Assessment of terminal heat stress tolerance in doubled haploids derived from synthetic hexaploid wheat (*Triticum aestivum* L.) using genetic variability and PCA-based cluster analyses

Abstract

A comprehensive study was conducted on germplasm derived from two synthetic wheats and two hexaploid wheats i.e., SHW14102 x BWL4444, SHW14102 x BWL3531, and SHW3761 x BWL4444 to identify the potential doubled haploids that can withstand high temperatures, particularly at the reproductive stage during crop seasons viz., 2020–2021 and 2021–2022. The doubled haploid lines were selected based on phenotypic characteristics using genetic variability analysis. In the preliminary field trial evaluation, 100 lines were selected based on high phenotypic uniformity for further testing against terminal heat stress conditions and high yield potential. Among these, 17 lines had relatively higher values for days to biomass yield, grain yield, thousand-grain weight, and harvest index and relatively lower values for maturity, and grain filling duration based on principal component and cluster analyses. Principal component analysis (PCA) based cluster analysis exhibited that, clusters II and III had clear separation compared to cluster I. All three clusters were analyzed according to their means and standard deviations. The mean values for GYP (385.07), BMYP (1162.37), and TGW (38.13) were relatively higher in cluster 1 than in other clusters. Cluster 2 exhibited a higher value for DH (89.14), SL with awns (18.21), and GFD (18.19) while cluster 3 showed a higher value for HI (41.02). Thus, it was concluded that cluster I demonstrated superior performance in grain yield per plant, biomass yield per plant, and thousand-grain weight, indicating its overall higher productivity compared to the other clusters under terminal heat stress conditions and suggesting them as ~~a~~ potential germplasm for future breeding programs.

Keywords: Doubled haploids, Genetic variability, Heat stress, PCA, Synthetics, Wheat

Introduction

To meet the increasing global food demand, projected to surpass 9 billion by 2050, wheat production (*Triticum aestivum* L.) needs to be raised by 70% (Neupane et al. 2022). According to agricultural statistics, India achieved a record wheat production of 108.75 million tons in 2020-21, exceeding the average production of 100.42 million tons by 8.32 million tons (source: https://pib.gov.in). Wheat is a strategic staple food crop providing a significant proportion of nutrition to the ever-burgeoning world population (Taha et al. 2021; Jones et al. 2023). It is crucial to enhance wheat production for long-term food security (Ahmed et al. 2018). Consequently, the limited timeframe available for the development of desired wheat varieties poses a challenge. Therefore, the doubled-haploid technique is a highly efficient approach for generating entirely homozygous lines within a single generation, thereby expediting the introduction of new wheat varieties with desired traits and minimizing associated costs as well as time. Further, as a cool-season crop, wheat requires optimal temperatures ranging from 12 to 25 °C for successful germination and seedling establishment, while the ideal temperature range for anthesis and grain filling is between 12 and 22 °C (Djanaguiraman et al. 2020). Elevated temperatures significantly constrain crop growth and productivity worldwide, leading to a substantial reduction in agricultural yield (Asseng et al. 2019). The global temperature is expected to rise by 1.5-4.5 °C by the end of the current century, and it is predicted that each 1 °C increase will result in a 3-17% decline in wheat yield in the South Asian wheat-growing regions, particularly in India and Pakistan (Al-Ashkar et al. 2020).

The ideal temperatures in the second week of November create optimal conditions for the successful germination, establishment of seedlings, and tillering of wheat in these regions. However, this timing often exposes the crop to heat stress towards the end of the growing season, resulting in reduced overall yield. Elevated temperatures (more than 27 °C) during the germination stage can lead to embryo death and a decline in the rate of seedling establishment (Sharma et al. 2022). Reduced photosynthesis in plants is caused by poor germination, smaller leaf areas, early leaf senescence, and impaired photosynthetic machinery linked to terminal heat stress ~~condition~~ conditions. Doubled haploids are suggested as breeding tools by which useful variation can be generated and time can be saved compared to conventional breeding (Eliby et al. 2022).

Therefore, to yearn for more yield and resistance to heat exposure at the grain-filling stage, the search ends with ~~the~~ selection of the doubled haploids that can germinate and establish themselves at seedling stage heat stress and grow into healthier plants. The present investigation was targeted to explore the grain-filling stage heat stress tolerance in doubled haploids based on their phenological response under timely and late sowing regimes.

2. Materials and methods

Plant material and experimental design

Two *Ae. tauschii* accessions viz., *Ae. tauschii* 14102 amphiploid and *Ae. tauschii* 3761 amphiploid were selected from the wild wheat germplasm available at Punjab Agricultural University, Ludhiana (India). The selection was based on their stay green character and higher grain weight (Chhuneja et al. 2010). These selected *Ae. tauschii* accessions were crossed with tetraploid wheat *T. durum*, PBW114, further selfing and selection leading to the development of synthetic hexaploid wheat PBW114-*Ae. tauschii* pau 14102 (SHW14102) and PBW114-*Ae. tauschii* pau 3761(SHW3761) (Kaur et al. 2021). The SHW14102 were then crossed with two elite hexaploid wheat lines BWL4444 and BWL3531, while SHW3761 crossed with BWL4444, as recurrent parents to develop BC1F1s (Kaur et al 2022). About 100 BC1F1 seeds from each cross were developed into doubled haploids of which 390 DHs were used in the current study (**Table 1**).

The experiment was conducted during 2020-21 and 2021-22 at Punjab Agricultural University, Ludhiana (30° 56 N latitude and 75° 48' E) on two dates of sowing (25th October as early sowing and 15th November as normal sowing). The 390 DHs along with five check cultivars (PBW869, DBW327, DBW303, PBW725, and PBW824) were sown in an augmented experimental design consisting of 15 blocks with 31 entries in each block. Each entry ~~was~~ sown in four rows of 1.5 m in length, with a row-to-row distance of 25 cm. The 100 selected DHs refer to the ~~supplemetary~~ supplementary files as (**Original**

resource 1) from the larger set of 390 were further evaluated for additional traits to screen out the best lines it for further analysis.

Data collection

Data on different phenological and yield contributing characters were recorded on 390 DH viz. seedling emergence percentage (SE), days to emergence (DTE), days to booting (DTB), days to heading (DTH), days to anthesis (DTA), days to maturity (DTM), grain filling duration (GFD) and thousand-grain weight (TGW; g). For 100 selected DHs, the additional data were recorded viz., spike length (with and without awns; cm), awn length (AL; cm), peduncle length (PL; cm), spikelets per spike (SpS), biomass yield per plot (BMYP; g plot^{-1}), grain yield per plot (GYP; g plot^{-1}) and harvest index (HI).

SE was calculated by dividing the emerged seedlings with the total number of seeds into 100. DE was defined to occur when 50% of the plant population had emerged. For EGV, plants were assigned ranks visually on the comparative basis of their growth and vigor at days to 50% emergence as rank 3 (good); rank 2 (average)**;** and rank 1 (poor)**.** The length of the spike was measured from the base of the first spikelet to the tip of the terminal spikelet, excluding awns (spike length without awns) and also till the tip of awns (spike length with awns). The AL was measured from the base of the awn till its tip. For SpS, five spikes on the main culm were tagged at random in each row. The number of spikelets was counted at the time of maturity. PL of the spikes on the main culm was measured in cm from the topmost culm node to the base of the spike.

DTE was defined to occur when 50% of the plant population had emerged. The length of the spike was measured from the base of the first spikelet to the tip of the terminal spikelet, excluding awns (spike length without awns) and also till the tip of awns (spike length with awns). The AL was measured from the base of the awn till its tip. For SpS, five spikes on the main culm were tagged at random in each row. The number of spikelets was counted at the time of maturity. PL of the spikes on the main culm was measured in cm from the topmost culm node to the base of the spike. DTB, DTH, and DTA were measured by counting the days from the date of sowing to the date when 50% of the plant population had completed these stages. Similarly, DTM was measured by counting days from the date of sowing to the date when grain growth ceases and grain becomes hard. It was visually determined when more than 50% of spikes and peduncles turned green to brown or golden yellow. Primarily, the complete loss of the green colour of the glumes served as an indication of physiological maturity. GFD was calculated by recording the number of days from anthesis to physiological maturity of the spike. At maturity, the crop was harvested manually the total dry weight excluding roots was recorded and BMYP was expressed in grams while the grains were harvested manually from each row and GYP was also obtained in grams. To measure TGW, the weight of a thousand grains from bulk seed produced from each plot was taken.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using Proc GLM procedure of SAS software (SAS 9.3.) as per augmented design. Genetic variability analysis was assessed by using the R studio (R Core Team 2020). Pearson’s rank correlation coefficients among the traits were estimated using the R package

"Metan" to find the degree of association among the correlated traits. Principal component analysis (PCA) based on the correlation matrix was performed using the "Factoextra" package built in the R studio to identify influential traits for selection. Ward's minimum variance method of hierarchical clustering was used for clustering and divided all the doubled haploids into two distinct clusters using R-studio.

3. Results and discussion

Under late sown conditions most of the traits had significant variation among introgression lines except for two traits i.e., EGV and awn length. However, when compared to check cultivars, EGV, spike length without awns, and grain yield per plot were at par (Table 2, 3). The significant adjusted block effects indicated heterogeneity of evaluation blocks. This heterogeneity of evaluation blocks was neutralized by estimating adjusted means for all the ILs using the checks. Adjusted means of ILs for different traits are added. The mean performance of all introgression lines derived from three crosses along with check cultivars under timely and late sown conditions varied significantly for selected morpho-physiological characters. The variation in the response of different lines to timely and late sown conditions for selected evaluated traits are discussed below:

3.1 Seedling emergence percentage (SE) and days to emergence (DE)

Under timely sown conditions, the seedling emergence percentage in check cultivars ranged from 75.33 to 80.16% % and 60.32 to 70.55% under late sown conditions. However, in doubled haploid introgression lines derived from cross 1 (BWL4444 × SHW14102) the emergence percentage ranged from 75.23 to 95.12% under timely and 60.65 to 75.81% under late sown conditions. In the ILs derived from cross 2 (BWL3531 × SHW14102) the emergence percentage ranged 65.23 to 80.06% under timely and 55.65 to 70.83% under late sown conditions. In ILs derived from cross 3 (BWL4444 × SHW3761) the emergence percentage ranged from 65.33 to 80.11% and 55.71 to 75.09% under timely and late sown conditions respectively (Table 4).

Under timely sown conditions, the ILs derived from cross 1 showed a maximum percentage (95.23%) while ILs derived from cross 3 and cross 2 showed 80.56% and 75.23% emergence respectively. The check cultivars had a mean emergence percentage of 75.38%. Under late sown conditions, the ILs derived from cross 1 showed a maximum percentage (70%) while ILs derived from cross 3 and cross 2 showed 65% and 60% emergence respectively. The check cultivars had a mean emergence percentage of 65%. Overall, cross 1 (BWL4444 × SHW14102) had a comparatively higher emergence percentage under both timely and late sown conditions.

The days to 50% emergence in check cultivars ranged from 10.23 to 15.55 DAS under timely and 07.23 to 10.56 DAS under late sown conditions. In ILs derived from cross 1 (BWL4444 × SHW14102) the days to 50% emergence ranged from 08.88 to 14.25 DAS and 08.32 to 11.58 DAS under timely and late sown conditions respectively. The ILs derived from cross 2 (BWL3531 × SHW14102) showed days to 50% emergence ranging from 13.23 to 16.11 DAS under timely and 07.84 to 10.78 DAS under late sown conditions. Cross 3 (BWL4444 × SHW3761) derived ILs showed days to 50% emergence ranging from 11.25 to 14.44 DAS under timely and 08.85 to 12.12 DAS under late sown conditions (Table 4).

Under timely sown conditions, the minimum mean number of days to 50% emergence in ILs derived from ~~the~~ cross 1 ~~were~~ was 08 DAS, in ILs from cross 2 ~~were~~ was 10 DAS, in ILs from cross 3 were 11 DAS and in check cultivars, it was 11 DAS. Under timely sown conditions, the minimum mean number of days to 50% emergence in ILs derived from ~~the~~ cross 1 were 08 DAS, in ILs from cross 2 were 09 DAS, in ILs from cross 3 were 09 DAS and in check cultivars, it was 08 DAS. Overall, ILs derived from cross 1 (BWL4444 × SHW14102) took comparatively ~~less~~ fewer days to 50% emergence under both timely and late sown conditions.

4.3.2 Early growth vigour (EGV)

Analysis of variance revealed that there was a non-significant difference between the check cultivars and ILs for early growth vigour under both timely and late sown conditions. However, the ILs derived from the three crosses showed significant ~~difference~~ differences among themselves. In ILs derived from cross 1 (BWL4444 × SHW14102), cross 2 (BWL3531 × SHW14102) and cross 3 (BWL4444 × SHW3761) derived ILs early growth vigour ranged from 01 to 03 while, the check cultivars showed average (02) growth vigour under both timely and poor (01) under late sown conditions.

4.3.3 Spike length (cm)

In the present study, the spike length with awns in check cultivars ranged from 10.98 to 20.02 cm under timely and 9.69 to 15.55 cm under late sown conditions. However, in doubled haploid introgression lines derived from cross 1 (BWL4444 × SHW14102) the spike length with awns ranged from 13.33 to 22.21 cm under timely and 12.52 to 18.56 cm under late sown conditions. In the ILs derived from cross 2 (BWL3531 × SHW14102) the spike length with awns ranged from 09.22 to 19.11 cm under timely and 09.69 to 15.55 cm under late sown conditions. In ILs derived from cross 3 (BWL4444 × SHW3761) the spike length with awns ranged from 12.36 to 21.19 cm timely and 12.11 to 16.88 cm under late sown conditions (Table 4). Overall, the maximum average spike length was observed in the ILs derived from ~~the~~ cross 1 under both timely (18.12 cm) and late (15.65 cm) sown conditions (Table 4).

Further, the spike length without awns in check cultivars ranged from 06.66 to 12.65 cm under timely and 05.56 to 10.25 cm under late sown conditions. However, in doubled haploid introgression lines derived from cross 1 the spike length without awns ranged from 10.21 to 14.21 cm under timely and 08.56 to 11.11 cm under late sown conditions. In the ILs derived from cross 2, the spike length without awns ranged from 06.23 to 13.52 cm under timely and 06.55 to 10.25 cm under late sown conditions. In ILs derived from cross 3, the spike length without awns ranged 08.08 to 13.89 cm timely and 07.84 to 12.52 cm under late sown conditions (Table 4). Overall, the maximum average spike length without awns was observed in the ILs derived from ~~the~~ cross 1 under timely (12.25 cm) and late (10.55 cm) sown conditions (Table 4).

4.3.4 Awn length (AL) (cm)

The check cultivars showed AL ranging from 04.23 to 08.21 cm and 04.13 to 5.28 cm under timely and late sown conditions respectively. In the ILs derived from the cross 1 (BWL4444 × SHW14102) the AL ranged 03.11 to 08.25 cm under timely and 04.22 to 07.43 cm under late sown conditions. In the ILs derived from cross 2 (BWL3531 × SHW14102) the AL ranged from 03.23 to 07.14 cm under timely and 03.55 to 05.87

cm under late sown conditions. In ILs derived from cross 3 (BWL4444 × SHW3761) the AL ranged from 04.98 to 07.14 cm timely and 04.27 to 04.36 cm under late sown conditions (Table 4).

Under timely sown conditions, the ILs derived from cross 1 showed average AL (07.88 cm) while ILs derived from cross 3 and cross 2 showed AL 06.81 cm and 05.22 cm respectively. The check cultivars had an average AL as 06.89 cm. Under late sown conditions, the ILs derived from cross 1 showed average AL (06.89 cm) while ILs derived from cross 3 and cross 2 showed AL 06.32 cm and 04.44 cm respectively. The check cultivars had an average AL as 04.69 cm. Overall, the maximum average AL was observed in the ILs derived from ~~the~~ cross 1 under both timely and late sown conditions.

4.3.5 Peduncle length (PL)

The check cultivars showed PL ranging from 08.55 to 20.17 cm and 07.14 to 18.45 cm under timely and late sown conditions respectively. In the ILs derived from the cross 1 (BWL4444 × SHW14102) the PL ranged from 09.41 to 23.38 cm under timely and 06.66 to 20.14 under late sown conditions. In the ILs derived from cross 2 (BWL3531 × SHW14102) the PL ranged from 08.32 to 18.25 cm under timely and 06.11 to 15.41 cm under late sown conditions. In ILs derived from cross 3 (BWL4444 × SHW3761) the PL ranged from 10.32 to 21.11 cm timely and 06.56 to 17.26 cm under late sown conditions (Table 4).

Under timely sown conditions, the ILs derived from cross 1 showed average PL (18.18 cm) while ILs derived from cross 2 and cross 3 showed PL 16.16 cm and 16.02 cm respectively. The check cultivars had an average AL as 16.36 cm. Under late sown conditions, the ILs derived from cross 1 showed average PL (16.28 cm) while ILs derived from cross 2 and cross 3 showed PL 14.16 cm and 11.02 cm respectively. The check cultivars had an average AL as 12.22 cm. Overall, the maximum average PL was observed in the ILs derived from ~~the~~ cross 1 under both timely and late sown conditions.

4.3.6 Spikelets per spike (SpS)

In the present study, mean value of spikelets per spike in check cultivars ranged from 19.36 to 23.88 under timely sown and 11.23 to 15.25 under late sown conditions. In the ILs derived from the cross 1 (BWL4444 × SHW14102) the SpS ranged from 13.25 to 25.31 under timely sown and 15.23 to 19.22 under late sown conditions. In the ILs derived from cross 2 (BWL3531 × SHW14102) the SpS ranged from 11.11 to 21.16 under timely sown and 09.01 to 13.25 under late sown conditions. In ILs derived from cross 3 (BWL4444 × SHW3761) the SpS ranged from 13.96 to 23.25 under timely sown and 09.25 to 15.55 under late sown conditions (Table 4).

Under timely sown conditions, the ILs derived from cross 1 showed an adjusted mean value for SpS (21.41) while ILs derived from cross 2 and cross 3 showed SpS 19.21 and 17.11 cm respectively. The check cultivars had a mean value for SpS of 17.25. Under late sown conditions, the ILs derived from cross 1 showed an adjusted mean value for SpS (15.23) while ILs derived from cross 3 and cross 2 showed SpS 11.25 and 13.36 cm respectively. The check cultivars had a mean value for SpS of 13.88. Overall, the maximum mean value for SpS was observed in the ILs derived from ~~the~~ cross 1 under both early and timely sown conditions. In the present study, the number of spikelets per spike showed significant variation in timely versus late sown crop. This suggests that shifting from the optimum sowing date to late sowing has a significant impact on the number of spikelets and eventually grain yield.

4.3.7 Days to booting (DTB)

In the present study, the check cultivars, days to 50% booting ranged from 73.33 to 81.11 DAS under timely and 70.23 to 78.11 DAS under late sown conditions respectively. However, in ILs derived from cross 1 (BWL4444 × SHW14102), the days to 50% booting ranged from 63.33 to 88.23 DAS under timely and 60.22 to 75.12 DAS under late sown conditions. In ILs derived from cross 2 (BWL3531 × SHW14102), the days to 50% booting ranged from 60.23 to 89.12 DAS under timely and 55.25 to 78.23 DAS under late sown conditions. For ILs obtained from the cross 3 (BWL4444 × SHW3761) days to 50% booting ranged from 63.23 to 86.11 DAS under timely and 60.12 to 75.11 DAS under late sown conditions (Table 4).

Under timely sown conditions, ILs derived from cross 2 took maximum mean days to 50% booting (80.25 DAS) followed by cross 3 (75.23 DAS) and cross 1 (70.52 DAS). The check cultivars took 76.95 DAS**.** Under late sown conditions, ILs derived from cross 2 took maximum mean days to 50% booting (75.11 DAS) followed by cross 3 (73.03 DAS) and cross 1 (68.25 DAS). The check cultivars took 73.54 DAS. Overall, cross 2 (BWL3531 × SHW14102) derived ILs took comparatively more days to reach 50% booting under both the sowing regimes.

4.3.8 Days to heading (DTH)

In the present study, the days to heading in check cultivars ranged from 83.23 to 87.12 DAS under timely and 77.23 to 85.11 DAS under late sown conditions. However, for ILs derived from ~~the~~ cross 1 (BWL4444 × SHW14102) the days to 50% heading ranged from 68.23 to 98.11 DAS and 63.33 to 81.66 DAS under timely and late sown conditions respectively. In ILs derived from cross 2 (BWL3531 × SHW14102), the days to 50% heading ranged from 69.22 to 108.23 DAS under timely and 72.23 to 85.66 DAS under late sown conditions. For ILs obtained from the cross 3 (BWL4444 × SHW3761) the days to 50% heading ranged from 73.23 to 102.21 DAS and 60.88 to 83.56 DAS under timely and late sown conditions respectively (Table 4).

Under timely sown conditions, the ILs derived from cross 2 (93.15 DAS) took the maximum mean days to reach 50% heading followed by cross 3 (85.23 DAS) and cross 1 (80.11 DAS). The check cultivars took 85.23 DAS. Under late sown conditions, the ILs derived from cross 2 (88.63 DAS) took the maximum mean days to reach 50% heading followed by cross 3 (77.45 DAS) and cross 1 (73.12 DAS). The check cultivars took 85 DAS. Overall, cross 2 (BWL4444 × SHW14102) derived ILs took comparatively more days to reach 50% heading under both the sowing conditions and the lines derived from cross 1 took the least time for heading.

4.3.9 Days to anthesis (DTA)

In check cultivars, the days to 50% anthesis ranged from 91.21 to 101.25 DAS and 88.23 to 96.96 DAS under timely and late sown conditions respectively. However, for ILs derived from the cross 1 (BWL4444 × SHW14102) the days to 50% anthesis ranged from 86.12 to 102.22 DAS under timely and

81.23 to 98.66 DAS under late sown conditions. In ILs derived from cross 2 (BWL3531 × SHW14102) the days to 50% anthesis ranged from 75.23 to 113.11 DAS and 80.63 to 105.25 DAS under timely and late sown conditions respectively. Cross 3 (BWL4444 × SHW3761) derived ILs showed days to 50% anthesis ranging from 85.55 to 109.23 DAS under timely and 78.41 to 101.11 DAS under late sown conditions (Table 4).

Under timely sown ILs derived from cross 2 showed maximum mean days to 50% anthesis (103.11 DAS) followed by cross 3 (96.15 DAS) and cross 1 (94.23 DAS). The check cultivars took 96.25 DAS. Under late sown conditions, the ILs derived from cross 2 showed maximum mean days to 50% anthesis (98.25 DAS) followed by cross 3 (93.21 DAS) and cross 1 (90.01 DAS). The check cultivars took 88.23 DAS. Thus, it is concluded that the ILs derived from cross 2 (BWL4444 × SHW14102) took more days to attain 50% anthesis under both the sowing conditions.

4.3.10 Days to ~~maturity~~ Maturity (DTM)

In the present study, the check cultivars days to maturity ranged from 108.22 to 126.12 DAS and 103.23 to 115.56 DAS under timely and late sown conditions respectively. However, for ILs derived from the cross 1 (BWL4444 × SHW14102) the days to maturity ranged from 102.22 to 135.12 DAS under timely and 101.23 to 120.55 DAS under late sown conditions. In ILs derived from cross 2 (BWL3531 × SHW14102), the days to maturity ranged from 96.12 to 135.11 DAS under timely and 98.23 to 128.11 DAS under late sown conditions. For ILs obtained from the cross 3 (BWL4444 × SHW3761), the days to maturity ranged from 107.11 to 128.25 DAS under timely and 95.25 to 118.14 DAS under late sown conditions (Table 4).

Under timely sown conditions, the ILs derived from cross 1 showed a maximum mean number of days to maturity (127.11 DAS) followed by cross 2 (123.32 DAS) and cross 3 (120.23 DAS). The check cultivars took 118.11 DAS. Under late sown conditions, the ILs derived from cross 1 showed the maximum mean number of days to maturity (118.21 DAS) followed by cross 2 (115.02 DAS) and cross 3 (113.25 DAS). The check cultivars took 108.66 DAS. Overall, ILs derived from cross 1 (BWL4444 × SHW14102) took comparatively more days to reach 50% physiological maturity under both the sowing regimes.

4.3.11 Grain filling duration (GFD)

In the present study, the duration of grain filling ranged from 15.23 to 20.11 days under timely and 11.23 to 18.85 days under late sown conditions for check cultivars. However, for ILs derived from the cross 1 (BWL4444 × SHW14102) duration of grain filling ranged from 22.23 to 28.12 days under timely and 18.58 to 21.12 days under late sown conditions. In ILs derived from cross 2 (BWL3531 × SHW14102) the days of grain filling duration ranged from 16.25 to 22.52 days under timely and 12.63 to 16.54 days under late sown conditions. Cross 3 (BWL4444 × SHW3761) derived ILs days for grain filling duration ranged from 14.12 to 21.21 days and 13.54 to 18.87 days under timely and late sown conditions respectively (Table 4).

Under timely sown conditions, the ILs derived from cross 1 showed a longer duration of grain filling (27.25 days) as compared to check cultivars (21.11 days) and other crosses viz., cross 2 (16.16 days) and cross 3 (20.08 days). Under late sown conditions, the ILs derived from cross 1 showed a longer duration of grain filling (20.23 days) as compared to check cultivars (15.25 days) and other crosses viz., cross 2 (13.45 days) and cross 3 (16.25 days). Overall, cross 1 (BWL4444 × SHW14102) derived ILs had comparatively longer grain filling duration under both the sowing regimes.

4.3.12 Biomass yield per plot (BMYP)

In check cultivars, biomass yield per plot ranged from 550.32 to 1650.55 g and 450.52 to 1100.23 g under timely and late sown conditions respectively. In the ILs derived from cross 1 (BWL4444 × SHW14102), the BMYP ranged from 450.22 to 1850.18 g and 350.52 to 1250.66 g under timely and late sown conditions. In the ILs derived from cross 2 (BWL3531 × SHW14102), the BMYP ranged from 550.55 to 1600.78 g under timely and 350.78 to 1100.45 g under late sown conditions. In the ILs obtained from cross 3 (BWL4444 × SHW3761), the BMYP ranged from 560.66 to 1950.11 g under timely and 500.47 to 1250.77 g under late sown conditions (Table 4).

Under timely sown conditions, ~~the~~ cross 1 showed the highest BMYP (1500.25 g) followed by cross 3 (1650.63 g) and cross 2 (1150.29 g). The check cultivars had a BMYP of 1100.25 g. Under late sown conditions, ~~the~~ cross 1 showed the highest BMYP (1100.89 g) followed by cross 3 (1050.14 g) and cross 2 (855.63 g). The check cultivars had a BMYP of 800.14 g. Overall, the lines derived from cross 1 (BWL4444 × SHW14102) had higher BMYP under both the sowing dates.

4.3.13 Grain yield per plot (GYP)

In check cultivars, grain yield per plot ranged from 250.12 to 556.23 g under timely and 200.85 to 350.68 g under late sown conditions respectively. In the ILs derived from cross 1 (BWL4444 × SHW14102), the GYP ranged from 250.22 to 558.14 g under timely and 258.85 to 455.32 g under late sown conditions. In the ILs derived from cross 2 (BWL3531 × SHW14102), the GYP ranged from 255.11 to 500.45 g under timely and 253.23 to 369.66 g under late sown conditions. In the ILs obtained from cross 3 (BWL4444 × SHW3761), the GYP ranged from 400.66 to 650.78 g under timely and 352.25 to 405.22 under late sown conditions (Table 4).

Under timely sown conditions, ~~the~~ cross 1 showed the highest GYP (500.63 g) followed by cross 3 (450.55 g) and cross 2 (300.23 g). The check cultivars had a GYP of 358.23 g. Under late sown conditions, ~~the~~ cross 1 showed the highest GYP (485.25 g) followed by cross 3 (400.63 g) and cross 2 (320.85 g). The check cultivars had a GYP of 280.23 g. Overall, the lines derived from cross 1 (BWL4444 × SHW14102) had higher GYP under both the sowing dates.

4.3.14 Thousand grain weight (TGW)

In check cultivars, ~~thousand grain~~thousand-grain weight ranged from 25.11 to 37.02 g and 20.12 to 31.23 g under timely and late sown conditions respectively. In the ILs derived from cross 1 (BWL4444 × SHW14102), the TGW ranged from 31.12 to 37.22 g and 22.22 to 33.33 g under timely and late sown conditions. In the ILs derived from cross 2 (BWL3531 × SHW14102), the TGW ranged from 28.11 to 31.25 g under timely and 20.25 to 28.65 under late sown conditions. In the ILs obtained from cross 3 (BWL4444 × SHW3761), the TGW ranged from 32.02 to 36.08 g under timely and 21.12 to 31.56 g under late sown conditions (Table 4).

Under timely sown conditions, ~~the~~ cross 1 showed the highest TGW (38.22 g) followed by cross 3 (36.66 g) and cross 2 (32.22 g). The check cultivars had TGW 36.12 g. Under late sown conditions, ~~the~~ cross 1 showed the highest TGW (30.06 g) followed by cross 3 (28.26 g) and cross 2 (25.85 g). The check cultivars had TGW 36.12 g. Overall, the lines derived from cross 1 (BWL4444 × SHW14102) had higher TGW under both the sowing conditions.

4.3.15 Harvest ~~index~~ Index (HI)

In check cultivars, HI ranged from 33.71 to 45.45 and 31.86 to 44.48 under timely and late sown conditions respectively. In the ILs derived from cross 1 (BWL4444 × SHW14102), the HI ranged from 30.16 to 55.57 and 36.45 to 73.71 under timely and late sown conditions. In the ILs derived from cross 2 (BWL3531 × SHW14102), the HI ranged from 31.25 to 72.23 under timely and 33.56 to 72.28 under late sown conditions. In the ILs obtained from cross 3 (BWL4444 × SHW3761), the HI ranged from 33.33 to 71.48 under timely and 32.44 to 70.42 under late sown conditions (Table 4).

Under timely sown conditions, the cross 3 derived ILs showed the average highest HI (56.25) followed by cross 2 (54.11) and cross 1 (50.36). The check cultivars had an average HI value ~~as~~ of 43.21. Under late sown conditions, ~~the~~ cross 1 showed the highest HI (61.63) followed by cross 3 (57.02) and cross 2 (55.23). The check cultivars had an average HI value ~~as~~ of 35.96. The lines derived from cross 3 (BWL4444 × SHW3761) and cross 1 (BWL4444 × SHW14102) had higher HI ~~value~~ values under timely and late sown conditions respectively.

Correlation and principal component biplot analysis to evaluate the selected doubled haploid introgression lines.

Correlation heat map and principal component analysis (PCA) were used to identify the key subcomponents of terminal heat stress tolerance. Under timely sown conditions, TGW showed a significant positive correlation with SpS (r=0.44), PL (r=0.55), EGV (r=0.43), DA (r=0.40), DH (r=0.38), DB (r=0.37) and DM (r=0.34). For late sown crop, the TGW showed a significant positive correlation with PL (r=0.38), DB (r=0.34), SpS (r=0.34), DA (r=0.33), DH (r=0.32), DM (r=0.28) and EGV (r=0.28). However, GYP showed a significant positive correlation with BMYP (r=0.77 and r=0.72) under both timely and late sown conditions respectively (Fig. 1).

Further, only for ~~late sown~~sown late-sown 100 doubled haploid ILs, the data were analysed by using PCA ~~in order to~~to find out the largest contributor to the total variation among a set of traits under reproductive stage heat stress conditions. In this study, out of ~~a~~ total of seventeen ~~principle~~ principal components (PCs), seven components i.e., PC1 to PC7, had ~~eigen values~~eigenvalues greater than one. These seven PCs explained 78.28% of the total variability. The other eleven components accounted for nearly 21.72% of the variation in the introgression lines. Suggesting these PC scores might be used to summarize the original 17 variables in any further analysis of the data. The lines were dispersed in different ordinates based on the dissimilarity among them (Fig. 2).

The PC1 which accounted for about 26.07% of the total variation was strongly related with DH

(0.424), DA (0.421), DB (0.407), PL (0.309), DM (0.286), TGW (0.242), SpS (0.216), EGV (0.208), HI (0.117), DE (0.037) and GYP (0.007), with positive loadings and rest exhibited the negative loadings. The PC2 (13.01% of total variation) exhibited a positive relation with SL with awns (0.430), BMYP (0.389), DE (0.369), GYP (0.354), SL without awns (0.326), DH (0.173), DB (0.160), DA (0.156) SpS (0.081) and TGW (0.003).

The PC3 explained 09.54% of the total variation among introgression lines for GYP (0.567), BMYP (0.469), PL (0.176), GFD (0.159), HI (0.153), DE (0.017), TGW (0.051) and DM (0.041). The PC4 has a significant loading factor for TGW (0.276), GYP (0.140), and BMYP (0.019). These factors accounted for about 8.75% of the variation. The PC5, accounted for about 8.08% of the variation. In this component, BMYP (0.378), TGW (0.286), GYP (0.284), AL (0.211), PL (0.188), and EGV (0.069) showed positive loadings. The PC6 explained 6.61% of the variation~~,~~ and exhibited a positive association with the factors viz., HI (0.700), GYP (0.288), SE (0.215), EGV (0.198), TGW (0.082), DH (0.082), DA (0.083), BMYP (0.078), DB (0.042) and SL without awns (0.025). The PC7 (6.21% of total variation) exhibited a positive relation with GFD (0.440), DE (0.341), AL (0.338), DA (0.186) and DM (0.146).

According to the PCA, days to heading was selected for the first group which means that days to heading had the largest loading for component one (PC1), spike length with awns was selected for the second group which had the largest loading for the second component (PC2), grain yield per plot for the third group which had the largest loading for the third component (PC3), ~~thousand grain~~thousand-grain weight was selected for the fourth group which had the largest loading for the fourth component (PC4), biomass yield per plot was selected for the fifth group which had the largest loading for the fifth component (PC5), harvest index for the sixth group which had the largest loading for the sixth component (PC6) and grain filling duration for the seventh group (PC 7) which had the largest loading for the seventh component (Table 6).

Cluster analysis

The doubled haploid ILs (100) were statistically ~~analysed~~ analyzed and clustered based on the yield traits ~~analysed~~ analyzed in PCA viz., DH, SL with awns, GYP, TGW BMYP, HI, and GFD. Ward’s minimum variance method of hierarchical clustering was used for clustering and divided all the selected introgression lines into two distinct groups (Fig. 3). The critical examination of the dendrogram revealed three clusters and were divided into two groups; Group A and Group B. Group A consisted of only one cluster i.e., cluster 1. Group B contained two clusters: cluster 2 and cluster 3. Cluster analysis showed that cluster 1 included 17 ILs while, cluster 2 and ~~cluster~~ Cluster 3 comprises of 29 and 54 ILs respectively. Group A consisting of cluster 1, which represents 17% of the total population and group B consisting of cluster 2 and 3 accounts for 83% of the population (Table 7). All three clusters were analysed according to their means and standard deviations. The mean values for GYP (385.07), BMYP (1162.37) and TGW (38.13) were relatively higher in the cluster 1 than in other clusters. The cluster 2 exhibited ~~the~~ a higher value for DH (89.14), SL with awns (18.21) and GFD (18.19) while~~,~~ cluster 3 showed a higher value for HI (41.02)

(Table 8).

DISCUSSION

Temperature plays a significant role in every phase of wheat growth, such as germination, tillering, booting, ear emergence, anthesis, and maturity. It serves as a crucial factor in determining the availability of water and other essential elements required for proper development. However, the effect of temperature varies depending on the wheat variety and the specific stage of growth. At high temperatures, wheat completes its life cycle much faster than under normal temperatures (Hossain et al. 2013). An increase in temperature will shorten the duration of the crop growing season, intensify evaporation rates of both crops and soil and increase the probability of heat stress during crucial reproductive stages, consequently impacting crop production (Luo et al. 2017). This implies that delayed planting takes ~~lesser~~ fewer days to complete phenological stages and eventually ~~reduced~~ reduces yield and yield components.

Significant differences were revealed by the analysis of variance among the evaluated lines for each of the traits. The mean values obtained after statistical analysis revealed that cross 1 derived introgression lines outperformed ~~than~~ the check cultivars and other crosses for the traits. This in turn suggested sufficient inherent genetic variability. The data were then subjected to genetic variability analysis. The values of PCV and GCV were quite significant in the expression of all the traits indicated a wide range of genetic variability along with the influence of environmental factors. High GCV and PCV were recorded in TGW followed by SE. The remaining traits recorded moderate to low GCV. High GCV and PCV for TGW have also been reported by several studies under different sowing conditions (Singh et al. 2012, Kumar et al. 2013, Dhakar et al. 2012, Kumar et al. 2017, Sapi and Bahttacharjee 2017, Bhanu et al. 2018, Raaj et al. 2018). Phenotypic variances (σ^2p) and genotypic variances (σ^2g) in a crop population are important for successful plant breeding. Broad-sense heritability (h^2) is expressed as the percentage of the ratio between the genotypic variance and phenotypic variance. The high heritability in addition to high genetic advance as a percent of the mean was shown in the traits SE followed by TGW, DH, DA, and DB. High heritability indicated that the selection for these characters would be effective, being less influenced by environmental effects (Hossain et al. 2021). High heritability estimated for 1000-seed weight along with other traits (grain yield and number of seeds per spike) have also been reported by ~~other study~~another study (Sachan and Singh 2003) which supported the present findings. The higher the heritability estimates, the simpler the selection procedures (Khan and Naqvi 2011). In general, it is considered that if a trait is governed by non-additive gene action, it may give high heritability but low genetic advance, whereas if the trait is governed by additive gene action, both heritability and genetic advance would be high (Eid 2009; Hossain et al. 2021).

Correlation analysis helps to ~~determination~~ determine effective traits ~~in order to~~to indirect selection superior genotypes. On the other ~~hands~~hand, principal component analysis is a suitable multivariate technique in ~~identify~~ identifying and determination of independent principal components that are effective on plant traits separately. Therefore, correlation and principal component analysis assist breeders in genetically improving traits such as yield that have low heritability specifically in early generations via indirect selection for traits effective on this (Beheshtizadeh et al. 2013; Adilova et al. 2020). In this study, correlation studies revealed an association of GYP with TGW and BMYP under early and timely sown conditions respectively. TGW showed a significantly positive correlation with PL, SpS, DH, DB, DA, DM, HI, and EGV under early sown conditions. Under timely sown conditions, TGW reflected a positive correlation with EGV, DM, DA, DB, DH, SpS, and PL. This suggested the TGW is the most important yield trait. The importance of this trait has been quoted by another study conducted in the double haploid population (Liu et al. 2020). In addition, TGW is more stably inherited than the overall final production (Mengistu et al. 2012). The results of PC analysis revealed the main components that contributed greatly to the evaluation of yield traits in DH introgression lines. Hence, days to heading is the best choice, which had the largest loading for PC1, spike length with awns for the second (PC2), grain yield per plot for the third group (PC3), days to booting for the fourth group (PC4) and spikelets per spike for the fifth group (PC5).

Cluster analysis based on squared Euclidean distance and Ward's method categorized the introgression lines into three clusters. Based on PCA, the first seven components explained over 78.28% of genetic variation. Cluster analysis based on PCA using the first five PCs indicated three separate clusters of DH introgression lines, with the genetic distance observed between cluster 1 and cluster 3. Such differences in components of traits studied in this manuscript can be applied as a new source of variation in other breeding programs and crossing nurseries for wheat improvement. The assessment of genetic diversity using quantitative traits has been of major importance in self-pollinated crops like wheat. Selecting the parents for the breeding program in such crops is critical because, the success rate depends upon the transgressive segregants derived between the parents, particularly when the aim is to improve the quantitative trait like yield. In this study, there is significant genetic variability among DH introgression lines which indicates the presence of an excellent opportunity to bring about improvement in future breeding ~~programmes~~ programs to obtain potential germplasm adapted to heat stress conditions. With a similar aim, a multivariate cluster and PCA were conducted on the Uzbek bread wheat cultivars (Adilova et al. 2021). Further studies on the influence of terminal heat stress and agronomic practices on the genetic potential of the DH introgression lines derived from synthetics is necessary to be evaluated ~~in order to~~to unravel the overall performance under both ~~the s~~tress conditions i.e., at the juvenile stage and reproductive stage. This may assist in stratifying the environments based on quality and yield suitability.

Declarations:

Supplementary Information The online version contains supplementary material available at

Code availability: NA

Ethical approval: NA.

Consent to participate: NA

Consent for publication: All the authors have agreed to the publication of this manuscript.

Availability of data and materials: Relevant data are included in this paper.

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Table 1. Doubled haploid lines tested for juvenile heat tolerance

	Cross (Parentage/Pedigree)	**Large set of DH (390 entries)**	**Small set of DH (100 entries)**
Cross 1	**BWL4444 x SHW14102** BWL4444 (HD2967+Yr10) x SHW14102 (PBW114-*Ae. tauschii* 14102 amphiploid)	140	32
Cross 2	**BWL3531 x SHW14102** BWL3531 (PBW343 Yr17+Yr70+Lr76) x SHW14102 (PBW114-*Ae. tauschii* 14102 amphiploid)	80	11
Cross 3	**BWL4444 x SHW3761** BWL4444 (HD2967+Yr10) x SHW3761 (PBW114-*Ae. tauschii* 3761 amphiploid)	170	57
	Total	**390**	**100**

Table 2. ANOVA and Coefficient of Variation (CV%) for 100 ILs under late sown conditions evaluated for various traits in the growing season 2021-2022

Source of Variation	Treatment (ignoring Blocks)	Treatment: Check	Treatment: Test vs. Check	Treatment: Test	Block (ignoring Treatments)	Residuals	CV%
Df	**104**	**4**	**1**	**99**	**3**	**12**	
SE	70**	136.25**	1066.67**	57.26**	44.58*	11.25	4.33
EGV	0.28 ns	0.07 ns	0.74 ns	0.28 ns	0.67 ns	0.21	18.82
SL w/ awns	9.38**	0.58 ns	67.34**	9.15**	1.8 ns	1.51	9.61
SL w/o awns	4.61**	2.33 ns	1.60 ns	4.73**	1.73 ns	1.19	14.87
AL	5.69 ns	4.25 ns	89.71**	4.9 ns	3.53 ns	3.45	34.19
PL	10.13**	0.67**	2.51**	10.59**	0.05 ns	0.04	1.72
SpS	10.00**	2.37 ns	53.40**	9.86**	6.6 ns	2.31	8.44
DE	8.24**	1.70**	43.74**	8.15**	7.38**	0.3	8.17
DB	94.33**	135.47 **	367.98**	89.91**	2.35 ns	2.43	1.92
DH	93.83**	57.72**	646.22**	89.71**	0.04 ns	3.03	1.86
DA	137.55**	35.74**	470.35**	138.30**	3.18 ns	2.34	1.4
DM	122.74**	146.16**	497.49**	118.01**	3.70 ns	12.68	2.62
GFD	125.19**	194.87**	1935.3**	104.09**	11.29 ns	16.98	14.59
BMYP	267731.15 ns	5777151.25 *	138096.37 *	114691.66 *	561451.25 ns	477701.25	83.74
GYP	13275.87 **	62498.68 **	849.66 ns	11412.58 **	3382.8 *	911.01	12.59
TGW	30.62 **	63.84 **	18.27 *	29.48 **	0.11 ns	12.52	2.11

HI	2302.51 ***	15.24 ns	1136.02 ***	2399.84 ***	19.66 ns	75.67	24.77

Df: Degree of freedom; SE: Seedling emergence percentage; SL w/ awns: Spike length with awns; SL w/o awns: Spike length without awns; AL: Aw length; PL: Peduncle length; SpS: Spikelets per spike; DE: Days to emergence; DB: Days to booting; DH; Days to heading; DA: Days to anthesis; DM: Days to maturity; GFD: Grain filling duration; BMYP: Biomass yield per plot; GYP; Grain yield per plot; TGW: Thousand grain weight and HI: Harvest index; SPAD-I: SPAD value at booting stage; SPAD-II: SPAD value at heading stage; SPAD-III: SPAD value at anthesis stage; SPAD-IV: SPAD value at grain filling stage; NDVI-I: NDVI value at booting stage; NDVI-II: NDVI value at heading stage; NDVI-III: NDVI value at anthesis stage; NDVI-IV: NDVI value at grain-filling stage.
ns: non-significant (P > 0.05)
*Significant at P=0.05; **Significant at P=0.01; ***Significant at P=0.001

Table 3. ANOVA and Coefficient of Variation (CV%) for 100 ILs under timely sown conditions evaluated for various traits in the growing season 2021-2022

Source of Variation	**Treatment (ignoring Blocks)**	**Treatment: Check**	**Treatment: Test vs. Check**	**Treatment: Test**	**Block (ignoring Treatments)**	**Residuals**	**CV%**
Df	**104**	**4**	**1**	**99**	**3**	**12**	
SE	60 ns	141.87*	1504.17**	42.11 ns	74.58 ns	27.71	6.14
EGV	0.23 ns	0.25 ns	0.43 ns	0.23 *	0.73*	0.15	14.71
SL w/ awns	8.91**	2.45 ns	116.16**	8.09**	6.58 ns	2.25	9.46
SL w/o awns	5.67 ns	3.83 ns	23.21*	5.57 ns	4.13 ns	3.59	17.91
AL	3.62 ns	1.55 ns	35.53**	3.38 ns	1.78 ns	1.95	26.51
PL	9.87 **	6.39 **	1.11 **	10.11 **	0.04 ns	0.06	1.57
SpS	7.9 ns	8.5 ns	5.41 ns	7.9 ns	2.33 ns	3.59	11.35
DTE	6.77 **	0.7 ns	16.67 **	6.92**	15.27**	0.6	7.96
DTB	94.77 **	118.2 **	416.96 **	90.57**	7.44*	1.59	1.5
DTH	96.95 **	102.56**	672.6 **	90.91**	0.8 ns	4.67	2.24
DTA	133.6**	43.6**	85.02**	137.72**	10.29 ns	4.55	1.91
DTM	164.8*	12.33 ns	363.73**	168.95**	4.69 ns	9.65	2.07
GFD	87.68**	82.14*	800.46**	80.7**	28.86 ns	17.04	10.75
BMYP	147978.16**	905557.84**	7490.9**	118787.94**	1287.78*	249.52	1.69
GYP	13695.33 **	95548.68**	25.26 ns	10526.2**	39.66 ns	38.18	2.2
TGW	26.71**	20.58**	21.27**	27.01**	0.28 ns	0.78	2.44
HI	1053.67**	12.82**	296.98**	1103.37**	2.01 ns	0.64	2.22

Df: Degree of freedom; SE: Seedling emergence percentage; SL w/ awns: Spike length with awns; SL w/o awns: Spike length without awns; AL: Aw length; PL: Peduncle length; SpS: Spikelets per spike; DE: Days to emergence; DB: Days to booting; DH; Days to heading; DA: Days to anthesis; DM: Days to maturity; GFD: Grain filling duration; BMYP: Biomass yield per plot; GYP; Grain yield per plot; TGW: Thousand grain weight and HI: Harvest index; SPAD-I: SPAD value at booting stage; SPAD-II: SPAD value at heading stage; SPAD-III: SPAD value at anthesis stage; SPAD-IV: SPAD value at grain filling stage; NDVI-I: NDVI value at booting stage; NDVI-II: NDVI value at heading stage; NDVI-III: NDVI value at anthesis stage; NDVI-IV: NDVI value at grain-filling stage.
ns: non-significant (P > 0.05)
*Significant at P=0.05; **Significant at P=0.01; ***Significant at P=0.001

Table 4. Descriptive analysis of phenotypic traits in 100 doubled haploid introgression lines derived from three crosses (BWL4444 x SHW14102, BWL4444 SHW3761, BWL3531 x SHW14102) and check cultivars (PBW869, DBW327, DBW303, PBW725 and PBW824) under timely and late sown conditions

Traits	Check cultivars		100 Doubled haploids (DH) Population Range					
			Cross 1		Cross 2		Cross 3	
	Timely Sown	Late Sown	Timely Sown	Late Sown	Timely Sown	Late Sown	Timely Sown	Late Sown
SE (%)	75.33-80.16	60.32-70.55	75.23-95.12	60.65-75.81	65.23-80.06	55.65-70.83	65.33-80.11	55.71-75.09
EGV	02 to 02	01 to 02	02 to 03	01 to 03	01 to 03	01 to 03	01 to 03	01 to 02
SL awns (cm)	10.98 to 20.02	9.69 to 15.55	13.33 to 22.21	12.52 to 18.56	09.22 to 19.11	09.69 to 15.55	12.36 to 21.19	12.11 to 16.88
SL w/o awns (cm)	06.66 to 12.65	05.56 to 10.25	10.21 to 14.21	08.56 to 11.11	06.23 to 13.52	06.55 to 10.25	08.08 to 13.89	07.84 to 12.52
AL (cm)	04.23 to 08.21	04.13 to 5.28	03.11 to 08.25	04.22 to 07.43	03.23 to 07.14	03.55 to 05.87	04.98 to 07.14	04.27 to 04.36
PL (cm)	08.55 to 20.17	07.14 to 18.45	09.41 to 23.38	06.66 to 20.14	08.32 to 18.25	06.11 to 15.41	10.32 to 21.11	06.56 to 17.26
SpS	19.45 to 23.33	11.23 to 15.25	13.44 to 25.36	15.23 to 19.22	11.32 to 21.25	09.01 to 13.25	13.25 to 23.36	09.25 to 15.55
DE (DAS)	10.23-15.55	07.23-10.56	08.88-14.25	08.32-11.58	13.23-16.11	07.84-10.78	11.25-14.44	08.85-12.12
DB (DAS)	73.33-81.11	70.23-78.11	63.33-88.23	60.22-75.12	60.23-89.12	55.25-78.23	63.23-86.11	60.12-75.11
DH (DAS)	83.23-87.12	77.23-85.11	68.23-98.11	63.33-81.66	69.22-108.23	72.23-85.66	73.23-102.21	60.88-83.56
DA (DAS)	91.21-101.25	88.23-96.96	86.12-102.22	81.23-98.66	75.23-113.11	80.63-105.25	85.55-109.23	78.41-101.11
DM (DAS)	108.22-126.12	103.23-115.56	102.22-135.12	101.23-120.55	96.12-135.11	98.23-128.11	107.11 to 128.25	95.25-118.14

GFD (d)	15.23-20.11	11.23-18.85	22.23-28.12	18.58-21.12	16.25-22.52	12.63-16.54	14.12-21.21	13.54-18.87
BMYP (g)	550.3-1650.5	450.52-1100.2	450.2-1850.1	350.52-1250.6	550.5-1600.7	350.78-1100.45	560.6-1950.11	500.47-1250.77
GYP (g)	250.12-556.23	200.85-350.68	250.22-558.14	258.85-455.32	255.11-500.4	253.23-369.66	400.66-650.78	352.25-405.22
TGW (g)	25.55 to 36.12	20.12 to 31.23	23.33 to 38.22	22.22 to 33.33	22.11 to 32.22	20.25 to 28.65	20.02 to 36.66	21.12 to 31.56
HI	33.71 to 45.45	31.86 to 44.48	30.16 to 55.57	36.45 to 73.71	31.25 to 72.23	33.56 to 72.28	33.33 to 71.48	32.44 to 70.42

SE: Seedling emergence percentage; SL w/ awns: Spike length with awns; SL w/o awns: Spike length without awns; AL: Awn length; PL: Peduncle length; SpS: Spikelets per spike; DE: Days to emergence; DB: Days to booting; DH; Days to heading; DA: Days to anthesis; DM: Days to maturity; GFD: Grain filling duration; BMYP: Biomass yield per plot; GYP; Grain yield per plot; TGW: Thousand grain weight and HI: Harvest index; SPAD-I: SPAD value at booting stage; SPAD-II: SPAD value at heading stage; SPAD-III: SPAD value at anthesis stage; SPAD-IV: SPAD value at grain filling stage; NDVI-I: NDVI value at booting stage; NDVI-II: NDVI value at heading stage; NDVI-III: NDVI value at anthesis stage; NDVI-IV: NDVI value at grain-filling stage; CTD-I: CTD value at booting stage; CTD-II: CTD value at anthesis stage; CTD-III: CTD value at grain filling stage

Table 5: Genetic parameters for 9 characters in 390 ILs along with check cultivars under early (E) and timely (T) sown conditions

Characters	Sowing	σ^2 g	σ^2 p	GCV	PCV	H^2 (Broad sense) %	GA as % of mean
SE	E	76.51	111.16	11.6	15.52	68.83	22.97
	T	77.56	119.26	12.6	15.63	65.03	20.97
DTE	E	12.25	16.65	4.01	5.3	73.57	9.23
	T	11.58	17.11	3.79	5.5	67.67	9.12
EGV	E	0.01	0.18	5.11	21.05	5.56	2.11
	T	0.01	0.16	5.05	21.02	6.25	2.51
DTB	E	10.15	17.93	3.99	5.3	56.61	6.19
	T	11.05	16.13	3.69	5.8	68.51	7.09
DTH	E	25.32	38.61	5.54	6.84	65.58	9.25
	T	27.06	35.32	5.48	6.26	76.61	9.89
DTA	E	24.45	41.13	4.37	5.67	59.45	6.96
	T	23.15	40.06	5.33	5.77	57.79	7.16
DTM	E	20.15	41.03	5.37	5.11	49.11	7.91
	T	22.15	42.16	5.63	5.27	52.53	8.01
GFD	E	10.25	15.23	6.23	7.11	67.31	7.75
	T	10.02	15.21	6.11	7.05	66.71	7.54
TGW	E	38.32	50.93	11.38	13.12	75.20	42.05
	T	36.66	50.22	11.11	12.25	73.00	41.74

σ^2 g= Genotypic variance; σ^2 p= Phenotypic variance; GCV= Genotypic coefficient of Variance; PCV= Phenotypic coefficient of Variance; h^2 = Heritability; GA= Genetic advance

Table 6. Vector loadings and principle component analysis explained variation by the seven PCs for late sown trial

Characters	**Eigen vectors**						
	PC1	PC2	PC3	PC4	PC5	PC6	PC7
SE	-0.050	-0.170	-0.128	-0.138	-0.040	0.215	-0.099
EGV	0.208	-0.119	-0.095	-0.218	0.069	0.198	-0.116
SL w/ awns	-0.195	0.430	-0.386	-0.299	-0.059	0.025	-0.120
SL w/o awns	-0.124	0.326	-0.133	-0.175	-0.378	-0.218	-0.519
AL	-0.141	0.260	-0.387	-0.230	0.211	0.242	0.338
PL	0.309	-0.034	0.176	-0.115	0.188	-0.152	-0.109
SpS	0.216	0.081	-0.100	-0.156	0.378	-0.309	-0.225
DE	0.037	0.369	0.017	-0.028	-0.129	-0.184	0.341
DB	0.407	0.160	-0.059	0.119	-0.114	0.042	-0.070
DH	0.424	0.173	-0.090	0.087	-0.146	0.082	0.066
DA	0.421	0.156	-0.102	0.068	-0.063	0.083	0.186
DM	0.286	-0.107	0.041	-0.484	-0.155	-0.109	0.146
GFD	-0.177	-0.289	0.159	-0.584	-0.091	-0.207	0.440
BMYP	-0.169	0.389	0.469	-0.019	0.378	0.078	0.044
GYP	0.007	0.354	0.567	-0.140	0.284	0.288	-0.046
TGW	0.242	0.003	0.051	-0.276	0.286	0.082	-0.364
HI	0.117	-0.050	0.153	-0.156	-0.576	0.700	-0.073
Principle component analysis							
Eigen value	4.432	2.212	1.622	1.488	1.374	1.124	1.056
Individual percentage	26.07	13.01	9.54	8.75	8.08	6.61	6.21
Cumulative percentage of variance	26.07	39.08	48.62	57.37	65.46	72.07	78.28

SE: Seedling emergence percentage; SL w/ awns: Spike length with awns; SL w/o awns: Spike length without awns; AL: Aw length; PL: Peduncle length; SpS: Spikelets per spike; DE: Days to emergence; DB: Days to booting; DH; Days to heading; DA: Days to anthesis; DM: Days to maturity; GFD: Grain filling duration; BMYP: Biomass yield per plot; GYP; Grain yield per plot; TGW: Thousand grain weight and HI: Harvest index

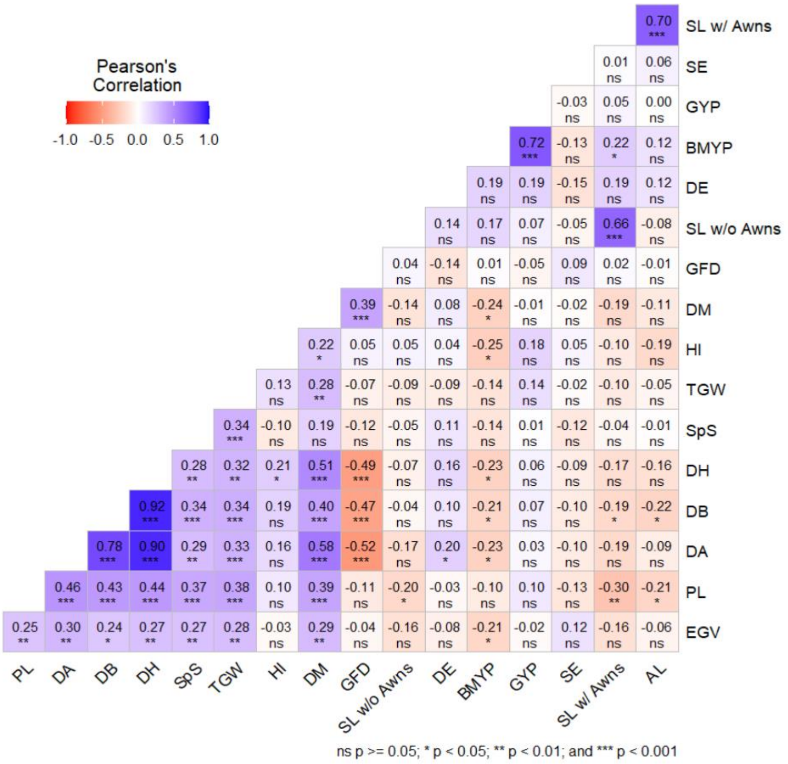
Table 7: Clustering of doubled haploids in clusters and groups based on yield traits analysed in principle component analysis

Doubled haploid introgression lines	Number	Clusters	Groups
DH41, DH63, DH96, DH79, DH85, DH103, DH3, DH46, DH4, DH45, DH121, DH12, DH104, DH30, DH31, DH14, DH126	17	Cluster 1	A
DH88, DH53, DH128, DH73, DH101, DH33, DH18, DH20, DH133, DH150, DH84, DH92, DH9, DH51, DH144, DH94, DH129, DH6, DH16, DH63, DH57, DH143, DH146, DH115, DH120, DH65, DH40, DH100, DH106	29	Cluster 2	B
DH87, DH55, DH72, DH60, DH90, DH25, DH26, DH131, DH123, DH76, DH113, DH42, DH44, DH68, DH81, DH91, DH111, DH38, DH58, DH135, DH147, DH49, DH71, DH117, DH82, DH124, DH141, DH136, DH138, DH52, DH77, DH97, DH11, DH27, DH109, DH29, DH61, DH1, DH15, DH47, DH23, DH114, DH149, DH 8, DH21, DH66, DH69, DH74, DH108, DH118, DH132, DH35, DH98, DH140	54	Cluster 3	

Table 8. Means and standard deviations for clusters based on yield and yield contributing traits

Traits	Cluster 1		Cluster 2		Cluster 3	
	Mean	SD	Mean	SD	Mean	SD
DH	84.14	2.6	89.14	3.2	79.64	2.2
SL w/ awns	13.19	1.6	18.21	1.2	13.55	1.5
GFD	16.11	1.8	18.19	1.5	13.25	2.3
BMYP	1162.37	31.1	1005.31	38.1	921.04	33.2
GYP	385.07	34.2	201.05	21.6	345.85	26.2
TGW	38.13	1.9	30.61	2.8	28.22	2.3
HI	35.12	1.5	35.87	2.1	41.02	2.8

DH: Days to heading; SL w/ awns: Spike length with awns; GFD: Grain filling duration; BMYP: Biomass yield per plot; GYP: Grain yield per plot: TGW: Thousand grain weight; HI: Harvest index; SD: Standard deviation



	PL	DA	DB	DH	SpS	TGW	HI	DM	GFD	SL w/o Awns	DE	BMYP	GYP	SE	SL w/ Awns	AL
SL w/ Awns																0.70 ***
SE															0.01 ns	0.06 ns
GYP														-0.03 ns	0.05 ns	0.00 ns
BMYP													0.72 ***	-0.13 ns	0.22 *	0.12 ns
DE												0.19 ns	0.19 ns	-0.15 ns	0.19 ns	0.12 ns
SL w/o Awns											0.14 ns	0.17 ns	0.07 ns	-0.05 ns	0.66 ***	-0.08 ns
GFD										0.04 ns	-0.14 ns	0.01 ns	-0.05 ns	0.09 ns	0.02 ns	-0.01 ns
DM									0.39 ***	-0.14 ns	0.08 ns	-0.24 *	-0.01 ns	-0.02 ns	-0.19 ns	-0.11 ns
HI								0.22 *	0.05 ns	0.05 ns	0.04 ns	-0.25 *	0.18 ns	0.05 ns	-0.10 ns	-0.19 ns
TGW							0.13 ns	0.28 **	-0.07 ns	-0.09 ns	-0.09 ns	-0.14 ns	0.14 ns	-0.02 ns	-0.10 ns	-0.05 ns
SpS						0.34 ***	-0.10 ns	0.19 ns	-0.12 ns	-0.05 ns	0.11 ns	-0.14 ns	0.01 ns	-0.12 ns	-0.04 ns	-0.01 ns
DH					0.28 **	0.32 **	0.21 *	0.51 ***	-0.49 ***	-0.07 ns	0.16 ns	-0.23 *	0.06 ns	-0.09 ns	-0.17 ns	-0.16 ns
DB				0.92 ***	0.34 ***	0.34 ***	0.19 ns	0.40 ***	-0.47 ***	-0.04 ns	0.10 ns	-0.21 *	0.07 ns	-0.10 ns	-0.19 *	-0.22 *
DA			0.78 ***	0.90 ***	0.29 **	0.33 ***	0.16 ns	0.58 ***	-0.52 ***	-0.17 ns	0.20 *	-0.23 *	0.03 ns	-0.10 ns	-0.19 *	-0.09 ns
PL		0.46 ***	0.43 ***	0.44 ***	0.37 ***	0.38 ***	0.10 ns	0.39 ***	-0.11 ns	-0.20 *	-0.03 ns	-0.10 ns	0.10 ns	-0.13 ns	-0.30 **	-0.21 *
EGV	0.25 **	0.30 **	0.24 *	0.27 **	0.27 **	0.28 **	-0.03 ns	0.29 **	-0.04 ns	-0.16 ns	-0.08 ns	-0.21 *	-0.02 ns	0.12 ns	-0.16 ns	-0.06 ns

ns p >= 0.05; * p < 0.05; ** p < 0.01; and *** p < 0.001

Fig. 1 Correlation heat-maps: Pearson correlation coefficient among various phenological, morphological, and yield traits of 100 DH introgression lines derived from three crosses (synthetics hexaploid wheat and hexaploid bread wheat). SE: Seedling emergence percentage; SL w/ awns: Spike length with awns; SL w/o awns: Spike length without awns; AL: Awn length; PL: Peduncle length; SpS: Spikelets per spike; DE: Days to emergence; DB: Days to booting; DH; Days to heading; DA: Days to anthesis; DM: Days to maturity; GFD: Grain filling duration; BMYP: Biomass yield per plot; GYP; Grain yield per plot; TGW: Thousand grain weight and HI: Harvest index

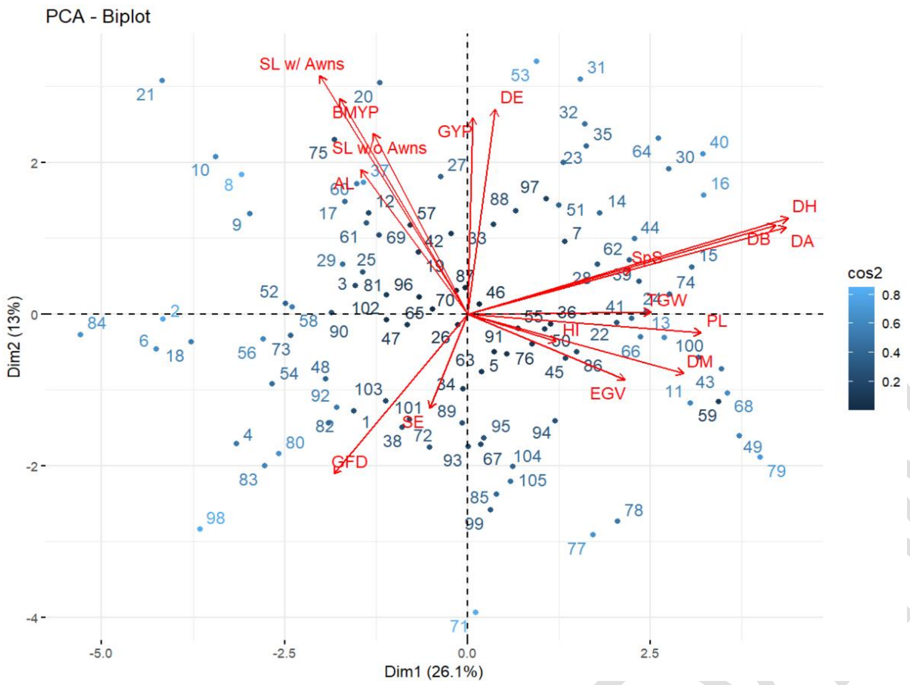


Fig 2. PCA biplot graphical display of the measured traits in 100 doubled haploid introgression lines derived from the three crosses (BWL4444 x SHW14102, BWL4444 x SHW3761 and BWL3531 x SHW14102)

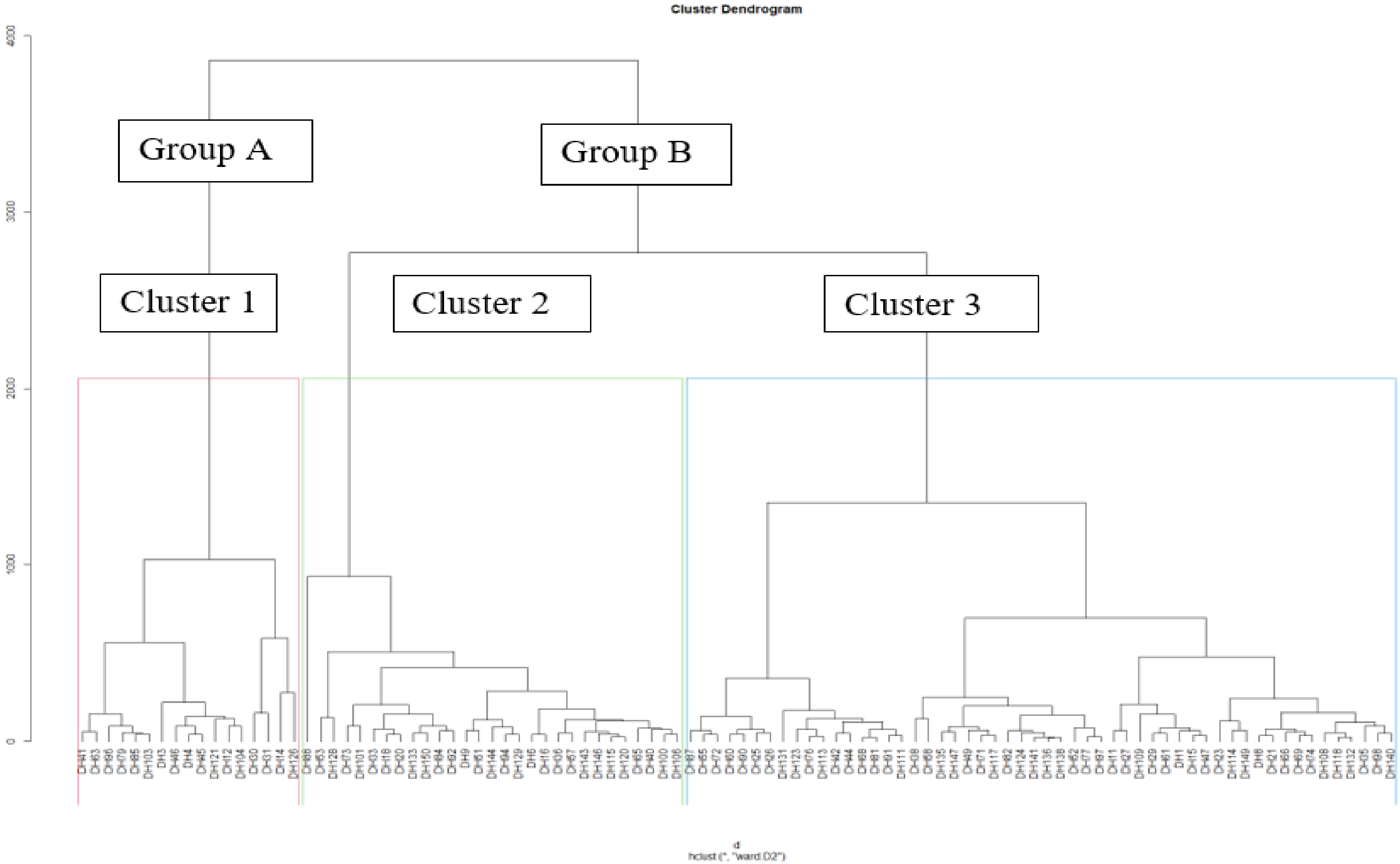


Fig. 3 Dendrogram of 100 doubled haploid introgression lines based on the selected traits from PCA viz., days to heading, spike length with awns, grain filling duration, biomass yield per plot, grain yield per plot, thousand grain weight and harvest index