Heat stress is detrimental to wheat (Triticum aestivum L.) productivity. In this study, we aimed to select heat-tolerant plants from a multiple synthetic derivatives (MSD) population and evaluate their agronomic and physiological traits. We 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. We grew these lines with N61 in the field and growth chamber. In the field, we used optimum and late sowings to ensure plant exposure to heat. In the growth chamber, in addition to N61, we used the heat-tolerant cultivars ‘Gelson’ and ‘Bacanora’. We confirmed that MNH2 and MNH5 lines acquired heat tolerance. These lines had higher photosynthesis and stomata conductance and exhibited no reduction in grain yield and biomass under heat stress compared to N61. We noticed that N61 had relatively good adaptability to heat stress. Our results indicate that the MSD population includes the diversity of Aegilops tauschii and is a promising resource to uncover useful quantitative traits derived from this wild species. Selected lines could be useful for heat stress tolerance breeding.

Key Words: heat stress, multiple synthetic derivatives, Aegilops tauschii, drought.
variation in drought stress-related characteristics both among the accessions of *Ae. tauschii* and SHWs. However, they found low correlation between the performance of the accessions and that of the corresponding SHW 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 the 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, we 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 (Gorafi *et al.* 2016, Tsujimoto *et al.* 2015).

Here, we used an MSD population (BC$_1$F$_4$) derived from the bread wheat cultivar ‘Norin 61’ and selected six plants showing good performance under heat stress in Sudan. We took the seeds separately from each of the six plants and developed six heat stress-tolerant lines. We 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, we confirmed that two are heat-tolerant genotypes and can be used to breed heat stress-tolerant high-yielding wheat varieties.

**Materials and Methods**

**Plant materials**

We 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) (Kajimura *et al.* 2011, Matsuoka and Nasuda 2004). To produce the initial population (4300 grains), we mixed 10 grains (BC$_1$F$_2$) from each of the 10 BC$_1$F$_1$ plants. We cultivated the plants as a bulk in Tottori, Japan and harvested the BC$_1$F$_3$ seeds; they were sown at Gezira Research Farm, Agricultural Research Corporation, Sudan (14°24′N, 33°29′E, 407 m above sea level) in the 2014/2015 season. The MSD population showed various phenotypes in spike, leaf, and plant traits at maturity time. We selected six plants that we felt may be heat-tolerant. These plants showed vigorous growth and remaining green leaves at maturity compared to the adjacent plants which were completely dry. We named these lines MNH1 to MNH6 for “MSD population of ‘Norin 61’ selected as heat stress-tolerant”. In addition, we also used two heat-tolerant, highly productive varieties, ‘Bacanora’ and ‘Gelenson’, from the International Maize and Wheat Improvement Center (CIMMYT) in this study.

**Evaluation of MNH lines in the field**

We cultivated the selected six plants as a bulk in Tottori, Japan and harvested the BC$_1$F$_3$ seeds. In the 2015/2016 season, we 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; which was used to expose plants to heat stress at the reproductive stage). We obtained data on the 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°C; min., 23°C) than in optimum sowing (max., 37°C; min., 15°C) (Fig. 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
We 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. 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).

**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., *PstI/HpaII* and *PstI/HhaI* were used to select a subset of *PstI-HpaII* and *PstI-HhaI* fragments, respectively (Sansaloni et al. 2011). At the DArT facility, the DArT soft marker extraction pipeline was used to filter the markers on the basis of reproducibility (the percentage of technical replicate pairs scoring identically for a given marker), call rate (the percentage of samples for which a given marker was scored), and the average read depth (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, we 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...
Results

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 2–7).

1. 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 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

| Table 2. Days to heading (DH) and grain filling duration (GFD) of ‘Norin 61’ and MNH lines in optimum sowing (OS), late sowing (LS) and the difference between LS and OS |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Line            | OS DH | LS DH | LS–OS DH | OS GFD | LS GFD | LS–OS GFD |
| 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 ** 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 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)

| Line   | PH (cm) OS | PH (cm) LS | PH (cm) RP (%) OS | PH (cm) LS | PH (cm) 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       | 91.0             | 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 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)

| Line   | KSP OS | KSP LS | KSP RP (%) OS | KSP LS | KSP RP (%) |
|--------|--------|--------|---------------|--------|------------|
| Norin 61 | 47     | 53     | 113.0         | 26.0   | 25.4       | 98.0         |
| MNH1   | 52     | 49     | 94.0          | 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     | 97.0          | 29.1   | 27.6       |
| G ** ** ** |          |        |              | **      |          |
| LSD (G) | 6.896   | 6.857  | 4.272         | 4.438  |            |
| SD ** | 1.650    |        |              | **      |            |
| G × SD ** NS |        |        |              | **      |            |
| 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 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)

| Line   | BIO (g/m²) OS | BIO (g/m²) LS | BIO (g/m²) RP (%) OS | BIO (g/m²) LS | BIO (g/m²) 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                  | 523           | 420              |
| G ** ** ** |          |              | **                   | **           | **               |
| LSD (G) | 9.128     | 172.6        | 53.41                | 96.69        |                |
| SD ** |          |              |                     | **           |            |
| G × SD ** NS |        |              |                     | **           |            |
| 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.
The DH values of MNH3, MNH4, and MNH6 were lower (by 9–14 days) in late sowing than in optimum sowing; this response is favoring the escape of impact of high temperature by shortening the DH.

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

| Lines   | CT-h (°C) | CT-gf (°C) |
|---------|-----------|------------|
|         | 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 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.

2. Plant height and peduncle length

Plant height (PH) values of MNH3, MNH5, and MNH6 were not affected by sowing conditions (Table 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 3). PL of N61 was also significantly reduced to 73%, whereas the decrease in MNH1 89% was not significant.

3. 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 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 N61 was reduced to 80% (Table 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 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 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.

4. 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 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 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 were higher in MNH lines than in N61 in both conditions. In late sowing, NDVI-gf was reduced to 71% in N61 and to 83% in MNH4 and increased to 120% in MNH2 (Table 7).

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

**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. 2A). Stomatal conductance was not affected by heat stress in N61, ‘Bacanora’, and MNH4, but was increased in MNH1, MNH2, and MNH5 (Fig. 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 stomata conductance than all other cultivars under heat stress. The increase in stomatal conductance suggests that these three lines might have a mechanism for drought tolerance and we need to evaluate them under drought conditions.

**Genotyping**

Using DArT-seq markers, we identified the parental synthetic lines of each line and found that the six lines originated from different synthetic lines (Table 8). Common chromosomal regions were not found among the six MNH lines. However, we noticed that the MNH2 and MNH5 tolerant lines were different from the other MNH lines at some chromosomal regions (Fig. 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. We 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 subgenomes indicating that the performance of the SHW lines depends also 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. 3). This result indicates that the MNH lines carry many A-genome genes from the synthetic wheat parents.

**Table 8. Pedigrees of the six synthetic derivative 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. 2. Photosynthetic rate (A) and stomatal conductance (B) of the six wheat genotypes and ‘Norin 61’, ‘Gelenson’, and ‘Bacanora’ as heat-tolerant lines grown 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. 3. Graphical genotyping maps of six MNH lines drawn 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 top (1) to bottom (7) in each sub-genome.
The wheat ancestor *Ae. tauschii*, which provided the D genome, is a rich source of stress resistance genes (Mujeeb-Kazi and Rajaram 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 contains a large amount of genetic variation for useful genes or traits of biotic and abiotic stress for bread wheat breeding and is usually genetically unstable for the numbers of chromosomes after crossing with the common wheat; to solve this problem we cross and backcross SHW with N61. On the other hand, the morphology of the six MNH lines is similar to the morphology of common wheat in spike traits and grain 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, we 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).

Our 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 2). 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. Our present study is in good agreement with their results.

All lines except MNH3 had longer peduncles than N61 in late sowing (Table 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 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 to maintain 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 7), indicating that these lines have genes to stay green during the grain filling and are able to perform efficient and stable grain filling and are adapted to high temperature.

MNH1, MNH2, and MNH5 had better photosynthesis under heat stress than N61 and the heat-tolerant check cultivars ‘Bacanora’ and ‘Gelsonon’ in term of no reduction and also net photosynthesis and stomatal conductance under heat stress (Fig. 3). On the other hand, unlike N61, MNH2 and MNH5 showed increased BIO and GY in late sowing compared to the optimum sowing (Table 5), indicating that they are more tolerant than N61. However, their GY and BIO values were lower than those 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. 2).

In this study, we unexpectedly found that N61 is relatively adaptable to heat stress conditions when we compared its performance between the control and heat stress conditions. However, this finding needs 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, we 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. We 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. 3). Although we cannot assess the consistency between our 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. 3). Thus, we have detailed information on the pedigree (Table 8). Our 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. 3), we can conclude that the performance of the tolerant MNH lines is a result of the N61 and *Ae. tauschii* genes and their interaction.
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 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, we found rich genetic diversity in the MSD population, which is available for wheat breeding for heat stress tolerance.

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