



Genomic dissection reveals QTLs for grain biomass and correlated traits under drought stress in Ethiopian durum wheat (*Triticum turgidum ssp. durum*)

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Funding information

(BMEL); Bundesministerium für Ernährung und Landwirtschaft; Federal Ministry of Food and Agriculture

Communicated by: Jens Léon

Abstract

Drought stress seriously challenges wheat production and productivity. Grain biomass (GB) and related traits were assessed under drought stress and non-stress for 285 and 185 durum wheat genotypes, respectively, in field and climate chamber experiments to identify quantitative trait loci (QTL). Significant correlations between traits estimated in field and climate chamber trials were observed ($p < .001$). Genotyping with the wheat 90 K iSelect single nucleotide polymorphism (SNP) array revealed 11,919 polymorphic SNP markers distributed across the durum wheat genome. The FarmCPU (Fixed and random model Circulating Probability Unification) method was used for genome-wide association studies (GWAS). A total of 191 significant ($-\log_{10}p \geq 4$) marker-trait associations (MTAs) were detected at a linkage disequilibrium (LD, $r^2 \geq .2$) at 4.78 Mb and were clustered into 70 QTLs. A total of 69 (36%) of the MTAs passed a false discovery rate (FDR) of 5%. The numbers of QTLs detected were 21, 31, 9 and 9 under field drought stress (FDS), field non-stress (FNS), climate chamber drought stress (CCDS) and climate chamber non-stress (CCNS) conditions, respectively. About 43% and 57% of the QTLs were located on the A and B genomes, respectively. Some of the detected QTLs were in agreement with previously reported QTLs, while others are novel ones for the traits investigated. QTLs on 1A between 495694477 and -501944537 bp, on 3B between 416256124 and 430507900 bp, on 3B between 745357158 and 759608934 bp, on 4B between 593416763 and 605142497 bp and on 4B between 658785890 and 670511624 bp were selected for validation and may be used to increase grain yield under drought stress in marker-assisted selection (MAS) schemes.

Key message

Genome-wide association studies (GWAS) enabled detecting novel quantitative trait loci (QTLs) and identifying single nucleotide polymorphism (SNP) markers associated with grain biomass and correlated traits for drought stress tolerance in durum wheat based on a combination of climate chamber and multiyear and multilocation field experiments.

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KEYWORDS

drought stress, durum wheat, Ethiopia, genomics, GWAS, QTL

1 | INTRODUCTION

Drought is one of the most serious abiotic factors challenging wheat production and quality internationally and especially in sub-Saharan Africa (Mwadingeni et al., 2017; Zampieri et al., 2017). In the worst scenario, it leads to plant death which results in a total yield loss (Nakashima et al., 2014). Grain yield reduction due to drought at 40% water reduction has been reported to be 20.6% in wheat and 39.3% in maize (Daryanto et al., 2016). On the other hand, it has been published that due to high population pressure by 2050, the demand for wheat is estimated to increase by 60% (FAO, 2013). Furthermore, in the developing world, more than 50% of wheat (50 million ha) is produced under a rain-fed system where rainfall is highly erratic (Gupta et al., 2017). Additionally, drought in combination with inherently low-fertile soils aggravates the impact of drought stress resulting in higher wheat yield losses (Mapfumo et al., 2017; Nezhadahmadi et al., 2013).

In Ethiopia, durum wheat nearly accounts for 15%–20% of the wheat production and covers 30% of the acreage grown with wheat (Alemu et al., 2019; Negassa et al., 2012). It is of prime importance for agricultural production in Ethiopia, as durum wheat is not only a staple crop for food security but also is becoming a major cash crop having 10% to 20% higher prices than bread wheat (Sall et al., 2019). This is accounted for the unique characteristics of durum wheat for making food products such as pasta, burghul and couscous. Nevertheless, despite the presence of over 10 million ha of land potential for wheat production, Ethiopia still imports wheat to meet the national wheat requirements (<https://www.indexmundi.com/agriculture/?country=et&commodity=wheat&graph=imports>). Water availability is the major limiting factor for the expansion of the production of wheat from the traditionally known growing areas in the highland to the lowland. Despite the availability of fertile soil in the lowland, this region, in general, has a moisture deficit and is prone to drought. In Ethiopia, only less than 1% of the cereal acreage has access to irrigation (Mann & Warner, 2015; Taffesse et al., 2011). Hence, the production of important crops like wheat is limited to the highland areas, only. One way of overcoming this problem is developing drought-tolerant wheat varieties that are used not only to expand wheat production to drought-prone areas but also are important to combat the recurrence of drought in the major wheat-growing regions. Ethiopia is considered the centre of diversity or secondary origin for durum wheat (Kabbaj et al., 2017), which offers the great potential to identify landraces that are tolerant to various stresses as evidenced for pathogens such as stem rust (Klindworth et al., 2007). This may also hold for drought.

Phenotyping of quantitative traits in the field, representing realistic environmental conditions and in growth chambers, which is better to control, has paramount importance in crop breeding. Phenotyping and genotyping of populations or landrace collections are crucial to

further define and understand traits of interest concerning complex abiotic stresses like drought via genetic mapping (Lopes et al., 2015). The two common genetic mapping methods are (1) linkage mapping and (2) association mapping or linkage disequilibrium (LD) mapping (Xu et al., 2017). Association mapping has the advantages of high resolution, high allelic richness, no need for developing mapping populations and is used as a powerful tool to detect natural variation underlying complex traits in several crop species (Wehner et al., 2015; Xu et al., 2017; Zhu et al., 2008). The magnitude of LD and its decay with genetic or physical distance determines the resolution of association mapping and is useful for assessing the desired numbers of SNPs on arrays (Vos et al., 2017). With the advent of rapid genotyping and next-generation sequencing technologies, GWAS has become a routine strategy for mapping genotype–phenotype associations in many species (Liu & Yan, 2019). For instance, in durum wheat, quantitative trait loci (QTLs) were detected under drought stress for grain yield on chromosomes 1A, 4A, 5B and 7B and days to heading (DH), days to maturity (DM) and thousand-kernel weight (TKW) on chromosome 2B (Sukumaran et al., 2018) and for seeds per spike (SPS) on chromosome 2B (Mengistu et al., 2016). In this study, we used a wheat 90K iSelect SNP array facilitating the analyses (Wang et al., 2014) with a high-density SNP-based consensus map and a physical reference sequence of tetraploid wheat (Maccaferri et al., 2015, 2019). The objectives of this study were (i) to identify marker-trait association (MTAs) and QTLs for yield and related traits under field and climate chamber conditions for drought stress and non-stress conditions and (ii) to assess the correlation between respective traits under field and climate chamber conditions.

2 | MATERIALS AND METHODS

2.1 | Study panel (SP)

A SP of 285 durum wheat accessions was used for the analyses of drought stress tolerance in field experiments in Ethiopia (supporting information Table S1). The genetic diversity and population structure were described in Negisho et al. (2021). From the SP, a set of 185 durum wheat accessions was selected for phenotyping in climate chamber experiments conducted at the Julius Kühn Institute (JKI), Germany (supporting information Table S1), based on the drought susceptibility index (DSI) calculated from least squares means (lsmeans) of field data as described by Fischer and Maurer (1978). Accordingly, based on the DSI results, a 1:2:1 ratio was used to select drought-tolerant, medium and susceptible accessions, respectively. A selection was needed representing the varying genotypes in the size limited climate chambers and was based on choosing characteristic genotypes from each group.

2.2 | Field experiments

Field phenotyping experiments were conducted at four sites for three seasons (2016–2018) in Ethiopia (Table 1). Biplot analysis for the relationship among environments explained 84.4% of the variation by PCA1 and PCA2. Drought stress sites and high-potential sites were clustered separately (Figure S1). The three years of data from the four sites were combined into two representing the two drought stress scenarios. Dera and Melkassa are sites for field drought stress (FDS), and Holeta and Debre Zeit are sites for non-drought stress (FNS) conditions. The FDS sites are located in the rift valley and were selected for screening genotypes for drought stress tolerance by the Ethiopian Institute of Agricultural Research (EIAR) (personal communication). Accessions were randomized in an incomplete block alpha lattice design with three replications per location and accession. Plots were arranged in rows of 1 m (Figure 1). The spacing between rows was 0.2 m, and sowing density was calculated based on a seeding rate of 395 seeds/m².

2.3 | Climate chamber experiments

A total of 185 accessions were planted in two replications for two scenarios. The climate chamber drought stress (CCDS) variant was 20% of the maximum soil water capacity (SWC), and the non-stress (CCNS) variant was 70% SWC. Pots with 15 cm × 15 cm × 20 cm capacity filled with 1500 g of ED73 soil containing 70% white soil and 30% clay with pH around 6 (H. Nitsch and Sohn GmbH and Co.KG, Germany) were used for the climate chamber experiments. Five seeds of durum wheat per accession were planted in each pot. Subsequently, germinated plants were thinned to three plants per pot. Watering was performed by weighing each pot every other day to maintain 70% SWC for both soil moisture variants until flowering time. At the time of flowering (BBCH 65), the CCDS treatment abstained from water supply until it reached 20% SWC. Then, CCNS and CCDS treatments were maintained at 70% and 20% SWC until maturity. The climate chamber temperature was set to 24°C during daytime

and 18°C during the night at the time of planting, with 13/11 light/dark hours, respectively. Then, to simulate the field conditions, from the time of flowering until harvest, it was readjusted to 26°C during day time and 20°C at night (Figure 1).

2.4 | Phenotyping

The durum wheat panel was evaluated for 10 agro-physiological traits under drought stress and non-stress for field and climate chamber experiments (Table 2). Hence, grain biomass (GB), DH, grain filling duration (DGF), DM, plant height (PH), SPAD, spike length (SL), SPS, harvest index (HI), and TKW were investigated under field and climate chamber conditions.

We recorded DH per pot for the climate chamber and per plot for the field at 50% spike emergence (Zadoks stage 65), and DM when 50% of spikes turned yellow or lost green colour (Zadoks stage 87) (Zadoks et al., 1974). We calculated DGF as the difference between DM and DH. At physiological maturity, PH was determined by measuring from the soil surface to the tip of the plant excluding awns, and SL was obtained by measuring the spike from its base to its tip excluding the awns. The number of SPS was recorded as an average seed count from 10 spikes per plot after harvesting spikes from ten randomly selected main culms under field and from three spikes per pot for the climate chamber. GB in gramme per plot for field and gramme per pot for climate chamber was determined based on the weight of harvested grain. We measured TKW by taking the weight of 1000 grains for the field experiments and it was estimated from 100 seeds for the climate chamber experiments. HI was calculated from total GB per plot to aboveground total biomass per plot for the field experiments. Similarly, for the climate chamber experiments, HI was determined from total GB per pot to aboveground total biomass of three plants per pot. Leaf colour was obtained indirectly with a Soil Plant Analysis Development (SPAD) chlorophyll metre after 10 to 15 days of flowering using a SPAD-502 Plus instrument (Minolta, Co. Ltd, Japan). Hence, three flag leaves were selected and five readings per selected leaf were acquired to get mean SPAD readings (Wehner et al., 2016).

TABLE 1 Summary of rainfall, minimum and maximum temperature as well as geositions of the field experimental sites in Ethiopia for the three growing seasons

Location	Treatment ^a	Latitude	Longitude	Altitude	Rainfall (mm)		Temperature (°C)						
								2016		2017		2018	
					2016	2017	2018	Min	Max	Min	Max	Min	Max
Dera	FDS	8°24'N	39° 21'E	1620	467	397	422	15	27	16	26	15	27
Melkassa	FDS	8°20'N	39° 19'E	1500	371	475	401	15	28	15	29	15	29
Holeta	FNS	8°10'N	38°30'E	2400	615	792	629	8	22	7	23	8	22
Debre Zeit	FNS	8°44'N	38° 58'E	1900	374	368	299	12	26	13	26	13	26

Abbreviations: FDS, field drought stress; FNS, field non-stress.

^aTreatment: FDS and FNS.

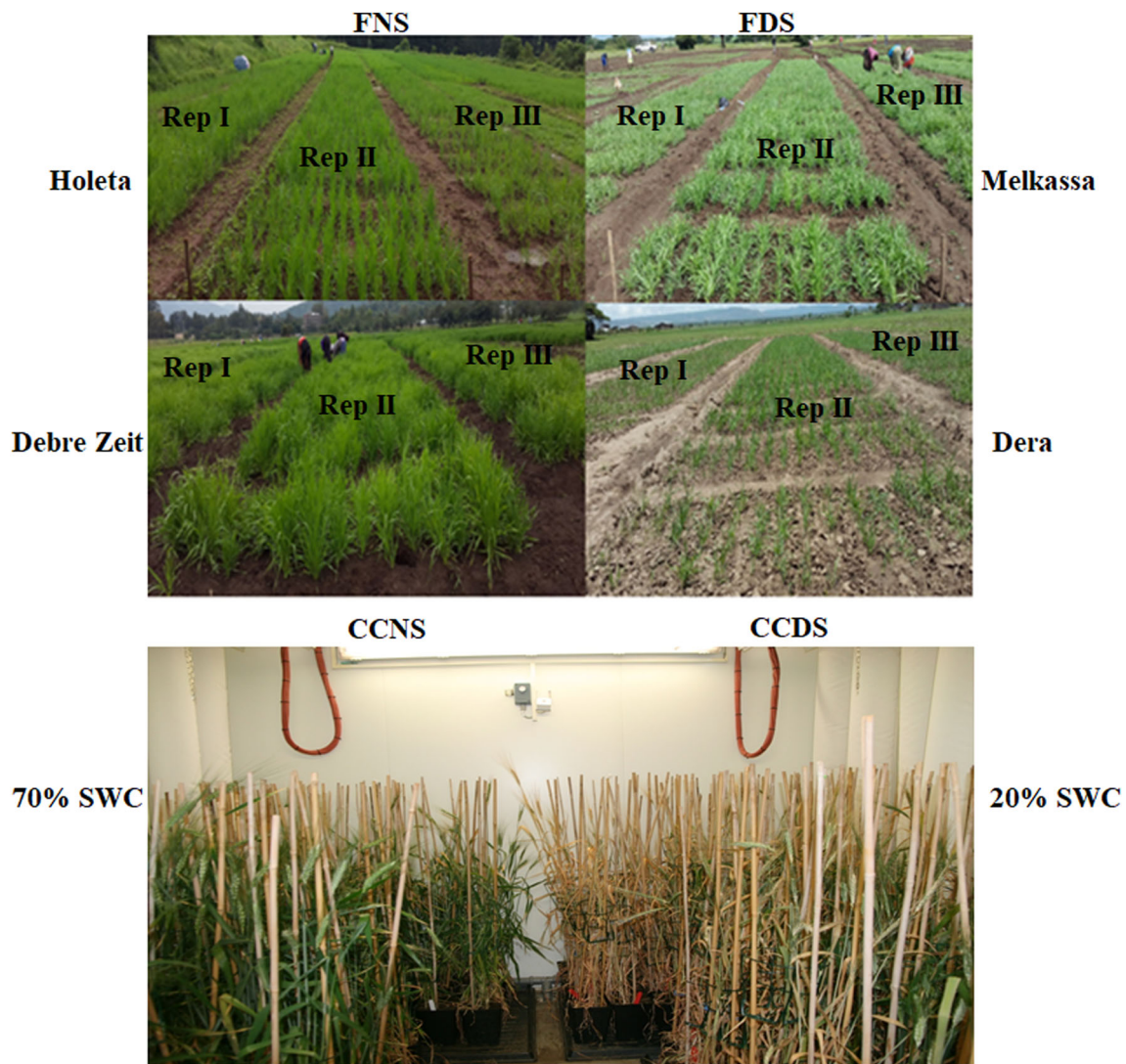


FIGURE 1 Field and climate chamber experiments. FNS: field non-stress (Holeta and Debre Zeit), FDS: field drought stress (Melkassa and Dera), CCNS: climate chamber non-stress = 70% soil water capacity (SWC), CCDS: climate chamber drought stress = 20% SWC. The numbers of replications under field conditions were indicated

2.5 | Statistical analysis of phenotypic data

The least squares means (lsmeans) were calculated by the lsmeans package in R (Russell, 2016) and were used for further analyses. Genotype was fixed, and years, locations, replications and blocks were considered random. Descriptive statistics were conducted based on the lsmeans by Rcmdr (Fox, 2017) in the R statistical computing environment (R Core Team, 2019/URL <http://www.R-project.org/>). Pearson's correlation coefficients (r) among different traits under drought stress and non-stress conditions were calculated using the corstars function in R, and the corrplot package was used to visualize the results (Graffelman, 2013; R Core Team, 2019/URL <http://www.R-project.org/>). For the 10 agro-physiological traits, selected correlations between the same traits were also analysed for FDS versus CCDS, as well as for FNS versus CCNS treatments.

The following linear mixed model was used for the combined analysis of variance (ANOVA) by the lmer function in the lme4 package for R (Bates et al., 2015):

$$Y_{ilyr} = \mu + G_i + L_l + Y_y + R_r(L_l Y_y) + B(L_l Y_y R_r) + GLY_{ily} + \varepsilon_{ilyr}$$

where y_{ilyr} is the trait of interest in the i th accession, l th location y th year, r th replication, μ is the overall mean, G_i is the effect of the i th accession, L_l is the effect of the l th location (i.e., Dera and Melkassa for FDS) and Y_y is the effect of the y th year. $R_r(L_l Y_y)$ is the r th replication within l th location and y th year, $B(L_l Y_y R_r)$ is the effect of k th incomplete block within l th location, y th year and r th replication, GLY_{ily} is the effect of the interaction among the i th accession, l th location and the y th year and ε_{ilyr} is the effect of residual. The effect of year was excluded from the climate chamber experiments. The distribution was assumed normal with mean zero and effect-specific variances.

TABLE 2 Descriptive statistics, heritability, and the number of significant ($-\log_{10}p \geq 4$) MTA for traits analysed under drought stress and non-stress conditions for field and climate chamber experiments

Trait	Unit	Trt	Mean	Min	Max	SD (%)	CV (%)	LSD	h^2 (%)	% Reduction = ($Y_{ns}-Y_s/Y_{ns}$)*100	MTA
GB	g/plot	FNS	77.10	33.00	115.00	15.60	20.25	6.62	69.60	35.79	4
		FDS	49.50	24.00	80.00	11.50	23.21	4.60	61.57		6
	g/pot	CCNS	2.91	0.00	5.00	1.04	35.77	0.21	NA	52.41	3
		CCDS	1.39	0.00	5.00	0.50	36.01	0.09	NA		8
DH	days	FNS	72.20	62.00	88.00	3.98	5.51	0.90	80.33	9.58	4
		FDS	65.30	57.00	77.00	4.27	6.55	0.96	80.37		5
		CCNS	73.00	64.00	85.00	4.58	6.28	0.72	NA	1.37	10
		CCDS	72.00	57.00	96.00	6.07	8.41	0.93	NA		5
DGF	days	FNS	47.70	39.00	57.00	3.17	6.65	1.29	56.58	35.56	10
		FDS	30.70	23.00	40.00	2.99	9.72	1.23	59.36		6
		CCNS	43.58	24.00	58.00	5.88	13.50	1.05	NA	44.10	0
		CCDS	24.36	12.00	40.00	5.55	22.80	1.04	NA		0
DM	days	FNS	120.00	113.00	129.00	3.00	2.51	1.34	48.20	19.91	7
		FDS	96.00	90.00	105.00	2.56	2.67	0.99	42.00		6
		CCNS	116.57	98.00	130.00	5.20	4.46	0.94	NA	17.35	1
		CCDS	96.34	84.00	109.00	6.29	6.23	0.97	NA		0
SPAD	free	FNS	40.40	31.00	53.00	4.84	11.98	1.55	72.79	9.69	12
		FDS	36.50	27.00	50.00	4.94	13.53	1.51	70.82		4
		CCNS	36.90	3.00	57.00	13.40	36.37	2.78	NA	36.71	6
		CCDS	23.40	4.00	58.00	12.40	52.96	2.48	NA		5
PH	cm	FNS	101.60	40.70	160.00	21.60	21.30	1.92	89.11	24.57	5
		FDS	76.60	35.00	125.00	15.70	20.40	2.13	75.72		6
		CCNS	85.30	48.00	113.00	11.70	13.74	1.73	NA	3.66	0
		CCDS	82.10	46.00	120.00	11.10	13.46	1.64	NA		0
SL	cm	FNS	7.90	5.00	12.00	1.15	14.57	0.19	72.32	28.62	7
		FDS	5.64	4.00	8.00	0.85	14.99	0.20	83.37		5
		CCNS	7.16	4.00	11.00	1.46	20.45	0.15	NA	1.47	5
		CCDS	7.06	4.00	11.00	1.30	18.47	0.18	NA		4
HI	%	FNS	50.00	35.00	70.00	6.47	12.95	2.69	52.25	21.15	9
		FDS	39.40	24.00	59.00	6.29	15.95	3.29	51.40		9
		CCNS	0.31	0.00	0.47	0.07	22.96	0.01	NA	28.63	6
		CCDS	0.22	0.00	0.08	0.08	37.88	0.00	NA		3
SPS	count	FNS	28.40	20.00	42.00	4.60	16.19	1.48	74.91	27.72	7
		FDS	20.60	14.00	31.00	3.96	19.26	1.19	77.56		5
		CCNS	18.10	2.00	32.00	5.82	32.16	1.19	NA	13.29	0
		CCDS	15.70	1.00	31.00	5.28	33.64	0.97	NA		5
TKW	g/1000	FNS	38.20	29.00	49.00	4.24	11.10	1.25	70.49	22.75	3
		FDS	29.50	20.00	41.00	3.34	11.31	1.22	64.70		6
		CCNS	23.39	6.00	53.00	7.90	33.80	1.15	NA	29.07	3
		CCDS	16.59	5.00	49.00	7.58	45.20	1.13	NA		1
Total										191	

Abbreviations: CCDS, climate chamber drought stress; CCNS, climate chamber non-stress; CV, coefficient of variation (standard deviation divided by mean)*100; DGF, grain filling duration; DH, days to heading; DM, days to maturity; FDS, field drought stress; FNS, field non-stress; GB, grain biomass; h^2 , heritability; HI, harvest index; LSD, least significant difference; mean, minimum, maximum; MTA, marker-trait association; NA, not applicable (heritability was not calculated for the climate chamber experiments since it was conducted only once with two replications for each drought variant); PH, plant height; SD, standard deviation; SL, spike length; SPAD, Soil Plant Analysis Development; SPS, seed per spike; TKW, thousand-kernel weight. Trt, treatment.

Broad sense heritability (h^2) of traits analysed in field experiments was calculated from variance components of location (l), year (y) and replication (r):

$$H^2 = \sigma^2_g / (\sigma^2_g + \sigma^2_{gl} / l + \sigma^2_{gy} / y + \sigma^2_{gly} / ly + \sigma^2_e / lyr),$$

where σ^2_g , σ^2_{gl} , σ^2_{gy} , σ^2_{gly} and σ^2_e are accession variance, accession \times location, accession \times year and accession \times location \times year interaction and error variance, respectively, and l , y , ly and r refer to the number of locations, years, interaction of locations and years and replications, respectively (Falconer & Mackay, 1996; Vargas-Reeve et al., 2013).

2.6 | Genotyping

Genotyping was conducted by SGS TraitGenetics GmbH (Gatersleben, Germany) using the wheat 90K iSelect SNP array (Wang et al., 2014). The consensus linkage map of tetraploid wheat (Maccaferri et al., 2015) and the IWGSC RefSeq v1.0 genomic assembly (International Wheat Genome Sequencing Consortium, 2018) were applied to assign a genomic location to each SNP marker. SNP markers with minor allele frequency (MAF) of <5%, missing data >10% and heterozygosity >12.5% were excluded, and SNP markers were imputed by the Beagle method in R (Browning & Browning, 2007). Physical distance positions were aligned to the recent Durum Wheat (cv. 'Svevo') RefSeq Rel. 1.0 (Maccaferri et al., 2019). Finally, a total of 11,919 high confident SNP markers were used to construct HapMap files for further MTA analyses. The genetic population structure was estimated with STRUCTURE 2.3.4 software (Falush et al., 2003, 2007; Hubisz et al. 2009; Pritchard et al., 2000) implementing a model-based Bayesian cluster analysis as described in (Negisho et al., 2021).

LD, LD decay and LD plots of the durum wheat genomes (A and B) were analysed using R packages genetics, LDheatmap and trio (R Core Team, 2014; Shin et al., 2006; Warnes, 2013). Thus, inter-marker genetic distances were assessed using the consensus physical distance position of the respective SNP markers (Maccaferri et al., 2019). LD critical value was set at $r^2 \geq .2$ (Oyiga et al., 2017; Voss-Fels et al., 2015).

2.7 | GWAS

GWAS was conducted using the genome association and prediction integrated tool (GAPIT) in R (Lipka et al., 2012). A mixed linear model was employed for each trait by including lsmeans, and drought treatments-based combined analysis was done for years, locations and replications. SNP markers, kinship matrix, and q-matrix were used as cofactors for MTA analysis (Yu et al., 2006). FarmCPU method, which is iteratively using the fixed-effect model and the random effect model for powerful and efficient GWAS (Liu et al., 2016), was used.

In the present study, Bonferroni–Holm correction for multiple testing was too strict to select significant MTAs (Gaetano, 2018;

Holm, 1979). Therefore, in this study, the threshold for associated markers was adjusted to $-\log_{10}p \geq 4$ (Bai et al., 2016; Bhatta et al., 2018; Ma et al., 2016). We also tested the MTA at FDR 5% as $p = 1/\text{total number of SNP} \times 0.05 = 4.19498E-06$ (LOD score = 5.4) (Benjamini & Hochberg, 1995). The phenotypic variance explained (PVE) was calculated based on sample size, MAF, effect size and standard error of effect size for each SNP following Teslovich et al. (2010). Identified MTAs were clustered into QTL using the critical LD decay value, and MTAs not in LD were considered as an independent QTL (Kidane et al., 2017; Negro et al., 2019). An MTA, which was similarly associated with a trait or several traits under the various treatments (FDS, FNS, CCDS and CCNS) on the same chromosome and at the same position, was considered as an overlapping MTA (Ahmad et al., 2014). Likewise, a QTL detected for a trait or several traits under the various treatments (FDS, FNS, CCDS and CCNS) on the same chromosome and within the same interval was considered an overlapping QTL (Tricker et al., 2018). A QTL that relates to two or more traits within the same treatment was considered as co-located QTL, while a QTL associated with a single trait was considered as an individual QTL (Ma et al., 2019; Sukumaran et al., 2018).

In the current study, the interval of the identified QTL was used as input in the *Triticum turgidum* Durum Wheat 'Svevo' (RefSeq Rel. 1.0, Maccaferri et al., 2019) in the GrainGenes database to compare these with previously reported QTLs. If the detected QTL did not match with any of the reported QTLs for the trait of interest, it is reported as likely new QTL detected in this study. Graphical representation of linkage groups and QTLs was carried out using MapChart 2.32 software (Voorrips, 2002).

3 | RESULTS

3.1 | Phenotyping

The durum wheat panel displayed broad phenotypic differences for each of the traits under field and climate chamber drought conditions, indicating the broad genetic diversity in the panel (supporting information Table S2, Table 2 and supporting information Figures S3 and S4, respectively). For all studied traits, mean values of the drought stress treatments were lower than the mean values of the non-stress treatments both under field and climate chamber conditions. In the climate chamber experiments, the mean value of GB was reduced by drought stress treatment by 52.4%, which was higher than mean GB reduction due to drought in the field (35.79%). Similarly, a higher reduction due to drought stress was observed for SPAD and HI under climate chamber conditions (36.71%, 28.63%) as compared with the field (9.69%, 21.15%). Notably, in the current study, a higher reduction was obtained under field (24.57%) as compared with the climate chamber conditions (3.66%) for PH. Boxplots illustrate the mean value reduction for all studied traits (Figure S2 and S3). The dispersion of the data from the mean was expressed in percentage of standard deviation (SD%) and was comparable under field as well as climate chamber conditions for all tested traits (Table 2). Under field

conditions, higher dispersion from the mean was observed for GB and HI as compared with the climate chamber experiment. In contrast, for SPAD and SPS, the SD% was higher under climate chamber as compared with field conditions (Table 2).

Under FNS conditions, heritability of traits analysed varied between 48.2% for DM and 89.11% for PH. Similarly, under FDS, heritability ranged between 42% for DM and 83.37% for SL. The current study showed higher heritability for GB, PH, SPAD, HI and TKW under FNS as compared with FDS conditions. However, heritability for DH, DGF, SL and SPS was higher under FDS as compared with FNS conditions.

The coefficient of variation (CV) was comparable for drought stress and non-stress conditions for all same traits, except for SL, HI and SPS under CCDS as compared with CCNS conditions.

ANOVA revealed significant ($p < .0001$) effects for genotype and treatment and accession by treatment interactions for GB, DH, DGF, DM, PH, SL, SPS and TKW under field conditions (supporting information Table S3). Similarly, significant ($p < .0001$) effects were observed among accessions and between treatments for GB, SPAD, PH, HI and SPS for the climate chamber experiment. These results indicate high genetic variability in the SP. However, under field conditions, no

significant difference was observed for HI between treatments and accession by treatment interactions. Likewise, accession by treatment interactions was non-significant for PH, SPAD, SPS and HI for the climate chamber experiment (supporting information Table S3).

3.2 | Correlation analysis

Correlations between traits investigated under FDS and CCDS conditions (above diagonal) and FNS and CCNS conditions (below diagonal) are shown in Table 3A,B. Under FDS and FNS conditions, GB was positively and significantly ($p < .001$) correlated with DGF ($r = .46, .21$), SPAD ($r = .29, .31$), SPS ($r = .47, .39$), HI ($r = .54, .44$) and TKW ($r = .47, .55$), respectively (Table 3A). Similarly, under CCDS and CCNS conditions, GB was positively and significantly ($p < .001$) correlated with PH ($r = .48, .60$), SL ($r = .32, .40$), SPS ($r = .50, .52$) and HI ($r = .39, .66$), respectively (Table 3B). However, under FDS conditions, GB was negatively and significantly ($p < .001$) associated with DH ($r = -.48$), DM ($r = -.27$) and SL ($r = -.24$) (Table 3A). In the current study, TKW was significantly ($p < .05$) correlated with GB under all the experimental conditions except under CCDS condition (Table 3A, B).

TABLE 3 Pearson's correlation coefficients (r) between traits under FDS and FNS conditions (A) and CCDS and CCNS conditions (B)

FDS/FNS		DH	DM	DGF	PH	SL	SPAD	SPS	HI	TKW	GB
A	DH		0.73	-0.81	0.23	0.37	-0.18	-0.51	-0.60	-0.37	-0.48
	DM	0.62		-0.20	-0.06	-0.07	0.26	-0.12	-0.27	-0.07	-0.27
	DGF	-0.66	0.17		-0.37	-0.59	0.48	0.62	0.63	0.48	0.46
	PH	0.47	-0.05	-0.63		0.63	-0.57	-0.46	-0.49	0.06	-0.04
	SL	0.52	-0.03	-0.69	0.75		-0.71	-0.62	-0.61	-0.38	-0.24
	SPAD	-0.21	0.32	0.58	-0.62	-0.64		0.71	0.53	0.38	0.29
	SPS	-0.31	0.18	0.57	-0.54	-0.57	0.69		0.68	0.38	0.47
	HI	-0.47	0.00	0.59	-0.63	-0.58	0.64	0.73		0.40	0.54
	TKW	-0.19	0.17	0.41	-0.11	-0.37	0.55	0.43	0.46		0.47
	GB	-0.14	0.04	0.21	0.10	-0.01	0.31	0.39	0.44	0.55	
CCDS/CCNS		DH	DM	DGF	PH	SL	SPAD	SPS	HI	TKW	GB
B	DH		0.61	-0.28	0.00	0.10	-0.21	0.07	-0.04	0.06	0.00
	DM	0.33		0.54	-0.11	-0.10	-0.10	0.08	0.19	0.31	0.10
	DGF	-0.59	0.57		-0.15	-0.24	0.14	0.05	0.27	0.30	0.11
	PH	0.10	-0.03	-0.11		0.48	-0.01	0.59	0.00	-0.33	0.48
	SL	0.15	-0.23	-0.33	0.43		-0.05	0.39	-0.09	-0.35	0.32
	SPAD	-0.17	0.16	0.28	0.18	0.07		0.14	0.14	-0.06	-0.04
	SPS	0.00	-0.06	-0.05	0.59	0.36	0.06		0.25	-0.54	0.50
	HI	-0.34	-0.11	0.21	0.39	0.14	0.30	0.53		0.09	0.39
	TKW	-0.20	0.02	0.19	0.00	-0.06	0.40	-0.38	0.14		0.01
	GB	-0.14	-0.12	0.02	0.60	0.40	0.40	0.52	0.66	0.35	

Note: Above diagonal indicates the correlation between traits under drought stress treatments and below diagonal shows correlation between traits under non-stress treatments. Correlations ≥ 0.15 were significant at $p < .05$ and highlighted.

Abbreviations: CCDS, climate chamber drought stress; CCNS, climate chamber non-stress; DGF, grain filling duration; DH, days to heading; DM, days to maturity; FDS, field drought stress; FNS, field non-stress; GB, grain biomass; HI, harvest index; PH, plant height; SL, spike length SPAD, Soil Plant Analysis Development; SPS, seeds per spike; TKW, thousand-kernel weight. Colors indicate the degree of correlation between the trait.

Under FDS and FNS conditions, SPAD was positively and significantly ($p < .001$) correlated with GB, DM, DGF, SPS, HI and TKW and negatively and significantly ($p < .01$) correlated with DH, PH and SL (Table 3A). Likewise, under CCNS conditions, SPAD was positively and significantly ($p < .001$) correlated with GB, DGF, HI and TKW. But under CCDS and CCNS conditions, SPAD was negatively and significantly ($p < .05$) correlated with DH (Table 3B).

DH and DM were positively and significantly ($p < .001$) correlated under all conditions (Table 3A,B). Under FDS and FNS conditions, DH was negatively and significantly ($p < .001$) correlated with DGF, SPAD, SPS, HI and TKW. Under CCDS and CCNS conditions, DH was negatively correlated with DGF and SPAD, while under CCNS conditions DH, was negatively and significantly ($p < .01$) correlated with HI and TKW.

Correlation between same traits was positive for field versus climate chamber ranging from non-significant to significant ($p < .001$) (Table 4A,B). Accordingly, the correlations between GB for FDS versus CCDS and FNS versus CCNS were positive and significant with $r = .17$ and $.32$, respectively. Similarly, the correlations between the same traits, namely DH, DM, DGF, PH and SL, were positive and significant ($p < .001$) for FDS versus CCDS and FNS versus CCNS. Positive and significant ($p < .01$) correlation was also observed for SPAD with $r = .19$ between FDS and CCDS, but it was non-significant for FNS versus CCNS. Positive and significant ($p < .001$) correlation was detected between SPS for FNS versus CCNS with $r = .26$, but the correlation was non-significant for FDS versus CCDS. Correlations between TKW for FDS versus CCDS and FNS versus CCNS were positive and significant with $r = .18$ and $.45$, respectively. In this study, HI showed a non-significant correlation for FDS versus CCDS and FNS versus CCNS conditions. Generally, ANOVA, descriptive analysis, boxplots and correlations between the same traits tested under similar drought treatment for field versus climate chamber indicate a similar trend.

3.3 | LD decay

The significant ($-\log_{10}p \geq 4$) MTA was clustered into QTL by the critical LD decay value ($r^2 \geq .2$) at 4.78 Mb. The highest LD decay was calculated for the A-genome on chromosome 4A and the B genome on chromosome 6B. Therefore, in the sets, chromosomes 4A and 3A had the highest and the lowest decay rates, respectively. Similarly, chromosomes 6B and 2B had the highest and the lowest decay rates in the set, respectively. Chromosome 2B had a notably slower decay rate than the others (supporting information Table S3).

3.4 | MTAs

A total of 191 significant ($-\log_{10}p \geq 4$) MTAs were detected across the whole durum wheat genome (supporting information Table S4). The numbers of detected significant MTAs were 58, 68, 36 and 29 for FDS, FNS, CCDS and CCNS, respectively. The highest number of MTAs (25) was detected on chromosome 1B, and the lowest number was detected on chromosome 3A (5). Concerning the traits analysed, the highest numbers of MTAs detected were 27 each for SPAD and HI, followed by 24 for DH. The lowest number of MTAs obtained was 11 for PH (Table 2 and supporting information Table S4). In the current study, no MTA was detected for PH under climate chamber conditions.

In this study, eight overlapping MTAs were detected associated with multiple phenotypic traits for drought stress and non-stress conditions and highlighted in yellow colour (supporting information Table S4). Three overlapping MTAs were detected on chromosome 1B at 10778560 bp associated with DH and DM under FDS, at 534692879 bp for GB and DH under CCNS and at 381876470 bp associated with DH and DGF under CCNS and FDS conditions, respectively. One overlapping MTA was detected for SPS on

A		B	
Drought stress treatments	r (p value)	Non-stress treatments	r (p value)
DH_FDS vs DH_CCDS	0.47***	DH_FNS vs DH_CCNS	0.56***
DM_FDS vs DM_CCDS	0.29***	DM_FNS vs DM_CCNS	0.35***
DGF_FDS vs DGF_CCDS	0.31***	DGF_FNS vs DGF_CCNS	0.32***
PH_FDS vs PH_CCDS	0.53***	PH_FNS vs PH_CCNS	0.66***
SL_FDS vs SL_CCDS	0.56***	SL_FNS vs SL_CCNS	0.48***
SPAD_FDS vs SPAD_CCDS	0.19**	SPAD_FNS vs SPAD_CCNS	0.12
SPS_FDS vs SPS_CCDS	0.07	SPS_FNS vs SPS_CCNS	0.26***
HI_FDS vs HI_CCDS	0.12	HI_FNS vs HI_CCNS	0.03
TKW_FDS vs TKW_CCDS	0.18*	TKW_FNS vs TKW_CCNS	0.45***
GB_FDS vs GB_CCDS	0.17*	GB_FNS vs GB_CCNS	0.32***

TABLE 4 Pearson's correlation coefficients (r) for the same traits tested under FDS versus CCDS conditions (A) and under FNS versus CCNS conditions (B) for those traits which MTAs were analysed

Abbreviations: CCDS, climate chamber drought stress; CCNS, climate chamber non-stress; DH, days to heading; DM, days to maturity; DGF, grain filling duration; FDS, field drought stress; FNS, field non-stress; GB, grain biomass; HI, harvest index; PH, plant height; SL, spike length; SPAD, Soil Plant Analysis Development; SPS, seed per spike; TKW, thousand-kernel weight; vs, versus.

*** $p < .001$. ** $p < 0.01$. * $p < 0.05$.

chromosome 3B at 25269809 bp both under FDS and FNS conditions. One overlapping MTA was detected on chromosome 4B at 485705797 bp associated with DM and HI under FNS and FDS conditions, respectively. Two overlapping MTAs were detected on chromosome 5A at 112213041 bp for SL under FDS and for DGF under FNS and at 110830599 bp for GB under FDS and for HI under FNS. Similarly, one overlapping MTA was detected on chromosome 7A at 616616464 bp associated with DH and SL both under CCDS condition. Therefore, from the eight overlapping MTAs associated with multiple phenotypic traits, regardless of the traits associated with, five markers (Excalibur_c7964_1290 on chromosome 4B at 485705797 bp, Kukri_rep_c116526_98 on chromosome 5A at 112213041 bp, Ra_c18323_183, RAC875_c60169_200 on chromosome 1B at 381876470 bp and Tdurum_contig76578_537 on chromosome 5A at 110830599 bp) were detected under contrasting drought treatment conditions, indicating that these markers are potentially stable. One stable marker, detected for SPS (RAC875_c60169_200) located on chromosome 3B at 25269809 bp, was detected under FDS and FNS (supporting information Table S4). Notably, after clustering into QTL, just one overlapping QTL remained (see below).

The percentage of PVE by each MTA varied from 0.03% to 11.83%. The highest PVE detected was 11.83% for SL under FNS followed by 10.61% PVE for HI under CCDS, while others showed lower than 10% PVE indicating the polygenic nature of the quantitative traits evaluated (supporting information Table S4). Manhattan plots for all investigated traits under FNS, FDS, CCNS and CCDS are visualized in Figures S5, S6, S7 and S8, respectively. In the current study, out of 191 detected MTAs, 69 MTAs associated with GB, DH, DGF, DM, PH, SPAD, SPS, HI and TKW were significant at FDR 5% highlighted in grey colour (supporting information Table S4) and with black, red, green and blue colours for FNS, FDS, CCNS and CCDS, respectively (Figure 2).

Using critical LD ($r^2 \geq .2$), the detected MTAs were clustered into 70 QTLs (Figure 3 and supporting information Table S5).

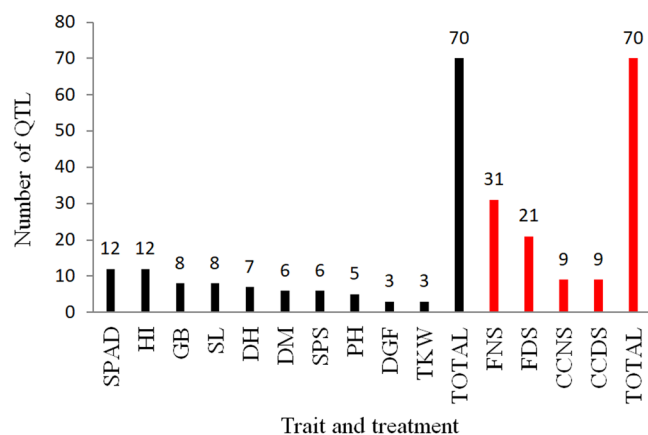


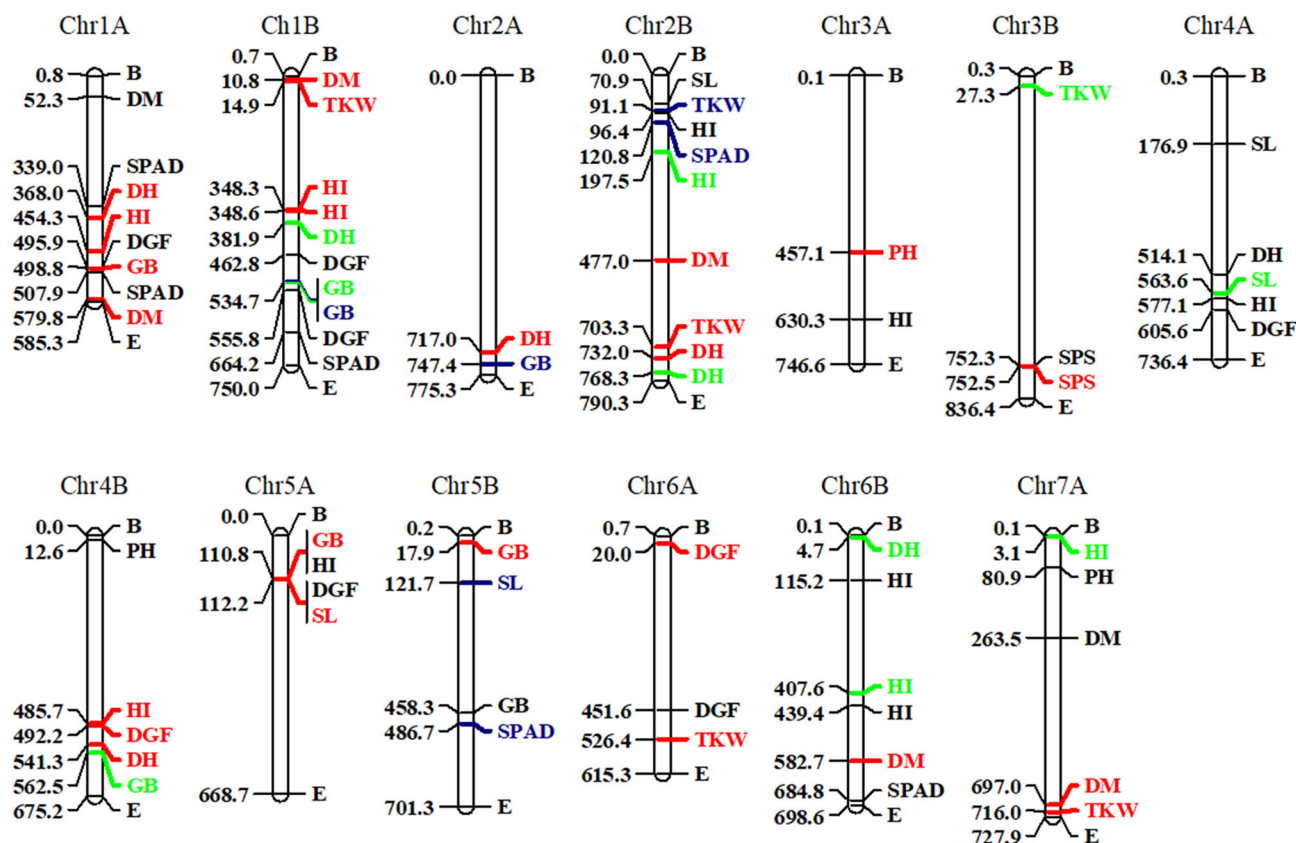
FIGURE 2 Number of detected quantitative trait loci (QTLs) for the investigated traits (black bar) and under field non-stress (FNS), field drought stress (FDS), climate chamber non-stress (CCNS), and climate chamber drought stress (CCDS) treatments (red bar)

Consequently, 31, 21, 9 and 9 QTLs were identified under FNS, FDS, CCDS and CCNS, respectively. The highest numbers of QTLs obtained were 12 each for SPAD and HI, followed by 8 QTLs each for GB and SL. The lowest number of QTLs detected was three for DGF and TKW, each (Figure 3). In the SP, 30 QTLs were identified on the A-genome and 40 QTLs on the B genome. The largest number of QTLs was detected on chromosomes 6B (10 QTLs), followed by 1A (eight QTLs) and 2B (eight QTLs). The smallest numbers of QTL detected were one on chromosome 6A, followed by 3A (two QTLs) (supporting information Table S5). In this study, only one QTL overlapping between the two watering regimes was detected on chromosome 1B between 620250467–627873395 bp for HI under FDS and for SPAD and HI under FNS.

The eight detected QTLs for GB, which are independent from DH were located on chromosomes 1A between 495694477 and 501944537 bp; 3B between 416256124 and 430507900 bp and 7A between 745357158 and 759608934 bp; 4B between 561075112 and 572800846, 593416763 and 605142497 and 658785890 and 670511624 bp; 6B between 505703728 and 510449994 bp and on 7A between 637937043 and 645127159 bp with PVE ranging from 1.92% to 4.24%. These QTLs for GB were co-located with DGF, DM, SL, SPS, SPAD and HI traits. Six out of the eight QTLs for GB were previously reported and two were likely new (supporting information Table S5). All the seven detected QTLs for DH were previously reported, and out of these, four QTLs turned out to be co-located with TKW, SL, SPAD, GB and HI. Two out of the three detected QTLs for DGF were co-located with SL, SPS, SPAD and TKW traits. One QTL detected for DGF was previously reported, and two are novel. Four out of the six detected QTLs for DM are co-located with DH, DGF, PH, SL, SPAD, HI and SPS. From the detected QTLs for DM, four are likely new. Five previously reported QTLs were detected for PH, and out of these, four QTLs are co-located with SL, DH, GB, DGF, SPS and SPAD. All the twelve QTLs detected for SPAD were previously reported, and eight QTLs are co-located with DGF, DM, SPS, HI, DH, PH and SL traits. Six out of the eight detected QTLs for SL are co-located with DGF, PH, DM, GB, HI, SPAD, TKW and DH traits and one QTL detected for SL is novel. Four out of the five detected QTLs for SPS are co-located with DGF, GB, HI, SPAD, PH and TKW traits, and one QTL for SPS is likely new. Six out of the 12 detected QTLs for HI are co-located with DGF, SPS, TKW, SPAD, DH, GB and SL, and two QTLs for HI are novel. The three detected QTLs for TKW were previously reported, and one QTL out of the three is co-located with DH and HI.

4 | DISCUSSION

Drought stress alters the morphological, physiological and molecular responses of plants. In the current study, GB reduction due to drought stress ranged from 35.79% to 52.41% for field and climate chamber experiments, respectively. Other recent reports revealed that grain yield reduction due to drought was up to 60% on durum wheat and more than 40% and 30% for bread wheat, and rice, respectively



Color key

Black = FNS, Red = FDS, Green = CCNS, Blue = CCDS

FIGURE 3 Physical linkage map of the durum wheat genome in Mb by MapChart (Voorrips, 2002). A total of 69 significant quantitative trait loci (QTLs) at false discovery rate (FDR) 5% under field non-stress (FNS), field drought stress (FDS), climate chamber non-stress (CCNS), and climate chamber drought stress (CCDS) elucidated by black, red, green, and blue colours, respectively. GB: grain biomass, DH: days to heading, DGF: grain filling duration, DM: days to maturity, PH: plant height, SL: spike length, SPAD, SPS: seed per spike, HI: harvest index, and TKW: thousand-kernel weight

(Sukumaran et al., 2018; Zhang et al., 2018). Field experiments in our study were reliable, showing moderate to high broad sense heritability. Higher heritability values were obtained for most of the evaluated traits under FNS compared with FDS conditions (Table 2). ANOVA results indicate the broad genetic diversity of the SP enabling the dissection of the embedded genetic diversity.

The negative correlation of GB with DH ($r = -.48$) and DM ($r = -.27$) under FDS conditions indicates that early maturing accessions had a yield advantage. This is in agreement with the finding of Millet et al. (2016), Sukumaran et al. (2018) and Qaseem et al. (2019). GB was also negatively correlated with SL ($r = -.24$) under FDS, which is in line with results on durum wheat (Pour-Aboughadareh et al., 2020). The negative correlation of GB with SL under FDS indicated a reduced seed set due to prolonged terminal drought under field conditions. A positive and significant correlation was observed between GB and DGF under FDS and FNS conditions. However, there was no correlation between GB and DGF under CCDS treatment. A report by Sukumaran et al. (2018) also indicated no association between GB and DGF in durum wheat genotypes studied under well-watered and drought conditions. In the current study, under field

conditions, GB did not show a correlation with PH but a positive and significant ($r = .6$) correlation with GB and PH under CCDS conditions. This is in agreement with Qaseem et al. (2017) who suggested that under drought stress environments, tall genotypes accumulate and mobilize more resources to grain and thus had a higher yield than shorter genotypes. In the current study, TKW was significantly ($p < .05$) correlated with GB except under CCDS under which the association between these traits was positive but non-significant. A recent study on durum wheat also reported a non-significant correlation between GB and TKW under drought stress and non-stress conditions (Sukumaran et al., 2018). However, studies revealed a significant association between GB and TKW in wheat (del Pozo et al., 2019; Mohammadi et al., 2018). This may be due to the complex nature of GB and that GB and TKW are affected by several factors under different environmental conditions.

GWAS is a powerful tool for the identification of quantitative trait loci and to exploit the differential decay of LD between marker loci and genes of interest in natural and domesticated populations (Laidò et al., 2014). The basic principle behind LD is to detect and cluster the detected MTAs during GWAS analysis. If the distance between two

identified MTAs is less than critical ($r^2 \geq .2$) LD decay value, it shows 95% confidence that the two MTAs stay tighter and they are assigned as one QTL; otherwise, they are in linkage equilibrium (Kidane et al., 2017). In crop plants, several QTLs have been reported on agronomic, physiology, and root traits using linkage mapping and genome-wide association studies (Gupta et al., 2020). In our SP, the LOESS curve crossed the critical LD ($r^2 \geq .2$) at 4.78 Mb. Similarly, other studies reported LD decay values of 4.5 Mb (Maccaferri et al., 2019) and 5.71 Mb (Taranto et al.,) at critical LD ($r^2 = .2$) for the durum wheat SP. Hence, based on the critical LD value, the identified 191 MTAs were grouped into 70 QTLs. LD pattern of a SP is important for selecting the marker density required for GWAS and for defining identified QTLs (Sjol et al., 2017). The PVE varied from 0.03%–11.83%, and only for two MTAs, a r value higher than 10% for PVE was calculated, demonstrating the polygenic control of the traits measured in this study. This is also reported in other studies in durum wheat (Wang et al., 2019) and bread wheat (Liu et al., 2018). In our study, two major MTAs were detected for traits HI under CCDS and SL under FNS with 10.6% and 11.83% PVE, respectively (supporting information Table S4). Interestingly, similar results were reported from GWAS analysis for the trait PH in durum wheat explaining 16% to 39% total PVE (Wang et al., 2019) and in bread wheat explaining 10.10% to 30.68% of the phenotypic variation (Jin et al., 2020).

In our study, QTLs were detected for yield and yield-related traits and matched with previously reported results from durum wheat in the GrainGenes database (supporting information Table S5). Grain yield is the main target in wheat breeding and it is a complex trait due to high GxE interaction and low to intermediate heritability (Börner et al., 2002). Identification and use of QTLs associated with valuable agronomic traits at early generation selection in wheat breeding programmes enhance the development of improved cultivars (Collard & Mackill, 2008). A QTL that relates to two or more traits is considered as co-located QTL, while a QTL associated with a single trait is considered as an individual QTL (Ma et al., 2019; Sukumaran et al., 2018). Thus, in this study, eight co-located QTLs were detected associated with GB (supporting information Table S5). QTLs for GB were found to be also linked with DGF between 495694477 and 501944537 bp; DM between 416256124 and 430507900 bp; with SL and SPS between 745357158 and 759608934 bp; SL between 561075112 and 572800846, 593416763 and 605142497 and 637937043 and 645127159 bp; with HI, DM and SPAD between 658785890 and 670511624 bp; as well as with SPAD between 505703728 and 510449994 bp under drought stress and non-stress conditions. Interestingly, in our study, QTLs detected for GB were not linked with DH, which hints to a limited effect of flowering time on grain yield as also reported in other studies for durum wheat (Zaïm et al., 2020) and wheat (Ullah et al., 2021). The detected GB QTLs were located on chromosomes 1A, 3B (2), 4B (3), 6B and 7A. Except two QTLs located on chromosomes 1A and 4B, which we believe to be reported for the first time, the other six QTLs have been reported in previous studies (Maccaferri et al., 2008; Mengistu et al., 2016; Soriano et al., 2017) on chromosome 3B, (Milner et al., 2016; Patil et al., 2013) on chromosome 4B, (Marcotuli et al., 2017) on 6B and on

7A (Mengistu et al., 2016). The current identification of QTLs within similar QTL intervals in our study and the mentioned previous studies confirms the findings and the power of GWAS (supporting information Table S5). Therefore, QTLs located on chromosomes 3B and 4B can be considered constitutive QTLs linked to GB whose selection may help to increase yield under drought stress. Similarly, recent findings identified QTLs for GB in the durum wheat genome (Arif et al., 2020; Mangini et al., 2018, 2021; Zaïm et al., 2020). Conversely, in our study and the report by Mangini et al. (2021), no QTL associated with GB were detected on chromosome 2B, which was reported to carry QTLs for GB by Zaïm et al. (2020). More important, in our study, GB QTLs detected under drought stress including newly detected QTLs showed a positive effect on yield with significant LOD value ranging between 4.11 and 7 and with up to 4.24% PVE, indicating that they could have the potential in increasing grain yield in durum.

DH provides the basis for plant adaptation and is a major trait in plant breeding (Zaïm et al., 2020). Also, under terminal drought, early flowering time and a shorter vegetative phase is important for wheat production (Shavrukov et al., 2017). In the present study, seven (four co-located and three individual) QTLs for DH were located on chromosomes 1A, 1B, 2A, 4A, 4B and 6B. Interestingly, five of the identified QTLs were positioned within reported QTL intervals on chromosomes 1A and 1B (Milner et al., 2016), 2A (Giunta et al., 2018), 4A (Maccaferri et al., 2011; Milner et al., 2016) and on chromosome 6B (Maccaferri et al., 2011; Roncallo et al., 2018). In addition, affirming the finding of our study, QTLs on chromosomes 2A, 2B, 4B, 5B and 7B were also reported for this phenology trait (Zaïm et al., 2020). Maccaferri et al. (2015), using durum wheat elite cultivars reported one QTL on chromosome 4A out of the 43 QTLs associated with DH across the durum wheat genome except for chromosome 6A. Genomic regions on chromosomes 2A and 2B were reported to be associated with the major photoperiod sensitivity loci Ppd-A1 and Ppd-B1 (Arjona et al., 2018; Maccaferri et al., 2008, 2011). Notably, our study detected a QTL on chromosome 2A under FDS that is located very close to the position of Ppd-1A, but no QTL was detected in the vicinity of Ppd-B1. Ppd1genes affect the time of heading and other traits, and play an important role in modifying source-sink equilibrium, thus affecting wheat growth and development (Foulkes et al., 2004; Kamran et al., 2014; Würschum et al., 2018).

The duration between heading and maturity is an important yield component in wheat. Here, three QTLs were detected for DGF located on chromosomes 1B between 378,065,006–385,687,934 bp, on 3B between 732,882,447–747,134,223 bp, and on 7B between 543,578,199–552,021,963 bp. The three detected QTLs have not been reported before and thus represent new QTLs linked to DGF in durum wheat.

QTLs for DGF on chromosome 7B cluster with two or more traits, for example, SPS, TKW, and SPAD. This may suggest the linear relationship between DGF and the traits or may be due to pleiotropic effects (Bhoite et al., 2018). A total of six (three co-located and three individual) QTLs associated with DM were located on chromosomes 1A, 2B, 4A, 5B, and 7A. Interestingly, two QTLs on chromosomes 4A

and 7A were detected in the same intervals in Ethiopian durum wheat landraces and modern varieties on chromosome 4A (Kidane et al., 2017) and in Ethiopian durum wheat landraces on chromosome 7A (Mengistu et al., 2016). When compared with the GrainGenes database, the QTLs detected on chromosomes 1A, 2B, and 5B are likely to be novel QTLs associated with DM in durum wheat.

Plant height is frequently altered when water is limiting to overcome deleterious effects of drought (Arif et al., 2020). Five (co-located with other traits) QTLs were identified associated with PH located on chromosomes 1A, 2B, 6B, 7A, and 7B. The identified QTL on chromosome 6B was previously reported associated with PH in Ethiopian durum wheat landraces (Mengistu et al., 2016). Similarly, the QTL on chromosome 7B linked with PH is located within a previously reported QTL region for PH in Mediterranean durum wheat landraces (Soriano et al., 2017) and close to a QTL region identified in Elite durum cultivars (Maccaferri et al., 2011). Similarly, Mangini et al. (2021) reported QTLs associated with PH on chromosomes which we also identified, except on chromosome 1A. However, opposite to our findings, Zaïm et al. (2020) reported QTLs associated with PH on chromosomes 4A, 4B and 5B. The Green Revolution resulted in the release of short, high-yielding cultivars, which is mainly related to genes controlling PH in wheat. The introduction of semidwarf genes into bread wheat resulted in the replacement of tall cultivars with semidwarf cultivars with high response to inputs (e.g., fertilizers) and resistance to lodging. Thereby, a significant increase in yield was achieved in many national breeding programmes (Xynias et al., 2020). In agreement with our study, Chai et al. (2021) mentioned different alleles responsible for dwarfing genes in wheat located on chromosomes 2B, 7A and 7B. In the current study, out of two QTLs for PH under FDS, the one located on chromosome 1A showed a high reducing effect with 1.47% PVE.

SPAD values serve as a valuable indicator for the photosynthetic capacity of plants (Fiorentini et al., 2019; Lopes & Reynolds, 2012). A total of 12 (eight co-located and four individual) QTLs were identified for SPAD readings from flag leaves and were mapped on chromosomes 1A, 2A, 2B, 3A, 5B, 6B and 7B. A recent study reported QTLs for flag leaf chlorophyll content for the durum wheat genome but on chromosomes 1B and 3B (Huang et al., 2018). GWAS results also indicated QTLs linked to leaf chlorophyll content under drought stress located on chromosomes 1B, 2A, 2B, 3B, 6B and 7B in winter wheat seedlings (Maulana et al., 2020). These results highlight the potential of exploring QTLs associated with leaf chlorophyll content in durum wheat as a key factor for photosynthesis by which 80% of wheat yield is realized in canopy leaves (Ghosh et al., 2003; Hussain et al., 2015).

SL is a yield-related trait in wheat. Eight (six co-located with other traits DGF, PH, DM, GB, HI, SPAD, TKW and HI and two individual) QTLs were detected for SL on chromosomes 2B, 4A, 5A, 5B, 7A and 7B. All QTLs were detected under drought stress conditions except a QTL located on chromosome 7B. Similarly, Hu et al. (2015) identified eight QTLs associated with the length of the main spike in durum wheat located on chromosomes 1B, 2B, 4A, 5A, 5B, 7A and 7B (Hu et al., 2015). The QTL detected on chromosome 7B is located in a QTL region reported by Thanh et al. (2013). There was no QTL for SL reported yet on chromosome 2B suggesting this QTL is novel. Six (five

co-located with DGF, GB, HI, SPAD, and TKW and one individual) QTLs were detected associated with SPS located on chromosomes 2B, 3B, 6A, 6B and 7A, of which five were reported earlier (Giunta et al., 2018; Mangini et al., 2018; Mengistu et al., 2016; Roncallo et al., 2018). One of the QTLs on 6B linked to SPS was reported here for the first time. Four of the six QTLs detected for SPS were detected under FDS and CCDS with a positive effect on the trait. Interestingly, these QTLs identified under drought stresses were also identified for traits such as DGF, PH, TKW and SPAD.

HI is an important trait directly associated with yield. Twelve QTLs were detected for HI located on chromosomes 1A, 1B, 2A, 2B, 3A, 5A, 6B and 7A. Recently, a study on association mapping of QTLs for yield and yield-related traits revealed QTLs associated with HI on chromosomes 1B, 2B, 3B, 4B, 5B, 7A and 7B (Arif et al., 2020). Similarly, in the current study, the two detected QTLs on chromosomes 2A and 6B were close to the reported QTL interval by Roncallo et al. (2018) and within the reported QTL interval on chromosome 6A (Peleg et al., 2009). QTLs for HI obtained in this study located on chromosomes 1A, 3A and 5A are reported for the first time in durum wheat. Three (two individual and one co-located with DH and HI) QTLs were detected associated with TKW located on chromosomes 2B, 3B and 4A. These three QTLs were also identified in multilocations in tetraploid wheat in segregating populations and germplasm collections for TKW (Mangini et al., 2018). The QTLs on chromosome 2B detected under CCDS and on 3B under CCNS and on 4A under FNS were found to be in close vicinity and within the already detected QTL intervals, respectively (Mangini et al., 2018). Recent studies also identified QTLs for TKW on chromosomes 1A, 1B, 2A, 3A, 4B, 5A, 5B and 6B in durum wheat (Giancaspro et al., 2019; Mangini et al., 2021), which we lack to identify in our study.

Despite the detection of significant positive correlations between climate chamber and field results for several traits, we did not observe common QTLs for any trait under the two environments (greenhouse and field). In our study, we mainly investigated polygenetic quantitative traits. This phenomenon has been explained as a quantitative trait being controlled by numerous genes, with each gene having a relatively small effect, and readily affected by environments (Zhang et al., 2020). Supported by our ANOVA results showing strongest effects for the environment, we concluded that the missing overlap of detected associated loci for a certain trait in the two environments may be explained by the effect of the environment leading to varying regulatory scenarios for the various traits under the two watering conditions. As a result, genetic selection for drought stress has to be conducted in the target environment and ideally may include the design of ideotypes for certain growth scenarios (Senapati & Semenov, 2019).

5 | CONCLUSIONS

The experimental setting revealed the impact of drought on the durum wheat SP. Traits such as DGF, SPAD, SPS, HI and TKW showed a strong and significant ($p < .0001$) correlation with GB under FDS. Heritability of the traits analysed varied between 48.2% for DM

and 89.11% for PH under FNS. Similarly, under FDS, heritability ranged between 42% for DM and 83.37% for SL. A significant ($p < .01$) positive correlation was detected between GB for FNS versus CCNS, as well as for FDS versus CCDS conditions. Similarly, significant ($p < .001$) positive correlations were observed between the same traits (DH, DM, DGF, PH and SL) for FDS versus CCDS and for FNS versus CCNS conditions. However, the correlation between HI was not significant for FDS versus CCDS and FNS versus CCNS conditions. GWAS is a powerful tool to pinpoint the association between traits and markers. Critical ($r^2 > .2$) LD decay value identified 70 QTLs, of which 50 QTLs were linked to several traits and where 20 QTLs were associated with any one of the traits under the drought treatments. Many of the QTLs were detected within or at close vicinity of previously reported QTL intervals, a fact highlighting the consistency of our study. In addition, we have identified several novel QTLs for some of the tested traits referring to the GrainGenes database for durum wheat and literature reports. QTLs with a positive effect size that were detected under drought stress conditions for GB and for traits co-located with GB may have high potential in increasing grain yield in durum wheat. These include, for example, QTL on 1A between 495694477 and 501944537 bp, on 3B between 416256124 and 430507900 bp, on 3B between 745357158 and 759608934 bp, on 4B between 593416763 and 605142497 bp and on 4B between 658785890 and 670511624 bp. We concluded that our SP is showing reasonable genetic evidence for adaptations under drought stress environments. In the future, genomic selection markers may be developed and validated for QTL markers with relevance for yield stability and yield improvement under drought stress conditions. While the environment and selected germplasm will strongly depend on the breeding strategy, the novel QTL markers identified in our study likely represent potential candidates for MAS in wheat breeding programmes for drought tolerance.

ACKNOWLEDGEMENTS

The authors wish to thank the Ethiopian Biodiversity Institute (EBI), Debre Zeit Agricultural Research Center (DZARC) and CIMMYT for sharing durum wheat accessions for the study panel. We would like to thank the technical staff of EIAR at respective experimental sites for providing experimental plots and assistance. We would also like to thank Julius Kühn Institute technical, greenhouse and laboratory staff for their guidance and support, particularly Dr. Heike Lehnert for her support in data analysis. Furthermore, we acknowledge the Federal Ministry of Food and Agriculture (Bundesministerium für Ernährung und Landwirtschaft BMEL) for funding of the project (FKZ 2813F501).

CONFLICT OF INTEREST

All authors have no conflicts of interest to declare that are relevant to the content of this article.

AUTHOR CONTRIBUTIONS

The research idea was developed by FO and GW. All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by KN, SS and GW. The

first draft of the manuscript was written by KN and amended by AM, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

Data are available within the article or its supporting information.

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REFERENCES

- Ahmad, M. A., Khan, S. H., Khan, A. S., Kazi, A. M., & Basra, S. M. A. (2014). Identification of QTLs for drought tolerance traits on wheat chromosome 2A using association mapping. *International Journal of Agriculture and Biology*, 16(2), 862–870. <https://doi.org/10.32404/rian.v6i2.3323>
- Alemu, T., Zegeye, H., Kassa, D., Asnake, D., Tafesse, S., & Asefa, A. (2019). *Wheat Production Concepts Validation and Assessment of Dissemination and Utilization Constraints*. Ethiopian Institute of Agricultural Research. Research report No 126
- Arif, A. R., Attaria, F., Shokat, A. S., Waheed, M. Q., Arif, A., & Börner, A. (2020). Mapping of QTLs associated with yield and yield related traits in durum wheat (*Triticum durum Desf.*) under irrigated and drought conditions. *International Journal of Molecular Sciences*, 21(7), 1–25. <https://doi.org/10.3390/ijms21072372>
- Arjona, J. M., Royo, C., Dreisigacker, S., Ammar, K., & Villegas, D. (2018). Effect of Ppd-A1 and Ppd-B1 allelic variants on grain number and thousand kernel weight of durum wheat and their impact on final grain yield. *Frontiers in Plant Science*, 9(888), 1–13. <https://doi.org/10.3389/fpls.2018.00888>
- Bai, X., Zhao, H., Huang, Y., Xie, W., Han, Z., Zhang, B., Guo, Z., Yang, L., Dong, H., Xue, W., Li, G., Hu, G., Hu, Y., & Xing, Y. (2016). Genome-wide association analysis reveals different genetic control in panicle architecture between indica and japonica rice. *Plant Genome*, 9, 1–10. <https://doi.org/10.3835/plantgenome2015.11.0115>
- Bates, D., Maechler, M., & Bolker, B. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1), 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Benjamini, Y., & Hochberg, Y. (1995). "Controlling the false discovery rate: A practical and powerful approach to multiple testing" (PDF). *Journal of the Royal Statistical Society, Series B*, 57(1), 289–300. <https://doi.org/10.1111/j.2517-6161.1995.tb02031.x>
- Bhatta, M., Morgounov, A., Belamkar, V., & Baenziger, P. S. (2018). Genome-wide association study reveals novel genomic regions for grain yield and yield-related traits in drought-stressed synthetic hexaploid wheat. *International Journal of Molecular Sciences*, 19(10), 1–10. <https://doi.org/10.3390/ijms19103011>
- Bhoite, R., Onyemaobi, I., Si, P., Siddique, K. H. M., & Yan, G. (2018). Identification and validation of QTL and their associated genes for pre-emergent metribuzin tolerance in hexaploid wheat (*Triticum aestivum* L.). *BMC Genetics*, 19(102), 1–11. <https://doi.org/10.1186/s12863-018-0690-z>
- Börner, A., Schumann, E., Fürste, A., Cöster, H., Leithold, B., Röder, M. S., & Weber, W. E. (2002). Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, 105, 921–936. <https://doi.org/10.1007/s00122-002-0994-1>
- Browning, B. L., & Browning, S. R. (2007). Efficient multilocus association mapping for whole genome association studies using localized haplotype clustering. *Genetic Epidemiology: The Official Publication of the International Genetic Epidemiology Society*, 31(5), 365–375. <https://doi.org/10.1002/gepi.20216>

- Chai, S., Yao, Q., Zhang, X., Xiao, X., Fan, X., Zeng, J., Sha, L., Kang, H., Zhang, H., Li, J., Zhou, Y., & Wang, Y. (2021). The semi-dwarfing gene Rht-dp from dwarf polish wheat (*Triticum polonicum* L.) is the "green revolution gene" Rht-B1b. *BMC Genomics*, 22(63), 1–15. <https://doi.org/10.1186/s12864-021-07367-x>
- Collard, B. C., & Mackill, J. D. (2008). Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B Biological Sciences*, 363(557–572), 557–572. <https://doi.org/10.1098/rstb.2007.2170>
- Daryanto, S., Wang, L., & Jacinthe, P. A. (2016). Global synthesis of drought effects on maize and wheat production. *PLoS ONE*, 11(5), e0156362. <https://doi.org/10.1371/journal.pone.0156362>
- del Pozo, A., Matus, I., Ruf, K., Castillo, D., Méndez-Espinoza, A. M., & Serret, M. D. (2019). Genetic advance of durum wheat under high yielding conditions: The case of Chile. *Agronomy*, 9(8), 1–14. <https://doi.org/10.3390/agronomy9080454>
- Falconer, D. S., & Mackay, T. F. (1996). *Introduction to Quantitative Genetics* (p. 464). Longman Group Ltd.
- Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics*, 164, 1567–1587. <https://doi.org/10.1093/genetics/164.4.1567>
- Falush, D., Stephens, M., & Pritchard, J. K. (2007). Inference of population structure using multilocus genotype data: Dominant markers and null alleles. *Molecular Ecology Notes*, 7(4), 574–578. <https://doi.org/10.1111/j.1471-8286.2007.01758.x>
- FAO-United Nations Food and Agricultural Organization. (2013) Wheat: Post-harvest operations: http://www.fao.org/fileadmin/user_upload/inpho/docs/Post_Harvest_Compndium_WHEAT.pdf Accessed on June 22, 2020.
- Fiorentini, M., Zenobi, S., Giorgini, E., Basili, D., Conti, C., Pro, C., Monaci, E., & Orsini, R. (2019). Nitrogen and chlorophyll status determination in durum wheat as influenced by fertilization and soil management: Preliminary results. *PLoS ONE*, 14(11), e0225126. <https://doi.org/10.1371/journal.pone.0225126>
- Fischer, R. A., & Maurer, R. (1978). Drought resistance in spring wheat cultivars. I. Grain yield response. *Australian Journal of Agricultural Research*, 29, 897–907. <https://doi.org/10.1071/AR9780897>
- Foulkes, M., Sylvester-Bradley, R., Worland, A., & Snape, J. (2004). Effects of a photoperiod response gene Ppd-D1 on yield potential and drought resistance in UK winter wheat. *Euphytica*, 135, 63–73. <https://doi.org/10.1023/B:EUPH.0000009542.06773.13>
- Fox, J. (2017). *Using the R Commander: A Point-and-Click Interface for R*. Chapman and Hall/CRC Press. <https://socialsciences.mcmaster.ca/jfox/Books/RCommander/>
- Gaetano, J. (2018). Holm-Bonferroni sequential correction: An Excel calculator-version 1.3.
- Ghosh, M., Pal, A. K., & Pal, S. K. (2003). Relationship of leaf area and chlorophyll content with yield of aromatic rice. *Indian Journal of Plant Physiology*, 8, 199–200.
- Giancaspro, A., Giove, S. L., Zacheo, S. A., Blanco, A., & Gadaleta, A. (2019). Genetic variation for protein content and yield-related traits in a durum population derived from an inter-specific cross between Hexaploid and tetraploid wheat cultivars. *Frontiers in Plant Science*, 10(1509), 1–13. <https://doi.org/10.3389/fpls.2019.01509>
- Giunta, F., De Vita, P., Mastrangelo, A. M., Sanna, G., & Motzo, R. (2018). Environmental and genetic variation for yield-related traits of durum wheat as affected by development. *Frontiers in Plant Science*, 9(00008), 1–19. <https://doi.org/10.3389/fpls.2018.00008>
- Graffelman, J. (2013). Linear-angle correlation plots: New graphs for revealing correlation structure. *Journal of Computational and Graphical Statistics*, 22(1), 92–106. <https://doi.org/10.1080/15533174.2012.707850>
- Gupta, P. K., Balyan, H. S., & Gahlaut, V. (2017). QTL analysis for drought tolerance in wheat: Present status and future possibilities. *Agronomy*, 7(5), 1–21. <https://doi.org/10.3390/agronomy7010005>
- Gupta, P. K., Balyan, H. S., Sharma, S., & Kumar, R. (2020). Review: Genetics of yield, abiotic stress tolerance and biofortification in wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, 133(5). <https://doi.org/10.1007/s00122-020-03583-3>
- Holm, S. (1979). A simple sequential rejective method procedure. *Scandinavian Journal of Statistics*, 6(2), 65–70.
- Hu, X., Ren, J., Ren, X., Huang, S., Sabiel, S. A. I., Luo, M., Nevo, E., Fu, C., Peng, J., & Sun, D. (2015). Association of agronomic traits with SNP markers in durum wheat (*Triticum turgidum* L. durum (Desf.)). *PLoS ONE*, 10(6), e0130854. <https://doi.org/10.1371/journal.pone.0130854>
- Huang, S., Sun, L., Hu, X., Wang, Y., Zhang, Y., Nevo, E., Peng, J., & Sun, D. (2018). Associations of canopy leaf traits with SNP markers in durum wheat (*Triticum turgidum* L. durum (Desf.)). *PLoS ONE*, 13(10), e0206226. <https://doi.org/10.1371/journal.pone.0206226>
- Hubisz, M. J., Falush, D., Stephens, M., & Pritchard, J. K. (2009). Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*, 9(5), 1322–1332. <https://doi.org/10.1111/j.1755-0998.2009.02591.x>
- Hussain, S., Ahmad, M., Iqbal, J., Qasim, G., Ali, A., Abbas, G., Mahpara, S., Khan Khattak, J. Z. (2015). Quantification of wheat (*Triticum Aestivum* L.) leaves contribution to grain yield through defoliation technique. *Canadian Journal of Bioinformatics & Biosciences* 2015, 1(15), 23–35.
- International Wheat Genome Sequencing Consortium. (2018). Shifting the limits in wheat research and breeding using a fully annotated reference genome. *Science*, 361(6403), 1–13. <https://doi.org/10.1126/science.aar7191>
- Jin, J., Liu, D., Qi, Y., Ma, J., & Zhen, W. (2020). Major QTL for seven yield-related traits in common wheat (*Triticum aestivum* L.). *Frontiers in Genetics*, 11(01012). 1–11. <https://doi.org/10.3389/fgene.2020.01012>
- Kabbaj, H., Sall, A. T., Al-Abdallat, A., Geleta, M., Amri, A., Filali-Maltouf, A., Belkadi, B., Ortiz, R., & Bassi, F. M. (2017). Genetic diversity within a global panel of durum wheat (*Triticum durum*) landraces and modern germplasm reveals the history of alleles exchange. *Frontiers in Plant Science*, 8(01277), 1–13. <https://doi.org/10.3389/fpls.2017.01277>
- Kamran, A., Iqbal, M., & Spaner, D. (2014). Flowering time in wheat (*Triticum aestivum* L.): A key factor for global adaptability. *Euphytica*, 197, 1–26.
- Kidane, Y. G., Mancini, C., Mengistu, D. K., Frascaroli, E., Fadda, C., Pè, M. E., & Dell'Acqua, M. (2017). Genome wide association study to identify the genetic base of smallholder farmer preferences of durum wheat traits. *Frontiers in Plant Science*, 8(01230). 1–11. <https://doi.org/10.3389/fpls.2017.01230>
- Klindworth, D. L., Miller, J. D., Jin, Y., & Xu, S. S. (2007). Chromosomal locations of genes for stem rust resistance in monogenic lines derived from tetraploid wheat accession ST464. *Crop Science*, 47(4), 1441–1450. <https://doi.org/10.2135/cropsci2006.05.0345>
- Laidò, G., Marone, D., Russo, M. A., Colecchia, S. A., Mastrangelo, A. M., De Vita, P., & Papa, R. (2014). Linkage disequilibrium and genome-wide association mapping in tetraploid wheat (*Triticum turgidum* L.). *PLoS ONE*, 9(4), e95211. <https://doi.org/10.1371/journal.pone.0095211>
- Lipka, A. E., Tian, F., Wang, Q., Peiffer, J., Li, M., Bradbury, P. J., Gore, M. A., Buckler, E. S., & Zhang, Z. (2012). GAPIT: Genome association and prediction integrated tool. *Bioinformatics*, 28(18), 2397–2399. <https://doi.org/10.1093/bioinformatics/bts444>
- Liu, H. J., & Yan, J. (2019). Crop genome-wide association study: A harvest of biological relevance. *The Plant Journal*, 97, 8–18. <https://doi.org/10.1111/tpj.14139>
- Liu, K., Xu, H., Liu, G., Guan, P., Zhou, X., Peng, H., Yao, Y., Ni, Z., Sun, Q., & du, J. (2018). QTL mapping of flag leaf-related traits in wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, 131, 839–849. <https://doi.org/10.1007/s00122-017-3040-z>
- Liu, X., Huang, M., Fan, B., Buckler, E. S., & Zhang, Z. (2016). Iterative usage of fixed and random effect models for powerful and efficient

- genome-wide association studies. *PLOS Genetics*, 12(2), e1005767. <https://doi.org/10.1371/journal.pgen.1005767>
- Lopes, M. S., & Reynolds, M. P. (2012). Stay-green in spring wheat can be determined by spectral reflectance measurements (normalized difference vegetation index) independently from phenology. *Journal of Experimental Botany*, 63(10), 3789–3798. <https://doi.org/10.1093/jxb/ers071>
- Lopes, S. M., el-Basyoni, I., Baenziger, P. S., Singh, S., Royo, C., Ozbek, K., Aktas, H., Ozer, E., Ozdemir, F., Manickavelu, A., Ban, T., & Vikram, P. (2015). Exploiting genetic diversity from landraces in wheat breeding for adaptation to climate change. *Journal of Experimental Botany*, 66, 3625–3638. <https://doi.org/10.1093/jxb/erv122>
- Ma, J., Zhang, H., Li, S., Zou, Y., Li, T., Liu, J., Ding, P., Mu, Y., Tang, H., Deng, M., Liu, Y., Jiang, Q., Chen, G., Kang, H., Li, W., Pu, Z., Wei, Y., Zheng, Y., & Lan, X. (2019). Identification of quantitative trait loci for kernel traits in a wheat cultivar Chuannong16. *BMC Genetics*, 20(77), 1–12. <https://doi.org/10.1186/s12863-019-0782-4>
- Ma, X. S., Feng, F. J., Wei, H. B., Mei, H. W., Xu, K., Chen, S. J., Li, T., Liang, X., Liu, H., & Luo, L. (2016). Genome-wide association study for plant height and grain yield in rice under contrasting moisture regimes. *Frontiers in Plant Science*, 7(01801), 1–13. <https://doi.org/10.3389/fpls.2016.01801>
- Maccaferri, M., Harris, N. S., Twardziok, S. O., Pasam, R. K., Gundlach, H., Spannagl, M., Ormanbekova, D., Lux, T., Prade, V. M., Milner, S. G., Himmelbach, A., Mascher, M., Bagnaresi, P., Faccioli, P., Cozzi, P., Lauria, M., Lazzari, B., Stella, A., Manconi, A., ... Cattivelli, L. (2019). Durum wheat genome s past domestication signatures and future improvement targets. *Nature Genetics*, 51(5), 885–895. <https://doi.org/10.1038/s41588-019-0381-3>
- Maccaferri, M., Ricci, A., Salvi, S., Milner, S. G., Noli, E., Martelli, P. L., Casadio, R., Akhunov, E., Scalabrin, S., Vendramin, V., Ammar, K., Blanco, A., Desiderio, F., Distelfeld, A., Dubcovsky, J., Fahima, T., Faris, J., Korol, A., Massi, A., ... Tuberosa, R. (2015). A high-density, SNP-based consensus map of tetraploid wheat as a bridge to integrated durum and bread wheat genomics and breeding. *Plant Biotechnology Journal*, 13, 648–663. <https://doi.org/10.1111/pbi.12288>
- Maccaferri, M., Sanguineti, M. C., Corneti, S., Ortega, J. L. A., Salem, M. B., Bort, J., DeAmbrogio, E., Del Moral, L., Demontis, A., el-Ahmed, A., Maalouf, F., Machlab, H., Martos, V., Moragues, M., Motawaj, J., Nachit, M., Nserallah, N., Ouabbou, H., Royo, C., ... Tuberosa, R. (2008). Quantitative trait loci for grain yield and adaptation of durum wheat (*Triticum durum* Desf.) across a wide range of water availability. *Genetics*, 178, 489–511. <https://doi.org/10.1534/genetics.107.077297>
- Maccaferri, M., Sanguineti, M. C., Demontis, A., El-Ahmed, A., Del Moral, L. G., Maalouf, F., Nachit, M., Nserallah, N., Ouabbou, H., Rhouma, S., Royo, C., Villegas, D., & Tuberosa, R. (2011). Association mapping in durum wheat grown across a broad range of water regimes. *Journal of Experimental Botany*, 62, 409–438. <https://doi.org/10.1093/jxb/erq287>
- Mangini, G., Blanco, A., Nigro, D., Signorile, M. A., & Simeone, R. (2021). Candidate genes and quantitative trait loci for grain yield and seed size in durum wheat. *Plants*, 10(312), 1–17. <https://doi.org/10.3390/plants10020312>
- Mangini, G., Gadaleta, A., Colasuonno, P., Marcotuli, I., Signorile, A. M., Simeone, R., De Vita, P., Mastrangelo, A. M., Laidò, G., Pecchioni, N., & Blanco, A. (2018). Genetic dissection of the relationships between grain yield components by genome-wide association mapping in a collection of tetraploid wheats. *PLoS ONE*, 13(1), e0190162. <https://doi.org/10.1371/journal.pone.0190162>
- Mann, M., & Warner, J. (2015). Ethiopian wheat yield and yield gap estimation: A Small area integrated data approach. In *Research for Ethiopia's Agricultural Policy*. Addis Ababa.
- Mapfumo, P., Onyango, M., Honkonou, S. K., El, M. E. H., Githeko, A., Rabeharisoa, L., Obando, K., Omolo, N., Majule, A., Denton, F., Ayers, J., & Agrawal, A. (2017). Pathways to transformational change in the face of climate impacts: An analytical framework. *Climate and Development*, 9(5), 439–451. <https://doi.org/10.1080/17565529.2015.1040365>
- Marcotuli, I., Gadaleta, A., Mangini, G., Signorile, A. M., Zacheo, S. A., Blanco, A., Simeone, R., & Colasuonno, P. (2017). Development of a High-Density SNP-Based Linkage Map and Detection of QTL for β -Glucans, Protein Content, Grain Yield per Spike and Heading Time in Durum Wheat. *International Journal of Molecular Sciences*, 18(6), 1329. <https://doi.org/10.3390/ijms18061329>
- Maulana, F., Huang, W., Anderson, J. D., & Ma, X.-F. (2020). Genome-wide association mapping of seedling drought tolerance in winter wheat. *Frontiers in Plant Science*, 11, 573786. <https://doi.org/10.3389/fpls.2020.573786>
- Mengistu, D. K., Kidane, Y. G., Catellani, M., Frascaroli, E., Fadda, C., Pè, M. E., & Dell'Acqua, M. (2016). High-density molecular characterization and association mapping in Ethiopian durum wheat landraces reveals high diversity and potential for wheat breeding. *Plant Biotechnology Journal*, 14, 1800–1812. <https://doi.org/10.1111/pbi.12538>
- Millet, E. J., Welcker, C., Kruijer, W., Negro, S., Coupel-Ledru, A., Nicolas, S. D., Laborde, J., Bauland, C., Praud, S., Ranc, N., Presterl, T., Tuberosa, R., Bedo, Z., Draye, X., Usadel, B., Charcosset, A., Van Eeuwijk, F., & Tardieu, F. (2016). Genome-wide analysis of yield in Europe: Allelic effects as functions of drought and heat scenarios. *Plant Physiology*, 172, 749–764. <https://doi.org/10.1104/pp.16.00621>
- Milner, S. G., Maccaferri, M., Huang, B. E., Mantovani, P., Massi, A., Frascaroli, E., & Salvi, S. (2016). A multiparental cross population for mapping QTL for agronomic traits in durum wheat (*Triticum turgidum* ssp. durum). *Plant Biotechnology Journal*, 14, 735–748. <https://doi.org/10.1111/pbi.12424>
- Mohammadi, R., Etmnan, A., & Shoshtari, L. (2018). Agro-physiological characterization of durum wheat genotypes under drought conditions. *Cambridge University Press*, 55(3), 484–499. <https://doi.org/10.1017/S0014479718000133>
- Mwadzingeni, L., Shimelis, H., Rees, D. J. G., & Tsilo, T. J. (2017). Genome-wide association analysis of agronomic traits in wheat under drought-stressed and non-stressed conditions. *PLoS ONE*, 12, e0171692. <https://doi.org/10.1371/journal.pone.0171692>
- Nakashima, K., Yamaguchi-Shinozaki, K., & Shinozaki, K. (2014). The transcriptional regulatory network in the drought response and its crosstalk in abiotic stress responses including drought, cold, and heat. *Frontiers in Plant Science*, 5(170), 1–7. <https://doi.org/10.3389/fpls.2014.00170>
- Negassa, A., Koo, J., Sonder, K., Shiferaw, B., Smale, M., Braun, H. J., Gbgebegbe, S., Guo, Z., Hodson, D. P., Wood, S., Payne, T. S., & Geleta, A. B. (2012). The potential for wheat production in sub-Saharan Africa: analysis of biophysical suitability and economic profitability. In *International Maize and Wheat Improvement Center (CIMMYT)*. Addis Ababa.
- Negisho, K., Shibru, S., Pillen, K., Ordon, F., & Wehner, G. (2021). Genetic diversity of Ethiopian durum wheat landraces. *PLoS ONE*, 16(2), e0247016. <https://doi.org/10.1371/journal.pone.0247016>
- Negro, S. S., Millet, E. J., Madur, D., & Nicolas, S. D. (2019). Genotyping-by-sequencing and SNP-arrays are complementary for detecting quantitative trait loci by tagging different haplotypes in association studies. *BMC Plant Biology*, 19(318), 1–22. <https://doi.org/10.1186/s12870-019-1926-4>
- Nezhadahmadi, A., Prodhon, Z. H., & Faruq, G. (2013). Drought tolerance in wheat. *Science World Journal*, 2013(610721), 1–12. <https://doi.org/10.1155/2013/610721>
- Oyiga, B. C., Sharma, R. C., Baum, M., Ogonnaya, F. C., Léon, J., & Ballvora, A. (2017). Allelic variations and differential expressions detected at quantitative trait loci for salt stress tolerance in wheat. *Plant Cell and Environment*, 41, 919–935. <https://doi.org/10.1111/pce.12898>

- Patil, R. M., Tamhankar, S. A., Oak, M. D., Raut, A. L., Honrao, B. K., Rao, V. S., & Misra, S. C. (2013). Mapping of QTL for agronomic traits and kernel characters in durum wheat (*Triticum durum* Desf.). *Euphytica*, 190, 117–129. <https://doi.org/10.1007/s10681-012-0785-y>
- Peleg, Z., Fahima, T., Krugman, T., Abbo, S., Yakir, D., Korol, A. B., & Saranga, Y. (2009). Genomic dissection of drought resistance in durum wheat X wild emmer wheat recombinant inbred line population. *Plant, Cell and Environment*, 32(7), 758–779. <https://doi.org/10.1111/j.1365-3040.2009.01956.x>
- Pour-Aboughadareh, A., Mohammadi, R., Etmnan, A., Shoostari, L., Maleki-Tabrizi, N., & Poccai, P. (2020). Effects of drought stress on some agronomic and morpho-physiological traits in durum wheat genotypes. *Sustainability*, 12(5610), 1–14. <https://doi.org/10.3390/su12145610>
- Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–959. <https://doi.org/10.1093/genetics/155.2.945>
- Qaseem, M. F., Qureshi, R., Illyas, N., & Jalal-Ud-Din Shabbir, G. (2017). Multivariate statistical analysis for yield and yield components in bread wheat plants under rainfed conditions. *Pakistan Journal of Botany*, 49(6), 2445–2450.
- Qaseem, M. F., Qureshi, R., Shaheen, H., & Shafiqat, N. (2019). Genome-wide association analyses for yield and yield-related traits in bread wheat (*Triticum aestivum* L.) under pre-anthesis combined heat and drought stress in field conditions. *PLoS ONE*, 14(3), e0213407. <https://doi.org/10.1371/journal.pone.0213407>
- R Core Team. (2014). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. <http://www.R-project.org/>
- R Development Core Team. (2019). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. <http://www.R-project.org/>
- Roncallo, P. F., Akkiraju, P. C., Cervigni, G. L., & Echenique, V. C. (2018). QTL mapping and analysis of epistatic interactions for grain yield and yield-related traits in *Triticum turgidum* L. var. durum. *Euphytica*, 57, 2627–2637. <https://doi.org/10.1007/s10681-017-2058-2>
- Russell, V. L. (2016). Least-squares means: The R package lsmeans. *Journal of Statistical Software*, 69(1), 1–33. <https://doi.org/10.18637/jss.v069.i01>
- Sall, A. T., Chiari, T., Legesse, W., Ahmed, S., Ortiz, R., van Ginkel, M., & Bassi, F. M. (2019). Durum wheat (*Triticum durum* Desf.) origin, cultivation, and potential expansion in sub-Saharan Africa. [https://doi.org/10.20944/preprints04.0149.v19\(263\)](https://doi.org/10.20944/preprints04.0149.v19(263))
- Senapati, N., & Semenov, M. A. (2019). Assessing yield gap in high productive countries by designing wheat ideotypes. *Scientific Reports*, 9(5516), 1–12. <https://doi.org/10.1038/s41598-019-40981-0>
- Shavrukov, Y., Kurishbayev, A., Jatayev, S., Shvidchenko, V., Zotova, L., Koekemoer, F., De Groot, S., Soole, K., & Langridge, P. (2017). Early flowering as a drought escape mechanism in plants: How can it aid wheat production? *Frontiers in Plant Science*, 8(01950), 1–8. <https://doi.org/10.3389/fpls.2017.01950>
- Shin, J. H., Blay, S., McNeney, B., & Graham, J. (2006). LDheatmap: An R functions for graphical display of pairwise linkage disequilibria between single nucleotide polymorphisms. *Journal of Statistical Software*, 16, 1–10. <https://doi.org/10.18637/jss.v016.c03>
- Siol, M., Jacquin, F., Chabert-Martinello, M., Smýkal, P., Le Paslier, M.-C., Aubert, G., & Burstin, J. (2017). Patterns of genetic structure and linkage disequilibrium in a large collection of pea germplasm. *G3 Genes|Genetics*, 7(8), 2461–2471. <https://doi.org/10.1534/g3.117.043471>
- Soriano, J. M., Malosetti, M., Rosello, M., Sorrells, M. E., & Royo, C. (2017). Dissecting the old Mediterranean durum wheat genetic architecture for phenology, biomass and yield formation by association mapping and QTL meta-analysis. *PLoS ONE*, 12(5), e0178290. <https://doi.org/10.1371/journal.pone.0178290>
- Sukumaran, S., Reynolds, M. P., & Sansaloni, C. (2018). Genome-wide association analyses identify QTL hotspots for yield and component traits in durum wheat grown under yield potential, drought, and heat stress environments. *Frontiers in Plant Science*, 9(81), 1–16. <https://doi.org/10.3389/fpls.2018.00081>
- Taffesse, A., Dorosh, P., & Asrat, S. (2011). *Crop Production in Ethiopia: Regional Patterns and Trends ESSP II Working Paper No. 0016*. Addis Ababa.
- Taranto, F., D'Agostino, N., Rodriguez, M., Pavan, S., Minervini, A. P., Pecchioni, N., Papa, R., & De Vita, P. (2020). Whole genome scan reveals molecular signatures of divergence and selection related to important traits in durum wheat germplasm. *Frontiers in Genetics*, 11(217). <https://doi.org/10.3389/fgene.2020.00217>
- Teslovich, T. M., Musunuru, K., Smith, A. V., Edmondson, A. C., Stylianou, I. M., Koseki, M., Pirruccello, J. P., Ripatti, S., Chasman, D. I., Willer, C. J., Johansen, C. T., Fouchier, S. W., Isaacs, A., Peloso, G. M., Barbalic, M., Ricketts, S. L., Bis, J. C., Aulchenko, Y. S., Thorleifsson, G., ... Kathiresan, S. (2010). Biological, clinical and population relevance of 95 loci for blood lipids. *Nature*, 466, 707–713. <https://doi.org/10.1038/nature09270>
- Thanh, P. T., Vladutu, C. I., Kianian, S. F., Thanh, P. T., Ishii, T., Nitta, M., Nasuda, S., & Mori, N. (2013). Molecular genetic analysis of domestication traits in emmer wheat. I: Map construction and QTL analysis using an F2 population. *Biotechnology*, 27, 3627–3637.
- Tricker, P. J., ElHabti, A., & Fleury Schmidt, J. D. (2018). The physiological and genetic basis of combined drought and heat tolerance wheat. *Journal of Experimental Botany*, 69(13), 3195–3210. <https://doi.org/10.1093/jxb/ery081>
- Ullah, N., Christopher, J., Frederiks, T., Ma, S., Tan, D. K. Y., & Chenu, K. (2021). A robust field-based method to screen heat tolerance in wheat. *bioRxiv*. <https://doi.org/10.1101/2021.06.09.447803>
- Vargas-Reeve, F., Mora, F., Perret, S., & Scapim, C. A. (2013). Heritability of stem straightness and genetic correlations in Eucalyptus cladocalyx in the semi-arid region of Chile. *Crop Breeding and Applied Biotechnology*, 13, 107–112. <https://doi.org/10.1590/S1984-70332013000200002>
- Voorrips, R. E. (2002). MapChart: Software for the graphical presentation of linkage maps and QTLs. *The Journal of Heredity*, 93(1), 77–78. <https://doi.org/10.1093/jhered/93.1.77>
- Vos, P. G., Paulo, M. J., Voorrip, R. E., Visser, R. G. F., van Eck, H. J., & Van Eeuwijk, F. A. (2017). Evaluation of LD decay and various LD-decay estimators in simulated and SNP-array data of tetraploid potato. *Theoretical and Applied Genetics*, 130, 123–135. <https://doi.org/10.1007/s00122-016-2798-8>
- Voss-Fels, K., Frisch, M., Qian, L., Kontowski, S., Friedt, W., Gottwald, S., & Snowden, R. J. (2015). Sub genomic diversity patterns caused by directional selection in bread wheat gene pools. *Plant Genome*, 8, 1–13. <https://doi.org/10.3835/plantgenome2015.03.0013>
- Wang, S., Wong, D., Forrest, K., Allen, A., Chao, S., Huang, B. E., Maccaferri, M., Salvi, S., Milner, S. G., Cattivelli, L., Mastrangelo, A. M., Whan, A., Stephen, S., Barker, G., Wieseke, R., Pleske, J., International Wheat Genome Sequencing Consortium, Lillemo, M., Mather, D., ... Akhunov, E. (2014). Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. *Plant Biotechnology Journal*, 12, 787–796. <https://doi.org/10.1111/pbi.12183>
- Wang, S., Xu, S., Chao, S., Sun, Q., Liu, S., & Xia, G. (2019). A genome-wide association study of highly heritable agronomic traits in durum wheat. *Frontier in Plant Sciences*, 10(00919), 1–13. <https://doi.org/10.3389/fpls.2019.00919>
- Warnes, G. (2013). *Genetics: population genetics*. R Package Version 1.3.8.1. <https://cran.r-project.org/package=genetics>

- Wehner, G., Balko, C., & Ordon, F. F. (2016). Experimental design to determine drought stress response and early leaf senescence in barley (*Hordeum vulgare* L.). *Bio-Protocol*, 6(5), 1–17. <https://doi.org/10.21769/BioProtoc.1749>
- Wehner, G. G., Balko, C. C., Enders, M. M., Humbeck, K. K., & Ordon, F. F. (2015). Identification of genomic regions involved in tolerance to drought stress and drought stress induced leaf senescence in juvenile barley. *BMC Plant Biology*, 15(1), 1–15. <https://doi.org/10.1186/s12870-015-0524-3>
- Würschum, T., Langer, S. M., Longin, C. F. H., Tucker, M. R., & Leiser, W. L. (2018). A three-component system incorporating Ppd-D1, copy number variation at Ppd-B1, and numerous small-effect QTL facilitates adaptation of heading time in winter wheat cultivars of worldwide origin. *Plant Cell & Environment*, 41, 1407–1416. <https://doi.org/10.1111/pce.13167>
- Xu, Y., Li, P., Yang, Z., & Xu, C. (2017). Genetic mapping of quantitative trait loci in crops. *The Crop Journal*, 5(5), 646–650. <https://doi.org/10.1016/j.cj.2016.06.003>
- Xynias, I. N., Mylonas, I., Korpatis, E. G., Ninou, E., Tsaballa, A., Avdikos, I. D., & Mavromatis, A. G. (2020). Review: Durum wheat breeding in the Mediterranean region: Current status and future prospects. *Agronomy*, 10(432), 1–27. <https://doi.org/10.3390/agronomy10030432>
- Yu, J., Pressoir, G., Briggs, W. H., Vroh Bi, I., Yamasaki, M., Doebley, J. F., McMullen, M. D., Gaut, B. S., Nielsen, D. M., Holland, J. B., Kresovich, S., & Buckler, E. S. (2006). A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nature Genetics*, 38, 203–208. <https://doi.org/10.1038/ng1702>
- Zadoks, J. C., Chang, T. T., & Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed Research*, 14, 415–421. <https://doi.org/10.1111/j.1365-3180.1974.tb01084.x>
- Zaïm, M., Kabbaj, H., Kehel, Z., Gorjanc, G., Filali-Maltouf, A., Belkadi, B., Nachit, M. M., & Bassi, F. M. (2020). Combining QTL analysis and genomic predictions for four durum wheat populations under drought conditions. *Frontiers. Genetics*, 11(00316), 1–15. <https://doi.org/10.3389/fgene.2020.00316>
- Zampieri, M., Ceglar, A., Dentener, F., & Toreti, A. (2017). Wheat yield loss attributable to heatwaves, drought, and water excess at the global, national and subnational scales. *Environmental Research Letters*, 12, 064008. <https://doi.org/10.1088/1748-9326/aa723b>
- Zhang, J., Zhang, S., Cheng, M., Jiang, H., Zhang, X., Peng, C., Lu, X., Zhang, M., & Jin, J. (2018). Effect of drought on agronomic traits of Rice and wheat: A Meta-analysis. *International Journal of Environmental Research and Public Health*, 15(839), 1–14. <https://doi.org/10.3390/ijerph15050839>
- Zhang, M., Liu, Y. H., Xu, W., Smith, C. W., Murray, S. C., & Zhang, H. B. (2020). Analysis of the genes controlling three quantitative traits in three diverse plant species reveals the molecular basis of quantitative traits. *Scientific Reports*, 10(10074), 1–14. <https://doi.org/10.1038/s41598-020-66271-8>
- Zhu, C., Gore, M. A., Buckler, E. S., & Yu, J. (2008). Status and prospects of association mapping in plants. *The plant. Genome*, 1(1), 1–16. <https://doi.org/10.3835/plantgenome2008.02.0089>

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How to cite this article: Negisho, K., Shibru, S., Matros, A., Pillen, K., Ordon, F., & Wehner, G. (2022). Genomic dissection reveals QTLs for grain biomass and correlated traits under drought stress in Ethiopian durum wheat (*Triticum turgidum* ssp. *durum*). *Plant Breeding*, 1–17. <https://doi.org/10.1111/pbr.13010>