# Transfer of quantitative trait loci for heat tolerance from *Triticum durum – Aegilops speltoides* introgression lines to bread wheat (*Triticum aestivum* L.) using marker assisted selection and QTL mapping for disease resistance

**A Thesis**

*Submitted in partial fulfillment of the requirements for the degree of*

**DOCTOR OF PHILOSOPHY**

in

**BIOTECHNOLOGY**

THAPAR INSTITUTE
OF ENGINEERING & TECHNOLOGY
(Deemed to be University)

**Submitted by:**
**Guriqbal Singh Dhillon**
(Regn. No. 901400012)

**Under the guidance of:**
**Dr. Niranjan Das**
Professor
Department of Biotechnology
(Supervisor)

**Under the guidance of:**
**Dr. Parveen Chhuneja**
Senior Molecular Geneticist
SOAB, PAU, Ludhiana
(Co-supervisor, External)

**Department of Biotechnology**
**Thapar Institute of Engineering & Technology**
**Patiala-147004, Punjab, INDIA**
**April 2022**

# DECLARATION

I, **Guriqbal Singh Dhillon**, hereby declare that the work being represented in the thesis entitled **"Transfer of quantitative trait loci for heat tolerance from *Triticum durum – Aegilops speltoides* introgression lines to bread wheat (*Triticum aestivum* L.) using marker assisted selection and QTL mapping for disease resistance"** in the partial fulfilment of requirements for the award of the degree of Doctor of Philosophy (PhD) in Biotechnology at Thapar Institute of Engineering and Technology, Patiala is an authentic record of my own research carried out under the guidance and supervision of **Dr. Niranjan Das**, Professor, Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala and **Dr. Parveen Chhuneja**, Senior Molecular Geneticist cum Director, School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, India. I also declare that this thesis's scientific content has not been the basis for the award of any degree, diploma or any other similar title of any University or Institute within or outside the country. I further attest that this work is original and I am fully responsible for my thesis's content. Any material being obtained from other sources have been duly acknowledged in the thesis.

**Date: 25th August, 2021**
**Place:** Patiala

**Guriqbal Singh Dhillon**
Regd. No.: 901400012
Department of Biotechnology,
TIET, Patiala, India

# CERTIFICATE

This is to certify that the PhD thesis "**Transfer of quantitative trait loci for heat tolerance from *Triticum durum* – *Aegilops speltoides* introgression lines to bread wheat (*Triticum aestivum* L.) using marker assisted selection and QTL mapping for disease resistance**" submitted by Guriqbal Singh Dhillon (Regn. No. 901400012) in the partial fulfilment of requirements for the award of the degree of Doctor of Philosophy (PhD) in Biotechnology at Thapar Institute of Engineering and Technology, Patiala is an authentic record of students' own research carried out under our guidance and supervision. The thesis's scientific content has not been the basis for the award of any degree, diploma or any other similar title of any University or Institute within or outside the country.

23 August 2021

**Dr. Niranjan Das**
Professor
Department of Biotechnology
(Supervisor)

23/8/2021

**Dr. Parveen Chhuneja**
Senior Molecular Geneticist
SOAB, PAU, Ludhiana
(Co-supervisor, External)

# Acknowledgment

It is my pleasure and privilege to acknowledge my supervisors **Dr. Niranjan Das**, Professor, Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala and **Dr. Parveen Chhuneja**, Senior Molecular Geneticist cum Director, School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, for providing me an opportunity to work under their guidance and supervision, assisting with all kinds of support, inspiration, wide counsel, constant encouragement, prudent advice, valuable suggestions, comments, and expertise throughout. I extend my heartful thanks to them. It has been a great privilege to experience sustained enthusiasm and involved interest, constructive criticism, and meticulous supervision from his side throughout my work.

I would like to express my sincere thanks to my Doctoral Committee members, **Dr. M.S. Reddy** (Head & Chairperson, Doctoral Committee of the Department), **Dr. Anil Kumar**, **Dr. B.N. Chudasama** (Ph.D. committee members) for their constant motivation, insightful comments, and ideas. I express my gratitude to **Dr. Rafat Siddique**, Dean (Research and Sponsored Projects), TIET, Patiala, for his encouragement and support during my research work. I would like to extend my sincere thanks to **Dr. Satinder Kaur**, Molecular Geneticist, SOAB, PAU, for her constant guidance and encouragement.

I wish to express my thanks to our respected Director, **Prof. Prakash Gopalan**, TIET, Patiala, for providing infrastructure and help during my research work. My sincere thanks to all the faculty members of Department of Biotechnology, TIET, Patiala, and School of Agricultural Biotechnology, PAU, Ludhiana, for constant motivation, invaluable suggestions, and support during my research work. I would like to acknowledge the Department of Biotechnology, TIET-Patiala, School of Agricultural Biotechnology, PAU-Ludhiana, Indian Institute of Wheat and Barley Research Station, Flowerdale, Shimla, and Kansas State University, USA for providing the infrastructural support, resources, assistance and co-operation.

I would like to acknowledge my colleagues and friends **Rajneesh Kumar, Gurpreet Kaur, Lakhwinder Singh, Bharat Yadav and Vipin Tomar** for their co-operation and help. I pay my sincere thanks to non-teaching staff members at Department of Biotechnology, TIET, Patiala, SOAB, PAU, Ludhiana, and Off-season Research station, PAU, Keylong, Lahaul Spiti, HP. I owe a word of thanks to all the research scholars, and students at Department of Biotechnology, TIET, Patiala, and SOAB, PAU, Ludhiana for constant support, encouragement, and an excellent working atmosphere along with necessary time to rejuvenate.

I feel a deep sense of gratitude for my mother, **Mrs. Jeevan Kaur**, and my fathe,r Late **Inderpal Singh**, for their unconditional love, support, encouragement, sacrifice and words of advice. I would like to mention my sisters, **Mrs. Rupinder Kaur** and **Mrs. Gurjit Kaur** for their love, support and encouragement. And above all, I am grateful to the Almighty God for his divine grace that enabled me to pursue a successful path in life.

Guriqbal Singh Dhillon

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(Note: Published research articles are attached.)

# List of Abbreviations

| **Abbreviations** | **Definitions** |
|---|---|
| % | Percentage |
| °C | Degree Celsius |
| µL | Microlitre |
| µmol | Micro-mole |
| ANOVA | Analysis of variation |
| APR | Adult plant resitance |
| ARF | Auxin response factor |
| ASED | Average standard error of differences |
| ASR | All stage resistance |
| BIL | Backcross introgression line |
| BLUP | Best linear unbiased prediction |
| CT | Canopy temperature |
| CTAB | Cetyl trimethyl ammonium bromide |
| CTD | Canopy temperature depression |
| CV | Coefficient of variation |
| CWI | Cell wall invertase |
| DNA | Deoxy-ribo nucleic acid |
| DP3 | Read depth 3 |
| DS-BIL | *Triticum durum – Aegilops speltoides* backcross introgression lines |
| EDTA | Ethylenediamine tetraacetic acid |
| ETI | Effector triggered immunity |
| EV | Enviornmental variation |
| FAM | Fluorescein amidites |
| FLL | Flag leaf length |
| FRET | fluorescence resonance energy transfer |
| g | Gram |
| GBS | Genotyping by sequencing |
| GFD | grain filling duration |
| GNpS | Grain number per spike |
| GNpS | grain number per spike |
| GS | Genomic selection |
| GST | glutathione S transferase |
| GV | genotypic variation |
| GW | grain weight |
| GWAS | genome-wide association study |
| h | Hour |
| $h^2$ | Heritability broad sense |
| HCl | Hydrochloric acid |
| HEX | Hexachloro-fluorescein |

| **Abbreviations** | **Definitions** |
|---|---|
| HSE | heat stress environment |
| HSP | heat shock protein |
| HT | Heat toleance |
| HTI | Heat tolerance index |
| HTIL | Heat tolerant introgression line |
| IAA | indol-acetic acid |
| K2015, K2016 | Keylong off season |
| KASP | Kompetitive allele specific PCR |
| L2015, L2016 | Ludhiana main season |
| LD | Linkage disequilibrium |
| LOD | logarithm of odds |
| LRR | leucine-rich repeat |
| LRT | likelihood ratio test |
| LSD | Significant Difference |
| MABC | marker assisted backcrossing |
| MACC | marker assisted complex or convergent crossing |
| MAGP | marker assisted gene pyramiding |
| MAP | mitogen activated protein |
| MARS | marker assisted recurrent selection |
| MAS | marker assisted selection |
| mg/L | Milligram per litre |
| min | Minute |
| mL | Millilitre |
| MLM | mixed linear model |
| mM | Milli molar |
| NaCl | Sodium chloride |
| NB | nucleotide-binding |
| NBS | nucleotide-binding site |
| NGS | next generation sequencing |
| NN | nearest neighbours |
| OE | optimum enviornment |
| PAMP | pathogen-associated molecular pattern |
| PCR | polymerase chain reaction |
| PH | plant height |
| pH | Potential of Hydrogen |
| PM | powdery mildew |
| PMC | pollen mother cell |
| PMD | plasma membrane |
| PRR | pattern recognition receptors |
| PTI | PAMP triggered immunity |
| PVE | Phenotypic variation explained |
| QTL | Quantitative trait loci |
| $R^2$ | coefficient of determination |

| **Abbreviations** | **Definitions** |
|---|---|
| RAD | restriction associated DNA |
| RBD | randomized block design |
| RCBD | randomized complete block design |
| RIL | recombinant inbred lines |
| RLK | Receptor like kinases |
| RLP | receptor like proteins |
| ROS | reactive oxygen species |
| ROX | 6-carboxy-X-rhodamine |
| RSTEP | stepwise regression |
| SAR | systematic acquired resistance |
| SCC | SPAD chlorophyll content |
| SCR | spike compaction ratio |
| SIM | simple interval mapping |
| SL | spike length |
| SMA | single marker analysis |
| SN | spiklet number |
| SNP | single nucleotide polymorphisms |
| SSR | simple sequence repeats |
| STS | Sequence-Tagged Sites |
| TE | Tris-EDTA |
| TERF | transcription termination factor |
| TF | transcription factors |
| TGW | Thousand grains weight |
| TKW | Thousand kernel weight |
| TMD | thylakoid membrane damage |
| TN | tiller number |
| TNpM | tiller number per meter |
| TTC | triphenyl tetrazolium chloride |
| UPS | ubiquitin-proteasome system |
| v/v | Volume per volume |
| w/w | Weight/weight |
| YD | yield per plot |
| YR | yellow rust |

# ABSTRACT

Wheat is one of the major food crops providing 21% of calories and proteins to the world population. Wheat yields are being hampered by losses induced by numerous biotic and abiotic stresses. The majority of modern-day spring wheat cultivars developed in India are vulnerable to severe temperature stress, particularly during reproductive phases. The most likely option for mitigating the impacts of global warming on wheat yield is to breed for heat-tolerant genotypes. Using marker assisted selection, seven heat tolerance QTLs were transferred to three distinct hexaploid backgrounds using *Triticum durum – Aegilops speltoides* backcross introgression lines (DS-BILs). A total of 164 $BC_2F_3$ progenies with various QTL combinations were developed, and 40 of these progenies were tested in replicated trials over the course of two years in both normal (OE) and heat stress conditions (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 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 the highest yield of 1.6kg/plot under HSE. The progenies developed during this study can further be used for developing heat-tolerant wheat varieties.

Likewise, biotic stressors produced by diseases such as rusts and powdery mildew have a substantial economic impact on production. Because the causative organisms are always evolving, the search for novel genes/quantitative trait loci (QTLs) for resistance to regulate the impact is never-ending. At both the seedling and adult plant stages, DS-BILs were screened for stripe rust and powdery mildew. At the adult plant stage, DS-BILs demonstrated full to moderate resistance, with variable resistance and susceptibility at the seedling stage. For QTL mapping, 1095 single-nucleotide polymorphisms (SNPs) were identified on 14 chromosomes of *T. durum* utilising genotyping by sequencing. Using a stepwise regression-based likelihood ratio test for additive effect of markers and single-marker analysis, eleven unique QTLs for resistance were identified across six chromosomes (chr1B, chr2A, chr2B, chr3B, chr6B, and chr7B), four QTLs for field mixture of stripe rust pathotypes, two QTLs for stripe rust pathotype 78S84, and five QTLs for field mixture of powdery mildew pathotypes Eleven DS-BILs carrying multiple QTLs have been identified, which will be useful in transferring resistance to susceptible cultivars in order to develop all-stage resistant elite cultivars where QTL for stripe rust resistance *QYrAs.pau-2A.1* (LOD 3.8, PVE 24.51 linked to SNP S2A_16016633) and QTL for powdery mildew resistance *QPmAs.pau-6B* (LOD 3.2, PVE 17.75 linked to SNP S6B_26793381) are major targets of the transfer. Also, putative candidate genes liked to the SNPs of the mapped QTLs were identified which would help in studying the function pathways or modes of action of the associated genes.

# CHAPTER-1 INTRODUCTION

Wheat, rice and maize are major food grains that provide approximately 60% of energy requirements to the world population (Todorovska et al. 2009). Wheat is a winter season crop and is grown in tropical and subtropical India. In 2019-20, wheat production in India was around 103.6 million tonnes with cultivated area of 29.32 million hectares (https://apps.fas.usda.gov/psdonline/circulars/production.pdf). Common bread wheat (*Triticum aestivum* L.) being one of the first crops to be domesticated provides 21% of total calories and 21% protein globally (Braun et al. 2010).

Cereal production must increase by 4.2% per year to keep pace with the increasing population (Gaur et al. 2017). However, over the past 20 years, the average growth in wheat production has been 1% per year, emphasizing a need to increase annual wheat production to feed the growing population. This slow growth in production is further compounded by depleting resources, global warming and the emergence of new pathogenic races. Immediate attention is required to overcome such type of challenges. Wheat production is continuously under threat from several biotic (disease and pest) and abiotic factors which are responsible for 15-20% loss of crop yield, with severe conditions could lead to complete loss of crop yield (Lobell and Gourdji 2012). Among biotic factors cereals rusts (leaf, stripe and stem rust), and powdery mildew are the most devastating, while drought and heat stresses are more prominent among the abiotic stresses.

Global air temperature rises about 0.2°C per decade, which cumulatively will lead to around 1.8–4°C higher average global temperature by 2100 (IPCC 2007). Global warming will be a significant contributory factor to high temperature stresses to both plant and animal life. In crops, high temperature is one of the limiting abiotic factors for yield losses and low grain quality. Therefore, to overcome this limitation and improve production efficiency, more stress-tolerant varieties must be developed (Khush 1999). High temperature during the reproductive stage of wheat is most detrimental to its productivity leading to high losses (Sadat et al. 2013). It has been demonstrated that an increase of 1-2°C in optimum temperature during grain filling results in shorter grain filling periods, thus reducing yield in the cereals (Yang and Zhang 2006). It has been apprehended that India is bound to lose 0.45 tonnes/ha due to a small rise of 0.5°C in average global temperature by the year 2050 (Easterling et al. 2007; Challinor et al. 2016). 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 (Dubey et al.

2020). High temperature also results in a reduction in the number of tillers with promoted shoot elongation in wheat and also reduced green leaf area. According to Lobell et al., (2011), the reproductive cycle is the most sensitive stage in the plant life cycle and stress during flowering can result in loss of entire grain crop. Higher temperature promotes early flowering, grain set and physiological maturity, which are collectively responsible for low yields (Prasad et al. 2008). The higher temperature at anthesis decreases grain number per spike (Yang et al. 2002) and grain size (Viswanathan and Khanna-Chopra 2001). The increase in grain filling rate during high-temperature stress is not adequate to compensate for the decrease in grain filling duration (Acevedo et al. 1990; Pimentel et al. 2015). High temperature during grain filling affects final grain weight by the reduced duration of grain filling due to suppression of photosynthesis and by direct inhibition of starch biosynthesis in endosperm that adversely affects processing quality of wheat making it unsuitable for bread making.

Similarly, the significant fungal foliar diseases, like stripe rust and powdery mildew caused by *Puccinia striiformis* and *Blumeria graminis,* are two significant constraints of sustainable wheat production, negatively affects both yield and quality (Elkot et al. 2015; Bariana et al. 2016; Bansal et al. 2017; Lan et al. 2017). Diseases and pests have attributed to substantial wheat yield losses ranging from 50% to 100% in epidemic conditions (Figueroa et al. 2018). The significant negative impact of long-term use of chemical pesticides on both the environment and human health has already been a significant issue. Thus, a holistic approach of the introduction of disease resistance genes is the best alternative to counter them. Characterisation and deployment of various genes from various sources to confer resistance against these diseases have already been reported by McIntosh et al. (2017).

Improvement of the wheat cultivars only can combat terminal heat stress or post-anthesis heat stress along with diseases that largely limits wheat productivity. Genetic variations in cultivated germplasm of *Triticum aestivum* L. are limited. The high complexity of stress-tolerant trait, the low genetic variance of yield components and the continuous evolution of disease-causing pathogens are significant limitations. Therefore, various progenitor and non-progenitor wild species which are rich sources of various useful traits can be used to transfer traits to the commonly used high-yielding wheat cultivars.

So, there is a need to screen various wild species to study, select and transfer components against both biotic and abiotic stresses to cultivated varieties. *Aegilops* species have been reported and exploited for resistance against both the stresses. The potential of *Aegilops* species to transfer genes for resistance have been reported in various studies (Monneveux et al., 2000; Colmer et al., 2006; Rawat et al., 2008; Pradhan et al., 2012; Liu et al., 2015; Awlachew et al., 2016; Kaur et al., 2018). Kishii (2019) has reported a compilation of various genes identified or transferred from various *Aegilops* species, including *Ae. speltoides*. According to Pradhan et al. (2012), various *Aegilops* species have a high potential for heat tolerance traits. Out of 52 accessions of *Aegilops* belonging to 5 different species, *Aegilops speltoides* showed higher heat tolerance index than others, making it suitable for transfer to known cultivars. Also, *Ae. speltoides* the putative B genome donor harbours various genes that are epistatic to Ph1 locus resulting in homeologous pairing in $F_1$ plants of the crosses of *Ae. speltoides* with wheat (Millet, 2008). However, multivalent formation between homeologous chromosomes leads to chromosome abnormalities making precise transfer difficult.

At Punjab Agricultural University (PAU), Ludhiana, using a heat-tolerant *Ae. speltoides* accession, backcross introgression lines with high thermo-tolerance have been developed in *T. durum* background. These lines have been screened under normal, and heat-stressed environments and various heat tolerance QTLs have been mapped (Awlachew & Chhuneja, 2013; Awlachew et al., 2016). The present study majorly focusses on an attempt to transfer the already mapped heat tolerance QTLs from these lines to the hexaploid wheat background using markers linked to the QTLs. Also identifying QTLs of disease resistance in the source material would help in mobilizing these disease resistance QTLs to hexaploid wheat for future breeding programmes.

# CHAPTER-2 REVIEW OF LITERATURE

## 2.1 Wheat an important food crop

With production of around 704.08 million tonnes (https://apps.fas.usda.gov/psdonline/circulars/production.pdf), and providing 21% calories and 21% protein requirements to the world population, wheat is an essential food crop (Braun et al. 2010). Broadly, 250 perennial and 75 annual species in the grass group (*Poaceae*) of wheat, and its related species constitute this important crop group. Bread wheat, durum wheat, rye, barley and triticale (*Triticosecale*, an artificially synthesized species) are being utilized for staple food, beverages and forage in several ways since the history of mankind (Wang et al. 2010). Modern wheat cultivars include two species: hexaploid bread wheat, *Triticum aestivum* ($2n = 6x = 42$, AABBDD) accounting for 95% of world wheat production, and tetraploid, durum wheat, *Triticum durum* ($2n = 4x = 28$, AABB) accounting for 5% of world wheat production used for macaroni and low-rising bread (Peng et al. 2011). Wild diploid wheat (*T. urartu*, $2n = 2x = 14$, genome AA) hybridized with the B genome ancestor that is the closest relative of goatgrass (*Aegilops speltoides*, $2n = 2x = 14$, genome SS) to produce wild emmer wheat (*T. dicoccoides*). Subconscious selection gradually created cultivated emmer (*T. dicoccum*), that spontaneously hybridized with another goatgrass (*Ae. tauschii*, $2n = 2x = 14$, genome DD) to produce the free-threshing ears of durum wheat (*T. durum*) and bread wheat (*T. aestivum*).

## 2.2 Heat Stress: a challenge in Wheat production

Owing to long-term breeding programs coupled with bottleneck events during wheat domestication genetic diversity of common wheat had been narrowed down, leaving it vulnerable to various abiotic and biotic stresses. Wheat wild relatives constitute a vital source for improving biotic and abiotic stress tolerance, and consequently, productivity under these stress conditions (Hussain and Qamar 2007). The world population is escalating at a rapid rate is expected to increase by 60% by 2050 (Dixon et al. 2009) and being one of the major food crops, demand for wheat is bound to follow. Thus, emphasizing the need for a significant increase in annual wheat production which has been looming around 1.0% from the past decade. This challenge will be further compounded by depleting resources, the shift in the weather pattern and biotic and abiotic stresses. A constant threat of biotic and abiotic stresses does not allow present-day cultivars to realize their full genetic potential. Global climate is changing with a steady rise in average global temperature. Considering the issue of global food security, increasing crop productivity and simultaneously avoiding fluctuations in production due to climatic variations is a big challenge for breeders.

Linear trends in the increase in average temperatures per decade, 0.3°C $T_{max}$ and 0.2°C $T_{min,}$ have been reported by Lobell and Gourdji (2012) resulting in alterations in plant life cycles within and across various plant species (Li et al. 2014). Increasing heat stress has become a challenge for the breeders because of the complex nature of heat tolerance trait, as stated by Cossani and Reynolds (2012). Prolonged heat stress has resulted in low seed set in both heat susceptible and hybrid mid-season rice genotypes, as noticed in China (Wang et al. 2019). There is apprehension that India will also lose 0.45 tonnes/ha due to rise of 0.5°C temperature by 2050 (Easterling et al., 2007). Heats stress has detrimental effects on crop growth and development since the physiological processes such as photosynthesis and respiration are adversely affected. Stress hinders overall reproductive processes and thus causing substantial yield losses (Zinn et al. 2010). According to Joshi et al. (2007), more than 13.5 million ha of wheat growing area in India is under stress becoming a significant concern in cooler wheat-growing regions. Severe damages at grain filling stage by disruption of the function of enzymes associated with the starch breakdown can result in up to 50% loss of yield in Australia if temperature varies even 2°C during the growing season (Asseng et al. 2011). During flowering, the vulnerability is key to unusual ovary development, pollen sterility, disruption in starch biosynthesis during early grain filling period and low biomass production (Farooq et al. 2011). Higher than optimal temperatures along with increased respiration, reduction in photosynthesis and inhibition of starch synthesis in developing kernels, there is a reduction in spike number per plant with reduced kernel per spike and kernel weight and also accelerated senescence (Ayeneh et al. 2002). Early sowing, judicious use of fertilizers with timely irrigation and temperature highly affect yield and thus directly affect wheat production. Plant height, days to heading, days to maturity, spikelets per spike, grains per spike and grain yield are highly reduced when two sowing extremes are compared. Shorter crop duration and grain filling duration, highly influenced by high temperatures, are critical for grain development. Change in ambient temperature leads to perturbations in metabolism, membrane fluidity, protein conformation and assembly of the cytoskeleton, which lead to plant detection of the change in temperature (Ruelland and Zachowski 2010). Acceleration in plant development and an overall reduction in plant size and growth cycle is because of high respiration rates and lower photosynthetic activity. Heat stress during seedling and leaf development causes improper pigmentation and inhibition of chloroplast function in wheat (Dash and Mohanty 2001).

Accelerated growth but reduced phenology results in reduced yields because of less time to capture resources (Zahedi and Jenner 2003). Photosynthesis is the most sensitive process of plants which is inhibited by inhibition of photo-system II activity when exposed to high temperatures (Camejo et al. 2005).

## 2.3 *Aegilops*: a novel source for tolerance

The *Aegilops* genus comprises of 22 species that include diploids, tetraploids and hexaploids (van Slageren 1994). Some *Aegilops* species participated in wheat evolution and played a significant role in wheat domestication thus the genus *Aegilops* represents the largest part of the secondary gene pool of wheat, and several species have been used in crop improvement programs (Kilian et al. 2009). *Aegilops speltoides* Tausch ($2n = 2x = 14$) has the highest genetic affinity to the B and G genomes of wheat. It is native to the eastern Mediterranean and Middle East region and exists as two varieties, *speltoides* and *ligustica*. *Ae. speltoides* is a valuable reservoir for agronomically useful genes, and it is the source for the resistance genes, *Lr28*, *Sr32*, *Lr35/ Sr39*, *Lr36*, *Pm12* and *Gb5* which have been transferred to wheat, *Triticum aestivum* L. (Adonina et al. 2004). Kishii (2019) listed genes identified or transferred from various *Aegilops* species. Till date, *Ae. speltoides* has contributed to various resistance genes including leaf rust resistance genes (*Lr28*, *Lr 35*, *Lr36*, *Lr37*, *Lr47*, *Lr51*, and *Lr66*), stem rust resistance genes (*Sr32*, *Sr39*, and *Sr47*), powdery mildew resistance genes (*Pm1d*, *Pm12*, *Pm32*, and *Pm53*), and green bug resistance gene (*Gb5*). Several wheat–*Aegilops* addition and translocation lines have been developed, and many agronomically useful traits have been incorporated into the wheat genome (Schneider et al. 2010). *Aegilops* species have been considered a genetic resource for increasing the genetic potential of cultivated wheat to withstand biotic and abiotic stresses (Pradhan et al. 2012). As two of the three genomes of bread wheat came from *Aegilops*, it has been an important source for disease- and insect-resistant genes. Wild wheats are also sources for abiotic stress-tolerance genes. Some accessions of *Ae. tauschii, Ae. speltoides,* and *Ae. geniculata* Roth have shown the capability to withstand drought (Baalbaki et al. 2006). A few heat stress-tolerant accessions belonging to *Ae. geniculata, Ae. speltoides, Ae. searsii* Feldman & Kislev ex K. Hammer, and *Ae. longissima* Schweinf. & Muschl. also have been reported (Zaharieva et al. 2001). Pradhan et al. (2012) studied fifty-two accessions of different *Aegilops* species and reported the common adverse effects of high temperature such as decreased chlorophyll, grain number per spike, individual grain weight, and grain yield per plant by 38, 40, 56, and 70%, respectively. Based on grain yield, Ae. *speltoides* Tausch and *Ae. geniculata* Roth were found to be the most heat tolerant; on the contrary, *Ae. longissima* Schweinf. & Muschl. was very susceptible to heat stress. Higher grain number per spike and/or heavier grains were the major indicators in assessing heat tolerance.

## 2.4 Parameters to determine heat tolerance

Heat tolerance is a complex trait controlled by various genes involved in various metabolic pathways. Various heat tolerance indexes and related traits like membrane thermo-stability, canopy temperature depression, TTC cell viability, chlorophyll content, photoperiod, flag leaf stomatal conductance and vernalization etc. can be used for selection under field conditions and to study the effect of post-anthesis heat stress on the wheat crop when early and late sowings are used (Mohammadi et al. 2004).

As compiled by Kumar et al. (2013), the following table shows the traits measuring heat tolerance for wheat:

| Traits | Parameters for study |
|---|---|
| Yield traits | Plot yield |
| | Thousand grains weight (TGW) |
| | Grain filling duration (GFD) |
| | Number of effective tiller per plant |
| Morphological traits | Early ground cover |
| | Stay-green |
| | Epicuticular wax/leaf glaucousness |
| | Leaf rolling |
| | Biomass |
| Physiological traits | Canopy temperature |
| | Photosynthetic rate |
| | Chlorophyll content |
| | Stomata conductance |
| | Stem reserve |
| | Membrane thermostability |

Leaf temperature is depressed below air temperature when water evaporates; therefore, canopy temperature (CT) is an indirect measure of (instantaneous) transpiration at the whole- crop level and of plant water status. Cooler canopy and high stomatal conductance at grain filling period would be assumed as the basic morpho-physiological criteria for higher grain yield under heat-stressed conditions (Munjal and Rana 2003; Gautam et al. 2015; Nautiyal 2017; Kumar et al. 2017b; Sofi et al. 2020). These reports recommended that CT could be used to identify plants with cooler canopies with the aim of yield increasing under non-stressed conditions. CT frequently shows a better association with yield and grain number than it does with total above-ground biomass. CT has shown a clear association with yield in warm environments which shows its association with heat stress tolerance. CT shows a high genetic correlation with yield and high values of the proportion of direct response to selection, indicating that the trait is heritable and therefore amenable to early generation selection. Nowadays, canopy temperature is directly used as a measure of heat tolerance. Lower the canopy temperature higher heat tolerance.

Reduction of triphenyl tetrazolium chloride (TTC) in mitochondria may also be used as an indicator of heat tolerance. Here TTC solution is vacuum-infiltrated into leaf tissues exposed to high temperature. The relative level of TTC reduction quantifies cell viability to formazan, which is detected by spectrophotometer (Yildiz and Terzi 2008). The dehydrogenase systems are responsible for TTC reduction. TTC reduction assay can be used to quantify acquired high-temperature tolerance differences in winter wheat cultivars. Sud and Bhagwat (2010) reported a significant correlation of TTC assay with tiller number, grain yield and plant weight on per meter basis.

The chlorophyll content is one of the significant factors affecting photosynthetic capacity. Reduction or no-change in chlorophyll content of plant under heat stress has been observed in different plant species, and its intensity depends on stress rate and duration (Jagtap et al., 1998). Selection for genotypic differences in photosynthetic capacity may depend on the amount of photosynthetic tissue per leaf area unit. Thus, single structural parameters such as total chlorophyll content per leaf unit area may be a good indicator of the strength of photosynthetic tissue (Fotovat et al. 2007).

Mass screening of wheat genotypes for heat tolerance may also be done for the stay-green character (Reynolds et al. 2001). A visual rating of stay-green is a quick and easy way for the plant breeders to screen on a mass scale (Xu et al. 2000). Kumar et al. (2010) have reported that stay-green or delayed senescence is considered to play a crucial role in grain development in wheat when assimilates are limited, and stay green cultivars are well adapted to heat-stressed conditions. Tewolde et al. (2006) reported that early-heading varieties performed better than later-heading varieties because they (1) produced fewer leaves per tiller and retained more green leaves, (2) had longer grain-filling periods, and (3) completed grain filling earlier in the season when air temperatures were lower.

## 2.5 Molecular basis of heat tolerance

Stimuli related to the abiotic stresses trigger various integrated signalling cascade, especially by receptors embedded in the plasma membrane (Los and Murata 2004). Calcium ions and calcium-dependent protein kinases act as a calcium sensor which switches mitogen-activated protein (MAP) kinases which in turn activate transcription factors (TF) and subsequently heat shock proteins (HSP) (Sangwan et al. 2002). A novel class of proteins called heat shock proteins activates and express during high temperature to compensate for the structural damages of cellular proteins which are essential for survival in stressed conditions. This is a very well-studied molecular response under heat stress (Rampino et al. 2009). Various molecular chaperones save cellular proteins from heat-induced aggregation and facilitate protein folding during recovery (Wang et al. 2004). During stress, stress-responsive genes are regulated by numerous transcription factors via binding to the *cis*-acting elements, usually in the promoter of a gene (Mizoi et al. 2013). When exposed to high-temperature normal protein synthesis is reduced, but heat shock proteins are produced in various tissues of tolerant lines, especially in developing parts like grain. HSP 100 (Sumesh et al. 2008), HSP 90 (Kotak et al. 2007), HSP 70 (Gupta et al. 2010a, b), HSP 60 (Al-Whaibi 2011) and HSP 18 (Sharma-Natu et al. 2010) have been observed in tolerant lines at elevated temperatures.

At the genomic level, response to the stress conditions is demonstrated by a set of candidate genes known as stress-responsive genes (or Transcription factors). Under stress conditions, TFs operate as molecular controls to regulate gene expression (Lata and Prasad 2011). Jia et al. (2013) identified 1,489 TFs (grouped into 56 families) in *Ae. tauschii*, of which the Myeloblastosis -related TFs which are involved in stress-tolerance (Garg et al. 2012) and in activating the expression of stress-tolerance genes, constituted a significant proportion. In another study, a TF, namely TaASR1 (abscisic acid-, stress-, and ripening-induced) derived from wheat was used to introduce drought stress tolerance in transgenic tobacco (Hu et al. 2013). The role of TFs as molecular controls under environmental stresses in various model crops, based on conserved gene orthology has also been demonstrated (Jia et al. 2013).

## 2.6 Heat tolerance as a quantitative trait

Heat tolerance is a quantitative trait and many QTLs (quantitative trait loci) linked to heat tolerance have been identified in wheat. A QTL linked to grain filling duration was reported on the short arms of chromosomes 1B and 5A by Yang et al. (2002b). Butler (2002) detected QTL for heat tolerance under hot and dry conditions on chromosomes 2B and 5B in a spring wheat population. Several QTLs for heat susceptibility indexes of various morphological and yield traits were found on chromosome 1A, 1B, 2A, 2B, 3B, 5A and 6D under short-term reproductive stage heat stress (Mason et al. 2011). QTL for thousand grain weight, grain fill duration and canopy temperature depression have been mapped on chromosome 2B, 7B and 7D, respectively by Paliwal et al. (2012). In addition, QTL with significant effects on grain yield, grain weight, grain filling, stay-green and senescence-associated traits were also reported on 2A, 3A, 4A, 6A, 6B and 7A under post-anthesis high-temperature stress in wheat (Vijayalakshmi et al. 2010). (Talukder et al. 2014) identified five QTLs regions significantly associated with response to heat stress. They identified associations for plasma membrane damage (PMD) on chromosomes 7A, 2B and 1D, SPAD chlorophyll content (SCC) on 6A, 7A, 1B and 1D and thylakoid membrane damage (TMD) on 6A, 7A and 1D. By mining de novo transcriptome data generated from heat shock (HS) treated wheat, Singh et al. 2019 discovered 38 new heat-responsive transcription factor genes from wheat cv. HD2985. A putative transcript (TaHD97) of 1.1 kbas was amplified and cloned from wheat cv. HD2985 using digital gene expression (DGE). The amino acid sequence revealed the existence of a heat stress transcription factor (HSF) DNA binding domain. Under heat stress, there was a difference in TaHD97 expression between HD2985 (thermotolerant) and HD2329 (thermosensitive).

### 2.7 Marker Assisted Selection (MAS): an approach for precise transfer of genetic loci

Marker Assisted Selection (MAS) refers to a procedure that integrates genetic information obtained from DNA markers to accelerate the detection and selection of desired genotypes in a traditional breeding program. In most of the breeding methods such as bulk and single seed descent, where screening for target loci is performed at $F_5$ or $F_6$ generations when most of the loci are homozygous. However, in MAS genotypic evaluation is performed at the early growth phase of plants, aiding in detection and subsequent selection of only those plants that carry the desired target loci and can fix specific alleles in their homozygous state as early as the $F_2$ generation. Key components that are required for an efficient MAS system include (1) suitable genetic markers and their characterization, (2) high-density molecular maps, (3) established marker-trait associations for the traits of interest, (4) high-throughput genotyping systems, and (5) functional data analysis and delivery systems.

The number of generations of MAS required depends upon the number of target loci involved, the number of linked markers used for detection, the association of trait and the marker and status of marker alleles. Breeding for quantitative traits like yield and abiotic stress tolerance is difficult because it is a complex trait involving the interaction of a large number of genetic loci each having small phenotypic effects and variable expression over time and space due to environmental conditions. Due to exponential increase in population size with an increase in the number of target QTLs, the involvement of a large number of genetic loci which may or may not be linked and limitation of resources and facilities, it is almost impossible to select for a single genotype incorporating all the target genetic loci. Therefore, repeated field tests are required to characterize the effects of the QTLs accurately and to evaluate the stability across environments. Different forms of MAS include: marker assisted backcrossing (MABC), marker assisted gene pyramiding (MAGP), marker assisted recurrent selection (MARS), marker assisted complex or convergent crossing (MACC) and genomic selection (GS) or genome wide selection (GWS).

Knapp (1998) estimated the efficiency of MAS as a strategy for increasing the probability of selecting superior genotypes. He reported cost efficiency of MAS relative to phenotypic selection ranging from 1.0 to 16.7. Therefore, a breeder using phenotypic selection must test 1.0 to 16.7 times more progeny than a breeder using MAS to be assured of selecting one or more superior genotypes. MAS has been implemented to introgress QTLs for resistance to biotic and abiotic stresses in cereals as well as other crop plants. In a study conducted by Watson (2008) MAS strategy was used for successful introgression of four QTLs for quality traits like PPO activity and Xanthophyll content from Australian wheat into Chinese wheat. In a study done by Lecomte et al. (2004) as many as five QTLs were used in the improvement of fruit quality traits in tomato via marker-assisted introgression. Sadat et al. (2013) used MAS strategy for screening 25 bread wheat genotypes tolerant to heat stress. Gautam et al. (2020) reported using MAS for pyramiding up to 12 genes/QTLs for various rust resistance genes against leaf rust, stripe rust and stem rust of wheat along with genes/QTLs for grain quality traits like grain weight, grain protein content, and high molecular weight glutenin subunit genes.

## 2.8 Genotyping by sequencing (GBS)

To perform various genotypic studies based on single nucleotide polymorphisms (SNP), a reduced representation genome sequencing technique called Genotyping by Sequencing (GBS) was developed by Elshire et al. (2011). This reduces the effective cost per marker per genotype by coupling restriction enzymes digestion with NGS sequencing methods. It has become a flexible and rapid option with simultaneous marker discovery and genotyping. With appropriate restriction enzymes and DNA coupled with DNA-barcoded adapters, it has a high capacity of multiplexing in a highly cost-effective manner without limiting the number of tags per genotype. The first basis of GBS were reported by Baird et al. (2008) through restriction associated DNA (RAD) tagging with GBS overcoming the limitations of RAD.

Since restriction enzymes have conserved sites across the species barrier, GBS can be efficiently used for different uncharacterized and new species or germplasms for de novo SNP discovery (Poland and Rife 2012). The basic flow of GBS includes using restrictions enzymes for genome complexity reduction, ligation of barcoded adapters to enzyme-cut sites, multiplexing, NGS sequencing and SNP discovery by bioinformatic pipelines. Fu et al. (2014) stated that the SNP discovery of complex plant genomes could be done using GBS without any prior knowledge about the genome. The critical component of this system includes rapid low costs, reduction in sample handling eased by few PCR and purification steps, no size fractionation, no reference sequence limits, efficient barcoding system easing multiplexing and scale-up with simultaneous detection of SNPs and genotypes (Davey et al. 2011). Thus, it becomes a powerful tool for utilizing high-density genotypic data for genetic linkage analysis, genetic map construction and mapping, molecular marker discovery, genome-wide association study (GWAS) and genomic selection.

Initially, GBS was demonstrated with maize (IBM), and barley (Oregon Wolfe Barley) recombinant inbred populations developed using single restriction enzyme (ApeK1) and two adaptors (barcoded and common adaptor) (Elshire et al. 2011). However, it can be multiplexed for 48, 96 or 384 samples by ligation of barcoded adaptors further reducing its cost. Further extension of the technique to two restriction enzymes with methylation sensitivity with rare cutter and frequent cutter enzymes by Poland et al. (2012) helps in generation of uniform libraries consisting of a forward (barcoded) adaptor and a reverse (Y) adaptor on alternate ends of each fragment (to avoid amplification of more common fragments). This is highly effective and efficient for large and complex genomes like wheat.

Library complexity, multiplexing level, and the output of the NGS platform used to decide the sequencing coverage per sample and thus decide the amount of missing data (Andolfatto et al. 2011). The missing data can be handled by either imputations or by increasing the sequencing depth, i.e. by reduction of the number of samples in the multiplex or multiple sequencing runs of the library (Poland and Rife 2012). However, these increases the total costs multiple times which can be worth when the genotypes have been extensively phenotyped and may serve as a community resource. Since the inception of the GBS technique (within ten years), a large number of reports across various species have been documented utilizing the critical aspects of time and cost-effectiveness. Hence various SNP calling methods like TASSEL GBSv2 pipeline in TASSEL (Glaubitz et al. 2014) and imputation methods like LD-kNNi in LinkImpute (Money et al. 2015) have been designed to overcome missing data limitation.

## 2.9 QTL Mapping for disease resistance

In wheat, disease resistance has been characterised in two categories based on their action across the lifecycle of the plant (Chen 2005). Firstly, the all-stage resistance (ASR) caused by seedling stage resistance genes, which start acting from the seedling stage and remain active throughout the plant life. These genes are mostly race-specific. Secondly, the adult plant resistance (APR) caused by genes that are primarily responsible for moderate to complete resistance at the adult plant stage only. APRs may confer hypersensitive reaction or provide more durable resistance to plants by providing slow rusting type resistance (Venkata et al. 2008; Niks et al. 2015). This type of durable resistance is crucial as it does not exert evolutionary stress on the causal organism by retarding the development of disease progression. Although APR is a durable form of resistance, only a small number of resistance genes have been known to confer this particular type of resistance (Kankwatsa et al. 2017).

Efficient strategies for selection can be improved using molecular marker technology. The detection of sequence variation between various genotypes closely linked to a trait of interest pinpoints that presence or absence of the variant could predict the trait. These variations may occur within genes or between genes of traits of interest and molecular marker. The power of detection is directly proportional to the linkage, i.e. the closeness of the genetic linkage between the sequence variant and the target locus. These variations are commonly called polymorphism of markers. Quantitative trait loci (QTLs) are the genomic regions which are linked to traits controlled by several genes, and thus these traits are perceived as a quantitative trait (Nishant et al. 2016).

QTL mapping is the identification of markers linked to the gene(s) or QTLs underlying the trait screened, which assists efficient improvement of specific target traits (Tanksley 1993). Linked markers can be successfully used to enhance the genetic advance through marker assisted selection. Identification and utilization of various significant traits are now possible with the availability of high-density markers and powerful statistical analysis tools. Establishing linkage or association between the phenotype and genotype are prime objectives of QTL analysis. The requirements for QTL mapping are mapping population (structured or unstructured for genetic mapping or GWAS, respectively, precise phenotyping for the trait, genotyping of the mapping populations with polymorphic markers, construction of genetic maps (for genetic mapping) and QTL mapping using both genotypic and phenotypic data (Tanksley 1993; Semagn et al. 2010; Chamarthi et al. 2011).

Single-marker analysis, simple interval mapping and composite interval mapping are the three widely-used methods for detecting QTLs (Tanksley 1993; Liu 1998). Single-marker analysis is the detection of QTLs associated with single markers. This includes statistical methods like t-tests, analysis of variance (ANOVA) and linear regression for the analysis. Among these, linear regression is the most commonly used one because in this the coefficient of determination ($R^2$) from the marker and explains the phenotypic variation arising from the QTL linked to the marker. Whereas, simple interval mapping (SIM) instead of analyzing single markers utilizes linkage maps taking up one marker interval at a time and analyses intervals between adjacent pairs of linked markers along chromosomes simultaneously. SIM has become the standard method for mapping QTL as use of linked markers for analysis compensates for recombination between the markers and the QTL and has been put into practice in several freely distributed software packages. Once the candidate gene or the markers associated with the trait of interest has been identified, the next step is their utilization in the breeding programme.

# CHAPTER-3 ORIGIN OF PROBLEM

Global warming and changing climatic conditions have resulted in higher optimum temperatures and shorter winter duration, affecting primarily the Rabi crops like wheat. Due to global warming and the rapidly growing population, there is a need to increase crop production at a much higher rate. Development and selection of crop varieties are aimed at improving yields under existing climatic conditions. With changing climate physiological, morphological and molecular traits unique for heat tolerance are required (Semenov 2009). Conventional breeding is not sufficient in selecting genotypes for high yield under heat stress conditions. Adaptability to heat stress can be caused by escape, avoidance and tolerance mechanisms which are contributed by various physiological mechanisms like higher photosynthetic rate, stay-green, canopy temperature depression, leaf chlorophyll, leaf conductance, spike number etc. Terminal heat stress is a significant drawback which adversely affects photosynthesis during grain filling (Feng et al. 2014).

Most of the traits related to yield and heat tolerance are controlled by several genes, each with minor individual effects but the significant effect when acting together. In wheat, QTL analyses are partly hindered by large genome size and partly due to repetitive DNA sequences. Natural genetic variation for heat stress can be used by direct selection under heat stress during a reproductive stage or through QTL mapping, and subsequent marker assisted selection, which is a more reliable method for transfer. To address the problem of heat stress various wild species of wheat were screened for heat tolerance at Punjab Agricultural University (PAU), Ludhiana and *Aegilops speltoides* accession pau3809 was found to be most heat tolerant and selected for transferring heat tolerance to the cultivated wheat varieties. This diploid species was crossed with a tetraploid wheat cultivar PDW274 and backcross introgression lines were developed. $BC_2F_8$ introgression library was evaluated for introgression level, heat tolerance traits and its association with yield components. Various QTLs belonging to traits such as chlorophyll content, canopy temperature, TTC cell viability and stay-green character were mapped. These were present in *T. durum* (tetraploid) background, which is not suitable for bread making and accounts only for 5% of total wheat cultivated in India. So, there was a need to transfer these QTLs to the hexaploidy wheat background the elite cultivars.

In this study, we proposed the transfer of the aforesaid QTLs of *Aegilops speltoides* origin to common bread wheat through marker assisted selection and then validation of the transfer by evaluating the heat tolerance traits. This study is novel since there are no similar reports available in the literature of transfer of heat tolerance QTLs from *Aegilops speltoides* background. Canopy temperature is an easily measured manifestation of crop metabolic and physiological response to the environment. It can be used to distinguish between stress tolerant wheat and intolerant wheat. Plant leaf temperature is affected by radiational, conventional and transpirational processes. Leaf temperatures are depressed below air temperature when water evaporates from its surface, thus giving a measure of plant response to its environment. Canopy temperature is lower in tolerant lines (Reynolds et al. 2001). The chlorophyll content is one of the major factors affecting photosynthetic capacity. The higher chlorophyll content is a desirable trait as it indicates a low degree of photoinhibition of photosynthesis by a reduction in chlorophyll content at high temperature or heat stress (Talebi 2011). A visual rating of stay-green character is a quick and easy way for screening and selection on a mass scale (Xu et al. 2000). Stay green with lower canopy temperatures could be used as effective selection criteria for heat stress tolerance. Stay-green or delayed senescence play a crucial role in grain development as it elongates the duration of grain filling even at higher temperatures. However, since heat tolerance is a complex trait governed by a large number of genes and QTLs for various traits it can only be estimated from the 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 the huge complexity of the bread wheat genome, it is difficult to dedicate a single trait as a representation of tolerance to heat stress (Deb and Khaleque 2009). However, tiller number, TGW and yield are the best estimates for heat stress tolerance.

Despite of introduction of QTLs for heat tolerance, disease resistance is a key trait without which the introgression lines could not actualize their yield potential. With the changing environment and introduction of new pathogenic races the major disease resistance genes become ineffective within short duration of time. Thus, there is a need to introduce new genes/QTLs for disease resistance from various sources and pyramiding of various genes/QTLs help to achieve effective combinations for longer duration of time.

The present study was proposed with four primary objectives, which are

i) Transfer of QTLs for heat tolerance from *Triticum durum – Aegilops speltoides* introgression lines to bread wheat (*Triticum aestivum* L.) background

ii) Marker assisted selection (MAS) in $BC_1F_1$, $BC_2F_1$ and $BC_2F_2$ progenies

iii) Evaluation of $BC_2F_3$ introgression lines for heat tolerance and its components

iv) Mapping QTLs for stripe rust and powdery mildew disease resistance in *Triticum durum – Aegilops speltoides* introgression lines

# CHAPTER-4 MATERIALS AND METHODS

The present study aimed at transfer of mapped heat tolerance QTLs (HT QTLs) in *T. durum* - *Ae. speltoides* backcross introgression lines (DS-BILs) to hexaploid wheat background, which were followed up by marker assisted selection (MAS) using the linked markers. The effect of these HT QTLs was validated in the developed hexaploid introgression lines for yield related traits under stress. Alongside transfer and validation of heat tolerance QTLs, the DS-BILs were also screened for disease resistance against two major wheat diseases, stripe rust and powdery mildew, and QTL/genes were mapped using SNPs developed through genotyping by sequencing.

## 4.1 Transfer of QTLs for heat tolerance from *Triticum durum* – *Aegilops speltoides* introgression lines to bread wheat (*Triticum aestivum* L.) background

From a set of 360 $BC_2F_8$ introgression lines of *Triticum durum* - *Aegilops speltoides*, six lines (namely DS-BIL23, DS-BIL25, DS-BIL31, DS-BIL37, DS-BIL44, and DS-BIL628) showing introgression of various heat tolerance QTLs mapped were selected for the present study. The development of these DS-BILs has been detailed in Awlachew et al. (2016). The DS-BILs were used as donors for transferring HT QTLs to stripe rust resistant versions of three important widely used cultivars of hexaploid wheat, namely BWL3558 (PBW550+*Yr5*), BWL4444 (HD2967+*Yr10*), and BWL5185 (PBW621+*Yr10*+*Yr15*). 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), $BC_2F_{3:5}$ heat tolerant introgression lines (HTILs) were developed in the background of hexaploid wheat following markers linked to respective HT QTLs using marker assisted selections at $BC_1F_1$, $BC_2F_1$, and $BC_2F_2$ generations. The crossing strategy and summary of the development and selection of the plant genetic material is given in Figure 4.1.1 and 4.1.2, respectively.

Figure 4.1.1: Crossing strategy of wheat. a) immature spike of DS-BIL, b-d) Preparation of spike for emasculation, e) Emasculation of a spike, f) Emasculated spike and g) Pollinated plants of DS-BILs.

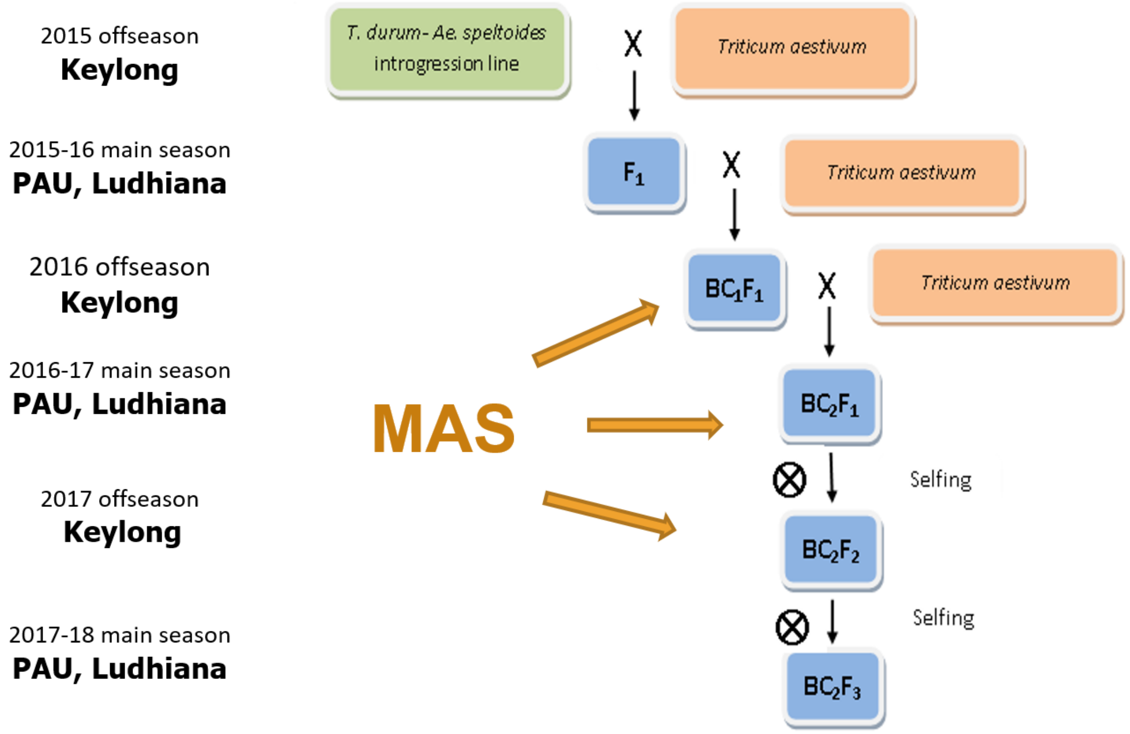

Figure 4.1.2: Development and selection strategy for the development of *T. durum-Ae. speltoides* X hexaploid wheat derived heat tolerant introgression lines across various seasons

## 4.2 Marker assisted selection (MAS) in $BC_1F_1$, $BC_2F_1$ and $BC_2F_2$ progenies

### 4.2.1 DNA Isolation

Genomic DNA isolation of $BC_1F_1$, $BC_2F_1$, and $BC_2F_2$ progenies along with donor and recurrent parents was done using a modified CTAB method. Using CTAB (Cetyl trimethyl ammonium bromide) method of Saghai-Maroof et al. (1984) with some modifications. Young leaves were collected from each of $BC_1F_1$, $BC_2F_1$ and $BC_2F_2$ progenies/plants. The leaf samples were grounded by QIAGEN Tissue Lyser II (Germany) with the help of liquid nitrogen in 2ml eppendorf tubes and then 800µl 2XCTAB extraction buffer was added to the powdered samples. Then tubes were incubated at 65ºC for 45 minutes in the water bath. After incubation, chloroform: isoamyl alcohol (24:1) was added to each sample for direct solvent extraction by mixing on a rotary shaker for 30 minutes. The tubes were then centrifuged at 10,000 rpm for 15 minutes at room temperature in a fixed angle rotor centrifuge. After centrifugation, the supernatant (aqueous phase) was transferred into 1.5ml micro-centrifuge eppendorf tube. 5µl of DNase free RNase (10mg/ml) was added and incubated at 37ºC in a water bath for half an hour. DNA was precipitated with the help of chilled isopropanol. DNA was pelleted after centrifuging at 10,000 rpm for 15 minutes. After discarding the supernatant, one washing with 70% ethanol was given, and DNA was air-dried for about one hour. Finally, depending on the quantity of DNA precipitate, 50-100µl of 1X TE (Tris EDTA pH 8.0) buffer was added and kept in a refrigerator for further work.

**Table 4.2.1: Composition of 2X CTAB Extraction Buffer**

| Component | Final concentration |
|---|---|
| CTAB | 2.0% |
| Tris HCl (pH 8.0) | 100mM |
| NaCl | 1.4M |
| EDTA | 20mM |
| β -Mercaptoethanol | 0.2% |

### 4.2.2 Assessing the quality and quantity of DNA

Quality of DNA was checked on 0.8% agarose gel by agarose gel electrophoresis where quality was judged by whether the DNA band is a single high molecular weight band (good quality) or smear (bad quality) with the help of gel documentation system by comparing λ DNA loaded alongside DNA samples. The genomic DNA was quantified using Thermo Scientific Nanodrop$^{TM}$8000 spectrophotometer by loading 1µl of DNA sample at the top of fibre optic pedestals. The source fibre optic cables are then brought into contact with the receiving fibres with the sample in between the two fibre optic cables. A pulsed Xenon flash lamp provided the light source and spectrophotometer utilizing linear charged couple device to analyse the light after passing through the sample. The readings of samples quantity were recorded. The quantified DNA was normalized to the concentration of 500ηg/µl by adding the appropriate amount of 1X TE buffer. The stock DNA was further diluted to a concentration of 20ηg/µl with sterile ddH2O for further use.

### 4.2.3 Marker Assisted Selection (MAS)

DNA molecular markers associated with QTLs mapped in DS-BILs were used as linked markers (Awlachew et al. 2016; Awlachew and Chhuneja 2013) to track various QTLs in $BC_1F_1$, $BC_2F_1$ and $BC_2F_2$ progenies. Along with these QTLs, stripe rust resistance genes *Yr5, Yr10* and *Yr15* from recurrent parents were also tracked using MAS in $BC_2F_1$, and $BC_2F_2$. The list of QTLs and genes followed up during the present study are detailed in Table 4.2.2. $BC_2F_5$ progenies were subjected to haplotype/ introgression profiling using the markers linked to the HT QTLs.

### 4.2.4 Analyses through PCR

SSR markers selected as linked markers were amplified on various DNA samples of $BC_1F_1$, $BC_2F_1$ and $BC_2F_2$ progenies for selection through polymerase chain reaction (PCR) in 384 well microtiter plate in an Applied Biosciences 384 thermal cyclers. PCR reaction was carried out in 10µl reaction volume. The components of PCR are given in Table 4.2.3 and amplification profile in Table 4.2.4. PCR products were resolved using 6% non-denaturing polyacrylamide gel by polyacrylamide gel electrophoresis and visualized and photographed using a gel documentation system. EmeraldAmp® GT PCR Master Mix (Cat # RR310A)was obtained from Takara Bio Inc, Japan and primers were synthesised from Integrated DNA Technologies Inc. (IDT), USA.

**Table 4.2.2: 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 | Marker ID | Trait associated | Donor line harbouring QTLs/ genes | QTL donor genome | 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 & Chhuneja, 2013 |
| *QTtc.pau-1B* | 2.70 | 14.20 | *Xwmc269* | TTC% | DS-BIL25, 31, 37, 44 | *Ae. speltoides* | Awlachew & Chhuneja, 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 | | |
| *Yr10* | | | *Xps3000* | Stripe rust resistance | BWL4444, BWL5185 | | |
| *Yr15* | | | *Xgwm498* | Stripe rust resistance | BWL5185 | | |

# 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

**Table 4.2.3: Composition of the PCR reaction mixture for SSR markers**

| PCR components | Concentration | Quantity |
|---|---|---|
| PCR Master Mix | 2X | 5.00 µl |
| Forward Primer | 5 µM | 0.75 µl |
| Reverse Primer | 5 µM | 0.75 µl |
| Template DNA | 20 ηg/µl | 2.50 µl |
| Molecular Grade distilled water | 4.6 µl | 1.00 µl |
| **Total** | | 10.00 µl |

**Table 4.2.4: Amplification profile for PCR reaction of SSR markers**

| Activity | Temperature (${}^{0}$C) | Duration (min.) | Cycles |
|---|---|---|---|
| Initial denaturation | 94 | 5 | 1 |
| Denaturation | 94 | 1 | 35 |
| Annealing | 51-61 | 1 | |
| Extension | 72 | 1.5 | |
| Final extension | 72 | 7 | 1 |

The KASP genotyping assay included primer mix, KASP master mix and sample DNA. The primer mix included three oligos, i.e. two allele-specific oligos and one common primer. The 2X KASP master mix carried FAM and HEX specific FRET cassette, Taq polymerase and an optimised buffer. The sample DNA was loaded first of all in 384 well microtitre plate. A cocktail for each primer was prepared to carry the 2X KASP master mix and primer mix. The cocktail was dispensed into each well already loaded with sample DNA. The constituent reagent volumes used in the PCR reaction is shown in Table 4.2.5. The scoring and genotyping of samples was done in a high throughput TECAN infinite F200 PRO plate reader. The 384 well plate was placed inside the plate reader where the fluorescent dyes specific filters measured the amount of fluorescence in each well. Kluster Caller software was used to view the FAM and HEX data in an x-y plot. The homozygous allele's signals were plotted near the x and y-axis for FAM and HEX dyes, respectively whereas heterozygotes (FAM/HEX) were plotted in between the two axis. ROX dye was used for normalization.

**Table 4.2.5: Cocktail used for KASP PCR analysis**

| Constituent | Volume (1X) (µl) |
|---|---|
| DNA | 2.000 µl |
| Primer mix | 0.056 µl |
| KASP mix | 1.944 µl |
| Total | 4 µl |

**Table 4.2.6: Amplification profile for PCR reaction of KASP markers**

| Activity | Temperature ($^0$C) | Duration | Cycles |
|---|---|---|---|
| Initial denaturation | 94 | 15 mins | 1 |
| Denaturation | 94 | 20 sec | 10 |
| Annealing | 65 (-ΔT 0.6$^0$C per cycle) | 60 sec | |
| Denaturation | 94 | 20 sec | 30 |
| Annealing | 55 | 60 sec | |

### 4.2.5 Meiotic studies

The F$_1$ plants were sampled for selection of true F$_1$ plants, by identifying pentaploids, for further backcrossing. The ploidy difference of tetraploid donor DS-BILs and hexaploid recurrent parent leads to the development of pentaploids F$_1$s. Inflorescence were collected from

$F_1$ plants from the field, during early to mid-booting stages. The inflorescences were fixed in Carnoy's fixative containing 60:30:10 ethanol–chloroform–acetic acid in jam jar bottles covered with aluminium foil and kept in the dark for 12-18hrs for fixation. After fixation, the Carnoy's fixative was drained, and 70% ethanol was added. These were stored at 4$^{0}$C till further use. The anthers from the spike were separated to prepare slides by squash method extruding pollen mother cells (PMCs). The PMCs were observed under a compound microscope after staining with 2% acetocarmine staining solution in order to count chromosome number.

## 4.3 Evaluation of $BC_2F_3$ introgression lines for heat tolerance and its components

### 4.3.1 Trial design and traits studied

The heat tolerant introgression lines developed (through MAS) during this study were evaluated during $BC_2F_3$ and $BC_2F_5$ generations in field trials during 2017-19 seasons for various agro-morphological traits. During the evaluation of $BC_2F_3$ HTILs in season 2017-18, the experiment was divided into two trials. The first trial contained 40 HTILs having sufficient seed to be planted as a replicated trial across two different sowing regimes. The second trial contained remaining 124 HTILs with less seed was planted as augmented trial. The replicated trial of 40 HTILs was planted with three recurrent parents and seven checks in 3m X 4rows plots in a randomized complete block design (RCBD) with two replications each at two different sowing dates with a gap of 30 days. The first set was sown during mid-November as normal sowing or optimum environment (OE) and the second set during mid-December as late sowing or heat stress environment (HSE) to impart terminal heat stress to the second set. The remaining 124 HTILs of the augmented trial were sown during mid-November as normal sowing or optimum environment (OE) with no replicates.

The replicated trial from $BC_2F_3$ HTILs was advanced to generate $BC_2F_5$ HTILs using the off-season nursery in 2018 summer season. The 40 $BC_2F_5$ HTILs were again sown with three recurrent parents and seven checks in 3m X 4rows plots in a randomized complete block design (RCBD) with two replications each at two different sowing dates with a gap of 30 days. The first set was sown during mid-November as normal sowing or optimum environment (OE) and the second set during mid-December as late sowing or heat stress environment (HSE) to impart terminal heat stress to the second set. The various agro-morphological traits studied during evaluation are:

1. Plant height (PH): Height of the plant from the ground to primary tiller (without awns) measured in cm of five randomly selected plants per genotype
2. Spike length (SL): Length of the spike measured in cm (without awns) of five randomly selected plants per genotype
3. Spikelet number per spike (SN): Number of spikelets per spike of the five spikes
4. Flag leaf length (FLL): Length of the flag leaf of primary tiller from peduncle to tip of leaf measured in cm of five randomly selected plants per genotype
5. Canopy temperature (CT): Canopy temperature of plants using handheld infra-red thermometer during grain filling duration, measured in degree Celsius randomly from five different positions in a plot
6. Grain filling duration (GFD): Duration of days from flowering to morphological maturity of plants
7. Grain number per spike (GNpS): Number of grains per spike counted for five spikes of each genotype after harvesting
8. Thousand grain weight (TGW): Weight of thousand grains measured in grams after harvesting
9. Yield /yield per plot (YD): Weight of total grains measured in kgs after harvesting

### 4.3.2 Statistical Analysis and Correlation

For the trials in replicated design across $BC_2F_3$ and $BC_2F_5$ generations of HTILs the analysis of variation (ANOVA), variability analysis and calculation of adjusted means (BLUPs) was done using META-R version 6.0 (Alvarado et al. 2016). The adjusted means of replicates in the trial were obtained by fitting mixed linear models (MLM) using the equation

$$Y_{ik} = \mu + R_i + G_k + \epsilon_{ik}$$

where $Y_{ik}$ is the trait of interest, $\mu$ is the mean effect, $R_i$ is the effect of the *i*th replicate, $G_k$ is the effect of the *k*th genotype, $\epsilon_{ik}$ is the error associated with the *i*th replication and the *k*th genotype which is assumed to be normally and independently distributed, with mean zero and homoscedastic variance $\sigma^2$. The genotypes were selected as random effects and replicates as fixed effects to calculate adjusted means across the replicates of different environments and generations. The broad sense heritability was calculated using the formula

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2 / n\, reps}$$

where $\sigma_g^2$ is genotypic variance and $\sigma_g^2$ is error variance and $n\,reps$ is the number of replications. The broad-sense heritability estimated the quality of a breeding program for the traits and the environments. The LSD with type I error, α = 0.05 of the level of significance, was calculated using formula

$$LSD = t_{(1-0.05,dferror)} \times ASED$$

where *t* is the cumulative student's t-test distribution, *dferror* is the degrees of freedom for the variance of error, and ASED is the average standard error of the differences between pairs of means. And the coefficient of variation is calculated using the formula

$$CV = 100 \times \frac{ASED}{grand\ mean}$$

The correlations were calculated as simple pairwise Pearson's correlations among traits. The correlations were calculated among all traits evaluated during the experiments but were studied in pairs against TGW and YD, as they are highly affected due to heat stress (Acevedo et al. 1990; Awlachew et al. 2016; Dias and Lidon 2009; Farooq et al. 2011; Flohr et al. 2017; Iqbal et al. 2017; Kumar et al. 2017). Thus, correlation of the traits provides an indirect effect on the genotypes due to heat stress. Comparisons were made between genotypes to the respective recurrent parent separately for both the environments.

For descriptive analysis package *summarytools* v0.9.4 was used. Analysis of variation, variability analysis and calculation of adjusted values of the augmented design was done using package *augmented.RCBD* v0.1.1. The trait values of $BC_2F_5$ progenies across the environments were used to perform multivariate analysis to identify the traits contributing to yield using FactoMineR v2.4 (Lê, Josse & Husson, 2008) and visualization was done via biplot using factoextra v1.0.7 (Kassambara & Mundt, 2020). The selected traits were used to model the contribution of the traits towards yield of $BC_2F_5$ HTILs under heat stress by structural equation modelling (SEM) in the package lavaan v 0.6-7 (Rosseel, 2012) and visualized using package semPlot v1.1.2 (Epskamp et al., 2019).

### 4.3.3 Heat tolerance index

Heat tolerance index was calculated for SN, FLL, TNpM, GNpS, GFD, TGW and YD. The heat tolerance index was calculated as the percentage of one minus change in trait values across the two environments to trait values in OE which is as under

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

where $HTI$ is heat tolerance index for a trait, $Trait\ OE$ is the trait value in the optimum environment, and $Trait\ HSE$ is the trait value in the heat susceptible environment.

### 4.3.4 Validation of effect of QTLs in HTILs

The presence or absence of different HT QTLs in the HTILs was correlated with the trait values of $BC_2F_5$ HTILs for validating the effect of these QTLs in the genotypes generated in the study in the hexaploid background of wheat. Trait data of SS, FLL, TNpM, GFD, TGW and YD under both OE and HSE was used to study the effect of the presence of HT QTLs in the respective environments. Effect of QTLs for CT and TTC was 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 on different traits. 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

$$eta^2 = (H - k + 1)/(n - k)$$

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).

## 4.4 Mapping QTLs for stripe rust and powdery mildew disease resistance in *Triticum durum – Aegilops speltoides* introgression lines

### 4.4.1 Plant material and disease screening

A set of 89 $BC_2F_{10}$ DS-BILs along with parental genotype PDW274 and susceptible check WL711 were used for the study. WL711 is a hexaploid wheat cultivar used as the susceptible check for both the diseases, stripe rust (YR), and powdery mildew (PM). For screening 10 seeds of each genotype were planted in bread boxes with each bread box containing one row of susceptible check WL711. Three different sets of plant material were sown for screening against three different inoculums. The bread boxes were then kept in two different temperature and moisture-controlled glasshouses. These glasshouses have been specifically maintained for conditions suitable for the development of respective diseases. The germinated seedlings (around 7th day) bearing the first leaves were inoculated with respective disease spores. For inoculation and screening against YR, two sets were inoculated, one with *Puccinia striiformis* (*Pst*) pathotype 78S84 and other with a mixture of pathotypes collected from the open field. For screening against PM, a mixture of *Blumeria graminis* (*Bg*) pathotypes collected from the open field was used for inoculation. The inoculated bread boxes for each inoculum were placed in respective glasshouses in water-filled trays covered with a black sheet for incubation of 24 hours to maintain 100% relative humidity for initiation of infection. After the incubation was complete, the bread boxes were maintained in the respective glasshouses till the development of the disease. The scoring for diseases was done after 14 days or after the susceptible check showed complete susceptibility. For scoring disease score against YR, Stakamans' scale was used (Visioni et al. 2018). Similarly, for scoring disease score against PM, a linear scale of 0-9 was used (Yang et al. 2017).

### 4.4.2 Molecular analysis

Using the CTAB method, the DNA extraction of 89 DS-BILs and parental genotypes PDW274 and *Ae. speltoides* acc. pau3809 was done. The protocol of DNA extraction and quantification is given in section 4.2.1 and 4.2.2, respectively. Genotyping by sequencing (GBS) using two enzymes *PstI* and *MspI* was performed by courtesy of Jesse Poland, Director, Wheat Genetics Resource Centre, Kansas State University, USA using the protocol of Poland et al. (2012). The raw reads generated by Illumina sequencing of multiplexed GBS protocol were used to call SNPs using the TASSEL GBSv2 pipeline in TASSELv5.2 (Glaubitz et al. 2014). Since the DS-BILs were in the tetraploid background, only A and B genomes of wheat reference genome refseqV1.0 was used for raw sequence TAG mapping and calling physically mapped SNPs. Brief description of the protocol is given in Figure 4.4.1. The results from the pipeline were saved in vcf file (variant call format) which was filtered at read depth 3 (DP3) and was further converted to HapMap format. The genotypic file was then filtered for homozygous SNPs for the parental lines, and further only the polymorphic SNPs between the two parental lines were selected. Finally, the SNPs were filtered for missing data, keeping only SNP markers with a maximum of 20% data missing which were then used for mapping.

### 4.4.3 Introgression profiling and QTL mapping

The physical location of the SNPs was used to check the coverage of SNP markers across the tetraploid genome of the DS-BILs and introgression profiling of *Ae. speltoides* fragments in *T. durum* background of 89 DS-BILs were done using GGT2 (van Berloo 2008). The QTL mapping for identifying loci imparting disease resistance in the DS-BILs was conducted in QTL IciMapping V4.1. (Meng et al. 2015). Using the conversion method by Yang et al. (2017), the disease reaction scores were converted from Stakamans' scale into the linear scale for YR to use for mapping, while the scores for PM were already in linear scale. A total of 1095 filtered SNP markers were used for mapping. Single marker analysis and likelihood ratio test based on stepwise regression (RSTEP-LRT) for additive effect (Wang et al. 2016) were employed as two basic functionalities for detecting disease resistance linked markers.

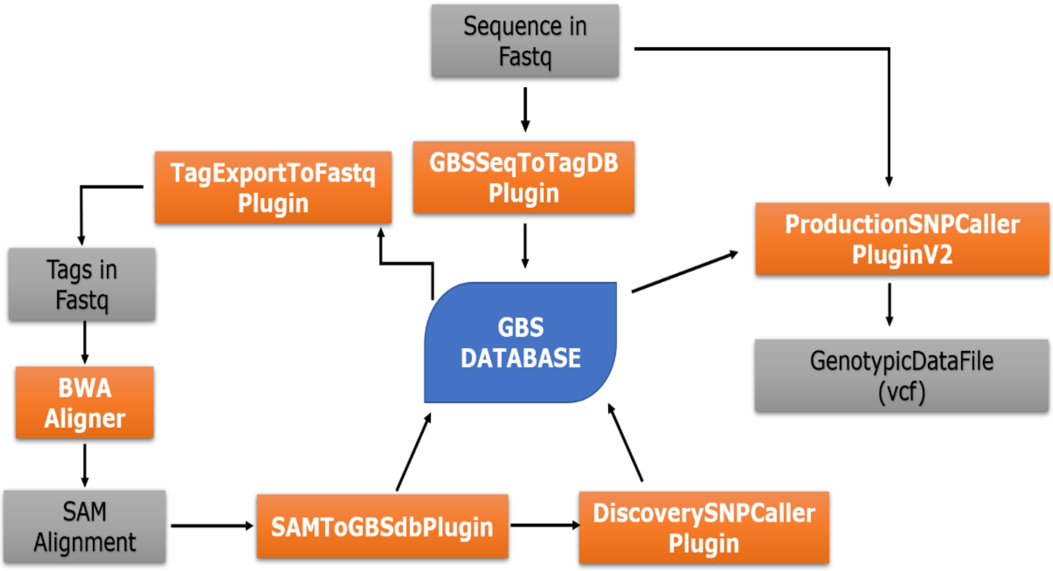

Figure 4.4.1: Protocol for SNP calling using the TASSEL GBSv2 pipeline

### 4.4.4 Postulation of Candidate Genes

The physical positions of the mapped SNPs in the introgressed segments were used to identify the candidate genes conferring resistance to diseases in the annotated wheat genome present at https://wheat-urgi.versailles.inra.fr/Seq-Repository/Annotations. Jbrowse functionality was used to manually hunt for the candidate genes in the designated regions of the annotated wheat genome. Gene names and functions were identified from https://web.persephonesoft.com/?data=genomes.

# CHAPTER-5 RESULTS AND DISCUSSION

The overall study of utilizing the *T. durum - Ae. speltoides* backcross introgression lines (DS-BILs) for the development of heat tolerant advanced breeding lines of hexaploid wheat has been the main object of the study. The transfer and marker assisted selection of the heat tolerance traits to the elite hexaploid background is presented into four sections. The first section (5.1) covers the breeding program of the selected DS-BILs aimed at transferring previously reported HT QTLs. The second section (5.2) details using marker assisted selection (MAS) during the backcrossing generations to help in selection of progenies with HT QTLs. The third section (5.3) aimed at evaluation of the hexaploid heat tolerant introgression lines (HTILs), generated in section 5.1 and 5.2, to study the effect of terminal heat stress. The third section (5.3) also includes validation of the effect of the HT QTLs to the yield related components the HTILs. Finally, the fourth section (5.4) covers the mapping of two economically significant fungal foliar diseases of wheat, to study the importance of the DS-BILs for developing the disease resistance elite cultivars.

## 5.1 Transfer of QTLs for heat tolerance from *Triticum durum – Aegilops speltoides* introgression lines to bread wheat (*Triticum aestivum* L.) background

For the generation of the $F_1$s, the six DS-BILs were crossed as the female parent with three recurrent parents during 2015 in the off-season nursery at Keylong, Lahaul Spiti, Himachal Pradesh. Ten to fifteen spikes of each DS-BILs was emasculated for pollination with each of the recurrent parents and resulting $F_1$ seed set varied in different cross combinations with the lowest seed set of 28 for DS-BIL44 X BWL5185 to highest seed set of 190 for DS-BIL25 X BWL3558 (Table 5.1.1). The resultant 1445 seeds of $F_1$s were planted in the main-season 2015-16, and the $F_1$s were screened visually aided by meiotic chromosome counting for selection of true $F_1$s (pentaploid) (Figure 5.1.1). The durum type plants were roughed out, and the remaining true pentaploid plants were backcrossed to their respective hexaploid parents to generate $BC_1F_1$ seeds. The $BC_1F_1$ seeds were sown as single plant progenies of $BC_1F_{1s}$ in off-season nursery during 2016 at Keylong. Using markers linked to the HT QTLs single plants were selected with multiple HT QTL introgressions. The selected plants were back-crossed and cross seed was harvested generating $BC_2F_1$ seeds. At $BC_2F_1$, the single plants were selected, using MAS, for selfing to generate seeds $BC_2F_2$ which were further evaluated and selfed to generated $BC_2F_3$ seeds.

Heat stress is a significant constraint in a winter crop like wheat, especially when temperature rises during the reproductive stage, affecting the pollen viability, fertilization capacity, grain filling processes and finally the total yield of the crop (Akter and Rafiqul 2017; Cramer 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 the hexaploid background as there is a 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). The 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 a good amount of seed set was observed in the $F_1$ and back cross generations but germination and further establishment of the plants were significantly less (~5% to 15%), which was expected in crosses between tetraploid and hexaploid parental lines. Also, donor DS-BILs carried alien introgressions other than targeted ones, which might have affected plant survival (Rezaei et al. 2010; Arabbeigi et al. 2010). Crossing between tetraploid donor genotypes and hexaploid recipient parents resulted in pentaploid $F_1$s 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 (Millet 2007; Colas et al. 2008) which can also result in a large number of multivalent formations, which can cause poor germination and plant establishment.

## 5.2 Marker assisted selection (MAS) in $BC_1F_1$, $BC_2F_1$ and $BC_2F_2$ progenies

Using markers linked to HT QTLs single plant selections were done, and a total of 192 $BC_1F_1$ plants (Table 5.1.1) with multiple HT QTL introgressions were selected (Fig. 5.2.1 and 5.2.2). The selected progenies were then backcrossed with their respective hexaploid recurrent parents to generate $BC_2F_1$ seed. A total of 2322 $BC_2F_1$ single seeds were sown in the main-season 2016-17, and single plants were screened for desired introgressions through MAS. The $BC_2F_1$ plants with introgressions were selected for one or more HT QTL introgressions and were further visually selected for plant vigour to aid selection leading to 122 single plant selections. The 2235 $BC_2F_2$ seeds from 122 plants were planted in offseason 2017, and 164 plants positive for target QTLs were selected. $BC_2F_3$ progenies/HTILs were again sown in main wheat season 2016-2017, and five plants of each progeny were analyzed for the confirmation of the presence of HT QTLs.

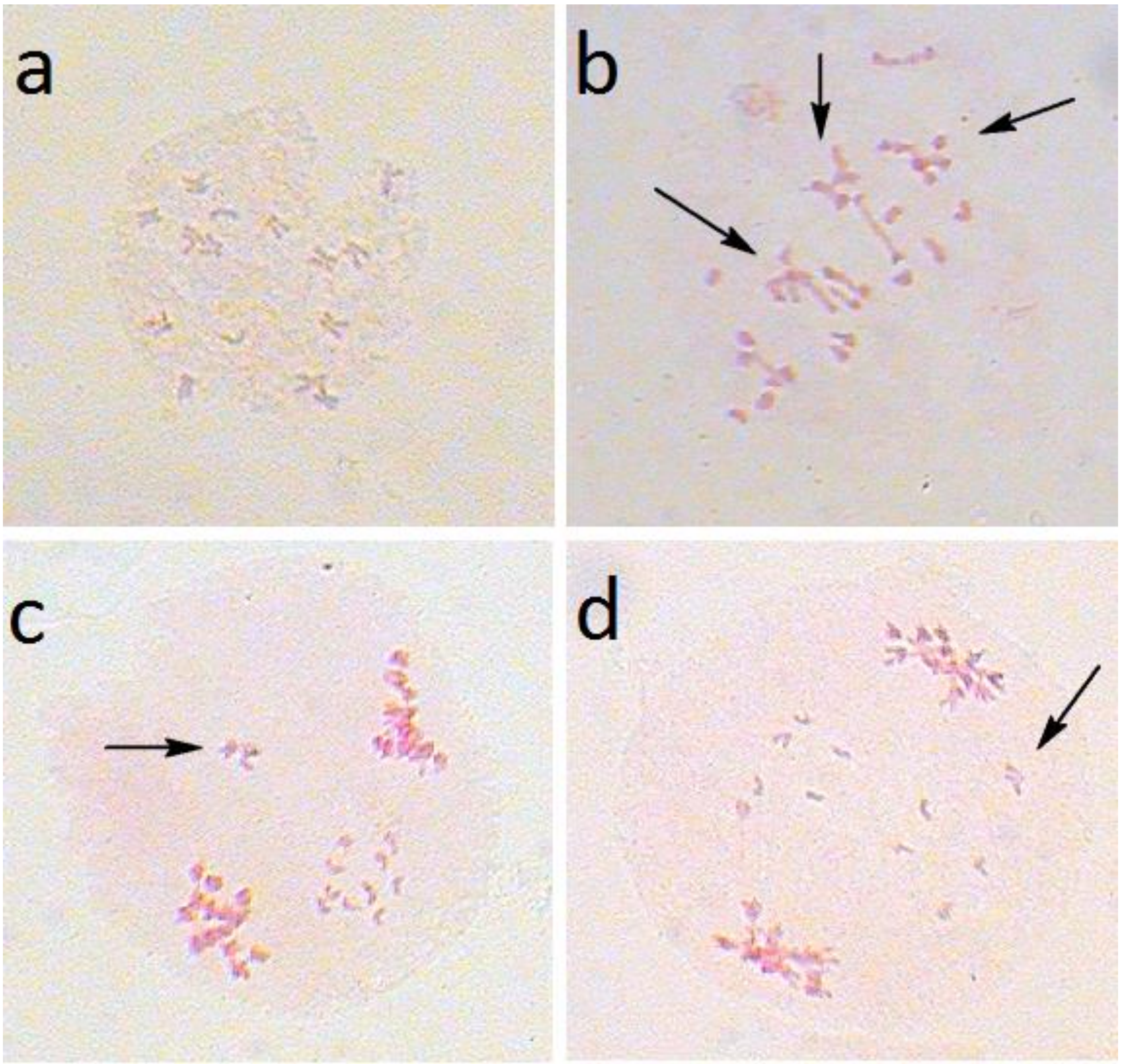

**Figure 5.1.1:** Pollen mother cells at various meiotic stages from the $F_1$s developed from crosses of *T. durum-Ae. speltoides* backcross introgression lines with hexaploid recurrent parents a) tetraploid donor parent DS-BIL23 b) metaphase with 2n=35 from cross DS-BIL23/BWL3558 showing multivalents 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

**Table 5.1.1: Summary of Marker Assisted Selection conducted for introgression of heat tolerance QTLs from *T. durum* - *Ae. speltoides* introgression lines to cultivated wheat**

| Recurrent parent | Donor DS-BIL ID | K2015# | L2015-16 | | K2016 | | L2016-17 |
|---|---|---|---|---|---|---|---|
| | | $F_1$ | $F_1$ | $BC_1F_1$ | $BC_1F_1$ | $BC_2F_1$ | $BC_2F_2$ |
| | | 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 |
| Total | | 1445 | 192 | 2322 | 122 | 2235 | 164 |

#K represents Offseason nursery at Keylong, Himachal Pardesh, India and L represents main campus, Ludhiana, India

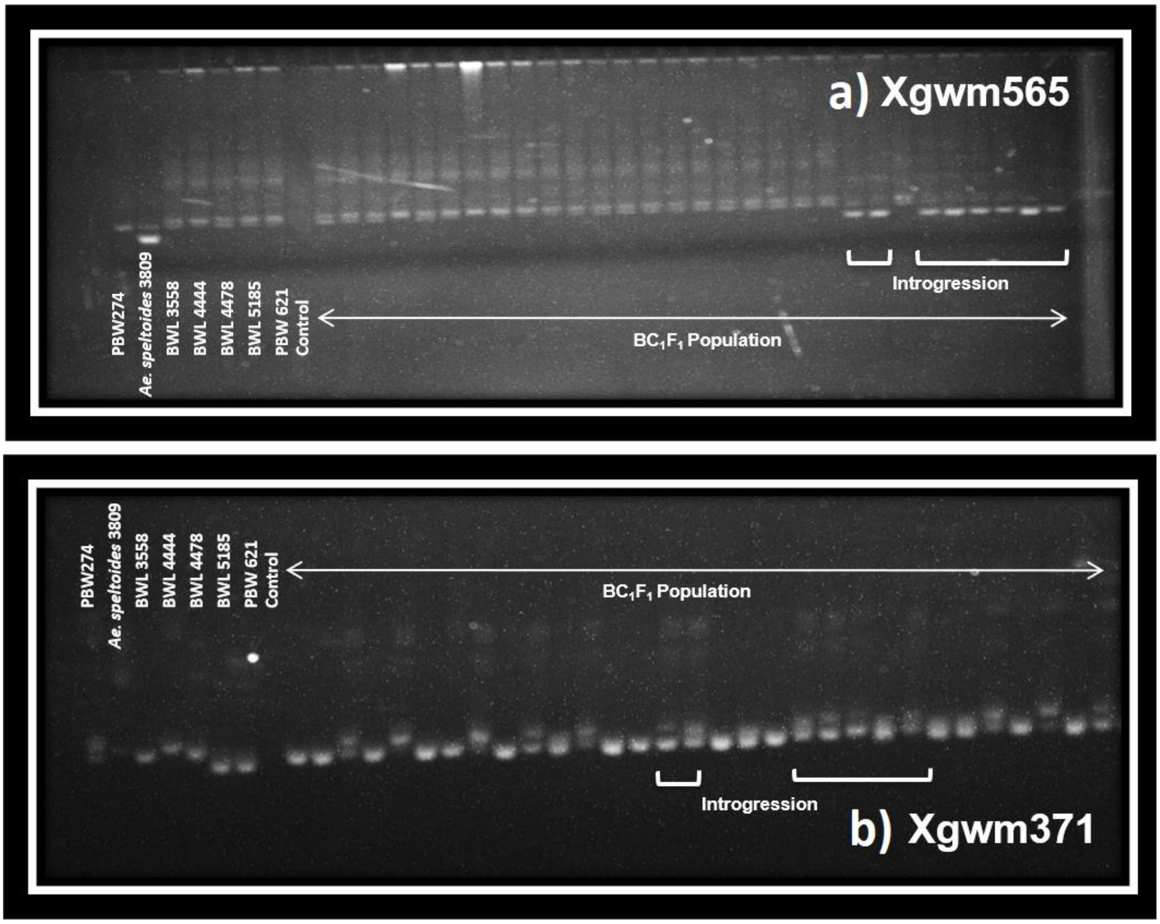

Figure 5.2.1: Marker assisted selection of $BC_1F_1$ plants using gel based SSR markers a) *Xgwm565* b) *Xgwm371*

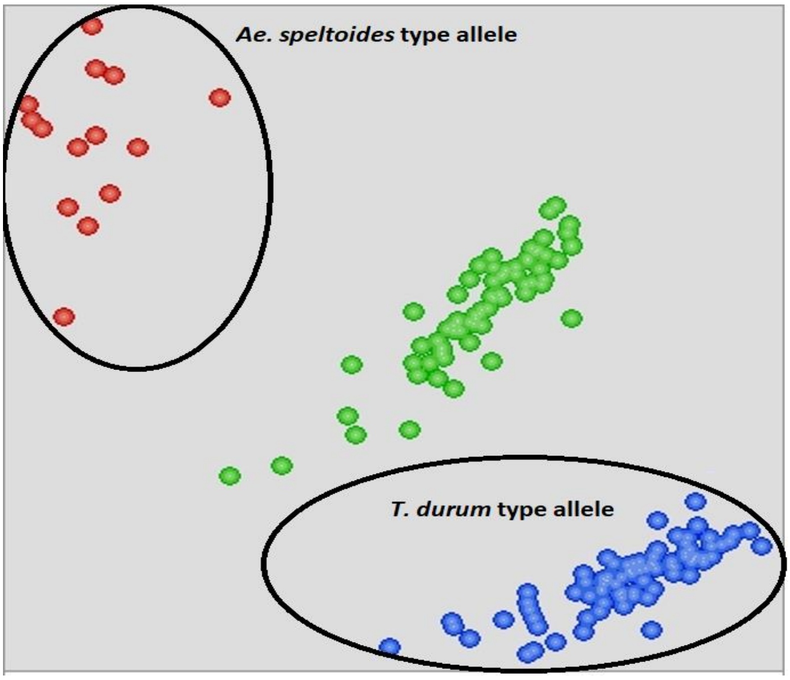

Figure 5.2.2: Marker assisted selection of $BC_1F_1$ plants using KASP based SNP marker *KASP_HT1*

In pre-breeding programmes, marker-assisted selection (MAS) is often utilised. Many studies using MAS to achieve leaf rust resistance (Cox et al. 1995; Nocente et al. 2007; Singh et al. 2010), powdery mildew resistance (Ma et al. 2018; Zhao et al. 2020), and fusarium head blight resistance (Shen and Ohm 2006; Gururani et al. 2012; Buerstmayr et al. 2020) have reported. MAS provides a number of advantages over phenotypic selection, including more flexibility, which allows breeders to deal with smaller populations, allowing them to make better use of field trial capacity.

To summarise, molecular markers should not be considered an exclusive selection technique, but rather as a supplement to phenotypic selection (Lammerts van Bueren et al. 2010; van Bueren et al. 2018). Gene specific markers derived from functional gene sequences are suitable markers for marker-assisted selection (MAS) in breeding because they reliably distinguish alleles at a single locus. Because functional markers are diagnostic of the target trait allele, they appear to have an advantage over random DNA markers (Shen and Ohm 2006). Polygenes influence wheat thousand grain weight (TGW), which is a key component of grain production.

Zhang et al. (2014) found that by combining the TaGS-D1 and TaCwi-A1 marker detections, the association between genotypes and phenotypes became more significant, and that the combination of these two STS markers increased the capacity of marker-assisted breeding to boost TGW. TKW is linked to the genes Sucrose synthase 2 (Sus2), grain width 2 (GW2), and cell-wall invertase (CWI) in rice (Huang et al. 2006; Song et al. 2007; Wang et al. 2011; Jin et al. 2020). In silico cloning techniques were utilised to clone these genes in common wheat, and functional markers were created and used to evaluate Chinese wheat cultivars (Su et al. 2011; Jiang et al. 2011; Ma et al. 2012).

In the present study, marker assisted selection aided to conduct marker assisted transfer of HT QTLs from the DS-BILS to hexaoplid lines. Marker assisted selection was used in the backcross generations to select for desirable plants carrying QTL for our traits of interest. Phenotypic selections were also conducted based on leaf color, leaf type, plant height, plant type, spike type, spike color, and awns along with MAS which aided in selecting for recurrent type plant phenotype. All the introgressed QTL were present in homozygous form in the selected $BC_2F_5$ HTILs. 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.

### 5.3 Evaluation of $BC_2F_3$ introgression lines for heat tolerance and its components

The overall summary of the marker assisted breeding program and evaluation of the HTILs across $BC_2F_3$ (Trial 1 in RBD design and Trial 2 in Augmented design), and $BC_2F_5$ ( RBD trial design) is summarized in Figure 5.3.1. The 164 plants in $BC_2F_3$ were divided into two trials, i.e. the trial 1 in RBD design containing 40 HTILs, 3 recurrent parents, and 7 check genotypes and trial 2 in Augmented design containing the remaining 120 HTILs with 3 recurrent parents repeated in each block. The trial 1 was advanced to $BC_2F_5$ and was planted in RBD design containing 40 HTILs, 3 recurrent parents, and 7 check genotypes.

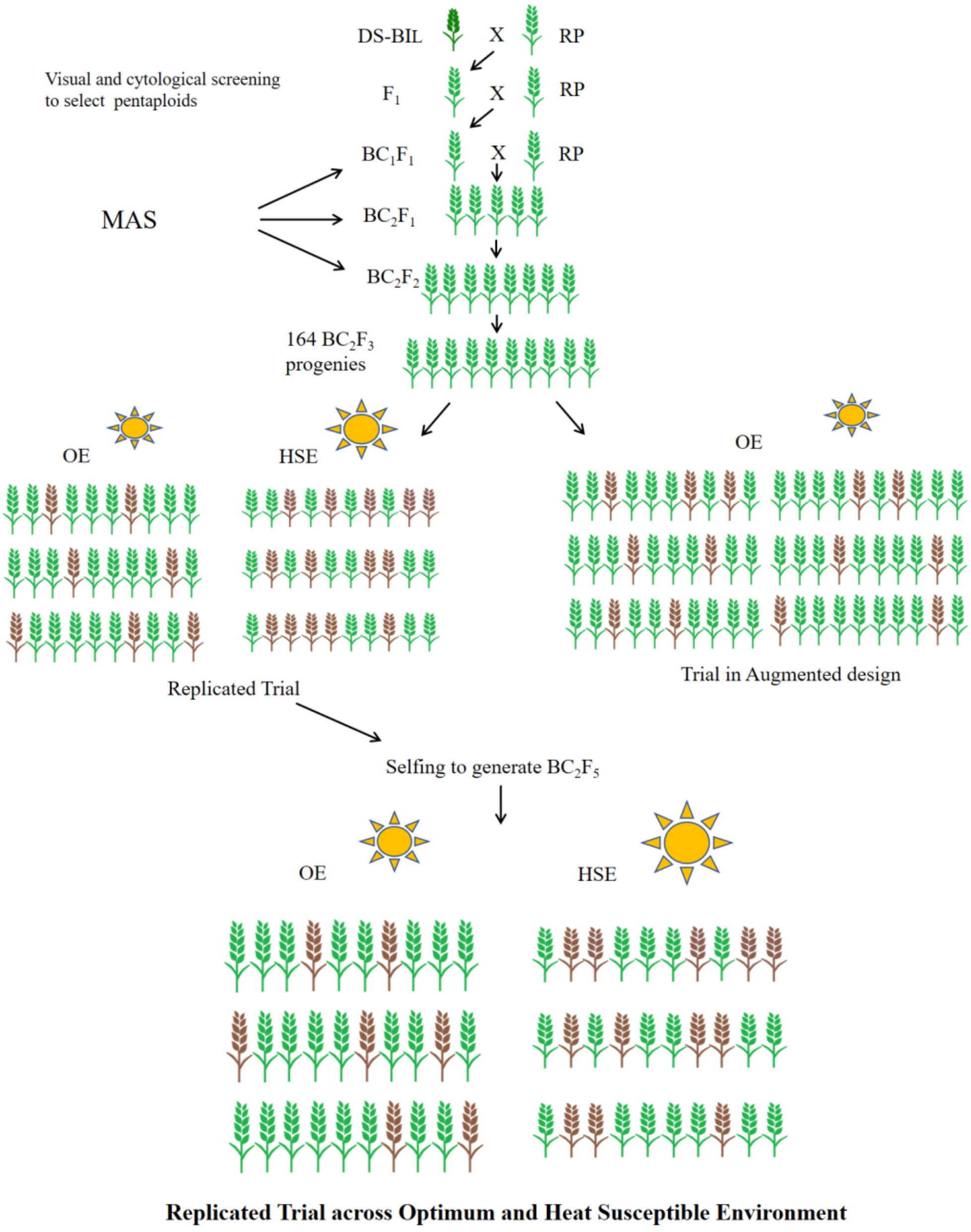

Figure 5.3.1: Development and screening strategy for *T. durum-Ae. speltoides* X hexaploid wheat derived $BC_2F_{3-5}$ HTILs

### 5.3.1 Phenotypic evaluation of $BC_2F_3$ HTILs (Trial 1- RBD design) during season 2017-18

During season 2017-18 evaluation of the 40 $BC_2F_3$ HTILs, a significant increase in TGW (~10%), TNpM (>20%), and YD (>20%) was observed in both OE and HSE than their respective recurrent parents. The variation range of different traits is shown along with $BC_2F_5$ trial in Figure 5.3.2 and Table 5.3.1. Overall large range of variation was observed for most of the traits including 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 the environments.

Significant decrease in trait values was observed in the heat stressed environment. Across the environments, among all the traits, SN (17.65-23.21 in OE and 18.70-22.28 in HSE) was least affected by the heat stress whereas the highest reduction of ~50% was observed in FLL. Overall plant growth was highly hindered in plants grown in HSE due to a sudden rise in temperature during the first week of April, 2018 causing forced maturation of plants in both the environments especially HSE (including HTILs, recurrent parental lines and checks) (Figure 5.3.3). This could be easily observed by high reductions of GFD in the range of 25-40 percent across the environments.

Overall reduced PH, TNpM, and FLL with a large number of plants showing retarded growth and a complete change of leaf architecture. High broad-sense heritability ($h^2>0.70$) was observed in both the environments for SN, TNpM, GNpS, GFD, TGW, and YD showing fitness of replications in the trials. However, coefficient of variation was the highest for YD (CV = 14.65) in HSE followed by FLL (CV=09.64) showing that the tested HTILs were differentially affected by heat stress for these traits (Table 5.3.1). This set of HTILs was planted at off-season nursery at Keylong during 2018 for generation advancement and was again planted in the replicated trial in season 2018-19 in RBD design across two environments for further evaluation.

**Table 5.3.1: Phenotypic data and summary statistics of HTILs in OE (Optimum Environment) and HSE (Heat Stress Environment) for two years 2017-18 ($BC_2F_3$) and 2018-19 ($BC_2F_5$)**

| Trait | Year | Env | Checks | BWL 3558 | HTIL/ BWL3558 | BWL 4444 | HTIL/ BWL4444 | BWL 5185 | HTIL/ BWL5185 | Population | $h^2$ | LSD | CV | GV | EV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PH | 2017-18 | 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 |
| | 2018-19 | 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 | 2017-18 | 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 |
| | 2018-19 | 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 | 2017-18 | 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 |
| | 2018-19 | 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 | 2017-18 | 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 |
| | 2018-19 | 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 | 2017-18 | 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 |
| | 2018-19 | 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 | 2017-18 | 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 |

| Trait | Year | Env | Checks | BWL 3558 | HTIL/ BWL3558 | BWL 4444 | HTIL/ BWL4444 | BWL 5185 | HTIL/ BWL5185 | Population | $h^2$ | LSD | CV | GV | EV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2018-19 | 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 | 2017-18 | 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 |
| | 2018-19 | 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 | 2017-18 | 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 |
| | 2018-19 | 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 (g) | 2017-18 | 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 |
| | | 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 |
| | 2018-19 | 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 (kg) | 2017-18 | 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 |
| | | 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 |
| | 2018-19 | 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 |

#Plant Height (PH), spikelet no. 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), and yield per plot (YD).

## 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

#### The variation components were significant at 1% level of significant.

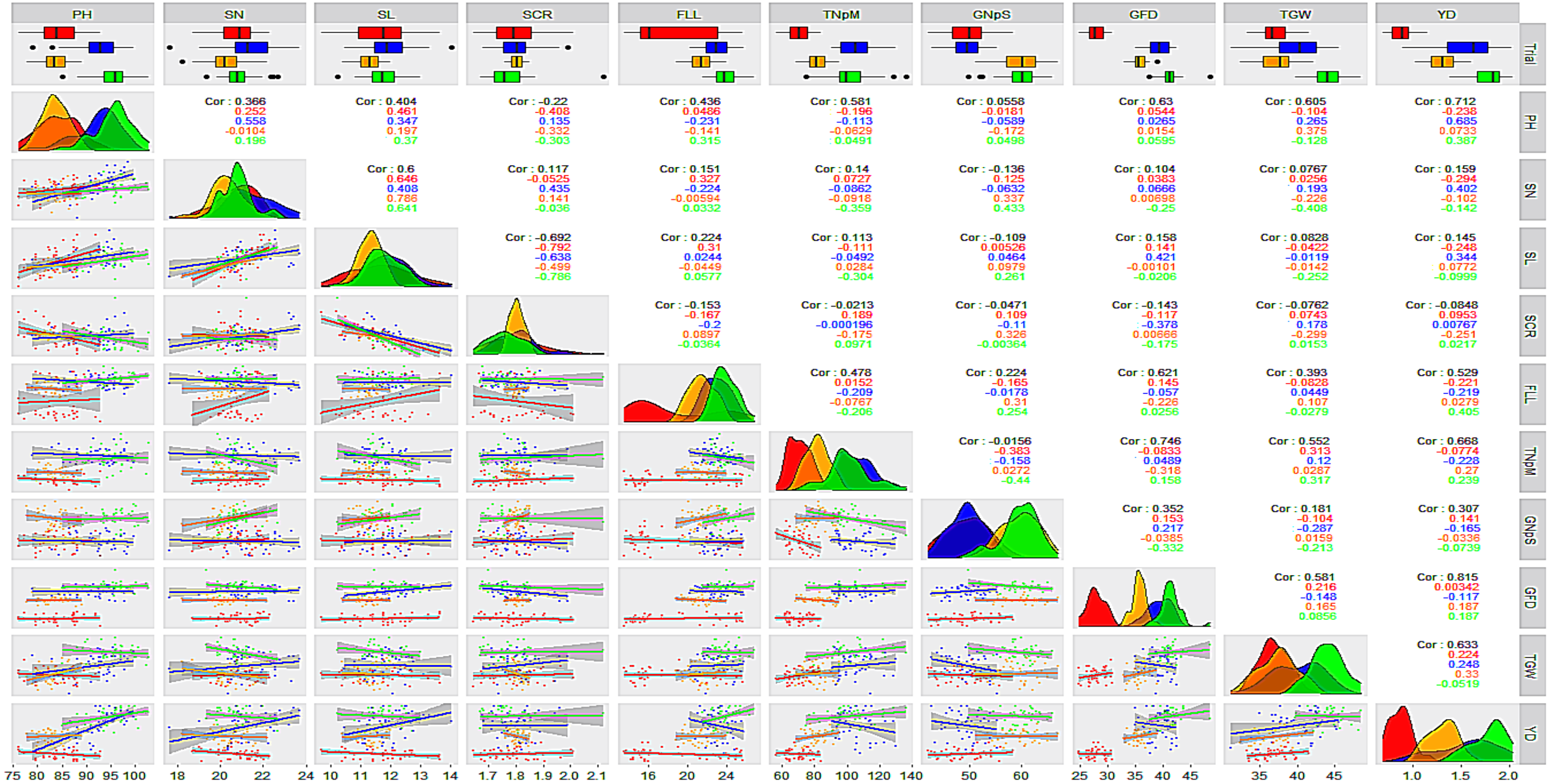

Figure 5.3.2: Evaluation of BC$_2$F$_3$ and BC$_2$F$_5$ progenies of HTILs developed through marker assisted introgression of the heat tolerance QTLs from crosses of *T. durum-Ae. speltoides* introgressions lines with different hexaploid wheat genotypes across two seasons 2017-18 and 2018-19.

#Blue colour represents trait response in optimum environment (OE) and red coloured boxes represent trait response in heat stress environment (HSE) of replicated trial in season 2017-18, green colour represent trait response in OE and orange colour represent trait response in HSE of replicated trial 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)

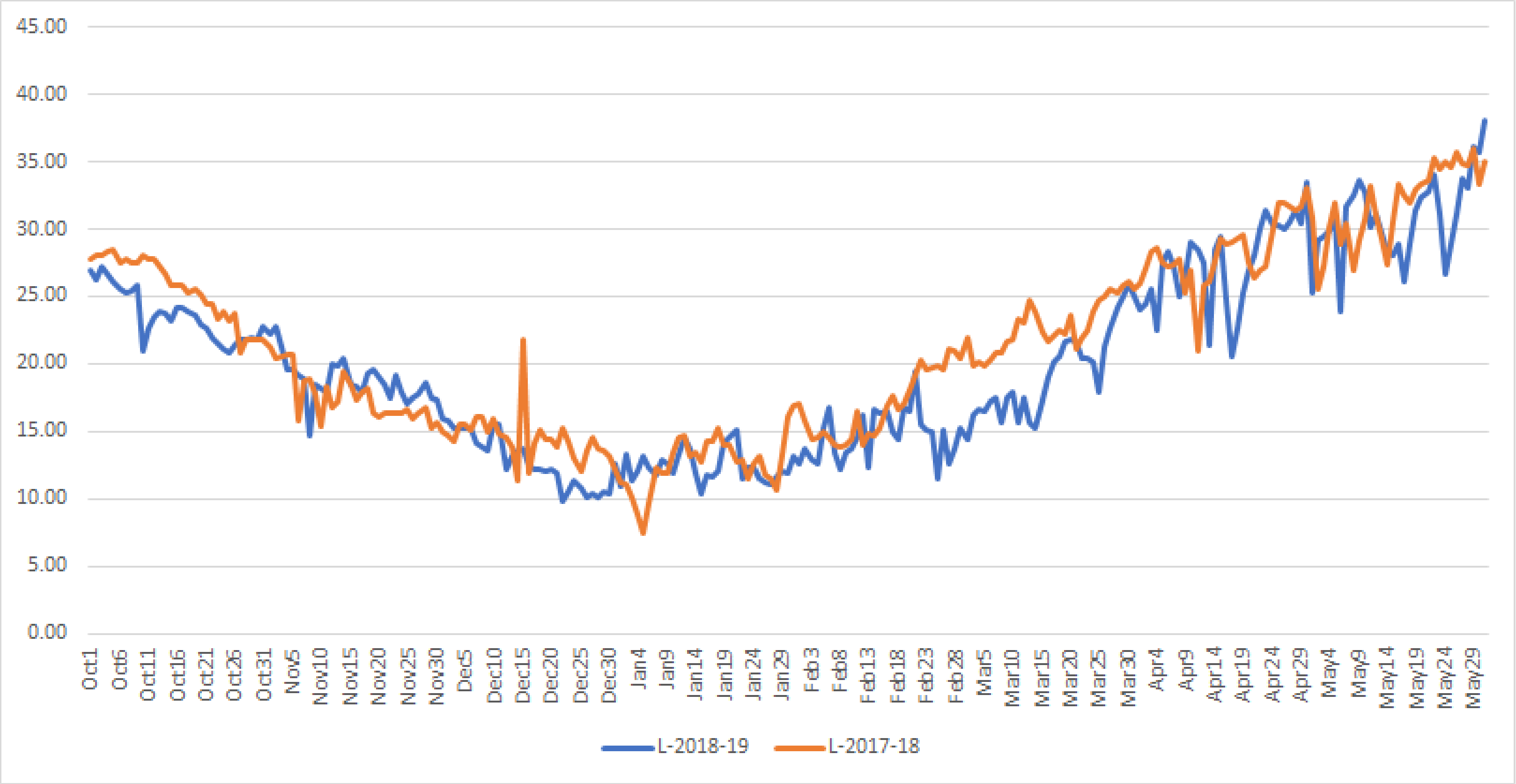

Figure 5.3.3: Temperature variation from October to May in the year 2017-18 and 2018-19

### 5.3.2 Phenotypic evaluation of $BC_2F_3$ HTILs (Trial 2- Augmented design) during season 2017-18

SN, SL, FLL, TNpM, GFD, TGW and YD showed wide variation in the trial 2 planted in the augmented trial in OE with many progenies showing better performance in FLL (>25cm), TNpM (>120), GFD (>45), TGW (>46gms) and YD (>400gms) than their respective recurrent parents (Table 5.3.2 and Figure 5.3.4). Some progenies (pau_HT96, 150, 154, 156, 165, 168 and 170) showed substantial increment (>15%) with respect to recurrent parents in TNpM, GFD and YDpP. PauHT_96, 146, 147 and 156 showed consistently better performance for most of the test traits than their recurrent parents. Overall analysis showed significant variability among traits YD, TGW, TNpM and GFD (>10%) in 120 $BC_2F_3$ progenies. All the traits studied for these progenies showed medium to high broad sense heritability (except TNpM due to very high variability) as per categories described by Aravind et al. (2019) and Kumar et al. (2017).

Table 5.3.2: Summary of 120 lines of $BC_2F_3$ progenies planted in augmented design

| **Trait** | **BWL 3558** | **BWL 4444** | **BWL 5185** | **Population** | **$h^2$** | **CV** | **GV** | **EV** |
|---|---|---|---|---|---|---|---|---|
| PH(cm) | 92.98 | 91.73 | 88.32 | 81.79-98.68 | 0.88 | 1.38 | 3.77 | 1.38 |
| SN | 22.20 | 24.20 | 24.60 | 19.4-27.8 | 0.69 | 4.14 | 6.25 | 4.14 |
| SL(cm) | 13.86 | 16.59 | 13.58 | 8.8-16.59 | 0.99 | 0.46 | 11.18 | 0.47 |
| SCR | 01.60 | 01.46 | 01.81 | 1.46-2.36 | 0.89 | 3.38 | 9.96 | 3.37 |
| FLL(cm) | 25.37 | 25.42 | 19.59 | 17.24-30.5 | 0.97 | 1.77 | 10.25 | 1.78 |
| TNpM | 153.0 | 159.5 | 134.0 | 77.17-222.83 | - | 26.83 | - | 26.83 |
| GFD | 43.50 | 44.00 | 41.50 | 30.33-46.67 | 0.91 | 2.71 | 8.71 | 2.72 |
| TGW(g) | 41.68 | 42.57 | 47.97 | 28.06-50.02 | 0.52 | 7.13 | 7.49 | 7.14 |
| YD(g) | 403.5 | 468.0 | 444.5 | 327.67-720.33 | 0.98 | 2.14 | 19.11 | 2.11 |

#Plant Height (PH), spikelet no. per spike (SN), Spike length (SL), spike compaction ratio (SCR), Flag leaf length (FLL), tiller number per meter (TNpM), grain filling duration (GFD), thousand grain weight (TGW), and yield per plot (YD).

## Heritability broad sense($h^2$), Genotypic Variance (GV), Residual/Environmental Variance (EV), Least Significant Difference (LSD) and Coefficient of variation (CV)

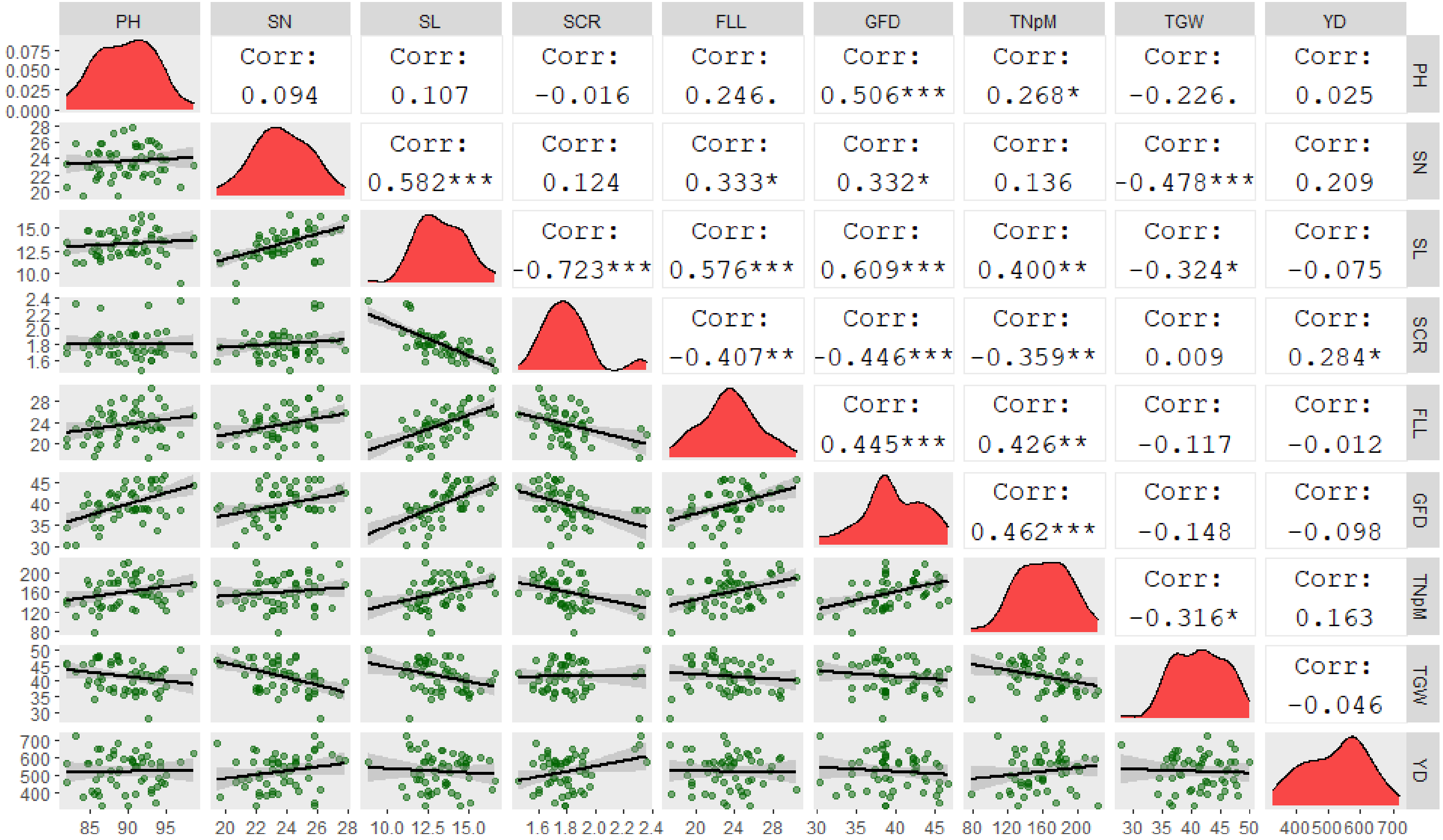

Fig 5.3.4: Evaluation of $BC_2F_3$ progenies of HTILs developed through marker assisted introgression of the heat tolerance QTLs from crosses of *T. durum-Ae. speltoides* introgressions lines with different hexaploid wheat genotypes in season 2017-18 planted as augmented design in optimum environment (OE).

#Upper right triangle shows pairwise correlation values. 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)

### 5.3.3 Phenotypic evaluation of $BC_2F_5$ HTILs (RBD design) during season 2018-19

The HTILs from RBD design of trial 1 in season 2017-18 were advanced and planted in the same design for evaluation in $BC_2F_5$ generation. Most of the HTILs outperformed their respective parental lines (for most traits studies) in OE and also showed lesser penalty across the traits due to heat stress in HSE (Table 5.3.1 and Figure 5.3.2). A considerable variation of trait values was observed both within and across the environments among the HTILs for various traits (mostly across environments) including for 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). For variability within the environment, TGW showed higher variation in HSE (32.47-42.16) than OE (39.69-48.45). This results in the conclusion that a large number of HTILs had a lesser reduction in TGW in HSE. Despite most of the traits showing the overall negative impact of heat stress with a reduction in trait values in HSE compared to OE, higher SN and GNpS in HSE signify increment due to stress (Figure 5.3.3). Yield 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 (1.602kg/plot) and pauHTIL_27 (1.564kg/plot) having the higher yield in HSE and pauHTIL_10, 11, and 12 showed yields higher than 2.0 kgs/plot in OE. Seven progenies (pauHTIL_9, 11, 12, 13, 18, 19, 20, 27, 30, and 31) maintained higher tiller numbers (TNpM) higher 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.

### 5.3.4 Correlation and multivariate analysis

Correlation analysis was performed among various agro-morphological traits with the main emphasis on their relationship with TGW and YD across and within the environments for trials in RBD design (Figure 5.3.2). A significant positive correlation of FLL, TNpM, GNpS, GFD, and TGW with YD was observed among all the traits studied. The overall GFD showed the highest correlation of 0.815 to YD, followed by 0.668 for TNpM and 0.633 for TGW. This showed that across the environments and trials, YD was highly dependent on TNpM and TGW. Similarly, TGW was highly correlated to GFD by 0.581. The regression curves between the traits also observed pairwise comparisons of the traits presented in Figure 5.3.2 (lower triangle), and the following conclusions were made. Longer GFD in both OE and HSE led to higher TGW in both generations of the HTILs. Similarly, increment in TGW and TNpM lead to an overall increase in YD.

Using the multivariate analysis across the environments, it was observed that PH, FLL, GFD, TGW, and TNpM contributed significantly to yield (Fig. 5.3.5A). These traits were then used to study their effect towards yield in the heat stress environment using SEM by fitting the model yield ~ PH + FLL + GFD + TGW+ TNpM. The model showed that the three main contributors to yield were TGW (0.262), GFD (0.278) and TNpM (0.358) (Fig. 5.2.5B). These effects were used as weights for each specific trait for calculating the weighted HTI.

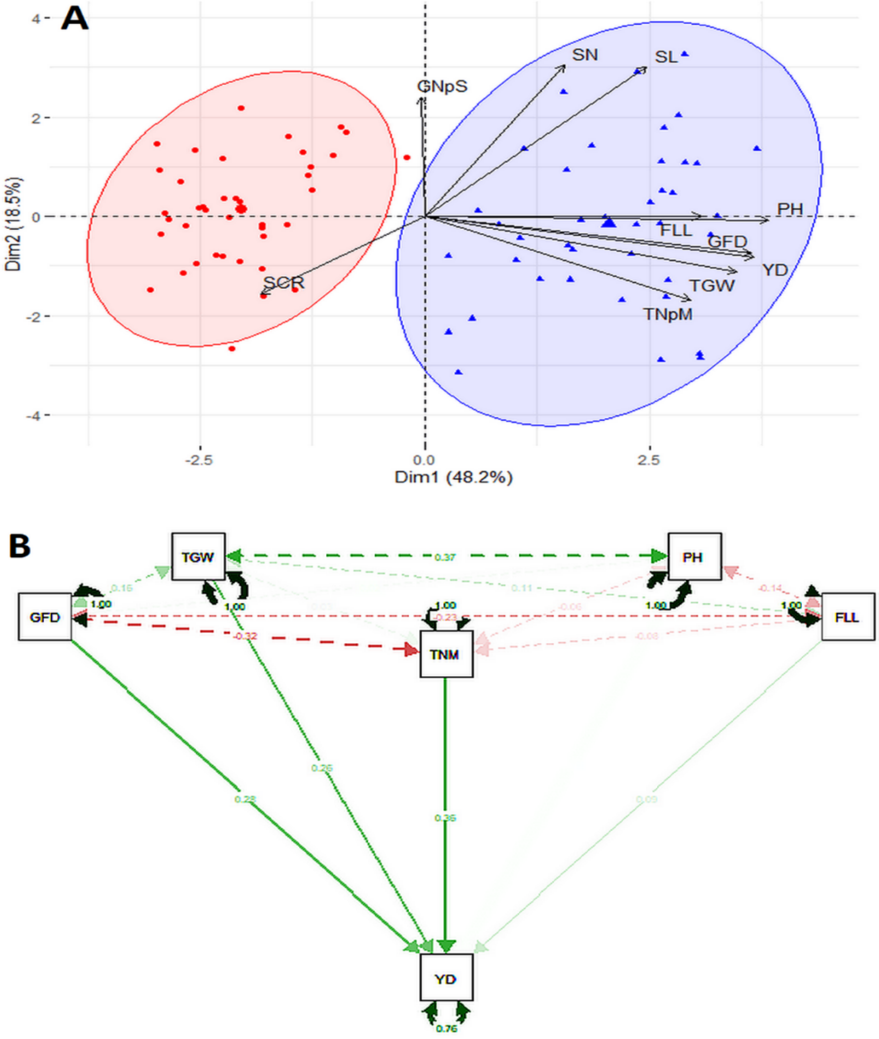

Fig 5.3.5: (A) Multivariate analysis using first two principal components as biplot for traits studied under optimum environment (blue) and heat stress environment (red); (B) Structural equation modelling of linked traits for identification of respective contribution towards yield in heat stress environment #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)

### 5.3.6 Heat Tolerance Index

Heat tolerance index (HTI) was calculated as the percent change in trait values in HSE with respect to the trait values in OE for each individual lines. The heat tolerance index can directly be compared to other lines to identify which of the lines had lower reductions due to heat stress. A heat tolerance index higher than 100 showed that the trait values increased in HSE as compared to OE. Most of the HTILs showed higher HTI index than their respective recurrent parents for one or more traits. Around 50% of the HTILs showed higher HTI for TGW and YD. This signified the positive effect of the selection in previous generations. Heat tolerance index calculated for various traits of the selected $BC_2F_5$ HTILs is given in Table 5.3.3. Considering yield (YD) and highly correlated traits of TGW and TNpM most of the lines not only performed better than their respective recurrent parents but also than the average HTI for the check elite varieties. 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). This clearly suggested that some genomic components transferred from DS-BILs have been activated during heat stress which helped them not only in maintaining expression of the phenotypic trait but also in some cases increased the trait values.

### 5.3.7 Validation of effect of QTLs in HTILs

For the validation of the positive effect of the presence of HT QTLs transferred in the HTILs, traits showing significant positive correlation to YD, i.e. FLL, TNpM, GFD, and TGW along with SN were studied. Firstly, the HTILs were subjected to introgression profiling to identify precisely the HT QTL composition of these lines (Figure 5.3.6). The presence or absence of the HT QTLs was correlated to the trait phenotypic values of the $BC_2F_5$ HTILs for both the environments (OE and HSE). This correlation of the phenotypic traits and the presence or absence of the HT QTLs is represented as a heatmap in Figure 5.3.7. Effect of the presence or absence of the donor alleles of the HT QTL in HTILs on these traits is also depicted as boxplots in both environments (Figure 5.3.8). As shown in these figures, a positive effect on SN was observed with the presence of QTL *QSs.pau-4A*. This effect was more prominent in HSE than OE.

**Table 5.3.3: 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 | 81.9 | 79.54 |
| BWL5185 | | 101.17 | 85.6 | 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_1 | *QTtc.pau-1B, QCt.pau-3B, QTgw.pau-2B* | 98.01 | 90.65 | **76.72** | 105.72 | 84.56 | 86.97 | 64.79 |
| 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_3 | *QTgw.pau-5B* | 98.6 | 90.72 | 68.28 | 93.45 | **89.32** | 78.95 | **73.13** |
| pauHTIL_4 | *QSs.pau-4A, QTtc.pau-1B, QCt.pau-3B, QTgw.pau-5B* | 97.42 | 90.91 | 66.01 | 94.92 | 85.99 | 75.7 | 57.43 |
| 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 | 61.8 |
| 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** | 85.7 | **90.39** | **111.06** | 86.83 | 79.86 | **86.12** |
| pauHTIL_19 | *QTtc.pau-1B, QCt.pau-3B, QTgw.pau-5B, QTgw.pau-2B* | 95.3 | 86.68 | **88.56** | 96.92 | 87.37 | 84.88 | 64.86 |
| pauHTIL_20 | *QSs.pau-4A, QTtc.pau-1B* | **102.6** | **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** |
| pauHTIL_31 | *QTgw.pau-5B* | 96.56 | **95.36** | **94.72** | 95.86 | 80.95 | 80.54 | 59.65 |
| HTIL(BWL4444) | | | | | | | | |
| pauHTIL_6 | *QTtc.pau-1B, QCt.pau-3B, QTgw.pau-5B* | 94.97 | 89.74 | 64.38 | 103.7 | 83.75 | 77.64 | **81.39** |
| pauHTIL_7 | *QTgw.pau-2B* | 97.4 | 91.41 | 70.62 | 95.5 | **90.42** | **96.78** | 66.07 |
| pauHTIL_8 | *QSs.pau-4A* | **104.84** | **96.85** | 69.73 | 104.47 | 86.67 | 81.16 | 62.2 |
| pauHTIL_9 | *QSs.pau-4A, QTtc.pau-1B, QCt.pau-3B* | **100.9** | 88.36 | **90.05** | 100.53 | 82.42 | 81.81 | 65.61 |
| pauHTIL_10 | *QSs.pau-4A, QCt.pau-3B, QTgw.pau-5B* | **101.32** | 90.22 | 75.61 | 102.92 | 80.94 | 76.67 | 51.9 |
| pauHTIL_11 | *QCt.pau-3B, QTgw.pau-5B* | 96.77 | 86.07 | **93.13** | 88.76 | 84.69 | 83.01 | **73.34** |

| **Genotype** | **HT QTLs introgresssed** | **SN** | **FLL** | **TNpM** | **GNpS** | **GFD** | **TGW** | **YD** |
|---|---|---|---|---|---|---|---|---|
| pauHTIL_12 | *QCt.pau-3B, QTgw.pau-5B* | 90.03 | 88.4 | **95.28** | 100.79 | 86.36 | 87.28 | 61.72 |
| 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** | 103.9 | **89.04** | 84.18 | **84.63** |
| pauHTIL_15 | *QSs.pau-4A, QTtc.pau-1B, QCt.pau-3B, QTgw.pau-5B* | 98.51 | 80.19 | 68.27 | 82.34 | 87.49 | 80.38 | 64.63 |
| pauHTIL_23 | *QCt.pau-3B, QTgw.pau-5B* | 92.65 | 78.7 | **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_26 | *QSs.pau-4A, QCt.pau-3B, QTgw.pau-5B, QTgw.pau-2B* | **101.05** | 79.95 | 75.3 | 97.12 | 86.38 | 83.36 | **74.34** |
| pauHTIL_27 | *QCt.pau-3B, QTgw.pau-5B, QTgw.pau-2B* | 94.9 | 86.1 | **81.95** | 90.46 | **88.82** | 84.2 | **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_35 | *QSs.pau-4A, QTgw.pau-5B, QTgw.pau-2B* | 94.89 | 85.45 | 68.77 | 93.69 | **88.1** | 81.8 | 71.2 |
| pauHTIL_36 | *QSs.pau-4A, QCt.pau-3B* | 92.18 | **99.58** | **78.2** | 93.18 | **90.09** | **88.35** | 70.38 |
| pauHTIL_37 | *QCt.pau-3B, QTgw.pau-5B* | 99.1 | 81.07 | 58.98 | 106.75 | 82.99 | 87.6 | 61.45 |
| 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** | **121.9** | 80.36 | 74.11 | **82.02** |
| pauHTIL_40 | *QCt.pau-3B* | 91.59 | 82.93 | 65.61 | 85.34 | 87.12 | **88.78** | 72.02 |
| 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 | **88.1** | **102.05** | **76.97** |
| pauHTIL_32 | *QSs.pau-4A* | 95.31 | 90.47 | **115.98** | **106.19** | 84.49 | **89.9** | **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 | **90.5** | 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 |

# spikelet no. per spike (SN), 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). Underlined values show performance better than respective recurrent parents and bold values shows performance better than checks.

With a similar effect in both the environments increment in overall FLL was observed with the presence of HT QTL *QTtc.pau-1B*. Similarly, increment in overall FLL was observed with the presence of HT QTL *QCt.pau-3B* in both environments with effect in OE being more prominent than in HSE. Both the QTLs for TGW, *QTgw.pau-5B,* and *QTgw.pau-2B* showed a large positive effect on TGW but in different environments where *QTgw.pau-5B* contributed more in OE while *QTgw.pau-2B* in HSE. All these QTLs (except *QSs.pau-4A*) showed a positive contribution to the overall yield.

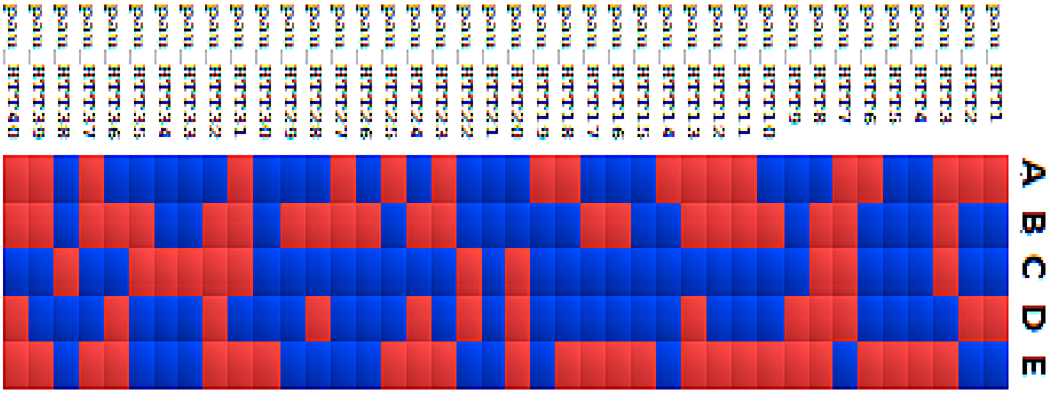

Figure 5.3.6: Haplotypic view of marker profile of the selected heat tolerant lines ($BC_2F_5$) developed in the present study. Molecular markers linked to various heat tolerant QTLs transferred in *T. durum-Ae. speltoides* introgression lines and reported by Awlachew et al. (2016) were used for marker 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.

Using the H statistics, the presence of QTL *QSs.pau-4A* showed positive effect on SN (Table 5.3.4) 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%).

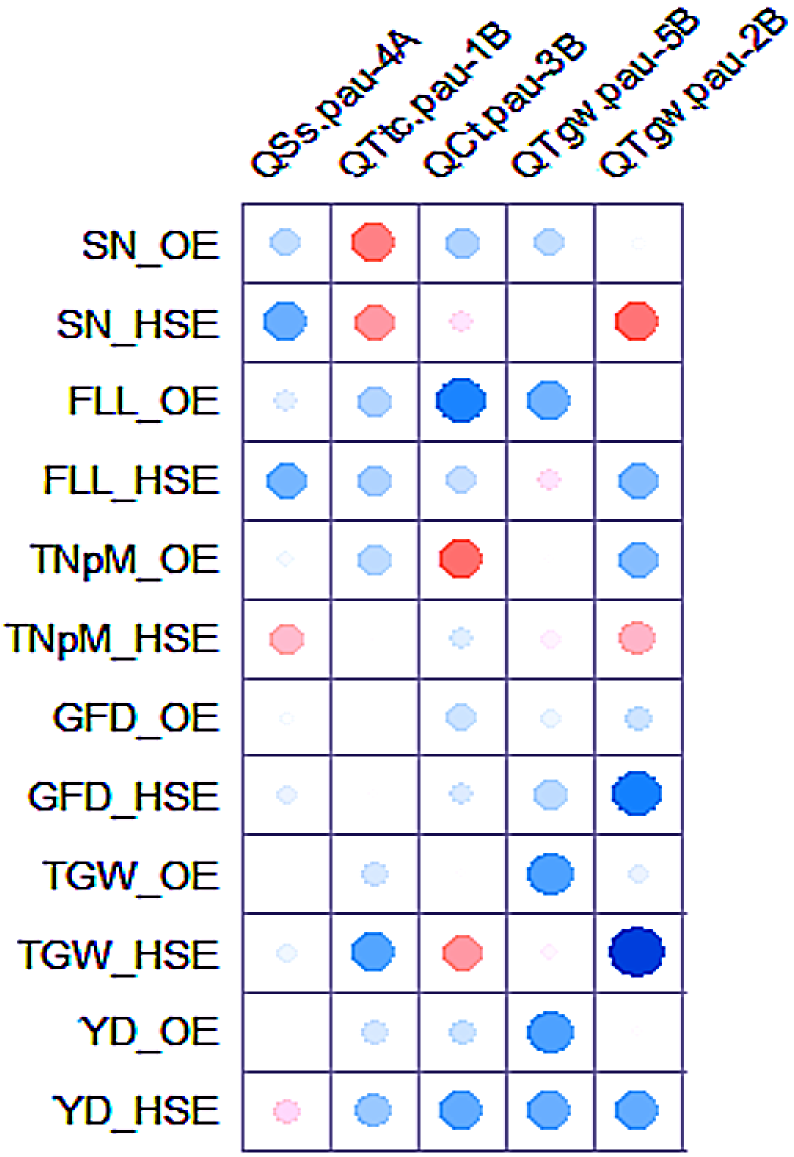

Figure 5.3.7: Heatmap of effect of HT QTLs present across the HTILs to various traits. Blue colour represents positive effect (increment) on trait value across both OE (Optimum environment) and HSE (Heat stress environment) while red colour represents negative effect (decrement) of trait values. The 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)

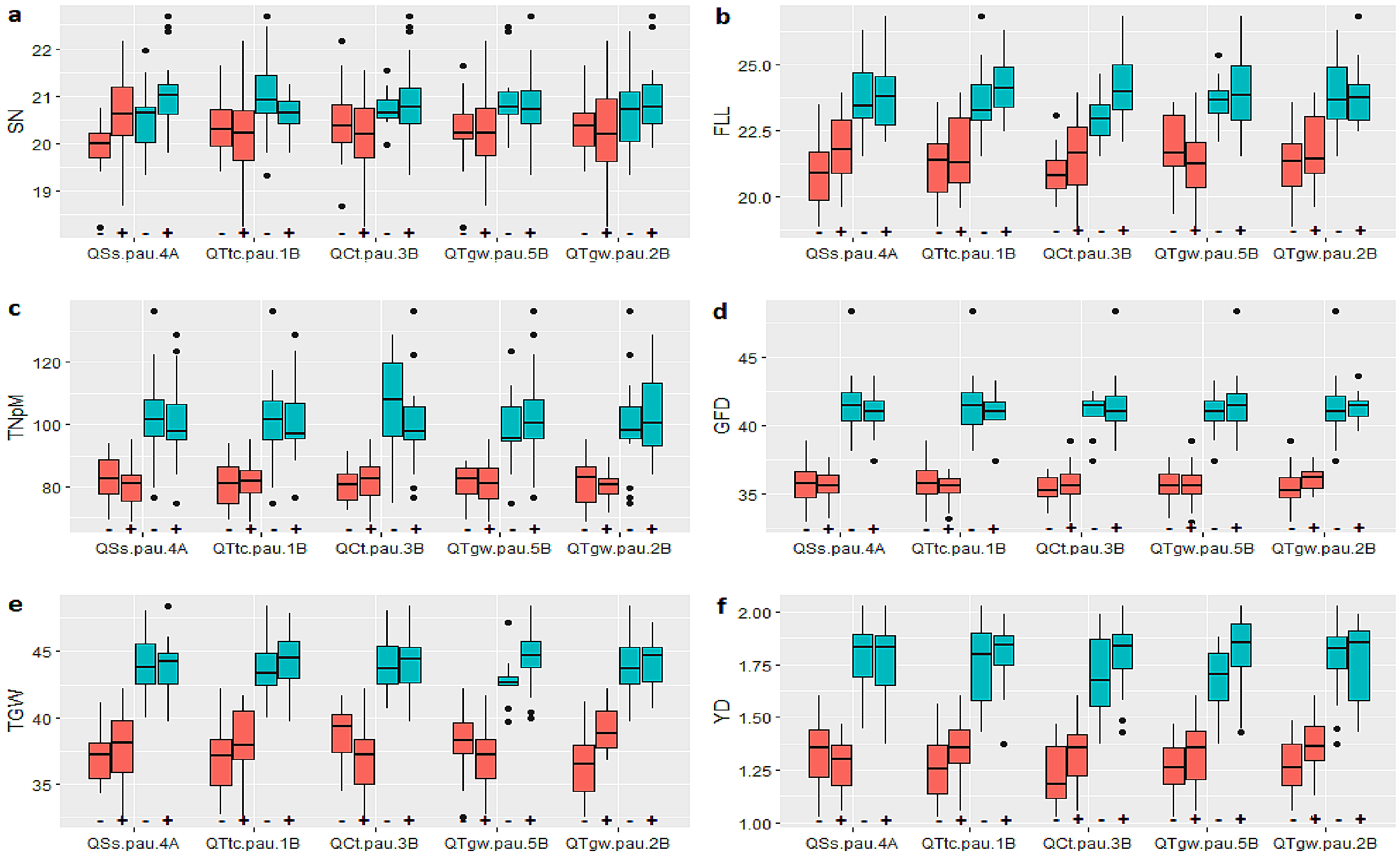

Figure 5.3.8: Effect of various QTL introgressions in HTILs on important yield related traits (a-f). Blue color represents trait response in OE (Optimum environment) and red color represents trait response in HSE (Heat stress environment). The positive (+) and negative (-) signs represents the distribution of trait response in presence and absence of the QTL, respectively. Number of lines positive for QTL *QSs.pau-4A*, *QTtc.pau-1B*, *QCt.pau-3B*, *QTgw.pau-5B*, and *QTgw.pau-2B* are 22, 18, 29, 17, and 15, respectively.

**Table 5.3.4: 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 |

† spikelet no. per spike (SN), Flag leaf length (FLL), grain filling duration (GFD), thousand grain weight (TGW), yield per plot (YD), environment (Env)

†† level of significance; p-value < 0.001 (***), p-value < 0.01 (**), p-value < 0.05 (*), and p-value < 0.10 (.)

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. There is a sudden rise in day temperature starting from mid to end of March which causes forced maturation of plants reducing their grain filling duration significantly, especially for those grown in HSE, as reported by Acevedo et al. (1990), and Pimentel et al. (2015). Late sown plants experience this stress more than those in OE as their anthesis time coincides with the period of heat stress (Flohr et al. 2017). In the present study, the daily temperatures varied from 17.4-34.0$^{o}$C during the day and 6.8-17.0$^{o}$C during the night from October to May (Figure 5.2.3). Higher temperature causes a reduction in overall plant phenology in HSE, i.e. higher rates of senescence. The inhibition of photosynthesis at high-temperature results in a drop of carbohydrate reserves, which in turn reduce production as a result of the reduction in crop cycle (Pimentel et al. 2015).

Different traits showed a differential response to heat stress in these HTILs. All the traits studied in this investigation, including CT, 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 a strong negative correlation between CT and

GFD in both the environments, especially in HSE where progenies with higher CT showed an overall reduction in GFD which in return reduced TGW and YD. HTIL pauHTIL_30 showed an increase in TGW in HSE than OE despite a reduction in GFD and YD which may be attributed to increase in the 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 a 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 the complex genome of bread wheat where alien introgressions are involved (Kaur et al. 2018b; King et al. 2018a; Hao et al. 2020; Al-Ashkar et al. 2020).

Heat tolerance is a complex trait that can only be estimated from the 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 (Guerra et al. 2015). Compounded by the huge complexity of the bread wheat genome, it is difficult to dedicate a single trait as a representation of tolerance to heat stress (Deb and Khaleque 2009). Since the overall effect of stress is manifested in the form of yield losses in the stressed conditions, the traits contributing to yield under stress could be used to study the tolerance in the genotypes. As shown by the structural equation modelling for yield in stress condition, tiller number, TGW and yield played an essential role in heat stress tolerance, reducing the overall yield penalty and are the best estimates for heat stress tolerance (Singh et al. 2018; Gupta et al. 2020; Mir et al. 2021). 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, 33, and 34 have shown higher yield than tested cultivars under OE and pauHTIL_14 under HSE. 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.

## 5.4 Mapping QTLs for stripe rust and powdery mildew disease resistance in *Triticum durum – Aegilops speltoides* introgression lines

### 5.4.1 Evaluation for stripe rust and powdery mildew resistance

The panel of 89 DS-BILs along with the recurrent parent PDW274 and susceptible check WL711 were first screened for diseases at the adult plant stage under artificial epiphytotic conditions in the field. The panel was moderate to completely resistant with PDW274 showing moderate resistance for both the diseases suggesting APR type gene in the recipient parent itself. The study was then focused on screening and identifying for the presence of seedling stage resistance (ASR) in the panel. On screening with stripe rust (YR) pathotype *Pst78S84* and mixture of pathotypes from open field the parental genotype *Ae. speltoides* acc. pau3809 showed complete resistance both at seedling stage and adult plant stage while PDW274 showed complete susceptibility at seedling stage and complete resistance at adult plant stage. This concluded that PDW274 might carry an adult plant resistance (APR) gene for stripe rust and *Ae. speltoides* acc. pau3809 harbours all stage resistance (ASR) gene(s). Seedling screening against *Pst78S84*, the DS-BILs showed varied reaction scores ranging from complete resistance to complete susceptibility (Figure 5.4.1 and Figure 5.4.2). A susceptible type of reaction score was recorded for most of the DS-BILs, with more than 60 DS-BILs showing a score of 8 (on a scale of 0-8) with 13 DS-BILs with moderate susceptibility score of 5.33 and remaining completely resistant to the YR pathotype with a score less than 3.0. Similarly, screening with a mixture of stripe rust pathotypes, the more than 60 DS-BILs recorded high susceptibility score (YR score of 8), 12 DS-BILs recorded a moderate susceptibility score of 5.33 and only two DS-BILs (DS-BIL6 and DS-BIL16) as completely resistant (YR score of 2.67). For both the inoculums *Ae. speltoides* showed complete resistance with a score of 0.

When the panel was screened for powdery mildew with a mixture of powdery mildew (*Bg*) pathotypes collected from the open field, almost half of the set, i.e. 40 DS-BILs recorded a disease score of 0 to 3.3 and were completely to moderately resistant. The remaining half of the panel of the DS-BILs along with PDW274 was highly susceptible, while *Ae. speltoides* showed complete resistance with a score of 0 (Figure 5.4.1b, 5.4.2c).

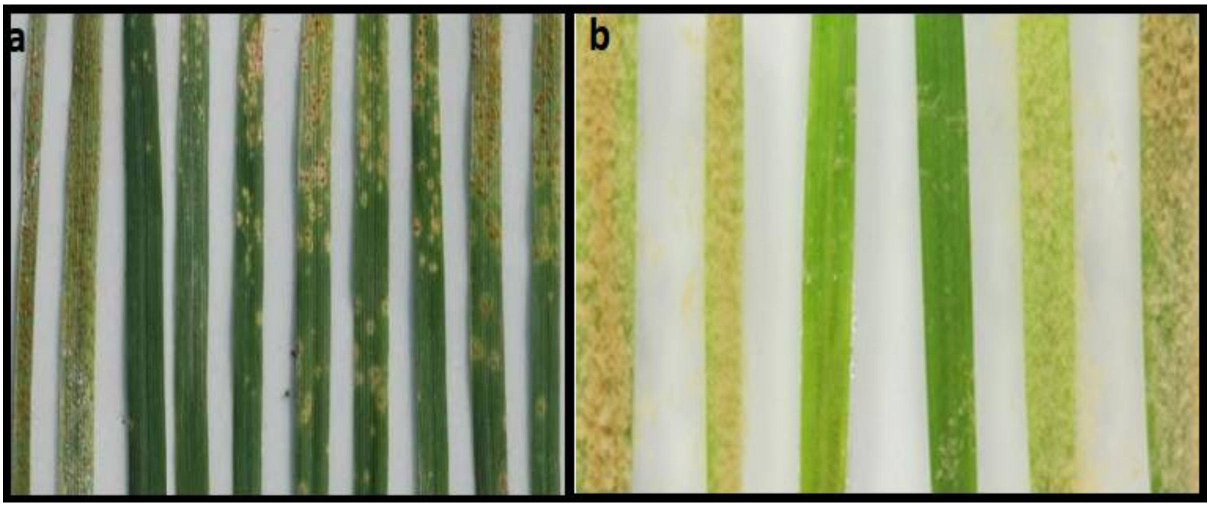

Figure 5.4.1: Disease reaction of tested genotypes for a) stripe rust pathotypes, and b) powdery mildew pathotypes. The first three leaves represent susceptible check *T. aestivum* cv. WL711, *T. durum* cv. PDW274, and *Ae. speltoides* acc pau3809, serially. Succeeding leaves represent reactions of DS-BILs.

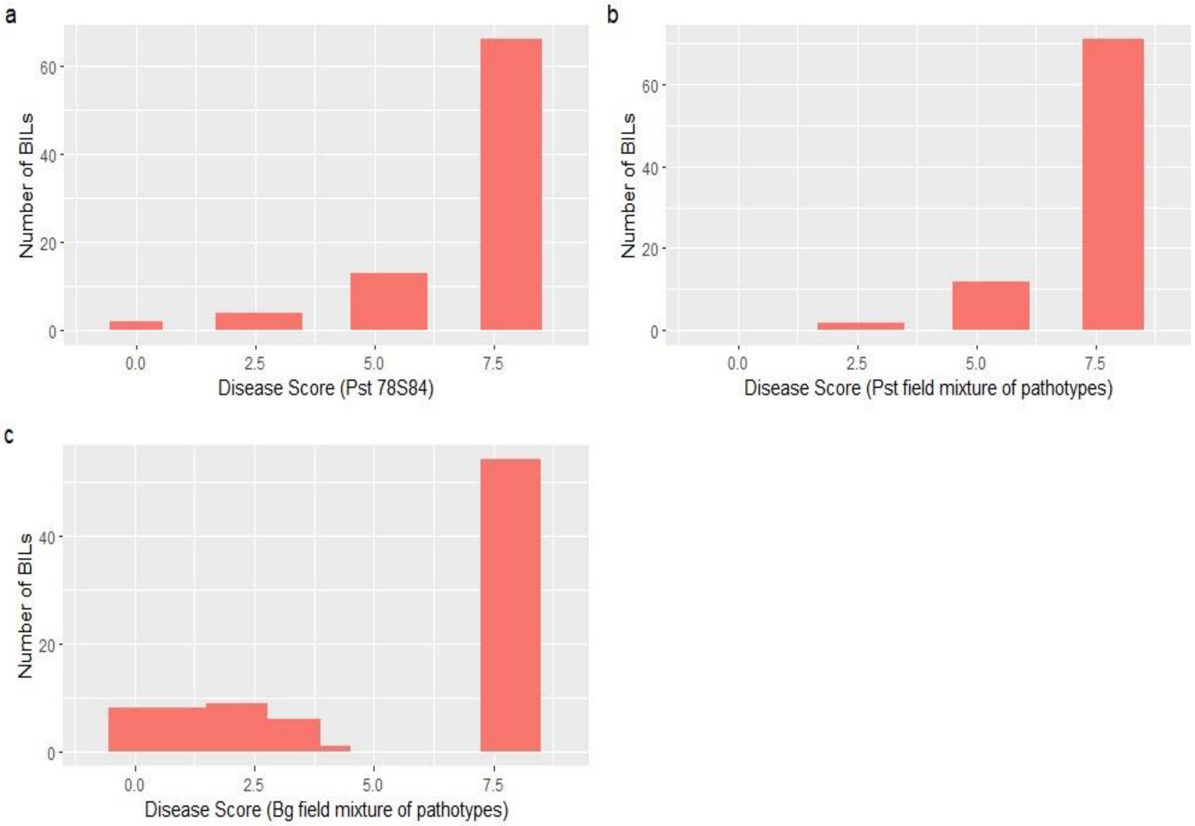

Figure 5.4.2: Frequency distribution of the disease score of *T. durum-Ae. speltoides* DS-BILs for a) stripe rust pathotype Pst 78S84 at the seedling stage, b) stripe rust mixture of field pathotypes and c) powdery mildew mixture of field pathotypes. Reaction score of recurrent parent PDW274 was eight for all the pathotypes.

### 5.4.2 Introgression profiling of DS-BILs

The 1095 physically mapped SNPs were used to draw the physical map of the DS-BILs, identifying the density of the SNPs across various chromosomes (Table 5.4.1). As shown in figure 5.4.3, the polymorphic SNPs were mostly located on distal ends of the chromosomes with very few and sparsely placed SNPs near the centromeric region of the chromosomes. The chromosome group 4, i.e. chr4A and chr4B were the least genotyped chromosomes with chr2B with the highest number of 172 markers. The introgression profile of the DS-BILs showed few lines had large segments of the introgression from *Ae. speltoides* with chromosomes 5A and 5B had the greatest number of large segment introgression throughout the panel. Similarly, the least number of introgressions were observed in the chr7B (Figure 5.4.3). The introgression profile clearly depicts the introgression of *Ae. speltoides* segments to both A and B genome of *T. durum* despite of SS genome of *Ae. speltoides* having high affinity to recombine with B genome of wheat. This is due to the fact that *Ae. speltoides* carries genes epistatic to Ph1 loci of wheat (Millet 2007; Colas et al. 2008; King et al. 2018b), called *Ph* suppressors, which lead to homoeologous recombination of the alien genome with wheat chromosomes.

**Table 5.4.1: Distribution of SNPs across the different chromosomes of *T. durum***

| Chr | No. of SNPs | First SNP Pos | Last SNP Pos | Coverage(inMp) | Density |
|---|---|---|---|---|---|
| 1A | 46 | 07.81 | 592.66 | 584.85 | 12.71 |
| 1B | 83 | 17.57 | 684.91 | 667.34 | 08.04 |
| 2A | 91 | 01.53 | 771.51 | 769.98 | 08.46 |
| 2B | 172 | 08.28 | 793.33 | 785.05 | 04.56 |
| 3A | 55 | 06.75 | 730.38 | 723.63 | 13.16 |
| 3B | 96 | 08.63 | 826.65 | 818.02 | 08.52 |
| 4A | 34 | 381.21 | 739.13 | 357.92 | 10.53 |
| 4B | 20 | 80.00 | 667.90 | 587.9 | 29.40 |
| 5A | 98 | 29.18 | 708.12 | 678.94 | 06.93 |
| 5B | 83 | 35.55 | 708.95 | 673.40 | 08.11 |
| 6A | 64 | 05.13 | 615.71 | 610.58 | 09.54 |
| 6B | 105 | 07.13 | 720.91 | 713.78 | 06.80 |
| 7A | 58 | 03.85 | 724.59 | 720.74 | 12.43 |
| 7B | 90 | 20.63 | 739.41 | 718.78 | 07.99 |
| GenomeA | 446 | 435.46 | 4882.1 | 4446.64 | 09.97 |
| GenomeB | 649 | 177.79 | 5142.06 | 4964.27 | 07.65 |
| Total | 1095 | 613.25 | 10024.16 | 9410.91 | 08.59 |

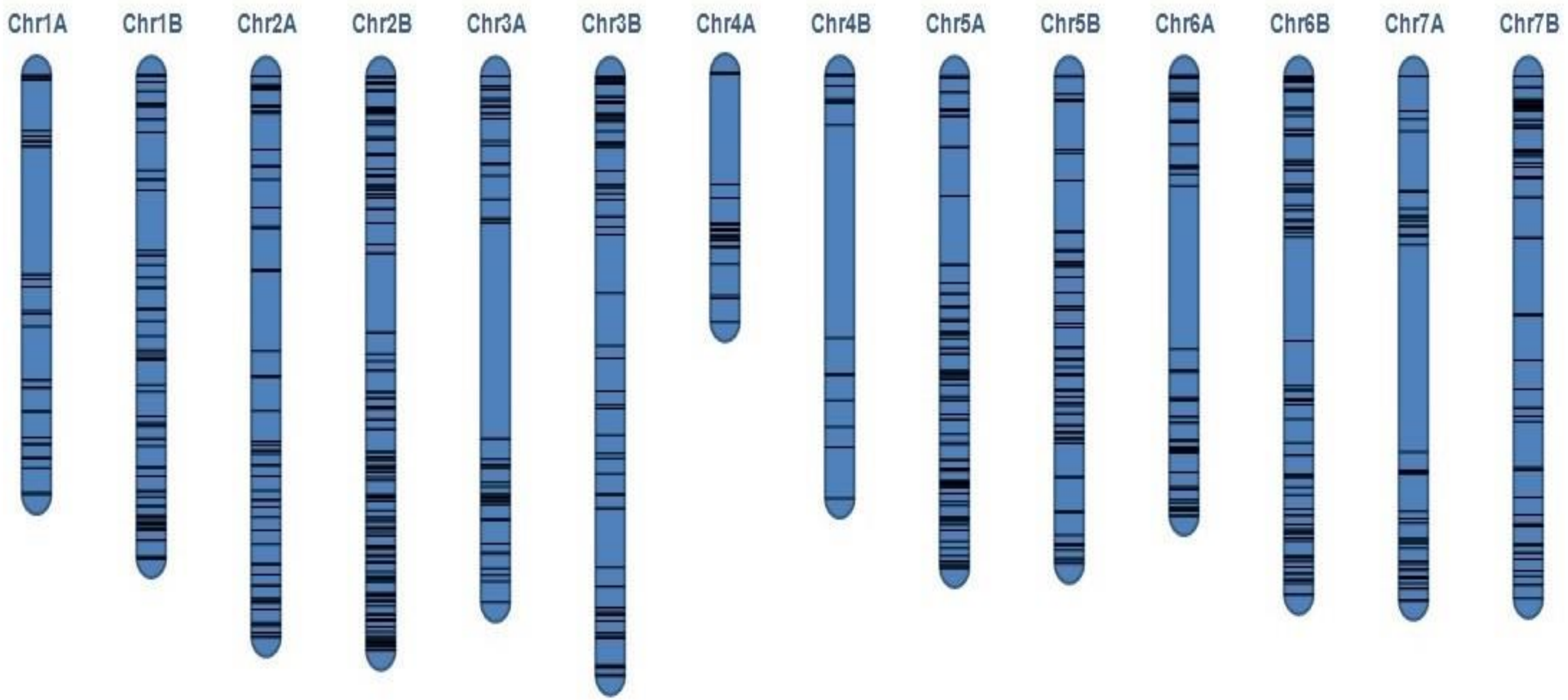

Figure 5.4.3: Coverage of filtered 1095 SNPs used for mapping QTLs for various diseases along A and B genome chromosomes. The black lines represent physical positions of the SNPs according to wheat RefSeqv1.0 on blue background of recurrent parent PDW274.

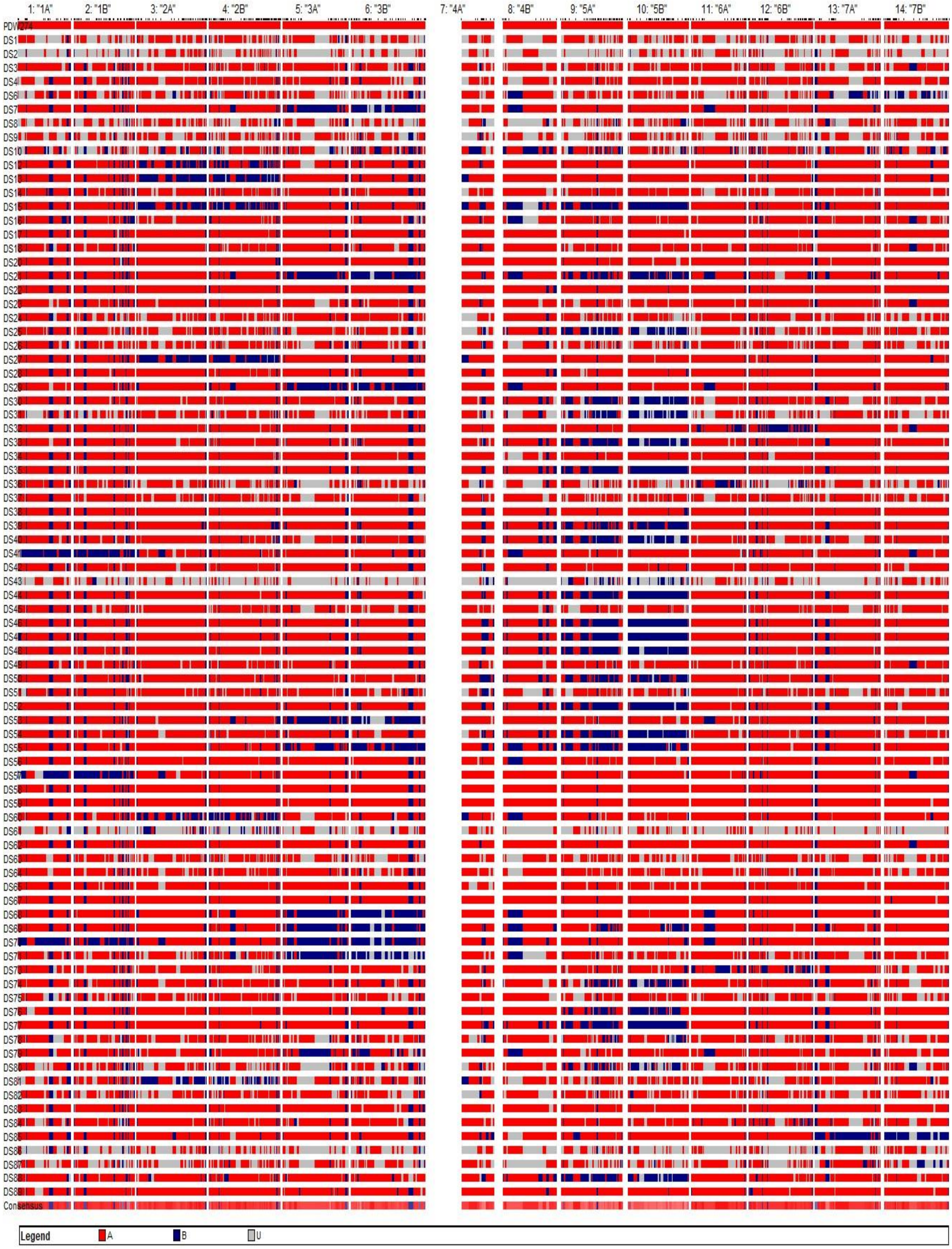

Figure5.4.4: Introgression profile of 89 lines with recurrent parent PDW274 (on top) is given with red colour represents the segment from PDW274 and blue represent the introgression of *Ae. speltoides* in the DS-BILs

### 5.4.3 QTL mapping for disease resistance

The mapping of QTLs for disease resistance using 1095 polymorphic SNP markers and disease reaction scores of the three different pathogenic inoculums used for disease screening, resulted in identifying a total of 11 QTLs. Four QTLs were mapped each for resistance against PM and YR field mixture of pathotypes, and two QTLs were mapped for YR pathotype *Pst* 78S84 using stepwise regression-based Likelihood Ratio Test (RSTEP-LRT) for the additive effect of markers and single-marker analysis (SMA) (Table 5.4.2, Figure 5.4.5). For stripe rust against field mixture of pathotypes, QTLs *QYrAs.pau-2A.1 and QYrAs.pau-7B* were mapped on chromosome 2A and 7B with both the algorithms. Using SMA QTL *QYrAs.pau-2A.1*. was mapped with a LOD score 3.8 (logarithm of odds) and PVE 24.51% (phenotypic variation explained) and with LOD score 2.6 and PVE 13.83% using RSTEP-LRT. Similarly, QTL *QYrAs.pau-7B* was mapped with a LOD score of 2.4 and PVE 10.06% using SMA and using RSTEP-LRT with a LOD score of 2.4 and PVE 10.06%. In both of these QTLs, the resistance allele was contributed by *Ae. speltoides*. Two other QTLs detected using the same inoculum were QTL *QYrAs.pau-1B* on chr1B and QTL *QYrAs.pau-2B* on chr2B. These two QTLs were detected only using SMA algorithm with LOD score 2.0 and PVE 11.64% for QTL *QYrAs.pau-1B* and with a LOD score of 2.2 and PVE 12.8% for QTL *QYrAs.pau-2B*. Similar to the previous two QTLs, the source of resistance was *Ae. speltoides* introgression.

Mapping with the disease reaction score of DS-BILs against stripe rust pathotype *Pst* 78S84, lead to mapping of two QTLs, QTL *QYrTd.pau-2A.2* mapped on chr2A and QTL *QYrTd.pau-3B* mapped on chr3B with both algorithms. QTL *QYrTd.pau-2A.2* was mapped with a LOD score of 2.9 and PVE 14% using SMA, and with a LOD score of 2.1 and PVE 9.4% using RSTEP-LRT. QTL *QYrTd.pau-3B* was mapped with a LOD score of 4.1 with PVE 19.29% using both SMA and RSTEP-LRT. Unlike the QTLs mapped using field mixture of pathotypes of stripe rust, the QTLs detected by mapping using pathotype *Pst* 78S84 the contributor of disease resistant allele was PDW274.

Mapping for disease resistance QTLs against PM field mixture of pathotypes at the seedling stage identified five QTLs located on chr2A, 2B, 3B, and 6B using both SMA and RSTEP-LRT. Algorithm SMA identified three QTLs, namely *QPmAs.pau-2A.2*, *QPmAs.pau-2B*, and *QPmAs.pau-6B*. QTL *QPmAs.pau-2A.2* was mapped at chr2A with a LOD score of 2.02 and PVE 11.49%, QTL *QPmAs.pau-2B* at chr2B with a LOD score of 2.3

Table 5.4.2: Summary of the QTL mapping using single marker analysis SMA and stepwise regression-based Likelihood Ratio Test (RSTEP-LRT) for additive effect of markers algorithms of QTL ICI Mapping

| | TraitName | QTL | SNP Marker Name | Marker position | | LOD | PVE(%) | Add |
|---|---|---|---|---|---|---|---|---|
| | | | | Chr. | Phy. Position (Mb) | | | |
| RSTEP-LRT | Pst field pathotypes | *QYrAs.pau-2A.1* | S2A_16016633 | S2A | 16.01 | 2.6 | 13.83 | 0.6621 |
| | Pst field pathotypes | *QYrAs.pau-7B* | S7B_708445814 | S7B | 708.44 | 2.4 | 10.06 | 0.8519 |
| | Pst 78S84 | *QYrTd.pau-2A.2* | S2A_766158316 | S2A | 766.15 | 2.2 | 09.42 | -0.6615 |
| | Pst 78S84 | *QYrTd.pau-3B* | S3B_743818730 | S3B | 743.81 | 4.1 | 19.29 | -1.2189 |
| | Bg field pathotypes | *QPmAs.pau-2A.1* | S2A_43146710 | S2A | 43.15 | 2.0 | 09.16 | 1.7895 |
| | Bg field pathotypes | *QPmAs.pau-3B* | S3B_775092221 | S3B | 775.09 | 2.4 | 12.89 | 2.4370 |
| | Bg field pathotypes | *QPmAs.pau-6B* | S6B_26793381 | S6B | 26.79 | 3.2 | 17.75 | 2.2547 |
| SMA | Pst field pathotypes | *QYrAs.pau-1B* | S1B_626229235 | S1B | 626.22 | 2.0 | 11.64 | 0.4094 |
| | Pst field pathotypes | *QYrAs.pau-2A.1* | S2A_16016633 | S2A | 16.01 | 3.8 | 24.51 | 0.8815 |
| | Pst field pathotypes | *QYrAs.pau-2B* | S2B_27896451 | S2B | 27.89 | 2.2 | 12.80 | 0.4638 |
| | Pst field pathotypes | *QYrAs.pau-7B* | S7B_708445814 | S7B | 708.44 | 2.4 | 10.06 | 0.8518 |
| | Pst 78S84 | *QYrTd.pau-2A.2* | S2A_766158316 | S2A | 766.15 | 2.8 | 14.00 | -0.8065 |
| | Pst 78S84 | *QYrTd.pau-3B* | S3B_743818730 | S3B | 743.81 | 4.1 | 19.29 | -1.2189 |
| | Bg field pathotypes | *QPmAs.pau-2A.2* | S2A_771507864 | S2A | 771.507 | 2.1 | 11.49 | 2.1681 |
| | Bg field pathotypes | *QPmAs.pau-2B* | S2B_791958961 | S2B | 791.958 | 2.3 | 14.05 | 2.2938 |
| | Bg field pathotypes | *QPmAs.pau-6B* | S6B_26793381 | S6B | 26.793 | 3.2 | 17.75 | 2.2547 |

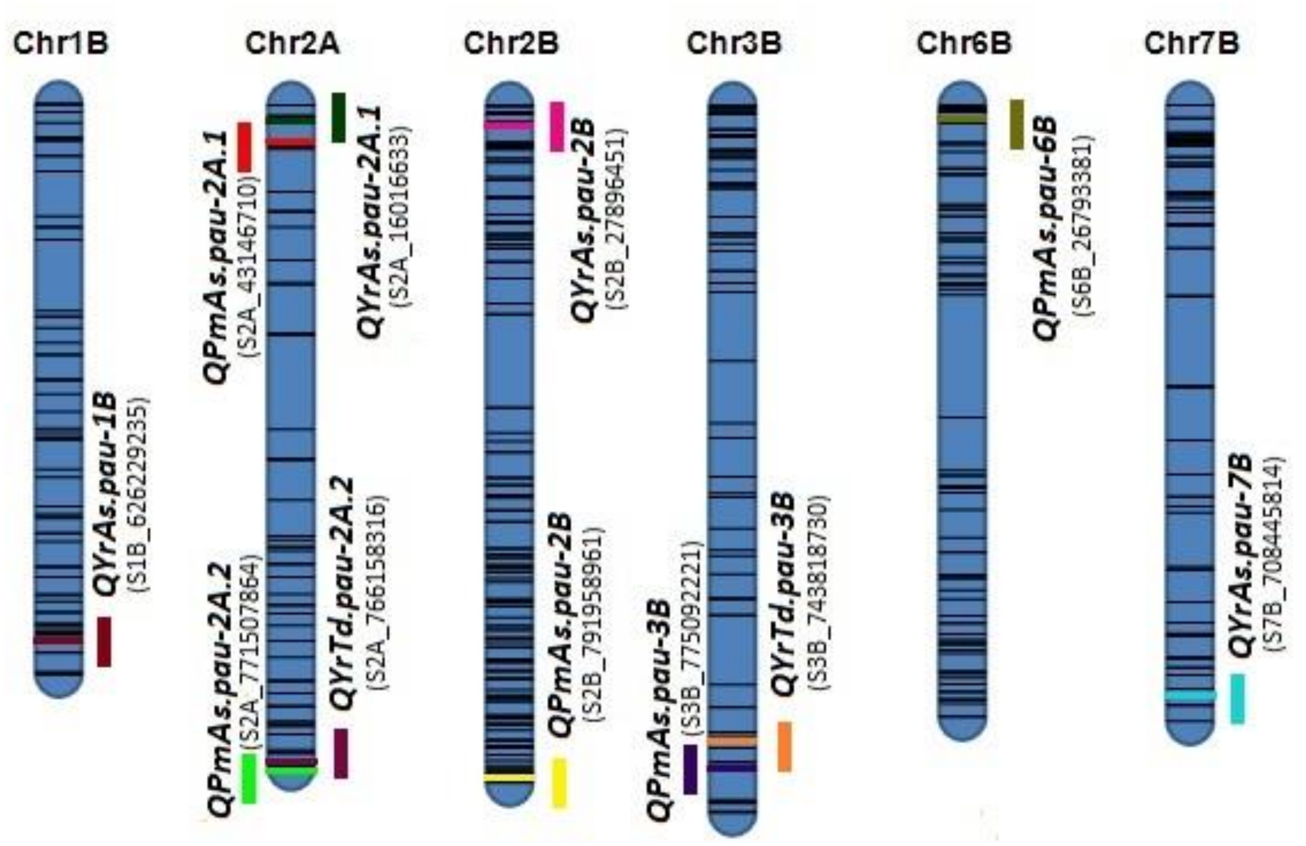

Figure 5.4.5: Summary of the mapped QTLs for different diseases in the *T. durum-Ae. speltoides* introgression lines. Mapped QTL are represented as bars alongside the carrier chromosomes. The black lines represent the SNP positions along the length of the chromosomes. The colored lines on chromosomes and bars alongside represent the positions of QTLs. QTL names are presented alongside the colored bars along with the SNPs linked to the QTLs.

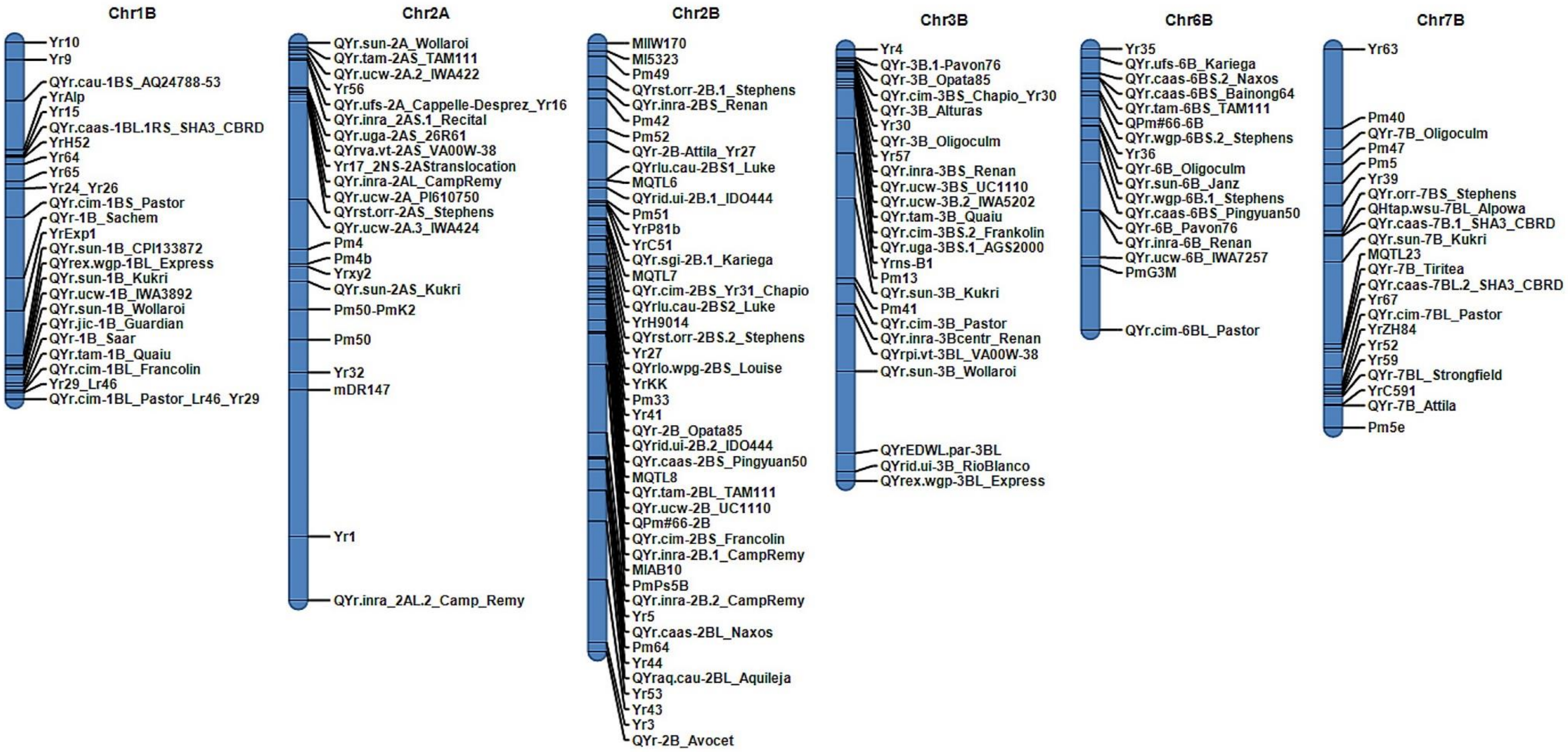

Figure 5.4.6: Summary of the designated genes, preliminary reported genes and QTLs on chromosomes harbouring QTL in the present study. The mapped genes/QTL, their linkage positions were retrieved from www.wheat.pw.usda.gov as has been reported by Maccaferri et al. (2015) for stripe rust and Ben-David et al. (2014) for powder mildew along with other powder mildew genes reported and chromosome maps were generated in IciMapping V4.1.0.0

and PVE 14.05%, and QTL *QPmAs.pau-6B* at chr6B with a LOD score of 3.2 and PVE 17.75%. QTL *QPmAs.pau-6B* was also mapped with algorithm RSTEP-LRT with a LOD score of 3.2 with PVE 17.75%. Other QTLs were mapped with RSTEP-LRT algorithm are *QPmAs.pau-2A.1,* and *QPmAs.pau-3B.* QTL *QPmAs.pau-2A.1,* was mapped with a LOD score of 2.0 with PVE 9.16% and QTL *QPmAs.pau-3B* with a LOD score of 2.4 and PVE 12.89%. All the QTLs mapped for resistance against PM had resistance allele contributed by *Ae. speltoides.*

Studying the disease score and the introgression profiles of the lines along the QTL sites mapped in this study, a set of 11 DS-BILs was identified carrying various combinations of these QTLs (Table 5.4.3). Six of these DS-BILs carried three to four loci for resistance against YR and PM diseases, namely DS-BIL6, DS-BIL8, DS-BIL16, DS-BIL18, DS-BIL20, and DS-BIL53 while other selected DS-BILs had two loci each. All four loci against field mixture of pathotypes of YR (*QYrAs.pau-1B, QYrAs.pau-2A.1, QYrAs.pau-2B,* and *QYrAs.pau-7B*) were identified in DS-BIL6 and DS-BIL16. Both DS-BILs showed a score of 0, i.e. complete resistance against stripe rust. However, lines with two or three loci showed moderate susceptibility. While for PM, complete resistance was observed in DS-BILs bearing either of three PM QTLs, viz. *QPmAs.pau-2A.1,* QPmAs.pau-2B, and *QPmAs.pau-3B.* The remaining two QTLs for PM, however, conferred moderate resistance in the DS-BILs only in combination with each other or other QTLs.

Table 5.4.3. Summary of the resistant DS-BILs along with QTLs present in these DS-BILs and disease score of the respective lines for different inoculms

| **Genotype** | ***Pst* *78s84*** | ***Pst* *mix.*** | ***PM* *mix.*** | **QTLs present** | | | |
|---|---|---|---|---|---|---|---|
| DS-BIL6 | 8 | 3 | 8 | *QYrAs.pau-1B* | *QYrAs.pau-2A.1* | *QYrAs.pau-2B* | *QYrAs.pau-7B* |
| DS-BIL8 | 8 | 8 | 1 | *QPmAs.pau-2A.2* | *QPmAs.pau-2B* | *QPmAs.pau-6B* | |
| DS-BIL16 | 8 | 3 | 8 | *QYrAs.pau-1B* | *QYrAs.pau-2A.1* | *QYrAs.pau-2B* | *QYrAs.pau-7B* |
| DS-BIL18 | 8 | 8 | 2 | *QPmAs.pau-2A.2* | *QPmAs.pau-2B* | *QPmAs.pau-6B* | |
| DS-BIL20 | 6 | 6 | 2 | *QPmAs.pau-2A.2* | *QPmAs.pau-2B* | *QPmAs.pau-6B* | |
| DS-BIL32 | 8 | 6 | 3 | *QPmAs.pau-2B* | *QPmAs.pau-6B* | | |
| DS-BIL40 | 8 | 6 | 4 | *QPmAs.pau-2A.2* | *QPmAs.pau-6B* | | |
| DS-BIL45 | 3 | 8 | 8 | *QYrTd.pau-3B* | *QYrTd.pau-2A.2* | *QPmAs.pau-6B* | |
| DS-BIL53 | 8 | 8 | 3 | *QPmAs.pau-2A.2* | *QPmAs.pau-2B* | | |
| DS-BIL55 | 3 | 8 | 2 | *QYrTd.pau-2A.2* | *QPmAs.pau-3B* | | |
| DS-BIL56 | 3 | 8 | 2 | *QYrTd.pau-3B* | *QYrTd.pau-2A.2* | | |

# mixture of pathotypes from open field

### 5.4.3 Postulation of Candidate Genes

To postulate and identify the candidate genes in the regions of QTLs mapped in this study, high confidence genes as per annotation v1.1 of wheat reference sequence RefSeqV1.0 were scanned. A general approach of screening 50kb regions on either side of the SNP markers with these QTLs was done. The genes found in these regions are summarized in table 5.4.4 along with the regions, gene ids and functions. Out of all these genes, the genes marked with an asterisk (*) are the ones which are, in the literature, known to be involved in different pathways of pathogen-host interactions and pathogenesis. Since in the region of QTL *QPmAs.pau-3B* no gene has been annotated yet, a broader region of 500kb was screened and reported. The functions of these genes, pathways of action in the pathogenesis or pathogen-host interactions have been searched in the literature and have been detailed in the discussion section. These genes are targets for future studies involved in breeding and transfer of the identified QTLs to elite cultivars.

Stripe rust and powdery mildew are major constraints to wheat production worldwide and in order to counter these wheat breeding programs need continuous identification and introgression of new disease resistance genes from diverse sources including wild species. The present manuscript describes transfer and mapping of new genes/QTL for stripe rust and powdery mildew resistance from *Ae. speltoides*. During this study on a set of *T. durum-Ae. spletoides* introgression lines, two major genic loci effective against stripe rust pathotype *Pst* 78S84 were identified. Five genic loci were found against field mixture of *Bg* pathotypes and four loci against mixture of *Pst* pathotypes collected from field. These QTLs were present on the terminal ends of the respective chromosomes (Figure 5.4.5) which have been reported to have recombination hot spots of chromosomes. The phenotypic data of the DS-BILs showed that a large number of loci may be responsible for resistance reaction as the variability of different reaction types show involvement of additive effects of multiple loci. Despite this, 11 loci were identified to provide resistance when mapping was done using stepwise regression-based Likelihood Ratio Test (RSTEP-LRT) for additive effect of markers and single marker analysis (SMA). Since there were few sites on different chromosomes where there was low coverage/density of SNPs which hindered identifying QTLs (if present) in these locations. While all the QTLs detected in the present study had good marker density in the genomic regions harboring target QTL. In order to explain the residual resistance which was not mapped in this study higher density of SNPs providing more coverage of genome and bigger set of the DS-BILs can be used. All the selected DS-BILs with different QTL combinations can be used

Table 5.4.4: Postulation of genes present in the survey sequence of wheat genome refseqV1.0

| QTL | Trait | Distance from SNP | Chr | Reference gene Gene ID | Function |
|---|---|---|---|---|---|
| *QYrAs.pau-1B* | Pst field pathotypes | +4.598 | chr1B | *TraesCS1B01G393400* | RING/U-box superfamily protein (Zinc finger) * |
| | | -1.251 | chr1B | *TraesCS1B01G393500* | Serine/threonine-protein phosphatase |
| | | -14.764 | chr1B | *TraesCS1B01G393600* | Disease resistance protein (NBS-LRR class) family* |
| | | -21.334 | chr1B | *TraesCS1B01G393700* | Reticulon-like protein |
| *QYrAs.pau-2A.1* | Pst field pathotypes | 0 | chr2A | *TraesCS2A01G038100* | L-gulonolactone oxidase* |
| | | -8.225 | chr2A | *TraesCS2A01G038200* | Vascular endothelial growth factor receptor 3 |
| | | -49.03 | chr2A | *TraesCS2A01G038300* | Beta-glucosidase |
| *QPmAS.pau-2A.1* | Bg field pathotypes | +13.059 | chr2A | *TraesCS2A01G090000* | 26S protease regulatory subunit* |
| | | +1.31 | chr2A | *TraesCS2A01G090100* | Leucine-rich repeat receptor-like protein kinase* |
| | | -33.072 | chr2A | *TraesCS2A01G090200* | Zinc-finger protein* |
| *QYrTd.pau-2A.2* | Pst 78S84 | +6.454 | chr2A | *TraesCS2A01G567300* | Auxin response factor* |
| | | 0 | chr2A | *TraesCS2A01G567400* | CAA30371.1 protein |
| *QPmAS.pau-2A.2* | Bg field pathotypes | +21.662 | chr2A | *TraesCS2A01G577300* | Protein kinase family protein, putative, expressed* |
| | | +2.653 | chr2A | *TraesCS2A01G577400* | Kinase family protein* |
| | | 0 | chr2A | *TraesCS2A01G577500* | Pentatricopeptide repeat-containing family protein |
| | | -4.614 | chr2A | *TraesCS2A01G577600* | 3-isopropylmalate dehydrogenase |
| | | -7.998 | chr2A | *TraesCS2A01G577700* | UPF0235 protein |
| | | -30.782 | chr2A | *TraesCS2A01G577800* | Cytochrome P450 family protein, expressed |
| | | -39.245 | chr2A | *TraesCS2A01G577900* | Glutathione S-transferase* |
| *QYrAs.pau-2B* | Pst field pathotypes | +8.029 | chr2B | *TraesCS2B01G057200* | ELMO domain-containing protein, putative |
| | | 0 | chr2B | *TraesCS2B01G057300* | Zinc finger family protein* |
| *QPmAS.pau-2B* | Bg field pathotypes | +1.911 | chr2B | *TraesCS2B01G612700* | Dihydroflavonol-4-reductase |

| QTL | Trait | Distance from SNP | Chr | Reference gene Gene ID | Function |
|---|---|---|---|---|---|
| *QYrTd.pau-3B* | Pst 78S84 | +5.51 | chr3B | *TraesCS3B01G499200* | Glutathione S-transferase* |
| *QPmAs.pau-3B* | Bg field pathotypes | -145.056 | chr3B | *TraesCS3B01G535100* | GRAS family transcription factor containing protein |
| | | +83.639 | chr3B | *TraesCS3B01G535200* | F-box protein |
| | | +352.758 | chr3B | *TraesCS3B01G535300* | Glutathione S-transferase* |
| | | +383.528 | chr3B | *TraesCS3B01G535400* | Peroxiredoxin |
| | | +388.745 | chr3B | *TraesCS3B01G535500* | Protein ENHANCED DISEASE RESISTANCE 2* |
| | | +444.521 | chr3B | *TraesCS3B01G535600* | F-box SKIP23-like protein |
| | | +469.715 | chr3B | *TraesCS3B01G535700* | Glutathione S-transferase* |
| *QPmAS.pau-6B* | Bg field pathotypes | +24.086 | chr6B | *TraesCS6B01G044800* | receptor kinase 1* |
| | | 0 | chr6B | *TraesCS6B01G044900* | Mitochondrial transcription termination factor-like* |
| | | -47.402 | chr6B | *TraesCS6B01G045000* | Mitochondrial transcription termination factor-like* |
| *QYrAs.pau-7B* | Pst field pathotypes | +29.704 | chr7B | *TraesCS7B01G443600* | RING/U-box superfamily protein (Zinc finger) * |
| | | -30.142 | chr7B | *TraesCS7B01G443700* | Coatomer subunit beta'-1 |

* represent genes/ functions which have been well reported to be a part of plant-pathogen interaction or plant-pathogenesis

In column distance from SNP represent the distance of start site of the gene to SNP linked with QTL, where + sign represents the gene was found downstream of the SNP and – sign gene was found upstream, 0 represent the SNP was present inside the gene, and all distances are in kilobase in breeding programs for marker assisted transfer of the respective QTLs to the hexaploid backgrounds providing a good source of resistance.

Various designated genes, temporary designated genes and QTLs for YR and PM reported from various studies have been compiled in figure 5.4.6 (source: www.wheat.pw.usda.gov). QTL *QYrAs.pau-1B* reported in this manuscript has been found in vicinity of designated genes *Yr29/Lr46* and QTL *QYr.cim-1BL* (Lan et al. 2014). Similarly, QTL *QYrAs.pau-2A.1* was found in vicinity of *Yr56* where some QTLs for YR have also been reported like *QYr.sun-2A_Wollaroi*, *QYr.tam-2AS_TAM111*, *QYr.ucw-2A.2(IWA422)*, *QYr.ufs-2A_Cappelle-Desprez_Yr16*, *QYr.inra_2AS.1_Recital* (Maccaferri et al. 2015). QTL *QYrAs.pau-7B* has been physically located in the vicinity of stripe rust resistance genes *Yr52* and *Yr59* (McIntosh et al. 2017). For PM, QTL *QPmAS.pau-6B* is located in the region harbouring QTL *QPm#66-2B* (Ben-David et al. 2014). Other QTLs reported in this study could also be traced to same arm or close vicinity of genes/QTLs reported in literature clearly depicted by figure 5.4.6. It was not possible to compare exact locations of the other QTL mapped in this study with reported genes or QTL as most of the reported genes or QTL have been mapped based on linkage. While in the present study, genes/QTL have been physically mapped to specific chromosome regions.

Studying the annotated reference of wheat genome refseqV1.0 showed the genes present in the regions of QTLs mapped. Various categories of these genes are NBS-LRR protein family responsible for disease resistance, receptor or receptor-like proteins/motifs like Zinc finger, ubiquitin pathway proteins like ubiquitin regulator units and glutathione S transferase, antioxidant pathway enzymes like L-gulonolactone oxidase, various kinases, auxin response factors and mitochondrial transcription termination factor-like proteins (Table 5.4.4). All these are known for their action in pathogen recognition, reactions, being involved in various biotic and abiotic stresses or plant-pathogenesis pathways. of other genes/proteins in plant pathogenesis is either yet not reported or not well documented.

*QYrAs.pau-1B* and *QPmAS.pau-2A.1* loci harboured genes with NBS-LRR and zinc finger motifs. NBS-LRR genes are the most abundant disease resistance gene family in plant genomes and zinc finger motifs have been reported to be major motif linked with response of plants to various biotic and abiotic stresses (McHale et al. 2006; DeYoung and Innes 2006; Lee and Yeom 2015; Dubey and Singh 2018). Zinc finger motif was also found in region of QTL *QYrAs.pau-2B*, and *QYrAs.pau-7B.* PM QTL *QPmAs.pau-3B* was found to be located in the genomic region having the gene coding for protein enhanced disease resistance 2 which induces resistance by negative regulation of salicylic acid in biotrophic pathogens like PM (Tang et al. 2005). Zhang et al. (2019) suggested that in wheat pathogen resistance genes can be activated by alternate splicing regulators in salicylic pathways, down-regulating its synthesis. L-gulonolactone oxidase (in region of QTLs *QYrAs.pau-2A.1*) is a key enzyme in formation of ascorbate. Thus the regulation of this enzyme is important in regulation of ascorbate formation in plants (Gullner and Kômíves 2007; Kuźniak 2010b). Ascorbate is one of the major antioxidant of plants (Potters et al. 2010; Paciolla et al. 2016) and second being glutathione against reactive oxygen species (ROS) produced under biotic stresses (Kuźniak 2010a). Being part of ascorbate-glutathione cycle it takes part in signal transduction in biotic stress besides regulating expression of nuclear genes as a response to invading pathogen providing both local and systematic defense (Sarowar et al. 2005; Kuźniak 2010a).

QTL *QPmAS.pau-2A.1* region also annotated gene for regulatory subunit of 26S proteasome subunit. Proteasome which is a part of ubiquitin-proteasome system (UPS) functions by removal of misfolded and defective proteins along with eliminating short lived proteins (Vierstra 2009). Along with this various pathways are controlled by UPS which include response to biotic and abiotic stresses (Sadanandom et al. 2012) and acts as one of major system in plant immunity (Üstün et al. 2016). Besides immunity their role in defense responses by production of ROS and forming hypersensitive reaction was reported (Marino et al. 2012). Üstün et al. (2016) showed that proteasome mutants have impaired/reduced systematic acquired resistance (SAR) on secondary infection and concluded that for pathogen-associated molecular pattern (PAMP) triggered immunity (PTI) and SAR, proteasome is essential for action. In a study with Arabidopsis with loss of function mutants Yao et al. (2012) reported that 26S regulatory subunit of proteasome, RPN1a, is essential for resistance and induced cell death when Arabidopsis was infected by powdery mildew, concluding its effect on basal defense and resistance protein mediated defense. Dielen et al. (2010) in a review on UPS (26S) highlighted involvement of the system in defense mechanism regardless of pathogen type.

Auxin response factor (ARF) (in the region of QTL *QYrTd.pau-2A.2*) in various studies have been explained as mediator of auxin to biotic and abiotic stresses (Ghanashyam and Jain 2009; Fu and Wang 2011; Bouzroud et al. 2018). Bouzroud et al. (2018) reported that ARFs have vital role in alteration (activation or repression) of rate of transcription of auxin responsive genes. Both biotic and abiotic stress responsive genes are enriched in *cis*-elements of 5' regulatory units in ARFs. They showed that under stress conditions ARFs are actively regulated at post transcriptional level. Besides this Fu and Wang (2011) reported that pathogen produced indole acetic acid (IAA) by action of ARFs can cause either resistance to necrotrophic pathogen (through ethylene signaling and/or camalexin biosynthesis), susceptibility by cell wall expansion or stomatal opening (through host IAA biosynthesis and/or IAA conjugation), basal resistance by IAA conjugation (differential regulation) or resistance to biotrophic pathogen (through indole glucosinolate biosynthesis and/or salicylic acid signaling).

High inductibility of glutathione S transferase (GST) in biotic stress in response to bacterial, fungal or viral infection by up-regulation of key defense enzymes has been reported under various studies (Gullner and Kômíves 2007; Taylor et al. 2012; Gullner et al. 2018). In our study QTLs *QPmAS.pau-2A.2*, *QYrTd.pau-3B*, and *QPmAs.pau-3B* were found to be linked to GST gene/s. These authors have reported that besides its role in detoxification of various toxic substances and as antioxidative in reaction in infected cells, it also regulates expression of various protective genes. Change in expression of GSTs are reported to be modifying symptoms of a disease and sometimes rate of multiplication of pathogens. Some GSTs with peroxidase activity are also known to detoxify lipid hydroperoxidases.

QTL *QPmAs.pau-6B* region which was found to carrythree genes, one of receptor kinase 1 and two of Mitochondrial transcription termination factor-like (mTERF) gene. Receptor kinases are known to be modulating plant defense responses. Receptor like kinases (RLKs) and receptor like proteins (RLPs) act as pattern recognition receptors pattern recognition receptors (PRRs) (Tang et al. 2017) and thus lead to first defense response. Multi-protein immune complexes of PRRs and other RLKs are formed at the surface of interaction. The two broad classes of receptors are, one in cytoplasm with NB-LRR and other on cell surface with RLKs and RLPs (Jones and Dangl 2006; Jones et al. 2016). In wheat, *TaRLK-R1,2,3* (Zhou et al. 2007) and *LRK10* (Feuillet et al. 1997) have been involved in plant immunity where *TaRLK-R1* have also been cloned (Qin et al. 2012). Wang and Bouwmeester (2017) suggested that PRRs not only recognize invading organism’s surface effectors but also damage-associated molecular patterns. PTI act as primary defense and ETI as secondary immunity by recognition of by-products of effector specific resistance genes (Shi et al. 2016). Thus, they both result in biotrophic pathogen’s growth reduction. While mTERFs are best known to act against abiotic stresses and since only eight plant mTERFs are known to be characterized, very little is known of their action against biotic stresses (Babiychuk et al. 2011; Ghulam et al. 2015; Chen et al. 2017; Pan et al. 2019). But mTERFs are known to show changed nuclear gene expression which could support their role in various stresses.

The inference from this outcome requires studying the functions, activation, deactivation or alteration in rate of expression of these loci in the process of development of resistance to specific diseases. However, regions having *Ae. speltoides* specific introgression, may carry novel genes. Either the case, there is need to study the regions countering resistance at transcriptional level to evaluate the true cause of resistance in DS-BILs which would further lead to identify unique pathways of development of disease resistance genotypes.

While several lines did contain large segment substitutions from *Ae. speltoides*, it is difficult to detect the QTLs that are close to each other with opposite effects. Hence transferring them to other backgrounds can identify some additional genes or QTL. To conclude despite the selected 11 DS-BILs being good source of resistance to YR and PM the functional study of the regions could only elaborate the effect of these loci/QTLs in providing disease resistance.

# CHAPTER-6 SUMMARY

Heat stress is a significant constraint in a winter crop like wheat, especially when temperature rises during the reproductive stage, affecting the pollen viability, fertilization capacity, grain filling processes and finally the total yield of the crop (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 the hexaploid background as there is a limited variation for heat stress tolerance in bread wheat cultivars. *Ae. speltoides* has been used to transfer HT QTLs to durum wheat (Awlachew et al. 2016; Awlachew and Chhuneja 2013). The 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.

- Crossing between tetraploid donor genotypes and hexaploid recipient parents resulted in pentaploid $F_1$s 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 (Millet 2007; Colas et al. 2008) which can also result in a large number of multivalent formations, which can cause poor germination and plant establishment.
- Marker assisted selection was used in the backcross generations to select for desirable plants carrying QTL for our traits of interest. Phenotypic selections were also conducted along with MAS which aided in selecting for recurrent type plant phenotype. All the introgressed QTL were present in homozygous form in the selected $BC_2F_5$ HTILs.
- Evaluation of these HTILs over two years across normal and heat stress environments showed that the introgressed of heat tolerance QTL imparteds heat stress tolerance to these lines.
- Different traits showed a 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. Progenies with reduction in GFD showed reduced TGW and YD.
- HTIL pauHTIL_30 showed an increase in TGW in HSE than OE despite a reduction in GFD and YD which may be attributed to increase in the rate of translocation of the photosynthates

and stem reserves to the seed when heat stress influenced the reproductive cycle (Dias and Lidon 2009)(Dias and Lidon 2009)(Dias and Lidon 2009)(Dias and Lidon 2009)(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 a 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 the complex genome of bread wheat where alien introgressions are involved.
- 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, 33, and 34 have shown higher yield than tested cultivars under OE and pauHTIL_14 under HSE.
- 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.

Despite of introduction of QTLs for heat tolerance, disease resistance is a key trait without which the introgression lines could not actualize their yield potential. With the changing environment and introduction of new pathogenic races the major disease resistance genes become ineffective within short duration of time. Thus, there is a need to introduce new genes/QTLs for disease resistance from various sources and pyramiding of various genes/QTLs help to achieve effective combinations for longer duration of time.

- During this study on a set of *T. durum-Ae. spletoides* introgression lines, two major genic loci effective against stripe rust pathotype *Pst* 78S84 were identified.
- Five genic loci were found against field mixture of *Bg* pathotypes and four loci against mixture of *Pst* pathotypes collected from field.
- These QTLs were present on the terminal ends of the respective chromosomes which have been reported to have recombination hot spots of chromosomes.

- The phenotypic data of the DS-BILs and the variability of different reaction types showed involvement of additive effects of multiple loci. Despite this, 11 loci were identified to provide resistance when mapping was done using stepwise regression-based Likelihood Ratio Test (RSTEP-LRT) for additive effect of markers and single marker analysis (SMA).
- All the selected DS-BILs with different QTL combinations can be used in breeding programs for marker assisted transfer of the respective QTLs to the hexaploid backgrounds providing a good source of resistance.
- Various designated genes, temporary designated genes and QTLs for YR and PM reported from various studies have been compiled (source: www.wheat.pw.usda.gov). QTL *QYrAs.pau-1B* reported in this study has been found in vicinity of designated genes *Yr29/Lr46* and QTL *QYr.cim-1BL* (Lan et al. 2014).
- QTL *QYrAs.pau-2A.1* was found in vicinity of *Yr56* where some QTLs for YR have also been reported like *QYr.sun-2A_Wollaroi*, *QYr.tam-2AS_TAM111*, *QYr.ucw-2A.2(IWA422)*, *QYr.ufs-2A_Cappelle-Desprez_Yr16*, *QYr.inra_2AS.1_Recital* (Maccaferri et al. 2015).
- QTL *QYrAs.pau-7B* has been physically located in the vicinity of stripe rust resistance genes *Yr52* and *Yr59*.
- For PM, QTL *QPmAS.pau-6B* is located in the region harbouring QTL *QPm#66-2*.
- Other QTLs reported in this study could also be traced to same arm or close vicinity of genes/QTLs reported in literature.
- Studying the annotated reference of wheat genome refseqV1.0 showed the genes present in the regions of QTLs mapped.
- Various categories of these genes are NBS-LRR protein family responsible for disease resistance, receptor or receptor-like proteins/motifs like Zinc finger, ubiquitin pathway proteins like ubiquitin regulator units and glutathione S transferase, antioxidant pathway enzymes like L-gulonolactone oxidase, various kinases, auxin response factors and mitochondrial transcription termination factor-like proteins.
- All these genes are known for their action in pathogen recognition, reactions, being involved in various biotic and abiotic stresses or plant-pathogenesis pathways.
- Selected 11 DS-BILs being good source of resistance to YR and PM, the functional study of the regions could elaborate the effect of these loci/QTLs in providing disease resistance.

## 7. References

Acevedo E, Nashit M, Ferrara GO (1990) Effects of heat stress on wheat and possible selection tools for use in breeding for tolerance. In: International Conference on Wheat for 1990

Adonina IG, Salina EA, Efremova TT, Pshenichnikova TA (2004) The study of introgressive lines of Triticum aestivum x Aegilops speltoides by in situ and SSR analyses. Plant Breed 123:220–224. https://doi.org/10.1111/j.1439-0523.2004.00932.x

Akter N, Rafiqul Islam M (2017) Heat stress effects and management in wheat. A review. Agron Sustain Dev 37:37(1–17). https://doi.org/10.1007/s13593-017-0443-9

Al-Ashkar I, Alotaibi M, Refay Y, Ghazy A, Zakri A, Al-Doss A (2020) Selection criteria for high-yielding and early-flowering bread wheat hybrids under heat stress. PLoS One 15:e0236351 (1-15)

Al-Whaibi MH (2011) Plant heat-shock proteins: A mini review. J King Saud Univ - Sci 23:139–150. https://doi.org/10.1016/j.jksus.2010.06.022

Alvarado G, Lopez M, Vargas M, Pacheco A, Rodríguez F, Burgueño J, Crossa J (2016) META-R (Multi Environment Trial Analysis whit R for Windows). 1–34

Andolfatto P, Davison D, Erezyilmaz D, Hu TT, Mast J, Sunayama-Morita T, Stern DL (2011) Multiplexed shotgun genotyping for rapid and efficient genetic mapping. Genome Res 21:610–617. https://doi.org/10.1101/gr.115402.110

Arabbeigi M, Arzani A, Saeidi G (2010) Meiotic Behavior of Wild, Synthetic and Cultivated Wheats. Cytologia (Tokyo) 75:169–175. https://doi.org/10.1508/cytologia.75.169

Aravind J, Sankar MS, Wankhede DP, Kaur V (2021) augmentedRCBD: Analysis of Augmented Randomised Complete Block Designs. R package. 1–42

Asseng S, Foster I, Turner NC (2011) The impact of temperature variability on wheat yields. Glob Chang Biol 17:997–1012. https://doi.org/10.1111/j.1365-2486.2010.02262.x

Awlachew ZT, Chhuneja P (2013) Molecular Characterization of Triticum durum – Aegilops speltoides Introgression lines and Evaluation for thermo–tolerance and its components. Punjab Agricultural University, Ludhiana-141004

Awlachew ZT, Singh R, Kaur S, Bains NS, Chhuneja P (2016) Transfer and mapping of the heat tolerance component traits of Aegilops speltoides in tetraploid wheat Triticum durum. Mol Breed 36:78–92. https://doi.org/10.1007/s11032-016-0499-2

Ayeneh A, Van Ginkel M, Reynolds MP, Ammar K (2002) Comparison of leaf, spike, peduncle and canopy temperature depression in wheat under heat stress. F Crop Res 79:173–184. https://doi.org/10.1016/S0378-4290(02)00138-7

Baalbaki R, Hajj-Hassan N, Zurayk R (2006) Aegilops species from semiarid areas of Lebanon: Variation in quantitative attributes under water stress. Crop Sci 46:799–806. https://doi.org/10.2135/cropsci2005.0120

Babiychuk E, Vandepoele K, Wissing J, Garcia-Diaz M, De Rycke R, Akbari H, Joubès J, Beeckman T, Jänsch L, Frentzen M, Van Montagu MCE, Kushnir S (2011) Plastid gene expression and plant development require a plastidic protein of the mitochondrial transcription termination factor family. Proc Natl Acad Sci U S A 108:6674–6679. https://doi.org/10.1073/pnas.1103442108

Baird NA, Etter PD, Atwood TS, Currey MC, Shiver AL, Lewis ZA, Selker EU, Cresko WA, Johnson EA (2008) Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers. PLoS One 3:e3376(1-7)

Bansal M, Kaur S, Dhaliwal HS, Bains NS, Bariana HS, Chhuneja P, Bansal UK (2017) Mapping of Aegilops umbellulata-derived leaf rust and stripe rust resistance loci in wheat. Plant Pathol 66:38–44. https://doi.org/10.1111/ppa.12549

Bariana H, Forrest K, Qureshi N, Miah H, Hayden M, Bansal U (2016) Adult plant stripe rust resistance gene Yr71 maps close to Lr24 in chromosome 3D of common wheat. Mol Breed 36:98. https://doi.org/10.1007/s11032-016-0528-1

Ben-David R, Peleg Z, Dinoor A, Saranga Y, Korol AB, Fahima T (2014) Genetic dissection of quantitative powdery mildew resistance loci in tetraploid wheat. Mol Breed 34:1647–1658. https://doi.org/10.1007/s11032-014-0178-0

Bouzroud S, Gouiaa S, Hu N, Bernadac A, Mila I, Bendaou N, Smouni AA, Bouzayen M, Zouine M (2018) Auxin response factors (ARFs) are potential mediators of auxin action in tomato response to biotic and abiotic stress (Solanum lycopersicum). PLoS One 13:e0193517:1-20. https://doi.org/10.1371/journal.pone.0193517

Braun HJ, Atlin G, Payne T (2010) Multi-location testing as a tool to identify plant response to global climate change. In: Reynolds MP (ed) Climate Change and Crop Production. International Maize and Wheat Improvement Center (CIMMYT), Apdo, Mexico, pp 115–138

Buerstmayr M, Steiner B, Buerstmayr H (2020) Breeding for Fusarium head blight resistance in wheat—Progress and challenges. Plant Breed 139:429–454. https://doi.org/10.1111/pbr.12797

Butler JM (2002) Quantitative trait locus evaluation for agronomic and morphological traits in a spring wheat population. Colorado State University, Fort Collins

Camejo D, Rodríguez P, Morales MA, Dell’Amico JM, Torrecillas A, Alarcón JJ (2005) High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. J Plant Physiol 162:281–289. https://doi.org/10.1016/j.jplph.2004.07.014

Challinor AJ, Koehler AK, Ramirez-Villegas J, Whitfield S, Das B (2016) Current warming will reduce yields unless maize breeding and seed systems adapt immediately. Nat Clim Chang 6:954–958. https://doi.org/10.1038/nclimate3061

Chamarthi SK, Kumar A, Vuong TD, Blair MW, Gaur PM, Nguyen HT, Varshney RK (2011) Trait mapping and molecular Breeding in legumes: concepts and examples in soybean, common bean and chickpea. In: Pratap A (ed) Biology and breeding of food legumes. CABI International, Oxfordshire, UK, pp 296–313

Chen X, Feng F, Qi W, Xu L, Yao D, Wang Q, Song R (2017) Dek35 Encodes a PPR Protein that Affects cis-Splicing of Mitochondrial nad4 Intron 1 and Seed Development in

Maize. Mol Plant 10:427–441. https://doi.org/10.1016/j.molp.2016.08.008

Chen XM (2005) Epidemiology and control of stripe rust [Puccinia striiformis f. sp. tritici] on wheat. Can J Plant Pathol 27:314–337. https://doi.org/10.1080/07060660509507230

Colas I, Shaw P, Prieto P, Wanous M, Spielmeyer W, Mago R, Moore G (2008) Effective chromosome pairing requires chromatin remodeling at the onset of meiosis. Proc Natl Acad Sci U S A 105:6075–6080. https://doi.org/10.1073/pnas.0801521105

Colmer TD, Flowers TJ, Munns R (2006) Use of wild relatives to improve salt tolerance in wheat. J Exp Bot 57:1059–1078. https://doi.org/10.1093/jxb/erj124

Cossani CM, Reynolds MP (2012) Physiological traits for improving heat tolerance in wheat. Plant Physiol 160:1710–1718. https://doi.org/10.1104/pp.112.207753

Cox TS, Sears RG, Bequette RK, Martin TJ (1995) Germplasm Enhancement in Winter Wheat × Triticum tauschii Backcross Populations. Crop Sci 35:913–919. https://doi.org/10.2135/cropsci1995.0011183X003500030047x

Cramer GR, Urano K, Delrot S, Pezzotti M, Shinozaki K (2011) Effects of abiotic stress on plants: A systems biology perspective. BMC Plant Biol 11:163(1–14). https://doi.org/10.1186/1471-2229-11-163

Dash S, Mohanty N (2001) Evaluation of assays for the analysis of thermo-tolerance and recovery potentials of seedlings of wheat (Triticum aestivum L.) cultivars. J Plant Physiol 158:1153–1165. https://doi.org/10.1078/0176-1617-00243

Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM, Blaxter ML (2011) Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nat Rev Genet 12:499–510. https://doi.org/10.1038/nrg3012

Deb A, Khaleque MA (2009) Nature of gene action of some quantitative traits in chickpea. World J Agric Sci 5:361–368

DeYoung BJ, Innes RW (2006) Plant NBS-LRR proteins in pathogen sensing and host defense. Nat Immunol 7:1243–1249. https://doi.org/10.1038/ni1410

Dias AS, Lidon FC (2009) Evaluation of Grain Filling Rate and Duration in Bread and Durum Wheat, under Heat Stress after Anthesis. J Agron Crop Sci 195:137–147. https://doi.org/10.1111/j.1439-037X.2008.00347.x

Dielen A-S, Badaoui S, Candresse T, German-Retana S (2010) The ubiquitin/26S proteasome system in plant-pathogen interactions: a never-ending hide-and-seek game. Mol Plant Pathol 11:293–308. https://doi.org/10.1111/j.1364-3703.2009.00596.x

Dixon J, Braun HJ, Crouch JH (2009) Overview: transitioning wheat research to serve the future needs of the developing world. In: Dixon J, Braun HJ, Kosina P, Crouch JH (eds) Wheat Facts and Futures 2009. CIMMYT, Apdo, Mexico, pp 1–25

Dubey N, Singh K (2018) Role of NBS-LRR Proteins in Plant Defense. In: Singh A, Singh IK (eds) Molecular Aspects of Plant-Pathogen Interaction. Springer, Singapore, pp 115–138

Dubey R, Pathak H, Chakrabarti B, Singh S, Gupta DK, Harit RC (2020) Impact of terminal heat stress on wheat yield in India and options for adaptation. Agric Syst 181:102826 (1–10). https://doi.org/10.1016/j.agsy.2020.102826

Easterling WE, Aggrawal PK, Batima P, Brander K, Erda L, Howden SM, Kirilenko A, Morton J, Soussana J-F, Schmidhuber J, Tubiello FN (2007) Food, fibre and forest products. In: Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. pp 273–313

Elkot AFA, Chhuneja P, Kaur S, Saluja M, Keller B, Singh K (2015) Marker assisted transfer of two powdery mildew resistance genes PmTb7A.1 and PmTb7A.2 from Triticum boeoticum (Boiss.) to Triticum aestivum (L.). PLoS One 10:e0128297(1-16). https://doi.org/10.1371/journal.pone.0128297

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE (2011) A Robust, Simple Genotyping-by-Sequencing (GBS) Approach for High Diversity Species. PLoS One 6:e19379(1-10)

Farooq M, Bramley H, Palta JA, Siddique KHM (2011) Heat stress in wheat during reproductive and grain-filling phases. CRC Crit Rev Plant Sci 30:491–507. https://doi.org/10.1080/07352689.2011.615687

Feng B, Liu P, Li G, Dong ST, Wang FH, Kong LA, Zhang JW (2014) Effect of Heat Stress on the Photosynthetic Characteristics in Flag Leaves at the Grain-Filling Stage of Different Heat-Resistant Winter Wheat Varieties. J Agron Crop Sci 200:143–155. https://doi.org/10.1111/jac.12045

Feuillet C, Schachermayr G, Keller B (1997) Molecular cloning of a new receptor-like kinase gene encoded at the Lr10 disease resistance locus of wheat. Plant J 11:45–52. https://doi.org/10.1046/j.1365-313X.1997.11010045.x

Figueroa M, Hammond-kosack KIME, Peter S (2018) A review of wheat diseases – a field perspective. Mol Plant Pathol 19:1523–1536. https://doi.org/doi: 10.1111/mpp.12618

Flohr BM, Hunt JR, Kirkegaard JA, Evans JR (2017) Water and temperature stress define the optimal flowering period for wheat in south-eastern Australia. F Crop Res 209:108–119. https://doi.org/10.1016/j.fcr.2017.04.012

Fotovat R, Valizadeh M, Toorchi M (2007) Association between water-use efficiency components and total chlorophyll content (SPAD) in wheat (Triticum aestivum L.) under well-watered and drought stress conditions. J Food, Agric Environ 5:225–227

Fu J, Wang S (2011) Insights into auxin signaling in plant-pathogen interactions. Front Plant Sci 2:74:1–7. https://doi.org/10.3389/fpls.2011.00074

Fu YB, Cheng B, Peterson GW (2014) Genetic diversity analysis of yellow mustard (Sinapis alba L.) germplasm based on genotyping by sequencing. Genet Resour Crop Evol 61:579–594. https://doi.org/10.1007/s10722-013-0058-1

Garg B, Lata C, Prasad M (2012) A study of the role of gene TaMYB2 and an associated SNP in dehydration tolerance in common wheat. Mol Biol Rep 39:10865–10871. https://doi.org/10.1007/s11033-012-1983-3

Gaur DK, Kaushal SK, Rani U, Singh S (2017) Agricultural Situation in India. Directorate of

Economics and Statastics, Department of Agriculture, Coorporation and Farmers Wellfare, Ministary of Agriculture, Government of India

Gautam A, Sai Prasad S V., Jajoo A, Ambati D (2015) Canopy Temperature as a Selection Parameter for Grain Yield and Its Components in Durum Wheat Under Terminal Heat Stress in Late Sown Conditions. Agric Res 4:238–244. https://doi.org/10.1007/s40003-015-0174-6

Gautam T, Dhillon GS, Saripalli G, Rakhi, Singh VP, Prasad P, Kaur S, Chhuneja P, Sharma PK, Balyan HS, Gupta PK (2020) Marker-assisted pyramiding of genes/QTL for grain quality and rust resistance in wheat (Triticum aestivum L.). Mol Breed 40:49–62. https://doi.org/10.1007/s11032-020-01125-9

Ghanashyam C, Jain M (2009) Role of auxin-responsive genes in biotic stress responses. Plant Signal Behav 4:846–8. https://doi.org/10.4161/psb.4.9.9376

Ghulam MM, Kousar S, Vardhan H (2015) Plant Mitochondrial Omics: State-of-the-Art Knowledge. In: Barh D, Khan MS, Davies E (eds) PlantOmics: The Omics of Plant Science. Springer India, New Delhi, pp 573–613

Glaubitz JC, Casstevens TM, Lu F, Harriman J, Elshire RJ, Sun Q, Buckler ES (2014) TASSEL-GBS: A high capacity genotyping by sequencing analysis pipeline. PLoS One 9:1–12. https://doi.org/10.1371/journal.pone.0090346

Guerra D, Crosatti C, Khoshro HH, Mastrangelo AM, Mica E, Mazzucotelli E (2015) Post-transcriptional and post-translational regulations of drought and heat response in plants: a spiderâ€™s web of mechanisms. Front Plant Sci 6:57 (1–14). https://doi.org/10.3389/fpls.2015.00057

Gullner G, Kômíves T (2007) Defense Reactions of Infected Plants: Roles of Glutathione and Glutathione S-Transferase Enzymes. Acta Phytopathol Entomol Hungarica 41:3–10. https://doi.org/10.1556/aphyt.41.2006.1-2.1

Gullner G, Komives T, Király L, Schröder P (2018) Glutathione S-transferase enzymes in plant-pathogen interactions. Front Plant Sci 9:1836:1–19. https://doi.org/10.3389/fpls.2018.01836

Gupta PK, Balyan HS, Sharma S, Kumar R (2020) Genetics of yield, abiotic stress tolerance and biofortification in wheat (Triticum aestivum L.). Theor Appl Genet 133:1569–1602. https://doi.org/10.1007/s00122-020-03583-3

Gupta S, Kaur S, Sehgal S, Sharma A, Chhuneja P, Bains NS (2010a) Genotypic variation for cellular thermotolerance in Aegilops tauschii Coss., the D genome progenitor of wheat. Euphytica 175:373–381. https://doi.org/10.1007/s10681-010-0185-0

Gupta SC, Sharma A, Mishra M, Mishra RK, Chowdhuri DK (2010b) Heat shock proteins in toxicology: How close and how far? Life Sci 86:377–384. https://doi.org/10.1016/j.lfs.2009.12.015

Gururani MA, Venkatesh J, Upadhyaya CP, Nookaraju A, Pandey SK, Park SW (2012) Plant disease resistance genes: Current status and future directions. Physiol Mol Plant Pathol 78:51–65. https://doi.org/10.1016/j.pmpp.2012.01.002

Hao M, Zhang L, Ning S, Huang L, Yuan Z, Wu B, Yan Z, Dai S, Jiang B, Zheng Y, Liu D

(2020) The Resurgence of Introgression Breeding, as Exemplified in Wheat Improvement. Front. Plant Sci. 11:252 (1–11)

Hemantaranjan A, Bhanu NA, Singh MN, Yadav DK, Patel PK, Singh R, Katiyar D (2014) Heat Stress Responses and Thermotolerance. Adv Plants Agric Res 1:1–10. https://doi.org/10.15406/apar.2014.01.00012

Hu W, Huang C, Deng X, Zhou S, Chen L, Li Y, Wang C, Ma Z, Yuan Q, Wang Y, Cai R, Liang X, Yang G, He G (2013) TaASR1, a transcription factor gene in wheat, confers drought stress tolerance in transgenic tobacco. Plant, Cell Environ 36:1449–1464. https://doi.org/10.1111/pce.12074

Huang XQ, Cloutier S, Lycar L, Radovanovic N, Humphreys DG, Noll JS, Somers DJ, Brown PD (2006) Molecular detection of QTLs for agronomic and quality traits in a doubled haploid population derived from two Canadian wheats (Triticum aestivum L.). Theor Appl Genet 113:753–766. https://doi.org/10.1007/s00122-006-0346-7

Hussain S, Qamar R (2007) Wheat genomics: challenges and alternative strategies. Proc Pakistan Acad Sci 44:305–327

IPCC (2007) Climate Change 2007: The Physical Science Basis. Cambridge University Press, Cambridge, United Kingdom and New York

Iqbal M, Raja NI, Yasmeen F, Hussain M, Ejaz M, Shah MA (2017) Impacts of Heat Stress on Wheat: A Critical Review. Adv Crop Sci Technol 5:1–9. https://doi.org/10.4172/2329-8863.1000251

Jha UC, Bohra A, Singh NP (2014) Heat stress in crop plants: Its nature, impacts and integrated breeding strategies to improve heat tolerance. Plant Breed 133:679–701. https://doi.org/10.1111/pbr.12217

Jia J, Zhao S, Kong X, Li Y, Zhao G, He W, Appels R, Pfeifer M, Tao Y, Zhang X, Jing R, Zhang C, Ma Y, Gao L, Gao C, Spannagl M, Mayer KFX, Li D, Pan S, et al. (2013) Aegilops tauschii draft genome sequence reveals a gene repertoire for wheat adaptation. Nature 496:91–95. https://doi.org/10.1038/nature12028

Jiang Q, Hou J, Hao C, Wang L, Ge H, Dong Y, Zhang X (2011) The wheat (T. aestivum) sucrose synthase 2 gene (TaSus2) active in endosperm development is associated with yield traits. Funct Integr Genomics 11:49–61. https://doi.org/10.1007/s10142-010-0188-x

Jin J, Liu D, Qi Y, Ma J, Zhen W (2020) Major QTL for Seven Yield-Related Traits in Common Wheat (Triticum aestivum L.). Front Genet 11:01012 (1–23). https://doi.org/10.3389/fgene.2020.01012

Jones JDG, Dangl JL (2006) The plant immune system. Nature 444:323–329. https://doi.org/10.1038/nature05286

Jones JDG, Vance RE, Dangl JL (2016) Intracellular innate immune surveillance devices in plants and animals. Science (80- ) 354:6316:1–8. https://doi.org/10.1126/science.aaf6395

Joshi AK, Ortiz-Ferrara G, Crossa J, Singh G, Sharma RC, Chand R, Parsad R (2007) Combining superior agronomic performance and terminal heat tolerance with resistance to spot blotch (Bipolaris sorokiniana) of wheat in the warm humid Gangetic Plains of South Asia. F Crop Res 103:53–61. https://doi.org/10.1016/j.fcr.2007.04.010

Kankwatsa P, Singh D, Thomson PC, Babiker EM, Bonman JM, Newcomb M, Park RF (2017) Characterization and genome-wide association mapping of resistance to leaf rust, stem rust and stripe rust in a geographically diverse collection of spring wheat landraces. Mol Breed 37:113–123. https://doi.org/10.1007/s11032-017-0707-8

Kaur S, Jindal S, Kaur M, Chhuneja P (2018a) Utilization of Wild Species for Wheat Improvement Using Genomic Approaches. In: Gosal S, Wani S (eds) Biotechnologies of Crop Improvement, Volume 3, Volume 3. Springer, CHAM, pp 105–150

Kaur S, Jindal S, Kaur M, Chhuneja P (2018b) Utilization of Wild Species for Wheat Improvement Using Genomic Approaches. In: Gosal SS, Wani SH (eds) Biotechnologies of Crop Improvement, Volume 3: Genomic Approaches. Springer International Publishing, Cham, pp 105–150

Khush GS (1999) Green revolution: Preparing for the 21st century. Genome 42:646–655. https://doi.org/10.1139/g99-044

Kilian B, Özkan H, Pozzi C, Salamini F (2009) Domestication of the Triticeae in the Fertile Crescent. In: Muehlbauer GJ, Feuillet C (eds) Genetics and Genomics of the Triticeae. Springer US, New York, NY, pp 81–119

King J, Grewal S, Yang CY, Hubbart Edwards S, Scholefield D, Ashling S, Harper JA, Allen AM, Edwards KJ, Burridge AJ, King IP (2018a) Introgression of Aegilops speltoides segments in Triticum aestivum and the effect of the gametocidal genes. Ann Bot 121:229–240. https://doi.org/10.1093/aob/mcx149

King J, Grewal S, Yang CY, Hubbart Edwards S, Scholefield D, Ashling S, Harper JA, Allen AM, Edwards KJ, Burridge AJ, King IP (2018b) Introgression of Aegilops speltoides segments in Triticum aestivum and the effect of the gametocidal genes. Ann Bot 121:229–240. https://doi.org/10.1093/aob/mcx149

Kishii M (2019) An update of recent use of Aegilops species in wheat breeding. Front Plant Sci 10:585(1–19). https://doi.org/10.3389/fpls.2019.00585

Knapp SJ (1998) Marker-assisted selection as a strategy for increasing the probability of selecting superior genotypes. Crop Sci 38:1164–1174. https://doi.org/10.2135/cropsci1998.0011183X003800050009x

Kotak S, Larkindale J, Lee U, von Koskull-Döring P, Vierling E, Scharf KD (2007) Complexity of the heat stress response in plants. Curr Opin Plant Biol 10:310–316. https://doi.org/10.1016/j.pbi.2007.04.011

Kumar A, Kumar P, Singh G, Kumar R, Kumar S (2017a) Genetic parameters and characters association analysis for yield components and heat tolerance in bread wheat (Triticum aestivum L.). Environ Ecol 35:1087–1092

Kumar M, Govindasamy V, Rane J, Singh AK, Choudhary RL, Raina SK, George P, Aher LK, Singh NP (2017b) Canopy temperature depression (CTD) and canopy greenness associated with variation in seed yield of soybean genotypes grown in semi-arid environment. South African J Bot 113:230–238.

https://doi.org/10.1016/j.sajb.2017.08.016

Kumar S, Kumari P, Kumar U, Grover M, Singh AK, Singh R, Sengar RS (2013) Molecular approaches for designing heat tolerant wheat. J Plant Biochem Biotechnol 22:359–371. https://doi.org/10.1007/s13562-013-0229-3

Kumar U, Joshi AK, Kumari M, Paliwal R, Kumar S, Röder MS (2010) Identification of QTLs for stay green trait in wheat (Triticum aestivum L.) in the “Chirya 3” × “Sonalika” population. Euphytica 174:437–445. https://doi.org/10.1007/s10681-010-0155-6

Kuźniak E (2010a) The ascorbate-gluathione cycle and related redox signals in plant-pathogen interactions. In: Ascorbate-Glutathione Pathway and Stress Tolerance in Plants. Springer Netherlands, pp 115–136

Kuźniak E (2010b) The Ascorbate–Gluathione Cycle and Related Redox Signals in Plant–Pathogen Interactions. In: Anjum NA, Umar S, Chan MT (eds) Ascorbate-Glutathione Pathway and Stress Tolerance in Plants. Springer, New York, pp 115–136

Lammerts van Bueren ET, Backes G, de Vriend H, Østergård H (2010) The role of molecular markers and marker assisted selection in breeding for organic agriculture. Euphytica 175:51–64. https://doi.org/10.1007/s10681-010-0169-0

Lan C, Hale IL, Herrera-Foessel SA, Basnet BR, Randhawa MS, Huerta-Espino J, Dubcovsky J, Singh RP (2017) Characterization and Mapping of Leaf Rust and Stripe Rust Resistance Loci in Hexaploid Wheat Lines UC1110 and PI610750 under Mexican Environments. Front Plant Sci 8:1–11. https://doi.org/10.3389/fpls.2017.01450

Lan C, Rosewarne GM, Singh RP, Herrera-Foessel SA, Huerta-Espino J, Basnet BR, Zhang Y, Yang E (2014) QTL characterization of resistance to leaf rust and stripe rust in the spring wheat line Francolin#1. Mol Breed 34:789–803. https://doi.org/10.1007/s11032-014-0075-6

Lata C, Prasad M (2011) Role of DREBs in regulation of abiotic stress responses in plants. J Exp Bot 62:4731–4748. https://doi.org/10.1093/jxb/err210

Lecomte L, Duffé P, Buret M, Servin B, Hospital F, Causse M (2004) Marker-assisted introgression of five QTLs controlling fruit quality traits into three tomato lines revealed interactions between QTLs and genetic backgrounds. Theor Appl Genet 109:658–668. https://doi.org/10.1007/s00122-004-1674-0

Lee HA, Yeom SI (2015) Plant NB-LRR proteins: Tightly regulated sensors in a complex manner. Brief Funct Genomics 14:233–242. https://doi.org/10.1093/bfgp/elv012

Li MY, Wang F, Jiang Q, Ma J, Xiong AS (2014) Identification of SSRs and differentially expressed genes in two cultivars of celery (Apium graveolens L.) by deep transcriptome sequencing. Hortic Res 1:10. https://doi.org/10.1038/hortres.2014.10

Liu B (1998) Statistical Genomics: Linkage. Mapping and QTL Analysis. CRC Press, Boca Raton

Liu Y, Wang L, Deng M, Li Z, Lu Y, Wang J, Wei Y, Zheng Y (2015) Genome-wide association study of phosphorus-deficiency-tolerance traits in Aegilops tauschii. Theor Appl Genet 128:2203–2212. https://doi.org/10.1007/s00122-015-2578-x

Lobell DB, Gourdji SM (2012) The Influence of Climate Change on Global Crop Productivity. Plant Physiol 160:1686–1697. https://doi.org/10.1104/pp.112.208298

Lobell DB, Schlenker W, Costa-Roberts J (2011) Climate trends and global crop production since 1980. Science (80- ) 333:616–620. https://doi.org/10.1126/science.1204531

Los DA, Murata N (2004) Membrane fluidity and its roles in the perception of environmental signals. Biochim Biophys Acta - Biomembr 1666:142–157. https://doi.org/10.1016/j.bbamem.2004.08.002

Ma D, Yan J, He Z, Wu L, Xia X (2012) Characterization of a cell wall invertase gene TaCwi-A1 on common wheat chromosome 2A and development of functional markers. Mol Breed 29:43–52. https://doi.org/10.1007/s11032-010-9524-z

Ma P, Xu H, Xu Y, Song L, Liang S, Sheng Y, Han G, Zhang X, An D (2018) Characterization of a Powdery Mildew Resistance Gene in Wheat Breeding Line 10V-2 and Its Application in Marker-Assisted Selection. Plant Dis 102:925–931. https://doi.org/10.1094/PDIS-02-17-0199-RE

Maccaferri M, Zhang J, Bulli P, Abate Z, Chao S, Cantu D, Bossolini E, Chen X, Pumphrey M, Dubcovsky J (2015) A genome-wide association study of resistance to stripe rust (Puccinia striiformis f. sp. tritici) in a worldwide collection of hexaploid spring wheat (Triticum aestivum L.). G3 Genes, Genomes, Genet 5:449–465. https://doi.org/10.1534/g3.114.014563

Marino D, Peeters N, Rivas S (2012) Ubiquitination during plant immune signaling. Plant Physiol 160:15–27. https://doi.org/10.1104/pp.112.199281

Mason RE, Mondal S, Beecher FW, Hays DB (2011) Genetic loci linking improved heat tolerance in wheat (Triticum aestivum L.) to lower leaf and spike temperatures under controlled conditions. Euphytica 180:181–194. https://doi.org/10.1007/s10681-011-0349-6

McHale L, Tan X, Koehl P, Michelmore RW (2006) Plant NBS-LRR proteins: Adaptable guards. Genome Biol 7:212:1–11. https://doi.org/10.1186/gb-2006-7-4-212

McIntosh RA, Dubcovsky J, Rogers WJ, Morris C, Xia XC (2017) CATALOGUE OF GENE SYMBOLS FOR WHEAT : 2017 SUPPLEMENT. 1–20

McMaster GS (1997) PHENOLOGY, DEVELOPMENT. Adv Agron 59:63

Meng L, Li H, Zhang L, Wang J (2015) QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. Crop J 3:269–283. https://doi.org/10.1016/j.cj.2015.01.001

Millet E (2007) Exploitation of Aegilops species of section Sitopsis for wheat improvement. Isr J Plant Sci 55:277–287. https://doi.org/10.1560/IJPS.55.3-4.277

Mir RR, Kumar S, Shafi S (2021) Genetic Dissection for Yield and Yield-Related Traits in Bread Wheat (Triticum aestivum L.). In: Physiological, Molecular, and Genetic Perspectives of Wheat Improvement. Springer International Publishing, Cham, pp 209–227

Mizoi J, Ohori T, Moriwaki T, Kidokoro S, Todaka D, Maruyama K, Kusakabe K, Osakabe

Y, Shinozaki K, Yamaguchi-Shinozaki K (2013) GmDREB2A;2, a canonical DEHYDRATION-RESPONSIVE ELEMENT-BINDING PROTEIN2-type transcription factor in soybean, is posttranslationally regulated and mediates DEHYDRATION-RESPONSIVE ELEMENT-dependent gene expression. Plant Physiol 161:346–361. https://doi.org/10.1104/pp.112.204875

Mohammadi V, Qannadha M, Zali A, Yazdi-Samadi B (2004) Effect of Post Anthesis Heat Stress on Head Traits of Wheat. Int J Agric Biol 6:42–44

Money D, Gardner K, Migicovsky Z, Schwaninger H, Zhong GY, Myles S (2015) LinkImpute: Fast and accurate genotype imputation for nonmodel organisms. G3 Genes, Genomes, Genet 5:2383–2390. https://doi.org/10.1534/g3.115.021667

Monneveux P, Zaharieva M, Rekika D (2000) The utilisation of Triticum and Aegilops species for the improvement of durum wheat. In: Araus JL, Di Fonzo N, Nachit M, Royo C (eds) Durum wheat improvement in the Mediterranean region: New challenges. Zaragoza : CIHEAM, pp 71–81

Munjal R, Rana R (2003) Evaluation of Physiological traits in wheat (Triticum aestivum L.) for terminal high temperature tolerance Proceedings of the Tenth International Wheat Genetics Symposium, Poestum, Italy, Class. Mol Breed 2:804–805

Nautiyal P (2017) Agronomic and physiological characterization of RILs for drought tolerance in wheat ( Triticum aestivum L .). Green Farming 8:41–46

Niks RE, Qi X, Marcel TC (2015) Quantitative Resistance to Biotrophic Filamentous Plant Pathogens: Concepts, Misconceptions, and Mechanisms. Annu Rev Phytopathol 53:445–470. https://doi.org/10.1146/annurev-phyto-080614-115928

Nishant BA, Singh MN, Srivastava K, Hemantaranjan A (2016) Molecular Mapping and Breeding of Physiological Traits. Adv Plants Agric Res 3:193–206. https://doi.org/10.15406/apar.2016.03.00120

Nocente F, Gazza L, Pasquini M (2007) Evaluation of leaf rust resistance genes Lr1, Lr9, Lr24, Lr47 and their introgression into common wheat cultivars by marker-assisted selection. Euphytica 155:329–336. https://doi.org/10.1007/s10681-006-9334-x

Paciolla C, Paradiso A, de Pinto MC (2016) Cellular Redox Homeostasis as Central Modulator in Plant Stress Response. In: Gupta DK, Palma JM, Corpas FJ (eds) Redox State as a Central Regulator of Plant-Cell Stress Responses. Springer International Publishing, Cham, pp 1–23

Paliwal R, Röder MS, Kumar U, Srivastava JP, Joshi AK (2012) QTL mapping of terminal heat tolerance in hexaploid wheat (T. aestivum L.). Theor Appl Genet 125:561–575. https://doi.org/10.1007/s00122-012-1853-3

Pan Z, Ren X, Zhao H, Liu L, Tan Z, Qiu F (2019) A Mitochondrial Transcription Termination Factor, ZmSmk3 , Is Required for nad1 Intron4 and nad4 Intron1 Splicing and Kernel Development in Maize. G3 Genes Genomes Genet 9:2677–2686. https://doi.org/10.1534/g3.119.400265

Peng J, Sun D, Nevo E (2011) Wild emmer wheat, Triticum dicoccoides, occupies a pivotal position in wheat domestication process. Aust J Crop Sci 5:1127–1143

Pimentel AJB, de Carvalho Rocha JR do AS, de Souza MA, Ribeiro G, Silva CR, Oliveira ICM (2015) Characterization of heat tolerance in wheat cultivars and effects on production components. Rev Ceres 62:191–198. https://doi.org/10.1590/0034-737X201562020009

Poland JA, Brown PJ, Sorrells ME, Jannink J-L (2012) Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. PLoS One 7:e32253(1-13). https://doi.org/10.1371/journal.pone.0032253

Poland JA, Rife TW (2012) Genotyping-by-Sequencing for Plant Breeding and Genetics. Plant Genome 5:92–102. https://doi.org/10.3835/plantgenome2012.05.0005

Potters G, Horemans N, Jansen MAK (2010) The cellular redox state in plant stress biology - A charging concept. Plant Physiol Biochem 48:292–300. https://doi.org/10.1016/j.plaphy.2009.12.007

Pradhan GP, Prasad PVV, Fritz AK, Kirkham MB, Gill BS (2012) High temperature tolerance in Aegilops species and its potential transfer to wheat. Crop Sci 52:292–304. https://doi.org/10.2135/cropsci2011.04.0186

Prasad PVV, Pisipati SR, Ristic Z, Bukovnik U, Fritz AK (2008) Impact of nighttime temperature on physiology and growth of spring wheat. Crop Sci 48:2372–2380. https://doi.org/10.2135/cropsci2007.12.0717

Qin B, Chen T, Cao A, Wang H, Xing L, Ling H, Wang D, Yu C, Xiao J, Ji J, Chen X, Chen P, Liu D, Wang X (2012) Cloning of a Conserved Receptor-Like Protein Kinase Gene and Its Use as a Functional Marker for Homoeologous Group-2 Chromosomes of the Triticeae Species. PLoS One 7:e49718:1-11. https://doi.org/10.1371/journal.pone.0049718

Rampino P, Mita G, Pataleo S, De Pascali M, Di Fonzo N, Perrotta C (2009) Acquisition of thermotolerance and HSP gene expression in durum wheat (Triticum durum Desf.) cultivars. Environ Exp Bot 66:257–264. https://doi.org/10.1016/j.envexpbot.2009.04.001

Rawat N, Tiwari VK, Singh N, Randhawa GS, Singh K, Chhuneja P, Dhaliwal HS (2008) Evaluation and utilization of Aegilops and wild Triticum species for enhancing iron and zinc content in wheat. Genet Resour Crop Evol 56:53. https://doi.org/10.1007/s10722-008-9344-8

Reynolds MP, Nagarajan S, Razzaque S, Ageeb OAA (2001) Heat tolerance. In: Reynolds MP, Ortiz-Monasterio JI, McNab A (eds) Application of Physiology in Wheat Breeding. CIMMYT, Mexico, pp 124–135

Rezaei M, Arzani A, Sayed-Tabatabaei BE (2010) Meiotic behaviour of tetraploid wheats (Triticum turgidum L.) and their synthetic hexaploid wheat derivates influenced by meiotic restitution and heat stress. J Genet 89:401–407. https://doi.org/10.1007/s12041-010-0058-2

Ruelland E, Zachowski A (2010) How plants sense temperature. Environ Exp Bot 69:225–232. https://doi.org/10.1016/j.envexpbot.2010.05.011

Sadanandom A, Bailey M, Ewan R, Lee J, Nelis S (2012) The ubiquitin-proteasome system:

Central modifier of plant signalling. New Phytol 196:13–28. https://doi.org/10.1111/j.1469-8137.2012.04266.x

Sadat S, Saeid KA, Bihamta MR, Torabi S, Hoseini Salekdeh SG, Lotfali Ayeneh GA (2013) Marker assisted selection for heat tolerance in bread wheat. World Appl Sci J 21:1181–1189. https://doi.org/10.5829/idosi.wasj.2013.21.8.2866

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci U S A 81:8014–8018. https://doi.org/10.1073/pnas.81.24.8014

Sangwan V, Örvar BL, Beyerly J, Hirt H, Dhindsa Rajinder S (2002) Opposite changes in membrane fluidity mimic cold and heat stress activation of distinct plant MAP kinase pathways. Plant J 31:629–638. https://doi.org/10.1046/j.1365-313X.2002.01384.x

Sarowar S, Eui NK, Young JK, Sung HO, Ki DK, Byung KH, Jeong SS (2005) Overexpression of a pepper ascorbate peroxidase-like 1 gene in tobacco plants enhances tolerance to oxidative stress and pathogens. Plant Sci 169:55–63. https://doi.org/10.1016/j.plantsci.2005.02.025

Schneider A, Molnár I, Molnár-Láng M (2010) Production and fish identification of wheat-Aegilops biuncialis addition lines and their use for the selection of U and M genome-specific molecular (SSR) markers. Acta Agron Hungarica 58:151–158. https://doi.org/10.1556/AAgr.58.2010.2.6

Semagn K, Bjørnstad Å, Xu Y (2010) The genetic dissection of quantitative traits in crops. Electron J Biotechnol 13:1–14. https://doi.org/10.2225/vol13-issue5-fulltext-14

Semenov MA (2009) Impacts of climate change on wheat in England and Wales. J R Soc Interface 6:343–350. https://doi.org/10.1098/rsif.2008.0285

Sharma-Natu P, Sumesh K V., Ghildiyal MC (2010) Heat shock protein in developing grains in relation to thermotolerance for grain growth in wheat. J Agron Crop Sci 196:76–80. https://doi.org/10.1111/j.1439-037X.2009.00390.x

Shen X, Ohm H (2006) Fusarium head blight resistance derived from Lophopyrum elongatum chromosome 7E and its augmentation with Fhb1 in wheat. Plant Breed 125:424–429. https://doi.org/10.1111/j.1439-0523.2006.01274.x

Shi G, Zhang Z, Friesen TL, Raats D, Fahima T, Brueggeman RS, Lu S, Trick HN, Liu Z, Chao W, Frenkel Z, Xu SS, Rasmussen JB, Faris JD (2016) The hijacking of a receptor kinase-driven pathway by a wheat fungal pathogen leads to disease. Sci Adv 2:e1600822:1-9. https://doi.org/10.1126/sciadv.1600822

Singh B, Bansal UK, Forrest KL, Hayden MJ, Hare RA, Bariana HS (2010) Inheritance and chromosome location of leaf rust resistance in durum wheat cultivar Wollaroi. Euphytica 175:351–355. https://doi.org/10.1007/s10681-010-0179-y

Singh G, Setter TL, Singh MK, Kulshreshtha N, Singh BN, Stefanova K, Tyagi BS, Singh JB, Kherawat BS, Barrett-Lennard EG (2018) Number of tillers in wheat is an easily measurable index of genotype tolerance to saline waterlogged soils: evidence from 10 large-scale field trials in India. Crop Pasture Sci 69:561–78.

https://doi.org/10.1071/CP18053

Singh P, Kumar R, Goswami S, Rai G, Sakh A, Singh P, Kumar R, Goswami S, Rai G, Sakhare A, Kumar S, Singh S, Bakshi S, Jamhulkar S, Sareen S, Singh G, Chinnusamy V, Praveen S, Kumar S, et al. (2019) A putative heat-responsive transcription factor (TaHD97) and its targets in wheat (Triticum aestivum) providing thermotolerance. Indian J Biotechnol 18:213–223

Sofi P, Ara A, Gull M, Rehman K (2020) Canopy Temperature Depression as an Effective Physiological Trait for Drought Screening. In: Ondrasek G (ed) Drought - Detection and Solutions. IntechOpen, London, pp 1–16

Song X-J, Huang W, Shi M, Zhu M-Z, Lin H-X (2007) A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nat Genet 39:623–630. https://doi.org/10.1038/ng2014

Su Z, Hao C, Wang L, Dong Y, Zhang X (2011) Identification and development of a functional marker of TaGW2 associated with grain weight in bread wheat (Triticum aestivum L.). Theor Appl Genet 122:211–223. https://doi.org/10.1007/s00122-010-1437-z

Sud S, Bhagwat SG (2010) Assessment of acquired thermotolerance in Indian bread wheat and association with yield and component traits under heat stress environment. J Food, Agric Environ 8:622–627

Sumesh K V., Sharma-Natu P, Ghildiyal MC (2008) Starch synthase activity and heat shock protein in relation to thermal tolerance of developing wheat grains. Biol Plant 52:749–753. https://doi.org/10.1007/s10535-008-0145-x

Talebi R (2011) Evaluation of chlorophyll content and canopy temperature as indicators for drought tolerance in durum wheat (Triticum durum Desf.). Aust J Basic Appl Sci 5:1457–1462

Talukder SK, Babar MA, Vijayalakshmi K, Poland J, Prasad PVV, Bowden R, Fritz A (2014) Mapping QTL for the traits associated with heat tolerance in wheat (Triticum aestivumL.). BMC Genet 15:97(1–13). https://doi.org/10.1186/s12863-014-0097-4

Tang D, Ade J, Frye CA, Innes RW (2005) Regulation of plant defense responses in Arabidopsis by EDR2, a PH and START domain-containing protein. Plant J 44:245–257. https://doi.org/10.1111/j.1365-313X.2005.02523.x

Tang D, Wang G, Zhou JM (2017) Receptor kinases in plant-pathogen interactions: More than pattern recognition. Plant Cell 29:618–637. https://doi.org/10.1105/tpc.16.00891

Tanksley SD (1993) Mapping Polygenes. Annu Rev Genet 27:205–233. https://doi.org/10.1146/annurev.ge.27.120193.001225

Taylor P, Street M, Wt L, Signaling P, Dubreuil-maurizi C, Poinssot B, Dubreuil-maurizi C, Poinssot B (2012) stress Role of glutathione in plant signaling under biotic stress © 2012 Landes Bioscience . https://doi.org/10.4161/psb.18831

Tewolde H, Fernandez CJ, Erickson CA (2006) Wheat cultivars adapted to post-heading high temperature stress. J Agron Crop Sci 192:111–120. https://doi.org/10.1111/j.1439-037X.2006.00189.x

Todorovska E, Christov N, Slavov S, Christova P, Vassilev D (2009) Biotic stress resistance in wheat - Breeding and genomic selection implications. Biotechnol Biotechnol Equip 23:1417–1426. https://doi.org/10.2478/V10133-009-0006-6

Tomczak M, Tomczak E (2014) The need to report effect size estimates revisited. An overview of some recommended measures of effect size. Trends Sport Sci 1:19–25

Üstün S, Sheikh A, Gimenez-Ibanez S, Jones A, Ntoukakis V, Börnke F (2016) The Proteasome Acts as a Hub for Plant Immunity and Is Targeted by Pseudomonas Type III Effectors. Plant Physiol 172:1941–1958. https://doi.org/10.1104/pp.16.00808

van Berloo R (2008) GGT 2.0: Versatile Software for Visualization and Analysis of Genetic Data. J Hered 99:232–236. https://doi.org/10.1093/jhered/esm109

van Bueren ETL, Struik PC, van Eekeren N, Nuijten E (2018) Towards resilience through systems-based plant breeding. A review. Agron Sustain Dev 38:42–78. https://doi.org/10.1007/s13593-018-0522-6

van Slageren MW (1994) Wild wheats: a monograph of Aegilops L. and Amblyopyrum (Jaub. & Spach) Eig (Poaceae). ICARDA, Aleppo, Syria and Wageningen Agricultural University, The Netherlands, Wageningen (Netherlands)

Venkata B, bullet U, Bansal U, bullet R, Singh M, Robert bullet, Park R, Harbans bullet, Bariana H (2008) Genetic Analyses of Durable Adult Plant Resistance to Stripe Rust and Leaf Rust in CIMMYT Wheat Genotype 11IBWSN50. Int J Plant Breed 2:64–68

Vierstra RD (2009) The ubiquitin-26S proteasome system at the nexus of plant biology. Nat Rev Mol Cell Biol 10:385–397. https://doi.org/10.1038/nrm2688

Vijayalakshmi K, Fritz AK, Paulsen GM, Bai G, Pandravada S, Gill BS (2010) Modeling and mapping QTL for senescence-related traits in winter wheat under high temperature. Mol Breed 26:163–175. https://doi.org/10.1007/s11032-009-9366-8

Visioni A, Gyawali S, Selvakumar R, Gangwar OP, Shekhawat PS, Bhardwaj SC, Al-Abdallat AM, Kehel Z, Verma RPS (2018) Genome Wide Association Mapping of Seedling and Adult Plant Resistance to Barley Stripe Rust (Puccinia striiformis f. sp. hordei) in India. Front Plant Sci 9:1–13. https://doi.org/10.3389/fpls.2018.00520

Viswanathan C, Khanna-Chopra R (2001) Effect of heat stress on grain growth, starch synthesis and protein synthesis in grains of wheat (Triticum aestivum L.) varieties differing in grain weight stability. J Agron Crop Sci 186:1–7. https://doi.org/10.1046/j.1439-037X.2001.00432.x

Wang J, Li H, Zhang L, Meng L (2016) Intigrated Software for Linkage Analysis and Genetic Mapping in Biparental Populations. 1–274

Wang J, Liu W, Wang H, Li L, Wu J, Yang X, Li X, Gao A (2011) QTL mapping of yield-related traits in the wheat germplasm 3228. Euphytica 177:277–292. https://doi.org/10.1007/s10681-010-0267-z

Wang J, Qi P, Wei Y, Liu D, Fedak G, Zheng Y (2010) Molecular characterization and functional analysis of elite genes in wheat and its related species. J Genet 89:539–554. https://doi.org/10.1007/s12041-010-0074-2

Wang W, Vinocur B, Shoseyov O, Altman A (2004) Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends Plant Sci 9:244–252. https://doi.org/10.1016/j.tplants.2004.03.006

Wang Y, Bouwmeester K (2017) L-type lectin receptor kinases: New forces in plant immunity. PLoS Pathog 13:e1006433:1-7. https://doi.org/10.1371/journal.ppat.1006433

Wang Y, Wang L, Zhou J, Hu S, Chen H, Xiang J, Zhang Y, Zeng Y, Shi Q, Zhu D, Zhang Y (2019) Research Progress on Heat Stress of Rice at Flowering Stage. Rice Sci 26:1–10. https://doi.org/https://doi.org/10.1016/j.rsci.2018.06.009

Watson B (2008) Use of Marker Assisted Selection for the Introgression of Quality Traits from Australian into Chinese Wheats. University of Southern Queensland

Xu W, Subudhi PK, Crasta OR, Rosenow DT, Mullet JE, Nguyen HT (2000) Molecular mapping of QTLs conferring stay-green in grain sorghum (*Sorghum bicolor* L. Moench). Genome 43:461–469. https://doi.org/10.1139/gen-43-3-461

Yang J, Sears RG, Gill BS, Paulsen GM (2002a) Quantitative and molecular characterization of heat tolerance in hexaploid wheat. Euphytica 126:275–282. https://doi.org/10.1023/A:1016350509689

Yang J, Sears RG, Gill BS, Paulsen GM (2002b) Growth and senescence characteristics associated with tolerance of wheat-alien amphiploids to high temperature under controlled conditions. Euphytica 126:185–193. https://doi.org/10.1023/A:1016365728633

Yang J, Zhang J (2006) Grain filling of cereals under soil drying. New Phytol 169:223–236. https://doi.org/10.1111/j.1469-8137.2005.01597.x

Yang L, Zhang X, Zhang X, Wang J, Luo M, Yang M, Wang H, Xiang L, Zeng F, Yu D, Fu D, Rosewarne GM (2017) Identification and evaluation of resistance to powdery mildew and yellow rust in a wheat mapping population. PLoS One 12:e0177905:1-18. https://doi.org/10.1371/journal.pone.0177905

Yao C, Wu Y, Nie H, Tang D (2012) RPN1a, a 26S proteasome subunit, is required for innate immunity in Arabidopsis. Plant J. https://doi.org/10.1111/j.1365-313X.2012.05048.x

Yildiz M, Terzi H (2008) Evaluation of acquired thermotolerance in wheat (Triticum aestivum and T. Durum) cultivars grown in Turkey. Pakistan J Bot 40:317–327

Zaharieva M, Gaulin E, Havaux M, Acevedo E, Monneveux P (2001) Drought and heat responses in the wild wheat relative Aegilops geniculata roth: Potential interest for wheat improvement. Crop Sci 41:1321–1329. https://doi.org/10.2135/cropsci2001.4141321x

Zahedi M, Jenner CF (2003) Analysis of effects in wheat of high temperature on grain filling attributes estimated from mathematical models of grain filling. J Agric Sci 141:203–212. https://doi.org/10.1017/S0021859603003411

Zhang H, Mao R, Wang Y, Zhang L, Wang C, Lv S, Liu X, Wang Y, Ji W (2019) Transcriptome-wide alternative splicing modulation during plant-pathogen interactions in wheat. Plant Sci 288:110160. https://doi.org/10.1016/j.plantsci.2019.05.023

Zhao F, Li Y, Yang B, Yuan H, Jin C, Zhou L, Pei H, Zhao L, Li Y, Zhou Y, Xie J, Shen Q-H (2020) Powdery mildew disease resistance and marker-assisted screening at the Pm60 locus in wild diploid wheat Triticum urartu. Crop J 8:252–259. https://doi.org/https://doi.org/10.1016/j.cj.2019.09.007

Zhou H, Li S, Deng Z, Wang X, Chen T, Zhang J, Chen S, Ling H, Zhang A, Wang D, Zhang X (2007) Molecular analysis of three new receptor-like kinase genes from hexaploid wheat and evidence for their participation in the wheat hypersensitive response to stripe rust fungus infection. Plant J 52:420–434. https://doi.org/10.1111/j.1365-313X.2007.03246.x

Zinn KE, Tunc-Ozdemir M, Harper JF (2010) Temperature stress and plant sexual reproduction: Uncovering the weakest links. J Exp Bot 61:1959–1968. https://doi.org/10.1093/jxb/erq053

## Published articles in peer-review journals

Dhillon, G. S., Das, N., Kaur, S., Srivastava, P., Bains, N. S., & Chhuneja, P. (2021). Marker-assisted mobilization of heat tolerance QTLs from *Triticum durum-Aegilops speltoides* introgression lines to hexaploid wheat. *Indian Journal of Genetics and Plant Breeding*, 81(2): 186-198. https://doi.org/10.31742/IJGPB.81.2.2

Dhillon, G. S., Kaur, S., Das, N., Singh, R., Poland, J., Kaur, J., & Chhuneja, P. (2020). QTL mapping for stripe rust and powdery mildew resistance in *Triticum durum - Aegilops speltoides* backcross introgression lines. *Plant Genetic Resources: Characterisation and Utilisation*, 18(4): 211–221. https://doi.org/10.1017/S1479262120000222

## Conferences attended

Young Scientist Award for best oral presentation at National Symposium on "Trends in Plant Biotechnology and Agriculture" held at Thapar Institute of Engineering and Technology, Patiala, India, February 6-8,2020

Poster Presentation at "International Conference on Food Security: Challenges and Opportunities" held at Thapar Institute of Engineering and Technology, Patiala, India, December 7-8,2018

Participated in "4th International Group Meeting" held at CSK Himachal Pradesh Agricultural University, Palampur, HP, India, February 14 -16, 2019

*Indian J. Genet*., 81(2): 186-198 (2021)
DOI: 10.31742/IJGPB.81.2.2

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

**Guriqbal Singh Dhillon#, Niranjan Das[2], Satinder Kaur, Puja Srivastava[1], Navtej Singh Bains[1], Parveen Chhuneja***

School of Agricultural Biotechnology, [1]Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana 141 004; [2]Dept. of Biotechnology, Thapar Institute of Engineering and Technology, Patiala 147 004

(Received: November 2020; Revised: April 2021; Accepted: May 2021)

## 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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*Corresponding author's e-mail: pchhuneja@pau.edu

[2]Present address: Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala 147 004

Published by the Indian Society of Genetics & Plant Breeding, A-Block, F2, First Floor, NASC Complex, IARI P.O., Pusa Campus, New Delhi 110 012; Online management by www.isgpb.org; indianjournals.com

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 $F_1$s were planted in main-season 2015-16, and initial screening of all $F_1$s was done removing durum type plants. Pentaploid $F_1$s (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 $F_1$s 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

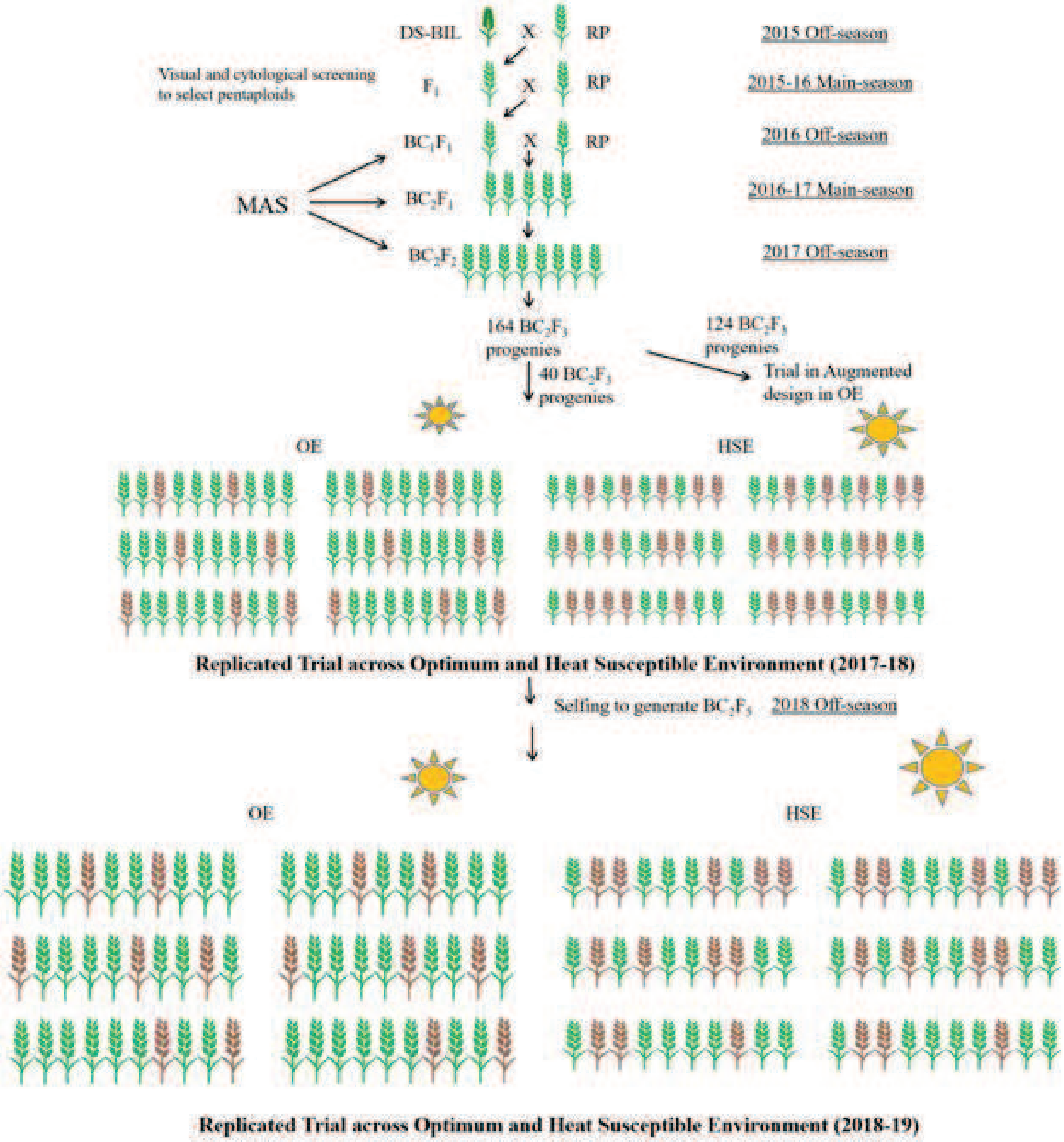


**Fig. 1.** **Development, screening and experimental design of *T. durum-Ae. speltoides*/hexaploid wheat derived $BC_2F_{3\text{-}5}$ HTILs. Selected lines were crossed with recurrent parent (RP), backcrossed and selfed till $BC_2F_3$ along with selections. At $BC_2F_3$, 40 lines with sufficient seeds 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).

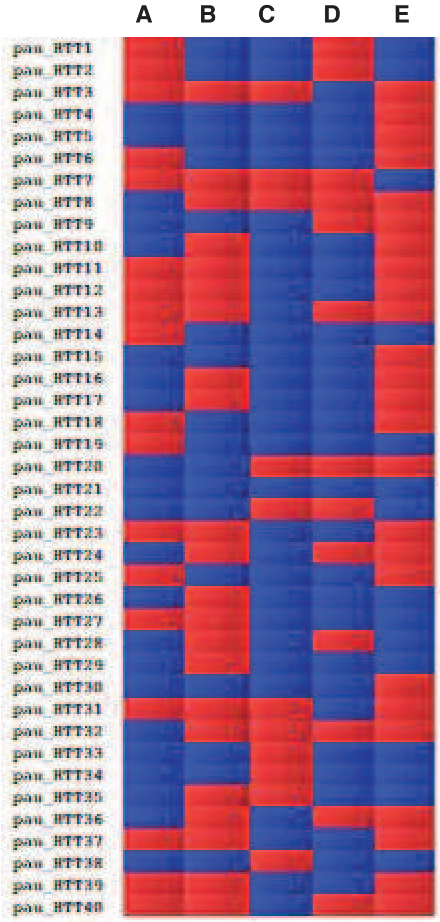
HTILs evaluated in $BC_2F_5$, not only out-performed their respective parental lines in OE but also showed less penalty in HSE (Fig. 3, Table 2). Alarge variation for various agro-morphological traits was observed in both the environments including TNpM (74.60-136.56 in OE and 68.56-95.33 in HSE), for 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 $BC_2F_3$HTILs 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 (cm) | $BC_2F_3$ | 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 |
| | | 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 (cm) | $BC_2F_3$ | 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 |
| | | 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 (cm) | $BC_2F_3$ | 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 |
| | | 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 (g) | $BC_2F_3$ | 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 |
| | | 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 (kg) | $BC_2F_3$ | 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 |
| | | 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



| | A | B | C | D | E |
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| pau_HTT3 | 🟥 | 🟥 | 🟥 | 🟦 | 🟥 |
| pau_HTT4 | 🟦 | 🟦 | 🟦 | 🟦 | 🟥 |
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| pau_HTT40 | 🟥 | 🟥 | 🟦 | 🟥 | 🟥 |

**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

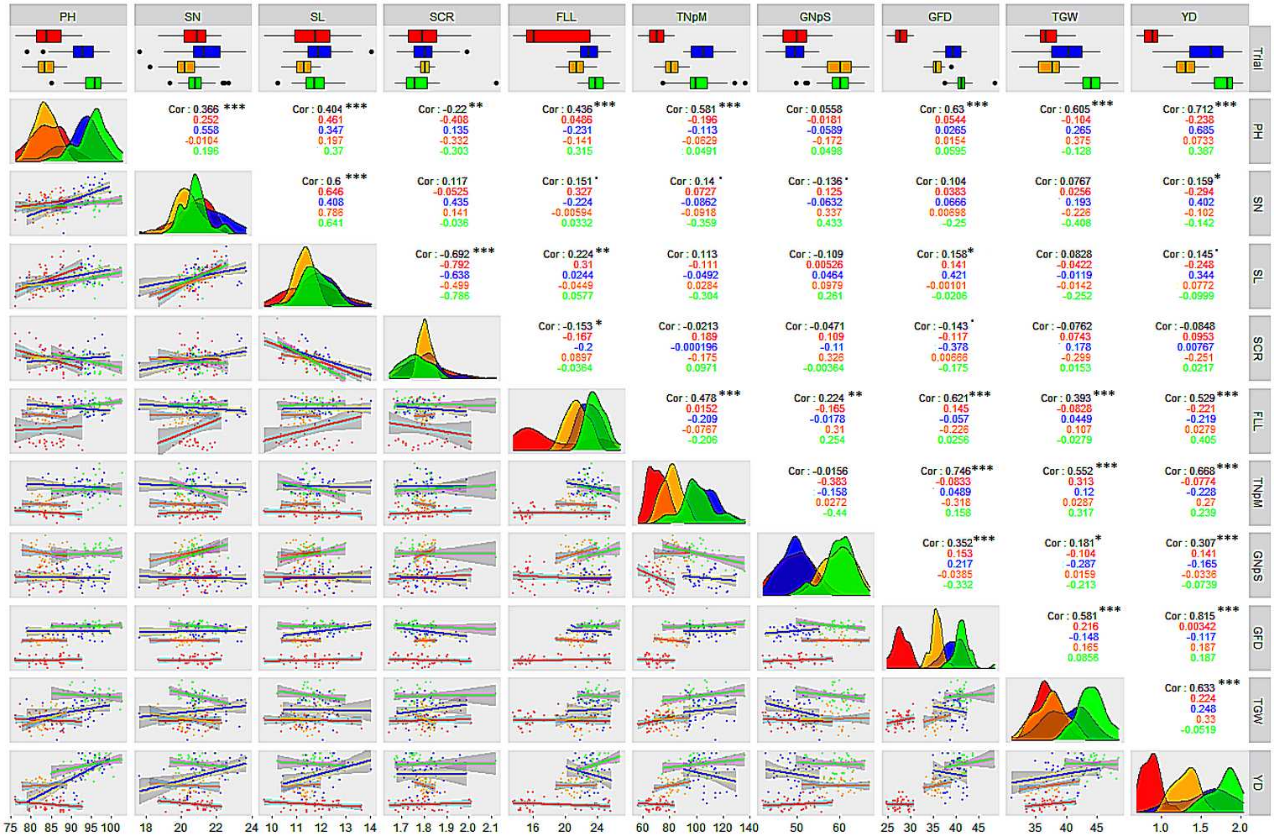


**Fig. 3. Evaluation of $BC_2F_3$ and $BC_2F_5$ progenies of HTILs developed across seasons 2017-18 and 2018-19. Blue colour represents trait response in optimum environment (OE) and red coloured boxes represent trait response in heat stress environment (HSE) in season 2017-18, green colour represent 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 $F_1$s 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 |

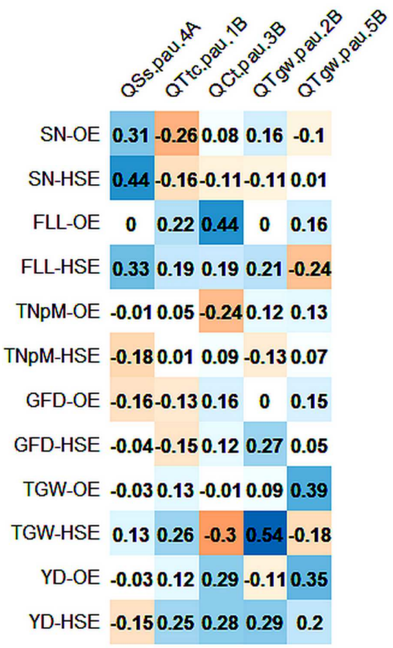
SN= Spikelet no. per spike, FLL =Flag leaf length , TNpM= Tiller number per meter , GNpS =Grain number per spike , GFD =Grain filling duration , TGW= Thousand grain weight, and YD= Yield per plot

††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 $BC_2F_5$ 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).

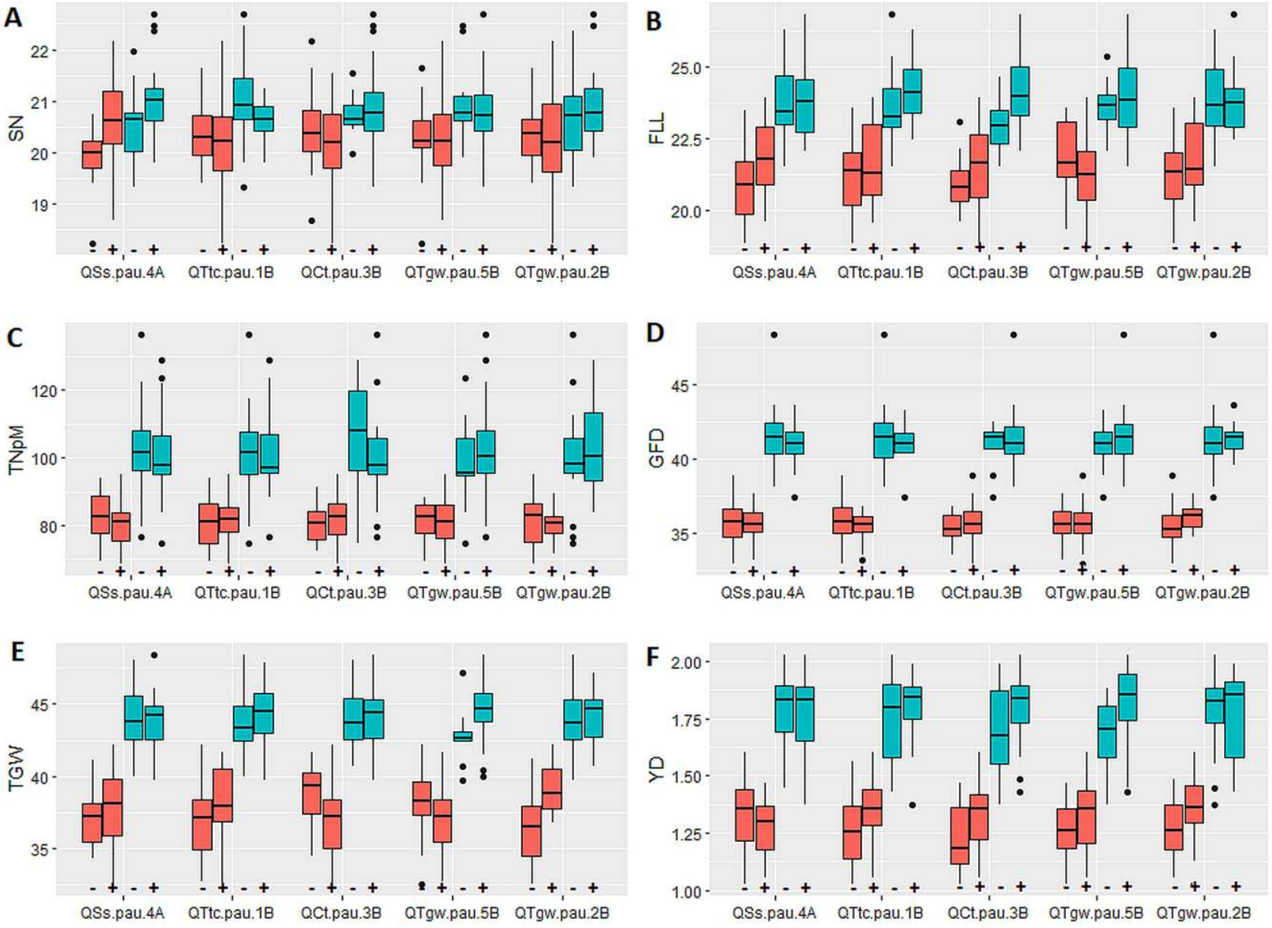


**Fig. 5. Effect of various QTL introgressions in HTILs on important yield related traits (a-f). Blue color represents trait response in OE (Optimum environment) and red color represents trait response in HSE (Heat stress environment). The positive (+) and negative (-) signs represents the distribution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

## Acknowledgements

The financial support provided by the Department of Biotechnology, Ministry of Scienceand Technology, Government of India in the form of grant No. BT/PR/5468/AGR/02/851/2012 is gratefully acknowledged.

## References

Acevedo E., Nashit M. and Ferrara G. O. 1990. Effects of heat stress on wheat and possible selection tools for use in breeding for tolerance., in: International Conference on Wheat for 1990.

Akter N. and Rafiqul Islam M. 2017. Heat stress effects and management in wheat. A review. Agron. Sustain. Dev. **37**: 37(1-17). https://doi.org/10.1007/s13593-017-0443-9.

Alvarado G., Lopez M., Vargas M., Pacheco A., Rodríguez F., Burgueño J. and Crossa J. 2016. META-R (Multi Environment Trial Analysis whit R for Windows).

Arabbeigi M., Arzani A. and Saeidi G. 2010. Meiotic Behavior of Wild, Synthetic and Cultivated Wheats. Cytologia (Tokyo). **75**: 169-175. https://doi.org/10.1508/cytologia.75.169.

Aravind J., Sankar M. S., Wankhede D. P. and Kaur V. 2019. augmentedRCBD: Analysis of Augmented Randomised Complete Block Designs. R package.

Awlachew Z. T. and Chhuneja P. 2013. Molecular Characterization of Triticum durum – Aegilops speltoides Introgression lines and Evaluation for thermo–tolerance and its components. Punjab Agricultural University, Ludhiana-141004.

Awlachew Z. T., Singh R., Kaur S., Bains N. S. and Chhuneja P. 2016. Transfer and mapping of the heat tolerance component traits of Aegilops speltoides in tetraploid wheat Triticum durum. Mol. Breed. **36**: 78-92. https://doi.org/10.1007/s11032-016-0499-2.

Colas I., Shaw P., Prieto P., Wanous M., Spielmeyer W., Mago R. and Moore G. 2008. Effective chromosome pairing requires chromatin remodeling at the onset of meiosis. Proc. Natl. Acad. Sci. U. S. A. **105**: 6075-6080. https://doi.org/10.1073/pnas.0801521105.

Comtois D. 2020. summarytools: Tools to Quickly and Neatly Summarize Data. R-package version 0.9.6.

Cramer G. R., Urano K., Delrot S., Pezzotti M. and Shinozaki K. 2011. Effects of abiotic stress on plants: A systems biology perspective. BMC Plant Biol., **11**: 163(1-14). https://doi.org/10.1186/1471-2229-11-163.

Deb A. and Khaleque M. A. 2009. Nature of gene action of some quantitative traits in chickpea. World J. Agric. Sci., **5**: 361-368.

Dias A. S. and Lidon F. C. 2009. Evaluation of Grain Filling Rate and Duration in Bread and Durum Wheat, under Heat Stress after Anthesis. J. Agron. Crop Sci., **195**: 137-147. https://doi.org/10.1111/j.1439-037X.2008.00347.x.

Dubey R., Pathak H., Chakrabarti B., Singh S., Gupta D. K. and Harit R. C. 2020. Impact of terminal heat stress on wheat yield in India and options for adaptation. Agric. Syst., **181**: 102826. https://doi.org/10.1016/j.agsy.2020.102826.

Farooq M., Bramley H., Palta J. A. and Siddique K. H. M. 2011. Heat stress in wheat during reproductive and grain-filling phases. CRC. Crit. Rev. Plant Sci., **30**: 491-507. https://doi.org/10.1080/07352689.2011.615687

Flohr B. M., Hunt J. R., Kirkegaard J. A. and Evans J. R. 2017. Water and temperature stress define the optimal flowering period for wheat in south-eastern Australia. F. Crop. Res., **209**: 108-119. https://doi.org/10.1016/j.fcr.2017.04.012.

Gaur D. K., Kaushal S. K., Rani U. and Singh S. 2017. Agricultural Situation in India. Directorate of Economics and Statastics, Department of Agriculture, Coorporation and Farmers Wellfare, Ministary of Agriculture, Government of India.

Hemantaranjan A., Bhanu N. A., Singh M. N., Yadav D. K., Patel P. K., Singh R. and Katiyar D. 2014. Heat Stress Responses and Thermotolerance. Adv. Plants Agric. Res., **1**: 1-10. https://doi.org/10.15406/apar.2014.01.00012.

Iqbal M., Raja N. I., Yasmeen F., Hussain M., Ejaz M. and Shah M. A. 2017. Impacts of Heat Stress on Wheat: A Critical Review. Adv. Crop Sci. Technol., **5**: 1-9. https://doi.org/10.4172/2329-8863.1000251.

Jha U. C., Bohra A. and Singh N. P. 2014. Heat stress in crop plants: Its nature, impacts and integrated breeding strategies to improve heat tolerance. Plant Breed., **133**: 679-701. https://doi.org/10.1111/pbr.12217.

Kaur S., Jindal S., Kaur M. and Chhuneja P. 2018. Utilization of Wild Species for Wheat Improvement Using Genomic Approaches, in: Gosal, S., Wani, S. (Eds.), Biotechnologies of Crop Improvement, Volume 3. Springer, CHAM, pp. 105-150. https://doi.org/10.1007/978-3-319-94746-4_6.

King J., Grewal S., Yang C. Y., Hubbart Edwards S., Scholefield D., Ashling S., Harper J. A., Allen A. M., Edwards K. J., Burridge A. J. and King I. P. 2018. Introgression of Aegilops speltoides segments in Triticum aestivum and the effect of the gametocidal genes. Ann. Bot., **121**: 229-240. https://doi.org/10.1093/aob/mcx149.

Kumar A., Kumar P., Singh G., Kumar R. and Kumar S. 2017. Genetic parameters and characters association analysis for yield components and heat tolerance in bread wheat (*Triticum aestivum* L.). Environ. Ecol., **35**: 1087-1092.

McMaster G. S. 1997. PHENOLOGY, DEVELOPMENT. Adv. Agron., **59**: 63.

Millet E. 2007. Exploitation of Aegilops species of section Sitopsis for wheat improvement. Isr. J. Plant Sci., **55**: 277-287. https://doi.org/10.1560/IJPS.55.3-4.277

Pimentel A. J. B., de Carvalho Rocha J. R. do A. S., de Souza M. A., Ribeiro G., Silva C. R. and Oliveira I. C. M. 2015. Characterization of heat tolerance in wheat cultivars and effects on production components. Rev. Ceres, **62**: 191-198. https://doi.org/10.1590/0034-737X201562020009.

Pradhan G. P., Prasad P. V. V., Fritz A. K., Kirkham M. B. and Gill B. S. 2012. High temperature tolerance in Aegilops species and its potential transfer to wheat. Crop Sci., **52**: 292-304. https://doi.org/10.2135/cropsci2011.04.0186.

Rezaei M., Arzani A. and Sayed-Tabatabaei B. E. 2010. Meiotic behaviour of tetraploid wheats (Triticum turgidum L.) and their synthetic hexaploid wheat derivates influenced by meiotic restitution and heat stress. J. Genet., **89**: 401-407. https://doi.org/10.1007/s12041-010-0058-2.

Sadat S., Saeid K. A., Bihamta M. R., Torabi S., Hoseini Salekdeh S. G. and Lotfali Ayeneh G. A. 2013. Marker assisted selection for heat tolerance in bread wheat. World Appl. Sci. J., **21**: 1181-1189. https://doi.org/10.5829/idosi.wasj.2013.21.8.2866

Saghai-Maroof M. A., Soliman K. M., Jorgensen R. A. and Allard R. W. 1984. Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. U. S. A., **81**: 8014-8018. https://doi.org/10.1073/pnas.81.24.8014.

Sonkar G., Mall R. K., Banerjee T., Singh N., Kumar T. V. L. and Chand R. 2019. Vulnerability of Indian wheat against rising temperature and aerosols. Environ. Pollut., **254**: 1129-1146. https://doi.org/10.1016/j.envpol.2019.07.114.

Tomczak M. and Tomczak E. 2014. The need to report effect size estimates revisited. An overview of some recommended measures of effect size. Trends Sport Sci., **1**: 19-25.

ISSN 1479-2621

Plant Genetic Resources: Characterization and Utilization (2020) **18**(4); 211–221
doi:10.1017/S1479262120000222

# QTL mapping for stripe rust and powdery mildew resistance in *Triticum durum–Aegilops speltoides* backcross introgression lines

Guriqbal Singh Dhillon[1,2], Satinder Kaur[1], Niranjan Das[2], Rohtas Singh[1], Jesse Poland[3], Jaspal Kaur[4] and Parveen Chhuneja[1]*

[1]*School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, India,* [2]*Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala, India,* [3]*Wheat Genetics Resource Centre, Kansas State University, Manhattan, KS, USA and* [4]*Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India*

**Received 31 October 2019; Accepted 18 July 2020 – First published online 17 August 2020**

## Abstract

Wheat, a major food crop, faces significant yield constraints due to losses caused by various diseases, especially rusts and powdery mildew. Since the causal organisms are always evolving, there is a never-ending hunt for new genes/quantitative trait loci (QTLs) for resistance to control the damage. For this purpose, *Triticum durum–Aegilops speltoides* backcross introgression lines (DS-BILs) developed in our wide hybridization programme were screened against stripe rust and powdery mildew at both seedling and adult plant stages. DS-BILs showed complete to moderate resistance at the adult plant stage while varying resistance and susceptibility at the seedling stage. A total of 1095 single-nucleotide polymorphisms (SNPs) identified on 14 chromosomes of *T. durum*, using genotyping by sequencing, were used for QTL mapping. Eleven unique QTLs, across six chromosomes (chr1B, chr2A, chr2B, chr3B, chr6B and chr7B) were identified for resistance, four QTLs for field mixture of stripe rust pathotypes, two QTLs for stripe rust pathotype 78S84 and five QTLs for field mixture of powdery mildew pathotypes using stepwise regression-based likelihood ratio test for additive effect of markers and single-marker analysis. Eleven DS-BILs carrying multiple QTLs were identified which will serve as a useful resource to transfer the respective resistance to susceptible cultivars to develop all stage resistant elite cultivars where QTL for stripe rust resistance *QYrAs.pau-2A.1* (LOD 3.8, PVE 24.51 linked to SNP S2A_16016633) and QTL for powdery mildew resistance *QPmAs.pau-6B* (logarithm of the odds (LOD) 3.2, phenotypic variation explained (PVE) 17.75 linked to SNP S6B_26793381) are major targets of the transfer.

**Keywords:** *Aegilops speltoides*, crop wild relatives, disease resistance, powdery mildew, QTL, stripe rust, *Triticum durum*, wheat

## Introduction

Stripe rust and powdery mildew caused by *Puccinia striiformis* and *Blumeria graminis* are two economically significant fungal foliar diseases of wheat which are a significant constraint for sustainable wheat production, hampering both yield and quality (Elkot *et al.*, 2015; Bariana *et al.*, 2016; Bansal *et al.*, 2017; Lan *et al.*, 2017). In recorded history, diseases and pests have caused substantial wheat yield losses ranging from 50 to 100% under epidemic conditions (Figueroa *et al.*, 2018). The long-term use of chemical pesticides has had a significant negative impact on the environment as well as on human health.

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*Corresponding author. E-mail: pchhuneja@pau.edu

Thus, a holistic approach of introduction of disease resistance genes is much more lucrative and sustainable. McIntosh *et al.* (2017) have reported characterization and deployment of more than 70 genes for each of these diseases from elite wheat genotypes, landraces and crop wild relatives to counter the problem of biotic stress on wheat production.

In wheat, disease resistance has been characterized into two categories based on their action across the lifecycle of the plant (Chen, 2005). Firstly, the all-stage resistance (ASR) caused by seedling stage resistance genes, which start acting from the seedling stage and remain active throughout the plant life. These genes are mostly race-specific. Secondly, the adult plant resistance (APR) caused by genes that are primarily responsible for moderate to complete resistance at the adult plant stage only. APRs may confer hypersensitive reaction or provide more durable resistance to plants by providing slow rusting type resistance (Venkata *et al.*, 2008; Niks *et al.*, 2015). This type of durable resistance is crucial as it does not exert evolutionary stress on the causal organism by retarding the development of disease progression. Although APR is a durable form of resistance, only a small number of resistance genes have been known to confer this particular type of resistance (Kankwatsa *et al.*, 2017).

Several *Aegilops* species have been exploited to transfer genes for resistance to wheat, against various insect pests, powdery mildew and rust diseases that have been commercially used (Kaur *et al.*, 2018). Kishii (2019) has compiled a list of genes identified or transferred from various *Aegilops* species, including *Aegilops speltoides*, which have been the sources of leaf rust resistance genes (*Lr28*, *Lr35*, *Lr36*, *Lr37*, *Lr47*, *Lr51* and *Lr66*), stem rust resistance genes (*Sr32*, *Sr39* and *Sr47*), powdery mildew resistance genes (*Pm1d*, *Pm12*, *Pm32* and *Pm53*) and green bug resistance gene (*Gb5*). Apart from disease resistance, *Aegilops* species have been reported to possess resistance to abiotic stresses like heat, salinity and drought tolerance (Monneveux *et al.*, 2000; Colmer *et al.*, 2006; Rawat *et al.*, 2008; Liu *et al.*, 2015; Awlachew *et al.*, 2016).

During field examination of various crop wild relatives of wheat (at the School of Agricultural Biotechnology, Punjab Agricultural University) for more than 20 years, selected accessions of *Ae. speltoides*, the putative wheat B genome donor (Zhang *et al.*, 2018), were found to be resistant to most of the diseases prevailing in the field. In this study, *Triticum durum–Ae. speltoides* backcross introgression lines (DS-BILs), developed through limited backcrossing followed by selfing, were evaluated for resistance against the two major wheat diseases, stripe rust and powdery mildew, and quantitative trait locus (QTL)/genes were mapped using single-nucleotide polymorphisms (SNPs) developed through genotyping by sequencing (GBS).

## Materials and methods

### Plant material

Eighty-nine $BC_2F_{10}$ DS-BILs developed from durum wheat cultivar PDW274 and *Ae. speltoides* accession #pau3809 were used in the study. Details of the development of the DS-BILs can be found in Awlachew *et al.* (2016). *Ae. speltoides* acc. pau3809 was crossed with *T. durum* cv. PDW274 as female and $F_1$ was backcrossed to *T. durum*. All the $BC_1$ plants were backcrossed and $BC_2F_1$s selfed to generate homozygous BILs of *Ae. speltoides* in *T. durum* background. This set of *T. durum–Ae. speltoides* backcross introgression lines will be denoted as DS-BILs henceforth in this paper.

### Seedling screening

For screening against stripe rust (YR), and powdery mildew (PM), 89 DS-BILs along with recurrent parent PDW274 and susceptible check WL711, a hexaploid wheat cultivar susceptible to both the diseases, were planted in bread boxes with 10 seeds of each genotype (with one row of control check each) and were kept in different temperature- and moisture-controlled glass houses maintained specifically for each disease. The first leaves of 7-day germinated seedlings were inoculated with respective disease spores. For YR, two sets of the same BILs were inoculated, one with *P. striiformis* (*Pst*) pathotype 78S84 and other with a mixture of pathotypes collected from open field. For PM, a mixture of *B. graminis* (*Bg*) pathotypes collected from open field was used for inoculation. The inoculated bread boxes for each disease were placed separately in water-filled trays covered with a black sheet for 24 h at 100% relative humidity. After the incubation, the bread boxes were maintained in separate glasshouses for disease development. For YR, disease scoring was done using Stakamans' scale (Visioni *et al.*, 2018) after 14 days when susceptible control showed complete susceptibility for respective pathotypes. Similarly, for scoring disease in PM, a linear scale of 0–9 was used (Yang *et al.*, 2017).

### Molecular analysis

DNA extraction of 89 DS-BILs along with PDW274 and *Ae. speltoides* acc. pau3809 was done using the cetyl trimethylammonium bromide (CTAB) method (Saghai-Maroof *et al.*, 1984). DNA was genotyped using GBS. The raw reads generated by GBS were subjected to SNP calling using the TASSELGBSv2 pipeline in TASSELv5.2 (Glaubitz *et al.*, 2014). The SNPs were called against the A and B genomes of wheat reference genome refseqV1.0. The vcf file generated using the pipeline was filtered for depth at 3

(DP3) and converted to HapMap format. The TASSEL output was then filtered for homozygous SNPs for each parental line, and the polymorphic SNPs between the two parental lines were selected. Furthermore, the SNPs were filtered for 20% missing data, and remaining SNPs were used for mapping. The distribution of SNPs along 14 chromosomes is presented in online Supplementary Fig. S1.

### QTL mapping

For mapping, the disease resistance, scoring of the diseases was converted into linear scale as per Yang *et al.* (2017) and 1095 SNPs were used for mapping using the CSL functionality of QTL IciMapping V4.1.0.0 employing single marker analysis (SMA) and stepwise regression-based likelihood ratio test (RSTEP-LRT) (Wang *et al.*, 2016). QTLs detected at LOD (logarithm of odds) score ≥ 2.0 and PVE (phenotypic variation explained) > 9.0 were considered to be significant.

### Introgression profile of DS-BILs

Introgression profiling of *Ae. speltoides* fragments in *T. durum* background of 89 DS-BILs were done using GGT2 (van Berloo, 2008).

### Postulation of candidate genes

The physical positions of the mapped SNPs in the introgressed segments were used to identify the candidate genes conferring resistance to diseases in the annotated wheat genome present at https://wheat-urgi.versailles.inra.fr/Seq-Repository/Annotations. Jbrowse functionality was used to manually hunt for the candidate genes in the designated regions of the annotated wheat genome. Gene names and functions were identified from https://web.persephonesoft.com/?data=genomes.

## Results

### Evaluation of rust and powdery mildew resistance

Recipient parent PDW274 was completely susceptible at the seedling stage against stripe rust pathotype Pst78S84 and mixture of pathotypes while donor *Ae. speltoides* acc. pau3809 showed complete resistance. PDW274 depicted moderate resistance against stripe rust at the adult plant stage under artificial epiphytotic conditions. PDW274 thus might carry an APR gene for stripe rust while *Ae. speltoides* acc. pau3809 harbours ASR gene(s). Seedling screening of the DS-BILs against Pst78S84 showed wide variation ranging from complete resistance to complete susceptibility (Figs. 1a and 2a) with most of the DS-BILs showing susceptible reaction. Similarly, when DS-BILs were tested at the seedling stage with a mixture of stripe rust pathotypes collected from open field, most genotypes were highly susceptible (YR score of 8) and 12 DS-BILs were moderately susceptible (YR score of 5.33) with only two DS-BILs (DS-BIL6 and DS-BIL16) as completely resistant (YR score of 2.67). However, the DS-BIL panel was completely resistant at the adult plant stage under artificial epiphytotic conditions in the field.

Screening for powdery mildew with a mixture of powdery mildew (*Bg*) pathotypes collected from open field, identified 40 DS-BILs to be completely to moderately resistant with a score ranging from 0 to 3.3 while rest of the DS-BILs were highly susceptible, whereas recipient parent PDW274 showed complete susceptibility while *Ae. speltoides* showed complete resistance with a score of 0 (Figs. 1b and 2c).

### QTL mapping

The results of QTL mapping using RSTEP-LRT for the additive effect of markers and single-marker analysis (SMA) are presented in Table 1. For stripe rust against field mixture of pathotypes, two QTLs (*QYrAs.pau-2A.1* and *QYrAs.pau-7B*) were mapped on chromosomes 2A and 7B with SMA (LOD score 3.8 with PVE 24.51% and LOD score 2.4 with PVE 10.06%) and RSTEP_LRT (LOD score 2.6 with PVE 13.83% and LOD score 2.4 with PVE 10.06%) with resistance allele contributed by *Ae. speltoides*. Also, for the same field mixture of pathotypes, two QTLs were mapped with only SMA, QTL *QYrAs.pau-1B* on chromosome 1B with LOD score 2.0 with PVE 11.64% and QTL *QYrAs.pau-2B* on chromosome 2B with LOD score of 2.2 and PVE 12.8%. These QTLs also had resistance allele contributed by *Ae. speltoides*. For stripe rust, two QTLs, *QYrTd.pau-2A.2* and *QYrTd.pau-3B* were mapped against *Pst* 78S84 on chromosomes 2A and 3B with both algorithms SMA (LOD score 2.9 with PVE 14% and LOD score 4.1 with PVE 19.29%) and RSTEP-LRT (LOD score 2.1 with PVE 9.4% and LOD score 4.1 with PVE 19.29%). PDW274 contributed resistance alleles for both the QTLs. However, the mapping could not be conducted for adult plant data as the whole of the population was resistant.

Similarly, for powdery mildew against field pathotypes at the seedling stage, five QTLs located on chromosomes 2A, 2B, 3B and 6B were detected using both algorithms. With SMA, QTLs *QPmAs.pau-2A.2*, *QPmAs.pau-2B* and *QPmAs.pau-6B* at a LOD score of 2.02 with PVE 11.49%, LOD score of 2.3 with PVE 14.05% and LOD score of 3.2 with PVE 17.75% were detected, respectively. With RSTEP-LRT, QTLs *QPmAs.pau-2A.1*, *QPmAs.pau-3B* and *QPmAs.pau-6B* were detected, respectively at an LOD score of 2.0 with PVE 9.16%, LOD score of 2.4 with PVE 12.89% and LOD score of 3.2 with PVE 17.75%. *Ae.*

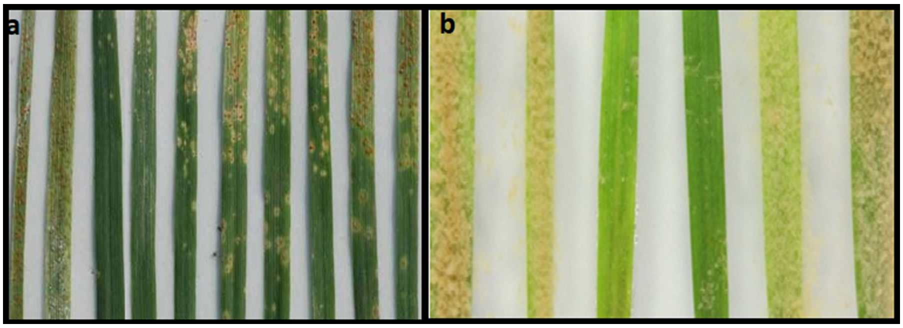

**Fig. 1.** Disease reaction of tested genotypes for (a) stripe rust pathotypes, and (b) powdery mildew pathotypes. The first three leaves represent susceptible check *T. aestivum* cv WL711, *T. durum* cv. PDW274 and *Ae. speltoides* acc pau3809, serially. Succeeding leaves represent reactions of DS-BILs.

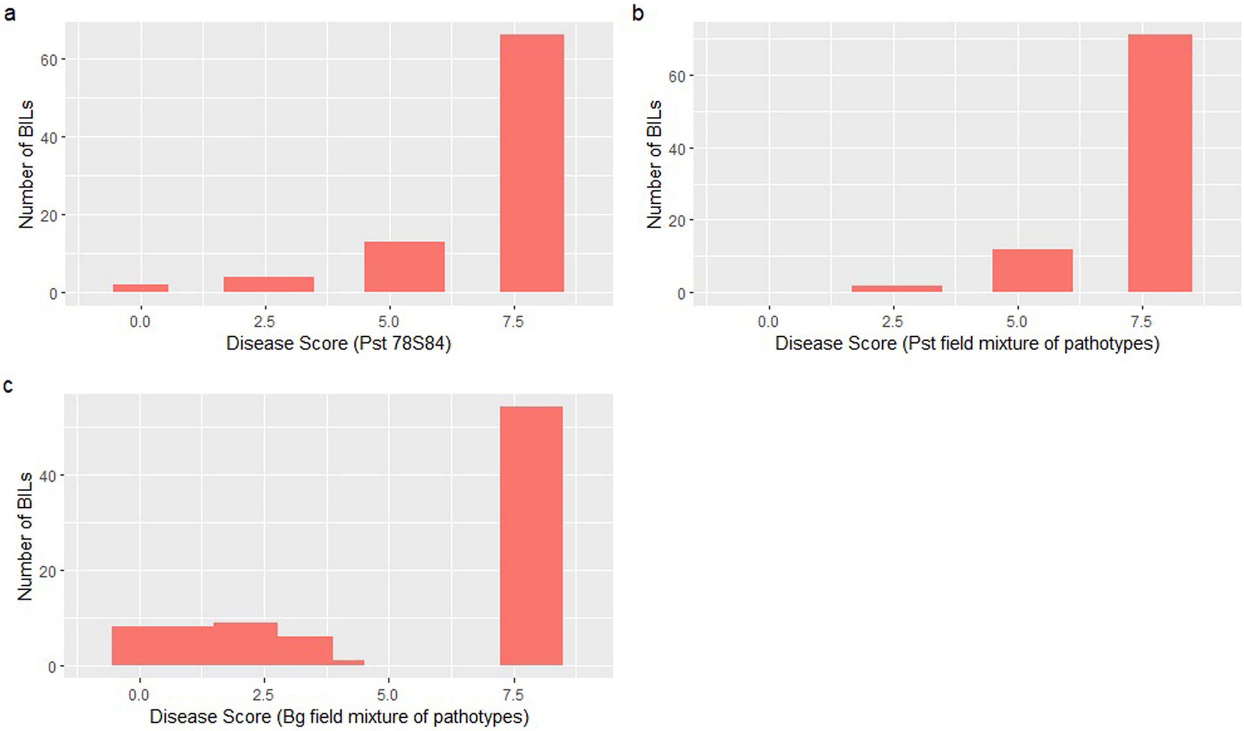

**Fig. 2.** Frequency distribution of the disease score of *T. durum–Ae. speltoides* DS-BILs for (a) stripe rust pathotype Pst 78S84, (b) mixture of pathotypes of stripe rust and (c) mixture of pathotypes of powdery mildew. PDW274 was highly susceptible with reaction score of 8 for all the stripe rust and powdery mildew pathotypes. *Ae. speltoides* acc. pau3809 was highly resistant with reaction score of 0.

*speltoides* contributed all the resistance alleles in these QTLs. Fig. 3 summarizes the mapped QTLs along with the linked markers on the physical map of the DS-BILs.

Since both the parents contributed to resistance for these diseases, QTLs mapped on segments donated by respective parents were studied. Studying the introgression in the resistant lines and the resistance donor fragments, 11 lines carrying multiple QTLs, mapped during this study, were identified. These lines are summarized in Table 2. Out of these six lines, namely DS-BIL6, DS-BIL8, DS-BIL16, DS-BIL18, DS-BIL20 and DS-BIL53 had three or four loci, for YR and PM, whereas other lines had two loci each. DS-BIL6 and DS-BIL16 had all the four loci (*QYrAs.pau-1B*, *QYrAs.pau-2A.1*, *QYrAs.pau-2B* and *QYrAs.pau-7B*) mapped for YR using field mixture of pathotypes and were completely resistant to stripe rust. However, lines with two or three loci showed moderate susceptibility. For PM, *QPmAs.pau-2A.1*, *QPmAs.pau-2B*

**Table 1.** Summary of the QTL mapping using single marker analysis (SMA) and RSTEP-LRT for additive effect of markers algorithms of QTL ICI mapping

| | | | | Marker position | | | | |
|---|---|---|---|---|---|---|---|---|
| | Trait name | QTL | SNP marker name | Chr. | Phy. position (Mb) | LOD | PVE (%) | Add |
| RSTEP-LRT | Pst field pathotypes | *QYrAs.pau-2A.1* | S2A_16016633 | S2A | 16.01 | 2.6 | 13.83 | 0.6621 |
| | Pst field pathotypes | *QYrAs.pau-7B* | S7B_708445814 | S7B | 708.44 | 2.4 | 10.06 | 0.8519 |
| | Pst 78S84 | *QYrTd.pau-2A.2* | S2A_766158316 | S2A | 766.15 | 2.2 | 09.42 | −0.6615 |
| | Pst 78S84 | *QYrTd.pau-3B* | S3B_743818730 | S3B | 743.81 | 4.1 | 19.29 | −1.2189 |
| | Bg field pathotypes | *QPmAs.pau-2A.1* | S2A_43146710 | S2A | 43.15 | 2.0 | 09.16 | 1.7895 |
| | Bg field pathotypes | *QPmAs.pau-3B* | S3B_775092221 | S3B | 775.09 | 2.4 | 12.89 | 2.4370 |
| | Bg field pathotypes | *QPmAs.pau-6B* | S6B_26793381 | S6B | 26.79 | 3.2 | 17.75 | 2.2547 |
| SMA | Pst field pathotypes | *QYrAs.pau-1B* | S1B_626229235 | S1B | 626.22 | 2.0 | 11.64 | 0.4094 |
| | Pst field pathotypes | *QYrAs.pau-2A.1* | S2A_16016633 | S2A | 16.01 | 3.8 | 24.51 | 0.8815 |
| | Pst field pathotypes | *QYrAs.pau-2B* | S2B_27896451 | S2B | 27.89 | 2.2 | 12.80 | 0.4638 |
| | Pst field pathotypes | *QYrAs.pau-7B* | S7B_708445814 | S7B | 708.44 | 2.4 | 10.06 | 0.8518 |
| | Pst 78S84 | *QYrTd.pau-2A.2* | S2A_766158316 | S2A | 766.15 | 2.8 | 14.00 | −0.8065 |
| | Pst 78S84 | *QYrTd.pau-3B* | S3B_743818730 | S3B | 743.81 | 4.1 | 19.29 | −1.2189 |
| | Bg field pathotypes | *QPmAs.pau-2A.2* | S2A_771507864 | S2A | 771.507 | 2.1 | 11.49 | 2.1681 |
| | Bg field pathotypes | *QPmAs.pau-2B* | S2B_791958961 | S2B | 791.958 | 2.3 | 14.05 | 2.2938 |
| | Bg field pathotypes | *QPmAs.pau-6B* | S6B_26793381 | S6B | 26.793 | 3.2 | 17.75 | 2.2547 |

and *QPmAs.pau-3B* provided complete resistance individually. The other two PM QTLs conferred moderate resistance only in combination.

### Introgression profile of DS-BILs

Introgression profile of DS-BILs was studied to visually identify the regions of introgression harbouring QTLs which would finally help in the selection of BILs as a pre-breeding material for rust resistance. The introgression profile is given in online Supplementary Fig. S2. Since *Ae. speltoides* is known to carry genes epistatic to *Ph1* locus of wheat (Millet, 2007; Colas *et al.*, 2008; King *et al.*, 2018), called *Ph* suppressors, which lead to homoeologous recombination of the alien genome with wheat chromosomes and are responsible for introgression of *Ae. speltoides* segments to both A and B genomes of *T. durum*, as seen in online Supplementary Fig. S2.

### Postulation of candidate genes

The regions of 50 kb on both sides of the linked markers with respective QTLs were scanned to identify the candidate genes. The identified genes for each of the QTL mapped in this study are listed in Table 3. All the genes identified were high confidence genes as per annotation v1.1. For each target locus, genes known to be involved in different pathways of pathogen–host interactions and pathogenesis were identified and will be validated by developing mapping populations from the selected DS-BILs. No gene was detected in 50 kb region harbouring QTL *QPmAs.pau-3B*, hence a region of 500 kb was scanned on both sides of the linked SNP and candidate genes around the target loci were identified (Table 3).

## Discussion

Stripe rust and powdery mildew are major constraints to wheat production worldwide. Wheat breeding programmes, to counter these hindrances, need continuous identification and introgression of new disease resistance genes from diverse sources including crop wild relatives. The work in hand describes the transfer and mapping of new genes/QTL for stripe rust and powdery mildew resistance from *Ae. speltoides*. During this study on a set of *T. durum*–*Ae. speltoides* introgression lines, two major genic loci effective against stripe rust pathotype *Pst* 78S84 were identified. Five genic loci were identified against field mixture of *Bg* pathotypes. Four loci were identified against field mixture of *Pst* pathotypes collected from the field. These QTLs were present on the terminal ends of the respective chromosomes (Fig. 3) which have been reported to have recombination hot spots of the chromosomes. The phenotypic data of the DS-BILs showed that a large number of loci might be responsible for resistance reaction as the variability of different reaction types shows the

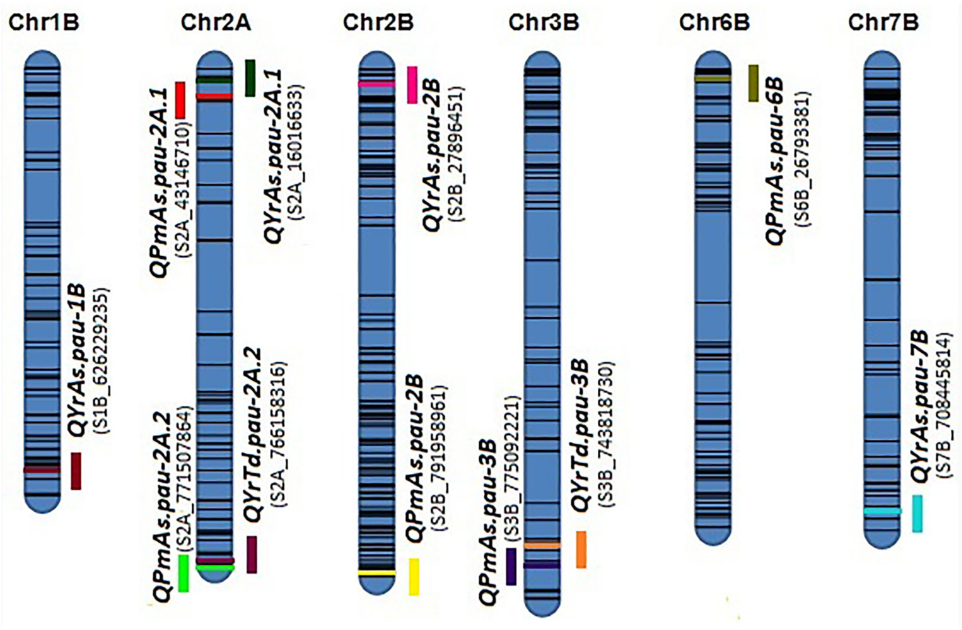

**Fig. 3.** Summary of the mapped QTLs for stripe rust and powdery mildew in the *T. durum–Ae. speltoides* introgression lines. Mapped QTLs are represented as bars alongside the carrier chromosomes. The black lines represent the SNP positions along the length of the chromosomes. The coloured lines on chromosomes and bars alongside represent the positions of QTLs. QTL names, and SNPs linked to the QTLs are presented alongside the coloured bars.

involvement of additive effects of multiple loci. Despite this, 11 loci were identified to provide resistance when mapping was done using RSTEP-LRT for the additive effect of markers and SMA. Since there were few sites on different chromosomes where there was low coverage/density of SNPs, and this hindered identifying QTLs (if present) in these locations. All the QTLs detected in the current study had good marker density (one SNP per 2.03 Mb against genomic average of one SNP per 8.60 Mb) in the genomic regions harbouring target QTL. To explain the residual resistance, which was not mapped in this study, a higher density of SNPs providing more coverage of the genome and a bigger set of the DS-BILs can be used. All the selected DS-BILs with different QTL combinations can be used in breeding programmes for marker-assisted transfer of the respective QTLs to the hexaploid backgrounds providing a good source of resistance.

Various designated genes, temporarily designated genes and QTLs for YR and PM reported from various studies have been compiled in online Supplementary Fig. S3 (source: www.wheat.pw.usda.gov). QTL *QYrAs.pau-1B* reported herein has been found in the vicinity of designated

**Table 2.** Summary of the resistant DS-BILs along with QTLs present in these DS-BILs

| Genotype | QTLs present | | | |
|---|---|---|---|---|
| DS-BIL6 | *QYrAs.pau-1B* | *QYrAs.pau-2A.1* | *QYrAs.pau-2B* | *QYrAs.pau-7B* |
| DS-BIL8 | *QPmAs.pau-2A.2* | *QPmAs.pau-2B* | *QPmAs.pau-6B* | |
| DS-BIL16 | *QYrAs.pau-1B* | *QYrAs.pau-2A.1* | *QYrAs.pau-2B* | *QYrAs.pau-7B* |
| DS-BIL18 | *QPmAs.pau-2A.2* | *QPmAs.pau-2B* | *QPmAs.pau-6B* | |
| DS-BIL20 | *QPmAs.pau-2A.2* | *QPmAs.pau-2B* | *QPmAs.pau-6B* | |
| DS-BIL32 | *QPmAs.pau-2B* | *QPmAs.pau-6B* | | |
| DS-BIL40 | *QPmAs.pau-2A.2* | *QPmAs.pau-6B* | | |
| DS-BIL45 | *QYrTd.pau-3B* | *QYrTd.pau-2A.2* | *QPmAs.pau-6B* | |
| DS-BIL53 | *QPmAs.pau-2A.2* | *QPmAs.pau-2B* | | |
| DS-BIL55 | *QYrTd.pau-2A.2* | *QPmAs.pau-3B* | | |
| DS-BIL56 | *QYrTd.pau-3B* | *QYrTd.pau-2A.2* | | |

**Table 3.** Postulation of genes present in the survey sequence of wheat genome refseqV1.0

| QTL | Trait | Distance from SNP | Chr | Reference gene/gene ID | Function |
|---|---|---|---|---|---|
| *QYrAs.pau-1B* | Pst field pathotypes | +4.59 | chr1B | *TraesCS1B01G393400* | RING/U-box superfamily protein (zinc finger) |
| | | −14.76 | chr1B | *TraesCS1B01G393600* | Disease resistance protein (NBS-LRR class) |
| *QYrAs.pau-2A.1* | Pst field pathotypes | 0 | chr2A | *TraesCS2A01G038100* | L-gulonolactone oxidase |
| *QPmAs.pau-2A.1* | Bg field pathotypes | +13.05 | chr2A | *TraesCS2A01G090000* | 26S protease regulatory subunit |
| | | +1.31 | chr2A | *TraesCS2A01G090100* | Leucine-rich repeat receptor-like protein kinase |
| | | −33.07 | chr2A | *TraesCS2A01G090200* | Zinc-finger protein |
| *QYrTd.pau-2A.2* | Pst 78S84 | +6.45 | chr2A | *TraesCS2A01G567300* | Auxin response factor |
| *QPmAs.pau-2A.2* | Bg field pathotypes | −39.24 | chr2A | *TraesCS2A01G577900* | Glutathione S-transferase |
| *QYrAs.pau-2B* | Pst field pathotypes | 0 | chr2B | *TraesCS2B01G057300* | Zinc finger family protein |
| *QYrTd.pau-3B* | Pst 78S84 | +5.51 | chr3B | *TraesCS3B01G499200* | Glutathione S-transferase |
| *QPmAs.pau-3B* | Bg field pathotypes | +352.75 | chr3B | *TraesCS3B01G535300* | Glutathione S-transferase |
| | | +388.74 | chr3B | *TraesCS3B01G535500* | Protein enhanced disease resistance 2 |
| | | +469.71 | chr3B | *TraesCS3B01G535700* | Glutathione S-transferase |
| *QPmAs.pau-6B* | Bg field pathotypes | +24.08 | chr6B | *TraesCS6B01G044800* | receptor kinase 1 |
| | | 0 | chr6B | *TraesCS6B01G044900* | Mitochondrial transcription termination factor-like |
| | | −47.40 | chr6B | *TraesCS6B01G045000* | Mitochondrial transcription termination factor-like |
| *QYrAs.pau-7B* | Pst field pathotypes | +29.70 | chr7B | *TraesCS7B01G443600* | RING/U-box superfamily protein (zinc finger) |

Distance from SNP represents the distance of start site of the gene to SNP linked with QTL, where + sign represents the gene was found downstream of the SNP and – sign gene was found upstream, 0 represents the SNP was present inside the gene, and all distances are in kilobases.

genes *Yr29/Lr46* and QTL *QYr.cim-1BL* (Lan *et al*., 2014). Similarly, QTL *QYrAs.pau-2A.1* was found in the vicinity of *Yr56* where some QTLs for YR have also been reported like *QYr.sun-2A_Wollaroi*, *QYr.tam-2AS_TAM111*, *QYr.ucw-2A.2(IWA422)*, *QYr.ufs-2A_Cappelle-Desprez_Yr16* and *QYr.inra_2AS.1_Recital* (Maccaferri *et al*., 2015). QTL *QYrAs.pau-7B* has been physically located in the vicinity of stripe rust resistance genes *Yr52* and *Yr59* (McIntosh *et al*., 2017). Interestingly, QTL *QYrTd.pau-3B* mapped from tetraploid donor PDW274 in the current study was found in the same genomic region as QTL *QYrEDWL.par-3BL* which was mapped in the tetraploid background of Ethiopian spring wheat by Liu *et al*. (2017). For PM, QTL *QPmAs.pau-6B* is located in the region harbouring QTL *QPm#66-2B* (Ben-David *et al*., 2014). Other QTLs reported in this study could also be traced to the same arm, or close vicinity of genes/QTLs reported in the literature, as is depicted in online Supplementary Fig. S3. It was not possible to compare exact locations of the other QTLs mapped in this study with reported genes or QTL as most of the reported genes or QTLs have been mapped on the based-on linkage; whereas in the current study, genes/QTLs have been physically mapped to specific chromosome regions.

Studying the annotated reference of wheat genome refseqV1.0 showed the genes present in the genomic regions of mapped QTLs. Various categories of these genes are NBS-LRR protein family responsible for disease resistance, receptor or receptor-like proteins/motifs like Zinc finger, ubiquitin pathway proteins like ubiquitin regulator units and glutathione S transferase (GST), antioxidant pathway enzymes like L-gulonolactone oxidase, various kinases, auxin response factors (ARFs) and mitochondrial transcription termination factor-like (mTERF) proteins (Table 3). All these are known for their action in pathogen recognition, reactions, being involved in various biotic and abiotic stresses or plant-pathogenesis pathways. A complete list of genes is given in online Supplementary Table S1. The role of other genes/proteins in plant pathogenesis is either yet not reported or not well documented.

*QYrAs.pau-1B* and *QPmAs.pau-2A.1* loci harboured genes with NBS-LRR and zinc finger motifs. NBS-LRR genes are from the most abundant disease resistance gene family in plant genomes, and zinc finger motifs have been reported to be major motifs linked with the response of plants to various biotic and abiotic stresses (DeYoung and Innes, 2006; McHale *et al.*, 2006; Lee and Yeom, 2015; Dubey and Singh, 2018). Zinc finger motif was also found in the region of QTL *QYrAs.pau-2B* and *QYrAs. pau-7B*. PM QTL *QPmAs.pau-3B* was found to be located in the genomic region having the gene coding for protein-enhanced disease resistance 2 which induces resistance by negative regulation of salicylic acid in biotrophic pathogens like PM (Tang *et al.*, 2005). Zhang *et al.* (2019) suggested that in wheat, pathogen resistance genes can be activated by alternate splicing regulators in salicylic pathways, down-regulating its synthesis. L-gulonolactone oxidase (in the region of QTLs *QYrAs.pau-2A.1*) is a key enzyme in the formation of ascorbate. Thus, the regulation of this enzyme is essential in the regulation of ascorbate formation in plants (Gullner and Kômíves, 2007). Ascorbate is one of the major antioxidants of plants (Potters *et al.*, 2010; Paciolla *et al.*, 2016) and second, being glutathione, both act against reactive oxygen species (ROS) produced under biotic stresses (Kuźniak, 2010). Being part of the ascorbate–glutathione cycle, it takes part in signal transduction in biotic stress besides regulating the expression of nuclear genes as a response to invading pathogen providing both local and systematic defence (Sarowar *et al.*, 2005; Kuźniak, 2010).

QTL *QPmAs.pau-2A.1* region also annotated gene for regulatory subunit of 26S proteasome subunit. Proteasome, which is a part of the ubiquitin-proteasome system (UPS), functions by removal of misfolded and defective proteins along with eliminating short-lived proteins (Vierstra, 2009). Along with this, various pathways are controlled by UPS which include response to biotic and abiotic stresses (Sadanandom *et al.*, 2012), and acts as one of the major systems in plant immunity (Üstün *et al.*, 2016). Besides immunity, their role in defence responses by the production of ROS and forming hypersensitive reactions were reported (Marino *et al.*, 2012). Üstün *et al.* (2016) showed that proteasome mutants impaired/reduced systematic acquired resistance (SAR) on secondary infection and concluded that proteasome is essential for the pathogen-associated molecular pattern (PAMP) triggered immunity (PTI) and SAR. In a study involving *Arabidopsis* with loss of function mutants, Yao *et al.* (2012) reported that 26S regulatory subunit of proteasome, RPN1a, is essential for resistance. It induced cell death when *Arabidopsis* was infected by powdery mildew, concluding its effect on basal defence and resistance protein-mediated defence. Dielen *et al.* (2010) in a review on UPS (26S) highlighted the involvement of the system in defence mechanisms regardless of pathogen type.

ARF (in the region of QTL *QYrTd.pau-2A.2*) in various studies has been explained as a mediator of auxin to biotic and abiotic stresses (Ghanashyam and Jain, 2009; Fu and Wang, 2011; Bouzroud *et al.*, 2018). Bouzroud *et al.* (2018) reported that ARFs have a vital role in alteration (activation or repression) of the rate of transcription of auxin-responsive genes. Both biotic and abiotic stress-responsive genes are enriched in *cis*-elements of 5′-regulatory units in ARFs. They showed that under stress conditions, ARFs are actively regulated at the post-transcriptional level. Besides this, Fu and Wang (2011) reported that pathogen produced indole acetic acid (IAA) by the action of ARFs can cause either resistance to a necrotrophic pathogen (through ethylene signalling or camalexin biosynthesis), susceptibility by cell wall expansion or stomatal opening (through host IAA biosynthesis or IAA conjugation), basal resistance by IAA conjugation (differential regulation) or resistance to biotrophic pathogen (through indole glucosinolate biosynthesis and/or salicylic acid signalling).

High inducibility of GST in biotic stress in response to bacterial, fungal or viral infection by up-regulation of key defence enzymes has been reported in various studies (Gullner and Kômíves, 2007; Taylor *et al.*, 2012; Gullner *et al.*, 2018). In our study, QTLs *QPmAs.pau-2A.2*, *QYrTd.pau-3B* and *QPmAs.pau-3B* were found to be linked to GST gene/s. These authors have reported that besides its role in detoxification of various toxic substances and as antioxidative in reaction in infected cells, it also regulates the expression of various protective genes. Changes in expression of GSTs are reported to be modifying symptoms of a disease and sometimes the rate of multiplication of pathogens. Some GSTs with peroxidase activity are also known to detoxify lipid hydroperoxidases.

QTL *QPmAs.pau-6B* region was found to carry three genes, one of receptor kinase 1 and two of mTERF gene. Receptor kinases are known to be modulating plant defence responses. Receptor-like kinases (RLKs) and receptor-like proteins (RLPs) act as pattern recognition receptors (PRRs) (Tang *et al.*, 2017) and thus lead to first defence response. Multi-protein immune complexes of PRRs and other RLKs are formed at the surface of interaction. The two broad classes of receptors are, one in the cytoplasm with NB-LRR and the other on the cell surface with RLKs and RLPs (Jones and Dangl, 2006; Jones *et al.*, 2016). In wheat, *TaRLK-R1,2,3* (Zhou *et al.*, 2007) and *LRK10* (Feuillet *et al.*, 1997) have been involved in plant immunity where *TaRLK-R1* has also been cloned (Qin *et al.*, 2012). Wang and Bouwmeester (2017) suggested that PRRs recognize not only the invading organism's surface effectors but also damage-associated molecular patterns. PTI acts as a primary defence, and ETI acts as a secondary defence by recognition of by-products of effector specific resistance genes (Shi *et al.*, 2016). Thus, they both result in biotrophic pathogens' growth reduction. While mTERFs are best known to act against abiotic stresses and since only eight plant mTERFs are known to be characterized, very little is known about their action against biotic stresses (Babiychuk *et al.*, 2011; Vardhan and Kousar, 2015; Chen *et al.*, 2017; Pan *et al.*, 2019). However, mTERFs are known to show changed nuclear gene expression, which could support their role in various stresses.

The inference from this outcome requires studying the functions, activation, deactivation or alteration in the rate of expression of these loci in the process of development of resistance to specific diseases. However, regions having *Ae. speltoides* specific introgression may carry novel genes. In either case, there is a need to study the regions countering resistance at the transcriptional level to evaluate the actual cause of resistance in DS-BILs which would further help in the identification of unique pathways of development of disease resistance genotypes.

While several lines did contain large segment substitutions from *Ae. speltoides*, it is difficult to detect the QTLs that are close to each other with opposite effects. Hence, transferring them to different backgrounds can identify some additional genes or QTL. To conclude, despite the selected 11 DS-BILs being a good source of resistance to YR and PM, only the functional study of the regions could elaborate on the effect of these loci/QTLs in providing disease resistance.

### Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S1479262120000222.

## Acknowledgements

The financial support provided by the Department of Biotechnology, Ministry of Science and Technology, Government of India in the form of DBT Programme Support (grant no. BT/PR/5468/AGR/02/851/2012 dated 15.5.2012) is gratefully acknowledged. The supply of the rust inoculum by the Indian Institute of Wheat and Barley Research Station, Flowerdale, Shimla, India is also gratefully acknowledged.

### Author contribution

GSD conducted disease screening, QTL mapping and manuscript writing; SK, JK and RS helped in the development of material and disease screening; ND supervised the study, helped in writing the manuscript; JP conducted genotyping by sequencing; PC designed the study, provided the basic genetic material, supervised the study and finalised the manuscript. All the authors have read the manuscript and approved it.

### Conflict of interest

The authors declare that they have no conflict of interest.

## References

Awlachew ZT, Singh R, Kaur S, Bains NS and Chhuneja P (2016) Transfer and mapping of the heat tolerance component traits of *Aegilops speltoides* in tetraploid wheat *Triticum durum*. *Molecular Breeding* 36: 78–92.

Babiychuk E, Vandepoele K, Wissing J, Garcia-Diaz M, De Rycke R, Akbari H, Joubès J, Beeckman T, Jänsch L, Frentzen M, Van Montagu MCE and Kushnir S (2011) Plastid gene expression and plant development require a plastidic protein of the mitochondrial transcription termination factor family. *Proceedings of the National Academy of Sciences of the United States of America* 108: 6674–6679.

Bansal M, Kaur S, Dhaliwal HS, Bains NS, Bariana HS, Chhuneja P and Bansal UK (2017) Mapping of *Aegilops umbellulata*-derived leaf rust and stripe rust resistance loci in wheat. *Plant Pathology* 66: 38–44.

Bariana H, Forrest K, Qureshi N, Miah H, Hayden M and Bansal U (2016) Adult plant stripe rust resistance gene Yr71 maps close to Lr24 in chromosome 3D of common wheat. *Molecular Breeding* 36: 98.

Ben-David R, Peleg Z, Dinoor A, Saranga Y, Korol AB and Fahima T (2014) Genetic dissection of quantitative powdery mildew resistance loci in tetraploid wheat. *Molecular Breeding* 34: 1647–1658.

Bouzroud S, Gouiaa S, Hu N, Bernadac A, Mila I, Bendaou N, Smouni AA, Bouzayen M and Zouine M (2018) Auxin response factors (ARFs) are potential mediators of auxin action in tomato response to biotic and abiotic stress (*Solanum lycopersicum*). *PLoS ONE* 13: e0193517 1–20. doi: 10.1371/journal.pone.0193517.

Chen XM (2005) Epidemiology and control of stripe rust [*Puccinia striiformis* f. sp. tritici] on wheat. *Canadian Journal of Plant Pathology* 27: 314–337.

Chen X, Feng F, Qi W, Xu L, Yao D, Wang Q and Song R (2017) Dek35 encodes a PPR protein that affects *cis*-splicing of mitochondrial nad4 intron 1 and seed development in maize. *Molecular Plant* 10: 427–441.

Colas I, Shaw P, Prieto P, Wanous M, Spielmeyer W, Mago R and Moore G (2008) Effective chromosome pairing requires chromatin remodeling at the onset of meiosis. *Proceedings of the National Academy of Sciences of the United States of America* 105: 6075–6080.

Colmer TD, Flowers TJ and Munns R (2006) Use of wild relatives to improve salt tolerance in wheat. *Journal of Experimental Botany* 57: 1059–1078.

DeYoung BJ and Innes RW (2006) Plant NBS-LRR proteins in pathogen sensing and host defense. *Nature Immunology* 7: 1243–1249.

Dielen A-S, Badaoui S, Candresse T and German-Retana S (2010) The ubiquitin/26S proteasome system in plant-pathogen interactions: a never-ending hide-and-seek game. *Molecular Plant Pathology* 11: 293–308.

Dubey N and Singh K (2018) Role of NBS-LRR proteins in plant defense. In: Singh, A. & I. K. Singh (eds.) *Molecular Aspects of Plant-Pathogen Interaction*. Singapore: Springer, pp. 115–138.

Elkot AFA, Chhuneja P, Kaur S, Saluja M, Keller B and Singh K (2015) Marker assisted transfer of two powdery mildew resistance genes PmTb7A.1 and PmTb7A.2 from *Triticum boeoticum* (Boiss.) to *Triticum aestivum* (L.). *PLoS ONE* 10: e0128297.

Feuillet C, Schachermayr G and Keller B (1997) Molecular cloning of a new receptor-like kinase gene encoded at the Lr10 disease resistance locus of wheat. *Plant Journal* 11: 45–52.

Figueroa M, Hammond-kosack KIME and Peter S (2018) A review of wheat diseases – a field perspective. *Molecular Plant Pathology* 19: 1523–1536.

Fu J and Wang S (2011) Insights into auxin signaling in plant? Pathogen interactions. *Frontiers in Plant Science* 2: 1–7. doi: 10.3389/fpls.2011.00074.

Ghanashyam C and Jain M (2009) Role of auxin-responsive genes in biotic stress responses. *Plant Signaling & Behavior* 4: 846–848.

Glaubitz JC, Casstevens TM, Lu F, Harriman J, Elshire RJ, Sun Q and Buckler ES (2014) TASSEL-GBS: a high capacity genotyping by sequencing analysis pipeline. *PLoS ONE* 9: 1–12.

Gullner G and Kômíves T (2007) Defense reactions of infected plants: roles of glutathione and glutathione S-transferase enzymes. *Acta Phytopathologica et Entomologica Hungarica* 41: 3–10.

Gullner G, Komives T, Király L and Schröder P (2018) Glutathione S-transferase enzymes in plant-pathogen interactions. *Frontiers in Plant Science* 9: 1–19. doi: 10.3389/fpls.2018.01836.

Jones JDG and Dangl JL (2006) The plant immune system. *Nature* 444: 323–329.

Jones JDG, Vance RE and Dangl JL (2016) Intracellular innate immune surveillance devices in plants and animals. *Science* 354: 1–8. doi: 10.1126/science.aaf6395.

Kankwatsa P, Singh D, Thomson PC, Babiker EM, Bonman JM, Newcomb M and Park RF (2017) Characterisation and genome-wide association mapping of resistance to leaf rust, stem rust and stripe rust in a geographically diverse collection of spring wheat landraces. *Molecular Breeding* 37: 113.

Kaur S, Jindal S, Kaur M and Chhuneja P (2018) Utilisation of wild species for wheat improvement using genomic approaches. In: *Biotechnologies of Crop Improvement*, vol. 3, Switzerland: Springer International Publishing, pp. 105–150.

King J, Grewal S, Yang CY, Hubbart Edwards S, Scholefield D, Ashling S, Harper JA, Allen AM, Edwards KJ, Burridge AJ and King IP (2018) Introgression of *Aegilops speltoides* segments in *Triticum aestivum* and the effect of the gametocidal genes. *Annals of Botany* 121: 229–240.

Kishii M (2019) An update of recent use of *Aegilops* species in wheat breeding. *Frontiers in Plant Science* 10: 585.

Kuźniak E (2010) The ascorbate-gluathione cycle and related redox signals in plant-pathogen interactions. In: Anjum, N.A., S. Umar, & M.T. Chan (eds.) *Ascorbate-Glutathione Pathway and Stress Tolerance in Plants*. Netherlands: Springer, pp. 115–136.

Lan C, Rosewarne GM, Singh RP, Herrera-Foessel SA, Huerta-Espino J, Basnet BR, Zhang Y and Yang E (2014) QTL characterisation of resistance to leaf rust and stripe rust in the spring wheat line francolin#1. *Molecular Breeding* 34: 789–803.

Lan C, Hale IL, Herrera-Foessel SA, Basnet BR, Randhawa MS, Huerta-Espino J, Dubcovsky J and Singh RP (2017) Characterisation and mapping of leaf rust and stripe rust resistance loci in hexaploid wheat lines UC1110 and PI610750 under Mexican environments. *Frontiers in Plant Science* 8: 1–11.

Lee HA and Yeom SI (2015) Plant NB-LRR proteins: tightly regulated sensors in a complex manner. *Briefings in Functional Genomics* 14: 233–242.

Liu Y, Wang L, Deng M, Li Z, Lu Y, Wang J, Wei Y and Zheng Y (2015) Genome-wide association study of phosphorus-deficiency-tolerance traits in *Aegilops tauschii*. *TAG. Theoretical and Applied Genetics. Theoretische Und Angewandte Genetik* 128: 2203–2212. doi: 10.1007/s00122-015-2578-x.

Liu W, Maccaferri M, Rynearson S, Letta T, Zegeye H, Tuberosa R, Chen X and Pumphrey M (2017) Novel sources of stripe rust resistance identified by genome-wide association mapping in Ethiopian durum wheat (*Triticum turgidum* ssp. durum). *Frontiers in Plant Science* 8: 774–788.

Maccaferri M, Zhang J, Bulli P, Abate Z, Chao S, Cantu D, Bossolini E, Chen X, Pumphrey M and Dubcovsky J (2015) A genome-wide association study of resistance to stripe rust (*Puccinia striiformis* f. sp. tritici) in a worldwide collection of hexaploid spring wheat (*Triticum aestivum* L.). *G3: Genes, Genomes, Genetics* 5: 449–465.

Marino D, Peeters N and Rivas S (2012) Ubiquitination during plant immune signaling. *Plant Physiology* 160: 15–27. doi: 10.1104/pp.112.199281.

McHale L, Tan X, Koehl P and Michelmore RW (2006) Plant NBS-LRR proteins: adaptable guards. *Genome Biology* 7: 1–11. doi: 10.1186/gb-2006-7-4-212.

McIntosh RA, Dubcovsky J, Rogers WJ, Morris C and Xia XC (2017) Catalogue of Gene Symbols for Wheat: 2017 Supplement, 1–20.

Millet E (2007) Exploitation of *Aegilops* species of section Sitopsis for wheat improvement. *Israel Journal of Plant Sciences* 55(3–4): 277–287. doi: 10.15607.

Monneveux P, Zaharieva M, and Rekika D (2000) The utilisation of Triticum and Aegilops species for the improvement of durum wheat. In: C. Royo, M. Nachit, N. Di Fonzo, & J. L. Araus, (eds.) *Durum wheat improvement in the Mediterranean region: New challenges*, Zaragoza: CIHEAM, pp. 71–81. (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 40).

Niks RE, Qi X and Marcel TC (2015) Quantitative resistance to biotrophic filamentous plant pathogens: concepts, misconceptions, and mechanisms. *Annual Review of Phytopathology* 53: 445–470.

Paciolla C, Paradiso A and De Pinto MC (2016) Cellular redox homeostasis as central modulator in plant stress response. In: Gupta, D. K., J. M. Palma, & F. J. Corpas (eds.) *Redox State as a Central Regulator of Plant-Cell Stress Responses*, Cham, Switzerland: Springer International Publishing. pp. 1–23.

Pan Z, Ren X, Zhao H, Liu L, Tan Z and Qiu F (2019) A mitochondrial transcription termination factor, ZmSmk3, is required for nad1 Intron4 and nad4 Intron1 splicing and kernel development in maize. *G3 : Genes Genomes Genetics* 9: 2677–2686.

Potters G, Horemans N and Jansen MAK (2010) The cellular redox state in plant stress biology – a charging concept. *Plant Physiology and Biochemistry* 48: 292–300.

Qin B, Chen T, Cao A, Wang H, Xing L, Ling H, Wang D, Yu C, Xiao J, Ji J, Chen X, Chen P, Liu D and Wang X (2012) Cloning of a conserved receptor-like protein kinase gene and its use as a functional marker for homoeologous group-2 chromosomes of the Triticeae species. *PLoS ONE* 7. e49718: 1–11. doi: 10.1371/journal.pone.0049718.

Rawat N, Tiwari VK, Singh N, Randhawa GS, Singh K, Chhuneja P and Dhaliwal HS (2008) Evaluation and utilisation of *Aegilops* and wild *Triticum* species for enhancing iron and zinc content in wheat. *Genetic Resources and Crop Evolution* 56: 53.

Sadanandom A, Bailey M, Ewan R, Lee J and Nelis S (2012) The ubiquitin-proteasome system: central modifier of plant signalling. *New Phytologist* 196: 13–28. doi: 10.1111/j.1469-8137.2012.04266.x.

Saghai-Maroof MA, Soliman KM, Jorgensen RA and Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences* 81: 8014 LP–8018.

Sarowar S, Eui NK, Young JK, Sung HO, Ki DK, Byung KH and Jeong SS (2005) Overexpression of a pepper ascorbate peroxidase-like 1 gene in tobacco plants enhances tolerance to oxidative stress and pathogens. *Plant Science* 169: 55–63.

Shi G, Zhang Z, Friesen TL, Raats D, Fahima T, Brueggeman RS, Lu S, Trick HN, Liu Z, Chao W, Frenkel Z, Xu SS, Rasmussen JB and Faris JD (2016) The hijacking of a receptor kinase-driven pathway by a wheat fungal pathogen leads to disease. *Science Advances* 2: 1–9. doi: 10.1126/sciadv.1600822.

Tang D, Ade J, Frye CA and Innes RW (2005) Regulation of plant defense responses in Arabidopsis by EDR2, a PH and START domain-containing protein. *Plant Journal* 44: 245–257.

Tang D, Wang G and Zhou JM (2017) Receptor kinases in plant-pathogen interactions: more than pattern recognition. *Plant Cell* 29: 618–637.

Taylor P, Street M, Wt L, Signaling P, Dubreuil-maurizi C, Poinssot B, Dubreuil-maurizi C and Poinssot B (2012) Stress role of glutathione in plant signaling under biotic stress© 2012 Landes Bioscience. doi: 10.4161/psb.18831.

Üstün S, Sheikh A, Gimenez-Ibanez S, Jones A, Ntoukakis V and Börnke F (2016) The proteasome acts as a hub for plant immunity and is targeted by *Pseudomonas* type III effectors. *Plant Physiology* 172: 1941–1958.

van Berloo R (2008) GGT 2.0: versatile software for visualization and analysis of genetic data. *Journal of Heredity* 99: 232–236.

Vardhan H and Kousar S (2015) Plant mitochondrial omics: state-of-the-art knowledge. In: Barh D., M. Khan, E. Davies (eds) *PlantOmics: The Omics of Plant Science*. India: Springer, pp. 573–614.

Venkata B, Bullet U, Bansal U, bullet R, Singh M, Robert B, Park R, Harbans B and Bariana H (2008) Genetic analyses of durable adult plant resistance to stripe rust and leaf rust in CIMMYT wheat genotype 11IBWSN50. *International Journal of Plant Breeding* 2: 64–68.

Vierstra RD (2009) The ubiquitin-26S proteasome system at the nexus of plant biology. *Nature Reviews Molecular Cell Biology* 10: 385–397. doi: 10.1038/nrm2688.

Visioni A, Gyawali S, Selvakumar R, Gangwar OP, Shekhawat PS, Bhardwaj SC, Al-Abdallat AM, Kehel Z and Verma RPS (2018) Genome wide association mapping of seedling and adult plant resistance to barley stripe rust (*Puccinia striiformis* f. sp. hordei) in India. *Frontiers in Plant Science* 9: 1–13.

Wang Y and Bouwmeester K (2017) L-type lectin receptor kinases: new forces in plant immunity. *PLoS Pathogens* 13: 1–7. doi: 10.1371/journal.ppat.1006433.

Wang J, Li H, Zhang L and Meng L (2016) Integrated Software for Linkage Analysis and Genetic Mapping in Biparental Populations, Institute of Crop Science Chinese Academy of Agricultural Sciences (CAAS) Beijing 100081, China and Genetic Resources Program International Maize and Wheat Improvement Center (CIMMYT) Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico, pp. 1–274.

Yang L, Zhang X, Zhang X, Wang J, Luo M, Yang M, Wang H, Xiang L, Zeng F, Yu D, Fu D and Rosewarne GM (2017) Identification and evaluation of resistance to powdery mildew and yellow rust in a wheat mapping population. *PLoS ONE* 12: 1–18. doi: 10.1371/journal.pone.0177905.

Yao C, Wu Y, Nie H and Tang D (2012) RPN1a, a 26S proteasome subunit, is required for innate immunity in Arabidopsis. *Plant Journal* 71: 1015–1028. doi: 10.1111/j.1365-313X.2012.05048.x.

Zhang W, Zhang M, Zhu X, Cao Y, Sun Q, Ma G, Chao S, Yan C, Xu SS and Cai X (2018) Molecular cytogenetic and genomic analyses reveal new insights into the origin of the wheat B genome. *Theoretical and Applied Genetics* 131: 365–375.

Zhang H, Mao R, Wang Y, Zhang L, Wang C, Lv S, Liu X, Wang Y and Ji W (2019) Transcriptome-wide alternative splicing modulation during plant-pathogen interactions in wheat. *Plant Science* 288: 110160.

Zhou H, Li S, Deng Z, Wang X, Chen T, Zhang J, Chen S, Ling H, Zhang A, Wang D and Zhang X (2007) Molecular analysis of three new receptor-like kinase genes from hexaploid wheat and evidence for their participation in the wheat hypersensitive response to stripe rust fungus infection. *Plant Journal* 52: 420–434.