS2A01G213800LC.1 2A | 174,045,147 | 4255 | NADH dehydrogenase [ubiquinone] iron-sulfur protein 3. mitochondrial |
|        |         | TraesCS2A01G213900LC.1 2A | 174,063,484 | 6101 | Phosphate carrier protein. mitochondrial |
| SNP1153 | 98.551 | TraesCS2A01G493000LC.1 2A | 582,628,952 | −7722 | Retrotransposon protein. putative. Ty3-gypsy subclass |
|        |         | TraesCS2A01G493100LC.1 2A | 582,629,900 | −6774 | Retrovirus-related Pol polyprotein from transposon gypsy |
|        |         | TraesCS2A01G493200LC.1 2A | 582,630,406 | −5695 | Retrotransposon protein. putative. Ty3-gypsy subclass |
|        |         | TraesCS2A01G493300LC.1 2A | 582,634,003 | −2671 | RAN guanine nucleotide release factor |
|        |         | TraesCS2A01G493400LC.1 2A | 582,637,903 | 1202 | Nucleosome assembly protein 1-like 1 |
|        |         | TraesCS2A01G493500LC.1 2A | 582,638,542 | 4894 | 1-phosphatidylinositol-3-phosphate 5-kinase FAB1A |
|        |         | TraesCS2A01G493600LC.1 2A | 582,639,127 | 4555 | Phosphate carrier protein. mitochondrial |
|        |         | TraesCS2A01G493700LC.1 2A | 582,648,355 | −38,849 | Zeaxanthin epoxidase. chloroplastic |
|        |         | TraesCS2A01G493800LC.1 2A | 582,649,684 | −37,520 | Zeaxanthin epoxidase. chloroplastic |
|        |         | TraesCS2A01G493900LC.1 2A | 582,650,147 | −37,057 | Zeaxanthin epoxidase. chloroplastic |
|        |         | TraesCS2A01G494000LC.1 2A | 582,652,662 | −34,542 | AUGMIN subunit 6 |
|        |         | TraesCS2A01G494100LC.1 2A | 582,654,847 | −32,357 | Mitochondrial carrier protein |
|        |         | TraesCS2A01G494200LC.1 2A | 582,658,154 | −29,050 | Phosphatase 2C family protein |
|        |         | TraesCS2A01G494300LC.1 2A | 582,668,655 | −549 | transmembrane protein |
|        |         | TraesCS2A01G494400LC.1 2A | 582,679,624 | −29,044 | Retrovirus-related Pol polyprotein LINE-1 |
|        |         | TraesCS2A01G494500LC.1 2A | 582,680,406 | −6774 | Retrovirus-related Pol polyprotein LINE-1 |
|        |         | TraesCS2A01G494600LC.1 2A | 582,681,024 | −5695 | Retrotransposon protein. putative. Ty3-gypsy subclass |
|        |         | TraesCS2A01G494700LC.1 2A | 582,682,542 | −2671 | RAN guanine nucleotide release factor |
|        |         | TraesCS2A01G494800LC.1 2A | 582,683,127 | 1202 | Nucleosome assembly protein 1-like 1 |
|        |         | TraesCS2A01G494900LC.1 2A | 582,684,703 | 4894 | 1-phosphatidylinositol-3-phosphate 5-kinase FAB1A |
|        |         | TraesCS2A01G495000LC.1 2A | 582,685,284 | 4555 | Phosphate carrier protein. mitochondrial |
|        |         | TraesCS2A01G495100LC.1 2A | 582,686,847 | −38,849 | Zeaxanthin epoxidase. chloroplastic |
|        |         | TraesCS2A01G495200LC.1 2A | 582,688,184 | −37,520 | Zeaxanthin epoxidase. chloroplastic |
|        |         | TraesCS2A01G495300LC.1 2A | 582,689,684 | −37,057 | Zeaxanthin epoxidase. chloroplastic |
|        |         | TraesCS2A01G495400LC.1 2A | 582,691,262 | −34,542 | AUGMIN subunit 6 |
|        |         | TraesCS2A01G495500LC.1 2A | 582,693,484 | −32,357 | Mitochondrial carrier protein |
|        |         | TraesCS2A01G495600LC.1 2A | 582,695,154 | −29,050 | Phosphatase 2C family protein |
|        |         | TraesCS2A01G495700LC.1 2A | 582,696,703 | −549 | transmembrane protein |

4. **Note:** The table continues with similar entries for various markers and transcripts, providing detailed information on the identities, transcripts, chromosome locations, physical positions, distances, and descriptions of the markers.
### Table 5. Cont.

| Marker   | Identity            | Transcript Chr | Physical Position | Distance | Description                                      |
|----------|---------------------|----------------|-------------------|----------|--------------------------------------------------|
| **DArT3172**  | 100                 | TraesCS2A01G333900.1  | 2A | 567,725,771 | ~8576 | RNA-dependent RNA polymerase                        |
|          |                     | TraesCS2A01G334000.1  | 2A | 567,735,196 | 849   | MLP protein                                         |
| **DArT3174**  | 100                 | TraesCS2A01G333300.1  | 2A | 566,454,172 | ~2950 | F-box/RNII-like superfamily protein                  |
|          |                     | TraesCS2A01G481800LC.1 | 2A | 566,461,411 | 4289  | Transposon Ty3-G Gag-Pol polyprotein                |
|          |                     | TraesCS2A01G481900LC.1 | 2A | 566,462,897 | 5775  | Craniofacial development protein 2                   |
|          |                     | TraesCS2A01G482000LC.1 | 2A | 566,478,234 | 21,112 | Retrotransposon protein. putative. unclassified     |
| **DArT3175**  | 100                 | TraesCS2A01G334000.1  | 2A | 544,359,272 | ~32,496 | target of AVRB operation1                          |
|          |                     | TraesCS2A01G460000LC.1 | 2A | 544,395,881 | 4113  | Retrotransposon protein. putative. unclassified     |
| **DArT3176**  | 98.182              | TraesCS2A01G464500LC.1 | 2A | 546,477,078 | 31,281 | Transposon Ty3-I Gag-Pol polyprotein                |
|          |                     | TraesCS2A01G464600LC.1 | 2A | 546,478,437 | 32,640 | Transposon Ty3-I Gag-Pol polyprotein                |
|          |                     | TraesCS2A01G464700LC.1 | 2A | 546,479,163 | 33,366 | Transposon Ty3-I Gag-Pol polyprotein                |
| **DArT3180**  | 100                 | TraesCS2A01G333900.1  | 2A | 567,725,771 | ~10,352 | RNA-dependent RNA polymerase                        |
|          |                     | TraesCS2A01G334000.1  | 2A | 567,735,196 | 927   | MLP protein                                         |
| **DArT3182**  | 100                 | TraesCS2A01G483800LC.1 | 2A | 569,357,751 | ~46,773 | autoinhibited Ca(2+)-ATPase. isoform 4              |
|          |                     | TraesCS2A01G335600.1  | 2A | 569,363,189 | ~41,335 | Zinc finger family protein                          |
| **DArT3187**  | 98.551              | TraesCS2A01G460800LC.1 | 2A | 541,301,644 | ~458   | 1-phosphatidylinositol-3-phosphate 5-kinase FAB1A   |
| **DArT3198**  | 98.305              | TraesCS2A01G457200LC.1 | 2A | 535,217,367 | ~18,493 | Acetylglutamate kinase-like protein                  |
|          |                     | TraesCS2A01G457300LC.1 | 2A | 535,221,879 | ~13,981 | LINE-1 reverse transcriptase like                    |
|          |                     | TraesCS2A01G457400LC.1 | 2A | 535,222,237 | ~13,623 | LINE-1 reverse transcriptase                        |
|          |                     | TraesCS2A01G311500.1  | 2A | 535,240,748 | 4888   | NAC domain protein.                                  |
| **DArT3201**  | 100                 | TraesCS2A01G483800LC.1 | 2A | 569,357,751 | ~46,711 | autoinhibited Ca(2+)-ATPase. isoform 4              |
|          |                     | TraesCS2A01G335600.1  | 2A | 569,363,189 | ~41,273 | Zinc finger family protein                          |
| **DArT10906** | 98.551              | TraesCS2A01G482600LC.1 | 2A | 566,976,225 | 12,025 | RNA-directed DNA polymerase (Reverse transcriptase) |
|          |                     | TraesCS2A01G336000.1  | 2A | 566,986,482 | 22,282 | Gibberellin-regulated protein 2                     |
| **SNP895**   | 96.296              | TraesCS2A01G309400.1  | 2A | 532,849,120 | ~4840  | Pentatricopeptide repeat-containing protein         |
|          |                     | TraesCS2A01G309500.1  | 2A | 532,854,936 | ~976   | Smr domain containing protein                      |
|          |                     | TraesCS2A01G309600.1  | 2A | 532,859,077 | 5117   | Acyl-CoA N-acyltransferase isoform 2                |
|          |                     | TraesCS2A01G309700.1  | 2A | 532,865,797 | 11,837 | Acyl-CoA N-acyltransferase isoform 2                |
| **DArT20759** | 97.619              | TraesCS2A01G460800LC.1 | 2A | 541,301,644 | ~573   | 1-phosphatidylinositol-3-phosphate 5-kinase FAB1A   |
| **DArT20961** | 100                 | TraesCS2A01G309000.1  | 2A | 532,040,483 | ~40,124 | Translocase of chloroplast                         |
|          |                     | TraesCS2A01G309000.1  | 2A | 532,077,143 | ~3464  | GTpase Der                                          |
|          |                     | TraesCS2A01G309100.1  | 2A | 532,085,578 | 4971   | Protein NRT1/PTR FAMILY 1.1                       |
|          |                     | TraesCS2A01G309100.2  | 2A | 532,085,909 | 5302   | Protein NRT1/PTR FAMILY 1.1                       |
4. Discussion

The maintenance of crop production is a current and pressing need given growing populations and reduced availability of arable land [76]. There is an increasing need for breeding programs to improve yield potential and the adaptation of new varieties to different biotic and abiotic stresses [77]. Abiotic stresses, including drought and heat, are impacting productivity on the million hectares of wheat grown worldwide each year [78]. Detailed molecular and phenotypic characterization are valuable tools in the dissection of complex traits [79], and especially those that are influenced by water availability [14].

The improvement of key traits is essential for better end-use production quantity and quality in wheat [80]. In this study, we analysed a set of 10 agronomic and quality traits under full irrigation conditions (FI), with an additional six traits also assessed under low water availability (RI) in order to understand trait variation under contrasting water regimes in the CIMMYT durum wheat breeding programme. Irrigation conditions influenced some important yield and yield-related traits such as GY and TKW, as well as adaptive traits including DTH and PH (Table 3). The RI treatments had decreased final yields in line with previous observations [6,81]. Previous reports have also shown TKW to be reduced by high temperatures [17], most likely related to water availability. Groos et al. [82] assessed the genetic basis of grain yield and protein content in a segregating population of wheat RILs grown at six locations and also identified effects from GxE interactions involving protein content and yield. Our mean trait values corroborated this, with the highest values for GY recorded for FI blocks across panels (see Table 3). A similar trend was shown for DTH, PH and TKW, which decreased under low-water regimes.

Correlations between the assessed traits showed that GY was positively correlated with two different phenology traits (PH and DTH). This is in agreement with Maccaferri et al. [6], who showed important positive and negative correlations for GY and DTH, and also positive correlations for GY and PH in several environments with different water regimes. DTH and PH were also positively correlated (Supplementary Materials Table S5), with taller plants having a longer time period to the emergence of the tip of the spike stage.

Wheat TKW is an important yield component with a direct effect on grain yield [83,84]. In line with this, our results showed a significant and positive correlation between TKW and GY. However, the previously reported negative correlation between TKW and DTH [6] was not observed, potentially as result of temperatures and water availability from emergence to heading, and also from heading to harvest. Rharrabti et al. [17] previously highlighted a positive correlation between protein content and TKW, which is in agreement with the results obtained in the present study. They highlighted that warm temperatures during grain filling could negatively affect this correlation.

Significant associations between endosperm proteins (gliadin and glutenin subunits) and SV have been previously highlighted [85,86]. Here we found a positive correlation \( r = 0.15 \) between SV and GPC. This correlation is thought to be the result of grain protein content influencing the sedimentation volume value [87], which depends on the degree of protein hydration and oxidation [88]. Finally, for sedimentation index (SDS) analysis, we observed a negative correlation with protein content, in agreement with results presented by Rharrabti et al. [17,45]. This is also in agreement with Oelofse et al. [89] who highlighted the significant influence of protein content on the SDS sedimentation test [90–92].

The SNP and DArT markers used to analyze patterns of genetic structure (Figure 2) and LD (Figure 3) for the durum wheat lines revealed no detectable genetic structure and consistent patterns of LD across chromosomes (LD was estimated to extend ~20 cM). These results support the suitability of durum elite line sets currently in use in breeding programmes for the practical application of GWAs analysis. The rate of unmapped markers was lower for SNP than for DArT markers (27.5% vs. 42.0%, Table 2), indicating higher precision in genetically mapping SNP markers, probably as a result of co-dominance.
In the assessment of MTAs for quality and yield-related traits, different AM analyses were performed on subsets of the dataset. Several MTAs for SV and TKW were detected across years and water regimes, located on chromosomes 1B and 2A, respectively. All GWAS analyses corroborated the major QTLs previously detected, and reported two new QTLs, one for YR in chromosome 1B, and another for SV in chromosome 4A.

Associations on chromosome 1B were significant for wheat quality. There are known loci including Gli-B1/Glu-B3 on this chromosome, which are of great importance for some gliadin and glutenin subunits [93]. In fact, the candidate gene analysis reported the presence of a high molecular-weight glutenin subunit (HMW-GS) gene in the proximity of marker DArT1744 (found to be significantly associated with SV and SDS), which was previously described by Mérida-García et al. [73] in association with specific weight. In line with this, Pogna et al. [93] highlighted the importance of Glu-B3 for determining protein quality with these endosperm proteins showing significant effects on SV, which also showed a high and positive correlation with SDS in our study (Figure 1 and Supplementary Materials Table S5). Likewise, Blanco et al. [86] reported three QTLs on chromosomes 1B, 6A and 7B (based on the analysis of 259 polymorphic markers) associated with SDS and SV in a recombinant inbred line population. In the present study, we found a SNP marker (SNP620) associated with SV, showing a positive additive effect of 1.26 (see Table 4) and also with SDS (marker effect of 0.11) (Supplementary Materials Table S6). This marker was previously placed on chromosome 1B, in the same location as MTAs for gluten index and sedimentation index [73]. Other previous studies, such as Reif et al. [94] and Fiedler et al. [95], also reported markers associated with SV on chromosome 1B, but with differing genetic positions. The additional locus for SV found on chromosome 4A (marker DArT9459) has not been previously reported.

Marker SNP620, significantly associated with SV, is located within a cluster of homoeolog gene triads coding for blue copper proteins (Table 5 and Supplementary Materials Figure S6). These proteins have been described containing a copper atom, and participate in redox processes [96], with a crucial role in the electron shuttle in plants [97]. In addition, Yao et al. [98] described the blue copper protein genes as the targets of miR408 in wheat, which is involved in the regulation of gene transcription required for heading time [99]. In our study SNP620 was also found in proximity to a gene coding for an ubiquinone biosynthesis O-methyltransferase. Liu et al. [100] highlighted its crucial role as an electron transporter in the electron transport chain of the aerobic respiratory chain. This ubiquinone gene is involved in plant growth and development, is implied in chemical compounds biosynthesis and metabolism which are involved in plant responses to stress, and also participates in gene expression regulation and cell signal transduction [100].

On chromosome 1B we also found a significant MTA for yellow rust, in agreement with previous studies in durum and bread wheat, which placed different markers significantly associated with this trait, but in differing genetic positions [101–104]. The candidate gene analysis revealed the proximity of this marker (SNP809) to sugar transporter protein genes. Sugars are formed during the photosynthetic process and are essential for plant nutrition. The sucrose transport has been considered of great importance for plant productivity [105]. In line with this, the sucrose is involved in the gene expression regulation of the supposedly sugar-sensing pathway [106,107].

The majority of MTAs for TKW were located on chromosome 2A, showing both positive and negative effects. Previous studies have reported different markers in association with this quality trait, including a number mapped on chromosome 2A [38,108–110]. One of the markers found by Yao et al. [38] (SSR marker gwm445 on chromosome 2A (68.2 cM), belongs to the same QTL found in this study for the marker SNP1153 (chromosome 2A, 68.6 cM), and also found by Juliana et al. [111] in bread wheat lines from CIMMYT’s first year-yield trials. Sukumaran et al. [43] analysed a durum wheat panel of 208 lines under yield potential, heat and drought stress conditions, and identified markers on chromosome 2A with a similar position to those detected in this study (4 markers at 70 cM and 6 markers at 69 cM) under heat stress conditions. They highlighted that several SNP markers were related to transmembranes or were uncharacterized proteins. We found several candidate genes for this
important TKW QTL (Table 5) among which the most striking feature is the presence of four reductase 1 genes (NADPH-dependent 6'-deoxychalcone synthase) and a type A response regulator 1 (Figure 4). These genes are both related with photosynthesis. Hu et al. [112] highlighted that NADPH plays a crucial role in biological processes in plants, such as the regulation of the production of reactive oxygen species (ROS) for the stress tolerance [113,114]. Additional GWAS analyses using reduced datasets revealed other interesting genes for this QTL (chromosome 2A, Supplementary Materials Table S8), encoding for the Acyl-CoA N-acyltransferase and the chloroplastic zeaxanthin epoxidase. The first gene has several functions in signaling and metabolic pathways [115]. The zeaxanthin epoxidase plays an important role in the xanthophyll cycle and abscisic acid (ABA) biosynthesis. The xanthophyll cycle has a main function in the dissipation of light energy excess and also increasing the photosynthetic system stability [116].

The proposed approach has successfully detected genetic markers with significant associations with TKW, SV, SDS and YR. These are of potential use in durum wheat breeding programs, and can be further interrogated to the candidate gene level using the RefSeqv1 bread wheat genome reference [69] and the durum wheat genome reference [71].

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/1/144/s1:

Figure S1: Rainfall data for Ciudad Obregon (Mexico) for the growing seasons 2012–2015; Figure S2: Manhattan plots for durum wheat mapped DARt markers. DTH: days to heading; PH: plant height; GY: grain yield; TKW: thousand kernel weight; YC: yellow color; SV: sedimentation volume; SDS: sedimentation index; and GPC: grain protein content; Figure S3: Manhattan plots for durum wheat mapped SNP markers. DTH: days to heading; PH: plant height; GY: grain yield; TKW: thousand kernel weight; YC: yellow color; SV: sedimentation volume; SDS: sedimentation index; and GPC: grain protein content; Figure S4: Quantile quantile-plots from genome-wide association studies (GWAS) analysis for durum wheat DARt markers (mapped and unmapped). DTH: days to heading; PH: plant height; LOD: lodging; GY: grain yield; TKW: thousand kernel weight; YC: yellow color; SV: sedimentation volume; SDS: sedimentation index; and GPC: grain protein content; Figure S5: Quantile quantile-plots from GWAS analysis for durum wheat SNP markers (mapped and unmapped). DTH: days to heading; PH: plant height; LOD: lodging; GY: grain yield; TKW: thousand kernel weight; YC: yellow color; SV: sedimentation volume; SDS: sedimentation index; and GPC: grain protein content; Figure S6: Blue copper protein gene cluster on durum wheat chromosome 1B. High confidence genes are shown in green colour, low confidence genes are shown in yellow; Figure S7: Cluster tree of blue copper protein gene homoeologs in bread wheat (RefSeqv1 [69]). For chromosome 1A, high confidence (HC) and low confidence (LC) genes are shown in brown and orange colour, respectively; for chromosome 1B, HC and LC genes are shown in dark and light green colour, respectively; for chromosome 1D, HC and LC genes are shown in dark and light blue colour, respectively; Table S1: Durum wheat elite lines assessed; Table S2: Best Linear Unbiased Estimates (BLUESs) outputs for all assessed traits and the association mapping analyses performed in durum wheat: [i] across years and blocks; [ii] across years for each block (FI and RI); [iii] across years and blocks for a reduced dataset (years 2013 and 2014); and [iv] across the reduced dataset for each block. DTH: days to heading; GPC: grain protein content; GY: grain yield; PH: plant height; SDS: sedimentation index; SV: sedimentation volume; TKW: thousand kernel weight; and YC: yellow colour; YR: yellow rust; LOD: lodging; Table S3: Kinship matrix for durum wheat DARt markers; Table S4: Kinship matrix for durum wheat SNP markers; Table S5: Phenotypic correlations between the assessed traits in durum wheat and their corresponding p values. YR: yellow rust; DTH: days to heading; PH: plant height; LOD: lodging; GY: grain yield; TKW: thousand kernel weight; YC: yellow color; SV: sedimentation volume; SDS: sedimentation index; and GPC: grain protein content; Table S6: Marker-trait associations found for the association mapping analyses performed in durum wheat: [i] across years and blocks; [ii] across years for each block (FI and RI); [iii] across years and blocks for a reduced dataset (years 2013 and 2014); and [iv] across the reduced dataset for each block. SV: sedimentation volume; TKW: thousand kernel weight; SDS: sedimentation index; YR: yellow rust; “-“: unmapped marker; Table S7: Homoeolog triads for blue copper protein genes mapped in the wheat reference assembly RefSeqv1 [69]; Table S8: Candidate genes for GWAS analyses performed in durum wheat: [i] across years for each block (FI and RI); [ii] across years and blocks for a reduced dataset (years 2013 and 2014); and [iii] across the reduced dataset for each block. Molecular markers mapping positions are shown both in the durum wheat genome [71] and the wheat reference assembly RefSeqv1 [69]; Supplementary Material S1. R script used to perform the GWAS analysis; Supplementary Material S2. R script used to perform the LD analysis.

Author Contributions: P.H., I.S. and K.A. conceived the experiment. K.A. and I.S. selected the plant materials and agronomic traits. K.A. managed the field trials and contributed the phenotypic data. I.S., P.H., G.D., S.G., R.M.-G. and A.R.B. analysed the genotypic and phenotypic data. R.M.-G. isolated the DNA. R.M.-G., A.R.B. and P.H. carried out the AM analyses. R.M.-G., A.R.B. and P.H. drafted the manuscript. All authors have read and agreed to the published version of the manuscript.

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