

Durum Wheat Heat Tolerance Loci Defined via a North-South Gradient

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Research Article

Keywords: Grain yield, GWAS, Heat gradient, Heat stress susceptibility index, KASP marker, QTL

Posted Date: October 14th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-2124092/v1>

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Version of Record: A version of this preprint was published at The Plant Genome on December 7th, 2023. See the published version at <https://doi.org/10.1002/tpg2.20414>.

Abstract

The global production of durum wheat (*Triticum durum* Desf.) is hindered by a constant rise in the frequency of severe heat stress. Three different germplasm panels were investigated under a range of heat stress to identify tolerant germplasm. The 'discovery panel' was field tested in the south of Morocco and along the Senegal River implementing a mild-to-strong North-South heat stress gradient. A subset of this same panel was also exposed to simulated terminal heat stress by applying plastic tunnels at the time of flowering. The 'investigation panel' and a 'validation panel' were field tested along the Senegal River under >32°C temperature throughout the season. Grain yield and its components were recorded at each site and a heat stress susceptibility index (HSI) was calculated. A total of 7,652 polymorphic single nucleotide polymorphisms (SNPs) defined the diversity of the first panel, while 5,642 SNPs defined the 'investigation panel'. Genome-wide association study revealed that 36 quantitative trait loci were associated with the target traits in the 'discovery' panel, of which five were confirmed in the 'subset' tested with plastic tunnels and in the 'investigation panel'. A haplotype study confirmed that Q.icd.Heat.003-1A, Q.icd.Heat.007-1B and Q.icd.Heat.016-3B are additive in nature and carrying the positive alleles at all three loci resulted in a 17% higher grain yield under heat stress. The underlying SNPs were converted into Kompetitive Allele Specific PCR (KASP) markers and tested on the 'validation' panel, confirming that each explained 4 to 9% of the phenotypic variation for grain yield under heat stress.

Key Message

Q.icd.Heat.003-1A, Q.icd.Heat.007-1B and Q.icd.Heat.016-3B carry positive alleles, which resulted in 17% higher grain yield under heat. Underlying SNPs were converted into KASPs that can assist breeding in heat prone areas.

Introduction

Durum wheat (*Triticum durum* Desf.) is grown on over 17 million hectares worldwide and accounts for around 6% of total wheat production (Sall et al. 2019). Durum wheat is a staple crop in North Africa and West Asia (Elias and Manthey 2005) and it is slowly becoming a strategic crop in sub-Saharan Africa and South Asia (Sall et al. 2019). Heat is one of the most important abiotic stresses affecting this crop, with an estimated 58% of global production undergoing mild to severe losses due to high temperatures each year (Kosina et al. 2007). For instance, in France the wheat yield has been predicted that it will suffer a 3.5 to 12.9% reduction in the medium term (Wang et al. 2018). In China, researchers reported that wheat productivity could be reduced by 3 to 10% due to a 1°C increase in temperature during the growing period (You et al. 2009). Similar predictions are also supported by Eruygur et al. (2016), suggesting the loss of 8–23% of global wheat production by the end of 2100. Furthermore, temperatures are expected to rise in the near future due to climate change (IPCC 2018; Ortiz et al. 2008). Hence, technological solutions must be converted rapidly to actions. Breeding heat tolerant crops remains one of the most viable options to maintain adequate production. For that, a key step is the identification of the loci responsible for the adaptive response of tolerant genotypes. Once identified, these loci can then be pyramided using targeted

crossing and marker-assisted selection schemes. Genome-wide association mapping (GWAS) is a valuable tool to pinpoint the genomic regions involved in the control of useful traits, even for traits with complex inheritance such as grain yield under stress (Cossa et al. 2007; McIntyre et al. 2010; Maccaferri et al. 2011; Charmet et al. 2009; Rehman-Arif et al. 2012). In durum wheat, quantitative trait loci (QTL) for heat tolerance have been identified on chromosomes 1B, 2B, 3A, 3B, 5A, 5B, 7A, and 7B (Mohammadi et al. 2008b; Maccaferri et al. 2011; Awlachev et al. 2016). However, their deployment in breeding has been thus far limited, and the environment utilized to investigate the heat stress did not strictly represent the expected dreadful scenario that climate change will impose by the end of the century.

Recently, a panel of durum cultivars and elite breeding lines (Kabbaj et al. 2017) was evaluated for two years at two sites under extremely warm conditions along the Senegal Basin, where the maximum daily temperature remains above 30 °C throughout the growing season (Sall et al. 2018a, b). Several entries have been identified as showing good response to this severe heat stress. By identifying the genomic region associated with heat tolerance in durum wheat, the work presented here aimed to further advance the results obtained in that study. To that scope, a North-South heat gradient was implemented by combining the field performances along the Senegal River (Sall et al. 2018b) with those obtained from field trials in Morocco, 14 parallels further North. The QTL were first discovered in the larger ‘discovery panel’, then further confirmed in a different ‘investigation panel’, and ultimately these were converted into Kompetitive Allele Specific PCR (KASP) technology and ‘validated’ on a third panel. These KASPs are now ready to be deployed by marker-assisted selection (MAS) across durum wheat breeding programs to improve its heat tolerance globally.

Materials And Methods

Plant material

The present study was conducted using three germplasm panels. The first panel was defined as ‘discovery panel’ and it includes 216 durum wheat modern lines selected from a larger panel of 384 entries described by Kabbaj et al. (2017). A further subset of this panel comprising 42 durum wheat modern lines selected for their similarity in flowering time and genetic diversity as described by El Hassouni et al. (2019) was also used. A second germplasm set defined as ‘investigation panel’ included 144 advanced durum wheat elite lines from the breeding program of the International Center for Agricultural Research in the Dry Areas (ICARDA). These elite lines were selected because at least one of the top heat tolerant entries identified by Sall et al (2018b) via phenotypic screening of the ‘discovery panel’ was incorporated into their pedigree. The third set of entries defined as ‘validation panel’ included 94 ICARDA’s elite lines that constituted the 40th International Durum Observation Nurseries (IDON40), which were provided to over 50 partners around the World for testing.

Field stations

Two irrigated field stations were used for phenotyping in the South of Morocco: Melk Zhar (MKZ, 30.019832, -9.486629) during seasons 2014–2015 and 2015–2016, and Tessaout (TES, 29.8348302,

-8.5786262) during season 2015–2016. Melk Zhar has sandy soil with low water holding capacity while Tessaout has red silt soil with high water holding capacity. Details on the growing conditions along the Senegal River can be found in Sall et al. (2018b). Briefly, Kaedi in Mauritania (KED, 15.999592, -13.071842) and Fanaye in Senegal (FAN, 16.538348, -15.221948) were used during 2014–2015, 2015–2016, 2018–2019, and 2019–2020 seasons. Fanaye has sandy-clay soil with higher organic matter and good water holding capacity, while Kaedi has lighter sandy-loam-clay soils with intermediate water holding capacity. At all locations, irrigation was guaranteed to avoid any moisture stress. In Melk Zhar during the 2014-15 season, 26 drip irrigations of 13 mm each were applied in addition to the 297 mm of rainfall for a total amount of 635 mm of water.

During season 2015–2016, the number of irrigations was increased to 30 to compensate for the extremely low rainfall (47 mm) to achieve a total of 437 mm of moisture. In Tessaout, nine gravity irrigations were provided for an estimated amount of 360 mm of water in addition to 95 mm of rainfall for a total of 455 mm. In Kaedi, gravity irrigations are performed every seven days for ten irrigations in total over a three-month period, which provides a total moisture amount of 320 mm. In Fanaye, the same number of irrigations was applied except that the estimated total moisture level was a higher (380 mm). At all stations, the same amount of fertilizer was provided. Pre-sowing 50 units of nitrogen (N), phosphate (P), and potassium (K) were applied. After sowing, all stations except Melk Zehr received an additional 100 units of N via two split applications, the first one at fourth leaf stage and the second just before the flowering stage. In Melk Zehr, the additional 100 units of N were applied at four different plant growth stages (25 units at each stage). Planting was conducted during the third and fourth week of November at Melk Zehr and Tassaout, while it was carried out during the second week of December along the Senegal River.

Phenotypic characterization

The ‘discovery panel’ was tested at Melk Zehr (two seasons), Tessaout (one season), Kaedi and Fanaye (two seasons). The sub-set of the ‘discovery panel’ was tested as explained in El Hassouni et al. (2019) applying plastic tunnels at the time of flowering to increase the temperatures by up to 15°C. The ‘investigation panel’ was tested at Fanaye during the 2018–2019 and 2019–2020 crop seasons. The ‘validation panel’ was tested at Fanaye during the 2017–2018 crop season. All panels were field-tested in a partially replicated (augmented) design with blocks of size 24 including 4 replicated checks each. Phenology traits, yield components and grain yield (GY) were recorded for all genotypes. The days to heading (DtH) was recorded as the number of days elapsed from sowing to the moment that 50% of the plot showed spikes emerging from the flag leaf at stage 59 in the Zadoks scale (Z59: [Zadoks et al. (1974)]). Before maturity (Z83-87), the number of fertile spikes per meter square (Spk.m²) were counted. Days to maturity (DtM) was recorded when 50% of the spikes turned yellow (Z91-92). The length of the grain filling period (GFP) was then computed as the difference between DtM and DtH. Plant height (PLH) was measured in cm from the ground to the top of a representative ear excluding its awns. For each plot, only the middle rows that cover an area of 2.7 m² were harvested, dried and the biomass (Biom) weighted before threshing. The weight of the threshed grains was converted into yield (GY) expressed as kg ha⁻¹.

The ratio between the GY and Biom was expressed as harvest index (HI). One thousand grains were weighted in grams as 1000-kernel weight (TKW). The number of grains per meter square (Gr.m²) was calculated using the weight of the grains harvested from 2.7 m² area and the weight of one kernel derived from the TKW value, as per:

$$\text{Gr. m}^2 = \frac{\text{Harvested weight of plot}}{2.7 \text{ m}^2 \times \frac{\text{TKW}}{1000}}$$

The number of grains per spike (Gr.spk) was derived from dividing the calculated number of grains per unit area by the number of spikes recorded for the same area, as follows:

$$\text{Gr. spk} = \frac{\text{Gr. m}^2}{\text{Spk. m}^2}$$

Statistical analysis of phenotypic data

Each location for each season was defined as a single environment, thereby resulting in 10 environments (KED15, KED16, FAN15, FAN16, FAN18, FAN19, FAN20, MKZ15, MKZ16, and TES16). Analyses of variance were performed, and best linear unbiased estimators (BLUEs) were obtained for each environment individually using the 'lme4' package in R Studio V 4.0.1 (R Development Core Team, 2020). Combined analyses for the 'discovery panel' was conducted using best linear unbiased estimator (BLUE) considering all sources of variations as fixed effects. For the 'investigation panel', the two environments (FAN19 and FAN20) were considered individually without combined analysis, and the same was done for the 'validation panel' since only one environment was available (FAN18).

The climatic information for maximum and minimum temperatures was collected from weather stations located at the research farms. The average maximum and minimum temperatures were then calculated for each major plant stage (vegetative stage, tillering, flowering time, and grain filling period) for each environment. This climate matrix was then used to conduct regression analysis against grain yield to determine the most significant climatic factor affecting yield performance of the germplasm. At the same time, the climate matrix was used to cluster environments using 'Dcluster' package in R Studio V 4.0.1, via hierarchical clustering based on Euclidean distance determined through principal component analysis (PCA) (Table 1). Using the two environmental clusters, genotypes tolerant to heat stress were identified implementing the heat susceptibility index (HSI) as suggested by El Hassouni et al. (2019) by the equation:

$$HSI = \frac{1 - \frac{Y_s}{Y_p}}{1 - \frac{Y_{s_{max}}}{Y_{p_{max}}}}$$

where Y_s and Y_p are values of the wheat lines evaluated under stressed (s) and non-stressed (p) conditions, respectively, and $\bar{Y}_s$ and $\bar{Y}_p$ are the mean values of all lines for the specific trait obtained under stress and non-stress conditions, respectively. Based on environmental clustering, the Moroccan sites were considered as non-stressed (p), while those of Senegal River as heat stressed (s). HSI was determined for GY, TKW and Gr.spk^{-1} . The individuals achieving an HSI score of less than 0.70 were considered as heat “tolerant”, while values of less than 1.2 as “non-tolerant”, and scores above 1.2 defined “susceptible” entries.

Genotyping, population structure and LD pattern analysis

For the ‘discovery panel’, a complete description of the genotyping methodology and definition of population kinship was previously presented in Kabbaj et al. (2017). Briefly, the Axiom 35K array was used to screen entries of the ‘discovery panel’. A total of 7,652 high quality polymorphic SNPs with minor allele frequency (MAF) of above 5% were retained for downstream analysis. The sequences of these markers were aligned to the Svevo genome assembly (Maccaferri et al. 2019) using a cut-off of above 97% sequence identity. A selection of 500 highly informative ($\text{PIC} > 0.25$) and evenly spaced markers were used to determine that modern lines of this panel are separated into six sub-groups (Kabbaj et al. 2017) representing the breeding programs of origin. Linkage disequilibrium (LD) decay was estimated using the “Neanderthal” method (Jujuman 2017) at 51.3 Mbp as presented in Bassi et al. (2019) for the ‘discovery panel’. The ‘investigation panel’ was genotyped with a 23K array chip developed by SGS – Institut Fresenius Trait Genetics section (Germany) which incorporates 14.5K SNPs from the 90K Infinium Array, 8.5K SNPs from the Axiom array, and 265 SNPs that were reported to be linked to genes. Marker curation was conducted as for the ‘discovery panel’, which resulted in 5,642 polymorphic SNPs. These were also aligned to the Svevo genome assembly (Maccaferri et al. 2019), and linkage analysis revealed that the LD decay was 36.3 Mbp. Kinship analysis by STRUCTURE identified three clusters among these elites.

Kompetitive Allele Specific PCR (KASP) assays were designed by providing LGC Genomics (UK) the array sequences of the most representative markers underlying the QTL of interest. The assays comprised three primers (one common primer, and one primer for each of the two alleles) for each marker and were then used to screen the ‘validation panel’. All genotyping was done using their property complete genotyping service. The primer sequences of the markers are protected by commercial rights and cannot be disclosed here. However, they can be purchased by users as a service from LGC by providing the marker names given here as references.

Genome-Wide Association Study (GWAS)

GWAS was carried out in TASSEL 5.0 software (Bradbury et al. 2007) using the mixed linear model (MLM), population structure coefficients (Q), and kinship matrix (K) for the ‘discovery panel’. While the generalized linear model (GLM) was used for ‘investigation panel’ as it better fit the data. DTH was used as a covariate for all studies. The traits GY, TKW, and Gr.spk were investigated via GWAS at each environment individually, and as combined across environments using HSI. The significance of marker-trait association (MTA) was assessed by Bonferroni corrected equation as suggested by Duggal et al.

(2008) at $P < 0.05$. Hence, the significant LOD threshold was set at 2.69 and 2.83 for the 'discovery panel' and 'investigation panel', respectively. MTAs located within less than two times the LD decay distance were considered as underlying the same haplotype block and hence merged into one QTL. For correction, regression analysis was performed between haplotype scores of flanking markers under each QTL. Flanking markers with values $r^2 < 0.2$ were considered unlinked and therefore new QTL boundaries were defined. Pearson's critical value (Pearson 1895) for correlation was squared to obtain a critical $r^2 = 0.032$ at $P < 0.05$ for both panels, which was used as the threshold to determine QTL that explained a significant part of the phenotypic variance. To further remove the effect of phenology from QTL discovery, any QTL overlapping with a significant MTA identified for DTH (see Gupta et al. 2020) was removed from all downstream considerations. Haplotype analysis was conducted on the 'discovery panel' to define which QTL were additive in nature. Classes of alleles at the main QTL were defined for each genotype. A two-way analysis of variance (ANOVA) and the least significant difference (LSD) test were used to determine significantly superior classes of alleles using genotypes within the same class of allele as replications. Those QTL that were identified in the same genomic region by the 'discovery panel', and its 'subset' using plastic tunnels (El Hassouni et al. 2019), as well as in the 'investigation panel' were considered the most critical and were then further validated using KASP method.

Markers validation by KASP

The conversion of Axiom markers to KASP markers was achieved by submitting the sequences containing the array markers associated with the desirable traits to LGC Genomics for in-silico designing of KASP primers using their proprietary software. Those that passed the in-silico criteria were purchased and used to genotype the 'validation panel'. For each amplified marker that showed polymorphism, the correlation significance cutoff between the phenotype and haplotype was set at $r = 0.105$ following Pearson's critical value for $P < 0.05$ (Pearson 1985). In addition, the top 20 and bottom 20 lines in terms of yield performances in environment FAN18 were considered as the true positive and true negative for heat tolerance. Hence, the accuracy was calculated as the ratio of the correct allelic call among all, sensitivity as the ratio of the correct positive allelic calls among the top 20 high-yielding lines, and specificity as the ratio of the correct negative allelic calls among the 20 lowest yielding lines.

Results

A North-South heat gradient

There are 14 parallels between the Moroccan stations (Melk Zehr and Tessaout) and the two stations along the Senegal River of Kaedi (Mauritania) and Fanaye (Senegal). This results in an average temperature difference of 10°C during the crop season (Table 1). The average min and max temperatures across the season at the stations located along the Senegal River basin were 17°C and 35°C, respectively, while the average min and max temperatures at the Moroccan stations were 8°C and 22°C, respectively. Temperatures varied across the sites and years with much warmer temperatures during the season 2015–2016, mainly during the flowering windows. The maximum temperature at flowering in Tessaout

station was 21°C during the season 2015–2016, in Melk Zhar was 22°C, in Fanaye it was 37°C while in Kaedi it was 39°C. Furthermore, at all the sites, the moisture was kept beyond the level of stress for the crop to complete the cycle, making temperature the main limiting factor for the plants. The average maximum and minimum temperatures were recorded for each crop stage (vegetative, tillering, flowering, and grain filling) for each environment, and these were regressed against grain yield (**Table S1**). The maximum temperature was the most critical factor affecting grain yield at all stages of the crop. The clustering pattern of the nine environments was then determined through principal component analysis (PCA) based on the significant climatic factors affecting the grain yield. The analysis generated two clusters based on the occurrence of temperature stress during the vegetative, flowering, and grain filling stages (Table 1). Environments Melk Zhar 2015 and 2016 were clustered together with Tassaout 2016 and constituted the non-heat stressed environments, whereas the other six environments along the Senegal River formed the second cluster, which was defined as the heat-stressed cluster.

Yield component performance under North-South heat gradient

The effect of temperature led to highly significant differences ($P < 0.001$) for genotype and genotype $\times$ environment effects for GY, TKW and Gr.spk across sites (**Table S2**). As expected, the site exposed to the highest temperatures (FAN16) yielded the least with an average grain yield of $1,943 \text{ kg ha}^{-1}$, whereas the highest grain yield of $8,710 \text{ kg ha}^{-1}$ and $8,890 \text{ kg ha}^{-1}$ were recorded at Tassaout and at Melk Zehr, respectively (**Fig S1a**). The top grain yields achieved in the heat stressed environments were $4,800 \text{ kg ha}^{-1}$ and $5,700 \text{ kg ha}^{-1}$ at Kaedi and Fanaye, respectively, despite a condensed crop season of just 95 days. The average GY at Fanaye was 35% and 52% lower compared to Melk Zhar during 2015 and 2016, respectively, and 63% less compared to Tessaout in 2016. While in Kaedi, the average yield was reduced by 35% and 46% compared to Melk Zhar (during 2015 and 2016, respectively) and by 59% compared to Tessaout in 2016. The regression at each environment against GY confirmed that the number of grains per spike was the most critical trait to ensure higher yields under heat stressed conditions (Table 2).

Heat susceptibility index (HSI)

A heat susceptibility index was defined based on the contrasting performances of the entries across the two heat stressed (Kaedi and Fanaye) and non-stressed (Tessaout and Melk Zhar) clusters, knowing that the temperature difference between the clusters was up to 10°C before flowering and up to 12°C after flowering. Since good HSI scores can be also achieved by very low yielding entries, this score was plotted against the GY calculated across all heat stressed environments (Fig. 1). Twenty-seven lines (12.5% of total) were identified as heat tolerant, with the Australian cultivar ‘Wollaroi’ achieving the overall best HSI = 0.25 (Table S5). Intersecting the HSI scores with GY revealed three ICARDA’s elites as the best performing, namely, ‘Berghouata1’ ($3,806 \text{ kg ha}^{-1}$ and HSI = 0.54), ‘ADYT_097’ ($3,545 \text{ kg ha}^{-1}$ and HSI = 0.54), and third ‘Arislahn5’ ($3,500 \text{ kg ha}^{-1}$ and HSI = 0.66).

Marker-Trait Associations (MTAs) of the ‘discovery’ and ‘investigation’ panel

A genome-wide association study (GWAS) was carried out for each trait (GY, TKW and Gr.spk⁻¹) at each environment individually. GWAS was also performed for the heat susceptibility index (HSI) for the ‘discovery panel’. The significant MTAs identified were merged into 36 significant QTL spread across all durum wheat chromosomes (**Table S3**), after removing any QTL with associated with the control of phenology (Gupta et al. 2020). Comparing these QTL to those identified in the heat stress study by El Hassouni et al. (2019) run using a subset of the ‘discovery panel’, confirmed 17 QTLs as cross-validated.

Conducting GWAS on the ‘investigation panel’ confirmed eleven of the initial 36 loci as cross-validated, of which five QTL were also identified by El Hassouni et al. (2019) (Fig. 2). These five consistent QTL were identified on the long arm of chromosomes 1A (Q.icd.Heat.003) and 7A (Q.icd.Heat.033), on the short arm of 2B (Q.icd.Heat.011) and 3B (Q.icd.Heat.016), and in the pericentromeric region of 1B (Q.icd.Heat.007). These loci were associated with GY, TKW, Gr.spk⁻¹ and their HSI.

Haplotype additive effects

Haplotype analysis was carried out for the three QTL (Q.icd.Heat.003, Q.icd.Heat.007, Q.icd.Heat.016) influencing GY (Fig. 3). Five haplotype classes were identified that resulted in a significant ($P < 0.05$) effect on GY calculated across the heat stressed environments along the Senegal River. The combination of three positive alleles (Hap1: CAC) resulted in the highest average GY (2,966 kg ha⁻¹), while the absence of any positive allele (Hap5: TGG) resulted in the lowest average GY (2,558 kg ha⁻¹) equal to -408 kg ha⁻¹ or a 16% decrease. The most critical QTL appears to be Q.icd.Heat.016 on chr. 3B, since not carrying the positive allele here caused a GY reduction of -197 kg ha⁻¹ (8%) even when positive alleles at the other two QTL remained. Q.icd.Heat.007 on chr 1B appears as the least critical, since lines that carried only the positive allele for it had average GY non significantly different than those not having any positive alleles. Interestingly, the top performing elites ‘Berghouata1’ and ‘ADYT_097’ have the Hap2 type, while ‘Arislahn5’ the Hap3 (**Table S5**). Among the best eight entries, only the ICARDA’s elite ‘Icavicre’ harbors Hap1 and it achieved a GY of 3,237 kg ha⁻¹ and a HSI = 0.53.

Markers validation by KASP

Marker validation is a critical step required to convert QTL discovery in actual usable solutions for breeders via MAS or genomic selection. In the present study, a total of 211 DNA sequences containing markers associated with important agronomical and spike fertility traits were submitted for designing KASP primers. Among these, 88 resulted as polymorphic in the ‘validation panel’. A total of 16 markers were validated for the relevant traits (**Table S4**). Interestingly, the three that tag the main QTLs confirmed significant ($P < 0.05$) correlation to grain yield under heat stress (Fig. 4). Marker AX-94583506 tags Q.icd.Heat.003 on chromosome 1A, marker AX-94507963 underlines Q.icd.Heat.007 on chromosome 1B, and marker AX-94887053 tags Q.icd.Heat.016 on chromosome 3B. AX-94583506 explains 4% of GY

variation under severe heat, at 80% sensitivity, 43% accuracy, and 5% specificity. AX-94887053 explains 9% of GY variation at 20% sensitivity, 40% accuracy, and 80% specificity. AX-94507963 reached 6% of GY variation at 80% sensitivity, 60% accuracy, and 40% specificity.

Discussion

Performances of the durum wheat panel across a North-South heat Gradient

The Senegal River Basin is strongly affected by heat stress with temperatures constantly above 30°C throughout the wheat growing cycle (Sall et al. 2018). The 14 parallels difference between the Moroccan northern sites and the southern ones along the River generated a 10°C temperature difference, ideal to assess the performance of the genotypes under severe temperatures. Highly significant differences could be identified for the genetic factor for GY, TKW, and Gr.spk^{-1} across sites, confirming the suitability of the experiment conducted.

The heat stress environments witnessed a reduction by 46–63% of the average GY compared to the non-stressed conditions. Similar results were reported previously by El Hassouni et al. (2019) that also indicated a mean yield loss of 54% when the temperatures were raised by 10° C. This represents a rate of 4.6–6.3% reduction for each 1°C temperature rise, which fall well within the 4.1–6.4% interval suggested by Liu et al. (2016). The number of grains per spike was the most affected trait in the heat-stressed environments. This is in accordance with what reported by Sall et al. (2018 a, b) and El Hassouni et al. (2019), indicating that seed setting is the most sensitive parameter to heat stress, with a noticeable influence on yield. The most logical explanation for this is that the high temperatures cause a rapid dehydration of the pollen or the accelerated dehiscence of the ovaries. Regardless, tolerant genotypes tend to maintain higher grain number and hence appear more capable of coping with the stress during sexual reproduction.

A positive result was the excellent yield achieved along the Senegal River reaching up to 5,700 kg ha⁻¹ in Fanaye in a cropping season that lasted only 92 days from sowing to harvest. This suggests that cultivation of wheat along the Senegal River might indeed be suitable and therefore it should be promoted to local farmers. In fact, the irrigated conditions of the Senegal River promoted the production of large kernels, which represents a critical factor for the determination of GY in durum wheat. This is in line with the study by Kumar et al. (2013) that identified TKW as a substantial contribution to GY under heat stress tolerance. However, some care should be placed when distinguishing the contribution of a trait to heat tolerance vis-a-vis its contribution to grain yield per se. In fact, our regression study, identified a mild TKW contribution to GY under both heat stressed and non-stressed, so it seems that this trait contributed to GY overall, but it is not specific for heat tolerance.

To dissect these type of complex interactions, two environmental clusters were defined based on temperature and then used to estimate HSI corrected for genotype · environment effects. Most of the investigations to date have reported HSI using simulated heat approaches –such as the contrasting of

early and late sown trials (Ayeneh et al. 2002; Kirigwi et al. 2007; Mohammadi et al. 2008; Pinto et al. 2010; Mason et al. 2010, 2011)– but we applied it to actual heat-stressed environment using the generated gradient. HSI utilizes rate of variations in GY in stressed vs non-stressed conditions to define a proxy for heat tolerance, as suggested by Mason et al. (2010). As such, it cannot distinguish between genotypes that maintain similar performances because of low yields under both conditions from those achieving top yields in both. For instance, the Australian cultivar ‘Wollaroi’ was identified as the best for HSI, but it only achieved average yields of 3,019 kg ha⁻¹ under stressed conditions, nearly 800 kg less than the top yielder ‘Berghouata1’. Hence, imposing a second level of selection for the average GY across stressed environment is a better approach to find germplasm truly useful for breeders. It is then unsurprisingly that ‘Berghouata1’ was released in Sudan in 2020 as a heat tolerant cultivar under the name ‘Basatna’.

Critical loci for durum wheat tolerance against severe heat

GWAS is the most popular approach for dissecting the genetic basis of complex traits (Sukumaran et al. 2015), but this approach is prone to the detection of false positives due to confounding population structure, the strong effect of phenology genes, and the tendency to overestimate significance (Yu et al. 2006; Zhang et al. 2010). Our study used a multi-layer approach with three germplasm set to maximize the control false positives. In exchange, we have certainly increased the risk of incurring in false negative. Because of the need of breeders to move ahead on solid grounds, we consider that ensuring the identification of truly useful alleles is more critical than losing potentially useful ones.

In this study, 36 QTLs were identified across all durum wheat chromosomes ensuring their independence from loci involved in the control of phenology (**Table S3**). The use of two additional GWAS run on a ‘subset’ of germplasm and an independent ‘investigation panel’ confirmed five QTL as cross-validated. These were detected on the long arm of chromosomes 1A (Q.icd.Heat.003), 7A (Q.icd.Heat.033), on the short arm of 2B (Q.icd.Heat.011), and 3B (Q.icd.Heat.016), on the pericentromeric region of 1B (Q.icd.Heat.007). Our results are in good agreement with Cossani and Reynolds (2012). Sukumaran et al. (2018) also identified loci associated with heat tolerance indices for grain yield (GY) in durum wheat on chromosomes 2B and 3B. Similarly, Tadesse et al. (2019) also confirmed the presence of a major QTL on chromosome 3B associated with GY in spring bread wheat tested under heat-stress in Sudan and Egypt. Acuña-Galindo et al. (2015) in a comprehensive meta-analysis also identified loci involved in heat and drought tolerance in wheat on chr 1B and 7A. Only the QTL we have identified on the long arm of chr 1A did not appear in our extensive literature search and it might therefore be considered as novel.

Breeding value of the identified loci in the fight against climate change

Breeding for heat tolerance remains a strategic solution to adapt crops to unpredictable environmental stresses. In that sense, the results of this study provide a good steppingstone to breed for better adaptation of durum wheat to heat stress. The analysis of additive effect of three QTL (Q.icd.Heat.003,

Q.icd.Heat.007, Q.icd.Heat.016) confirmed that the combination of positive alleles (Hap1) accounted for an 16% gain in GY.

Among the top three entries identified by phenotypic values, none carried Hap1, with just 'Arislahn5' harboring two positive alleles (Hap3). This suggests that our conservative approach to QTL discovery, investigation and validation has potentially prevented us from identifying additional loci responsible for 'Bergouata1' GY superiority under stress. In particular, Q.icd.Heat.033 on chr 7A and Q.icd.Heat.011 on chr. 2B are good candidates to explain these top performances. Nevertheless, 'Icavicre' having Hap1 is among the top eight entries. Also, the ICARDA's elite 'Ouassara' harbors Hap1 and it has been released in 2020 in Senegal under the cultivar name 'Dioufissa'. This further confirms the usefulness of Hap1 for heat tolerance. Hence, combining the alleles of 'Bergouata1' with those of Hap1 represents a possibility to further improve genetic gain for heat tolerance. These entries are readily available to many breeders worldwide as part of the Global Durum Panel (Oussara = GDPv2-163 = GDPv1-162, and Berghouata = GDPv2-168 = GDPv1-167; Mazzucotelli et al. 2021) and their use for improving heat tolerance is highly advisable.

In addition, three KASP markers have been validated in yet another independent germplasm panel of ICARDA's elites. These markers can now be used by breeders to follow the introgression of these alleles and ensure their rapid integration into their program.

In conclusion, this work has established a new standard for the identification of truly useful alleles when tackling by GWAS complex traits such as heat tolerance, via the deployment of three separate germplasm sets: discovery, investigation, and validation. In addition, it has presented a new methodology for assessing heat tolerance using a North-South gradient that can now be deployed by others. Furthermore, it has confirmed the critical importance of Gr.spk to guide heat tolerance mechanism, and it has delivered novel germplasm sources to introgress tolerance, while also providing the markers to facilitate their deployment. Overall, the present work has provided a new steppingstone to facilitate the fight of durum wheat breeders against climate change.

Declarations

ACKNOWLEDGEMENTS

We are grateful to the Swedish Research Council (Vetenskapsrådet) for its financial support via U-Forsk2013-6500: "Deployment of molecular durum breeding to the Senegal Basin: capacity building to face global warming" U-Forsk2017-05522: "Genomic prediction to deliver heat tolerant wheat to the Senegal River basin", and UForsk2021-04956 "Delivering heat tolerant alleles to raise farm income along the Senegal River". The "Accelerated Breeding Initiative" of the OneCGIAR also contributed to financing the staff time of H.K. and F.M.B. for the completion of this article. The authors wish also to extend recognition to the technical staff that carried out the field activities at both stations.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

Funding

This work was supported by Vetenskapsrådet [the Swedish Research Council] through grants U-Forsk2013-6500, U-Forsk2017-05522, and U-Forsk2021-04956]. HK and FMB. have received research support from “Accelerated Breeding Initiative” of the OneCGIAR.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by A.T.S., H.K. and S.O.E.M. under the supervision of F.M.B. and R.O. The first draft of the manuscript was written by A.T.S and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during and analyzed during the current study are available from the first author on reasonable request.

References

1. Acuña-Galindo MA, Mason RE, Subramanian NK, Hays DB (2015) Meta-analysis of wheat QTL regions associated with adaptation to drought and heat stress. *Crop Science* 55(2):477-92.
2. Ain Q, Rasheed A, Anwar A, Mahmood T, Xia X, He Z, Quraishi UM (2015) Genome-wide association for grain yield under rainfed conditions in historical wheat cultivars from Pakistan. *Frontiers in Plant Science* 6:743. <https://doi.org/10.3389/fpls.2015.00743>
3. Awlchew ZT, Singh R, Kaur S (2016) Transfer and mapping of the heat tolerance component traits of *Aegilops speltoides* in tetraploid wheat *Triticum durum*. *Molecular Breeding* 36-78. <https://doi.org/10.1007/s11032-016-0499-2>
4. Ayeneh, A, Van Ginkel M, Reynolds MP, Ammar K (2002) Comparison of leaf, spike, peduncle, and canopy temperature depression in wheat under heat stress. *Field Crops Research* 79: 173–184. [https://doi.org/10.1016/S0378-4290\(2\)00138-7](https://doi.org/10.1016/S0378-4290(2)00138-7)
5. Bassi F, Brahmi H, Sabraoui A, Amri A, Nsarellah N, Nachit MM, Al-Abdallat A, Chen MS, Lazraq A, El Bouhssini M (2019) Genetic identification of loci for Hessian fly resistance in durum wheat. *Molecular Breeding* 39: 24. <https://doi.org/10.1007/s11032-019-0927-1>
6. Boy J, Wilcke W (2008) Tropical Andean Forest derives calcium and magnesium from Saharan dust. *Global Biogeochemical Cycles* 22: GB1027. <https://doi.org/10.1029/2007GB002960>

7. Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES (2007) TASSEL, Software for association mapping of complex traits in diverse samples. *Bioinformatics* 23: 2633-2635.
8. Charmet G, Masood-Quraishi U, Ravel C et al (2009) Genetics of dietary fibre in bread wheat. *Euphytica* 170: 155–168. <https://doi.org/10.1007/s10681-009-0019-0>
9. Cossani CM, Reynolds MP (2012) Physiological Traits for Improving Heat Tolerance in Wheat. *Plant Physiology* 160:1710–1718. <https://doi.org/10.1104/pp.112.207753>
10. Crossa J, Burgueño J, Dreisigacker S et al (2007) Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. *Genetics* 177: 1889–1913.
11. Duggal P, Gillanders EM, Holmes TN, Bailey-Wilson JE (2008) Establishing an adjusted p-value threshold to control the family-wide type 1 error in genome wide association studies. *BMC Genomics* 9:516. <https://doi.org/10.1186/1471-2164-9-516>
12. El Hassouni K, Belkadi B, Filali-Maltouf A, Sall AT, Al-Abdallat A, Nachit M, Bassi FM (2019) Loci controlling adaptation to heat stress occurring at the reproductive stage in durum wheat. *Agronomy* 9:414. <https://doi.org/10.3390/agronomy9080414>.
13. Elias EM, Manthey FA (2005) End products, present and future uses. In : Royo C, Nachit MM, DiFonzo N, Araus JL, Pfeiffer WH, Slafer GA (eds) durum wheat breeding current approaches and future strategies, food products. Press, New York, pp 63-86
14. Eruygur O, Özokcu S (2016) Impacts of climate change on wheat yield in Turkey, a heterogeneous panel study. *Ekonomik Yaklasim*, 27: 219-255.
15. Gupta P, Kabbaj H, El hassouni K, Maccaferri M, Sanchez-Garcia M, Tuberosa R, Bassi FM (2020) Genomic regions associated with the control of flowering time in durum wheat. *Plants* 9:1628. [doi:10.3390/plants9121628](https://doi.org/10.3390/plants9121628)
16. [IPCC] Intergovernmental Panel on Climate Change (2018) Global warming of 1.5°C, an IPCC special report on the impacts of global warming of 1.5 °C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty.
17. International Grains Council (2014, October). Report activities. Retrieved from <http://www.igc.int/en/Default.aspx>.
18. Jujumaan. (2017, July 15). Estimating and plotting the decay of linkage disequilibrium. Retrieved from <https://jujumaan.com/2017/07/15/linkage-disequilibrium-decay-plot/>.
19. Kabbaj H, Sall AT, Al-Abdallat A, Geleta M, Amri A, Filali-Maltouf A, Bassi FM (2017) genetic diversity within a global panel of durum wheat (*Triticum durum*) landraces and modern germplasm reveals the history of allele exchange. *Frontiers in Plant Science* 8 :1277. <https://doi.org/10.3389/fpls.2017.01277>
20. Kosina P, Reynolds M, Dixon J, Joshi A (2007) Stakeholder perception of wheat production constraints, capacity building needs and research partnerships in developing countries. *Euphytica* 157: 475–483. <https://doi.org/10.1007/s10681-007-9529-9>

21. Kirigwi FM, Ginkel M, Brown-Guedira G et al (2007) Markers associated with a QTL for grain yield in wheat under drought. *Molecular Breeding* 20: 401–413
22. Liu B, Asseng S, Muller C, Ewert F, Elliott J, Lobell DB, Rosenzweig C (2016) Similar estimates of temperature impacts on global wheat yield by three independent methods. *Nat Clim Chang* 6:1130–1136
23. Maccaferri M, Harris NS, Twardziok SO, Pasam RK, Gundlach H, Spannagl M, Himmelbach A (2019) Durum wheat genome highlights past domestication signatures and future improvement targets. *Nature Genetics* 51: 885–895. <https://doi.org/10.1038/s41588-019-0381-3>
24. Maccaferri M, Corinna M, Demontis SA, El-Ahmed A, Garcia del Moral L, 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>
25. Mangini G, Gadaleta A, Colasuonno P, Marcotuli I, Signorile AM, Simeone R et al. (2018) Genetic dissection of the relationships between grain yield components by genome-wide association mapping in a collection of tetraploid wheats. *PLoS ONE* 13: e0190162. <https://doi.org/10.1371/journal.pone.0190162>
26. Mason RE, Mondal S, Beecher FW, Hays DB (2011) Genetic loci linking improved heat tolerance in wheat (*Triticum aestivum* L) to lower leaf and spike temperature under controlled conditions. *Euphytica* 180: 181–194. <https://doi.org/10.1007/s10681-011-0349-6>
27. Mason RE, Mondal S, Beecher FW, Pacheco A, Jampala B, Ibrahim AMH, Hays DB (2010) QTL associated with heat susceptibility index in wheat (*Triticum aestivum* L) under short-term reproductive stage heat stress. *Euphytica* 174: 423–436. <https://doi.org/10.1007/s10681-010-0151-x>
28. Mazzucotelli E, Sciara G, Mastrangelo AM, Desiderio F, Xu SS, Faris J, Hayden MJ, Tricker PJ, Ozkan H, Echenique V, Steffenson BJ, Knox R, Niane AA, Udupa SM, Longin FCH, Marone D, Petruzzino G, Corneti S, Ormanbekova D, Pozniak C, Roncallo PF, Mather D, Able JA, Amri A, Braun H, Ammar K, Baum M, Cattivelli L, Maccaferri M, Tuberosa R, Bassi FM (2020) The Global Durum Wheat Panel (GDP): An International Platform to Identify and Exchange Beneficial Alleles. *Frontiers in Plant Science* 11:569905. <https://doi.org/10.3389/fpls.2020.569905>. PMID: 33408724; PMCID: PMC7779600.
29. McIntyre CL, Mathews KL, Rattey A et al (2010) Molecular detection of genomic regions associated with grain yield and yield-related components in an elite bread wheat cross evaluated under irrigated and rainfed conditions. *Theoretical and Applied Genetics* 120: 527–541.
30. Mohammadi V, Zali AA, Bihamta MR (2008b) Mapping QTLs for heat tolerance in wheat. *Journal of Agricultural Science and Technology* 10: 261–267.
31. Mohler V, Albrecht T, Hartl L (2014) Inventory and effects of causal genes for thousand grain weight in winter wheat. In: Österreichs (Eds) Tagung der Vereinigung der Pflanzensüchter und Saatgutkaufleute, Raumberg-Gumpenstein (pp. 47).

32. Ortiz R, Sayre B, Govaerts R, Gupta G, Subbarao V, Ban T (2008) Climate change, can wheat beat the heat? *Agriculture, Ecosystem & Environment* 126 : 46–58. <https://doi.org/101016/j.agee200801019>
33. Pearson K (1985) Notes on regression and inheritance in the case of two parents. *Proceedings of the Royal Society of London* 58: 240–242
34. Pinto RS, Reynolds MP, Mathews KL, McIntyre CL, Olivares-Villegas JJ, Chapman SC (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/101007/s00122-010-1351-4>
35. Ramya P, Chaubal A, Kulkarni K, Gupta L, Kadoo N., Dhaliwal HS, Chuneja P, Lagu M, Gupta V (2010) QTL mapping of 1000-kernel weight, kernel length, and kernel width in bread wheat (*Triticum aestivum* L.). *Journal of Applied Genetics*. 51: 421-429.
36. Rehman-Arif MA, Nagel N, Newmann K, Kobiljski B, Lohwasser U, Borner A (2012) Genetic studies of seed longevity in hexaploidy wheat using segregation and association mapping approaches. *Euphytica* 1869: 1–13. <https://doi.org/101007/s10681-011-0471-5>
37. R Core Team (2020) A language and environment for statistical computing R Foundation for Statistical Computing, Vienna, Austria Retrieved from <http://www.R-project.org>
38. Sall AT, Chiari T, Legesse W, Seid-Ahmed K, Ortiz R, Van Ginkel M, Bassi FM (2019) Durum Wheat (*Triticum durum* Desf), Origin, Cultivation and Potential Expansion in Sub-Saharan Africa. *Agronomy* 9: 263. <https://doi.org/103390/agronomy9050263>
39. Sall AT, Bassi FM, Cisse M, Gueye H, Ndoeye I, Filali-Maltouf A, Ortiz R (2018b) Durum wheat breeding , In the heat of the Senegal river. *Agriculture* 8: 99. <https://doi.org/103390/agriculture8070099>
40. Sall AT, Kabbaj H, Cisse M, Gueye H, Ndoeye I, Maltouf AF, Ortiz R, Bassi FM (2018a) Heat tolerance of durum wheat (*Triticum durum* Desf) elite germplasm tested along the Senegal River. *Journal of Agricultural Science* 10: 217. <https://doi.org/105539/jasv10n2p217>
41. Sukumaran S, Dreisigacker S, Lopes, M (2015) 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
42. Sukumaran S, Reynolds MP, Sansaloni C (2018) Genome-wide association analysis identify QTL hotspots for yield and component trait in durum wheat grown under yield potential, drought and heat stress environments. *Frontiers in Plant Science* 9:81. <https://doi.org/10.3389/fpls.2018.00081>
43. Tadesse W, Suleiman S, Tahir I, Sanchez-Garcia M, Jighly A, Hagraas A, Sherif A, 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/102135/cropsci2018060389>
44. Wang J, Vanga S K, Saxena R, Orsat V, Raghavan V (2018) Effect of climate change on the yield of cereal crops. A review *Climate* 6: 41.
45. You L, Rosegrant, MW, Wood S, Sun D (2009) Impact of growing season temperature on wheat productivity in China. *Agricultural and Forest Meteorology* 149: 1009-1014.

46. Yu J, Pressoir G, Briggs WH, Vroh Bi I, Yamasaki M, Doebley JF et al (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nature Genetics* 38: 203–208. <https://doi.org/101038/ng1702>
47. Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. *Weed Research* 14: 415–421
48. Zhang Z, Ersoz E, Lai C, Todhunter RJ, Tiwari HK, Gore MA et al (2010) Mixed linear model approach adapted for genome-wide association studies. *Nature Genetics* 42: 355–360. <https://doi.org/101038/ng546>
49. Zhang H, Zhang F, Li G, Zhang S, Zhang Z, Ma L (2017) Genetic diversity and association mapping of agronomic yield traits in eighty-six synthetic hexaploid wheat. *Euphytica* 213: 111

Tables

Table 1. Temperature minimum and maximum per stage of growth (vegetative, tillering, flowering, and grain filling) across the environments, ordered following hierarchical clustering for temperature.

Stage env/Climatic factor	Vegetative Stage		Tellinging		Flowering time		Grain filling Period	
	Min temperature	Max temeprature*	Min temperature	Max temeprature*	Min temperature	Max temeprature*	Min temperature	Max temeprature*
Tessaout-2016	5.2	22.9	4.8	20.3	4.9	20.9	11.2	27.4
Melk zher-2015	7.5	21.2	5.6	20.9	6.7	20.4	12.2	23.7
Melk zher-2016	8.0	24.0	8.2	21.1	7.5	22.3	11.9	22.8
Fanaye- 2019	11.9	32.3	12.0	31.5	15.8	34.6	14.5	33.4
Fanaye-2016	14.2	32.4	14.5	33.0	14.4	37.1	17.5	35.7
Kaedi-2015	21.7	33.0	23.1	35.3	18.0	41.0	23.5	37.8
Kaedi-2016	21.1	32.0	22.1	34.3	21.6	39.0	22.5	36.8
Fanaye- 2020	13.6	31.5	13.6	31.1	13.6	36.5	15.4	36.8
Fanaye-2015	14.6	30.5	15.8	32.4	15.0	37.9	15.3	33.4

* = highly significant effect of the temperature for the environments; $p < 0.01$.

Table 2. Regression (r^2) for days to heading (DtH), grains per spike (Gr.SpK), 1,00-kernel weight (TKW) against grain yield (GY) at each environment and average across cluster of heat environments.

Environment	DtH	Gr.SpK	TKW
	r ² against GY		
FAN15	0.10**	-	0.04*
FAN16	0.14**	0.04*	-
FAN19	0.01	0.35**	-
FAN20	-	0.41**	-0.01
KED15	0.08**	-	0.05*
KED16	0.01	0.53**	-
<i>Heat stress avg</i>	<i>0.06**</i>	<i>0.33**</i>	<i>0.02</i>
TES16	-0.00	-0.00	0.01
MKZ15	0.00	-	0.01
MKZ16	0.10**	-0.00	0.29*
<i>Non stress avg</i>	<i>0.03**</i>	<i>-0.00</i>	<i>0.10</i>

* = p<0.05; ** = p <0.001

Figures

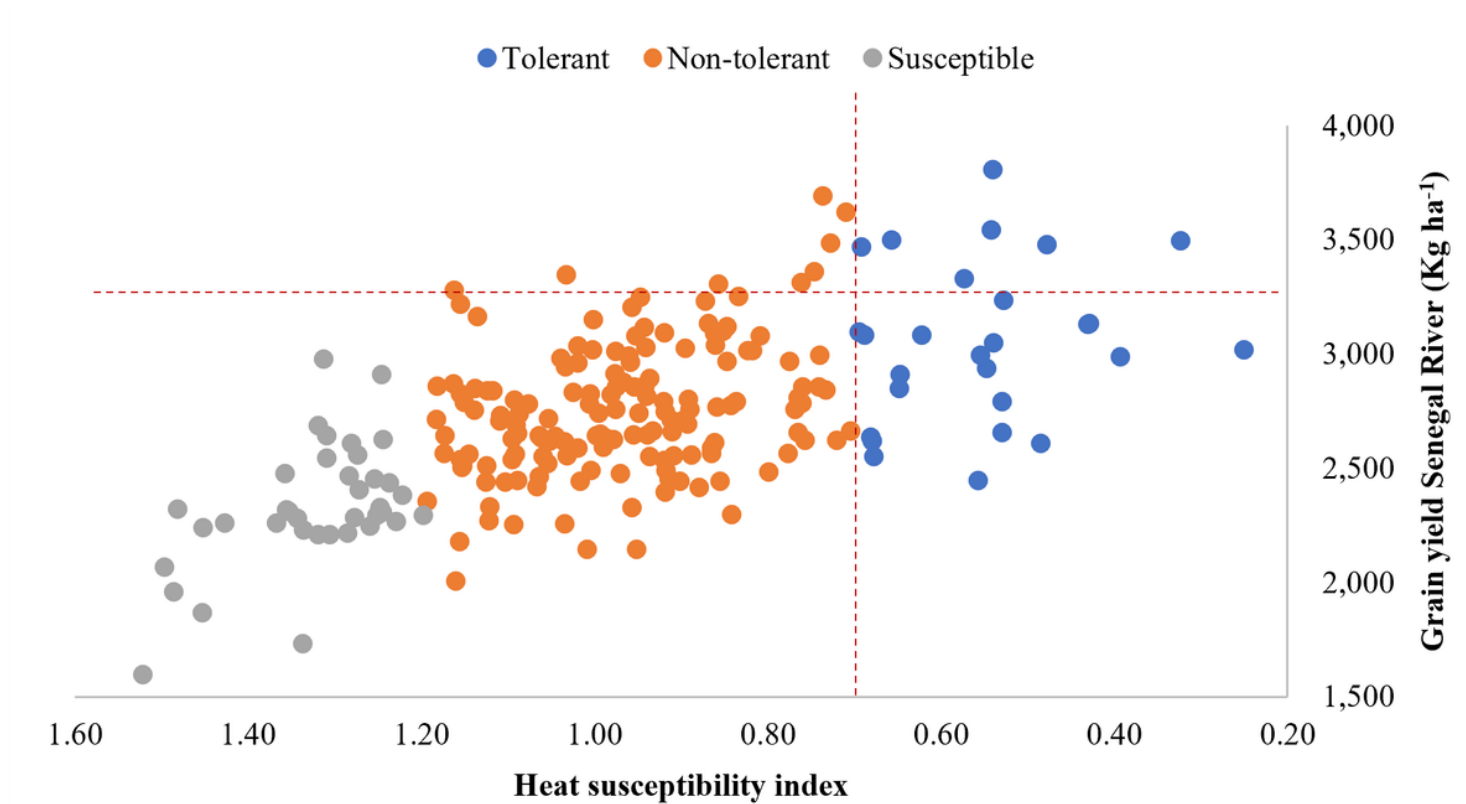
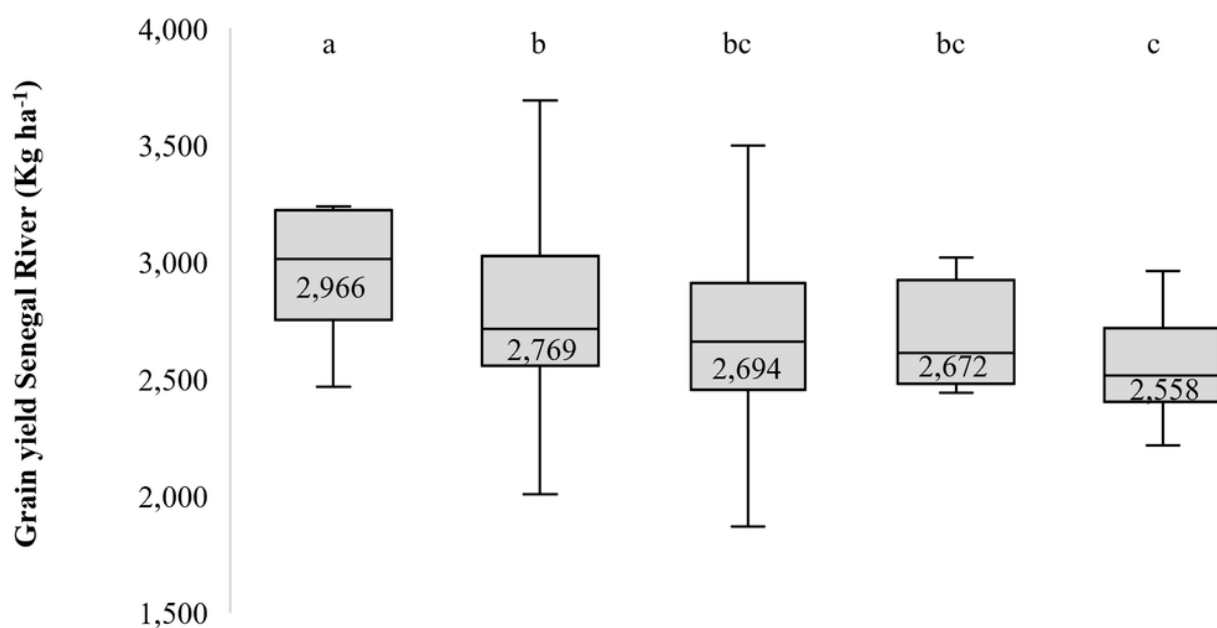


Figure 1

Heat tolerance response of entries tested across locations for heat susceptibility index (HSI) vs grain yield combined across heat stressed environments of the Senegal River. Color coding is based on the HSI. The dashed lines mark the boundaries for 'tolerant' HSI and top grain yield.



Locus	Chr	Hap1	Hap2	Hap3	Hap4	Hap5
AX-94583506	1A	CC	CC	CC	TT	TT
AX-94507963	1B	AA	GG	AA	AA	GG
AX-94887053	3B	CC	GG	GG	GG	GG

Figure 3

Haplotype analysis for three major QTLs involved in grain yield based on the allele of their most representative marker: Q.icd.Heat.003 chr 1A, Q.icd.Heat.007 chr 1B, and Q.icd.Heat.016 on chr 3B. The box whiskers represent the quartiles of the performances for grain yield combined across the Senegal River heat stressed environments for entries of the 'discovery panel' segregated by their haplotype. The actual average is reported inside the box as number of kg ha⁻¹. The significant classes based on LSD are reported above the boxes as letters. The positive allele is identified with a green background in the haplotypes table.

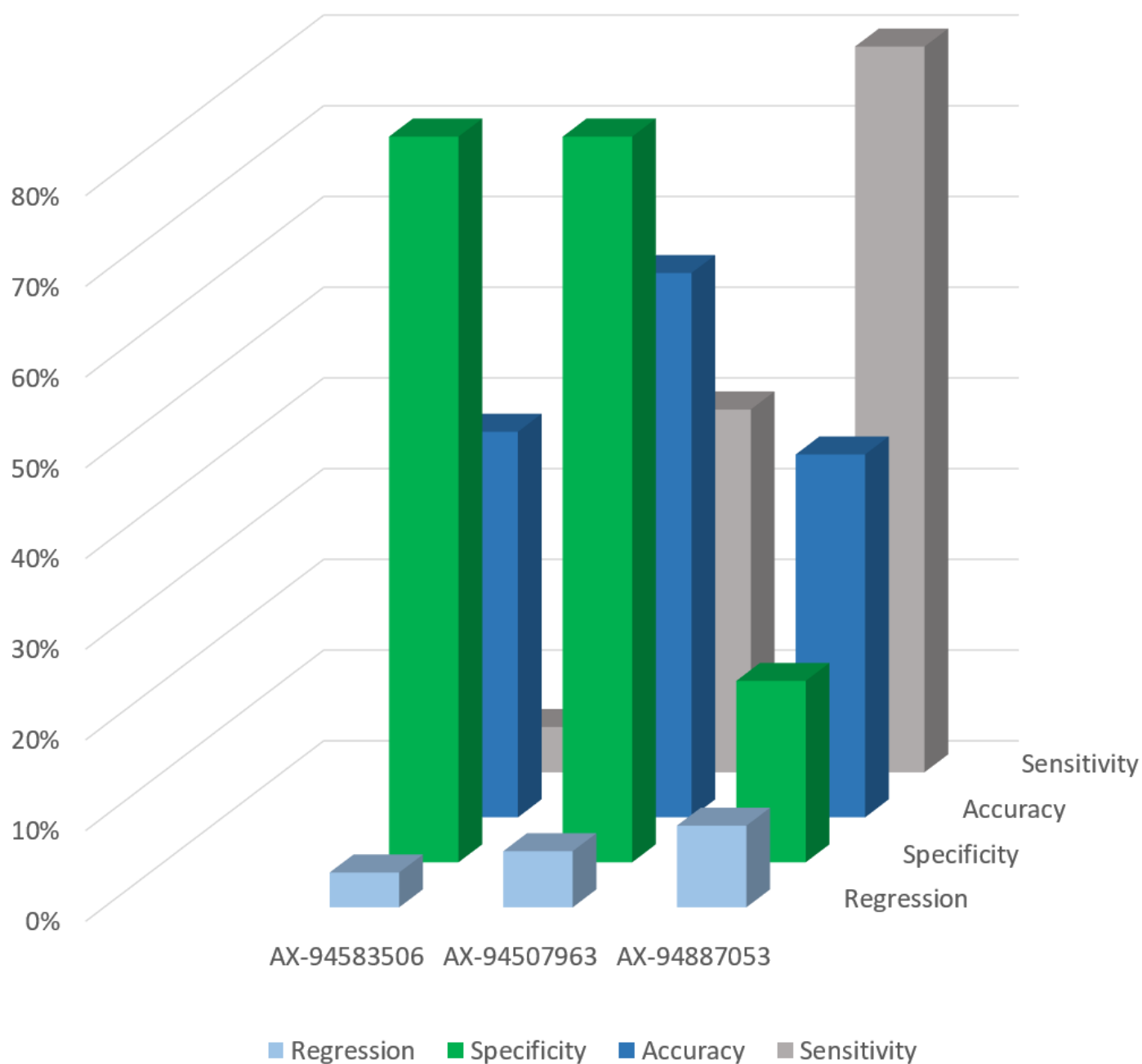


Figure 4

Validation of Kompetitive Allele Specific PCR (KASP) markers on an independent set of 94 elite lines of ICARDA tested under severe heat for grain yield. Correlation was measured between the BLUE for grain yield recorded along the Senegal River and the haplotype score. Accuracy, sensitivity, and specificity were determined using only the 20 top-performing and 20 least performing lines.

Supplementary Files

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