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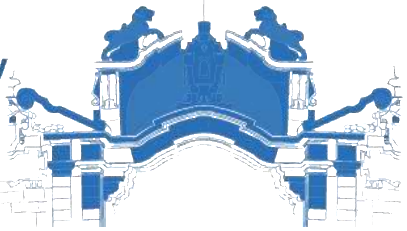

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Genetic and Virulence Variability of *Zymoseptoria tritici* Populations; and Genome-Wide Association Studies for Septoria Tritici Bloch Resistance and Yield Potential in Wheat (*Triticum aestivum* L.) in Ethiopia

PhD Dissertation

Tilahun Mekonnen Negassa

August, 2021

Addis Ababa, Ethiopia

Genetic and Virulence Variability of *Zymoseptoria tritici* Populations; and Genome-Wide Association Studies for Septoria Tritici Bloch Resistance and Yield Potential in Wheat (*Triticum aestivum* L.) in Ethiopia

Tilahun Mekonnen Negassa

A Thesis Submitted to the Institute of Biotechnology of the Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Biotechnology

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August, 2021
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ADDIS ABABA UNIVERSITY
INSTITUTE OF BIOTECHNOLOGY
PhD THESIS APPROVAL SHEET

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By

Tilahun Mekonnen Negassa

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STATEMENT OF AUTHOR

I, Tilahun Mekonnen Negassa, hereby declare and affirm that this dissertation and its entirety is an authentic record of my own work and has been submitted in partial fulfillment of the requirements for a PhD degree in Biotechnology at Addis Ababa University. Any source material used in the dissertation has been duly acknowledged. I solemnly assert that this dissertation or its part has not been submitted to any other institution anywhere for an award of any academic degree. Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgement is made. Requests for permission for extended quotation from or reproduction of this dissertation in whole or in part may be granted by the director of the Institute of Biotechnology when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

05 August, 2021

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Finally, I wish to extend a warm thanks to everybody who was involved directly or indirectly in my work.

LIST OF ACRONYMS

AC	Accessory Chromosomes
ADT	Adaptation trial
ANOVA	Analysis of variance
BIC	Bayesian information criterion
BLINK	Bayesian-information and linkage-disequilibrium iteratively nested keyway
CC	Core Chromosomes
CIMMYT	International Maize and Wheat Improvement Center
cM	Centi Morgan
CMLM	Compressed Mixed Linear Mode
CTAB	Cetyltrimethylammonium bromide
DMSO	Dimethyl sulfoxide
ECMLM	Enriched compressed mixed linear model
FarmCPU	Fixed and random model circulating probability unification
FHB	Fusarium head blight
GAPIT	Genome Association and Prediction Integrated Tools
GBS	Genotyping-by-Sequencing
GLM	General linear model
GWAS	Genome-wide association study
HR	Hypersensitive response
HRWSN	high-rain wheat screening nursery
HRWYT	high rain wheat yield trial
IBWSN	International bread wheat screening nursery
ICGEB	International Centre for Genetic Engineering and Biotechnology
ISEPON	International Septoria observation nursery
IWGSC	International Wheat Genome Sequencing Consortium
LD	Linkage disequilibrium
MAPK	Mitogen-activated protein kinase
MAS	Marker Assisted Selection

Mbp	Mega base pair
MLM	Mixed liner model
NLA	Necrosis leaf area
NVT	National variety trial
PC	Pycnidia coverage
PCA	Principal component analysis
PCR	Polymerase chain reaction
PVT	Preliminary variety trial
Q-Q	Quantile-Quantile
QTL	Quantitative trait locus
R gene	Resistant gene
ROS	Reactive oxygen species
SA	Salicylic acid (SA)
SAR	Systemic acquired resistance
SNP	Single nucleotide polymorphism
SPL	Scientific Phytopathological Laboratory
SSR	Simple sequence repeat
STB	Septoria tritici blotch
Stb genes	Septoria resistance genes
SUPER	Settlement of mixed linear model under progressively exclusive relationship
TASSEL	Trait Analysis by Association, Evolution and Linkage
WGS	Whole genome sequence

DEDICATION

This PhD dissertation is dedicated to GOD Almighty for giving me this opportunity which would not have been possible otherwise. It is also dedicated to all members of my family, especially to my: spouse, Fikade Lulu; Kids, Latera, Kenassa, Feneti and Ebassa Tilahun; father, Mekonnen Negassa; and mother, Abdene Feyissa.

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DECLARATION

I confirm that this is my own work and all materials used from other sources have been properly and fully acknowledged.

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Date……..

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Genetic and Virulence Variability of Zymoseptoria tritici Populations; and Genome-Wide Association Studies for Septoria Tritici Bloch Resistance and Yield Potential in Wheat (*Triticum aestivum* L.) in Ethiopia

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Addis Ababa University, 2021

ABSTRACT

Septoria tritici blotch (STB) caused by the fungus *Zymoseptoria titici* is the major bottleneck to wheat production in Ethiopia and worldwide. The present study was targated to investigate genetic and virulence variability of *Zymoseptoria tritici* populations; and Genome-Wide association studies for STB resistance and yield potential in bread wheat in Ethiopia. Genetic structure analysis of 182 *Z. tritici* isolates representing eight populations from major wheat growing areas of Ethiopia using 14 polymorphic microsatellite markers confirmed the presence of high genetic diversity (h = 0.45) where 92% of the total genetic variation resides within populations. Cluster (UPGMA), PCoA and STRUCTURE analyses did not group the *Z. tritici* populations into sharply genetically distinct clusters according to their geographical areas of sampling. All individual samples shared alleles from two subgroups (K=2). Pathogenicity assay conducted with eight *Z. tritici* isolates on a set of 45 bread wheat genotypes showed highly significant differences for isolate-by-genotype interaction on both percentage of necrosis leaf area (NLA) and pycnidia coverage (PC). The isolates showed significantly higher differences in virulence patterns. Isolate I_1 sourced from Bale was found to be the most virulent (93%) and aggressive (NLA = 58%) isolate. Among tested *Stb* genes, *Stb16* conferred broad-spectrum resistance to 75% of the isolates. Three genotypes (MURGA, Km7 and Hidase) conferred the greatest level of seedling resistance to the tested isolates. Molecular screening for major *Stb* genes in 180 genotypes using 16 tightly linked markers identified a number of *Stb* genes ranging from 6.67% for *Stb12* to 96.1% for *Stb13* gene. Field evaluation conducted on 180 wheat genotypes across six environments revealed a very highly significant difference among the wheat genotypes for most traits. Top 5% selected genotypes showed 10.8 - 21.99% and 52.25-74.25% superior grain yield performance and STB resistance to standard check (King-bird), respectively. Moreover, population structure, linkage disequilibrium (LD), and GWAS for adult-plant STB resistance, yield, yield- related and phenological traits were conducted for 178 wheat genotypes using 7,776 SNPs resulted from Illumina HiSeq2500 Genotype-by-sequencing.

STRUCTURE and PCA confirmed two sub-populations with greater degree of genetic admixture. In all chromosomes, LD between pairs of SNPs declined at $r^2 = 0.2$ within physical distance of 2.26 - 105.62 Mbp, with an overall mean of 31.44 Mbp.The association analysis identified 53 loci significantly associated with STB resistance pointing to 33 putative QTLs. Most of these shared similar chromosome locations with already published Septoria resistance genes and QTLs, and were distributed across chromosomes 1B, 1D, 2A, 2B, 2D, 3A, 3B, 3D, 4A, 5A, 5B, 6A, 7A, 7B and 7D. However, five of the putative QTLs identified on chromosomes 1A, 5D and 6B appeared to be novel. Predicting the possible functions of the detected loci from the IWGSC RefSeq Annotation v2.1 revealed the existence of disease resistance-associated genes in the identified QTL regions which are involved in plant defense responses. Seven of the QTLs for STB resistance also co-localized with MTAs for agronomic traits. In conclusion, the study provides new insights into the genetic structure and virulence variability of *Z. tritici* in Ethiopia to design effective and durable management strategies. The identified *Stb* genes, QTLs and resistance source materials could be used in wheat genome-assisted resistance breeding against STB. Therefore, the information generated in the present study is useful to improve STB resistance and grain yield performance in wheat which ultimately leads to increased and stable wheat production to ensure food security in Ethiopia and the region at large.

Key Words: DArTSeq, Genetic diversity, GWAS, LD, PCA, QTL, SSR marker, *Stb* genes, STB, Virulence variability, Wheat, *Zymoseptoria titici*

CHAPTER 1

1. Introduction

1.1 General Introduction

By 2050, the world population is predicted to be over nine billion (Jaggard *et al*., 2010), and food production needs to increase by 25 –70% above the present level to meet the rising demand (FAO, 2009). Currently, one in nine people are undernourished globally, and ensuring access to food by all people and ending malnutrition demands achieving the 2030 targeted "Global Goal 2: Zero Hunger!" through doubling agricultural productivity and incomes of small-holder farmers (UNDP, 2015). This can be achieved through ensuring access to advanced agricultural technologies, agricultural inputs, and solving other production constraints.

Wheat (*Triticum aestivum* L. 2n = 6x = 42, AABBDD) is the major staple food crop of the world, supplying almost 21% of the total calories and 20% of the protein demand for about 4.5 billion people globally (Braun *et al.*, 2010; Arzani and Ashraf, 2017; IWGSC, 2018; Ramadas *et al*., 2019). It is grown on more land area (219 million ha) than any other food crop followed by maize (197 million ha) and then rice (167 million ha) (FAOSTAT, 2019). In 2019, its production was 765.8 million tonnes making the crop the second most-produced cereal crop after maize (FAOSTAT, 2019). China, India, Russia, USA, France and Canada are the world's top five wheat-producing countries (FAOSTAT, 2019).

Since the mid 1990's, wheat consumption in all Africa countries has increased considerably faster than any other major food grain likely due to population growth, rapid urbanization and changing dietary preferences from cereal grains like maize, sorghum, and millet, and tubers to wheat (Morris and Byerlee, 1993). This has resulted in an increasing reliance on wheat imports, as the domestic wheat production in Africa has failed to meet the growing demand. Sub-Saharan African (SSA) countries also experience a huge gap between wheat production and consumption, and hence, it is vital for the region to increase its domestic wheat production to ensure sustainable growth (Asfaw Negassa *et al.*, 2013).

In Ethiopia, wheat is one of the major staple and strategic food security crop (Eyob Bezabeh *et al.*, 2015) ranking fourth after Teff (*Eragrostis tef*), Maize (*Zea mays*) and Sorghum (*Sorghum bicolor*) in area coverage and third after Maize and Teff in total production (CSA, 2019). Both bread wheat and durum wheat are widely cultivated for multiple uses including as food, animal feed and income generation with the former representing about 80% of wheat areas of the country (Asfaw Negassa *et al*., 2013). Oromia, Amhara, Tigray, and Southern Nations, Nationalities and Peoples' Region (SNNPR) represent 99% of the country's wheat area. Arsi, Bale and Shewa zones of Oromia alone account for 75.5% of the national wheat production (Tesfaye Gemechu and Fiseha Tadese, 2018).

During the last 15 years, wheat harvest area, production and productivity in Ethiopia had increased from 1.40 million ha, 2.18 million metric tonnes and 1.56 t ha^{-1} in 2005 to 1.79 million ha, 5.32 million metric tonnes and 2.97 t ha^{-1} in 2019, respectively (FAOSTAT, 2019). However, the current national average wheat productivity of 2.97 t ha^{-1} is

considerably lower than the global average of 3.5 t ha^{-1}, resulting in production deficit to meet the rising demand (Tesfaye Gemechu and Fiseha Tadese, 2018). The low productivity could be due to limited access to advanced production technologies, low agricultural inputs, and biotic and abiotic stresses (Alemar Said, 2016) exacerbated by climate change.

Among biotic stress, Septoria tritici blotch (STB) caused by a hemibiotrophic haploid fungus *Mycosphaerella graminicola* (Fuckel) J. Schröt in Cohn (anamorph *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous, formerly called *Septoria tritici*) is one of the major bottleneck for wheat production worldwide (Dalvand *et al.,* 2018; Odilbekov *et al*., 2019). Globally, wheat yield losses of 30 – 60% (Shipton *et al*., 1971; Eyal *et al*., 1987) have been attributed to Septoria infestations with the diseases affecting grain yield by causing reduced tillering, poor seed set, shriveled kernels, death of leaves, spikes or the entire plant (van Ginkel and Rajaram, 1999). STB is also a major threat to wheat production in Ethiopia (Stewart and Dagnachew Yirgou, 1967; Yosef Geberehawariat *et al*., 2017; Tilahun Mekonnen *et al.,* 2019, 2020) causing 25- 82 % grain yield loss in the worst seasons (Getinet Gebeyehu *et al.,* 1990; Mengistu Huluka *et al*., 1991). STB is mainly a foliar disease and the primary infections may arise from airborne or rain-splashed asexual pycnidiospores and sexual ascospores from infested crop debris or susceptible grass species (Eyal *et al.,* 1987).

Genetic resistance is the best strategy, durable, economical and environmentally friendly method for controlling cop diseases including STB (Ghanei *et al*., 2012; Miedaner *et al*., 2013; Makhdoomi *et al*., 2015; Odilbekov *et al*., 2019). Conventional, phenotype-based

selection predominates in Ethiopia making the crop improvement program very slow and inefficient. There is also a dearth of information on the genetic structure and virulence patterns of the *Z. tritici* populations in Ethiopia. Moreover, screening of large sets of wheat germplasm in the target environment to identify best sources of STB resistance is limited. Furthermore, the application of cutting-edge genomic tools to uncover the genetic architecture of STB resistance in bread wheat is seriously lacking. Therefore, the present study was conducted to investigate the genetic structure and virulence variability of the *Z. tritici* populations, and also to identify the genetic architecture of STB resistance and yield potential in bread wheat (*T. aestivum* L.) association panel in Ethiopia.

1.2 General Objective:

- To investigate the genetic and virulence variability of *Z. tritici* populations of Ethiopia, and to identify source of resistance to STB and yield potential in bread wheat (*Triticum aestivum* L.) through integrated application of phenotype, SSR and GWAS approaches.

1.2.1 Specific Objectives

- To assess the genetic diversity and population structure of *Z. tritici* populations of major wheat-growing areas of Ethiopia using microsatellite markers.
- To investigate virulence variability of Ethiopian *Z. tritici* isolates and efficacy of wheat genotypes and *Stb* resistance genes against the isolates.
- To evaluate larger set of wheat genotypes for yield, yield components and other agronomic traits at multiple locations and years to identify high yielding and stable genotypes.
- To search for *Stb* genes in 180 wheat genotypes using tightly linked SSR markers.
- To elucidate population structure, Linkage Disequilibrium (LD) and genomic architecture of adult-plant resistances to STB and important agronomic traits in bread wheat association panel using high-density SNP markers.

CHAPTER 2

2. Literature Reviews

2.1 Origin, Domestication and Distributions of Wheat

Bread wheat *(Triticum aestivum* L.) is thought to have originated in the Fertile Crescent of Middle East presently occupied by Syria, Jordan, Iraq, Iran and Turkey through two rounds of hybridizations (Feldman,1976**;** Feldman *et al*., 1995; Ozkan *et al.,* 2001; Peng *et al.,* 2011). The first round of polyploidization occurred about 0.5 – 0.36 million years ago between *Triticum urartu* (2n = 2x = 14, A genome source) with unknown source of the B genome (most likely *Aegilops speltoides,* 2n = 2x = 14) which resulted the wild emmer wheat *Triticum turgidum ssp. dicocoides* (2n = 4x = 28, AABB) which later domesticated as emmer wheat and gave rise to the modern durum wheat cultivars. The second round of hybridization occurred between the wild grass *Aegilops tauschii* (2n = 2x = 14, D genome source) and *T. turgidum ssp. dicocoides* (AABB) about 10,000 years ago to give rise to the modern hexaploid bread wheat (*T. aestivum* L.) (Kihara, 1944; McFadden and Sears, 1946; Feldman *et al*., 1995; Huang *et al*., 2002).

Various archeological findings evidenced that modern wheat domestication had begun in the Middle East. About 9,000 years old primitive relatives of present day bread wheat were discovered in eastern Iraq (Herlan, 1981; Heun, 1997; Gibson and Benson, 2002; Salamini, 2002; Balfourier *et al.,* 2019). Moreover, grains of wheat were discovered in archaeological remains dating back to 6 500 BC and 5,500 BC in Ali Koshi in Iranian Khusistan and in Anatolia in Turkey, respectively. There are also evidences that bread wheat was grown in

the Nile Valley, India, China, and England at about the same time about 5,000 B.C (Gibson and Benson, 2002).

From Middle East, bread wheat spread to Mediterranean countries, North Africa, Asia, Europe, and Russia (Herlan, 1981). It spread to Latin America, South Africa and North America in 1,500 AD and then to Australia in 1,790 AD (Bonjean and Angus, 2001). Today, wheat is one of the most widely cultivated cereal food crop in the world extending from 67° North to 45° South under varied agro-ecological conditions and altitudes (from sea level to 4500m) confirming its wide adaptability. It is grown in more than 85 countries on about 219 million hectares with 765.8 million megatons production each year (FAOSTAT, 2019).

From1970s onwards, wheat production made a quantum jump that triggered the "Green Revolution" due to the introduction of semidwarf high yielding wheat varieties, establishment of a free and unrestricted distribution of improved varieties, large investment on fertilizers, irrigation and transportation infrastructure, strong commitment of developing nations for food self-sufficiency, establishment of conducive agricultural policy environment, and good crop management practices (Pal and Byerlee, 2005). Due to their significant increas in productivity, more than 70% of wheat cultivars grown globally by the late 1990s possessed one of the original semi-dwarfing genes (Evans, 1998). Its broader adaptability, higher productivity, responsiveness to agricultural inputs and multiple uses made wheat crop of choice to be widely cultivated in Ethiopia. In Ethiopia, it grows between 6 and 16°N and 35 and 42°E at an altitude ranging from 1500 to 3000 m above sea level.

The most suitable agro-ecological zones, however, are between the 1900 and 2700 m.a.s.l (Hailu Gebre-Mariam, 1991).

2.2 Morphology and Reproductive Biology of Wheat

Bread wheat is a monocot grass plant that has a 0.7 to 1.2 m long single main stem (culm) plus 2 - 3 tillers per plant that arise from the axils of the basal leaves. The tillers number can vary based on genetic feature, growing conditions, seedling depth and crop density. Only developed tillers can produce an ear and others may die before maturity. The hallow culms have five to seven nodes, and elongated internodes, with its diameter reducing gradually towards the peduncle which bears the spike. A leaf arises at each node, and a culm can produce three to four foliage leaves. The leaf consists of a tubular leaf sheath that wraps the culm and extends from the node to which it is attached to the next higher node, and a long, narrow, flat and parallel veinated blade (lamina). At the junction of the sheath and lamina, there is a small membranous structure called the auricles wrapping around the stem (Abou-Taleb and Abd – El, 2013).

Wheat produces two types of roots: the seminal and nodal roots. The seminal roots develop from the seed. About six root primordia are present in the embryo. At germination, the primary root bursts through the coleorhiza, followed by the emergence of four or five lateral seminal roots. These form the seminal root system, which may grow to 2 m in depth and support the plant until the nodal roots appear. The nodal, crown or adventitious roots

develop from the lower nodes, and are associated with tillers and become increasingly important as the plant grows (Kirby and Appleyard, 1987).

A wheat plant develops a spike (head) on its top having spikelets (florets) arranged on opposite sides of the flat main axis or rachis. Spikelets are separated by short internodes. Each spikelet is a condensed reproductive shoot consisting of two sterile bracts (glumes) that enclose 3-5 florets. The glumes enclose two to five florets that are born on a short axis, or rachilla. The florets consist of two bract-like structures, the lemma and the palea, which covers the reproductive organs. The lemma extends to form the awns that may be short, long or absent (awnless). Wheat florets contain three stamens with large anthers and a pistil that bears a single ovary, with a single ovule, two styles, and two branching plumose stigmas at the end of each style. Wheat is predominantly (91%) self-pollinated plant with less than nine percent of out-crossing (Lersten, 1987; Hucl, 1996).

On the base of their growing seasons and commercial uses, there are several kinds of wheat. Based on growing seasons wheat can be spring wheat, winter wheat and facultative types. Spring wheat is sown in the spring when winter is over, continue growth with no inactive period and flowers in the same year yielding grain in about 90 days. It cannot resist lower temperatures below -10°C for more than 12 hours. It is semi-erect or erect and insensitive to photoperiod. Winter wheat requires a cold treatment (vernalization) at 5°C – 10°C for 3-6 weeks after germination in order to set flowering stems and to produce grain. It is sown in the fall, appears grassy during early growth stages and starts to grow before winter sets in, and remains inactive in winter. The plant again continues its growth as the temperature rises

during the next spring and thus, harvesting is carried out in the following year. Winter types cover about 80% of the world's wheat cultivation. Whereas, facultative wheat tolerates cold more than spring wheat but less than winter wheat, and does not require extended exposure to cold temperatures to reproduce.

Commercially, both spring and winter wheat could be divided into either hard or soft, with each further divided into white and red types (https://www.breadexperience.com/types-of-wheat/). Harder winter wheat usually contains higher protein content than spring wheat and is suitable for making pasta and bread. Spring wheat is used for products that do not require high-protein content, such as tender pastries and cakes (https://www.yourarticlelibrary.com/ difference/difference-between-winter-wheat-and-spring-wheat/25490).

2.3 Wheat Taxonomy

Common wheat (*Triticum aestivum* L. ssp. *aestivum*) belongs to the grass family *Poaceae*, tribe: *Triticeae*, subtribe: *Triticinae* and genus: *Triticum* L. (Bálint *et ai.*,2000). The genus comprises of species that are diploid, tetraploid and hexaploid. The *T. aestivum* L. comprises of five subspecies including Common wheat: *T. aestivum* L. subsp. *Aestivum*, Club wheat: *T. aestivum* subspecies *compactum* (Host) MacKey, Macha wheat: *T. aestivum* subsp. *macha* (Dek. and Men.) MacKey, Vavilovi wheat: *T. aestivum* subsp. *vavilovi* (Tuman) Sears and Shot wheat: *T. aestivum* subsp. *sphacrococcum* (Perc.). MacKey. Sub-specie *T. aestivum* L. subsp. *Aestivum* could be either spring or winter type and is the most cultivated form in USA (Magness *et al.*,

1971). *T. aestivum* subsp. *Macha* and *T. aestivum* subsp. *Spelta* are hulled wheat groups. Whereas, *T. aestivum* subsp. *aestivum*, *T. aestivum* subsp. *compactum* and *T. aestivum* subsp. *sphaerococcum* are hulless and thus, free-threshing.

2.4 The Wheat Genome

Bread wheat (*T. aestivum* L.) is an allohexaploid comprised of three genomes (A, B and D sub-genomes with 2n=6x=42; AABBDD) (Griffiths *et al.*,2006) each with the size of barely genome (~5,100 Mb) (Mayer *et al.,* 2012). The spontaneous polyploidization combination of the three genomes resulted in bread wheat with ~ 17.30 Gb (Brenchley *et al.*,2012), which is approximately five times, seven times, 38 times and 110 times larger than that of human, maize (*Zea mays* L.), rice (*Oryza sativa* L.) and Arabidopsis genome, respectively (Hussain and Rivandi, 2007; WGSC, 2018). The International Wheat Genome Sequencing Consortium (IWGSC) (2018) revealed that repetitive DNA elements account for approximately 85% of bread wheat genome. Wheat has about 164,000 - 334,000 genes which is by far larger than a human genome (20,000 -25,000).

Genome sequence information is vital for precisely dissecting genomic loci underlying useful agronomic traits including yield, disease resistance and tolerance to abiotic stresses (WGSC, 2018). IWGSC (2018) reported reference genome sequence of bread wheat cultivar Chinese Spring (CS), IWGSC RefSeq v1.0 in 2018. They released sequence and annotation assembly (IWGSC Annotation v2.1) of the 21 chromosomes of the *T. aestivum* cv. Chinese

Spring (Figure 2.1) which are now publicly available at data repository of IWGSC hosted by URGI (https://wheat-urgi.versailles.inra.fr/Seq-Repository/Assemblies), Ensembl Plants (https://plants.ensembl.org/Triticum_aestivum/Info/Index), WheatIS (http://wheatis.org/), and Graingenes (https://wheat.pw.usda.gov/GG3/), and Persephone (https://web.persephon-esoft.com/?data=genomes/IWGSCv1.0).

In the IWGSC RefSeq v1.0 genome assembly, about 97% (14.1 Gb) of the sequences have been assigned and ordered along the 21 chromosomes. While, about 481 Mb (2.8% of the assembly length) unanchored scaffolds have formed the "unassigned chromosome" (ChrUn) bin (IWGSC, 2018). The RefSeq v1.0 assembly identified several molecular markers useful for wheat research and breeding program including 504 single-stranded repeats (SSRs), 3025 diversity array technologies (DArTs), 6689 expressed sequence tags (ESTs), 205,807, single nucleotide polymorphisms (SNPs), and 4,512,979 insertion site–based polymorphism (ISBPs) (IWGSC, 2018).

Analysis of the genome sequence components also identified 3,968,974 copies (85% of the genome) of transposable element (TE) with relatively identical distribution across the three sub-genomes, 112,744 long terminal repeat (LTR)–retrotransposons, non-coding RNAs, microRNA, nuclear-inserted plastid DNA segments (8000), 11,000 nuclear-inserted mitochondrial DNA segments, 107,891 high-confidence (HC) protein-coding loci with the A, B, and D sub-genomes containing 35,345, 35,643, and 34,212 loci, respectively, about 161,537 low-confidence (LC) protein coding genes (51,585, 58,359 and 44,835 for A, B, D sub-genomes, respectively), and 290,760 pseudogenes with significantly fewer in D sub-

genome (81,905) as compared to the A (999,754) and B (109,097) sub-genomes (IWGSC, 2018).

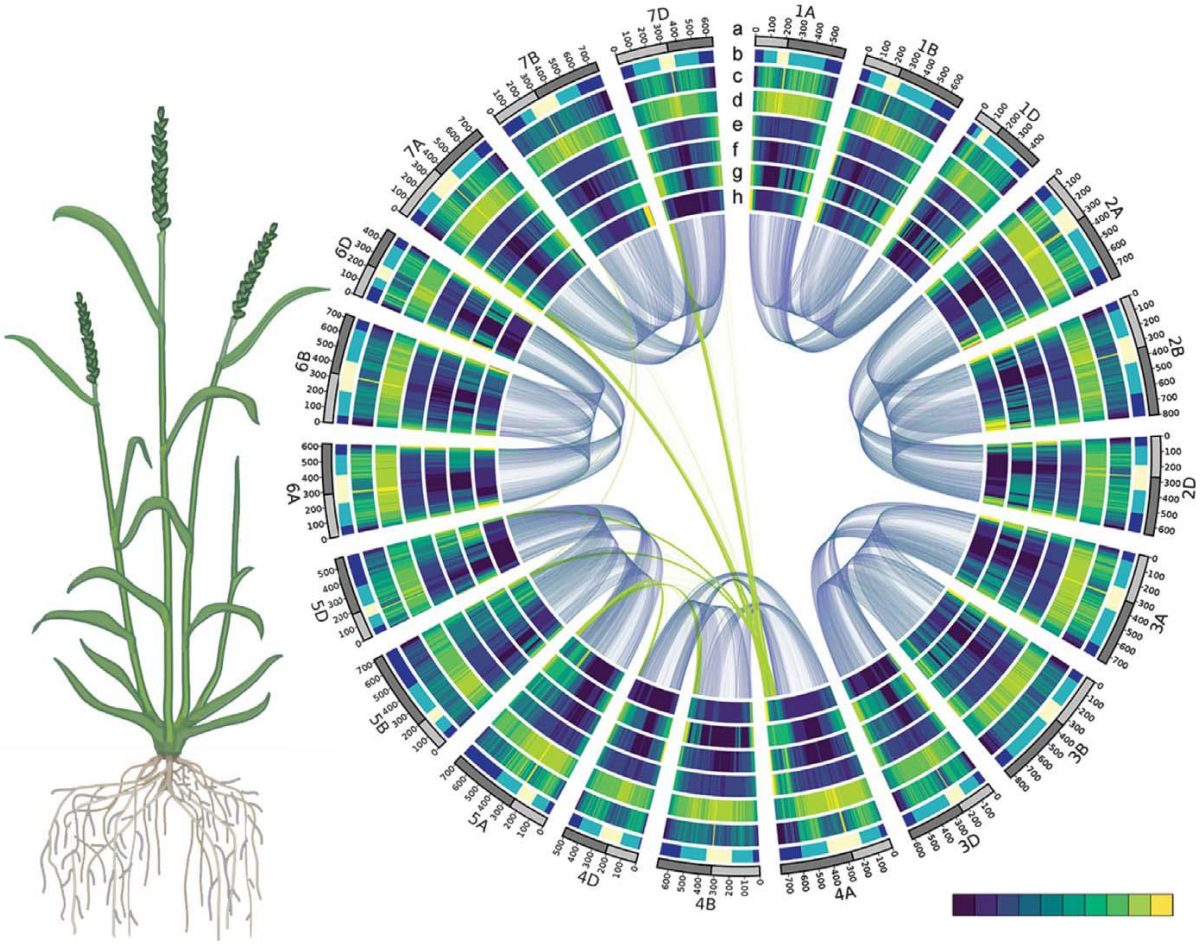

Figure 2. 1 Circular diagram showing genomic features of wheat.

a) Chromosome name and size (100-Mb tick size; light gray bar indicates the short arm and dark gray indicates the long arm of the chromosome); b) dimension of chromosomal segments R1, R2a, C, R2b, and R3; c) K-mer 20-frequencies distribution; d) long terminal repeat (LTR)-retrotransposons density; e) pseudogenes density (0 to 130 genes per Mb); f) density of high-confidence (HC) gene models (0 to 32 genes per Mb); g) density of recombination rate; and h) SNP density. Connecting lines in the center of the diagram highlight homologous relationships of chromosomes (blue lines) and translocated regions (green lines). Source: IWGSC (2018).

2.5 Uses of Wheat

Wheat is used as food, feed for livestock and income generation. It is the most widely consumed and traded cereal food crop contributing about 21% of the total global calories and 20% protein intake worldwide (Braun *et al.*,2010; Arzani and Ashraf, 2017; IWGSC, 2018; Ramadas *et al*., 2019). This is substantially higher than that of maize which contributes about 5% of the dietary calories and 4% of the protein, and rice which accounts 9% of the calories and 13% of the proteins (Bekele Shiferaw, 2013). A 100 grams of whole-grain wheat flour can provide 340 calories of energy, 13.2 g protein, 11% water, 72 g Carbs, 0.4 g sugar, 10.7 g fiber and 2.5 g fat (USDA, 2019). Gluten which provides unique elasticity and stickiness to wheat dough accounts for 80% of the protein content (Godfrey *et al.,* 2010). Moreover, wheat is good source of several vitamins like thiamin, niacin, riboflavin, vitamin B6 (pyridoxine), folate and pantothenic acid, and vitamin E. It is also important source of minerals such as iron, zinc, magnesium, potassium, phosphorus, copper, iodine and selenium (Godfrey *et al.,* 2010).

Industrially, wheat is used for the production of a wide range of foods including bread, muffins, noodles, crumpets, pasta, biscuits, cakes, pastries, cereal bars, sweet and savory snack foods, crackers, crisp-breads, sauces and confectionery (e.g. liquorices). Some wheat is also used for the production of starch, glue, malt, dextrose, gluten, alcohol, and other products (https://www.britannica.com/plant/wheat).

Wheat consumption differs between developing and developed countries. In developed countries, wheat accounts for about 75% and 81% of the cereal-based calories and proteins, respectively. The corresponding contributions in developing countries are about 35% and 44%, respectively. Central Asia is the leading for annual per capita (171 kg) wheat demand followed by North Africa (165 kg), West Asia (122 kg), and Eastern Europe and Russia (120 kg) (Bekele Shiferaw *et al*., 2013). Wheat consumption in SSA has significantly increased since 1991's, likely due to the growing urbanization and income which led to a shift in dietary preferences from coarse grains like maize, sorghum, and millet, and tuber crops such as cassava, sweet potato, and yam to wheat and rice. Consequently, SSA region anually imports more than 15 million tons of wheat grain (Listman, 2019), as the domestic wheat production in this region was not sufficient to meet the increasing demand. Ethiopia satisfies 25% of its domestic wheat demand through commercial import and food aids (Eyob Bezabeh *et al.*, 2015). Therefore, to ensure sustainable economic growth and political stability in the region, SSA countries need to maintain reliable sources of food supplies at affordable prices (Asfaw Negassa *et al.*, 2013).

2.6 Global Wheat Production

Wheat is one of the most produced and traded commercial crop globally (Akter and Islam, 2017). Because it is highly adaptable, nutritious, easily stored and transported, and easily processed. Bread and durum wheat are the two most cultivated types where they account for 90% and 5% of the total global wheat production, respectively (Randhawa *et al*., 2013; Getachew Gudero and Biruk Kedir, 2018). In 2019, about 216 million ha of land was grown

with wheat globally (Table 2.1), followed by maize (197 M ha) and then rice (162 M ha) (FAOSTAT, 2019). In the same marketing year, global wheat production was about 765.8 million metric tonnes (Table 2.1) making the crop the second most-produced food crop in the world preceded by maize (1116.3 million metric tonnes) (FAOSTAT, 2019).

The 2019 global average wheat production showed an increase of over 139 and 32.4 million metric tonnes compared to the 2005 and 2018 marketing years, respectively (Table 2.1). China, India, Russian Federation, USA, France, Canada, Pakistan, Germany, Australia, and Ukraine represent the world's top 10 wheat producing countries contributing about 71% of the global production. Russia, Canada, and Australia are the largest wheat exporters accounting for nearly half of the total global wheat export (Nag, 2019).

Table 2. 1 Global wheat harvested area, production and productivity from 2005-2019.

Year	Production (tonnes)	Area harvested (ha)	Yield (tonne ha^{-1})
2005	627020838	221665780	2.83
2006	614381122	212558976	2.89
2007	606595141	215449041	2.82
2008	680294442	222130343	3.06
2009	683639173	225199135	3.04
2010	640802664	215602998	2.97
2011	696898368	220263250	3.16
2012	673729410	217917932	3.09
2013	710398667	218870212	3.25
2014	728757761	219755320	3.32
2015	742029713	223413527	3.32
2016	748494478	219018037	3.42
2017	772295897	218299828	3.54
2018	733386177	213981968	3.43
2019	765769635	215901958	3.55

Source: FAOSTAT: 2005-2019. [Accessed on 26 December 2020].

Africa accounts for only 3.5% of the total global production (FAOSTAT, 2019). The low wheat production in the continent could be due to lack of improved varieties, biotic factors like pests and diseases, and abiotic factors such as drought, heat, salinity, acidity, etc. (Asfaw Negassa *et al.,* 2013). As a result, the continent heavily depends on imported wheat, a burden on the scarce foreign exchange reserves. During 2009-2011, Ethiopia was the leading SSA country in terms of wheat cultivated area (1.6 million ha) with total production and productivity of 2.8 million metric tonne and 1.8 tonne ha^{-1}, respectively. South Africa follows with wheat grown area of about 0.6 million ha that resulted in similar production (2.8 million metric tonne) and more productivity (3.0 tonne ha^{-1}), respectively (Asfaw Negassa *et al.,* 2013). In 2019, about 2.12 million ha of land was covered with wheat in East Africa which gave a total of 6.07 million metric tonne and 2.86 tonnes ha^{-1}, respectively (FAOSTAT, 2019).

2.7 Wheat Production in Ethiopia

Wheat is one of the major staple and strategic food security crops in Ethiopia (Tesfaye Letta *et al*., 2013; Eyob Bezabeh *et al.,* 2015) ranking fourth after Teff (*Eragrostis tef*), Maize (*Zea mays*) and Sorghum (*Sorghum bicolor*) in area coverage, and third after Maize and Teff in total production (CSA, 2019). Both bread and durum wheat are widely cultivated in Ethiopia with the former representing about 80% of the total wheat areas of the country (Asfaw Negassa *et al*., 2013). In Ethiopia, wheat is cultivated for its various uses including as food, animal feed, income generation and also for hatching roofs (the straw). It is grown

in wide range of altitudes from 1500 m to 3200 m between 6 - 14^{o} N; and between 35 - 42^{o} E. The most suitable regions, however, fall between 1900 m and 2700 m (Hailu Gebre-Mariam, 1991). Rain-fed production system that includes the short rains (belg) falling from February to April and the main rains (meher) extending from June to September is the main characteristics of wheat cultivation in Ethiopia (Asfaw Negassa *et al.*, 2013).

Four regions in Ethiopia, Oromia, Amhara, Tigray, and Southern Nations, Nationalities and Peoples' Region (SNNPR) account for 99% of the country's wheat production. From these regional states, 17 zones are identified to be major wheat growing areas. These include nine zones from Oromia (East Shewa, West Shewa, North Shewa, South West Shewa, Arsi, West Arsi, East Bale, West Bale and Horoguduro Wellega), six zones from Amhara region (South Wollo, North Gonder, South Gonder, East Gojjam, West Gojjam and North Shewa), South West Tigray zone from Tigray region and Hadiya zone from SNNPR region. Arsi, Bale and Shewa zones alone account for 75.5% of the national wheat production (Tesfaye Gemechu and Fiseha Tadese, 2018).

Smallholder farmers are major producers and suppliers of wheat in Ethiopia although there are some sort of mechanized extensive farming practices in Arsi and Bale zones. Analysis of the last 15 years wheat cultivation in Ethiopia revealed that wheat harvest area, production and productivity had increased from 1.40 million ha, 2.18 million metric tonnes and 1.56 t ha^{-1} in 2005 to 1.79 million ha, 5.32 million metric tonnes and 2.97 t ha^{-1} in 2019, respectively (Table 2.2) (FAOSTAT, 2019). However, the current national average wheat productivity 2.97 t ha^{-1} is far lower than the global average of 3.55 t ha^{-1}, resulting in production deficit to meet the rising local demand (Tesfaye Gemechu and Fiseha Tadese,

2018). The local production can satisfy only 75% of the domestic wheat demand, and hence, the country loses millions of dollars annually for commercial import of wheat to meet the increasing local demand. The lower productivity could be due to various production constraints including limited access to advanced production technologies (eg. mechanized chaltivations), low agricultural inputs, and biotic and abiotic stresses (Alemar Said, 2016).

Table 2. 2 Wheat harvested area, production and productivity in Ethiopia (2005 -2019).

Year	Production (tonnes)	Area harvested (ha)	Yield (tonnes/ha)
2005	2176603	1398215	1.56
2006	2219075	1459540	1.52
2007	2463064	1473917	1.67
2008	2314489	1424719	1.62
2009	3075644	1683565	1.83
2010	2855682	1553240	1.84
2011	2916334	1437485	2.03
2012	3434706	1627647	2.11
2013	3925174	1605654	2.44
2014	4231589	1663845	2.54
2015	4650934	1664565	2.79
2016	4537852	1696083	2.68
2017	4642966	1696907	2.74
2018	4838074	1747939	2.77
2019	5315270	1789372	2.97

Source: FAOSTAT: 2005-2019. [Accessed on 26 December 2020].

2.8 Wheat Production Constraints

2.8.1 Abiotic Factors

Even though wheat occupies the largest proportion of the total cultivated area (38.8%) among cereals, its overall productivity remains the lowest globally. The major abiotic factors that influence wheat production and productivity include drought, flood, soil

pollution, salinity, acidity, high and low temperature, and radiations (Lawlor and Cornic, 2005; Abhinandan *et al.,* 2018).

Wheat is a cold-loving crop that needs an optimal temperature of 12–30 °C to give maximum grain yield. The rising of temperature (heat stress) beyond the optimal adversely affect wheat productivity by altering physiological, biological and biochemical processes of the plant (Asseng *et al*., 2015). Heat stress causes poor seed germination, reduced leaf area, reduced grain number, reduced grain filling duration, reduced nutrient translocation, premature leaf senescence, damage of photosynthetic machineries, and decreased chlorophyll content, all of which ultimately contribute to reduced production and productivity (Suresh, 2020). A 1°C rise of temperature beyond the optimal may cause global wheat production loss of about 6% (42 million tonnes) (Senthold *et al.,* 2011).

Drought or water stress is also the most important abiotic factor adversely affecting wheat production globally. It affects grain yield potential in various ways including impairing seed germination and poor stand establishment, reducing time to anthesis and shortens grain-filling durations (Tiwari *et al.*, 2017). It is responsible for 9-10% global yield reductions, and thus, it is the major challenge to food security worldwide (Daryanto *et al*., 2017). Drought affects morphological traits like leaf (shape, area, expansion, size, senescence, pubescence, waxiness, and cuticle tolerance) and root (length, dry weight, and density) which ultimately leads to reduced yield potential (Tiwari *et al.*, 2017).

Soil contamination with heavy metals is another abiotic factor severely affecting crop productivity. The soil receives considerable amount of heavy metals from various sources every year (Wannaz *et al.*, 2019), and recently, accumulation of increased level of heavy metals in the soil is reported world-wide (Bolan *et al*., 2011). As a result, high accumulation in plant tissues affects crop productivity by influencing the biochemical, physiological and morphological functions in plants (Shahid *et al.* 2015). The excess accumulation of heavy metals reduce crop production and productivity by inducing toxic effects to various physiological processes in plants including: accumulation, seed germination, and remobilization of seed reserves during germination, plant growth, and photosynthesis (Shahid *et al.*, 2015).

2.8.2 Biotic Factors

Being sessile organisms, plants are often exploited as a source of food and shelter by a wide range of biotic factors including viruses, bacteria, fungi, nematodes, insect-pests and even other plants (Gachomo *et al.*, 2003). Nearly 85% of plant diseases are caused by fungal or fungal like organisms (Isleib, 2020).

Some of the major wheat fungal diseases that have global significance include Brown rust (*Puccinia recondita)*, Common bunt (aka Covered smut) (*Tilletia caries*), Ergot Claviceps (*purpurea*), Eyespot (*Pseudocercosporella herpitrichoides*), Glume blotch (*Septoria nodorum*), Barley yellow dwarf virus or BYDV, Septoria leaf blotch (*Mycosphaerella graminicola*, synonyms: *Septoria tritici* or *Zymoseptoria tritici*), Powedery

Mildew (*Erysiphe graminis*), *Fusarium* head blight (*Fusarium graminearum*), Seedling blight (*Fusarium spp., Septoria nodorum*), Sharp eyespot (*Rhizoctonia cerealis*), Spot blotch (*Biplolaris sorokiana*),Take-all (*Gaeumannomyces graminis*), Tan spot (*Pyrenophora tritici-repentis*), Yellow rust (*Puccinia striiformis*), Stem (*Puccinia graminis*), and Leaf rust (*Puccinia triticina*) (Prescott *et al.,* 1986).

2.8.2.1 Common Fungal Diseases of Wheat in Ethiopia

In Ethiopia, wheat cultivation is persistently affected by more than 30 fungal diseases (Eshetu Bekele, 1985). Among these, stem rust (*Puccinia graminis* Pers. f.sp. *tritici* Eriks and Hann), leaf rust (*Puccinia triticina* Eriks), stripe or yellow rust (*Puccinia striiformis* Westend. f.sp. tritici), Septoria tritici blotch (*Zymoseptoria tritici* (Desm.) Quaedvlieg and Crous), Fusarium head blight (FHB) (*Fusarium graminearum*) and tan spot (*Pyrenophora tritici repentis*) are the most frequently reported ones (Eshetu Bekele, 1985; Ayele Badebo, 2002; CIMMYT, 2005; Misgana Mitiku and Yesuf Eshete, 2016). They are the major constraints of wheat production in the major wheat growing areas of Ethiopia.

2.8.2.1.1 Septoria Tritici Blotch (STB) of Wheat

Septoria tritici blotch (STB) caused by the fungus *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous (teleomorph *Mycosphaerella graminicola* (Fuckel) J. Schr¨ot. in Cohn or *Septoria tritici* Roberge in Desmaz) (Quaedvlieg *et al*. 2011), is one of the most economically damaging diseases of wheat worldwide (Testa *et al.,* 2015; Kettles and Kanyuka, 2016). It is mainly a foliar disease of wheat (Schultz and French, 2008; Ponomarenko *et al*., 2011). Its

potential threat to wheat production received international attention after the very devastating epidemics that occurred in North Africa in 1968–1969 (Brown *et al.,* 2015) following the introduction of semi-dwarf varieties and increased use of nitrogen fertilizers (Saari and Wilcoxson, 1974). Its economic importance increased largely due to the introduction and wide cultivation of early-maturing, semi-dwarf susceptible cultivars in place of the local wheat cultivars that were resistant to the pathogen (Eyal *et al.,* 1987; Wiese, 1987).

STB infection of the flag leaf, which contributes nearly half of the carbohydrates at the grain-filling stage, can result in low grain quality and substantial yield loss if not controlled (Eyal, 1987). Globally, wheat yield losses of 30 – 60% (Shipton *et al*., 1971; Eyal *et al*., 1987) have been attributed to Septoria disease with the diseases substantially affecting grain yield by causing poor seed set, reduced tillering, poor grain fill (shriveled kernels), death of leaves, spikes or the entire plant (van Ginkel and Rajaram, 1999; Simón *et al*., 2002; Goodwin, 2007; Ponomarenko *et al*., 2011; Miedaner *et al*., 2013). In Europe and the United States, the annual yield losses due to STB are estimated to be $400 million and $275 million, respectively. Besides, the cost incurred for controlling STB is significantly high and accounts for about 70% of annual fungicide usage in Europe (Fones and Gurr, 2015).

Severe outbreaks of STB have occurred in high-rainfall areas such as South America, semiarid countries along the Mediterranean Coast and in Australia. In Ethiopia, STB is a major threat to wheat production causing 25- 82% yield loss in the worst-affected areas with increasing incidence and severity in the major wheat belts of the country (Bekele Abate *et*

al., 2011; Abera Takele *et al.*, 2015; Endale Hailu and Getaneh Woldeab, 2015; Tilahun Mekonnen *et al.*, 2019, 2020). Therefore, STB is considered to be one of the most important economically devastating diseases of wheat in Ethiopia and worldwide (Ghaffary *et al.*, 2011; Ponomarenko *et al.*, 2011; Brown *et al.*, 2015; Makhdoomi *et al.*, 2015).

2.8.2.1.1.1 Origin and Distribution of Z. tritici

Zymoseptoria tritici (synonym *Mycosphaerella graminicola or Septoria tritici*) is believed to have originated from closely related *Zymoseptoria* species colonizing wild grasses in the Fertile Crescent of the Middle East, overlapping the center of origin of its main host, the bread wheat (*T. aestivum* L.) (Stukenbrock *et al*., 2007; 2010; Sanchez-Vallet *et al.*, 2015). Its expansion is also correlated with the distribution of its host-plant as well (Stukenbrock *et al*., 2007). The pathogen population structure analysis using molecular markers like restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSR) that targeted nuclear and mitochondrial genome as well as sequence diversity analysis based on housekeeping genes evidenced that the Middle East is hot spots of the pathogen genetic diversity from where it spread to the other parts of the world (Zhan *et al.*, 2003; Banke *et al.*, 2004). Moreover, studies on new collections from wheat fields and three generas of wild grasses in Iran confirmed that the Middle East is a hot spot of Z*ymoseptoria* diversity, and also led to the discovery of closely related *Zymoseptoria* species found exclusively on wild grasses which were unable to infect wheat (Stukenbrock *et al*., 2007, 2010). Analysis of the DNA sequences shared among wild relatives and *Z. tritici* revealed that their common ancestor lived 10,000 years ago (Stukenbrock *et al*., 2007).

Septoria diseases are important in all wheat growing areas of the world including Mediterranean areas, South America, highlands of East Africa, Australia and Europe (Figure 2.2) (Saari and Wilcoxson, 1974; Rajaram and Dubin, 1977; Goodwin, 2007; Ponomarenko *et al*., 2011; Miedaner *et al*., 2013; Fones and Gurr, 2015). There are two types of Septaria diseases in wheat in the world. These are *Septaria tritici* blotch (syn. Septaria leaf blotch, *speckled* leaf blotch) caused by the fungus *Z. tiritici* (teleomorph *M. graminicola*) and *Septaria nodorum* blotch (syn. Septaria glume blotch) caused by the fungus *Septoria nodorum* (sexual state: *Leptosphaeria nadorum)* (van Ginkel *et al.* 1999).

Septoria tritici blotch is the most economically important disease of wheat reported from more than 50 countries, specifically in major wheat producing countries including the USA and Canada (Eyal *et al*., 1985). Several studies revealed that STB is one of the most common type, and major threat to wheat production in Ethiopia as well, more specifically in the major wheat-growing areas of the country (Eshetu Bekele, 1985, Abera Takele *et al.*, 2015; Endale Hailu and Getaneh Woldeab, 2015; Tilahun Mekonnen *et al.*, 2019, 2020).

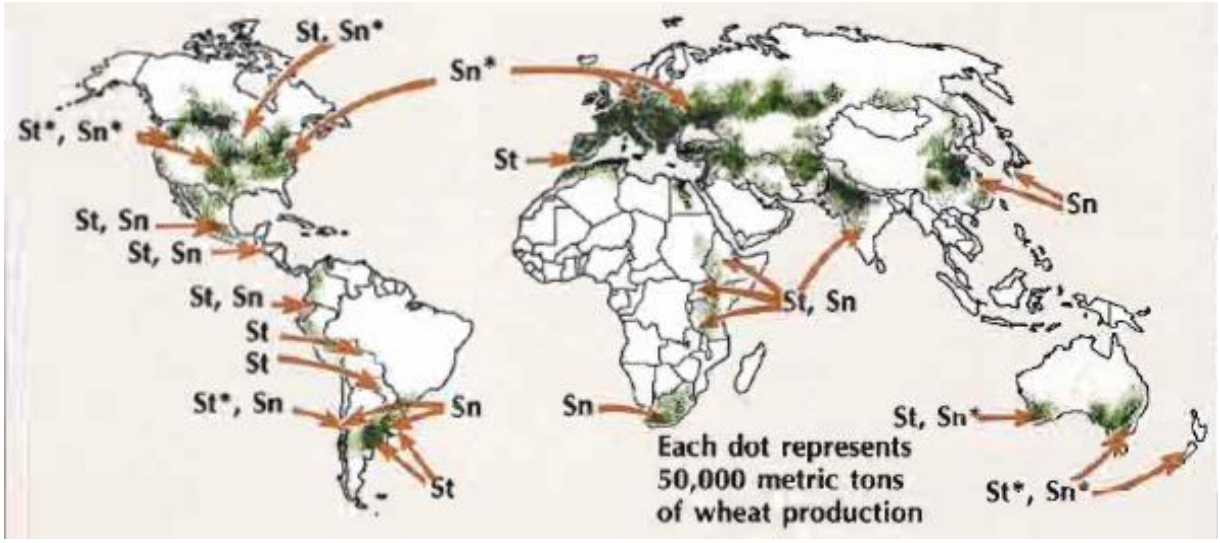

Figure 2. 2 Global distribution of *Septoria spp*. on wheat.
St= *Z. tritici* (formerly or *Septoria tritici*), and Sn= *Septoria nodorum*. An asteriks indicates locations where the sexual state (pseudothecia and ascospores) has been reported. Source: Ginkel *et al.* (1987).

2.8.2.1.1.2 Biology of Z. tritici

Morphologically, *Z. tritici* exhibits three vegetative growth forms (Steinberg, 2015). Under laboratory condition, it shows a "yeast-like" growth form referred as the macropycnidiospore (Figure 2.3 A). But, unlike yeast which is unicellular, the macropycnidiospore is composed of 4-8 elongated cells each with 1.5–3.5 µm wide and 40–100 µm long with 3-5 septa (Sanderson *et al*., 1985; Wiese, 1987). The fungus also produces a small growth cells (with ~1 µm width and 5–10 µm length) called the micropycnidiospores (Eyal *et al*., 1987) that laterally grow from macropycnidiospores or hyphae (Figure 2.3 A and C, arrowheads) (Steinberg, 2015). Both micropycnidiosporse and macropycnidiosporse can initiate infection. They are dispersed by rain splash (Sanderson *et al*., 1985). Up on contact with wheat leaf surface, the macropycnidiospores germinate to

produce a vegetative structure called hyphae (Figure 2.3 B) that enters plant tissue through the stomata (Gow, 1995). These pycnidiospores are important for local dispersion of STB in the same growing season (Sanderson *et al.*, 1985).

Pycnidia develop on necrotic leaf lesions, and remain embedded in the leaf epidermal and messophyl tissue on both side of the leaf with ostiole (an opening) on top (Eyal *et al.*, 1987). Depending on the fungal strain, infection density (Eyal and Brown, 1975; Kema and Annone, 1991), and the size of the stomata of wheat cultivars, the pycnidia size can range from 60- 200 µm (Sanderson *et al*., 1985).

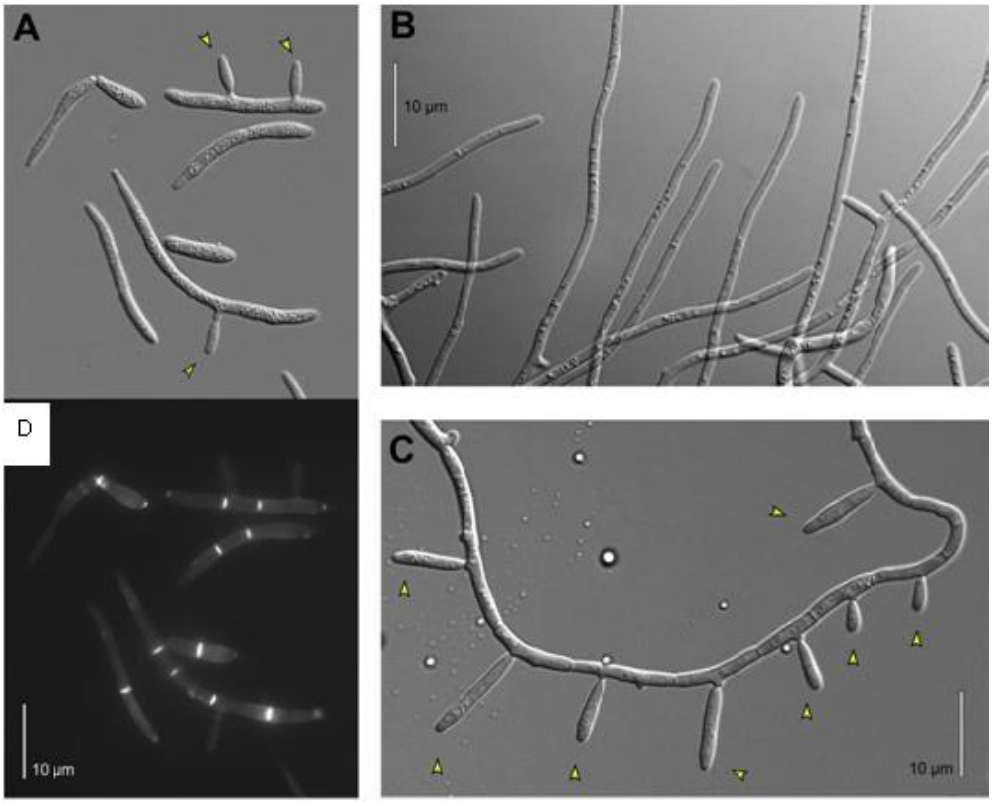

Figure 2. 3 Growth forms of *Zymoseptoria tritci.*
A) Pycnidiospores, B) Hyphae C) Micropycnidia grown from hyphal cells, and D) the septa. *Source:* Steinberg (2015).

Z. tritici also produces sexual spores (ascospores) in fungal fruiting bodies called the perithecia. Ascospors are composed of two unequal cells. They are important for over-seasoning of the pathogen in the crop stubbles and debris from where they will spread to over hundreds of kilometers by air in the next growing season (Eyal *et al*., 1987).

2.8.2.1.1.3 Genome of Z. tritici

Z. tritici is a haploid organism (Wittenberg *et al*., 2009) with 21 chromosomes. Goodwin *et al*. (2011) reported a full genome sequence of the reference strain IPO323 in 2011, and it is the first filamentous fungus whose complete genome sequence has been reported. Its genome size is about 39.9 Mb which is organized into 21 chromosomes. The size of the chromosomes range from 0.39 - 6.09 Mb (Wittenberg *et al*., 2009; Goodwin *et al*., 2011). The first 13 chromosomes (1–13) are called core chromosomes (CCs), and are involved in growth of the pathogen. The remaining eight chromosomes designated as 14–21 chromosomes are not involved in saprophytic growth and could be lost during meiosis with no visible effect on the fungus. These chromosomes are called non-essential, dispensable or accessory chromosomes (AC) (Goodwin *et al*., 2011; McDonald *et al*., 2015). The ACs are smaller (0.39 - 0.77 Mb) than the core chromosomes. The ACs are thought to have originated through horizontal transfer from an unknown source, which is followed by degeneration and extensive recombination with the core chromosomes (Goodwin *et al*., 2011).

Although the biological function of the ACs in *Z. tritici* remains unclear, they carry genes involved in Pathogenicity in some other pathogenic fungi (Han *et al*., 2001; Hatta *et al*., 2002). The ACs of *Z. tritici* contain a high number of repetitive sequences (Dhillon *et al*., 2014) and also show frequent evolutionary changes (Stukenbrock *et al*., 2010), suggesting that they could facilitate rapid adaptation of the pathogen to changing environments (Wittenberg *et al*., 2009) or could be involved in the development of fungicide resistance (Torriani *et al*., 2009).

2.8.2.1.1.4 Taxonomy of Z. tritici

*Z. tritici (*formerly *Septoria tritici*) was reported for the first time in 1842 by Desmazi`eres (Desmazi`eres, 1842; Sprague, 1938). Originally, it was considered as a variety of *Septoria graminum.* However, Sprague (1938) confirmed that *S. tritici* differs from *S. graminum* in its conidia length (40-75 µm) and number of septa (3-7). *Mycosphaerella graminicola* was first identified in Europe in 1894 (Cohn, 1894). Sanderson (1972) reported for the first time in New Zealand that *Mycosphaerella graminicola* (Fuckel) J. Schröt is the sexual form (telomorph) of *Z. tritici*. Taxonomically, the fungus belongs to Phylum: *Ascomycota*; Class: *Dothideomycetes*; Subclass: *Dothideomycetidae*; Order: *Capnodiales* and family: *Mycosphaerellaceae.* The genus *Mycosphaerella* is one of the largest plant-pathogenic fungi in Dothideales containing at least 500 species. It includes other plant pathogens such as *M. fijiensis* (a banana black Sigatoka pathogen), and many other significant pathogens of important agricultural crops. *Septoria passerinii* which causes the speckled leaf blotch in barley is one of the closest relatives of *M. graminicola*.

2.8.2.1.1.5 Life Cycle of Z. tritici and Infection Process

Reproduction in *Z. tritici* can be both in sexual and asexual forms (Shaw and Royle, 1993; Kema *et al*., 1996a). The life cycle begins soon after the emergence of the seedlings (Dooley, 2015) and the primary infections may arise from airborne sexual ascospores released from infested crop debris and volunteer grass (Shaner, 1981; Eyal, 1999; Hunter *et al*., 1999; Gilchrist and Dubin, 2002). The sexual reproduction form requires coming together of the two separate mating types (*mat 1-1* and *mat 1-2*) (Kema *et al*., 1996a). The resulting sexual fruiting bodies (pseudothecia) develop under the leaf epidermis and appear as dark brown or black spot. The pseudothecia contain asci which encloses eight two-celled ascospores (Eyal *et al*., 1987).

The sexual stage plays a major role in the disease cycle. It is important for most of the initial infection in the next growing season (Cordo *et al.*, 1999) (Figure 2.4). During the next growing seasons, ascospores maintained in pseudothecia are released into the air following absorption of moisture and increased relative humidity (Ponomarenka *et al*., 2011). The ascospores can travel in air over long distances (10-200 km) (Linde *et al*., 2002). *Z. tritici* can carry out several cycles of sexual reproduction in a growing season. This has resulted in high within populations genetic diversity, and also the development of potentially virulent strains (Welch *et al*., 2018). The high genetic diversity (which facilitates the evolution of novel traits) and the ability to spread over large geographical areas made *Z. titici* as a very successful pathogen (Tiley *et al*., 2018).

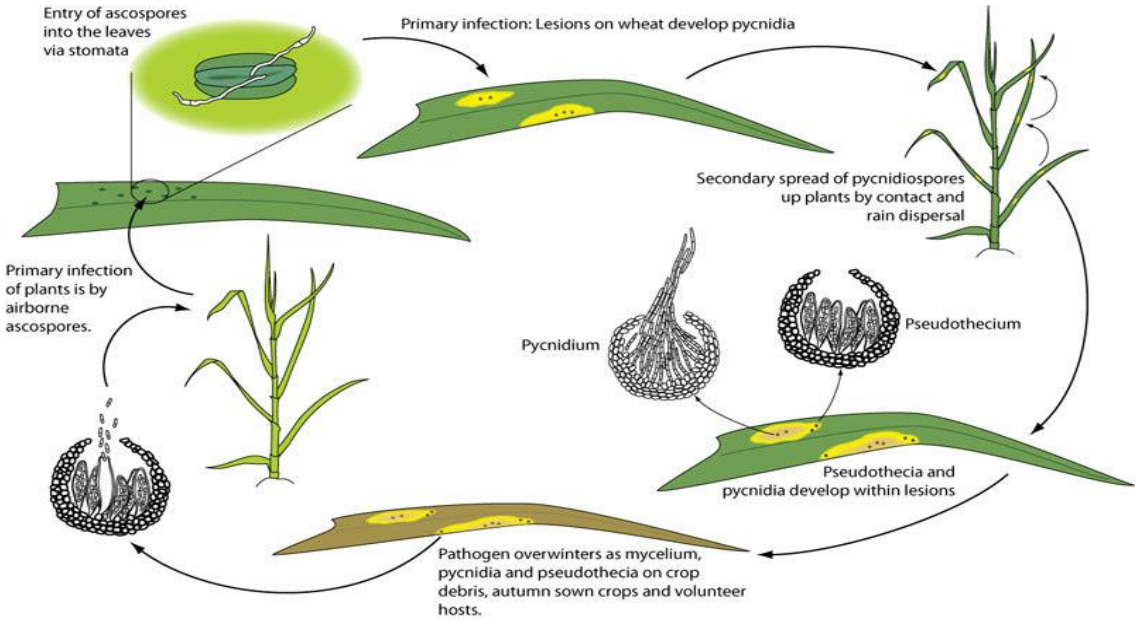

Figure 2. 4 Disease cycle of Septoria tritici blotch.
Source: Ponomarenko *et al*. (2011).

STB infection process begins with the landing of windblown sexual ascospores or rain splashed asexual pycnidiospores on wheat leaf surface. Following contact with wheat leaf, the spores begin to germinate to produce germ tube and hyphae. The hyphae extend along the leaf surface and enter into the host cells exclusively through the stomata within one- two days post-infection (Figure 2.5) (Duncan and Howard, 2000). After penetrating into the host cell, between 3-14 days the fungus proceeds colonizing the mesophyll tissue by growing inter-cellularly (Figure 2.5) without causing major damage on the host cells (Goodwin *et al*., 2011) which is termed to be biotrophic or latent phase (Kema *et al*., 1996b; Duncan and Howard, 2000). During this period, there is no symptom, the fungus does not develop any intracellular feeding structures like haustoria, nor does it induce changes in host metabolism and nor in the composition of the apoplastic fluid (Sánchez-Vallet *et al.*, 2015). The fungus

lives on the nutrient reserves in the germinating spore and also nutrients already present in the apoplast (Sánchez-Vallet *et al.*, 2015).

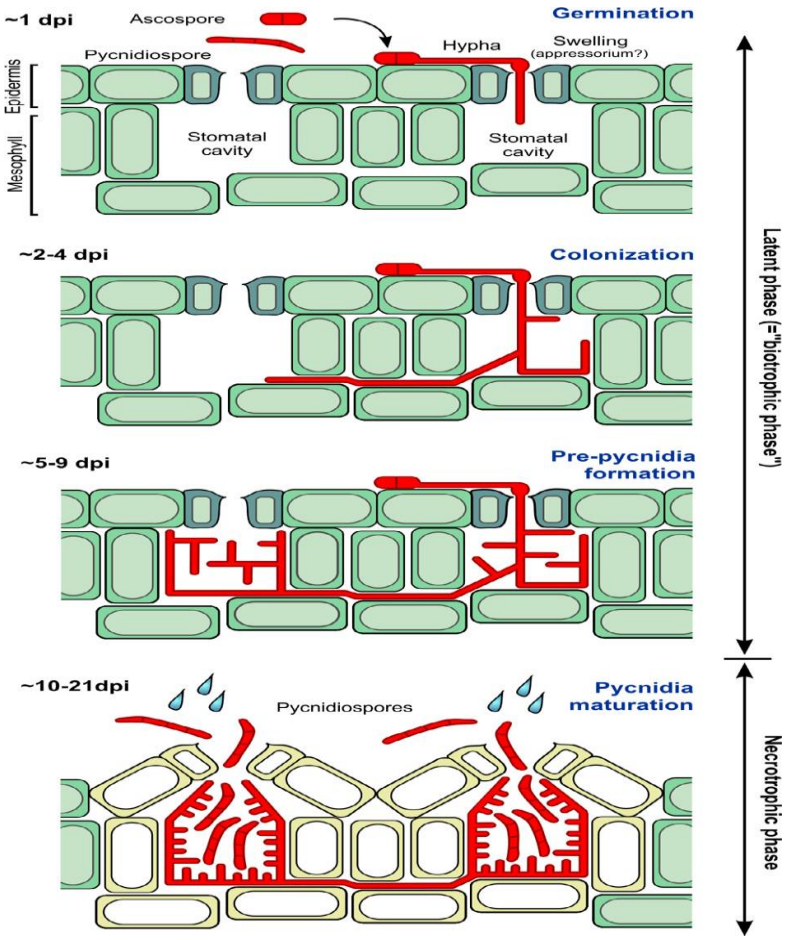

Figure 2. 5 Summary of plant infection stages of *Z. tritici.*
Source: Steinberg (2015).

Following the asymptomatic biotrophic phase, *Z. tritici* switches to necrotrophic phase at 14-21 days post-infection. This stage is characterized by massive mycelial growth, collapse of host cells and the development of chlorotic and necrotic lesions on the leaves (Figure 2.5) (Kema *et al.*, 1996b; Duncan and Howard, 2000). RNA-Seq analysis confirmed the increased expression of genes coding for plant cell degrading enzymes during the

necrotrophic phase (Rudd *et al*., 2015; Palma-Guerrero *et al*., 2016). During this period, both pseudothecia (sexual fruiting bodies) and pycnidia (asexual fruiting bodies) that produce ascospores and pycnidiospores, respectively develop on the necrotic lesions of the leaves. From the ostiole of each pycnidium, about 10,000 pycnidiospores are exuded in a thick gelatinous matrix containing proteins and sugars, termed cirrhus (Eyal *et al*., 1987).

The spores spread vertically to upper leaves of the same plant or horizontally to a nearby plant through rain-splash, contact and/or via wind to initiate a secondary infection (Figure 2.4) (Eyal *et al*., 1987; McDonald *et al*. 1999). The ascospores play major role for long distance spread of the disease (Eyal *et al*., 1987; Eyal, 1999). The fungus survives the harsh off- season conditions as pseudothecia and pycnidia on crop debris, on off-season grown hosts and volunteer plants (Tiley *et al.*, 2015). *Z. tritici* can survive for several years in the form of vegetative strands (mycelium) and pycnidia in wheat residues.

2.8.2.1.1.6 Diagnostic Symptoms of STB

The initial symptoms of *Septoria tritici* blotch are small yellowish or chlorotic lesions usually detected on the lower leaves soon after the seedlings emerge usually in five to six days of infection. As they mature, the lesions become light tan, irregularly shaped ranging from elliptical to long and narrow delimited by leaf veins. Necrotic lesions (dead tissue) will develop at the chlorotic sites. Moreover, the pycnidia (fruiting bodies) develop at the necrotic lesion as small, round, black spots (Figure 2.6) scattered on both lower and upper surfaces of the leaf (Eyal *et al*., 1987). The development of black pycnidia in lesions area is

the most reliable and striking symptom for identifying STB disease (Figure 2.6) (Ponomarenko *et al.,* 2011).

Figure 2. 6 Symptoms of Septoria tritici blotch on wheat leaves.
Photo captured by Tilahun Mekonnen (2016).

The time for the development of first symptom may vary depending on the environmental conditions (moisture and temperature), inoculums concentration and the host during the infection process. Generally, STB development becomes higher at temperature of 15 to 20°C (Wiese, 1987; Magboul *et al*., 1992) coupled with longer wetness period (Magboul *et al*., 1992) and higher inoculums concentration. Chungu *et al*. (2001) demonstrated that an increase of the temperature from 18°C day/15°C night to 22°C day/15°C night or the inoculums concentration from $1x10^6$ spores/ml to $1x10^7$ spores/ml may cause the pycnidia to develop four days earlier. In Ethiopia STB infection is very severe in the main growing season that extends from June to December (Tewodros T. Wakie *et al.,* 2016).

The size of the developed pycnidia can vary among cultivars depending on its density (becomes smaller when the pycnidia is dense) (Djerbi *et al.,* 1976). Moreover, the spore production vary depending on the cultivar response, and lower spore production could occur on resistant cultivars (Gough, 1978). Furthermore, the vertical spread of the disease could be limited by plant height (Ma *at el*., 2018; Miedaner *et al*., 2013).

Sometimes STB infection can occur with other diseases, causing the phenotypic disease diagnosis difficult. In many countries such as UK, Brazil, northern U.S.A., Western Australia, Uruguay, and other areas, STB and SNB often occur together on the same leaf. Sometimes, even other fungi that form similar spore, fruiting structures, and other symptoms can occur to complicate identification. Therefore, the field STB diagnosis need to be coupled with microscopic examination of pure culture of the pathogen at l00x or 400x (Eyal *et al.,* 1987), and/or using molecular tools which are accurate and rapid detection methods even at early growth stage (Cao *et al*., 2016). The internal transcribed spacer (ITS) sequences within the ribosomal RNA genes (ribosomal DNA) are widely used as a molecular probe to identify fungal pathogens (Beck and Ligon 1995). They are the prime fungal default region for species identification and it can be retrieved using diagnostic primers that target the sequence (Das and Deb, 2015).

Several PCR assays have been developed and widely used for detection of *Z. tritici.* Beck and Ligon (1995) developed a primer pair ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and JB446 (5'-CGAGGCTGGAGTGGTGT-3') that specifically produce a 345-bp product in *Z. tritici* but, do not result in any amplification in other fungal species. Besides, other *Z. tritici*

specific PCR primers were developed including E1/STSP2R and STIF2/BAF4ST, E1/STSP2R and STIF2/BAF4ST (Fraaije *et al*., 1999, 2001), ST-rRNA F/R (Guo *et al*., 2006), A(f)/A(r) (Consolo *et al.*, 2009), and LidSt11/12, LidSt15/16 and LidSt19/20 (Kuzdralińsk *et al.,* 2020). Moreover, primers for quantitative detection (qPCR) of *Z. tritici* without electrophoresis step have been reported (Bearchell *et al*., 2005). Furthermore, high throughput sequencing of nuclear ITS rDNA region is becoming a method of choice for fungal identification including *Z. tritici* (Das and Deb, 2015). Therefore, integrating molecular tools with field symptomological assay would make the pathogen identification efforts accurate and hence, helps to design and implement durable and effective management strategies.

2.8.2.1.1.7 STB Evaluation Methods

Assessment of disease infection is important for timely implementation of appropriate disease management measures, for pathogen genetic and virulence variability analysis, for screening germplasm for resistance, and for other host-pathogen interaction studies. Percentage of necrotic leaf area (affected leaf area), pycnidia density or the combination of the two are used for STB rating (Eyal *et al*., 1987). James (1971) and Eyal and Brown (1976) used an electronic scanner and a television scanner to determine the amount of necrotic leaf area and pycnidial coverage on affected leaves, respectively. Although the methods offer correct estimate of the disease severity, the image acquisition process is very time consuming, labor intensive and costly (Eyal *et al.*, 1987).

Nowadays, several types of visual rating scales have been developed and widely employed for estimation of STB severity (Eyal *et al.,* 1987). The double digit (00-99) scoring scale improved from the Saari and Prescott (1975) (0-9), which simultaneously considers the vertical progress of the disease and its severity in the assessment process, is the most widely used method for STB evaluation (Eyal *et al.*, 1987). The first digit (0-9) indicates the relative height of the blotch on the plant using the original 0-9 Saari-Prescott scoring scale as a measure (for instance 5 if the disease reached at the middle (50%) of the plant height, 8 for flag leaf and 9 for spike), while the second digit represents the disease severity as a percentage but, in terms of 0-9 (0 = 0%, 1= 10%, 2= 20% and 9 = 90%) (Eyal *et al*., 1987). Septoria disease severity percentage (SDS%) could be computed from the double digit score using the following formula as described by Sharma and Duveiller (2007):

$$\text{SDS} = \left[\left(\frac{D1}{9}\right)\left(\frac{D2}{9}\right)\right] x100$$

where D1 and D2 are the first and the second digits of double-digit scores, respectively. SDS values range from 0 to 100, where 0 would indicate complete resistance, and 100 would indicate complete susceptibility. This method is very suitable for rapid screening of breeding lines in the field (Eyal *et al.,* 1987).

Likewise, Rosielle (1972) developed a six point STB severity scoring scale that combined the amount of necrotsis area and pycnidia density to classify the plants as immune, highly resistant, resistant, intermediate, susceptible and very susceptible (Eyal *et al.*, 1987). Moreover, Eyal and Ziv (1974) developed an evaluation method known as Septoria Progress Coefficient (SPC) that describes the pycnidia position relative to the plant height regardless

of pycnidial coverage. It is the ratio of disease height to plant height (in cm), and its values range from 0.0 to 1.0, where *SPC* = 0 means no disease at all, while *SPC* =1 indicates that the disease covered the entire plant (Eyal *et al.* 1987). Furthermore, Eyal and Brown (1976) developed a diagrammatic disease severity rating scale based on the actual pycnidial density per unit leaf area. Based on the average pycnidia density on four uppermost leaves, the germplasms could be considered as very resistant (0-5%), resistant (5-15%), moderately resistant (15-30%), moderately susceptible (30-40%), and susceptible when the pycnidial density is > 40%.

2.10 Pathogenic Specialization in *Z. tritici*

Understanding the physiologic specialization of a pathogen and identification of potential resistance sources are prerequisite for designing durable management strategies (Eyal *et al.*, 1983). In the wheat-*Z. titici* pathosystem, there have been contradicting reports on the presence of virulence differences among *Z. tritici* isolates. Some studies assert that the variations among *Z. tritici* populations are only in the degree of pathogenicity (aggressiveness) ruther than true virulence differences (Marshall, 1985; Ginkel and Rajaram, 1995). On the other hand, several other previous studies confirmed the existence of true virulence variability among different *Z. tritici* isolates (Eyal *et al*. 1973; Eyal and Levy, 1987; Saadaoui, 1987; Ballantyne, 1995; Ackerman *et al*., 1995; Jlibene *et al*., 1995; Kema *et al*., 1995; Kema *et al*., 1996a, 1996b; Kema and van Silfhout, 1997; Brading *et al*., 2002; Grieger, 2005; Tilahun Mekonnen *et al.* 2020). The presence of virulence differences among *Z. tritici* isolates was first reported in Israel (Eyal *et al*., 1973).

Eyal *et al*. (1985) analyzed the virulence pattern of 97 isolates of *M. graminicola* from 22 countries on seedlings of 35 wheat and triticale cultivars. They observed significant isolate x cultivar interactions, suggesting the existence of cultivar-specific virulence genes among isolates. Similarly, Saadaoui (1987) in Morocco and Ballantyne (1995) in Australia reported the presence of true physiological races and differences in the degree of pathogenicity (aggressiveness) among *Z. tritici*. Kema *et al.,* (1995) observed significant cultivar x isolate interactions at both seedling and adult-plant stages using 78 isolates of *M. graminicola* isolates tested on 22 differential cultivars, indicating the presence of cultivar specificity and virulence variability among the isolates. Ackerman *et al.,* (1995) in South America also reported significant isolates x cultivar interaction with a study carried out on 10 *Z. tritici* isolates.

Moreover, Jlibene *et al*. (1995) in Morocco reported the presence of physiological specialization in *M. graminicola i.e* bread wheat *Z. tritici* isolates infect bread wheat and durum wheat isolates to durum wheat, respectively. They also revealed that *Z. tritici* isolates from bread wheat show differential virulence reaction on bread wheat differential lines. Likewise, Kema *et al.* (1996a) analyzed the genetic variation for virulence among 63 bread wheat and durum wheat *M. graminicola* isolates on bread and durum wheat seedlings separately, and observed significant cultivar x isolate interaction in both experiments, confirming the existence of variations in virulence among the isolates. Kema and van Silfbout (1997) also revealed the existence of significant interaction between host cultivars and *Z. tritici* isolates in both seedling and adult plant stages. In line with this, based on their reaction on the wheat line ST6, Grieger (2005) differentiated isolates of *Z. tritici* from

western Canada into two physiological races: physiological race 1 was virulent on ST6, whereas race 2 was virulent on ST6.

The occurrence of differential isolate x cultivar interactions between the wheat genotypes and the *Z. tritici* isolates as reported by earlier studies (Eyal *et al*., 1985; Kema *et al*., 1996a, 1996b; McCartney *et al*., 2002; Brading *et al.,* 2002; Ghaneie *et al.,* 2012; Hosseinnezhad *et al.,* 2014; Makhdoomi *et al.,* 2015) indicates that a gene-for-gene model operates in the wheat-*Z. tritici* pathosystem. With this principle, differential wheat genotypes carrying known *Stb* genes could be used for estimating the virulence diversity of *M. graminicola* populations (Czembor *et al.,* 2011). Field and/or growth room/greenhouse testes could be used to determine the differential host-pathogen interactions. In line with this, Hanson *et al.* (2016) revealed that greenhouse/growth room tests where plants are inoculated with specific pathogen strains, and defined inoculums concentrations could help to rapidly determine disease reactions under controlled environmental conditions. However, in the field where the cultivars are exposed to mixture of isolates, it is difficult to notice virulence variability (van Ginkel and Rajaram, 1995). This report contradicts Kema and van Silfhout (1997) who noticed significant isolate x cultivar interactions under field conditions.

2.11 Genetic Structure of *Z. tritici*

Genetic structure refers to the amount and distribution of genetic variation within and among populations. Pathogen populations that have high levels of genetic variability are more likely to adapt to resistant cultivars than populations with less genetic variability (Razavi, 2003, McDonald *et al*., 1995). *Z. tritici* exhibits high genetic diversity with low geographical

genetic differentiation (Bruce *et al*., 2016), in which an average of 92% of the global pathogen diversity could be found within a single wheat field and 84% of global diversity can be found within a one square area of any wheat field (Linde *et al.,* 2002; Zhan *et al.,* 2003). Only about 3% of the global genetic diversity of the pathogen is distributed among continents. This is attributed to the higher degree of sexual recombination and high rate of gene flow exhibited by the pathogen. Several previous studies also evidenced the presence of lower among populations genetic diversity (8- 31%) with low to moderate population genetic differentiations (Medini and Hamza, 2008; Abrinbana *et al.,* 2010; El Chartouni *et al.,* 2011; Boukef *et al.,*2012; Samia *et al*., 2013; Dalvand1*et al.,*2018; Siah *et al.,* 2018; Tilahun Mekonen *et al.,* 2020).

The long-distance movement of ascospores by wind (Boeger *et al*.,1993; Linde *et al*. 2002; McDonald and Mundt, 2016), exchange of infected seeds through marketing (Brokenshire, 1975; Dalvand *et al.,* 2018) and movement of plant parts like straws have facilitated for the high gene flow, and hence contributed for lower among-populations genetic differentiation (Consolo *et al*., 2009; Dalvand *et al.,* 2018).

Knowledge on the distribution of genetic variability within pathogen populations is vital and could have direct implications for designing suitable resistance breeding program to deploy resistance genes (Schnieder *et al*., 2001; McDonald and Linde, 2002; Dalvand *et al.,* 2018). Understanding the genetic structure of *Z. tritici* is useful for designing and implementing efficient and sustainable disease management strategies (Abrinbana *et al.,* 2010). Similarly, McDonald and Linde (2002) indicated that understanding the genetic structure of a pathogen

population is useful for deploying successful resistance breeding program, and for optimizing the management of resistant genes and fungicides.

The genetic structure of *Z. tritici* populations derived from different geographical locations can be easily evaluated with a variety of markers. Historically, phenotypic markers such as virulence variability analysis on a set of wheat differential lines carrying different resistant genes (Razavi, 2003; Dalvand *et al.*, 2018), pycindia density, total necrotic leaf area, latent period and disease progress have been used to characterize the genetic structure of *Z. tritici* populations (Brown *et al*., 2015). However, as virulent genes represent a small fraction of the pathogen genome and may not represent the random samples of genes, virulence pattern analysis alone may not provide sufficient insights into the true genetic structure of the pathogen populations. Moreover, phenotypic markers are highly influenced by the interaction of the pathogen with the host plant and also by the environmental conditions, and thus, they are less reliable to explain the true genetic structure of the pathogen (Brown *et al*., 2015). They should be complemented by more reliable and neutral genetic markers that are distributed randomly throughout the genome (Razavi, 2003).

Protein polymorphism or isozymes markers are the first molecular markers used for analysis of the genetic structure of populations (Scandalios 1969; Brown, 1978; Hamrick *et al.*, 1979). However, their lower rate of polymorphism and developmental stage specificity made protein markers less reliable to uncover the true genetic structure of pathogen populations. Nowadays, DNA polymorphisms became markers of choice for molecular-based surveys of genetic variation. They are the most preferred methods because they are

highly reproducible, highly polymorphic, neutral, abundant, and are not tissue or developmental stage specific (Park *et al*., 2009).

Several DNA marker systems have been developed and widely used to describe the genetic structure of *Z. tritici* populations. Each genetic tool exhibits its own advantage and limitations, and it is up to the researcher to select the appropriate marker that suites to the research objective. The marker selection can depend on their information content, reproducibility, polymorphism, degree of genetic resolution, degree of automation and technological and financial constraints (Nagata *et al.*, 2005). The following sections will briefly present some of molecular marker systems used for genetic analysis of *Z. tritici*.

2.11.1 Low-Throughput Markers for Z. tritici Genetic Analysis

Restriction fragment length polymorphism (RFLP) is the first generation of DNA marker system exploited (Botstein *et al.*, 1980) to analyze the genetic structure of *Z. tritici* populations originating from different geographical regions (Abrinbana *et al.*, 2010). They are co-dominant, robust, reliable and highly reproducible markers. They have been used to explore the genetic structure of *Z. tritici* populations in various parts of the world including in USA (McDonald *et al.*, 1995; Chen and McDonald, 1996), and Tunisia, Algeria and Canada (Medini and Hamza, 2008). However, RFLPs are costly, time consuming, labor intensive, need larger amounts of DNA and less polymorphic especially in closely related lines (Govindaraj *et al*., 2015; Dubcovsky *et al*., 2001), and hence, rarely used now for crop improvement (Lörz and Wenzel, 2005).

2.11.2 Medium-throughput Markers for Z. tritici Genetic Analysis

The second-generation DNA markers for genetic analyses include those derived from polymerase chain reaction (PCR) (Mullis *et al.,* 1986). Randomly Amplified Polymorphic DNA (RAPDs), amplified fragment length polymorphisms (AFLP) and Simple Sequence Repeats (SSRs) are some of the major PCR based marker systems widely used for genetic analysis of *Z. tritici* populations (Park *et al.,* 2009).

RAPDs are genetic markers which resulted from random amplification of genomic sequences recognized by primers of arbitrary nucleotide sequence (Williams *et al*., 1990). Several previous studies (Tingey and del Tufo, 1993; Czembor and Arseniuk, 1996; Razavi and Hoghes, 2004) used RAPD markers to explore the genetic structure of *Z. tritici* populations. However, they have lower transferability between laboratories, and hence, less preferred for genetic analysis. Moreover, the dominant marker AFLPs which combine both RFLP and PCR methods (Mohan *et al*., 1997) have been employed to explore the genetic structure of *Z. tritici* populations in Kansas, USA (Kabbage *et al.,* 2008), Iran (Abrinbana *et al.,* 2010) and Germany (Schnieder *et al.,*2001). However, nowadays AFLP technology lost its importance in genetic analysis due to its dominance nature and time-consuming protocol (Lörz and Wenzel, 2005).

Simple sequence repeats (SSRs) or microsatellites are another most commonly used marker technologies in genetic analysis of *Z. tritici* populations (Mohan *et al*., 1997). They are short tandem repeats of two to five nucleotides length widely and evenly distributed throughout

the genome of eukaryotes (Hamada *et al*., 1982; Rosewich and McDonald, 1994; Mohan *et al*., 1997). Polymorphism among the microsatellite loci is mainly due to difference in the number of repeating units that could be detected through PCR amplification of the variable region using a pair of primers targeting the conserved sequences flanking the variable region (Mohan *et al*., 1997; Govindaraj *et al*., 2015). Microsatellites are marker of choice for genetic analyses because of their higher polymorphism, multi-allelic nature, reproducibility, co-dominant inheritance, easily automated, relative abundance, ease of identification from genomic sequences and good analytic resolution (Winter and Kahl, 1995; Medini and Hamza, 2008; Gautier *et al*., 2014). Microsatellite markers have been used to analyze the genetic structure of *Z. tritici* populations in different countries including England (Owen *et al*., 1998), Tunisia (Boukef *et al.*, 2012; Samia *et al.*, 2013; Berraies *et al.* 2013), France (El Chartouni *et al*., 2011; Siah *et al*., 2018) and Ethiopia (Tilahun Mekonnen *et al.*, 2019).

Besides, SSR genetic tools are widely used in plants for genetic mapping, seed purity assessment and germplasm conservation, genotype identification and variety protection, diversity studies, pedigree analysis and paternity determination, gene and quantitative trait locus analysis, and marker-assisted breeding. They are very helpful to identify or track the positions/introgression of genes on chromosomes in several crops. SSR markers tightly linked to target genes could serve as chromosomal landmark, "sign" or "flag" for hunting genomic regions controlling desirable agronomic traits (Singh *et al.*, 2015). The same authors screened 192 rice genotypes for blast resistance genes using 10 closely mapped SSRs. Similarly, Yan *et al.* (2007) and Imam *et al.* (2014) used molecular technology to screen rice germplasms for rice blast resistance genes.

2.11.3 High-Throughput Markers for Z. tritici Genetic Analysis

A SNP represents a single nucleotide differences among DNA sequences or individuals at a specific locus in the genome (Park *et al.,* 2009; Lateef, 2015). They are the most abundant type of variations in the genome (Scherer and Christensen, 2016) occurring at a range of one SNP in every 100-300 bp in plants (Lateef, 2015). The advent of next-generation sequencing technology has enabled whole-genome comparison of fungal pathogens to dissect their host specializations (Thynne *et al.,* 2015). In line with this, McDonald *et al.* (2016) identified over 800,000 SNPs by comparing the genome of 13 Australian *Z. tritici isolates* with the reference isolate IPO323 genome of which ~10% had an effect on the coding regions of the genome. The authors also revealed over 1700 probable presence/absence of polymorphisms in genes across the Australian isolates using *de novo* assembly. Similarly, Stukenbrock and Dutheil (2018) identified about 1.48 million SNPs in *Z. tritici* which can be used for population genomic analysis of the pathogen. In *Arabidopsis thaliana*, about 37,344 SNPs have been documented. In wheat about 205,807 SNPs have been reported (IWGSC, 2018).

SNPs are typically bi-allelic markers that resulted from point mutations, and can be detected by aligning similar genomic regions from different genotypes of the same or different species (Xu, 2010). Set of alleles (SNPs) found together on a single chromosome, or part of a chromosome, is called a haplotype. In the absence of recombination, selection and mutation, the haploypes remain together. Such nonrandom co-occurrence of alleles within a chromosome or haplotype is called linkage disequilibrium. Its bi-allelic character, abundance and stability from generation to generation make SNPs amenable to automated,

high-throughput, and therefore, a suitable tool for diversity analysis, quantitative trait loci (QTL) mapping studies and marker-assisted selection in plant breeding programs (Lörz and Wenzel, 2005; Varshney *et al*., 2009).

2.12 STB Management Methods

Effective Septoria tritici blotch management requires an integrated approaches that includes crop management practices, chemical fungicides applications and the use of resistant cultivars (host resistance) (Ponomarenko *et al.,* 2011).

2.12.1 Crop Management Practices

Cultural disease management strategies include those practices that help to remove the possible sources of the spores from the field. Rotating wheat fields with non-host cultivars also helps to remove the ascospores which remained in crop residuals. Rotating a wheat field with other non-host cultivars for about 3-5-year could considerably decrease the incidence of STB (Eyal, 1981). Fernandez *et al*.(1998 also reported that two years of spring wheat followed by two years of a non-cereal crop or a cereal crop and summer fallow results in substantial reduction of STB infection. Brown and Rosielle (1980) also stated that wheat field sanitation practices like removal of crop debris and deep plowing could considerably reduce the amount of inoculums available to initiate a new disease cycle. Moreover, late planting is another basic agronomic practice that can reduce STB severity by avoiding the availability of the host during early season (reducing the 'green-bridge' between crops) when more spores are released (Gladders *et al*., 2001).

Furthermore, several previous reports (Broscious *et al*., 1984; Leitch and Jenkins, 1995; Simón *et al*., 2003) demonstrated that high nitrogen (N) fertilizer application could significantly increase STB severity. Hence, appropriate soil N management practices could contribute to reduced STB infection. Another crop management method useful for reducing STB severity is good management of the crop architecture by controlling the seed rate (Baccar *et al*., 2011). Obviously, higher seeding rate results in dense growth of the plants with their canopy very close to each other. This leads to increased STB infection by facilitating easy transfer of spores both vertically and horizontally through contact (Broscious *et al*. (1984) and also provides a suitable conditions for the development of the disease (Tompkins *et al*., 1993). Nevertheless, a sparser open canopy could result in easy vertical transfer of the spores through rain splash (Eyal, 1981). Although late planting, low seeding rate and low nitrogen fertilization may reduce STB infection, they may also contribute to lower yielding (Green and Ivins, 1985) and thus, less used by wheat growers.

2.12.2 Fungicide Application

Reports from previous studies (Dunne *et al*., 2008; Torriani *et al*., 2015) revealed that the use of chemical fungicides against STB improves wheat yield performance. If properly applied at right growth stages, agrochemical fungicides can provide sufficient protection against fungal diseases including *Z. tritici* (Christ and Frank 1989; Paveley *et al*., 2000). The first fungicide shall be applied immediately when the third leaf has fully emerged (approximately at GS 32) (Zadoks *et al*., 1974). This could provide sufficient protection to the third leaf and to some extent to leaf 2. The second fungicide application should be

conducted at about growth stage 39 (Zadoks *et al*., 1974) which is just when the flag leaf has fully emerged to provide protection for leaf two and the flag leaf (Sylvester-Bradley *et al*., 2008). But, a delay in fungicide treatment against *Z. tritici* beyond growth stage 37 can lead to great wheat yield loss of up to17 kg per hectare each day (Cook *et al.*, 1999).

Some of the most common chemical fungicides used for seed treatment to control STB in wheat includes thiabendazole (Dinoor, 1977), triadimenol and difenconazole (Sundin *et al*., 1999). Among the foliar fungicides, the triazoles group are not only effective for controlling STB, Fusarium head blight and brown rust, but also in delaying flag leaf senescence, and thus, leads to increased grain protein content and yield performance (King *et al*., 1983; Shaw and Royle, 1989; Blandino and Reyneri, 2009; Watson *et al.,* 2010; Muqaddasi *et al*., 2019). There is no agrochemical fungicide that could control STB once the pycnidia had developed, and thus, the flag leaf protection need to be performed before disease symptoms appear (Lucas *et al.*, 1999). The three major groups of chemical fungicides used to control *Z. tritici* are single-site fungicides like succinate dehydrogenase inhibitors (SDHIs), demethylation inhibitors (DMIs) and multisite fungicides including chlorothalonil (Torriani *et al*., 2015).

However, nowadays there is less interest to use chemical fungicides against crop diseases (Vincelli, 2016) including STB as it needs more than one fungicide application, which resource poor small-scale farmers could not afford (Brading *et al*., 2002). In Europe, the fungicide expenditure for STB control is about €1 billion per year (Kettles and Kanyuka, 2016). Besides, chemical fungicides are not durable and also have negative impact on human health and the environment. Furthermore, the consecutive and high input of chemical

fungicides in combating STB may lead to the development of fungicide resistant strains (Miedaner *et al.,* 2013; Vincelli, 2016; Tiley *et al.*, 2018). For instance, quinine outside inhibitors (Qols) or strobilurin were the best fungicide to control cereal pathogens including *Z. tritici* infections in the 1990s (Fraaije *et al*., 2005). However, spontaneous single amino acid substitution in *cytochrome b* of the fungus has resulted in the development of strobilurin resistant *Z. tritici* strains in 2002 (Tiley *et al.*, 2018). This has ultimately resulted in widespread of the fungicide resistant strains through sexual- ascospores by wind (Torriani *et al*., 2015), after which the fungicide became no longer effective against STB (Tiley *et al.*, 2018).

Other fungicides for which resistance has been developed include triadimenol, triadimefon, tebuconazole, propiconazole and epoxiconazole. However, the recently released fungicide: Fenpicoxamid is very efficient against *Z. tritici* (Owen *et al*., 2017). As frequent use of single fungicide can lead to new resistance strains to evolve, it is important to use combinations of fungicides with different mode-of actions. Furthermore, the use of fungicides should be coupled with other disease management methods like good crop management practices and host resistance.

2.12.3 Host Resistance

Because of the various limitations of chemical fungicides, currently host resistance is considered to be the most effective, cheapest, durable and environmentally friendly method to control cop diseases including STB (Eyal and Levy, 1987; Eyal, 1999; Ponomarenko *et al.*, 2011; Ghaneie *et al*., 2012; Miedaner *et al.*, 2013; Ragimekula *et al.*, 2013; Makhdoomi

et al., 2015; Hanson *et al.*, 2016; Odilbekov *et al*., 2019; Tilahun Mekonnen *et al*., 2019). Resistance to *Z. tritici* became a significant target trait in wheat breeding program much more recently than diseases such as the rusts and powdery mildew (Brown *et al.*, 2015). In wheat, two types of host genetic resistances to *Z. tritici* have been reported: qualitative and quantitative (Arraiano and Brown, 2006; Arraiano *et al*., 2009).

2.12.3.1 Qualitative Resistance to STB

Qualitative resistance, specific resistance also called complete resistance is a condition in which one or a few major *Stb* genes provide resistance to specific avirulent pathogens but not against other virulent *Z. tritici* isolates (Brown *et al*., 2015). It follows a gene-for-gene pattern of interaction with *Z. tritici* (Brading *et al*., 2002). So far, about 18 (*Stb1-18*) major resistance (R) genes (*Stb* genes) to STB and closely linked microsatellite markers have been identified and mapped in wheat (Ghaffary *et al.,* 2011a; Simón *et al.,* 2012; Brown *et al*., 2015). The *Stb1* was mapped on chromosome 5B (Adhikari *et al.,* 2004a), *Stb2*, *Stb3* and *Stb4* genes on chromosome 3B, 7A and 7D, respectively (Adhikari *et al.,* 2004b, c; Goodwin *et al.,* 2007), *Stb5* on 7D (Arraiano *et al.,* 2001), *Stb6* on 3A (Brading *et al.,* 2002), *Stb7* on 4A (McCartney *et al.,* 2003), *Stb8* on 7B (Adhikari *et al.,* 2003), *Stb9* on 2B (Chartrain *et al.,* 2009), *Stb10* and *Stb12* genes on 1D and 4A, respectively (Chartrain *et al.,* 2005a; b), *Stb11* on 1B (Chartrain *et al*., 2005c), *Stb13* and *Stb14* on 7B and 3B, respectively (McIntosh *et al.,* 2007), *Stb15* on 6A (Arraiano and Brown, 2007; Arraiano *et al*., 2007), *Stb16* on 3D and *Stb17* on 5A (Ghaffary *et al.,* 2012), and *Stb18* on chromosome 6D (Ghaffary *et al.,* 2011). Some other reported major genes conferring resistance to STB are

StbSm3 on 3A (Cuthbert, 2011), *StbWW* on 1B (Raman *et al.*, 2009), and *TmStb1* on 7A (Jing *et al*., 2008).

All of the reported *Stb* genes provide protection against individual or few groups of *Z. tritici* isolates except *Stb16q* gene that confers a broad resistance spectrum to a panel of 20 *Z. tritici* isolates collected from different parts of the world (Ghaffary *et al*., 2012; Kettles and Kanyuka, 2016). As the *Z. tritici* changes its genome rapidly leading to the development of new virulent strains, host resistance based on single major gene may not be durable. The best example for this is the breakdown of *Stb4* gene in the wheat cultivar Gene just after 5 years of its introduction (Cowger *et al*., 2000). Therefore, pyramiding or stacking of multiple resistance genes into single wheat cultivar may provide durable resistance to a range of pathogen races including *Z. tritici*. For instance, the wheat lines TE9000 and Kavkaz-K4500 possess multiple *R* genes providing them sufficient resistance against several *Z. tritici* isolates (Chartrain *et al*., 2004).

2. 12.3.2 Quantitative Resistance

Quantitative resistance is also called adult-plant resistance or generalized resistances to a range of pathogens which resulted from many genes expressions with minor additive effects. As it provides control-over a broad range of pathogen races in the field, quantitative resistance is the most effective, durable and preferred method to manage the rapidly evolving wheat pathogens including *Z. tritici* (Long *et al*., 2019).

So far, about 89 quantitative trait loci (QTLs) that confer resistance to STB have been reported in wheat among which 27 were identified at the seedling growth stage, 48 at adult stage and 14 at both growth stages (Brown *et al.*, 2015). Eriksen *et al*. (2003) identified five QTLs on chromosomes 3A, 6B, 2B and 7B that are effective for seedling and adult plant resistances to *Z. tritici*. Risser *et al.* (2011) also detected effective QTLs against *Z. tritici* on chromosomes 3B and 6D from "Floret" and 4B and 6B from "Tuareg". Similarly, Chartrain *et al*. (2004b) reported that a QTL on chromosome 6B was effective for *M. graminicola* resistance in the variety Riband. Simón *et al*. (2004) described three QTLs effective for seedling resistance on chromosomes 1D, 2D and 6B, and two adult plant resistances against *M. graminicola* in the synthetic hexaploid wheat `W7984. He *et al.* (2020) identified six QTLs effective for STB resistance on chromosome 2B, 2D, 3A, 3B, 3DL and 5B of the wheat line Murga. Among these, the major QTL on chromosome 3DL explained a total phenotypic variation for STB resistance of 27.5-40.3% in Uruguay and 41.2-62.5% in Mexico. While, the other five QTLs were identified to have minor effect on phenotypic variance for STB resistance. As a rule, a combination of major (R) genes and minor genes or QTLs for resistance against a pathogen is the most desirable makeup for any plant variety.

2.14 QTL Mapping for STB Resistance

The major scientific challenge in the process of crop improvement is understanding the genomic architecture of desired traits (Pasam *et al.*, 2012). Identification of genes/ quantitative trait loci (QTLs) of agronomic importance and its effective utilization in crop improvement program requires mapping their positions in the genome of the crop species

using molecular markers (Wuletaw Tadesse *et al.*, 2014; Sehgal *et al.*, 2016; Yano *et al.*, 2016). Nowadays, the advent and application of molecular marker technology made it easier to identify, map and efficiently transfer genes/QTLs underlying trait of interest in crop plants. Molecular markers closely mapped to the desired gene/QTLs can serve as chromosomal landmark to track the presence or introgression of the desired chromosomal region in breeding processes (Asad *et al*., 2012).

2.14.1 Linkage Mapping

Linkage mapping and association mapping are the two most widely used techniques for dissecting complex traits and identifying genomic regions underlying trait variation in plants, animals, and human (Sehgal *et al*., 2016; Yano *et al*., 2016; Burghard *et al*., 2017). Linkage mapping or bi-parental mapping is based on mapping population derived from crosses between two parents with contrasting characters of a trait under investigation. The method has been used to identify the number, effect size and chromosomal position of QTLs underlying agriculturally important quantitative traits in various crops (Yadav *et al.*, 2015).

Briefly, the work-flow for bi-parental mapping includes selection of two parental lines that exhibit sufficient polymorphisms for the trait of interest (eg. pure susceptible and pure resistant parental lines for disease analysis); developing mapping populations which can be double haploid lines (DHLs), Backcross (BC) population, F_2 population or recombinant inbred lines (RILs); phenotyping the mapping population for the target trait(s) under appropriate condition like growth chamber, screen-house, greenhouse, and/or open field;

molecular profiling of the population with adequate number of uniformly spaced polymorphic markers; constructing a linkage map; and identifying the QTLs underlying the target trait using suitable computer programs (Sehgal *et al*., 2016). Some of the advantages associated with linkage mapping include no population structure, the need for relatively fewer markers for genome coverage and ability to locate QTL regions along chromosomes (Sorrells and Yu, 2009). Numerous QTLs effective for STB resistance have been identified based on bi-parental mapping. For instance, Miedaner *et al.* (2013) mapped 26 QTL for STB, with the phenotypic variance explained by the individual QTL ranging from 3 to 21%.

Although linkage mapping will continue being as a useful tool for tagging important genes of crops, it suffers from a number of limitations (Sehgal *et al*., 2016). Due to limited number of recombination events, the genetic diversity and genetic mapping resolution in bi-parental mapping is limited (Zhu *et al.,* 2008; Brachi *et al*. 2011; Burghard *et al.,* 2017). Besides, bi-parental mapping incurs more cost and time for developing mapping population (Yano *et al* 2016; Burghard *et al.,* 2017). Moreover, it is less applicable for forest trees where mapping population development is either impossible or takes several years (Sehgal *et al*., 2016).

2.14.2 Association Mapping

Unlike traditional QTL mapping, association mapping uses several, diverse and unrelated lines to identify marker–trait associations, reducing the time and cost of developing mapping populations (Wuletaw Tadesse *et al*., 2014; Burghard *et al.,* 2017). It exploits ancestral recombination events that occurred in the population and takes into account all major alleles

present in the population to identify marker-trait associations (Pasam *et al.,* 2012). During the long historical time, several recombination can occur, and hence, only closely located alleles can be co-inherited (Weising *et al.,* 2005). Such non-random co-segregation of alleles at nearby loci is called linkage disequilibrium (LD) (Xu, 2010). LD declines with increasing physical and genomic distance. Mating system, population structure and recombination hot spots are all factors affecting LD. Association mapping uses LD between alleles within diverse populations to determine marker–trait association at whole genome scale (Pasam *et a.l,* 2012; Burghard *et al.,* 2017).

There are two types of association mapping: 1) candidate-gene association mapping, and 2) genome-wide association mapping or Genome wide association studies (GWAS) (Erena Aka, 2013). The former mapping approach aimed at linking phenotypic variation with allelic variation in candidate genes. It identifies marker-trait association in the candidate genes rather than scanning the association at whole genome scale. While, Genome-wide association studies survey genetic variation across the whole genome linking it to various traits, thereby identifying DNA markers of potential use for plant breeding. So far, several plant species have been explored for quality, yield, yield related traits, and resistance to biotic and abiotic stresses (Zhu *et al*., 2008; Scherer and Christerensen, 2016; Yano *et al.* 2016; Burghard *et al.,* 2017).

2.14.2.1 Genome-Wide Association Mapping and Procedures

The science of Genome-Wide Association Mapping (GWAM) or GWAS has emerged about a decade ago as a powerful scientific tool to identify genes associated with the outward traits of an organism (Scherer and Christensen, 2016; Fan *et al.,* 2016). It is becoming a promising approach to dissect genomic regions underlying complex quantitative trait loci of interest in crops (Maccaferri *et al*., 2015; Yano *et al.,* 2016). It is a powerful and robust strategy to genetically fine-map multiple traits such as plant morphology, yield, yield-components, diseases resistance and quality traits simultaneously. The advent and use of high-throughput sequencing technologies have enabled GWAS to scan the entire genome to determine markers associated with desired traits (Rafalski *et al.,* 2010). GWAS has become a widely accepted strategy for decoding genotype-phenotype associations in many species thanks to technical advances in next-generation sequencing applications (Xiao *et al.,* 2017).

GWAS has been used to elucidate the genetic architectures of important agronomic traits in several crops including Barley (Pasam *et al*., 2012; Tian *et al.,* 2012; Fan *et al.,* 2016; Rashid *et al*., 2018), rice (Huang *et al*., 2012), sugarcane (Racedo *et al.,* 2016) and sorghum (Morris *et al.,* 2012; Gezahegn Girma *et al*., 2019). In wheat, GWAS has been successfully used to determine genomic regions associated with phenological, yield and yield-related traits (Jamil *et al.,* 2019; Wang *et al*., 2019; Ward *et al*., 2019), drought tolerance (Mathew *et al*., 2019), resistance to diseases such as strip rust (Long *et al*., 2019; Yao *et al*., 2019; Cheng *et al*., 2020), stem rust (Kankwatsa *et al*., 2017; Erena Aka *et al*., 2018) and Septoria tritici blotch (Yosef Geberehawariat *et al*., 2017; Odilbekov *et al*., 2019). Vagndorf *et al.*

(2017) mapped four QTLs associated with STB resistance on chromosomes 1B, 2A, 5D and 7A in 164 North European winter wheat varieties and breeding lines. Kollers *et al*. (2013) also reported numerous marker-trait associations for STB resistance that were co-localized with previously mapped major genes such as *Stb*1, 3, 4, 6 and 8 and other new associations on chromosome 2A, 2D, 3A, 5B, 7A and 7D in 372 European wheat cultivars. Likewise, Yosef Geberehawariat *et al*. (2017) identified putative QTLs associated with STB resistance on chromosome 1A, 2B, 3A, 4A and 5A in 318 Ethiopian durum wheat mapping panel. Moreover, Muqaddasi *et al.* (2018) detected potential QTLs effective for STB resistance on chromosomes 1A, 1B, 2D, 4A, 5A, 6A, 6D, 7A, and 7B in 371 European wheat. Furthermore, Odilbekov *et al.* (2019) mapped QTLs responsible for STB resistance on chromosome 1B, 1A, 2B, 3A, and 5A in 175 winter wheat landraces and old cultivars of Nordic origin.

Therefore, GWAS is a very powerful and promising technique to identify and locate genomic regions underlying agronomically desired traits, and hence, there is a significant increase in number of GWA studies and publications over time. The upcoming sections present the logical steps of GWAS (Sehgal *et al.,* 2016).

2.14.2.1.1 Selection of Association Mapping Panels

The first and most important step in association analysis is the choice of study subjects (Flint-Garcia *et al.,* 2005; Breseghello and Sorrells, 2006). This is because, the genetic diversity, the extent of LD as well as the level of population structure and relatedness in the

population under investigation determine the mapping resolution, the association analysis method and the statistical power to detect traits associations (Stich and Melchinger, 2010). Generally, four types of mapping populations can be used for association analysis (Stich and Melchinger, 2010) including: natural populations, gene bank collections, synthetic populations, and elite breeding material. The association-mapping panel is expected to differ considerably with respect to genotypic and phenotypic diversity, extent of LD and population structure and familial relatedness (Stich and Melchinger, 2010).

If the association-mapping panel is from the gene bank, the core collections are expected to represent most of the genetic variability with a manageable number of accessions. In such cases, selecting a minimum sample size with maximum variation can reduce population structure and decrease LD, and thus, become very suitable for association mapping. Core collections are useful for mapping qualitative traits, such as disease resistance or quality characteristics. However, their broad genetic variability makes them often unsuitable for analysis of quantitative traits because accessions are usually unadapted to growing conditions, resulting in poor precision of trait measurement. In such case, elite lines might be more desirable materials for mapping low-heritability traits such as grain yield (Sehgal *et al.*, 2016). Therefore, selecting association panel that composed of individual lines or cultivars with wide genetic diversity is vital for successful GWAS.

2.14.2.1.2 Measuring the Phenotype

The target of GWAS is to determine the association between genome-wide scanned SNPs marker and a phenotype of interest that has been scored across a large number of individuals

(Korte and Farlow, 2013). The phenotypic data for association studies should come from a relatively large number, diverse accessions, with adequate replications across locations over years (Ingvarsson and Street, 2011; Burghard *et al.,* 2017). With regard to field design, the use of incomplete block design (e.g. α-lattice) has the potential to increase the mapping power (Piepho *et al*., 2006). However, if the field design is unbalanced, proper statistical modeling of the experimental design and the consideration of genotype x environment as well as marker x environment interactions (Malosetti *et al.,* 2008) can increase the mapping power (Stich *et al.,* 2008).

Generally, traits which are controlled by fewer number of loci with large effect sizes (have a simple genetic architecture) are highly amenable to GWAS (Louthan and Kay, 2011; Burghard *et al.,* 2017), and in such case use of small sample size can serve the purpose of association study. However, polygenic traits that possess more complex genetic architectures present difficulties for GWAS and in such cases increasing the sample size will improve the power to recover meaningful marker-phenotype associations (Burghard *et al.,* 2017). In line with this, Miedaner *et al.*(2013) determined the genetic architecture of resistance to STB in 1,142 bread wheat genotypes evaluated in unreplicated field trials at two locations. Likewise, Kollers *et al.* (2013) identified resistance to STB in 372 wheat genotypes evaluated at single location for two years with three replications per year. Similarly, Vagndorf *et al.* (2017) explored the genetic architecture of STB resistance in 164 wheat genotypes evaluated at three locations over three years. Moreover, Muqaddasi *et al.* (2018) and Yates *et al.* (2019) dissected the genetic bases of STB resistance using 371 and 335 wheat genotypes evaluated at single location for two years and one year, respectively.

These all evidenced that well designed and replicated environments phenotype scoring in large sample size is helpful for precise dissection of the genetic architecture of STB resistance through GWAS approach.

2.14.2.1.3 Genome-Wide Marker Profiling of Mapping Population

To determine the association of phenotypic variation with genomic variation, the entire panel involved in the association study need to be genotyped (Burghard *et al.,* 2017). Scanning single nucleotide polymorphisms (SNPs) at whole-genome scale in all individuals can ensure a high probability of finding a causative variant. The advent of different sequencing technologies have enabled the whole genome sequencing and re-sequencing of the genome of several organisms, and thus, facilitated the identification of large numbers of SNPs in any plant species (Ossowski *et al*., 2008; Ganal *et al*., 2009). The availability of ultra-high density SNP markers simplified genetic analysis related to linkage mapping, population structure, association studies, marker-assisted plant breeding, map-based cloning, and functional genomics (Kumar, 2012). This confirms that dense SNPs genotyping is the core component of every genetic association study, and thus, selecting appropriate genotyping platform is important. An ideal SNP genotyping platform should be rapid, robust, fully automatic, highly accurate and affordable (Syvänen, 2001). The advent of next generation sequencing (NGS) technologies has dramatically reduced the time and cost of SNP genotyping, and hence, enhanced the use of several SNPs in plant breeding programs (Kim *et al*., 2016).

2.14.2.1.3.1 SNP Discovery through Next-Generation Sequencing

Knowing the genome sequence of a species is of paramount importance in crop breeding. As the gold standard of DNA sequencing, Sanger sequencing method has been exclusively employed in sequencing DNA of different origins in the early days of genomics research. However, its low throughput and high genotyping cost greatly limited the application of Sanger sequencing in research. This caused life-science researchers to develop alternative DNA sequencing techniques – the next-generation sequencing (NGS) techniques. Roche 454 Genome Sequencer (Roche Diagnostics Corp., Branford, CT, USA), HiSeq 2000 (Illumina Inc., San Diego, CA, USA), AB SOLiD_System (Life Technologies Corp., Carlsbad, CA, USA) and Ion Personal Genome Machine (Life Technologies, South San Francisco, CA, USA) are the first four commercially available NGS platforms. They involve PCR amplification of genome fragments prior sequencing. The other category that does not involve the PCR amplification step prior to sequencing are called 'single molecule' sequencing (SMS) technologies. It includes HeliScope (Helicos Bio- Sciences Corp., Cambridge, MA, USA) and PacBio RS SMRT system (Pacific Biosciences, Menlo Park, CA, USA) (Shokralla *et al*., 2012).

NGS is still subjected to high cost per sample analysis (Srinivasan and Batra, 2014), PCR amplification biases (Kumar *et al.,* 2012), and also weak to characterize repetitive elements and structural variants (Salzberg *et al.,* 2005; Treangen *et al.,* 2011). These have necessitated the development of higher levels of sequencing, third-generation and fourth-generation; which involve single DNA molecule sequencing without a prior PCR

amplification step (Srinivasan and Batra, 2014). The third-generation sequencing uses single-molecule templates without involving PCR amplification step. It is beneficial to the previous sequencing platforms in that it needs less starting material, it is very fast and less prone to error (Ku and Roukos, 2013; Bleidorn *et al*., 2016). It generates long reads with average read lengths ranging from 5,000bp - 15,000bp (Lee *et al.,* 2016) with some reads lengths exceeding 150, 000 bp (Bleidorn *et al*., 2016). There are three commercially available third-generation sequencing technologies including Illumina Tru-seq Synthetic Long-Read technology, Pacific Biosciences (PacBio) Single Molecule Real Time (SMRT) sequencing and the Oxford Nanopore Technologies sequencing platform (Mignardi and Nilsson, 2014).

The fourth-generation sequencing platform involves massive parallel sequencing of nucleic acids directly within tissues or cells (Mignardi and Nilsson, 2014). This *in situ* sequencing (ISS) method exploits second-generation NGS chemistry to read nucleic acid composition directly in fixed cells and tissues, and allows fast and robust detection of SNPs (Ke *et al.,* 2016). The nanopore sequencing technology (Roche/IBM and Oxford) is the fourth-generation sequencing platform. This technology is advantages in that it does not need sample preparation, and the transduction and recognition occur in real time. Besides, it generates long reads (up to 10,000 bp) which could be are capable of de novo sequencing (Ke *et al.,* 2016).

2.14.2.1.3.2 Genotyping by Sequencing as SNP genotyping technology

The evolution of NGS technologies contributed to the completion of the whole genome sequences (WGS) of several plant species (Elshir *et al.,* 2011; Kim *et al.,* 2016). The WGS data is ideal for the discovery of large sets of SNP markers important for genetic diversity analysis, linkage mapping, QTL analysis, and genomic selection (Spindel *et al.,* 2013). However, the high sequencing cost per data point and low coverage became the major bottlenecks for WGS approach (Kim *et al.,* 2016). In species with large genome size, removing the repetitive regions of the genome using restriction enzymes (REs) (genome complexity reduction), and sequencing only the lower copy regions of the genome makes the NGS-based genotyping very simple, specific, quick, highly reproducible, and ensure sufficient overlap in sequence coverage (Elshir *et al.*, 2011).

The relatively new and highly multiplexed GBS platform conducts SNP discovery and genotyping simultaneously in much less time and cost (Kumar *et al.,* 2012; Kim *et al.,* 2016; Bhattacharjee *et al.,* 2020). GBS is a truly revolutionary technology in providing a powerful method for developing high-density SNP markers to the vast majority of plant species including those species with no reference genome sequence (Poland *et al.,* 2012). The GBS platform made SNP discovery, phylogenetic study, bi-parental mapping, candidate gene mapping, GWAS, and genome selection accessible to all plant researchers at reasonable cost (Kumar *et al.,* 2012).

Briefly, the GBS protocol begins with library construction that involves plating and drying of genomic DNA of each study sample, unique or barcode adaptor, and common adapter on an Illumina flow cell. This is followed by endonuclease digestion with suitable REs usually using two restriction enzymes – one methylation sensitive (eg. *Ape*KI), and one that is not. Ligation of one common and one barcode adaptor to the sticky ends of the DNA fragments in the presence of T4 ligase. Then, T4 ligase is inactivated through heating followed by removing unreacted adapters by pooling and applying the sample to a size exclusion column. This is followed by PCR amplification using primers having complementary sequences to the adapters. The PCR products constitute a sequencing "library" which will be further cleaned to reduce adapter dimers. The GBS libraries are then sequenced using Illumina platform like HiSeq 2500 platform. During sequencing, reads are associated with an individual by the unique adapter that serves as an ID tag (Elshir *et al.*, 2011). Generally, use of frequent cutter enzymes generate higher number of fragments, show more genome coverage and hence, results in more SNPs identification (Poland *et al.*, 2012).

2.14.2.1.3.3 GBS Analysis Bioinformatics Pipelines

GBS generates large raw datasets of sequence reads that needs bioinformatics pipelines to analyze and interpret the datasets. There are two groups of automated bioinformatics pipelines used for analyzing GBS datasets: reference-based and de novo-based (Torkamaneh *et al.*, 2017). The former category refers to a SNP calling by aligning reads from reduced-representation sequencing against the reference genome, and a SNP is identified when a nucleotide from the read differs from the reference genome sequence at the same nucleotide

position. Some of the most widely used reference-based GBS analysis bioinformatics pipelines include TASSEL-GBS (TASSEL 3.0 and TASSEL 5.0) (Bradbury *et al.,* 2007; Glaubitz *et al.,* 2014), Stacks (both *de novo* and reference-based) (Kumar *et al.,* 2012), IGST (IBIS Genotyping-by-Sequencing Tool), and Fast-GBS) (Catchen *et al.,* 2013; Torkamaneh *et al*., 2017). Among these pipelines, TASSEL-GBS v1 and Fast-GBS are the fastest, and IGST is the slowest pipelines running for ~1h45 and ~13h, respectively to complete the analysis (Torkamaneh *et al.,* 2017).

In the absence of reference genome sequence, the *de novo* GBS analysis pipelines are employed for genotype calling. Universal Network Enabled Analysis Kit (UNEAK), and Stacks are the most widely used *de novo* SNPs calling methods (Catchen *et al*., 2013; Lu *et al*., 2013). Among *de novo* GBS pipelines, UNEAK (1h11) is identified to be three times faster than Stacks (vs 3h07), and proved to be the fastest of all pipelines (Torkamaneh *et al.,* 2017).

The GBS technology has been used for high-density SNP discovery and genotyping in various plant species for use in diversity analysis (Baloch *et al.,* 2017; Robbana *et al.,* 2019; Akohoue *et al*.,, 2020), linkage mapping (Raman *et al.,* 2020;), MAS (He *et al*., 2014*),* GWAS for pest resistance (Ando *et al.,* 2018; Visioni *et al.,* 2018; Gezahegn Girma *et al.,* 2019; Long *et al.,* 2019; Murube *et al.,* 2019; Yao *et al.,* 2019) and genomic selection prediction (Akohoue *et al.*,2020).

2.14.2.1. 4 Imputation of Missing Genotype Data

Missing genotype data significantly affect accurate dissection of marker- trait association. Thus, in GWAS, any missing genotype data need to be imputed as the average of the marker scores across all taxa (Li *et al.,* 2009). This allows researchers to explore association at markers that are not directly genotyped. Imputing missed data greatly increases the number of SNPs that can be tested for association, increases the power of analysis, and facilitates analysis of GWAS data (VanRaden *et al.,* 2015). Besides, imputation save cost and avoids re-sequencing of the study sample (Ingvarsson and Street, 2011). The imputation tools attempts to identify sharing of stretches of short distantly inherited chromosomal sequence between the haplotype of the study material and the haplotypes in the reference set and use these sharing to fill the missing alleles in the study individuals (Li *et al.,* 2009). Family members share long stretches of chromosome between the individuals (show "identity-by-descent"). In such case, modest number of markers present in some individuals of the family can be used to infer most of the missing genetic markers in other members of the family sharing the same haplotypes (Li *et al.,* 2009). The same approach can be used for apparently unrelated samples. However, as the common ancestors are more distant, the stretches of haplotypes shared between apparently unrelated samples will be shorter (Li *et al.,* 2009)

There are two categories of genotype imputation tool: computationally intensive tools like MACH (Li, 2006), IMPUTE (Marchini *et al.,* 2007) and fastPHASE/BIMBAM (Scheet and Stephens, 2006; Servin and Stephens, 2007) which take into account all observed genotypes when imputing each missing genotype, and computationally more efficient tools such as

TUNA (Nicolae, 2006), LD-kNNi (Money *et al.,* 2015), PLINK (Purcell *et al.,* 2007), BEAGLE (Browning, 2006) and WHAP (Zaitlen *et al.,* 2007). The former can be further categorized into two: i) those which compare the potential haplotypes for each individual with all other observed haplotypes (e.g. MACH and IMPUTE,), and ii) those that compare potential haplotypes for each individual to a representative set of haplotypes (e.g. fastPHASE) (Li *et al.,* 2009). In TASSEL 5, there are two missing genotype imputation methods: a generalized approach suitable for all types of populations but optimized for those with higher inbreeding coefficients (FILLIN) and the other is specifically optimized for finding recombination break points in FSFHap (Full--Sib Family Haplotype Imputation) (Bradbury *et al.,* 2007). Some software programs like MaCH, Minimac, IMPUTE2 (Howie *et al.,* 2012) and Beagle (Browning and Browning, 2009) automatically impute missing genotypes during the haplotype estimation process (Li *et al.,* 2009).

Each tool has its own specific cons and pros, in terms of speed and accuracy (Howie *et al.,* 2012). Comparison analysis showed that there is no significant difference (only discrepancy of <1.5%) between genotyped and imputed SNPs (Li *et al.,* 2009) in genotype calls, estimated allele frequencies and test statistics. This implies that genotype imputation is cost effective and also fast approach to predict un-typed portions of the genome for GWAS.

2.14.2.1.5 Linkage Disequilibrium Estimation in Wheat

Linkage disequilibrium (LD) refers to the non-random association of alleles in different loci in a population (Flint-Garcia *et al.,* 2003). Over long generations, there will be high likely for recombination to takes place between any pairs of alleles in specie, resulting in reduced

LD distance between loci. Consequently, only physically close linked loci can co-occur (in LD) for many generations (Morton *et al*., 2001). GWAS uses this concept of LD to identify and map genetic markers that are near to the causal variants or loci controlling important agronomic traits with high resolution. A marker allele in LD with the causative variant can signify an association with the trait of interest by proxy. LD between pairs of loci depends on various factors in which small population size, inbreeding, genetic isolation between lineages, population subdivision, low recombination rate, admixture, and genetic drift increase LD. While, out-crossing, high recombination rate, high mutation rate, *etc*., lead to disruption of LD (LD decay) (Sorkheh *et al.,* 2008).

LD decay is a function of genetic or physical distance. It may decay over a short or long distance based on the population and species under consideration and the region of the chromosome. Hence, determining the extent and pattern of LD between all pairs of SNPs at the population level is vital for high resolution mapping of genomic regions controlling the target traits (Sorkheh *et al.,* 2008). The two most commonly used statistics to measure LD are D' (disequilibrium coefficient) and r^2 (square of the correlation coefficient or coefficient of determination) (Gupta, 2005). All LD statistics measure the difference between the observed and expected haplotype frequencies (Flint-Garcia, 2003; Mackay and Powell, 2007). For two loci with alleles "A" and "a" at locus X, and alleles "B" and "b" at the second locus Y with their respective allelic frequencies of π_A, π_a, π_B, and π_b, the obtained haplotype frequencies will be π_{AB}, π_{Ab}, π_{aB}, and π_{ab}. Then, the LD measure r^2 can be computed as:

$$r^2 = \frac{(Dab)^2}{\pi A \pi a \pi B \pi b} \text{ , where } \quad Dab = \pi_{AB} - \pi_A \pi_B$$

Where π_{AB} is the observed frequency of gametes AB, and π_A and π_B are the expected frequencies of allele A and B at X and Y, respectively. The value of r^2 ranges from 0 – 1, where $r^2 = 0$ implies the two loci are inherited independently, and the value of r^2 near to 1 indicates the frequencies of co-segregation of alleles at two loci is higher (Ersoz *et al*., 2009).

Alternatively, the LD measure *D*' can be calculated (Lewontin, 1964) as:

$$|D'| = \frac{(Dab)^2}{\min(\pi A \pi b, \pi a \pi B)}, \qquad \text{for } Dab < 0;$$

$$|D'| = \frac{(Dab)^2}{\min(\pi A \pi B, \pi a \pi b)}, \qquad \text{for } Dab > 0.$$

D' values range between 0 and 1 and it will only be less than 1 if all four possible haplotypes are observed; hence, a presumed recombination event has occurred between the two loci (Flint-Garcia, 2003). Relatively, r^2 is less sensitive to small sample size and low allele frequencies, and hence, it is better in describing how markers might be correlated with QTL of interest (Flint-Garcia *et al*., 2003; Martinez *et al*., 2006). Hence, the r^2 is preferred among researchers to describe the pattern and extent of LD than the D' statistics.

There are several freely available software packages such as TASSEL (Version 3 and Version 5) (Bradbury *et al.*, 2007), GAPIT (Zhiwu Zhang Lab, 2020), GOLD (Abecasis and Cookson, 2000) and Powermarker (Liu and Muse, 2005) that are used to determine the extent and pattern of LD.

2.14.2.1.5.1 LD Decay in wheat

The r^2 (alternatively, the *D'*) values for all pairs-wise combinations of alleles are plotted against the genetic (cM) or physical (bp) distance among the alleles at a chromosome level or entire genome to generate LD decay plot or disequilibrium matrices. The graph summarizes the rate at which LD declines with distance for a chromosome or for the whole-genome (Gupta *et al*., 2005). A nonlinear logarithmic curve called LOESS fitting curve is produced to model the LD decay. The point at which the LOESS curve intercepts the critical $r^2 = 0.1$ is considered to be the average LD decay of a population (Pasam *et al*., 2012). For more accurate estimates, $r^2 \geq 0.2$ are used as cutoff point for association between all pairs of loci and to describe the maximum distance at which LD is significant (Zhu *et al*., 2008).

The strength and pattern of LD in wheat differ among chromosomes and genomes. Chao *et al*. (2007) reported that for 43 U.S wheat cultivars, the intra-chromosome LD decay below $r^2 < 0.2$ within 10 cM. However, for the same wheat cultivars, long distance (over 30 cM) LD decay was observed for chromosome 3DL, 4DL and 6AL. Among the three genomes, the B genome exhibited the highest proportion of significant LD (Chao *et al*., 2007). Similarly, with a study conducted on 96 soft winter wheat association panel using SSR markers, Breseghello and Sorrells (2006) confirmed that the LD decayed within 1 cM and 5 cM for chromosome 2D and 5A, respectively.

Likewise, Chao *et al*. (2010) confirmed the presence of significant difference in LD decay rate among wheat genomes of 478 spring and winter wheat assessed using 394 SNP markers. They reported that LD declined to half of its initial value within 6 - 7 cM for the three wheat genomes. Ando *et al* (2018) reported that LD declined to half of its initial value within an average distance of 5.1 cM for 395 wheat lines genotyped using iSelect 9K. LD analysis for 120 China winter wheat association panel assayed with 90K SNP arrays revealed that on average the LD declined below $r^2 < 0.2$ at 12 cM (Cheng *et al.,* 2020). Similarly, for 152 China wheat landraces genotyped with DArT-seq technology, the LD decayed below $r^2 = 0.3$ at 12 cM (Long *et al.,* 2019). It was reported that the LD in 318 Ethiopian durum wheat (*Triticum durum* Desf.) genotyped with 16,223 SNPs declined below $r^2 = 0.2$ within 1.5–6 cM in all chromosomes (Yosef Geberehawariat *al*., 2017). Similarly, Sisay Kidane *et al.* (2021) revealed that the LD between all SNP marker pairs in 300 Ethiopian durum wheat genotypes assayed using 35K Axiom Array at 7,093 positions declined below $r^2 = 0.2$ at 69.1 Mbp. Since there is considerable difference among studies on association mapping population size, LD significance threshold (r^2) value and marker type, it is difficult to give overall conclusion regarding the patterns and extent of LD in wheat. The remark that could be drawn is that LD analysis should be conducted at chromosomal level and entire genome for each association panel.

2.14.2.1.6 Controlling Population Structure

Population structure is one of the major hurdle for dissecting complex traits through GWAS (Kim, 2019). Therefore, controlling for population structure shall be a standard procedure in

GWAS (Brachi *et al*., 2011). One of the several methods used to control for the effect of population structure is conducting structured associations (SA) (Pritchard *et al*., 2000). The method first searches a population for closely related clusters using Bayesian model-based algorithm and then uses the clustering matrices (Q) as covariate in association analysis (Sehgal *et al*., 2016). STRUCTURE program (Pritchard *et al*., 2000) can effectively estimate the population structure and kinship (shared co-ancestry coefficients between individuals of subdivisions of a population) using several models. More recently, the principal component analysis (PCA) method where only few of the PCs can be included to account for population structure is considered to be the fast and effective way to control population structure (Zhu and Yu, 2009). The optimal number of PCs/covariates to be included in the GWAS model can be determined based on Bayesian information criterion (BIC) or based on a screen plot (Zhiwu Zhang Lab, 2020).

However, in the presence of some degree of familial relationships among sampled individuals, considering only population structure information (Q-matrix or PCA) in the analysis may not sufficiently control spurious association. Thus, it is important to use powerful statistical models that combine both population structure (Q-matrix or PCA) and familial relatedness (kinship-matrix) information in association analysis. Several statistical models have been developed and widely used in GWAS (Yu *et al*., 2006). These include general linear model (GLM), mixed linear model (MLM), compressed MLM (CMLM), enriched CMLM (ECMLM), settlement of MLM under progressively exclusive relationship (SUPER), fixed and random model circulating probability unification (FarmCPU), and Bayesian-information and linkage-disequilibrium iteratively nested keyway (BLINK). The

GWAS models differ in their statistical power which are ordered as GLM < MLM< CMLM< ECMLM < SUPER < FarmCPU< BLINK (Zhiwu Zhang Lab, 2020).

GLM reduces for false-positive association by including the Q-matrix to account for the population structure. The MLM includes the kinship (K) matrix to account for familial relatedness among individuals in addition to the Q- matrix. The MLMM, improves the model fitting by including the associated markers as cofactors for marker test, maintaining both Q and K unchanged. SUPER eliminate the confounding effect of familial relatedness by using the kinship derived from associated markers. FarmCPU uses both fixed effect model and the random effect model iteratively. BLINK improved the computational speed and statistical power by using Bayesian Information Content (BIC) of a fixed effect model to approximate the maximum likelihood of a random effect model to select the associated markers among the markers remained the exclusion based on LD (Zhiwu Zhang Lab, 2020).

Various levels of population structure have been reported in wheat. Zheng *et al*. (2009) detected eight subpopulations in 96 Great Plains winter wheat cultivars and advanced lines analyzed for population structure using 60 SSR. Likewise, a population structure analysis in 120 China winter wheat association panel genotyped with 90K SNP arrays revealed the presence of six subgroups in the panel (Cheng *et al.*, 2019). Moreover, Long *et al.* (2019) reported two subpopulations in 152 China wheat accessions assayed with DArT-seq SNP markers. Furthermore, a population structure analysis conducted on 395 wheat lines genotyped using iSelect 9K detected two subpopulations (Ando *et al*. 2018).

2.14.2.1.7 Computational Software Programs for GWAS

Corresponding to the advances in sequencing and genotyping technologies, several software programs have been developed to efficiently use sequence information to dissect the genetic architecture of complex traits, and enhance the crop improvement programs. Some of the software programs useful for association analysis are TASSEL (Trait Analysis by Association, Evolution and Linkage) (Bradbury *et al.,* 2007), GAPIT (Genomic Association and Prediction Integrated Tool) (Zhiwu Zhang Lab, 2020), ASREML (Gilmour *et al*., 2002), JMP Genomics (Lovell, 2010), SAS, PLATO software (Hall *eta l.,* 2017) and KDCompute PlugIn system (http://www.kddart.org/ kdcompute. html). TASSEL is the most commonly used public free software package for trait association analysis (Bradbury *et al.,* 2007; Sehga *et al.,* 2016). The program implements different statistical method like GLM, MLM, CMLM, and P3D (population parameters previously determined) for marker-trait association analysis (Bradbury *et al.,* 2007). Besides, TASSEL is used for imputation of missing data, LD analysis, and data visualization (Sukumaran and Yu, 2014; Sehga *et al.,* 2016). The latest version of TASSEL (TASSEL 5.0) has CMLM for computing large data sets with up to 500,000 markers (Sehga *et al.,* 2016).

GAPIT is a new and user-friendly tool in the R package that is widely used for genome-wide association study and genomic selection and prediction (Lipka *et al*., 2012). The package implements state-of-the-art computational methods like MLM, CMLM, MLMM, ECMLM, SUPER, FarmCPU, BLINK, and genomic best linear unbiased prediction (gBLUP) (Zhiwu Zhang Lab, 2020) to carry out GWAS, genomic selection and predictions (Lipka *et al.,*

2012). By subdividing the genotypic dataset into multiple files, the GAPIT package can handle huge datasets of over 10,000 individuals and 1 million SNPs with low computational time. The program produces several tables and graphs in publication ready formats to interpret results (Lipka *et al*., 2012).

ASReml is a powerful tool that fits LMM using Residual Maximum Likelihood (REML) to estimate the parameters (Gilmour *et al*., 2002). It can handle complex datasets generated in quantitative genetics, breeding, environmental sciences and medical sciences (Isik *et al.*, 2017). JMP® Genomics is also advanced software program useful for GWAS, expression analysis, predictive modeling and plant breeding (Lovell, 2010). Statistical Analysis Software (SAS) is also a software system commonly used for association mapping. PLATO (PLatform for the Analysis, Translation, and Organization of large-scale data) software package contains functions for quality control, filtering and GWAS (Hall *et al.,* 2017). KDCompute is a web based analysis platform within a secure, customizable and cooperative plugin framework. It can carry out analytical, imputation or data processing tasks efficiently leaving your computer free to perform other tasks. Some other GWAS data analytical tools include PLINK (Purcell, *et al.,* 2007), SUGEN (Lin *et al.,* 2014), SNPtest (Wellcome Trust Case Control Consortium, 2007), GenABEL (Karssen *et al.,* 2016); GWASTools (Gogarten *et al.,* 2012), EMMAX (Kang *et al.,* 2010), GMMAT (Chen *et al.,* 2016) implemented via GENESIS (Conomos, *et al.,* 2017).

2.14.2.1.8 Significance Thresholds and Corrections for Multiple Testing

In experimental hypothesis testing, a *P*-values (α value) is used to describe the likelihood that a given result from a test is due to chance or false positive, and it is usually set to 5%. This implies that at a = 0.05 significance level, one marker has 5% probability of false-positive association by chance (Burghardt *et al*., 2017). However, as GWAS tests several genetic markers simultaneously, there could be high accumulations of false-positive association by chance. This needs use of adjusted *P*-value (α value) threshold for statistical significance to lower the number of false-positive associations.

The two most commonly used statistical methods to control false-positive association in GWAS are the Bonferroni correction and the false discovery rate (FDR) correction. The former is a stringent method used to control the family-wise error rate (FWER) i.e the probability of incorrectly rejecting the true null hypothesis. For m number of genetic markers (SNPs) tested simultaneously, the Bonferroni corrected significance threshold is determined as α/m. This make almost all markers non-significantly associated to the target trait, and only few marker-trait associations that surpassed the Bonferroni-correction significance threshold at the nominal *p*-value are considered as statically significantly associated (Bradbury *et al.,* 2007). Accordingly, the Bonferroni corrected significance threshold for GWAS of 100,000 SNPs at α = 5% will be $p = 5x\ 10^{-7}$, and hence, MTA that surpassed $-log_{10}(5x\ 10^{-7}) \geq 6.3$ are considered to be significant. Yosef Geberehawariat *et al.* (2017) considered marker-trait associations at Bonferroni corrected significance threshold of $9.25\ x10^{-6}$ determined at α = 0.15 significance level. Ando *et al.* (2018) used an association significance threshold of $p = 0.001$ *i.e* $-log_{10}(p\text{-value}=0.001) \geq 3$. They also considered the association at Bonferroni adjusted significance threshold calculated at *P* <

0.1. Similarly, Kollers *et al.* (2013) analyzed MTAs at significance threshold of $-log_{10}(P$ value$) \geq 3.0$ and at Bonferroni corrected significance threshold of $-log10\ (P\ value) \geq 4.82$.

The False discovery rate (FDR) significant threshold cutoff correction method developed by Benjamini and Hochberg (1995) is less stringent than the Bonferroni method (Qu *et al.,* 2012). This means a FDR = 0.05 allows 5% of reported positives are false positives, while the Bonferroni correction $\alpha = 0.05$ requires the whole family of positives to be true positives with the certainty of 95% (Qu *et al.,* 2012). The FDR correction method determines the significance cutoff each *p*-value according to the following formula first by ranking all p-values in an increasing order.

$$FDR\quad corrected\quad p=\frac{Number\quad of\quad tests}{p\quad Rank}\times p$$

Several previous studies (Miedaner *et al.,* 2013; Ando *et al.,* 2018; Muqaddasi*et al.,* 2018; Visioni *et al.,* 2018; Gezahegn Girma *et al.,* 2019) declared MTAs based on significance threshold calculated using FDR correction method. On the other hand, Ye *et al.* (2019), Yao *et al.* (2019) and Long *et al.*(2019) declared significant associations at significance cutoff $p = 0.001$ or $-log_{10}(P) \geq 3.0$ without accounting for the error accumulated by the multiple tests .

2.14.2.1.9 GWAS Data Visualization

Quantile-Quantile plot (Q-Q plot)

One of the most widely used methods to visualize whether or not the fitted statistical model has adequately controlled the confounding factors is the Quantile-Quantile (Q-Q) plot. It is generated by plotting the observed *p-value* or $-log_{10}$ (*p*-values) on the *y-axis* against the expected uniform distribution of *p-values* (–log10 *p*-values) on the *x-axis* under the null hypothesis of no association (Chen *et al.,* 2016). In GWAS, it is expected that the vast majority of SNPs are not associated with the trait (WTCCC, 2007). Hence, in the absence of confounding factors the Q-Q plot shows a solid line matching x = y until it sharply curves at the end (representing the small number of true associations) (Barrett, 2010). The early deviation of the observed *p*-values from null expectations indicates presence of confounding factors, and hence inclusion of covariates in the model to control for the confounding factors can improve the observed versus expected distributions (Burghardt *et al*., 2017).

The first possible reason for the high deviations of observed distribution from the expectations could be problem in phenotypic data (the data might not full fill the used model assumptions), where data transformation can correct the Q-Q distribution (Goh and Yap, 2009). The second could be presence of heavily genotyped locus *i.e* genotyping errors leading to inflated false positive associations (WTCCC, 2007). In such case, removing of some SNPs from such locus can correct by allowing few marker-trait association (Hafler *et al*., 2007). The third possible reason for the inflated false-positive association could be

presence of population structure (Q) and relatedness (K) in which inclusion of these factors as covariates in the model can correct the Q-Q distribution (Wen *et al.,* 2014).

Manhattan plots

A Manhattan plot is a scatter plot used to visualize where the signal SNP is coming from and how strong it is at whole genome scale (McCarthy *et al.,* 2008). It is constructed by potting the negative log of the *p* –values *i.e.* $-log_{10}$ (*P-value*) of each SNPs on the y-axis against their corresponding genomic positions on chromosomes colored in alternating colors in the x-axis (Greg, 2010). Each dot on the plot represents a SNP, and SNPs that have strong association have the smallest *p*-values, where their negative logarithms becomes the largest. On the plot, highly associated SNPs appear as "skyscrapers" along the plot and their position can be tracked on the x-axis. Usually horizontal line is produced to represent SNPs that surpassed the employed sigificance thresholed.

CHAPTER 3

3. Genetic diversity and population structure of *Zymoseptoria tritici* in Ethiopia as revealed by microsatellite markers

Abstract

Septoria tritici blotch (STB), caused by *Zymoseptoria tritici* is one of the most devastating diseases of wheat globally. Understanding genetic diversity of the pathogen has supreme importance in developing best management strategies. However, there is dearth of information on the genetic structure of *Z. tritici* populations in Ethiopia. Therefore, the present study was targeted to uncover the genetic diversity and population structure of *Z. tritici* populations from the major wheat-growing areas of Ethiopia. A total of 182 *Z. tritici* isolates representing eight populations were analyzed with 14 microsatellite markers. All the microsatellite loci were polymorphic and highly informative, and hence, useful genetic tools to depict the genetic diversity and population structure of the pathogen. A high within-populations genetic diversity was confirmed with gene diversity index and PPL values ranging from 0.34 - 0.58 and 79 - 100% with overall mean of 0.45 and 94%, respectively. Analysis of molecular variance (AMOVA) revealed a moderate genetic differentiation where 92 % of the total genetic variation resides within populations, leaving only 8% to among populations. Cluster (UPGMA), PCoA and STRUCTURE analyses did not group the populations into sharply genetically distinct clusters according to their geographical origins, likely due to high gene flow (Nm = 5.66) and reproductive biology of the pathogen. All individual samples shared alleles from two subgroups (K=2) evidencing high potential of genetic admixture. In conclusion, the microsatellite markers used in the present study were highly informative and thus, helped to dissect the genetic structures of *Z. tritici* populations in Ethiopia. Among the studied populations, those of East Shewa, Arsi, South West Shewa and Bale showed a high genetic diversity, and hence these areas can be considered as hot spots for investigations planned on the pathogen and host-pathogen interactions. Therefore, the present study not only enriches missing information in Ethiopia but also provides new insights into the epidemiology and genetic structure of *Z. tritici* in Africa where the agro-climatic conditions and the wheat cropping systems are different from other parts of the world. Such baseline information is useful for designing and implementing durable and effective management strategies.

3.1 Introduction

Zymoseptoria tritici, the causal agent of *Septoria tritici* blotch (STB) of wheat (McDonald *et al*., 2015; Bruce and Christopher, 2016; Dalvand *et al.*, 2018) is a hemibiotrophic (Ziming *et al*., 2017; Xin *et al*., 2018) haploid fungus (with 21 chromosomes: 13 core and 8 dispensable) (Goodwin *et al*., 2011; Croll and McDonald, 2012) belonging to the class Dothideomycetes (Testa *et al.*, 2015). Globally, wheat yield losses of 30 – 54% (Eyal *et al*., 1987), and even > 60% (Shipton *et al*., 1971) have been attributed to Septoria infestations with the diseases substantially affecting yield by causing reduced tillering, poor seed set, poor grain fill or shriveled kernels, death of leaves, spikes or the entire plant (van Ginkel and Rajaram, 1999; Simón *et al*., 2002; Goodwin, 2007; Ponomarenko *et al*., 2011; Miedaner *et al*., 2013). Moreover, the cost incurred for STB control is significantly high and accounts for about 70% of annual fungicide usage in Europe (Fones and Gurr, 2015). STB is mainly a foliar disease (Schultz and French, 2008) and the primary infections may arise from airborne or rain-splashed asexual pycnidospores and sexual ascospores from infested crop debris (Shaner, 1981; Hunter *et al*., 1999; Gilchrist and Dubin, 2002).

Wheat is one of the major staple and strategic food security crops in Ethiopia (Tesfaye Letta *et al*., 2013; Eyob Bezabeh *et al*., 2015) cultivated by half a million house holders on about 1.7 million ha. In spite of its incredible contributions to food and nutritional security of the country, the national average wheat productivity is 2.77 t/ ha far below the global average of 3.47 t/ha (FAOSTAT, 2018). Septoria tritici blotch has emerged as a major constraint to wheat production in the country causing 25 to 82% production loss in the worst-affected areas with increasing incidence and severity in the major wheat belts (Eshetu Bekele *et al*.,

2011; Abera Takele *et al*., 2015; Endale Hailu and Getaneh Woldeab, 2015; Tilahun Mekonnen, 2019, 2020).

To adopt controlling practices or to design novel management strategies, knowledge of the pathogen genetic diversity and population structure are of paramount importance (Sebei and Harrabi, 2008). Plant pathogen populations that have high levels of genetic variability are more likely to adapt to resistant cultivars than populations with less genetic variability (McDonald *et al*., 1995).

So far, different DNA marker systems have been developed and widely used to uncover the genetic structure of *Z. tritici* populations in different parts of the world. McDonald *et al.* (1995) and Chen and McDonald (1996) used restriction fragment length polymorphisms (RFLP) to study the populations of *Z. tritici* in the USA. Medini and Hamza (2008) also used RFLP to analyze the genetic diversity of *Z. tritici* populations of Tunisia, Algeria and Canada. Czembor and Arseniuk (1996) used random amplified polymorphic DNA (RAPD) markers to explore the genetic similarity of three fungal species, *Septoria tritici*, *Stagonospora nodorum* and *Stagonospora avenae* f sp. *triticea.* Amplified fragment length polymorphisms (AFLP) were used to evaluate the genetic structure of *Z. tritici* populations of Germany (Schnieder *et al.*, 2001), Kansas, USA (Kabbage *et al.*, 2008) and Iran (Abrinbana *et al.*, 2010). Similarly, simple-sequence repeats (SSR) or microsatellite markers were used to analyze the genetic diversity of *Z. tritici* isolates from England (Owen *et al*., 1998), Tunisia (Boukef *et al.*, 2012; Samia *et al.*, 2013), France (El Chartouni *et al*., 2011; Siah *et al*., 2018). SSR markers are preferred for genetic analyses because of their higher

rate of polymorphism, in formativeness, reproducibility, multiallelic nature, co-dominant inheritance, relative abundance and ease of identification from genomic sequences (Winter and Kahl, 1995; Medini and Hamza, 2008; Gautier *et al*., 2014).

Irrespective of the huge economic importance of the STB disease, there is a dearth of information about the genetic diversity and population structure of *Z. tritici* populations of Ethiopia. Moreover, the applications of molecular tools in plant pathogen studies and resistance breeding are greatly missing. Therefore, the present study was targeted to uncover the genetic diversity and population structure of *Z. tritici* populations collected from the major wheat-growing areas of Ethiopia using microsatellite markers so as to generate useful information important for making informed decision in designing best management strategies.

3.2 Materials and Methods

Sample Collection Procedure

The study was conducted on *Z. tritici* isolates recovered from STB-infected wheat leaf samples collected from eight major wheat-growing zones of Ethiopia: East Shewa (ESH), West Shewa (WSH), South West Shewa (SWSH), North Shewa (NSH), Oromia Special Zone surrounding Finfine (OSZ), ARSI, West Arsi (WARSI) and BALE (Figure 3.1), where 75% of the total wheat production area of the country is located. The collections were made during the 2016 and 2017 main cropping seasons following the main roads and accessible routes in a randomly selected district, and stops were made at 5 - 10 km intervals based on

vehicle odometers where wheat fields were accessible. The collection was made when the crop growth stage (GS) was on average between the medium milk (60 GS) and early dough (75 GS) stages according to Zadoks *et al*. (1974). During collection, green leaves with symptoms of STB were cut with 70% ethanol-cleaned scissors, and placed in paper envelopes labeled with the sample code. Sample passport data including administrative region, geographical location and elevations were also recorded (Appenix I and Table 3.1).

Table 3. 1 Summary information of the eight populations of *Z. tritici* sampled in Ethiopia

Populations[a]		Isolates code	Geographic position (UTM)[b]		Elevation range (m.a.s.l)
Code	Size		Latitude range	Longitude range	
ESH	6	ZTET001 - ZTET006	08° 46' 319 - 08° 46' 322	039° 00' 043 - 039° 00' 582	1879 -1882
ARSI	61	ZTET007 - ZTET067	07° 01' 525 -08° 06' 100	039 07 479 - 039 27 128	2137- 2993
WARSI	12	ZTET068- ZTET079	07° 03' 628-07° 16' 580	039° 00' 620 - 039° 16' 263	2374 -2654
SWSH	8	ZTET080 -ZTET087	08° 37' 954- 08° 40' 813	037° 54' 162 - 038° 02' 516	2196 - 2739
WSH	9	ZTET088 -ZTET96	08° 54' 024 - 09° 01' 594	037° 26' 856- 037° 43' 483	2256 -2533
NSH	9	ZTET097 -ZTET105	09° 13' 693 - 9° 47' 258	038° 31' 623 - 038° 4 358	2586 -2905
BALE	63	ZTET106 -ZTET168	07° 00' 023 - 07° 22' 763	039° 52' 971 - 040° 27' 635	2048- 2904
OSZ	14	ZTET169 -ZTET182	09° 03' 278 - 09° 03' 596	038° 30' 295-038° 30' 686	2377- 2391

[a] ESH = East Shewa; WARSI = West Arsi; SWSH = South West Shewa; WSH = West Shewa; NSH = North Shewa; OSZ = Oromia Special Zone Surrounding Finfine.

[b] UTM = Universal Transverse Mercator coordinate system of Ethiopia. m.a.s.l= Meters above sea level

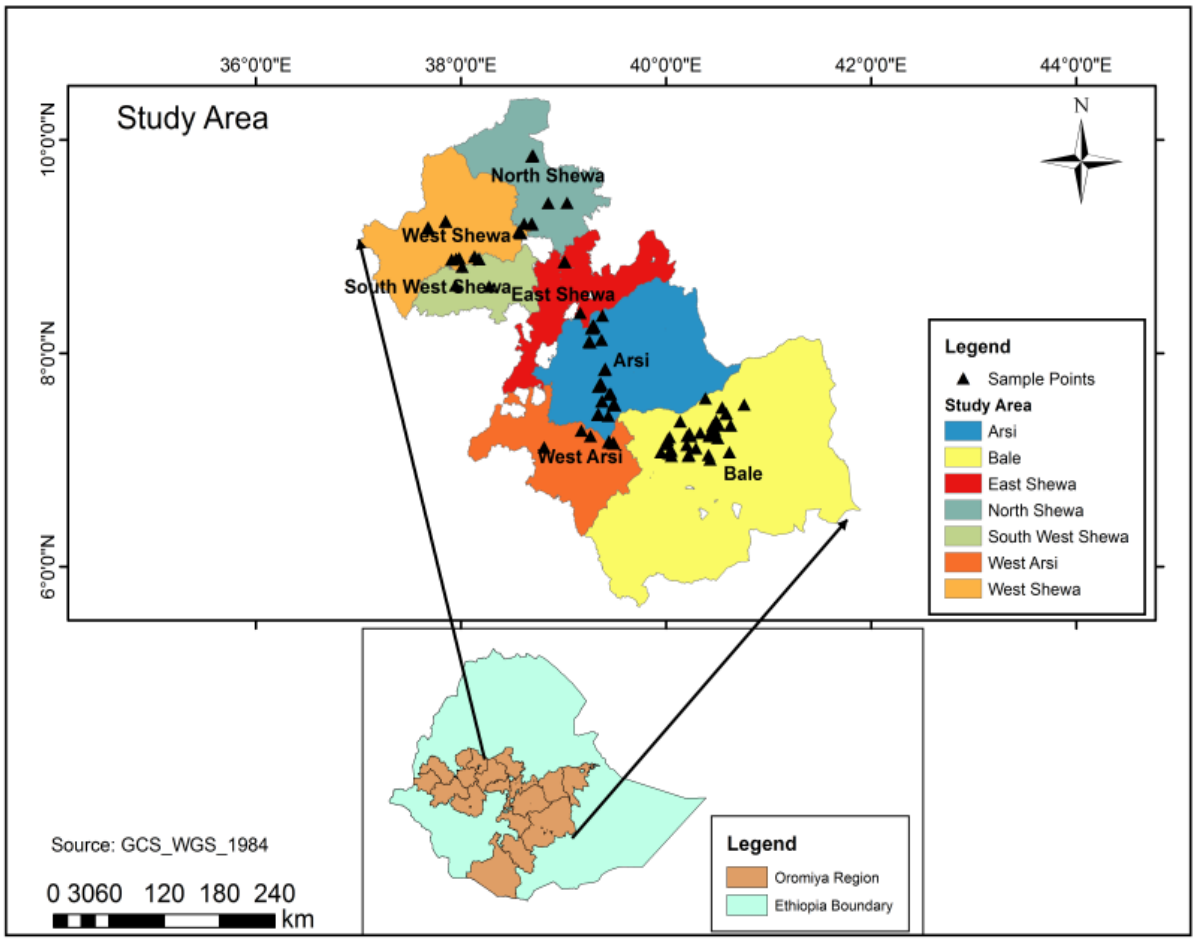

Figure 3. 1 Map showing the eight *Z. tritici* study populations sampling sites.

As it is a recently established administrative zone, we couldn't indicate Oromia Special Zone Surrounding Finfine on the Map. It is one of the zones of Oromia Region in Ethiopia that surrounds Addis Ababa or Finfine.

Spore Isolation Procedure

The spore isolation and subsequent laboratory works were carried out at the National Agricultural Biotechnology Research Center (NABRC), Holetta, Ethiopia, which is located 29 km west of Addis Ababa. During isolation, collected leaves with pycnidia were cut into about 10 cm lengths and placed on sterile filter paper in Petri plates wetted with distilled

water as described in Eyal *et al.* (1987*)*. The Petri dishes with specimen were placed in polyethylene plastic bags and then incubated for 3 - 4 hr at 24°C. The samples were periodically checked under a stereoscopic dissecting microscope for the formation of cloudy ooze on the top of pycnidia. Using a flame-sterilized fine needle, the mono-pycnidial oozing drops were transferred onto potato dextrose agar (PDA; potato 200 g/l, dextrose 20 g/l, agar 15 g/l) plates supplemented with 250 mg of chloramphenicol after autoclaving. Inoculated Petri plates were kept at 24°C for 7 - 10 days until fungal growth was observed. Once the right colony morphology was confirmed under a microscope (40x), developed pinkish-orange colonies were streaked onto new PDA supplemented with chloramphenicol, and then kept at the same conditions for growth. Single spore-derived colonies were transferred into a liquid medium composed of 1% (w/v) yeast extract powder + 1% (w/v) sucrose for further spore propagation for use as inoculum, to store as a stock culture and for extraction of genomic DNA. Cultures were maintained on an orbital shaker at 130 rpm for two to three weeks for spore multiplication. A total of 182 single-spore-derived isolates were successfully recovered (Appendix I) and used for molecular diversity analysis. The stock cultures were preserved in 30% glycerol at −80 °C for further studies.

Genotyping

For genomic DNA extraction, isolates grown in liquid medium were centrifuged at 10,000 rpm for five minutes to collect the fungal pellet. DNA extraction was carried out using the plant DNA extraction protocol described in Diversity Arrays Technology (DArT) with some modifications. To facilitate the crushing process, mortars, pestles and the spore samples

were kept overnight at -80 °C. Extracted DNA quality was checked by loading 5µl of DNA + 2 µl of 6x loading dye with gel red on a 1% agarose gel and separated at 100 V for 40 minutes. The isolates were confirmed using a *Z. tritci* specific diagnostic marker developed by Beck and Ligon (1995). The primer pair sequence involved ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and JB446 (5'- CGAGGCTGGAGTGGTGT-3') that targets the internal transcribed spacer (ITS) sequences within the ribosomal RNA genes (ribosomal DNA). The PCR reaction was performed using 96 well PCR plate in a volume of 12.5 µl containing 6.25 µl One *Taq* 2x Master mix with Standard Buffer (New England BioLabs), 0.4 µM each of forward (ITS1) and reverse (JB446) primers, 3.25µl nuclease free water and 1 µl of genomic DNA using BIORAD Thermal cycler. Amplification condition involved an initial denaturation at 94°C for 3 min followed by 35 cycles with 1 min denaturation at 94°C, 1 min annealing at 60°C, primer extension at 72°C for 2 min followed by final extension step of 10 min at 72°C. Genomic DNA from the reference isolate (IPO323) obtained from Professor S. B. Goodwin's lab., Purdue University, USA was included as positive control, and nucleic acid free water was used as negative control.

For genetic structure analysis, a total of 14 microsatellite markers (Table 3.2) were used. The primers' annealing temperatures were gradient optimized. The PCR program for the first seven primer pairs involved an initial denaturation at 94 ^{o}C for 3 minutes followed by 45 cycles of denaturation at 94 ^{o}C for 1minute, optimized annealing temperature of 65 or 58 ^{o}C for 1 minute (Table 3.2), and primer extension at 72 ^{o}C for 2 minutes which was followed by a final extension at 72 ^{o}C for 10 minutes and holding at a temperature of 4^{o}C. The PCR conditions for the remaining primer pairs (8 to 14 in Table 3.2) involved an initial

denaturation at 94 °C for 2 minutes followed by 35 cycles with 30 s of denaturation at 93 °C, 2 minutes annealing at 53 °C, and primer extension at 72 °C for 2 minutes, followed by a final extension step of 10 minutes at 72 °C before holding at 4 °C. The PCR products were fractionated by 3% agarose gel electrophoresis using 1× TAE buffer at 100V for 3 hr. The gel was stained in gel red (2 µl), visualized under UV light and subsequently photographed using a BioDoc-It TM Imaging System. A 50 + 100 bp DNA ladder was used to estimate the amplification size.

Table 3.2 SSR primers used for genetic analysis of *Z. tritici* populations in Ethiopia.

Locus	Forward primer (5′ to 3′)	Reverse primers (5′ to 3′)	Annealing temperature (°C)	Range of Fragment Size (bp)
ST1E7 (MGR 7038)	GATCTCGAGCAGGGCGGAAGT	TCACACGCTGGTCTGTGAATC	58	86-98
ST1G7 (MGR 7037)	ATGCTGAGAAGTTCGGTGAGG	CGTTCTTCCACCTCCAACACT	65	96-103
ST2E4 (MGR 7034)	GAAGATCAACAGCATGGGCGG	CTCCAGAGGGATCACAAAGGC	58	54-110
ST1A4 (MGR 7032)	GGTTCGATGGAGAGATTT	TCACCTCCTCATCGCAGA	58	98-100
ST1D7 (MGR 7039)	TTGAAGTGGCATCCTCCATT	AACTCGGCTGGTGGAACA	61	95-105
ST1B3 (MGR 7033)	CGCGCACTAGTAGACGCTCT	TCTACCTTAATCCTCACCGCC	58	54-121
ST2C10(MGR 7036)	AGGCGAGAAACTTGCTTGCAG	AATGAACGTCCCATGGACGTG	58	75-98
ag-0003	ACTTGGGGAGGTGTTGTGAG	ACGAATTGTTCATTCCAGCG	53	230-258
ac-0001	CACCACACCGTCGTTCAAG	CGTAAGTTGGTGGAGATGGG	53	171-200
ag-0009	GACTCCATTTACCTGTGGCG	TGTGAAGGACACGCAAAGAG	53	192-200
ggc-0001	GATACCAAGGTGGCCAAGG	CACGTTGGGAGTGTCGAAG	53	181-298
ac-0002	TGAACATCAACCTCACACGC	AGAAGAGGACGACCCACGAG	53	172-210
tcc-0009	TCAATTGCCAATAATTCGGG	AGACGAGGCAGTTGGTTGAG	53	142-177
caa-0005	AAGAATCCCACCACCCAAAC	’ 5CACACGGCTCCTTTGACAC’	53	262-321

Data scoring and analysis

Sizes of all PCR-amplified SSR regions were estimated in reference to the size marker using PyElph software package (Pavel and Vasile, 2012). Each primer pair was assumed to

amplify a single genetic locus where bands of different molecular weight were considered to be different alleles of a particular locus. Genetic diversity and population structure analyses were computed on the basis of the scored marker data. Various statistical software packages were employed to compute the standard indices of genetic diversity. Locus-based diversity indices across the entire populations including major allele frequency (MAF) and Polymorphic information content (PIC) were computed using PowerMarker v3.25 software (Liu and Muse, 2005).

Population differentiation (Gst) and Gene flow (Nm = 0.5 (1 - Gst)/Gst) across populations were determined using POPGENE version 1.31(Yeh and Yang, 1999). Allelic frequency, the number of observed alleles (Na), gene diversity (h), effective number of alleles (Ne) and Shannon's Information index (I) over the entire populations, and other population diversity indices over all loci in each population including Na, Ne, I, the number of private alleles (NPA), Nei's gene diversity (h) and Percentage of polymorphic loci (PPL) were computed using GenAlEx ver. 6.501 software (Peakall and Smouse, 2006, 2012). The same software was used to compute pairwise population genetic distances and gene flow, and to perform the genetic differentiation test (PhiPT and *p*- values) over 999 bootstrap replications. Analysis of molecular variance (AMOVA) and estimate of the variance components were computed using Arlequin ver. 3.5.2.2 (Excoffier and Lischer, 2010). Gene flow (Nm) among populations was estimated using the formula, Nm (Haploid) = [(1 / PhiPT) - 1] / 2, where PhiPT = the variance among populations/total genetic variations. A genetic dissimilarity matrix was computed based on the continuous Euclidian dissimilarity index and Neighbor-Joining (NJ) and Nei's standard genetic distance (D_{ST}, corrected) (Nei, 1972) based

Unweighted Pair Group Method with Arithmetic Mean (UPGMA) trees were generated using DARwin ver. 6.0.14 (Perrier and Jacquemoud-Collet, 2006) and PowerMarker v3.25 (Liu and Muse, 2005), respectively; significance was tested based on 1000 bootstrap replications (Felsenstein, 1985). The resulting trees were visualized using FigTree ver. 1.4.3 (Andrew, 2016) and TreeView (built in PowerMarker v3.25).

Population structure and admixture patterns were determined using STRUCTURE software ver. 2.3.4 with a Bayesian model-based clustering algorithm (Pritchard *et al.*, 2000). To estimate the true number of population cluster (K), a burn-in period of 100,000 was used in each run, and data were collected over 250,000 Markov Chain Monte Carlo (MCMC) replications for K = 1 to K = 10 using 20 iterations for each K. The optimum K value was predicted following the simulation method of Evanno *et al.* (2005) using the web-based STRUCTURE HARVESTER ver. 0.6.92 (Earl and Von Holdt, 2012). A bar plot for the optimum K was determined using Clumpak beta version (Kopelman *et al*., 2015).

3.3 Results

PCR based detection of *Z. tritici*

The two ITS (ITS1 and ITS2) sequences located between 18S (SSU) and 28S (LSU) genes surrounding 5.8S-coding sequence are frequently used to discriminate fungi at the genus and species level. Sequencing the ITS region of *Z. tritici* isolate ATCC #26517, Beck and Ligon (1995) developed a *Z. tritici* specie specific markers (ITS1 and JB446 primer). In the present study, the use of same primer pair (ITS1 and JB446 primer) resulted in the amplification of

about 345 bp fragment from all of the isolates. Similar amplification size was observed in the positive control (PC) reference isolate (IPO323), while no amplification was observed in the negative control (NC) using the same diagnostic primers (Figure 3.2).

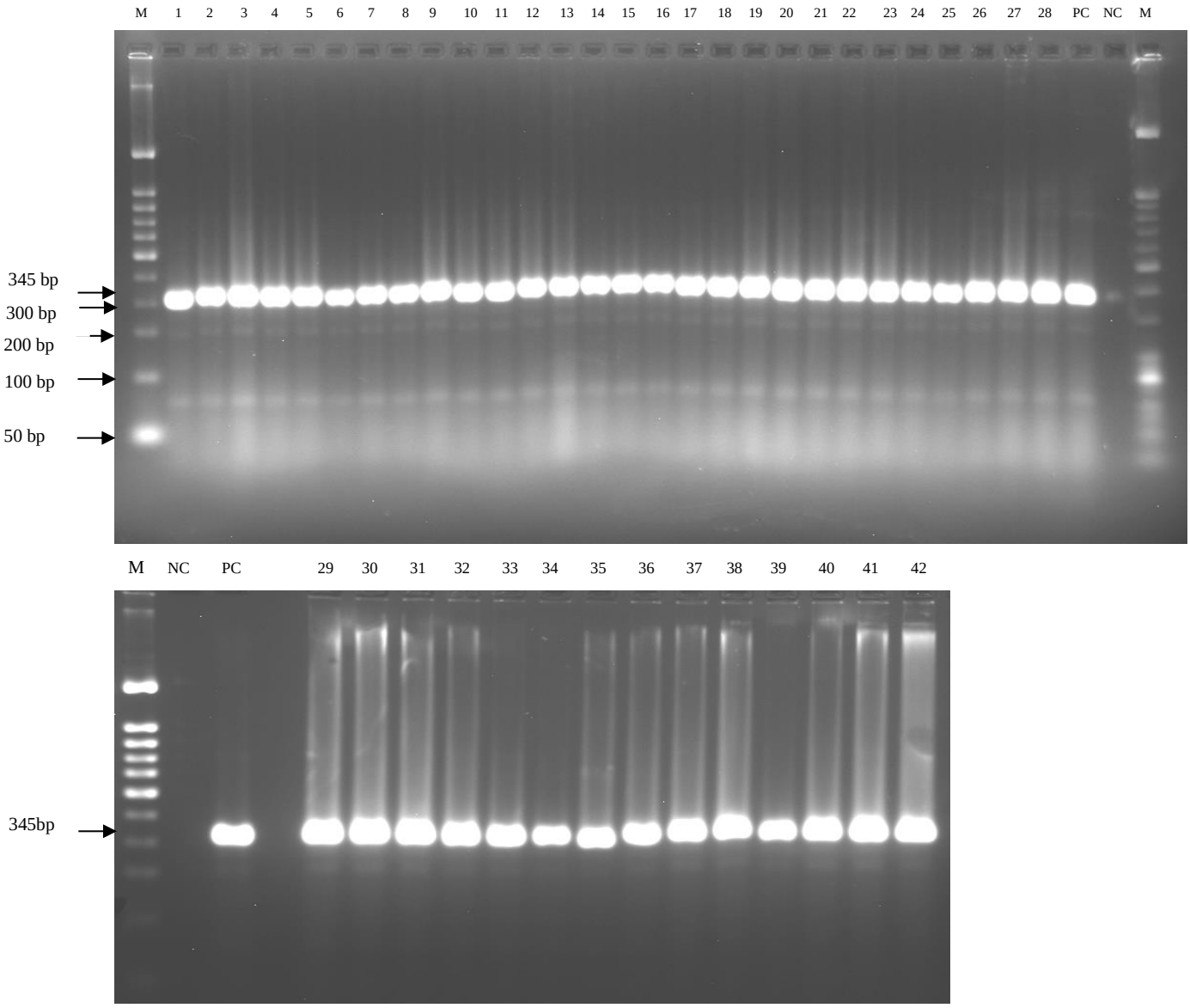

Figure 3. 2 Agarose gel image of PCR product using *Z. tritici* specific primers.
The numbers 1- 42 represent the *Z. tritici* isolates ZTET001 to ZTET042 (Appendix I). M is a 50 +100 bp size marker while PC and NC are positive and negative control, respectively.

The Genetic structure analysis of *Z. tritici*

Microsatellite Markers Level of Polymorphism

The analysis revealed that all 14 SSR loci were polymorphic and produced a total of 48 alleles with an average of 3.43 alleles per locus (Appendix II, Table 3.3), out of which six were scarce (frequency between 0.01 and 0.05). The frequency of five alleles was between 0.05 and 0.1 and the remaining 37 alleles had a frequency higher than 0.1 (Appendix II). For each locus, the frequency of the most common allele was less than 0.95 or 0.99 confirming the high polymorphism of the markers. The number of alleles per locus ranged from three for the locus ac-0001 to five for the locus ST2E4 (Appendix II, Table 3.3). The reaming four loci (28.57%) had four alleles per locus (Table 3.3). In the entire populations, major allele frequencies ranged from 0.40 for locus caa-0005 to 0.77 for ST1E7 with an overall mean of 0.59 (Table 3.3). The largest MAF and the lowest Ne, I, gene diversity and PIC were observed for the microsatellite loci ST1E7 (Table 3.3). Conversely, the highest Ne, I and gene diversity were recorded for caa-0005 (Table 3.3). Microsatellite loci ag-0003 (h = 0.59) and ac-0002 (h = 0.57) resulted in the second and third highest gene diversity across the entire populations, respectively. Locus ag-0003 had moderate genetic differentiation (Gst = 0.07, PhiPT = 0.00) and the highest gene flow (Nm = 7.09) among the populations (Table 3.3). The informativeness of individual loci as measured by polymorphic information content ranged from 0.34 (ST1E7) to 0.60 (ac-0002) with an overall mean of 0.49. Five (35.71 %) of the loci were moderately informative ($0.25 \leq PIC < 0.5$) and the remaining nine (64.29 %) were highly informative ($PIC \geq 0.5$) (Table 3.3).

Table 3. 3 Diversity summary statistics[a] for SSR loci across eight populations of *Z. tritici*.

Locus	MAF	Na	Ne	I	H	Gst	PhiPT	*P* value	Nm	PIC
ST1E7	0.77	3.00	1.45	0.45	0.26	0.23	0.12	0.001	1.65	0.34
ST1G7	0.62	3.00	1.86	0.65	0.40	0.15	0.10	0.001	2.7	0.48
ST2E4	0.76	5.00	1.59	0.55	0.32	0.10	0.03	0.047	4.41	0.37
ST1A4	0.42	3.00	1.99	0.73	0.46	0.22	0.11	0.001	1.73	0.58
ST1D7	0.64	3.00	1.73	0.60	0.36	0.23	0.11	0.001	1.62	0.47
ST1B3	0.70	4.00	1.79	0.66	0.38	0.15	0.04	0.030	2.79	0.43
ST2C10	0.46	4.00	2.34	0.93	0.56	0.14	0.07	0.003	3.00	0.58
ag-0003	0.50	3.00	2.47	0.94	0.59	0.07	0.00	0.536	7.09	0.55
ac-0001	0.65	3.00	1.96	0.75	0.45	0.22	0.08	0.001	1.72	0.46
ag-0009	0.72	3.00	1.79	0.66	0.40	0.10	0.02	0.175	4.38	0.40
ggc-0001	0.49	4.00	2.15	0.80	0.51	0.15	0.07	0.001	2.74	0.56
ac-0002	0.47	4.00	2.47	0.96	0.57	0.15	0.06	0.006	2.73	0.60
tcc-0009	0.66	3.00	1.88	0.74	0.44	0.22	0.18	0.001	1.79	0.45
caa-0005	0.40	3.00	2.56	1.00	0.60	0.09	0.02	0.112	5.10	0.59
Mean	0.59	3.43	2.00	0.74	0.45	0.16	0.07	0.001	2.64	0.49

[a] MAF = Major allele frequency; Na = Observed number of alleles; Ne = Effective number of alleles; I = Shannon's Information statistic; h = Nei's gene diversity; Gst and PhiPT= Genetic differentiation statistics by locus; Nm = estimate of the number of migrants (gene flow) from Gst where Nm = 0.5(1 - Gst)/Gst; *p* = Differentiation statistics probabilities; and PIC = Polymorphic information content.

Genetic Variability Within and Among the Populations

A summary of the different genetic diversity estimates over all loci across populations was presented in Table 3.4. The overall genetic diversity estimates within the populations had a mean number of effective alleles of 2.00 (range1.66 - 2.47), number of alleles unique to a single population (private alleles) 0.02 (range 0.0 - 0.14), Shannon's Information index of 0.74 (range 0.55 - 1.00), and gene diversity value of 0.45 (range 0.34 - 0.58) (Table 3.4). The ARSI population showed the highest Ne, NPA, NLCA, I, and genetic diversity (Table 3.4). The BALE population ranked second in terms of I and third in Ne and gene diversity index (Table 3.4). Except for the population of ARSI where NPA = 0.14, there was no

private allele unique to a single population. NLCA was the highest in both ARSI and BALE populations. The lowest and highest Nei's gene diversity was observed in the populations of OSZ and ARSI, respectively. Fifty percent of the study populations showed a genetic diversity greater than the mean value of 0.45 (Table 3.4). The percentage of polymorphic loci per population ranged from 79% (WARSI) to 100% (ESH, ARSI, SWSH, NSH and BALE) with an average of 94% (Table 3.4).

Table 3. 4 Diversity indices[a] across populations averaged over the 14 SSR loci.

Population[b]	Size	Ne	NPA	NLCA	I	h	PPL
ESH	6	2.01	0.00	0.14	0.77	0.47	100%
ARSI	61	2.47	0.14	0.43	1.00	0.58	100%
WARSI	12	1.85	0.00	0.00	0.59	0.37	79%
SWSH	8	2.11	0.00	0.14	0.79	0.5	100%
WSH	9	1.99	0.00	0.14	0.7	0.44	86%
NSH	9	1.91	0.00	0.14	0.73	0.44	100%
BALE	63	2.03	0.00	0.43	0.82	0.48	100%
OSZ	14	1.66	0.00	0.14	0.55	0.34	86%
Mean	23	2	0.02	0.20	0.74	0.45	94%

[a] Na = Observed number of alleles; Ne = Number of effective alleles; NPA = Number of Private Alleles (i.e., the number of alleles unique to a single population); I = Shannon's information statistic; h = Nei's genetic diversity; PPL = Percentage of Polymorphic Loci; NPA= Number of private alleles; and NLCA = Number of Locally Common Alleles (frequency !5%) found in 25% or fewer populations. ESH = East Shewa; WARSI = West Arsi; SWSH = South West Shewa; WSH = West Shewa; NSH = North Shewa; OSZ = Oromia Special Zone Surrounding Finfine.

Genetic Relationships Between the Populations

Table 3.5 presents pairwise population measures of Nei's genetic distance (below diagonal) and gene flow (Nm) (above diagonal). The pairwise Nei's standard genetic distance of each population from the other populations ranged from 0.07 to 0.43. The lowest genetic distance

was observed between the populations of ESH and BALE (Table 3.5), which was followed by the relationship between BALE and WARSI populations that scored a distance of 0.08. The highest measure of genetic distance (0.43) with relatively low gene flow (Nm =1.32) was observed between the populations of SWSH and OSZ (Table 3.5). The second-highest genetic distance was observed between the populations of ESH and SWSH. The lowest gene flow was observed between the populations of ESH and ARSI, ESH and NSH, and ESH and BALE (Table 3.5).

Table 3. 5 Pairwise genetic distance and gene flow (Nm) among eight *Z. tritici* populations.

Population[a]	ESH	ARSI	WARSI	SWSH	WSH	NSH	BALE	OSZ
ESH	----	0.00	15.69	3.40	5.72	0.00	0.00	3.60
ARSI	0.09	----	7.52	5.05	6.03	9.70	7.53	3.18
WARSI	0.13	0.11	----	2.72	3.86	8.63	10.69	2.75
SWSH	0.39	0.24	0.27	----	3.11	2.99	2.58	1.32
WSH	0.24	0.18	0.18	0.32	----	4.23	5.43	1.86
NSH	0.12	0.14	0.12	0.33	0.22	----	13.57	3.77
BALE	0.07	0.09	0.08	0.29	0.15	0.10	----	3.31
OSZ	0.19	0.20	0.17	0.43	0.27	0.16	0.16	----

Nei's genetic distance (below diagonal) and gene flow (Nm) values (above diagonal).
[a] ESH = East Shewa; WARSI = West Arsi; SWSH = South West Shewa; WSH = West Shewa; NSH = North Shewa; OSZ = Oromia Special Zone Surrounding Finfine.
---- = not applicable.

Populations of ESH and WARSI exhibited the highest (Nm =15.69) gene flow (Table 3.5) with low (0.03) and statistically non-significant (p >0.05*)* genetic differentiation (Table 3.6). The pairwise coefficient of genetic differentiation between the populations ranged from 0.00 (ESH vs ARSI, ESH vs NSH, and ESH and BALE) to 0.27 (between OSZ and SWSH) (Table 3.6). The highest and statistically significant genetic differentiation (PhiPT = 0.27, p=0.001) was observed between populations of OSZ and SWSH (Table 3.6), implying the

lowest gene flow between them (Table 3.5). The second-highest genetic differentiation with a gene flow rate of 1.86 was observed between the populations of OSZ and WSH. The genetic differentiations between populations of ESH and ARSI, ESH and WARSI, ESH and WSH, ESH and NSH, and ESH and BALE, NSH and WARSI, and BALE and ARSI were not statistically significant ($p > 0.05$) (Table 3.6).

Table 3. 6 Genetic differentiation (below the diagonal) between the eight *Z. tritici* populations with *p*-values above the diagonal.

PhiPT/*p value*	ESH	ARSI	WARSI	SWSH	WSH	NSH	BALE	OSZ
ESH	----	0.43	0.20	0.02	0.08	0.46	0.39	0.00
ARSI	0.00	----	0.01	0.00	0.00	0.02	0.00	0.00
WARSI	0.03	0.06	----	0.00	0.01	0.06	0.02	0.00
SWSH	0.13	0.09	0.16	----	0.03	0.01	0.00	0.00
WSH	0.08	0.08	0.12	0.14	----	0.02	0.01	0.00
NSH	0.00	0.05	0.06	0.14	0.11	----	0.06	0.00
BALE	0.00	0.06	0.05	0.16	0.08	0.04	----	0.00
OSZ	0.12	0.14	0.15	0.27	0.21	0.12	0.13	----

ESH = East Shewa; WARSI = West Arsi; SWSH = South West Shewa; WSH = West Shewa; NSH = North Shewa; OSZ = Oromia Special Zone Surrounding Finfine.
---- = not applicable.

Analysis of Molecular Variance (AMOVA)

Analysis of molecular variance (AMOVA) was computed without and with grouping of the populations according to their geographical locations. AMOVA based on PhiPT values revealed that 92% of the genetic variation occurred within populations (Table 3.7). The variability among populations and among geographical regions only accounted for 8% and 0%, respectively, of the total genetic diversity (Table 3.7). The overall genetic

differentiation coefficient among the populations was moderate (PhiRT = 0.08, p = 0.001) with high gene flow (≥ 5.66) (Table 3.7).

Table 3. 7 Analysis of Molecular variance (AMOVA**)**

Source of variation	Degree of freedom	Sum of squares	Mean squares	Estimate of variance	Percent of variation	Genetic differentiation[a]	*P* value[b]
Among populations	7	68.47	9.78	0.317	8	PhiPT:0.08	0.001
Within populations	174	625.59	3.60	3.595	92	FIS:0.92	0.000
Total	181	694.07		3.913	100		
Nm (haploid)[c] populations	5.66						
Among regions[d]	1	8.242	8.24	0.000	0%	PhiRT: -0.008	0.973
Among populations	6	57.117	9.52	0.310	8%	PhiPR: 0.08	0.001
Within populations	174	628.706	3.61	3.613	92%	PhiPT: 0.07	0.001
Total	181	694.066		3.923	100%		
Nm (haploid) regions	6.50						

[a] PhiPT= the coefficient of genetic differentiation, calculated as = AP / (WP + AP), where AP = the variance among populations and WP = the variance within populations.
[b] Based on 999 permutations.
[c] Gene flow calculated as [(1 / PhiPT) - 1] / 2.
[d] The central highlands (Shewa) versus the southeast parts of Ethiopia.

Cluster Analysis, PCoA and Population Structure

The neighbor joining- based clustering of the 182 individuals identified four major clusters (C1, C2, C3 and C4), each of which were further grouped into two sub clusters (i and ii) (Figure 3.2). Ninety-eight (53.8%) of the isolates were assigned into C1 which was followed by C3. The lowest (3) number of isolates were assigned to cluster C2 (Figure 3.2). None of the major clusters and/or sub-clusters is composed of exclusively isolates from a

particular population or collection zone, confirming the existence of considerable intermixing of the isolates.

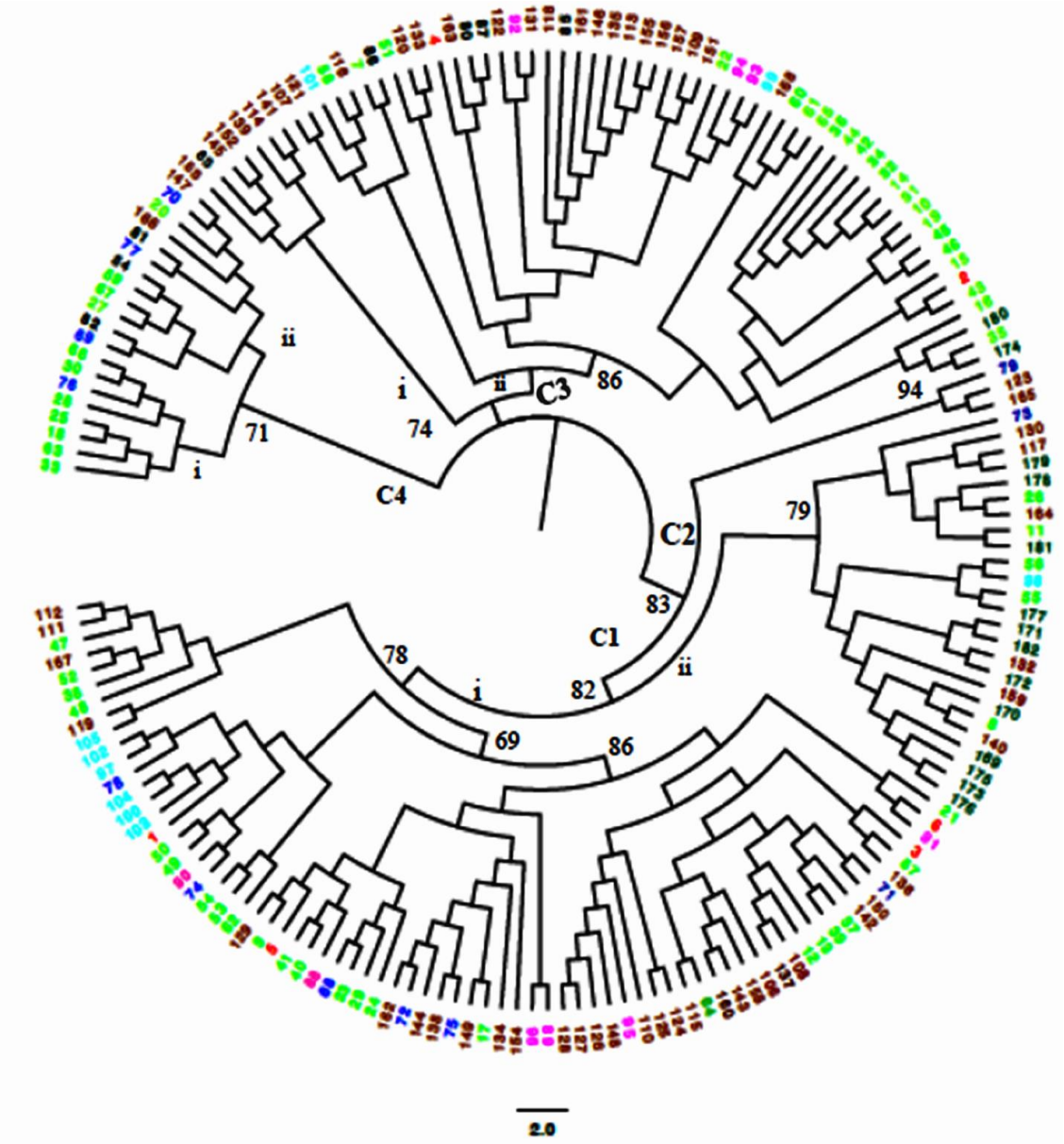

Figure 3. 3 Neighbor-joining tree for 182 *Z. tritici* isolates from the eight populations.
Numbers at the roots of the branches represent percentages of bootstrap values, and values less than 60% were not indicated. Each color represents a population: Red = East Shewa; Light green = Arsi; Blue = West Arsi; Black = South west Shewa; Pink = West Shewa; Cyan = North Shewa; Brown = Bale and deep green = Oromia Special Zone Surrounding Finfine. C1 to C4 = major clusters while i and ii indicate sub-clusters. Numbers at the roots of the branches represent bootstrap values, and bootstrap values less than 60% were not indicated.

The dendrogram generated using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) weakly grouped the eight populations into four major clusters (I, II, III and IV) (Figure 3.4), where the first three clusters were composed of only a single population, and cluster IV was composed of five populations, which further formed more sub-clusters (Figure 3. 3).

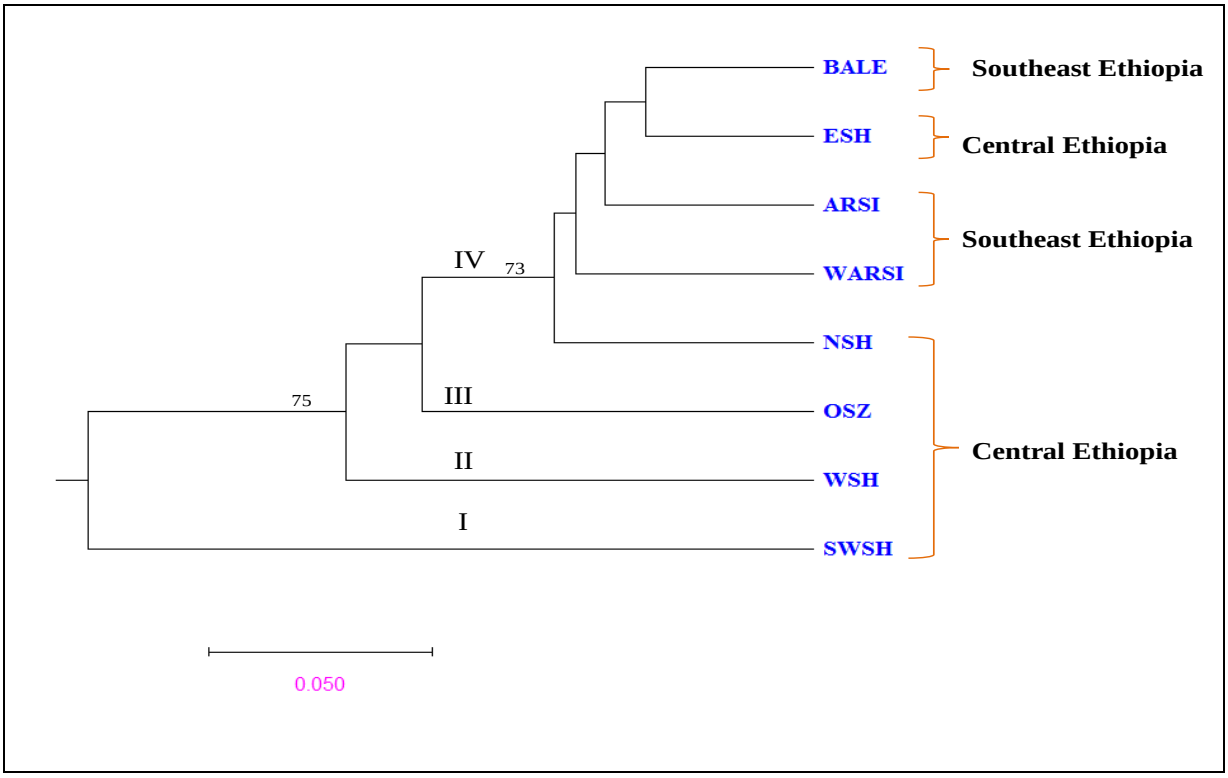

Figure 3. 4 A dendrogram showing genetic relationships among 8 *Z. tritici* populations.
Numbers above braches represent percentage of bootstrap values, and values less than 60% were not indicated. ESH = East Shewa; WARSI = West Arsi; SWSH = South West Shewa;WSH = West Shewa; NSH = North Shewa; OSZ = Oromia Special Zone Surrounding Finfine.

The scatter plot obtained from PCoA revealed almost a uniform distribution of the isolates of all populations around the center of the two-dimensional coordinate plane with poor population clustering (Figure 3.4). The first three principal coordinate axes explained 28.34

% of the total variation (Figure 3.4) with each of the dimensions (1, 2 and 3) accounting for only 13.84%, 7.81%, and 6.71%, respectively.

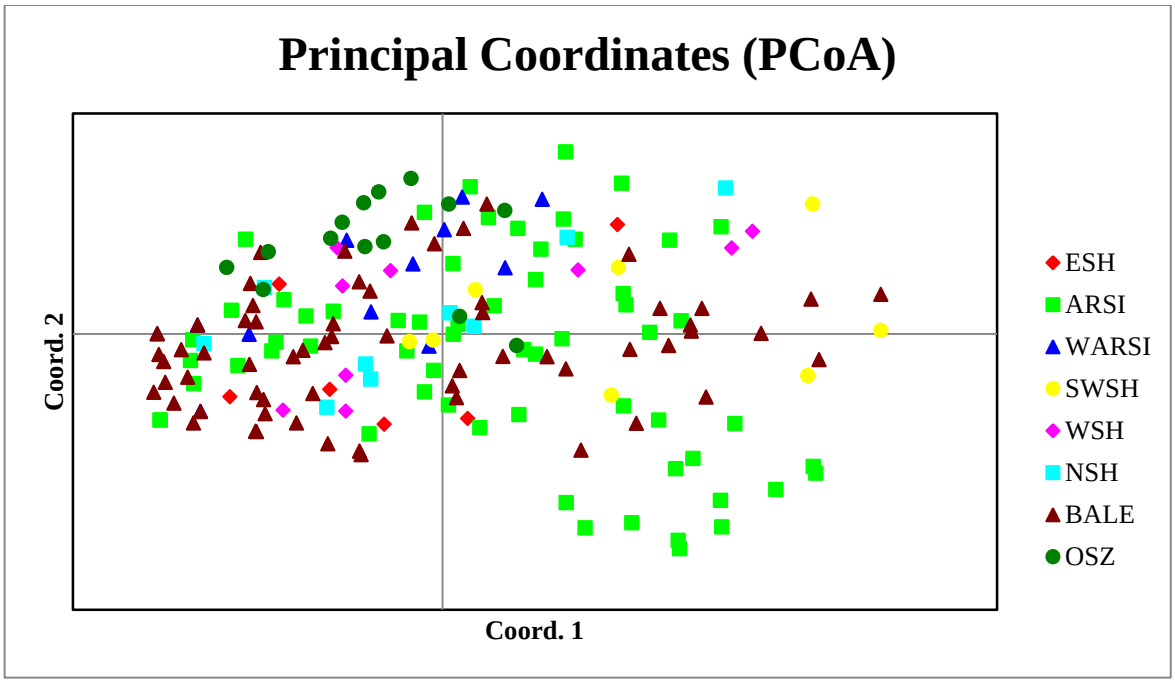

Figure 3. 5 PCoA of the 182 *Z. tritici* isolates as revealed by 14-microsatellite loci. Samples coded with the same symbol and color belongs to the same population. PCoA explained 28.34 % of the total variations and the first three axes (1, 2 and 3) accounted for 13.84%, 7.81%, and 6.71%, respectively. Population abbreviations are: ESH = East Shewa; WARSI = West Arsi; SWSH = South West Shewa; WSH = West Shewa; NSH = North Shewa; OSZ = Oromia Special Zone Surrounding Finfine.

Bayesian model-based population structure of the 182 isolates was inferred using Structure v 2.3.4 software, and the *K* value was used to estimate the number of clusters of the isolates. Output of the Structure harvester revealed that the delta *K* (ΔK) values reached a sharp peak at $K = 2$ (Figure 3.5a), confirming that the pathogen isolates can be clustered into two subpopulations. The Clumpak result (bar plot) detected a greater degree of genetic

admixture between the two subpopulations and hence there was no clear geographic origin-based structuring in the *Z. tritici* populations of Ethiopia (Figure 3.5b).

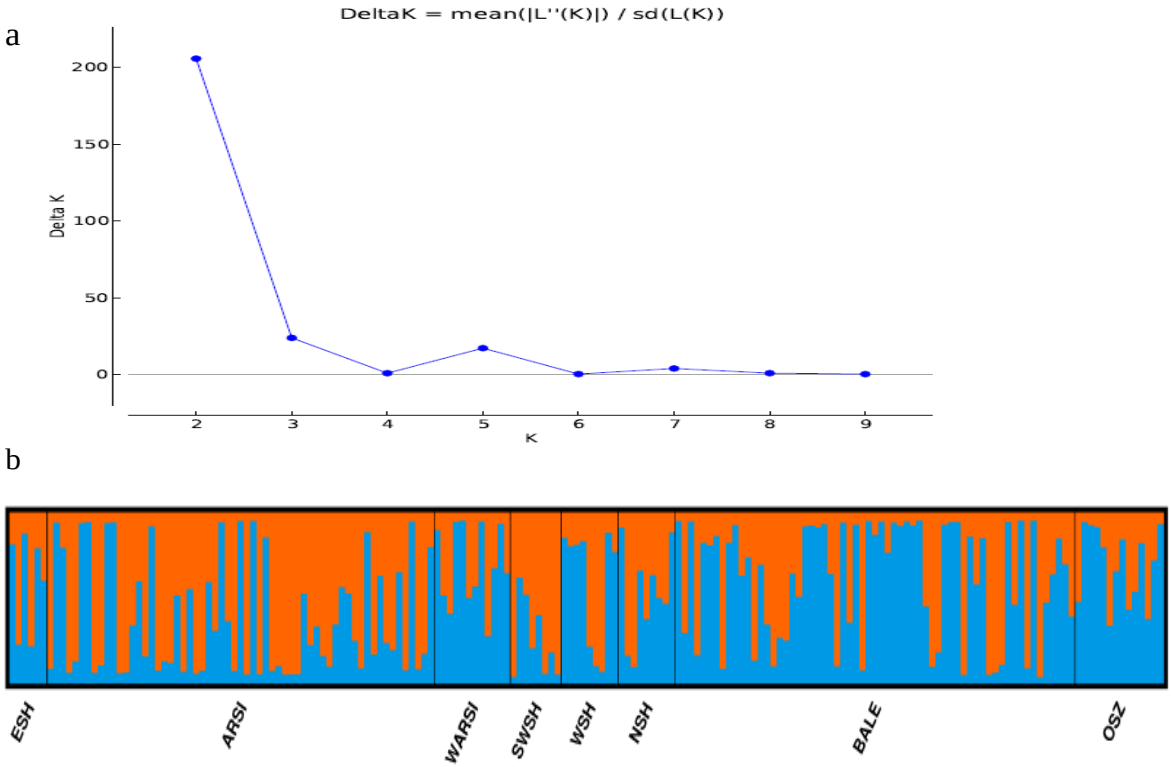

Figure 3. 6 Population structure of 182 *Z. tritici* isolates representing the 8 populations.

a) Best delta K value estimated using the method of Evano et al. (2005); and b) Estimated population structure for K = 2 according to geographical locations. The different (blue and orange) colures represent genetic groups or sub-populations designated by Structure Harvester: the x-axis represents individual samples and y-axis represents the proportion of ancestry to each cluster. Population abbreviations are: ESH = East Shewa; WARSI = West Arsi; SWSH = South West Shewa; WSH = West Shewa; NSH = North Shewa; OSZ = Oromia Special Zone Surrounding Finfine.

3.4 Discussion

Identification of *Z. tritici isolates*

Microscopic diagnosis method is less specific and less sensitive, and it can lead to wrong conclusion. Hence, the isolates were further confirmed using molecular technique. The *Z. tritici* specie-specific diagnostic markers (ITS1 and JB446) had successfully amplified about 345 bp fragment size from all the isolates, and also from the positive control. The successful amplification from all the sample isolates and from the reference (IPO323), but not from the negative control indicated that all the study materials were *Z. tritici*. Similar to our result, Beck and Ligon (1995) reported a PCR product of 345 bp from *Z. tritici* using same marker pair. Using same technique, the authors successfully distinguished *Z. titici* from other fungal species including *Stagonospora nodurum, Septoria glycines, Septoria passerinii, Pseudocercosporella herpotrichoides, Pseudocercosporella aestiva, Ceratobasidium cereale, and Drechslera sorokiniana*. PCR assay is a very sensitive, fast, accurate and economical plant pathogen diagnosis technique that likely to be used for the benefit of modern agriculture. Therefore, the present study has successfully detected and identified the wheat devastating pathogen, *Z. tritici* isolates collected from the major wheat growing areas of Ethiopia.

Polymorphism Level of the Microsatellite Markers

Z. tritici is a haploid, and amplification with all 14 SSR primer sets resulted in one allele per individual isolate at each locus, confirming that they are all single copy. For each locus, the frequency of the most common allele was less than 0.95 or 0.99 (Appendix II) confirming

that all were polymorphic, and hence useful genetic tools for population genetics analyses. Each locus had from three to five alleles with an average of 3.43 alleles over all 14 loci. The average number of alleles observed in the present study is significantly higher than that reported by Medini and Hamza (2008), who described an average of 2.5 alleles per locus among 42 isolates of *Z. tritici,* but less than the 4.2 identified for populations in northern France over eight SSR loci (Siah *et al*., 2018). Except for genetic differentiation, the average values for gene diversity (h = 0.45) and gene flow (Nm =2.64) over all loci for the total population in Ethiopia were all significantly higher than the comparable statistics reported by Dalvand *et al.*(2018), who reported mean genetic diversity of 0.38 and gene flow of 1.12 for 75 *Z. tritici* isolates collected from seven wheat-producing provinces in Iran; these low values were confirmed through analysis of an additional 221 isolates from Iran by Abrinbana *et al.* (2010).

In previous analysis using SSR markers, Owen *et al*. (1998), Razavi and Hughes (2004b) and El Chartouni *et al*. (2011) reported average genetic diversities of 0.49, 0.44 and 0.54 for 12 UK, 90 Canadian and 363 French isolates of *Z. tritici*, respectively. The high genetic diversity of *Z. tritici* populations of Ethiopia could be due to genetic recombination during sexual reproduction (Zhan *et al*., 1998; Zhan and McDonald, 2004) and spontaneous mutations (Samia *et al*., 2013). The high percent of polymorphism and Nei's gene diversity coupled with higher value of PIC observed in Ethiopia indicates the power and informativeness of the markers in revealing the genetic diversity of *Z. tritici* populations to deliver useful information for designing durable management strategies against the disease.

Population Genetic Diversity

Statistical analysis of population genetic variability across all loci confirmed high genetic diversity within the *Z. tritici* populations in Ethiopia. This is evidenced by a high mean number of observed alleles (3.43), number of effective alleles (2.00), Shannon's information index (0.74), Nei's gene diversity (0.45), and percentage of polymorphic loci (94%) across the eight populations. Similar, but generally lower numbers have been obtained in analysis of the genetic diversity of *Z. tritici* in various parts of the world. In Iran, Dalvand *et al.* (2018) reported a mean number of alleles of 2.0, number of effective alleles of 1.6, Shannon's information index of 0.53, Nei's genetic diversity of 0.35, and percentage of polymorphic loci value of 64.72%. Higher estimates of gene diversity ranging from 0.31 to 0.70 were reported for *Z. tritici* populations from various regions of the United States of America (Gurung *et al*., 2011), Tunisia (Boukef *et al*., 2011) and northern France (El Chartouni *et al*., 2011; Siah *et al*., 2018). In contrast, lower values of gene diversity (0.24) and percentage of polymorphism (91.12 %) were reported for *Z. tritici* populations from Iran (Abrinbana *et al.,* 2010).

As indicated by diversity parameters such as Shannon's information index and Nei's gene diversity, relatively all of the populations in Ethiopia showed higher genetic diversity. Among the studied populations, those of ESH, ARSI, SWSH and BALE were more diverse showing a mean gene diversity greater than the grand mean (h = 0.45), thus areas representing these populations could be considered as hot spots for *Z. tritici* diversity and hence ideal for studies on the pathogen, host-pathogen interactions and screening breeding

lines for resistance. Overall, the ARSI population had the highest values for all of the genetic diversity estimators such as Na =3.36, Ne =2.47, I=1.00, h = 0.58, and private alleles =0.14, suggesting that it could be a source population where diversity was maintained through cultivation of a broad spectrum of wheat cultivars (Medini and Hamza, 2008).

Ethiopia is considered to be center of diversity and center of origin for durum wheat (*Triticum turgidum* var *durum* Desf) (Vavilov, 1951). Wheat has been cultivated in the country since ancient times, and thus the long years of exposure of the pathogen to possible resistance genes in the host plants might have forced it to evolve to overcome host resistance (Zhan *et al*., 2002). In line with this, Medini and Hamza (2008) stated that the exclusive and widespread use of major resistance genes to control Septoria tritici blotch can possibly lead to the rapid emergence of new virulent strains within pathogen populations. Zhan *et al.* (1998) stated that sexual recombination is considered to be the major source of genetic variation in populations of the pathogen, which provides optimum conditions for the emergence of new virulence and/or fungicide resistance alleles. Similarly, Zhan and McDonald (2004) described how regular sexual reproduction provides a mechanism for this pathogen to rapidly generate novel allele combinations while asexual reproduction, gene flow, and natural selection ensure the maintenance and rapid dissemination of those combinations with the highest fitness.

Population Genetic structure

AOMVA revealed that *Z. tritici* populations in Ethiopia showed moderate genetic differentiation, with the among populations variation accounting for only 8% of the total genetic variation (3.91). Similar lower proportions of among population genetic variations of 31%, 12.5%, 23 % and 12 % were reported for *Z. tritici* populations in Iran (Dalvand1*et al.*,2018), Tunisia, Algeria and Canada (Medini and Hamza, 2008), Canada (Razavi and Hughes, 2004a) and USA (Linde *et al*., 2002), respectively. McDonald and Mundt (2016) and Naouari *et al*. (2016) also reported high within population genetic variation in *Z. tritci*. For the same pathogen, variable rates of genetic differentiation were reported from different parts of the world. A statistically non-significant genetic differentiation and high gene flow was reported for *Z. tritici* populations of Tunisia (Boukef *et al.*,2012), while moderate to high among-population genetic differentiations were described for those from northern France (El Chartouni *et al.*, 2011; Siah *et al.*, 2018) and Iran (Abrinbana *et al.*, 2010), respectively. In the present study, the high within-population genetic variability could be attributed to sexual recombination and spontaneous mutation (Samia *et al*., 2013). Long-distance movement of ascospores by wind (Linde *et al*. 2002; Boeger *et al*.,1993; Donald and Mundt, 2016), exchange of infected seeds through marketing (Brokenshire, 1975; Dalvand *et al.,* 2018) and movement of plant parts like straws have facilitated high gene flow (Nm =5.66) among isolates from different geographical zones leading to reduced among-populations genetic differentiation (Consolo *et al*., 2009; Dalvand1 *et al.,* 2018). This is supported by the non- significant effect of geographical locations of the populations on genetic variation.

Neighbourjoing-based cluster analysis did not sharply group the populations according to their geographical origins confirming the presence of high gene flow among populations and also low associations of the genetic background of *Z. tritici* populations with their sites of sampling. Likewise, UPGMA clustering based on Nei's genetic distance resulted in limited grouping of populations corresponding to their geographic origins. The clustering pattern is weak enough to support the concept of "isolation by distance". The lack of a sharp, genetically distinct clustering patter was also supported by the results of PCoA and STRUCTURE analyses. PCoA failed to sharply cluster the populations according to their geographical areas of sampling, and detected a high genetic intermixing among *Z. tritici* populations of different administrative zones.

STRUCTURE analysis also weakly inferred two sub-groups (K=2), in which all analyzed individual isolates share genetic background (alleles) inherited from both clusters, confirming the presence of high potential of genetic admixture and also the close relationship among isolates of the different sampling sites as a result of high gene flow. In line with this, STRUCTURE analyses did not reveal any population substructure in *Z.tritici* populations of Tunisia (Boukef *et al*., 2012). In contrast, a more structured *i.e* with 5 - 6 genetically distinct groups in accordance with their geographical area of sampling was reported for *Z. tritici* populations from northern France (El Chartouni *et al*., 2011; Siah *et al.* 2018) and Iran (Abrinbana *et al*., 2010), respectively.

3.5 Conclusions

Zymoseptoria tritici is a world-wide-distributed fungal pathogen causing Septoria tritici blotch (STB) on wheat. STB poses significant yield losses globally, and currently is a critical problem for wheat production in Ethiopia. Knowledge of the genetic structure of the pathogen is very helpful for developing durable management strategies for controlling the disease. In the present study, 182 *Z. tritici* isolates collected from eight major wheat-growing zones of Ethiopia were analyzed for allelic diversity at 14 SSR loci. The analysis revealed that all the loci were highly polymorphic and informative to describe the genetic diversity and population structure of the pathogen populations. The study revealed a very high genetic diversity within *Z. tritici* populations of Ethiopia, where 92% of the total genetic diversity resides within populations. Mutation and sexual recombination are likely the main sources of this genetic variability. All of the study populations showed relatively high gene diversities ranging from 0.34 to 0.58 confirming their suitability of the study areas for investigations planned on the pathogen, host and their interactions. Among the tested populations, those from East Shewa, Arsi, South West Shewa and Bale displayed gene diversity greater than the overall mean value of 0.45 suggesting that these locations are hot spots for *Z. tritici* diversity, and also can serve as excellent locations to screen germplasm for resistance against STB. All the *Z. tritici* populations shared genetic background originated from two sub-populations confirming the presence of high gene flow among locations. Disease management strategies like host resistance, fungicide-treated seed, and removing crop debris can limit gene flow and reduce STB severity. As genetic resistance is suitable, economical and effective method to manage crop diseases, screening of large sets of wheat germplasms and breeding materials against the genetically diverse isolates needs to

be targeted. Generally, the present study has provided a first stab to comprehend the genetic structure of *Z. tritici* populations of the central highlands (Shewa) and Southeastern (Arsi and Bale) parts of Ethiopia, where 75% of the national wheat production is located. This baseline information is very helpful for wheat breeders and pathologists to design effective management strategies so as to reverse wheat production losses due to STB. The results also provide new insight into the epidemiology and genetic structure of *Z. tritici* in Africa where the agro-climatic conditions and the wheat cropping systems are different from the remaining parts of the world. Lastly, we suggest additional studies that include populations from the remaining parts of the country to generate a nationwide picture of the disease and also the integration of molecular marker techniques with post-genomics technologies to unfold the molecular mechanisms of wheat resistance to *Z. tritici* for effective control of the disease.

CHAPTER 4

4. Virulence variability of Ethiopian *Zymoseptoria tritici* isolates and efficacy of wheat genotypes and *Stb* resistance genes against the isolates

Abstract

Septoria tritici blotch caused by the fungus *Zymoseptoria tritici* is a serious threat to wheat production worldwide. Knowledge of physiologic specialization of the pathogen and identification of potential source of resistance are prerequisite for designing durable management strategies. The present study was targeted to determine the virulence pattern of eight bread wheat derived Ethiopian *Z. tritici* isolates, and efficacy of known *Stb* resistance genes and wheat genotypes against the isolates. Disease severity analysis confirmed the presence of specific interaction in the pathogen. Pathogenecity assay identified 60 isolate-specific resistances among all (n=360) interactions. Of 45 wheat genotypes, 40% showed no isolate-specific resistance responses and were susceptible to all isolates. Tested *Z. tritici* isolates showed significantly different virulence patterns with virulence on 71% to 93% wheat genotypes. Isolate I_1 was found to be the most virulent (on 93% of the tested genotypes), and hence, useful for germplasm screening. Among tested known *Stb* genes, *Stb13/Stb14* in Salamouni did not confer resistance to any of the isolates, while *Stb16* gene conferred broad spectrum resistance to 75% of them, and thus, promising source of resistance to STB in Ethiopia. Among tested 20 commercial cultivars, 45% did not confer resistance to all the isolates. Three genotypes (MURGA, Km7 and the cultivar Hidase) conferred the greatest level of resistance to the tested Ethiopian *Z. tritici* isolates. The information is very useful for wheat breeders and the wheat farming community in making informed decisions to manage STB disease in Ethiopia.

4.1 Introduction

Wheat, a staple food of more than 50% of the world's population, is severely threatened by various biotic and abiotic stresses. Septoria tritici blotch (STB) caused by the fungus *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous (telomorph: *Mycosphaerella graminicola* (Fuckel) J. Schr¨ot. in Cohn or *Septoria tritici* Roberge in Desmaz), is one of the most economically damaging diseases of wheat worldwide (Quaedvlieg *et al*., 2011; Hosseinnezhad *et al.,* 2014; Testa *et al.,* 2015; Kettles and Kanyuka, 2016). Under favorable disease conditions, it can result in significant global wheat yield loss of 30 – 54% (Eyal *et al*., 1987), 35 to 50% (Ponomarenko *et al*., 2011) and even > 60% (Shipton *et al*., 1971). The economic impact of STB on wheat is getting increasing due to the introduction of high yielding susceptible varieties that are improved and selected for resistance to other biotic and abiotic stresses.

In Ethiopia, the occurrence of Septoria tritici blotch has been indicated by a number of reports (Stewart and Dagnachew Yirgou, 1967; Eshetu Bekele, 1985; Getinet Gebeyehu *et at*., 1990; Ayele Badebo *et al.* 2008; Endale Hailu and Getaneh Woldeab, 2015; Abera Takele *et al.* 2015; Tilahun Mekonnen *et al.* 2019). Currently, it becomes one of the most wheat devastating disease in the country (Endale Hailu and Getaneh Woldeab, 2015; Teklay Abebe *et al.,* 2015; Abera Takele *et al.,* 2015) with increasing incidence and severity in the major wheat-growing areas (Teklay Abebe *ét al.,* 2015; Tilahun Mekonnen *et al.,* 2019). It causes considerable (25- 82%) production loss (Getinet Gebeyehu*et al.,* 1990; Mengistu Huluka *et al*., 1991; Ayele Badebo *et al.* 2008). Endale Hailu and Getaneh Woldeab (2015)

also reported the wide distribution (100%) of STB disease in West and South West Shewa zones of the country. This is likely due to the frequent occurrence of rust epidemics that made the wheat breeding program to focus on the introduction and development of wheat genotypes resistant to rusts, but STB susceptible commercial cultivars (Belayneh Admassu *et al*., 2012).

Genetic resistance is currently seen as the most effective, cheapest and environmentally safe method to control cop diseases including Septoria (Eyal and Levy, 1987; Eyal, 1999; Ponomarenko *et al.,* 2011; Ghaneie *et al*., 2012; Miedaner *et al.,* 2013; Ragimekula *et al.,* 2013; Makhdoomi *et al.,* 2015; Hanson *et al.,* 2016). Successful resistance breeding requires consistent characterization of the pathogen virulence patterns, and identification and deployment of new resistance genes that overcome the prevailing virulent races (Belayneh Admasu *et al.,* 2012*)*. In this regard, knowledge of the pathogen virulence pattern is of paramount importance for designing best and durable resistance breeding strategies (Eyal *et al*., 1983; Sebei and Harrabi, 2008). In line with this, Gieco *et al.* (2004) stated that pathogenicity tests verifying the behavior of *S. tritici* isolates should be considered as a priority in the selection of resistant wheat materials to this pathogen.

Various reports evidenced the existence of differential interaction between the wheat genotypes and the different *Z. tritici* isolates, confirming the presence of physiological specialization of the pathogen (Eyal *et al*., 1973; King *et al*., 1983; Saadaoui, 1987; Eyal and Levy, 1987; Ballantyne, 1989; Ackerman *et al*., 1995; Jlibene *et al*., 1995; Kema *et al*., 1995; Kema *et al*., 1996a, b; Boughalleba and Harrabi, 1997; Kema and van Silfhout, 1997;

Grieger, 2001; Brading *et al*., 2002; Sebei and Harrabi, 2008; Ghaneie *et al.*, 2012; Hosseinnezhad *et al.,* 2014; Makhdoomi *et al.,* 2015). The term "pathotpyes" refers to populations within a fungal species that are recognized as morphologically similar but have different infection behavior that can be identified by their reaction to a set of test host cultivars known as "differentials" (Sidhu and John, 1981). Czembor *et al.* (2011) sated that differential wheat genotypes carrying known *Stb* genes could be used for determining virulence diversity of *M. graminicola* populations. Seedling screening offers the opportunity to identify the efficacy of resistance to a wide panel of isolates (Tabib Ghaffari *et al*., 2011). Hanson *et al.* (2016) stated that greenhouse/growth room screening procedures in which plants are inoculated with specific pathogen strains, and defined inoculum concentrations can assess disease reactions quickly reducing some sources of environmental variations and other confounding effects due to other pests or diseases.

However, in Ethiopia, irrespective of the increasing economic significance of Septoria disease on wheat production, information on the pathogen virulence patterns which is vital for designing and implementing durable management strategies is greatly missing. Moreover, knowledge on the resistance status of commercial wheat cultivars which is important to identify potential sources of resistance to STB, and also to replace the susceptible cultivars by resistant genotypes is lacking. Therefore, the present study was undertaken to investigate the virulence variability of eight Ethiopian *Z. tritici* isolates collected from the major wheat-growing areas of the country and also explore the efficacy of known *Stb* genes against the isolates. It also targeted to determine the resistance spectra of bread wheat genotypes cultivated in Ethiopia to identify broad effective genetic materials

that can be grown in STB hot spots and also serve as source of resistant genes against STB in wheat breeding programs.

4.2 Materials and methods

Plant materials

In this study, a set of 45 bread wheat genotypes including 25 introduced materials (among which eight were differential lines, each carrying one or more known *Stb* gene/s) (Table 4.1) and 20 selected commercial bread wheat cultivars of Ethiopia (Table 4.2) were used to determine the infection behavior of eight randomly selected *Zymoseptoria tritci* isolates collected from the major wheat growing areas of Ethiopia at seedling stage under greenhouse condition. The introduced wheat genotypes were obtained from International Maize and Wheat Improvement Center (CMMYT), Mexico, while the commercial cultivars were obtained from wheat breeding program of Holetta Agricultural Research Center (HARC), Ethiopia. Fungal isolates pathotyping was conducted by determining the reaction of the differential lines against the isolates. Cultivar Lakech was used as a susceptible check.

Table 4. 1 Introduced wheat genotypes and differential lines used for virulence analysis.

Cultivar source	Origen	*Stb* Gene	Reference
Salamouni	Canada	Stb13 (7BL) + Stb14 (3BS)	(McIntosh et al. 2007)
6B662	CIMMYT, Mexico	*	Pawan SK CIMMYT, Mexico
COULTER	Canada	*	Pawan SK CIMMYT, Mexico
ERIK	CIMMYT, Mexico	*	Pawan SK CIMMYT, Mexico
GLENKEA	CIMMYT, Mexico	*	Pawan SK CIMMYT, Mexico
ND-495	*USA*	*	Pawan SK CIMMYT, Mexico
Veranopolis	Brazil	Stb2 (3BS) + Stb6	(Adhikari *et al.*, 2004b)
Israel 493	Israel	Stb3 (7AS) + Stb6	Adhikari *et al*., 2004b; Chartrain *et al*. 2005b)
Tadina	USA	Stb4 (7DS)+ Stb6	(Adhikari *et al*., 2004a; Chartrain *et al*. 2005b; Somasco *et al*. 1996)
Shafir	Israel	Stb6 (3AS)	(Brading *et al*., 2002)
Estanzuela Federal	Uruguay	Stb7 (4AL)	(McCartney *et al*., 2003)
Kavkaz-K4500	Iran	Stb10 (1D) + Stb12 (4AL) + Stb6 + Stb7	(Chartrain *et al*., 2005a)
KM7	Iran	*Stb16*	(Dalvand *et al*.,2017)
GONDO/CBRD	CIMMYT, Mexico	*	Pawan SK CIMMYT, Mexico
BR 18	CIMMYT, Mexico	*	Pawan SK CIMMYT, Mexico
CATBIRD	CIMMYT, Mexico	*	Pawan SK CIMMYT, Mexico
BR 34	CIMMYT, Mexico	*	Pawan SK CIMMYT, Mexico
FRONTANA	Brazil	*	Pawan SK CIMMYT, Mexico
MURGA	CIMMYT, Mexico	Stb16	Pawan SK CIMMYT, Mexico
BLOUK #1	CIMMYT, Mexico	*	Pawan SK CIMMYT, Mexico
CHIBIA//PRLII/CM65531/ 3/SKAUZ/BAV92	CIMMYT, Mexico	*	Pawan SK CIMMYT, Mexico
CIANO T 79	CIMMYT, Mexico	*	Pawan SK CIMMYT, Mexico
SOKOLL//W1592/WBLL1	CIMMYT, Mexico	*	Pawan SK CIMMYT, Mexico
WAXWING*2/CIRCUS	CIMMYT, Mexico	*	Pawan SK CIMMYT, Mexico

*= the specific *Stb* genes were not clearly defined from the seed source- CIMMYT (International Maize and Wheat Improvement Center).

Fungal Isolates

In this study, eight single- spore derived *Z. tritici* isolates were used. They were randomly selected from the isolates recovered in section 3.2. Half of the isolates were from Bale while three were from Arsi, and one was from East Showa administrative zones (Table 4.3). To use as an inoculum, single spore-derived isolates were multiplied in a liquid medium composed of 1% (w/v) yeast extract powder + 1% (w/v) sucrose. The cultures were maintained on an orbital shaker of 130 rpm for two to three weeks.

Table 4. 2 List of Ethiopian released bread wheat cultivars tested for STB resistance.

Wheat Genotype	Origin	Selection center	Pedigree	Year of Release
Lakeck	Mexico	Debre Zeit	*PJ"S"/GB55*	1967
Et-13 A2	Ethiopia	Holetta	UQ 105 SEL. X ENKOY	1981
Kubsa	CIMMYT, Mexico	Kulumsa	ATTILA	1994
Mararo	Ethiopia	Kulumsa	*M/4/HAR 1709/ 3/M//24/E*	2005
Tay	Ethiopia	Adet	*ET12D4/4777(2)//FKN/GB/3/PVN"S*	2005
Digalu	CIMMYT, Mexico	Kulumsa	*SHA7/KAUZ*	2005
Alidoro	USA	Holetta	*HK-14-R251*	2007
Millinum	CIMMYT, Mexico	Kulumsa	*ALD/CEP75630//CEP75234/PT7219/3/BUC/BIY/4/*	2007
Gassay	CIMMYT, Mexico	Adet	*PASTOR*	2009
Kakaba	CIMMYT, Mexico	Kulumsa	*KIRITATI//SERI/RAYON*	2010
Danda'a	CIMMYT, Mexico	Kulumsa	*KIRITATI//2*PBW65/2*SERI.1B*	2010
Hoggena	CIMMYT, Mexico	Kulumsa	*PYN/BAU//MILAN*	2011
Hidase	CIMMYT, Mexico	Kulumsa	*YANAC/3/PRL/SARA//TSI/VEE#5/4/CROC-1/AE…*	2012
Ogalcho	CIMMYT, Mexico	Kulumsa	*WORRAKATTA/2*PASTOR*	2012
Huluka	ICARDA	Kulumsa	*UTQUE96/3/PYN/BAU//MILAN*	2012
Liben	NA	NA	NA	2015
Dembal	NA	NA	NA	2015
King Bird	NA	NA	NA	2015
Wane	NA	Kulumsa	SOKOLL/EXCALIBUR	2016
Lemu	NA	Kulumsa	WAXWING*2/HEILO	2016

Note: CIMMYT = International Maize and Wheat Improvement Center, ICARDA=International Center for Agricultural Research in the Dry Areas, and NA= Not available.

Table 4. 3 *Z. tritici* isolates used in races analysis, and efficacy test for *Stb* genes.

Z tritici Isolate		Collection		Geographic position (UTM)[a]		Altitude (m)
Code	Name	Zone	District	Latitude	Longitude	
I_1	ZTET158	Bale	Sinana	040° 17' 178	07 05' 621	2359
I_2	ZTET26	Arsi	Lemu Bilbilo	039° 15' 325	07° 32' 552	2810
I_3	ZTET119	Bale	Goba	039° 59' 077	07° 03' 365	2557
I_4	ZTET107	Bale	Dinsho	039° 52' 972	07° 07' 884	2691
I_5	ZTET012	Arsi	Hetosa	039° 09' 475	08° 00' 919	2211
I_6	ZTET159	Bale	Sinana	040° 14' 973	07° 10' 141	2400
I_7	ZTET006	East Showa	Ada'a	039° 00' 045	08° 46' 320	1880
I_8	ZTET067	Arsi	Adaba	039° 27' 126	07° 01' 525	2459

[a]UTM = Universal Transverse Mercator coordinate system.

Inoculation and Pathogenicity Assay

The seedling test was conducted in the greenhouse of National Agricultural Biotechnology Research center; Holetta, 28 km west of Addis Ababa, Ethiopia. Figure 4.1 presnents the pattogenecity assay. For each wheat genotype, a 5 - 7 seedlings were grown in plastic pots of 10cm diameter and 15cm depth filled with dry sterilized mixture of sieved river sand, forest soil and well decomposed farm yard manure (FYM) in a 1:1:1 ratio. The experiment was set up in a randomized complete block factorial design with three replications. Multiplied spores concentration was adjusted at 107spore per milliliter using hemacytometer and supplemented with 0.15% Tween- 20. Ten- day- old wheat seedlings were hand sprayed with the Z. tritici spore suspensions until it run-off (Perello et al., 1991, Ganeile et al., 2011). To avoid cross contamination and to provide high humidity for infection development, the inoculated seedlings were covered with plastic bags and rapped with aluminum foil for 96 hours. Afterwards, removing the foil and the plastic bags, the seedlings were maintained in the same greenhouse conditions of 20°C /17°C (at day/ night), 12 hours photoperiod and relative humidity of 25-80%. The seedlings were continuously monitored for STB symptom development for a period of three weeks.

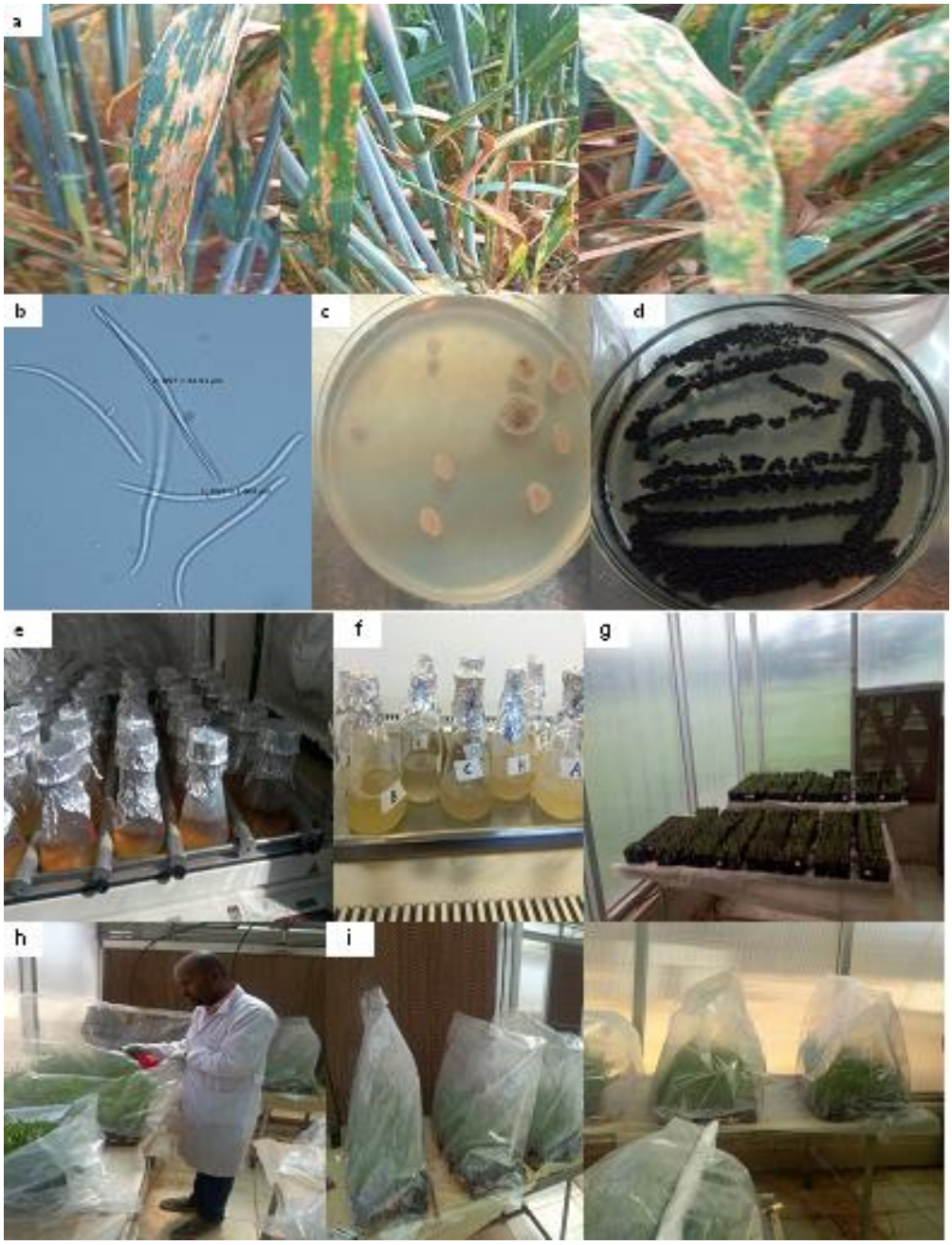

Figure 4. 1 *Z. tritici* isolates' physiological race analysis on wheat genotypes.

a) Septoria tritici blotch symptomatic wheat b) *Z. tritici* spores detected at magnification power of 40x objective, c) *Z. tritici* spore colonies on PDA, d) fungal culture streak plated on PDA, e) spore culture multiplication in liquid media on orbital shaker, f) Spore concentration adjusted to 10^7spore/ml, g) Seedling of wheat differential to be used for pathogecity test, h) artificial inoculation of wheat differentials with single race of *Z. tritici* spores, and i) inoculated seedlings sealed in plastic bag to avoid cross inoculation.

Data Collection and Statistical Analysis

In various *Z. tritici* isolates – host cultivar interaction studies, percentage of leaf area with necrosis, pycnidia coverage and their combinations were used to estimate disease severity (Eyal *et al*., 1987; Kema *et al*., 1996a). Hence, in the present study disease severity scoring on the tested wheat lines was carried out 21 days post inoculation from 15 plants per isolate * cultivar combinations by visual estimation of the percentage of necrotic leaf area (NLA) and pycnidia coverage (PC) as disease parameters (Eyal and Ziv, 1974; Kema *et al.*, 1996a). These values were averaged per pot for further analysis of the disease parameters. Percentage data were normalized using arcsine square root-transformation (Sokal and Rohlf, 1995). Normalized data were subjected to analysis of variance (ANOVA) using SAS software version 9.2 according to linear mixed model (LMM) by considering the effects of isolate, wheat and their two-way interactions as fixed effects, and the block effect as random.

Specific interactions between wheat genotypes and the isolates were determined by computing least significant differences (LSD) of means of wheat genotypes-by-isolate interaction values at α = 1% and 5% significance levels (Wilson *et al*., 2004; Cherif *et al*., 2007). The lowest mean of transformed disease severity (zero value) was used as resistant control. While, interaction mean values lower than the LSD values at α = 1% and 5% significant levels were considered as resistant and highly resistant genotypes, respectively (Ghaneie *et al.*, 2012; Hosseinnezhad *et al*., 2014). The pathogens virulence spectrum was determined based on their infection rate on the tested wheat genotypes. A pathotype that

could infect more than one resistant gene in the differential line was considered to be a broad spectrum virulent. Mean disease severity values of the genotypes-by-isolate were subjected to a hierarchical cluster analysis using the average linkage method and Euclidean distance in the statistical package Meet MINITAB Release 14 for Window. The back-transformed data for pycnidial coverage and necrosis leaf areas are presented in Tables 5.

4.3 Results

Combined Analysis of Variance

The disease severity data analysis showed a very highly significant differences (p<0.0001) among the wheat genotypes, the isolates, and their two-way interactions for both percentage of necrosis leaf area and pycnidia coverage (Table 4.4). ANOVA revealed that the main effect due to isolates was large (Table 4.4), indicating presence of significant virulence variability among Ethiopian *Z. tritici* isolates, and it is the major source of variation as well. Likewise, the very highly significant differences (P<0.0001) for the wheat genotypes indicated that the wheat genotypes differ greatly in their reactions against the inoculated *Z. titici* isolates. Moreover, the very highly significance differences for the interaction effect of isolate-by-genotype indicated the existence of cultivar specificity and variation for virulence among isolates. This implies that the genotypes of the isolates interacted differentially with the genotypes of the host.

Table 4.4 ANOVA of PC and NLA% by eight *Z. tritici* on 45 wheat genotypes.

Source of Variations	DF	Mean Square	
		PC	NLA
Genotype	44	3956.8^{2***}	3607.49***
Isolate	7	10536.73***	12458.70***
Genotype*Isolate	308	1049.40***	944.00***
R^2		98.95	98.17
CV (%)		7.304	9.92
Grand mean		2.91	3.72

PC= Pycnida coverage, NLA= ecrosis leaf area, *** = very highly significant (p<0.0001) and DF= degree of freedom.

Aggressiveness Variability Among *Zymoseptoria tritici* Isolates

Table 4.5, presents the back transformed data for mean percentage of pycnidia coverage and necrosis leaf area for the isolate-by- genotype interactions. The isolates aggressiveness analysis revealed that mean disease severity varied significantly among the tested wheat genotypes. Among tested isolates, I_1 was found to be the most aggressive with the highest mean pycnidia coverage (58%) and necrosis leaf area (58%) (Table 4.5). It was followed by I_8 with mean pycnidia coverage and necrosis leaf area of 58% and 53%, respectively. Conversely, isolate I_2 and I_5 were identified to be the least aggressive isolates with mean pycnidia density and necrosis leaf area of 28% and 21%, respectively (Table 4.5).

Table 4. 5 Percentage of pycnidia coverage and necrosis leaf area on wheat genotypes due to *Z. tritici* infection.

	Zymoseptoria tritici Isolates																	
	I_1		**I_2**		**I_3**		**I_4**		**I_5**		**I_6**		**I_7**		**I_8**		Mean[a]	
Wheat genotypes	**PC**	**NLA**	**PC**	**NLA**	**PC**	**NLA**	**PC**	**NLA**	**PC**	**NLA**	**PC**	**NLA**	**PC**	**NLA**	**PC**	**NLA**	**PC**	**NLA**
SALAMOUNI	77	67	14	16	34	19	43	36	30	29	72	53	52	49	53	55	47	41
6B662	79	80	34	53	4	9	1**	1**	1**	3*	23	9	37	32	85	82	33	34
COULTER	64	56	28	29	33	15	36	18	1**	1**	1**	1**	28	27	0**	0**	24	18
ERIK	95	98	18	14	1**	0**	20	31	29	19	77	57	29	23	90	77	45	40
GLENKEA	83	95	78	84	28	33	67	41	34	11	28	26	1**	1**	81	76	50	46
ND-495	77	87	48	44	5	16	90	79	28	20	80	71	53	49	94	98	60	58
VERANOPOLIS	84	68	29	30	53	44	3	5	13	1**	28	28	1**	0**	54	53	33	29
ISRAEL-493	54	87	27	32	44	49	3	4	52	9	54	51	30	32	84	77	43	43
TADINIA	54	28	1**	1**	21	26	1**	1**	39	10	29	27	1**	1**	97	75	31	21
SHAFIR	89	98	55	52	78	65	86	82	15	2**	54	75	21	13	83	67	60	57
ESTANZUELA FEDERAL	85	85	27	21	84	85	94	79	23	21	76	59	0**	1**	98	98	61	56
Kavkaz-K4500	87	97	0**	0**	44	47	77	54	1**	0**	62	54	23	23	95	97	49	47
KM7	1**	2**	1**	1**	28	20	1**	0**	1**	0**	0**	1**	68	67	1**	0**	13	12
GONDO/CBRD	78	76	35	31	0**	0**	77	68	34	28	39	30	24	20	98	65	48	40
BR 18	93	95	76	76	64	58	87	87	37	14	28	27	38	36	84	81	63	59
CATBIRD	95	98	29	46	30	28	57	56	22	0**	1**	1**	1**	2**	95	96	41	41
BR 34	88	95	53	65	55	62	88	88	49	29	94	91	2*	1**	95	96	65	66
FRONTANA	65	50	29	27	38	29	88	78	26	31	1**	1**	54	47	83	77	48	43
MUTUS	93	88	53	47	67	56	19	16	72	38	61	64	28	31	95	95	61	55
MURGA	30	28	1**	2**	1**	1**	1**	1**	44	32	1**	0**	1**	1**	24	14	13	10
BLOUK=1	73	86	28	42	44	36	94	73	50	28	1**	2**	1**	2**	97	97	49	46
CHIBIA	30	28	30	38	28	26	87	75	25	14	93	89	33	23	95	92	53	48
CIANO T 79	54	50	28	27	15	17	12	10	77	53	74	50	78	69	96	96	54	47
SOKOLL//W15.92/WBLL1	85	84	28	50	75	58	80	86	22	14	66	64	30	28	94	90	60	60
WAXWING*2/CIRCUS	66	73	38	38	79	71	82	71	53	32	76	73	63	54	82	78	67	61
Lakech	95	94	35	27	77	76	73	71	64	59	65	49	97	97	66	50	72	65
Et-13A2	78	87	4	6	47	45	28	61	51	45	24	25	74	75	23	23	41	46
Mararo	48	52	27	21	12	7	2*	3*	1**	2**	14	12	45	64	12	15	20	22
Liben	30	31	35	38	88	85	92	77	16	14	2*	2**	96	93	3	3*	45	43
Gassay	41	41	36	42	82	77	85	84	35	31	47	35	67	58	45	34	55	51

Table 4.5 Continued

Alidoro	36	37	1**	2**	2*	3*	14	14	1**	1**	28	30	84	81	27	33	24	25
Hoggena	18	21	0**	1**	39	37	1**	2**	20	18	32	22	2*	2**	34	20	19	15
Dembal	21	20	8	9	30	25	65	49	30	25	7	9	47	52	7	10	27	25
Kubsa	74	75	44	25	72	69	89	68	27	29	2*	1**	89	81	1**	1**	50	44
Tay	66	56	18	14	39	29	34	28	76	68	67	56	95	78	70	56	58	48
Wane	39	42	29	32	32	35	83	84	33	18	4	4	97	95	4	3*	40	39
Hidase	1**	0**	1**	1**	1**	2**	2*	2**	1**	1**	5	5	28	31	5	6	5	6
Digalu	26	27	21	24	13	13	1**	2**	2*	2**	36	26	42	36	34	30	22	20
Ogalcho	51	47	14	47	24	25	96	84	32	24	41	30	83	58	42	30	48	43
Huluka	24	27	65	50	36	30	76	67	3	3*	24	20	63	55	23	20	39	34
Kakaba	80	79	39	26	73	75	73	66	80	68	64	58	91	78	66	59	71	64
Danda'a	22	29	25	18	25	23	2*	3*	31	31	72	70	53	55	73	71	38	38
King Bird	32	34	14	15	74	67	67	59	40	39	37	33	92	79	36	30	49	45
Lemu	1**	0**	2*	2**	35	30	27	27	1**	2*	14	12	43	37	13	11	17	15
Millinum	27	26	43	44	46	36	46	50	34	25	64	48	76	74	64	50	50	44
Mean	58	58	28	29	40	37	50	45	30	21	39	35	46	43	58	53	44	40
S.E	2	1.37	1.99	1.47	1.79	1.58	1.37	1.77	1.67	2.21	1.95	2.86	2.76	2.62	2.34	1.99	1.871	

PC	$LSD^{b}_{5\%}$ =1.65	NLA	$LSD^{b}_{5\%}$ = 2.32
	$LSD^{b}_{5\%}$ =2.2		$LSD^{b}_{1\%}$ =3.05

[a] Mean disease scores were calculated by omitting data for specific interactions

[b] Least significant difference between means of disease scores

[*] Highly resistant: means not significantly different from zero (according to LSD5 %)

[**] Resistant: means not significantly different from zero (according to LSD1 %)

Virulence Variability of Ethiopian *Zymoseptoria tritici* Isolates

Virulence variability analysis revealed that the tested *Z. tritici* isolates showed a very highly significantly ($p<0.0001$) different virulence patterns on the tested wheat genotypes. The tested Ethiopian *Z. tritici* isolates were found to be broad spectrum virulent with virulence on 71% to 93% of the tested wheat genotypes. Isolate I_1 obtained from Bale Zone (Sinana) was found to be the most virulent isolate with virulence on 42 (93%) of the tested genotypes (Table 4.5), suggesting that it might have the lowest number of avirulent genes compared to the other isolates. It was avirulent only on one introduced differential genotype (KM7) and two commercial cultivars (Hidase and Lemu) (Table 4.5). Isolate I_8 was the second most virulent isolate with virulence on 41 (91%) wheat genotypes. In contrast, isolate I_5 was detected to be the lowest virulent isolate with virulence on 32 (71%) genotypes (Table 4.5), suggesting that it may possess the greatest number of avirulence genes. Isolate I_2, I_3 and I_6 also were high virulent on wheat genotypes with virulence to 37 (82%), 40 (89%) and 37 (82%), respectively. Two isolates, I_4 and I_5 were found to be virulent on 36 (80%) of the tested genotypes (Table 4.5).

Cluster Analysis Revealed Two Major Clusters of *Z. trictici* Isolates

Hierarchical cluster analysis based on mean disease severities on 45 bread wheat genotypes poorly grouped the tested *Z. tritici* isolates into two virulence clusters (Figure 4.2). Cluster A consisted of two isolates (I_1 and I_8) that were from geographically adjoining zones (Bale and Arsi, respectively) (Figure 4.2). They were virulent on 42 (I_1) and 40 (I_8) wheat genotypes and grouped to the same cluster because of their relatively similar higher

aggressiveness (Table 4.5). They became avirulent to the same wheat genotype KM7 (Table 4.5). Besides, I_1 was avirulant to the commercial cultivars Lemu and Hidase, while I_8 was avirulant to COULTER, Kubsa and Wane (Table 4.5). Cluster B comprised of six isolates among which one (I_7) from East Showa, two (I_2 and I_5) from Arsi and three (I_3, I_1 I_4, and I_6) from Bale zones (Figure 4.2). All the isolates of this cluster were virulent to the same 19 (42%) wheat genotypes and were avirulant on none of the tested genotype. Isolate I_3 was found to be the most virulent isolate of this cluster with virulence on 40 (89%) wheat genotypes. Whereas, isolate I_5 from Arsi zone became the least virulent (71%) and aggressive (21%) isolate placed in cluster B (Figure 4.2). With the same number of virulence (82%), isolate I_2 and I_6 were placed in different sub-clusters of the main cluster B due to their difference in aggressiveness, which was 29% and 35%, respectively. The same principle holds true for isolate I_4 and I_7 which were recovered from Bale and East Showa zones, respectively (Table 4.5).

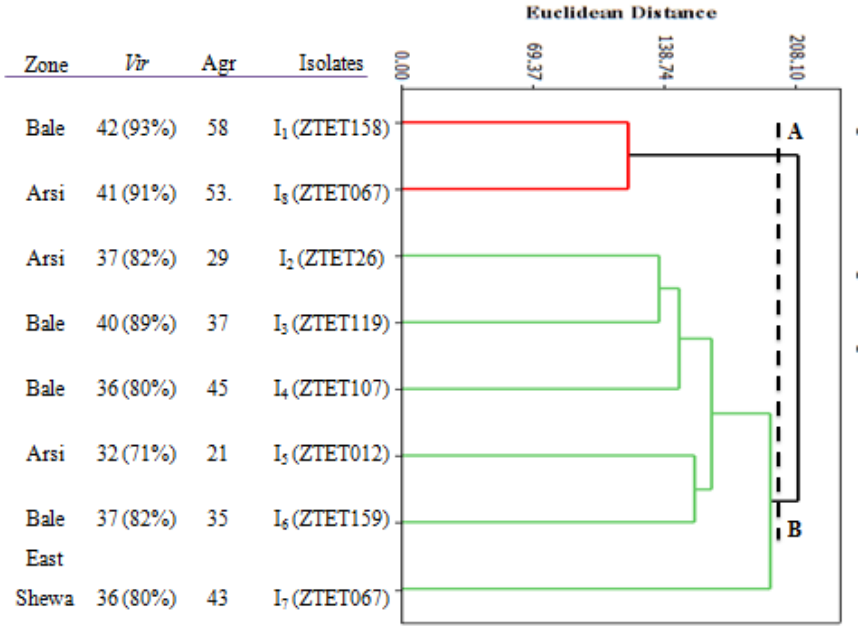

Figure 4. 2 Clustering of 8 *Z. tritici* isolates based on their mean severity on wheat genotype. *Vir* = Virulent and Agr = Aggressiveness.

Isolate- specific- Response of the Wheat Genotypes

Statistical analysis revealed that disease severity (pycnidia coverage and necrosis leaf area percentage) varied significantly among the tested wheat genotypes, the isolates and their two way interactions. Among 360 isolates-by-wheat genotype interactions, 53 (15%) and 60 (17%) showed mean PC and NLA values lower than the LSD values at $P<0.01$ and $P<0.05$ levels, respectively, and thus, they can be considered to be resistant to one or more of the tested *Z. triici* isolates (Table 4.5). Among the wheat genotypes used in this study, KM7 showed six (75%) isolate-specific resistances to the tested Ethiopian *Z. tritici* isolates. The introduced genotype MURGA and the commercial cultivar Hidase showed five (62.2%) isolate-specific resistances. Likewise, 10 (22%) wheat genotypes showed one isolate-specific resistance, eight (17%) genotypes showed two isolate-specific resistance, and six (13%) genotypes showed specific resistance to three *Z. tritici* isolates (Table 4.5).

The study revealed that 18 wheat genotypes (144 or 40% of the combinations) showed no isolate-specific- resistance response, and were susceptible to all the tested *Z. tritici* isolates (Table 4.5). None of the tested wheat genotypes were resistant to all the study *Z. tritici* isolates. Of 45 tested wheat genotypes, 27 (60%) of them showed significant specific resistance to one or more of the tested *Z. tritici* isolates (Table 4.5).

Cluster Analysis of the Wheat Genotypes

Hierarchical cluster analysis based on mean disease severity had grouped the 45 bread wheat genotypes into two clusters (Figure 4.3). Cluster A composed of 33 (73.3%) wheat genotypes among which 18 (54.55%) showed no isolate-specific resistance response, and thus considered to be susceptible genotypes (Table 4.5; Figure 4.3). From the same cluster, 10 (27.3%), four (12.12%), and one genotypes showed one, two and three isolate-specific resistance reactions, respectively (Table 4.5). The mean disease severity in Cluster A varied from 25% (Dembe) to 65% (Lakech) with overall mean of 47.97%. Five (39%) of the genotypes of this cluster showed disease severity values higher than the cluster mean, while 61% of the group members showed severity values lower than the cluster mean severity value.

Cluster B was composed of 12 (26.67%) wheat genotypes that showed two to five isolates-specific resistance reactions to the tested Ethiopian *Z. tritici* isolates (Table 4.5; Figure 4.3). The mean disease severities of this cluster ranged from 6% (Hidase) to 34% (6B662) with overall mean of 18.9%. About 50% of the genotypes in this cluster showed mean severity values higher than the cluster mean, and same percentage were identified to have severity values lower than the group mean. About 41.67% of the genotypes of Cluster B were commercial cultivars (Mararo, Alidoro, Hoggana, Hidase and Digalu) (Figure 4.3). Moreover, most (67%) of the genotypes of this cluster showed a 3 - 5 isolates-specific resistance to the tested isolates. The cultivar Hidase was found to be the most resistant genotype among tested commercial cultivars.

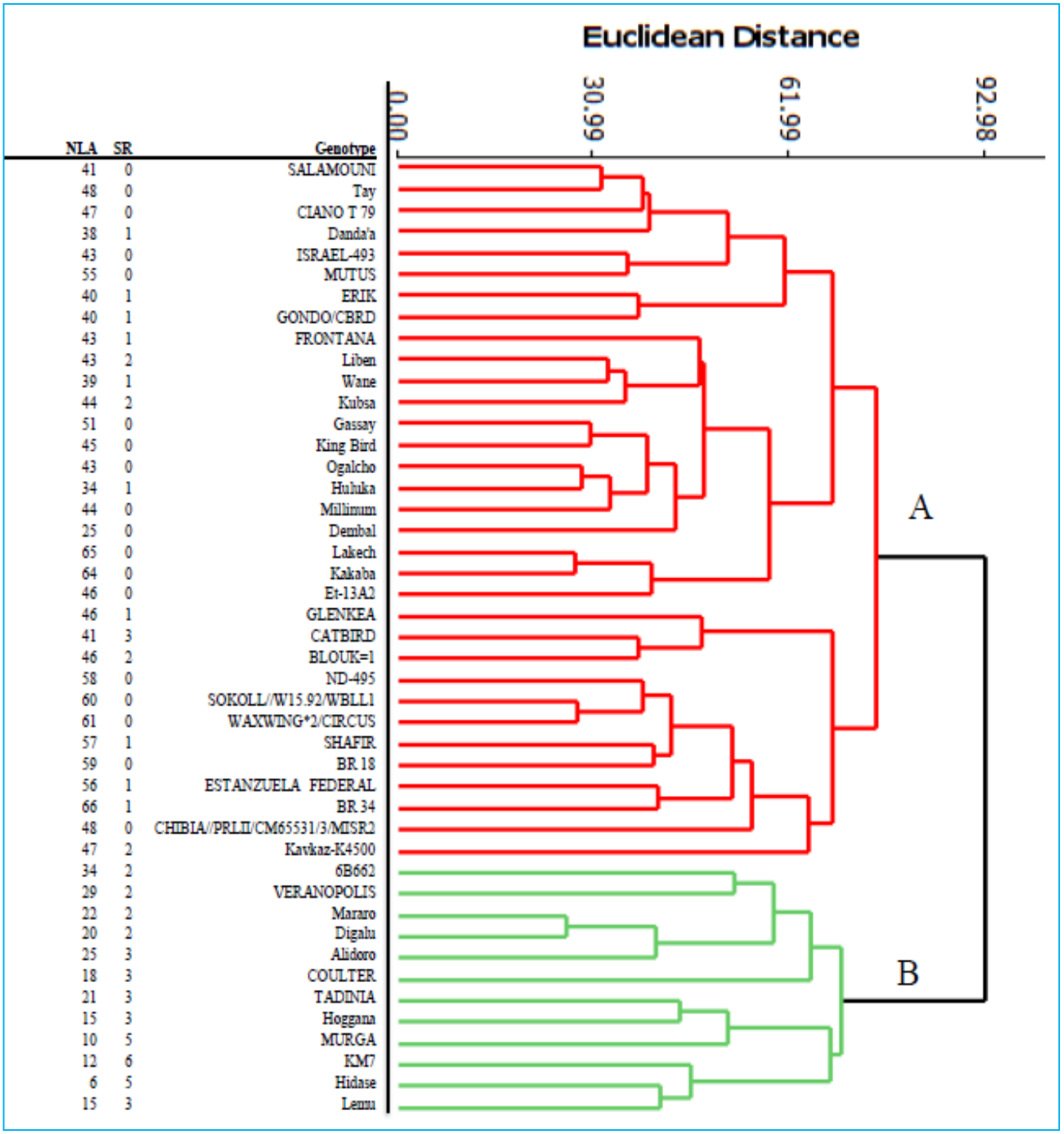

Figure 4.3 Clustering of 45 wheat genotypes based on their NLA induced by 8 *Z.tritici* isolates.
The positions of the nodes correspond with Euclidian distance on the horizontal axis at the top. The means disease severity (NLA) and the number of specific resistance (SR) for each wheat genotype are indicated.

Efficacy of the *Stb* Resistant Genes against Ethiopian *Z. tritici* Isolates

Race analysis on differential lines carrying mapped *Stb* genes indicated that Ethiopian *Z. tritici* isolates show significant variability in their infection behavior. Efficacy analysis on *Stb* genes revealed that I_3 was the most virulent isolates causing Septoria tritici blotch on all the tested differential lines carrying known *Stb* genes (Table 4.6). Whereas, I_5 was identified to be virulent in fewest cultivars like Salamouni and Estanzuela Federal that carry *Stb13/Stb14,* and *Stb7* genes, respectively (Table 4.6). Similarly, I_1 was observed to be virulent in most of the differential cultivars except Tadinia and Km7 that carry *Stb4/Stb6* and *Stb16,* respectively (Table 4.6). Isolate I_2 was a broad spectrum virulent on most of the wheat differential lines except Tadinia, Estanzuela Federal, Kavkaz-K4500 and Km7 that possessed *Stb4/Stb6, Stb7,* Stb10/Stb12/*Stb*6/Stb7 and *Stb16* genes, respectively (Table 4.6). The study revealed that Km7 (carrying *Stb16)* conferred resistance to all the isolates except I_3 and I_7. While, the *Stb13/ Stb14* present in Salamouni did not confer resistance to any of the tested isolates (Table 4.6). Salamouni, Israel-493, Kavkaz-K4500, and Km7 carry respectively *Stb13/Stb14*, *Stb3/Stb6, Stb10 /Stb12/ Stb6 /Stb7*, and *Stb16*, and all did not conferred resistance to I_7. Isolate I_8 was found to be virulent on all the *Stb* genes of the wheat differentials except *Stb16* in Km7. Veranopolis carry *Stb2/Stb6* and was effective against I_4, I_5 and I_7, but it was susceptible to the rest of the isolates. Israel-493 contains *Stb3/Stb6* conferred resistance to two isolates only (I_4 and I_5). Among the differentials, KM7 and Tadinia that possessed *Stb16* and *Stb4/Stb6* were the most resistant genotypes that conferred resistance to six and four of tested *Z, tritici* isolates, respectively (Table 4.6).

Table 4. 6 Reaction of known *Stb* differentials to eight Ethiopian *Z. tritici* isolates.

Wheat deferential lines	*Stb* genes	*Zymoseptoria tritici* isolates							
		I_1	I_2	I_3	I_4	I_5	I_6	I_7	I_8
Salamouni	*Stb13+ Stb14*	S	S	S	S	S	S	S	S
Veranopolis	*Stb2 +Stb6*	S	S	S	R	R	S	R	S
Israel-493	*Stb3 +Stb6*	S	S	S	R	R	S	S	S
Tadinia	*Stb4 +Stb6*	S	R	S	R	R	S	R	S
Shafir	*Stb6*	S	S	S	S	R	S	R	S
Estanzuela Federal	*Stb7*	S	R	S	S	S	S	R	S
Kavkaz-K4500	Stb10 +Stb12+ Stb6 +Stb7	S	R	S	S	R	S	S	S
Km7	*Stb16*	R	R	S	R	R	R	S	R

Stb = Septoria resistant genes in the differential lines, S = Susceptible response, and R = Resistant response.

4.4 Discussion

Wheat, a staple food of more than 50% of the world's population, has been cultivated in Ethiopia since ancient time. In spite of its tremendous contributions to improved livelihood of large segments of population in Ethiopia, the current average wheat productivity in the country (2.97 t ha^{-1}) is far below the global average of 3.5 t/ha (FAOSTAT, 2019). STB is one of the major bottlenecks of wheat production in Ethiopia. Use of resistant cultivars is considered to be the most effective, economical and environmentally safe method to control Septoria disease. Knowledge of the pathogen virulence pattern and germplasm screening are prerequisite for designing durable management strategies. In the present study the virulence pattern of eight *Z. tritici* isolates collected from major-wheat growing areas of Ethiopia were tested on 45 bread wheat genotypes at seedling stage by considering percentage of pycnidia coverage (PC) and necrosis leaf area (NLA) as disease parameter (Eyal *et al.,* 1987; Kema *et al.,* 1996a). We expect that the study had generated valuable

information on virulence variability of Ethiopian *Z. tritici* isolates, *Stb* genes that have been defeated and wheat cultivars against the isolates that help wheat breeders and growers to make informed decisions about breeding and planting proper cultivars.

The combined analysis of variance for both parameters (PC and NLA) revealed highly significant differences ($p< 0.0001$) for the interaction effect confirming the presence of specific interactions in the tested isolates (Perello *et al*., 1991). It confirmed the existence of cultivar specificity and variation for virulence among Ethiopian *Z. tritici* isolates. Among 360 isolates-by-wheat genotype interactions, 53 (15%) and 60 (17%) showed mean PC and NLA values lower than the LSD values at $P<0.01$ and $P<0.05$ significant levels, indicating their resistance to one or more of the tested *Z. tritici* isolates. Significant number (60%) of the tested wheat genotypes showed significant resistance response to one or more tested *Z. tritici* isolates. Among tested wheat genotypes, KM7 which carries *Stb16* showed the highest (75%) isolate-specific resistance response. It was followed by MURGA and Hidase which had five (62.5%) isolates-specific resistances. Conversely, considerable (18 or 40%) of the study wheat genotypes showed no isolate-specific-resistance response, and were susceptible to all the tested *Z. tritici* isolates. In line with this, He *et al.* (2020) reported six QTLs effective for STB resistance on chromosome 2B, 2D, 3A, 3B, 3DL and 5B of the wheat line Murga. This implies that the detected resistant genotypes could be reliable source of resistance genes against Ethiopian *Z. tritici* populations. The cultivars BR-34 and Lakech were identified to be the most susceptible genotypes with mean NLA of 66% and 65%, respectively. Hence, they can serve as susceptible local checks in the national wheat resistance breeding program.

The presence of isolate-specific resistance in wheat-*Z. tritici* pathosystem had been also evidenced in various studies. Makhdoomi *et al.* (2014) tested six Iranian *Z. tritici* isolates on 70 wheat genotypes and reported 116 (28%) isolates-specific resistance of the wheat genotypes. The same authors also reported that 26 (37%) of the study genotypes didn't show isolate-specific responses and were susceptible to all the tested isolates. Likewise, Ghaneie *et al.* (2012) tested eight Iranian *Z. tritici* isolates on 45 durum wheat, and found 138 isolate-specific resistances among all interactions (n=360). They reported that 17 (38%) of the tested genotypes showed no isolate-specific responses to all the tested isolates, while 11% (n=5) showed specific responses to all tested isolates. Moreover, Hosseinnezhad *et al.* (2014) observed 224 (26%) isolate-specific resistances among 931 wheat genotype– *Z. tritici* isolate interactions in Iran.

The study confirmed the presence of greater degree of variability in aggressiveness among the tested *Z. tritici* isolates. Isolate I_1 was identified to be the most aggressive with the highest mean pycnidia coverage (58%) and necrosis leaf areas (58%). Conversely, isolate I_5 was detected to be the least aggressive (21%) and lowest virulent (71%) isolate indicating that it may have an avirulence gene that is recognized by a common resistance gene present in most of the tested wheat genotypes or this isolate may produce several avirulence proteins that are recognized by the corresponding cognate resistance proteins presented in different genotypes (Ghaneie *et al.*, 2012).

Tested Ethiopian *Z. tritici* isolates showed significantly different virulence patterns on the wheat genotypes used in this study. They were identified to have broad spectrum virulence with a virulence on 71% to 93% of the wheat genotypes. Isolate I_1 showed the greatest virulence spectrum towards 93% of the tested wheat genotypes, indicating that it might have the least number of avirulence genes compared to the other isolates. Similarly, Hosseinnezhad *et al.* (2014) reported the existence of significantly different virulence patterns among Iranian *Z. tritici* isolates with virulence spectra ranging from 40% of the tested genotypes (for isolates RM189) to 90% of the tested genotypes (isolate RM46). Ghaneie *et al.* (2012) also evidenced the presence of significant virulence variability among eight Iranian Z. *tritici* isolates tested on 45 durum wheat landraces. The isolates showed virulence spectrum ranging from 42% to 82% of the tested wheat genotypes. Therefore, the present study confirmed that Ethiopian *Z. tritici* isolates have broad spectrum virulence and greater degree of aggressiveness implying the need to device a gene management strategy that incorporates a combination of genes (gene pyramiding) that can provide sufficient protection and durable control of Septoria tritici blotch in the country. Moreover, the highly virulent isolates (I_1 and I_8) identified in the present study can be used for germplasm screening in wheat resistance breeding program.

Hierarchical clustering of the tested *Z. tritici* isolates based on their mean disease severities on the wheat genotypes poorly grouped the isolates according to their geographical areas of sampling, likely due to the presence of high gene flow between populations of different areas. In this regard, Linde *et al.* (2002) confirmed the presence of high gene flow between continents possibly through long distance movement of ascospores, contaminated seeds and

plant materials like straw. Similar heterogeneous clustering of *Z. tritici* isolates based on mean disease severities on wheat genotypes have been reported in various studies (Sebei and Harrabi 2008; Hosseinnezhad *et al.* 2014).

Stb genes efficacy analysis indicated that Ethiopian *Z. tritici* isolates showed significantly different virulence pattern on the tested *Stb* genes. The study on known *Stb* genes revealed that isolate I_3 was found to be the most virulent isolate causing Septoria tritici blotch on Salamouni, Veranopolis, Israel-493, Tadinia, Shafir, Estanzuela Federal, Kavkaz-K4500, and Km7 that carry the resistant genes *Stb13/Stb14*, *Stb2 /Stb6*, *Stb3/Stb6*, *Stb4/Stb6*, *Stb6*, *Stb7*, Stb10/Stb12/ Stb6 /Stb7, and *Stb16*, respectively. This suggests that more studies on other *Stb* genes, gene pyramiding and adult-plant resistance test (Brown *et al*., 2015; Muqaddasi *et al.* 2019) shall be conducted to manage such broad spectrum virulent isolates. Contrastingly, the differential line Salamouni (carrying *Stb13/Stb14*) did not confer resistance to any Ethiopian *Z. tritici* isolates suggesting that it cannot be reliable source of major résistance gene. Similarly, Hosseinnezhad *et al.* (2014) reported that Salamoni is susceptible to all Iranian *Z. tritici* isolates.

In the present study Shafir (carrying *Stb6*) showed a specific resistance against some (isolate I_5 and I_7) Ethiopian *Z. tritici* isolates which contradicted to the report of Abrinbana *et al.* (2012) and Hosseinnezhad *et al.* (2014) who described Shafir as completely susceptible to *M. graminicola* in Iran. In this study, Israel 493 (carrying *Stb3* and *Stb6*) and Tadinia (carrying *Stb4* and *Stb6*) showed specific resistance against two and four Ethiopia *Z. tritici* isolates, respectively. Likewise, Hosseinnezhad *et al.* (2014) identified that these two

differential lines showed specific resistance against four and five Iranian isolates, respectively. In the present study, Shafir (carrying *Stb6*) showed resistance to isolate I_5 and I_7, while Estanzuela Federal (carrying *Stb7*) was resistant to isolate I_2 and I_7. However, Kavkaz-K4500 (carrying *Stb10 +Stb12+Stb6 +Stb7*) showed specific resistances to I_2 and I_5 which could be accounted by *Stb7* and *stb6*, respectively. This indicates that *Stb10* or *Stb12* in Kavkaz-K4500 was not effective against any of the tested *Z. tritici* isolates. Susceptibility of Kavkaz-K4500 to I_7 which both Shafir and Estanzuela Federal were resistant indicates that the later two could have unknown resistant genes which the former is lacking. Abrinbana *et al*. (2012) also observed one isolates-specific resistance in Kavkaz-K4500, and stated that this cultivar cannot be reliable source of vertical resistance genes against *M. graminicola*, although its genes can be useful for gene pyramiding to obtain cultivars with general resistance to STB.

In the present study, the differential line Km7 (carrying *Stb16*) conferred resistance to 75% of the tested *Z. tritici* isolates. The broad spectrum resistance of this genotype to Ethiopian isolates with very diverse virulence spectra indicates that *Stb16* is a broad spectrum resistant gene to Ethiopian *Z. tritici* isolates, and thus it can be a suitable source of resistance for breeders who are interested in the improvement of resistance of bread wheat to STB. In line with this, Tabib Ghaffari *et al.* (2012) confirmed that *Stb16* confers broad-spectrum resistance to *Z. tritici*. More recently, an international consortium working on this pathogen has succeeded in cloning *Stb16q*-gene and successfully introduced it into wheat (Kema, 2021). The cloned gene is identified to offer a unique broad-spectrum resistance to great majority of *Z. tritici*. It can also easily transferred to commercial wheat varieties through

breeding, and thus, in the future, growers will have less loss of harvest and less need to apply chemical fungicides against the disease (Kema, 2021). Among tested differential lines, three genotypes (Veranopolis, Tadinia and Km7) conferred resistant to ≥ 38 % of Ethiopian *Z. tritici* isolates, suggesting that these genotypes could be also proper source of major resistance genes to be deployed into the susceptible but high yielding commercial cultivars to reverse wheat yield loss due to STB.

The present study is the first of its kind to evaluate the responses of Ethiopian bread wheat cultivars to STB resistance through artificial inoculation. Among tested released wheat cultivars, 45% (Lakech, Et-13A2, Gassay, Dembal, Tay, Ogalcho, Kakaba, King Bird and Millinum) did not confer resistance to any of the tested *Z. tritici* populations suggesting that they may not be proper genotypes for cultivating in the severe epidemics of STB even if they may have other desirable agronomic characters. Sixteen (80%) of the commercial cultivars showed 0 to 2 resistance response to the tested isolates indicating that most wheat cultivars in Ethiopia are selected for other biotic and abiotic stress resistances regardless of their wide cultivations in STB hot spot areas as well. Only 20% (Alidoro, Hoggena, Lemu and Hidase) of the tested commercial cultivars showed 3 to 5 resistance responses to a representative number of *Z. tritici* isolates recovered from STB hot spots areas of the country, and thus these genotypes could be good source of major resistance genes against STB and also proper genotypes for growing in STB hot spots areas of Ethiopia. Cluster analysis based on mean disease severity clearly grouped these and some other eight bread wheat genotypes with relatively broad spectrum resistance into the same cluster (Cluster B), indicating that genotypes of this cluster could be suitable source of resistance genes for

improving bread wheat resistance to STB. In general, the study confirmed that three genotypes (MURGA, KM7 and the cultivar Hidase) which showed the greatest level of resistance to representative Ethiopian *Z. tritici* isolates could be the best sources of resistance for breeders to improve the resistance of Ethiopian bread wheat cultivars against STB.

4.5 Conclusion

In the present study, the virulence patters of eight Ethiopian *Z. tritici* isolates recovered from representative STB hot spots were explored. The study disclosed that Ethiopian *Z. tritici* isolates show specific-interactions, broad spectrum virulence patterns and greater degrees of aggressiveness on bread wheat genotypes, suggesting the need to device a resistance breeding strategy that incorporates a combination of genes (gene pyramiding) that can provide sufficient protection and durable control of the disease in the country. The detected highly virulent isolates can be used for germplasm screening in resistance breeding program. The study also confirmed that *Stb16* gene in KM7 conferred broad spectrum resistance to Ethiopian *Z. tritici* isolates, and thus, can be the best gene to be targeted by breeders to improve STB resistance in high yielding, but STB susceptible wheat cultivars. Moreover, MURGA and the cultivar Hidase which showed the greatest level of resistance to the tested *Z. tritici* isolates could be the best sources of resistance to improve the resistance of Ethiopian bead wheat cultivars against STB. In general, the study had generated valuable information on the virulence spectrum of Ethiopian *Z. tritici* populations, efficacy of known *Stb* genes, and resistance status of wheat genotypes that can help wheat breeders and

the wheat farming community in making informed decisions to manage STB. As the pathogen genome changes frequently, we also suggest continuous survey of the pathogen populations and a deep investigation in understanding the molecular mechanisms underlying the pathogen-host interactions.

CHAPTER 5

5. Molecular Screening of Septoria tritici Bloch Resistance Genes in Wheat (*Triticum aestivum* L.) using Tightly Linked SSR Markers

Abstract

Septoria tritici blotch is becoming the major bottleneck to wheat production throughout the world including Ethiopia. Resistance breeding is currently seen as the best strategy, durable, economical and environmentally friendly method to control the disease. Nowadays, molecular marker technology is widely used in plant breeding program to promote the speed and efficiency of resistance breeding. So far, several Septoria resistant genes and tightly linked simple sequence repeat markers (SSR) have been identified in wheat. These markers can serve as chromosomal land mark in hunting the resistance genes in diverse breeding materials. However, in Ethiopia, the application of molecular tools in resistance breeding is greatly missing. Therefore, the present study was aimed at searching for Septoria resistant genes (Stb genes) in 180 bread wheat genotypes using 16 tightly linked SSR markers. The result revealed greater degree of genetic variability among the tested genotypes for *Stb* genes. The genetic frequency of the resistance genes ranged from 6.67% (*Stb*12 gene) to 96.1% for stb13 gene. Three wheat genotypes (G21, G63, and G64) possessed the maximum (13) Septoria resistance genes. While 14 of the genotypes were found to have 12 *Stb* genes. Four genotypes were found to have the minimum (four) *Stb* genes. Thi study identified 17 wheat genotypes harboring 12 to 13 Septoria resistance genes. The information is very relevant for breeders in developing broad spectrum and/or durable resistant cultivars through gene pyramiding, and for fast-track release of the resistant genotypes to contribute to food and nutritional security in Ethiopia.

5.1 Introduction

Wheat (*Triticum aestivum* L.) is the second largest cereal commodity grown worldwide next to rice, with ever-increasing land allocation and production. It provides 20% of the total calories of the world's population, making the crop an important component of food security at the global level. Wheat is one of the major staple and strategic food security crops in Ethiopia (Eyob Bezabeh *et al.,* 2015). Both bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum* ssp. *durum*) are widely cultivated in Ethiopia for their multiple purposes such as for food, animal feed and income generation. In spite of its multiple importance, the average wheat productivity of the country is 2.97 t/ha; far below the global average of 3.5 t/ha (FAOSTAT, 2019). Numerous biotic and abiotic limiting factors are known to contribute to this low yield. The fungal diseases Septoria tritici blotch caused by the Zymos*eptoria tritici* (sexual state: *Mycosphaerella graminicola*) (Brown *et al.,* 2001; Ghaneie, 2012; Miedaner *et al.,* 2013) is among the major wheat production limiting factors across the world including Ethiopia (Yosef Geberehawariat*al.,* 2017).

Under sever epidemics, a global wheat yield losses of 31 – 54% have been attributed to Septoria diseases (Eyal *et al.,* 1987). The disease affects yield by causing reduced tillering, poor seed set, poor grain fill or shriveled kernels, death of leaves, spikes or the entire plant (Eyal and Levy, 1987; Ponomarenko *et al.,* 2011; Miedaner *et al.,* 2013). Losses are dependent on the growth stage of the plant at which infection occurs, level of resistance and prevailing environmental conditions. It occurs more frequently in rain-fed areas in wet season due to favorable environmental conditions for disease development.

Septoria diseases are important in warm and humid wheat growing areas of the world especially in the coastal areas of the Mediterranean, South America, and the highlands of East Africa, Australia and Western Europe (Rajaram and Dubin, 1977; Saari and Wilcoxson, 1974). The occurrence of Septoria diseases in Ethiopia has been reported by a number of researchers (Stewart and Dagnachew Yirgou, 1967; Eshetu Bekele, 1985; Getinet Gebeyehu *et at*., 1990; Ayele Badebo *et al.* 2008; Endale Hailu and Getaneh Woldeab, 2015; Abera Takele *et al.* 2015). In Ethiopia, higher (>25%) wheat production losses have been reported due to Septoria tritici blotch, and currently the disease prevalence has increased in major wheat growing areas of the country (Bekele Abate *et al*., 2011). Early-maturing and semi-dwarf wheat cultivars are most susceptible to the pathogen (van Ginkel *et al*., 1999).

Chemical control of the diseases requires more than one fungicide application, which is difficult to afford by resource poor small-scale farmers (Brading *et al*., 2002). Besides, fungicide application is a strategy that is not durable, and also has negative environmental consequences. Moreover, the high input of fungicides in combating the disease may lead to the development of fungal strains showing resistance to fungicides (Miedaner *et al.,* 2013; Vincelli, 2016). Hence, the best strategy, durable, economical and environmentally friendly method to manage wheat yield loss due to STB is using the resistant cultivars (Eyal and Levy, 1987; Eyal, 1999; Ponomarenko *et al*., 2011; Miedaner*et al*., 2013; Makhdoomi *et al*., 2015) and breeding for Septoria resistance becomes therefore a priority to improve wheat productivity in Ethiopia (Bekele Abate *et. al*., 2011).

In Ethiopia, the resistance breeding method under use is totally conventional which is very slow despite the successes so far (Wieczorek, 2003; Choudhary *eta l.,* 2008; Malav *et al.,* 2016). This calls for technological interventions that can promote the speed and effciency of resistance breeding, and hence reduce the time and cost needed to develope and release broad spectrum and/or durable resistant varieties. In this regard, the advent and applications of biotechnological tools like molecular markers techniques are already changing the global plant breeding approaches. Molecular markers are widely used for precise identification and efficient transfer of chromosomal regions underlying useful traits. DNA markers mapped to be in close linkage with genomic regions controlling important traits can serve as a chromosomal landmark to track the presence or introgression of the target regions in breeding materials. Of the several marker systems, simple sequence repeats (SSR) (Rafalski, 2002) and single nucleotide polymorphism (SNP) (Zhu, 2003) are the most preferable marker system widely used for genome assisted resistance breeding.

Septoria resistance genes also called *Stb* – genes are among the most remarkable examples of wheat genes that are mapped to be in close linkage to several microsatellite markers. So far, about 18 (*Stb 1-18)* major (R) resistant genes to Septoria tritici blotch and closely linked microsatellite markers have been identified in wheat (Ghaffary *et al.,* 2011a; Simón *et al.,* 2012). These include: *Stb1* (Adhikari *et al.,* 2004a), *Stb2*, *Stb3* and *Stb4* (Adhikari *et al.,* 2004b,c), *Stb5* (Arraiano *et al.,* 2001), *Stb6* (Brading *et al.,* 2002), *Stb7* (McCartney *et al.,* 2003), *Stb8* (Adhikari *et al.,* 2003), *Stb9* (Chartrain *et al.,* 2009), *Stb10*, *Stb11* and *Stb12* (Chartrain *et al.,* 2005a; b), *Stb13* and *Stb14* (McIntosh *et al.,* 2007), *Stb15* (Arraiano and

Brown, 2007), *Stb17* (Ghaffary *et al.,* 2012) and *Stb16, Stb18* (Ghaffary *et al.,* 2011a; b). Hence, the tightly linked SSR markers can be used as chromosomal landmark for hunting the resistant genes in diverse wheat breading materials or gene bank collections (Singh *et al.,* 2015).

In Ethiopia, most of the released bread wheat cultivars are susceptible to septoria tritici blotch (Abera Takele *et al.,* 15). Moreover, the resistance breeding method underuse is quite traditional which made the crop improvement process very slow and inefficient. The use of modern molecular tools in assessing the resistance genes in the available wheat gene pool and their transfer into the susceptible but high yielding cultivars or for the development of new cultivars with durable resistance is greatly missing. Therefore, the present study was aimed at marker assisted searching of Septoria resistant (*Stb*) genes in 180 bread wheat genotypes using tightly linked SSR markers to initiate genome-assisted resistance breeding in Ethiopia. The information is very relevant for wheat breeders to integrate up-to-date technology in wheat breeding for fast development of high yielding and broad spectrum Septoria disease resistant wheat varieties.

5.2 Materials and Methods

Plant Materials

The study involved 180 bread wheat genotypes (167 recombinant inbred lines and 13 commercial bread wheat cultivars) (Appenix II). The inbred lines were obtained from the International Maize and Wheat Improvement Center, (CIMMYT) while the commercial

cultivars were obtained from Holetta and Kulumsa Agricultural research centers, Ethiopia. Genotyping was conducted at International Center for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India in 2016 with the support of Non-aligned and Other Developing Countries (NAM S&T Centre) under its program Research Training Fellowship for Developing Country Scientists.

Genotyping

Genomic DNA was extracted from greenhouse grown 2-week-old pooled leaf samples from five plants per line using modified CTAB procedure (Ogbonnaya *et al.*, 2001). The extracted DNA concentration and quality were checked with Nanodrop Spectrophotometer and on 1% agarose gel at 100V for 1 hr, respectively. For genotyping, 16 SSR markers (Table 5.1) closely linked to the respective *Stb* genes were used. The PCR program was set as described by Tilahun Mekonnen *et al.* (2017) with some modifications. Accordingly, the PCR reaction was carried out in 25 µl reaction mixture that composed of 10×PCR buffer with $MgCl_2$, 10 mM dNTPs, 0.4 µM each of forward and reverse primers (Table 5.2), 1 µl dimethyl sulfoxide (DMSO), 1 µl Taq DNA polymerase (5 Units), 1 µl template DNA and 16.5 µl double distilled sterile H_2O using a thermal cycler (Applied Biosystems Veriti 96 Well Thermal Cycler, Thermo Fisher Sciuentific, USA). Primer annealing temperature was optimized using gradient program.

Table 5. 1 List of STB resistance genes and closely linked molecular markers in wheat.

Locus/Gene	Marker	Linkage	Chr
Stb1	*barc74*	2.7 cM prox	5BL
Stb1	*gwm335*	7.4 cM prox	5BL
Stb2	*barc008*	5 cM	1BS
Stb2	*wmc230*	Tight	1BS
Stb2	*wmc406*	6 cM	1BS
Stb3	*wmc83*	3cM	7A
Stb4	*gwm111*	0.7cM	7DS
Stb5	*gwm44*	6-7cM	7DS
Stb6	*gwm369*	Flanking	3AS
Stb7/Stb12	*wmc313*	0.3cM distal	4AL
Stb7/Stb12	*wmc219*	0.8cM distal	4AL
Stb8	*gwm146*	3.5cM	7BL
Stb8	*gwm577*	5.3 cM	7BL
Stb9	*wmc317*	7cM	6AS
Stb10	*gwm848*	Flanking	1D
Stb11	*barc008*	Flanking	1BS
Stb11	*barc137*	Flanking	1BS
Stb13	*wmc396*	7-9cM	7B
Stb14	*wmc623*	5cM	3B
Stb14	*wmc500*	2cM	3B
Stb16	*wmc494*	1-5 cM	3D
Stb17	*hbg247*	1-5 cM	5A
Stb18	*gpw5176*	1-5 cM	6DS
Stb18	*gpw3087*	1-5 cM	6DS

Note*: Stb 1-18= Septoria tritici blotch resistance genes*. A, B, and D = A, B and D genomes of bread wheat, S= short arm, and L= long arm of a chromosome (Simon *et al.*, 2012*).*

Table 5. 2 List of SSR primers used to target *Stb* genes of wheat.

Stb genes	Oligo Name	Sequence 5' to 3'	Expected PCR product size (bp)
Stb1	barc74 F	5'GCGCTTGCCCCTTCAGGCGAG3'	175
	barc74 R	5'CGCGGGAGAACCACCAGTGACAGAGC3'	
Stb2	gwm389 F	5'ATCATGTCGATCTCCTTGACG3'	117
	gwm389 R	5'TGCCATGCACATTAGCAGAT3'	
Stb3	wmc83 F	5'TggAggAAAcAcAATggATgcc3'	160
	wmc83 R	5'gAgTATcgccgAcgAAAgggAA3'	
Stb4	gwm111 F	5'TCTGTAGGCTCTCTCCGACTG3'	206
	gwm111 R	5'ACCTGATCAGATCCCACTCG3'	
Stb5	gwm44 F	5'GTTGAGCTTTTCAGTTCGGC3'	178
	gwm44 R	5'ACTGGCATCCACTGAGCTG3'	
Stb6	gwm369 F	5'CTGCAGGCCATGATGATG3'	184
	gwm369 R	5'ACCGTGGGTGTTGTGAGC3'	
Stb7/12	gwm313 F	5'gcAgTcTAATTATcTgcTggcg3'	197
	gwm313 R	5'gggTccTTgTcTAcTcATgTcT3'	
Stb12	wmc219 F	5'TgcTAgTTTgTcATccgggcgA3'	204
	wmc219 R	5'cAATcccgTTcTAcAAgTTccA3'	
Stb8	gwm146 F	5'CCAAAAAAACTGCCTGCATG3'	174
	gwm146 R	5' CTCTGGCATTGCTCCTTGG3'	
Stb9	wmc317 F	5'TgcTAgcAATgcTccgggTAAc3'	139
	wmc317 R	5'TcAcgAAAccTTTTccTccTcc3'	
Stb11	barc137 F	5'GGCCCATTTCCCACTTTCCA3'	260
	barc137 R	5'CCAGCCCCTCTACACATTTT3	
Stb13	wmc396 F	5'TgcAcTgTTTTAccTTcAcggA3'	146
	wmc396 R	5'cAAAgcAAgAAccAgAgccAcT3'	
Stb14	wmc623 F	5'ACGATTGGCCACAGAGGAG3'	192
	wmc623 R	5'CAGTGACCAATAGTGGAGGTCA3'	
Stb16	wmc494 F	5'ggATcgAgTcTcAAgTcTAcAA3'	218
	wmc494 R	5'AgAAggAAcAAgcAAcATcATA3'	
Stb17	hbg247 F	5'acatgcggggatgatgattt3'	259
	hbg247 R	5'gcggacccatgataaaatgtct3'	
Stb18	gpw3087 F	5'CTCTAAGAAGTCCAATGCAACA3'	166
	gpw3087R	5'AATTGCAGAAACTGGATGCC3'	

Note: *Stb* = Septoria tritici blotch resistant genes of wheat, F=Forward primer and R= Reverse primer

PCR amplification program involved an initial denaturation at 94°C for 3 min followed by 45 cycles with 1 min denaturation at 94°C, optimized annealing temperature for each primer at 1minute, primer extension at 72°C for 2 min followed by final extension step of 10 min at

72°C. The PCR product was fractionated in 3% agarose gel electrophoresis using 1× TAE buffer at 100V for 3 h. The gel was stained with ethidium bromide and visualized under UV light and subsequently photographed. A 50 bp ladder was used to determine the amplification size.

5.3 Results

Allelic diversity of Septoria Resistance (*Stb*) Genes in Bread Wheat

The results of SSR based genotypic screening of the 180 bread wheat lines for the presence or absence of 16 major *Septoria tritici* resistance genes are indicated in Table 5.3. Appendix III also shows the electrophoresis patterns of the SSR markers linked to the resistance *Stb* genes for few of the markers and wheat lines. The presence of the resistance genes were estimated based on their respective expected fragment size on the gel. Accordingly, presence of the 16 *Septoria* resistance genes (*Stb1, Stb2, Stb3, Stb4, Stb5, Stb6, Stb7, Stb12, Stb8, Stb9, Stb11, Stb13, Stb14, Stb16, Stb17* and *Stb*18) were determined by observing the PCR product on a 3% agarose gel at the expected size of 175bp, 117 bp, 160 bp, 206 bp, 178bp, 184 bp, 197bp, 204bp, 174 bp, 139bp, 260bp, 146bp, 192bp, 218 bp, 259 bp and 166 bp, respectively. Figure 5. 1 also presents summary of *Stb* genes distribution (%) among the tested 180 bread wheat genotypes.

A greater degree of variation was observed among the tested genotypes. The genetic frequency of *Stb* genes ranged from 6.67% to 96.1, and *Stb13* was the most widely

distributed gene occurring in 96.1% of the tested genotypes. It was followed by *Stb5* (92.22%*), Stb3* (85%), *Stb2* (82.77%*), Stb9* (73.33 %*), Stb8* (66.11%*), Stb14* (58.33%*), Stb7* (56.7%*), Stb4* (55.56%*), Stb18* (54.44%*), Stb6* (47.22%*), Stb17* (43.89%*), Stb16* (26.11%*), Stb11* (25%), *Stb1* (19.44%*) and Stb12* (6.67%*).* It was observed that three genotypes (G21, G63, and G64) contain 13 of the resistance genes while 14 of the genotypes possessed 12 of the resistance genes, and 19 genotypes had 11 genes, 29 of the genotypes had 10 *Stb* genes, 36 lines possessed 9 genes, 30 genotypes possessed 8 genes, 32 genotypes possessed 7 genes, 12 genotypes had 6 genes and the remaining four genotypes possessed 5 of the major *Stb* genes (Table 5.3 and Appendix III).

Table 5. 3 Result of 180 bread wheat lines screened for STB resistance gene using tightly linked SSR markers

Bread wheat Genotype		*Septoria tritici* resistance genes																
		Stb1	*Stb2*	*Stb3*	*Stb4*	*Stb5*	*Stb6*	*Stb7*	*Stb12*	*Stb8*	*Stb9*	*Stb11*	*Stb13*	*Stb14*	*Stb16*	*Stb17*	*Stb18*	Total Number of *Stb genes*
Name	Code	barc74	gwm389	wmc83	gwm111	gwm44	gwm369	gwm313	wmc219	gwm146	wmc317	barc137	wmc396	wmc623	wmc494	hbg247	gpw3087	
EBW001	G1	0	0	1	1	1	1	0	1	1	0	0	1	1	1	1	0	10
EBW002	G2	1	0	1	1	1	0	1	0	0	1	0	1	1	0	1	0	9
EBW003	G3	1	1	1	1	1	0	1	0	1	0	0	1	0	1	1	0	10
EBW004	G4	0	1	1	0	1	0	1	0	0	0	0	1	1	0	0	1	7
EBW005	G5	0	1	0	1	1	1	0	0	1	1	0	1	0	0	1	1	9
EBW006	G6	0	1	1	1	1	1	1	0	1	1	0	1	1	0	0	1	11
EBW007	G7	0	0	1	0	1	1	1	0	1	1	0	1	0	0	0	1	8
EBW008	G8	1	0	0	0	1	1	0	0	1	1	0	1	1	0	1	1	9
EBW009	G9	0	0	1	1	1	0	1	0	1	0	0	1	0	0	0	0	6
Bika	G10	0	1	1	0	1	0	1	0	1	1	0	0	1	0	0	1	8
EBW011	G11	0	1	1	1	1	0	0	0	1	0	0	1	1	0	1	1	9
EBW012	G12	0	1	1	0	1	0	1	0	1	1	1	1	0	0	0	1	9
EBW013	G13	0	1	1	1	1	1	0	1	1	1	0	1	1	1	0	0	11
Meraro	G14	0	0	0	0	1	1	1	0	1	0	1	1	0	1	1	0	8
EBW015	G15	0	1	1	1	1	1	1	0	1	0	0	1	1	0	1	0	10
EBW016	G16	0	1	1	0	1	0	1	0	1	1	0	1	0	0	0	1	8
EBW017	G17	0	0	1	0	1	1	1	0	0	0	1	1	0	1	1	0	8
EBW018	G18	0	1	1	1	1	0	0	0	0	0	0	1	1	0	0	1	7
EBW019	G19	0	1	1	1	1	0	0	0	1	0	0	1	0	1	0	1	8
EBW020	G20	0	1	1	0	1	0	1	0	1	0	0	1	1	1	0	1	9
Alidoro	G21	0	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	13
Medawolabu	G22	0	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	12
EBW023	G23	0	1	1	0	1	1	1	0	1	1	0	1	1	0	1	0	10
EBW024	G24	0	1	1	1	1	1	0	0	1	1	0	1	0	0	0	1	9
EBW025	G25	0	0	1	0	1	0	1	0	1	0	0	1	1	0	0	1	7
EBW026	G26	0	1	1	0	1	1	0	0	0	1	0	1	1	1	1	1	10
EBW027	G27	0	0	1	0	1	0	0	0	1	1	0	1	1	0	0	1	7

Table 5.3 Continued

EBW028	G28	0	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	12
EBW029	G29	0	1	1	1	1	1	1	0	1	1	0	1	1	0	0	1	11
EBW030	G30	0	1	1	1	1	1	1	0	1	0	0	1	1	0	0	0	9
EBW031	G31	0	1	0	0	1	1	0	0	1	0	0	1	0	1	1	0	7
EBW032	G32	1	1	1	1	0	0	1	0	1	1	0	1	0	0	1	0	9
Huluka	G33	0	0	1	1	1	0	1	0	1	0	0	1	0	0	0	0	6
EBW034	G34	0	1	1	1	1	1	0	0	0	0	1	1	0	0	0	0	7
EBW035	G35	0	1	1	0	1	1	1	0	1	0	0	1	0	0	0	1	8
EBW036	G36	0	1	1	1	1	1	0	0	1	1	0	1	1	1	0	1	11
EBW037	G37	1	1	1	1	1	0	1	0	1	1	0	1	0	0	0	0	9
EBW038	G38	0	1	0	0	1	1	0	0	1	1	0	1	1	1	1	1	10
EBW039	G39	0	1	1	1	1	0	0	0	1	1	1	0	1	1	1	1	11
King-bird	G40	1	1	1	1	1	0	1	0	1	0	1	1	0	0	1	0	10
EBW041	G41	0	1	1	1	1	0	0	1	1	1	0	1	1	0	0	1	10
EBW042	G42	0	1	0	0	1	1	0	0	0	1	0	1	1	1	1	0	8
EBW043	G43	0	1	1	0	1	1	1	0	1	1	0	1	1	0	0	1	10
EBW044	G44	0	1	1	1	1	0	1	0	1	1	0	1	1	0	1	0	10
EBW045	G45	0	1	0	0	1	0	1	0	1	0	0	1	1	0	1	1	8
EBW046	G46	0	1	1	1	1	0	1	0	1	1	1	1	0	1	0	1	11
EBW047	G47	0	1	1	0	1	0	1	0	1	0	1	1	1	0	1	0	9
EBW048	G48	0	1	0	0	1	1	0	0	1	1	0	1	0	0	1	0	7
EBW049	G49	0	1	1	0	1	0	1	0	0	1	0	1	0	0	0	1	7
EBW050	G50	0	1	0	0	1	0	1	0	0	1	1	1	0	1	0	1	8
EBW051	G51	1	1	1	0	1	1	0	0	0	1	0	1	1	0	1	0	9
EBW052	G52	0	1	0	1	1	0	0	0	0	0	0	1	1	0	0	0	5
EBW053	G53	0	1	1	1	0	0	1	0	1	1	0	1	1	1	1	0	10
EBW054	G54	0	1	1	1	1	1	1	0	1	1	0	1	0	0	0	0	9
EBW055	G55	0	1	1	0	1	0	0	0	1	0	0	1	1	0	0	1	7
EBW056	G56	0	1	1	1	1	0	0	0	0	1	0	1	0	0	0	0	6
EBW057	G57	0	1	1	0	1	0	0	0	1	0	0	1	0	0	0	0	5
EBW058	G58	0	1	1	0	1	0	1	0	1	0	0	1	1	0	1	1	9
EBW059	G59	0	0	1	1	1	1	0	1	1	0	1	1	0	0	1	0	9
EBW060	G60	0	1	1	1	1	0	1	0	1	1	0	1	0	0	0	0	8
EBW061	G61	1	1	1	0	1	0	1	0	0	1	1	1	1	0	0	0	9
EBW062	G62	0	0	0	1	0	0	1	0	1	1	1	1	1	1	1	1	10
EBW063	G63	1	1	1	1	1	0	1	0	1	1	1	1	1	0	1	1	13
EBW064	G64	0	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	13
EBW065	G65	0	0	1	0	1	0	0	0	1	1	0	1	1	0	0	0	6
EBW066	G66	1	0	1	1	1	0	0	0	1	0	0	1	1	0	0	0	7
EBW067	G67	1	1	0	1	0	0	1	0	1	1	1	1	1	0	1	0	10
EBW068	G68	0	1	1	0	1	0	1	0	0	0	0	1	0	0	1	0	6
EBW069	G69	0	1	0	1	1	1	1	0	0	1	1	1	1	0	0	0	9
EBW070	G70	1	1	1	1	1	1	1	0	1	1	0	1	1	0	0	1	12
Danda'a	G71	0	1	1	1	0	0	1	0	0	1	0	1	1	0	0	1	8
EBW072	G72	0	0	1	1	1	0	0	1	0	0	0	1	1	1	1	1	9
EBW073	G73	0	1	1	0	1	0	1	0	0	1	0	1	0	0	0	1	7
EBW074	G74	0	1	1	0	1	0	1	0	0	1	0	1	0	0	0	0	6
EBW075	G75	0	1	0	0	1	0	0	0	0	1	0	1	0	1	1	1	7
EBW076	G76	0	1	1	1	1	0	0	0	0	1	0	1	1	0	0	1	8
EBW077	G77	1	1	1	1	1	0	0	0	0	1	0	1	0	1	1	1	10
EBW078	G78	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0	1	5
EBW079	G79	0	1	1	1	1	0	0	0	0	0	0	1	0	1	0	0	6
Et-13A2	G80	0	0	1	1	1	1	0	0	0	1	0	1	1	0	0	0	7
EBW081	G81	0	0	1	1	1	0	0	0	0	0	0	1	1	0	0	1	6
EBW082	G82	0	0	1	0	1	0	0	0	0	0	0	1	1	0	0	1	5
EBW083	G83	0	1	1	1	1	1	0	0	0	0	0	1	0	0	0	1	7
EBW084	G84	0	1	1	1	1	0	0	0	1	0	1	1	0	1	0	0	8
EBW085	G85	0	1	0	1	1	0	0	0	1	0	0	1	1	0	1	1	8
EBW086	G86	0	1	1	1	1	1	0	0	0	0	0	1	0	0	1	0	7
EBW087	G87	0	1	0	1	1	1	0	0	0	0	0	1	1	1	0	0	7
EBW088	G88	0	0	1	0	1	1	0	0	1	0	0	1	1	0	0	1	7
EBW089	G89	0	1	1	1	1	0	0	0	1	1	1	1	0	0	0	1	9
EBW090	G90	0	1	1	1	1	1	0	0	1	0	0	1	0	0	0	0	7
EBW091	G91	0	0	1	0	1	1	1	0	0	1	0	1	1	0	0	1	8
EBW092	G92	0	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	12

Table 5.3 Continued

EBW093	G93	0	1	0	1	0	0	1	0	1	1	0	1	0	0	1	0	7
EBW094	G94	0	1	1	1	1	1	1	0	1	1	0	1	1	1	0	1	12
EBW095	G95	0	1	1	0	1	1	0	0	0	1	0	1	1	0	0	1	8
EBW096	G96	0	1	1	1	0	0	1	0	0	1	0	0	1	0	0	1	7
EBW097	G97	0	1	1	0	1	1	0	0	0	0	0	1	1	0	1	0	7
EBW098	G98	0	1	1	0	1	1	0	0	0	1	0	1	1	0	0	0	7
EBW099	G99	0	1	1	0	1	1	0	0	0	1	0	1	1	0	0	1	8
EBW100	G100	0	1	0	1	0	0	1	0	1	1	0	1	0	0	1	0	7
EBW101	G101	0	1	1	0	1	1	1	0	0	1	1	1	1	0	1	1	11
EBW102	G102	0	1	0	1	0	0	1	0	0	1	1	1	1	1	1	0	9
EBW103	G103	0	1	1	1	0	1	0	0	0	1	0	1	0	0	0	0	6
EBW104	G104	1	1	1	0	1	0	0	1	0	1	1	1	0	0	1	0	9
EBW105	G105	1	1	1	1	1	0	1	0	0	1	0	1	0	0	1	0	9
ETBW106	G106	0	1	1	1	1	0	1	0	1	1	0	1	1	1	0	1	11
EBW107	G107	1	1	1	1	1	0	1	0	1	0	0	1	0	1	0	0	9
EBW108	G108	1	1	1	1	1	1	1	0	1	0	0	1	1	0	0	0	10
EBW109	G109	0	1	0	1	1	1	1	0	1	1	0	1	1	0	1	1	11
EBW110	G110	0	1	0	1	1	1	1	0	1	1	0	1	1	0	1	1	11
EBW111	G111	1	1	0	0	1	1	1	0	0	1	0	1	1	0	0	1	9
EBW112	G112	0	1	1	0	1	0	1	0	1	1	0	1	0	0	0	1	8
EBW113	G113	1	1	1	0	1	0	1	0	0	1	1	1	1	1	0	0	10
EBW114	G114	1	1	1	1	1	0	1	0	1	1	0	1	1	1	0	1	12
EBW115	G115	0	1	1	1	1	0	0	0	0	1	0	1	0	1	0	1	8
EBW116	G116	0	0	1	0	1	0	0	0	0	1	0	1	1	0	0	1	6
EBW117	G117	0	1	1	0	1	1	1	0	1	1	0	1	1	1	1	0	11
EBW118	G118	1	1	1	1	1	1	1	0	0	1	1	1	1	0	0	1	12
EBW119	G119	0	1	0	0	1	0	0	1	1	1	0	1	1	0	0	1	8
EBW120	G120	0	1	1	1	1	1	0	1	1	1	0	1	0	0	0	1	10
EBW121	G121	0	1	1	1	1	0	0	0	1	1	0	1	0	1	0	1	9
EBW122	G122	0	1	1	1	1	0	0	0	1	1	0	1	0	0	1	0	8
EBW123	G123	0	1	0	1	1	1	1	0	1	1	0	1	1	0	0	0	9
EBW124	G124	0	1	1	1	1	1	1	0	1	0	0	1	1	0	1	1	11
EBW125	G125	0	1	1	0	1	1	1	0	1	0	1	1	1	1	1	0	11
EBW126	G126	0	1	1	0	1	1	1	0	0	1	0	1	1	0	0	0	8
Paven-76	G127	0	0	1	0	1	1	0	1	1	1	1	1	1	0	1	1	11
EBW128	G128	0	1	1	0	1	0	0	0	1	1	0	1	0	0	0	0	6
EBW129	G129	0	1	1	0	1	0	0	0	0	1	0	1	0	1	0	1	7
EBW130	G130	1	1	1	0	1	1	1	0	0	1	1	1	0	0	1	1	11
EBW131	G131	1	1	1	0	1	0	1	0	1	1	0	1	0	0	0	0	8
EBW132	G132	0	1	1	0	1	0	1	0	1	1	0	1	0	0	0	1	8
EBW133	G133	0	1	1	1	1	1	1	0	1	1	1	0	0	0	1	0	10
EBW134	G134	0	1	1	0	1	0	1	0	1	0	0	1	0	0	0	1	7
EBW135	G135	1	1	1	1	1	1	1	0	1	1	0	1	0	0	0	1	11
EBW136	G136	0	1	1	1	1	1	0	0	0	1	0	1	0	0	1	1	9
EBW137	G137	0	1	1	0	1	0	0	0	1	1	0	1	1	0	0	0	7
EBW138	G138	0	1	1	0	1	1	1	0	1	1	1	1	1	1	1	0	12
EBW139	G139	0	1	1	0	1	0	1	0	1	1	0	1	0	0	0	0	7
EBW140	G140	0	1	1	1	1	0	1	0	1	1	0	0	1	0	0	0	8
EBW141	G141	0	1	1	0	1	1	0	0	1	1	0	1	1	0	0	1	9
EBW142	G142	0	0	1	1	1	1	0	0	0	1	0	1	1	0	1	1	9
EBW143	G143	0	1	0	1	0	1	1	0	1	1	0	1	1	0	0	1	9
EBW144	G144	1	1	1	1	1	0	0	0	1	1	1	1	1	0	1	1	12
EBW145	G145	0	1	1	1	1	1	1	0	1	1	0	1	1	0	1	1	12
EBW146	G146	1	1	1	0	1	0	1	0	1	1	0	1	1	0	0	0	9
EBW147	G147	0	1	1	1	1	0	1	0	1	1	0	1	0	0	1	0	9
EBW148	G148	0	1	1	0	1	1	0	0	1	0	0	1	0	0	1	0	7
Sofomor	G149	0	1	1	0	0	0	1	0	1	1	0	1	1	0	1	0	8
EBW150	G150	1	1	1	1	1	0	0	0	0	1	0	1	1	1	0	1	10
EBW151	G151	0	1	1	1	1	1	1	0	0	1	0	1	0	0	1	1	10
KI6295-4A	G152	1	1	1	0	1	0	0	0	1	1	1	1	1	1	1	1	12
EBW153	G153	0	1	1	1	1	1	1	0	0	1	1	1	0	0	1	0	10
EBW154	G154	0	0	1	0	1	1	1	0	1	0	0	1	1	0	1	1	9

Table 5.3 Continued

EBW155	G155	1	1	1	0	1	0	1	0	1	1	1	1	1	1	0	1	12
EBW156	G156	0	1	1	0	1	0	1	0	0	1	0	1	0	0	1	1	8
EBW157	G157	0	0	1	1	1	0	0	0	1	1	0	1	1	0	0	1	8
EBW158	G158	0	1	1	0	1	0	1	0	0	0	0	1	1	0	1	0	9
EBW159	G159	0	1	1	1	1	1	0	1	0	1	1	1	1	0	0	0	11
EBW160	G160	0	0	0	1	1	0	0	0	0	1	0	1	1	0	0	1	9
EBW161	G161	0	1	1	0	1	1	1	0	1	1	1	1	0	0	0	0	10
EBW162	G162	0	1	1	1	1	1	1	0	1	1	0	1	0	0	1	0	11
EBW163	G163	0	1	1	1	1	1	1	0	1	1	0	1	0	0	0	0	11
EBW164	G164	0	0	1	1	1	1	0	0	1	1	0	1	0	0	0	1	9
EBW165	G165	1	1	1	1	1	1	1	0	0	1	0	1	0	0	0	1	8
EBW166	G166	0	1	1	1	1	1	1	0	0	1	1	1	0	1	1	1	10
EBW167	G167	0	0	1	0	1	1	1	0	0	1	0	0	0	1	1	0	12
EBW168	G168	1	1	1	0	1	1	1	0	1	1	1	1	1	0	1	1	8
EBW169	G169	0	1	1	0	1	1	0	0	1	1	1	1	1	0	0	1	6
EBW170	G170	1	1	1	1	1	0	0	0	1	1	0	1	0	1	0	1	11
EBW171	G171	0	1	0	0	1	1	0	1	1	1	0	1	1	0	1	0	12
EBW172	G172	1	1	1	0	1	0	0	0	1	1	0	1	0	0	0	0	8
K-6290-Bulk	G173	0	1	1	1	1	1	0	0	1	1	1	1	1	0	1	0	10
EBW174	G174	0	1	1	1	0	1	1	0	1	1	0	1	1	1	1	1	9
Hogana	G175	0	1	1	0	1	0	1	0	1	1	1	1	1	0	1	0	8
EBW176	G176	0	1	1	1	1	1	1	0	1	1	0	1	1	0	0	1	9
EBW177	G177	0	1	1	1	1	0	1	0	1	1	0	1	1	0	1	0	11
EBW178	G178	1	1	1	1	0	0	1	0	1	1	1	1	0	0	1	1	11
EBW179	G179	0	0	1	0	1	0	0	0	1	1	1	1	1	0	0	0	8
EBW180	G180	0	1	1	1	1	1	1	0	1	1	1	1	0	0	0	0	11
Frequency (%)		19.4	82.8	85	55.6	92.2	47.2	56.7	6.67	66.1	73.3	25	96.1	58.3	26.1	43.9	54.4	
Appr. size (bp)		175	117	160	206	178	184	197	204	174	139	260	146	192	218	259	166	

Note: Septoria tritici blotch resistance genes in bread wheat genotypes scored as the presence (1) or absence (0) of amplicon linked to 16 of allele specific SSR markers.

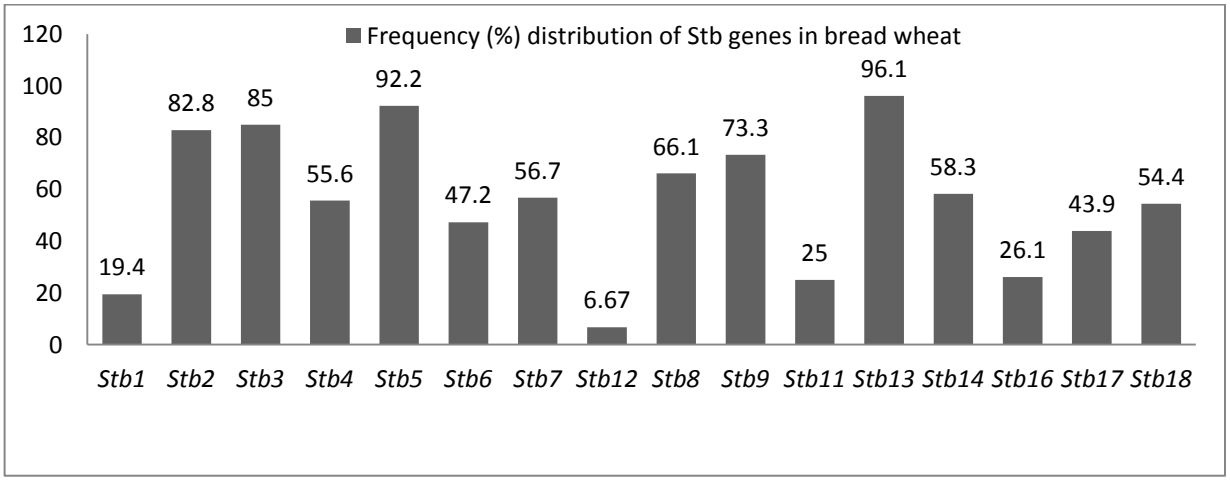

Figure 5. 1 Summary of *Stb* genes distribution (%) in 180 bread wheat genotypes

Screening for the *Stb* genes

PCR based screening of *Stb1* gene on chromosome 5BL revealed that only 35 of the tested genotypes resulted in positive band size of 175 bp confirming presence of the resistance gene in those genotypes. It was observed that 145 (80.56%) of the genotypes did not show product of the expected size by the same marker confirming the absence of the *Stb1* gene in close vicinity. *Stb1* was found to be the most rarely (19.44%) distributed gene among the genotypes next to *Stb12* (6.67%). Similarly, PCR amplification of the SSR marker *gwm389* resulted in a band size of 117 bp in 149 (82.8%) genotypes indicating presence of *Stb2* gene in close linkage. Lack of the expected product size in 31 of the study materials reveled absence of the resistance gene in them.

Moreover, screening for *Stb3* found on chromosome 7A (3cM) using the SSR marker *wmc83* resulted in positive band size of 160 bp in 153 wheat genotypes. Whereas, scanning for *Stb4* on chromosome 7DS (0.7cM) using *gwm111* marker resulted in the expected product size of 206 bp in 100 (55.6 %) genotypes implying the presence of the target gene. Similarly, screening for *Stb5* using *gwm44* (on chromosome 7DS) had produced positive band size of 178 bp. It was observed that 92.2% of the study materials amplified the marker confirming presence of the gene in nearby, while 14.04% of the genotypes didn't produce fragment of the expected size to show absence of the gene. Assessment for S*tb6* genes using the primer sequence targeting *gwm369* marker on (on chromosome 3AS) also resulted positive band size of 184 bp in 47.2 % of the genotypes implying their possession of the desired gene.

Screening for the closely located resistance genes *Stb7* and *Stb12* using the SSR marker *gwm313* and *wmc219* produced positive amplification size of 197 bp and 204 bp in 102 and 12 genotypes, respectively. No genotype resulted in positive amplicon of the expected size for the two markers. Also it was observed that 66 of the genotypes did not amplify either of the two markers indicating their absence in the study materials. *Stb12* was found to be the least (6.67%) frequently distributed gene among the study materials. Presence of the Septoria resistance genes *Stb8* on chromosome 7BL (5.3cM) and *Stb9* on chromosome 6AS (7cM) were identified in 119 and 132 wheat genotypes using the closely linked SSR markers *gwm146* and *wmc317*, respectively. The positive band size of 174bp by *gwm146* confirmed the presence of *Stb8* gene in close vicinity on the same chromosome, while an amplicon of 139 bp size by *wmc317* indicated the presence of *Stb9* gene. The analysis showed that 87 of the wheat genotypes resulted in positive band for both genes, while 16 genotypes did not amplify any of these genes. Moreover, PCR amplification of the SSR marker *barc137* produced amplicon size of 260 bp in 45 genotypes to confirm the presence of *Stb11* on chromosome 1B. The analysis revealed that screening of the study materials for *Stb13* using *wmc396* resulted in positive band of 146 bp in 173 genotypes. It was observed that 42 of the lines amplified both SSR markers, 134 lines amplified only one of the markers, while the remaining 4 didn't amplify any of the two SSR markers which showed negative for the two genes.

Assessment with the microsatellite marker *wmc623* resulted in positive amplicon size of 192 bp in 105 genotypes to indicate the presence of the resistance gene *stb14* on chromosome

3B. While scanning the genotypes with *wmc494* marker gave positive band size of 218 bp in 47 genotypes confirming presence of *Stb16* gene on chromosome 3D. It was found that 29 of the genotypes resulted in positive amplification with both markers, while 94 of the materials didn't show any amplification indicating the absence of both genes. Similarly, screening for S*tb17* and *Stb18* genes using the tightly linked SSR markers hbg247 and gpw3087 resulted in positive amplification size of 259 bp and 166 bp in 79 and 98 genotypes, respectively, confirming presence of the genes. The analysis indicated that 37 genotypes amplified both markers to indicate presence of the two resistance genes in the same materials, while 40 lines did not amplify either of the two markers confirming their absence.

5.4 Discussion

Septoria tritici blotch is the major bottleneck to wheat production across the world including Ethiopia. Because of its various undesirable effects, currently the interest on the use of chemicals against the disease is decreasing. Resistance breeding is the preferred and durable strategy to control biotic stresses including Septoria disease. Nowadays, molecular markers are widely used in wheat breeding programs to facilitate the identification and deployment of the resistance genes. DNA markers tightly linked (< 5cM) to agronomically useful genomic regions serve as a chromosomal land marks, "signs" or "flags" to track the presence or introgression of the target gene controlling the desired trait in breeding materials. In this study, 180 bread wheat lines were screened for the possible presence of 16 STB resistance genes namely *Stb1, Stb2, Stb3, Stb4, Stb5, Stb6, Stb7, Stb12, Stb8, Stb9,*

Stb11, Stb13, Stb14, Stb16, Stb17 and Stb18 using closely linked SSR markers *barc74, gwm389, wmc83, gwm111, gwm44, gwm369, gwm313, wmc219, gwm146, wmc317, barc137, wmc396, wmc623, wmc494, hbg247* and *gpw3087,* respectively.

The study revealed presence of significant genetic variability among the tested bread wheat genotypes for their possession of *Stb genes.* The *Stb* genes genetic frequency ranged from 6.67% to 96.1, and *Stb13* was found to be the most widely distributed gene occurring in 96.1% of the tested genotypes. It was followed by *Stb5* (92.22%*), Stb3* (85%), *Stb2* (82.77%*), Stb9* (73.33 %*), Stb8* (66.11%*), Stb14* (58.33%*), Stb7* (56.7%*), Stb4* (55.56%*), Stb18* (54.44%*), Stb6* (47.22%*), Stb17* (43.89%*), Stb16* (26.11%*), Stb11* (25%*), Stb1* (19.44%*) and Stb12* (6.67%*).* Similarly, Singh *et al.* (2015) reported a genetic frequency of 19.79% to 54.69% in 192 for blast resistance genes using 10 SSR markers liked to the blast resistance genes. This implies that molecular markers are very helpful to assess the presence of the target genomic regions controlling useful agronomic traits in large sets of germplasm collections.

5.5 Conclusion

Septoria tritici blotch is one of the major wheat production limiting factors in Ethiopia and worldwide. Host resistance is the most durable and economical method to control the disease, and hence, breeding efforts for developing new resistant cultivars continue to be a priority for wheat breeding programs. Identification of proper resistance source is prerequisite for resistance breeding. The predominant traditional disease assays to evaluate

resistance source are less precise, laborious and time-consuming. Nowadays, the advent of molecular marker technology and their applications in plant breeding offer breeders to directly select genotypes carrying target genes using tightly linked molecular markers. In the present study, 16 microsatellite markers finely mapped with *Stb* genes in wheat were used to screen the presence of STB resistance genes in 180 bread wheat genotypes. The study has identified several major Septoria resistance genes. About 17 bread wheat genotypes were detected to harbor about 12 to 13 *Stb* genes. The information is very relevant for the development of durable and/or broad-spectrum Septoria resistant varieties through gene pyramiding. Moreover, genotypes identified to have many *Stb* genes could also be advanced for multi-location yield trial test for fast-track release leading to increased wheat production and productivity in the country. Therefore, the technique used in the present study has offered an efficient, straightforward, economical and fast way to tag Septoria resistance genes in the available wheat materials, which otherwise may take several seasons and years in field or greenhouse germplasm evaluations.

CHAPTER 6

6. Genotype × Environment interaction and GGE Biplot analysis for grain yield, yield components and other agronomic traits in wheat (*Triticum aestivum* L.)

Abstract

The average wheat productivity in Ethiopia, 2.97 ha^{-1}, is lower than the global average of 3.5 t ha^{-1}, resulting in an increasing reliance on wheat import to keep up with the local demand. Understanding the genetic variability, heritability, genotype-by-environment interaction and associations between agronomic traits is vital for wheat improvement. In the present study, 180 bread wheat genotypes were evaluated at six environments to identify high yielding and stable genotypes. The experiment was laid in an alpha lattice design with two replications. The tested genotypes exhibited wide genetic variability for grain yield and other agronomic traits, suggesting large possibility for genetic improvement through selection. The moderate to high heritability for most agronomic traits indicates the presence of alleles of breeding relevances. Grain yield and related traits were highly significantly (p<0.0001) affected by genotype, environment and their interactions. GGE-biplot analysis confirmed the existence of significant GxE interaction on yield performance, and the first two principal components explained 59.59% of the total variation. Grain yield showed variable associations with the tested agronomic traits. Top 5% selected genotypes showed 10.8 - 21.99% superior grain yield performance to the used standard check. Bekoji 2016 was found to be an ideal environment for grain yield performance evaluation. Among tested genotypes, G85, G159, G169 and 174 represent the top four best yielding and highly stable genotypes to be used for broad adaptation. Environmental clustering identified three mega-environments. This information is very helpful for breeders to select appropriate genotypes for specific and broad adaptations in wheat breeding program.

6.1 Introduction

Wheat is the most widely grown cereal crop globally (Osundwa *et al*., 2013; Randhawa *et al*., 2013) serving as a staple of nearly one third of global population (Ramadas *et al*., 2019). Globally, it is grown on more land area (219 M ha) than any other food crop followed by maize (197 M ha) and then rice (167 M ha). In 2019, its production was 765.8 million tonnes making the crop the second most-produced cereal crop after maize (FAOSTAT, 2019). Common or bread wheat (*Triticum aestivum L.*, 2n = 6x = 42, AABBDD) is the most (95%) widely cultivated species of wheat worldwide followed by durum (*Triticum turgidum* L. subsp. *durum* Desf., AABB; 2n=4x=28) representing nearly 5% of the total production (Arzani and Ashraf, 2017; Ramadas *et al*., 2019).

In Ethiopia, wheat is cultivated for multiple purposes such as for food, animal feed and income generation, and it ranks fourth after Teff (*Eragrostis tef*), Maize (*Zea mays*) and Sorghum (*Sorghum bicolor*) in area coverage and third after Maize and Teff in total production (CSA, 2017, 2019). Both bread wheat and durum wheat account for 80% and 20% of the total wheat cultivation of the country (Asfaw Negassa *et al.,* 2013), respectively, although emmer wheat is also grown to a small extent (Tesfaye Letta *et al.,* 2013; Eyob Bezabeh *et al.,* 2015). In Ethiopia, wheat is grown between 6 - 14^{o} N; and 35 - 42^{o}E with altitude ranging from 1500 to 3200 m.a.s.l. The most appropriate regions, however, fall between 1900 - 2700 m.a.s.l (Hailu Gebre-Mariam, 1991). Oromia, Amhara, Tigray, and Southern Nations, Nationalities and Peoples (SNNP) are the leading wheat growing regions of the country accounting for more than 90% of national wheat production. Arsi, Bale and

Shewa zones of Oromia regional state alone account for 75.5 % of the national wheat production (Hailu Gebre-Mariam ,1991; Tesfaye Gemechu and Fiseha Tadese, 2018).

During the last 15 years, wheat harvest area, production and productivity in Ethiopia had increased from 1.17 M ha, 1.39 M metric tonnes and 1.6 t ha^{-1} in 2003 to 1.72 M ha, 4.83 M metric tonnes and 2.81 t ha^{-1} in 2017, respectively (FAOSTAT, 2019). However, the current national average wheat productivity is substantially lower than the global average of 3.5 t ha^{-1} (FAOSTAT, 2019), resulting in an increasing reliance on wheat import (25%) to meet the increasing domestic demand due to rapid population growth, increased urbanization and change in food preferences (Asfaw Negassa *et al.,* 2013). Numerous factors are known to contribute for this low productivity including limited access to advanced production technologies, lack of agricultural inputs (improved varieties, fertilizer and herbicides), and biotic and abiotic stresses (Alemar Said, 2016).

Field evaluation is one of the most effective germplasm selection techniques among large sets of genotypes (Hanson *et al.* 2016). Hence, reducing the yield gap and boosting wheat production and productivity in Ethiopia requires the development and use of improved and stably high yielding cultivars with their recommended technological packages. This requires breeders to find out sufficient genetic variability for grain yield performance in the available germplasm as well as information on genetic control of the traits (Phuke *et al.*, 2017). Thus, accurate estimation of genetic parameters of yield and yield-related, and other agronomic

traits are of great significance to wheat breeding and selection (Reddy *et al*., 2010; Phuke *et al.,* 2017).

In general, the observed phenotypic variation is the combined effects of genotype (G), environment (E) and their interaction (GxE) (Farshadfar *et al.,* 2013). Although most of the total phenotype variations are explained by the environment (Gauch and Zobel, 1996; Yan, 2002), it is irrelevant in cultivar evaluation and thus, only the very relevant components (G and GxE) shall be considered simultaneously in making meaningful selection decisions (Yan and Kang, 2003; Farshadfar *et al.,* 2013). The presence of significant GxE interaction reduces the association between genotype and phenotype value, complicating the process of selecting genotypes with superior performance. Conducting multi-environment trials (METs) enable breeders to determine the different responses of genotypes across a range of environments (Kang, 1998), assisting the selection of best genotypes with high and stable yield to a wide range of environments (Delacy *et al*., 1996; Farshadfar, 2011).

So far, numerous multi- location trial (MLTs) analysis methods have been developed and widely used including analysis of variance (ANOVA), principal component analysis (PCA), linear regression models and additive main effects and multiplicative interaction (AMMI) (Zobel *et al.,* 1988). Being an additive model, ANOVA is efficient in describing the main effect due to genotype and the environment than the interaction effect (Snedecor and Cochran 1980); it tests the significance of the G × E interaction but does not provide insight into particular pattern of genotype or environment that give rise to G × E interaction (Phuke

et al., 2017). Likewise, being a multiplicative model, PCA is more capable of describing the interaction effect. However, it does not describe the main effects (Zobel *et al.,* 1988). Although AMMI model integrates both the main and interaction effects, it is misleading in identifying 'which won-where' (Yan *et al*., 2007).

Hence, to fully explore the MET data, Yan *et al.* (2000) has developed a new METs data analysis methodology called GGE- biplot that integrates the G and GxE (*i.e* G+ GE), removing the environmental component from the model. GGE- biplot allows researchers to concentrate on the part of the MET data that is most useful for cultivar evaluation. The tool is most effective, useful, and elegant to reveal 1) the "which-won-where" pattern of a MET data set whereby specific genotypes can be recommended to specific mega-environments (Yan and Kang, 2003; Yan and Tinker, 2006), 2) genotype evaluation (mean performance and stability), and 3) environmental evaluation (the power to discriminate among genotypes in target environments) (Yan *et al*., 2007). The technique is super to address and visualize the relationship among test environments, genotypes and the GxE interactions (Gabriel, 1971; Rakshit *et al.*, 2012; Farshadfar *et al.*, 2012; 13). The technique has been widely used to analyze METs data sets in many crop species including rice (Akter *et al.,* 2015), wheat (Kaya *et al*., 2006; Roozeboom *et al*., 2008), barley (Dehghani *et al*., 2006; Mohammadi *et al*., 2009), Lentil (Sabaghnia *et al*., 2008), oat (Yan *et al*., 2010), sorghum (Phuke *et al.,* 2017) and others.

However, the application of GGE-biplot in deciding superior genotypes and test environments in bread wheat through METs in Ethiopia is limited (Muez Mehari *et al.,* 2015; Zerihun Tadesse *et al.,* 2016). Therefore, the present study was targeted to assess genetic variability, Genotype-by- environment interaction, association of grain yield and other phenological traits in bread wheat genotypes, and also apply GGE biplot technique to examine the possible existence of different mega- environments in wheat- growing regions of Ethiopia, to identify ideal test environment, and to select superior yielding and stable bread wheat genotypes.

6.2 Materials and Methods

Experimental Materials

In this study, a total of 180 bread wheat (*T. aestivum* L.) panel (Appendix IV) were used: 167 were obtained from International Maize and Wheat Improvement Center (CIMMYT-Mexico) while, 13 were commercial cultivars grown in Ethiopia. The 167 CIMMYT genotypes included 49 from a set of the international bread wheat screening nursery (IBWSN), 56 were from the international Septoria observation nursery (ISEPON), 14 were from the high rain wheat yield trial (HRWYT), 34 were from the high-rain wheat screening nursery (HRWSN), five were from an adaptation trial, six came from the national variety trial (NVT), and the remaining three genotypes were from a preliminary variety trial (PVT).

Multi-Environment Field Trials

The study materials were evaluated for two consecutive years (2015 and 2016) in the main cropping seasons that exends from June to December across three locations: Holetta Agricultural Research Center (HARC) located at 9° 3' N and 38° 30' E with an elevation of 2400 above sea level, Bekoji Agricultural Research Sub-station situated at 7° 32'N and 39° 15'E with an elievation of 2780 m above sea level, and Kulumsa Agricultural research center (KARC) located at 8° 02' N and 39° 15 ' E, Ethiopia (Table 6.1). The experiment was laid in an alpha lattice design with two replications and six sub-blocks within replication.

Table 6. 1 Field layouts and sowing for each location in two consecutive years.

Code	Location-Year	Plots/ rep	Design	Average rainfall	Temperature Max.& mini	Planting date	Soil type
E1	Holetta-2015	180	(30 entries/block × 6 blocks) × 2 reps	1055ml	22^0C & 6^0C	3Jul 2015	Eutric Nitisol
E2	Bekoji-2015	180	(30 entries/block × 6 blocks) × 2 reps	1020ml	20^0C to 8^0C	26Jun 2015	Eutric Nitosol
E2	Kulumsa-2015	180	(30 entries/block × 6 blocks) × 2 reps	788 ml	22.2 & 6.1	7 Jul 2015	Xerosols
E4	Holetta-2016	180	(30 entries/block × 6 blocks) × 2 reps	1055ml	22^0C& 6^0C	10Jul2016	Eutric Nitisol
E5	Bekoji-2016	180	(30 entries/block × 6 blocks) × 2 reps	1020ml	20^0C & 8^0C	30Jun2016	Eutric Nitosol
E6	Kulumsa-2016	180	(30 entries/block × 6 blocks) × 2 reps	788 ml	22.2 & 6.1^{0}C	5 Jul2016	Xerosols

Agronomic practices and data recording

The trial was planted by hand with each entry sown in four rows of 2.5 m long, 20 cm spacing between rows and 40 cm between entries. Spaces between blocks and replications were maintained to 1.50 m long. A seed rate of 150 kg ha^{-1} and fertilizer rates of 100 and 75 kg ha^{-1} N and P_2O_5, respectively, were applied to all experiments. Weeding was done three times by hand. Data were recorded on grain yield, yield components and phenology traits including days to 50% heading (DH), days to 50% flowering (DF), grain filling duration

(GFD), days to 90% maturity (DM), grain yield (kg), thousand kernel weight (TKW = as the weight of 1,000 kernels, in grams), plant height (PH), panicle length (PL), spike length (SL), flag leaf length (FLL), flag leaf width (FLW), flag leaf area (FLA), number of spikelets per spike (SPS), number of kernels per spikelete (NKPS) and number of kernel per spike (NKS). Days to flowering and days to maturity were recorded for whole plots when 50% of the plants reached the corresponding Zadock's growth stages. Yield data were taken from the four rows of each plot and converted to kilograms per hectare (kg ha^{-1}) at 12.5% moisture content using the plot size as factor. Measurements of Plant height, panicle length and spike length were taken at physiological maturity from five randomly selected and tagged plants from middle rows of each entry. Spike of the same plants were collected in separate medicinal bags to determine the number of spikelets per spike, number of kernels per spikelet and total number of kernels per spike.

Analysis of Variance (ANOVA)

The analysis of variance at each location was computed using SAS software version 9.2 (SAS Institute Inc., 2008). The combined ANOVA was executed by using mixed effect model by considering genotype as fixed effect and the location and year as random effect. Error variance homogeneity test was performed using the F-max method of Hartley (1950), which is based on the ratio of the larger mean square of error (MSE) from the separate analysis of variance to the smaller mean square of error as:

$$\mathrm{F-ratio} = \frac{\text{Larger MSE}}{\text{Smaller MSE}}$$

If the larger error mean square is not three-fold larger than the smaller error mean square, the error variance was considered homogeneous (Gomez and Gomez, 1984). Combined analyses were performed to know average genotypic effects, environment effects, and genotype x environment effects; as well as to get the estimates of environmental and genotype x environment interaction variances. Significant means were separated using least significant difference (LSD) procedure at 5% significance level.

Estimation of Genetic Parameters

Estimation of variance components such as genotypic variance, phenotypic variance, genotype by environment interaction variance, and coefficient of phenotypic and genotypic variability were computed from ANOVA table as follows:

$$\text{Genotypic variance } (\delta^2 g) = \frac{MSg - MSe}{lr},$$

where, MS_g = genotype mean square variance, MS_e = error mean square, l = number of location and r = replication.

$$\text{Genotype by location interaction variance} (\delta^2 gl) = \frac{MSgl - MSe}{r},$$

where, MS_{gl} = mean square of genotype by location interaction variance, MS_e = error mean square, and r = replication. Mean square error is an estimator of the environmental variance. Thus, environmental variance = Mean square error = MS_e. From this the total phenotypic variance was computed as:

$$\delta^2 p = \delta^2 g + \delta^2 gy + \delta^2 gl + \delta^2 gyl + \delta^2 e$$

Where, $\delta^2 p$ is the total phenotypic variance, $\delta^2 g$ is genotypic variance, $\sigma^2 gy$ is the genotype by year interaction variance, $\sigma^2 gl$ is the genotype-by-location interaction variance, $\sigma^2 gyl$ is the genotype-by-year-by location interaction variance, and $\delta^2 e$ is environmental variance.

Phenotypic and Genotypic Coefficient of Variation

The traits estimate of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were calculated using the Singh and Choudhary (1985) formula as follows:

$$\text{PCV} = \frac{\sqrt{\delta^2 p}}{\bar{x}} X100 \text{ and the GCV} = \frac{\sqrt{\delta^2 g}}{\bar{x}} X100,$$

where $\delta^2 p$ and $\delta^2 g$ are the total phenotypic and genotypic variations, respectively and $\bar{x}$ = grand mean of the characters under study. Computed GCV and PCV values were categorized as low (<10%), moderate (10-20%) and high (>20% and above) as suggested by Sivasubramanian and Madhavamenon (1973).

Heritability estimate

In the present study, heritability (H^2) in broad sense across environments were estimated by the formula:

$$H^2 = \frac{\delta^2 g}{\delta^2 g + \delta^2 gy/y + \delta^2 gl/l + \delta^2 gyl/yl + \delta^2 \varepsilon/ylr}$$

Where l, r, y, represents the number of locations, replicates and years, respectively. Because heritability is a proportion, its numerical value ranges from 0.0 (genes do not contribute at all to phenotypic individual differences) to 1.0 (genes are the only reason for individual

differences). The heritability percentage was categorized as low (<30%), moderate (30-60%) and high (≥ 60%) as described by Robinson *et al*. (1949).

Genetic advance under selection (GA): The predicted response to selection or the expected genetic advance (GA) was computed following the formula described by Johnson *et al*. (1955), assuming the selection intensity of 5%, as:

$$GA = K\delta P\, H^2 ,$$

where, GA = Expected genetic advance from selection, H^2 = Heritability in broad sense, δp = phenotypic standard deviation and k = the standardize selection differential at 5% selection intensity (K = 2.063) (Singh and Chaudhary, 1985). The calculated GA was categorized as low (<10), moderate (10 - 20%), and high (>20%) based on Johnson *et al.* (1955). Genetic advance as percent of mean (GAM was also calculated following the formula described by Robinson and Comstock (1949) as:

$$GAM = \frac{GA}{\bar{x}} x\ 100$$

where, GAM = Genetic advance as percent of mean, GA = Genetic advance under selection and $\bar{x}$ = grand mean of the trait. Computed GAM was categorized as low (<10%), moderate (10 - 20%, and high (>20%) (Falconer and Mackay, 1996).

Association Analysis between Agronomic Traits

Relationship between agronomic traits like days to 50% heading, days to 50% flowering, grain filling duration, days to maturity, 1000 kernel weight, plant height, grain yield, panicle length, spike length, flag leaf length, flag leaf width, flag leaf area, number of spikelets per

spike, number of kernels per spikelet and number of kernels per spike were computed using Pearson's correlation. The correlation analysis was carried out using pooled data from the six environments termed as best linear unbiased estimator (BLUE) values. The correlation coefficients between every pair of traits were computed using PROC CORR procedure in SAS based on the standard procedure as:

$$r = \frac{\mathrm{Cov}(x, y)}{\sqrt{\delta^2 x + \delta^2 y}}$$

Where r = correlation coefficients, Cov ($_{x,y}$) = co-variance of traits x and y, δ^2_x = variance of x and δ^2_y = variance of y. The correlation analysis for each trait was carried out using the pooled mean of all genotypes. The level of significance of correlation coefficients was determined from r table (correlation table) at appropriate degrees of freedom and probability level (Gomez and Gomez, 1984).

GGE-biplot analysis

In this study, mean yield performance based GGE biplot analysis was conducted to identify the genotype-by-environment (GxE) interaction, identify superior performing genotypes and determine representativeness and discrimination power of the test environments. The analysis was conducted using GenStat 15th Edition software. The model for a GGE biplot analysis based on singular value decomposition (SVD) of first two principal components is given by:

$$Y_{ij} - \mu - \beta_j = \lambda_1 \xi_{i1} \eta_{1j} + \lambda_2 \xi_{i2} \eta_{2j} + \varepsilon_{ij}$$

where Y_{ij} = the mean yield of genotype i (=1,2,…,180) in environment j (=1,2,…e), μ = the grand mean, β_j = the main effect of environment j, ($\mu+\beta$) = the mean yield of all genotypes across environment j, λ_1 and λ_2 = singular value of the first and second largest principal components, PC1 and PC2, respectively; ξ_{i1} and ξ_{i2} = the eigenvectors of genotype i for PC1 and PC2, respectively; η_{1j} and η_{2j} = the eigenvectors of environment j for PC1 and PC2, respectively; and ε_{ij} is the residual associated with genotype i in environment j (Yan and Kang, 2003).

6.3 Results

Mean Performance Analysis

Table 6.2. presents the genotypes mean performance, standard deviations, range and significance level for phenotypic traits measured in all six environments. The analysis revealed that the mean performance of the genotypes for grain yield, spike length and flag leaf length were highest in E2 (Bekoji-2015) but, lowest in E3 (Kulumsa-2015) (Table 6.2). Highest and lowest mean performance for 1000 kernels weight was recoreded in E2 and E6 (Kulumsa-2016), respectively. The mean performance of the genotypes for days to 50% heading, days to 50% flowering, days to maturity, grain filling duration and plant height were higher in E5 (Table 6.2). Similarly, the

Table 6. 2 Descriptive statistics of phenotypic values in wheat genotypes evaluated at six environments.

Trait	Environment	Mean	Range	SD	R^2	CV	Pr > F	Trait	Environment	Mean	Range	SD	R^2	CV	Pr > F
DH	E1 (HARC 2015)	68.03	64-68.5	2.99	84	2.7	***	FLL	E1 (HARC 2015)	25.31	19.8-29.6	1.77	74	6	***
	E2 (Bekoji 2015)	71.31	63-94.5	5.61	97	2.2	***		E2 (Bekoji 2015)	26.34	19.2-34.8	2.54	86	5.7	***
	E3 (Kulumsa 2015)	65.26	61-78	3.31	94	1.9	***		E3 (Kulumsa 2015)	14.02	9.1-18.2	1.84	87	7.4	***
	E4 (HARC 2016)	65.78	58.5-80.5	2.95	95	1.5	***		E4 (HARC 2016)	25.44	18.73-33.82	2.2	87	4.8	***
	E5 (Bekoji 2016)	80.5	73.5-88	2.78	88	1.9	***		E5 (Bekoji 2016)	26.02	20.8-33.3	1.81	88	3.7	***
	E6 (Kulumsa 2016)	65.62	59-89	3.54	96	1.7	***		E6 (Kulumsa 2016)	20.98	17.46-26.12	0.97	91	2.1	***
DF	E1 (HARC 2015)	76.03	70-88.5	2.99	84	2.4	***	FLW	E1 (HARC 2015)	1.86	1.22-2.58	0.17	84	7.7	***
	E2 (Bekoji 2015)	79.16	69.5-99.5	5.42	96	2.1	***		E2 (Bekoji 2015)	1.6	1.2-1.96	0.17	84	6.6	***
	E3 (Kulumsa 2015)	72.8	69-82	2.58	84	2.2	***		E3 (Kulumsa 2015)	1.68	1.24-2.09	0.14	74	7.2	***
	E4 (HARC 2016)	74.44	65-86	3.22	91	1.9	***		E4 (HARC 2016)	1.82	1.21-2.91	0.16	64	9.7	***
	E5 (Bekoji 2016)	86.65	80-93.5	2.96	84	2.2	***		E5 (Bekoji 2016)	1.54	1.2-1.96	0.15	80	7.2	***
	E6 (Kulumsa 2016)	69.87	63-83	3.16	75	3.8	***		E6 (Kulumsa 2016)	1.66	1.24-1.95	0.13	88	4.2	***
DM	E1 (HARC 2015)	124.8	103-146	6.46	93	2.15	***	FLA	E1 (HARC 2015)	35.28	21.14-51.21	3.87	79	10	***
	E2 (Bekoji 2015)	144.2	134.5-16	4.97	90	1.7	***		E2 (Bekoji 2015)	30.8	20.98-45.23	4.85	87	8.9	***
	E3 (Kulumsa 2015)	104.5	95.5-116.5	4.18	91	1.8	***		E3 (Kulumsa 2015)	17.69	10.55-27.78	2.76	84	9.8	***
	E4 (HARC 2016)	136.9	125.5-151	3.71	89	1.4	***		E4 (HARC 2016)	33.61	16.73-45.64	5.4	96	4.6	***
	E5 (Bekoji 2016)	161.7	157.5-182	2.6	49	2.4	**NS**		E5 (Bekoji 2016)	30.11	22.46-42.14	3.96	88	7.1	**NS**
	E6 (Kulumsa 2016)	112.1	94-125	5.02	93	1.8	***		E6 (Kulumsa 2016)	27.21	16.73-38.30	4.04	84	9.4	***
GDF	E1 (HARC 2015)	56.81	30-75	5.54	87	5.47	***	SN	E1 (HARC 2015)	17.58	14-22	1.3	85	4.5	***
	E2 (Bekoji 2015)	73.12	57-84	4.49	71	6.1	***		E2 (Bekoji 2015)	16.87	14.3-19.5	1.03	81	4.4	***
	E3 (Kulumsa 2015)	39.27	30-47	2.5	84	4	***		E3 (Kulumsa 2015)	16.38	14.4-18.3	0.79	73	4.6	***
	E4 (HARC 2016)	71.6	54-115.5	5.36	88	4	***		E4 (HARC 2016)	15.9	13.1-19.2	0.95	63	6.7	***
	E5 (Bekoji 2016)	81.24	74-109-5	3.7	66	4.8	***		E5 (Bekoji 2016)	16.3	11.6-19.5	1.34	81	5.7	***
	E6 (Kulumsa 2016)	46.39	20-57.5	5.01	97	2.9	***		E6 (Kulumsa 2016)	16.68	13-24	1.16	81	4.9	***
Yield	E1 (HARC 2015)	4846	2445-7081.5	897.1	81	12.9	***	NKPS	E1 (HARC 2015)	3.18	2.45-4.15	0.28	61	11	***
	E2 (Bekoji 2015)	5538	2630-7840	1113	657	21.3	***		E2 (Bekoji 2015)	3.19	2.00-4.50	0.63	69	20	***
	E3 (Kulumsa 2015)	3787	2342.5-5065	523.3	76	11.3			E3 (Kulumsa 2015)	3.18	2.50-3.91	0.29	73	9.3	**
	E4 (HARC 2016)	4420	2104.75-6860	809.1	91	8.5	***		E4 (HARC 2016)	3.3	2.60-3.80	0.28	72	7.8	***
	E5 (Bekoji 2016)	4171	1572.5-6622.5	788	87	10.9	***		E5 (Bekoji 2016)	1.85	0.50-3.3	0.49	63	30	***
	E6 (Kulumsa 2016)	4181	2410-6300	715.3	91	8.1	***		E6 (Kulumsa 2016)	3.26	2.44-4.2	0.29	82	6.1	***
HLW	E1 (HARC 2015)	78.46	68.6-83.6	2.19	82	1.9	***	NKS	E1 (HARC 2015)	46.21	35.00-59.5	4.9	81	7.5	***
	E2 (Bekoji 2015)	34.38	30.77-36.46	1.1	76	2.7	***		E2 (Bekoji 2015)	45.24	31.1-65.9	6.44	77	12	***
	E3 (Kulumsa 2015)	34.55	31.67-36.7	0.88	57	3.3	**NS**		E3 (Kulumsa 2015)	41.86	32-49	3.2	70	7.6	**NS**
	E4 (HARC 2016)	74.71	65.05-80.1	3.09	81	3.1	***		E4 (HARC 2016)	47.19	33.3-68.3	5.97	93	5.2	***
	E5 (Bekoji 2016)	31.45	14.68-36.33	4.72	91	23.6	***		E5 (Bekoji 2016)	28.34	4.5-48.1	7.83	94	10	***
	E6 (Kulumsa 2016)	34.37	28.94-36.92	1.27	84	2.4	***		E6 (Kulumsa 2016)	47.19	35-63	5.06	81	7.5	***
TKW	E1 (HARC 2015)	35.94	24.6-46.6	4.95	98	3.2	***	PL	E1 (HARC 2015)	39.5	31.45-51.34	2.76	76	5.8	***
	E2 (Bekoji 2015)	44.36	30.46-55.06	5.03	91	5	***		E2 (Bekoji 2015)	43	32.15-56.4	3.85	93	3.7	***
	E3 (Kulumsa 2015)	37.42	25.26-51-01	3.95	72	9.7	***		E3 (Kulumsa 2015)	36.1	26.25-48.39	3.56	97	2.4	***
	E4 (HARC 2016)	35.13	17.5-47.2	6.07	92	7.2	***		E4 (HARC 2016)	48	35.8-59.21	2.89	93	2.5	***
	E5 (Bekoji 2016)	35.1	11.93-48.18	7.76	98	4.8	***		E5 (Bekoji 2016)	40.3	33.347.3	2.8	87	3.8	***
	E6 (Kulumsa 2016)	35.05	23.64-42.24	3.44	82	6.8	***		E6 (Kulumsa 2016)	37.7	30.65-44.96	2.89	66	8.1	*
PH	E1 (HARC 2015)	94.36	78.5-111.8	4.87	91	2.3	***								
	E2 (Bekoji 2015)	96.34	82.5-123.5	5.52	92	2.4	***								
	E3 (Kulumsa 2015)	92.92	82.8-114.9	4.46	91	2.2	***								
	E4 (HARC 2016)	95.99	83.5-119.6	4.57	91	2.1	***								
	E5 (Bekoji 2016)	99.69	81.8-114.5	6.24	100	0.66	***								
	E6 (Kulumsa 2016)	95.51	78.4-115.4	5.73	99	0.76	***								

DH = days to 50% heading; DF = days to 50% flowering; DM = days to 90% maturity; GDF = Grain filling duration; HLW = Hectoliter weight; TKW = 1000 kernel weight; PH = Plant height (cm); PL= Panicle length (cm); SL= Spike length (cm); FLL= Flag leaf length; FLW = Flag leaf width; FLA = Flag leaf area; SN = number of spikelet per spike; NKPS= Number of kernel per spikelet; and NKS = Number of kernel per spike

mean performance of the panel for hectoliter weight, number of spikelets per spike, flag leaf area and flag leaf width were highest for E1, whereas mean performance of the genotypes for hectoliter weight and flag leaf width, flag leaf area and number of spikelets per spike were the lowest in E5, E3 and E4, respectively. The mean performance of the genotypes for panicle length, number of kernels per spikelet and kernels per spike were significantly higher at E4 (Table 6.2).

Analysis of Variance

The genotypes showed highly significant diffrences for all phenotypic traits meaured in all six environments except for days to maturity, hectoliter weight, number of kernels per spike, and flag leaf area which were non-significant in E5, E3, E3 and E5, respectively (Table 6.2). All the agronomic traits showed very highly significant genetic variances (δ^2g) in all individual environments (Table 6.3). Days to heading, days to flowering, panicle length, and flag leaf length showed highest genotypic variance in E2, while days to maturity, grain filling duration, grain yield, number of spikelets per spike had the highest genotypic variance in E1 (Table 6.3). Spike length showed the highest genetic variance in E3, whereas hectoliter weight, 1000-kernel weight and flag leaf area showed the largest genotypic variance in E4. Plant height and number of kernels per spike had greatest genetic variance in E5, while only number of kernels per spikelet showed largest genotypic variance in E6 (Table 6.3).

Table 6. 3 Genotypic variance and heritability for agronomic traits at six test environments**.**

	Holetta -2015 (E1)		Bekoji-2015 (E2)		Kulumsa-2015 (E3)		Holetta -2016(E4)		Bekoji-2016 (E5)		Kulumsa -2016(E6)	
Trait	**σ^2g**	**H^2**	**σ^2g**	**H^2**	**σ^2g**	**H^2**	**σ^2g**	**H^2**	**σ^2g**	**H^2**	**σ^2g**	**H^2**
DH	7.57***	0.85	30.32***	0.96	10.21***	0.93	8.18***	0.94	14.73***	0.95	11.92***	0.95
DF	7.21***	0.81	27.98***	0.95	5.36***	0.81	9.36***	0.90	16.25***	0.93	6.55***	0.66
DM	39.09***	0.94	21.57***	0.87	15.76***	0.90	11.93***	0.87	8.72***	0.65	23.24***	0.92
GDF	26.7***	0.87	10.28***	0.51	5.03v	0.81	24.66***	0.86	22.35***	0.82	24.17***	0.96
Yield	6134838***	0.76	541069.1***	0.44	182518.4***	0.67	583358***	0.89	1174827***	0.95	454200***	0.89
HLW	3.66***	0.77	0.80***	0.66	0.8***	0.66	6.97***	0.73	1.58E+10***	0.90	1.28***	0.79
TKW	23.88***	0.97	22.85***	0.90	9.06***	0.58	33.61***	0.91	119.5***	0.99	9.05***	0.76
PH	21.31***	0.90	27.75***	0.91	17.75***	0.89	18.75***	0.90	38.72***	0.99	32.62***	0.99
PL	4.96***	0.65	13.54***	0.91	12.32***	0.97	7.64***	0.91	6.63***	0.85	3.72***	0.45
SL	0.2***	0.45	0.36***	0.32	0.4***	0.82	0.18***	0.59	0.32***	0.70	0.23***	0.57
FLL	2.22***	0.73	5.34***	0.83	2.86***	0.84	4.11***	0.84	2.82***	0.86	0.84***	0.90
FLW	0.02***	0.64	0.02***	0.80	0.01***	0.62	0.01***	0.28	0.02***	0.75	0.01***	0.86
FLA	8.67***	0.58	19.85***	0.84	6.13***	0.80	28.01***	0.96	13.43***	0.85	13.05***	0.80
SN	1.38***	0.81	0.80***	0.75	0.34***	0.54	0.33***	0.37	1.37***	0.76	0.49***	0.38
NKPS	0.02***	0.27	0.20***	0.50	0.04***	0.49	0.04***	0.57	0.09***	0.38	19.35***	0.76
NKS	18.88***	0.79	27.88***	0.67	5.20***	0.51	32.66***	0.92	57.18***	0.93	24.76***	0.80

σ^2g = Genotypic variance; H^2= heritability in broad-sense; DH = days to 50% heading; DF = days to 50% flowering; DM = days to 90% maturity; GDF = Grain filling duration; HLW = Hectoliter weight; TKW = 1000 kernel weight; PH = Plant height (cm); PL= Panicle length (cm); SL= Spike length (cm); FLL= Flag leaf length; FLW = Flag leaf width; FLA = Flag leaf area; SN = number of spikelet per spike; NKPS= Number of kernel per spikelet; and NKS = Number of kernel per spike; *** = very highly significant at p<0.0001, ** = highly significant at p< 0.001, * = significant at p< 0.05, and ns = non significant at p= 0.05 significance level.

The analysis of variance based on pooled data revealed that the main effects due to genotype, blocking, year and location were highly significant ($p < 0.0001$) for all agronomic traits (Table 6.4). Likewise, the interaction effects due genotype x year, genotype x location, location x year, and their three way interactions (genotype x year x location) were highly significant ($p < 0.0001$) on all the agronomic traits except flag leaf width where the effect of genotype-by-year and the three way interactions were non-significant (Table 6.4). All agronomic traits showed very highly significant ($p < 0.0001$) and largest genetic variance (δ^2g) across environments except grain filling duration, 1000 kernel weight, plant height, number of spikelets per spike, number of kernels per spikelet, and number of kernels per spike, in which the variance due to three way interactions (σ^2gyl) was the largest (Table 6.5). Moreover, except for flag leaf width (where genotype x year variance was non-significant), for all agronomic traits the genotype x year (σ^2gy), genotype x location (σ^2gl), and year x location (σ^2yl) interaction were highly significant, but lower in magnitude as compared to genotypic variance (Table 6.5).

For all agronomic traits, the values for phenotypic variation were higher in magnitude than the corresponding values of genotypic variance (Table 6.5), and the highest genotypic and phenotypic variances were observed in grain yield. Likewise, for all agronomic traits the magnitude of phenotypic coefficient of variation (PCV) was slightly larger than the corresponding values of genotypic coefficient of variation (GCV) (Table 6.5), which could be due to the larger environmental effect in the expression of the traits. The PCV values ranged from 4.33 (grain filing duration) to 22.54% for grain yield (Table 6.5). Of the 16 agronomic traits, 43.75% showed low (< 10), 50% showed moderate (10-20%) and 6.25% showed higher (>20%) PCV. Likewise, the values of GCV varied from 0.78% (grain filing duration) to 10.79%

Table 6. 4 Combined Analysis of variance for 16 traits across six environmentds.

Traits	Mean Square								
	G (df=179)	R (df=1)	Y (df =1)	L (df =2)	B(YL) (df =5)	G*Y (df =179)	G*L (df =358)	Y*L (df =2)	G*Y*L (df =358)
DH	74.12***	11.41*	3185.24***	23120.29***	53.71***	21.87***	16.85***	6464.23***	15.52***
DF	79.64***	3.75ns	530.05***	24957.46***	48.82***	15.68***	13.99***	5795.46***	12.42***
DM	103.98***	8.07ns	83005.60***	358688.64***	171.79***	35.23***	32.31***	4315.13***	27.80***
GDF	47.14***	82.05*	54170.14***	216648.40***	129.023***	36.61***	45.75***	3130.95***	36.96***
Yield	2515597.8***	10703404.9***	117230923.4***	147443029.4***	4822885.5***	1180855.2***	1444683.1***	139694193.4***	810402.4***
HLW	11.47***	104.38***	1848.34***	434989.56***	18.13***	7.19***	7.23***	581.36***	6.33***
TKW	96.33***	114.35***	9276.47***	3632.84***	101.38***	76.60***	42.70***	3636.96***	45.24***
PH	133.36***	0.12ns	3437.64***	2814.01***	136.19***	40.68***	40.13***	132.13***	39.59***
PL	71.56***	40.34***	3377.25***	8930.28***	113.59***	12.39***	7.64363	5796.80***	10.01***
SL	2.79***	0.21ns	4.16***	233.65***	2.43**	0.76**	0.73***	100.79***	0.71**
FLL	21.73***	4.19ns	2747.90***	16570.26***	35.43***	4.27***	4.07***	2986.35***	5.00***
FLW	0.12***	1.00***	0.83***	13.41***	0.06*	0.01ns	0.06***	0.11*	0.01ns
FLA	94.18***	460.43***	3105.36***	26834.04***	70.25***	19.10***	34.96***	6882.75***	15.78***
SN	3.69***	1.59ns	228.54***	8.07***	2.56*	2.26***	2.55***	176.26***	1.91***
NKPS	0.38***	2.47**	77.35***	121.04***	1.25***	0.31***	0.34***	125.69***	0.29***
NKS	95.85***	24.51ns	6729.95***	19533.67***	91.79***	64.45***	64.55***	24971.79***	53.58***

Note: G = Genotype; R= replication; Y = Year; B = incomplete block; G*Y = Genotype-by-year interaction; G*L = Genotype-by- location interaction; Y*L= Year-by-location interaction; G*Y*L = Genotype-by-year-by location interaction; DH = days to 50% heading; DF = days to 50% flowering; DM = days to 90% maturity; GDF = Grain filling duration; HLW = Hectoliter weight; TKW = 1000 kernel weight; PH = Plant height (cm); PL= Panicle length (cm); SL= Spike length (cm); FLL= Flag leaf length; FLW = Flag leaf width; FLA = Flag leaf area; SN = number of spikelet per spike; NKPS= Number of kernel per spikelet; and NKS = Number of kernel per spike; *** = very highly significant at p<0.0001, ** = highly significant at p< 0.001, * = significant at p< 0.05, ns = non significant at p= 0.05 significance level, and df = degree of freedom. Numbers in parentheses are degrees of freedom associated with the corresponding source of variation.

(flag leaf area). Except for grain yield and flag leaf area that showed moderate GCV (10.79%) (Sivasubramanian and Madhavamenon, 1973), all other agronomic traits showed low GCV (< 10) (**Table 6.5**).

Broad-sense heritability and expected genetic gain from selection

As per the scale of Robinson (1949), all the agronomic traits showed moderate (0.30 - 0.60) to high heritability ($H^2 > 0.60$) in all individual environments except for flag leaf width ($H^2 = 27\%$) in E1 and number of kernels per spikelet ($H^2 = 28\%$) in E4 (Table 6.3). Heritability of the agronomic traits at individual environments ranged from 27% to 99%, and traits like days to heading, days to flowering and days to maturity, hectoliter weight, plant height, flag leaf length and flag leaf area were identified to be highly heritable ($H^2 > 60\%$) (Table 6.3). The broad sense heritability was comparatively high in the second year (28 - 99%) than first year (27 – 97%). Environmental wise, lowest heritability value was in E2 for number of kernels per spike, which also scored lowest genotypic variance in the same environment (Table 6.3). Broad-sense heritability of the agronomic traits across the entire environments ranged from 6% (grain filling duration) to 93% (panicle length) (Table 6.5). High broad-sense heritability ($H^2 > 60\%$) values were observed for days to heading (83%), days to flowering (88%), days to maturity (78%), plant height (82%), panicle length (93%), spike length (84%), flag leaf length (91%), flag leaf width (64%), and flag leaf area (76%) (Table 6.5). Moderate heritability ($H^2 = 30 - 60\%$) values were exhibited by grain yield (54%), hectoliter weight (51%), 1000 kernel weight (59%), number of spikelet per spike (44%) and number of kernels per spike (45%), whereas grain filling duration and number of kernels per spikelet were identified to be low heritable traits (Table 6.5).

Table 6. 5 Variance components, Heritability, Phenotypic and Genotypic coefficient of variations, and Genetic advance for 16 traits in 180 wheat genotypes based on pooled data.

Trait	σ^2g	σ^2gy	σ^2gl	σ^2gyl	σ^2e	PV	GCV	PCV	H^2	GA	GAM
DH	9.55***	1.065***	0.33***	6.64***	2.25***	19.82	4.45	6.41	0.83	7.64	12
DF	10.94***	0.54***	0.39***	4.33***	3.7^{6}***	19.97	4.32	5.84	0.88	8.15	11
DM	11.94***	1.24***	1.13***	10.12***	7.56***	32.00	2.64	4.33	0.78	9.14	7
GDF	0.23***	-0.06***	2.20***	12.7***	11.52***	26.61	0.78	8.4	0.06	0.62	1
Yield	178485.8***	61742.13***	158570.2***	185165.0***	440073.0***	1024036	10.41	22.54	0.54	1130.01	26
HLW	0.70***	0.14***	0.24***	1.44***	3.45***	5.98	1.73	5.07	0.51	2.56	6
TKW	8.94***	5.23***	-0.63***	19.11***	7.02***	39.66	8.04	16.94	0.59	7.69	21
PH	15.54***	0.18***	0.13***	17.3***	4.99***	38.15	4.11	6.45	0.82	10.44	11
PL	10.65***	0.40***	-0.59***	3.15***	3.70***	17.32	8.01	10.21	0.93	7.97	20
SL	0.34***	0.01**	0.01**	0.09***	0.53**	0.98	6.51	10.95	0.84	1.72	19
FLL	2.94***	-0.12***	-0.23***	1.80***	1.41***	5.8	7.45	10.46	0.91	4.54	20
FLW	0.01***	-0.00***	0.01^{ns}	-0.01***	0.02***	0.05	5.79	11.82	0.64	0.27	16
FLA	9.87***	0.55***	4.80***	3.60***	8.59***	27.41	10.79	17.98	0.76	8.17	29
SN	0.19***	0.06***	0.16***	0.56***	0.80***	1.76	2.63	7.98	0.44	1.21	8
NKPS	0.01***	0.0***	0.01***	0.06***	0.18***	0.26	2.87	16.81	0.2	0.22	8
NKS	5.22***	1.8***	2.74***	19.67***	14.24***	43.69	5.35	15.49	0.45	6.19	15

******* = very highly significant at p<0.0001, ** = highly significant at p< 0.001, * = significant at p< 0.05, ns = non significant at p= 0.05 significance level; DH = days to 50% heading; DF = days to 50% flowering; DM = days to 90% maturity; GDF = Grain filling duration; HLW = Hectoliter weight; TKW = 1000 kernel weight; PH = Plant height (cm); PL= Panicle length (cm); SL= Spike length (cm); FLL= Flag leaf length(cm); FLW = Flag leaf width(cm); FLA = Flag leaf area ($cm^{2)}$; SN = number of spikelet per spike; NKPS= Number of kernel per spikelet; and NKS = Number of kernel per spike.

At 5% selection intensity, genetic advance of the studied morphological traits varied from 0.22 for number of kernel per spikelet to 1130.01 for grain yield, while the magnitude of expected genetic gain as percent of mean ranged from 1 -29% (Table 6.5). Grain yield (26%), 1000 kernels weight (21%), and flag leaf area (29%) showed high genetic gains from selection as percent of mean. Whereas, days to heading, days to flowering, plant height, panicle length, spike length flag leaf length, flag leaf width, and number of kernels per spike showed moderate genetic gains from selection as percent of mean. Days to 90% maturity, grain filing duration, hectoliter weight, number of spikelet per spike and number of kernels per spikelet exhibited low genetic gains from selection, although some of them had moderate to high broad-sense heritability (Table 6.5).

Grain yield and yield related performances

The very highly significant differences for the genotypic effect confirmed the existence of substantial genetic variability among the tested genotypes suggesting heritability of the traits. The genotypes grain yield and yield related performance varied from 2874.13 - 5705.92 $kgha^{-1}$ in grain yield (mean = 4490.53 $kgha^{-1}$), 43.89 - 50.29 $kghL^{-1}$ in hectoliter weight (mean = 48.21 $kghL^{-1}$), 9.36 - 44.63g in1000 kernel weight (mean = 37.17g), 2.56 - 3.34 kernels per spikelet (mean = 3) and 34.2 - 51.32 kernels per spike (mean = 42.67) (Appendix V). Among tested genotypes, genotype G174 and G12 were found to be the highest (5705.92 $kgha^{-1}$) and lowest (2874.13 $kgha^{-1}$) performing genotypes, respectively (Appendix V). Comparing the test genotypes with a recently released bread wheat variety King-bird (a standard check) and with the overall mean performance of 13 released

commercial cultivars (K6295-4A, K-6290-Bulk, ET-13A2, Medawolabu, Alidoro, Pavon-76, Sofumar, Biqa, Danda'a, Huluka, Hogana, Meraro and King-bird) revealed that considerable number of tested genotypes had superior performances in grain yield and yield components and other desirable agronomic traits.

Among the tested genotypes, 68 (38%) genotypes had superior grain yield performance over the standard check, King-bird, while 32% were found to perform lower. Figure 6.1 presents the proportion by number of the genotypes with superior and inferior agronomic performance to the standard check, King-bird. The best 5% of the genotypes for their grain yield performance include G174, G176, G81, G116, G169, G56, G51, G121 and G159 (Table 6.6). These genotypes showed superior grain yield performance ranging from 10.8 - 21.99% over the standard check (King-bird) and 3.99 - 26.86% superior to the average yield performance of the commercial cultivars (Table 6.6). The top 5% genotypes for 1000 kernel weight (g) were found to be superior by 13.97 - 22.25% to King-bird and by 0.52 - 22.88% to the 1000 kernels weight mean performance of the released varieties. Likewise, the comparative advantage of the best genotypes over the standard check and over the mean performance of the released varieties ranged from 11.05 - 13.6 and 10.23 - 12.76% for kernels per spikelet, and 18.78 - 29.92 % and 12.08 - 22.6% for kernels per spike, respectively (Table 6. 6).

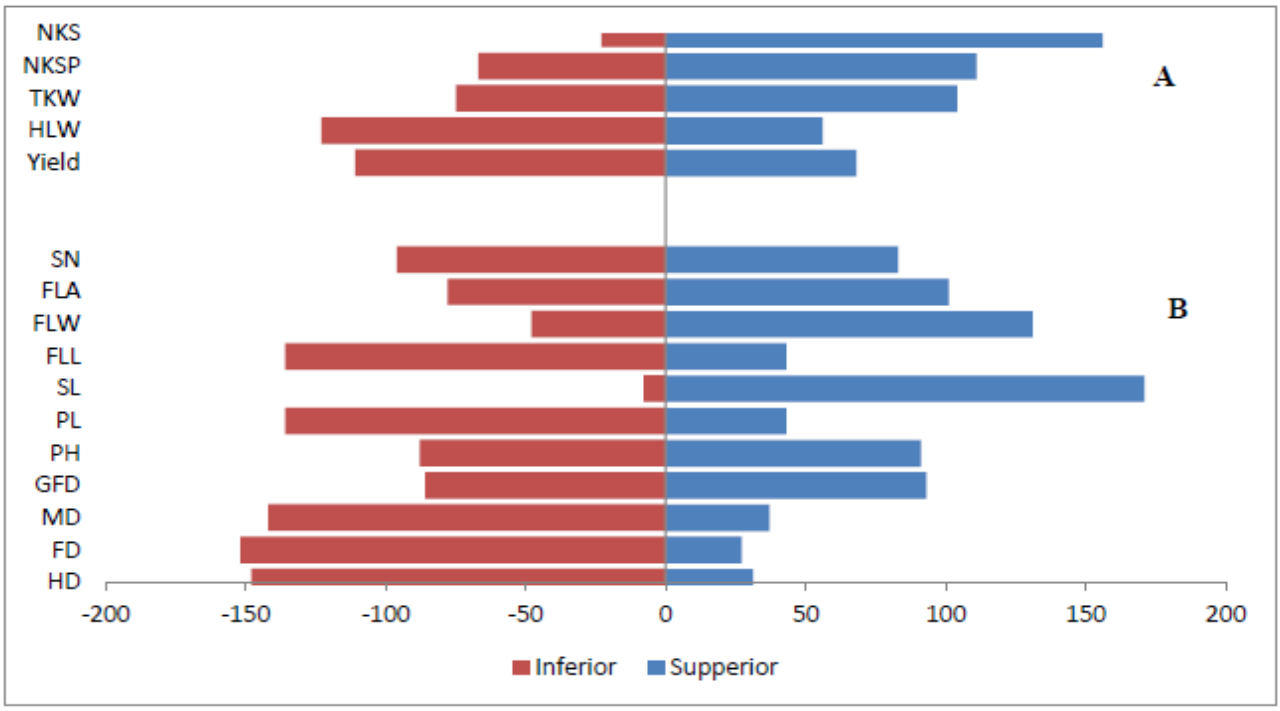

Figure 6.1 Proportion of the wheat genotypes superior and inferior to the check, King-bird.

DH = days to 50% heading; DF = days to 50% flowering; DM = days to 90% maturity; GDF = Grain filling duration; HLW = Hectoliter weight; TKW = 1000 kernel weight; PH = Plant height (cm); PL= Panicle length (cm); SL= Spike length (cm); FLL= Flag leaf length(cm); FLW = Flag leaf width(cm); FLA = Flag leaf area ($cm^{2)}$; SN = number of spikelet per spike; NKPS= Number of kernel per spikelet; and NKS = Number of kernel per spike.

Table 6. 6 Comparison of mean performances of top 5% selected genotypes for grain yield and related traits performance with King-bird, and with mean of 13 released varieties.

Genotype	Mean of selected Genotypes	Comparative advantage (% over)		Genotypes	Mean of selected Genotypes	Comparative advantage (% over)	
		King-bird	MRV*			King-bird	MRV*
Grain Yield (kg ha^{-1})				Number of Kernels per spikelet			
G174	5705.92	21.99	26.86	G165	3.35	13.6	12.76
G176	5613.5	20.02	24.8	G138	3.34	13.32	12.48
G81	5369.5	14.8	19.38	G155	3.33	13.04	12.2
G116	5329.3	13.94	18.49	G124	3.33	13.04	12.2
G169	5289.42	13.09	17.6	G88	3.3	11.9	11.08
G56	5285.25	13	17.51	G172	3.3	11.9	11.08
G51	5275.71	12.8	17.29	G7	3.28	11.34	10.51
G121	5265.25	12.57	17.06	G58	3.28	11.34	10.51
G159	5183.3	10.82	15.24	G114	3.27	11.05	10.23
King-bird	4677.42	---	3.99	King-bird	2.95	---	-0.74
MRV*	4498.03	-3.84	---	MRV*	2.97	0.75	---
Hectoliter weight				Kernels per spike			
G35	50.3	3.31	3.66	G176	51.32	29.92	22.6
G170	50.17	3.05	3.41	G177	49.75	25.95	18.85
G51	50.11	2.92	3.27	G171	49.74	25.91	18.81
G39	50.07	2.85	3.2	G59	49.57	25.49	18.41
G56	49.93	2.56	2.92	G174	48.82	23.59	16.62
G144	49.84	2.36	2.72	G138	48.6	23.04	16.11
G38	49.75	2.19	2.54	G7	48.32	22.33	15.43
G41	49.74	2.16	2.51	G93	47.14	19.33	12.6
G96	49.57	1.83	2.17	G23	46.92	18.78	12.08
King-bird	48.69	---	0.35	King-bird	39.5	---	-5.64
MRV*	48.52	-0.35	---	MRV*	41.87	5.98	---
1000 kernels weight (g)							
G159	44.64	22.25	22.88	Note: *MRV = Mean of 13 selected released varieties. .			
G144	43.21	18.34	18.95				
G97	43.08	17.98	18.59				
G174	42.55	16.53	17.14				
G61	41.82	14.54	15.13				
G131	41.68	14.16	14.75				
G156	41.67	14.12	14.71				
G154	41.66	14.1	14.69				
G153	41.62	13.97	14.56				
King-bird	36.52	---	0.52				
MRV*	36.33	-0.52	---				

Association between agronomic traits

Pearson's correlation among agronomic traits was computed based on across environments data. Most traits showed weak association, some were strongly correlated, while others were not-significantly associated (Table 6.7). Very highly significant ($p < 0.0001$) and nearly perfect positive correlation ($r \geq 0.9$) was observed between days to heading and days to flowering ($r = 0.96$) (Table 6.7). Grain yield showed significant positive association with hectoliter weight ($r= 0.31$; $p < 0.0001$), 1000 kernels weight ($r= 0.38$; $p < 0.0001$), number of kernels per spike ($r = 0.33$; $p < 0.0001$), days to 90% maturity ($r = 0.21$; $p < 0.001$), grain filling duration ($r = 0.24$; $p < 0.001$), plant height ($r = 0.18$; $p < 0.05$), flag leaf area ($r = 0.23$; $p < 0.05$) and number of kernels per spikelet ($r= 0.23$; $p < 0.05$) (Table 6.7). Similarly, number of kernels per spikelet showed significant positive correlation with 1000 kernels weight ($r= 0.18$; $p < 0.05$) and kernels per spike ($r= 0.49$; $p < 0.0001$). Number of kernels per spike was significantly positively correlated with days to 50% flowering ($r= 0.17$; $p < 0.05$), days to 90% maturity ($r= 0.23$; $p < 0.05$), grain filling durations ($r= 0.17$; $p < 0.05$), spike length ($r = 0.25$; $p < 0.001$), flag leaf width ($r=0.26$; $p < 0.001$), flag leaf area ($r = 0.32$; $p < 0.0001$) and number of kernels per spikelet ($r= 0.49$; $p < 0.0001$). Moreover, thousand kernel weight was significantly positively related with grain filling duration ($r = 0.23$; $p < 0.05$), hectoliter weight ($r = 0. 0.39$; $p < 0.0001$), plant height ($r = 0.29$; $p < 0.0001$), panicle length ($r = 0.2$; $p < 0.05$), flag leaf width ($r = 0.21$; $p < 0.05$), flag leaf area ($r = 0.24$; $p < 0.001$), and kernels per spikelet ($r = 0.18$; $p < 0.05$).

Table 6. 7 Association between agronomic traits in 180 bread wheat genotypes

	DH	DF	DM	GDF	Yield	HLW	TKW	PH	PL	SL	FLL	FLW	FLA	SN	NKSP	NKS
DH	1	0.96***	0.78***	0.01^{ns}	-0.06*	-0.06^{ns}	-0.15*	-0.03^{ns}	-0.21**	0.06^{ns}	-0.05^{ns}	0.05^{ns}	0.05^{ns}	0.22**	-0.03^{ns}	0.13^{ns}
DF		1	0.79***	0.04^{ns}	-0.07*	-0.11^{ns}	-0.14^{ns}	-0.04^{ns}	-0.23**	0.09^{ns}	-0.08^{ns}	0.11^{ns}	0.06^{ns}	0.26**	-0.01^{ns}	0.17*
DM			1	0.57***	0.21**	-0.06^{ns}	0.02^{ns}	0.05^{ns}	-0.13^{ns}	0.21*	0.07^{ns}	0.15^{ns}	0.19*	0.36***	0.03^{ns}	0.23*
GDF				1	0.24**	0.07^{ns}	0.23*	0.1^{ns}	0.07^{ns}	0.22*	0.15^{ns}	0.15*	0.24*	0.25**	0.08^{ns}	0.17*
Yield					1	0.31***	0.36***	0.18*	0.11^{ns}	0.08^{ns}	0.07^{ns}	0.17*	0.23*	0.12^{ns}	0.23*	0.33***
HLW						1	0.39***	0.13^{ns}	0.12^{ns}	-0.15^{ns}	0.07^{ns}	-0.02^{ns}	0.05^{ns}	-0.19*	0.02^{ns}	-0.04^{ns}
TKW							1	0.29***	0.2*	0.14^{ns}	0.09^{ns}	0.21*	0.24**	-0.09^{ns}	0.18*	0.1^{ns}
PH								1	0.62***	0.14^{ns}	0.27**	0.07^{ns}	0.29***	0.05^{ns}	0.07^{ns}	-0.02^{ns}
PL									1	0.14^{ns}	0.53***	-0.1^{ns}	0.33***	-0.19*	-0.07^{ns}	-0.14^{ns}
SL										1	0.29***	0.31***	0.44***	0.39***	0.12^{ns}	0.25**
FLL											1	-0.1^{ns}	0.64***	-0.02^{ns}	0.01^{ns}	0.13^{ns}
FLW												1	0.63***	0.21*	0.15^{ns}	0.26**
FLA													1	0.17*	0.15*	0.32***
SN														1	0.09^{ns}	0.43***
NKPS															1	0.49***
NKS																1

DH = days to 50% heading; DF = days to 50% flowering; DM = days to 90% maturity; GDF = Grain filling duration; HLW = Hectoliter weight; TKW = 1000 kernel weight; PH = Plant height (cm); PL= Panicle length (cm); SL= Spike length (cm); FLL= Flag leaf length(cm); FLW = Flag leaf width(cm); FLA = Flag leaf area ($cm^{2)}$; SN = number of spikelet per spike; NKPS= Number of kernel per spikelet; and NKS = Number of kernel per spike.***=very highly significant ($p<0.0001$), **= highly significant and *=significant, ns=non significant at α=5% significance level, (-) = negative correlation. The magnitude of the correlation coefficient indicates the strength of the correlation.

GGE Biplots Analysis

The combined analysis of variance revealed that there was significant differential agronomic performance among tested genotypes over years and across locations. The genotypes showed significant performance fluctuation across locations over years confirming the presence of GxE interaction resulting in crossover *i.e.* change in the relative rank of the genotypes performance from one environment to another. GGE biplot analysis was

employed to display the GxE interaction pattern of grain yield data to visualize the interrelationships among test environments, to identify powerful test environments and also to select superior yielding and stable genotypes. It was observed that the first two principal components (PC1 and PC2) explained 59.59% (PC1= 36.745% and PC2= 22.85%) of the total GGE variations. Thus, the model containing PC1 and PC2 is best for explaining the GxE interaction patterns, and for rejecting noises from the data. In addition, PC1 and PC2 can be readily displayed in a two dimensional biplot so that the interaction between each genotype and each environment can be visualized (Yan and Hunt, 2002).

Interrelationship among test environments

Figure 6.2 presents the interrelationship among the test environments which is important to avoid location redundancy and hence reduce research cost. The lines that connect the environment markers to the biplot origin are called vectors. The cosine of the angle between the vectors of two environments approximates the correlation coefficient between them (Kroonenberg, 1995; Yan and Hunt, 2002). Generally, acute angles have positive correlation, obtuse angles have a negative correlation and right angles no correlation (Yan and Kang, 2003). Environments that have small angles between them could have highly positive correlation. Accordingly, E1, E3, E4, E5 and E6 lie in the same quadrant and are acute angles, suggesting that they are highly positively correlated. The angle between E4 and E5 was considered to be the smallest, implying that they are highly positively correlated and thus, inclusion of one of them in the test environment can give sufficient information

about the genotype. Whereas, as the angle between E2 and E3, E4, E5 or E6 is large enough, implying no or negative correlation between them.

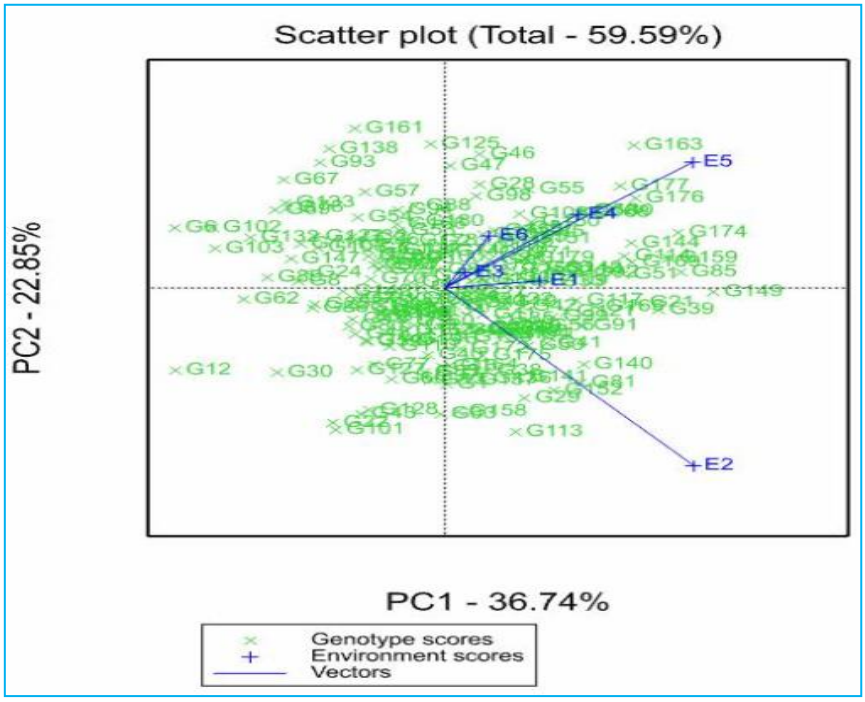

Figure 6. 2 The GGE biplot showing the interrelationships among the six environments. E1 = Holleta in 2015, E2 = Bekoji in 2015, E3 = Kulumsa in 2015, E4= Holleta in 2016, E4 = Bekoji in 2016 and E6 = Kulumsa in 2016. G1-180 represent the 180 tested wheat genotypes

Discriminating ability of the Test Environments

Another important measure of a test environment is its power to discriminate genotypes. A test environment that lacks good discriminating power will no longer provide relevant information on the genotype, and thus its inclusion in the METs is useless. In Figure 6.2, the length of the vector is related to the power of the environment to discriminate the genotypes *i.e* the longer the vector, the more powerful the environment is to discriminate the genotypes

and vice versa. Accordingly, E2 and E5 were observed to have the highest discriminating ability among the test genotypes. Hence, Bekoji is found to be the best environment to test genotypes for grain yield performance. E2 and E5 were followed by E4 which in turn followed by E1. It was observed that E3 (Kulumsa) has the least discriminating ability of the genotypes tested for yield under natural Septoria disease infestations.

Winning Genotype and Mega-environment: The "which-won-where" patterns

The polygon-view of a GGE biplot (Figure 6.3A) provides an effective and elegant means of visualizing the "which-won-where" pattern of a MET dataset (Yan and Hunt, 2001). It is ideal technique to identify the best yielding genotype(s) in each environment and groups of environments (Yan and Hunt, 2002). The polygon was drawn by joining genotypes located farthest away from the biplot origin in various directions so that all genotypes are contained within the resulting polygon. Perpendicular lines (rays) drawn from the biplot origin to each side of the polygon divided the biplot into seven sectors (numbered as 1 to 7), and environments that fall in the same sector constituted a mega environment (environmental group which has similarity to support performance of some genotypes simultaneously) (Yan , 2002). The vertex cultivar in each sector is the highest-yielding cultivar in environments that fell in the particular sector. Accordingly, the six experimental sites were clustered into three mega environments. E3, E4, E5 and E6 fall into the same mega environment indicated by the large circle and genotype G163 was the winning genotype in this mega Environment. Similarly, E1 and E2 made another two separate mega environments in which G149 and

G113 were the wining genotypes in each mega environments, respectively. No environment fell in the sectors with vertex genotype G101, G12, G6 and G161 implying that these genotypes were not the best in any of the test environments; they were the poorest genotypes at all or some environments (Yan and Hunt, 2001).

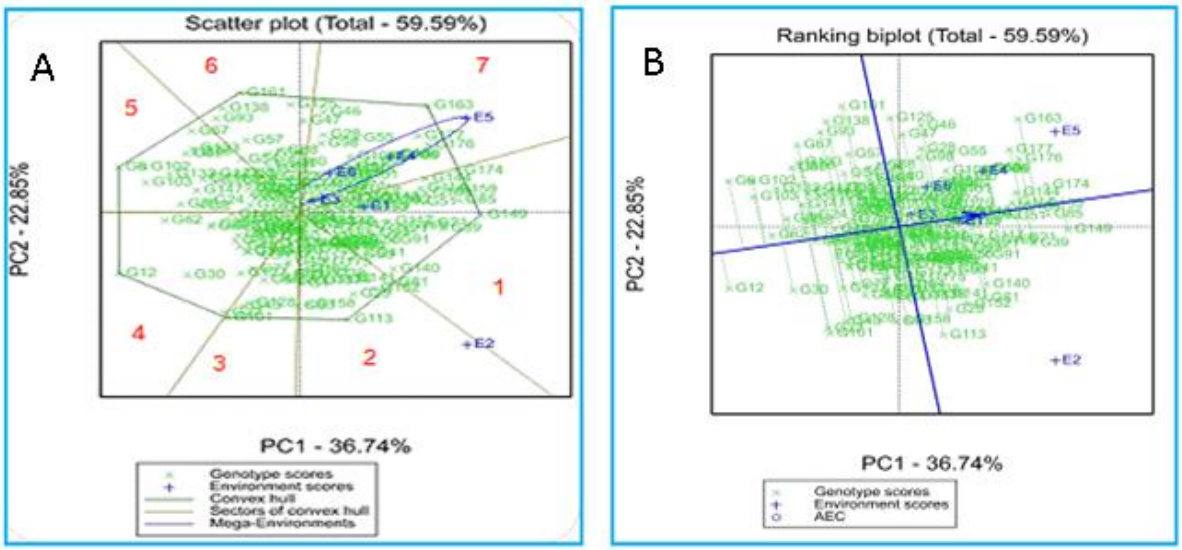

Figure 6. 3 A) The "which-won-where" pattern, and B) Ranking of tested genotypes for yield and stability performance.
E1 = Holleta in 2015, E2 = Bekoji in 2015, E3 = Kulumsa in 2015, E4= Holleta in 2016, E4 = Bekoji in 2016 and E6 = Kulumsa in 2016. G1-180 represent the 180 tested wheat genotypes.

Mean performance and stability of the genotypes across locations

The GGE biplot methodology provides a superior way to breeders to make selection decisions based on both mean performance and stability. Figure 6.3B presents the ranking of

180 bread wheat genotypes based on their mean grain yield performance and stability using an average environment coordinate (AEC) on the genotype-focused biplot. The line that passes through the biplot origin and the small circle (average environment) is called the average environment axis- AEC axis. It represents genotype mean yield performance, and genotypes are ranked along the axis with the arrow pointing towards higher mean values. Accordingly, the genotypes G174, G149, G85, G159, G163, G176, G39, G177 and so on showed the highest mean yield performance. Whereas, genotypes G12, G6 and the like which are located on the left margin of the graph showed the poorest mean yield performance. The line that passes through the GGE biplot origin and is perpendicular to the average environment axis indicates stability of the genotypes. In both directions away from the biplot origin, on this line, displays greater GxE interaction and thus lower stability (Yan, 2002). An ideal genotype should have the highest mean performance and be absolutely stable. It is located close to the origin and has the shortest vector from the AEC axis. Moreover, genotypes on the right side of the vertical line are considered to have yield performance greater than mean yield and the genotypes on the left side of this line had yields less than the mean. Thus, genotypes G85, G159, G169, G174, G149 and so on were identified to be high yielding and stable genotypes. Conversely, G163, G176 and G177 and so on that projected far away from the AEC axis had high mean yield and low stability. Whereas, genotypes G12, G30, G62 and so on which are found on the left margin of the perpendicular axis close to the AEC axis had low mean yield and high stability, while genotype G161 showed low mean grain yield and low stability than the other cultivars (Figure 6.3B).

Evaluation of the genotypes relative to the ideal genotype

Another importance of the GGE biplot is its application in identifying the stable and highly performing genotypes in reference to the ideal genotype. An ideal genotype is the one that has the highest mean yield and stability in all the environments (Farshadfar *et al.,* 2013). Although such an ideal genotype rarely exist in reality, it can be used as a reference for real cultivar evaluation. Concentric circles are drawn using the ideal genotype at the center with an arrow pointing into it. Test genotypes are ranked based on their distance from the ideal genotype, and those closest to the ideal genotype are the most desirable genotypes with highest mean and stability (Yan and Kang, 2003). Figure 6.4A illustrates the ranking of 180 bread wheat genotypes relative to an ideal genotype. Accordingly, genotype G174 was the closest to the ideal genotype, and therefore, it is the most desirable genotype among the tested genotypes with highest yielding ability and stability. It was followed by genotypes G159, G185, G149. G144, G169 and G114 which were indicated in the second smallest concentric circle. Conversely, G12 and G6 were located at farthest distance from the ideal genotype, and thus, they are the undesirable genotypes with poorest yielding ability and highest instability.

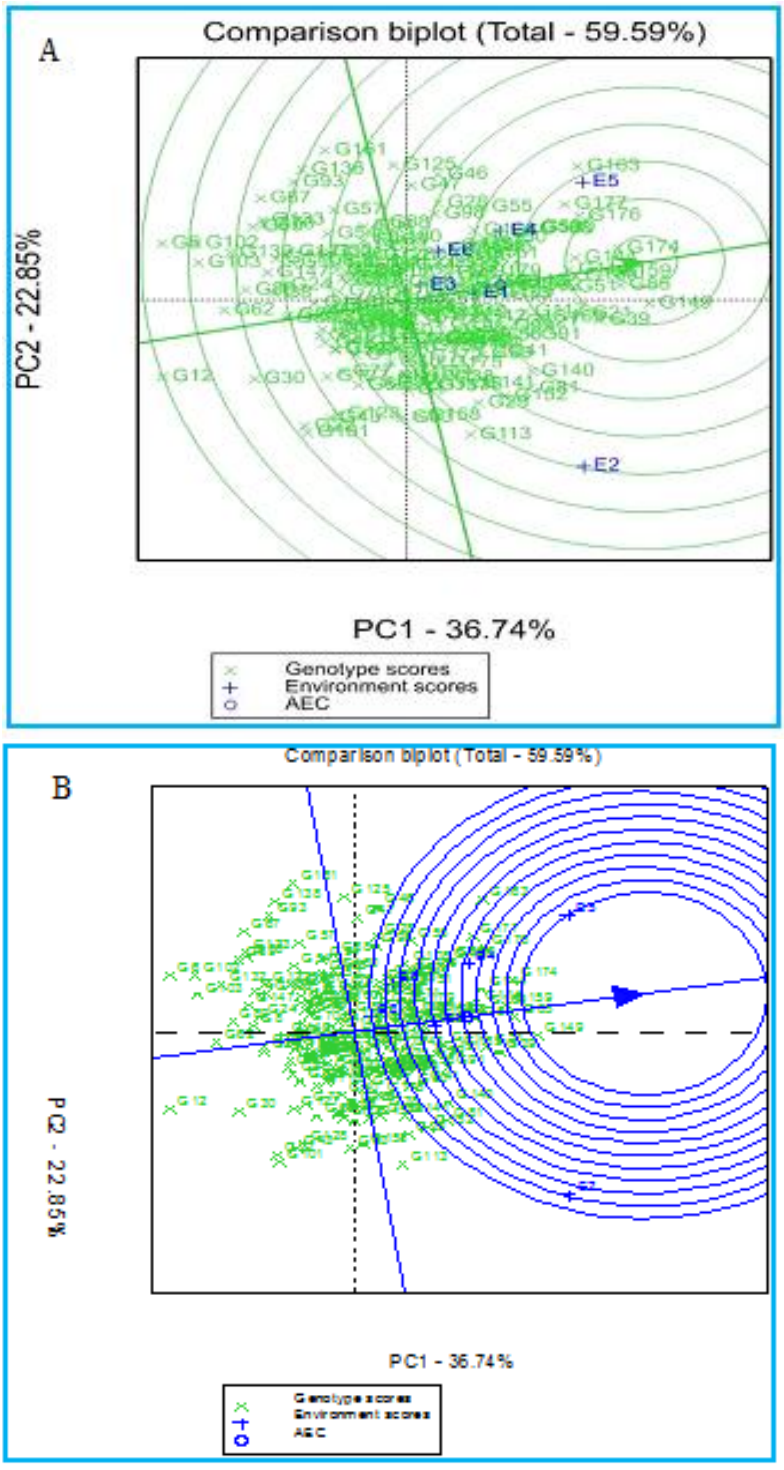

Figure 6. 4 Ranking of the A) test wheat genotypes, and B) test environments relative to the ideal cultivar and environment, respectively.
E1 = Holleta in 2015, E2 = Bekoji in 2015, E3 = Kulumsa in 2015, E4= Holleta in 2016, E4 = Bekoji in 2016 and E6 = Kulumsa in 2016. G1-180 represent the 180 tested wheat genotypes.

Ranking Test Environments based on Representativeness

A test environment should be representative of the target environments; otherwise, it may provide biased information on the test genotypes leading to wrong conclusions (Yan and Kang, 2003). Desirable environments are those environments that are most effective in selecting superior genotypes. Figure 6.4B presents ranking of the test environments relative to the ideal environment based on environment-focused scaling. The average environment is indicated by the small circle at the center of the concentric circles with the arrow pointing to it. The line that passes through the biplot origin and the average environment is the average environment axis. The angle between this axis and the environment vector is a good estimate of representativeness of the test environment. The smaller the angle, the more the representativeness of the environment, and the higher the angle the less representative the test environment is. Hence, the angle between the AEC axis and E1 was found to be the smallest, and thus, Holetta was the most representative test environment followed by E5 which in turn followed by E4 (Figure 6.4B). Whereas, E2 was identified to be the least representative of the average environment. An ideal environment is the one that has the most discriminating ability and absolute representativeness of all test environments. It is indicated by the small circle on the AEC axis, with an arrow pointing to it. Test environments are ranked based on their distance from the ideal environment in which the closest is the most desirable of all the test environments. Accordingly, E5 is the closest environment to the ideal environment, and thus, it is the most desirable of all six environments. It is the most effective environment for selecting superior genotypes -positive selection. It was followed by E4 which in turn is followed by E1. E2, E3 and E6 were found to be the least desirable

test environments; they were not good environments for selecting superior cultivars. Ruther they can be used for culling inferior genotypes, negative selection.

6.4 Discussion

The present investigation was undertaken with multiple purposes including to identify genotypic variability among tested bread wheat genotypes for yield, yield related performances and other agronomic traits and to determine the effect of GxE interaction on these agronomic traits. It was also targeted to explore the association between agronomic traits for direct and indirect selections, to determine high yielding and stable genotypes, and to identify appropriate test environment for germplasm evaluation.

The analysis of variance revealed highly significant differences among tested genotypes for all agronomic traits at all individual environments except hectoliter weight and number of kernels per spike in environment Kulumsa in 2015, and days of 90% maturity and flag leaf area in Bekoji in 2016, respectively. The combined ANOVA also confirmed that grain yield, yield components and other agronomic traits were highly significantly influenced by genotypes, locations, year and their interactions. Similarly, Alemu Dabi *et al.* (2019) reported substantial genetic variability for yield, yield components and other agronomic traits among bread wheat genotypes in Ethiopia. The highly significant ($p<0.0001$) genotypic differences for the studied traits at individual and across locations indicate existence of inherent genetic variability among tested genotypes, suggesting greater possibility of genetic improvement through selection.

The pooled data analysis confirmed that for all agronomic traits, a large proportion of the total phenotypic variance was accounted by the location effect followed by year effect except plant height and spike number where the effect of the later was the largest. This is in line with Gonçalves *et al.* (2003) who reported that a large proportion of the phenotypic variation is caused by the locations effect and their interaction with years, followed by the year effect. The very highly significant and large proportions of location effect indicates possible existence of different mega-environments (test environments are diverse), suggesting the need to either develop specific varieties for different environments or broadly adapted varieties that can perform well under variable conditions. Likewise, the highly significant and large year effect indicates seasonal fluctuation of genotypes performance, and thus, it is important to develop stable genotypes that can perform reasonably well under a range of conditions. Moreover, the very highly significant genotype by location effects for all agronomic traits indicates that the wheat genotypes responded differently to the different environmental conditions suggesting the importance of the assessment of genotypes under different environments in order to identify better performing genotypes for a particular environment (Alemu Dabi *et al.*, 2019).

Moreover, the very highly significant and large genotype by location x year (δ^2gyl) interaction effects for some agronomic traits like grain filling duration, grain yield, hectoliter weight, 1000 kernels weight, number of spikelet per spike and number of kernels per spike indicates that their expression is largely influenced by the environmental interaction effects. This confirms the importance of conducting multi-location and multi-year germplasm

evaluation in order to identify superior yielding and stable wheat genotypes. The significant roles of genotype, environment and their interaction on agronomic traits of wheat were also reported in various studies (Kaya and Akcura 2014; Studnicki *et al.*, 2016). However, for some agronomic traits, the variance due to genotype x location x year (δ^2gyl) was substantially lower than the genotypic variance, suggesting that they are largely under genetic control and the effect of environment is limited (Phuke *et al.*, 2017).

The existence of sufficient genetic variability among tested bread wheat genotypes was evidenced by wide ranges of grain yield and yield components mean performances at individual and across environments. The present grain yield mean performance (1572.5 - 7840 kg ha^{-1}) at individual environments is significantly higher than earlier reports by Gezahegn Fikre *et al.* (2015) and Alemu Dabi *et al.* (2019) who described grain yield mean performances of 1284.4 - 4683.3 kg ha-1 and 2115 kg ha-1 - 5955 kg ha-1, respectively. Although all the study locations represent wheat cultivation belts in Ethiopia, environment Bekoji 2015 resulted in the highest grain yield performance. Moreover, considerable variation was observed for pooled grain yield (2874.13- 5705.92 kg), yield components like hectoliter weight (43.89 -50.29 kghL^{-1}), 1000 kernel weight (36 - 44.63g), kernels per spikelet (2.56 - 3.34), kernels per spike (34.2 - 51.32), and other agronomic traits, confirming the existence of genetic variability among genotypes and hence, the possibility of genetic improvement of these traits through exploiting the variability existing within the materials (Phuke *et al.*, 2017). Similar substantial genetic variability for grain yield and yield component traits performances have been reported for wheat in Ethiopia (Mohammed

Abinasa *et al*., 2011; Obsa Chimdesa *et al.*, 2017) and elsewhere in the world (Khan *et al*., 2013; Mohammad *et al.*, 2013; Khan *et al.*, 2019).

Among 180 tested genotypes, genotype G174 and G12 were the highest (5705.92 $kgha^{-1}$) and lowest (2874.13 $kgha^{-1}$) yielding genotypes across environments, respectively. Best 5% tested genotypes including G174, G176, G81, G116, G169, G56, G51, G121 and G159 showed 10.8 - 21.99% and 3.99 - 26.86% super grain yield performance compared to the standard check variety King-bird, and to the average performance of 13 commercial cultivars in Ethiopia, respectively. This confirms the importance of genetic variability analysis for yield and related traits across locations and over years to identify supper performing and stable genotypes among available genetic resources for successful plant breeding.

Heritability estimates helps to understand the extent to which a specific trait can be transferred to the subsequent generations (Al-Otayk, 2019). It refers to the proportion of phenotypic variance attributed to genetic variance (Ogunniyan and Olakojo, 2014). In line with previous studies, the highest heritability values were obtained for days to heading (Lohithaswa *et al*., 2011), days to maturity (Al-Otayk, 2019), grain filling duration, plant height (Lohithaswa *et al*., 2011; Al-Otayk, 2019) and spike length (Alemu Dabi *et al*., 2019). The high heritability coupled with high genetic advance as percent of mean exhibited by panicle length, flag leaf length and flag leaf area indicates that these traits are largely under additive genetic control, and hence, selection based on any of these traits is highly

desirable and effective for improvement of the traits (Berhanu Meles *et al.,* 2017; Al-Otayk, 2019). Moderate heritability was observed for grain yield, hectoliter weight, 1000 kernels weight, flag leaf width, number of spikelet per spike and number of kernels per spike (Alemu Dabi *et al.,* 2019). The high heritability accompanied by moderate genetic advance as percent of mean observed for days to heading (Berhanu Meles *et al.,* 2017; Alemu Dabi *et al.,* 2019), days to flowering, plant height (Sohail *et al.,* 2018), spike length (Al-Otayk, 2019), flag leaf width, and number of kernels per spikelet confirms that the traits are mostly influenced by interaction of non-additive (dominance and epistatic) gene action and environment (Kumar *et al.,* 2016). The observed high heritability (0.78) coupled with low genetic advance (7%) for days to maturity indicate that the trait is under the influence of non-additive gene action (Kumar *et al.,* 2016). However, some traits like grain filling duration and number of kernels per spikelet exhibited low heritability and low genetic advance as percent of mean, suggesting that they are largely influenced by environment with minor contribution from genetic control, and hence, less likely or virtually impractical to improve through selection (Akinwale *et al*., 2011).

The study revealed that the studied agronomic traits exhibited wide range of phenotypic and genotypic coefficient of variations. For all the agronomic traits the magnitudes of phenotypic coefficient of variations (PCV) are relatively larger than the respective values of genotypic coefficient of variation (GCV) (Aharizad *et al*., 2012; Al-Otayk, 2019; Taneva *et al.*,2019), likely due to larger environmental effect in the expression of the traits. The larger differences between PCV and GCV observed in grain filling duration, grain yield, TKW,

flag leaf area, number of kernels per spike and number of kernels per spike could be associated with larger environmental than genotypic impact on the variation of this traits (Taneva *et al.*,2019). Similar to previous studies (Aharizad *et al*., 2012; Al-Otayk, 2019; Alemu Dabi *et al.,* 2019) grain yield showed the highest (22.54%) PCV. In agreement with our results, moderate PCV was reported for 1000 kernels weight and number of kernels per spike (Alemu Dabi *et al.,* 2019; Misganaw Ferede and Fisseha Worede, 2020). Some agronomic traits like days to heading, days to flowering, days to maturity, grain filling duration, hectoliter weight, plant height and spike number exhibited low (< 10 %) PCV and GCV, indicating lack of sufficient genetic variability, and hence, less scope of selection for improvement. The smaller differences between PCV and GCV for days to heading, days to flowering, days to maturity, plant height, panicle length, flag leaf length and flag leaf area indicates the less influence of the environment on the phenotypic expression of the respective trait (Taneva *et al.,* 2019). In the present study the relatively larger GCV and PCV for grain yield and flag leaf area encourage wheat breeders to use these parameters in selection of suitable genotypes for crossing or further improvements (Ogunniyan and Olakojo, 2015).

In breeding program, the selection of desirable agronomic trait (eg. yield) depends on several other contributing agronomic traits, and hence, understanding the complex chain of associations among agronomic traits make the selection process simple and successful. In line with this the studied agronomic traits exhibited wide range of associations from highly significant and nearly perfect positive correlation ($r = 0.96$; $p < 0.0001$) between days to

heading and days to 50% flowering to highly significant and negative correlation (r = - 0.23; $p< 0.001$) between days to flowering and panicle length. Similar to our results, previous studies confirmed significant and positive association of grain yield with hectoliter weight, 1000 kernels weight, number of kernels per spike, days to maturity, grain filling duration, plant height, flag leaf area, number of kernels per spikelet and number of kernels per spike (Sohail *et al.,* 2018), indicating that grain yield performance in wheat can be indirectly selected for, by considering any of these agronomic traits (Wani *et al*., 2018).

Pooled data revealed highly significant differences for grain yield among tested genotypes across locations and over years. The highly significant GxE interaction effect caused considerable change in ranking order of the tested genotypes over environments, confirming the relevance of multi-environment than a single environment trials to identify and select best genotypes (Yan and Kang, 2003). The presence of crossover interaction indicates that both G and GxE must be simultaneously considered in wheat genotype evaluation and selection for grain yield performance. Similar to reports by previous authors (Mohamed *et al.*, 2013; Verma *et al.*, 2015; Muez Mehari *et al.,* 2015) the GGE biplot analysis disclosed the relevance of G + GE on tested genotypes yield performance. The GGE biplot explained 59.59% of the total G+ GE variations.

The GGE biplot displayed the interrelationships present between the test environments. Except Bekoji 2015, all the test environments exhibited positive correlation, suggesting that similar information on the genotypes grain yield performance could be generated by

considering only few of the test environments if budget is a limiting factor (Yan and Kang, 2003). In line with this, Farshadfar *et al*.(2011) stated that if two test environments are correlated consistently across years, one of them can be dropped without loss of much information about the genotypes. Environment Bekoji 2016 was identified to have high discrimination power and representatives and hence, it is ideal or the most desirable and effective environment for selecting superior genotypes (positive selection) under the presence of Septoria disease and other limiting factors. Similarly, Zerihun Tadesse *et al*. (2016) reported the higher grain yield performance at Bekoji location. Holetta 2016 was identified to be the second best environment for grain yield performance evaluation. On the other hand, environment Bekoji 2015 which showed negative correlation with other test environments (Farshadfar and Sadeghi, 2014), and lower representativeness is found to be useful for removing unstable wheat genotypes (Phuke *et al.,* 2017). While, Kulumsa 2015 and Kulumsa 2016 showed lower discriminating power for yield performance evaluation under natural Septoria disease infestations. Hence, among tested environments, Bekoji 2015, Kulumsa 2015 and Kulumsa 2016 were the least desirable environments for selecting superior yielding cultivars. Rather they can be used for negative selection (culling inferior genotypes). The present result contradicts the report of Zerihun Tadesse *et al.* (2016) who reported high grain yield performance at Kulumsa.

Similar to earlier studies (Ding *et al.,* 2008; Frashadfar *et al.,* 2012; 2013; Farshadfar and Sadeghi, 2014; Phuke *et al.,* 2017) the "which-won-where" pattern analysis identified three environmental clusters with their corresponding wining genotypes (Yan and Hunt 2002).

Kulumsa 2015, Kulumsa 2016, Holetta 2016, and Bekoji 2016 constituted same mega environment where genotype G163 was the winning genotype and hence, recommended for this specific mega-environment (Yan and Hunt 2002). Likewise, Holetta 2015 and Bekoji 2015 formed another two environmental clusters where G149 and G113 were the corresponding best genotypes at each environment, respectively. Whereas, some genotypes like G101, G12, G6 and G161 were identified to be the poorest at all tested environments (Yan, 2001).

The GGE biplot methodology is also super for visualizing the interrelationship among genotypes based on mean performance and stability (Yan and Kang, 2003). Like earlier reports by Ding *et al.*(2008) the GGE biplot clustering of the genotypes based on mean performance and stability identified four groups. The first group consisted of highly desirable genotypes with high yield and high stability. The genotypes G21, G51, G85, G114, G144, G159, G169, and G174 are some of the genotypes in this cluster. Genotypes ranking in reference to ideal genotype revealed that G159, G169 and G174 are the most desirable genotypes with high yield and high stability across all environments (Farshadfar *et al.*, 2013). The second group composed of high yielding but, less stable genotypes like G29, G46, G47, G55, G58, G81, G98, G113, G125, G140, G152, G163 *etc.* which may be useful for specific selections. The third genotype cluster includes low yielding and low stable genotypes such as genotype G67, G93, G101, G128, G138, G161 *etc.* which could be desirable for other breeding purposes like selection for drought. The fourth cluster includes the most undesirable genotypes like G6, G8, G12, G14, G24, G30, G62, G102, G103 and *etc*

which are with low yield but, high stability. The present result is in consistent with previous studies (Ding *et al*., 2008; Mohammed, 2013; Mohammed *et al*., 2013, Ding *et al*., 2015).

6.5 Conclusion

The present study on evaluation of bread wheat genotypes in multi- locations and years confirmed the presence of wide genetic variability among tested genotypes for grain yield, yield components and other agronomic traits, indicating presence of promising possibilities for improvement of the traits by exploiting the available variations. Genetic variability is prerequisite for selection of potential parental lines for crossing or genotypes for further trait improvements through breeding. Except some traits like days to maturity, grain filling duration, number of spikelet per spike and number of kernels per spike, all the studied agronomic traits showed moderate to high heritability indicating the scope of selection for improvement. The study revealed significant and positive association of grain yield with hectoliter weight, 1000 kernels weight, number of kernels per spike, days to maturity, grain filling duration, flag leaf area, number of kernels per spikelet and number of kernels per spike, indicating that yield performance in wheat can be simultaneously improved indirectly by considering any of these traits. The present study identified genotypes that have 10.8 - 21.99 % and 3.99 - 26.86% superior grain yield performance as compared to the standard check variety King-bird, and to the average performance of commercial cultivars grown in Ethiopia, respectively. The very highly significant genotype by location by year interaction effect on grain yield, yield related and other agronomic traits indicates the significance of multi location and years germplasm evaluation to identify the higher yielding and stable

cultivars. The biplot analysis also confirmed the existence of crossover interaction for grain yield performance, suggesting that breeders need to make special care like use of checks and appropriate experimental design to minimize environmental errors. The analysis also confirmed existence of different mega-environments for grain yield performance. Kulumsa 2015, Kulumsa 2016, Holetta 2016, and Bekoji 2016 formed similar mega-environment where genotype G163 was the winning genotype. Likewise, Holetta 2015 and Bekoji 2015 formed two environmental groups where G149 and G113 were the best genotypes at each environment, respectively. Bekoji 2016 is identified to be ideal test environments (representative and discriminating) for selecting superior yielding and stable genotypes. The genotypes clustering based on mean grain yield performance and stability identified four clusters, among which the genotypes G85, G159, 169 and G174 are the top four most desirable genotypes performing near to the "ideal" genotype. Therefore, they could be considered as promising high yielding and highly stabile genotypes to develop new cultivars to boost wheat production and productivity in Ethiopia and the region at large.

CHAPTER 7

7. Genome-wide association study of quantitative resistance to Septoria tritici blotch in wheat (*T. aestivum* L.) association panel in Ethiopia

Abstract

Septoria tritici blotch (STB) caused by the fungus *Zymoseptoria tritici* poses serious and persistent challenges to wheat cultivation in Ethiopia and worldwide. Deploying resistant cultivars is a major component of controlling STB. Our objective was to elucidate the genomic architecture of STB resistance in an association panel of 178 bread wheat genotypes. The association panel was phenotyped for STB resistance, phenology, yield and yield-related traits at three locations for two years. The panel was genotyped for SNP markers using DArTseq high-throughput genotyping platform, and a total of 7,776 polymorphic SNPs were used in subsequent analysis. Marker-trait associations were computed using GAPIT. Broad sense heritability for STB resistance ranged from 0.58 - 0.97 and 0.72 - 0.81 at individual and across environments, respectively, indicating the presence of STB resistance alleles in the association panel. Population structure and principal component analysis detected two sub-groups with greater degree of admixture. LD analysis confirmed the existence of significant ($p \leq 0.01$) linkage between 27.61% marker pairs and that LD declined within overall mean of 31.44 Mbp. The association analysis identified 53 loci significantly (FDR <0.05) associated with STB resistance pointing to 33 putative QTLs. Most of these putative QTLs had shared similar chromosome with already published Septoria resistance genes and QTLs which were distributed across chromosomes 1B, 1D, 2A, 2B, 2D, 3A, 3B, 3D, 4A, 5A, 5B, 6A,7A,7B and 7D. However, five of the putative QTLs identified on chromosomes 1A, 5D and 6B appeared to be novel. Dissecting the detected loci on IWGSC RefSeq Annotation v2.1 revealed the existence of disease resistance associated genes in the identified QTL regions which are involved in plant defense system. The putative QTLs explained between 2.70 and 13.20% of the total phenotypic variation. Seven of the QTLs for STB resistance also co-localized with MTAs for agronomic traits. Overall, the present analysis reports on putative QTLs for adult-plant resistance to STB and some important agronomic traits. Previously reported and novel QTL were identified indicating the possibility to improve STB resistance by pyramiding the QTLs using marker-assisted selection.

7.1 Introduction

Common wheat (*Triticum aestivum* L.) is the most widely cultivated and the major staple food crop in the world consumed by human, providing almost 20% of the total calories and 20 % protein demand globally (Arzani and Ashraf, 2017; IWGSC, 2018; Ramadas *et al*., 2019). By 2050, the world's human population is projected to reach nine billion, and we will need to increase wheat production by 70% to feed this projected growth (FAO, 2009; Ray *et al*., 2013; Marcussen *et al*., 2014). Hence, boosting the wheat harvest is very pertinent to achieve zero-hunger by 2050.

Septoria tritici blotch (STB) caused by the fungus *Zymoseptoria tritici* (teleomorph *Mycosphaerella graminicol*; anamorph: *Septoria tritici*) is an ever-existing bottleneck to wheat cultivation worldwide (Dalvand *et al.,* 2018; Odilbekov *et al*., 2019) accounting for 30 - 54% global wheat yield loss annually (Eyal *et al*., 1987). STB is one of the major threat to wheat production in Ethiopia (Getinet Gebeyehu *et al.,* 1990; Abera Takele *et al.,* 2015; Yosef Geberehawariat *et al*., 2017; Tilahun Mekonnen *et al*., 2019, 2020) causing up to 82% yield loss in the worst seasons (Getinet Gebeyehu *et al*., 1990; Mengistu Huluka *et al*., 1991; Ayele Badebo *et al*., 2008).

Developing and implementing effective and durable management strategies to control STB is important (Fones and Gurr, 2015; Talbot, 2015). Host-plant resistance is a durable, economical and environmentally friendly strategy for STB management (Ghanei *et al*., 2012; Odilbekov *et al.*, 2019; Tilahun Mekonnen *et al*., 2019). Qualitative and quantitative

resistances to STB have been reported in wheat (Arraiano and Brown, 2006; Arraiano *et al*., 2009). The former refers to a condition where one or a few major *Stb* genes provide resistance to specific *Z. tritici* isolate (Brown *et al*., 2015). Quantitative resistance results from expression of many genes with minor effects and is not isolate specific: quantitative resistance is the most effective, durable and preferred method to manage rapidly evolving wheat pathogens including *Z. tritici* (Long *et al*., 2019).

Conventional, phenotype-based selection predominates in Ethiopia making the crop improvement program very slow and inefficient. The development and use of modern genomic tools have revolutionized crop breeding by facilitating precise identification, mapping and introgression of genomic regions controlling useful agronomic traits. Genome-Wide Association Studies (GWAS) is a powerful approach to elucidate the genomic architecture of many traits (Long *et al*., 2019). The development of high-throughput sequencing and bioinformatics technologies (Huang *et al*., 2017) have enabled GWAS to scan SNPs associated with a desirable trait at the whole-genome scale (Rafalski *et al*, 2010). Genotyping-by-sequencing (GBS) which involves sequencing of a subset of a complex genome, has enabled the generation of high-density SNPs in wheat at reasonably low cost (Bernardo *et al*., 2015).

GWAS has been successfully applied to many crop species (Xiao *et al.*, 2017) including maize (Rashid *et al*., 2018), rice (Huang *et al*., 2012), wheat (Long *et al*., 2019; Cheng *et al*., 2020) and sorghum (Gezehagn Girma *et al*., 2019). GWAS has been used in wheat to

analyze several traits including resistance to stripe rust (Long *et al*., 2019; Yao *et al*., 2019; Cheng *et al*., 2020), stem rust (Kankwatsa *et al*., 2017; Erena Aka *et al*., 2018) and Septoria tritici blotch (Yosef Geberehawariat *et al*., 2017; Odilbekov *et al*., 2019), drought tolerance (Mathew *et al*., 2019) and other phenological characteristics, yield and yield-related traits (Jamil *et al*, 2019; Wang *et al*., 2019; Ward *et al*., 2019). Our objectives were to elucidate the population structure, linkage disequilibrium and genomic architecture of adult-plant resistance to STB in Ethiopia to test the hypothesis that the tested wheat cultivars contain new sources of resistance that could be useful in Ethiopian wheat breeding.

7.2 Materials and Methods

Association mapping panel

This study used an association panel of 180 bread wheat (*Triticum aestivum* L.) (**Appendix IV**) genotypes: 167 were obtained from International Maize and Wheat Improvement Center (CIMMYT-Mexico) while, and 13 were commercial cultivars grown in Ethiopia. The 167 CIMMYT genotypes included 49 from a set of the international bread wheat screening nursery (IBWSN), 56 were from the international Septoria observation nursery (ISEPON), 14 were from the high rain wheat yield trial (HRWYT), 34 were from the high-rain wheat screening nursery (HRWSN), five were from an adaptation trial, six came from the national variety trial (NVT), and the remaining three genotypes were from a preliminary variety trial (PVT).

Multi Environment Trials

Field evaluation were carried out under natural STB infestation during 2015 and 2016 main cropping seasons across three STB hotspots: Holetta Agricultural Research Center (HARC) (9° 3' N/38° 30' E), Bekoji Agricultural Research Sub-center (7° 32'N/39° 15'E) and Kulumsa Agricultural Research Center (KARC) (8° 02’ N/ 39° 15 ' E) . The experimental design was an alpha lattice with two replications, six incomplete blocks and 30 entries per sub-block per replication. The trial was sown by hand with each entry sowed in four rows of 2.5 m length, 20 cm spacing between rows and 40 cm between entries. The susceptible cv. "Lakech" was planted as a spreader row along the length of the blocks to create adequate disease pressure. Spaces between blocks and replications were 1.50 m long. A seeding rate of 150 kg ha-1 and fertilizer rates of 100 and 75 kg ha^{-1} N and P_2O_5, respectively, were used in all experiments. Weeding was done three times each season by hand.

Septoria tritici blotch Evaluation

Septoria tritici blotch disease severity (SDS) was visually estimated plot-wise by considering the percentage of necrotic leaf area (NLA) on the four uppermost infected leaves of 10 - 20 plants (Eyal *et al*., 1987) at three growth stages: at heading (SDH), medium milk stage (SDMM) and at maturity stages (SDM) using a double-digit 00 - 99 scoring scale (Eyal *et al.* 1987). The first digit (0 - 9) represents the blotch development up the plant height (for instance 5 if the disease reached at the middle (50%) of the plant height, 8 for flag leaf and 9 for spike), and the second digit stands for disease severity as a percentage but

in terms of 0 - 9 (1 = 10%, 2 = 20% and 9 = 90 %). For each stage, Septoria disease severity percentage (SDS %) was computed from the 00 - 99 score using the following formula as described by Sharma and Duveiller (2007):

$$\text{SDS} = \left[\left(\frac{D1}{9}\right)\left(\frac{D2}{9}\right)\right] x100$$

where D1 and D2 are the first and the second digits of double-digit scores, respectively. SDS values range from 0 to 100, where 0 would indicate complete resistance, and 100 would indicate complete susceptibility (Yosef Geberehawariat*et al.,* 2017).

In addition, the Septoria progress coefficient (SPC) developed by Eyal and Ziv (1974) was computed to indicate the position of pycnidia relative to plant height according to the following equation.

$$SPC = \left(\frac{SDH}{PH}\right)$$

where *SDH* (Septoria disease height) is the maximum height (cm) above ground at which the pycnidia of the pathogen could be found on the plant at the maturity stage and *PH* is the average height of the genotype from ground to the tip of its awn. The SPC coefficient indicates the position of pycnidia relative to plant height, regardless of pycnidial coverage, and allows the comparison of infection placement on cultivars with different plant stature. *SPC* values range from 0.0 to 1.0 where *SPC* = 0 means there is no disease, while *SPC* =1 mean pycnidia are produced to the top of the plant (Eyal *et al.,* 1987).

DNA Extraction and Genotyping by Sequencing

Wheat plants of the association panel were grown at the National Agricultural Biotechnology Research Center, Holetta, under greenhouse conditions. Two week-old leaf samples were collected into 96 deep-well sample collection plates, oven-dried overnight at 50 °C and sent to Integrated Genotyping Service and Support (IGSS) located at the Biosciences Eastern and Central Africa (BecA-ILRI) Hub in Nairobi, Kenya for high-density genotyping using Diversity Arrays Technology sequencing (DArTseq™ technology). DNA extraction was carried out using the Nucleomag Plant Genomic DNA extraction kit (MACHEREY-NAGEL GmbH & Co. KG, Germany). Extracted DNA quality and quantity were checked on a nanodrop spectrophotometer and on 0.8% agarose gels. The extracted genomic DNA concentration ranged from 50-100 ng μl^{-1}. Whole-genome profiling was carried out using genotype-by-sequencing (GBS) platform as described by Elshire *et al*. (2011). It involved library construction following the DArTSeq complexity reduction method via digestion of genomic DNA using *ApeKI* (a type II restriction endonuclease that recognizes a degenerate 5-bp sequence (GCWGC, where W is A or T), ligation of one common and one barcode adaptor to the sticky ends of each fragment followed by PCR amplification of adapter-ligated fragments. Libraries were sequenced using single-read sequencing runs for 77 bases. Next generation sequencing of the GBS library was carried out using an Illumina HiSeq2500 lane following the manufacturer's protocol.

Quality Control and SNP Calling

Technical quality of sequencing was checked using *Sequencing Analysis Viewer*. DArTSeq markers were scored using *DArTsoft*14 software implemented in the KDCompute plug-in system developed by Diversity Arrays Technology (http://www.kddart.org/ kdcompute. html) based on alignment with the reference genome of Chinese Spring Wheat RefSeq v1.0 (IWGSC, 2018), obtained from the International Wheat Genome Sequencing Consortium database (https://urgi.versailles.inra.fr/download/iwgsc/) at minimum base identity of 90% and e-value of 5e-10. Two types of markers were scored: SilicoDArT markers and SNP markers, which were both scored in the binary fashion (1/0) indicating the presence or absence of a marker in the genomic representation of each sample as described by Akbari *et al*. (2006). Marker quality was maintained by removing mono-morphic markers and those with lower call rate (>30% missing) and MAF (minor allele frequencies) < 5% using *ArTSoft14* software.

Phenotypic Data Analysis

We conducted an analysis of variance (ANOVA) for each location in each year using SAS software version 9.2 (SAS Institute Inc., 2008) by considering genotype and the block as fixed and random factors, respectively. At an individual environment, the observed phenotypic response of the i^{th} genotype in j^{th} replication and l^{th} sub-block was computed using the following model:

$$y_{ijl} = \mu + g_i + \gamma_j + bl_{(j)} + \varepsilon_{ij}$$

where y_{ijl} = observed phenotype, μ = grand mean, g_i = fixed effect of the i^{th} genotype, γ_j = effect of the j^{th} replication, $bl_{(j)}$ = random effect of the l^{th} block nested within the j^{th} replication, and ε_{ijl} = random error term.

The ANOVA over all seasons and locations was executed by considering genotype as a fixed effect and the block, location and year as random effect according to the following model:

$$Y_{ijklm} = \mu + g_m + \gamma_{ijk} + y_{ij} + e_j + b_{ijkl} + (gy)_{im} + (ge)_{jm} + (ye) + (yeg)_{ijm} + \varepsilon_{ijklm}$$

Where Y_{ijklm} = observed response of genotype m, replication k of block l of location j and year i; μ = grand mean; g_m = fixed effect of genotype m; r_{ijk} = effect of replication k in location j and year i; y_{ij} = random effect of year i at location j and is ~ Normally and Independently Distributed (NID) (0, δ^2_y); e_j = random effect of location j and is ~ NID(0, δ^2_e); b_{ijkl} = random effect of block l nested with replication k in location j and year i and is ~ NID(0, δ^2_b); $(gy)_{im}$ = random effect of the interaction between genotype m and year i and is ~ NID(0, δ^2_{gy}); $(ge)_{jm}$ = random effect of the interaction between genotype m and location j and is ~ NID(0, δ^2_{ge}); $(ye)_{ij}$ = random effect of the interaction between year i and location j and is ~ NID(0, δ^2_{ye}); $(yeg)_{ijm}$ = random effect of the three-way interaction of genotype m in location j and year i and is ~ NID(0, δ^2_{gey}), and ε_{ijklm} = random residual effect and ~ NID(0,δ^2_{ε}).

Variance components were computed. Broad sense heritability (H^2) within an environment level was estimated for the STB resistance traits from analysis of variance using the following formula: $H^2 = \frac{\delta^2 g}{\delta^2 g + \delta^2 \varepsilon / r}$ .Broad-sense heritabilities across environments were estimated by the formula-

$$H^2 = \frac{\delta^2 g}{\delta^2 g + \delta^2 gy/y + \delta^2 ge/l + \delta^2 gye/yl + \delta^2 \varepsilon/ylr}$$

Where, $\delta^2 g$ is the genotypic variance, $\sigma^2 gy$ is the genotype-by-year interaction variance, $\sigma^2 ge$ is the genotype-by-location interaction variance, $\sigma^2 gye$ is the genotype-by-year-by-location interaction variance, and $\delta^2 e$ is the location variance and l, r, and y represent the numbers of locations, replicates and years, respectively. The heritability percentage was categorized as low (<30%), moderate (30- 60%) or high (≥ 60%) as described by Robinson *et al*. (1949). The relationship between agronomic traits was determined using Pearson's correlation using SAS software version 9.2 (SAS Institute Inc., 2008).

Population Structure Analysis

Population stratification of the association panel was visualized using principal components analysis (PCA) using KDCompute plugins system version 1.0.1 (https://kdcompute.seqart.net/kd_compute/plugins). Population admixture patterns were determined using a Bayesian model-based clustering algorithm implemented in STRUCTURE software v.2.3.4 (Pritchard *etal.*, 2000). The STRUCTURE program was run with the admixture model, correlated allele frequencies, a burn-in period of 10,000, and 50,000 Markov Chain Monte Carlo (MCMC) replications after burn in for the hypothetical

sub-population K from 1 to 10 with 10 iterations. The optimum K value was predicted based on Evanno *et al.* (2005) using STRUCTURE HARVESTER ver. 0.6.92 (Earl and Von Holdt, 2012). A bar plot for the optimum K was determined using Clumpak beta version (Kopelman *et al*., 2015).

Genome-Wide Association Study

Association mapping of phenotypic traits with Genome-wide Scand SNPs was conducted using Genome Association and Prediction Integrated Tools (GAPIT) packages (Lipka *et al*., 2012) in R software (R Core Team, 2017). GWAS was carried out for four Septoria disease traits including SDSH, SDSMM, SDSM and SPC, and some important agronomic traits such as days to 50% heading (DH), days to 50% flowering (DF), grain filling duration (GFD), days to 90% maturity (DM), grain yield, thousand-kernel weight (TKW) and plant height (PH) in each individual environment and using means across all environments (The Best Linear Unbiased Estimates or BLUE values). The analysis involved a total of 7,776 robust SNPs with call rate > 70% and MAF > 5%. Missing SNPs data were imputed using optimal impute ver. 1.0.0 in the KDcompute_plugin system based on the KNN imputation method. The markers distribution on each chromosome was determined using LD measure R^2 ver.0.2.2 in KDcompute_plugin. Pairwise LD measures (r^2 and *P*-value) between markers on each chromosome were computed using TASSEL Ver. 5 (Bradbury *et al*., 2007). A genome- wide LD decay scatter plot was produced by plotting the r^2 values against physical distance (bp) using GAPIT software. $r^2 = 0.2$ was considered as a cutoff point for no LD between pairs of markers.

GWAS was conducted using the Fixed and Random model Circulating Probability Unification (FarmCPU) algorithm (Liu *et al*., 2016) implemented in the GAPIT R package (2.0) (Tang *et al.*, 2016). The model uses both a fixed-effects model and the random-effects model iteratively to control spurious marker-trait association due to population structure and family relatedness (Lipka *et al*., 2012). A kinship (K) matrix was computed using the method of VanRaden (2008). Principal components describing the population stratification were computed using R/GAPIT and iteratively added to the fixed part of the model. Quantile-quantile (QQ)-plot generated from –log10 *p-values* were visually assessed to determine how well the model accounted for population structure and family relatedness among study samples. Statistically significant marker-trait- associations were declared using a false discovery rate- adjusted $p < 0.05$ as implemented in GAPIT. Further, the Bonferroni correction rate at a significance threshold of $p < 0.15$ or –log10 (*p-values*) = 4.71 was also included in the analysis for comparison. Q–Q and Manhattan plot were visualized using the R package qqman (Turner, 2014). High confident candidate genes within the identified resistance associated regions were extracted from the recently released IWGSC RefSeq Annotation v2.1 available on URGI Seq repository(https://wheat-urgi.versailles.inrae.fr/Seq-Repository/Annotations).

7.3 Results

Adult-plant responses to Septoria tritici blotch and broad-sense heritability

The genotypes effect was significant ($p < 0.0001$) for STB resistance at all growth stages in all test environments. Genotypic variance ($\sigma^2 g$) was the major contributor to STB resistance variability among the tested wheat genotypes. STB infestation showed seasonal fluctuation, and it was higher during the 2015 growing season (Table 7.1). Moreover, the disease severity showed increasing trend from heading to maturity stages. At each environment, mean SDS values at heading and mid-maturity stages ranged from 18.16 - 31.23% and 21.73-37.6 %, respectively and the highest severity values were registered at Holetta in 2015. The mean disease severity at maturity and its vertical progress varied from 30.04 - 50.77 % and 0.41 – 0.69, respectively, and were the highest at Bekoji in 2015 growing season. The lowest Septoria severity was recorded at Kulumsa in 2016. Broad-sense heritability for Septoria resistance in each environment ranged from moderate ($h^2 = 0.58$) to high ($h^2 = 0.99$) (Table 7.1).

At maturity, the mean disease severity and its vertical progress varied from 30.04 - 50.77 % and 0.41 – 0.69, respectively, and the highest values were recorded at Bekoji during the 2015 growing season (Table 7.1). The lowest Septoria severity was recorded at Kulumsa in 2016. Broad-sense heritability for Septoria resistance traits in each environment ranged from moderate ($h^2 = 0.58$) to high ($h^2 = 0.99$) (Table 7.2).

Table 7. 1 SDS values in wheat genotypes evaluated in six individual environments.

Trait	Environment	Mean	Range	SD	Pr > F	Trait	Environment	Mean	Range	SD	Pr > F
SDSH	E1(HARC2015)	31.23	72.84	8.88	***	SDSM	E1 (HARC 2015)	46.9	95.68	24.07	***
	E2 (Bekoji 2015)	24.48	63.58	6.8	***		E2 (Bekoji 2015)	50.77	77.16	19.05	***
	E3(Kulumsa 2015)	23.47	40.74	8.00	***		E3 (Kulumsa 2015)	32.38	70.99	9.44	***
	E4 (HARC 2016)	20.09	39.81	4.88	***		E4 (HARC 2016)	33.07	65.44	1.8	***
	E5 (Bekoji 2016)	19.36	44.71	9.08	***		E5 (Bekoji 2016)	34.28	55.56	10.41	***
	E6(Kulumsa 2016)	18.16	38.89	6.87	***		E6(Kulumsa 2016)	30.04	46.94	9.61	***
SDSMM	E1 (HARC 2015)	37.6	82.72	22.7	***	SPC	E1 (HARC 2015)	0.65	0.87	0.2	***
	E2 (Bekoji 2015)	34.56	74.07	17.2	***		E2 (Bekoji 2015)	0.69	0.74	0.12	***
	E3(Kulumsa 2015)	32.13	49.51	8.2	***		E3(Kulumsa 2015)	0.41	0.60	0.09	***
	E4 (HARC 2016)	27.13	58.95	5.29	***		E4 (HARC 2016)	0.6	0.62	0.09	***
	E5 (Bekoji 2016)	25.6	47.03	9.51	***		E5 (Bekoji 2016)	0.58	0.61	0.12	***
	E6(Kulumsa 2016)	21.73	39.51	8.35	***		E6(Kulumsa 2016)	0.58	0.46	0.08	***

SDSH= Septoria disease severity at heading, SDSMM = Septoria disease severity at mid- maturity, SDSM= Septoria disease severity at maturity, SPC= Septoria progress coefficient, SD = standard deviation, ***= very highly significant ($p \leq 0.0001$), * = significant, and "sn"= Non significant at $\alpha = 5\%$ significant level.

Table 7. 2 Genotypic variance (σ^2g) and heritability estimate for SDS traits measured in 180 bread wheat genotypes at six different environments.

	Holetta -2015 (E1)		Bekoji-2015 (E2)		Kulumsa -2015 (E3)		Holetta - 2016(E4)		Bekoji-2016 (E5)		Kulumsa - 2016(E6)	
Trait	$\boldsymbol{\sigma^2_g}$	$\mathbf{H^2}$	$\boldsymbol{\sigma^2_g}$	$\mathbf{H^2}$	$\boldsymbol{\sigma^2_g}$	$\mathbf{H^2}$	$\boldsymbol{\sigma^2_g}$	$\mathbf{H^2}$	$\boldsymbol{\sigma^2_g}$	$\mathbf{H^2}$	$\boldsymbol{\sigma^2_g}$	$\mathbf{H^2}$
SDSH	344.51^{***}	0.90	212.66^{***}	0.90	39.14^{***}	0.61	42.56^{***}	0.78	79.41^{***}	0.96	44.67^{***}	0.95
SDSMM	485.36^{***}	0.94	255.28^{***}	0.86	38.7^{***}	0.58	92.23^{***}	0.90	88.63^{***}	0.98	66.48^{***}	0.95
SDSM	516.40^{***}	0.89	275.44^{***}	0.76	331.16^{***}	0.88	127.99^{***}	0.99	105.24^{***}	0.97	86.59^{***}	0.94
SPC	0.04^{***}	0.88	0.01^{***}	0.70	0.02^{***}	0.87	0.01^{***}	0.62	0.01^{***}	0.83	0.00^{***}	0.67

SDSH= Septoria disease severity at heading, SDSMM = Septoria disease severity at mid- maturity, SDSM= Septoria disease severity at maturity, SPC= Septoria progress coefficient, σ^2_g = Genotypic variance, H^2 = Heritability in broad-sense (H^2), ***= very highly significant ($p \leq 0.0001$), * = significant, and "sn"= Non significant at $\alpha = 5\%$ significant level.

The combined ANOVA revealed the effect of genotype, year, location and their two (genotype x year, genotype x location, year x location) and three way interactions (genotype x year x locations) were significant for SDS traits except for the Septoria progress coefficient where the effect of year was not significant (Table 7.3). Analysis of pooled data revealed that genotypic variance (σ^2g) was the highest for all SDS parameters except SDSMM, where the environmental effect was the highest (Table 7.4). Broad-sense

heritability of Septoria resistance traits were highly heritable (h^2 = 72- 81%) (Table 7.4) (Robinson, 1949). The phenotypic coefficient of variation and genotypic coefficient of variation for SDS traits ranged from 32.37% (SPC) to 68.26% (SDSH), and 22.51% (SPC) to 41.41% (SDSM), respectively.

Table 7. 3 Combined ANOVA for Septoria disease severity traits.

Source of variation	DF	Mean Square			
		SDSH	SDSMM	SDSM	SPC
Genotype	179	699.53***	874.84***	1650.29***	0.12***
Replication	1	662.48*	35.41ns	5498.56***	0.87***
Year	1	29489.27***	44295.09***	64059.86***	0.01ns
Location	2	5257.27***	2734.03 ***	24849.55***	4.57***
Incomplete block (nested)	5	1468.79***	2167.8***	2955.71***	0.31***
Genotype*year	179	310.41***	392.85***	1008.41***	0.06***
Genotype*location	358	207.74***	288.83***	172.91***	0.02***
Year*location	2	1987.73***	358.46ns	10452.82***	3.78***
Genotype*Year*Location	358	148.97***	213.74***	152.76*	0.02***

SDSH= Septoria disease severity at heading, SDSMM = Septoria disease severity at mid- maturity, SDSM= Septoria disease severity at maturity, SPC= Septoria progress coefficient, SDS= Septoia disease severity, *** = very highly significant at p<0.0001, ** = highly significant at p< 0.001, * = significant at p< 0.05, ns = non significant at p= 0.05 significance level, and DF = degree of freedom.

Table 7. 4 Variance components, Heritability, and Genetic advances of SDS traits of 180 wheat genotypes based on pooled data from six environments.

Trait	σ^2g	σ^2gy	σ^2gl	σ^2gyl	σ^2e	H^2	GCV	PCV	GA	GAM
SDSH	81.97***	26.91***	14.7***	34.63***	79.71***	0.73	40.07	68.26	102.39	453.06
SDSMM	97.67***	29.86***	18.78***	40.46***	132.82***	0.72	32.62	59.01	87	287.15
SDSM	246.24***	142.61***	5.04***	15.32***	122.13***	0.75	41.41	60.83	93.09	245.65
SPC	0.02***	0.01***	-0.01***	0.01***	0.01***	0.81	22.51	32.37	53.7	9180.34

σ^2g = Genotypic variance estimate, σ^2gL = Genotype × Year interaction variance estimate, σ^2gL = Genotype × Location interaction variance estimate, σ^2gyl = Genotype × Year × Location interactions variance estimate, σ^2e = residual variance estimate, GCV = Genotypic coefficient of variance, PCV = Phenotypic coefficient of variance, GA= Genetic advance, GAM = Genetic advance as percent mean (GAM), *** = very highly significant at p<0.0001, ** = highly significant at p< 0.001, * = significant at p< 0.05, ns = non significant at p= 0.05 significance level.

At 5% selection intensity, genetic advance for SDS traits ranged from 53.7 (SPC) to 102.39% (SDSH), while the magnitude of the expected genetic gain as a percent of the mean varied from 245.65% (SDS at maturity) to 9180.34% SPC (Table 7.4).

Acros all environments, the average SDS of individual wheat genotypes ranged from 5.25 - 39.81% at heading, 8.18 - 48.46% at mid-maturity, and 10.60 - 65.33% at the maturity (Appendix VI). The average SPC of individual environment ranged from 0.37 - 0.79. The most resistant genotype at all growth stages was G174, while G104 (39.81%), G76 (48.46%) and G127 (65.33%) were the most susceptible genotypes at the heading, mid-maturity and maturity stages, respectively (Appendix VI). Comparative severity analysis with the standard check King-bird (G40) and the mean performance of the released varieties confirmed the presence of superior STB resistant genotypes among the tested materials. Of the 180 tested genotypes, 56 (31%) genotypes at heading, 75 (42%) at mid-maturity and 105 (59%) at maturity stage had numerically superior STB resistance compared to King-bird (Appendix VI). The top 5% best genotypes at maturity had 47.58 -70.95% greater resistance than King-bird, and 11.88- 74.4% greater resistance compared to the mean performance of the released varieties (Table 7.5).

Pearson's correlation analysis of the means over all environments revealed that STB resistance traits were significantly negatively associated with important agronomic traits. Except for SPC, all SDS traits showed non-significant and negligible negative correlation ($r < -0.3$) with plant height. Disease traits also showed no or little negative association with

HD, FD, GFD, NKPS and NKS. Significant weak negative association (r= -0.25 to -0.48) was observed between SDS traits with MD, grain yield, hectoliter weight and TKW (Table7.6).

Table 7. 5 Comparison of top 5% of the selected wheat genotypes for STB resistance with King-bird, and mean performances of 13 released varieties.

Genotypes	Mean of selected Genotypes	Comparative advantage for STB resistance (% over)		Genotypes	Mean of selected Genotypes	Comparative advantage for STB resistance (% over)	
		King-bird	MRV*			King-bird	MRV*
Septoria disease severity at heading (%)				Septoria disease severity at maturity (%)			
G174	5.25	70.95	74.4	G174	10.6	74.25	71.83
G153	5.82	67.81	71.63	G144	14.21	65.49	62.24
G144	5.87	67.53	71.38	G3	15.23	63	59.51
G150	6.07	66.39	70.38	G153	15.95	61.25	57.6
G151	8.45	53.22	58.78	G156	18.73	54.5	50.21
G141	8.9	50.72	56.57	G133	18.83	54.25	49.94
G133	8.9	50.72	56.57	G151	19.04	53.75	49.4
G3	9.32	48.44	54.56	G155	19.45	52.75	48.3
G156	9.47	47.58	53.81	G97	19.66	52.25	47.75
King-bird	18.06	---	11.88	King-bird	41.15	---	-9.43
MRV*	20.49	13.48	---	MRV*	37.61	8.62	---
Septoria disease severity at mid-maturity (%)				Septoria progress coefficient			
G174	8.18	70.4	72.74	G174	0.31	50.44	45.59
G153	10.55	61.83	64.86	G144	0.35	44.51	39.09
G144	14.15	48.79	52.85	G133	0.37	40.82	35.03
G3	14.46	47.68	51.83	G3	0.37	40.31	34.48
G151	15.28	44.7	49.08	G155	0.37	40.3	34.46
G155	15.75	43.02	47.54	G151	0.38	38.97	33.01
G92	16.31	40.97	45.65	G154	0.39	38.03	31.97
G150	16.36	40.79	45.48	G97	0.39	37.76	31.68
G81	17.03	38.37	43.25	G47	0.4	36.88	30.71
King-bird	27.63	---	7.93	King-bird	0.62	---	9.78
MRV*	30.01	-8.61	---	MRV*	0.57	8.91	---

Note: *MRV = Mean of 13 selected released varieties. Negative values for comparative advantage indicate less STB resistance (inferior performance) of the genotype.

Table 7. 6 Association analyses among Septoria resistance traits and other agronomic traits.

	HD	FD	MD	GFD	Yield	HLW	TKW	PH	NKPS	NKS
SDSH	-0.04^{ns}	-0.05^{ns}	-0.19*	-0.21*	-0.43***	-0.32***	-0.42***	-0.1^{ns}	-0.19*	-0.19*
SDSMM	-0.1^{ns}	-0.1^{ns}	-0.25*	-0.24**	-0.48***	-0.33***	-0.51***	-0.08^{ns}	-0.25**	-0.21*
SDSM	-0.21**	-0.22*	-0.35***	-0.26**	-0.47***	-0.32***	-0.44***	-0.06^{ns}	-0.16*	-0.14^{ns}
SPC	-0.15*	-0.14^{ns}	-0.29***	-0.22*	-0.38***	-0.25**	-0.44***	-0.21*	-0.12^{ns}	-0.1^{ns}

DH = days to 50% heading; DF = days to 50% flowering; DM = days to 90% maturity; GDF = Grain filling duration; HLW = Hectoliter weight; TKW = 1000 kernel weight; PH = Plant height (cm); NKPS= Number of kernel per spikelet; and NKS = Number of kernel per spike***=*very highly significant (p<0.0001),* SDSH= Septoria disease severity at heading, SDSMM = Septoria disease severity at mid- maturity, SDSM= Septoria disease severity at maturity, SPC= Septoria progress coefficient, ***= *highly significant and *=significant, ns=non significant at α=5% significance level, (-) = negative correlation.* The magnitude of the correlation coefficient indicates the strength of the correlation.

The SNPs Statistics

Illumina HiSeq 2500 sequencing failed to generate SNPs data for two genotypes (Genotype 9 and 95) and hence, a total of 178 bread wheat genotypes were successfully DArTSeq genotyped. Initially, a total of 35, 672 SNPs were discovered of which 31,052 (87%) were mapped to known chromosomal positions of the reference used, and 828 (2%) of the sequences were mapped to the unknown chromosomes in the references. Approximately, 11% (3,792) of the SNPs did not align to any of the chromosomes of the wheat reference genome. The discovered DArTSeq SNPs were not evenly distributed among the sub-genomes with the A, B and D genomes accounting for 10,317, 10,979 and 9,756 SNPs, respectively (Figure 7.1). Among the 21 wheat chromosomes, the highest (2065) and the

lowest (833) numbers of SNPs were mapped to chromosomes 7D and 4D, respectively (Figure 7.2), and on average each chromosome harbored about 1479 SNPs. Maintaining SNPs with higher call rate (> 70%) and MAF > 0.05 resulted in 7, 776 SNP markers, among which 87.29 % had a known chromosome position in the wheat reference genome. Among the filtered SNPs, 2,410 were distributed on the A-genome, 2,872 were on the B genome, and 1506 were distributed on the D genome. The remaining 988 SNPs were assigned to a hypothetical chromosome "0" for the sake of analysis. Hence, 7,776 SNPs were used in subsequent analyses including principal component analysis (PCA), population clustering, population structure, linkage disequilibrium (LD), and GWAS.

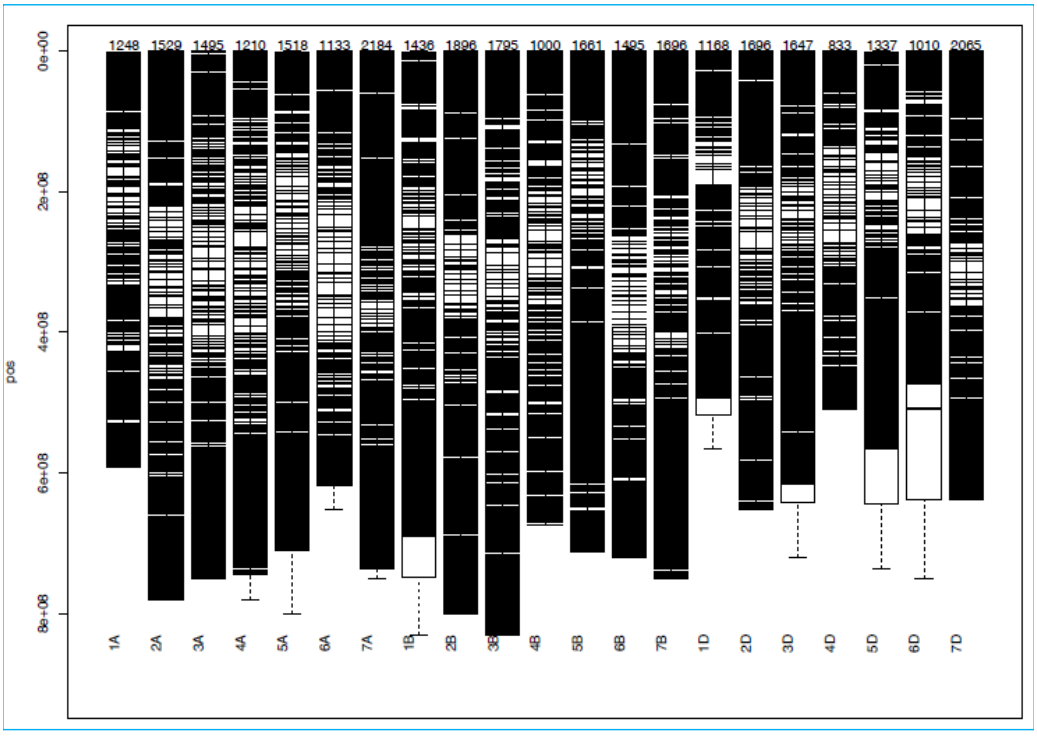

Figure 7. 1 Distribution of DArTSeq SNPs on 21 bread wheat chromosomes.

Population Structure Analysis

The STRUCTURE analysis indicated two sub-populations in the association panel (Figure 7.2A). About 43% (76) of the genotypes were assigned to cluster one and 57% (101) were assigned to cluster two. Figure 7.2B presnts the Q plot of the two sub-populations (SP1 and SP2) is the association pannel. The Clumpak result detected a greater degree of genetic admixture between the two sub-populations (Figure 7.2C) where all the individual genotypes shared alleles inherited from both subgroups confirming the presence of close relationship among the study materials.

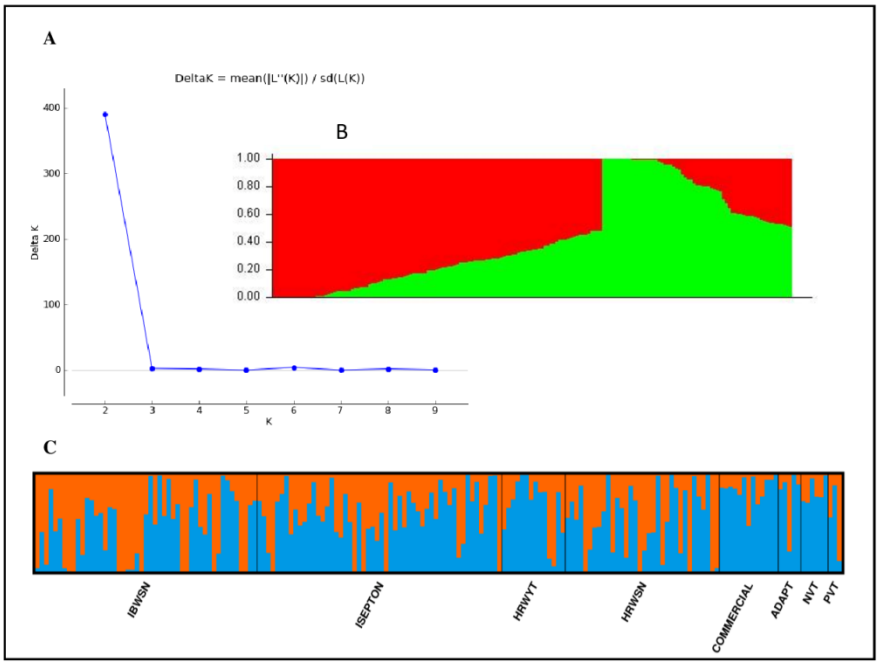

Figure 7. 2 Population structure of 178 bread wheat genotypes representing 8 populations.
(A) Best delta K value estimated using the method of Evano *et al*. (2005), and the pick at k = 2 indicates the number of subpopulations in our collection, (B) Population Structure plot and SP1 and SP2 represent subpopulations 1 and 2, respectively, (C) Estimated population structure for K = 2 according to the breeding materials. The different (blue and orange) colures represent genetic groups or sub-populations designated by Structure Harvester: the x-axis represents individual samples and y-axis represents the proportion of ancestry to each cluster. Population abbreviations are: IBWSN = International Bread Wheat Screening Nursery; ISEPTON = International Septoria Observation Nursery; HRWYT = High Rain Wheat Yield Trial; HRWSN = High Rain Wheat Screening Nursery; ADAPT = Adaptation trial; NVT = National Verification Trial, and PVT = Preliminary Verification trial.

The PCA results also suggested the presence of two sub-population (Figure 7.3). The first two principal components explained 65% (PC1 = 50% and PC2 = 15 %) of the total variances contained in the data (Figure 7.3). None of the clusters is composed of genotypes exclusively from a particular population confirming the presence of considerable genotypes admixture. A scree plot used to display the proportion of variation captured by each of the 10 principal components also depicted that the first two principal components (PC1 and PC2) explained the highest proportion of the total variation in the panel (Figure 7.4 A). The analysis also confirmed the presence of kinship in the association panel (Figure 7.4 C), suggesting the importance of using a powerful statistical GWAS model that accounts for the population structure and familial relatedness in the association study.

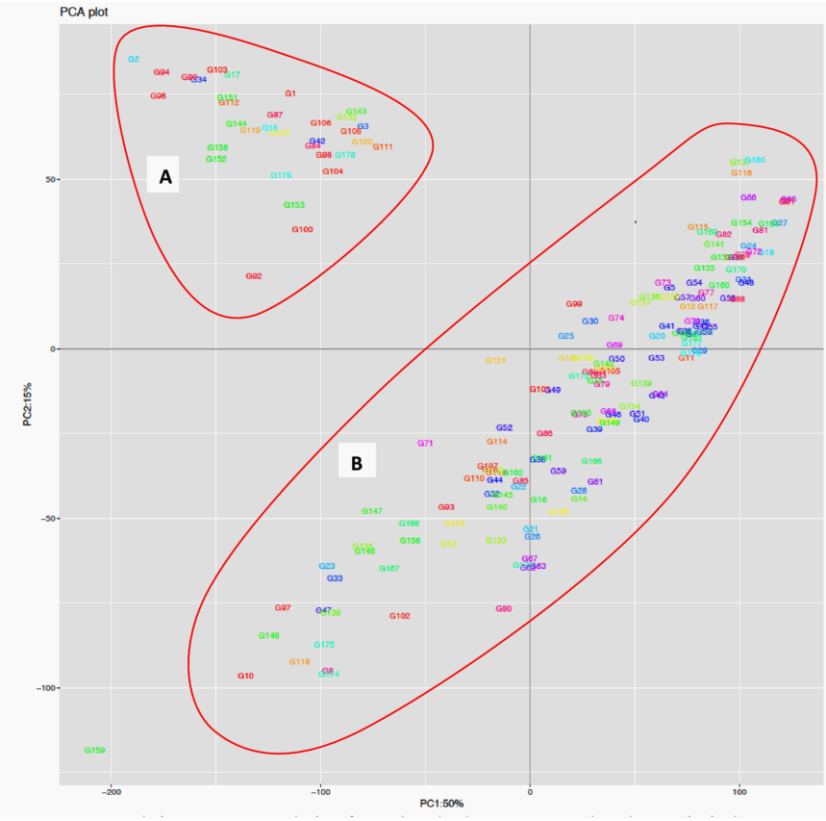

Figure 7. 3 Population structure analysis of 178 wheat genotypes based on PC clustering. Samples coded with the same color belong to same population. Cluster (A) composed of 33 (18.54 %) genotypes while Cluster (B) possessed 145 (81.46%) genotypes.

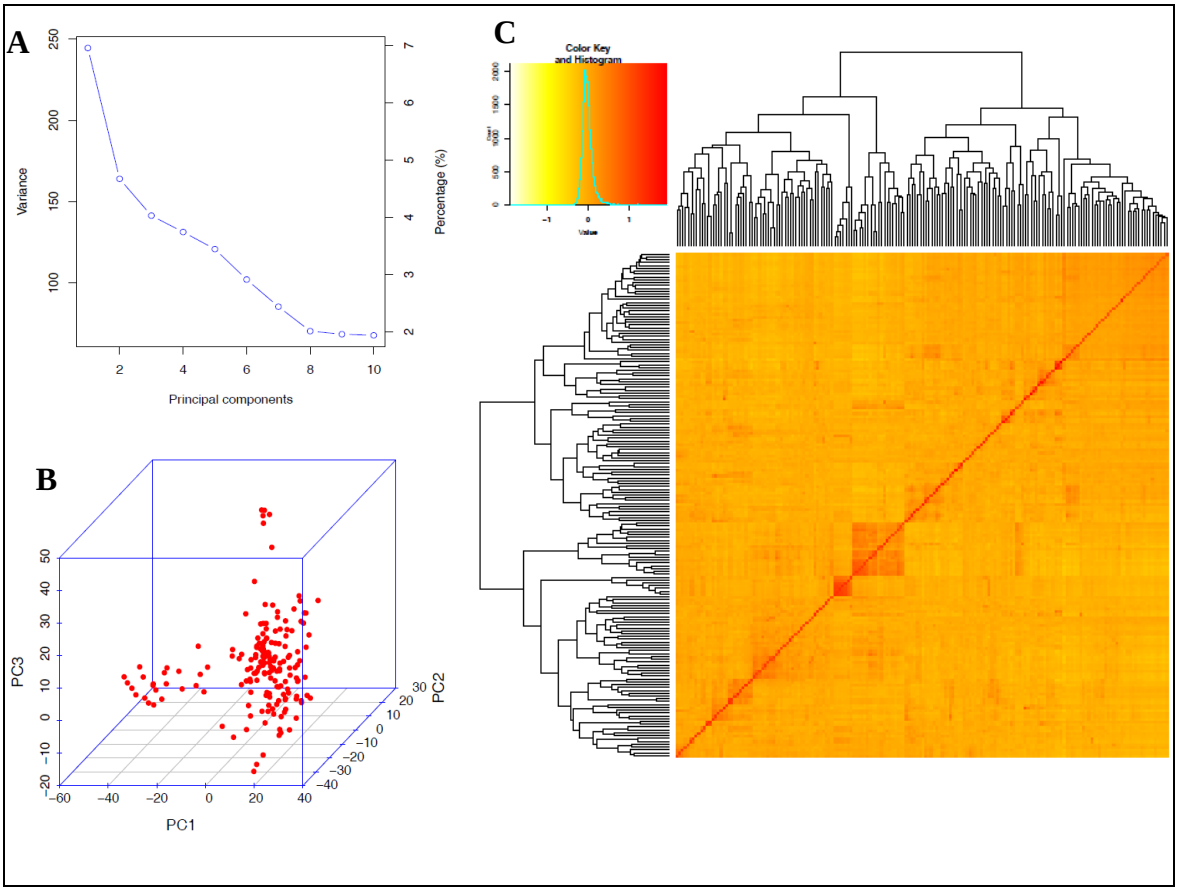

Figure 7. 4 Principal component and familiar relatedness analysis of 178 wheat genotypes. (A) A scree plot displaying the first10 principal components with their corresponding fraction of variation explained, (B) 3D plots of the first three principal components to depict the samples' relationship in space, and (C) Kinship displayed through heat map and a tree. The kinship values showed a normal distribution (turquoise curve), and orange represents weak correlation between pairs of individuals in the panel while, red shows high correlation. The resulted clustering tree is indicated outside of the matrix.

Linkage Disequilibrium (LD) Analysis

The linkage disequilibrium of alleles at different loci varied considerably across each chromosome, among chromosomes and sub-genomes (Table 7.7). A scatter plot of genome-wide pairwise LD r^2 values between SNPs on each chromosome against inter-marker physical distance is represented in Figure 7.5.

There was a total of 338,125 marker pairs with average LD values of $r^2 = 0.11$ with 97,723 (27.61%) pairs showing significant linkage at $p \leq 0.01$ (Table 7.7). The B Genome harbored the highest (143,600 or 42.47%) number of marker pairs, followed by the A genome with 119225 (35.53%) marker pairs (Table 7). The D genome harbored the lowest number (75,300 or 22.27%) of marker pairs. Relatively the SNPs on the B genome showed the strongest LD with mean value of $r^2 = 0.1187$. Over all the chromosomes, LD between SNPs declined to $r^2 = 0.2$ within physical distance of 31.44 Mbp: this ranged from 2.26 to 105.6 Mbp by chromosome. The weakest and strongest LD values were observed between marker pairs on chromosomes 4D ($r^2 = 0.0251$) and 2D ($r^2 = 0.211$), respectively (Table 7.7). The physical distance (bp) at which the LD decayed to the critical r^2 (0.2) value was used to determine the confidence interval for declaring distinct QTL for each chromosome. Significant SNP markers from the same chromosome were assigned to the same QTL if the distance between the significant markers was less than the critical physical distance specific to that linkage group or the r^2 value between the markers was > 0.2 (Appendix VII).

Table 7. 7 Summary of LD analysis among marker pairs per chromosome and genome.

Chromosomes	TNMP	r^2	Distance (Mbp)	Significant marker pairs (P<0.01)
1A	12,475	0.13	58.05	4,374 (35.06)
1B	19,900	0.10	44.93	5,910(29.7)
1D	10,250	0.128	57.08	1,783 (17.40)
2A	21,200	0.15	48.03	7,342 (34.63)
2B	28,100	0.11	36.88	9,511 (33.85)
2D	14,550	0.21	57.45	4,771 (32.79)
3A	17,450	0.09	57.38	4,259 (24.41)
3B	22,000	0.12	49.85	6,795 (30.89)
3D	14,650	0.12	56.41	3,940 (26.90)
4A	13,050	0.11	75.78	3,830 (29.35)
4B	9,600	0.14	96.54	3,463 (36.08)
4D	2,700	0.03	218.80	1,71 (6.34)
5A	17,600	0.09	52.97	4,897 (27.83)
5B	22,750	0.14	40.22	8,193 (36.01)
5D	11,100	0.12	59.51	2,273 (20.48)
6A	14,700	0.08	57.51	3,928 (26.72)
6B	18,800	0.11	50.53	6,163 (32.78)
6D	7,950	0.05	87.75	911 (11.46)
7A	22,750	0.09	42.53	6,382 (28.05)
7B	22,450	0.11	42.47	7,492 (33.37)
7D	14,100	0.08	60.62	2,246 (15.93)
A genome	119,225	0.11	56.04	35,012 (29.44)
B genome	143,600	0.12	51.63	47,527 (33.24)
D genome	75,300	0.11	65.70	15,184 (20.16)
Total	338,125	0.11	57.79	97,723 (27.61)

TNM P= Total no. of marker pairs; Mbp = Mega base pairs

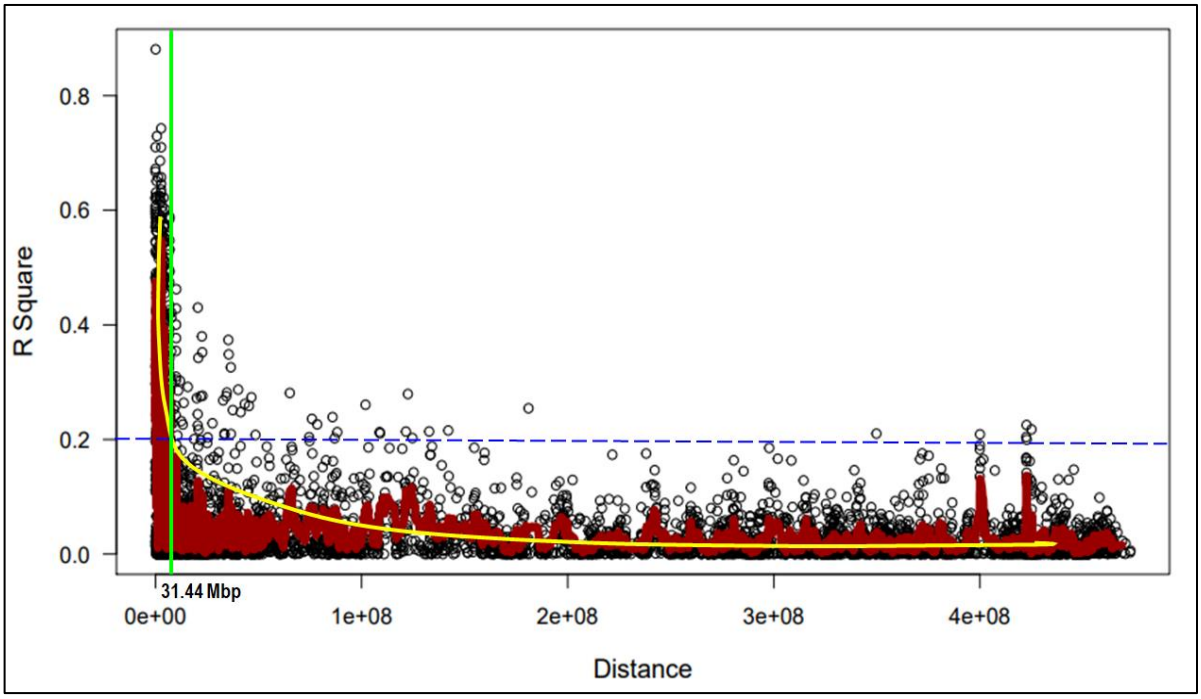

Figure 7. 5 Genome-wide LD decade plot over physical distance based on 7,776 SNP markers. The yellow curve represents the model fits to LD decay. The blue dash-line represents the arbitrary threshold for no LD (r^2 =0.2). The light green line indicates the intersection between critical r^2 and the average map distance to determine QTL confidence intervals.

Genome-Wide Association Study (GWAS) for STB resistance

GWAS using a highly powerful statistical model, FarmCPU identified 53 SNPs that were significantly (FDR < 0.05) associated with STB resistance at any growth stages. The report included only marker-trait-associations (MTAs) that surpassed a Bonferroni-correction significance threshold of 0.15. Table 7.8 presents only significant GWAS results for STB resistance at the heading, mid-maturity and maturity stages, and also for Septoria progress

coefficient. The table reports allele identity, marker position, MAF, *p*-values, FDR corrected q values and additive effects of the identified MTAs.

Among 53 identified MTAs, three did not have a chromosomal position on the bread-wheat physical map (Table 7.8). Ten (18.87%) MTAs conferred STB resistance at heading, four (7.55%) were effective at mid-maturity, four (7.55%) were effective at the maturity stage, and 35 (66.94%) MTAs were associated with resistance to the disease development up the plant height (Table 7.8). The percentage of phenotypic variance explained by the markers varied considerably from 2.7% for SDS measured at maturity at Bekoji in 2016 to 13.20% for severity data measured at the mid-maturity stage at the same location in 2015 (Table 7.8). The proportion of phenotypic variation (R^2) explained by SDS MTAs at heading ranged from 2.92% for the allele 1195254|F|0-31:C>T-31:C>T on chromosome 3A to 11.09% for 1087857|F|0-41:T>C-41:T>C on 7D. Likewise, the R^2 for MTAs for SDS at mid-maturity, maturity and SPC ranged from 8.6 - 13.01%, 2.67 - 2.73% and 5.98% - 10.80%, respectively (Table 7.8).

Table 7. 8 Summary of individual Marker-Trait Associations for STB resistance.

Trait	Allele ID	Chromo-some	Position	Allele	P.value	FDR_Adjusted_P-values	Add effect	R2 (%)
SDSH_Holetta_2015	1016309|F|0-30:A>T-30:A>T	3A	710771071	30:A>T	7.23E-07	0.003517647	8.06	11.08
SDSH_Holetta_2015	2333359|F|0-32:G>C-32:G>C	2A	514858369	32:G>C	9.87E-07	0.003517647	6.78	10.63
SDSH_Holetta_2015	1087857|F|0-41:T>C-41:T>C	7D	531439751	41:T>C	1.75E-06	0.003517647	11.2	11.09
SDSH_Holetta_2015	3023856|F|0-37:A>T-37:A>T	3D	593664469	37:A>T	1.81E-06	0.003517647	8.05	10.43
SDSH_Holetta_2015	2351081|F|0-57:A>G-57:A>G	5A	688359748	57:A>G	1.52E-05	0.023597278	5.57	10.44
SDSH_Holetta_2015	1085095|F|0-25:G>C-25:G>C	1D	463434850	25:G>C	3.33E-05	0.043105524	-5.14	10.44
SDSH_Bekoji_2016	1217828|F|0-18:G>C-18:G>C	3D	42679365	18:G>C	2.48E-06	0.00977402	5.13	3.48
SDSH_Bekoji_2016	1095723|F|0-50:A>C-50:A>C	0	0	50:A>C	2.51E-06	0.00977402	-3.15	2.95
SDSH_Bekoji_2016	1361071|F|0-37:T>G-37:T>G	7A	691722567	37:T>G	1.08E-05	0.027621167	3.19	3.02
SDSH_Bekoji_2016	1195254|F|0-31:C>T-31:C>T	3A	203418249	31:C>T	1.42E-05	0.027621167	-2.67	2.92
SDSMM_Holetta_2015	1082842|F|0-8:A>G-8:A>G	1B	587138312	8:A>G	8.09E-14	6.29E-10	-10.78	8.68
SDSMM_Holeta_2015	3023856|F|0-37:A>T-37:A>T	3D	593664469	37:A>T	1.79E-08	6.98E-05	9.84	13.01
SDSMM_Holeta_2015	982125|F|0-45:T>A-45:T>A	7B	686989852	45:T>A	3.24E-06	0.00839702	5.53	8.71
SDSMM_Holeta_2015	1164032|F|0-20:G>C-20:G>C	0	0	20:G>C	9.88E-06	0.019202181	-6.22	10.75
SDSM_Bekoji_2016	980448|F|0-68:G>C-68:G>C	6A	609480220	68:G>C	8.31E-07	0.006464556	3.8	2.72
SDSM_Bekoji_2016	1030239|F|0-17:C>T-17:C>T	4A	619375783	17:C>T	2.59E-06	0.010082733	-5.54	2.7
SDSM_Bekoji_2016	2249152|F|0-50:C>A-50:C>A	1D	3324483	50:C>A	7.95E-06	0.020619331	2.95	2.73
SDSM_Bekoji_2016	1008083|F|0-38:G>A-38:G>A	7D	21667638	38:G>A	2.69E-05	0.052212203	3.2	2.67
SPC_Holetta_2015	3023856|F|0-37:A>T-37:A>T	3D	593664469	37:A>T	2.01E-07	0.001396764	0.09	10.8
SPC_Holetta_2015	1082842|F|0-8:A>G-8:A>G	1B	587138312	8:A>G	4.23E-07	0.001396764	-0.06	9.67
SPC_Holetta_2015	982125|F|0-45:T>A-45:T>A	7B	686989852	45:T>A	5.39E-07	0.001396764	0.06	9.71
SPC_Holetta_2015	2244530|F|0-34:A>T-34:A>T	1D	375956648	34:A>T	3.02E-06	0.005864081	0.13	9.66
SPC_Holetta_2015	1016309|F|0-30:A>T-30:A>T	3A	710771071	30:A>T	9.47E-06	0.014727727	0.07	9.92
SPC_Holetta_2015	1058879|F|0-41:C>T-41:C>T	2B	700740191	41:C>T	3.27E-05	0.042438412	-0.05	9.84
SPC_Kulumsa_2015	3023856|F|0-37:A>T-37:A>T	3D	593664469	37:A>T	9.29E-08	0.000722731	0.09	10.2
SPC_Kulumsa_2015	3064880|F|0-36:C>T-36:C>T	1A	474702375	36:C>T	1.00E-05	0.03890377	0.08	9.21
SPC_Kulumsa_2015	982125|F|0-45:T>A-45:T>A	7B	686989852	45:T>A	1.59E-05	0.041084625	0.04	9.18
SPC_Bekoji_2016	4009281|F|0-26:A>G-26:A>G	7D	528900507	26:A>G	9.08E-12	7.06E-08	0.1	6.63
SPC_Bekoji_2016	2244141|F|0-19:G>C-19:G>C	7D	63471279	19:G>C	8.34E-08	0.000324444	-0.06	6.02
SPC_Bekoji_2016	2280416|F|0-6:T>C-6:T>C	7A	690377106	6:T>C	1.06E-06	0.002754288	0.05	7.26
SPC_Bekoji_2016	1005662|F|0-23:C>T-23:C>T	2D	593032041	23:C>T	1.50E-06	0.002907847	0.07	6.01
SPC_Bekoji_2016	1058162|F|0-27:C>T-27:C>T	5B	538706298	27:C>T	2.95E-06	0.003907101	-0.03	6.92
SPC_Bekoji_2016	2369156|F|0-8:G>A-8:G>A	2D	450991087	8:G>A	3.01E-06	0.003907101	-0.06	7.35
SPC_Bekoji_2016	1351797|F|0-16:C>G-16:C>G	6B	708272196	16:C>G	3.05E-05	0.033204738	-0.04	6.13
SPC_Bekoji_2016	100688619|F|0-22:A>G-22:A>G	1B	558551443	22:A>G	3.42E-05	0.033204738	0.04	5.98
SPC_Kulumsa_2016	1002704|F|0-50:T>C-50:T>C	2B	243083729	50:T>C	2.13E-10	1.66E-06	-0.04	6.63
SPC_Kulumsa_2016	4540934|F|0-13:T>C-13:T>C	0	0	13:T>C	1.08E-07	0.000421212	-0.03	6.02
SPC_Kulumsa_2016	979640|F|0-16:A>G-16:A>G	7A	116530515	16:A>G	3.25E-06	0.008414496	-0.02	7.26
SPC_Kulumsa_2016	1090518|F|0-44:T>A-44:T>A	6A	607427728	44:T>A	9.59E-06	0.017998052	-0.03	6.01
SPC_Kulumsa_2016	989092|F|0-5:A>G-5:A>G	5B	487460716	5:A>G	1.50E-05	0.017998052	0.03	6.92
SPC_Kulumsa_2016	2252418|F|0-22:C>G-22:C>G	2D	598728762	22:C>G	1.52E-05	0.017998052	0.03	7.35
SPC_Kulumsa_2016	1233107|F|0-31:A>G-31:A>G	5D	541603929	31:A>G	1.62E-05	0.017998052	0.04	6.13
SPC_Kulumsa_2016	1091861|F|0-32:A>G-32:A>G	1A	566369413	32:A>G	2.00E-05	0.019399692	-0.02	5.98
SPC_Kulumsa_2016	1000905|F|0-46:A>G-46:A>G	3B	17785833	46:A>G	3.33E-05	0.028734661	-0.04	6.07
SPC_Kulumsa_2016	4393733|F|0-28:G>C-28:G>C	1A	366278319	28:G>C	5.13E-05	0.039874389	-0.03	6.91
SPC_Combined	3023856|F|0-37:A>T-37:A>T	3D	593664469	37:A>T	3.12E-09	2.43E-05	0.04	10.67
SPC_Combined	982125|F|0-45:T>A-45:T>A	7B	686989852	45:T>A	3.49E-07	0.001357275	0.03	9.66
SPC_Combined	1082842|F|0-8:A>G-8:A>G	1B	587138312	8:A>G	8.85E-07	0.002293203	-0.03	9.65
SPC_Combined	1090511|F|0-27:C>G-27:C>G	3A	8862385	27:C>G	1.83E-06	0.003549739	-0.02	9.82
SPC_Combined	1058892|F|0-53:C>G-53:C>G	2D	288602370	53:C>G	4.90E-06	0.007618496	-0.05	9.68
SPC_Combined	1009941|F|0-45:A>T-45:A>T	7D	28074523	45:A>T	2.92E-05	0.037801544	-0.05	10.79
SPC_Combined	3022065|F|0-21:G>C-21:G>C	6B	675620421	21:G>C	3.58E-05	0.039728943	0.03	9.91
SPC_Combined	2262270|F|0-28:C>G-28:C>G	3B	59645976	28:C>G	6.85E-05	0.066597949	-0.03	9.75

Note: The table reports SDS traits, Allele ID, Chromosome, phyiscal position, SNP position, significance value (*p* value and FDR), addative effect, and "R^2" value = proportion of phenotypic variation explained by marker. "Chromosome = 0" reports chromosomes for unmapped.

The GWA scan for SDS at the individual-environment level in a single-year identified considerable (45) MTAs conferring resistance to STB at different growth stages (Table 7.8). The analysis for disease data measured in 2015 at Holetta identified six MTAs for STB resistance at heading stage on chromosomes 1D, 2A, 3A, 3D, 5A and 7D, four MTAs effective for STB resistance at the mid-maturity stage on chromosome 1B, 3D, 7B and one MTA with unknown position on the bread wheat physical map at Holetta, and nine MTAs for SPC (six at Holetta and three at Kulumsa) on chromosomes 1A, 1B, 1D, 2B, 3A, 3D and 7B (Table 7.8, Figure 7.6). However, no MTA was observed for SDS data measured at the maturity stage in the same year. The quantile-quantile plots indicate how well the GWAS model accounted for population structure and kinship for each of the disease traits.

Likewise, association analysis for SDS data measured in 2016 identified 26 MTAs: four for STB resistance at heading at Bekoji on chromosomes 3A, 3D, 7A and one MTA with unknown position on the bread wheat physical map, four for maturity stage resistance on chromosomes1D, 4A, 6A and 7D at the same location, and 18 MTAs for SPC which are mapped to chromosomes 1A, 1B, 2B, 2D, 3B, 5B, 5D, 6A, 6B, 7A, 7D plus one MTA with unknown position on the bread wheat physical map (Table 7.8, Figure 7.7).

The combined measure of SDS at the heading, mid-maturity and maturity stages did not provide any significant associations at the used threshold. However, the combined measure of SPC identified eight MTAs at the stringent Bonferroni significance threshold on chromosomes 1B, 2D, 3A, 3B, 3D, 6B, 7B, 7D and one MTA that was unmapped on the bread wheat physical map (Table 7.8, Figure 7.7).

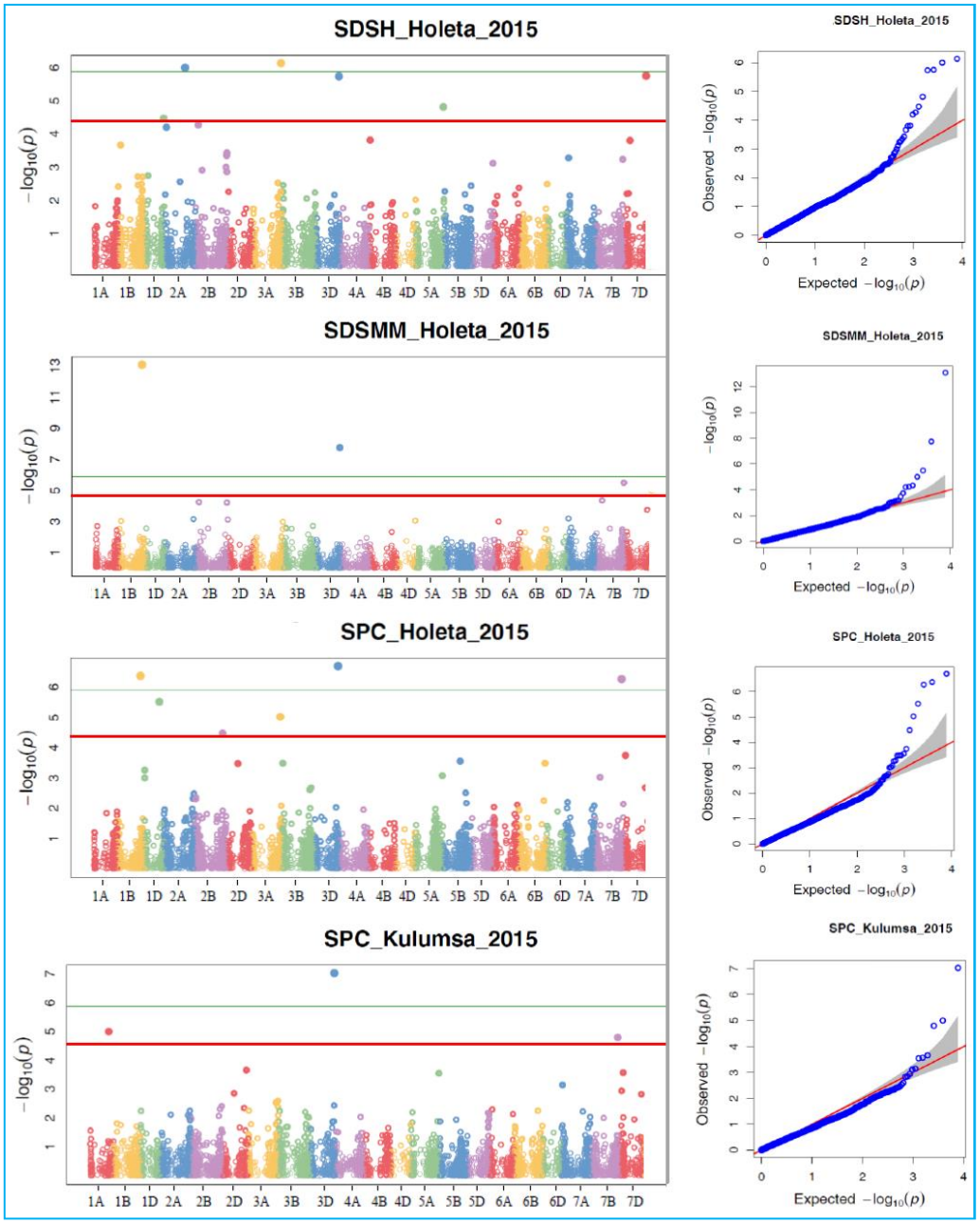

Figure 7. 6. Manhattan plots for Septoria disease severity traits measured in 2015.

Only GWA scans resulting in significant associations are reported. Each dot represents a SNP. On the X-axis is the genomic position of the SNPs on the corresponding chromosomes indicated in diffrent colours.Y-axis is the *-log10* of the *P*-value depicting the significance of the association test. The horizontal red line is the Bonferroni correction significance threshold used in the assotion studies of SDSH (Septoria disease severity at heading), SDSMM (Septoria disease severity at mid-maturity), and SPC (Septoria progress cofficient) at Holeta and Kulumsa locations. The quantile-quantile plots indicate how well the GWAS model accounted for population structure and kinship for each of the disease traits. In each plot, the observed *–log (P values)* from the fitted GWAS models (y-axis) are compared with their expected value (x-axis) under the null hypothesis of no association with the trait. Each blue dot represents a single nucleotide polymorphism, the red line is the model for no assosaation.

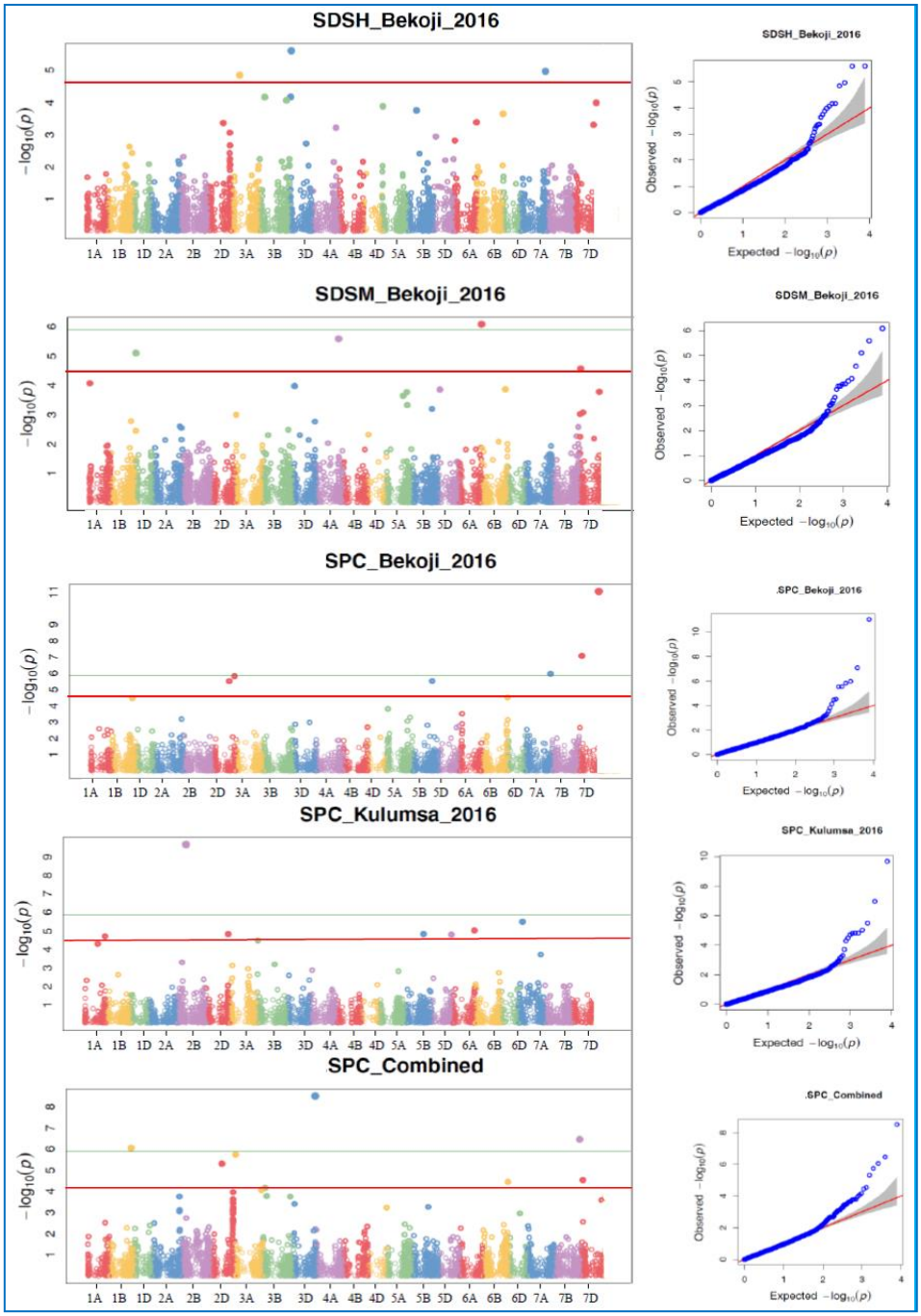

Figure 7.7 Manhattan plots for Septoria severity data measured in 2016 and combined data.

Only GWA scans resulting in significant associations are presented. Each dot represents a SNP. On the X-axis is the genomic position of the SNPs on the corresponding chromosomes indicated in different colours. On the Y-axis is the *-log10* of the *P*-value depicting the significance of the association test. The horizontal red line is the Bonferroni significance threshold used in the association studies of SDSH, Septoria disease severity at heading; SDSM, Septoria disease severity at maturity; and SPC, Septoria progress coefficient at Bekoji and Kulumsa locations.

Putative QTLs for STB resistance were identified by combining the MTAs based on their genomic positions using a window of physical distance (in Mbp) determined through pair wise LD analysis of the Genome-wide scanned SNPs. MTAs falling on the same linkage group within the physical distance for LD decay specific for that chromosome were assigned to the same putative QTL. Hence, based on our LD criteria, the 53 markers were assigned to 33 QTLs (Table 7.9, Figure 7.8).

The association analysis for STB resistance at heading at individual environments identified nine putative QTLs localized on the chromosomes 1D (qSTB7), 2A (qSTB8), 3A (qSTB16 and qSTB17), 3D (qSTB20 and qSTB21), 5A (qSTB23), 7A (qSTB30) and 7D (Table 7.9, Figure 7.8). The combined measure of SDS at the mid-maturity stage did not reveal any QTL. However, measuring the same trait in 2015 at Holetta identified three putative QTLs on the chromosomes 1B (qSTB4), 3D (qSTB21 and 7B (qSTB31) (Table 7.9) which are effective for STB resistance at the mid-maturity stage. Likewise, SDS measured at maturity in 2016 at Bekoji provided four putative QTLs on chromosomes1D (qSTB5), 4A (qSTB22), 6A (qSTB27) and 7D (qSTB32) (Table 7.9, Figure 7.8). However, the same phenotype measured across all environments did not provide any putative QTLs effective for STB resistance.

We identified seven QTLs for SPC in the analysis of means overall six environments: qSTB11 on 2D, qSTB15 on 3A, qSTB19 on 3B, qSTB21 on 3D, qSTB28 on 6B, qSTB31

on 7B, and qSTB32 on 7D. These seven QTL modeled 9.7 to 13.0% of the phenotypic variation. Three of these QTL (qSTB11 on 2D, qSTB14 on 3A, and qSTB19 on 3D) were not significant in the analyses of the six environments, two (qSTB28 and qSTB32) were significant in one of the environments, while the other two (qSTB21 and qSTB31) were significant in two environments (Table 7.9, Figure 7.8). Measuring the same trait in 2015 identified seven putative QTLs on chromosomes 1A (qSTB1), 1B (qSTB4), 1D (qSTB6), 2B (qSTB10), 3A (qSTB17), 3D (qSTB21) and 7B (qSTB31) (Table 7.9, Figure 7.8). Similarly, association analysis for the 2016 measured SPC data identified effective putative QTLs on 1A (qSTB1 and qSTB3), 1B (qSTB4), 2B (qSTB9), 2D (qSTB12-14), 3B (qSTB18, 5B (qSTB24 and qSTB25), 5D (qSTB26), 6A (qSTB27), 6B (qSTB28), 7A (qSTB29) and 7D (qSTB32 and qSTB33) (Table 7.9, Figure 7.8).

Functional association of the identified QTLs on STB resistance was further investigated by annotating genes found in the QTLs region on the recently released IWGSC RefSeq Annotation v2. The analysis resulted in several résistance associated genes that are involved in plant defense system (Appendix VIII). For instance, the high confidence candidate genes: *TraesCS1A02G279300* on 1A, *TraesCS1B02G332400* on 1B,*TraesCS1D02G001700* on 1D and *TraesCS2A02G297500* on 2A are highly involved in systemic acquired resistance (SAR): a long-lasting broad spectrum resistance of plants to pathogen infections. The high confidence gene detected in qSTB8 on 2A (*TraesCS2A02G297500*) controls Mitogen-activated protein kinase (MAPK) cascades which are involved in signaling multiple defense responses of plants against pathogen attacks (Meng and Zhang, 2013).

Table 7. 9 Putative QTLs identified across bread wheat chromosomes for STB resistance.

Putative QTL	Chr	Map Position (bp)	No.of MTAs	Flanking Markers			Phenotypes _Location and Year	R^2
				Position* (Mbp)	Left /Right	Position** (Mbp)		
qSTB1	1A	366278319	1	366.28	9766808|F|0-10:A>G-10:A>G /1087379|F|0-64:G>A-64:G>A	374.57	SPC_Kulumsa_2016	6.91
qSTB2	1A	474702375	1	474.56	4409931|F|0-10:T>C-10:T>C/2263809|F|0-17:G>C-17:G>C	475.71	SPC_Kulumsa_2015	9.21
qSTB3	1A	566369413	1	565.20	987669|F|0-11:T>G-11:T>G/1863565|F|0-7:G>A-7:G>A	566.56	SPC_Kulumsa_2016	5.98
qSTB4	1B	558551443-587138312	4	556.11	3948637|F|0-11:A>G-11:A>G/1276419|F|0-52:A>C-52:A>C	587.28	SPC and SDS at mid-maturity	5.98-9.67
qSTB5	1D	3324483	1	2.95	2248863|F|0-53:G>T-53:G>T/1863120|F|0-61:T>C-61:T>C	4.82	SDSM_Bekoji_2016	273
qSTB6	1D	375956648	1	363.25	1217216|F|0-11:G>C-11:G>C/1119123|F|0-11:C>G-11:C>G	377.58	SPC_Holetta_2015	9.66
qSTB7	1D	463434850	1	462.07	1034027|F|0-63:C>G-63:C>G/1398976|F|0-52:G>C-52:G>C	464.86	SDSH_Holetta_2015	10.44
qSTB8	2A	514858369	1	513.83	1181149|F|0-28:G>A-28:G>A/1102718|F|0-68:C>A-68:C>A	21.69	SDSH_Holetta_2015	10.63
qSTB9	2B	243083729	1	237.98	100665389|F|0-10:A>G-10:A>G/3064852|F|0-14:G>A-14:G>A	249.19	SPC_Kulumsa_2016	6.63
qSTB10	2B	700740191	1	698.10	1127049|F|0-20:T>C-20:T>C/1220715|F|0-13:A>G-13:A>G	701.09	SPC_Holetta_2015	9.84
qSTB11	2D	288602370	1	215.60	3025921|F|0-19:G>T-19:G>T/1107980|F|0-6:T>C-6:T>C	331.55	SPC_Combined	9.68
qSTB12	2D	450991087	1	443.24	2262159|F|0-55:C>A-55:C>A/991014|F|0-5:G>C-5:G>C	461.85	SPC_Bekoji_2016	7.35
qSTB13	2D	593032041	1	591.60	2251911|F|0-13:A>G-13:A>G/1078056|F|0-40:C>T-40:C>T	594.54	SPC_Bekoji_2016	6.01
qSTB14	2D	598728762	1	595.56	5324627|F|0-45:G>C-45:G>C/2246647|F|0-7:T>C-7:T>C	598.73	SPC_Kulumsa_2016	7.35
qSTB15	3A	8862385	1	8.74	2256311|F|0-9:C>G-9:C>G/1088933|F|0-37:C>T-37:C>T	12.87	SPC_Combined	9.82
qSTB16	3A	203418249	1	161.44	12470406|F|0-23:A>G-23:A>G/992022|F|0-9:G>A-9:G>A	222.04	SDSH_Bekoji_2016	2.92
qSTB17	3A	710771071	2	710.34	989196|F|0-7:A>T-7:A>T/4989102|F|0-40:G>A-40:G>A	711.04	SDSH_Holetta_2015 SPC_Holetta_2015	9.92
qSTB18	3B	17785833	1	17.11	1244651|F|0-19:A>G-19:A>G/998652|F|0-18:C>T-18:C>T	18.45	SPC_Kulumsa_2016	6.07
qSTB19	3B	59645976	1	59.53	1263371|F|0-58:G>A-58:G>A/1110947|F|0-39:T>C-39:T>C	60.37	SPC_Combined	9.75
qSTB20	3D	42679365	1	42.63	981546|F|0-39:T>C-39:T>C/4911094|F|0-6:T>C-6:T>C	45.94	SDSH_Bekoji_2016	3.48
qSTB21	3D	593664469	5	593.66	1102020|F|0-37:G>A-37:G>A/992091|F|0-53:G>C-53:G>C	595.02	SDSH_Holetta_2015,SDSMM_Holeta_2015,SPC_Holetta_2015,SPC_Kulumsa_2015& SPC_Combined	2.67-13.01
qSTB22	4A	619375783	1	619.16	2263956|F|0-45:T>C-45:T>C/994022|F|0-52:G>C-52:G>C	620.75	SDSM_Bekoji_2016	2.7
qSTB23	5A	688359748	1	685.96	3938163|F|0-43:T>C-43:T>C/2278701|F|0-37:A>T-37:A>T	689.42	SDSH_Holetta_2015	10.44
qSTB24	5B	487460716	1	487.44	1696148|F|0-16:C>G-16:C>G/2281586|F|0-67:A>G-67:A>G	491.07	SPC_Kulumsa_2016	6.92
qSTB25	5B	538706298	1	538.31	5582250|F|0-47:T>C-47:T>C/1097026|F|0-40:C>A-40:C>A	539.08	SPC_Bekoji_2016	6.92
qSTB26	5D	541603929	1	539.15	1696148|F|0-16:C>G-16:C>G/6038202|F|0-6:C>T-6:C>T	541.68	SPC_Kulumsa_2016	6.13
qSTB27	6A	607427728-609480220	2	607.43	2328288|F|0-13:G>C-13:G>C/1231806|F|0-13:G>C-13:G>C	608.28	SPC_Kulumsa_2016 and SPC_Kulumsa_2016	2.72-6.01
qSTB28	6B	708272196	2	706.98	995556|F|0-65:C>T-65:C>T/1091698|F|0-30:C>G-30:C>G	708.02	SPC_Combined and SPC_Bekoji_2016	6.13-9.91
qSTB29	7A	116530515	1	116.12	3064815|F|0-27:A>G-27:A>G/1151957|F|0-24:T>G-24:T>G	123.28	SPC_Kulumsa_2016	7.26
qSTB30	7A	690377106-691722567	2	688.71	3064770|F|0-20:G>A-20:G>A/3532952|F|0-38:G>A-38:G>A	690.86	SPC_Bekoji_2016 and SDSH_Bekoji_2016	3.02-7.26
qSTB31	7B	686989852	4	686.00	3023327|F|0-28:G>A-28:G>A/7340828|F|0-11:C>G-11:C>G	687.06	SDSMM_Holeta_2015,SPC_Holetta_2015, SPC_Kulumsa_2015 and SPC_Combined	8.71-9.71
qSTB32	7D	21667638-63471279	3	18.92	1072451|F|0-9:C>T-9:C>T/981671|F|0-20:G>C-20:G>C	64.24	SDSM_Bekoji_2016,SPC_Combined and SPC_Bekoji_2016	2.67-10.79
qSTB33	7D	528900507-531439751	2	526.49	1004225|F|0-30:G>A-30:G>A/5579572|F|0-19:G>A-19:G>A	540.62	SPC_Bekoji_2016 and SDSH_Holetta_2015	6.63-11.09

QTL= Quantitative trait locus; Chr = Chromosomes, MTAs = Marker trait associations, position* = position (Mbp) of the left flanking marker, Position** = position (Mbp) of right flanking marker, Phenotype_Location_year = Septoria disease severity measured at individual or across location (Holetta, Bekoji and Kulumsa) in 2015 and 2016, R^2 = percentage of total phenotypic variance explained by the identified putative QTL. SDSH= Septoria disease severity at heading, SDSMM = Septoria disease severity at mid-maturity, SDSM = Septoria disease severity at maturity, and SPC = Septoria progress coefficient.

Putative QTLs for STB Resistance

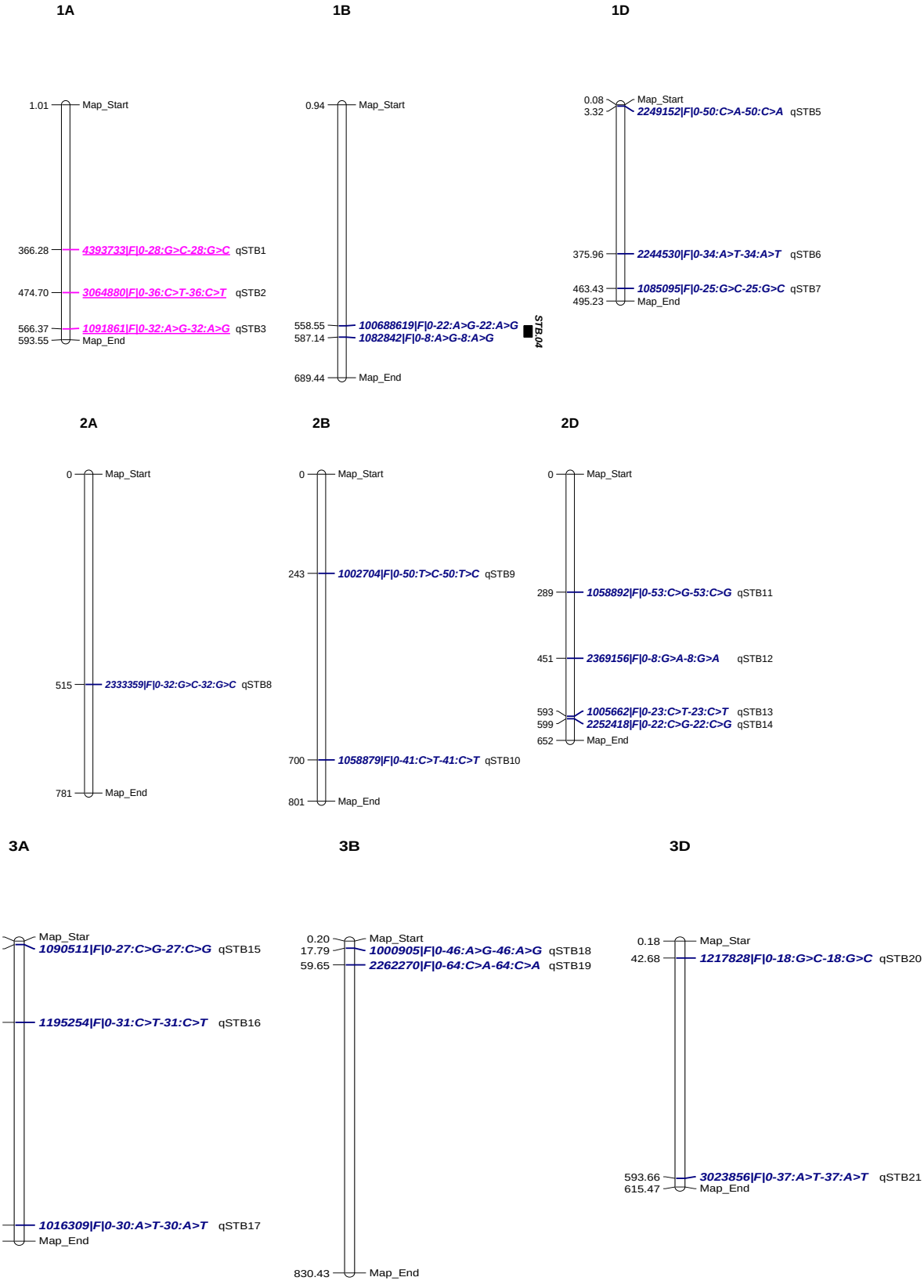

Figure 7. 8 Genomic positions of detected putative QTLs effective for STB resistance. Significant DArTSeq SNPs are presented according to their physical positions on chromosomes in millions base pairs. The putative QTLs identified in this study for the MTAs are indicated on the right sides of the bars. Underlined MTAs (marked in pink) on the right sides of the chromosomes could be potentially novel loci in this study.

Marker-Trait Associations (MTAs) for Agronomic Traits

The combined measures of the agronomic traits such as days to heading, days to flowering, days to maturity, grain-filling duration, grain yield and 1000-kernel weight did not provide any MTAs at the stringent Bonferroni significance threshold used except for plant height that resulted in one MTA on chromosome 7A at the 514.43 Mbp position (Appendix IX). However, dissections of these traits at individual environments in separate years identified considerable MTAs at the significance threshold utilized. A GWA scan for days to heading in 2015 at Holetta provided six MTAs on chromosomes 1A, 5A, 5B, 6A, 6B and 7B (Appendix IX, Figure 7.9). The same trait measured in 2016 at Kulumsa identified three significant (FDR <0.05) SNPs on chromosome 2B, 5D and 6A (Appendix IX, Figure 7.10). The total phenotypic variance explained by the SNPs for this trait ranged from 8.54% for the allele 987806|F|0-16: A>G-16:A>G on chromosome 5D at 77.02 Mbp to 24.07% for the SNP1204551|F|0-57:C>T-57:C>T positioned on 1A at 499.84 Mbp (Appendix IX).

The GWA scan for days to flowering at individual environments identified six MTAs for the data measured in 2015 at Holetta on chromosomes 1A, 5A, 5B, 6A, 6B and 7B (Appendix IX, Figure 7.9). The detected markers could explain 22.82 – 24.97% of the phenotypic variation for days to flowering, and the SNP marker 1000134|F|0-15:T>C-15:T>C on chromosome 6B and 1204551|F|0-57:C>T-57:C>T on chromosome 1A accounted for the lowest and the highest phenotypic variation in days to flowering in the association panel, respectively (Appendix IX). Likewise, association analysis for days to maturity data measured in 2016 at Bekoji identified 15 MTAs pointing to nine putative QTLs on 1A, 2A,

3A, 3B, 5B, 6D and 7A (Appendix IX**,** Figure 7.10). Three of these significant SNPs were not mapped on the bread wheat physical map. The detected markers explained 11.60 - 15.6% of the phenotypic variation for days to maturity, and the lowest and highest values were reported for the SNP 2278215|F|0-18:A>G-18:A>G on 2A and for 7332831|F|0-9:T>C-9:T>C on 1A, respectively (Appendix IX).

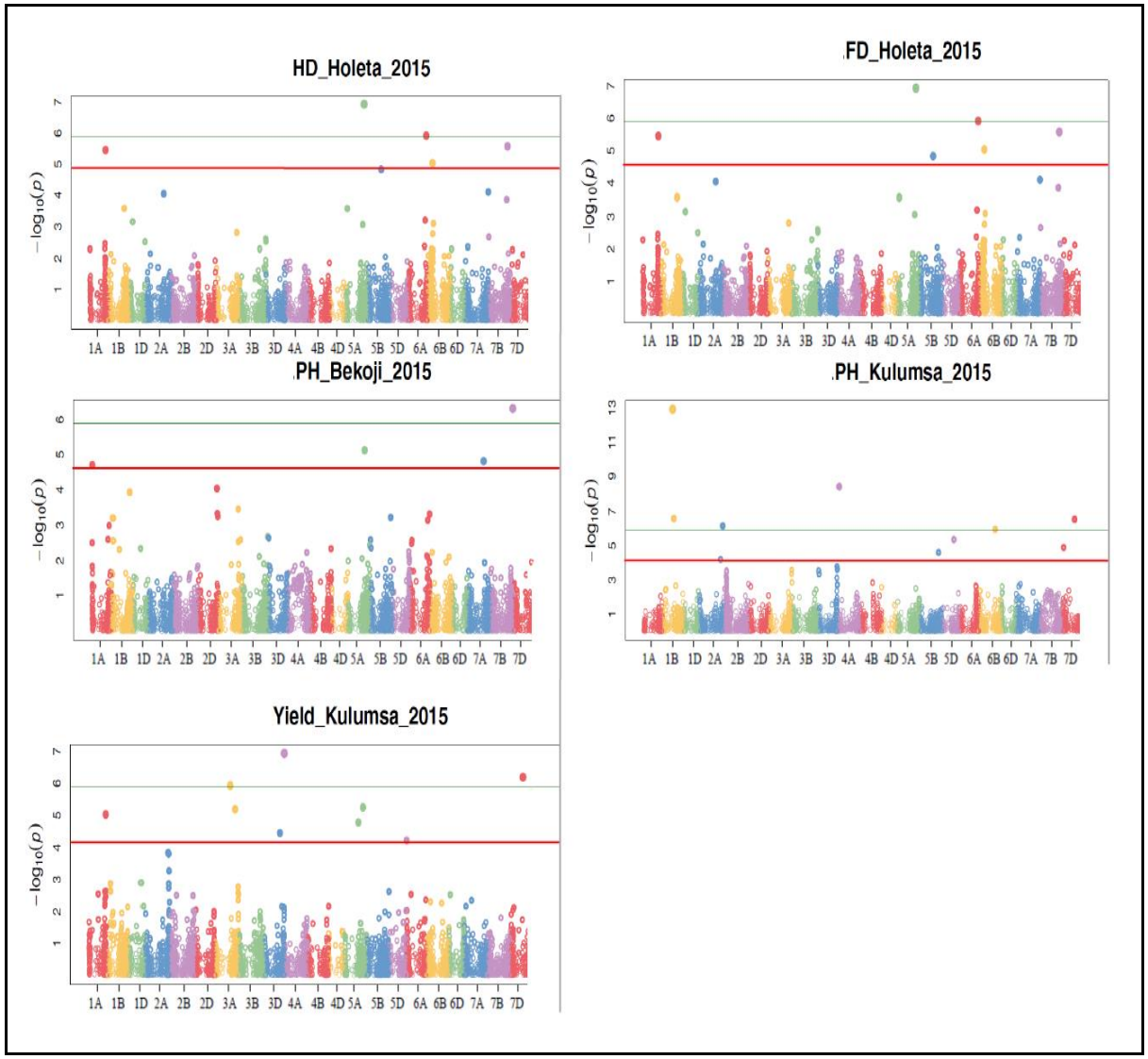

Figure 7. 9 Manhattan plots for the GWA scan on agronomic traits measured in 2015. HD= Days to 50 % heading, FD = Days to 50% flowering, and PH = Plant height

Moreover, a GWA scan on grain filling duration data measured in 2016 revealed 15 MTAs among which 11 were identified from the data collected at Holetta on chromosome 1B, 2B, 3A, 3B, 6D, 7D, and one MTA with unknown position on the bread wheat physical map (Appendix IX; **Figure 7.10**). Four of the significant associations for this trait were obtained from data measured at Kulumsa on chromosome 2B, 3B, 5B and one MTA with an unknown chromosomal position in bread wheat genetics. The total phenotypic variation for GFD explained by the SNPs ranged from 0.49% for the allele on chromosome 3B (5325155|F|0-26:G>T-26:G>T) to 7.89% for the SNP marker on 3A at 250.82 Mbp (5325155|F|0-26:G>T-26:G>T) (Appendix IX).

Association analysis for pooled plant height data identified one MTA on chromosome 7A, and 24 MTAs for the data measured at individual environments (Appendix IX, Figure 7.10). The plant height data measured in 2015 at Bekoji resulted in four MTAs on chromosomes1A, 5A, 7A and 7B, and 10 MTAs for Kulumsa on the chromosomes 1B, 2A, 5B, 5D, 6B and 7D (Appendix IX**,** Figure 7.9). The same trait measured in 2016 at Kulumsa provided 10 MTAs on chromosomes 3B, 5A, 5B, 6B, 6D, 7D and two MTAs that were unmapped on the bread wheat physical map (Appendix IX**,** Figure 7.10). The identified SNPs accounted for 3.29-12.32% of the total variation in plant height, and the SNP marker on 6B (SNP 2276919|F|0-10:G>T-10:G>T) at 521.99 Mbp accounted the highest (Appendix IX).

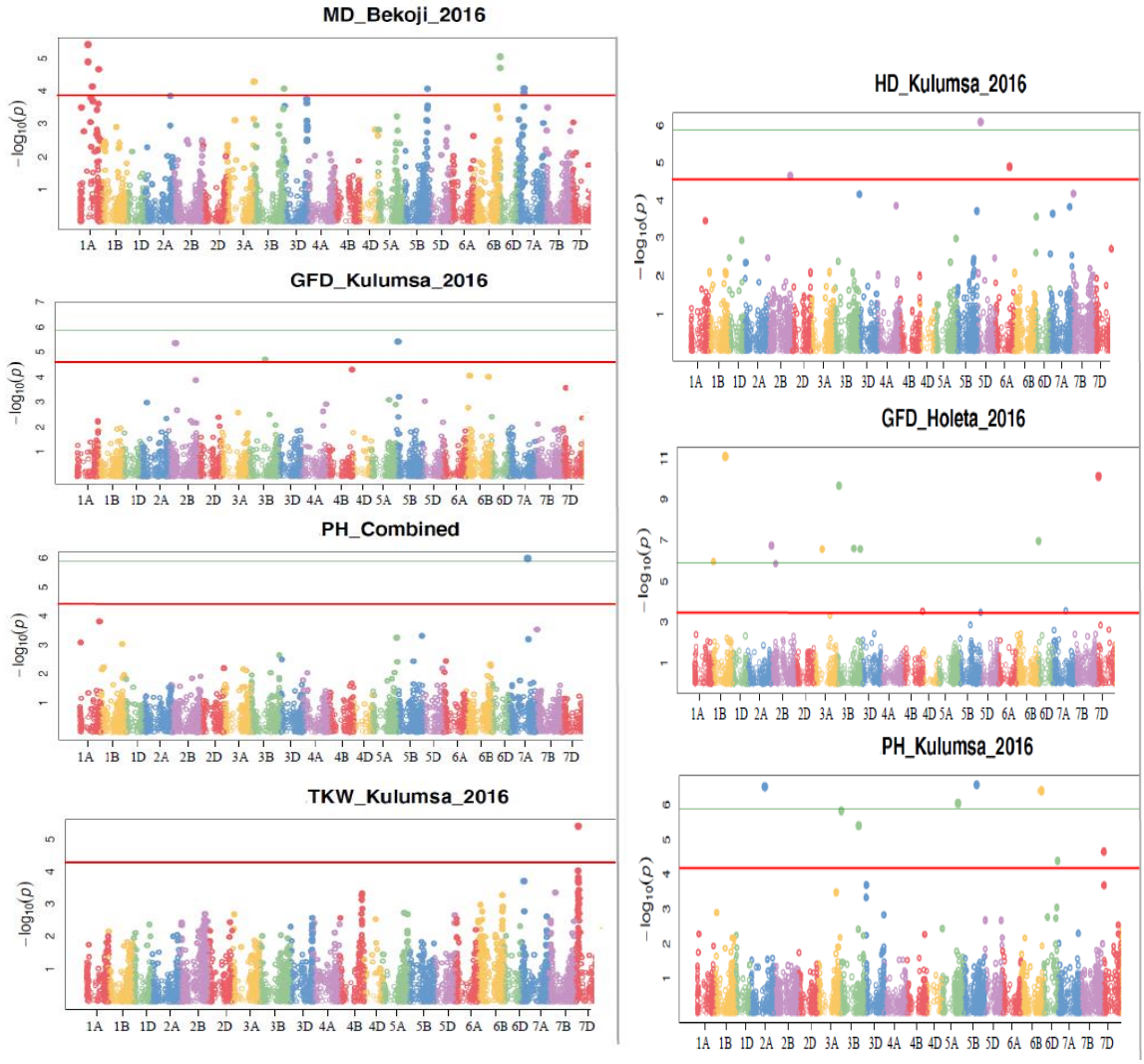

Figure 7. 10 Manhattan plots for agronomic and yield related traits of 2016 and pooled data.

The study revealed that grain yield and yield-related attributes measured overall environments did not provide any significant SNPs. However, their association analysis based on mean value at individual environments identified numerous MTAs. Grain yield measured in 2015 at Kulumsa identified 10 significant MTAs on chromosomes 1A, 3A, 3D, 4A, 5A, 5D, 7D and one SNP that was not mapped in bread wheat genome (Appendix IX**,** Figure 7.9). Similarly, a GWA scan for TKW measured in 2016 at Kulumsa provided one

MTA pointing to the putative QTL on 7D at 79.52 Mbp (Appendix IX, Figure 7.10). The phenotypic variations explained by the significant SNPs for yield and related traits varied considerably based on the trait and the marker, with the lowest being 0.5% for grain yield on 3D (1045011|F|0-60:T>A-60:T>A) and the highest being 15.71% for TKW on 7D (4262368|F|0-28:A>G-28:A>G) (Appendix IX).

The analysis revealed that some putative QTLs identified by SDS data overlapped with putative QTLs determined for agronomic data. For instance, the putative QTL determined for plant height measured in 2016 at Kulumsa on chromosome 6B is co-mapped with qSTB28, identified for the combined SPC. Similarly, the putative QTL identified on 7B for plant height measured at Bekoji in 2015 was co-mapped with qSTB31, identified for combined SPC. Besides, the putative QTL mapped on chromosome 7D for plant height measured in 2015 and 2016 at Kulumsa, for GFD and TKW data measured in 2016 at Holetta and Kulumsa, respectively were co-mapped with qSTB32, identified for SDS data measured at the maturity stage at Bekoji and for pooled SPC data. Appendix X presents the genomic positions (in Mbp) of the detected loci underlying important agronomic traits including yield, yield components and phenological traits. It was observed that some traits eg. days to heading and flowering are controlled by similar loci (Appendix X).

7.4 Discussion

Phenotypic Variability

The phenotypic evaluation revealed considerable genetic variability for STB resistance among the tested wheat genotypes. High broad sense heritability within individual environments (h^2 = 0.58 - 0.99) and across-environments (h^2 = 0.72 – 81) indicated strong genetic signal in the data and that selection using this data could improve resistance. High heritability (H^2 = 0.78) for STB resistance has been reported for European bread wheat varieties in Germany (Muqaddasi *et al*., 2019) and in Tunisia (H^2 = 0.55) (Berraies *et al*., 2014). The study also confirmed that STB infestation was significantly influenced by year, location, and all interaction effects, confirming the importance of multi-locations and over-years germplasm evaluations at hot spots to identify durable and stable STB-resistant genotypes.

The correlation analysis revealed that Septoria disease rating had negligible negative correlation with plant height, indicating that tallness had weak contribution for reducing STB infection. Moreover, SDS traits showed no or little negative association with days to heading and days to flowering. This implies that these agronomic traits have no or little contribution for STB resistance, and hence, presenting these traits as "escape" strategy for STB resistance is not logical. Muqaddasi *et al*. (2019) also reported negative associations between STB infection with plant height and heading date. STB infection also showed significant negative correlation with yield and related traits like hectoliter weight, TKW

NKPS and NKS. This is most likely because STB infection of the flag and second leaves at the grain-filing stage influenced the photosynthesis process and reduced grain yield and yield-related traits. Negative associations of SDS with days to flowering, days to maturity, number of seeds per spike, and thousand-grain weights was also reported for Ethiopian durum wheat (Yosef Geberehawariat*et al*., 2017). The strong negative associations of some phenological and agronomic traits with SDS traits indicate the role that these traits can play and that then should be considered in indirect selection for STB resistance.

Population Stratification and LD

STRUCTURE and principal analysis revealed the presence of population stratifications as wells as admixture. The analyses suggested two sub-groups in the population. The used model (FarmCPU) has sufficiently accounted for population stratification, familial relatedness and marker effects, and thus, reduced the confounding effects that could result in false-positive MTAs. This was confirmed by visualizing the Q-Q and Manhattan plots. Similar in-distinct population stratification, higher admixture and weak population sub-structuring were reported among 371 European wheat genotypes based on 35k and 90k SNP marker arrays (Muqaddasi *et al*., 2019).

Like previous reports, the present study confirmed an unequal distribution of the SNP markers among the wheat genomes where most were harbored by the A (10,317) and B genomes (10,979), and relatively small numbers of SNPs (9,756) were harbored by the D

genome (Berkman *et al*., 2013; Erena Aka *et al*., 2015; Rahimi *et al*., 2019). This has been attributed to their evolutionary history (Dvorak *et al*., 2006; Jordan *et al*., 2015).

The study revealed that the LD between the markers and genes contributing to STB resistance declined to $r^2 < 0.2$ within physical distance of 1.26 – 105.61 Mbp in all chromosomes, with overall mean of 31.44 Mbp. This is much lower than the average physical distance (69.1 Mbp) at which Genome-wide LD between SNP pairs in Ethiopian durum wheat declined at the critical threshold of $r^2 = 0.2$ (Sisay Kidane *et al*., 2021). Although LD across the genome for all the sub-genomes was nearly similar ($r^2 = 0.11$), the marker distances across the older sub-genomes (A and B) were relatively lower than those for the D sub-genome, which could be due to the long evolutionary history of the A and B genomes as compared to the D genome. LD between alleles can decay due to a number of factors including selection, recombination, mating system, genetic drift, mutation and/or population relatedness (Stich and Melchinger, 2010). Hence, it is likely that the shorter selection history of the D sub-genome did not allow linkage breakdown due to recombination to occur between SNPs located at longer physical distances.

Marker-trait Associations and Identification of Candidate Genes

The GWAS analysis identified 53 MTAs pointing to 33 QTLs for STB resistance and 82 MTAs for agronomic traits where markers had an FDR $p \leq 0.05$ and a Bonferroni correction significance threshold of 0.15. The number of SDS MTAs identified in the present study is significantly lower than the findings of Rahimi *et al*. (2019) who reported 313 – 394 MTAs

for an Iranian bread wheat association panel, but substantially higher than the report of Yosef Geberehawariat *et al*. (2017) who identified 35 significant associations for an Ethiopian durum wheat panel. Just seven QTL for SPC were identified in an analysis of the mean over environments and none were detected for SDS. Yosef Geberehawariat *et al*. (2017) also reported no QTLs for SDS in an analysis of means.

More QTL were noted in the analysis of data from individual environments, though none were detected in more than four of the six environments and 24 of 33 were detected in just one environment. The failure to generally not repeat QTL effects over environments could be due to seasonal specific QTL effects, disease pressure, or the use of a very stringent FDR level that controls type I error but leads to increased type II error, e.g., declaring a QTL as not significant when it is real. The 2015 growing season at Holetta was characterized by extended and heavy rainfall that resulted in the highest STB natural infestations across all growth stages, and 12 QTLs were detected (five for SPC and 7 for SDS): three QTL affected both SPC and SDS in Holeta 2015. Heavy rainfall and prolonged moisture experienced at Bekoji in 2016 produced the highest SDS and 12 QTLs were identifed using that data: six for SPC, four for SDS and two QTL affecting both traits. Climatic conditions such as persistent crop moisture and prolonged heavy rain favor successful infection and spread of the disease throughout the crop canopy (Fones and Gurr, 2015; Helen and Sarah, 2015). No QTL for SDS were detected for Kulumsa in either year. Though a total of 11 QTL for SPC were detected in the same environment, none were repeated over years. The different climatic conditions may have caused a later onset of the disease in both growing

seasons. One QTL, qSTB21, had the most repeatable effect and was significant for SPC overall, and for SPC in two environments, and for SDS at heading and mid-maturity stages in 2015 at Holetta.

In the present study, GWA scan on SDS data measured at heading stage at Holetta in 2015 and at Bekoji in 2016 identified putative QTLs on chromosomes 1D, 2A, 3A, 3D, 5A, 7A and 7D. Moreover, association analysis for SDS at mid-maturity at Holetta in 2015 reported three effective putative QTLs on chromosomes 1B, 3D and 7B, which were not reported for the trait at this stage so far. Likewise, dissecting the disease data measured at the maturity stage identified putative QTLs on chromosomes 1D, 4A, 6A and 7D. Furthermore, a GWA scan for SPC identified putative QTLs on chromosomes 1A, 1B, 1D, 2B, 2D, 3A, 3B, 3D, 5B, 5D, 6A, 6B,7A,7B and 7D.

Although the different mapping methods, marker system and populations used can make the comparison of QTLs position from different studies, some QTLs detected in our study coincided with the mapping positions of previously reported major STB resistant genes. Hence, our putative QTL on 1B (qSTB4) may represent *Stb2* (Liu *et al*., 2013) and/or *Stb11* (Chartrain *et al.*, 2009), the QTLs on 1D (qSTB5-7) may represent *Stb10* (Chartrain *et al*., 2005c), the QTL on 2B may represent *Stb9* (Chartrain *et al*., 2009), the QTLs on 3A (qSTB15-17) may represent *Stb6* (Brading *et al*., 2002) and/or *StbSm3* (Cuthbert, 2011), the QTLs on 3B may be *Stb14* (Cowling, 2006), the QTLs on 3D represent *Stb16q* (Tabib Ghaffary *et al*., 2012), the QTL on 4A may be *Stb7* (McCartney *et al*., 2003) and *Stb12*

(Chartrain *et al*., 2005c), the QTLs on 5A represents *Stb17* (Tabib Ghaffary *et al*., 2012), the QTLs on 5B may represent *Stb1* (Adhikari *et al*., 2004a), the QTL on 6A may be *Stb15* (Arraiano *et al*., 2007b), the QTLs on 7A may be *Stb3* (Goodwin and Thompson, 2011) and *TmStb1* (Jing *et al*., 2008), the QTL on 7B may represent *Stb8* (Adhikari *et al*., 2003) and *Stb13* (Cowling, 2006), and the putative QTLs on 7D may represent *Stb4* (Adhikari *et al*., 2004c) and *Stb5* (Arraiano *et al*., 2001b). Similar to our result, Kollers *et al* . (2013) reported significant MTAs for STB resistance on chromosome 2A and 2D. The present result also agrees with Muqaddasi *et al*. (2018) who reported an adult-plant stage STB resistance QTL on chromosome 4A.

The identification of defense related candidate genes such as *TraesCS1A02G279300, TraesCS1B02G332400,TraesCS1D02G278400,TraesCS2B02G233600,TraesCS2A02G297500, TraesCS2D02G497400, TraesCS2D02G506300* and *TraesCS4A02G341300* in the vicinity of the significant markers indicate the functional association of detected QTL regions in plant defense system against pathogen infections. For instance, the translates of the genes found in qSTB2 (*TraesCS1A02G279300*) and qSTB4 (*TraesCS1B02G332400*) on chromosome 1A and 1B, respectively, are involved in jasmonic acid and ethylene-dependent systemic acquired resistances of plants to pathogen infections. Systemic acquired resistance (SAR) is a broad-spectrum long-lasting resistance acquired after initial localized infection of plants by pathogens (Lawton *et al*., 1995). The gene *TraesCS2A02G297500* found in *qSTB8* on chromosome 2A controls MAPK cascades which are involved in signaling multiple defense responses, including the biosynthesis or signaling of plant stress/

defense hormones, reactive oxygen species (ROS) generation, stomatal closure, defense gene activation, phytoalexin biosynthesis, cell wall strengthening, and hypersensitive response (HR) cell death. Moreover, most of the genes in proximity to the detected significant markers are inferred to involve in salicylic acid (SA) biosyntheis: an important plant hormone that is best known for mediating host responses upon pathogen infection (Lefevere *et al*., 2020).

We have discovered some STB resistance QTLs that appear novel. These include the putative QTLs on chromosomes 1A (qSTB1-3), 5D (qSTB26), and 6B (qSTB28) that explained > 5% of the genetic variations, suggesting their relevance for wheat resistance breeding against STB. To the best of our knowledge, none of the known major STB resistance genes published in the literature have been mapped to these regions of the wheat chromosomes, and therefore, these QTLs could be considered as novel.

Some of the putative STB resistance QTLs were co-located with MTAs for agronomic trait. For instance, the putative QTL derived from plant height measured in 2016 at Kulumsa on chromosome 6B and in 2015 at Bekoji on chromosome 7B were co-mapped with qSTB28 and qSTB31, identified for combined SPC. Likewise, the putative QTLs mapped on chromosome 7D for grain filling duration and at 1000-kernels weight measured in 2016 at Holetta and Kulumsa, respectively were co-mapped with qSTB32, identified for SDS data measured at the maturity stage at Bekoji and for pooled SPC data. STB is the most destructive foliar disease and hence, infection of the flag and second leaves, which

contributes most to photosynthetic assimilates at the grain-filling stage (King *et al*., 1983; Muqaddasi *et al*., 2019), can result in substantial loss of grain weight and yield. This is consistent with Yosef Geberehawariat *et al*. (2017), who reported co-mapping of putative QTLs for 1000-kernel weight with SDS data. Moreover, the putative QTL on chromosome 1A identified for grain yield measured at Kulumsa in 2015 was co-mapped with qSTB3, identified for SPC measured in the same environment. It is expected that the vertical progression rate of the disease could affect grain yield by influencing grain filling and the number of seeds produced per spike.

Most of the QTLs (24) identified for agronomic and phenological traits did not overlap with those detected for SDS traits likely due to the lack of a common genetic effects for STB resistance and these traits. Many of the correlations of STB traits with agronomic traits were non-significant, and had negligible to weak negative coefficients indicating that the traits were independent. However, some level of co-localization was observed for the putative QTL for days to heading and days to flowering measured at Holetta in 2015 on chromosome 6B with the putative QTL qSTB28 on 6B, identified for SPC measured at Bekoji in 2016 and for pooled data. Another possible complicating factor in the analysis could be disease escape. Traits that facilitate escape such as tallness and wide leaf spacing reduce the disease severity by limiting or delaying the vertical spread of the spores up the plant, leading them to be scored as resistant, while they are inherently not resistant to disease (Eyal e*t al.,* 1987; Miedaner *et al*., 2012; Miedaner *et al.,* 2013). Other previous reports also revealed that late

heading, flowering and maturation are important disease escape traits contributing to apparent resistance to Septoria (Miedaner *et al.,* 2013; Muqaddasi *et al*., 2019).

7.5 Conclusion

Association genetics based on linkage disequilibrium is a powerful and promising technique widely used to dissect the genetic bases of disease resistances including the worldwide wheat devastating disease Septoria tritici blotch (STB). In the present study, the genetic architecture of adult-plant resistance to STB was explored in bread wheat using high-density Genome-wide SNP markers and multi environment-derived phenotype data. The study revealed that the association panel possessed considerable STB resistance alleles that could be deployed to improve wheat resistance to the prevailing *Zymoseptoria tritici* populations in Ethiopia. Several genotypes with better resistance than the moderate resistant check King-bird were identified. The GWAS identified 33 putative QTLs which are associated with 53 SNPs. Most of the QTLs(24) were detected in just one environment suggesting the presence of resistance gene/genes effective to location specific *Z. tritici* races. The low reproducibility of QTL effects over environments indicates that future wheat durable resistance breeding programs shall take into consideration race-specific resistances. The detected QTLs explained 2.70 - 13.20% of the total phenotypic variance for STB resistance. Several disease resistance associated gene/s such as *TraesCS1A02G279300, TraesCS1B02G332400, Traes-CS1D02G393000, TraesCS2B02G233600, TraesCS2D02G497-400, and TraesCS2D02-G506300* have been identified in the proximity of the detected SNPs which can be targeted in efforts to understand the actual causative genes in the associated loci. Most of the

detected putative QTLs shared similar chromosomal positions with previously reported genes and QTLs. Among the detected alleles, five were potentially novel accounting for > 5% of STB resistance. The effect of these QTLs needs to be validated before being deployed in MAS. Combined with the conclusion that STB resistance is controlled by genes for small effect, the results suggest that genomic selection may be the preferred breeding technology for resistance to STB in this population. In general, the identified stable resistant wheat genotypes and the identified QTLs could be deployed in the wheat breeding program for the development of durable and broad spectrum resistant varieties against *Z. tritici*.

CHAPTER 8

8. Conclusions and Recommendations

8.1 Conclusion and implications of the study to improve the productivity of wheat in Ethiopian

Wheat is one of the major strategic food security crops in Ethiopia. The high population growth, rapid urbanization, changing food preferences and its wide industrial applications have increased wheat demand in Ethiopia resulting in a huge gap between production and consumption. Thus, reversing the increasing wheat import to meet the domestic demand requires enhancing production and productivity through use of advanced production technologies including mechanization, efficient agricultural inputs with appropriate applications of improved seeds and fertilizers, irrigation farming, and also addressing and effective control of the biotic and abiotic production limiting factors. Septoria tritici blotch caused by *Z. tritici (telomorph M. graminicola*) is the major wheat production-limiting factor in Ethiopia and beyond. Genetic resistance is considered to be the best strategy, durable, economical and environmentally friendly method to control the disease. Designing successful resistance breeding requires knowledge of the pathogen's genetic structure, their infection pattern, and identification and deployment of resistance sources.

The first objective of the present study was to profile the extent and patterns of genetic diversity and population structure of 182 *Z. tritici* isolates representing eight populations collected from major wheat growing areas of Ethiopia using 14 SSR loci. All the microsatellite loci were highly polymorphic in the studied population, and found to be very

useful tool to successfully depict the genetic structure of the pathogen populations. The analysis revealed a very high genetic diversity within *Z. tritici* populations of Ethiopia, where 92% of the total genetic diversity resides within populations. All the study populations exhibited high gene diversity ranging from 0.34 to 0.58 indicating the suitability of the study areas for investigations planned on the pathogen, host and their interactions. Among tested populations, those from Arsi, Bale, Southwest Shewa and East Shewa displayed gene diversity greater than the overall mean gene diversity, suggesting that these locations are hot spots for *Z. tritici* diversity, and hence, ideal experimental locations for germplasm screening for resistance to STB.

The analysis revealed that all the *Z. tritici* populations shared genetic background originated from two sub-populations confirming the presence of high gene flow among locations. Adopting integrated disease management strategies that include host resistance, use of fungicide-treated seed, and removing crop debris can limit the gene flow and reduce STB severity in Ethiopia. As host resistance is a durable and effective method to manage crop diseases, screening of large sets of wheat germplasms and breeding materials against the genetically diverse isolates needs to be targeted. Therefore, the present study has provided a first stab to comprehend the genetic structure of *Z. tritici* populations of the central highlands (Shewa) and Southeastern (Arsi and Bale) parts of Ethiopia, where 75% of the national wheat production is located. This baseline information is very helpful for wheat breeders and pathologists to design effective management strategies so as to minimize wheat production losses due to STB. The results also provide new insight into the epidemiology

and genetic structure of *Z. tritici* in Africa where the agro-climatic conditions and the wheat cropping systems are different from the remaining parts of the world. However, the present pathogen genetic structure analysis did not include all potential wheat growing areas of the country, and thus, a future study that include populations from the remaining parts of the country can generate data to provide a nationwide picture of the disease.

The second part of this study attempted to assess the virulence patters of eight Ethiopian *Z. tritici* isolates recovered from representative STB hot spots. The study disclosed that Ethiopian *Z. tritici* isolates show specific-interactions, broad spectrum virulence patterns and greater degrees of aggressiveness on bread wheat genotypes, suggesting the need to device a resistance breeding strategy that incorporates a combination of genes that can provide sufficient protection and durable control of the disease in the country. Hence, national wheat breeders should follow gene pyramiding approach to combine all known resistant genes.

The pathogenicity analysis detected highly virulent isolates that can be used for germplasm screening in resistance breeding program. The study also confirmed that the internationally reported *Stb16* gene conferred broad spectrum resistance to Ethiopian *Z. tritici* isolates, and thus, it can be the best candidate gene to be targeted by breeders to improve STB resistance in high yielding, but STB susceptible commercial wheat cultivars of Ethiopia. Moreover, MURGA, KM7 and the cultivar Hidase which showed the greatest level of resistance to most tested *Z. tritici* isolates could serve as good source of resistance against STB. However, as the pathogen changes its genome frequently, continuous survey of the pathogen

populations and deep investigation on the molecular mechanisms underlying the pathogen-host interactions are important.

The present study also aimed at searching for Septoria resistant genes (*Stb* genes) in 180 bread wheat genotypes using 16 tightly linked SSR markers. Tested wheat genotypes showed greater degree of genetic variability for *Stb* genes. The frequency of the qualitative resistance genes ranged from 6.67% for *Stb*8 gene to 96.1% for *Stb13* gene. The study identified 17 wheat genotypes harboring 12 to 13 Septoria resistance genes. The information is vital for national breeders in developing broad spectrum and/or durable resistant cultivars through multiple genes pyramiding. Moreover, those genotypes identified to have many *Stb* genes can also be advanced for multi-location yield trial test (for grain yield performance and stability) for fast track release that leads to increased wheat production and productivity in the country. The technique is very efficient, economical and fast to select the resistant genotypes which may take several seasons and years in field or greenhouse germplasm evaluations.

In this study, attempt was also made to identify stable and high yielding bread wheat genotypes through multi locations and years field evaluation of 180 bread wheat genotypes for grain yield, yield components and other agronomic traits. Tested genotypes exhibited wide genetic variability for grain yield and other agronomic traits, suggesting large possibility for genetic improvement through selection. Most studied agronomic traits showed moderate to high heritability, indicating the presence of alleles of breeding

relevance. The study revealed significant and positive association of grain yield with hectoliter weight, 1000 kernels weight, number of kernels per spike, days to maturity, grain filling duration, flag leaf area, number of kernels per spikelet and number of kernels per spike, indicating that yield performance in wheat can be simultaneously improved indirectly by considering any of these traits. The present study also identified genotypes that have 10.8 - 21.99 % and 3.99 - 26.86 % super grain yield performance to the recently released standard check variety King-bird, and to the average performance of 13 commercial cultivars grown in Ethiopia, respectively. The very highly significant genotype by location by year interaction effect on grain yield, yield related and other agronomic traits indicates the significance of multi location and years germplasm evaluation to identify high yielding and highly stable cultivars.

The GGEbiplot analysis also confirmed the existence of crossover interaction for grain yield performance, suggesting that breeders need select genotypes carefully after making comparison with standard checks and implementation of appropriate experimental design to minimize environmental errors. The biplot analysis also confirmed existence of different mega-environments for grain yield performance where Bekoji (2016) was found to be ideal test environments with high discriminating and representative power for selecting superior yielding and stable genotypes. The genotypes clustering based on mean grain yield performance and stability identified four clusters, among which the genotypes G21, G51, G85, G114, G144, G159, G169, and G174 are the most desirable with high grain yield and high stability. Among these genotypes, G85, G159, 169 and G174 represent the top four

most desirable genotypes performing near to the "ideal" genotype. These genotypes could be considered as promising high yielding and highly stabile genotypes to develop new cultivars to boost wheat production and productivity in Ethiopia and the region at large.

Nowadays, association genetics based on linkage disequilibrium is becoming a promising technique widely used to dissect the genetic bases of disease resistances in crop plants. In the present study, high-density DArTSeq SNPs were used to explore the population structure, LD and genetic architecture of adult-plant resistance to STB in 178 bread wheat genotypes. The association panel was phenotyped for STB resistance, phenology, yield and yield-related traits at three locations for two years. The genotypes exhibited significant genetic variations to STB infestation with broad-sense heritability of 0.58 - 0.97 and 0.72 - 0.81 at individual and across environments, respectively, suggesting the presence of STB resistance alleles in the association panel. SDS showed an increasing trend from heading to maturity. The genotypes G174, G144, G3, G153, G156, G133, G151, G155 and G97 were among the top 5% best STB-resistant genotypes that registered 52.25- 74.25% super resistance to the moderately resistant standard check King-bird, and 47.75 -71.83% over the average of 13 released varieties in Ethiopia.

The STRUCTURE analysis indicated two sub-populations in the association panel with a greater degree of genetic admixture. The PCA results also suggested the presence of two sub-population where the first two PCs explained 65% (PC1 = 50% and PC2 = 15 %) of the total variances contained in the data. The analysis also confirmed the presence of kinship in

the association panel, suggesting the importance of using a powerful statistical GWAS model that accounts for the population structure and familial relatedness in the association study.

LD analysis revealed that the LD between the markers and genes contributing to STB resistance declined to $r^2 < 0.2$ within physical distance of 1.26 – 105.61 Mbp in all chromosomes, with overall mean of 31.44 Mbp.

GWAS revealed that the association panel harbored considerable STB resistance alleles that could be deployed to improve wheat resistance to the prevailing *Z. tritici* populations in Ethiopia. The analysis identified 53 MTAs pointing to 33 QTLs for STB resistance and 82 MTAs for agronomic traits where markers had an FDR $p \leq 0.05$ and a Bonferroni correction significance threshold of 0.15. Most of the detected putative QTLs shared similar chromosomal positions with previously reported genes and QTLs. Most detected QTLs had shared similar chromosome with previously reported Septoria resistance genes and QTLs which were distributed across chromosomes 1B, 1D, 2A, 2B, 2D, 3A, 3B, 3D, 4A, 5A, 5B, 6A,7A,7B and 7D. However, five of the putative QTLs identified on chromosomes 1A, 5D and 6B appeared novel. Dissecting the detected loci on IWGSC RefSeq Annotation v2.1 revealed the existence of disease resistance associated genes in the identified QTLs regions which are involved in plant defense system. Seven of the QTLs detected for STB resistance were found to be co-localized with MTAs for agronomic trait. In general, the association study successfully revealed the genetic architecture of adult-plant resistance to

STB resistance. The identified stable resistant wheat genotypes and QTLs could be deployed in the wheat breeding program for the development of durable and broad spectrum resistant varieties against *Z. tritici*.

In conclusion, the present piece of work reports the extent and patterns of genetic diversity and population structure and virulence variability of the wheat devastating pathogen *Z. tritici* populations in Ethiopia. It also reports promising high yielding and stable STB resistance sources, qualitative resistance genes (*Stb* genes) and QTLs important for durable and effective control of STB disease of wheat in Ethiopia that results in increased and stable wheat production and productivity there by contributing to food and nutritional security and overall economic progress in the country. Moreover, any future studies on the pathogen genetic dynamics need to consider multiple isolates from the present identified hot spots areas including East Shewa, Arsi, South West Shewa and Bale Zones. Furthermore, future national and international wheat germplasm screening programs for STB resistance shall include Bekoji, Holetta and other previously reported Septoria hot spots. To guarantee their use for marker-assisted selection, the diversity and effectiveness of the newly identified QTLs need to be functionally validated.

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APPENDICES

Appendix **I .** Passport data for 182 *Z.tritici* isolates representing the eight populations in Ethiopia.

Isolate* Code	Population Code	Administrative Locations				Geographical Positions (UTM)[a]		Altitude (m)	Geographical Location in Ethiopia	Disease Symptom	Host Plant
		Region	Zone	District	Kebele/Station	Latitude	Longitude				
ZTET001	ESH	Oromia	East Shoa	Adaa	DZARC	08° 20' 164	039° 00' 581	1881	Central Highland	STB	Bread Wheat
ZTET002	ESH	Oromia	East Shoa	Adaa	DZARC	08° 20' 165	039° 00' 582	1881	Central Highland	STB	Bread Wheat
ZTET003	ESH	Oromia	East Shoa	Adaa	DZARC	08° 46' 322	039° 00' 027	1882	Central Highland	STB	Bread Wheat
ZTET004	ESH	Oromia	East Shoa	Adaa	DZARC	08° 46' 329	039° 00' 029	1881	Central Highland	STB	Bread Wheat
ZTET005	ESH	Oromia	East Shoa	Adaa	DZARC	08° 46' 319	039° 00' 043	1879	Central Highland	STB	Bread Wheat
ZTET006	ESH	Oromia	East Shoa	Adaa	DZARC	08° 46' 320	039° 00' 045	1880	Central Highland	STB	Bread Wheat
ZTET007	ARSI	Oromia	Arsi	Hetosa	Shagi Sharara	08° 06' 100	039° 13' 553	2202	South East	STB	Bread Wheat
ZTET008	ARSI	Oromia	Arsi	Hetosa	Shagi Sharara	08° 05' 968	039° 13' 584	2171	South East	STB	Bread Wheat
ZTET009	ARSI	Oromia	Arsi	Hetosa	Shagi Sharara	08° 05' 968	039° 13' 584	2171	South East	STB	Bread Wheat
ZTET010	ARSI	Oromia	Arsi	Hetosa	KARC	08° 00' 927	039° 09' 486	2211	South East	STB	Bread Wheat
ZTET011	ARSI	Oromia	Arsi	Hetosa	KARC	08° 00' 927	039° 09' 486	2211	South East	STB	Bread Wheat
ZTET012	ARSI	Oromia	Arsi	Hetosa	KARC	08° 00' 919	039° 09' 475	2211	South East	STB	Bread Wheat
ZTET013	ARSI	Oromia	Arsi	Hetosa	KARC	08° 00' 930	039° 09' 504	2212	South East	STB	Bread Wheat
ZTET014	ARSI	Oromia	Arsi	Hetosa	KARC	08° 00' 917	039° 09' 479	22137	South East	STB	Bread Wheat
ZTET015	ARSI	Oromia	Arsi	Hetosa	KARC	08° 00' 922	039° 09' 521	2213	South East	STB	Bread Wheat
ZTET016	ARSI	Oromia	Arsi	Hetosa	KARC	08° 00' 890	039° 09' 524	2213	South East	STB	Bread Wheat
ZTET017	ARSI	Oromia	Arsi	Hetosa	KARC	08° 00' 837	039° 09' 488	2213	South East	STB	Bread Wheat
ZTET018	ARSI	Oromia	Arsi	Hetosa	KARC	08° 00' 858	039° 09' 481	2214	South East	STB	Bread Wheat
ZTET019	ARSI	Oromia	Arsi	Lemu Bilbilo	Lemu Dima	08° 00' 844	039° 09' 441	2211	South East	STB	Bread Wheat
ZTET020	ARSI	Oromia	Arsi	Lemu Bilbilo	Lemu Dima	07° 36' 053	039° 13' 823	2606	South East	STB	Bread Wheat
ZTET021	ARSI	Oromia	Arsi	Lemu Bilbilo	Lemu Dima	07° 36' 058	039° 13' 821	2607	South East	STB	Bread Wheat
ZTET022	ARSI	Oromia	Arsi	Lemu Bilbilo	Lemu Dima	07° 36' 058	039° 13' 841	2606	South East	STB	Bread Wheat
ZTET023	ARSI	Oromia	Arsi	Lemu Bilbilo	Lemu Dima	07° 36' 074	039° 13' 873	2608	South East	STB	Bread Wheat
ZTET024	ARSI	Oromia	Arsi	Lemu Bilbilo	Bokoji Station	07° 32' 552	039° 15' 325	2810	South East	STB	Bread Wheat
ZTET025	ARSI	Oromia	Arsi	Lemu Bilbilo	Bokoji Station	07° 32' 568	039° 15' 333	2812	South East	STB	Bread Wheat
ZTET026	ARSI	Oromia	Arsi	Lemu Bilbilo	Bokoji Station	07° 32' 579	039° 15' 340	2815	South East	STB	Bread Wheat
ZTET027	ARSI	Oromia	Arsi	Lemu Bilbilo	Bokoji Station	07° 32' 587	039° 15' 343	2815	South East	STB	Bread Wheat
ZTET028	ARSI	Oromia	Arsi	Lemu Bilbilo	Bokoji Station	07° 32' 585	039° 15' 352	2817	South East	STB	Bread Wheat
ZTET029	ARSI	Oromia	Arsi	Lemu Bilbilo	Bokoji Station	07° 32' 580	039° 15' 359	2816	South East	STB	Bread Wheat
ZTET030	ARSI	Oromia	Arsi	Lemu Bilbilo	Bokoji Station	07° 32' 593	039° 15' 363	2816	South East	STB	Bread Wheat
ZTET031	ARSI	Oromia	Arsi	Lemu Bilbilo	Bokoji Station	07° 32' 598	039° 15' 358	2820	South East	STB	Bread Wheat
ZTET032	ARSI	Oromia	Arsi	Lemu Bilbilo	Bokoji Station	07° 32' 600	039° 15' 363	2815	South East	STB	Bread Wheat
ZTET033	ARSI	Oromia	Arsi	Lemu Bilbilo	Bokoji Station	07° 32' 602	039° 15' 372	2816	South East	STB	Bread Wheat
ZTET034	ARSI	Oromia	Arsi	Lemu Bilbilo	Bokoji Station	07° 32' 598	039° 15' 380	2817	South East	STB	Bread Wheat
ZTET035	ARSI	Oromia	Arsi	Lemu Bilbilo	Bokoji Station	07° 32' 619	039° 15' 367	2813	South East	STB	Bread Wheat
ZTET036	ARSI	Oromia	Arsi	Lemu Bilbilo	Bokoji Station	07° 32' 628	039° 15' 374	2812	South East	STB	Bread Wheat
ZTET037	ARSI	Oromia	Arsi	Lemu Bilbilo	Bokoji Station	07° 32' 641	039° 15' 386	2811	South East	STB	Bread Wheat
ZTET038	ARSI	Oromia	Arsi	Lemu Bilbilo	Bokoji Station	07° 32' 648	039° 15' 390	2814	South East	STB	Bread Wheat

Appenix **I** Continued

ZTET039	ARSI	Oromia	Arsi	Lemu Bilbilo	Bokoji Station	07° 32' 648	039° 15' 397	2814	South East	STB	Bread Wheat
ZTET040	ARSI	Oromia	Arsi	Lemu Bilbilo	Bokoji Station	07° 32' 661	039° 15' 407	2812	South East	STB	Bread Wheat
ZTET041	ARSI	Oromia	Arsi	Lemu Bilbilo	Bokoji Station	07° 32' 626	039° 15' 395	2812	South East	STB	Bread Wheat
ZTET042	ARSI	Oromia	Arsi	Lemu Bilbilo	Mararo	07° 24' 455	039° 14' 925	2991	South East	STB	Bread Wheat
ZTET043	ARSI	Oromia	Arsi	Lemu Bilbilo	Mararo	07° 24' 452	039° 14' 923	2993	South East	STB	Bread Wheat
ZTET044	ARSI	Oromia	Arsi	Lemu Bilbilo	Mararo	07° 24' 450	039° 14' 916	2991	South East	STB	Bread Wheat
ZTET045	ARSI	Oromia	Arsi	Lemu Bilbilo	Mararo	07° 24' 443	039° 14' 918	2990	South East	STB	Bread Wheat
ZTET046	ARSI	Oromia	Arsi	Lemu Bilbilo	Mararo	07° 24' 422	039° 14' 913	2992	South East	STB	Bread Wheat
ZTET047	ARSI	Oromia	Arsi	Lemu Bilbilo	Mararo	07° 24' 419	039° 14' 928	2987	South East	STB	Bread Wheat
ZTET048	ARSI	Oromia	Arsi	Lemu Bilbilo	Mararo	07° 24' 415	039° 14' 941	2988	South East	STB	Bread Wheat
ZTET049	ARSI	Oromia	Arsi	Lemu Bilbilo	Mararo	07° 24' 403	039° 14' 946	2990	South East	STB	Bread Wheat
ZTET050	ARSI	Oromia	Arsi	Lemu Bilbilo	Mararo	07° 24' 407	039° 14' 949	2993	South East	STB	Bread Wheat
ZTET051	ARSI	Oromia	Arsi	Lemu Bilbilo	Mararo	07° 24' 419	039° 14' 956	2990	South East	STB	Bread Wheat
ZTET052	ARSI	Oromia	Arsi	Lemu Bilbilo	Mararo	07° 24' 430	039° 14' 947	2989	South East	STB	Bread Wheat
ZTET053	ARSI	Oromia	Arsi	Lemu Bilbilo	Mararo	07° 18' 894	039° 16' 388	2792	South East	STB	Bread Wheat
ZTET054	ARSI	Oromia	Arsi	Lemu Bilbilo	Mararo	07° 18' 893	039° 16' 395	2792	South East	STB	Bread Wheat
ZTET055	ARSI	Oromia	Arsi	Lemu Bilbilo	Mararo	07° 18' 911	039° 16' 387	2791	South East	STB	Bread Wheat
ZTET056	ARSI	Oromia	Arsi	Tiyo	Haro Bilalo	07° 52' 864	039° 07' 479	2531	South East	STB	Bread Wheat
ZTET057	ARSI	Oromia	Arsi	Adaba	Soboro	07° 01' 498	039° 27' 128	2460	South East	STB	Bread Wheat
ZTET058	ARSI	Oromia	Arsi	Adaba	Soboro	07° 01' 526	039° 27' 131	2457	South East	STB	Bread Wheat
ZTET059	ARSI	Oromia	Arsi	Tiyo	Haro Bilalo	07° 52' 863	039° 07' 489	2528	South East	STB	Bread Wheat
ZTET060	ARSI	Oromia	Arsi	Tiyo	Haro Bilalo	07° 52' 857	039° 07' 501	2533	South East	STB	Bread Wheat
ZTET061	ARSI	Oromia	Arsi	Tiyo	Haro Bilalo	07° 52' 847	039° 07' 511	2539	South East	STB	Bread Wheat
ZTET062	ARSI	Oromia	Arsi	Tiyo	Haro Bilalo	07° 52' 874	039° 07' 480	2531	South East	STB	Bread Wheat
ZTET063	ARSI	Oromia	Arsi	Tigona Digelu	Goba Lencha	07° 41' 580	039° 09' 895	2458	South East	STB	Bread Wheat
ZTET064	ARSI	Oromia	Arsi	Tigona Digelu	Goba Lencha	07° 41' 598	039° 09' 906	2458	South East	STB	Bread Wheat
ZTET065	ARSI	Oromia	Arsi	Tigona Digelu	Goba Lencha	07° 41' 619	039° 09' 920	2460	South East	STB	Bread Wheat
ZTET066	ARSI	Oromia	Arsi	Tigona Digelu	Goba Lencha	07° 41' 596	039° 09' 914	2460	South East	STB	Bread Wheat
ZTET067	ARSI	Oromia	Arsi	Adaba	Soboro	07° 01' 525	039° 27' 126	2459	South East	STB	Bread Wheat
ZTET068	WARSI	Oromia	West Arsi	Asassa	Asassa Station	07° 07' 177	039° 11' 936	2374	South East	STB	Bread Wheat
ZTET069	WARSI	Oromia	West Arsi	Asassa	Asassa Station	07° 07' 175	039° 11' 927	2375	South East	STB	Bread Wheat
ZTET070	WARSI	Oromia	West Arsi	Asassa	Asassa Station	07° 07' 185	039° 11' 929	2375	South East	STB	Bread Wheat
ZTET071	WARSI	Oromia	West Arsi	Asassa	Asassa Station	07° 07' 194	039° 11' 933	2374	South East	STB	Bread Wheat
ZTET072	WARSI	Oromia	West Arsi	Asassa	Asassa Station	07° 07' 216	039° 11' 934	2373	South East	STB	Bread Wheat
ZTET073	WARSI	Oromia	West Arsi	Asassa	Kakawa Walkita	07° 13' 709	039° 14' 733	2531	South East	STB	Bread Wheat
ZTET074	WARSI	Oromia	West Arsi	Asassa	Edo Naga	07° 16' 561	039° 16' 242	2620	South East	STB	Bread Wheat
ZTET075	WARSI	Oromia	West Arsi	Asassa	Edo Naga	07° 16' 580	039° 16' 245	2622	South East	STB	Bread Wheat
ZTET076	WARSI	Oromia	West Arsi	Asassa	Edo Naga	07° 16' 591	039° 16' 263	2624	South East	STB	Bread Wheat
ZTET077	WARSI	Oromia	West Arsi	Asassa	Huruba Hanto	07° 03' 628	039° 10' 345	2369	South East	STB	Bread Wheat
ZTET078	WARSI	Oromia	West Arsi	Kofale	Wabe	07° 00' 990	039° 00' 620	2539	South East	STB	Bread Wheat
ZTET079	WARSI	Oromia	West Arsi	Kofale	Mamo: Station	07° 05' 136	038° 47' 093	2654	South East	STB	Bread Wheat
ZTET080	SWSH	Oromia	SouthWest Shoa	Woliso	Adami Gotu	08° 37' 954	038° 02' 516	2399	Central Highland	STB	Bread Wheat
ZTET081	SWSH	Oromia	SouthWest Shoa	Woliso	Adami Gotu	08° 37' 960	038° 02' 505	2397	Central Highland	STB	Bread Wheat
ZTET082	SWSH	Oromia	SouthWest Shoa	Woliso	Adami Gotu	08° 37' 037	038° 01' 931	2320	Central Highland	STB	Bread Wheat
ZTET083	SWSH	Oromia	SouthWest Shoa	Wenchi	Sonkole Kake	08° 38' 014	037° 54' 162	2196	Central Highland	STB	Bread Wheat

Appenix **I** Continued

ZTET084	SWSH	Oromia	SouthWest Shoa	Wenchi	Fite Wato	08° 39' 585	037° 53' 458	2295	Central Highland	STB	Bread Wheat
ZTET085	SWSH	Oromia	SouthWest Shoa	Wenchi	Fite Wato	08° 40' 788	037° 53' 241	2505	Central Highland	STB	Bread Wheat
ZTET086	SWSH	Oromia	SouthWest Shoa	Wenchi	Fite Wato	08° 40' 813	037° 53' 353	2517	Central Highland	STB	Bread Wheat
ZTET087	SWSH	Oromia	SouthWest Shoa	Wenchi	Waldo Talfam	08° 43' 579	037° 53' 085	2738	Central Highland	STB	Bread Wheat
ZTET088	WSH	Oromia	West Shoa	Ambo	Bilo	08° 54' 029	037° 52' 949	2532	Central Highland	STB	Bread Wheat
ZTET089	WSH	Oromia	West Shoa	Ambo	Bilo	08° 54' 024	037° 52' 954	2533	Central Highland	STB	Bread Wheat
ZTET090	WSH	Oromia	West Shoa	Toke Kutaye	Albaf Anchabi	08° 58' 960	037° 43' 483	2256	Central Highland	STB	Bread Wheat
ZTET091	WSH	Oromia	West Shoa	Toke Kutaye	Albaf Anchabi	08° 58' 969	037° 43' 480	2258	Central Highland	STB	Bread Wheat
ZTET092	WSH	Oromia	West Shoa	Toke Kutaye	Albaf Anchabi	08° 58' 981	037° 43' 475	2257	Central Highland	STB	Bread Wheat
ZTET093	WSH	Oromia	West Shoa	Chelia	Gedo 02	09° 01' 548	037° 26' 856	2470	Central Highland	STB	Bread Wheat
ZTET094	WSH	Oromia	West Shoa	Chelia	Gedo 02	09° 01' 572	037° 26' 885	2470	Central Highland	STB	Bread Wheat
ZTET095	WSH	Oromia	West Shoa	Chelia	Gedo 02	09° 01' 559	037° 26' 873	2470	Central Highland	STB	Bread Wheat
ZTET096	WSH	Oromia	West Shoa	Chelia	Gedo 02	09° 01' 594	037° 26' 886	2467	Central Highland	STB	Bread Wheat
ZTET097	NSH	Oromia	North Shewa	Degam	Degam	09° 13' 693	038° 45' 358	2586	Central Highland	STB	Bread Wheat
ZTET098	NSH	Oromia	North Shewa	Degam	Degam	09° 24' 039	038° 50' 722	2615	Central Highland	STB	Bread Wheat
ZTET099	NSH	Oromia	North Shewa	Degam	Degam	09° 47' 222	038° 31' 629	2898	Central Highland	STB	Bread Wheat
ZTET100	NSH	Oromia	North Shewa	Degam	Degam	09° 47' 257	038° 31' 664	2905	Central Highland	STB	Bread Wheat
ZTET101	NSH	Oromia	North Shewa	Degam	Degam	09° 47' 258	038° 31' 665	2905	Central Highland	STB	Bread Wheat
ZTET102	NSH	Oromia	North Shewa	Degem	Anajiru Bishandima	09° 47' 219	038° 31' 623	2897	Central Highland	STB	Bread Wheat
ZTET103	NSH	Oromia	North Shewa	Degem	Anajiru Bishandima	09° 47' 222	038° 31' 629	2898	Central Highland	STB	Bread Wheat
ZTET104	NSH	Oromia	North Shewa	Degem	Anajiru Bishandima	09° 47' 249	038° 31' 659	2899	Central Highland	STB	Bread Wheat
ZTET105	NSH	Oromia	North Shewa	Degem	Anajiru Bishandima	09° 47' 257	038° 31' 664	2905	Central Highland	STB	Bread Wheat
ZTET106	BALE	Oromia	Bale	Dinsho	Abukara	07° 07' 882	039° 52' 971	2693	South East	STB	Bread Wheat
ZTET107	BALE	Oromia	Bale	Dinsho	Abukara	07° 07' 884	039° 52' 972	2691	South East	STB	Bread Wheat
ZTET108	BALE	Oromia	Bale	Dinsho	Homa	07° 08' 196	039° 55' 349	2529	South East	STB	Bread Wheat
ZTET109	BALE	Oromia	Bale	Dinsho	Homa	07° 08' 287	039° 55' 423	2520	South East	STB	Bread Wheat
ZTET110	BALE	Oromia	Bale	Dinsho	Homa	07° 08' 286	039° 55' 421	2523	South East	STB	Bread Wheat
ZTET111	BALE	Oromia	Bale	Dinsho	Homa	07° 08' 286	039° 55' 421	2524	South East	STB	Bread Wheat
ZTET112	BALE	Oromia	Bale	Dinsho	Homa	07° 08' 286	039° 55' 421	2525	South East	STB	Bread Wheat
ZTET113	BALE	Oromia	Bale	Sinana	Horu Boka	07° 10' 220	039° 58' 978	2408	South East	STB	Bread Wheat
ZTET114	BALE	Oromia	Bale	Sinana	Horu Boka	07° 10' 211	039° 58' 972	2408	South East	STB	Bread Wheat
ZTET115	BALE	Oromia	Bale	Sinana	Horu Boka	07° 10' 211	039° 58' 972	2409	South East	STB	Bread Wheat
ZTET116	BALE	Oromia	Bale	Sinana	Horu Boka	07° 10' 211	039° 58' 972	2410	South East	STB	Bread Wheat
ZTET117	BALE	Oromia	Bale	Sinana	Horu Boka	07° 10' 207	039° 58' 985	2406	South East	STB	Bread Wheat
ZTET118	BALE	Oromia	Bale	Sinana	Horu Boka	07° 05' 517	039° 59' 800	2510	South East	STB	Bread Wheat
ZTET119	BALE	Oromia	Bale	Goba	Waltai Tosha	07° 03' 365	039° 59' 077	2557	South East	STB	Bread Wheat
ZTET120	BALE	Oromia	Bale	Goba	Waltai Tosha	07° 03' 365	039° 59' 079	2554	South East	STB	Bread Wheat
ZTET121	BALE	Oromia	Bale	Goba	Waltai Tosha	07° 03' 365	039° 59' 079	2555	South East	STB	Bread Wheat
ZTET122	BALE	Oromia	Bale	Goba	Waltai Tosha	07° 03' 365	039° 59' 079	2556	South East	STB	Bread Wheat
ZTET123	BALE	Oromia	Bale	Goba	Waltai Tosha	07° 03' 358	039° 59' 073	2552	South East	STB	Bread Wheat
ZTET124	BALE	Oromia	Bale	Goba	Waltai Tosha	07° 02' 779	039° 58' 934	2573	South East	STB	Bread Wheat
ZTET125	BALE	Oromia	Bale	Goba	Waltai Tosha	07° 02' 758	039° 58' 934	2576	South East	STB	Bread Wheat
ZTET126	BALE	Oromia	Bale	Goba	Sinja	07° 02' 759	039° 58' 934	2577	South East	STB	Bread Wheat
ZTET127	BALE	Oromia	Bale	Goba	Sinja	07° 02' 056	039° 58' 908	2610	South East	STB	Bread Wheat
ZTET128	BALE	Oromia	Bale	Goba	Sinja	07° 02' 056	039° 58' 908	2611	South East	STB	Bread Wheat

Appenix **I** Continued

ZTET129	BALE	Oromia	Bale	Goba	Sinja	07° 02' 052	039° 58' 918	2612	South East	STB	Bread Wheat
ZTET130	BALE	Oromia	Bale	Goba	Sinja	07° 02' 047	039° 58' 924	2613	South East	STB	Bread Wheat
ZTET131	BALE	Oromia	Bale	Goba	Sinja	07° 02' 017	039° 58' 901	2616	South East	STB	Bread Wheat
ZTET132	BALE	Oromia	Bale	Gasara	Balo Amiyna	07° 22' 763	040° 12' 659	2406	South East	STB	Bread Wheat
ZTET133	BALE	Oromia	Bale	Ginir	Adano	07° 13' 794	040° 21' 847	2350	South East	STB	Bread Wheat
ZTET134	BALE	Oromia	Bale	Ginir	Adano	07° 13' 774	040° 21' 834	2350	South East	STB	Bread Wheat
ZTET135	BALE	Oromia	Bale	Ginir	Kara Adano	07° 13' 390	040° 23' 471	2324	South East	STB	Bread Wheat
ZTET136	BALE	Oromia	Bale	Ginir	Kara Adano	07° 13' 391	040° 23' 451	2324	South East	STB	Bread Wheat
ZTET137	BALE	Oromia	Bale	Ginir	Kara Adano	07° 13' 993	040° 25' 463	2280	South East	STB	Bread Wheat
ZTET138	BALE	Oromia	Bale	Ginir	Kara Adano	07° 13' 984	040° 25' 472	2281	South East	STB	Bread Wheat
ZTET139	BALE	Oromia	Bale	Ginir	Kara Adano	07° 14' 481	040° 26' 190	2280	South East	STB	Bread Wheat
ZTET140	BALE	Oromia	Bale	Ginir	Kara Adano	07° 14' 481	040° 26' 190	2281	South East	STB	Bread Wheat
ZTET141	BALE	Oromia	Bale	Ginir	Delosebro	07° 14' 470	040° 26' 199	22 82	South East	STB	Bread Wheat
ZTET142	BALE	Oromia	Bale	Ginir	Delosebro	07° 15' 268	040° 27' 647	2189	South East	STB	Bread Wheat
ZTET143	BALE	Oromia	Bale	Ginir	Delosebro	07° 15' 264	040° 27' 635	2190	South East	STB	Bread Wheat
ZTET144	BALE	Oromia	Bale	Ginir	Delosebro	07° 15' 264	040° 27' 635	2191	South East	STB	Bread Wheat
ZTET145	BALE	Oromia	Bale	Ginir	Delosebro	07° 15' 264	040° 27' 635	2192	South East	STB	Bread Wheat
ZTET146	BALE	Oromia	Bale	Ginir	Afaba	07° 18' 792	040° 29' 997	2048	South East	STB	Bread Wheat
ZTET147	BALE	Oromia	Bale	Ginir	Karmu	07° 13' 385	040° 24' 197	2311	South East	STB	Bread Wheat
ZTET148	BALE	Oromia	Bale	Sinan	Selka	07° 04' 266	040° 11' 065	2435	South East	STB	Bread Wheat
ZTET149	BALE	Oromia	Bale	Sinan	Selka	07° 04' 151	040° 13' 265	2465	South East	STB	Bread Wheat
ZTET150	BALE	Oromia	Bale	Sinan	Selka	07° 03' 837	040° 13' 821	2496	South East	STB	Bread Wheat
ZTET151	BALE	Oromia	Bale	Goro	Wlseide	07° 11' 136	040° 20' 502	2163	South East	STB	Bread Wheat
ZTET152	BALE	Oromia	Bale	Goro	Wlseide	07° 00' 171	040° 18' 407	2242	South East	STB	Bread Wheat
ZTET153	BALE	Oromia	Bale	Goro	Wlseide	07° 00' 171	040° 18' 407	2243	South East	STB	Bread Wheat
ZTET154	BALE	Oromia	Bale	Goro	Wlseide	07° 00' 171	040° 18' 407	2244	South East	STB	Bread Wheat
ZTET155	BALE	Oromia	Bale	Goro	Wlseide	07° 00' 023	040° 18' 462	2237	South East	STB	Bread Wheat
ZTET156	BALE	Oromia	Bale	Goro	Wlseide	06° 59' 894	040° 18' 438	2236	South East	STB	Bread Wheat
ZTET157	BALE	Oromia	Bale	Goro	Wlseide	06° 59' 867	040° 18' 438	2234	South East	STB	Bread Wheat
ZTET158	BALE	Oromia	Bale	Sinana	W/Arjo	07° 05' 621	040° 17' 178	2359	South East	STB	Bread Wheat
ZTET159	BALE	Oromia	Bale	Sinana	Hisu	07° 10' 141	040° 14' 973	2400	South East	STB	Bread Wheat
ZTET160	BALE	Oromia	Bale	Sinana	Hisu	07° 10' 288	040° 13' 884	2393	South East	STB	Bread Wheat
ZTET161	BALE	Oromia	Bale	Sinana	Hisu	07° 10' 296	040° 13' 883	2392	South East	STB	Bread Wheat
ZTET162	BALE	Oromia	Bale	Goba	Sinja	07° 02' 019	039° 59' 243	2904	South East	STB	Bread Wheat
ZTET163	BALE	Oromia	Bale	Goba	Sinja	07° 02' 179	039° 59' 231	2600	South East	STB	Bread Wheat
ZTET164	BALE	Oromia	Bale	Goba	Sinja	07° 02' 197	039° 59' 216	2600	South East	STB	Bread Wheat
ZTET165	BALE	Oromia	Bale	Goba	Sinja	07° 02' 235	039° 59' 225	2597	South East	STB	Bread Wheat
ZTET166	BALE	Oromia	Bale	Goba	Sinja	07° 02' 113	039° 59' 151	2604	South East	STB	Bread Wheat
ZTET167	BALE	Oromia	Bale	Goba	Sinja	07° 02' 142	039° 96' 058	2607	South East	STB	Bread Wheat
ZTET168	BALE	Oromia	Bale	Goba	Sinja	07° 02' 142	039° 56' 058	2608	South East	STB	Bread Wheat
ZTET169	OSZ	Oromia	OSZ	Wolmara	HARC	09° 03' 268	038° 30' 298	2373	Central Highland	STB	Bread Wheat
ZTET170	OSZ	Oromia	OSZ	Wolmara	HARC	09° 03' 273	038° 30' 303	2376	Central Highland	STB	Bread Wheat
ZTET171	OSZ	Oromia	OSZ	Wolmara	HARC	09° 03' 278	038° 30' 299	2377	Central Highland	STB	Bread Wheat
ZTET172	OSZ	Oromia	OSZ	Wolmara	HARC	09° 03' 287	038° 30' 295	2377	Central Highland	STB	Bread Wheat
ZTET173	OSZ	Oromia	OSZ	Wolmara	HARC	09° 03' 299	038° 30' 273	2376	Central Highland	STB	Bread Wheat

Appenix **I** Continued

ZTET174	OSZ	Oromia	OSZ	Wolmara	HARC	09° 03' 316	038° 30' 255	2375	Central Highland	STB	Bread Wheat
ZTET175	OSZ	Oromia	OSZ	Wolmara	HARC	09° 03' 332	038° 30' 230	2374	Central Highland	STB	Bread Wheat
ZTET176	OSZ	Oromia	OSZ	Wolmara	HARC	09° 03' 596	038° 30' 674	2379	Central Highland	STB	Bread Wheat
ZTET177	OSZ	Oromia	OSZ	Wolmara	HARC	09° 03' 590	038° 30' 682	2382	Central Highland	STB	Bread Wheat
ZTET178	OSZ	Oromia	OSZ	Wolmara	HARC	09° 03' 597	038° 30' 410	2392	Central Highland	STB	Bread Wheat
ZTET179	OSZ	Oromia	OSZ	Wolmara	HARC	09° 03' 583	038° 30' 686	2382	Central Highland	STB	Bread Wheat
ZTET180	OSZ	Oromia	OSZ	Wolmara	HARC	09° 03' 593	038° 30' 686	238	Central Highland	STB	Bread Wheat
ZTET181	OSZ	Oromia	OSZ	Wolmara	HARC	09° 03' 572	038° 30' 682	2381	Central Highland	STB	Bread Wheat
ZTET182	OSZ	Oromia	OSZ	Wolmara	HARC	09° 03' 586	038° 30' 674	2380	Central Highland	STB	Bread Wheat

* In the Neighbor-Joining tree the isolates were indicated as 1 to 182, ESH = East Shewa, WARSI = West Arsi, SWSH = South West Shewa, WSH = West Shewa, NSH = North Shewa, OSZ = Oromia Special Zone Surrounding Finfine, and [a]UTM = Universal Transverse Mercator coordinate system.

Appendix **II.** Allelic frequencies of 14 microsatellite loci of *Z. tritici* within eight locations of the major wheat growing areas of Ethiopia

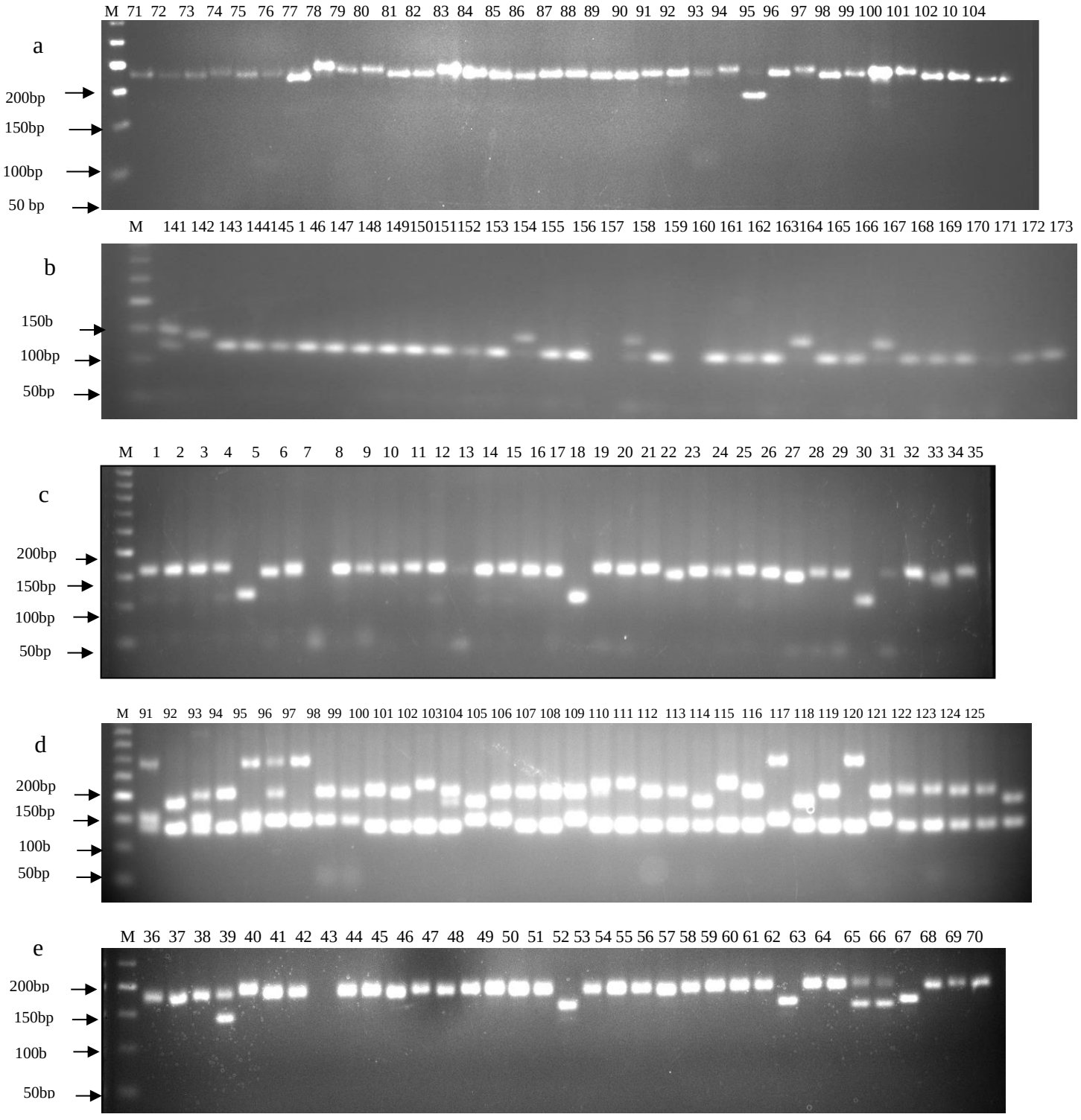
Locus	Allele	Locations								Mean
		ESH	ARSI	WARSI	SWSH	WSH	NSH	BALE	OSZ	
ST1E7	85	0.00	0.10	0.00	0.00	0.11	0.11	0.08	0.00	0.05
	93	0.83	0.62	1.00	0.38	0.78	0.89	0.84	1.00	0.79
	98	0.17	0.28	0.00	0.63	0.11	0.00	0.08	0.00	0.16
ST1G7	90	0.67	0.41	0.58	0.38	1.00	0.78	0.73	0.86	0.67
	96	0.33	0.43	0.42	0.38	0.00	0.22	0.08	0.07	0.24
	100	0.00	0.16	0.00	0.25	0.00	0.00	0.19	0.07	0.08
ST2E4	54	0.00	0.07	0.00	0.00	0.00	0.11	0.00	0.00	0.02
	75	0.67	0.64	1.00	0.63	0.78	0.78	0.79	1.00	0.79
	98	0.33	0.20	0.00	0.25	0.22	0.11	0.11	0.00	0.15
	102	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.01
	110	0.00	0.00	0.00	0.13	0.00	0.00	0.10	0.00	0.03
ST1A4	98	0.83	0.26	0.50	0.13	0.67	0.56	0.54	0.21	0.46
	99	0.00	0.39	0.50	0.88	0.33	0.44	0.16	0.64	0.42
	100	0.17	0.34	0.00	0.00	0.00	0.00	0.30	0.14	0.12
ST1D7	95	0.00	0.20	0.00	0.00	0.44	0.00	0.14	0.00	0.10
	100	0.83	0.46	0.92	0.63	0.11	0.89	0.71	0.93	0.68
	105	0.17	0.34	0.08	0.38	0.44	0.11	0.14	0.07	0.22
ST1B3	54	0.67	0.66	0.92	0.38	1.00	0.56	0.70	0.86	0.72
	56	0.17	0.21	0.00	0.50	0.00	0.22	0.10	0.14	0.17
	121	0.00	0.08	0.08	0.13	0.00	0.22	0.21	0.00	0.09
	n	0.17	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.03
ST2C10	75	0.50	0.56	0.50	0.25	0.11	0.33	0.41	0.64	0.41
	84	0.33	0.36	0.08	0.13	0.67	0.44	0.19	0.36	0.32
	90	0.17	0.07	0.42	0.63	0.22	0.22	0.38	0.00	0.26
	98	0.00	0.02	0.00	0.00	0.00	0.00	0.02	0.00	0.00
ag-0003	198	0.50	0.56	0.58	0.63	0.44	0.33	0.46	0.43	0.49
	230	0.33	0.28	0.42	0.38	0.22	0.22	0.27	0.14	0.28
	258	0.17	0.16	0.00	0.00	0.33	0.44	0.27	0.43	0.23

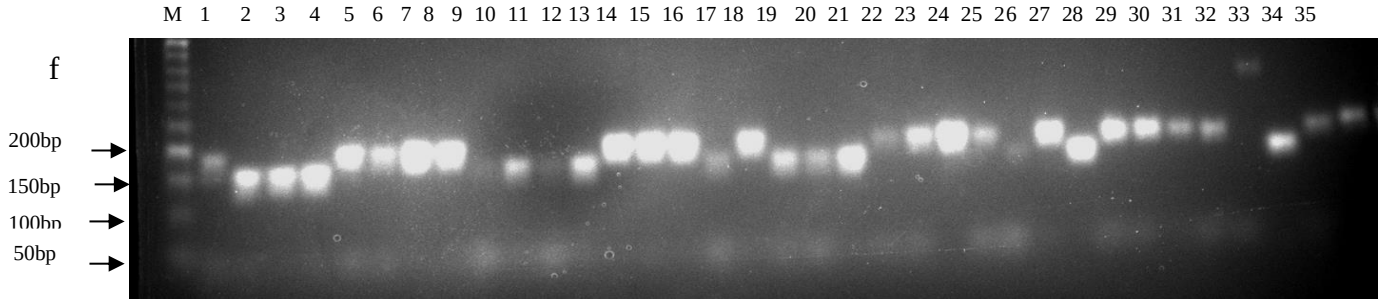
Appendix **II** Continued

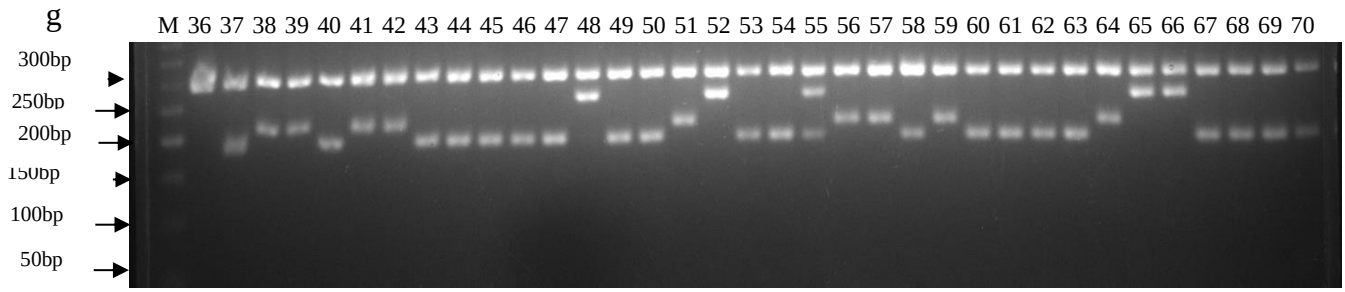
ac-0001	151	0.67	0.66	0.50	0.00	0.33	0.89	0.71	0.86	0.58
	171	0.17	0.15	0.25	0.25	0.33	0.00	0.11	0.14	0.18
	200	0.17	0.20	0.25	0.75	0.33	0.11	0.17	0.00	0.25
ag-0009	192	0.67	0.66	1.00	0.50	0.67	0.67	0.75	0.86	0.72
	197	0.33	0.23	0.00	0.25	0.00	0.22	0.10	0.07	0.15
	200	0.00	0.11	0.00	0.25	0.33	0.11	0.16	0.07	0.13
ggc-0001	181	0.67	0.34	0.17	0.00	0.00	0.11	0.38	0.43	0.26
	232	0.33	0.31	0.50	0.75	0.56	0.67	0.60	0.57	0.54
	256	0.00	0.28	0.33	0.25	0.44	0.22	0.02	0.00	0.19
	298	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.01
ac-0002	172	0.33	0.43	0.33	0.75	0.67	0.22	0.59	0.21	0.44
	182	0.33	0.28	0.50	0.00	0.33	0.11	0.33	0.43	0.29
	206	0.17	0.11	0.17	0.25	0.00	0.67	0.06	0.36	0.22
	210	0.17	0.18	0.00	0.00	0.00	0.00	0.02	0.00	0.05
tcc-0009	142	0.67	0.64	0.75	0.50	0.56	0.78	0.84	0.00	0.59
	161	0.17	0.31	0.25	0.25	0.33	0.11	0.05	0.21	0.21
	179	0.17	0.05	0.00	0.25	0.11	0.11	0.11	0.79	0.20
caa-0005	229	0.67	0.49	0.25	0.38	0.22	0.56	0.35	0.29	0.40
	263	0.17	0.33	0.42	0.25	0.33	0.33	0.27	0.07	0.27
	299	0.17	0.18	0.33	0.38	0.44	0.11	0.38	0.64	0.33

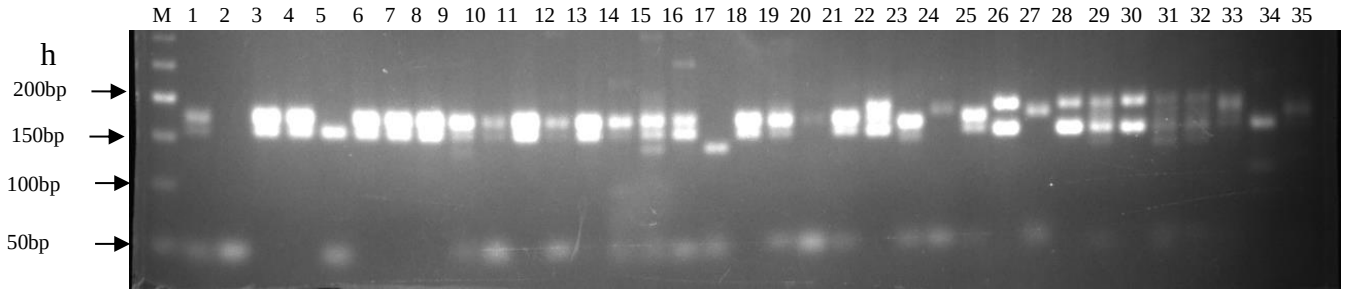
ESH = East Shewa, WARSI = West Arsi, SWSH = South West Shewa, WSH = West Shewa, NSH = North Shewa, OSZ = Oromia Special Zone Surrounding Finfine.
n= Missing genotypes frequency.

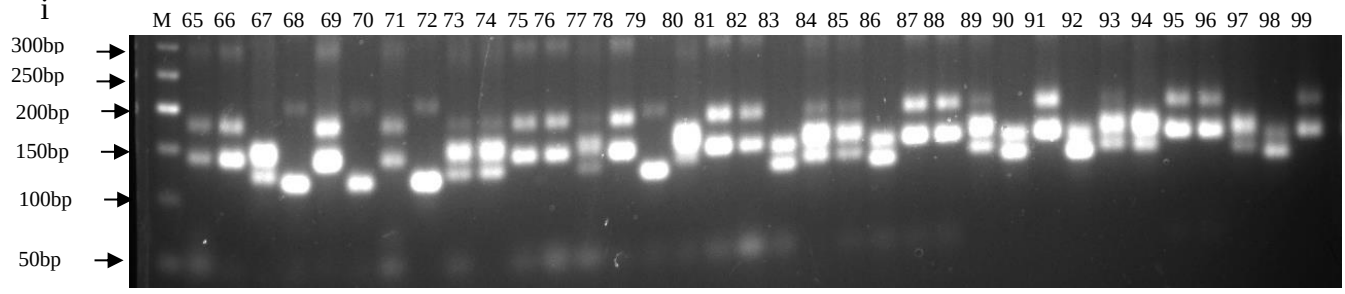
Appendix **III.** Result of 180 bead wheat lines screened for *Zymoseptoria* resistance gene using tightly linked SSR markers

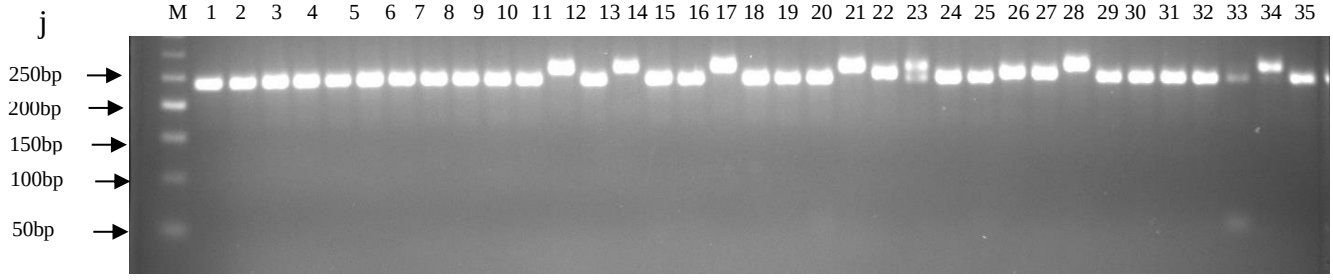


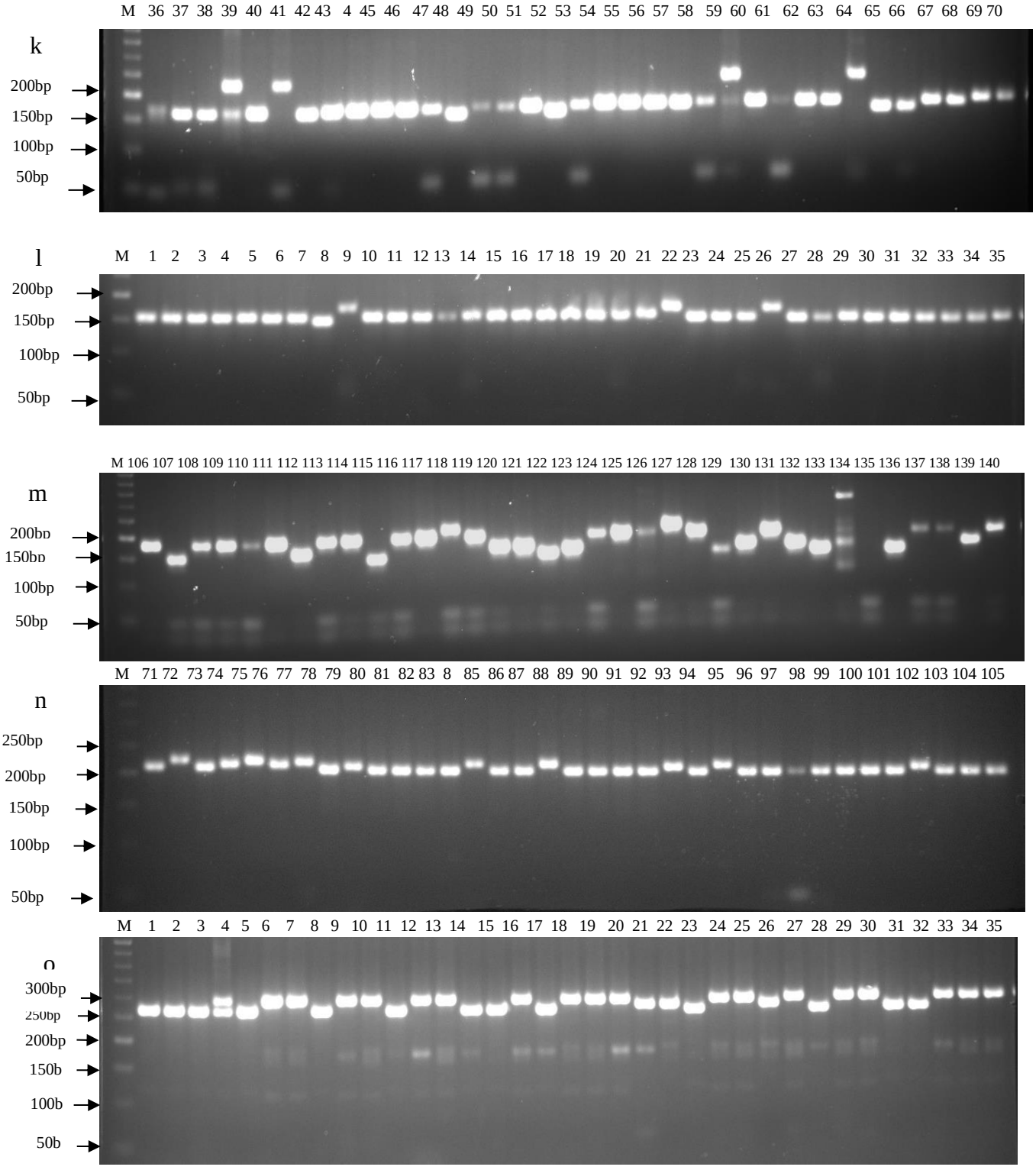

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
f
200bp
150bp
100bp
50bp

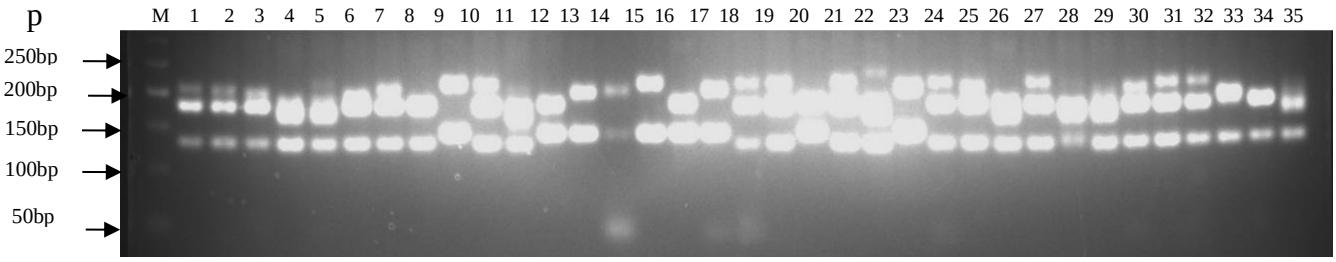

M 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70
g
300bp
250bp
200bp
150bp
100bp
50bp


M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
h
200bp
150bp
100bp
50bp


M 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99
i
300bp
250bp
200bp
150bp
100bp
50bp


M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
j
250bp
200bp
150bp
100bp
50bp


M 36 37 38 39 40 41 42 43 4 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70
k
200bp
150bp
100bp
50bp
l
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
200bp
150bp
100bp
50bp
M 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140
m
200bp
150bp
100bp
50bp
M 71 72 73 74 75 76 77 78 79 80 81 82 83 8 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
n
250bp
200bp
150bp
100bp
50bp
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
o
300bp
250bp
200bp
150b
100b
50b



Appendix **III.** Agarose gel electrophoretic patterns of SSR markers generated from selected bread wheat genotypes. Pairs of primers used include *xbarc*74 (a), *gwm389* (b), *wmc*83 (c), *gwm*111 (d), *gwm*449 (e), *gwm369* (f), *gwm313(g), gwm* 146 (h), *xgwm*317 (i), *xbarc*137 (j), w*mc219* (k), *Wmc396* (l), *wmc623* (m), *gwm*494 (n), *hbg*247 (o), and *gpw*3087 (p). Where M is a 50bp size marker and the numbers 1-180 indicates the wheat genotypes.

Appendix **IV.** List of 180 wheat association panel used for SSR screening, GxE evaluation and GWAS .

S.N	Selection History	Origion	Genotype	
			Name	Code
1	CMSS08Y00491S-099Y-099M-099NJ-37WGY-0B	MXI12-13\M47IBWSN\194	EBW001	G1
2	CMSA07Y00597S-040ZTY-040ZTM-040Y-46ZTM-010Y-0B	MXI12-13\M24ISEPTON\56	EBW002	G2
3	CMSA07M00022T-032(B=WMC221SR25HET)Y-040ZTM-074 (WMC0221SR25HOM+HET)Y-55ZTM-010Y-01B-0Y	MXI12-13\M24ISEPTON\25	EBW003	G3
4	CMSS07Y01253T-099TOPM-099Y-099M-099Y-16M-0RGY	MXI11-12\M21HRWYT\24	EBW004	G4
5	CMSS08Y00924T-099TOPM-099Y-099M-099NJ-099NJ-10RGY-0B	MXI12-13\M25HRWSN\1053	EBW005	G5
6	CMSS08Y00224S-099Y-099M-099NJ-099NJ-1WGY-0B	MXI12-13\M47IBWSN\81	EBW006	G6
7	CMSS08Y00704T-099TOPM-099Y-099Y-11M-0WGY	MXI12-13\M47IBWSN\247	EBW007	G7
8	CMSS08B00861T-099TOPY-099M-099Y-13M-0WGY	MXI12-13\M47IBWSN\722	EBW008	G8
9	CMSA06M00637S-040ZTM-040ZTY-33ZTM-0Y-0B	MXI11-12\SELC23ISEPTO\95	EBW009	G9
10	Commercial Cultivar	Bika	Bika	G10
11	CMSA08M00458S-040ZTM-050Y-50ZTM-010Y-0B	MXI12-13\M25HRWSN\1174	EBW011	G11
12	CMSS93B00686S-12Y-010M-010Y-010M-7Y-1M-0Y-3SJ-0Y-2PZ-0Y	MXI12-13\M24ISEPTON\102	EBW012	G12
13	Addaptation rial	Adap	EBW013	G13
14	Commercial Cultivar	Meraro	Meraro	G14
15	CMSA08M00432S-040M-0NJ-10Y-0B	MXI12-13\M25HRWSN\1166	EBW015	G15
16	CMSA08M00466S-040ZTM-050Y-47ZTM-010Y-0B	MXI12-13\M25HRWSN\1175	EBW016	G16
17	LOCAL CHECK	\\0	EBW017	G17
18	CMSS08B00911T-099TOPY-099M-099NJ-12WGY-0B	MXI12-13\M47IBWSN\779	EBW018	G18
19	CMSS08B00422S-099M-099NJ-5RGY-0B	MXI12-13\M25HRWSN\1100	EBW019	G19
20	CMSS08Y00611T-099TOPM-099Y-099M-099Y-2M-0WGY	MXI12-13\M47IBWSN\208	EBW020	G20
21	Commercial Cultivar	Alidoro	Alidoro	G21
22	Commercial Cultivar	Medawolabu	Medawolabu	G22
23	CMSS06Y00586T-099TOPM-099Y-099ZTM-099Y-099M-5WGY-0B	MXI11-12\SELC23ISEPTO\22	EBW023	G23
24	CMSS08B00772T-099TOPY-099M-099Y-21M-0WGY	MXI12-13\M47IBWSN\655	EBW024	G24
25	CMSS08Y01110T-099M-099Y-099M-099NJ-7WGY-0B	MXI12-13\M47IBWSN\415	EBW025	G25
26	Addaptation rial	Adap	EBW026	G26
27	CMSS08Y00869T-099TOPM-099Y-099M-099Y-22M-0WGY	MXI12-13\M47IBWSN\335	EBW027	G27
28	CMSS07Y00299S-0B-099Y-099M-099Y-2M-0WGY	MXI12-13\M24ISEPTON\69	EBW028	G28
29	CMSS08Y00477S-099Y-099M-099NJ-7WGY-0B	MXI12-13\M47IBWSN\185	EBW029	G29
30	CMSS08Y00483S-099Y-099M-099Y-11M-0WGY	MXI12-13\M47IBWSN\188	EBW030	G30
31	CMSS08B00777T-099TOPY-099M-099NJ-099NJ-5RGY-0B	MXI12-13\M25HRWSN\1127	EBW031	G31
32	CMSA07M00376S-040ZTM-040ZTY-63ZTM-010Y-02B-0Y	MXI11-12\M31SAWSN\266	EBW032	G32
33	Commercial Cultivar	Huluka	Huluka	G33
34	CMSS07B00311S-099M-099Y-099M-4WGY-0B	MXI12-13\M24ISEPTON\89	EBW034	G34
35	CMSS07Y00774T-099TOPM-099Y-099M-099NJ-099NJ-6WGY-0B	MXI12-13\M24ISEPTON\74	EBW035	G35
36	CMSS08B00966T-099TOPY-099M-099Y-099B-17RGY-0B	MXI12-13\M25HRWSN\1138	EBW036	G36
37	CMSA08Y00045T-024(CRE1)B-050ZTY-067(CRE1)ZTM-03Y-01B-0Y	MXI12-13\M24ISEPTON\32	EBW037	G37
38	National Variety Trial 3	NVT3	EBW038	G38
39	CMSS08B00337S-099M-099NJ-099NJ-29WGY-0B	MXI12-13\M47IBWSN\492	EBW039	G39
40	Commercial Cultivar	King-bird	King-bird	G40
41	CMSS08B00381S-099M-099Y-1M-0RGY	MXI12-13\M25HRWSN\1099	EBW041	G41
42	CMSS07B00191S-099M-099Y-099M-22WGY-0B	MXI12-13\M24ISEPTON\85	EBW042	G42
43	CMSS08Y00872T-099TOPM-099Y-099M-099NJ-099NJ-9RGY-0B	MXI12-13\M25HRWSN\1041	EBW043	G43
44	CMSS07Y00815T-099TOPM-099Y-099M-099Y-7M-0RGY	MXI11-12\M21HRWYT\16	EBW044	G44
45	CMSA07M00634S-040ZTM-040ZTY-54ZTM-010Y-01B-0Y	MXI12-13\M24ISEPTON\12	EBW045	G45
46	CMSS08B00974T-099TOPY-099M-099Y-099B-38WGY-0B	MXI12-13\M47IBWSN\811	EBW046	G46
47	CMSA08Y00065T-099B-050Y-050ZTM-050Y-14BMX-010Y-0B	MXI12-13\M25HRWSN\1141	EBW047	G47
48	Addaptation rial	Adap	EBW048	G48
49	CMSS08Y00127S-099Y-099M-099NJ-30WGY-0B	MXI12-13\M47IBWSN\25	EBW049	G49
50	CMSS08B00475S-099M-099Y-17M-0WGY	MXI12-13\M47IBWSN\517	EBW050	G50
51	National Variety Trial 4	NVT4	EBW051	G51
52	CMSS08B00283S-099M-099NJ-099NJ-8WGY-0B	MXI12-13\M47IBWSN\470	EBW052	G52
53	CMSA07M00371S-040ZTM-040ZTY-19ZTM-010Y-02B-0Y	MXI12-13\M24ISEPTON\2	EBW053	G53
54	CMSA08Y00469S-050Y-040M-0NJ-20Y-0B	MXI12-13\M25HRWSN\1147	EBW054	G54
55	CMSS08Y00627T-099TOPM-099Y-099M-099NJ-5WGY-0B	MXI12-13\M47IBWSN\220	EBW055	G55
56	Plimenary Variety Trial	PVT	EBW056	G56
57	CMSA07M00441S-040ZTM-040ZTY-20ZTM-010Y-01B-0Y	MXI12-13\M24ISEPTON\4	EBW057	G57
58	CMSS07B00236S-099M-099Y-099M-13WGY-0B	MXI11-12\M46IBWSN\202	EBW058	G58
59	CMSS06Y01171T-099TOPM-099Y-099ZTM-099Y-099M-8WGY-0B	MXI11-12\M34ESWYT\218	EBW059	G59
60	CMSS07Y00346S-0B-099Y-099M-099NJ-099NJ-3WGY-0B	MXI12-13\M24ISEPTON\70	EBW060	G60
61	Plimenary Variety Trial	PVT	EBW061	G61

Appendix **IV** Continued

62	National Variety Trial 2	NVT2	EBW062	G62
63	Addaptation rial	Adap	EBW063	G63
64	CMSS06Y00631T-099TOPM-099Y-099ZTM-099Y-099M-2WGY-0B	MXI11-12\SELC23ISEPTO\31	EBW064	G64
65	CMSS08Y00871T-099TOPM-099Y-099M-099NJ-099NJ-2WGY-0B	MXI12-13\M47IBWSN\345	EBW065	G65
66	CMSS08Y00182S-099Y-099M-099NJ-099NJ-2WGY-0B	MXI12-13\M47IBWSN\69	EBW066	G66
67	CMSS93B00686S-12Y-010M-010Y-010M-7Y-1M-0Y-3SJ-0Y-2PZ-0Y	MXI10-11\C22ISEPTON\102	EBW067	G67
68	CMSA07M00443S-040ZTM-040ZTY-47ZTM-010Y-02B-0Y	MXI11-12\M21HRWYT-RF\2	EBW068	G68
69	CMSA07Y01090T-045B-040Y-040ZTM-040Y-20ZTM-010Y-0B	MXI12-13\M24ISEPTON\34	EBW069	G69
70	CMSS07B00264S-099M-099NJ-099NJ-8WGY-0B	MXI12-13\M24ISEPTON\88	EBW070	G70
71	Commercial Cultivar	Danda'a	Danda'a	G71
72	CMSS08Y00491S-099Y-099M-099NJ-099NJ-11WGY-0B	MXI12-13\M47IBWSN\196	EBW072	G72
73	CMSS08Y00627T-099TOPM-099Y-099M-099NJ-6WGY-0B	MXI12-13\M47IBWSN\221	EBW073	G73
74	CMSA08Y00109T-099B-050Y-050ZTM-050Y-42BMX-010Y-0B	MXI12-13\M47IBWSN\823	EBW074	G74
75	CMSS08B00777T-099TOPY-099M-099NJ-099NJ-12RGY-0B	MXI12-13\M25HRWSN\1128	EBW075	G75
76	CMSS07Y00213S-0B-099Y-099M-099Y-20M-0WGY	MXI12-13\M24ISEPTON\65	EBW076	G76
77	CMSA07M00022T-032(B=WMC221SR25HET)Y-040ZTM-074 (WMC0221SR25HOM+HET)Y-9ZTM-010Y-01B-0Y	MXI11-12\M31SAWSN\93	EBW077	G77
78	CMSS07Y00672T-099TOPM-099Y-099M-099Y-36M-0WGY	MXI12-13\M24ISEPTON\71	EBW078	G78
79	CMSS07B00614T-099TOPY-099M-099Y-099M-7WGY-0B	MXI12-13\M24ISEPTON\92	EBW079	G79
80	Commercial Cultivar	Et-13A2	Et-13A2	G80
81	CMSS08Y01116T-099M-099Y-099M-099NJ-099NJ-14WGY-0B	MXI12-13\M47IBWSN\418	EBW081	G81
82	CMSA07Y00273S-040ZTY-040ZTM-040Y-30ZTM-010Y-0B	MXI12-13\M24ISEPTON\55	EBW082	G82
83	CMSA07M00455S-040M-0NJ-0NJ-2Y-0B	MXI12-13\M24ISEPTON\42	EBW083	G83
84	CMSS06B00001S-0Y-099ZTM-099Y-099M-8WGY-0B	MXI12-13\M24ISEPTON\99	EBW084	G84
85	CMSS07Y00174S-0B-099Y-099M-099Y-22M-0WGY	MXI11-12\M34ESWYT\18	EBW085	G85
86	CMSA08M00446S-040ZTM-050Y-5ZTM-010Y-0B	MXI12-13\M25HRWSN\1170	EBW086	G86
87	CMSS07B00151S-099M-099Y-099M-7WGY-0B	MXI12-13\M24ISEPTON\83	EBW087	G87
88	CMSS08Y00224S-099Y-099M-099NJ-099NJ-16RGY-0B	MXI12-13\M25HRWSN\1011	EBW088	G88
89	CMSS07B00231S-099M-099Y-099M-47WGY-0B	MXI12-13\M24ISEPTON\86	EBW089	G89
90	CMSS07Y01307T-099Y-7M-0Y-3B-0Y	MXI11-12\M46IBWSN\320	EBW090	G90
91	CMSS97M03642T-040Y-020Y-030M-020Y-040M-7Y-3M-0Y	MXI12-13\M24ISEPTON\101	EBW091	G91
92	CMSA07WM00080S-050Y-040ZTM-040ZTY-78ZTM-010Y-02B-0Y	MXI11-12\M1STSWYPT\22	EBW092	G92
93	CMSA07M00065T-029(AWW5L7BO1HET&CRE1M19NEG) Y-040ZTM-040Y-36ZTM-010Y-02B-0Y	MXI12-13\M24ISEPTON\23	EBW093	G93
94	CMSS06Y00920T-099TOPM-099Y-099ZTM-099Y-099M-16WGY-0B	MXI11-12\SELC23ISEPTO\36	EBW094	G94
95	CMSS06Y00706T-099TOPM-099Y-099ZTM-099NJ-099NJ-7WGY-0B	MXI12-13\M24ISEPTON\96	EBW095	G95
95	MXI12-13\M25HRWSN\1148	CMSA08Y00469S-050Y-040M-0NJ-21Y-0B	EBW096	G96
97	CMSS08Y00224S-099Y-099M-099NJ-099NJ-8RGY-0B	MXI12-13\M25HRWSN\1009	EBW097	G97
98	CMSA07Y00598S-040ZTY-040ZTM-040Y-5ZTM-010Y-0B	MXI12-13\M24ISEPTON\36	EBW098	G98
99	CMSS08Y00224S-099Y-099M-099NJ-099NJ-19RGY-0B	MXI12-13\M25HRWSN\1012	EBW099	G99
100	CMSS08Y00174S-099Y-099M-099NJ-099NJ-17WGY-0B	MXI12-13\M47IBWSN\64	EBW100	G100
101	CMSA08Y00530S-050Y-040M-0NJ-7Y-0B	MXI12-13\M47IBWSN\847	EBW101	G101
102	CMSS08B00771T-099TOPY-099M-099NJ-099NJ-21WGY-0B	MXI12-13\M47IBWSN\653	EBW102	G102
103	CMSA07M00043T-050Y-040ZTM-040ZTY-92ZTM-010Y-02B-0Y	MXI12-13\M24ISEPTON\13	EBW103	G103
104	CMSS07Y00032S-0B-099Y-099M-099Y-2M-0WGY	MXI12-13\M24ISEPTON\63	EBW104	G104
105	CMSS07Y00963T-099TOPM-099Y-099M-099Y-32M-0WGY	MXI11-12\M34ESWYT\72	EBW105	G105
106	CMSS07Y00255S-0B-099Y-099M-099NJ-099NJ-14WGY-0B	MXI12-13\M24ISEPTON\66	EBW106	G106
107	CMSS06Y00117S-0B-099Y-099ZTM-099Y-099M-12WGY-0B	MXI11-12\SELC23ISEPTO\12	EBW107	G107
108	CMSS08B00391S-099M-099Y-10M-0WGY	MXI12-13\M47IBWSN\496	EBW108	G108
109	Plimenary Variety Trial	PVT	EBW109	G109
110	CMSS06Y00946T-099TOPM-099Y-099ZTM-099Y-099M-13WGY-0B	MXI12-13\M24ISEPTON\97	EBW110	G110
111	CMSS08Y00220S-099Y-099M-099NJ-6RGY-0B	MXI12-13\M25HRWSN\1007	EBW111	G111
112	CMSA07M00644S-040M-0NJ-0NJ-5Y-0B	MXI12-13\M24ISEPTON\44	EBW112	G112
113	CMSS07Y00815T-099TOPM-099Y-099M-099Y-7M-0RGY	MXI11-12\M21HRWYT\16	EBW113	G113
114	CMSA08Y00378S-050Y-050ZTM-050Y-2BMX-010Y-0B	MXI12-13\M47IBWSN\830	EBW114	G114
115	CMSS08Y00627T-099TOPM-099Y-099M-099NJ-37WGY-0B	MXI12-13\M47IBWSN\224	EBW115	G115
116	CMSS08Y00182S-099Y-099M-099NJ-099NJ-9WGY-0B	MXI12-13\M47IBWSN\74	EBW116	G116
117	CMSS08Y00623T-099TOPM-099Y-099M-099NJ-099NJ-7WGY-0B	MXI12-13\M47IBWSN\217	EBW117	G117
118	CMSA07Y00950T-8B-040Y-040ZTM-040Y-10ZTM-010Y-0B	MXI12-13\M24ISEPTON\62	EBW118	G118
119	CMSS07Y00704T-099TOPM-099Y-099M-099NJ-099NJ-9WGY-0B	MXI12-13\M24ISEPTON\73	EBW119	G119
120	CMSA07M00158T-056(CSLV34HET)Y-040ZTM-055 (CSLV34HOM+HET)Y-17ZTM-010Y-01B-0Y	MXI12-13\M24ISEPTON\26	EBW120	G120
121	CMSS08B00196S-099M-099NJ-099NJ-36RGY-0B	MXI12-13\M25HRWSN\1074	EBW121	G121

Appendix **IV** Continued

122	CMSS06Y00920T-099TOPM-099Y-099ZTM-099Y-099M-36WGY-0B	MXI11-12\SELC23ISEPTO\37	EBW122	G122
123	CMSS08B00776T-099TOPY-099M-099NJ-099NJ-21RGY-0B	MXI12-13\M25HRWSN\1124	EBW123	G123
124	CMSA08M00424S-040ZTM-050Y-13ZTM-010Y-0B	MXI12-13\M25HRWSN\1162	EBW124	G124
125	CMSA07WM00080S-050Y-54ZTM-08Y-0B-03Y-(01B)-0Y	MXI11-12\M1STSWYPT\43	EBW125	G125
126	CMSS06Y00952T-099TOPM-099Y-099ZTM-099Y-099M-5WGY-0B	MXI11-12\SELC23ISEPTO\44	EBW126	G126
127	Commercial Cultivar	Paven-76	Paven-76	G127
128	CMSA07M00470S-040ZTM-040ZTY-15ZTM-010Y-02B-0Y	MXI12-13\M24ISEPTON\20	EBW128	G128
129	CMSS08B00776T-099TOPY-099M-099NJ-099NJ-14RGY-0B	MXI12-13\M25HRWSN\1123	EBW129	G129
130	CMSA07M00443S-040ZTM-040ZTY-47ZTM-010Y-02B-0Y	MXI12-13\M24ISEPTON\51	EBW130	G130
131	CMSS07B00248S-099M-099Y-099M-4WGY-0B	MXI11-12\M46IBWSN\215	EBW131	G131
132	CMSS08Y00351S-099Y-099M-099NJ-099NJ-4RGY-0B	MXI12-13\M25HRWSN\1023	EBW132	G132
133	CMSS08Y00872T-099TOPM-099Y-099M-099NJ-099NJ-4RGY-0B	MXI12-13\M25HRWSN\1040	EBW133	G133
134	CMSS08Y00627T-099TOPM-099Y-099M-099NJ-16WGY-0B	MXI12-13\M47IBWSN\222	EBW134	G134
135	CMSA08Y00556S-050Y-050ZTM-050Y-45BMX-010Y-0B	MXI12-13\M25HRWSN\1150	EBW135	G135
136	CMSS08B00919T-099TOPY-099M-099NJ-099NJ-7RGY-0B	MXI12-13\M25HRWSN\1137	EBW136	G136
137	CMSS08Y00182S-099Y-099M-099NJ-099NJ-8WGY-0B	MXI12-13\M47IBWSN\73	EBW137	G137
138	CMSA07WM00080S-050Y-54ZTM-08Y-0B-08Y-(03B&04B)-0Y	MXI11-12\M1STSWYPT\8	EBW138	G138
139	CMSA08M00445S-040ZTM-050Y-47ZTM-010Y-0B	MXI12-13\M25HRWSN\1169	EBW139	G139
140	CMSS07B00869T-099TOPY-099M-099Y-099M-1WGY-0B	MXI12-13\M24ISEPTON\95	EBW140	G140
141	CMSS08Y00224S-099Y-099M-099NJ-099NJ-4RGY-0B	MXI12-13\M25HRWSN\1008	EBW141	G141
142	CMSS08Y00234S-099Y-099M-099NJ-099NJ-20WGY-0B	MXI12-13\M47IBWSN\95	EBW142	G142
143	CMSA07WM00080S-050Y-040ZTM-040ZTY-8ZTM-010Y-01B-0Y	MXI11-12\M1STSWYPT\20	EBW143	G143
144	CMSS07B00024S-099M-099Y-099M-21RGY-0B	MXI11-12\M24HRWSN\32	EBW144	G144
145	CMSS08Y00220S-099Y-099M-099Y-11M-0WGY	MXI12-13\M47IBWSN\78	EBW145	G145
146	CMSS08B00763T-099TOPY-099M-099NJ-19WGY-0B	MXI12-13\M47IBWSN\644	EBW146	G146
147	CMSA07M00445S-040ZTM-040ZTY-75ZTM-010Y-01B-0Y	MXI12-13\M24ISEPTON\18	EBW147	G147
148	CMSA07M00403S-040ZTM-040ZTY-21ZTM-010Y-02B-0Y	MXI12-13\M24ISEPTON\16	EBW148	G148
149	Commercial Cultivar	Sofomor	Sofomor	G149
150	CMSA08M00124T-050Y-040ZTM-050Y-8ZTM-010Y-0B	MXI12-13\M25HRWSN\1154	EBW150	G150
151	CMSA08M00052T-050Y-040M-0NJ-10Y-0B	MXI12-13\M25HRWSN\1152	EBW151	G151
152	Commercial Cultivar	KI6295-4A	KI6295-4A	G152
153	CMSA08Y00083T-099B-050Y-050ZTM-050Y-41BMX-010Y-0B	MXI12-13\M25HRWSN\1144	EBW153	G153
154	CMSS06B00201S-0Y-099ZTM-099Y-099M-15WGY-0B	MXI11-12\SELC23ISEPTO\1	EBW154	G154
155	CMSS06Y00672T-099TOPM-099Y-099ZTM-099Y-099M-10WGY-0B	MXI11-12\SELC23ISEPTO\34	EBW155	G155
156	CMSA07M00365S-040M-0NJ-0NJ-4Y-0B	MXI12-13\M24ISEPTON\58	EBW156	G156
157	CMSA07M00453S-040ZTM-040ZTY-13ZTM-010Y-01B-0Y	MXI11-12\M21HRWYT-RF\4	EBW157	G157
158	CMSA07WM00080S-050Y-23ZTM-03Y-0B-07Y-(01B&02B)-0Y	MXI11-12\M1STSWYPT\34	EBW158	G158
159	National Variety Trial 6	NVT6	EBW159	G159
160	CMSS08B00233S-099M-099NJ-099NJ-25RGY-0B	MXI12-13\M25HRWSN\1075	EBW160	G160
161	CMSS07B00326S-099M-099Y-099M-16WGY-0B	MXI11-12\M46IBWSN\229	EBW161	G161
162	CMSS07Y00066S-0B-099Y-099M-099Y-43M-0WGY	MXI11-12\M46IBWSN\13	EBW162	G162
163	CMSA08Y00025T-095(1A1R)B-050ZTY-075(1A1R)ZTM-03Y-02B-0Y	MXI12-13\M24ISEPTON\31	EBW163	G163
164	CMSS08Y00235S-099Y-099M-099NJ-1WGY-0B	MXI12-13\M47IBWSN\96	EBW164	G164
165	National Variety Trial 2	NVT2	EBW165	G165
166	National Variety Trial 5	NVT5	EBW166	G166
167	LOCAL CHECK	\\0	EBW167	G167
168	Addaptation rial	Adap	EBW168	G168
169	CMSS08Y00224S-099Y-099M-099NJ-099NJ-11WGY-0B	MXI12-13\M47IBWSN\82	EBW169	G169
170	CMSS08B00776T-099TOPY-099M-099NJ-099NJ-6RGY-0B	MXI12-13\M25HRWSN\1122	EBW170	G170
171	CMSS06Y00672T-099TOPM-099Y-099ZTM-099Y-099M-8WGY-0B	MXI11-12\SELC23ISEPTO\33	EBW171	G171
172	CMSA08Y00061T-079(1A1RSR26)B-050ZTY-026 (1A1RSR26)ZTM-02Y-01B-0Y	MXI12-13\M24ISEPTON\33	EBW172	G172
173	Commercial Cultivar	K-6290-Bulk	K-6290-Bulk	G173
174	CMSA05M00149S-040ZTM-040ZTY-11ZTM-02Y-0B	MXI11-12\SELC23ISEPTO\81	EBW174	G174
175	Commercial Cultivar	Hogana	Hogana	G175
176	CMSS06B00201S-0Y-099ZTM-099Y-099M-8WGY-0B	MXI11-12\M34ESWYT\224	EBW176	G176
177	CMSS08Y00627T-099TOPM-099Y-099M-099NJ-38WGY-0B	MXI12-13\M47IBWSN\225	EBW177	G177
178	CMSA07M00376S-040ZTM-040ZTY-15ZTM-010Y-02B-0Y	MXI12-13\M24ISEPTON\50	EBW178	G178
179	CMSS08Y00182S-099Y-099M-099NJ-099NJ-6WGY-0B	MXI12-13\M47IBWSN\72	EBW179	G179
180	CMSS07Y00963T-099TOPM-099Y-099M-099Y-32M-0WGY	MXI12-13\M24ISEPTON\78	EBW180	G180

Note: Genotype 9 and 95 failed the quality standered for genotyping and thus, were not included in the assocation study.

Appendix **V.** Combined mean performance of wheat genotypes for yield, yield-components and phenological traits measured across three locations over two years in Ethiopia.

Genotype		DH	DF	DM	GFD	Yield (kg)	HLW	TKW	PH (cm)	PL (cm)	SL(cm)	FLL(cm)	FLW (cm)	FLA(cm)	SN	NKSP	NKS
Name	Code	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
EBW001	G1	67.17	74.25	127.75	60.5	4219.54	48.31	35.93	90.84	42.61	8.82	22.25	1.7	28.18	16.58	2.98	41.2
EBW002	G2	66.5	72.08	126.5	59.67	4054.88	49.02	37.67	99.22	44.6	10.27	22.47	1.65	27.6	16.13	3.16	42.33
EBW003	G3	70.75	77.75	131.83	60.17	4748.63	49.06	37.35	99.8	44.58	8.45	24.08	1.45	25.45	16.82	3.14	46.02
EBW004	G4	67.08	73.42	129.42	62.33	4395	48.69	41.5	91.36	41.25	8.88	23.26	1.78	30.61	16.58	2.89	41.2
EBW005	G5	73.83	82.08	129.92	56.08	4059.67	46.67	34.45	92.27	38.07	9.48	22.81	1.84	31.82	16.85	2.96	41.83
EBW006	G6	66.92	74.08	126.17	59.5	3371.92	48.31	36.89	95.73	42.06	8.9	23.53	1.64	29.1	15.52	2.93	36.23
EBW007	G7	68.25	75.58	128.25	60	3557.08	45.51	29.36	92.38	39.27	8.95	23.1	1.75	31.68	17.18	3.28	48.32
EBW008	G8	67.5	74.08	127.75	60.5	3716.29	48.16	34.79	94.13	44.61	8.46	22.94	1.54	24.87	16.87	2.9	38.8
EBW009	G9	66.58	71.67	125.83	59	3788.58	46.76	35.76	93.68	42.57	8.79	23.95	1.51	26.58	16.48	2.8	40.83
Bika	G10	65	71.67	129.75	64.75	4074	49.67	39.43	95.33	42.14	8.92	24.48	1.6	27.31	15.75	3.06	43.02
EBW011	G11	68.08	75.58	131.17	63.08	4729.42	46.67	40.5	99.63	43.49	9.16	22.38	1.61	27.38	16.6	3.13	45.95
EBW012	G12	67.92	75.08	126.67	59	2874.13	46.52	34.77	91.88	36.99	8.18	19.25	1.74	24.67	16.52	2.69	39.35
EBW013	G13	71.67	73.42	129.08	63.83	4077.21	48.93	38.11	93.75	41	9.29	25.06	1.52	28.88	16.48	3.01	39.92
Meraro	G14	76.08	83	135.17	59.33	4097.96	50	33.05	90.47	36.61	9.09	22.86	1.53	25.72	17.47	3.23	44.47
EBW015	G15	68.92	76.67	127.67	58.75	3896.08	49.15	38.12	95.95	40.53	9.3S8	22.29	1.98	27.71	16.75	2.81	42.98
EBW016	G16	75.5	82	136.25	60.75	4378.29	47	37.57	98.81	40.8	8.43	21.29	1.78	28.15	17.3	2.93	41.65
EBW017	G17	75.17	81.75	136.92	61.5	3960.58	48.65	33.98	90.45	36.6	8.68	21.55	1.62	27.19	17.2	2.88	42.33
EBW018	G18	68.08	76	130.08	62.75	4612.25	48.57	37.2	97.25	41.81	9.08	23.51	1.7	30.6	16.78	2.88	42.63
EBW019	G19	68.25	75.25	129	60.75	4678.29	47.23	35.27	95	39.59	9.58	21.48	1.59	26.24	16.38	3.21	40.93
EBW020	G20	70.17	77.33	133.92	63.42	4449.67	46.96	36.51	96.24	41.76	8.58	22.06	1.62	27.09	16.97	3.04	43.52
Alidoro	G21	71.58	80.42	137.92	66.33	5368.13	48.69	36.37	102.58	43.84	10.63	25.36	2.03	39.04	17.88	3.14	47.07
Medawo--labu	G22	69	76.42	132	63.08	3576.38	46.32	38.88	99.3	44.96	9.31	27.84	1.58	33.21	16.65	2.93	44.65
EBW023	G23	74	79	134.67	61.17	4680.71	48.07	41.42	98.47	41.12	10.04	23.73	1.78	32.76	17.38	3.21	46.92
EBW024	G24	71.58	78.42	135	63.17	4286.38	45.61	34.45	94.29	39.36	8.96	22.71	1.72	29.37	17.42	2.75	43.75
EBW025	G25	67.5	74.58	130.17	62.67	3783.46	47.72	36.8	90.38	40.25	9.03	24.79	1.77	32.15	16.73	3.13	44.35
EBW026	G26	67.25	74.33	129	61.58	4891.21	48.31	39.19	94.17	44.43	8.93	23.74	1.81	32.25	15.95	2.99	37.3
EBW027	G27	66.58	73.75	130.08	63.5	4511.67	46.11	31.64	90.49	39.43	8.52	23.77	1.56	26.87	15.73	2.86	45.22
EBW028	G28	71	78.33	131.08	59.33	4620.33	45.09	33.02	90.13	37.94	8.65	22.77	1.69	28.84	16.43	3.23	45.87
EBW029	G29	68.58	75.5	134.67	65.17	4714.42	48.85	35.45	92.12	39.67	8.96	20.82	1.71	26.23	16.82	3.19	44.07
EBW030	G30	68.67	75.33	130.33	61.58	3709.75	48.82	36.64	94.93	42.01	9.11	22.8	1.58	26.56	16.43	3.01	43.71
EBW031	G31	69	75.67	131	62.5	4549.71	48.05	31.31	92.26	39.73	9.39	22.9	1.76	30.66	17.25	2.66	43.05
EBW032	G32	67.75	74.33	129.42	61.42	3847	45.52	32.94	90.78	39.87	8.78	23.82	1.65	29.29	16.83	3.09	42.47
Huluka	G33	72.58	80.17	135.83	62.67	4756.54	49.79	37.17	97.3	39.96	8.69	22.13	1.64	28.28	16.77	3.11	44.78
EBW034	G34	68.42	76.83	125.25	55.83	4340.38	48.94	38.23	99.33	42.22	9.37	22.18	1.75	29.01	16.55	3.09	42.28
EBW035	G35	67.75	72.5	127	59.75	4791	50.29	37.93	94.87	41.14	8.94	21.48	1.76	28.01	16.05	3.14	43.93
EBW036	G36	69.58	76.67	130	60.42	5043.67	48.18	34.26	95.14	40.91	9.5	19.99	1.71	24.76	16.47	2.76	42.65
EBW037	G37	68.25	75.17	128.5	60.25	4558.79	47.4	35.16	95.43	40.34	8.74	23.48	1.8	31.92	15.7	3.03	41.15
EBW038	G38	68	74.92	125.25	57.25	4654	49.74	36.44	94.13	42.62	8.61	25.02	1.76	33.06	15.87	2.74	43.5
EBW039	G39	69.75	76.75	133.17	63.75	5124.5	50.07	39.24	97.04	42.01	8.93	24.62	1.71	31.11	16.87	3.03	45
King-bird	G40	71.58	79	132.83	61.33	4677.42	48.68	36.51	95.67	42.19	8.24	23.87	1.64	28.79	16.72	2.94	39.5
EBW041	G41	67.75	74.83	131.17	64.67	5091.75	49.73	38.45	93.38	40.79	8.5	22.84	1.69	28.62	15.65	2.71	38.02
EBW042	G42	67.42	74.17	127.67	60.25	5077.42	48.51	37.09	92.76	36.59	8.48	19.57	1.76	25.31	16.18	2.64	39.13
EBW043	G43	69.08	76.17	129.58	60.5	4191.67	48.69	31.27	94.75	40.49	8.36	22.78	1.59	27.01	15.82	2.86	34.27
EBW044	G44	69.67	77.33	131.75	62.25	4828.17	48.36	37.17	90.98	37.41	8.7	20.51	1.82	28.53	16.5	2.98	38.8
EBW045	G45	68.33	75.67	131.58	63.33	4829.38	47.72	34.87	91.77	41.49	8.93	22.3	1.81	30.43	16.65	3.23	44.47
EBW046	G46	68.08	76.5	130	61.92	4783.5	47.84	36.26	92.35	40.33	9.12	21.64	1.66	26.28	16.77	3.13	42.82
EBW047	G47	68.08	74.58	131	63.17	4427.92	49.27	36.24	96.01	43.84	8.93	22.94	1.67	28.5	15.9	3.11	39.53
EBW048	G48	69.25	77.08	129.5	60.25	4089.13	46.79	32.53	96.85	42.12	9.12	23	1.78	30.61	16.28	2.86	43.57
EBW049	G49	71.92	78.75	131.67	59.58	4140.13	46.87	30.03	93.58	36.96	8.8	23.46	1.68	31.46	17.17	2.88	41.58
EBW050	G50	71.67	78.25	132.83	61.67	4671	48.71	37.91	95.24	39.77	9.26	22.87	1.74	29.55	16.63	3.04	45.48
EBW051	G51	71.33	77.33	130.08	59	5275.71	50.1	37.99	95.6	42.46	8.61	24.68	1.81	33.61	15.72	2.94	44.42
EBW052	G52	67.83	74.25	129.33	61.5	4326.54	48.71	34.11	97.43	41.53	9.21	24.38	1.68	31.2	17	2.86	40.32
EBW053	G53	64.75	71.58	129.17	65.25	4218.63	47.95	38.5	96.08	44.48	8.63	23.67	1.8	31.03	16.75	2.73	41.27
EBW054	G54	68.25	75.5	129.42	61	4387.88	48.69	37.09	94.42	40.66	9.01	22.55	1.63	25.62	16.53	2.93	43.63
EBW055	G55	68.92	75.92	130.17	60.83	4938.63	47.97	37.99	92.35	41.18	8.78	21.42	1.65	26.39	16.58	2.83	43.23
EBW056	G56	68.58	76.25	132.25	63.67	5285.25	49.93	38.86	95.53	38.37	8.08	20.44	1.69	26.18	16.62	3.04	42.62
EBW057	G57	66.75	73.08	127.75	61.25	4366.88	48.88	39.81	95.8	40.17	8.85	22.9	1.68	27.7	16.07	2.74	39.73
EBW058	G58	67.5	75	129.42	62.58	5052.17	49.15	39.86	93.45	40.87	9.19	22.63	1.75	30.18	17.12	3.28	45.88
EBW059	G59	72.5	80.33	134.17	61.92	4649.88	49.48	41.34	93.18	38.97	8.9	21.65	1.8	28.71	16.4	3.09	49.57
EBW060	G60	71.75	79	132.83	62.58	4348.63	48.48	37.91	93.48	40.16	9.22	22.19	1.76	29.11	17	2.86	43.13
EBW061	G61	72.83	80.33	136.17	63.33	4812.21	46.4	41.82	94.15	42.78	9.35	23.41	1.7	30.29	16.53	3.08	41.28
EBW062	G62	69.42	76.17	131.75	62.92	3730.25	48.21	40.75	94.6	41.43	9.3	24.11	1.7	31.68	16.43	2.94	39.45
EBW063	G63	73.42	80.5	130.83	56.58	4403.29	47.07	35.98	92.73	42.43	9.46	23.54	1.6	27.23	17.42	2.81	39.38
EBW064	G64	72.75	80.17	136	63.33	4745.04	47.48	36.41	93.18	40.98	9.68	24.32	1.81	33.64	16.75	2.88	41.52
EBW065	G65	70.83	77.83	130.5	60.25	4608.13	47.92	34.19	93.43	39.39	9.19	23.49	1.82	32.65	16.48	2.89	46.63
EBW066	G66	68.17	75.42	129.83	61.25	4780.5	48.05	34.27	92.28	40.38	9.13	24.37	1.63	30.43	16.05	2.98	42.53

Appendix V Continued

EBW067	G67	73.08	82	131.58	57.67	3690.38	47.85	37.89	91.05	37.4	9.6	22.21	1.71	28.64	16.67	2.59	41.28
EBW068	G68	71.17	77.33	132.5	60.25	4787.17	46.86	37.47	98.6	41.63	9.25	23.48	1.69	30.03	16.88	3.09	44.2
EBW069	G69	68.33	76	130.08	61.75	4342.88	47.56	37.1	96.75	42.29	9.23	23.74	1.59	29.2	16.8	2.63	41.62
EBW070	G70	72.08	79.83	133.5	61.08	3953.21	47.48	37.15	100.88	42.19	9.75	23.44	1.72	30.97	16.75	2.86	42.78
Danda'a	G71	69.75	75.08	129.25	57.92	5072.88	48.63	40.95	97.15	41.18	9.14	24.13	1.83	33.64	16.25	3.08	43.27
EBW072	G72	65.25	73.08	127.17	61.92	4735.38	47.05	38.45	95.33	44.39	9.53	23.13	1.87	33.39	17.08	2.76	41.2
EBW073	G73	68.08	75.08	128.67	60.58	4594.29	48.01	37.62	97.22	40.95	9.15	22.27	1.81	30.49	16.53	2.99	41.72
EBW074	G74	68.83	76.83	127.75	58.92	4088	48.94	36.76	97.88	38.78	8.82	20.65	1.71	26.45	16.2	3.01	43.58
EBW075	G75	69.17	77.08	130.67	61.5	4701.38	46.23	32.58	99.33	40.81	9.52	23.91	1.7	30.6	16.73	3.19	43.7
EBW076	G76	68.83	76.33	129	59.83	3926.42	45.25	34.94	99.08	40.03	8.99	22.79	1.58	27.3	16.73	3.23	42.78
EBW077	G77	70.42	78.25	132.5	62	4341.25	47.39	35.93	95.57	41.19	8.82	24.03	1.74	30.75	16.88	2.94	43.6
EBW078	G78	69.58	76.83	132.83	63.25	4481.38	47.46	37.09	98.87	40.86	9.35	23.48	1.75	32.18	16.8	2.81	41.45
EBW079	G79	73.17	79.92	134.83	61.58	4157.96	47.73	36.12	94.63	37.85	8.41	20.71	1.85	29.34	17.03	2.83	42.37
Et-13A2	G80	75.08	80.75	134.08	60.42	4116.5	48.96	37.09	106.47	46.68	8.44	23.08	1.66	30.94	16.02	2.69	39.3
EBW081	G81	69.17	73.5	131.58	61.67	5369.5	49.38	39.31	100.84	41.89	8.9	23.61	1.71	30.61	16.93	3.04	42.82
EBW082	G82	67.92	74.25	127.67	59.42	4524	48.67	38.52	93.67	39.24	8.95	23.23	1.71	30.44	16.95	2.88	42.85
EBW083	G83	68	74.92	132.75	64.92	4571.58	47.52	35.78	95.73	40.41	8.97	22.26	1.74	27.96	16.48	3.19	42.33
EBW084	G84	70.17	77.33	129.92	62.33	4368.38	48.93	36.15	90.82	38.48	9.18	23.96	1.66	30.15	17.35	2.78	43.13
EBW085	G85	70	77.42	131.92	61.08	5069.63	48.35	38.82	93.77	41.84	8.83	24.01	1.77	30.32	16.15	2.81	42.23
EBW086	G86	71.42	78.17	128.67	58.5	4062.67	48.47	37.33	98.04	46.44	8.45	25.56	1.64	31.76	15.35	3.04	41.22
EBW087	G87	69.83	76.5	130	60.25	4199.71	47.29	36.22	98.77	41.92	9.96	23.44	1.68	30.1	16.57	3.08	45.25
EBW088	G88	68.17	75.25	128.67	60.75	4385.96	47.12	38.19	96.73	40.07	9.25	23.18	1.69	31.56	15.8	3.29	42.13
EBW089	G89	70	77.42	131.42	61.42	3783.58	47.17	36.76	95.13	40.53	8.43	22.09	1.74	28.58	16.67	2.73	37.47
EBW090	G90	71.25	78.75	130.92	59.67	4316.42	48.09	35.33	94.28	42.48	8.49	22.86	1.67	28.84	16.62	3.11	44.1
EBW091	G91	69.08	76	130.5	61.92	4710.75	48.19	36.17	92.87	40.03	8.77	24.21	1.65	28.63	16.4	3.23	45
EBW092	G92	67.75	74.33	128.83	61.08	4381.96	48.85	39.34	90	36.08	7.95	24.08	1.67	28.98	16.13	3.24	45.95
EBW093	G93	68.67	75.75	127.33	57.92	4211.88	46.4	35.5	89.4	37.44	8.11	21.5	1.67	26.64	16.32	3.16	47.13
EBW094	G94	70.92	78.17	131.25	60.33	4269.54	46.92	34.9	99.28	43.18	9.08	24.62	1.77	31.24	16.6	3.19	42.97
EBW095	G95	70.33	77.17	131.17	60.58	4753.75	47.89	35.76	97.5	40.41	9.21	24.11	1.83	34.17	16.92	3.04	46.13
EBW096	G96	67.67	74.92	128.33	60.67	4289.5	49.57	40.88	98.47	41.64	9.29	23.75	1.74	30.87	15.95	3.14	40.7
EBW097	G97	70.33	77.5	133.25	62.08	4502.71	48.28	43.07	96.5	36.01	10.1	22.85	1.86	32	17.02	3.19	43.32
EBW098	G98	69.25	76.17	131.58	62.33	4558.38	48.51	37.56	97.78	40.15	8.87	24.08	1.68	29.31	16.79	2.96	43.18
EBW099	G99	68.42	74.58	130.08	61.75	4970.17	47.47	35.62	98.13	41.6	8.7	23.8	1.68	30.52	16.52	2.86	42.28
EBW100	G100	70.42	77.5	133.42	63.17	4730.79	48.68	38	94.95	41.89	10.16	25.77	1.69	34.67	16.9	3.13	45.67
EBW101	G101	73.08	79.75	131.83	60.75	4069.83	47.61	34.49	93.9	41.77	9.1	23.31	1.7	29.26	16.08	2.99	42.62
EBW102	G102	75.33	82.08	138.5	63.17	3931.5	46.44	36.21	90.08	35.48	9.7	23.07	1.7	27.61	16.32	2.86	41.8
EBW103	G103	67.67	74.92	131.33	61.92	3467.42	46.67	36.96	97.1	39.66	9.57	23.19	1.73	26.96	16.68	3.04	39.93
EBW104	G104	72.58	79.5	130.5	58.17	3995.71	47.12	35.44	95.02	38.3	9.3	22.52	1.78	29.89	16.78	3.21	42.23
EBW105	G105	70.67	75.92	128	59.33	4215.5	47.95	31.69	94.98	37.65	8.76	22.67	1.61	26.11	16.52	2.91	41.13
EBW106	G106	68.67	76.17	130.17	60	3790.71	47.36	32.01	93.95	37.19	8.93	23.06	1.64	27.01	17.27	2.84	39.62
EBW107	G107	66.5	73.25	126.17	60.5	4454.21	46.81	35.37	100.1	42.73	8.68	22.8	1.68	26.33	16.13	3.21	39.22
EBW108	G108	70.08	77	129.83	59.75	4823.42	48.49	38.69	96.7	39.11	8.68	22.62	1.77	30.37	16.97	3.21	44.08
EBW109	G109	68.67	75.58	125.92	57.67	4982.71	49.16	36.16	96.05	38.37	8.54	21.6	1.63	25.09	16.32	3.13	44.48
EBW110	G110	71.33	78.67	133.08	62.17	4292.75	46.67	37.71	95.88	39.37	8.84	21.68	1.68	27.57	14.78	2.69	36.9
EBW111	G111	71.67	78.42	131.75	59.25	4011.13	47.04	35.71	98.43	41.54	9.24	21.37	1.77	28.35	16.07	2.71	42.78
EBW112	G112	68	75.17	130.33	62.17	4474.58	46.39	38.16	101.28	41.66	8.93	22.57	1.71	29.05	17.23	3.04	44.18
EBW113	G113	72.75	79.42	134.08	61.42	4783.08	48.94	36.51	100.98	41.33	8.9	23.72	1.6	28.08	16.52	3.26	42.2
EBW114	G114	70.75	76.67	128.83	58.83	4769.17	48.83	38.49	98.45	42.65	8.64	22.48	1.76	28.89	16.07	3.27	43.43
EBW115	G115	68.08	74.83	128.17	60.08	4698.75	47.8	37.59	96.63	37.74	9.21	21.33	1.75	27.11	17.35	2.98	42.8
EBW116	G116	68.83	75.42	130.08	59.58	5329.29	47.61	35.98	95.78	40.06	9.08	23.13	1.77	29.54	16.15	3.03	45.53
EBW117	G117	66.75	73.75	127.33	60.75	4951.38	47.67	36.67	99.33	44.86	9.16	22.47	1.67	27.68	16.38	2.97	39.92
EBW118	G118	67.17	74.08	129.33	62.42	4893.33	48.83	41.07	98.35	42.19	8.98	24.71	1.76	31.72	16.77	3.22	44.97
EBW119	G119	69.25	76.08	129.42	60.17	4120.46	45.12	34.3	95.37	38.48	9.26	22.12	1.7	28.37	16.95	3.08	41.53
EBW120	G120	69.92	77.25	133.42	63.5	4885.92	48.22	40.98	96.45	40.47	10	23.58	1.69	30.28	16.42	3.24	45.6
EBW121	G121	68.33	76.33	131.67	63	5265.25	49.16	37.88	95.78	41.49	9.18	23.04	1.68	29.81	16.82	3.16	42.78
EBW122	G122	66.83	74.58	127.17	60.33	4598.29	46.51	40.04	93.83	39.98	8.91	23.13	1.74	30.94	17.03	3.14	43.23
EBW123	G123	66.33	73.92	128.08	61.75	4048.5	43.89	33.85	93.72	40.86	9.88	23.02	1.73	28.76	17.17	2.93	44.73
EBW124	G124	67.58	74.83	130.33	62.75	4223.92	48.55	39.84	95.65	42.5	9.3	22.88	1.68	29.09	16.37	3.33	44.73
EBW125	G125	70.08	77.17	131.75	61.67	4287.88	47.89	36.68	92.82	34.47	8.27	21.54	1.67	26.34	17.02	3.13	46.2
EBW126	G126	68.83	76.08	130.33	61.25	4377.17	47.1	33.37	91.77	36.34	8.9	21.2	1.62	22.85	18.08	2.89	42.62
Paven-76	G127	68.17	75.5	128.92	60.58	3729.25	47.45	30.26	90.93	42.74	8.86	24.2	1.61	27.25	15.95	3.08	39.83
EBW128	G128	66.17	73.83	126.58	60.42	3691.71	47.22	33.93	101.84	42.88	8.51	22.03	1.67	26.26	16.13	2.86	38.22
EBW129	G129	65.83	73.08	126.42	60.58	4506.75	46.07	39.9	99.33	41.08	9.05	20.68	1.73	25.6	16.9	3.11	43.15
EBW130	G130	68.5	76.08	130.42	61.83	4801.04	48.79	38.54	101.1	43.72	8.87	23.55	1.72	31.25	17.26	3.14	46.5
EBW131	G131	69.67	77.08	132.08	61.58	4460.29	48.16	41.68	100.27	41.04	9.22	23.54	1.82	31.95	16.83	2.79	43.4
EBW132	G132	66.75	74	126.25	59.5	4099.17	46.47	30.2	90.78	38.95	8.74	21.74	1.56	24.85	16.43	3.01	44.38
EBW133	G133	68.33	75.92	131.33	63	4037.54	47.97	38.83	99.93	45.48	9.03	23.93	1.62	29.75	15.92	3.03	43.82
EBW134	G134	68.92	77	129.25	60.33	4659.08	48.75	37.39	92.47	34.63	8	19.12	1.7	21.28	16.3	3.03	37.33
EBW135	G135	64.83	72	125.17	60.33	4150.5	47.81	41.62	93.55	42.14	8.8	24.1	1.6	29.32	15.6	2.86	35.93
EBW136	G136	70	78.08	133	62.58	4767.17	46.59	36.15	99.07	40.33	9.65	23.13	1.69	27.32	17.13	2.99	43.43
EBW137	G137	68.08	75.83	129.92	61.83	4541.67	47.12	35.87	96.08	41.68	9.25	24.05	1.55	28.48	16.3	3.06	40.55
EBW138	G138	71.33	78.83	131.08	59.75	4055.33	48.08	36.75	90.56	36.5	8.12	22.43	1.74	29.32	16.48	3.33	48.6

Appendix V Continued

EBW139	G139	75.92	82.17	138.25	65.92	4445.21	47.58	38.17	95.98	38.12	8.13	20.2	1.66	23.31	16.1	2.99	42.12
EBW140	G140	68.67	75.83	130.33	61.75	4738.92	49.15	41.11	99.53	41.22	9.17	22.41	1.8	30.11	15.88	2.94	39.98
EBW141	G141	67	74.67	128.5	61.5	4711.83	49.22	40.18	93.42	40.5	8.49	23.55	1.72	30.64	16.15	2.68	41.5
EBW142	G142	68	76.08	133.5	65.5	3792.71	47.33	32.01	93.45	38.83	9.73	23.96	1.81	33.07	17.48	2.89	43.67
EBW143	G143	67.67	74.92	126.42	58.75	4613.67	47.97	35.43	97.3	37.42	9.12	22.16	1.44	22.88	16.88	2.69	41.33
EBW144	G144	68.33	75.17	129.75	61.83	4809.75	49.83	43.21	99.13	42.26	9.21	24.57	1.66	31.8	17.13	3.04	43.48
EBW145	G145	65.75	72.33	126.75	61.42	4235.67	47.33	37.53	97.23	43.02	9.28	22.23	1.47	24.52	16.98	2.63	40.73
EBW146	G146	66.33	73.5	127.5	61.17	4646.79	47.99	39.98	96.85	41	10.11	24.3	1.76	32.23	16.72	2.84	41.87
EBW147	G147	66.58	73.92	129.42	61.58	3843.42	48.88	39.08	98.45	40.81	8.86	23.47	1.74	31.32	16.75	3.16	44.77
EBW148	G148	68.42	75.42	127.33	58.92	4361.13	48.83	35.22	95.55	39.93	8.93	22.7	1.55	26.11	15.88	2.94	37.27
Sofomor	G149	68.92	75.58	129.33	59.58	5140.08	49.87	39.22	101.67	42.33	8.94	23.37	1.42	23.93	17.1	3.08	42.2
EBW150	G150	70.58	77.17	126.83	57.75	4263.08	49.17	41.67	95.62	41.92	8.27	20	1.63	23.64	15.43	2.89	36.77
EBW151	G151	66.42	72.92	126.25	59.83	4819.21	49.43	40.01	93.25	40.52	8.77	23.31	1.46	25.25	16.27	3.04	43.62
KI6295-4A	G152	71.08	78.25	133.42	62.25	4787.63	48.49	34.6	102.05	48.96	8.48	27.1	1.42	29.16	15.63	2.56	37.47
EBW153	G153	66.75	73.58	129.75	63	4492.63	48.92	41.61	101.6	41.75	9.19	24.48	1.77	32.6	16.4	2.98	41.67
EBW154	G154	67.5	74.58	130.67	63.17	4754.63	45.85	41.66	98.17	42.9	9.34	24.81	1.65	31.92	16.9	3.19	42.12
EBW155	G155	67.92	75.58	132.17	64.17	5105.04	47.12	41.45	97.08	41.76	9.82	24.02	1.79	33.3	16.07	3.33	45.65
EBW156	G156	69.42	77	132.67	63.42	4875.88	49.28	41.67	101.55	45.57	8.76	25.08	1.62	30.43	16.37	2.86	41.5
EBW157	G157	65.83	72.83	129.08	63.25	4600.71	48.03	41	97.03	43.49	8.55	23.83	1.78	32.04	17.1	2.98	42.37
EBW158	G158	67.83	75.17	128.5	60.67	4622.92	49.1	36.46	89.22	34.74	7.55	23.1	1.56	26.69	14.9	2.88	42
EBW159	G159	68.25	75.83	132	63.75	5183.29	48.85	44.63	96.49	41.82	8.83	21.83	1.93	31.85	16.38	3.03	41.28
EBW160	G160	67	73.92	130.33	63.33	4564.21	48.88	34.83	99.43	42.23	9.26	23.71	1.67	29.97	17.17	2.84	44.13
EBW161	G161	70.25	78.33	134	63.75	4130.96	47.44	39.23	98.98	41.05	9.63	20.83	1.8	27.59	17.13	3.09	37.43
EBW162	G162	66.42	75	129.33	62.92	4813.29	48.41	41.71	89.73	34.44	9.21	20.78	1.64	24.35	17.12	3.13	45.43
EBW163	G163	72.5	81.08	133.75	61.25	5180	45.91	39.27	94.53	38.12	8.41	21.44	1.73	28.6	17.37	3.01	46.68
EBW164	G164	68.5	75.33	130	61.5	4281.38	48.19	35.17	93.82	39.21	8.73	23.2	1.67	29	17.05	2.89	42.35
EBW165	G165	73.75	82	135.92	65.5	5105.96	48.5	35.93	95.93	39.74	8.27	20.56	1.68	27.32	16.7	3.34	45.57
EBW166	G166	70.5	78.58	134.08	63.5	4884.17	48.87	35.44	96.63	41.49	9.53	22.89	1.7	30.11	18.27	2.98	46.4
EBW167	G167	75.67	82.75	140.42	64.75	4060.63	48.63	35.31	92.48	36.42	9.21	23.11	1.56	26.14	17.33	2.63	41.15
EBW168	G168	71.08	78.42	133.17	62.5	4833.75	49.28	37.24	95.33	42.53	9.21	25.1	1.57	28.21	16.55	2.63	40.42
EBW169	G169	69.58	77	130	60.75	5289.42	48.44	35.58	96.9	40.53	9.14	23.09	1.83	32.76	17.38	3.26	45.58
EBW170	G170	65.83	73.25	127.5	61.67	4317.79	50.17	42.17	96.72	40.36	9.05	22.67	1.57	26.12	16.15	3.24	45.3
EBW171	G171	74.17	82.08	139.33	70.17	4995.96	48.32	40.31	100.35	37.36	10.36	23.19	2.01	36.76	18.32	3.18	49.73
EBW172	G172	70	77.5	131.17	61.17	4791.92	48.68	39.96	96.65	39.91	8.78	23.1	1.65	29.08	16.07	3.29	39.7
K-6290-Bulk	G173	68.33	75.08	128.92	60.58	4500	48.67	36.48	110.72	47.37	8.73	25.16	1.62	32.61	16.68	2.88	40.75
EBW174	G174	70.58	78.67	134.42	64	5705.92	48.22	42.55	95.38	42.08	9.44	25.14	1.62	31.8	17.37	3.06	48.82
Hogana	G175	79.17	85.67	139.83	60.67	4577.58	45.49	32.19	92.1	39.57	8.74	22.5	1.43	22.08	16.9	2.78	37.88
EBW176	G176	74.75	81.67	135.25	60	5613.5	47.52	39.5	97.88	42.64	9.83	25.37	1.87	35.83	17.03	3.23	51.32
EBW177	G177	69	75.83	131.17	62.17	5061.58	46.12	38.56	96.05	41.65	9.2	22.95	1.62	27.49	17.15	3.01	49.75
EBW178	G178	67.08	72.58	128.92	62.17	4501.83	49.35	39.72	95.85	41.79	8.63	22.33	1.73	29.3	16.57	2.98	40.6
EBW179	G179	68.25	76.17	130	62	4779.88	47.75	35.91	96.78	40.8	9.18	23.2	1.73	29.97	17.48	3.06	46.25
EBW180	G180	70.92	77.83	132.25	61.17	4915.38	48.03	37.84	97.28	39.96	9.12	23.43	1.73	31.16	16.95	3.14	42.23

DH = days to 50% heading; DF = days to 50% flowering; DM = days to 90% maturity; GDF = Grain filling duration; HLW = Hectoliter weight; TKW = 1000 kernel weight; PH = Plant height (cm); PL= Panicle length (cm); SL= Spike length (cm); FLL= Flag leaf length; FLW = Flag leaf width; FLA = Flag leaf area; SN = number of spikelet per spike; NKPS= Number of kernel per spikelet; and NKS = Number of kernel per spike.

Appendix **VI.** Mean performance of bread wheat genotypes for SDS traits across six environments in Ethiopia

Genotypes		SDSH	SDSMM	SDSM	SPC	Genotypes		SDSH	SDSMM	SDSM	SPC
Name	Code	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Name	Code	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
EBW001	G1	25.1±4.9	34.31±11.37	44.5517.82	0.66±0.11	EBW091	G91	16.82±9.54	21.71±10.33	23.679.9	0.43±0.14
EBW002	G2	27.93±12.15	43.62±26.96	51.2323.58	0.72±0.11	EBW092	G92	10.75±6.57	16.31±4.12	24.075.59	0.51±0.1
EBW003	G3	9.31±7.03	14.45±9.01	15.235.11	0.37±0.12	EBW093	G93	31.84±16.74	39.66±25.76	57.226.88	0.73±0.15
EBW004	G4	15.59±5.56	27.52±7.56	26.236.73	0.61±0.11	EBW094	G94	22.84±7.53	31.38±7.23	40.0214.92	0.65±0.16
EBW005	G5	31.69±9.8	32.36±13.12	34.5715.69	0.61±0.18	EBW095	G95	16.41±6.41	24.69±5.22	34.186.91	0.54±0.07
EBW006	G6	27.31±10.25	39.35±16.67	44.2417.42	0.68±0.15	EBW096	G96	13.58±7.18	21.5±6.38	24.3810.86	0.48±0.16
EBW007	G7	18.67±5.09	39.3±8.58	50.6218.92	0.69±0.12	EBW097	G97	15.28±6.78	21.09±11.14	19.6510.59	0.39±0.17
EBW008	G8	33.9±14.21	45.73±18.1	50.8214.06	0.66±0.11	EBW098	G98	25±9.6	34.1±21.57	45.9923.46	0.64±0.19
EBW009	G9	28.29±15.55	41.05±25.66	60.0224.93	0.72±0.2	EBW099	G99	18.93±7.71	28.14±16.53	23.6616.23	0.48±0.24
Bika	G10	24.43±2.01	35.96±5.19	41.365.47	0.62±0.11	EBW100	G100	16.82±3.95	25.15±6.07	32.829.33	0.55±0.08
EBW011	G11	24.54±15.35	36.21±18.45	44.7620.31	0.65±0.09	EBW101	G101	30.04±10.9	38.27±19.06	55.0422.2	0.65±0.12
EBW012	G12	30.92±17.11	36.88±18.57	51.6428.11	0.71±0.12	EBW102	G102	21.3±15.76	28.4±17.24	27.2619.88	0.49±0.27
EBW013	G13	29.27±6.94	34.57±8.56	42.87.5	0.67±0.1	EBW103	G103	32.41±12.01	43.11±19.56	57.4922.97	0.74±0.14
Meraro	G14	20.94±6.1	20.63±8.33	25.216.81	0.52±0.12	EBW104	G104	39.81±16.64	44.29±22.92	55.2516.36	0.75±0.13
EBW015	G15	27.16±9.65	41.41±12.73	50.3114.13	0.68±0.1	EBW105	G105	26.65±8.21	45.83±15.38	54.9415.99	0.75±0.11
EBW016	G16	25.05±7.16	36.01±9.33	42.397.25	0.64±0.1	EBW106	G106	33.23±15.02	45.11±22.55	58.3325.37	0.71±0.13
EBW017	G17	16.31±6.33	28.7±4.84	26.237.77	0.53±0.12	EBW107	G107	18.56±8.5	20.88±11.57	30.7621.92	0.46±0.25
EBW018	G18	27.67±9.72	31.58±15.48	29.8311.67	0.65±0.1	EBW108	G108	18.98±10.41	24.69±10.43	28.0912.64	0.52±0.14
EBW019	G19	24.07±3.66	39.92±238.51	49.5911.82	0.64±0.1	EBW109	G109	33.69±12.68	38.68±10.76	45.1714.76	0.63±0.09
EBW020	G20	23.56±7.04	37.86±7.14	41.6712.92	0.61±0.18	EBW110	G110	35.34±9.7	47.17±10.56	54.0211.86	0.65±0.1
Alidoro	G21	18.52±5.83	26.85±11.91	27.2511.21	0.49±0.14	EBW111	G111	38.63±11.19	44.39±13.08	50.5216.86	0.62±0.09
Medawolabu	G22	34.77±8.71	42.7±11.12	59.0612.41	0.65±0.09	EBW112	G112	30.97±5.76	33.54±16.49	49.0822.72	0.66±0.08
EBW023	G23	22.48±5.91	22.89±6.57	34.3912.36	0.55±0.14	EBW113	G113	19.96±4.14	24.74±6.19	29.739.2	0.58±0.1
EBW024	G24	24.59±3.69	31.07±6.11	32.5112.42	0.57±0.2	EBW114	G114	10.65±1.35	25.77±7.48	34.169.86	0.53±0.1
EBW025	G25	21.76±5.72	31.94±10.14	38.6811.69	0.64±0.12	EBW115	G115	22.58±11.46	25.77±7.09	35.0817.82	0.55±0.14
EBW026	G26	20.32±7.13	24.28±14.74	25.3320.97	0.54±0.24	EBW116	G116	16.46±5.75	28.09±7.31	37.148.54	0.57±0.1
EBW027	G27	20.52±10.07	30.25±7.69	45.378.01	0.66±0.16	EBW117	G117	25.36±12.52	37.14±11.95	46.519.19	0.62±0.1
EBW028	G28	24.43±7.49	39.2±10.84	40.3321.5	0.68±0.12	EBW118	G118	12.24±2.63	19.19±7.15	27.379.09	0.46±0.1
EBW029	G29	19.86±5.58	27.31±15.26	43.4110.9	0.69±0.09	EBW119	G119	22.48±13.83	29.63±15.24	40.8119.05	0.58±0.16
EBW030	G30	16.2±7.41	32.51±10.22	38.689.85	0.62±0.11	EBW120	G120	19.82±6.24	29.78±4.42	34.875.28	0.58±0.11
EBW031	G31	24.8±4.65	38.37±7.64	46.614.61	0.68±0.11	EBW121	G121	18.72±2.81	31.07±10.72	40.3314.85	0.62±0.12
EBW032	G32	17.03±10.51	22.79±10.41	43.4211.11	0.59±0.14	EBW122	G122	21.19±7.31	35.96±14.34	47.6318.78	0.67±0.12
Huluka	G33	24.33±10.98	24.79±10.76	28.176.71	0.57±0.12	EBW123	G123	29.99±20.74	44.14±25.63	53.432.94	0.72±0.18
EBW034	G34	29.68±11.43	38.27±19.5	48.6621.54	0.69±0.1	EBW124	G124	16.2±6.67	30.25±9.11	35.57.88	0.58±0.09
EBW035	G35	24.69±4.38	25.82±5.69	36.7315.73	0.63±0.09	EBW125	G125	10.34±6.68	21.4±11.43	21.1911.28	0.4±0.16
EBW036	G36	19.5±6.07	34.26±15.12	47.0218.79	0.65±0.09	EBW126	G126	20.37±10.01	36.06±17.14	33.7517.14	0.51±0.2
EBW037	G37	24.49±12.27	38.73±24.08	54.4225.75	0.7±0.12	Paven-76	G127	26.49±5.75	47.69±15.26	65.3313.24	0.74±0.13
EBW038	G38	17.28±2.82	27.06±12.77	36.6316.68	0.64±0.14	EBW128	G128	32.82±8.47	42.95±21.21	53.3924.75	0.68±0.14
EBW039	G39	18.7±6.82	25.21±15.63	29.6313.82	0.58±0.11	EBW129	G129	23.2±8.77	29.48±7.91	49.6918.66	0.62±0.13
King-bird	G40	18.06±2.91	27.62±7.89	41.1511.85	0.62±0.1	EBW130	G130	13.17±6.36	20.27±8.45	20.215.85	0.41±0.18
EBW041	G41	11.83±2.77	18.36±7.42	20.419.34	0.51±0.14	EBW131	G131	19.8±7.72	29.53±7.5	35.715.58	0.58±0.09
EBW042	G42	23.82±19.1	40.28±22.22	49.5925.65	0.65±0.13	EBW132	G132	36.16±9.38	46.24±24.64	57.6122.16	0.79±0.13
EBW043	G43	23.61±9.03	33.64±12.7	38.5814.06	0.62±0.15	EBW133	G133	8.9±4.61	19.14±12.9	18.8320.86	0.37±0.27
EBW044	G44	17.44±12.39	23.51±14.64	20.6814.99	0.43±0.25	EBW134	G134	27.67±13.57	38.89±20.25	51.4421.21	0.72±0.14
EBW045	G45	31.38±13.29	34.72±10.69	41.0713.01	0.64±0.13	EBW135	G135	21.6±12.16	30.71±9.98	44.7517.57	0.7±0.1
EBW046	G46	22.48±9.56	33.23±12.43	45.1717.65	0.69±0.11	EBW136	G136	21.45±15.91	32.82±11.14	32.8614.17	0.49±0.15
EBW047	G47	14.61±10.8	17.23±12.51	21.9117	0.39±0.2	EBW137	G137	14.15±8.23	22.48±4.1	27.677.56	0.51±0.15
EBW048	G48	19.55±5.86	27.57±8.33	38.9913.68	0.58±0.14	EBW138	G138	16.46±3.86	21.04±7.59	27.310.55	0.54±0.17
EBW049	G49	28.7±2.97	44.39±9.82	59.6711.8	0.7±0.09	EBW139	G139	24.02±3.89	32.15±7.2	35.69.03	0.65±0.1
EBW050	G50	26.08±5.14	25.87±9.56	33.8512.11	0.57±0.12	EBW140	G140	14.97±2.51	24.02±7.12	26.0312.68	0.45±0.17
EBW051	G51	18.42±5.69	28.7±10.2	38.9915.14	0.64±0.11	EBW141	G141	8.9±7.01	17.59±7.22	22.127.26	0.44±0.15
EBW052	G52	27.31±3.86	36.78±7.58	40.1211.07	0.64±0.09	EBW142	G142	23.05±5.36	36.11±8.29	42.289.32	0.65±0.11
EBW053	G53	23.77±6.65	36.93±14.97	54.0722.63	0.69±0.11	EBW143	G143	37.5±11.77	45.16±15.27	46.8119.21	0.64±0.12
EBW054	G54	21.09±6.86	26.13±14.14	32.8227.42	0.54±0.21	EBW144	G144	5.86±3.24	14.15±8.1	14.26.86	0.34±0.17
EBW055	G55	25.26±15.38	32.05±16.65	41.6721.09	0.62±0.14	EBW145	G145	28.65±7.11	37.29±11.31	49.0818.7	0.65±0.11
EBW056	G56	28.4±9.68	29.48±8.99	34.2616.83	0.6±0.14	EBW146	G146	18.42±3.37	35.55±4.47	43.2110.54	0.59±0.13
EBW057	G57	34.52±20.45	41.05±19.26	51.6518.87	0.71±0.11	EBW147	G147	26.18±16.72	40.84±20.52	55.6622.04	0.66±0.13
EBW058	G58	25.93±11.69	25±6.39	39.1911.39	0.63±0.13	EBW148	G148	31.58±17.62	42.75±20.75	53.2121.04	0.73±0.13
EBW059	G59	16.46±3.35	25.77±9.99	31.598.64	0.57±0.1	Sofomor	G149	9.1±6.74	26.18±15.23	43.7216.34	0.61±0.12
EBW060	G60	25.31±9.74	34.47±15.34	46.617.49	0.62±0.13	EBW150	G150	6.07±3.52	16.36±5.61	28.1712.78	0.43±0.15
EBW061	G61	15.74±3.92	20.88±8.79	26.6514.12	0.53±0.19	EBW151	G151	8.45±8.38	15.28±8.39	19.039.49	0.38±0.16

Appendix VI Continued

EBW062	G62	22.74±9.6	22.27±4.12	31.387.88	0.52±0.15	KI6295-4A	G152	14.81±6.17	30.3±13.57	27.5716.78	0.48±0.19
EBW063	G63	21.4±11.31	26.8±7.37	28.0915.66	0.53±0.23	EBW153	G153	5.81±4.33	10.55±6.39	15.9510.09	0.4±0.15
EBW064	G64	21.6±15.46	27.42±14.29	25.3115.7	0.49±0.19	EBW154	G154	13.89±6.35	20.01±9.21	22.226.53	0.38±0.11
EBW065	G65	29.58±3.08	33.69±6.59	36.219.82	0.63±0.13	EBW155	G155	10.24±7.49	15.74±7.69	19.4423.14	0.37±0.23
EBW066	G66	21.45±3.97	30.35±9.96	37.556.06	0.57±0.12	EBW156	G156	9.47±3.19	17.75±3.9	18.726.84	0.41±0.13
EBW067	G67	15.07±10.98	26.03±15.69	26.5420.43	0.47±0.28	EBW157	G157	19.44±5.67	25.51±8	37.2412.35	0.63±0.1
EBW068	G68	19.86±5.3	22.69±7.58	24.4914.63	0.5±0.11	EBW158	G158	17.85±5.63	31.79±7.25	33.448.81	0.58±0.14
EBW069	G69	31.48±13.5	46.19±21.6	54.0119.84	0.67±0.14	EBW159	G159	14.25±6.19	18.72±9.45	23.8712.51	0.45±0.17
EBW070	G70	25.15±12.21	34.36±12.16	47.3315.24	0.61±0.08	EBW160	G160	26.44±5.4	33.49±9.65	45.377.33	0.61±0.08
Danda'a	G71	14.04±3.68	19.24±8.39	22.435.71	0.45±0.12	EBW161	G161	26.9±9.27	30.71±6.81	37.4611.36	0.57±0.12
EBW072	G72	20.94±8.85	26.39±7.37	34.056.9	0.62±0.09	EBW162	G162	18.06±5.11	26.9±7.19	38.5814.76	0.65±0.15
EBW073	G73	31.74±14.82	41.87±14.46	52.2617.35	0.65±0.11	EBW163	G163	13.94±3.87	26.39±11.41	37.1411.84	0.64±0.15
EBW074	G74	23.15±13.14	37.45±21.15	55.5615.75	0.73±0.13	EBW164	G164	13.27±6.62	19.91±5.94	22.729.59	0.47±0.19
EBW075	G75	21.97±8.42	33.49±9.06	46.314.5	0.65±0.11	EBW165	G165	21.86±8.06	34.67±9.08	34.059.58	0.6±0.11
EBW076	G76	35.55±12.94	48.46±21.75	55.619.92	0.7±0.13	EBW166	G166	9.57±4.86	24.18±3.46	30.865.95	0.58±0.12
EBW077	G77	12.65±7.98	21.71±13.6	31.7923.08	0.46±0.26	EBW167	G167	19.24±7.84	26.85±9.06	325.46	0.56±0.13
EBW078	G78	14.71±2.45	30.45±7.16	35.497.97	0.59±0.09	EBW168	G168	19.34±7.8	28.34±8.61	31.596.7	0.53±0.12
EBW079	G79	22.27±11.16	35.6±23.24	53.6823.34	0.66±0.13	EBW169	G169	15.33±6.94	18.21±3.92	26.0310.55	0.51±0.17
Et-13A2	G80	15.02±10.56	28.29±13.14	34.6712.16	0.47±0.17	EBW170	G170	19.96±6.7	29.17±12.14	45.7823.92	0.6±0.11
EBW081	G81	10.75±8.32	17.03±4.21	22.6410.36	0.41±0.15	EBW171	G171	18.26±7.92	23.61±9.67	28.3911.3	0.52±0.12
EBW082	G82	13.53±4.24	19.6±4.95	26.758.03	0.55±0.11	EBW172	G172	12.45±6.1	22.89±4.57	26.545.91	0.58±0.14
EBW083	G83	24.18±10.3	29.99±15.28	42.5917.56	0.62±0.1	K-6290-Bulk	G173	24.18±6.73	33.44±13.03	48.7615.51	0.59±0.14
EBW084	G84	32.25±8.5	41.1±16.64	52.2612.43	0.77±0.1	EBW174	G174	5.25±5.68	8.18±9.36	10.69.08	0.31±0.17
EBW085	G85	17.39±7.38	18.72±9.12	22.4313.3	0.45±0.24	Hogana	G175	21.66±10.4	26.34±8.51	24.1815.3	0.52±0.24
EBW086	G86	24.38±13.3	39.3±21	42.0815.03	0.64±0.15	EBW176	G176	17.75±2.01	25.67±6.67	33.79.06	0.58±0.11
EBW087	G87	35.75±22.86	42.7±22.58	61.4223.87	0.71±0.11	EBW177	G177	24.28±11.82	26.85±9.75	36.4216.98	0.62±0.11
EBW088	G88	14.45±1.61	18.42±7.62	24.6910.23	0.48±0.17	EBW178	G178	17.54±13.63	22.63±13.51	26.4416.08	0.45±0.21
EBW089	G89	28.91±10.53	32.3±14.83	43.9317.66	0.61±0.12	EBW179	G179	10.55±5.6	28.19±12.54	41.6612.35	0.6±0.11
EBW090	G90	25.72±8.65	38.32±14.58	49.811.75	0.68±0.11	EBW180	G180	20.52±7.53	29.01±12.42	42.5919.4	0.61±0.11

Appendix **VII.** For each chromosome, physical distance at which mean LD calculated in rolling windows on pairwise data drops below $r^2 = 0.2$.

Chromosome	Dist_bp	Mbp
1A	18289767	18.29
1B	96181598	96.19
1D	34801332	34.81
2A	22585488	22.59
2B	14734203	14.74
2D	1260605	1.26
3A	16767098	10.37
3B	2250448	2.26
3D	24495183	24.5
4A	5152895	5.15
4B	4016183	4.02
4D	29499474	29.5
5A	59438391	59.44
5B	15781567	15.79
5D	2953194	2.96
6A	4074779	4.08
6B	89313712	89.32
6D	23050239	23.06
7A	53261067	53.27
7B	36793383	36.8
7D	105610385	105.61
Mean	31443380.52	31.44

Appendix **VIII**. High confidence genes and gene models detected within the identified STB resistance associated QTL regions.

Putative QTL	Chromosome	SNP Position	Gene ID [a]	Gene/gene model position Gene names			Gene Ontology (Biological process)
				Start	End	strand	
qSTB.01	1A	366278319	TraesCS1A02G201700	363006018	363007340	-	Defense response/ethylene biosynthetic process.
			TraesCS1A02G203900	365617809	365619048	-	Indoleacetic acid biosynthetic process/Regulation of defense response
			TraesCS1A02G202000	363047773	363052288	-	Immune system process.
qSTB.02	1A	474702375	TraesCS1A02G279300	475309490	475313192	+	Systemic acquired resistance/ jasmonic acid mediated signaling pathway/regulation of plant-type hypersensitive/ defense response to fungus/defense response to bacterium
qSTB.03	1A	566369413	TraesCS1A02G403900	567318284	567327266	+	cellular response to stimulus, Hessian fly resistance
qSTB.04	1B	558551443-587138312	TraesCS1B02G333500	560018875	560021191	-	Response to biotic stress
			TraesCS1B02G332400	558550247	558552802	-	Jasmonic acid metabolic process/jasmonic acid and ethylene-dependent systemic resistance/induced systemic resistance
qSTB.05	1D	3324483	TraesCS1D02G001700	447129	462647	+	Systemic acquired resistance/regulation of defense response.
qSTB.06	1D	375956648	TraesCS1D02G276100	372147878	372155789	+	defense response
			TraesCS1D02G278400	375954117	375957575	-	Jasmonic acid mediated signalling pathway/ethylene-activated signaling pathway.

Appendix VIII Continued

qSTB.07	1D	463434850	TraesCS1D02G389000	460147069	460151558	-	Response to biotic stress
			TraesCS1D02G387600	460147069	460151558	-	Defense response to biotic stresses
			TraesCS1D02G393000	462739002	462746635	-	Systemic acquired resistance/regulation of plant-type hypersensitive response /defense response to fungus.
			TraesCS1D02G390300	461536153	461539106	-	Response to biotic stress
			TraesCS1D02G390100	461536153	461539106	-	Response to abiotic stress, response to biotic stress
			TraesCS1D02G387600	460147069	460151558	-	Defense response.
qSTB.08	2A	514858369	TraesCS2A02G295900	509070654	509076530	+	Response to biotic stress
			TraesCS2A02G299900	515452029	515453761	+	Programmed cell death/ induction of programmed cell death.
			TraesCS2A02G298600	514231854	514233503	+	Defense response to fungus.
			TraesCS2A02G298100	513286315	513287713	+	Defense response.
			TraesCS2A02G297500	511474142	511477203	+	Defense response to fungus, plant-type hypersensitive response, systemic acquired resistance/ salicylic acid mediated signaling pathwa/ jasmonic acid mediated signaling pathway/ **MAPK cascades** are involved in signaling multiple **defense** responses, including the biosynthesis/signaling of **plant** stress/ **defense** hormones, reactive oxygen species (ROS) generation, stomatal closure, **defense** gene activation, phytoalexin biosynthesis, cell wall strengthening, and hypersensitive response (HR) cell death.

Appendix VIII Continued

qSTB.09	2B	243083729	TraesCS2B02G233600	232266510	232273397	+	Systemic acquired resistance/Jasmonic acid biosynthetic process/ resistance response to pathogenic bacteria
			TraesCS2B02G492500	690682191	690683084	-	Obsolete pathogen-associated molecular pattern dependent induction by symbiont of host innate immune response
			TraesCS2B02G500100	695688695	695689617	+	Abscisic acid-activated signaling pathway/response to biotic stimulus/defense response.
			TraesCS2B02G499300	695449352	695451719	+	Response to stress
qSTB.11	2D	288602370	TraesCS2D02G246800	289157650	289160782	+	Response to biotic stress
qSTB.12	2D	450991087	TraesCS2D02G350500	448501026	448502188		Response to biotic stress
			TraesCS2D02G348700	446640621	446640884	-	Response to biotic stress
			TraesCS2D02G351600				Jasmonic acid mediated signaling pathway.
qSTB.13	2D	593032041	TraesCS2D02G489700	588676152	588677693	-	Response to biotic stress
			TraesCS2D02G486800	586957083	586961710	-	Defense response to fungus.
			TraesCS2D02G488100	587696143	587696631	+	Response to biotic stress
			TraesCS2D02G488200	587697352	587701032	-	Response to biotic stress
			TraesCS2D02G497700	593408267	593410141	+	Response to biotic stress
			TraesCS2D02G497600	593370558	593373483	+	Response to biotic stress
			TraesCS2D02G497400	593270400	593275755	-	Salicylic acid mediated signaling pathway/defense response to fungus/abscisic acid-activated signaling pathway/ jasmonic acid mediated signaling pathway.
			TraesCS2D02G498000	593523002	593524197	-	Methyl jasmonate esterase activity
qSTB.14	2D	598728762	TraesCS2D02G504500	598812395	598819350	+	Biotic defense response.
			TraesCS2D02G504200	598733638	598735164	_	Salicylic acid-dependent systemic acquired resistance.
			TraesCS2D02G504000	598714158	598720273	-	Response to biotic stress
			TraesCS2D02G503900	598712612	598713618	+	Response to biotic stress
			TraesCS2D02G503800	598610615	598613095	+	Defense response: defense/immunity protein activity
			TraesCS2D02G506300	600161338	600164249	-	Salicylic acid mediated response to a pathogen which confers broad spectrum resistance/salicylic acid-dependent systemic resistance
			TraesCS2D02G506200	600131157	600134165	-	Salicylic acid mediated response to a pathogen which confers broad spectrum resistance
			TraesCS2D02G505700	599916155.	599919148	+	Jasmonic acid biosynthetic process.

Appendix VIII Continued

qSTB.15	3A	8862385	TraesCS3A02G007800	7788947	7791684	-	Response to biotic stress
			TraesCS3A02G007000	7432258	7432761	-	Response to biotic stress
			TraesCS3A02G005500	6891942	6899514	-	Immune system process: defense response
			TraesCS3A02G016500	7469796	7474094	-	Response to biotic stress
			TraesCS3A02G010900	9064431	9066825	-	Defense response to fungus
			TraesCS3A02G009900	8837590	8840754	+	Defense response
			TraesCS3A02G009800	8794573	8800596	-	Defense response to fungus
			TraesCS3A02G009300	8549930	8550907	+	Defense response.
			TraesCS3A02G009200	8323975	8330044	-	Defense response
			TraesCS3A02G019400	11800250	11808293	-	Response to biotic stress
			TraesCS3A02G020700	11808293	11808293	-	Salicylic acid mediated signaling pathwa
qSTB.16	3A	203418249	TraesCS3A02G479600	711077944	711078848	-	Response to biotic stress.
qSTB.17	3A	710771071	TraesCS3A02G479400	711056566	711061191	+	Defense response
			TraesCS3A02G479300	711040605	711041129	+	Response to biotic stress
			TraesCS3A02G479200	710946876	710949891	+	Defense response and response to biotic stimulus
			TraesCS3A02G479100	710935516	710940717	+	Defense response
			TraesCS3A02G478900	710837017	710838334	+	Defense response
qSTB.18	3B	17785833	TraesCS3B02G035100	17081352	17087675	-	Defense response
			TraesCS3B02G035000	16983166	16984121	+	Plant-type hypersensitive response.
			TraesCS3B02G034700	16692513	16694199	+	Plant-type hypersensitive response.
			TraesCS3B02G034200	16333353	16334691	+	Response to biotic stress.
			TraesCS3B02G034000	16202219	16202482	-	Response to biotic stress.
			TraesCS3B02G033400	16078276	16079730	-	Plant-type hypersensitive response.
			TraesCS3B02G033400	16078276	6079730	-	Regulation of plant-type hypersensitive response
			TraesCS3B02G040100	19394360	19396966	-	Defense response
			TraesCS3B02G039200	18858691	8865152	-	Defense response
			TraesCS3B02G038600	18558602	18559871	-	Response to biotic stress
			TraesCS3B02G038500	18542363	18542847	+	Defense response
			TraesCS3B02G037700	18306039	18306470	+	Defense response to fungi
			TraesCS3B02G037900	18393856	18394281	+	Defense response to fungi
			TraesCS3B02G03750	18243443	18248804	+	Defense response
qSTB.19	3B	59645976	TraesCS3B02G08140	51244017	51245393	+	Defense response

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qSTB.20	3D	42679365	TraesCS3D02G080600	40526100	40531620	+	Response to biotic stress
			TraesCS3D02G080000	39921031	39931494	+	Response to biotic stress.
			TraesCS3D02G088300	45015144	45018965	+	Plant-type hypersensitive response.
			TraesCS3D02G088100	44966972	44967619	+	Defense response to fungi/regulation of plant-type hypersensitive response/systemic acquired resistance/ jasmonic acid mediated signaling pathway.
qSTB.21	3D	593664469	TraesCS3D02G509900	595062529	595067402	-	Jasmonic acid mediated signaling pathway and defense response to bacterium.
			TraesCS3D02G508900	594802578	594803164	+	Response to stress.
			TraesCS3D02G508700	594769919	594770140	+	Any immune system process that functions in the calibrated response of an organism to a potential internal or invasive threat.
			TraesCS3D02G508400	594573981	594583032	+	Defense response
			TraesCS3D02G506300	593235701	593238423	+	defense response
			TraesCS4A02G337400	619582627	619584158		Response to biotic stress
			TraesCS4A02G341300	621796765	621806512	-	Regulation of plant-type hypersensitive response, systemic acquired resistance, defense response by callose deposition in cell wall and about induced systemic resistance.
			TraesCS4A02G335500	618085607	618089160	+	Defense response, physiological defense response, antimicrobial peptide activity, defense/immunity protein activity
			TraesCS4A02G335600	618121544	618125040	+	Defense response /immunity protein activity
			TraesCS4A02G335700	618158574	618175725	+	Defense response/immunity protein activity
			TraesCS4A02G337700	619948780	619950150	-	Response to biotic stress
qSTB.23	5A	688359748	TraesCS5A02G525600	685875882	685876097	+	Defense response to fungi/resistance response to pathogenic fungi
			TraesCS5A02G525500	685869126	685870602	+	Regulation of defense response
			TraesCS5A02G521400	682617294	682617626	-	Regulation of non-apoptotic programmed cell death
			TraesCS5A02G528100	688297677	688302598	-	response to biotic stress.
			TraesCS5A02G528000	688292625	688295351	+	Plant-type hypersensitive response/ he rapid/localized death of plant cells in response to invasion by a pathogen/ immune response-regulating signaling pathway.
			TraesCS5A02G527600	688137629	688142671	-	Defense response to fungi
			TraesCS5A02G526500	687275788	687281990	-	Defense response/immunity protein activity
qSTB.24	5B	487460716	TraesCS5B02G299200	482423553	482432342	-	Response to biotic stress.
			TraesCS5B02G299000	482288012	482291300	+	Defense response by callose deposition
			TraesCS5B02G299000	482288012	482291300	+	Defense response to fungi.
			TraesCS5B02G302800	487344161	487346328	-	Any immune system process that functions in the calibrated response of an organism to a potential internal or invasive hreat

Appendix VIII Continued

qSTB.25	5B	538706298	TraesCS5B02G356900	536476323	536476925	+	Defense response/immunity protein activity
			TraesCS5B02G359800	539460745	539469556	-	MAPK cascade/systemic acquired resistance/salicylic acid mediated signaling pathway/jasmonic acid mediated signaling pathway/defense response to fungus/regulation of plant-type hypersensitive response.
			TraesCS5B02G356800	536384212	536385111	+	defense response/immunity protein activity
			TraesCS5B02G360300	539933883	539935311	+	Response to stress.
qSTB.26	5D	541603929	TraesCS1A02G356800	539754423	539758138	+	Defense response /immunity protein activity
			TraesCS1A02G356600	539720660	539724029	+	Response to biotic stress.
			TraesCS1A02G355300	537966441	537967220	-	Salicylic acid-dependent systemic resistance/defense response to bacterium
			TraesCS1A02G354300	537345702	537347810	-	Response to biotic stress.
			TraesCS1A02G354000	537347810	537083849	+	Defense response
			TraesCS1A02G358100	540191129	540192273	+	Response to stress.
qSTB.27	6A	607427728-609480220	TraesCS6A02G385200	603135847	603136814	+	Respiratory system process.
			TraesCS6A02G385000	60309120	603095476	+	Immune response-regulating signaling pathway.
			TraesCS6A02G385000	603091205	603095476	+	Plant-type hypersensitive response/defense response to bacterium
			TraesCS6A02G384900	603080652	603085053	+	Immune response-regulating signaling pathway/plant-type hypersensitive response.
			TraesCS6A02G384700	603045257	603046126	+	Response to biotic stress
			TraesCS6A02G389000	604607404	604610875	-	Defense response to fungi/defense response to fungus, resistance response to pathogenic fungus/response to parasitic fungi/response to parasitic fungus
			TraesCS6A02G391700	606435973	606436281	-	defense response to fungus.
			TraesCS6A02G391300	606094973	606095368	+	Defense response to fungus/ defense response to bacterium.
			TraesCS6A02G391100	606035615	606041135	+	Response to biotic stress.
			TraesCS6A02G390300	605324997	605339666	+	Defense response to fungus/salicylic acid mediated signaling pathway
			TraesCS6B02G436100	703463862	703466298	-	Response to stress.
			TraesCS6B02G431700	700953964	700954293	-	Defense response to fungus/defense response to bacterium.

Appendix VIII Continued

qSTB.28	6B	708272196	TraesCS6B02G430800	698974724	698978209	+	Defense response to fungus and regulation of plant-type hypersensitive response.
			TraesCS6B02G430000	697752877	697760334	-	response to biotic stress.
			TraesCS6B02G428600	696636476	696641207	+	Defense response
			TraesCS6B02G428500	696429438	696482513	-	regulation of plant-type hypersensitive response/response to stress.
			TraesCS6B02G428200	696344464	696345417	-	Response to stress.
			TraesCS6B02G428000	696148391	696152567	-	Defense response.
			TraesCS6B02G427100	695809523	695811508	-	Response to fungus, response to stress.
			TraesCS6B02G425300	694340532	.694342363	+	Innate immune response.
			TraesCS6B02G424000	693923562	693930572	+	immune response-regulating signaling pathway/ defense response to bacterium.
			TraesCS6B02G424200	693945124	693946318	+	response to stress.
qSTB.29	7A	116530515	TraesCS7A02G154900	10786890	107870802	+	Jasmonic acid mediated signaling pathway/ethylene-activated signaling pathway regulation of plant-type hypersensitive response/defense response to bacterium. Defense response to fungus /systemic acquired resistance,
			TraesCS7A02G160700	116774158	116775320	-	Response to fungus/ response to jasmonic acid, jasmonic acid biosynthetic process.
			TraesCS7A02G159400	115815406	115818288	-	Systemic acquired resistance.
			TraesCS7A02G159300	115803096	115807251	-	Defense response to fungus/ systemic acquired resistance/obsolete pathogen-associated molecular pattern dependent induction by symbiont of host innate immune response
			TraesCS7A02G159200	115502958	115504909	-	Defense response to fungus/systemic acquired resistance, salicylic acid mediated signaling pathway/jasmonic acid mediated signaling pathway/regulation of plant-type hypersensitive response
			TraesCS7A02G158800	114413214	114414446	-	Systemic acquired resistance
			TraesCS7A02G158000	112263526	112266127	+	Response to fungus, response to biotic stress.
			TraesCS7A02G156900	109825167	109826129	+	Defense response to fungus/regulation of plant-type hypersensitive response/ salicylic acid biosynthetic process/systemic acquired resistance/ Jasmonic acid mediated signaling pathway.
qSTB.30	7A	690377106-691722567	TraesCS7A02G502500	691521202	691522644	-	Defense response to bacterium.
			TraesCS7A02G501800	691522644	691290122	-	Defense response to bacterium/Cellular response to stress.
			TraesCS7A02G501100	690929758	690930261	-	Response to biotic stress.
			TraesCS7A02G501000	690917988	690918491	-	Response biotic stress
			TraesCS7A02G500100	690202537	690206037	-	Defense response/(R)-limonene synthase activity. response to jasmonic acid.

Appendix VIII Continued

qSTB.31	7B	686989852	TraesCS7A02G49960	689919674	689926140	-	Defense response.
			TraesCS7A02G498600	688993023	688999806	-	Defense response.
			TraesCS7A02G498200	688685229	688694372	-	Defense response to bacterium.
			TraesCS7A02G496900	686683155	686684065	+	SCF ubiquitin ligase complex.
			TraesCS7A02G496200	686155127	686162048	+	Response to fungus.
			TraesCS7A02G495400	685581322	685586908	+	Jasmonic acid biosynthetic process/ regulation of defense response.
			TraesCS7A02G492900	681785989	681791135	-	Defense response to fungus.
			TraesCS7B02G413800	681849784	681852313	+	Response to salicylic acid
			TraesCS7B02G412900	680540509	680541684	-	Response to biotic stress.
			TraesCS7B02G412400	680217732	680217971	-	Response to stress
			TraesCS7B02G411500	680066562	680071534	+	Positive regulation of programmed cell death
qSTB.32	7D	21667638-63471279	TraesCS7D02G041100	20573622	20578504	+	Defense response
			TraesCS7D02G040900	20547371	20549251	+	Defense response
			TraesCS7D02G040700	20442479	20463194	+	Defense response
			TraesCS7D02G039500	20057020	20069082	-	Defense response
			TraesCS7D02G038200	19404885	19410860	-	Systemic acquired resistance/regulation of plant-type hypersensitive response.
			TraesCS7D02G038100	19399535	19401331	+	Defense response.
			TraesCS7D02G037800	19207405	19209126	-	Methyl jasmonate methylesterase activity.
			TraesCS7D02G037700	19049652	19053785	+	Systemic acquired resistance.
			TraesCS7D02G037400	18926487	18927411	+	Immune response-regulating cell surface receptor signaling pathway/ defense response.
			TraesCS7D02G035900	18401249	18403992	-	Defense response.
			TraesCS7D02G035600	18244409	18246083	+	Defense response.
qSTB.33	7D	528900507-531439751	TraesCS7D02G411100	529956128	529956538	-	Immune response.
			TraesCS7D02G41070	529772570	529772953	+	Defense response.
			TraesCS7D02G410400	529681686	529682069	-	Defense response.
			TraesCS7D02G409800	528891729	528896252	+	Defense response
			TraesCS7D02G409600	528130780	528133540	+	Systemic acquired resistance, jasmonic acid mediated signaling pathway, salicylic acid biosynthetic process , and regulation of plant-type hypersensitive response and defense response to fungus.

[a] High confident genes annotated from IWGSC CS RefSeq ver. 1.0 IWGSC Annotation v2.1 available at https://wheat-urgi.versailles.inrae.fr/Seq-Repository/Annotations. Retrieved on May 24,2021.

Appendix **IX**. GWAS result for Marker-Trait Associations for selected agronomic traits.

The table reports SDS traits, Allele ID, Chromosome, physical position, significance value (p. value, FDR and Bonferroni *P*-value), additive effect, MAF= Minor allele frequency, SNP position, and "R2" value = proportion of phenotypic variation explained by marker.

Trait_Location_Year	AlleleID	Chromosome	Position	P.value	maf	FDR_Adjusted _P-values	Bonferroni P.value (-log(*p* value)	Add effect	R2 (%)	Agronomic Trait
HD_Holetta_2015	1145956|F|0-9:A>G-9:A>G	5A	5.81E+08	1.15E-07	0.039326	0.000897454	6.94	-1.97	23.06	Days to heading
HD_Holetta_2015	1006311|F|0-25:C>T-25:C>T	6A	5.74E+08	1.20E-06	0.339888	0.004658898	5.92	-0.79	23.01	Days to heading
HD_Holetta_2015	977335|F|0-19:C>A-19:C>A	7B	6.11E+08	2.62E-06	0.351124	0.006715652	5.58	0.8	23.04	Days to heading
HD_Holetta_2015	1204551|F|0-57:C>T-57:C>T	1A	5E+08	3.45E-06	0.370787	0.006715652	5.46	-0.7	24.07	Days to heading
HD_Holetta_2015	1000134|F|0-15:T>C-15:T>C	6B	1.53E+08	9.10E-06	0.418539	0.014147355	5.04	0.59	22.82	Days to heading
HD_Holetta_2015	7940358|F|0-58:A>T-58:A>T	5B	4.2E+08	1.46E-05	0.311798	0.018980106	4.83	0.65	22.86	Days to heading
HD_Kulumsa_2016	987806|F|0-16:A>G-16:A>G	5D	77015512	8.01E-07	0.179775	0.006232399	6.1	-1.28	8.54	Days to heading
HD_Kulumsa_2016	1251525|F|0-49:G>A-49:G>A	6A	4.74E+08	1.27E-05	0.030899	0.049549824	4.89	-2.88	8.97	Days to heading
HD_Kulumsa_2016	1163416|F|0-7:A>G-7:A>G	2B	7.49E+08	2.24E-05	0.351124	0.057980166	4.65	-0.91	8.77	Days to heading
FD_Holetta_2015	1145956|F|0-9:A>G-9:A>G	5A	5.81E+08	1.15E-07	0.039326	0.000897454	6.94	-1.97	23.06	Days to Flowering
FD_Holetta_2015	1006311|F|0-25:C>T-25:C>T	6A	5.74E+08	1.20E-06	0.339888	0.004658898	5.92	-0.79	23.01	Days to Flowering
FD_Holetta_2015	977335|F|0-19:C>A-19:C>A	7B	6.11E+08	2.62E-06	0.351124	0.006715652	5.58	0.8	23.04	Days to Flowering
FD_Holetta_2015	1204551|F|0-57:C>T-57:C>T	1A	5E+08	3.45E-06	0.370787	0.006715652	5.46	-0.7	24.07	Days to Flowering
FD_Holetta_2015	1000134|F|0-15:T>C-15:T>C	6B	1.53E+08	9.10E-06	0.418539	0.014147355	5.04	0.59	22.82	Days to Flowering
FD_Holetta_2015	7940358|F|0-58:A>T-58:A>T	5B	4.2E+08	1.46E-05	0.311798	0.018980106	4.83	0.65	22.86	Days to Flowering
MD_Bekoji_2016	7332831|F|0-9:T>C-9:T>C	1A	2.2E+08	3.72E-06	0.044944	0.022994893	5.43	-2.39	15.86	Days to Maturity
MD_Bekoji_2016	4537441|F|0-8:A>G-8:A>G	0	0	7.96E-06	0.047753	0.022994893	5.1	-2.17	14.95	Days to Maturity
MD_Bekoji_2016	5324623|F|0-26:C>T-26:C>T	6D	1372781	8.87E-06	0.078652	0.022994893	5.05	-1.7	14.82	Days to Maturity
MD_Bekoji_2016	3026844|F|0-5:C>G-5:C>G	1A	2.22E+08	1.26E-05	0.036517	0.024561638	4.9	-2.46	14.39	Days to Maturity
MD_Bekoji_2016	1020044|F|0-10:C>T-10:C>T	6D	1414317	1.95E-05	0.101124	0.027930729	4.71	-1.47	13.88	Days to Maturity
MD_Bekoji_2016	1062638|F|0-63:G>A-63:G>A	1A	5.12E+08	2.16E-05	0.042135	0.027930729	4.67	-2.13	13.76	Days to Maturity

Appendix **IX** Continued

MD_Bekoji_2016	3936661|F|0-23:T>C-23:T>C	0	0	4.52E-05	0.047753	0.04996193	4.34	2.57	14.76	Days to Maturity
MD_Bekoji_2016	3954380|F|0-34:T>C-34:T>C	3A	7.38E+08	5.14E-05	0.098315	0.04996193	4.29	-1.76	12.74	Days to Maturity
MD_Bekoji_2016	5971568|F|0-8:C>A-8:C>A	0	0	6.85E-05	0.036517	0.051346445	4.16	2.34	13.74	Days to Maturity
MD_Bekoji_2016	1094246|F|0-48:A>G-48:A>G	1A	3.38E+08	7.27E-05	0.05618	0.051346445	4.14	-1.79	12.34	Days to Maturity
MD_Bekoji_2016	1218776|F|0-51:C>T-51:C>T	7A	1.76E+08	8.30E-05	0.05618	0.051346445	4.08	-1.96	12.19	Days to Maturity
MD_Bekoji_2016	1077671|F|0-25:C>T-25:C>T	3B	8.11E+08	8.50E-05	0.036517	0.051346445	4.07	-2.76	12.16	Days to Maturity
MD_Bekoji_2016	1003602|F|0-66:T>C-66:T>C	5B	6.38E+08	8.58E-05	0.042135	0.051346445	4.07	-1.96	12.15	Days to Maturity
MD_Bekoji_2016	1092581|F|0-53:C>T-53:C>T	7A	1.71E+08	0.000113	0.036517	0.062855357	3.95	-2.1	11.83	Days to Maturity
MD_Bekoji_2016	2278215|F|0-18:A>G-18:A>G	2A	6.91E+08	0.000142	0.087079	0.073419553	3.85	-1.41	11.57	Days to Maturity
GFD_Holetta_2016	1126869|F|0-21:A>G-21:A>G	1B	4.34E+08	8.20E-12	0.044944	6.38E-08	11.09	-5.14	4.38	Grain filling duration
GFD_Holetta_2016	1012066|F|0-21:C>A-21:C>A	7D	42519818	7.55E-11	0.073034	2.94E-07	10.12	-3.43	4.69	Grain filling duration
GFD_Holetta_2016	2262270|F|0-28:C>G-28:C>G	3B	59645976	2.17E-10	0.115169	5.63E-07	9.66	3.33	4.41	Grain filling duration
GFD_Holetta_2016	2279882|F|0-54:G>A-54:G>A	0	60288624	4.24E-08	0.070225	8.25E-05	7.37	-3.19	3.99	Grain filling duration
GFD_Holetta_2016	2257595|F|0-52:T>C-52:T>C	6D	9054221	1.09E-07	0.033708	0.000168785	6.96	3.58	4.11	Grain filling duration
GFD_Holetta_2016	987584|F|0-22:C>T-22:C>T	2B	7648615	1.84E-07	0.042135	0.000234186	6.74	-3.32	4.01	Grain filling duration
GFD_Holetta_2016	1087828|F|0-8:G>C-8:G>C	3B	5.53E+08	2.50E-07	0.050562	0.000234186	6.6	3.58	4.54	Grain filling duration
GFD_Holetta_2016	1059250|F|0-13:A>G-13:A>G	3B	7.64E+08	2.66E-07	0.087079	0.000234186	6.58	2.51	4.32	Grain filling duration
GFD_Holetta_2016	1073870|F|0-52:G>T-52:G>T	3A	2.51E+08	2.71E-07	0.117978	0.000234186	6.57	2.46	7.89	Grain filling duration
GFD_Holetta_2016	1107243|F|0-40:C>T-40:C>T	1B	38225318	1.10E-06	0.050562	0.000857489	5.96	3.25	4.01	Grain filling duration
GFD_Holetta_2016	1186401|F|0-34:C>G-34:C>G	2B	1.47E+08	1.40E-06	0.398876	0.000990316	5.85	1.53	3.94	Grain filling duration
GFD_Kulumsa_2016	5583042|F|0-5:T>C-5:T>C	0	0	1.85E-07	0.070225	0.001435183	6.73	3.3	1.12	Grain filling duration
GFD_Kulumsa_2016	2255872|F|0-45:T>G-45:T>G	5B	28386599	3.83E-06	0.073034	0.011444294	5.42	2.99	1.08	Grain filling duration
GFD_Kulumsa_2016	1079882|F|0-51:C>T-51:C>T	2B	1.83E+08	4.42E-06	0.182584	0.011444294	5.36	-1.72	1.44	Grain filling duration
GFD_Kulumsa_2016	5325155|F|0-26:G>T-26:G>T	3B	4.63E+08	2.04E-05	0.044944	0.039652851	4.69	3.84	0.49	Grain filling duration
PH_Bekoji_2015	2253061|F|0-65:C>G-65:C>G	7B	7.06E+08	4.92E-07	0.042135	0.003823697	6.31	-4.47	8.88	Plant height
PH_Bekoji_2015	1051311|F|0-7:T>C-7:T>C	5A	5.3E+08	7.58E-06	0.044944	0.029458432	5.12	-3.36	8.69	Plant height
PH_Bekoji_2015	3222186|F|0-13:A>G-13:A>G	7A	5.14E+08	1.54E-05	0.300562	0.038631495	4.81	1.56	9	Plant height

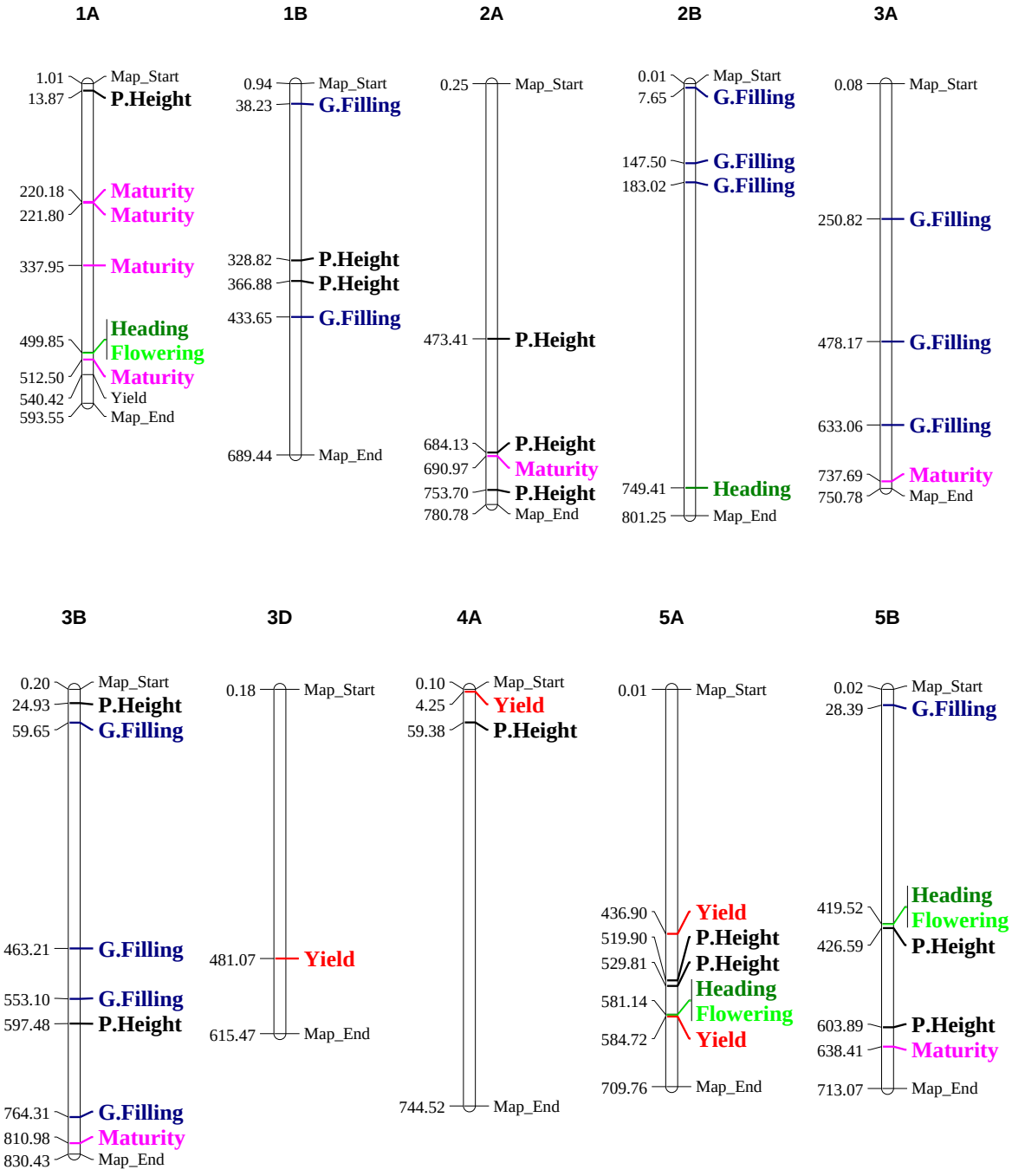
Appendix **IX** Continued

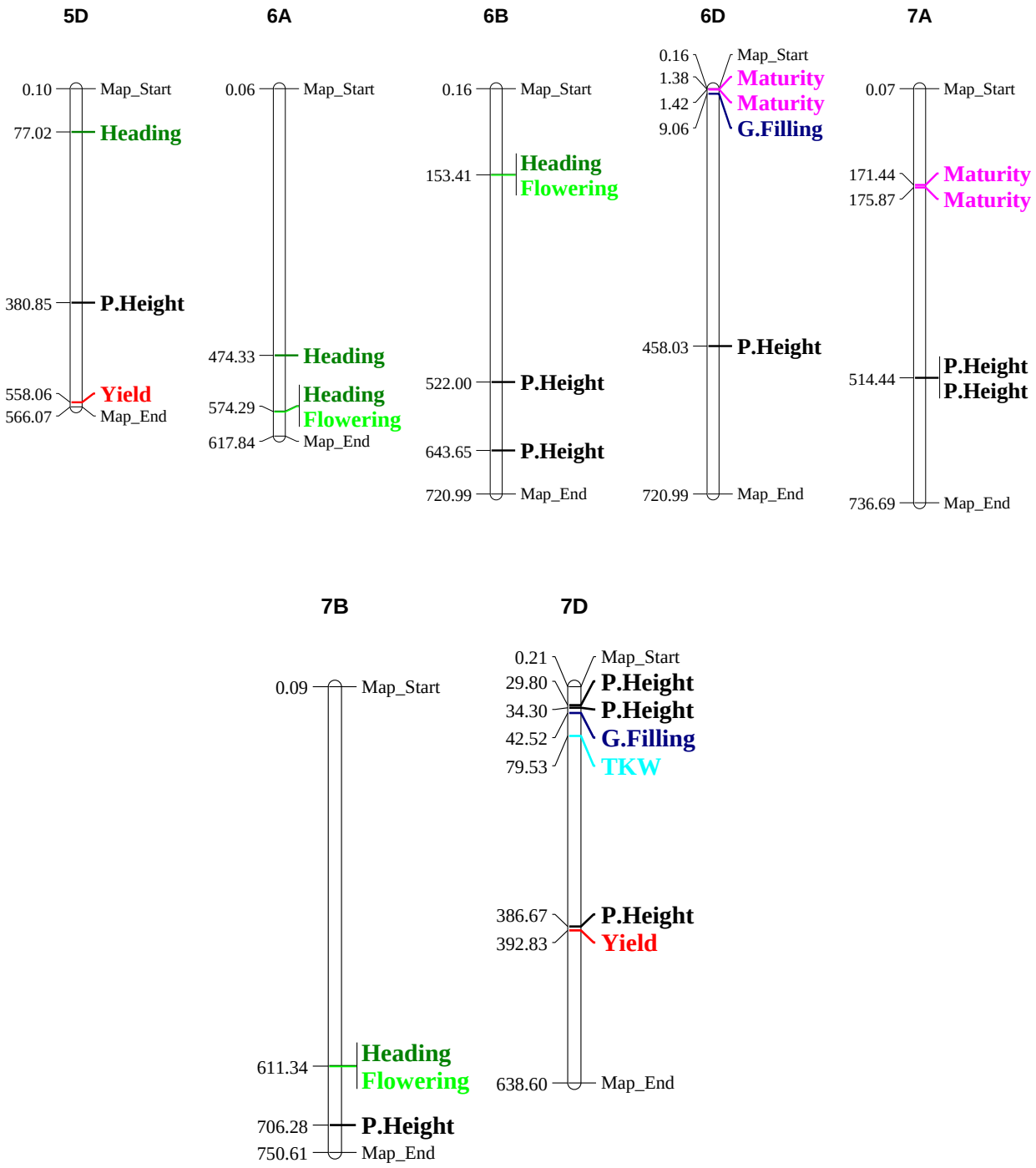
PH_Bekoji_2015	1100955|F|0-33:C>T-33:C>T	1A	13860047	1.99E-05	0.41573	0.038631495	4.7	1.17	10.24	Plant height
PH_Combined	3222186|F|0-13:A>G-13:A>G	7A	5.14E+08	1.02E-06	0.300562	0.007929671	5.99	0.9	3.29	Plant height
PH_Kulumsa_2015	2257474|F|0-58:C>T-58:C>T	1B	3.29E+08	1.17E-13	0.064607	9.13E-10	12.93	-5.53	10.64	Plant height
PH_Kulumsa_2015	2259716|F|0-19:G>A-19:G>A	4A	59371930	4.02E-09	0.182584	1.56E-05	8.4	-1.48	10.05	Plant height
PH_Kulumsa_2015	1062366|F|0-9:C>T-9:C>T	1B	3.67E+08	2.56E-07	0.132022	0.000553148	6.59	2.51	10.52	Plant height
PH_Kulumsa_2015	1050317|F|0-58:C>T-58:C>T	7D	3.87E+08	2.85E-07	0.070225	0.000553148	6.55	2.95	9.69	Plant height
PH_Kulumsa_2015	1081594|F|0-26:C>A-26:C>A	2A	7.54E+08	6.87E-07	0.081461	0.001068837	6.16	-2.46	9.95	Plant height
PH_Kulumsa_2015	2276919|F|0-10:G>T-10:G>T	6B	5.22E+08	1.08E-06	0.474719	0.001402064	5.97	1.13	12.32	Plant height
PH_Kulumsa_2015	2260283|F|0-23:A>G-23:A>G	5D	3.81E+08	4.38E-06	0.196629	0.004860753	5.36	-1.72	10.74	Plant height
PH_Kulumsa_2015	1204683|F|0-30:T>C-30:T>C	7D	34297014	1.26E-05	0.047753	0.012246934	4.9	-2.22	9.81	Plant height
PH_Kulumsa_2015	3064914|F|0-34:A>G-34:A>G	5B	6.04E+08	2.47E-05	0.075843	0.021297653	4.61	1.49	9.7	Plant height
PH_Kulumsa_2015	1079734|F|0-46:G>C-46:G>C	2A	6.84E+08	6.37E-05	0.311798	0.049556023	4.2	-1.15	9.99	Plant height
PH_Kulumsa_2016	1068423|F|0-44:C>T-44:C>T	5B	4.27E+08	2.66E-07	0.174157	0.001028789	6.57	-2.67	9.86	Plant height
PH_Kulumsa_2016	4993093|F|0-35:A>G-35:A>G	2A	4.73E+08	3.04E-07	0.179775	0.001028789	6.52	-2.03	9.77	Plant height
PH_Kulumsa_2016	991424|F|0-8:A>C-8:A>C	6B	6.44E+08	3.97E-07	0.390449	0.001028789	6.4	-1.62	11.36	Plant height
PH_Kulumsa_2016	1862541|F|0-21:G>C-21:G>C	5A	5.2E+08	9.15E-07	0.370787	0.001779553	6.04	-1.85	11.54	Plant height
PH_Kulumsa_2016	1234768|F|0-5:T>C-5:T>C	3B	24929087	1.49E-06	0.092697	0.002318835	5.83	2.99	9.9	Plant height
PH_Kulumsa_2016	1125248|F|0-66:C>T-66:C>T	3B	5.97E+08	4.01E-06	0.297753	0.00519245	5.4	1.54	9.73	Plant height
PH_Kulumsa_2016	1221660|F|0-37:A>G-37:A>G	0	82177106	5.53E-06	0.109551	0.006143434	5.26	2.16	9.7	Plant height
PH_Kulumsa_2016	9721834|F|0-58:G>T-58:G>T	7D	29796264	2.26E-05	0.365169	0.021956125	4.65	1.41	9.97	Plant height
PH_Kulumsa_2016	1101947|F|0-8:A>G-8:A>G	6D	4.58E+08	4.12E-05	0.075843	0.035616115	4.38	2.84	11.87	Plant height
PH_Kulumsa_2016	1165350|F|0-26:G>C-26:G>C	0	0	6.40E-05	0.16573	0.04973888	4.19	1.39	9.7	Plant height
Yield_Kulumsa_2015	3064595|F|0-11:G>A-11:G>A	4A	4240767	1.14E-07	0.466292	0.000888987	6.94	168	1.83	Grain yield
Yield_Kulumsa_2015	987784|F|0-55:T>G-55:T>G	7D	3.93E+08	6.34E-07	0.449438	0.002466692	6.2	171	0.57	Grain yield
Yield_Kulumsa_2015	1711331|F|0-12:A>T-12:A>T	3A	4.78E+08	1.15E-06	0.300562	0.002971043	5.94	178	0.53	Grain yield
Yield_Kulumsa_2015	1112465|F|0-25:G>T-25:G>T	0	0	1.98E-06	0.123596	0.003843107	5.7	245	1.21	Grain yield
Yield_Kulumsa_2015	1135154|F|0-9:G>A-9:G>A	5A	5.85E+08	5.59E-06	0.064607	0.008275483	5.25	-313	3.34	Grain yield

Appendix **IX** Continued

Yield_Kulumsa_2015	996906|F|0-56:G>A-56:G>A	3A	6.33E+08	6.39E-06	0.294944	0.008275483	5.19	161	0.53	Grain yield
Yield_Kulumsa_2015	1166094|F|0-38:G>A-38:G>A	1A	5.4E+08	9.33E-06	0.33427	0.01036203	5.03	-128	0.79	Grain yield
Yield_Kulumsa_2015	1703104|F|0-27:T>C-27:T>C	5A	4.37E+08	1.65E-05	0.342697	0.016028919	4.78	144	4.3	Grain yield
Yield_Kulumsa_2015	1045011|F|0-60:T>A-60:T>A	3D	4.81E+08	3.51E-05	0.073034	0.030324073	4.45	207	0.52	Grain yield
Yield_Kulumsa_2015	1074542|F|0-12:G>C-12:G>C	5D	5.58E+08	6.01E-05	0.05618	0.046728927	4.22	253	0.86	Grain yield
TKW_Kulumsa_2016	4262368|F|0-28:A>G-28:A>G	7D	79520808	4.00E-06	0.053371	0.0310725	5.4	3.03	0	Thousad Kernels Weight

Appendix X. Genomic positions (Mbp) of identified loci underlying yield, yield components and phenological traits in wheat.




5D
0.10
Map_Start
77.02
Heading
380.85
P.Height
558.06
Yield
566.07
Map_End
6A
0.06
Map_Start
474.33
Heading
574.29
Heading
Flowering
617.84
Map_End
6B
0.16
Map_Start
153.41
Heading
Flowering
522.00
P.Height
643.65
P.Height
720.99
Map_End
6D
0.16
Map_Start
1.38
Maturity
1.42
Maturity
9.06
G.Filling
458.03
P.Height
720.99
Map_End
7A
0.07
Map_Start
171.44
Maturity
175.87
Maturity
514.44
P.Height
P.Height
736.69
Map_End
7B
0.09
Map_Start
611.34
Heading
Flowering
706.28
P.Height
750.61
Map_End
7D
0.21
Map_Start
29.80
P.Height
34.30
P.Height
G.Filling
42.52
TKW
79.53
386.67
P.Height
392.83
Yield
638.60
Map_End