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Marker assisted mobilization of heat tolerance QTLs from *Triticum durum-Aegilops speltoides* introgression lines to hexaploid wheat

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Abstract

Most of the modern-day cultivars of spring wheat cultivated in the Indian sub-continent are susceptible to high-temperature stress during reproductive stages, and breeding for heat-tolerant genotypes is the plausible solution to mitigate effects of global warming on wheat productivity. *Triticum durum – Aegilops speltoides* backcross introgression lines were used for transferring seven heat tolerance QTLs to three different hexaploid backgrounds using marker assisted selection. A total of 164 BC_2F_3 progenies with different combinations of QTLs were generated and 40 progenies were evaluated in replicated trials across two years under normal (OE) and heat stress environments (HSE). Phenotypic evaluation and heat tolerance index (HTI) analysis over two environments showed that grain filling duration, spikelets/spike, tiller number, thousand grain weight, and yield showed were enhanced due to the introgression of heat stress tolerance QTLs. Progenies pauHTIL_10, 11, 12, 33, and 34 have shown higher yield than tested cultivars under OE with pauHTIL_10, 11, and 12 showed yields higher than 2.0 kgs/plot under OE and pauHTIL_14 showed yield of 1.6kg/plot under HSE. The progenies developed during this study can further be used for developing heat-tolerant wheat varieties.

Key words: *Aegilops speltoides*, HT QTL, heat tolerance marker assisted selection, hexaploid wheat

Introduction

The green revolution, not only in India but across most of the second and third world countries, has allowed the population to have access to food. Ever-increasing human population requires matching growth in food production to equalize the demand-production reatio..Cereal production must increase by 4.2% per year to keep pace with the increasing population (Gaur et al. 2017) but the rate of yield gain is too slow to ensure food for all. The changing environment has further added another dimension to an existing complex situation. Plant growth and productivity are severely restricted by heat stress, and it has been touted a ssignificant constraint leading to substantial losses in wheat yield if stress strikes during the reproductive stage (Sadat et al. 2013). There is apprehension that India will also lose 11.1% in wheat yield in forcasted scenerios of 2050 (Dubey et al. 2020). About 9 million hectares of a total of 29.8 million hectares of area under wheat cultivation in India has been anticipated to be highly prone to sudden heat stress (Sonkar et al. 2019).

Developing heat stress tolerant wheat varieties in combination with smart agriculture can help in mitigating the effect of heat stress on wheat productivity. In India, some wheat genotypes with a moderate level of heat stress tolerance have been identified and are being used in the wheat breeding programmes. Wild species, on the other hand, have the arsenal to withstand heat stress and various accessions of species like *Aegilops speltoides, Ae. tauschii* and *Ae. geniculata* accessions have been identified to possess thermotolerance (Awlachew et al. 2016; Pradhan et al. 2012).

Ae. speltoides putative B genome donor of wheat, possessing SS diploid genome, has originated from

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South-west Asia (Tigris Eupharates region). It is a rich genetic source to increase genetic variability of modern-day cultivars of wheat for various traits of economic importance, and it has been used in introgression breeding to a limited scale (Kaur et al. 2018; King et al. 2018). It has also been reported to possess genetic variation for terminal heat stress tolerance (Awlachew et al. 2016; Pradhan et al. 2012).

From several years of phenotypic assessment of wild wheat gene pool at Punjab Agricultural University (Ludhiana), India, *Ae. speltoides* has shown normal growth, full pollen fertility, and normal seed set with no apparent negative effects of high temperature. In order to transfer the terminal heat stress tolerance from *Ae. speltoides*, an accession pau3809 was crossed with *T. durum* cultivar PBW114 and tetraploid *Triticum durum – Ae. speltoides* backcross introgression (DS-BILs) lines were developed (Awlachew et al. 2016). QTLs for various component traits of heat stress tolerance have been mapped in these DS-BILs after several years of screening under heat stressed environments (Awlachew et al. 2016). In the present study heat stress tolerance QTLs from selected DS-BILs have been transferred to hexaploid background using marker assisted selection (MAS) along with validation of the effect of these QTLs in hexaploid wheat background.

Materials and methods

Development of plant genetic material

Development of the *T. durum - Ae. speltoides* backcross introgression lines (DS-BILs) has been described in detail in Awlachew et al. (2016). Six selected DS-BILs (namely, DS-BIL23, DS-BIL25, DS-BIL31, DS-BIL37, DS-BIL44, and DS-BIL628) having heat stress tolerance quantitative trait loci (HT QTLs) were used as donors for transferring seven HT QTLs to stripe rust resistant versions of three important hexaploid wheat varieties, BWL3558 (PBW550+*Yr5*), BWL4444 (HD2967+*Yr10*), and BWL5185 (PBW621+*Yr10*+*Yr15*). PBW550 has been a unique cultivar released for special mid-November planting, its popularity in the state is adjudged by facts that it was grown over 27% area at its crux and had highest breeder seed indent for consecutive year 2011, 2012. HD2967 has been the queen variety since 2011, from 2013-15 it was grown over 80% area of the state and even after becoming susceptible to stripe rust races it has ~30% area under its cultivation PBW621 is an internationally famous line KACHU, its four sibs have been released in India PBW621, DBW50, HD3059, DBW88. These popular varieties have been resurrected against susceptibility to yellow rust by incorporation of stripe rust resistance genes. BC_2F_5 was generated with marker assisted selections at BC_1F_1, BC_2F_1, and BC_2F_2 generations using markers linked to respective QTLs using shuttle breeding between main season at Punjab Agricultural University, Ludhiana, Punjab (November-May) and off-season at Punjab Agricultural University Regional station, Keylong, Himachal Pradesh (May-October). Strategy used for the development, of *T. durum - Ae. speltoides* X hexaploid wheat-derived $BC_2F_{3:5}$ progenies are summarised in Fig. 1 (called HTILs or heat-tolerant introgression lines from here onwards).

Marker assisted introgression of heat tolerance QTL to hexaploid wheat

Genomic DNA isolation of BC_1F_1, BC_2F_1, and BC_2F_2 progenies along with donor parent and recurrent parent was done using modified CTAB method (Saghai-Maroof et al. 1984). The samples were subjected to marker assisted selection (MAS) using set of markers linked with heat stress tolerance QTLs (HT QTLs) in *T. durum – Ae.speltoides* backcross introgression library (Awlachew et al. 2016; Awlachew and Chhuneja 2013). Along with the seven QTLs, stripe rust resistance genes *Yr5, Yr10* and *Yr15* from recurrent parents were also tracked using MAS in BC_2F_1, and BC_2F_2. PCR reactions were carried out as reported (Awlachew et al. 2016), and PCR profile of SNP marker *Kasp_HT1* (inhouse designed) was used as reported by LGC genomics at https://www.lgcgroup.com. BC_2F_5 progenies were subjected to haplotype/introgression profiling using the markers linked to the HT QTLs.

Phenotypic evaluation for various agro-morphological traits

Forty selected BC_2F_3 HTILs were evaluated across two different sowing dates each, normal sowing or optimum environment (OE) in mid of November, 2017 and late sowing or heat stress environment (HSE) in mid of December, 2017 in randomized complete block design (RCBD) with three recurrent parents and seven checks in 3m X 4 rows plots (plant to plant distance-10cm and row to row distance-23cm), while remaining 124 HTILs (with low seed numbers) were evaluated at OE in augmented design (data not given). A difference of 30 days was maintained between two sowing dates to impart terminal heat stress. Various agronomic traits studied include plant height (PH), grain filling duration

(GFD), canopy temperature (CT) using handheld infrared thermometer, spike length (SL), flag leaf length (FFL), spikelet number per spike (SN), tiller number per meter (TNpM), grain number per spike (GNpS), thousand grain weight (TGW) and yield per plot (YD). Besides yield and tiller number, all other traits were recorded for five plants of each progeny. Forty HTILs from replicated trial, were advanced by sowing in off-season 2018 and were again evaluated at BC_2F_5.

Correlation and statistical analysis

Correlation of different traits was studied with main emphasis on TGW and YD, as they are highly affected due to heat stress. Descriptive analysis and variability studies were done using various packages in R-studio (Aravind et al. 2019; Comtois 2020). For descriptive analysis package *summary tools* v0.9.4 was used. Analysis of variation,variability analysis and calculation of adjusted means (BLUPs) was done using META-R version 6.0 (Alvarado et al. 2016). Comparisons were made between genotypes to the respective recurrent parent separately for both the environments. Adjusted means (BLUPs) of replications were used for comparisons for respective environments.Heat tolerance index was calculated for SN, FLL, TNpM, GNpS, GFD, TGW and YD.The heat tolerance index was calculated as percentage of one minus change in trait values across the environments to trait values in OE which is as under

$$\text{HTI} = \left(1 - \frac{\text{Trait OE - Trait HSE}}{\text{Trait OE}}\right) \times 100$$

Validation of effect of QTLs in HTILs

For validating effect of QTLs in HTIL, the presence or absence of QTLs was correlated to the trait values of BC_2F_5 HTILs. For this purpose, trait data of SS, FLL, TNpM, GFD, TGW and YD studied under both OE and HSE were used. Effect of QTLs for CT and TTC were studied against FLL as both CT and TTC are dependent on leaf architecture and coverage with a significant effect on GFD and TGW. Besides this, boxplots were plotted for each trait to study the effect of the presence or absence of different HT QTLs in HTILs. Significant difference on the phenotypes due to the presence or absence of different HT QTLs in HTILs was tested using Kruskal-Wallis test and the effect size, based on H-statistic, was calculated by squared eta using the equation

$$\text{eta}^2 = (H - k + 1)/(n - k)$$

where H is the value obtained in the Kruskal-Wallis test, k is the number of alternate alleles, n is the total number of observations (Tomczak and Tomczak 2014). The effects are characterized as small effect (0.01 to < 0.06), moderate effect (0.06 to < 0.14), and large effect (>= 0.14).

Results

Marker assisted introgression of heat tolerance QTLs to hexaploid wheat

Six DS-BILs were crossed as female parent with three recurrent parents during off-season 2015. F_1 seed set varied from 28 (DS-BIL44 BWL5185) to 190 (DS-BIL25 X BWL3558) in different cross combinations. The F_1s were planted in main-season 2015-16, and initial screening of all F_1s was done removing durum type plants. Pentaploid F_1s (checked cytologically, Supplementary Fig. S1) were backcrossed to the hexaploid parents to generate BC_1F_1 seed. BC_1F_1 single plant progenies were sown in 2016 at Keylong (off-season nursery), and single plant selections were made for HT QTLs using linked markers (Supplementary Table S1). The number of seeds and plant survival in F_1s and subsequent backcross generations is summarized in Table 1. Selected progenies (144 plants) with introgressions of the HT QTLs were backcrossed to generate BC_2F_1 seed (Fig. 1, Supplementary Fig. S2). BC_2F_1 single plant progenies were sown in the main season 2016-17, and single plants with desired introgressions were selected through MAS. BC_2F_1 plants with one or more than one HT QTL introgressions along with stripe rust resistance genes were further visually selected for plant vigour and checked cytologically for stable chromosome number i.e., 2N= 42 (Supplementary Fig. S3), selecting a total of 122 single plant progenies. BC_2F_2 progenies were planted in offseason 2017, and 164 plants positive for target QTLs along with stripe rust resistance genes were selected. BC_2F_3 progenies/HTILs were again sown in main wheat season 2017, and five plants of each progeny were analysed for the confirmation of the presence of HT QTLs. Forty HTILs were advanced by sowing in off-season 2018 and were again evaluated at BC_2F_5 in the crop season 2018-19. Haplotyping/ Introgression profiling was done using the markers linked to the QTLs to evaluate the presence of different QTL combinations across selected lines (Fig. 2). The haplotyping showed that the selected 40 progenies contained only five out of the seven targeted HT QTLs (*QSs.pau-4A, QTtc.pau-1B, QCt.pau-3B, QTgw.pau-5B,* and *QTgw.pau-2B*) which were present in

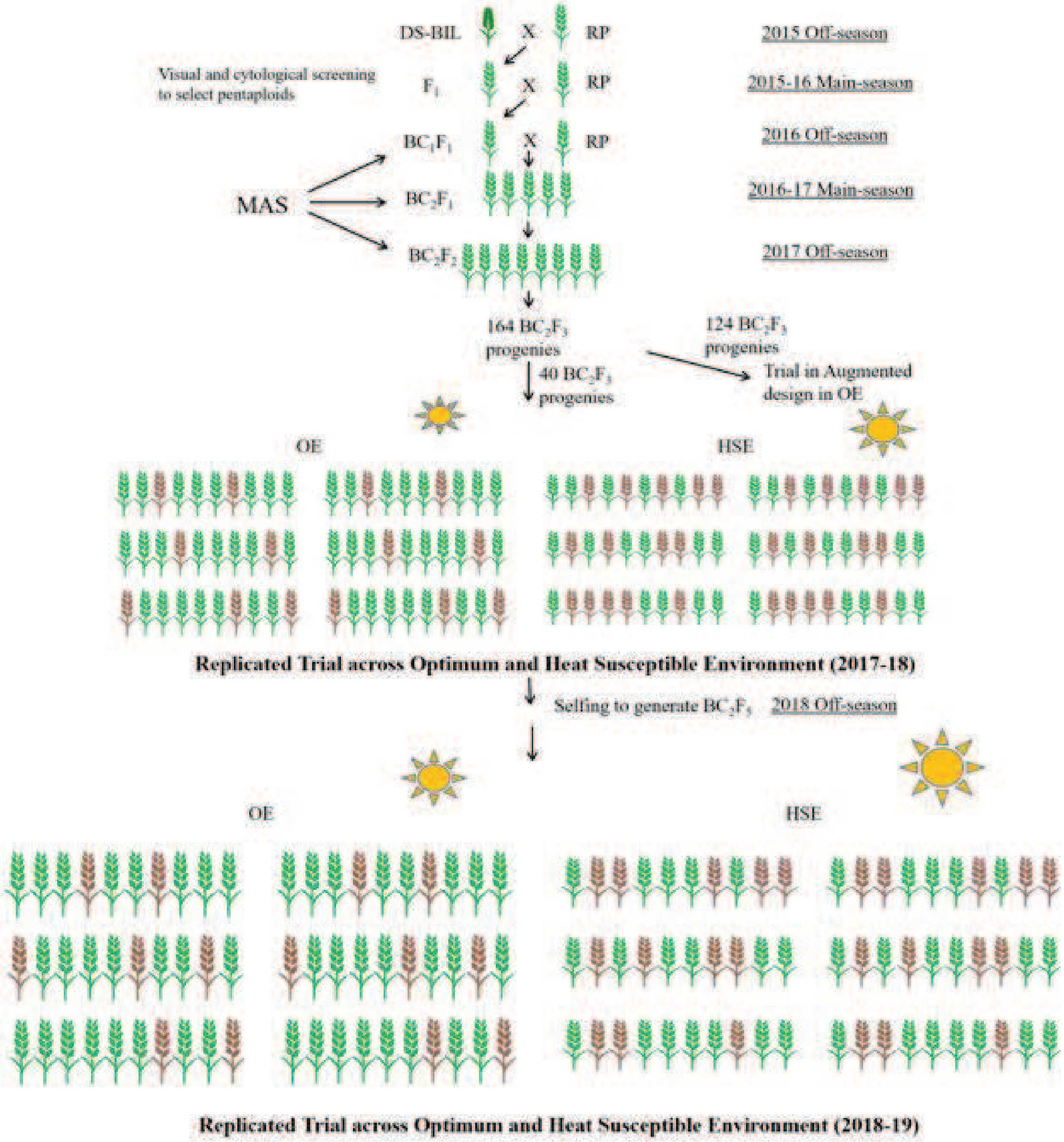


Fig. 1. Development, screening and experimental design of *T. durum-Ae. speltoides*/hexaploid wheat derived $BC_2F_{3\text{-}5}$ HTILs. Selected lines were crossed with recurrent parent (RP), backcrossed and selfed till BC_2F_3 along with selections. At BC_2F_3, 40 lines with sufficient seeds were sown in two replications each in OE (Optimum environment) and HSE (Heat stress environment) and remaining 124 in augmented design in OE. Plants from replicated trial were selfed till BC_2F_5 which were planted again in two replications each in OE (Optimum environment) and HSE (Heat stress environment). Green plants in figure represent tolerant plants while brown represent susceptible plants

homozygous state. The number of HT QTLs ranged from one HT QTL in seven HTILs to five HT QTLs in one HTIL (pauHTIL_21).

Phenotypic evaluation of $BC_2F_{3:5}$ HTILs

BC_2F_3 HTILs, showed significant increase in TGW (around 10%), YD(>20%) and TNpM(>20%), in both OE and HSE with respect to recurrent parents (Table 2). The range of variation for various traits is depicted in Fig.3 and Table 2. Large variations in FLL (20.20-25.74 in OE and 14.20-25.58 in HSE), GFD (35.01-42.37 in OE and 24.81-30.82 in HSE), TGW (31.25-45.51 in OE and 33.62-41.56 in HSE), TNpM (92.79-130.15 in OE and 61.78-79.45 in HSE) and YD (00.89-02.02 in OE and 00.69-01.16 in HSE) was observed across both environments with large reductions in HSE.

Table 1. Marker Assisted Selection for introgression of heat tolerance QTLs from *T. durum - Ae. speltoides* introgression lines to cultivated wheat across various generations

Recurrent parent	Donor DS-BIL ID	K2015	L2015-16		K2016		L2016-17
		F_1	BC_1F_1		BC_2F_1		BC_2F_2
		Seed generated	Plants survived	Seed generated	Plants selected	Seed generated	Plants selected
BWL3558	DS-BIL23	113	15	368	21	376	46
	DS-BIL25	190	17	203	8	134	23
	DS-BIL31	47	3	61	2	34	-
	DS-BIL37	97	23	97	2	42	5
	DS-BIL44	59	4	135	6	59	1
	DS-BIL628	86	6	70	6	103	9
BWL4444	DS-BIL23	136	30	440	34	696	31
	DS-BIL25	53	8	211	11	45	4
	DS-BIL31	34	10	78	-	-	-
	DS-BIL37	102	18	85	3	20	1
	DS-BIL44	159	-	-	-	-	-
	DS-BIL628	89	3	93	9	154	6
BWL5185	DS-BIL23	30	7	55	1	8	-
	DS-BIL25	49	14	96	2	73	-
	DS-BIL31	63	7	112	1	160	4
	DS-BIL37	64	23	110	7	5	-
	DS-BIL44	28	-	-	-	-	-
	DS-BIL628	46	4	108	9	326	34

K = Off-season nursery at Keylong, Himachal Pradesh and L = Main campus, Ludhiana

SN (17.65-23.21 in OE and 18.70-22.28 in HSE) was least affected by heat stress and FLL showed the highest reduction upto 50% in HSE. Due to sudden rise in temperature during first week of April in 2018 overall plant phenology was highly affected for HTILs sown in HSE (Supplementary Fig.S4). SN, TNpM, GNpS, GFD, TGW, and YD showed high broad-sense heritability (>0.70) in both the environments. However, YD in HSE showing maximum coefficient of variation (CV = 14.65) (Table 2).

HTILs evaluated in BC_2F_5, not only out-performed their respective parental lines in OE but also showed less penalty in HSE (Fig. 3, Table 2). Alarge variation for various agro-morphological traits was observed in both the environments including TNpM (74.60-136.56 in OE and 68.56-95.33 in HSE), for SN (19.33-22.69 in OE and 18.23-22.17 in HSE), GNpS (49.68-66.02 in OE and 51.08-67.05 in HSE), GFD (37.40-48.36 in OE and 32.93-38.86 in HSE) and YD (01.38-02.03 in OE and 01.03-01.60 in HSE). TGW on the other hand, showed higher variability in HSE (32.47-42.16) than OE (39.69-48.45), showing many genotypes had lesser reduction in TGW in HSE. Many HTILs showed higher SN and GNpS in HSE, while other traits were observed to be negatively affected by heat stress imposed in HSE (Fig. 3). Yield overall decreased in HSE as compared to OE but some HTILs such as pauHTIL_6, 11, 13, 14, 17, 21, 22, 23, 25, 26, 30, 33, and 34 showed high yields in both the environments (higher than all the recurrent parents), with pauHTIL_14 (1.602 kg/plot) and pauHTIL_27 (1.564 kg/plot) having the higher yield in HSE and pauHTIL_10, 11, and 12 showed yields higher than 2.0 kgs/plot in OE. Ten progenies (pauHTIL_9, 11, 12, 13, 18, 19, 20, 27, 30, and 31) maintained higher tiller numbers (TNpM) than all the parental genotypes in both the environment where pauHTIL_37 showed TNpM of 136.56 in OE and pauHTIL_30 had 95.33 in HSE.

Table 2. Phenotypic and genotypic variabilities in BC_2F_3HTILs and BC_2F_5 HTILs under OE (Optimum Environment) and HSE (Heat Stress Environment) during 2017-18 and 2018-19, respectively

Trait	Gen	Env	Checks	BWL 3558	HTIL/ BWL3558	BWL 4444	HTIL/ BWL4444	BWL 5185	HTIL/ BWL5185	Population	h^2	LSD	CV	GV	EV
PH	BC_2F_3	OE	78.9-87.9	84.4	78.9-99.7	88.7	85.5-97.2	89.5	84.3-97.7	78.9-99.7	0.72	9.70	6.28	42.00	32.48
(cm)		HSE	76.0-88.3	82.5	76.0-89.5	88.5	77.3-91.8	87.4	79.6-87.4	76.0-92.9	0.74	7.08	4.87	24.29	16.82
	BC_2F_5	OE	88.1-102.9	94.5	88.1-102.8	98.4	85.0-102.5	92.7	90.0-97.2	85.0-102.8	0.77	6.00	3.59	19.16	11.73
		HSE	79.7-84.9	79.7	77.7-89.1	94.4	78.9-88.4	79.1	79.9-86.6	77.7-89.0	0.79	4.32	2.92	10.91	5.92
SN	BC_2F_3	OE	16.8-20.8	20.8	17.6-**23.2**	22.0	19.6-**22.7**	21.3	20.5-**22.5**	17.6-23.2	0.84	1.67	4.29	2.20	0.82
		HSE	19.3-22.2	20.3	18.7-21.6	21.8	20.1-**22.2**	21.2	20.0-21.3	18.7-22.2	0.70	1.64	4.69	1.13	0.95
	BC_2F_5	OE	18.5-21.4	19.6	19.8-21.2	21.2	19.3-**22.4**	20.5	19.9-**22.6**	19.3-22.6	0.80	1.06	2.87	0.71	0.35
		HSE	19.0-20.8	20.8	18.2-20.7	21.3	19.3-**22.1**	20.7	18.6-**21.1**	18.2-22.1	0.65	1.50	4.60	0.80	0.87
SL	BC_2F_3	OE	10.1-11.0	11.7	10.4-12.5	12.5	11.5-13.3	11.6	11.1-12.3	10.4-13.3	0.70	1.53	7.75	0.99	0.83
(cm)		HSE	10.1-11.8	11.1	9.6-11.7	12.4	10.7-13.6	10.8	10.3-11.9	09.6-13.6	0.83	1.18	5.58	1.05	0.42
	BC_2F_5	OE	10.8-11.6	11.4	10.2-11.9	12.1	11.2-13.0	11.5	11.2-12.7	10.2-13.0	0.66	1.11	5.86	0.46	0.47
		HSE	10.4-11.6	11.1	10.3-11.6	12.1	10.6-11.9	11.3	10.9-11.5	10.3-11.9	0.51	1.09	6.81	0.30	0.59
SCR	BC_2F_3	OE	1.75-1.95	1.79	1.71-1.99	1.77	1.68-1.83	1.82	1.81-1.84	1.68-1.99	0.47	0.21	8.34	0.01	0.02
		HSE	1.75-1.97	1.82	1.79-2.01	1.78	1.66-1.94	1.94	1.79-1.91	1.64-2.01	0.65	0.19	6.48	0.01	0.01
	BC_2F_5	OE	1.62-1.92	1.74	1.68-2.12	1.73	1.67-1.84	1.79	1.72-1.85	1.67-2.12	0.72	0.14	4.56	0.01	0.01
		HSE	1.75-1.89	1.84	1.79-1.85	1.76	1.76-1.85	1.82	1.76-1.81	1.76-1.85	0.34	0.11	5.32	0.00	0.01
FLL	BC_2F_3	OE	20.1-25.7	24.9	20.9-25.7	24.3	21.6-24.7	20.8	20.2-22.8	20.2-25.7	0.65	3.26	8.85	3.75	4.10
(cm)		HSE	19.8-25.9	19.9	15.0-25.5	19.3	14.2-25.4	18.3	15.6-21.2	14.2-25.5	0.91	3.51	9.64	17.81	3.35
	BC_2F_5	OE	20.0-25.2	25.0	21.5-**25.3**	23.7	22.0-**26.8**	21.5	22.1-23.3	21.5-26.8	0.73	2.26	5.60	2.32	1.75
		HSE	18.5-23.6	22.5	19.5-**23.9**	22.1	18.8-23.5	18.4	20.0-22.6	18.8-23.9	0.71	2.54	7.07	2.74	2.28
TNpM	BC_2F_3	OE	89.7-114.9	97.5	92.7-**117.9**	103.4	93.2-**130.1**	98.4	95.4-**128.8**	92.7-130.1	0.87	10.6	5.46	107.9	32.63
		HSE	56.4-84.0	73.6	64.8-79.4	75.9	61.7-78.6	77.5	64.8-73.3	61.7-79.4	0.77	10.9	8.80	64.65	39.07
	BC_2F_5	OE	94.4-132.2	94.4	94.8-123.6	91.3	76.7-**136.5**	89.2	74.6-128.8	74.6-136.5	0.86	14.5	7.62	189.8	61.57
		HSE	74.5-91.1	84.0	68.5-**91.4**	84.7	69.2-**93.9**	79.1	81.2-**95.3**	68.5-95.3	0.70	11.5	8.36	55.72	46.77
GNpS	BC_2F_3	OE	42.6-49.5	44.4	44.6-**55.3**	47.6	47.8-**55.3**	50.5	43.3-**52.7**	43.3-55.3	0.77	4.91	5.70	13.30	7.76
		HSE	45.7-58.3	47.4	43.3-53.7	49.9	41.9-54.1	51.3	44.0-55.8	41.9-55.8	0.84	5.08	5.55	20.02	7.65
	BC_2F_5	OE	47.4-56.0	53.5	56.7-**63.8**	59.2	49.6-**66.0**	54.3	52.2-**62.1**	49.6-66.0	0.76	7.08	7.01	26.39	16.34
		HSE	51.2-61.3	59.8	54.9-**65.1**	66.6	51.0-**63.6**	63.8	57.1-**67.0**	51.0-67.0	0.75	6.46	6.33	20.49	13.92
GFD	BC_2F_3	OE	38.7-41.6	38.9	35.0-41.2	40.3	37.6-**42.3**	38.9	38.0-41.6	35.0-42.3	0.72	3.50	5.21	5.46	4.22
		HSE	27.6-29.4	28.3	24.8-**30.4**	28.1	26.5-**30.8**	29.3	25.1-**30.1**	24.8-30.8	0.71	2.68	5.70	3.07	2.54
	BC_2F_5	OE	39.9-42.5	39.2	37.4-41.7	41.7	38.1-**48.3**	41.7	39.5-42.5	37.4-48.3	0.73	2.79	3.96	3.61	2.66
		HSE	34.7-37.9	35.9	33.5-36.1	36.4	32.9-**38.8**	36.7	35.0-36.7	32.9-38.8	0.59	2.68	4.87	2.20	3.03
TGW	BC_2F_3	OE	35.4-40.7	39.8	34.8-**45.5**	40.0	31.2-**42.8**	40.0	35.5-38.6	31.2-45.5	0.87	3.45	4.64	11.64	3.39
(g)		HSE	34.6-39.1	38.1	34.1-**41.5**	37.4	34.5-**39.8**	39.0	33.6-37.3	33.6-41.5	0.74	3.23	5.05	4.93	3.52
	BC_2F_5	OE	42.6-47.0	44.5	42.4-**48.4**	42.7	39.6-**47.8**	43.1	40.4-46.1	39.6-48.4	0.72	3.34	4.44	5.04	3.83
		HSE	35.3-43.3	36.0	32.6-41.1	35.0	32.4-42.1	38.6	38.2-41.7	32.4-42.1	0.79	3.69	5.49	7.98	4.31
YD	BC_2F_3	OE	1.43-1.79	1.48	0.98-**2.02**	1.50	0.89-**1.92**	1.47	1.15-1.73	0.89-2.02	0.92	0.23	7.67	0.08	0.01
(kg)		HSE	0.94-1.19	1.09	0.72-1.16	0.83	0.74-0.96	1.04	0.69-0.90	0.69-1.16	0.73	0.23	14.6	0.02	0.02
	BC_2F_5	OE	1.55-1.90	1.67	1.38-**1.97**	1.71	1.49-**2.03**	1.73	1.43-**1.99**	1.38-2.03	0.81	0.22	6.77	0.03	0.01
		HSE	1.16-1.39	1.09	1.05-**1.47**	1.36	1.03-**1.60**	1.14	1.18-**1.41**	1.03-1.60	0.73	0.23	10.2	0.02	0.02

PH= Plant height SN= Spikelet no. per spike, SL =Spike length , SCR= Spike compaction ratio , FLL =Flag leaf length , TNpM= Tiller number per meter , GNpS =Grain number per spike (), GFD =Grain filling duration , TGW= Thousand grain weight (), and YD= Yield per plot (). Generation of heat tolerant introgression lines (Gen), Heritability broad sense (h^2), Genotypic Variance (GV), Residual/Environmental Variance (EV), Least Significant Difference (LSD) and Coefficient of variation (CV)

††† Underlined values show performance better than respective recurrent parents and bold values shows performance better than check

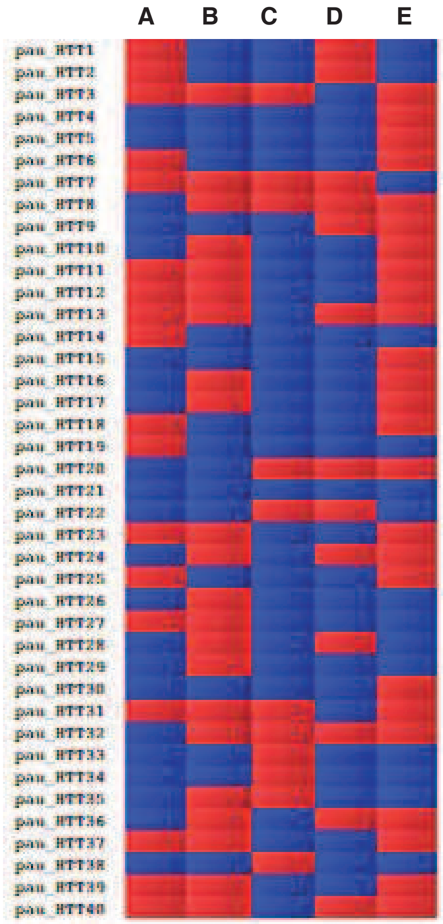


Fig. 2. Haplotypic view of marker profile of the selected heat tolerant lines (BC_2F_5) developed in the present study. Molecular markers linked to various heat tolerant QTLs transferred in *T. durum-Ae. speltoides* introgression lines and reported by Awlachew et al. (2016) were used for marker assisted transfer of selected QTL to hexaploid wheat genotypes. Blue colour square represents presence of marker allele associated with QTL; red colour square represents recipient parent type marker allele. A to E represent markers associated with QTLs: *QSs.pau-4A, QTtc.pau-1B, QCt.pau-3B, QTgw. pau-5B,* and *QTgw.pau-2B, r*espectively

Correlation analysis

Correlation studies between various agro-morphological traits were carried out to establish their relationship with TGW and YD for both OE and HSE (Fig.3).The HTILs showed significant positive correlation of FLL, TNpM, GNpS, GFD, and TGW with YD, where GFD had the highest correlation followed by TNpM and TGW. Pairwise comparisons of the most important traits were made and have been presented in Fig. 3 (lower triangle). Longer GFD led to higher TGW in OE and HSE across both BC_2F_3 and BC_2F_5 generations of HTILs. Similar trend was observed when TGW and TNpM was compared to yield.

Heat Tolerance Index

HTILs showed higher HTI index than their recurrent parents for one or the other trait, mostly for TGW and YD, which showed positive effect of the selection in previous generations. A summary of heat tolerance index calculated for various traits of the selected BC_2F_5 lines (with higher HTI of at least three traits) is given in Table 4 and detailed HTI for full set of HTILs is presented in Supplementary Table S3. HTI for yield was high for pauHTIL_27, 29, 20, 38, 18, 2, and 28 with pauHTIL_27 having the highest HTI (> 100). For TGW, HTI for pauHTIL_30 was 102.05. Similarly, for TNpM, HTI was high for pauHTIL_25 (119.61), pauHTIL_32 (115.98), pauHTIL_28 (105.55), and pauHTIL_30 (100.07).

Validation of effect of QTLs in HTILs

Traits showing significant positive correlation to YD i.e., FLL, TNpM, GFD, and TGW along with SN were used to study the effect of specific QTLs in HTILs. The presence or absence of QTLs were correlated to the trait phenotypes of BC_2F_5 HTILs under both OE and HSE which has been presented as heat map in Fig. 4. Effect of the presence of the donor alleles of the HT QTL on the traits under study has been depicted as boxplots in both environments (Fig. 5, Table 4). Presence of QTL *QSs.pau-4A* showed positive effect on SN across the HTILs with more prominent effect in HSE (19.47%) than OE (9.24%). QTL *QCt.pau-3B* also showed positive effect on FLL in OE (18.49%) and TGW in HSE (8.05%). Both the QTLs for TGW *QTgw.pau-5B,* and *QTgw.pau-2B* showed large positive effect on TGW but in different environments where *QTgw.pau-5B* contributed more in OE (15.74%) while *QTgw.pau-2B* in HSE (26.70%). All these QTLs (except *QSs.pau-4A*) had significant effect on overall yield with a significant contribution of *QTgw.pau-5B* in OE (13.06%) while *QTgw.pau-2B* in HSE (6.45%).

Discussion

Heat stress is a major constraint in a winter crop like wheat, especially when temperature rises during reproductive stage, affecting the pollen viability, fertilization capacity, grain filling processes and finally the total yield of crop (Akter and Rafiqul 2017; Cramer

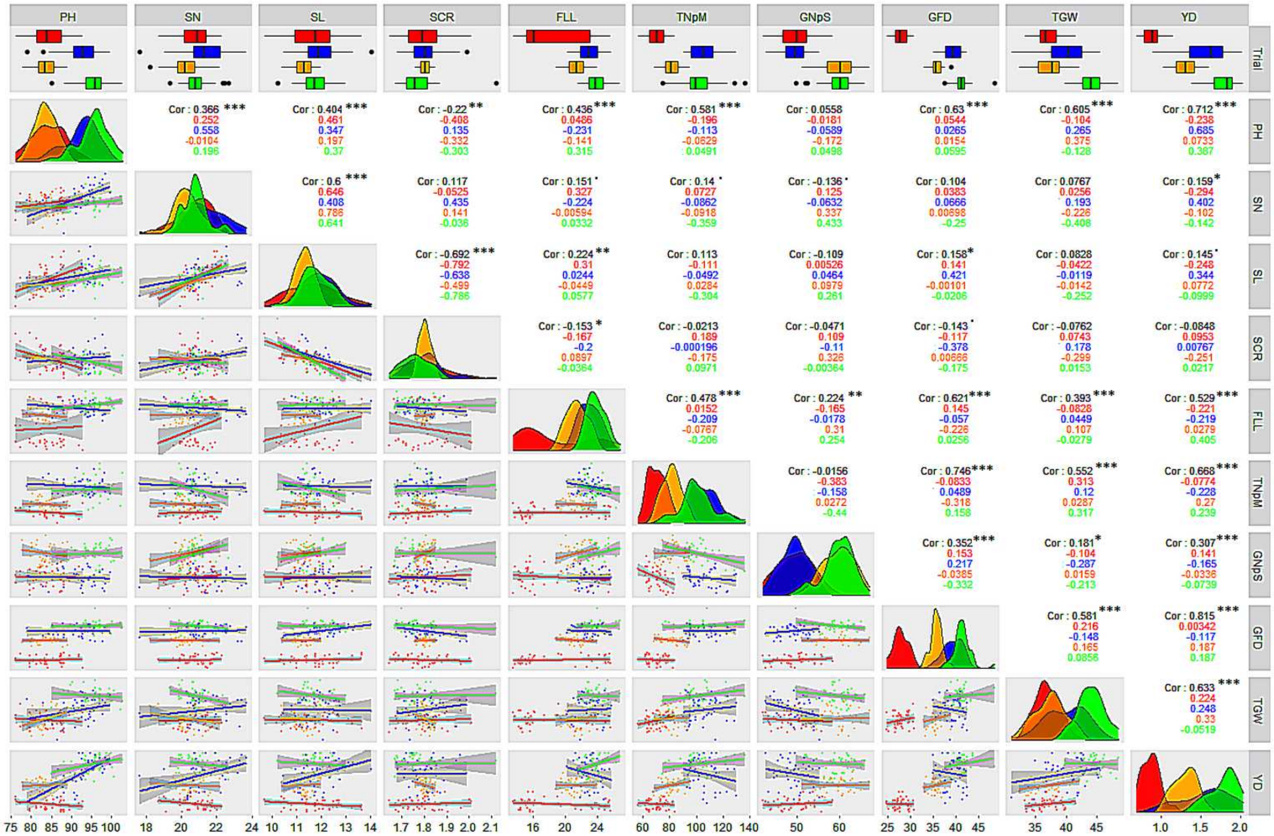


Fig. 3. Evaluation of BC_2F_3 and BC_2F_5 progenies of HTILs developed across seasons 2017-18 and 2018-19. Blue colour represents trait response in optimum environment (OE) and red coloured boxes represent trait response in heat stress environment (HSE) in season 2017-18, green colour represent trait response in OE and orange colour represent trait response in HSE in season 2018-19. Top row represents the distribution of traits as boxplots. Upper right triangle shows pairwise correlation values as overall correlation in black colour while other colours are represented individually as explained above. The diagonal represents pairwise density distribution plots of relative traits. Lower triangle represents individual trait values in pairwise dotplots with regression lines. #Plant height (PH), Spikelet number per spike (SN), spike length (SL), spike compaction ratio (SCR), flag leaf length (FLL), tiller number per meter (TNpM), grain number per spike (GNpS), grain filling duration (GFD), thousand grain weight (TGW), yield per plot (YD)

et al. 2011; Farooq et al. 2011; Hemantaranjan et al. 2014; Iqbal et al. 2017). The main objective of the study was to transfer heat tolerance components from *T. durum-Ae.speltoides* backcross introgression lines to hexaploid background as there is limited variation for heat stress tolerance in bread wheat cultivars. *Ae. speltoides* has been used to transfer HT QTLs to durum wheat by our group (Awlachew et al. 2016; Awlachew and Chhuneja 2013). Present study reports the transfer of HT QTL to hexaploid wheat using these DS-BILs as donors. Marker assisted selection was used for tracking the heat tolerance QTLs exploiting the codominance nature of markers to distinguish between the homozygous and heterozygous donor alleles.

Although good amount of seed set was observed in the F_1 and back cross generations but germination and further establishment of the plants was very less (~5% to 15%), which was expected in crosses between tetraploid and hexaploid parental lines (Table 1). Also, donor DS-BILs carried alien introgressions other than targeted ones, which might have affected plant survival (Arabbeigi et al. 2010; Rezaei et al. 2010). Crossing between tetraploid donor genotypes and hexaploid recipient parents resulted in pentaploid F_1s in which were backcrossed to recipient parent (s) for recovering normal chromosome complement. *Ae. speltoides* has been reported to carry genes that are epistatic to *Ph1* locus resulting in homeologous pairing in F_1 plants of the crosses of *Ae. speltoides* with wheat (Colas et al.

Table 3. Heat tolerance index of BC_2F_5 HTILs of various traits calculated based on phenotypic evaluation under OE and HSE

Genotype	HT QTLs introgresssed	SN	FLL	TNpM	GNpS	GFD	TGW	YD
BWL3558		106.26	90.32	89.05	111.65	91.51	80.95	64.99
BWL4444		100.71	93.42	92.76	112.48	87.34	81.90	79.54
BWL5185		101.17	85.60	88.68	117.53	88.06	89.66	65.53
Check		99.46	92.05	75.77	105.98	87.76	87.98	72.98
HTIL(BWL3558)								
pauHTIL_2	*QTtc.pau-1B, QCt.pau-3B, QTgw.pau-2B*	91.65	**97.19**	**83.07**	97.96	86.62	87.22	**85.99**
pauHTIL_5	*QSs.pau-4A, QTtc.pau-1B, QCt.pau-3B, QTgw.pau-5B*	95.34	**92.67**	**85.54**	101.64	**88.16**	83.53	68.82
pauHTIL_16	*QSs.pau-4A, QCt.pau-3B, QTgw.pau-5B*	**103.49**	87.42	**76.35**	99.47	**88.29**	67.33	61.80
pauHTIL_17	*QSs.pau-4A, QCt.pau-3B, QTgw.pau-5B*	**104.24**	**96.72**	**77.23**	**110.67**	86.86	**80.11**	**74.23**
pauHTIL_18	*QTtc.pau-1B, QCt.pau-3B, QTgw.pau-5B*	**101.16**	85.70	**90.39**	**111.06**	86.83	79.86	**86.12**
pauHTIL_20	*QSs.pau-4A, QTtc.pau-1B*	**102.60**	**92.22**	**89.32**	105.46	**89.65**	**93.65**	**94.55**
pauHTIL_21	*QSs.pau-4A, QTtc.pau-1B, QCt.pau-3B, QTgw.pau-5B, QTgw.pau-2B*	92.75	**98.68**	**78.45**	**110.21**	**88.29**	84.46	71.06
pauHTIL_22	*QSs.pau-4A, QTtc.pau-1B, QTgw.pau-2B*	94.13	**93.74**	62.85	98.99	87.49	**93.29**	**78.09**
HTIL(BWL4444)								
pauHTIL_13	*QCt.pau-3B*	**101.06**	88.41	**82.62**	97.91	86.83	81.03	72.82
pauHTIL_14	*QTtc.pau-1B, QCt.pau-3B, QTgw.pau-5B, QTgw.pau-2B*	**101.67**	**94.04**	**87.55**	103.90	**89.04**	84.18	**84.63**
pauHTIL_23	*QCt.pau-3B, QTgw.pau-5B*	92.65	78.70	**93.46**	99.45	86.54	87.53	**73.38**
pauHTIL_24	*QSs.pau-4A, QCt.pau-3B*	97.77	**98.14**	**87.13**	100.54	86.57	87.93	**82.34**
pauHTIL_25	*QTtc.pau-1B, QCt.pau-3B, QTgw.pau-5B*	97.59	81.32	**119.61**	96.69	82.33	86.14	**79.73**
pauHTIL_27	*QCt.pau-3B, QTgw.pau-5B, QTgw.pau-2B*	94.90	86.10	**81.95**	90.46	**88.82**	84.20	**105.32**
pauHTIL_28	*QSs.pau-4A, QCt.pau-3B, QTgw.pau-2B*	94.74	97.51	**105.55**	96.54	87.34	**98.97**	**85.01**
pauHTIL_36	*QSs.pau-4A, QCt.pau-3B*	92.18	**99.58**	**78.20**	93.18	**90.09**	**88.35**	70.38
pauHTIL_38	*QSs.pau-4A, QTtc.pau-1B, QTgw.pau-5B, QTgw.pau-2B*	**104.53**	87.41	**80.44**	93.88	86.04	**89.92**	**86.92**
pauHTIL_39	*QCt.pau-3B, QTgw.pau-5B*	**107.35**	89.11	**76.74**	**121.90**	80.36	74.11	**82.02**
HTIL(BWL5185)								
pauHTIL_29	*QSs.pau-4A, QCt.pau-3B, QTgw.pau-5B, QTgw.pau-2B*	88.01	**100.13**	**90.45**	101.07	**88.43**	**92.08**	**95.31**
pauHTIL_30	*QSs.pau-4A, QTtc.pau-1B, QCt.pau-3B, QTgw.pau-5B*	98.76	87.36	**100.07**	100.69	**88.10**	**102.05**	**76.97**
pauHTIL_32	*QSs.pau-4A*	95.31	90.47	**115.98**	**106.19**	84.49	**89.90**	**76.18**
pauHTIL_33	*QSs.pau-4A, QTtc.pau-1B, QTgw.pau-5B, QTgw.pau-2B*	**103.37**	89.75	67.78	**128.08**	86.54	**90.50**	70.19
pauHTIL_34	*QSs.pau-4A, QTtc.pau-1B, QTgw.pau-5B, QTgw.pau-2B*	93.54	**94.07**	63.07	**109.32**	84.49	**90.42**	68.86

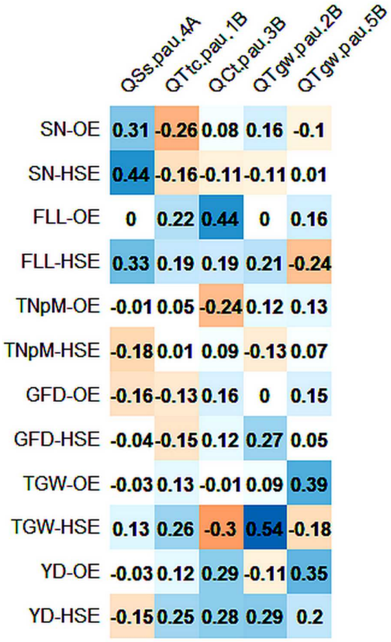
SN= Spikelet no. per spike, FLL =Flag leaf length , TNpM= Tiller number per meter , GNpS =Grain number per spike , GFD =Grain filling duration , TGW= Thousand grain weight, and YD= Yield per plot

††Underlined values show performance better than respective recurrent parents and bold values shows performance better than checks.

Table 4. Effect of various QTL introgressions in HTILs on important yield related traits in OE (Optimum environment) and HSE (Heat stress environment) with significant difference based on Kruskal-Wallis test

Trait	QTL	Env	H-statistic	Effect	magnitude
SN	*QSs.pau-4A*	OE	04.511	09.24*	moderate
	QSs.pau-4A	HSE	08.400	19.47**	large
FLL	*QCt.pau-3B*	OE	08.027	18.49**	large
	QSs.pau-4A	HSE	04.327	08.76*	moderate
GFD	*QTgw.pau-2B*	HSE	03.536	06.67*	moderate
TGW	*QTgw.pau-5B*	OE	06.982	15.74**	large
	QTgw.pau-2B	HSE	11.146	26.70***	large
	QCt.pau-3B	HSE	04.057	08.05*	moderate
YD	*QTgw.pau-5B*	OE	05.962	13.06*	moderate
	QTgw.pau-2B	HSE	03.452	06.45.	moderate

SN= Spikelet no. per spike, FLL =Flag leaf length, GFD =Grain filling duration, TGW= Thousand grain weight, YD= Yield per plot and Env= Environment, †† level of significance; p-value < 0.001 (***), p-value < 0.01 (**), p-value < 0.05 (*), and p-value < 0.10 (.)



	QSs.pau.4A	QTtc.pau.1B	QCt.pau.3B	QTgw.pau.2B	QTgw.pau.5B
SN-OE	0.31	-0.26	0.08	0.16	-0.1
SN-HSE	0.44	-0.16	-0.11	-0.11	0.01
FLL-OE	0	0.22	0.44	0	0.16
FLL-HSE	0.33	0.19	0.19	0.21	-0.24
TNpM-OE	-0.01	0.05	-0.24	0.12	0.13
TNpM-HSE	-0.18	0.01	0.09	-0.13	0.07
GFD-OE	-0.16	-0.13	0.16	0	0.15
GFD-HSE	-0.04	-0.15	0.12	0.27	0.05
TGW-OE	-0.03	0.13	-0.01	0.09	0.39
TGW-HSE	0.13	0.26	-0.3	0.54	-0.18
YD-OE	-0.03	0.12	0.29	-0.11	0.35
YD-HSE	-0.15	0.25	0.28	0.29	0.2

Fig. 4. Heatmap of effect of HT QTLs present across the HTILs to various traits. Blue colour represents positive effect (increment) on trait value across both OE (Optimum environment) and HSE (Heat stress environment) while red colour represents negative effect (decrement) of trait values. The size and intensity of circles represents the association effect with larger and darker circles representing higher effect on trait by presence of a QTL and smaller and lighter colour intensity represent lower association or effect. # Spiklet number per spike (SN), flag leaf length (FLL), tiller number per meter (TNpM), grain filling duration (GFD), thousand grain weight (TGW), yield per plot (YD)

2008; Millet 2007) which can also result in large number of multivalent formations, which can cause poor germination and plant establishment (Supplementary Fig. S1).

Marker assisted selection was used in the backcross generations to select for desirable plants carrying QTL for traits of interest. Phenotypic selections were also conducted along with MAS which aided in selecting for recurrent type plant phenotype. All the introgressed QTLs were present in homozygous form in the selected BC$_2$F$_5$ HTILs (Fig. 2). Evaluation of these HTILs over two years across normal and heat stress environments showed that the introgressed heat tolerance QTL imparted heat stress tolerance to these lines (Table 3, 4).

There is sudden rise in day temperature starting from mid to end of March which cause forced maturation of plants reducing their grain filling duration significantly, especially for those grown in HSE, as reported by Acevedo et al. (1990), McMaster (1997) and Pimentel et al. (2015). Late sown plants experience this stress more than those in OE as their anthesis time coincides with theperiod of heat stress (Flohr et al. 2017). In the present study, the daily temperatures varied from 17.4-34.0°C during day and 6.8-17.0°C during night from October to May (Supplementary Fig. S4). Higher temperature causes reduction in overall plant phenology in HSE i.e., higher rates of senescence. The inhibition of photosynthesis at high-temperature results in drop of carbohydrate reserves, which in turn reduce production as a result of reduction in crop cycle (Pimentel et al. 2015).

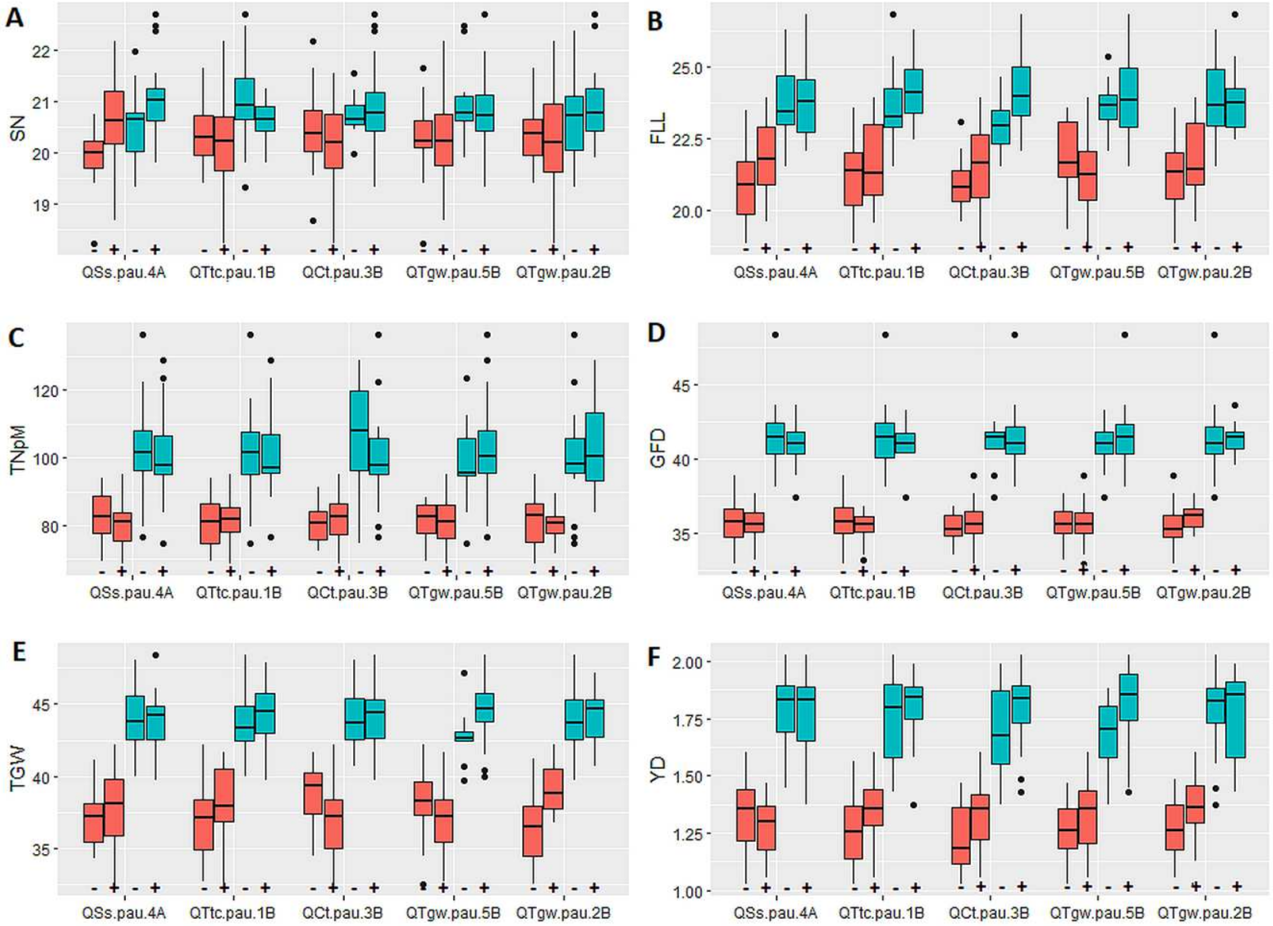


Fig. 5. Effect of various QTL introgressions in HTILs on important yield related traits (a-f). Blue color represents trait response in OE (Optimum environment) and red color represents trait response in HSE (Heat stress environment). The positive (+) and negative (-) signs represents the distribution of trait response in presence and absence of the QTL, respectively

Different traits showed differential response to heat stress in these HTILs. All the traits studied in this investigation, including TNpM, GFD, TGW, and YD, were negatively affected by heat stress except SN and GNpS. Plants maintain lower CT by increasing rates of transpiration and photosynthesis leading to less yield losses in HSE (Awlachew et al. 2016). This has been depicted in the present study by strong negative correlation between CT and GFD in both the environments, especially in HSE where progenies with higher CT showed overall reduction in GFD which in return reduced TGW and YD. HTILpauHTIL_30 with introgression of QTLs *QSs.pau-4A, QTtc.pau-1B, QCt.pau-3B,* and *QTgw.pau-5B* showed an increase in TGW in HSE than OE despite reduction in GFD and YD which may be attributed to increase in rate of translocation of the photosynthates and stem reserves to the seed when heat stress influenced the reproductive cycle (Dias and Lidon 2009).

The most important contributing factor to yield increase was found to be increase in tiller number increasing the number of grains per plant which showed positive correlation for both the environments in most of the progenies as also been suggested by Kumar et al. (2017). Progenies developed during this investigation showed higher tolerance to heat stress than their respective recurrent parents as depicted from overall higher HTI for most of the yield related traits. HTI (>100) for TGW showed that TGW was higher in HSE than in OE which may be due to *Ae. speltoides* specific genetic elements which expressed during higher temperatures. This kind of transgressive behaviour has also been reported earlier in complex genome of bread wheat where alien introgressions are involved.

Heat tolerance is a complex trait that can only be estimated from change in yield-related traits under heat stress (Jha et al. 2014; Kumar et al. 2017). These traits being quantitative in nature vary significantly based on genes or pathways involved. Compounded by huge complexity of the bread wheat genome, it is difficult to dedicate a single trait as representation of tolerance to heat stress (Deb and Khaleque 2009). However, tiller number, TGW and yield are the best estimates for heat stress tolerance.In the present study large variations were observed among genotypes in different backgrounds and different environments, which can be attributed to G X E interactions. Introgression of the favourable QTLs from the donor parent led to the development of heat stress-tolerant progenies. From the 40 selected lines pauHTIL_10, 11, 12, 34, 37 and 19 have shown higher yield than tested cultivars under OE (Supplementary Table S4) and pauHTIL_14 under HSE. pauHTIL_14 had introgression for 4 QTLs (*QTtc.pau-1B, QCt.pau-3B, QTgw.pau-5B*, and *QTgw.pau-2B*) where QTL *QTgw.pau-5B* and *QTgw.pau-2B* have shown significant effect of yield (Table 4). These progenies are now being further evaluated under multiple environments at state level trials and these can further be used for developing heat-tolerant wheat varieties.

Authors' contribution

Conceptualization of research (PC, ND); Designing of the experiments (GSD, ND, SK, PC); Contribution of experimental materials (PC); Execution of field/lab experiments and data collection (GSD, SK, NDB, PS); Analysis of data and interpretation (GSD); Preparation of manuscript (GSD, ND, PC).

Declaration

The authors declare no conflict of interest.

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Supplementary Table S1. Summary of heat tolerance QTLs from *T. durum-Ae. speltoides* introgression lines and rust resistance genes of the recurrent parent genotypes followed through MAS in the present study

Heat tolerance QTLs/ rust resistance genes	LOD score	PVE name	Marker associated	Trait genes	Donor line harbouring QTLs/genome	QTL donor	Source
QCt.pau-3B	4.20	19.73	*Xgwm264*	CT	DS-BIL25, 31, 37, 44, 628	*T. durum*	Awlachew et al., 2016
QTgw.pau-5B	4.92	17.22	*Xgwm371*	TGW	DS-BIL23, 25, 31, 37, 44, 628	*Ae. speltoides*	unpublished
QSs.pau-4A	4.10	18.60	*Xgwm565*	SN	DS-BIL23, 25, 31, 37, 44, 628	*Ae. speltoides*	Awlachew & Chhuneja, 2013
QTgw.pau-2B	3.50	17.82	*Xwmc31*	TGW, GW	DS-BIL23, 25, 44	*T. durum*	Awlachew et al., 2013
QTtc.pau-1B	2.70	14.20	*Xwmc269*	TTC%	DS-BIL25, 31, 37, 44	*Ae. speltoides*	Awlachew et al., 2013
QSs.pau-7B	4.61	13.65	*Xwmc517*	SN	DS-BIL25, 31, 37	*T. durum*	unpublished
QSs.pau-1A	3.20	14.68	*Kasp_HT1*	SN	DS-BIL44, 628	*T. durum*	unpublished
Yr5			*Xwmc175*	Stripe rust resistance	BWL3558		*https://wheat.pw.usda.gov/*
Yr10			*Xps3000*	Stripe rust resistance	BWL4444, BWL5185		*https://wheat.pw.usda.gov/*
Yr15			*Xgwm498*	Stripe rust resistance	BWL5185		*https://wheat.pw.usda.gov/*

† Phenotypic variation explained during mapping (PVE), Canopy Temperature (CT), spikelet no. per spike (SN), thousand grain weight (TGW), grain weight (GW), percent TTC converted (TTC%)

† † *Kasp_HT1*is inhouse designed SNP marker

Supplementary Table S2. Heat tolerance QTL (HT QTL) composition of derived Heat tolerant introgression lines (HTILs)

Genotype	Pedigree	HT QTLs
pau_HTT1	HTIL/BWL3558	*QTtc.pau-1B, QCt.pau-3B, QTgw.pau-2B*
pau_HTT2	HTIL/BWL3558	*QTtc.pau-1B, QCt.pau-3B, QTgw.pau-2B*
pau_HTT3	HTIL/BWL3558	*QTgw.pau-5B*
pau_HTT4	HTIL/BWL3558	*QSs.pau-4A, QTtc.pau-1B, QCt.pau-3B, QTgw.pau-5B*
pau_HTT5	HTIL/BWL3558	*QSs.pau-4A, QTtc.pau-1B, QCt.pau-3B, QTgw.pau-5B*
pau_HTT6	HTIL/BWL4444	*QTtc.pau-1B, QCt.pau-3B, QTgw.pau-5B*
pau_HTT7	HTIL/BWL4444	*QTgw.pau-2B*
pau_HTT8	HTIL/BWL4444	*QSs.pau-4A*
pau_HTT9	HTIL/BWL4444	*QSs.pau-4A, QTtc.pau-1B, QCt.pau-3B*
pau_HTT10	HTIL/BWL4444	*QSs.pau-4A, QCt.pau-3B, QTgw.pau-5B*
pau_HTT11	HTIL/BWL4444	*QCt.pau-3B, QTgw.pau-5B*
pau_HTT12	HTIL/BWL4444	*QCt.pau-3B, QTgw.pau-5B*
pau_HTT13	HTIL/BWL4444	*QCt.pau-3B*
pau_HTT14	HTIL/BWL4444	*QTtc.pau-1B, QCt.pau-3B, QTgw.pau-5B, QTgw.pau-2B*
pau_HTT15	HTIL/BWL4444	*QSs.pau-4A, QTtc.pau-1B, QCt.pau-3B, QTgw.pau-5B*
pau_HTT16	HTIL/BWL3558	*QSs.pau-4A, QCt.pau-3B, QTgw.pau-5B*
pau_HTT17	HTIL/BWL3558	*QSs.pau-4A, QCt.pau-3B, QTgw.pau-5B*
pau_HTT18	HTIL/BWL3558	*QTtc.pau-1B, QCt.pau-3B, QTgw.pau-5B*
pau_HTT19	HTIL/BWL3558	*QTtc.pau-1B, QCt.pau-3B, QTgw.pau-5B, QTgw.pau-2B*
pau_HTT20	HTIL/BWL3558	*QSs.pau-4A, QTtc.pau-1B*
pau_HTT21	HTIL/BWL3558	*QSs.pau-4A, QTtc.pau-1B, QCt.pau-3B, QTgw.pau-5B, QTgw.pau-2B*
pau_HTT22	HTIL/BWL3558	*QSs.pau-4A, QTtc.pau-1B, QTgw.pau-2B*
pau_HTT23	HTIL/BWL4444	*QCt.pau-3B, QTgw.pau-5B*
pau_HTT24	HTIL/BWL4444	*QSs.pau-4A, QCt.pau-3B*
pau_HTT25	HTIL/BWL4444	*QTtc.pau-1B, QCt.pau-3B, QTgw.pau-5B*
pau_HTT26	HTIL/BWL4444	*QSs.pau-4A, QCt.pau-3B, QTgw.pau-5B, QTgw.pau-2B*
pau_HTT27	HTIL/BWL4444	*QCt.pau-3B, QTgw.pau-5B, QTgw.pau-2B*
pau_HTT28	HTIL/BWL4444	*QSs.pau-4A, QCt.pau-3B, QTgw.pau-2B*
pau_HTT29	HTIL/BWL5185	*QSs.pau-4A, QCt.pau-3B, QTgw.pau-5B, QTgw.pau-2B*
pau_HTT30	HTIL/BWL5185	*QSs.pau-4A, QTtc.pau-1B, QCt.pau-3B, QTgw.pau-5B*
pau_HTT31	HTIL/BWL3558	*QTgw.pau-5B*
pau_HTT32	HTIL/BWL5185	*QSs.pau-4A*
pau_HTT33	HTIL/BWL5185	*QSs.pau-4A, QTtc.pau-1B, QTgw.pau-5B, QTgw.pau-2B*
pau_HTT34	HTIL/BWL5185	*QSs.pau-4A, QTtc.pau-1B, QTgw.pau-5B, QTgw.pau-2B*
pau_HTT35	HTIL/BWL4444	*QSs.pau-4A, QTgw.pau-5B, QTgw.pau-2B*
pau_HTT36	HTIL/BWL4444	*QSs.pau-4A, QCt.pau-3B*
pau_HTT37	HTIL/BWL4444	*QCt.pau-3B, QTgw.pau-5B*
pau_HTT38	HTIL/BWL4444	*QSs.pau-4A, QTtc.pau-1B, QTgw.pau-5B, QTgw.pau-2B*
pau_HTT39	HTIL/BWL4444	*QCt.pau-3B, QTgw.pau-5B*
pau_HTT40	HTIL/BWL4444	*QCt.pau-3B*

Supplementary Table S3. Heat tolerance index of BC_2F_5 HTILs for various traits calculated based on phenotypic evaluation under OE and HSE

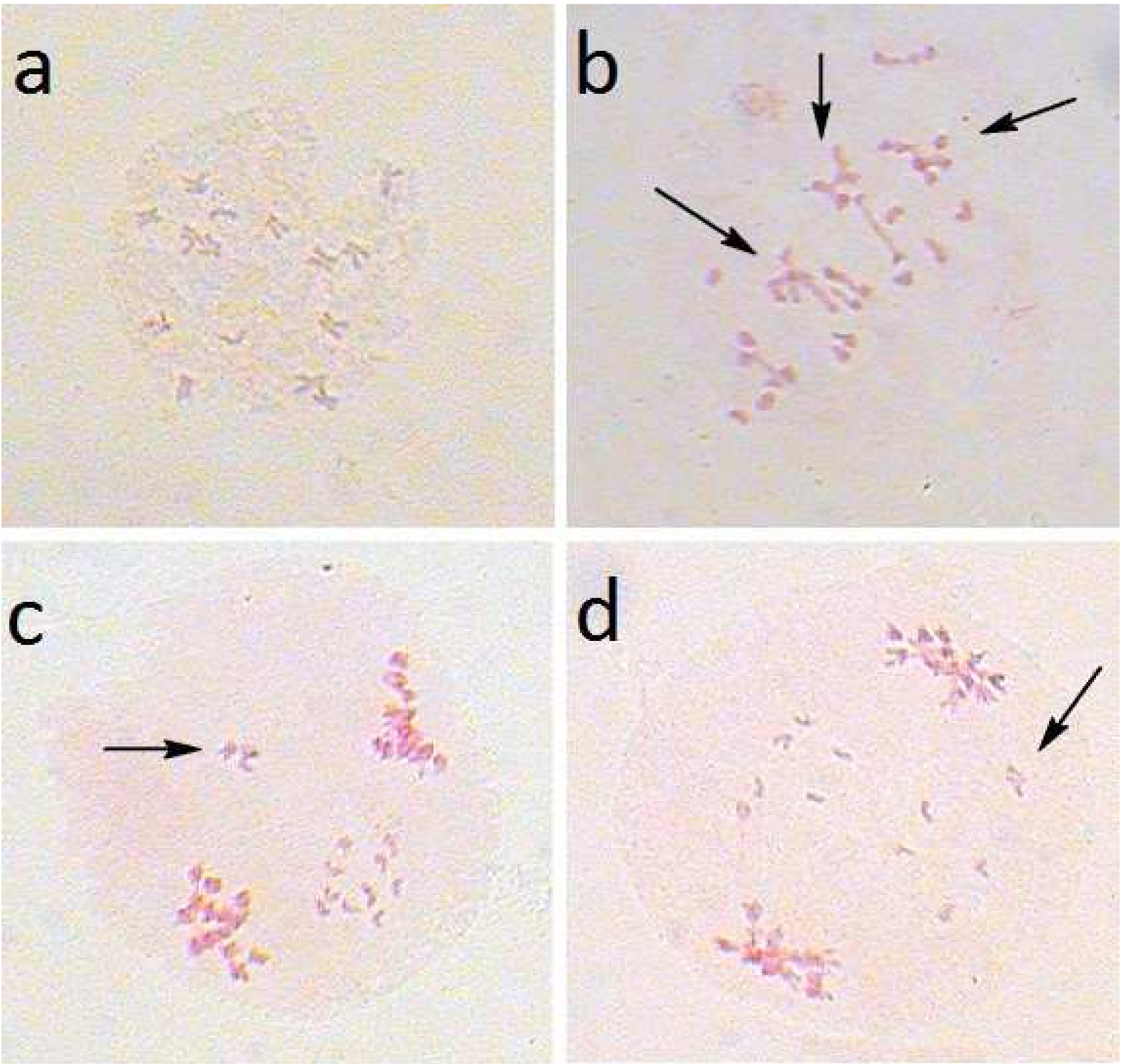
Genotype	Pedigree	SN	FLL	TNpM	GNpS	GFD	TGW	YD
BWL3558	BWL3558	106.26	90.32	89.05	111.65	91.51	80.95	64.99
BWL4444	BWL4444	100.71	93.42	92.76	112.48	87.34	81.90	79.54
BWL5185	BWL5185	101.17	85.60	88.68	117.53	88.06	89.66	65.53
Check	Check	99.46	92.05	75.77	105.98	87.76	87.98	72.98
pauHTT_1	HTIL(BWL3558)	98.01	90.65	**76.72**	105.72	84.56	86.97	64.79
pauHTT_2	HTIL(BWL3558)	91.65	**97.19**	**83.07**	97.96	86.62	87.22	**85.99**
pauHTT_3	HTIL(BWL3558)	98.60	90.72	68.28	93.45	**89.32**	78.95	**73.13**
pauHTT_4	HTIL(BWL3558)	97.42	90.91	66.01	94.92	85.99	75.70	57.43
pauHTT_5	HTIL(BWL3558)	95.34	**92.67**	**85.54**	101.64	**88.16**	83.53	68.82
pauHTT_16	HTIL(BWL3558)	**103.49**	87.42	**76.35**	99.47	**88.29**	67.33	61.80
pauHTT_17	HTIL(BWL3558)	**104.24**	**96.72**	**77.23**	**110.67**	86.86	**80.11**	**74.23**
pauHTT_18	HTIL(BWL3558)	**101.16**	85.70	**90.39**	**111.06**	86.83	79.86	**86.12**
pauHTT_19	HTIL(BWL3558)	95.30	86.68	**88.56**	96.92	87.37	84.88	64.86
pauHTT_20	HTIL(BWL3558)	**102.60**	**92.22**	**89.32**	105.46	**89.65**	**93.65**	**94.55**
pauHTT_21	HTIL(BWL3558)	92.75	**98.68**	**78.45**	**110.21**	**88.29**	84.46	71.06
pauHTT_22	HTIL(BWL3558)	94.13	**93.74**	62.85	98.99	87.49	**93.29**	**78.09**
pauHTT_31	HTIL(BWL3558)	96.56	**95.36**	**94.72**	95.86	80.95	80.54	59.65
pauHTT_6	HTIL(BWL4444)	94.97	89.74	64.38	103.70	83.75	77.64	**81.39**
pauHTT_7	HTIL(BWL4444)	97.40	91.41	70.62	95.50	**90.42**	**96.78**	66.07
pauHTT_8	HTIL(BWL4444)	**104.84**	**96.85**	69.73	104.47	86.67	81.16	62.20
pauHTT_9	HTIL(BWL4444)	**100.90**	88.36	**90.05**	100.53	82.42	81.81	65.61
pauHTT_10	HTIL(BWL4444)	**101.32**	90.22	75.61	102.92	80.94	76.67	51.90
pauHTT_11	HTIL(BWL4444)	96.77	86.07	**93.13**	88.76	84.69	83.01	**73.34**
pauHTT_12	HTIL(BWL4444)	90.03	88.40	**95.28**	100.79	86.36	87.28	61.72
pauHTT_13	HTIL(BWL4444)	**101.06**	88.41	**82.62**	97.91	86.83	81.03	72.82
pauHTT_14	HTIL(BWL4444)	**101.67**	**94.04**	**87.55**	103.90	**89.04**	84.18	**84.63**
pauHTT_15	HTIL(BWL4444)	98.51	80.19	68.27	82.34	87.49	80.38	64.63
pauHTT_23	HTIL(BWL4444)	92.65	78.70	**93.46**	99.45	86.54	87.53	**73.38**
pauHTT_24	HTIL(BWL4444)	97.77	**98.14**	**87.13**	100.54	86.57	87.93	**82.34**
pauHTT_25	HTIL(BWL4444)	97.59	81.32	**119.61**	96.69	82.33	86.14	**79.73**
pauHTT_26	HTIL(BWL4444)	**101.05**	79.95	75.30	97.12	86.38	83.36	**74.34**
pauHTT_27	HTIL(BWL4444)	94.90	86.10	**81.95**	90.46	**88.82**	84.20	**105.32**
pauHTT_28	HTIL(BWL4444)	94.74	97.51	**105.55**	96.54	87.34	**98.97**	**85.01**
pauHTT_35	HTIL(BWL4444)	94.89	85.45	68.77	93.69	**88.10**	81.80	71.20
pauHTT_36	HTIL(BWL4444)	92.18	**99.58**	**78.20**	93.18	**90.09**	**88.35**	70.38
pauHTT_37	HTIL(BWL4444)	99.10	81.07	58.98	106.75	82.99	87.60	61.45
pauHTT_38	HTIL(BWL4444)	**104.53**	87.41	**80.44**	93.88	86.04	**89.92**	**86.92**
pauHTT_39	HTIL(BWL4444)	**107.35**	89.11	**76.74**	**121.90**	80.36	74.11	**82.02**
pauHTT_40	HTIL(BWL4444)	91.59	82.93	65.61	85.34	87.12	**88.78**	72.02
pauHTT_29	HTIL(BWL5185)	88.01	**100.13**	**90.45**	101.07	**88.43**	**92.08**	**95.31**
pauHTT_30	HTIL(BWL5185)	98.76	87.36	**100.07**	100.69	**88.10**	**102.05**	**76.97**
pauHTT_32	HTIL(BWL5185)	95.31	90.47	**115.98**	**106.19**	84.49	**89.90**	**76.18**
pauHTT_33	HTIL(BWL5185)	**103.37**	89.75	67.78	**128.08**	86.54	**90.50**	70.19
pauHTT_34	HTIL(BWL5185)	93.54	**94.07**	63.07	**109.32**	84.49	**90.42**	68.86

SN= Spikelet no. per spike, FLL= Flag leaf length, TNpM= Tiller number per meter, GNpS= Grain number per spike, GFD= Grain filling duration, TGW= Thousand grain weight and YD= Yield per plot

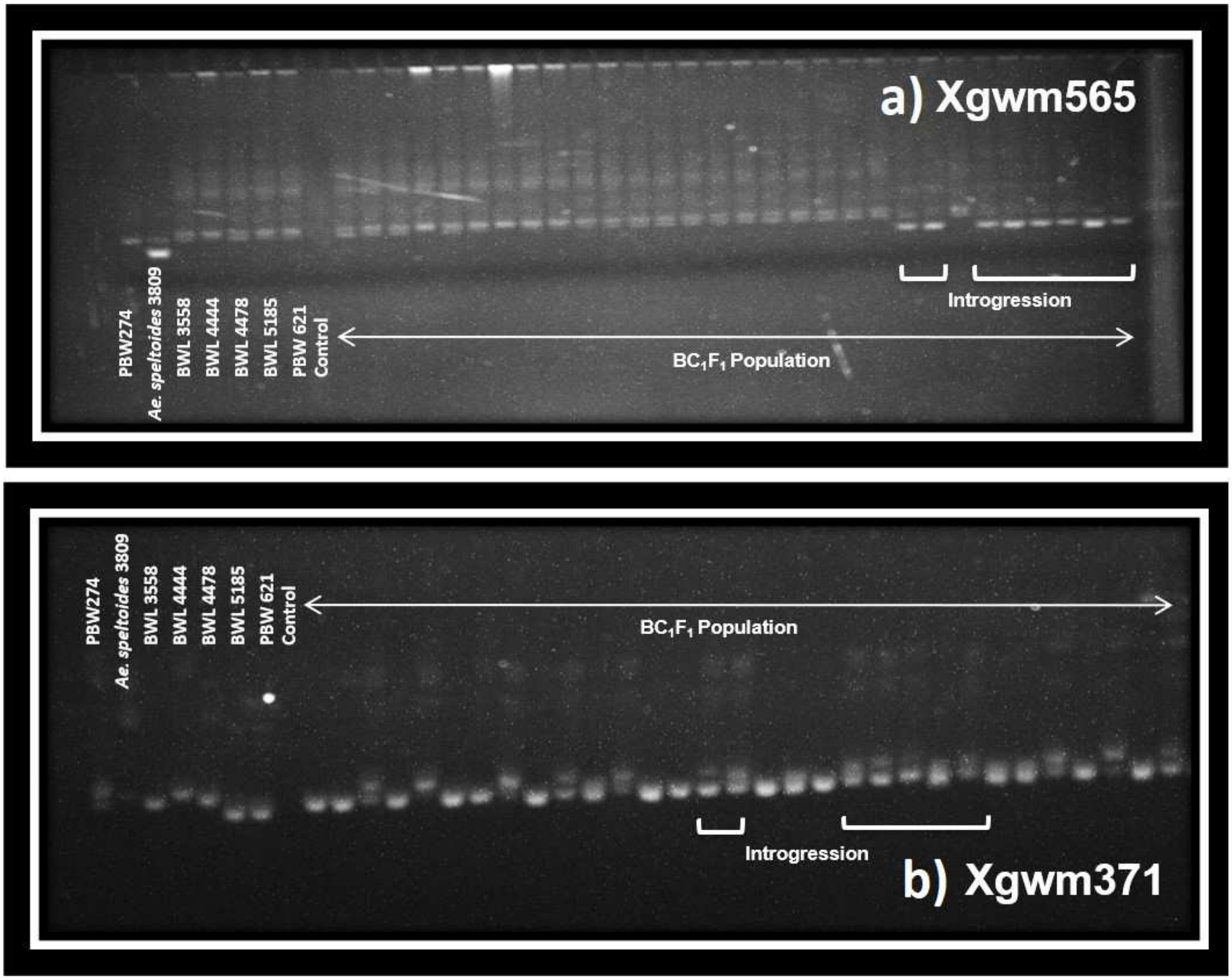
Supplementary Table S4. Top performing BC_2F_5 HTILs showing yield higher then tested cultivars

Genotype	Pedigree	SN	FLL	TNpM	GFD	TGW	YD
pau_HTT10	HTIL/BWL4444	21.25	24.33	105.58	43.61	44.4	2.029
pau_HTT11	HTIL/BWL4444	20.77	25.34	100.85	39.59	41.44	2.029
pau_HTT12	HTIL/BWL4444	21.97	24.91	97.84	38.13	42.38	2.027
pau_HTT19	HTIL/BWL3558	20.41	24.18	96.12	41.42	44.92	1.972
pau_HTT34	HTIL/BWL5185	19.97	22.58	128.81	41.78	46.12	1.988
pau_HTT37	HTIL/BWL4444	19.89	23.24	136.56	43.61	43.95	1.974

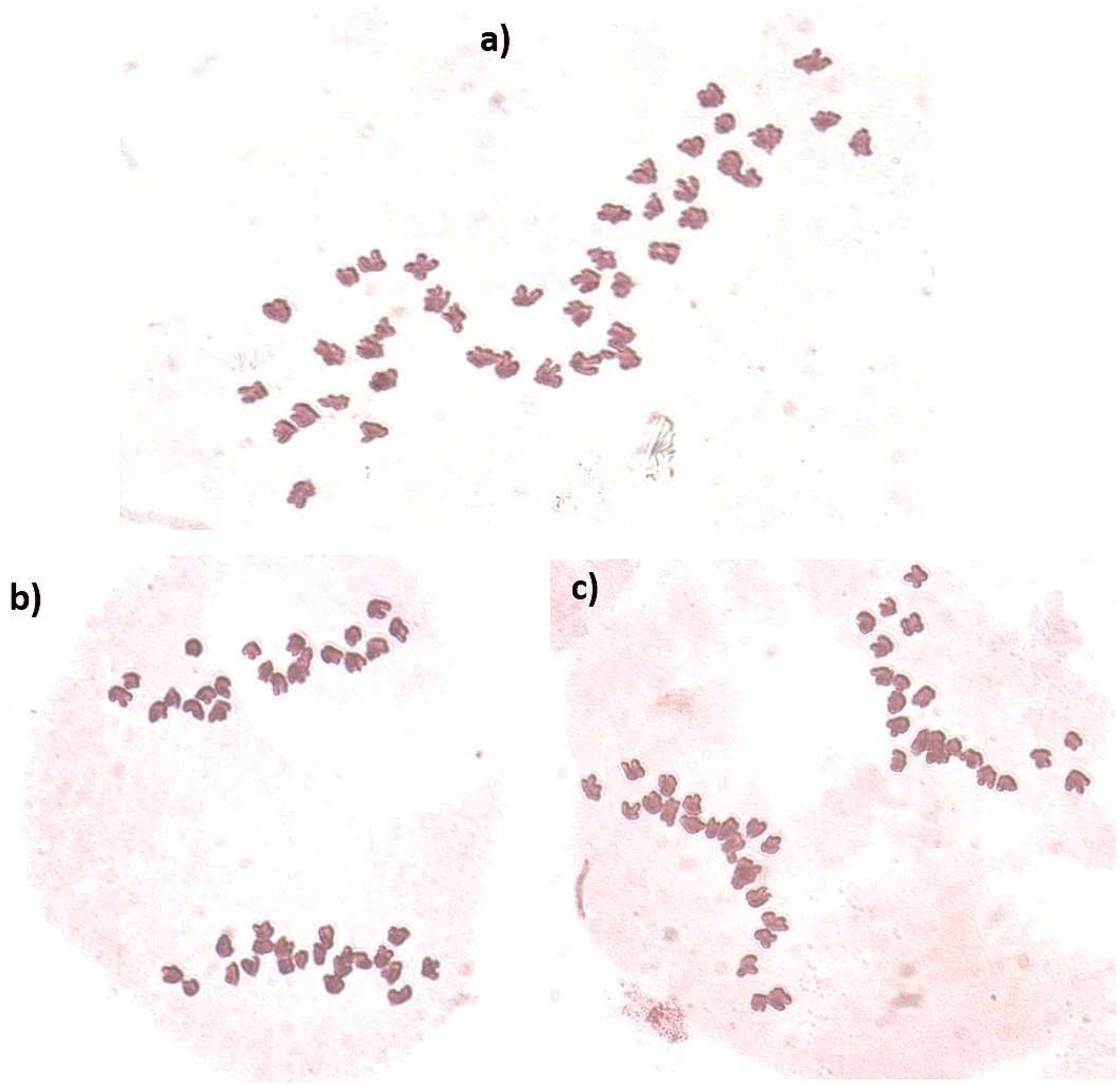
SN= Spikelet no. per spike, FLL= Flag leaf length, TNpM= Tiller number per meter, GFD= Grain filling duration, TGW= Thousand grain weight and YD= Yield per plot



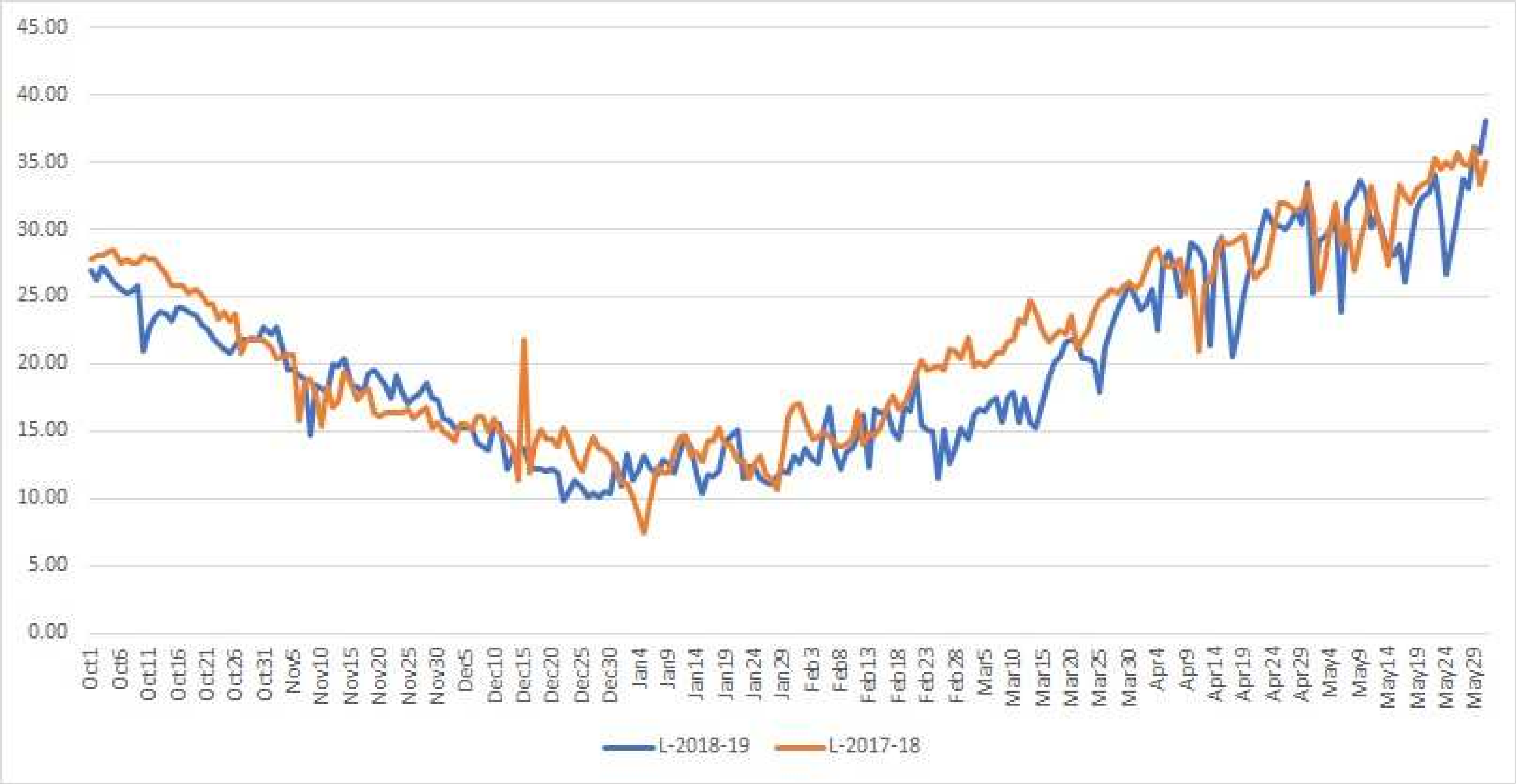
Supplementary Fig. S1. Pollen mother cells at various meiotic stages from the F_1s developed from crosses of *T. durum-Ae. speltoides* backcross introgression lines with hexaploid recurrent parents a) tetraploid donor parent DS-BIL23 b) metaphase with 2n=35 from cross DS-BIL23/BWL3558 showing multivalents from A and B genomes of DS-BILs and hexaploid parent. D genome chromosome are seen as univalent c-d) Late anaphase with unpaired chromosomes lying as laggards on the metaphase plate



Supplementary Fig. S2. Marker assisted selection of BC_1F_1 plants using gel based SSR markers a) *Xgwm565* b) *Xgwm371*



Supplementary Fig. S3. Pollen mother cells at various meiotic stages from the BC_2F_2s developed from crosses of *T. durum-Ae. speltoides* backcross introgression lines with hexaploid recurrent parents a) hexaploid recurrent parent BWL3558b-c) anaphase with chromosomes with n=21



Supplementary Fig. S4. Temperature variation from October to May in the year 2017-18 and 2018-19