

Genome Wide Association Study of Yield and Yield-Related Traits in Elite Spring Bread Wheat Genotypes Grown under High Temperature Environment in Sudan

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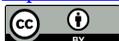
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Abstract

Genetic improvement of wheat for heat tolerance is the main breeding objective in Sudan as it is the principal limiting factor for wheat production. To this end, the availability of wheat genetic diversity is key for success. Genetic diversities and genome-wide association studies were conducted to parse the complex genetic composition of yield-related traits of 250 bread wheat genotypes which were selected from ICARDA's advanced materials. The genotypes were evaluated under heat stress environment for two seasons (2016/17 and 2017/18) in two sowing dates (optimum and late) at the Gezira Research Farm (GRF), of the Agricultural Research Corporation (ARC), Wad Medani, Sudan. Yield levels ranged from 4016 - 5120 kg/ha for optimum and late sowing dates respectively in the first season and from 5386 - 3926 kg/ha for optimum and late sowing dates respectively in the second season. The top 20 genotypes were better than the best check in the two seasons except, Imam which ranked number 18 in the second season. Two genotypes, genotypes 27 and 37, produced high grain yield in both seasons. Wide ranges of variations among genotypes were found for yield and yield-related traits, including days to heading and maturity, plant height, number of seeds/spike, 1000 seed weight, biomass and harvest index. Genome wide association analyses were performed using 15 k SNP markers and the phenotypic data. A total of 282, 355 and 475 significant ($P < 0.05$) markers associated with grain yield were observed in the first season, second season and across seasons, respectively. Markers (6B/Kukri_c27662, 6B/.BobWhite_c23771_525, 6B/Excalibur_c32219_491, 6B/RAC875_c31299_13021302, 6B/BS00046264_51,

6A/wsnp_Ex_rep_c69373_68312188, 6A/Tdurum contig12123_1650, 6BKukri_c27662_675 and 6A/BobWhite_c15802_72) on chromosomes 6B and 6A for grain yield were highly significantly associated and stable in every season and across seasons. Highly significant Marker-traits associations (MTAs) were observed for many traits including days to heading and maturity, plant height, number of seeds/spike, 1000 seed weight, biomass and harvest index. The currently identified MTAs should be further validated using other elite sets of genotypes so that they can be used for MAS in the breeding program. The high yielding and heat tolerant genotypes identified in this study will be evaluated across key locations in Sudan for potential release in Sudan. Furthermore, they will be used as parents in the breeding program for heat tolerance.

Keywords

Wheat, High Temperature, Tolerance, Genome, Chromosome, Marker, Phenotyping and Genotyping

1. Introduction

Heat stress caused by high temperature is one of the most important wheat yield limiting factor affecting more than 30 million hectares of wheat production area annually in the world and thereby causing significant grain yield reduction [1]. The effect of high temperature is even expected to be more detrimental stress in the future because of a rise in global temperature by 1°C - 4.5°C over the next 50 years associated with climate change [2]. In addition to lowering grain yield, the effect of climate change is also evident in the quality of wheat as increased heat results in shriveled wheat grains [3]. Heat stress can cause partial or total breakdown of anatomy, morphology, biochemistry and physiology of the crop. It is a function of the magnitude and rate of temperature increase, as well as the duration of exposure to the raised temperature [4]. Genetic variabilities for heat stress have been previously reported [5]. Association mapping using phenotypic and genotypic data of association panels has become an important approach in identifying molecular markers linked to traits of interest for potential use in marker selection. Its advantage over the more conventional bi-parental mapping approach lies mainly in the greater extent of allelic variation which can be surveyed, while also avoiding the need to establish a customized mapping population [6]. Association mapping or genome-wide association studies (GWAS) may be a suitable complementary approach to bi-parental populations for identifying genetic markers associated with traits of interest [7]. Compared to other marker types, single nucleotide polymorphisms (SNPs) are generally more abundant, stable, efficient, high-res genetic maps and genomic selection and cost effective [8]. The objectives of this study were to 1) Genetic diversities of 250 bread wheat genotypes and identify heat tolerant genotypes; 2) Identify significant MTAs and

QTLs for heat tolerance in Sudan; 3) Select the best high yielding and heat tolerant genotypes.

2. Materials and Methods

2.1. Phenotyping

2.1.1. Experimental Site

The experiments were conducted in two seasons (2016/17 and 2017/18) using two dates for planting; first sowing date (1st SD) on 20 November (optimum) and second sowing date (2nd SD) on 12 December (late) at the Gezira Research Station Farm (GRSF) of the Agricultural Research Corporation (ARC), Wad Medani, Sudan. (GRSF) is located at latitude of 14°-24'N and longitude of 29°-33'E and altitude of 407 masl. The soil at this station is classified as heavy clay soil, pH of about 8.0 - 8.4, low organic matter (0.05), deficient in nitrogen (380 ppm), and phosphorus (ESP, 4 ppm).

2.1.2. Plant Materials and Experimental Design

Experiments were conducted using 250 genotypes selected from different spring bread wheat advanced yield trials introduction from ICARDA, including three checks (namely Imam, Goumria and Nebta). Experiments were arranged in an alpha lattice design with two replications. Plots consisted of four rows, 3 m long and 0.2 m apart. Seeds were sown at the rate of 120 kg/ha.

2.1.3. Cultural Practices

The recommended dose of fertilizer (43 kg P₂O₅/ha) was applied prior to sowing and 86 kg N/ha as urea was applied with the second and fourth irrigations. The experiments were irrigated (flood irrigation) at 10 - 12 days interval for a total of 9 irrigations.

2.1.4. Measurements and Statistical Analysis

Data were recorded for days to heading and maturity, plant height, number of spikes/m², number of seeds/spike, 1000 seed weight (gm), biomass (kg/ha), harvest index and grain yield (kg/ha). The phenotypic data were statistically analyzed using Genstat software version 18.

2.1.5. Metrological Data

In the first season, the temperature in the 1st SD was higher than the 2nd SD, while in the second season it was the opposite (**Figure 1**). In the first season, the mean temperature in the first sowing date at the critical period of growth (from 20 December -20 January) was higher than the second sowing date at the critical period (from end of January - end of February). In the first season, the maximum and minimum temperatures were lower by 3°C in the second sowing date respectively when compared to the first sowing date of the critical stage of crop growth, while in the second season, maximum and minimum temperatures were lower by 5°C in the first sowing date when compared to the second sowing date.

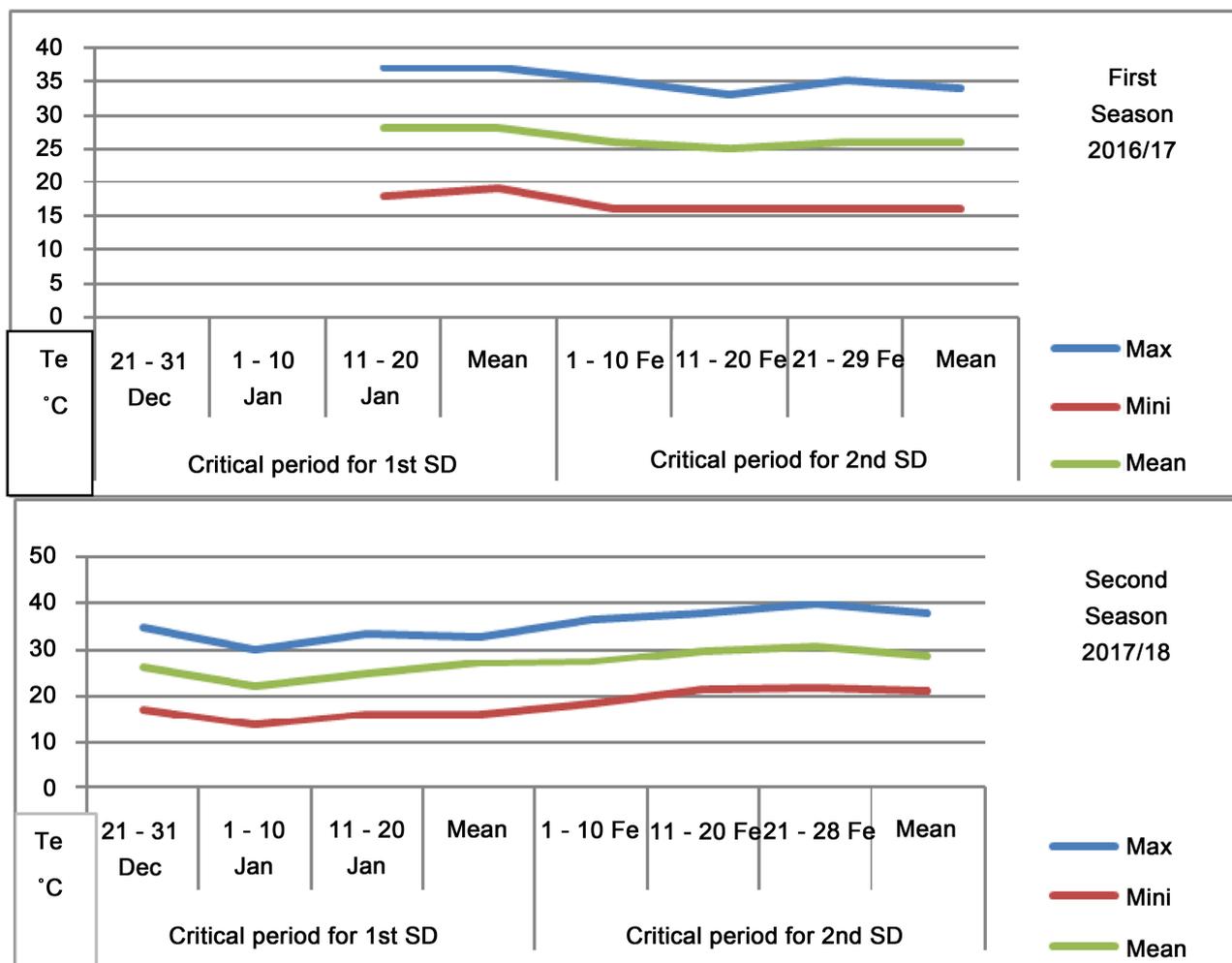


Figure 1. Maximum, minimum and mean temperatures (°C) at the critical stages of wheat genotypes grown at two sowing dates for two seasons (2016/17 and 2017/18) at Gezira Research Station Farm (GRSF), Wad Medani, Sudan.

2.2. Genotyping

2.2.1. DNA Extraction

DNA extraction was carried out following standard protocol. Genome-wide markers were obtained for the genotypes using wheat 15 k Single Nucleotide Polymorphism (SNP) markers for yield and traits in 231 bread wheat genotypes. Analysis was carried out at Trait Genetics Company, Germany (specialized in the development and analysis of molecular markers for biodiversity and plant breeding research).

2.2.2. Genome-Wide Association Analysis (GWAS)

A GWAS was performed using the mixed linear models (MLM) in TASSEL (version 5.2. 37). The significant between 15K SNPs and traits were identified using a model with a Q matrix as the fixed effect and a kinship matrix as the random effect (Q + K) as well as a model with a principal component (PC) matrix as the fixed effect and a kinship matrix as the random effect (PCA + K), at a threshold of $P < 0.001$.

The likelihood distribution (LD) is the nonrandom association of alleles at different sites. For association studies the appropriate marker density depends on the background levels of LD. The low LD population need higher marker densities and high LD population require low marker densities. LD among markers was calculated using observed vs. expected allele frequencies of the markers in TASSELv.5.2.37. Only mapped markers were used for LD calculation both for the entire panel and for model-based subgroups. The critical r^2 -value beyond which LD is due to true physical linkage was determined by taking the 95th percentile of the square root transformed r^2 data of unlinked markers [9]. The percentage of marker pairs significant at different critical r^2 -values (0.2) and $P < 0.001$ was determined for each chromosome to compare the degree of LD among genes.

3. Results and Discussion

3.1. Phenotypic Variation

3.1.1. Yield and Yield Components Variations

Highly significant genotypes differences were found for yield and yield components for two seasons in two sowing. Generally were found wide range between maximum and minimum values of yield and yield components. These variations among genotypes for their traits reflect their different genetic backgrounds. These results were in agreement with those of [10] [11] [12]. Maximum grain yield ranged from 4016 - 5120 kg/ha for 1st SD and 2nd SD respectively in first season and from 5386 - 3926 kg/ha for 1st SD and 2nd SD respectively in second season and the biomass ranged from 11,032 - 13,625 kg/ha for 1st SD and 2nd SD respectively in first season and from 15,625 - 11,161 kg/ha for 1st SD and 2nd SD respectively in second season (**Table 1**). The maximum difference values among above traits due to furthermore genetic backgrounds to the difference temperature in sowing date in each environment (in the first season, generally the temperature in the first sowing date higher than the second sowing date, while the second season the opposite). Significant phenotypic variability for different traits such number of grain per spike and harvest index. Genotypes differed significantly for these traits among these experiments indicating the presence of sufficient genetic variability for selection to identify the best genotypes.

3.1.2. Morphological Traits Variations

Highly significant genotypes differences were found for days to heading, days to maturity, plant height and number of spike/m² for two seasons in two sowing dates (**Table 2**). Wide range variations in these traits, days to heading ranged from 41 - 70 days for 1st SD and 2nd respectively in first season and from 46 - 79 days for 1st SD and 2nd respectively in second season, days to maturity ranged from 73 - 102 days for 1st SD and 2nd respectively in first season and from 76 - 116 days for 1st SD and 2nd respectively in second season, plant height from 44 - 87 cm for 1st SD and 2nd respectively in first season and from 66 - 100 cm for 1st SD and 2nd respectively in second season and number of spike/m² ranged from

Table 1. Mean, minimum and maximum values for yield and yield components of the 250 elite spring bread wheat genotypes grown in two sowing dates in GRF in Wad Medani, Sudan for seasons 2016/17 and 2017/18.

Season 2016/17										
	Grain yield kg/ha		Biomass kg/ha		Harvest Index %		1000 seed weight (g)		Number seed/spike	
	1 st SD	2 nd SD	1 st SD	2 nd SD	1 st SD	2 nd SD	1 st SD	2 nd SD	1 st SD	2 nd SD
Maximum	4016	5120	11,032	13,625	46.6	48.9	47.6	45	53	55
Minimum	1057	1192	3193	3137	26.1	23.3	29.6	28.8	22	23
Mean	2221.6	2939.3	6103	8287	36.4	35.5	36.8	37.4	35	39
SE±	514.5	531.4	1302	1636	5.2	4.5	3.9	3.2	5	4.6
CV %	23.2	18.1	21.3	19.8	14.3	12.8	10.7	8.5	14.7	11.9
Sig Level	***	***	***	***	*	***	Non	***	***	***
Season 2017/18										
Maximum	5386	3926	15,625	11,161	48.6	40.2	49.4	47.6	72	60
Minimum	1337	1602	4911	5804	24.1	20.3	25.7	24.2	29	27
Mean	3742.5	2783.7	10,039	9063	37.6	30.9	36.2	34.5	46	43
SE±	667.4	530.2	1800	1429	4.9	4.9	4.2	4.5	5.7	7.8
CV %	17.8	19.1	17.9	15.8	13.3	15.9	11.6	11.7	11.6	16.2
Sig Level	***	***	***	***	***	***	***	***	***	***

Sig Level = significant level and *** = very highly significant at P < 0.001.

Table 2. Mean, minimum and maximum values for morphological traits of the 250 elite spring bread wheat genotypes grown in two sowing dates in GRF in Wad Medani, Sudan season 2016/17.

Season 2016/17									
	Days to heading		Days to maturity		Plant height (cm)		Number spikes/m ²		
	1 st SD	2 nd SD	1 st SD	2 nd SD	1 st SD	2 nd SD	1 st SD	2 nd SD	
Maximum	71	70	102	101	87	86	586	544	
Minimum	41	42	73	73	45	44	222	202	
Mean	55	52	87	86	64	67	386	429	
SE±	2.8	2.7	2.8	3.7	4.7	7.8	58	49.7	
CV %	5.2	5.5	6.1	4.3	6.5	11.5	15.1	11.6	
Sig Level	***	***	***	***	***	***	***	***	***
Season 2017/18									
Maximum	79	70	116	98	100	89	595	500	
Minimum	46	46	82	76	66	63	355	280	
Mean	61	56	94	87	83	76	460	398	
SE±	3.9	3.8	3.8	3.7	3.8	3.5	60.5	63.5	
CV %	6.4	6.1	3.7	3.9	4.6	4.7	13.1	15.9	
Sig Level	***	***	***	***	***	***	***	***	***

Sig Level = significant level and *** = very highly significant at P < 0.001.

202 - 586 for 1st SD and 2nd respectively in first season and from 280 - 595 for 1st SD and 2nd respectively in second season. This variation depends on genotypes. Very large variation in the degree of response of bread wheat to heat stress was observed for various traits including days to heading and maturity plant height [11].

3.2. Association Mapping

Genome wide association analysis was performed using 15 k SNPs markers for yield and traits in 231 bread wheat genotypes (13 missing for genotypic data) grown for two seasons. The associations between genotype and phenotype were worked out using the mixed linear models (MLM) in TASSEL. The significance between 15 K SNPs and traits was identified using a model with a Q matrix as the fixed effect and a kinship matrix as the random effect (Q + K) as well as a model with a principal component (PC) matrix as the fixed effect and a kinship matrix as the random effect (PCA + K), at a threshold of $P < 0.001$.

3.2.1. Population Structure

STRUCTURE analysis showed that the number of sub-populations ranged from zero to ten when using the diploid genotyping model. Population structure analysis of the genotypes indicated that the likely number of sub populations was four (Figure 2). The cluster analysis also provided evidence for the presence of four subpopulations (Figure 3). The similarity between clusters and the sub-groups of model-based analysis in STRUCTURE indicated that higher genetic

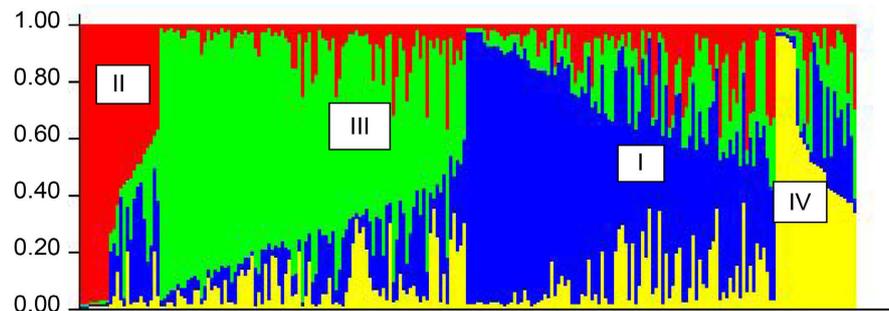


Figure 2. Population structure for 231 genotypes in a spring wheat association mapping.

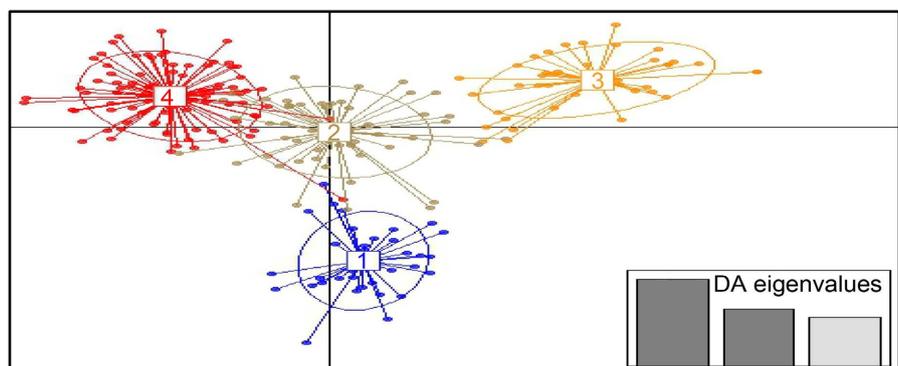


Figure 3. Cluster for 231 genotypes in a spring wheat association mapping panel.

variance among population. Population structure caused by admixture of different sub-population should be in consideration because GWAS has become more complicated due to complex breeding history of germplasm along with limited gene flow between different sub populations; this can be reduced by taking population structure as covariant in GWAS studies [13] [14].

3.2.2. Linkage Disequilibrium (LD)

In Genome-Wide Association Studies (GWAS), the concept of linkage disequilibrium is important as it allows identifying genetic markers that tag the actual causal variants. LD was estimated as squared allele frequency correlations (R^2), and only P-values ≥ 0.01 for each pair of loci were considered significant analysis using TASSEL software. LD decay distance increases with minor allelic frequency, the increase is very significant at minor allelic frequency ≥ 0 . In this study LD decay rate was determined on chromosome level, however, a clear and rapid decline in LD with distance were observed (Figure 4) also showed (Figure 5). LD

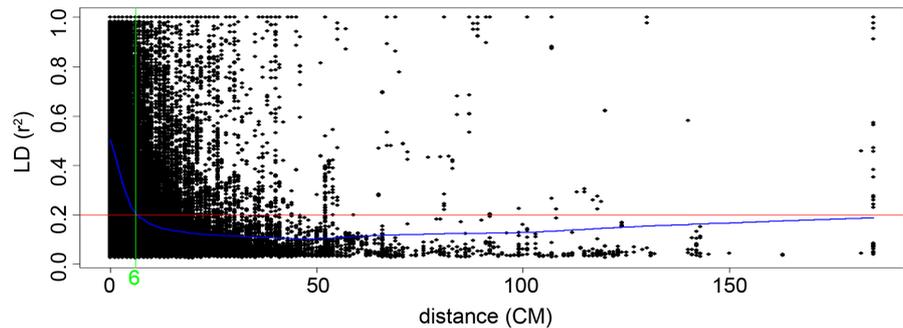


Figure 4. Linkage disequilibrium (r^2) plot of all chromosomes of the genome in 231 genotypes of a spring wheat association mapping panel. Plot of pair-wise single-nucleotide polymorphism LD r^2 values as a function of intermarker map distance (Mbp). The blue curve represents the model fit to LD decay. The green line represents the 51.26 (Mbp) confidence interval for the quantitative trait loci regions in which LD $r^2 = 0.2$, beyond which LD is likely due to linkage.

■ A1 ■ B1 ■ D1 ■ A2 ■ B2 ■ D2 ■ A3 ■ B3 ■ D3 ■ A4 ■ B4
 ■ D4 ■ A5 ■ B5 ■ D5 ■ A6 ■ B6 ■ D6 ■ A7 ■ B7 ■ D7



Figure 5. Linkage disequilibrium (r^2) of all chromosomes with highest significant comparison of marker (LD at $P < 0.001$) for 231 genotypes in across seasons grown at GRF, Wad Medani, Sudan.

decay rate evaluation was conducted at the genome and individual chromosome level. The genome level LD decayed below $r^2 = 0.2$ at about 6 cM for the A genome, 5 cM for the B genome and 16 cM for D genome (Figures 6-8, respectively). The results showed extended LD in the D genome than in the A and B genomes. Similar finding using 394 genetically mapped SNP markers on 478 spring and winter wheat cultivars and reported more extended LD in the D genome than in the A and B genomes could be the introduction of new haplotypes, which can increase the extent of LD from *Aegilops tauschii* (D genome donor) into the D genome of hexaploid wheat germplasm through synthetic wheats [15]. Extent of LD depends upon genetic distance and it determines the number of markers required for association mapping, the low LD population need higher

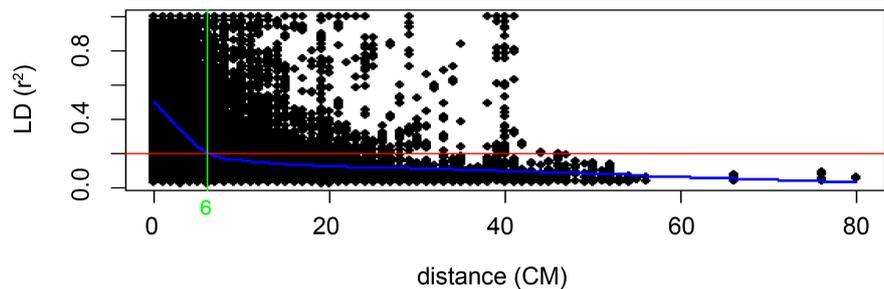


Figure 6. Linkage disequilibrium (r^2) plot of A genome.

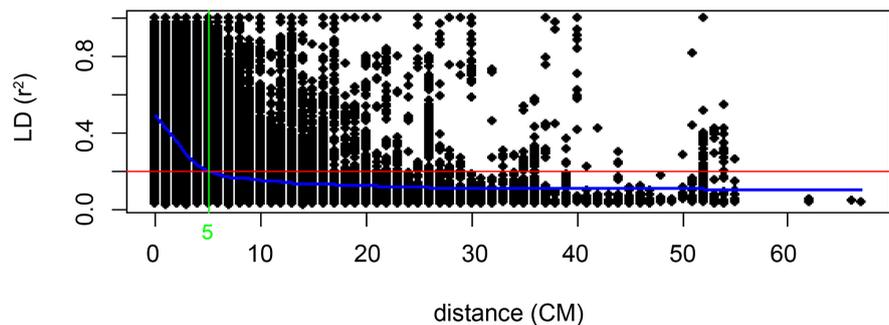


Figure 7. Linkage disequilibrium (r^2) plot of B genome.

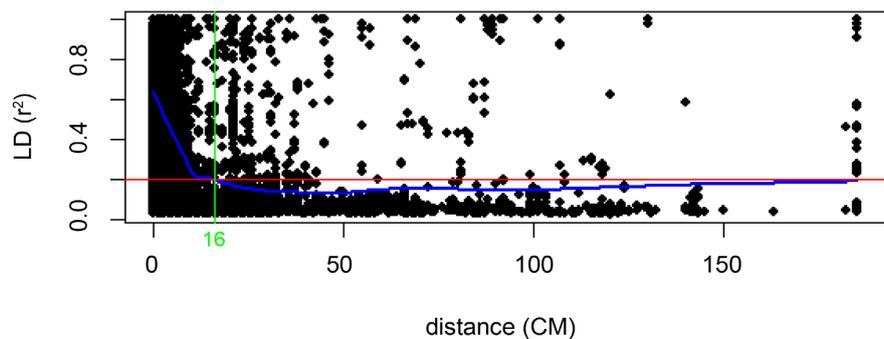


Figure 8. Linkage disequilibrium (r^2) plot of D genome. The green line represents the 6 (CM) for A genome 5 (CM) for B genome and 16 (CM) for D genome confidence interval for the quantitative trait loci regions in which LD $r^2 = 0.2$, beyond which LD is likely due to linkage.

marker densities and high LD population require low marker densities [16]. This indicates that fewer markers are needed for GWAS in the D genome than for the A and B genomes.

3.2.3. Marker-Traits Associations (MTAs) of Grain Yield

A total of 282, 355 and 475 significant ($P \leq 0.05$) markers associated with grain yield were observed in the first, second and across seasons, respectively. MTAs for grain yield were highly significantly associated with the chromosomes 6B, 4B and 6A in the first season, were 6B, 6A, 7A and 3A in the second season and 6B and 6A in the across seasons (**Table 3**). Chromosomes 6B and 6A were stable in

Table 3. Top markers with high association with yield of 231 wheat genotypes evaluated under heat stress in each season and across seasons at GRF, Wad Medani, Sudan.

Yield seasons	Marker	Chr	Position	P value	R ²
2016/17	Kukri_c27662_645	6B	110	5.57E-05	0.07164
	wsnp_Ex_c4125_7456528	4B	115	8.10E-05	0.08355
	BobWhite_c23771_525	6B	114	1.03E-04	0.08272
	Excalibur_c32219_491	6B	114	1.20E-04	0.08255
	wsnp_Ex_rep_c69373_68312188	6A	110	1.42E-04	0.08009
	RAC875_c31299_1302	6B	110	1.55E-04	0.07747
	Tdurum_contig12123_1650	6A	114	2.71E-04	0.07234
	BobWhite_c5324_168	6A	1	3.87E-04	0.07056
	BS00046264_51	6B	110	4.09E-04	0.06905
	wsnp_BE638137B_Ta_2_2	4B	115	4.50E-04	0.06863
2017/18	Kukri_c27662_675	6B	110	1.31E-04	0.08153
	BobWhite_c15802_72	6A	27	2.00E-04	0.07761
	Kukri_c102502_261	6A	27	2.80E-04	0.07437
	RAC875_c31299_1215	6B	110	3.13E-04	0.07374
	RAC875_rep_c106238_237	6B	110	3.92E-04	0.07119
	Kukri_c27662_645	6B	110	5.61E-04	0.05367
	BS00023673_51	7A	128	6.53E-04	0.06673
	RAC875_c14887_829	6B	110	7.58E-04	0.06546
	BS00085589_51	6B	110	7.69E-04	0.06509
	RAC875_c15003_377	3A	123	7.73E-04	0.06496
across season	Kukri_c27662_645	6B	110	8.45E-06	0.08842
	RAC875_c31299_1302	6B	110	2.41E-05	0.09495
	BobWhite_c23771_525	6B	114	4.01E-05	0.09133
	wsnp_Ex_rep_c69373_68312188	6A	110	4.69E-05	0.09066
	Excalibur_c32219_491	6B	114	8.53E-05	0.08513
	BS00046264_51	6B	110	1.49E-04	0.07878
	wsnp_Ex_c3025_5587183	6B	110	1.96E-04	0.07548
	BobWhite_c15802_72	6A	27	2.20E-04	0.07457
	Kukri_c27662_675	6B	110	2.29E-04	0.07405
	Tdurum_contig12123_1650	6A	114	2.49E-04	0.0733

Chro = chromosome number.

all seasons. This result was in agreement with [17] reported that chromosomes 6B and 6A associated with yield under temperature irrigated environments. Chromosome 6B was significantly associated with yield under drought stress [18]. Manhattan plots of the GWAS results are shown in **Figures 9-11** for yield in the first, second and across seasons, respectively. Marker associated with grain yield in chromosomes 6B, 4B and 6A in the first season, 6B and 6A in the second season and 6B, 6A and 2B across seasons. The grain yields were highly significantly ($P \leq 0.001$) associated with the markers 6B/Kukri_c27662_645 in each season and across seasons (**Table 3**), with markers 6B/ BobWhite_c23771_525, 6B/Excalibur_c32219_491, 6B/RAC875_c31299_13021302, 6B/BS00046264_51, 6A/wsnp_Ex_rep_c69373_68312188 and 6A/Tdurum-contig12123_1650 in the first season and across seasons and with markers 6BKukri_c27662_675 and 6A/BobWhite_c15802_72 in the second season and across seasons. Chromosome 6B was significantly associated with yield under rain-fed conditions [19].

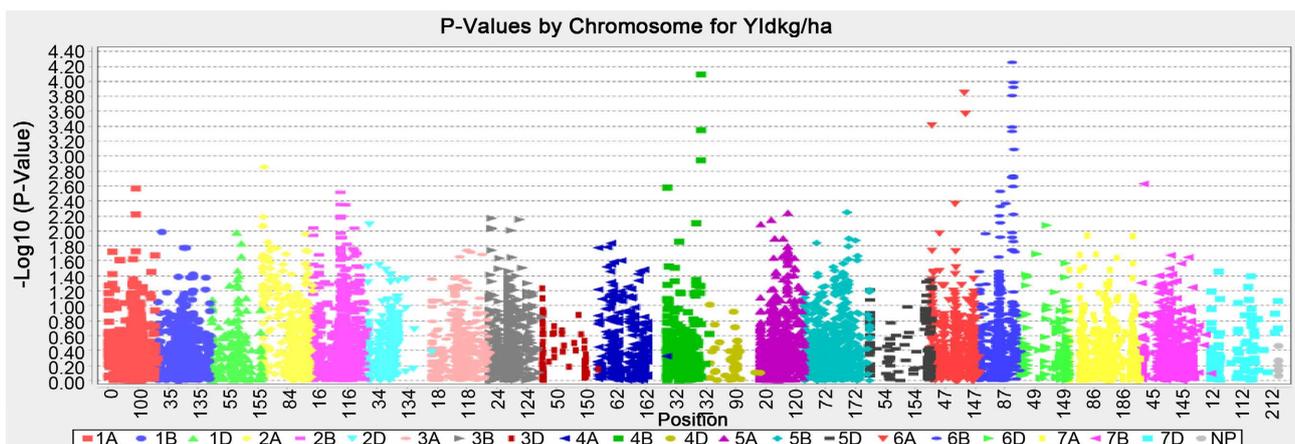


Figure 9. Manhattan plots of the GWAS results using 15 K SNPs markers in grain yield kg/ha for 231 wheat genotypes in first season grown at GRF, Wad Medani, Sudan.

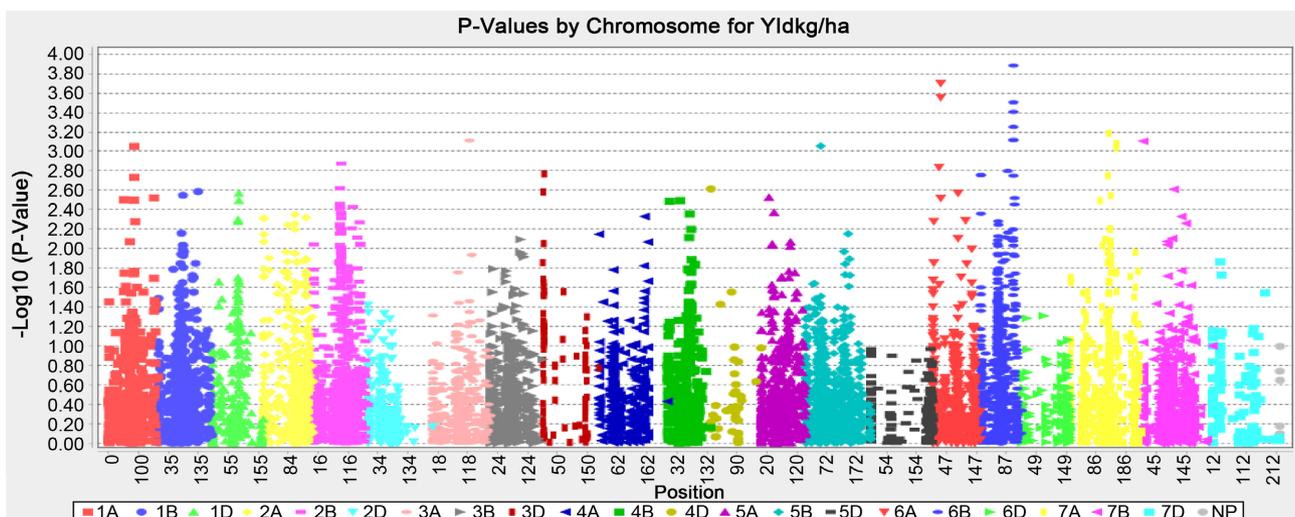


Figure 10. Manhattan plots of the GWAS results using 15 K SNPs markers in grain yield kg/ha for 231 wheat genotypes in second season.

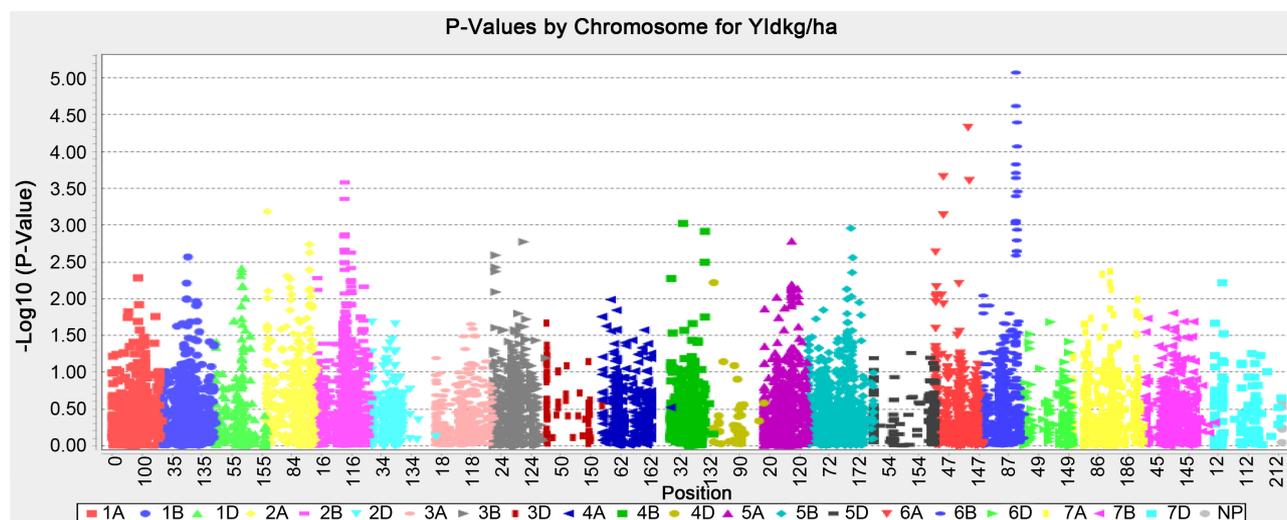


Figure 11. Manhattan plots of the GWAS results using 15 K SNPs markers in grain yield kg/ha for 231 genotypes in across seasons grown at GRF, Wad Medani, Sudan.

3.2.4. MTAs of Morphological Traits in Optimum and Late Sowing Dates

Days to heading (DH) was associated with two markers located on chromosome 3B as well as with three chromosomes 4B, 6B, and 5A in optimum sowing dates in both seasons. Chromosome 3B was significantly associated with DH under rain-fed conditions [20]. Several QTL were identified by multi-environment single trait analysis for DH on some chromosome including 6B (Lopes *et al.*, 1013). QTL that affect flowering time in wheat have been reported on chromosomes 5A, and 3B by [21]. In the late sowing dates DH associated with markers on chromosomes 1A and 1B (Table 4), Several chromosomes associated with days to maturity (DM) in optimum and late sowing dates but markers on chromosomes 2B associated in late in two seasons. Earlier studies have reported QTLs for maturity on chromosome 2B in a parental population [22]. The MTAs for DH and DM on chromosome 2B corresponded to a previously identified chromosomal region [23]. Plant height was associated with markers on chromosomes 6B, 1A, and 2A in optimum sowing dates in two seasons. Chromosome 1A was significantly associated with plant height under irrigated environments [17] and chromosome 2A is most likely the QTL reported by [24]. Plant height in late sowing dates was associated with 6B in two seasons. Many markers on chromosomes were associated with number of seed/spike but the chromosome 2A was stable under optimum and late. Chromosome 2A, and most of the associated markers were located near QTL for multiple traits such as number of spikelets per spike and number of grains per spike [25] [26]. Several chromosomes like 6A, 2A, 7A, 3B, 4B, 5B, 6B, 1B, 4D and 6D associated with number of spikes/m² [19] found QTL on chromosomes 1B, 2A, 3B, 4D, 5D, 6D and 7A for tiller number per m². The thousand kernel weight associated with four markers located on chromosome 6B in optimum and late sowing dates and also the trait associated with chromosomes 4B, 6D, 3B, 4A, 3A, 1B, 5A, 2D, 7A and 2B. QTL for kernel weight on chromosomes 6B, 2B, 2D, 3B, 3A, 5A and 1B [19].

Table 4. Chromosomes with high association with agronomic traits of 131 wheat genotypes evaluated under heat stress seasons 2016/17 and 2017/18 at GRSF, Wad Medani, Sudan.

Traits	Optimum Sowing Date		Late Sowing Date	
	Chromosome		Chromosome	
	2016/17	2017/18	2016/17	2017/18
DH	3D, 3B (2) , 4B , 1A, 6B , 5A , 7A (2)	4B (2) , 6B (2) , 3A, 5A , 2A, 7B, 3B (2)	4B (3), 2B, 1A, 1B, 7B (2)	1A (3), 6B (4), 3B, 1B (2)
DM	3B (7), 3D, 2A, 3A	6B (2), 7D, (2), 4B, 1B, 7B (3), 1A (2), 4B (2), 2B , 7B, 1B	2B (2) , 5B (3), 3B (3), 7B	
PH	6B (4) , 2A , 1B (2) , 1A, 3A	2A (2) , 4A, 1A (3) , 7D, 6A, 6B	1A (4), 6B (3) , 1B, 3B	6B (2) , 7A (2), 5B (4), 4A (2),
Spi/m ²	6A, 2A (3), 5B, 7A (2), 4B, 3A	3B (6), 3A, 4B (2), 5B	7B, 1B, 2A, 3B (2), 4D, 6D	5B (4), 6A (3), 6B (2)
Se/Spi	7D, 1A (3), 1B (3), 2B, 2A , 4A	2B, 5A, 2A , 7B (2), 5A, 4A	7A (3), 6D, 4B (2), 2A , 5D	1B (4), 5B, 2A (2) , 6D, 7A, 3A
TKW	4B (2), 6B (4) , 6D, 3B	4A (4), 3A, 7B, 2A, 5A (2)	6B (4) , 1B (4), 3A	2D (4), 7A, 3B (2), 2B, 1B
Bio kg/ha	2A (4), 4B (2), 5A , 6B	4A (4), 3A, 7B, 5A (2) , 2A	5A (2) , 6B (4), 7B, 2A, 2D	5B (3), 1B (4), 5A (2)
HI	7A , 2B , 3B (4), 5B, 1B, 1A, 5A	2B (6) , 2D, 7A , 2A (2)	6B (2) , 5B (4) , 7B , 2B (2), 7D	2A, 3D, 7B, 7A, 3B, 2D, 5B , 6B , 1B

DH = days to heading, DM = days to maturity, PH = plant height, Spi/m² = spikes/m², Se/Spi = number of seed/spike, TKW = thousand seed weight, Bio kg/ha = biomass kg/ha and HI = harvest index.

Multi-environment single trait QTL analysis for kernel weight detected significant QTL on chromosomes 6B and 6D [27]. Biomass was associated with chromosome 5A and was stable in optimum and late, [24] reported that chromosome 5A associated with biomass under drought and heat conditions. Chromosomes 2B and 7A were associated with harvest index in optimum in two seasons while, in late the trait was associated with chromosomes 5B, 6B and 7B. QTL for harvest index on chromosome 5B [28].

3.2.5. Yield Covariate with Days to Heading

Heading time is an important trait for adaptation of wheat to target environments [29]. It is regulated by three well-characterized groups of loci namely, vernalization (VRN), photoperiod (Ppd) and earliness (Es) [30]. The VRN genes are dominant for the spring growth habit whereas the *VRN2* genes are dominant for the winter growth habit [29]. Days to heading as covariate to identify QTLs which is independent and more reliable reported by [5]. When used as covariate of yield days to heading in this study, markers on chromosome 2B associated with yield and were stable in the first, second and across seasons (Table 5). The marker of (Tdurum_contig71387_65) on chromosome 2B more stable in the first, second and across season and markers (Tdurum_contig10994_617 and RFL_Contig1483_1765) were found on chromosome 2B in the first season and

Table 5. Chromosomes with high association yield covariate with days to heading in wheat genotypes evaluated in seasons 2016/17 and 2017/18 and across seasons at GRSF, Wad Medani, Sudan.

Days to heading with Yield	Marker	Chr	Position	P value	R ²
First season	Tdurum_contig71387_65	2B	93	1.46E-04	0.05863
	BS00022940_51	2B	92	2.77E-04	0.05392
	Tdurum_contig10994_617	2B	92	3.82E-04	0.05169
	tplb0031k24_254	2B	92	6.34E-04	0.04778
	IAAV6156	2B	93	6.34E-04	0.04778
	RFL_Contig1483_1765	2B	93	6.34E-04	0.04778
	w SNP_Ra_c16822_25566950	2B	93	6.34E-04	0.04778
	Kukri_c6973_344	2B	93	7.77E-04	0.04662
	IAAV4434	2B	92	7.89E-04	0.04749
	w SNP_Ex_c13351_21042379	2B	93	7.89E-04	0.04769
Second season	BS00023673_51	7A	128	5.29E-04	0.0637
	BobWhite_c15802_72	6A	27	6.59E-04	0.06154
	w SNP_CAP12_c1960_972031	1A	85	7.14E-04	0.06141
	Kukri_c102502_261	6A	27	9.31E-04	0.0585
	BS00082412_51	7A	134	0.00108	0.05728
	RAC875_c106584_747	6A	21	0.00144	0.05476
	RAC875_rep_c106238_237	6B	110	0.00164	0.05363
	w SNP_JD_c20555_18262260	7A	153	0.00209	0.05153
	w SNP_JD_c20555_18262317	7A	153	0.00209	0.05153
	Tdurum_contig71387_65	2B	93	0.0021	0.052
Across seasons	Tdurum_contig71387_65	2B	93	2.99E-04	0.05248
	BS00022940_51	2B	92	3.11E-04	0.05192
	Tdurum_contig10994_617	2B	92	6.43E-04	0.04708
	Kukri_c6973_344	2B	93	7.03E-04	0.04622
	tplb0031k24_254	2B	92	8.93E-04	0.04446
	IAAV6156	2B	93	8.93E-04	0.04446
	RFL_Contig1483_1765	2B	93	8.93E-04	0.04446
	w SNP_Ra_c16822_25566950	2B	93	8.93E-04	0.04446
	w SNP_Ex_c13351_21042379	2B	93	9.73E-04	0.04523
	w SNP_Ku_c13905_22034406	2B	93	9.90E-04	0.04492

Chro = chromosome number.

across season. Days to heading was one trait specific SNP on chromosome 2B presented on unmapped chromosome were found under heat and drought stress [30]. In the second season markers on chromosomes 7A, 6A, 2B, 6B and 1A were found. MTAs on 6B were associated with yield. QTL cluster for yield with

heading were detected on chromosome 2B, 7A, 5A, 6B, and 1A [29]. Yield under stress is mainly controlled by the QTLs controlling heading and maturity time because the duration of the crop life cycle affects the timing and intensity of the stress experienced by the plants [31].

4. Conclusion

The results of phenotypic variations for all the traits studied, indicated that the variability among the genotypes considered in this study. Results of genome-wide association study (GWAS) indicated that several chromosomes were detected for yield and related traits in this population. The most important chromosomes findings were:

- The chromosomes 6B and 6A were important for grain yield in two seasons and in across season.
- More than 10 markers on chromosomes were highly associated with grain yield at least at two seasons but marker (Kukri_c27662_645) was stable in the two seasons and across seasons. The markers (wsnp_Ex_c4125_7456528, BobWhite_c23771_525, Excalibur_c32219_491, wsnp_Ex_rep_c69373_68312188, RAC875_c31299_1302, RAC875_c31299_1302, Tdurum_contig12123_1650, BS00046264_51, BobWhite_c15802_72, RAC875_c14887_829 and wsnp_Ex_c3025_5587183) are associated with grain yield in this study can be important targets for marker-based breeding and fine mapping of functional genes in the future.
- Using yield as covariate with days to heading, chromosome 2B was associated with yield and more stable in the two seasons and across seasons.
- The study showed that there were more than 10 genotypes showed good performance with high yield potential and heat tolerance and the pedigree of the best 10 genotypes are (WBLL1*2/BRAMBLING//SHAMIEKH-4 (27), ZARAF-5/FLAG-6//MILAN/PASTOR (37), CHAM-10/3/PASTOR//MUNIA/ALTAR 84/4/PFAU/MILAN (242), HUBARA-15/ICBW 206971/(4) EALME4SA-464 (139), ABU-REYAA-1/LEITH-1 (192), CHAM-10/3/PASTOR//MUNIA/ALTAR84/4/PFAU/MILAN (152), DEBEIRA//MILAN/ASTOR/4/URES/BOW//OPATA/3/HD2206/HORK'S' (20), WAXWING*2 (WAXWING*2/KUKUNA//SHUHA-4/CHAM-8 (94), KAUZ//KAUZ/STAR/3/ETBW4919/4/TEMPORALERAM87*2/KONK and CHAMRAN/4/OPATA/BOW//BAU/3/OPATA/BOW/5/SAMIRA-9.

5. Recommendation

- The chromosomes 6B and 6A were associated for grain yield in two seasons and in across season.
- More than 10 markers on chromosomes highly associated with grain yield, can be use these markers for fine mapping of functional genes in the future.
- 10 genotypes showed good performance with high yield potential and heat

tolerance, it recommended to evaluate these genotypes in advanced trials to the release some of them.

The currently identified MTAs should be further validated using other elite set of genotypes so that they can be used for MAS in the breeding program. The high yielding and heat tolerant genotypes identified in this study will be evaluated across key locations in Sudan for potential release in Sudan. Furthermore, they will be used as parents in the breeding.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Battisti, D.S. and Naylor, R.L. (2010) Historical Warnings of Future Food Insecurity with Unprecedented Seasonal Heat. *Science*, **323**, 240-244. <https://doi.org/10.1126/science.1164363>
- [2] Hansen, J., Sato, M., Ruedy, R., Lo, K., Lea, D.L. and Medina-Elizade, M. (2006) Global Temperature Change. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 14288-14293. <https://doi.org/10.1073/pnas.0606291103>
- [3] Tadesse, W., Morgounov, A.I., Braun, H.J., Akin, B., Keser, M., *et al.* (2013) Breeding Progress for Yield in Winter Wheat Genotypes Targeted to Irrigated Environments of the CWANA Region. *Euphytica*, **194**, 177-185. <https://doi.org/10.1007/s10681-013-0903-5>
- [4] Wahid, A., Gelani, S., Ashraf, M. and Foolad, M.R. (2007) Heat Tolerance in Plants: An Overview. *Environmental and Experimental Botany*, **61**, 199-223. <https://doi.org/10.1016/j.envexpbot.2007.05.011>
- [5] Tadesse, W., Suleiman, S., Tahir, I., Sanchez-Garcia, M., Jighly, A., Hagra, A., Thabet, S. and Baum, M. (2019) Heat-Tolerant QTLs Associated with Grain Yield and Its Components in Spring Bread Wheat under Heat-Stressed Environments of Sudan and Egypt. *Crop Science*, **59**, 199-211. <https://doi.org/10.2135/cropsci2018.06.0389>
- [6] Hamblin, M.T., Buckler, E.S. and Jannink, J.-L. (2011) Population Genetics of Genomics-Based Crop Improvement Methods. *Trends in Genetics*, **7**, 98-106. <https://doi.org/10.1016/j.tig.2010.12.003>
- [7] Risch, N. and Merikangas, K. (1996) The Future of Genetic Studies of Complex

- Human Diseases. *Science*, **273**, 1516-1517.
<https://doi.org/10.1126/science.273.5281.1516>
- [8] Rafalski, J.A. (2002) Novel Genetic Mapping Tools in Plants: SNPs and LD-Based Approaches. *Plant Science*, **162**, 329-333.
[https://doi.org/10.1016/S0168-9452\(01\)00587-8](https://doi.org/10.1016/S0168-9452(01)00587-8)
- [9] Breseghello, F. and Sorrells, M.E. (2006) Association Mapping of Kernel Size and Milling Quality in Wheat (*Triticum aestivum* L.) Cultivars. *Genetics*, **172**, 1165-1177. <https://doi.org/10.1534/genetics.105.044586>
- [10] Reynolds, M.P., Balota, M., Delgado, M.I.B., Amani, I. and Fischer, R.A. (1994) Physiological and Morphological Traits Associated With Spring Wheat Yield Under Hot, Irrigated Conditions. *Australian Journal of Plant Physiology*, **21**, 717-730.
<https://doi.org/10.1071/PP9940717>
- [11] Elahmadi, A.B., Ageeb, O.A., Solh, M. and Saxena. (1995) Review of Wheat Production and Improvement in the Sudan. *Proceedings of the Research Review Workshop*, Wad Medani, 27-30 August 1995.
- [12] Slafer, G.A., Araus, J.L., Royo, C. and Del Moral, L.F.G. (2005) Promising Eco-physiological traits for genetic improvement of cereal yields in Mediterranean environments. *Annals of Applied Biology*, **146**, 61-67.
<https://doi.org/10.1111/j.1744-7348.2005.04048.x>
- [13] Yu, J. and Buckler, E.S. (2006) Genetic Association Mapping and Genome Organization of Maize. *Current Opinion in Biotechnology*, **17**, 155-160.
<https://doi.org/10.1016/j.copbio.2006.02.003>
- [14] Pritchard, J.K. and Donnelly, P. (2001) Case-Control Studies of Association in Structured or Admixed Populations. *Theoretical Population Biology*, **60**, 227-237.
<https://doi.org/10.1006/tpbi.2001.1543>
- [15] Chao, S., Dubcovsky, J., Dvorak, J., Luo, M.-C. Baenziger, S.P. Matnyazov, R., Clark, D.R., Talbert, L.E., Anderson, J.A., Dreisigacker, S., Glover, K., Chen, J.L., Campbell, K., Bruckner, P.L., Rudd, J.C., Haley, S., Carver, B.F., Perry, S., Sorrells, M.E. and Akhunov, E.D. (2010) Population- and Genome-Specific Patterns of Linkage Disequilibrium and SNP Variation in Spring and Winter Wheat (*Triticum aestivum* L.). *BMC Genomics*, **11**, Article No. 727. <https://doi.org/10.1186/1471-2164-11-727>
- [16] Maccaferri, M., Sanguineti, M.C., Corneti, S., Ortega, J.L.A., Ben Salem, M., Bort, J., DeAmbrogio, E., del Moral, L.F.G., Demontis, A., El-Ahmed, A., Maalouf, F., Machlab, H., Martos, V., Moragues, M., Motawaj, J., Nachit, M., Nserallah, N., Ouabbou, H., Royo, C., Slama, A. and Tuberosa, R. (2008) Quantitative Trait Loci for Grain Yield and Adaptation of Durum Wheat (*Triticum durum*.) Across a Wide Range of Water Availability. *Genetics*, **178**, 489-511.
<https://doi.org/10.1534/genetics.107.077297>
- [17] Sukumaran, S., Dreisigacker, S., Lopes, M., Chavez, P. and Reynold, M.P. (2014) Genome-Wide Association Study for Grain Yield and Related Traits in an Elite Spring Wheat Population Grown in Temperate Irrigated Environments. *Theoretical and Applied Genetics*, **128**, 353-363. <https://doi.org/10.1007/s00122-014-2435-3>
- [18] Mwadzingeni, L., Shimelis, H., Rees, D.J.G. and 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>
- [19] Yan, L., Loukoianov, A., Tranquilli, G., Helguera, M., Fahima, T. and Dubcovsky, J. (2003) Positional Cloning of the Wheat Vernalization Gene *VRN1*. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 6263-6268.
<https://doi.org/10.1073/pnas.0937399100>

- [20] Tadesse, W., Ogonnaya, F.C., Jighly, A., Sanchez-Garcia, M., Sohail, Q., Rajaram, S. and Baum, M. (2015) Genome-Wide Association Mapping of Yield and Grain Quality Traits in Winter Wheat Genotypes. *PLOS ONE*, **10**, e0141339. <https://doi.org/10.1371/journal.pone.0141339>
- [21] Wang, R.X., Hai, L., Zhang, X.Y., You, G.X., Yan, C.S. and Xiao, S.H. (2009) QTL Mapping for Grain Filling Rate and Yield-Related Traits in RILs of the Chinese Winter Wheat Population Heshangmai × Yu8679. *Theoretical and Applied Genetics*, **118**, 313-325. <https://doi.org/10.1007/s00122-008-0901-5>
- [22] Pinto, R.S., Reynolds, M.P., Mathews, K.L., *et al.* (2010) Heat and Drought Adaptive QTL in a Wheat Population Designed to Minimize Confounding Agronomic Effects. *Theoretical and Applied Genetics*, **121**, 1001-1021. <https://doi.org/10.1007/s00122-010-1351-4>
- [23] Chu, C.-G., Xu, S.S., Friesen, T.L. and Faris, J.D. (2008). Whole Genome Mapping in a Wheat Doubled Haploid Population Using SSR and TRAPs and the Identification of QTL for Agronomic Traits. *Molecular Breeding*, **22**, 251-226. <https://doi.org/10.1007/s11032-008-9171-9>
- [24] Qaseem, M.F., Qureshi, R., Shaheen, H. and Shafqat, 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**, e0213407. <https://doi.org/10.1371/journal.pone.0213407>
- [25] Ain, Q., Rasheed, A., Anwar, A., Imtiaz, M., Mahmood, T., Xia, X., He, Z. and Quraishi, U.M. (2015) Genome-Wide Association for Grain Yield under Rainfed Conditions in Historical Wheat Cultivars from Pakistan. *Frontiers in Plant Science*, **6**, Article 743. <https://doi.org/10.3389/fpls.2015.00743>
- [26] Huang, X.Q., Cöster, H., Ganai, M.W. and Röder, M.S. (2003) Advanced Backcross QTL Analysis for the Identification of Quantitative Trait Loci Alleles from Wild Relatives of Wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, **106**, 1379-1389. <https://doi.org/10.1007/s00122-002-1179-7>
- [27] Lopes, M.S., Reynolds, M.P., McIntyre, C.L., Mathews, K.L., Jalal Kamali, M.R., Mossad, M., Feltaous, Y., Tahir, I.S.A., Chatrath, R., Ogonnaya, F. and Baum, M. (2013) QTL for Yield and Associated Traits in the Seri/Babax Population Grown across Several Environments in Mexico, in the West Asia, North Africa, and South Asia Regions. *Theoretical and Applied Genetics*, **126**, 971-984. <https://doi.org/10.1007/s00122-012-2030-4>
- [28] Cuthbert, J.L., Somers, D.J., Brülé-Babel, A.L., Brown, P.D. and Crow, G.H. (2008) Molecular Mapping of Quantitative Trait Loci for Yield and Yield Components in Spring Wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, **117**, 595-608. <https://doi.org/10.1007/s00122-008-0804-5>
- [29] Erena Aka, E. (2013) Association Mapping for Yield, Yield Components and Drought Tolerance-Related Traits in Spring Wheat Grown under Rained and Irrigated Condition. Colorado State University, Fort Collins.
- [30] Bennett, D., Izanloo, A., Edwards, J., Kuchel, H., Chalmers, K., Tester, M., Reynolds, M., Schnurbusch, T. and Langridge, P. (2012) Identification of Novel Quantitative Trait Loci for Days to Ear Emergence and Flag Leaf Glauconess in Bread Wheat (*Triticum aestivum* L.) Population Adapted to Southern Australian Conditions. *Theoretical and Applied Genetics*, **124**, 697-711. <https://doi.org/10.1007/s00122-011-1740-3>
- [31] Reynolds, M. and Tuberosa, R. (2008) Translational Research Impacting on Crop Productivity in Drought-Prone Environments. *Current Opinion in Plant Biology*, **11**, 171-179. <https://doi.org/10.1016/j.pbi.2008.02.005>