Harnessing genetic diversity of the wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) for wheat breeding

コムギ育種のための野生エンマーコムギ（*Triticum turgidum* ssp. *dicoccoides*）の遺伝的多様性の利用

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2022

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A thesis submitted to the United Graduate School of Agricultural Sciences, Tottori University in partial fulfillment of the requirements for the award of Doctoral of Philosophy (PhD) in Dryland Agriculture (Plant Molecular Breeding)

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DEDICATION

I dedicate this work to souls of my dearest mother and father. To my dearest brothers and sisters.
To all my teachers, instructors, colleagues, and friends.
To all those who have contributed to this work in one way or another
I dedicate this work with great love and esteem

Mohammed Balla

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Table of Contents

List of Figures

List of Tables

General Introduction

Among all cultivated wheat, bread wheat (*Triticum aestivum*, $2n = 6x = 42$, DDBBAA) and durum wheat (*Triticum turgidum* ssp. *durum* Desf., $2n = 4x = 28$, BBAA) are the most important food crops in the world. Common wheat, or bread wheat, is a major crop for more than 40% of the world population, particularly in Europe, North America, and Western and North Asia (Giraldo et al., 2019). It occupies a central place in human nutrition and provides 20% of the daily proteins and food calories. Roughly 90 to 95% of the wheat produced in the world is bread wheat. This is likely because of high agronomic adaptability, ease of grains storage, and ease of converting grains into flour for making many different foods (Mastrangelo and Cattivelli, 2021).

On the other hand, durum wheat is a crop grown commercially only on 5 to 10% of the total wheat cultivated area. The world durum wheat acreage and production are concentrated in West Asia, North and East Africa, the North America Great Plains, India, Eastern and Mediterranean Europe (Elias, 1995; Kabbaj et al., 2017). The importance of durum is derived from its unique seed characteristics. Compared with bread wheat, durum wheat seeds are known for their hardness, high protein content, and golden amber color, making them most suitable for manufacturing a unique and diverse range of food products. Its final uses vary between industrial production of pasta, couscous, and other semolina products and traditional handmade foods such as *frike*, bourghul, and unleavened bread (Kabbaj et al., 2017).

During their evolution, plants in wheat have gained adaptive mechanisms to overcome different biotic and abiotic stresses. However, the domestication process and high selection pressure applied in modern breeding programs are associated with the massive erosion of genetic diversity, resulting in the vulnerability and susceptibility of modern genotypes or cultivars to biotic and abiotic environmental stresses (Peng et al., 2013; Maccaferri et al., 2019). This narrow genetic base in modern wheat cultivars may be a major limiting factor in identifying QTLs or genes necessary for wheat improvement. Therefore, broadening the genetic base of this crop using the adaptive capacity resources of wild progenitors is one of the key principles of improvement that involves utilizing genetic variation in the gene pool and further enhancements in tolerance to environmental stresses (Ullah et al., 2018). One of such resources is wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*), a direct progenitor of domesticated durum wheat (*T. turgidum* ssp. *durum*) and the A and B genomes of bread wheat (*T.* ssp. *aestivum*). Wild emmer wheat harbours a rich allelic repertoire for various morpho-physiological traits conferring biotic resistance and abiotic tolerance (Peng et al., 2013).

Since wheat is susceptible to a temperature beyond the optimum, the current situation of the world climate involving the warming trend from year to year and the expected climate change scenarios position wheat production in the danger zone and warn about the future food security. The natural variation of wild emmer wheat encompasses

important agronomic, physiological, and yield-related traits associated with heat stress tolerance (Peng et al., 2013). Thus, this diversity in wild emmer wheat is needed to sustain and improve wheat tolerance against heat stress to cope with climate change.

This study developed a new strategy to efficiently utilize the natural variation of the wild emmer wheat and examine its potential to enhance wheat against heat stress. This study established a new durum wheat population by crossing and backcrossing nine wild emmer wheat with cultivated durum wheat cultivar to harness the diversity of wild emmer wheat for wheat improvement. The lines in this population are named Multiple Derivative Lines (MDL).

This thesis has three chapters. Chapter 1 describes the MDL development strategy, its suitability and potential for durum and bread wheat genetic improvement, genome-wide association (GWA) analysis, and gene mining from wild emmer wheat. Chapter 2 evaluated the MDL population in four environments, including optimum and heat stress, to identify germplasm and QTLs associated with heat stress tolerance from wild emmer wheat diversity. Finally, chapter 3 is a general discussion and summary. Figure 1.0 is a graphical abstract that summarizes the study.

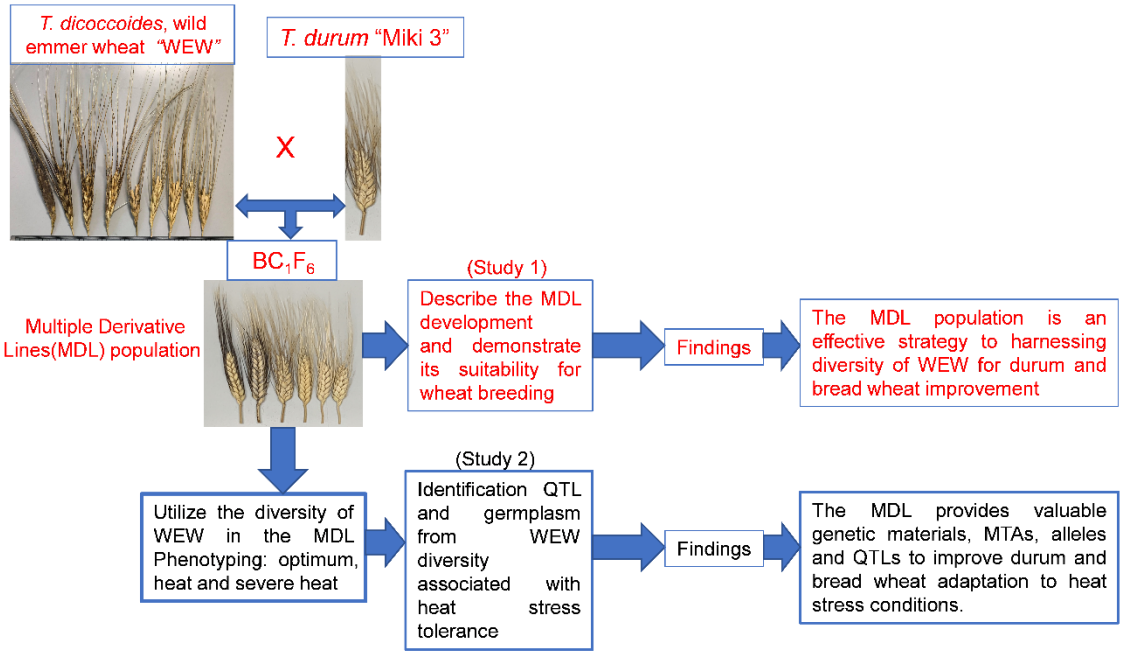

Figure 1.0. Graphical abstract showing the sequence of the studies of the multiple derivative lines (MDLs) from its development (highlighted by red color) and the evaluation under field conditions (highlighted by black and blue color).

Chapter 1

Harnessing the diversity of wild emmer wheat for genetic improvement of durum wheat

1. 1 Introduction

Durum wheat (*Triticum turgidum* ssp. *durum*, genome BBAA) is a tetraploid wheat species ($2n$ = 28), mainly used for human consumption in the form of pasta, bulgur, couscous, and some bread types (Al-Khayri et al., 2019). In terms of consumption and area under cultivation, durum wheat ranks second after bread wheat with 5% of the total cultivated wheat area (Mastrangelo and Cattivelli, 2021). Although the Mediterranean region accounts for about half of the total world durum wheat production, it still remains the largest importer and consumer of durum wheat (Royo et al., 2021). Durum wheat, which evolved from wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*), shares two of the three sub-genomes with common wheat (*T. aestivum*). The best accepted scenario for its evolution involved two successive domestication events; with the first event leading to the replacement of the brittle type of wild emmer wheat with a non-brittle type, which produced the first domesticated forms of emmer wheat (*T. turgidum* ssp. *dicoccum*). The second event accounted for the further domestication of emmer forms into the modern free-threshing durum wheat (*T. turgidum* ssp. *durum*) (Gioia et al., 2015; Kabbaj et al., 2017; Maccaferri et al., 2019). During these two events, population sizes reduced, resulting in limited genetic diversity, now explained as bottlenecks (Lopes et al., 2015). In durum wheat, 84% of the nucleotide diversity in wild emmer wheat has been lost during the domestication events (Haudry et al., 2007). Furthermore, Maccaferri et al. (2019) reported that a great reduction in diversity occurred during recent breeding activities. The loss of genetic diversity in modern durum wheat cultivars restricts the improvement of durum wheat for high productivity and increases its vulnerability to biotic and abiotic stresses (Abdurakhmonov and Abdukarimov, 2008). This narrow genetic diversity hinders the identification of efficient QTLs and genes necessary for its genetic improvement. However, despite the impressive results achieved during the Green Revolution (Borlaug, 2007), major equity problems encountered by the farmers, stability, and sustainability led to calls for a 'new phase' of agricultural research and development (Conway and Barbie, 1988). The Green Revolution impacted negatively on sustainable agriculture through the increased use of fertilizers and mono-cropping systems, decreasing soil quality and reducing crop biodiversity (Eliazer Nelson et al., 2019). In addition, we now face the urgent need of doubling productivity for a projected human population of 9.2×10^9 by 2050 (Rasheed et al., 2018). Hence, breeders have adopted the application of genomics, phenotyping technologies, and analytical tools in breeding diverse populations to maximize opportunities for better selection towards the improvement of staple food crops such as wheat. To this end, wheat breeders now focus

on enriching the gene pool by reintroducing valuable wild alleles that were changed, modified, lost, or left behind during the domestication process (Tsujimoto et al., 2015; Merchuk-Ovnat et al., 2016; Gorafi et al., 2018). The diversity in wild emmer wheat needs to be explored as it played a central role in the domestication of durum wheat.

wild emmer wheat is considered as a source of valuable genetic diversity that offers important agronomic, biotic, and abiotic stress-related traits (Matsuoka, 2011; Peng et al., 2011; Rahman et al., 2020). The similarity of the wild emmer wheat genomes to the durum and a part of bread wheat facilitates the transfer of any gene of interest into cultivated wheats. Wild emmer wheat has been shown to have two lineages of diversity which could be exploited for wheat improvement via genetic introgression : the western lineage, colonizing Israel, Syria, Lebanon, and Jordan and central-eastern lineage, dominating in Turkey, Iraq, and Iran (Mori, 2003; Ozkan et al., 2005; Matsuoka, 2011; Peng et al., 2011).

Substantial research efforts have been devoted to developing diverse durum wheat populations to exploit the wild emmer wheat diversity via the advantage of genomic tools (Peleg et al. 2008, 2009; Avni et al. 2014; Merchuk-Ovnat et al. 2016; Jorgensen et al. 2017). However, this effort needs the integration of representatives of the two lineages of wild emmer wheat diversity in one population. Using such a strategy, a population harboring the diversity of the two wild emmer wheat lineages was developed by crossing and backcrossing nine *T. turgidum* ssp. *dicoccoides* accessions with the common durum wheat cultivar 'Miki 3'. Collectively, the lines in this population were named multiple derivative lines (MDLs). This work describes the MDL development strategy, its suitability and potential for durum and bread wheat genetic improvement, genome-wide association (GWA) analysis, and gene mining from wild emmer wheat. This population is available to the wheat community upon request from the Laboratory of Arid Land Plant Resources of the Arid Land Research Center of Tottori University, Japan.

1. 2 Materials and methods

1. 2. 1 Plant materials

Nine wild emmer wheat accessions provided by the National BioResource Project – Wheat, at Kyoto University; durum wheat (*T. turgidum* ssp. *durum*) cultivar 'Miki 3' provided by Dr. M. Nachit, International Center for Agricultural Research in the Dry Areas (ICARDA); and 43 elite Sudanese durum wheat lines were used in this study. The MDL population was bred from 'Miki 3' and nine wild emmer accessions, namely KU-108-1, KU-108-4, and KU-108-5, of unknown origin (UN); KU-8808, KU-8810, KU-8814, and KU-8815, from Iraq (IQ); and KU-14474 and KU-14532, from Israel (IL) (Table 1.1). 'Miki 3' was chosen because it is a leading durum wheat cultivar in some Mediterranean countries including Lebanon and Syria where it is known as 'Berdawni' and 'Cham 7', respectively, and has high yield, high resilience in irrigated environments, and resistance to yellow rust and leaf rust (Afifi and Sastry, 2013). Sudanese cultivars

included five released cultivars ('Cham 1', 'Zaidab', 'Argu', 'Basatna', and 'Wadelbur') and 38 advanced lines as checks.

1. 2. 2 Production of the MDL population

First, the crossing between the nine wild emmer wheat accessions as males and the 'Miki 3' as female was used to produce nine F_1 hybrids in 2011. In 2012, nine F_1 plants as females was backcrossed with 'Miki 3' and obtained nine BC_1F_1 families consisting of 236 plants. Ten self-pollinated seeds from each of 10 BC_1F_1 plants in the nine families were mixed and planted as a population of 900 BC_1F_2 plants. Of the 900 plants, 369 (41%) showed hybrid necrosis and died. The bulked seeds from the remaining 531 plants were harvested and named this population Multiple Derivative Lines (MDLs) BC_1F_2. All plants showed diverse morphology; 453 plants had a waxy stem and 78 were waxy-less. In 2014, the seeds from the 531 bulked plants were sowed and obtained BC_1F_3 MDLs from 466 surviving plants. In 2015, randomly 1000 seeds from the BC_1F_3 MDLs were selected and produced BC_1F_4 MDLs. All these activities were conducted at the Arid Land Research Center, Tottori University. In 2016, based on the agronomically desired traits (heading, non-shattering, and free-threshing) 501 plants from the 1000 BC_1F_4 population was selected and evaluated at the Agricultural Research Corporation (ARC), Wad Medani, Sudan, as separate lines and selected 225 potential lines. In 2018, the 225 BC_1F_5 lines was re-evaluated at Wad Medani and selected 178 BC_1F_6 with good agronomic performance. A diagram illustrated breeding scheme of the 178 MDL lines is shown in Fig. 1.1. These 178 MDLs were used as a validation panel for the MDL platform.

1. 2. 3 DNA extraction, DArTseq genotyping, and genetic analysis

Total genomic DNA was isolated using the CTAB method (Saghai-Maroof et al., 1984), and DNA samples (20 µL; 50–100 ng μL^{-1}) were sent to Diversity Array Technology (DArT) Pty. Ltd., Australia (http://www.diversityarrays.com), for whole-genome scanning with DArTseq (DArT sequencing) markers. Restriction fragments from each sample were sequenced and aligned to durum wheat cv. 'Svevo' RefSeq v. 1.0 (Maccaferri et al., 2019).

Pedigree analysis, principal component analysis (PCA), phylogenetic analysis, estimation of Nei's genetic diversity index, and analysis of molecular variance (AMOVA) were conducted in Flapjack v. 1.20.10.07 (Milne et al., 2010), R, PowerMarker v. 3.25 (Liu and Muse, 2005), MEGA X (Kumar et al., 2018), and GenAlex v. 6.5 software (Peakall and Smouse, 2012), respectively. In the pedigree analysis, I chose each of the nine wild emmer wheat accessions as the first parent and 'Miki 3' as the second parent, and then selected each of the MDL progeny with ≥75% similarity to the first parent as putative progeny. From AMOVA, the pairwise population (Phipt) and Nm (haploid number of migrants) within the population were obtained from GenAlexv.65. Marker deviation from the expected Mendelian segregation ratio of 3:1 was evaluated by using the following equation:

$$\chi = -(O_w - 0.25\,n) / 0.25\,n + (O_m - 0.75\,n) / 0.75\,n$$

where n is total number of lines in a family, and O is observed number of lines with a (w) wild emmer wheat or (m) 'Miki 3' allele in the family. The χ value is the deviation from the expected ratio; a large value indicates deviation to 'Miki 3' and a small value to wild emmer wheat.

The MDL individuals were clustered with a discriminant analysis of principal component (DAPC) implemented in R/adegenet (Jombart et al., 2010) to identify genetic similarity between MDL families. A clustering algorithm based on Bayesian information criteria (BIC) was used to determine the number of clusters in the MDLs.

1. 2. 4 Phenotypic evaluation of the MDL population

To test the usefulness and suitability of the MDL population for durum wheat breeding and genome-wide association analysis, I measured days to heading (DH) and plant height (PHT) in Dongola (Sudan) and in Tottori (Japan) because these traits are extensively studied in durum wheat. In both locations, DH was measured as the number of days from the first irrigation or transplanting until 50% of the plant headed. PHT was recorded at maturity by measuring the distance between the ground and the top spike excluding awns.

In Sudan, the 178 MDLs and 'Miki 3' were grown during the winter season (2019–2020) at Dongola Research Station Farm (19°08′N, 30°27′E, and 239 m a.s.l.), Agricultural Research Corporation (ARC), Sudan. The soil is high terrace soil (pH 8.0−8.4) with low organic matter content <5% (Elbashir et al., 2017). Seeds were dressed with insecticide/fungicide mixture of Gaucho (Imidacloprid 39% WP, Bayer Crop Science, Kansas City, MO, USA) at 0.75 g kg^{-1}. Sowing was performed manually at the rate of 120 kg ha^{-1} during the 1st week of December. Fertilization was done using DAP (Diammonium phosphate) or triple superphosphate by furrow placement before planting at the rate of 43 kg ha^{-1} of P_2O_5, whereas urea was split-applied by broadcasting before the second and fourth irrigation at the rate of 86 kg ha^{-1}. Irrigation was carried out at 10−14 days intervals following the ARC recommendation (wheat water requirement is about 400 mm) to avoid water stress. Weeding was done manually at least twice in both locations. All the cultural practices were conducted according to the ARC recommendations for wheat production. The average minimum and maximum temperatures during the season were 11.4 °C and 28.3 °C, respectively.

In Japan, seeds of the genetic materials were germinated on tray pots and transferred to the field of the Arid Land Research Center (35°32′N, 134°13′E, 11 a.s.l.), Tottori in the second week of December and harvested in mid-June. This location has a high cold with rain-fed field conditions, and the average minimum and maximum temperatures during the season were 7.1°C and 16.2 °C, respectively, and the rainfall amount was about 930 mm (Arid Land Research Center weather station). Before sowing, three commercial fertilizer mixtures—Kumiai Fukugo PKN 366 (MC Ferticom Co., Ltd.; Tokyo. Japan; 60kg), Hitachi Fukugo 1 (Htachi-Fukugo Co. Hitachi, Ibaraki, Japan, Ltd.; 40 kg), and

granular carbonated magnesium lime (Shimizu Kogyo Co., Ltd. Tokyo, Japan; 100 kg) ―were spread onto soil (Elhadi et al. 2021).

Each field experiment was arranged in an alpha-lattice design with two replicates. The plot size was four rows, 1 m long, 0.2 m apart in Dongola, and one row with five plants 0.2 m apart in Tottori.

1. 2. 5 Genome-wide association (GWA) analysis

GWA analysis was performed with the genotyping data (DArTseq markers) and phenotypic data, using a mixed linear model (MLM) incorporating the population structure as fixed effect and kinship matrix as random effect among the individuals, in TASSEL v. 5.2.66 software (Bradbury et al., 2007). In total, 13,312 SNPs markers with a call rate of 90% (10% missing data) and MAF (minor allele frequency) > 0.05 were used in the analysis. The threshold of $P < 0.0001$ ($-\log_{10}(p) > 4$) indicated the degree of association between each SNP marker and a trait, and R^2 was the variation explained by the significantly associated markers. The MLM product from TASSEL was used in R v. 4.0.3 with custom scripts in the developed GWAS package rMVP (R Core Team, 2020) to draw Manhattan plots and quantile–quantile plots (Yin et al., 2021).

1. 3 Results

1. 3. 1 Genotyping of the MDL population

The DArTseq genotyping platform provides two types of markers: Silico-DArT markers (SiD), scored as presence or absence, and SNP markers. I obtained 54,712 SiD and 64,817 SNP markers. The genetic positions of 628 SiD and 7,275 SNP markers with a call rate of 100% (no missing data) were determined on the 14 durum wheat chromosomes (Fig. 1. 2). As SNP markers are codominant and are used widely, I used them for most of my analysis with no missing data. By SNP genotyping, the total length of the physical map was 9 939 Mbp. The longest chromosomes were 3B (832 Mbp) and 2B (788 Mbp), and the shortest was 1A (583 Mbp). The average physical distance between SNPs was 1.4 Mbp. The length of the A genome was 4 845 Mbp and B genome 5 088 Mbp. Chromosome 2B had the most SNPs (715), and 6A had the fewest (318). The SNP markers were denser in the telomeric regions than in the centromeric regions.

1. 3. 2 Comparison of geographical origin and genetic relationship between population parents and Sudanese cultivars

Phylogenetic analysis using 7,275 SNPs markers placed the Sudanese cultivars and ‘Miki 3’ in one group and the nine wild emmer wheat accessions in another group (Fig. 2). The latter was further divided into two sub-groups, one with an accession from Israel and one of unknown origin, and the other with the remaining seven accessions. This latter group was further divided into two sub-groups, one with an accession from Israel and two of unknown origin, and the other with four accessions from Iraq (Fig. 1. 3). Accessions KU-8814 and KU-8815 from Iraq have substantial genetic similarity. The passport data

revealed that they are derived from the same line, so they are probably separated because of their distinct characteristics.

1. 3. 3 Pedigree of the MDL lines

Since each of 178 MDLs comes from bulked population, I sought the pedigree analysis of these lines. Each wild emmer wheat accession produced from 7 to 37 MDLs (Fig. 1. 4). In contrast, there was no similarity of the five Sudanese cultivars to the nine wild emmer wheat accessions.

Following one backcross event, the expected frequencies of 'Miki 3' and wild emmer wheat genomes in the MDLs are 75% 'Miki 3' and 25% wild emmer wheat. The χ-test for each chromosome found a deviation from the expected ratio toward one or other parent (mainly the recurrent parent) on most of the chromosomes in both A and B genomes in all families (Fig. 1. 5).

1. 3. 4 Genetic diversity of the MDL population

The PCA was performed to estimate genetic diversity among the MDL lines, their parents, and the 43 Sudanese durum wheat cultivars using 7,275 SNPs (Fig. 1. 6). The groups formed three clear clusters (Fig. 1. 6). PCA divided the nine wild emmer wheat accessions into two groups, with two and seven accessions, in agreement with the phylogenetic analysis. The Sudanese cultivars and 'Miki 3' were clustered together. However, the MDL lines were divided into two groups, one closer to the upper seven wild emmer wheat accessions, the other closer to the lower two. The MDL lines were placed between the Sudanese group (including 'Miki 3') and the wild emmer wheat accessions. The MDL lines explained much more genetic diversity than the Sudanese cultivars. However, the PCA showed low variance in principal components, PC 1 (6.69%) and PC 2 (6.33%) (Fig. 1. 6), and 26 PCs were necessary to capture 50% of the molecular variance (Fig. 1. 7), suggesting limited structure in the population. Discriminant analysis of principal components (DAPC) showed nine genetic groups in the MDL population (Fig. 1. 8a). However, Bayesian information criterion (BIC) provided five main clusters (Fig. 1. 8b). The overlap between some families is consistent with the degree of similarity observed among the nine wild emmer wheat accessions (Fig. 1. 3). Although the PCA explained low variation, the MDL population had difference phenotypic variation in spike length, size and shape, awn colour and length, and glume colour (Fig. 1. 9).

I used Nei's gene diversity index and the polymorphic information content (PIC) to evaluate genetic diversity within the MDL population. Nei's index indicates the probability that two randomly chosen alleles from a population are different (Xu and Vayena, 2015). PIC values provide an estimate of the likelihood of finding polymorphism between two random samples of germplasm. Numbers of SNP and polymorphic markers, Nei's genetic diversity index, and PIC values estimated for each chromosome and genome are listed in Table 1. 2. Out of the 7,275 SNP markers, 2,093 were highly polymorphic across the MDLs. The Nei's genetic diversity was 0.2476. The A genome had a genetic diversity of 0.2559 and a PIC of 0.2333; the B genome had a genetic

diversity of 0.2384 and a PIC of 0.2182. The differences between A and B genomes in genetic diversity and PIC were not significant (paired *t*-test = 2.126, *P* = 0.0775, and *t*-test = 2.255, *P* = 0.0649, respectively).

Differentiation between the MDLs and the nine wild emmer wheat accessions was assessed by AMOVA based on PhiPT values, which found 9% of variance among and 91% of genetic diversity within populations (Nm=5.31, Phipt= 0.086), indicating a high gene exchange (low genetic differentiation) between the two groups (Table 1. 3).

1. 3. 5 GWA analysis

To validate the usefulness of the MDL population for mapping traits and gene mining, I performed GWA analyses of DH and PTH in Dongola and Tottori.

Highly significant differences ($P \leq 0.001$) among the MDL lines for both traits was identified. DH had a wide range of variation, from 62 to 90 days in Dongola (Fig. 1. 10a) and 123 to 147 days in Tottori (Fig. 1. 10b). PHT ranged from 73.5 to 131.5 cm in Dongola and 38.3 to 90.9 cm in Tottori (Fig. 1. 11a, b). GWA analysis identified three significant marker-trait associations (MTAs) for DH in Dongola, on chromosomes 1A (1 MTA) and 5A (2 MTAs) (Fig. 1. 10c). These MTAs explained 13.8 to 14.3% of the genetic variance (Table 1. 4). In Tottori, GWA analysis revealed one significant MTA for DH, on chromosome 3B (Fig 1. 10d), which explained 13.9% of the genotypic variance. GWAS detected 28 significant MTAs for PHT at three genomic regions: 26 MTAs in Dongola on chromosomes 4A, 4B, and 7B; and 2 MTAs in Tottori on chromosomes 4A and 4B (Fig. 1. 11c, d; Table 1. 4). The MTAs explained 13.7 to 32.0% of the genetic variance in Dongola and 10.8 to 14.9% in Tottori. I identified two stable markers (rs2252536 on chromosome 4A and rs2278767 on chromosome 4B) associated with PHT in both locations (Table 1. 4). Alleles contributing to early heading and short PHT were found to be derived from the recurrent parent ‘Miki 3’ in both locations (Fig. 1. 12).

1. 4 Discussion

Although durum wheat was domesticated about 10 000 years ago (Shewry, 2009), the official breeding program does not exceed 120 years (Taranto et al., 2020). A robust genetic bottleneck occurred during this gap as the domestication process caused substantial genetic erosion (Maccaferri et al., 2019). However, to meet the needs of a growing human population and the increasing climate change scenario, crop production would need to be further improved especially through the use of genetic resources of wild progenitors to introgress agronomically superior and adaptive traits.

In this study a new population of multiple derivative lines (MDLs) that harbor fragments of wild emmer wheat diversity in its gene pool was developed. This study elucidated the genetic potential of this population by identifying novel traits and MTAs from the wild relative progenitor *T. turgidum* ssp. *dicoccoides,* and the suitability of this population for wheat breeding.

The wild emmer wheat accessions from Iraq clustered separately from those

originating from Israel (Fig. 1. 3). Two wild emmer wheat lineages exist in its distribution area: the western lineage, found in Jordan, Syria, Lebanon, and Israel, and the central-eastern lineage, found in Turkey, Iran, and Iraq (Mori, 2003; Ozkan et al., 2005; Matsuoka, 2011; Peng et al., 2011). As the nine wild emmer wheat accessions used in this study represent the western lineage (Israel) and the central-eastern lineage (Iraq), I speculate that they cover the spectrum of diversity present in wild emmer wheat, although the number is limited.

Although MDL is a mixed population, I could identify the pedigree of each of the 178 lines using DArTseq markers. Such analyses allow me to track the origin of useful traits and use the corresponding accessions for further crossing in the breeding program. Although the MDL population was created by mixing an equal number of seeds from each cross, the nine wild emmer wheat accessions contributed different numbers of individuals among the lines (Fig. 1. 4). I attributed this imbalance to both natural and artificial selection during the production of the MDL population, which is in agreement with a previous study in bread wheat by Gorafi et al. (2018).

The MDL population (BC_1F_6) has an expected contribution of 75% from 'Miki 3' and 25% from the donor wild emmer wheat accessions. All MDLs showed a deviation from the expected ratio toward one or the other parent, especially the recurrent parent (Fig. 1. 5). This deviation can be attributed to the fact that the 178 accessions used here were selected for good agronomic performance under the Sudanese environment. This selection removed all individuals with unsuitable wild emmer wheat traits such as brittle rachis, glume tenacity, and non-free-threshing type, and consequently reduced the contribution of wild emmer wheat alleles. The chromosomes within families that showed deviation toward one parent could be a result of competition between gametes for preferential fertilization or from gamete or zygote abortion. The number of individuals within each family was low, ranging from 7 to 37 (Fig. 1. 4). Therefore, distortion could be due to non-biological factors derived from low population size and genotyping errors (Alheit et al., 2011).

Phylogenetic analysis showed a difference between the nine wild emmer wheat accessions and modern Sudanese durum wheat cultivars including 'Miki 3' (Fig. 1. 3). This result revealed the loss of genetic diversity in the Sudanese cultivars caused by domestication and breeding (Maccaferri et al., 2019). The PCA placed the MDLs between the wild emmer wheat accessions and modern cultivars (Fig. 1. 6). The MDL families grouped by DAPC analysis (Fig. 1. 8a) reflect the genetic makeup of the nine wild emmer wheat. Although the DAPC analysis showed the nine genetic groups, the Bayesian information criterion (BIC) revealed five clusters in the MDLs (Fig. 1. 8b). This result could be due to the high similarity among some wild emmer wheat accessions. For instance, accessions KU-8814 and KU-8815 were derived from the same line, and their progenies (families six and seven) are highly overlapped (Fig. 1. 8a). Interestingly, the DAPC grouping seems to reflect the geographical origin of the nine wild emmer wheat,

in agreement with phylogenetic analysis (Fig. 1. 3). These results revealed that the MDLs provide an effective platform with which to harness the wild emmer wheat diversity.

I evaluated days to heading and plant height in Dongola and Tottori to validate the suitability of the MDL population for MTA identification and to dissect the wild emmer wheat genes. GWA analysis identified two genomic loci on chromosomes 1A and 5A with relevant DH effects in Dongola (Fig. 1. 10c). Flowering time of wheat is controlled by a network of genes integrating major vernalization genes located on chromosomes 5A (*Vrn-1* and *Vrn-2*) and 7BS (*Vrn-3*); a series of homoeologous photoperiod response genes on group 2 chromosomes; and earliness genes on chromosomes 1A, 3A, and 3B (Pánková et al., 2008; Fowler et al., 2016). The significant MTAs for DH identified in Dongola are located on the chromosomes reported to harbor major genes associated with DH. GWA analysis for DH showed one MTA on chromosome 3B (Fig. 1. 10d) positioned at the earliness *per se* locus detected in Tottori (Pánková et al., 2008). Kobayashi et al. (2016) evaluated 96 Japanese wheat cultivars (JWC) for DH in autumn and spring sowing and found significant MTAs on chromosome 3B associated with DH in autumn sowing. The differences in the GWA results between Dongola and Tottori arose from the different climatic conditions during the period of evaluation. Although Dongola is regarded as a relatively cooler location than other places in Sudan, it is warmer than Tottori, and this difference may explain the detection of the vernalization loci in Dongola. Distelfeld et al. (2009) reported that *Vrn-1* genes regulate the transition from vegetative to reproductive phase in response to temperature and thus determine the spring and winter growth habit. Therefore, evaluation of DH in the MDL population revealed the three genomic loci reported to control flowering time in wheat (Pánková et al., 2008; Kobayashi et al., 2016; Gupta et al., 2020).

GWA analysis for PHT revealed two common genomic loci in Dongola and Tottori on chromosomes 4A and 4B (Fig. 1. 11c, d). These MTAs correspond to *Reduced height* alleles *Rht-A1* and *Rht-B1* (Wilhelm et al., 2013). The introduction of *Rht-1* in the 1960s during the Green Revolution led to improved lodging resistance and yield. Similar results of GWA analysis for PHT between Dongola and Tottori indicated that the specific environment did not restrict the occurrence of *Rht-1*. On the other hand, the MTAs detected on chromosome 7B that coincided with the location of the *Rht13* allele appeared only in Dongola (Fig. 1. 11c, Ellis et al. 2005).

Most of the earlier and shorter genotypes contain alleles derived from the ‘Miki 3’ (Fig. 1. 12). Bentley et al. (2011) reported that mutations associated with the early heading phenotype are absent from wild tetraploid wheat but were predominate on chromosome 2A in modern durum wheat, suggesting that they originated after domestication and were selected for the improvement of adaptation. Also, more than 70% of the modern wheat cultivars incorporate one of the original semi-dwarfing genes defining the characteristics of the ‘Green Revolution’ (Jobson et al., 2019).

A significant advantage for plant geneticists comes from creating diverse experimental populations that enable the genetic dissection of complex traits to support

plant breeding. To this end, Gorafi et al. (2018) proposed an efficient platform in bread wheat named multiple synthetic derivative (MSD) lines that possess a large diversity of *Aegilops tauschii* in a modern bread wheat cultivar. This method facilitates the exploration of the diversity of wild wheat progenitors in one population. The MDL platform is similar to that of the MSDs: the MSD harnesses the diversity of *Ae. tauschii* (the D-genome donor of hexaploid wheat), and the MDL platform exploits the diversity of wild emmer wheat A and B genomes. Moreover, compared with multi-parental advanced-generation inter-cross and nested association-mapping strategies, the MDL/MSD platforms allow us to save time by starting evaluation and selection of desired phenotypes at an early generation. Combining new advances in speed-breeding methods (Hickey et al., 2019; Wanga et al., 2021), the MDL/MSD strategy could offer a rapid way to utilize the diversity of wild relatives for wheat improvement.

The MDL lines are being tested under heat stress conditions in Sudan to further evaluate the MDLs potential. My preliminary findings showed several potential heat-tolerant lines with good agronomical performance (data showed in chapter 2). I believe that the MDL platform could provide valuable materials for different breeding purposes such as drought tolerance, salinity tolerance, and end-use quality improvements just as the MSD population of bread wheat is a useful source of heat and drought tolerance (Elbashir et al. 2017; Itam et al. 2020; Elhadi et al. 2021). The uniform genetic backgrounds of these platforms allow accurate evaluation of quantitative traits of wild species (*Ae. tauschii* or wild emmer wheat) as traits of cultivated wheat species (*T. aestivum* or *T. turgidum* ssp. *durum*). Furthermore, diversity in the MDL has a potential to improve the diversity of A and B genomes of bread wheat. Efforts are currently underway to accumulate these platforms' diversity by intercrossing selected MDL/MSD lines and developing lines with traits linked to heat and combined heat-drought stress tolerance.

Table 1. 1 Accessions of wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides* used in this study and their origin.

Code	Accession number	Origin
1	KU-108-1	Unknown (UN)
2	KU-108-4	Unknown (UN)
3	KU-108-5	Unknown (UN)
4	KU-8808	Iraq (IQ)
5	KU-8810	Iraq (IQ)
6	KU-8814	Iraq (IQ)
7	KU-8815	Iraq (IQ)
8	KU-14474	Israel (IL)
9	KU-14532	Israel (IL)

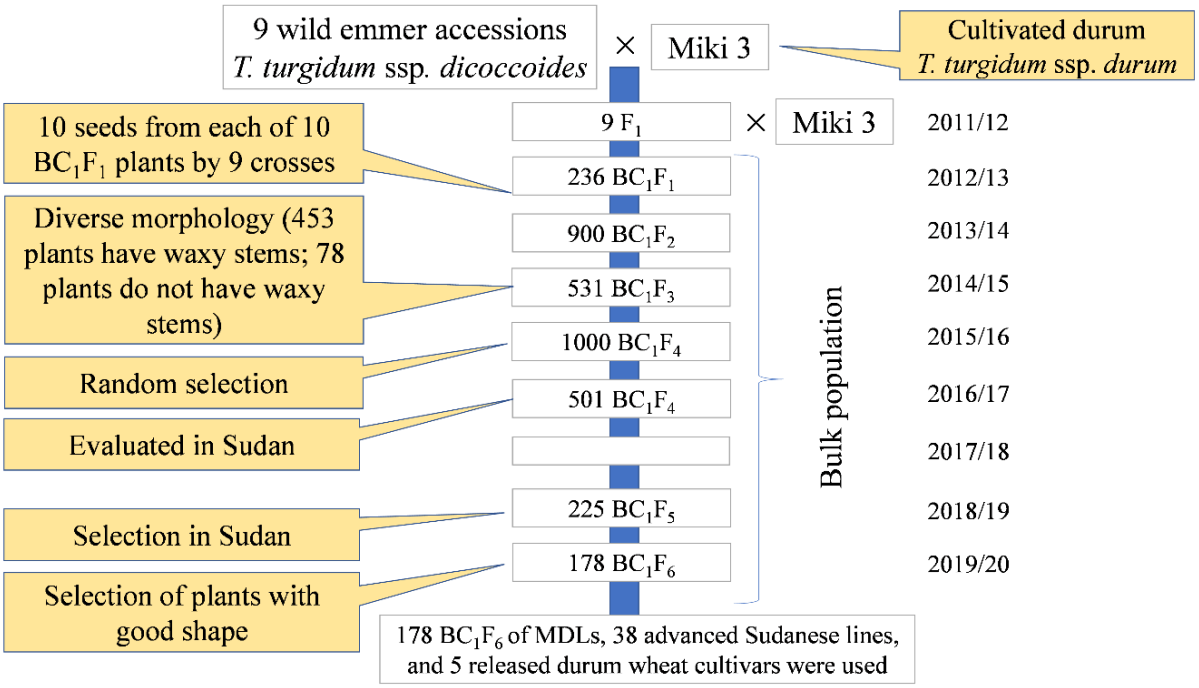

Figure 1. 1 Pedigree of multiple derivative lines of durum wheat (*Triticum turgidum* ssp. *durum*) incorporating diversity of wild emmer wheat (*T. turgidum* ssp. *dicoccoides*).

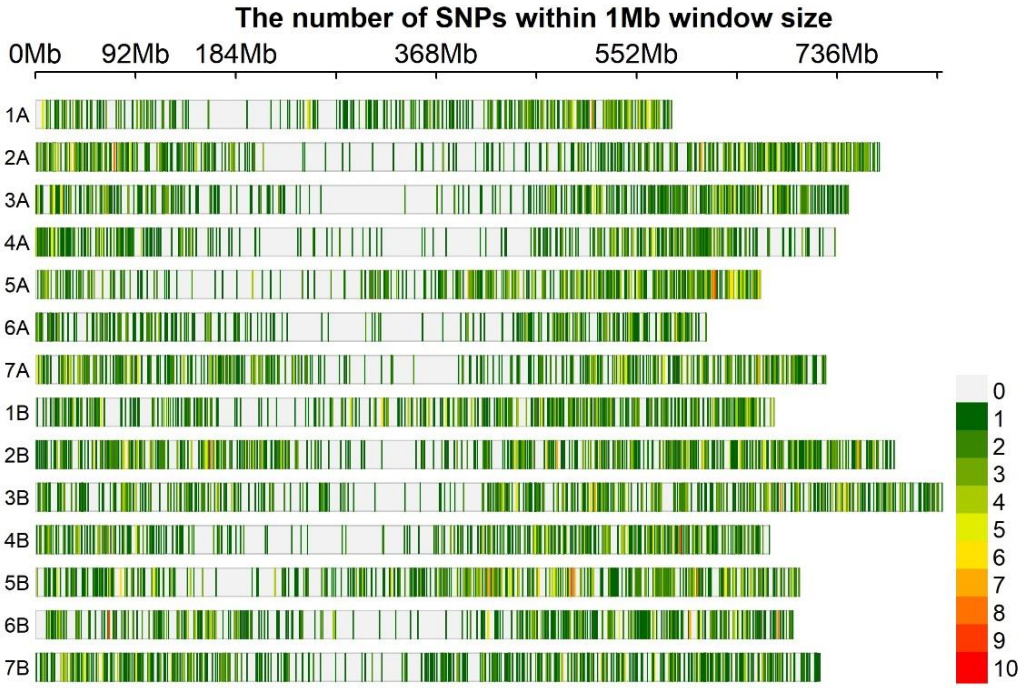

Figure 1. 2 Distribution of SNPs of 7275 DArTseq markers among 178 multiple derivative lines, nine wild emmer wheat accessions, and 43 Sudanese durum wheat cultivars. Redder markers have higher density; greener markers have lower density; gray areas have no markers.

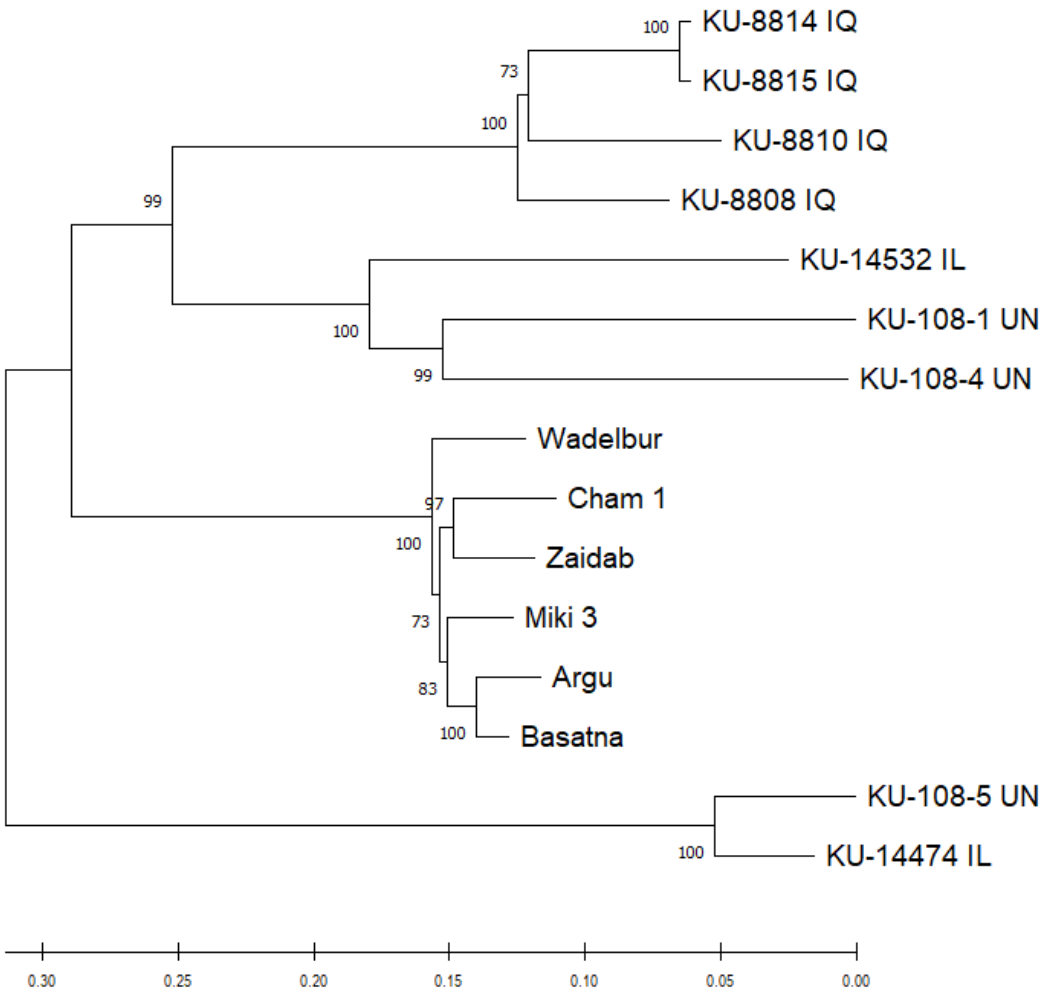

Figure 1. 3 Phylogenetic analysis to elucidate the genetic relationship between the Sudanese cultivars and parents used to generate the multiple derivative lines. The recurrent parent 'Miki 3' is a durum wheat cultivar; the other nine parents (with code KU) are wild emmer wheat wild emmer wheat accessions, country abbreviation code IL indicate Israel, IQ indicate Iraq and UN indicate unknown origin. Sudanese cultivars are five released cultivars ('Cham 1', 'Zaidab', 'Argu', 'Basatna', and 'Wadelbur'). All Sudanese cultivars and 'Miki 3' are clustered in one group, whereas the nine wild emmer wheat are placed together in another groups.

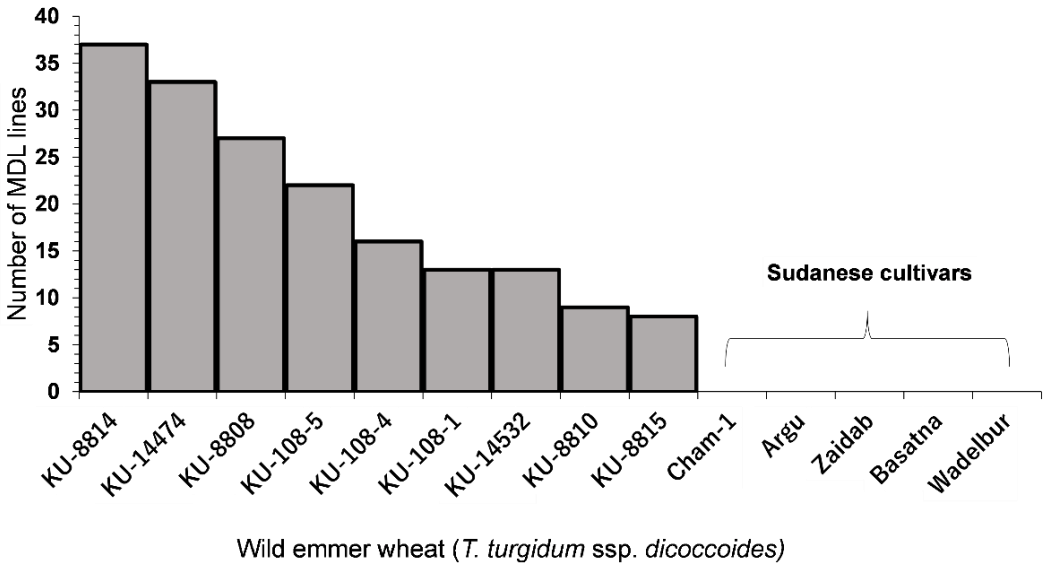

Figure 1. 4 Number of progenies from each of the nine wild emmer wheat accessions (range, 7 to 37). Five Sudanese cultivars were used as checks.

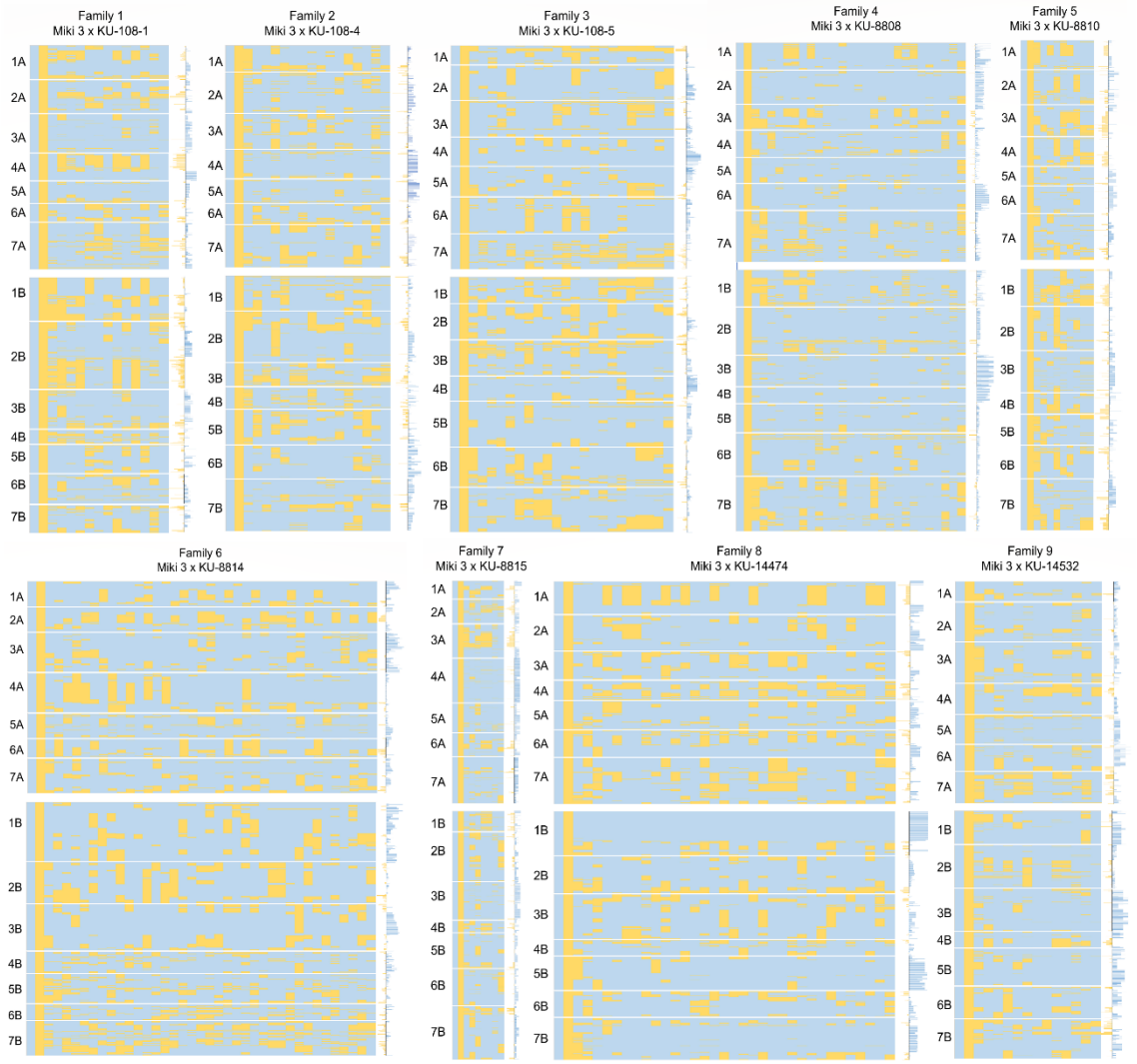

Figure 1. 5 Graphical genotyping describes recombination between wild emmer wheat and recurrent parent 'Miki 3' genomes in the nine multiple derivative line families. Conditional formatting in Microsoft Excel generated the plots from polymorphic markers between 'Miki 3' (pale blue) and wild emmer wheat (yellow). Blue and yellow colors spread in each family indicate the 'Miki 3' and wild emmer wheat genomes, respectively. Letters Codes on the left indicate chromosome numbers. The leftmost column in each family indicates the 'Miki 3' genome and the following columns indicate wild emmer wheat genomes. Marker deviation from the expected Mendelian segregation ratio of 3:1 is plotted to the right of each family plot: blue, toward 'Miki 3'; yellow, toward wild emmer wheat donor parent. The middle blackline indicates no deviation from the expected 3:1 ratio.

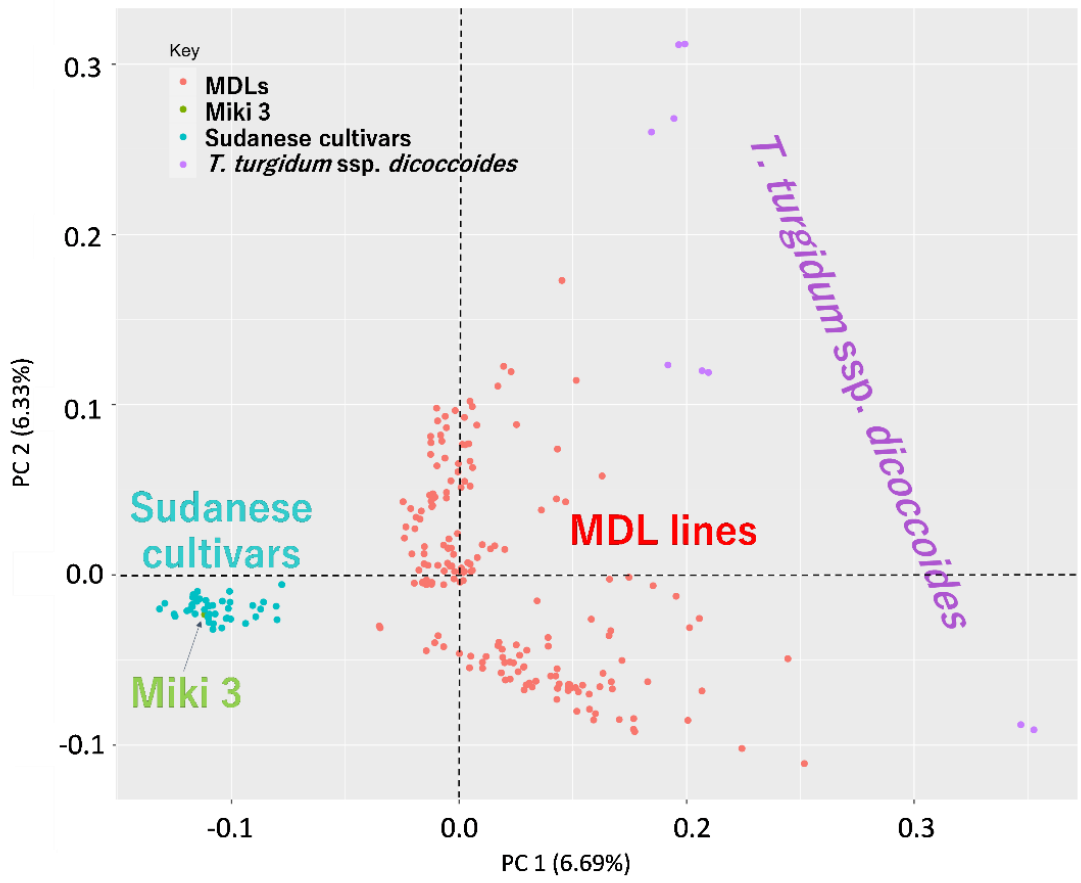

Figure 1. 6 Principal component analysis (PCA) of diversity in multiple derivative lines, parental lines and Sudanese varieties conducted using 7275 SNP markers.

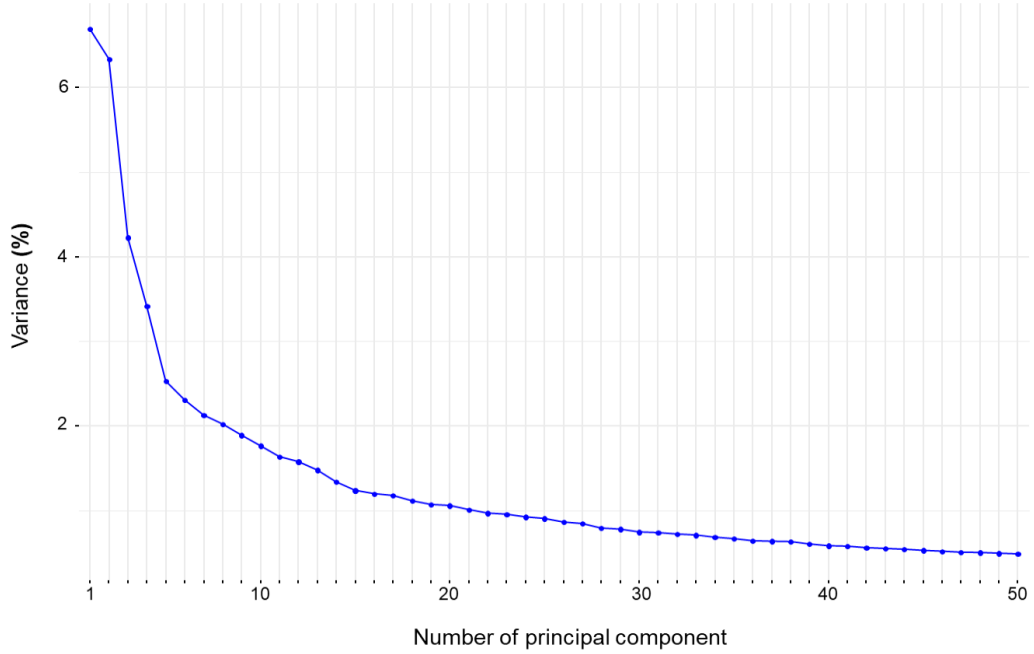

Figure 1. 7 Number of principal components to explain the diversity of the multiple derivative lines.

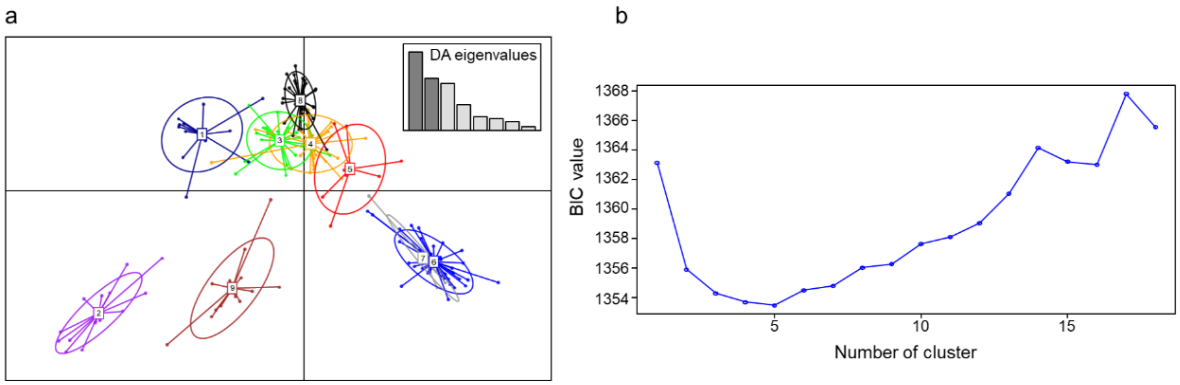

Figure 1. 8 Discriminate analysis of principal components (DAPC) and Bayesian information criterion (BIC) to elucidate the structure of genetic diversity in multiple derivative lines (MDLs). (a) DAPC exhibited nine genetic groups. 1 to 9 correspond to the MDL families. (b) Value of BIC versus number of clusters showed five main clusters for the diversity in the MDL population.

Figure 1. 9 Diversity of shape among some multiple derivative lines: (a) variation of spike length, awn length, and color; (b) variation of glume color; (c) variation of spike shape. Scale bars, 1 cm.

Table 1. 2 Total number of markers, number of polymorphic markers, Nei's genetic diversity index, and PIC in each chromosome in the 178 multiple derivative lines (MDLs)

Genome	No. of markers	No. of polymorphic markers	Nei's genetic diversity index	PIC†
A genome				
1A	407	145	0.2973	0.2685
2A	585	152	0.2269	0.2091
3A	485	173	0.2655	0.2411
4A	389	119	0.2636	0.2383
5A	577	159	0.2419	0.2220
6A	318	91	0.2418	0.2215
7A	552	172	0.2544	0.2329
Mean in A genome	**473.3**	**144.4**	**0.2559**	**0.2333**
B genome				
1B	525	153	0.2531	0.2315
2B	715	208	0.2259	0.2073
3B	604	182	0.2384	0.2206
4B	466	123	0.2369	0.2199
5B	635	135	0.2119	0.1906
6B	472	107	0.2275	0.2095
7B	545	174	0.2756	0.2481
Mean in B genome	**566.0**	**154.6**	**0.2385**	**0.2182**
Total / Mean	**7,275**	**2,093**	**0.2476**	**0.2264**

† PIC: Polymorphic information content.

Table 1. 3 Analysis of molecular variance between nine wild emmer wheat accessions and 178 multiple-derivative lines (MDLs) using 7275 SNPs markers

Source of variation	df†	Sum of squares	Mean of Squares	Estimate variance	Percentage of variance
Among population	1	1116.321	1116.321	10.194	9%
Within population	186	80686.553	426.913	216.913	91%
Total	187	81802.874		467.107	
Pairwise population (Phipt)	0.086				
Nm (haploid)	5.31				

† Degree of freedom

† Nm (haploid number of migrants)

Table 1. 4 Marker–trait associations of days to heading (DH) and plant height (PHT) in multiple derivative lines (MDLs) grown under two environments, Dongola, and Tottori.

Chromosome	Marker	Position	Trait	Environment	*P*-value	Marker R^2
1A	2262791	576975023	DH	Dongola	4.27×10^{-5}	0.142
3B	1033938	828896388	DH	Tottori	8.90×10^{-5}	0.139
4A	2252536	572495965	PHT	Dongola	5.80×10^{-6}	0.146
		572495965		Tottori	8.01×10^{-5}	0.108
	12776290	567028621		Dongola	6.31×10^{-6}	0.172
	1087149	569604831		Dongola	7.44×10^{-6}	0.143
	1220382	575450397		Dongola	4.67×10^{-5}	0.143
4B	2371505	29297345	PHT	Dongola	8.55×10^{-10}	0.321
	1216462	24384093		Dongola	4.24×10^{-9}	0.306
	1064354	26614161		Dongola	5.47×10^{-8}	0.259
	1863400	38841165		Dongola	3.52×10^{-7}	0.218
	2278767	30576288		Dongola	4.55×10^{-7}	0.220
		30576288		Tottori	2.92×10^{-5}	0.149
	991096	38841231		Dongola	1.53×10^{-6}	0.197
	1300855	39949802		Dongola	3.65×10^{-6}	0.180
	2283875	37707737		Dongola	4.80×10^{-6}	0.176
	1088389	39319967		Dongola	5.76×10^{-6}	0.173
	1212987	21575326		Dongola	8.14×10^{-6}	0.168
	55408728	28796166		Dongola	1.32×10^{-5}	0.163
	1003062	38154454		Dongola	1.91×10^{-5}	0.159
	1092216	12250336		Dongola	2.01×10^{-5}	0.154
	4010028	36999359		Dongola	2.43×10^{-5}	0.152
	1091494	39054980		Dongola	3.14×10^{-5}	0.156
	984917	21287734		Dongola	3.50×10^{-5}	0.147
	3958247	35840364		Dongola	4.04×10^{-5}	0.147
	1101888	17488180		Dongola	8.42×10^{-5}	0.133
5A	1081408	557659428	DH	Dongola	3.11×10^{-5}	0.143
	1074046	557542905		Dongola	5.22×10^{-5}	0.138
7B	1017437	585126775	PHT	Dongola	1.85×10^{-5}	0.168
	1214796	609747127		Dongola	2.12×10^{-5}	0.160
	1058067	581541708		Dongola	4.45×10^{-5}	0.142
	2257383	611276698		Dongola	8.56×10^{-5}	0.137

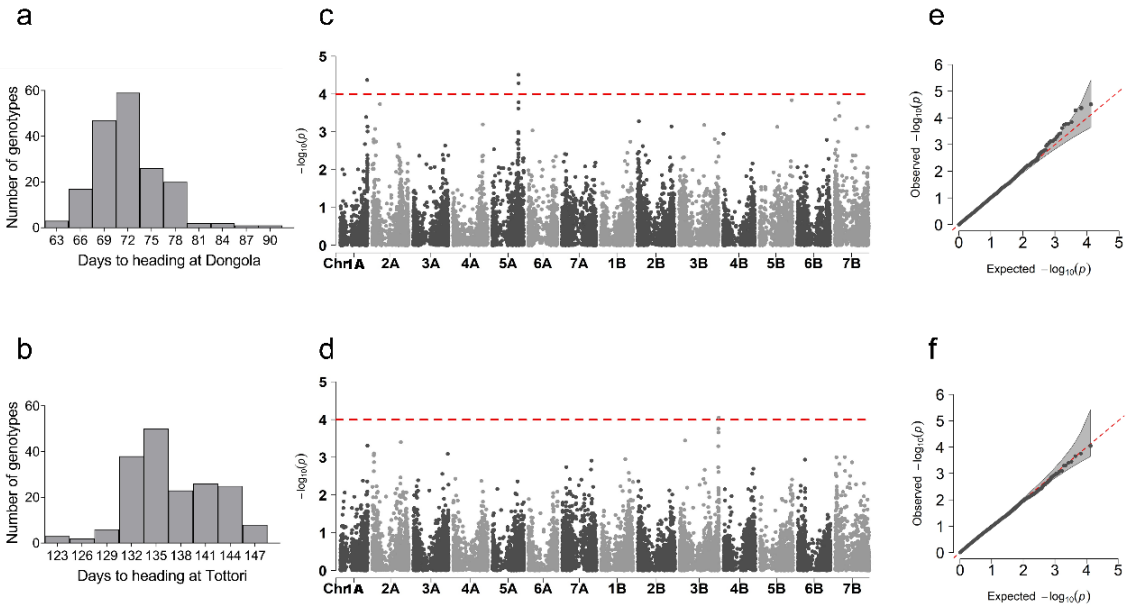

Figure 1. 10 Genome-wide association analysis of days to heading at (a, c, e) Dongola and (b, d, f) Tottori in the multiple derivative line population: (a, b) frequency distribution; (c, d) Manhattan plots (dashed red line indicates significance threshold); (e, f), quantile–quantile plots.

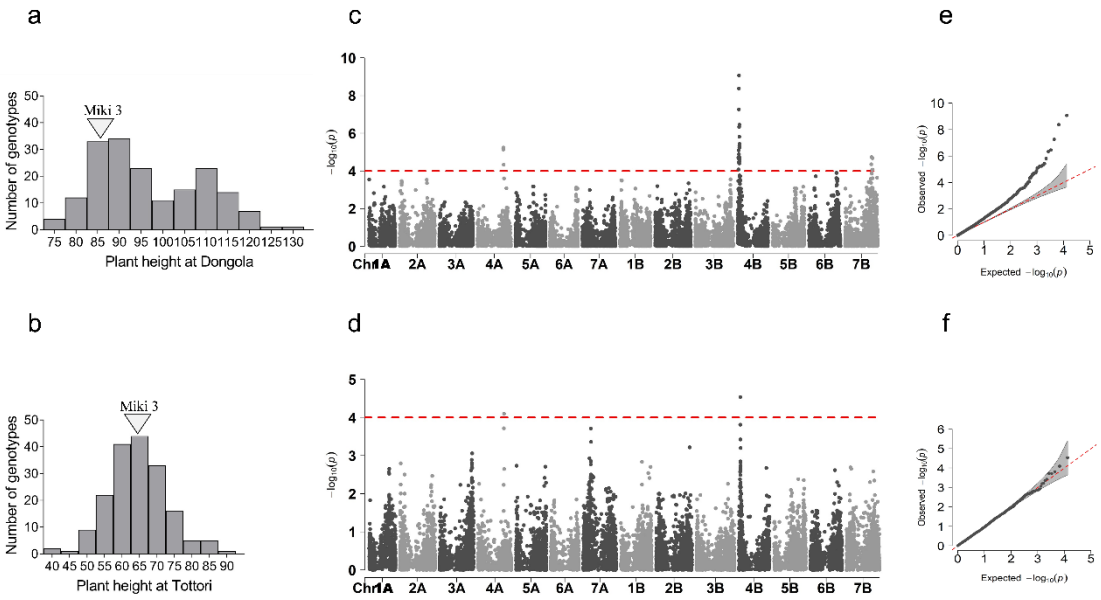

Figure 1. 11 Genome-wide association analysis of plant height at (a, c, e) Dongola and (b, d, f) Tottori in the multiple derivative line population: (a, b) frequency distribution; (c, d) Manhattan plots (dashed red line indicates significance threshold); (e, f), quantile–quantile plots.

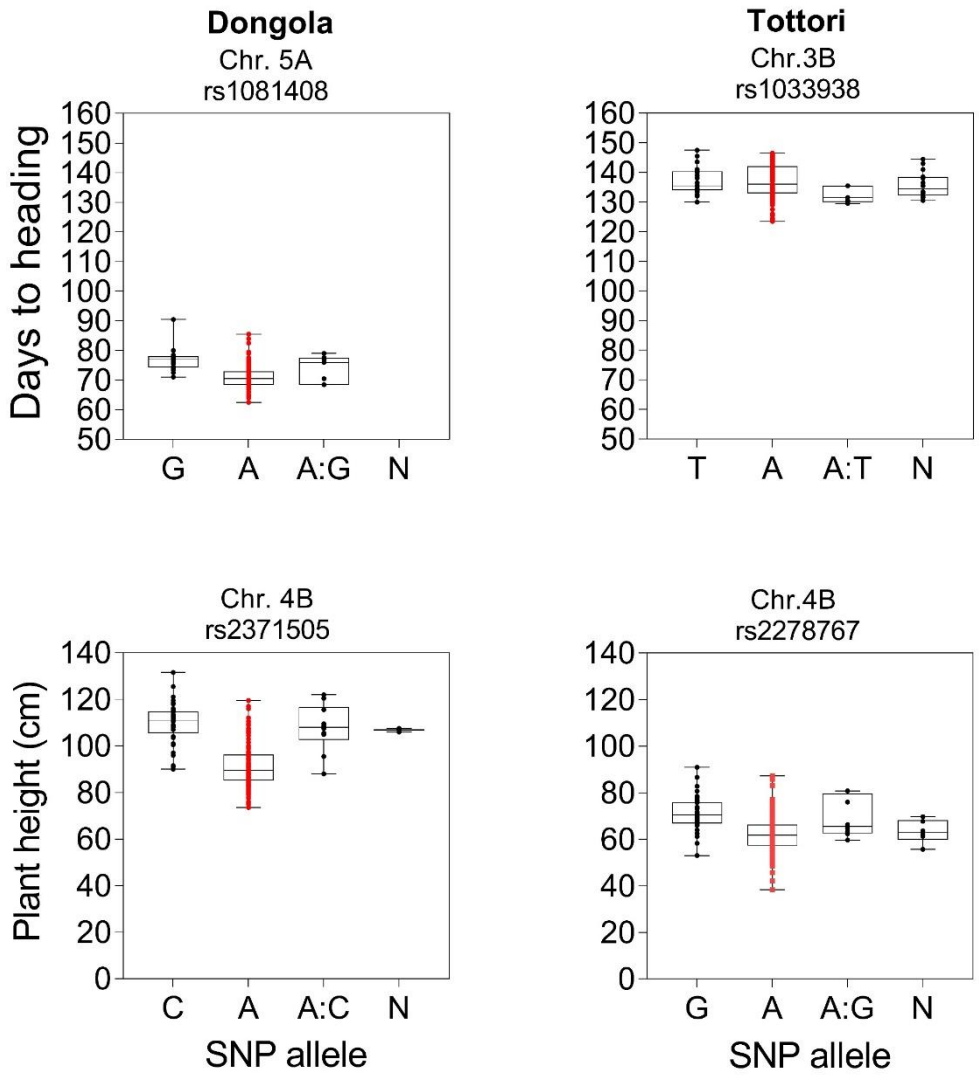

Figure 1. 12 Effect of marker-trait associations on days to heading and plant height in MDL population evaluated in Dongola or Tottori. A, adenine; C, cytosine; G, guanine; T, thymine; N, unknown. Red dots are the allele of 'Miki 3'.

Chapter 2

Exploiting wild emmer wheat diversity to improve wheat A and B genomes in breeding for heat stress adaptation

2. 1 Introduction

Durum wheat (*Triticum turgidum* L. ssp. *durum*, genome BBAA) is a common tetraploid wheat (2n = 28) grown commercially worldwide. Although durum wheat ranks second after bread wheat (*Triticum aestivum* L. ssp. *aestivum*, genome AABBDD), occupying 5% of the total wheat cultivated area (Mastrangelo and Cattivelli, 2021), it has a high market demand because of unique seed characteristics and versatile end uses such as pasta, macaroni, and other semolina products. Moreover, the A and B genomes in durum wheat are identical to those of bread wheat, which can be utilized with the D-genome of *Aegilops tauschii* to improve the plasticity of hexaploid wheat, conferring higher adaptation to improve abiotic tolerance and biotic resistance (Rosyara et al., 2019).

Global warming is projected to negatively impact crop growth, development, and productivity in a wide range of environments, posing a threat to global food security (Mishra et al., 2021). Therefore, improving staple food crops to thrive under stressful heat conditions is essential. Wheat is a cold−loving crop that needs daytime temperatures ranging from 17−23 °C with a nighttime temperature of ≤14 °C to give maximum yield potential (John and Megan, 1999; Prasad et al., 2008). Heat above the threshold of 23 °C alters various physiological, biological, and biochemical processes in wheat and ultimately decreases grain yield (Akter and Rafiqul Islam, 2017; Djanaguiraman et al., 2020). Grain filling stage considered the most sensitive stage, where high temperature stress of 35/20 °C (day/night) from 10 days after anthesis until maturity decreased grain yield by 78%, grain number by 63%, and grain weight by 29% (Gibson and Paulsen, 1999). Climate will warm in the coming few decades. An increase of 1 °C in temperature is predicted to reduce the average wheat grain yield by 4.1−6.4% (Liu et al., 2016; Hassouni et al., 2019). Already more than 40% of wheat−growing regions are experiencing increased temperatures above the optimum (Narayanan, 2018; Djanaguiraman et al., 2020). Nevertheless, world wheat production needs to increase by 60% to meet future demand (Zhang et al., 2021). This challenging situation generally requires at least 1.6% annual wheat yield increases (Narayanan, 2018) and specifically 2.7% increases in semiarid regions where heatwaves are prevalent (Iizumi et al., 2021). Therefore, breeding for heat stress tolerance is essential to improve wheat grain yield and adaptation.

Crop breeders usually utilize genetic variation to improve crops against environmental stresses. However, most wheat cultivars have relatively narrow genetic diversity associated with selection on yield *per se*, restricting the potential to breed for heat stress tolerance (Pinto et al., 2017). These constraints imply that the production level of current wheat cultivars cannot fulfill the world demand for food since global population

constantly increases. Therefore, a practical solution is to expand the genetic base of wheat by using the adaptive capacity resources of wild progenitors. One of the potential resources is wild emmer wheat (*T. turgidum* ssp. *dicoccoides*), a direct progenitor of domesticated durum wheat (*T. durum*), and the A and B genomes of bread wheat (*T. aestivum*). Wild emmer wheat has been shown to have two lineages that could be exploited for wheat improvement via genetic introgression: the western lineage, distributed in Israel, Syria, Lebanon, and Jordan, and the central eastern lineage, distributed in Turkey, Iraq, and Iran (Mori, 2003; Ozkan et al., 2005; Matsuoka, 2011; Peng et al., 2011). Wild emmer wheat is a good resource for improving wheat against environmental stresses (Peng et al., 2013; Tsujimoto et al., 2015; El Haddad et al., 2021). Interestingly, the natural variation of wild emmer wheat encompasses important agronomic, physiological, and yield-related traits associated with heat stress tolerance (Peng et al., 2013). Thus, this diversity in wild emmer wheat is needed to sustain and improve wheat tolerance against heat stress.

Heat stress tolerance is a complex trait strongly affected by the environment, and genotype-by-environment interaction seriously restricts its selection for sustainable breeding. Hence, breeding to improve heat stress tolerance should be conducted in multiple environments to provide evidence of the stability of the key traits (Liu et al., 2019a; Ma'arup et al., 2020).

Most of the previous research targeted exploiting the diversity in wild wheat progenitors to improve heat stress tolerance in hexaploid wheat (Elbashir et al., 2017; Liu et al., 2019b; Ma'arup et al., 2020; Elhadi et al., 2021a, 2021b; Itam et al., 2021a, 2021b; Ullah et al., 2021). However, in durum wheat, only a pre-breeding set of 77 advance lines has been developed and evaluated for heat stress tolerance (Aberkane et al., 2020, 2021). Also, a field evaluation for heat stress tolerance has been conducted on a diverse set of elite lines and landraces (Sall et al., 2018; El Haddad et al., 2020). On the other hand, genetic information from tetraploid wheat growing under field conditions is rare (Sukumaran et al., 2018; Hassouni et al., 2019; Wang et al., 2019). Although previous work identified many quantitative trait loci (QTLs) associated with heat stress tolerance in tetraploid wheat evaluated under various climatic conditions (Sukumaran et al., 2018; Hassouni et al., 2019), such QTLs associated with wild emmer wheat diversity have not been fully explored in a modern durum wheat background. In the chapter 1 I have described the creation and development of multiple derivative lines (MDLs) by crossing and backcrossing nine wild emmer wheat accessions with the durum wheat cultivar 'Miki 3'. This population harbors in its gene pool genomic fragments from both wild emmer wheat lineages. With the expectation that the diversity in the MDLs could be used to improve heat stress tolerance in tetraploid wheat, in the current study, in this chapter, I evaluated a diverse set of 178 MDLs under four environments, including optimum and heat stress conditions. The objective was to identify QTLs linked to heat stress tolerance from the wild emmer wheat diversity in the MDLs, and germplasm to be used in breeding for heat stress tolerance. The MDL platform used in this study provides valuable genetic

materials and QTLs to improve both bread and durum wheats adaptation to heat stress conditions.

2. 2 Materials and Methods

2. 2. 1 Plant material

I used a population of multiple derivative lines (MDLs) consisting of 178 BC_1F_6 durum wheat lines, and their recurrent parent 'Miki 3'. The 178 BC_1F_6 lines were developed by crossing and backcrossing nine wild emmer wheat accessions with the common durum wheat cultivar 'Miki 3'. Detailed information for MDL development and population structure are available in chapter 1.

2. 2. 2 Field evaluation and experimental design

Field experiments were conducted during the winter season (2019−2020) at four sites: one in Japan and three in Sudan. All experiments were arranged in an alpha lattice design with two replications. In Japan, the experiment was conducted in Tottori at the field of the Arid Land Research Center, Tottori University (35°32′N, 134°13′E, 11 m a.s.l.), hereinafter abbreviated TOT. Before sowing, three commercial fertilizer mixtures—Kumiai Fukugo PKN 366 (MC Ferticom Co., Ltd.; Tokyo. Japan; 60kg), Hitachi Fukugo 1 (Htachi-Fukugo Co. Hitachi, Ibaraki, Japan, Ltd.; 40 kg), and granular carbonated magnesium lime (Shimizu Kogyo Co., Ltd. Tokyo, Japan; 100 kg) —were spread onto soil. Seeds were planted and germinated in tray pots in November and transferred to the field during the first week of December and harvested in mid-June. Each genotype was planted in one row of five plants with 0.2 m spacing between plants and 0.8 m spacing between rows. This location has a cold winter with rain-fed field conditions, and the total rainfall amount during the experiment was 930 mm (Arid Land Research Center weather station).

In Sudan, the first experiment was conducted at Dongola Research Station Farm, in North Sudan (19°08′N, 30°27′E, 239 m a.s.l.; abbreviated DON). The second and third experiments were conducted at the Gezira Research Station Farm (GRSF), Agricultural Research Corporation, Wad Medani (14°24′N, 29°33′E, 407 m a.s.l.) with optimum (MED/SD1) and late sowing (MED/SD2), respectively. The late sowing was performed to ensure exposure of the plants to heat stress during the reproductive stage. The GRSF is located in the central clay plain in Gezira State.

In all sites, seeds of each genotype were sown manually in a plot consisting of four rows of 1.0 m length with 0.2 m spacing between rows (the total number of plants per row was about 60 plants). In Dongola, the sowing was during the first week of December, while in Wad Medani, the optimum or first sowing date was in the 4th week of November and the late or second sowing date was in the 4th week of December. All field descriptions, management, seed treatments, irrigation, and fertilization were the same as described in

chapter 1 in the materials and methods section. All cultural practices followed the Agricultural Research Corporation's recommendations for wheat production in Sudan.

2. 3. 3 Measurement of phenological, leaf physiological, and grain yield traits

Phenological traits included days to heading (DH) observed as the number of days from first irrigation or transplanting until 50% of the plant reached heading. Days to maturity (DM) was recorded when 90% of plants lost green color from the glumes. Grain filling duration (GFD) was calculated as the difference in days between DH and DM. Plant height (PHT, cm) was recorded at maturity by measuring the distance between the ground and the top of the spike, excluding awns.

Leaf physiological traits included chlorophyll content at heading (CHLH) and chlorophyll content at maturity (CHLM) measured from three random flag leaves per plot using the Minolta SPAD-502 chlorophyll meter (Konica-Minolta, Japan). Chlorophyll content degradation (CHLD) is calculated as the ratio between CHLM and CHLH (100*CHLM/CHLH). Canopy temperature at heading (CTH) was measured from the canopy of each plot from 13:00 to 14:00 using a handheld infrared thermometer (Everest Interscience, Tucson, AZ, USA) only in Sudanese environments.

Yield and its component traits include grain yield (GY), biomass (BIO), thousand kernel weight (TKW), harvest index (HI), and seed number/spike (SN). GY was determined as grain weight per plot and then converted to kg ha^{-1} for further analysis. BIO was measured for the above-ground dry weight per plot and converted to kg ha^{-1} for further analysis. (TKW, g) and SN were determined from random samples of 10 spikes taken from the central rows. HI was measured as the ratio between BIO and GY (GY/BIO*100).

2. 2. 4 Statistical analysis of phenotypic data

Analysis of variance (ANOVA) for alpha lattice design of all studied traits in each location was performed in GenStat 18th edition (http://genstat.co.uk). I used Tukey's honestly significant difference (HSD) test for environment–environment comparisons. Pearson's correlation coefficient between traits in each environment was calculated in IBM SPSS Statistics for Windows v. 25 (IBM Corp., Armonk, NY, USA). Broad-sense heritability was estimated in Plant Breeding Tools v. 1.4 (http://bbi.irri.org).

To identify heat stress-tolerant genotypes, heat tolerance efficiency (HTE) was calculated as $100*(Y_{si}/Y_{pi})$, where Y_{si} is GY under stress or in a hot environment, and Y_{pi} is GY under an optimum or cold environment (Elbashir et al., 2017). In the first HTE (HTE1), I have used the GY values from DON as the cold environment and MED/SD1 as the hot environment. In the second HTE (HTE2), I considered GY values from MED/SD1 as the cold environment and MED/SD2 as the hot environment.

2. 2. 5 SNP genotyping and data analysis

Total genomic DNA of all genotypes was extracted following a modified CTAB method (Saghai-Maroof et al., 1984). DNA samples (20 µL; 50–100 ng μL^{-1}) were sent to Diversity Array Technology (DArT) Pty., Ltd., Australia (http://www.diversityarrays.com) for whole-genome scanning with the DArTseq (DArT sequencing) platform. Restriction fragments from each sample were sequenced and aligned to durum wheat cv. 'Svevo' RefSeq v.1.0 to generate Silico DArT or SNP markers (Maccaferri et al., 2019). I obtained 32,942 mapped SNP markers scored as "0" (homozygous reference allele), "1" (homozygous SNP allele), or "2" (heterozygous) with a call rate of 90% (10% missing genotype). After removing markers with minor allele frequency of <0.05, I have obtained 13,312 SNPs markers and used them for genome-wide association (GWA) analysis.

2. 2. 6 GWA analysis and candidate genes identification

GWA analysis was performed for each location separately using a mixed linear model (MLM) implemented in TASSEL v. 5.6 (Bradbury et al., 2007). The MLM approach was used to control population structure and relatedness effects and reduce the rate of false-positive associations. The significant marker-trait associations (MTAs) were detected at the threshold $-\log_{10}(P) > 3$. The *P*-values were adjusted for multiple testing using two-levels false discovery rate (FDR) at 0.05 and 0.2 (Benjamini and Hochberg, 1995). The MLM product from TASSEL was used in R v. 4.0.3 with custom scripts in the developed GWAS package rMVP to draw Manhattan plots and quantile−quantile plots for GY (Yin et al., 2021). MTAs were considered stable when found in two or more environments and considered pleiotropic when detected for two or more traits. The favorable alleles for each QTL region were identified by comparing the significant MTAs with extreme phenotypic values in the MDL panel.

For candidate genes analysis, I selected the highly significant markers of the important traits evaluated under the optimum condition at DON, moderate heat (MED/SD1) and severe heat (MED/SD2) stress. I searched for candidate genes in a ± 0.5 Mbp window size from the position of the significant marker by blasting the sequence of the significant markers against IWGSC RefSeq v 2.1 database (https://urgi.versailles.inra.fr/blast). I used Ensemble Plant database https://urgi.versailles.inra.fr/jbrowseiwgsc/gmod_jbrowse/ to determine the number and name of the known genes. The functions of the putative genes were identified using UniProtKB, (https://www.uniprot.org/dataset/identifier).

2. 3 Results

2. 3. 1 Climate condition

Temperature data of the four environments used to evaluate the MDLs during the 2019−2020 growing season are shown in Fig. 2. 1. At TOT, the average daily maximum and minimum air temperatures were 17.0 °C and 7.8 °C, respectively (Fig. 2. 1A). At DON, the average daily maximum and minimum air temperatures were 29.3 °C and 11.4 °C, respectively (Fig. 2.1B). However, at MED/SD1, the average daily maximum and minimum temperatures were 34.9 °C and 16.3 °C (Fig. 2. 1C), while at MED/SD2, they were 36.2 °C and 17.4 °C, respectively. MED/SD2 experienced more heat stress mainly at the reproductive stage (Fig. 2. 1D). In TOT there was no heat stress during the growing season. DON was the coolest among the Sudanese environments and MED/SD1 was cooler than MED/SD2. Therefore, I considered TOT and DON as favorable environments, and MED/SD1 and MED/SD2 as moderate and severe heat stress environments, respectively.

2. 3. 2 Effect of heat stress on MDL performance

The mean GY was 4838 kg ha^{-1} in TOT, 3691 kg ha^{-1} in DON, 2018 kg ha^{-1} in MED/SD1, and 848 kg ha^{-1} in MED/SD2, indicating the severity of heat stress in Wad Medani (Fig. 2. 2 and Table 2. 1). Heat stress (from TOT or DON to MED/SD1 or MED/SD2) caused significant (P<0.001) decreases in GY, BIO, TKW, SN, HI, PHT, DH, DM, GFD, CHLH, CHLM, and significant (P<0.001) increases in CTH (Fig. 2. 3). Late sowing at Wad Medani significantly (P<0.001) reduced CHLD and increased CHLM compared with DON (Fig. 2. 2). The ANOVA showed a highly significant (P<0.001) genotype (G) effect for most of the studied traits in all environments (Table 2. 2). The genotype-by-environment (G×E) interaction effects were highly significant (P<0.0001) for all studied traits except CTH and HI. The broad-sense heritability estimates were high (0.91 to 0.68) for DH, DM, TKW, GFD, and PHT, indicating significant genetic control for these traits (Table 2. 2). However, GY, BIO, SN, and HI showed moderate heritability estimates ranging from 0.38 for GY to 0.48 for SN. In contrast, the lowest heritability estimates of 0.10 was observed for CHLM. Despite the heat stress severity in Wad Medani, some MDLs showed higher GY than their recurrent parent ‘Miki 3’ in MED/SD1 and MED/SD2 (Fig. 2. 3).

I calculated HTE to identify heat tolerant genotypes. As grain yield is important for breeding, I performed regression analysis between GY and HTE to identify the heat-tolerant genotypes with respect to their yield potential (Fig. 2. 4). In the first HTE (HTE1; calculated from GY of DON and MED/SD1), nine genotypes (5%) had a higher HTE1 than their recurrent parent ‘Miki 3’ (Fig. 2. 4A). However, in the second HTE (HTE2; calculated from GY of MED/SD1 and MED/SD2), 25% of the MDLs had a higher HTE2 than their recurrent parent ‘Miki 3’, indicating high genetic gain (Fig. 2. 4B). Most of the MDLs that showed higher HTE in both HTE1 and HTE2 had a lower GY than ‘Miki 3’ except for four genotypes (Fig.2. 4A and Fig. 2. 4B).

GY correlated significantly (P<0.05) with most of the studied traits in the four environments (Table 2. 3−2. 6). In all environments, GY showed a consistent correlation

(P<0.01) with BIO and SN with correlation (r) values ranging from 0.225 to 0.836. GY was positively correlated with HI in all environments except DON. In contrast, no leaf physiological traits were correlated with GY except CTH in MED/SD2, which revealed a significant negative correlation of −0.220 (Table 2. 6). DH and GFD were negatively correlated in all environments, with correlation (r) values ranging from –0.382 to –0.899 (Table 2. 3−2. 6). Under both heat stress conditions, MED/SD1 and MED/SD2, HTE values were positively correlated with GY, BIO, HI, as well as with CHLM in MED/SD2 (Table 2. 5 and Table 2. 6).

2. 3. 3 Detection of MTAs

At $-\log_{10}(P) > 3$, a total of 287, 427, 299, and 406 MTAs were detected in TOT, DON, MED/SD1, and MED/SD2, respectively (Appendix 1−4). To reduce false positives, I have used a more stringent FDR threshold of 0.05 and 215 MTAs were identified across environments (Appendix 5). However, because this FDR threshold is stringent and many potentially important MTAs were excluded, I reduced the threshold up to 0.2, and an identified an additional 90 MTAs (Appendix 5). In total, 35 highly significant MTAs were identified in TOT (30 MTAs with FDR of 0.05 and 5 with FDR of 0.2), 117 in DON (64 MTAs with FDR of 0.05 and 53 with FDR of 0.2), 102 in MED/SD1 (101 MTAs with FDR of 0.05 and one MTA with FDR of 0.2), and 51 in MED/SD2 (21 MTAs with FDR of 0.05 and 30 with FDR of 0.2) (Fig. 2. 5 and Appendix 5). The MTAs for each trait varied, with the highest number observed for GFD (59), followed by TKW (45), HI (44), and PHT (30) (Fig. 2. 6A). In all environments, the number of MTAs observed on the A genome (156) and B genome (149) were almost similar (Fig. 2. 6B). The highest numbers of MTAs were observed on chromosomes 2A (51) and 2B (41), and the lowest number on chromosome 1B (9) (Fig. 2. 6B). To avoid confounding effects of phenological genes on different traits, GWA analysis was performed for GY and other traits for each location using DH as a covariate (Sukumaran et al., 2018). All the identified MTAs were independent of the effect of phenological genes except HTE2.

2. 3. 4 MTAs under favorable conditions (TOT and DON)

In TOT, significant MTAs were detected for HI and PHT (Fig. 2. 5 and Appendix 5), whereas no significant associations were detected for the other traits at the FDR thresholds. The 32 MTAs identified for HI were scattered on 10 chromosomes, and 20 of them were collocated. Five MTAs collocated on chromosomes 2B (635−765 Mbp), 3A (598−672 Mbp), 4B (626−658 Mbp), and 5B (664−671 Mbp) explained on average 13.7, 16.3, 13.9, and 12.0% of the phenotypic variation, respectively. Three MTAs were detected for PHT on chromosomes 4A (one MTA at 572.5 Mbp) and 4B (2 MTAs at 30.5−31.3 Mbp), which explained 10.9−15.2% of the variation (Fig. 2. 5 and Appendix 5).

In DON, 57.0% of the MTAs detected were for CTH, PHT, CHLD, GFD, and HI (Appendix 5). The 29 MTAs detected for PHT were distributed on chromosomes 2B, 4A, 4B, 6B, and 7B; these MTAs explained from 11.1 to 26.6% of the phenotypic variation. Of these MTAs, 21 were collocated on chromosome 4B (12.2−57.5 Mbp) (Fig. 2. 5 and Appendix 5). Among 36 MTAs for CTH, 13 were collocated on chromosome 2B (608.7−781.3 Mbp) and explained 8.6−10.5% of the phenotypic variation (Fig. 2. 5 and Appendix 5).

The eight MTAs for GY were located on chromosomes 1B (2 MTAs at 565.8−590.4 Mbp), 2A (1 MTA at 749.6 Mbp), 2B (4 MTAs at 629.4−705.2 Mbp), and 3A (1 MTA at 473.7 Mbp) that explained from 11.2 to 18.3% of the phenotypic variation (Fig. 2. 5 and Fig. 2.7). A region on chromosome 2A (563.6−755.9 Mbp) had significant MTAs for CHLH, CTH, GY, and HI. A region on chromosome 2B (629.4−781.3 Mbp) harbored MTAs that control CHLD, CTH, overlapped with the GY region spanning 629.4−705.2 Mbp. We found another region on chromosome 3A at 395.8 Mbp that contained MTA for CTH close to the GY MTA at 473.7 Mbp (Fig. 2. 5 and Fig. 2. 8).

I have detected two stable markers on chromosomes 4A (rs2252536 at 572.5 Mbp) and 4B (rs2278767 at 30.6 Mbp) for PHT at TOT and DON, respectively. These MTAs explained 11.9−19.3% of the phenotypic variation. An MTA on chromosome 4B (rs1278393 at 32.8 Mbp) had a pleiotropic effect for HI at TOT and CHLD at DON, that explained 13.7 and 13.2% of the phenotypic variation, respectively (Table 2. 7).

2. 3. 5 MTAs under moderate heat (MED/SD1) and severe heat (MED/SD2) stress

In MED/SD1, out of 102 MTAs, 94 (92.0%) were for TKW, GFD, or DM. The 37 MTAs identified for TKW explained from 11.2 to 17.3% of phenotypic variation, and four of them were collocated between 496.5 and 522.7 Mbp on chromosome 1A (Fig. 2. 5 and Appendix 5). Nine MTAs were detected for DM on chromosomes 1B, 2A, 2B, and 7B that explained on average 17.1% of the phenotypic variation. A region on chromosome 1B (629.4 Mbp) harbored an MTA (SNP rs4406564) that affected DH and DM, explaining 12.4 and 20.3% of the phenotypic variation, respectively. Similarly, two of these MTAs collocated on chromosome 2A (35.8−36.0 Mbp) associated with DH and DM, explaining 15.5 and 18.1% of the phenotypic variation, respectively (Fig. 2. 5 and Appendix 5).

In MED/SD2, I have detected 51 MTAs for nine traits, and 38 (74.5%) of them were for BIO, CHLM, DH, or DM (Fig. 2. 5 and Appendix 5). The eight MTAs for CHLM were located on chromosomes 1A, 2A, 5B, and 6A. Six of these MTAs were collocated with each other: two MTAs on chromosome 1A (522.6−522.9 Mbp) and four on chromosome 2A (754.6−757.1 Mbp). These MTAs explained, on average, 24.4 and 23.3% of the phenotypic variation, respectively (Fig. 2. 5 and Appendix 5). However, strong MTAs for CHLM were identified on chromosomes 1A (522.9 Mbp) and 5B (528.8 Mbp) that explained 28.8 and 26.8% of the phenotypic variation, respectively (Appendix

5). Furthermore, the MTA for CHLM on chromosome 1A was also associated with DH (Table 2. 7). Among six MTAs for BIO, four were collocated on chromosome 3A (708.3−713.4 Mbp); these explained 11.5−15.4% of the phenotypic variation (Fig. 2. 5 and Appendix 5). Five MTAs (SNPs rs1071015, rs982956, rs2252351, rs5970682, rs1277633) collocated on chromosome 2A (32.7−62.0 Mbp) controlled DH and DM and explained on average 17.1 and 17.4% of the variation, respectively (Fig. 2. 5 and Appendix 5). Two MTAs were identified on chromosome 6B, one each for SN (24.6 Mbp) and GFD (160.9 Mbp), explaining 15.1 and 15.0% of the variation, respectively (Fig. 2. 5 and Appendix 5). Three MTAs for GY were identified on chromosomes 2A (35.6 Mbp), 3A (638.4 Mbp), and 3B (795.3 Mbp); these explained 12.1−15.8% of the phenotypic variation (Fig. 2. 5 and Fig. 2. 7).

A common region on chromosome 2A (32.6−119.8 Mbp) had significant MTAs for DH, DM, HI and GY. A region on chromosome 3A (590.4−713.3 Mbp) contained MTAs that controlled BIO, DM, GY, and TKW; these MTAs explained 9.0−15.8% of the variation. I have identified a region on chromosome 3B (744.0−795.2 Mbp) common to BIO and GY, explaining 12.7 and 14.0% of the allelic variation, respectively (Fig. 2. 5 and Fig. 2. 8).

Several MTAs associated with two or more traits between MED/SD1 and MED/SD2 were identified, indicating a high degree of pleiotropic effects (Table 2. 7). A locus on chromosome 1A (522.6 Mbp) controlled TKW in MED/SD1 and CHLM in MED/SD2. An MTA on chromosome 1B (SNP rs4406564 at 629.4 Mbp) had multiple pleiotropic effects on TKW, DH, and DM in MED/SD1, and on DH and DM in MED/SD2; it explained 12.4−20.3% of the variation in these traits (Table 2. 7).

MTAs associated with multiple traits across environments were also identified in this study (Table 2. 7). A common region on chromosome 2A (33.0−62.0 Mbp) affected CHLD, DH, and DM in DON, MED/SD1, and MED/SD2, respectively, and explained 12.6−22.5% of the phenotypic variation. These traits also showed pleiotropic associations on chromosomes 2B (1 MTA) and 7B (2 MTAs) in DON, MED/SD1, and MED/SD2 with phenotypic variation ranging from 12.5−22.5%. I have identified another locus on chromosome 3A (713.3 Mbp) that had a pleiotropic effect on CTH in DON and BIO in MED/SD2, explaining 9.5 and 11.5% of the variation, respectively (Table 2.7). The region on chromosome 1A (358.9−522.6 Mbp) contained MTAs controlled TKW in DON, MED/SD1, and MED/SD2 with phenotypic variation ranging from 11.2−16.3%, whereas the region on 6B (81.3−146.7 Mbp) harbored MTAs for TKW in DON and MED/SD1, explaining 17.9 and 15.6% of the allelic variation, respectively (Fig. 2. 5 and Appendix 5).

2. 3. 6 MTAs for heat tolerance efficiency

I calculated HTE based on the GY values under optimum, moderate heat stress, and severe heat stress conditions (Fig. 2. 4). For the first HTE (HTE1; calculated from GY of DON and MED/SD1), three MTAs were identified, one located on each of chromosomes

2A (695.9 Mbp), 2B (705.1 Mbp), and 5A (622.3 Mbp); these explained 11.2−20.0% of the phenotypic variation (Fig. 2. 5 and Appendix 5). For the second HTE (HTE2; calculated from GY of MED/SD1 and MED/SD2), one MTA was detected on chromosome 3A (16.9 Mbp), by using DH as a covariate; it explained 13.3% of the variation (Fig. 2. 5 and Appendix 5). The locus on chromosome 2B (705.1 Mbp) had a pleiotropic effect on GY in DON and HTE1; it explained 16.0 and 18.3% of the phenotypic variation, respectively (Table 2. 7).

2. 3. 7 Effects of wild emmer wheat alleles in different environments

Strong and pleiotropic MTAs were selected to investigate their haplotype diversity across environments (Fig. 2. 9). The MTA rs982956 on chromosome 2A had favorable alleles from the wild emmer wheat genome that reduced CHLD in DON, increased DH in MED/SD1, and increased DM in MED/SD2 (Fig. 2. 9 and Table 2. 7). Under the severe heat stress condition (MED/SD2), I found two MTAs that had strong positive wild emmer wheat alleles on chromosomes 1A and 5B that increased CHLM: these MTAs explained 28.8 and 26.8% of the phenotypic variation, respectively (Fig. 2. 9). Another MTA on chromosome 3A showed a positive SNP allele from the wild emmer wheat genome: it increased BIO and explained 15.4% of the phenotypic variation. I found two MTAs, one each on chromosomes 2B (705.1 Mbp) and 3A (16. 9 Mbp), that had positive SNP alleles from wild emmer wheat, increasing HTE1 and HTE2, respectively, compared with the recurrent parent ‘Miki 3’ allele (Fig. 2. 9). An MTA located on chromosome 3B had positive SNP allele from the wild emmer wheat genome that increased TKW in MED/SD1 (Fig. 2. 9). To examine the presence of the favorable wild emmer wheat alleles in the elite durum wheat germplasm, I genotyped 43 elite Sudanese durum wheat genotypes and investigated the frequency of the favorable wild emmer wheat alleles in these elite lines (Table 2. 8). Three alleles associated with BIO, CHLM, and TKW were absent from the elite durum lines and the frequency of the other alleles ranged from 2 to 9%. Since the wild emmer wheat has two different lineages, we sought to identify which lineages confer favorable alleles to the MDLs (Table 2. 8). I found that the MDLs carrying a favorable wild emmer wheat allele for CHLM belong to the western lineage, whereas those carrying a favorable allele for BIO belong to the eastern lineage. In contrast, both western and eastern lineages contribute favorable wild emmer wheat alleles for HTE1, HTE2, TKW, CHLD, DH, and DM (Table 2. 8). In this study I evaluated 43 different phenotypic traits (traits × environments). Out of these 43 traits, only eight (19%) had the positive alleles from the wild emmer wheat whereas the remaining 35 had their positive alleles from the recurrent parent ‘Miki 3’. This ratio (19%) is very close to the theoretical ratio of 25% expected from the one backcross event involved in the development of the MDL population as described in chapter 1.

2. 3. 8 Effect of allele combination on GY under severe heat stress

Regions on chromosomes 2A, 3A, and 3B regulate multiple traits under severe heat stress, such as DH, DM, GY, BIO, HI, and TKW. I investigated the haplotype diversity at these loci for GY under severe heat stress. Three haplotype classes with different allelic combinations were identified (Fig. 2. 10). Combining the positive alleles of these loci showed a wide range of GY from 180 to 2215 kg ha^{-1}. Genotypes with haplotype classes containing two positive SNP alleles on chromosomes 2A and 3A and one negative allele on chromosome 3B had GY ranging from 309 to 1423 kg ha^{-1}. The third class, containing one genotype with two negative alleles on chromosomes 2A and 3A and one positive allele on chromosome 3B, had GY of 60 kg ha^{-1} (Fig. 2. 10).

2. 3. 9 Candidate genes analysis

I searched for the candidate genes of the most important markers identified in the different environments (Appendix 6). Most of the identified genes were related to abiotic stress tolerance, especially heat stress tolerance in bread wheat. A candidate gene *TraesCS2B02G521800* in the region of the pleiotropic marker rs5412116 associated with HTE1 in MED/SD1 and GY in DON on chromosome 2B encodes serine/threonine-protein kinase. It regulates hyperosmotic stress responses and ABA signaling (Mao et al., 2010). A candidate gene *TraesCS3A03G0073100* on chromosome 3A for HTE2 was characterized as gene related to zinc finger family protein and regulates heat stress tolerance in bread wheat (Agarwal and Khurana, 2018). Strong significant MTAs in this study were identified for CHLM on chromosomes 1A and 5B with a phenotypic variation of 28.8 and 26.8%, respectively (Fig. 2. 5 and Appendix 5). The deep search around sequences of these MTAs for candidate genes showed 136 genes (Appendix 6). Among them, the genes *TraesCS1A02G341400* on chromosome 1A, *TraesCS5B02G351600* and *TraesCS5B02G350900* on chromosome 5B encode for proteins involved in regulating stay-green traits (Tyagi et al., 2017; Zhang et al., 2020) (Appendix 6). The candidate genes associated with other yield traits such as BIO, TKW, SN, and HI were also identified (Appendix 6).

2. 4 Discussion

2. 4. 1 MDL responses to heat stress and wild emmer wheat contribution to heat tolerance

The mechanism of heat stress tolerance is not well studied in durum wheat. I evaluated MDLs over four environments to identify QTLs linked to heat stress tolerance. In the present study, the late sowing environment reduced GY by 82, 77, and 57% compared with TOT, DON, and MED/SD1 and revealed a consistent decrease with the increase in temperature from TOT to DON and MED/SD1 (Fig. 2. 1); thus, selecting these environments for evaluation was appropriate as they represent different levels of heat stress. Although heat stress resulted in a severe reduction in GY, some MDL lines were

superior and produced higher GY than their recurrent parent 'Miki 3' under moderate and severe heat stress (Fig. 2. 3), indicating that useful genetic variation was introduced from the wild emmer wheat. The MDLs exhibited highly significant differences for most of the studied traits in all environments as well as G×E interaction, indicating the presence of genetic variability in this panel. The MDLs would respond positively to selection because all traits except CHLM had moderate to high heritability, indicating firm genetic control for these traits. Similar results for most traits under optimum and heat stress conditions were reported in bread and durum wheat (Elbashir et al., 2017; Sukumaran et al., 2018; Ullah et al., 2021; Aberkane et al., 2021a; Itam et al., 2021b). Low heritability estimates observed for CHLM may be due to a relatively higher error variance in measurements at different environments (Ogbonnaya et al., 2017). Although heat stress drastically reduced most of the studied traits, it significantly increased CHLM compared to the favorable conditions at DON (Fig. 2. 2). These results indicate that plants in this population maintain high greenness during the maturity stages in response to heat stress.

GY was positively correlated with BIO and SN in all environments, suggesting that selection based on BIO or SN will be adequate to improve GY under optimum or heat stress conditions. Similar results have been reported under similar field conditions except for TOT (Elbashir et al., 2017; Aberkane et al., 2021b; Itam et al., 2021b). GY correlated with HI and TKW only under Sudanese environments indicating their importance in yield determination under heat stress. I identified a significant negative correlation between GY and CTH. As a similar result was previously reported under field conditions (Elbashir et al., 2017; Itam et al., 2021b), CTH would be a practical selection criterion to improve GY in dry, hot environments such as Sudan.

In the current study, I have used HTE (an indicator of yield loss under heat stress) to identify heat-tolerant genotypes among the MDLs (Fig. 2. 4). GY is very important for breeders; therefore, I regressed the HTE to GY to be able to select the tolerant genotypes with good yield performance. For both HTE1 in MED/SD1 and HTE2 in MED/SD2, 5 and 25% of the MDLs, respectively, showed higher HTE than their recurrent parent 'Miki 3'. However, except for four genotypes (MDL36, MDL48, MDL87, and MDL117), most of the genotypes with higher HTE had a lower GY than 'Miki 3' (Fig. 2. 4). Moreover, HTE showed a positive correlation with GY, BIO, HI, in both moderate and severe heat stress environments and with CHLM only under severe heat stress. Therefore, I attribute the high ratio of tolerant genotypes in MED/SD2 (25%) to the positive correlation between HTE2 and CHLM (Table 2. 6). This finding confirms the importance of the stay-green trait as an essential heat tolerance mechanism in wheat (Kamal et al., 2019).

The four high-yielding heat-tolerant MDL genotypes identified are promising and can be integrated into breeding programs to improve heat stress tolerance in durum and bread wheats. The other heat-tolerant genotypes with low yield potential could also be used as a good source of tolerance.

2. 4. 2 GWAS and dissection of the heat-associated MTAs

Multi-environment GWA analysis is a practical approach to elucidate the genetic basis of complex traits such as GY and stress tolerance. However, this approach is influenced by the effects of phenology genes on other agronomic traits (Sukumaran et al., 2018). In this study, the identified MTAs for all traits were independent of the effects of plant phenology genes except HTE2. The MDL panel was pre-selected based on heading under Sudanese conditions (short hot dry growing season); it had a relatively homogenous heading time (Fig. 2. 2), making it ideal to screen for heat stress tolerance. Several MTAs were identified in all environments with phenotypic variation ranging from 7.3−28.8%. A and B genomes had similar number of MTAs, consistent with recent findings (Ullah et al., 2021). In this study, the highest numbers of MTAs were observed on chromosomes 2A and 2B, consistent with the previous findings (Sukumaran et al., 2018) in durum wheat materials. To highlight the usefulness of the MTAs identified for some traits in this study, I compared my findings with the previous GWAS studies in bread wheat (Kumar et al., 2020; Itam et al., 2021b; Ullah et al., 2021) and durum wheat (Sukumaran et al., 2018; Hassouni et al., 2019). The MTAs position on chromosome 3A (473.7−638.4 Mbp) in DON and MED/SD2 for GY indicate the stability of these regions and its importance under favorable and heat stress conditions; for these reasons, this region could be a target for marker-assisted selection. The other MTAs for GY were located in the distal parts of chromosome 2A (35.6 Mbp in DON and 749.7 Mbp in MED/SD2); thus, the environment affected the position of these QTLs on this chromosome. Similar results were reported for the same chromosomes in bread wheat under heat stress (Kumar et al., 2020; Ullah et al., 2021), and this the first such report in durum wheat on this chromosomes for the optimum and heat stress environments.

The MTA for GY on chromosome 3B (795.3 Mbp) was found only under the severe heat stress conditions, indicating that this region may provide opportunities to improve GY under heat stress conditions and should be further investigated to validate its suitability for breeding. A QTL affecting GY on the same chromosome was previously reported under late sowing conditions (Bennett et al., 2012; Kumar et al., 2020).

Under severe heat stress, I found GY regions that harbored MTAs clustered with other important yield-related traits (Fig. 2. 8). For instance, a locus on chromosome 2A (35.6−119.8 Mbp) controlled GY and HI; a locus on chromosome 3A (631.6−713.4 Mbp) controlled TKW, GY, and BIO; and a locus on chromosome 3B (774.1−795.3 Mbp) controlled BIO and GY. In this study, GY under severe heat stress was positively correlated with BIO, HI, and TKW (Table 2. 6). These results indicate that associated traits are likely mapped to similar locations (Liu et al., 2019a) and suggest that BIO, HI, and TKW are effective selection criteria for GY improvement in a heat stress environment. Under the same severe heat stress, I found an independent MTA on chromosome 6B (24.6 Mbp) that controls SN. A similar result was obtained by Hassouni et al. (2019), who found a QTL associated with grain number per spike under heat stress on the same chromosome in durum wheat inbred lines.

There was a high degree of similarity between the MTAs for DH and DM under heat stress on chromosomes 1B, 2A, 2B, and 7B. A similar observation was reported by Ullah et al. (2021), who concluded that, under heat stress, the effect of phenology genes is prominent as significant MTAs mapped on chromosomes 2A and 2B, corresponding to the photoperiod genes *Ppd-A1* and *Ppd-B1*. The locations of MTAs on chromosomes 1B and 7B for DH are similar to those reported previously under optimum, heat, and combined heat and drought stresses in bread wheat (Tahmasebi et al., 2016; Schmidt et al., 2020; Ullah et al., 2021), and under optimum conditions for durum wheat materials (Sukumaran et al., 2018).

A strong association among traits was observed within and across environments. This indicates a high level of pleiotropic effects and stability of the QTLs identified across environments in this study, as found by Ullah et al. (2021) in emmer-derived materials. I identified an MTA on chromosome 1A (522.6 Mbp) that controlled TKW in MED/SD1 and CHLM in MED/SD2; this finding suggests that high chlorophyll content at maturity would result in high TKW and greater yield under heat stress. Although I found no positive association of CHLM with other yield−related traits (Table 2. 3−2. 6), I found a positive trend between CHLM and HTE2 (Table 2. 6); thus, the presence of stay-green in these materials led to improved heat stress tolerance. QTLs for CHLM on chromosomes 1A and 5B and HTE2 on 3A explained a great phenotypic variation in this study, and a combination between them will likely increase the degree of tolerance and ultimately improve GY and its component traits. Furthermore, the favored alleles for these QTLs were derived from the wild emmer wheat genome and might not be well represented in elite durum wheat germplasm (Table 2. 8, Fig. 2. 9). Therefore, these QTLs for CHLM and HTE2 should be carefully validated in recombinant mapping populations to understand their combined effects on GY and its related traits. It is worth noting that the MTAs for HTE2 on chromosome 3A and CHLM on chromosome 5B were linked to important genes (Appendix 8). For instance, the gene *TraesCS3A03G0073100* for HTE2 on chromosome 3A is annotated as a zinc finger family protein (Appendix 8). This family includes C4HC3-type zinc finger *TaZnF* from bread wheat, its overexpression was reported to increase tolerance to heat, oxidative, and cold stress (Agarwal and Khurana, 2018). Similarly, the gene *TraesCS5B02G351600* identified for CHLM on chromosome 5B encodes superoxide dismutase (Appendix 7). The superoxide dismutase gene from bread wheat *TaSOD* stimulates antioxidant enzymes such as catalase, peroxidase, and ascorbate peroxidase, which are involved in various defenses against reactive oxygen species activities resulted from oxidative stress (Tyagi et al., 2017).

Similarly, markers found on chromosomes 2A, 2B, and 7B, had pleiotropic effects on CHLD, DH, and DM in DON, MED/SD1, and MED/SD2. This result indicates that low chlorophyll degradation is associated with longer DH and DM and is supported by haplotype diversity analysis of chromosome 2A (SNP rs982956), which shows that genotypes with a positive G allele from the wild emmer wheat genome reduced CHLD

and consequently increased DH and DM (Fig. 2. 9). Interestingly, this wild emmer wheat allele is not abundant in the elite durum germplasm (Table 2. 8).

A marker on chromosome 2B (705.1 Mbp) had a pleiotropic effect on GY and HTE1 in DON and MED/SD1, respectively. Chromosome 2B was previously reported for GY and tolerance indices (heat susceptibility index) under optimum and heat stress conditions in bread wheat (Qaseem et al., 2019). Although MTAs associated with heat stress index in durum wheat were reported on the same chromosome (Sukumaran et al., 2018), this is the first report of an MTA with pleiotropic effects on GY and stress tolerance index in durum wheat. Interestingly the candidate gene *TraesCS2B02G521800* associated with this marker encodes for serine/threonine-protein kinase, and regulates hyperosmotic stress response (Appendix 7). Mao et al., (2010) found that the overexpression of *TaSnRK2.4*, an SNF1-type serine/threonine-protein kinase of wheat, delayed seedling establishment, promoted longer primary roots, and increased yield under normal growing conditions. Moreover, the *TaSnRK2.4* overexpression enhanced the tolerance to drought, salt, and freezing stress conditions.

I observed an MTA with pleiotropic effects on CTH and BIO in DON and MED/SD2 on chromosome 3A (713.3 Mbp); genotypes with low CTH tend to produce higher BIO. Itam et al. (2021b) reported an association between canopy temperature and BIO located on chromosomes 3D, 5D, and 7D under drought and combined heat and drought stresses in hexaploid wheat. To the best of my knowledge, this is the first report of this genomic region in durum wheat having pleiotropic effects on canopy temperature and BIO. This region could be a potential target for marker-assisted selection after careful validation because both CTH and BIO are associated with GY.

I identified stable MTAs on chromosomes 4A (572.5 Mbp) and 4B (30.5 Mbp) for PHT in TOT and DON. This result indicates the effect of reduced height alleles (*Rht-1*) are prominent in optimum conditions, as these MTAs mapped on chromosomes 4A and 4B are at the same location as reduced height genes *Rht-A1* and *Rht-B1* (Wilhelm et al., 2013). Similar results on the same chromosomes were reported under optimum, heat and combined heat and drought conditions in bread wheat (Itam et al., 2021b; Ullah et al., 2021), and under optimum and heat stress conditions in durum wheat (Sukumaran et al., 2018). Another stable MTA on chromosome 6B (160.9 Mbp) controlled GFD under moderate heat stress (MED/SD1) and severe heat stress (MED/SD2). Similar results were obtained by Sharma et al. (2016), who identified stable QTLs associated with GFD on chromosomes 1B, 2B, 3B, 5A, and 6B under timely and late sowing conditions. This marker could be exploited for molecular wheat breeding programs targeting GFD under heat stress.

The MTAs position on chromosomes 1A (359.9−522.6 Mbp) in DON, MED/SD1, and MED/SD2, and on 6B (81.3−146.7 Mbp) in DON and MED/SD1 for TKW indicates stability of these regions under optimum, moderate and severe heat stress environments. Since TKW is associated with the GY in DON, MED/SD1 and MED/SD2 (Table 2. 3−2. 6), these regions could be a target for marker-assisted selection for improving TKW and

GY under optimum and heat stress environments. The MTAs mapped on chromosome 6B for TKW are equivalent to the hexaploid wheat grain weight gene *$TaGW_2$-6B* location (Qin et al., 2014). Similar results were reported for TKW on chromosomes 1A and 6B under optimum, heat and combined heat-drought stress in bread wheat (Elhadi et al., 2021b; Ullah et al., 2021), and this the first time such gene reported in durum wheat on this chromosomes under the optimum and heat stress environments.

Under severe heat stress, three regions on chromosomes 2A, 3A, and 3B regulate multiple traits: DH, DM, BIO, HI, GY, and TKW, indicating that these regions are critical for heat stress tolerance. Investigation of haplotype diversity for GY at these loci revealed that the combination of favorable alleles facilitates high GY. Among the MDLs, the top five high-yielding genotypes were confirmed to carry three favorable alleles. These genotypes can be used for potential direct release or used as parents in crossing schemes to incorporate their favorable alleles to improve GY under heat stress conditions. The positive combination of MTAs on chromosomes 2A (SNP rs9724899) and 3A (SNP rs1017738) efficiently increase GY under severe heat stress (Fig. 2. 10). Further analysis to validate these MTAs in recombinant mapping populations would be needed to understand their effects on GY.

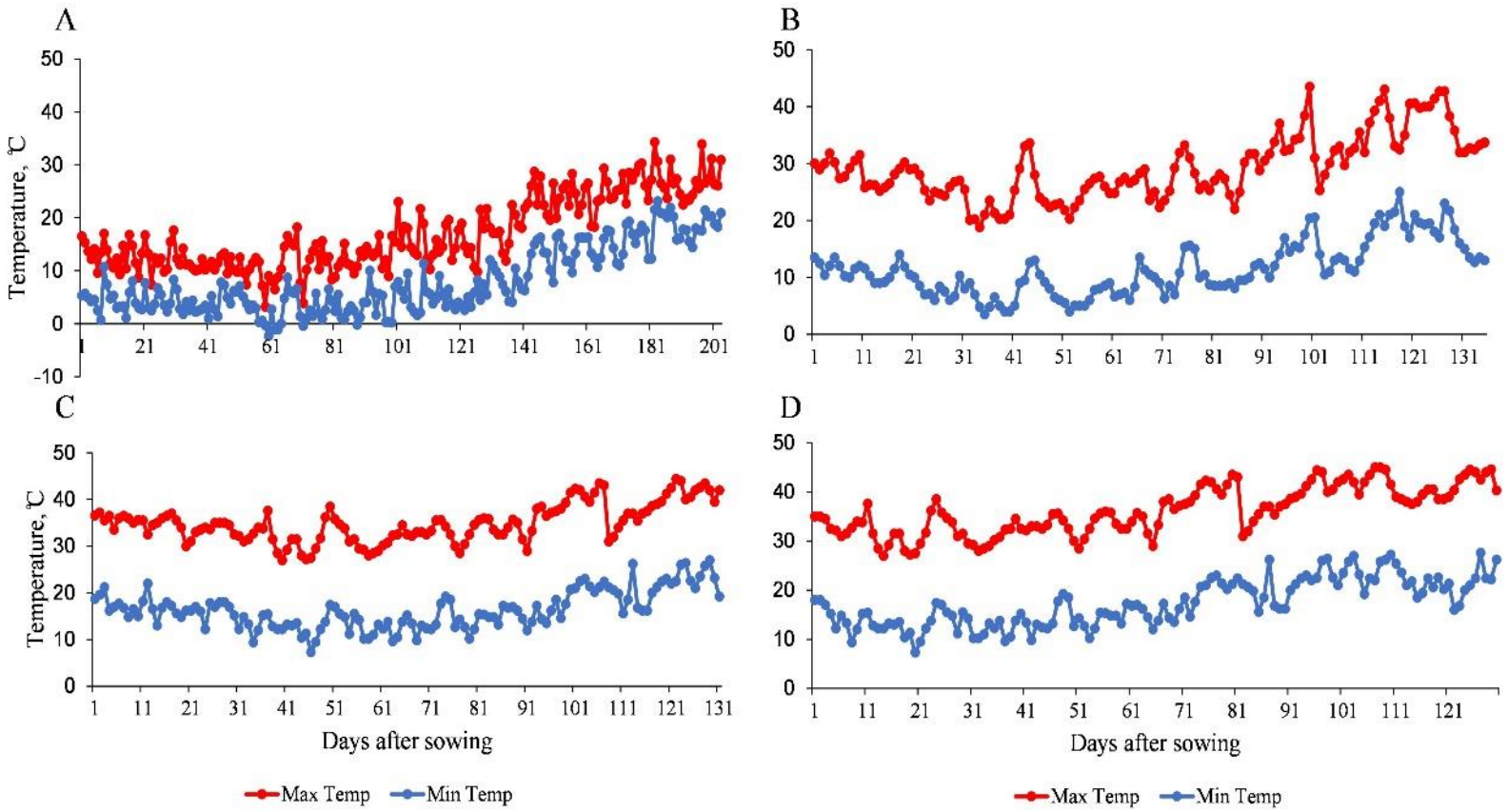

Figure 2. 1 Daily maximum and minimum air temperature of the four environments used to evaluate the multiple derivative lines (MDLs): (A) Tottori (TOT); (B) Dobgola (DON); (C) Wad Medani first sowing date (MED/SD1); and (D) Wad Medani second sowing date (MED/SD2).

Table 2. 1 Means and range of the 15 traits measured for the multiple derivatives lines (MDLs) and their recurrent parent 'Miki 3' evaluated at Tottori (TOT), Dongola (DON), Wad Medani first sowing date (MED/SD1), and Wad Medani second sowing date (MED/SD2) during the 2019–20 season.

	TOT			DON			MED/SD1			MED/SD2		
Trait	Range	Mean	Miki 3	Range	Mean	Miki 3	Range	Mean	Miki 3	Range	Mean	Miki 3
DH	123.0 − 147.0	136.7	144	62.0 − 90.0	71.7	68	57.0 − 94.0	70.4	67.5	58.0 − 84.0	67	67
DM	172.0 − 184.0	178.2	177	104.0 − 126.0	112.1	110.5	91.0 − 126.0	104.6	104	90.0 − 114.0	97	97.5
GFD	31.0 − 54.0	41.5	33.5	19.0 − 50.0	40.3	42.5	21.0 − 49.0	34.1	36.5	22.0 − 33.0	30	30.5
CHLH	43.2 − 62.9	53.3	50.5	21.8 − 62.4	47.1	47.8	45.9 − 59.1	53	56.7	37.8 − 53.7	44	45
CHLM	25.7 − 69.1	48.1	49.4	3.1 − 53.1	15	11.5	30.0 − 42.1	35.1	31.2	30.1 − 53.7	32.4	33
CHLD	-29.5−519	10	1.9	5.1 − 93.9	68.6	74.1	3.5 − 39.6	27.1	34.5	19.1 − 36.7	26.2	26.1
CTH	NA	NA	NA	12.5 − 29.3	17.3	17.6	20.7 − 31.6	25.4	25.5	22.0 − 32.0	25.4	25.3
GY	1763 − 8156	4838.1	4171	906 − 6031	3691	2844	322 − 4173	2018.1	2747	60 − 2215	848.8	1449
BIO	7538 − 28497	17229.1	1451	3750 − 23125	12849.7	6875	2623 − 14347	8282	10626	750 − 11106	5191.7	7106
TKW	33.8 − 63.2	48.7	52.5	16.3 − 56.5	43.3	44.4	20.2 − 54.7	41	46.4	14.6 − 46.0	33.8	35.4
SN	16.2 − 67.6	40.2	43	16.5 − 47.9	32.5	38.1	21.0 − 58.5	25.2	26.5	6.2 − 47.5	17.1	20.4
HI	12.4 − 42.3	28.1	26.9	8.2 − 87.8	30.9	35.8	5.5 − 49.0	31	39	5.6 − 36.5	22	28.6
PHT	38.3 − 91.0	64.3	64.5	73.1 − 131.5	96.4	83	65.0 − 110.1	82.8	75	57.5 − 92.5	75.5	76.7
HTE1	NA	NA	NA	NA	NA	NA	7.6 −238.0	58.1	96.5	NA	NA	NA
HTE2	NA	NA	NA	NA	NA	NA	NA	NA	NA	3.9 −262.9	47.6	52.7

DH, days to heading; DM, days to maturity; GFD, grain filling duration; CHLH, chlorophyll at heading; CHLM, chlorophyll at maturity; CTH, canopy temperature at heading; GY, grain yield; BIO, biomass; TKW, thousand kernel weight; SN, seed number per spike; HI, harvest index; PHT, plant height; HTE1, heat tolerance efficiency evaluated at MED/SD1; HTE2, heat tolerance efficiency evaluated in MED/SD2.

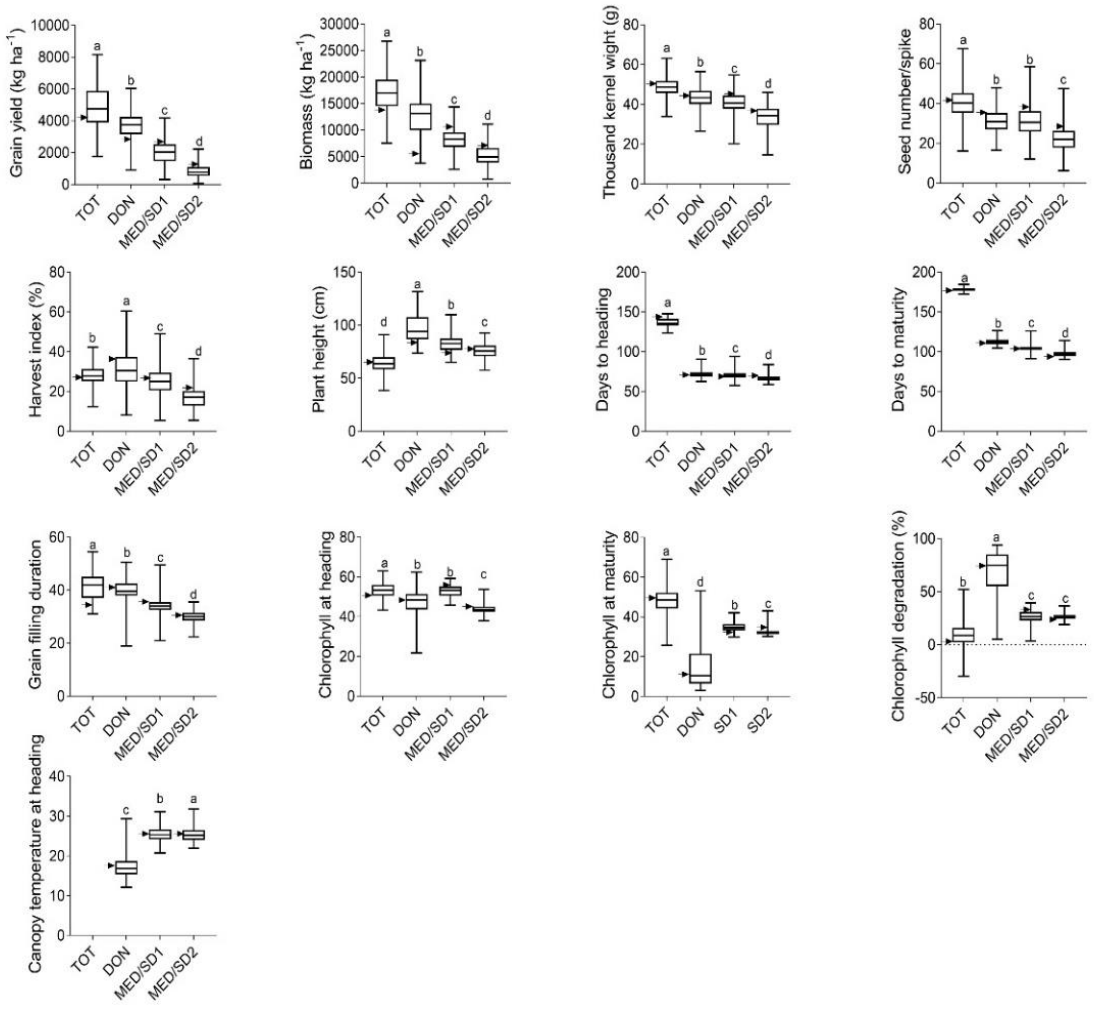

Figure 2. 2 Box plots of the environmental effects on the 13 evaluated traits of the multiple derivatives lines evaluated in four environments: Tottori (TOT); Dongola (DON); Wad Medani first sowing date (MED/SD1); and Wad Medani second sowing date (MED/SD2). Boxes show medians and interquartile range, and whiskers show range. Environments were compared using Tukey's honestly significant difference test.

Table 2. 2 Mean sum of squares of the 13 evaluated traits in the multiple derivative lines grown at Tottori (TOT), Dongola (DON), Wad Medani first sowing date (MED/SD1) and Wad Medani second sowing date (MED/SD2).

Trait	TOT	DON	MED/SD1	MED/SD2	Combined			
	G	G	G	G	G	E	G × E	h^2
DH	48.6***	32.0***	62.9***	32.5***	148.0***	402276.2***	18.9***	0.91
DM	8.6***	29.5***	42.1***	27.5***	90.8***	497786.7***	15.9***	0.85
GFD	43.5***	22.8***	17.4***	8.1**	39.5***	10456.7***	17.9***	0.70
CHLH	28.74ns	97.9**	13.7***	8.7*	47.9.0***	7290.0***	35.3*	0.51
CHLM	67.75**	208.7***	9.6*	5.1ns	78.6***	67064.5***	76.2***	0.10
CHLD	273.2ns	750.7**	64.5ns	15.1ns	283.3*	226344.3***	294.7***	
CTH		7.6ns	9.2*	5.2*	12.4ns	4622.7***	13.4ns	0.49
GY	3626957.0***	1482059.0**	983349.0***	321533.0***	2058936.0***	1114870046.0***	1650547.0***	0.38
BIO	23033923.0***	21186611.0*	8935496.0***	7139663.0***	21380000.0***	9861000000.0***	15890000.0***	0.43
TKW	41.0***	74.1**	47.65**	52.2**	83.4***	13565.2***	43.3***	0.72
SN	136.6***	74.5***	91.9***	92.0***	128.8***	19273.8***	94.0***	0.48
HI	42.35***	233.9ns	103.8***	56.0***	133.5***	15123.1***	102.5ns	0.47
PHT	132.5***	282.9***	114.6*	89.5ns	256.5***	64867.7***	128.9***	0.68

*, **, ***, and ns indicate F-probability values less than 0.05, 0.01, 0.001 and non-significant, respectively. DH, days to heading; DM, days to maturity; GFD, grain filling duration; CHLH, chlorophyll at heading; CHLM, chlorophyll at maturity; CHLD, chlorophyll degradation; CTH, canopy temperature at heading; GY, grain yield; BIO, biomass; TKW, thousand kernel weight; SN, seed number/spike; HI, harvest index; PHT, plant height. G, indicates the main genotype effect at each environment or combined environment; E, environment main effect; G × E, genotype by environment interaction; h^2 heritability estimate.

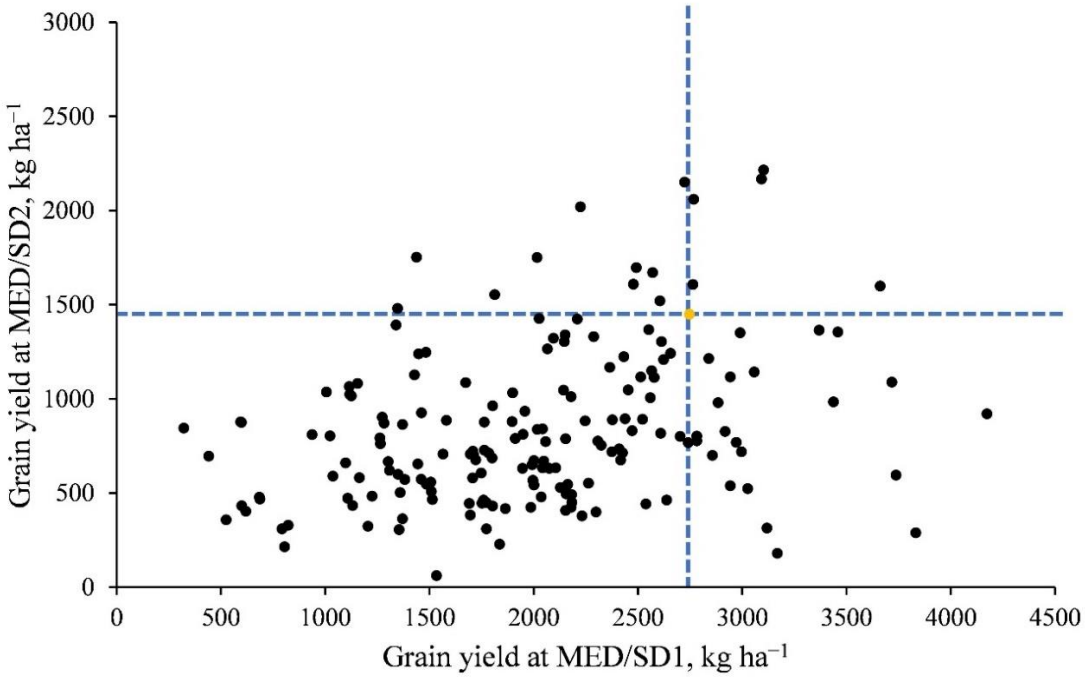

Figure 2. 3 Average grain yield of the multiple derivative lines (MDLs) in Wad Medani for the second sowing date (MED/SD2) versus that in Wad Medani for the first sowing date (MED/SD1). The dashed blue lines intersect on the recurrent parent 'Miki 3' (yellow circle). A few MDLs showed higher grain yield than their recurrent parent 'Miki 3' for both MED/SD1 and MED/SD2.

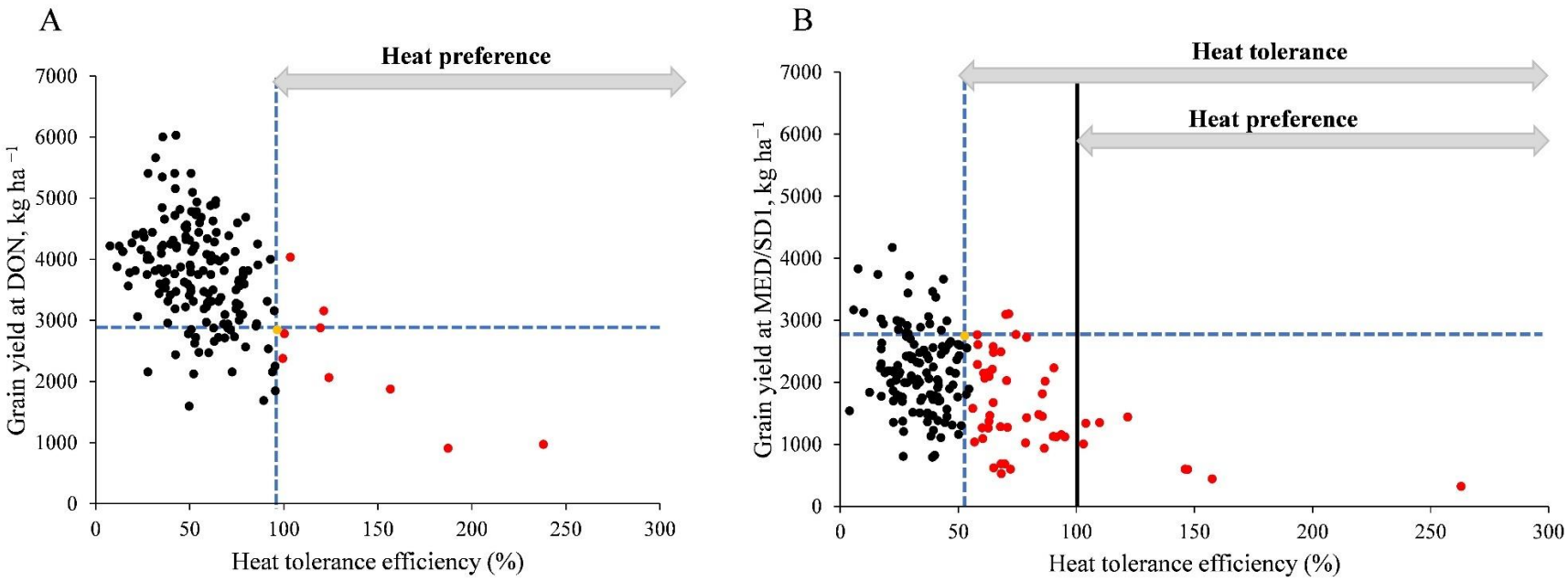

Figure 2. 4 Grain yield (GY) versus heat tolerance efficiency (HTE). (A) GY at Dongola (DON) versus HTE calculated using the GY of DON as the cool environment and that of Wad Medani for the first sowing date (MED/SD1) as the hot environment (referred to as HTE1 in the chapter 2). (B) GY at MED/SD1 and HTE calculated using the GY of MED/SD1 as the cool environment and that of Wad Medani for the second sowing date (MED/SD2) as the hot environment (referred to as HTE2 in the chapter 2). The yellow dot indicates the values for 'Miki 3'. Black dots denote MDLs with HTE less than that of 'Miki 3'. Red dots denote MDLs with HTE higher than that of 'Miki 3'. Dashed blue lines intersect on 'Miki 3'. Vertical black line marks HTE = 100. Horizontal grey arrows denote the ranges of heat tolerance and heat preference.

Table 2. 3 Correlation coefficients between the traits measured in the multiple derivative lines evaluated at Tottori.

Trait	DH	DM	GFD	CHLH	CHLM	CHLD	GY	BIO	TKW	SN	HI	PHT
DH	1	0.361**	-0.899**	0.230**	0.162*	-0.029	0.172*	0.331**	0.210**	0.251**	-0.098	0.212**
DM	0.361**	1	0.083	0.133	0.346**	-0.265**	-0.144	-0.053	0.424**	-0.061	-0.188*	-0.038
GFD	-0.899**	0.083	1	-0.183*	-0.011	-0.093	-0.252**	-0.378**	-0.026	-0.297**	0.016	-0.244**
CHLH	0.230**	0.133	-0.183*	1	0.215**	0.376**	0.125	0.190*	0.102	0.060	-0.017	-0.031
CHLM	0.162*	0.346**	-0.011	0.215**	1	-0.816**	0.040	0.038	0.249**	0.142	0.007	-0.054
CHLD	-0.029	-0.265**	-0.093	0.376**	-0.816**	1	0.024	0.062	-0.184*	-0.089	-0.023	0.040
GY	0.172*	-0.144	-0.252**	0.125	0.040	0.024	1	0.836**	0.138	0.486**	0.649**	0.084
BIO	0.331**	-0.053	-0.378**	0.190*	0.038	0.062	0.836**	1	0.141	0.355**	0.198**	0.213**
TKW	0.210**	0.424**	-0.026	0.102	0.249**	-0.184*	0.138	0.141	1	-0.036	0.070	0.188*
SN	0.251**	-0.061	-0.297**	0.060	0.142	-0.089	0.486**	0.355**	-0.036	1	0.441**	0.140
HI	-0.098	-0.188*	0.016	-0.017	0.007	-0.023	0.649**	0.198**	0.070	0.441**	1	-0.124
PHT	0.212**	-0.038	-0.244**	-0.031	-0.054	0.040	0.084	0.213**	0.188*	0.140	-0.124	1

*and ** significant at the 0.05 and 0.01 levels, respectively (2-tailed). DH, days to heading; DM, days to maturity; GFD, grain filling duration; CHLH, chlorophyll at heading; CHLM, chlorophyll at maturity; CHLD, chlorophyll degradation; GY, grain yield; BIO, biomass; TKW, thousand kernel weight; HI, harvest index; SN, seed number/spike; PHT, plant height.

Table 2. 4 Correlation coefficients between the traits measured in the multiple derivative lines evaluated at Dongola.

Trait	DH	DM	GFD	CHLH	CHLM	CHLD	CTH	GY	BIO	TKW	SN	HI	PHT
DH	1	0.690**	-0.459**	0.329**	0.538**	-0.471**	-0.212**	0.019	0.118	-0.084	-0.089	0.114	0.108
DM	0.690**	1	0.260**	0.363**	0.612**	-0.549**	-0.297**	0.155*	0.218**	0.042	-0.075	0.099	0.083
GFD	-0.459**	0.260**	1	-0.004	-0.024	0.013	-0.027	0.166*	0.183*	0.104	0.055	0.027	-0.022
CHLH	0.329**	0.363**	-0.004	1	0.404**	-0.138	-0.027	0.097	0.132	0.003	-0.065	0.067	-0.171*
CHLM	0.538**	0.612**	-0.024	0.404**	1	-0.940**	-0.401**	0.138	0.158*	0.010	-0.118	0.108	-0.057
CHLD	-0.471**	-0.549**	0.013	-0.138	-0.940**	1	0.434**	-0.089	-0.100	0.016	0.100	-0.075	0.020
CTH	-0.212**	-0.297**	-0.027	-0.027	-0.401**	0.434**	1	-0.053	-0.030	-0.169*	0.011	-0.016	-0.0181
GY	0.019	0.155*	0.166*	0.097	0.138	-0.089	-0.053	1	0.450**	0.180*	0.342**	0.117	0.081
BIO	0.118	0.218**	0.183*	0.132	0.158*	-0.100	-0.030	0.450**	1	0.076	-0.504**	0.029	0.118
TKW	-0.084	0.042	0.104	0.003	0.010	0.016	-0.169*	0.180*	0.076	1	0.018	-0.068	0.218**
SN	-0.089	-0.075	0.055	-0.065	-0.118	0.100	0.011	0.342**	-0.504**	0.018	1	0.121	-0.079
HI	0.114	0.099	0.027	0.067	0.108	-0.075	-0.016	0.117	0.029	-0.068	0.121	1	-0.0621
PHT	0.108	0.083	-0.022	-0.171*	-0.057	0.020	-0.0181	0.081	0.118	0.218**	-0.079	-0.0621	1

*and ** significant at the 0.05 and 0.01 levels, respectively (2-tailed). DH, days to heading; DM, days to maturity; GFD, grain filling duration; CHLH, chlorophyll at heading; CHLM, chlorophyll at maturity; CHLD, chlorophyll degradation; CTH, canopy temperature; GY, grain yield; BIO, biomass; TKW, thousand kernel weight; HI, harvest index; SN, seed number/spike; PHT, plant height.

Table 2. 5 Correlation coefficients between the traits measured in the multiple derivative lines evaluated at Wad Medani optimum sowing date (MED/SD1).

Trait	DH	DM	GFD	CHLH	CHLM	CHLD	CTH	GY	BIO	TKW	HI	SN	PHT	HTE1
DH	1	0.848**	-0.567**	0.117	0.071	0.171*	-0.137	-0.085	-0.055	-0.386**	-0.025	-0.031	0.021	-0.039
DM	0.848**	1	-0.046	0.227**	0.028	0.180*	-0.146	-0.092	-0.071	-0.226**	-0.033	-0.015	0.027	-0.064
GFD	-0.567**	-0.046	1	0.113	-0.090	-0.043	0.031	0.017	-0.007	0.377**	-0.005	0.035	0.003	-0.025
CHLH	0.117	0.227**	0.113	1	0.007	0.265**	-0.058	-0.062	-0.070	-0.013	0.011	0.073	-.231**	0.016
CHLM	0.071	0.028	-0.090	0.007	1	-0.741**	0.182*	0.010	0.023	-0.126	-0.019	-0.044	-0.056	0.081
CHLD	0.171*	0.180*	-0.043	0.265**	-0.741**	1	-0.171*	-0.035	-0.053	0.037	0.015	0.051	-0.054	-0.094
CTH	-0.137	-0.146	0.031	-0.058	0.182*	-0.171*	1	-0.093	-0.052	0.093	-0.065	-0.113	-0.103	-0.078
GY	-0.085	-0.092	0.017	-0.062	0.010	-0.035	-0.093	1	0.585**	0.297**	0.642**	0.298**	0.177*	0.626**
BIO	-0.055	-0.071	-0.007	-0.070	0.023	-0.053	-0.052	0.585**	1	0.178*	-0.202**	0.170*	0.229**	0.430**
TKW	-0.386**	-0.226**	0.377**	-0.013	-0.126	0.037	0.093	0.297**	0.178*	1	0.181*	0.056	0.350**	0.065
HI	-0.025	-0.033	-0.005	0.011	-0.019	0.015	-0.065	0.642**	-0.202**	0.181*	1	0.232**	0.022	0.359**
SN	-0.031	-0.015	0.035	0.073	-0.044	0.051	-0.113	0.298**	0.170*	0.056	0.232**	1	0.021	0.120
PHT	0.021	0.027	0.003	-.231**	-0.056	-0.054	-0.103	0.177*	0.229**	0.350**	0.022	0.021	1	0.057
HTE1	-0.039	-0.064	-0.025	0.016	0.081	-0.094	-0.078	0.626**	0.430**	0.065	0.359**	0.120	0.057	1

*and ** significant at the 0.05 and 0.01 levels, respectively (2-tailed). DH, days to heading; DM, days to maturity; GFD, grain filling duration; CHLH, chlorophyll at heading; CHLM, chlorophyll at maturity; CHLD, chlorophyll degradation; CTH, canopy temperature; GY, grain yield; BIO, biomass; TKW, thousand kernel weight; HI, harvest index; SN, seed number/spike; PHT, plant height; HTE1, heat tolerance efficiency evaluated in MED/SD1.

Table 2. 6 Correlation coefficients between the traits measured in the multiple derivative lines evaluated at Wad Medani second late sowing date (MED/SD2).

Trait	DH	DM	GFD	CHLH	CHLM	CHLD	CTH	GY	BIO	TKW	HI	SN	PHT	HTE2
DH	1	0.868**	-0.382**	0.477**	0.152*	0.014	-0.0132	-0.274**	-0.309**	-0.108	0.002	-0.129	0.052	-0.181*
DM	0.868**	1	0.127	0.488**	0.139	0.006	-0.087	-0.181*	-0.193*	-0.018	-0.024	-0.112	0.057	-0.138
GFD	-0.382**	0.127	1	-0.045	-0.046	-0.018	-0.136	0.211**	0.259**	0.183*	-0.050	0.050	0.002	0.106
CHLH	0.477**	0.488**	-0.045	1	0.334**	0.217**	0.008	-0.090	-0.116	0.078	-0.002	-0.016	0.022	-0.001
CHLM	0.152*	0.139	-0.046	0.334**	1	-0.415**	-0.006	0.049	0.096	-0.005	0.058	-0.030	0.054	0.309**
CHLD	0.014	0.006	-0.018	0.217**	-0.415**	1	-0.050	0.019	-0.044	0.120	-0.020	0.007	-0.064	-0.050
CTH	-0.0132	-0.087	-0.136	0.008	-0.006	-0.050	1	-0.220**	-0.238**	-0.116	0.068	-0.078	0.003	-0.078
GY	-0.274**	-0.181*	0.211**	-0.090	0.049	0.019	-0.220**	1	0.791**	0.326**	0.467**	0.225**	0.084	0.466**
BIO	-0.309**	-0.193*	0.259**	-0.116	0.096	-0.044	-0.238**	0.791**	1	0.287**	-0.082	0.125	0.063	0.403**
TKW	-0.108	-0.018	0.183*	0.078	-0.005	0.120	-0.116	0.326**	0.287**	1	0.121	0.031	0.019	0.058
HI	0.002	-0.024	-0.050	-0.002	0.058	-0.020	0.068	0.467**	-0.082	0.121	1	0.1303	0.0675	0.220**
SN	-0.129	-0.112	0.050	-0.016	-0.030	0.007	-0.078	0.225**	0.125	0.031	0.1303	1	0.142	0.046
PHT	0.052	0.057	0.002	0.022	0.054	-0.064	0.003	0.084	0.063	0.019	0.0675	0.142	1	0.036
HTE2	-0.181*	-0.138	0.106	-0.001	0.309**	-0.050	-0.078	0.466**	0.403**	0.058	0.220**	0.046	0.036	1

*and ** significant at the 0.05 and 0.01 levels, respectively (2-tailed). DH, days to heading; DM, days to maturity; GFD, grain filling duration; CHLH, chlorophyll at heading; CHLM, chlorophyll at maturity; CHLD, chlorophyll degradation; CTH, canopy temperature; GY, grain yield; BIO, biomass; TKW, thousand kernel weight; HI, harvest index; SN, seed number/spike; PHT, plant height; HTE2, heat tolerance efficiency evaluated in MED/SD2.

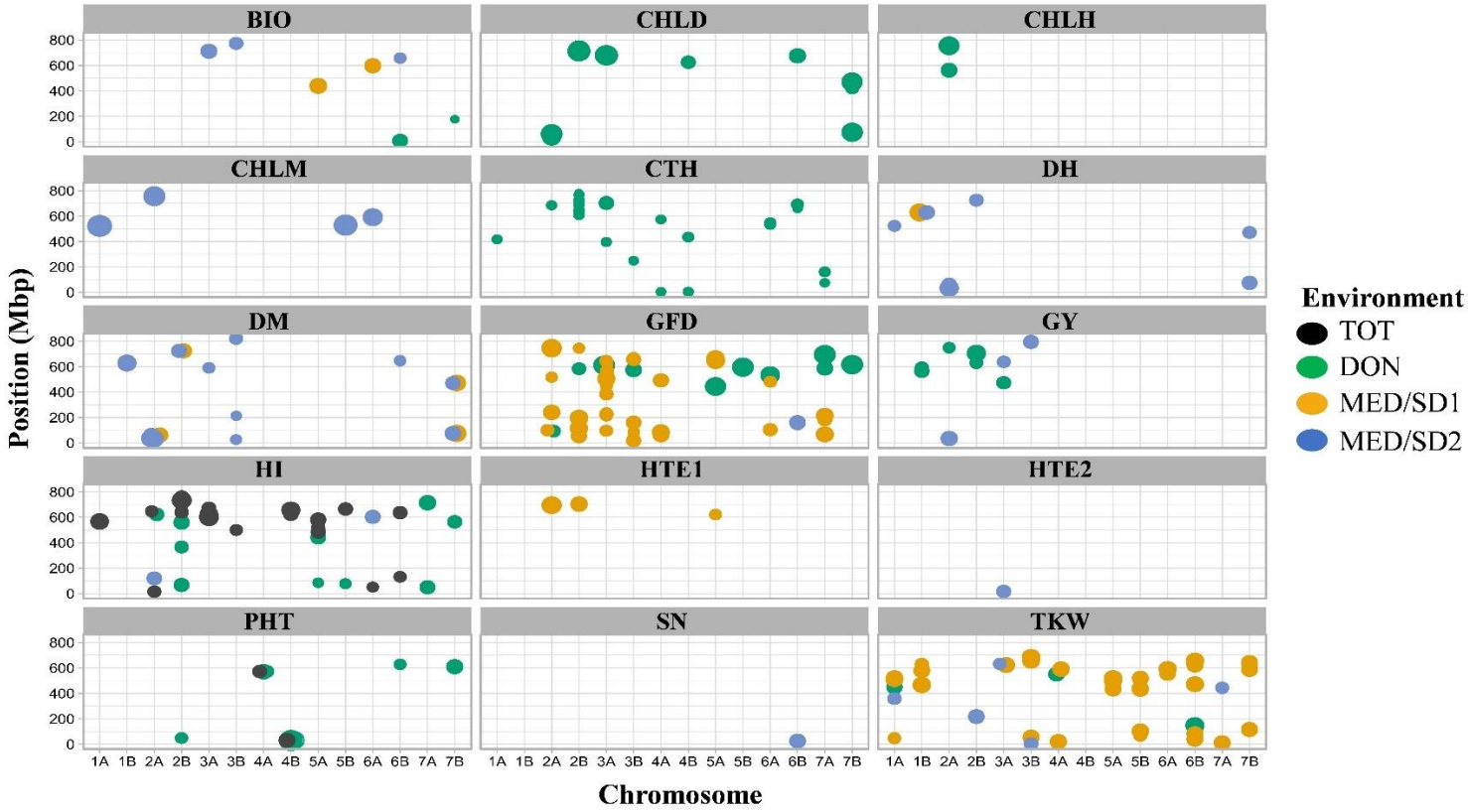

Figure 2. 5 Physical positions of markers associated with evaluated traits in the four environments; Tottori (TOT); Dongola (DON); Wad Medani first sowing date (MED/SD1); and Wad Medani second sowing date (MED/SD1). BIO, biomass; CHLD, chlorophyll degradation; CHLH, chlorophyll at heading; CHLM, chlorophyll at maturity; CTH, canopy temperature at heading; DH, days to heading; DM, days to maturity; GFD, grain filling duration; GY, grain yield; HI, harvest index; HTE1, heat tolerance efficiency evaluated in MED/SD1; HTE2, heat tolerance efficiency evaluated in MED/SD2; PHT, plant height; SN, seed number/spike; TKW, thousand kernel weight. Symbol size corresponds to the allelic effect of each MTA.

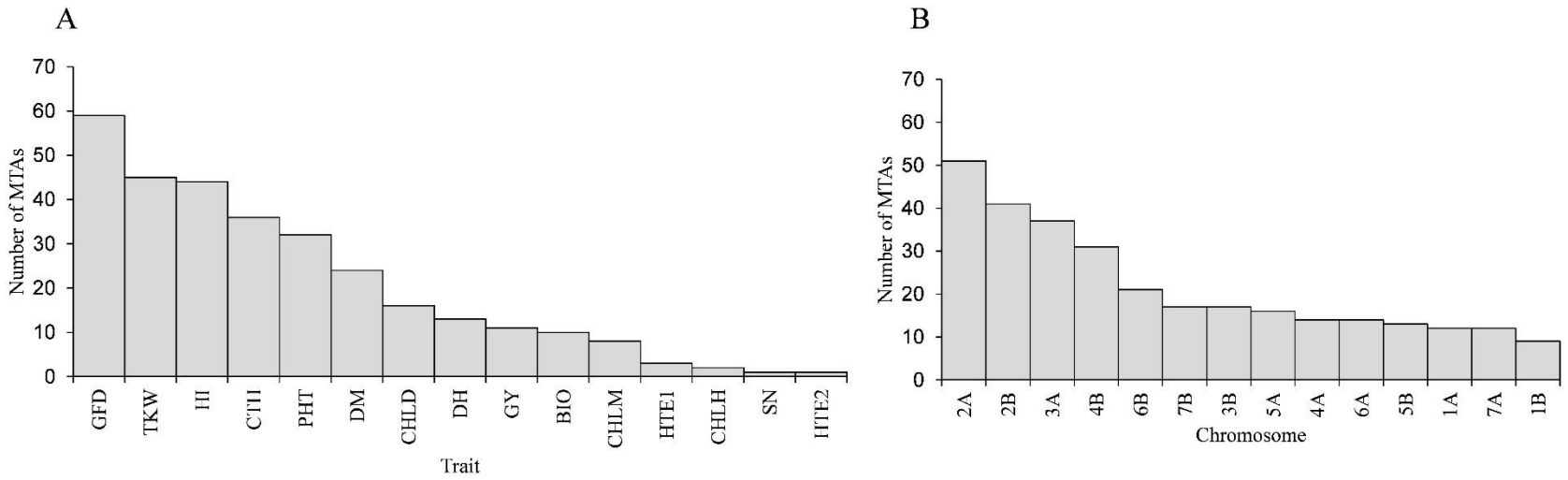

Figure 2. 6 Number of marker−trait associations (MTAs) explained by (A) evaluated traits in all environments or (B) chromosomes in all environments. BIO, biomass; CHLD, chlorophyll degradation; CHLH, chlorophyll at heading; CHLM, chlorophyll at maturity; CTH, canopy temperature at heading; DH, days to heading; DM, days to maturity; GFD, grain filling duration; GY, grain yield; HI, harvest index; PHT, plant height; SN, seed number/spike; TKW, thousand kernel weight; HTE1, heat tolerance efficiency evaluated in MED/SD1; HTE2, heat tolerance efficiency evaluated in MED/SD2.

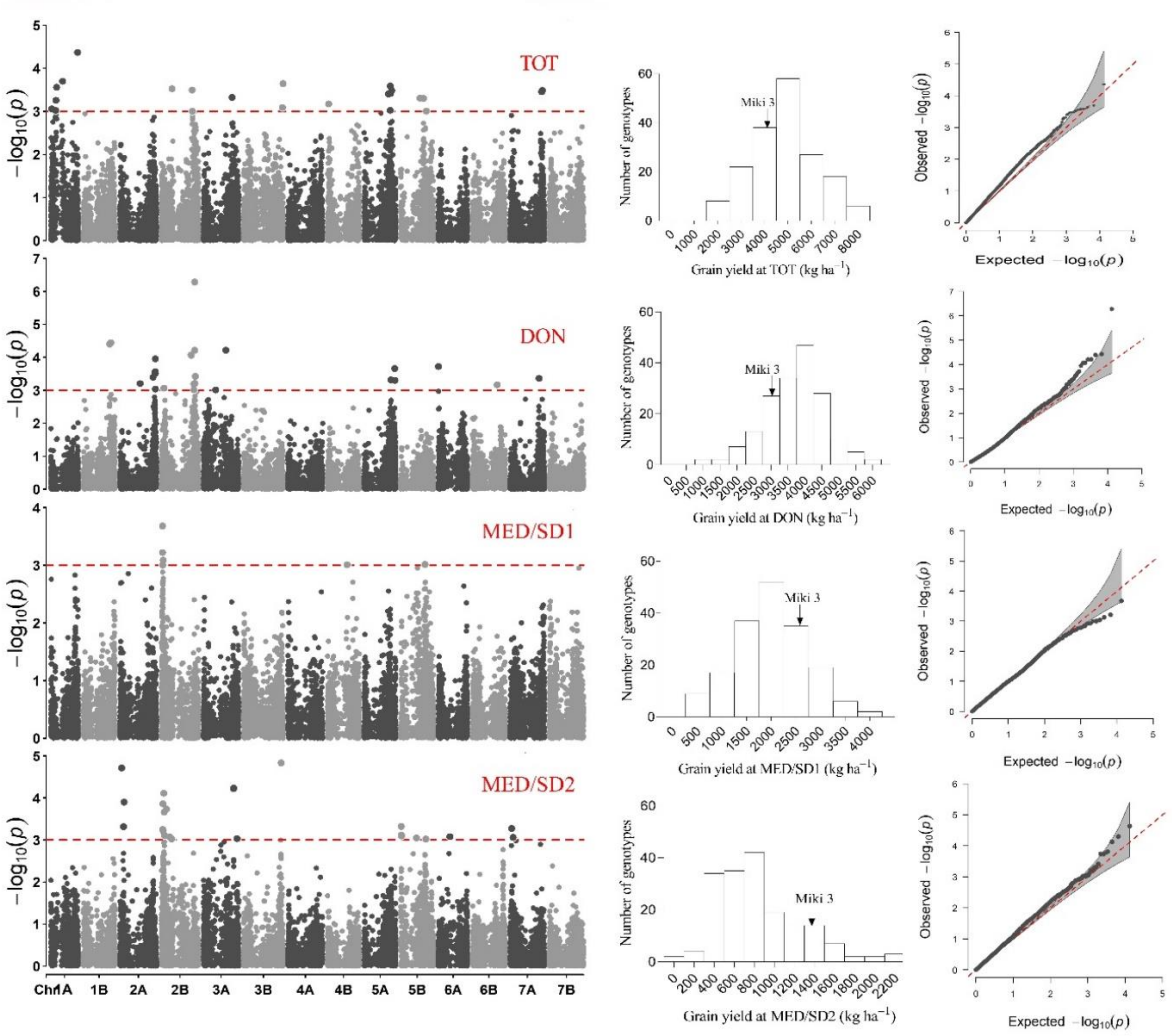

Figure 2. 7 Representative Manhattan plots for grain yield of the genome−wide analysis showing marker−trait association in the four environments; Tottori (TOT), Dongola (DON), Wad Medani first sowing date (MED/SD1) and Wad Medani second sowing date (MED/SD2). Frequency distributions and quantile−quantile plots for grain yield are shown for each environment.

Table 2. 7 Stable and pleiotropic marker-trait associations in the four environments; Tottori (TOT), Dongola (DON), Wad Medani first sowing date (MED/SD1) and Wad Medani second sowing date (MESD/SD2) used to evaluate the multiple derivative lines (MDLs) during the 2019–20 growing season.

Marker	Chromosome	Position (Mbp)	FDR threshold	TOT	DON	MED/SD1	MED/SD2	Allelic effect (%)
rs1062872	1A	522.682366	0.05			TKW	CHLM	15.4−20.0
rs7336178	1A	522.966035	0.05				CHLM, DH	11.8−28.7
rs4406564	1B	629.363030	0.20, 0.05			TKW, DH, DM	DH, DM	12.4−20.3
rs1071015	2A	62.009636	0.05		CHLD	DH	DH, DM	13.3−22.5
rs982956	2A	36.038265	0.05		CHLD	DH, DM	DH, DM	15.5−8.1
rs2252351	2A	35.846102	0.05		CHLD	DH, DM	DH, DM	15.5−18.0
rs1277633	2A	32.676657	0.05		CHLD	DM	DH, DM	15.8−18.8
rs5970682	2A	32.888573	0.20, 0.05		CHLD, DM	DM	DH, DM	12.6−18.6
rs5412116	2B	705.194705	0.05		GY	HTE1		16.0−18.3
rs1151045	2B	724.923696	0.05		CHLD	DM	DH, DM	12.5−21.9
rs984212	3A	713.346253	0.2		CTH		BIO	9.5−11.5
rs2278767	4B	30.576288	0.1, 0.05	PHT	PHT			15.2−19.3
rs1278393	4B	32.888570	0.05	HI	CHLD			13.7−13.2
rs2252536	4A	572.496000	0.05	PHT	PHT			12.9−11.9
rs1018411	6B	160.902903	0.05			GFD	GFD	12.8−15.0
rs1111512	7B	471.484999	0.05, 0.20		CHLD	DM	DH, DM	12.4−21.8
rs1255650	7B	74.684763	0.1, 0.05		CHLD	DM	DH, DM	14.1−22.5

BIO, biomass; CHLD, chlorophyll degradation; CHLM, chlorophyll at maturity; CTH, canopy temperature at heading; DH, days to heading; DM, days to maturity; GFD, grain filling duration; GY, grain yield; PHT, plant height; TKW, thousand kernel weight.

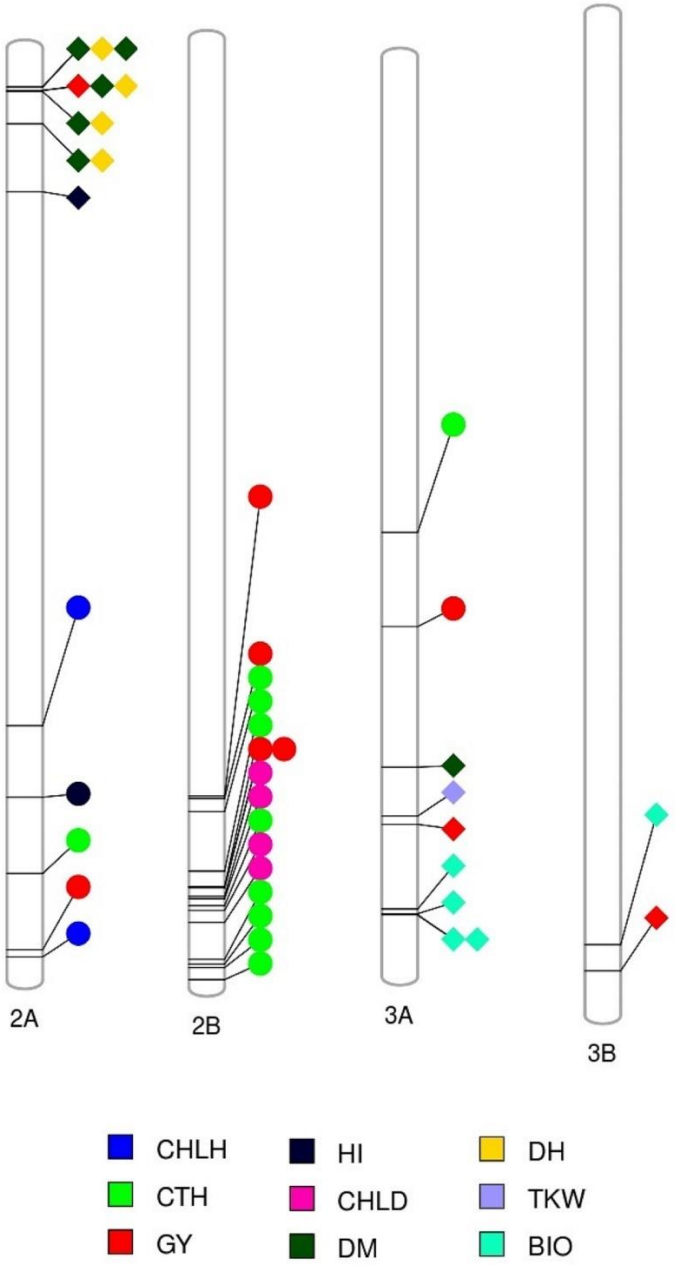

Figure 2. 8 Markers associated with multiple traits (see color key legend in the figure) identified under favored environment at Dongola (circles) or severe heat stress environment at Wad Medani (MED/SD2; diamonds). BIO, biomass; CHLD, chlorophyll degradation; CHLH, chlorophyll at heading; CTH, canopy temperature at heading; DH, days to heading; DM, days to maturity; GY, grain yield; HI, harvest index; TKW, thousand kernel weight.

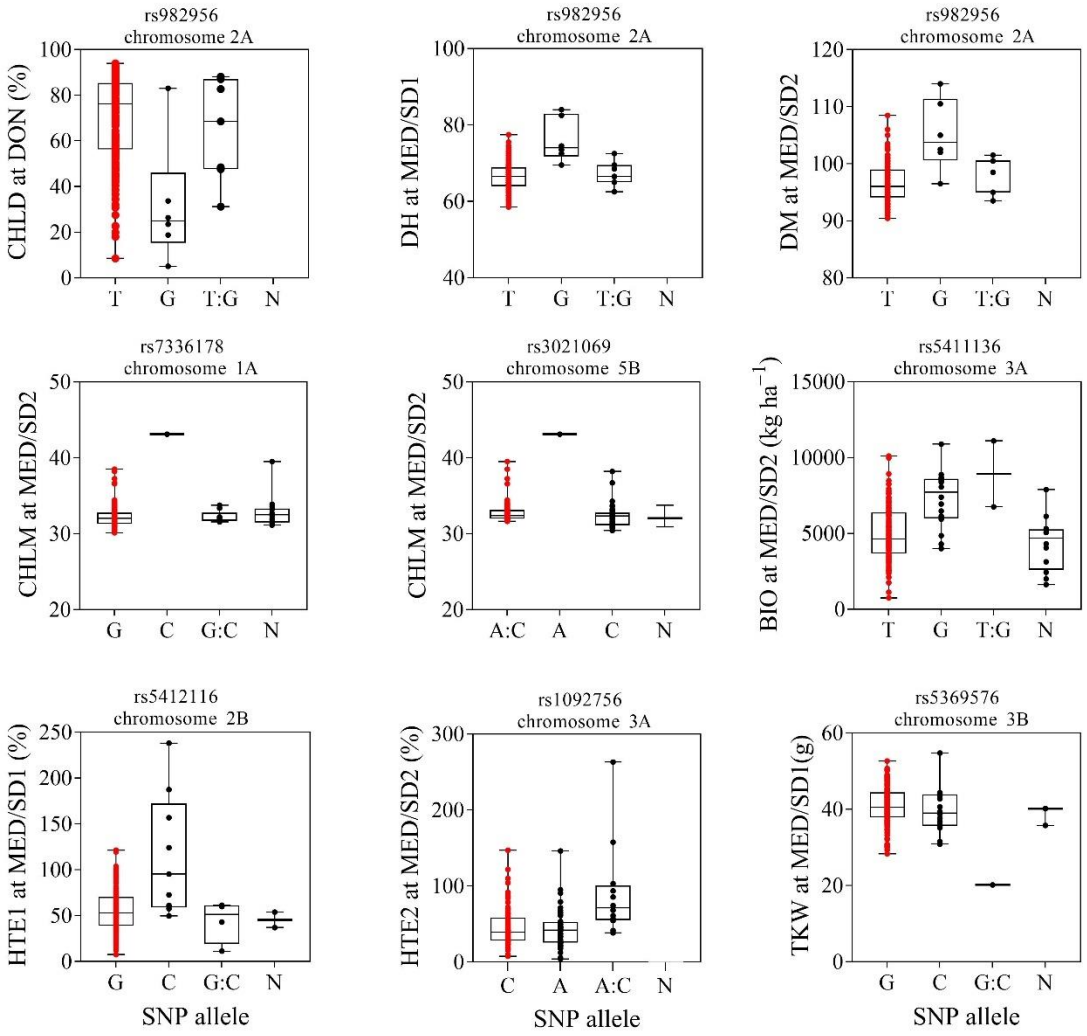

Figure 2. 9 Effect of selected marker−trait associations for different traits evaluated at Dongola (DON), Wad Medani first sowing date (MED/SD1) or Wad Medani second sowing date (MED/SD2). BIO, biomass; CHLD, chlorophyll degradation; CHLM, chlorophyll at maturity; DH, days to heading; DM, days to maturity; HTE1, heat tolerance efficiency evaluated in MED/SD1; HTE2, heat tolerance efficiency evaluated in MED/SD2; TKW, thousand kernel weight. Boxes show median and interquartile range and whiskers show the range. Significant marker and chromosome identified for each trait are displayed on the top box. Red dots indicate genotypes with 'Miki 3' allele. A, adenine; C, cytosine; T, thymine; G, guanine; N, unknown.

Table 2. 8. Investigation of wild emmer wheat (WEW) alleles on Sudanese cultivars for some traits showed positive SNP alleles from WEW.

Marker	Chromosome	Trait	Target allele from WEW	Number of Sudanese cultivars that share WEW allele	Number of Sudanese cultivars that share 'Miki 3' allele	Number of MDLs belonging to each WEW lineage	
rs7336178	1A	CHLM	C	0	37 (G), 1 (S), 5 (N)	1 Western	
rs5411136	3A	BIO	G	0	43 (T)		16 Eastern
rs5369576	3B	TKW	C	0	41 (G), 2 (N)	5 Western	10 Eastern
rs1092756	3A	HTE2	A:C	1 (A:C)	36 (C), 6 (A)	4 Western	7 Eastern
rs982956	2A	CHLD	G	2 (G)	41 (T)	6 Western	2 Eastern
rs982956	2A	DH	G	2 (G)	41 (T)	4 Western	2 Eastern
rs982956	2A	DM	G	2 (G)	41 (T)	4 Western	2 Eastern
rs5412116	2B	HTE1	C	4 (C)	39 (G)	5 Western	5 Eastern
rs3021069	5B	CHLM	A	1 (A)	32 (M), 10 (C)	1 Western	

BIO, biomass; CHLD, chlorophyll degradation; DH, days to heading; DM, days to maturity; HTE1, heat tolerance efficiency evaluated in MED/SD1; HTE2, heat tolerance efficiency evaluated in MED/SD2; TKW, thousand kernel weight. A, adenine; C, cytosine; T, thymine; G, guanine; N, unknown.

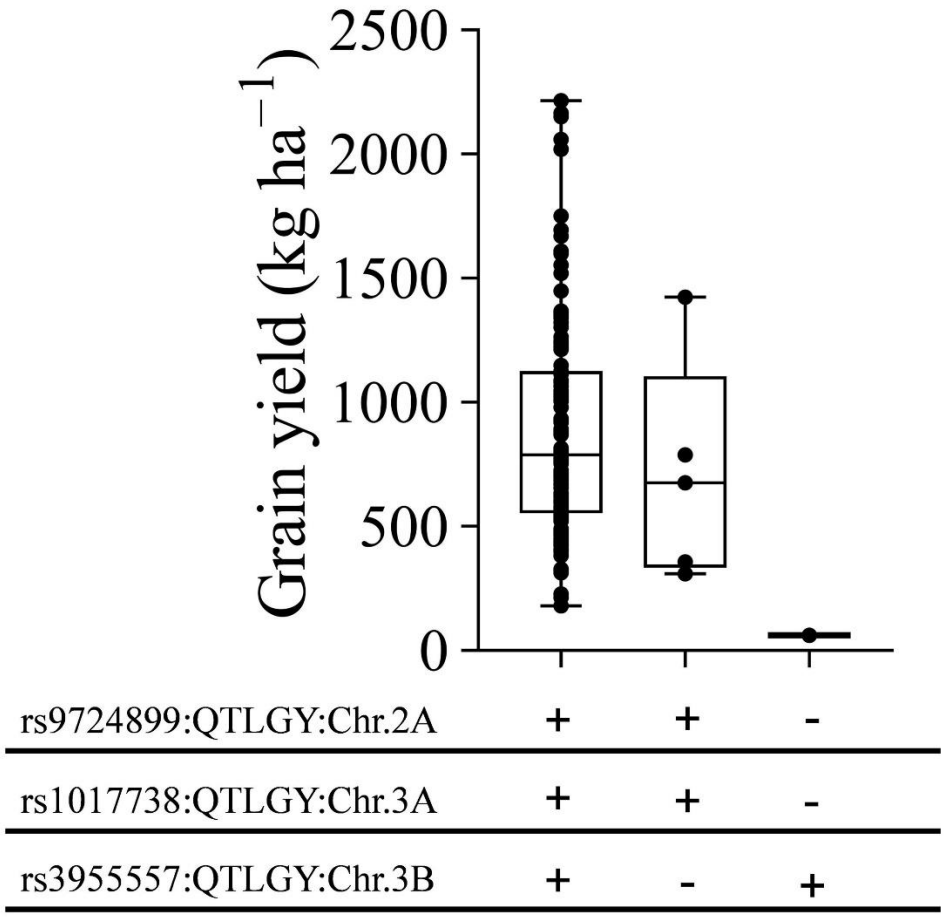

Figure 2. 10 Effect of different allele combinations of significant marker−trait associations on grain yield performance at Wad Medani under late sowing date. The MDLs were divided into three classes based on haplotype diversity analysis for three significant MTAs. Black circles indicate average grain yield by each genotype. '+' marks indicate positive alleles, '–' marks indicate negative alleles. rs9724899, rs1017738, and rs3955557 denote significant MTAs for grain yield on chromosomes 2A, 3A, and 3B, respectively.

Chapter 3

General discussion and Summary

3. 1 General Discussion

Durum wheat evolved from the two successive domestication events conducted by the ancient farmers. The first event was applied in the wild emmer wheat (*T. turgidum* ssp. *dicoccoides*), and the second one on a domesticated form of emmer wheat (*T. dicoccoum*) (Ozkan et al., 2005; Matsuoka, 2011; Peng et al., 2011). Although these events removed unsuitable wild wheat traits, it contributes negatively to reducing genetic diversity in the whole genome of modern durum wheat cultivars. Moreover, a great reduction in genetic diversity has occurred during breeding activities (Maccaferri et al., 2019). This narrow genetic diversity exposed modern durum wheat cultivars to the challenges posed by biotic and abiotic environmental stresses. Moreover, the continuous growth of the global population put the wheat breeders in another challenge. Hence, improving the phenotypic plasticity of durum and bread wheat to meet such challenges is necessary for sustainable production under changing climate. Wheat breeders usually compensate for the loss of diversity by reintroducing the valuable wild alleles that were changed, lost, modified, or left behind during domestication processes (Tsujimoto et al., 2015; Gorafi et al., 2018).

In this study, a durum wheat population of multiple derivative lines (MDLs) was developed by crossing and backcrossing nine wild emmer wheat with cultivated durum wheat cultivar.

The strategy of the MDL development allows us to explore the diversity of a nine wild emmer wheat in one population. Accordingly, the MDL population was described deeply to see how much the nine wild emmer wheat's diversity can affect individuals in this population. Interestingly, the MDL population captured most of the diversity present in the nine wild emmer wheat, and high gene exchange between the MDL and nine wild emmer wheat was identified. Moreover, the nine wild emmer wheat studied here represent the two main diversity lineages (Western lineage) and (Central lineage). The distribution of the MDL individuals reflects the genetic makeup of the nine wild emmer wheat. These findings indicate that the MDL population harbored both lineage's diversity in its gene pool, which can be utilized to improve bread and durum wheat. In addition, common traits with known genes position (plant height) and (days to heading) were used to validate this population, and the MDL population identified similar genomic locations. These findings prove that the MDL population is an effective strategy to harness wild emmer wheat diversity.

As a cool-season crop, wheat has an excessive sensitivity to temperature over the optimum. More challenging, more than 40% of wheat-growing regions are experiencing heat above the optimum range. Therefore, breeding for heat stress tolerance adaptation is

indispensable. Since the MDL population explored the diversity of the wild emmer wheat, this diversity can be utilized to improve wheat heat stress adaptation. In this study, the MDL panel was evaluated in four environments. Two optimum environments at Tottori, Japan, and Dongola, Sudan, one moderate heat stress and one severe heat stress environment at Wad Medani, Sudan. The MDL population exhibited high genetic variation for all evaluated traits in all locations. The MDL would respond positively to selection because most of the evaluated traits exhibited moderate to high heritability values. In addition, grain yield showed a consistent correlation with biomass and seed number/spike across environments indicating that these traits can be used as selection criteria to improve grain yield under optimum or heat stress conditions. Moreover, some genotypes with high heat tolerance and grain yield were identified in this study. These genotypes can be used for direct release or as parents in a crossing scheme to improve grain yield under heat stress conditions. One of the interesting findings, under severe heat stress, grain yield showed a strong positive correlation with chlorophyll content at maturity, confirming the presence of the stay-green in these materials, which is known as an essential heat stress tolerance mechanism. Since the genetic information from tetraploid wheat grown under field conditions is very rare, the identified candidate genes, germplasm, MTAs, alleles, and QTLs will potentially serve as reliable genetic information to improve bread and durum wheat adaptation to heat stress. Also, alleles identified by the MDL population are absent or very rare in the elite durum wheat being bred under heat stress conditions. Genetic information from this study has been fully explored to improve the diversity of the A and B genomes of bread wheat. Currently, some MDL lines with positive allele from wild emmer wheat has been selected to intercrossing with some bread wheat lines to develop new population with traits linked to heat and combined heat-drought stress tolerance.

The sequence of these studies in this thesis reflects the power of gene mining from the wild emmer wheat and utilizing the genetic diversity of wild emmer wheat to improve wheat A and B genomes adaptation to heat stress. It also evaluated the valuable diversity of the wild emmer wheat for heat stress resilience. Accordingly, global wheat breeders can use the genetic diversity in the MDL platform for different breeding purposes such as drought, salinity, and quality improvement. Also, this diversity can be utilized for biotic stress resistance. This population is currently available to the wheat community upon request from the Laboratory of Arid Land Plant Resources of the Arid Land Research Center of Tottori University, Japan.

3. 2 Summary in English

Substantial research efforts have been devoted to developing a diverse durum wheat population to exploit the wild emmer wheat diversity. However, the combined diversity of both wild emmer wheat lineages in one population has not been fully explored. Also, there is a shortage of genetic information from tetraploid wheat grown under natural field conditions and a lack of knowledge on heat stress tolerance mechanisms. This study used a population of multiple derivative lines (MDL) containing genomic fragments from nine wild emmer wheat (*Triticum dicoccoides*) to improve durum and bread wheat. Its suitability for harnessing the diversity of wild emmer wheat for bread and durum wheat improvement was explored. The genomic regions (including candidate genes, QTLs, MTAs, and alleles) regulating heat stress tolerance were highlighted. Genotypes sharing positive alleles from wild emmer wheat under heat stress were identified. Also, the value of utilizing the diversity of the wild emmer wheat for heat stress tolerance adaptation was evaluated.

In chapter 1, a durum wheat population consisting of 178 BC_1F_6 was developed by crossing and backcrossing nine wild emmer wheat (*Triticum. dicoccoides*) with cultivated durum wheat (*T. durum*) cultivar 'Miki 3". I described the development of this population, which was named multiple derivative lines (MDL), and demonstrated its suitability for durum wheat breeding. I genotyped the MDL population, the parents, and 43 Sudanese durum wheat cultivars on a Diversity Array Technology sequencing platform. I evaluated days to heading and plant height in Dongola (Sudan) and in Tottori (Japan) for MDL validation. The physical map length of the MDL population was 9,939 Mbp, with an average of 1.4 SNP/Mbp. The MDL population had greater diversity than the Sudanese cultivars. I found high gene exchange between the nine wild emmer accessions and the MDL population, indicating that the MDL captured most of the diversity in the wild emmer accessions. Genome-wide association analysis identified three loci for days to heading on chromosomes 1A and 5A in Dongola and one on chromosome 3B in Tottori. For plant height, common genomic loci were found on chromosomes 4A and 4B in both locations, and one genomic locus on chromosome 7B was found only in Dongola. The results revealed that the MDLs are an attractive resource with which to uncover the genes of wild emmer wheat and facilitate their use for bread and durum wheat improvement.

In chapter 2, the diverse set of the multiple derivative lines (MDL) was evaluated under four environments: two optimum environments at Tottori, Japan, and Dongola, Sudan, and one moderate heat stress environment and one severe heat stress environment at Wad Medani, Sudan. This study aimed to identify germplasm and QTLs/alleles associated with heat stress tolerance from wild emmer wheat diversity. Genome-wide association analysis was conducted with 13,312 SNP markers. Strong marker-trait associations (MTAs) were identified for chlorophyll content at maturity on chromosomes 1A and 5B: these MTAs explained 28.8 and 26.8% of the variation, respectively. A region on chromosome 3A (473.7−638.4 Mbp) contained MTAs controlling grain yield under optimum and severe heat stress. The region on chromosome 1A (358.9−522.6 Mbp)

contained MTAs controlled thousand kernel weight under optimum conditions at Dongola, moderate heat, and severe heat stresses with phenotypic variation ranging from 11.2−16.3%, whereas the region on 6B (81.3−146.7 Mbp) harbored MTAs for thousand kernel weight in Dongola and moderate heat stress, explaining 17.9 and 15.6% of the allelic variation, respectively. Under severe heat stress, regions on chromosomes 3A (590.4−713.3 Mbp) controlled grain yield, biomass, days to maturity, and thousand kernel weight, and on 3B (744.0−795.2 Mbp) grain yield and biomass. Heat tolerance efficiency (HTE) was controlled by three MTAs, one each on chromosomes 2A, 2B, and 5A under moderate heat stress and one MTA on chromosome 3A under severe heat stress. Some of the MTAs found here were previously reported, but the new ones originated from the wild emmer wheat genomes. The favorable alleles identified from wild emmer wheat were absent or rare in the elite durum wheat germplasm being bred for heat stress tolerance. Further analysis revealed candidate genes, including serine/threonine-protein kinase, include *TaSnRK2.4* from bread wheat regulates hyperosmotic responses and ABA signaling, C4HC3-type zing finger *TaZnF* regulating heat stress tolerance in bread wheat, and superoxide dismutase type *TaOSD* stimulates antioxidant enzymes in bread wheat. This study provides potential genetic materials, candidate genes, alleles, MTAs, and quantitative trait loci for enhancing wheat adaptation to heat stress. The derivative lines studied here could be investigated to enhance other stress tolerance such as drought and salinity.

Overall, this work illustrates the importance of wild emmer wheat (*T. dicoccides*) for improving modern durum wheat. It provides potential germplasms and genomic regions (including candidate genes, MTAs, alleles, and QTLs) in response to heat stress adaptation. Stable loci across environments were identified for important agronomic traits such as grain yield and thousand kernel weight. These loci can be further explored for use in marker-assisted selection and gene discovery. Some of the MTAs identified in this study are specific to heat stress and could be targeted in selection to improve heat stress tolerance. Identification of genotypes with favorable alleles and candidate genes from *T. turgidum ssp. dicoccoides* for different traits such as biomass, thousand kernel weight, and chlorophyll content at maturity demonstrate that the MDLs are an effective strategy for exploring wild emmer wheat diversity to adapt wheat to heat stress. Such germplasm with positive wild emmer wheat alleles has been used to improve the diversity of bread wheat A and B genomes by intercrossing some MDL lines with some bread wheat lines. Crossing tetraploid wheat and hexaploidy wheat, together with appropriate strategies for evaluating and selecting desirable lines under heat and combined heat-drought stress, will aid in the genetic improvement of both durum and bread wheat.

In the future, validation of these findings will enable to serve as effective knowledge for wheat breeding under changing climate.

3. 3 日本語要旨

野生エンマコムギ（*Triticum dicoccoides*）の多様性を利用するために、多様なデュラムコムギ（*T. durum*）の集団開発に多大な研究努力が払われてきた。しかし、1 つの集団に含まれる野生エンマコムギの両系統の複合的多様性については、十分に検討されていない。また、自然圃場条件下で栽培された 4 倍体コムギの遺伝情報が不足しており、高温ストレス耐性機構に関する知見も不足している。本研究では、9 つの野生エンマコムギのゲノム断片を含む多重派生系統（MDL）の集団を用いて、デュラムコムギとパンコムギの改良を行った。パンコムギおよびデュラムコムギの改良のために野生エンマコムギの多様性を利用することへの適性を探索した。高温ストレス耐性を制御するゲノム領域（候補遺伝子、QTL、MTA、対立遺伝子を含む）を明らかにした。また、高温ストレス下で野生エンマコムギから正の対立遺伝子を共有する遺伝子型を同定した。また、野生エンマコムギの多様性を高温ストレス耐性適応に利用する際の有用性を評価した。

第 1 章では，9 系統の野生エンマコムギとデュラムコムギ品種「Miki 3」を交配・戻し交配し、178 の BC_1F_6からなるデュラムコムギ集団を作出した。この集団は多重派生系統(multiple derivative lines、MDL)と名付けられ、デュラムコムギの育種に適していることを説明した。MDL 集団、親、およびスーダンのデュラムコムギ 43 品種の遺伝子型を Diversity Array Technology のシークエンシングプラットフォームで解析した。MDL の検証のため、スーダンのドンゴラと日本の鳥取で、出穂日と草丈を評価した。MDL 集団の物理的地図長は 9,939 Mbp で、平均 1.4 SNP/Mbp であった。MDL 集団はスーダンの品種よりも大きな多様性を持っていた。9 つの野生エンマコムギ系統と MDL 集団の間で高い遺伝子交換が認められ、MDL が野生エンマコムギ系統の多様性のほとんどを捉えていることが示された。ゲノムワイド関連解析により、ドンゴラでの栽培の 1A 染色体および 5A 染色体に出穂日に関する 3 つの遺伝子座が、鳥取栽培の 3B 染色体に 1 つの遺伝子座が同定された。また、草丈については、両地域の 4A 染色体および 4B 染色体に共通のゲノム遺伝子座が、ドンゴラでは 7B 染色体に 1 つのゲノム上の座位が見つかった。これらの結果から、MDL は野生エンマコムギの遺伝子を明らかにし、パンやデュラムコムギの改良に利用しやすくするための魅力的なリソースであることが明らかとなった。

第 2 章では、鳥取とスーダンのドンゴラにおける 2 つの最適環境、スーダンのワッドメダニにおける中程度の高温ストレス環境と極度な高温ストレス環境の 4 環境下で、多様な MDL を評価した。本研究では、野生エンマコムギの多様性から、高温ストレス耐性に関連する生殖質および QTL/対立遺伝子を同定することを目的とした。13,312 個の SNP マーカーを用いてゲノムワイド関連解析が行われた。1A 染色体および 5B 染色体上の成熟期クロロフィル含量について強いマーカー・形質間相関（MTA）が同定され、これらの MTA はそれぞれ 28.8 および 26.8%の変異を説明した。3A 染色体上の領域（473.7-638.4Mbp）には、最適環境および厳しい熱ストレス下での穀物収量を制御する MTA が存在した。

1A 染色体上の領域（358.9-522.6Mbp）は、ドンゴラの最適条件、中程度の暑さ、および極度な高温ストレス下で千粒重を制御する MTA を持ち、表現型の変異は 11.2-16.3%の範囲にあった。6B の領域（81-346.7Mbp）には、ドンゴラおよび中程度の暑さのストレス下で千粒重に対する MTA があり、変異の 17.9%と 15.6%をそれぞれ説明した。極度な高温ストレス下では、3A 染色体 (590.4-713.3 Mbp) 上の領域が穀物収量、バイオマス、成熟日数、千粒重を、3B 染色体 (744.0-795.2 Mbp) 上の領域が穀物収量とバイオマスを支配していた。高温耐性指数（HTE）は、中程度の高温ストレス下では 2A、2B、5A 染色体上にそれぞれ 1 つ、極度な高温ストレス下では 3A 染色体上の 1 つの MTA によって制御されていた。今回見つかった MTA のいくつかは既報のものであったが、新たに見つかったものは野生エンマコムギゲノムに由来するものであった。野生エンマコムギから同定された有利な対立遺伝子は、高温ストレス耐性を求めて育種されている優秀なデュラムコムギの生殖質には存在しないか希であった。さらに、候補遺伝子として見つかった、セリン・スレオニン・プロテインキナーゼ *TaSnRK2.4* は、パンコムギにおいて、高浸透圧応答と ABA シグナルを制御することが、C4HC3 型ジングフィンガー *TaZnF* はパンコムギにおいて高温ストレス耐性を制御することが、スーパーオキシドディスムターゼ型 *TaOSD* はパンコムギにおいて抗酸化酵素を活性化することが知られている。本研究は、コムギの高温ストレス適応能を高めるための潜在的な遺伝物質、候補遺伝子、アリル、MTA、量的形質座位を提供するものである。本研究で得られた派生系統は、乾燥や塩害などの他のストレス耐性を向上させるために研究される可能性がある。

全体として、本研究は、現在のデュラムコムギを改良するための野生エンマコムギの重要性を示すものである。また、高温ストレス適応に対応する生殖細胞やゲノム領域（候補遺伝子、MTA、アリル、QTL を含む）を提供する。穀物収量や千粒重などの重要な農作物形質について、複数の環境間で安定した遺伝子座が同定された。これらの遺伝子座は、マーカーアシスト選抜や遺伝子探索に利用するためにさらに探索することができる。本研究で同定された MTA のいくつかは高温ストレスに特異的であり、高温ストレス耐性を向上させるための選抜で標的となり得る。野生エンマコムギからバイオマス、千粒重、成熟期クロロフィル量などの異なる形質について、好ましい対立遺伝子と候補遺伝子をもつ遺伝子型を同定したことは、MDL がコムギを高温ストレスに適応させるための野生エンマコムギ多様性の探索に有効な戦略であることを示している。このような野生エンマコムギの対立遺伝子が正の効果を与える生殖質を用いて、いくつかの MDL 系統といくつかのパンコムギ系統を交配することにより、パンコムギ A および B ゲノムの多様性を向上させることに成功した。四倍体コムギと六倍体コムギの交雑は、高温・乾燥複合ストレス下で望ましい系統を評

価・選抜する適切な戦略とともに、デュラムコムギとパンコムギの両方の遺伝的改良に役立つと思われる。

今後、これらの知見を検証すれば、気候変動下でのコムギ育種に有効な知見を与えることが期待される。

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Appendices

Appendix 1 Summary of significant (P<0.001) marker–trait associations (MTAs) detected in MDLs evaluated in Tottori during the 2019–20 growing season.

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
BIO	rs1057035	3A	51.435656	0.00033371	0.11694
BIO	rs12419741	2B	27.535089	0.00059706	0.09841
CHLD	rs1231125	4A	592.792448	0.0001379	0.12055
CHLD	rs2289613	2A	683.286562	0.00021388	0.11075
CHLD	rs5372028	7A	169.677952	0.00040697	0.10967
CHLD	rs1255327	1B	604.102235	0.00030038	0.10721
CHLD	rs3942476	2A	585.852169	0.00044674	0.10695
CHLD	rs1023505	1B	521.823064	0.00028014	0.10549
CHLD	rs1262830	4A	287.886114	0.0007804	0.09616
CHLD	rs1162520	1B	520.418529	0.00058504	0.09577
CHLD	rs5576912	5A	417.225674	0.00055582	0.09464
CHLD	rs1091478	2A	686.725151	0.00061282	0.09428
CHLD	rs3956169	5B	65.506235	0.00071319	0.09142
CHLD	rs2275417	5B	91.306234	0.00084452	0.08979
CHLD	rs992248	5B	201.988463	0.00094174	0.08874
CHLD	rs1134949	6B	485.889451	0.00097459	0.08798
CHLD	rs4412031	7B	716.841951	0.00096726	0.08733
CHLH	rs2258700	3B	66.549272	0.00031126	0.11243
CHLH	rs1093026	6A	561.426099	0.00061303	0.09415
CHLH	rs2315117	3A	638.957082	0.00067746	0.09247
CHLH	rs991366	2B	11.991490	0.00094267	0.09144
CHLM	rs1231125	4A	592.792448	2.9588E-05	0.1394
CHLM	rs5576912	5A	417.225674	0.00010007	0.11758
CHLM	rs1044196	2B	22.348668	0.00032509	0.11341
CHLM	rs2289613	2A	683.286562	0.00032023	0.10737
CHLM	rs5369576	3B	656.171285	0.000353	0.10184
CHLM	rs1049247	6B	499.155843	0.00035541	0.10023
CHLM	rs1126662	4A	596.733159	0.00067241	0.09617
CHLM	rs12770698	2A	685.864106	0.00073397	0.09348
CHLM	rs2279888	2A	720.025114	0.00081903	0.09197
CHLM	rs1091478	2A	686.725151	0.00087067	0.09166
DH	rs995752	3B	823.146127	7.3488E-05	0.12593
DH	rs995752	3B	823.146127	0.00010399	0.11868
DH	rs1265475	2A	606.617353	0.00018538	0.11448
DH	rs1104842	7B	9.447880	0.00081568	0.10447

Appendix 1 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
DH	rs999837	6B	152.693759	0.00085	0.09994
DH	rs2279038	7B	27.272831	9.9E-05	0.09914
DH	rs6026246	4B	632.413268	0.00077	0.09614
DH	rs2280813	7B	201.138769	0.00068	0.09593
DH	rs1033938	3B	828.896388	0.00085	0.09592
DH	rs1094075	7B	37.408757	0.00075	0.09196
DH	rs1243904	3B	820.364494	0.00093	0.09016
DH	rs982956	2A	36.038265	0.00095	0.08881
DH	rs991533	7A	87.180847	0.00022	0.08735
DH	rs3027023	3B	114.434189	0.00048	0.07763
DM	rs3944005	3A	679.822517	0.00051	0.1106
DM	rs1097630	2B	677.154846	0.0003	0.10188
DM	rs1210857	6B	200.245095	0.00045	0.09965
DM	rs1233374	2B	712.100845	0.00043	0.09784
DM	rs4411627	6B	207.331271	0.00046	0.09663
DM	rs38280097	2B	676.661197	0.00061	0.09512
DM	rs3064420	2B	699.560725	0.00091	0.08914
DM	rs1088598	6B	138.474386	0.00094	0.08882
DM	rs1228193	2B	684.915040	0.00086	0.08874
DM	rs3941346	3B	122.809655	0.00054	0.07448
DM	rs1128688	2B	457.867581	0.00067	0.07187
GFD	rs995752	3B	823.146127	5.9E-05	0.1281
GFD	rs2294920	3A	731.880383	0.00084	0.09096
GFD	rs2279038	7B	27.272831	0.00026	0.08686
GFD	rs991533	7A	87.180847	0.00045	0.07818
GY	rs989816	1A	567.433748	2.2E-05	0.15585
GY	rs2316835	1A	466.333196	0.0007	0.09326
GY	rs1008272	7A	664.917226	0.00012	0.12732
GY	rs3028623	3B	836.305274	0.00017	0.12107
GY	rs4412046	5A	515.072810	0.00021	0.11234
GY	rs3023370	2B	224.926906	0.00022	0.10912
GY	rs1408530	7A	665.197977	0.00031	0.11946
GY	rs1129236	4B	618.745721	0.00032	0.10379
GY	rs1102601	5A	590.444505	0.00036	0.10756
GY	rs1761198	7A	675.012773	0.00037	0.10311

Appendix 1 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
GY	rs23884330	4B	661.740441	0.00038349	0.10402
GY	rs5581306	5A	582.482538	0.00038488	0.10949
GY	rs1191576	1A	251.007380	0.00010199	0.12197
GY	rs1125460	1B	665.163350	0.00052	0.10769
GY	rs984449	5A	355.590114	0.00052569	0.09792
GY	rs7336242	3B	821.859897	0.00052722	0.1064
GY	rs2295838	1A	124.001638	0.00049621	0.09791
GY	rs1361071	7A	685.283439	0.00081122	0.09702
GY	rs1046082	7A	29.430305	0.00088902	0.09374
GY	rs55408751	1A	116.147337	0.00093494	0.09126
HI	rs1126577	3A	603.064325	2.5904E-07	0.19044
HI	rs2276009	3A	622.127589	5.7521E-07	0.18042
HI	rs5581032	2B	733.424860	7.0908E-07	0.19876
HI	rs1013793	3A	598.247640	8.092E-07	0.18266
HI	rs12771929	4B	658.574530	1.1352E-06	0.17956
HI	rs989816	1A	567.433748	5.0438E-06	0.17503
HI	rs1147034	2B	726.672510	1.0513E-05	0.14113
HI	rs5581306	5A	582.482538	1.2978E-05	0.14431
HI	rs999525	3A	606.358602	1.4447E-05	0.13818
HI	rs1126368	4B	655.053847	1.5457E-05	0.13539
HI	rs1278393	4B	626.244814	1.6695E-05	0.13732
HI	rs989478	4B	650.571576	2.8707E-05	0.13358
HI	rs1220965	5A	513.083177	3.4137E-05	0.12502
HI	rs7903755	3A	672.279726	3.4741E-05	0.1282
HI	rs5579491	5A	567.766228	5.4014E-05	0.12627
HI	rs1097392	2B	699.569503	5.7432E-05	0.12054
HI	rs1219329	2B	765.688256	5.8798E-05	0.11802
HI	rs2283966	6B	636.615129	5.8812E-05	0.13221
HI	rs1202815	2B	639.888656	6.1501E-05	0.12515
HI	rs1240031	5A	484.918482	6.4351E-05	0.13052
HI	rs2291303	2A	647.762868	6.4714E-05	0.11779
HI	rs1160820	5B	664.842303	6.7056E-05	0.11907
HI	rs2275286	5B	669.775874	6.9509E-05	0.11687
HI	rs1102693	5B	670.641570	7.2358E-05	0.11781
HI	rs4005420	2A	15.083630	0.00007294	0.1216

Appendix 1 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
HI	rs1054888	4B	658.918162	8.2006E-05	0.1141
HI	rs39466056	5B	665.553225	8.3267E-05	0.13335
HI	rs3064725	2B	755.294011	8.4366E-05	0.12183
HI	rs1213694	5B	671.415925	8.6893E-05	0.11728
HI	rs2293274	3B	500.376801	9.4276E-05	0.1156
HI	rs1240043	6A	51.200421	0.0001183	0.10925
HI	rs4003428	6B	131.646455	0.00012439	0.11468
HI	rs5364006	3A	134.125533	0.00014627	0.10692
HI	rs4397594	5A	664.443844	0.00015095	0.106
HI	rs4411296	2B	720.595556	0.00015788	0.10566
HI	rs1093715	2B	720.593711	0.00016306	0.10579
HI	rs12775296	4B	40.176073	0.00017205	0.10434
HI	rs1095442	2B	720.564272	0.0001721	0.10434
HI	rs1158665	2B	720.673763	0.0001721	0.10434
HI	rs2277196	1A	276.530634	0.00017845	0.10551
HI	rs981803	1A	92.460261	0.00018349	0.10367
HI	rs5582892	2B	720.564206	0.00018909	0.10417
HI	rs1761198	7A	675.012773	0.00021697	0.10318
HI	rs1264455	7B	666.874178	0.00021918	0.10356
HI	rs23884330	4B	661.740441	0.0002294	0.10437
HI	rs1028377	2B	720.742456	0.00023645	0.10422
HI	rs4663968	3A	11.017166	0.00023795	0.11105
HI	rs992176	1A	47.277643	0.00024917	0.10224
HI	rs1091839	4B	27.200934	0.00025291	0.09947
HI	rs1145893	3B	668.740667	0.00025461	0.10559
HI	rs1126519	4B	658.205039	0.0002557	0.10593
HI	rs998506	4B	668.219710	0.00025724	0.10305
HI	rs1408530	7A	665.197977	0.00025915	0.10524
HI	rs1129236	4B	618.745721	0.00026534	0.09887
HI	rs3064863	3B	502.301963	0.00028867	0.0981
HI	rs1125460	1B	665.163350	0.00029701	0.11224
HI	rs5970682	2A	32.888573	0.00030598	0.10067
HI	rs1129092	4B	655.053847	0.00033258	0.10335
HI	rs9724999	2A	93.411204	0.00034467	0.09671
HI	rs3020804	3A	622.144891	0.00035172	0.09532

Appendix 1 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
HI	rs1058753	3A	623.711540	0.00035172	0.09532
HI	rs1091769	4A	584.893894	0.00036688	0.07556
HI	rs1111233	4A	643.708983	0.00037008	0.09739
HI	rs5341701	5A	509.566173	0.00038582	0.09461
HI	rs4411089	3B	669.265294	0.00038912	0.09406
HI	rs12768779	5A	47.629513	0.00039856	0.09538
HI	rs998637	4B	600.119884	0.00039872	0.09987
HI	rs1129203	4B	665.669342	0.00040217	0.095
HI	rs1112974	3B	677.942853	0.00040583	0.09353
HI	rs1081242	3A	629.370594	0.0004089	0.09453
HI	rs1863018	5A	46.211028	0.00042978	0.0935
HI	rs1235285	5A	468.008713	0.00043266	0.09906
HI	rs983787	3A	660.994024	0.00043947	0.09313
HI	rs1099593	3A	658.021729	0.00044033	0.09251
HI	rs2364747	3A	672.556882	0.00044454	0.09936
HI	rs1394339	5A	485.292401	0.00044667	0.09305
HI	rs3956335	3A	659.181708	0.00046305	0.09463
HI	rs1102601	5A	590.444505	0.00046405	0.09543
HI	rs3939990	5A	661.369571	0.00046774	0.07344
HI	rs1229114	3A	738.344986	0.00047912	0.0916
HI	rs7913640	3B	636.087759	0.00048432	0.0917
HI	rs1088702	3B	684.027945	0.00050659	0.0936
HI	rs1038058	2A	69.063238	0.00051557	0.09994
HI	rs12774649	2B	158.260506	0.00051985	0.0932
HI	rs4909962	4B	42.281913	0.00052049	0.09101
HI	rs13196373	4B	26.871407	0.00053937	0.09536
HI	rs2277021	4A	4.870584	0.00054002	0.09047
HI	rs1270366	4A	692.757160	0.00054894	0.09319
HI	rs5338376	3A	604.636678	0.00055464	0.09469
HI	rs3955378	1A	40.684643	0.00056189	0.0905
HI	rs1021974	3B	512.435829	0.00057914	0.09991
HI	rs1133136	4B	667.892917	0.00058972	0.09001
HI	rs1064354	4B	26.614161	0.00059465	0.09463
HI	rs12766204	5A	483.632198	0.00063204	0.08799
HI	rs1237956	7B	108.313273	0.00063587	0.09335

Appendix 1 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
HI	rs1092528	4B	19.264755	0.00066655	0.10118
HI	rs1219555	5A	659.150134	0.00069259	0.08707
HI	rs4536076	4B	38.159001	0.00069688	0.08677
HI	rs1054901	7B	657.369807	0.00070636	0.0899
HI	rs1243032	1A	124.389399	0.00071082	0.08742
HI	rs1331748	2B	635.477684	0.00072175	0.09577
HI	rs55408710	1A	51.402776	0.00072761	0.08691
HI	rs1015063	2A	647.488464	0.00073691	0.06793
HI	rs1110164	3B	798.006060	0.00074621	0.09254
HI	rs3023370	2B	224.926906	0.00077561	0.08544
HI	rs1126997	2B	750.937843	0.00082762	0.08538
HI	rs1031939	4A	4.321095	0.00082873	0.0859
HI	rs12764092	4B	50.342659	0.00084469	0.08875
HI	rs4260627	2B	715.967431	0.00085107	0.08771
HI	rs4909926	5B	637.298461	0.00087142	0.08423
HI	rs1026367	2A	62.009870	0.00088072	0.08624
HI	rs1030075	1A	567.388744	0.0009191	0.09205
HI	rs4396104	1B	490.255870	0.00094416	0.08365
PHT	rs2278767	4B	30.576288	9.194E-06	0.15272
PHT	rs1004850	4B	31.292927	0.00015942	0.1127
PHT	rs2371505	4B	29.297345	0.00018517	0.11243
PHT	rs1021727	7A	168.479564	0.00025179	0.11202
PHT	rs2252536	4A	572.495965	4.3991E-05	0.10656
PHT	rs3941361	3A	717.533722	0.0003305	0.10209
PHT	rs4010028	4B	36.999359	0.00042302	0.09858
PHT	rs3952582	5A	14.801152	0.00055539	0.09493
PHT	rs7492354	3A	714.507257	0.00067442	0.09312
PHT	rs1265612	4B	30.655464	0.00067171	0.09244
PHT	rs986158	1B	635.701901	0.00089851	0.09225
PHT	rs1087149	4A	569.604831	0.00014739	0.09134
PHT	rs1118805	7A	169.676793	0.00077326	0.09105
PHT	rs1071544	2A	708.884129	0.00085442	0.08923
SN	rs1103766	2B	194.373459	5.4326E-05	0.13061
SN	rs2291198	5A	569.144487	0.00019086	0.12802
SN	rs984449	5A	355.590114	6.3884E-05	0.12639

Appendix 1 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
SN	rs1361668	1A	256.200117	0.00010268	0.12299
SN	rs1863261	2A	721.169420	0.00013615	0.11729
SN	rs983421	1A	177.981317	0.00038964	0.11683
SN	rs1322080	2B	217.092494	0.00055636	0.11526
SN	rs3954049	1A	237.316162	0.00019972	0.11031
SN	rs1071946	2A	720.497198	0.00021454	0.11019
SN	rs1231850	2A	721.813206	0.0001911	0.10991
SN	rs1127725	3A	128.514845	0.00004668	0.10868
SN	rs1102601	5A	590.444505	0.00032909	0.10776
SN	rs984789	2B	231.474068	0.00029757	0.10688
SN	rs1103641	7A	666.306403	0.00031112	0.10638
SN	rs1068770	1B	403.201649	0.00041312	0.10453
SN	rs2276937	1A	105.711652	0.0003118	0.10331
SN	rs992176	1A	47.277643	0.00034532	0.10263
SN	rs1163499	7A	36.165817	0.00041812	0.1025
SN	rs12768683	7A	679.718979	0.00036772	0.10234
SN	rs1696117	5A	551.057957	0.000619	0.10234
SN	rs1125449	3A	618.058813	0.00047796	0.10158
SN	rs1075382	7A	39.965754	0.00045536	0.10145
SN	rs12419741	2B	27.535089	0.00059017	0.10091
SN	rs1081539	2B	413.399512	0.00054031	0.1007
SN	rs2260336	5A	624.027954	0.00038751	0.10039
SN	rs4989890	5A	0.283436	0.00070359	0.1002
SN	rs1078069	1B	455.618695	0.00063274	0.09988
SN	rs39655107	4A	662.205858	0.00049582	0.09985
SN	rs3064594	1B	480.266362	0.00075648	0.09784
SN	rs2259728	2B	655.921188	0.00050384	0.09693
SN	rs1185112	1B	406.127666	0.00050543	0.09683
SN	rs1275924	2A	716.953189	0.00053413	0.09658
SN	rs2276835	2A	724.000196	0.00056146	0.09652
SN	rs1147034	2B	726.672510	0.00063429	0.09645
SN	rs981803	1A	92.460261	0.0005419	0.09632
SN	rs1104858	6A	514.490118	0.00062496	0.09591
SN	rs1089548	1A	114.508465	0.00080143	0.09508
SN	rs55408890	7B	722.870770	0.00067457	0.09478

Appendix 1 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
SN	rs1717893	2B	244.007695	0.00085533	0.09462
SN	rs989800	6A	525.298518	0.00087886	0.09446
SN	rs1088087	2B	730.734742	0.00060601	0.09441
SN	rs2275228	1A	116.175691	0.00060751	0.09437
SN	rs1067084	1A	216.562926	0.00060751	0.09437
SN	rs982059	2B	230.226223	0.00062013	0.0941
SN	rs1028377	2B	720.742456	0.00084325	0.09386
SN	rs1130392	2B	204.183890	0.00065563	0.09365
SN	rs992116	2B	211.586619	0.00066677	0.09313
SN	rs1066264	2A	726.762431	0.0007766	0.09275
SN	rs3064789	6A	447.354218	0.00075364	0.09263
SN	rs1108884	2B	726.767223	0.00095834	0.09258
SN	rs4411296	2B	720.595556	0.00082025	0.0919
SN	rs1083365	6A	514.489428	0.00087677	0.09187
SN	rs1147429	2B	733.559458	0.00075185	0.09154
SN	rs1092489	2A	723.188962	0.00085298	0.09142
SN	rs5582892	2B	720.564206	0.00076396	0.09138
SN	rs5579583	6A	519.212699	0.00089784	0.08959
SN	rs12773433	6A	500.450174	0.00087412	0.08953
SN	rs5346700	6A	521.316664	0.0009512	0.08945
SN	rs1054888	4B	658.918162	0.00097484	0.08917
SN	rs2275227	6A	511.247458	0.00090051	0.08914
SN	rs1126009	2B	650.531046	0.0009032	0.0891
SN	rs1082485	2B	232.879342	0.00090287	0.0891
SN	rs1158665	2B	720.673763	0.00099249	0.08785
SN	rs1095442	2B	720.564272	0.00099249	0.08785
SN	rs1065780	1B	155.755652	0.00027673	0.08407
SN	rs3959631	7A	44.591960	0.00066882	0.07324
SN	rs5324717	2B	66.637230	0.00091984	0.07207
TKW	rs32940537	3A	662.440429	0.00035525	0.10809
TKW	rs3940326	6A	526.970188	0.00099254	0.08733
TKW	rs3064370	3A	646.901140	0.00053949	0.09906
TKW	rs1718307	6A	492.967767	0.00094188	0.09069
TKW	rs12779479	3A	647.244695	0.00080347	0.09673
TKW	rs1021673	3A	648.237627	0.00052359	0.09647

Appendix 1 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
TKW	rs4909821	6A	478.264117	0.00064615	0.09413
TKW	rs2267419	3A	641.355150	0.00076997	0.09391
TKW	rs1035771	7A	182.493646	0.00084066	0.09205
TKW	rs1151704	6A	451.301358	0.00056651	0.10204
TKW	rs1401396	5A	572.628527	0.00098699	0.08744
TKW	rs1236523	6A	432.272692	0.00081371	0.09851
TKW	rs1051400	6B	515.102600	0.00095412	0.06849

BIO, biomass; CHLD, chlorophyll degradation; CHLH, chlorophyll at heading; CHLM, chlorophyll at maturity; CTH, canopy temperature at heading; DH, days to heading; DM, days to maturity; GFD, grain filling duration; GY, grain yield; HI, harvest index; PHT, plant height; SN, seed number/spike; TKW, thousand kernel weight. R^2 indicate phenotypic contribution by marker.

Appendix 2 Summary of significant (P<0.001) marker–trait associations (MTAs) detected in MDLs evaluated in Dongola during the 2019–20 growing season.

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
BIO	rs1217219	6B	8.384405	1.9446E-05	0.1382
BIO	rs2279388	7A	202.252640	0.00011451	0.11167
BIO	rs2342855	7A	183.038716	0.00020425	0.1102
BIO	rs1218234	7A	664.671736	0.00018563	0.10484
BIO	rs1234945	3A	612.003903	0.00028721	0.10483
BIO	rs1089029	7A	664.052969	0.0001898	0.10455
BIO	rs2254730	6B	15.176185	0.00032403	0.1038
BIO	rs1024404	5B	597.038079	0.00024856	0.10348
BIO	rs1071836	7A	662.598662	0.00026135	0.10344
BIO	rs3948739	7A	663.595209	0.00029134	0.10109
BIO	rs1093097	7B	617.187497	0.00025835	0.10061
BIO	rs1205251	7A	694.414248	0.00029859	0.10034
BIO	rs1052671	7A	664.132160	0.00037165	0.09967
BIO	rs2277813	7A	183.188097	0.00031634	0.09963
BIO	rs991978	5A	444.857070	0.000353	0.09839
BIO	rs1027069	7A	205.503561	0.0003902	0.09679
BIO	rs1100271	7A	208.295298	0.00036788	0.0961
BIO	rs1068473	7A	179.294987	0.00040794	0.09545
BIO	rs1091695	5B	528.445522	0.00047602	0.09432
BIO	rs1278437	7A	202.252574	0.00044303	0.09424
BIO	rs4404836	7A	186.510510	0.00043038	0.09411
BIO	rs3956705	7A	178.227367	0.00045042	0.09353
BIO	rs3934069	7A	178.797222	0.00045042	0.09353
BIO	rs5354930	7A	664.022344	0.0004737	0.09326
BIO	rs12770820	7A	193.952568	0.00055673	0.09308
BIO	rs4411956	7A	165.716637	0.00049068	0.09271
BIO	rs1151117	7B	177.178294	0.0005005	0.09219
BIO	rs1200675	6B	11.483913	0.00080761	0.08879
BIO	rs1040910	2A	599.665183	0.00089328	0.08771
BIO	rs5354535	7B	144.774228	0.00090791	0.06611
CHLD	rs3944005	3A	679.822517	5.6743E-09	0.25375
CHLD	rs1233374	2B	712.100845	4.2139E-09	0.25105

Appendix 2 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
CHLD	rs1255650	7B	74.684763	3.3215E-08	0.22536
CHLD	rs1071015	2A	62.009636	2.3931E-08	0.2253
CHLD	rs1151045	2B	724.923696	2.658E-08	0.21952
CHLD	rs1111512	7B	471.484999	2.7464E-08	0.21877
CHLD	rs4911119	2B	714.178077	6.6341E-06	0.15045
CHLD	rs982956	2A	36.038265	4.3201E-07	0.18048
CHLD	rs2252351	2A	35.846102	4.3258E-07	0.18046
CHLD	rs1277633	2A	32.676657	2.5802E-06	0.15858
CHLD	rs1016684	6B	676.034823	1.3575E-05	0.15374
CHLD	rs1131740	2B	734.423108	6.9664E-06	0.14463
CHLD	rs1076670	2B	131.420448	9.3869E-05	0.11719
CHLD	rs3064420	2B	699.560725	8.6881E-05	0.1119
CHLD	rs1097392	2B	699.569503	0.00019959	0.10839
CHLD	rs1278393	4B	626.244814	2.0086E-05	0.13217
CHLD	rs5970682	2A	32.888573	4.4244E-05	0.12635
CHLD	rs3064420	2B	699.560725	0.00012449	0.10729
CHLD	rs1667549	2B	429.833561	0.00018159	0.10546
CHLD	rs1151891	7B	423.281420	6.0366E-05	0.12257
CHLD	rs1228193	2B	684.915040	0.00015626	0.10429
CHLD	rs1150497	2B	467.831531	0.00018361	0.10369
CHLD	rs7915837	3A	679.925205	4.7328E-05	0.11922
CHLD	rs2261913	2B	666.738099	0.00059795	0.10022
CHLD	rs2283390	2B	728.576863	0.00040568	0.09835
CHLD	rs2243154	2B	713.235204	0.00031331	0.09551
CHLD	rs1090230	2B	712.450394	0.00035559	0.09436
CHLD	rs4406564	1B	629.36303	0.00018738	0.11034
CHLD	rs21718380	2B	710.031770	0.00036085	0.09389
CHLD	rs2279574	3A	16.853596	0.00016602	0.10745
CHLD	rs1166235	2B	743.936781	0.00043463	0.09181
CHLD	rs1119237	1A	92.460311	0.00012475	0.1072
CHLD	rs1051075	6B	625.079410	0.0001485	0.10688
CHLD	rs3021936	2A	42.408207	0.00012685	0.10678
CHLD	rs3033178	2B	737.726221	0.00047353	0.09039
CHLD	rs3025448	2B	699.891564	0.00052723	0.08955

Appendix 2 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
CHLD	rs2308550	2B	699.555095	0.00053655	0.08885
CHLD	rs1694640	7A	592.278526	0.00020011	0.10491
CHLD	rs1095412	7A	588.934330	0.00015186	0.10453
CHLD	rs3222127	2B	54.740118	0.00080474	0.08421
CHLD	rs1133136	4B	667.892917	0.0001702	0.104
CHLD	rs1102480	2B	696.278477	0.00088283	0.08273
CHLD	rs5325339	2B	12.371247	0.00092185	0.0822
CHLD	rs5324886	3B	26.867808	0.00016688	0.10335
CHLD	rs1092601	1B	654.289440	0.00022043	0.10031
CHLD	rs3950894	3A	395.823261	0.00024712	0.09887
CHLD	rs3021074	7A	542.557187	0.00024414	0.09872
CHLD	rs1862312	3A	629.499707	0.0004621	0.09841
CHLD	rs1046611	7A	611.129453	0.00039572	0.09504
CHLD	rs9724999	2A	93.411204	0.00047723	0.09286
CHLD	rs992176	1A	47.277643	0.00041643	0.09214
CHLD	rs1019408	7B	175.020861	0.00044408	0.09119
CHLD	rs1220113	6B	2.060565	0.00053264	0.0909
CHLD	rs2266323	4B	11.675233	0.00075539	0.08981
CHLD	rs1128824	4A	630.203220	0.00050592	0.08958
CHLD	rs1064694	6A	594.040966	0.00053266	0.08928
CHLD	rs2278367	4A	626.243359	0.00054198	0.08873
CHLD	rs5581324	1B	650.21818	0.00057635	0.08842
CHLD	rs12773563	5A	459.51274	0.00059546	0.08793
CHLD	rs1008794	7A	605.111703	0.00069676	0.08563
CHLD	rs2289271	2A	85.554724	0.00071907	0.08525
CHLD	rs1129092	4B	655.053847	0.00099322	0.08482
CHLD	rs1091951	7A	629.925280	0.00080143	0.08437
CHLD	rs2283342	7B	621.875222	0.00099804	0.08406
CHLD	rs3956183	7B	68.277868	0.00090472	0.08384
CHLH	rs5372757	2A	755.881404	5.09E-08	0.21465
CHLH	rs1010893	2A	563.637353	6.976E-06	0.1492
CHLH	rs5969666	3A	652.967523	4.8716E-05	0.13719
CHLH	rs26673568	4A	687.839228	1.5827E-05	0.13528
CHLH	rs2262346	5A	10.293188	0.00007393	0.13442

Appendix 2 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
CHLH	rs1092210	2A	763.212623	0.00012273	0.11649
CHLH	rs2277084	3A	714.340120	8.6968E-05	0.11627
CHLH	rs1199287	3A	706.85239	0.00012782	0.11597
CHLH	rs2260638	4A	717.408703	0.00018223	0.11051
CHLH	rs100021349	1B	645.949797	0.00033956	0.10417
CHLH	rs1088484	3B	32.018800	0.00018875	0.10298
CHLH	rs1009838	6B	126.051923	0.00026917	0.09833
CHLH	rs4706031	5A	486.907029	0.00040647	0.09789
CHLH	rs1121960	7A	265.673551	0.00027892	0.09783
CHLH	rs1215835	4A	592.081486	0.00032736	0.0976
CHLH	rs12419942	5A	621.676800	0.00034033	0.09526
CHLH	rs1216462	4B	24.384093	0.00045456	0.09356
CHLH	rs1091029	5A	621.534395	0.00077608	0.09189
CHLH	rs2293162	4A	589.315575	0.00059193	0.08974
CHLH	rs1204740	4A	698.878376	0.0005976	0.08886
CHLH	rs1128278	4A	599.016192	0.00072738	0.08871
CHLH	rs3064698	5A	309.390531	0.00069091	0.08622
CHLH	rs1216996	4A	592.024295	0.00075198	0.08517
CHLH	rs1338445	3B	11.585282	0.00075952	0.08505
CHLH	rs1100882	4A	41.446949	0.00094163	0.0848
CHLH	rs2276147	4A	698.877661	0.00095592	0.0823
CHLM	rs1081408	5A	557.659428	0.00033629	0.09011
CHLM	rs1074046	5A	557.542905	0.00046888	0.08754
CHLM	rs1131350	5A	557.677843	0.00060907	0.08524
CTH	rs1225592	3A	702.365848	2.8384E-05	0.13593
CTH	rs4989102	3A	702.365848	2.8384E-05	0.13593
CTH	rs1086318	3A	705.688411	3.6144E-05	0.12195
CTH	rs998626	3A	644.964259	8.7186E-05	0.11775
CTH	rs1209110	6B	692.178142	0.0001165	0.11496
CTH	rs1079045	3A	713.365311	9.1497E-05	0.11248
CTH	rs1044473	6A	546.002435	0.00013072	0.1085
CTH	rs986855	4B	435.281281	0.00018058	0.1065
CTH	rs1081192	6B	690.695552	0.00026105	0.10563
CTH	rs55408881	7A	161.115530	0.00027986	0.10547

Appendix 2 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
CTH	rs1229379	7A	700.674705	0.00017789	0.10529
CTH	rs3943257	6A	534.062068	0.00014682	0.10414
CTH	rs5971304	2B	720.291613	0.00020586	0.10497
CTH	rs1195353	2B	642.664564	0.00027891	0.10497
CTH	rs3064889	2B	608.686784	0.00028291	0.10308
CTH	rs1151135	2B	691.371823	0.00029482	0.10123
CTH	rs4989353	2A	685.431141	0.00029876	0.10158
CTH	rs1136890	2B	704.963343	0.00026429	0.10069
CTH	rs980362	2B	769.126812	0.00028351	0.09895
CTH	rs3950894	3A	395.823261	0.00019937	0.10067
CTH	rs1166538	4B	4.105760	0.00022703	0.10065
CTH	rs2292620	2B	626.971383	0.00037323	0.09832
CTH	rs5364104	3A	717.632745	0.00019673	0.1004
CTH	rs12769200	2B	613.614041	0.00028497	0.09807
CTH	rs2333203	6A	534.392116	0.00024162	0.09993
CTH	rs5357574	1A	417.371125	0.00023051	0.0999
CTH	rs1201070	4A	573.878504	0.00022875	0.09938
CTH	rs2263385	2B	765.696137	0.00029948	0.09699
CTH	rs1036517	2B	625.826747	0.00027502	0.0962
CTH	rs983997	2B	613.24129	0.00027993	0.09598
CTH	rs7353090	2B	781.294311	0.00041648	0.09125
CTH	rs1323308	3B	249.16521	0.00024574	0.09806
CTH	rs1213856	4A	4.560956	0.00025519	0.09806
CTH	rs2302976	7A	75.368757	0.00029077	0.09774
CTH	rs1209574	2B	772.734885	0.00066359	0.08624
CTH	rs1128807	2B	765.555447	0.00084033	0.08317
CTH	rs5324565	6B	659.697904	0.00037483	0.0969
CTH	rs3955500	2B	769.833848	0.00082268	0.0828
CTH	rs984212	3A	713.346253	0.00030013	0.09513
CTH	rs1147138	3A	705.739615	0.00045706	0.09318
CTH	rs5581017	6B	657.112472	0.00046706	0.09205
CTH	rs3026545	6B	660.848974	0.00083029	0.08435
CTH	rs1088670	3A	674.459633	0.0008917	0.08263
CTH	rs1206589	3A	721.328926	0.00087533	0.08231

Appendix 2 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
DH	rs1104842	7B	9.447880	0.00015209	0.12611
DH	rs1081408	5A	557.659428	9.7969E-05	0.11027
DH	rs3939520	2A	563.385712	0.00015835	0.10423
DH	rs2262791	1A	576.975023	0.00023505	0.10133
DH	rs5577883	2A	536.500977	0.00028212	0.09702
DH	rs1110112	2A	570.465134	0.00028778	0.09677
DH	rs1322080	2B	217.092494	0.00057888	0.10266
DH	rs5971036	2B	30.183090	0.00046166	0.09191
DH	rs1074046	5A	557.542905	0.00041892	0.0936
DH	rs5011113	7B	704.448195	0.00038944	0.09301
DH	rs7478525	4A	635.192899	0.00074986	0.09217
DH	rs1131350	5A	557.677843	0.00059134	0.0911
DH	rs3950827	7B	27.481531	0.00081271	0.08648
DH	rs1114145	6A	546.823173	0.00068417	0.08621
DH	rs1009771	7B	22.959680	0.00081058	0.08554
DH	rs1673778	2A	604.673920	0.00086412	0.08377
DH	rs3945948	6A	86.740837	0.00096697	0.08345
DH	rs2252351	2A	35.846102	0.00089866	0.08269
DH	rs981123	2A	76.938668	0.00099041	0.0815
DH	rs1018473	5B	376.372512	0.00028656	0.07773
DH	rs980365	3B	757.165049	0.00066438	0.06799
DM	rs5970682	2A	32.888573	1.7557E-05	0.1513
DM	rs2252351	2A	35.846102	4.7057E-05	0.12176
DM	rs982956	2A	36.038265	4.9521E-05	0.1211
DM	rs39664419	6B	121.511999	0.00015134	0.11316
DM	rs1091004	7A	209.023264	0.00027707	0.10629
DM	rs2279474	7A	169.099064	0.00032181	0.10183
DM	rs1277633	2A	32.676657	0.00026558	0.1017
DM	rs3934093	3B	783.010618	0.00081816	0.09962
DM	rs1104842	7B	9.447880	0.00080907	0.09722
DM	rs1086815	6B	133.045678	0.00042229	0.09672
DM	rs1213183	2A	51.462915	0.00066625	0.09633
DM	rs4009130	2B	26.834053	0.00084216	0.09254
DM	rs984078	2B	505.536683	0.00059323	0.08973

Appendix 2 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
DM	rs6044639	7B	69.346078	0.00047298	0.09445
DM	rs4405228	7A	127.546806	0.00058817	0.09278
DM	rs981123	2A	76.938668	0.00051581	0.09119
DM	rs2276337	2A	577.685932	0.00077043	0.08974
DM	rs5000974	1A	555.329015	0.00072399	0.08955
DM	rs4003428	6B	131.646455	0.00090971	0.089
DM	rs1081408	5A	557.659428	0.00089445	0.08428
DM	rs1018473	5B	376.372512	0.00082226	0.06839
GFD	rs1093097	7B	617.187497	7.1548E-09	0.22989
GFD	rs1205251	7A	694.414248	1.4072E-08	0.22931
GFD	rs1234945	3A	612.003903	2.0124E-08	0.22844
GFD	rs1024404	5B	597.038079	1.4363E-08	0.2269
GFD	rs991978	5A	444.857070	2.126E-08	0.21772
GFD	rs1062457	6A	534.996979	2.4525E-07	0.1901
GFD	rs987950	3B	574.927631	6.0302E-06	0.14772
GFD	rs4989260	7A	587.961622	7.2733E-06	0.14747
GFD	rs9724999	2A	93.411204	3.1856E-05	0.12522
GFD	rs1140490	3A	624.069251	5.1463E-05	0.11831
GFD	rs12777160	4A	5.002882	5.0809E-05	0.11689
GFD	rs1015512	5B	475.807449	5.9583E-05	0.11475
GFD	rs1130302	6A	51.885466	9.8796E-05	0.11459
GFD	rs1021420	2A	752.280072	8.2318E-05	0.10894
GFD	rs1217883	5B	631.108990	0.0002013	0.09806
GFD	rs1077141	3A	625.441235	0.00035867	0.09623
GFD	rs1040910	2A	599.665183	0.00026858	0.09555
GFD	rs4009328	3A	631.799888	0.00031974	0.0948
GFD	rs14394261	2B	584.573613	3.9424E-05	0.12374
GFD	rs3957202	1A	559.661555	0.00027995	0.09407
GFD	rs2283390	2B	728.576863	0.00021734	0.10375
GFD	rs3222352	2B	629.460103	0.00017984	0.10316
GFD	rs1315604	2B	631.826858	0.00017744	0.10054
GFD	rs5580582	2B	737.688589	0.00033793	0.0967
GFD	rs2300488	2B	641.775930	0.00040224	0.09083
GFD	rs1100646	2B	707.554210	0.00099925	0.08888

Appendix 2 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
GFD	rs999196	2B	595.336786	0.00056896	0.08555
GFD	rs12772354	2B	691.501755	0.00058539	0.08549
GFD	rs3026254	3A	629.509715	0.00040736	0.09062
GFD	rs3958113	1B	388.085045	0.00051591	0.09035
GFD	rs1045033	1B	623.755006	0.0009502	0.08781
GFD	rs1002968	5A	544.771658	0.00054795	0.086
GFD	rs1093781	1B	388.089569	0.00055694	0.08581
GFD	rs2289267	5B	632.601840	0.00057722	0.08538
GFD	rs978268	5B	632.835245	0.00062231	0.08448
GFD	rs5581987	1B	527.381377	0.00080906	0.08231
GFD	rs6041859	6B	477.629403	0.00087637	0.0804
GFD	rs1077769	7B	546.713374	0.00091364	0.08018
GFD	rs1125497	3A	629.316266	0.00091211	0.08005
GFD	rs1269980	5B	526.690971	0.0009225	0.07979
GFD	rs1340844	3A	626.244848	0.0009452	0.07973
GFD	rs994004	1B	464.819849	0.00096916	0.0792
GFD	rs1104236	1B	473.324428	0.00096916	0.0792
GFD	rs2344677	1B	476.891194	0.00096916	0.0792
GFD	rs1092694	1B	486.538015	0.00096916	0.0792
GFD	rs1093901	1A	358.962977	0.00024644	0.07681
GFD	rs2252203	1A	342.502548	0.00085333	0.06335
GY	rs1124360	1B	565.822858	3.9716E-05	0.13674
GY	rs2304706	3A	473.715596	6.1164E-05	0.13101
GY	rs2282538	1B	590.437281	3.6262E-05	0.1252
GY	rs5356783	2A	749.687256	0.00011175	0.11291
GY	rs1081529	6A	0.9326580	0.00019015	0.10429
GY	rs1081171	2A	746.622201	0.00027368	0.10418
GY	rs2331235	5A	647.857289	0.00022035	0.10279
GY	rs4993845	2A	749.627323	0.00031	0.0993
GY	rs1046611	7A	611.129453	0.00043626	0.09657
GY	rs1145598	2A	695.975263	0.00040297	0.09465
GY	rs5368115	5A	561.973975	0.00048372	0.0941
GY	rs1163336	5A	650.454653	0.00050546	0.09242
GY	rs982129	2B	54.880411	0.00087035	0.08666

Appendix 2 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
GY	rs3222352	2B	629.460103	8.4779E-05	0.11962
GY	rs1315604	2B	631.826858	8.6811E-05	0.11732
GY	rs2289488	2B	684.913945	0.00064877	0.09366
GY	rs981317	2B	685.294349	0.00097246	0.0836
GY	rs4411213	2A	426.206501	0.00063108	0.08869
GY	rs6050235	2B	705.089008	6.1414E-05	0.12076
GY	rs5412116	2B	705.194705	5.2193E-07	0.18349
GY	rs12763993	2B	707.616210	0.00062608	0.09128
GY	rs3533636	2B	720.596370	0.00038305	0.09662
GY	rs1019030	3A	256.623676	0.00098166	0.08449
GY	rs2329479	2A	747.001075	0.00092948	0.07237
GY	rs3946260	6B	525.349933	0.00069079	0.06882
HI	rs997120	7A	714.316843	5.9479E-06	0.15733
HI	rs979953	7A	49.329526	0.00001341	0.14096
HI	rs2260348	5A	441.667556	1.4949E-05	0.13858
HI	rs2251024	2A	622.406808	2.0608E-05	0.134
HI	rs3030640	7B	564.269567	4.0868E-05	0.13181
HI	rs1052542	5B	77.997394	0.00012243	0.10786
HI	rs4993355	5A	84.512583	0.00017868	0.10403
HI	rs2298076	2A	597.034740	0.00040586	0.09785
HI	rs1143742	4A	658.454844	0.00049012	0.09753
HI	rs3024257	5B	506.377603	0.00035979	0.09614
HI	rs100105587	3B	779.656998	0.00056456	0.0911
HI	rs4993440	3B	780.909897	0.00052913	0.09069
HI	rs1094660	5A	80.596299	0.0006551	0.08849
HI	rs1179041	5A	58.658934	0.00063608	0.08726
HI	rs7341214	5A	375.667312	0.00063664	0.08725
HI	rs4991396	2B	560.187876	8.8569E-06	0.14565
HI	rs979357	2B	67.091013	1.6286E-05	0.13879
HI	rs1139873	2B	367.097515	6.3083E-05	0.12535
HI	rs2256701	2B	612.75935	0.00028012	0.09746
HI	rs1018785	4A	611.881643	0.00050939	0.07126
PHT	rs2371505	4B	29.297345	1.6089E-09	0.2659
PHT	rs1216462	4B	24.384093	1.1014E-08	0.24751

Appendix 2 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
PHT	rs1064354	4B	26.614161	1.3337E-08	0.24079
PHT	rs1863400	4B	38.841165	4.3805E-08	0.21681
PHT	rs2278767	4B	30.576288	3.2362E-07	0.19369
PHT	rs984917	4B	21.287734	1.1002E-06	0.1729
PHT	rs1088389	4B	39.319967	1.3597E-06	0.16918
PHT	rs1212987	4B	21.575326	1.4695E-06	0.16813
PHT	rs991096	4B	38.841231	2.6406E-06	0.16112
PHT	rs1300855	4B	39.949802	3.2855E-06	0.15729
PHT	rs55408728	4B	28.796166	3.9242E-06	0.15689
PHT	rs1214796	7B	609.747127	6.8049E-06	0.15339
PHT	rs12776290	4A	567.028621	5.2453E-06	0.15104
PHT	rs1091494	4B	39.054980	9.6026E-06	0.15059
PHT	rs2283875	4B	37.707737	6.3721E-06	0.14845
PHT	rs4010028	4B	36.999359	9.6058E-06	0.14355
PHT	rs1220382	4A	575.450397	1.3168E-05	0.14021
PHT	rs1087149	4A	569.604831	2.0488E-06	0.13992
PHT	rs2252536	4B	32.412506	3.1702E-05	0.13639
PHT	rs1003062	4B	38.154454	2.5926E-05	0.13302
PHT	rs2257383	7B	611.276698	2.7663E-05	0.13221
PHT	rs1102155	4B	41.327653	3.7167E-05	0.13139
PHT	rs4910062	4B	39.950204	0.00009161	0.12461
PHT	rs2252536	4A	572.495965	9.9751E-06	0.11981
PHT	rs2268367	7B	609.828045	0.00020038	0.11797
PHT	rs3958247	4B	35.840364	9.5373E-05	0.11721
PHT	rs2242043	4B	57.529163	6.9739E-05	0.11706
PHT	rs1092216	4B	12.250336	8.9778E-05	0.1138
PHT	rs1210626	2A	598.663437	0.00013766	0.11378
PHT	rs1204523	6B	628.082889	0.00010962	0.11125
PHT	rs1014382	4B	47.500339	0.00022125	0.11096
PHT	rs998647	4B	50.276118	0.00012473	0.10957
PHT	rs1095899	2A	39.796874	0.00013654	0.10933
PHT	rs985411	6B	618.761928	0.00025567	0.10931
PHT	rs1258394	2A	46.117554	0.00027903	0.10827
PHT	rs980754	4B	42.873407	0.0002519	0.10718

Appendix 2 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
PHT	rs1017437	7B	585.126775	0.00028887	0.1062
PHT	rs2300741	2A	600.84341	0.00024516	0.10595
PHT	rs4404282	6B	621.500476	0.00026584	0.10558
PHT	rs1095859	4B	42.690693	0.00019365	0.10393
PHT	rs1064591	6B	629.33206	0.00024497	0.10269
PHT	rs3064670	7B	614.403099	0.00042866	0.10269
PHT	rs1260541	1A	6.385201	0.00025287	0.10261
PHT	rs1065785	7B	551.134013	0.0002533	0.10237
PHT	rs1100780	6B	622.384371	0.00023745	0.10193
PHT	rs1051075	6B	625.07941	0.00027833	0.10123
PHT	rs7337269	6B	622.685159	0.00024757	0.10111
PHT	rs2276241	6B	622.927418	0.00024757	0.10111
PHT	rs1092528	4B	19.264755	0.00062044	0.10102
PHT	rs3385425	7B	708.680058	0.00025809	0.10091
PHT	rs1058549	7B	614.421085	0.00034085	0.10087
PHT	rs993648	5A	401.828190	0.0003168	0.10035
PHT	rs7940659	6B	617.850404	0.00043021	0.10023
PHT	rs1090186	7B	663.179342	0.00061384	0.09993
PHT	rs1145018	6B	151.493952	0.00071805	0.09969
PHT	rs1115336	3B	778.318206	0.00027227	0.09962
PHT	rs1088735	5A	388.149467	0.00027629	0.09951
PHT	rs1329221	2A	42.508991	0.00037706	0.09892
PHT	rs2261971	6B	121.496932	0.00070588	0.09803
PHT	rs1050081	2A	41.670794	0.00036283	0.09782
PHT	rs5579533	7B	661.372916	0.00080652	0.09771
PHT	rs1101888	4B	17.488180	0.00034153	0.09718
PHT	rs4910989	6B	608.835350	0.00041845	0.09688
PHT	rs1263824	4B	12.576710	0.00035194	0.09636
PHT	rs3954996	6A	605.003107	0.0005482	0.09603
PHT	rs7171130	4B	42.281847	0.00076385	0.09591
PHT	rs1685028	6A	577.322310	0.00050899	0.0957
PHT	rs1265612	4B	30.655464	0.00037425	0.09562
PHT	rs1004850	4B	31.292927	0.0004206	0.0953
PHT	rs1238487	4B	72.900839	0.0005089	0.09485

Appendix 2 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
PHT	rs1220886	4A	637.464954	0.00054264	0.09393
PHT	rs12777429	6B	625.627099	0.00056238	0.09378
PHT	rs1118334	6A	574.630152	0.00064894	0.09338
PHT	rs1059804	6B	608.170362	0.00069112	0.09295
PHT	rs1097943	7B	614.396869	0.00092227	0.09236
PHT	rs3940103	6B	120.452759	0.00049364	0.09204
PHT	rs3021523	7B	668.867524	0.00051072	0.09161
PHT	rs3034480	4B	48.038118	0.00057384	0.09111
PHT	rs987979	4B	31.573760	0.00063561	0.09013
PHT	rs4536076	4B	38.159001	0.00057797	0.09005
PHT	rs3943815	4B	81.466495	0.00059176	0.08997
PHT	rs3020680	4B	102.899421	0.0006793	0.08801
PHT	rs1002274	4B	30.655464	0.00084707	0.08764
PHT	rs5324879	7B	670.925358	0.00074952	0.08756
PHT	rs1058067	7B	581.541708	0.00076982	0.08644
PHT	rs12779268	2A	42.489135	0.00087846	0.08532
PHT	rs1094174	3B	772.661714	0.00092435	0.08414
PHT	rs6045349	2B	48.673741	8.4733E-05	0.11515
PHT	rs3024851	2B	46.856303	0.00020355	0.10347
PHT	rs2278320	2B	736.277979	0.00094183	0.08424
PHT	rs1673680	4B	63.396877	0.00065695	0.0696
PHT	rs1091769	4A	584.893894	0.00077098	0.06776
SN	rs4989776	4B	638.634909	0.00031712	0.10715
SN	rs7331622	7A	510.368383	0.00024333	0.10494
SN	rs1076768	7A	474.910796	0.00050979	0.10152
SN	rs2276134	7A	506.048310	0.0002441	0.10056
SN	rs3935193	3A	479.863689	0.00029063	0.09835
SN	rs1027448	7A	480.216891	0.00043018	0.09755
SN	rs2298324	3A	471.632101	0.0004287	0.09476
SN	rs1242104	1B	505.680503	0.00079499	0.09406
SN	rs3064562	7A	83.395819	0.00053328	0.0926
SN	rs1394030	7A	506.064852	0.0006828	0.09139
SN	rs1019955	3A	477.078639	0.00062309	0.08884
SN	rs3953389	7B	634.863837	0.00064773	0.0883

Appendix 2 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
SN	rs2335012	7A	589.454863	0.00072955	0.0875
SN	rs996096	7A	500.246119	0.00071896	0.08694
SN	rs2283095	7B	716.842017	0.00088187	0.08692
SN	rs1029059	3B	531.339324	0.00076898	0.0861
SN	rs2255021	7A	508.406019	0.00082092	0.08528
SN	rs1082094	6A	420.890375	0.0009022	0.0848
SN	rs5582316	7A	493.35208	0.00091789	0.08452
SN	rs4910825	7A	444.654334	0.00098451	0.08311
TKW	rs5580545	6B	146.771110	6.0271E-07	0.17931
TKW	rs1099328	1A	450.210011	6.2987E-06	0.14819
TKW	rs2293174	4A	550.867303	1.5002E-05	0.14675
TKW	rs3934012	7B	685.354715	0.00022849	0.11437
TKW	rs1153320	7A	100.348524	0.00014159	0.10927
TKW	rs1090835	5A	370.119224	0.00013602	0.10753
TKW	rs3945282	4A	169.707886	0.00050581	0.09589
TKW	rs3947559	6B	145.48259	0.00056149	0.0925
TKW	rs7332924	5A	3.2431500	0.00089014	0.09189
TKW	rs1694762	6B	557.606008	0.00063967	0.08801
TKW	rs1106830	6B	559.851063	0.0006709	0.08769
TKW	rs999758	2A	763.665081	0.00081803	0.08703
TKW	rs4004391	5A	4.0538900	0.00071676	0.08661

BIO, biomass; CHLD, chlorophyll degradation; CHLH, chlorophyll at heading; CHLM, chlorophyll at maturity; CTH, canopy temperature at heading; DH, days to heading; DM, days to maturity; GFD, grain filling duration; GY, grain yield; HI, harvest index; PHT, plant height; SN, seed number/spike; TKW, thousand kernel weight. R^2 indicate phenotypic contribution by marker.

Appendix 3. Summary of significant (P<0.001) marker–trait associations (MTAs) detected in MDLs evaluated in Wad Medani first sowing date (MED/SD1) during the 2019–20 growing season

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
BIO	rs2277132	5A	439.286596	2.947E-06	0.16333
BIO	rs1218943	6A	598.584406	5.436E-06	0.15072
BIO	rs5577001	5B	368.583784	9.854E-05	0.11504
BIO	rs7913978	5A	10.133473	0.0002711	0.09972
BIO	rs12774887	3B	719.683512	0.0002939	0.10777
BIO	rs4394333	5A	10.133116	0.0003111	0.09796
BIO	rs2305675	4A	25.889139	0.000382	0.10243
BIO	rs1041490	5B	232.657559	0.0003865	0.10819
BIO	rs1021257	1A	565.568101	0.0004402	0.07429
BIO	rs3026324	6B	15.717932	0.0004755	0.09279
BIO	rs3954363	4A	8.255676	0.0005216	0.09141
BIO	rs55408837	3B	22.175074	0.0007273	0.08906
BIO	rs1285620	3B	655.984576	0.0008166	0.09536
BIO	rs7171629	1A	564.198028	0.0008211	0.06979
BIO	rs4009024	5A	435.717558	0.0008415	0.08884
CHLD	rs2278019	7B	689.329520	0.0001774	0.10934
CHLD	rs996362	5A	407.576673	0.0002674	0.10603
CHLD	rs1338445	3B	11.585282	0.0002704	0.10022
CHLD	rs2340117	7B	689.729444	0.0004855	0.09761
CHLD	rs4909590	2A	21.275555	0.0005544	0.09632
CHLD	rs1066723	2B	3.510352	0.0006131	0.09248
CHLD	rs1138588	2B	10.253484	0.0007501	0.08999
CHLD	rs1020490	2A	2.206662	0.000862	0.08548
CHLH	rs3940729	7A	0.1330740	5.103E-05	0.13174
CHLH	rs1088004	7A	0.1913130	0.0002016	0.11194
CHLH	rs2277967	7A	9.099850	0.0003324	0.11674
CHLH	rs1153322	7A	9.099721	0.0003595	0.11553
CHLH	rs7352817	7A	9.275729	0.0006212	0.10172
CHLM	rs1107198	2B	24.755206	0.0008764	0.09408
CTH	rs1042061	6B	159.712735	0.0001473	0.11256
CTH	rs1071948	7A	100.655847	0.0002476	0.08219
CTH	rs1382924	4A	690.432563	0.0003382	0.09827
CTH	rs1697150	4A	601.564226	0.0003412	0.10656
CTH	rs1120737	5B	627.393662	0.0003875	0.07763

Appendix 3 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
CTH	rs1092612	7A	94.288723	0.0003988	0.0763
CTH	rs2264223	6B	155.056436	0.0008883	0.08989
CTH	rs3961443	7B	58.048470	0.0009061	0.0855
DH	rs4406564	1B	629.36303	5.842E-07	0.20305
DH	rs982956	2A	36.038265	4.434E-06	0.15577
DH	rs2252351	2A	35.846102	4.63E-06	0.15519
DH	rs1111512	7B	471.484999	2.048E-05	0.13599
DH	rs1277633	2A	32.676657	3.405E-05	0.13137
DH	rs5970682	2A	32.888573	3.65E-05	0.15552
DH	rs1255650	7B	74.684763	6.378E-05	0.15241
DH	rs1151045	2B	724.923696	7.626E-05	0.11935
DH	rs1071015	2A	62.009636	0.0001039	0.11914
DH	rs1101637	2B	72.716032	0.0002895	0.10547
DH	rs3948727	3A	454.808149	0.0002981	0.08004
DH	rs3534443	2B	5.039403	0.0003739	0.11244
DH	rs4404388	2A	82.776144	0.0004135	0.10238
DH	rs26673017	3A	649.258185	0.0004675	0.1052
DH	rs1125767	3B	567.799099	0.0005355	0.10056
DH	rs2261913	2B	666.738099	0.0006242	0.0939
DH	rs4991340	3B	19.277093	0.0006734	0.10569
DH	rs994060	3B	19.463482	0.000716	0.10063
DH	rs4911031	3B	19.353712	0.000979	0.09263
DH	rs1237928	3B	18.834849	0.0009927	0.09541
DM	rs982956	2A	36.038265	6.259E-07	0.18196
DM	rs2252351	2A	35.846102	8.189E-07	0.17824
DM	rs1111512	7B	471.484999	9.303E-07	0.17681
DM	rs4406564	1B	629.363030	2.477E-06	0.17788
DM	rs1277633	2A	32.676657	3.113E-06	0.1635
DM	rs1151045	2B	724.923696	5.292E-06	0.15394
DM	rs1255650	7B	74.684763	6.887E-06	0.18733
DM	rs1071015	2A	62.009636	9.386E-06	0.15628
DM	rs5970682	2A	32.888573	9.682E-06	0.16835
DM	rs1039177	3B	287.163002	0.000208	0.10527
DM	rs4404388	2A	82.776144	0.0002535	0.10526

Appendix 3 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
DM	rs1125767	3B	567.799099	0.0003153	0.10458
DM	rs1222839	2B	5.314801	0.000351	0.09803
DM	rs1694640	7A	592.278526	0.0004236	0.09715
DM	rs12774320	3B	821.859831	0.0004315	0.10263
DM	rs1101637	2B	72.716032	0.0004631	0.10061
DM	rs1064694	6A	594.040966	0.0005355	0.09701
DM	rs1016684	6B	676.034823	0.0005812	0.09851
DM	rs1101823	3B	212.456261	0.0006462	0.08977
DM	rs1066723	2B	3.510352	0.000662	0.09406
DM	rs2261913	2B	666.738099	0.0006743	0.09273
DM	rs1410611	3B	568.847507	0.0006869	0.08897
DM	rs3534443	2B	5.0394030	0.0006881	0.10292
DM	rs1009771	7B	22.959680	0.0007014	0.08978
DM	rs5324886	3B	26.867808	0.0009097	0.08534
DM	rs3222252	7A	617.145728	0.0009975	0.0872
GFD	rs5411471	2A	746.602058	1.302E-07	0.20108
GFD	rs1207505	2A	747.35873	2.044E-07	0.2003
GFD	rs1218138	5A	646.887838	1.183E-06	0.17477
GFD	rs5581489	2B	196.580196	1.225E-06	0.17186
GFD	rs1062525	2A	748.542814	1.392E-06	0.1787
GFD	rs1062525	2A	748.542814	1.392E-06	0.1787
GFD	rs4993789	2A	739.547772	1.668E-06	0.1654
GFD	rs7331595	2A	754.832675	2.017E-06	0.16904
GFD	rs1092232	5A	666.32089	2.32E-06	0.16954
GFD	rs1064949	2A	741.311789	2.602E-06	0.18556
GFD	rs1235793	4A	84.950236	2.713E-06	0.16475
GFD	rs1209601	2B	115.222949	2.972E-06	0.16859
GFD	rs1007206	7A	214.898594	3.125E-06	0.15928
GFD	rs12768999	2A	240.35004	3.255E-06	0.15889
GFD	rs1262375	7A	67.908800	3.372E-06	0.16607
GFD	rs1175159	3A	505.092874	3.582E-06	0.16736
GFD	rs7331604	4A	66.008955	1.363E-05	0.15643
GFD	rs55408748	4A	492.703686	1.643E-05	0.14231
GFD	rs2284541	3B	657.649520	2.392E-05	0.13229

Appendix 3 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
GFD	rs982129	2B	54.880411	2.448E-05	0.14378
GFD	rs1018411	6B	160.902903	2.755E-05	0.12882
GFD	rs2277813	7A	183.188097	3.449E-05	0.12792
GFD	rs1091144	6A	104.962204	4.261E-05	0.12935
GFD	rs3946006	2A	732.808255	5.247E-05	0.12054
GFD	rs5355208	3A	227.0979290	5.392E-05	0.12446
GFD	rs1010498	2A	732.866411	6.117E-05	0.11846
GFD	rs4394467	3B	19.277062	7.006E-05	0.1319
GFD	rs1105202	3A	385.097595	7.318E-05	0.12446
GFD	rs1166451	3B	161.091147	8.793E-05	0.13614
GFD	rs1095667	2A	100.750287	9.147E-05	0.12029
GFD	rs1089113	3A	96.428649	9.217E-05	0.11762
GFD	rs29307918	3A	449.764102	9.589E-05	0.11248
GFD	rs1102568	6A	483.033474	0.0001036	0.11607
GFD	rs12772208	3A	227.336130	0.0001133	0.1101
GFD	rs1215366	2A	732.750304	0.0001231	0.10999
GFD	rs983761	3A	214.174130	0.0001265	0.11207
GFD	rs1207119	2B	745.945636	0.0001283	0.10942
GFD	rs4993296	3A	565.377461	0.0001298	0.12341
GFD	rs1202383	3B	672.805051	0.0001463	0.10683
GFD	rs1051462	3A	645.300869	0.000165	0.11746
GFD	rs1101685	3A	215.220130	0.0001665	0.10519
GFD	rs1092043	3A	239.636424	0.0001665	0.10519
GFD	rs3024561	6B	140.424481	0.0001667	0.10517
GFD	rs2281198	7A	186.219703	0.000167	0.10649
GFD	rs12763810	3B	87.335976	0.0001703	0.10832
GFD	rs1261539	2A	517.428072	0.000182	0.10934
GFD	rs1156906	5A	664.858430	0.0001852	0.10383
GFD	rs1086479	3A	619.745389	0.0001912	0.10346
GFD	rs984538	3B	676.197473	0.0002108	0.10393
GFD	rs4993877	2A	742.578938	0.0002273	0.10143
GFD	rs1049135	3A	380.94806	0.000229	0.11393
GFD	rs1108172	3A	643.906409	0.0002312	0.1016
GFD	rs3934470	7B	11.837362	0.0002322	0.10096

Appendix 3 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
GFD	rs977387	7A	155.897840	0.0002348	0.10519
GFD	rs55408760	3A	389.303472	0.0002392	0.10247
GFD	rs2248581	5B	668.157324	0.000244	0.10968
GFD	rs1396824	7A	62.940473	0.0002455	0.10148
GFD	rs32939420	7B	611.681939	0.0002922	0.10456
GFD	rs39571704	4A	601.791497	0.0003508	0.10056
GFD	rs1167866	6B	195.801253	0.0004119	0.09372
GFD	rs1001699	3B	19.724602	0.000417	0.09471
GFD	rs1040607	2A	746.220675	0.0004263	0.09329
GFD	rs1206846	6A	104.781721	0.0004276	0.09325
GFD	rs5577300	6B	693.998366	0.0004675	0.09213
GFD	rs1055322	3A	596.721939	0.0004682	0.09211
GFD	rs12770820	7A	193.952568	0.0004731	0.09731
GFD	rs1064165	2A	765.166974	0.0004989	0.0756
GFD	rs2269445	7B	3.455371	0.0005106	0.09795
GFD	rs977146	6B	213.350708	0.0005114	0.091
GFD	rs6046585	3B	87.189464	0.000526	0.0907
GFD	rs997388	6B	139.466150	0.0005332	0.09468
GFD	rs1123319	3A	638.403193	0.0005577	0.09044
GFD	rs2278208	2A	742.564624	0.0005648	0.09019
GFD	rs1090377	6B	670.644094	0.0005837	0.10852
GFD	rs5576947	6B	145.550759	0.0006187	0.10102
GFD	rs1039780	3A	472.711496	0.0006202	0.08858
GFD	rs1233176	2A	757.586915	0.000644	0.07217
GFD	rs5325506	3B	160.164089	0.0006563	0.08864
GFD	rs7921861	6A	592.039928	0.0006644	0.08967
GFD	rs2275549	3B	150.345373	0.0006668	0.09046
GFD	rs3027270	2A	749.627389	0.0006673	0.08769
GFD	rs2279707	3B	625.664086	0.0007752	0.08611
GFD	rs12694466	2A	748.472407	0.0007802	0.08571
GFD	rs1009447	6B	183.415201	0.0008285	0.08528
GFD	rs1123760	5A	610.415674	0.0008359	0.09097
GFD	rs1099788	3B	796.827932	0.0008528	0.09174
GFD	rs1113371	6B	203.362219	0.0008875	0.08873

Appendix 3 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
GFD	rs2267186	6B	206.153470	0.0008948	0.084
GFD	rs1073118	3B	786.702990	0.0009842	0.08281
GY	rs4992694	2B	23.928090	0.0002132	0.10438
GY	rs983831	2B	24.905985	0.0006159	0.07088
GY	rs1065263	2B	39.901519	0.000754	0.08825
GY	rs3021975	2B	26.331832	0.0009159	0.0849
HI	rs5363115	2A	534.959029	0.0001562	0.10974
HI	rs3021975	2B	26.331832	0.0002836	0.09756
HI	rs1697382	2B	23.879504	0.0003405	0.09527
HI	rs1023239	1A	524.603012	0.0003666	0.09811
HI	rs7334847	2A	31.547273	0.000386	0.07533
HI	rs1136582	2B	39.475307	0.0004688	0.09128
HI	rs4993724	2B	24.723090	0.0005623	0.08905
HI	rs100105743	7A	703.491585	0.0005656	0.09147
HI	rs1125454	2B	26.135216	0.0006937	0.0897
HI	rs1112265	7A	686.094204	0.0007122	0.08608
HI	rs6048251	7B	115.199560	0.0008123	0.09788
HI	rs1207458	1A	513.819177	0.0008436	0.08478
HI	rs981649	2B	33.241587	0.0009744	0.0829
HTE1	rs1145598	2A	695.975263	1.489E-07	0.20004
HTE1	rs5412116	2B	705.194705	2.362E-06	0.16
HTE1	rs2290854	5A	622.323117	9.888E-05	0.11201
HTE1	rs2304706	3A	473.715596	0.0001666	0.11361
HTE1	rs5411126	2B	193.044214	0.0001272	0.10934
HTE1	rs1124360	1B	565.822858	0.0001506	0.11911
HTE1	rs1202590	1B	18.747099	9.733E-05	0.11112
HTE1	rs42781071	2B	302.144804	0.0002468	0.09937
HTE1	rs3064766	5A	577.680783	0.0002856	0.09966
HTE1	rs1162363	7B	666.201399	9.44E-05	0.1136
HTE1	rs6050235	2B	705.089008	0.0004057	0.09374
HTE1	rs3533636	2B	720.596370	0.0003788	0.09437
HTE1	rs4989260	7A	587.961622	0.0004886	0.09791
HTE1	rs4910809	2A	577.688557	0.0006075	0.09105
HTE1	rs1070992	7A	585.348093	0.0007683	0.08549

Appendix 3 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
HTE1	rs3222352	2B	629.460103	0.00089	0.08434
HTE1	rs1315604	2B	631.826858	0.0008557	0.08562
PHT	rs2281794	7B	576.866202	6.226E-05	0.11922
PHT	rs4910062	4B	39.950204	6.758E-05	0.12243
PHT	rs1030723	7B	565.143457	9.037E-05	0.11799
PHT	rs1003062	4B	38.154454	0.0001239	0.11573
PHT	rs1091976	7B	562.535253	0.0001947	0.10759
PHT	rs1863400	4B	38.841165	0.0002347	0.10512
PHT	rs3957644	7B	568.604891	0.0002509	0.1012
PHT	rs1088389	4B	39.319967	0.0002542	0.10104
PHT	rs2293413	5A	411.837411	0.0002694	0.10726
PHT	rs3955524	7B	551.108438	0.0002925	0.09924
PHT	rs1123681	7B	559.717804	0.0003563	0.10168
PHT	rs1002274	4B	30.655464	0.0004071	0.10124
PHT	rs978206	7B	568.532529	0.0005812	0.09049
PHT	rs2289784	5A	415.60265	0.000594	0.09294
PHT	rs4396638	7B	568.558954	0.0006588	0.0889
PHT	rs1010120	5B	546.668873	0.0007274	0.06946
PHT	rs5581022	7B	569.362802	0.0007477	0.0912
PHT	rs1667384	7B	495.673853	0.0007516	0.08723
PHT	rs2283875	4B	37.707737	0.0007917	0.08658
PHT	rs991096	4B	38.841231	0.0008998	0.0862
PHT	rs1091494	4B	39.054980	0.0009226	0.09157
SN	rs1764478	5B	693.783006	0.000236	0.10643
SN	rs49101029	5B	693.803785	0.0002981	0.10684
SN	rs1700335	5A	666.667638	0.0003708	0.10028
SN	rs1013369	5B	23.174590	0.0003714	0.10482
SN	rs1014436	2B	549.022584	0.0004404	0.09719
SN	rs2288384	2B	65.681175	0.0005651	0.09178
SN	rs977573	7B	173.593001	0.0006832	0.08832
SN	rs5372608	7A	674.776696	0.0007668	0.06875
SN	rs1035293	2B	522.942272	0.0007847	0.09113
SN	rs5372607	7A	674.938100	0.0009485	0.08497
TKW	rs1207784	1B	466.335185	1.15E-06	0.17341

Appendix 3 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
TKW	rs3027075	5A	518.591473	0.0000013	0.17173
TKW	rs7336027	3B	684.281096	1.407E-06	0.17374
TKW	rs1251962	3A	622.277119	2.045E-06	0.16553
TKW	rs3955857	6B	657.218787	2.346E-06	0.17008
TKW	rs3958186	5A	497.059379	2.451E-06	0.16347
TKW	rs5369576	3B	656.171285	2.749E-06	0.16269
TKW	rs5361434	6A	591.769617	2.968E-06	0.16117
TKW	rs1182251	5B	102.551855	3.85E-06	0.15727
TKW	rs3029186	6B	624.032694	3.871E-06	0.15686
TKW	rs4008147	4A	590.792171	3.952E-06	0.15837
TKW	rs3022648	5B	433.530091	4.168E-06	0.15585
TKW	rs990162	5B	438.041352	4.199E-06	0.15575
TKW	rs5411637	6B	81.349881	4.219E-06	0.15569
TKW	rs7921861	6A	592.039928	4.348E-06	0.15769
TKW	rs3955875	7B	589.465397	4.717E-06	0.15418
TKW	rs41940126	6B	471.361761	4.882E-06	0.16532
TKW	rs2279254	1A	511.880067	4.955E-06	0.16301
TKW	rs5359584	1B	580.221133	4.972E-06	0.15398
TKW	rs3025986	7B	115.30835	5.233E-06	0.15382
TKW	rs1122111	5A	434.264347	5.359E-06	0.15425
TKW	rs1062872	1A	522.682366	5.364E-06	0.1549
TKW	rs1004245	7B	642.993887	5.449E-06	0.15321
TKW	rs4990976	5B	518.984458	5.549E-06	0.15199
TKW	rs12770832	6B	39.689026	5.563E-06	0.15195
TKW	rs3954041	4A	21.601904	5.581E-06	0.15191
TKW	rs2256600	4A	17.859875	5.696E-06	0.15163
TKW	rs1076647	6A	557.760387	5.717E-06	0.15158
TKW	rs4261108	7A	10.440166	5.73E-06	0.15163
TKW	rs1120723	5A	501.012601	6.187E-06	0.1529
TKW	rs39658776	3B	54.989738	6.59E-06	0.15497
TKW	rs1114608	6A	591.765186	8.215E-06	0.16548
TKW	rs1096399	5B	68.352798	5.323E-05	0.1219
TKW	rs992176	1A	47.277643	7.565E-05	0.11743
TKW	rs4406564	1B	629.36303	0.0001023	0.12438

Appendix 3 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
TKW	rs1213102	1A	518.722321	0.0001185	0.11229
TKW	rs14925014	1A	496.462045	0.0001201	0.11684
TKW	rs1091278	5A	320.03586	0.0001701	0.11567
TKW	rs3574331	5A	624.860500	0.000178	0.08609
TKW	rs1079731	7B	634.206872	0.000203	0.10447
TKW	rs1141497	7B	635.139115	0.000203	0.10447
TKW	rs1107154	5A	12.193586	0.0002266	0.10305
TKW	rs2317268	4B	548.001773	0.0002374	0.10288
TKW	rs5369866	2A	747.066853	0.0003002	0.07957
TKW	rs55408893	7B	589.46628	0.0003583	0.10019
TKW	rs4405963	7B	81.063248	0.0003684	0.09738
TKW	rs1863928	7A	109.116844	0.000405	0.10024
TKW	rs4002603	2A	704.626367	0.0004309	0.09612
TKW	rs5582801	4A	707.164545	0.0004605	0.09394
TKW	rs5582800	5B	607.548725	0.0004605	0.09394
TKW	rs1092904	5A	5.357914	0.000497	0.09786
TKW	rs2259625	4B	622.270570	0.0005092	0.09846
TKW	rs1117295	5B	532.601300	0.0006197	0.09183
TKW	rs5372381	5A	37.288484	0.0006267	0.09602
TKW	rs4004816	5B	533.556899	0.0006445	0.09248
TKW	rs1071279	3B	809.028449	0.0007318	0.09001
TKW	rs7333346	5B	535.442122	0.0007424	0.09746
TKW	rs1253329	2B	683.012408	0.0007988	0.09559
TKW	rs1218943	6A	598.584406	0.0008298	0.08643
TKW	rs1208237	5A	18.010357	0.0008327	0.09998
TKW	rs4005079	6A	587.828411	0.0008417	0.08625
TKW	rs3955313	1A	526.791936	0.0008851	0.09256

BIO, biomass; CHLD, chlorophyll degradation; CHLH, chlorophyll at heading; CHLM, chlorophyll at maturity; CTH, canopy temperature at heading; DH, days to heading; DM, days to maturity; GFD, grain filling duration; GY, grain yield; HI, harvest index; HTE1, heat tolerance efficiency evaluated in MED/SD1; PHT, plant height; SN, seed number/spike; TKW, thousand kernel weight. R^2 indicate phenotypic contribution by marker.

Appendix 4. Summary of significant (P<0.001) marker–trait associations (MTAs) detected in MDLs evaluated in Wad Medani second sowing date (MED/SD2) during the 2019–20 growing season.

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
BIO	rs5411136	3A	712.302663	9.6811E-06	0.15434
BIO	rs1001418	3A	713.396583	1.8716E-05	0.13549
BIO	rs2290456	3A	708.34637	3.0915E-06	0.13425
BIO	rs1016105	3B	774.067507	6.2146E-05	0.12775
BIO	rs984212	3A	713.346253	7.7509E-05	0.11549
BIO	rs7353920	6B	657.523940	0.00010376	0.11191
BIO	rs1044703	3B	800.439517	0.00016057	0.08596
BIO	rs7927015	6B	651.839962	0.00016329	0.10593
BIO	rs981534	3B	67.138519	0.00017541	0.10801
BIO	rs1079045	3A	713.365311	0.00023451	0.10667
BIO	rs1207485	1A	535.052305	0.00019964	0.11046
BIO	rs1279802	2B	760.414435	0.00020565	0.10681
BIO	rs1277234	3A	707.860582	0.00019381	0.10405
BIO	rs1023494	3A	727.200245	0.00029063	0.10276
BIO	rs1378232	6A	603.409528	0.00030173	0.11104
BIO	rs1026927	3A	714.868966	0.00034938	0.09999
BIO	rs1206236	3B	769.701013	0.00040591	0.10213
BIO	rs5350239	6B	648.041208	0.00041614	0.09403
BIO	rs999768	2B	143.195223	0.000535	0.09086
BIO	rs2282478	6B	652.295742	0.00059613	0.09701
BIO	rs1138009	6B	465.205319	0.00071234	0.09179
BIO	rs1229403	1A	348.562397	0.0007334	0.08937
BIO	rs1059004	6A	443.433380	0.00074393	0.08801
BIO	rs1095722	3A	717.458537	0.00079277	0.09359
BIO	rs3064794	7A	132.601826	0.00081761	0.08939
BIO	rs3570011	1A	563.815509	0.00081984	0.08672
BIO	rs5324565	6B	659.697904	0.0008283	0.08738
BIO	rs12768606	2B	765.065657	0.00082921	0.08715
BIO	rs4988943	2B	117.563732	0.00084243	0.08927
BIO	rs1699577	1A	563.337754	0.00085651	0.08826
BIO	rs39604672	6B	281.452062	0.00086125	0.08917
BIO	rs1089334	6B	649.376261	0.00087232	0.08471
BIO	rs4910116	5B	512.724634	0.00089233	0.0875
BIO	rs2277084	3A	714.34012	0.00089764	0.09019

Appendix 4 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
BIO	rs1233068	2A	626.348022	0.0008993	0.09037
BIO	rs1203258	2B	471.872308	0.00094221	0.09366
BIO	rs3953702	5B	692.847946	0.0009647	0.08356
BIO	rs4393896	5A	433.092647	0.00098576	0.08318
BIO	rs1164950	2B	152.955807	0.00099124	0.09216
BIO	rs1094592	2B	145.431855	0.0009941	0.0831
CHLD	rs4991129	2A	752.743096	3.5478E-05	0.12347
CHLD	rs1209389	2A	758.457529	0.00024272	0.09982
CHLD	rs32940777	7B	703.145862	0.00031723	0.09938
CHLD	rs7336062	6B	515.861537	0.00062382	0.08703
CHLD	rs1215945	1B	505.034299	0.00071575	0.08533
CHLD	rs12776838	1B	652.354553	0.00088682	0.08637
CHLD	rs1863625	6B	132.129747	0.00095849	0.08195
CHLH	rs3939092	7B	64.298515	9.8152E-05	0.11265
CHLH	rs2288384	2B	65.681175	0.00014421	0.10907
CHLH	rs1695956	3B	531.995245	0.00015805	0.0864
CHLH	rs5582607	7B	104.572739	0.00016151	0.10625
CHLH	rs3945038	1B	545.334612	0.00016888	0.10945
CHLH	rs3936723	5A	30.122108	0.00028689	0.11056
CHLH	rs4990317	7A	714.94923	0.00029716	0.10879
CHLH	rs5331903	4B	586.267344	0.00037524	0.09551
CHLH	rs1086242	2A	519.993303	0.00039299	0.0987
CHLH	rs3022648	5B	433.530091	0.00041707	0.09417
CHLH	rs4397808	7B	85.650601	0.00042486	0.09393
CHLH	rs1122111	5A	434.264347	0.00046276	0.09351
CHLH	rs7333375	5A	639.351827	0.00047946	0.0924
CHLH	rs1410611	3B	568.847507	0.00048467	0.09239
CHLH	rs1073641	2A	504.274095	0.00048648	0.10816
CHLH	rs3021069	5B	528.801618	0.00050136	0.09409
CHLH	rs1863405	2A	162.815866	0.00052012	0.09142
CHLH	rs1107107	6B	14.014446	0.00065619	0.10309
CHLH	rs1321605	4B	574.600170	0.00066208	0.08833
CHLH	rs1233721	3B	16.513940	0.00069021	0.08781
CHLH	rs1700056	1A	577.244584	0.00074751	0.09027

Appendix 4 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
CHLH	rs981123	2A	76.938668	0.00081912	0.08565
CHLH	rs980685	5B	687.445911	0.00082907	0.0855
CHLH	rs2293642	6A	569.373155	0.00088624	0.08683
CHLH	rs1202743	1B	597.339819	0.00098944	0.08328
CHLM	rs7336178	1A	522.966035	4.1963E-09	0.28788
CHLM	rs3021069	5B	528.801618	1.2649E-09	0.26812
CHLM	rs1228050	2A	763.757597	0.00010084	0.12754
CHLM	rs1092210	2A	763.212623	0.00094916	0.09742
CHLM	rs1267702	2A	759.784583	0.00073951	0.08647
CHLM	rs1209389	2A	758.457529	3.6274E-05	0.12401
CHLM	rs1114608	6A	591.765186	2.2959E-07	0.20271
CHLM	rs1062872	1A	522.682366	2.1245E-07	0.20033
CHLM	rs2290054	5B	678.381108	4.8261E-06	0.16984
CHLM	rs2288384	2B	65.681175	5.6543E-06	0.16428
CHLM	rs1089212	3A	701.769542	7.6885E-06	0.15857
CHLM	rs12769106	5B	647.00716	9.5805E-05	0.15579
CHLM	rs1220492	4A	646.119886	2.1989E-05	0.15568
CHLM	rs1213102	1A	518.722321	5.6361E-06	0.15532
CHLM	rs3064546	2A	757.654508	0.00089913	0.09252
CHLM	rs1052066	2A	757.175798	1.1059E-08	0.2341
CHLM	rs2242043	4B	57.529163	4.9449E-06	0.1499
CHLM	rs1120723	5A	501.012601	0.0001269	0.14945
CHLM	rs1128442	6A	550.296341	9.6925E-05	0.14944
CHLM	rs1215945	1B	505.034299	5.1484E-06	0.14937
CHLM	rs4405026	2A	756.891858	5.4485E-06	0.15441
CHLM	rs4405026	2A	756.891858	6.2405E-06	0.14684
CHLM	rs1218562	2A	755.656996	1.1509E-08	0.23268
CHLM	rs12854643	5B	679.870814	5.1453E-05	0.1435
CHLM	rs1139314	2A	755.649440	4.9689E-05	0.13395
CHLM	rs1079731	7B	634.206872	1.2035E-05	0.13825
CHLM	rs1141497	7B	635.139115	1.2035E-05	0.13825
CHLM	rs1120468	3A	703.842077	0.00020141	0.13806
CHLM	rs3936723	5A	30.122108	2.7705E-05	0.13406
CHLM	rs1092904	5A	5.357914	0.00098415	0.134

Appendix 4 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
CHLM	rs986208	2A	755.601095	1.1509E-08	0.23268
CHLM	rs1394377	7B	656.144090	0.00004181	0.1339
CHLM	rs1165147	2B	666.718089	9.5724E-05	0.13358
CHLM	rs1235374	3A	698.255461	0.00071485	0.13303
CHLM	rs1388360	3A	695.646312	2.1272E-05	0.13263
CHLM	rs1088739	7B	706.949474	2.0247E-05	0.13204
CHLM	rs1062544	1A	507.655411	2.2329E-05	0.13158
CHLM	rs1090186	7B	663.179342	5.1704E-05	0.13059
CHLM	rs5579533	7B	661.372916	5.3608E-05	0.13018
CHLM	rs1258373	3A	694.227338	0.00034538	0.13016
CHLM	rs1208237	5A	18.010357	5.2085E-05	0.12996
CHLM	rs1102296	4A	11.175527	2.8339E-05	0.12919
CHLM	rs1060943	2A	755.600528	0.00029852	0.09717
CHLM	rs44298360	1A	525.171179	6.0704E-05	0.12852
CHLM	rs1166302	1A	520.770718	7.9242E-05	0.12846
CHLM	rs40729395	2B	676.599011	7.9579E-05	0.12791
CHLM	rs3064387	2B	690.686132	7.7639E-05	0.1279
CHLM	rs984393	2A	755.261393	1.6198E-05	0.14683
CHLM	rs1082368	2A	754.666972	1.4291E-08	0.22963
CHLM	rs2290095	6A	550.308910	8.3126E-05	0.12665
CHLM	rs14925016	2B	675.647256	8.2426E-05	0.12619
CHLM	rs3025062	5B	687.019772	9.4503E-05	0.12607
CHLM	rs4009271	2A	752.992114	7.1716E-05	0.12684
CHLM	rs1115154	6A	15.178865	6.1121E-05	0.12437
CHLM	rs4991129	2A	752.743096	5.9423E-06	0.1485
CHLM	rs4009225	6B	49.555414	9.3511E-05	0.1239
CHLM	rs3024830	3A	702.744322	4.0017E-05	0.12377
CHLM	rs996462	5A	15.387505	9.7693E-05	0.12341
CHLM	rs1311487	2A	751.661373	0.00043155	0.12899
CHLM	rs1127943	2B	681.905919	8.9466E-05	0.12263
CHLM	rs2256874	5B	674.049774	7.2906E-05	0.1224
CHLM	rs4002603	2A	704.626367	4.2039E-05	0.12232
CHLM	rs5581909	5A	17.624563	9.1442E-05	0.12232
CHLM	rs1095427	1B	519.876082	6.5728E-05	0.12075

Appendix 4 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
CHLM	rs1217551	5B	650.905207	5.7477E-05	0.1191
CHLM	rs49461883	7A	483.159528	0.00012214	0.1191
CHLM	rs5582221	2B	691.855594	0.00005785	0.11872
CHLM	rs14925014	1A	496.462045	6.4728E-05	0.11785
CHLM	rs1091278	5A	320.035860	0.0001094	0.11729
CHLM	rs1237690	5B	601.093774	0.00006511	0.11688
CHLM	rs986158	1B	635.701901	8.3466E-05	0.1163
CHLM	rs3934394	4B	118.575005	7.2181E-05	0.11561
CHLM	rs1690605	6A	596.198717	0.00010351	0.11545
CHLM	rs4911104	3A	702.164192	7.0969E-05	0.11542
CHLM	rs3023373	6A	598.564867	7.4232E-05	0.11478
CHLM	rs5582801	4A	707.164545	0.00007472	0.1147
CHLM	rs5582800	5B	607.548725	0.00007472	0.1147
CHLM	rs1124915	2B	85.748866	8.0379E-05	0.11377
CHLM	rs1107154	5A	12.193586	9.0663E-05	0.11223
CHLM	rs3023364	2A	509.283770	1.1421E-05	0.15064
CHLM	rs1205801	4A	505.355228	0.0001022	0.11121
CHLM	rs5579533	7B	661.372916	0.00020359	0.11089
CHLM	rs1202743	1B	597.339819	0.00013496	0.10718
CHLM	rs4005883	7B	372.422980	0.00014158	0.10657
CHLM	rs3027978	1B	599.145518	0.00017566	0.10427
CHLM	rs2268359	6A	594.671045	0.00018346	0.10424
CHLM	rs4008147	4A	590.792171	0.00040082	0.10251
CHLM	rs2277412	5B	604.186688	0.00030667	0.10218
CHLM	rs1073641	2A	504.274095	0.00083609	0.09458
CHLM	rs1112946	4A	672.322922	0.00031928	0.1009
CHLM	rs1696673	7B	640.723374	0.00027029	0.10021
CHLM	rs1069895	4A	8.254592	0.00038067	0.0991
CHLM	rs38283832	1B	563.771810	0.00028831	0.09896
CHLM	rs1862701	7B	624.562845	0.00026312	0.09876
CHLM	rs1100714	5B	670.867739	0.00038789	0.09848
CHLM	rs1207784	1B	466.335185	0.00027254	0.09831
CHLM	rs4009243	3A	705.247482	0.0002991	0.09797
CHLM	rs1115511	5B	118.117012	0.00031397	0.09765

Appendix 4 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
CHLM	rs3064427	5B	533.724323	0.00063309	0.09764
CHLM	rs3222542	1B	120.591422	0.0003662	0.09754
CHLM	rs1187807	2A	479.705575	0.00016047	0.12297
CHLM	rs1115318	4A	9.391235	0.00030885	0.09734
CHLM	rs2317268	4B	548.001773	0.00030036	0.09718
CHLM	rs2253618	2A	418.839514	0.00066588	0.08715
CHLM	rs1111300	2A	414.723909	0.00055315	0.09064
CHLM	rs1052776	5A	12.925965	0.00039533	0.09592
CHLM	rs1129829	2A	350.280672	0.00026974	0.11199
CHLM	rs1125575	1B	667.261738	0.00039095	0.09554
CHLM	rs1103901	2B	70.610438	0.00051781	0.0952
CHLM	rs1076978	6A	15.772277	0.00038215	0.095
CHLM	rs5411637	6B	81.349881	0.00035607	0.09496
CHLM	rs3959280	3B	596.753299	0.00037304	0.09488
CHLM	rs12778966	2A	214.998173	8.2079E-05	0.12495
CHLM	rs1057647	5B	540.079803	0.00080026	0.09444
CHLM	rs5324318	3A	701.771043	0.00086246	0.09423
CHLM	rs4002889	5B	599.122374	0.00038507	0.09398
CHLM	rs1229072	2B	749.609438	0.00050127	0.09355
CHLM	rs1128194	1A	490.371135	0.000433	0.09345
CHLM	rs1034859	5B	600.825982	0.00040281	0.09341
CHLM	rs2268563	2A	196.218395	0.00084034	0.08456
CHLM	rs2276329	1B	664.101988	0.00042421	0.09304
CHLM	rs1255451	1B	476.524246	0.00043108	0.09275
CHLM	rs1696617	2A	182.533773	0.0004426	0.09224
CHLM	rs3024851	2B	46.856303	0.00043963	0.09244
CHLM	rs2264931	2A	179.511679	0.00040425	0.09709
CHLM	rs4009227	2A	163.97503	0.00046145	0.09205
CHLM	rs4009206	1B	518.950963	0.00047431	0.0918
CHLM	rs1341985	1B	630.969761	0.00046938	0.09178
CHLM	rs981336	2A	162.705739	0.00021136	0.1016
CHLM	rs1237398	3A	577.097934	0.00051229	0.09041
CHLM	rs1667800	1B	672.088969	0.0007119	0.09006
CHLM	rs1240536	5B	575.815351	0.00053173	0.08995

Appendix 4 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
CHLM	rs1028725	1A	522.919236	0.00093185	0.08993
CHLM	rs1034732	4A	647.996193	0.00060729	0.08864
CHLM	rs3385469	5B	680.468116	0.00072001	0.08815
CHLM	rs1020682	5A	578.319132	0.00062146	0.08801
CHLM	rs2255863	5B	607.253597	0.00062146	0.08801
CHLM	rs2277083	5A	23.781320	0.00063545	0.08788
CHLM	rs1122111	5A	434.264347	0.000857	0.08786
CHLM	rs3064776	2A	76.914785	1.1096E-05	0.13931
CHLM	rs12776037	1B	563.732843	0.00073295	0.08675
CHLM	rs1095939	6A	16.752265	0.0007491	0.08664
CHLM	rs1329221	2A	42.508991	0.0003814	0.09561
CHLM	rs3027075	5A	518.591473	0.00070604	0.08642
CHLM	rs39658776	3B	54.989738	0.0008311	0.08642
CHLM	rs13879421	3A	708.346436	0.0007343	0.08593
CHLM	rs1268209	5A	553.551798	0.00025117	0.08583
CHLM	rs2279254	1A	511.880067	0.00098122	0.08528
CHLM	rs1182251	5B	102.551855	0.00078277	0.08525
CHLM	rs5359584	1B	580.221133	0.00084748	0.08498
CHLM	rs3022648	5B	433.530091	0.00081371	0.08466
CHLM	rs12779268	2A	42.489135	0.00043456	0.09327
CHLM	rs4992627	5B	573.746780	0.00086253	0.08438
CHLM	rs4990976	5B	518.984458	0.0008356	0.08433
CHLM	rs979392	5A	10.367893	0.00089453	0.08348
CHLM	rs3020431	5A	557.662484	0.0002078	0.08299
CHLM	rs5361434	6A	591.769617	0.0009736	0.08294
CHLM	rs7927015	6B	651.839962	0.00093711	0.08291
CHLM	rs7923595	5B	647.574283	0.00099338	0.08284
CHLM	rs3025986	7B	115.308350	0.00097539	0.08283
CHLM	rs3064906	5B	584.092657	0.00099059	0.08266
CHLM	rs3027446	5B	604.755393	0.0004154	0.07396
CHLM	rs3026584	5B	607.213357	0.00051469	0.07295
CTH	rs1672554	3B	772.064449	0.00039008	0.09488
CTH	rs1077507	6A	590.721173	0.0005312	0.09097
CTH	rs1204907	6A	590.978517	0.00060888	0.09164

Appendix 4 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
DH	rs981123	2A	76.938668	0.00046373	0.09329
DH	rs1071015	2A	62.009636	3.4841E-05	0.13355
DH	rs982956	2A	36.038265	7.5687E-07	0.17807
DH	rs1111512	7B	471.484999	4.2943E-05	0.12419
DH	rs1151045	2B	724.923696	4.4571E-05	0.1257
DH	rs4406564	1B	629.36303	4.4896E-05	0.14886
DH	rs1255650	7B	74.684763	5.0318E-05	0.14138
DH	rs7336178	1A	522.966035	0.00013114	0.11598
DH	rs1695956	3B	531.995245	0.00026535	0.08238
DH	rs3936723	5A	30.122108	0.00026643	0.10485
DH	rs1260019	3B	417.725124	0.00032551	0.11062
DH	rs2278531	3B	256.280713	0.00036022	0.10173
DH	rs1101823	3B	212.456261	0.00037791	0.09703
DH	rs3021069	5B	528.801618	0.00040419	0.10054
DH	rs1128824	4A	630.20322	0.00050233	0.09228
DH	rs991533	7A	87.180847	0.0006666	0.06978
DH	rs1396188	2B	70.715786	0.00069104	0.09777
DH	rs2252351	2A	35.846102	7.6272E-07	0.17796
DH	rs2288384	2B	65.681175	0.00083229	0.08798
DH	rs1113878	5B	448.071174	0.00091403	0.08681
DH	rs3064800	2A	35.627791	0.00069213	0.08824
DH	rs5970682	2A	32.888573	2.824E-06	0.18012
DH	rs1277633	2A	32.676657	5.2108E-07	0.18809
DM	rs1071015	2A	62.009636	1.6726E-05	0.14416
DM	rs1067817	2A	58.658895	0.00061903	0.08983
DM	rs982956	2A	36.038265	6.0146E-07	0.18154
DM	rs2252351	2A	35.846102	7.6613E-07	0.17821
DM	rs3064800	2A	35.627791	0.00090948	0.08516
DM	rs4406564	1B	629.363030	4.3342E-06	0.18053
DM	rs1111512	7B	471.484999	1.4465E-05	0.13864
DM	rs5970682	2A	32.888573	1.4811E-06	0.18691
DM	rs1255650	7B	74.684763	1.8888E-05	0.151
DM	rs1151045	2B	724.923696	1.9143E-05	0.13836
DM	rs1205083	3B	820.888340	0.00013291	0.12255
DM	rs1273483	3A	590.415143	0.00015252	0.11067

Appendix 4 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
DM	rs5324886	3B	26.867808	0.00016615	0.10661
DM	rs5350239	6B	648.041208	0.00018636	0.11018
DM	rs1101823	3B	212.456261	0.00024721	0.10241
DM	rs1695956	3B	531.995245	0.00030123	0.07985
DM	rs1087897	6B	633.908242	0.00031244	0.10032
DM	rs6027352	6B	633.518871	0.00042984	0.09818
DM	rs1243734	7B	435.145036	0.00044302	0.09404
DM	rs3958791	6B	630.232123	0.00049112	0.09272
DM	rs7336178	1A	522.966035	0.00050878	0.10014
DM	rs1039177	3B	287.163002	0.00058669	0.0925
DM	rs3936723	5A	30.122108	0.0006263	0.09577
DM	rs13196478	6B	176.491603	0.00070664	0.09459
DM	rs3024454	6B	634.596438	0.00071992	0.0883
DM	rs1202743	1B	597.339819	0.00082148	0.0862
DM	rs1009771	7B	22.959680	0.00084677	0.08829
DM	rs1032131	6B	633.855852	0.00089	0.08776
DM	rs3570130	5B	38.575235	0.00090753	0.09769
DM	rs1277633	2A	32.676657	9.7866E-07	0.17924
DM	rs1064591	6B	629.332060	0.0009585	0.08753
GFD	rs1018411	6B	160.902903	5.4733E-06	0.15007
GFD	rs1117688	6B	147.205960	0.00010667	0.11056
GFD	rs3029090	5B	486.503789	0.00011586	0.11653
GFD	rs3024561	6B	140.424481	0.0001161	0.10948
GFD	rs1128408	3B	749.39004	0.00018101	0.10877
GFD	rs12775953	6B	165.538506	0.00020801	0.10613
GFD	rs5576947	6B	145.550759	0.00035342	0.10406
GFD	rs1101982	6B	182.364536	0.00023984	0.10262
GFD	rs1057316	6B	189.379060	0.00029299	0.09778
GFD	rs991702	6B	176.498293	0.00035776	0.09547
GFD	rs1081427	3B	739.970220	0.00036359	0.09503
GFD	rs1009447	6B	183.415201	0.00038882	0.09478
GFD	rs1108263	7B	23.668562	0.00046949	0.09182
GFD	rs1066552	6B	163.046131	0.00050935	0.094
GFD	rs3020808	3B	761.432624	0.00051043	0.09078

Appendix 4 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
GFD	rs4004295	3B	734.01566	0.00054959	0.0916
GFD	rs982140	6B	153.097569	0.00060884	0.09314
GFD	rs997388	6B	139.466150	0.0006047	0.08977
GFD	rs1667468	6B	183.415957	0.00061422	0.08846
GFD	rs1167866	6B	195.801253	0.00062761	0.08819
GFD	rs1104514	5B	452.109387	0.00073715	0.08674
GFD	rs3222195	6B	199.305328	0.00081505	0.08554
GFD	rs3934470	7B	11.837362	0.00092866	0.08331
GY	rs3955557	3B	795.294089	1.8142E-05	0.13762
GY	rs9724899	2A	35.606534	0.00002909	0.15205
GY	rs2279722	2B	112.186167	0.00027267	0.11786
GY	rs1103801	3A	638.066037	8.4352E-05	0.1159
GY	rs1017738	3A	638.449148	8.7751E-05	0.11671
GY	rs4003161	2B	66.291810	0.00096537	0.09005
GY	rs1218953	2A	87.954018	0.00021223	0.10738
GY	rs5324076	2B	60.136622	0.00032609	0.11893
GY	rs1026385	2B	50.291611	8.1949E-05	0.11833
GY	rs1022661	2A	76.709885	0.0005588	0.09005
GY	rs1355469	7A	31.354877	0.00058042	0.09455
GY	rs1125300	5B	14.789189	0.0005906	0.09205
GY	rs1152238	2B	38.978498	0.00088458	0.08696
GY	rs3943920	7A	65.000392	0.00093619	0.08354
GY	rs1053125	2B	33.065672	0.00017463	0.11306
GY	rs1090166	7A	638.609684	0.00098973	0.08285
HI	rs4009240	6A	604.182912	1.0311E-05	0.14081
HI	rs4008104	2A	119.891666	1.0562E-05	0.13806
HI	rs1183203	7A	683.845156	0.00014083	0.11723
HI	rs12777318	2B	691.369195	0.00023146	0.10135
HI	rs1673314	2A	5.154274	0.0002622	0.10242
HI	rs1214239	6A	492.445442	0.00026225	0.09954
HI	rs2276468	2B	616.729745	0.00035203	0.0987
HI	rs1092938	6A	605.006033	0.00044198	0.09522
HI	rs2265145	5A	7.840108	0.00052331	0.09163
HI	rs1026385	2B	50.291611	0.00054766	0.09233

Appendix 4 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
HI	rs1086430	6A	601.495617	0.00055361	0.09865
HI	rs1069184	2B	28.721241	0.00056351	0.08846
HI	rs979640	7A	113.033216	0.00068401	0.08993
HI	rs1089242	2B	27.836960	0.00085853	0.08329
HI	rs7928794	2B	33.175201	0.00088401	0.08352
HI	rs1129883	7B	392.112902	0.00093808	0.08295
HI	rs2277064	2B	63.970756	0.00096617	0.08155
HTE2	rs1092756	3A	16.907515	8.0254E-06	0.13346
HTE2	rs2317268	4B	548.001773	5.0625E-05	0.11132
HTE2	rs13881055	6B	650.311839	0.00013882	0.10127
HTE2	rs1132777	4B	538.743351	0.00017978	0.1051
HTE2	rs1062872	1A	522.682366	0.000218	0.09611
HTE2	rs1114608	6A	591.765186	0.00022103	0.09999
HTE2	rs5361434	6A	591.769617	0.00029406	0.09089
HTE2	rs1213102	1A	518.722321	0.00034473	0.09092
HTE2	rs1223619	3A	512.068689	0.00038848	0.08731
HTE2	rs7921861	6A	592.039928	0.00039112	0.08843
HTE2	rs3029186	6B	624.032694	0.00042488	0.08617
HTE2	rs4008147	4A	590.792171	0.00049874	0.08692
HTE2	rs3955857	6B	657.218787	0.00050618	0.08964
HTE2	rs1697382	2B	238.795040	0.00059411	0.08228
HTE2	rs1103801	3A	638.066037	0.00062265	0.08332
HTE2	rs1355469	7A	313.548770	0.00063326	0.08259
HTE2	rs1089334	6B	649.376261	0.00066173	0.08103
HTE2	rs44298360	1A	525.171179	0.00071084	0.08848
HTE2	rs1017738	3A	638.449148	0.00072525	0.0806
HTE2	rs3954041	4A	21.601904	0.000727	0.07995
HTE2	rs1125917	1A	35.527402	0.00073688	0.08076
HTE2	rs7336027	3B	684.281096	0.00080359	0.08385
HTE2	rs1076647	6A	557.760387	0.00082526	0.07848
HTE2	rs1274619	7A	75.535830	0.00087502	0.08411
HTE2	rs9724899	2A	35.606534	0.00089115	0.08672
HTE2	rs990162	5B	438.041352	0.0008935	0.07757
HTE2	rs1009485	3A	736.635784	0.00091529	0.08296

Appendix 4 continued

Trait	Marker	Chromosome	Position (Mbp)	P-value	MarkerR2
HTE2	rs1091278	5A	32.003586	0.0009172	0.08093
HTE2	rs1717147	3A	468.276351	0.0009229	0.07733
HTE2	rs41940126	6B	471.361761	0.00093555	0.08011
HTE2	rs1097738	3A	468.276366	0.00095321	0.07682
HTE2	rs1719885	3A	459.974121	0.00099786	0.0763
SN	rs3940546	6B	24.658095	1.4396E-05	0.15139
SN	rs1218234	7A	664.671736	0.00022763	0.10155
SN	rs1089029	7A	664.052969	0.0002462	0.10055
SN	rs3948739	7A	663.595209	0.00031956	0.09807
SN	rs1071836	7A	662.598662	0.0003374	0.09852
TKW	rs1322080	2B	217.092494	1.1497E-05	0.14871
TKW	rs3938259	3B	2.612423	2.5253E-05	0.12855
TKW	rs4411987	1A	358.963043	3.7246E-05	0.12366
TKW	rs41420864	3A	631.665602	4.8794E-05	0.12149
TKW	rs7332747	7A	443.443948	8.6595E-05	0.12068
TKW	rs1103414	4A	54.50859	0.00016736	0.1053
TKW	rs1150455	7A	425.947551	0.0007231	0.09027
TKW	rs3024458	7A	424.54628	0.00025286	0.08009
TKW	rs1136246	7A	399.493632	0.00040097	0.09342
TKW	rs2276976	7A	289.398733	0.00027712	0.1052
TKW	rs2283405	7A	284.620678	0.00099275	0.09059
TKW	rs12774606	7A	241.541564	0.00016775	0.1105
TKW	rs1067925	7B	302.778802	0.0004797	0.07227
TKW	rs1094036	7A	223.738827	0.00051718	0.09028
TKW	rs1090493	7B	357.797874	0.00052134	0.07132
TKW	rs7928199	7A	198.633887	0.00028475	0.09771
TKW	rs5324851	5B	359.410957	0.00064509	0.0878
TKW	rs1004769	7A	189.356885	0.00087575	0.0837
TKW	rs2291431	7A	121.893577	0.00029153	0.0978
TKW	rs3935447	6B	633.928834	0.00096004	0.08365
TKW	rs4262592	7A	11.782323	0.00063136	0.0953

BIO, biomass; CHLD, chlorophyll degradation; CHLH, chlorophyll at heading; CHLM, chlorophyll at maturity; CTH, canopy temperature at heading; DH, days to heading; DM, days to maturity; GFD, grain filling duration; GY, grain yield; HI, harvest index; HTE2, heat tolerance efficiency evaluated in MED/SD2; PHT, plant height; SN, seed number/spike; TKW, thousand kernel weight. R^2 denote to the phenotypic variation explained by marker.

Appendix 5. Marker-trait associations that passed the false discovery rate (FDR) test at 0.05 and 0.2 levels detected in four environments: Tottori, (TOT); Dongola, (DON); Wad Medani first sowing date (MED/SD1); Wad Medani second sowing date (MESD/SD2) in the 2019–20 growing season.

Environment	Trait	Marker	Chromosome	Position (Mbp)	*P*-value	FDR value	Allelic effect (%)
DON	BIO	rs1217219	6B	8.384405	1.945E-05	0.20	13.8
DON	BIO	rs1151117	7B	177.178294	0.0005005	0.20	9.2
DON	CHLD	rs1071015	2A	62.009636	2.393E-08	0.05	22.5
DON	CHLD	rs982956	2A	36.038265	4.32E-07	0.05	18.0
DON	CHLD	rs2252351	2A	35.846102	4.326E-07	0.05	18.0
DON	CHLD	rs1277633	2A	32.676657	2.58E-06	0.05	15.9
DON	CHLD	rs5970682	2A	32.888573	4.424E-05	0.05	12.6
DON	CHLH	rs5372757	2A	755.881404	5.09E-08	0.05	21.5
DON	CHLH	rs1010893	2A	563.637353	6.976E-06	0.05	14.9
DON	CTH	rs4989353	2A	685.431141	0.0002988	0.12	10.2
DON	DM	rs5970682	2A	32.888573	1.756E-05	0.20	15.1
DON	GFD	rs9724999	2A	93.411204	3.186E-05	0.05	12.5
DON	GY	rs5356783	2A	749.687256	0.0001118	0.20	11.3
DON	HI	rs2251024	2A	622.406808	2.061E-05	0.05	13.4
DON	TKW	rs1099328	1A	450.210011	6.299E-06	0.10	14.8
DON	CTH	rs5364104	3A	717.632745	0.0001967	0.20	10.0
DON	CTH	rs1079045	3A	713.365311	9.15E-05	0.20	11.2
DON	CTH	rs7353090	2B	781.294311	0.0004165	0.20	9.1
DON	CTH	rs1209574	2B	772.734885	0.0006636	0.20	8.6
DON	CTH	rs980362	2B	769.126812	0.0002835	0.14	9.9
DON	CTH	rs2263385	2B	765.696137	0.0002995	0.12	9.7
DON	CTH	rs5357574	1A	417.371125	0.0002305	0.20	10.0
DON	CTH	rs5971304	2B	720.291613	0.0002059	0.20	10.5
DON	CTH	rs1136890	2B	704.963343	0.0002643	0.20	10.1
DON	CTH	rs1151135	2B	691.371823	0.0002948	0.13	10.1
DON	CTH	rs1195353	2B	642.664564	0.0002789	0.20	10.5
DON	CTH	rs2292620	2B	626.971383	0.0003732	0.14	9.8
DON	CTH	rs984212	3A	713.346253	0.0003001	0.12	9.5
DON	CTH	rs1147138	3A	705.739615	0.0004571	0.20	9.3
DON	CTH	rs1086318	3A	705.688411	3.614E-05	0.20	12.2
DON	CTH	rs4989102	3A	702.365848	2.838E-05	0.20	13.6
DON	CTH	rs1323308	3B	249.16521	0.0002457	0.20	9.8
DON	CTH	rs1201070	4A	573.878504	0.0002288	0.20	9.9
DON	CTH	rs1213856	4A	4.560956	0.0002552	0.20	9.8

Appendix 5 continued

Environment	Trait	Marker	Chromosome	Position (Mbp)	*P*-value	FDR value	Allelic effect (%)
DON	CTH	rs986855	4B	435.281281	0.0001806	0.20	10.7
DON	CTH	rs1166538	4B	4.105760	0.000227	0.20	10.1
DON	CTH	rs1044473	6A	546.002435	0.0001307	0.20	10.9
DON	CTH	rs3943257	6A	534.062068	0.0001468	0.20	10.4
DON	CTH	rs2333203	6A	534.392116	0.0002416	0.20	10.0
DON	CTH	rs1209110	6B	692.178142	0.0001165	0.20	11.5
DON	CTH	rs1081192	6B	690.695552	0.0002611	0.20	10.6
DON	CTH	rs5324565	6B	659.697904	0.0003748	0.20	9.7
DON	CTH	rs5581017	6B	657.112472	0.0004671	0.20	9.2
DON	CTH	rs55408881	7A	161.115530	0.0002799	0.15	10.5
DON	CTH	rs2302976	7A	75.368757	0.0002908	0.13	9.8
DON	CTH	rs1036517	2B	625.826747	0.000275	0.20	9.6
DON	CTH	rs12769200	2B	613.614041	0.000285	0.13	9.8
DON	CTH	rs983997	2B	613.24129	0.0002799	0.14	9.6
DON	CTH	rs3064889	2B	608.686784	0.0002829	0.14	10.3
DON	CTH	rs3950894	3A	395.823261	0.0001994	0.20	10.1
DON	CHLD	rs1131740	2B	734.423108	6.966E-06	0.05	14.5
DON	CHLD	rs1151045	2B	724.923696	2.658E-08	0.05	22.0
DON	CHLD	rs4911119	2B	714.178077	6.634E-06	0.05	15.0
DON	CHLD	rs1233374	2B	712.100845	4.214E-09	0.05	25.1
DON	CHLD	rs7915837	3A	679.925205	4.733E-05	0.05	11.9
DON	CHLD	rs3944005	3A	679.822517	5.674E-09	0.05	25.4
DON	CHLD	rs1278393	4B	626.244814	2.009E-05	0.05	13.2
DON	GFD	rs1234945	3A	612.003903	2.012E-08	0.05	22.8
DON	GFD	rs987950	3B	574.927631	6.03E-06	0.05	14.8
DON	GFD	rs991978	5A	444.857070	2.126E-08	0.05	21.8
DON	GFD	rs1024404	5B	597.038079	1.436E-08	0.05	22.7
DON	GFD	rs1062457	6A	534.996979	2.453E-07	0.05	19.0
DON	GFD	rs1205251	7A	694.414248	1.407E-08	0.05	22.9
DON	GFD	rs4989260	7A	587.961622	7.273E-06	0.05	14.7
DON	GFD	rs1093097	7B	617.187497	7.155E-09	0.05	23.0
DON	GFD	rs14394261	2B	584.573613	3.942E-05	0.05	12.4
DON	GY	rs2282538	1B	590.437281	3.626E-05	0.20	12.5
DON	GY	rs1124360	1B	565.822858	3.972E-05	0.20	13.7

Appendix 5 continued

Environment	Trait	Marker	Chromosome	Position (Mbp)	*P*-value	FDR value	Allelic effect (%)
DON	GY	rs5412116	2B	705.19471	5.219E-07	0.05	18.3
DON	GY	rs6050235	2B	705.08901	6.141E-05	0.20	12.1
DON	GY	rs1315604	2B	631.82686	8.681E-05	0.20	11.7
DON	GY	rs3222352	2B	629.4601	8.478E-05	0.20	12.0
DON	GY	rs2304706	3A	473.7156	6.116E-05	0.20	13.1
DON	HI	rs4991396	2B	560.18788	8.857E-06	0.05	14.6
DON	HI	rs2260348	5A	441.66756	1.495E-05	0.05	13.9
DON	HI	rs4993355	5A	84.512583	0.0001787	0.20	10.4
DON	HI	rs1052542	5B	77.997394	0.0001224	0.20	10.8
DON	HI	rs997120	7A	714.31684	5.948E-06	0.10	15.7
DON	HI	rs979953	7A	49.329526	1.341E-05	0.05	14.1
DON	HI	rs3030640	7B	564.26957	4.087E-05	0.10	13.2
DON	HI	rs1139873	2B	367.09752	6.308E-05	0.10	12.5
DON	HI	rs979357	2B	67.091013	1.629E-05	0.05	13.9
DON	PHT	rs6045349	2B	48.673741	8.473E-05	0.05	11.5
DON	PHT	rs12776290	4A	567.02862	5.245E-06	0.05	15.1
DON	PHT	rs1220382	4A	575.4504	1.317E-05	0.05	14.0
DON	PHT	rs1087149	4A	569.60483	2.049E-06	0.05	14.0
DON	PHT	rs2252536	4A	572.49597	9.975E-06	0.05	12.0
DON	PHT	rs2371505	4B	29.297345	1.609E-09	0.05	26.6
DON	PHT	rs1216462	4B	24.384093	1.101E-08	0.05	24.8
DON	PHT	rs1064354	4B	26.614161	1.334E-08	0.05	24.1
DON	PHT	rs1863400	4B	38.841165	4.381E-08	0.05	21.7
DON	PHT	rs2278767	4B	30.576288	3.236E-07	0.05	19.4
DON	PHT	rs984917	4B	21.287734	1.1E-06	0.05	17.3
DON	PHT	rs1088389	4B	39.319967	1.36E-06	0.05	16.9
DON	PHT	rs1212987	4B	21.575326	1.47E-06	0.05	16.8
DON	PHT	rs991096	4B	38.841231	2.641E-06	0.05	16.1
DON	PHT	rs1300855	4B	39.949802	3.286E-06	0.05	15.7
DON	PHT	rs55408728	4B	28.796166	3.924E-06	0.05	15.7
DON	PHT	rs1091494	4B	39.05498	9.603E-06	0.05	15.1
DON	PHT	rs2283875	4B	37.707737	6.372E-06	0.05	14.8
DON	PHT	rs4010028	4B	36.999359	9.606E-06	0.05	14.4
DON	PHT	rs2252536	4B	32.412506	3.17E-05	0.05	13.6

Appendix 5 continued

Environment	Trait	Marker	Chromosome	Position (Mbp)	*P*-value	FDR value	Allelic effect (%)
DON	PHT	rs1003062	4B	38.154454	2.593E-05	0.05	13.3
DON	PHT	rs1102155	4B	41.327653	3.717E-05	0.05	13.1
DON	PHT	rs4910062	4B	39.950204	9.161E-05	0.05	12.5
DON	PHT	rs3958247	4B	35.840364	9.537E-05	0.05	11.7
DON	PHT	rs2242043	4B	57.529163	6.974E-05	0.05	11.7
DON	PHT	rs1092216	4B	12.250336	8.978E-05	0.05	11.4
DON	PHT	rs1204523	6B	628.08289	0.0001096	0.05	11.1
DON	PHT	rs1214796	7B	609.74713	6.805E-06	0.05	15.3
DON	PHT	rs2257383	7B	611.2767	2.766E-05	0.05	13.2
DON	CHLD	rs1016684	6B	676.03482	1.358E-05	0.01	15.4
DON	CHLD	rs1111512	7B	471.485	2.746E-08	0.05	21.9
DON	CHLD	rs1255650	7B	74.684763	3.322E-08	0.05	22.5
DON	TKW	rs2293174	4A	550.8673	1.5E-05	0.20	14.7
DON	CHLD	rs1151891	7B	423.28142	6.037E-05	0.05	12.3
DON	TKW	rs5580545	6B	146.77111	6.027E-07	0.05	17.9
MED/SD1	BIO	rs2277132	5A	439.2866	2.947E-06	0.05	16.3
MED/SD1	BIO	rs1218943	6A	598.58441	5.436E-06	0.05	15.1
MED/SD1	DH	rs4406564	1B	629.36303	5.842E-07	0.05	20.3
MED/SD1	DM	rs4406564	1B	629.36303	2.477E-06	0.05	17.8
MED/SD1	DH	rs982956	2A	36.038265	4.434E-06	0.05	15.6
MED/SD1	DH	rs2252351	2A	35.846102	4.63E-06	0.05	15.5
MED/SD1	DM	rs1071015	2A	62.009636	9.386E-06	0.05	15.6
MED/SD1	DM	rs982956	2A	36.038265	6.259E-07	0.05	18.2
MED/SD1	TKW	rs2279254	1A	511.88007	4.955E-06	0.05	16.3
MED/SD1	DM	rs2252351	2A	35.846102	8.189E-07	0.05	17.8
MED/SD1	DM	rs5970682	2A	32.888573	9.682E-06	0.05	16.8
MED/SD1	DM	rs1277633	2A	32.676657	3.113E-06	0.05	16.4
MED/SD1	GFD	rs7331595	2A	754.83268	2.017E-06	0.05	16.9
MED/SD1	GFD	rs1062525	2A	748.54281	1.392E-06	0.05	17.9
MED/SD1	TKW	rs1062872	1A	522.68237	5.364E-06	0.05	15.5
MED/SD1	TKW	rs992176	1A	47.277643	7.565E-05	0.05	11.7
MED/SD1	TKW	rs1213102	1A	518.72232	0.0001185	0.05	11.2
MED/SD1	TKW	rs14925014	1A	496.46205	0.0001201	0.05	11.7
MED/SD1	GFD	rs1062525	2A	748.54281	1.392E-06	0.05	17.9

Appendix 5 continued

Environment	Trait	Marker	Chromosome	Position (Mbp)	*P*-value	FDR value	Allelic effect (%)
MED/SD1	GFD	rs1207505	2A	747.35873	2.044E-07	0.05	20.0
MED/SD1	GFD	rs5411471	2A	746.60206	1.302E-07	0.05	20.1
MED/SD1	GFD	rs1064949	2A	741.31179	2.602E-06	0.05	18.6
MED/SD1	TKW	rs5359584	1B	580.22113	4.972E-06	0.05	15.4
MED/SD1	TKW	rs4406564	1B	629.36303	0.0001023	0.05	12.4
MED/SD1	TKW	rs1207784	1B	466.33519	1.15E-06	0.05	17.3
MED/SD1	GFD	rs4993789	2A	739.54777	1.668E-06	0.05	16.5
MED/SD1	GFD	rs1010498	2A	732.86641	6.117E-05	0.05	11.8
MED/SD1	GFD	rs3946006	2A	732.80826	5.247E-05	0.05	12.1
MED/SD1	GFD	rs1215366	2A	732.7503	0.0001231	0.05	11.0
MED/SD1	DM	rs1151045	2B	724.9237	5.292E-06	0.05	15.4
MED/SD1	GFD	rs1261539	2A	517.42807	0.000182	0.05	10.9
MED/SD1	GFD	rs12768999	2A	240.35004	3.255E-06	0.05	15.9
MED/SD1	GFD	rs1095667	2A	100.75029	9.147E-05	0.05	12.0
MED/SD1	HTE1	rs1145598	2A	695.97526	1.49E-07	0.05	20.0
MED/SD1	GFD	rs5581489	2B	196.5802	1.225E-06	0.05	17.2
MED/SD1	GFD	rs1209601	2B	115.22295	2.972E-06	0.05	16.9
MED/SD1	GFD	rs982129	2B	54.880411	2.448E-05	0.05	14.4
MED/SD1	GFD	rs1207119	2B	745.94564	0.0001283	0.05	10.9
MED/SD1	GFD	rs1175159	3A	505.09287	3.582E-06	0.05	16.7
MED/SD1	GFD	rs5355208	3A	227.09793	5.392E-05	0.05	12.4
MED/SD1	GFD	rs1105202	3A	385.0976	7.318E-05	0.05	12.4
MED/SD1	GFD	rs1089113	3A	96.428649	9.217E-05	0.05	11.8
MED/SD1	GFD	rs29307918	3A	449.7641	9.589E-05	0.05	11.2
MED/SD1	GFD	rs12772208	3A	227.33613	0.0001133	0.05	11.0
MED/SD1	GFD	rs983761	3A	214.17413	0.0001265	0.05	11.2
MED/SD1	GFD	rs4993296	3A	565.37746	0.0001298	0.05	12.3
MED/SD1	GFD	rs1051462	3A	645.30087	0.000165	0.05	11.7
MED/SD1	GFD	rs1101685	3A	215.22013	0.0001665	0.05	10.5
MED/SD1	GFD	rs1092043	3A	239.63642	0.0001665	0.05	10.5
MED/SD1	GFD	rs1086479	3A	619.74539	0.0001912	0.05	10.3
MED/SD1	GFD	rs2284541	3B	657.64952	2.392E-05	0.05	13.2
MED/SD1	GFD	rs4394467	3B	19.277062	7.006E-05	0.05	13.2
MED/SD1	GFD	rs1166451	3B	161.09115	8.793E-05	0.05	13.6

Appendix 5 continued

Environment	Trait	Marker	Chromosome	Position (Mbp)	*P*-value	FDR value	Allelic effect (%)
MED/SD1	GFD	rs1202383	3B	672.80505	0.0001463	0.05	10.7
MED/SD1	GFD	rs12763810	3B	87.335976	0.0001703	0.05	10.8
MED/SD1	GFD	rs1235793	4A	84.950236	2.713E-06	0.05	16.5
MED/SD1	GFD	rs7331604	4A	66.008955	1.363E-05	0.05	15.6
MED/SD1	GFD	rs55408748	4A	492.70369	1.643E-05	0.05	14.2
MED/SD1	GFD	rs1218138	5A	646.88784	1.183E-06	0.05	17.5
MED/SD1	GFD	rs1092232	5A	666.32089	2.32E-06	0.05	17.0
MED/SD1	GFD	rs1156906	5A	664.85843	0.0001852	0.05	10.4
MED/SD1	GFD	rs1091144	6A	104.9622	4.261E-05	0.05	12.9
MED/SD1	GFD	rs1102568	6A	483.03347	0.0001036	0.05	11.6
MED/SD1	GFD	rs1018411	6B	160.9029	2.755E-05	0.05	12.9
MED/SD1	GFD	rs3024561	6B	140.42448	0.0001667	0.05	10.5
MED/SD1	GFD	rs1007206	7A	214.89859	3.125E-06	0.05	15.9
MED/SD1	GFD	rs1262375	7A	67.908800	3.372E-06	0.05	16.6
MED/SD1	GFD	rs2277813	7A	183.1881	3.449E-05	0.05	12.8
MED/SD1	GFD	rs2281198	7A	186.2197	0.000167	0.05	10.6
MED/SD1	HTE1	rs5412116	2B	705.19471	2.36E-06	0.05	16.0
MED/SD1	HTE1	rs2290854	5A	622.32312	9.89E-05	0.20	11.2
MED/SD1	TKW	rs1251962	3A	622.27712	2.045E-06	0.05	16.6
MED/SD1	TKW	rs7336027	3B	684.2811	1.407E-06	0.05	17.4
MED/SD1	TKW	rs5369576	3B	656.17129	2.749E-06	0.05	16.3
MED/SD1	TKW	rs39658776	3B	54.989738	6.59E-06	0.05	15.5
MED/SD1	TKW	rs4008147	4A	590.79217	3.952E-06	0.05	15.8
MED/SD1	TKW	rs3954041	4A	21.601904	5.581E-06	0.05	15.2
MED/SD1	TKW	rs2256600	4A	17.859875	5.696E-06	0.05	15.2
MED/SD1	TKW	rs3958186	5A	497.05938	2.451E-06	0.05	16.3
MED/SD1	TKW	rs1122111	5A	434.26435	5.359E-06	0.05	15.4
MED/SD1	TKW	rs1120723	5A	501.0126	6.187E-06	0.05	15.3
MED/SD1	TKW	rs3027075	5A	518.59147	0.0000013	0.05	17.2
MED/SD1	TKW	rs1182251	5B	102.55186	3.85E-06	0.05	15.7
MED/SD1	TKW	rs3022648	5B	433.53009	4.168E-06	0.05	15.6
MED/SD1	TKW	rs990162	5B	438.04135	4.199E-06	0.05	15.6
MED/SD1	TKW	rs4990976	5B	518.98446	5.549E-06	0.05	15.2
MED/SD1	TKW	rs1096399	5B	68.352798	5.323E-05	0.05	12.2

Appendix 5 continued

Environment	Trait	Marker	Chromosome	Position (Mbp)	*P*-value	FDR value	Allelic effect (%)
MED/SD1	TKW	rs5361434	6A	591.76962	2.968E-06	0.05	16.1
MED/SD1	TKW	rs7921861	6A	592.03993	4.348E-06	0.05	15.8
MED/SD1	TKW	rs1076647	6A	557.76039	5.717E-06	0.05	15.2
MED/SD1	TKW	rs1114608	6A	591.76519	8.215E-06	0.05	16.5
MED/SD1	TKW	rs3955857	6B	657.21879	2.346E-06	0.05	17.0
MED/SD1	TKW	rs3029186	6B	624.03269	3.871E-06	0.05	15.7
MED/SD1	TKW	rs5411637	6B	81.349881	4.219E-06	0.05	15.6
MED/SD1	TKW	rs41940126	6B	471.36176	4.882E-06	0.05	16.5
MED/SD1	TKW	rs12770832	6B	39.689026	5.563E-06	0.05	15.2
MED/SD1	TKW	rs4261108	7A	10.440166	5.73E-06	0.05	15.2
MED/SD1	DM	rs1111512	7B	471.485	9.303E-07	0.05	17.7
MED/SD1	DM	rs1255650	7B	74.684763	6.887E-06	0.05	18.7
MED/SD1	TKW	rs3955875	7B	589.4654	4.717E-06	0.05	15.4
MED/SD1	TKW	rs3025986	7B	115.30835	5.233E-06	0.05	15.4
MED/SD1	TKW	rs1004245	7B	642.99389	5.449E-06	0.05	15.3
MED/SD2	BIO	rs1016105	3B	774.06751	6.215E-05	0.20	12.8
MED/SD2	HTE2	rs1092756	3A	16.907515	8.025E-06	0.1	13.3
MED/SD2	BIO	rs7353920	6B	657.52394	0.0001038	0.20	11.2
MED/SD2	CHLM	rs1062872	1A	522.68237	2.125E-07	0.05	20.0
MED/SD2	CHLM	rs7336178	1A	522.96604	4.196E-09	0.05	28.8
MED/SD2	CHLM	rs3021069	5B	528.80162	1.265E-09	0.05	26.8
MED/SD2	CHLM	rs1114608	6A	591.76519	2.296E-07	0.05	20.3
MED/SD2	DH	rs7336178	1A	522.96604	0.0001311	0.20	11.6
MED/SD2	DH	rs4406564	1B	629.36303	4.49E-05	0.10	14.9
MED/SD2	DM	rs1273483	3A	590.41514	0.0001525	0.20	11.1
MED/SD2	DH	rs1151045	2B	724.9237	4.457E-05	0.10	12.6
MED/SD2	DH	rs1111512	7B	471.485	4.294E-05	0.10	12.4
MED/SD2	DH	rs1255650	7B	74.684763	5.032E-05	0.10	14.1
MED/SD2	DM	rs4406564	1B	629.36303	4.334E-06	0.10	18.1
MED/SD2	TKW	rs41420864	3A	631.6656	4.879E-05	0.20	12.1
MED/SD2	GY	rs1017738	3A	638.44915	6.066E-05	0.20	12.1
MED/SD2	DM	rs1151045	2B	724.9237	1.914E-05	0.10	13.8
MED/SD2	BIO	rs2290456	3A	708.34637	3.092E-06	0.10	13.4
MED/SD2	DM	rs1205083	3B	820.88834	0.0001329	0.20	12.3

Appendix 5 continued

Environment	Trait	Marker	Chromosome	Position (Mbp)	*P*-value	FDR value	Allelic effect (%)
MED/SD2	DM	rs5324886	3B	26.867808	0.0001662	0.20	10.7
MED/SD2	DM	rs1101823	3B	212.45626	0.0002472	0.20	10.2
MED/SD2	DM	rs1277633	2A	32.676657	9.787E-07	0.05	17.9
MED/SD2	DM	rs5970682	2A	32.888573	1.481E-06	0.05	18.7
MED/SD2	GY	rs9724899	2A	35.606534	1.947E-05	0.20	15.8
MED/SD2	DH	rs1277633	2A	32.676657	5.211E-07	0.05	18.8
MED/SD2	DM	rs2252351	2A	35.846102	7.661E-07	0.05	17.8
MED/SD2	DH	rs5970682	2A	32.888573	2.824E-06	0.05	18.0
MED/SD2	DM	rs982956	2A	36.038265	6.015E-07	0.05	18.2
MED/SD2	DM	rs1071015	2A	62.009636	1.673E-05	0.05	14.4
MED/SD2	DH	rs2252351	2A	35.846102	7.627E-07	0.05	17.8
MED/SD2	HI	rs4008104	2A	119.89167	1.056E-05	0.10	13.8
MED/SD2	DH	rs982956	2A	36.038265	7.569E-07	0.05	17.8
MED/SD2	DM	rs5350239	6B	648.04121	0.0001864	0.20	11.0
MED/SD2	DH	rs1071015	2A	62.009636	3.484E-05	0.10	13.4
MED/SD2	DM	rs1111512	7B	471.485	1.447E-05	0.05	13.9
MED/SD2	DM	rs1255650	7B	74.684763	1.889E-05	0.05	15.1
MED/SD2	GFD	rs1018411	6B	160.9029	5.473E-06	0.05	15.0
MED/SD2	CHLM	rs1082368	2A	754.66697	1.429E-08	0.05	23.0
MED/SD2	BIO	rs5411136	3A	712.30266	9.681E-06	0.05	15.4
MED/SD2	CHLM	rs986208	2A	755.6011	1.151E-08	0.05	23.3
MED/SD2	CHLM	rs1218562	2A	755.657	1.151E-08	0.05	23.3
MED/SD2	CHLM	rs1052066	2A	757.1758	1.106E-08	0.05	23.4
MED/SD2	GY	rs3955557	3B	795.29409	1.482E-05	0.20	14.0
MED/SD2	HI	rs4009240	6A	604.18291	1.031E-05	0.20	14.1
MED/SD2	BIO	rs984212	3A	713.34625	7.751E-05	0.20	11.5
MED/SD2	SN	rs3940546	6B	24.658095	1.44E-05	0.20	15.1
MED/SD2	TKW	rs4411987	1A	358.96304	3.725E-05	0.20	12.4
MED/SD2	TKW	rs1322080	2B	217.09249	1.15E-05	0.20	14.9
MED/SD2	BIO	rs1001418	3A	713.39658	1.872E-05	0.20	13.5
MED/SD2	TKW	rs3938259	3B	2.612423	2.525E-05	0.20	12.9
MED/SD2	TKW	rs7332747	7A	443.44395	8.66E-05	0.20	12.1
TOT	HI	rs1202815	2B	639.88866	6.15E-05	0.10	12.5
TOT	HI	rs1097392	2B	699.5695	5.743E-05	0.10	12.1

Appendix 5 continued

Environment	Trait	Marker	Chromosome	Position (Mbp)	*P*-value	FDR value	Allelic effect (%)
TOT	HI	rs989816	1A	567.43375	5.044E-06	0.10	17.5
TOT	HI	rs1147034	2B	726.67251	1.051E-05	0.10	14.1
TOT	HI	rs5581032	2B	733.42486	7.091E-07	0.05	19.9
TOT	HI	rs3064725	2B	755.29401	8.437E-05	0.05	12.2
TOT	HI	rs1219329	2B	765.68826	5.88E-05	0.05	11.8
TOT	HI	rs7903755	3A	672.27973	3.474E-05	0.05	12.8
TOT	HI	rs2276009	3A	622.12759	5.752E-07	0.05	18.0
TOT	HI	rs999525	3A	606.3586	1.445E-05	0.05	13.8
TOT	HI	rs1126577	3A	603.06433	2.59E-07	0.05	19.0
TOT	HI	rs1013793	3A	598.24764	8.092E-07	0.05	18.3
TOT	HI	rs2293274	3B	500.3768	9.428E-05	0.05	11.6
TOT	HI	rs1054888	4B	658.91816	8.201E-05	0.05	11.4
TOT	HI	rs12771929	4B	658.57453	1.135E-06	0.05	18.0
TOT	HI	rs1126368	4B	655.05385	1.546E-05	0.05	13.5
TOT	HI	rs989478	4B	650.57158	2.871E-05	0.05	13.4
TOT	HI	rs1278393	4B	626.24481	1.67E-05	0.05	13.7
TOT	HI	rs5581306	5A	582.48254	1.298E-05	0.05	14.4
TOT	HI	rs5579491	5A	567.76623	5.401E-05	0.05	12.6
TOT	HI	rs1220965	5A	513.08318	3.414E-05	0.05	12.5
TOT	HI	rs1240031	5A	484.91848	6.435E-05	0.05	13.1
TOT	HI	rs1213694	5B	671.41593	8.689E-05	0.05	11.7
TOT	HI	rs1102693	5B	670.64157	7.236E-05	0.05	11.8
TOT	HI	rs2275286	5B	669.77587	6.951E-05	0.05	11.7
TOT	HI	rs39466056	5B	665.55323	8.327E-05	0.05	13.3
TOT	HI	rs1160820	5B	664.8423	6.706E-05	0.05	11.9
TOT	HI	rs1240043	6A	51.200421	0.0001183	0.05	10.9
TOT	HI	rs2283966	6B	636.61513	5.881E-05	0.05	13.2
TOT	HI	rs4003428	6B	131.64646	0.0001244	0.05	11.5
TOT	HI	rs2291303	2A	647.76287	6.471E-05	0.05	11.8
TOT	PHT	rs2252536	4A	572.49597	4.30E-06	0.05	12.9
TOT	PHT	rs2278767	4B	30.576288	9.194E-06	0.05	15.3
TOT	PHT	rs1004850	4B	31.292927	1.27E-04	0.20	10.9
TOT	HI	rs4005420	2A	15.083630	7.294E-05	0.05	12.2

BIO, biomass; CHLD, chlorophyll degradation; CHLH, chlorophyll at heading; CHLM, chlorophyll at maturity; CTH, canopy temperature at heading; DH, days to heading; DM, days to maturity; GFD, grain filling duration; GY, grain yield; HI, harvest index; HTE1, heat tolerance efficiency evaluated in MED/SD1; HTE2, heat tolerance efficiency evaluated in MED/SD2; PHT, plant height; SN, seed number/spike; TKW, thousand kernal weight. FDR indicate false discovery rate.

Appendix 6. Candidate genes for strong significant markers identified for some important agronomic traits evaluated under optimum (DON), moderate heat (MED/SD1) or severe heat (MED/SD2) stress.

Marker	Environment	Chr.	Trait	R^2	Gene	Protein	Function
rs9724899	MED/SD2	2A	GY	0.15	TraesCS2A03G0151600	UDP-Glycosyltransferase	Regulates grain size and abiotic stress tolerance in rice
rs1001418	MED/SD2	3A	BIO	0.13	TraesCS3A03G1146600	Protein kinase	Regulates wheat growth and stress tolerance
rs5412116	MED/SD1	2B	HTE1	0.16	TraesCS2B02G521400	Serine/threonine protein kinase, Hyperosmotic stress respons	Regulates hyperosomtic stress responses and ABA signaling
	DON		GY	0.18			
rs1092756	MED/SD2	3A	HTE2	0.13	TraesCS3A03G0073100	Zinc finger family protein	Regulates heat stress tolerance in wheat
rs5369576	MED/SD1	3B	TKW	0.16	TraesCS3B03G1029000	Myb transcription factor	Regulates oxidative and heat stress responses through calcium signaling
rs4008104	MED/SD2	2A	HI	0.14	TraesCS2A03G0349200	Sucrose synthase	Convert sucrose to starch in endosperm in wheat

Appendix 6 continued

Marker	Environment	Chr.	Trait	R^2	Gene	Protein	Function
rs3940546	MED/SD2	6B	SN	0.15	TraesCS6B02G046400	Expansion protein	Regulates crop growth and development as well as ultimate yield formation in wheat
rs1062872	MED/SD2	1A	CHLM	0.28	TraesCS1A02G341400	RING/U-box superfamily protein	Regulates to delay leaf senescence through jasmonic acid pathway in Arabidopsis
rs3021069	MED/SD2	5B	CHLM	0.26	TraesCS5B02G351600	Superoxide dismutase [Cu-Zn]	Regulates response to the oxidative stress
					TraesCS5B02G350900	Plant regulator RWP-RK family protein, putative	Regulates nitrogein use efficiency in wheat

BIO, biomass; CHLM, chlorophyll at maturity; GY, grain yield; HI, harvest index; HTE1, heat tolerance efficiency evaluated in MED/SD1; HTE2, heat tolerance efficiency evaluated in MED/SD2; SN, seed number/spike; TKW, thousand kernel weight. R^2 denote to the phenotypic variation explained by marker.

List of publications

Chapter 1

Title: Harnessing the diversity of wild emmer wheat for genetic improvement of durum wheat

Authors: Balla, M.Y., Gorafi, Y.S.A., Kamal, N.M., Abdalla, M.G.A., Tahir, I.S.A. and Tsujimoto, H

Journal: Theoretical and Applied Genetic, 135:1671-1684
https://doi.org/10.1007/s00122-022-04062-7

Published online: March 7

Chapter 2

Title: Exploiting wild emmer wheat diversity to improve wheat A and B genomes in breeding for heat stress adaptation

Authors: Balla, M.Y., Gorafi, Y.S.A., Kamal, N.M., Abdalla, M.G.A., Tahir, I.S.A. and Tsujimoto, H

Journal: Frontier in Plant Science, 13:895742
https://doi.org/10.3389/fpls.2022.895742