Exploitation and utilization of multiple synthetic derivatives population for breeding wheat with heat stress tolerance

（高温ストレス耐性コムギの育種のための多重合成派生集団の開拓と利用）

By

Awad Ahmed Elawad Elbashir

September 2017

The United Graduate School of Agricultural Sciences

Tottori University, Japan

Exploitation and utilization of multiple synthetic derivatives population for breeding wheat with heat stress tolerance

（高温ストレス耐性コムギの育種のための多重合成派生集団の開拓と利用）

Awad Ahmed Elawad Elbashir

2017

Exploitation and utilization of multiple synthetic derivatives population for breeding wheat with heat stress tolerance

（高温ストレス耐性コムギの育種のための多重合成派生集団の開拓と利用）

A Thesis Submitted to the United Graduate School of Agricultural Sciences, Tottori University, in Partial Fulfillment of Requirements for the Doctoral Degree (Ph.D.) in Agricultural Sciences, Plant Molecular Breeding

By

Awad Ahmed Elawad Elbashir

Approved by:

Prof. Dr. Nitaro Maekawa ………………

Dean, United Graduate School of Agricultural Sciences, Tottori University

Prof. Dr. Hisashi Tsujimoto ……………

Chairman of Supervisory Committee

The United Graduate School of Agricultural Sciences

Tottori University

2017

Table of contents

Chapter 1 Wheat multiple synthetic derivatives: a new source for heat stress tolerance adaptive traits

Chapter 2 Genetic variation in heat tolerance-related traits in a population of wheat multiple synthetic derivatives

Chapter 3 Genome-wide association study of adaptive traits under high temperature environments

Acknowledgements

Foremost, I am grateful to Allah for the good health and wellbeing that were necessary to complete this study.

I would like to express my sincere gratitude to my supervisor Prof. Hisashi Tsujimoto for his continuous supervision to complete my Ph.D. study; his patience; motivation and immense knowledge; his guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my Ph.D.

Next, I am very grateful to my co-supervisors Dr. Prof. Masayoshi Shigyo and Dr. Prof. Kinya Akashi for their advice and suggestions during my study and all their efforts to follow-up my progress.

Especial thanks to Dr. Izzat Sidahmed Ali Tahir, is the person who strongly nominated and encouraged me to have this valuable scholarship, and he also was the team leader during the implementation time of my field experiment.

My deepest thanks to Dr. Yasir Serag Alnor Gorafi for his kind support fruitful advice, suggestions teaching and helping me during my study period. His support was not only academic but it was also social support, which made my stay in Japan much easy.

Also, I would like to thank Dr. June-Sik Kim for analyzing the genotypic data and valuable comments.

My thanks also extended to my colleagues in Plant Molecular Breeding Laboratory specially, Dr. Nasrin for her support and teaching me many things during my study period and Ms. Higashida, Koji, Nanasato, Nishimura, Inagaki, Shimizu and the technical staffs in Arid Land Research Center. Mr. Shimizu for helping in setting the growth chamber and teaching the maintenance and operation of the physiological equipment.

I am very grateful to the staffs of Wheat Research Program, Agricultural Research Corporation in Sudan for the management and data collection of my experiments from the field, especially Mr. Mohammed Abdalla (Jiha).

Also, I would like to thank all the staffs of the Tottori University especially administration office in the Arid Land Research Center for their kind collaboration.

My gratitude and thanks to the Ministry of Education, Science, Sports and Culture of Japan for providing the financial support that effectively contributed to the rapid, smooth completion of this study.

Last and deep thanks to my parents Zeinab and Ahmed, for giving birth to me at the first place and supporting me spiritually throughout my life, my brother, sisters, and in-laws. My wife Dalia for her understanding, support and encouragement to making this dissertation possible and my children, Mohammed and Mahmoud.

Awad Ahmed Elawad Elbashir

General introduction

Wheat (*Triticum aestivum* L.) is one of the major cereals in the world. It is grown on 222 million hectares, and its production increased from 235 million tons in 1961 to an estimated 733 million tons in 2015 worldwide (USDA, 2016 and FAOSTAT, 2014). Wheat is one of the main sources of calories and protein. Approximately 85% and 82% of the global population depends on wheat as a basic source of calories and proteins, respectively (Chaves *et al.* 2013). Moreover, wheat is used in the production of a variety of products, such as leavened bread, flat and steamed bread, cakes, pasta, biscuits, noodles, couscous and beer (Curtis and Halford 2014). Beyond its use for human consumption, wheat is also used for the development of non-food products such as fuel.

In recent years, changes in population trends, food behaviors and economic condition in Africa and Asia, have caused an increase in global wheat demand. Wheat has a high level of adaptation, for that is cultivated in tropical and subtropical regions and under both rain-fed and irrigated cultivation. However, wheat production is severely affected by adverse environmental stresses (Rahaie *et al.* 2013). Several crop models have estimated 6-13% yield reduction in wheat for each ºC increase in temperature (Mondal *et al*. 2016). The increased climatic changes like high temperature, decreased amount of rainfall, increased percentage of the saline soils, new pests, pathogens, and diseases will affect the future wheat linear productivity growth. Breeding wheat for tolerance to climatic variability and disease resistance combined with good agronomy can increase wheat yield to fill the gap in future demand. The contribution of new modern wheat cultivars is little to the genetic potential in improvement and development of new cultivars with increased heat, drought and salinity, due to their low within-species variability. One strategy for enhancing modern wheat cultivars is to use wild relative species. Wild relatives of common wheat have an old history in wheat breeding for heat, drought, salinity and cold tolerance (Zaharieva *et al.* 2001, Yasayan *et al.*

2009, Zamani-Babgohari *et al.* 2013, Arabbeigi *et al.* 2014, Kiani *et al.* 2015, Masoomi-Aladizgeh *et al.* 2015).

Zamani-Babgohari *et al*. (2013) reported wide genetic variation response to edaphic stress within the same species in wild wheat relatives. Several researchers reported the importance of the wild emmer (*Triticum dicoccoides*) in biotic and abiotic stresses (Huang *et al*. 2016, Nevo and Chen 2010, Peng *et al*. 2011). It has large physiological differences under salt stress, it is a rich gene source for *Fusarium* head blight resistance, and it is a source of high protein content that is placed on wheat chromosome 6B *(Gpc-B1*) region by controlling the zinc and iron movement through the seed before maturation (Shavrukov *et al*. 2010, Oliver *et al*. 2007, Distelfeld *et al*. 2006). Three accessions of Jordanian *Ae. crassa* (*Ae. crassa* 1, *Ae. crassa* 2 and *Ae. crassa* 3) were identified to enhance chlorophyll content, chlorophyll fluorescence and biomass accumulation under drought conditions (Harb *et al.* 2013). On the other hand, *Ae. tauschii* is widely distributed and contained some species used for drought and salt tolerance enhancement in wheat (Dubcovsky *et al*. 1996, Sohail *et al.* 2011), also, there are new wheat cultivars developed through crosses with *Ae. tauschii*, *Ae. geniculate, Ae. speltoides* and *Ae. variabilis* for resistant to Hessian fly, powdery mildew and rust (Suszkiw 2005, Stoilova and Spetsov 2006, Schneider *et al*. 2008, Barloy *et al*. 2007). A commercial bread wheat cultivar named Chuanmai 42 with 20-30% yield increase in season 2003 was developed through hybridization between hexaploid bread wheat and synthetic wheat (CIMMYT, 2004).

Tsujimoto *et al.* (2011) produced a new population containing a big diversity of the wild relative species, *Ae. tauschii* Coss. (2n=2x=14, DD) by crossing and backcrossing 43 synthetic wheat lines derived from crosses between tetraploid wheat cultivar Langdon and 43 *Ae. tauschii* accessions, to the Japanese elite wheat cultivar Norin 61. After crossing and backcrossing the 43 synthetic wheat lines to Norin 61, the BC_1F_1 plants from each cross were self-pollinated and BC_1F_2 seeds were produces. From each cross ten seeds from each ten plants were collected and then all seeds were mixed and a mixture of 4300 seeds was created. This population named multiple synthetics derivatives population (MSD). Usually the breeders use one *Ae. tauschii* accession and cross it with common wheat and hence transfer a few number of genes to the new genotypes. Here the MSD population was developed mainly to provide as much as possible of the genetic variation of *Ae. tauschii* in one population to ease and facilitate the utilization of the wide variation of *Ae. tauschii* in wheat breeding and improvement.

In this study, I used this new population to;

1. Select heat-tolerant plants from multiple synthetic derivatives (MSD) population and evaluate their agronomic and physiological traits.
2. Examine the genetic variability of heat stress–adaptive traits and to identify new sources of heat tolerance to be used in wheat breeding programs.
3. Identify QTLs/genes associated with heat stress-adaptive traits.

Chapter 1

Wheat multiple synthetic derivatives: a new source for heat stress tolerance adaptive traits

1.1 Abstract

Heat stress is detrimental to wheat (*T. aestivum* L.) productivity. In this study, I aimed to select heat-tolerant plants from multiple synthetic derivatives (MSD) population and evaluate their agronomic and physiological traits. I selected six tolerant plants from the population with the background of the cultivar ‘Norin 61’ (N61) and established six MNH (MSD population of N61 selected as heat stress tolerant) lines. I grew these lines with N61 in the field and growth chamber. In the field, I used optimum and late sowings to ensure plant exposure to heat. In the growth chamber, in addition to N61, I used the heat tolerant cultivars ‘Gelenson’ and ‘Bacanora’. I confirmed that MNH2 and MNH5 lines acquired heat tolerance. These lines had higher photosynthesis and stomatal conductance and exhibited no reduction in grain yield and biomass under heat stress compared to N61. I noticed that N61 had the relatively good adaptability to the heat stress. The results indicate that the MSD population includes the diversity of *Ae. tauschii*, is a promising resource to uncover useful quantitative traits derived from this wild species. Selected lines could be useful for heat stress tolerance breeding.

1.2 Introduction

Heat stress is one of the major constraints on the production of bread wheat (*Triticum aestivum* L.) in many areas worldwide. Nearly 40% of the total irrigated area where wheat is grown is affected by heat stress (Reynolds *et al.* 1994). Heat stress adversely affects morphological, phenological, and physiological traits at all stages, in particular, it decreases chlorophyll content and photosynthetic capacity of leaves (Prasad *et al.* 2008).

The optimum temperature for wheat growth and yield is 18–24°C and exposure to 28–32°C for only 5–6 days reduces yield by up to 20% (Stone and Nicolas 1994). Wheat grain yield can be evaluated by two major components: the number of grains/m^2 and individual grain weight. In a crop exposed to high temperatures before anthesis, grain number is reduced via a reduction in spike/m^2 and grains/spike (Shpiler and Blum 1991).

Morphological and physiological traits, including yield and yield components, are used as selection criteria to determine the heat tolerance of wheat. Photosynthetic rate, stomatal conductance, canopy temperature, chlorophyll content, chlorophyll fluorescence, and normalized difference vegetation index are physiological traits used widely to evaluate heat stress tolerance in wheat (Al-khatib and Paulsen 1990, Moffate *et al.* 1990, Pinto *et al.* 2010, Reynolds *et al*. 1994, Smillie and Hetherington 1983). However, the genetic diversity of heat stress tolerance is limited in the elite wheat gene pool (Ogbonnaya *et al*. 2013).

Ae. tauschii, the diploid wild ancestral species and the D genome donor of bread wheat, is a promising genetic resource for breeding (Feldman 2001, Helbaek 1959, Kihara 1944, Mujeeb-Kazi *et al*. 1996), and many *Ae. tauschii* genes have been introduced to bread wheat through amphidiploids between *Ae. tauschii* and tetraploid wheat; these amphidiploids are named synthetic hexaploid wheat (SHW) or primary synthetic wheat (Gill *et al.* 2008, Halloran *et al.* 2008, Ogbonnaya *et al.* 2013). *Ae. tauschii* grows widely in harsh environments in Central Asia and the Middle East and thus may carry useful genes for biotic and abiotic stress tolerance. Sohail *et al*. (2011) found a significant variation in drought stress–related characteristics both among the accessions of *Ae. tauschii* and SHWs. However, they found a low correlation between the performance of the accessions and that of the corresponding SHWs lines, especially under stress; that is, SHWs derived from drought-tolerant *Ae. tauschii* accessions are not always drought-tolerant. This fact indicates that we cannot predict the level of drought stress tolerance in hexaploid wheat from the performance of *Ae. tauschii* because the morphology of this wild diploid species is too different from that of bread wheat. SHWs retain wild morphology such as tough glumes (Okamoto *et al*. 2012), which precludes threshing and thus measuring of yield-related characteristics (Tsujimoto *et al*. 2015). Synthetic derivative lines, which originate from crosses between SHWs and bread wheat cultivars, are a better choice to uncover the variation in *Ae. tauschii* that may be used for breeding. However, most synthetic derivative lines originated from crosses between bread wheat varieties and a limited number of SHWs. To investigate and use comprehensively the intraspecific variation of *Ae. tauschii* for bread wheat breeding, and produced multiple synthetic derivatives (MSD) populations, in which the interspecific variation of *Ae. tauschii* was compiled in the genetic background of a certain bread wheat cultivar (Tsujimoto *et al*. 2015, Gorafi *et al*. unpublished).

Here, I used an MSD population (BC_1F_4) derived from the bread wheat cultivar 'Norin 61' and selected six plants showing good performance under heat stress in Sudan. I took the seeds separately from each of the six plants and developed six heat stress–tolerant lines. I evaluated these lines in the field and in a controlled environment to verify their tolerance and examine their potential for wheat breeding. Out of the six selected plants, I confirmed that two are heat-tolerant genotypes and can be used to breed heat stress–tolerant high-yielding wheat varieties.

1.3 Materials and Methods

1.3.1 Plant materials

I used an MSD population produced by crossing and backcrossing of the Japanese bread wheat cultivar ‘Norin 61’ (hereafter referred to as N61) with 43 synthetic hexaploid wheat lines derived from crosses between 43 accessions of *Ae. tauschii* and *T. turgidum* var. *durum* cv. ‘Langdon’ (LDN) (Matsuoka and Nasuda, 2004, Kajimura *et al.* 2011). To produce the initial population (4300 grains), I mixed 10 grains (BC_1F_2) from each of the 10 BC_1F_1 plants. I cultivated the plants as a bulk in Tottori, Japan and harvested the BC_1F_3 seeds; they were sown at Gezira Research Farm, Agricultural Research Corporation, Sudan (14°24'N, 33°29'E, 407 m above the sea level) in the 2014/2015 season. The MSD population showed various phenotypes in spike, leaf, and plant traits at maturity time. I selected six plants that we feel they might be heat tolerant. Those plants showed vigorous growth and remaining green leaves at maturity compared to the adjacent plants that were completely dry. I named these lines MNH1 to MNH6 for “MSD population of ‘Norin 61’ selected as heat stress tolerant”. In addition, I also used two heat tolerant, highly productive varieties, ‘Bacanora’ and ‘Gelenson,' from International Maize and Wheat Improvement Center (CIMMYT) in this study.

1.3.2 Evaluation of MNH lines in the field

I cultivated the selected six plants as a bulk in Tottori, Japan and harvested the BC_1F_4 seeds. In the 2015/2016 season, I grew the selected lines and their bread wheat parent N61 in the same field in Sudan. Seeds were sown either in the third week of November (optimum sowing) or in the second week of December (late sowing; it was used to expose plants to heat stress at the reproductive stage). I obtained the data on weekly maximum, minimum, and mean temperatures during the crop cycle from a meteorological station located 500–750 m from the experimental site. During heading and grain filling, the temperatures were higher in late sowing (max., 41^{o}C; min., 23^{o}C) than in optimum sowing (max., 37^{o}C; min., 15^{o}C) (Fig. 1-1A). The soil (cracking clay vertisols) had very low water permeability, a pH of 8.5, and was poor in organic matter (0.5%) and deficient in nitrogen (300–400 ppm) and available phosphorus (4–5 ppm). Seeds were manually sown (12 g/m^2) in rows 0.2 m apart in plots of four 0.5-m-long rows. Seeds were treated with the insecticide Gaucho (imidacloprid 35% WP) (Bayer Crop Science, USA) to control pests, especially termites and aphids. Triple superphosphate (at a rate of 43 kg/ha of P_2O_5) was applied at sowing, and urea (86 kg/ha of N) was applied before the second irrigation. Irrigation was carried out at 10–12-day intervals to avoid any water stress. Weeding was performed manually at least twice. The trial was conducted in a randomized complete block design with three replications. The experimental site was free from major wheat diseases.

Abbreviations used for phenotypic and physiological traits and methods of measurement are listed in Table 1-1. Grain yield was measured for each plot. Analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD) test at $P < 0.05$ was performed using GenStat Discovery Edition 3 software (v. 7.2.2.222; VSN International, UK) for each sowing date separately and then combined to quantify the interactive effects of traits and sowing date. The relative performance (RP%) was calculated as described by Asana and Williams (1965) and was expressed as a percentage using the equation: RP (%)= performance of genotype in late sowing/performance of genotype in optimum sowing.

1.3.3 Evaluation of MNH lines in environment-controlled chambers

I evaluated the six lines plus N61 and the two-heat tolerant, highly productive varieties, 'Bacanora' and 'Gelenson,' in optimum and heat-stress conditions using growth chambers at the Arid Land Research Center, Tottori, Japan. Seeds were sown directly in pots (9 cm) containing 1 kg organic soil and kept at a light intensity of 80,000 lux under a 14/10 h day/night photoperiod (22/18°C, 40/50% relative humidity). The pots (one seedling in each) were kept in a completely randomized design with three replications for 60 days; then half of them were transferred to another chamber set at 38/18°C day/night (Fig. 1-1B). Stressed plants were watered every day and control plants every two days. Photosynthetic rate and stomatal conductance were measured at 21 days using upper fully expanded leaves with an LC Pro Console Photosynthesis Meter (model EN11 ODB, ADC Bioscientific Ltd., UK).

1.3.4 Graphical genotyping of the selected lines

Total genomic DNA was extracted using the CTAB method (Saghai-Maroof *et al.* 1984), and DNA samples (20 µl; 50–100 ng μl^{-1}) were sent to Diversity Arrays Technology Pty. Ltd, Australia (http://www.diversityarrays.com) for a whole-genome scan using DArT-seq markers. The sequencing-based DArT genotyping applies two complexity reduction methods optimized for several plant species at DArT PL, i.e., *Pst*I/ *Hpa*II and *Pst*I/ *Hha*I were used to select a subset of *PstI-Hpa*II and *Pst*I-*Hpa*I fragments, respectively (Sansaloni *et al.* 2011). At the DArT facility, the DArTsoft marker extraction pipeline was used to filter the markers on the basis of reproducibility (that is, the percentage of technical replicate pairs scoring identically for a given marker), call rate (that is, the percentage of samples for which a given marker was scored), and the average read depth (that is, the average number of sequence ‘tag’ counts contributing to the genotype calls for a given marker). The minimum threshold value for reproducibility was 95%, and the minimum threshold value for call rate was 85%. The minimum threshold value for average read depth for SNPs was 7, whereas, for silicoDArTs, it was 8.

In total, I obtained 15,616 polymorphic DArT-seq markers between the primary SHW, MNH lines and N61. The chromosomal location and map positions of 4,539 markers out of the 15,616 were provided by the company according to Li *et al.* (2015). These 4,539 markers were used in the graphical genotyping. The markers were aligned by their genetic positions from top to bottom. I assigned different colors to the markers (genotypes) according to their similarity or dissimilarity to N61 and SHW using Microsoft Excel. The missing data also had a different color. The frequency of SHW alleles in the six MNH lines (S freq.) was calculated for each marker.

1.4 Results

1.4.1 Evaluation of agronomic and physiological traits of MNH lines under field conditions

The analysis of variance indicated that the genotypes effect was significant for all traits except CT-gf, and the sowing date effect was significant for all traits except TKW and NDVI-gf. The genotype and sowing date interaction effect was significant for all traits except GFD and PH (Tables 1-2 to 1-7).

1.4.2 Heading and grain filling duration

All MNH lines except MNH5 headed 8–29 days later than N61 in optimum sowing, and all except MNH4 and MNH5 headed 4–10 days later than N61 in late sowing (Table 1-2). The number of days to heading (DH) of N61 did not differ significantly from those of MNH4 (late sowing) and MNH5 (both conditions). No line headed earlier than N61 in both conditions, probably because initially only late plants (stay green) were selected from the MSD population. The DH values of N61, MNH1, MNH2, and MNH5 were not considerably affected by sowing conditions (Table 1-2). The DH values of MNH3, MNH4, and MNH6 were lower by 5–24 days in late sowing than in optimum sowing, indicating that these lines escape the impact of the high temperature by shortening the DH.

Grain filling duration (GFD) of N61 was not significantly affected by sowing conditions, whereas those of all MNH lines were shorter than that of N61 in both conditions, especially in late sowing (Table 1-2). GFD values of MNH1, MNH2, and MNH6 were significantly lower (by 9–14 days) in late sowing than in optimum sowing; this response is favorable for adaptation to terminal heat stress and would allow seed production even in hot seasons.

1.4.3 Plant height and peduncle length

Plant height (PH) values of MNH3, MNH5, and MNH6 were not affected by sowing conditions (Table 1-3), but PH of MNH1 decreased to 85% and that of MNH2 to 92% in late sowing in comparison with optimum sowing. MNH1 was significantly shorter than N61 in late sowing, whereas MNH2, MNH3, MNH5, and MNH6 were significantly taller in both conditions.

Peduncle length (PL) values of all MNH lines were significantly reduced to 77–90% in late sowing in comparison with optimum sowing (Table 1-3). PL of N61 was also significantly reduced to 73%, whereas the decrease in MNH1 89% was not significant.

1.4.4 Yield and yield components

Kernel number/spike (KSP) of MNH3 was significantly reduced to 71% in late sowing, but those of N61 and all other lines were not affected (Table 1-4). The KSP values of MNH2, MNH3, MNH5, and MNH6 were significantly higher than that of N61 in optimum sowing but not in late sowing.

Thousand kernel weight (TKW) of MNH1 increased to 121% in late sowing, whereas that of MNH6 was reduced to 80% (Table 1-4); TKW of other lines did not differ significantly between conditions.

Biomass (BIO) values of all MNH lines were significantly lower than that of N61 in both conditions, except MNH2 in late sowing (Table 1-5). BIO values of MNH1, MNH4, and MNH6 were significantly smaller in late sowing than in optimum sowing. BIO was increased in MNH2 to 122% and in MNH3 to 123% in late sowing. BIO of N61 and MNH5 was unaffected by sowing conditions.

Grain yield (GY) was slightly and not significantly decreased in N61 but was increased in MNH2 and MNH5 in late sowing (Table 1-5). GY values of other MNH lines were significantly reduced in late sowing. GY values of all MNH lines were lower than that of N61 in both conditions, but the difference was not significant in MNH2 or MNH5.

1.4.5 Canopy temperature, Normalized difference vegetation index and leaf area

Canopy temperature at heading (CT-h) values of N61 and all lines except MNH3 were significantly higher in late sowing than in optimum sowing (Table 1-6). Interestingly, the CT-h of MNH3 was highest among the lines in both conditions. Canopy temperature at grain filling (CT-gf) was highly increased in late sowing in N61 (by 7°C) and MNH5 (by 9°C) but not in the other lines (Table 1-6), suggesting that most of these lines are able to keep the canopy cool until grain filling in late sowing. CT-gf was significantly lower in MNH1 than in N61 in late sowing.

Normalized difference vegetation index at grain filling (NDVI-gf) values was higher in MNH lines than in N61 in both conditions. In late sowing, NDVI-gf it was reduced to 71% in N61 and to 83% in MNH4 and increased to 120% in MNH2 (Table 1-7).

Leaf area (LA) values were significantly decreased by late sowing in N61 and MNH lines except for MNH4 and MNH5, where it was not affected by sowing conditions (Table 1-7).

1.4.6 Evaluation under growth chamber conditions

In growth chamber conditions, photosynthetic rates were significantly reduced by heat stress in N61, ‘Gelenson,' ‘Bacanora,' MNH3, MNH4, and MNH6, but were significantly increased in MNH1, MNH2, and MNH5 (Fig. 1-2A). Stomatal conductance was not affected by heat stress in N61, ‘Bacanora,' and MNH4, but was increased in MNH1, MNH2, and MNH5 (Fig. 1-2B). The results showed that MNH1, MNH2, and MNH5 are more tolerant than N61 and the heat tolerant cultivars Bacanora and Gelenson as they did not show any reduction in photosynthesis due to heat stress and had higher stomatal conductance than the all checks under heat stress. The increase in stomatal conductance suggests that these three lines might have a mechanism for drought tolerance and I need to evaluate them under drought conditions.

1.4.7 Genotyping

Using DArT-seq markers, I identified the parental synthetic lines of each line and found that the six lines originated from different synthetic lines (Table 1-8). Common chromosomal regions were not found among the six MNH lines. However, I noticed that the MNH2 and MNH5 tolerant lines were different from the other MNH lines at some chromosomal regions (Fig. 1-3). MNH2 was a different form at 6A, 5B and 5D whereas MNH5 was different at 1A, 6A, 3B, 4B, 6B, 3D, 4D and 6D. I did not find any common chromosomal region between the two tolerant lines indicating that they acquired different tolerance genes. Most of the putative tolerance regions observed were in A and B sub-genomes indicating that the performance of the SHW lines also depends on the genes of the durum wheat parent and their interaction with the D genome genes from the *Ae. tauschii* parent. The allelic frequency of the alleles derived from the parental synthetic lines in these lines was high on chromosomes 2A (Fig. 1-3). This result indicates that the MNH lines carry many A-genome genes from the synthetic wheat parents.

1.5 Discussion

The wheat ancestor *Ae. tauschii*, which provided the D genome, is a rich source of stress resistance genes (Mujeeb-Kazi and Rajaram *et al*. 2002, Ogbonnaya *et al.* 2013, Sehgal *et al*. 2015). Primary synthetic wheat, an amphidiploid between durum wheat and *Ae. tauschii* has a robust morphology and hard spikes, which causes difficulties in the measurements of agronomic traits. However, SHW produced by crosses between *Triticum durum* with *Ae. tauschii* generally SHW contains a large amount of genetic variation for useful genes or traits of biotic and abiotic stress for bread wheat breeding, but the SHW usually genetically unstable for the numbers of chromosomes after crossing with the common wheat, to solve this problem I cross and backcross SHW with N61. On the other hand, the morphology of the six MNH lines is similar to the morphology of the common wheat in spike traits, and grains shape. Thus, synthetic derivative lines, the offspring of crosses between primary synthetic wheat and cultivated wheat, are a better choice to evaluate the useful traits of the wild species. However, many of the synthetic derivatives originated from a limited number of *Ae. tauschii* accessions. Here, I evaluated the materials selected from the MSD population created by crosses between cultivated wheat and 43 synthetic wheat lines derived from *Ae. tauschii* (Tsujimoto *et al*. 2015).

The study showed the different responses of N61 and MNH lines to high temperature in different traits. These traits are regulated by genes derived from *Ae. tauschii* or LDN. All MNH lines headed later than N61, except MNH5 and earlier in late sowing than in optimum sowing, except, MNH1, MNH2, and MNH5 (Table 1-2). The GFD, of MNH1, MNH2, and MNH6 was shorter in late sowing than in optimum sowing. Plasticity allows plants to adapt to changing conditions. When the temperature is not elevated, plants delay heading to use favorable conditions for grain filling, whereas at high temperature they accelerate heading to escape stress. This adaptation can be used in breeding to improve heat tolerance and achieve high yield. Mondal *et al*. (2015) reported that early maturing genotypes could escape heat stress and use available resources efficiently to produce high grain yield under heat stress. The present study is in good agreement with their results.

All lines except MNH3 had longer peduncles than N61 in late sowing (Table 1-3). Many studies have reported the importance of PL in wheat breeding for heat stress tolerance: peduncle lengthening enhances photosynthesis and grain filling and improves grain yield in many wheat crosses (Chowdhry *et al.* 2001, Ivans *et al.* 1996, Kumar and Ganguli 1993). Large PL may contribute to the stabilization of GFD in MNH4 and MNH5 in late sowing

(Table 1-2).

Amani *et al.* (1996) and Reynolds *et al.* (1998) reported that CT is a good selection criterion to identify heat-tolerant genotypes in wheat breeding. Some of the MNH lines were lower than N61 in the CT from the OS to LS indicating their better ability than N61 in maintaining their CT under heat stress and hence their better adaptation to heat stress. On the other hand, Shahnoza *et al.* (2012) reported that NDVI is an indicator of stay-green traits and heat-tolerant genotypes. NDVI-gf values of all MNH lines were higher than that of N61 in both conditions (Table 1-7), indicating that these lines have genes to stay green during the grain filling and able to perform efficient and stable grain filling, and they are adapted to high temperature.

The MNH1, MNH2, and MNH5 had better photosynthesis under heat stress than N61 and the heat tolerant check cultivars Bacanora and Gelenson in term of no reduction and also net photosynthesis and stomatal conductance under heat stress (Fig. 1-3). On the other hand, unlike N61, MNH2 and MNH5 showed increased BIO and GY in late sowing comparing to the optimum sowing (Table 1-5), indicating that they are more tolerant than N61. However, their GY and BIO values were lower than that of N61 in optimum and late sowings. This result may be attributable to the increase in photosynthetic rate and stomatal conductance under heat stress condition (Fig. 1-2).

In this study, I unexpectedly found that N61 is relatively adaptable to heat stress–conditions when I compared its performance between the control and heat stress conditions. However, this finding need to be validated through intensive evaluation, it is encouraging to evaluate all the Japanese wheat varieties for their heat stress tolerance and adaptation.

Using molecular markers, I found that six MNH lines are genetically different and acquired different genes from their synthetic parents. Therefore, the variation of phenological and physiological traits observed in these lines is attributed to the genes of the different *Ae.*

tauschii accessions used to develop the primary synthetic parents. I identified several chromosome locations unique to the tolerant lines MNH2 (6A, 5B, and 5D) and MNH5 (1A, 6A, 3B, 4B, 6B, 3D, 4D and 6D) and might be related to their heat stress tolerance.

The high allelic frequency of SHW alleles in our material enhanced positively their adaptation to heat stress (Fig. 1-3). Although, I cannot assess the consistency between my data and previously published data on the basis of our graphical maps, this marker information may be used in genome-wide association studies to identify QTLs for heat stress tolerance. In addition, the markers are important for variety registration. At present, cultivar registration requires information on the pedigree. Molecular markers clearly revealed the parental synthetic wheat lines, although the MNH lines originated from a population derived from N61 and 43 different primary synthetic lines (Fig. 1-3). Thus, I have detailed information on the pedigree (Table 8). The phenotypic data highlighted the relative adaptation of N61 to the heat stress conditions. Taking this into consideration in addition to the large contribution of N61 genes in the MNH lines (Fig. 1-3). I can conclude that the performance of the tolerant MNH lines is a result of the N61 and *Ae. tauschii* genes and their interaction.

In conclusion, this study revealed that MNH lines had different responses to heat stress in comparison with N61, producing a long peduncle (all MNH lines except MNH3), increasing the photosynthetic rate and stomatal conductance (MNH1, MNH2, and MNH5), and increasing biomass and grain yield (MNH2 and MNH5). Crosses will be made between MNH2 and MNH5, and a heat stress sensitive line to facilitate identification of the QTLs associated with the tolerance of the two lines. In this study, I found rich genetic diversity in the MSD population, which is available for wheat breeding for heat stress tolerance.

Table 1-1. Phenotypic and physiological traits examined.

Trait	Abbreviation	Method of measurement
Days to heading	DH	Days from sowing to the time when 50% of plants headed
Grain filling duration	GFD	Days from heading to maturity
Plant height	PH	Height (cm) from the soil surface to the top of the spike excluding the awns at physiological maturity
Peduncle length	PL	Distance (cm) from the upper node to the basal node of the spike
Kernel number/spike	KSP	Number of kernels per spike
1000-kernel weight	TKW	Calculated from the weight (g) of 200 randomly sampled grains
Biomass	BIO	Dry weight (g) per plot after removing roots
Grain yield	GY	Grain weight (g) per plot after drying for >10 days
Canopy temperature	CT	Temperature (°C) measured remotely with a hand-held infrared thermometer (Everest Interscience, Tucson, AZ, USA). Measurement started when plots had sufficient ground cover such that the thermometer could be angled with a field of view that did not include any bare soil between rows. Readings were taken in the afternoon (13:00–14:00) 4 or 5 days after irrigation on clear calm days. Measurements were taken at heading stage (CT-h), and during grain filling (CT-gf).
Normalized difference vegetation index	NDVI	Measured using leaves during grain filling with Hand Held optical sensor unit (GreenSeeker), 2002 NTech Industries, Inc., Ukiah, CA, USA). The instrument records the reflectance in the middle for each plot and the distance between the the GreenSeeker and canopy of the plant was kept constant at around 50 cm itself and , and the plot was kept constant at around 50 cm.
Leaf area	LA	Area (cm^2) of five leaves per plot measured using a portable leaf area meter (CI-202; CID Bio-Science, Washington, USA).

Table 1-2. Days to heading (DH) and grain filling duration (GFD) of 'Norin 61' and MNH lines in optimum sowing (OS) and late sowing (LS) and the difference between LS and OS.

	DH			GFD		
Line	OS	LS	LS−OS	OS	LS	LS−OS
Norin 61	60	61	1	47	51	4
MNH1	72**	71**	−1	39**	25**	−14*
MNH2	68**	68**	0	36**	27**	−9*
MNH3	89**	65**	−24**	29**	21**	−8
MNH4	72**	64	−8**	37**	32**	−5
MNH5	63	63	0	40**	36**	−4
MNH6	76**	71**	−5**	32**	19**	−13*
Mean	73	67		35	30	
G	**	**		**	**	
LSD (G)	3.404	3.545		4.94	10.96	
SD		**			**	
G×SD		**			NS	
CV%	2.7	3.1		8.1	21.5	

G and SD denote the genotypes and sowing date, respectively. * and ** next to OS and LS values indicate significant differences from 'Norin 61' at the 0.05 and 0.01 levels of probability, respectively. Asterisks next to LS−OS values indicate significant differences between the LS and OS values.

Table 1-3. Plant height (PH), peduncle length (PL) and the relative performance (RP) of 'Norin 61' and MNH lines in optimum sowing (OS) and late sowing (LS).

	PH (cm)			PL (cm)		
Line	OS	LS	RP (%)	OS	LS	RP (%)
Norin 61	78.3	76.0	97.0	30.0	22.0	73.3
MNH1	74.2	63.0**	85.0	28.0**	25.0**	89.2
MNH2	96.0**	88.0**	92.0	35.0**	27.0**	77.1
MNH3	88.3**	87.0**	99.0	29.2	24.0	82.1
MNH4	81.0	78.0	96.2	35.0	27.0**	77.1
MNH5	95.0**	95.0**	100	34.0	27.0**	79.4
MNH6	87.0**	84.2**	97.0	30.0	27.0**	90.0
Mean	84.1	80.0		30.7	25.5	
G	**	**		**	**	
LSD (G)	4.564	3.485		3.467	2.737	
SD		**		1.650	**	
G×SD		NS			*	
CV%	3.2	5.3		6.6	6.3	

G and SD denote the genotypes and sowing date, respectively. * and ** indicate significant differences from 'Norin 61' at the 0.05 and 0.01 levels of probability, respectively.

Table 1-4. Kernel number/spike (KSP), 1000-kernel weight (TKW) and the relative performance (RP) of 'Norin 61' and MNH lines in optimum sowing (OS) and late sowing (LS).

	KSP			TKW		
Line	OS	LS	RP (%)	OS	LS	RP (%)
Norin 61	47	53	113.0	26.0	25.4	98.0
MNH1	52	49	94.2	29.0	35.0**	121.0
MNH2	54**	59	109.2	32.2*	29.1	90.3
MNH3	56**	40	71.4	26.0	25.0	96.1
MNH4	50	49	98.0	30.0	28.0	93.3
MNH5	57**	54	95.0	27.4	27.2	99.2
MNH6	57**	53	93.0	30.3*	24.3	80.1
Mean	54	52		29.1	27.6	
G	**	**		*	**	
LSD (G)	6.896	6.857		4.272	4.438	
SD		*			NS	
G×SD		**			*	
CV%	7.4	7.7		8.5	9.4	

G and SD denote the genotypes and sowing date, respectively. * and ** indicate significant differences from 'Norin 61' at the 0.05 and 0.01 levels of probability, respectively.

Table 1-5. Biomass (BIO), grain yield (GY) and the relative performance (RP) of ‘Norin 61’ and MNH lines in optimum sowing (OS) and late sowing (LS).

	BIO (g/m^2)			GY (g/m^2)		
Lines	OS	LS	RP (%)	OS	LS	RP (%)
Norin 61	1938	2050	106.0	719	639	89.0
MNH1	1025**	462**	45.0	409**	308**	75.3
MNH2	1800**	2188	122.0	575**	601	105.0
MNH3	1338**	1650**	123.3	307**	211**	69.0
MNH4	1650**	988**	60.0	618**	318**	51.4
MNH5	1688**	1762**	104.3	569**	603	106.0
MNH6	1213**	988**	81.4	461**	258**	56.0
Mean	1522	1361		523	420	
G	**	**		**	**	
LSD (G)	91.28	172.6		53.41	96.69	
SD		*			*	
G×SD		**			**	
CV%	3.7	7.4		6.4	14.1	

G and SD denote the genotypes and sowing date, respectively. * and ** indicate significant differences from ‘Norin 61’ at the 0.05 and 0.01 levels of probability, respectively.

Table 1-6. Canopy temperature at heading (CT-h) and grain filling (CT-gf) of 'Norin 61' and MNH lines in optimum sowing (OS) and late sowing (LS) and the difference between LS and OS.

	CT-h (°C)			CT-gf (°C)		
Lines	OS	LS	LS–OS	OS	LS	LS–OS
Norin 61	16.2	26.0	10.0*	23.0	30.2	7.2**
MNH1	20.1**	28.4**	8.3*	28.0	28.0	0.0
MNH2	17.0	25.1	8.1*	29.0	30.0	1.0
MNH3	27.0**	29.2**	2.2	29.3	31.1	2.0
MNH4	20.3**	25.0	5.0*	28.0	29.4	1.4
MNH5	16.4	24.3	8.0*	22.0	31.0	9.0**
MNH6	21.4**	26.0	5.0*	27.0	28.3	1.3
Mean	19.7	26.2		26.6	29.7	
G	**	**		**	NS	
LSD (G)	2.838	2.742		1.693		
SD		**			**	
G×SD		**			**	
CV%	8.2	6.1		3.8	4.2	

G and SD denote the genotypes and sowing date, respectively. * and ** next to OS and LS values indicate significant differences from 'Norin 61' at the 0.05 and 0.01 levels of probability, respectively. Asterisks next to LS−OS values indicate significant differences between the LS and OS values.

Table 1-7. Normalized difference vegetation index at the grain-filling time (NDVI-gf), leaf area (LA) and the relative performance (RP) of 'Norin 61' and MNH lines in optimum sowing (OS) and late sowing (LS).

	NDVI-gf			Leaf area (LA)		
Lines	OS	LS	RP (%)	OS	LS	RP (%)
Norin 61	0.48	0.34	71.0	83	70	84.3
MNH1	0.69**	0.66**	96.0	94**	65	69.1
MNH2	0.61**	0.73**	120	85	63	74.1
MNH3	0.70**	0.76**	109.0	126**	77	61.1
MNH4	0.66**	0.55**	83.3	77	75	97.4
MNH5	0.58**	0.58**	100	66**	67	102.0
MNH6	0.61**	0.61**	100	78	66	85.0
Mean	0.62	0.60		87	69	
G	**	**		**	**	
LSD (G)	0.628	0.602		7.940	7.475	
SD		NS			**	
G×SD		*			**	
CV%	7.3	1.1		5.3	6.4	

G and SD denote the genotypes and sowing date, respectively. * and ** indicate significant differences from 'Norin 61' at the 0.05 and 0.01 levels of probability, respectively.

Table 1-8. Pedigrees of the six synthetic derivatives wheat lines.

Line	Pedigree
MNH1	Norin 61/*T. durum* cv. Langdon × *Ae. tauschii* KU-2124//*Norin 61
MNH2	Norin 61/*T. durum* cv. Langdon × *Ae. tauschii* KU-2156//*Norin 61
MNH3	Norin 61/*T. durum* cv. Langdon × *Ae. tauschii* KU-2092//*Norin 61
MNH4	Norin 61/*T. durum* cv. Langdon × *Ae. tauschii* AE-929//*Norin 61
MNH5	Norin 61/*T. durum* cv. Langdon × *Ae. tauschii* IG126387//*Norin 61
MNH6	Norin 61/*T. durum* cv. Langdon × *Ae. tauschii* AT55//*Norin 61

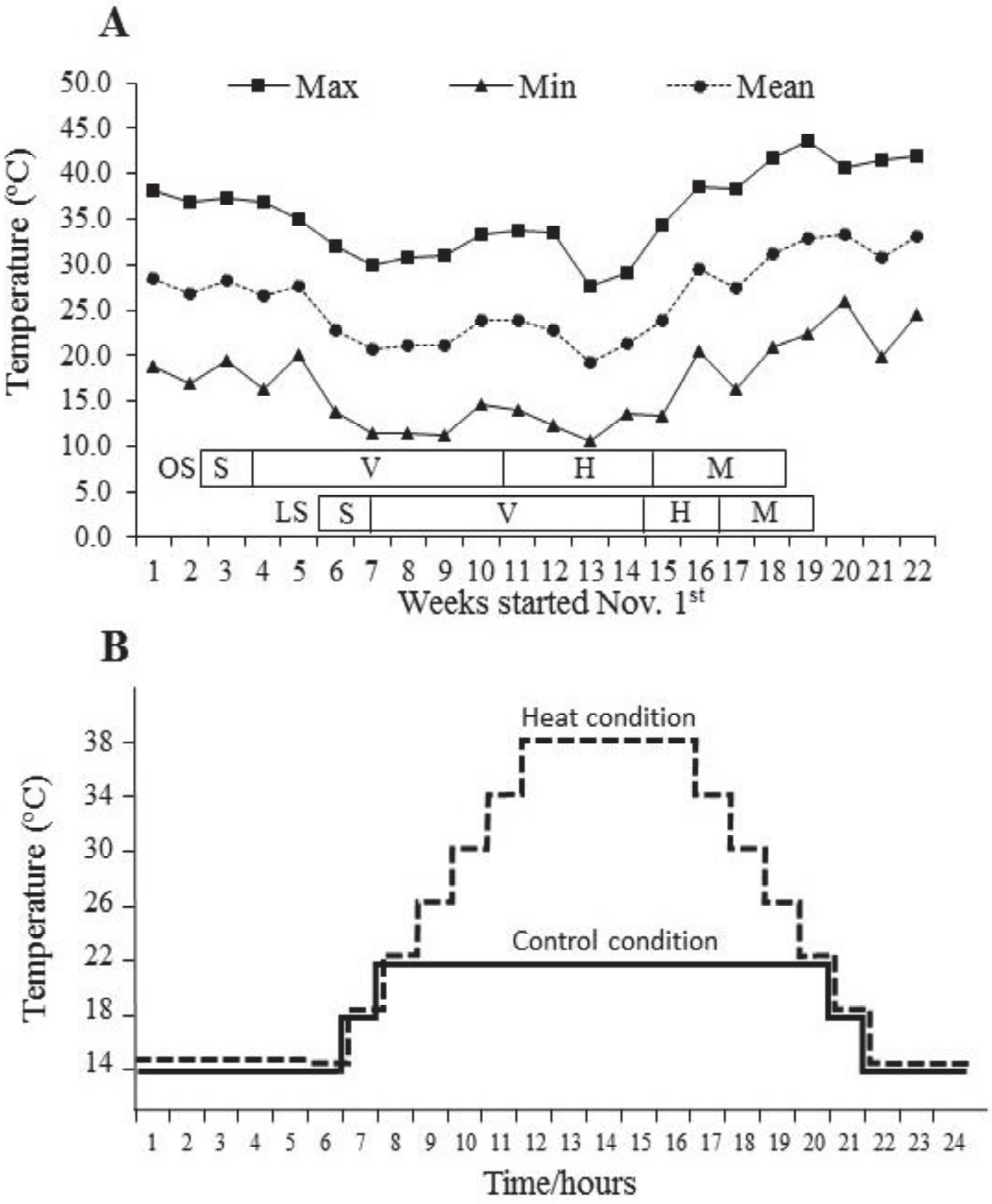

Fig. 1-1. Temperature in the field and growth chamber. A) Maximum, minimum, and mean temperature at Gezira Experimental Farm during the wheat cultivation season, which begins on weeks from Nov. 1. OS, optimum sowing; LS, late sowing; S, sowing time; V, vegetative stage; H, heading stage; M, maturing stage. B). Temperature in the growth chamber under the control and heat-stress conditions.

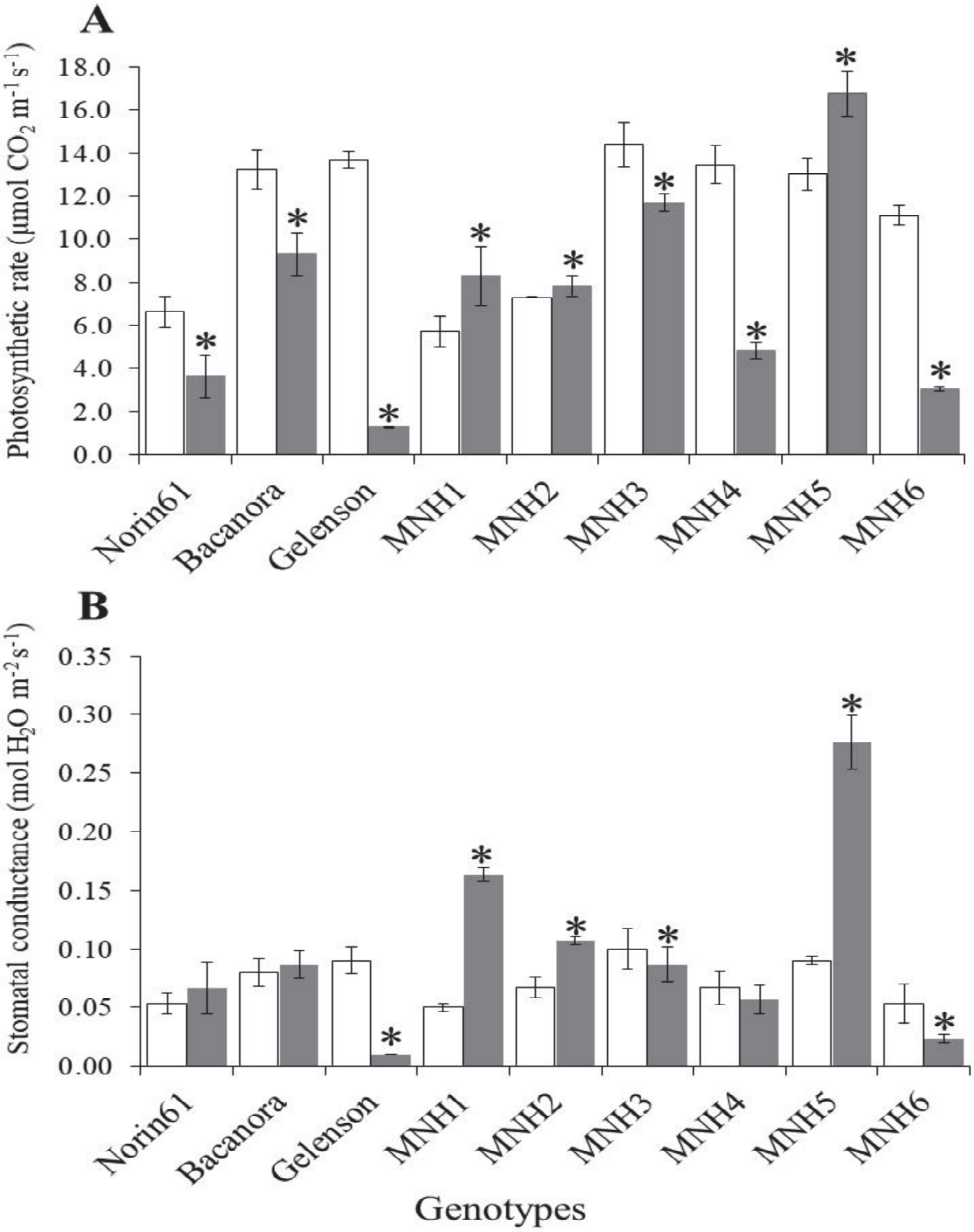

Fig 1-2. Photosynthetic rate (A) and stomatal conductance (B) of the six wheat genotypes and 'Norin 61', 'Gelenson' and 'Bacanora' as heat tolerant lines measured at 21 days in a growth chamber in control (white bars) and heat-stress (gray bars) conditions. The data represent means ±SE (n= 3). Asterisks denote significant differences from control condition to heat stress condition (*P*<0.05, using LSD).

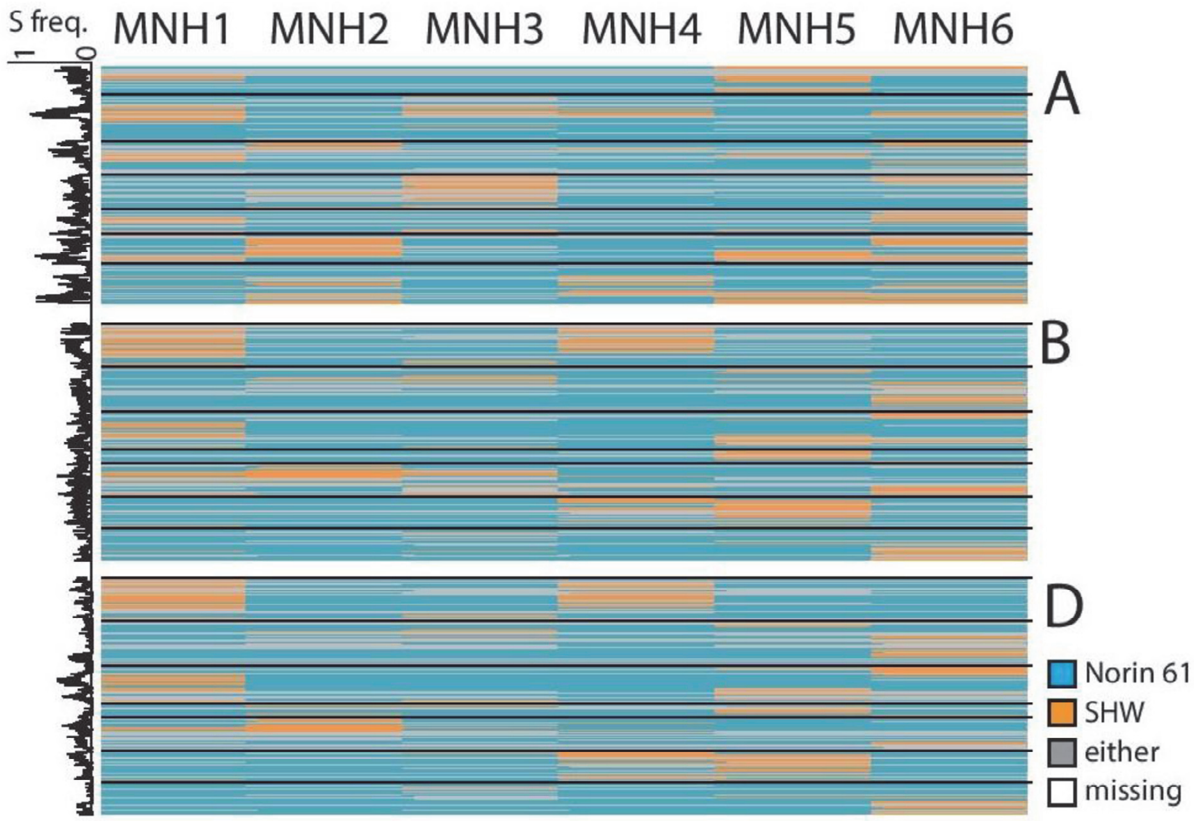

Fig 1-3. Graphical genotyping maps of six MNH lines drawn by using DArT-seq markers. A, B and D on the right of the figure indicate the three wheat genomes. Blue, genotypes identical to those of 'Norin 61'; orange, genotypes identical to those of synthetic wheat; gray, genotypes identical to both Norin 61' and synthetic wheat. S Freq; indicates the effects of genomic regions of synthetic wheat on six MNH lines on each chromosome. The solid black horizontal line denotes the border between chromosomes. The chromosomes arranged from the top (1) to bottom (7) in each sub-genome.

Chapter 2

Genetic variation in heat tolerance-related traits in a population of wheat multiple synthetic derivatives

2.1 Abstract

High temperature (≥30°C) during wheat (*Triticum aestivum* L.) grain filling is a major cause of yield reduction. I studied 400 lines randomly selected from the wheat multiple synthetic derivative (MSD) population constructed from *Triticum aestivum*, *Triticum turgidum* subsp. durum and *Aegilops tauschii* to examine the genetic variability of heat stress–adaptive traits and to identify new sources of heat tolerance to be used in wheat breeding programs. The experiment was arranged in an augmented randomized complete block design in four environments in Sudan. A wide range of genetic variability was found in most of the traits in all environments. For all traits examined, I found MSD lines that showed better performance than their parent 'Norin 61' and two adapted Sudanese cultivars. Using the heat tolerance efficiency, I identified 13 highly heat-tolerant lines and several lines with intermediate heat tolerance and good yield potential. I also identified lines with alleles that can be used to increase wheat yield potential. The study revealed that the use of the MSD population could be the best way to explore the genetic variation in *Ae. tauschii* for wheat breeding and improvement.

2.2 Introduction

High temperature is an important constraint to bread wheat productivity in tropical and subtropical environments; it causes morphological and physiological changes at all stages and results in considerable yield losses (Al-khatib and Paulsen 1990, Tahir and Nakata 2005, Tahir *et al*. 2006, Tewolde *et al.* 2006). Wheat is adapted to perform well at 18−24°C. High temperatures during flowering decrease grain number, and temperatures above 34°C after anthesis lead to reduce yield potential (Asseng *et al.* 2011, Ferris *et al.* 1998, Lobel *et al.* 2012). Asseng *et al.* (2014) reported that an increase of 1°C reduces grain yield by 6%. Physiological parameters such as chlorophyll content, canopy temperature, and normalized difference vegetation index are widely used as indicators of wheat heat tolerance (Al-khatib and Paulsen 1990, Amani *et al.* 1996, Hazratkulova *et al.* 2012).

Although the genetic variation for heat stress tolerance breeding is limited in elite wheat germplasms, considerable genetic variation is present in wild relatives (Li *et al.* 2014). *Ae. tauschii* (2n=2x=14, DD) and durum wheat (*Triticum turgidum* subsp. *durum*, 2n=4x=28, AABB), both ancestors of bread wheat (*T. aestivum* L., 2n=6x=42, AABBDD), are secondary gene pools useful for improving the resistance or tolerance of bread wheat to biotic and abiotic stresses. Synthetic hexaploid wheat (SHW) is usually produced by crossing durum wheat and *Ae. tauschii* to enable the use of these genetic resources. Sharma *et al.* (2014) found four highly heat-tolerant lines among 24 SHW lines. A synthetic wheat derivative from the International Maize and Wheat Improvement Center (CIMMYT) with early maturity, a short grain filling period, and good grain quality was found to be suitable for late planting in southern Spain and to produce a higher yield than commercial varieties. In 2003, this derivative was registered as a new cultivar, ‘Carmona’ (Kazi and Van Gikel 2004). Another new SHW line, ‘Super wheat’, developed at the National Institute of Agricultural Botany, Cambridge University, could increase wheat productivity up to 30%

(http://www.niab.com/news_and_events/article/281). Furthermore, SHWs are tolerant to high temperatures (35−40°C) during the grain filling stage (Van Ginkel and Ogbonnaya 2007).

Despite these developments, only a limited number of *Ae. tauschii* lines, and therefore only a small portion of the high diversity of this species, are used in breeding programs. In other words, only small or limited portion of the huge diversity of *Ae. tauschii* has been utilized. For the breeding program to overcome this problem and to ensure proper and extensive the utilization of this tremendous diversity, a multiple synthetic derivatives (MSD) has been developed (Tsujimoto *et al.* 2015, Gorafi *et al.* unpublished). This population was developed by crossing and backcrossing the Japanese wheat cultivar 'Norin 61' to 43 SHW lines produced by crossing the durum wheat cultivar 'Langdon' and 43 different *Ae. tauschii* accessions (Matsuoka and Nasuda 2004). In 2013, I grew 1000 plants from the MSD population under heat stress in Sudan, out of which six plants were selected visually as heat tolerant, and two of them were then confirmed to be heat tolerant (Elbashir *et al.* 2017).

In this study, to identify new sources for the development of heat-tolerant high-yielding wheat cultivars, I randomly selected 400 BC_1F_4 plants from the MSD population, and evaluated them in four environments in Sudan. I detected considerable genetic variation for heat stress tolerance–related traits and identified several heat-tolerant lines suitable for use in breeding programs.

2.3 Materials and Methods

2.3.1 Plant materials

In this study, I used 400 BC_1F_4 lines randomly selected from the MSD population, their backcross parent 'Norin 61' (hereafter N61) as a control to show the effect of the wild genes on the improvement of N61 heat stress adaptability, and the two heat-tolerant Sudanese cultivars 'Goumria' and 'Imam' as checks to evaluate the heat stress adaptability of the MSD lines. The 400 MSD lines were selected in 2014/2015 at the Arid Land Research Center, Tottori University, Japan (35°32′N, 134°13′E, 6 masl).

2.3.2 Experimental sites, design and field management

I evaluated the plants in four agro-ecological environments at three sites in Sudan (Dongola, Hudeiba, and Wad Medani) in 2015/2016. Dongola Research Farm (DON) is located in Northern State (19°08′N, 30°27′E, 239 masl) and has a high-terrace soil (pH 8) with low organic matter content (<5%). The texture is sandy clay loam at 0–30 cm and silty clay loam at 30–60 cm. Hudeiba Research Farm (HUD) is located in River Nile State (14°40′N, 33°50′E, 409 masl) and has a middle-terrace soil (Karu; pH 8). Wad Medani (MED) is located in the central clay plain of Gezira State (14°24′N, 29°33′E, 407 masl) and has a heavy clay soil (pH 8.0–8.4) with low organic matter content (<5%) and low levels of nitrogen (380 ppm) and phosphorus. I expected the temperatures to be low or optimum for wheat cultivation at DON and high at MED. At all three sites, I used an augmented randomized complete block design. At DON and HUD, seeds were sown in the 4th week of November. At MED, seeds were sown in the 4th week of November (optimum sowing; MED/OS) and in the 2nd week of December (late sowing; MED/LS) to ensure plant exposure to high temperatures at the grain filling stage.

Before sowing, seeds were treated with the insecticide Gaucho (imidacloprid, 35% WP, Bayer Crop Science, USA) at 1 g kg^{-1} seed, mainly to control termites and aphids. Seeds were sown at 120 kg ha^{-1} manually. Each line was planted in a plot of four rows, 0.5 m long and 0.2 m apart. Phosphorus was applied as superphosphate by furrow placement before sowing at 18.8 kg P ha^{-1}. Nitrogen was split-applied as urea at the three-leaf stage (second irrigation) and the tillering stage (fourth irrigation) at 86 kg N ha^{-1}. Irrigation was applied every 10–12 days. The fields were hand-weeded at least twice.

2.3.3 Evaluated traits

The following morphological and physiological traits were evaluated: days to heading (DH), days to maturity (DM), grain filling duration (GFD), plant height (PH), peduncle length (PL), biomass (BIO), grain yield (GY), harvest index (HI), kernel number per spike (KS), thousand-kernel weight (TKW), and grain number per m^2 (Gm^{-2}). At the grain filling stage, I measured the following physiological parameters: canopy temperature (CT), chlorophyll content (SPAD), and normalized difference vegetation index (NDVI). The measurements were performed according to Pask *et al.* (2012). Abbreviations used for phenotypic and physiological traits and methods of measurement are listed in Table 2-1. The heat tolerance efficiency (HTE) of each line was calculated as HTE = 100*Ysi/Ypi (Fischer and Wood 1981), where Ysi is GY under high temperature and Ypi is GY under low temperature. The first HTE calculation was performed using the GY values at DON (considered a cool environment) and at MED/LS (hot environment); the second HTE calculation was performed using the GY values at MED/OS (cool environment) and MED/LS (hot environment).

2.3.4 Statistical analysis

Analysis of variance was carried out using Plant Breeding Tools software (PBTools, version 1.4. International Rice Research Institute, Los Baños, The Philippines; http://bbi.irri.org/products). Simple correlation coefficients between GY and other traits were calculated for all genotypes in each environment. To identify heat-tolerant lines, high-yielding lines, and heat-tolerant high-yielding lines, I performed regression analysis in two ways: first, by using the GY data at DON as the optimum condition and the HTE data calculated using DON vs. MED/LS; second, by using the GY data at MED/OS as the optimum condition and the HTE data calculated using MED/OS and MED/LS. I calculated the heritability in PBTools considering genotype as a random effect. The heritability was classified as low (0–0.30), moderate (>0.30–0.60), or high (>0.60) (Allard 1960).

2.4 Results

2.4.1 Temperature at three experimental sites

The weekly maximum and minimum temperatures during the cropping season in the four environments are shown in Figure 1. DON was the coolest environment during sowing, vegetative growth, and heading (up to the 12th week) and after the 16th week (late maturity), whereas MED/OS and MED/LS were the hottest during these time periods. HUD was the hottest and MED/OS and MED/LS were the coolest environments between the 12th week (late heading) and the 16th week (early maturity). Overall, I considered DON and MED/OS as cool environments and HUD and MED/LS as hot environments.

2.4.2 Performance of N61, the genetic background of MSD lines, under Sudanese conditions

First, investigated the adaptability of N61 in comparison with the two Sudanese cultivars adapted to high temperature. The DH values of N61 were similar to those of the Sudanese cultivars at DON but were significantly lower at HUD and MED/LS (Fig. 2-2A and Table 2-5). The DM values were similar between N61 and the Sudanese cultivars in all environments (Fig. 2-2B and Table 2-5). N61 was significantly taller in PH than 'Goumria' at MED/LS, and its PL was similar between all the environments (Fig. 2-3 and Table 2-5). TKW of N61 was significantly lower than that of 'Imam' at HUD (Fig. 2-5B). All other parameters measured were similar between N61 and the Sudanese cultivars in all environments (Table 2-5). All these data indicate that N61 is adapted to heat stress.

2.4.3 Days to heading and maturity and grain filling duration in MSD lines

MSD lines showed high variability in DH, DM, and GFD, and 8% of the 400 lines at DON, 5% at MED/OS, 39% at HUD, and 38% at MED/LS did not head (Fig. 2-2). Among these lines, 16 were common between the cool environments DON and MED/OS, 110 between the hot environments HUD and MED/LS, and 16 between all environments.

The DH values were 62–104 days at DON, 53–115 at MED/OS, 56–96 at HUD and 50–75 at MED/LS (Table 2-1). The mean DH value was lowest at MED/LS followed by HUD, MED/OS, and DON. This result reflected the differences in temperature (cool at DON to hot at MED/LS; Fig. 2-1). I observed the same trend for DM. The DM values were lowest at MED/LS and highest at DON, whereas the GFD values were highest at HUD and lowest at MED/LS (Table 2-1).

The variation in DH, DM, and GFD was greater in the MSD lines than in N61, and the two adapted Sudanese cultivars (Fig. 2-2 and Table 2-5). In comparison with N61, the DH values were significantly lower in 0.3% of the MSD lines at DON, 5% at MED/OS, 3% at HUD, and 4% at MED/LS, and were significantly higher in 40% of the MSD lines at DON, 36% at MED/OS, 55% at HUD, and 15% at MED/LS (Fig. 2-2A). In comparison with N61, the DM values were significantly lower in 1% of the MSD lines at DON and MED/OS, and in 5% at HUD, and significantly higher in 35% of the MSD lines at DON, 27% at MED/OS, and 34% at HUD (Fig.2-2B). In comparison with N61 and the two Sudanese cultivars, the GFD values were significantly higher in 5% of the MSD lines at MED/OS and in 11% at HUD, and significantly lower in 8% at MED/OS and in 19% at HUD (Fig. 2-2C).

The heritability of DH (0.87), DM (0.74), and GFD (0.76) was high across the four environments (Table 2-1). The genotype effect (G) was significant for the three traits in all environments except for DM at MED/LS and GFD at DON and MED/LS (Table 2-6). The environment effect (E) and the G × E effect were significant for the three traits.

2.4.4 Plant height and peduncle length

The PH values were 47–136 cm at DON, 65–113 at MED/OS, 55–110 at HUD, and 56–106 at MED/LS (Table 2-1). The mean PH values were highest at DON followed by

MED/OS, MED/LS and HUD.

The variation in PH and PL was greater in the MSD lines than in N61 and the two adapted Sudanese cultivars. In comparison with N61, the PH values were significantly higher in 14% of the MSD lines at DON, 28% at MED/OS, 41% at HUD, and 36% at MED/LS, and were significantly lower in 2% of the MSD lines at DON, 0.3% at MED/OS and 1% at MED/LS (Fig. 2-3A). In comparison with N61, the PL values were significantly higher in 8% of the MSD lines at DON, 29% at MED/OS, 18% at HUD, and 23% at MED/LS, and were significantly lower in 5% of the MSD lines at DON, 2% at MED/OS, and 6% at MED/LS (Fig. 2-3B). The heritability of PH (0.71) and PL (0.76) was high (Table 2-1). The G, E, and G × E effects were significant for both traits (Table 2-6).

2.4.5 Canopy temperature, chlorophyll content, and normalized difference vegetation index

The CT values were 15–31°C at DON, 13–31°C at MED/OS, 25−36°C at HUD, and 21−37°C at MED/LS (Table 2-1). The mean CT values were lowest at MED/OS followed by DON, MED/LS, and HUD. The SPAD values were 30–54 at DON, 27−68 at MED/OS, 33−58 at HUD, and 38−60 at MED/LS (Table 2-1). The mean SPAD values were highest at HUD followed by MED/OS, MED/LS, and DON. The NDVI values were higher at MED/LS than at MED/OS. The variation in these three traits was higher in the MSD lines than in N61, ‘Goumria,' and ‘Imam’ (Table 2-1 and Table 2-5). In comparison with N61, the CT values were significantly lower in 10% of the MSD lines at HUD and were significantly higher in 23% at MED/OS and in 8% at HUD (Fig. 4A). At HUD, the MSD lines that had lower CT values than N61 also had lower CT values than the Sudanese cultivars. In comparison with N61, the SPAD values were significantly higher in 7% of the MSD lines at DON, 3% at MED/OS, and 2% at MED/LS, and were significantly lower in 3% of the MSD lines at DON, 10% at MED/LS, and 7% at MED/OS and HUD (Fig. 2-4B). In comparison with N61, the NDVI values were significantly higher in 30% of the MSD lines at MED/OS and in 6% at

MED/LS, and were significantly lower in 5% of the MSD lines at MED/OS and MED/LS (Fig. 4C). Comparing to the adapted Sudanese cultivars, the MSD lines that had cooler canopy than N61 also had cooler canopy than the adapted Sudanese cultivars at HUD. The heritability of SPAD (0.65) and CT (0.70) was high, whereas the heritability of NDVI (0.24) was low (Table 2-1). The G effect was significant for SPAD in all environments, for CT at MED/OS and HUD, and for NDVI at MED/OS and MED/LS (Table 2-6). The E and G × E effects were significant for the three traits.

2.4.6 Kernel number per spike, thousand-kernel weight, and grain number per m^2

The KS values were 10–30 at DON, 10–65 at MED/OS, 15–55 at HUD, and 10–65 at MED/LS (Table 2-1). The mean KS value was highest at MED/OS followed by those at MED/LS, HUD, and DON. In comparison with N61, KS values were higher in 2% of the MSD lines at DON and significantly lower in 14% (DON), 27% (MED/OS), 5% (HUD), and 26% (MED/LS) (Fig. 2-5A).

The TKW values were 15–55 g at DON, 10–55 at MED/OS, 15–40 at HUD, and 10–40 at MED/LS (Table 2-1). The mean TKW value was highest at DON followed by MED/OS, HUD and MED/LS. In comparison with N61, TKW values were significantly higher in 6% (DON) and 15% (HUD) of the MSD lines, and were significantly lower in 16% (DON) and 6% (HUD) (Fig. 2-5B). These results indicate the impact of the genes from the different synthetic wheat in these lines. The MSD lines that were higher in TKW than N61 were also higher than the adapted Sudanese cultivars. The heritability of KS was high (0.81) and TKW (0.88) were high (Table 2-1).

The Gm^{-2} values were 2838–23268 at DON, 2250–48000 at MED/OS, 2704–23874 at HUD, and 2400–35200 at MED/LS (Table 2-1). The mean Gm^{-2} value was highest at MED/OS, followed by those at MED/LS, HUD, and DON. In comparison with N61, Gm^{-2} values were significantly higher in 3% (MED/OS), 6% (HUD), and 5% (MED/LS) of the

MSD lines, and were significantly lower in 42% (MED/OS), 12% (HUD), and 25% (MED/LS) (Fig. 2-5C). The MSD lines that had higher values of all three traits than N61 also had higher values than the Sudanese cultivars. The heritability of all three traits was high: 0.81 for KS, 0.88 for TKW, and 0.68 for Gm^{-2} (Table 2-1).

The G effect was significant for the three traits in all environments except for TKW at MED/OS and MED/LS and Gm^{-2} at DON (Table 2-6). The E and G × E effects were significant for the three traits.

2.4.7 Grain yield, biomass, and harvest index

The GY values were 41–1172 g m^{-2} at DON, 13–1053 g m^{-2} at MED/OS, 18–950 g m^{-2} at HUD, and 8–891 g m^{-2} at MED/LS (Table 2-1). The mean GY value was highest at DON followed by MED/OS, HUD, and MED/LS. This result was consistent with the low temperature at DON and high temperature at MED/LS (Fig. 2-1). The mean BIO value was greatest at DON and lowest at HUD. The mean HI value was highest at HUD followed by DON, MED/OS and MED/LS (Table 2-1). The lowest BIO at HUD and lowest HI at MED/LS can be attributed to their high temperatures compared to those of DON and MED/OS (Table 2-1 and Fig. 2-1).

In comparison with N61, GY values were significantly higher in 10% of the MSD lines (28 lines) at HUD, and were significantly lower in 38% at MED/OS and 7% at HUD (Fig. 2-6A). In comparison with N61, BIO values were significantly higher in 3% of the MSD lines at MED/OS and 10% at HUD, and were significantly lower in 4% at MED/OS and 8% at HUD (Fig. 2-6B). In comparison with N61, HI values were significantly lower in 0.6% of the MSD lines at MED/OS and in 5% at HUD (Fig. 2-6C).

In comparison with ‘Goumria’ and ‘Imam’, GY values were significantly higher in 3% of the MSD lines at HUD; BIO values were significantly higher in 3% of the MSD lines at MED/OS and in 4% at HUD; and HI values were significantly higher in 2% of the MSD lines at MED/OS and in 3% at HUD. The heritability of GY (0.88) and HI (0.89) was high (Table 2-1) and that of BIO (0.60) was moderate. The G effect was significant for the three traits at HUD and MED/OS (Table 2-6). The E and G × E effects were significant for the three traits.

2.4.8 Heat tolerance efficiency

The HTE values calculated from GY at DON and MED/LS were 14–151% (first calculation; N61, 90%) and 15–173% when calculated from GY at MED/OS and MED/LS (second calculation; N61, 85%) (Table 2-3). In comparison with N61, HTE was higher in 38 MSD lines (17%) in the first calculation (range, 93–151%; Fig. 2-7A) and in 56 MSD lines (24%) in the second calculation (range, 88–173%; Fig. 7B). I considered the lines with HTE above 100% as highly heat tolerant and found 13 lines (Table 2-7) consistent between both calculations.

To classify the MSD lines according to their GY and HTE, I performed a regression analysis of the relationship between HTE from the first calculation and GY at DON (cool environment), and between HTE from the second calculation and GY at MED/OS (cool environment). In both analyses, no MSD lines showed higher GY with HTE than those of N61 (Fig. 2-7). Higher HTE values and lower yield than N61 were found in 17% of the MSD lines in the first analysis (Fig. 2-7A) and in 24% in the second analysis (Fig. 2-7B). These MSD lines also had higher HTE than the Sudanese cultivars in both analyses.

2.4.9 Association of grain yield with morphological, phenological, and physiological traits

The correlation of GY with some heat tolerance–related traits among the MSD lines is shown in Table 2-2. GY was negatively correlated with DH in all environments. GY was negatively correlated with DM and positively correlated with GFD in all environments except for MED/LS, where correlations between GY and these two traits were not significant. PL was positively correlated with GY at MED/OS and MED/LS, was negatively correlated at

DON, and did not correlate with GY at HUD.

BIO, HI, KS, TKW, and Gm^{-2} were positively correlated with GY in all environments, except that TKW did not correlate with GY at DON. At MED/OS, CT was negatively correlated with GY, whereas SPAD was positively correlated. NDVI was positively correlated with GY at MED/OS and weakly negatively correlated at MED/LS. HTE showed a negative correlation with GY of DON and MED/OS.

2.5 Discussion

In this study, I used a unique population of diverse lines derived from 43 *Ae. tauschii* accessions in a hexaploid wheat background (N61). I evaluated this population in four different environments with N61 and two adapted Sudanese cultivars, and found considerable genetic variation in all traits examined (Table 2-1). All traits except NDVI had moderate to high heritability, indicating that the selection for all these traits within the MSD population will be effective.

The responses of DH and DM depended on the differences in temperature and environments (Table 2-1 and Fig. 2-2A and 2-2B); the average DH values were higher in the cool environments (DON and MED/OS) than in the hot environments (HUD and MED/LS) (Table 2-1). The GFD values were higher in some lines than in N61 and the Sudanese cultivars, and lower in some other lines (Table 2-1, Table 2-5 and Fig. 2-2C). These results indicate the ability of the MSD lines to adapt to heat stress by changing life-cycle length depending on the temperature. In each environment, one group of lines headed and matured earlier than N61, another group headed and matured later than N61, and the third group was intermediate. This grouping is very important for wheat breeding under heat stress because of the temperature fluctuations during the crop cycle. These lines might also be suitable for drought tolerance breeding because of the fluctuation of the timing of rain between different locations and years. The variation in DH allows wheat genotypes to optimize their use of resources (Laurie 1997). Early-maturing genotypes are preferable for Sudanese environments, where grain yield is limited by high temperatures and the wheat cycle is short (Reynolds *et al.* 1994, Lopes *et al.* 2012). Most of the MSD lines headed later than N61 and some lines were unable to head (winter growth habit) in any of the four environments (Table 2-1, Fig. 2-2A). Most of the 43 SHW parental lines did not head at MED. Thus, I attributed late heading and the winter growth habit of the MSD lines to the vernalization requirement inherited from their SHW parents (unpublished data). The number of the MSD lines that did not flower was highest at HUD and MED/LS, whereas the number of the MSD lines that headed was highest in MED/OS, likely because the minimum temperatures from the mid-vegetative growth stage to the beginning of heading (middle of the 6th week – 8th week) at MED/OS were lower than those at HUD and MED/LS at late vegetative growth and beginning of heading. The low night temperature at MED/OS might be suitable for wheat to become flowering competent.

Blum (1998) reported that PL plays a role in the storage of water-soluble carbohydrates under stress when GFD is short. Therefore, longer peduncles are preferable under heat stress to support grain filling. In this study, PL was correlated with GY (Table 2-2). The MSD lines exhibited wide variation in PL (Table 2-1), and PL values were higher in some lines than in N61 and the Sudanese cultivars. These lines are promising in programs aimed to improve GY under heat stress.

Gm^{-2} greatly contributes to high yield in heat stress environments (Fischer 2008). Selection for BIO and HI is an efficient method for selecting high-yielding genotypes under heat stress (He and Rajaram 1994, Tahir and Nakata 2005). In this study, KS, TKW, Gm^{-2}, BIO, and HI were correlated with GY, and their values were higher in a number of MSD lines than in N61 and the Sudanese cultivars in each environment (Fig. 2-5 and Fig. 2-6). These lines could serve as an excellent resource to improve these traits and hence GY under heat stress conditions.

CT, SPAD, and NDVI varied widely among the MSD lines, and their values were better in some lines than in N61 (Fig. 2-4). Low CT, high SPAD, and high NDVI are reliable indicators of heat-tolerant and high-yielding genotypes in wheat (Olivares-Villegas *et al.* 2007, Lopes *et al.* 2012, Shahnoza *et al.* 2012). However, in this study, I found a weak correlation between these traits and GY (Table 2-2). These traits are highly affected by the phenology and PH (Lopes *et al.* 2012). This population exhibited wide variation in heading and maturity (Table 2-1), and therefore I attribute the weak correlation to the inconsistency in the time of measurements for different plants.

I found 29 MSD lines with significantly higher GY than that of N61 at HUD and a number of other MSD lines that tended to have higher GY than N61, indicating a high frequency of favorable alleles for GY in the population. These lines can be used in breeding programs to increase yield potential without major concerns about the presence of alleles with negative effects. The 29 high-yielding MSD lines responded differently to heat stress, and each of them had different characteristics that increased productivity (Table 2-8). For example, line MSD215 headed early, and some other lines had lower CT or higher PL, KS, TKW, Gm^{-2}, BIO, or HI values.

Using HTE, I was able to estimate the heat tolerance of each line. In 13 lines, HTE values were consistently higher than in N61 (Table 2-7). The negative correlation between GY and HTE indicates that the heat tolerance is associated with lower yielding ability, which is expected in this BC_1 population, as the lines are not expected to perform as well as N61. The HTE of some lines was intermediate between those of N61 and highly tolerant lines with good yield potential (Fig. 2-7); these lines are a good source to improve both GY and heat tolerance. The characteristics of highly tolerant lines were similar to those of N61, but with low GY (Table 2-7). More detailed physiological analysis is needed to explain the heat tolerance of these lines. In comparison with N61, some of these lines showed insignificantly lower CT and higher PL, KS, TKW, and BIO values (Table 2-7). Some of them had significantly higher CT than N61, indicating that their mechanism of tolerance differs from CT reduction through high transpiration, which would make these lines suitable for cultivation under drought. The pedigree of these lines (Table 2-6) indicated that 9 of the 43 SHW lines contributed to the tolerance.

GY could be boosted by combining traits conferring agronomic and physiological tolerance under heat stress in the same cultivar. Reynolds *et al.* (2009) reported that "strategic trait-based crossing" results in progeny that has superior yield and yield-related physiological traits in comparison with conventionally bred advanced lines. Strategic trait-based crossing requires characterization of large sets of germplasm to find lines with desirable traits; such germplasm can be crossed to introduce positive alleles for each trait into new genetic backgrounds (Lopes *et al.* 2012).

In conclusion, this study revealed several MSD lines had significantly associated with adaptation to heat stress. These lines are an excellent source of alleles to improve wheat adaptation and tolerance to heat stress through the strategic trait-based crossing. In this study, only a small number of lines excelled in several traits studied in comparison with N61. These results indicate that this approach is very effective and could be the best way to explore and use the variation in heat tolerance in *Ae. tauschii* in a practical, fast, and economical way. I performed genomic analysis of these 400 MSD lines to conduct a genome-wide association study and identify the quantitative trait loci (QTLs) associated with each trait; this information would facilitate the introgression of these QTLs through marker-assisted selection.

Table 2-1. Phenotypic and physiological traits examined.

Trait	Abbreviation	Method of measurement
Days to heading	DH	Days from sowing to the time when 50% of plants headed.
Days to maturity	DM	Days from sowing to physiological maturity (loss of green color from the glumes).
Grain filling duration	GFD	Days from heading to maturity.
Plant height	PH	Height (cm) from the soil surface to the top of the spike excluding the awns at physiological maturity.
Peduncle length	PL	Distance (cm) from the upper node to the basal node of the spike
Kernel number/spike	KS	Counted after reaching physiological maturity, measured by counting the number of seeds after threshing each spike individually by hand.
1000-kernel weight	TKW	Calculated from the weight (g) of 200 randomly sampled grains.
Grains number/m^2	Gm^{-2}	Calculated from number of kernel/spike×number of tillers/m^2.
Biomass	BIO	Dry weight (g) per plot after removing roots.
Grain yield	GY	Grain weight (g) per plot after drying for >10 days.
Harvest index	HI	Calculated from grain yield/biomass.
Canopy temperature at grain filling	CT	Temperature (°C) measured remotely with a hand-held infrared thermometer (Everest Interscience, Tucson, AZ, USA). Measurement started when plots had sufficient ground cover such that the thermometer could be angled with a field of view that did not include any bare soil between rows readings were taken in the afternoon (13:00–14:00) 4 or 5 days after irrigation on clear calm days Measurements were taken at during grain filling.
Normalized difference vegetation index at grain filling	NDVI	Measured using leaves during grain filling with Hand Held optical sensor unit (GreenSeeker), 2002 NTech Industries, Inc., Ukiah, CA, USA). The instrument records the reflectance in the middle for each plot and the distance between the the GreenSeeker and canopy of the plant was kept constant at around 50 cm itself and , and the plot was kept constant at around 50 cm.
Chlorophyll content at grain filling	SPAD	The total amount of graeen pigment in the flag leaf during the mid-grain filling stage, estimated in SPAD units using a chlorophyll meter (SPAD-502, Minolta, Japan).

Table 2-2. Morphological, phenological, physiological, yield, and yield component traits of the 400 MSD lines and their parent ‘Norin 61’ (N61) evaluated for heat stress tolerance at Dongola (DON), Hudeiba (HUD), Wad Medani with optimum sowing (MED/OS), and Wad Medani with late sowing (MED/LS) in Sudan.

Trait	DON			MED/OS			HUD			MED/LS			Heritability
	Range	Mean	N61	Range	Mean	N61	Range	Mean	N61	Range	Mean	N61	
DH	62−104	79	72	53−115	72	64	56−96	66	61	50−75	63	62	0.87
DM	99−135	110	106	88−135	108	101	94−125	107	103	85−111	97	92	0.74
GFD	10−52	32	33	11−61	36	37	19−61	40	42	20−45	33	30	0.76
PH	47−136	102	97	65−113	91	84	55−110	80	69	56−106	84	79	0.71
PL	20−53	35	34	13−57	32	82	8−32	15	13	14−39	28	26	0.76
CT	15−31	23	32	13−31	20	17	25−36	30	30	21−37	26	25	0.65
SPAD	30−54	44	43	27−68	49	50	33−58	51	51	38−60	49	50	0.70
NDVI	0.50−0.80	0.68	0.71	0.27−0.84	0.62	0.55	0.40−0.58	0.65	0.63	0.54−0.89	0.69	0.69	0.24
KS	10−30	20	23	10−65	46	56	15−55	41	45	10−65	42	54	0.81
TKW	15−55	32	33	10−55	30	30	15−40	29	27	10−40	27	26	0.88
Gm^{-2}	2838−23268	9142	10965	2250-48000	21330	27181	2704−23874	9269	10357	2400−35200	15726	21283	0.68
GY	41 −1172	637	784	13−1053	501	823	18−950	421	383	8−891	415	703	0.88
BIO	500−4850	23 98	2366	300−3975	2006	2038	99−2326	1284	1279	310−3400	1833	1089	0.60
HI	2−63	27	33	1−66	25	40	6−60	33	31	2−61	23	39	0.89

DH, days to heading; DM, days to maturity; GFD, grain filling duration (days); PH, plant height (cm); PL, peduncle length (cm); CT, canopy temperature (°C); SPAD, chlorophyll content (SPAD units); NDVI, normalized difference vegetation index; KS, kernel number per spike; TKW, thousand-kernel weight (g); Gm^{-2}, grain number per m^2; GY, grain yield ($g\ m^{-2}$), BIO, biomass ($g\ m^{-2}$); HI, harvest index (%).

Table 2-3. Correlation coefficients between grain yield and the phenological, morphological, physiological, and yield component traits of the 400 MSD lines at Wad Medani with optimum sowing (MED/OS), Wad Medani with late sowing (MED/LS), Dongola (DON), and Hudeiba (HUD).

Trait	MED/OS	MED/LS	DON	HUD
DH	−0.52***	−0.33***	−0.17***	−0.29***
DM	−0.50**	-0.10^{ns}	−0.15***	−0.22***
GFD	0.27***	-0.01^{ns}	0.17**	0.13*
PH	0.26***	0.04^{ns}	−0.17**	-0.09^{ns}
PL	0.32***	0.13*	−0.13*	-0.03^{ns}
CT	−0.11*	-0.07^{ns}	0.006^{ns}	-0.10^{ns}
SPAD	0.14*	0.11^{ns}	-0.05^{ns}	0.03^{ns}
NDVI	0.39***	−0.18**	0.02^{ns}	0.03^{ns}
KS	0.41***	0.21*	0.21***	0.28***
TKW	0.41***	0.26***	0.09^{ns}	0.39***
Gm^{-2}	0.31***	0.39***	0.33***	0.54***
BIO	0.42***	0.38***	0.47***	0.59***
HI	0.81***	0.74***	0.60***	0.52***
HTE	−0.25***		−0.54***	

*, **, *** significant at the 0.05, 0.01, 0.001 probability levels, respectively; ns, non-significant; DH, days to heading; DM, days to maturity; GFD, grain filling duration; PH, plant height; PL, peduncle length, CT, canopy temperature; SPAD, chlorophyll content; NDVI, normalized difference vegetation index; KS, kernel number per spike; TKW, thousand-kernel weight; Gm^{-2}, grain number per m^2; BIO, biomass; HI, harvest index; HTE, heat tolerance efficiency.

Table 2-4. Range of heat tolerance efficiency calculated from the grain yield of the 400 MSD lines, their parent 'Norin 61' (N61), and two adapted Sudanese cultivars 'Goumria' and 'Imam' at Dongola (DON) and Wad Medani with optimum sowing (MED/OS) and late sowing (MED/LS).

Environment	Range (%)	Mean (%)	N61 (%)	Goumria (%)	Imam (%)
DON and MED/LS	14–151	62	90	61	90
MED/OS and MED/LS	15–173	71	85	53	81

Table 2-5. Morphological, phenological, physiological, yield, and yield component traits of ‘Norin 61’ (N61) and the Sudanese cultivars ‘Goumria’ and ‘Imam’ evaluated for heat stress tolerance at Dongola (DON), Hudeiba (HUD), Wad Medani with optimum sowing (MED/OS), and Wad Medani with late sowing (MED/LS).

Trait	DON			HUD			MED/OS			MED/LS		
	N61	Goumria	Imam	N61	Goumria	Imam	N61	Goumria	Imam	N61	Goumria	Imam
DH	72	75	78	61	63	73	64	63	73	62	65	71
DM	106	109	109	103	107	108	101	104	107	92	97	102
GFD	33	34	30	42	39	35	37	40	34	30	32	31
PH	97	83	89	69	64	75	84	74	86	79	70	83
PL	34	32	32	13	8	12	28	28	34	26	25	28
CT	32	22	21	30	30	30	17	17	23	25	25	27
SPAD	43	38	40	51	51	52	50	50	51	50	51	48
NDVI	0.71	0.73	0.71	0.63	0.55	0.62	0.55	0.58	0.59	0.69	0.67	0.73
KS	23	24	21	45	54	45	56	55	55	54	50	48
TKW	33	33	32	27	31	33	30	32	25	26	32	25
Gm^{-2}	10965	10532	9787	10357	11531	11284	27181	21502	21335	21283	23142	20893
GY	784	683	614	383	555	514	823	784	685	703	413	555
BIO	2366	2459	2016	1279	1434	1457	2038	2109	2109	1089	1428	2244
HI	33	30	31	31	40	35	40	39	32	39	31	24

DH, days to heading; DM, days to maturity; GFD, grain filling duration (days); PH, plant height (cm); PL, peduncle length (cm); CT, canopy temperature (°C); SPAD, chlorophyll content (SPAD units); NDVI, normalized difference vegetation index; KS, kernel number per spike; TKW, thousand-kernel weight (g); Gm^{-2}, grain number per m^2; GY, grain yield ($g\ m^{-2}$), BIO, biomass ($g\ m^{-2}$); HI, harvest index (%).

Table 2-6. The effect of genotype (G), environment (E), and genotype–environment interaction (G×E) on morphological, phenological, physiological, yield, and yield component traits of the 400 MSD lines, 'Norin 61' (N61), and the Sudanese cultivars 'Goumria' and 'Imam' evaluated for heat stress tolerance at Dongola (DON), Hudeiba (HUD), Wad Medani with optimum sowing (MED/OS), and Wad Medani with late sowing (MED/LS) in Sudan.

Trait	DON	HUD	MED/OS	MED/LS		
	G	G	G	G	E	G×E
DH	93.15***	71.94***	318.50***	24.38**	***	138.36***
DM	92.85***	47.81***	119.96***	NS	***	799.93***
GFD	NS	62.52***	64.83***	NS	***	228.63***
PH	146.79*	95.08*	91.59***	86.56***	***	565.86***
PL	30.07*	20.59**	36.21***	17.86***	***	82.20***
CT	NS	2.02*	17.84*	NS	***	103.26***
SPAD	13.74*	29.41*	16.00**	11.77*	***	221.91***
NDVI	NS	NS	0.0154***	0.0028*	***	0.0248***
KS	20.41**	195.67***	151.25***	137.47*	***	407.36***
TKW	30.31**	83.43***	NS	NS	***	289.96***
Gm^{-2}	NS	17728694***	136882698***	44834708*	***	49961421***
GY	NS	34838.94**	73552.02**	NS	***	112179.5***
BIO	NS	142111.7*	587.59*	NS	***	510362.3***
HI	NS	134.42*	167.13***	NS	***	384.08***

DH, days to heading; DM, days to maturity; GFD, grain filling duration (days); PH, plant height (cm); PL, peduncle length (cm); CT, canopy temperature (°C); SPAD, chlorophyll content (SPAD units); NDVI, normalized difference vegetation index; KS, kernel number per spike; TKW, thousand-kernel weight (g); Gm^{-2}, grain number per m^2; GY, grain yield ($g\ m^{-2}$), BIO, biomass ($g\ m^{-2}$); HI, harvest index (%).

Table 2-7. Heat tolerance efficiency (HTE), morphological, phenological, physiological, yield, and yield component traits of 13 heat-tolerant MSD lines and their parent 'Norin 61' at Wad Medani with late sowing (MED/LS) and their combined yield at Dongola, Wad Medani with optimum sowing, Hudeiba, and MED/LS.

Line	Cross name	DH	DM	GFD	PL	CT	SPAD	NDVI	KS	TKW	Gm^{-2}	BIO	GY^a	GY^b	HI	HTE
MSD006	N61/Syn65//*N61	60	94	34	29	26	51	0.68	48	32	25200	2300	743	478	32	118
MSD054	N61/Syn26//*N61	61	96	35	24	26	50	0.66	48	28	31440	1900	705	614	38	125
MSD084	N61/Syn30//*N61	58	92	32	30	27	48	0.62	52	28	14983	2150	746	670	34	109
MSD108	N61/Syn45//*N61	60	100	38	24	23	46	0.72	44	35	10418	2225	639	535	28	107
MSD186	N61/Syn30//*N61	62	98	35	30	27	51	0.73	31	32	11500	2475	759	608	31	148
MSD208	N61/Syn26//*N61	61	92	31	26	22	52	0.67	41	29	16267	2175	891	766	41	124
MSD265	N61/Syn44//*N61	59	90	30	31	28	51	0.64	28	33	10625	2200	712	590	33	127
MSD296	N61/Syn32//*N61	59	94	35	33	27	44	0.69	52	29	21580	2550	758	538	29	126
MSD303	N61/Syn68//*N61	57	91	33	28	26	44	0.54	40	29	18842	1875	562	475	30	122
MSD345	N61/Syn48//*N61	65	98	35	24	27	54	0.69	47	32	17267	2275	629	504	28	110
MSD360	N61/Syn57//*N61	59	93	34	28	30	53	0.89	40	32	21175	2375	763	588	32	115
MSD367	N61/Syn57//*N61	61	100	37	34	26	49	0.69	68	25	21080	2575	644	577	25	115
MSD453	N61/Syn50//*N61	58	90	34	29	24	49	0.65	37	31	12467	2375	827	660	34	117
Norin 61		62	92	30	26	25	50	0.69	54	25	21283	1809	703	659	39	90
Grand mean		**63**	**96**	**33**	**27**	**26**	**49**	**0.69**	**40**	**26**	**15363**	**1842**	**417**	**396**	**23**	**62**

DH, days to heading; DM, days to maturity; GFD, grain filling duration (days); PL, peduncle length (cm); CT, canopy temperature (°C); SPAD, chlorophyll content (SPAD units); NDVI, normalized difference vegetation index; KS, kernel number per spike; TKW, thousand-kernel weight (g); Gm^{-2}, grain number per m^2; BIO, biomass ($g\ m^{-2}$); GY^a, grain yield ($g\ m^{-2}$) at MED/LS; GY^b, combined grain yield combine in all environments ($g\ m^{-2}$); HI, harvest index (%).

Table 2-8. Morphological, phenological, physiological, yield, and yield component traits of the 29 high-yielding MSD lines and the parent 'Norin 61' at Hudeiba, and their combined yield evaluated at Dongola, Wad Medani with optimum sowing, Wad Medani late with sowing and Hudeiba.

Line	Cross name	DH	DM	GFD	PL	CT	SPAD	NDVI	KS	TKW	Gm^{-2}	BIO	GY^{a}	HI	GY^{b}
MSD044	N61/Syn71//*N61	63	105	43	12	33	52	0.62	53	27	14826	1688	749	44	783
MSD132	N61/Syn34//*N61	62	104	42	18	28	50	0.80	40	28	10787	1771	722	41	693
MSD136	N61/Syn31//*N61	69	104	35	17	30	51	0.57	47	**34**	4795	1595	635	40	632
MS140	N61/Syn68//*N61	67	104	38	10	31	54	0.60	46	**35**	11124	1852	676	36	630
MSD157	N61/Syn40//*N61	66	104	38	12	**26**	51	0.78	45	25	**15788**	**2114**	685	32	649
MSD163	N61/Syn31//*N61	59	104	45	**19**	30	55	0.67	39	**36**	13132	**2174**	752	35	796
MSD166	N61/Syn39//*N61	66	108	42	17	**24**	53	0.73	42	**34**	8915	1445	636	44	671
MSD187	N61/Syn68//*N61	66	**97**	32	9	30	56	0.63	31	**39**	7881	1652	701	42	562
MSD195	N61/Syn27//*N61	60	118	**58**	16	28	54	0.79	50	32	12751	1215	666	**55**	556
MSD208	N61/Syn26//*N61	60	104	44	14	28	53	0.74	45	28	11148	1224	658	**54**	766
MSD215	N61/Syn68//*N61	**56**	116	**61**	**19**	28	51	0.63	49	27	10293	1694	675	40	624
MSD225	N61/Syn48//*N61	68	104	36	21	30	53	0.76	43	29	12840	**2064**	785	38	587
MSD267	N61/Syn42//*N61	68	107	40	16	29	55	0.62	42	**34**	10192	**1871**	771	41	662
MSD278	N61/Syn62//*N61	61	104	43	11	27	53	0.66	46	**34**	14167	**1878**	682	36	564
MSD289	N61/Syn30//*N61	61	106	45	10	28	44	0.56	26	**43**	8190	974	679	**70**	463
MSD300	N61/Syn35//*N61	62	104	42	17	27	51	0.80	38	**33**	14036	**1967**	737	37	657
MSD304	N61/Syn62//*N61	67	103	36	**19**	**26**	55	0.70	45	**36**	**16413**	881	708	**80**	600
MSD307	N61/Syn68//*N61	59	104	45	16	31	54	0.60	43	26	6474	1047	636	**61**	572
MSD320	N61/Syn58//*N61	66	104	38	17	28	52	0.68	39	31	8166	1775	744	42	602
MSD326	N61/Syn62//*N61	67	108	41	12	30	55	0.62	0	19	**23874**	1804	785	44	699
MSD367	N61/Syn57//*N61	64	**97**	34	**20**	31	50	0.59	35	**33**	8775	1820	641	35	577
MSD368	N61/Syn48//*N61	63	106	43	11	29	52	0.66	50	29	13673	**2221**	888	40	680

MSD377	N61/Syn27//*N61	63	108	45	**21**	**25**	53	0.78	48	34	10101	**1908**	638	33	582
MSD410	N61/Syn27//*N61	60	104	45	16	32	49	0.64	**59**	29	**17093**	**2228**	916	41	640
MSD413	N61/Syn48//*N61	68	102	35	16	31	57	0.64	43	32	10325	**2166**	950	44	663
MSD434	N61/Syn52//*N61	68	112	44	12	28	52	0.60	45	26	12808	**2027**	634	31	587
MSD491	N61/Syn37//*N61	65	108	43	14	29	38	0.80	41	**33**	10675	**1964**	772	39	582
MSD499	N61/Syn66//*N61	76	108	32	9	29	55	0.54	49	31	**15746**	1277	818	**64**	482
MSD500	N61/Syn34//*N61	60	103	43	**21**	33	57	0.60	31	32	5947	1327	422	32	478
Norin 61		61	103	42	13	30	50	0.63	41	26	10357	1279	383	30	659
Grand mean		**66**	**107**	**40**	**15**	**30**	**51**	0.60	**37**	**26**	**8458**	**1283**	**429**	**32**	**396**

DH, days to heading; DM, days to maturity; GFD, grain filling duration (days); PL, peduncle length (cm); CT, canopy temperature (°C); SPAD, chlorophyll content (SPAD units); NDVI, normalized difference vegetation index; KS, kernel number per spike; TKW, thousand-kernel weight (g); Gm^{-2}, grain number per m^2; BIO, biomass (g m^{-2}); GY^a, grain yield (g m^{-2}) at HUD; HI, harvest index (%); GY^b, grain yield combine for all environments (g m^{-2}). Bold letters indicate values that are significantly difference than those of Norin 61.

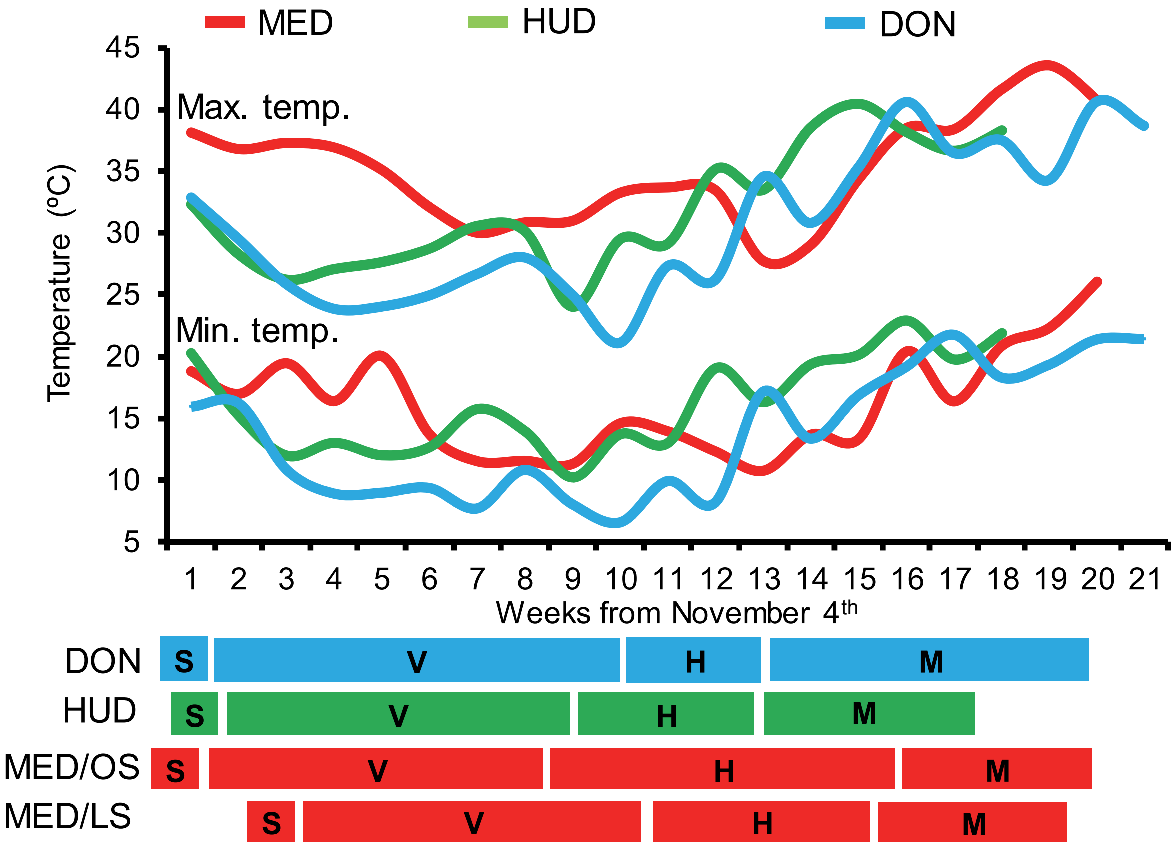

Fig. 2-1. Weekly maximum and minimum temperatures at Dongola (DON), Hudeiba (HUD), and Gezira (Wad Medani; MED) Research Farms starting from the 4th week of November. OS, optimum sowing; LS, late sowing; S, sowing time; V, vegetative stage; H, heading stage; M, maturity stage.

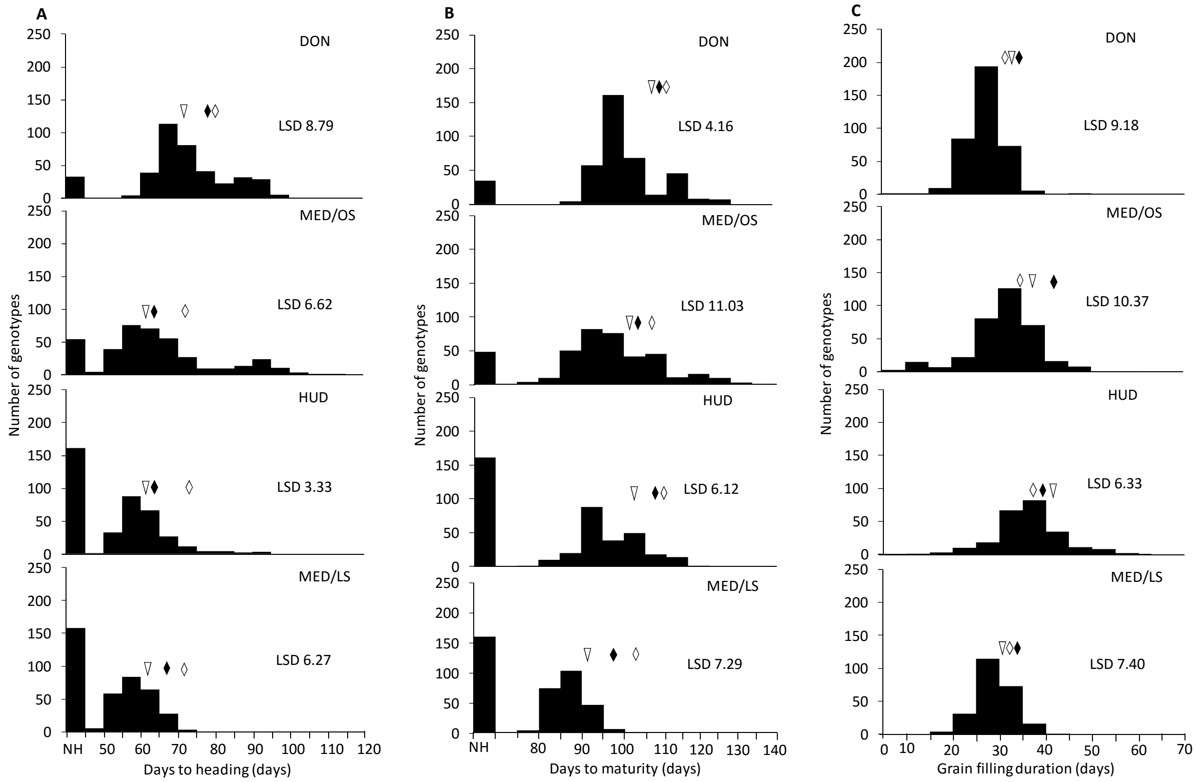

Fig. 2-2. Frequency distribution of days to heading (A), days to maturity (B) and grain filling duration (C) of the 400 MSD lines their parent 'Norin 61' (triangle) and the adapted Sudanese cultivars 'Goumria' (black rhombus) and 'Imam' (white rhombus) of four environments of Dongola (DON), Wad Medani optimum sowing (MED/OS), Hudieba (HUD) and Wad Medani late sowing (MED/LS) in 2015/16.

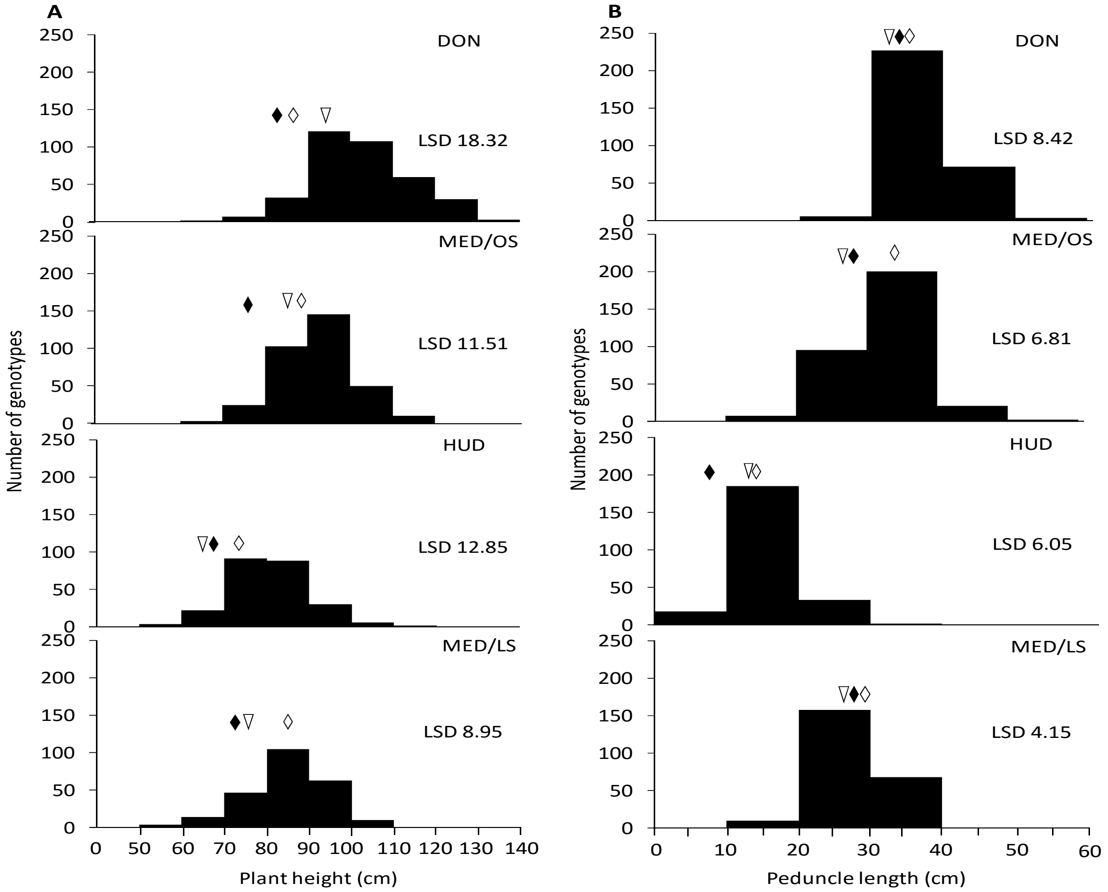

Fig. 2-3. Frequency distribution of plant height (A) and peduncle length (B) of the 400 MSD lines their parent 'Norin 61' (triangle) and the adapted Sudanese cultivars 'Goumria' (black rhombus) and 'Imam' (white rhombus) of four environments of Dongola (DON), Wad Medani optimum sowing (MED/OS), Hudieba (HUD) and Wad Medani late sowing (MED/LS) in 2015/16.

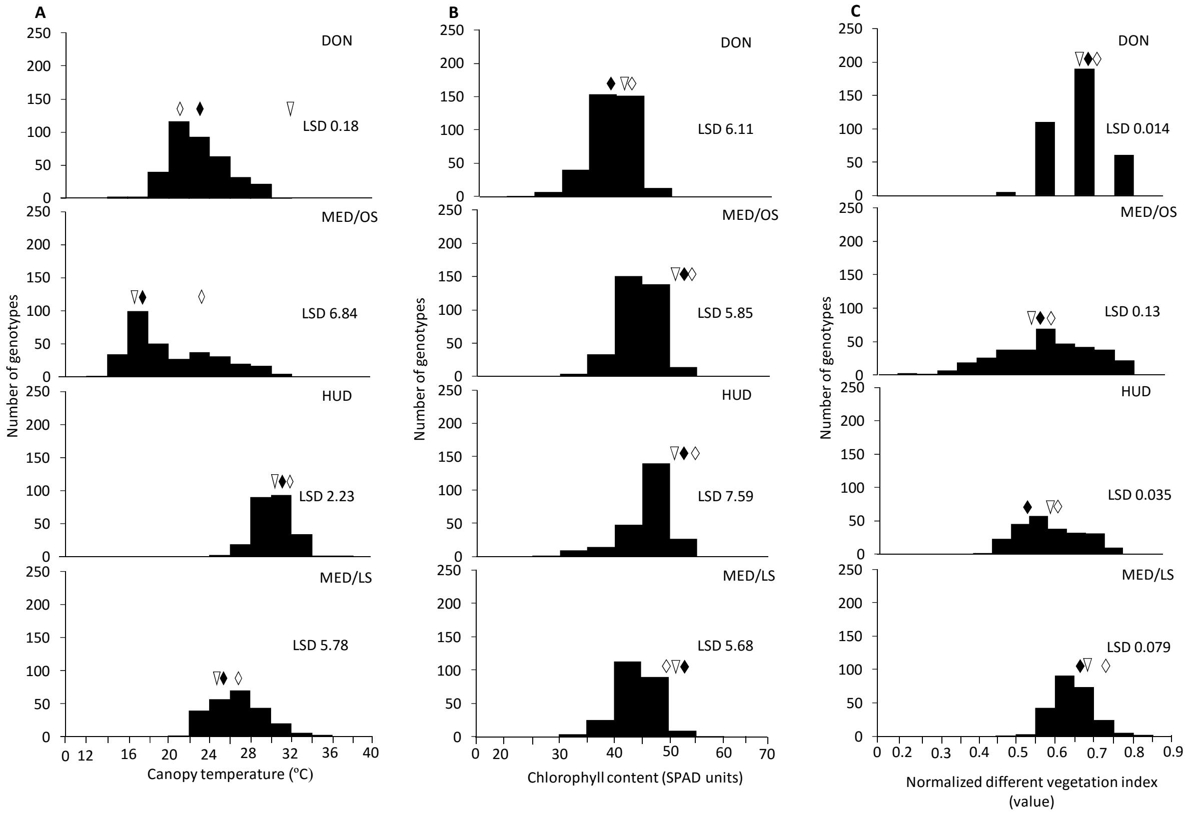

Fig. 2-4. Frequency distribution of canopy temperature (A) chlorophyll content (B) and normalized difference vegetation index (C) of the 400 MSD lines their parent 'Norin 61' (triangle) and the adapted Sudanese cultivars 'Goumria' (black rhombus) and 'Imam' (white rhombus) of four environments of Dongola (DON), Wad Medani optimum sowing (MED/OS), Hudieba (HUD) and Wad Medani late sowing (MED/LS) in 2015/16.

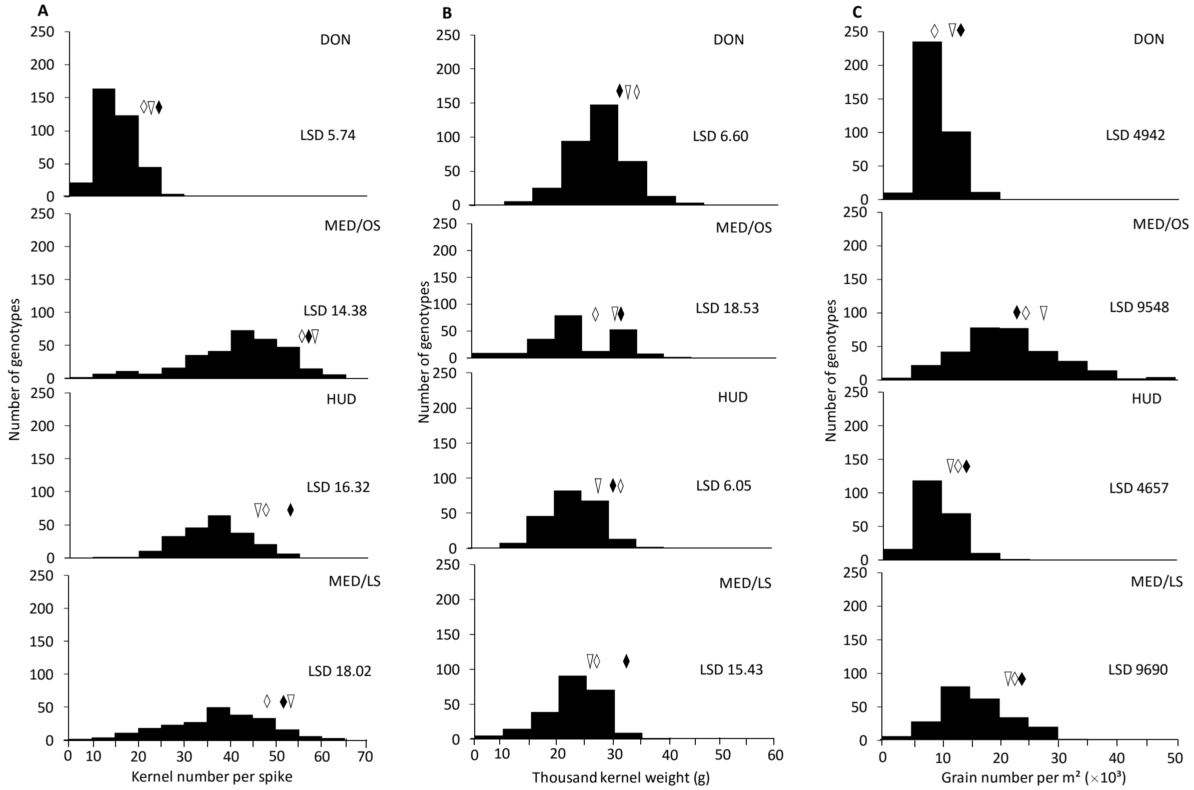

Fig. 2-5. Frequency distribution of kernel number per spike (A), thousand-kernel weight (B) and grain number per m^2 (C) of the 400 MSD lines their parent ‘Norin 61’ (triangle) and the adapted Sudanese cultivars ‘Goumria’ (black rhombus) and ‘Imam’ (white rhombus) of four environments of Dongola (DON), Wad Medani optimum sowing (MED/OS), Hudieba (HUD) and Wad Medani late sowing (MED/LS) in 2015/16.

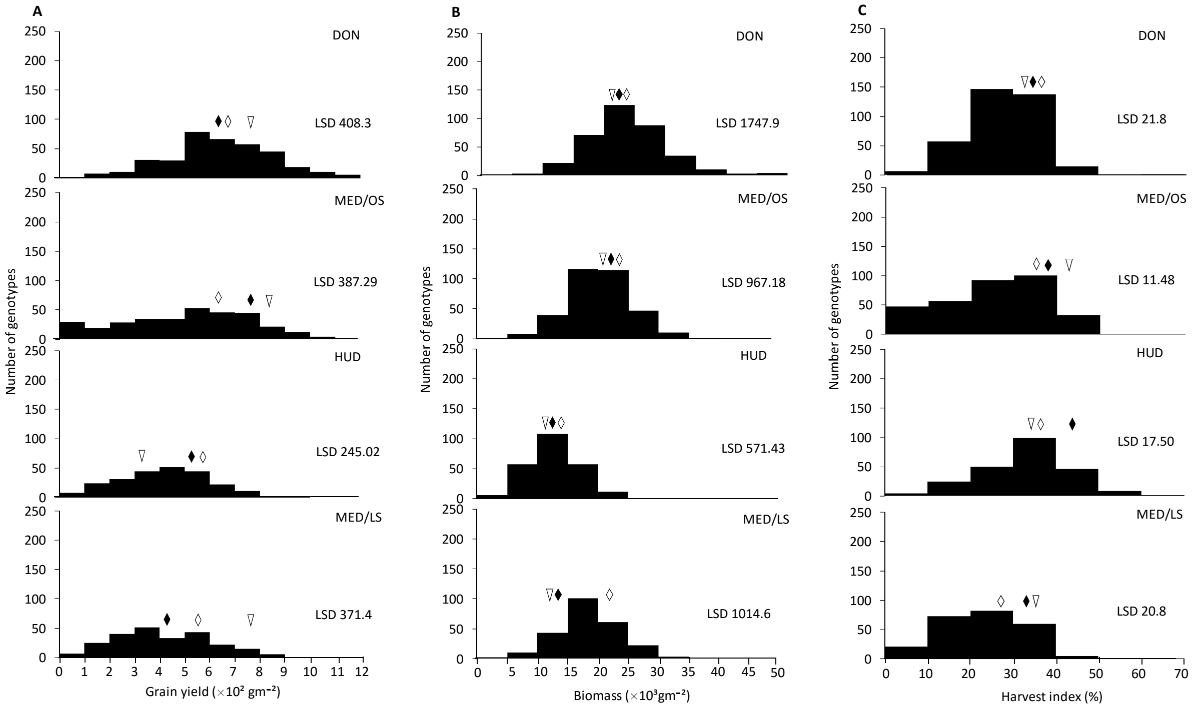

Fig. 2-6. Frequency distribution of grain yield (A), biomass (B) and harvest index (C) of the 400 MSD lines their parent ‘Norin 61’ (triangle) and the adapted Sudanese cultivars ‘Goumria’ (black rhombus) and ‘Imam’ (white rhombus) of four environments of Dongola (DON), Wad Medani optimum sowing (MED/OS), Hudieba (HUD) and Wad Medani late sowing (MED/LS) in 2015/16.

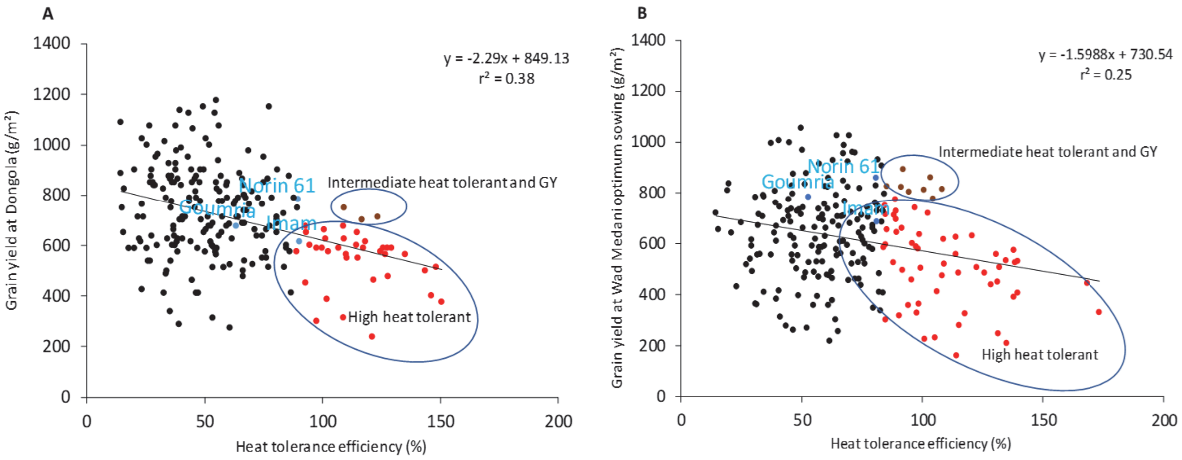

Fig. 2-7. Regression analysis of the relationship between grain yield at DON and heat tolerance efficiency DON (considered a cool environment) and at MED/LS (hot environment) (A) and relationship between grain yield at MED/OS and heat tolerance efficiency MED/OS (considered a cool environment) and at MED/LS (considered a hot environment) (B) for the 400 MSD lines, their parent 'Norin 61' (control), and the adapted Sudanese cultivars 'Goumria' and 'Imam'. Red dots, highly heat-tolerant lines; dark orange dots, moderately heat-tolerant lines with good grain yield.

Chapter 3

Genome-wide association study of adaptive traits under high temperature environments

3.1 Abstract

Heat stress has become a major threat to international food security in bread wheat (*T. aestivum* L.). Genetic variation for heat tolerance-adaptive traits is limited in the elite wheat germplasm, whereas, in the secondary gene pool, considerable genetic variability for heat tolerance has been found in both *Aegilops tauschii* and *T. turgidum*. Recently Multiple Synthetic Derivatives (MSD) population possessing large variation of *Ae. tauschii* has been developed. This study aimed to identify QTLs/genes associated with heat stress-adaptive traits. I conducted a genome-wide association study (GWAS) using 15,616 DArT-seq and SNPs markers across 400 MSD population lines. Phenotyping was carried out under heat stress in Sudan at three locations. The 400 lines showed considerable genetic variation in most of the traits studied. Several lines were earlier than the check cultivar 'Norin 61,' and others showed low canopy temperature. Three QTLs were identified for days to heading on chromosomes 2A, 2B and 2D. Two QTLs were detected for growth habit on chromosomes 2D and 5D. Two QTLs were identified for canopy temperature at heading stage on chromosomes 2A and 2D. In heading trait, the associated genes might be the photoperiod alleles *Ppd-A1, Ppd-B1* and *Ppd-D1*. The variation observed and QTLs detected in this population could be useful for wheat breeding and improvement for heat stress tolerance. This population could be evaluated for other abiotic stresses such as salinity and drought.

3.2 Introduction

Wheat (*T. aestivum* L.) is one of the most important food crops in the world. Its productivity is often reduced by both biotic and a biotic stress, and heat stress is one of the major constraints. However, genetic variation for heat stress tolerance is limited in the elite wheat germplasm. Because wild species in the secondary gene pool *(Ae. tauschii* and *T. turgidum*) has considerable genetic variability for potentially adaptive traits, increased biomass, total photosynthesis and thousand kernel weight, those should be exploited in wheat breeding (Gill *et al.* 2008; Halloran *et al.* 2008; Lopes *et al*. 2015).

Many scientists are using few wild relatives and synthetic wheats (SW) in breeding programs, this limits the diversity of germplasm or, in other words, there will be few good genes coming from few lines. Tsujimoto *et al*. (2011) produced Multiple Synthetic Derivative (MSD) populations by crossing 43 synthetic wheat (SW) with Norin 61 (Japanese spring wheat variety). Synthetic wheat is the amphidiploid between *T. turgidum* var. *durum* cv. 'Langdon' and 43 accessions of *Ae. tauschii* collected from the whole distribution of this species. Paliwal *et al.* (2012) reported that plant biomass, harvest index, thousand kernel weight, grain filling duration, grains per m^2, spikes per m^2 and canopy temperature could be used as selection criteria for heat stress.

QTL mapping is a key approach for understanding the genetic architecture of complex traits in plants (Holland, 2007). In wheat, classical QTL mapping locates genomic regions of the QTLs that originated from both parents crossed to produce the segregation population, and thus (Navakode *et al*. 2014). As a complement to this QTL mapping, genome-wide association studies (GWAS) provide a powerful approach for identifying genetic markers associated with complex traits of interest (Lopes *et al*. 2014; Risch and Merikangas, 1996). However, GWAS for spring wheat are limited and represents a challenge due to the large genome, incomplete genome sequence, and hexaploidy, which makes it difficult to assign the markers to individual (A, B and D) genomes (Sukumaran and Yu, 2014).

In this study, I used 400 MSD lines genotyped with 68,516 silico-DArT and SNPs markers to identify a new source of germplasm for heat tolerance and to identify markers associated with heat tolerance-related traits using GWAS. I identified several promising lines and QTLs which can be used to breed well-adapted heat tolerant wheat cultivars.

3.3 Materials and Methods

3.3.1 Plant material

I used 400 plants selected randomly from MSD population (Tsujimoto *et al.* 2011) developed from crosses between 'Norin 61' (N61, Japanese spring wheat variety) and 43 SW lines (Matsuoka et al. 2013). These lines in addition to their parent N61, two Sudanese varieties ('Imam' and 'Gomeria') and an Afghanistan landrace 'Safedak Ishkashim'.

3.3.2 Experimental sites, design and field management

The plants were evaluated in three different environments in Sudan using Augmented Randomized Complete Block Design. The three environments represent gradient temperatures from low or optimum in Dongola (DON, 19°08'N, 30°27'E, 239 masl) in Northern State, Hudeiba (HUD, 14°40'N, 33°50'E, 409 masl) in River Nile State, and to high temperature in Wad Medani (MED, 14°24'N, 29°33'E, 407 masl) in Gezira State. In MED, I used two planting dates; optimum and late planting (4th week of November and second week of December, respectively).

The seeds were treated with imidacloprid (35% WP) at 1 g/kg seeds to control pests, mainly termites and aphids. The seeds were sown manually at 120 kg ha^{-1}. Each line was sown in a plot of four rows, 0.5 m long and 0.2 m apart. Superphosphate as phosphorus was applied by furrow placement before sowing at 18.8 kg P/ha. Urea as nitrogen was split-applied at the three-leaf stage (second irrigation) and the tillering stage (fourth irrigation) at 86 kg N/ha. Irrigation was applied every 10–12 days. The fields were hand-weeded at least twice.

I evaluated three phenotypic traits including growth habit, days to heading and canopy temperature at heading stage. Abbreviations used for phenotypic and physiological traits and methods of measurement are listed in Table 3-1. Metrological data, in particular, the weekly maximum, minimum and mean temperatures, during the cropping season was obtained from metrological stations located at the three experimental sites (Fig. 1A, 1B, 1C).

3.3.3 Statistical analysis

Analysis of variance was carried out using Plant Breeding Tools software (PBTools, version 1.4. International Rice Research Institute, Los Baños, The Philippines; http://bbi.irri.orig/products).

3.3.4 Genotyping

DNA extraction was carried out according to CTAB procedure Saghai-Maroof et al. (1984). Two µg of total genomic DNA in 20 µl from each sample was sent to Diversity Arrays Technology Pyt. Ltd, Australia (http://www.diversityarrays.com/) as a commercial service provider for whole genome scan using DArT-seq markers. Totally 68,516 DArT markers were used to genotype the 400 synthetic wheat derivatives.

3.4 Results

3.4.1 Temperature and three experimental sites

Weekly maximum, minimum and mean temperatures during the cropping season at the three sites are given in (Fig. 1A, 1B, 1C). The temperature in the second planting date was higher than in the first planting date at MED. The temperature at MED was higher than DON and HUD (Fig. 1A, 1B, 1C). The high temperature of the second planting date in MED reduced the mean of all traits studied (Table 3-1).

3.4.2 Heading and growth habit

The genotypic effect was significant for all traits studied at the three sites except tillers number per plant at DON (Table 3-2). The frequency distribution of MSD population for growth habit 14.7 %, 18.4%, 21.7%, 22.2 and 23.0% were erect, semi-erect, intermediate, semi-prostrate and prostrate, respectively. The days to heading (DH) of N61 were 63 and 62 under optimum planting and late planting at MED, respectively (Table 3-2). The DH of MSD population ranged from 53 days to 126 days under optimum planting and from 50 days to 111 days under late planting (Table 3-2). I observed 93 genotypes were earlier than N61 under optimum planting, whereas under late planting we found 90 and 228 genotypes earlier than N61 and Imam, respectively. DH of the MSD lines ranged from 62 to 104 days and from 54 to 119 days at DON and HUD, respectively (Table 3-2). At DON, I observed 83 genotypes were earlier than N61, the 157 genotypes earlier than the other checks and 21 genotypes later than the latest check. DH of the MSD lines ranged from 54 to 119 days at HUD, furthermore 72 genotypes had earlier heading than N61 (Table 3-2).

3.4.3 Canopy temperature at heading and tiller number per m^2

The canopy temperature at heading stage (CT-h) was cooler in optimum planting (12.4−29.6 °C) than in late planting (10.5−34.9 °C) at MED (Table 3-2) under late planting, 25.5% of the MSD population had a cooler canopy than the cooler check N61.

The tiller number per m^2 (TN) was ranged from 175 to 995 and from 125 to 860 under optimum and late planting at MED, respectively (Table 3-2). The 145 and 140 MSD genotypes had more TN than N61 under optimum and late planting, respectively. The TN at DON was ranged from 187 to 797, furthermore, I observed 86 genotypes had the higher TN than N61. On the other hand, the TN was ranged from 100 to 487 at HUD, and, furthermore, I found 50 genotypes had higher TN than N61 (Table 3-2).

3.4.4 Marker-trait associations

For this analysis, I used only the phenotypic data of the late planting date at MED as it represents the most stressed environment (Fig. 3-1A). After the markers filtration, I used 14,300 SNP markers with a minor allele frequency (MAF) greater than 0.05 for maker traits associations (MTAs). Two QTLs were detected for GH on chromosomes 2D and 5D with 19 significant markers (Fig. 3-2A). For DH, I detected three QTLs on chromosomes 2A, 2B and 2D with 41 significant SNP markers (Fig. 3-2B). Two QTLs were identified for CT-h on chromosomes 2A and 2D with 52 significant SNP markers (Fig. 3-2C).

3.5 Discussion

The results indicated that all traits are highly affected by heat stress. The MSD population showed wide genetic variation under heat stress−irrigated conditions of Sudan. Previous study reported the canopy temperature, days to heading, spikes per m^2 are indirect selection criteria for increasing grain yield under heat and drought stresses (Serious *et al.* 2014; Khan *et al.* 2007).

Of seven QTL identified in this study, four QTLs (2A, 2B, 2D and 5D) were significantly associated with traits related to heat tolerance. (Fig. 3-2A, 3-2B, 3-2C). Many authors have reported days to heading and canopy temperature were genetically controlled by quantitative trait loci. Heidari *et al.* (2012) identified two QTLs on chromosomes 2D and 2B made the largest contribution to the expression of days to heading. Lopes *et al.* (2013) identified QTLs for canopy temperature in the Seri/Babax bread wheat population on chromosomes (2B, 5D-b, 7D-b) under drought, hot irrigated and non-stress conditions.

In conclusion, the results showed the presence of ample genetic variability in growth habit, days to heading, canopy temperature at heading stage and tiller number per m^2. This variation can be used and utilized for breeding heat tolerant and adapted genotypes. Through GWAS, I identified several QTLs which might have an impact on wheat improvement and lead to higher yield. In addition, the combining of GWAS and phenotypic analysis was a powerful approach to describe genetic variation for heat stress tolerance. The identified QTL can be used for marker assisted selection in breeding wheat for improved heat tolerance. The MSD population it can be the good source of genes for improving wheat varieties under high temperatures stresses and with any wheat breeding objectives.

Table 3-1. Traits measured, their abbreviations and definitions.

Trait	Abbreviation	Method of measurement
Growth habit	GH	Visual score from (1-5) 1=erect, 2=semi erect, 3=intermediate, 4=semi prostrate, 5=prostrate during stem elongation.
Days to heading	DH	Days from sowing to the time when 50% of plants headed.
Canopy temperature at heading stage	CT-h	Temperature (°C) measured remotely with a hand-held infrared thermometer (Everest Interscience, Tucson, AZ, USA). Measurement started when plots had sufficient ground cover such that the thermometer could be angled with a field of view that did not include any bare soil between rows readings were taken in the afternoon (13:00–14:00) 4 or 5 days after irrigation on clear calm days Measurements were taken at heading stage (CT-h).
Tiller number	TN	Number of culms with spikes per m^2 or in a specific area within the plot.

Table 3-2. Growth habit (GH), days to heading (DH), canopy temperature at heading stage (CT-h) and tiller number per m^2 (TN) of the MSD population and their parent N61 plus three checks for heat stress tolerance at optimum planting (OP) and late planting (LP) at Gezira Research Farm, Wad Medani (MED), Dongola Research Farm, Dongola (DON) and Hudeiba Research Farm, Hudeiba (HUD), Sudan season (2015/2016).

Check	GH	DH				CT-h		TN			
		MED	MED	DON	HUD	MED	MED	MED	MED	DON	HUD
		OP	LP			OP	LP	OP	LP		
Norin 61	1	63	62	72	61	15.1	18.3	488	398	464	252
Gomeria	3	63	65	75	68	15.3	18.1	393	426	445	212
Imam	2	72	70	78	73	16.7	19.0	386	448	470	252
Safedak	2	100	100	95	96	28.1	31.7	465	514	508	320
Range	1−5	53−126	50−111	62−104	54−119	12.4−29.6	10.5−34.9	70−995	125−860	187−797	100−487
Mean	3.0	76.0	72.0	79	71	18.9	20.9	463.0	379.0	448	209
G	*	***	***	***	***	***	***	***	*	NS	***
SE	1.3	4.0	3.7	5.3	1.8	4.4	3.6	119.8	224.8	115.1	59.4

G, genotypes. NS, not significant. *, *** Significant at the 0.05 and 0.001 probability levels, respectively.

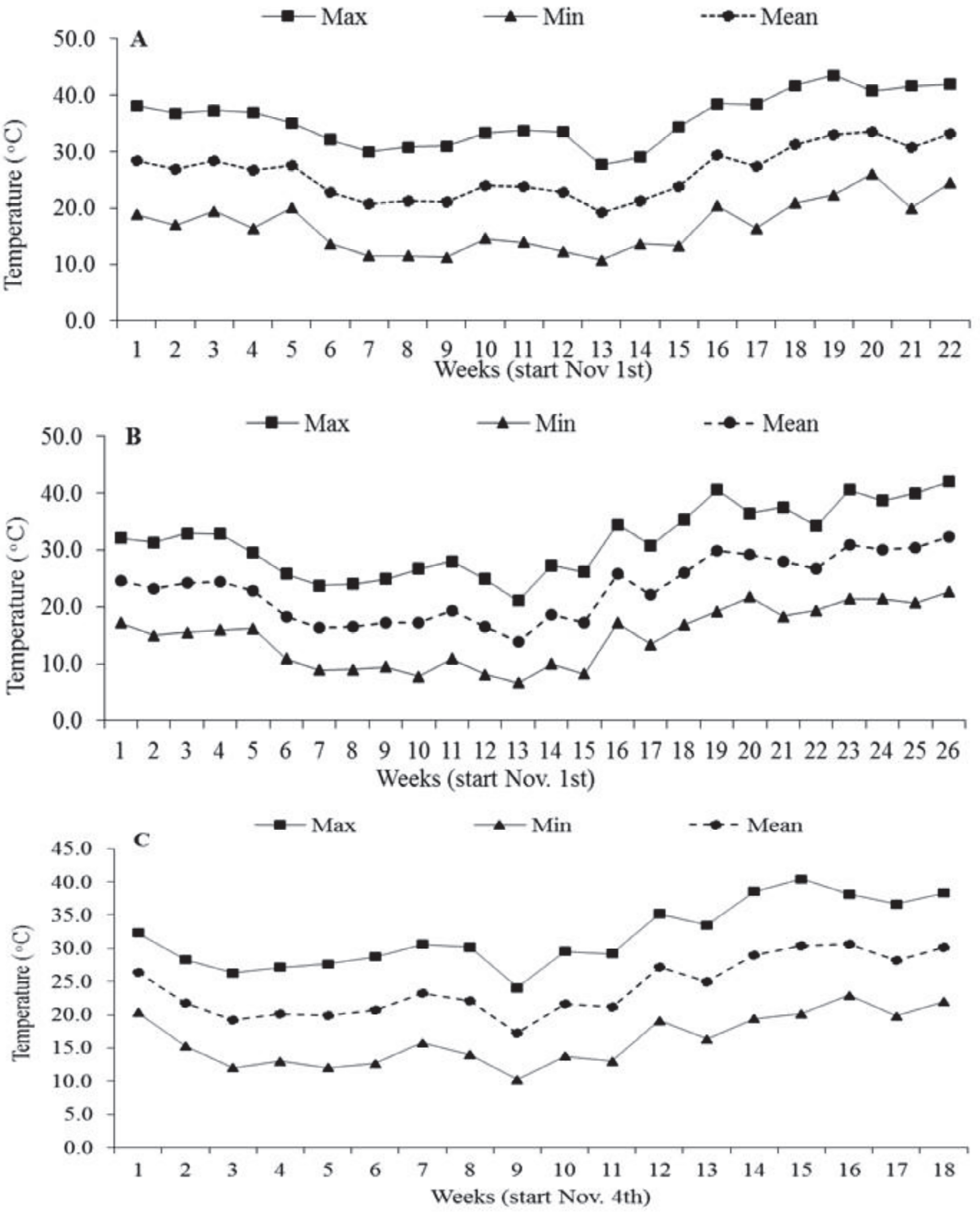

Fig. 3-1. Weekly, maximum, minimum and average temperatures at Gezira Research Station, Wad Medani (A) Farm, Dongola Research Farm, Dongola (B) and Hudeiba Research Farm, Hudeiba (C), respectively during the 2015/2016 cropping season.

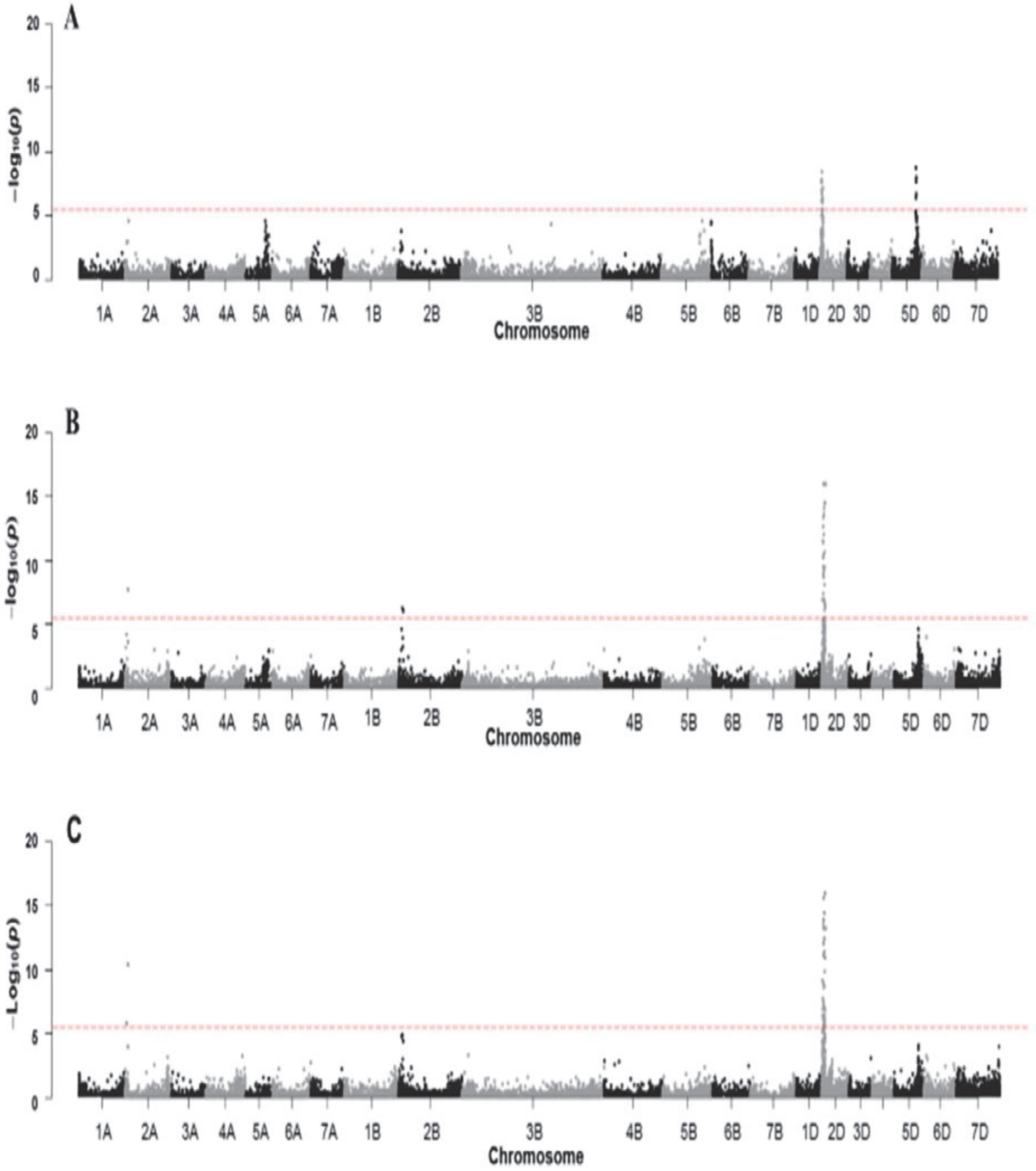

Fig. 3-2. GWAS results using 14,300 SNPs markers in MSD population for growth habit (A), day to heading (B) and canopy temperature at heading stage (C) at Gezira Research Farm, Wad Medani, season 2015/16.

General discussion and conclusion

The climate changes are expected to increase the severity of the biotic and abiotic stresses such as diseases, insect, heat, salt and drought, and hence reduce the crop production of the world. On the other hand, *Ae. tauschii* accessions that were involved in the origin of common wheat has a wide genetic variability and are rich resource for the common wheat enhancements against biotic and abiotic stresses. However, only limited portion of this huge and tremendous resources has been utilized in the breeding and improvement of the wheat crop. The population used in this study produced by crossing and backcrossing the Japanese wheat cultivar N61 and 43 *Ae. tauschii* accessions, is harboring the genetic variation of these 43 accessions, and thus provides a better chance in a more efficient way to explore the genetic variation of heat tolerant related traits in a wide range of *Ae. tauschii* accessions. I observed wide genetic variation and different responses to the high-temperature among the MSD lines in morphological, physiological, yield and its components traits. In this study, some MSD lines had better performance than that of N61 and the adapted Sudanese cultivars. These results indicating the impact of 43 *Ae. tauschii* accessions used to produce the 43 different SHW parental lines of the MSD population.

In this study, I identified several lines with significant traits associated with adaptation of wheat to heat stress. These lines represent an excellent source of alleles to improve wheat adaptation and tolerance to heat stress through strategic trait-based crossing. On the other hand, the QTLs detected in this study could be useful for wheat breeding and improvement for heat stress tolerance. The MSD population is very effective to utilize the rare variation of heat tolerance in *Ae. tauschii* in short time and more practical less expensive way.

Summary

Wheat (*Triticum aestivum* L.) is the most important staple food of the world. It is cultivated widely in a diverse environments in the globe and exposed to different biotic and abiotic stresses. The growth and yield of wheat are adversely affected by environmental stresses such as high temperature, drought, salinity, etc. High temperature is one of the most important abiotic stress affecting wheat production. Breeding for heat stress tolerance is a big challenge because of the complex and unpredictable nature of heat stress and the complex interaction of the plant with the environment. Few genes are known which may increase tolerance to heat stress.

Wild relatives of wheat have adapted to various environments. Some of these wild relatives are expected to have heat stress tolerance genes. *Aegilops tauschii* Coss. is a wild relative of wheat and widely distributed around the world. This species is one of the direct ancestors of bread wheat, the D-genome donor to hexaploid bread wheat and is a rich genetic resource for wheat breeding. Many *Ae. tauschii* genes have been introduced to bread wheat through amphidiploids between *Ae. tauschii* and tetraploid wheat; these amphiploids are named synthetic hexaploid wheat (SHW). SHW retain wild morphology, such as tough glumes, which precludes threshing and thus the measuring of yield-related characteristics. Synthetic derivative lines, which originate from crosses between SHWs and bread wheat cultivars, are a better choice to uncover the variation in *Ae. tauschii* that may be used for breeding. However, most of the synthetic derivative lines originated from crosses between bread wheat cultivars and a limited number of SHWs. To investigate and use comprehensively the intraspecific variation of *Ae. tauschii* for wheat breeding, a multiple synthetic derivatives (MSD) population was produced, in which the intraspecific variation of *Ae. tauschii* was

compiled in the genetic background of bread wheat cultivar. Here, I select heat-tolerant plants from MSD population in terms of their agronomic and physiological traits. Also, I examined the genetic variability of heat stress–adaptive traits and to identify new sources of heat tolerance to be used in wheat breeding. In addition, I identified QTLs/genes associated with heat stress-adaptive traits.

I conducted two experiments. First, I grew 1000 BC_1F_3 plants from MSD population under heat stress in Sudan. I selected six BC_1F_4 plants showing good performance and I named these plants MNH lines (MSD population of N61 selected as heat stress tolerant). I grew these plants with Norin 61 (N61) in the field at Gezira Research Farm, Sudan and growth chambers in Tottori University, Arid Land Research Center, Japan in season 2015/16. In the field, I used optimum and late sowings to insure the the plants exposure to heat. The experiment was arranged in randomized complete block design with three replications. I used the six plants, plus N61 as control, I collected data on days to heading, grain filling duration, plant height, peduncle length, kernel number per spike, thousand kernel weight, biomass, grain yield, canopy temperature, normalized difference vegetation index and leaf area. On the other hand, in the growth chamber, I used two conditions control and hot conditions with the same six plants those I used in field plus N61 as control and two heat tolerant cultivars 'Gelenson' and 'Bacanora'. I collected data on photosynthetic rate and stomatal conductance.

In the second experiment I studied 400 BC_1F_4 lines randomly selected from MSD population. The experiment was arranged in an augmented randomized complete block design in four environments (Dongola, Hudieba, Wad Medani optimum sowing and Wad Medani late sowing) in Sudan in season 2015/2016. I used N61 as a control to show the effect of the wild genes on the improvement of N61 heat stress adaptability, and the two heat-tolerant Sudanese cultivars 'Goumria' and 'Imam' as checks to evaluate the heat tress adaptability of the MSD lines. I collected data on plants days to heading and maturity, grain filling duration, plant height, peduncle length, kernel number per spike, thousand kernel weight, grain number per m^2, biomass, grain yield, harvest index, canopy temperature, chlorophyll content and normalized difference vegetation index and heat tolerant efficiency (HTE). In addition, I performed genotyping of these 400 MSD lines to conduct a genome-wide association study using 15,616 DArT-seq markers for growth habit, days to heading, canopy temperature at heading and tiller numbers per m^2 in three environments in Sudan.

The results of the first experiment showed that the six plants had different responses to heat stress in comparison with N61, increasing the photosynthetic rate and stomatal conductance (MNH1, MNH2 and MNH5), and increasing biomass and grain yield (MNH2 and MNH5) in the filed and growth chamber, respectively. Also, I identified that MNH2 and MNH5 lines acquired heat tolerant. In addition, I noticed that N61 had relatively good adaptability to heat stress.

The results of second experiment showed wide range of genetic variability in most of the traits in all environments. In all traits examined, I found MSD lines with better performance than their parent N61 and the two adapted Sudanese cultivars. In comparison with N61 and two adapted Sudanese cultivars, some of the MSD lines showed significantly lower canopy temperature and higher peduncle length, kernel number per spike, thousand kernel weight and biomass values. Using the heat tolerance efficiency, I identified 13 highly heat-tolerant lines and several lines with intermediate heat tolerance and good yield potential. I also identified lines with alleles that can be used to increase wheat yield potential. For genome-wide association study, I detected three QTLs for heading date on chromosomes 2A, 2B and 2D and two QTLs for growth habit on chromosomes 2A and 2D.

The results of these studies indicated that the MSD population, including the diversity of *Ae. tauschii*, is a promising resource to uncover useful quantitative traits derived from this wild species. Also, these results indicated that the approach is very effective and could be the best way to explore and use the variation of heat tolerance in *Ae. tauschii* in a practical, fast, and economical way. The identified QTLs can be used for marker assisted selection in breeding wheat for improved heat tolerance after validation.

摘要 Summary (in Japanese)

コムギ（*Triticum aestivum* L.）は、世界で二番目に重要な主食であり、世界中のきわめて多様な環境において広く栽培され、様々な生物的および非生物的ストレスに曝されている。コムギの成長および収量は、高温、干ばつ、塩分などの環境ストレスによって悪影響を受けるが、高温は非生物的ストレスの中で、最も深刻なものの一つである。しかし、高温ストレス耐性を目的とした育種は、高温ストレスの複雑で予測不可能な性質および作物と環境との複雑な相互作用のために大きな挑戦である。これまで、高温ストレスに対する耐性を増加させる可能性のある遺伝子はほとんど知られていない。

パンコムギの近縁野生植物は様々な環境に適応している。これら野生種の中には、高温ストレス耐性遺伝子をもつものが期待できる。タルホコムギ(*Aegilops tauschii*)はコムギの近縁種であり広い適応性をもつ。この種はパンコムギの直接の先祖であり、6 倍性パンコムギのDゲノムの供与親である。タルホコムギ遺伝子の多くはタルホコムギと 4 倍性コムギの複二倍体を橋渡しと

してパンコムギに導入することができる。この複二倍体を合成六倍性コムギ(SHW)というが、SHW は野生植物の堅い外穎などの野性的な形質を留めており、そのために脱穀が不可能で、収量関連形質を調査することができない。SHW とパンコムギ品種の交配に起源する合成コムギ派生系統は、育種に利用できるタルホコムギ変異を見出すためには良い選択肢である。しかしながら、合成コムギ派生系統の多くは、パンコムギと限られた数の SHW の交配によるものである。タルホコムギの種内変異を広範に見出し利用するために、ここでは、多重合成コムギ派生集団(MSD)を提案します。この MSD 集団はタルホコムギの種内変異をパンコムギの遺伝的背景に詰め込んだものである。本研究は、MSD 集団から高温耐性植物を選抜し、コムギの育種プログラムに使うことのできる新しい高温耐性遺伝資源を同定することを目的として行った。さらに、高温ストレス形質に関連する QTL や遺伝子を同定することも目的であった。

本論文では、私は二つの研究を行った。まず、MSD 集団から取り出した 1000 の BC_1F_3 植物をスーダンの高温条件下で栽培した。私は、高い成績を示

す6つの BC_1F_4 植物を選抜し、これらの植物を農林 61 号と共に、スーダンのゲジラ研究試験圃場および鳥取大学乾燥地研究センターのグロースチャンバーにおいて 2015/2016 年に栽培した。圃場実験では、高温により強く暴露させるため適正播種に加え遅延播種を行った。実験は 3 反復を含む完全乱塊法によって行い、各反復は 6 個体とした。これらの植物を MNH（農林 61 号の MSD 集団から選抜した高温耐性系統）と呼び、これらと農林 61 号を対照として栽培し、到穂日数、登熟期間、草丈、穂首長、穂あたり粒数、千粒重、バイオマス、収量、葉温、正規化差植生指数(NDVI)を調査した。一方、グロースチャンバー内では、対象および高温条件を設定し、同一の 6 系統を栽培し、農林 61 号、高温耐性品「ゲレンソン」および「バカノラ」と比較した。これらの植物の光合成率と気孔開度を調査した。

第二の実験では、私は MSD 集団からランダムに選んだ 400 の BC_1F_4 系統を研究した。この実験は 2015/2016 年スーダンの 4 環境下（ドンゴラ、フダイバ、ワドメダニにおける適正播種および遅延は種）に、拡張完全乱塊法で栽培

した。この実験では農林 61 号を野生植物の遺伝子の効果を調べる対象として用い、さらにスーダンの高温耐性品種「ゴムリア」と「イマム」を MSD 系統の高温適応の比較として用いた。植物の到穂日数、登熟機関、草丈、穂首長、穂あたり粒数、千粒重、平米あたり粒数、バイオマス、種子収量、収穫指数、葉温、葉緑素量、正規化差植生指数、および高温耐性効率を調査した。さらに、400 の MSD 系統のスーダンの 3 カ所の環境で取った成長特性、到穂日数、出穂期の葉温および平米あたり稈数のデータと 15616 の DArT-seq マーカーによるジェノタイピングデータから、ゲノムワイド連関解析を行った。

最初の実験の結果から、農林 61 号に比較して 6 系統が高温に対し、光合成率および気孔開度の増加(MNH1, MNH2 and MNH5)、圃場およびグロースチャンバーにおけるバイオマスおよび種子収量の増加(MNH2 and MNH5)など異なる反応を示す事が明らかとなった。さらに、MNH2 および MNH5 が高温耐性を獲得していることを明らかにした。さらに、農林 61 号も比較的高温適応性をもつことを明らかにした。

第二の実験の結果から、すべての環境において形質のほとんどが大きい遺伝的変異をもつことが明かとなった。調査したすべての形質において、親である農林 61 号やスーダンに適応している 2 品種より、高い成績を示す系統のあることを見出した。いくつかの MSD 系統においては、有意に低い葉温、有意に高い穂首長、種子収量、千粒重、バイオマスをもつものが存在した。高温耐性効率を用いて、13 の極高温耐性系統、数系統の高温耐性系統、および高収量ポテンシャルをもつ系統を同定した。また、コムギの収量ポテンシャルを増加させる可能性のある遺伝子をもつ系統を同定することができた。ゲノムワイド連関解析により、到穂日数に関する QTL を、2A、2B、2D 染色体に、成長様態に関する QTL を 2A および 2D 染色体に同定した。

これらの研究の結果から、タルホコムギの多様性を含む MSD 集団が野生種に由来する有用な量的形質を見出すための有望な遺伝資源であることが明かとなった。また、このアプローチは非常に効果的であり、高温耐性を見つけるために最も現実的で早く、経済的な方法であることが明らかとなった。同定した

QTL は高温耐性コムギを育種するためのマーカー選抜に利用することができる。この研究において、MSD 集団の遺伝的多様性を見出し、コムギの高温耐性育種が可能可能であることを示すことができた。今回選抜した高温耐性系統と感受性系統の交配によって、これら高温耐性に関与する QTL を明らかにすることができるであろう。

References

Al-Khatib, K. and G.M. Paulsen (1990) Photosynthesis and productivity during high-temperature stress of wheat genotypes from major world regions. Crop Sci. 30: 1127–1132.

Allard, R.W. (1960) Principles of plant breeding, John Wiley and Sons, New York, USA.

Amani, I., R.A. Fisher and M.P. Reynolds (1996) Canopy temperature depression association with yield of irrigated spring wheat cultivars in hot climates. J. Agric. Crop Sci. 176: 119–129.

Arabbeigi, M., A. Arzani, M.M. Majidi, R. Kiani, B.E.S. Tabatabaei, F. Habibi (2014) Salinity tolerance of *Aegilops cylindrica* genotypes collected from hyper-saline shores of Uremia Salt Lake using physiological traits and SSR markers. Acta Pysiol. Plant 36: 2243−2251.

Asana, R.D. and R.F. Williams (1965) The effect of temperature stress on grain development in wheat. Aust. J. Agric. Res. 16: 1–3.

Asseng, S., F. Ewert, P. Martre, R.P. Rötter, D.B. Lobell, D. Cammarano, B.A. Kimball, M.J. Ottman, G.W. Wall, J.W. White *et al.* (2014) Rising temperatures reduce global wheat production. Nat. Clim. Change 5: 143–147.

Asseng, S., I. Foster and N.C. Turner (2011) The impact of temperature variability on wheat yields. Glob. Change Biol. 17: 997–1012.

Barloy, D., J. Lemoine, P. Abelard, A.M. Tanguy, R. Rivoal and J. Jahier (2007) Marker-assisted pyramiding of two cereal cyst nematode resistance genes from Aegilops variabilis in wheat. Mol. Breed. 20: 31–40.

Blum, A. (1998) Improving wheat grain filling under stress by stem reserve mobilization. Euphytica 100: 77–83.

Chaves, M.S., J.A. Martinelli, C. Wesp-Guterres, F.A.S. Graichen, S. Brammer, S.M. Scagliusi, P.R. Da Sliva, P. Wietholter, G.A.M. Tores, E.Y. Lau et al. (2013) The importance for food security of maintaining rust resistance in wheat. Food Security 5:157−176.

Chowdhry, M.A., M.A. Chaudhry, S.M.M. Gilani and M. Ahsan (2001) Genetic control of some yield attributes in bread wheat. Pak. J. Biol. Sci 4: 980–982.

CIMMYT (2004) Wild Wheat Relatives Help Boost Genetic Diversity, Mexico City.

Curtis, T. and N.G. Halford (2014) Food Security: the challenge of increasing wheat yield and the importance of not compromising food safety. Ann. App. Biol. 164:354-372.

Distelfeld, A., I. Cakmak, Z. Pelega, L. Ozturk, A.M. Y.H. Budak, Y. Saranga, and T. Fahima (2006) Multiple QTLs-effects of wheat Gpc-B1 locus on grain protein and micronutrient concentrations. Physiol. Planta. 0: 1–9.

Dubcovsky, J., G. Sant Maria, E. Epstein, M.-C. Luo, J. Dvorak (1996) Mapping of the K+/Na+ discrimination locus Kna1 in wheat. Theo. App. Gen. 92: 448−454.

Elbashir, A.A.E., Y.S.A. Gorafi, I.S.A. Tahir, J.-S. Kim and H. Tsujimoto (2017) Wheat multiple synthetic derivatives: a new source for heat stress tolerance adaptive traits. Breed. Sci. (in press).

FAOSTAT (2014) Food and Agriculture Organization of the United Nations Statistics Division. Rome: FAO.

Feldman M. (2001) Origin of cultivated wheat. *In*: Bonjean A.P. and W.J. Angus (eds.) The World Wheat Book: A History of Wheat Breeding, Lavoisier Publishing, Paris, pp. 3–53.

Ferris, R., R.H. Ellis, T.R. Wheeler and P. Hadley (1998) Effect of high temperature stress at anthesis on grain yield and biomass of field-grown crops of wheat. Ann. Bot. 82: 631– 639.

Fischer, K.S and G. Wood (1981) Breeding and selection for drought tolerance in tropical maize. *In*: Fischer, K.S., E.S. Jonson, G.O. Edmeades (eds.) Principles and Methods in Crop Improvement for Drought Resistance with Emphasis on Rice. Proceeding of the Symposium. IRRI, Philippines, pp 31−46.

Fischer, R. A (2008) The importance of grain or kernel number in wheat: a reply to Sinclair and Jamieson. Field Crops Res. 105: 15–21.

Gill, B.S., L. Huang, V. Kuraparthy, W.J. Raupp, D.L. Wilson and B. Friebe (2008) Alien genetic resources for wheat leaf rust resistance, cytogenetic transfer and molecular analysis. Aust.J. Agr. Res. 59: 197–205.

Gorafi, Y.S.A and H. Tsujimoto (2016) *Leymus racemosus*: A potential species of gene pool enrichment for wheat improvement. *In:* Rajpal, V.R., S.R. Rao and S.N. Raina (eds.) Sustainable Development and Biodiversity, Springer. Rev. 10: 1–15.

Halloran, G.M., F.C. Ogbonnaya and E.S. Lagudah (2008) *Triticum* (*Aegilops*) *tauschii* in the natural and artificial synthesis of hexaploid wheat. Aust. J. Agr. Res. 59: 457– 490.

Harb, A.M. and L.N. Jamil (2013) Response of three accessions of Jordanian (Aegilops crassa Bioss). and durum wheat to controlled drought. Jordan J. of Biol. Sci. 6:151–230.

Hazratkulova, S., R. Sharma, S. Alikulov, S. Islomov, T. Yuldashev, Z. Ziyaev, Z. Khalikulov, Z. Ziyadullaev and J. Turok (2012) Analysis of genotypic variation for normalized difference vegetation index and its relationship with grain yield in winter wheat under terminal heat stress. Plant Breed. 131: 716–721.

He, Z. and S. Rajaram (1994) Differential responses of bread wheat characters to high temperature. Euphytica 72:197–203.

Heidari, B., G. Saeidi, B.E. Sayed Tabatabaei and K. Suenaga (2012) QTLs involved in plant height, peduncle length and heading date of wheat (Triticum aestivum L.). Journal of Agricultural Sciences Technology 14:1093–1104.

Helbaek H. (1959) Domestication of food plants in the old world. Science 130: 365–372.

Holland, J. B. (2007) Genetic architecture of complex traits in plant. Current Opinion in Plant Biology 10:156–161.

Huang, L., D. Raats, H. Sela, V. Klymiuk, G. Lidzbarsky, L. Feng, T. Krugman and T. Fahima (2016) Evolution and adaptation of wild emmer wheat populations to biotic and abiotic stresses. Annual Rev. of Phyto. 54:279−301.

Ivans, L.T., I.F. Wardlaw, E. Zamski and A.A. Schaffer (1996) Photoassimilate distribution in plants and crops, Marcel Dekker, New York, 501–518.

Kajimura, T., K. Murai and T. Shigeo (2011) Distinct genetic regulation of flowering and grain filling period based on empirical study of D-genome diversity in synthetic hexaploid wheat lines. Breed. Sci. 61: 130–141.

Kazi, M. and M. Van Ginkel (2004) Wild wheat relatives help boost genetic diversity. CIMMYT News.

Khan, M.I., T. Mohammad, F. Subhan, M. Amin and S.T. Shah (20079 Agronomic evaluation of different bread wheat (Triticum aestivum L.) genotypes for terminal heat stress. Pakistan Journal of Botany 39: 2415−2425.

Kiani, R., A. Arzani, F. Habibi (2015) Physiology of salinity tolerance in Aegilops cylindrica. Acta Physiol. Plant 37:135−145.

Kihara H. (1944) Discovery of the DD-analyser, one of the ancestors of *Triticum Vulgare*. Agric. Hort. 19: 13–14.

Kruse, A. (1967) Intergeneric hybrids between *Hordeum vulgare* L. ssp. *distichum* (v Pallas 2n=14) and *Secale cereale* L. (v Petkus 2n=14). pp. 82–92. *In*: Royal Veterinary and Agricultural College Yearbook 1967. Copenhagen.

Kruse, A. (1969) Intergeneric hybrids between *Triticum aestivum* L. (v Koga, 2n=42) and *Avena sativa* L. (v Stal 2n=42) with pseudogamous seed formation. pp. 188–200. *In*: Royal Veterinary and Agricultural College Yearbook 1967. Copenhagen.

Kumar, S. and D.K. Ganguli (1993) Heterosis and inbreeding depression in bread wheat. *In*: Verma, M.M., D.S. Virk and G.S. Chahal (eds.) Heterosis breeding in crop plants: Theory and Application, Crop Improvement Society Symposium, Ludhiana, India, pp 62–3.

Laurie, D.A. (1997) Comparative genetics of flowering time. Plant Mol. Biol. 35: 167–177.

Li, H., P. Vikram, R. P. Singh, A. Kilian, J. Carling, J. Song, J. A. Burgueno-Ferreira, S. Bhavani, J. Huerta-Espino, T. Payne, D. Sehgal, P. Wenzl and S. Singh JA et al (2015) A high density GBS map of bread wheat and its application for dissecting complex disease resistance traits. BMC Genomics 16:216.

Li, J., H.-S, Wan and W.-Y. Yang (2014) Synthetic hexaploid wheat enhances variation and adaptive evolution of bread wheat in breeding processes. J. systematics Evo. 52: 735–742.

Lobell, D.B., A. Sibley and J.I. Ortiz-Monasterio (2012) Extreme heat effects n wheat senescence in India. Nat. Climate Chan. 2: 186 –189.

Lopes, M.S., M.P. Reynolds, C.L. McIntyre, K.L. Mathew, M.R. Jalal Kamali, M. Mossad, Y. Feltaous, I.S.A. Tahir, R. Chatrath, F. Ogbonnaya and M. Baum (2013) QTL for yield and associated traits in the Seri/Babax population grown across several environments in Mexico, in the West Asia, North Africa and South Asia regions. Theoretical Applied Genetics. 126:971−984.

Lopes, M.S., M.P. Reynolds, M.R. Jalal-Kamali, M. Moussa, Y. Feltaous, I.S.A. Tahir, N. Barma, M. Vargas, Y. Mannes and M. Baum (2012) The yield correlations of selectable physiological traits in a population of advanced spring wheat lines grown in warm and drought environments. Field Crops Res. 128: 129–136.

Lopes, M.S., S. Dreisigacker, R.J. Pena, S. Sukumaran and M.P. Reynolds (2014) Genetic characterization of Wheat Association Mapping Initiative (WAMI) panel for dissection of complex traits in spring wheat. Theoretical Applied Genetics. 128:453−464.

Lopes, S.M., I. El-basyoni, P.S. Baenziger, S. Singh, C. Royo, K. Ozbek, H. Aktas, E. Ozer, F. Ozdemir, A. Manickavelu, T. Ban and P. Vikram (2015) Exploiting genetic diversity from landraces in wheat breeding for adaptation to climate change. Journal of Experimental Botany 66:3477–3486.

Masoomi-Aladizgeh, F., A. Aalami, M. Esfahani, M.J. Aghaei, K. Mozaffari (2015) Identification of CBF14 and NAC2 genes in Aegilpos tauschii associated with resistance to freezing stress. Appl. Biochem. Biotech. 176: 1059-1070.

Matsuoka, Y., and S. Nasuda (2004) Durum wheat as a candidate for the unknown female progenitor of bread wheat: an empirical study with a highly fertile F_1 hybrid with *Aegilops tauschii* Coss. Theor. Appl. Genet. 109: 1710 –1717.

Moffate, J.M., R.G. Sears and G.M. Paulsen (1990) Wheat high temperature tolerance during reproductive growth. 1. Evaluation by chlorophyll fluorescence. Crop Sci. 30: 881–885.

Mondal, S., A.K. Joshi, J. Huerta-Espino and R.P. Singh (2015) Early maturity in wheat for adaptation to high temperature stress. *In*: Ogihara, Y., S. Takumi and H. Handa (eds.) Advances in Wheat Genetics: From Genome to Field. Proceedings of the 12th International Wheat Genetics Symposium. Yokohama, Japan Springer, pp 239−246.

Mujeeb-Kazi A., V. Rosas and S. Roldan. (1996) Conservation of the genetic variation of *Triticum tauschii* in synthetic hexaploid wheats and its potential utilization for wheat improvement. Genet. Resour. Crop Evol. 43: 129–134.

Mujeeb-Kazi, A and S. Rajaram (2002) Transferring alien genes from related species and genera from wheat improvement. *In*: Curtis B.C., S. Rajaram and H. Gomez Macpherson, (eds.) Bread Wheat: Improvement and Production, FAO, Rome, Italy, pp.199–215.

Navakode, S., K. Neumann, B. Kobiljski, U. Lohwasser and A. Borner (2014) Genome wide association mapping to identify aluminum tolerance loci in bread wheat. Euphytica 198:401−411.

Nevo, E and G. Chen (2010) drought and salt tolerances in wild relatives for wheat and barley improvements. Plant Cell Environ. 33:670−685.

Ogbonnaya, F.C., O. Abdalla, A. Mujeeb-Kazi, A. Kazi, G.N. Gosnian and E.S. Lagudah (2013) Synthetic hexaploid: harnessing species of the primary gene pool for wheat improvement. Plant Breed. Rev. 37: 35–122.

Okamoto, Y., T. Kajimura, T. M. Ikeda and S. Takumi (2012) Evidence from principal component analysis for improvement of grain shape-and spikelet morphology-related traits after hexaploid wheat speciation. Genes Genet. Syst. 87: 299–310.

Olivares-Villegas, J.J., M.P. Reynolds, and G.K. McDonald (2007) Drought adaptive attributes in the Seri/Babax hexaploid wheat population. Funct. Plant Biol. 34: 189–203.

Oliver, R.E., R.W. Stack, J.D. Miller, X. Cai (2007) Reaction of wild emmer wheat accessions to Fusarium head blight. Crop. Sci. 47:893–899.

Paliwal, R., M.S. Roder, U. Kumar, J.P. Srivastava and A.K. Joshi (2012) QTL mapping of terminal heat tolerance in hexaploid wheat (T. aestivum L.). Theoretical Applied Genetics 125: 561–575.

Peng, J.H., D. Sun and E. Nevo (2011) Domestication evolution, genetics and genomics in wheat. Mol. Breed. 28:281–301.

Prasad, P.V., S.R. Pisipati, R.N. Mutava and M.N. Tuinistra (2008) Sensitivity of grain sorghum to high temperature stress during reproductive development. Crop Sci. 48: 1911–1917.

Rahaie, M., G.P. Xue, and P.M. Schenk (2013) The role of transcription factors in wheat under different abiotic stresses. In: K. Vahdati and C. Leslie (eds) Abiotic Stress-Plant Responses and Applications in Agriculture. InTech, Rijeka, Croatia, pp 367-385.

Reynolds, M.P., C.S. Pierre, A.S.I. Saad, M. Vargas and A.G. Condon (2007) Evaluating potential genetic gains in wheat associated with stress-adaptive trait expression in elite genetic resources under drought and heat stress. Crop Science 47: S–172–S–189.

Reynolds, M.P., M. Balota, M.I.B. Delgado, I. Amani and R. A. Fischer (1994) Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. Aust. J. Plant Physiol. 21: 717–730.

Reynolds, M.P., R.P. Singh, A. Ibrahim, O.A. Ageeb, and J.S. Quick (1998) Evaluating physiological traits to complement empirical selection for wheat in warm environments. Euphytica 100: 85–94.

Reynolds, M.P., Y. Manes, A. Izanloo, A and P. Langridge (2009) Phenotyping for physiological and morphological breeding and gene discovery in wheat. Ann. Appl. Bio1. 155: 309–320.

Risch. N and K. Merikangas.1996. The future of genetic studies of complex human diseases. Science 273:1516−1517.

Saghai-Maroof, M.A., K.M. Soliman R.A. Jorgensen and R.W. Allard (1984) Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. USA 24: 8014–8018.

Sansaloni, C., C. Petroli, D. Jaccoud, J. Carling, F. Detering, D. Grattapaglia and A. Kilian (2011) Diversity Array Technology (DArT) and next-generation sequencing combined: genome wide, high-throughput, highly informative genotyping for molecular breeding of eucalyptus. BMC Proceed. 5:54.

Schneider, A., I. Molnar, and M. Molnar-Lang (2008) Utilization of Aegilops (goat grass) species to widen the genetic diversity of cultivated wheat. Euphytica 163:1–9.

Sehgal, D., P. Vikram, C.P. Sansaloni, C. Ortiz, C.S. Pierre and T. Payne, M. Ellis, A Amri, C.D. Petroli, P. Wenzl and S. Singh *et al.* (2015) Exploring and Mobilizing the Gene Bank Biodiversity for Wheat Improvement. Plos One 10: 1–18.

Serious, T., B. Heidari, H. Pakniyat, and M. Kamali (2014) Independent and combined effects of heat and drought stress in the Seri M82×Babax bread wheat population. Plant Breeding. 133: 702 –711.

Shahnoza, H., R.C. Sharma, S. Alikulov, S. Islomov, T. Yuldashev, Z. Ziyaev, Z. Khalikulov, Z. Ziyadullaev and J. Turok (2012) Analysis of genotypic variation for normalized difference vegetation index and its relationship with grain yield in winter wheat under terminal heat stress. Plant Breed. 131: 716–721.

Sharma, P., S. Sareen, M. Saini, A. Verma, B.S. Tyagi and I. Sharma (2014) Assessing genetic variation for heat tolerance in synthetic wheat lines using phenotypic data and molecular markers. Aust. J. Crop Sci. 8: 515–522.

Shavrukov, Y., P. Langridge, M. Tester, E. Nevo (2010) Wide genetic diversity of salinity tolerance, sodium exclusion and growth in wild emmer wheat, Triticum dicoccoides. Breed. Sci. 60: 426–435.

Shpiler, L and A. Blum (1991) Heat tolerance to yield and its components in different wheat cultivars. Euphytica 51:257–263.

Smillie, R.M. and S.E. Hetherington (1983) Stress tolerance and stress induced injury in crop plants measured by chlorophyll fluorescence in vivo. Chilling, freezing, ice cover, heat and high light. Plant Physiol. 74: 1043–1050.

Sohail, Q., T. Inoue, H. Tanaka, A. Elsadig Eltayeb, Y. Matsuoka and H. Tsujimoto (2011) Applicability of *Aegilops tauschii* drought tolerance traits to breeding of hexaploid wheat. Breed. Sci. 61: 347–357.

Sohail, Q., T. Inoue, H. Tanaka, A.E. Eltayeb, Y. Matsuoka, H. Tsujimouka (2011) Applicability of Aegilops tauschii drought tolerance traits to breeding of hexaploid wheat. Breed. Sci. 61:347−357.

Stoilova, T. and P. Spetsov (2006) Chromosome 6U from (Aegilops geniculata Roth) carrying powdery mildew resistance in bread wheat. Breed. Sci. 56:351–357.

Stone, P.J. and M. E. Nicolas (1994) Wheat cultivars very widely in their responses of grain yield and quality to short periods of post-anthesis heat stress. Aust. J. Plant Physiology. 21: 887–900.

Sukumaran, S and J. Yu (2014) Association mapping of genetic resources: achievements and future perspectives. In: Genomics of plant genetic resources, Springer, Netherlands, pp 207−235. doi: 10.1007/978−94−007−7572−5−9.

Suszkiw, J. (2005) Hessian Fly-Resistant Wheat Germplasm Available Agricultural Research Service, News and Events, United States Department of Agriculture.

Tahir I.S.A. and N. Nakata (2005) Remobilization of nitrogen and carbohydrate from stems of bread wheat in response to heat stress during grain filling. J. Agron. Crop Sci. 191: 105–116.

Tahir I.S.A., N. Nakata, A.M. Ali, H.M. Mustafa, A.S.I. Saad, K. Takata N. Ishikawa and O. S. Abdalla (2006) Genotypic and temperature effects on wheat grain yield and quality in a hot-irrigated environment. Plant Breed. 125: 323–330.

Tahir, I.S.A., A.B. Elahmadi, O.S. Abdalla, A.S. Ibrahim, O.E. Mohammed and N. Nakata (2005) Potential selection criteria for yield of bread wheat under early and late heat stress in a dry irrigated environment. Wheat Information Service 99: 35–40.

Tewolde, H., C.J. Fernandez and C.A. Erickson (2006) Wheat cultivars adapted to postheading high stress. J. Agron. Crop Sci. 192: 111–120.

Tsujimoto, H., Q. Sohail and Y. Matsuoka. (2011) Broadening the genetic diversity of common and durum wheat for abiotic stress tolerance breeding. Chapter 25: 233–238. Proceeding of the 12th International Wheat Genetic Symposium. 8–14 September 2013, Yokohama, Japan.

Tsujimoto, H., Q. Sohail and Y. Matusoka (2015) Broadening the genetic diversity of common and durum wheat for abiotic stress tolerance breeding. *In*: Ogihara, Y., S. Takumi and H. Handa (eds.) Advances in Wheat Genetics: From Genome to Field. Proceedings of the 12th International Wheat Genetics Symposium. Yokohama, Japan Springer, pp 233−238.

USDA (2016) World Agricultural Production. USDA Foreign Agricultural Service, Washington DC 20250. Available at: http://apps.fas.usda.gov/psdonline/circulars/grain-wheat.pdf

Van Ginkel, M. and F. Ogbonnaya (2007) Novel genetic diversity from synthetic wheats in breeding cultivars for changing production conditions. Field Crops Res. 104: 86−94.

Yesayan, A.H., K.V. Grigorin, A.M. Danielian, N.A. Hovhannisyan (2009) Determination of salt tolerance in wild einkorn wheat (Triticum boeoticum Bioss.) under in vitro conditions. Crop Wild Relat. 7:4−7.

Zaharieva, M., E. Gaulin, M. Havaux, E. Acevedo, P. Monneveux (2001) Drought and heat responses in wild wheat relatives Aegilops geniculata Roth: potential interest for wheat improvement. Crop Sci. 41:1321−1329.

Zamani-Bangohari, M., A. Niazi, A.A. Moghaddam, T. Deihimi, E. Ebrahimie (2013) Genome-wide analysis of key salinity tolerance transporter (HKT;5) in wheat and wild wheat relatives (A and D genomes). In Vitro Cell Dev Biol Plant 49:97−106.

Zhu, C.M., E.S. Gore-Buckler and J. Yu (2008) Status and prospects of association mapping in plants. Plant Genome Journal 1:5. do: 10.3835/plant genome2008.02.0089

List of published papers

Chapter 1:

Title: Wheat multiple synthetic derivatives: a new source for heat stress tolerance adaptive traits

Authors: Awad Ahmed Elawad Elbashir, Yasir Serag Alnor Gorafi, Izzat Sidahmed Ali Tahir, June-Sik Kim, and Hisashi Tsujimoto

Journal with volume number and pages: Breeding Science 67 (3): 248−256.

Published Online: June 14, 2017

Chapter 2:

Title: Genetic variation in heat tolerance–related traits in a population of wheat multiple synthetic derivatives

Authors: Awad Ahmed Elawad Elbashir, Yasir Serag Alnor Gorafi, Izzat Sidahmed Ali Tahir, Ashraf Mohamed Ahmed Elhashimi, Modather Glal Abdeldaim Abdalla and Hisashi Tsujimoto

Journal, Breeding Science, advance online publication. Doi:10.1270/jsbbs.17048.

Published Online: December 1, 2017

Appendix

Pedigree of 399 lines of multiple synthetic derivatives population

Entry no.	Cross name
MSD001	N61/Syn32//*N61
MSD002	N61/Syn27//*N61
MSD003	N61/Syn42//*N61
MSD004	N61/Syn28//*N61
MSD005	N61/Syn65//*N61
MSD006	N61/Syn38//*N61
MSD007	N61/Syn55//*N61
MSD008	N61/Syn57//*N61
MSD009	N61/Syn32//*N61
MSD010	N61/Syn71//*N61
MSD011	N61/Syn33//*N61
MSD012	N61/Syn57//*N61
MSD013	N61/Syn32//*N61
MSD014	N61/Syn52//*N61
MSD015	N61/Syn52//*N61
MSD016	N61/Syn42//*N61
MSD017	N61/Syn42//*N61
MSD018	N61/Syn54//*N61
MSD019	N61/Syn29//*N61
MSD020	N61/Syn29//*N61
MSD021	N61/Syn64//*N61
MSD022	N61/Syn71//*N61
MSD023	N61/Syn30//*N61
MSD024	N61/Syn37//*N61
MSD025	N61/Syn57//*N61
MSD026	N61/Syn58//*N61
MSD027	N61/Syn57//*N61
MSD028	N61/Syn38//*N61
MSD029	N61/Syn29//*N61
MSD030	N61/Syn32//*N61
MSD031	N61/Syn32//*N61
MSD032	N61/Syn71//*N61
MSD033	N61/Syn36//*N61
MSD034	N61/Syn29//*N61

Entry no.	Cross name
MSD035	N61/Syn27//*N61
MSD036	N61/Syn38//*N61
MSD037	N61/Syn71//*N61
MSD039	N61/Syn60//*N61
MSD040	N61/Syn35//*N61
MSD041	N61/Syn39//*N61
MSD042	N61/Syn40//*N61
MSD043	N61/Syn44//*N61
MSD044	N61/Syn34//*N61
MSD045	N61/Syn68//*N61
MSD046	N61/Syn26//*N61
MSD047	N61/Syn51//*N61
MSD048	N61/Syn44//*N61
MSD049	N61/Syn28//*N61
MSD050	N61/Syn32//*N61
MSD051	N61/Syn26//*N61
MSD052	N61/Syn35//*N61
MSD053	N61/Syn27//*N61
MSD054	N61/Syn55//*N61
MSD055	N61/Syn54//*N61
MSD056	N61/Syn31//*N61
MSD057	N61/Syn26//*N61
MSD058	N61/Syn37//*N61
MSD059	N61/Syn26//*N61
MSD060	N61/Syn57//*N61
MSD061	N61/Syn42//*N61
MSD062	N61/Syn42//*N61
MSD063	N61/Syn32//*N61
MSD064	N61/Syn68//*N61
MSD065	N61/Syn40//*N61
MSD066	N61/Syn49//*N61
MSD067	N61/Syn42//*N61
MSD068	N61/Syn44//*N61
MSD069	N61/Syn47//*N61
MSD070	N61/Syn30//*N61
MSD071	N61/Syn33//*N61
MSD072	N61/Syn68//*N61

Entry no.	Cross name
MSD073	N61/Syn47//*N61
MSD074	N61/Syn54//*N61
MSD075	N61/Syn52//*N61
MSD076	N61/Syn72//*N61
MSD077	N61/Syn29//*N61
MSD078	N61/Syn36//*N61
MSD079	N61/Syn32//*N61
MSD080	N61/Syn39//*N61
MSD081	N61/Syn38//*N61
MSD082	N61/Syn27//*N61
MSD083	N61/Syn32//*N61
MSD084	N61/Syn59//*N61
MSD085	N61/Syn29//*N61
MSD086	N61/Syn51//*N61
MSD087	N61/Syn65//*N61
MSD088	N61/Syn45//*N61
MSD089	N61/Syn32//*N61
MSD090	N61/Syn32//*N61
MSD091	N61/Syn44//*N61
MSD092	N61/Syn52//*N61
MSD093	N61/Syn28//*N61
MSD094	N61/Syn28//*N61
MSD095	N61/Syn30//*N61
MSD096	N61/Syn37//*N61
MSD097	N61/Syn26//*N61
MSD098	N61/Syn42//*N61
MSD099	N61/Syn26//*N61
MSD100	N61/Syn32//*N61
MSD101	N61/Syn28//*N61
MSD102	N61/Syn26//*N61
MSD103	N61/Syn50//*N61
MSD104	N61/Syn30//*N61
MSD105	N61/Syn33//*N61
MSD106	N61/Syn42//*N61
MSD107	N61/Syn67//*N61
MSD108	N61/Syn50//*N61
MSD109	N61/Syn50//*N61

Entry no.	Cross name
MSD110	N61/Syn47//*N61
MSD111	N61/Syn34//*N61
MSD112	N61/Syn72//*N61
MSD113	N61/Syn26//*N61
MSD114	N61/Syn31//*N61
MSD115	N61/Syn48//*N61
MSD116	N61/Syn39//*N61
MSD117	N61/Syn32//*N61
MSD118	N61/Syn68//*N61
MSD119	N61/Syn62//*N61
MSD120	N61/Syn44//*N61
MSD121	N61/Syn31//*N61
MSD122	N61/Syn64//*N61
MSD123	N61/Syn26//*N61
MSD124	N61/Syn65//*N61
MSD125	N61/Syn42//*N61
MSD126	N61/Syn26//*N61
MSD127	N61/Syn51//*N61
MSD128	N61/Syn36//*N61
MSD129	N61/Syn32//*N61
MSD130	N61/Syn68//*N61
MSD131	N61/Syn40//*N61
MSD132	N61/Syn26//*N61
MSD133	N61/Syn63//*N61
MSD134	N61/Syn32//*N61
MSD135	N61/Syn71//*N61
MSD136	N61/Syn39//*N61
MSD137	N61/Syn31//*N61
MSD138	N61/Syn28//*N61
MSD139	N61/Syn62//*N61
MSD140	N61/Syn39//*N61
MSD141	N61/Syn37//*N61
MSD142	N61/Syn28//*N61
MSD143	N61/Syn32//*N61
MSD144	N61/Syn42//*N61
MSD145	N61/Syn40//*N61
MSD146	N61/Syn30//*N61

Entry no.	Cross name
MSD147	N61/Syn44//*N61
MSD148	N61/Syn68//*N61
MSD149	N61/Syn60//*N61
MSD150	N61/Syn68//*N61
MSD151	N61/Syn42//*N61
MSD152	N61/Syn33//*N61
MSD153	N61/Syn32//*N61
MSD154	N61/Syn26//*N61
MSD155	N61/Syn30//*N61
MSD156	N61/Syn68//*N61
MSD157	N61/Syn57//*N61
MSD158	N61/Syn28//*N61
MSD159	N61/Syn32//*N61
MSD160	N61/Syn42//*N61
MSD161	N61/Syn30//*N61
MSD162	N61/Syn26//*N61
MSD163	N61/Syn27//*N61
MSD164	N61/Syn38//*N61
MSD165	N61/Syn60//*N61
MSD166	N61/Syn60//*N61
MSD167	N61/Syn72//*N61
MSD168	N61/Syn38//*N61
MSD169	N61/Syn42//*N61
MSD170	N61/Syn54//*N61
MSD171	N61/Syn30//*N61
MSD172	N61/Syn26//*N61
MSD173	N61/Syn32//*N61
MSD174	N61/Syn50//*N61
MSD175	N61/Syn32//*N61
MSD176	N61/Syn44//*N61
MSD177	N61/Syn68//*N61
MSD178	N61/Syn33//*N61
MSD179	N61/Syn29//*N61
MSD180	N61/Syn42//*N61
MSD181	N61/Syn39//*N61
MSD182	N61/Syn55//*N61
MSD183	N61/Syn58//*N61

Entry no.	Cross name
MSD184	N61/Syn64//*N61
MSD185	N61/Syn61//*N61
MSD186	N61/Syn48//*N61
MSD187	N61/Syn28//*N61
MSD188	N61/Syn39//*N61
MSD189	N61/Syn60//*N61
MSD190	N61/Syn61//*N61
MSD191	N61/Syn49//*N61
MSD192	N61/Syn34//*N61
MSD193	N61/Syn51//*N61
MSD194	N61/Syn26//*N61
MSD195	N61/Syn26//*N61
MSD196	N61/Syn51//*N61
MSD197	N61/Syn27//*N61
MSD198	N61/Syn49//*N61
MSD199	N61/Syn30//*N61
MSD200	N61/Syn52//*N61
MSD201	N61/Syn63//*N61
MSD202	N61/Syn47//*N61
MSD203	N61/Syn26//*N61
MSD204	N61/Syn32//*N61
MSD205	N61/Syn31//*N61
MSD206	N61/Syn69//*N61
MSD207	N61/Syn45//*N61
MSD208	N61/Syn60//*N61
MSD209	N61/Syn56//*N61
MSD210	N61/Syn55//*N61
MSD211	N61/Syn42//*N61
MSD212	N61/Syn48//*N61
MSD213	N61/Syn29//*N61
MSD214	N61/Syn39//*N61
MSD215	N61/Syn44//*N61
MSD216	N61/Syn47//*N61
MSD217	N61/Syn42//*N61
MSD218	N61/Syn28//*N61
MSD219	N61/Syn65//*N61
MSD220	N61/Syn63//*N61

Entry no.	Cross name
MSD221	N61/Syn37//*N61
MSD222	N61/Syn30//*N61
MSD223	N61/Syn35//*N61
MSD224	N61/Syn60//*N61
MSD225	N61/Syn40//*N61
MSD226	N61/Syn28//*N61
MSD227	N61/Syn62//*N61
MSD228	N61/Syn32//*N61
MSD229	N61/Syn42//*N61
MSD230	N61/Syn48//*N61
MSD231	N61/Syn37//*N61
MSD232	N61/Syn59//*N61
MSD233	N61/Syn26//*N61
MSD234	N61/Syn31//*N61
MSD235	N61/Syn71//*N61
MSD236	N61/Syn30//*N61
MSD237	N61/Syn55//*N61
MSD238	N61/Syn32//*N61
MSD239	N61/Syn32//*N61
MSD240	N61/Syn68//*N61
MSD241	N61/Syn35//*N61
MSD242	N61/Syn33//*N61
MSD243	N61/Syn68//*N61
MSD244	N61/Syn62//*N61
MSD245	N61/Syn28//*N61
MSD246	N61/Syn68//*N61
MSD247	N61/Syn60//*N61
MSD248	N61/Syn68//*N61
MSD249	N61/Syn32//*N61
MSD250	N61/Syn27//*N61
MSD251	N61/Syn57//*N61
MSD252	N61/Syn44//*N61
MSD253	N61/Syn31//*N61
MSD254	N61/Syn53//*N61
MSD255	N61/Syn35//*N61
MSD256	N61/Syn42//*N61
MSD257	N61/Syn58//*N61

Entry no.	Cross name
MSD258	N61/Syn29//*N61
MSD259	N61/Syn56//*N61
MSD260	N61/Syn26//*N61
MSD261	N61/Syn64//*N61
MSD262	N61/Syn34//*N61
MSD263	N61/Syn62//*N61
MSD264	N61/Syn26//*N61
MSD265	N61/Syn64//*N61
MSD266	N61/Syn28//*N61
MSD267	N61/Syn55//*N61
MSD268	N61/Syn33//*N61
MSD269	N61/Syn68//*N61
MSD270	N61/Syn42//*N61
MSD271	N61/Syn52//*N61
MSD272	N61/Syn59//*N61
MSD273	N61/Syn42//*N61
MSD274	N61/Syn68//*N61
MSD275	N61/Syn27//*N61
MSD276	N61/Syn72//*N61
MSD277	N61/Syn27//*N61
MSD278	N61/Syn33//*N61
MSD279	N61/Syn37//*N61
MSD280	N61/Syn48//*N61
MSD281	N61/Syn35//*N61
MSD282	N61/Syn26//*N61
MSD283	N61/Syn40//*N61
MSD284	N61/Syn40//*N61
MSD285	N61/Syn26//*N61
MSD286	N61/Syn34//*N61
MSD287	N61/Syn31//*N61
MSD288	N61/Syn51//*N61
MSD289	N61/Syn26//*N61
MSD290	N61/Syn44//*N61
MSD291	N61/Syn42//*N61
MSD292	N61/Syn36//*N61
MSD293	N61/Syn57//*N61
MSD294	N61/Syn64//*N61

Entry no.	Cross name
MSD295	N61/Syn69//*N61
MSD296	N61/Syn26//*N61
MSD297	N61/Syn26//*N61
MSD298	N61/Syn66//*N61
MSD299	N61/Syn57//*N61
MSD300	N61/Syn48//*N61
MSD301	N61/Syn62//*N61
MSD302	N61/Syn40//*N61
MSD303	N61/Syn29//*N61
MSD304	N61/Syn68//*N61
MSD305	N61/Syn72//*N61
MSD306	N61/Syn68//*N61
MSD307	N61/Syn27//*N61
MSD308	N61/Syn39//*N61
MSD309	N61/Syn42//*N61
MSD310	N61/Syn26//*N61
MSD311	N61/Syn32//*N61
MSD312	N61/Syn29//*N61
MSD313	N61/Syn27//*N61
MSD314	N61/Syn48//*N61
MSD315	N61/Syn72//*N61
MSD316	N61/Syn32//*N61
MSD317	N61/Syn63//*N61
MSD318	N61/Syn53//*N61
MSD319	N61/Syn39//*N61
MSD320	N61/Syn32//*N61
MSD321	N61/Syn48//*N61
MSD322	N61/Syn30//*N61
MSD323	N61/Syn27//*N61
MSD324	N61/Syn30//*N61
MSD325	N61/Syn62//*N61
MSD326	N61/Syn56//*N61
MSD327	N61/Syn30//*N61
MSD328	N61/Syn44//*N61
MSD329	N61/Syn47//*N61
MSD330	N61/Syn51//*N61
MSD331	N61/Syn44//*N61

Entry no.	Cross name
MSD332	N61/Syn60//*N61
MSD333	N61/Syn36//*N61
MSD334	N61/Syn57//*N61
MSD335	N61/Syn53//*N61
MSD336	N61/Syn27//*N61
MSD337	N61/Syn29//*N61
MSD338	N61/Syn48//*N61
MSD339	N61/Syn68//*N61
MSD340	N61/Syn32//*N61
MSD341	N61/Syn55//*N61
MSD342	N61/Syn26//*N61
MSD343	N61/Syn71//*N61
MSD344	N61/Syn68//*N61
MSD345	N61/Syn68//*N61
MSD346	N61/Syn58//*N61
MSD347	N61/Syn32//*N61
MSD348	N61/Syn68//*N61
MSD349	N61/Syn62//*N61
MSD350	N61/Syn42//*N61
MSD351	N61/Syn58//*N61
MSD352	N61/Syn57//*N61
MSD353	N61/Syn26//*N61
MSD354	N61/Syn52//*N61
MSD355	N61/Syn33//*N61
MSD356	N61/Syn28//*N61
MSD357	N61/Syn40//*N61
MSD358	N61/Syn60//*N61
MSD359	N61/Syn42//*N61
MSD360	N61/Syn29//*N61
MSD361	N61/Syn68//*N61
MSD362	N61/Syn28//*N61
MSD363	N61/Syn60//*N61
MSD364	N61/Syn26//*N61
MSD365	N61/Syn64//*N61
MSD366	N61/Syn39//*N61
MSD367	N61/Syn32//*N61
MSD368	N61/Syn50//*N61

Entry no.	Cross name
MSD369	N61/Syn31//*N61
MSD370	N61/Syn40//*N61
MSD371	N61/Syn54//*N61
MSD372	N61/Syn31//*N61
MSD373	N61/Syn32//*N61
MSD374	N61/Syn68//*N61
MSD375	N61/Syn42//*N61
MSD376	N61/Syn60//*N61
MSD377	N61/Syn26//*N61
MSD378	N61/Syn64//*N61
MSD379	N61/Syn32//*N61
MSD380	N61/Syn50//*N61
MSD381	N61/Syn26//*N61
MSD382	N61/Syn30//*N61
MSD383	N61/Syn61//*N61
MSD384	N61/Syn26//*N61
MSD385	N61/Syn58//*N61
MSD386	N61/Syn42//*N61
MSD387	N61/Syn59//*N61
MSD388	N61/Syn30//*N61
MSD389	N61/Syn68//*N61
MSD390	N61/Syn28//*N61
MSD391	N61/Syn54//*N61
MSD392	N61/Syn64//*N61
MSD393	N61/Syn37//*N61
MSD394	N61/Syn37//*N61
MSD395	N61/Syn60//*N61
MSD396	N61/Syn29//*N61
MSD397	N61/Syn68//*N61
MSD398	N61/Syn66//*N61
MSD399	N61/Syn34//*N61