SNP-based association study of kernel architecture in a worldwide collection of durum wheat germplasm

Longqing Sun¹, Sisi Huang¹, Genlou Sun², Yujuan Zhang¹, Xin Hu¹, Eviatar Nevo³, Junhua Peng⁴, Dongfa Sun¹,5*

¹ College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, China, 2 Biology Department, Saint Mary’s University, Halifax, Nova Scotia, Canada, 3 Institute of Evolution, University of Haifa, Mount Carmel, Haifa, Israel, 4 Germplasm Enhancement Department, Huazhi Biotech Institute, Changsha, Hunan, China, 5 Hubei Collaborative Innovation Center for Grain Industry, Jingzhou, Hubei, China

* sundongfa1@mail.hzau.edu.cn

Abstract

Durum wheat, genetic resource with favorable alleles is considered as natural gene pool for wheat breeding. Kernel size and weight are important factors affecting grain yield in crops. Here, association analysis was performed to dissect the genetic constitution of kernel-related traits in 150 lines collected from 46 countries and regions using a set of EST-derived and genome-wide SNP markers with five consecutive years of data. Total 109 significant associations for eight kernel-related traits were detected under a mix linear model, generating 54 unique SNP markers distributed on 13 of 14 chromosomes. Of which, 19 marker-trait associations were identified in two or more environments, including one stable and pleiotropic SNP BE500291_5_A_37 on chromosome 5A correlated with six kernel traits. Although most of our SNP loci were overlapped with the previously known kernel weight QTLs, several novel loci for kernel traits in durum were reported. Correlation analysis implied that the moderate climatic variables during growth and development of durum are needed for the large grain size and high grain weight. Combined with our previous studies, we found that chromosome 5A might play an important role in durum growth and development.

Introduction

Wheat is the most extensively grown commercial crop in the world [1]. The global demand for wheat is predicted to increase by 60% as the global population is estimated to be over nine billion by 2050 [2]. Therefore, genetic improvement of grain yield will still be the principal aim of wheat breeding. Considering the complex, polygenic inheritance, low heritability and significant influence of environment, yield improvement is faced with daunting challenges [3]. One of the important facets to achieve this goal is to explore novel genetic resources to discover genes that affect grain yield [4]. As a result, durum wheat (*Triticum turgidum* L. ssp. *Durum* Desf.) is often used as a bridge for transferring favorable alleles into bread wheat [5]. Wheat
Grain yield is a complex trait and determined by three main components, including spike number per unit area, kernel number per spike and kernel weight [6]. Kernel size, a key factor determining kernel weight and therefore grain yield [7], is also a quantitative trait with a complex genetic basis.

Comparative genomics approaches provide a powerful tool for gene discovery in wheat. Several QTLs and genes contributing to grain size and weight have gradually been isolated from wheat by using homology-based cloning of the orthologs in other cereal crops, including TaGW2 [8], TaSus2-2B [9], TaCwi-A1 [10], TaCKX6-D1 [11], TaGS-D1 [12], TaGS5 [13], TaTEGW6-A1 [14], and TaFlo2-A1 [15]. In recent years, more and more researchers have been attracted to study the functions of these genes. For instance, analysis of the function of TaGW2 using CRISPR/Cas9 showed that mutation of TaGW2 homoeologs resulted in the decrease of grain weight by affecting the grain size in bread wheat [16], which was in accordance with down-regulation of all the three homoeologs of TaGW2 by RNA interference [17]. Haplotype TaTGW6-A1a associated with high grain weight was observed in approximately 80% of cultivars, indicating that it was a positively selected allele in wheat breeding [14]. TaGS5-A1 haplotype was positively associated with high thousand-kernel weight in Chinese modern wheat [18]. In general, these results have helped us to understand the mechanism for kernel development in wheat.

High-density genetic linkage maps had been constructed to detect qualitative and quantitative trait loci for identifying candidate genes of many important traits in many species. A stable QTL qKW-6A was detected in both RIL population and DH population, suggesting that qKW-6A plays an important role in kernel width of wheat [19]. TaTGW-7A, a major QTL explaining 21.7–27.1% of phenotypic variance for thousand-kernel weight contributed significantly to wheat grain yield [20]. A single nucleotide polymorphism (SNP) in the promoter region of 1-FEH w3 gene was identified to be associated with thousand-kernel weight under drought conditions [21]. Six stable QTL were identified for controlling kernel size and weight in a recombinant inbred line population (RIL) [22]. qKnps-4A, a major stable QTL for kernel number per spike was identified by using the Affymetrix Wheat-660K single-nucleotide polymorphism (SNP) array [23].

However, linkage mapping has limitations including the basic requirement to create a biparental population segregating for target traits [24]. Another approach for identifying loci of traits is to employ association analysis with a large germplasm resources, known as linkage disequilibrium (LD) mapping, association mapping or genome-wide association studies (GWAS) [25], based on linkage disequilibrium (LD) or the non-independence of alleles in a natural population [26]. Association mapping has been proven to be successful in identifying marker-trait associations in plant [27]. Recent study has identified 26 quantitative trait loci (QTL) for kernel width and 27 QTL for kernel length in a historical United States wheat population [28]. A comprehensive genome-wide analysis using microsatellite markers and 90K iSELECT array identified TaGW-6A underlying thousand grain weight in a panel of European winter wheat varieties [29]. Twenty-seven markers were found to be associated with grain weight in a set of 230 elite Indian bread wheat cultivars [30]. Association analysis of 231 synthetic hexaploid wheats revealed that the loci associated with grain morphology were mainly distributed on homoeologous group 2, 3, 6 and 7 chromosomes [31]. Based on GBS markers, 17 grain size-associated SNPs were found in wild wheat Aegilops tauschii [32].

In common wheat, GWAS approach has been successfully employed to identify numerous candidate genes controlling a series of traits. Nevertheless, limited studies have utilized GWAS in durum wheat to dissect the genetic basis controlling kernel size and weight. In this study, we analyzed architecture of kernel characters in a panel of 150 durum lines collected from 46 countries and regions. As the result, a number of candidate genes were identified, which
provides a useful resource for further functional studies to understand the molecular mechanism underlying grain development.

**Materials and methods**

**Plant materials and field trials**

One hundred fifty durum wheat accessions, consisting of 51 landraces and 99 cultivars from 46 countries and regions around the world, were used for association analysis in the study. This set of durum wheat was classified into 11 groups based on their geographic origins: East Asia (15), Central Asia (2), South Asia (6), Middle East (32), North America (33), Latin America (12), Oceania (7), Western Europe (14), Eastern Europe (5), South Africa (4), and North Africa (12). Details information was given in previous study [33]. All the accessions were cultivated in the experimental plot of Huazhong Agricultural University, Wuhan, Hubei of China (N30°32' and E114°20') in five consecutive years. During the 2013/2014, 2014/2015, 2015/2016, 2016/2017 and 2017/2018 cropping seasons, the durum wheat accessions were planted in late October of first year and harvested in June of next year for each cropping season. Each accession was sown in four rows with 1 m in length and 20 cm between rows, 8 plants in each row. The experimental field belongs to the type of heavy loam with PH value of about 6.2. Water was sprayed evenly after sowing by sprinkling irrigation system. The soil moisture for durum seedling was about 70% of field water capacity. Compound fertilizer (825 kg/ha) was used as base fertilizer and 150 kg/ha of urea fertilizer was used as top dressing. Each field trial was conducted in a randomized complete block design with three replications.

**Phenotypic evaluation**

The kernel traits were measured at maturity. Thirty spikes from the individual plant of each line were randomly collected from the middle row in each plot and sundried. Then, all spikes from three different field experimental trials were mixed together for threshing. About 300 fully filled seeds of every line were randomly selected to obtain kernel parameters using SC-G phenotyping system (Wanshen Detection Technology Co., Ltd., China) [34]. In total, 8 traits were measured or calculated: kernel area (KA), kernel circumference (KC), kernel diameter (KD), kernel length (KL), kernel roundness (KR), kernel width (KW), length/width ratio (L/W), thousand kernel weight (TKW).

**Phenotypic data and correlation analysis**

Descriptive statistical analysis and analysis of variance (ANOVA) of phenotypic data, broad-sense heritability ($H^2$) for each trait, and Pearson correlation coefficients analysis among different traits were calculated by using SPSS 21.0 (https://www.ibm.com/support/pages/node/213045). Kolmogorov-Smirnov test was performed to test normal distribution of each trait. Origin Pro2017 (http://www.chem.ox.ac.uk/origin/) was used to draw figures of frequency distribution for the examined traits. In order to calculate the mean values of each trait, the best linear unbiased prediction (BLUP) method was estimated using a mixed-effects model implemented in the lme4 package [35]. Correlation analysis between eight evaluated kernel traits and three climate factors was performed by using SPSS 21.0. The critical developmental stages for durum wheat after overwintering were divided into three important growth stages (I, II, and III). Stage I represented the growth period of regreening for durum around the time in February, and stage II corresponded with the growth of jointing stage in March. Due to the growth rate vary with different lines of the durum population at the heading and flowering, grain filling, and ripening phases, stage III was the combination period from heading to
ripening (April-June period). The average values of temperature, sunlight and rainfall precipitation for each of the three stages, which were collected from weather station in Hubei province from 2014 to 2018, were set as the climate parameters. Correlation coefficients of kernel phenotypes with climate factors were based on the mean values of the kernel traits and climate parameters.

Association analysis

SNP genotyping was performed on Illumina Bead Array platform and Golden Gate Assay (Illumina, San Diego, CA) at the Genome Center of the UC Davis according to the manufacturer’s protocols. The SNP markers used in this study were developed from the EST database. After the process of quality management, 1366 single nucleotide polymorphisms (SNP) markers covering the whole genome of durum were used to genotype the durum accessions. The rate of change in the Napierian logarithm probability relative to standard deviation (ΔK) suggested that the best structure was K = 2. More details were described in previous study [33]. The mean marker density of 95–96 markers per chromosome, ranging from 66 (3B) to 130 (7A) for all the 14 chromosomes, were used to calculate the extent of LD. At the chromosomal level, the LD decay distance ranged from 1.90 Mb (1A) to 96.05 Mb (2A) [33, 36]. The associations were estimated under the mixed linear model (MLM) using software TASSEL 3.0 (http://www.Misogynistic.net/tassel), accounting for Q-Matrix of the population structure as a covariate and pair-wise kinship coefficients (K matrix) as random effects [33]. Significance of associations between markers and traits was evaluated by P-value, and the QTL effects were estimated using marker-R². P = 0.01 was used to declare the significant association signals according to our previous study [36].

The Physical Position Identification and Candidate Gene Prediction

The EST sequence of each significantly associated SNP marker was analyzed using translated nucleotide BLAST software from NCBI (http://www.ncbi.nlm.nih.gov/) for candidate genes prediction. Their functions were predicted based on the level of homology identity with the other species. The durum genome was downloaded from the International Durum Wheat Genome Sequencing Consortium (Triticum turgidum Durum Wheat Svevo, RefSeq Rel. 1.0). To obtain the physical positions of the our SNP sequences, and search previously identified QTLs which overlapped with our markers in durum, all EST sequences of the significant SNP markers were analyzed using Nucleotide BLAST to gain information in durum reference genome (https://wheat.pw.usda.gov/GG3/jbrowse_Durum_Svevo).

Results

Phenotypic Variation

In total, eight kernel-related traits were measured: kernel area (KA), kernel circumference (KC), kernel diameter (KD), kernel length (KL), kernel roundness (KR), kernel width (KW), length/width ratio (L/W), thousand kernel weight (TKW). The frequency distribution of these traits was showed in Fig 1. A large range of variation for each investigated trait was detected in this natural population. Moreover, the trait distribution pattern was similar among five years for most traits (Fig 1A–1H). Therefore, the kernel traits showing the typical quantitative in heritance were used for association mapping analysis. The phenotypic values for each of the six kernel-related traits (KA, KC, KD, KL and KW) in the years from 2014 to 2016 were all lower than those in the years from 2017 to 2018 (S1 Fig). The descriptive statistics of the investigated traits for the population were shown in Table 1.
The coefficients of variation (CV) among genotypes for all the phenotypic traits in each environment ranged from 5.53 to 28.45%. However, the variation of each trait was different among years. For instance, the variation for the KA ranged from 7.61 to 17.16 mm (mean ± SD = 12.54 ± 1.60 mm) in year 2014, but from 11.96 to 23.47 mm (17.15 ± 2.01 mm) in 2018. TKW had the highest CV among these traits, whereas KD had the lowest CV (Table 1). Most of the traits have high broad-sense heritability ($H^2 > 60\%$), indicating that a large portion of phenotypic variance for kernel traits were stable and mainly contributed by genotypic effects.
Table 1. Descriptive statistics of phenotypic performance and broad-sense heritability for the evaluated kernel traits.

| Trait   | Year | Mean       | Range | SD  | CV (%) | $H^2$ (%) |
|---------|------|------------|-------|-----|--------|-----------|
|         |      |            | Minimum | Maximum |        |           |
| KA (mm²) | 2014 | 12.54      | 7.61   | 17.16 | 1.60   | 12.76     | 62.43     |
|         | 2015 | 12.01      | 7.84   | 19.90 | 1.93   | 16.07     |           |
|         | 2016 | 12.19      | 8.05   | 17.38 | 1.58   | 12.96     |           |
|         | 2017 | 16.14      | 9.96   | 21.31 | 1.84   | 11.40     |           |
|         | 2018 | 17.15      | 11.96  | 23.47 | 2.01   | 11.72     |           |
| KC (mm) | 2014 | 15.28      | 12.21  | 18.39 | 1.15   | 7.53      | 76.40     |
|         | 2015 | 15.05      | 12.03  | 20.75 | 1.29   | 8.57      |           |
|         | 2016 | 15.00      | 11.40  | 18.87 | 1.11   | 7.40      |           |
|         | 2017 | 17.32      | 12.72  | 21.05 | 1.20   | 6.93      |           |
|         | 2018 | 17.59      | 14.03  | 22.14 | 1.19   | 6.77      |           |
| KD (mm) | 2014 | 3.98       | 3.10   | 4.67  | 0.25   | 6.28      | 59.88     |
|         | 2015 | 3.89       | 3.15   | 5.02  | 0.31   | 7.97      |           |
|         | 2016 | 3.92       | 3.23   | 4.69  | 0.25   | 6.38      |           |
|         | 2017 | 4.52       | 3.70   | 5.19  | 0.25   | 5.53      |           |
|         | 2018 | 4.66       | 3.07   | 5.45  | 0.26   | 5.58      |           |
| KL (mm) | 2014 | 6.30       | 4.86   | 7.88  | 0.53   | 8.41      | 80.91     |
|         | 2015 | 6.22       | 4.74   | 8.78  | 0.58   | 9.32      |           |
|         | 2016 | 6.13       | 4.45   | 7.94  | 0.51   | 8.32      |           |
|         | 2017 | 7.05       | 4.93   | 8.92  | 0.56   | 7.94      |           |
|         | 2018 | 7.08       | 5.43   | 9.20  | 0.53   | 7.49      |           |
| KR      | 2014 | 0.41       | 0.31   | 0.51  | 0.04   | 9.76      | 74.14     |
|         | 2015 | 0.40       | 0.30   | 0.51  | 0.04   | 10.00     |           |
|         | 2016 | 0.42       | 0.32   | 0.53  | 0.04   | 9.52      |           |
|         | 2017 | 0.41       | 0.32   | 0.54  | 0.03   | 7.32      |           |
|         | 2018 | 0.43       | 0.34   | 0.53  | 0.03   | 6.98      |           |
| KW (mm) | 2014 | 2.57       | 2.06   | 3.09  | 0.18   | 7.00      | 50.50     |
|         | 2015 | 2.48       | 1.95   | 3.09  | 0.23   | 9.27      |           |
|         | 2016 | 2.54       | 1.93   | 3.13  | 0.20   | 7.87      |           |
|         | 2017 | 2.92       | 2.33   | 3.36  | 0.19   | 6.51      |           |
|         | 2018 | 3.08       | 2.60   | 3.75  | 0.20   | 6.49      |           |
| L/W     | 2014 | 2.49       | 2.01   | 3.34  | 0.23   | 9.24      | 73.68     |
|         | 2015 | 2.57       | 2.02   | 3.36  | 0.23   | 8.95      |           |
|         | 2016 | 2.46       | 1.94   | 3.12  | 0.23   | 9.35      |           |
|         | 2017 | 2.45       | 1.87   | 3.08  | 0.20   | 8.16      |           |
|         | 2018 | 2.32       | 1.98   | 2.88  | 0.18   | 7.33      |           |
| TKW (g) | 2014 | 35.03      | 13.76  | 52.43 | 6.95   | 19.85     | 40.52     |
|         | 2015 | 29.60      | 11.15  | 56.66 | 8.42   | 28.45     |           |
|         | 2016 | 28.66      | 11.28  | 44.85 | 6.30   | 22.00     |           |
|         | 2017 | 32.43      | 12.17  | 47.74 | 6.68   | 20.59     |           |
|         | 2018 | 35.17      | 14.44  | 52.85 | 6.18   | 17.58     |           |

*KA kernel area, KC kernel circumference, KD kernel diameter, KL kernel length, KR kernel roundness, KW kernel width, L/W length/width ratio, TKW thousand kernel weight.

bSD standard deviation.

cCV coefficient of variation.

d$H^2$ broad-sense heritability.

https://doi.org/10.1371/journal.pone.0229159.t001
The 150 durum accessions were divided into 11 geography of origins based on their sources and locations [33]. The potential relationship between kernel traits and geographical regions was estimated to explore the effect of regional characteristics on phenotypic traits. Phenotypic variability varied from the different regions (Fig 2). The values of KA, KC, KD, KL, KW, L/W and TKW for durum accessions from Middle East were almost all higher than those in the other 10 geographical regions (Fig 2A, 2B and 2D–2H). The values of KR, KW and TKW for durum wheat from Latin America were lower than those in other regions except Central Asia (Fig 2C, 2E and 2H), but the value of L/W of durum germplasm in Latin America was the highest (Fig 2F). The values of KA, KC, KD, KW, and TKW for durum from Central Asia were the lowest (Fig 2A, 2B, 2D, 2E, 2G and 2H). However, it is unlikely to be typical case due to only two durum wheat accessions from this region included in this study.

Comparison between landraces and cultivars did not show significant rule and trend of the kernel-related traits (Fig 2I), and significant differences for KA, KC, KD, KL, KR, KW, L/W and TKW between landraces and cultivars of durum (S2 Fig).

Correlation among the observed traits

Correlation analysis was performed among eight evaluated kernel traits. Out of the 28 possible correlation pairs, there were 23 highly significant (p < 0.01) and two significant (p < 0.05) correlations. Moreover, as shown in Fig 1I, the correlations between phenotypic traits were relatively high, most of them achieved over 80%. Highly positive correlations were observed among KA, KC, KD and KL (r = 0.840–0.999). KA, KC, KL and L/W showed significantly

https://doi.org/10.1371/journal.pone.0229159.g002
negative correlations with KR, while KR was significantly positive correlated with KW. KW showed significantly positive correlations with other kernel traits except for significantly negative correlations with L/W. KA, KC and KL showed significantly positive correlations with L/W, while KR and KW showed significantly negative correlations with L/W (Fig 1I). The correlation between KR and KD was very low, which indicated that the genetic determinant of these two parameters were relatively independent. KR had significantly negative correlation with KA, which suggested tradeoffs between them. Interestingly, TKW was positively correlated with five kernel traits, KA, KC, KD, KL and KW. However, no significant correlation was found for TKW with either KR or L/W (Fig 1I).

Associations for kernel-related traits

In our study, 1366 single nucleotide polymorphisms (SNP) markers covering the whole genome of durum were used to genotype 150 durum germplasm accessions. Details on the population structure and the linkage decay were described in our previous study [33]. Here, association analyses were performed on the 8 kernel traits and SNP markers. In total, 109 trait-marker associations (MTAs) were identified by MLM for the all kernel traits across five consecutive years. It was found that the numbers of MTAs in the year 2014, 2017 and 2018 were similar (S1 Table). The complete list of MTAs was shown in Table 2. The number of SNPs detected for a trait varied among the years. A total of 17 SNP markers were detected for KR in year 2016, which was the maximum amount of SNPs in single year for single trait (S3A Fig). About 94.5% of the significant SNPs for kernel traits exhibited marker-\( R^2 < 10\% \), only a few SNPs associated with KA, KC, KL, KR and L/W showed \( R^2 \geq 10\% \) (S3B Fig). The results implied that the kernel-related traits in durum are mainly controlled by many loci with minor

| Trait | Marker | Allele | Environment | P value | \( R^2 \) |
|-------|--------|--------|-------------|---------|--------|
| KA    | BE500291_5_A_37 | T/C | 2015 | 0.0003 | 0.1100 |
|       |        |       | 2016 | 0.0016 | 0.0702 |
|       |        |       | 2017 | 0.0094 | 0.0471 |
|       |        |       | 2018 | 0.0043 | 0.0568 |
|       |        |       | Blup | 0.0016 | 0.0703 |
|       | BE445667_6_B_Y_285 | A/C | 2016 | 0.0095 | 0.0469 |
|       | CD452967_5_B_Y_229 | T/C | 2017 | 0.0058 | 0.0534 |
| KC    | BE500291_5_A_37 | T/C | 2015 | 0.0000 | 0.1250 |
|       |        |       | 2016 | 0.0006 | 0.0839 |
|       |        |       | 2017 | 0.0039 | 0.0585 |
|       |        |       | 2018 | 0.0008 | 0.0789 |
|       |        |       | Blup | 0.0005 | 0.0871 |
|       | BF483039_7_A_Y_202 | A/G | 2017 | 0.0058 | 0.0723 |
| KD    | BE500291_5_A_37 | T/C | 2015 | 0.0004 | 0.0899 |
|       |        |       | 2016 | 0.0030 | 0.0630 |
|       |        |       | 2018 | 0.0060 | 0.0535 |
|       |        |       | Blup | 0.0023 | 0.0665 |
|       | CD453605_6_B_427 | A/G | 2016 | 0.0088 | 0.0678 |
|       |        |       | 2018 | 0.0060 | 0.0732 |
|       | BF292193_7_B_N_78 | A/C | 2014 | 0.0086 | 0.0492 |
|       | CD452967_5_B_Y_229 | T/C | 2017 | 0.0008 | 0.0816 |

(Continued)
Table 2. (Continued)

| Trait | Marker                | Allele | Environment | P value | R²   |
|-------|-----------------------|--------|-------------|---------|------|
| KL    | BE500291_5_A_37       | T/C    | 2015        | 0.0000  | 0.1265 |
|       |                       |        | 2016        | 0.0007  | 0.0812 |
|       |                       |        | 2017        | 0.0039  | 0.0583 |
|       |                       |        | 2018        | 0.0004  | 0.0893 |
|       |                       |        | Blup        | 0.0004  | 0.0896 |
|       | BQ168780_5_B_995      | C/G    | 2014        | 0.0025  | 0.0847 |
|       |                       |        | 2015        | 0.0065  | 0.0709 |
|       | BF483039_7_A_Y_202    | A/G    | 2017        | 0.0070  | 0.0697 |
|       | BG274985_5_A_Y_267    | T/C    | 2018        | 0.0096  | 0.0467 |
|       | BE403211_5_A_Y_601    | A/G    | 2014        | 0.0044  | 0.0930 |
|       |                       |        | Blup        | 0.0027  | 0.0642 |
|       |                       |        | 2015        | 0.0009  | 0.0780 |
|       |                       |        | 2016        | 0.0036  | 0.0605 |
|       |                       |        | 2017        | 0.0079  | 0.0501 |
|       |                       |        | Blup        | 0.0032  | 0.0992 |
|       | BE474023_3_A_Y_425    | T/C    | 2014        | 0.0043  | 0.0577 |
|       |                       |        | 2015        | 0.0009  | 0.0701 |
|       |                       |        | 2016        | 0.0036  | 0.0733 |
|       |                       |        | 2017        | 0.0078  | 0.0692 |
|       | BE404377_4_B_Y_333    | T/C    | 2014        | 0.0071  | 0.0861 |
|       |                       |        | 2016        | 0.0017  | 0.1094 |
|       |                       |        | Blup        | 0.0032  | 0.0992 |
|       | BE352626_4_A_Y_110    | T/C    | 2014        | 0.0091  | 0.0480 |
|       |                       |        | 2015        | 0.0061  | 0.0529 |
|       | BM140538_2_B_133      | A/G    | 2014        | 0.0061  | 0.0530 |
|       |                       |        | 2016        | 0.0096  | 0.0475 |
|       | BE404912_6_B_Y_488    | T/G    | 2017        | 0.0009  | 0.1201 |
|       | BE405604_2_A_Y_353    | A/T    | 2015        | 0.0065  | 0.0522 |
|       | BE425301_4_A_Y_160    | A/G    | 2016        | 0.0058  | 0.0541 |
|       | BE444480_2_A_N_24     | A/G    | 2015        | 0.0093  | 0.0475 |
|       | BE488358_2_B_N_620    | T/G    | 2015        | 0.0085  | 0.0487 |
|       | BE517914_3_A_Y_81     | T/G    | 2015        | 0.0023  | 0.0866 |
|       | BE591739_4_A_Y_131    | T/C    | 2015        | 0.0065  | 0.0520 |
|       | BF482356_4_B_Y_504    | A/C    | 2015        | 0.0095  | 0.0658 |
|       | BG262421_6_A_87       | A/G    | 2015        | 0.0073  | 0.0695 |
|       | BG263233_1_A_Y_836    | A/G    | 2016        | 0.0074  | 0.0508 |
|       | BG605368_2_A_156      | T/C    | 2016        | 0.0036  | 0.0976 |
|       | BQ161779_6_B_Y_185    | C/G    | 2016        | 0.0074  | 0.0508 |
|       | CD452413_3_B_Y_189    | T/C    | 2014        | 0.0095  | 0.0816 |
| KW    | BF291774_6_B_519      | A/G    | 2014        | 0.0057  | 0.0733 |
|       | BF292193_7_B_N_78     | A/C    | 2014        | 0.0072  | 0.0509 |
|       | BE497375_7_A_Y_191    | A/G    | 2016        | 0.0033  | 0.0617 |
|       | CD452967_5_B_Y_229    | T/C    | 2017        | 0.0006  | 0.0833 |
|       | RE637838_7_A_Y_208    | A/G    | 2018        | 0.0054  | 0.0739 |
|       | BE499652_7_A_Y_391    | A/G    | 2018        | 0.0058  | 0.0727 |
|       | BE495175_3_B_Y_443    | A/T    | 2018        | 0.0060  | 0.0723 |
|       | BE517872_2_A_N_504    | A/G    | 2018        | 0.0064  | 0.0712 |
|       | BE498763_6_A_Y_318    | A/G    | 2018        | 0.0064  | 0.0712 |
|       | BE499248_7_B_Y_63     | A/T    | 2018        | 0.0070  | 0.0857 |
|       | BG604507_4_B_383      | T/C    | 2018        | 0.0071  | 0.0856 |

(Continued)
| Trait  | Marker              | Allele | Environment | P value | R^2  |
|--------|---------------------|--------|-------------|---------|------|
|        |                     |        | 2014    | 0.0048  | 0.0559 |
|        |                     |        | 2015    | 0.0007  | 0.0815 |
|        |                     |        | 2016    | 0.0020  | 0.0672 |
|        |                     |        | 2017    | 0.0038  | 0.0589 |
|        |                     |        | 2018    | 0.0040  | 0.0580 |
|        | Blup                |        |          | 0.0015  | 0.0715 |
|        |                     |        |          | 0.0086  | 0.0482 |
|        |                     |        | 2016    | 0.0096  | 0.0469 |
|        |                     |        | 2017    | 0.0095  | 0.0470 |
|        |                     |        |          | 0.0069  | 0.0858 |
|        |                     |        |          | 0.0088  | 0.0665 |
|        |                     |        | 2015    | 0.0060  | 0.0714 |
|        |                     |        |          | 0.0081  | 0.0677 |
|        |                     |        | 2014    | 0.0002  | 0.1047 |
|        |                     |        | 2016    | 0.0096  | 0.0806 |
|        |                     |        |          | 0.0054  | 0.0541 |
|        |                     |        | 2014    | 0.0072  | 0.0506 |
|        |                     |        | 2015    | 0.0043  | 0.0566 |
|        |                     |        | 2015    | 0.0012  | 0.0949 |
|        |                     |        |          | 0.0064  | 0.0711 |
|        |                     |        | 2014    | 0.0016  | 0.0704 |
|        |                     |        | 2018    | 0.0054  | 0.0541 |
|        |                     |        | 2014    | 0.0068  | 0.0861 |
|        |                     |        | 2016    | 0.0028  | 0.0627 |
|        |                     |        | 2016    | 0.0028  | 0.0627 |
|        |                     |        | 2016    | 0.0080  | 0.0492 |
|        |                     |        | 2016    | 0.0087  | 0.0668 |
|        |                     |        | 2014    | 0.0078  | 0.0683 |
|        |                     |        | 2016    | 0.0038  | 0.0587 |
|        |                     |        | 2016    | 0.0060  | 0.0722 |
|        |                     |        | 2014    | 0.0080  | 0.0492 |
|        |                     |        | 2016    | 0.0080  | 0.0492 |
|        |                     |        | 2016    | 0.0080  | 0.0492 |
|        |                     |        | 2016    | 0.0080  | 0.0492 |
|        |                     |        | 2016    | 0.0080  | 0.0492 |
|        |                     |        | 2015    | 0.0073  | 0.0686 |
|        |                     |        | 2016    | 0.0041  | 0.0776 |
|        |                     |        | 2016    | 0.0062  | 0.0525 |
|        |                     |        | 2016    | 0.0080  | 0.0492 |
|        |                     |        | 2018    | 0.0031  | 0.0612 |
|        |                     |        | 2017    | 0.0078  | 0.0839 |
|        |                     |        | 2016    | 0.0025  | 0.0852 |
|        |                     |        | 2018    | 0.0066  | 0.0515 |
|        |                     |        | 2016    | 0.0080  | 0.0492 |
|        | TKW                 |        | 2015    | 0.0029  | 0.0625 |
|        |                     |        | 2018    | 0.0071  | 0.0507 |
|        |                     |        |          | 0.0090  | 0.0476 |
|        |                     |        | 2014    | 0.0048  | 0.0753 |
|        |                     |        | 2015    | 0.0093  | 0.0472 |
|        |                     |        | 2016    | 0.0036  | 0.0595 |
|        |                     |        | 2017    | 0.0083  | 0.0673 |
|        |                     |        | 2017    | 0.0015  | 0.0713 |
|        |                     |        | 2018    | 0.0083  | 0.0828 |

https://doi.org/10.1371/journal.pone.0229159.t002
As the most reliable method in detecting significant associations, BLUP was calculated to minimize the errors caused by simple means of all the years [37]. Only 11 significant MTAs for evaluated traits of kernel were obtained based on BLUP values, and no significant associations were found for KW (S2 Table). No SNP with $R^2 > 10\%$ was detected for all measured traits under BLUP model (S3C Fig).

Only three SNP markers for KA were detected across the five years. These QTLs were located on chromosomes 5A, 5B and 6B. Out of these 3 SNPs, one repeatable SNP BE500291_5_A_37 was detected for four consecutive years (Fig 3A), and explained 4.71–11.0% of the phenotypic variation with the highest contribution value in year 2015 (Table 2).

Only two SNP markers for KC were identified in five consecutive years. BE500291_5_A_37 was detected from year 2015 to 2018 (Fig 3B), accounting for 5.85–12.50% of the phenotypic variance. The highest–log10 (p) value for KC was obtained from this SNP, with–log10 (p) of 4.49 in 2015 (S4 Fig). The other associated SNP marker BF483039_7_A_Y_202 was only detected in year 2017, explaining 7.23% phenotypic variation (Table 2).

A total of 4 SNP markers for KD were obtained in five consecutive years, and located on chromosome 5A, 5B, 6B and 7B, respectively. The phenotypic variance explained (PVE) values were from 4.92% to 8.99% (Table 2). Two markers BE500291_5_A_37 and CD453605_6_B_427 were detected in multiple years, and the other two were year-specific markers (Fig 3C).

In total, 5 SNP markers were detected for KL, individually contributed to 4.67–12.65% of the phenotypic variance (Table 2). The highest–log10 (p) value for KL was obtained from the SNP marker BE500291_5_A_37 in 2015 (S4 Fig). Moreover, the stable SNP BE500291_5_A_37

---

**Fig 3.** The Venn diagram of significant associations for the kernel traits in five years. (A) kernel area (KA), (B) kernel circumference (KC), (C) kernel diameter (KD), (D) kernel length (KL), (E) kernel roundness (KR), (F) kernel width (KW), (G) length/width ratio (L/W), (H) thousand kernel weight (TKW).

https://doi.org/10.1371/journal.pone.0229159.g003
was significantly associated with KL in four consecutive years, and the SNP marker BQ168780_5_B_995 in two consecutive years were detected (Fig 3D).

Eighteen SNPs for KR were identified in five consecutive years, and were distributed more evenly on A subgenome than that on B subgenome. They individually explained 4.75–12.01% of the phenotypic variance, with BE404912_6_B_Y_488 detected in year 2017 displaying the highest contribution value (Table 2). Five repeatable SNPs were detected in multiple years and the other 13 environment-specific SNPs were not monitored repeatedly (Fig 3E). The stable marker, BF474023_3_A_Y_425 explained 5.01–7.80% PVE, and was observed in four consecutive years (Table 2).

Eleven SNPs for KW were obtained in five consecutive years. These SNPs were distributed on eight chromosomes, and explained 5.09–8.57% of the phenotypic variance. No SNP for KW was repeatedly detected (Table 2, Fig 3f). The SNP marker BE499248_7_B_Y_63 on chromosome 7B was detected in year 2017 displaying the highest contribution value.

In total, 41 significant associations between L/W and SNPs were detected in five consecutive years. The SNPs markers were located in almost all chromosomes, accounting for 4.69–10.47% of the phenotypic variance. Six repeatable SNPs were mapped in multiple years. Obviously, one stable marker BF474023_3_A_Y_425 was observed in all the five consecutive years, explaining 5.59–8.15% PVE (Table 2, Fig 3G).

Seven SNPs influencing TKW were found in five consecutive years (Fig 3H), which were relatively equally distributed on six chromosomes, and explained 4.72–9.35% of the phenotypic variance, with the highest contribution value from BE405269_4_B_84 (Table 2).

In general, after the deletion of duplicated SNPs in Table 2, 54 unique SNP markers were found (Table 3), which distributed unevenly across almost all chromosomes except chromosomes 1A (Table 3 and S5 Fig). About half of the SNP markers were derived from four chromosomes, 2A, 6A, 4B and 6B (S5 Fig). Association analysis also showed that only 5 significant SNPs were obtained for all traits using their BLUP values (S2 Table), including BE517914_3_A_Y_81 and BF292264_7_A_779 associated with L/W, BF474023_3_A_Y_425 and BF474862_5_A_762 associated with both KR and L/W, BE500291_5_A_37 associated with KA, KC, KD, KL and TKW (S2 Table). Combining all significant associations identified from annual data and BLUP data together, 19 repeatable associations, each of which was detected in two or more environments, were identified from different evaluated traits (S3 Table). For example, BF474023_3_A_Y_425 associated with L/W was observed in all environments, and other SNPs were detected in two to five environments.

Based on the association study using BLUP values across the five consecutive years, the number of SNP markers associated with L/W was relatively higher than other kernel traits, most of which only have one marker (S2 Table). Thus, haplotype study was carried out on the L/W trait with the number of favored alleles. Seven haplotypes were identified across four significant SNPs (S4 Table). Among lines having 1 to 2 favorable alleles, the values of L/W were relatively higher, while with increasing numbers of favorable alleles the values were decreased. Accordingly, it was shown negative correlation between the L/W and the number of favorable alleles (R² = 0.527) using linear regression analysis (S3D Fig).

Combination Analysis of Loci Identified here with Previously Known QTL

In previous studies of kernel traits in durum, only kernel weight-related QTLs have been identified using traditional linkage mapping and genome-wide association mapping [38–54]. After searching QTL identified here with previously reported QTL in durum wheat genome, most of the SNPs identified from kernel-related traits in this study were close to or overlapped with the positions of kernel weight-related QTL reported in previous studies. The loci of twelve SNPs
Table 3. Candidate SNP loci identified in this study overlapping the regions of previously known QTLs in durum genome.

| Marker* | Position of markerb | Known QTL of kernel-relative traitc |
|---------|---------------------|-----------------------------------|
| chrB    | Start (bp)          | End (bp)                          | Name             | Position (bp) | Physical distance (bp) | Trait                | Reference          |
|         |                     |                                   |                  |              |                       |                     |
| BG263233_1_A_Y_836 | chr1B 600671684 | 600671642 | QTL1735_1B | chr1B:33966133–594220377 | 560234244 | kernel weight | Peng et al. [38] |
| BG056044_2_A_Y_353 | chr2A 461924254 | 461924495 | QTL0677_TKW | chr2A:194985905–605115332 | 410129427 | kernel weight | Mangini et al. [39] |
| BE446480_2_A_N_24 | chr2A 106406344 | 106406383 | n.d       |                     |                       |                     |                   |
| BE488358_2_B_N_620 | chr2A 767209359 | 767209599 | QTL1745_2A | chr2A:761218533–775446234 | 14230401 | kernel weight | Peng et al. [38] |
| BE406551_2_B_Y_100 | chr2B 493058796 | 493059015 | QTL1130_2B | chr2B:196546476–537614490 | 341068014 | kernel weight | Faris et al. [42] |
| BG274985_5_A_995 | chr2A 57497976 | 57497981 | n.d       |                     |                       |                     |                   |
| BG189892_2_A_208 | chr2A 755759658 | 755759898 | QTL1515_2A | chr2A:75536060–775446234 | 223389624 | kernel weight | Maccferri et al. [40] |
| BE403597_2_B_Y_552 | chr2B 489898967 | 489899208 | QTL1130_2B | chr2B:196546476–537614490 | 341068014 | kernel weight | Faris et al. [42] |
| BE404332_2_B_29 | chr2B 346924954 | 346925043 | QTL1130_2B | chr2B:196546476–537614490 | 341068014 | kernel weight | Faris et al. [42] |
| BE466087_3_B_Y_750 | chr3B 125801022 | 125801223 | n.d       |                     |                       |                     |                   |
| BE495175_3_B_Y_443 | chr3B 72255163 | 72255404 | QTL1134_3B | chr3B:736501649–772286011 | 35784362 | kernel weight | Faris et al. [42] |
| BE352626_4_A_Y_110 | chr4A 102124871 | 102125100 | n.d       |                     |                       |                     |                   |
| BE423501_4_A_Y_160 | chr4A 36377171 | 36377412 | n.d       |                     |                       |                     |                   |
| BE495116_4_A_Y_239 | chr4A 179488832 | 179488832 | QTL0962_TKW | chr4A:103274341–576057502 | 472783161 | kernel weight | Mangini et al. [39] |
| BE517913_4_A_Y_131 | chr4A 265664 | 265667 | n.d       |                     |                       |                     |                   |
| BE404377_4_B_Y_333 | chr4B 185966672 | 185966964 | QTL1725_4B | chr4B:38526374–526354820 | 490528446 | kernel weight | Peleg et al. [41] |
| BE442666_4_B_Y_327 | chr4B 25926747 | 25926988 | QTL0905_TKW | chr4B:38526374–49274630 | 41348256 | kernel weight | Soriano et al. [45] |
| BE442666_4_B_Y_327 | chr4B 25926747 | 25926988 | QTL1676_4B | chr4B:180052042–504276765 | 324224723 | kernel weight | Patil et al. [46] |
| BE40977_4_B_Y_227 | chr4B 26664293 | 26664534 | QTL2013_4B | chr4B:212262807–774081325 | 31452620 | kernel weight | Mangini et al. [39] |
| BE405269_4_B_84 | chr4B 643901320 | 643901523 | QTL1406_4B | chr4B:624077361–653894380 | 29817019 | kernel weight | Graziani et al. [44] |
| BE443253_4_B_Y_414 | chr4B 396507651 | 396507832 | QTL1725_4B | chr4B:38526374–526354820 | 490528446 | kernel weight | Peleg et al. [41] |
| BF482356_4_B_Y_504 | chr4B 338755473 | 338755655 | QTL0905_TKW | chr4B:38526374–49274630 | 41348256 | kernel weight | Soriano et al. [45] |
| BG604507_4_B_583 | chr4B 120944366 | 120944607 | QTL1725_4B | chr4B:38526374–526354820 | 490528446 | kernel weight | Peleg et al. [41] |
| BG604507_4_B_583 | chr4B 120944366 | 120944607 | QTL1676_4B | chr4B:180052042–504276765 | 324224723 | kernel weight | Patil et al. [46] |
| BE500291_5_A_Y_37 | chr5A 149303640 | 149303797 | QTL0201_TKW | chr5A:43811436–321137020 | 277325584 | kernel weight | Kidane et al. [48] |
| BE403211_5_A_Y_601 | chr5A 584269900 | 584270062 | n.d       |                     |                       |                     |                   |
| BG274985_5_A_Y_267 | chr5A 312216001 | 312216242 | QTL0201_TKW | chr5A:43811436–321137020 | 277325584 | kernel weight | Kidane et al. [48] |

(Continued)
identified in this study were not detected previously (Table 3). In addition, each of the eight SNP markers **BE483358_2_B_N_620**, **BE404377_4_B_Y_333**, **BE443253_4_B_Y_414**, **BF482356_4_B_Y_504**, **BG604507_4_B_383**, **BF291774_6_B_519**, **BE443010_7_B_354** and **CD453605_6_B_427** represents the SNP markers overlapped with two or more known QTLs.

Table 3. (Continued)

| Marker* | Position of markerb | Known QTL of kernel-relative traitc |
|---------|---------------------|-------------------------------------|
| Chr | Start | End | Name | Position | Physical distance | Trait | Reference |
| CD452967_5_B_Y_229 | chr5B | 121165117 | 121165358 | QTL0204_TKW | chr5B:461476–132851290 | 86703687 | kernel weight | Kidane et al. [48] |
| | | | | QTL0205_TKW | chr5B:5757235–370924682 | 313352130 | kernel weight | Kidane et al. [48] |
| | | | | QTL2045_5B | chr5B:68504586–470496688 | 42854102 | kernel weight | Thanh et al. [49] |
| | | | | QTL2044_5B | chr5B:68504586–467639351 | 399134765 | kernel weight | Thanh et al. [49] |
| BE496986_6_A_110 | chr6A | 536591248 | 536591477 | n.d. | | |
| BE498763_6_A_Y_318 | chr6A | 459213165 | 459213406 | QTL1416_6A | chr6A:129338–480593476 | 35122509 | kernel weight | Graziani et al. [44] |
| | | | | QTL0719_TKW | chr6A:52609610–455718431 | 293142233 | kernel weight | Roncallo et al. [51] |
| BE636872_6_A_119 | chr6A | 609352251 | 609352013 | QTL1361_6A | chr6A:2579–608245286 | 82589324 | kernel weight | Golabadi et al. [50] |
| BF483091_6_A_357 | chr6A | 598092326 | 598092567 | QTL1361_6A | chr6A:2579–608245286 | 82589324 | kernel weight | Golabadi et al. [50] |
| BG262421_6_A_87 | chr6A | 83401625 | 83401831 | QTL0719_TKW | chr6A:7213818–493284322 | 421145604 | kernel weight | Mangini et al. [39] |
| BE404912_6_B_Y_488 | chr6B | 53251449 | 53251690 | QTL1965_6B | chr6B:152609610–455718431 | 303142233 | kernel weight | Roncallo et al. [51] |
| BEQ161779_6_B_Y_185 | chr6B | 551413872 | 551414113 | QTL1416_6B | chr6B:129338–480593476 | 35122509 | kernel weight | Tzarfati et al. [52] |
| BF4829164_7_A_779 | chr7A | 18762196 | 18762437 | QTL0160_TKW | chr7A:2374395–29299532 | 5554579 | kernel weight | Giraldo et al. [54] |
| BF499248_7_B_Y_63 | chr7B | 26282735 | 26282917 | QTL0982_TKW | chr7B:411621786–496126441 | 84499255 | kernel weight | Roncallo et al. [51] |
| | | | | QTL0979_TKW | chr7B:459321833–517442227 | 58120394 | kernel weight | Blanco et al. [43] |
| BE499652_7_A_Y_391 | chr7A | 157545898 | 157546139 | QTL1684_7A | chr7A:106152757–131796856 | 25644099 | kernel weight | Patil et al. [46] |
| BE637838_7_A_Y_208 | chr7A | 689303442 | 689303683 | QTL0731_TKW | chr7A:694638997–718753890 | 23214893 | kernel weight | Mangini et al. [39] |
| BF292264_7_A_779 | chr7A | 18762196 | 18762437 | QTL0160_TKW | chr7A:2374395–29299532 | 5554579 | kernel weight | Giraldo et al. [54] |
| BF292193_7_B_N_78 | chr7B | 576058434 | 576058629 | QTL0737_TKW | chr7B:496126667–578067640 | 82480073 | kernel weight | Mangini et al. [39] |
| BE443010_7_B_354 | chr7B | 503240339 | 503240580 | QTL0737_TKW | chr7B:496126667–578067640 | 82480073 | kernel weight | Mangini et al. [39] |
| | | | | QTL1982_7B | chr7B:411621786–496126441 | 84499255 | kernel weight | Roncallo et al. [51] |
| | | | | QTL0979_7B | chr7B:459321833–517442227 | 58120394 | kernel weight | Blanco et al. [43] |
| BF483039_7_A_Y_202 | chrUn | 153138960 | 153139201 | n.d. | | |
| BM140538_2_B_133 | chrUn | 34717974 | 34718218 | QTL1733_7B | chr7B:3257279–7257942 | 4900663 | kernel weight | Peleg et al. [41] |
| BF474862_5_A_762 | chrUn | 68232437 | 68232678 | n/a | | |

*a* represents the SNP markers overlapped with two or more known QTLs.

*b* Candidate SNP markers identified in this study.

*c* The position of marker in durum genome.

*d* QTLs for kernel-relative traits reported in previous studies.

Chr, chromosome. n.d, not denoted; n/a, not applicable.
BE499248_7_B_Y_63, was overlapped with more than three known QTLs detected for kernel-weight traits in previous reports (Table 3). Interestingly, half of them were located on chromosome 4B.

Moreover, all four SNPs BE403597_2_B_Y_552, BE404332_2_B_29, BE406351_2_B_Y_100 and BE517872_2_A_N_504 from chromosome 2B were located on the same known QTL of QTL1130_2B with physical distance of 341 Mb between chr2B-196546476 and chr2B-537614490 (Table 3). Two adjacent SNPs, BE404977_4_B_Y_227 and BE442666_4_B_Y_327, were found in the same QTL region of QTL2013_4B on chromosome 4B. It also has two SNP markers close to both sides of the same QTL region of QTL1361_6A with BE636872_6_A_119 and BF483091_6_A_357 on each side. Meanwhile, the physical areas for these two QTL were relative narrow, only about 10 Mb of chromosome regions for both QTL2013_4B and QTL1361_6A (Table 3). In addition, the two QTLs, QTL0160_TKW flanked by BF292264_7_A_779 and QTL1733_7B overlapped with BE499248_7_B_Y_63, have the minimum physical interval of about 5 Mb (Table 3).

Identification of candidate genes

Since the resolution was very low and LD were significantly large in this study, it would be rather difficult to define candidate genes. As the SNP markers used in this study were developed from the EST database, so these SNPs were actually expressed genes in wheat. Thus, the EST sequences related to candidate SNP markers were analyzed by using BLAST at the NCBI for gene function prediction. As the result, a total of 54 candidate genes supposed to be important for kernel traits were annotated from the significantly associated markers in this study (S5 Table). The candidate genes were divided into several categories, most of them encoded metabolism related enzymes, and some of them involved in kernel development. A comparison of SNPs detection by using five-years BLUP values also indicated the most consistent association for kernel traits was the same SNP of BE500291_5_A_37 (S3 Table). The sequence of this stable marker was derived from wheat pre-anthesis spike cDNA library, whose functional annotation was best matched with 1-acyl-sn-glycerol-3-phosphate acyltransferase (PLS1). Thus, PLS1 gene might play a core role in grain development in durum wheat. Another important SNP locus, BF474023_3_A_Y_425 located on chromosome 3A was simultaneously detected in all of the five environments for L/W (Table 2), whose functional annotation is abscisic acid insensitive like1 protein (ABIL1) (S5 Table). It can be considered that PLS1 and ABIL1 are two of the most important genes that determine grain architecture in durum.

Relationship between climatic variables and kernel traits

Climate variability is one of the most important factors for crop production. In order to evaluate the potential impact of climatic factors on kernel growth, a preliminary analysis has been performed on their association. We collected five years of meteorological information from weather station in China’s central Hubei province (S6 Table). Correlation analysis showed that there was no significant correlation between climatic variables and kernel traits at stage I (Fig 4A). However, the significant and positive correlations were found between temperature and five kernel traits except KR, and L/W and TKW at stage II (Fig 4B). Furthermore, the correlation analysis indicated that significant and positive correlations were presented between temperature and both KR and KW traits, while significant and negative correlation between temperature and L/W at stage III (Fig 4C). In addition, the average of rainfall precipitation was negatively correlated with almost all of the kernel traits, and exhibited significant and negative correlations with TKW at stage III (Fig 4C).
Discussion

GWAS is the most popular approach for dissecting the genetic constitution of the heritable complex traits [55]. So far, it has been successfully used in the exploration of candidate genes in durum [56]. However, few genes/QTLs associated with kernel traits have been identified in durum wheat through the association mapping approach. In this study, we intended to reveal the genetic architecture of kernel characters in a panel of 150 durum lines collected from 46 countries and regions. A lot of SNPs associated with kernel-related traits were identified. Our results provide a useful resource for further functional studies to understand the molecular mechanism of the regulation involved in grain development.

The stable SNPs for controlling a trait or different traits

The effect of association analysis could be impacted by genetic and environmental factors [57]. In order to increase the reliability of SNPs identified, a total of five year data were used to identify associations for kernel traits in our study. A considerable number of SNP markers were detected in more than two environments and exhibited obvious environmental stability (Table 3 and S3 Table). Therefore, the more consistency of obtained a SNP for a kernel trait across different environments implied, the more importance of itself in kernel development. For instance, BF474023_3_A_Y_425 was repeatedly detected for L/W in all environments. Thus, this locus may play an important potential role in kernel development. However, all SNP markers identified for KW in wheat had poor stability, which were detected only in a single year. The results implied that kernel width might be controlled and modified by more minor effect genes. The multiple effects of a single gene on different phenotypic traits are the phenomenon of gene pleiotropy [58]. Many candidate genes tagged by SNP markers may control multiple kernel-related traits in this study. Observably, sixteen important pleiotropic loci were further identified by overlapping analysis (S7 Table). In particular, BE500291_5_A_37, one stable SNP for KA, KC, KD, KL, L/W and TKW, was repeatedly detected in two or more environments for each associated trait with less environmental interactions (Table 3).
Therefore, the candidate gene marked by \textit{BE500291\_5\_A\_37} may be a critical regulator for kernel development.

\textbf{Molecular mechanisms underlying kernel-related traits}

It is difficult to define candidate genes as the low resolution and large LD in this study. Nevertheless, the SNP markers used in this study was developed from the EST database, these ESTs might be candidate genes. As this study shown, the stable SNP marker \textit{BE500291\_5\_A\_37}, concurrently associated with six of kernel-related traits, which provided a candidate for further studying its function on grain development. Moreover, some other genes tagged by the EST-derived SNP markers may play roles in kernel development of durum wheat. The EST of \textit{BF482356\_4\_B\_Y\_504} was shown very high homology with the ubiquitin carboxyl-terminal hydrolase 12 (S5 Table). The E3 Ubiquitin ligase OsGW2 is associated with rice grain development by influencing kernel width and weight. Its homologue gene, located on the homologous group 6 chromosomes in wheat [59], was also identified and considered as a candidate gene related to grain weight and width [60]. Thus, a new ubiquitin-mediated pathway contributed to kernel development in durum might be controlled by the gene marked by \textit{BF482356\_4\_B\_Y\_504} on chromosome 4B. Because of the role of auxin in regulating grain size, plant productivity could be improved by altering auxin transport and distribution [61]. Consequently, the low expression of \textit{TaTGW6} was associated with low auxin content that was considered to be the main influence factor for grain development of wheat [62]. In this study, the SNP marker \textit{BE497375\_7\_A\_Y\_191}, significantly associated with KW (Table 3), was found to be very high homology with auxin-responsive protein IAA21 (S5 Table). Therefore, we speculated that the contribution of \textit{BE497375\_7\_A\_Y\_191} to KW might be attributed to the role of IAA signaling pathway. Abscisic acid-response genes have effects on accumulation of storage proteins and participate in seed development, such as in Arabidopsis and soybean [63, 64]. The EST of \textit{BF474023\_3\_A\_Y\_425} has very high homology with an ABA insensitive protein encoded by \textit{ABIL1} (Abscisic acid insensitive like 1). Therefore, the responsive gene involved in ABA signaling pathway might be correlated with grain development of durum.

\textbf{Syntenic regions of candidate genes in 5A chromosome}

In the present study, many SNP markers were identified for kernel-related traits in different years in durum wheat. In which, a stable and multi-traits associated locus \textit{BE500291\_5\_A\_37} was mapped on chromosome 5A (Table 3 and S5 Fig), which can be further explored for discovering candidate genes and for function analysis across traits and environments. Integrated with our published studies [36, 65, 66], we further picked out all of those significant SNPs which we had previously found in chromosome 5A. In total, 23 unique significant SNPs were associated with 41 evaluated traits at different developmental stages of vegetative and reproductive growth in durum (S8 Table). According to the durum genome sequence information, several of them were clustered on 5A region with a short physical distance of 31 Mb (S6 Fig), implying that this region might be SNP hotspots. Meanwhile, co-localizing SNPs were identified among seedling traits, canopy leaf traits, agronomic traits, and kernel traits. Especially, a SNP \textit{BE443538\_5\_A\_1436} associated with 19 traits was deemed to be a super pleiotropic marker that was highly related with the growth and development of durum. Therefore, the candidate genes close to \textit{BE443538\_5\_A\_1436} might affect multi-phenotypes in durum. Therefore, this region from 129–160 Mb encompassed by six SNP markers on chromosome 5A was supposed to be the crucial candidate region for gene discovery in our future work.
The impact of climate variability on kernel traits

In this study, phenotypes of some kernel traits seemed to be affected by environments, presenting different trends in different years, the values in the years from 2014 to 2016 were all significantly lower than those from 2017 to 2018 (S1 Fig). Previous research has demonstrated that the final grain yield is controlled by a network of genes and environment factors [67], such as temperature, sunlight, and rainfall precipitation. Especially, temperature was the major governing factor during crop growth period [68]. The variation in average growing-season temperatures of ±2°C can cause reduction in grain production up to 50% for wheat in Australia [69]. It was proposed that global wheat production will change by −2.3% to 7.0% under the 1.5°C warming and −2.4% to 10.5% under the 2.0°C warming [70]. There were few reports about the relationship between grain size and climate in wheat. Previous study showed that the low average temperature in March and April greatly increased grain number per spike, and the longer sunshine duration could increase grain weight in north China [71]. Similarly, the longer sunshine duration at II stage could ultimately increase KA, KC, KD, KL and KW of grain in durum. This result suggested that the role of sunshine duration is quite important in durum growth at jointing stage (Fig 4B). Moreover, temperature showed significant correlations with both KR and KW in the period from heading to ripening, but there were no significant correlations between KL and climate factors in this stage (Fig 4C). Therefore, KL had more climate stability than other evaluated traits. Furthermore, the average precipitation was negatively correlated with almost all of the kernel traits, as well as exhibited significant negatively correlation with TKW (Fig 4C). This indicated the larger amount of precipitation, the less kernel dimension and especially the kernel weight. The research about the adaptation of wheat to areas of Europe indicated that the hotter and drier climate was concerned with quicker maturation, but resulting in lower yields [72]. Similarly, our study implied that colder and moister climate might particularly contribute to lower grain quality of wheat in Middle-lower Yangtze River area in China. Conclusively, the large grain dimension and high grain weight needed a longer sunshine duration, a moderate temperature and certain amount of precipitation at different developmental stages in durum.

Conclusions

To increase yield is still the main goal in common wheat breeding until now. One of the important facets to achieve this goal is to explore novel genetic resources to discover genes that affect grain yield. In this study, association analysis for kernel characters in a natural population of durum wheat was conducted using genome-wide of EST-derived SNP markers. Consequently, 54 significantly unique SNP markers were identified from 109 marker-trait association pairs. Especially, the stable SNP BE500291_5_A_37 was repeatedly detected in two or more environments for each associated trait. The candidate loci identified for controlling kernel traits in durum will provide candidates for studying the genetic architecture of grain quality in common wheat.

Supporting information

S1 Fig. Boxplot of the phenotypic data of eight evaluated kernel traits for the durum wheat natural population in five years. Analysis of variance (ANOVA) was applied to examine the difference of traits among different years. Different numbers indicate statically significant difference at P ≤ 0.05. Phenotypic differences observed for each trait under five consecutive years of 2014–2018, respectively. (A) KA; (B) KC; (C) KD; (D) KL; (E) KR; (F) KW; (G) L/W; (H) TKW. (TIF)
S2 Fig. Comparison of kernel-related traits between landraces and cultivars. Analysis of variance (ANOVA) was applied to examine the difference of traits between landraces and cultivars. There was no significant difference between the two groups (P values > 0.05).

(TIF)

S3 Fig. Associations for kernel-related traits and haplotype study for L/W. (A) SNP numbers for every kernel-related trait in different years. (B) The range of associated R^2-values (variation explained by SNP markers) distributed for each kernel trait detected under five years of 2014–2018. (C) The distribution of R^2-values for each kernel trait evaluated by using five-years best linear unbiased prediction (BLUP) values. (D) Linear regressions between number of favorable alleles and mean phenotypic effect on L/W.

(TIF)

S4 Fig. Manhattan plots for kernel traits and summary of several important SNP markers identified from association analysis. (A) Manhattan plots of P values indicating SNP markers associated with KC in 2015. (B) Manhattan plots of P values indicating SNP markers associated with KL in 2015. The horizontal line indicated P = 0.01 thresholds for significant associations.

(TIF)

S5 Fig. Chromosomal locations of significant SNPs for kernel-related traits identified in this study. Positions of significant markers projected to the durum wheat genome (Triticum turgidum Durum Wheat Svevo, RefSeq Rel. 1.0).

(TIF)

S6 Fig. A physical region of the associated SNP markers on 5A chromosome segment from 129 to 160 Mb.

(TIF)

S1 Table. Number of SNP marker-trait associations for the observed traits in different years.

(XLS)

S2 Table. Significant association pairs between SNP markers and kernel traits detected by using five-years BLUP values.

(XLS)

S3 Table. Significant association pairs between SNP markers and kernel traits detected at least in two environments.

(XLS)

S4 Table. Haplotypes analysis using four SNPs and their phenotypic effects. Marked base representing favorable alleles.

(XLS)

S5 Table. The putative functions of candidate genes for each significant SNPs analyzed by BLAST alignment using their EST sequences.

(XLS)

S6 Table. The average values of rainfall precipitation, temperature and sunlight during three important growth stages in Hubei from 2014 to 2018.

(XLS)
S7 Table. SNP markers associated with multiple kernel traits in durum wheat.
(XLS)

S8 Table. SNP markers on 5A chromosome associated with multiple traits for different developmental stages of vegetative and reproductive growth in durum wheat.
(XLS)

Author Contributions
Conceptualization: Dongfa Sun.
Data curation: Longqing Sun.
Formal analysis: Longqing Sun.
Funding acquisition: Longqing Sun, Dongfa Sun.
Investigation: Longqing Sun, Yujuan Zhang.
Project administration: Dongfa Sun.
Resources: Eviatar Nevo, Junhua Peng.
Software: Sisi Huang, Xin Hu.
Supervision: Dongfa Sun.
Validation: Longqing Sun.
Writing – original draft: Longqing Sun.
Writing – review & editing: Genlou Sun, Dongfa Sun.

References
1. Chenu K, Porter JR, Martre P, Basso B, Chapman SC, Ewert F, et al. Contribution of crop models to adaptation in wheat. Trends Plant Sci. 2017; 22(6): 472–490. https://doi.org/10.1016/j.tplants.2017.02.003 PMID: 28389147
2. Figueroa M, Hammond-Kosack KE, Solomon PS. A review of wheat diseases—a field perspective. Mol Plant Pathol. 2018; 19(6): 1523–1536. https://doi.org/10.1111/mpp.12618 PMID: 29045052
3. Li F, Wen W, He Z, Liu J, Jin H, Cao S, et al. Genome-wide linkage mapping of yield-related traits in three Chinese bread wheat populations using high-density SNP markers. Theor Appl Genet. 2018; 131(9): 1903–1924. https://doi.org/10.1007/s00122-018-3122-6 PMID: 29858949
4. Reynolds M, Foulkes J, Furbank R, Griffiths S, King J, Murchie E, et al. Achieving yield gains in wheat. Plant Cell Environ. 2012; 35(10): 1799–1823. https://doi.org/10.1111/j.1365-3040.2012.02588.x PMID: 22860982
5. Klymiuk V, Fatiukha A, Huang L, Wei ZZ, Kis-Papo T, Saranga Y, et al. Durum wheat as a bridge between wild emmer wheat genetic resources and bread wheat. Appl Genet Genom Res. Cereal, 2019; 201–230.
6. Xiao Y, Qian Z, Wu K, Liu J, Xia X, Ji W, et al. Genetic gains in grain yield and physiological traits of winter wheat in Shandong Province, China, from 1969 to 2006. Crop Sci. 2012; 52(1): 44–56.
7. Sinclair T, Jamieson P. Grain number, wheat yield, and bottling beer: an analysis. Field Crops Res. 2006; 98(1): 60–67.
8. Su Z, Hao C, Wang L, Dong Y, Zhang X. Identification and development of a functional marker of TaGW2 associated with grain weight in bread wheat (Triticum aestivum L.). Theor Appl Genet. 2011; 122(1): 211–223. https://doi.org/10.1007/s00122-010-1437-z PMID: 20938758
9. Jiang Q, Hou J, Hao C, Wang L, Ge H, Dong Y, et al. The wheat (T. aestivum) sucrose synthase 2 gene (TaSus2) active in endosperm development is associated with yield traits. Funct Integr Genomic. 2011; 11(1): 49–61.
10. Ma D, Yan J, He Z, Wu L, Xia X. Characterization of a cell wall invertase gene TaCwi-A1 on common wheat chromosome 2A and development of functional markers. Mol Breed. 2012; 29(1): 43–52.
11. Zhang L, Zhao YL, Gao LF, Zhao GY, Zhou RH, Zhang BS, et al. TaCKX6-D1, the ortholog of rice OsCKX2, is associated with grain weight in hexaploid wheat. New Phytol. 2012; 195(3): 574–584. https://doi.org/10.1111/j.1469-8137.2012.04194.x PMID: 22670578

12. Zhang Y, Liu J, Xia X, He Z. TaGS-D1, an ortholog of rice OsGS3, is associated with grain weight and grain length in common wheat. Mol Breed. 2014; 34(3): 1097–1107.

13. Ma L, Li T, Hao C, Wang Y, Chen X, Zhang X. TaGS5-3A, a grain size gene selected during wheat improvement for larger kernel and yield. Plant Biotechnol. J. 2016; 14(5): 1269–1280. https://doi.org/10.1111/pbi.12492 PMID: 26480952

14. Hanif M, Gao F, Liu J, Wen W, Zhang Y, Rasheed A, et al. TaTGW6-A1, an ortholog of rice TGW6, is associated with grain weight and yield in bread wheat. Mol Breed. 2016; 38(1): 1.

15. Sajjad M, Ma X, Khan SH, Shoaib M, Song Y, Yang W, et al. TaFlo2-A1, an ortholog of rice Flo2, is associated with thousand grain weight in bread wheat (Triticum aestivum L.). BMC Plant Biol. 2017; 17(1): 164. https://doi.org/10.1186/s12870-017-1114-3 PMID: 29037166

16. Zhang Y, Li D, Zhang D, Zhao X, Cao X, Dong L, et al. Analysis of the functions of TaGW2 homeologs in wheat grain weight and protein content traits. Plant J. 2018; 94(5): 857–866. https://doi.org/10.1111/tpj.13903 PMID: 29570880

17. Hong Y, Chen L, Du LP, Su Z, Wang J, Ye X, et al. Transcript suppression of TaGW2 increased grain width and weight in bread wheat. Funct Integr Genomic. 2014; 14(2): 341–349.

18. Wang S, Yan X, Wang Y, Liu H, Cui D, Chen F. Haplotypes of the TaGS5-A1 gene are associated with thousand-kernel weight in Chinese bread wheat. Front Plant Sci. 2016; 7: 783. https://doi.org/10.3389/fpls.2016.00783 PMID: 27375643

19. Chen W, Sun D, Yan X, Li R, Wang S, Shi Y, et al. QTL analysis of wheat kernel traits, and genetic effects of qKW-6A on kernel width. Euphytica. 2019; 215(2): 11.

20. Hu MJ, Zhang HP, Liu K, Cao JJ, Wang SX, Jiang H, et al. Cloning and characterization of TaTGW-7A gene associated with grain weight in wheat via SLAF-seq-BSA. Front Plant Sci. 2016; 7: 1902. https://doi.org/10.3389/fpls.2016.01902 PMID: 28066462

21. Zhang J, Xu Y, Chen W, Deli B, Vergauwen R, Biddulph B, et al. A wheat 1-FEH w3 variant underlies enzyme activity for stem WSC remobilization to grain under drought. New Phytol. 2015; 205(1): 293–305. https://doi.org/10.1111/nph.13030 PMID: 25250511

22. Su Q, Li J. QTL detection for kernel size and weight in bread wheat (Triticum aestivum L.) using a high-density SNP and SSR-based linkage map. Front Plant Sci. 2018; 9: 1484. https://doi.org/10.3389/fpls.2018.01484 PMID: 30364249

23. Cui F, Zhang N, Fan X-i, Zhang W, Zhao C-h, Yang L-j, et al. Utilization of a Wheat660K SNP array-derived high-density genetic map for high-resolution mapping of a major QTL for kernel number. Sci Rep. 2017; 7(1): 3788. https://doi.org/10.1038/s41598-017-04028-6 PMID: 28630475

24. Xu L, Hu K, Zhang Z, Guan C, Chen S, Hua W, et al. Genome-wide association study reveals the genetic architecture of flowering time in rapseed (Brassica napus L.). DNA Res. 2015; 23(1): 43–52. https://doi.org/10.1093/dnares/dsv035 PMID: 26659471

25. Pearson TA, Manolio TA. How to interpret a genome-wide association study. Jama. 2008; 299(11): 1335–1344. https://doi.org/10.1001/jama.299.11.1335 PMID: 18349094

26. Qiu X, Pang Y, Yuan Z, Xing D, Xu J, Dingkuhn M, et al. Genome-wide association study of grain appearance and milling quality in a worldwide collection of indica rice germplasm. PloS One. 2015; 10(12): e0145577. https://doi.org/10.3389/fpls.2015.00145 PMID: 26714258

27. Rahim MS, Sharma H, Parveen A, Roy JK. Trait Mapping Approaches Through Association Analysis in Plants. Adv Biochem Eng Biotechnol. 2018; 83–108.

28. Daba SD, Tyagi P, Brown-Guedira G, Mohammad MM. Genome-wide association studies to identify loci and candidate genes controlling kernel weight and length in a historical US wheat population. Front Plant Sci. 2018; 9: 1045. https://doi.org/10.3389/fpls.2018.01045 PMID: 30123226

29. Zanke CD, Ling J, Plesko J, Kollers S, Ebmeyer E, Korzon V, et al. Analysis of main effect QTL for thousand grain weight in European winter wheat (Triticum aestivum L.) by genome-wide association mapping. Front Plant Sci. 2015; 6: 644. https://doi.org/10.3389/fpls.2015.00644 PMID: 26388777

30. Mir R, Kumar N, Jaiswal V, Girdharwal N, Prasad M, Balyan H, et al. Genetic dissection of grain weight in bread wheat through quantitative trait locus interval and association mapping. Mol Breed. 2012; 29(4): 963–972.

31. Rasheed A, Xia X, Ogbonnaya F, Mahmood T, Zhang Z, Mujeeb-Kazi A, et al. Genome-wide association for grain morphology in synthetic hexaploid wheats using digital imaging analysis. BMC Plant Biol. 2014; 14(1): 128.
32. Arora S, Singh N, Kaur S, Bains NS, Uauy C, Poland J, et al. Genome-wide association study of grain architecture in wild wheat Aegilops tauschii. Front Plant Sci. 2017; 8: 886. https://doi.org/10.3389/fpls.2017.00886 PMID: 28620398

33. Ren J, Sun D, Chen L, You F, Wang J, Peng Y, et al. Genetic diversity revealed by single nucleotide polymorphism markers in a worldwide germplasm collection of durum wheat. Int J Mol Sci. 2013; 14(4): 7061–7088. https://doi.org/10.3390/ijms140407061 PMID: 23538839

34. Yin C, Li H, Li S, Xu L, Zhao Z, Wang J. Genetic dissection on rice grain shape by the two-dimensional image analysis in one japonica × indica population consisting of recombinant inbred lines. Theor Appl Genet. 2015; 126(10): 1969–1986. https://doi.org/10.1007/s00122-015-2560-7 PMID: 26137332

35. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. J Stat Softw. 35.

36. Hu X, Ren J, Ren X, Huang S, Sabiel SA, Luo M, et al. Association of agronomic traits with SNP markers in durum wheat (Triticum turgidum L. durum (Desf.)). PloS One 2015; 10(6): e0130854. https://doi.org/10.1371/journal.pone.0130854 PMID: 26110423

37. Kumar V, Paillard S, Fopa-Foméju B, Falentin C, Deniot G, Baron C, et al. Multi-year linkage and association mapping confirm the high number of genomic regions involved in oilseed rape quantitative resistance to blackleg. Theor Appl Genet. 2018; 131(8): 1627–1643. https://doi.org/10.1007/s00122-018-3103-9 PMID: 29728747

38. Peng J, Ronin Y, Fahima T, Röder MS, Li Y, Nevo E, et al. Domestication quantitative trait loci in Triticum dicoccoides, the progenitor of wheat. Proc Natl Acad Sci USA. 2003; 100(5): 2489–2494. https://doi.org/10.1073/pnas.252763199 PMID: 12604784

39. Mangini G, Gadaleta A, Colasuonno P, Marcotuli I, Signorile AM, Simeone R, et al. Genetic dissection of the relationships between grain yield components by genome-wide association mapping in a collection of tetraploid wheats. PloS One. 2018; 13(1): e0190162. https://doi.org/10.1371/journal.pone.0190162 PMID: 29324803

40. Maccaferri M, El-Feki W, Nazemi G, Salvi S, Canê MA, Colalongo MC, et al. Prioritizing quantitative trait loci for root system architecture in tetraploid wheat. J Exp Bot. 2011; 62(14): 5051–5061. https://doi.org/10.1093/jxb/err206 PMID: 21778183

41. Faris JD, Zhang Q, Chao S, Zhang Z, Xu SS. Analysis of agronomic and domestication traits in a durum × cultivated emmer wheat population using a high-density single nucleotide polymorphism-based linkage map. Theor Appl Genet. 2014; 127(11): 2333–2348. https://doi.org/10.1007/s00122-014-2380-1 PMID: 25186168

42. Bianco A, Mangini G, Giancaspro A, Giove S, Colasuonno P, Simeone R, et al. Relationships between grain protein content and grain yield components through quantitative trait locus analyses in a recombinant inbred line population derived from two elite durum wheat cultivars. Mol Breed. 2012; 30(1): 79–92.

43. Graziani M, Maccaferri M, Royo C, Salvatorelli F, Tuberosa R. QTL dissection of yield components and morpho-physiological traits in a durum wheat elite population tested in contrasting thermo-pluviometric conditions. Crop and Pasture Sci. 2014; 65(1): 80–95.

44. Soriano JM, Malosetti M, Roselli M, Sorrells ME, Royo C. Dissecting the old Mediterranean durum wheat genetic architecture for phenology, biomass and yield formation by association mapping and QTL meta-analysis. PloS One. 2017; 12(5): e0178290. https://doi.org/10.1371/journal.pone.0178290 PMID: 28542488

45. Patil R, Tamhankar S, Oak M, Raut A, Honrao B, Rao V, et al. Mapping of QTL for agronomic traits and kernel characters in durum wheat (Triticum durum Desf.). Euphytica. 2013; 190(1): 117–129.

46. Russo MA, Picco DBM, Laiò G, Marone D, Papa R, Blanco A, et al. A dense durum wheat × T. dicoccum linkage map based on SNP markers for the study of seed morphology. Mol Breed. 2014; 34(4): 1579–1597.

47. Kidane YG, Mancini C, Mengistu DK, Frascaroli E, Fadda C, Pê ME, et al. Genome wide association study to identify the genetic base of smallholder farmer preferences of durum wheat traits. Front Plant Sci. 2017; 8: 1230. https://doi.org/10.3389/fpls.2017.01230 PMID: 28769945

48. Thanh PT, Vladutu CJ, Kianian SF, Thanh PT, Ishii T, Nitta M, et al. Molecular genetic analysis of domestication traits in emmer wheat. I: Map construction and QTL analysis using an F2 population. Biotechnol Biotec Eq. 2013; 27(2): 3627–3637.

49. Gobletti A, Arzani A, Maibody SM, Tabatabaee BS, Mohammadi S. Identification of microsatellite markers linked with yield components under drought stress at terminal growth stages in durum wheat. Euphytica. 2011; 177(2): 207–221.
51. Roncallo PF, Akkiraju PC, Cervigni GL, Echenique VC. QTL mapping and analysis of epistatic interactions for grain yield and yield-related traits in *Triticum turgidum* L. var. durum. Euphytica. 2017; 213(12): 277.

52. Tzarfati R, Barak V, Krugman T, Fahima T, Abbo S, Saranga Y, et al. Novel quantitative trait loci underlying major domestication traits in tetraploid wheat. Mol Breed. 2014; 34(4): 1613–1628.

53. Elouafi I, Nachit M. A genetic linkage map of the Durum × *Triticum dicoccoides* backcross population based on SSRs and AFLP markers, and QTL analysis for milling traits. Theor Appl Genet. 2004; 108(3): 401–413. https://doi.org/10.1007/s00122-003-1440-8 PMID: 14676946

54. Giraldo P, Royo C, González M, Carrillo JM, Ruiz M. Genetic diversity and association mapping for agromorphological and grain quality traits of a structured collection of durum wheat landraces including subsp. durum, turgidum and dicoccoides. PloS One. 2016; 11(11): e0166577. https://doi.org/10.1371/journal.pone.0166577 PMID: 27846306

55. Zhang YM, Jia Z, Dunwell JM. The Applications of New Multi-locus GWAS Methodologies in the Genetic Dissection of Complex Traits. Front Plant Sci. 2019; 10.

56. Fiedler JD, Salsman E, Liu Y, Michalak de Jiménez M, Hegstad JB, Chen B, et al. Genome-wide association and prediction of grain and semolina quality traits in durum wheat breeding populations. Plant Genome. 2017; 10(3).

57. Rellstab C, Gugerli F, Eckert AJ, Hancock AM, Holderegger R. A practical guide to environmental association analysis in landscape genomics. Mol Ecol. 2015; 24(17): 4348–4370. https://doi.org/10.1111/mec.13322 PMID: 26184487

58. Cichon S, Ripke S. Insights From Genome-Wide Association Studies (GWAS). The Neurobiol Schizophren. 2016; 39–50.

59. Qin L, Hao C, Hou J, Wang Y, Li T, Wang L, et al. Homologous haplotypes, expression, genetic effects and geographic distribution of the wheat yield gene *TaGW2*. BMC Plant Biol. 2014; 14(1): 107.

60. Simmonds J, Scott P, Leverington-Waite M, Turner AS, Brinton J, Korzun V, et al. Identification and independent validation of a stable yield and thousand grain weight QTL on chromosome 6A of hexaploid wheat (*Triticum aestivum* L.). BMC Plant Biol. 2014; 14(1): 191.

61. Liu L, Tong H, Xiao Y, Che R, Xu F, Hu B, et al. Activation of Big Grain1 significantly improves grain size by regulating auxin transport in rice. Proc Natl Acad Sci USA. 2015; 112(35): 11102–11107. https://doi.org/10.1073/pnas.1512748112 PMID: 26283354

62. Hu MJ, Zhang HP, Cao JJ, Zhu XF, Wang SX, Jiang H, et al. Characterization of an IAA-glucose hydrolase gene *TaTGW6* associated with grain weight in common wheat (*Triticum aestivum* L.). Mol Breed. 2016; 36(3): 25.

63. Du R, Qiao Y, Wang X, Lv X, Wang J. Establishment and analysis of the mRNA expression patterns of *ABI3*-like and storage protein genes during soybean seed development. Emir J Food Agr. 2016: 10.1111/tpj.13118 PMID: 26729600

64. Dekkers BJ, He H, Hanson J, Willems LA, Jamar DC, Cueff G, et al. The Arabidopsis DELAY OF GERMINATION 1 gene affects *ABSCISIC ACID INSENSITIVE 5 (ABI 5)* expression and genetically interacts with *ABI 3* during Arabidopsis seed development. Plant J. 2016; 85(4): 451–465. https://doi.org/10.1111/tpj.13118 PMID: 26729600

65. Sabiel SA, Huang S, Hu X, Ren X, Fu C, Peng J, et al. SNP-based association analysis for seedling traits in durum wheat (*Triticum turgidum* L. *durum*). Breeding Sci. 2017: 16074.

66. Huang S, Sun L, Hu X, Wang Y, Zhang Y, Nevo E, et al. Associations of canopy leaf traits with SNP markers in durum wheat (*Triticum turgidum* L. *durum*). PloS one. 2018; 13(10): e0206226. https://doi.org/10.1371/journal.pone.0206226 PMID: 30352102

67. Zhang Z, Liu Z, Cui Z, Hu Y, Wang B, Tang J. Genetic analysis of grain filling rate using conditional QTL mapping in maize. PloS One. 2013; 8(2): e56344. https://doi.org/10.1371/journal.pone.0056344 PMID: 23441180

68. Li K, Yang X, Tian H, Pan S, Liu Z, Lu S. Effects of changing climate and cultivar on the phenology and yield of winter wheat in the North China Plain. Int J Biometeorol. 2016; 60(1): 21–32. https://doi.org/10.1007/s00484-015-1002-1 PMID: 25962358

69. Asseng S, Foster I, Turner NC. The impact of temperature variability on wheat yields. Global Change Biol. 2011; 17(2): 997–1012.

70. Liu B, Martre P, Ewert F, Porter JR, Challinor AJ, Müller C, et al. Global wheat production with 1.5 and 2.0˚C above pre-industrial warming. Global Change Biol. 2019; 25(4): 1428–1444.

71. Lv L, Yao Y, Zhang L, Dong Z, Jia X, Liang S, et al. Winter wheat grain yield and its components in the North China Plain: irrigation management, cultivation, and climate. Chin J Agr Res. 2013; 73(3): 233–242.

72. Mohammad M. Effects of kernel weight and source-limitation on wheat grain yield under heat stress. Afr J Biotechnol. 2012; 11(12): 2931–2937.