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RESEARCH

Heat-Tolerant QTLs Associated with Grain Yield and Its Components in Spring Bread Wheat under Heat-Stressed Environments of Sudan and Egypt

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ABSTRACT

Heat stress decreases photosynthesis, pollen viability, and grain number and weight and hence lowers yield and quality of wheat (*Triticum aestivum* L.) by variable amounts among different cultivars and genotypes. The present study was performed to determine genetic variability of spring bread wheat genotypes for yield and other agronomic traits under heat-stressed (Wad Medani, Sudan) and high-yielding (Sids, Egypt) environments and to identify linked single nucleotide polymorphism (SNP) markers through association mapping. A heat association panel of 197 spring wheat genotypes from ICARDA was evaluated for yield and agronomic traits at Wad Medani and Sids stations for 2 yr (2014–2015). A total of 111 significant marker-trait associations were detected. The wsnp_Ex_c12812_20324622 marker on chromosome 4A was significantly correlated with yield at both locations. At Wad Medani, wsnp_Ex_c2526_4715978 on chromosome 5A was significantly correlated with grain yield. Wheat genotypes carrying the cytosine base at the wsnp_Ex_c12812_20324622 and wsnp_Ex_c2526_4715978 markers outyielded the ones carrying the alternative bases by 15%, whereas genotypes carrying the cytosine base at only one of the two markers increased their yield by 7.9 to 10%, suggesting the importance of using these markers for marker-assisted selection in breeding programs to increase yield under heat stress. The top 20 high-yielding and heat-tolerant genotypes identified in this study have been distributed to the national research systems of Central and West Asia and North Africa (CWANA) and sub-Saharan Africa (SSA) for potential direct release and/or use as parents after local adaptation trials.

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Abbreviations: BIC, Bayesian information criterion; CWANA, Central and West Asia and North Africa; GWAS, genome-wide association study; HAP, heat association panel; LD, linkage disequilibrium; LOD, logarithm of odds; MTA, marker-trait association; PIC, polymorphism information content; QTL, quantitative trait locus; SNP, single nucleotide polymorphism; SSA, sub-Saharan Africa; TKW, thousand-kernel weight.

Wheat (*Triticum aestivum* L.) is one of the most important and widely adapted strategic food crop. It provides ~19% of the calories, 55% of carbohydrates, and 21% of protein needs of daily human requirements at the global level (Gupta et al., 1999; Braun et al., 2010). According to FAO (2018), ~749.5 Tg of wheat were produced globally in 2017 on ~220 million ha with a grain yield level of 3.4 t ha^{-1}. In 2050, the world's population is expected to reach 9 billion and the demand for wheat will increase to >900 Tg. Fulfilling this demand will be challenging, especially in developing countries where the effect of climate change is more pronounced through the aggravation of both abiotic and biotic stresses (Dixon et al., 2009; Lobell et al., 2011).

High temperature during reproductive stages of wheat causes significant loss in both grain yield and quality in the tropical and subtropical climates of West and South Asia, North Africa, and sub-Saharan Africa (SSA) (Ortiz et al., 2008; Talukder et al., 2014). This is mainly due to reductions in the duration of

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developmental stages, lower biomass, early leaf senescence, and adverse physiological and biochemical changes (Lobell et al., 2012; Chand et al., 2014). Recently, Asseng et al. (2015) estimated a reduction of global wheat production by 6% for every 1°C rise in temperature. In China, You et al. (2009) estimated wheat yield reductions of up to 10% for every 1°C temperature increase during the growing season.

Nonetheless, considerable variability in thermotolerance has been observed in wheat (Lopes et al., 2013, 2014; Chand et al., 2014; Tadesse et al., 2015). Precision phenotyping of germplasm at key locations, supported by molecular approaches, would help to improve breeding efficiency and increase genetic gains (Gupta et al., 1999; Tadesse et al., 2015).

Association mapping is an approach for identification of marker-trait associations (MTAs) that can utilize diverse sets of germplasm (landraces, cultivars, elite breeding lines, etc.) and exploit the high marker coverage and higher resolution of the new genotyping technologies without the time and effort needed to develop biparental mapping populations (Crossa et al., 2007). Marker-trait associations using association mapping have been identified in different crops (Zhu et al., 2008) using different types of molecular markers. Breseghello and Sorrells (2006) and Tadesse et al. (2015), among many others, identified significantly associated markers with kernel size and milling quality in winter wheat. Similarly, association mapping studies have been performed in wheat for grain yield (Crossa et al., 2007; Edae et al., 2014; Lopes et al., 2014; Ogbonnaya et al., 2017), resistance to foliar diseases (Crossa et al., 2007; Tadesse et al., 2014; Sukumaran et al., 2015, 2016; Jighly et al., 2015), soil-borne pathogens (Mulki et al., 2013), and resistance to major insect pests (Joukhadar et al., 2013).

The present study was performed using a heat association panel (HAP) of 197 spring wheat genotypes from the ICARDA to determine linkage disequilibrium (LD) decay rate in the panel, analyze the population structure, and identify closely associated markers with grain yield, yield components, and other morphological and phenological traits under heat-stressed environments.

MATERIALS AND METHODS

Mapping Population

A collection of 197 spring bread wheat genotypes from ICARDA was composed as a HAP with the intention of identifying quantitative trait loci (QTLs) for heat tolerance. The HAP consists of synthetic derived lines, cultivars from the Central and West Asia and North Africa (CWANA) region, and elite breeding lines. The elite lines were developed following ICARDA's shuttle breeding approach (Tadesse et al., 2016), which has been designed to develop high-yielding and widely adapted germplasm with resistance or tolerance to the major biotic and abiotic stresses prevailing in the CWANA and SSA regions.

Phenotyping

The HAP was evaluated at Wad Medani station in Sudan and at Sids station in Egypt. Wad Medani station (14°24′04″ N, 33°31′11″ E, 410 m asl) is a global platform for heat tolerance research managed by the Agricultural Research Corporation of Sudan in collaboration with CIMMYT and ICARDA. The temperature ranges from 22 to 40°C with average of 37°C temperature in March during the flowering and grain-filling stages. The wheat cropping cycle is short at this station (from December to March). The soil is a calcareous Vertisol with a pH level of 8.5 and is deficient in N (300 mg L^{-1}) and P (4–6 mg L^{-1}). The Sids station in Egypt is a high-yielding station located in Benisuef governorate at 29°3′58.06″ N, 31°5′57.79″ E, 32.2 m asl. The soil is highly fertile clay loam with a pH level of 7.8. The temperature ranges from 20 to 35°C with average of 30°C during the cropping cycle (from December to April). Both stations are irrigated, and there is no rainfall during the growing season.

All 197 genotypes were planted around the first week of December in plots of 2.5-m length and six rows with 0.2-m spacing between rows at a seeding rate of 100 kg ha^{-1} using an α-lattice design with two replications both at Wad Medani and Sids stations for 2 yr (2014–2015). The trials were fully irrigated through gravity flood irrigation. At regular intervals, seven and nine irrigation events during the cropping cycle were applied at Wad Medani and Sids stations, respectively. Days to heading and maturity were recorded for each experiment when 50 and 90% of the plants in a plot reached the heading and maturity stages, respectively. Additionally, aboveground biomass was determined by cutting the whole wheat plants close to the ground (∼1–2 cm) in a 1-m^2 area from the central part of each plot at the time of harvesting. From this sample, the number of spikes and the number of grains were counted to calculate the number of spikes per square meter and the number of grains per spike. Thousand-kernel weight (TKW) was calculated by weighing 1000 grains of the sample. The sample grain yield and aboveground biomass at each plot were used to calculate the harvest index. Plant height was recorded as the distance from the ground to the tip of the spike excluding the awns.

Statistical Analysis

GenStat 17 (VSN International, 2014) was used to carry out ANOVA and descriptive statistics for yield and yield components of the HAP at each locations for each year (genotype × year) and across years and locations (genotype × location × year). A mixed-model approach for multiyear α-lattice designs was used in which genotype, year, and genotype × year were considered fixed factors, while the interactions between the year and the replication and block were considered random. Best linear unbiased estimations for the genotype factor were calculated following the abovementioned approach. To determine significant differences between genotypes, an $LSD_{0.05}$ test was used. Different variance components were calculated following the procedure indicated in Fehr (1993) as follows: genotypic variance (σ^2_G) = (genotype mean square − error mean square)/number of replications; phenotypic variance (σ^2_P) = environmental variance + genetic variance; and residual (error) variance (σ^2_E) = environmental variance. Broad-sense heritability or repeatability = (genetic variance/phenotypic variance).

Genotyping and SNP Filtering

DNA extraction was performed from 2-wk-old seedlings using pooled leaf samples from five individual plants frozen in liquid N and stored at −80°C before DNA extraction (Ogbonnaya et al., 2001). DNA from the 197 genotypes was sent for 15K single nucleotide polymorphism (SNP) genotyping to a service provider company (TraitGenetics). After discarding SNPs with minor allele frequency of <5%, and missing values, 13,007 SNPs were used for the analysis.

STRUCTURE Analysis

The genetic structure of the 197 genotypes was investigated following Pritchard et al. (2000) using 102 unlinked SNPs distributed across the wheat genome (TraitGenetics). The markers were selected to be at least 10 cM apart according to the consensus map (Wang et al., 2014). The resulting genome coverage consisted on an average of 4.85 markers per chromosome with a minimum of one marker for chromosome 4D and a maximum of eight markers for chromosome 5B. Bayesian clustering method was applied to identify clusters of genetically similar individuals using the software STRUCTURE version 2.3 (Pritchard et al., 2000). A burn-in length of 10^4 cycles was used to minimize the effect of starting configuration. This was followed by a simulation run of 10^6 cycles. Then, the best run was selected and the admixture level of the genotypes was extracted. We chose cluster values (K) ranging from 2 to 24 and six independent runs for each value. Additionally, the results were further confirmed by using the Bayesian information criterion (BIC) for different number of populations obtained by using the *k*-means algorithm implemented in adegenet package (Jombart, 2008) for R statistical software.

Linkage Disequilibrium

From the set of 13,007 SNP markers, 5350 markers with known position (TraitGenetics) were randomly selected to perform the LD analysis using Trait Analysis by Association, Evolution and Linkage (TASSEL) 4.3.1 software (Bradbury et al., 2007). Linkage disequilibrium was estimated, from a sample of random markers at each genome, as squared allele frequency correlations (R^2) and only P values $\leq$0.01 for each pair of loci were considered significant. Gene diversity and polymorphism information content (PIC) values were calculated using PowerMarker software version 3.25 (Liu and Muse, 2005).

Association Mapping

Best linear unbiased estimations for the genotypic factor for grain yield and yield components (biomass, grains per spike, spikes per square meter, and TKW), days to heading, and plant height at each location of the 197-genotype HAP and the corresponding SNP data were used for the association mapping. The GAPIT package for R (Lipka et al., 2012) was used to perform genome-wide association study (GWAS) analysis. To avoid population structure and major physiological traits confounding effect on the resulting QTL, the first three axes of the principal component analysis of the genotypic data, the STRUCTURE data for all subpopulation, and the average days to heading (DH) and plant height (PH) were used as covariables in the analysis. Thus, seven different mixed models were fitted for each dependent variable using, in addition to the kinship matrix, the aforementioned covariates and the combination of the two later ones (MLM+PCA, MLM+STRUCTURE, MLM+DH, MLM+PH, and MLM+DH+PH). The best model for each variable was selected based on the BIC results that were obtained from the analysis performed using the kinship matrix and the covariates. The model with the highest BIC was selected for each trait and location. False discovery rate values were calculated at r = 0.05 according to Benjamini and Hochberg (1995). Marker alleles with P values $\leq$0.001 were declared as suggestive QTLs for yield and yield-related traits.

RESULTS

Agronomic Performance of the 197 Lines

The means, minimums, maximums, variance parameters, and repeatability estimates of the agronomic traits for the 197 genotypes at Wad Medani and Sids stations are indicated in Table 1. Significant differences among the genotypes were observed for grain yield, TKW, days to heading, and maturity at both locations. The mean grain yield at Wad Medani station ranged from 1.6 to 4.5 t ha^{-1} with average yield of 2.87 t ha^{-1}. At Sids station (Egypt), the average yield was 9.98 t ha^{-1}, which is 3.5 times higher than at Wad Medani in Sudan. Synthetic derived genotypes such as PASTOR-2/HUBARA-5, LAKTA-1/QFZAH-21, SHIHAB-16, QADANFER-11, and ZEMAMRA-1 were among the highest yielding genotypes at both stations. The harvest index at both locations was comparable, with averages of 0.35 and 0.37 in Sudan and Egypt, respectively. On the other hand, the aboveground biomass was 3.26 times higher in Egypt than in Sudan (Table 2). The number of grains per spike and the grain weight were 1.32 and 1.21 times higher in Egypt than in Sudan, respectively. There was no significant difference among genotypes at both locations for the number of spikes per square meter. Varieties required 42 more days to reach heading in Egypt than in Sudan, and their grain-filling period was 1.4 times longer. In Egypt, the average height of the varieties was also 1.5 times higher than in Sudan.

Correlation among Agronomic Traits

The correlations between yield and other agronomic traits in the high-yielding (Sids, Egypt) and the heat-stressed (Wad Medani, Sudan) environments are indicated in Table 1. Under heat stress, grain yield was positively and significantly correlated with spikes per square meter (r = 0.35), grains per spike (r = 0.36), aboveground biomass (r = 0.60), harvest index (r = 0.51), and days to heading (r = 0.36). Similarly, aboveground biomass showed a strong and positive correlation with the number of spikes per square meter (r = 0.42), grains per spike (r = 0.15), and days to heading (r = 0.44, Table 2). In the high-yielding environment, grain yield was positively correlated to grain weight (r = 0.36), aboveground biomass (r = 0.36), and harvest

index ($r = 0.36$). Similar to the heat-stress scenario, aboveground biomass was positively correlated to spikes per square meter ($r = 0.38$), days to heading ($r = 0.26$), and plant height ($r = 0.30$). Grain yield performance in Wad Medani and Sids was also significantly correlated ($r = 0.66$, Fig. 1).

Polymorphism, Linkage Disequilibrium, and Population Structure

Out of the 15,000 markers obtained from genotyping, 13,007 were selected for analysis. A total of 4862 markers were located on the A genome, whereas 6411 and 1707 polymorphic markers were distributed on the B and D genomes, respectively. The position of 127 polymorphic markers was unknown. The average gene diversity, PIC, and average LD values were 0.30, 0.24 and 0.08, respectively (Table 3). Low values of gene diversity and PIC were observed in the D genome as compared with the A and B genomes. Similarly, the LD decay was slower in the D genome than the A and B genomes (Table 3, Fig. 2).

Structure analysis performed using 102 unlinked markers (located at least 10 cM apart) from all chromosomes and genomes showed that a model with three populations was the most accurate to explain the structure of the population (Fig. 3).

Table 1. Pearson's correlation between yield, yield components, and associated traits in Sudan (above diagonal) and Egypt (below diagonal).

Trait†	YLD	SM	GSP	TKW	HI	BM	DH	PH
YLD	–	0.35***	0.36***	−0.11	0.51***	0.60***	0.36***	0.16*
SM	<0.01	–	−0.04	−0.46***	0.01	0.42***	0.33***	−0.01
GSP	0.13	−0.05	–	−0.21**	0.25***	0.15*	0.16*	0.05
TKW	0.19**	−0.44***	−0.16*	–	0.03	−0.18*	−0.38***	0.11
HI	0.49***	−0.23**	0.10	0.14*	–	−0.34***	−0.04	−0.14*
BM	0.37***	0.38***	0.08	−0.06	−0.53***	–	0.44***	0.33***
DH	0.05	0.25***	0.13	−0.35***	−0.13	0.26***	–	0.36***
PH	0.10	−0.16*	0.02	0.22**	−0.20**	0.30***	0.15*	–

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

† YLD, grain yield; SM, spikes per square meter; GSP, grains per spike; TKW, thousand-kernel weight; HI, harvest index; BM, aboveground biomass; DH, days to heading; PH, plant height.

Table 2. Descriptive statistics and variance parameters estimated for yield and yield-associated traits of the 197 lines of the heat association panel grown in Wad Medani (Sudan) and Sids (Egypt) during the 2013–2014 and 2014–2015 crop seasons.

		Descriptive statistic			Variance parameter†			
Location	Trait‡	Mean	Min.	Max.	σ^2_G	$\sigma^2_{G\times Y}$	σ^2_{Err}	Heritability
Wad Medani	Yield, t ha^{-1}	2.87	1.59	4.48	1.09	0.265	0.301	0.84
	TKW, g	35.2	25.9	45.7	60.8	16.9	14.6	0.83
	BM, t ha^{-1}	8.51	5.67	12.8	6.80	3.5	3.49	0.72
	HI	0.35	0.19	0.52	0.01	0.006	0.006	0.69
	DH, d	56.8	46.8	80.8	89.2	11.96	4.73	0.93
	DM, d	89.7	78.8	111	76.1	8.44	7.05	0.93
	GFP, d	32.9	28.5	49.3	28.7	11.8	7.18	0.79
	PH, cm	70.1	59.3	84.3	95.2	21.5	15.5	0.87
	SM	467	337	578	6872	4632	4984	0.66
	GSP	39.6	26.1	56.4	112	52.7	56.5	0.74
	SSP	18.5	15.8	23.3	6.49	3.89	5.01	0.67
Sids	Yield, t ha^{-1}	9.98	7.06	12.5	3.32	2.61	1.93	0.65
	TKW, g	42.7	31.6	55.5	97.2	33.1	12.4	0.83
	BM, t ha^{-1}	27.8	19.9	35.7	32.3	29.1	24.8	0.61
	HI	0.37	0.23	0.50	0.009	0.007	0.007	0.61
	DH, d	97.4	86.3	108	58.6	14.3	13.6	0.85
	DM, d	144	137	151	25	14.3	13	0.71
	GFP, d	46.8	40.5	54.0	37.4	0.242	8.30	0.94
	PH, cm	105	91.3	127	156	35.6	30	0.86
	SM§	466	295	641	26,940	–	9173	–
	GSP	52.3	33.3	70.9	219	114	65.3	0.75
	SSP§	19.3	16.3	22.5	8.68	–	4.16	–

† σ^2_G, genotypic variance; $\sigma^2_{G\times Y}$, genotype × year variance; σ^2_{Err}, residual variance.

‡ TKW, thousand-kernel weight; BM, biomass; HI, harvest index; DH, days to heading; PH, plant height; SM, spikes per square meter; GSP, grains per spike; SSP, spikelets per spike.

§ Only data for the 2013–2014 season was available.

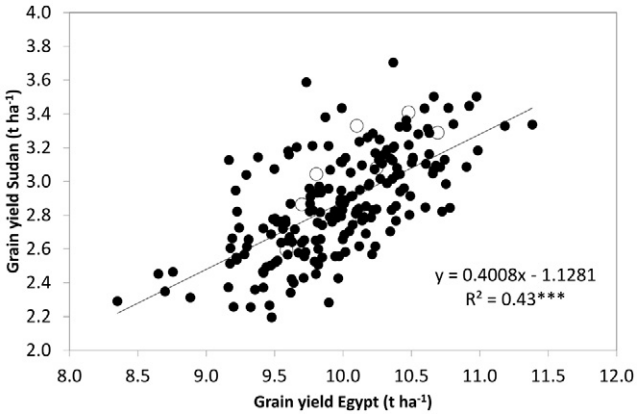

Fig. 1. Scatterplot of average yield performance of the heat association panel of spring bread wheat varieties in Sudan and Egypt during the 2013–2014 and 2014–2015 cropping seasons. Open symbols represent the checks used.

Table 3. Gene diversity, polymorphism information content (PIC), number of markers), chromosome size, marker density, and average linkage disequilibrium (LD) for all chromosomes, genomes, and overall means, calculated using single nucleotide polymorphism (SNP) markers in the 197 lines of heat association panel population.

Chr.	Gene diversity	PIC	No. of markers	Chr. size	SNP density	Avg. LD (R^2)†
				cM	no. loci cM^{-1}	
1A	0.28	0.23	700	111.7	6.27	0.08
1B	0.32	0.25	1016	111.7	9.10	0.08
1D	0.32	0.26	365	139.7	2.61	0.14
2A	0.32	0.26	687	120.5	5.70	0.08
2B	0.32	0.26	1208	144.2	8.38	0.07
2D	0.31	0.25	483	141.2	3.42	0.17
3A	0.30	0.24	619	165	3.75	0.08
3B	0.26	0.21	1007	137	7.35	0.06
3D	0.19	0.16	170	164.1	1.04	0.13
4A	0.30	0.24	496	161.8	3.07	0.08
4B	0.31	0.25	425	107	3.97	0.08
4D	0.30	0.24	62	112.9	0.55	0.11
5A	0.34	0.27	771	117.7	6.55	0.06
5B	0.32	0.26	1038	179.6	5.78	0.05
5D	0.24	0.20	237	167.8	1.41	0.11
6A	0.31	0.25	822	122.2	6.73	0.06
6B	0.29	0.24	949	110.4	8.60	0.09
6D	0.29	0.23	204	122.3	1.67	0.09
7A	0.29	0.23	767	167.5	4.58	0.04
7B	0.31	0.25	768	142	5.41	0.07
7D	0.23	0.18	186	195.1	0.95	0.07
Genome A	0.31	0.25	4862	NA‡	NA	0.07
Genome B	0.30	0.25	6411	NA	NA	0.07
Genome D	0.28	0.23	1707	NA	NA	0.13
Avg.	0.30	0.24	NA	NA	NA	0.08

† The LD was calculated on a random sample of markers for each chromosome.

‡ NA, data not available.

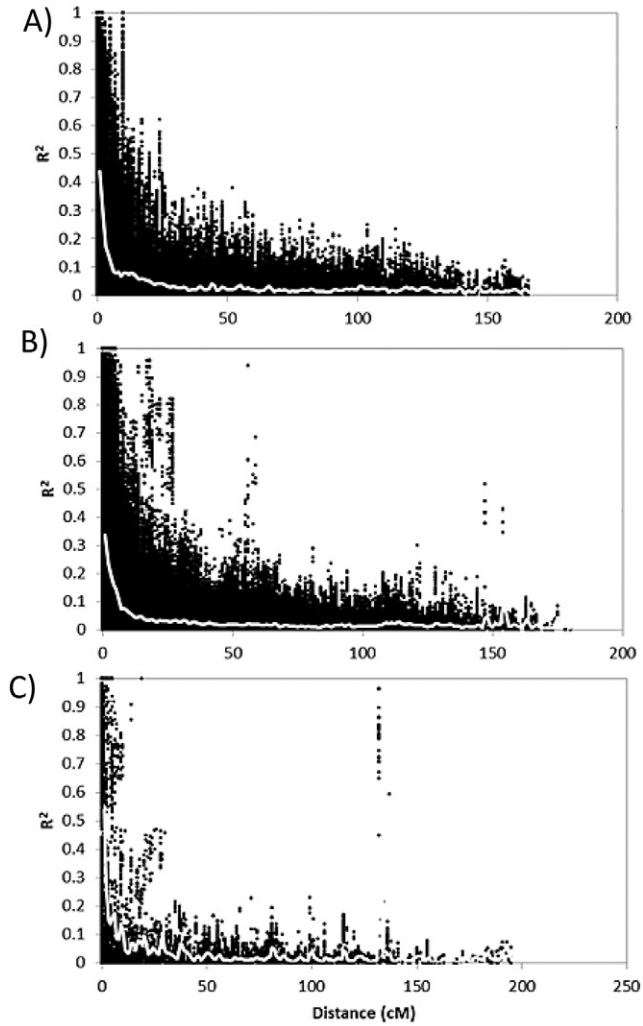

Fig. 2. Decline of linkage disequilibrium in 197-genotype heat association panel as measured by R^2 against genetic distance for (A) Genome A, (B) Genome B, and (C) Genome D.

Marker-Trait Associations

More than 200 significant MTAs were identified using the kinship matrix as random factor in the analysis. However, additional GWAS analysis were performed using population structure, plant height and heading date as covariables, and the best models were selected according to the BIC (Supplemental Tables S1a and S1b, Table 4). As a result, a total of 111 significant MTAs, independent from population structure, phenology, and plant height bias, were detected. Thirty-nine of the 111 MTAs were identified in Sids station (Egypt), whereas the remaining 72 were identified under severe heat stress in Wad Medani (Sudan).

The results showed that in both Egypt and Sudan, days to heading was mostly controlled by genes located on chromosome 5A. Additionally, significant markers for this trait were also found on chromosomes 2A and 6A at Sids station, Egypt (Fig. 4). In both Egypt and Sudan, the most significant marker was TG00019, which explained up to 19% of the variance in both environments and is labelled as a candidate for *Vrn-A1* by the genotyping service provider.

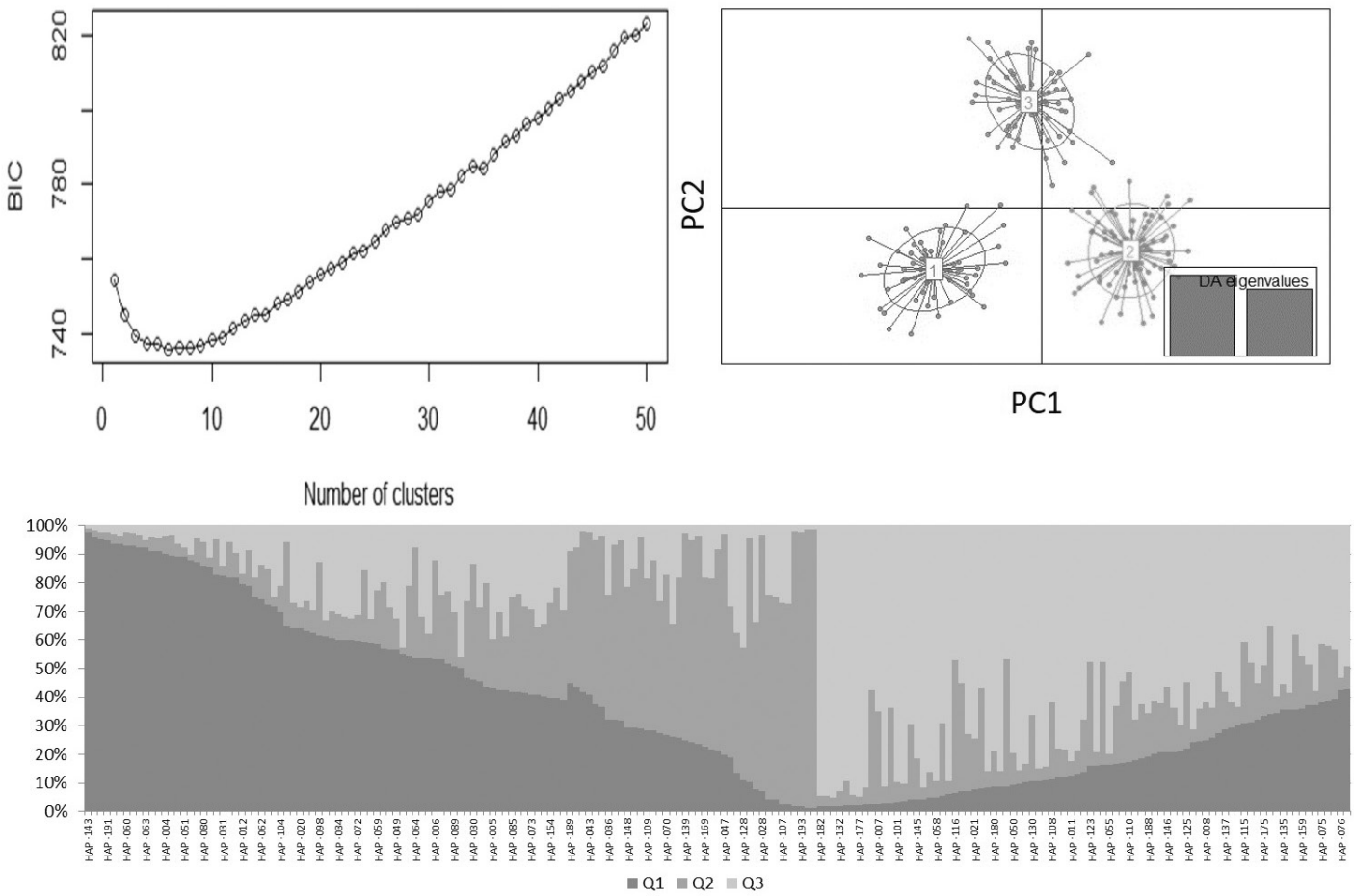

Fig. 3. Population structure among genotypes: (A) plot of the Bayesian information criterion (BIC) for each population number from 1 to 50 using a *K* means cluster, (B) biplot of the first two principal components (PCs) of the genotypic data, and (C) the proportion of the genome of each individual originating from each inferred population (a total of three, and each color represents a single population).

RAC875_c13931_205, wsnp_AJ612027A_Ta_2_1, and BS00022071_51 were also highly significant.

Three significant MTAs were found for yield performance in Egypt. One of the three markers was located on chromosome 4A (wsnp_Ex_c12812_20324622), and the other two were located on chromosome 3B (Table 4, Supplemental Tables S1a and S1b, Fig. 5) and were tightly linked to each other. Figure 6 depicts the average yield of the lines according to their base–pair combination at two unlinked SNP of the three identified (KukRi_c3243_1065 and wsnp_Ex_c12812_20324622). The results showed that the wheat genotypes carrying the thymine and cytosine bases at the KukRi_c3243_1065 and wsnp_Ex_c12812_20324622 markers, respectively, significantly outyielded the ones carrying the alternative base by 0.6 t ha^{-1} (6.8%, Fig. 6). All four combinations showed similar average heading date.

At Wad Medani (Sudan), the best model was the one adjusted by heading date (Supplemental Table S1). This is in line with the highly significant phenotypic correlation ($r = 0.36$) between grain yield and days to heading (Table 1). Seven MTAs were identified for yield (Fig. 4, Supplemental Tables S1a and S1b). One significant QTL was identified in chromosome 4A (wsnp_Ex_c12812_20324622) and accounted for >5% of the variance. This marker was also identified in Egypt. Another six markers were identified in chromosome 5A and located within 10 cM (Table 4, Supplemental Tables S1a and S1b). The marker with the highest logarithm of odds (LOD) was wsnp_Ex_c2526_4715978 (Table 4, Supplemental Tables S1a and S1b). The results showed that the genotypes carrying the cytosine base at the wsnp_Ex_c12812_20324622 and wsnp_Ex_c2526_4715978 markers outyielded the ones carrying the alternative bases by 15% (Fig. 6). The genotypes carrying the cytosine base at only one of the two markers increased their yield by 7.9 to 10% (Fig. 6). All four combinations showed similar average phenology (days to heading and maturity).

The best models identified for aboveground biomass GWAS in both environments involved phenology and plant height as covariables. The results of these models showed two MTAs in Egypt collocated on chromosome 7A and two MTAs on chromosomes 3B and 5B in Sudan (Table 4, Supplemental Tables S1a and S1b). The number of MTAs identified for TKW and number of spikes per square meter was more than for other traits. Up to 31 MTAs were identified for the number of spikes per square

meter, 26 in Sudan and five in Egypt. However, most of these MTAs were colocated and tightly linked, resulting in seven individual QTLs identified in Sudan—on chromosomes 3B, 5A, and 7A and two on 5B and 6A, in addition to five QTLs with unknown location—and two QTLs identified in Egypt on chromosomes 2A and 7A. The markers identified on chromosome 7A were detected at both locations (Wad Medani and Sids).

The TKW also showed polygenic control in both Sudan and Egypt, with 33 and 24 MTAs identified, respectively. The final number of QTLs identified after accounting for markers colocated was 14 in Sudan—on chromosomes 1A, 1B, 3A, 3B, 4A, 5A, 5B, 6A, 6B, 6D, 7A, and 7D and two on 7B, in addition to one with unknown location—and five in Egypt located on chromosomes 1B, 3B, 4A, 5A, and 6A. The markers located

Table 4. Summary of unique loci from the genome-wide association analysis performed on the average yield and yield components obtained in Wad Medani, Sudan, and Sids, Egypt, during the 2012–2013 and 2013–2014 seasons.

Location	Trait†	Marker	Chr.	Position	MAF‡	Simple model LOD§	Best model covariables¶	Best model LOD	Marker R^2
				cM					
Sudan	Yield	wsnp_Ex_c12812_20324622	4A	66.3	0.29	3.91	Kin+DH	3.54	0.05
		RAC875_Rep_c113313_607	5A	90.0	0.34	6.11	Kin+DH	3.30	0.05
		wsnp_Ex_c2526_4715978	5A	99.6	0.29	6.06	Kin+DH	3.89	0.06
	BM	KukRi_c23752_659	5B	71.6	0.40	3.58	Kin+DH+PH	3.52	0.05
		BS00086093_51	3B	134.8	0.34	2.21	Kin+DH+PH	3.31	0.05
	SM	RAC875_c2340_616	3B	125.4	0.35	2.84	Kin+DH	3.20	0.05
		wsnp_Ex_c7487_12808011	5A	68.9	0.31	3.98	Kin+DH	4.05	0.07
		ExcalibuR_c71712_180	5B	184.4	0.30	3.38	Kin+DH	3.68	0.06
		RAC875_c2253_1255	6A	37.1	0.08	2.93	Kin+DH	3.40	0.05
		wsnp_BE403818A_Ta_2_1	6A	84.7	0.39	3.13	Kin+DH	3.31	0.05
		Ku_c5938_4231	7A	101.5	0.43	2.53	Kin+DH	3.40	0.05
		TduRuM_contig46877_84	7B	56.9	0.07	3.14	Kin+DH	3.48	0.06
	GSP	KukRi_c19751_873	2B	108.0	0.29	3.35	Kin+DH	3.49	0.06
		ExcalibuR_c47996_509	2B	159.7	0.42	3.49	Kin+DH	3.10	0.05
		IAAV622	6A	82.4	0.35	2.67	Kin+DH	3.04	0.05
	TKW	ExcalibuR_c6255_1119	1A	70.1	0.09	2.81	Kin+DH	3.57	0.04
		RAC875_Rep_c119728_146	1B	75.1	0.46	3.56	Kin+DH	3.34	0.04
		BS00049032_51	3A	47.2	0.16	3.67	Kin+DH	3.83	0.05
		CAP8_Rep_c4453_136	3B	63.8	0.08	3.15	Kin+DH	3.14	0.04
		KukRi_c27648_350	4A	135.1	0.07	4.17	Kin+DH	4.09	0.05
		TA004832–0873	5A	8.7	0.43	4.03	Kin+DH	3.90	0.05
		RAC875_c68530_59	5A	23.5	0.47	3.55	Kin+DH	3.09	0.04
		BS00024993_51	5B	3.9	0.08	3.20	Kin+DH	3.56	0.04
		wsnp_Ex_c22089_31269531	6A	72.0	0.47	2.95	Kin+DH	3.17	0.04
		IAAV8704	6B	39.1	0.10	2.76	Kin+DH	3.25	0.04
		RAC875_c25839_225	6D	82.8	0.28	3.48	Kin+DH	4.34	0.06
		BobWhite_c8680_918	7A	51.4	0.07	3.36	Kin+DH	3.38	0.04
		ExcalibuR_c8486_419	7B	10.1	0.09	3.41	Kin+DH	3.27	0.04
		wsnp_Ex_c20320_29383733	7D	112.4	0.06	3.37	Kin+DH	3.73	0.05
Egypt	Yield	wsnp_Ex_c12812_20324622	4A	66.3	0.29	3.24	Kin	3.24	0.06
		KukRi_c3243_1065	3B	102.5	0.15	3.24	Kin	3.24	0.06
	BM	wsnp_Ex_c39221_46569987	7A	125.5	0.33	2.80	Kin+DH+PH	3.13	0.05
	SM	TduRuM_contig8350_243	2A	151.3	0.37	2.90	Kin+DH	3.35	0.06
		Ku_c5938_4231	7A	101.5	0.43	2.85	Kin+DH	3.34	0.06
	GSP	ExcalibuR_c47996_509	2B	159.7	0.42	3.79	Kin+DH	3.44	0.06
		KukRi_c19751_873	2B	108.0	0.29	3.15	Kin+DH	3.23	0.06
		CAP8_c3273_136	4B	71.5	0.19	2.94	Kin+DH	3.13	0.06
	TKW	JD_c3116_778	1B	75.1	0.49	3.03	Kin+DH+PH	3.56	0.05
		CAP8_Rep_c4453_136	3B	63.8	0.08	3.03	Kin+DH+PH	3.11	0.04
		GENE-0689_30	4A	135.2	0.07	3.63	Kin+DH+PH	3.25	0.04
		RAC875_c68530_59	5A	23.5	0.47	3.54	Kin+DH+PH	3.05	0.04
		TA004832–0873	5A	8.7	0.43	4.09	Kin+DH+PH	4.13	0.05
		wsnp_Ex_c25300_34566908	6A	71.7	0.47	3.24	Kin+DH+PH	3.22	0.04

† BM, biomass; SM, spikes per square meter; GSP, grains per spike; TKW, thousand-kernel weight.

‡ MAF, minor allele frequency.

§ LOD, logarithm of odds.

¶ Kin, kinship; DH, days to heading; PH, plant height.

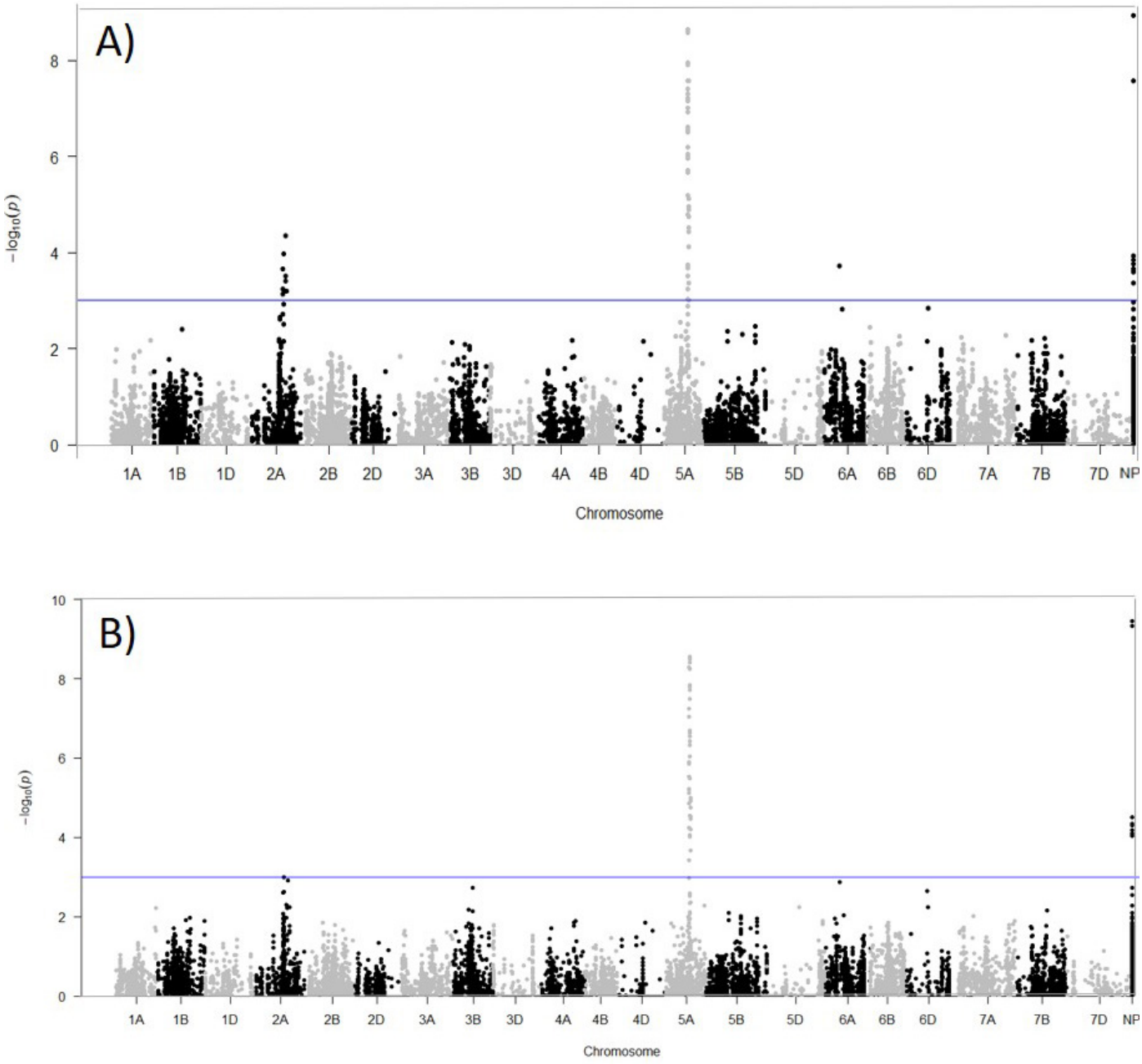

Fig. 4. Genome-wide association study results using 10,577 single nucleotide polymorphism markers in a 197 spring bread wheat population for the average days to heading in (A) Sids, Egypt, and (B) Wad Medani, Sudan, during the 2013–2014 and 2014–2015 cropping seasons. The horizontal line shows the $-\log_{10}(p) = 3$ threshold.

on chromosomes 1B, 3B, 4A, 5A, and 6A were significant at both environments. Finally, the number of grains per spike was controlled by five MTAs at each location. Four of the five markers were located on chromosome 2B, and three of them (KukRi_c19751_873, wsnp_Ex_Rep_c67543_66165372, and ExcalibuR_c47996_509) were significant both in Sudan and Egypt. Additionally, significant MTAs for both yield and number of grains per spike ($p < 0.005$) were found colocated at ~102 cM on chromosome 3B (Fig. 7).

DISCUSSION

Combining Heat Tolerance with Yield Potential

As heat stress becomes a very important yield-limiting factor, ICARDA's wheat breeding program aims to combine heat tolerance with high yield potential to maximize yield gains during heat stress and good seasons, respectively. To this end, the wheat breeding program at ICARDA, in collaboration with the national agricultural research centers of Sudan and Egypt, has made rigorous efforts to identify elite spring bread wheat genotypes combining high yield potential and heat tolerance

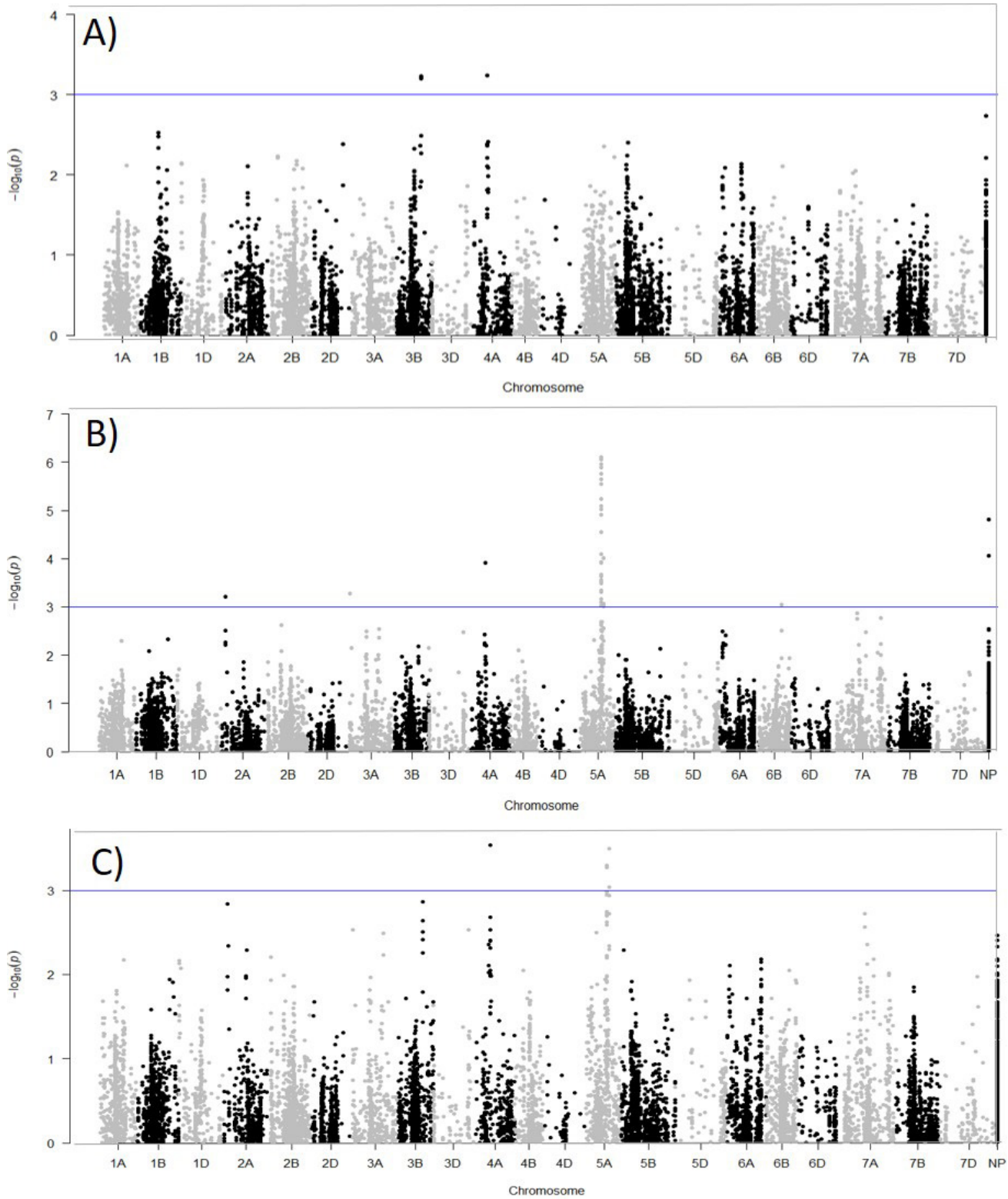

Fig. 5. Genome-wide association study results using 10,577 single nucleotide polymorphism markers in a 197 spring bread wheat population for the average yield in (A) Sids, Egypt, (B) Wad Medani, Sudan, and (C) in Wad Medani using the days to heading as a covariable during the 2013–2014 and 2014–2015 cropping seasons. The horizontal line shows the $-\log_{10}(p) = 3$ threshold.

with acceptable grain quality and resistance to yellow rust (*Puccinia striiformis* Westend.) (Tadesse et al., 2016). Phenotypic correlations between yield and related agronomic traits showed that in both Sudan and Egypt, yield is positively correlated with number of grains per spike, number of spikes per square meter, and biomass and negatively correlated with TKW. Additionally, heading date was positively correlated with yield, indicating that late varieties outyielded the early ones. These results contradict, to some extent, the ones obtained in previous studies

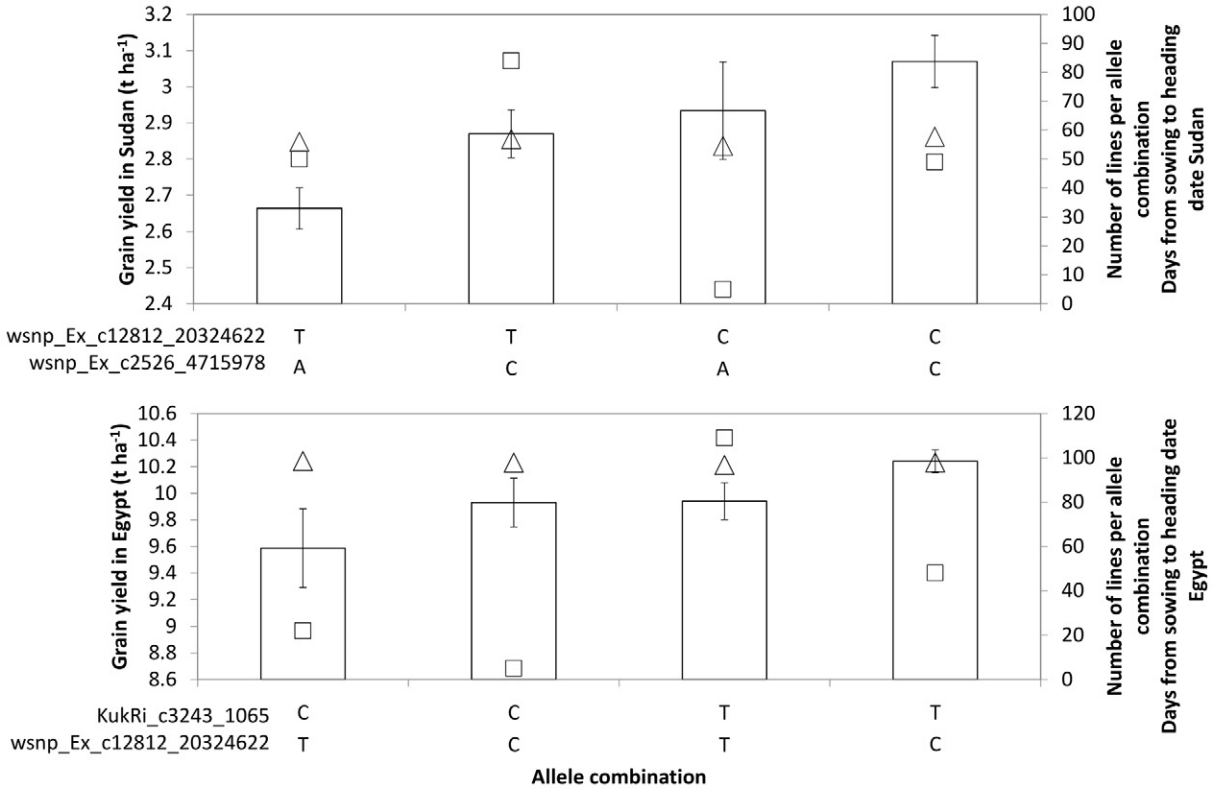

Fig. 6. Average grain yield (bars) from two field experiments performed in (A) Wad Medani, Sudan (2013–2014 and 2014–2015) and (B) Sids, Egypt (2013–2014 and 2014–2015) with 182 bread wheat genotypes having four base–pair combinations of two independent single nucleotide polymorphism markers associated with grain yield (logarithm of odds > 3.0): wsnp_Ex_c12812_20324622 and wsnp_Ex_c2526_4715978 in Sudan (A) and KukRi_c3243_1065 and wsnp_Ex_c12812_20324622 in Egypt (B). The number of lines and the average days to heading date per base–pair combination are indicated in open squares (□) and triangles (△), respectively. Confidence level at α = 0.05 is also shown for each combination.

performed under heat stress (Pinto et al., 2010), reporting a positive correlation of yield with TKW and a negative correlation with days to heading. The differences could be attributed to the different types of mapping populations used and the difference in the environments. In the case of Pinto et al. (2010), a biparental population was used, reducing the potential number of strategies to cope with high temperatures. Ideally, it is the interest of breeders to have high-yielding genotypes with large seed size. Some breeders have used indirect selection for grain size to improve yield, whereas others have used biomass or number of seeds per square meter. At ICARDA, our approach is based on obtaining yield increases with acceptable seed size by improving the number of grains per square meter. This increase is attained by increasing biomass or number of spikes per square meter because vigorous genotypes cover the ground early and close the canopy tightly reducing evapotranspiration and excessive heating. Additionally, clear differences can be identified between the heat-stressed environments selected by Pinto et al. (2010) and the ones used in the present study. Early flowering in late heat- and drought-stress environments is a widely used strategy to perform the grain-filling process under milder conditions that usually result in higher grain weights (Sanchez-Garcia et al., 2012, 2013). However, the environment used in the present study (Wad Medani) is characterized by high temperatures not only at late stages (terminal heat stress), but throughout most of the cropping cycle. Therefore, under these conditions, accelerating flowering to escape late heat-stress conditions would be less useful. In fact, the results obtained in the present study confirm the findings of a previous study also performed in Sudan (Lopes et al., 2012), highlighting the specific conditions of Sudan as a heat-stressed environment.

The negative correlation of grain yield with grain-filling period and canopy temperature depression indicates that the high-yielding genotypes have cooler canopies and fill their grain rapidly (Tadesse et al., 2015; Ogbonnaya et al., 2017). Pedigree analysis of the high-yielding genotypes indicated that some of them are synthetic derived lines (e.g., PASTOR-2/HUBARA-5, LAKTA-1/QFZAH-21, SHIHAB-16, QADANFER-11, and ZEMAMRA-1), whereas others are derived from genotypes such as Kauz, which is well known for its drought tolerance (Zhang et al., 2009). The top-yielding genotypes reported here under heat stress conditions were also among the highest yielding genotypes in Morocco under moisture stress environments, indicating the confounding effects of

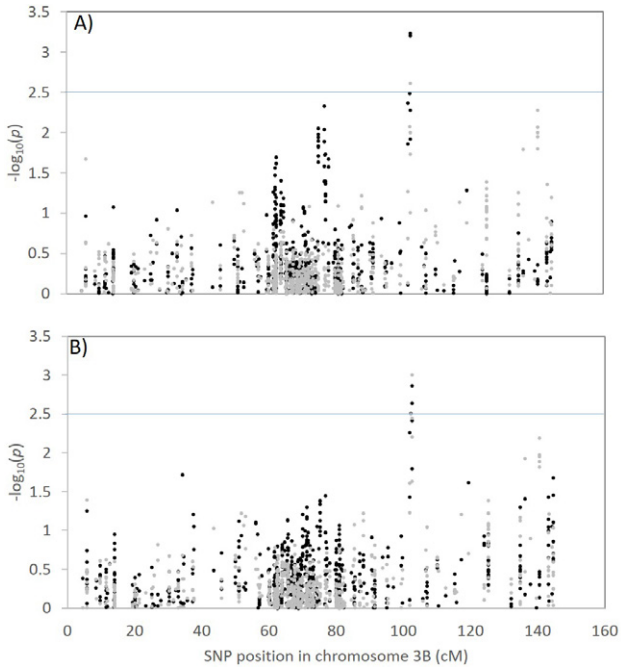

Fig. 7. Genome-wide association study results using 714 single nucleotide polymorphism (SNP) markers on chromosome 3B in a 197 spring bread wheat population for the average grain yield (gray dots) and grains per spike (black dots) in (A) Wad Medani, Sudan, and (B) Sids, Egypt, during the 2013–2014 and 2014–2015 cropping seasons. The horizontal line shows the $-\log_{10}(p) = 2.5$ threshold.

genes for drought and heat stress tolerance (Tadesse et al., 2016; Ogbonnaya et al., 2017). Similar results of such confounding effects of genes for heat and drought tolerance have been reported by other authors (Pinto et al., 2010; Edae et al., 2014; Ogbonnaya et al., 2017).

Marker-Trait Associations

Several MTA studies have been performed in wheat to identify closely linked molecular markers that could be used for marker-assisted selection and thereby increase the efficiency of conventional breeding efforts in pyramiding genes of interest in desired cultivars (Pinto et al., 2010; Edae et al., 2014; Tadesse et al., 2015; Ogbonnaya et al., 2017). However, it is well known that traits controlled mostly by major genes such as plant height and phenology can have a confounding effect over wheat performance-related traits (Olivares-Villegas et al., 2007; Lopes et al., 2012). Therefore, in the present study, we used plant height and days to heading as covariables to identify QTLs that are independent for these two traits and, presumably, more reliable. Three QTLs correlated to grain yield under heat-stressed environments of Sudan and Egypt were identified in the current study. One of them (linked to *wsnp_Ex_c12812_20324622*) was significant in both locations. Previous studies have also reported significant markers located on chromosome 4A associated with yield and yield components under heat stress (Pinto et al., 2010; Sukumaran et al., 2015).

A significant QTL for yield performance located in the long arm of chromosome 3B was also found in Egypt. At the same chromosomal position (~120 cM), although with a lower level of certitude (LOD > 2.5), other significant MTAs for yield and grains per spike were found both in Egypt and Sudan. This chromosomal region was already associated with yield and grains per unit area in previous studies performed under heat stress (Pinto et al., 2010; Sukumaran et al., 2015).

Another environment-specific QTL for grain yield was found on chromosome 5A at Wad Medani, Sudan. The significant markers identified on chromosome 5A were located between 99 and 90 cM, and the latter ones are linked to the *Vrn-A1* locus. Previously, markers such as BS00022071_51 were identified *as Vrn-A1* (Sukumaran et al., 2016), which is located at 90 cM (Wang et al., 2014). However, the results showed that these markers are independent of the flowering date of the varieties. Similar results have been found also under heat stress in Mexico with a different population (Sukumaran et al., 2016, suggesting the presence of a heat-tolerance-related gene situated close to *Vrn-A1*. Genes controlling the response to frost damage and osmotic adjustment have been found on chromosome 5A, such as alternative alleles of the *Vrn-A1* locus (Snape et al., 2001; Eagles et al., 2011) or other genes like *Fr2* (Båga et al., 2007).

Pinto et al. (2010) identified cultivar SERI as the carrier of negative alleles for grain yield on chromosomes 3B and 4A in a SERI/BABAX RIL population. SERI is a cultivar grown worldwide that is also present in the pedigrees of several lines in the present study, and it provides wide adaptation and disease resistance (*Yr9* and *Pm8*) (Rajaram et al., 1983). Additionally, SERI and its descendant Attila have also been found to carry the *Vrn-A1w* allele on chromosome 5A (Eagles et al., 2011). This allele, originated from the Russian cultivar Kavkaz and present in cultivars adapted to very cold conditions, has been associated with osmotic regulation under frost and drought conditions (Eagles et al., 2011). The high significance of markers situated close to the *Vrn-A1* locus in the present study could indicate that this osmotic regulation could also be useful under heat conditions. However, further specific research is needed to confirm this hypothesis.

Several MTAs for spikes per unit area and grain weight were identified in the present study. However, none of them showed an impact on yield performance. One possible explanation is the well-documented tradeoffs between yield components. Increases in one of the components have a negative impact on the others, cancelling their effect on final yield performance. In spite of this, improving our knowledge of the genetic control of the yield components might help to identify linked markers that could potentially assist in improving the breeding process. Of particular interest would be the MTAs that are

consistent across locations and therefore have more probability of being useful in different environments. Several MTAs were identified in both Egypt and Sudan on chromosome 7A. The number of spikes per unit area is an important yield component that has contributed to wheat genetic gains and adaptation during the 20th century (Sanchez-Garcia et al., 2012, 2013). However, this trait usually gets neglected due to the time and energy required to measure it. Therefore, new research aiming to increase the knowledge of its genetic control can be of great help to the breeding community, especially those investigating yield performance in multiple environments.

Thousand-kernel weight is an essential grain yield component and is often considered an important indicator of the terminal stress suffered by the crop. In the present study, several MTAs were found to be associated with TKW, of which five were detected both at Sids and Wad Medani. The significant markers in both environments were located on chromosomes 1B, 3B, 4A, 5A, and 6A. Previous studies reported QTLs for TKW on the same chromosomes (Börner et al., 2002; Pinto et al., 2010; Sukumaran et al., 2016), suggesting that these chromosomes would play a role in controlling kernel weight.

CONCLUSION

The present results indicate the presence of both common and environment-specific QTLs in ICARDA elite spring bread wheat germplasm for yield and yield components under heat-stressed environments in Sudan and Egypt, indicating the potential of these MTAs to improve these traits. We also reinforce the previously reported negative impact on yield performance of two QTL on chromosome 3B and 4A possibly associated with the presence of the cultivar SERI in the pedigree. The associated markers detected in this study can be used to select against these QTLs in breeding programs. Additionally, our results identified a stress-tolerant QTL on chromosome 5A, independent from flowering time. Our results indicate that under severe heat stress, genotypes carrying the positive allele of the markers wsnp_Ex_c12812_20324622 and wsnp_Ex_c2526_4715978, located on chromosomes 4A and 5A, respectively, have 15% greater yield on average. Therefore, these markers, irrespective of their specific effect, could be used in breeding programs to increase yield under heat stress. The top high-yielding and heat-tolerant genotypes identified in this study have been distributed to the national research systems in the CWANA and SSA regions through our international nursery system for potential direct release and/or use as parents after local adaptation trials by the National Agricultural Research Systems of the respective countries.

Conflict of Interest

The authors declare that there is no conflict of interest.

Supplemental Material

Supplemental material is available online for this article.

Acknowledgments

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