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Genome-wide association mapping for grain shape and color traits in Ethiopian durum wheat (*Triticum turgidum* ssp. *durum*)

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ABSTRACT

Grain shape and color strongly influence yield and quality of durum wheat. Identifying QTL for these traits is essential for transferring favorable alleles based on selection strategies and breeding objectives. In the present study, 192 Ethiopian durum wheat accessions comprising 167 landraces and 25 cultivars were genotyped with a high-density Illumina iSelect 90K single-nucleotide polymorphism (SNP) wheat array to conduct a genome-wide association analysis for grain width (GW), grain length (GL), CIE (Commission Internationale l'Eclairage) L* (brightness), CIE a* (redness), and CIE b* (yellowness) traits. The accessions were planted at Sinana Agricultural Research Center, Ethiopia in the 2015/2016 cropping season in a complete randomized block design with three replications. Twenty homogeneous and healthy seeds per replicate were used for trait measurement. Digital image analysis of seeds with GrainScan software package was used to generate the phenotypic data. Analysis of variance revealed highly significant differences between accessions for all traits. A total of 46 quantitative trait loci (QTL) were identified for all traits across all chromosomes. One novel major candidate QTL ($-\lg P \geq 4$) with pleiotropic effects for grain CIE L* (brightness) and CIE a* (redness) was identified on the long arm of chromosome 2A. Eighteen nominal QTL ($-\lg P \geq 3$) and 26 suggestive QTL ($-\lg P \geq 2.5$) were identified. Pleiotropic QTL influencing both grain shape and color were identified.

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1. Introduction

Durum wheat (*Triticum turgidum* ssp. *durum*) is an allotetraploid wheat comprising an A genome from the diploid species *T. urartu* and a B genome from *Aegilops speltoides* or a relative that may now be extinct [1]. Durum wheat is the main source of semolina for production of pasta (particularly in Europe and North America) and couscous and bread in the Middle East and North Africa. Ethiopia is a center of durum genetic diversity. This high diversity is associated with the wide range of agro-ecological conditions and farmer cultural diversity [2–4] and is a focus of genetic studies and a source of novel alleles [5,6]. Ethiopian durum wheat kernels include several color types [4]. In Ethiopia, the crop is used mainly for local consumption. Despite the high genetic diversity and suitable conditions for durum production, the country depends on imported durum, owing to a lack of high-quality cultivars [7].

Grain shape traits (grain length and width) strongly influence yield and milling quality [8,9]. These traits are quantitative traits controlled by several genes [10] and their genetic improvement is a target of durum breeding [11].

The grain color of durum wheat comes from its pigments, consisting of carotenoids and anthocyanins. A yellowish-amber color, which is an essential quality parameter of durum semolina, is due to the carotenoid pigment/yellow pigment content (YPC) in the whole kernel and is commercially identified as the yellow index [12]. Anthocyanins, in contrast, accumulate in the pericarp or aleurone of durum wheat and provide the blue, purple, and red colors of the grain [13]. In addition to their visual appeal, both carotenoids and anthocyanins influence human health and nutrition [14–16].

Red grain color of wheat increases dormancy and has been used as a marker for resistance to preharvest sprouting in wheat breeding programs [17]. Carotenoids have been a target of plant breeders, mainly in response to the needs of pasta producers. However, because of their role in health and nutrition and a corresponding consumer demand, anthocyanins have become a target of genetic improvement [13,18]. Grain color is controlled by genetic and environmental factors and cultivation practices. Genes involved in pigment accumulation and proteins with regulatory roles have been identified [13].

Digital image analysis is increasingly used in plant science, especially in phenomics [19]. Novel software packages based on digital image analysis allows detailed imaging of grain features including shape and color [20,21].

Elucidating the genetic basis of complex quantitative traits controlling agronomic traits of economic importance is key to modern plant breeding. Genome-wide association mapping (GWAS) involves genotyping a large collection of accessions with numerous single-nucleotide polymorphisms (SNPs) distributed throughout the genome and testing their association with phenotypic traits. SNP genotyping platforms have been constructed to permit genome-assisted selection by identifying quantitative trait loci (QTL) [22]. The 90K wheat SNP array by Illumina allows genome-wide scans for this purpose [23,24].

Numerous QTL for whole grain or flour color of both bread and durum wheat have been identified [25–31]. The unexploited Ethiopian durum wheat landraces are rich in genetic diversity for many traits including grain shape and color. The objective of this study was to perform a genome-wide association analysis for grain shape and color traits using Ethiopian durum wheats.

2. Materials and methods

2.1. Plant materials

A panel of 192 Ethiopian spring durum wheats comprising 167 landraces and 25 cultivars (Table S1) was assembled. Accessions were collected and maintained by Debre-Zeit Agricultural Research Center (DZARC) and Sinana Agricultural Research Center (SARC), Ethiopia as single-seed descent (SSD) progenies. From this panel, Liu et al. [5] used 182 (160 landraces and 22 cultivars) to identify QTL for stripe rust resistance. Landrace accessions were originally collected from major wheat-producing areas of Ethiopia, including Bale, Gondar, Gojjam, Shewa, Tigray, and Wollo and included 12 lines currently cultivated in the USA. Cultivars were released by DZARC and SARC from 1994 to 2010 and have been cultivated in Ethiopia.

2.2. Phenotypic data

All accessions were planted in consecutive growing seasons (2013/2014 and 2014/2015) at Sinana Agricultural Research Center (SARC), located between 07°06′12″ N and 07°07′29″ N and 40°12′40″ E to 40°13′52″ E, for purification by ear-to-row planting. Purified and homogeneous accessions were planted in 2015/2016 in a completely randomized block design with three replications. All accessions were grown in two-row plots 1 m long with 15 cm between rows. The field was managed following local agricultural practices. Twenty homogeneous and healthy seeds were selected from each replicate for digital image analysis. Digital images were acquired with a flatbed CanoScan LiDE 120 F (Canon Inc., Tokyo, Japan), with a true optical resolution of 4800 dpi and 48-bit internal color depth. Seeds were spread on the scanner glass and spaced for accurate measurement. Black cardboard was used to cover the scanner to increase contrast and reduce reflection. All images were scanned at 24-bit with 300 dpi resolution and 2250 × 3705 pixels and recorded in a JPEG format.

2.3. Digital image analysis

Digital image measurements were collected with GrainScan [21], a software package developed for high-throughput phenotyping of cereal grains. The default-automated threshold was used to measure grain length (GL) and grain width (GW). The mean value of 20 seeds per replicate was recorded. Commission Internationale l'Eclairage (CIE) Lab, a three-dimensional (L*a*b*) color space method, was used to measure the color of accessions. Calibration with a color checker, which is scanned under the same setting as the seeds, was used to change the raw RGB value measured by the scanner to standardized CIE L*a*b* values. CIE L* values represent brightness, CIE a* indicates redness (positive values) and

greenness (negative values) and CIE b* represents yellowness (positive value) and blueness (negative value).

2.4. Phenotypic data analysis

Analyses of variance (as a general linear model) were fitted for all grain shape and color traits including blocks, replicates, and genotypes. ANOVA and other descriptive statistics were analyzed using Minitab 18 (Minitab Ltd., Coventry, UK). Seed weight was used as a covariate to test for any possible effect of maternal etiology. Broad-sense heritability (H^2) was calculated from the means of the three replications using the formula $H^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_E^2)$; where σ_G^2 (genetic variance) was calculated as $(MS_{genotype} - MS_{residual}) / r$; σ_E^2 (residual variance) = $MS_{residual}$; r is the number of replications and MS is the mean square value.

2.5. Genotypic data

For each accession, 25 one-week-old plants from the same seed source used for digital image analysis were pooled for genomic DNA extraction using a DNeasy 96 Plant Kit (Qiagen GmbH, Hilden, Germany). An Illumina high-density 90K wheat SNP array [23] was used for genotyping accessions. SNP calling and clustering were performed with GenomeStudio 2011.1 (Illumina, San Diego, CA, USA). Calls showing residual heterozygosity were assigned as missing values. SNP markers with minor-allele frequency < 0.05 and >0.1 missing values per accession were excluded. After filtering, imputation of missing data was performed with Beagle 4.0 [32]. Considering the higher levels of homozygosity exist in durum wheat, imputation was made without any phased reference populations. Twenty five markers were considered in the imputation rolling window with an overlap of a single marker, the typical number of markers included in a 0.5 cM interval. Since tuning of other parameters did not improve imputation accuracy, default values were kept.

A high-density consensus map of tetraploid wheat generated by Maccaferri et al. [24] was used to assign chromosome positions of SNPs and markers with unknown positions were excluded.

2.6. Population structure and kinship analysis

A Bayesian model-based (Markov chain Monte Carlo, MCMC) clustering approach was used for population structure analysis by STRUCTURE 2.3 [33]. The Haploview Tagger function (based on analysis of marker pairwise r^2 values) was used to select tag-SNPs with a tagger filter set at $r^2 = 0.5$ in HAPLOVIEW 4.2 [34] and 1496 tag-SNPs were selected for population structure analysis. The most likely subgroup number was inferred by the log probability (ln *PD*) and an ad-hoc statistic (ΔK) based on the rate of change of ln *PD* between runs using successive *K*-values as described by Evanno et al. [35]. Based on this, the ΔK shows a clear peak at the ideal number of subgroups. Ten subgroups with 20 independent runs for each subgroup were done under an admixture model of population structure with correlated allele frequencies and a burn-in period of 50,000 iterations and 100,000 MCMC iterations after burn-in were calculated for each run.

The Haploview Tagger function was also used to select tag-SNPs for kinship matrix (*K*) analysis with a tagger filter set at $r^2 = 0.1$ in HAPLOVIEW and 4842 tag-SNPs were selected to compute the *K* and incorporated in the mixed linear model (MLM) along with the population structure (*Q*) value for GWAS analysis by TASSEL v.5.2 [36].

2.7. Linkage disequilibrium and genome-wide association analysis

Linkage disequilibrium (LD) was calculated as r^2 values between pairwise SNPs using TASSEL. The specific critical r^2 value beyond which LD is due to true physical linkage was determined by taking the 95th percentile of r^2 data of unlinked marker pairs [37]. LD decay was estimated as physical distance using the procedure of Hill and Weir [38] in R [39] and an LD decay curve was fitted with a smoothing spline regression line at the genome level.

An MLM [40] was used for genome-wide association (GWAS) analysis with population structure (*Q*) and kinship (*K*) covariates. Population structure was estimated as the first 10 principal components (PCs) calculated from tag-SNPs (r^2 = 0.5) and kinship was measured as similarity matrix from tag-SNPs (r^2 = 0.1) in TASSEL software package. Three levels of significance were introduced for reporting GWAS QTL: (i) experiment-wise $P \leq .05$ ($-\lg P \geq 4.00$) for "major" QTL, (ii) marker-wise $P \leq .001$ ($-\lg P \geq 3.00$) for "nominal" QTL, and (iii) marker-wise $P \leq 0.005$, ($-\log P \geq 2.5$) for "suggestive" QTL. The experiment-wise threshold was established from the number of independent SNP tests estimated in HAPLOVIEW using the tagger function of $r^2 = 0.3$ [41] and the total number of tag-SNPs was 816. Bonferroni adjustment for multiple GWAS tests ($P \leq .05$) was equal to $-\lg P = 4.04$ (rounded to 4). Hence the experiment-wise, Bonferroni-corrected significance threshold at $P = 0.05$ matched a marker-wise threshold of $-\lg P \geq 4.00$. Significance intervals of identified QTL were reported as the intervals after inclusion of all SNPs associated with the trait with $P \leq 0.01$ (marker-wise) and in LD of $r^2 \geq 0.3$. The confidence interval of a QTL was defined based on ±2.25 cM (LD decay of this panel) from the GWAS QTL peak (QTL-tag SNP).

3. Results

3.1. Phenotypic variation

Highly significant variation was observed among accessions for all grain shape and color traits (Table 1, Fig. 1). Maximum values of 8.84 and 3.57 mm and minimum values of 5.21 and 2.44 mm were recorded for grain length and width with mean values of 7.50 and 3.05 mm, respectively. CIE L* (brightness) ranged from 41.38 to 68.51 with a mean of 57.26, CIE a* ranged from 6.48 to 14.15 with a mean of 9.56, and CIE b* ranged from 8.78 to 21.43 with a mean of 16.14. The phenotypic means of accessions are presented in Table S2. A normal distribution was observed for GL and b*, whereas GW, L* and a* showed bimodal distributions (Fig. 2). CIE L* showed negative correlation with a* and positive correlation with b* (Fig. 3). CIE a* showed negative correlations with GW,

b*, and GL. Thousand-grain weight showed no correlation with either grain shape or color-related traits, indicated that the variation in color and shape traits was not due to seed size. The trait was accordingly not included in GWAS analysis.

3.2. Population structure

The optimal subgroup of the panel was inferred using the second-order rate of change of the likelihood in STRUCTURE. The result showed a clear peak at $K = 3$ indicating that three is the optimal number of subgroups in the panel. Subgroups 1, 2, and 3 contained respectively 75, 27, and 90 accessions, (Table S1). Of the 25 cultivars, 24 were clustered in subgroup 2 with two landraces from Bale and Gojjam. Most of the landraces (44) collected from Sinana Agricultural Research Center were grouped in subgroup 1 along with one cultivar (selam), 12 landraces collected from DZARC, 8 landraces from Wollo, and landraces from Gojjam, Shewa and Tigray. The remaining 90 landraces were clustered in subgroup 3.

Accessions did not cluster by geographical background. Eight landraces from northern Ethiopia (Wollo) were grouped in subgroup 1, whereas 25 landraces with the same origin were clustered in subgroup 3. Landraces from central

Fig. 1 – Images of accessions with highest values of CIE L* (EP150), b* (EP121), a* (EP182), GL (EP130), GW (EP029) and with amber (EP004).

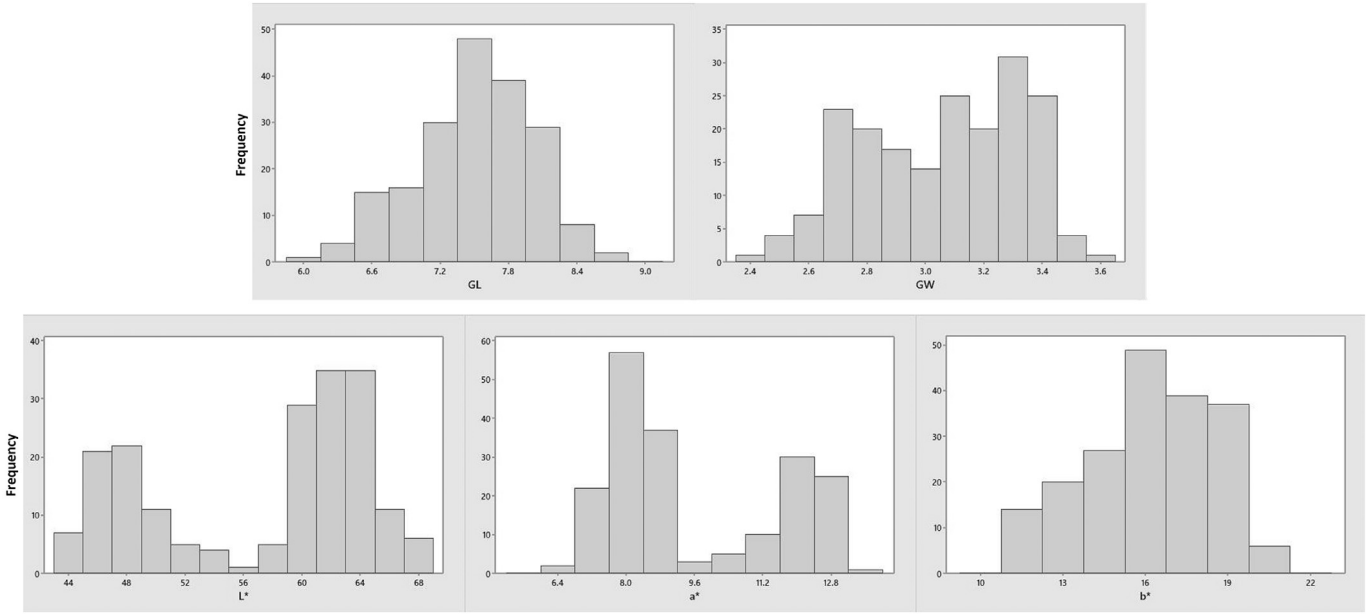

Fig. 2 – Frequency distributions of grain shape and color traits in Ethiopian durum wheat accessions. GL, grain length; GW, grain width; L*, brightness; a*, redness; b*, yellowness.

Fig. 3 – Correlation coefficients and levels of significance among phenotypic mean values of kernel shape and color traits in Ethiopian durum wheat accessions. GL, grain length; GW, grain width; TGW, thousand-grain weight; L*, brightness; a*, redness; b*, yellowness. *, ** and *, significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.**

Table 1 – ANOVA for kernel shape and color-traits of Ethiopian durum wheat accessions.

	GL (mm)	GW (mm)	L*	a*	b*
Maximum	8.84	3.57	68.51	14.15	21.43
Median	7.83	3.09	60.5	8.69	16.23
Minimum	5.21	2.44	41.38	6.48	8.78
H^2	0.8	0.87	0.91	0.88	0.87
CV	1.78	2.30	1.51	7.12	5.47
Accessions	***	***	***	***	***
Replicates	NS	*	NS	NS	*

Kernel shape and color trait acronyms: GL, grain length; GW, grain width; L*, brightness; a*, redness; b*, yellowness.
*, **, and ***, significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

Ethiopia (Akaki and Shewa) grouped in both subgroups 1 (four landraces) and 3 (19 landraces). All 12 Ethiopian landraces now cultivated in the USA were grouped in subgroup 3.

3.3. Linkage disequilibrium

The genome-wide LD of the panel decayed below $r^2 = 0.3$ (the standard critical threshold) at 2.25 cM (Fig. 4). This means that ±2.25 cM would be the confidence interval of a given QTL from the QTL-tag SNP (a SNP found at the peak of the corresponding QTL). The specific critical r^2 value beyond which LD is due to true physical linkage was 0.15 and the intersection of the threshold and the LD decay curve was at 5.75 cM.

3.4. Genome-wide association analysis

An MLM with population structure and kinship matrix (MLM + Q + K) was chosen for marker trait association (MTA) analysis, as the quantile-quantile (Q-Q) plot showed that the observed MTA *P*-values were close to the expected distribution (Fig. S1). Genotypes of 75,010 missing SNPs were imputed. After filtering followed by imputation, 10,789 polymorphic SNP markers (4591 from the A and 6198 from the B genome) and phenotypic values for grain shape and color traits were used for MTA analysis. The Population structure data estimated as the first 10 PCs resulting from 1496 tag-SNPs and kinship matrix data resulting from 4842 tag-SNPs were also incorporated in the MLM-GWAS analysis.

A total of 46 QTL were identified for grain shape and color traits. The only two QTL with $-\lg P \geq 4$ were *EPdwa*-2A* and *EPdwL*-2A.2*, with $-\lg P$ values of 5.39 and 5.23 and phenotypic variance of 11.4% and 11.0%, respectively. Eighteen nominal QTL reached the $-\lg P \geq 3$ threshold, of which five QTL each were identified for b* and GL and four QTL each for L* and GW with phenotypic variance ranging from 5.5% to 6.8% (Table 2).

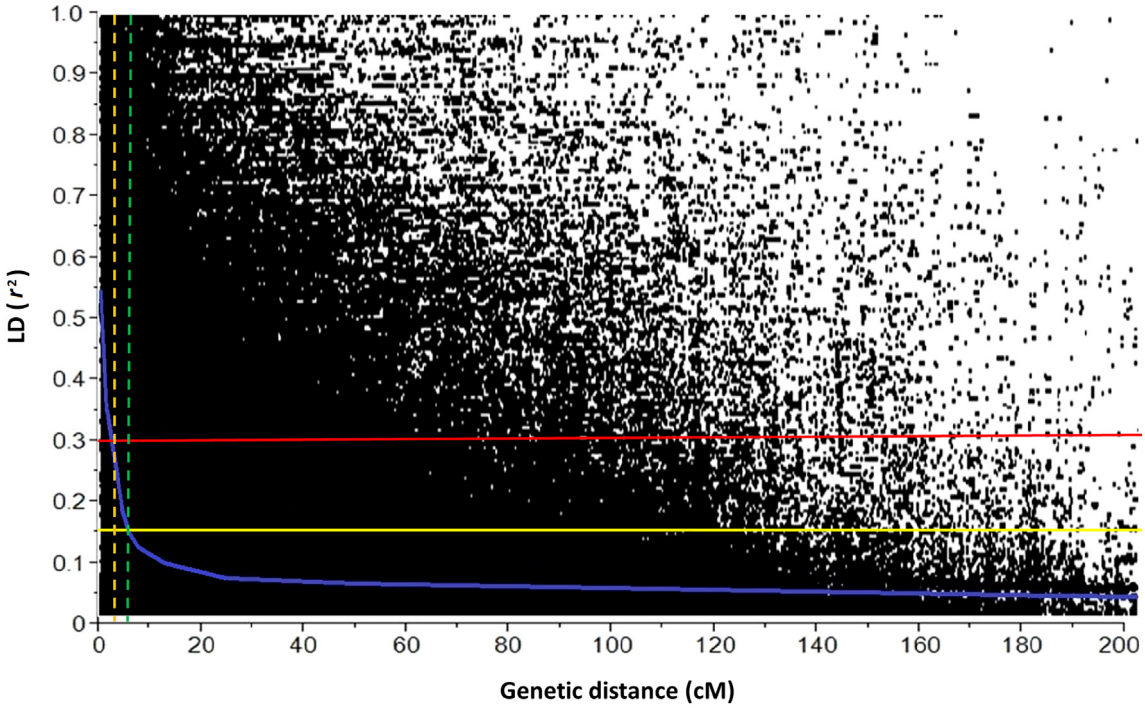

Fig. 4 – A scatter plot of pairwise SNPs showing genome-wide linkage disequilibrium (LD) decay in 192 Ethiopian durum wheat accessions. Genetic distance in cM is plotted against the LD estimate (r^2) for pairs of markers. The blue solid curve represents the smoothing spline regression model fitted to LD decay. The horizontal red line represents the standard critical r^2 value of the genome ($r^2 = 0.30$), the horizontal yellow line represents the 95th percentile unlinked r^2 value of the genome ($r^2 = 0.15$), the vertical orange dashed line represents the genetic distance (2.25 cM) at which the standard critical r^2 intersects with the LD decay curve, and the green dashed line represents the genetic distance (5.75 cM) at which the 95th percentile unlinked r^2 intersects with the LD decay curve.

The other 26 QTL were identified as suggestive QTL with –lg $P \geq 2.5$ (Table S3).

3.5. QTL for grain length and width

Five nominal QTL (*EPdwGL-2B*, *EPdwGL-4B*, *EPdwGL-5A*, *EPdwGL-6A*, and *EPdwGL-7B*) for grain length were identified on chromosomes 2B, 4B, 5A, 6A, and 7B, respectively (Table 2, Fig. S2). *EPdwGW-2A.1*, *EPdwGW-2A.2*, *EPdwGW-5A*, and *EPdwGW-7B* were the four nominal QTL detected for grain width on chromosomes 2A (2), 5A and 7B, respectively. Eleven suggestive QTL for GL and GW were identified (Table S3). Allelic distributions and effects for QTL-tagging SNPs are described in Tables S4 and S5 for GL and GW, respectively.

3.6. QTL for color traits

A major QTL with two alleles having a positive effect for a* (redness) (*EPdwa*-2A) and a negative effect for L* (brightness) (*EPdwL*-2A.2*) was identified at a locus (*IWB72154*, QTL-tag SNP) on the long arm of chromosome 2A with a slightly more significant MTA for a* (–lg $P = 5.39$) than for L* (–lg $P = 5.23$) and explained respectively 11.4% and 11.0% of phenotypic variance. This QTL also showed a pleotropic effect for grain width (Fig. 5). Adjacent to this major QTL, another locus (*IWB8286*, QTL-tag SNP) made a nominal QTL for L* (*EPdwL*-2A.1*) with negative effect and a suggestive QTL for a* with positive effect (Table S3). Four further nominal QTL (*EPdwL*-1A*, *EPdwL*-2A.1*, *EPdwL*-7A*, and *EPdwL*-7B*) for L* were detected on chromosomes 1A, 2A, 7A and 7B, respectively (Table 2). Five nominal QTL, *EPdwb*-1B*, *EPdwb*-3A*, *EPdwb*-4B*, *EPdwb*-5A*, and *EPdwb*-7B*, for b* (yellowness) were detected on chromosomes 1B, 3A, 4B, 5A, and 7B, respectively. Ten further suggestive QTL for L*, a*, and b* traits were identified (Table S2). Allelic distributions and effects of QTL-tagging SNPs for b*, L*, and a* traits are described in Tables S6, S7, and S8, respectively.

4. Discussion

A panel of 192 Ethiopian durum wheat accessions comprising 167 landraces collected from major wheat-growing areas of Ethiopia and 25 improved varieties released between 1994 and 2010 and cultivated in the country was assembled to map QTL for shape and color traits using GWAS analysis. High variation recorded in grain shape and color trait values suggested the potential of GWAS for identifying QTL.

Because of nonrandom mating, isolation, or artificial selection, structured populations are present in most plant populations. Consequently, genetic loci may be spuriously associated with a trait when there is no true association. The probability of a false positive, or type I error, increases in association mapping studies if population structure is not appropriately accounted for [33]. For this reason, assessment of population structure is crucial for any GWAS analysis. The present panel was stratified into three subgroups. Liu et al. [5] reported the same subgroups using these accessions, and 10 more genotypes were added for the present study. Subgroup 2 was composed of cultivars. Landraces made up the other two subgroups, irrespective of their geographic origin. This admixture could be accounted for by seed exchange between farmers in local markets throughout the country [42]. Understanding the linkage disequilibrium (LD) pattern in germplasm is essential for reasons including selection of the marker density and experimental design to be used for GWAS analysis and for defining identified QTL regions [43].

Both GW and GL showed significant positive correlations with L* and b* and negative correlations with a*. To our knowledge, no published report has described the relationship between grain shape and color traits in wheat. CIE b* (yellowness) showed a significant negative correlation with a* (redness), in agreement with a previous finding [44] but unlike that study, showed a significant positive correlation with L* (brightness). However, Goriewa-Duba et al. [45] also reported a highly significant positive correlation between b* and L*. This difference might have arisen from the samples taken for analysis in which, unlike in the present study, the flour (endosperm) was used. For instance, Humphries et al. [46] found higher values of a* and b* but lower L* in wheat bran than in flour. However, they reported a significant correlation between values of color traits in grain and flour across genotypes and concluded that color values could be extrapolated from the easily accessible part of the grain. A strong positive correlation between b* and a* has been reported [47]. CIE b* has been used as an alternative descriptor of yellow pigment content (YPC), an essential quality parameter in the pasta industry, given their strong correlation [29]. Red wheat kernels have been identified as resistant to preharvest sprouting and this relationship could be due either to the pleiotropic effect of genes controlling red-testa pigmentation (*R*) or to linkage between these genes and other genes affecting preharvest sprouting [17].

Grain shape and color are important for many reasons. Identifying QTL for these traits will facilitate the transfer of favorable alleles based on selection strategies and breeding objectives. Grain shape and color traits are crucial quality parameters with various uses and have been used as selection criteria in durum wheat breeding. Previous studies identified QTL on all chromosomes and genomes for grain length, grain width and color traits. In the present study, the QTL regions associated with GL and GW were distinct except for a QTL on chromosome arm 5AL indicated in previous reports [10]. QTL were detected in chromosome regions reported previously. Chen et al. [48] reported QTL for GL on chromosome arms 1BS and 5AL and for GW on 4BS, 7BS, and 7BL. Gao et al. [49] identified a stable TGW QTL, *QTKW.caas-7AL*, in all tested environments using an F8 recombinant inbred line population of Chinese spring wheat. Ma et al. [50] identified the *TaCYP78A3* gene of the *CYP78A* family, encoding cytochrome *CYP78A3 P450*, on the short arms of bread wheat chromosome group 7 (7AS, 7BS, and 7DS), which was associated with grain size and shape, and silencing of this gene reduced the values of these traits. Yan et al. [51] identified the *TaGW8* gene, with *TaGW8-B1a* and *TaGW8-B1b* alleles, on chromosome 7B, which had a significant effect on both grain size and shape traits. Wang et al. [52] cloned a *TaGS5* gene found on chromosomes 3AS and 3DS of Chinese bread wheat that controls thousand-grain weight and grain width. In agreement with these reports, QTL for GL and GW on the same regions of chromosomes 7A and 7B were detected in the present study. QTL

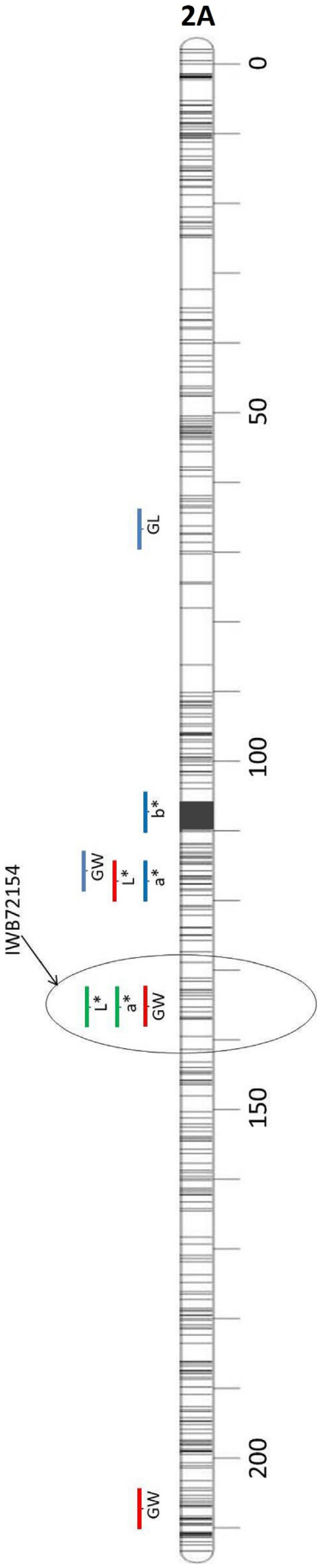
2A
0
50
100
150
200
GL
b*
GW
L*
a*
IWB72154
L*
a*
GW
GW

Table 2 – List of identified QTL for grain shape and color traits in Ethiopian durum wheat.

QTL	Trait	Marker	Chr.	Position (cM)	P-value	–lg *P*	r^2 (%)	Sig. SNPs	CI (cM)	Allele (SNP base)	Effect	Allele count (No.)	Allele (SNP base)	Effect	Allele count (No.)
EPdwa-2A*	a*	IWB72154	2A	5.39	0.000004	5.39	11.4	1	133.79–138.29	C	1.36	104	T	0	88
EPdwb-1B*	b*	IWB11958	1B	115.74	0.000395	3.40	6.3	2	113.49–117.99	C	1.32	147	T	0	45
EPdwb-3A*	b*	IWB11992	3A	141.52	0.000828	3.08	5.6	1	139.27–143.77	A	1.46	61	G	0	131
EPdwb-4B*	b*	IWB73411	4B	39.47	0.000401	3.40	6.3	4	37.22–41.72	C	–1.45	22	T	0	170
EPdwb-5A*	b*	IWA3196	5A	16.83	0.000802	3.10	5.7	0	14.58–19.08	C	4.66	26	T	0	166
EPdwb-7B*	b*	IWB34768	7B	67.34	0.000721	3.14	5.8	11	65.09–69.59	C	1.28	55	T	0	137
EPdwL-1A*	L*	IWB65551	1A	87.00	0.000683	3.17	6.0	3	84.75–89.25	C	5.19	115	T	0	77
EPdwL-2A.1*	L*	IWB8286	2A	117.05	0.000501	3.30	6.3	1	114.8–119.3	C	–5.35	43	T	0	149
EPdwL-2A.2*	L*	IWB72154	2A	136.04	0.000005	5.23	11.0	1	133.79–138.29	C	–4.96	104	T	0	88
EPdwL-7A*	L*	IWB72494	7A	143.19	0.000729	3.14	5.9	1	140.94–145.44	G	7.48	167	T	0	25
EPdwL-7B*	L*	IWB62029	7B	165.05	0.000545	3.26	6.2	0	162.8–167.3	C	6.66	182	T	0	10
EPdwGL-2B	GL	IWB3865	2B	108.92	0.000723	3.14	6.1	4	106.67–111.17	c	–0.37	102	T	0	90
EPdwGL-4B	GL	IWB9672	4B	66.43	0.000381	3.42	6.8	0	64.18–68.68	C	0.67	38	T	0	154
EPdwGL-5A	GL	IWB43493	5A	72.73	0.000985	3.01	5.8	1	70.48–74.98	C	–0.45	50	T	0	142
EPdwGL-6A	GL	IWB67412	6A	1.06	0.000955	3.02	5.8	14	0–3.31	A	–0.39	141	G	0	51
EPdwGL-7B	GL	IWA2568	7B	20.75	0.000877	3.06	5.9	1	18.5–23.0	A	–0.55	159	G	0	33
EPdwGW-2A.1	GW	IWB72154	2A	136.04	0.000258	3.59	6.8	0	133.78–138.29	C	–0.15	104	T	0	88
EPdwGW-2A.2	GW	IWB23912	2A	208.76	0.000985	3.01	5.5	0	206.51–211.01	C	–0.32	31	T	0	161
EPdwGW-5A	GW	IWB46350	5A	84.77	0.000300	3.52	6.6	8	82.52–87.07	A	–0.20	114	G	0	78
EPdwGW-7B	GW	IWB36189	7B	66.36	0.000874	3.06	5.6	7	64.11–68.61	A	–0.15	137	G	0	55

GL, grain length; GW, grain width; L*, brightness; a*, redness; b*, yellowness.
Marker, a SNP found at the peak of corresponding QTL (QTL-tag SNP); Sig. SNPs, the number of significant SNPs lying in the significance interval; CI, confidence interval to the left and right of a QTL-tag SNP based on the tetraploid wheat consensus map [25].

for grain shape traits located close to positions found in a previous study [8] were identified on all chromosomes except 3A.

Many QTL for color traits have been identified in both bread and durum wheat [27,53]. The *Psy1* gene, a gene coding for the enzyme phytoene synthase 1, which is considered the rate-limiting molecule in the carotenoid biosynthetic pathway, cosegregated with yellow pigment content (YPC) and CIE b* on chromosomes 7BL [27] and 7AL [54]. A second Psy gene (*Psy2*) is located on group 5 chromosomes of durum wheat [27]. Crawford and Francki [55] identified a SNP in the gene encoding *lycopene-cylcase* that controls phenotypic variation for flour b* color on chromosome 3A. In agreement with these findings, QTL for b* were detected on the same chromosome arms: 3AL, 5AS, and 7BL. Additional b* QTL were identified on

Fig. 5 – A major QTL (*IWB72154*, the QTL-tag SNP) identified on the long arm of chromosome 2A with a positive effect on CIE a* and a negative effect on CIE L*. Identified QTL are shown at the left of the chromosome with blue representing –lg $P \geq 2.5$ (suggestive QTL); red representing –lg $P \geq 3$ (nominal QTL), and green representing –lg $P \geq 4$ (major QTL). Lengths of bars indicate confidence intervals of QTL and horizontal lines indicate positions of QTL-tagging SNPs. GL, grain length; GW, grain width; L*, brightness; a*, redness; b*, yellowness.

chromosome arms 1BL, 2AL, 2BL, 4BS, and 6AL. A major QTL controlling both L* (brightness) and a* (redness) at a single locus (*IWB72154*) was identified on the long arm of chromosome 2A. Another locus (*IWB8286*) made a nominal QTL for L* (*EPdwL*-2A.1) with negative effect and a suggestive QTL for a* with positive effect. Since it has a positive effect on a* and negative effect on L*, this QTL could be a locus with two alleles. Sherman et al. [56] identified a locus on chromosome group 3 in bread wheat for grain color with red as dominant and white as recessive alleles and reported that the degree of red color is additive, with genotypes homozygous dominant at all three loci (*R-A1b*, *R-B1b*, and *R-D1b*) having the darkest red color and only those homozygous recessive at all three genes being white (*R-A1a*, *R-B1a*, and *R-D1a*). Seven QTL were identified for L* on chromosome arms 1AS, 2AS, 5AL, 6AL, 7AL (2) and 7BL and four QTL for a* on chromosome arms 1AS, 2AL, 5AS, and 7AL. Most grain color QTL identified in the present study overlapped with those identified in previous studies. For instance, Zahi et al. [31] identified a major QTL for a* (*QFa.caas-2AL.2*) on the long arm of chromosome 2A and for b* (*QFb.caas-1BL.2*) on the long arm of chromosome 1B and *QFb.caas-5AS.1* on the short arm of chromosome 5A near to the respective QTL *EPdwa*-2A, *EPdwb*-1B, and *EPdwb*-5A detected in the present study.

Similar to the significant correlation observed among grain shape and color related traits in the phenotypic data, a pleiotropic effect or tight linkage was observed in the MTA analysis of these traits (Fig. 6). Five GW QTL having a pleiotropic effect or tight linkage with color traits on chromosome arms 2AL (two), 4BS, 5AL, and 7BL were identified. A single GL QTL was also identified as having a pleiotropic effect or tight linkage with b* and a* on the short arm of chromosome arm 5A. Further investigation of this pleotropic effect may shed light on the responsible genetic architecture.

5. Conclusions

Novel QTL were detected in a panel of genetically rich and untapped Ethiopian durum wheat landraces in a single-environment-single-location study. Use of multi-environment, multi-year phenotypic data could reveal QTL stable across environments. A major QTL controlling both redness and brightness traits on chromosome arm 2AL was detected. QTL for grain shape and color traits were identified in chromosome regions in which major QTL or/and genes were detected in previous studies. Digital image analysis is an inexpensive and non-invasive alternative to trait evaluations.

Supplementary data for this article can be found online at https://doi.org/10.1016/j.cj.2020.01.001.

Conflict of interest

Authors declare that there are no conflicts of interest.

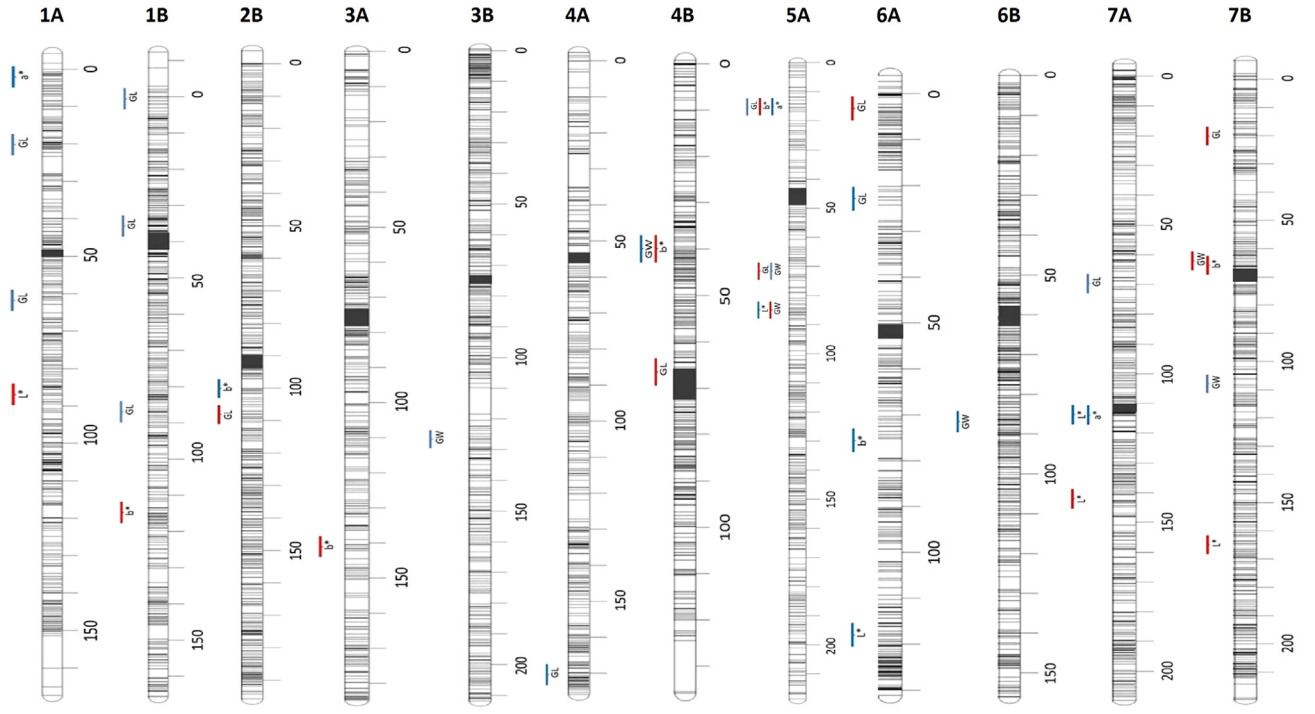

Fig. 6 – Genetic map of QTL for grain shape and color traits identified in 192 Ethiopian durum wheat, projected on the SNP-based tetraploid consensus map [24]. Identified QTL are shown at the left of the chromosome with blue representing –lg $P \geq 2.5$ (suggestive QTL); red representing –lg $P \geq 3$ (nominal QTL), and green representing –lg $P \geq 4$ (major QTL). Lengths of bars indicate confidence intervals of QTL and horizontal lines indicate positions of QTL-tagging SNPs. GL, grain length; GW, grain width; L*, brightness; a*, redness; b*, yellowness.

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Author contributions

AA, RT, and TF conceived and designed the study. TL and BA assembled the panel. MM conducted the fingerprinting of accessions. AA, TL and GS conducted phenotyping and data analysis. AA prepared the manuscript. TF and RT edited the manuscript. All authors read and approved the final manuscript.

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