QTL MAPPING OF STEM RUST RESISTANCE LOCI IN DURUM WHEAT POPULATIONS

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ABSTRACT

Stem rust caused by *Puccinia graminis* f. sp. *tritici* Eriks. & Henn is the most destructive disease of durum and common wheat. The main focus of this study is to identify loci associated with stem rust resistance in durum wheat using association mapping and linkage mapping. A panel of 283 lines and 224 recombinant inbred lines (RILs) from a cross between 'Reichenbachii' and 'DAKIYE' developed by the durum wheat breeding program of the International Maize and Wheat Improvement Center (CIMMYT) were used for the study. The panel was evaluated against races TTKSK, TKTTF, JRCQC and TTRTF at the seedling stage and TKTTF and JRCQC in the field in Ethiopia from 2018 to 2019 for two seasons. The same panel was evaluated against bulk of multiple stem rust races prevalent in Ethiopia and Kenya from 2018 to 2019 in five environments. Genome-wide association study (GWAS) was conducted using 26,439 single nucleotide polymorphism (SNP) markers for seedling response (280 lines) and field response (283 lines) to stem rust. The RILs along with the two parents were evaluated for response to bulk of multiple stem rust races in Ethiopia and Kenya for two seasons from 2019 to 2020. Linkage analyses were conducted using 843 SNP markers for 175 lines. For GWAS of seedling response, a mixed linear model (MLM) identified 17 quantitative trait loci (QTL) of which eight were putatively novel while FarmCPU identified 20 QTL and 12 were likely novel. For field resistance to races

TKTTF and JRCQC, MLM detected 19 QTL of which 12 were likely novel while FarmCPU detected 16 QTL and seven were putatively novel. For resistance to multiple *Pgt* races in East Africa, 160 significant marker-trait associations (MTAs) grouped into 42 QTL were identified using MLM and FarmCPU and 21 QTL were likely novel. From previously reported *Sr* genes, the regions of *Sr7a*, *Sr8a*, *Sr8155B1, Sr11, Sr12,* alleles of *Sr13, Sr17, Sr22/Sr25,* and *Sr49* were identified. For the biparental population, composite interval mapping (CIM) identified three QTL on chromosomes 3B (*QSr.cnl-3B)*, 4B (*QSr.cnl-4B*) and 7B (*QSr.cnl-7B*). These three QTL contributed by the resistant parent explained 4.7% to 15.3% of the phenotypic variation and all match previously reported loci. Lines with multiple-race stem rust resistance can be used as parents in durum wheat resistance breeding to stem rust and markers identified in the GWAS can be used in marker-assisted selection (MAS) once validated in a different population. Further study on the validation of allele specific markers and allelism tests in the *Sr13* region of chromosome 6A is needed. Future evaluation of large numbers of durum wheat lines and searching for durable adult plant resistance gene is crucial in resistance breeding of durum wheat.

BIOGRAPHICAL SKETCH

Shitaye Homma Megerssa was born and grew up in Sebeta town, 25 km from the capital of Ethiopia, Addis Ababa. Shitaye received a diploma in General Agriculture from Jimma University, the former Jimma College of Agriculture. After graduation Shitaye was employed by the Ethiopian Institute of Agricultural Research (EIAR), Debre Zeit Agricultural research Center (DZARC) as a technical assistant. Shitaye then enrolled in Haramaya University, then former Alemaya University, where she earned a BSc degree with distinction. She was re-employed by EIAR as a highland pulse breeder. After two years she joined Wageningen University, The Netherlands, for her MSc study and received an MPS degree in plant science with a specialization in greenhouse horticulture. After working for some time, she travelled to Sweden to join family and returned to Ethiopia in 2013 and joined EIAR, DZARC as a wheat breeder. Then she joined Cornell University for her PhD study in 2017 as a student of Prof. Mark E. Sorrells. Shitaye was supported by the DGGW project funded by the UK Aid from the British People and the Bill & Melinda Gates Foundation. Her project was focused on mapping of QTL for stem rust resistance in Durum Wheat.

DEDICATION

I am dedicating this thesis to three beloved people. My parents, Tezeru Teferi and Homma Megerssa who supported and encouraged me since my childhood, their memory is always in my heart although they are no longer in this world. “Aba” and “Gashe”, I love you and miss you a lot, May God rest you in heaven. Next, my beloved husband Hailemariam Teklewold, who is a model father in handling the responsibility at home and working hard on his research by ignoring the cultural barrier about women education in my country. Hailu, I lack words to express my love and respect to you.

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LIST OF ABBREVIATIONS

APR: Adult plant resistance

BLUPs: Best linear unbiased predictions

BGRI: Borlaug Global Rust Initiative

BSL3: Biosafety level-3

CI: Coefficient of infection

CIM: Composite interval mapping

CIMMYT: International Maize and Wheat Improvement Center

CMLM: Compressed Mixed Linear Model

CSA: Central statistical authority

CTAB: Cetyl trimethylammonium bromide

DArT: Diversity arrays technology

FarmCPU: Fixed and random model Circulating Probability Unification

FDR: False Discovery Rate

GBS: Genotyping-by-sequencing

GAPIT: Genomic Association and Prediction Integrated Tool

GID: Genotype identification

GWAS: Genome-wide association analysis

IT: Infection type

LD: Linkage Disequilibrium

LMM: Linear mixed model

LOD: Logarithm of odds

LOESS: Locally estimated scatterplot smoothing

MAF: Minor Allele Frequency

MAMPS: Microbial-associated molecular patterns

MAS: Marker-assisted selection

MLMM: Multi-locus Mixed Linear Model

MTA(s): Marker trait association(s)

NB-LRR: Nucleotide binding leucine rich repeats

PAMPS: Pathogen-associated molecular patterns

PBC: Pseudo-black chaff

PC(A): Principal component (analysis)

PRRs: Pattern-recognition receptors

PTI: PAMP-triggered immunity

QTL: Quantitative trait locus/loci

Q-Q: Quantile-quantile

RIL(s): recombinant inbred line(s)

SNP: Single nucleotide polymorphism

SSRs: Simple sequence repeats

TASSEL: Trait Analysis by aSSociation, Evolution and Linkage

CHAPTER 1.

GENERAL INTRODUCTION

Wheat is the most widely grown cereal and among the most important global food security crops. It provides about 21% of the total calories and 20% of the protein demand to more than 4.5 billion people in several developing countries (Singh et al., 2011; Shiferaw et al., 2013). Wheat covers about 240 Mha of area and an estimated annual production of about 750 Mt in the world (Bhavani et al., 2019). In the Sub-Saharan Africa, Ethiopia is the second largest producer of wheat following South Africa (FAO, 2016). In Ethiopia, wheat covered 1.7 million hectares of land in the 2017 cropping season and a production of 4.64 million metric tons was reported in the season from the two common species (CSA , 2017).

Wheat has different ploidy levels. The commonly cultivated species are the hexaploid wheat species (*Triticum aestivum* L., 2n=6x=48; AABBDD genome) known as common wheat; and the tetraploid wheat (*Triticum turgidum* L., 2n=4x=28; AABB genome) (Shewry and Hey, 2015). Durum wheat (*Triticum turgidum* L., ssp. Durum (Desf.) Husnot) is a tetraploid wheat species used for the processing of pasta and other traditional food recipes (Laidò et al., 2014; Shewry and Hey, 2015; Kabbaj et al., 2017). Durum wheat is cultivated in the highlands of Ethiopia and bread/common wheat occupies the largest area. Durum wheat occupies only 40% of the total area covered by wheat but the area is expected to increase due to emerging food industries in the country and urbanization driven demand for pasta (Letta et al., 2014; Hailu et al., 2015).

The demand for wheat in the developing world is projected to increase by 60% in 2050 due to the rapidly growing world population (Singh et al., 2011). However, several biotic and abiotic factors are expected to reduce wheat production and worsen the challenge of feeding the growing population. Among the biotic factors the emergence of new virulent pathogen races such as the rusts threaten the global wheat production. The three rust species, *Puccinia graminis* f.sp. *tritici* Eriks. & E. Henn. (stem rust), *Puccinia triticina* Eriks (leaf rust) and *Puccinia striiformis* f.sp. *tritici* Eriks. & E. Henn. (yellow/stripe rust) are among the most economically important fungal diseases of wheat which can cause significant yield losses globally (Hodson, 2011; Aktar-Uz-Zaman et al., 2017).

Among the three rusts, the current study focuses on stem rust of wheat. Stem rust is the most damaging fungal disease of both common and durum wheat (Roelfs et al., 1992). Stem rust can occur in all areas where wheat is produced and the environment is conducive for disease development (Singh et al., 2008; Olivera et al., 2015). The stem rust fungus is heteroecious, i.e., it needs two hosts to complete its life cycle and it has a complex life cycle with all five fungal spores. The spores of stem rust have the ability to disperse long distance through wind flow and cause epidemics in neighboring regions (Olivera et al., 2015). The stem rust fungus has a short generation interval that can form a large population size favoring mutation and evolution of new races to attack the wheat crop (Kolmer et al., 2015). It can cause complete yield loss under wide epidemics when susceptible varieties are grown (Dean et al., 2012). The fungus draws nutrients from the vascular system of the wheat plant resulting in the harvest of shriveled seed which downgrades kernel quality and end use

product quality (Leonard and Szabo, 2005; Laidò et al., 2015). Moreover, a stem rust-infected wheat crop can easily lodge due to damaged stems, caused by the pathogen, that makes mechanical harvest difficult (Schumann and Leonard 2000; Leonard and Szabo, 2005). In some regions of the world, the narrow genetic base of stem rust resistance favors the extensive production of cultivars with single resistance genes and exposes the crop to severe damage by an epidemic under environmental conditions suitable for stem rust development (Newcomb et al., 2013; Olivera et al., 2015; Nirmala et al., 2016). New virulent races including the Ug99 group, 'Digalu'(TKTTF); the virulent races identified on durum in Ethiopia, race JRCQC; a race identified in Italy and Georgia, TTRTF; and other races threatening wheat production and food security due to their broad virulence to several resistance genes deployed in commercial wheat cultivars and breeding lines across the world (Olivera et al., 2012a; Singh et al., 2015).

The commonly applied management options to control stem rust are spraying fungicide and genetic resistance. Applying the former as a management option is sometimes costly, it can be environmentally unsafe if applied improperly and the fungicide supply could be unsustainable (Edae and Rouse, 2020). Furthermore, pathogens may develop fungicide resistance during long term application of narrow-spectrum fungicides (Ellis et al., 2014; Aktar-Uz-Zaman et al., 2017). However, under conditions of no available genetic resistance, fungicide application is the only alternative to control stem rust (Dangl et al., 2013; Oliver, 2014).

In the presence of genetic variability, genetic resistance is an environmentally friendly and economically feasible method to mitigate the damage caused by stem rust

on wheat (Schumann and Leonard 2000; Singh et al., 2013). More than 60 stem rust resistance genes are cataloged and about 34 of them are in the A and B sub-genomes. However, most of these genes are major gene resistances (R-genes) and are effective against specific races. Many of the effective major gene resistances that originated from alien species and landraces are associated with undesirable effects on agronomic traits (McIntosh et al.,1995, 2017) which needs extra effort to break the linkage drag that could be introduced to breeding lines. Nevertheless, the genetic characterization and identification of available sources of resistance in the germplasm pool is a continuous process to manage the threat posed by constantly emerging stem rust races. The general objective of the current study was therefore to evaluate a durum wheat panel and recombinant inbred line (RIL) population developed by the CIMMYT durum wheat breeding program against multiple virulent races of stem rust at the seedling and adult plant stages and map genomic regions associated with seedling and adult plant resistances through association mapping and linkage mapping. The specific objectives were:

- to evaluate seedlings of a durum wheat panel against four *Pgt* races (TTKSK, JRCQC, TKTTF and TTRTF) and conduct GWAS analysis to identify genomic regions associated with seedling resistance.
- to evaluate adult plants of a durum wheat panel against two single races of *Pgt* (JRCQC and TKTTF) and conduct GWAS analysis to identify genomic regions associated with field resistance.
- to evaluate adult plants of a durum wheat panel against multiple races of stem rust across multiple seasons in East Africa (Ethiopia and Kenya) and conduct

GWAS analysis to identify genomic regions associated with field resistances to East African *Pgt* races.

- to evaluate adult plants of durum wheat RIL populations against multiple *Pgt* races in East Africa and identify genomic regions associated with field resistance.

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CHAPTER 2.

LITERATURE REVIEW

The domestication of durum wheat

Durum wheat is among the tetraploid wheat species and the domestication of tetraploid wheat dates back to about 12, 000 years in the Fertile Crescent. Durum wheat has passed through two domestication incidents. The first was the period ancient farmers in the Fertile Crescent selected non-shattering cultivated emmer wheat (*Triticum turgidum* ssp. *dicoccum* (Scharank ex Schübl.) Thell.) from wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) (Gioia et al., 2015) and the next was the time durum wheat has been selected from cultivated emmer wheat for easy threshability about 6,500 to 7,500 years ago. In the process of domestication, durum wheat has been selected for improved agronomic features such as loss of spike shattering, easy threshability, larger seed size, reduced number of tillers, erect growth habit and reduced dormancy (Dubcovsky and Dvorak, 2010; Gioia et al., 2015). After the domestication, continued evolution driven by natural and artificial selection resulted in the development of landraces that are adapted to specific regions and considered as the source of diversity for several agronomic traits (Nazco et al., 2012).

Ethiopia is one of the centers of diversity for tetraploid wheat (Vavilov,1951) and durum wheat has been cultivated in the highlands of Ethiopia since ancient times (Dejene and Mario, 2016). Currently, the national gene bank of Ethiopia has reserved over 7,000 accessions of durum wheat landraces. However, these landraces were not well characterized for traits of agronomic importance and they need additional effort to limit the expression of undesirable agronomic features. As a result, they are

underutilized for breeding purposes (Dejene and Mario, 2016). Reports indicated that the first durum wheat breeding program was launched in Italy through pure line selection from landraces around the early 1900s and a cultivar was released from hybridization around 1915 (Laidò et al., 2014; Kabbaj et al., 2017). Gradually, modern cultivars replaced the landraces as a consequence of the Green Revolution in the early 1970s (Ortiz et al., 2007).

Importance of durum wheat

Durum wheat has been cultivated as an important crop since around 1,500 to 2,000 years ago. The migration of humans and expansion of agriculture from the Fertile Crescent across Europe and Asia have been reported as the main drivers for the production of this crop (Maccaferri et al., 2019). However, the recent global area share of durum wheat is only 5% of the total wheat production area (Ranieri, 2015; Taylor D and Koo, 2015). The major producers of durum wheat in the world are Canada, the Mediterranean basin (Algeria, Italy, Morocco, Tunisia, Turkey, Spain, Portugal and Greece), the North American plains, Mexico and Australia (Loladze et al., 2014 Ranieri, 2015; Bond and Liefert, 2017; Kthiri et al., 2018). About 75% of the world durum wheat is produced in the Mediterranean basin due to the broad adaptation to the semiarid climates (Cakmak et al., 2010; Letta et al., 2013). The North African countries (Algeria, Morocco, Tunisia, and Libya) are the major importers of durum wheat as this crop constitutes the traditional recipes mainly consumed in these countries (Taylor and Koo, 2015).

Durum wheat is used for the processing of different food recipes. It is mainly used for the processing of pasta consumed in different parts of the world and,

traditional recipes largely consumed in the Mediterranean countries including couscous, bulgur, frike, and unleavened bread (Kabbaj et al., 2017; Soriano et al., 2018). For the production of quality pasta and other end use products from durum wheat, both grain yield and quality are important (Montesinos-López et al., 2019). These traits can be negatively affected by several factors (biotic and abiotic) and stem rust is among the biotic factors that has caused significant damage to wheat production across the world.

Stem rust biology and taxonomic classification

The causal agent of stem rust, *Puccinia graminis*, infects wheat, barley, oat, rye and several perennial grasses. Stem rust has been identified since 1300 B. C. in Israel (Schumann and Leonard 2000; Leonard and Szabo, 2005). The fungus *Puccinia graminis* taxonomically belongs to the phylum Basidiomycota, class Urediniomycetes, order Uredinales and family Pucciniaceae. The family Pucciniaceae has been further classified in to 17 genera and about 4,121 species with the majority being a member of the genus *Puccinia*. The species *Puccinia graminis* has been subdivided into forma specialis (f. sp.) based on the host species specificity which further subdivided into races based on resistance genotype specificity within a host species (Leonard and Szabo, 2005).

Puccinia graminis is an obligate biotroph, i.e., it needs a living host tissue for nutrient acquisition and growth (Duplessis et al., 2011; Schumann and Leonard 2000; Singh et al., 2006). It is a heteroecious fungus, i.e., it needs two different hosts to complete its life cycle. The host species that belong to the Berberidaceae are known as aecial hosts (alternate hosts for sexual cycle) and the species in the Poaceae family are

known as uredinial and telial hosts (main hosts for asexual cycle) (Abbasi et al., 2005). The wheat stem rust (*Puccinia graminis* f.sp. *tritici*) has 28 species as its natural host (Leonard and Szabo, 2005). For forma specialis *tritici,* wheat and common barberry (*Berberis vulgaris* L.) are the main and alternate host to complete the lifecycle of this pathogen*,* respectively (Schumann and Leonard 2000; Leonard and Szabo, 2005; Jin, 2011).

Life cycle of the stem rust

The life cycle of the stem rust fungus has both sexual and asexual spores. In the presence of the main and alternate host, *Puccinia graminis* produces all five fungal spore stages in its life cycle (Fig. 2.1) (Schumann and Leonard 2000). Dormant spores called teliospores will be produced on the straw close to the maturity of the main host to escape an environment without nutrient supply. Teliospores are the only spore types that can survive in the absence of a living host for a limited time in the field (Schumann and Leonard 2000). In spring, teliospores begin to germinate and develop a structure called a basidium where sexual spores called basidiospores are produced. Basidiospores carried by wind flow can infect nearby alternate host, common barberry where the sexual cycle is taking place. *Mahonia repens* (Lindl.) G. Don, *Mahonia aquifolium* (Pursh) Nutt and over 70 species of *Berberis* were reported as other alternate hosts of *Puccinia graminis*; however, reports indicated that spores identified from these species may not infect wheat (Abbasi et al., 2005; Jin, 2011). Then pycniospores form inside the pycnium. The pycniospores have two coupling types that serve as female and male gametes. These mating types undergo nuclear division and pairing that produce aecium. Inside the aecium, sexual spores called aeciospores that

infect small grains and other grass hosts are produced. Asexual spores called urediniospores are responsible for plant to plant spread of the pathogen. Then the urediniospores turn to teliospores (dormant spores) and the cycle resumes (Leonard and Szabo, 2005).

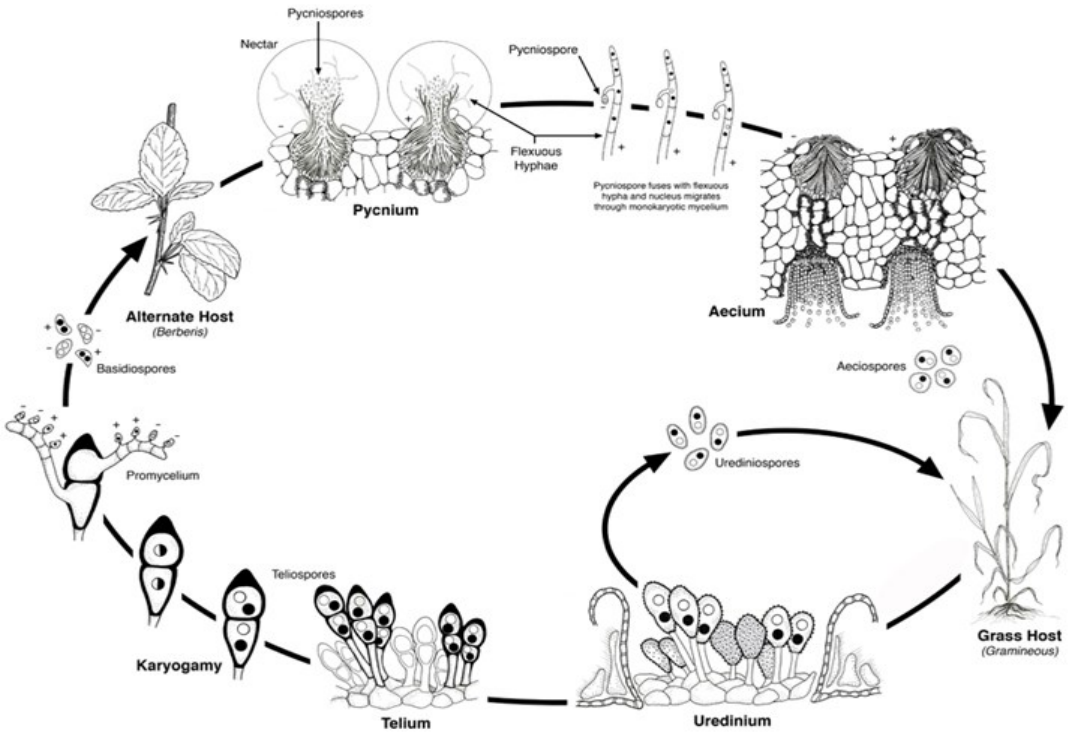


Figure 2.1. Life cycle of *Puccinia graminis*

(from https://www.ars.usda.gov ; accessed on September 24, 2020)

Conditions favoring stem rust in wheat and sources of inoculum

Infection of stem rust occurs through the stomata of the host. Post-infection, symptoms can develop within one to two weeks if the environment is suitable for the development of the pathogen. Infection and germination of spores can be favored by temperature ranging from 25-30 ^{o}C (77-86 ^{o}F) during the day and 15-20 ^{o}C (59-68 ^{o}F) at night, and moisture on the surface of leaves or stems. After infection, masses of

hyphae will develop under the host epidermis and produce urediniospores that spread the disease from plant to plant. Stem rust symptoms can be observed on leaf sheaths and on stems that can rupture the epidermis. Occasionally, symptoms can also be observed on leaf blades and glumes (Schumann and Leonard 2000; Leonard and Szabo, 2005).

The primary source of inoculum for stem rust varies for different climatic regions. In the tropical regions where the climate is warmer, urediniospores on volunteer wheat plants near wheat fields or spores that survived due to a green bridge provided by year-round cultivation of wheat are the primary sources of inoculum (Harder et al., 1972; Schumann and Leonard 2000). In the absence of an alternate host, urediniospores are the sole infecting spores of the main host. However, urediniospores are incapable of surviving harsh environmental conditions. In temperate regions where both winter and spring wheat are produced, stem rust can be severe on both. The winter wheat is known to perform better than the spring wheat because severe winters are unfavorable for the survival of the pathogen and the crop is already established in the spring when the weather is conducive to growth. In the presence of an alternate host near the surrounding, the primary source of inoculum can be aeciospores, or it can be wind-blown urediniospores from neighboring regions (Schumann and Leonard 2000).

The main source of genetic variation in the pathogen population differs in the presence and absence of the alternate host. In the presence of the alternate host genetic recombination is the main source of variation while in its absence mutation is the main source of variation in the pathogen population (Schumann and Leonard 2000). The

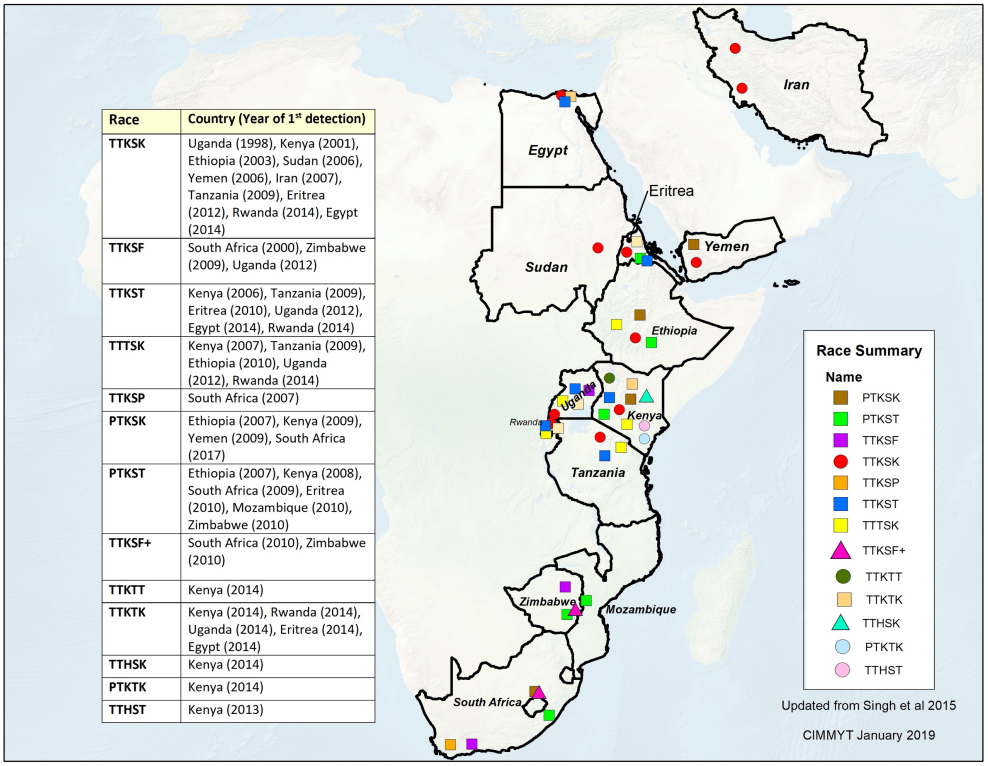
East African highlands have been proven as a suitable environment for a year-round survival of a large stem rust inoculum that increases the chance of evolution of new races through mutation (Singh et al., 2006). Due to the continuously evolving races, varying levels of damages have been reported in this region and other parts of the world at different times.

Global damage of stem rust races on wheat production

Stem rust has caused substantial damage on wheat production across the world. A crop that appeared healthy at some point can turn into a crop with ruptured stems covered with dark spores three weeks before harvest (Singh et al., 2006; Leonard and Szabo, 2005). Some of the races have initiated epidemics in different regions of the world at different times and caused varying levels of yield loses (Nirmala et al., 2017). In the United States, stem rust epidemics that happened in the early and mid 1900s caused an average yield loss of 19 % to 25% in Minnesota, North Dakota, and South Dakota (Dean et al., 2012; Singh et al., 2015). The spread of the disease has been controlled through the use of genetic resistance and eradication of the alternate host, barberry, near wheat fields (Kolmer et al., 1991; Schumann and Leonard 2000; Leonard and Szabo, 2005; Jin and Singh, 2006; Jin, 2011; Singh et al., 2015). The utilization of genetic resistance has been reported to be an effective control measure to stem rust in different parts of the world beginning from the 1950s (McIntosh et al.,1995). However, the continuous emergence of virulent races including the Ug99 race group and other unrelated races threatened the global wheat production and food security and many of the commercially deployed major resistance genes (R-genes) in wheat varieties grown across the globe have been defeated (Singh et al., 2006).

In Ethiopia, an epidemic that was reported prior to the emergence of Ug99 occurred in 1993 and 1994. During this epidemic, huge losses were reported on a popular wheat variety of that time called 'Enkoy' (Shank, 1994). Ug99 was first identified in Uganda in 1999 and spread across the rest of East Africa, Yemen, Iran and South Africa (Nirmala et al., 2017; rusttracker.cimmyt.org). An estimated loss of USD 3 billion was reported due to this race (Aktar-Uz-Zaman et al., 2017). Ug99, that was previously named as TTKS, defeated the resistance gene transferred from rye to wheat (*Sr31*). *Sr31* was reported as the source of resistance that was effective for more than three decades in wheat cultivars across the world (Jin and Singh, 2006; Wanyera et al., 2006). Due to the additional virulence of the Ug99 on *Sr38*, TTKS was renamed as TTKSK according to the North American Stem Rust Nomenclature system (Jin et al., 2007). Based on the past survey, TTKSK was reported as one of the predominant races in the major wheat growing regions of Ethiopia (Hailu et al.; 2015). Until present, Ug99 has evolved to 13 races identified in different countries which overcame more resistance genes (rusttracker.cimmyt.org; Nirmala et al., 2017, Bhavani et al., 2019). Among the variants of Ug99, TTKST has evolved through mutation within the Ug99 lineages. This race was identified in Kenya in 2006 and has combined virulence to widely deployed resistance genes in common wheat, *Sr24* and *Sr31* (Jin et al., 2006). The resistance conferred by *Sr36* was defeated by race TTTSK identified in 2007 (Singh et al., 2015). However, *Sr24* is effective against races reported in Ethiopia (Hailu et al., 2015) and this gene was originally introgressed to bread wheat from *Thinopyrum elongatum* (McIntosh et al., 1995; Singh et al., 2006). Moderate to high susceptibility of many of the global wheat breeding lines and varieties to the Ug99

race group has been reported due to the broad virulence of this race to commercially deployed resistance genes (Bajgain et al., 2015; Singh et al., 2015).



Race	Country (Year of 1st detection)
TTKSK	Uganda (1998), Kenya (2001), Ethiopia (2003), Sudan (2006), Yemen (2006), Iran (2007), Tanzania (2009), Eritrea (2012), Rwanda (2014), Egypt (2014)
TTKSF	South Africa (2000), Zimbabwe (2009), Uganda (2012)
TTKST	Kenya (2006), Tanzania (2009), Eritrea (2010), Uganda (2012), Egypt (2014), Rwanda (2014)
TTTSK	Kenya (2007), Tanzania (2009), Ethiopia (2010), Uganda (2012), Rwanda (2014)
TTKSP	South Africa (2007)
PTKSK	Ethiopia (2007), Kenya (2009), Yemen (2009), South Africa (2017)
PTKST	Ethiopia (2007), Kenya (2008), South Africa (2009), Eritrea (2010), Mozambique (2010), Zimbabwe (2010)
TTKSF+	South Africa (2010), Zimbabwe (2010)
TTKTT	Kenya (2014)
TTKTK	Kenya (2014), Rwanda (2014), Uganda (2014), Eritrea (2014), Egypt (2014)
TTHSK	Kenya (2014)
PTKTK	Kenya (2014)
TTHST	Kenya (2013)

Figure 2.2. Races in the Ug99 group and their distribution in different regions. (From https://rusttracker.cimmyt.org; accessed on February 26, 2021)

Apart from the Ug99 race group, other stem rust races different from the Ug99 lineage and with virulences to previously effective resistance genes were continuously emerging in different regions of the world. Among those, race TRTTF is virulent to *SrTmp*, *Sr1RS* and *Sr13* which are effective against the Ug99 groups, and *Sr36* and *Sr9e* effective against TTKSK (Olivera et al., 2012b). TRTTF was reported as the first known race that defeated the resistance conferred by the 1AL-1RS rye translocation

(Olivera et al., 2012b) and caused susceptibility of all the winter wheat varieties and durum wheat lines in the United States carrying these genes (Singh et al., 2015).

Race TKTTF is another virulent race unrelated to the Ug99 race group. TKTTF was identified in Ethiopia after the severe epidemic in the southeastern parts of the country during the 2013/14 cropping season (Olivera et al., 2015). This epidemic has caused nearly 100% yield loss on 100,000 hectares of land covered by a popular high yielding variety called 'Digalu' which has the *SrTmp* gene and was widely adopted after a stripe rust epidemics in 2010 (Olivera et al., 2015; Singh et al., 2015). A loss assessment from ten years (2010 to 2019) of wheat rust survey data in Ethiopia revealed an estimated total loss due to stem rust varying between ~ 170 million USD during the year of severe epidemic (2014, a year of epidemic due to race TKTTF) and ~ 40 million USD during the year of mild epidemic (2011) (Meyer et al., 2021).

Race TKTTF was currently reported in several European countries including Sweden, Denmark and Germany (Rahmatov et al., 2016; Olivera Firpo et al., 2017). Race TKTTF was the second predominant race in Ethiopia according to a past stem rust survey (Hailu et al., 2015). TKTTF has also broad virulence with high infection responses reported on differential lines carrying *Sr5, Sr6, Sr7b, Sr8a, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr17, Sr21, Sr30, Sr36, Sr38, SrTmp,* and *SrMcN* (Olivera et al., 2015). Durum wheat carries some of these genes. However, the all-stage resistance gene, *Sr13*, and its alleles confer resistance against race TKTTF.

Race JRCQC is also unrelated to the Ug99 group and is virulent on durum wheat. This race was identified after previously resistant durum wheat germplasm of

the North America and CIMMYT were found to be susceptible to races in Ethiopia (Olivera et al., 2012a). The combined virulence of JRCQC to the most common resistance genes (*Sr9e* and *Sr13b*) in CIMMYT and North American durum germplasm, and durum cultivars produced worldwide was reported by Olivera et al. (2012a). Moreover, a high infection type was reported on differential lines carrying *Sr6*, *Sr9a*, *Sr9g*, *Sr11*, *Sr13/17* and *SrMcN* (Olivera et al., 2012b). Recently, Zhang et al. (2017) reported three haplotypes named R1, R2 and R3. Lines carrying R1 and R3 were reported to be resistant to races TTKSK, TKTTF, TRTTF and JRCQC under controlled conditions and designated as *Sr13a* while those carrying R2 were susceptible to JRCQC and was designated as *Sr13b* (Zhang et al., 2017). Although this allele (*Sr13a*) is effective against the races stated, there is always a chance to be defeated by an emerging race unless properly deployed in combination with other resistance genes.

Race TTRTF is another virulent race on durum wheat detected in Sicily, Italy after a sever epidemic in 2016. It is known that Italy is among the major producers of durum wheat in the world. A survey report indicated that many of the popular varieties produced in this country were susceptible to race TTRTF (Bhattacharya, 2017; Randazzo, 2016). TTRTF was first observed in Georgia in 2014 and has broad virulence to resistance genes including *Sr13b, Sr35* and *Sr37* that are valuable in breeding for resistance to Ug99 (Olivera et al., 2019). As indicated in the previous paragraphs, virulent races are continuously emerging which may cause ineffectiveness of more resistance genes. Therefore, the search for sources of resistance and proper

deployment of the available sources of resistances should be a continuous process to mitigate the losses caused by stem rust.

Types of resistance

Types of resistance to wheat rust can be grouped into two classes based on the plant growth stage i.e. seedling resistance and adult plant resistance (APR). Both seedling and adult plant resistances are important in managing stem rust (Ellis et al., 2014). Deploying them in combination or pyramiding several qualitative/seedling resistance genes is suggested as a strategy to increase the durability of resistance. However, the mechanisms of resistance are different between the two types of resistance (Bhavani et al., 2011; Ellis et al., 2014; Mago et al., 2011;Yu et al., 2014).

Mechanisms of seedling resistance

Seedling resistance is expressed at the seedling stage and persists through all growth stages (Ellis et al., 2014). Seedling resistance to stem rust can be evaluated in a greenhouse and allows screening of large numbers of lines in a short period of time (Letta et al., 2014). This type of resistance is race specific and qualitative in nature with simple inheritance (Laidò et al., 2014). Qualitative resistance is known to be controlled by a few major genes with large effects and is also known as vertical resistance, monogenic resistance, R-gene resistance, all stage resistance, or major-gene resistance. The mechanism of resistance in this type of resistance is based on Flor's gene-for-gene concept which assumes a resistance gene in the host interacts with an avirulence gene in the pathogen resulting an incompatible interaction and a hypersensitive response by the host. Hence, hypersensitive response is the outcome of

the interaction between receptors in the plant immune system and pathogenicity factors in the pathogen (Flor, 1971).

The plant immune system consists of two interconnected receptors each located inside and outside the plant cell. Those receptors located outside on the plant cell surface (at the plasma membrane) are called pattern-recognition receptors (PRRs). PRRs are triggered by the pathogen or microbial-associated molecular patterns (PAMPS or MAMPS) of the pathogen and they are involved in pathogen perception (Dangl et al., 2013; Ellis et al., 2014; Andolfo and Ercolano, 2015). Once the PRRs are activated, they induce signaling via the phytohormones such as salicylic acid (SA) within the plant cell that inhibits further colonization of the pathogen known as PAMP-triggered immunity (PTI). However, some effective pathogens have the fitness to inhibit PTIs through their effectors (molecules that disrupt the hormone signaling). Plants have mechanisms to recognize and counteract the effectors of pathogens that trigger plant receptors encoded by R-genes called the nucleotide binding leucine rich repeats (NB-LRR) proteins and result in a hypersensitive response in plants (Dangl et al., 2013; Andolfo and Ercolano, 2015).

Mechanisms of adult plant resistance

APR is usually non-race specific and is a quantitatively inherited type of resistance (Knott, 1982; Bhavani et al., 2011; Ellis et al., 2014). APR is controlled by several genes, each with small effects. It is expressed at the adult plant stage and is identified by evaluating germplasm under field condition. Lines susceptible at the seedling stage but resistant at the adult plant stage are expected to carry APR genes, otherwise it can

be masked by R-genes and can result in ineffective selection for APR (Ellis et al., 2014; Laidò et al., 2015).

APR is more durable than seedling resistance (Singh et al., 2011; Ellis et al., 2014; Yu et al., 2014). It is also known as slow rusting, horizontal-resistance, and polygenic resistance. This type of resistance is often characterized by extended latent periods with few small sized uredinia and restricted production of urediniospores (Bhavani et al., 2011). APR is known to provide incomplete protection under high disease pressure or severe epidemics. The ability to attain close to immune response through combining four to five minor (small effect) genes was previously reported in common wheat (Ellis et al., 2014; Singh et al., 2014) however, the need to develop a large population size and the lack of diagnostic markers were described as challenges for the practical application (Ellis et al., 2014; Singh et al., 2006, 2008).

Utilization of resistance sources for the control of stem rust

Proper utilization of resistance genes is needed for effective control of stem rust. Deploying a single qualitative resistance gene over a large area (monoculture) can increase the selection pressure on the pathogen which results in the breakdown of resistance genes by constantly evolving pathogen races with new virulence factors (Jin et al., 2009; Ellis et al., 2014). Pyramiding of several R-genes in a single cultivar or combining R-genes with APR genes is reported as a valuable strategy that can improve durability of resistance to stem rust in wheat (Ellis et al., 2014). Sometimes, qualitative resistance can also be durable. Among the known qualitative resistance genes that provided prolonged protection, the 1BL.1RS translocated R-gene, *Sr31* has

been effective for more than three decades until the resistance was defeated by Ug99 (Schumann and Leonard 2000; Singh et al., 2011; Ellis et al., 2014; Yu et al., 2014).

Careful utilization of resistance genes requires information on the types and frequency of pathogen races present in a given region (Ellis et al., 2014). Following the emergence of Ug99, an initiative to combat the global damage of stem rust (and/or the three rusts) on wheat production and food security was coordinated by an international consortium known as The Borlaug Global Rust Initiative (BGRI) (Singh et al., 2011). On the effort to fight the damage caused by stem rust and the two other rusts, the BGRI managed by Cornell University was organizing global collaboration on searching for sources of resistances mainly durable adult plant resistance (Rutkoski et al., 2011), developing markers for marker-assisted selection, pyramiding of resistance genes; rust surveillance, monitoring and early warning, and information sharing and training (Schumann and Leonard 2000). Due to this global collaboration, a significant impact has been reported on wheat production across the world mainly in the developing world through the use of resistant varieties and an early warning system for the control of the disease.

Documented stem rust resistance genes utilized in durum wheat

More than 60 stem rust resistance (*Sr*) genes have been cataloged (McIntosh et al., 1995, 2017; Yu et al., 2014) and the sources of many of the major-gene resistances are alien species (Singh et al., 2011). Among the documented *Sr* genes, only five of them are APR genes named *Sr2* (*Yr30/Lr27/pbc1*), *Sr55* (*Lr67/Yr46/Pm39*), *Sr56, Sr57* (*Lr34/Yr18/Pm38*) and *Sr58* (*Lr46/Yr29/Pm39*) (Bansal et al., 2014; Herrera-Foessel et al., 2014; Lagudah et al., 2006; Singh et al., 2015; Yu et al., 2014). All except *Sr56*

are known for pleiotropic effects with multiple disease resistances i.e. yellow rust, leaf rust and powdery mildew resistances (Singh et al., 2014). The APR genes *Sr2* (*Yr30/Lr27/pbc1*), *Sr56* and *Sr58* (*Lr46/Yr29/Pm39*) are located on chromosomes 3BS, 5BL and 1BL, respectively and they are expected to be present both in tetraploid and hexaploid wheat while *Sr55* (*Lr67/Yr46/Pm39*) and *Sr57 (Lr34/Yr18/Pm38)* are expected to be present in hexaploid wheat because of their location on the D-subgenome.

Tetraploid wheat is the source of several stem rust resistance genes. *Sr2, Sr9d, Sr9e, Sr9g, Sr11, Sr12, Sr13, Sr14* and *Sr17* are among the *Sr* genes originated from tetraploid wheat (Singh et al., 2011). *Sr2* has been known for providing APR for wheat cultivars in most parts of the world for more than five decades. This gene is tightly linked to the pseudo- black chaff (PBC) phenotype on the glume and this trait can sometimes be used as a morphological marker for the presence of *Sr2*. A yield penalty or undesirable agronomic performance due to high expression of the PBC trait was reported (Ellis et al., 2014; Laidò et al., 2014). The strong effect of the environment on the expression of this trait was also reported by Singh et al. (2006). However, selection of genotypes with a low level of PBC has been suggested to limit the undesirable effect (Singh et al., 2014). Reports indicated that *Sr2* is not fully protectective when used alone under high disease pressure (epidemics). However, enhanced resistance when combined with other R-genes has been previously reported (Ellis et al., 2014; Basnet et al., 2015). In durum wheat, combined utilization of qualitative resistance genes is commonly practiced.

Sr13 is an all-stage resistance gene located on chromosome 6AL. This gene is present in several durum wheat cultivars around the world conferring resistance to the Ug99 group of races and other unrelated races (Simons et al., 2011). The Ethiopian landrace, 'ST464', and 'Leeds' are the sources of *Sr9e* and *Sr13* (Simons et al., 2011; Olivera et al., 2012b). The domesticated emmer wheat cultivar 'Khapli' is the source of *Sr13*, *Sr7a* and *Sr14*. *SrWeb/Sr9h* is an allele of *Sr9* effective against race TTKSK and the sources of this gene are cultivars 'Gabo' and 'Webster' (Hiebert et al., 2010; Rouse et al., 2014). The source of *Sr11* is the durum wheat cultivar 'Gaza' (McIntosh et al.,1995) and this gene is effective against race TKTTF (Nirmala et al., 2017). The source of *Sr12* and *Sr9g* is the durum wheat cultivar 'Iumillo' and that of *Sr17* is an emmer cultivar 'Yarsolav' (McIntosh et al.,1995). Oftentimes, more than one *Sr* gene can be carried in the same genetic background of durum wheat. These effective qualitative resistance genes are at high risk of being defeated by emerging races. Therefore, identification of sources of resistances through molecular markers linked to QTL enhances breeding for resistance to stem rust.

Opportunities and methods for identifying sources of genetic resistance

At present, there are several possibilities to undertake successful genetic studies in different species. The development of high-throughput and dense-marker technologies, and the improvement of statistical approaches are among the great advancements and opportunities for understanding the genetic basis of agronomically important traits (Poland et al., 2012; Zhu et al., 2008). Moreover, the efficient cost to provide genome-wide marker coverage mainly the single nucleotide polymorphism (SNP) markers discovered through platforms such as genotyping-by-sequencing

(GBS) (Poland et al., 2012; Xu et al., 2017) promotes the extensive application of marker technology in resistance breeding and other genetic studies (Ellis et al., 2014).

Identification of accurate markers linked to a QTL of interest through the use of dense-markers can facilitate marker development for MAS (Collard et al., 2005). It also facilitates pyramiding of resistance genes in adapted lines (Laidò et al., 2015) and improves the gain from selection per unit time by including the identified markers in genomic selection models (Eathington et al., 2007; Rutkoski et al., 2011; Gutierrez-Gonzalez et al., 2019). The known methods for identification of markers linked to a QTL of interest are linkage mapping and association (linkage disequilibrium) mapping (Zhu et al., 2008). The two approaches differ in the design of the mapping population to be used, but they complement each other and their combined application was described as a means of validating mapping results (Nordborg and Weigel, 2008).

Linkage mapping

Linkage mapping is a common method to identify marker trait association or QTL associated with various agronomic traits. In this method, linkage disequilibrium is generated by developing populations using biparental crosses (Laidò et al., 2014). The population to apply linkage mapping could be F_2, backcrosses, doubled haploids, recombinant inbred lines and near-isogenic lines (Xu et al., 2017). The co-segregation of markers and phenotype of an agronomic trait of interest helps to identify linked markers in this mapping method. The identified markers can be used in MAS, fine mapping and cloning (Wen et al., 2017). The main limitation of linkage mapping is the low resolution of QTL mapping due to the limited number of meiotic/recombination events happening during the development of the mapping population (Flint-Garcia et

al., 2003; Zhu et al., 2008; Laidò et al., 2014). Reports indicated that linkage mapping has a high power in identifying rare alleles that have large effect, but sometimes the effect on the phenotype could be undesirable (Nordborg and Weigel, 2008; Xu et al., 2017). Unlike association mapping which samples a substantial amount of the potential alleles from existing diverse lines, linkage mapping samples a small proportion of the potential alleles from a population where the parents utilized for crossing are selected (Laidò et al., 2014; Xu et al., 2017). In order to identify QTL, the parents used to develop the bi-parental population for linkage mapping should be diverse for an agronomic trait of interest. Once the mapping population is developed, phenotyping, genotyping with appropriate marker technology and analysis using proper statistical models will be used to identify QTL (Xu et al., 2017).

Different statistical approaches are used in biparental mapping. The powerful method developed by Knott and Haley (1992) that analyzes multiple QTL at the same time by combining regression and interval mapping is known as composite interval mapping (CIM). This method uses flanking markers in QTL identification and assumes a QTL to be contolled by multiple loci unlike interval mapping that assumes a QTL to be controlled by a single locus (Xu et al., 2017). The regression approach that uses flanking markers was recommended as the best method to estimate QTL effects and position of a QTL in biparental mapping (Knott and Haley, 1992).

Association (linkage disequilibrium) mapping

Association mapping is a technique applied to dissect the genetic bases of complex traits in different species. It is an efficient approach to identify MTAs in several crop and animal species (Zhu et al., 2008; Xu et al., 2017). Association mapping identifies

marker-trait associations (MTAs) that can be grouped into QTL by assessing the level of linkage disequilibrium between markers and casual polymorphism in diverse populations (Zhu et al., 2008). This method is known for its power of detecting MTAs responsible for the variation in a phenotype of interest by applying robust statistical tools (Flint-Garcia et al., 2003; Zhu et al., 2008; Chao et al., 2010).

Association mapping can be applied on a diverse panel of lines or on elite breeding lines unlike linkage mapping which needs a designed population (Chao et al., 2010; Laidò et al., 2014). It leverages the recombination events that occurred over a prolonged period of time among lines in the population and results in higher resolution mapping that leads to fine mapping of QTL (Breseghello and Sorrells, 2006; Nordborg and Weigel, 2008; Laidò et al., 2014; Chao et al., 2017). However, structured populations can lead to false associations by increasing the level of linkage disequilibrium (LD) between loosely linked or unlinked loci if not properly taken into account in GWAS (Maccaferri et al., 2005; Chao et al., 2010). The chance of false positive associations can be reduced by using appropriate GWAS models and validation of identified MTAs (Laidò et al., 2014). MLM that include population structure (Q-matrix) derived from principal component analysis (PCA) or structure analysis as a fixed effect and the relationship between individuals using a marker-based kinship matrix (K-matrix) as a random effect can correct false positive associations resulting from a structured population (Xu et al., 2017).

Linkage disequilibrium (LD) is key in association mapping. LD is the non-random association between alleles at different loci. Tightly linked loci are expected to have higher LD than unlinked loci where recombination reduces the LD (Laidò et al.,

2014). In GWAS, the extent of LD determines the marker density needed and the resolution of mapping a marker linked to a casual polymorphism (Chao et al., 2010). The extent of LD varies among species with different mating types and the type of population selected for study (Flint-Garcia et al., 2003). Species with extended (slower) decay of LD (selfing species) need lower marker density than species with faster decay of LD (outcrossing species). The resolution of mapping is lower in species with lower marker coverage due to slower decay of LD compared to species with faster LD decay that need higher marker density (Xu et al., 2017). With regard to the type of mapping population to be used, a higher and extended level of LD was reported in improved cultivars and breeding lines than landraces in wheat (Maccaferri et al., 2005; Laidò et al., 2014).

LD can be affected by a number of factors including selection for favorable alleles, genetic drift, mutation, recombination, and admixture (gene-flow) (Flint-Garcia et al., 2003; Chao et al., 2010; Xu et al., 2017). Selection, genetic drift and admixture can increase the LD between alleles. Recombination reduces within chromosomal LD or it can eliminate LD between unlinked loci (Flint-Garcia et al., 2003; Laidò et al., 2014).

LD is measured using two statistics. One of the statistics is r^2, the squared allele frequency correlation between two loci, and the other is D′ that scales the difference between the observed and expected haplotype frequencies based on the observed allele frequencies (Flint-Garcia et al., 2003; Xu et al., 2017). The values of LD vary between 0 to 1 indicating linkage equilibrium and perfect LD, respectively. Among the two measures of LD, the r^2 statistics that indicates the correlation between

markers and the causal loci is suggested as a measure to evaluate the resolution of GWAS mapping (Flint-Garcia et al., 2003).

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CHAPTER 3.

GENOME-WIDE ASSOCIATION MAPPING OF SEEDLING AND ADULT PLANT RESPONSE TO STEM RUST IN A DURUM WHEAT PANEL

ABSTRACT

Many of the major stem rust resistance genes deployed in commercial wheat cultivars and breeding lines become ineffective over time due to the continuous emergence of virulent races. A genome-wide association study (GWAS) was conducted using 26,439 single nucleotide polymorphism (SNP) markers and 280 durum wheat lines from CIMMYT to identify genomic regions associated with seedling resistance to races TTKSK, TKTTF, JRCQC and TTRTF and field resistance to TKTTF and JRCQC. The phenotypic data analysis across environments revealed 61% to 91% and 59% to 77% of phenotypic variation explained by the genotypic component for seedling and adult plant response of lines, respectively. For seedling resistance, mixed linear model (MLM) identified eight novel and nine previously reported quantitative trait loci (QTL) while a Fixed and random model Circulating Probability Unification (FarmCPU) detected 12 novel and eight previously reported QTL. For field resistance, MLM identified 12 novel and seven previously reported loci while FarmCPU identified seven novel and nine previously reported loci. The regions of *Sr7a*, *Sr8155B1, Sr11,* alleles of *Sr13, Sr17, Sr22/Sr25,* and *Sr49* were identified. Novel loci on chromosomes 3B, 4A, 6A, 6B, 7A and 7B could be used as sources of resistance to the races virulent on durum wheat. Two large effect markers on chromosome 6A could potentially be used to differentiate resistant haplotypes of *Sr13* (R1, R3). Allelism tests for *Sr13*, breaking the deleterious effect associated with *Sr22/Sr25* and

retaining the resistance allele at the *Sr49* locus, are needed to protect future varieties from emerging races.

INTRODUCTION

Durum wheat (*Triticum turgidum* L., ssp. *durum* (Desf.) Husnot) is a tetraploid wheat species grown in different parts of the world with the major production region being the Mediterranean Basin (Letta et al., 2013; Shewry and Hey, 2015; Kabbaj et al., 2017). Stem rust of wheat caused by *Puccinia graminis* Pers.f.sp. *tritici* Eriks. and Henn., is among the most damaging fungal diseases of common wheat (*Triticum aestivum* L.) and durum wheat worldwide. Stem rust can occur in all wheat production areas where the environment is favorable for disease development (Singh et al., 2008). Susceptible varieties in these areas can incur a total yield loss under severe epidemics (Yu et al., 2014). The stem rust pathogen interferes with the transport of nutrients through the vascular system and results in shriveled seeds at harvest, stem breakage and lodging (Bhavani et al., 2019). Shriveled seeds harvested from stem rust infected wheat degrade end use product quality (Singh et al., 2006).

Stem rust epidemics have occurred in several regions of the world at different periods and caused varying levels of yield loss (Bajgain et al., 2015a; Nirmala et al., 2017). This damage is attributed to the narrow genetic base of stem rust resistance of cultivars and breeding lines in some regions of the world (Fu and Somers, 2009; Newcomb et al., 2013). During the epidemics of stem rust in the United States, disease occurrence has been effectively controlled by the utilization of resistance genes in wheat cultivars (McIntosh et al.,1995) and eradication of the alternative host common barberry (*Berberis vulgaris* L.) near wheat growing areas (Kolmer et al., 1991; Jin and

Singh, 2006; Singh et al., 2015; Nirmala et al., 2017). However, the emergence of new virulent races like TTKSK (Ug99) that defeated the resistance conferred by *Sr31* (Singh et al., 2011; Bajgain et al., 2015b) and other virulent races unrelated to Ug99 with broad virulence to commercially deployed resistance genes have continued to limit global production of both common and durum wheat. Race TTKSK was identified in Uganda in 1999 and spread to East Africa and the Middle East (Singh et al., 2006). This race with thirteen variants has been recognized as a severe threat to worldwide wheat production and food security due to its broad virulence to several resistance genes mainly deployed in commercial wheat varieties and germplasm (Singh et al., 2011, 2015; Olivera et al., 2012a; Bajgain et al., 2015b; Newcomb et al., 2016; Chao et al., 2017). Race TKTTF is unrelated to the Ug99 group of races and it is predominant in Ethiopia with broad virulence to several *Sr* genes. This race caused severe yield loss during the epidemics of 2013/2014 and devastated the popular bread wheat variety 'Digalu' grown over 100,000 ha (Olivera et al., 2015; Singh et al., 2015). Race TKTTF defeated the resistance conferred by *SrTmp* gene in 'Digalu'. Pathogen races outside of the Ug99 race group and with relevant virulence on durum wheat have also been reported in the past decade. Race JRCQC is unrelated to the Ug99 lineage and it was identified in Ethiopia in 2009. JRCQC has a combined virulence to *Sr9*e and *Sr13b*, alleles of commonly deployed resistance genes in durum wheat (Olivera et al., 2012b ; Zhang et al., 2017). This race was identified upon evaluation of durum wheat germplasm from North America and CIMMYT that were mostly resistant to races in Kenya at that time but became highly susceptible when evaluated in the field nursery in Ethiopia (Olivera et al., 2012b; Singh et al., 2015).

TTRTF is another virulent race on durum wheat that caused a severe epidemic on durum wheat in Sicily, Italy in 2016 (Bhattacharya, 2017). This race was observed for the first time in Georgia in 2014 and carries broad virulence to several resistance genes in durum and common wheat including *Sr9e*, *Sr13b, Sr35, Sr36, Sr37, Sr38, Sr45* and *SrTmp* (Olivera et al., 2019). A pathogen survey report from Sicily, Italy indicated that race TTRTF is virulent on 25 durum wheat varieties and breeding lines including major varieties grown in the region (Randazzzo et al., 2016). Among the resistance genes most deployed in durum wheat in different regions of the world, *Sr13a* is still effective against the *Pgt* races virulent on durum, including TTRTF and JRCQC (Zhang et al., 2017; Olivera et al., 2019).

The stem rust pathogen evolves continuously, producing new races with virulences to resistance genes commonly deployed in commercial varieties and breeding lines. The narrow genetic base of stem rust resistance in durum wheat compared to common wheat exposes the crop to a risk of resistance being defeated by an emerging virulent race. Nevertheless, the application of genetic resistance is a preferred method to control stem rust due to environmental safety and cost efficiency; and broadening the genetic base of resistance is paramount. In an attempt to manage stem rust through the application of genetic resistance, over 60 stem rust resistant genes and alleles have been cataloged. However, most of them are major-effect gene resistances (R-genes) which are most often effective against specific races (McIntosh et al.1995, 2017). Therefore, the continuous evaluation and identification of new sources of resistances to stem rust, characterization of the available sources of resistance in the germplasm pool and their proper utilization is crucial to mitigate the

risk posed by stem rust on global wheat production. Although there is a possibility of incorporating novel sources of resistances in breeding materials from wild relatives or landraces, breaking the linkage drag is often challenging. The current study utilizes a panel of breeding lines from CIMMYT to evaluate and characterize sources of resistance to virulent races of the stem rust pathogen through association mapping.

Association mapping (linkage disequilibrium mapping) is an efficient approach to identify marker-trait associations (MTAs) (Zhu et al., 2008). This technique exploits genetic recombination that occurred over generations in the population used for study (Zhu et al., 2008; Chao et al., 2017) and is a powerful method for studying simple and complex traits in many crop species (Kumar et al., 2017). Mapping resolution is higher in association mapping than linkage mapping due to a higher level of polymorphism on using a population composed of diverse lines. However, population structure must be taken into account in GWAS analysis models if the population under study has a stratification which otherwise can result in false positive associations (Yu and Buckler, 2006).

Genetic studies to identify and map sources of stem rust resistance in durum wheat using dense marker coverage is limited compared to that of common wheat. Moreover, the panel of CIMMYT durum wheat lines used in the current study have not previously been evaluated for seedling response to TTKSK, TKTTF, the durum virulent races (JRCQC and TTRTF), or field response against single races. Therefore, the objectives of the current study were to 1) evaluate seedlings of a panel of durum wheat lines for resistance to four virulent *Pgt* races (TTKSK, JRCQC, TKTTF and TTRTF) and field resistance to races JRCQC and TKTTF and 2) conduct GWAS

analysis using SNP markers to identify genomic regions associated with seedling and field resistances against these races.

MATERIALS AND METHODS

Plant materials and phenotyping

Seedling evaluation

A panel of 283 spring durum wheat lines representing the germplasm pool of the CIMMYT durum wheat breeding program was evaluated against four *Pgt* races in a biosafety level-3 (BSL3) greenhouse facility at the University of Minnesota in January 2019. The four races were: TTKSK (isolate 04KEN156/04), JRCQC (isolate 09ETH08-1), TKTTF (isolate 13ETH18-1) and TTRTF (isolate 14GEO189-1). These races were selected based on their broad virulence on commercially deployed resistance genes and their damage on global wheat production. Six seeds of each line were planted in trays filled with vermiculite and replicated twice for each race. Seven-day old seedlings were inoculated with urediniospores of each race following the procedure by Rouse et al. (2011). Seedlings were scored 14 days post inoculation using the 0 to 4 scale described by Stakman et al. (1962). Accordingly, infection types (ITs) ";", "0", "1^-", "1", "1^+", "2^- ", "2", and "2^+" were considered resistant whereas "3^-", " 3", " 3^+" and "4" considered as susceptible. This scale was linearized to 0-9 scale according to Zhang et al. (2011) as ';' and '0' = 0, '1^- ' = 1, '1' = 2, '1^+' = 3, '2^-' = 4, '2' = 5, '2^+' = 6, '3^-' = 7, '3' = 8, '3^+' = 9, '4' = 9 for statistical analysis. Lines with linearized scale ≤ 6 (IT $\leq 2^+$) and > 6 (IT $> 2^+$) were considered seedling resistant and susceptible, respectively.

Field evaluation

The same panel used for seedling evaluation was tested for responses to races TKTTF and JRCQC at the adult plant stage in single race nurseries at the Debre Zeit Agricultural Research Center, Ethiopia from 2018 to 2020. The response to race JRCQC was evaluated during main-season 2019 (JRCQC_MS19) and off-season 2020 (JRCQC_OS20) while that of race TKTTF was evaluated during the main-season 2018 (TKTTF_MS18) and main-season 2019 (TKTTF_MS19). The TKTTF_MS18 nursery was inoculated with bulk of isolates ETH-9TZaTX25, SR-BA-14, SR-BA-28, AM-S, AM-14, AM#-a1, Am-03 while TKTTF_MS19 was inoculated with bulk of isolates AM-A4, Am-A17, AM-B28, DZ-A-8, DZ-A25, Gonder-A-2. The JRCQC_MS19 and JRCQC_MS20 trials were inoculated with bulk of isolates Ku#3, Ku#22, Ku#30, Am#6 and BD#30 identified in 2015 and 2016. The main and off-seasons in Ethiopia are from June to November and from January to May, respectively. The nurseries were established in isolation from the international screening nursery where germplasm screening is done against a bulk of multiple races to avoid potential contamination. Moreover, the two single race nurseries were also isolated by distance (~1 km apart) to control contamination. The lines were planted in double rows (1m X 0.2 m) using a randomized incomplete block design and two replications. One moderately resistant ('Mangudo') and two susceptible ('Local Red' and 'Arendato') checks were planted after every 50 lines. The 20 stem rust differential lines were planted at the start and end of each nursery. The cultivar 'Leeds', carrying *Sr13/Sr13b* and variety 'Digalu' carrying *SrTmp* were planted perpendicular to the plots and surrounding the nursery as spreader rows to initiate infections on the trials of

JRCQC and TKTTF, respectively. Moreover, the nurseries were surrounded by oat (non-host for *Puccinia graminis* f.sp. *tritici)* to act as a physical barrier to potential spore contaminations. Spores of the bulk of isolates of each race were mixed with distilled water and a drop of Tween 20 was added to reduce surface tension of water (one drop/0.5 L). Each nursery was inoculated twice with this mixture at stem elongation (Zadok's growth stage =31) (Zadoks et al., 1974).

Disease severity was scored according to the modified Cobb's scale by estimating the proportion of the stem area (0-100%) covered by rust pustules (Peterson et al., 1948). Infection response was scored according to Roelfs et al.(1992) based on the size of pustules and amount of chlorosis and necrosis on the stem. The responses classes are: '0' for no visible infection, 'R' for resistant, 'MR' for moderately resistant, 'MS' for moderately susceptible and 'S' for susceptible. The nursery was scored three times for JRCQC_MS19 and TKTTF_MS19 and four times for TKTTF_MS18 and JRCQC_OS20. The severity and response were combined to a value called coefficient of infection (CI) by multiplying the severity with a 0 to 1 scale assigned for each response class. The scale was assigned as: immune = 0.0, R = 0.2, MR = 0.4, MS = 0.8 and S = 1.0, and the mean of the scale of responses was used to calculate CI in the cases where combinations of infection responses were scored for a given genotype (Stubbs et al., 1986). Then, the CI was used for further statistical analysis and the last scoring was considered to calculate the CI in all except TKTTF_MS18 where the third scoring was used.

Statistical analysis of phenotype data

Seedling response

The linearized scale of the seedling response against the four races was used to apply statistical analysis. R statistical software Version 3.6.1 (R Core Team, 2019) was used to plot the distributions of the responses and analyze the correlation between responses against the four races. A linear mixed model (LMM) described in equation-3.1 was fitted using the lmer() function of the R package *lme4* (Bates et al., 2015) considering the genotype and replication as random.

$$y_{ij} = \mu + g_i + r_j + \varepsilon_{ij} \quad (3.1)$$

Where: y_{ij} is the response of the i^{th} line at the j^{th} replication, μ is the overall mean response g_i is the random effect of the i^{th} genotype (line), r_j is the random effect of the j^{th} replication and εij is the residual associated with the model. Variance components estimated from equation (3.1) above were used to calculate broad sense heritability (H^2) Holland et al.(2003):

$$H^2 = V_g/V_p \quad (3.2)$$

Where: H^2 is the broad sense heritability, V_g is the variance due to the genotype (line), V_P is the variance due to the phenotype, ($V_p = V_g + V_e$) and V_e is the residual variance. The race by genotype (line) effect was estimated from LMM described in equation-3.3 using the lmer() function of R considering genotype/line, race, replication and line by race interaction as random effects.

$$y_{ijk} = \mu + g_i + r_j + (gr)_{ij} + R_k + \varepsilon_{ijk} \quad (3.3)$$

Where: y_{ijk} is the response of the i^{th} line in the j^{th} race and k^{th} replication, μ is the overall mean response, g_i is the random effect of the i^{th} genotype (line), r_j is the random effect of the j^{th} race, gr_{ij} is the interaction effect of the i^{th} line and the j^{th} race as

random, R_k is the random effect of the k^{th} replication, ε_{ijk} is the residual associated with the model. The variance components estimated from equation (3.3) was used to calculate broad sense heritability (H^2) (Tsilo et al., 2014):

$$H^2 = \frac{V_g}{V_g + \frac{V_{gr}}{n(r)} + \frac{V_e}{(n(r)*n(rep)}} \qquad (3.4)$$

Where: H^2 is broad sense heritability, V_g is the variance due to the genotype (line),V_{gr} is the variance due to the interaction of genotype and race, V_e is the variance due to the error (residual), n(r) is number of races, n(rep) is number of replications.

Adult plant response

The LMM was fitted on the CI as a response variable for the JRCQC_MS19, TKTTF_MS19 and JRCQC_OS20 while the square root transformed CI was used for TKTTF_MS18. For JRCQC_MS19 and TKTTF_MS19 the following model (equation-3.5) was fit using the lmer() function of the R package *lme4* to estimate the variance components.

$$y_{ijk} = \mu + g_i + C_j + r_k + \varepsilon_{ijk} \qquad (3.5)$$

Where: y_{ijk} is the response of the i^{th} line in the j^{ith} column and the k^{th} replication, g_i is the random effect of the i^{th} line, C_j is the fixed effect of the j^{th} column, and r_k is the random effect of k^{th} replication and ε_{ijk} is the residual associated with the model.

For TKTTF_MS18 and JRCQC_OS20, the models described in equation-3.6 and equation-1 were fit using ASReml, respectively (Gilmour et al., 2009) to estimate the variance components. Best linear unbiased predictions (BLUPs) were calculated from the respective models and the broad-sense heritability was calculated using equation-3.2 for each race across seasons. R_j in eqution-3.6 is the fixed effect of the j^{th} row and the remaining descriptions were same as equation-3.5.

$$y_{ijk} = \mu + g_i + R_j + r_k + \varepsilon_{ijk} \qquad (3.6)$$

Genotyping, population structure and linkage disequilibrium analyses

The same panel of 283 lines from the CIMMYT durum wheat breeding germplasm pool used for adult plant evaluation against multiple-races in East Africa (Ethiopia and Kenya) was genotyped using genotyping-by-sequencing following the protocol described by Poland et al. (2012). Single nucleotide polymorphism (SNP) genotype calling, data filtering and data imputation were performed as described in Megerssa et al. (2020) on a GWAS study of the same panel for response to bulk of multiple *Pgt* races prevalent in East Africa. A total of 26,439 SNP markers for 280 lines were retained for GWAS analysis. The linkage disequilibrium (LD) between pairs of SNPs was calculated as the squared allele frequency correlation (r^2) using TASSEL software version 5 (Bradbury et al., 2007) as described in Megerssa et al. (2020). The presence of population structure was assessed using principal component analysis. The extent of LD and population structure was previously reported for this panel (Megerssa et al., 2020).

Genome Wide Association Analysis

GWAS analysis was conducted using GAPIT (Lipka et al., 2012) by fitting three models: MLM (Yu et al., 2006), Compressed Mixed Linear Model (CMLM) (Zhang et al., 2010) and FarmCPU (Liu et al., 2016). The mean linearized scale of the two replications for the seedling response to the four races, and the BLUPs calculated from the respective models for the adult plant response against the two single races (JRCQC and TKTTF) were used as a response in the fitted GWAS models. The first two PCA scores and the kinship matrix were fitted as fixed and random effects, respectively.

The results of GWAS were visualized using Manhattan and quantile-quantile (Q-Q) plots produced using the R package *qqman* (Turner, 2017) applied on the -log10 *P-value*. The three models were compared based on the deviation of the distribution of the observed -log10 *P-value* from the expected in the Q-Q plots and results were interpreted from MLM and FarmCPU. Significant markers on the same chromosome were grouped into QTL based on their LD. A false discovery rate (FDR) of 5% was used for multiple comparison adjustment and as a threshold to declare significant MTAs (Benjamini and Hochberg, 1995). GAPIT calculates the FDR adjusted P.values and markers with P.values < 0.05 were taken as significant MTAs. The FDR threshold value was calculated using a vector of the P.values from the GWAS output sorted from the most significant to the least. Then using a function formed in R a cutoff was calculated for each test using the formula: cutoff = (1:N)/N)*FDR, where N was the total number of tests (Numbers of markers). Then the numbers of significant markers (n) (P.values < 0.05) with the numbers of tests (N) and FDR threshold (0.05) were used to calculate the threshold value using the formula: FDR threshold value = ((0:N/N)*FDR[n+1] and the -log10(threshold value) was used to mark the threshold line on the Manhattan plot. Consistent MTAs between races and race/seasons in the field were visualized using the R package *Venndiagram* (Chen and Boutros, 2011). Markers reported in previous QTL mapping studies on durum and common wheat were gathered and their sequences were searched from the GrainGenes database. The fasta file of the sequences was searched using the blastn program of the IWGSC database. Then the alignment of physical positions of the significant markers identified in the current study with the chromosomal positions of the 'Svevo'

reference assembly were compared and resistance genes/alleles were proposed based on the similarity of positions and race specificity of known stem rust resistance genes/alleles**.**

RESULTS

Phenotypic data analysis

Seedling response to the four races

We evaluated a panel of lines representing the durum wheat breeding germplasm pool of CIMMYT for seedling responses to four *Pgt* races virulent to durum wheat. The distributions of the seedling response of the lines against the four *Pgt* races was skewed towards the resistant scores (linearized response ≤ 6 or IT ≤ 2^{+}) (Supplemental Fig. S3.1). The percentage of resistant lines varied from 56.4% against race TTRTF to 73% against race TKTTF (Table 3.1). Moreover, the lines exhibited resistance to combinations of races that ranged from 50.9% to 58.3% for combinations of three races and 52.3% to 67.1% for combinations of two races (Table 3.2). Of the lines evaluated, 50.2% (142 lines) were resistant to all four races, while 19.4% (55 lines) were susceptible to all four races. Based on the infection type and race specificity, 8.6% of the lines (24 lines) were postulated to carry *Sr13b*. These lines showed low infection types for response to TTKSK (2^{-}) and TKTTF (2^{-}, to 2^{+}) while high infection type was scored for response to JRCQC and TTRTF (3 to 4) (Supplementary Table S3.13). One line (genotype identification (GID) 7147182) and two lines (GID 7147179 and 7147180) showed an immune seedling response against all four races and three races (TTKSK, TKTTF, JRCQC), respectively. The broad-sense heritability for seedling responses to the four races varied from 0.61 for race TTRTF to 0.91 for

race TKTTF (Table 3.1). The phenotypic correlation coefficients between the responses to the four races ranged from moderate (r = 0.47) between JRCQC and TTKSK to high (r = 0.76) between TKTTF and TTKSK (Fig. 3.1).

Table 3.1. Summary of the percent resistant and susceptible lines against the four Pgt races and broad-sense heritability of seedling response. Values are percentages and counts in parenthesis.

Race	Resistant	Susceptible	Heritability (H^2)
TTKSK	70.6 (197)	29.4 (82)	0.86
TKTTF	73.1 (204)	26.9 (75)	0.91
JRCQC	67.1 (188)	32.8 (92)	0.90
TTRTF	56.4 (159)	43.6 (123)	0.61

Table 3. 2. Number and percentage of lines resistant at the seedling stage against different combinations of the four races.

Race combination	Total No. lines	Number of resistant lines	Percentage of resistant lines
TTKSK+TKTTF+JRCQC+TTRTF	283	142	50.2
TTKSK +TKTTF+JRCQC	283	165	58.3
TTKSK +JRCQC+TTRTF	283	144	50.9
TTKSK +TKTTF+TTRTF	283	145	51.1
JRCQC+TKTTF+TTRTF	283	148	52.3
TTKSK +TKTTF	283	190	67.1
TTKSK +JRCQC	283	168	59.36
TTKSK +TTRTF	283	148	52.29
JRCQC+TKTTF	283	176	62.19
TKTTF+TTRTF	283	151	53.36
JRCQC+TTRTF	283	151	53.36

Table 3. 3.Summary of descriptive statistics, genetic variance and broad sense heritability of coefficient of infection for field responses to races JRCQC and TKTTF across seasons.

Statistic	JRCQC_MS19	JRCQC_OS20	TKTTF_MS18	TKTTF _MS19
Mean	36.3	39.0	23.5	38.3
Range	0-70	0-80	0-80	0-70
$V_{g\dagger}$	154.8	207.9	3.4	227.1
H^{2*}	0.67	0.59	0.74	0.77

* Broad-sense heritability
† Genetic variance

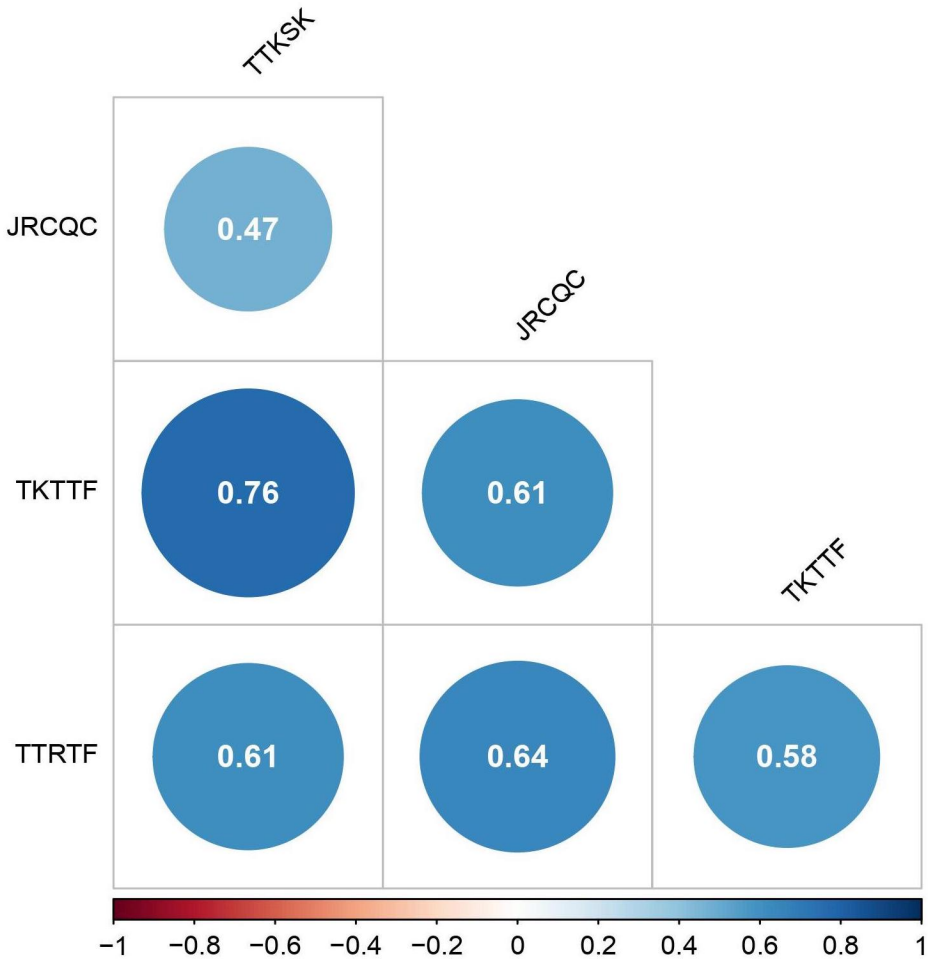


Figure 3. 1. Correlation between seedling responses of durum wheat lines against four races. Large circle indicates the magnitude of the correlation while dark blue color indicates the strength (intensity) of the correlation.

Adult plant response to the two races

The panel of lines were evaluated for field responses against two races (JRCQC and TKTTF) for two seasons from main-season 2018 to off-season 2020. The frequency distribution of the CI of lines was normal for JRCQC_MS19, JRCQC_OS20 and TKTTF_MS19 but skewed towards resistance for TKTTF_MS18 (Supplemental Fig.S3.2). The normality of the CI for TKTTF_MS18 was improved after square root transformation and the transformed CI was used for further analysis. The broad-sense

heritability for the adult plant responses ranged from 0.59 for JRCQC_OS20 to 0.77 for TKTTF_MS19 (Table 3.3). Moderate correlations were observed between seedling and field responses to the two races (0.37 to 0.53 for JRCQC and 0.55 to 0.61 for TKTTF) (Data not shown).

Genome Wide Association Analysis

Marker trait association analysis for seedling responses to the four *Pgt* races (TTKSK, TKTTF, JRCQC and TTRTF), and field responses to the two single races (JRCQC and TKTTF) were conducted using GAPIT by fitting three different models (MLM, CMLM and FarmCPU). The Q-Q plots of MLM and FarmCPU fitted the data well for all race-season combinations and results were interpreted from these two models.

GWAS for seedling response to the four *Pgt* races

The mean linearized scale of the two replications for the seedling responses of lines against the four races was used as a response variable for GWAS analysis. A total of 114 significant markers distributed along the 14 chromosomes and unaligned contigs were identified for seedling resistance against the four *Pgt* races using MLM (Supplemental Table S3.1 to S3.4). Among those, 1%, 16.6%, 30.7%, 51.7% were associated with seedling resistance against the four races, three of the four races, two of the four races and a single race, respectively (Fig. 3.6). Five of the MTAs were on unaligned contigs and the remaining 109 were grouped into 17 QTL represented by single and multiple adjacent markers with known chromosomal locations (Supplemental Table S3.1 to S3.4; Fig. 3.2). The numbers of QTL identified using MLM were six, seven, two and eight for seedling resistance against races TTKSK, TKTTF, JRCQC and TTRTF, respectively. This study is the first to report GWAS

analysis of durum wheat for response to race TTRTF. FarmCPU identified 34 significant MTAs that were grouped into 20 QTL with known chromosomal locations (Fig. 3.3; Supplemental Table S3.5). Among the 34 MTAs, a single marker for each was associated with seedling resistance against combinations of two and three races while 32 markers were associated with seedling resistance to single races. Six QTL located on chromosomes 2B (89 Mb to 97 Mb), 3A (565 Mb and 614 Mb), 6A (205 Mb, and 602 Mb to 615 Mb) and 7A (686 Mb to 721 Mb) were consistent between the two models (Table 3.4).

On chromosome 1A, an MTA was identified at 258 Mb for seedling response to race TTKSK (Supplemental Table S3.1). On chromosome 1B, six significant markers representing five putative QTL were identified (Figs. 3.2, 3.3). The 11 Mb locus was associated with seedling resistance to race TKTTF while the regions at 550 Mb, 551 Mb and 587 Mb were associated with seedling resistance to race TTRTF (Fig. 3.2; Supplementary Table S3.2, S3.4). The markers at 550 Mb and 551 Mb were in strong LD ($r^2 = 0.95$) and represent the same QTL that explained 5.1% of the phenotypic variation on average. The remaining two MTAs, at 22 Mb and 166 Mb identified by FarmCPU were associated with seedling resistance to races TTKSK and JRCQC, respectively (Supplemental Table S3.1, S3.2).

On chromosome 2B, a QTL represented by eight significant markers spanning from 89 Mb to 97 Mb (LD, $r^2 = 0.81$ to 0.98) was identified for seedling resistance against race TKTTF (Fig. 3.2; Supplemental Table S3.2). This QTL was consistent between MLM and FarmCPU, and it explained 4.2% to 5.8% of the phenotypic variation (Table 3.4).

On chromosome 3A, two MTAs consistent between the MLM and FarmCPU models were identified at 565 Mb and 614 Mb regions. The 565 Mb locus was associated with seedling resistance to races TKTTF and TTRTF and explained 3.9% and 7.4% of the phenotypic variation, respectively while the 614 Mb region was identified for seedling resistance to race TKTTF and explained 3.1% of the phenotypic variation (Supplemental Tables S3.1 to S3.5, Table 3.4). On chromosome 3B, significant associations were identified using FarmCPU at 40 Mb and 139 Mb (FDR adjusted *p-value* = 0.04) regions for resistance against races JRCQC and TTRTF, respectively (Supplementary Table S3.3, S3.4).

Four significant markers (17 Mb, 619 Mb, 651 Mb, 718 Mb) were identified on chromosome 4A (Supplemental Tables S3.1 S3.5; Figs. 3.2, 3.3). The MTAs at 17 Mb and 619 Mb were identified using MLM for seedling resistance against race TTRTF and explained 5.3% and 4.2% of the phenotypic variation, respectively. The 651 Mb region was associated with seedling resistance to race TTKSK and explained 5.2% of the phenotypic variation. The 718 Mb locus was detected by FarmCPU for seedling resistance against race TKTTF. On chromosome 4B, one MTA (444 Mb) was identified using FarmCPU for seedling resistance to race JRCQC (Supplemental Table S3.5).

On chromosome 5A, a significant marker (581 Mb) was identified for seedling resistance to race JRCQC using FarmCPU (Supplemental Table S3.4; Fig. 3.3). On chromosome 5B, MTAs were detected at 287 Mb and 396 Mb using FarmCPU for seedling resistance against race TTRTF (Supplemental Table S3.4; Fig. 3.3) while two

MTAs, at 61 Mb and 691 Mb were identified for seedling resistance against race TKTTF using MLM and FarmCPU, respectively (Supplemental Tables S3.1 to S3.5).

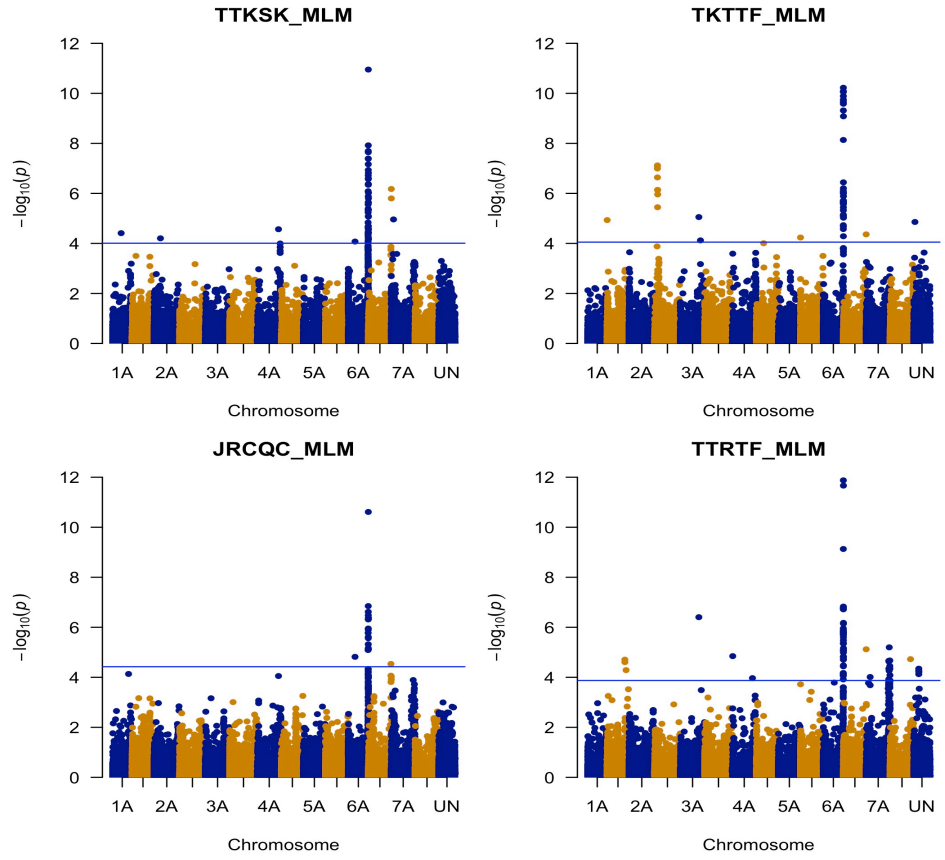


Figure 3. 2. Manhattan plots of GWAS analyses for seedling response of durum wheat lines against four *Pgt* races identified using MLM.

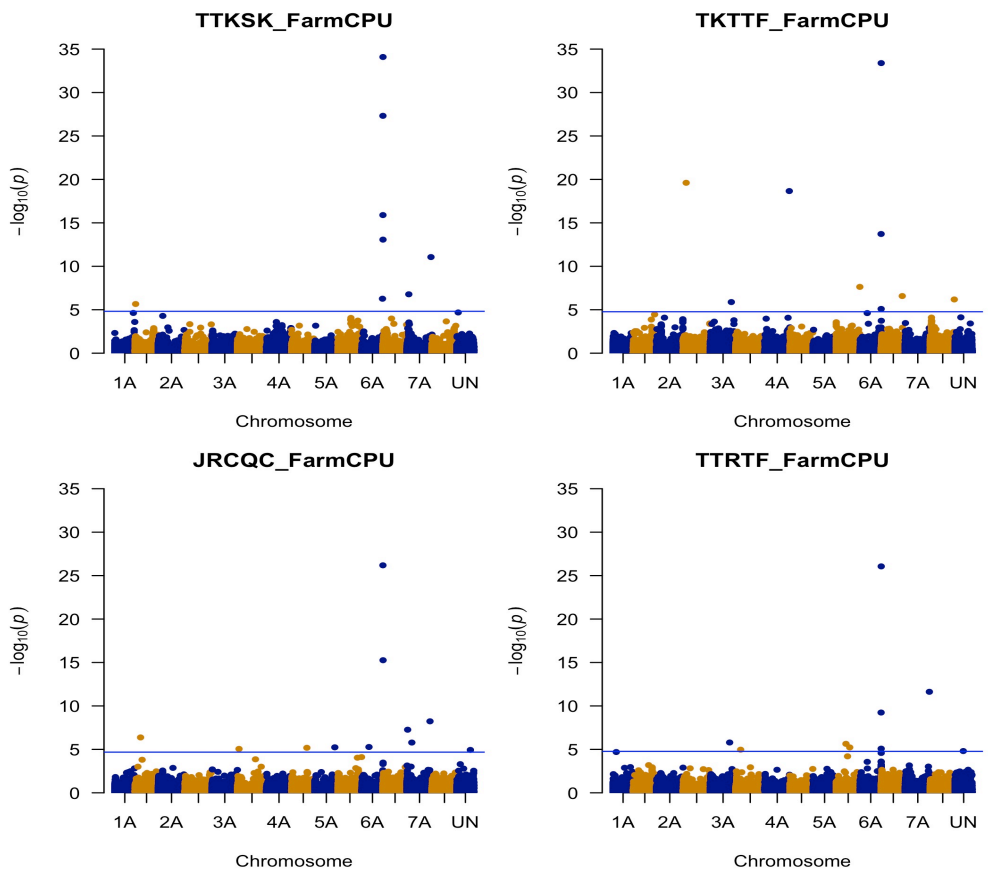


Figure 3. 3. Manhattan plots of GWAS analyses for seedling response of durum wheat lines against four Pgt races identified using FarmCPU.

Table 3. 4. Lists of consistent significant markers between MLM and FarmCPU for seedling resistance against four races and field resistance against the two races across seasons.

Type of resistance	Position	Chr.	Trial
Seedling resistance	89523302	2B	TKTTF
	565464709	3A	TKTTF, TTRTF
	614332431	3A	TKTTF
	205649407	6A	TTKSK, JRCQC
	609635640	6A	TTKSK, TKTTF, JRCQC, TTRTF
	611495915	6A	TTKSK, TKTTF, JRCQC, TTRTF
	612003938	6A	TTKSK, TKTTF, TTRTF
	612043936	6A	TTKSK, TKTTF, TTRTF
	612802438	6A	TTKSK, TKTTF, TTRTF
	613131839	6A	TTKSK, TKTTF, TTRTF
	613294106	6A	TTKSK, TTRTF

	613748730	6A	TTKSK, TKTTF, TTRTF
	615619215	6A	TTKSK, TTRTF, JRCQC
	697030516	7A	TTRTF, TTKSK
	700805183	7A	TTRTF, TTRTF
Field resistance	689821784	5B	TKTTF_MS19, JRCQC_MS19, JRCQC_OS20
	615604035	6A	TKTTF_MS18, TKTTF_MS19, JRCQC_MS19, JRCQC_OS20
	700805183	7A	TKTTF_MS18, TKTTF_MS19, JRCQC_MS19, JRCQC_OS20
	717518884	7A	TKTTF_MS18, TKTTF_MS19, JRCQC_MS19 JRCQC_OS20

Chromosome 6A had the highest number of significant markers (70 markers) with the largest contribution to phenotypic variation (Supplemental Tables S3.1 to S3.5; Figs. 3.2, 3.3). These MTAs were identified using MLM and FarmCPU and grouped into two QTL based on their position and LD. A QTL at 205 Mb identified by both models explained 4.6% of the phenotypic variation for seedling responses to races TTKSK and JRCQC (Supplementary Tables S3.1, S3.3, S3.5). The significant markers that extended from 602 Mb to 615 Mb may represent a single QTL. The phenotypic variation explained by these markers ranged from 4.5% to 14.5% for race TTKSK, 3.2% to 8.8% for race TKTTF, 4.9% to 11.5 for race JRCQC, and 4.2 to 17.1% for race TTRTF. A marker at 611 Mb (611495915 bp) was associated with seedling resistances to all four races and was detected by both MLM and FarmCPU (Table 3.4, Supplementary Tables S3.1 to S3.5). This marker (611 Mb) contributed the most to the phenotypic variation for the seedling response of lines to races TTKSK (R^2 = 14.5%) and JRCQC (R^2 = 11.5%) (Supplemental Tables S3.1, S3.3). Moreover, the 611 Mb marker was in weak to strong LD (r^2 = 0.13 to 0.75) with the significant markers extending from 602 to 610 Mb except one at 608 Mb (Fig. 3.8). Markers at

612 Mb (612832613 bp) and 613 Mb (613131839 bp) contributed the most to the phenotypic variation for the seedling response to races TKTTF (R^2 = 8.8%) and TTRTF (R^2 = 17.1%), respectively (Supplementary Table S3.2, S3.4). These two markers were consistent between these two races and were in strong LD (r^2 = 0.94). They were in weak to strong LD (r^2 = 0.12 to 0.98) with 36 significant markers extending from 612 Mb to 615 Mb (Figs. 3.6, 3.10). All the significant markers on chromosome 6A extending from 602 Mb to 615 Mb except 21 markers were in weak to moderate LD with the *Sr13* marker (r^2 = 0.10 to 0.40) (Fig. 3.10). On chromosome 6B, five significant MTAs representing three putative QTL were identified (Supplemental Tables S3.1 to S3.5). A QTL tagged by two markers at 698 Mb (LD, r^2 = 0.93) identified using MLM was associated with seedling resistance to race TTKSK and explained 7.2% of the phenotypic variation on average. A region at 693 Mb identified using MLM for seedling resistance against races TKTTF and TTRTF explained 3.3% and 5.7% of the phenotypic variation, respectively (Supplemental Table S1). An MTA at 609 Mb was detected using FarmCPU for seedling resistance to TKTTF (Supplemental Table S3.5).

On chromosome 7A, 19 significant markers representing five putative QTL were identified using MLM and FarmCPU (Supplemental Tables S3.1 to S3.5; Figs. 3.2, S3.3). Four of the QTL represented by single markers were associated with seedling resistance to races TTKSK (51 Mb, 67 Mb) and JRCQC (17 Mb, 139 Mb). The fifth QTL represented by 14 significant markers extending from 668 Mb to 721 Mb was associated with seedling resistance to races TTKSK, JRCQC and TTRTF. These 14 markers were in moderate to strong LD (r^2 = 0.29 to 0.98) and explained

3.3% to 5.8% of the phenotypic variation (Fig. 3.9). On chromosome 7B, significant MTAs were identified for seedling resistance against races TTRTF at 622 Mb using MLM and TKTTF at 698 Mb using FarmCPU (Supplemental Tables S3.1 to S3.5). For race JRCQC, MLM identified the QTL on chromosomes 6A only (Supplemental Table S3.3, Fig. 3.2) while FarmCPU identified additional QTL on chromosomes 1B, 3B, 4B, 5A and 7A, albeit represented by single markers (Fig.3.3; Supplemental Table S3.5).

GWAS for field response to JRCQC and TKTTF

The BLUPs estimated from the respective models fitted on field responses were used as response variables to fit GWAS models. A total of 108 significant markers distributed on the 14 chromosomes and unaligned contigs were identified using MLM for field resistance against JRCQC and TKTTF across two seasons (Supplemental Table S3.6, Fig. 3.4). Among the significant markers, 12%, 23.2% and 23.1% were associated with field resistance to four, three and two of the four race-season combinations, respectively and 41.7% were associated with field resistance to different single race-season combinations (non-overlapped region on the Venn diagram) (Fig. 3.7). The consistently significant markers across two to four race-season combinations were located on chromosomes 1B, 3B, 4A, 5B, 6A, 6B, 7A and on unaligned contigs (Fig. 3.7, Supplemental Table S3.11). Among the total MTAs identified by MLM, 101 were on known chromosomal regions and grouped into 19 QTL represented by single and multiple nearby markers (Supplemental Table S3.6, Fig. 3.4). FarmCPU identified 19 significant MTAs on nine chromosomes (none on 1B, 2A, 2B, 3A and 4A) that were grouped into 16 QTL (Supplemental Table S3.10;

Fig. 3.5). Among those, three QTL on chromosomes 5B (689 Mb), 6A (615 Mb), and 7A (700 Mb and 717 Mb), were consistent between MLM and FarmCPU (Table 3.4; Supplemental Table S3.12; Fig. 3.7).

On chromosome 1A, an MTA was identified at 566 Mb for field resistance in TKTTF_MS18 using FarmCPU (Supplemental Table S3.10; Fig.3.5). On chromosome 1B, three significant markers (11 Mb, 551Mb, 587 Mb) were identified using MLM. The regions at 11 Mb and 551 Mb were associated with field resistance in JRCQC_OS20 and TKTTF_MS19, respectively. The 587 Mb locus was associated with field resistance in JRCQC_MS19 and TKTTF_MS19 and it explained 6.7% and 5.7% to the phenotypic variation, respectively (Supplemental Table S3.6, S3.7, Fig. 3.4). On chromosome 2A, FarmCPU identified significant MTA at 728 Mb for field resistance in TKTTF_MS19 (Supplemental Table S3.6).

On chromosome 3B, four significant MTAs (38 Mb, 55 Mb, 97 Mb, 669 Mb) were identified (Supplemental Tables S3.6 to S3.10). The 55 Mb and 97 Mb regions, representing two QTL, were identified using MLM for field resistance in JRCQC_MS19 and TKTTF_MS19. These two QTL explained 11.7% and 10.5% of the phenotypic variation for field response to races JRCQC and TKTTF, respectively (Supplemental Table S3.6, S3.9). The MTAs at 38 Mb and 669 Mb regions identified by MLM and FarmCPU, respectively were associated with field resistance in JRCQC_MS19 (Supplemental Tables S3.6 to S3.10).

On chromosome 4A, an MTA at 619 Mb identified by MLM explained 8.6% of the phenotypic variation in JRCQC_MS19 and on average 5.9% of the phenotypic variation in TKTTF_MS18 and TKTTF_MS19 (Supplemental Table S3.8, S3.9). On

chromosome 4B, an MTA at 470 Mb was identified using FarmCPU for field resistance in JRCQC_OS20 (Supplemental Table S3.10; Fig. 3.5).

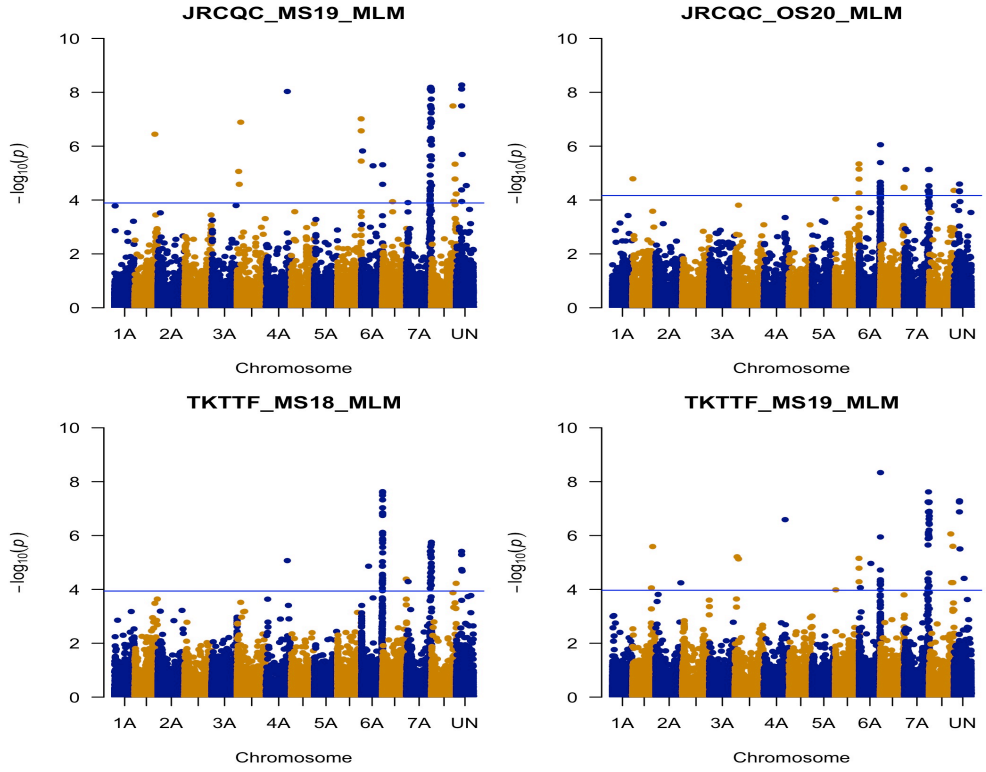


Figure 3. 4. Manhattan plot of GWAS analyses for field response of durum wheat lines against two *Pgt* races identified using MLM.

On chromosome 5A, MTAs were identified using FarmCPU at 429 Mb and 527 Mb for field resistance in JRCQC_OS20 and TKTTF_MS19, respectively (Supplemental Table S3.10; Fig. 3.5). Seven significant markers were identified on chromosome 5B using both models. Three MTAs from 689 Mb to 692 Mb (LD, r^2 = 0.85 to 0.98) representing the same QTL were consistently identified for field resistance against JRCQC across the two seasons and TKTTF_MS19. The 689 Mb locus identified by both MLM and FarmCPU contributed 5.2% to 7.4% to the phenotypic variation for field response against the two races (Supplemental Tables

S3.6, S3.7, S3.9). Two loci identified by FarmCPU at 7 Mb (TKTTF_MS18) and 345 Mb (TKTTF_MS19) were associated with field resistance to race TKTTF (Supplemental Tables S3.10).

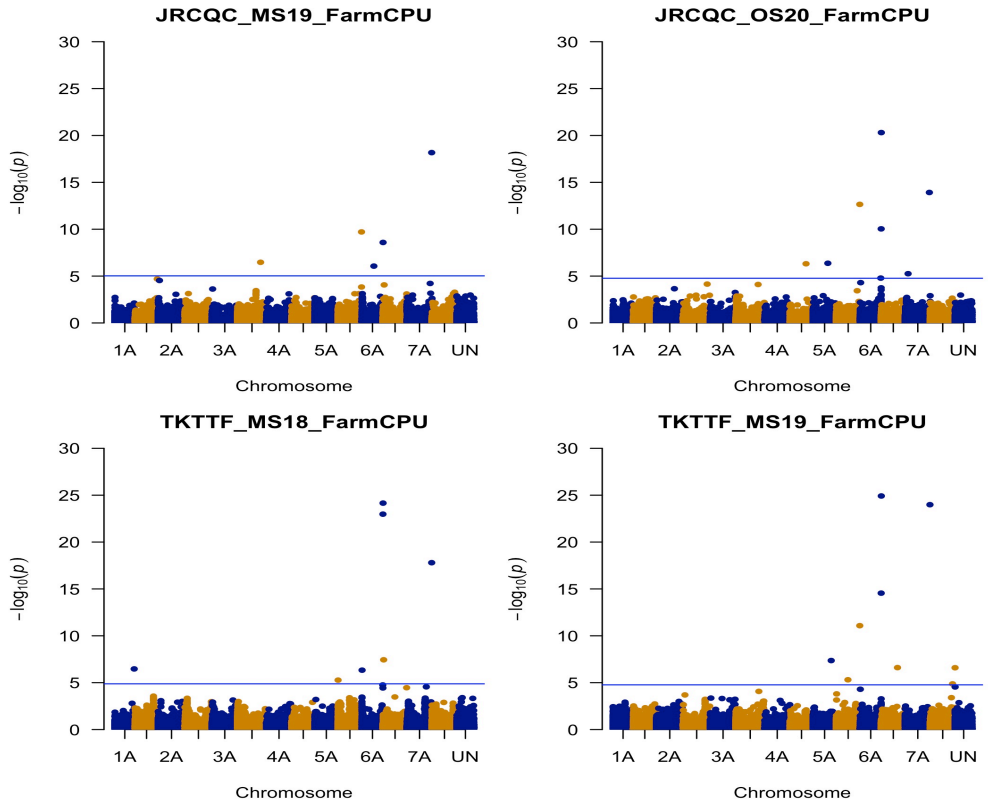


Figure 3. 5. Manhattan plot of GWAS analyses for field response of durum wheat lines against two *Pgt* races identified using FarmCPU.

On chromosome 6A, 39 distinct significant markers representing six QTL were identified using MLM and FarmCPU. Five QTL, at 5 Mb (TKTTF_MS18), 28 Mb (JRCQC_MS19 and TKTTF_MS19), 205 Mb (TKTTF_MS18), 334 Mb (TKTTF_MS19) and 347 Mb (JRCQC_MS19) were represented by single markers. One QTL represented by 34 significant markers spanning from 603 Mb to 615 Mb explained 3.7% to 9.1% of the phenotypic variation (Supplemental Tables S3.6 to

S3.10). For this QTL (603 Mb to 615 Mb), the marker with the highest contribution to the phenotypic variation was located at 615 Mb (615604035 bp) for JRCQC_MS19 (R^2 = 5.3%), TKTTF_MS19 (R^2 = 9.1%), and JRCQC_OS20 (R^2 = 6.5%). This region (615 Mb) was consistently identified by the two models for all race-season combinations and was in LD with markers extending from 612 Mb to 614 Mb and *Sr13* (Table 3.4; Fig. 3.8). For TKTTF_MS18, a marker at 613 Mb (613256520 bp) contributed the most to the phenotypic variation (R^2 = 8.0%) and the 615 Mb region explained 7.0% of the phenotypic variation (Supplemental Table S3.8). These two markers (613 Mb and 615 Mb) were in weak LD (r^2 = 0.13) (Fig. 3.8). On chromosome 6B, FarmCPU identified significant MTAs at 17 Mb and 471 Mb for field resistances in TKTTF_MS18 and TKTTF_MS19, respectively (Supplemental Table S3.10, Fig. 3.5). In the same chromosome, MLM identified a QTL represented by two significant markers (686 Mb and 687 Mb) for field resistance in TKTTF_MS18 and JRCQC_OS20 and it explained 4.2% and 4.5% of the phenotypic variation, respectively (Supplemental Table S3.7, S3.8; Fig. 3.4).

Chromosome 7A harbored the largest number (44) of significant markers representing three putative QTL identified by MLM and FarmCPU (Figs. 3.4, 3.5).

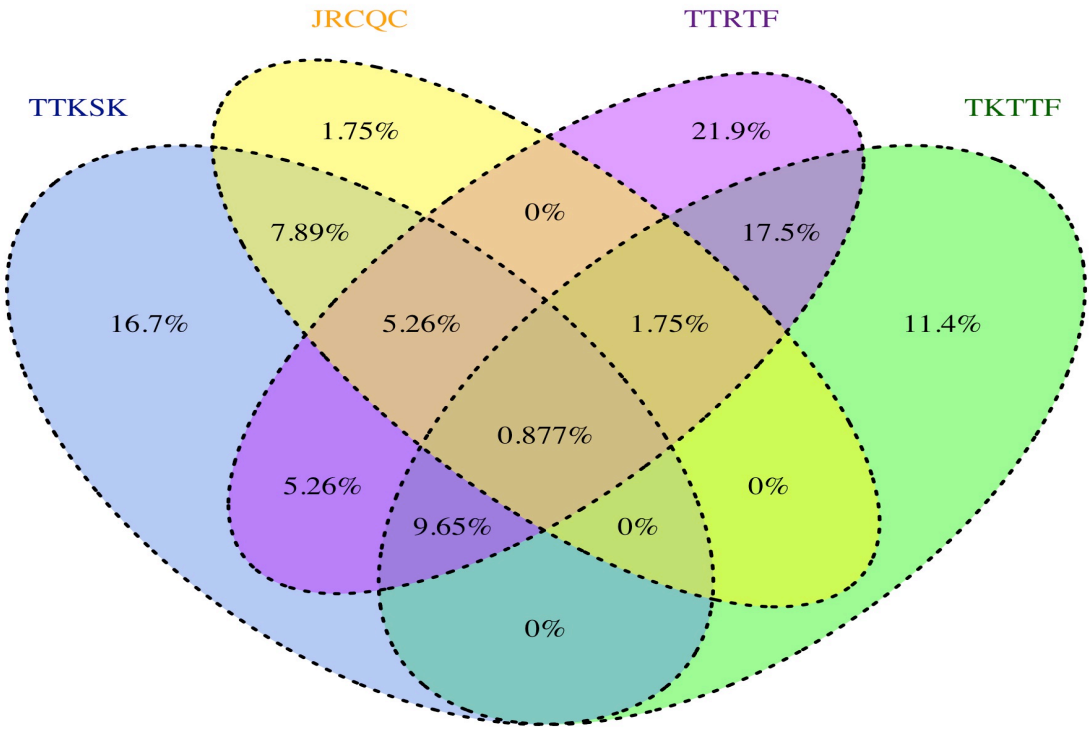


Figure 3. 6. Percentage of common significant markers among seedling responses of lines against four *Pgt* races identified using MLM.

The MTA at 43 Mb identified using MLM was associated with field resistance in JRCQC_OS20 and TKTTF_MS18 (Supplemental Table S3.7, S3.8), while the 81 Mb region identified using FarmCPU was associated with field resistance in JRCQC_OS20 (Supplemental Table S3.10). The remaining 42 MTAs extending from 673 Mb to 727 Mb explained 3.7% to 8.8 % of the phenotypic variation for field responses to races JRCQC and TKTTF across seasons. The markers with the highest contributions to the phenotypic variation were in the 700 Mb region (700805183 bp

and 700727874 bp; R^2 = 5.3 to 8.8%) for field resistance in JRCQC_MS19, JRQC_OS20 and TKTTF_MS19 (Supplemental Tables S3.6, S3.7, S3.9). For TKTTF_MS18, a significant marker at 721 Mb (721720978 bp) contributed the most to the phenotypic variation (R^2 = 5.8%). This marker (721 Mb) was in strong LD (average r^2 = 0.88) with the consistently identified significant markers (700 Mb and 717 Mb) by MLM and FarmCPU across all race-season combinations (Fig. 3.9).

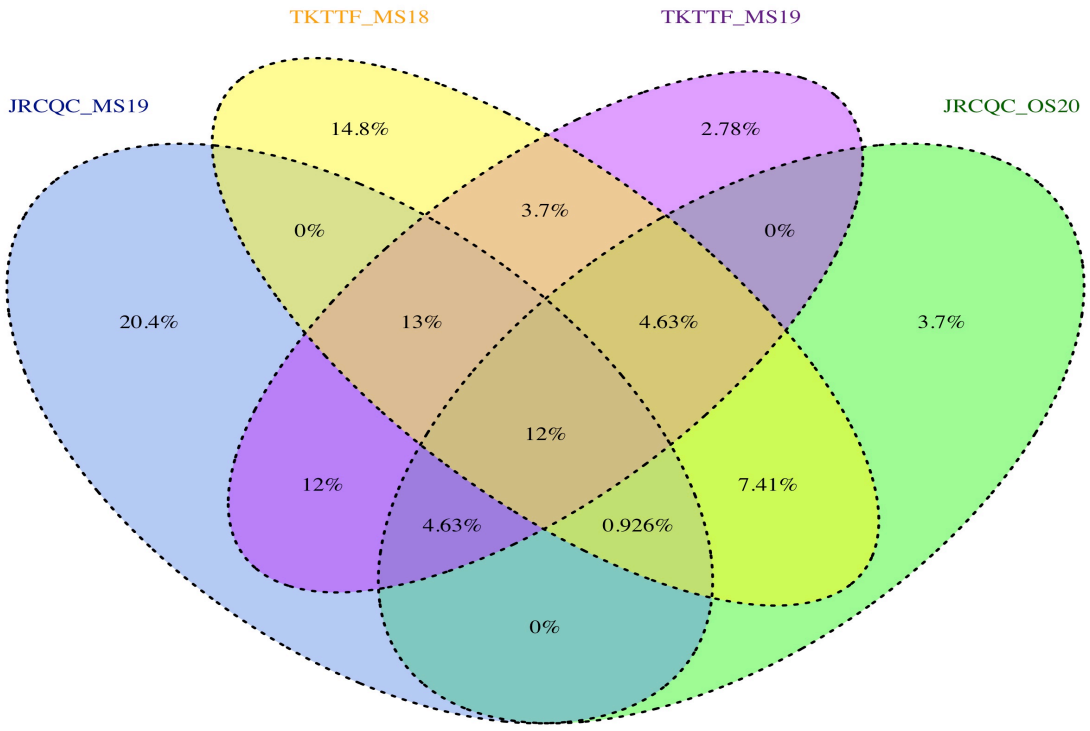


Figure 3. 7. Percentage of common significant markers among field responses of lines against two *Pgt* races across two seasons identified using MLM.

On chromosome 7B, seven significant MTAs were identified using MLM and FarmCPU and five of them represent four QTL (Supplemental Tables S3.6 to S3.10). A locus at 622 Mb (622041448 bp) explained 7.9% and 6.3% of the phenotypic

variation in JRCQC_MS19 and TKTTF_MS19, respectively. This marker (622 Mb) was in strong LD ($r^2 = 0.64$) with a significant marker at 644 Mb and the two may represent the same QTL. Two MTAs at 681 Mb and 683 Mb regions were consistently identified in JRCQC_MS19 and TKTTF_MS19 using MLM (Supplemental Table S3.11). The markers at 281 Mb and 283 Mb regions were physically close but were not in LD and the two QTL explained 4.2% to 5.7% of the phenotypic variation across the two race-season combinations. A QTL at 721 Mb identified using FarmCPU was associated with field resistance in TKTTF_MS19 (Supplemental Table S3.10). Novel loci were consistently identified across races and seasons on chromosomes 3B, 4A, 6A and 7B. Lines that lack *Sr13* and *Sr58* (*Lr46*) on marker screening of the same durum panel with KASP markers designed in the genotyping laboratory and previously reported in Megerssa et al. (2020), carried single to multiple favorable alleles at these novel loci (Supplemental Table S3.13).

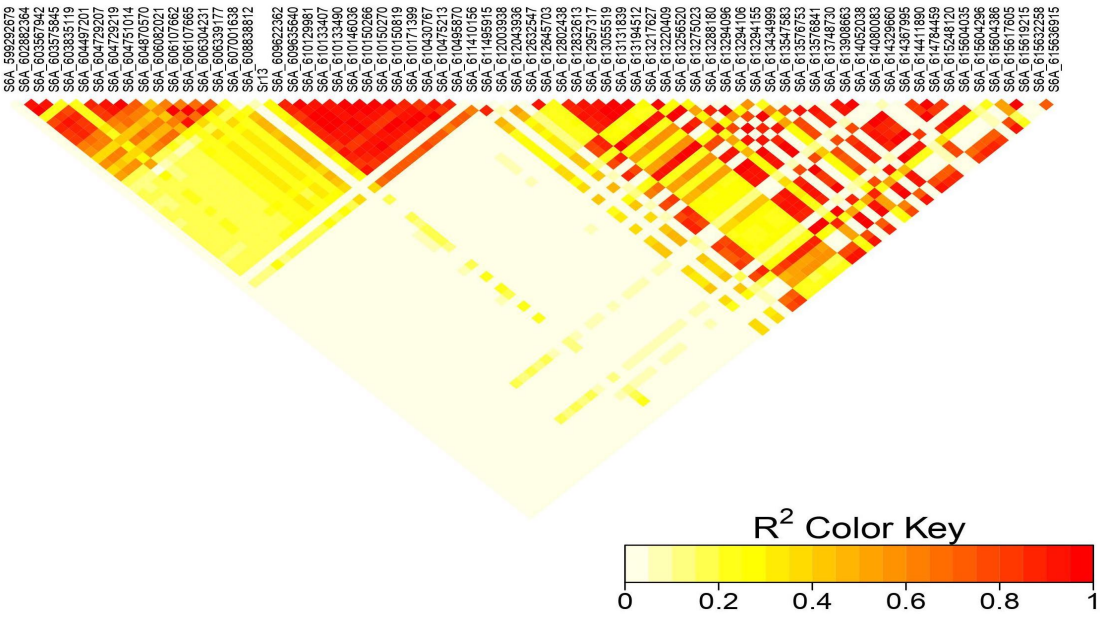


Figure 3. 8. LD heatmap of significant markers on chromosome 6A identified using MLM and FarmCPU for seedling resistance against four Pgt races and field resistance against two races.

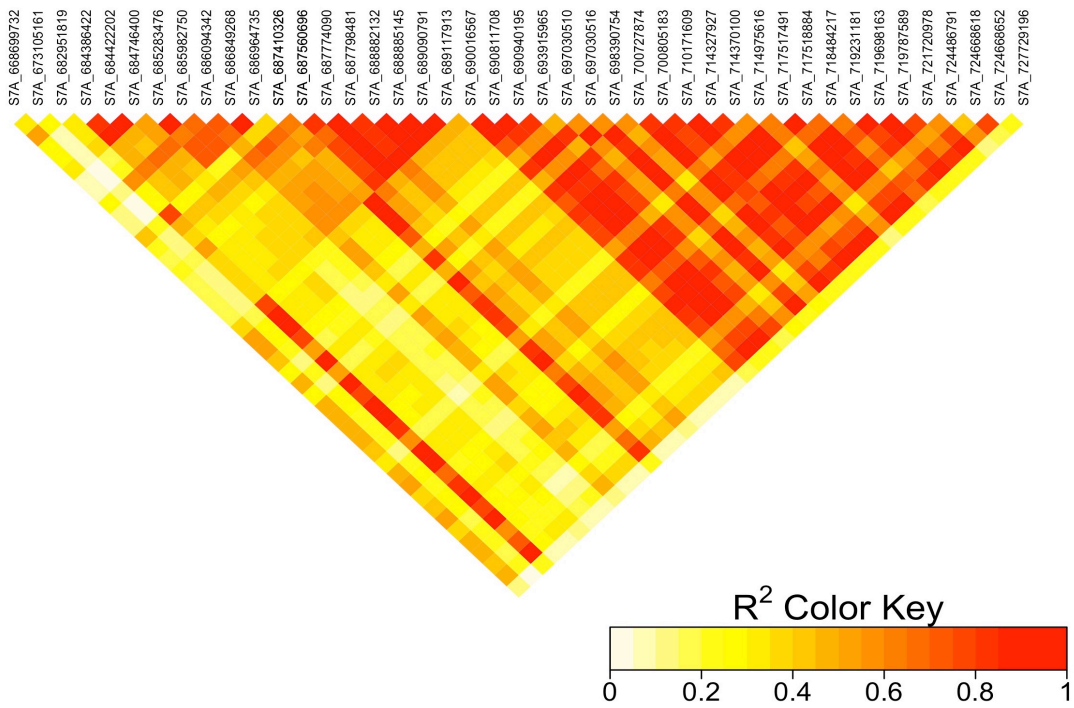


Figure 3. 9. LD heatmap of significant markers on chromosome 7A identified using MLM and FarmCPU for seedling resistance against four Pgt races and field resistance against two races.

DISCUSSION

The utilization of genetic resistance is an ecological and economical approach to manage wheat stem rust in different parts of the world. In the current study, we evaluated a panel of spring durum wheat lines representing the CIMMYT durum wheat germplasm pool for the response to four virulent races of the stem rust pathogen (TTKSK, TKTTF, JRCQC, TTRTF) at the seedling stage and against two of the races (JRCQC and TKTTF) at the adult plant stage. High-density SNP markers were used to fit three GWAS models (MLM, CMLM and FarmCPU) and genomic regions associated with seedling and field resistances were identified for future utilization in resistance breeding.

Phenotypic data analysis

Seedling response to the four *Pgt* races

The high frequency of resistant lines and percentage of phenotypic variance explained by the genotypic component (61% for race TTRTF to 91% for race TKTTF) for response to the four races agrees with the qualitative nature of seedling resistance (Supplemental Fig.S3.1; Table 3.1). However, seedling resistance should be consistent with the field responses to be protective. The relatively lower percentage of lines resistant to races JRCQC (67.1%) and TTRTF (56.4%) compared to races TTKSK (70.6%) and TKTTF (73.1%) is expected because of the documented virulence of the former two races on durum wheat (Olivera et al., 2012b; Olivera Firpo et al., 2019). The seedling resistances observed in the population ranged from single to multiple race resistance indicating the effectiveness of the same resistance source against multiple races (Table 3.2). Our finding of the moderate (0.47) to strong (0.76) correlation among the responses of the lines to the four races further verify this result (Fig. 3.1).

Field response to races JRCQC and TKTTF

Seedling evaluation is the fastest and the cheapest method for screening large number of lines. However, seedling evaluation should be confirmed by field evaluation for resistance to be reliable. Considering CI ≤ 18 (30MSMR) as resistant in the field, the high frequency of susceptible lines for response to race JRCQC and the low frequency for response to TKTTF (TKTTF_MS18) was not surprising as JRCQC is more virulent to *Sr13* than TKTTF which is avirulent on *Sr13* (Supplemental Fig. S3.2). The higher proportion of susceptible lines against race JRCQC compared to race TKTTF (TKTTF_MS18) agrees with the findings of Hundie et al. (2019) on evaluation of 14

durum wheat cultivars against four single races. *Sr13a* is moderately effective against JRCQC however, the high frequency of susceptible lines to this race could also be explained by the reduced effect of this gene under field conditions (Olivera, *unpublished*), or the temperature dependence of *Sr13* effectiveness as reported by Zhang et al. (2017) in greenhouse evaluation of wheat lines which may apply in the field due to the expected seasonal variation in temperature. The low frequency of resistant lines in TKTTF_MS19 was unusual as durum wheat is known to have better resistance against race TKTTF. The lower percentage of phenotypic variance explained by the genotypic component for race JRCQC (59% to 67%) than race TKTTF (74% to 77%) across the two seasons indicates the presence of less variation for resistance to race JRCQC than for TKTTF in the population (Table 3.3). The moderate correlation between the seedling and field response to race JRCQC (0.37 for MS19 and 0.53 for OS20) and TKTTF (0.55 for MS19 and 0.61 for MS18) may indicate that only some of the lines resistant at the seedling stage are consistently resistant in the field. Thus, the lines which showed consistent resistance in the seedling assay and in the field can be deployed as sources of resistance in durum breeding programs and can also be used for combining with known adult plant resistance genes to increase durability of resistance.

Comparison of seedling and field resistance loci with previously published QTL studies and known stem rust resistance genes

Many of the QTL identified in the current study co-located with previously reported QTL markers on tetraploid and hexaploid wheat, and cataloged stem rust resistance genes. On chromosome 1A, a QTL at 566 Mb for field resistance in TKTTF_MS18 may tag a region close to regions reported by Edae et al. (2018) (*IWB45411,* 9 Mb away) and Mihalyov et al. (2017) (*IWA4897,* 11 Mb away) (Fig. 3.5; Supplemental Table S3.10). On chromosome 1B, an MTA at 11 Mb for seedling resistance to race TKTTF and field resistance in JRCQC_OS20 is close to (4.5 Mb away) the *Sr31* locus (Edae and Rouse, 2020). *Sr31* is located on the short arm of chromosome 1B and transferred from rye to hexaploid wheat. This gene has been effective for more than three decades until defeated by the Ug99 race TTKSK (Jin and Singh, 2006; Wanyera et al., 2006). Although *Sr31* is effective against races TKTTF and JRCQC (Olivera et al., 2015), this gene is not expected in the durum panel. So, the 11 Mb locus is a novel region close to *Sr31* (Supplemental Tables S3.2, S3.7). A region at 22 Mb (22978945 bp) associated with seedling resistance against race TTKSK may represent the same region as (2 Mb away) QTL tagging markers *IWB72495* reported by Bajgain et al.(2015b) and *IWA64* reported by Chao et al. (2017) (Fig. 3.3; Supplemental Table S3.5). A QTL at 550 Mb and 551 Mb regions for seedling resistance against race TTRTF and field resistance in TKTTF_MS19 co-locates with (1 Mb to 2 Mb away) a QTL linked marker *barc61* reported by Letta et al. (2014) and is expected to be the same QTL. An MTA at 587 Mb for seedling resistance against race TTRTF, field resistance to JRCQC and TKTTF in the main-season 2019 may map the same region as a QTL tagging marker *IWB40197* (1 Mb away) reported by Edae et al. (2018) (Figs.

3.2, 3.4; Supplemental Tables S3.4, S3.6, S3.9). Chromosome 1BL is known to harbor *Sr14* and the pleiotropic APR gene *Sr58* (*Lr46/Yr29/Pm39*) that are known to be effective against several races (McIntosh et al., 1995; Bhavani et al., 2011). However, none of the loci we detected on chromosome 1B are close to markers associated with *Sr14* (*barc8, wPt1876*) and *Sr58* (*wmc44)* previously reported by Letta et al. (2013).

A single marker (728 Mb) representing a QTL on chromosome 2A associated with field resistance in TKTTF_MS19 is far away from markers reported by Bajgain et al. (2015b) and Mihalyov et al. (2017) and could be a novel locus (Supplemental Table S3.9; Fig. 3.4). Chromosome 2A hosts *Sr38* (transferred from *T.ventricosum*) (Bariana and McIntosh, 1994) which is ineffective against race TKTTF (Olivera et al., 2015; Flath et al., 2018). Eight of the lines in the panel are expected to possess *Sr38* (Ammar, personal communication, 2020) but this region was undetected because it was below the MAF threshold. On chromosome 2B, a QTL associated with markers ranging from 89 Mb to 97 Mb identified for seedling resistance against race TKTTF may map the same locus as a QTL marker *IWA8599* (1 kb to 7 Mb away) reported by Gao et al. (2017) (Supplemental Tables S3.2, S3.5; Figs. 3.2, 3.3).

On chromosome 3A, two QTL identified for seedling resistance to races TTRTF (565 Mb) and TKTTF (565 Mb and 614 Mb) (Supplemental Tables S3.4, S3.2, Figs 3.2, 3.3) were further away from *wmc264* reported by Letta et al. (2014) in the regions of *Sr27* and *Sr35*, and no other nearby regions were previously reported. Moreover, *Sr27* and *Sr35* orginated from *S. cereale* and *T.monococcum,* respectively (McIntosh et al., 1995) and are unlikely to be present in the durum panel suggesting that these two QTL are likely novel. On chromosome 3B, no nearby marker is

previously reported for loci at 40 Mb, 55 Mb, 97 Mb, and 38 Mb (Supplemental Tables S3.5, S3.6, S3.9, Figs. 3.3 to 3.5). Chromosome 3BS harbors the known adult plant resistance gene (*Sr2*) that originated from tetraploid wheat (*T.turgidum* var. dicoccum) (McIntosh et al., 1995) however, screening of the panel of lines with an *Sr2* linked marker reported in a different study on the same panel (Megerssa et al., 2020) indicated that this gene was absent in the panel. So, these four QTL are likely to be novel.

An MTA at 17 Mb (17308554 bp) on chromosome 4A associated with seedling resistance to race TTRTF co-locates (789 kb away) with a QTL marker *IWB40004* reported by Bajgain et al. (2015b) (Supplemental Table S3.4; Fig. 3.2). None of the markers previously reported by several authors (Yu et al., 2011; Letta et al., 2013, 2014; Bajgain et al., 2015b; Chao et al., 2017; Gao et al., 2017) were close to a QTL at 619 Mb region of chromosome 4A that was associated with seedling resistance against race TTRTF and field resistance in JRCQC_MS19, TKTTF_MS18, and TKTTF_MS19 (Supplemental Tables S3.4, S3.6, S3.8, S3.9; Figs. 3.2, 3.4). Therefore, the 619 Mb (619746683 bp) locus could be novel for multiple-race specific resistance including the durum virulent races. A QTL at 651 Mb region associated with seedling resistance against race TTKSK maps a region close to a QTL flanking marker (*wPt5857,* 1Mb away) reported by Yu et al. (2012) and a region associated with *barc78* (4 Mb away) reported by Letta et al. (2014) (Fig. 3.2; Supplemental Table S3.1). A region at 718 Mb (718944322 bp) associated with seedling resistance to race TKTTF co-locates with several markers reproted by Bajgain et al. (2015b) including *IWB34733, IWB3569, IWB61312* (809 kb away) for seedling resistance of spring

wheat collections against TKTTF, marker *IAAV3545* (809 kb) reported by Edae et al. (2018) for seedling resistance of spring wheat against race RCRSC, several markers reported by Edae and Rouse (2020) for resistnace of spring wheat against races TKTTF isolate from Ethiopia, (TKTTF-ETH, the closest marker is 5.6 Mb away) and TTRTF (2 Mb away), marker *IWA4651* (324 kb) linked to *Sr7a* reported by Gao et al. (2017) for seedling resistance of spring wheat against race TTTTF (Fig. 3.3; Supplemental Table S3.5). Olivera et al. (2015) and Bajgain et al. (2015b) reported that *Sr7a* is effective against race TKTTF isolate from Ethiopia but not against the isolate from Germany (Olivera Firpo et al., 2017). So, based on the proximity to previously reported loci and the race specificity the 718 Mb region likely maps to the *Sr7a* locus. No markers close to the MTAs at 444 Mb (JRCQC) and 740 Mb (JRCQC_OS20) on chromosome 4B were previously reported. These two loci are possibly novel, but they were only identified at the seedling stage and in one season (Supplemental Tables S3.3, S3.10; Figs. 3.3, 3.5).

On chromosome 5A, an MTA at 527 Mb associated with field resistance in TKTTF_MS19 may be close to a QTL marker *IWA2836* (9 Mb away) reported by Bajgain et al. (2015b). A QTL linked marker for resistance of spring wheat against race TTRTF reported by Edae and Rouse (2020) match the 581 Mb locus (5.3 Mb away) associated with seedling resistance to race JRCQC (Supplemental Table S3.5; Figs. 3.3). A QTL represented by significant markers at 689 Mb, 691 Mb and 692 Mb on chromosome 5B co-locate with simple sequence repeat (SSR) markers flanking the region of an all stage resistance gene *Sr49* reported by Bansal et al. (2015) (Supplemental Tables S3.5 to S3.7, S3.9, S3.10; Figs.3.3 to 3.5). The consistency of

this QTL (689 Mb to 692 Mb) across races (JRCQC and TKTTF), seasons, growth stages (seedling and adult) and the two GWAS models suggests the reliability of the QTL and the association with multiple-race specific resistance at all growth stages although limited by the low MAF (0.05) (Table 3.4, Figs. 3.6, 3.7). Increasing the frequency of the favorable allele at this locus in the durum breeding lines and incorporating them in future varieties with other resistance genes may prolong the protection against the virulent race JRCQC.

Chromosome 6A harbored six QTL represented by single and multiple markers (Figs. 3.2 to 3.7). A QTL at 5 Mb (5058172 bp) region associated with field resistance in TKTTF_MS18 is very close to QTL tagging markers *IWA7913* (138 kb) and *IWB23519* (146 kb) reported by Bajgain et al. (2015b), *IWB72958* (138 kb) reported by Nirmala et al. (2017) as a predictive marker for *Sr8155B1,* markers *IWA7913* (138 kb) and *S6A_PART1_3015737*/*S6A_PART1_3206675* (2Mb away) associated with *Sr8a* reported by Guerrero-Chavez et al. (2015) and Edae and Rouse (2020), respectively. *Sr8155B1* is effective against several races but not TTKSK and JRCQC at the seedling stage (Nirmala et al., 2017) and *Sr8a* is ineffective against race TKTTF (Olivera et al., 2015). Thus, the 5 Mb region likely represent *Sr8155B1* or a new allele of *Sr8* (Supplemental Table S3.10; Fig. 3.5). No marker close to the QTL at 28 Mb, 205 Mb, 334 Mb and 347 Mb was previously reported, and these four QTL are likely to be novel. In addition, consistency of the QTL at 28 Mb, 205 Mb and 334 Mb across races, races and models, and races, respectively suggests the reliability of the QTL and the association with multiple-race specific resistance including the durum virulent race JRCQC (Supplemental Tables S3.1, S3.3, S3.6, S3.8, S3.9). However, further study

and validation of these loci is needed. A QTL represented by the markers spanning 602 Mb to 615 Mb (69 markers) collocated with several previously reported markers in the region of *Sr13* including *CD926040* and *barc104* (Simons et al., 2011; Letta et al., 2013, 2014), *BE471213, BE403950, CK207347* (Simons et al., 2011; Bhavani et al., 2019), *CJ641478*, *CJ6719993* and *CJ666008* (Zhang et al., 2017), *IWA4918* (Chao et al., 2017), *IWA7495* (Simons et al., 2011). Moreover, screening of the same panel of lines with a marker linked to *Sr13* reported in Megerssa et al. (2020) indicated that 69% of the lines in the panel carry *Sr13*. It is known that *Sr13* with its alleles are the mainly used stem rust resistance genes in durum wheat cultivars and germplasm worldwide (Qamar et al., 2009; Olivera et al., 2015; Singh et al., 2015). Different alleles of *Sr13* are expected to be present in the durum panel based on the race specificity and the weak to strong LD with the *Sr13* linked marker (Supplemental Tables S3.1, S3.2; Fig.3.8). *Sr13a* (R1 and R3 haplotypes in Zhang et al., 2017) is effective against races TTKSK, TKTTF, JRCQC and TTRTF (Zhang et al., 2017; Olivera Firpo et al., 2019), whereas *Sr13b* (R2 haplotype in Zhang et al., 2017) is effective against the former two races but not against the latter two (Olivera et al, 2012b; Olivera et al., 2019; Zhang et al., 2017; Randhawa et al., 2018). Accordingly, the SNP at 611 Mb (6A_611495915) that was consistently detected for seedling resistance to the four races may identify allele *Sr13a.* Moreover, a marker at 615 Mb (6A_615604035) was consistent across races TKTTF, JRCQC and TTRTF at the seedling stage and all race-season combinations in the field. However, differences were observed in the direction of the effect on the response and the allele frequency of markers in LD with 6A_615604035 indicating that this region could be novel or the

region of *Sr13a* based on the effectiveness against the four races that might be originated from different sources (Supplemental Tables S3.1, S3.3, S3.4). There was no significant SNP specifically shared between TTKSK and TKTTF only (0%, Fig. 3.6), but based on the race specificity and infection types (IT) on 24 lines we were able to postulate *Sr13b* (Supplemental Table S3.13). A marker at 612 Mb (6A_612003938) that was identified using FarmCPU for seedling resistance against race TTKSK may map the region of *Sr13b*. The detection of the favorable allele at this locus (6A_612003938) in 18 of the 24 lines that showed low IT to races TTKSK and TKTTF may support our postulation of *Sr13b* (Supplemental Table S3.13). The identification of three markers (606107662 bp, 606304231 bp, 607001638 bp) that were in LD with SNPs from 602 Mb to 611 Mb (*Sr13a* region) (Fig. 3.8) for response to JRCQC in the off-season 2020 only could be in agreement with the results reported by Zhang et al. (2017) which indicated the effectiveness of *Sr13* at high temperature, but additional season data is needed to confirm the result. The 615 Mb (6A_615604035) region identified across all race-season combinations may indicate the effectiveness of the resistance at this locus regardless of the temperature variation in the main and off-seasons. Nevertheless, the *Sr13* region on chromosome 6A needs further study to survey the presence of other alleles and develop markers that are reliably allele-specific.

Several markers (108.9 cM to 119 cM) reported by Bajgain et al. (2015b) are very close (195 kb to 4 Mb) to a QTL at 686 Mb and 687 Mb regions on chromosome 6B (Supplemental Table S3.3, S3.7; Figs. 3.2, 3.4). The closest markers that map the location of *Sr11*, *IWB59175.2* and *IWA4246* are 195 kb and 501 kb away from the

QTL markers 6B_687598497 and 6B_686489689, respectively. Olivera et al. (2015) reported low infection response (2) of lines carrying *Sr11* against TKTTF and high for JRCQC (3^{+}) at the seedling stage, but the MTA we detected was at both growth stages for JRCQC and field resistance against TKTTF (Supplemental Tables S3.3, S3.7, S3.8). This region is close to the *Sr11* locus but could very well be novel given the known effects of *Sr11*. A QTL at 693 Mb identified for seedling resistance against races TKTTF and TTRTF is close to (492 kb to 1 Mb away) several markers (120.3 cM to 122.9 cM) associated with *Sr11* reported by Bajgain et al. (2015b). The closest marker (*IWB46893*) is 492 kb away suggesting that the 693 Mb (693829939 bp) region may be the *Sr11* locus. Further study on the effectiveness of *Sr11* against the durum virulent race (TTRTF) in the field is needed (Supplemental Tables S3.2, S3.4; Fig. 3.2).

Chromosome 7A harbored seven QTL represented by single and multiple markers (Supplemental Tables S3.1 to S3.4; Figs. 3.2 to 3.5). QTL markers *wmc479* reported by Letta et al. (2013) and *IWA7200* reported by Chao et al. (2017) match loci at 17 Mb (17624367 bp, 2 Mb away) associated with seedling resistance to JRCQC and at 67 Mb (67384663 bp, 6 Mb away) associated with seedling resistance against race TTKSK, respectively (Supplemental Tables S3.1, S3.5; Figs. 3.2, 3.3). No QTL marker close to the loci at 43 Mb, 51 Mb, 81 Mb and 139 Mb has been reported previously but only the 43 Mb locus could be a true association as it was consistent between JRCQC_OS20 and TKTTF_MS18 (Supplemental Tables S3.5, S3.7, S3.8, S3.10). For a QTL represented by the significant markers spanning 668 Mb to 727 Mb (43 markers), the most significant markers (700 Mb and 717 Mb) that were in LD with

the rest of the MTAs co-locate with the region of *Sr22* (Fig. 3.9). Markers *IWB5070, IWB1874, IWB1830, IWB62560* reported by (Bajgain et al., 2015b) are 2 Mb away from the 700 Mb locus while *IWB48466* is 5 Mb away from the 717 Mb region. The origins of *Sr22* are *T. boeoticum* and *T. monococcum* (Periyannan et al., 2011) and this gene is effective against several stem rust races including the Ug99 groups of races, JRCQC, TTRTF and several other races in North America (Olivera et al., 2012b; Olivera Firpo et al., 2019). Similarly, we detected this QTL for seedling resistance against all four races and field resistance against the two races using the two GWAS models (Table 3.4, Supplemental Table S3.11, S3.12). The 721 Mb region in the same QTL co-locates (718 kb away) with a marker in the region of *Sr25* (*BF145935*) (Liu et al., 2010) and 15 lines are known to carry *Sr25* (Ammar, personal communication, 2020).

On chromosome 7B, a QTL at 622 Mb and 644 Mb identified for seedling resistance against race TTRTF, field resistance in TKTTF_MS19 and JRCQC_MS19 is close to (between 7 Mb and 14 Mb) marker *wmc517* at the *Sr17* locus reported by Letta et al. (2014) (Supplemental Tables S3.4, S3.6, S3.8). Low infection type to race TKTTF ($< 2^+$) (Olivera et al., 2015) and high infection type to race JRCQC ($> 2^+$) (Olivera et al., 2012b) were reported at the seedling stage on differential lines carrying *Sr17*, however we detected the association at the adult plant stage for both races which indicates that the region could be close to *Sr17* but novel. Letta et al. (2013) also reported a QTL flanking marker *wPt4045* as *Sr17* locus and a QTL at 698 Mb identified for seedling resistance against race TKTTF is 873 kb away from this marker and may represent the *Sr17* region (Supplemental Table S3.5; Fig.3.3). An MTA at

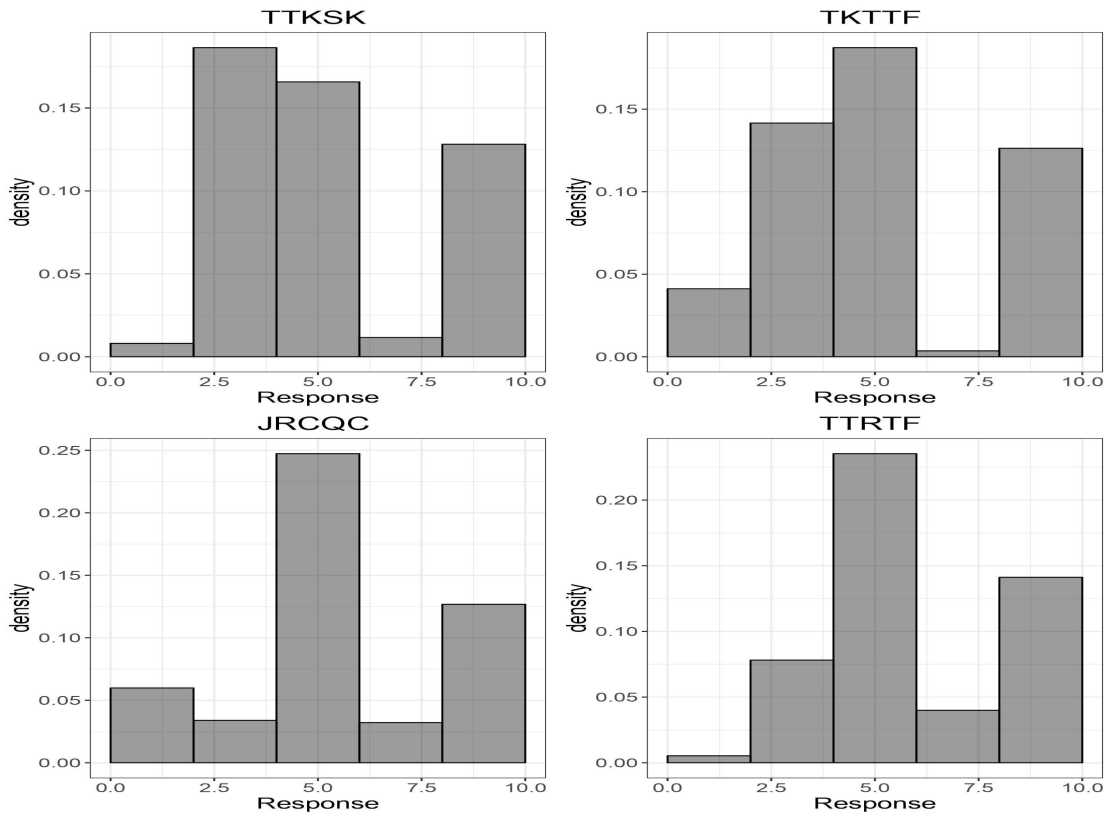
681 Mb associated with field resistance in TKTTF_MS19 and JRCQC_MS19 is 4 Mb away from a QTL flanking marker *wPt4258* reported by Yu et al. (2014) and may be the same locus. A QTL at 683 Mb (not in LD with 681 Mb marker) associated with field resistance in TKTTF_MS19 and JRCQC_MS19 may represent the same regions as a QTL identified by markers *wPt1715*, *wPt4298* and *wPt7191* (3 Mb away) reported by Letta et al. (2013) (Fig. 3.4; Supplemental Table S3.6, S3.9). A QTL flanking marker (*wpt8007)* reported by Yu et al. (2014) (2.6 Mb away) and a locus associated with resistance of spring wheat against race TKTTF-ETH reported by Edae and Rouse (2020) may map the same region as the 721 Mb locus identified in TKTTF_MS19 (Supplemental Table S3.10). We were unable to determine the position of nine significant MTAs that were identified on unaligned contigs.

CONCLUSION

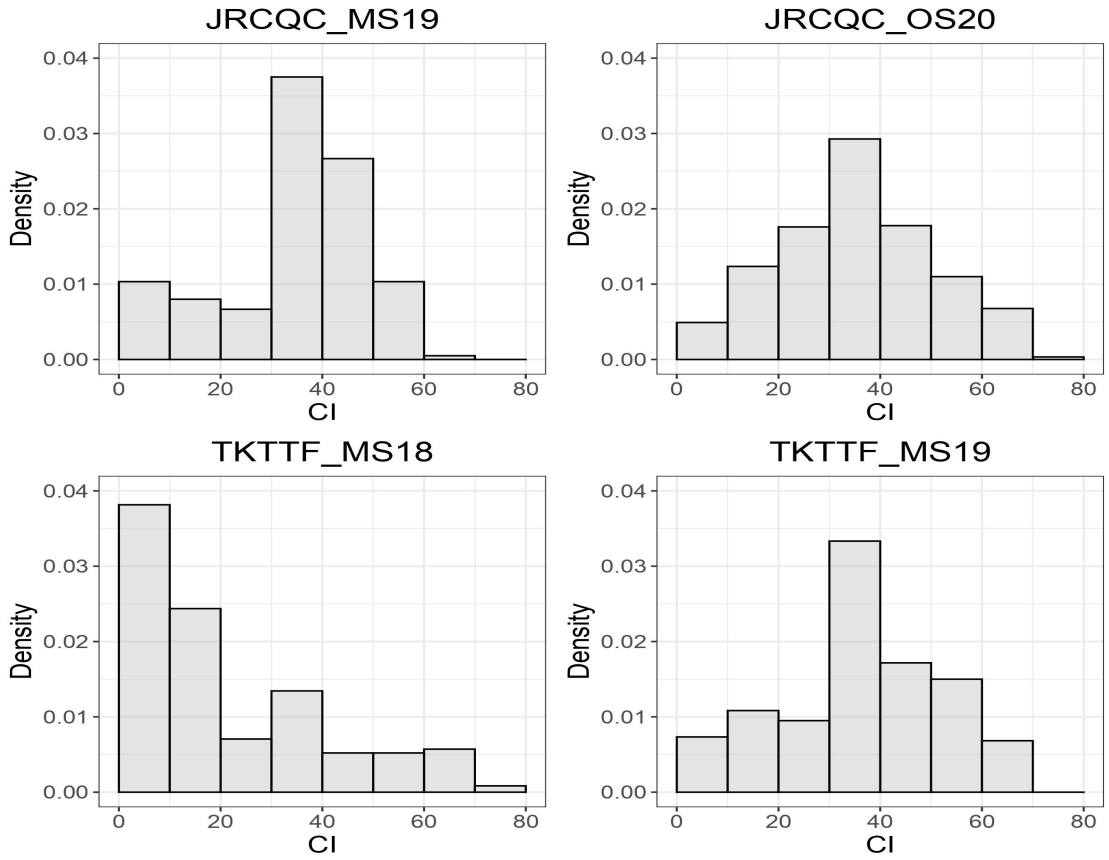
This study revealed that the CIMMYT durum wheat breeding lines harbor race-specific and multiple-race resistance to virulent *Pgt* races at the seedling and adult plant stages. Lines consistently resistant in the seedling assay and in the field are being used as sources of resistance in the durum wheat breeding program. We have identified several QTL for resistance to virulent stem rust races at the seedling stage and in the field. Among the 17 QTL identified using MLM for seedling resistance against the four races, eight are putatively novel and among the 20 QTL identified using FarmCPU, 11 are putatively novel. Among the 19 QTL identified using MLM for field resistance against races JRCQC and TKTTF, 12 are putatively novel and among the 16 QTL identified by FarmCPU, seven are putatively novel. Therefore, the stem rust resistance in this study population is controlled by multiple genes. QTL

represented by single markers that were not consistent across races and seasons should be verified before use in future resistance breeding. The markers linked to the six QTL for seedling resistance and three QTL for field resistance that were consistent between the two models can be reliably used in MAS once validated in different populations. Two large effect markers in the region of *Sr13* on chromosome 6A that were consistent between races, seasons and models may identify the *Sr13* haplotypes in different population or *Sr13a* and novel region effective against multiple races. Since the resistance allele at the *Sr49* locus was rare in the population and this gene is effective against multiple races, this gene should be retained in future selections if no known linkage drag is associated with it. The contribution of the *Sr22/Sr25* region on chromosome 7A to the phenotypic variance was comparable to the *Sr13* region however, these genes are associated with undesirable agronomic features such as low kernel weight and reduced yield. New recombinant lines less defective in such traits but harboring these genes, either individually or together, are being developed for further evaluation. The evaluation of a panel of lines against virulent races of *Pgt* at the seedling stage and in the field enabled us to identify novel QTL regions specific to the durum virulent races that are consistently identified for other races. Therefore, the novel loci on chromosomes 3B, 4A, 6A, 6B, 7A and 7B are regions to be validated for use as novel sources of resistance and strategically used in breeding programs. Identification of sources of adult plant resistance is also very important in future resistance breeding of durum wheat against stem rust.

Lists of supplemental figures



Supplemental Figure 3. 1. Distribution of seedling responses of durum wheat lines against four Pgt races. Data was the linearized scale of the 0-4 IT score to 0-9 scale.



Supplemental Figure 3. 2. Distribution of field responses of durum wheat lines against two Pgt races. Data was the coefficient of infection (CI). JRCQC_MS19 and JRCQC_OS20 refer to JRCQC main-season 2019 and off-season 2020, respectively while TKTTF_MS18 and TKTTF_MS19 refer to TKTTF main-season 2018 and 2019, respectively.

Lists of supplemental tables

Supplemental Table S3.1**.** Lists of SNPs significantly associated with seedling resistance to TTKSK identified using MLM.

SNP	Chr.	Position	P.value	MAF	FDR.Adj. P.value	Effect	R2	Proposed gene/allele
S1A_258973737	1A	258973737	3.89E-05	0.056	0.02433907	1.21626151	5.03579733	Likely novel
S2A_194023441	2A	194023441	6.26E-05	0.051	0.03593269	1.31329603	4.76022417	_
S4A_651298931	4A	651298931	2.71E-05	0.076	0.01934862	1.46763626	5.24792999	Yu et al. (2012);Letta et al.(2014)
S6A_205649407	6A	205649407	8.44E-05	0.257	0.04375249	0.63210517	4.58722923	Likely novel
S6A_602882364	6A	602882364	4.71E-05	0.486	0.02831573	-0.6245292	4.92475227	Sr13
S6A_603567942	6A	603567942	9.01E-05	0.480	0.04579339	-0.6073627	4.5497345	Sr13
S6A_603575845	6A	603575845	2.20E-05	0.486	0.01712337	-0.6482586	5.36906516	Sr13
S6A_604497201	6A	604497201	1.46E-05	0.451	0.01205718	-0.6606369	5.6109981	Sr13
S6A_604729207	6A	604729207	3.57E-05	0.486	0.02372762	-0.6487849	5.08603798	Sr13
S6A_604729219	6A	604729219	2.30E-05	0.498	0.01734868	0.66507995	5.34439656	Sr13
S6A_604751014	6A	604751014	3.96E-05	0.496	0.02433907	-0.6418925	5.02619739	Sr13
S6A_604870570	6A	604870570	8.04E-05	0.440	0.04250448	0.6149627	4.61536253	Sr13
S6A_606082021	6A	606082021	5.72E-06	0.464	0.00540054	0.66408167	6.16602377	Sr13
S6A_606107662	6A	606107662	8.20E-07	0.366	0.00127585	0.80060826	7.33432927	Sr13
S6A_606107665	6A	606107665	2.76E-06	0.467	0.00303895	0.68326237	6.60196705	Sr13
S6A_606304231	6A	606304231	4.63E-06	0.371	0.00453238	0.74315416	6.29220247	Sr13
S6A_606339177	6A	606339177	4.15E-06	0.451	0.00438809	0.67517344	6.35749251	Sr13
S6A_608838812	6A	608838812	5.58E-05	0.170	0.03275734	0.70035087	4.8270892	Sr13
S6A_609622362	6A	609622362	1.92E-07	0.168	0.00050864	0.96468272	8.22164231	Sr13
S6A_609635640	6A	609635640	2.03E-08	0.150	0.00014952	1.11145443	9.62142948	Sr13
S6A_610129981	6A	610129981	6.83E-08	0.156	0.00025806	1.05002415	8.8624855	Sr13
S6A_610133407	6A	610133407	4.12E-08	0.163	0.00018149	1.06726638	9.17802144	Sr13
S6A_610133490	6A	610133490	2.66E-07	0.159	0.0005864	1.00662587	8.02198841	Sr13
S6A_610146036	6A	610146036	1.17E-07	0.156	0.00038577	1.04188201	8.53016582	Sr13
S6A_610150266	6A	610150266	1.32E-06	0.150	0.00183844	0.95840258	7.04548882	Sr13
S6A_610150270	6A	610150270	4.53E-07	0.154	0.00079851	1.00225375	7.69613524	Sr13
S6A_610150819	6A	610150819	2.26E-08	0.159	0.00014952	1.08661762	9.55342224	Sr13
S6A_610171399	6A	610171399	4.06E-08	0.178	0.00018149	1.00245433	9.18691553	Sr13
S6A_610430767	6A	610430767	2.24E-06	0.145	0.00269395	0.96739826	6.72664547	Sr13
S6A_610475213	6A	610475213	4.24E-07	0.156	0.00079851	0.99091862	7.73725519	Sr13

S6A_610495870	6A	610495870	1.20E-08	0.178	0.00014952	1.03451441	9.95399965	Sr13
S6A_611495915	6A	611495915	1.12E-11	0.150	2.95E-07	1.34964231	14.4949398	Sr13
S6A_612043936	6A	612043936	8.14E-06	0.299	0.00742072	-0.6612643	5.95626265	Sr13
S6A_612802438	6A	612802438	2.43E-06	0.286	0.0027934	0.67473648	6.67812496	Sr13
S6A_612832613	6A	612832613	6.39E-05	0.261	0.03593269	0.59072292	4.74826203	Sr13
S6A_612957317	6A	612957317	2.68E-05	0.264	0.01934862	0.61853947	5.25423311	Sr13
S6A_613055519	6A	613055519	3.93E-05	0.263	0.02433907	0.60397452	5.03067991	Sr13
S6A_613131839	6A	613131839	3.59E-05	0.261	0.02372762	0.61226913	5.08318442	Sr13
S6A_613194512	6A	613194512	6.66E-05	0.261	0.0366837	0.58956002	4.72409837	Sr13
S6A_613256520	6A	613256520	4.58E-06	0.274	0.00453238	0.67344101	6.29805857	Sr13
S6A_613288180	6A	613288180	2.53E-07	0.170	0.0005864	0.89265007	8.05430863	Sr13
S6A_613294106	6A	613294106	1.53E-07	0.167	0.00045073	0.92421493	8.36115996	Sr13
S6A_613294155	6A	613294155	2.05E-05	0.264	0.0164274	0.62528167	5.41093884	Sr13
S6A_613547583	6A	613547583	4.36E-07	0.168	0.00079851	0.87846186	7.71895542	Sr13
S6A_613576841	6A	613576841	8.51E-06	0.179	0.00749984	0.76757217	5.92986305	Sr13
S6A_614329660	6A	614329660	2.78E-05	0.205	0.01936807	0.65538133	5.23173753	Sr13
S6A_615604386	6A	615604386	6.88E-05	0.308	0.03709748	0.58012049	4.7056762	Sr13
S6A_615617605	6A	615617605	1.57E-06	0.178	0.00200135	0.80592315	6.94085514	Sr13
S6A_615619215	6A	615619215	9.39E-07	0.174	0.00137918	0.83781672	7.25233976	Sr13
S6B_698318152	6B	698318152	6.66E-07	0.120	0.00109979	1.15590082	7.46151014	likely novel
S6B_698318155	6B	698318155	1.59E-06	0.127	0.00200135	1.08426387	6.93373368	likely novel
S7A_67384663	7A	67384663	1.10E-05	0.072	0.00941372	1.19710598	5.77585598	Chao et al.(2017)

Supplemental Table S3.2. Lists of SNPs significantly associated with seedling resistance to TKTTF identified using MLM.

SNP	Chr.	Position	P.value	MAF	FDR.Adj. P.value	Effect	R2	Proposed gene/allele
S1B_11134567	1B	11134567	1.17E-05	0.054	0.00793677	-1.58	3.77	Likely novel
S2B_89523302	2B	89523302	1.01E-07	0.070	0.00020629	-1.71	5.67	Gao et al.(2017)
S2B_90108250	2B	90108250	8.20E-08	0.069	0.00018058	-1.75	5.75	Gao et al.(2017)
S2B_90262508	2B	90262508	7.62E-08	0.070	0.00018058	-1.72	5.78	Gao et al.(2017)
S2B_90783099	2B	90783099	2.30E-07	0.070	0.0004343	-1.69	5.33	Gao et al.(2017)
S2B_93795309	2B	93795309	7.28E-07	0.072	0.00106985	-1.54	4.87	Gao et al.(2017)
S2B_94322394	2B	94322394	3.58E-06	0.074	0.0030024	-1.41	4.24	Gao et al.(2017)
S2B_96407116	2B	96407116	7.28E-07	0.072	0.00106985	-1.54	4.87	Gao et al.(2017)
S2B_97210200	2B	97210200	1.10E-06	0.070	0.00131951	-1.52	4.71	Gao et al.(2017)

S3A_565464709	3A	565464709	8.85E-06	0.079	0.00632452	1.13	3.88	Likely novel
S3A_614332431	3A	614332431	7.57E-05	0.058	0.04257365	-1.00	3.06	Likely novel
S5B_6135976	5B	6135976	5.81E-05	0.074	0.03337811	1.09	3.16	Likely novel
S6A_611410156	6A	611410156	8.08E-07	0.085	0.00112403	1.12	4.83	Sr13
S6A_611495915	6A	611495915	1.87E-05	0.150	0.01202965	0.86	3.59	Sr13
S6A_612003938	6A	612003938	7.31E-09	0.097	1.93E-05	1.24	6.75	Sr13
S6A_612043936	6A	612043936	2.66E-05	0.301	0.01633476	-0.63	3.46	Sr13
S6A_612632547	6A	612632547	1.95E-06	0.076	0.00210129	1.13	4.48	Sr13
S6A_612645703	6A	612645703	7.15E-06	0.083	0.00555931	1.00	3.97	Sr13
S6A_612802438	6A	612802438	8.74E-11	0.283	7.70E-07	0.96	8.63	Sr13
S6A_612832613	6A	612832613	5.98E-11	0.258	7.70E-07	1.02	8.79	Sr13
S6A_612957317	6A	612957317	4.84E-10	0.264	1.60E-06	0.95	7.89	Sr13
S6A_613055519	6A	613055519	1.29E-10	0.262	8.56E-07	0.99	8.46	Sr13
S6A_613131839	6A	613131839	8.34E-11	0.260	7.70E-07	1.01	8.65	Sr13
S6A_613194512	6A	613194512	2.20E-10	0.260	9.70E-07	0.98	8.23	Sr13
S6A_613217627	6A	613217627	2.31E-06	0.094	0.00220457	1.02	4.41	Sr13
S6A_613220409	6A	613220409	4.73E-06	0.096	0.00378939	0.98	4.13	Sr13
S6A_613256520	6A	613256520	1.81E-10	0.271	9.55E-07	0.97	8.32	Sr13
S6A_613275023	6A	613275023	2.67E-06	0.096	0.00234896	1.01	4.35	Sr13
S6A_613294096	6A	613294096	9.90E-07	0.088	0.00124578	1.10	4.75	Sr13
S6A_613294155	6A	613294155	2.60E-10	0.264	9.84E-07	0.97	8.16	Sr13
S6A_613434999	6A	613434999	2.13E-05	0.067	0.01339542	1.12	3.54	Sr13
S6A_613576753	6A	613576753	7.81E-06	0.099	0.00590157	0.94	3.93	Sr13
S6A_613576841	6A	613576841	5.17E-05	0.181	0.03039814	0.71	3.20	Sr13
S6A_613748730	6A	613748730	3.63E-06	0.096	0.0030024	0.98	4.23	Sr13
S6A_613908663	6A	613908663	9.59E-07	0.088	0.00124578	1.11	4.76	Sr13
S6A_614052038	6A	614052038	6.28E-07	0.087	0.00103764	1.13	4.93	Sr13
S6A_614080083	6A	614080083	3.62E-07	0.247	0.00063883	-0.77	5.15	Sr13
S6A_614367995	6A	614367995	2.34E-06	0.087	0.00220457	1.07	4.41	Sr13
S6A_614411890	6A	614411890	2.42E-06	0.105	0.00220457	0.99	4.39	Sr13
S6A_614784459	6A	614784459	8.20E-06	0.105	0.0060217	0.92	3.91	Sr13
S6A_615248120	6A	615248120	1.99E-06	0.090	0.00210129	1.07	4.47	Sr13
S6A_615604035	6A	615604035	1.29E-06	0.280	0.0014784	-0.71	4.64	Sr13
S6A_615604296	6A	615604296	2.18E-06	0.096	0.00220457	1.01	4.43	Sr13
S6A_615604386	6A	615604386	8.28E-10	0.307	2.43E-06	0.93	7.67	Sr13
S6A_615636915	6A	615636915	9.38E-06	0.099	0.00652879	0.92	3.86	Sr13

S6B_693829939	6B	693829939	4.35E-05	0.079	0.02612874	0.97	3.27	Sr11
SUN_34199795	UN	34199795	1.39E-05	0.058	0.00919888	-1.37	3.71	

Supplemental Table S3.3. Lists of SNPs significantly associated with seedling resistance to JRCQC identified using MLM.

SNP	Chr.	Position	P.value	MAF	FDR.Adj. P.value	Effect	R2	Proposed gene/allele
S6A_205649407	6A	205649407	1.52E-05	0.257	0.021093	0.748	4.62	Likely novel
S6A_603835119	6A	603835119	8.12E-06	0.205	0.01192971	0.892	4.92	Sr13
S6A_606107662	6A	606107662	1.68E-06	0.366	0.00443734	0.861	5.70	Sr13
S6A_609622362	6A	609622362	7.79E-06	0.168	0.01192971	0.892	4.94	Sr13
S6A_609635640	6A	609635640	1.42E-07	0.150	0.00183714	1.127	6.95	Sr13
S6A_610129981	6A	610129981	2.42E-07	0.156	0.00183714	1.082	6.68	Sr13
S6A_610133407	6A	610133407	1.24E-06	0.163	0.00364974	1.014	5.85	Sr13
S6A_610133490	6A	610133490	3.33E-07	0.159	0.00183714	1.077	6.52	Sr13
S6A_610146036	6A	610146036	4.86E-07	0.156	0.00183714	1.067	6.32	Sr13
S6A_610150266	6A	610150266	4.80E-06	0.150	0.00976293	0.977	5.18	Sr13
S6A_610150270	6A	610150270	2.73E-06	0.154	0.00602294	1.003	5.46	Sr13
S6A_610150819	6A	610150819	1.12E-06	0.159	0.00364974	1.014	5.91	Sr13
S6A_610171399	6A	610171399	6.82E-06	0.178	0.01192971	0.883	5.01	Sr13
S6A_610430767	6A	610430767	7.56E-06	0.145	0.01192971	0.988	4.96	Sr13
S6A_610475213	6A	610475213	7.41E-06	0.156	0.01192971	0.943	4.97	Sr13
S6A_610495870	6A	610495870	2.45E-06	0.178	0.00588843	0.917	5.51	Sr13
S6A_611495915	6A	611495915	2.45E-11	0.150	6.48E-07	1.420	11.54	Sr13
S6A_614080083	6A	614080083	4.20E-07	0.248	0.00183714	-0.811	6.40	Sr13
S6A_615604035	6A	615604035	4.36E-07	0.281	0.00183714	-0.792	6.38	Sr13
S6B_686489689	6B	686489689	2.92E-05	0.188	0.03861086	0.869	4.30	Likely novel

Supplemental Table S3.4. Lists of SNPs significantly associated with seedling resistance to TTRTF identified using MLM.

SNP	Chr.	Position	P.value	MAF	FDR.Adj. P.value	Effect	R2	Proposed gene/allele
S1B_550850202	1B	550850202	1.97E-05	0.052	0.01213452	1.04	5.16	Letta et al. (2014)
S1B_551557383	1B	551557383	2.47E-05	0.054	0.01391494	1.01	5.03	Letta et al. (2014)
S1B_587942809	1B	587942809	5.18E-05	0.050	0.02443391	-1.29	4.63	Edae et al.(2018); Bajgain et al.(2015)
S3A_565464709	3A	565464709	3.95E-07	0.079	0.00069584	1.11	7.39	Likely novel
S4A_17308554	4A	17308554	1.43E-05	0.079	0.01019175	0.96	5.34	Bajgain et al.,(2015)
S4A_619746683	4A	619746683	0.00010804	0.054	0.04200599	-1.30	4.22	Likely novel
S6A_609635640	6A	609635640	7.29E-05	0.152	0.03109537	0.68	4.44	Sr13
S6A_610133407	6A	610133407	8.25E-05	0.165	0.0337209	0.66	4.37	Sr13
S6A_610150819	6A	610150819	8.74E-05	0.161	0.03502592	0.66	4.34	Sr13
S6A_610171399	6A	610171399	7.28E-05	0.179	0.03109537	0.63	4.44	Sr13
S6A_610475213	6A	610475213	0.00012146	0.158	0.04587622	0.65	4.16	Sr13
S6A_610495870	6A	610495870	6.70E-05	0.179	0.02954332	0.63	4.48	Sr13
S6A_611410156	6A	611410156	1.44E-06	0.084	0.00190737	0.94	6.64	Sr13
S6A_611495915	6A	611495915	1.12E-06	0.152	0.00163776	0.83	6.79	Sr13
S6A_612003938	6A	612003938	1.65E-07	0.097	0.00035947	0.96	7.90	Sr13
S6A_612043936	6A	612043936	7.40E-10	0.303	1.96E-06	-0.81	11.14	Sr13
S6A_612645703	6A	612645703	3.62E-05	0.082	0.01915615	0.79	4.82	Sr13
S6A_612802438	6A	612802438	9.28E-14	0.285	4.91E-10	0.96	16.86	Sr13
S6A_612832613	6A	612832613	1.33E-12	0.256	4.39E-09	0.95	15.12	Sr13
S6A_612957317	6A	612957317	6.31E-13	0.262	2.38E-09	0.95	15.60	Sr13
S6A_613055519	6A	613055519	7.06E-14	0.260	4.91E-10	0.99	17.04	Sr13
S6A_613131839	6A	613131839	6.82E-14	0.258	4.91E-10	1.00	17.06	Sr13
S6A_613194512	6A	613194512	1.26E-13	0.258	5.56E-10	0.99	16.65	Sr13
S6A_613217627	6A	613217627	1.33E-06	0.093	0.00184479	0.89	6.69	Sr13
S6A_613220409	6A	613220409	1.71E-06	0.095	0.00199341	0.88	6.54	Sr13
S6A_613256520	6A	613256520	7.99E-14	0.271	4.91E-10	0.99	16.96	Sr13
S6A_613275023	6A	613275023	1.98E-06	0.095	0.00209624	0.87	6.46	Sr13
S6A_613288180	6A	613288180	1.88E-07	0.168	0.00035947	0.79	7.82	Sr13
S6A_613294096	6A	613294096	1.52E-05	0.088	0.01028784	0.83	5.31	Sr13
S6A_613294106	6A	613294106	1.90E-07	0.165	0.00035947	0.80	7.81	Sr13
S6A_613294155	6A	613294155	7.56E-14	0.262	4.91E-10	0.99	16.99	Sr13
S6A_613434999	6A	613434999	4.25E-06	0.066	0.00400904	1.05	6.02	Sr13

S6A_613547583	6A	613547583	1.49E-07	0.167	0.00035834	0.80	7.96	Sr13
S6A_613576753	6A	613576753	1.48E-05	0.099	0.01028784	0.78	5.32	Sr13
S6A_613576841	6A	613576841	1.97E-06	0.179	0.00209624	0.71	6.46	Sr13
S6A_613748730	6A	613748730	1.73E-06	0.095	0.00199341	0.87	6.53	Sr13
S6A_613908663	6A	613908663	3.30E-06	0.088	0.00323321	0.90	6.17	Sr13
S6A_614052038	6A	614052038	1.02E-05	0.086	0.00746449	0.85	5.53	Sr13
S6A_614080083	6A	614080083	4.52E-06	0.246	0.00411829	-0.59	5.99	Sr13
S6A_614329660	6A	614329660	3.35E-05	0.203	0.01844536	0.56	4.87	Sr13
S6A_614367995	6A	614367995	1.97E-05	0.086	0.01213452	0.83	5.16	Sr13
S6A_614411890	6A	614411890	2.50E-06	0.106	0.00254275	0.85	6.32	Sr13
S6A_614784459	6A	614784459	8.86E-06	0.104	0.00669185	0.79	5.61	Sr13
S6A_615248120	6A	615248120	1.84E-05	0.090	0.01213452	0.82	5.20	Sr13
S6A_615604035	6A	615604035	8.01E-06	0.278	0.00642027	-0.56	5.66	Sr13
S6A_615604296	6A	615604296	7.16E-07	0.095	0.0011139	0.91	7.04	Sr13
S6A_615604386	6A	615604386	2.17E-12	0.305	6.36E-09	0.92	14.80	Sr13
S6A_615617605	6A	615617605	1.57E-06	0.176	0.00197416	0.70	6.59	Sr13
S6A_615619215	6A	615619215	6.66E-07	0.172	0.00110062	0.74	7.08	Sr13
S6A_615632258	6A	615632258	8.41E-06	0.056	0.00653655	1.21	5.64	Sr13
S6A_615636915	6A	615636915	6.90E-06	0.099	0.00588444	0.80	5.75	_
S6B_693829939	6B	693829939	7.59E-06	0.079	0.00627235	0.92	5.70	Sr11
S7A_117505003	7A	117505003	9.73E-05	0.054	0.03840983	1.12	4.28	_
S7A_686094342	7A	686094342	0.00012001	0.091	0.04587622	-0.84	4.16	Sr22
S7A_687410326	7A	687410326	2.19E-05	0.050	0.01288431	-1.30	5.10	Sr22
S7A_690016567	7A	690016567	2.34E-05	0.052	0.01345394	-1.28	5.06	Sr22
S7A_690811708	7A	690811708	4.53E-05	0.057	0.02216573	-1.26	4.70	Sr22
S7A_690940195	7A	690940195	4.53E-05	0.057	0.02216573	-1.26	4.70	Sr22
S7A_697030516	7A	697030516	5.49E-05	0.056	0.02502578	-1.24	4.59	Sr22
S7A_700727874	7A	700727874	5.06E-05	0.059	0.02431665	-1.23	4.64	Sr22
S7A_700805183	7A	700805183	6.34E-06	0.077	0.00559185	-1.28	5.80	Sr22
S7A_717517491	7A	717517491	3.54E-05	0.056	0.01908917	-1.28	4.84	Sr22
S7A_717518884	7A	717518884	2.13E-05	0.059	0.01280992	-1.30	5.12	Sr22
S7A_719698163	7A	719698163	4.53E-05	0.057	0.02216573	-1.26	4.70	Sr22
S7A_719787589	7A	719787589	0.0001317	0.052	0.04904174	-1.27	4.11	Sr22
S7A_721720978	7A	721720978	8.29E-05	0.065	0.0337209	-1.15	4.37	Sr22/Sr25
S7B_622041448	7B	622041448	1.88E-05	0.075	0.01213452	-1.21	5.19	Novel close to Sr17
SUN_151516737	UN	151516737	7.47E-05	0.124	0.03134797	-0.71	4.42	_

SUN_151742792	UN	151742792	5.49E-05	0.056	0.02502578	-1.24	4.59	_
SUN_151847140	UN	151847140	4.53E-05	0.057	0.02216573	-1.26	4.70	_
SUN_153928527	UN	153928527	6.13E-05	0.056	0.0274907	-1.27	4.53	_

Supplemental Table S3.5. Lists of SNPs significantly associated with seedling resistance to four *Pgt* races identified using FarmCPU.

Race	Position	Chr.	P-value	Allele	AF	Effect	Proposed gene/allele
TTKSK	22978945	1B	2.19E-06	A/**T**	0.069	-0.58	Bajgain et al. (2015); Chao et al. (2017)
	599292679	6A	5.40E-07	**G**/C	0.888	0.55	-
	611495915	6A	4.80E-28	**G**/A	0.850	1.53	*Sr13a*
	612003938	6A	1.27E-16	G/**A**	0.098	-1.25	*Sr13*
	613294106	6A	8.15E-35	**C**/T	0.833	1.39	*Sr13*
	613748730	6A	8.51E-14	**T**/A	0.904	1.06	*Sr13*
	51332135	7A	1.67E-07	**A**/T	0.571	0.28	Likely novel
	697030516	7A	8.66E-12	G/**A**	0.050	-0.93	*Sr22*
TKTTF	89523302	2B	2.45E-20	A/**C**	0.070	-1.54	Gao et al. (2017)
	614332431	3A	1.31E-06	C/**T**	0.058	-0.68	Likely novel
	718944322	4A	2.19E-19	C/**T**	0.108	-1.07	*Sr7a*
	691693264	5B	2.34E-08	T/**A**	0.050	-0.99	*Sr49*
	609635640	6A	1.91E-14	**G**/A	0.851	0.85	*Sr13*
	611495915	6A	8.07E-06	**G**/A	0.851	0.88	*Sr13a*
	612802438	6A	4.09E-34	**A**/C	0.717	1.10	*Sr13*
	609817335	6B	2.65E-07	A/**G**	0.090	-0.54	Likely novel
	698482081	7B	6.54E-07	**G**/A	0.867	0.45	Likely *Sr17*
JRCQC	166695897	1B	4.23E-07	**T**/C	0.931	0.79	Likely novel
	40946146	3B	8.80E-06	**T**/C	0.576	0.30	Likely novel
	444117468	4B	6.59E-06	C/**T**	0.300	-0.32	Likely novel
	581150219	5A	5.79E-06	**A**/C	0.944	0.73	Likely novel
	205649407	6A	5.32E-06	**T**/C	0.743	0.48	Likely novel
	611495915	6A	6.53E-27	**G**/A	0.850	1.60	*Sr13a*
	615619215	6A	5.47E-16	**G**/A	0.830	0.87	*Sr13*
	17624367	7A	5.48E-08	C/**T**	0.143	-0.67	Letta et al. (2013)
	139258774	7A	1.64E-06	A/**T**	0.159	-0.49	Likely novel
	668699732	7A	5.88E-09	A/**G**	0.072	-0.95	*Sr22*
	393754818	UN	1.16E-05	G/**A**	0.054	-0.83	-
TTRTF	565464709	3A	1.62E-06	**G**/A	0.921	0.35	Likely novel
	139104893	3B	1.09E-05	**A**/T	0.866	0.38	-
	287211519	5B	2.30E-06	T/**A**	0.081	-0.57	Likely novel
	396874801	5B	5.93E-06	T/**C**	0.088	-0.50	Likely novel
	609635640	6A	5.75E-10	**G**/A	0.848	0.59	*Sr13*
	612043936	6A	8.29E-06	T/**C**	0.303	-0.25	*Sr13c*
	613131839	6A	8.68E-27	**G**/A	0.742	0.95	*Sr13*
	700805183	7A	2.36E-12	A/**T**	0.077	-0.88	*Sr22*
	237571373	UN	1.56E-05	**C**/T	0.923	0.60	-

Supplemental Table S3.6. Lists of SNPs significantly associated with field resistance in JRCQC_MS19 identified using MLM.

SNP	Chr.	Position	P.value	MAF	FDR.Adj. P.value	Effect	R2	Proposed gene/allele
S1B_587942809	1B	587942809	3.60E-07	0.050	0.00036612	-9.24	6.66	Bajgain et al.(2015); Edae et al.(2018)
S3B_38937548	3B	38937548	8.67E-06	0.064	0.00546035	-6.98	5.03	Likely novel
S3B_55889860	3B	55889860	2.60E-05	0.064	0.0144029	-6.89	4.48	Likely novel
S3B_97870708	3B	97870708	1.29E-07	0.055	0.00015516	-9.83	7.20	Likely novel
S4A_619746683	4A	619746683	9.31E-09	0.054	2.46E-05	-11.56	8.60	Likely novel
S5B_689821784	5B	689821784	9.67E-08	0.050	0.00012902	-8.48	7.35	Sr49
S5B_691693264	5B	691693264	2.70E-07	0.048	0.00028544	-8.50	6.81	Sr49
S5B_692277095	5B	692277095	3.59E-06	0.055	0.00263332	-7.05	5.48	Sr49
S6A_28859024	6A	28859024	1.51E-06	0.052	0.00128683	-9.29	5.92	Likely novel
S6A_334834338	6A	334834338	5.36E-06	0.052	0.00352377	-7.99	5.28	Likely novel
S6A_614080083	6A	614080083	2.61E-05	0.245	0.0144029	-3.07	4.48	Sr13
S6A_615604035	6A	615604035	4.91E-06	0.277	0.0034152	-3.27	5.32	Sr13
S6B_287433588	6B	287433588	0.00011488	0.055	0.04533131	-6.03	3.75	_
S7A_39254359	7A	39254359	0.0001244	0.061	0.04836759	4.97	3.71	_
S7A_673105161	7A	673105161	0.00011035	0.130	0.04533131	-3.75	3.77	Sr22
S7A_682951819	7A	682951819	2.33E-06	0.054	0.00181139	-9.21	5.70	Sr22
S7A_684386422	7A	684386422	9.21E-05	0.141	0.03992654	-3.69	3.86	Sr22
S7A_684422202	7A	684422202	9.68E-05	0.121	0.04126744	-3.83	3.84	Sr22
S7A_684746400	7A	684746400	3.78E-05	0.129	0.01887755	-4.14	4.30	Sr22
S7A_685283476	7A	685283476	6.09E-05	0.077	0.02826349	-4.87	4.06	Sr22
S7A_685982750	7A	685982750	7.27E-05	0.079	0.03202802	-4.90	3.98	Sr22
S7A_686094342	7A	686094342	5.46E-06	0.091	0.00352377	-5.75	5.27	Sr22
S7A_686849268	7A	686849268	3.59E-05	0.073	0.01860139	-5.16	4.32	Sr22
S7A_686964735	7A	686964735	1.17E-05	0.075	0.0072073	-5.40	4.88	Sr22
S7A_687410326	7A	687410326	6.33E-07	0.050	0.00057687	-8.87	6.37	Sr22
S7A_687560696	7A	687560696	3.73E-05	0.079	0.01887755	-5.31	4.30	Sr22
S7A_687774090	7A	687774090	2.23E-05	0.095	0.0128206	-5.06	4.56	Sr22
S7A_687798481	7A	687798481	6.28E-05	0.079	0.02863031	-5.43	4.05	Sr22
S7A_688882132	7A	688882132	4.51E-05	0.102	0.02169401	-4.81	4.21	Sr22
S7A_688885145	7A	688885145	0.00010783	0.098	0.04525109	-4.52	3.78	Sr22
S7A_689090791	7A	689090791	6.54E-05	0.096	0.02931873	-4.70	4.03	Sr22
S7A_689117913	7A	689117913	2.23E-05	0.095	0.0128206	-5.06	4.56	Sr22

S7A_690016567	7A	690016567	1.97E-07	0.052	0.00021685	-9.12	6.98	Sr22
S7A_690811708	7A	690811708	7.58E-09	0.057	2.46E-05	-10.80	8.71	Sr22
S7A_690940195	7A	690940195	7.58E-09	0.057	2.46E-05	-10.80	8.71	Sr22
S7A_693915965	7A	693915965	5.32E-06	0.071	0.00352377	-6.92	5.28	Sr22
S7A_697030510	7A	697030510	9.76E-08	0.093	0.00012902	-7.14	7.35	Sr22
S7A_697030516	7A	697030516	3.20E-08	0.055	5.66E-05	-10.13	7.94	Sr22
S7A_698390754	7A	698390754	3.13E-08	0.096	5.66E-05	-7.44	7.95	Sr22
S7A_700727874	7A	700727874	8.08E-09	0.059	2.46E-05	-10.51	8.68	Sr22
S7A_700805183	7A	700805183	6.52E-09	0.077	2.46E-05	-9.81	8.79	Sr22
S7A_710171609	7A	710171609	3.65E-08	0.055	6.04E-05	-10.09	7.87	Sr22
S7A_714327927	7A	714327927	1.41E-07	0.066	0.00016203	-9.26	7.15	Sr22
S7A_714370100	7A	714370100	5.79E-08	0.052	8.51E-05	-10.01	7.62	Sr22
S7A_714975616	7A	714975616	5.22E-07	0.091	0.00051114	-6.72	6.47	Sr22
S7A_717517491	7A	717517491	1.79E-08	0.055	4.30E-05	-10.33	8.25	Sr22
S7A_717518884	7A	717518884	7.33E-09	0.059	2.46E-05	-10.57	8.73	Sr22
S7A_718484217	7A	718484217	2.30E-06	0.098	0.00181139	-6.91	5.71	Sr22
S7A_719231181	7A	719231181	4.06E-08	0.064	6.32E-05	-9.15	7.81	Sr22
S7A_719698163	7A	719698163	7.58E-09	0.057	2.46E-05	-10.80	8.71	Sr22
S7A_719787589	7A	719787589	6.23E-07	0.052	0.00057687	-9.73	6.38	Sr22
S7A_721720978	7A	721720978	9.04E-09	0.064	2.46E-05	-10.11	8.61	Sr22/Sr25
S7A_724486791	7A	724486791	3.19E-06	0.105	0.00241144	-6.13	5.54	Sr22
S7A_724668618	7A	724668618	9.08E-07	0.077	0.00080012	-7.36	6.19	Sr22
S7A_724668652	7A	724668652	1.20E-07	0.052	0.00015115	-9.66	7.24	Sr22
S7A_727729196	7A	727729196	2.85E-05	0.209	0.01536515	-3.26	4.44	Sr22
S7B_622041448	7B	622041448	3.21E-08	0.075	5.66E-05	-9.14	7.94	likely novel close to Sr17
S7B_644041948	7B	644041948	0.00011147	0.059	0.04533131	-6.43	3.77	likely novel close to Sr17
S7B_681996206	7B	681996206	1.66E-05	0.063	0.00996873	-7.38	4.71	Yu et al. (2014)
S7B_683438364	7B	683438364	4.65E-06	0.107	0.0033237	-5.15	5.35	Letta et al., 2013
S7B_714275296	7B	714275296	5.96E-05	0.463	0.02813229	-3.68	4.07	_
SUN_151516737	UN	151516737	4.20E-05	0.123	0.02058088	-4.21	4.25	_
SUN_151742792	UN	151742792	3.20E-08	0.055	5.66E-05	-10.13	7.94	_
SUN_151847140	UN	151847140	7.58E-09	0.057	2.46E-05	-10.80	8.71	_
SUN_153093563	UN	153093563	0.00011336	0.096	0.04533131	-4.37	3.76	_
SUN_153928527	UN	153928527	5.35E-09	0.055	2.46E-05	-11.19	8.90	_
SUN_166522707	UN	166522707	2.03E-06	0.054	0.00167945	-9.07	5.77	_
SUN_288369273	UN	288369273	2.91E-05	0.075	0.01536515	-5.53	4.43	_

Supplemental Table S3.7. Lists of SNPs significantly associated with seedling resistance in JRCQC_OS20 identified using MLM.

SNP	Chr.	Position	P.value	MAF	FDR.Adj. P.value	Effect	R2	Proposed gene/allele
S1B_11134567	1B	11134567	1.63E-05	0.054	0.04395755	-8.65	4.91	Likely Novel
S5B_689821784	5B	689821784	4.56E-06	0.051	0.02786357	-8.21	5.58	Sr49
S5B_690450778	5B	690450778	5.49E-05	0.056	0.04395755	10.04	4.29	_
S5B_691693264	5B	691693264	7.14E-06	0.049	0.02786357	-8.36	5.35	Sr49
S5B_692277095	5B	692277095	1.67E-05	0.056	0.04395755	-7.41	4.90	Sr49
S6A_606107662	6A	606107662	2.20E-05	0.368	0.04395755	3.97	4.76	Sr13
S6A_606304231	6A	606304231	3.14E-05	0.374	0.04395755	3.91	4.57	Sr13
S6A_607001638	6A	607001638	5.40E-05	0.188	0.04395755	4.39	4.29	Sr13
S6A_612802438	6A	612802438	2.20E-05	0.283	0.04395755	3.42	4.76	Sr13
S6A_613055519	6A	613055519	3.84E-05	0.258	0.04395755	3.45	4.47	Sr13
S6A_613131839	6A	613131839	2.86E-05	0.256	0.04395755	3.54	4.62	Sr13
S6A_613256520	6A	613256520	3.94E-05	0.269	0.04395755	3.42	4.46	Sr13
S6A_613288180	6A	613288180	3.82E-05	0.170	0.04395755	4.00	4.47	Sr13
S6A_613294106	6A	613294106	6.17E-05	0.166	0.04657885	3.96	4.23	Sr13
S6A_613294155	6A	613294155	4.86E-05	0.260	0.04395755	3.40	4.35	Sr13
S6A_613547583	6A	613547583	4.63E-05	0.168	0.04395755	3.98	4.37	Sr13
S6A_614080083	6A	614080083	4.08E-06	0.245	0.02786357	-3.89	5.64	Sr13
S6A_615604035	6A	615604035	8.84E-07	0.280	0.02337702	-4.05	6.46	Sr13
S6A_615604386	6A	615604386	6.36E-05	0.303	0.04673699	3.33	4.21	Sr13
S6B_686489689	6B	686489689	3.61E-05	0.191	0.04395755	4.52	4.50	likely novel close to Sr11
S6B_687598497	6B	687598497	3.32E-05	0.148	0.04395755	5.11	4.54	likely novel close to Sr11
S7A_43311031	7A	43311031	7.34E-06	0.092	0.02786357	6.30	5.33	Novel
S7A_690811708	7A	690811708	4.57E-05	0.058	0.04395755	-8.42	4.38	Sr22
S7A_690940195	7A	690940195	4.57E-05	0.058	0.04395755	-8.42	4.38	Sr22
S7A_697030516	7A	697030516	4.85E-05	0.056	0.04395755	-8.30	4.35	Sr22
S7A_700805183	7A	700805183	7.38E-06	0.078	0.02786357	-8.43	5.33	Sr22
S7A_714370100	7A	714370100	2.95E-05	0.052	0.04395755	-8.65	4.61	Sr22
S7A_717518884	7A	717518884	6.09E-05	0.060	0.04657885	-8.13	4.23	Sr22
S7A_718484217	7A	718484217	5.02E-05	0.097	0.04395755	-6.76	4.33	Sr22
S7A_719698163	7A	719698163	4.57E-05	0.058	0.04395755	-8.42	4.38	Sr22
S7A_721720978	7A	721720978	5.49E-05	0.065	0.04395755	-7.86	4.29	Sr22/Sr25
S7A_724668618	7A	724668618	7.33E-06	0.076	0.02786357	-7.70	5.33	Sr22

S7B_714275296	7B	714275296	4.39E-05	0.457	0.04395755	-4.23	4.40	_
SUN_151742792	UN	151742792	4.85E-05	0.056	0.04395755	-8.30	4.35	_
SUN_151847140	UN	151847140	4.57E-05	0.058	0.04395755	-8.42	4.38	_
SUN_153928527	UN	153928527	2.55E-05	0.056	0.04395755	-8.93	4.68	_

Supplemental Table S3.8**.** Lists of SNPs significantly associated with seedling resistance in TKTTF_MS18 identified using MLM.

SNP	Chr.	Position	P.value	MAF	FDR.Adj. P.value	Effect	R2	Proposed gene/allele
S4A_619746683	4A	619746683	8.54E-06	0.054	0.00753012	-1.36	5.00	Likely Novel
S6A_205649407	6A	205649407	1.38E-05	0.257	0.01042083	0.53	4.76	Likely novel
S6A_603835119	6A	603835119	5.56E-05	0.205	0.02775042	0.57	4.07	Sr13
S6A_606107662	6A	606107662	1.38E-05	0.370	0.01042083	0.55	4.76	Sr13
S6A_606107665	6A	606107665	8.95E-05	0.478	0.04012725	0.45	3.84	Sr13
S6A_606304231	6A	606304231	4.99E-05	0.375	0.02585915	0.52	4.12	Sr13
S6A_609622362	6A	609622362	0.00011199	0.172	0.04853801	0.54	3.73	Sr13
S6A_609635640	6A	609635640	9.37E-06	0.154	0.0079905	0.66	4.95	Sr13
S6A_610129981	6A	610129981	2.76E-05	0.159	0.01737032	0.61	4.41	Sr13
S6A_610133407	6A	610133407	4.51E-05	0.167	0.02485824	0.60	4.17	Sr13
S6A_610133490	6A	610133490	6.39E-05	0.163	0.02964042	0.59	4.00	Sr13
S6A_610146036	6A	610146036	4.81E-05	0.159	0.02543897	0.60	4.14	Sr13
S6A_610150819	6A	610150819	3.06E-05	0.163	0.01882804	0.60	4.36	Sr13
S6A_610171399	6A	610171399	5.70E-05	0.181	0.02791179	0.55	4.06	Sr13
S6A_610495870	6A	610495870	1.36E-05	0.181	0.01042083	0.59	4.77	Sr13
S6A_611495915	6A	611495915	3.22E-08	0.154	0.00028384	0.82	7.87	Sr13
S6A_612802438	6A	612802438	2.57E-08	0.288	0.00028384	0.60	7.99	Sr13
S6A_612832613	6A	612832613	4.75E-08	0.259	0.0003139	0.62	7.66	Sr13
S6A_612957317	6A	612957317	1.51E-07	0.264	0.00052881	0.59	7.06	Sr13
S6A_613055519	6A	613055519	9.33E-08	0.263	0.0004932	0.60	7.31	Sr13
S6A_613131839	6A	613131839	1.80E-07	0.261	0.00052881	0.59	6.97	Sr13
S6A_613194512	6A	613194512	7.92E-07	0.261	0.00190428	0.56	6.20	Sr13
S6A_613256520	6A	613256520	2.35E-08	0.274	0.00028384	0.63	8.03	Sr13
S6A_613288180	6A	613288180	1.32E-06	0.170	0.00268296	0.63	5.94	Sr13
S6A_613294106	6A	613294106	9.27E-07	0.167	0.00204315	0.64	6.12	Sr13
S6A_613294155	6A	613294155	1.45E-07	0.264	0.00052881	0.59	7.08	Sr13
S6A_613547583	6A	613547583	1.53E-06	0.168	0.00269524	0.62	5.87	Sr13

S6A_613576841	6A	613576841	1.87E-06	0.181	0.00291461	0.61	5.76	Sr13
S6A_614080083	6A	614080083	7.65E-07	0.246	0.00190428	-0.55	6.22	Sr13
S6A_614329660	6A	614329660	3.85E-05	0.201	0.02212333	0.48	4.25	Sr13
S6A_615604035	6A	615604035	1.63E-07	0.274	0.00052881	-0.58	7.02	Sr13
S6A_615604386	6A	615604386	1.48E-06	0.308	0.00269524	0.54	5.88	Sr13
S6A_615617605	6A	615617605	4.39E-06	0.178	0.00464255	0.57	5.33	Sr13
S6A_615619215	6A	615619215	2.73E-06	0.174	0.0036149	0.60	5.57	Sr13
S6B_686489689	6B	686489689	4.14E-05	0.192	0.02327622	0.61	4.22	likely novel close to Sr11
S7A_43311031	7A	43311031	5.16E-05	0.092	0.02622492	0.72	4.11	Likely novel
S7A_687410326	7A	687410326	9.14E-05	0.051	0.04029458	-1.05	3.83	Sr22
S7A_690016567	7A	690016567	5.88E-05	0.053	0.02815431	-1.06	4.04	Sr22
S7A_690811708	7A	690811708	3.88E-06	0.058	0.00427403	-1.32	5.39	Sr22
S7A_690940195	7A	690940195	3.88E-06	0.058	0.00427403	-1.32	5.39	Sr22
S7A_697030510	7A	697030510	3.41E-05	0.094	0.02048709	-0.84	4.31	Sr22
S7A_697030516	7A	697030516	5.12E-06	0.056	0.00501845	-1.27	5.25	Sr22
S7A_698390754	7A	698390754	2.45E-05	0.098	0.0157818	-0.86	4.47	Sr22
S7A_700727874	7A	700727874	2.62E-06	0.060	0.0036149	-1.31	5.59	Sr22
S7A_700805183	7A	700805183	4.72E-05	0.078	0.02543897	-1.04	4.15	Sr22
S7A_710171609	7A	710171609	1.59E-05	0.056	0.0113605	-1.20	4.69	Sr22
S7A_714327927	7A	714327927	6.26E-06	0.067	0.00591339	-1.22	5.15	Sr22
S7A_714975616	7A	714975616	6.73E-06	0.092	0.00613702	-0.92	5.12	Sr22
S7A_717517491	7A	717517491	1.07E-05	0.056	0.00885947	-1.22	4.88	Sr22
S7A_717518884	7A	717518884	2.17E-06	0.060	0.00318439	-1.32	5.69	Sr22
S7A_719231181	7A	719231181	7.05E-05	0.065	0.03211895	-1.01	3.96	Sr22
S7A_719698163	7A	719698163	3.88E-06	0.058	0.00427403	-1.32	5.39	Sr22
S7A_719787589	7A	719787589	1.44E-05	0.053	0.01055591	-1.29	4.74	Sr22
S7A_721720978	7A	721720978	1.78E-06	0.065	0.00291461	-1.28	5.79	Sr22/Sr25
S7A_724668618	7A	724668618	3.75E-05	0.078	0.02202342	-0.94	4.26	Sr22
S7A_724668652	7A	724668652	2.15E-05	0.053	0.01421463	-1.17	4.54	Sr22
S7B_714275296	7B	714275296	5.96E-05	0.466	0.02815431	-0.57	4.04	_
SUN_151742792	UN	151742792	5.12E-06	0.056	0.00501845	-1.27	5.25	_
SUN_151847140	UN	151847140	3.88E-06	0.058	0.00427403	-1.32	5.39	_
SUN_153928527	UN	153928527	1.83E-05	0.056	0.01276242	-1.25	4.62	_
SUN_166522707	UN	166522707	2.08E-05	0.054	0.01410851	-1.23	4.55	_

Supplemental Table S3.9. Lists of SNPs significantly associated with seedling resistance in TKTTF_MS19 identified using MLM.

SNP	Chr.	Position	P.value	MAF	FDR.Adj. P.value	Effect	R2	Proposed gene/allele
S1B_551557383	1B	551557383	8.81E-05	0.054	0.04235542	6.81	3.94	Letta et al. (2014)
S1B_587942809	1B	587942809	2.57E-06	0.050	0.00234313	-10.94	5.73	Bajgain et al. (2015); Edae et al.(2018)
S2A_728226059	2A	728226059	5.70E-05	0.093	0.03203907	-6.22	4.15	Likely Novel
S3B_55889860	3B	55889860	6.16E-06	0.064	0.00525392	-9.57	5.28	Novel
S3B_97870708	3B	97870708	7.38E-06	0.055	0.00591566	-10.70	5.19	Novel
S4A_619746683	4A	619746683	2.57E-07	0.054	0.00045386	-13.44	6.93	Novel
S5B_13939811	5B	13939811	0.00010402	0.088	0.04824845	-6.23	3.85	_
S5B_689821784	5B	689821784	7.04E-06	0.050	0.00581709	-9.23	5.21	Sr49
S5B_691693264	5B	691693264	1.64E-05	0.048	0.01235395	-9.22	4.78	Sr49
S5B_692277095	5B	692277095	5.20E-05	0.055	0.03183537	-7.98	4.20	Sr49
S6A_28859024	6A	28859024	8.64E-05	0.052	0.04235542	-9.77	3.94	Likely novel
S6A_334834338	6A	334834338	1.09E-05	0.052	0.00846145	-9.93	4.99	Likely novel
S6A_611495915	6A	611495915	6.62E-05	0.152	0.03502722	4.92	4.08	Sr13
S6A_612802438	6A	612802438	4.99E-05	0.288	0.03183537	3.67	4.22	Sr13
S6A_612832613	6A	612832613	0.00010156	0.259	0.04795078	3.71	3.86	Sr13
S6A_612957317	6A	612957317	6.62E-05	0.264	0.03502722	3.77	4.08	Sr13
S6A_613055519	6A	613055519	6.30E-05	0.263	0.03472285	3.79	4.10	Sr13
S6A_613131839	6A	613131839	1.91E-05	0.261	0.01403003	4.09	4.70	Sr13
S6A_613194512	6A	613194512	6.81E-05	0.261	0.03530916	3.79	4.06	Sr13
S6A_613256520	6A	613256520	4.38E-05	0.273	0.02897502	3.84	4.28	Sr13
S6A_613294155	6A	613294155	5.30E-05	0.264	0.03183537	3.82	4.19	Sr13
S6A_614080083	6A	614080083	1.13E-06	0.245	0.00124693	-4.62	6.16	Sr13
S6A_615604035	6A	615604035	4.61E-09	0.277	0.00012177	-5.51	9.10	Sr13
S7A_682951819	7A	682951819	4.11E-05	0.054	0.02785866	-10.41	4.32	Sr22
S7A_686964735	7A	686964735	8.78E-05	0.075	0.04235542	-6.23	3.94	Sr22
S7A_687410326	7A	687410326	2.25E-06	0.050	0.00220513	-10.90	5.80	Sr22
S7A_690016567	7A	690016567	1.31E-06	0.052	0.00132822	-10.96	6.08	Sr22
S7A_690811708	7A	690811708	5.67E-08	0.057	0.00019718	-13.23	7.74	Sr22
S7A_690940195	7A	690940195	5.67E-08	0.057	0.00019718	-13.23	7.74	Sr22
S7A_693915965	7A	693915965	5.15E-05	0.071	0.03183537	-7.97	4.20	Sr22
S7A_697030510	7A	697030510	8.77E-07	0.093	0.00110395	-8.55	6.29	Sr22
S7A_697030516	7A	697030516	1.33E-07	0.055	0.00030809	-12.56	7.29	Sr22

S7A_698390754	7A	698390754	7.64E-07	0.096	0.00106282	-8.63	6.36	Sr22
S7A_700727874	7A	700727874	2.40E-08	0.059	0.00019718	-13.28	8.20	Sr22
S7A_700805183	7A	700805183	9.45E-07	0.077	0.00113515	-10.73	6.25	Sr22
S7A_710171609	7A	710171609	5.97E-08	0.055	0.00019718	-12.91	7.71	Sr22
S7A_714327927	7A	714327927	1.08E-06	0.066	0.0012452	-11.18	6.18	Sr22
S7A_714370100	7A	714370100	3.43E-07	0.052	0.00056759	-12.19	6.78	Sr22
S7A_714975616	7A	714975616	2.47E-05	0.091	0.01762722	-7.31	4.57	Sr22
S7A_717517491	7A	717517491	2.44E-07	0.055	0.00045386	-12.26	6.96	Sr22
S7A_717518884	7A	717518884	1.40E-07	0.059	0.00030809	-12.50	7.26	Sr22
S7A_718484217	7A	718484217	7.35E-05	0.098	0.03736587	-7.50	4.03	Sr22
S7A_719231181	7A	719231181	6.28E-07	0.064	0.00092178	-10.80	6.46	Sr22
S7A_719698163	7A	719698163	5.67E-08	0.057	0.00019718	-13.23	7.74	Sr22
S7A_719787589	7A	719787589	1.24E-06	0.052	0.00131071	-12.32	6.11	Sr22
S7A_721720978	7A	721720978	1.27E-07	0.064	0.00030809	-12.09	7.31	Sr22/Sr25
S7A_724668618	7A	724668618	3.82E-07	0.077	0.00059393	-9.91	6.73	Sr22
S7A_724668652	7A	724668652	1.90E-07	0.052	0.00038726	-12.33	7.09	Sr22
S7B_622041448	7B	622041448	8.73E-07	0.075	0.00110395	-10.51	6.29	likely novel close to Sr17
S7B_644041948	7B	644041948	5.60E-05	0.059	0.03203907	-8.67	4.16	likely novel close to Sr17
S7B_681996206	7B	681996206	5.59E-05	0.063	0.03203907	-8.93	4.16	Yu et al. (2014)
S7B_683438364	7B	683438364	2.52E-06	0.107	0.00234313	-6.86	5.74	Letta et al., 2013
SUN_151742792	UN	151742792	1.33E-07	0.055	0.00030809	-12.56	7.29	_
SUN_151847140	UN	151847140	5.67E-08	0.057	0.00019718	-13.23	7.74	_
SUN_153928527	UN	153928527	5.15E-08	0.055	0.00019718	-13.60	7.79	_
SUN_166522707	UN	166522707	3.15E-06	0.054	0.00277579	-11.55	5.62	_
SUN_288369273	UN	288369273	3.91E-05	0.075	0.02718542	-6.97	4.34	_

Supplemental Table S3.10. Lists of SNPs significantly associated with field resistance to two *Pgt* races identified using FarmCPU.

Race/Season	Position	Chr.	P-value	Allele	AF	Effect	Proposed gene/allele
JRCQC_MS19	669183691	3B	3.38E-07	**C**/G	0.832	2.60	Bajgain et al. (2015)
	689821784	5B	1.95E-10	C/**G**	0.050	-6.41	*Sr49*
	347960291	6A	8.63E-07	**C**/T	0.825	2.41	Likely novel
	615604035	6A	2.58E-09	A/**C**	0.277	-2.40	*Sr13*
	717518884	7A	6.74E-19	T/**C**	0.059	-8.97	*Sr22*
JRCQC_OS20	470658058	4B	4.84E-07	**T**/C	0.600	2.16	Likely Novel
	429077415	5A	4.29E-07	A/**C**	0.070	-4.16	Likely Novel
	689821784	5B	2.24E-13	C/**G**	0.050	-8.24	*Sr49*
	612043936	6A	9.17E-11	T/**C**	0.305	-3.62	*Sr13*
	615604035	6A	4.92E-21	A/**C**	0.280	-5.55	*Sr13*
	81338498	7A	5.57E-06	G/**A**	0.258	-2.15	Likely Novel
	700805183	7A	1.21E-14	A/**T**	0.078	-7.56	*Sr22*
TKTTF_MS18	566000158	1A	3.41E-07	**G**/T	0.908	0.54	Mihalyove et al. (2017)
	7669679	5B	5.36E-06	**G**/A	0.418	0.24	Likely Novel
	5058172	6A	4.63E-07	A/**G**	0.219	-0.30	*Sr8155B1*
	612043936	6A	1.05E-23	T/**C**	0.143	-0.85	*Sr13*
	615604035	6A	7.02E-25	A/**C**	0.274	-0.88	*Sr13*
	17572564	6B	3.62E-08	G/**A**	0.150	-0.42	Yu et al. (2014)
	717518884	7A	1.59E-18	T/**C**	0.060	-1.06	*Sr22*
TKTTF_MS19	527339451	5A	4.36E-08	A/**G**	0.111	-3.87	Bajgain et al. (2015)
	345123955	5B	4.90E-06	A/**C**	0.193	-3.04	Likely Novel
	689821784	5B	8.29E-12	C/**G**	0.050	-8.45	Sr49
	612043936	6A	2.81E-15	T/**C**	0.146	-4.86	Sr13
	615604035	6A	1.22E-25	A/**C**	0.277	-6.64	Sr13
	471287983	6B	2.47E-07	C/T	0.057	-6.90	Novel
	717518884	7A	1.03E-24	T/C	0.059	12.68	Sr22
	647958825	7B	1.34E-05	C/T	0.082	-3.89	-
	721015179	7B	2.54E-07	C/T	0.114	-3.49	Yu et al. (2014)

Supplemental Table S3.11. Lists of common significant markers between races for seedling resistance of lines to the four *Pgt* races and/or between race-season combinations for field resistance to two *Pgt* races identified using MLM.

Position	Chr.	MAF	Seedling resistance to Races	Adult plant resistance to race/Season
11134567	1B	0.054	TKTTF	JRCQC_OS20
551557383	1B	0.054	TTRTF	TKTTF_MS19
587942809	1B	0.050	TTRTF	TKTTF_MS19, JRCQC _MS19
565464709	3A	0.079	TKTTF, TTRTF	-
55889860	3B	0.064	-	TKTTF_MS19, JRCQC _MS19
97870708	3B	0.055	-	TKTTF_MS19, JRCQC _MS19
619746683	4A	0.054	TTRTF	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19
689821784	5B	0.05	-	TKTTF_MS19, JRCQC _MS19, JRCQC_OS20
691693264	5B	0.05	TKTTF	TKTTF_MS19, JRCQC _MS19, JRCQC_OS20
692277095	5B	0.05	-	TKTTF_MS19, JRCQC _MS19, JRCQC_OS20
28859024	6A	0.05	-	TKTTF_MS19, JRCQC _MS19
205649407	6A	0.257	TTKSK, JRCQC	TKTTF_MS18
334834338	6A	0.052	-	TKTTF_MS19, JRCQC _MS19
603835119	6A	0.204	JRCQC	TKTTF_MS18
606107662	6A	0.366	TTKSK, JRCQC	TKTTF_MS18, JRCQC_OS20
606107665	6A	0.478	TTKSK	TKTTF_MS18
606304231	6A	0.371	TTKSK	TKTTF_MS18, JRCQC_OS20
609622362	6A	0.168	TTKSK, JRCQC	TKTTF_MS18
609635640	6A	0.150	TTKSK, JRCQC, TTRTF	TKTTF_MS18
610129981	6A	0.156	TTKSK, JRCQC	TKTTF_MS18
610133407	6A	0.163	TTKSK, JRCQC, TTRTF	TKTTF_MS18
610133490	6A	0.159	TTKSK, JRCQC	TKTTF_MS18
610146036	6A	0.156	TTKSK, JRCQC	TKTTF_MS18
610150266	6A	0.150	TTKSK, JRCQC	-
610150270	6A	0.154	TTKSK, JRCQC	-
610150819	6A	0.159	TTKSK, JRCQC, TTRTF	TKTTF_MS18

610171399	6A	0.177	TTKSK, JRCQC, TTRTF	TKTTF_MS18
610430767	6A	0.145	TTKSK, JRCQC	-
610475213	6A	0.156	TTKSK, JRCQC, TTRTF	-
610495870	6A	0.177	TTKSK, JRCQC, TTRTF	TKTTF_MS18
611410156	6A	0.085	TKTTF, TTRTF	-
611495915	6A	0.150	TTKSK, TKTTF, JRCQC, TTRTF	TKTTF_MS18, TKTTF_MS19
612003938	6A	0.097	TKTTF, TTRTF	-
612043936	6A	0.301	TTKSK, TKTTF, TRTTF	-
612645703	6A	0.083	TKTTF, TTRTF	-
612802438	6A	0.285	TTKSK, TKTTF, TRTTF	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19
612832613	6A	0.260	TTKSK, TKTTF, TTRTF	TKTTF_MS18, TKTTF_MS19
612957317	6A	0.264	TTKSK, TKTTF, TTRTF	TKTTF_MS18, TKTTF_MS19
613055519	6A	0.262	TTKSK, TKTTF, TTRTF	TKTTF_MS18, TKTTF_MS19, JRCQC _OS20
613131839	6A	0.260	TTKSK, TKTTF, TTRTF	TKTTF_MS18, TKTTF_MS19, JRCQC _OS20
613194512	6A	0.260	TTKSK, TKTTF, TTRTF	TKTTF_MS18, TKTTF_MS19
613217627	6A	0.094	TKTTF, TTRTF	-
613220409	6A	0.095	TKTTF, TTRTF	-
613256520	6A	0.273	TTKSK, TKTTF, TTRTF	TKTTF_MS18, TKTTF_MS19, JRCQC _OS20
613275023	6A	0.095	TKTTF, TTRTF	TKTTF_MS18, JRCQC _OS20
613288180	6A	0.170	TTKSK, TTRTF	TKTTF_MS18, JRCQC _OS20
613294096	6A	0.088	TKTTF, TTRTF	-
613294106	6A	0.167	TTKSK, TTRTF	TKTTF_MS18, JRCQC _OS20
613294155	6A	0.264	TTKSK, TKTTF, TTRTF	TKTTF_MS18, TKTTF_MS19, JRCQC _OS20
613434999	6A	0.067	TKTTF, TTRTF	-
613547583	6A	0.168	TTKSK, TTRTF	TKTTF_MS18, JRCQC _OS20
613576753	6A	0.099	TKTTF, TTRTF	-
613576841	6A	0179	TTKSK, TKTTF, TTRTF	TKTTF_MS18
613748730	6A	0.095	TTKSK, TKTTF, TTRTF	-
613908663	6A	0.088	TKTTF, TTRTF	-
614052038	6A	0.086	TKTTF, TTRTF	-
614080083	6A	0.247	TKTTF, JRCQC, TTRTF	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19, JRCQC _OS20

614329660	6A	0.205	TTKSK, TTRTF	TKTTF_MS18
614367995	6A	0.087	TKTTF, TTRTF	-
614411890	6A	0.105	TKTTF, TTRTF	-
614784459	6A	0.104	TKTTF, TTRTF	-
615248120	6A	0.090	TKTTF, TTRTF	-
615604035	6A	0.279	TKTTF, JRCQC, TTRTF	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19, JRCQC _OS20
615604296	6A	0.095	TKTTF, TTRTF	-
615604386	6A	0.307	TTKSK, TKTTF, TTRTF	TKTTF_MS18, JRCQC _OS20
615617605	6A	0.177	TTKSK, TTRTF	TKTTF_MS18
615619215	6A	0.173	TTKSK, TTRTF	TKTTF_MS18
615636915	6A	0.099	TTKSK, TTRTF	-
686489689	6B	0.191	JRCQC	TKTTF_MS18, JRCQC _OS20
693829939	6B	0.079	TKTTF, TTRTF	-
43311031	7A	0.092	-	TKTTF_MS18, JRCQC _OS20
682951819	7A	0.054	-	TKTTF_MS19, JRCQC _MS19
686094342	7A	0.091	TTRTF	JRCQC _MS19
686964735	7A	0.075	-	TKTTF_MS19, JRCQC _MS19
687410326	7A	0.050	TTRTF	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19
690016567	7A	0.052	TTRTF	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19
690811708	7A	0.057	TTRTF	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19, JRCQC _OS20
690940195	7A	0.057	TRTTF	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19, JRCQC _OS20
693915965	7A	0.071	-	TKTTF_MS19, JRCQC _MS19
697030510	7A	0.093	-	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19
697030516	7A	0.056	TRTTF	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19, JRCQC _OS20
698390754	7A	0.096	-	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19
700727874	7A	0.059	TRTTF	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19
700805183	7A	0.077	TRTTF	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19, JRCQC _OS20
710171609	7A	0.055	-	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19
714327927	7A	0.067	-	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19
714370100	7A	0.052	-	TKTTF_MS19, JRCQC _MS19, JRCQC _OS20
714975616	7A	0.091	-	TKTTF_MS19, JRCQC _MS19, JRCQC _OS20

717517491	7A	0.056	TRTTF	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19
717518884	7A	0.059	TRTTF	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19, JRCQC _OS20
718484217	7A	0.098	-	TKTTF_MS19, JRCQC _MS19, JRCQC _OS20
719231181	7A	0.064	-	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19
719698163	7A	0.057	TRTTF	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19, JRCQC _OS20
719787589	7A	0.052	TRTTF	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19
721720978	7A	0.065	TRTTF	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19, JRCQC _OS20
724668618	7A	0.077	-	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19, JRCQC _OS20
724668652	7A	0.052	-	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19
681996206	7B	0.063	-	JRCQC _MS19, TKTTF_MS19
683438364	7B	0.107	-	JRCQC _MS19, TKTTF_MS19
151516737	UN	0.123	TRTTF	JRCQC _MS19
151742792	UN	0.056	TRTTF	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19, JRCQC _OS20
151847140	UN	0.057	TRTTF	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19, JRCQC _OS20
153928527	UN	0.056	TRTTF	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19, JRCQC _OS20
166522707	UN	0.054	-	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19
288369273	UN	0.075	-	TKTTF_MS19, JRCQC _MS19

Supplemental Table S3.12. Lists of common significant markers between races for seedling resistance of a durum wheat panel to the four *Pgt* races and/or between race-season combinations for field resistance to two *Pgt* races identified using FarmCPU.

Position	Chr.	MAF	Seedling resistance	Field resistance
689821784	5B	0.050	-	TKTTF_MS19, JRCQC _MS19, JRCQC _OS20
609635640	6A	0.149	TKTTF, TTRTF	-
611495915	6A	0.150	TTKSK, TKTTF, JRCQC	-
612043936	6A	0.301	TTRTF	TKTTF_MS18, JRCQC _MS19, JRCQC _OS20
615604035	6A	0.277	-	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19, JRCQC _OS20
700805183	7A	0.077	TTRTF	JRCQC _OS20
717518884	7A	0.059	-	TKTTF_MS18, TKTTF_MS19, JRCQC _MS19

Supplemental Table S3.13. Lists of durum wheat lines postulated to carry *Sr13b* based on race specificity and lines carrying favorable allele (FA) at the region of *Sr13b* (612003938).

GID	REP	TTKSK	TKTTF	JRCQC	TTRTF	FA
7145382	1	2-	2	3+	3+	+
7145526	1	2-	2	3+	3+	+
7383504	1	2-	2-	3+	3+	+
7383636	1	2-	2-	3+	3+	+
7383851	1	2-	1	3+	4	-
7383862	1	3+	2	3+	3+	+
7407103	1	2-	2-	3+	3+	+
7407855	1	2-	2	3+	2	+
7409307	1	2-	2+	3	3+	-
7409435	1	2-	2	3	2-	-
7409461	1	2-	2-	3	3+	+
7409573	1	2-	2	3+	3+	-
7409575	1	2-	2-	3+	3+	+
7410487	1	2-	2	3+	3+	+
7410802	1	2-	2-	3+	3+	+
7606753	1	2-	2	4	3+	+
7606811	1	2-	2	3+	3+	+
7606825	1	2-	2-	3+	3+	+
7384200	1	2-	2	3+	3+	+
7384201	1	2-	2	3+	3+	+
7384216	1	2	2-	2	2	-
7384219	1	2-	2-	3+	3+	+
7405994	1	2-	2+	3+	3	+
7406012	1	2-	NA	3	3	-
7145382	2	2-	2	3+	3+	+
7145526	2	2-	2	3+	3	+
7383504	2	2-	2-	3+	3+	+
7383636	2	2-	2-	3+	3+	+
7383851	2	2-	2	3+	3+	-
7383862	2	2-	2	3+	3	+
7407103	2	2	2-	3+	3+	+
7407855	2	2-	2	3+	4	+
7409307	2	2-	2	3+	2	-
7409435	2	2-	2	2	2+	-
7409461	2	2	2-	3+	3+	+
7409573	2	2-	2-	3+	3+	-
7409575	2	2-	2-	3+	3	+
7410487	2	2-	2-	3+	2	+
7410802	2	2-	2-	3	3+	+
7606753	2	2	2	3+	3+	+
7606811	2	2-	2-	4	3+	+
7606825	2	2-	2-	3+	2	+
7384200	2	2	2-	3+	3+	+
7384201	2	2-	2	3+	2	+
7384216	2	2	2	3+	3+	-
7384219	2	2-	2-	3+	3+	+
7405994	2	2-	2-	3+	2-	+
7406012	2	2-	NA	3+	2	-

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CHAPTER 4.

MULTIPLE-RACE STEM RUST RESISTANCE LOCI IDENTIFIED IN DURUM WHEAT USING GENOME-WIDE ASSOCIATION MAPPING

ABSTRACT

Stem rust of wheat caused by *Puccinia graminis* Pers.f.sp. *trtici* Eriks and E. Henn., is the most damaging fungal disease of both common (*Triticum aestivum* L.) and durum (*Triticum turgidum* L., ssp. Durum) wheat. Continuously emerging races virulent to many of the commercially deployed qualitative resistance genes have caused remarkable loss worldwide and threaten global wheat production. The objectives of this study were to evaluate the response of a panel of 283 durum wheat lines assembled by the International Maize and Wheat Improvement Center (CIMMYT) to multiple races of stem rust in East Africa at the adult plant stage and map loci associated with field resistance. The lines were evaluated in Debre Zeit, Ethiopia and Njoro, Kenya from 2018 to 2019 in five environments (year x season). The panel was genotyped using genotyping-by-sequencing. After filtering, 26,439 Single Nucleotide Polymorphism (SNP) markers and 280 lines and three checks were retained for analysis. Population structure was assessed using principal component analysis. Genome-wide association analysis (GWAS) was conducted using Genomic Association and Prediction Integrated Tool (GAPIT). The broad-sense heritability of the phenotype data revealed that 64% to 83% of the variation in stem rust response explained by the genotypes and lines with multiple race resistance were identified. GWAS analysis detected a total of 160 significant marker trait associations representing 42 quantitative trait loci. Of those, 21 were potentially novel and 21 were mapped to the same regions as previously reported loci. Known stem rust resistance

genes/alleles were postulated including *Sr8a*, *Sr8155B1, SrWeb/Sr9h, Sr11, Sr12, Sr13/Sr13 alleles, Sr17, Sr28/Sr16, Sr22* and *Sr49.* Lines resistant to multiple races in East Africa can be utilized as parents in durum wheat breeding programs. Further studies are needed to determine if there are new alleles at the *Sr13* locus and potential markers for the known *Sr13* alleles.

INTRODUCTION

Durum wheat (*Triticum turgidum* L., ssp. Durum (Desf.) Husnot, 2n=4x=28; AABB genome) is among the tetraploid wheat species used for making pasta, couscous and other traditional recipes mainly consumed in the Mediterranean regions (Shewry and Hey, 2015). The European Union, Canada, the Mediterranean basins, the North American plains, and Mexico are the major producers of durum wheat in the world (Bond and Liefert, 2017). A number of biotic and abiotic stress factors challenge the production of durum wheat. Among the biotic factors, stem rust of wheat caused by *Puccinia graminis* f.sp. *tritici* Eriks. & E. Henn (*Pgt*) is the most destructive fungal disease of both common and durum wheat (Roelfs et al., 1992). Stem rust can occur in all wheat growing areas and can cause complete yield loss under severe epidemics when susceptible cultivars are grown (Dean et al., 2012). The shriveling of grain due to stem rust can also downgrade the quality of the harvest and resulting end use products.

East African highlands are considered as hot spots for the emergence of new stem rust pathogen races. The emergence of new virulent races in East Africa and other parts of the world caused severe losses and continue to pose a threat to global wheat production and food security (Singh et al., 2015; Olivera et al., 2015; Bhavani

et al., 2019). Many of the races evolve with corresponding virulence to commercially deployed resistance genes and some have broad virulence spectrum. The races in East Africa including Ug99 (TTKSK) and its lineage, TKTTF('Digalu'), TRTTF and JRCQC defeated the resistance conferred by many major/R-genes in breeding lines and commercial cultivars. Stem rust race Ug99 was identified in Uganda in 1999 and spread across other countries in East Africa, the Middle East and South Africa. To date, 13 races identified from different countries with broad virulence to commercially deployed resistance genes, are considered part of the of the Ug99 lineage (Singh et al., 2015; Nirmala et al., 2017; Bhavani et al., 2019). Due to the continuously evolving races in the Ug99 group, most of the worldwide wheat germplasm were found to be moderately to highly susceptible to this group of races (Bajgain et al., 2015b; Singh et al., 2015).

Breeders in different regions of the world are incorporating resistance genes effective against the Ug99 lineages in their germplasm. However, the continuously emerging virulent races unrelated to Ug99 such as TKTTF, TRTTF and JRCQC in East Africa (Olivera et al., 2015) and the rest of the world, continue to defeat major resistance genes effective against the Ug99 race groups, threatening global production of both common and durum wheat. Race TKTTF identified in Ethiopia during the 2013/14 epidemics caused close to 100% yield loss on 10,000 hectares of land planted with the wheat variety 'Digalu'. This race defeated the resistance conferred by *SrTmp* which was effective against the Ug99 lineages. TKTTF has broad virulence to several other major genes (Olivera et al., 2015). Races JRCQC and TRTTF have combined virulence to the most frequent resistance genes/alleles in durum wheat, namely *Sr13b*

and *Sr9e* that are effective against TTKSK and other races from the same lineage (Olivera et al., 2012). Due to the emergence of JRCQC, a very large proportion of the global durum wheat germplasm including many of the CIMMYT and North American durum wheat germplasm which were protected by *Sr9e* and *Sr13b* became susceptible in Ethiopia where this race is predominant. These two races also have broad virulence to other major *Sr* genes deployed in commercial cultivars. TRTTF is virulent to *SrTmp* and *Sr36* and was the first to defeat the resistance conferred by the 1AL-1RS rye translocation (*Sr1RS)* (Olivera et al., 2012). As a result all spring and winter wheat varieties carrying these genes became susceptible to *Pgt* races identified in Africa and Asia (Olivera et al., 2012; Singh et al., 2015). Among the alleles of *Sr13, Sr13a* is effective against races TTKSK, TKTTF, TRTTF, JRCQC and the race recently identified in Italy and Georgia (TTRTF) while *Sr13b* is effective only against TTKSK and TKTTF (Zhang et al., 2017; Olivera et al., 2019). These resistance alleles, unless deployed properly in combination with other genes, are likely to be defeated by an emerging race.

More than 60 stem rust resistance genes have been cataloged and about 34 of them are located in the A and B sub-genomes. However, most of them are R-gene/major-gene resistances and many are effective against specific races only (McIntosh et al.,1995, 2017). Among the catalogued *Sr* genes, only five confer adult plant resistance (APR), namely *Sr2, Sr55*(*Lr67/Yr46/Pm39*), *Sr56, Sr57(Lr34/Yr18/Pm38),* and *Sr58 (Lr46/Yr29/Pm39)* (Singh et al., 2015). Adult plant resistance (APR) is quantitative in nature and is thought to be more durable than the qualitative major gene-based resistance. Quantitative resistance is generally expressed

at the adult plant stage and identified through field evaluations of seedling susceptible lines (Laidò et al., 2015). Conversely, evaluation of lines for field response regardless of their seedling response can be applied to identify all stage resistance genes but selection for APR can be challenging due to the masking by major or R-genes. Deploying combinations of several APR genes or in combination with effective major genes is a possible strategy to increase the durability of resistance in stem rust management (Bhavani et al., 2011). The genetic characterization and identification of available sources of resistance in a given germplasm pool is important for the judicious use of different resistance sources and subsequent deployment of gene combinations with proper stewardship. Genetic studies characterizing sources of resistance to stem rust are more limited in durum wheat than in common wheat (Chao et al., 2017). The limited genetic studies in the past used low density markers such as simple sequence repeats (SSRs) and Diversity arrays technology (DArT) (Haile et al., 2012; Letta et al., 2013) and very few used high density SNP markers. The lines used in the current study were not previously characterized for their field responses to the multiple stem rust races currently prevailing in East Africa and their genetic basis of resistance was not well understood. In the current study, a panel of lines from the CIMMYT germplasm pool were evaluated against multiple races of stem rust in Ethiopia and Kenya, and we used high density SNP markers discovered through the genotyping-by-sequencing (GBS) approach to identify genomic regions associated with the field responses of the genotypes.

MATERIALS AND METHODS

Plant materials and phenotyping

A panel of 283 spring durum wheat genotypes composed of a wide collection of advanced breeding lines and some cultivars that represent the current CIMMYT durum wheat germplasm was evaluated for adult plant response to stem rust for three seasons in Ethiopia (Debre Zeit Agricultural Research Center); off-season (January to May) 2018 and 2019, main season (June to November) 2018; and two seasons in Kenya (KARI, Njoro Station) during the main season (June to October) 2018 and 2019; hereafter abbreviated as ETOS18, ETOS19, ETMS18, KNMS18 and KNMS19, respectively. Among the 283 genotypes included in the panel, ten harbor *Sr25* (translocation from *Thinopyrum ponticum* onto chromosome 7A), six carry the *Sr25* + *Sr22 (Sr22* is a translocation from *T. boeticum* onto chromosome 7A) and eight have *Sr38* (a translocation from *T. ventricosum* onto chromosome 2A) that were developed through marker-assisted selection and represent resistances that are not present in any of the durum germplasm pools worldwide (Ammar, personal communication, 2020). In the Debre Zeit nursery, lines were planted in dual rows of 1 m length with 0.2 m inter-row spacing arranged in randomized incomplete block design with two replications. Two susceptible (‘Arendato’ and ‘Local red’) and one moderately resistant (‘Mangudo’) checks were repeated after every 50 plots. In addition, the 20 stem rust differential lines with known stem rust resistance genes (Fetch et al., 2009) were planted at the beginning and end of the nursery in Debre Zeit, Ethiopia. The plots were surrounded by spreader rows planted with a mixture of susceptible lines, namely ‘Arendato’, ‘PBW 343’, ‘Morocco’ and ‘Digalu’ in equal proportions. In the Njoro nursery, plots consisted of two rows of 0.7 m with 0.3 m

inter-row spacing arranged using the same design as in Ethiopia. The plots and the experimental field were surrounded by spreader rows planted as hill plots with an equal proportion mixture of the stem rust susceptible cultivars 'Cacuke' and 'Robin', and six lines carrying *Sr24* (Genotype identification number (GID) = 5391050, 5391052, 5391056, 5391057, 6391059, and 5391061).

Disease infection was initiated by artificial inoculation of the spreader rows with a bulk of stem rust urediniospores collected at each specific location from the previous season to ensure uniform disease distribution in the trials. Spreaders were inoculated with a mixture of field collection of stem rust races TTKSK, TKTTF, JRCQC, TTTTF and TRTTF in Debre Zeit, Ethiopia; and races TTKSK, TTKST, TTKTT and TTTTF in Njoro, Kenya. Inoculation was done by suspension of urediniospores in distilled water and adding a drop of Tween 20 (a drop/0.5 L) and syringe-injection of the spreader rows (at ~ 30 cm interval per meter) at stem elongation (~ Zadok's growth scale 31, first node detectable) (Zadoks et al., 1974) and repeated two to three times. Then urediniospores prepared with a similar protocol were sprayed one to two times on the spreader rows to enhance infection and disease development. In the off-season nurseries, furrow irrigation was applied for the establishment of the nursery and for providing a humid environment for proper disease development.

Disease severity was scored according to the modified Cobb's scale by estimating the proportion of the stem area (0-100%) covered by rust pustules (Peterson et al., 1948). Infection response was scored according to Roelfs et al. (1992) based on the size of pustules and amount of chlorosis and necrosis on the

stem. The responses classes are: '0' for no visible infection, 'R' for resistant, 'MR' for moderately resistant, 'MS' for moderately susceptible and 'S' for susceptible. A combination of responses was scored in the case of an overlap of infection responses on a single genotype by taking the most frequent response first followed by the less frequent. Stem rust was scored two to four times in each environment at 8 to 10-day intervals and the final scoring was considered for analysis. The stem rust severity and response were combined in a value called coefficient of infection (CI) calculated by multiplying the severity values with a linearized scale of 0 to 1 assigned to the respective responses. The scale was assigned as: immune = 0.0, R = 0.2, MR = 0.4, MS = 0.8 and S = 1.0, and the mean of the scale of responses was used to calculate CI in the cases where combinations of infection responses were scored for a given genotype (Stubbs et al., 1986).

Statistical analysis of phenotype data

The CI was used in the statistical analysis using R statistical software version 3.6.1 (R Core Team 2019) and ASReml-R version-3 for spatial correction (Gilmour et al., 2009). We fitted different models and finally chose a model which resulted in the highest estimate of broad-sense heritability. In some cases, a model with a significant Wald test for fixed effect was considered when the row and column effects were fitted as fixed (Gilmour et al., 2009). For the off-season 2018 nursery in Ethiopia, a linear mixed model (LMM) described in equation-4.1 was fitted on the CI using ASReml-R to extract the best linear unbiased predictions (BLUPs).

$$y_{ijk} = \mu + g_i + C_j + r_k + \varepsilon_{ijk} \qquad (4.1)$$

Where: y_{ijk} is the response of the i^{th} line in the j^{ith} column and the k^{th} replication, g_i is the random effect of the i^{th} line, C_j is the fixed effect of the j^{th} column, and r_k is the random effect of k^{th} replication and ε_{ijk} is the residual associated with the model.

For the main season 2018 nursery in Ethiopia, the LMM described in equation-4.2 was fitted on the square-root-transformed CI using the lmer() function of the R package *lme4* (Bates et al., 2015) and extracted genotypic BLUPs (R Core Team 2019).

$$y_{ij} = \mu + g_i + r_j + \varepsilon_{ij} \qquad (4.2)$$

Where: y_{ij} is the response of the i^{th} line at the j^{th} replication, g_i is the random effect of the i^{th} genotype (line), r_j is the random effect of the j^{th} replication, ε_{ij} the residual associated with the model.

For the off-season 2019 nursery in Ethiopia, the LMM described in equation-4.3 with the residual variance (ε_{ij}) fitted as ar1(row):ar1(column), the first order autoregressive correlation of the residuals of the row and column, as random effects, which assumes the residuals could be correlated (Gilmour et al., 2009) was fitted on the square-root transformed CI using ASReml-R and BLUPs were extracted. For the nursery in Kenya during the main season 2018, the LMM described in equation-4.3 was fitted on the square-root-transformed CI using ASRreml-R (Gilmour et al., 2009) and genotypic BLUPs were extracted.

$$y_{ijkl} = \mu + g_i + R_j + C_k + r_l + \varepsilon_{ijkl} \qquad (4.3)$$

Where:y_{ijkl} is the response of the i^{th} line in the j^{th} row, in the k^{th} column and l^{th} replication, g_i is the random effect of the i^{th} line, R_j the fixed effect of the j^{th} row, C_k is the fixed effect of the k^{th} column, r_l is the random effect of the l^{th} replication and ε_{ijkl} is the residual associated with the model.

For the main season 2019 nursery in Kenya, the MLM described in equation-4.2 was fitted on the square-root transformed CI using the lmer() function of the R package *lme4* and genotypic BLUPs were extracted. From the variance components estimated from each model, broad sense heritability was calculated following the method by Holland et al. (2003).

$$H^2 = V_g/V_p \quad (4.4)$$

Where: H^2 is the broad sense heritability, V_g is the variance due to the genotype (line), V_p is the variance due to the phenotype, $V_p = V_g + V_e$, V_e is the residual variance.

Genotyping and data filtering

Two cm of young leaf tissue were collected and frozen at -80^{o}C for two weeks. The frozen leaf samples were then lyophilized and shipped to the USDA-ARS Eastern Regional Small Grains Genotyping Laboratory in Raleigh, NC for genotyping. Genomic DNA was isolated from the lyophilized tissue samples using a sbeadex plant DNA isolation kit (LGC Genomics, Middlesex, UK) according to manufacturer's instructions. Genomic DNA was then fragmented using a *PstI-MspI* double restriction digest following the GBS protocol of Poland et al. (2012). Sequencing adapters were ligated to DNA fragments, and single-ended 100bp short read sequencing was then performed on an Illumina (San Diego, CA) Novaseq instrument. SNP genotype calling was done using TASSEL software version 5 (Glaubitz et al., 2014) and the recently published durum wheat reference genome of cultivar 'Svevo' (Maccaferri et al., 2019) was used to assign a physical position to each SNP marker. Thereafter, SNP markers with missing data above 50%, minor allele frequency (MAF) below 5%, and heterozygous call rates above 15% were filtered out. Missing data was then imputed

using Beagle 5 (Browning et al., 2018). Following imputation, PLINK 1.9 (Chang et al., 2015) was used to remove all but one SNP in groups of SNPs in perfect linkage disequilibrium (LD) with each other ($r^2 = 1$), using a sliding window of 250 SNPs, advancing by 10 SNPs per step. In total, 26,439 SNPs were called in 283 lines (including three checks) and retained for genome-wide association analysis.

All lines were also screened with kompetitive allele-specific PCR (KASP) assays developed around SNP linked to the resistance genes *Sr2* and *Lr46/Sr58*. For *Sr2*, lines were evaluated with marker *Sr2_ger93p* (Mago et al., 2011). For *Sr58,* lines were evaluated for SNP CIMwMAS0085 tightly linked leaf rust APR gene, *Lr46* (https://www.integratedbreeding.net). Lines were also evaluated with a KASP assay targeting *Sr13,* the major gene most frequent in durum wheat which provides effective resistance to the Ug99 lineage. The *Sr13* assays was designed around the mutation at amino acid W743R (Zhang et al. 2017). Lines having the 734R amino acid associated with resistance to TTKSK were noted as having an *Sr13* allele for resistance. KASP assay primer sequences are noted in Supplemental Table 4.9.

Population structure and linkage disequilibrium analyses

If not taken into account, population structure results in false positive marker trait associations (MTA) in GWAS analyses. In the current study, the presence of population structure was assessed using principal component analysis (PCA) using the R function 'prcomp' and visualized for the clustering of PC scores. The extent of LD in a population is useful for determining the resolution of association mapping. The LD between pairs of markers for the 26,439 markers was calculated as the squared allele frequency correlation (r^2) by applying a sliding window of 50 markers using

TASSEL software version 5 (Bradbury et al., 2007). The r^2 values of pairs of loci were plotted against the physical distances in Megabases (Mb) after randomly sampling 10% of the total loci pairs. A locally estimated scatterplot smoothing (LOESS) curve was fitted using 'geom_smooth' in R package *ggplot2* (Wickham, 2016) to visualize the decay of LD in each of the 14 chromosomes. The r^2 threshold to verify that LD was likely to be due to linkage was estimated from the 95th percentile of the distribution of the square-root-transformed r^2 of unlinked markers (Breseghello and Sorrells, 2006). The point at which the horizontal line at the r^2 critical value and the LOESS curve on the LD scatter plot intersected was treated as the estimate of the extent of LD for each chromosome in our study population.

Genome-wide association analyses

The BLUPs derived from the respective models fitted on the phenotypic data were considered as the response to fit GWAS models. The analysis was conducted using GAPIT (Lipka et al., 2012) by fitting four models; Mixed Linear Model (MLM) (Yu et al., 2006), Compressed Mixed Linear Model (CMLM) (Zhang et al., 2010), Multi-locus Mixed Linear Model (MLMM) (Segura et al., 2012) and Fixed and random model Circulating Probability Unification (FarmCPU) (Liu et al., 2016). MLM is a single locus model that fits one marker at a time as a fixed effect, population structure as a fixed effect (Q) and marker based additive relationship matrix or Kinship (K) as a random effect in the model (Q+K model). CMLM fits MLM after clustering individuals to estimate kinship and reduces computational time (Zhang et al., 2010). MLMM estimates variance components using a stepwise forward-backward linear mixed-model regression and fits the significant SNP as a covariate for the following

step (Lipka et al., 2012), and FarmCPU uses both Fixed Effect and Random Effect models iteratively. It fits one marker at a time in the Fixed Effect Model with significant markers as covariates. Then the kinship of the significant markers is used to fit the Random Effect Model (Liu et al., 2016). The first two PC scores were used to account for population structure in all models. A false discovery rate (FDR) of 5% was applied for multiple comparison adjustment and as a threshold to declare significant MTAs (Benjamini and Hochberg, 1995). The deviation of the observed -log10p-value distribution from the expected distribution in the quantile-quantile (Q-Q) plots was used to compare the models and results were interpreted from MLM and FarmCPU. Manhattan plots of -log10 p-values were generated using the R package *qqman* (Turner, 2017). A linkage disequilibrium heatmap was plotted for significant markers on chromosome 6A and the *Sr13* marker, and the significant markers on chromosome 7A using the R package *LDheatmap* applied on the square matrix of the squared allele frequency correlation between pairs of markers (Shin et al., 2006). Significant markers tagging quantitative trait loci/locus (QTL) were gathered from previous QTL studies on durum and common wheat. The sequences of these markers were searched from the GrainGenes database. Then the fasta file of the sequences was aligned against the respective chromosomes of the 'Svevo' reference sequence using the blastn program of the IWGSC database for similarity of physical positions with the significant markers identified in the current study and for postulation of resistance genes/alleles.

RESULTS

Phenotypic data analyses

The distributions of the CI were skewed towards resistance in all environments except ETOS18 which was close to normal distribution (Figure 4.1). The percentage of resistant lines (CI <= 18) varied from 10 % in ETOS18 with a mean CI of 40 to 65 % in KNMS18 with a mean CI of 18.3 (Table 4.1). The broad-sense heritabilities estimated from the variance components of each model fitted were 0.71 for ETOS18, 0.64 for ETMS18, 0.83 for ETOS19, 0.77 for KNMS18 and 0.69 for KNMS19 indicating that most of the variation in the response (64% to 83%) was explained by the genotypic component.

Table 4. 1. Summary of descriptive statistics, genetic variance and broad-sense heritability of coefficient of infection (CI) of the 283 durum wheat lines across the five environments.

Statistic	ETOS18	ETMS18	ETOS19	KNMS18	KNMS19
Mean	40.0	28.7	24.4	18.3	25.1
Range	0-80	0-80	0-80	0-90	1-100
Resistant (%)	10	35	46	65	55
Susceptible (%)	90	65	54	35	45
V_g	241	2.58	2.36	3.44	3.39
H^2	0.71	0.64	0.83	0.77	0.69

H^2 : Broad-sense heritability
V_g :genetic variance

Screening of the lines with markers linked to *Sr2*, *Sr13* and *Sr58 (*using *Lr46* linked marker*)* revealed that 69% of the total number of lines evaluated were likely to carry *Sr13,* 46% were likely to have *Lr46* (*Sr58*), 30% (85 lines) were likely to have both genes (*Sr13* and *Lr46/Sr58*) and 15% (43 lines) were lacking both genes. Among the lines positive to *Sr13* and *Lr46/Sr58,* 14.3% showed resistance (CI <= 18) in all the five environments, 16.7% in four environments, 32.1% in three environments 21.4% in two environments and 15.5% in a single environment (Supplemental Table 4.1). Three lines with an Origin GID 7147179, 7147180, 7147182 showed immune responses in most environments (Supplemental Table 4.1). None of the lines from the current panel was found to carry *Sr2*. Among the 43 lines that lack *Sr13* and

Lr46/Sr58 based on the marker screening, a line with GID 7145241 was consistently resistant in all the five testing environments, line GID 6951159 was resistant in four environments except ETOS19, line GID 5928165 was resistant in three environments, line GID 7408527 was resistant in ETOS19 and KNMS18, line GID 7409573 was resistant in KNMS18 and KNMS19. Lines with GID 7383430, 7407575 and 7384241 were resistant in KNMS18 while GID 7408885 was resistant in KNMS19 (data not shown).

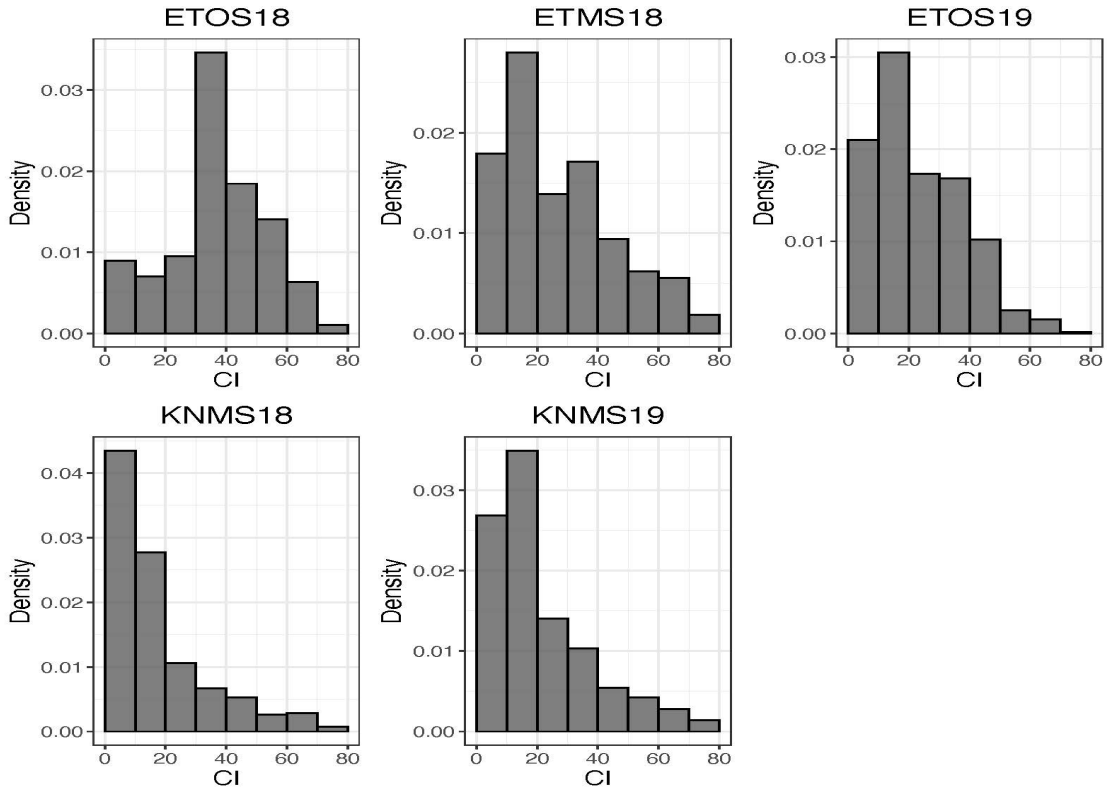


Figure 4. 1. Distribution of coefficient of infection (CI) calculated as the product of severity and a linearized scale for response across five environments.

Population structure and linkage disequilibrium analyses

The scatter plot of the first two PC scores indicated two putative groups although the clustering was not clear. The first and the second PC scores explained 3.79% and 2.78% of the genetic variation in the panel, respectively (Figure 4.2). The genome-wide LD calculated for the 26,439 markers resulted in a total of 1,320,675 pairwise comparisons of loci. Out of the total pairs of loci compared, 37.4% (494,449) were in significant LD ($p < 0.001$). The mean genome-wide LD (r^2) for the population was 0.39. Of the total loci pairs, 1.28% (16,860) of the loci pairs were in wide range LD on different chromosomes, and 1.09% (184) of those on different chromosomes were in significant LD ($p < 0.001$). The LD threshold for the population estimated from the 95th percentile of the distribution of square root transformed r^2 of unlinked markers (markers located on different chromosomes) was 0.16, the critical value beyond which LD was likely due to physical linkage. The decay of LD for the linked markers varied across chromosomes in both sub-genomes (Supplemental Figure 4.1). The LOESS curve crossed the horizontal line of threshold value at approximately 4 Mb in all chromosomes of the A genome except chromosomes 2A (8 Mb), 3A (3 Mb) and 5A (5 Mb) with an average of 4.5 Mb. For the B genome, the LOESS curve crossed with the horizontal line of the critical value at 5 Mb for chromosomes 1B, 2B and 7B, at 4 Mb for chromosomes 3B and 5B, at 8 Mb for 4B, and at 4.5 Mb for 6B with an average of 4.6 Mb. The decay of LD in chromosome 2A and 4B was slower (8Mb) than the rest of the chromosomes (Supplemental Figure 4.1).

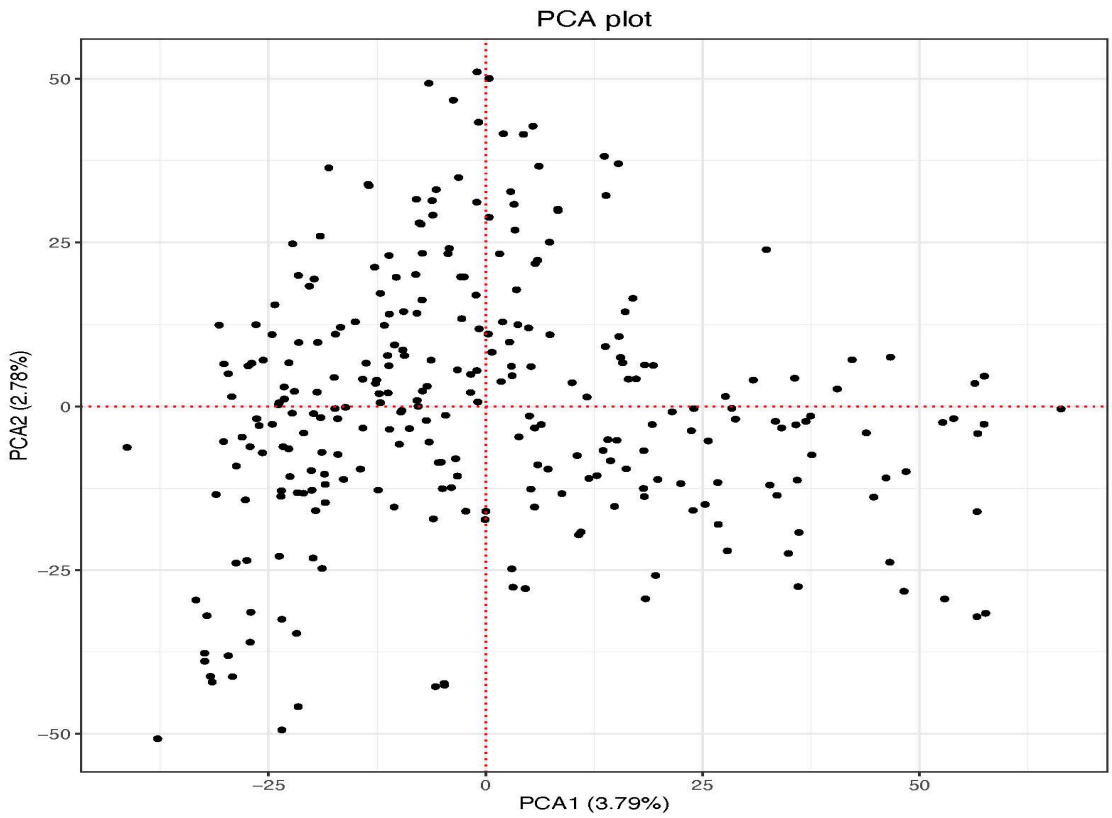


Figure 4. 2. Principal component-1 (PC1) plotted against principal component-2 (PC2) of the panel.

Genome-wide association analyses

GWAS analysis was conducted by fitting four models (MLM, CMLM, MLMM and FarmCPU) for each of the evaluation environments. Based on the Q-Q plots and the power of FarmCPU to limit potential false positive and false negative associations, we limited the interpretation of results to those from MLM and FarmCPU models. Many of the significant MTAs identified by MLM were confirmed by FarmCPU and the unconfirmed MTAs were assessed for consistency across environments to determine if they were reliable MTAs (Supplemental Tables 4.2 to 4.7). FarmCPU selected the most significant marker from linked markers falling within the same QTL, such as for chromosomes 6A and 7A in the GWAS results of the MLM. FarmCPU also identified

novel as well as previously reported MTAs unidentified by MLM (Supplemental Tables 4.2). The results of the CMLM and MLMM were not considered further for the following reasons: the Q-Q plot of CMLM fitted the data well only for ETOS18, ETOS19 and KNMS18 and under such circumstances, the significant MTAs identified by MLM and CMLM were the same. Although MLMM had an acceptable Q-Q plot, this model identified the fewest significant MTAs in all the five environments (data not shown).

MLM identified a total of 135 significant MTAs for field resistance to multiple *Pgt* races in Ethiopia and Kenya across the five testing environments. From these,14.1% were detected in all the five environments, 7.4% in four environments, 5.2% in three environments, 16 .3% in two environments and 57% in only one environment (Supplemental Tables 4.3 to 4.7). Among the 57% (77 markers) identified in a single testing environment, most were on chromosomes 6A and 7A and they were in LD with other nearby markers identified across multiple environments (Figures 4.5 and 4.6). From the total MTAs identified by MLM, 9.6% were confirmed by FarmCPU (Supplemental Tables 4.2 and 4.8) and most of the significant markers on chromosome 6A and 7A identified by MLM were in LD with the those identified by FarmCPU on the same chromosome. FarmCPU identified a total of 47 significant MTAs (Supplemental Table 4.2). Among the total, 4% were identified in three testing environments, 11% in two environments and the remaining 85% in a single testing environment (Table 4.2). Out of the total MTAs identified by the two models, nine MTAs were on unaligned contigs (Supplemental Tables 4.2 to 4.8).

Table 4. 2. Lists of consistent significant markers between environments identified using FarmCPU.

Position	Chr.	MAF	Environment	Proposed gene

724805496	3B	0.104	ETOS18, KNMS18	*Sr12*
691693264	5B	0.051	ETOS18, ETMS18	*Sr49*
692277095	5B	0.058	ETOS19, KNMS18	*Sr49*
592006	6A	0.228	ETOS18, KNMS19	*Novel/Sr8155B1*
612043936	6A	0.302	ETMS18, KNMS18, KNMS19	*Sr13*
700805183	7A	0.076	ETOS18, ETOS19, KNMS19	*Sr22/Sr25*
717518884	7A	0.058	ETMS18, KNMS18	*Sr22/Sr25*

Three significant MTAs were identified on chromosome 1A at 95 Mb, 144 Mb and 485 Mb (Figure 4.3; Supplemental Figure 4.2). The QTL at 95 Mb and 485 Mb explained 3% and 3.73% of the phenotypic variation, respectively and the MTA at 144 Mb was close to the threshold (FDR adjusted p-value = 0.04) (Supplemental Tables 4.2, 4.5). On chromosome 1B, four significant MTAs were identified at 183 Mb, 546 Mb, 587 Mb and 620 Mb (Supplemental Figure 4.2, Figure 4.4). The three MTAs on chromosome 1B except the 183 Mb (FDR adjusted *p-value* = 0.045) represented three QTL that explained 3.43 to 4.59% of the phenotypic variation (Supplemental Tables 4.2-4.4). Seven significant MTAs (20 Mb, 67 Mb, 78 Mb, 135 Mb, 699 Mb, 728 Mb and 770 Mb) were detected on chromosome 2A (Figures 4.3 and 4.4). Six of the MTAs represented putatively six QTL and one at 699 Mb had an FDR adjusted p-value close to the threshold (0.049) (Supplemental Table 4.3). Four MTAs (56 Mb, 456 Mb, 759 Mb, 780 Mb) were identified on chromosome 2B (Supplemental Figure 4.2; Figures 4.3 and 4.4). The three MTAs represented three QTL that explained 2.37% to 3.93 % of the phenotypic variation while the 56 Mb region was close to the threshold (FDR adjusted *p-value* = 0.046) (Supplemental Table 4.3). Three putative QTL represented by three significant MTAs (9 Mb, 313 Mb, 344 Mb) were identified on chromosome 3A using FarmCPU (Figures 4.3 and 4.4). The phenotypic variance explained by the two MTAs at 313 Mb and 344 Mb was 3.25% and 2.98%, respectively and was very low for the 9 Mb region (data not shown). All the

significant MTAs identified on chromosomes 1A, 1B, 2A and 2B were identified at a single testing environment and using either one of the two models.

Five significant MTAs (38 Mb, 55 Mb, 97 Mb, 213 Mb, 724 Mb) representing three QTL were detected on chromosome 3B. The MTA at 55 Mb was identified at a single environment using MLM and it explained 4.04 % of the phenotypic variation. The 97 Mb region identified using MLM was consistent across four (ETOS18, ETMS18, ETOS19, KNMS18) of the five testing environments and it explained 3.91% to 4.81% of the phenotypic variation (Supplemental Tables 4.2 to 4.6). The QTL at 724 Mb was consistent across two testing environments (ETOS18 and KNMS18) and the two models (Table 4.2). This QTL (724 Mb) explained 3.28% of the phenotypic variation on average (Supplemental Table 4.3). The two MTAs at 38 Mb and 213 Mb were close to the FDR threshold (FDR adjusted *p-value* =0.04) (Supplemental Table 4.3). Two significant MTAs representing two putative QTL were identified on chromosome 4A using MLM. The 619 Mb region was consistent in all the five testing environments and explained 5% to 7.84% of the phenotypic variation while the association at 651 Mb region was detected in a single environment and explained 3.99 % of the phenotypic variation (Supplemental Tables 4.3 to 4.8). Two significant MTAs (8 Mb and 35 Mb) representing two putative QTL were detected on chromosome 5A using FarmCPU. These two MTAs were identified in one testing environment (Supplemental Table 4.2) and explained only 2.66% and 1.71% of the phenotypic variation, respectively (data not shown).

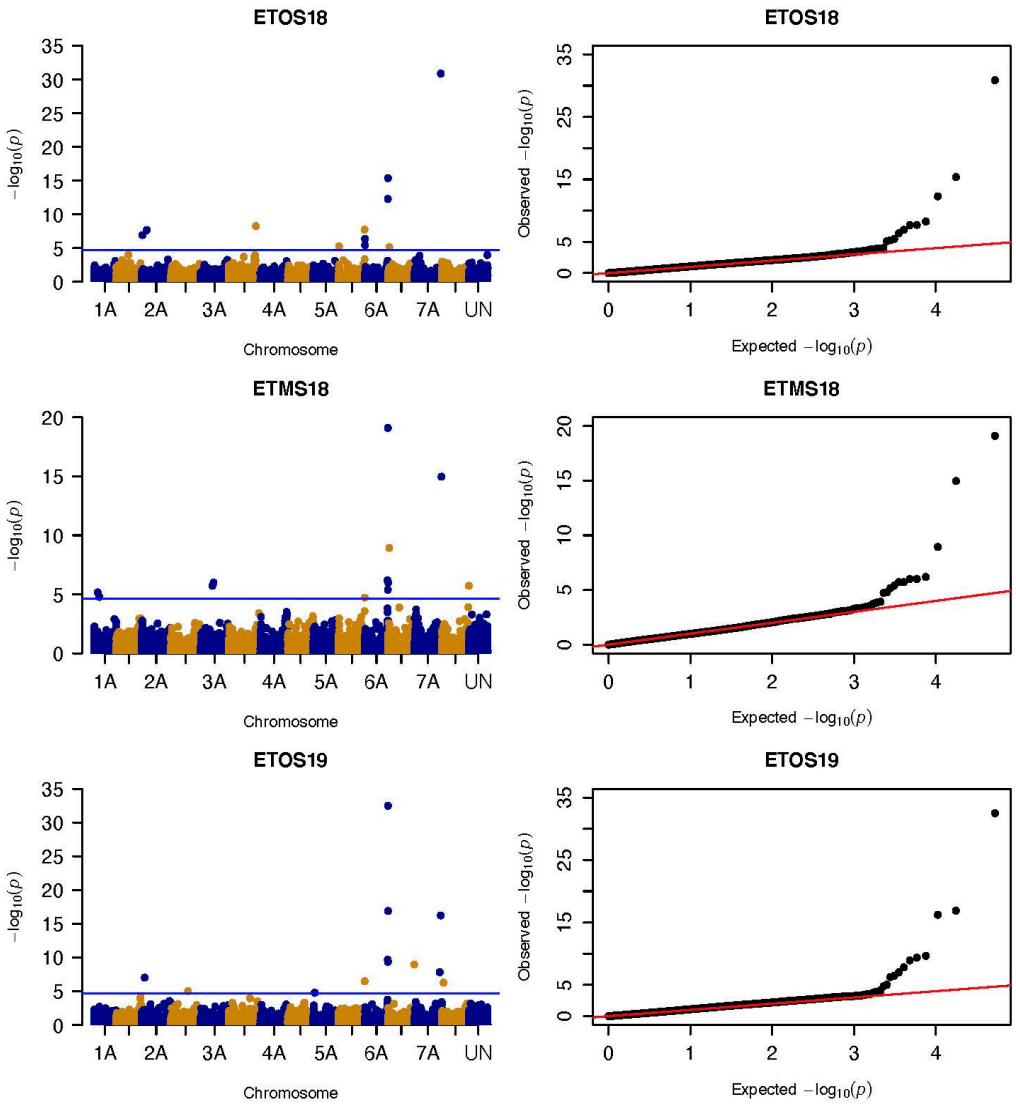


Figure 4. 3. Manhattan and QQ-plots of GWAS results of field resistance of durum wheat lines to multiple races in Ethiopia across three seasons identified using FarmCPU.

Seven MTAs (at 12 Mb, 13 Mb, 581 Mb, 671 Mb, 688 Mb, 691 Mb, 692 Mb) representing five QTL were identified on chromosome 5B (Figures 4.3 and 4.4; Supplemental Figures 4.2 and 4.3). The QTL represented by the MTAs at 12 Mb and 13 Mb (LD, $r^2 = 0.46$) was identified using FarmCPU in KNMS18 and ETOS18, respectively (Supplemental Table 4.2). This QTL explained 2.6 % of the phenotypic

variation on average (data not shown). The QTL at 581 Mb was consistently identified by MLM and FarmCPU in KNMS19 and explained 5.56% of the phenotypic variation. Two QTL represented by single markers at 671 Mb and 688 Mb regions explained 3.17% and 3.63% of the phenotypic variation, respectively and both were identified in one testing environment and one of the two models (Supplemental Tables 4.2 and 4.3). The QTL at 691 Mb and 692 Mb identified by FarmCPU (LD, $r^2 = 0.86$) was consistent across four of the five testing environments (Table 4.2).

On chromosome 6A, 52 significant MTAs representing five putative QTL were identified using MLM and FarmCPU (Supplemental Tables 4.2 to 4.8). The MTA at 592 kb identified using FarmCPU was consistent across two environments (Table 4.2) and explained 2.68% of the phenotypic variation on average (data not shown). This marker (592006 bp) was in strong LD ($r^2 = 0.89$) with a significant marker at 4 Mb (4914394 bp) identified using FarmCPU which explained 3.18% of the phenotypic variation. An MTA identified by FarmCPU in a single environment at 1.4 Mb explained 3.18% of the phenotypic variation (data not shown). A QTL at 28 Mb was consistently identified at two testing environments and explained 4.42% of the phenotypic variation on average while the 334 Mb region was consistent across all the five testing environments and explained 3.52% to 7.39% of the phenotypic variation (Supplemental Table 4.4). Forty-five MTAs extending from 606 Mb to 615 Mb represented one putative QTL on chromosome 6A that explained 3.38% to 9.79% of the phenotypic variation. All significant markers identified on chromosome 6A that extended from 598 Mb to 615 Mb except one marker at 612 Mb were in LD with the *Sr13* marker ($r^2 = 0.10$ to 0.40) (Figure 4.5). The 598 Mb region was identified in a

single environment and contributed less to the variation in the phenotype (R^2 = 1.62%). Twenty-three MTAs identified by MLM extending from 609 Mb to 615 Mb were consistent across two to four testing environments (Supplemental Table 4.4) whereas nine MTAs from 606 Mb to 615 Mb were consistently identified by MLM and FarmCPU (Supplemental Tables 4.2 to 4.7). One MTA at 612 Mb was consistently identified across three testing environments using FarmCPU (Table 4.2). From the MTAs on chromosome 6A that extended from 606 Mb to 615 Mb, the most significant markers were located at 612 Mb (612802438 bp) (p-value = 1.01E-07) for ETOS18, at 611 Mb (611495915 bp) for ETMS18 (p-value = 8.47E-07) and ETOS19 (p-value = 5.61E-10), at 612 Mb (612043936 bp) for KNMS18 (p-value = 3.13E-09) and KNMS19 (p-value = 3.71E-09). The marker at 611 Mb (611495915 bp) was consistent across two testing environments and the two models. This MTA explained 5.31% to 9.49% of the phenotypic variation and this marker was in weak to strong LD (r2 = 0.12 to 0.75) with 22 significant markers that extended from 598 Mb to 610 Mb (Figure 4.5). The MTA at 612 Mb (612043936 bp) was consistently identified across four environments using MLM and three testing environments using FarmCPU (Supplemental Table 4.4; Table 4.2). This MTA explained 3.44% to 9.79% of the phenotypic variation across the test environments. The other most significant marker at 612 Mb (612802438 bp) was consistent across three environments and the two models; it explained 4.94 to 9.29% of the phenotypic variation. This marker was in weak to strong LD (r2 = 0.14 to 0.96) with 20 significant markers that extended from

612 Mb to 615 Mb on chromosome 6A (Figure 4.5).

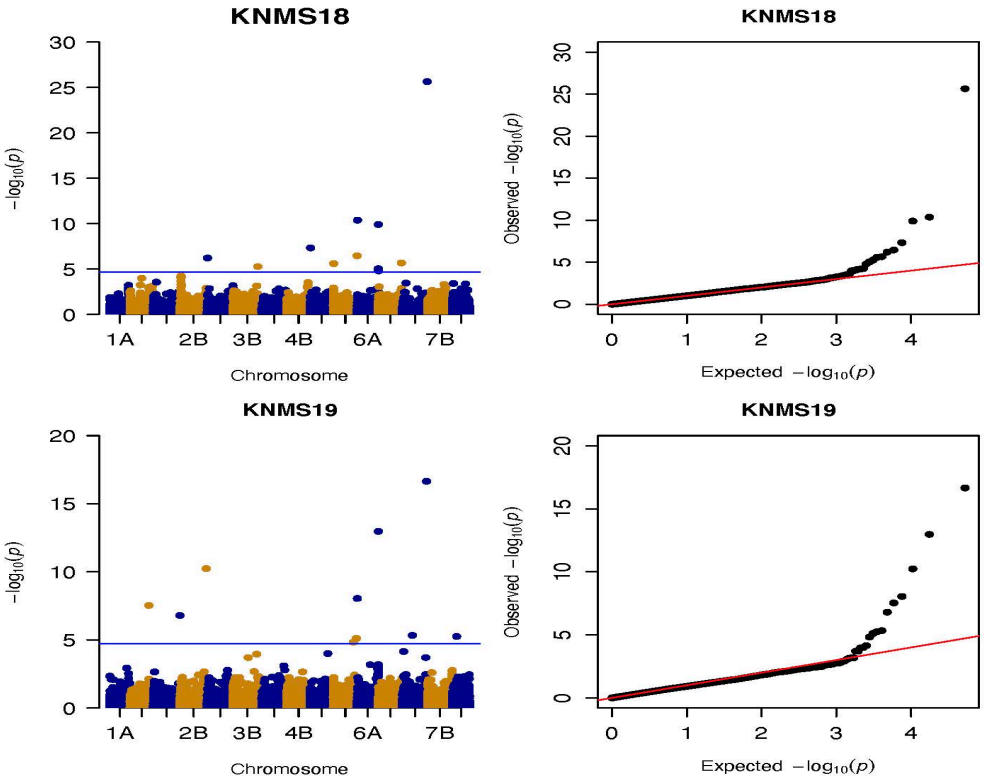


Figure 4.4. Manhattan and QQ-plots of GWAS results of field resistance of durum wheat lines to multiple races in Kenya across two seasons identified using FarmCPU.

Six significant MTAs were detected on chromosome 6B (Figures 4.3 and 4.4; Supplementary Figure 4.2). A QTL at 30 Mb and 31 Mb (LD, $r^2 = 0.33$) identified using FarmCPU was consistent across two seasons in Ethiopia (Table 4.2) and explained only 2.36% of the phenotypic variation on average (data not shown). The MTAs at 666 Mb and 692 Mb were identified in single environments using FarmCPU (Supplemental Table 4.2).

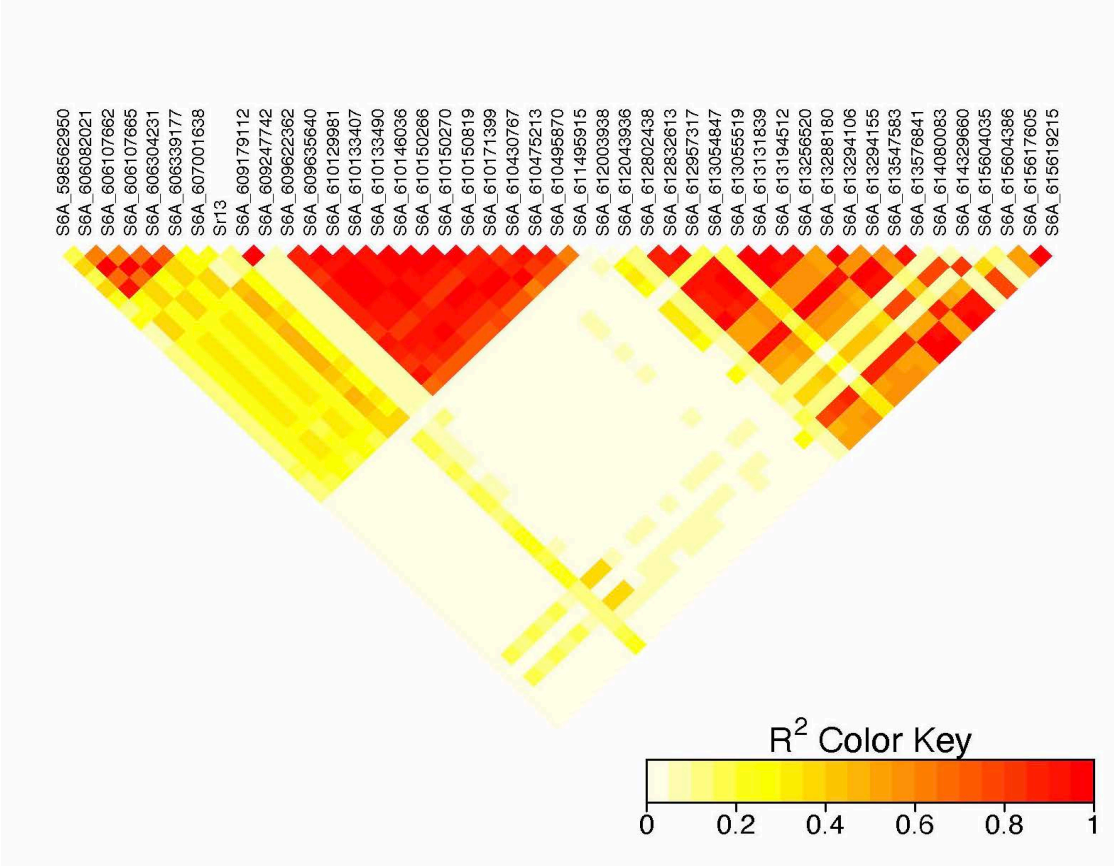


Figure 4. 5. Linkage disequilibrium heatmap of the *Sr13* marker and nearby significant markers on chromosome 6A.

The QTL at 666 Mb explained 2.35% of the phenotypic variation while the 692 Mb region contributed very low to the phenotypic variation (data not shown) and had low MAF (0.053). A QTL at 686 Mb and 687 Mb (LD, $r^2 = 0.64$) was identified using MLM in ETOS19 and explained 3.72% of the phenotypic variation on average Supplemental Table 4.5).

On chromosome 7A, 60 significant MTAs were identified using MLM and FarmCPU (Figures 4.3 and 4.4; Supplemental Figures 4.2 and 4.3). Four MTAs at 43 Mb, 117 Mb, 139 Mb and 285 Mb regions were inconsistent across the testing environments and the two models. The remaining MTAs that extended from 668 Mb to 727 Mb (55 Markers) explained 3.42% to 10.38 % of the phenotypic variation

(Supplemental Tables 4.3 to 4.7). These markers were in weak to strong LD and may represent the same QTL (Figure 4.6).

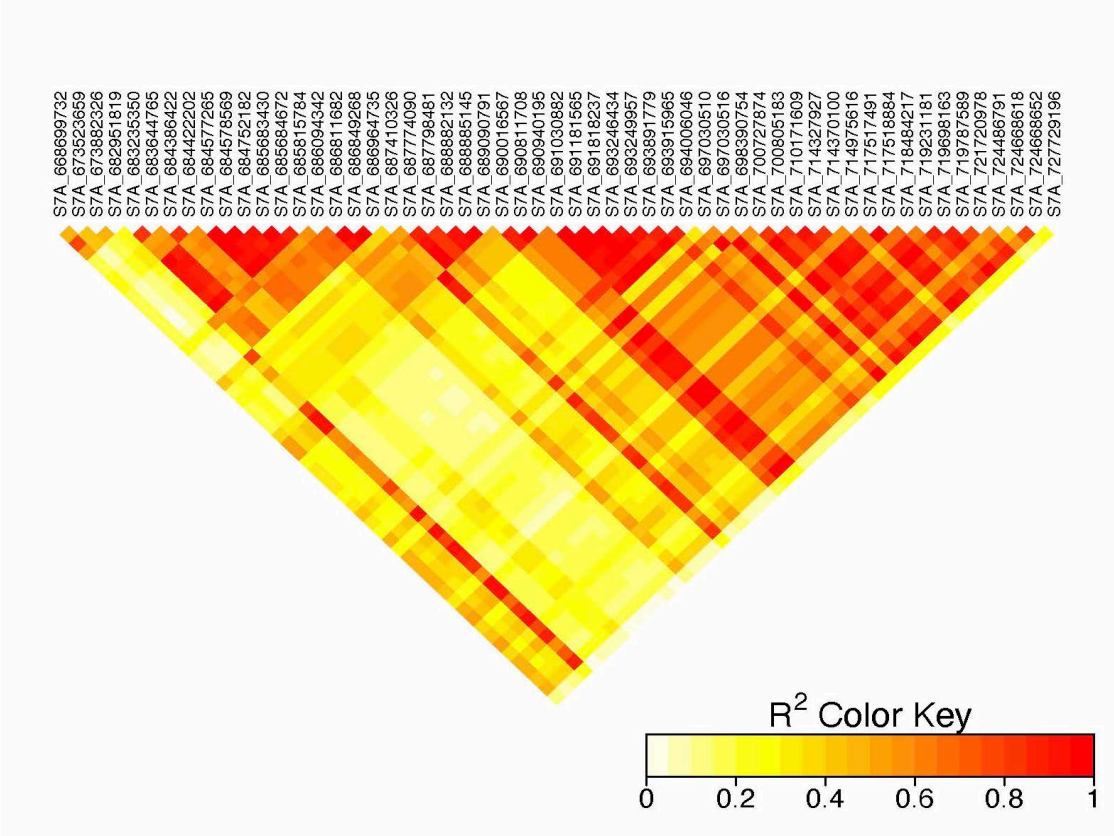


Figure 4. 6. Linkage disequilibrium heatmap of adjacent significant markers on chromosome 7A.

On chromosome 7A, 23 MTAs that extended from 690 Mb to 724 Mb identified using MLM were consistent across two to five testing environments (Supplemental Table 4.4). Two MTAs (700 Mb and 717 Mb) were consistently identified by MLM and FarmCPU in all the five testing environments (Table 4.2). The markers at 700 Mb (700805183 bp) and 717 Mb (717518884 bp) were identified as the most significant markers in each of the testing environments using MLM and FarmCPU (Supplemental Tables 4.2 to 4.8). The MTA at 700 Mb explained 5.25% to 9.05% the phenotypic variation across the five testing environments (average =

7.13%) while the one at 717 Mb explained 5.06% to 10.38% of the phenotypic variation across the five testing environments (average = 7.66%). These two markers (700 Mb and 717 Mb) were in strong LD ($r^2 = 0.83$) (Figure 4.6). Five MTAs representing four QTL were identified on chromosome 7B. Two QTL at 46 Mb and 717 Mb detected by FarmCPU and one QTL at 707 Mb detected by MLM were identified in single environments. A QTL at 622 Mb and 644 Mb (LD, $r^2 = 0.64$) identified by MLM was consistent across four of the five environments and explained 3.78% to 5.77% of the phenotypic variation (Supplemental Tables 4.2 to 4.7).

DISCUSSION

The characterization and identification of widely effective resistance available in a breeding program's elite pool is valuable for addressing the stem rust problem in durum wheat. In the current study, we evaluated the reaction of a panel of 283 elite durum wheat lines and cultivars representing the CIMMYT germplasm pool to multiple races of stem rust in East Africa and mapped a number of previously reported and novel genomic regions associated with field resistance to the locally prevailing races (Lists of Pedigrees: Appendix).

Phenotypic data analysis

The skewed distribution of the lines towards the resistance side in all testing environments except in ETOS18 could be due to the differences in race compositions across the testing environments (Figure 4.1). In contrast to races in Kenya which are less virulent on durum wheat, those in Ethiopia are composed of races such as the JRCQC with combined virulence to the most deployed stem rust resistance genes/alleles (*Sr13b* and *Sr9e*) in worldwide durum wheat germplasm and cultivars

(Olivera et al. 2012). The similar frequency distribution of the CI of the lines in ETMS18 and ETOS19 to that of the two seasons in Kenya is not expected (Figure 4.1). The possible explanation for this result is that the spores collected in the previous season to inoculate the ETMS18 and ETOS19 trials are possibly composed of high frequency of durum avirulent races than virulent ones. Among the resistant lines across the five testing environments, 85 lines were likely carrying *Sr13* and *Lr46* which showed resistance against multiple stem rust races in single testing environment (15.5%) and all the five testing environments (14.3%) (Supplemental Table 4.1). This inconsistency in the response across environments while carrying these two genes could be due to the seasonal variation in race composition, race specificity of R-genes/alleles such as the alleles of *Sr13* since the marker used for screening of the lines for this gene was not allele specific and the subjectivity of disease scoring may also contribute. Lines lacking *Sr13* and *Lr46* that showed resistance to multiple-races across the testing environments may carry other resistance genes. These lines harboring widely effective field resistance would represent potentially useful parents that can be utilized in durum wheat breeding programs. Moreover, the risk of introducing linked undesirable alleles in utilizing these lines as sources of resistance in durum wheat breeding programs is unlikely since the study population is a collection of breeding lines from the CIMMYT durum wheat breeding program. Evaluating the multiple race resistant lines for agronomic performance and combining more resistance genes/alleles to the best performing lines can increase durability of resistance to stem rust in future varieties.

Population structure and linkage disequilibrium

The population structure in the current study panel was minimal indicated in the PCA plot and the variance explained by the two PCs (Figure 4.2). The heatmap of marker-based kinship matrix indicated in the supplemental figure also supports this result (Supplemental Figure 4.4). This could be because our study population was a panel of breeding lines sourced only from CIMMYT. The resolution of GWAS mapping relies on the level of LD, which can vary based on the population used for study (Chao et al., 2017). For our population, the decay of LD varied across chromosomes of both sub-genomes with an average of 4.5 Mb for the A sub-genome and 4.6 Mb for the B sub-genome (Supplemental Figure 4.1). The average LD of the A sub-genome ($r^2 = 0.39$) and B sub-genome ($r^2 = 0.40$) was not divergent (*p-value* = 0.6961) which may indicate comparable selection pressure for important agronomic traits in the two sub-genomes of the durum panel. Chromosomes 2A and 4B had the slowest in the rate of LD decay (~8 Mb) (Supplemental Figure 4.1) indicating that the mapping resolution on these chromosomes is low although chromosome 4B did not contain any significant MTAs. Studies on LD patterns in durum wheat were reported using low density markers (Letta et al., 2013, 2014) and some using relatively high density markers (SNP markers) (Mengistu et al., 2016; Chao et al., 2017) on worldwide durum wheat collections and landraces. Although the decay of LD in these studies was described in genetic distances which may be difficult to compare with our results, it was reported that LD can vary from 5 cM in diverse breeding lines to 20 cM in worldwide collections (Chao et al., 2017).

Comparison of significant markers with previous studies

The comparison of our results with previous linkage mapping and association mapping studies on resistance to multiple races in East Africa and few others from different regions of the world validated many of the significant MTAs identified in our study (Supplemental Tables 4.2 to 4.7). Many of the MTAs in our study were consistent across two to five seasons (Table 4.2; Supplemental Table 4.8) indicating the reliability of the results of our GWAS analyses and effectiveness of resistance to multiple stem rust races though seasonal variability in the frequency of race compositions is inevitable in the respective regions of evaluation as indicated in the differences in the mean responses of the population across the five environments (Table 4.1).

Three significant markers (95 Mb, 144 Mb and 485 Mb) were identified on chromosome 1A (Figure 4.3; Supplemental Figure 4.2). Markers *IWB57448* and *IWA8622* reported by Bajgain et al. (2015b), one of the flanking markers of a QTL identified by Bhavani et al. (2011) (*wPt-734078*), and markers *IWA2057* and *IWA5702* reported by Gao et al. (2017) tagging *Sr31* for resistance to TTTTF and TRTTF were not close to the markers we identified on 1A. These three markers were in linkage equilibrium. The MTAs at 95 Mb and 485 Mb may represent novel QTL while the 144 Mb region was on the threshold line (FDR adjusted *p-value* = 0.04) (Figure 4.3) which makes this association unreliable, and it could be false positive.

On chromosome 1B, four significant MTAs were detected (Figure 4.4; Supplemental Figure 4.2). The marker at 546 Mb is close to *barc61* (2.7 Mb away) reported by Letta et al. (2014) for seedling resistance of durum accessions to TRTTF,

TTTTF and TTKSK while the marker at 620 Mb region is 2.2 Mb away from *barc81* reported by the same author for seedling resistance to races TTTTF and TTKSK and may tag the same QTL. The MTA at 183 Mb is 3 Mb away from *IWB9794* reported by Bajgain et al. (2015b) for seedling resistance of spring wheat to TRTTF, but this marker had an FDR adjusted p-value close to threshold (0.045) while the MTA at 587 Mb is 1.5 Mb away from *IWB40197* reported by Edae et al. (2018) for seedling resistance of spring wheat to race QFCSC likely representing the same locus. Chromosome 1BL is known to harbor the adult plant leaf rust resistance gene *Lr46*, that is tightly linked to the APR gene for stem rust, *Sr58.* However, one of the flanking markers to *Lr46, wmc44* and the same marker reported by Letta et al. (2014) for seedling resistance of durum wheat to TTTTF and JRCQC are further away from the marker we detected. Screening of the lines with the KASP marker designed for *Lr46* (*CIMwMAS0085*, https://www.integratedbreeding.net; Supplemental Table 4.9) indicated that 46% of the lines are expected to carry *Lr46/Sr58* however, this locus was not significant in our study. This may be because of the confounding effect of major gene resistances in our population as the lines were evaluated for field response regardless of their seedling response or the *Lr46* marker may not be predictive.

We identified seven significant MTAs on chromosome 2A (Figures 4.3 and 4.4). The MTA at 20 Mb detected in ETOS18 is close to *wPt-5839* (386 kb away) reported by Letta et al. (2014) for seedling resistance of durum wheat accessions to TRTTF, TTTTF and TTKSK likely representing the same QTL. No known marker close to the QTL at 67 Mb, 78 Mb, 135 Mb, 728 Mb, and 770 Mb regions was reported previously. Therefore, these five markers are representing putatively novel

loci. One MTA at 699 Mb with an FDR adjusted *p-value* close to the threshold (0.049) is likely to be false positive (Supplemental Table 4.3). Chromosome 2A is known to host *Sr21* and *Sr38* transferred to hexaploid wheat from *Triticum monococcum* and *Triticum ventricosum*, respectively (Singh et al., 2011; Chen et al., 2018). About eight lines in the panel possess *Sr38* (Ammar, personal communication, 2020) but it is unlikely to be detected due to the MAF below the threshold. Both *Sr21* and *Sr38* are ineffective against the *Ug99* lineages (predominant in Kenya), TKTTF and JRCQC (predominant in Ethiopia) (Olivera et al., 2015).

On chromosome 2B, four significant markers were identified (Figures 4.3 and 4.4). The MTA at 759 Mb is close (8 Mb away) to marker *wmc361* reported by Letta et al. (2013) and Yadav et al. (2015) likely representing the region of *SrWeb/Sr9h. SrWeb/Sr9h* is effective against Ug99 (Jin et al., 2007; Rouse et al., 2014a) and this MTA (759 Mb) was identified in KNMS19 where Ug99 is predominant. The MTA at 780 Mb is 7.4 Mb away from *wmc356* reported by the same author for APR of durum wheat to Ug99 that co-locates with the region of *Sr28/Sr16*. Several markers were reported by a number of authors on chromosome 2B (Letta et al., 2013, 2014; Yu et al., 2014; Bajgain et al., 2015b; Chao et al., 2017; Gao et al., 2017; Edae et al., 2018), but none are close to the remaining two significant markers. The 456 Mb region may represent a novel locus but identified in one season only while the 569 Mb region had an FDR adjusted *p-value* close to the threshold (0.046) which may indicate unreliable association (Supplemental Tables 4.2 and 4.3). Chromosome 2B is known to carry the alleles of *Sr9* (*Sr9a*, *Sr9b*, *Sr9d*, *Sr9e*, *Sr9f*, *Sr9g*, *SrWeb/Sr9h*)*, Sr28, Sr36* and *Sr16.* Among the seven alleles of *Sr9,* five of them are ineffective against Ug99 while *Sr9e*

is reported to be inconclusive (Jin et al., 2007; Rouse et al., 2014a). *Sr9a*, *Sr9d*, *Sr9e* and *Sr9g* are ineffective against JRCQC and TKTTF (Olivera et al., 2012). *Sr28* is effective against Ug99 but *Sr16* is not (Rouse et al., 2014a). *Sr36* confers resistance to TTKSK and TTKST (Jin et al., 2007; Rouse et al., 2014a) but ineffective to TTTSK (Ug99 lineage), TTRTF and TKTTF (Jin et al., 2009; Olivera et al., 2012, 2015) and this gene was transferred to common wheat from *Triticum timopheevi* (Jin et al., 2009) and it is unlikely to exist in the durum wheat panel.

Three significant markers (9 Mb, 313 Mb, 344 Mb) were identified on chromosome 3A (Figures 4.3 and 4.4). Markers *wPt6854* and *barc12* reported by Letta et al. (2013) are close to the marker at 9 Mb (5 Mb away) indicating that this marker may represent the same region though identified in one season only. Markers *wmc264, wPt-8203, barc1177* and *wmc388* reported by Letta et al. (2013, 2014) are further away from the remaining two markers on 3A. So, the MTAs at 313 Mb and 344 Mb may represent novel loci for field resistance to *Pgt* races in Ethiopia albeit both were identified in one season. Chromosome 3A is known to host *Sr27* and *Sr35,* and both are effective against Ug99 (Jin et al., 2007; Rouse et al., 2014a). *Sr35* was transferred from *Triticum monococcum* to common wheat (Zhang et al., 2010) while *Sr27* was transferred from rye to common wheat (Jin et al., 2009; Letta et al., 2013). None of these wild relative-derived genes are known to have been introgressed into the CIMMYT durum germplasm.

Five significant MTAs were identified on chromosome 3B (Supplemental Tables 4.2 and 4.3). Markers *wPt-0365* and *wPt-6802* reported by Yu et al. (2014) tagging *Sr12* is 14 Mb away from the MTA at 724 Mb. Flanking markers of *Sr12*

(*wPt-0544* and *wPt-6047*) reported by Rouse et al. (2014b) are further away from the 724 Mb locus. However, this marker lies between the regions reported by Yu et al. (2014) and Rouse et al. (2014b) indicating that it could be representing *Sr12*. Rouse et al. (2014b) reported that *Sr12* confers resistance to Ug99 (TTKSK) at adult plant stage when combined with other resistance loci in a QTL study of Thatcher/McNeal RIL population. Although no significant interaction was observed with any of the known *Sr* genes postulated in our GWAS result, significant interactions were observed between the marker at 724 Mb region and QTL on chromosome1B (at 620 Mb) (*p-value* = 0.020903) and 5B (688 Mb) (*p-value* = 0.013911) for resistance to multiple races in Ethiopia and Kenya, respectively. The MTA at 9 Mb region that was consistently identified in four of the five testing environments using MLM was not close to any of the previously reported markers suggesting that it may represent a novel locus unidentified by FarmCPU (Supplemental Table 4.8). The remaining three MTAs were identified in one season only. One of the three markers at 213 Mb region had FDR adjusted *p-value* close to the threshold (0.042) (Supplemental Table 4.3) and this marker is close to *wmc43* (4.5 Mb away) reported by Letta et al. (2014) but less reliable. The MTA at 55 Mb region is 14 Mb away from *wPt-6945* reported by Yu et al. (2011) likely identified the same region. No known marker close to the MTA at 38 Mb region was reported previously and this marker had an FDR adjusted *p-value* close to the threshold (0.036) which makes this association less reliable. The short arm of chromosome 3B is known to harbor the known APR gene, *Sr2* but this gene is not present in the CIMMYT durum germplasm as confirmed by the screening of the panel using KASP marker designed for *Sr2* (*Sr2_ger93p*, Mago et al. 2011; Supplemental

Table 4.9) and the absence of the pseudo black chaff trait (morphological marker for *Sr2*) in any of the lines in greenhouse and field.

Two significant MTAs (619 Mb, 651 Mb) were identified on chromosome 4A (Supplemental Tables 4.3 to 4.7). The region at 651Mb is 1.5 Mb away from one of the flanking marker (*wPt-5857*) of a QTL on chromosome 4AL reported by Yu et al. (2014) on Ug99 resistance consensus map of wheat and likely identified the same locus. None of the markers reported by Letta et al. (2014), Bajgain et al. (2015b), Yu et al. (2011, 2014) are close to the marker at 619 Mb region indicating that this marker is likely representing a novel resistant locus. Chromosome 4A hosts the alleles of *Sr7* (*Sr7a*, *Sr7b*). *Sr7a* confers resistance against race TKTTF (Olivera et al., 2015) whereas *Sr7b* is effective against race JRCQC (Olivera et al., 2012).

Two significant markers were identified on chromosome 5A at 8 Mb and 35 Mb regions (Figures 3.3 and 3.4). Markers *IWA1062*, *IWA5040* and *IWA5368* reported by Chao et al. (2017) for seedling resistance of durum wheat accessions to races TTRTF, JRCQC and bulk races in Debre Zeit, Ethiopia; *IWB47184*, *IWA2224, IWA2836* and *IWB34927* reported by Bajgain et al. (2015b) for APR of spring wheat to Ug99 and seedling resistance to TKTTF; *barc165* reported by Letta et al. (2014) for seedling resistance of durum wheat accessions to race JRCQC are not close to the markers we detected on 5A. These two markers likely represent novel loci for field resistance to multiple races in Ethiopia and Ug99 lineages in Kenya, but they were identified in one season.

On chromosome 5B, seven significant MTAs were identified (Figures 4.3 and 4.4). Bansal et al.(2014) reported markers *sun209* and *sun479* flanking *Sr49* which is

effective against all the races in Australia. The MTA at 691 Mb co-locates with *sun479* (530 kb away) while 692 Mb region co-locates with *sun209* (485 kb away). These two markers (691 Mb and 692 Mb) were consistent across four of the five seasons though limited by the low MAF (0.053 on average) which indicates that this gene is rare in the panel (Supplemental Table 4.2). The 691 Mb locus was detected for resistance to TKTTF at the seedling stage (manuscript accepted) indicating that these two markers are representing an all stage multiple-race specific resistance gene likely *Sr49*. Bhavani et al.(2011) reported flanking markers *wPt0750* and *wPt5896* on chromosome 5BL in biparental mapping (PBW343/Juchi) for APR to Ug99 in hexaploid wheat. The MTA at 581 Mb identified in KNMS19 using both models, is close to these flanking markers (~ 5 to 6 Mb away) and was detected at the adult plant stage in Kenya only. Hence, this marker is likely tagging the same locus as Bhavani and Singh (2011). One of the flanking markers (*wPt8604)* of a QTL reported by Yu et al. (2014) on the Ug99 resistance consensus map of wheat is 7 Mb away from two MTAs identified at 13 Mb and 12 Mb regions likely representing the same QTL (Figures 4.3 and 4.4). A number of markers have been reported by several authors on chromosome 5B (Letta et al., 2013; Bansal et al., 2014; Yu et al., 2014; Bajgain et al., 2015a; Mago et al., 2015; Chao et al., 2017) but none of them are close to the markers at 688 Mb and 671Mb regions identified in ETOS18 and KNMS19, respectively (Supplemental Tables 4.2 and 4.3). The long arm of chromosome 5B hosts the adult plant resistance gene *Sr56* and an all stage resistance gene *Sr49* (Bansal et al., 2014, 2015). Both durum and common wheat can have *Sr56*. However, markers linked to *Sr56* reported by Bansal et al.(2014, 2015) are further away from the MTAs at 671Mb

and 688 Mb. Therefore, these two markers may represent novel loci for field resistance to races in Kenya and Ethiopia although detected in only one season.

On chromosome 6A, 52 significant MTAs representing five QTL mapped the regions of previously reported loci and novel loci (Supplemental Tables 4.2 to 4.7). None of the markers reported by Letta et al. (2013, 2014); Bajgain et al. (2015b) and Chao et al. (2017) are close to the MTA at 592 kb region. Markers *IWA7913*, *IWA7006*, *IWB23519* reported by Bajgain et al. (2015b) and Gao et al. (2017) for seedling resistance of spring wheat to race TRTTF and BCCBC are very close to an MTA at 4 Mb region (~3 to 5 kb away). Guerrero-Chavez et al. (2015) reported that these markers are linked to *Sr8a.* Marker *IWB72958* reported by Nirmala et al. (2017) is linked to *Sr8155B1* in durum wheat that is effective against TTKST and TRTTF and this marker is ~ 4.8 kb away from the marker at 4 Mb region. Moreover, *Sr8155B1* was reported effective against races in Njoro, Kenya but not effective against races in Debre Zeit, Ethiopia (Nirmala et al., 2017). Similarly, the MTA at 4 Mb region was identified for adult plant resistance of durum lines in Kenya only where race TTKST is predominant. This indicates that the MTA at 4 Mb likely maps the region of *Sr8155B1*. The marker at 592 kb was in strong LD ($r^2 = 0.89$) with the 4 Mb region. However, the 592 kb region was associated with resistances to races in Ethiopia where the virulent races to *Sr8155B1* (JRCQC and TTKSK) are predominant indicating that this MTA may represent a new allele at the *Sr8* locus, or a novel gene linked to the *Sr8* locus. The high LD between these two loci may indicate limited recombination rate in the regions or the resistance alleles might be selected together. Markers *wPt1742* and *wPt1377* reported by Letta et al. (2013) for field resistance of durum wheat accessions

to Ug99 are close to (~765 kb and 845 kb away) an MTA at 1.4 Mb identified for field resistance in ETOS18 (Supplemental Table 4.2). Markers *IWA272*, *IWB64917*, *IWB64918*, *IWB5029*, *IWB35595*, *IWB43808*, *IWB72956* reported by Bajgain et al. (2015b) for seedling resistance of spring wheat to TRTTF are 1Mb away from the MTA at 1.4 Mb indicating that this MTA likely maps the region of *Sr8a* though identified in one season only. It is known that the short arm of chromosome 6A hosts the alleles of *Sr8 (Sr8a and Sr8b*) and *Sr8a* confers resistance to the predominant races in Ethiopia, TRTTF (Jin et al., 2007; Nirmala et al., 2017) and JRCQC (Olivera et al., 2012) but both alleles are ineffective against TTKSK and TTKST at seedling and adult plant stage (Jin et al., 2007). No known marker close to the markers at 28 Mb, 189 Mb and 334 Mb regions of chromosome 6A (Supplemental Tables 4.3 to 4.7) was previously reported. The MTAs at 28 Mb and 334 Mb regions likely represent new loci whereas the one at 189 Mb was identified in one season only and is on the FDR threshold line (Supplemental Figure 4.2) which makes this association less reliable. All significant markers identified on chromosome 6A from 606 Mb to 615 Mb regions collocate with markers tagging *Sr13* region including *CD926040* and *barc104* reported by several authors (Simons et al., 2011; Letta et al., 2013, 2014), *IWA4918* reported by Chao et al. (2017), *IWA7495* reported by Gao et al. (2017) for seedling and adult plant resistance to multiple *Pgt* races, and the flanking markers of *Sr13, CJ671993* and *CJ641478* reported by Zhang et al. (2017). Therefore, the MTAs extended from 606 Mb to 615 Mb regions of chromosome 6A likely represent *Sr13*/alleles. It is known that *Sr13* is an all-stage resistance gene to the Ug99 lineages. The higher percentage of lines (69%) carrying *Sr13* on marker screening may indicate

the wide usage of this gene in CIMMYT durum wheat breeding program. This result is proven by the higher frequency (27% to 85%) of the favorable alleles at the *Sr13* locus. However, more than one allele is expected as indicated in the differences in allele frequencies and the LD between nearby markers (Supplemental Tables 4.3 to 4.7, Figure 4.5). The alleles, *Sr13a* and *Sr13c* confer resistance to the most virulent races of durum wheat including JRCQC and TTRTF and to the Ug99 lineages (Olivera et al., 2019, Olivera, personal communication, 2020) while *Sr13b* confers resistance against TTKSK, TKTTF, TRTTF (Randhawa et al., 2018; Zhang et al., 2017).) but is ineffective against JRCQC and TTRTF (Zhang et al., 2017). Three MTAs, at 611 Mb and 612 Mb (two at 612 Mb) identified as the most significantly associated markers for field resistance to multiple races (Supplemental Tables 4.2 and 4.3) in the different testing environments were also identified at the seedling stage (manuscript accepted). These markers could potentially be used to identify the different alleles of *Sr13* although further study and validation on different populations will be needed. In some cases, the LD between the significant markers identified on chromosome 6A at the *Sr13* region was slightly below the threshold or weak (Figure 4.5), suggesting that the region could be a recombination hotspot which can lead to low intra-chromosomal LD.

On chromosome 6B, six significant MTAs representing four putative QTL were identified (Supplemental Tables 4.2 and 4.5). Several markers (*IWB24880, IWB46893, IWB48548, IWB71190, IWB47075*) reported by Bajgain et al. (2015b) for seedling resistance of spring wheat to TKTTF, and *IWB35697* for adult plant resistance to Ug99 in Ethiopia and Kenya, are close to the MTA at 692 Mb (229 kb to

2 Mb away). Marker *KASP_6BL_IWB72471* reported by Nirmala et al. (2016) as a predictive marker for *Sr11* is 2 Mb away from this marker indicating that it is likely mapping the *Sr11* locus. However, *Sr11* is ineffective against TTKSK, JRCQC and TRTTF at the seedling stage and is effective against TKTTF (Jin et al., 2007; Olivera et al., 2012) which is among the predominant races in Ethiopia where the association was identified (ETOS19). It is known that residual effects of ineffective major gene resistances are among the possible mechanisms of field quantitative resistance. Two MTAs at 686 Mb and 687 Mb regions were in strong LD ($r^2 = 0.64$) and represent the same QTL (Supplemental Table 4.5). Several markers reported by Bajgain et al. (2015b) are close to these two markers. The closest markers, *IWA4245* and *IWA4246* are 502 kb away from the 686 Mb locus while *IWB59175.2* is 196 kb away from 687 Mb region indicating that the two markers may represent the same region as the one reported by Bajgain et al. (2015b). None of the markers reported by Bajgain et al. (2015b), and markers *wPt1541, barc79, wPt4930, wPt5333* and *wPt5037* reported by Yu et al. (2014) are close to the MTAs at 31 Mb, 30 Mb and 666 Mb regions. The two markers at 31 Mb and 30 Mb regions were in LD ($r^2 = 0.33$) indicating that they represent the same QTL in the short arm of 6B which is likely novel and the MTA at 666 Mb region could also be representing a novel locus (Supplemental Table 4.2).

We identified 60 significant MTAs on chromosome 7A (Supplemental Tables 4.2 to 4.7). The markers that extended from 668 to 727 Mb were in LD and may represent a single QTL (Figure 4.6). The 700 Mb and 717 Mb regions were identified in multiple seasons (Supplemental Table 4.8) suggesting that these markers are tagging a multiple-race resistance locus. Markers *IWB5070, IWB1874, IWB4830 and*

IWB62560 reported by Bajgain et al. (2015b) for adult plant resistance of spring wheat to Ug99 are 2 Mb away from the MTA at 700 Mb region. Marker *IWB48466* reported by the same author is 5 Mb away from the MTA at 717 Mb region. Marker *IWA2270* reported by Chao et al. (2017) for resistance of durum wheat accessions to race TTTTF tagging the *Sr22* locus co-locates with the MTA at 673 Mb (~ 5 kb away). These three markers (673 Mb, 700 Mb, 717 Mb) were in moderate to strong LD (r^2 = 0.37 to 0.83) indicating that these MTAs are representing the region of *Sr22.* This gene confer resistance to TTKSK (Jin et al., 2007), JRCQC and TRTTF (Olivera et al., 2012) and transferred from *T. monococcum* (Olson et al., 2010). The resistance allele at the *Sr22* locus is probably rare in the study population as observed in the frequency of the favorable alleles (Supplemental Tables 4.2 to 4.7). Some of the lines in the panel (~ 10 lines) possess *Sr25* (Ammar, personal communication, 2020). However, it is unlikely to identify the *Sr25* locus due to MAF below the threshold (0.05). *Sr25* and *Sr22* come with severe yield penalties in durum wheat (Ammar, personal communication, 2020). So, breeders should be prepared to conduct several cycles of selection to use these gene with minimal to no performance penalties. None of the markers listed earlier including markers *IWA7200* reported by Chao et al. (2017), *barc70* and *wmc479* reported by Letta et al. (2013), *Xbarc121* reported by Yu et al. (2014) are close to the MTAs at 43 Mb, 117 Mb, 139 Mb and 285 Mb regions of chromosome 7A and these MTAs were identified in one season only. Moreover, two of the regions had FDR adjusted *p-value* close to the threshold (Supplemental Table 4.2 and 4.5) indicating that these loci could be false positives.

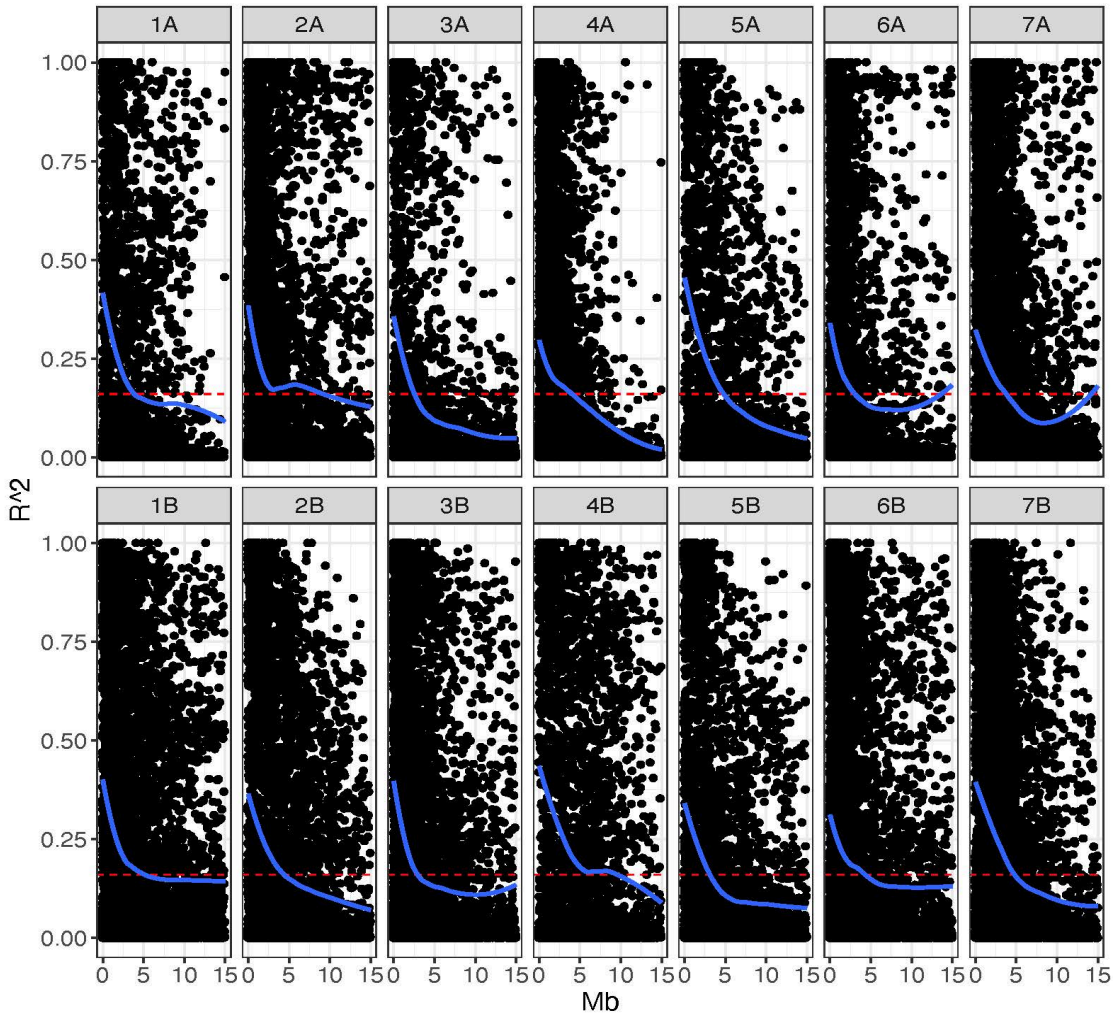
On chromosome 7B, we identified five significant MTAs (Supplemental Tables 4.2 to 4.7). The MTA at 717 Mb is 8 Mb away from *IWB47548* and *IWA4175* reported by Bajgain et al. (2015b) for adult plant resistance of spring wheat to Ug99 indicating that this MTA is likely representing the same locus. The MTA at 644 Mb is 7 Mb away from an SSR marker linked to *Sr17* (*wmc517*) reported by Letta et al. (2014) for seedling resistance of durum wheat accessions to races TTTTF and TTKSK. So, this MTA (644 Mb) and an MTA at 622 Mb (LD, $r^2 = 0.64$) likely represent *Sr17*. The consistency of these two MTAs across three seasons may indicate the reliability of association although the resistance allele at this locus is rare in the population (only 7% of the lines/19 lines carry the resistance allele on average). Markers *wmc182, wmc517, wPt1715, wPt4298, wPt7191, wPt4045* reported by Letta et al. (2013), and marker *wPt1149* reported by Yu et al. (2014) are further away from the MTA at 46 Mb region and this region is likely novel. The MTA at the 707 Mb is 2 Mb away from *IWB47548* and *IWB4175* reported by Bajgain et al. (2015b), but the FDR adjusted *p-value* was close to the threshold (0.047) which makes this association less reliable. We identified nine significant MTAs on an unknown chromosomal location (Supplemental Tables 4.2 to 4.7). Four of the nine MTAs were identified in one season only while the remaining five were identified in three to five seasons and we were unable to find a location for these markers.

CONCLUSION

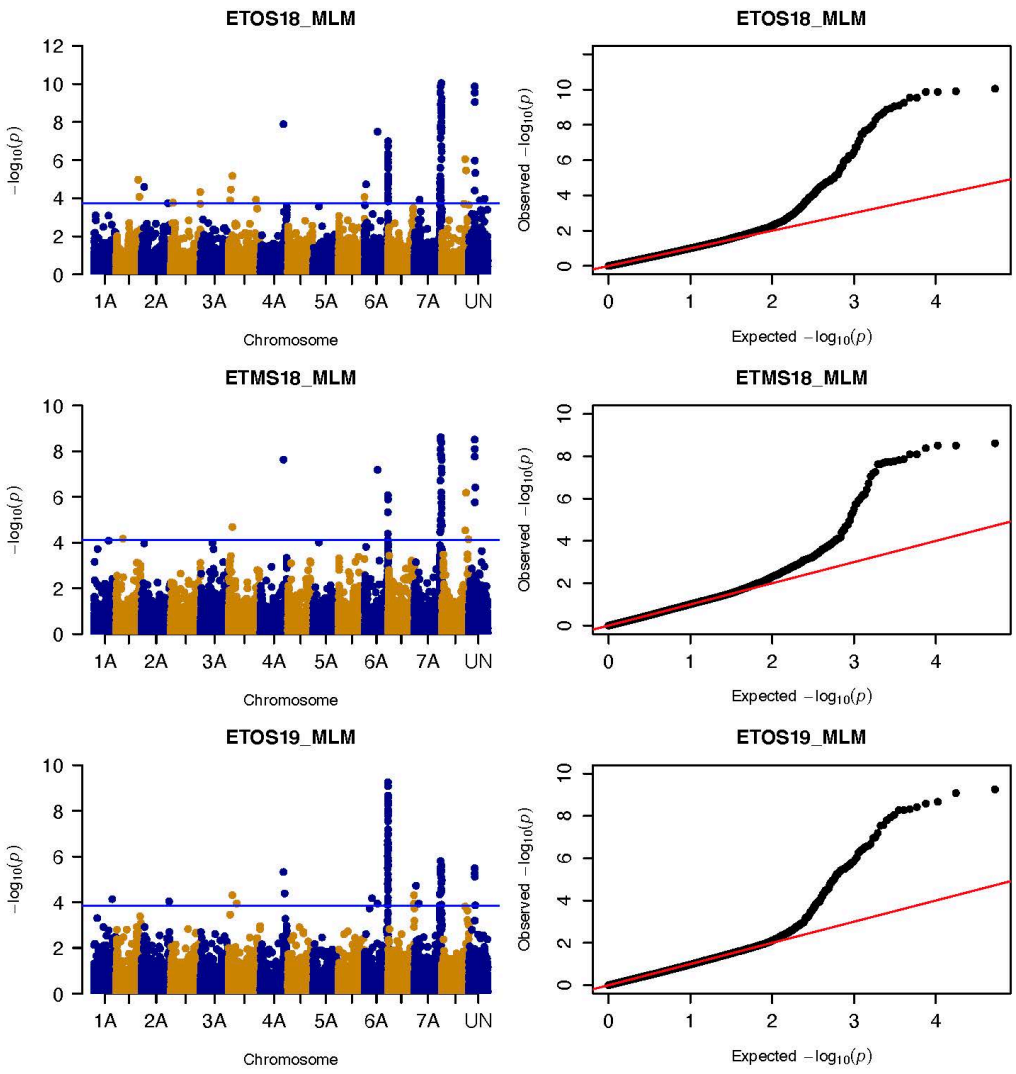
Overall, several lines were consistently resistant across the five seasons in the two hotspot regions (Ethiopia and Kenya) and can be used as sources of resistance to multiple stem rust races in East Africa. Once these lines are evaluated for agronomic

performance, combining more resistance alleles and/or genes to the best performing lines may increase durability of resistance to potentially emerging races. Among a total of 160 significant MTAs identified using MLM and FarmCPU with known chromosomal locations and grouped to 42 QTL, 21 QTL are putatively novel and the remaining 21 are mapped to previously reported regions. The regions representing *Sr12, Sr13/*alleles, *Sr17, Sr22* and *Sr49* are among the known resistant genes consistent in two to five seasons for resistance to multiple races in East Africa. *Sr13* was more frequent in the population while *Sr12*, *Sr17*, *Sr22* and *Sr49* were less frequent. Novel loci consistent across multiple seasons were also identified on chromosomes 3B, 4A, 6A and 6B and the resistance alleles at the loci on chromosomes 3B, 4A and 6A were less frequent. Therefore, breeders should try to retain these rare genes/alleles during the selection process in future breeding plans. The markers identified in the current study once validated and optimized for high-throughput platforms, can be used in marker- assisted selection to combine sources of resistance to stem rust in durum wheat. The information on the available sources of resistance in this panel is also useful for future deployment of the resistance sources in durum wheat breeding programs. The region of *Sr13* on chromosome 6AL is wider and the extent of LD is complex. Therefore, allelism tests and further studies on the validation of potential allele specific markers for *Sr13* are needed.

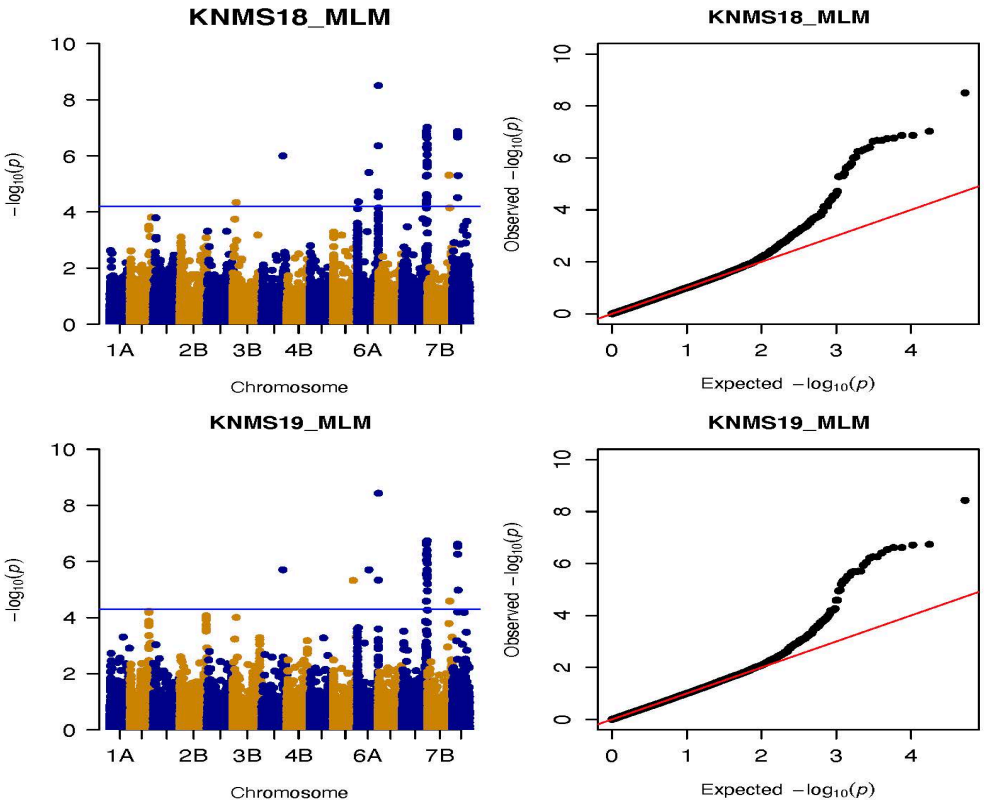
Lists of supplemental figures



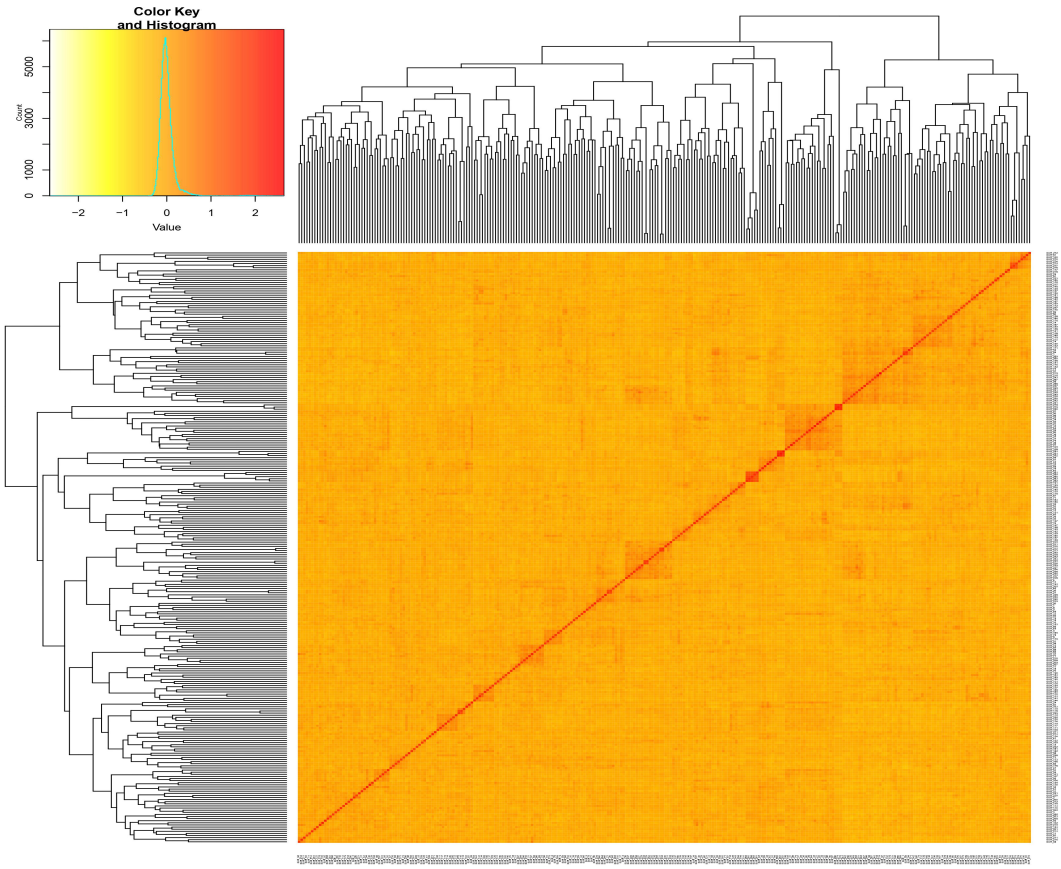
Supplemental Figure 4. 1. Scatter plot of squared allele-frequency correlations (r2) versus physical distance (Mb) between pairs of markers indicating the decay of linkage disequilibrium (LD) across the 14 chromosomes of the durum wheat panel.



Supplemental Figure 4. 2.Manhattan and QQ-plots of GWAS results of field resistance of durum wheat lines to multiple races in Ethiopia across three seasons identified using MLM.



Supplemental Figure 4.3. Manhattan and QQ-plots of GWAS results of field resistance of durum wheat lines to multiple races in Kenya across two seasons identified using MLM.



Supplemental Figure 4.4. Heatmap of marker-based kinship matrix of a panel of durum wheat lines.

Lists of supplemental tables

Supplemental Table 4.1. Mean coefficient of infection of lines positive to *Sr13* and *Lr46/Sr58* marker screening with multiple-race resistance at the adult plant stage.

Origin GID	ETOS18	ETMS18	ETOS19	KNMS18	KNMS19
7145228	25.5	12.0	22.5	10.0	15.0
7145451	43.0	47.5	27.8	5.0	21.0
7145526	40.0	38.3	23.5	15.0	13.5
7145583	31.3	13.5	9.8	7.5	3.8
7145599	39.0	23.0	12.5	13.5	18.0
7145651	21.8	5.8	3.3	4.8	6.0
7145664	27.0	15.0	27.0	7.5	16.5
7145707	8.8	7.0	6.0	18.0	5.5
7145713	22.5	27.0	11.3	40.5	22.5
7145733	48.5	7.5	14.0	13.5	12.0
7145764	19.5	12.0	21.0	5.5	12.0
7145770	36.0	30.0	13.0	18.0	10.5
7145771	11.5	11.0	7.5	6.5	5.5
7145779	31.5	13.5	15.0	7.0	10.0
7145795	32.0	18.0	16.5	2.5	21.0
7145800	38.0	18.0	13.8	13.0	21.0
7383281	22.0	10.0	18.0	12.0	12.0
7383291	45.0	40.5	34.0	17.0	12.0
7383456	38.0	25.5	36.0	3.8	7.5
7383862	40.5	24.0	21.5	12.8	24.0
7384046	14.0	11.5	12.3	5.3	12.0
7384063	27.0	16.0	9.8	13.0	21.0
7384071	20.3	9.0	17.5	6.8	13.5
7384072	36.0	10.5	7.5	10.5	39.0
7384079	31.5	11.8	10.8	26.0	18.0
7384096	38.0	15.0	20.0	12.0	15.0
7406259	36.0	0.0	5.0	5.0	9.0
7406303	21.8	10.3	10.0	8.0	15.0
7406313	31.5	33.5	27.0	5.5	12.0
7406340	43.0	11.0	20.3	5.5	7.5
7406449	38.0	16.0	20.0	5.5	2.3
7406486	30.0	14.0	13.5	3.3	2.8
7406533	45.0	11.0	20.5	8.5	24.0
7406594	27.0	12.5	10.0	2.3	10.3
7406684	40.5	21.0	18.3	13.0	34.5
7406808	34.0	9.8	24.0	8.5	11.0
7406899	24.3	18.5	12.0	7.5	10.5
7407025	31.5	8.5	6.3	9.0	7.5
7407092	36.0	23.5	7.5	18.0	15.0
7407117	24.8	19.0	13.3	1.0	3.0
7407174	27.0	19.0	27.0	1.0	9.0
7407242	31.5	26.0	5.8	5.5	16.5
7407561	31.5	9.8	10.0	3.0	7.5
7407611	36.0	34.0	18.5	7.0	21.5
7407689	65.0	55.0	50.0	12.0	53.0
7407740	47.5	22.0	13.8	9.0	21.5
7408065	38.0	25.5	16.5	13.5	25.5
7408683	43.0	24.0	18.0	31.5	39.0

7408843	36.0	24.0	8.3	15.0	18.0
7408925	36.0	32.0	17.8	34.5	45.0
7409002	43.0	34.5	16.3	17.0	27.0
7409071	43.5	30.0	19.5	8.0	3.8
7409080	43.0	26.0	10.5	7.5	16.0
7409188	43.0	19.0	15.0	9.0	19.5
7409275	43.0	32.5	19.5	9.0	24.0
7409314	55.0	43.0	16.5	37.5	36.0
7409395	48.0	18.0	25.0	24.0	22.0
7409461	60.0	48.0	31.5	33.0	15.0
7410092	45.0	30.0	29.3	12.0	11.0
7410242	38.0	18.0	19.5	9.0	14.0
7410277	29.5	27.0	22.5	28.5	16.0
7410549	55.0	20.0	26.0	12.0	12.0
7410632	36.0	44.0	12.5	9.1	21.0
7410795	45.0	24.0	13.8	5.0	9.0
7606811	55.0	25.5	20.0	7.0	13.0
7606825	48.0	30.0	16.5	10.5	19.5
7147237	36.0	17.5	10.5	7.0	33.0
7384203	43.0	36.0	18.0	25.5	15.0
7405994	33.5	13.0	27.0	6.0	9.0
7406012	40.0	16.5	15.8	12.0	24.0
7406016	43.0	9.5	25.8	10.5	18.0
7406050	27.0	25.0	16.5	4.8	6.0
7406069	31.3	25.5	16.5	15.0	34.5
6420695	6.3	4.0	8.8	4.0	1.0
6420696	5.8	2.0	6.3	0.8	1.0
6420697	2.0	3.0	7.0	4.0	1.3
6420699	19.0	3.0	11.3	2.3	1.3
6420704	3.0	3.3	5.0	0.2	1.3
6951168	7.0	2.0	3.3	2.3	2.8
5928162	5.8	10.0	6.3	6.8	6.5
6951195	30.0	18.0	5.8	12.0	14.0
7147179	0.6	0.0	0.0	0.0	1.0
7147180	0.6	0.0	1.2	0.0	1.0
7147182	1.2	0.0	0.0	0.0	1.3

Supplemental Table 4.2. Lists of SNPs significantly associated with field resistance to East African *Pgt* races across five seasons identified using FarmCPU.

Env.	Position (bp)	Chr.	P value	Allele	AF	Effect	Proposed gene
ETOS18	20977834	2A	1.17E-07	**C**/T	0.721	2.16	Letta et al. (2014)
	135744411	2A	2.12E-08	G/**A**	0.355	-2.72	Likely novel
	724805496	3B	5.57E-09	G/**A**	0.104	-4.35	*Sr12*
	13909625	5B	5.43E-06	**T**/C	0.751	2.36	Yu et al. (2014)
	691693264	5B	1.92E-08	T/**A**	0.051	-6.24	*Sr49*
	592006	6A	4.28E-07	G/**A**	0.228	-2.43	Novel/*Sr8155B1*
	1424376	6A	3.47E-06	**C**/G	0.906	3.79	*Sr8a*
	610171399	6A	5.10E-13	**G**/A	0.820	4.53	*Sr13*
	613576841	6A	4.27E-16	**G**/C	0.813	4.98	*Sr13*
	31294519	6B	7.81E-06	**C**/T	0.790	2.01	Likely novel
	700805183	7A	1.34E-31	A/**T**	0.076	-12.19	*Sr22*
ETMS18	95587608	1A	6.82E-06	**A**/G	0.937	0.38	Likely novel
	144772265	1A	1.63E-05	**A**/G	0.931	0.37	-
	313477146	3A	1.90E-06	**C**/T	0.841	0.21	Likely novel
	344594454	3A	1.02E-06	T/**G**	0.108	-0.33	Likely novel
	691693264	5B	1.90E-05	T/**A**	0.051	-0.37	*Sr49*
	598562950	6A	6.46E-07	**A**/G	0.544	0.16	Likely novel
	609346836	6A	4.04E-06	**C**/G	0.894	0.31	*Sr13 allele*
	612043936	6A	8.05E-20	T/**C**	0.302	-0.48	*Sr13*
	615604035	6A	1.02E-06	A/**C**	0.274	-0.20	*Sr13*
	30564627	6B	1.15E-09	**A**/G	0.562	0.23	Likely novel
	717518884	7A	1.08E-15	T/**C**	0.058	-0.83	*Sr22*
		7B		T/**G**	0.081		Bajgain et al.
	717849029		1.89E-06			-0.32	(2015b)
ETOS19	78492640	2A	9.73E-08	**A**/C	0.940	0.47	Likely novel
	456530846	2B	9.29E-06	**A**/G	0.913	0.35	-
	35001659	5A	1.65E-05	**T**/G	0.820	0.24	Likely novel
	692277095	5B	3.36E-07	T/**C**	0.058	-0.40	*Sr49*
	606107662	6A	2.21E-10	**G**/A	0.636	0.33	*Sr13*
	611495915	6A	1.29E-17	**G**/A	0.846	0.70	*Sr13*
	612003938	6A	4.42E-10	G/**A**	0.095	-0.50	*Sr13 allele*
	612802438	6A	3.25E-33	**A**/C	0.708	0.80	Novel/*Sr13b*
	692192009	6B	1.10E-09	A/**G**	0.053	-0.56	*Sr11*
	673523659	7A	1.51E-08	T/**A**	0.092	-0.44	Likely *Sr22*
	700805183	7A	5.95E-17	A/**T**	0.076	-0.87	*Sr22*
	46338417	7B	5.51E-07	C/**T**	0.417	-0.21	Likely novel
KNMS18	9819941	3A	6.15E-07	**A**/G	0.846	0.39	Letta et al. (2013)
	724805496	3B	5.46E-06	G/**A**	0.104	-0.43	*Sr12*
	8470400	5A	4.72E-08	T/**C**	0.416	-0.29	Likely novel
	12999566	5B	2.55E-06	**C**/T	0.878	0.44	Yu et al. (2014)
	692277095	5B	3.47E-07	T/**C**	0.058	-0.61	*Sr49*
	4914394	6A	4.22E-11	C/**G**	0.226	-0.45	*Sr8155B1*
	609622362	6A	9.13E-06	**T**/C	0.829	0.33	*Sr13 allele*
	612043936	6A	1.25E-10	T/**C**	0.302	-0.44	*Sr13*
	615619215	6A	1.61E-05	**G**/A	0.820	0.31	*Sr13*
	666439193	6B	2.17E-06	G/**A**	0.378	-0.26	Likely novel
	717518884	7A	2.26E-26	T/**C**	0.058	-1.48	*Sr22*
KNMS19	546977269	1B	2.92E-08	**C**/T	0.869	0.47	Letta et al. (2014)
	770363872	2A	1.60E-07	C/**G**	0.071	-0.67	Likely novel
	759454292	2B	5.78E-11	**A**/G	0.756	0.43	*SrWeb/Sr9h*

581703945	5B	1.48E-05	**G**/A	0.913	0.49	Reported APR
671134916	5B	7.69E-06	**C**/G	0.611	0.23	Likely novel
592006	6A	8.96E-09	G/**A**	0.228	-0.36	Novel/*Sr8155B1*
612043936	6A	1.06E-13	T/**C**	0.302	-0.49	*Sr13*
285980279	7A	4.64E-06	**A**/T	0.882	0.40	Likely novel
700805183	7A	2.22E-17	A/**T**	0.076	-1.08	*Sr22*
122277080	UN	5.55E-06	**G**/T	0.936	0.53	Unknown

AF= allele frequency, bold face written alleles are the favorable allele at each locus.

Supplemental Table 4.3: Lists of SNPs significantly associated with field resistance to Pgt races in Ethiopia during the off-season 2018 (ETOS18) identified using MLM.

SNP	Chr.	Position	P.value	Alleles	FAF	FDR.Adj.P	Effect	R^2	Proposed_Gene/Allele
S1B_587942809	1B	587942809	1.06E-05	T/**C**	5E-02	0.006	-9.20	4.59	Edae et al. (2018)
S1B_620602482	1B	620602482	8.40E-05	A/**G**	6E-02	0.027	-7.26	3.64	Letta et al. (2014)
S2A_67311951	2A	67311951	2.51E-05	C/**T**	5E-02	0.010	-7.93	4.19	Likely novel
S2A_699774613	2A	699774613	0.00018278	A/**C**	5E-02	0.049	-7.13	3.28	-
S2B_56938728	2B	56938728	0.00017043	T/**C**	5E-02	0.046	-7.23	3.32	-
S2B_780938491	2B	780938491	4.72E-05	C/**G**	6E-02	0.017	-8.50	3.90	*Sr28/Sr16*
S3B_38937548	3B	38937548	0.00012774	T/**G**	6E-02	0.036	-7.19	3.45	-
S3B_55889860	3B	55889860	3.49E-05	G/**C**	6E-02	0.013	-8.14	4.04	Yu et al. (2011)
S3B_97870708	3B	97870708	6.64E-06	A/**G**	5E-02	0.004	-10.07	4.81	Likely novel
S3B_724805496	3B	724805496	0.00012009	G/**A**	1E-01	0.035	-4.32	3.47	*Sr12*
S4A_619746683	4A	619746683	1.29E-08	A/**G**	5E-02	0.000	-14.00	7.84	Likely novel
S5B_688838898	5B	688838898	8.60E-05	G/**A**	6E-02	0.027	-7.42	3.63	Likely novel
S6A_28859024	6A	28859024	1.87E-05	G/**A**	5E-02	0.008	-10.02	4.33	Likely novel
S6A_334834338	6A	334834338	3.21E-08	G/**A**	5E-02	0.000	-11.81	7.39	Likely novel
S6A_609622362	6A	609622362	0.00013338	**T**/C	8E-01	0.037	4.22	3.43	*Sr13*
S6A_609635619	6A	609635619	9.78E-07	A/**G**	5E-02	0.001	-9.76	5.73	-
S6A_609635640	6A	609635640	0.00014932	**G**/A	8E-01	0.041	4.47	3.38	*Sr13*
S6A_610171399	6A	610171399	3.08E-05	**A**/G	8E-01	0.012	4.55	4.10	*Sr13*
S6A_610495870	6A	610495870	5.71E-05	**A**/T	8E-01	0.019	4.38	3.81	*Sr13*
S6A_612043936	6A	612043936	0.00013064	T/**C**	7E-01	0.037	-3.36	3.44	*Sr13*
S6A_612802438	6A	612802438	1.01E-07	A/**C**	3E-01	0.000	4.55	6.83	*Sr13*
S6A_612832613	6A	612832613	5.82E-07	C/**T**	3E-01	0.000	4.49	5.97	*Sr13*
S6A_612957317	6A	612957317	2.63E-06	**G**/A	7E-01	0.002	4.17	5.25	*Sr13*
S6A_613055519	6A	613055519	5.30E-07	**T**/C	7E-01	0.000	4.48	6.02	*Sr13*
S6A_613131839	6A	613131839	1.30E-06	**G**/A	7E-01	0.001	4.34	5.59	*Sr13*
S6A_613194512	6A	613194512	4.51E-07	**C**/T	7E-01	0.000	4.52	6.10	*Sr13*
S6A_613256520	6A	613256520	1.89E-07	**T**/C	7E-01	0.000	4.61	6.52	*Sr13*
S6A_613288180	6A	613288180	1.70E-05	**A**/G	8E-01	0.008	4.39	4.37	*Sr13*
S6A_613294106	6A	613294106	1.09E-05	**C**/T	8E-01	0.006	4.56	4.58	*Sr13*
S6A_613294155	6A	613294155	5.45E-07	**G**/T	7E-01	0.000	4.46	6.01	*Sr13*
S6A_613547583	6A	613547583	6.51E-06	**G**/C	8E-01	0.004	4.61	4.82	*Sr13*
S6A_613576841	6A	613576841	1.18E-05	**G**/C	8E-01	0.006	4.46	4.55	*Sr13*

S6A_614329660	6A	614329660	9.11E-05	**A**/T	8E-01	0.028	3.65	3.60	*Sr13*
S6A_615604386	6A	615604386	2.22E-06	**A**/T	7E-01	0.002	4.19	5.33	*Sr13*
S6A_615617605	6A	615617605	1.56E-05	**A**/G	8E-01	0.008	4.27	4.41	*Sr13*
S6A_615619215	6A	615619215	7.52E-06	**G**/A	8E-01	0.004	4.51	4.76	*Sr13*
S7A_139258774	7A	139258774	0.00011928	**A**/**T**	2E-01	0.035	-4.01	3.48	-
S7A_683235350	7A	683235350	1.51E-05	C/**A**	8E-02	0.008	-6.35	4.43	*Sr22*
S7A_683644765	7A	683644765	1.69E-05	A/**C**	8E-02	0.008	-6.17	4.38	*Sr22*
S7A_684386422	7A	684386422	0.00010082	C/**T**	1E-01	0.031	-4.45	3.55	*Sr22*
S7A_684422202	7A	684422202	8.33E-05	A/**G**	1E-01	0.027	-4.66	3.64	*Sr22*
S7A_684577265	7A	684577265	3.56E-05	A/**G**	8E-02	0.013	-5.96	4.03	*Sr22*
S7A_684578569	7A	684578569	1.63E-05	C/**G**	8E-02	0.008	-6.23	4.39	*Sr22*
S7A_684752182	7A	684752182	6.48E-05	G/**A**	8E-02	0.022	-5.78	3.76	*Sr22*
S7A_685683430	7A	685683430	3.21E-05	C/**T**	8E-02	0.012	-5.86	4.08	*Sr22*
S7A_685684672	7A	685684672	8.55E-05	C/**T**	9E-02	0.027	-5.42	3.63	*Sr22*
S7A_685815784	7A	685815784	8.62E-06	T/**G**	8E-02	0.005	-6.55	4.69	*Sr22*
S7A_686094342	7A	686094342	5.42E-05	A/**G**	1E-01	0.019	-6.13	3.84	*Sr22*
S7A_686811682	7A	686811682	4.67E-05	T/**C**	7E-02	0.017	-6.07	3.91	*Sr22*
S7A_686849268	7A	686849268	1.97E-05	G/**C**	8E-02	0.009	-6.41	4.31	*Sr22*
S7A_686964735	7A	686964735	1.16E-05	C/**G**	8E-02	0.006	-6.49	4.55	*Sr22*
S7A_687410326	7A	687410326	5.28E-05	A/**G**	6E-02	0.018	-8.36	3.85	*Sr22*
S7A_687774090	7A	687774090	1.74E-05	C/**T**	9E-02	0.008	-6.25	4.36	*Sr22*
S7A_687798481	7A	687798481	7.06E-06	A/**T**	8E-02	0.004	-7.44	4.79	*Sr22*
S7A_688882132	7A	688882132	2.53E-06	T/**G**	1E-01	0.002	-6.74	5.27	*Sr22*
S7A_688885145	7A	688885145	0.00010676	G/**A**	1E-01	0.032	-5.51	3.53	*Sr22*
S7A_689090791	7A	689090791	8.01E-05	T/**C**	1E-01	0.026	-5.66	3.66	*Sr22*
S7A_690016567	7A	690016567	1.72E-08	C/**T**	5E-02	0.000	-12.08	7.70	*Sr22*
S7A_690811708	7A	690811708	6.87E-08	A/**G**	6E-02	0.000	-11.87	7.02	*Sr22*
S7A_690940195	7A	690940195	2.87E-10	C/**A**	6E-02	0.000	-14.59	9.77	*Sr22*
S7A_691030882	7A	691030882	4.21E-05	G/**C**	9E-02	0.015	-6.24	3.95	*Sr22*
S7A_691181565	7A	691181565	2.06E-05	G/**T**	9E-02	0.009	-6.65	4.28	*Sr22*
S7A_691818237	7A	691818237	1.50E-05	G/**C**	8E-02	0.008	-6.77	4.43	*Sr22*
S7A_693246434	7A	693246434	2.75E-05	C/**A**	9E-02	0.011	-6.48	4.15	*Sr22*
S7A_693249957	7A	693249957	2.40E-05	G/**C**	9E-02	0.010	-6.39	4.21	*Sr22*
S7A_693891779	7A	693891779	2.86E-05	C/**A**	9E-02	0.011	-6.48	4.13	*Sr22*
S7A_693915965	7A	693915965	2.11E-08	A/**T**	7E-02	0.000	-10.51	7.60	*Sr22*
S7A_694006046	7A	694006046	3.21E-05	A/**G**	9E-02	0.012	-6.43	4.08	*Sr22*

S7A_697030510	7A	697030510	1.13E-05	A/**G**	9E-02	0.006	-7.15	4.57	*Sr22*
S7A_697030516	7A	697030516	1.35E-10	G/**A**	5E-02	0.000	-14.55	10.17	*Sr22*
S7A_698390754	7A	698390754	2.32E-05	T/**G**	1E-01	0.010	-6.91	4.23	*Sr22*
S7A_700727874	7A	700727874	1.24E-10	G/**C**	6E-02	0.000	-14.53	10.21	*Sr22*
S7A_700805183	7A	700805183	1.19E-09	A/**T**	8E-02	0.000	-12.62	9.05	*Sr22*
S7A_706027775	7A	706027775	0.00012352	A/**G**	5E-01	0.036	-3.93	3.46	*Sr22*
S7A_710171609	7A	710171609	8.20E-10	A/**G**	5E-02	0.000	-13.83	9.24	*Sr22*
S7A_714327927	7A	714327927	1.33E-09	G/**A**	7E-02	0.000	-13.23	8.99	*Sr22*
S7A_714370100	7A	714370100	5.14E-09	A/**G**	5E-02	0.000	-13.21	8.31	*Sr22*
S7A_714975616	7A	714975616	3.32E-08	C/**T**	9E-02	0.000	-9.11	7.37	*Sr22*
S7A_717517491	7A	717517491	2.00E-09	G/**A**	5E-02	0.000	-13.48	8.78	*Sr22*
S7A_717518884	7A	717518884	8.89E-11	T/**C**	6E-02	0.000	-14.65	10.38	*Sr22*
S7A_718484217	7A	718484217	1.83E-07	T/**C**	1E-01	0.000	-9.32	6.53	*Sr22*
S7A_719231181	7A	719231181	8.83E-07	G/**A**	7E-02	0.001	-9.81	5.77	*Sr22*
S7A_719698163	7A	719698163	3.34E-09	A/**C**	6E-02	0.000	-13.52	8.52	*Sr22*
S7A_719787589	7A	719787589	9.88E-09	T/**G**	5E-02	0.000	-13.82	7.98	*Sr22*
S7A_721720978	7A	721720978	2.71E-09	A/**T**	6E-02	0.000	-12.85	8.63	*Sr22*
S7A_724486791	7A	724486791	3.44E-07	G/**C**	1E-01	0.000	-8.20	6.23	*Sr22*
S7A_724668618	7A	724668618	1.95E-08	A/**G**	8E-02	0.000	-10.33	7.64	*Sr22*
S7A_724668652	7A	724668652	5.57E-10	A/**G**	5E-02	0.000	-13.91	9.43	*Sr22*
S7B_622041448	7B	622041448	8.89E-07	C/**T**	7E-02	0.001	-9.83	5.77	likely *Sr17*
S7B_644041948	7B	644041948	3.44E-06	C/**A**	6E-02	0.002	-9.41	5.13	likely *Sr17*
SUN_151516737	UN	151516737	3.96E-05	T/**C**	1E-01	0.015	-5.13	3.98	-
SUN_151742792	UN	151742792	1.35E-10	T/**C**	5E-02	0.000	-14.55	10.17	-
SUN_151847140	UN	151847140	2.87E-10	C/**A**	6E-02	0.000	-14.59	9.77	-
SUN_153093563	UN	153093563	1.05E-06	A/**G**	1E-01	0.001	-6.56	5.69	-
SUN_153928527	UN	153928527	8.88E-10	T/**A**	5E-02	0.000	-14.48	9.20	-
SUN_166522707	UN	166522707	4.69E-06	T/**C**	5E-02	0.003	-10.66	4.98	-
SUN_288369273	UN	288369273	0.0001265	G/**C**	8E-02	0.036	-5.97	3.45	-
SUN_412024226	UN	412024226	0.00010628	T/**C**	1E-01	0.032	-5.11	3.53	-

Bold face letters indicate favorable allele, FAF = Favorable allele frequency

Supplemental Table 4.4: Lists of SNPs significantly associated with field resistance to Pgt races in Ethiopia during the main-season 2018 (ETMS18) identified using MLM.

SNP	Chr.	Position	P.value	Alleles	FAF	FDR.Adj.P	Effect	R^2	Proposed Gene/Allele
S1B_183096071	1B	183096071	6.71E-05	**C**/T	9E-01	0.046	0.58	3.43	-
S3B_97870708	3B	97870708	2.07E-05	A/**G**	5E-02	0.016	-0.84	3.93	Likely novel
S4A_619746683	4A	619746683	2.35E-08	A/**G**	5E-02	0.000	-1.21	6.92	Likely novel
S6A_334834338	6A	334834338	6.50E-08	G/**A**	5E-02	0.000	-1.03	6.46	Likely novel
S6A_609635619	6A	609635619	4.61E-06	A/**G**	5E-02	0.004	-0.81	4.57	-
S6A_609635640	6A	609635640	7.44E-05	**G**/A	8E-01	0.048	0.41	3.38	*Sr13*
S6A_610133407	6A	610133407	4.08E-05	**A**/G	8E-01	0.028	0.42	3.64	*Sr13*
S6A_611495915	6A	611495915	8.47E-07	**G**/A	8E-01	0.001	0.50	5.31	*Sr13*
S6A_612043936	6A	612043936	1.28E-06	T/**C**	3E-01	0.001	-0.38	5.13	*Sr13*
S7A_682951819	7A	682951819	3.52E-05	C/**T**	6E-02	0.025	-0.83	3.70	*Sr22*
S7A_690811708	7A	690811708	1.93E-07	A/**G**	6E-02	0.000	-1.00	5.97	*Sr22*
S7A_690940195	7A	690940195	3.10E-09	C/**A**	6E-02	0.000	-1.20	7.85	*Sr22*
S7A_693915965	7A	693915965	1.03E-05	A/**G**	7E-02	0.009	-0.72	4.22	*Sr22*
S7A_697030510	7A	697030510	2.95E-05	A/**G**	9E-02	0.022	-0.60	3.77	*Sr22*
S7A_697030516	7A	697030516	8.10E-09	G/**A**	5E-02	0.000	-1.14	7.40	*Sr22*
S7A_698390754	7A	698390754	1.87E-05	T/**G**	1E-01	0.015	-0.61	3.97	*Sr22*
S7A_700727874	7A	700727874	2.43E-09	G/**C**	6E-02	0.000	-1.17	7.96	*Sr22*
S7A_700805183	7A	700805183	1.37E-08	A/**T**	8E-02	0.000	-1.03	7.16	*Sr22*
S7A_710171609	7A	710171609	1.82E-08	A/**G**	5E-02	0.000	-1.11	7.03	*Sr22*
S7A_714327927	7A	714327927	1.52E-08	G/**A**	7E-02	0.000	-1.08	7.11	*Sr22*
S7A_714370100	7A	714370100	6.38E-07	A/**G**	5E-02	0.001	-0.98	5.44	*Sr22*
S7A_714975616	7A	714975616	1.24E-05	C/**T**	9E-02	0.011	-0.63	4.14	*Sr22*
S7A_717517491	7A	717517491	2.06E-08	G/**A**	5E-02	0.000	-1.11	6.98	*Sr22*
S7A_717518884	7A	717518884	4.12E-09	T/**C**	6E-02	0.000	-1.16	7.71	*Sr22*
S7A_718484217	7A	718484217	5.76E-06	T/**C**	1E-01	0.005	-0.71	4.47	*Sr22*
S7A_719231181	7A	719231181	1.05E-06	G/**A**	7E-02	0.001	-0.85	5.21	*Sr22*
S7A_719698163	7A	719698163	1.82E-08	A/**C**	6E-02	0.000	-1.12	7.03	*Sr22*
S7A_719787589	7A	719787589	1.85E-06	T/**G**	5E-02	0.002	-1.00	4.97	*Sr22*
S7A_721720978	7A	721720978	5.45E-08	A/**T**	6E-02	0.000	-1.03	6.53	*Sr22*
S7A_724486791	7A	724486791	2.41E-08	G/**C**	1E-01	0.000	-0.80	6.90	*Sr22*
S7A_724668618	7A	724668618	3.14E-06	A/**G**	8E-02	0.003	-0.75	4.74	*Sr22*

S7A_724668652	7A	724668652	8.80E-08	A/**G**	5E-02	0.000	-1.05	6.32	*Sr22*
S7A_727729196	7A	727729196	1.75E-05	T/**A**	2E-01	0.014	-0.36	4.00	*Sr22*
S7B_622041448	7B	622041448	2.91E-05	C/**T**	7E-02	0.022	-0.73	3.78	likely *Sr17*
S7B_644041948	7B	644041948	6.53E-07	C/**A**	6E-02	0.001	-0.90	5.43	likely *Sr17*
S7B_707179085	7B	707179085	7.20E-05	T/**C**	1E-01	0.048	-0.48	3.40	-
SUN_151742792	UN	151742792	8.10E-09	T/**C**	5E-02	0.000	-1.14	7.40	-
SUN_151847140	UN	151847140	3.10E-09	C/**A**	6E-02	0.000	-1.20	7.85	-
SUN_153093563	UN	153093563	1.73E-06	A/**G**	1E-01	0.002	-0.57	5.00	-
SUN_153928527	UN	153928527	1.72E-08	T/**A**	5E-02	0.000	-1.16	7.06	-
SUN_166522707	UN	166522707	3.84E-07	T/**C**	5E-02	0.001	-1.05	5.66	-
S1B_183096071	1B	183096071	6.71E-05	**C**/T	9E-01	0.046	0.58	3.43	-

Supplemental Table 4.5: Lists of SNPs significantly associated with field resistance to Pgt races in Ethiopia during the off-season 2019 (ETOS19) identified using MLM.

SNP	Chr.	Position	P.value	Alleles	FAF	FDR Adj. P	Effect	R^2	Proposed Gene/Allele
S1A_485305123	1A	485305123	7.25E-05	**T**/C	0.908	0.029	0.62	3.73	Likely novel
S2A_728226059	2A	728226059	9.06E-05	C/**A**	0.095	0.035	-0.59	3.63	Likely novel
S3B_97870708	3B	97870708	4.90E-05	A/**G**	0.055	0.020	-0.96	3.91	Likely novel
S3B_213690656	3B	213690656	0.00011392	**T**/A	0.936	0.042	0.60	3.52	-
S4A_619746683	4A	619746683	4.77E-06	A/**G**	0.053	0.003	-1.18	5.00	Likely novel
S4A_651298931	4A	651298931	4.16E-05	**A**/G	0.926	0.018	0.87	3.99	Yu et al. (2014)
S6A_189134995	6A	189134995	6.69E-05	C/**T**	0.074	0.027	-0.57	3.77	-
S6A_334834338	6A	334834338	0.00011552	G/**A**	0.051	0.042	-0.87	3.52	Likely novel
S6A_606082021	6A	606082021	3.33E-06	**T**/A	0.532	0.002	0.45	5.18	*Sr13*
S6A_606107662	6A	606107662	2.09E-07	**G**/A	0.636	0.000	0.56	6.51	*Sr13*
S6A_606107665	6A	606107665	9.47E-07	**A**/G	0.530	0.001	0.48	5.78	*Sr13*
S6A_606304231	6A	606304231	3.04E-07	**T**/C	0.631	0.000	0.56	6.33	*Sr13*
S6A_606339177	6A	606339177	1.56E-05	**A**/C	0.546	0.008	0.42	4.44	*Sr13*
S6A_607001638	6A	607001638	0.00013047	**T**/C	0.816	0.046	0.47	3.46	*Sr13*
S6A_609179112	6A	609179112	6.33E-05	C/**T**	0.302	0.026	-0.37	3.79	*Sr13*
S6A_609247742	6A	609247742	8.66E-05	C/**T**	0.304	0.034	-0.36	3.65	*Sr13*
S6A_609622362	6A	609622362	2.11E-06	**T**/C	0.829	0.002	0.56	5.39	*Sr13*
S6A_609635640	6A	609635640	3.73E-07	**G**/A	0.846	0.000	0.64	6.23	*Sr13*
S6A_610129981	6A	610129981	1.11E-07	**T**/C	0.841	0.000	0.66	6.83	*Sr13*
S6A_610133407	6A	610133407	4.89E-07	**A**/G	0.834	0.001	0.62	6.10	*Sr13*
S6A_610133490	6A	610133490	5.73E-07	**A**/T	0.837	0.001	0.62	6.02	*Sr13*
S6A_610146036	6A	610146036	1.41E-06	**C**/T	0.845	0.001	0.61	5.59	*Sr13*
S6A_610150266	6A	610150266	3.00E-05	**C**/G	0.850	0.014	0.53	4.14	*Sr13*
S6A_610150270	6A	610150270	3.04E-05	**T**/G	0.846	0.014	0.53	4.13	*Sr13*
S6A_610150819	6A	610150819	2.13E-06	**T**/A	0.841	0.002	0.59	5.39	*Sr13*
S6A_610171399	6A	610171399	1.12E-06	**A**/G	0.820	0.001	0.57	5.70	*Sr13*
S6A_610430767	6A	610430767	6.00E-06	**A**/G	0.855	0.004	0.60	4.90	*Sr13*
S6A_610475213	6A	610475213	2.55E-05	**G**/A	0.845	0.013	0.53	4.21	*Sr13*
S6A_610495870	6A	610495870	1.65E-06	**A**/T	0.823	0.002	0.56	5.51	*Sr13*
S6A_611495915	6A	611495915	5.61E-10	**G**/A	0.846	0.000	0.77	9.49	*Sr13*
S6A_612802438	6A	612802438	8.34E-10	A/**C**	0.708	0.000	0.56	9.29	*Sr13*

S6A_612832613	6A	612832613	2.71E-08	C/**T**	0.739	0.000	0.53	7.53	*Sr13*
S6A_612957317	6A	612957317	5.37E-09	**G**/A	0.735	0.000	0.56	8.34	*Sr13*
S6A_613054847	6A	613054847	1.06E-05	**G**/A	0.943	0.006	0.69	4.63	*Sr13*
S6A_613055519	6A	613055519	1.19E-08	**T**/C	0.737	0.000	0.54	7.94	*Sr13*
S6A_613131839	6A	613131839	3.83E-09	**G**/A	0.739	0.000	0.57	8.51	*Sr13*
S6A_613194512	6A	613194512	5.34E-09	**C**/T	0.739	0.000	0.56	8.34	*Sr13*
S6A_613256520	6A	613256520	2.15E-09	**T**/C	0.726	0.000	0.57	8.80	*Sr13*
S6A_613288180	6A	613288180	2.87E-08	**A**/G	0.827	0.000	0.61	7.50	*Sr13*
S6A_613294106	6A	613294106	1.61E-08	**C**/T	0.830	0.000	0.63	7.79	*Sr13*
S6A_613294155	6A	613294155	8.89E-09	**G**/T	0.735	0.000	0.55	8.08	*Sr13*
S6A_613547583	6A	613547583	6.48E-08	**G**/C	0.825	0.000	0.59	7.09	*Sr13*
S6A_613576841	6A	613576841	4.12E-07	**G**/C	0.813	0.000	0.55	6.18	*Sr13*
S6A_614080083	6A	614080083	4.74E-09	G/**A**	0.242	0.000	-0.56	8.40	*Sr13*
S6A_614329660	6A	614329660	2.47E-06	**A**/T	0.797	0.002	0.47	5.32	*Sr13*
S6A_615604035	6A	615604035	2.63E-09	A/**C**	0.274	0.000	-0.56	8.70	*Sr13*
S6A_615604386	6A	615604386	2.57E-07	**A**/T	0.689	0.000	0.49	6.41	*Sr13*
S6A_615617605	6A	615617605	2.73E-07	**A**/G	0.816	0.000	0.54	6.38	*Sr13*
S6A_615619215	6A	615619215	1.04E-07	**G**/A	0.820	0.000	0.57	6.86	*Sr13*
S6B_686489689	6B	686489689	0.00011282	**C**/T	0.813	0.042	0.48	3.53	Bajgain et al. (2015b)
S6B_687598497	6B	687598497	4.94E-05	**C**/T	0.855	0.020	0.54	3.91	Bajgain et al. (2015b)
S7A_43311031	7A	43311031	1.89E-05	**T**/C	0.910	0.009	0.66	4.35	-
S7A_117458210	7A	117458210	0.00011444	**T**/A	0.882	0.042	0.45	3.52	-
S7A_668699732	7A	668699732	0.00014079	A/**T**	0.071	0.048	-0.84	3.42	*Sr22*
S7A_673882326	7A	673882326	4.50E-05	T/**C**	0.087	0.019	-0.75	3.95	*Sr22*
S7A_690940195	7A	690940195	5.53E-06	C/**A**	0.057	0.003	-1.09	4.93	*Sr22*
S7A_697030510	7A	697030510	1.57E-06	A/**G**	0.092	0.001	-0.83	5.54	*Sr22*
S7A_697030516	7A	697030516	3.24E-06	G/**A**	0.055	0.002	-1.10	5.19	*Sr22*
S7A_698390754	7A	698390754	3.71E-06	T/**G**	0.095	0.003	-0.80	5.12	*Sr22*
S7A_700727874	7A	700727874	3.64E-06	G/**C**	0.058	0.003	-1.09	5.13	*Sr22*
S7A_700805183	7A	700805183	2.84E-06	A/**T**	0.076	0.002	-1.01	5.25	*Sr25*
S7A_710171609	7A	710171609	2.68E-05	A/**G**	0.055	0.013	-0.99	4.19	*Sr22*
S7A_714327927	7A	714327927	1.44E-05	G/**A**	0.065	0.007	-0.98	4.48	*Sr22*
S7A_714370100	7A	714370100	1.36E-05	A/**G**	0.051	0.007	-1.03	4.51	*Sr22*
S7A_714975616	7A	714975616	3.41E-05	C/**T**	0.090	0.015	-0.72	4.08	*Sr22*
S7A_717517491	7A	717517491	8.57E-06	G/**A**	0.055	0.005	-1.05	4.73	*Sr22*
S7A_717518884	7A	717518884	4.26E-06	T/**C**	0.058	0.003	-1.08	5.06	*Sr22*

S7A_718484217	7A	718484217	0.00012734	T/**C**	0.097	0.045	-0.72	3.47	*Sr22*
S7A_719231181	7A	719231181	3.30E-05	G/**A**	0.067	0.015	-0.87	4.09	*Sr22*
S7A_719698163	7A	719698163	3.28E-05	A/**C**	0.058	0.015	-0.99	4.10	*Sr22*
S7A_721720978	7A	721720978	2.52E-06	A/**T**	0.064	0.002	-1.06	5.31	*Sr25*
S7A_724668618	7A	724668618	1.05E-05	A/**G**	0.076	0.006	-0.85	4.63	*Sr22*
S7A_724668652	7A	724668652	1.14E-05	A/**G**	0.051	0.006	-1.03	4.59	*Sr22*
SUN_151742792	UN	151742792	3.24E-06	T/**C**	0.055	0.002	-1.10	5.19	-
SUN_151847140	UN	151847140	5.53E-06	C/**A**	0.057	0.003	-1.09	4.93	-
SUN_153928527	UN	153928527	7.63E-06	T/**A**	0.055	0.004	-1.10	4.78	-
SUN_166522707	UN	166522707	0.00013665	T/**C**	0.053	0.048	-0.94	3.44	-

Supplemental Table 4.6: Lists of SNPs significantly associated with field resistance to *Pgt* races in Kenya during the main-season 2018 (KNMS18) identified using MLM.

SNP	Chr.	Position	P.value	Alleles	FAF	FDR.adj.P	R^2	Proposed Gene/Allele
S3B_97870708	3B	97870708	4.60E-05	A/**G**	0.055	0.037	4.48	Likely novel
S4A_619746683	4A	619746683	1.00E-06	A/**G**	0.053	0.002	6.54	Likely novel
S6A_28859024	6A	28859024	4.31E-05	G/**A**	0.051	0.036	4.51	Likely novel
S6A_334834338	6A	334834338	3.93E-06	G/**A**	0.051	0.005	5.79	Likely novel
S6A_609635619	6A	609635619	4.38E-07	A/**G**	0.053	0.001	6.99	-
S6A_612043936	6A	612043936	3.13E-09	T/**C**	0.302	0.000	9.79	*Sr13*
S6A_612802438	6A	612802438	1.93E-05	**A**/**C**	0.708	0.020	4.94	*Sr13*
S6A_613256520	6A	613256520	2.83E-05	**T**/C	0.726	0.026	4.73	*Sr13*
S7A_690016567	7A	690016567	5.26E-06	C/**T**	0.051	0.006	5.63	*Sr22*
S7A_690811708	7A	690811708	5.13E-05	A/**G**	0.060	0.040	4.42	*Sr22*
S7A_690940195	7A	690940195	1.35E-07	C/**A**	0.057	0.001	7.65	*Sr22*
S7A_697030510	7A	697030510	2.43E-05	A/**G**	0.092	0.024	4.82	*Sr22*
S7A_697030516	7A	697030516	2.13E-07	G/**A**	0.055	0.001	7.39	*Sr22*
S7A_698390754	7A	698390754	3.88E-05	T/**G**	0.095	0.033	4.57	*Sr22*
S7A_700727874	7A	700727874	1.79E-07	G/**C**	0.058	0.001	7.49	*Sr22*
S7A_700805183	7A	700805183	4.86E-07	A/**T**	0.076	0.001	6.94	*Sr22*
S7A_710171609	7A	710171609	2.28E-07	A/**G**	0.055	0.001	7.35	*Sr22*
S7A_714327927	7A	714327927	1.63E-06	G/**A**	0.065	0.003	6.27	*Sr22*
S7A_714370100	7A	714370100	9.19E-07	A/**G**	0.051	0.002	6.58	*Sr22*
S7A_714975616	7A	714975616	2.82E-05	C/**T**	0.090	0.026	4.74	*Sr22*
S7A_717517491	7A	717517491	3.90E-07	G/**A**	0.055	0.001	7.06	*Sr22*
S7A_717518884	7A	717518884	9.43E-08	T/**C**	0.058	0.001	7.85	*Sr22*
S7A_719231181	7A	719231181	4.90E-06	G/**A**	0.067	0.006	5.67	*Sr22*
S7A_719698163	7A	719698163	2.47E-06	A/**C**	0.058	0.003	6.04	*Sr22*
S7A_719787589	7A	719787589	5.62E-07	T/**G**	0.051	0.001	6.86	*Sr22*
S7A_721720978	7A	721720978	2.17E-06	A/**T**	0.064	0.003	6.11	*Sr22*
S7A_724668618	7A	724668618	2.05E-06	A/**G**	0.076	0.003	6.14	*Sr22*
S7A_724668652	7A	724668652	5.50E-07	A/**G**	0.051	0.001	6.87	*Sr22*
S7B_622041448	7B	622041448	4.90E-06	C/**T**	0.074	0.006	5.67	likely *Sr17*
SUN_151742792	UN	151742792	2.13E-07	T/**C**	0.055	0.001	7.39	-
SUN_151847140	UN	151847140	1.35E-07	C/**A**	0.057	0.001	7.65	-

SUN_153093563	UN	153093563	3.07E-05	A/**G**	0.099	0.027	4.69	-
SUN_153928527	UN	153928527	1.74E-07	T/**A**	0.055	0.001	7.51	-
SUN_166522707	UN	166522707	5.03E-06	T/**C**	0.053	0.006	5.66	-

Supplemental Table 4.7. Lists of SNPs significantly associated with field resistance to *Pgt* races in Kenya during the main-season 2019 (KNMS19) identified using MLM.

SNP	Chr.	Position	P.value	Alleles	FAF	FDR.adj.P	Effect	R^2	Proposed Gene/Allele
S4A_619746683	4A	619746683	1.98E-06	A/**G**	0.053	0.003	-1.37	6.02	Likely novel
S5B_581703945	5B	581703945	4.74E-06	**G**/A	0.913	0.006	0.80	5.56	Bhavani and Singh (2011)
S6A_334834338	6A	334834338	1.99E-06	G/**A**	0.051	0.003	-1.24	6.02	Likely novel
S6A_609635619	6A	609635619	4.63E-06	A/**G**	0.053	0.006	-1.14	5.57	-
S6A_612043936	6A	612043936	3.71E-09	T/**C**	0.302	0.000	-0.64	9.46	*Sr13*
S7A_690016567	7A	690016567	2.86E-06	C/**T**	0.051	0.004	-1.20	5.82	*Sr22*
S7A_690811708	7A	690811708	2.59E-05	A/**G**	0.060	0.025	-1.08	4.67	*Sr22*
S7A_690940195	7A	690940195	2.42E-07	C/**A**	0.057	0.001	-1.39	7.15	*Sr22*
S7A_697030516	7A	697030516	5.49E-07	G/**A**	0.055	0.002	-1.33	6.71	*Sr22*
S7A_700727874	7A	700727874	9.08E-07	G/**C**	0.058	0.002	-1.28	6.44	*Sr22*
S7A_700805183	7A	700805183	1.94E-07	A/**T**	0.076	0.001	-1.26	7.27	*Sr22*
S7A_710171609	7A	710171609	3.93E-07	A/**G**	0.055	0.001	-1.35	6.89	*Sr22*
S7A_714327927	7A	714327927	3.83E-06	G/**A**	0.065	0.005	-1.17	5.67	*Sr22*
S7A_714370100	7A	714370100	2.01E-06	A/**G**	0.051	0.003	-1.27	6.01	*Sr22*
S7A_717517491	7A	717517491	6.24E-07	G/**A**	0.055	0.002	-1.32	6.64	*Sr22*
S7A_717518884	7A	717518884	1.83E-07	T/**C**	0.058	0.001	-1.37	7.30	*Sr22*
S7A_718484217	7A	718484217	3.04E-06	T/**C**	0.097	0.004	-0.99	5.79	*Sr22*
S7A_719698163	7A	719698163	2.17E-06	A/**C**	0.058	0.003	-1.26	5.97	*Sr22*
S7A_719787589	7A	719787589	2.04E-06	T/**G**	0.051	0.003	-1.35	6.01	*Sr22*
S7A_721720978	7A	721720978	6.20E-06	A/**T**	0.064	0.007	-1.14	5.42	*Sr22*
S7A_724668618	7A	724668618	1.12E-05	A/**G**	0.076	0.012	-0.96	5.10	*Sr22*
S7A_724668652	7A	724668652	1.17E-06	A/**G**	0.051	0.003	-1.29	6.30	*Sr22*
S7B_644041948	7B	644041948	2.59E-05	C/**A**	0.058	0.025	-1.03	4.67	likely *Sr17*
SUN_151742792	UN	151742792	5.49E-07	T/**C**	0.055	0.002	-1.33	6.71	-
SUN_151847140	UN	151847140	2.42E-07	C/**A**	0.057	0.001	-1.39	7.15	-
SUN_153928527	UN	153928527	2.91E-07	T/**A**	0.055	0.001	-1.41	7.05	-
SUN_166522707	UN	166522707	1.05E-05	T/**C**	0.053	0.012	-1.22	5.14	-

Supplemental Table 4.8. Lists of consistent significant markers between testing environments identified using MLM.

Position	Chr.	MAF	Environment	Proposed gene
97870708	3B	0.055	ETOS18, ETMS18, ETOS19, KNMS18	Novel
619746683	4A	0.053	ETOS18, ETMS18, ETOS19, KNMS18, KNMS19	Novel
28859024	6A	0.051	ETOS18, KNMS18	Novel
334834338	6A	0.051	ETOS18, ETMS18, ETOS19, KNMS18, KNMS19	Novel
609622362	6A	0.171	ETOS18, ETOS19	*Sr13*
609635640	6A	0.154	ETOS18, ETMS18, ETOS19	
610133407	6A	0.166	ETMS18, ETOS19	
610171399	6A	0.180	ETOS18, ETOS19	
610495870	6A	0.177	ETOS18, ETOS19	
611495915	6A	0.154	ETMS18, ETOS19	
612043936	6A	0.302	ETOS18, ETMS18, KNMS18, KNMS19	
612802438	6A	0.292	ETOS18, ETOS19, KNMS18	
612832613	6A	0.261	ETOS18, ETOS19	
612957317	6A	0.265	ETOS18, ETOS19	
613055519	6A	0.263	ETOS18, ETOS19	
613131839	6A	0.261	ETOS18, ETOS19	
613194512	6A	0.261	ETOS18, ETOS19	
613256520	6A	0.274	ETOS18, ETOS19, KNMS18	
613288180	6A	0.173	ETOS18, ETOS19	
613294106	6A	0.169	ETOS18, ETOS19	
613294155	6A	0.265	ETOS18, ETOS19	
613547583	6A	0.175	ETOS18, ETOS19	
613576841	6A	0.187	ETOS18, ETOS19	
614329660	6A	0.203	ETOS18, ETOS19	
615604386	6A	0.311	ETOS18, ETOS19	
615617605	6A	0.184	ETOS18, ETOS19	
615619215	6A	0.180	ETOS18, ETOS19	
690016567	7A	0.051	ETOS18, KNMS18, KNMS19	*Sr22*
690811708	7A	0.060	ETOS18, ETMS18, KNMS18, KNMS19	
690940195	7A	0.057	ETOS18, ETMS18, ETOS19, KNMS18, KNMS19	
693915965	7A	0.071	ETOS18, ETMS18	
697030510	7A	0.092	ETOS18, ETMS18, ETOS19, KNMS18	
697030516	7A	0.054	ETOS18, ETMS18, ETOS19, KNMS18, KNMS19	
698390754	7A	0.095	ETOS18, ETMS18, ETOS19, KNMS18	
700727874	7A	0.058	ETOS18, ETMS18, ETOS19, KNMS18, KNMS19	
700805183	7A	0.076	ETOS18, ETMS18, ETOS19, KNMS18, KNMS19	
710171609	7A	0.055	ETOS18, ETMS18, ETOS19, KNMS18, KNMS19	
714327927	7A	0.065	ETOS18, ETMS18, ETOS19, KNMS18, KNMS19	
714370100	7A	0.051	ETOS18, ETMS18, ETOS19, KNMS18, KNMS19	
714975616	7A	0.901	ETOS18, ETMS18, ETOS19, KNMS18	
717517491	7A	0.054	ETOS18, ETMS18, ETOS19, KNMS18, KNMS19	
717518884	7A	0.058	ETOS18, ETMS18, ETOS19, KNMS18, KNMS19	
718484217	7A	0.097	ETOS18, ETMS18, ETOS19, KNMS19	
719231181	7A	0.067	ETOS18, ETMS18, ETOS19, KNMS18	
719698163	7A	0.058	ETOS18, ETMS18, ETOS19, KNMS18, KNMS19	
719787589	7A	0.051	ETOS18, ETMS18, KNMS18, KNMS19	
721720978	7A	0.064	ETOS18, ETMS18, ETOS19, KNMS18, KNMS19	
724486791	7A	0.104	ETOS18, ETMS18	
724668618	7A	0.059	ETOS18, ETMS18, ETOS19, KNMS18, KNMS19	
724668652	7A	0.051	ETOS18, ETMS18, ETOS19, KNMS18, KNMS19	

622041448	7B	0.074	ETOS18, ETMS18, KNMS18	*Sr17*
644041948	7B	0.058	ETOS18, ETMS18, KNMS19, KNMS19	
151742792	UN	0.054	ETOS18, ETMS18, ETOS19, KNMS18, KNMS19	Unknown
151847140	UN	0.056	ETOS18, ETMS18, ETOS19, KNMS18, KNMS19	
153093563	UN	0.099	ETOS18, ETMS18, KNMS19	
153928527	UN	0.054	ETOS18, ETMS18, ETOS19, KNMS18, KNMS19	
166522707	UN	0.053	ETOS18, ETMS18, ETOS19, KNMS18, KNMS19	

Supplemental Table 4.9. Information on KASP assays designed for screening lines for the presence of *Sr2*, *Sr13* and *Lr46*/*Sr58*.

Primer name	Primer sequence	Allele	Allele type	notes:
Sr13-Rev_ALT	GAAGGTGACCAAGTTCATGCTAGAAGTCATCATCATCATTCCCCCA	T	non-Sr13	
Sr13-Rev_ALC	GAAGGTCGGAGTCAACGGATTAAGTCATCATCATCATTCCCCCG	C	Sr13	
Sr13-Rev_C1	CGGTAAACTATGCACACAAAACCTTTGTT			
Lr46_JF2-2_AL1	GAAGGTGACCAAGTTCATGCTATTGTGTGAAGATAGAAGTTCTAATTGAAC	C	non-Lr46/Yr29	
Lr46_JF2-2_AL2	GAAGGTCGGAGTCAACGGATTGTGTGAAGATAGAAGTTCTAATTGAAG	G	Lr46	
Lr46_JF2-2_C1	CTTGTTCTCTCTTGGAGCGTTGGTA			
Sr2_ger9_3p_AL1	GAAGGTGACCAAGTTCATGCTGTGCGAGACATCCAACACTCAC	G	non-Sr2	known null allele, scored as non-Sr2
Sr2_ger9_3p_AL2	GAAGGTCGGAGTCAACGGATTGTGCGAGACATCCAACACTCAT	A	Sr2	
Sr2_ger9_3p_C1	CTCAAATGGTCGAGCACAAGCTCTA			
tail_AL1 (FAM)	GAAGGTGACCAAGTTCATGCT			
tail_AL2 (HEX)	GAAGGTCGGAGTCAACGGATT			

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CHAPTER 5

QTL MAPPING OF FIELD RESISTANCE TO MULTIPLE STEM RUST RACES IN EAST AFRICA IN DAKIYE /REICHENBACHII DURUM WHEAT POPULATION

ABSTRACT

Stem rust caused by the fungus *Puccinia graminis* f.sp. *tritici* Eriks. & E. Henn. (*Pgt*) threatens the global production of both durum wheat (*Triticum turgidum* L. ssp. *durum* (Desf.) Husnot**)** and common wheat (*Triticum aestivum* L.). The objective of this study was to evaluate adult plants of a durum wheat recombinant inbred line (RIL) population developed by the International Center for the Improvement of Maize and Wheat (CIMMYT) from a cross between a resistant parent 'Reichenbachii' and a susceptible parent 'DAKIYE' against multiple *Pgt* races prevalent in East Africa and map field resistance. A total of 224 lines along with the parents were evaluated in the fields of Ethiopia and Kenya for two seasons from 2019 to 2020 and genotyped using the genotyping-by-sequencing (GBS) approach. A total of 843 single nucleotide polymorphism (SNP) markers for 175 lines were used for quantitative trait loci/locus (QTL) analyses. Composite interval mapping (CIM) identified three QTL on chromosomes 3B, 4B and 7B that explained 4.7% to 15.3% of the phenotypic variation and were contributed by the resistant parent. The power to identify additional QTL in this population was limited by the number of high-quality markers. Future evaluations of large numbers of durum lines and identification of durable adult plant resistance sources is crucial in breeding for stem rust resistance of durum wheat in the future.

INTRODUCTION

Durum wheat (*Triticum turgidum* L. ssp. *durum* (Desf.) Husnot) is a tetraploid wheat species (2n = 4x =28, AABB) used for the industrial processing of pasta and other food recipes that mainly constitute the diets in the Mediterranean regions (Shewry and Hey, 2015; Kabbaj et al., 2017). The processing of these end-use products from durum wheat demands both grain yield and quality. However, several factors constrain these and other agronomically important traits. Among the factors, stem rust of wheat is one of the most devastating diseases of both common wheat and durum wheat (Roelfs, et al., 1992). The stem rust fungus draws assimilates from the vascular system and results in reduced grain yield and shriveled seeds that reduce end-use product quality (Schumann and Leonard 2000; Leonard and Szabo, 2005). This pathogen can also cause a complete yield loss when susceptible varieties are grown under environmental conditions conducive for disease development (Dean et al., 2012). The commonly used stem rust control methods are the use of genetic resistance and application of fungicide spray. In the presence of genetic variability for resistance, genetic resistance is the preferred method due to its advantage in environmental safety and cost efficiency.

Many of the commercially deployed stem rust resistance genes are qualitative or race specific. The extensive deployment of qualitative resistance is often challenged by continuously evolving virulent races causing resistance to be ineffective (Newcomb et al., 2013; Yu et al., 2014). Races in the Ug99 group and other virulent races unrelated to Ug99 with broad virulence to several *Sr* genes in wheat cultivars threaten global wheat production and food security (Wanyera et al., 2006; Singh et al., 2015).

Ug99 (TTKSK) overcame the resistance conferred by *Sr31*, a resistance gene that has been effective over three decades. Ug99 was first reported in Uganda in 1999 and spread to the rest of East Africa, Yemen, Iran and South Africa (Newcomb et al., 2013; Nirmala et al., 2017). Race TKTTF that broke the resistance confered by *SrTmp* caused the 2013/14 epidemic in Ethiopia. This race devastated a poplar variety called 'Digalu' planted on more than 100,000 ha of land. Durum wheat lines carrying *Sr13* are reported to be resistant to races TTKSK (the first identified race of Ug99) and TKTTF (Jin et al., 2007; Olivera et al., 2015). However, races JRCQC idenitifed in Ethiopia and TTRTF identified in Sicily, Italy have combined virulence to *Sr13b* and *Sr9e*. These alleles (*Sr13b* and *Sr9e*) are widely deployed in CIMMYT germplasm, North American durum wheat germplasm, and durum wheat cultivars produced in different parts of the world (Olivera et al., 2012; Olivera Firpo et al., 2019). Race TTRTF was identified in Georgia in 2014 and caused the 2016 epidemic in Italy. Races JRCQC and TTRTF have broad virulence to several commercially deployed resistance genes (Olivera Firpo et al., 2019). However, *Sr13a* is effective against these races (JRCQC and TTRTF) (Zhang et al., 2017; Olivera Firpo et al., 2019). Wide deployment of *Sr13a* due to the narrow genetic bases of resistance in durum wheat may risk breakdown by emerging virulent races. Therefore, broadening the genetic bases of stem rust resistance in durum wheat through introduction of new sources of resistance and identification of molecular markers linked to quantitative trait locus/loci (QTL) is important in improving the efficiency of resistance breeding to stem rust.

New variability can be introduced to breeding lines from wild relatives and/or landraces. Introduction of undesirable agronomic features to elite breeding lines is

expected in using resistance genes from landraces and/or wild species. However, the linkage drag from landraces could also be less than that of wild relatives (Babiker et al., 2017). Durum wheat variety 'Reichenbachii' is a landrace among the collections by Vavilov conserved in the United States National Plant Germplasm System. The resistance of this landrace to many of the older *Pgt* races prevalent all over the world has been reported in the past by Bechere et al. (1991). Considering the current *Pgt* races prevalent in East Africa (Ethiopia and Kenya), 'Reichenbachii' exhibited lower severity with moderately resistant to moderately susceptible responses (1MR to 10 MSMR). Identifying molecular markers linked to resistance against the current stem rust races in this cultivar may contribute to the efficient transfer of resistance into breeding lines and may also introduce new sources of resistance to the durum germplasm pool. The current advancement in dense marker or next generation sequencing technologies with low genotyping cost is a great opportunity for improving the resolution of mapping and identification of reliable markers tightly linked to QTL for stem rust resistance and other agronomically important traits (Zhou et al., 2010; Edwards et al., 2013; Wang et al., 2014). Therefore, the objective of this study was to evaluate adult plants of durum wheat RIL population derived from a cross between 'Reichenbachii' and 'DAKIYE' against the current multiple *Pgt* races prevalent in East Africa and map field resistance in this population.

MATERIALS AND METHODS

Plant Material

A total of 224 F_9 recombinant inbred lines (RILs) from a cross between a stem rust resistance donor parent 'Reichenbachii' (GID 30660) and a susceptible parent

'DAKIYE' (GID 6139553; Pedigree, CMOS_3//SOMAT_4/INTER_8/3/SOOTY_9/RASCON_37/4/SFAR_1) developed by the CIMMYT durum wheat breeding program was used in this study. The RILs were evaluated along with the parents for field response to multiple stem rust races in Ethiopia and Kenya for two seasons from 2019 to 2020. The trials were named after the country names, the seasons, and the years of evaluation. Hence, ETOS19 and ETMS19 refer to trials in Ethiopia during the off-season (January to May) and main-season (June to November) 2019, respectively while KNMS19 and KNMS20 refer to the trials in Kenya during the main-seasons (June to October) 2019 and 2020, respectively.

Experimental design and disease scoring

The RILs were planted using randomized incomplete block design in two replications in all testing environments. One moderately resistant ('Mangudo') and two susceptible ('Arendato' and 'Local Red') checks were planted after every 100 plots. In Debre Zeit, Ethiopia, lines were planted in 1 m long single rows with an inter-row spacing of 0.2 m. Spreader rows were planted between blocks and surrounding the experimental field with a mixture of equal proportions of stem rust susceptible cultivars 'Morocco', 'PBW343', 'Digalu', and 'Arendato'. In Njoro, Kenya, lines were planted in 0.7 m long single rows with an inter-row spacing of 0.3 m. The blocks and the experimental field were surrounded by spreader rows planted as hill plots with a mixture of stem rust susceptible cultivars 'Cacuke' and 'Robin', and six lines carrying *Sr24* (GID = 5391050, 5391052, 5391056, 5391057, 6391059, and 5391061) in equal proportions. Pathogen infection was initiated by artificial inoculation of spreader rows with a bulk

of urediniospores collected from the previous field season of each testing environments. Spreader rows were syringe-injected with a mixture of urediniospores, distilled water and a drop of Tween20 (one drop/0.5 L) at the stage of stem elongation (~ Zadok's growth scale 31) (Zadoks et al., 1974). The bulked mixture of races, distilled water and Tween20 was also sprayed on the spreader rows twice to favor uniform infection of the pathogen. The bulk of races were composed of TTKSK, TKTTF, JRCQC, TTTTF, and TRTTF in Debre Zeit, Ethiopia and TTKSK, TTKST, TTKTT, and TTTTF in Njoro, Kenya. However, these races were not the only races prevalent in the testing locations and there was variation in natural race composition.

Disease severity was scored using the modified Cobb's scale (0 to 100) by estimating the proportion of stem area covered with rust pustules (Peterson et al., 1948). Infection response was scored based on the size of pustules and the amount of chlorosis or necrosis surrounding the pustules on the stem as described in Roelfs et al. (1992). The response classes are scored as '0', 'R', 'MR', 'MS' and 'S' that designate no visible infection (immune), resistant, moderately resistant, moderately susceptible and susceptible reactions, respectively. Whenever different infection responses are observed on a single genotype, combinations of response classes can be scored by taking the most frequent first followed by the less frequent response. The disease severity and response classes were combined to a value called coefficient of infection (CI), the product of the disease severity and a 0 to 1 scale assigned to the response classes. The scale was specified as 0.0, 0.2, 0.4, 0.8 and 1.0 for immune, R, MR, MS, and S, respectively. In cases of combined responses per a single genotype, the mean of the scales was used to calculate CI (Stubbs et al.1986). The trials were scored at 7-to-

14-day intervals four times in ETOS19, three times in ETMS19 and KNMS20, and twice in KNMS19. In all trials the last scoring was used to calculate CI and apply further statistical analyses.

Statistical analyses of phenotypic data

The CI was used as a response variable to apply statistical analyses using R statistical software version 4.0.2 (R Core Team 2020) and ASReml-R version 3 for spatial correction (Glimour et al., 2009). A model that resulted in the highest estimate of broad-sense heritability and in some cases a model with significant Wald test for fixed effects was chosen to estimate BLUPs. For ETOS19, a linear mixed model (LMM) described in equation-5.1 was fitted using ASReml-R.

$$y_{ijk} = \mu + g_i + R_j + r_k + \varepsilon_{ijk} \quad \text{..... (5.1)}$$

Where: y_{ijk} is the response of the i^{th} genotype in the j^{th} row and in the k^{th} replication, μ is the overall mean response, g_i is the random effect of the i^{th} genotype, R_j is the fixed effect of the j^{th} row, r_k is the random effect of the k^{th} replication and ε_{ijk} is the residual associated with the model. For ETMS19, a LMM described in equation-5.2 was fitted on the square-root transformed CI as a response variable using the lmer() function of the R package *lme4* (Bates et al., 2015) and the BLUPs were estimated.

$$y_{ij} = \mu + g_i + r_j + \varepsilon_{ij} \quad \text{..... (5.2)}$$

Where: y_{ij} is the response of the i^{th} genotype and the j^{th} replication, g_i is the random effect of the i^{th} genotype, r_j is the random effect of the j^{th} replication and ε_{ij} is the residual. For KNMS19 and KNMS20, a model described in equation-5.2 was fitted on the data and BLUPs were extracted from this model. The broad-sense heritability for

each environment was calculated by applying equation-5.3 on the estimated variance components from the respective models fitted on the data (Holland et al.,2003).

$$H^2 = V_g / V_p \quad \text{..... (5.3)}$$

Where H^2 is the broad-sense heritability, V_g is the variance due to the genotype or line, V_p is the phenotypic variance $V_p = V_g + V_e$, V_e is the residual variance.

Genotyping and SNP calling

Leaf tissues were sampled in 1.1 ml tubes in 96-well plates from seedlings of the two parents and RILs (226 lines in total) grown in a greenhouse at CIMMYT, Mexico. Samples were frozen at -80 °C for three hours and lyophilized for 48 h. Lyophilized leaf samples were ground using a 2010 GenoGrinder (SPEX, SamplePrep, USA) for 2-3 minutes by placing stainless steel balls in the sampling tube. Genomic DNA was extracted using the modified cetyl trimethylammonium bromide (CTAB) protocol as described in Dreisigacker et al. (2016) and shipped to USDA-ARS Eastern Regional Small Grains Genotyping Lab. in Raleigh, NC for genotyping. The extracted DNA was quantified using the PicoGreen reagent in greiner flat bottom plates on a BMGLabTech PHERAstar Plus plate reader with MARS software. Then genotyping was done using the GBS protocol as described in Poland et al. (2012). The libraries were sequenced using an Illumina NovaSeq 6000, SP 100bp SE Lane. Single Nucleotide Polymorphism (SNP) genotypes were called using the TASSEL GBS software version 5 (Glaubitz et al., 2014) and the durum wheat assembly of cultivar 'Svevo' was used to assign SNP markers to physical positions and chromosomes (Maccaferri et al., 2019).

Genotype data filtering and linkage map construction

SNP markers were filtered by applying different filtering criteria. Markers with unknown chromosomal position, with missing data above 20%, those in perfect linkage disequilibrium (LD, $r^2 = 1$), and lines and markers ≥ 10% heterozygous calls were removed from the data. After filtering, 7418 SNPs for 201 lines were used as input data to generate the format needed by the R/*qtl* package (Broman et al., 2003).

The SNP markers were converted to the ABH-genotype format (A = allele from susceptible parent; B = allele from resistant parent and H = heterozygous) using TASSEL (Bradbury et al., 2007). On conversion of the SNP markers to ABH-genotypes using the *read.cross*() function of R/*qtl,* 929 marker genotypes for 198 lines were generated for the next filtering steps. Heterozygous calls from the *read.cross*() output were replaced by missing data that was imputed using the *fill.geno*() function in the R/*qtl* library version 1.46-2. In the *fill.geno*() function, the "*argmax*" method that uses the most likely sequence given the observed data was applied.

The data was further diagnosed for the presence of outlier genotypes for each line and marker, excessive proportion of shared alleles between lines, marker genotypes with segregation distortions and genotyping errors, markers with misaligned positions, and SNP markers and lines with excess double crossover/crossover counts. Chi-square tests were conducted to evaluate segregation distortion (deviation from the expected 1:1 ratio) at a Boneferroni threshold for multiple test correction (*P-value* < 5.38e-05). Linkage groups were formed at minimum LOD score value of six and maximum recombination frequency of 35%. The presence of markers grouped to a different linkage group than the alignment to the

durum wheat reference genome was assessed. Lines with more than 95% shared alleles, single markers misaligned to different positions/linkage groups, marker genotypes with double crossover counts above 10 and lines with crossover counts ≥ 60 were discarded from the dataset.

The genetic map was estimated at each filtering step. The *ripple*() function in R/*qtl* package was used for the likelihood ratio test that assesses all possible permutations of marker orders and recombination frequencies. All were converted to map distances (centiMorgans) using the Kosambi mapping function (Kosambi, 1943). The marker order with the highest LOD score and the shortest possible length was chosen for each chromosome. Then, genetic map was graphically represented using 843 quality markers for 175 RILs using *plotMap*() function in the R/*qtl* package.

QTL analysis

Before conducting QTL analyses, QTL genotype probabilities were calculated using *calc.genoprob*() function of the R/*qtl* package at a step of 2 cM with an assumed genotyping error rate of $1.0e^{-4}$ and using the Kosambi mapping function (Kosambi, 1943). QTL analysis was conducted using composite interval mapping (CIM) (Zeng et al., 1993) and the Haley-Knott regression method (Knott and Haley, 1992) by assigning three markers as covariates. The BLUPs estimated from the LMM fitted on the phenotypic data were fitted as response variables for the QTL analyses. No significant regions were identified on using LOD score thresholds identified by 1000 permutation tests at an experiment-wise $\alpha = 0.05$ and $\alpha = 0.10$ and a window size of 10 cM. Therefore, a LOD score of 2.5 was set as a threshold to declare the identification of significant QTL. Markers flanking the QTL were identified using

lodint() function in the R/*qtl* package that calculates the 1.5 LOD intervals. The effects of QTL on the phenotype and the percentage of variance in the phenotype explained by the QTL were identified by fitting linear model using the *fitqtl*() function of the R/*qtl* package. The donor of the identified QTL for resistance among the two parents and the QTL effect was visualized using *effectplot*() function in the R/*qtl* package. Then the presence of QTL by environment interaction was examined by fitting linear model using the BLUPs as a response variable and the QTL, environment, and QTL by environment interaction as explanatory variables.

RESULTS

Phenotypic data analyses

Field responses of RIL population to multiple stem rust races in Ethiopia and Kenya

The frequency distribution of the CI (the product of the severity and 0 to 1 scale for the response classes) of the RIL population to *Pgt* races in East Africa was close to normal for ETOS19 and KNMS19 but skewed towards the resistant score for ETMS19 and was near bimodal distribution for KNMS20 (Fig. 5.1). The broad-sense heritability estimated from the variance components was 0.58, 0.62, 0.85 and 0.84 in ETMS19, ETOS19, KNMS19 and KNMS20, respectively. The mean CI of the resistant parent ('Reichenbachii') ranged from 0 in KNMS20 to 6 in ETMS19 while that of the susceptible parent varied from 35.6 in ETMS19 to 85 in KNMS20 (Table 5.1). Assuming a disease score of 30MSMR as resistant in the field (CI = 30 x 0.6 = 18), 7%, 33%, 44.7% and 38.7% were resistant (CI ≤ 18) in ETOS19, ETMS19, KNMS19 and KNMS20, respectively. The proportion of susceptible lines ranged from 55.3% in KNMS19 to 93% in ETOS19. Among the total number of resistant lines in

each environment, none (0%) were transgressive segregants for resistance in ETOS19 and KNMS20 but 1% (one line, GID 8600910) and 15.7% (21 lines) were transgressive segregants for resistance in ETMS19 and KNMS19, respectively (Table 5.2). From the 21 transgressive segregants for resistance against races in Kenya, only four lines had marker data, but the remaining were missing (data not shown). Among the four, line GID 8600960 was a non-parental type.

Table 5.1. Mean, genetic variance and broad-sense heritability of CI of RIL population across four testing environments.

Statistic	ETOS19	ETMS19	KNMS19	KNMS20
Overall mean	43.9	26.3	25.4	39.9
Mean of resistant parent	3.6	6	0.6	0.0
Mean of susceptible parent	67.5	35.6	47.3	85.0
Genetic variance	207	2.08	448.5	1090
Heritability	0.62	0.58	0.85	0.84

Table 5.2. Percentage of resistant, susceptible and transgressive segregants of RILs evaluated for response to multiple stem rust races across four testing environments.

Environment	Percent resistant	Percent susceptible	Percent transgressive segregants	
			Resistant	Susceptible
ETOS19	7	93	0	19
ETMS19	33	67	1	86
KNMS19	44.7	55.3	15.7	66
KNMS20	38.7	61.3	0	35

Data filtering and linkage map construction

Several steps of filtering were undertaken before construction of the genetic linkage map and QTL analyses. The heatmap of the marker data before imputation, and after imputation and filtering is presented in Supplemental Fig. 5.1. No outlier line and marker genotypes were detected after imputation (Supplemental Fig. 5.2). Lines were compared for their shared proportion of alleles and 20 lines with > 95% shared alleles

were discarded (Fig.5.2). On a chi-square test of the deviation from a 1:1 segregation of marker genotypes, 47 marker showed significant segregation distortion at Boneferroni threshold (*P-value* < 5.38e-05) (Supplemental Table 5.1) and these markers were discarded.

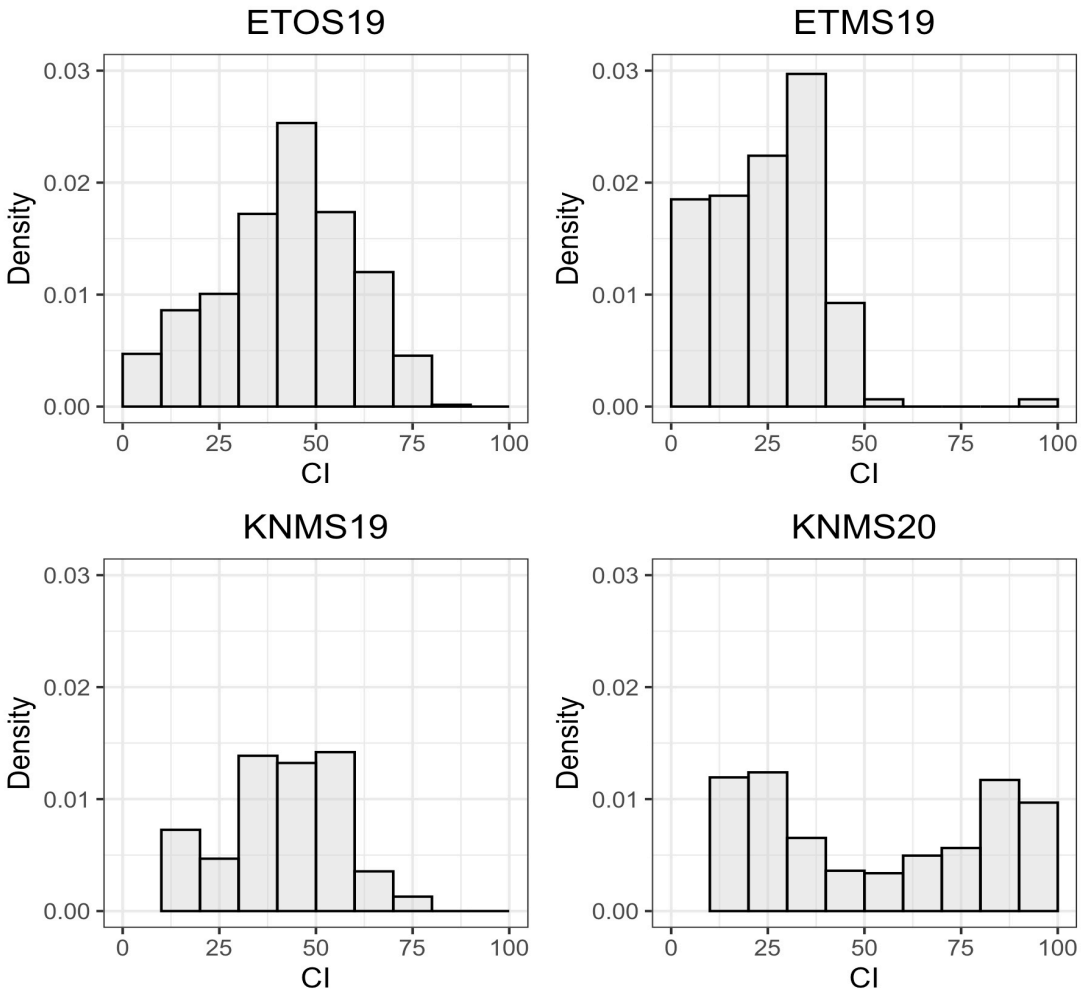


Figure 5.1. Distribution of CI of field responses of RIL populations derived from 'Reichenbachii' /DAKIYE cross in four testing environments.

Misaligned markers that mapped to a different linkage group and markers with switched alleles were omitted from the dataset based on the recombination fraction and LOD score heatmap (Fig. 5.3). Twenty-one markers with double crossover counts

above 10 and three lines with marker crossover counts ≥ 60 were removed from the dataset (Supplemental Fig. 5.3). SNP markers were tested for the presence of genotyping errors with an assumed error rate of 0.01 and no marker with genotyping error above the cutoff (error LOD score = 4) was identified.

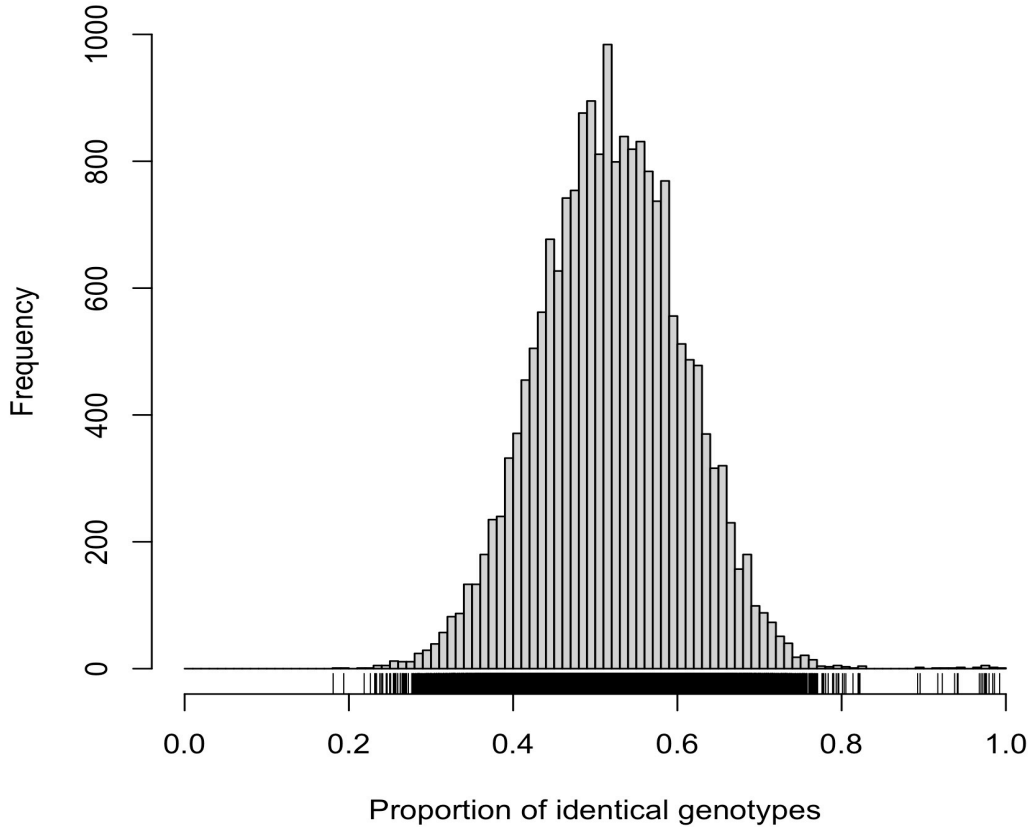


Figure 5.2. Proportion of shared alleles between RILs from 'Reichenbachii' /DAKIYE cross.

The heatmap of recombination fraction and LOD score of 843 quality markers for 175 RILs used to construct the genetic linkage map was shown in Supplemental Fig. 5.4. The 843 markers were distributed across 13 linkage groups representing all chromosomes of durum wheat except chromosome 7A (Figs. 5.4, 5.5). These markers covered 1458.1 cM of the genome with an average interval of 1.73 cM. The B sub-

genome had larger number of SNPs (535) than the A sub-genome (308) (Fig. 5.4). The A sub-genome covered 674.4 cM with an average interval of 2.19 cM while the B sub-genome covered 783.7 cM with an average interval of 1.23 cM. Chromosome 3B carried the largest number of SNPs covering a genetic distance of 207.3 cM followed by chromosome 7B (171.2 cM). SNPs on chromosome 7A were dropped during the filtering steps and chromosomes 2B and 4A were the lowest of all chromosomes in marker coverage with 4.4 cM and 12.6 cM, respectively (Figs. 5.4, 5.5).

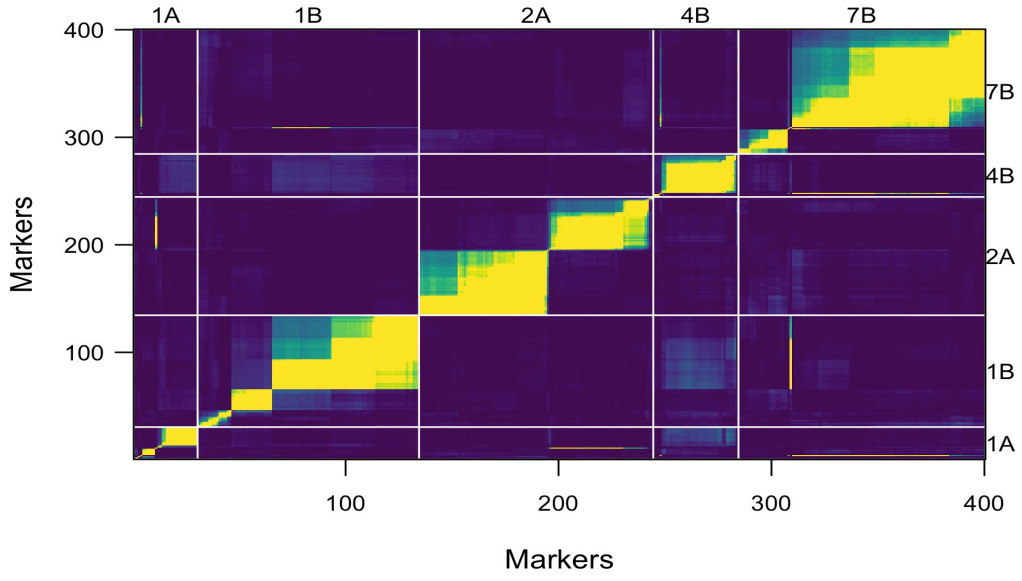


Figure 5.3. Heatmap of recombination fraction (upper left triangle) and LOD score (lower right triangle) of selected chromosomal regions with misaligned markers indicated by yellow strip on the blue background.

QTL mapping

Composite interval mapping detected three significant QTL (one per testing environment) on chromosomes 3B, 4B and 7B associated with field resistance to multiple stem rust races in Ethiopia and Kenya (Table 5.3). Sub-threshold QTL peaks were observed on chromosomes 1A and 5A (Fig. 5.6). The LOD scores of the QTL identified ranged from 2.52 to 4.29 (Table 5.3). The QTL *QSr.cnl-3B* (named

according to McIntosh et al., 2003) and located at 66 cM to 67 cM on chromosome 3B was significant in two of the four environments (ETMS19 and KNMS19). This QTL explained 6.4% and 15.3% of the phenotypic variation in ETMS19 and KNMS19, respectively. The peak marker for *QSr.cnl-3B* in ETMS19 (S3B_166187578) was mapped between markers S3B_91123277 (5.1 cM away) and S3B_259053349 (4.8 cM away). In KNMS19, the peak marker for the same QTL was c3B.loc66 and was mapped between markers S3B_343854 and S3B_196992709, 66 cM and 3.5 cM away, respectively. QTL on chromosome 4B (*QSr.cnl-4B*) identified in ETOS19 explained 4.7% of the phenotypic variation in this environment (Table 5.3). The peak marker of QTL *QSr.cnl-4B* (S4B_524068577) was mapped 37.9 cM away from marker S4B_8811137 and 6.8 cM away from marker S4B_550731907 (Table 5.3).

Table 5.3. Lists of QTL identified using composite interval mapping across four testing environments

Env.[a]	QTL name	SNP.ID	FM[b]		Pos[c] (cM)	LOD	R^{2d}
			Left	Right			
ETOS19	*QSr.cnl-4B*	S4B_524068577	S4B_8811137	S4B_550731907	38.7	2.5	4.7
ETMS19	*QSr.cnl-3B*	S3B_166187578	S3B_91123277	3B_259053349	67.0	2.84	6.4
KNMS19	*QSr.cnl-3B*	c3B.loc66	S3B_343854	S3B_196992709	66.0	4.3	15.3
KNMS20	*QSr.cnl-7B*	c7B.loc136	S7B_677752911	7B_688049535	143.0	2.7	7.2

[a] Environment, ETOS19 and ETMS19 = Ethiopia off-season2019 and main season 2019, respectively; KNMS19 and KNMS20 = Kenya main-season 2019 and 2020, respectively
[b] Flanking markers
[c] Position in cM
[d]Values indicate the percentage of phenotypic variance explained by the QTL

In KNMS20, a QTL was identified at 143 cM on chromosome 7B (*QSr.cnl-7B*). A marker linked to this QTL (c7B.loc136) was located 2.9 cM and 7.7 cM away from markers S7B_677752911 and S7B_688049535, respectively. QTL *QSr.cnl-7B* explained 7.2% of the phenotypic variation for field resistance to multiple stem rust races in KNMS20 (Table 5.3). All three QTL were contributed by the resistant parent, 'Reichenbachii' and the QTL on chromosome 3B (*QSr.cnl-3B*) explained the highest

percentage of the phenotypic variation in KNMS19 (15.3%) followed by the 7B locus (*QSr.cnl-7B*) in KNMS20 (7.2%) (Fig. 5.7; Table 5.3). The QTL by environment interaction was significant for *QSr.cnl-3B* (*P-value* = 6.705e^{-05}) and *QSr.cnl-7B* (*P-value* = 3.489e^{-04}) but not significant for *QSr.cnl-4B* (*P-value* = 0.10666) (data not shown).

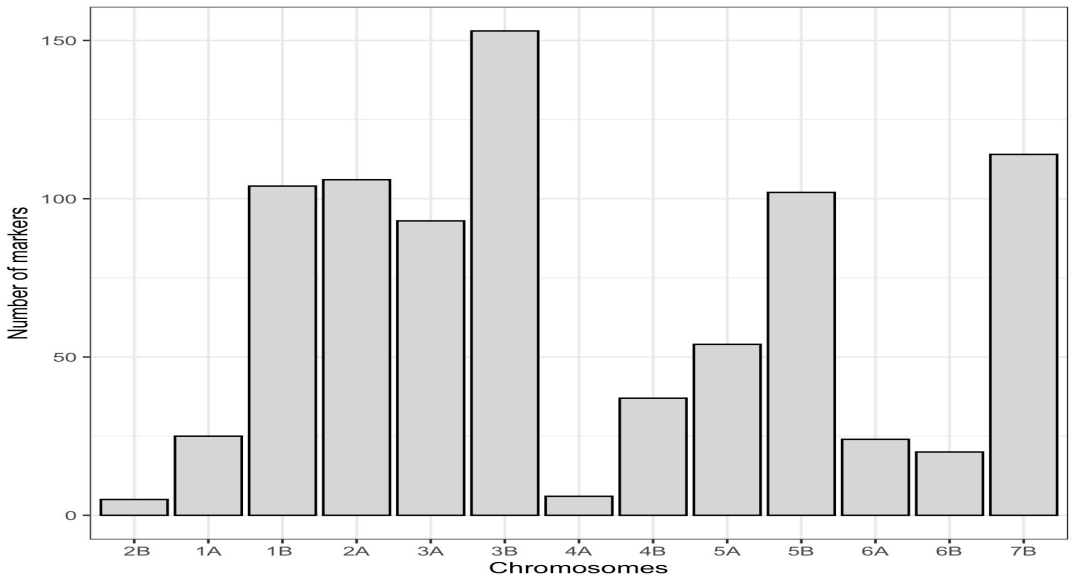


Figure 5.4. Distribution of SNP markers of RILs derived from genotyping-by-sequencing across linkage groups/chromosomes.

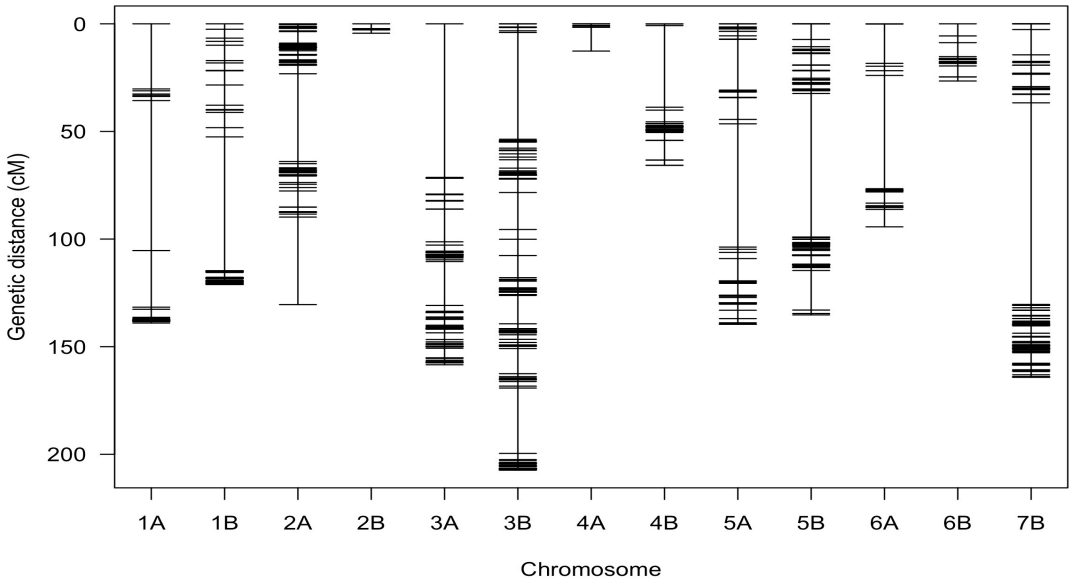


Figure 5.5. Genetic linkage map constructed from SNP markers derived from genotyping-by-sequencing in a recombinant inbred lines of a cross between Reichenbachii and DAKIYE.

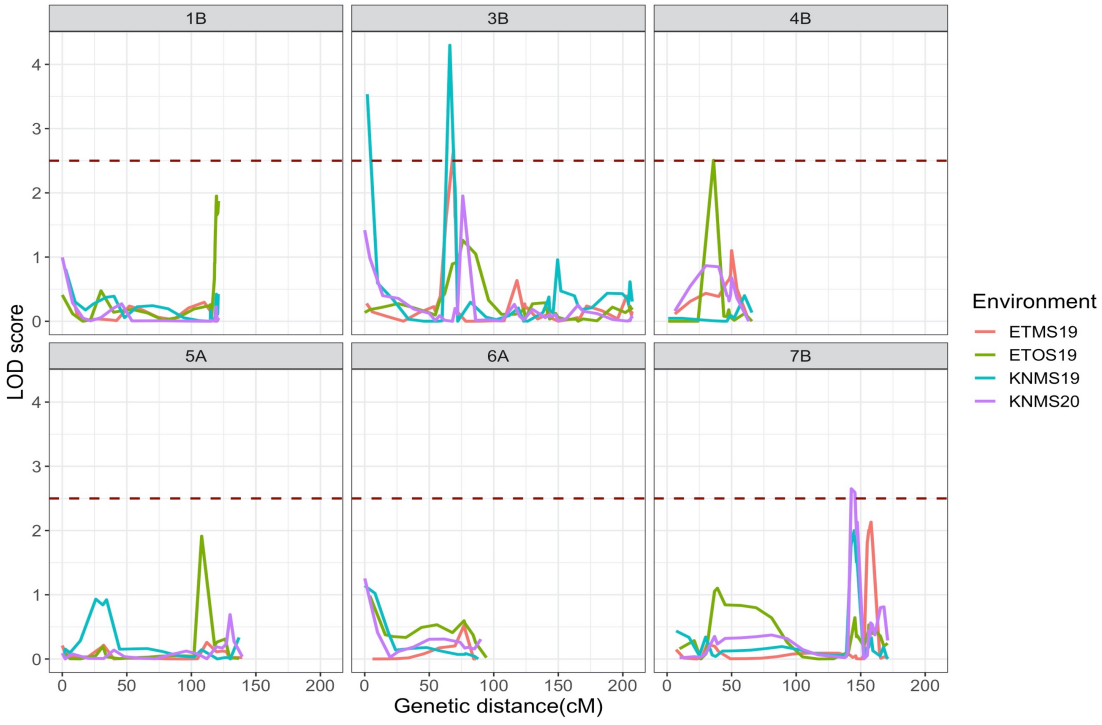


Figure 5.6. LOD score curves of selected chromosomes from composite interval mapping results across the four testing environments, the brown dotted line indicates the LOD threshold (2.5).

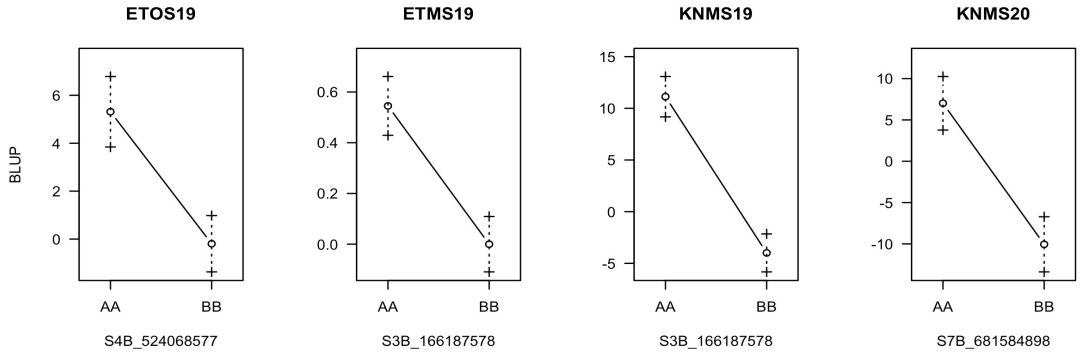


Figure 5.7. Effects of QTL on the response of RILs across the testing environments, the A allele was from the susceptible parent ('DAKIYE') and the B allele was from the resistant parent ('Reichenbachii').

DISCUSSION

In this study we report the field responses of a RIL population derived from the cross between a resistant parent 'Reichenbachii' and a susceptible parent 'DAKIYE' developed by the CYMMIT durum wheat breeding program and QTL identified using CIM for field resistance to multiple races of stem rust predominant in Ethiopia and Kenya.

Field responses of RIL population to multiple stem rust races in Ethiopia and Kenya

The RIL population responded differently to the stem rust races in different testing environments. The differences in the distribution of the CI of the RILs across the testing environments indicated the presence of variation in race composition. The skewed distribution towards the resistance scores in ETMS19 and the lower overall mean of the disease response (CI) indicated that there was a low frequency of virulent races. On the other hand, the overall mean CI (43.9) and the percentage of susceptible lines (93%) were highest in ETOS19 indicating that there was a high frequency of

virulent races in that nursery (Table 5.1). This could also be explained by a higher disease pressure favored by the warm and humid environment in the off-season than in the main season resulting in a better screening environment. The near bimodal distribution in KNMS20 suggested that there was a single resistance gene segregating in the population in this environment (Fig.5.1). Although no transgressive segregant was identified in the environment where virulent race composition was expected (ETOS19), evaluation of the transgressive segregants identified in ETMS19 and KNMS19 against multiple races at the seedling and adult plant stage may help to understand the type of resistance in these lines (Table 5.2).

Data filtering and linkage map construction

Several low-quality markers were discarded on applying different filtering criteria. To minimize the loss of information, imputation on marker data with a high proportion of missing data (≤ 50%) was attempted. However, this resulted in overestimation of recombination and extended genetic map distances were observed in this population (data not shown). Therefore, we used an imputed dataset with less missing data (20%) and the genetic map presented in Fig. 5.5 was generated. This genetic map was improved but still had an uneven distribution of markers in most of the linkage groups. This was due to the removal of 6,575 low-quality markers leaving 843 markers after filtering as indicated in the methods and results sections. The removal of many markers may also affect estimation of genetic distance, resolution of mapping and the power to identify QTL however, filtering out low quality markers is a critical step before construction of a linkage map and QTL analysis. The causes for the low-quality markers and difficulty in constructing the map are unknown. A selection from the

‘Reichenbachii’ landrace variety was the resistant parent and its origin is unknown. Variety ‘Reichenbachii’, the oldest accession in the USDA National Germplasm Repository, was collected in 1940 from Portugal. Genome structural rearrangements of alien introgressions can cause abnormal segregation and additional cytological investigations will be required to resolve this question.

QTL mapping

The continuous distribution of disease responses across the testing environments except in KNMS20 indicated the presence of more than one locus responsible for resistance to multiple races of stem rust in this RIL population (Fig. 5.1). However, only one QTL was detected per environment. This could be due to genotype by environment interaction and the low marker density in most of the linkage groups which reduced the power to detect additional QTL and the mapping resolution (Figs. 5.4, 5.5).

QTL *QSr.cnl-3B* (located at 67 cM and 66 cM) was associated with resistance to stem rust in ETMS19 and KNMS19 (Table 5.3). The short arm of chromosome 3B harbors the known adult plant resistance gene *Sr2* originating from emmer wheat (*Triticum dicoccum*). This gene is characterized by slow rusting and the pseudo black chaff trait. The pseudo black chaff trait cannot be identified at the seedling stage and is not always expressed in the adult plant (Mago et al., 2011). *Sr2* is recessive (McIntosh et al.,1995) and Bechere et al. (1991) reported that the resistance in ‘Reichenbachii’ was also recessive inheritance. However, the QTL we identified is 55 cM to 67 cM away from six markers reported by Bajgain et al.(2015a) and marker cs*Sr2* reported by Mago et al. (2011) and Yu et al. (2011) representing the *Sr2* locus. Thus, the region

identified on chromosome 3B is unlikely to be the *Sr2* locus. The all-stage resistance gene *Sr12* is also located on chromosome 3B. Rouse et al. (2014) reported that *Sr12* is involved in adult plant resistance as a result of complementary epistasis with other resistance genes. Markers *IWA4195*, *IWA4630, IWA4235, IWA3218, IWA610, IWA611* reported by Chao et al. (2017) for seedling resistance of diverse durum wheat lines to race BCCBC that map in the region of *Sr12* were located between 87 cM to 88 cM and this locus is 21 cM away from *QSr.cnl-3B* (Table 5.3). Moreover, the monogenic differential line carrying *Sr12* showed a susceptible response to race TTKSK at the seedling stage (3^{+}) and in field evaluation against races in Kenya (Disease score from 60S to 80S) where the QTL is detected (Jin et al., 2007). Hence, the locus detected on chromosome 3B is unlikely to be *Sr12.* The peak markers of *QSr.cnl-3B* (S3B_166187578, in ETMS19 and c3B.loc66, in KNMS19) collocate (0.5 cM to 1.5 cM away) with QTL tagging markers *IWB24497*, *IWB30621*, *IWB42046*, *IWB4823*, *IWB56471*, *IWB61425* (67.5 cM) reported by Bajgain et al. (2015b) for seedling resistance of diverse spring wheat lines to Ug99. It is known that the Ug99 group of races are predominant in Kenya. Furthermore, the skewed distribution of the RILs to the resistance score in ETMS19 may indicate inoculation of the nursery with a low frequency of durum virulent races, possibly the Ug99 group of races and the 'Digalu' race. Although the QTL on chromosome 3B was identified in the field, it is likely that this QTL is the same as the QTL identified by Bajgain et al. (2015b).

One of the flanking markers of a QTL in PBW343/Kingbird population reported by Bhavani et al. (2011) (*tPt-0602*) and a QTL in a durum wheat diversity panel reported by Letta et al. (2013) (*wPt-8543*) are further away from QTL *QSr.cnl-*

4B (132 Mb to 153 Mb away) identified for field resistance in ETOS19 (Table 5.3). QTL *QSr.cnl-4B* is close to *QSr.umn-4B.2* linked to marker *wsnp_Ku_c8075_13785546* (4.4 cM away) reported by Bajgain et al. (2015a) for adult plant resistance of the RBO7/MNO6113-8 RIL population to *Pgt* races in St. Paul, Minnesota (Table5.3). This QTL showed the lowest effect (R^2 = 4.74) and may identify the same region as the one reported by Bajgain et al. (2015a). Field screening of the RILs against races in Minnesota is needed to understand whether the same QTL is effective against races in Minnesota and Ethiopia. If this region is novel, it will be useful for breeding durum wheat resistant to virulent races predominant in Ethiopia.

QTL *QSr.cnl-7B* identified in KNMS20 is close to the *Sr17* locus identified by markers *wPt-1715, wPt-4298, wPt-7991, wPt-4045* reported by Letta et al. (2013) for field resistance of diverse durum wheat lines against races in Ethiopia. Marker *wmc517* reported by Letta et al., (2014) tagging the *Sr17* locus for seedling resistance against races TTTTF and TTKSK is 5 cM away from one of the flanking markers of *QSr.cnl-7B* (S7B_688049535). Although the marker platform is different, the QTL we identified on chromosome 7B could be the *Sr17* locus and the region was also physically close (1Mb to 4Mb away) to the markers linked to the *Sr17* locus.

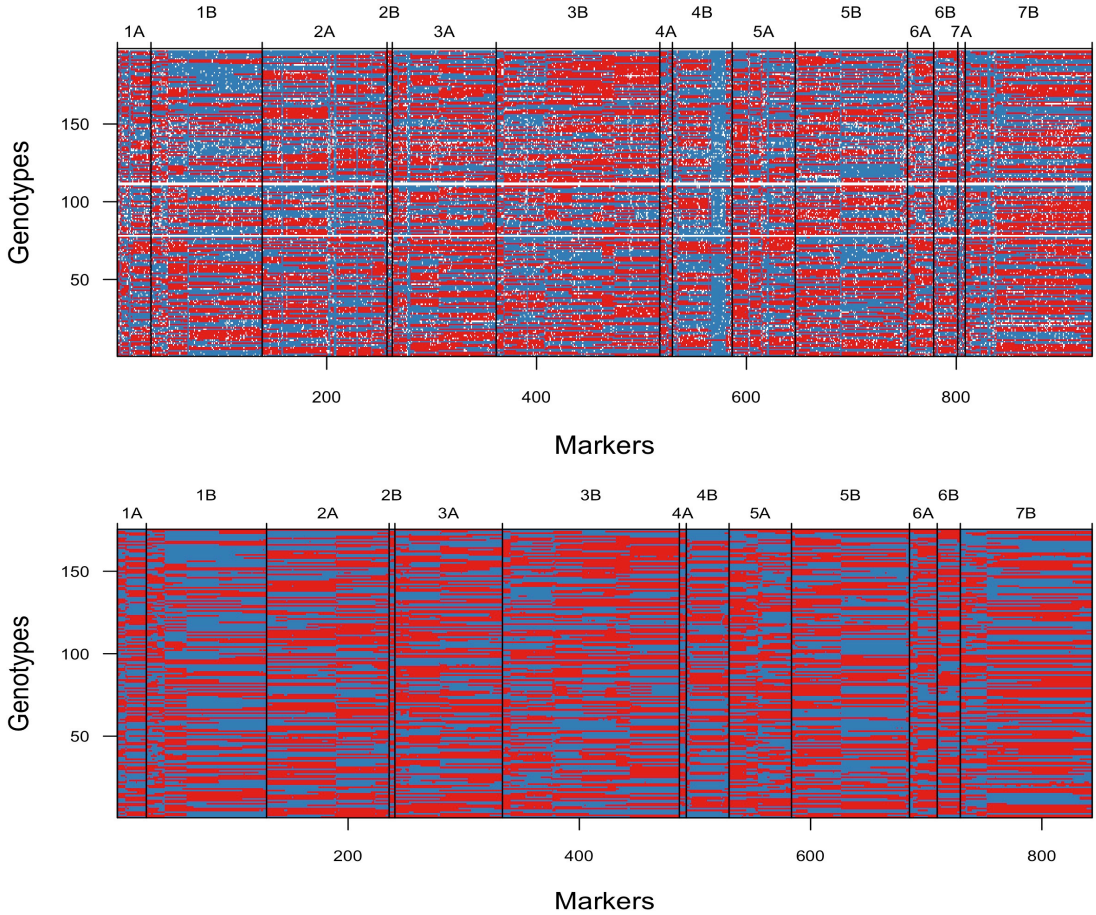
None of the QTL identified except the *QSr.cnl-3B* were consistent across evaluation environments. This could be explained by the significant QTL by environment interaction (data not shown). The QTL effect on disease reduction was larger against races in Kenya than races in Ethiopia which could indicate the presence of virulent races in Ethiopia and less virulent races in Kenya (Fig. 5.7). The interaction of the QTL with multiple-races prevalent in the testing environments could be another

reason for the lower effect of the QTL on the response. Therefore, evaluation of the RIL population against the responses to single races may elucidate the real effects of these QTL on the response.

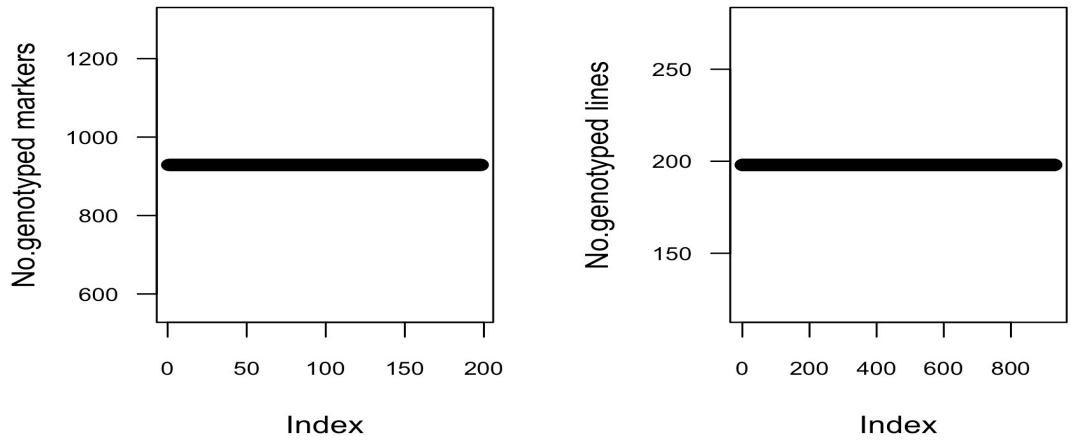
CONCLUSION

In summary, the three QTL contributed by 'Reichenbachii' (the resistance donor parent) identified in this study were previously reported. As the QTL effect on the response in the current study was generally small, evaluation of the RIL population against single races may uncover the specific effects of the QTLs on the response. Most of the RILs were very tall and susceptible to lodging. Therefore, evaluation of the RILs for the presence of other undesirable traits linked to the QTL that could potentially be transferred to elite lines prior to using this parent in resistance breeding will be needed. The power to identify additional QTL in this RIL population will be limited by abnormal segregation resulting in the removal of several low-quality markers. As many of the commercially deployed stem rust resistance genes in durum wheat are qualitative including those identified in this study, evaluation of large numbers of durum lines to identify sources of durable adult plant resistance to stem rust is crucial in the future resistance breeding of durum wheat against stem rust.

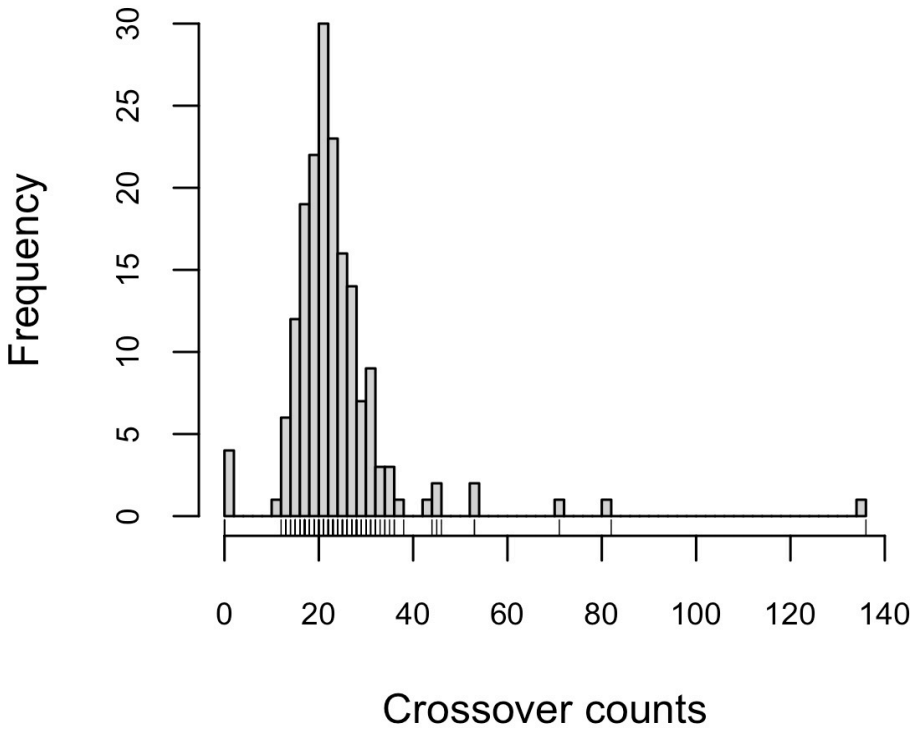
Lists of supplemental figures



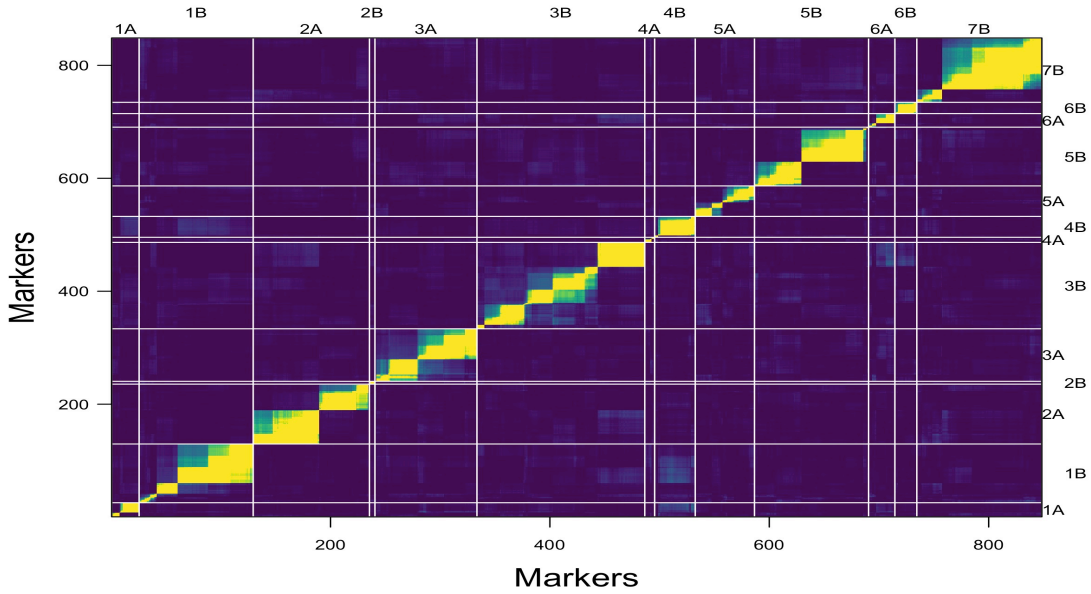
Supplemental Figure 5.1. Distribution of alleles from the susceptible parent (DAKIYE) coded as A and the resistant parent (Reichenbachii) coded as B. Red represents the allele from the susceptible parent and blue represents the allele from the resistant parent. The white spaces in the upper plot were missing data and the lower plot was after imputation and filtering. R-code adapted from Hussain et al. (2017).



Supplemental Figure 5.2. Plots of numbers of marker genotypes for each line (left) and numbers of lines genotyped for each marker (right) for diagnosis of outlier data.



Supplemental Figure 5.3. Distributions of crossover counts of RILs.



Supplemental Figure 5.4. Heatmap of recombination fraction and LOD score after filtering.

Lists of supplemental tables

Supplemental Table 5.1. Lists of marker genotypes with significant segregation distortion at Bonefferroni threshold.

Marker name	Chr.	No. missing	Genotype		*P.value*
			AA	BB	
S1A_539936987	1A	0	9	169	3.89E-33
S1A_540368269	1A	0	14	164	2.51E-29
S1B_647549343	1B	0	9	169	3.89E-33
S1B_653826081	1B	0	60	118	1.38E-05
S2A_9011801	2A	0	16	162	7.17E-28
S2A_10258583	2A	0	14	164	2.51E-29
S2A_58306939	2A	0	14	164	2.51E-29
S2A_58658896	2A	0	14	164	2.51E-29
S2A_73264522	2A	0	11	167	1.39E-31
S2A_73264854	2A	0	10	168	2.35E-32
S2A_79984294	2A	0	11	167	1.39E-31
S2A_124355541	2A	0	18	160	1.87E-26
S2A_479705642	2A	0	23	155	4.43E-23
S3A_629510529	3A	0	21	157	2.12E-24
S3A_630327049	3A	0	12	166	8.03E-31
S3A_633327219	3A	0	12	166	8.03E-31
S3A_729311968	3A	0	28	150	6.00E-20
S3B_213748627	3B	0	20	158	4.48E-25
S4B_16858295	4B	0	51	127	1.22E-08
S4B_50227616	4B	0	42	136	1.85E-12
S4B_583648902	4B	0	10	168	2.35E-32
S4B_587234956	4B	0	9	169	3.89E-33
S4B_587874996	4B	0	7	171	9.96E-35
S4B_592875786	4B	0	7	171	9.96E-35
S4B_593153840	4B	0	7	171	9.96E-35
S4B_594781678	4B	0	7	171	9.96E-35
S4B_599284312	4B	0	7	171	9.96E-35
S4B_600154291	4B	0	7	171	9.96E-35
S4B_600154369	4B	0	7	171	9.96E-35
S4B_603200542	4B	0	7	171	9.96E-35
S4B_604213919	4B	0	7	171	9.96E-35
S4B_604266753	4B	0	7	171	9.96E-35
S4B_605608964	4B	0	7	171	9.96E-35
S4B_605667151	4B	0	7	171	9.96E-35

S4B_605804494	4B	0	11	167	1.39E-31
S5A_222801444	5A	0	18	160	1.87E-26
S5A_639651884	5A	0	12	166	8.03E-31
S5A_639781876	5A	0	12	166	8.03E-31
S5B_457902655	5B	0	18	160	1.87E-26
S5B_660296496	5B	0	32	146	1.29E-17
S6B_655908347	6B	0	25	153	8.47E-22
S7A_631803925	7A	0	14	164	2.51E-29
S7B_47265059	7B	0	15	163	1.36E-28
S7B_59128773	7B	0	10	168	2.35E-32
S7B_60598849	7B	0	9	169	3.89E-33
S7B_60600901	7B	0	12	166	8.03E-31
S7B_221204263	7B	0	13	165	4.54E-30

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CHAPTER 6.

GENERAL CONCLUSION

The extensive deployment of qualitative resistance genes imposes a high selection pressure on the pathogen and leads to the evolution of new races. Consequently, many of the commercially deployed qualitative stem rust resistance genes are defeated by continuously emerging races. Utilizing genetic resistance for the control of stem rust requires characterization and identification of sources of resistance in the germplasm pool. The durum wheat panel and the RIL population used in the current study were not previously characterized at the seedling stage against virulent races and multiple stem rust races prevalent in the fields of East Africa. Moreover, genetic studies with dense marker coverage of the genome are limited in durum wheat compared to common wheat. In this study, evaluation of a panel of durum wheat lines and RIL populations developed by the CIMMYT durum wheat breeding program against single and multiple stem rust races at the seedling and adult plant stages, and genetic mapping of resistance to stem rust using SNP markers derived from the GBS protocol was reported.

GWAS analyses conducted using 26, 439 SNP markers for 280 to 283 lines identified several QTL for seedling resistance to races TTKSK, TKTTF, JRCQC and TTRTF, field resistance to single races of JRCQC and TKTTF, and field resistance to bulk multiple-stem rust races prevalent in East Africa (Ethiopia and Kenya). The regions of several previously reported stem rust resistance genes and alleles including

Sr7a, *Sr8155B1, Sr11,* alleles of *Sr13, Sr17, Sr22/Sr25, Sr49* and novel QTL were consistently identified in this durum wheat panel. The durum panel included few lines that carried the favorable alleles at all or most of the identified QTL. These lines, once evaluated for their agronomic performance, can be utilized as future varieties or as sources of resistance to stem rust that can possibly provide prolonged protection of durum wheat against stem rust. Consistently identified markers linked to the previously reported and newly identified QTL can be used in MAS after validation in a different population. These markers can also be used to design high-throughput markers and improve the efficiency of durum wheat stem rust resistance breeding.

Linkage mapping employed 843 quality markers for 175 lines from a cross between a resistant parent 'Reichenbachii' and a susceptible parent 'DAKIYE'. Three QTL for resistance to a bulk of multiple stem rust races in Ethiopia and Kenya that matched previously reported loci were identified in this population. In general, many of the resistance genes commercially deployed in durum wheat including those identified in the current study are qualitative. Sometimes breeders utilize two or more R-genes combined in the same genetic background which can improve durability of resistance compared to deploying a single resistance gene. Although the source of the known adult plant resistance gene (*Sr2*) is tetraploid wheat, this gene is underutilized in durum wheat production. Therefore, evaluation of large numbers of durum wheat lines against stem rust races to identify durable adult plant resistance to stem rust is critical in the future resistance breeding of durum wheat against the stem rust pathogen.

APPENDIX

Lists of pedigrees of a panel of durum wheat lines used for the study.		
ENTRY	GID	Pedigree
DUR_1	5077000	CIRNO C 2008
DUR_2	5080658	MUSK_1//ACO89/FNFOOT_2/4/MUSK_4/3/PLATA_3//CREX/ALLA/5/OLUS*2/ILBOR//PATKA_7/YAZI_1
DUR_3	5081059	SULA/AAZ_5//CHEN/ALTAR 84/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1
DUR_4	7145205	GUAYACAN INIA/GUANAY/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/6/SOMBRA_20/7/JUPARE C 2001/9/HUBEI//SOOTY_9/RASCON_37/3/2*SOOTY_9/RASCON_37/4/2*SOOTY_9/RASCON_37/10/SN TURK MI82-83 90//GLOSSY HUGENOT/6*FOCHA_1/
DUR_5	7145222	GEROMTEL-3/7/ALTAR 84/BINTEPE 85/3/STOT//ALTAR 84/ALD/4/POD_11/YAZI_1/5/VANRRIKSE_12/SNITAN/6/SOOTY_9/RASCON_37//WODUCK/CHAM_3
DUR_6	7145228	GEROMTEL-3/4/AJAIA/LOTUS_4/3/SOMAT_3/PHAX_1//TILO_1/LOTUS_4
DUR_7	7145230	GERUFTEL-1//GUAYACAN INIA/2*SNITAN
DUR_8	7145241	TUNSYR-2//GUAYACAN INIA/2*SNITAN
DUR_9	7145318	CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/SOOTY_9/RASCON_37//WODUCK/CHAM_3/9/SOOTY_9/RASCON_37//WODUCK/CHAM_3/3/SOMAT_3/PHAX_1//TILO_1/LOTUS_4/10/GUAYACAN INIA/2*SNITAN
DUR_10	7145376	GUAYACAN INIA/POMA_2//SNITAN/4/D86135/ACO89//PORRON_4/3/SNITAN/5/SHEWA 28/GDO//ZHONG ZUO/2*GREEN_3/3/BOOMER_18/LOTUS_4/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/7/ALTAR 84/BINTEPE 85/3/STOT//ALTAR 84/ALD/4/POD_11/YAZI_1/5/VANRRIKSE_12/SNITAN/6/SOOTY_9/RA
DUR_11	7145382	GUAYACAN INIA/GUANAY/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/6/SOMBRA_20/7/JUPARE C 2001/9/RCOL/THKNEE_2/3/SORA/2*PLATA_12//SOMAT_3/10/SOMAT_4/INTER_8/4/GODRIN/GUTROS//DUKEM/3/THKNEE_11/5/1A.1D 5+1-06/2*WB881
DUR_12	7145434	CNDO/VEE//PLATA_8/3/6*PLATA_11/6/PLATA_8/4/GARZA/AFN//CRA/3/GTA/5/RASCON/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/GUAYACAN INIA/POMA_2//SNITAN/4/D86135/ACO89//PORRON_4/3/SNITAN
DUR_13	7145451	CNDO/VEE//PLATA_8/3/6*PLATA_11/6/PLATA_8/4/GARZA/AFN//CRA/3/GTA/5/RASCON/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/ALTAR 84/BINTEPE 85/3/STOT//ALTAR 84/ALD/4/POD_11/YAZI_1/5/VANRRIKSE_12/SNITAN/6/SOOTY_9/RASCON_37
DUR_14	7145458	RANCO//CIT71/CII/3/COMDK/4/TCHO//SHWA/MALD/3/CREX/5/SNITAN/6/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/9/CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/SOOTY_9/RASCON_37//WODUCK/CHA
DUR_15	7145489	1A.1D 5+1-06/3*MOJO//RCOL/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/11/SOOTY_9/RASCON_37//WODUCK/CHAM_3/10/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT/12/ALTAR 84/STINT//SILVE
DUR_16	7145517	MOHAWK/10/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT/11/PLATA_6/GREEN_17//SNITAN/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/12/GUAYACAN INIA/GUANAY//PORRON_4/BEJAH_7/3/V
DUR_17	7145526	GUAYACAN INIA/POMA_2//SNITAN/4/D86135/ACO89//PORRON_4/3/SNITAN/12/CNDO/VEE//PLATA_8/3/6*PLATA_11/4/GUANAY/10/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT/11/ADAMAR_15//ALBI
DUR_18	7145583	BD96021.25/10/CNDO/VEE//CELTA/3/PATA_2/6/ARAM_7//CREX/ALLA/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/11/BD00010.504/4/CBC 509 CHILE/SOMAT_3.1/3/RASCON_37/TARRO_2//RASC
DUR_19	7145599	BYBLOS/6/TOPDY_18/FOCHA_1//ALTAR 84/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/4/SOMAT_3/GREEN_22/5/VRKS_3/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/7/GUAYACAN INIA/GUANAY//PORRON_4/BEJAH_7/3/VANRRIKSE_12/SNITAN
DUR_20	7145625	CEMEXI C 2008/10/CNDO/VEE//CELTA/3/PATA_2/6/ARAM_7//CREX/ALLA/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9
DUR_21	7145650	C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2/6/MOHAWK/3/GUANAY//TILO_1/LOTUS_4/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1
DUR_22	7145651	C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2/10/KOFA/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9
DUR_23	7145660	C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2/6/STORLOM/3/RASCON_37/TARRO_2//RASCON_37/4/D00003A/5/1A.1D 5+1-06/3*MOJO/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13
DUR_24	7145662	C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2/12/MOHAWK/10/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT/11/ARMENT//SRN_3/NIGRIS_4/3/
DUR_25	7145664	C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2/6/BCR/GUEROU_1/3/MINIMUS_6/PLATA_16//IMMER
DUR_26	7145669	C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2/6/MOHAWK/4/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1
DUR_27	7145699	BHA/5/MOHAWK/4/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1/6/C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2
DUR_28	7145704	MOHAWK/4/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1/5/KOFA/4/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1/6/C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2
DUR_29	7145707	MOHAWK/4/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1/5/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/3/SOMAT_3/PHAX_1//TILO_1/LOTUS_4/4/CANELO_8//SORA/2*PLATA_12/7/ODIN_15/WITNEK_1//ISLOM_1/5/TARRO_1/TISOMA_2//TARRO_1/3/COMB DUCK_2/ALAS//4*COMB DUCK_2/4/SHAG_9/BUTO_1
DUR_30	7145713	SNITAN*2/RBC/10/KOFA/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9
DUR_31	7145733	MARJANA/5/MOHAWK/4/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1
DUR_32	7145738	CMH83.2578/4/D88059//WARD/YAV79/3/ACO89/5/2*SOOTY_9/RASCON_37/6/1A.1D 5+1-06/3*MOJO/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/12/MOHAWK/10/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/HUI/POC//B
DUR_33	7145740	C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2/6/GUAYACAN INIA/GUANAY//PORRON_4/BEJAH_7/3/VANRRIKSE_12/SNITAN
DUR_34	7145746	C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2/10/KOFA/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9
DUR_35	7145749	C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2/6/KOFA/4/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1
DUR_36	7145752	C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2/11/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/RAFI97/9/MALMUK_1/SERRATOR_1/10/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1
DUR_37	7145764	C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2/6/MOHAWK/4/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1

DUR_38	7145767	STORLOM/3/RASCON_37/TARRO_2//RASCON_37/4/D00003A/5/1A.1D 5+1-06/3*MOJO/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/12/MOHAWK/10/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNF
DUR_39	7145770	KOFA/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/MOHAWK/4/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1
DUR_40	7145771	KOFA/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/INRAM_1805//SOMAT_4/INTER_8/3/SOOTY_9/RASCON_37//TILO_1/LOTUS_4
DUR_41	7145779	MOHAWK/3/GUANAY//TILO_1/LOTUS_4/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/5/INRAM_1805//SOMAT_4/INTER_8/3/SOOTY_9/RASCON_37//TILO_1/LOTUS_4
DUR_42	7145793	STORLOM/3/RASCON_37/TARRO_2//RASCON_37/4/D00003A/5/1A.1D 5+1-06/3*MOJO/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/12/MOHAWK/10/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNF
DUR_43	7145795	KOFA/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/MARJANA/11/INRAM_1805//SOMAT_4/INTER_8/3/SOOTY_9/RASCON_37//TILO_1/LOTUS_4
DUR_44	7145796	KOFA/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/KRONOS/11/C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2
DUR_45	7145800	STORLOM/3/RASCON_37/TARRO_2//RASCON_37/4/D00003A/5/1A.1D 5+1-06/3*MOJO/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/6/CHAM 1/11/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/RAFI97/9/MALMUK_1/SERRATOR_1/10/ARMENT//SRN_3/NIGRIS
DUR_46	7145802	BHA/10/CMH85.797//DUKEM_12/2*RASCON_21/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/11/GUAYACAN INIA/GUANAY//PORRON_4/BEJAH_7/3/VANRRIKSE_12/SNITAN
DUR_47	7383244	CEMEXI C 2008/5/ARMENT//2*SOOTY_9/RASCON_37/4/CNDO/PRIMADUR//HAI-OU_17/3/SNITAN/6/ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/5/SOMAT_4/INTER_8
DUR_48	7383253	CEMEXI C 2008/5/2*GUAYACAN INIA/POMA_2//SNITAN/4/D86135/ACO89//PORRON_4/3/SNITAN
DUR_49	7383256	CEMEXI C 2008/5/GUAYACAN INIA/POMA_2//SNITAN/4/D86135/ACO89//PORRON_4/3/SNITAN/7/ADAMAR_15//ALBIA_1/ALTAR 84/3/SNITAN/4/SOMAT_4/INTER_8/5/SOOTY_9/RASCON_37/6/BICHENA/AKAKI_7/4/LIS_8/FILLO_6/3/FUUT//HORA/JOR/5/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN
DUR_50	7383281	GEROMTEL-3/12/ARTICO/AJAIA_3//HUALITA/10/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT/11/CNDO/PRIMADUR//HAI-OU_17/3/SNITAN
DUR_51	7383291	TUNSYR-2/5/C94.52/3/2*AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/4/2*RASCON_37/2*TARRO_2
DUR_52	7383298	CIRNO C 2008/10/CHEN_1/TEZ/3/GUIL//CIT71/CII/4/SORA/PLATA_12/5/STOT//ALTAR 84/ALD/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9
DUR_53	7383306	CIRNO C 2008/10/TADIZ/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9
DUR_54	7383307	CIRNO C 2008/6/PLATA_6/GREEN_17/3/CHEN/AUK//BISU*2/5/PLATA_3//CREX/ALLA/3/SOMBRA_20/4/SILVER_14/MOEWE
DUR_55	7383312	CIRNO C 2008/5/CMH85.797//CADO/BOOMER_33/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1
DUR_56	7383344	GUAYACAN INIA/2*SNITAN/10/TADIZ/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9
DUR_57	7383372	HUBEI//SOOTY_9/RASCON_37/3/2*SOOTY_9/RASCON_37/4/SOOTY_9/RASCON_37/5/SOOTY_9/RASCON_37/10/CHEN_1/TEZ/3/GUIL//CIT71/CII/4/SORA/PLATA_12/5/STOT//ALTAR 84/ALD/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9
DUR_58	7383387	HUBEI//SOOTY_9/RASCON_37/3/2*SOOTY_9/RASCON_37/4/2*SOOTY_9/RASCON_37/10/CHEN_1/TEZ/3/GUIL//CIT71/CII/4/SORA/PLATA_12/5/STOT//ALTAR 84/ALD/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9
DUR_59	7383418	GUAYACAN INIA/GUANAY/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/6/SOMBRA_20/7/JUPARE C 2001/9/SILK_3/DIPPER_6/3/ACO89/DUKEM_4//5*ACO89/4/PLATA_7/ILBOR_1//SOMAT_3
DUR_60	7383430	GUAYACAN INIA/GUANAY/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/6/SOMBRA_20/7/JUPARE C 2001/9/ARMENT//2*SOOTY_9/RASCON_37/4/CNDO/PRIMADUR//HAI-OU_17/3/SNITAN
DUR_61	7383444	ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/5/SOMAT_4/INTER_8/6/SILK_3/DIPPER_6/3/ACO89/DUKEM_4//5*ACO89/4/PLATA_7/ILBOR_1//SOMAT_3
DUR_62	7383456	ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/5/SOMAT_4/INTER_8/6/CMH85.797//CADO/BOOMER_33/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1
DUR_63	7383471	SOMAT_4/INTER_8/4/GODRIN/GUTROS//DUKEM/3/THKNEE_11/5/1A.1D 5+1-06/2*WB881//1A.1D 5+1-06/3*MOJO/3/BISU_1/PATKA_3/4/GODRIN/GUTROS//DUKEM/3/THKNEE_11/10/CHEN_1/TEZ/3/GUIL//CIT71/CII/4/SORA/PLATA_12/5/STOT//ALTAR 84/ALD/9/USDA595/3/D67.3/RABI//CRA/4/ALO/
DUR_64	7383504	SWAHEN_2/KIRKI_8//PROZANA_1/4/ADAMAR_15//ALBIA_1/ALTAR 84/3/SNITAN/9/GUAYACAN INIA/GUANAY/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/6/SOMBRA_20/7/JUPARE C 2001/10/CHEN_1/TEZ/3/GUIL//CIT71/CII/4/SORA/PLATA_12/5/
DUR_65	7383514	SWAHEN_2/KIRKI_8//PROZANA_1/4/ADAMAR_15//ALBIA_1/ALTAR 84/3/SNITAN/9/GUAYACAN INIA/GUANAY/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/6/SOMBRA_20/7/JUPARE C 2001/10/PLATA_6/GREEN_17/3/CHEN/AUK//BISU*2/5/PLATA_3//
DUR_66	7383526	SWAHEN_2/KIRKI_8//PROZANA_1/4/ADAMAR_15//ALBIA_1/ALTAR 84/3/SNITAN/9/GUAYACAN INIA/GUANAY/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/6/SOMBRA_20/7/JUPARE C 2001/10/TOPDY_18/FOCHA_1//ALTAR 84/3/AJAIA_12/F3LOCAL(S
DUR_67	7383536	ADAMAR_15//ALBIA_1/ALTAR 84/3/SNITAN/4/SOMAT_4/INTER_8/5/SOOTY_9/RASCON_37/6/BICHENA/AKAKI_7/4/LIS_8/FILLO_6/3/FUUT//HORA/JOR/5/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/10/TADIZ/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/P
DUR_68	7383557	ADAMAR_15//ALBIA_1/ALTAR 84/3/SNITAN/4/SOMAT_4/INTER_8/5/SOOTY_9/RASCON_37/6/BICHENA/AKAKI_7/4/LIS_8/FILLO_6/3/FUUT//HORA/JOR/5/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/7/ARMENT//2*SOOTY_9/RASCON_37/4/CNDO/PRIMADUR//HAI-OU_17/3/SNITAN
DUR_69	7383561	ADAMAR_15//ALBIA_1/ALTAR 84/3/SNITAN/4/SOMAT_4/INTER_8/5/SOOTY_9/RASCON_37/6/BICHENA/AKAKI_7/4/LIS_8/FILLO_6/3/FUUT//HORA/JOR/5/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/7/TOPDY_18/FOCHA_1//ALTAR 84/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/4/SO
DUR_70	7383636	GUAYACAN INIA/GUANAY/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/6/SOMBRA_20/7/JUPARE C 2001/9/BICHENA/AKAKI_7/3/SOMAT_3/PHAX_1//TILO_1/LOTUS_4/7/CHEN_11/POC//TANTLO/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO
DUR_71	7383643	ALTAR 84/BINTEPE 85/3/STOT//ALTAR 84/ALD/4/POD_11/YAZI_1/5/VANRRIKSE_12/SNITAN/6/SOOTY_9/RASCON_37//WODUCK/CHAM_3/10/CHEN_1/TEZ/3/GUIL//CIT71/CII/4/SORA/PLATA_12/5/STOT//ALTAR 84/ALD/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79
DUR_72	7383690	GEROMTEL-3/12/ARTICO/AJAIA_3//HUALITA/10/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT/11/CNDO/PRIMADUR//HAI-OU_17/3/SNITAN/13/SWAHEN_2/KIRKI_8//PROZANA_1/4/ADAMAR_15//ALBIA
DUR_73	7383732	GEROMTEL-3/6/ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/5/SOMAT_4/INTER_8/7/AJAIA/LOTUS_4/3/SOMAT_3/PHAX_1//TILO_1/LOTUS_4

DUR_74	7383769	AMRIA/6/SOMAT_3/PHAX_1//TILO_1/LOTUS_4/3/GUANAY/5/NETTA_4/DUKEM_12//RASCON_19/3/SORA/2*PLATA_12/4/GREEN_18/FOCHA_1//AIRON_1/9/CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/SOOTY_9/
DUR_75	7383809	GEROMTEL-3/9/GUAYACAN INIA/GUANAY/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/6/SOMBRA_20/7/JUPARE C 2001/10/HUBEI//SOOTY_9/RASCON_37/3/2*SOOTY_9/RASCON_37/4/SOOTY_9/RASCON_37/5/SOOTY_9/RASCON_37
DUR_76	7383851	TUNSYR-2//GUAYACAN INIA/2*SNITAN/10/SWAHEN_2/KIRKI_8//PROZANA_1/4/ADAMAR_15//ALBIA_1/ALTAR 84/3/SNITAN/9/GUAYACAN INIA/GUANAY/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/6/SOMBRA_20/7/JUPARE C 2001
DUR_77	7383861	TUNSYR-2/8/STOT//ALTAR 84/ALD/3/THB/CEP7780//2*MUSK_4/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/RASCON_37/2*TARRO_2/4/ROK/FGO//STIL/3/BISU_1/5/MALMUK_1/SERRATOR_1/9/GUAYACAN INIA/GUANAY/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI
DUR_78	7383862	TUNSYR-2/4/AJAIA/LOTUS_4/3/SOMAT_3/PHAX_1//TILO_1/LOTUS_4/5/GUAYACAN INIA/2*SNITAN
DUR_79	7383871	LILE/6/C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2
DUR_80	7383901	ALAMO:DR/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/5/PLATA_6/GREEN_17//SNITAN/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/6/C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2
DUR_81	7383916	TRIDENT/3*KUCUK/7/CMH83.2578/4/D88059//WARD/YAV79/3/ACO89/5/2*SOOTY_9/RASCON_37/6/1A.1D 5+1-06/3*MOJO/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13
DUR_82	7383935	JUPARE C 2001*2/RBC/6/STORLOM/3/RASCON_37/TARRO_2//RASCON_37/4/D00003A/5/1A.1D 5+1-06/3*MOJO/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/7/C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2
DUR_83	7383957	JUPARE C 2001*2/KHAPLI/4/INRAM_1805//SOMAT_4/INTER_8/3/SOOTY_9/RASCON_37//TILO_1/LOTUS_4/12/MOHAWK/10/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT/11/ARMENT//SRN_3/NIGRIS_4
DUR_84	7384008	CMH83.2578/4/D88059//WARD/YAV79/3/ACO89/5/2*SOOTY_9/RASCON_37/6/1A.1D 5+1-06/3*MOJO/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/7/KOFA/4/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1/8/JUPARE C 2001*2/KHAPLI
DUR_85	7384019	STORLOM/3/RASCON_37/TARRO_2//RASCON_37/4/D00003A/5/1A.1D 5+1-06/3*MOJO/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/6/MOHAWK/3/GUANAY//TILO_1/LOTUS_4/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/7/JUPARE C 2001*2/KHAPLI
DUR_86	7384027	C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2/7/ODIN_15/WITNEK_1//ISLOM_1/5/TARRO_1/TISOMA_2//TARRO_1/3/COMB DUCK_2/ALAS//4*COMB DUCK_2/4/SHAG_9/BUTO_17/6/VANRRIKSE_6.2//1A-1D 2+12-5/3*WB881/5/TARRO_1/TISOMA_2/
DUR_87	7384033	C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2/6/KRONOS/11/AJAIA_16//HORA/JRO/3/GAN/4/ZAR/5/SOOTY_9/RASCON_37/6/JUPARE C 2001/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/ARMENT
DUR_88	7384039	JUPARE C 2001*2/IM/7/CMH83.2578/4/D88059//WARD/YAV79/3/ACO89/5/2*SOOTY_9/RASCON_37/6/1A.1D 5+1-06/3*MOJO/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/2*NETTA_4/DUKEM_12//RASCON_19/3/SORA/2*PLATA_12/4/GR
DUR_89	7384046	JUPARE C 2001*2/KHAPLI/4/GUAYACAN INIA/GUANAY//PORRON_4/BEJAH_7/3/VANRRIKSE_12/SNITAN/7/KOFA/4/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1/6/CHEN_1/TEZ/3/GUIL//CIT71/CII/4/SORA/PLATA_12/5/STOT//ALTAR 84/ALD
DUR_90	7384051	JUPARE C 2001*2/KHAPLI/6/C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2/7/LORITA/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/5/SORA/2*PLATA_12//RASCON_37/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN
DUR_91	7384062	CMH83.2578/4/D88059//WARD/YAV79/3/ACO89/5/2*SOOTY_9/RASCON_37/6/1A.1D 5+1-06/3*MOJO/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/7/STORLOM/3/RASCON_37/TARRO_2//RASCON_37/4/D00003A/5/1A.1D 5+1-06/3*MOJO/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13
DUR_92	7384063	CMH83.2578/4/D88059//WARD/YAV79/3/ACO89/5/2*SOOTY_9/RASCON_37/6/1A.1D 5+1-06/3*MOJO/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/7/KOFA/4/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1/8/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/2*NETTA_4/DUKEM_12//RAS
DUR_93	7384071	C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2/6/MOHAWK/4/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1/7/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/2*NETTA_4/DUKEM_12//RASCON_19/3/SORA/2*PLATA_12/4/GREEN_18/FOCHA_1
DUR_94	7384072	STORLOM/3/RASCON_37/TARRO_2//RASCON_37/4/D00003A/5/1A.1D 5+1-06/3*MOJO/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/6/MOHAWK/4/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1/7/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/2*NETTA_4/DUKEM_12//RASCON_19/3/SO
DUR_95	7384079	KOFA/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/MOHAWK/4/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1/11/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/2*NETTA_4/DUKEM_12//RASCON_19/3/SORA/2*PLATA_12/4/GREEN_18/FOCHA_1//A
DUR_96	7384096	WBDTBO/10/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT/11/MÂALI/10/ALTAR 84/CMH82A.1062//ALTAR 84/3/YAZI_10/4/SNITAN/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE
DUR_97	7384135	MOHAWK/6/LOTUS_5/F3LOCAL(SEL.ETHIO.135.85)/5/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT/7/SORA/2*PLATA_12/3/SORA/2*PLATA_12//SOMAT_3/4/AJAIA_13/YAZI//DIPPER_2/BUSHEN_3
DUR_98	7384142	MOHAWK/6/LOTUS_5/F3LOCAL(SEL.ETHIO.135.85)/5/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT/10/SWAHEN_2/KIRKI_8//PROZANA_1/4/ADAMAR_15//ALBIA_1/ALTAR 84/3/SNITAN/9/GUAYACAN INIA/GUANAY/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/
DUR_99	7406251	WBD881/3/PLANETA/PIQUERO//BERGAND/KNIPA/4/TRIDENT/3*KUCUK
DUR_100	7406259	WBD881/3/PIQUERO/AMIC//PLAYERO/PLANETA/4/TRIDENT/3*KUCUK

DUR_101	7406303	WBD881/6/C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2/7/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/2*NETTA_4/DUKEM_12//RASCON_19/3/SORA/2*PLATA_12/4/GREEN_18/FOCHA_1//AIRON_1
DUR_102	7406313	WBD881/6/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/2*NETTA_4/DUKEM_12//RASCON_19/3/SORA/2*PLATA_12/4/GREEN_18/FOCHA_1//AIRON_1/7/PH896-21/4/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1
DUR_103	7406319	WBD881/7/ODIN_15/WITNEK_1//ISLOM_1/5/TARRO_1/TISOMA_2//TARRO_1/3/COMB DUCK_2/ALAS//4*COMB DUCK_2/4/SHAG_9/BUTO_17/6/VANRRIKSE_6.2//1A-1D 2+12- 5/3*WB881/5/TARRO_1/TISOMA_2//TARRO_1/3/COMB DUCK_2/ALAS//4*COMB DUCK_2/4/SHAG_9/BUTO_17/12/MOHAWK/10/PLATA_
DUR_104	7406340	ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/10/CMH79.959/CHEN//SOOTY_9/RASCON_37/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/11/RCOL//SHAG_23/LAPDY_25/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/12/KOFA/9/USDA5
DUR_105	7406351	ALAS/5*SILVER_2/4/2*ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/6/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/2*NETTA_4/DUKEM_12//RASCON_19/3/SORA/2*PLATA_12/4/GREEN_18/FOCHA_1//AIRON_1/7/C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SH
DUR_106	7406436	MOHAWK/3/GUANAY//TILO_1/LOTUS_4/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/6/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/2*NETTA_4/DUKEM_12//RASCON_19/3/SORA/2*PLATA_12/4/GREEN_18/FOCHA_1//AIRON_1/7/C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/
DUR_107	7406449	PLATA_7/ILBOR_1//SOMAT_3/7/CHEN_11/POC//TANTLO/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/6/MINIMUS/COMB DUCK_2//CHAM_3/10/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/9/CHEN_11/POC//TANTLO/5/ENTE/MEXI_2//HUI/4/YAV_
DUR_108	7406486	ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/4/TOSKA_26/RASCON_37//SNITAN/5/PLAYERO/11/CLAUDIO/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/10/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/9/USDA595/3/D67.3/RABI //CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9
DUR_109	7406503	ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/4/TOSKA_26/RASCON_37//SNITAN/5/PLAYERO/11/E90040/MFOWL_13//LOTAIL_6/3/PROZANA/ARLIN//MUSK_6/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/TOSKA_26/RASCON_37//SNITAN/4/ARMENT//SRN_3/
DUR_110	7406516	BELLAROI/5/HUBEI//SOOTY_9/RASCON_37/3/2*SOOTY_9/RASCON_37/4/SOOTY_9/RASCON_37/11/BRONTE/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/10/RCOL/THKNEE_2/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9
DUR_111	7406533	BELLAROI/5/HUBEI//SOOTY_9/RASCON_37/3/2*SOOTY_9/RASCON_37/4/SOOTY_9/RASCON_37/11/CLAUDIO/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/10/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9
DUR_112	7406594	SIMETO/3/SORA/2*PLATA_12//SRN_3/NIGRIS_4/5/TOSKA_26/RASCON_37//SNITAN/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/11/CLAUDIO/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/10/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDE
DUR_113	7406605	SIMETO/3/SORA/2*PLATA_12//SRN_3/NIGRIS_4/5/TOSKA_26/RASCON_37//SNITAN/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/11/E90040/MFOWL_13//LOTAIL_6/3/PROZANA/ARLIN//MUSK_6/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/TOSKA_26/R
DUR_114	7406615	BRONTE/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/10/RCOL/THKNEE_2/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/11/CLAUDIO/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/10/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/9/USDA595/3/D67.3/RABI
DUR_115	7406684	1A.1D 5+1- 06/3*MOJO//RCOL/3/SNITAN/SOMAT_3//FULVOUS_1/MFOWL_13/10/AVILLO_1/3/CANELO_8//SORA/2*PLATA_12/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/11/CLAUDIO/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/10/TARRO_1/2*YUA
DUR_116	7406748	KALKA/10/MINIMUS/COMB DUCK_2//CHAM_3/3/CANELO_9/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/11/YALLAROI/6/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/3/POD_9/4/RASCON_37/TARRO_2//RASCON_37/5/ARMENT//SRN_3/NIGRIS_4/3/C
DUR_117	7406808	OROBEL//BUSHEN_4/2*GREEN_18/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/6/SOMBRA_20/7/JUPARE C 2001/11/CLAUDIO/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/10/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/9/USDA595/3/D67.3/RABI
DUR_118	7406881	P91.272.3.1/3*MEXI75//2*JUPARE C 2001/5/ARTICO/AJAIA_3//HUALITA/3/FULVOUS_1/MFOWL_13/4/TECA96/TILO_1/6/RISSA/GAN//POHO_1/3/PLATA_3//CREX/ALLA*2/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/11/CLAUDIO/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/10/TARRO_1/2*
DUR_119	7406899	P91.272.3.1/3*MEXI75//2*JUPARE C 2001/5/ARTICO/AJAIA_3//HUALITA/3/FULVOUS_1/MFOWL_13/4/TECA96/TILO_1/6/RISSA/GAN//POHO_1/3/PLATA_3//CREX/ALLA*2/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/11/E90040/MFOWL_13//LOTAIL_6/3/PROZANA/ARLIN//MUSK_6/9/USDA595/3/D67
DUR_120	7406952	MINIMUS/COMB DUCK_2//CHAM_3/3/CANELO_9/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/3/SOMAT_4/INTER_8/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/11/CLAUDIO/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/G
DUR_121	7407025	LILE/10/KOFA/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/11/PORTO_3//SORA/2*PLATA_12/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9
DUR_122	7407050	PLANETA/PIQUERO//BERGAND/KNIPA/6/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/2*NETTA_4/DUKEM_12//RASCON_19/3/SORA/2*PLATA_12/4/GREEN_18/FOCHA_1//AIRON_1/12/ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/10/CMH79.959/CHEN//SOOTY_9/RASCON_37
DUR_123	7407092	MOHAWK/3/GUANAY//TILO_1/LOTUS_4/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/6/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/2*NETTA_4/DUKEM_12//RASCON_19/3/SORA/2*PLATA_12/4/GREEN_18/FOCHA_1//AIRON_1/9/OROBEL//BUSHEN_4/2*GREEN_18/8/GEDIZ/FGO//GTA/3/SRN_1/4/T
DUR_124	7407103	PLATA_7/ILBOR_1//SOMAT_3/7/CHEN_11/POC//TANTLO/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/6/MINIMUS/COMB DUCK_2//CHAM_3/10/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/9/CHEN_11/POC//TANTLO/5/ENTE/MEXI_2//HUI/4/YAV_
DUR_125	7407117	CBC 509 CHILE/SOMAT_3.1//BOOMER_18/LOTUS_4/12/MOHAWK/10/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT/11/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/13/P91.272.3.1/3*MEXI75//2*JUPAR

DUR_126	7407130	CIT71/DIPPER_1//ARIZA_2/3/PROZANA/ARLIN//MUSK_6/4/TATLER_1/TARRO_1//HYDRANASSA30/SILVER_5/10/PLATA_3//CREX/ALLA/3/SORA/2*PLATA_12/4/RASCON_37/GREEN_2/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/11/ALTAR 84/STINT//SILVE
DUR_127	7407174	ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/10/CMH79.959/CHEN//SOOTY_9/RASCON_37/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/11/RCOL//SHAG_23/LAPDY_25/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/12/KOFA/9/USDA5
DUR_128	7407242	AINZEN_1/6/CMH82A.1062/3/GERARDO VZ 394//SBA81/PLC/4/AAZ_1/CREX/5/HUI//CIT71/CII/10/SELIM/9/ALTAR 84/860137//YAZI_1/4/LIS_8/FILLO_6/3/FUUT//HORA/JOR/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/6/SOMBRA_20/7/JUPAR
DUR_129	7407490	WBDTBO/7/AINZEN_1/6/CMH82A.1062/3/GERARDO VZ 394//SBA81/PLC/4/AAZ_1/CREX/5/HUI//CIT71/CII/8/STOT//ALTAR 84/ALD/3/PATKA_7/YAZI_1/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/5/NASR 99
DUR_130	7407511	CANNIZZO/6/SOMAT_3.1//WODUCK/CHAM_3/5/AJAIA_16//HORA/JRO/3/GAN/4/ZAR/7/STOT//ALTAR 84/ALD/3/PATKA_7/YAZI_1/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/5/NASR 99
DUR_131	7407561	WBDTBO/11/MAALI/10/ALTAR 84/CMH82A.1062//ALTAR 84/3/YAZI_10/4/SNITAN/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/12/SELIM/9/ALTAR 84/860137//YAZI_1/4/LIS_8/FILLO_6/3/FUUT//HORA/JOR/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/EN
DUR_132	7407575	ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/5/GUAYACAN INIA/YEBAS_8/3/TOPDY_18/FOCHA_1//ALTAR 84/9/CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/SOOTY_9/RASCON_37//WO
DUR_133	7407611	ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/5/GUAYACAN INIA/YEBAS_8/3/TOPDY_18/FOCHA_1//ALTAR 84/7/WID22256/5/ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/6/SN TURK MI83-84 503/LOTUS_4//MUSK_4/3/CANELO_9/4/YAZI_1/AKAKI_4//S
DUR_134	7407689	ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/5/GUAYACAN INIA/YEBAS_8/3/TOPDY_18/FOCHA_1//ALTAR 84/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/3/SOMBRA_20/4/SNITAN/5/SOMAT_4/INTER_8/6/GUAYACAN INIA/POMA_2//SNITAN
DUR_135	7407710	ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/5/GUAYACAN INIA/YEBAS_8/3/TOPDY_18/FOCHA_1//ALTAR 84/6/CBC 514 CHILE/3/AUK/GUIL//GREEN
DUR_136	7407713	CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/SOOTY_9/RASCON_37//WODUCK/CHAM_3/9/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/3/SOMBRA_20/4/SNITAN/5/SOMAT_4/INTER_8/6/GUAYACAN INIA
DUR_137	7407740	WID22256/5/ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/6/SN TURK MI83-84 503/LOTUS_4//MUSK_4/3/CANELO_9/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/3/SOMBRA_20/4/SNITAN/5/SOMAT_4/INTER_8/6/
DUR_138	7407855	CALERO/4/SOOTY_9/RASCON_37//JUPARE C 2001/3/SOOTY_9/RASCON_37//GUAYACAN INIA
DUR_139	7407885	GRECALE/9/CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/SOOTY_9/RASCON_37//WODUCK/CHAM_3
DUR_140	7407937	CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/SOOTY_9/RASCON_37//WODUCK/CHAM_3/12/SOOTY_9/RASCON_37//GUAYACAN INIA/11/BOOMER_33/ZAR/3/BRAK_2/AJAIA_2//SOLGA_8/10/PLATA_10/6/MQUE/4/U
DUR_141	7407946	CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/SOOTY_9/RASCON_37//WODUCK/CHAM_3/9/SOOTY_9/RASCON_37//JUPARE C 2001/3/SOOTY_9/RASCON_37//GUAYACAN INIA
DUR_142	7407978	CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/SOOTY_9/RASCON_37//WODUCK/CHAM_3/9/SOOTY_9/RASCON_37//GUAYACAN INIA/3/SOOTY_9/RASCON_37//STORLOM
DUR_143	7408065	WID22256/5/ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/6/SN TURK MI83-84 503/LOTUS_4//MUSK_4/3/CANELO_9/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/12/SOOTY_9/RASCON_37//GUAYACAN INIA/11/BOOMER_33/ZAR/3/BRAK_2/AJAIA_2//SOLGA_8/10/PLATA_
DUR_144	7408093	WID22256/5/ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/6/SN TURK MI83-84 503/LOTUS_4//MUSK_4/3/CANELO_9/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/7/SOOTY_9/RASCON_37//GUAYACAN INIA/3/SOOTY_9/RASCON_37//STORLOM
DUR_145	7408214	CIRNO C 2008/4/SOOTY_9/RASCON_37//JUPARE C 2001/3/SOOTY_9/RASCON_37//CAMAYO
DUR_146	7408527	WID22256/5/ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/6/SN TURK MI83-84 503/LOTUS_4//MUSK_4/3/CANELO_9/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/7/HUBEI//SOOTY_9/RASCON_37/3/2*SOOTY_9/RASCON_37/4/2*SOOTY_9/RASCON_37/8/ALTAR 84/BINTEP
DUR_147	7408588	CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/SOOTY_9/RASCON_37//WODUCK/CHAM_3/9/CBC 514 CHILE/3/AUK/GUIL//GREEN/10/ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/5/GUAY
DUR_148	7408625	WID22256/5/ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/6/SN TURK MI83-84 503/LOTUS_4//MUSK_4/3/CANELO_9/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/7/ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/5/SOMAT_4/INTER_8/8/SOOTY_9/
DUR_149	7408683	HUBEI//SOOTY_9/RASCON_37/3/2*SOOTY_9/RASCON_37/4/2*SOOTY_9/RASCON_37/6/SOMAT_3/PHAX_1//TILO_1/LOTUS_4/3/GUANAY/5/NETTA_4/DUKEM_12//RASCON_19/3/SORA/2*PLATA_12/4/GREEN_18/FOCHA_1//AIRON_1/7/ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/5/G
DUR_150	7408721	HUBEI//SOOTY_9/RASCON_37/3/2*SOOTY_9/RASCON_37/4/2*SOOTY_9/RASCON_37/5/GUAYACAN INIA/2*SNITAN/10/SELIM/9/ALTAR 84/860137//YAZI_1/4/LIS_8/FILLO_6/3/FUUT//HORA/JOR/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/6/SOMB
DUR_151	7408749	CIRNO C 2008/5/HUBEI//SOOTY_9/RASCON_37/3/2*SOOTY_9/RASCON_37/4/2*SOOTY_9/RASCON_37/7/ALTAR 84/BINTEPE 85/3/STOT//ALTAR 84/ALD/4/POD_11/YAZI_1/5/VANRRIKSE_12/SNITAN/6/SOOTY_9/RASCON_37//WODUCK/CHAM_3
DUR_152	7408787	CBC 514 CHILE/3/AUK/GUIL//GREEN/6/STORLOM/3/RASCON_37/TARRO_2//RASCON_37/4/KIRKI_1/HIMAN_9/5/GLAS_5/LOTUS_4//SOMBRA_20/7/SOOTY_9/RASCON_37//JUPARE C 2001/3/SOOTY_9/RASCON_37//GUAYACAN INIA

DUR_153	7408818	ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/5/SOMAT_4/INTER_8/6/STORLOM/3/RASCON_37/TARRO_2//RASCON_37/4/KIRKI_1/HIMAN_9/5/GLAS_5/LOTUS_4//SOMBRA_20/7/ALTAR 84/BINTEPE 85/3/STOT//ALTAR 84/ALD/4/POD_11/YAZI_1/5/VANRRIKSE_12/SNITAN/6/SOOT
DUR_154	7408843	ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/5/GUAYACAN INIA/YEBAS_8/3/TOPDY_18/FOCHA_1//ALTAR 84/6/SOMAT_3/GREEN_22//2*RASCON_37/2*TARRO_2
DUR_155	7408856	CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/SOOTY_9/RASCON_37//WODUCK/CHAM_3/9/SOMAT_3/GREEN_22//2*RASCON_37/2*TARRO_2
DUR_156	7408885	SILVER_14/MOEWE//BISU_1/PATKA_3/3/PORRON_4/YUAN_1/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/4/ARMENT//SRN_3/NIGRIS_4/3/ CANELO_9.1/11/CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4
DUR_157	7408925	SILVER_14/MOEWE//BISU_1/PATKA_3/3/PORRON_4/YUAN_1/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/4/ARMENT//SRN_3/NIGRIS_4/3/ CANELO_9.1/11/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/3/S
DUR_158	7408967	SILVER_14/MOEWE//BISU_1/PATKA_3/3/PORRON_4/YUAN_1/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/4/ARMENT//SRN_3/NIGRIS_4/3/ CANELO_9.1/11/ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//Y
DUR_159	7408983	SILVER_14/MOEWE//BISU_1/PATKA_3/3/PORRON_4/YUAN_1/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/4/ARMENT//SRN_3/NIGRIS_4/3/ CANELO_9.1/11/CBC 514 CHILE/3/AUK/GUIL//GREEN
DUR_160	7409002	SILVER_14/MOEWE//BISU_1/PATKA_3/3/PORRON_4/YUAN_1/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/4/ARMENT//SRN_3/NIGRIS_4/3/ CANELO_9.1/11/GUAYACAN INIA/2*SNITAN
DUR_161	7409071	E90040/MFOWL_13//LOTAIL_6/3/PROZANA/ARLIN//MUSK_6/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/SORA/2*PLATA_12//RASCON_37/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/ GUIL//GREEN/11/WID22256/5/ALTAR 84/STINT//SILVER_45/3/GUANAY/4
DUR_162	7409080	E90040/MFOWL_13//LOTAIL_6/3/PROZANA/ARLIN//MUSK_6/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/SORA/2*PLATA_12//RASCON_37/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/ GUIL//GREEN/11/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/3/
DUR_163	7409164	PLATA_6/GREEN_17/3/CHEN/AUK//BISU*2/5/PLATA_3//CREX/ALLA/3/SOMBRA_20/4/SILVER_14/MOEWE/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/3/SOMBRA_20/4/SNITAN/5/SOMAT_4/INTER_8/6/GUAYACAN INIA/POMA_2//SNITAN
DUR_164	7409181	1A.1D 5+1-06/3*WB881/6/CHEN_1/TEZ/3/GUIL//CIT71/CII/4/SORA/PLATA_12/5/STOT//ALTAR 84/ALD/7/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1/9/CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/
DUR_165	7409188	1A.1D 5+1-06/3*WB881/6/CHEN_1/TEZ/3/GUIL//CIT71/CII/4/SORA/PLATA_12/5/STOT//ALTAR 84/ALD/7/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1/8/WID22256/5/ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/6/SN TURK MI83-84 503/LOTUS_4//MUSK_4/3/CANELO_
DUR_166	7409275	AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/3/SOMBRA_20/4/SNITAN/5/SOMAT_4/INTER_8/6/GUAYACAN INIA/POMA_2//SNITAN/7/ODIN_15/WITNEK_1//ISLOM_1/5/TARRO_1/TISOMA_2//TARRO_1/3/COMB DUCK_2/ALAS//4*COMB DUCK_2/4/SHAG_9/BUTO_17/6/VANRRIKSE_6.2//1A-1D 2+12-
DUR_167	7409307	CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/SOOTY_9/RASCON_37//WODUCK/CHAM_3/10/CHEN_1/TEZ/3/GUIL//CIT71/CII/4/SORA/PLATA_12/5/STOT//ALTAR 84/ALD/9/USDA595/3/D67.3/RABI//CRA/4/AL
DUR_168	7409314	CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/SOOTY_9/RASCON_37//WODUCK/CHAM_3/9/TOPDY_18/FOCHA_1//ALTAR 84/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/4/SOMAT_3/GREEN_22/5/VRKS
DUR_169	7409323	CIRNO C 2008/5/SILK_3/DIPPER_6/3/ACO89/DUKEM_4//5*ACO89/4/PLATA_7/ILBOR_1//SOMAT_3/6/GUAYACAN INIA/POMA_2//SNITAN/4/D86135/ACO89//PORRON_4/3/SNITAN
DUR_170	7409351	CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/SOOTY_9/RASCON_37//WODUCK/CHAM_3/9/CMH85.797//CADO/BOOMER_33/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/10/ADAMAR_15//AL BIA_1/ALTAR 84/3/SN
DUR_171	7409379	HUBEI//SOOTY_9/RASCON_37/3/2*SOOTY_9/RASCON_37/4/CRAKE_10/RISSA/11/TATLER_1/TARRO_1/3/ALTAR 84/BISU_1//PLATA_2/10/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT/12/TOPDY_18/F
DUR_172	7409395	GUAYACAN INIA/GUANAY//PORRON_4/BEJAH_7/6/TOPDY_18/FOCHA_1//ALTAR 84/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/4/SOMAT_3/GREEN_22/5/VRKS_3/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/7/SOMAT_3/GREEN_22//2*RASCON_37/2*TARRO_2
DUR_173	7409435	GUAYACAN INIA/GUANAY//PORRON_4/BEJAH_7/3/VANRRIKSE_12/SNITAN/10/CHEN_1/TEZ/3/GUIL//CIT71/CII/4/SORA/PLATA_12/5/STOT//ALTAR 84/ALD/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/11/ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREE
DUR_174	7409445	GUAYACAN INIA/GUANAY//PORRON_4/BEJAH_7/3/VANRRIKSE_12/SNITAN/5/CMH85.797//CADO/BOOMER_33/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/6/HUBEI//SOOTY_9/RASCON_37/3/2*SOOTY_9/RASCON_37/4/2*SOOTY_9/RASC ON_37
DUR_175	7409461	SOMAT_3/PHAX_1//TILO_1/LOTUS_4/3/GUANAY/5/NETTA_4/DUKEM_12//RASCON_19/3/SORA/2*PLATA_12/4/GREEN_18/FOCHA_1//AIRON_1/6/TOPDY_18/FOCHA_1//ALTAR 84/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/4/SOMAT_3/GREEN_22/5/VRKS_3/3/AJAIA_12/F3LOCAL(SEL.ETHIO.1
DUR_176	7409493	ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/5/SOMAT_4/INTER_8/6/CMH85.797//CADO/BOOMER_33/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/7/GUAYACAN INIA/POMA_2//SNITAN/4/D86135/ACO89//PORRON_4/3/SNITAN
DUR_177	7409506	ADAMAR_15//ALBIA_1/ALTAR 84/3/SNITAN/4/SOMAT_4/INTER_8/5/SOOTY_9/RASCON_37/6/BICHENA/AKAKI_7/4/LIS_8/FILLO_6/3/FUUT//HORA/JOR/5/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/7/SILK_3/DIPPER_6/3/ACO89/DUKEM_4//5*ACO89/4 /PLATA_7/ILBOR_1//SOMAT_3/9/CBC 509
DUR_178	7409573	CALERO/6/BCRIS/BICUM//LLARETA INIA/3/DUKEM_12/2*RASCON_21/5/SILK_3/DIPPER_6/3/ACO89/DUKEM_4//5*ACO89/4/PLATA_7/ILBOR_1//SOMAT_3

DUR_179	7409575	CALERO/12/1A.1D 5+1-06/3*MOJO//RCOL/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/11/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/RAFI97/9/MALMUK_1/SERRATOR_1/10/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1
DUR_180	7409752	SOOTY_9/RASCON_37//GUAYACAN INIA/11/BOOMER_33/ZAR/3/BRAK_2/AJAIA_2//SOLGA_8/10/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT/12/WID22209/6/ADAMAR_15//ALBIA_1/ALTAR 84/3/SNIT
DUR_181	7409764	SOOTY_9/RASCON_37//GUAYACAN INIA/11/BOOMER_33/ZAR/3/BRAK_2/AJAIA_2//SOLGA_8/10/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT/12/P91.272.3.1/3*MEXI75//2*JUPARE C 2001/5/ARTIC
DUR_182	7409772	SOOTY_9/RASCON_37//GUAYACAN INIA/11/BOOMER_33/ZAR/3/BRAK_2/AJAIA_2//SOLGA_8/10/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT/12/SOOTY_9/RASCON_37//SOMAT_3.1/3/SOOTY_9/RASCON
DUR_183	7409774	SOOTY_9/RASCON_37//GUAYACAN INIA/11/BOOMER_33/ZAR/3/BRAK_2/AJAIA_2//SOLGA_8/10/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT/12/MÂALI/6/MUSK_1//ACO89/FNFOOT_2/4/MUSK_4/3/PLA
DUR_184	7409895	SOOTY_9/RASCON_37//JUPARE C 2001/3/SOOTY_9/RASCON_37//CAMAYO/4/SOOTY_9/RASCON_37//SOMAT_3.1/3/SOOTY_9/RASCON_37//STORLOM/10/SOMAT_4/INTER_8/5/AJAIA_16//HORA/JRO/3/GAN/4/ZAR/9/BOOMER_18/LOTUS_4/3/MINIMUS_6/PLATA_16//IMMER/8/GEDIZ/FGO//GTA/3/SRN_1/4/TO
DUR_185	7409905	SOOTY_9/RASCON_37//JUPARE C 2001/3/SOOTY_9/RASCON_37//CAMAYO/4/SOOTY_9/RASCON_37//SOMAT_3.1/3/SOOTY_9/RASCON_37//STORLOM/11/MÂALI/6/MUSK_1//ACO89/FNFOOT_2/4/MUSK_4/3/PLATA_3//CREX/ALLA/5/OLUS*2/ILBOR//PATKA_7/YAZI_1/10/SELIM/9/ALTAR 84/860137//YAZI_1
DUR_186	7409975	SARAGOLLA/12/SOOTY_9/RASCON_37/3/SOOTY_9/TARRO_1//AJAIA_2/10/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT/11/RISSA/GAN//POHO_1/3/PLATA_3//CREX/ALLA/4/JUPARE C 2001/5/ARMENT
DUR_187	7410092	CIRNO C 2008/5/CMH85.797//CADO/BOOMER_33/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/6/AJAIA_3/SILVER_16//AJAIA_13/YAZI/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/5/GODRIN/GUTROS//DUKEM/3/THKNEE_11
DUR_188	7410116	BCRIS/BICUM//LLARETA INIA/3/DUKEM_12/2*RASCON_21/6/PLATA_6/GREEN_17/3/CHEN/AUK//BISU*2/5/PLATA_3//CREX/ALLA/3/SOMBRA_20/4/SILVER_14/MOEWE/7/WID22256/5/ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/6/SN TURK MI83-84 503/LOTUS_4//MUSK_4/3/C
DUR_189	7410208	HUBEI//SOOTY_9/RASCON_37/3/2*SOOTY_9/RASCON_37/4/2*SOOTY_9/RASCON_37/10/CHEN_1/TEZ/3/GUIL//CIT71/CII/4/SORA/PLATA_12/5/STOT//ALTAR 84/ALD/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/12/SOOTY_9/RASCON_37//GUAYACAN INIA/
DUR_190	7410242	SOMAT_4/INTER_8/4/GODRIN/GUTROS//DUKEM/3/THKNEE_11/5/1A.1D 5+1-06/2*WB881//1A.1D 5+1-06/3*MOJO/3/BISU_1/PATKA_3/4/GODRIN/GUTROS//DUKEM/3/THKNEE_11/6/TOPDY_18/FOCHA_1//ALTAR 84/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/4/SOMAT_3/GREEN_22/5/VRKS_3
DUR_191	7410277	T.DIC 1460/MEXI75//MEXI75/T.MONOC.2433/3/CEMEXI C 2008/4/SOOTY_9/RASCON_37
DUR_192	7410332	SILVER_14/MOEWE//BISU_1/PATKA_3/3/PORRON_4/YUAN_1/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/11/CF4-JS 21//RASCON_37/2*TARRO_2/10/AAZ//ALTAR 84/
DUR_193	7410350	SILVER_14/MOEWE//BISU_1/PATKA_3/3/PORRON_4/YUAN_1/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/11/SOMAT_4/SILVER_1/4/STORLOM/3/RASCON_37/TARRO_2//
DUR_194	7410359	SILVER_14/MOEWE//BISU_1/PATKA_3/3/PORRON_4/YUAN_1/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/11/SOOTY_9/RASCON_37//GUAYACAN INIA/5/BRAK_2/AJAIA_
DUR_195	7410402	SILVER_14/MOEWE//BISU_1/PATKA_3/3/PORRON_4/YUAN_1/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/11/SOOTY_9/RASCON_37//JUPARE C 2001/3/SOOTY_9/RASCO
DUR_196	7410404	SILVER_14/MOEWE//BISU_1/PATKA_3/3/PORRON_4/YUAN_1/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/11/BICHENA/AKAKI_7/4/LIS_8/FILLO_6/3/FUUT//HORA/JOR
DUR_197	7410418	SILVER_14/MOEWE//BISU_1/PATKA_3/3/PORRON_4/YUAN_1/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/11/CF4-JS 21//RASCON_37/2*TARRO_2/10/AAZ//ALTAR 84/
DUR_198	7410419	BICHENA/AKAKI_7/4/LIS_8/FILLO_6/3/FUUT//HORA/JOR/5/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/6/RASCON_33/TISOMA_2/3/CANELO_8//SORA/2*PLATA_12/4/SOMAT_4/INTER_8/7/ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_10/AUK/5/GUAYACAN INIA/YEBAS_8/3/TOPD
DUR_199	7410448	SILVER_14/MOEWE//BISU_1/PATKA_3/3/PORRON_4/YUAN_1/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/11/SOOTY_9/RASCON_37//JUPARE C 2001/3/SOOTY_9/RASCO
DUR_200	7410487	AMMAR-1/6/CNDO/PRIMADUR//HAI-OU_17/3/SNITAN/4/PLATA_7/ILBOR_1//SOMAT_3/5/HESSIAN-F_2/3/STOT//ALTAR 84/ALD/7/EUPODA_3/SLA_2//MINIMUS/3/PLATA_7/ILBOR_1//SOMAT_3
DUR_201	7410498	SHAG_21/DIPPER_2//PATA_2/6/ARAM_7//CREX/ALLA/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/7/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/8/SORA/2*PLATA_12/3/SORA/2*PLATA_12//SOMAT_3/4/AJAIA_13/YAZI//DIPPER_2/BUSHEN_3/9/TARRO_1/2*YUAN_1//AJAIA_13/YAZI/3/SO
DUR_202	7410510	SHAG_21/DIPPER_2//PATA_2/6/ARAM_7//CREX/ALLA/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/7/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/8/SOMAT_3/3/STOT//ALTAR 84/ALD/4/FOCHA_1/MUSK_3/6/RANCO//CIT71/CII/3/COMDK/4/TCHO//SHWA/MALD/3/CREX/5/SNITAN/9/PLATA_6
DUR_203	7410526	SORA/2*PLATA_12/3/SORA/2*PLATA_12//SOMAT_3/4/AJAIA_13/YAZI//DIPPER_2/BUSHEN_3/5/NOK_23//PLATA_6/GREEN_17/3/SORA/2*PLATA_12//SRN_3/NIGRIS_4/8/SHAG_21/DIPPER_2//PATA_2/6/ARAM_7//CREX/ALLA/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/7/ARMENT//SRN_3
DUR_204	7410549	SNITAN/5/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/3/SOMAT_3/4/SOOTY_9/RASCON_37/6/SNITAN/9/GUAYACAN INIA/GUANAY/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/6/SOMBRA_20/7/JUPARE C 2001/10/NOK_23//PLATA_6/GREEN

DUR_205	7410557	SNITAN/5/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/3/SOMAT_3/4/SOOTY_9/RASCON_37/6/SNITAN/10/SWAHEN_2/KIRKI_8//PROZANA_1/4/ADAMAR_15//ALBIA_1/ALTAR 84/3/SNITAN/9/GUAYACAN INIA/GUANAY/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD
DUR_206	7410559	CNDO/PRIMADUR//HAI-OU_17/3/SNITAN/4/PLATA_7/ILBOR_1//SOMAT_3/5/HESSIAN-F_2/3/STOT//ALTAR 84/ALD/7/SOMAT_3/3/STOT//ALTAR 84/ALD/4/FOCHA_1/MUSK_3/6/RANCO//CIT71/CII/3/COMDK/4/TCHO//SHWA/MALD/3/CREX/5/SNITAN/10/SWAHEN_2/KIRKI_8//PROZANA_1/4/ADAMAR_15//A
DUR_207	7410621	GEROMTEL-1/10/SWAHEN_2/KIRKI_8//PROZANA_1/4/ADAMAR_15//ALBIA_1/ALTAR 84/3/SNITAN/9/GUAYACAN INIA/GUANAY/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/6/SOMBRA_20/7/JUPARE C 2001/11/GUAYACAN INIA/GUANAY/8/GEDIZ/FGO/
DUR_208	7410632	SHAG_21/DIPPER_2//PATA_2/6/ARAM_7//CREX/ALLA/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/7/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/8/MOHAWK/6/LOTUS_5/F3LOCAL(SEL.ETHIO.135.85)/5/CHEN/ALTA R 84/3/HUI/POC//BUB/RUFO/4/FNFOOT/9/NOK_23//PLATA_6/GREEN_17/3
DUR_209	7410646	SHAG_21/DIPPER_2//PATA_2/6/ARAM_7//CREX/ALLA/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/7/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1/9/GUAYACAN INIA/GUANAY/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/6/SOMBRA_20/7
DUR_210	7410659	MOHAWK/6/LOTUS_5/F3LOCAL(SEL.ETHIO.135.85)/5/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT/7/SNITAN/5/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/3/SOMAT_3/4/SOOTY_9/RASCON_37/6/SNITAN/10/SWAHEN_2/KIRKI_8//PROZANA_1/4/ADAMAR_15//ALBIA_1/ALTAR 84/3/SNIT
DUR_211	7410727	DWL5023/9/GUAYACAN INIA/GUANAY/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/6/SOMBRA_20/7/JUPARE C 2001/10/MOHAWK/6/LOTUS_5/F3LOCAL(SEL.ETHIO.135.85)/5/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT
DUR_212	7410795	DWL5023/10/SWAHEN_2/KIRKI_8//PROZANA_1/4/ADAMAR_15//ALBIA_1/ALTAR 84/3/SNITAN/9/GUAYACAN INIA/GUANAY/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/6/SOMBRA_20/7/JUPARE C 2001/11/SOMAT_3/3/STOT//ALTAR 84/ALD/4/FOCHA
DUR_213	7410802	WID22289/6/CNDO/PRIMADUR//HAI-OU_17/3/SNITAN/4/PLATA_7/ILBOR_1//SOMAT_3/5/HESSIAN-F_2/3/STOT//ALTAR 84/ALD/7/SORA/2*PLATA_12/3/SORA/2*PLATA_12//SOMAT_3/4/AJAIA_13/YAZI//DIPPER_2/BUSHEN_3
DUR_214	7410825	A 624/7/SOMAT_3/3/STOT//ALTAR 84/ALD/4/FOCHA_1/MUSK_3/6/RANCO//CIT71/CII/3/COMDK/4/TCHO//SHWA/MALD/3/CREX/5/SNITAN/8/MUSK_1//ACO89/FNFOOT_2/4/MUSK_4/3/PLATA_3//CREX/ALLA/5/OLUS*2/ILBOR//PATKA_7/YAZI_1
DUR_215	7410894	MOHAWK/6/LOTUS_5/F3LOCAL(SEL.ETHIO.135.85)/5/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT/7/ODIN_15/WITNEK_1//ISLOM_1/5/TARRO_1/TISOMA_2//TARRO_1/3/COMB DUCK_2/ALAS//4*COMB DUCK_2/4/SHAG_9/BUTO_17/6/VANRRIKSE_6.2//1A-1D 2+12-5/3*WB881/5/TARRO_1/TISOMA_
DUR_216	7606753	CALERO//SOOTY_9/RASCON_37
DUR_217	7606773	CARPIO//SOOTY_9/RASCON_37
DUR_218	7606790	SWABAA ELGIA,ITGC//SOOTY_9/RASCON_37
DUR_219	7606802	INRAT 69,ITGC//SOOTY_9/RASCON_37
DUR_220	7606807	PI 61111-GRIN//SOOTY_9/RASCON_37
DUR_221	7606811	T.DICOCCON, PI 94747//SOOTY_9/RASCON_37
DUR_222	7606821	T.DICOCCON PI 94749-GRIN//SOOTY_9/RASCON_37
DUR_223	7606825	T.CARTHLICUM PI 572849-GRIN//SOOTY_9/RASCON_37
DUR_224	7147198	CNDO/PRIMADUR//HAI-OU_17/3/SNITAN/4/JUPARE C 2001/5/CNDO/PRIMADUR//HAI-OU_17/3/SNITAN*2/6/PLANETA/PIQUERO//BERGAND/KNIPA
DUR_225	7147237	CHEN_1/TEZ/3/GUIL//CIT71/CII/4/SORA/PLATA_12/5/STOT//ALTAR 84/ALD/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/BAIRDS/11/PLANETA/PIQUERO//BERGAND/KNIPA
DUR_226	7147250	GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/6/SOMBRA_20/7/JUPARE C 2001/8/CS/TH.CU//GLEN/3/GEN/4/MYNA/VUL/5/2*DON87/6/2*BUSCA_3/9/BAIRDS/10/PLAYERO/AMIC//PIQUERO/KNIPA
DUR_227	7147285	HELLER/ORLU
DUR_228	7384182	PLAYERO/AMIC//PIQUERO/KNIPA/3/SOOTY_9/RASCON_37
DUR_229	7384191	HELLER #1/SVEVO
DUR_230	7384198	HELLER/CLAUDIO
DUR_231	7384199	PLAYERO/AMIC//PIQUERO/KNIPA/3/SVEVO
DUR_232	7384200	PLANETA/PIQUERO//BERGAND/KNIPA/3/CLAUDIO
DUR_233	7384201	PLANETA/PIQUERO//BERGAND/KNIPA/3/SVEVO
DUR_234	7384203	PLANETA/PIQUERO//BERGAND/KNIPA/3/CLAUDIO
DUR_235	7384209	PLANETA/AMIC//BERGAND/TRILE/3/CLAUDIO
DUR_236	7384213	BAIRDS/CLAUDIO
DUR_237	7384216	BAIRDS/CLAUDIO
DUR_238	7384219	BAIRDS/SVEVO
DUR_239	7384222	HELLER #1/2*CEMEXI C 2008
DUR_240	7384228	HELLER #1//2*SOOTY_9/RASCON_37
DUR_241	7384233	HELLER//2*SOOTY_9/RASCON_37
DUR_242	7384234	PLANETA/AMIC//BERGAND/TRILE/3/2*SOOTY_9/RASCON_37
DUR_243	7384237	BAIRDS//2*SOOTY_9/RASCON_37
DUR_244	7384241	BAIRDS/2*CEMEXI C 2008
DUR_245	7384248	PLAYERO/AMIC//PIQUERO/KNIPA/3/DAKTER/4/SOOTY_9/RASCON_37

DUR_246	7384250	PLANETA/AMIC//BERGAND/TRILE/3/DAKTER/4/CEMEXI C 2008
DUR_247	7384262	BAIRDS/DAKTER//SOOTY_9/RASCON_37
DUR_248	7606826	HELLER #1//SOOTY_9/RASCON_37
DUR_249	7606909	PLAYERO/AMIC//PIQUERO/KNIPA/3/SOOTY_9/RASCON_37
DUR_250	7606971	PLANETA/AMIC//BERGAND/TRILE/3/SOOTY_9/RASCON_37
DUR_251	7607064	BAIRDS//SOOTY_9/RASCON_37
DUR_252	7405994	PLANETA/AMIC//BERGAND/TRILE/3/SVEVO/4/PLANETA/PIQUERO//BERGAND/KNIPA
DUR_253	7406012	PLANETA/PIQUERO//BERGAND/KNIPA/3/SVEVO/4/PLANETA/AMIC//BERGAND/TRILE
DUR_254	7406016	BAIRDS/SVEVO//HELLER
DUR_255	7406021	BAIRDS/SVEVO/3/PLANETA/PIQUERO//BERGAND/KNIPA
DUR_256	7406050	VIVADUR//ATIL/HELLER #1
DUR_257	7406069	PI 352426//ATIL/HELLER #1
DUR_258	7406193	ASA DE CORVO//PLATINUM/HELLER #1
DUR_259	7406218	VIVADUR//PLATINUM/BAIRDS
DUR_260	6469777	AG 1-22/2*ACO89//2*UC1113/3/SOOTY_9/RASCON_37
DUR_261	6420695	BHA/3/SORA/2*PLATA_12//SRN_3/NIGRIS_4/4/AG 1-22/2*ACO89//2*UC1113
DUR_262	6420696	BHA/3/SORA/2*PLATA_12//SRN_3/NIGRIS_4/4/AG 1-22/2*ACO89//2*UC1113
DUR_263	6420697	BHA/3/SORA/2*PLATA_12//SRN_3/NIGRIS_4/4/AG 1-22/2*ACO89//2*UC1113
DUR_264	6420699	BHA/3/SORA/2*PLATA_12//SRN_3/NIGRIS_4/4/AG 1-22/2*ACO89//2*UC1113
DUR_265	6420704	BHA/3/SORA/2*PLATA_12//SRN_3/NIGRIS_4/4/AG 1-22/2*ACO89//2*UC1113
DUR_266	6951158	AG 1-22/2*ACO89//2*UC1113/3/5*SOOTY_9/RASCON_37/5/SILK_3/DIPPER_6/3/ACO89/DUKEM_4//5*ACO89/4/PLATA_7/ILBOR_1//SOMAT_3
DUR_267	6951159	AG 1-22/2*ACO89//2*UC1113/3/5*SOOTY_9/RASCON_37/5/ADAMAR_15//ALBIA_1/ALTAR 84/3/SNITAN/4/SOMAT_4/INTER_8
DUR_268	6951168	AG 1-22/2*ACO89//2*UC1113/3/5*RCOL/5/C94.52/3/2*AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/4/2*RASCON_37/2*TARRO_2
DUR_269	6701302	AG 1-22/2*ACO89//2*UC1113/8/AVTA/ALTAR 84/5/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT/6/SORA/2*PLATA_12//SOMAT_3/7/SOOTY_9/RASCON_37/11/7A.7S-S3/3*ACO89/10/TADIZ/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9
DUR_270	5928162	TRIDENT/3*KUCUK
DUR_271	5928165	TRIDENT//CADO/BOOMER_33/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/11/GODRIN/GUTROS//DUKEM/3/THKNEE_11/12/GLAS_5/LOTUS_4//SOMBRA_20
DUR_272	6951185	TRIDENT/3*KUCUK/13/TRIDENT//CADO/BOOMER_33/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/11/GODRIN/GUTROS//DUKEM/3/THKNEE_11/12/GLAS_5/LOTUS_4/
DUR_273	6951195	SLVS/GUANAY/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/TRIDENT/3*KUCUK/14/TRIDENT//CADO/BOOMER_33/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/PO
DUR_274	6951188	SLVS/5/AJAIA_16//HORA/JRO/3/GAN/4/ZAR/6/SOMAT_3/PHAX_1//TILO_1/LOTUS_4/11/CANELO_9.1/SNITAN/10/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/HUI/POC//BUB/RUFO/4/FNFOOT/12/TRIDENT/3*KUCUK/6/YAV79/4/ARM
DUR_275	6951189	SLVS/GUANAY/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/13/TRIDENT//CADO/BOOMER_33/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/
DUR_276	6951191	SLVS/GUANAY/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/13/TRIDENT//CADO/BOOMER_33/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/
DUR_277	6951193	SLVS/GUANAY/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/13/TRIDENT//CADO/BOOMER_33/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/6/ARDENTE/7/HUI/YAV79/8/POD_9/10/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV_1/
DUR_278	7147176	SW SR227.B (SR 22)/6/2*RASCON_22/RASCON_21//MOJO_2/3/GUANAY/4/RCOL/5/SORA/2*PLATA_12//SOMAT_3/7/C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2/8/AG 1-22/2*ACO89//2*UC1113/3/5*KOFA/5/KOFA/4/DUKEM_1//PATKA_7/YAZI
DUR_279	7147177	SW SR227.B (SR 22)/6/2*RASCON_22/RASCON_21//MOJO_2/3/GUANAY/4/RCOL/5/SORA/2*PLATA_12//SOMAT_3/7/C F4 20 S/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/GUIL//GREEN/5/CANELO_9.1//SHAKE_3/2*AJAIA_2/8/AG 1-22/2*ACO89//2*UC1113/3/5*KOFA/5/KOFA/4/DUKEM_1//PATKA_7/YAZI
DUR_280	7147178	AG 1-22/2*ACO89//2*UC1113/3/5*KOFA/5/KOFA/4/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1/8/SW SR227.B (SR 22)/6/2*RASCON_22/RASCON_21//MOJO_2/3/GUANAY/4/RCOL/5/SORA/2*PLATA_12//SOMAT_3/7/CMH83.2578/4/D88059//WARD/YAV79/3/ACO89/5/2*SOOTY_9/RASCON_37/6/1A.
DUR_281	7147179	AG 1-22/2*ACO89//2*UC1113/3/5*KOFA/5/KOFA/4/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1/8/SW SR227.B (SR 22)/6/2*RASCON_22/RASCON_21//MOJO_2/3/GUANAY/4/RCOL/5/SORA/2*PLATA_12//SOMAT_3/7/CMH83.2578/4/D88059//WARD/YAV79/3/ACO89/5/2*SOOTY_9/RASCON_37/6/1A.
DUR_282	7147180	AG 1-22/2*ACO89//2*UC1113/3/5*KOFA/5/KOFA/4/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1/8/SW SR227.B (SR 22)/6/2*RASCON_22/RASCON_21//MOJO_2/3/GUANAY/4/RCOL/5/SORA/2*PLATA_12//SOMAT_3/7/CMH83.2578/4/D88059//WARD/YAV79/3/ACO89/5/2*SOOTY_9/RASCON_37/6/1A.
DUR_283	7147182	AG 1-22/2*ACO89//2*UC1113/3/5*KOFA/5/KOFA/4/DUKEM_1//PATKA_7/YAZI_1/3/PATKA_7/YAZI_1/8/SW SR227.B (SR 22)/6/2*RASCON_22/RASCON_21//MOJO_2/3/GUANAY/4/RCOL/5/SORA/2*PLATA_12//SOMAT_3/7/CMH83.2578/4/D88059//WARD/YAV79/3/ACO89/5/2*SOOTY_9/RASCON_37/6/1A.