

**Sources of FHB Resistance, Genetics and Mapping of Stem Rust Resistance in  
Kenyan and Ethiopian Spring Wheat Germplasm**

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## **Dedication**

This is dedicated to my family members and friends for their support.

## Abstract

Fusarium head blight (FHB or scab) and stem rust are important diseases of wheat. *Fusarium graminearum* is considered the primary causal agent of FHB. This study evaluated 215 wheat genotypes from Kenya and Ethiopia for their response to *F. graminearum* in inoculated and mist-irrigated nurseries established in St. Paul, MN in 2016, St. Paul, MN in 2017 and Crookston, MN in 2017. Six genotypes with stable resistance across the three test locations were identified. Positive associations were identified between FHB index, VSK, and DON and negative associations were identified between FHB index, plant height, and heading dates. The response of select genotypes was confirmed under greenhouse conditions. These studies identified resistance sources that can be used to improve the resistance levels in Kenyan and Ethiopian wheat germplasm. Stem rust caused by *Puccinia graminis* f. sp. *tritici* remains a threat to wheat production in East African wheat growing regions. In this study, we characterized the genetics of stem rust resistance, identified QTLs and markers associated with the resistance in spring wheat line CI 14275. The RILs together with their parents were evaluated at the seedling stage in a biosafety level 3 greenhouse against *Pgt* races TTKSK and TRTTF and in the USDA-ARS Cereal Disease Lab greenhouse against *Pgt* races TPMKC, TTTTF, and RTQQC. Screening for resistance to *Pgt* races in the field was undertaken in Kenya, Ethiopia, and the US in 2016, 2017, and 2018. One and three complementary genes conferred resistance to races TTTTF and RTQQC, respectively. The QTL *QSr.cdl-2BS.2*, that conferred resistance in Kenya and Ethiopia was validated and the marker Excalibur\_c7963\_1722 was shown to have potential in marker assisted selection. This is the first study to both detect and validate an adult plant stem rust resistance QTL on chromosome arm 2BS. The *QSr.cdl-3B.1* is likely *Sr12*, *QSr.cdl-4AL.1* is postulated as *Sr7a*, *QSr.cdl-6BL.1* is likely *Sr11*, and *QSr.cdl-6AS.1* appears to be a new QTL. Combination of *QSr.cdl-2BS.2*, *QSr.cdl-3B.1*, and *QSr.cdl-6AS.1* has the potential to reduce stem rust severity in Africa. The work presented on FHB and stem rust provides resources for wheat improvement in East Africa.

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## **Chapter One**

# **General Introduction-Literature Review of Wheat, Stem Rust and Fusarium Head Blight**

## 1.1 Wheat history and genetics

Wheat (*Triticum aestivum* L.), is an annual crop belonging to the grass family *Poaceae*, tribe *Triticeae* and sub tribe *Triticineae* (Haider, 2013). Wheat has been an important crop since the ancient times, and it remains the most widely cultivated crop in the world followed by rice (*Oryza sativa*) and maize (*Zea mays*) (Haider, 2013). Wheat is cultivated on about 220.4 million hectares worldwide, with 756.8 million tonnes produced in 2018 and an estimated 736.1 million tonnes forecasted to be produced in 2019 (FAO, 2018). About 10,000 years ago, agriculture begun in the Pre-pottery Neolithic Near East (Nevo *et al.*, 2002). Einkorn (*Triticum monococcum*) and emmer (*Triticum dicoccum*) wheat were among the first founder agricultural crops when people were changing from hunting into agriculture (Lev-Yadun *et al.*, 2000; Nevo *et al.*, 2002; Shewry and Hey, 2015). Evidence for wheat utilization as a cereal crop in the ancient times was found at the Ohalo II site in Israel, where wild emmer wheat was discovered among other crops, and was believed to be 19,000 years old (Kislev *et al.*, 1992). Between 8000 to 3000 years B.C., cultivation of wheat had spread beyond the Fertile Crescent, into countries such as Greece, Cyprus, India, Egypt, Germany, Spain, England and Scandinavia (Cooper, 2015). Wheat is now grown throughout the world, in the tropical, subtropical, Mediterranean and temperate regions (Nevo *et al.*, 2002). The top five wheat-producing regions are; European Union, China, India, Russian Federation and the United States (FAO, 2018). With the currently estimated human population of 7.7 billion, there should be an increase in wheat production to meet the demands of estimated human population of 8.5 billion in 2030 and 9.7 billion in 2050 (United Nations, 2019).

Scientists recognize five or more species of cultivated wheat: common or bread wheat (*T. aestivum*), durum wheat (*T. durum*), emmer wheat (*T. dicoccum*), einkorn wheat (*T. monococcum*) and spelt wheat (*T. spelta*) (Haider, 2013; Cooper, 2015). However, many scientists categorize accessions of spelt wheat as one or more subspecies of *T. aestivum* and both durum and emmer wheat as various subspecies of *T. turgidum* (GRIN). The *Triticum* species have three ploidy levels; diploid with one set of chromosomes (e.g *T. monococcum*;  $2n = 2x = 14$ , AA), tetraploid with two sets of chromosomes (e.g *T. turgidum*, *T. dicoccum*, *T. durum*;  $2n = 4x = 28$ , AABB), and hexaploid, with three sets of

chromosomes (e.g *T. aestivum*, *T. spelta*;  $2n = 6x = 42$ , AABBDD) (Dubcovsky and Dvorak, 2007; Cooper, 2015). Hexaploid wheat is believed to have originated through hybridization between tetraploid *T. turgidum* (AABB) and *Aegilops tauschii* (DD). The diploid wild wheat *T. urartu* (AA,  $2n = 2x = 14$ ) has been accepted as the donor of the A genome (McFadden and Sears 1946; Riley *et al.*, 1958; Dvorak *et al.*, 2012). The B genome has been reported to be closely related to the S genome of the Sitopsis species (*Ae. speltoides*, *Ae. longissima*, *Ae. sharonensis*, *Ae. searsii*, and *Ae. bicornis*) and a positive evidence of *Ae. speltoides* (BB,  $2n = 2x = 14$ ) being the progenitor of B genome has been found (Haider, 2013). A wild goat grass *Aegilops squarrosa* (DD,  $2n = 2x = 14$ ) is the donor of D genome (McFadden and Sears 1946; Riley *et al.*, 1958; Dvorak *et al.*, 2012).

Hexaploid bread wheats and tetraploid durum wheats are the major types of wheat cultivated today (Cooper, 2015; Shewry and Hey, 2015). The hexaploid wheats are further sub-divided based on their different characteristics, classified as either winter or spring, hard or soft, red or white, and cultivated in different regions depending on climatic conditions and local market preferences. The ancient grains that are still cultivated, though in small areas, include diploid einkorn wheat, tetraploid emmer wheat and hexaploid spelt wheat (McFadden and Sears 1946; Shewry and Hey, 2015). The resurgence of cultivation of ancient grains is believed to be due to the health-related benefits of these wheats, where the ancient grains have been found to have higher nutritional benefits compared to the widely cultivated bread and durum wheats (Lachman *et al.*, 2013; Cooper, 2015; Shewry and Hey, 2015). One of the major differences between the widely cultivated (bread and durum) wheat species compared to the ancient (einkorn, emmer and spelt) species is their threshability, with the former species being easier to thresh.

## **1.2 Economic importance of wheat**

During the ancient times, wheat was the most consumed cereal crop, besides barley (*Hordeum vulgare*). At this modern age, wheat remains an important crop nutritionally, and wheat is relied upon by many people as a source of calories, providing ca. 20% of

daily calories where wheat is a staple. The wheat kernel is comprised of carbohydrates (60-80%; largely starch), proteins (8-15%), vitamins (B complex and E vitamins), fats (1.5-2%), minerals (1.5-2%), dietary fibre and the essential amino acids (except lysine, tryptophan, and methionine) (Nevo *et al.*, 2002; Shewry and Hey, 2015). Vitamin E, selenium, phytic acid and phenolic acids (ferulic acid and caffeic acid) are in abundance in wheat germ and bran, contributing to wheat flour's antioxidant properties (Adom and Liu, 2002; Cheng *et al.*, 2006; Yu *et al.*, 2013). Bread wheat, compared to other cereal crops, has unique baking quality due to the gluten proteins that facilitate dough development (Nevo *et al.*, 2002). Wheat has been found to be a good source of antioxidants, which prevent free radicals in the human body from damaging the body cells. Besides the nutritional importance of wheat to humans, wheat is also fed to livestock in different parts of the world, mainly as hay derived from wheat straw (Lindgren, 1949). Wheat straw has been utilized for bioethanol production in countries that lead in wheat production (Erdei *et al.*, 2010; Novy *et al.*, 2015).

### **1.3 Stem rust history and epidemiology**

Stem rust is caused by *Puccinia graminis* Pers. f. sp. *tritici* (*Pgt*), and its infection on cereals dates back 1300 B.C. (Kislev, 1982). The severity of the disease was evident by prayers made to rust god Robigo, during the Robigala ceremony for the protection of the crop from rust devastation (Roelfs *et al.*, 1992). The first scientific report of stem rust was made in 1767 by Fontana and Tozzetti, and the causal organism was later (1797) named by Persoon (Roelfs *et al.*, 1992). The primary hosts of *Puccinia graminis* Pers. f. sp. *tritici* include wheat, barley and triticale (*X Triticosecale* Wittmack), and the main alternate host is common barberry (*Berberis vulgaris*), although other *Berberis* species in addition to species in the *Mahonia* and *Mahoberberis* genera have been found susceptible to *P. graminis* (Roelfs *et al.*, 1992).

The minimum, optimum, and maximum temperatures for germination of urediniospores are 2, 15-24, and 30 °C, respectively along with free moisture for one to three hours in the presence of low light intensity (Roelfs *et al.*, 1992). The successful penetration of the host by the pathogen has minimum, optimum and maximum temperatures of 15, 29 and

35 °C, respectively, high light intensity and free water are also required (Roelfs *et al.*, 1992). The growth of the pathogen has minimum, optimum, and maximum temperatures of 5, 30, and 40 °C, respectively, and high light intensity, but free water is not necessary (Roelfs *et al.*, 1992). The sporulation of the pathogen requires minimum, optimum, and maximum temperatures of 15, 30, and 40 °C, respectively, along with high light intensities, though free water is not necessary for sporulation (Roelfs *et al.*, 1992). The urediniospores are dispersed long distances via wind and deposited on plants by rain events (Nagarajan *et al.*, 1976). Spores can remain viable for long periods, though they will lose viability when the moisture content of the spores is greater than 50% (Roelfs *et al.*, 1992). High humidity prevents more spores from being released from uredinia. Wind at high velocities results in the release of more spores and their dispersal over long distances (Roelfs *et al.*, 1992).

#### **1.4 *Puccinia graminis* Pers. f. sp. *tritici* life cycle**

The stem rust pathogen is a biotroph, obtaining nutrients from a living plant and on which it reproduces. The fungus is heteroecious, requiring wheat, its primary host, and common barberry, as the alternate host, to complete its life cycle. The life cycle of *Pgt* is macrocyclic, having five spore stages; urediniospores, teliospores, basidiospores, pycniospores and aeciospores that include haploid, diploid and dikaryotic nuclear types (Roelfs *et al.*, 1992; Kolmer *et al.*, 2009). On wheat straw, the dikaryotic (n+n) teliospores act as the surviving fungal structures, being found on dead or senescing tissues of host plants and become diploid (2n) after karyogamy takes place (Roelfs *et al.*, 1992; Kolmer *et al.*, 2009). Under favorable conditions, the teliospores germinate and meiosis takes place, giving rise to four haploid basidiospores (Roelfs *et al.*, 1992). The basidiospores (hyaline in color) are forcibly ejected from the basidium and are wind dispersed, though they can only travel short distances to the alternate host. There the basidiospores germinate and penetrate directly the host tissue, giving rise to haploid pycnium (n) fungal structure (Roelfs *et al.*, 1992). The pycnium then produces pycniospores in a sugary nectar, and receptive hyphae of a single mating type (+ or -) acting as male and female gametes, respectively (Roelfs *et al.*, 1992; Kolmer *et al.*, 2009). With the aid of rain, dew, or insects that are attracted to sugary nectar, the

pycniospores of one mating type are transferred to a receptive hyphae of a different mating type, in a process called plasmogamy (Roelfs *et al.*, 1992; Kolmer *et al.*, 2009), which results in the development of an aecium usually on the lower surface of the leaf of the alternate host. Dikaryotic (n+n) aeciospores are produced in chains within the aecium (Roelfs *et al.*, 1992; Kolmer *et al.*, 2009). The virulence and aggressiveness of aeciospores may differ because they are produced via genetic recombination. The aeciospores can travel for long distances on air currents and will infect leaves of the primary host, producing a uredinium in which dikaryotic urediniospores (n+n) develop (Roelfs *et al.*, 1992; Kolmer *et al.*, 2009). The uredinium converts to producing telia late in the growing season and the life cycle then repeats itself. The brick red urediniospores on the stems, leaf sheaths, leaf surfaces (upper and lower), and sometimes on the spikes, are observed 7-10 d after infection. A single rust spore produces a single pustule, capable of producing thousands of spores. New infections can be initiated on the same plant or new plants by each urediniospores and multiple cycles of infection can result in the development of an epidemic. Epidemics can be caused when multiple cycles of infection, sporulation and re-infection occur (Roelfs *et al.*, 1992; Kolmer *et al.*, 2009). In regions where the alternate host is present, the aeciospores infect the primary host initiating disease development, often early in the growing season. In regions without the alternate host, urediniospores play a major role in disease development, acting as initial inoculum, with new virulences occurring through mutation and selection (Roelfs *et al.*, 1992; Kolmer *et al.*, 2009).

### **1.5 Stem rust *Pgt* races (Ug99 and non-Ug99)**

The *Pgt* isolates virulent to *Sr31* were reported in Uganda in 1999, hence the acronym 'Ug99' (Pretorius *et al.*, 2000). Between 2003 and 2004, most Kenyan cultivars and CIMMYT germplasm with the stem rust resistance (*Sr*) gene *Sr31* were found to be susceptible to stem rust (Wanyera *et al.*, 2006). Testing of the isolates for virulence on the 16 North American stem rust race differentials led to identification of the race TTKS (Wanyera *et al.*, 2006). The race TTKS was later renamed as TTKSK following addition of a fifth set of genes (*Sr24*, *Sr31*, *Sr38*, and *SrMcN*) in the North American stem rust nomenclature system (Jin *et al.*, 2008). The *Pgt* race Ug99 was genotyped and grouped to

Clade I (Singh *et al.*, 2015). More variants of race TTKSK were thereafter reported, examples include race TTKST, identified in 2006, which possessed virulence to the *Sr24* gene and race TTTSK, identified in 2007, which possessed virulence to the *Sr36* gene (Jin *et al.*, 2008, 2009). In 2014, races TTKTK and TTKTT were identified to have virulence to the *SrTmp* gene in Robin (the most widely grown Kenyan wheat cultivar at the time) (Newcomb *et al.*, 2016; Patpour *et al.*, 2016).

*Pgt* races that do not belong to the Ug99 lineage have also been reported, causing epidemics. Race TKTTF, which falls in Clade IV of *Pgt*, is also known as the “Digalu race” (Singh *et al.*, 2015). The race TKTTF, believed to have originated from the Middle East, defeated the *SrTmp* gene in the most widely grown Ethiopian wheat cultivar at the time, Digalu, resulting in stem rust epidemics in Ethiopia in 2013 and 2014 (Olivera *et al.*, 2015; Olivera Firpo *et al.*, 2017). Stem rust outbreaks due to Clade IV races were reported in Germany and in the UK in 2014 (Meyer *et al.*, 2017; Olivera Firpo *et al.*, 2017). In 2016, there was an outbreak of stem rust in Sicily, Italy caused by *Pgt* Clade III, typified by race TTRTF (Bhattacharya, 2017). The race TTRTF and its variants have spread to Egypt, Eritrea, Ethiopia, Georgia and Kenya (Bhavani *et al.*, 2019). Another Clade III race (but classified in a different subclade), RRTTF, was detected in Ecuador (Barnes *et al.*, 2018).

### **1.6 Stem rust resistance types and resistance genes**

The resistance in wheat to *Pgt* races can be classified as race specific or race non-specific and can be observed in seedlings under greenhouse conditions and at the adult plant crop stage in the field (Vander Plank, 1968). Terms such as race specific resistance, all stage resistance, and vertical resistance have been used interchangeably to refer to seedling resistance which is mainly controlled by a single gene. However, there are examples where these types of resistances are not interchangeable such as seedling resistance genes for which not virulence has been detected and thus cannot be considered race specific (McIntosh *et al.*, 1995). Terms like adult plant resistance, field resistance, and horizontal resistance are sometimes used interchangeably with race non-specific resistance, generally controlled by minor-effect genes. Specific and non-specific interactions are the

two categories of host-pathogen interactions, with the specific interaction providing the basis for the gene-for-gene theory, which states that for every resistance gene in the host plant there is a corresponding avirulence gene in the pathogen (Flor, 1971; Roelfs *et al.*, 1992). The absence of either the host resistance gene or the pathogen avirulence gene will not trigger resistance (Flor, 1971). Seedling resistance, which is usually controlled by one gene is often race specific.

Plants with major-effect resistance genes (R genes) may express intermediate resistance to immunity when challenged by the pathogen. There are several major genes effective against the race Ug99, although some genes, such as *Sr32*, *Sr37*, *Sr39*, *Sr40*, and *Sr44*, have not been utilized in breeding programs because of their linkage with unwanted traits (Singh *et al.*, 2015). Some of the major genes currently utilized in breeding programs to confer resistance to *Pgt* are; *Sr13*, *Sr22*, *Sr23*, *Sr25*, *Sr26*, *Sr33*, *Sr38*, and *Sr50* (Helguera *et al.*, 2003; Mago *et al.*, 2005; Simons *et al.*, 2011; Mago *et al.*, 2013; Periyannan *et al.*, 2013; Mago *et al.*, 2015; Singh *et al.*, 2015). Genes with temporary designations, such as *SrHuw234*, *SrND643*, *SrNing* and *SrYanac*, are also being utilized to provide resistance against *Pgt* (Lopez-Vera *et al.*, 2014; Basnet *et al.*, 2015). Eight major genes, effective against Ug99 (*Sr13*, *Sr22*, *Sr33*, *Sr35*, *Sr45*, *Sr46*, *Sr50*, and *Sr60*) have been cloned (Periyannan *et al.*, 2013; Saintenac *et al.*, 2013; Zhang *et al.*, 2013; Mago *et al.*, 2015; Steuernagel *et al.*, 2016; Zhang *et al.*, 2017; Arora *et al.*, 2019; Chen *et al.*, 2019). The downfall of deploying these major-effect genes individually is that they can be easily overcome by the pathogen, resulting to what is referred to as “boom and bust” cycles. The Ug99 race group clearly highlights the “boom-bust” cycle by the defeat of important R-genes like *Sr24*, *Sr31*, *Sr36*, and *SrTmp* that were widely deployed worldwide (Pretorius *et al.*, 2000; Jin *et al.* 2008, 2009).

Race non-specific resistances confer resistance to all *Pgt* races, and the effect of such resistance is often minor relative to the major-effect genes. The race non-specific genes such as *Sr2* (pleiotropic to *Yr30*), *Sr55* (pleiotropic to *Lr67*, *Yr46*, *Pm46*), *Sr56*, *Sr57* (pleiotropic to *Lr34*, *Yr18*, *Pm38*), and *Sr58* (pleiotropic to *Lr46*, *Yr29*, *Pm39*) confer slow rusting resistance at the adult plant stage and thus may not provide adequate



resistance when present alone under high disease pressure (Bansal *et al.*, 2014; Herrera-Foessel *et al.*, 2014; Singh *et al.*, 2015). The gene *Sr2* was derived from *Triticum turgidum* (Yaroslav emmer) whereas *Sr55*, *Sr56*, *Sr57*, and *Sr58* were discovered in *Triticum aestivum* (Singh *et al.*, 2015). *Sr2* has been used for approximately 60 years as a source of durable resistance. Due to the recessive and minor-effect nature of the *Sr2* gene, traditional breeding with *Sr2* has been difficult. Pseudo-black chaff, a dark pigmentation mainly on stem internodes, glumes, and peduncles is a morphological trait linked to *Sr2* and it is still used as a marker to select for this gene (Singh *et al.*, 2015). The level of expression of this morphological marker has been found to vary with the environment. The resistance of *Sr2* can be enhanced when in combination with other unknown stem rust resistance genes. Molecular markers for *Sr2*, *Sr55*, *Sr57*, and *Sr58* are available and are currently utilized in breeding programs (Lagudah *et al.*, 2009; Kolmer *et al.*, 2015). Combinations of effective major-effect genes (R-genes) and/or minor-effect race non-specific resistance genes may result in durable resistance, or resistance that will remain effective for a longer period of time (Parlevliet and Ommeren, 1988; Priyamvada *et al.*, 2011).

### **1.7 Quantitative trait loci (QTL) mapping and mapping populations**

Before QTL mapping was introduced, classical quantitative genetics which described additive gene action, nonadditive gene action, gene number, and genotype by environment interaction was used to understand the complexity of some disease resistances, although these classical genetics could not unravel the physical location of disease resistance genes (Young, 1996). The association of a complex trait with a simple trait led to the idea of QTL mapping, as was first described in 1923 (Sax, 1923). QTL mapping has made it easier to understand complex disease resistances which are controlled by many genes or loci and has also led to successful cloning of such resistance genes (Tanksley, 1993; Young, 1996). Mapping populations such as doubled haploids (DH), F<sub>2</sub> generation, backcrosses, and recombinant inbred lines (RILs) are genotyped with molecular markers, and the markers associated with the trait of interest are identified (Collard *et al.*, 2005). QTL mapping depends on good phenotypic data of the mapping population, dense genome coverage with markers, adequate population size, and

statistical approaches to identify the loci (Lander and Botstein, 1988). The advantages of using RILs over F<sub>2</sub>s or early generation backcross-derived populations is that they are homozygous (genetically fixed), which means they can be replicated in trials, and increased crossover events during meiosis results in high mapping resolution in the RILs (Collard *et al.*, 2005). Developing RILs does however take longer than developing other bi-parental populations (Collard *et al.*, 2005). Mapping of the loci depends on genotyped markers, close enough to the loci of interest, in each RIL. The size of a RIL population affects the power of detecting QTL and mapping resolution. Population sizes greater than 100 lines having allele frequency drift that is negligible, have high power for QTL detection and resolution (Collard *et al.*, 2005).

### **1.8 Genotyping**

Multiple genes influence quantitative traits, and genomic positions of such genes can be identified and mapped using genome-wide molecular markers. Widely used markers include DNA (molecular) markers because of their abundance and ease of use. Morphological and biochemical markers can be influenced by plant developmental stage, environment, limited in number and can be more difficult in practice (Jones *et al.*, 1997). Based on the detection method, three classes of DNA markers exist: polymerase chain reaction (PCR)-based, DNA sequencing-based and hybridization-based (Winter and Kahl, 1995; Jones *et al.*, 1997; Gupta *et al.*, 1999).

The adoption of genotyping approaches such high-throughput DNA sequencing technologies, commonly referred as next generation sequencing (NGS), have led to a shift from conventional breeding techniques to new methods including genomic selection, association mapping, and marker assisted selection (Collard and Mackill, 2008). The NGS platforms 454 (Roche, <http://www.454.com>), Illumina (Illumina, <http://www.illumina.com>), and PacBio RS (Pacific Biosciences, <http://www.pacificbiosciences.com>), are examples of cost effective and time efficient genotyping platforms (Pérez-de-Castro *et al.*, 2012). The number of markers, their coverage across the genome, and the availability of characterized markers should be considered in selecting markers or a genotyping platform (Collard *et al.*, 2005). The

iSelect 90K SNP assay with 81,587 gene-associated SNPs was developed from SNPs identified in diverse hexaploid and tetraploid wheat populations (Wang *et al.*, 2014). From the 81,587 markers, 46,977 were genetically mapped, providing an important resource for genotyping studies among researchers (Wang *et al.*, 2014). The identified SNPs can be used in constructing linkage maps and to develop markers that will speed up the breeding process through their use in marker assisted selection.

### **1.9 Linkage map construction**

Linkage maps show the position of markers and the genetic distance between the markers along one or more chromosomes (Collard *et al.*, 2005). A linkage map enables one to identify the location of genes on the chromosome and quantitative trait loci (QTL) that are associated with markers on the linkage map (Collard *et al.*, 2005). When constructing a linkage map, linkage between markers is determined. Normally, a logarithm of odds (LOD; ratio of linkage versus no linkage) value of  $>3$  is used to determine significantly linked markers (i.e linkage is 1000 times more likely than no linkage) although this LOD value can be lowered if more markers need to be included in the map (Collard *et al.*, 2005). Linked markers are assigned to a linkage group, with the linkage group representing a physical chromosome (Collard *et al.*, 2005). Generally, closely linked markers in a chromosome have a lower recombination frequency, while distant markers have a higher recombination frequency. The genetic distance between markers is calculated using either the Kosambi mapping function or the Haldane mapping function, the former assumes that recombination events interfere with adjacent recombination events, while the latter function assumes a lack of interference among recombination events. Genetic distances are calculated by converting recombination fractions into to map units, referred as centi-Morgans (cM) (Collard *et al.*, 2005). Declaring a significant QTL is based on permutation tests and LOD scores (Churchill and Doerge, 1994; Collard *et al.*, 2005). For permutation, marker-trait association tests are repeated at least 500 times and based on the desired level of false positive marker-trait associations. From these permutations, the level of significance is determined (Collard *et al.*, 2005). A minimum LOD score between 2.0 and 3.0 can be used as a threshold for detecting a significant association, although LOD values of 3.0 or greater are preferred (Collard *et*

*al.*, 2005). Linkage maps can be constructed using software programs that include; Mapmaker/EXP, MapManager QTX, JoinMap, and ASMAP (Lander *et al.*, 1987; Stam, 1993; Manly *et al.*, 2001). A QTL can be declared as a minor or major QTL based on two things: i) based on  $R^2$  value (proportion of phenotypic variation that the QTL explains) i.e. QTLs with  $>10\%$   $R^2$  value can be referred as major QTLs whereas those with  $< 10\%$   $R^2$  value can be indicated as a minor QTL, or ii) based on the QTLs stability across the environments, major QTLs are stable across the environments whereas minor QTLs do not show stability across the environments (Collard *et al.*, 2005).

### **1.10 QTL mapping methods**

The commonly used methods for detecting QTLs are; single-marker analysis, simple interval mapping and composite interval mapping (Tanksley, 1993). Single-marker analysis does not require a complete linkage map, though QTL located far from the markers may not be detected if recombination frequently occurred between the QTL and the marker leading to an underestimation of the QTL effect with this method (Tanksley, 1993; Collard *et al.*, 2005). Simple interval mapping requires linkage maps and uses the intervals between markers in the analysis (Lander and Botstein, 1989). Composite interval mapping is a precise and effective method, that combines interval mapping and linear regression, fitting both QTL and covariate effects, hence controlling linked QTL effects (Collard *et al.*, 2005). The main factors that can affect QTL detection include; magnitude of the QTL effect (with detected QTLs being those that have large phenotypic effects), the distance between linked QTLs (with closely linked QTLs detected as a single QTL), environmental factors (the environment can affect the expression of phenotypic traits), population size (larger populations being more accurate, reliable, and allowing small effect QTLs to be detected), along with genotyping and phenotyping errors (Collard *et al.*, 2005).

### **1.11 Management of stem rust disease**

Methods for the control of stem rust include chemical control using fungicides, cultural practices and genetic control. Several different fungicides are effective in controlling of stem rust. For example, tebuconazole (Folicur 250 EC, Orius 25 EW), combinations of

tebuconazole and tridimenol (Silvacur 375 EC) and cyproconazole (AmistarXtra 280 SC) have been reported to be effective for controlling stem rust in Kenya (Wanyera *et al.*, 2009, 2010). Challenges in using fungicides include; the timely application of the right amount of effective chemicals, cost of fungicides, and concerns regarding environmental pollution arising from the continual application of these chemicals (Roelfs *et al.*, 1992). Some cultural practices that have been used in the control of stem rust include eradication of the alternate host near wheat cultivation, planting of early maturing cultivars, early planting, destroying “green bridge” plants, and controlling the frequency and amount of irrigation and fertilizer (Roelfs *et al.*, 1992; Todorovska *et al.*, 2009). Eradication of the alternate host, common barberry, which successfully reduced stem rust epidemics in parts of the US and Europe (Roelfs *et al.*, 1992), significantly limited the evolution of virulent races, which in turn resulted in the resistance genes being effective for longer periods (Roelfs *et al.*, 1992). Genetic resistance remains a feasible approach to control stem rust, and durable resistance can be achieved by combining effective major and/or minor genes. Planting of resistant varieties may eliminate or reduce the use of fungicides, which is costly to farmers. One of the major challenges for adoption of this approach is a possibility of the resistance gene(s) no longer being effective because of a virulence change in the pathogen population (Roelfs *et al.*, 1992).

### **1.12 Fusarium head blight (FHB) and *Fusarium* species distribution**

FHB, also referred as scab, was first reported in early 1880’s in England and in 1890’s in the US, caused by *Fusisporium culmorum* (Parry *et al.*, 1995). Today, FHB is recognized as being principally caused by *F. graminearum* Schwabe [teleomorph = *Gibberella zeae* (Schw.) Petch] with at least 16 different species, most of which belong to the *F. graminearum* complex (FGC) (Schroeder and Christensen, 1963; Sutton, 1982; Bai and Shaner, 1994; Aoki *et al.*, 2014). *Microdochium nivale* may also be one of the FHB agents when cool and wet conditions are present (Ferrigo *et al.*, 2016). The other species commonly reported in association with FHB in wheat are: *F. culmorum*, *F. avenaceum*, and *F. poae* (Parry *et al.*, 1995). The geographic distribution of these *Fusarium* species is related to temperature, with *F. graminearum* thriving well in the US, Canada, Australia, Central Europe and Southern China where climatic conditions are warmer and wet, while

*F. culmorum* and *F. avenacum* are more common in regions with cooler climatic conditions such as those experienced in Northwest Europe, *F. poae* survives in regions with drier and warmer climates and *Microdochium spp.* survive in regions with frequent rainfall and experiencing mild temperatures (Parry *et al.*, 1995). In Kenya, *F. graminearum* and *F. poae* have been reported as being the dominant species inciting FHB in wheat and barley (Muthomi *et al.*, 2012). In South Africa, *F. graminearum* was reported as the predominant species (Minnaar-Ontong *et al.*, 2017).

### **1.13 Economic importance of FHB**

FHB or scab is a devastating disease in most countries that produce small grain cereal crops such as wheat, barley, oats (*Avena sativa*), triticale, and rye (*Secale cereale*), although wheat and barley crops are the most affected. FHB epidemics have been reported in most wheat and barley producing countries worldwide (Parry *et al.*, 1995; Bai and Shaner, 2004). In the United States, the first FHB epidemics were from 1910s to 1930s, with later epidemics reported in the early 1990s (Parry *et al.*, 1995; McMullen *et al.*, 1997). Losses worth 2.492 billion USD due to FHB on small grain cereal crops were reported in the US from 1993 through 2001 (Nganje *et al.*, 2004). The losses incurred from FHB also resulted in a shift away from growing wheat and barley to oil crops, decreasing the production of wheat and affecting the supply of wheat to industries producing food and feed products (Windels, 2000). In China, FHB was first reported in 1936, and since then epidemics have been reported resulting to more than 1 million tons of yield losses (Qu *et al.*, 2008). Significant losses in cereal crops to FHB haven't been reported in East African countries, presumably the lack of favorable conditions for development and establishment of *Fusarium* species reduces the risk of the disease, though FHB infections have been reported in field surveys (Muthomi *et al.*, 2012). In South Africa, FHB epidemics have been reported since the early 1990s in regions that practice no tillage and the rotation of maize with wheat (Minnaar-Ontong *et al.*, 2017). FHB infected florets become sterile, affecting grain quality and yield. The market of the grain is affected by the bleached colour and withered kernels. In addition to the incurred yield losses due to FHB, the mycotoxins associated with FHB-infested kernels impact human and animal health.

### **1.14 Mycotoxins**

Mycotoxins are toxic secondary metabolites produced by fungi. Fungi in the genus *Fusarium* produce three classes of mycotoxins: trichothecenes, zearalenones, and fumonisins (Champeil *et al.*, 2004). Trichothecenes are the largest class of toxins produced by *Fusarium* species, and two types of trichothecenes exist: Type A (T2-toxins and HT-2 toxins) and type B [deoxynivalenol (DON), nivalenol (NIV), 3-acetylDON (3-ADON) and 15-acetylDON (15-ADON)] (Boenisch and Schafer, 2011). Type A trichothecenes are produced by *Fusarium* species including *F. poae*, *F. equiseti*, *F. sporotrichioides*, and *F. langsethiae* while type B trichothecenes are largely produced by *F. graminearum* and *F. culmorum* (Ferrigo *et al.*, 2016). DON is an important mycotoxin because it is the mostly commonly detected mycotoxin in small grain cereals.

Consumption of DON and NIV contaminated food may cause vomiting, haemorrhage, convulsions, and skin irritations (Krska *et al.*, 2001; Rocha *et al.*, 2005). Cases of mycotoxicoses have been reported in Asia, Europe, New Zealand, US and Africa (Cortinovis *et al.*, 2013). The consumption of feed contaminated with fumonisin B1, ZEA, DON, NIV, HT-2, and/or T-2 toxins can result to reproductive disorders, feed refusal, vomiting and retarded growth in pigs and cattle (Eriksen and Pettersson, 2004; Cortinovis *et al.*, 2013). Trichothecenes have been reported to inhibit protein synthesis by binding to the 60S ribosomal subunit (Rocha *et al.*, 2005; Kazan *et al.*, 2012). DON has been shown to be a virulence factor in FHB development, aiding the spread of fungus from infected florets to the rachis (Proctor *et al.*, 1995; Jansen *et al.*, 2005).

### **1.15 Types of FHB resistance**

Resistance to FHB in wheat is either morphological or physiological (Rudd *et al.*, 2001). Although physiological resistance is considered more important than morphological resistance, the latter is considered to influence FHB development (Rudd *et al.*, 2001). Morphological traits such as spike length, spike compactness, presence/absence of awns, plant height, and heading date have been associated with FHB resistance. Disease is reported to spread faster in genotypes with spikes that are more compact and awned (Rudd *et al.*, 2001). Similarly, plant height has been associated with FHB, with short

plants being more prone to disease than tall ones (Mesterhazy, 1995). The susceptibility of short plants has also been attributed to higher humidity around the spike, as more moisture is retained in shorter plants compared to their tall counterparts, that enhances FHB development (Buerstmayr *et al.*, 2000; Somers *et al.*, 2003; Klahr *et al.*, 2007).

There are multiple types of resistance in wheat to FHB reported in the literature, although two, type I, defined as resistance to initial infection, and type II, defined as resistance to spread of the fungus in head, are the most commonly measured (Shroeder and Christensen, 1963; Mesterhazy, 1995). Type I resistance is typically measured under field conditions following spray inoculation at anthesis, whereas type II is generally measured under greenhouse conditions following development of FHB from the point-inoculation of a single spikelet at the centre of a spike at anthesis. The other types of physiological resistance described to FHB include; resistance to kernel infection, resistance to DON toxin accumulation and resistance to yield loss despite the disease, the last of which is often referred to as tolerance (Mesterhazy, 1995).

FHB resistance QTLs have been reported in several studies (Anderson *et al.*, 2001: ND2603/Butte RIL population, Somers *et al.*, 2003: Wuhan-1/Maringa DH population, Shen *et al.*, 2003: Ning 894037/Alondra RIL population, Jia *et al.*, 2005: Wangshuibai/Alondra's' DH population, Liu *et al.*, 2007: Ernie/ MO 94-317 RIL population, Yu *et al.*, 2008: Wangshuibai/Wheaton RIL population, Wang *et al.*, 2017: GWAS study on spring wheat lines). There are five FHB genes recognized; *Fhb1* gene, which has been mapped on chromosome arm 3BS in cultivars Sumai 3 and Ning7840, *Fhb2* mapped on chromosome arm 6BS in Sumai 3, *Fhb3* mapped on chromosome arm 7AS in *Leymus racemosus*, *Fhb4* on chromosome 4B in Wuhan 1, *Fhb5* mapped on chromosome arm 5AS in cultivars Sumai 3, Frontana, and Wangshuibai (Somers *et al.*, 2003; Cuthbert *et al.*, 2007; Qi *et al.*, 2008; Xue *et al.*, 2011). Among these genes, *Fhb1* from Sumai 3 is the most widely used resistance because of its stability in different genetic backgrounds. The FHB resistance QTLs have been found to overlap with different morphological traits (Gervais *et al.*, 2003: FHB QTLs overlap with flowering time and plant height QTLs in a Renan/Recital population on chromosome 2B and 5A,



Somers *et al.*, 2003: DON content QTL overlaps with a plant height QTL on chromosome arm 2DS in a Wuhan-1/Maringa population, Paillard *et al.*, 2004: FHB and plant height/heading date QTLs overlap in an Arina/Forno population).

### **1.16 FHB disease cycle**

FHB species colonize living hosts and also have the ability to survive saprophytically on debris of wheat, barley, rice (*Oryza sativa*) and maize (*Zea mays*) in the soil surface (Sutton, 1982; Bai and Shaner, 2004). The debris of the previous crops acts as a source of inoculum (macroconidia, ascospores, chlamydospores, hyphal fragments) that may initiate infection whenever conditions are favorable (Bai and Shaner, 1994; Dill-Macky and Jones, 2000). Ascospores released from perithecia are considered the primary source of inoculum initiating FHB epidemics, although macroconidia are often used to initiate epidemics in inoculated field plots (Bai and Shaner, 1994; Osborne and Stein, 2007). Seedling blight may occur when FHB infected seeds are planted or when healthy seeds are planted into soil containing infected debris. The airborne spores, mainly ascospores and macroconidia, are wind dispersed and deposited on, or inside, wheat florets (Parry *et al.*, 1995; Bai and Shaner, 2004). The dispersal of ascospores may be aided by both wind and rain splash while macroconidia, are mainly rain splash dispersed (Champeil *et al.* 2004). The pathogen can enter the florets via the exposed anthers or through the spikelet opening between the lemma and palea. After infection, the glumes of the infected florets initially show water-soaked, dark-brown spots. The pathogen then spreads in the susceptible wheat via vascular bundles in the rachis from one floret to another and between spikelets, until the whole spike appears blighted or bleached (Ribichich *et al.*, 2000; Bai and Shaner, 2004). Additionally, when the vascular tissues are infected, the uninfected grains distal to the point of infection are deprived of water and nutrients and this results to premature death of the spike. The infected florets may not have any grain or may have shriveled and bleached grains in them at harvest. Wet, humid, and warm weather immediately before, during and shortly after the anthesis period, along with the amount of inoculum and the susceptibility of the host, ultimately determines the FHB severity. The relative aggressiveness and toxin production capacity of *F. graminearum* isolates does vary (Bai and Shaner, 2004).

### **1.17 Management of FHB**

Effective measures for the control of FHB include; the use of fungicides, cultural practices and host genetic resistance. Treatment of seeds with fungicides before planting prevents seed-borne inoculum and prevents seedling blight disease, application of foliar fungicides during anthesis has been shown to reduce FHB infection (Jones, 2000). The use of fungicides as a control measure is costly and fungicides may not be effective if the chemical is not applied at the optimal growth stage to prevent infection (Bai and Shaner, 2004). Another drawback of chemical use is that as much as it may reduce the direct yield loss due to FHB, it may not reduce mycotoxin levels in the contaminated grains to acceptable levels in human food and animal feed. Cultural practices which include crop rotation with non-cereal crops reduces the amount of inoculum source, practicing deep tillage to bury the infested residues, therefore eliminating or reducing the inoculum source and amount (Bai and Shaner, 1994). The most economical and effective measure is however the planting of FHB resistant varieties (Ban, 1997; Bai and Shaner, 2004). The complexity of FHB resistance, the limited number of resistance sources, many of which have poor agronomic traits, along with the large environmental influence on disease development, makes breeding for FHB resistance a hard and complicated process (Buerstmayr *et al.*, 2002). The most feasible approach to FHB management appears to be integrated management, that combines the three measures: use of effective fungicide, cultural practices and planting resistant cultivars.

## **Chapter Two**

### **Response of Kenyan and Ethiopian wheat genotypes to *Fusarium graminearum***

## 2.1 Introduction

Fusarium head blight (FHB or scab) is an important disease of small grain cereals including wheat, barley and oats and is caused by many *Fusarium* species, although *Fusarium graminearum* is considered the primary causal agent (Shroeder and Christensen, 1963; Mesterhazy, 1995; Bai and Shaner, 2004). Initial infection and development of FHB generally requires warm and moist conditions. The adoption of conservation tillage practices and the production of alternative hosts of *F. graminearum*, including maize (*Zea mays*), have been linked to increased FHB epidemics (Dill Macky and Jones, 2000). FHB infections result in the production of shriveled grains with reduced test weight that are of poor quality for use in food and feed products (Parry *et al.*, 1995). FHB may result in the development of seedling blight, also primarily attributed to *F. graminearum*, when infected seeds are planted in a subsequent growing season (Parry *et al.*, 1995; Champeil *et al.*, 2004). Additionally, *Fusarium* species produce mycotoxins that may adversely impact human and animal health. Type B trichothecenes including: deoxynivalenol (DON), the acetylated derivatives of DON (3-ADON, 15-ADON), and nivalenol (NIV), are the most important toxins produced by *F. graminearum* that impact the quality of wheat (Krska *et al.*, 2001; Boenisch and Schafer, 2011). Nivalenol has been reported to be more toxic to humans and animals than DON, although DON is of greater concern because it is more common and thus generally detected at higher concentrations in wheat.

Resistance to FHB in wheat is complex, with many minor resistance genes with additive effects reported (Bai and Shaner, 1994; Parry *et al.*, 1995). Sources of FHB resistance in wheat such as ‘Sumai 3’, ‘Frontana’, ‘Ning 7840’, and ‘Nobeoka-bozu’ among others have been identified to confer resistance and have been widely used as sources of resistance in wheat breeding programs (Rudd *et al.*, 2001; Bai and Shaner, 2004; Buerstmayr *et al.*, 2012). Type I (resistance to initial infection) and type II (resistance to spread of the fungus within the spike) resistances have been identified as the two most important types of FHB resistance to FHB on wheat (Schroeder & Christenson, 1963; Bai and Shaner, 1994). While type I and type II resistance have been the most widely examined, other mechanisms of resistance are reported in the literature including;

resistance to accumulation of DON toxin, resistance to kernel infection, and resistance to yield loss despite the presence of the disease (Mesterhazy, 1995; Malhipour *et al.*, 2016). Agronomic traits such as plant height, heading date, the presence/absence of awns have also been associated with FHB resistance (Mesterhazy, 1995; Rudds *et al.*, 2001; Somers *et al.*, 2003; Tamburic-Ilincic *et al.*, 2007). Typically, greenhouse screening for type resistance is conducted by examining disease spread following the point-inoculation of a central spikelet (Mesterhazy, 1995; Gilbert & Tekauz, 2000). The inoculation of field plots using macroconidial inoculum, as is frequently done in screening nurseries, is used to measure both type I and type II resistances. Accurate assessment of type I resistance alone in the field is challenging because it is typically confounded by type II resistance.

As resistance to FHB is not complete, the management of FHB generally relies on the use of control practices in addition to host resistance including; avoiding the planting of highly susceptible germplasm, the use of fungicides, tillage practice to bury infested crop residues, and crop rotations with non-host crops. Although integrated management is key to the control of FHB, host resistance is the cornerstone of FHB control (Anderson *et al.*, 2001; Wegulo *et al.*, 2015). The identification of sources of FHB resistance, and/or resistance in germplasm adapted for local conditions is key to the development of wheat lines with improved resistance to FHB. Currently, there is a lack of information on the reaction of Kenyan and Ethiopian wheat genotypes to FHB. This study aims to determine the reaction of a collection of East African germplasm to FHB with the aim of identifying both resistant and susceptible germplasm. The identification of susceptible lines should be useful in limiting the planting of germplasm that could increase the risk of FHB epidemics, while resistant lines may be utilized as parental material to improve FHB resistance in the wheat breeding programs of East Africa.

## **2.2 Materials and Methods**

### **2.2.1 Field Screening**

#### **2.2.1.1 *Fusarium graminearum* isolates**

The *F. graminearum* isolates used in this study were isolated from symptomatic spikes of wheat and barley collected from commercial crops in Minnesota. The 30 isolates used as

inoculum in 2016 were collected from 28 wheat and 2 barley fields between 2011 and 2015. The 40 isolates used in 2017 were collected from 38 wheat and 2 barley fields between 2009 and 2016.

### **Isolation of *F. graminearum* from seed**

The followed procedure of isolating *F. graminearum* is as described by Leslie and Summerell (2007). Seeds (~10) obtained from symptomatic spikes collected in the field were placed into a biopsy cassette (Fisherbrand Histosette II Biopsy Cassette, Cat. No. 15-182-702H) and surface disinfested by immersing the cassette sequentially in 70% ethanol for 15-20 s, sterile distilled water for ca. 10 s, 1% sodium hypochlorite for 90 s, then rinsing three times in sterile distilled water for ca. 10 s each rinse, and finally blotting dry on sterile filter paper. The surface disinfested seeds were then transferred to Petri plates (60 x 15 mm) containing Komada's medium agar (KMA: 1 g of K<sub>2</sub> HPO<sub>4</sub>, 0.5 g of MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.5 g of KCL, 0.01 g of Fe-Na-EDTA, 2 g of L-Asparagine, 20 g of D(+)-Galactose, 15 g of agar, 1 L distilled water, autoclaved at 115 °C for 20 min, medium cooled to 55-60 °C, 1 g of pentachloronitrobenzene (PCNB), 0.5 g of Oxgall [dissolved in 5 mL 100% EtOH], 1 g of Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>.10H<sub>2</sub>O, 6 mL of streptomycin sulfate solution [5 g streptomycin sulfate/100 mL sterile distilled water]) and incubated for 10-14 d at room temperature (20-23 °C). Colonies of *F. graminearum* on KMA typically had white-pale pink or pale-yellow aerial mycelium and a pink-red undersurface, with the pigments diffusing into the agar.

### **Purification and identification of *F. graminearum* isolates**

A tuft of mycelium from the edge of each *F. graminearum*-like colony was transferred aseptically to Petri plates of carnation leaf-piece agar (CLA: 15 g of agar, 1 L distilled water autoclaved at 115 °C for 20 min, and aseptically placing sterile carnation leaf pieces [ca. 3 pieces (4 mm<sup>2</sup>) per plate] onto the agar surface) and incubated for 10-14 d at 20 °C under lights (16 h light per day, cool white and blacklight fluorescent). After the incubation period, sterile distilled water (3 mL) was used to flood each CLA plate and the macroconidia dislodged from the agar surface with a sterile L-shaped glass rod. The resulting spore suspension was streaked onto the surface of 2% water agar plates (WA:

20 g of agar, 1 L distilled water, autoclaved at 115 °C for 20 min) using a sterile loop and the plates left for 16 to 20 h on the laboratory bench to allow the spores to germinate.

Germinating spores were located in each WA plate using a stereo microscope and a section of the media containing a single spore was cut from the agar, using a sterile scalpel, and transferred to a Petri plate of potato dextrose agar (PDA: 39 g of Potato Dextrose Agar, 1 L distilled water, autoclaved at 121 °C for 20 min). The PDA plates were incubated at room temperature (22 °C for 5-7 d (16 h light per day, cool white and blacklight fluorescent).

To confirm the identification of the isolates, a tuft of mycelium from the edge of each colony growing on PDA was transferred aseptically to a CLA plate and incubated at room temperature (22 °C) for 14 d (16 h of light per day, cool white and blacklight fluorescent). Perithecia formation on the CLA plates was considered diagnostic, confirming the identification of *F. graminearum*.

#### **2.2.1.2 Inoculum production - macroconidial suspensions**

CLA cultures of *F. graminearum* (14 days-old) were flooded with ca. 7 mL sterile distilled water and a sterile L-shaped glass rod was used to dislodge spores from the agar surface. Aliquots (1.5 mL) of the resulting spore suspension were pipetted into Petri plates containing mung bean agar (MBA: 40 g of mung beans, 1 L distilled water, boiled for 23 min [the point at which the beans just start to crack open], broth filtered through 2-4 layers of cheesecloth, adjusted to 1 L, 15 g of agar, autoclaved at 115 °C for 20 min) and spread across the surface of the media using a sterile L-shaped glass rod. The inoculated MBA plates were incubated at room temperature (22 °C) for 7 d (16 h of light per day, cool white and blacklight fluorescent). At 7 d, the macroconidia were washed from the MBA cultures using distilled water (ca. 10 mL per plate) using a CO<sub>2</sub>-powered sprayer. The resulting mycelia fragments and spore suspension was filtered through 2-3 layers of cheesecloth. The spore concentration was determined using a hemocytometer, adjusted to 800,000 macroconidia/mL, transferred to wide-mouthed one-liter Nalgene bottles, frozen and stored at -20 °C until needed.

### **2.2.1.3 Inoculum production - *Fusarium*-colonized corn grain**

Corn (*Zea mays*) grain (1 kg) was placed in stainless steel pans (45 × 25.5 × 7.5 cm) lined with two layers of aluminum foil. One liter of water was added to the corn in the pan, and the pan was then covered with two layers of aluminum foil and left overnight on the lab bench to allow the grain to imbibe the water. The following day, the pans of grain were autoclaved for 1 h at 121 °C. The pans of grain were autoclaved a second time, one day later. After the grain had cooled following the second autoclave cycle, each pan was inoculated with five Petri plates of 7-10-day old cultures (PDA or MBA) of a single *F. graminearum* isolate. Working aseptically, the agar and fungal colony were cut into pieces (>1 cm diameter) with a metal spatula and evenly distributed throughout the grain. The foil pan cover was replaced, and the inoculated grain incubated at room temperature for 2-3 weeks. Following the incubation period, the colonized grain, which had formed a solid block, was broken up manually. The colonized grain was either kept in cold room at 4 °C to be used fresh, or spread as a thin layer (ca. 1 cm deep) in shallow pans and dried in a fume hood for 2-4 d. The dried grain was stored at room temperature for up to 2 weeks before being used in the field experiments.

### **2.2.1.4 Plant materials**

Two hundred and fifteen spring wheat genotypes originating from Kenya (23 cultivars, 10 Kenya Seed lines), Ethiopia (24 cultivars) and the International Maize and Wheat Improvement Centre (CIMMYT) (66 lines selected in Kenya, 92 lines selected in Ethiopia) were examined for their reaction to *F. graminearum* in three field experiments, planted in St. Paul, MN in 2016 (STP16) and 2017 (STP17) and Crookston, MN in 2017 (CRK17). The term locations will henceforth be used in the document to refer to the three field experiments (STP16, STP17, CRK17). The wheat genotypes were part of a collection that were sent from Kenya and Ethiopia to the USDA-ARS, Cereal Disease Laboratory (CDL) to be tested for their reaction to *Puccinia graminis* f. sp. *tritici* at the seedling stage. The genotypes used in this study included only those genotypes in the larger collection where >5 g of seed was available. In addition to the Kenyan and Ethiopian genotypes, FHB checks, including the FHB-resistant variety Rollag (Anderson *et al.*, 2015), the FHB-moderately resistant varieties LCS Albany (PI 658002) and



Linkert (Anderson *et al.*, 2018), and the FHB-susceptible varieties Samson (PI 652923), WB-Mayville (PI 661061) and Wheaton (Busch *et al.*, 1984) were included in the experiments. Of these checks, five (those listed except Wheaton) were included in the 2016 experiment, while all were included in the 2017 experiments. More information on the accessions, including their pedigrees, is presented in Appendixes 2.1 and 2.2.

#### **2.2.1.5 Experimental design**

The St. Paul field screening nurseries were sown on May 20, 2016 and May 15, 2017 while the Crookston experiment was sown on May 18, 2017. At both locations, the experimental plots were 1.5 m single row plots and plots were planted 30 cm apart with a 0.5 m alley between adjacent beds. The experimental design in each experiment was a randomized complete block design (RCBD) with three replicates. Fertilizer (23 lb of actual nitrogen/acre) was applied pre-planting in both St. Paul experiments. Herbicide (Axial; 24 oz/A + Bromac; 16 oz/A) was applied at the 3-4 leaf stage (Zadok's growth stage 13-14) in the St. Paul 2016 experiment. In the Crookston experiment, fertilizer (46-0-0; 80 lb/A, 11-52-0; 100 lb/A) was applied during planting while herbicides (Axial XL+Bromac; 1 pint/A) were applied at the 3-leaf stage (Zadok's growth stage 13). No herbicide was applied at St. Paul in 2017, instead the plots were hand-weeded throughout the growing season.

#### **2.2.1.6. Inoculations**

##### **St. Paul inoculations - *F. graminearum* macroconidial suspensions**

In each year at St. Paul, the plots were inoculated with a suspension of *F. graminearum* macroconidia, with each plot inoculated twice. The first inoculations of any given plot in the 2016 experiment was applied on July 5, 8, 11, 14, 18, 21 or 28, whereas in the 2017 experiment, the first inoculations were applied on July 6, 10, 13, 17 and 20. The first application was applied to a given plot when ca. 50% of the spikes in the plot were at anthesis. The second inoculation was applied three days after the initial inoculation (d.a.i.). The inoculum was applied at a concentration of 100,000 macroconidia.ml<sup>-1</sup> with Tween 20 (polysorbate) added to the inoculum at 2.5 ml. L<sup>-1</sup> to act as a wetting agent. The inoculum was applied using a CO<sub>2</sub>-powered backpack sprayer (R & D sprayers

[Opelousas, LA], model T) fitted with a SS8003 TeeJet spray nozzle with an output of 10 ml.sec<sup>-1</sup> at a working pressure of 275 kPa. A mist-irrigation system was installed in the nursery to facilitate FHB development and operated from July 5 to August 1 in the 2016 experiment and from July 7 to July 27 in the 2017 experiment. The mist-irrigation system was programmed to run for 15 minutes eight times per day (0000 h, 0400 h, 0600 h, 1600 h, 1800 h, 2000 h) and served to keep free-moisture on the spikes during the overnight hours.

### **Crookston inoculation - *F. graminearum*-colonized corn**

The experiment was inoculated when the majority of the plots were at the flag leaf emergence growth stage (Zadok's growth stage 15) on June 27, 2017 by spreading the dried *Fusarium*-colonized corn in the nursery at a rate of 50 lb/acre. A mist-irrigation system, similar to that established in the St. Paul nurseries, ran from June 30 to August 1 and was programmed to mist the plots for 10 min every 70 min between 1700 and 0700 daily.

#### **2.2.1.7 Data collection**

##### **Agronomic traits**

In all experiments, heading dates (HD) for each plot were determined as the days from sowing to when at least 50% of the spikes had fully emerged (Table 2.1). Plant height (PH) was measured in each plot as the distance from the soil surface to the top of spikes, excluding the awns (Table 2.1). PH was only measured in the St. Paul 2016 trial. The presence (+) or absence (-) of awns was also noted for each genotype, only in St. Paul 2016 trial (Table 2.1). Based on observations in the field, genotypes with  $\leq 49$  HD were categorized as early-maturing, those with HD between 50 and 59 were considered as mid-maturing and those with  $\geq 60$  HD were categorized as late-maturing (Table 2.1). For PH, genotypes with a height  $\leq 79$  cm were categorized as short, whereas those with a height  $\geq 80$  cm were categorized as tall (Table 2.1).

### **FHB disease assessment**

FHB development was assessed visually at 19-21 d.a.i. in the St. Paul experiments, and at 16-26 d.a.i. in the Crookston experiment. Ten spikes were considered sufficient and were arbitrarily selected in each plot. The ten spikes were used to determine both the incidence of infected spikes (defined as the percentage of spikes with visible symptoms of the 10 spikes assessed) and the infected spike severity (defined as the average percentage of symptomatic spikelets per spike, of those spikes with visible FHB symptoms). These values were used to determine the FHB index (incidence of infected spikes x infected spike severity) for each plot. FHB index is reported in this study and used in all the analyses. Based on the performance of the resistant and susceptible checks, genotypes with an average FHB index from 0 to < 30% were categorized as resistant, genotypes with an FHB index from  $\geq 30\%$  and < 45% were categorized as intermediate, and genotypes with an FHB index of  $\geq 45\%$  were categorized as susceptible (Table 2.1).

### **Harvesting**

At maturity, approximately thirty heads from primary tillers were randomly selected from each plot, and were hand harvested. Harvesting was conducted September 7 & 8, 2016 (St. Paul 2016), August 23-25 & September 5, 2017 (St. Paul 2017) and September 6 & 7, 2017 (Crookston 2017). The harvested heads were threshed using a mechanical belt thresher, operated at a low fan speed to prevent the lightweight kernels from being lost, and the resulting seed was cleaned manually. The cleaned grain sample was used to determine the percentage of visually scabby kernels (VSK) and was then submitted for mycotoxin analysis.

### **Post-harvest assessments**

#### **Percent visually scabby kernels (VSK)**

The percentage of visually scabby kernels (VSK) was estimated by comparing the samples with comparison standards, created by mixing healthy seeds of hard red spring wheat with scabby kernels, that were hand selected, to create ratios equivalent to 0, 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45 and 50% on a 1,000-kernel count basis (Jones and Mirocha, 1999). VSK assessments were determined independently by two individuals,

and the average of the two values was reported. Based on the performance of the resistant and susceptible checks, genotypes with a VSK from 0 to < 30% were categorized as resistant, genotypes with a VSK  $\geq$  30% and < 45% were categorized as intermediate, and genotypes with a VSK of  $\geq$  45% were categorized as susceptible (Table 2.1).

### **Deoxynivalenol (DON) analysis**

The samples were ground using a laboratory mill (model 3310, Perten Instruments, Hagersten, Sweden). A 4 g sub-sample was weighed out and 16 mL of acetonitrile: water (84:16, v/v) was added. The samples were mixed by being placed on a shaker and allowed to mix for 1 hr. A 4 mL aliquot of the extract was then filtered through a column packed with C18 and aluminum oxide (1:3, w/w) and the filtrate was collected in a plastic test tube. A 1 mL aliquot of the filtrate was then transferred into a 1-dram glass vial and dried under nitrogen in a fume hood for ca. 1 h 20 min and derivatized using trimethylsilyl ether (TMS). The concentration of DON in each sample was determined using gas chromatography-mass spectrometry (GC-MS) (Shimadzu GCMSQP2010; Shimadzu Corporation, Kyoto, Japan). The mycotoxin analyses were conducted by Dr. Yanhong Dong (Department of Plant Pathology at the University of Minnesota). Based on the performance of resistant and susceptible checks, genotypes with DON levels ranging from 0 to < 5 ppm were categorized as resistant, genotypes with DON from  $\geq$  5 to < 10 ppm were categorized as intermediate, and genotypes with DON  $\geq$  10 ppm were categorized as susceptible (Table 2.1).

#### **2.2.1.8 Data analysis**

The ANOVA and Pearson correlation coefficients were determined using the statistical program R (version 3.3.3). Analysis of variance (ANOVA) was conducted to test for significant differences in FHB index, VSK, DON, HD and PH. Pearson correlation coefficients were used to examine the associations between FHB index, VSK, DON, HD and PH. Broad sense heritability was calculated on an entry mean basis according to Melchinger *et al.* (1998), using the formula:

$$\delta^2 G / (\delta^2 G + \frac{\delta^2 e}{reps})$$

Where  $\delta^2G$  is genotypic variance,  $\delta^2e$  is error variance and *reps* refer to the number of replicates

## **2.2.2 Greenhouse Evaluation**

### **2.2.2.1 *F. graminearum* isolates**

The two *F. graminearum* isolates (10116010 and 10116012) used in this study were isolated from symptomatic spikes of wheat collected from commercial crops in Minnesota. The two isolates were isolated, purified and identified to species as described previously (Section 2.2.1.1). The relative aggressiveness of the two isolates was examined in greenhouse inoculations of the hard red spring wheat varieties Alsen and Wheaton and mycotoxin production capacity were examined from rice cultures of the two *F. graminearum* isolates (Table 2.2).

### **2.2.2.2 Plant materials**

Eighty-two wheat genotypes were evaluated for their reaction to two *F. graminearum* isolates in each of two greenhouse experiments. Eighty of the genotypes were selected based on their performance in the field evaluations. The genotypes selected were generally those with an FHB index of < 30% in at least one test location. In addition to selecting genotypes with promising field performance, widely cultivated varieties in Kenya and Ethiopia were prioritized given their importance in the production environments from which they were sourced. The 80 genotypes selected included six genotypes with a low FHB index (< 30%) across the three test locations, 18 genotypes with a low FHB index (< 30%) in two of the three test locations, 26 genotypes with a low FHB index (< 30%) in one of the test locations, 24 genotypes with intermediate or susceptible (FHB index >30%) ratings in three of the test locations, and six FHB checks including the FHB-resistant variety Rollag, the FHB-moderately resistant varieties LCS Albany and Linkert, and the FHB-susceptible varieties Samson, WB-Mayville and Wheaton (Table 2.3). Kwale, one of the highest yielding cultivars in Kenya and the FHB-resistant variety Alsen, neither of which had been included in the field screening due to limited seed availability, were also included in this study.

### **2.2.2.3 Experimental design**

The selected genotypes were screened for their reaction to *F. graminearum* in two greenhouse experiments conducted in the University of Minnesota, Plant Growth Facility West, St. Paul in 2017 (GH2017) and 2018 (GH2018) each using a split-plot design, where isolate was considered the main plot and cultivar the sub-plot. Five seeds were planted per pot (13.34 cm w x 13.34 cm l x 13.97 cm d; Jumbo squares, Belden Plastics, St. Paul MN) and three replicates (pots) were planted of each entry for each isolate. The growing media used was Sungro Professional Growing Mix, Metro-Mix 852 (composted bark, Canadian Sphagnum peat moss, perlite, dolomite lime and a wetting agent; Sun Gro Horticulture, Agawam MA). After sowing, the pots were placed on the greenhouse bench with temperatures maintained between 18 and 20 °C from 0500 h to 2000 h and between 15 and 17 °C from 2000 h to 0500 h. Supplemental lighting was provided for 15 h daily (0600 h-1700 h) with 400 W high-pressure sodium lamps. Osmocote Classic slow release fertilizer (14-14-14, N-P-K; 2.5 g/pot) was applied at the 2-3 leaf stage and pots were watered as necessary, at least every two days.

### **2.2.2.4 Inoculum production, inoculation and assessment of FHB severity**

Macroconidial suspensions (100,000 macroconidia.ml<sup>-1</sup>) were prepared, as described previously, for each of the two *F. graminearum* isolates. When the majority of the plants in given pot reached anthesis, the middle floret in a central spikelet in each of the five primary spikes per pot (one spike/plant) were marked with a permanent marker pen and 10 µL of the macroconidial suspension was pipetted between the lemma and palea of each marked floret. Inoculated spikes were covered with clear, reclosable plastic bags (3 x 6-inch, 2 mil thick) for 72 h to maintain humidity. FHB severity (average percent symptomatic spikelets) was assessed visually 21 d.a.i. for each inoculated spike.

### **2.2.2.5 Data Analysis**

The statistical program R (version 3.3.3) was used in all analyses. The mean FHB severity of the five plants per replicate (pot) was determined and used as the replicate value. An ANOVA was conducted to test for significant differences among the genotypes inoculated with two *F. graminearum* isolates in GH2017 and GH2018 experiments.

Pearson correlation coefficients were used to determine the association between isolates 10116010 and 10116012 in GH2017 and GH2018, respectively, and the association between FHB severity in GH2017 and GH2018 following inoculation with isolate 10116010 and with isolate 10116012, respectively.

## **2.3 Results**

### **2.3.1 Field evaluations of Fusarium head blight - FHB index**

The ANOVA for FHB index in the three field experiments confirmed significant differences among the genotypes, replications, locations, and genotype by location interactions, and broad sense heritability was 0.74 (Table 2.4). The mean FHB index for all the genotypes in the STP16 experiment was higher (58.7%) than the mean FHB index in either the STP17 (36.9%) or CRK17 (40.2%) experiments (Table 2.1, Figure 2.1). The FHB indexes ranged from 9.2 to 97.5% in the STP16 experiment, 9.1 to 86.8% in the STP17 experiment, and from 1.2 to 82.5% in the CRK17 experiment (Table 2.1). Across the three test locations, the range of the FHB index for each of the resistant checks Rollag, LCS Albany, and Linkert, ranged from 2 1.1 to 25.1%, 19.7 to 32.7%, and 23.1 to 26.6%, respectively. The range of the FHB index for the susceptible checks Samson, WB-Mayville, and Wheaton ranged from 28.1 to 62.6%, 34.1 to 44.3%, and 34.1 to 46.1%, respectively (Table 2.1).

Among the 215 Kenyan and Ethiopian genotypes that were evaluated, six genotypes: Bollo, Kenya Eagle 10, ETBW 7213, Menze, Njoro BW II, and R 1286, appeared to have stable FHB resistance as each recorded an FHB index < 30% in all three test locations (Table 2.1). Eighteen additional genotypes displayed a low FHB index (< 30%) in two of the three test locations. Among the 18 genotypes were nine named cultivars; eight from Kenya (Bonza 63, Kenya Cheetah, Kenya Jay, Morris, P. Walker Munro, Tama, Trophy and 1012. B.1(L)) and one cultivar (Sofumar) from Ethiopia (Table 2.1). Forty-seven genotypes recorded a low FHB index (< 30%) in one of the three test locations. Among these 47 genotypes were six named cultivars from Kenya (Bailey, Beacon-Ken, Kenya Goblet, Kenya Civet, Kenya Korongo, and RFN) and four named cultivars from Ethiopia (Digelu, Gasay, Honqolo and Tay) (Table 2.1). Most of the tested genotypes (65%) were

susceptible to FHB in all three experiments (Table 2.1). The genotypes that were identified as FHB susceptible included six named cultivars from Kenya (Kenya Kingbird, Kenya Page, Kenya Paka, Kenya Wren, Lenana and Primex) and 18 named cultivars from Ethiopia (Alidoro, Biqa'a, Danda'a, Dure, Kenya Gabrino, Galama, Gambo, Hawi, Hidase, Hoggana, Hulluka, KBG-01, Kingbird, Kubsa, Mada-Walabu, Millenium, QulQullu and Shorima) (Table 2.1).

### **2.3.2 Post-harvest assessments**

#### **Visually Scabby Kernels (VSK)**

The ANOVA indicated significant differences among the genotypes, replications, locations and genotype by location interactions for VSK, and broad sense heritability was 0.86 (Table 2.4). The mean VSK for the genotypes in the STP16 experiment was 31.6%, 30.7% in the STP17 experiment and 33.3% in the CRK17 experiment (Table 2.1, Figure 2.1B). In the STP16 experiment, VSK ranged from 1.7 to 62.5%, while the VSK's ranged from 7.7 to 50.8% in the STP17 experiment, and from 4.3 to 70.8% in the CRK17 experiment (Table 2.1, Figure 2.1B). The VSK value for the resistant checks Rollag, LCS Albany, and Linkert ranged from 7.7 to 10%, 7.7 to 9.7%, and 9.5 to 19.2%, respectively. The range of values for the VSKs for the susceptible checks were 35 to 48.3% for Samson, 30 to 38.3% for WB-Mayville, and 32.5 to 39.2% for Wheaton (Table 2.1).

Twenty-eight genotypes that included; LCS Albany, Bollo, Digelu, Kenya Eagle 10, ETBW 7213, ETBW 7364, ETBW 7724, Gasay, Kenya Seed 2, Kenya Seed 5, Kenya Seed 6, Linkert, Menze, Mada-Walabu, P. Walker Munro, Ravi 11, Ravi 43, Ravi 44, Rollag, R 1286, R 1397, R 1408, R 1415, R 1424, R 1429, R 1460, Tay and Trophy had average VSK values less than 30% in all three of the test locations (Table 2.1). Among these 28 genotypes, seven genotypes (Bollo, Kenya Eagle 10, ETBW 7213, Linkert, Menze, Rollag and R 1286) had also recorded a low FHB index (< 30%) in all three test locations, six genotypes (Kenya Seed 5, Mada-Walabu, R 1397, R 1408, R 1415 and Ravi 44) had recorded a high FHB index (> 30%) in the three test locations, while the remaining 15 genotypes had recorded a low FHB index (< 30%) in one or two of the test locations (Table 2.1).



## **DON concentrations**

The ANOVA indicated significant differences among the genotypes, replications, locations and genotype by location interactions for the DON concentration of harvested grain, and broad sense heritability was 0.66 (Table 2.4). The DON levels were generally higher in the CRK17 experiment than in either the STP16 or STP17 experiments, with overall means of 26.6 ppm, 10 ppm, and 7.5 ppm, respectively (Table 2.1; Figure 2.1C). The DON concentrations for the entries in STP16 ranged from 0.7 to 27.8 ppm, while in STP17 the DON ranged from 1.2 to 21.9 ppm and in CRK17 the DON ranged from 6.2 to 70.8 ppm (Table 2.1). The DON concentrations for the resistant checks, LCS Albany, Linkert, and Rollag, ranged from 1.2 to 8.8 ppm, 3.2 to 12.9 ppm, and 1.6 to 6.9 ppm, respectively, while the DON concentrations for the susceptible checks, Samson, WB-Mayville, and Wheaton, were 3.8 to 24.0 ppm, 5.5 to 22.3 ppm, and 17.8 to 30.5 ppm, respectively (Table 2.1).

None of the evaluated genotypes had a DON concentration less than 5 ppm across all three experiments. However, 28 genotypes had a DON concentration < 5 ppm in two of the three experiments; St. Paul 2016 and St. Paul 2017. For the genotypes that were identified as resistant (FHB index < 30% across the three experiments), four genotypes, LCS Albany, Kenya Eagle 10, Linkert, and Rollag, had less than 5 ppm DON in two of the three experiments. Four genotypes (Bollo, ETBW 7213, Menze, and Njoro BW II) had a DON concentration < 5 ppm in only one experiment (STP16). By contrast, the genotype R 1286 had a DON concentration > 10 ppm in all three experiments (Table 2.1). For the genotypes that were considered having intermediate resistance or susceptible responses to FHB (FHB index > 30%), only 27 genotypes had a DON concentration < 5 ppm in at least one experiment (Table 2.1).

### **2.3.3 Agronomic traits**

#### **Heading dates**

The ANOVA indicated significant differences among the genotypes, replications, locations and genotype by location interactions for HD, and broad sense heritability was 0.94. All the genotypes that recorded a low FHB index (< 30%) in either one, two, or

three of the test locations were either early or mid-maturing, except for 12 genotypes (Bollo, Bonza 63, ETBW 6496, ETBW 7213, Kenya Cheetah, Kenya Jay, Menze, Njoro BW II, R 1286, R 1429, R 1462 and Kenya 1012-B-1-L that were late maturing, with heading dates ranging from 60 to 63 days (Table 2.1). All genotypes that were considered to be susceptible, having a high FHB index in all three experiments, were early or mid-maturing, except for the cultivar Hoganna from Ethiopia that was late in maturity (Table 2.1).

### **Plant height**

Of the 215 evaluated genotypes for their response to FHB, both resistant and susceptible genotypes were short or tall (Table 2.1). The resistant genotypes that were short included Kenya Eagle 10, ETBW 7213, Menze, Njoro BW II and R 1286 and the resistant genotypes that were tall included Bailey, Bollo, Kenya Goblet, Kenya Jay, Morris, P. Walker Munro, Tama and Trophy (Table 2.1).

### **Presence or absence of awns**

Ten of the 215 genotypes evaluated in the field were awnless; these were Bailey, Beaco-Ken, Bonza-63, Kenya Cheetah, Kenya Civet, Kenya Jay, Morris, Primex, Tama and Trophy (Table 2.1). These awnless genotypes were all FHB resistant in at least one of the test locations, except for Primex, which was susceptible in all three experiments (Table 2.1).

## **2.3.4 Correlations between the examined variables**

### **FHB Index and VSK**

Positive correlations were observed between FHB index and percent VSK in all three locations (STP16,  $r = 0.56$ ; STP17,  $r = 0.32$ ; and CRK17,  $r = 0.65$ ) (Figure 2.2, Table 2.5). The correlation between FHB index and VSK was however, less strong in the STP17 experiment compared to either STP16 or STP17.

### **FHB Index and DON**

Positive correlations were observed between FHB index and DON in the three experiments, although the relationship was stronger in the STP16 experiment ( $r = 0.54$ ) than in the CRK17 ( $r = 0.39$ ) and STP17 ( $r = 0.22$ ) experiments (Figures 2.3, Table 2.5).

### **VSK and DON**

Strong positive correlations were observed between VSK and DON in the STP16 ( $r = 0.73$ ) and CRK17 ( $r = 0.74$ ) experiment, while only a weak positive correlation was observed between VSK and DON in the STP17 ( $r = 0.48$ ) experiment (Figures 2.4, Table 2.5).

### **Heading date and FHB Index**

Negative correlations were observed between HD and FHB index in all three experiments, although the correlation was much weaker in the STP16 ( $r = -0.18$ ) and STP17 ( $r = -0.13$ ) experiments, compared to the CRK17 experiment ( $r = -0.50$ ) (Figures 2.5, Table 2.5).

### **Plant Height and FHB Index**

Negative correlations were observed between PH and HD in both the STP16 ( $r = -0.13$ ) and CRK17 ( $r = -0.20$ ) experiments. No significant correlation was observed between PH and HD in the STP17 experiment ( $r = -0.01$ ) (Figures 2.6, Table 2.5).

#### **2.3.4 Greenhouse evaluations - Fusarium head blight**

The greenhouse tests were carried out with the aim of confirming the responses of select genotypes (resistant and susceptible) that had been evaluated in the field experiments. Results from the ANOVA tests showed that tested genotypes varied in their response to *F. graminearum* (Tables 2.6 and 2.7). Isolate 10116012 of *F. graminearum* generally appeared more aggressive than isolate 10116010, with higher FHB severities observed among the genotypes inoculated with this isolate in both greenhouse seasons (Table 2.3). Positive correlations were observed between the FHB severities in GH2017 and in GH2018 following inoculation with the two *F. graminearum* isolates, indicating that the

relative ranking genotypes was much the same irrespective of which isolate was used (Appendixes 2.3, 2.4, 2.5 and 2.6).

Of the six Kenyan and Ethiopian genotypes selected as they had shown consistently low FHB in the field tests (FHB index < 30% across all three field experiments), tested along with the FHB-resistant check Rollag and the FHB-moderately resistant check Linkert, five (Kenya Eagle 10, ETBW 7213, Menze, Njoro BW II, and R 1286), along with Rollag and Linkert, demonstrated resistance to the two isolates, having FHB severities lower than the mean ( $\leq -1$  standard deviation (sd) from the mean). Only one of the six genotypes, the genotype Bollo, had an FHB severity higher than the mean (+1 sd above mean) to both isolates (Table 2.3).

Among the 18 genotypes that had demonstrated a low FHB index (< 30%) in two of the three field experiments, seven genotypes (Kenya Cheetah, Kenya Jay, Kenya Seed 7, P. Walker Munro, Ravi 43, Tama, and Trophy) and the moderately resistant check variety LCS Albany showed stable resistance to the two isolates, with average FHB severities more than 1 sd below the experimental mean (Table 2.3). Five of the 19 genotypes that appeared resistant in the field; Bonza 63, Morris, R 1454, R 1462, and Sofumar were recorded as susceptible to both the isolates in the greenhouse, having FHB severities higher than the mean ( $\geq +1$  sd from the mean) (Table 2.3). The remaining six genotypes selected based on their promising field performance (Kenya 1012-B-1-L, ETBW 6109, ETBW 8469, Kenya Seed 6, Ravi 46 and R 1404) showed unstable resistance in the greenhouse experiments, recording FBH severities than were both above and below the experimental means (Table 2.3).

Of the 47 genotypes that showed a low FHB index (< 30%) in only one of the three test locations, 26 genotypes were selected and tested in the greenhouse (Table 2.3). Of these 26 genotypes, nine (Digelu, ETBW 7364, Kenya Goblet, Honqolo, Kenya Korongo, Kenya Seed 2, R 1360, R 1403 and Tay) showed stable resistance to the two isolates, recording FHB severities lower than the experimental mean ( $\leq -1$  sd from the mean) in both greenhouse experiments (Table 2.3). Seventeen genotypes, of the 26 selected, did

not perform well in the greenhouse tests, and all recorded FHB severities above the experimental mean in the greenhouse (Table 2.3).

Of the 147 genotypes that were susceptible across the three test locations, only 27 genotypes, including the susceptible checks WB-Mayville, Samson, and Wheaton, were selected for further testing in the greenhouse (Table 2.3). Of the 27 selected genotypes, all were susceptible in the greenhouse, recording higher FHB severities than the experimental mean, except for five genotypes (Galama, Hawi, Kenya Kingbird, Kubsa and R 1441), that had lower FHB severities than then mean (0 to -1 sd from the mean) (Table 2.3).

Two genotypes, cultivar Kwale and the FHB-resistant check variety Alsen, were not evaluated in the field but were tested in the greenhouse. Both genotypes appeared resistant in the greenhouse, with lower FHB severities than the experimental mean for both the *F. graminearum* isolates with which they were challenged (Table 2.3).

## **2.4 Discussion**

The genotypes evaluated for their response to FHB in this study were part of a collection of wheat lines sent to the USDA-ARS Cereal Disease Laboratory (CDL) for stem rust screening at the seedling stage. The tested genotypes included cultivars that are currently grown in Kenya and Ethiopia and older cultivars including varieties that were released in the 1960s, but which are no longer in commercial production. Many of these older lines carry traits that are considered undesirable, including being tall. Also included in the collection examined were a number of CIMMYT-derived lines, that are presently under selection in Kenya and Ethiopia, and which therefore have the potential of being released as cultivars in East Africa.

All lines in the collection were assessed for their response to FHB in the field and selected lines were then assessed in the greenhouse. Field screening of the genotypes in the field involved two inoculation methods, spray inoculation method and *Fusarium*-colonized corn method used in St. Paul and Crookston field experiments, respectively.

The traits measured to quantify the response of the plants included: FHB index, percent VSK, DON concentration, HD and PH. FHB development has been reported to be influenced by environmental conditions (Mesterhazy, 1995, Parry *et al.*, 1995; Lacey *et al.*, 1999; De Wolf *et al.*, 2003; Xu *et al.*, 2008). Other than the environmental conditions, morphological traits of plants have been reported to confound FHB data recorded under field conditions (Rudd *et al.*, 2001). Factors influencing disease development include the inoculation method(s) used (i.e. macroconidia inoculation vs. *Fusarium*-colonized corn). The inoculation method and timing may also be confounded by maturity and this interaction likely contributed to the observed differences in FHB index and related variables among the genotypes in this study. Therefore, further screening of promising genotypes was carried out in the greenhouse using a point inoculation method, which measures the spread of the fungus within the spike and is presumably not influenced by morphological traits like height, maturity/heading time, or the presence or absence of awns.

All traits measured varied among the genotypes and differed from one location to the other, with the observed differences attributed to the combination of genetic and environmental influences. Other studies have reported differences in FHB severity/incidence/index and related variables among evaluated wheat (winter, spring, emmer), barley (winter, spring) and oats (Bonin and Kolb, 2009; Bai *et al.*, 2001; Buerstmayr *et al.*, 2003, 2004; Zhang *et al.*, 2008; Gagkaeva *et al.*, 2013; Berger *et al.*, 2014). A high heritability of 0.74, 0.86, 0.66 and 0.94 were observed on FHB index, percent VSK, DON concentration and HD, respectively, which suggests possibilities of improvement of these traits. High heritabilities in these traits have been observed in other studies (Draeger *et al.*, 2007: heritability of 0.75 for DON, 0.77 for FHB severity; Klahr *et al.*, 2007: heritability ranging from 58.9 to 82.3 for FHB severity; He *et al.*, 2013: heritability ranging from 0.63 to 0.86 for FHB index; Miedaner and Longin, 2014: heritability ranging from 0.72 to 0.74 for FHB severity, 0.76-0.87 for HD, 0.89 for PH; Brisco *et al.*, 2017: heritability of 0.84 for FHB severity).

The higher FHB severities recorded in the STP16 experiment, compared to other two field experiments, may have been caused by higher temperatures during the infection period that affected initial infection and symptom development. Similar findings have been reported and attributed to high temperatures and humidity (Bonin and Kolb, 2009; Berger *et al.*, 2014). The observed continuous distribution for the measured FHB index suggests quantitative inheritance of FHB resistance, this in agreement with statements and findings of other studies (Shroeder and Christensen, 1963; Buerstmayr *et al.*, 2000; Rudd *et al.*, 2001; Bonin and Kolb, 2009). In our study, 6 (3%) and 18 (8%) of 215 evaluated genotypes across all three and two of three field experiments, respectively, were resistant, which is in line with the proportion of resistant genotypes identified in other studies (Oliver *et al.*, 2007: 7% in wild emmer wheat collection; Zhang *et al.*, 2008: 7% in spring wheat accessions; Gagkaeva *et al.*, 2013: 8 to 4.8% in oat collection).

The selected genotypes were screened in the greenhouse using two isolates. Isolate 10116012 appeared to be more aggressive than isolate 10116010 as it resulted in higher FHB severities in both greenhouse experiments. It was expected in this study that isolate 10116010 may have been more aggressive as it had a higher capacity to produce mycotoxins, including DON and 15-ADON, compared to isolate 10116012. A study by Panthi *et al.* (2014) showed that the most aggressive isolate generally produced higher concentrations of DON in culture. The positive correlations observed between the FHB severities in GH2017 and in GH2018 indicated that the relative ranking of genotypes was much the same irrespective of which isolate was used and greenhouse results.

Of the 215 genotypes from Kenya and Ethiopia evaluated in the field, six genotypes appeared to have stable resistance in all three experiments. The six genotypes identified as having the best resistance include two cultivars from Kenya (Kenya Eagle 10 and Njoro BW II), two cultivars from Ethiopia (Bollo and Menze), and two CIMMYT lines (ETBW 7213 and R 1286). The CIMMYT lines ETBW 7213 and R 1286 were selected in Ethiopia and Kenya, respectively. The field performance of the lines as determined by FHB index was generally supported by the percentage of scabby kernels in the six genotypes, which was low across the three locations. The DON levels associated with

these six lines were not consistent across the three field experiments. Kenya Eagle 10 did have less than 5 ppm of DON in the grain harvested from two of the three field experiments, however, the DON levels in Bollo, ETBW 7213, Menze, and Njoro BW II were below 5 ppm in only one of the three field experiments (STP16). The sixth genotype to record consistently low FHB indexes in the three experiments was R 1286, and this line had higher DON levels (>10 ppm) in the grain harvested in all three field experiments. The agronomic traits of these six lines differed considerably, Kenya Eagle 10 was an early maturing cultivar (44.7 days), whereas the remaining five (Bollo, ETBW 7213, Menze, Njoro BW II, and R 1286) were late maturing. All the six genotypes were awned and short, except for Bollo that was tall. The greenhouse performance of five of these six genotypes (Kenya Eagle 10, ETBW 7213, Menze, Njoro BW II and R 1286) was generally similar to Rollag and Linkert, which served as FHB-resistant and FHB-moderately resistant checks, respectively. It appears then that the greenhouse tests helped to confirm the resistance in these five genotypes. The response of the cultivar Bollo in the greenhouse did not match the field performance, with Bollo recording high FHB severities in the greenhouse to both the isolates it was tested against. It may be that Bollo, which we note was tall, may have escaped FHB infection in the field. Tall lines have been reported to have either lower FHB infections or no infections at all (Buerstmayr *et al.*, 2000, 2004; He *et al.*, 2014). The FHB resistance in Njoro BW II has been reported before, with results ranging from resistant to intermediate. Muthomi *et al.* (2007), using mixture of isolates in a greenhouse study, observed an FHB severity of 9.0% while Okumu *et al.* (2016), from two field experiments, observed FHB severities ranging from 30 to 44.4%.

These five resistant genotypes, with acceptable height except for Bollo are therefore reported here as new sources of FHB resistance. The pedigree information of these genotypes is different from the commonly known sources of Chinese origin, including Sumai 3, Ning7840, Ning 894037, Wangshuibai, Wuhan-1, suggesting possibilities of different underlying resistance genes (Somers *et al.*, 2003; Cuthbert *et al.*, 2007; Yu *et al.*, 2008).



Of the genotypes that recorded low FHB index in two of the three field experiments, seven genotypes (Kenya Cheetah, Kenya Jay, Kenya Seed 7, P. Walker Munro, Ravi 43, Tama, and Trophy), and the moderately resistant check LCS Albany, also demonstrated resistance to the two isolates in the greenhouse tests. This appears to confirm that these lines have a useful level of genetic resistance. These lines differed considerably in the agronomic traits measured in this study including maturity (Kenya Seed 7, P. Walker Munro, Ravi 43, Tama, and Trophy were early maturing lines, while Kenya Cheetah, and Kenya Jay were late maturing), plant height (tall: P. Walker Munro, Tama, and Trophy; short: Kenya Seed 7 and Ravi 43), and the presence or absence of awns (awned: Kenya Seed 7, P. Walker Munro, and Ravi 43; awnless: Kenya Cheetah, Kenya Jay, Tama, and Trophy). The percentage of scabby kernels appeared to be low in P. Walker Munro, Ravi 43, Trophy and the moderately resistant check LCS Albany. These seven genotypes also recorded inconsistent DON levels, with higher DON levels observed in Crookston compared to the St. Paul experiments. The different environmental conditions, including the conditions between disease assessment and harvest, may have contributed to the observed differences in DON levels between the experiments. Similar findings were observed in other studies (He *et al.*, 2013; He *et al.*, 2014), and the existence of strong type I and/or type II resistance but weak resistances to DON accumulation in such genotypes has been observed. Such findings suggest that the genes controlling the FHB resistance mechanisms and related parameters are independent. Due to the stability in resistance of these genotypes under greenhouse conditions, the genotypes may still be of use as sources of resistance. Because the field response of these lines was not stable in all experiments, further testing of these genotypes is recommended before they are definitively classified and utilized in breeding programs.

The resistance of nine genotypes Digelu, ETBW 7364, Kenya Goblet, Honqolo, Kenya Korongo, Kenya Seed 2, R 1360, R 1403, and Tay, that had a low FHB index in only one of the three field experiments, is still of interest because these nine genotypes also appeared to be resistant to both isolates in the greenhouse experiments. Of these nine genotypes, only four (Digelu, ETBW 7364, Kenya Seed 2, and Tay) had low VSK

values. The observed DON levels in these nine genotypes were less than 10 ppm in St. Paul field experiments compared to that observed in the Crookston field experiment ( $\geq 10$  ppm), although the DON levels in ETBW 7364, Kenya Seed 2, Tay, R 1360, and R 1403 genotypes were lower than the experimental mean. The differences in DON levels across the field experiments may again largely be attributed to environmental conditions. Even though these genotypes show some potential as FHB sources, further screening in more locations is recommended to confirm the stability of their resistance under field conditions.

Genotypes that were susceptible across the three field experiments but recorded lower FHB severities than the experimental means in the greenhouse included Galama, Hawi, Kenya Kingbird, Kubsa, and R 1441. The FHB infection and progression may have been favored by the environmental conditions and further testing of these genotypes in different environments is recommended. These results suggest that these genotypes may lack type I resistance but have some type II resistance.

The susceptibility of genotypes Alidoro, ETBW 7872, Kenya Wren, Kingbird, Lenana, Primex, Ravi 1, Ravi 6, and R 1363 was confirmed to be stable across the three field experiments and to the two isolates in the greenhouse, and these genotypes are therefore candidates to be used as FHB susceptible checks in FHB screening nurseries in Kenya and Ethiopia. Cultivars from Ethiopia including; Biqa'a, Dandaa, Dure, Gambo, Hidase, Hoggana, Hulluka, KBG-01, Mada-Walabu, Millennium, and Shorima, and cultivars from Kenya including; Kenya Gabrino and Kenya Paka were also susceptible across the three field experiments. Because these genotypes were not among those tested in the greenhouse, further testing of these genotypes is recommended to ascertain their susceptibility if they were to be used in greenhouse experimentation. From the 215 evaluated genotypes, 147 (68%) appeared susceptible across the three field experiments and includes varieties that have been widely cultivated in Kenya and Ethiopia. These findings suggest there is the possibility of significant yield loss and mycotoxin contamination in wheat growing regions in Kenya and Ethiopia if FHB became prevalent.

The study therefore emphasizes on efforts to improve levels of FHB resistance in East African germplasm.

Kwale, one of the high yielding Kenyan cultivars that showed stable resistance to the two isolates in the greenhouse, still needs to be tested in the field to confirm the resistance. A greenhouse study by Muthomi *et al.* (2007) using a mixture of isolates reported the FHB severity of Kwale at 33.3%, although no information was given on aggressiveness of the isolates used. This suggests that the cultivar can be susceptible if the disease pressure is high.

The observed positive correlations between FHB index and percent VSK in all three experiments agree with the findings reported by others (He *et al.*, 2014; He *et al.*, 2015). The positive association between the two variables could suggest possible selection of genotypes with reduced kernel damage based on low visual estimate of disease symptoms in the field or vice versa. The correlation was less strong in the STP17 experiment compared to either STP16 or CRK17 and this could be due to the lower mean FHB index and lower mean VSK in STP17, compared to either the STP16 or CRK17 experiment.

Positive correlations were observed between FHB index and DON in the three field experiments, with most genotypes having a low FHB index also observed to have a lower level of DON, although this relationship was strongest in the STP16 experiment. Positive associations between FHB index and DON have been reported by other researchers (Bai *et al.*, 2001; Urrea *et al.*, 2002; Buerstmayr *et al.*, 2004; Bonin and Kolb, 2009; Khatibi *et al.*, 2012; Berger *et al.*, 2014; He *et al.*, 2014; He *et al.*, 2015), although there is also evidence of reduced associations between the two variables (Tekauz *et al.*, 2000; Lemmens *et al.*, 2005). Observations of low DON levels in inoculated experiments have been attributed to reduced fungal biomass or by the degradation of DON in resistant genotypes (Miller *et al.*, 1985). The weak correlation in STP17 appears to be largely related to the overall low level of disease (low mean FHB index) compared to the other experiments. Less conducive weather conditions, primarily temperature and moisture,

may contribute to a lower FHB index, as observed among the genotypes in the STP17 experiment.

Stronger correlations were observed between VSK and DON than between FHB index and either VSK or DON. The strong correlations between these variables (VSK and DON) is expected because the variables are measured on the harvested grain sample, while FHB index was measured approximately two weeks before harvest on whole head in the field plots. Environmental influences between disease assessment and harvest may also have influenced both post-harvest variables. Very strong correlations were observed between VSK and DON in the STP16 and CRK17 experiments, while only a weak positive correlation was observed between VSK and DON in the STP17 experiment. The weak correlation in STP17 may again have been due to the lower overall means of VSK and DON in STP17, compared to either the STP16 or CRK17 experiments. Similar findings were observed in other studies (Jones and Mirocha, 1999; Ma *et al.*, 2009; He *et al.*, 2014; Jin *et al.*, 2014). The stronger correlations between VSK and DON suggests that selecting genotypes with a lower VSK value could be more reliable in selecting for genotypes with low DON levels than using visual ratings in the field.

Negative correlations were observed between heading date and FHB index in all three experiments, with most early and late maturing genotypes recording lower FHB indexes across the three locations. The delay in maturity period for genotypes in the Crookston experiment, compared to the St. Paul experiments, may have contributed to the weaker correlations observed in the STP16 and STP17 experiments, compared to the CRK17 experiment. Other studies have reported negative associations between HD and FHB index (Choo *et al.*, 2004; Ma *et al.*, 2009; Khatibi *et al.*, 2012; He *et al.*, 2014). The reported negative associations between HD and FHB could be due to varying environmental factors such as temperature, humidity and precipitation around the flowering time allowing for the possibility of disease escape for the early and/or late maturing genotypes. The association may also be due to the overlap of QTL conferring heading time and response to FHB as reported by Dahleen *et al.* (2003), Mesfin *et al.* (2003), Buerstmayr *et al.* (2012) and He *et al.* (2014). However, positive associations

between HD and FHB development have also been reported (Ma *et al.*, 2000). In this study, the inoculation of the experimental field plots, especially the use of macroconidial inoculum applied in relation to the heading date of the plot, should have minimized the chances of any genotype escaping FHB infection, although the possibility of escapes is not completely ruled out. Since *Fusarium*-colonized corn grain were spread few weeks before anthesis in Crookston experiment, and serves to provide inoculum over a longer period of time, early and late maturing genotypes may have escaped the infection if inoculum was not available or if environmental conditions were not favorable for disease development as early or late maturing lines reached anthesis.

Few significant correlations were observed between PH and FHB index. The lack of a significant correlation implies that low and high FHB index were observed in both short and tall genotypes. Short genotypes are generally considered to be more susceptible than tall genotypes (Mesterházy, 1995), but the findings of this study do not support this statement. There were however no very short or very tall genotypes included in this study. The reduced height (*Rht*) genes including *Rht1*, *Rht2*, *Rht8*, *Rht9*, and *Rht10* control plant height as do several QTLs that have been identified (Cadalen *et al.*, 1998; Buerstmayr *et al.*, 2002; Ellis *et al.*, 2005). Mao *et al.* (2010) confirmed a negative association of FHB resistance with the *Rht1*, *Rht2*, and *Rht8* genes. A negative association between plant height and resistance to FHB has also been reported in other studies (Gervais *et al.*, 2003; Somers *et al.*, 2003; Paillard *et al.*, 2004; Klahr *et al.*, 2007; Talas *et al.*, 2011; Buerstmayr *et al.*, 2012; He *et al.*, 2015). The negative association between these two variables has been suggested to result from linkage, pleiotropy, or morphological escape (Mesterházy 1995; Buerstmayr *et al.*, 2000; Draeger *et al.*, 2007) although Gervais *et al.* (2003) concluded that pleiotropy did not explain an association between FHB and PH in their study. Yan *et al.* (2011), using near isogenic lines (NILs) for different *Rht* genes, reported better FHB resistance in tall isolines than in dwarf isolines.

An association between presence, or absence, of awns with FHB resistance have been reported (Mesterházy, 1995; Tamburic-Ilincic *et al.*, 2007). In this study, the awnless

genotypes Bailey, Beaco-Ken, Bonza-63, Kenya Cheetah, Kenya Civet, Kenya Jay, Morris, Tama, and Trophy all appeared to be FHB resistant in at least one of the field experiments, while one awnless cultivar, Primex, was susceptible in all three experiments. The greenhouse results for these genotypes, except for Beaco-Ken, that was not included in the greenhouse tests, showed variation in the results from the field results, with some genotypes maintaining their resistance while some appeared to lack stable resistance. Tamburic-Ilicic *et al.* (2007), using a spray inoculation method, observed a lower FHB index in awned lines compared to awnless ones in their study. The lower FHB index observed in the awned lines in this study was thought to be related to the awns interfering with the deposition of macroconidia onto the spikes. By contrast, Mesterházy (1995) in a study conducted under natural conditions, reported high FHB severities in awned wheats compared to awnless wheats, attributing his observations to the larger spike surface area of awned wheats that are exposed to airborne spores and the possibility of these wheats retaining more moisture compared to their awnless counterparts.

## **2.5 Conclusions**

This study identified resistance sources that can be used in improving the FHB resistance levels in Kenyan and Ethiopian wheat germplasm. Identification of underlying resistance genes in the identified sources is recommended. The identified resistant and susceptible genotypes will be useful as checks in FHB experiments in Kenya and Ethiopia. However, the susceptibility of a large percentage (68%) of the evaluated genotypes shows that Kenya and Ethiopia are FHB prone and that they are at risk to yield loss and mycotoxin contamination if FHB became prevalent. The study identified positive associations between FHB index, VSK and DON and negative associations between FHB index, PH and HD. The absence of awns in wheat spikes may have contributed more resistance in the field, although the association between the absence of awns with FHB resistance warrants more studies. In East African countries, Kenya and Ethiopia are among the leading countries that produce wheat. Findings of this study therefore emphasize on more research in FHB disease, in addition to the currently devastating stem rust disease. This step will help minimize yield losses in case of an epidemic, and also minimize or prevent mycotoxin contamination in grains. It is therefore recommended that screening for FHB

resistance should be initiated in East African region, and other methods like use of prediction models could also be considered as one of future FHB management strategy.

## 2.6 Tables

**Table 2.1.** Fusarium head blight (FHB) Index (%), visually scabby kernel (VSK) (%), deoxynivalenol (DON) concentration (%), heading dates (HD) (days after planting), plant height (cm), presence (+) or absence (-) of awns for 221 wheat genotypes inoculated with *Fusarium graminearum* in mist-irrigated nurseries conducted at St. Paul (2016 & 2017) and Crookston 2017 in Minnesota, USA. The genotypes are grouped based on their country of origin and then ranked alphabetically within the groups.

Genotype	ST. PAUL - 2016						ST. PAUL – 2017				CROOKSTON - 2017			
	FHB Index (%)	VSK (%)	DON (ppm)	HD (days)	Plant Height (cm)	Awns (+/-)	FHB Index (%)	VSK (%)	DON (ppm)	HD (days)	FHB Index (%)	VSK (%)	DON (ppm)	HD (days)
<b>Kenyan cultivars/lines<sup>a</sup></b>														
Bailey	58.1	32.5	5.5	49.0	89.3	-	39.3	30.8	5.3	53.0	25.5	13.0	9.5	52.7
Beacon-Ken	78.1	25.8	5.8	57.3	83.0	-	40.4	33.3	6.7	58.3	29.6	35.0	25.5	56.3
Bonza-63	-	-	-	-	74.7	-	24.0	44.2	6.1	59.7	7.2	19.2	14.0	62.3
K. Cheetah	-	-	-	-	-	-	9.1	24.2	8.6	63.7	4.7	7.0	12.5	60.7
K. Civet	-	-	-	-	-	-	-	-	-	-	12.9	24.2	23.2	62.3
K. Eagle 10	20.6	18.3	3.4	44.7	69.5	+	19.5	27.5	1.7	49.3	19.6	18.8	13.6	52.0
K. Gabrino	62.4	26.7	8.6	58.7	80.0	+	54.7	43.3	10.6	57.3	57.3	31.7	24.4	57.3
K. Goblet	33.3	23.3	5.0	44.7	82.7	+	40.6	22.5	10.0	56.0	21.1	30.8	14.1	53.3
K. Jay	22.1	2.2	2.3	64.7	87.5	-	31.8	12.3	5.0	59.3	1.2	5.7	9.4	63.7
K. Kingbird	81.9	36.7	12.2	53.0	74.0	+	36.8	40.8	10.5	50.0	54.9	38.3	30.9	52.7
K. Korongo	39.8	25.0	7.9	57.7	75.3	+	49.0	39.2	11.9	56.7	28.8	25.0	24.4	57.0
K. Page	84.4	35.8	7.8	49.3	80.0	+	67.2	24.2	4.2	53.3	51.5	16.7	10.0	55.7



Genotype	ST. PAUL - 2016						ST. PAUL – 2017				CROOKSTON - 2017			
	FHB		DON	HD	Plant		FHB		DON	HD	FHB		DON	HD
	Index	VSK			Height	Awns	Index	VSK			Index	VSK		
(%)	(%)	(ppm)	(days)	(cm)	(+/-)	(%)	(%)	(ppm)	(days)	(%)	(%)	(ppm)	(days)	
K. Paka	43.6	19.0	4.5	42.0	70.0	+	38.0	22.5	5.2	47.3	70.7	32.5	12.6	49.0
K. Wren	54.2	25.0	4.0	56.3	79.0	+	50.5	27.5	6.2	54.0	37.8	34.2	25.5	56.7
K.1012-B-1-L	-	-	-	-	73.0	+	11.1	10.2	2.1	61.3	9.3	7.5	16.7	63.7
K. Seed 1	45.1	25.8	5.3	57.7	63.3	+	37.0	33.3	7.6	58.0	60.6	31.7	25.0	55.0
K. Seed 2	32.1	16.7	3.0	58.0	77.5	+	32.5	22.5	3.7	55.7	28.4	20.0	17.2	57.7
K. Seed 3	50.7	34.2	3.7	55.7	77.7	+	62.7	38.3	4.8	52.0	31.7	34.2	19.0	55.3
K. Seed 4	71.4	37.5	4.7	54.0	71.7	+	43.2	40.8	5.9	55.3	56.2	39.2	21.7	52.0
K. Seed 5	49.9	20.0	5.5	58.0	79.0	+	32.1	23.3	4.3	56.0	33.4	21.7	13.0	56.3
K. Seed 6	40.1	10.8	1.1	54.0	87.5	+	24.6	15.0	2.7	54.0	14.3	19.2	11.6	55.0
K. Seed 7	42.8	16.7	4.2	59.3	84.3	+	25.3	34.2	5.0	53.3	25.8	24.2	20.8	55.0
K. Seed 8	77.7	32.5	13.6	51.3	77.0	+	44.1	28.3	5.5	53.3	65.9	43.3	29.3	55.0
K. Seed 9	85.6	41.7	18.1	49.3	68.3	+	35.9	33.3	10.8	50.0	48.7	36.3	24.2	53.7
K. Seed 10	71.3	39.2	14.0	48.7	67.5	+	43.5	28.3	7.7	51.0	54.5	33.3	25.5	52.7
Lenana	87.5	35.8	5.8	51.0	84.0	+	37.7	25.8	5.5	50.7	31.9	27.5	16.4	52.3
Morris	67.2	35.0	4.6	49.0	84.7	-	17.7	16.7	2.6	53.3	15.9	11.5	6.2	53.3
Njoro BW II	19.1	13.8	4.3	61.7	70.0	+	22.9	30.8	15.9	56.7	8.8	18.8	16.5	61.0
Primex	74.9	40.0	9.4	45.0	85.0	-	46.3	32.5	7.5	48.3	78.2	49.2	30.6	50.3

Genotype	ST. PAUL - 2016						ST. PAUL – 2017				CROOKSTON - 2017			
	FHB		DON	HD	Plant		FHB		DON	HD	FHB		DON	HD
	Index	VSK			Height	Awns	Index	VSK			Index	VSK		
(%)	(%)	(ppm)	(days)	(cm)	(+/-)	(%)	(%)	(ppm)	(days)	(%)	(%)	(ppm)	(days)	
<b>P. Walker</b>														
Munro	51.9	20.0	5.3	46.0	82.0	+	16.8	15.8	2.8	51.7	24.1	13.0	14.1	52.7
RFN	-	-	-	-	68.5	+	-	-	-	-	5.9	17.5	17.9	57.0
Tama	62.1	30.8	5.8	49.0	87.7	-	29.3	35.8	7.0	50.3	25.5	17.7	12.7	51.0
Trophy	48.1	12.5	7.7	51.3	90.3	-	28.6	17.5	3.0	54.0	20.3	22.5	17.4	54.3
<b>Ethiopian cultivars/lines<sup>a</sup></b>														
Alidoro	59.6	30.0	7.2	49.7	81.0	+	32.7	30.8	3.8	55.7	30.9	30.0	16.9	55.7
Biqa'a	77.0	40.8	12.1	51.3	73.3	+	43.6	25.8	9.6	54.3	41.1	33.3	24.1	55.0
Bollo	23.2	4.7	2.3	60.7	80.7	+	19.9	19.2	8.1	58.0	27.4	7.8	15.1	60.3
Danda'a	47.2	35.8	12.7	57.0	80.0	+	36.5	39.2	14.0	57.7	46.1	35.8	24.5	49.0
Digelu	9.2	7.0	3.4	62.0	84.0	+	46.6	26.7	7.6	52.3	49.0	27.5	24.6	53.7
Dure	87.0	57.5	27.5	51.0	67.5	+	40.2	41.7	15.0	55.7	50.3	30.0	29.4	54.7
Galama	52.4	31.7	10.6	52.3	77.0	+	31.8	13.0	9.2	59.0	35.0	20.0	16.4	57.0
Gambo	42.7	26.7	7.0	55.0	85.0	+	59.7	31.7	9.0	53.0	44.8	39.2	35.5	55.0
Gasay	39.3	19.2	5.6	55.3	78.5	+	33.7	26.7	4.0	54.7	20.5	26.7	28.3	54.3
Hawi	48.6	44.2	16.1	45.0	70.3	+	44.8	37.5	13.4	50.0	43.3	43.3	19.1	51.3
Hidase	58.8	41.7	17.3	44.7	69.3	+	67.3	34.2	15.8	52.3	53.1	53.1	20.5	52.7

Genotype	ST. PAUL - 2016						ST. PAUL – 2017				CROOKSTON - 2017			
	FHB		DON	HD	Plant		FHB		DON	HD	FHB		DON	HD
	Index	VSK			Height	Awns	Index	VSK			Index	VSK		
(%)	(%)	(ppm)	(days)	(cm)	(+/-)	(%)	(%)	(ppm)	(days)	(%)	(%)	(ppm)	(days)	
Hoggana	59.7	16.7	8.2	65.0	62.0	+	37.9	38.3	11.5	56.3	45.2	45.2	30.9	58.7
Honqolo	42.6	30.0	5.4	55.0	68.7	+	35.1	33.3	9.5	53.0	18.5	18.5	23.3	54.7
Hulluka	38.2	30.0	5.0	59.0	72.5	+	46.1	41.7	13.5	57.0	44.6	44.6	21.8	60.0
KBG-01	85.1	62.5	27.8	46.0	-	+	39.4	33.3	6.4	48.7	82.5	31.7	19.3	52.3
Kingbird	97.5	41.7	19.8	52.3	72.3	+	37.6	40.0	13.1	49.3	39.7	32.5	18.2	54.3
Kubsa	78.7	46.7	16.8	49.7	71.3	+	38.7	32.5	9.3	51.0	33.2	31.3	18.8	52.5
Mada-Walabu	90.9	27.5	9.0	53.3	76.7	+	51.5	26.7	3.9	53.3	46.3	24.2	14.2	53.3
Menze	21.6	7.7	4.6	61.0	76.5	+	20.4	23.3	7.7	59.0	7.6	4.3	9.3	61.3
Millennium	62.2	24.2	6.7	51.7	77.0	+	63.1	28.3	4.9	53.0	48.3	32.5	24.5	54.7
QulQullu	68.4	30.0	6.4	54.0	73.3	+	35.0	26.7	4.9	56.3	48.3	37.5	27.0	53.3
Shorima	80.1	42.5	14.1	50.0	77.3	+	62.5	40.8	9.3	51.7	52.3	40.0	38.6	55.3
Sofumar	78.1	31.7	16.7	51.0	82.7	+	26.9	24.2	6.4	52.3	28.5	27.5	18.7	53.3
Tay	42.5	14.0	6.4	55.0	85.0	+	49.3	23.3	5.7	54.0	28.3	26.7	17.4	55.0
<b>Advanced CIMMYT spring wheat lines<sup>a</sup></b>														
ETBW 6109	46.2	30.8	8.0	52.3	80.0	+	27.1	31.7	6.7	54.3	20.2	27.5	36.7	55.7
ETBW 6114	51.2	23.3	8.3	55.0	71.5	+	43.4	31.7	4.7	55.3	35.0	18.3	16.1	57.0

Genotype	ST. PAUL - 2016						ST. PAUL – 2017				CROOKSTON - 2017			
	FHB		DON	HD	Plant		FHB		DON	HD	FHB		DON	HD
	Index	VSK			Height	Awns	Index	VSK			Index	VSK		
(%)	(%)	(ppm)	(days)	(cm)	(+/-)	(%)	(%)	(ppm)	(days)	(%)	(%)	(ppm)	(days)	
ETBW 6496	56.4	28.3	10.8	64.0	78.3	+	38.3	41.7	12.6	59.3	17.9	33.3	44.2	64.0
ETBW 6696	80.4	30.8	9.3	55.0	72.3	+	49.2	32.5	6.3	55.0	21.1	31.3	27.8	54.5
ETBW 6832	54.4	35.8	9.8	54.7	75.0	+	45.4	39.2	8.6	52.0	36.5	38.3	29.9	55.0
ETBW 6850	68.0	41.7	16.1	49.7	75.0	+	30.7	36.7	9.2	50.3	44.5	34.2	29.1	54.0
ETBW 6875	49.5	21.7	6.9	51.0	80.0	+	26.5	15.8	5.5	53.7	46.9	34.2	27.0	55.7
ETBW 6965	80.1	44.2	18.5	47.0	70.0	+	36.6	26.3	7.0	49.0	47.7	32.5	24.1	50.3
ETBW 7058	97.2	30.8	10.3	56.3	74.3	+	63.4	32.5	6.2	54.0	32.7	25.8	25.2	56.3
ETBW 7101	75.8	46.7	10.8	48.7	73.5	+	46.8	45.8	4.7	51.7	55.1	65.0	49.5	55.3
ETBW 7213	19.1	7.8	4.4	65.0	72.3	+	19.3	27.5	7.1	57.3	8.9	6.7	14.1	62.7
ETBW 7258	78.3	40.0	21.7	49.3	71.5	+	20.2	22.5	4.3	49.7	38.9	24.2	15.8	53.0
ETBW 7364	36.8	18.3	4.5	54.7	72.3	+	30.6	16.7	6.3	54.3	17.4	19.2	22.8	56.0
ETBW 7698	72.9	41.7	18.4	50.7	76.5	+	54.8	28.3	7.8	52.3	44.6	40.0	39.2	55.3
ETBW 7724	65.8	18.3	9.2	55.3	70.0	+	49.6	27.5	5.3	53.3	22.8	21.3	17.8	55.7
ETBW 7730	64.1	40.8	9.3	55.0	75.0	+	49.2	32.5	7.4	54.3	60.3	50.0	46.9	55.0
ETBW 7872	57.2	25.0	8.4	58.7	80.7	+	47.1	30.0	8.0	54.7	31.3	30.0	21.6	57.0
ETBW 8469	52.4	41.7	15.4	46.0	73.5	+	20.6	34.2	11.3	50.0	18.2	29.2	21.9	51.3
R 1286	22.0	15.5	12.2	65.0	77.0	+	11.9	29.2	19.5	63.3	10.4	28.8	31.0	60.3
R 1301	41.5	30.0	4.0	54.7	88.7	+	40.3	32.5	4.2	52.3	32.9	40.8	37.2	55.0

Genotype	ST. PAUL - 2016						ST. PAUL - 2017				CROOKSTON - 2017			
	FHB		DON	HD	Plant		FHB		DON	HD	FHB		DON	HD
	Index	VSK			Height	Awns	Index	VSK			Index	VSK		
(%)	(%)	(ppm)	(days)	(cm)	(+/-)	(%)	(%)	(ppm)	(days)	(%)	(%)	(ppm)	(days)	
R 1317	53.5	31.7	10.8	52.0	88.7	+	68.6	32.5	9.2	52.0	35.0	26.7	24.5	55.0
R 1325	47.2	31.7	5.3	57.7	79.5	+	27.3	32.5	5.1	55.7	50.0	36.7	27.2	55.7
R 1331	87.9	45.8	7.6	52.3	80.0	+	41.6	45.8	6.5	52.3	47.2	66.7	42.2	54.0
R 1351	75.9	35.0	12.7	50.3	76.5	+	39.3	27.5	7.1	50.3	55.4	34.2	29.5	53.0
R 1352	56.5	62.5	18.6	49.3	74.7	+	35.1	43.3	12.0	50.0	56.4	50.0	42.1	49.7
R 1353	54.7	25.8	6.9	51.7	78.3	+	42.1	26.7	4.6	53.7	32.4	31.3	18.8	54.3
R 1354	52.4	21.7	9.9	53.3	77.0	+	39.0	33.3	5.4	52.3	49.6	42.5	29.1	54.3
R 1357	68.0	23.3	7.9	55.0	76.5	+	59.8	22.5	8.1	51.3	44.5	40.8	30.6	54.3
R 1360	65.4	35.0	7.3	50.3	77.3	+	23.4	33.3	4.8	53.3	32.2	35.0	27.4	54.3
R 1361	39.0	32.5	15.6	59.3	78.0	+	34.9	30.0	7.3	55.7	56.3	40.0	37.5	59.0
R 1362	68.2	25.0	10.7	61.0	78.3	+	25.4	37.5	13.5	58.0	39.3	33.3	33.7	59.0
R 1363	70.3	49.2	14.4	49.0	69.7	+	34.6	29.2	7.2	49.7	69.8	38.3	25.9	53.0
R 1364	69.6	55.0	20.5	51.7	85.0	+	47.1	33.3	8.3	52.7	49.8	42.5	41.1	55.0
R 1365	80.4	50.8	17.1	54.7	78.3	+	74.5	42.5	14.1	53.0	28.6	53.3	50.5	55.3
R 1366	72.8	39.2	7.4	48.3	75.3	+	34.4	29.2	5.7	51.0	49.5	42.5	26.8	53.0
R 1367	55.8	40.8	9.3	49.3	73.5	+	25.3	25.8	4.5	50.7	39.3	42.5	31.0	53.0
R 1370	50.1	31.7	12.2	44.0	74.7	+	31.6	26.7	7.0	49.3	52.4	39.2	33.4	50.7
R 1371	63.9	34.2	10.6	52.7	74.0	+	30.3	38.3	8.1	49.3	40.5	36.7	27.8	50.7

Genotype	ST. PAUL - 2016						ST. PAUL - 2017				CROOKSTON - 2017			
	FHB		DON	HD	Plant		FHB				FHB			
	Index (%)	VSK (%)			Height (cm)	Awns (+/-)	Index (%)	VSK (%)	DON (ppm)	HD (days)	Index (%)	VSK (%)	DON (ppm)	HD (days)
R 1372	44.8	29.2	8.0	50.3	76.3	+	38.2	18.0	4.3	51.7	28.9	32.5	23.8	54.7
R 1373	46.7	14.2	5.7	54.0	76.0	+	29.3	10.5	2.7	54.0	41.9	41.7	33.8	55.0
R 1374	54.1	32.5	13.9	51.3	84.3	+	28.7	33.3	5.7	50.7	64.6	45.8	35.9	53.0
R 1376	41.1	28.8	4.5	55.3	71.3	+	51.0	30.0	5.8	55.3	33.1	40.0	32.4	54.7
R 1378	65.6	34.2	8.5	54.7	73.0	+	23.3	44.2	11.5	51.0	40.2	40.8	42.4	52.3
R 1379	73.7	30.8	4.9	51.0	76.7	+	33.5	25.8	4.5	55.7	42.6	40.8	30.4	54.0
R 1380	67.0	30.0	10.7	54.3	76.0	+	42.6	30.0	8.5	55.7	35.8	33.3	30.6	57.0
R 1382	62.3	26.7	6.6	55.0	67.0	+	27.0	19.7	3.2	52.7	47.7	30.0	27.6	56.0
R 1387	67.1	44.2	10.5	44.3	68.3	+	24.7	44.2	6.5	49.7	44.6	32.5	15.5	51.0
R 1388	74.5	32.5	18.8	58.7	70.0	+	42.0	33.3	10.0	55.3	58.4	25.8	13.3	58.7
R 1389	68.7	34.2	12.0	53.3	79.7	+	65.1	30.8	11.1	51.3	63.2	37.5	18.4	54.7
R 1390	81.6	39.2	12.8	48.7	70.7	+	33.5	37.5	6.9	51.7	49.1	39.2	26.2	53.0
R 1391	75.4	25.0	13.3	55.0	71.3	+	66.9	28.3	10.5	53.3	56.9	35.8	33.6	54.7
R 1392	76.1	49.2	15.0	51.3	75.3	+	44.2	35.8	5.4	53.7	54.6	39.2	37.2	54.0
R 1393	77.0	46.7	17.3	51.3	74.7	+	34.5	29.2	6.9	51.3	44.8	41.7	28.1	53.3
R 1394	84.2	46.7	11.8	52.0	71.7	+	38.9	35.8	5.6	51.7	59.9	52.5	41.0	51.7
R 1395	66.1	29.2	12.1	51.0	74.0	+	36.0	35.0	8.4	51.0	55.7	32.5	18.6	51.7
R 1396	61.8	39.2	11.5	50.0	74.5	+	42.5	37.5	9.7	50.0	50.7	38.3	33.0	51.7

Genotype	ST. PAUL - 2016						ST. PAUL - 2017				CROOKSTON - 2017			
	FHB		DON	HD	Plant		FHB		DON	HD	FHB		DON	HD
	Index	VSK			Height	Awns	Index	VSK			Index	VSK		
(%)	(%)	(ppm)	(days)	(cm)	(+/-)	(%)	(%)	(ppm)	(days)	(%)	(%)	(ppm)	(days)	
R 1397	61.2	27.5	9.0	58.7	76.0	+	34.7	29.2	9.9	54.3	36.9	28.3	33.8	56.3
R 1398	46.6	40.0	7.8	51.3	79.0	+	34.6	25.8	6.5	53.0	41.6	42.5	31.7	53.0
R 1402	61.3	45.8	9.8	52.3	73.0	+	56.4	44.2	11.0	51.0	61.3	44.2	43.5	53.3
R 1403	60.5	30.8	3.0	53.3	79.3	+	33.2	30.8	7.3	56.7	28.0	22.5	13.0	55.3
R 1404	54.5	30.8	8.5	49.0	78.7	+	22.3	34.2	9.4	51.7	15.6	28.3	15.2	55.0
R 1406	63.7	17.5	3.9	52.3	81.0	+	59.8	30.8	6.0	53.0	37.4	30.0	25.8	54.7
R 1407	70.3	25.0	5.8	55.0	76.3	+	55.9	32.5	8.8	52.7	32.7	32.5	30.2	54.7
R 1408	58.2	25.8	8.1	60.3	73.0	+	36.8	27.5	8.3	56.0	38.0	23.3	20.2	58.3
R 1409	59.2	40.0	10.5	51.3	75.0	+	36.9	31.7	3.4	53.7	50.4	41.7	24.9	55.0
R 1410	53.7	51.7	11.7	48.7	67.7	+	41.9	42.5	6.0	50.0	65.4	58.3	22.2	50.0
R 1411	80.2	29.2	5.1	55.7	53.3	+	34.9	31.7	6.0	53.3	50.9	40.8	23.3	55.0
R 1412	63.4	26.7	2.5	54.7	75.3	+	33.1	31.7	3.8	53.0	36.8	35.8	19.9	55.0
R 1415	65.6	26.7	5.7	51.0	74.0	+	35.3	35.8	4.5	53.0	42.0	22.5	20.9	54.3
R 1416	54.5	33.3	6.6	50.0	69.3	+	42.2	21.7	6.5	54.7	45.6	23.3	17.7	55.3
R 1417	65.4	30.8	5.7	55.7	71.3	+	39.4	30.0	7.6	53.7	28.9	37.5	21.5	54.7
R 1418	62.9	25.0	5.8	59.0	67.5	+	49.2	24.2	7.1	54.0	48.7	40.0	28.2	53.7
R 1420	79.7	30.0	13.2	55.0	75.0	+	45.1	30.0	7.2	53.3	34.7	23.8	23.2	53.3
R 1421	41.6	34.2	8.5	44.3	71.0	+	46.0	31.7	4.8	54.0	41.1	35.0	23.6	54.3

Genotype	ST. PAUL - 2016						ST. PAUL - 2017				CROOKSTON - 2017			
	FHB		DON	HD	Plant		FHB		DON	HD	FHB		DON	HD
	Index	VSK			Height	Awns	Index	VSK			Index	VSK		
(%)	(%)	(ppm)	(days)	(cm)	(+/-)	(%)	(%)	(ppm)	(days)	(%)	(%)	(ppm)	(days)	
R 1422	82.4	20.5	6.9	54.3	75.0	+	38.6	27.5	3.8	52.7	56.8	32.5	15.1	54.0
R 1423	37.7	32.5	11.8	56.3	73.5	+	55.9	28.3	9.1	53.0	57.1	39.2	32.8	54.0
R 1424	62.7	10.5	3.0	63.0	71.5	+	18.9	20.8	4.6	55.3	43.2	17.5	19.3	57.7
R 1426	66.7	25.8	9.3	51.3	75.5	+	55.4	34.2	11.2	54.0	45.3	35.0	27.3	54.7
R 1427	55.9	30.8	12.4	57.3	74.3	+	72.5	30.0	11.1	56.0	51.9	36.7	43.0	58.3
R 1429	58.5	9.8	6.7	63.0	68.3	+	27.1	22.5	11.1	59.3	36.8	27.5	26.3	57.7
R 1430	60.5	58.3	15.6	51.0	76.3	+	63.7	50.8	9.0	49.3	78.9	60.8	38.7	53.0
R 1431	55.5	37.5	7.9	50.0	78.3	+	45.9	30.0	5.1	49.3	42.5	44.2	33.3	52.3
R 1432	75.4	38.3	11.5	51.3	80.3	+	44.2	26.7	5.6	53.3	56.6	40.8	30.5	54.3
R 1433	64.3	28.3	9.2	53.3	76.0	+	40.5	26.7	5.0	52.3	43.7	38.8	23.3	54.5
R 1434	64.9	27.5	8.9	55.0	73.3	+	47.6	34.2	7.9	53.7	52.9	37.5	36.8	54.7
R 1435	67.5	26.7	9.4	54.3	73.3	+	28.0	35.0	6.4	54.3	41.5	26.3	21.6	53.3
R 1436	66.6	61.7	8.4	55.0	71.3	+	59.0	41.7	6.0	53.3	32.3	41.7	32.3	56.3
R 1439	71.6	37.5	10.9	54.7	75.0	+	48.9	30.8	7.2	52.0	33.8	36.7	28.4	54.7
R 1440	59.4	45.0	19.8	57.3	73.3	+	40.5	38.3	11.2	54.0	40.7	40.0	37.4	55.0
R 1441	52.0	35.0	6.2	51.3	66.0	+	35.2	19.7	6.9	54.7	33.2	24.2	26.8	55.0
R 1442	62.6	47.5	12.5	49.3	76.0	+	43.5	40.8	3.9	53.0	31.7	34.2	30.4	54.3
R 1445	64.1	30.8	7.6	61.3	70.0	+	30.8	30.0	6.7	54.3	46.4	33.3	38.7	57.3



Genotype	ST. PAUL - 2016						ST. PAUL - 2017				CROOKSTON - 2017			
	FHB		DON	HD	Plant		FHB				FHB			
	Index	VSK			Height	Awns	Index	VSK	DON	HD	Index	VSK	DON	HD
	(%)	(%)	(ppm)	(days)	(cm)	(+/-)	(%)	(%)	(ppm)	(days)	(%)	(%)	(ppm)	(days)
R 1447	53.6	36.7	9.0	45.3	79.0	+	20.4	34.2	6.0	50.0	35.0	30.8	22.6	52.3
R 1448	48.0	25.0	4.5	55.0	82.3	+	44.4	21.7	4.9	53.7	35.0	31.7	20.5	55.0
R 1449	45.5	18.0	7.2	61.3	74.0	+	26.1	35.0	6.8	53.3	50.9	18.8	19.2	54.3
R 1450	67.3	35.8	6.6	49.0	71.5	+	33.8	39.2	4.9	51.0	54.0	31.7	19.3	52.7
R 1451	60.5	37.5	10.2	52.7	73.5	+	36.5	39.2	8.6	54.0	41.5	38.3	22.5	54.7
R 1452	54.7	14.2	4.2	60.3	71.0	+	30.5	41.7	10.0	53.3	35.3	45.8	30.3	54.3
R 1453	62.4	45.8	10.4	49.7	75.5	+	43.8	34.2	3.7	52.0	34.6	28.3	13.9	55.3
R 1454	45.6	36.7	9.8	47.7	67.5	+	16.1	24.2	3.1	50.0	29.1	24.2	14.6	54.3
R 1455	57.7	44.2	11.9	50.0	76.0	+	20.4	30.0	5.4	50.0	45.7	35.0	23.2	53.3
R 1456	48.3	29.2	6.7	43.0	79.0	+	30.6	29.2	5.1	49.0	42.3	31.7	17.3	50.3
R 1459	73.8	30.0	12.7	53.3	73.0	+	52.6	22.5	5.4	53.3	36.8	30.0	22.4	55.0
R 1460	36.2	15.8	5.1	58.7	73.0	+	33.6	29.2	4.6	54.7	24.5	25.8	24.1	56.0
R 1462	58.1	20.0	11.0	61.7	75.7	+	24.9	35.8	21.9	60.3	11.2	14.7	26.9	63.0
R 1463	66.2	43.3	20.4	55.3	74.7	+	56.0	46.7	9.1	55.7	36.0	70.8	45.6	55.7
R 1466	61.9	37.5	11.4	53.7	76.0	+	41.5	36.7	7.2	53.7	49.7	52.5	65.4	55.0
R 1467	61.2	44.2	14.0	52.0	76.7	+	57.0	35.8	9.1	52.7	42.4	40.0	30.2	53.7
R 1468	59.7	29.2	10.2	55.0	79.7	+	34.3	30.0	8.1	47.0	60.8	37.5	31.6	49.0
R 1471	68.6	20.8	6.1	53.3	70.0	+	52.7	21.3	5.6	53.3	45.1	37.5	31.5	55.0

Genotype	ST. PAUL - 2016						ST. PAUL - 2017				CROOKSTON - 2017			
	FHB		DON	HD	Plant		FHB		DON	HD	FHB		DON	HD
	Index	VSK			Height	Awns	Index	VSK			Index	VSK		
(%)	(%)	(ppm)	(days)	(cm)	(+/-)	(%)	(%)	(ppm)	(days)	(%)	(%)	(ppm)	(days)	
Ravi 1	88.1	58.3	21.7	54.7	75.0	+	72.4	32.5	7.6	51.7	56.2	39.2	28.3	53.0
Ravi 2	46.7	45.8	16.6	44.7	68.0	+	36.2	26.7	5.9	56.7	48.7	30.8	17.0	54.3
Ravi 3	68.8	29.2	14.8	50.3	78.0	+	44.7	28.3	7.6	52.0	47.8	38.3	28.2	52.3
Ravi 4	82.4	38.3	16.1	50.0	72.3	+	36.2	28.3	6.9	51.7	57.9	43.3	32.8	54.7
Ravi 5	65.7	35.8	14.9	50.3	67.0	+	35.3	36.7	11.5	50.0	46.8	37.5	29.5	52.3
Ravi 6	96.4	48.3	11.9	55.3	73.3	+	36.3	35.8	6.0	50.7	60.1	35.0	24.4	53.0
Ravi 7	73.5	45.0	18.6	49.7	71.0	+	44.5	23.0	7.1	53.0	49.7	30.0	26.6	52.0
Ravi 8	60.8	41.7	11.6	55.0	77.5	+	45.6	36.7	10.1	50.7	63.6	33.3	25.9	55.0
Ravi 9	74.2	45.0	18.6	51.3	77.0	+	44.8	30.8	8.5	50.7	58.1	44.2	31.0	54.3
Ravi 10	58.2	38.3	12.4	52.0	76.7	+	38.3	30.8	6.5	53.3	61.0	59.2	70.8	55.0
Ravi 11	43.4	19.2	7.2	58.3	75.0	+	20.2	23.3	4.7	49.7	40.6	29.2	36.2	59.0
Ravi 12	79.1	42.5	14.0	48.3	75.0	+	28.7	28.3	5.5	53.3	48.9	45.0	31.1	50.7
Ravi 13	61.9	42.5	16.7	51.7	84.7	+	49.3	35.8	11.7	51.3	36.7	40.0	32.4	53.0
Ravi 14	74.4	40.0	14.5	52.3	79.0	+	48.8	25.8	8.2	53.3	44.5	42.5	37.6	54.0
Ravi 15	86.1	44.2	18.5	51.3	76.7	+	55.5	30.0	8.0	52.0	50.1	37.5	36.2	54.0
Ravi 16	72.1	44.2	12.7	45.0	72.5	+	42.6	36.7	9.4	50.3	75.7	45.0	36.6	50.7
Ravi 17	52.0	44.2	13.8	44.7	76.3	+	41.5	39.2	9.7	52.7	58.5	44.2	29.1	51.0
Ravi 18	85.4	50.0	20.2	49.0	71.0	+	49.1	23.3	5.7	53.0	71.1	41.7	30.2	53.3

Genotype	ST. PAUL - 2016						ST. PAUL - 2017				CROOKSTON - 2017			
	FHB		DON	HD	Plant		FHB		DON	HD	FHB		DON	HD
	Index	VSK			Height	Awns	Index	VSK			Index	VSK		
(%)	(%)	(ppm)	(days)	(cm)	(+/-)	(%)	(%)	(ppm)	(days)	(%)	(%)	(ppm)	(days)	
Ravi 20	75.3	41.7	17.8	50.3	73.0	+	86.8	37.5	11.8	52.3	42.0	43.3	36.5	52.0
Ravi 24	69.9	45.8	13.8	54.7	76.7	+	31.9	30.8	7.4	54.0	52.1	50.0	32.3	55.0
Ravi 25	73.3	25.0	13.4	54.0	72.3	+	27.8	31.7	7.1	50.7	41.4	27.5	20.2	54.7
Ravi 26	54.8	40.0	9.7	48.3	75.7	+	29.4	30.0	8.7	49.3	58.0	37.5	27.3	50.0
Ravi 27	45.3	29.2	9.5	51.0	63.0	+	71.2	36.7	11.9	52.7	41.9	36.7	29.4	54.3
Ravi 29	87.8	43.3	16.3	51.0	73.3	+	37.5	30.0	7.5	52.7	55.4	38.3	23.0	51.7
Ravi 30	55.8	30.0	11.1	55.0	70.0	+	47.1	32.5	7.2	54.3	42.6	25.8	20.0	55.0
Ravi 33	50.9	12.5	4.9	63.0	73.5	+	58.2	30.8	9.5	52.0	8.0	14.0	19.0	62.3
Ravi 34	50.7	44.2	10.2	47.7	77.3	+	20.7	28.3	2.8	51.3	37.6	30.0	16.8	51.5
Ravi 35	55.9	43.8	10.1	52.0	76.0	+	49.7	37.5	6.6	51.0	43.7	34.2	25.9	53.0
Ravi 36	49.6	20.0	7.0	61.7	76.0	+	30.2	27.5	7.9	53.0	63.0	32.5	31.5	54.0
Ravi 37	65.0	37.5	13.6	54.0	74.0	+	63.0	36.7	7.0	51.7	56.6	40.0	40.2	52.0
Ravi 39	45.8	17.5	5.7	57.7	80.0	+	37.9	24.2	5.4	54.0	54.3	38.3	22.1	51.0
Ravi 40	74.8	30.0	9.4	51.7	75.0	+	62.0	31.7	10.7	51.0	57.0	34.2	29.7	53.0
Ravi 41	49.6	20.8	6.3	52.7	76.3	+	40.7	33.3	7.1	50.7	47.8	33.3	17.3	52.7
Ravi 42	22.8	39.2	11.1	42.0	70.0	+	39.4	35.8	10.2	48.7	53.4	36.7	30.8	50.0
Ravi 43	46.8	27.5	9.9	49.3	72.0	+	16.9	29.2	8.2	50.0	28.5	27.5	25.5	52.3
Ravi 44	65.1	27.5	9.8	49.3	75.0	+	43.8	29.2	8.0	51.0	40.9	26.7	29.0	52.3

Genotype	ST. PAUL - 2016						ST. PAUL – 2017				CROOKSTON - 2017			
	FHB		DON	HD	Plant		FHB		DON	HD	FHB		DON	HD
	Index	VSK			Height	Awns	Index	VSK			Index	VSK		
(%)	(%)	(ppm)	(days)	(cm)	(+/-)	(%)	(%)	(ppm)	(days)	(%)	(%)	(ppm)	(days)	
Ravi 45	81.7	44.2	17.2	50.7	76.0	+	31.7	28.3	8.2	51.0	67.1	50.0	39.5	53.0
Ravi 46	73.1	35.0	11.6	49.3	73.7	+	19.7	20.5	3.9	50.3	27.3	17.5	16.4	52.5
Ravi 47	52.4	24.2	9.5	59.3	75.7	+	29.4	34.2	12.0	58.0	36.5	35.0	39.7	59.0
Ravi 48	45.2	30.8	8.7	55.0	80.5	+	41.6	26.7	9.5	55.7	54.6	40.0	53.8	58.3
Ravi 49	91.6	31.8	17.5	50.3	68.3	+	51.8	38.3	14.0	50.7	66.8	40.8	43.8	56.3
Ravi 50	66.2	20.0	5.6	55.7	76.3	+	62.8	33.3	9.1	54.0	26.1	30.0	26.8	57.7
Ravi 51	84.1	37.5	11.2	51.3	71.3	+	56.4	30.0	7.6	52.7	50.4	37.5	24.7	54.7
Ravi 52	65.2	35.8	12.7	50.7	72.7	+	51.9	39.2	8.8	52.3	58.1	52.5	35.9	54.3
Ravi 53	76.1	24.2	9.8	54.3	70.7	+	35.6	24.3	4.3	52.7	60.6	41.7	34.3	53.7
Ravi 54	57.3	37.5	11.7	54.3	77.0	+	34.5	21.7	6.0	54.3	38.4	30.0	23.1	55.7
Ravi 55	48.1	20.0	10.5	60.3	78.3	+	33.6	30.8	11.6	56.0	15.8	35.0	57.5	59.0
Ravi 56	39.4	23.3	7.0	60.0	74.7	+	34.8	41.7	14.0	56.0	48.5	34.2	33.1	57.3
<b>Checks<sup>b</sup></b>														
LCS Albany														
(MR)	32.7	9.7	1.7	49.0	78.0	+	24.5	7.7	1.2	52.3	19.7	9.5	8.8	55.0
Linkert (MR)	25.1	9.5	3.8	42.0	84.0	+	26.6	14.3	3.2	49.3	23.1	19.2	12.9	52.0

Genotype	ST. PAUL - 2016						ST. PAUL – 2017				CROOKSTON - 2017			
	FHB		DON	HD	Plant		FHB		DON	HD	FHB		DON	HD
	Index	VSK			Height	Awns	Index	VSK			Index	VSK		
(%)	(%)	(ppm)	(days)	(cm)	(+/-)	(%)	(%)	(ppm)	(days)	(%)	(%)	(ppm)	(days)	
WB-Mayville														
(S)	45.6	37.5	7.7	42.0	65.0	+	34.1	30.0	5.5	48.0	44.3	38.3	22.3	49.0
Rollag (R)	22.1	10.0	1.6	45.0	74.0	+	25.1	7.7	1.7	49.0	21.1	9.2	6.9	50.7
Samson (S)	33.2	39.2	10.3	44.7	68.0	+	28.1	35.0	3.8	49.0	62.6	48.3	24.0	51.0
Wheaton (S)	-	-	-	-	-		34.1	32.5	17.8	61.0	46.1	39.2	30.5	56.3
<b>Mean</b>	58.7	31.6	10.0	53.1			36.9	30.7	7.5	53.2	40.2	33.3	26.6	54.6
<b>SD</b>	19.4	12.1	4.9	5.1			14.2	7.5	3.2	3.0	16.4	10.9	10.0	2.8

<sup>a</sup> K = Kenya, K. Seed = wheat lines from Kenya Seed Company, ETBW = Ethiopian bread wheat (advanced CIMMYT wheat lines selected in an Ethiopian environment), “Ravi” and “R” lines = advanced CIMMYT lines selected in a Kenyan environment, dash (-) symbol indicates missing data.

<sup>b</sup> Response of checks to Fusarium head blight are indicated in parentheses following the name of each check: R = resistant, MR = moderately resistant, S = susceptible.

**Table 2.2.** Aggressiveness, as determined by FHB severity (%) on two wheat cultivars (Alsen, FHB-moderately resistant; Wheaton, FHB-susceptible) and mycotoxin production [deoxynivalenol (DON), acetylated derivatives of DON (3-ADON, 15-ADON), nivalenol (NIV) and zearalenone (ZEA)] in 7-day old rice cultures for *F. graminearum* isolates 10116010 and 10116012. The isolates were both obtained from symptomatic wheat heads collected in commercial wheat fields in Minnesota in 2016.

Accession Number*	Location (County)	FHB Severity (%)		Mycotoxins in Rice Culture (ppm)				
		Alsen	Wheaton	DON	3-ADON	15-ADON	NIV	ZEA
10116010	Marshall	13.85	97.57	547.7	20.1	406.4	nd	22.7
10116012	Red Lake	14.28	95.24	214.7	407.4	2.5	nd	4.7

\*Accession numbers were assigned by the Small Grains Pathology Laboratory, University of Minnesota.

**Table 2.3.** Number of days to heading, plant height (cm), Fusarium head blight (FHB) index (%) (from field experiments) and FHB severity (%) (from greenhouse experiments) for 82 wheat genotypes that were selected from a collection of wheat lines largely from Kenya and Ethiopia. FHB index was evaluated in the field in each of three mist-irrigated nurseries (St. Paul 2016 (STP16), St. Paul 2017 (STP17) and Crookston 2017 (CRK17)) following inoculation with a mixture of *F. graminearum* isolates. FHB severity was determined in response to inoculation with each of two *F. graminearum* isolates (10116010 and 10116012) in two greenhouse experiments conducted in 2017 and 2018 (for each isolate, 2017 and 2018 data are combined). The genotypes are ranked alphabetically<sup>a</sup>.

Genotype	Days to Heading (days)			Plant Height (cm)	FHB Index (%)			FHB Severity (%)	
	STP16	STP17	CRK17	STP16	STP16	STP17	CRK17	Isolate	Isolate
								10116010	10116012
Alidoro	49.7	55.7	55.7	81.0	59.6	32.7	30.9	43.0	63.2
Alsen	-	-	-	-	-	-	-	8.5	25.2
Bailey	49.0	52.7	53.0	89.3	58.1	39.3	25.5	38.3	56.6
Bollo	60.7	60.3	58.0	80.7	23.2	19.9	27.4	40.8	63.5
Bonza 63	65.0	62.3	59.7	74.7	-	24.0	7.2	41.4	78.2
Digelu	62.0	53.7	52.3	84.0	9.2	46.6	49.0	35.7	50.3
ETBW 6109	52.3	55.7	55.7	80.0	46.2	27.1	20.2	60.4	84.9
ETBW 6496	64.0	64.0	59.3	78.3	56.4	38.3	17.9	77.1	80.8
ETBW 7213	65.0	62.7	57.3	72.3	19.1	19.3	8.9	27.5	48.2
ETBW 7258	49.3	53.0	49.7	73.0	78.3	20.2	38.9	36.2	70.1
ETBW 7364	54.7	56.0	54.3	72.3	36.8	30.6	17.4	22.3	48.3

Genotype	Days to Heading (days)			Plant Height (cm)	FHB Index (%)			FHB Severity (%)	
	STP16	STP17	CRK17	STP16	STP16	STP17	CRK17	Isolate	Isolate
								10116010	10116012
ETBW 7724	55.3	55.7	53.3	70.0	65.8	49.6	22.8	48.7	71.7
ETBW 7872	58.7	57.0	54.7	80.7	57.2	47.1	31.3	58.1	86.3
ETBW 8469	46.0	51.3	50.0	74.3	52.4	20.6	18.2	37.3	53.2
Galama	52.3	57.0	59.0	79.3	52.4	31.8	35.0	17.9	52.1
Gasay	55.3	54.3	54.7	78.7	39.3	33.7	20.5	45.7	62.4
Hawi	45.0	51.3	50.0	70.3	48.6	44.8	43.3	33.9	52.2
Honqolo	55.0	54.7	53.0	68.7	42.6	35.1	18.5	27.3	44.8
Kenya Cheetah	-	60.7	63.7	-	-	9.1	4.7	31.3	55.0
Kenya Civet	65.0	62.3	63.5	-	-	-	12.9	17.0	51.1
Kenya Eagle 10	44.7	52.0	49.3	69.5	20.6	19.5	19.6	28.7	54.3
Kenya Goblet	44.7	53.3	56.0	82.7	33.3	40.6	21.1	11.8	19.7
Kenya Jay	64.7	63.7	59.3	87.5	22.1	31.8	1.2	34.0	53.6
Kenya Kingbird	53.0	52.7	50.0	74.0	81.9	36.8	54.9	26.4	45.1
Kenya Korongo	57.7	57.0	56.7	75.3	39.8	49.0	28.8	11.6	49.9
Kenya Seed 2	58.0	57.7	55.7	78.7	32.1	32.5	28.4	18.4	42.4
Kenya Seed 5	58.0	54.0	56.0	78.0	49.9	32.1	33.4	31.0	52.5
Kenya Seed 6	54.0	55.0	54.0	87.0	40.1	24.6	14.3	40.2	33.8
Kenya Seed 7	59.3	55.0	53.3	84.3	42.8	25.3	25.8	16.6	35.4
Kenya Wren	56.3	57.5	54.0	79.0	54.2	50.5	37.8	64.8	80.4



Genotype	Days to Heading (days)			Plant Height (cm)	FHB Index (%)			FHB Severity (%)	
	STP16	STP17	CRK17	STP16	STP16	STP17	CRK17	Isolate	Isolate
								10116010	10116012
Kenya 1012-B-1-L	65.0	63.7	61.3	73.0	-	11.1	9.3	31.1	63.3
Kingbird	52.3	54.3	49.3	72.3	97.5	37.6	39.7	32.1	75.3
Kubsa	49.7	52.5	51.0	71.3	78.7	38.7	33.2	21.3	46.4
Kwale	-	-	-	-	-	-	-	18.3	55.8
LCS Albany	49.0	55.0	52.3	78.0	32.7	24.5	19.7	21.6	41.6
Lenana	51.0	52.3	50.7	84.0	87.5	37.7	31.9	51.6	89.3
Linkert	42.0	52.0	49.3	65.0	25.1	26.6	23.1	6.3	15.7
WB-Mayville	42.0	49.0	48.0	65.0	45.6	34.1	44.3	32.2	52.3
Menze	61.0	61.3	59.0	76.3	21.6	20.4	7.6	34.8	52.5
Morris	49.0	53.3	53.3	84.7	67.2	17.7	15.9	40.3	60.8
Njoro BW II	61.7	61.0	56.7	70.0	19.1	22.9	8.8	11.3	31.1
P. Walker Munro	46.0	52.3	51.7	84.3	51.9	16.8	24.1	6.3	21.7
Primex	45.0	50.3	48.3	85.0	74.9	46.3	78.2	83.9	91.0
R 1286	65.0	60.3	60.3	76.7	22.0	11.9	10.4	27.2	58.4
R 1301	54.7	55.0	52.3	88.7	41.5	40.3	32.9	49.7	72.2
R 1353	51.7	54.3	53.7	78.3	54.7	42.1	32.4	27.0	71.0
R 1360	50.3	54.3	53.3	77.3	65.4	23.4	32.2	23.3	45.7
R 1362	61.0	59.0	58.0	78.3	68.2	25.4	39.3	58.3	77.6
R 1363	49.0	53.0	49.7	69.7	70.3	34.6	69.8	69.7	75.9

Genotype	Days to Heading (days)			Plant Height (cm)	FHB Index (%)			FHB Severity (%)	
	STP16	STP17	CRK17	STP16	STP16	STP17	CRK17	Isolate	Isolate
								10116010	10116012
R 1365	54.7	55.3	53.0	78.3	80.4	74.5	28.6	90.9	87.2
R 1367	49.3	53.0	50.7	76.0	55.8	25.3	39.3	46.3	61.4
R 1372	50.3	54.7	51.7	76.3	44.8	38.2	28.9	33.1	66.3
R 1373	54.0	55.0	54.0	76.0	46.7	29.3	41.9	31.7	64.7
R 1397	58.7	56.3	54.3	76.0	61.2	34.7	36.9	50.0	72.0
R 1403	53.3	55.3	56.7	79.3	60.5	33.2	28.0	31.7	42.4
R 1404	49.0	55.0	51.7	78.7	54.5	22.3	15.6	29.7	65.5
R 1412	54.7	55.0	53.0	75.3	63.4	33.1	36.8	32.0	65.7
R 1429	63.0	57.7	59.3	68.3	58.5	27.1	36.8	28.8	60.7
R 1430	51.0	53.0	49.3	76.3	60.5	63.7	78.9	64.6	88.7
R 1441	51.3	55.0	54.7	68.7	52.0	35.2	33.2	25.1	54.7
R 1442	49.3	54.3	53.0	78.7	62.6	43.5	31.7	61.6	75.1
R 1447	45.3	52.3	50.0	79.7	53.6	20.4	35.0	52.8	76.2
R 1452	60.3	54.3	53.3	72.0	54.7	30.5	35.3	57.0	84.5
R 1454	47.7	54.3	50.0	67.5	45.6	16.1	29.1	38.9	61.2
R 1460	58.7	56.0	54.7	73.0	36.2	33.6	24.5	46.0	64.0
R 1462	61.7	63.0	60.3	75.7	58.1	24.9	11.2	58.2	81.6
Ravi 1	54.7	53.0	51.7	75.0	88.1	72.4	56.2	46.0	95.9
Ravi 6	55.3	53.0	50.7	73.3	96.4	36.3	60.1	51.3	84.6

Genotype	Days to Heading (days)			Plant Height (cm)	FHB Index (%)			FHB Severity (%)	
	STP16	STP17	CRK17	STP16	STP16	STP17	CRK17	Isolate	Isolate
								10116010	10116012
Ravi 25	54.0	54.7	50.7	72.3	73.3	27.8	41.4	44.7	75.7
Ravi 34	47.7	51.5	51.3	77.3	50.7	20.7	37.6	40.5	65.9
Ravi 43	49.3	52.5	50.0	72.0	46.8	16.9	28.5	13.0	21.6
Ravi 46	49.3	52.5	50.3	73.7	73.1	19.7	27.3	16.2	52.7
Ravi 47	59.3	59.0	58.0	75.7	52.4	29.4	36.5	58.4	84.6
Ravi 54	54.3	55.7	54.3	77.0	57.3	34.5	38.4	43.5	72.8
Ravi 55	60.3	59.0	56.0	78.3	48.1	33.6	15.8	54.7	76.1
Rollag	45.0	50.7	49.0	74.0	22.1	25.1	21.1	10.4	22.7
Samson	44.7	51.0	49.0	68.0	33.2	28.1	62.6	28.9	31.9
Sofumar	51.0	53.3	52.3	82.7	78.1	26.9	28.5	50.1	80.7
Tama	49.0	51.0	50.3	87.7	62.1	29.3	25.5	30.8	46.9
Tay	55.0	55.0	54.0	85.0	42.5	49.3	28.3	16.2	27.2
Trophy	51.3	54.3	54.0	90.3	48.1	28.6	20.3	29.4	55.6
Wheaton	-	56.3	61.0	-	-	34.1	46.1	65.3	84.7
<b>Mean</b>	53.8	55.4	53.9	76.9	52.1	31.9	30.2	37.3	59.9
<b>SD</b>	6.2	3.5	3.7	5.8	19.1	12.2	15.5	18.1	19.1

<sup>a</sup> conditional formatting



The dash (-) symbol indicates missing data.

**Table 2.4.** Analysis of variance and heritability for Fusarium head blight (FHB) index (%), visually scabby kernels (VSK) (%), deoxynivalenol (DON) concentration (ppm), heading days (HD) and plant height (PH) for 221 wheat genotypes evaluated in inoculated (*Fusarium graminearum*) and mist-irrigated nurseries at St. Paul in 2016, St. Paul in 2017 and in Crookston in 2017. Data for late genotypes, five in STP16 and two in STP17, were not collected.

Trait	Source	df <sup>a</sup>	MS <sup>b</sup>	F value <sup>c</sup>	Heritability <sup>d</sup>
FHB Index	Genotype	220	1247	5.93***	0.74
	Replications	2	1124	5.35**	
	Locations	2	77861	370.51***	
	Genotype x locations	432	431	2.05***	
	Error	1283	210		
VSK	Genotype	220	628.68	10.36***	0.86
	Replications	2	2238.53	36.89***	
	Locations	2	1272.04	20.96***	
	Genotype x locations	438	147.76	2.43***	
	Error	1281	60.67		
DON	Genotype	220	197	5.36***	0.66
	Replications	2	1852	50.43***	
	Locations	2	69318	1887.44***	
	Genotype x locations	437	99	2.68***	
	Error	1279	37		
HD	Genotype	220	93.59	42.76***	0.94
	Replications	2	31.73	14.49***	
	Locations	2	461.54	210.87***	
	Genotype x locations	438	14.36	6.56***	
	Error	1300	2.19		
PH	Genotype	216	74.79	5.04***	
	Replications	2	34.65	2.33	
	Error	358	14.84		

<sup>a</sup>df = degree of freedom

<sup>b</sup>MS = mean square,

<sup>c</sup>\*\*\*Significant at the 0.01 probability level, \*\* significant at the 0.001 probability level.

<sup>d</sup>Broad sense heritability calculated on an entry mean basis.

**Table 2.5.** Pearson correlation coefficients for Fusarium head blight (FHB) index (%) with visually scabby kernels (VSK) (%), deoxynivalenol (DON) concentration (ppm), heading dates (days) (HD) and plant height (PH) (cm). The five variables were assessed on 221 wheat genotypes evaluated in inoculated (*Fusarium graminearum*) and mist-irrigated nurseries at St. Paul in 2016, St. Paul in 2017 and in Crookston in 2017. Plant height was only measured in STP16 location. Data for late genotypes, five in STP16 and two in STP17, were not collected<sup>a</sup>.

ST. PAUL - 2016					
	FHB Index (%)	VSK (%)	DON (ppm)	HD (days) <sup>b</sup>	PH (cm)
FHB Index (%)	1				
VSK (%)	0.56 ***	1			
DON (ppm)	0.54***	0.73***	1		
HD (days)	-0.18***	-0.54***	-0.33***	1	
PH (cm)	-0.13	-0.12	-0.15*	-	1
ST. PAUL - 2017					
FHB Index (%)	1				
VSK (%)	0.32***	1			
DON (ppm)	0.22***	0.48***	1		
HD (days)	-0.13	-0.11	0.27***	1	
PH (cm)	0.01	-0.14*	-0.13	-	
CROOKSTON - 2017					
FHB Index (%)	1				
VSK (%)	0.65***	1			
DON (ppm)	0.39***	0.74***	1		
HD (days)	-0.50***	-0.39***	-0.01	1	
PH (cm)	-0.20**	-0.09	-0.02	-	

<sup>a</sup>\*, \*\* and \*\*\* indicate significance at  $P \leq 0.05$ , 0.01 and 0.001, respectively

<sup>b</sup>The dash (-) symbol indicates no correlation performed.

**Table 2.6.** Analysis of variance for FHB severity (%) for a greenhouse experiment conducted in 2017 examining 82 wheat genotypes for their response to *F. graminearum* isolates 10116010 and 10116012.

Source	df <sup>a</sup>	MS <sup>b</sup>	F value <sup>c</sup>
Genotype	81	2310	9.34***
Isolate	1	70791	286.10***
Replications	2	453	1.83
Genotype × Isolate	81	433	1.75***
Error	320	247	

<sup>a</sup>df = degree of freedom

<sup>b</sup>MS = mean square

<sup>c</sup>\*\*\* Significant at 0.001 probability level.

**Table 2.7.** Analysis of variance for FHB severity (%) for a greenhouse experiment conducted in 2018 examining 82 wheat genotypes for their response to *F. graminearum* isolates 10116010 and 10116012.

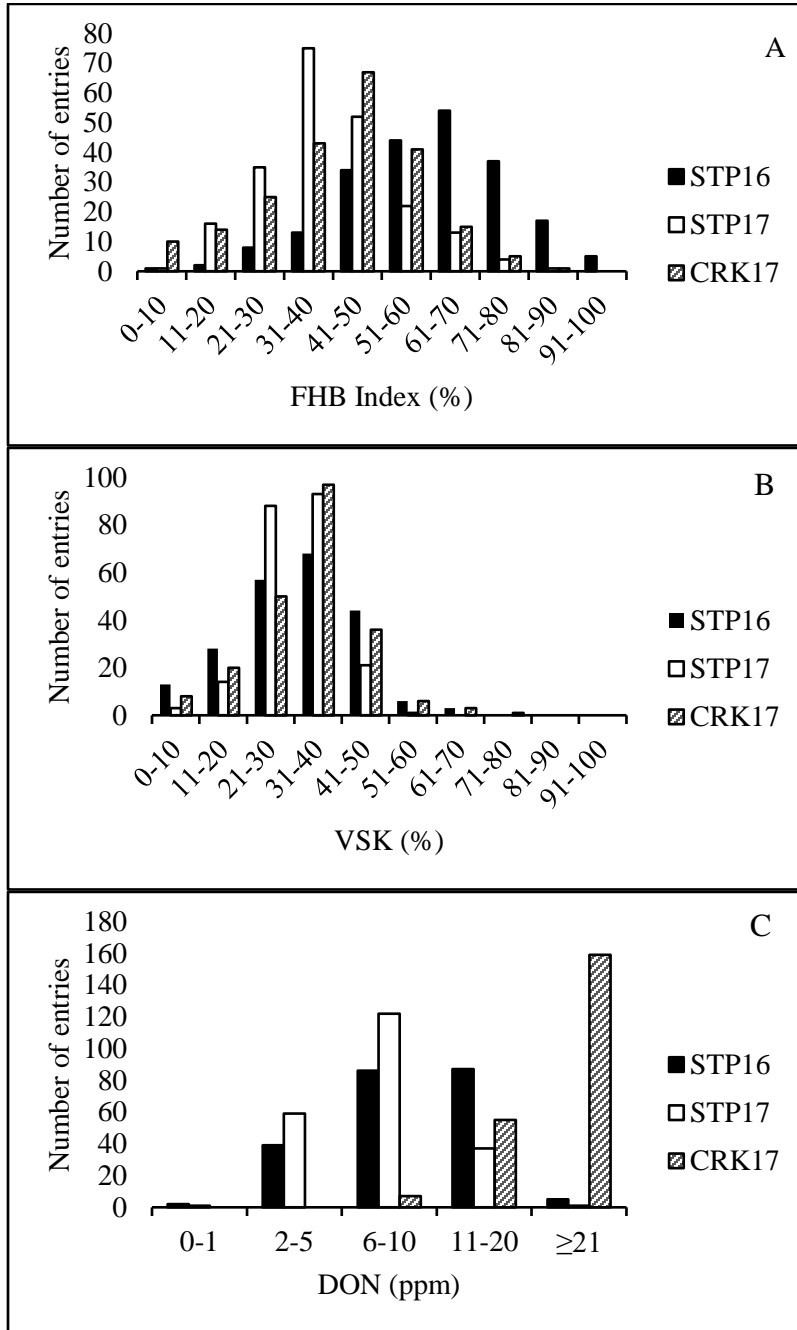
Source	df <sup>a</sup>	MS <sup>b</sup>	<i>F</i> value <sup>c</sup>
Genotype	81	2169	6.75***
Isolate	1	56577	176.00***
Replications	2	542	1.69
Genotype × Isolate	81	233	0.72
Error	322	321	

<sup>a</sup>df = degree of freedom

<sup>b</sup>MS = mean square

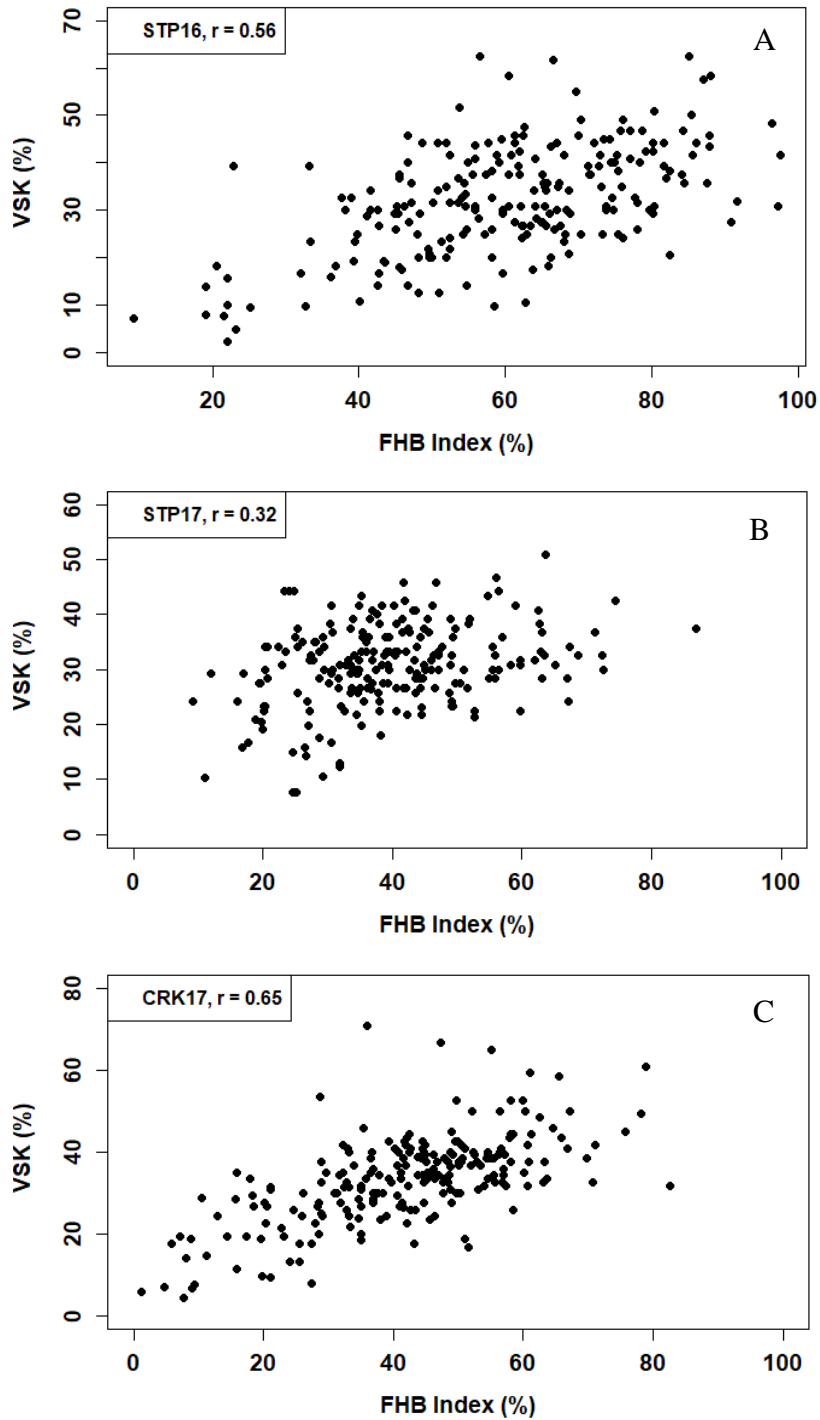
<sup>c</sup>\*\*\* significant at 0.001 probability level.

## 2.7 Figures

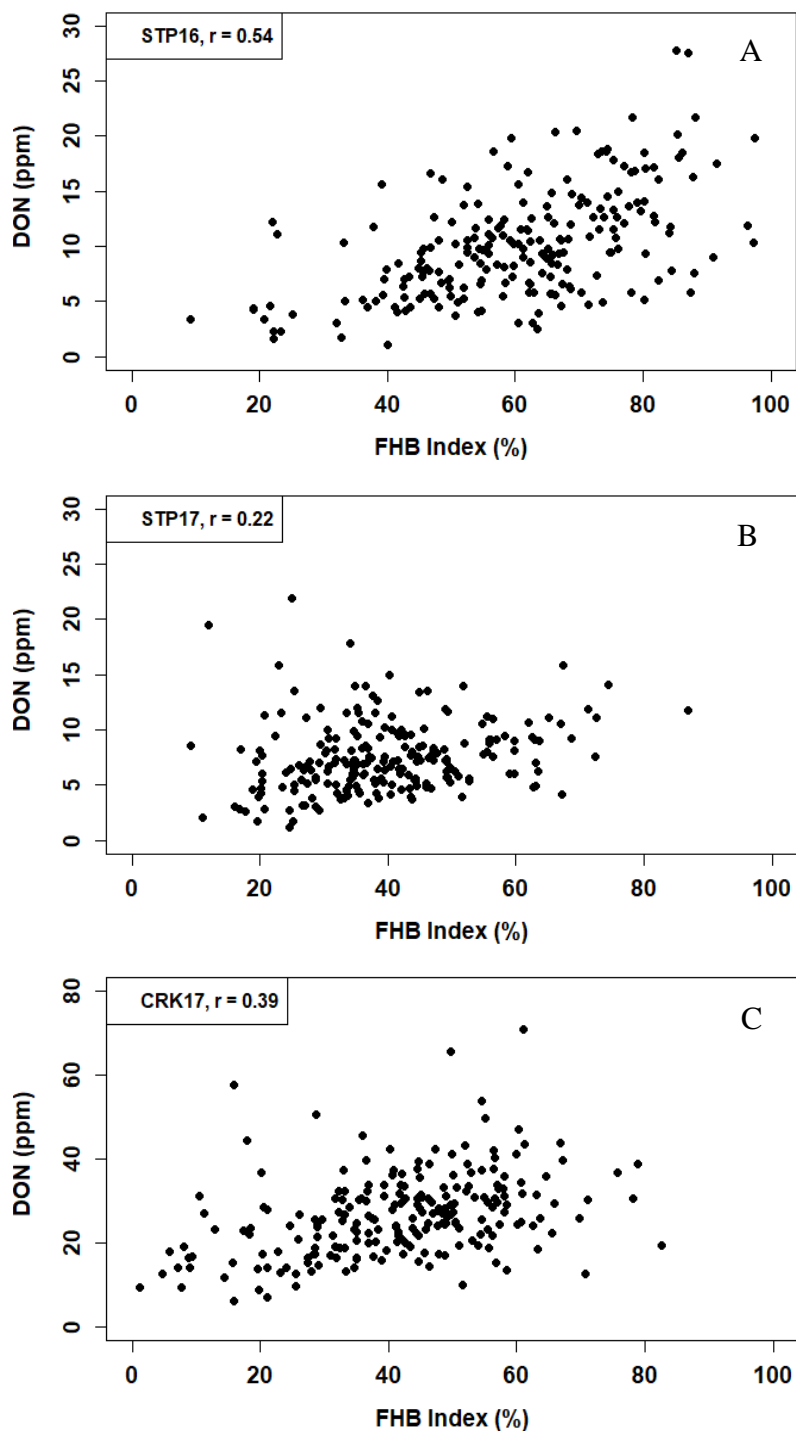


**Figure 2.1.** Frequency distribution for A) Fusarium head blight (FHB) index (%); B) visually scabby kernels (VSK) (%); and C) deoxynivalenol (DON) concentration (ppm) for 221 wheat genotypes evaluated in inoculated (*Fusarium graminearum*) and mist-irrigated nurseries at St. Paul in 2016 (STP16), St. Paul in 2017 (STP17) and in Crookston in 2017 (CRK17). Data for late genotypes, five in STP16 and two in STP17, were not collected.

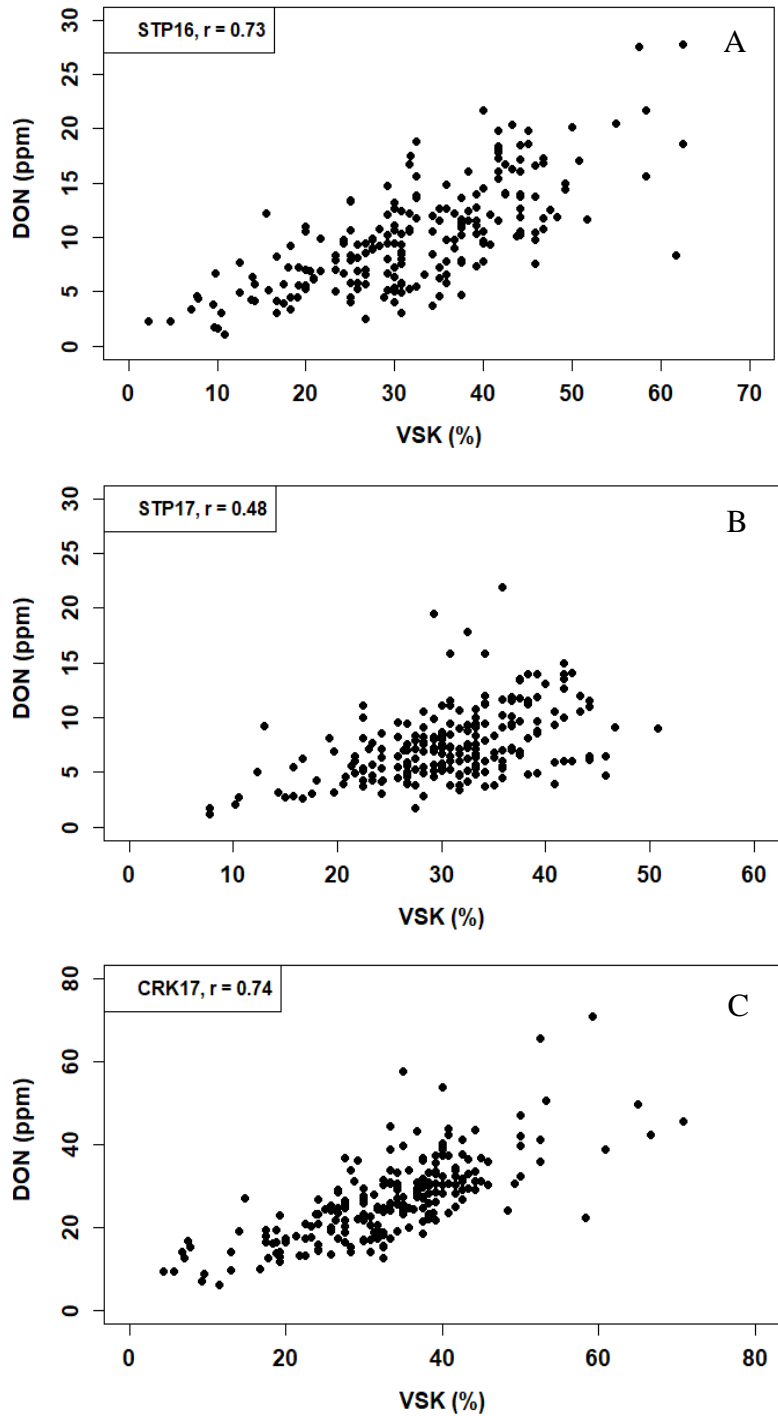




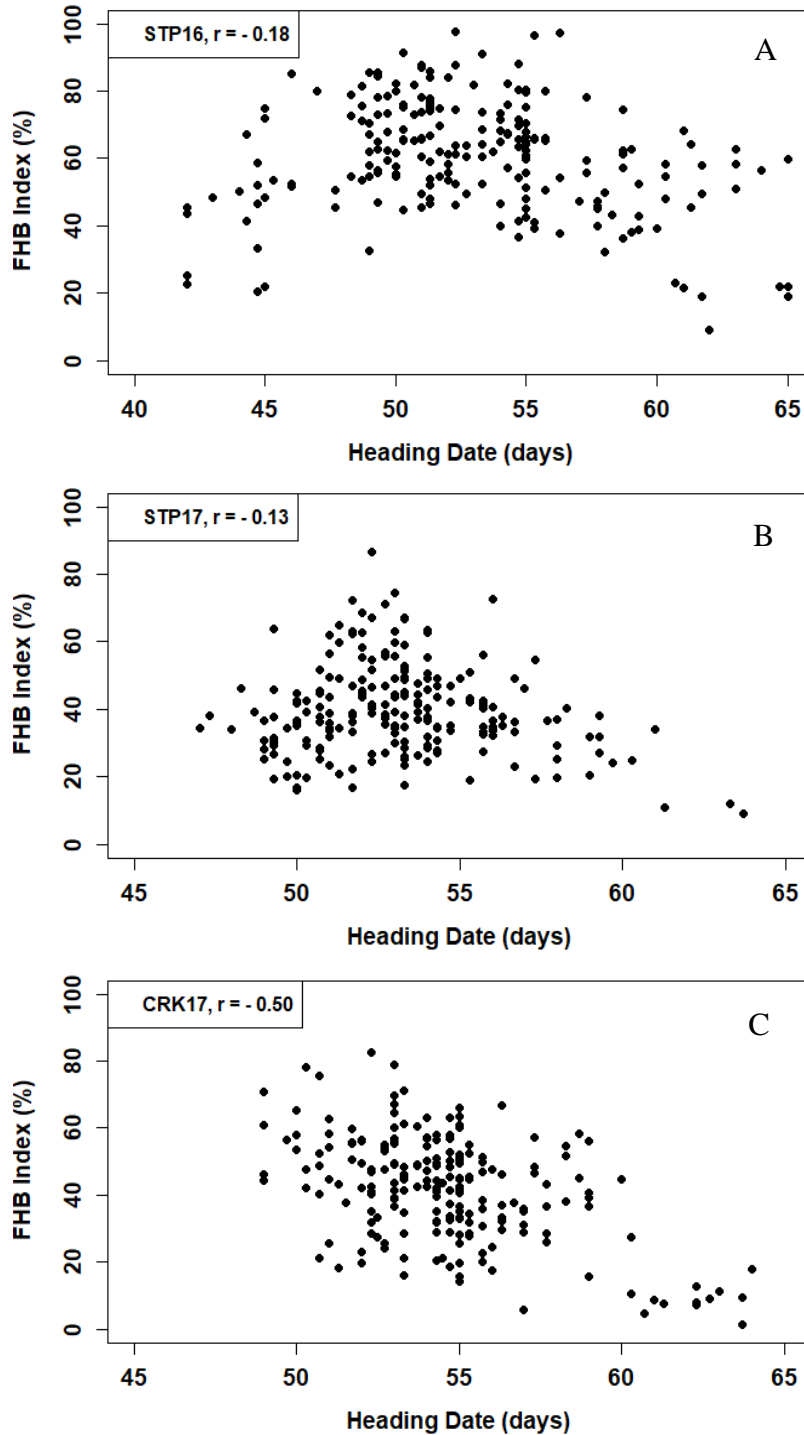
**Figure 2.2.** Correlations between Fusarium head blight (FHB) index (%) and visually scabby kernels (VSK) (%) for 221 wheat genotypes inoculated with *Fusarium graminearum* in mist-irrigated nurseries conducted at St. Paul (A) in 2016 (STP16) and (B) in 2017 (STP17), and (C) at Crookston in 2017 (CRK17). Data for late genotypes, five in STP16 and two in STP17, were not collected.



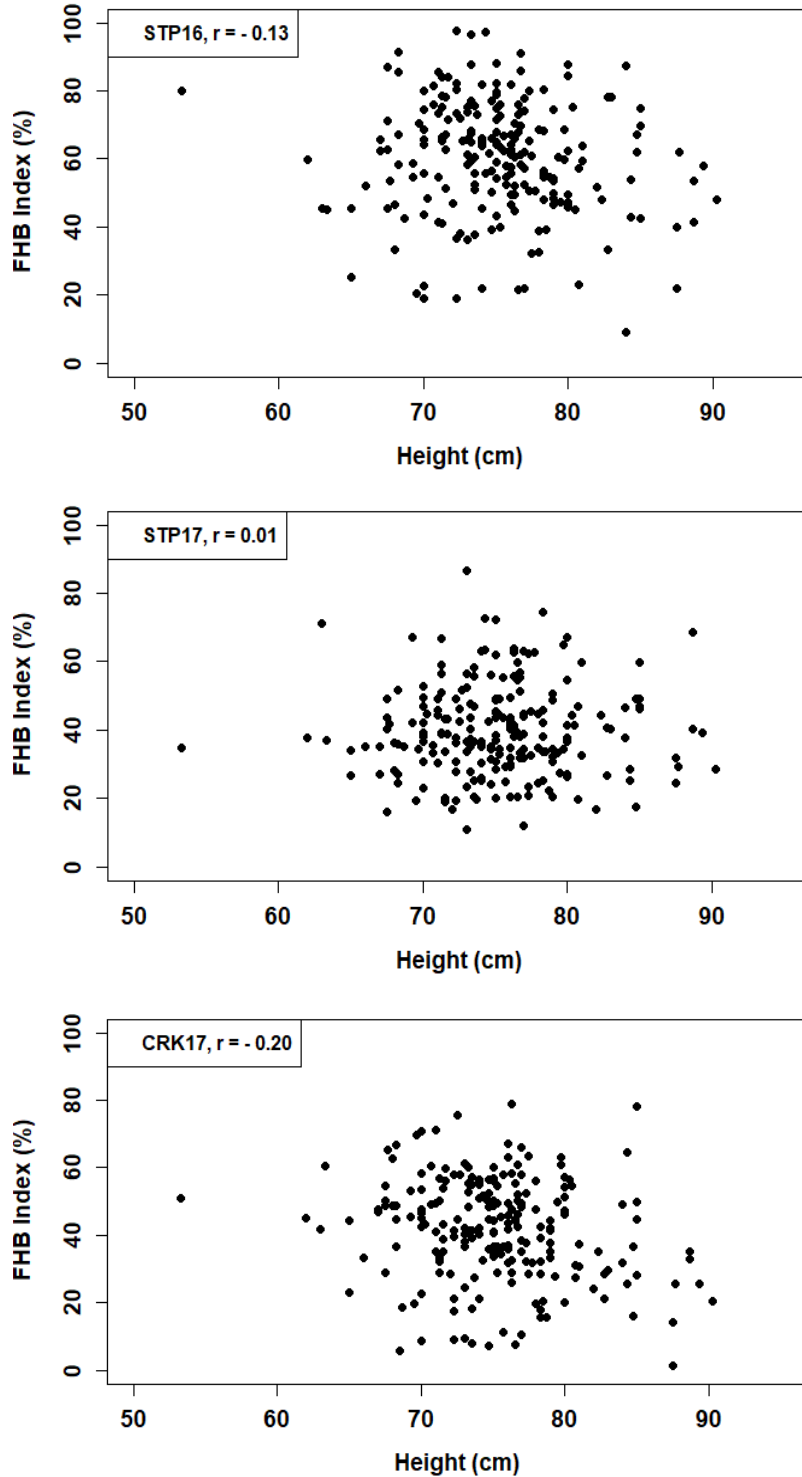
**Figure 2.3.** Correlations between Fusarium head blight (FHB) index (%) and deoxynivalenol (DON) concentration (ppm) for 221 wheat genotypes inoculated with *Fusarium graminearum* in mist-irrigated nurseries conducted at St. Paul (A) in 2016 (STP16) and (B) in 2017 (STP17), and (C) at Crookston in 2017 (CRK17). Data for late genotypes, five in STP16 and two in STP17, were not collected.



**Figure 2.4.** Correlation between visually scabby kernels (VSK) (%) and deoxynivalenol (DON) concentration (ppm) for 221 wheat genotypes inoculated with *Fusarium graminearum* in mist-irrigated nurseries conducted at St. Paul (A) in 2016 (STP16) and (B) in 2017 (STP17), and (C) at Crookston in 2017 (CRK17). Data for late genotypes, five in STP16 and two in STP17, were not collected.



**Figure 2.5.** Correlation between heading date (days from planting) and Fusarium head blight (FHB) index (%) for 221 wheat genotypes inoculated with *Fusarium graminearum* in mist-irrigated nurseries conducted at St. Paul (A) in 2016 (STP16) and (B) in 2017 (STP17), and (C) at Crookston in 2017 (CRK17). Data for late genotypes, five in STP16 and two in STP17, were not collected.



**Figure 2.6.** Correlation between plant height (cm) and FHB index (%) for 221 wheat genotypes inoculated with *Fusarium graminearum* in mist-irrigated nurseries conducted at St. Paul (A) in 2016 (STP16) and (B) in 2017 (STP17), and (C) at Crookston in 2017 (CRK17). Data for late genotypes, five in STP16 and two in STP17, were not collected.

**Chapter Three**  
**Genetics and Mapping of Stem Rust Resistance in Spring Wheat Line CI 14275**

### 3.1 Introduction

Wheat currently accounts for approximately 20% of the daily consumed calories, with hexaploid wheat [*Triticum aestivum* ( $2n = 6x = 42$ ; AABBDD)] being the most widely cultivated species (Gupta *et al.*, 2008). The European Union, China, India, Russia and the United States are the top five wheat producers (FAO, 2018). Production of wheat is faced with both biotic and abiotic challenges, stem rust caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) being one of the most devastating diseases that may result in 100% yield loss on susceptible varieties. Stem rust occurs wherever wheat is grown, and it is favored by high humidity and temperatures between 18 to 30 °C. *Pgt* race Ug99, first isolated from Uganda in the year 1999 (Pretorius *et al.*, 2000), hence the acronym “Ug99”, and later characterized using the North American race nomenclature as race TTKSK (Jin *et al.*, 2008), was the first *Pgt* race virulent to the *Sr31* stem rust resistance gene (Pretorius *et al.*, 2000; Wanyera *et al.*, 2006). Since then, multiple variants of TTKSK have been reported including TTKST, virulent to *Sr24* and identified in 2006 (Jin *et al.*, 2008), TTTSK, virulent to *Sr36* and identified in 2007 (Jin *et al.*, 2009), TTKTK and TTKTT, virulent to *SrTmp* and identified in 2014 (Newcomb *et al.*, 2016; Patpour *et al.*, 2016). The *Sr* genes that have been overcome by race TTKSK and its variants had been widely deployed in most cultivated wheat.

Stem rust can be controlled by fungicides and host resistance (Wanyera *et al.*, 2009). Fungicide use, however can be an expensive option, especially for farmers who are not financially stable. The efficacy of fungicides can be affected by crop stage and application rates; hence host resistance remains the most effective and economic approach of controlling stem rust. The resistance in wheat to stem rust is observed at the seedling stage and/or the adult plant stage (Priyamvada *et al.*, 2011). The resistance at the seedling stage, generally conferred by major genes, is also called “all stage resistance” because it is usually expressed at both the seedling and adult plant stages (Priyamvada *et al.*, 2011). Resistance that is expressed only at the adult plant stage, commonly referred to as “adult plant resistance”, is often conferred by multiple minor-effect genes (Priyamvada *et al.*, 2011). The deployment of single major genes in any wheat background is risky because the resistance may be overcome as has happened repeatedly, a good example

being the Ug99 race group that has overcome several important major genes such as *Sr24*, *Sr26*, *Sr31*, and *SrTmp* (Jin *et al.*, 2008, 2009; Singh *et al.*, 2011). To achieve durable resistance, multiple major and/or minor genes need to be deployed together (Roelfs *et al.*, 1992; Priyamvada *et al.*, 2011). Identification and deployment of new sources of resistance into wheat backgrounds is important to provide the components needed for the development of new resistance gene combinations.

The objectives of this study were; to characterize the genetics of stem rust resistance in the spring wheat line CI 14275, identify QTLs associated with the resistance, and develop markers linked to the identified resistance QTL. CI 14275 is a Canadian breeding line (Q 2331-34) that was a part of the 1966 USDA-ARS International Spring Wheat Rust Nursery and which appeared effective to the Ug99 race group in Kenya when screened in 2005, 2006 and 2007 (Rouse *et al.*, 2011). The USDA National Small Grains Collection lists the pedigree of CI 14275 as Thatcher\*6/Kenya Farmer//6\* Lee/Kenya Farmer. Thatcher has been reported to have *Sr5*, *Sr9g*, *Sr12*, and *Sr16* genes (McIntosh *et al.*, 1995). The effectiveness of resistance in Thatcher to stem rust at the adult plant stage was shown to involve *Sr12* (Rouse *et al.*, 2014b) and be enhanced when combined with *Sr57* (*Lr34*) (Kolmer *et al.*, 2011). Kenya Farmer has *Sr6*, *Sr7a*, *Sr9b*, *Sr10*, *Sr11*, and *Sr12* genes, whereas Lee has *Sr5*, *Sr9g*, *Sr11*, *Sr12*, and *Sr16* genes (Knott, 1957; McIntosh *et al.*, 1995). Screening of CI 14275 at the seedling stage to Kenyan (04KEN156/04 [TTKSK]), 06KEN19-V-3[TTKST]) and North American (74MN1409 [TPMKC], 01MN84A-1-2 [TTTTF]) isolates of *Pgt* resulted in low infection types of ;12, ;2, ;13, and 1+++LIF (low infection frequency, i.e low density of uredinia on the leaf) against races TTKSK, TTKST, TPMKC and TTTTF, respectively (Rouse *et al.*, 2011). CI 14275 was evaluated at the adult plant stage in the field to predominant race TTKSK in Njoro, Kenya and to a mixture of races QFCSC, TPMKC, RKQQC, RCRSC, QTHJC and MCCFC in St. Paul, MN (Rouse *et al.*, 2011). Responses of 20MR in 2005 and 5MR in 2006 were observed in Njoro, Kenya, and an immune (0) response was observed in the US in 2006 (Rouse *et al.*, 2011).



## **3.2 Materials and Methods**

### **3.2.1 Plant materials**

#### **Development of primary population**

One hundred-thirteen recombinant inbred lines (RILs) (F<sub>7:9</sub>) were developed from a cross between LMPG-6 [a stem rust susceptible spring wheat line (Knott, 1990)] and CI 14275 (LMPG-6/CI 14275) through single seed descent by Dr. Yue Jin of the USDA-ARS Cereal Disease Lab., Minnesota, USA.

#### **Development of validation population**

From the 113 RILs (F<sub>7:9</sub>) derived from LMPG-6/CI 14275, Line #162 was selected because it expressed a consistent level of resistance to stem rust across the three environments; Ethiopia (0-20MSS), Kenya (0-15SMS), St. Paul (5RMR-25RMR). Additionally, Line #162 was among the early maturing lines. Line #162 was crossed with Kwale, a susceptible Kenyan cultivar, to generate 180 F<sub>3:4</sub> families (Kwale/Line #162).

### **3.2.2 Phenotyping of the populations**

#### **Phenotyping in the greenhouse**

The LMPG-6/CI 14275 RIL population, together with the parents, were tested for their response to *Pgt* races TTKSK (04KEN156/04; Ug99) and TRTTF (06YEM34-1) in a Biosafety level 3 (BSL-3) greenhouse and for the reaction to *Pgt* US races TPMKC (74MN1409), TTTTF (01MN84A-1-2) and RTQQC (04MN74-1) at the USDA-ARS Cereal Disease Lab (CDL) greenhouse in Minnesota, United States, following a method described by Rouse *et al.* (2011). Square 6 × 6 cm diameter plastic pots were filled with vermiculite, five seeds for each line were planted in two replicates and placed in the growth chamber. *Pgt* isolates were derived from single pustules, increased in isolation and stored at -80 °C. When primary leaves were fully emerged, the inoculum was prepared by removing *Pgt* urediniospores from -80 °C, heat shocking for 15 min in a 45 °C water bath, rehydrating for approximately 2-4 h in a chamber maintained at 80% relative humidity by a KOH solution, suspending in light weight soltrol oil (Soltrol 70; ConocoPhillips Inc., Houston) and plants were inoculated by spraying the suspension with an atomizer. After inoculation, the plants were then placed in a fume hood for 30

min to allow the oil to evaporate and thereafter transferred to a dew chamber at 80% relative humidity for 14-16 h. After the dew chamber incubation, plants were returned to the greenhouse bench and maintained at  $18 \pm 2$  °C with a photoperiod of 16 h.

Two weeks post-inoculation, plants were assessed for seedling infection types (ITs) based on the 0-4 scale developed by Stakman *et al.* (1962). The description of infection types used to classify the reactions to *Pgt* are as follows: '0' = no uredinia or any other sign of infection, ';' = no uredinia but presence of hypersensitive necrotic flecks, '1' = small uredinia surrounded by yellow chlorotic or necrotic areas, '2' = small to medium-sized uredinia in a dark green island surrounded by a chlorotic area, '3' = medium-sized uredinia, surrounded by light green chlorosis, '4' = large uredinia with no or a limited amount of chlorosis. All observed infection types on the same leaf were recorded, with the infection type(s) listed in order according to their prevalence. A comma (,) symbol was used to separate multiple ITs observed on the same plant, with the most frequent IT recorded first. Whenever multiple infection types were observed on different plants of the same line, a forward slash (/) symbol was used to separate the infection types. A letter 'C' was indicated whenever extensive chlorosis was associated with the infection. The plus (+) and minus (-) symbols were used for the pustules that were relatively larger or smaller, respectively, than normal. Plants with ITs ranging from 0 to 2 were categorized as resistant, and those with 3-4 ITs were categorized as susceptible. The seedling infection types based on a 0 to 4 scale were then converted to a 0 to 9 linear scale according to Gao *et al.* (2019) and used for analyses (Appendix 3.1).

### **Phenotyping of the primary population in the field**

The experimental plots were evaluated for stem rust severity in three field locations: Njoro, Kenya; Debre Zeit, Ethiopia; and St. Paul, Minnesota in 2016, 2017, and 2018. Across the three locations, the experimental plots were randomized, and approximately 5g seed was sown in each of two replicates. In Njoro, Kenya, the field plots were two 0.7 m long rows separated by 0.3 m. Spreader plants, consisting of a mixture of susceptible cultivars Caccuke, Kenya Eagle 10, Robin, and six CIMMYT lines with *Sr24* gene were planted to surround the entries 1-2 weeks before the experimental plots were

planted. From the booting to heading growth stages (Zadok's growth stages 37-60), fresh urediniospores from predominant Ug99 *Pgt* races that include TTKSK, TTKST, TTKTK and TTKTT were bulked, suspended in distilled water, and approximately 1 mL of the suspension was injected into spreader plants using a hypodermic syringe. Spreader rows were also planted when the plots were planted and arranged in rows of hill plots perpendicular to the entries to facilitate the build-up and spread of the disease. In Debre Zeit, Ethiopia, the experimental plots were planted in double 1 m long rows. The spreader rows consisting of cultivars Arendeto, Digalu, Local Red, Morocco and PBW343 were planted perpendicular to the plots, 1-2 weeks prior to planting of the experimental plots, and spreader rows were artificially inoculated at Zadok's growth stage 37-60 with bulked urediniospores from predominant *Pgt* races TTKSK, TKTTF, TRTTF and JRCQC to initiate infection on the plots. In St. Paul, the experimental plots were single 1 m long rows, separated by 0.3 m. The spreader rows consisting of cultivars Baart, Morocco and Thatcher were planted 1-2 weeks earlier than entries and sown perpendicular to the experimental plots. At heading stage, the plots were spray-inoculated using an Ulva+ sprayer (Micron Sprayers Ltd., Bromyard, UK) with a light mineral oil suspension of bulked urediniospores of North American *Pgt* races QFCSC (isolate 06ND76C), TPMKC (isolate 74MN1409), RKRQC (isolate 99KS76A), RCRSC (isolate 77ND82A), QTHJC (isolate 75ND717C), and MCCFC (isolate 59KS19). The inoculated spreader rows initiated stem rust infection on the experimental plots.

When the spreaders had attained 50% severity in the three locations, stem rust severity was visually scored in the experimental plots based on the modified Cobb scale of 0-100, where 0 = immune; no uredinia or any other sign of infection and 100% = completely susceptible (Peterson *et al.*, 1948). Infection response was rated as either; resistant (R), small uredinia surrounded by necrosis; moderately resistant (MR), medium-sized uredinia surrounded by necrosis or chlorosis; moderately susceptible (MS), medium-sized uredinia without necrosis; susceptible (S), large uredinia without necrosis; or MRMS, infection response that included both the MR and MS categories (Roelfs *et al.*, 1992). Coefficient of infection (COI) values were generated by multiplying the stem rust severity value for each line by a constant value for each infection response: 0 = 0, R = 0.2, RMR = 0.3, MR

= 0.4, M = 0.6, MS = 0.8, S = 1.0 (Knott, 1989). Average coefficient of infection for the two replicates were determined and used for analyses.

### **Field evaluations of the validation population**

Twice-replicated plots of the 180 F<sub>3:4</sub> validation lines were evaluated for field response to the Ug99 race group in Njoro, Kenya in 2018. Planting, inoculation with *Pgt* races that include TTKSK, TTKST, TTKTK, TTKTT, and evaluation was conducted as previously mentioned.

### **3.2.3 DNA extraction**

Tissues were harvested from three-leaf stage single plants of the RIL population (F<sub>7:9</sub>), from single F<sub>3</sub> plants of the Kwale/Line #162 population, and their parents into Eppendorf tubes (1.5 mL). The harvested leaves were dried and ground for 1-2 min using a Genogrinder. Extraction buffer (300  $\mu$ l: 200mM Tris-HCL pH 8.0, 250mM NaCl, 25mM EDTA, 0.5% SDS, ddH<sub>2</sub>O) was added to each well, tubes shaken gently, and centrifuging completed at 2500 rpm. Working under a fume hood, 300  $\mu$ l of chloroform:isoamyl alcohol (24:1) was added to each well, plates were centrifuged for 20 min at 2500 rpm and the supernatant (200-300  $\mu$ l) was transferred to separate tubes. Cold isopropanol (300  $\mu$ l) was then added to each tube. Plates were shaken gently and placed in the fridge for 10-20 min. After decanting the supernatant, 300  $\mu$ l of 70% ethanol was added to each tube, the tubes were centrifuged for 20 min at 2500 rpm, the ethanol was poured out and the DNA pellets were air-dried. When the pellets were completely dry, 100  $\mu$ l of distilled H<sub>2</sub>O was added to each tube to resuspend the pellet and the tubes were left in the fridge overnight. DNA was quantified using a Nano-Drop spectrophotometer, and concentrations were diluted to 50 ng/ $\mu$ l.

### **3.2.4 Genotyping of the primary population**

Genotyping of the RILs and the parents was completed at the USDA-ARS small grains genotyping lab in Fargo, North Dakota using the iSelect 90k SNP assay developed by Wang *et al.* (2014). The 90k SNP assay contains 81,587 SNPs and was developed for allohexaploid and allotetraploid wheat populations. The raw 90K SNP data together with

the consensus map were uploaded onto illumina GenomeStudio genotyping software version 2.0. The SNPs were manually called, and the genotypes grouped as AA, AB, or BB. The missing genotypes were indicated as no call (NC). Following calculation of allele frequencies, a total of 12,243 polymorphic markers with AA and BB allele frequencies between 25% and 75% were selected.

### **3.2.5 QTL mapping**

A genome-wide genetic map was constructed using genotype calls from GenomeStudio. A total of 33 linkage groups, each with greater than five markers were generated. The 33 linkage groups included a total of 876 markers (Appendix 3.2). These 33 linkage groups were used for QTL analysis using the R/qtl software (Broman and Sen, 2009). The QTL that conferred resistance to races RTQQC, TTTTF, and TPMKC at the seedling stage and QTL that conferred resistance at the adult plant stage in the Kenya, Ethiopia, and St. Paul environments were identified separately. Using the scanone function, single-QTL genome scan via Haley-Knott regression was performed. The statistical significance thresholds of genome scan results were determined by running 1,000 permutation tests with a 0.05 significance level. Markers flanking the QTL were identified using the find.flanking function. Linkage groups were assigned to chromosomes based on the 90K consensus map. The physical positions of the QTL were identified by searching positions of the flanking markers in the T3/Wheat website: <https://triticeaetoolbox.org/wheat/> (Blake *et al.*, 2016). The probe sequence of the markers was determined by searching the GrainGenes website: <https://wheat.pw.usda.gov/GG3/> when marker information was missing in the T3/Wheat website, and the sequences were blasted to the reference genome at the BLAST website: <https://wheat-urgi.versailles.inra.fr/Seq-Repository/BLAST> to find the physical position of the markers (Alaux *et al.*, 2018).

### **3.2.6 Confirmation of presence/absence of Lr34/Sr57 in CI 14275 line**

Since the effectiveness of resistance in Thatcher to *Pgt* races in North America and Kenya is enhanced by the presence of *Lr34* (Kolmer *et al.*, 2011), the diagnostic marker, CsLV34, for the *Lr34/Sr57* gene was run on the parents (LMPG-6 and CI 14275). Chinese Spring was used as the positive control.

### **3.2.7 Data analyses**

Chi-square analysis was performed on the linearized seedling data to determine goodness-of-fit to the expected segregation ratios for different inheritance models. Using R software, analysis of variance (ANOVA) was performed using the average coefficient of infection (COI) values. Correlation of stem rust severity between the environments was determined using Pearson correlation coefficients.

## **3.3 Results**

### **3.3.1 Phenotyping in the greenhouse**

The RILs and the parents were all susceptible when tested against races TTKSK and TRTTF in two replicates each (Table 3.1). There were some inconsistencies of infection types observed between replicates in some lines. For race TTKSK, a 33+ infection type (IT) was observed on CI 14275 (resistant) and a 3/33+ IT was observed on LMPG-6 (susceptible) (Table 3.1). For race TRTTF, infection types of 33+ and 3+/33+ ITs were observed on the parents CI 14275 and LMPG-6, respectively (Table 3.1). For race TTTTF, infection types of ;1/1+3C and 3+ ITs were observed on the resistant and susceptible parents, respectively, and the observed frequency of the population fit into the expected ratio of 1:1 (one gene) with a  $\chi^2$  value of 0.704 ( $p = 0.40$ ) (Tables 3.1 and 3.2). Screening for race RTQQC resulted in 3/33+ and 3+/33+ ITs on CI 14275 and LMPG-6, respectively, and the observed frequency of the population fit into expected ratio of 15:1 (susceptible:resistant; three complementary genes) with a  $\chi^2$  value of 0.005 ( $p = 0.94$ ) (Tables 3.1 and 3.2). For race TPMKC, ITs of 23-/23+ and 3+/33+ were observed on the resistant and susceptible parents, respectively, and the observed frequency of the population did not fit any simple expected ratio (Tables 3.1 and 3.2).

### **3.3.2 Phenotyping in the field**

Stem rust ratings from 0 to 90S were observed in Kenya 2016 (KEN16), 0 to 80S in Kenya 2017 (KEN17), and TR to 60S in Kenya 2018 (KEN18) (Table 3.3). Ratings from TR (trace R) to 80S were observed in Ethiopia 2016 (ETH16), TMS (trace MS) to 50S in Ethiopia 2017 (ETH17) and TMR (trace MR) to 70S in Ethiopia 2018 (ETH18) (Table 3.4). Ratings from 5RMR to 100S were observed in St. Paul 2017 (STP17) and TR to

80S in St. Paul 2018 (STP18) (Table 3.5). Stem rust ratings from 0 to 70S were observed in the validation population (Appendix 3.3). An error occurred during planting of the experimental plots that led to a lot of missing data in the St. Paul 2016 experiment, leading to omission of the data from further analyses. In the three Kenyan environments: KEN16, KEN17 and KEN18, the distribution of stem rust severity was somewhat skewed towards resistance (Figure 3.1A-C). A symmetric distribution was observed in the ETH16, ETH17, and ETH18 environments (Figure 3.1D-F). A somewhat symmetric distribution was observed in STP17 and STP18 environments (Figure 3.1G-H). There were significant effects for lines, environment, a significant interaction between the RILs and the environments, and a non-significant effect for replication (Table 3.6). Stem rust severity over the different environments had statistically significant correlation coefficients, ranging between 0.55 and 0.85 for correlations among all environments except with ETH17, where the correlations were low but still statistically significant, ranging between 0.21 and 0.47 (Table 3.7).

### **3.3.3 Confirmation of presence/absence of *Lr34/Sr57* in CI 14275 line**

The parents were both negative for the *Lr34* gene-based marker, proving that the resistance in CI 14275 did not involve *Lr34/Sr57*. This result was confirmed by the lack of significant QTL on chromosome 7D, where *Lr34* is located.

### **3.3.4 Identified QTLs at seedling and adult plant stages**

#### **QTL on chromosome arm 2BS**

One QTL mapped to chromosome arm 2BS in the KEN16, ETH16, and ETH18 environments, and was designated *Q<sub>Sr.cdl-2BS.2</sub>* (Table 3.8). The QTL is flanked by the markers Tdurum\_contig54704\_176 and GENE-0592\_352 (Table 3.8). We designated this QTL with the suffix *2BS.2* since *Q<sub>Sr.cdl-2BS</sub>* was already designated in Rouse *et al.* (2014b). The variance explained by the QTL ranged from 8.3 to 14.0% in the three environments (Table 3.8).

### **QTL on chromosome 3B**

One QTL mapped to chromosome 3B in the KEN17, KEN18, STP17, and STP18 environments and was designated *Q<sub>Sr.cdl-3B.1</sub>* (Table 3.8). The markers BS00057988\_51, Excalibur\_c57658\_54, Excalibur\_c57658\_54, IAAV3838, RAC875\_c10595\_473, wsnp\_Ra\_c69\_149518 and Tdurum\_contig32277\_121 were linked to *Q<sub>Sr.cdl-3B.1</sub>* (Table 3.8). The variance explained by the QTL in the four environments ranged from 8.3 to 21.4% (Table 3.8).

### **QTL on chromosome arm 4AL**

One QTL mapped to chromosome arm 4AL for race TTTTF and was designated *Q<sub>Sr.cdl-4AL.1</sub>* (Table 3.8). The QTL *Q<sub>Sr.cdl-4AL.1</sub>* was flanked by markers Tdurum\_contig42019\_1714 and wsnp\_Ex\_rep\_c68677\_67531081 (Table 3.8). This QTL explained a large proportion (41.2%) of the variance observed (Table 3.8).

### **QTL on chromosome arm 5DL**

One QTL mapped to chromosome arm 5DL for race TTTTF and was designated *Q<sub>Sr.cdl-5DL.1</sub>* (Table 3.8). This QTL is flanked by the markers BS00062990\_51 and BS00021991\_51 (Table 3.8). The QTL explained only 3.8% of the phenotypic variance (Table 3.8).

### **QTL on chromosome arm 6AS**

One QTL mapped to chromosome arm 6AS in the KEN17, ETH16, and ETH18 environments and was designated *Q<sub>Sr.cdl-6AS.1</sub>* (Table 3.8). The QTL is flanked by BS00031178\_51 and IAAV3806 markers (Table 3.8). The variance explained by the QTL in the three environments tested ranged from 9.8 to 10.4% (Table 3.8).

### **QTL on chromosome 6B**

One QTL, *Q<sub>Sr.cdl-6BL.1</sub>*, mapped to chromosome arm 6BL. The QTL is flanked by BobWhite\_c47040\_185, BobWhite\_c27364\_296 and BS00109878\_51 markers (Table 3.8). The QTL explained phenotypic variance that ranged from 17.8 to 37.5% in the two



St. Paul field environments (STP17 and STP18) and to races RTQQC and TMPKC (Table 3.8).

### **Combined 2BS, 3B, and 6AS QTLs**

Three QTLs, *Q<sub>Sr.cdl-2BS.2</sub>*, *Q<sub>Sr.cdl-3B.1</sub>* and *Q<sub>Sr.cdl-6AS.1</sub>* were identified only at the adult plant stage in seven of the eight field environments, with no QTL reported in ETH17 because disease pressure was low, although significantly correlated with other environments. Based on the genotype data of the QTL peak markers, seven lines were found to have all three QTLs combined and eleven lines lacked the three QTLs. The t-test results from the two groups (with and without the three QTLs) showed statistically significant differences ( $p < 0.01$ ;  $0.001$ ) in seven environments (all environments except ETH17) (Table 3.9). The combined QTL provided large reductions in the stem rust severity in the observed environments and appeared to be a highly effective combination even when the disease pressure was high (Table 3.9).

### **3.3.5 Kompetitive allele-specific PCR (KASP) markers**

KASP markers were designed corresponding to the 90K SNP markers linked to QTL (Table 3.10). When assessed on the Kwale/Line #162 population, one marker, Excalibur\_c7963\_1722\_C2 was not polymorphic (Table 3.11). Simple ANOVAs for each marker were independently run to determine whether any of the KASP markers predicted the phenotype in the validation population. One marker, Excalibur\_c7963\_1722\_C1 ( $p$ -value =  $0.003$ ) was associated with reduced stem rust severity in Njoro, Kenya in 2018. This validated *Q<sub>Sr.cdl-2BS.2</sub>* that was identified in the LMPG-6/ CI 14275 population in the KEN16, ETH16, and ETH18 environments.

### **3.4 Discussion**

The RIL population together with the parents were susceptible at the seedling stage when tested with *Pgt* races TTKSK and TRTTF, an indication that the resistant parent, CI 14275, lacked an effective major gene conferring resistance to these races. This observation is different from that observed by Rouse *et al.* (2011) for the seedling response of CI 14275 to race TTKSK. Different environmental conditions between the

two studies, such as temperature, may explain the variable responses, although the differences in these findings may warrant further investigation. The observed inconsistencies of infection types between replicates in some lines could have been due to the temperature effect on the background genes. In this study, the seedling resistance to race TTTTF was conferred by a single gene. Based on map location and race-specificity, this gene is likely *Sr7a* (Turner *et al.*, 2016; Saini *et al.*, 2018). We observed transgressive segregation for response to races RTQQC and TPMKC. The distribution of disease severities for the RILs at the adult plant stage across all the environments was mostly continuous but somewhat skewed towards resistance in the Kenyan environments, suggesting a quantitative type of resistance. Lower correlation coefficients (0.21-0.32) were observed between ETH17 and the ETH16, KEN16, KEN17, STP17, and STP18 environments, suggesting genotype by environment interactions, and as indicated by the ANOVA results. Stem rust severities of the entire population were relatively low in ETH17. The differences in environmental conditions, different races used for inoculation, and the amount of inoculum used in different environments could have also contributed to the observed significant genotype by environment interactions.

### **QTL on chromosome arm 2BS**

The identified *Q<sub>Sr.cdl-2BS.2</sub>* on chromosome arm 2BS, with the explained phenotypic variance ranging from 8.3 to 14%, conferred adult plant resistance in the KEN16, ETH16, and ETH18 environments. The QTL was also validated in a separate population in Kenya in 2018. *Q<sub>Sr.cdl-2BS.2</sub>* is flanked by Tdurum\_contig54704\_176 and GENE-0592\_352 markers. Several genes have been mapped on chromosome arms 2BS and 2BL. The stem rust resistance genes effective to race TTKSK that mapped to chromosome arm 2BS are; *Sr36*, *Sr39*, *Sr40* (Wu *et al.*, 2009; Niu *et al.*, 2011; Rouse *et al.*, 2012). The gene *Sr36* was identified in *Triticum timopheevi*, while *Sr39* was identified in *Aegilops speltoides* and *Sr40* was identified in *Triticum araraticum*. The resistance provided by *Sr36* gene was overcome by race TTTSK (*Sr31+Sr36*) (Jin *et al.*, 2009), whereas *Sr39* and *Sr40* genes appear effective against the Ug99 race group but have not been utilized in breeding programs due to their linkage with undesirable traits. The possibility of *Sr39* and *Sr40* conferring the observed resistance in this study are ruled out because of both the

alien source of these genes and the susceptible seedling infection types observed in the RIL population to race TTKSK.

The mapped genes on chromosome arm 2BL are; *Sr9h*, *Sr16*, *Sr28*, and *Sr47* (Tsilo *et al.*, 2007; Hiebert *et al.*, 2011; Rouse *et al.*, 2014a). The *Sr9h*, *Sr16* and *Sr28* genes were identified in *Triticum aestivum* whereas the gene *Sr47* was identified in *A. speltoides*. The *Sr16* gene was shown to be ineffective against Ug99 races (Jin *et al.*, 2007). However, since *Sr16* and *QSr.cdl-2BS.2* are on different chromosome arms, they cannot be the same. The *Sr28* and *Sr47* genes have been found effective against the Ug99 race group (Jin *et al.*, 2007; Rouse *et al.*, 2012) but the QTL *QSr.cdl-2BS.2* identified in this study is unlikely either the *Sr28* or *Sr47* genes because of the observed susceptibility of CI 14275 at the seedling stage in response to race TTKSK. The *Sr9* alleles; *Sr9a*, *Sr9b*, *Sr9d*, *Sr9e*, *Sr9f* and *Sr9g* are ineffective against Ug99 isolates but the *Sr9h* allele has been found effective against race TTKSK and other Ug99 race group isolates with virulence to *Sr31* (Rouse *et al.*, 2014a). Based on the physical position of the flanking markers, the *QSr.cdl-2BS.2* reported in this study is located between 35.2 Mb and 58.3 Mb on the 90K consensus map (Wang *et al.*, 2014). This map position excludes the possibility that *QSr.cdl-2BS.2* could be conferred by stem rust resistance genes on the long arm of chromosome 2B. The *QSr.umn-2B.2* reported by Bajgain *et al.* (2015) in a RB07/MN06113-8 population is between 22.7 Mb to 40.7 Mb on 2B, and *QSr.cdl-2BS* reported by Rouse *et al.* (2014b) in Thatcher/McNeal population is located between 12.0 Mb and 31.7 Mb on 2B. The physical location of the reported *QSr.cdl-2BS.2* does overlap a total of 5.5 Mb with the QTL from Bajgain *et al.* (2015) but does not overlap with the QTL reported by Rouse *et al.* (2014b). Rouse *et al.* (2014b) found that the resistant allele of the reported *QSr.cdl-2BS* was contributed by Thatcher. Though there is not an overlap between *QSr.cdl-2BS.2* with *QSr.cdl-2BS*, the two QTL are only 3.5 Mb apart. Since Thatcher is one of the parents that derived the CI 14275 line, it is still possible that *QSr.cdl-2BS.2* is the same as the Thatcher allele from that reported by Rouse *et al.* (2014b). Tdurum\_contig54704\_176, one of the flanking markers of *QSr.cdl-2BS.2*, is located at 61 cM in the popseq consensus map, whereas the Excalibur\_c7963\_1722 marker that was picked up in the validation population is located at 60 cM in the popseq

consensus map, hence the two loci are detecting the same QTL (*QSr.cdl-2BS.2*). Several other studies have reported stem rust resistance QTLs on chromosome 2B (association mapping study QTL at 79.6 Mb on 2BL: Letta *et al.*, 2013; GWAS study QTL located between 11.3 Mb and 79.9 Mb: Prins *et al.*, 2016; Cacuke/Huhwa and Cacuke/Yaye population between 65.8 Mb and 73.9 Mb on 2BL: Randhawa *et al.*, 2018). Our study is the first to both detect and validate an adult plant stem rust resistance QTL on chromosome arm 2BS.

### **QTL on chromosome 3B**

*QSr.cdl-3B.1* explained between 8.3 and 21.4% of the phenotypic variance and conferred adult plant resistance in the KEN17, KEN18, STP17, and STP18 environments.

*QSr.cdl-3B.1* was flanked by markers BS00057988\_51, Excalibur\_c57658\_54, IAAV3838, RAC875\_c10595\_473, Tdurum\_contig32277\_121 and wsnp\_Ra\_c69\_149518 in the various environments. The *Sr2* gene is located on chromosome arm 3BS, whereas *Sr12* is located near the centromere on chromosome 3B. Both were derived from *Triticum turgidum*. *Sr2* is an adult plant resistance gene used in breeding as a source of durable resistance to stem rust, although the gene may not provide adequate resistance under high disease pressure. *Sr12* is present in all of the parents of CI 14275: cultivars Thatcher, Kenya Farmer and Lee, therefore, *Sr12* is almost certainly present in CI 14275 (unless there is a mistake in the pedigree or the gene postulations of the parents). The identified *QSr.cdl-3B.1* in this study is located between 58.6 Mb and 75.9 Mb on 3B and overlaps other reported QTLs on 3B (Thatcher/McNeal population located between 15.4 Mb and 85.8 Mb: Rouse *et al.*, 2014b; association mapping population located between 13.8 Mb and 59.5 Mb: Bajgain *et al.*, 2015; Spark/Rialto DH population located between 20.1 Mb and 73.8 Mb: Getie *et al.*, 2016; Prins *et al.*, 2016: GWAS study, located between 68.9 Mb and 71.0 Mb; LMPG-6/PI 362698-1 population located between 62.2 Mb and 72.9 Mb: Zurn *et al.*, 2018), and is likely the gene *Sr12*.

The QTL *QSr.cdl-3B.1* does not overlap with that reported by Haile *et al.* (2012) in Kristal/Sebatel population located at 29.1Mb. The *Sr12* gene alone did not provide strong adult plant resistance in three environments, suggesting a possible combination of *Sr12*

and other QTL(s). The gene *Lr34/Sr57* has been reported to enhance the effectiveness of Thatcher resistance in North America and Kenya (Kolmer *et al.*, 2011) but the parents (LMPG-6 and CI 14275) were both negative for the *Lr34* gene-based marker (CsLV34), indicating that the resistance in CI 14275 did not involve *Lr34/Sr57*. Lack of significant QTL on chromosome 7D in this study, where *Lr34* is located, also is a strong indication that *Lr34/Sr57* was not segregating in this population. The KASP markers linked to *Qsr.cdl-3B.1* were not associated with stem rust response in the validation population. To look into this further, we genotyped CI 14275, LMPG-6, Line #162, and Kwale with the *Sr12*-associated marker from Hiebert *et al.* (2016): NB-LRR3. We found that LMPG-6 did not possess the *Sr12*-positive genotype, but CI 14275 and both parents of the validation population (Line #162 and Kwale) did possess the *Sr12*-positive genotype. Therefore, *Sr12* may be fixed in the validation population.

#### **QTLs on chromosome arm 4AL**

*Qsr.cdl-4AL.1*, that explained a large proportion of the phenotypic variance (41.2%) for response to race TTTTF, is located at 73.3 Mb and mapped to chromosome arm 4AL. *Qsr.cdl-4AL.1* was flanked by the markers Tdurum\_contig42019\_1714 and wsnp\_Ex\_rep\_c68677\_67531081. *SrND643*, a temporary designated gene that is also located on chromosome arm 4AL, is reported to provide inadequate resistance under high disease pressure (Basnet *et al.*, 2015). Even though *SrND643* is located between 72.3 Mb and 73.7 Mb, at a similar location to identified *Qsr.cdl-4AL.1*, *SrND643* shows a low infection type of 2 to 22+ at the seedling stage against Ug99 races TTKSK and TTKST (Basnet *et al.*, 2015) but line CI 14275 showed a high infection type of 3 to 33+ against Ug99 race TTKSK, indicating that *Qsr.cdl-4AL.1* is not *SrND643*. The stem rust resistance gene *Sr7*, with two characterized alleles; *Sr7a* and *Sr7b*, is located on chromosome arm 4AL and the alleles confer resistance to some North American *Pgt* races (McIntosh *et al.*, 1995; Turner *et al.*, 2016). In this study, race TTTTF was virulent on *Sr7b* in the North American stem rust differential set, with infection types ranging from 3- to 3+, ruling out the possibilities of *Sr7b* being involved in the observed resistance. The *Qsr.rwg-4A* (believed to be *Sr7a*), at a location between 59.1 Mb and 73.9 Mb and specific to race TTTTF, was reported by Saini *et al.* (2018) in the durum

wheat ‘Lebsock’. The location of *Qsr.cdl-4AL.1* and *Qsr.rwg-4A* overlaps and it is therefore likely that *Qsr.cdl-4AL.1* identified in this study is *Sr7a*. *Sr7a* was demonstrated to be effective to race TTTTF by Turner *et al.* (2016).

#### **QTL on chromosome arm 5DL**

*Qsr.cdl-5DL*, located at 56.2 Mb, was associated with resistance to race TTTTF and was flanked by the markers BS00062990\_51 and BS00021991\_51. *Qsr.cdl-5DL* explained only 3.8% of the phenotypic variance. The gene *Sr53*, which was identified in *Aegilops geniculata*, has been mapped on chromosome arm 5DL and is effective to the Ug99 race group. A study by Olivera *et al.* (2018) showed that a higher percentage of accessions of *A. geniculata* showed resistance to race TTTTF, but since *A. geniculata* is not in the pedigree of CI 14275, the possibility of *Qsr.cdl-5DL* being *Sr53* is ruled out. *Sr30* was mapped to chromosome arm 5DL (Bariana *et al.*, 2001; Hiebert *et al.*, 2010). Even though one of the markers that was linked to the gene identified by Bariana *et al.* (2001) mapped at 58.2 Mb, at a similar position to that of *Qsr.cdl-5DL*, race TTTTF was virulent on *Sr30* in the North American stem rust differential set, ruling out the possibility of *Qsr.cdl-5DL* being *Sr30* gene. In a seedling stage study by Gireesh *et al.* (2015), a recessive gene was mapped at 47.2 Mb (believed to be an allele of *Sr30*) on 5DL in wheat line WR95. Since the 4AL QTL (*Qsr.cdl-4AL.1*), which was also found to confer resistance to race TTTTF, explained a larger percentage of the phenotypic variance (41.2%) compared to the observed phenotypic variance (3.8%) explained by *Qsr.cdl-5DL*, the identified *Qsr.cdl-5DL* could be involved in *Sr7a*-mediated resistance. The small phenotypic variance of *Qsr.cdl-5DL* may, however, not warrant further study of this QTL.

#### **QTL on chromosome arm 6AS**

*Qsr.cdl-6AS.1*, located between 51.4 Mb and 52.0 Mb, conferred adult plant resistance in the KEN17, ETH16, and ETH18 environments. *Qsr.cdl-6AS.1* explained phenotypic variance ranging from 9.8 to 10.38% and is flanked by the markers BS00031178\_51 and IAAV3806. The genes mapped to chromosome arm 6AL are *Sr13*, *Sr26*, and *Sr52*. The gene *Sr13*, which is derived from *T. turgidum* is effective to race TTKSK and its variants,

and it is generally restricted to durum wheats (Zhang *et al.*, 2017). The *Sr26* gene derived from *Thinopyrum elongatum* is effective to Ug99 races, whereas *Sr52* gene, derived from *Dasypyrum villosum*, is a wild relative of common wheat (Qi *et al.*, 2011). *QSr.cdl-6AS.1* is different from the QTL reported by Letta *et al.* (2013), that mapped between 60.6 Mb and 61.8 Mb on 6AL, but overlaps reported QTL on 6AS (Kankwatsa *et al.*, 2017: genome-wide association study located between 10.0 Mb and 59.6 Mb). The susceptibility of the resistant CI 14275 line at the seedling stage to race TTKSK and pedigree information, however, rule out the possibilities of these genes (*Sr13*, *Sr26*, *Sr52*) contributing to the observed resistance. The *Sr8* locus alleles; *Sr8a* and *Sr8b* have been reported on chromosome arm 6AS (Singh and McIntosh, 1986). A QTL identified by Bajgain *et al.* (2015) in an association mapping study is located at 52.3 Mb, a similar position to *QSr.cdl-6AS.1*. Prins *et al.* (2016) identified QTL on 6AS, though the physical location of linked markers was not found. A race-specific gene on 6A, likely *Sr8a*, was identified at the seedling stage for race TRTTF (Dunckel *et al.*, 2015). In Ethiopian nurseries, other than the predominance of race TTKSK, *Pgt* races including TRTTF and JRCQC have been reported (Olivera *et al.*, 2012; Hailu *et al.*, 2015). The races TRTTF and JRCQC are avirulent to *Sr8a* although race TTKSK, which is predominant in Kenyan and Ethiopian environments is virulent to *Sr8a* (Olivera *et al.*, 2012; Hailu *et al.*, 2015). The susceptibility of the RIL population to *Sr8a* rules out the possibility of *QSr.cdl-6AS.1* being *Sr8a*. Nirmala *et al.* (2017) mapped *Sr8155B1*, a gene conferring resistance to race TTKST on chromosome 6A. Although *Sr8155B1* is located between 6.7 and 10.9 Mb, a different location to the QTL *QSr.cdl-6AS.1* identified in this study, there is a possibility that *QSr.cdl-6AS.1* is *Sr8155B1*, although this hypothesis would require additional experiments to test. The KASP markers linked to *QSr.cdl-6AS.1* were not associated with stem rust resistance in the validation population. Unfortunately, Line #162 (the resistant parent in the validation population) possesses the susceptible alleles of the *QSr.cdl-6AS.1*-linked markers. Selecting Line #162 as a parent was undertaken based on the phenotypic data alone. So, the absence of *QSr.cdl-6AS.1* from Line #162 precluded the ability to validate this QTL in our validation population. Additional experiments are needed to validate the effectiveness of *QSr.cdl-6AS.1*.

### **QTL on chromosome 6B**

*QSr.cdl-6BL.1* was located between 61.3 Mb and 71.9 Mb on chromosome arm 6BL. *QSr.cdl-6BL.1* explained between 17.8 and 18.1% of the phenotypic variance in adult plant response in STP17 and STP18 environments, and between 20.6 and 37.5% of the phenotypic variance to races RTQQC and TMPKC at the seedling stage. *Sr11* is located on chromosome 6B (McIntosh *et al.*, 1995) and it is present in the cultivars Kenya Farmer and Lee from which the line CI 14275 was derived. The races TPMKC and RTQQC were virulent to *Sr11* in the North American stem rust differential set, with infection types of 3 and 33+, respectively. Although the races are virulent to *Sr11*, the observed effect to the races could be a residual effect of the defeated *Sr11*. Since a mixture of US races were used in field inoculations in St. Paul, US races that include RKQQC, RCRSC, QFCSC and MCCFC which are avirulent to *Sr11*, could have contributed to the observed field resistance. Additionally, the SNP markers identified by Nirmala *et al.* (2016) that predicted *Sr11* are located between 71.6 and 71.8 Mb, a similar location to the *QSr.cdl-6BL.1* identified in this study. Therefore, *QSr.cdl-6BL.1* is possibly *Sr11*. QTL on 6B have been reported in other studies (Bajgain *et al.*, 2015: association mapping study located at 71.6 Mb; Bajgain *et al.*, 2016: Nested association mapping study (physical location of markers not found); Kankwatsa *et al.*, 2017: genome-wide association study (physical location of markers not found). The value of the QTL on chromosome 6B is limited since these QTL were not effective in Africa.

### **3.5 Conclusions**

This study reports QTL for wheat stem rust resistance on chromosome 2BS, 3B, 4AL, 5DL, 6AS, and 6BL. We characterized the adult plant resistance of CI 14275 in Africa as the cumulative contribution of three QTL: *QSr.cdl-2BS.2*, *QSr.cdl-3B.1*, and *QSr.cdl-6AS.1*. The identified QTL *QSr.cdl-2BS.2*, that conferred adult plant resistance in the KEN16, ETH16, and ETH18 environments, was validated in a second population in KEN18, and can be selected for by the validated linked marker, Excalibur\_c7963\_1722. This QTL therefore has the potential of being used in marker assisted selection. Our study is the first to both detect and validate an adult plant stem rust resistance QTL on chromosome arm 2BS. *QSr.cdl-3B.1*, which conferred resistance in the KEN17, KEN18,



STP17, and STP18 environments is likely *Sr12*. *Q<sub>Sr.cdl-4AL.1</sub>* that conferred resistance to race TTTTF is postulated to be *Sr7a*. *Q<sub>Sr.cdl-5DL</sub>* that conferred resistance to race TTTTF explained only a small proportion of the phenotypic variance observed. *Q<sub>Sr.cdl-6AS.1</sub>* which conferred resistance in the ETH16, ETH18, and KEN17 environments is potentially a new QTL, but the QTL requires validation in another population before we would recommend using this QTL in breeding. *Q<sub>Sr.cdl-6BL.1</sub>* is possibly *Sr11*. Our study showed that combination of the three QTL: *Q<sub>Sr.cdl-2BS.2</sub>*, *Q<sub>Sr.cdl-3B.1</sub>*, and *Q<sub>Sr.cdl-6AS.1</sub>* result in a large resistance effect that can be used to reduce stem rust severity in Africa.

### 3.6 Tables

**Table 3.1.** Seedling infection types (ITs) of 113 recombinant inbred lines (RILs) and the parents of the cross LMPG-6/CI 14275 against five *Puccinia graminis* f. sp. *tritici* races: RTQQC, TTTTF, TPMKC, TRTTF, and TTKSK<sup>a</sup>

RILs/Parents	RTQQC		TTTTF		TPMKC		TRTTF		TTKSK	
	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II
1	3+ (9)	3+ (9)	3+ (9)	3+ (9)	3+2- (7)	3+ (9)	33+ (8)	33+ (8)	33+ (8)	3 (8)
2	33+ (8)	3+ (9)	1+3+C (5)	;13C (3)	2+3+ (7)	23- (6)	3+ (9)	3+ (9)	3+3 (9)	3+ (9)
3	3+ (9)	3+ (9)	3+2- (7)	3+ (9)	3+3- (8)	3+2+ (8)	3 (8)	3+ (9)	33+ (8)	3+ (9)
4	33+ (8)	33+ (8)	3+3- (8)	3+ (9)	3+3- (8)	33- (8)	3 (8)	3+ (9)	3+ (9)	3+ (9)
5	3+ (9)	23+ (6)	1+3C (5)	13- (4)	2+3+ (7)	23- (6)	33- (8)	3-C (7)	3+ (9)	3+ (9)
6	3 (8)	23+ (6)	3+ (9)	3 (8)	2+3- (6)	23- (6)	33+ (8)	33+ (8)	33+ (8)	3+ (9)
7	3 (8)	3+ (9)	3- (7)	;1 (1)	2+3+ (7)	23- (6)	3 (8)	33+ (8)	3 (8)	3+ (9)
8	3+ (9)	3+ (9)	1+3C (5)	33C (8)	3+3 (9)	3+3- (8)	33+ (8)	3+ (9)	3+ (9)	3+ (9)
9	3+ (9)	33+ (8)	3+3C (9)	3+ (9)	23+ (6)	23 (6)	33- (8)	3+ (9)	3+ (9)	3+ (9)
10	3 (8)	33+ (8)	3+3- (8)	3+ (9)	23- (6)	23 (6)	33+ (8)	33+ (8)	3 (8)	3+ (9)
11	3+ (9)	3+3 (9)	3+3- (8)	3+3- (8)	23+ (6)	3+3 (9)	3- (7)	3+ (9)	3 (8)	3+ (9)
12	3+ (9)	3+ (9)	0, 1+ (0)	13 (4)	3+ (9)	3+3 (9)	33C (8)	3-C (7)	3 (8)	3+ (9)
13	3+ (9)	33+ (8)	3+ (9)	3+ (9)	3+3 (9)	3+ (9)	3 (8)	3+ (9)	33+ (8)	3+ (9)
14	33+ (8)	33+ (8)	3+3- (8)	3+3- (8)	23- (6)	23- (6)	3 (8)	33+ (8)	33+ (8)	33+ (8)
15	3+ (9)	3+ (9)	3+ (9)	3+ (9)	32- (7)	33- (8)	3 (8)	33+ (8)	33+ (8)	3+ (9)
16	3+ (9)	3+ (9)	3+ (9)	3+ (9)	3+3- (8)	3+3 (9)	33+ (8)	3+ (9)	33+ (8)	3+ (9)
17	3+ (9)	3+ (9)	3+3- (8)	3+3- (8)	3+3- (8)	3+ (9)	33- (8)	3+3- (8)	3+ (9)	3+ (9)
18	3+ (9)	3+ (9)	3+ (9)	3+ (9)	33+ (8)	3+3 (9)	3- (7)	33- (8)	3+ (9)	3+ (9)
19	3+ (9)	3+ (9)	3+3- (8)	3+ (9)	3+ (9)	3+3 (9)	3- (7)	3- (7)	33+ (8)	3+ (9)

RILs/Parents	RTQQC		TTTTF		TPMKC		TRTTF		TTKSK	
	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II
			0/1+3-							
20	3+ (9)	33+ (8)	(0/4)	22+ (5)	3+3- (8)	33- (8)	33+ (8)	33+C (8)	3 (8)	-
21	3+ (9)	3+ (9)	3+3- (8)	3+ (9)	3+3 (9)	3+ (9)	33+ (8)	3+ (9)	33+ (8)	3+ (9)
22	3+3 (9)	33+ (8)	0/1+ (0/3)	33- (8)	33- (8)	23 (6)	33+ (8)	33+ (8)	3+ (9)	3+ (9)
23	3+3 (9)	33+ (8)	3+3- (8)	3+ (9)	23- (6)	23- (6)	33+ (8)	3+ (9)	3+ (9)	3+ (9)
24	33+ (8)	33+ (8)	3+3- (8)	3+3- (8)	23- (6)	3-3+ (8)	33+ (8)	33+ (8)	33+ (8)	3+ (9)
25	3+ (9)	3+ (9)	3+ (9)	3+ (9)	3+ (9)	3+3- (8)	3+ (9)	3+ (9)	3+ (9)	3+ (9)
26	33- (8)	3+ (9)	3+3- (8)	-	3+ (9)	3 (8)	-	-	33- (8)	33-C (8)
27	32+ (7)	33+ (8)	1+3+ (5)	;1 (1)	3+3- (8)	3+ (9)	3+ (9)	33+ (8)	33+ (8)	3C (8)
28	32- (7)	33+ (8)	3+1+C (7)	3C (8)	3+3- (8)	3+ (9)	33+ (8)	3+ (9)	33- (8)	3+ (9)
29	23- (6)	3- (7)	3+ (9)	3+ (9)	3+ (9)	23 (6)	33+ (8)	3+ (9)	3 (8)	3+ (9)
30	3+2- (7)	3+ (9)	3+1/0 (7)	;13 (3)	3+3- (8)	3+3- (8)	33+(8)	33+ (8)	3+3 (9)	3+ (9)
31	23- (6)	33+ (8)	33+C (8)	3+ (9)	3+3- (8)	3+3- (8)	33+ (8)	3+ (9)	3+ (9)	3+ (9)
32	23- (6)	33+ (8)	3+ (9)	13 (4)	2+3+ (7)	32+ (7)	33+ (8)	3+ (9)	3+ (9)	3+ (9)
33	3 (8)	3+ (9)	3+ (9)	;13 (3)	3+2 (8)	3+3- (8)	33+ (8)	3+3 (9)	3+ (9)	3 (8)
34	2+ (6)	3+ (9)	1+3C (5)	1 (2)	3+2 (8)	3- (7)	33+ (8)	33+ (8)	3+ (9)	3+ (9)
35	22+ (5)	3- (7)	1+3+C (5)	1 (2)	23+ (6)	23+ (6)	33+ (8)	3+C (9)	3+ (9)	3+ (9)
36	2+ (6)	3 (8)	13C (4)	13- (4)	2+3 (7)	23 (6)	33+ (8)	3+ (9)	3 (8)	3+ (9)
37	22+ (5)	3 (8)	13 (4)	;1 (1)	23+ (6)	32 (7)	33+ (8)	33+ (8)	3+ (9)	3+ (9)
38	22+ (5)	3- (7)	31C (6)	13- (4)	23- (6)	23- (6)	33C (8)	3-C (7)	3+ (9)	3+ (9)
39	3+ (9)	3+ (9)	1+3C (5)	1 (2)	3+3- (8)	32- (7)	33+ (8)	33+ (8)	3+ (9)	3+ (9)
40	2+3- (6)	3+ (9)	1+3+C (5)	33C (8)	3+3- (8)	23 (6)	33+ (8)	3+3 (9)	3+ (9)	3+ (9)

RILs/Parents	RTQQC		TTTTF		TPMKC		TRTTF		TTKSK	
	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II
41	1+ (3)	23- (6)	1+3+C (5)	31C (6)	23+ (6)	22+ (5)	33+ (8)	33+ (8)	3+ (9)	3+ (9)
42	33+ (8)	3+ (9)	3+ (9)	3+ (9)	33+ (8)	3+2 (8)	33+ (8)	33+ (8)	3+ (9)	3+ (9)
43	22+ (5)	3+ (9)	3+ (9)	32 (7)	33- (8)	32 (7)	33+ (8)	3+ (9)	3+ (9)	3+ (9)
44	23- (6)	3+ (9)	3+1C (7)	13C (4)	33+ (8)	3+2 (8)	3+3 (9)	3+ (9)	3+ (9)	3+ (9)
45	33+ (8)	3+ (9)	3+C (9)	;1 (1)	3+3- (8)	33+ (8)	3-C (7)	33+C (8)	3+ (9)	3+ (9)
46	23- (6)	3+ (9)	13+C (4)	31C (6)	3+ (9)	32+ (7)	3- (7)	3 (8)	3+ (9)	3+ (9)
47	22+ (5)	3+ (9)	1+3C (5)	;1 (1)	23 (6)	3+2 (8)	3-C (7)	3-C (7)	3+ (9)	3+ (9)
48	3+ (9)	3+3- (8)	1+3-C (4)	;1 (1)	23+ (6)	22+ (5)	33+ (8)	33+C (8)	33+ (8)	3+ (9)
49	3+ (9)	3+ (9)	0 (0)	1 (2)	3+3- (8)	3+2 (8)	3+3C (9)	3- (7)	3+ (9)	3+ (9)
50	3+ (9)	3+ (9)	; 13- (2)	;1 (1)	3+ (9)	3+3- (8)	3+3 (9)	3+ (9)	3 (8)	3+ (9)
51	33+ (8)	3- (7)	3+3C (9)	31C (6)	3+2 (8)	22+ (5)	3+ (9)	3+ (9)	33- (8)	3+ (9)
52	3+ (9)	3+ (9)	1+3C (5)	13- (4)	3+2 (8)	33+ (8)	3+ (9)	3+3C (9)	33+ (8)	3+ (9)
53	33+ (8)	3- (7)	1+3-C (4)	;13- (2)	23- (6)	2+ (6)	33+ (8)	33+ (8)	3+ (9)	3+ (9)
54	3+ (9)	3+ (9)	3+ (9)	3+ (9)	3+ (9)	3+3- (8)	3+ (9)	3+ (9)	3+ (9)	3+ (9)
55	3+ (9)	3+ (9)	1+3+ (5)	;1+ (1)	3+ (9)	33+ (8)	3+3 (9)	3+ (9)	3+3 (9)	3+ (9)
56	3+ (9)	3 (8)	3+ (9)	3+ (9)	23+ (6)	23+ (6)	3+ (9)	3+ (9)	33- (8)	3+ (9)
57	3+ (9)	3+ (9)	1+3- (4)	31C (6)	3+2 (8)	32- (7)	3-C (7)	3+ (9)	33+ (8)	3+ (9)
58	3+ (9)	3+ (9)	3+3C (9)	3+ (9)	3+3- (8)	32 (7)	33+ (8)	3+ (9)	3 (8)	3+ (9)
59	3+ (9)	3+ (9)	1+3C (5)	31C (6)	3+3- (8)	3+3 (9)	33+ (8)	3+ (9)	33-C (8)	3+ (9)
60	3- (7)	23- (6)	0/1+ (0/3)	;1 (1)	23- (6)	22+ (5)	3-C (7)	3-C (7)	3- (7)	33- (8)
61	3- (7)	23- (6)	1+3+C (5)	32- (7)	32 (7)	2+2 (6)	3 (8)	33+ (8)	33- (8)	3 (8)
62	3+3 (9)	3+ (9)	33+ (8)	3+ (9)	3+ (9)	32+ (7)	33+ (8)	3+ (9)	3 (8)	3+ (9)

RILs/Parents	RTQQC		TTTTF		TPMKC		TRTTF		TTKSK	
	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II
63	3+ (9)	3+ (9)	3+3- (8)	3+ (9)	3+ (9)	33+ (8)	33- (8)	3+ (9)	3 (8)	33+ (8)
64	3+ (9)	3+c (9)	1+3+C (5)	31C (6)	3+ (9)	33+ (8)	3- (7)	3+ (9)	3+ (9)	3+ (9)
65	3+ (9)	33+ (8)	1+3C (5)	31C (6)	33+ (8)	23+ (6)	33- (8)	33+C (8)	33+ (8)	3+ (9)
			0/1+C							
66	3+3- (8)	3- (7)	(0/3)	13 (4)	3+3- (8)	3+3 (9)	3-C (7)	3-C (7)	33+ (8)	3+ (9)
67	33+ (8)	13- (4)	3 (8)	3+ (9)	3+3- (8)	3+ (9)	33+ (8)	3+ (9)	3 (8)	3+ (9)
68	3+ (9)	3+ (9)	1+3C (5)	31C (6)	3+ (9)	33+ (8)	33- (8)	3-C (7)	3+ (9)	3+ (9)
69	3+ (9)	3-3+ (8)	1+3- (4)	13C (4)	3+3 (9)	3+3 (9)	3-3+ (8)	3-3+ (8)	3+ (9)	3+ (9)
70	3+ (9)	13- (4)	;1 (1)	13C (4)	33+ (8)	33+ (8)	33+C (8)	33+C (8)	3+ (9)	3+ (9)
71	33- (8)	;13- (2)	13C (4)	31C (6)	23- (6)	23- (6)	3- (7)	3C (8)	3 (8)	33+ (8)
72	33- (8)	;13- (2)	3+1 (7)	3+ (9)	3- (7)	32+ (7)	3 (8)	33+ (8)	3+ (9)	3+ (9)
73	3+ (9)	3+ (9)	3+3- (8)	3+ (9)	33+ (8)	33+ (8)	33+ (8)	33+ (8)	3 (8)	3- (7)
74	3+ (9)	3+ (9)	33+ (8)	33C (8)	3+3- (8)	23- (6)	33+ (8)	33+ (8)	3+ (9)	3+ (9)
75	3+3- (8)	33+ (8)	3+ (9)	3+ (9)	3+ (9)	3+3 (9)	3+ (9)	3+ (9)	3+ (9)	3+ (9)
76	33+ (8)	3+ (9)	33+ (8)	13C (4)	23+ (6)	23+ (6)	3+ (9)	3C (8)	3 (8)	3+ (9)
77	3+ (9)	3 (8)	13C (4)	31C (6)	3+ (9)	23- (6)	33+ (8)	33+ (8)	3+ (9)	3+ (9)
78	3+3 (9)	33+ (8)	1+3C (5)	13C (4)	3+3- (8)	32 (7)	3+ (9)	3C (8)	3+3 (9)	3+3 (9)
79	3+3- (8)	32- (7)	13-C (4)	13 (4)	3+ (9)	3- (7)	3-C (7)	3C (8)	3+ (9)	3+3 (9)
80	3+ (9)	2+ (6)	3+ (9)	3+ (9)	3-2+ (7)	2/3+ (6)	3+ (9)	31 (6)	3+ (9)	3+ (9)
81	33- (8)	3 (8)	3+3-C (8)	3+ (9)	3+ (9)	23- (6)	33+ (8)	3-C (7)	3+ (9)	3+ (9)
82	3 (8)	3+ (9)	3+ (9)	3+ (9)	3+ (9)	23+ (6)	3+ (9)	33+ (8)	3+ (9)	3+ (9)
83	3+3- (8)	3 (8)	13- (4)	13C (4)	3+2- (7)	23- (6)	3+ (9)	3C (8)	3+ (9)	3+ (9)

RILs/Parents	RTQQC		TTTTF		TPMKC		TRTTF		TTKSK	
	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II
84	3+ (9)	33+ (8)	33+ (8)	3 (8)	33+ (8)	23- (6)	3+ (9)	33+ (8)	3+ (9)	3+ (9)
85	3+3- (8)	33- (8)	3+1C (7)	3+ (9)	3+3- (8)	23+ (6)	3+ (9)	3+ (9)	3+ (9)	3+ (9)
86	3+ (9)	3+ (9)	3+ (9)	3+ (9)	3+ (9)	3+3 (9)	3+ (9)	3+ (9)	3+ (9)	3+ (9)
87	3+ (9)	3+ (9)	13C (4)	3+ (9)	33+ (8)	3+3 (9)	3+3- (8)	33- (8)	3+ (9)	3+ (9)
88	3+ (9)	3+ (9)	3+ (9)	3+ (9)	3+ (9)	33+ (8)	3+3- (8)	33+ (8)	3+ (9)	3+ (9)
89	3+3- (8)	33+ (8)	13+ (4)	;1 (1)	23+ (6)	23- (6)	33- (8)	3- (7)	3+ (9)	3+ (9)
90	3+ (9)	3+ (9)	3+ (9)	3+3 (9)	3+ (9)	33- (8)	33+ (8)	33+ (8)	3+ (9)	3+ (9)
91	3 (8)	13- (4)	13- (4)	13C (4)	33+ (8)	23- (6)	3+3C (9)	3-C (7)	3+ (9)	3+ (9)
92	3+ (9)	3 (8)	1+3C (5)	31C (6)	23+ (6)	32 (7)	3-C (7)	33C (8)	3+ (9)	3+ (9)
93	3+ (9)	3+ (9)	3+ (9)	3+ (9)	3+ (9)	32+ (7)	3C (8)	33+ (8)	3+ (9)	3+ (9)
94	3+3- (8)	33+ (8)	3+ (9)	3+ (9)	3+ (9)	3+3 (9)	3+ (9)	33+ (8)	3+ (9)	3+ (9)
95	3+ (9)	3+ (9)	3+3C (9)	;1 (1)	3+ (9)	3+ (9)	3+ (9)	33+ (8)	3+ (9)	3+ (9)
96	33+ (8)	3- (7)	13C (4)	13C (4)	23+ (6)	23- (6)	3C (8)	33+C (8)	3+ (9)	3+ (9)
97	33- (8)	3- (7)	31 (6)	13 (4)	3+ (9)	23+ (6)	3- (7)	3-C (7)	3+ (9)	3+ (9)
98	3+ (9)	3- (7)	33+C (8)	13 (4)	3+ (9)	23- (6)	3- (7)	33+ (8)	3+ (9)	33+ (8)
99	3+ (9)	3+ (9)	3+ (9)	;1 (1)	3+ (9)	3+ (9)	33+ (8)	33+ (8)	3+ (9)	3+ (9)
100	3+ (9)	3+ (9)	1+3C (5)	13 (4)	3+ (9)	3+2 (8)	3-C (7)	33+ (8)	3+ (9)	3+ (9)
101	3 (8)	3+ (9)	1+3+ (5)	31C (6)	3+2 (8)	3-3+ (8)	3- (7)	3- (7)	3+ (9)	3+ (9)
102	3+ (9)	3+ (9)	1+3 (5)	33C (8)	3+ (9)	3+ (9)	3- (7)	3 (8)	3+ (9)	3+ (9)
103	3+3- (8)	3+ (9)	3- (7)	13 (4)	3+2- (7)	32 (7)	3-C (7)	33+ (8)	33+ (8)	3+ (9)
104	3+ (9)	3+ (9)	3+ (9)	3+ (9)	3+ (9)	32- (7)	3+ (9)	3+ (9)	3+ (9)	3+ (9)
105	3+ (9)	3+ (9)	33+ (8)	3+3 (9)	3+3- (8)	32 (7)	33+ (8)	33+ (8)	3+ (9)	3+ (9)

RILs/Parents	RTQQC		TTTTF		TPMKC		TRTTF		TTKSK	
	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II
106	3 (8)	3- (7)	33+ (8)	31 (6)	3+ (9)	23- (6)	3- (7)	3+ (9)	3+ (9)	3+ (9)
107	33+ (8)	1+3C (5)	33+C (8)	31C (6)	3+3- (8)	23- (6)	3-3+ (8)	3+ (9)	3+ (9)	3+ (9)
108	3+ (9)	3+ (9)	31 (6)	31C (6)	3+3- (8)	3+2 (8)	33+ (8)	3+ (9)	-	3+ (9)
109	3+3- (8)	3 (8)	1+3- (4)	31C (6)	3+ (9)	3+ (9)	3- (7)	33- (8)	3+ (9)	3 (8)
110	3+ (9)	3+ (9)	3+ (9)	3+ (9)	3+ (9)	3+2 (8)	3+ (9)	3+ (9)	3+ (9)	3+ (9)
111	3+ (9)	1+3C (5)	1+3+C (5)	13 (4)	23- (6)	32 (7)	3- (7)	33- (8)	3+ (9)	3 (8)
112	33+ (8)	3- (7)	1+3- (4)	;1+ (1)	2+3- (6)	32 (7)	33+ (8)	33+ (8)	3+ (9)	3+ (9)
113	3+3- (8)	3+ (9)	1+3- (4)	13C (4)	3+ (9)	3+3- (8)	33+ (8)	3-3+ (8)	3+ (9)	3+ (9)
CI 14275	33+ (8)	3 (8)	1+3C (5)	;1 (1)	23- (6)	23+ (6)	33+ (8)	33+ (8)	33+ (8)	33+ (8)
LMPG-6	3+/3 (9)	3+ (9)	3+ (9)	3+ (9)	3+3 (9)	3+ (9)	3+ (9)	33+ (8)	33+ (8)	3 (8)

<sup>a</sup>Infection types were score based on the 0-4 scale developed by Stakman *et al.* (1962). A comma (,) symbol was used to separate multiple ITs observed on the same plant, with the most frequent IT recorded first. A forward slash (/) symbol was used to separate multiple infection types observed on different plants of the same line, with the most frequent IT recorded first. A “C” letter was recorded after the ITs, and denotes extensive chlorosis associated with infection. The plus (+) and minus (-) symbols were used for the pustules that were relatively larger or smaller, respectively, than normal. A dash (-) denotes missing data. Plants with ITs ranging from 0 to 2 were categorized as resistant, and those with 3-4 ITs were categorized as susceptible. The seedling infection types based on a 0 to 4 scale were then converted to a 0 to 9 linear scale (indicated in parentheses) according to Gao *et al.* (2019).

**Table 3.2.** Segregation of stem rust resistance in 113 recombinant inbred lines (RILs) along with response of parents of the cross LMPG-6/CI 14275 against five *Puccinia graminis* f. sp. *tritici* races<sup>a</sup>

Races	Number of Lines		Seedling Infection Types		Chi-square	P value	Postulated Segregating Genes <sup>b</sup>
	Resistant	Susceptible	Susceptible Parent - LMPG-6	Resistant Parent - CI 14275			
RTQQC	7	106	3+/33+	3/33+	0.005	0.94	3 comp.
TTTTF	57	56	3+	;1/1+3C	0.704	0.40	One
TPMKC	16	97	3+/3+3	23-/23+	-	-	Unknown
TRTTF	0	113	3+/33+	33+	-	-	-
TTKSK	0	113	3/33+	33+	-	-	-

<sup>a</sup>Infection types (ITs) follow Stakman *et al.* (1962) scale. The plus (+) and minus (-) symbols was used for the pustules that were relatively larger and smaller, respectively, than normal; A forward slash (/) symbol separates multiple infection types observed on different plants of the same line; Fleck (;) indicates no uredinia but presence of hypersensitive necrotic flecks; ITs ranging from 0 to 2 are categorized as resistant, and 3-4 ITs are categorized as susceptible; The dash (-) symbol indicates no generated data.

<sup>b</sup>Comp. = complementary; Unknown = segregation of RILs did not fit any expected ratio; The dash (-) symbol indicates no postulated genes.



**Table 3.3.** Stem rust severity (0-100 scale), infection response (resistant (R)-susceptible (S)), and coefficient of infection (COI) in parenthesis of 113 recombinant inbred lines (RILs) along with the parents of the cross LMPG-6/CI 14275 in the field in Kenya in 2016 (KEN16), 2017 (KEN17) and in 2018 (KEN18) to predominant Ug99 races. Presented data is for two replicates (rep)<sup>a</sup>.

RILs/Parents	KEN16		KEN17		KEN18	
	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II
1	20S (20)	30MS (24)	20MSS (18)	10MSS (9)	40S (40)	30SMS (27)
2	0 (0)	0 (0)	TR (0.2)	TSMS (0.2)	5R (1)	5R (1)
3	10S (10)	10S (10)	60S (60)	70S (70)	15MS (12)	20MSMR (12)
4	70S (70)	70MS (56)	TMS (0.2)	TR (0.2)	45S (45)	45SMS (40.5)
5	10S (10)	20MR (8)	5R (1)	5R (1)	10RMR (3)	15MRMS (9)
6	10S (10)	60S (60)	5R (1)	5MSS (4.5)	5RMS (1)	5RMR (1.5)
7	<sup>b</sup>	-	5R (1)	TMS (0.2)	10R (2)	15R (3)
8	0 (0)	60 (60)	20SMS (18)	50SMS (45)	45S (45)	45S (45)
9	70S (70)	10R (2)	15MS (12)	5R (1)	20RMRMS (6)	25RMRMS (15)
10	0 (0)	0 (0)	TSMS (0.2)	5R, TS (1)	10RMR (3)	20RMS (8)
11	0 (0)	40S (40)	30SMS (27)	20S (20)	10RMRMS (3)	25MSS (22.5)
12	20S (20)	10R (2)	5MSS (4.5)	5SMS (4.5)	15RMRMS (4.5)	5R (1)
13	TR (0.2)	10MS (8)	10SMS (9)	25SMS (22.5)	35S (35)	30MSS (27)
14	40S (40)	20MSMR (12)	15MSMR (9)	30MSMR (18)	15RMRMS (4.5)	15RMRMS (9)
15	10MS (8)	80S (80)	30SMS (27)	50MSMR (30)	25MSMR (15)	35MSMR (21)
16	60S (60)	70MS (56)	30SMS (27)	40S (40)	40MRMS (24)	30MSMR (18)
17	60S (60)	20MR (8)	25SMS (22.5)	15MSMR (9)	35MS (28)	25MRMS (15)
18	10MS (8)	30MS (24)	20SMS (18)	25S (25)	25S (25)	30S (30)

RILs/Parents	KEN16		KEN17		KEN18	
	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II
19	0 (0)	1R (0.2)	10S (10)	5S (5)	20MSMR (12)	20MS (16)
20	0 (0)	0 (0)	5MS (4)	5MSS (4.5)	5R (1)	5R (1)
21	90S (90)	80S (80)	30MR (12)	30MRMS (18)	35S (35)	50S (50)
22	0 (0)	10MR (4)	5R (1)	5R (1)	25RMS (10)	25MRMS (15)
23	0 (0)	0 (0)	10S (10)	TSMS (0.2)	25MS (20)	20RMR (6)
24	10MRMS (6)	1MR (0.4)	20S (20)	10SMS (9)	25MSS (22.5)	15MSS (13.5)
25	80S (80)	90S (90)	40MSMR (24)	50S (50)	55S (55)	40SMS (36)
26	10MS (8)	10MS (8)	TR (0.2)	10R (2)	55S (55)	45SMS (40.5)
27	0 (0)	TR (0.2)	5MSS (4.5)	TSMS (0.2)	15RMR (4.5)	15MRMS (9)
28	10MS (8)	0 (0)	5RMR (1.5)	10R (2)	15MS (12)	20MRMS (12)
29	10MSMR (6)	10MS (8)	5SMS (4.5)	20SMS (18)	25MSMR (15)	35MS (28)
30	70S (70)	80S (80)	60S (60)	40MSMR (24)	60S (60)	50S (50)
31	80S (80)	60S (60)	10R (2)	25MSMR (15)	40MSS (36)	40S (40)
32	30MS (24)	10MR (4)	30MRMS (18)	TSMS (0.2)	10MRMS (3)	25RMRMS (15)
33	10MRMS (6)	10MS (8)	40MSS (36)	40SMSMR (24)	25RMR(7.5)	35MSMR (21)
34	80S (80)	90S (90)	0 (0)	TS (0.2)	55S (55)	45SMS (40.5)
35	0 (0)	0 (0)	60S (60)	60S (60)	5R (1)	5R (1)
36	-	-	20S (20)	15SMS (13.5)	35SMS (31.5)	45SMS (40.5)
37	10S (10)	0 (0)	40MR (16)	25MRMS (15)	15MRMS (9)	15SMS (13.5)
38	40MS (32)	1R (0.2)	25S (25)	50SMS (45)	10MRMS (6)	5R (1)
39	40MSMR (24)	20MS (16)	50SMS (45)	10S (10)	35S (35)	40MS (32)
40	40SMS (36)	60S (60)	20RMR (6)	30MR (12)	40MSS (36)	40SMS (36)

RILs/Parents	KEN16		KEN17		KEN18	
	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II
41	30MRMS (18)	10R (2)	40SMS (36)	70S (70)	15RMRMS (4.5)	25RMRMS (15)
42	60SMS (54)	80SMS (72)	5MR (2)	50SMS (45)	45MSS (40.5)	45SMS (40.5)
43	20MS (16)	60S (60)	5R (1)	10S (10)	50S (50)	50S (50)
44	30MS (24)	40MRMS (24)	TSMS (0.2)	10S (10)	20MS (16)	20SMS (18)
45	20S (20)	10MS (8)	20SMS (18)	30SMS (27)	5RMS (2)	20S (20)
46	TR (0.2)	10MRMS (6)	30SMS (27)	30MR (12)	40S (40)	35MSS (31.5)
47	40MS (32)	40MS (32)	20MS (16)	30MSMR (18)	35MRMS (21)	40MSMR (24)
48	50MS (40)	0 (0)	15MS (12)	5MS (4)	5RMRMS (3)	5RMR (1.5)
49	0 (0)	TR (0.2)	15SMS (13.5)	40S (40)	20MSMR (12)	15MS (12)
50	20MSMR (12)	40MRMS (24)	80S (80)	60S (60)	30MRMS (18)	25MRMS (15)
51	70S (70)	40MS (32)	5SMS (4.5)	20MR (8)	50S (50)	45SMS (40.5)
52	30SMS (27)	30MS (24)	5R (1)	5R (1)	30SMS (27)	35SMSMR (21)
53	0 (0)	0 (0)	15S (15)	50S (50)	5RMR (1.5)	5RMS (2)
54	10S (10)	20MS (16)	20SMS (18)	10SMS (9)	30MS (24)	25MSMR (15)
55	0(0)	TR (0.2)	5SMS (4.5)	5R (1)	30SMS (27)	25SMS (22.5)
56	0 (0)	0 (0)	40S (40)	50MS (40)	10RMS (4)	5R (1)
57	40MS (32)	80S (80)	25MS (20)	40S (40)	55S (55)	45MS (36)
58	60S (60)	30MS (24)	40MRMS (24)	20MR (8)	30SMS (27)	40MSS (36)
59	50MS (40)	60MS (48)	30MSMR (18)	60S (60)	25MRMS (15)	25RMRMS (15)
60	60SMS (54)	0 (0)	30MSMR (18)	15RMR (4.5)	5RMR (1.5)	15RMS (6)
61	0 (0)	0 (0)	5R (1)	5R (1)	10RMRMS (3)	10R (2)
62	0 (0)	0 (0)	40MR (16)	40MRMS (24)	5R (1)	5R (1)

RILs/Parents	KEN16		KEN17		KEN18	
	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II
63	60S (60)	80S (80)	40SMS (36)	40S (40)	40MSMR (36)	35MSMR (21)
64	40SMS (36)	30S (30)	10MSS (9)	10S (10)	35S (35)	25MSS (22.5)
65	20S (20)	0 (0)	10SMS (9)	5MRMS (3)	5RMS (2)	15S (15)
66	0 (0)	TR (0.2)	20S (20)	30SMS (27)	20SMS (18)	20MS (16)
67	40MS (32)	10S (10)	-	-	25S (25)	30S (30)
68	20S (20)	80S (80)	30MRMS (18)	40MRMSS (24)	40MSS (36)	40SMS (36)
69	0 (0)	0 (0)	20SMS (18)	TR (0.2)	15RMRMS (4.5)	15RMS (6)
70	70S (70)	60S (60)	40SMS (36)	70S (70)	35MSS (31.5)	40MS (32)
71	10MS (8)	20MS (16)	TS (0.2)	10S (10)	30S (30)	20RMS (8)
72	30SMS (27)	60S (60)	30MR (12)	20MRMS (12)	35MSMR (21)	35MSSMR (21)
73	40MSMR (24)	0 (0)	20MSMR (12)	40SMS (36)	30MS (24)	30SMS (27)
74	20S (20)	60SMS (54)	5MR (2)	15MSS (13.5)	30MS (24)	25MSMR (15)
75	TR (0.2)	TR (0.2)	TMS (0.2)	TR (0.2)	15RMS (3)	15RMMS (13.5)
76	20MS (16)	20MRMS (12)	10RMR (3)	20RMR, 10 S (6)	20RMRMS (6)	25RMRMS (15)
77	0 (0)	70S (70)	40MRMS (24)	50MS (40)	45SMS (40.5)	40MSS (36)
78	10R (2)	TR (0.2)	10R (2)	50MSMR (30)	15RMRMS (4.5)	10RMS (4)
79	20MRMS (12)	0 (0)	5R (1)	5SR (1)	20RMR (6)	15R (3)
80	30MR (12)	20MR (8)	5R (1)	10RMR (3)	15RMR (4.5)	10R (2)
81	20MR (8)	20MS (16)	30MR (12)	50MSS (45)	45S (45)	30MSS (27)
82	40S (40)	40S (40)	40S (40)	40S (40)	40SMS (36)	35SMS (31.5)
83	30MRR (9)	30MR (12)	10RMR (3)	15MRR (4.5)	10RMRMS (3)	25RMRMS (15)
84	0 (0)	10MRMS (6)	15RMR (4.5)	20R (4)	15RMR (4.5)	15R (3)

RILs/Parents	KEN16		KEN17		KEN18	
	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II
85	60SMS (54)	70S (70)	30MR (12)	40MSMR (24)	25MS (20)	45SMS (40.5)
86	10S (10)	0 (0)	25SMS (22.5)	50SMS (45)	15RMRMS (4.5)	15RMS (6)
87	40MS (32)	20MS (16)	40SMS (36)	20SMS (18)	35MSS (31.5)	35S (35)
88	0 (0)	0 (0)	5R (1)	5R (1)	5R (1)	5R (1)
89	70S (70)	90S (90)	50S (50)	40S (40)	40S (40)	40MS (32)
90	60SMS (54)	40S (40)	40MSS (36)	50S (50)	40MSS (36)	35SMS (31.5)
91	0 (0)	10MS (8)	20S (20)	TR (0.2)	10MRMS (6)	30MS (24)
92	10S (10)	20S (20)	20MSS (18)	15S (15)	35MSS (31.5)	5MS (4)
93	0 (0)	0 (0)	5MS (4)	0 (0)	5RMRMS (1.5)	10MSS (9)
94	TR (0.2)	1S (1)	5SMS (4.5)	5S (5)	5RMRMS (1.5)	20RMS (8)
95	0 (0)	0 (0)	TR (0.2)	TSMS (0.2)	10RMR (3)	5RMS (2)
96	20MS (16)	20MRMS (12)	30S (30)	20SMS (18)	15S (15)	20MSMR (12)
97	10MS (8)	0 (0)	20MSSMR (12)	10S (10)	15RMRMS (4.5)	25MRMS (15)
98	70S (70)	80S (80)	30MRMS (18)	60S (60)	45MSS (40.5)	50S (50)
99	0 (0)	0 (0)	TR (0.2)	TSMS (0.2)	10RMR (3)	5R (1)
100	30MS (24)	10MR (4)	40S (40)	20S (20)	25MSS (22.5)	40MSMR (24)
101	30SMS (27)	10S (10)	-	30S (30)	35S (35)	20S (20)
102	20S (20)	20MS (16)	20SMS (18)	30S (30)	5MS (4)	15MS (12)
103	0 (0)	0 (0)	5R (1)	15SMS (13.5)	10RMR (3)	15RMR (4.5)
104	0 (0)	30S (30)	TR (0.2)	10S (10)	40S (40)	35S (35)
105	10S (10)	0 (0)	5RMR (1.5)	5MRMS (3)	20MSS (18)	20MSS (18)
106	0 (0)	0 (0)	0 (0)	TMS (0.2)	10RMR (3)	5R (1)

RILs/Parents	KEN16		KEN17		KEN18	
	Rep I	Rep II	Rep I	Rep II	Rep I	Rep II
107	10S (10)	10MS (8)	25SMS (22.5)	5SMS (4.5)	25S (25)	40SMS (36)
108	0 (0)	20S (20)	TR (0.2)	10MSS (9)	10R (2)	15MS (12)
109	20S (20)	30S (30)	10SMS (9)	20S (20)	20MS (16)	10RMR (3)
110	0 (0)	0 (0)	5MS (4)	0 (0)	5R (1)	15RMRMS (9)
111	0 (0)	1R (0.2)	20SMS (18)	10S (10)	20S (20)	20SMS (18)
112	10MS (8)	90S (90)	40S (40)	50S (50)	45S (45)	30SMS (27)
113	20S (20)	0 (0)	5R (1)	TSMS (0.2)	10R (2)	10R (2)
CI 14275	0 (0)	0	TR (0.2)	TR (0.2)	TR (0.2)	5R (1)
LMPG-6	70S (70)	90S (90)	70S (70)	60S (60)	55S (55)	45S (45)

<sup>a</sup>Stem rust severity was visually scored based on the modified Cobb scale of 0-100, where 0 = immune; no uredinia or any other sign of infection and 100% = completely susceptible (Peterson *et al.*, 1948). The infection responses were assigned as either; resistant (R), small uredinia surrounded by necrosis; moderately resistant (MR), medium-sized uredinia surrounded by necrosis or chlorosis; moderately susceptible (MS), medium-sized uredinia without necrosis; MRMS, infection response that included both the MR and MS categories; susceptible (S), large uredinia without necrosis; MSS infection responses that included both the MS and S (Roelfs *et al.*, 1992). Coefficient of infection (COI) values were generated by multiplying the stem rust severity value for each line by a constant value for each infection response: 0 = 0, R = 0.2, RMR = 0.3, MR = 0.4, M = 0.6, MS = 0.8, S = 1.0 (Knott, 1989). Average coefficient of infection for the two replicates were determined and used for analyses.<sup>b</sup>-Indicates missing data.

**Table 3.4.** Stem rust severity (0-100 scale), infection response (resistant (R)-susceptible (S)), and coefficient of infection (COI) in parenthesis of 113 recombinant inbred lines (RILs) along with the parents of the cross LMPG-6/CI 14275 in the field in Ethiopia in 2016 (ETH16), 2017 (ETH17) and in 2018 (ETH18). Presented data is for two replicates (rep)<sup>a</sup>

RILs/Parents	ETH16		ETH17		ETH18	
	Rep I	REP II	Rep I	Rep II	Rep I	Rep II
1	.. <sup>b</sup>	40SMS (36)	50SMS (45)	40S (40)	60S (60)	60S (60)
2	-	5R (1)	10MS (8)	20MS (18)	25MSMR (15)	20MRMS (12)
3	30SMS (27)	15MSS (13.5)	40SMS (36)	40S (40)	30S (30)	50SMS (45)
4	60S (60)	60S (60)	20MSS (18)	40S (40)	60S (60)	70S (70)
5	-	30MRMS (18)	20MSS (18)	30MSS (27)	50MSMR (30)	40MSS (36)
6	5MRMS (3)	15MSS (13.5)	40SMS (36)	40S (40)	50MS (40)	30MS (24)
7	-	-	15MS (12)	20S (20)	40MSMR (24)	40MSMR (24)
8	30MSS (27)	40S (40)	20MS (16)	30MS (24)	60S (60)	50S (50)
9	-	30MRMS (18)	20SMS (18)	20MS (16)	40SMS (36)	40MSS (36)
10	25MS (20)	30MSMR (18)	20MS (16)	30MSS (27)	40MSMR (24)	30MSS (27)
11	10MSS (9)	20MS (16)	50S (50)	40S (40)	40S (40)	40S (40)
12	15SMS (13.5)	15MS (12)	30MSS (27)	30MSS (27)	30MS (24)	40SMS (36)
13	-	25MSMR (15)	40MSS (36)	40S (40)	40MS (32)	50SMS (45)
14	30MSMR (18)	25MRMS (15)	20MSS (18)	10MS (8)	50MSS (45)	50SMS (45)
15	70S (70)	60S (60)	40SMS (36)	40S (40)	40S (40)	50S (50)
16	60S (60)	60S (60)	20MS (16)	10MS (8)	70S (70)	30MS (24)
17	30MRMS (18)	60S (60)	50SMS (45)	30MSS (27)	45SMS (40.5)	55S (55)
18	30SMS (27)	40MS (32)	40SMS (36)	40S (40)	60S (60)	60S (60)
19	20MSS (18)	20MSS (18)	40MSS (36)	40SMS (36)	50S (50)	60S (60)

RILs/Parents	ETH16		ETH17		ETH18	
	Rep I	REP II	Rep I	Rep II	Rep I	Rep II
20	TSMR (0.2)	-	10MS (8)	5MS (4)	20MS (16)	-
21	50S (50)	70S (70)	30MS (24)	40S (40)	60S (60)	70S (70)
22	-	15MRMS (9)	20MSS (18)	20SMS (18)	30MSMR (18)	40MSS (36)
23	-	-	30MSS (27)	15MS (12)	20MSS (18)	20SMS (18)
24	20SMS (18)	20MSS (18)	40S (40)	40MSS (36)	50SMS (45)	50SMS (45)
25	70S (70)	60S (60)	30MSS (27)	40S (40)	70S (70)	70S (70)
26	30SMS (27)	30S (30)	-	-	50S (50)	70S (70)
27	15SMS (13.5)	10MSS (9)	10MS (8)	10MS (8)	30MSS (27)	15MSS (13.5)
28	10SMS (9)	15MSS (13.5)	20MSS (18)	25MS (20)	30SMS (27)	20MS (16)
29	15SMS (13.5)	15MSS (13.5)	40MSS (36)	40MSS (36)	50SMS (45)	40SMS (36)
30	80S (80)	60S (60)	20MS (16)	30MS (24)	50S (50)	60S (60)
31	60S (60)	40S (40)	40SMS (36)	20MS (16)	55s (55)	50S (50)
32	30MSMR (18)	20MRMS (12)	15MS (12)	30MSS (27)	50SMS (45)	40MSS (36)
33	25MRMS (15)	35S (35)	40MSS (36)	40MSS (36)	50S (50)	60S (60)
34	50S (50)	70S (70)	40SMS (36)	40S (40)	50S (50)	40SMS (36)
35	-	5MS (4)	15MSS (13.5)	5MS (4)	10MS (8)	TMR (0.2)
36	-	-	10MS (8)	50S (50)	50S (50)	40SMS (36)
37	15MSS (13.5)	15MSS (13.5)	30SMS (27)	30MSS (27)	30MSS (27)	30SMS (27)
38	5MRMS (3)	20MRMS (12)	40SMS (36)	30SMS (27)	30MSMR (18)	40SMS (36)
39	-	-	50MSS (45)	40MSS (36)	60SMS (54)	40S (40)
40	30SMS (27)	-	20MSS (18)	30SMS (27)	60SMS (54)	40S (40)
41	40SMS (36)	50SMS (45)	5MS (4)	5MS (4)	40MRMS (24)	40SMS (36)



RILs/Parents	ETH16		ETH17		ETH18	
	Rep I	REP II	Rep I	Rep II	Rep I	Rep II
42	50S (50)	70S (70)	40MSS (36)	50S (50)	60S (60)	50S (50)
43	50S (50)	40S (40)	30MS (24)	50S (50)	50S (50)	60S (60)
44	25SMS (22.5)	-	30MSS (27)	30MSS (27)	25MS (20)	40SMS (36)
45	10MRMS (6)	-	20MSS (18)	20MS (16)	15MSS (13.5)	20MS (16)
46	20MSS (18)	30MSS (27)	15MSS (13.5)	30MSS (27)	30SMS (27)	40SMS (36)
47	40SMS (36)	30MSMR (18)	5MSS (4.5)	10MS (8)	60MSMR (36)	30SMS (27)
48	15MRMS (9)	15MSS (13.5)	20MS (16)	40MSS (36)	40MS (32)	40MSMR (24)
49	25MS (20)	50MSMR (30)	10MSS (9)	40SMS (36)	50SMS (45)	30MSS (27)
50	30MRMS (18)	30MRMS (18)	15MSS (13.5)	20MS (16)	40MSMR (24)	30MSS (27)
51	70S (70)	60S (60)	30MSS (27)	30MS (24)	60S (60)	50MSS (45)
52	20MSS (18)	20SMS (18)	30MSS (27)	30MSS (27)	50S (50)	40SMS (36)
53	5MR (2)	15MR, 10S (6)	15MS (12)	20MS (16)	25MSMR (15)	30MSMR (18)
54	25SMS (22.5)	30SMS (27)	50S (50)	40MSS (36)	40S (40)	60S (60)
55	20MSS (18)	25SMS (22.5)	10MS (8)	30MSS (27)	40SMS (36)	15MRM (9)
56	15MS (12)	5MS (4)	15MS (12)	30S (30)	50S (50)	40MSS (36)
57	-	30SMS (27)	40MSS (36)	40SMS (36)	50S (50)	60S (60)
58	30MSS (27)	30MSMR (18)	30MSS (27)	50S (50)	50SMS (45)	40SMS (36)
59	30SMS (27)	20SMS (18)	25MS (20)	25MS (20)	50SMS (45)	50MSMR (30)
60	-	-	5MS (4)	10MS (8)	10MRR (3)	10MSMR (6)
61	20MSMS (16)	30MRMS (18)	20MS (16)	20MSS (18)	-	40MSS (36)
62	TR (0.2)	5MS (4)	2SMS (20)	30MSS (27)	30MS (24)	20MSS (18)
63	60S (60)	50S (50)	40MSS (36)	30MSS (27)	40S (40)	60S (60)

RILs/Parents	ETH16		ETH17		ETH18	
	Rep I	REP II	Rep I	Rep II	Rep I	Rep II
64	30SMS (27)	15MSS (13.5)	30S (30)	20MS (16)	60S (60)	60S (60)
65	5MS (4)	-	20MS (16)	20MS (16)	5MS (4)	40MSMR (24)
66	10MSMR (6)	15SMS (13.5)	30MSS (27)	40SMS (36)	30SMS (27)	20MSMR (12)
67	5SMS (4.5)	15SMS (13.5)	40MSS (36)	40S (40)	40S (40)	30SMS (27)
68	-	50S (50)	30MSS (27)	30SMS (27)	50MSS (45)	60S (60)
69	25MSMR (15)	5SMS (4.5)	15MSS (13.5)	5MS (4)	30MSS (27)	10MSMR (6)
70	60S (60)	70S (70)	15MSS (13.5)	10MS (8)	60MSS (54)	60SMS (54)
71	15SMS (13.5)	30MR (12)	30MSS (27)	30MSS (27)	40MRMS (24)	25MSMR (15)
72	40SMS (36)	40MSMR (24)	15MSS (13.5)	15MS (12)	60S (60)	55S (55)
73	15SMS (13.5)	25SMS (22.5)	30MSS (27)	40SMS (36)	50MSS (45)	50SMS (45)
74	-	30MSMR (18)	30MSS (27)	40MSS (36)	50S (50)	50S (50)
75	10MS (8)	15MSS (13.5)	30MSS (27)	40S (40)	30MSS (27)	30MSS (27)
76	30SMS (27)	25MR (10)	10MS (8)	10MS (8)	50SMS (54)	40S (40)
77	50S (50)	50S (50)	5SMS (4.5)	30MSS (27)	60S (60)	45SMS (40.5)
78	-	15SMS (13.5)	20MSS (18)	10MS (8)	30MSMR (18)	30SMS (27)
79	25MRMS (15)	40MRMS (24)	20MS (16)	20MS (16)	40MSMR (24)	30MRMS (18)
80	30MS (24)	40SMS (36)	40MSS (36)	30MS (24)	50SMS (45)	30MSS (27)
81	30MSS (27)	30SMS (27)	20MSS (18)	25MSS (22.5)	50MS (40)	50SMS (45)
82	-	40SMS (36)	30MSS (27)	40S (40)	50S (50)	55S (55)
83	40MSS (36)	40SMS (36)	20MS (16)	20MS (16)	40MSMR (24)	50MSS (45)
84	50SMS (45)	30MSS (27)	25MSS (22.5)	30SMS (27)	40SMS (36)	40S (40)
85	60S (60)	60S (60)	25MSS (22.5)	25MSS (22.5)	60S (60)	50S (50)

RILs/Parents	ETH16		ETH17		ETH18	
	Rep I	REP II	Rep I	Rep II	Rep I	Rep II
86	TSMR (0.2)	20MR (8)	20MS (16)	10MS (8)	50SMS (45)	50SMS (45)
87	40SMS (36)	40MS (32)	10MS (8)	40MSS (36)	70SMS (63)	60S (60)
88	20MSMR (12)	-	20MSS (18)	30MSS (27)	40SMS (36)	30SMS (27)
89	40S (40)	60S (60)	30SMS (27)	40SMS (36)	60MSMR (36)	60S (60)
90	30S (30)	40S (40)	50SMS (45)	50S (50)	50S (50)	50S (50)
91	15MS (12)	5MRMS (3)	20MSS (18)	20MS (16)	30MS (24)	30MSS (27)
92	25MSS (22.5)	20MS (16)	40S (40)	20MSS (18)	40MSS (36)	15MS (12)
93	5MS (4)	15SMS (13.5)	40MSS (36)	40MSS (36)	30SMS (27)	15MSS (13.5)
94	10MSS (9)	20SMS (18)	10MSS (9)	30MSS (27)	30MSMR (18)	40S (40)
95	10MS (8)	30MSMR (18)	20MSS (18)	10MS (8)	35MSMR (21)	20MSMR (12)
96	30MRMS (18)	40MSMS (24)	30SMS (27)	40SMS (36)	50MSS (45)	60S (60)
97	20MS (16)	30MR, 5S (12)	40SMS (36)	30SMS (27)	50SMS (45)	30MS (24)
98	70S (70)	70S (70)	30MSS (27)	30MSS (27)	40MS (32)	70S (70)
99	5MS (4)	-	20MSS (18)	25MS (20)	20MSS (18)	15SMS (13.5)
100	15MSS (13.5)	15SMS (13.5)	40MSS (36)	25MS (20)	40S (40)	50S (50)
101	-	15S (15)	30MSS (27)	30MSS (27)	25MSS (22.5)	30S (30)
102	-	15MSS (13.5)	10MS (8)	40MSS (36)	30SMS (27)	30S (30)
103	-	10R (2)	5MS (4)	20MSS (18)	30SMS (27)	20MS (16)
104	30SMS (27)	30SMS (27)	40MSS (36)	40MSS (36)	50SMS (45)	50S (50)
105	15MSS (13.5)	-	20MSS (18)	20S (20)	20MSS (18)	30S (30)
106	0 (0)	-	20MSS (18)	30MSS (27)	20MS (16)	20MS (16)
107	15MSS (13.5)	15SMS (13.5)	40MSS (36)	40S (40)	40SMS (36)	30MSS (27)

RILs/Parents	ETH16		ETH17		ETH18	
	Rep I	REP II	Rep I	Rep II	Rep I	Rep II
108	-	10SMS (9)	25MSS (22.5)	20MSS (18)	25MRMS (15)	30S (30)
109	-	15MSS (13.5)	40S (40)	30MS (24)	40MSS (36)	50SMS (45)
110	15MSS (13.5)	15MS (12)	20SMS (18)	10SMS (9)	15MS (12)	25MS (20)
111	10MSS (9)	50SMS (45)	30SMS (27)	20SMS (18)	5MS (4)	20MS (16)
112	40MSMR (24)	-	20MS (16)	40S (40)	60SMS (54)	60S (60)
113	TRS (0.2)	TMRMS (0.2)	30SMS (27)	30S (30)	20MSMR (12)	15MSMR (9)
CI 14275	5RMR (1.5)	TMRMS (0.2)	TMS (0.2)	5MS (4)	10R-TMS (2)	15MSMR (9)
LMPG-6	70S (70)	70S (70)	50S (50)	40S (40)	70S (70)	40S (40)

<sup>a</sup>Stem rust severity was visually scored based on the modified Cobb scale of 0-100, where 0 = immune; no uredinia or any other sign of infection and 100% = completely susceptible (Peterson *et al.*, 1948). The infection responses were assigned as either; resistant (R), small uredinia surrounded by necrosis; moderately resistant (MR), medium-sized uredinia surrounded by necrosis or chlorosis; moderately susceptible (MS), medium-sized uredinia without necrosis; MRMS, infection response that included both the MR and MS categories; susceptible (S), large uredinia without necrosis; MSS infection responses that included both the MS and S (Roelfs *et al.*, 1992). Coefficient of infection (COI) values were generated by multiplying the stem rust severity value for each line by a constant value for each infection response: 0 = 0, R = 0.2, RMR = 0.3, MR = 0.4, M = 0.6, MS = 0.8, S = 1.0 (Knott, 1989). Average coefficient of infection for the two replicates were determined and used for analyses. <sup>b</sup>-Indicates missing data.

**Table 3.5.** Stem rust severity (0-100 scale), infection response (resistant (R)-susceptible (S)), and coefficient of infection (COI) in parenthesis of 113 recombinant inbred lines (RILs) along with the parents of the cross LMPG-6/CI 14275 in the field in St. Paul, MN in 2017 (STP17) and in 2018 (STP18). Presented data is for two replicates (rep)<sup>a</sup>

RILs/Parents	STP17		STP18 <sup>b</sup>	
	Rep I	Rep II	Rep I	Rep II
1	70S (70)	70S (70)	100S (100)	100S (100)
2	10MR (4)	20MRMS (12)	10RMR (3)	15RMR (4.5)
3	60S (60)	40S (40)	50S (50)	40M (24)
4	60S (60)	90S (90)	100S (100)	100S (100)
5	25MSMR (15)	20MSMR (12)	10M (6)	15M (9)
6	20MSMR (12)	20MRMS (12)	15RMR (4.5)	20M (12)
7	15MRMS (9)	15MRMS (9)	1RMR (0.3)	15RMR (4.5)
8	70S (70)	50S (50)	80S (80)	90S (90)
9	20MSMR (12)	40MSMR (24)	40M (24)	50S (50)
10	20MRMS (12)	15MRMS (9)	15M (9)	10RMR (3)
11	30MSS (27)	40S (40)	60S (60)	60S (60)
12	40MSMR (24)	40MSMR (24)	60S (60)	40M (24)
13	70S (70)	50MS (40)	60S (60)	90S (90)
14	20MRMS (12)	25MRMS (15)	40M (24)	30M (18)
15	70S (70)	70S (70)	100S (100)	100S (100)
16	90S (90)	70S (70)	100S (100)	100S (100)
17	60MSMR (36)	25MRMS (15)	100S (100)	90S (90)
18	15MSS (13.5)	40S (40)	60S (60)	40MS (32)
19	70S (70)	60S (60)	100S (100)	100S (100)
20	60S (60)	60S (60)	50S (50)	60S (60)
21	80S (80)	90S (90)	100S (100)	100S (100)
22	15MRMS (9)	25MRMS (15)	5RMR (1.5)	30M (18)
23	5RMR (1.5)	5MS (4)	TR (1)	10M (6)
24	60S (60)	60S (60)	80S (80)	60S (60)
25	70S (70)	80S (80)	80S (80)	100S (100)
26	15MS (12)	40MSMR (24)	-	-
27	50MRMS (30)	20MRMS (12)	10RMR (3)	25M (15)
28	30MSS (27)	40MS (32)	25MSS (22.5)	50S (50)
29	5RMR (1.5)	20MSMR (12)	15M (9)	30MSS (27)
30	90S (90)	70S (70)	100S (100)	100S (100)

RILs/Parents	STP17		STP18 <sup>b</sup>	
	Rep I	Rep II	Rep I	Rep II
31	70S (70)	50S (50)	100S (100)	80S (80)
32	30MRMS (18)	15MRMS (9)	30M (18)	30M (18)
33	40MSS (36)	30MSMR (18)	60S (60)	60S (60)
34	50S (50)	70S (70)	80S (80)	70S (70)
35	5MS (4)	5RMR (1.5)	5M (3)	5RMR (1.5)
36	70S (70)	60S (60)	80S (80)	90S (90)
37	25MSMR (15)	40MSMR (24)	15M (9)	25M (15)
38	25MRMS (15)	20MRMS (12)	30M (18)	25M (15)
39	70S (70)	70S (70)	100S (100)	100S (100)
40	60S (60)	50S (50)	100S (100)	90S (90)
41	40MS (32)	40MRMS (24)	60S (60)	20M (12)
42	60S (60)	90S (90)	100S (100)	100S (100)
43	30MSS (27)	30MS (24)	50S (50)	30MS (24)
44	90S (90)	80S (80)	100S (100)	100S (100)
45	25MSS (22.5)	40MS (32)	50S (50)	60S (60)
46	15MS (12)	15MS (12)	15M (9)	30MS (24)
47	70S (70)	50S (50)	100S (100)	100S (100)
48	30MSMR (18)	50MS (40)	30M (18)	20M (12)
49	60S (60)	50S (50)	60S (60)	50S (50)
50	40MSMR (24)	50MRMS (30)	60S (60)	70S (70)
51	60S (60)	40MSMR (24)	100S (100)	100S (100)
52	40MSMR (24)	20MS (16)	40MSS (36)	30M (18)
53	15MRMS (9)	20MRMS (12)	10RMR (3)	15M (9)
54	70S (70)	50S (50)	50S (50)	50S (50)
55	70S (70)	40MSMR (24)	100S (100)	100S (100)
56	25MSMR (15)	40MS (32)	60S (60)	40S (40)
57	50MS (40)	50MSMR (30)	80S (80)	100S (100)
58	50MSMR (30)	30MSMR (18)	20M (12)	30M (18)
59	80S (80)	40MS (32)	100S (100)	100S (100)
60	10RMR (3)	10MR (4)	1RMR (0.3)	15RMR (4.5)
61	40MRMS (24)	40MRMS (24)	50S (50)	30M (18)
62	25MSMR (15)	15MRMS (9)	15M (9)	25M (15)
63	80S (80)	60S (60)	100S (100)	100S (100)
64	25MS (20)	40S (40)	25MSS (22.5)	50S (50)
65	25MSMR (15)	40MSMR (24)	10RMR (3)	15M (9)

RILs/Parents	STP17		STP18 <sup>b</sup>	
	Rep I	Rep II	Rep I	Rep II
66	40MS (32)	40MS (32)	50S (50)	40M (24)
67	25MSS (22.5)	15M (9)	15M (9)	30MSS (27)
68	60S (60)	40S (40)	70S (70)	80S (80)
69	60S (60)	50MSMR (30)	90S (90)	80S (80)
70	80S (80)	50MS (40)	100S (100)	100S (100)
71	10MRMS (6)	15MS (12)	15M (9)	15M (9)
72	20MRMS (12)	30MRMS (18)	60S (60)	40M (24)
73	50MSMR (30)	60S (60)	70S (70)	100S (100)
74	70S (70)	50S (50)	60S (60)	50S (50)
75	50S (50)	50S (50)	90S (90)	50S (50)
76	50MS (40)	40MS (32)	30M (18)	50S (50)
77	60S (60)	70S (70)	80S (80)	100S (100)
78	15MRMS (9)	5RMR (1.5)	30M (18)	30M (18)
79	15MRMS (9)	40MRMS (24)	25M (15)	25M (15)
80	30MSMR (18)	40MSMR (24)	40M (24)	50S (50)
81	15MSMR (9)	15MRMS (9)	10RMR (3)	15RMR (4.5)
82	80S (80)	50S (50)	70S (70)	100S (100)
83	25MRMS (15)	15MRMS (9)	50S (50)	40M (24)
84	50MRMS (30)	30MSMR (18)	30M (18)	30M (18)
85	50MSMR (30)	40MRMS (24)	80S (80)	50S (50)
86	25MSMR (15)	40MSMR (24)	90S (90)	50M (30)
87	15MS (12)	40S (40)	50S (50)	30MSS (27)
88	25MSMR (15)	30MSMR (18)	15M (9)	60S (60)
89	70S (70)	90S (90)	40M (24)	100S (100)
90	80S (80)	80S (80)	100S (100)	100S (100)
91	50MSMR (30)	25MSMR (15)	50S (50)	50S (50)
92	50S (50)	25MSMR (15)	25M (15)	15M (9)
93	30MS (24)	25MS (20)	25MS (20)	15MSS (13.5)
94	20MRMS (12)	30MRMS (18)	15M (9)	15RMR (4.5)
95	60S (60)	40MS (32)	25M (15)	40M (24)
96	40MSMR (24)	40MSMR (24)	30M (18)	50M (30)
97	30MS (24)	40S (40)	30M (18)	40M (24)
98	80S (80)	90S (90)	100S (100)	100S (100)
99	15MS (12)	30S (30)	15M (9)	40MS (32)
100	70S (70)	60S (60)	50S (50)	50S (50)

RILs/Parents	STP17		STP18 <sup>b</sup>	
	Rep I	Rep II	Rep I	Rep II
101	5RMR (1.5)	5MS (4)	20M (12)	15MSS (13.5)
102	30S (30)	40S (40)	60S (60)	50S (50)
103	10MRMS (6)	10MRMS (6)	10RMR (3)	25M (15)
104	40MS (32)	20MS (16)	50S (50)	50S (50)
105	50S (50)	60S (60)	30M (18)	30MSS (27)
106	5RMR (1.5)	5RMR (1.5)	5RMR (1.5)	5RMR (1.5)
107	15MS (12)	15MS (12)	10M (6)	25M (15)
108	50MS (40)	25MSMR (15)	40M (24)	40M (24)
109	70MSS (63)	50S (50)	50S (50)	60S (60)
110	15MSS (13.5)	15MS (12)	5RMR (1.5)	5RMR (1.5)
111	5MS (4)	15MS (12)	15M (9)	15M (9)
112	15MS (12)	25MS (20)	15M (9)	25M (15)
113	60S (60)	40M (24)	40M (24)	25M (15)
CI 14275	5RMR (1.5)	5RMR (1.5)	0 (0)	TR (1)
LMPG-6	100S (100)	90S (90)	90S (90)	100S (100)

<sup>a</sup>Stem rust severity was visually scored based on the modified Cobb scale of 0-100, where 0 = immune; no uredinia or any other sign of infection and 100% = completely susceptible (Peterson *et al.*, 1948). The infection responses were assigned as either; resistant (R), small uredinia surrounded by necrosis; moderately resistant (MR), medium-sized uredinia surrounded by necrosis or chlorosis; moderately susceptible (MS), medium-sized uredinia without necrosis; MRMS, infection response that included both the MR and MS categories; susceptible (S), large uredinia without necrosis; MSS infection responses that included both the MS and S (Roelfs *et al.*, 1992). Coefficient of infection (COI) values were generated by multiplying the stem rust severity value for each line by a constant value for each infection response: 0 = 0, R = 0.2, RMR = 0.3, MR = 0.4, M = 0.6, MS = 0.8, S = 1.0 (Knott, 1989). Average coefficient of infection for the two replicates were determined and used for analyses

<sup>b</sup>-Indicates missing data.



**Table 3.6.** Analysis of variance for stem rust severity of 113 recombinant inbred lines (RILs) and the parents of the cross LMPG-6 /CI 14275 tested in eight environments (Kenya - 2016, 2017 and 2018; Ethiopia - 2016, 2017 and 2018; St. Paul - 2017 and 2018).

Source	Degree of freedom	Mean Square	F-value
Lines	114	3653.2	30.7*** <sup>a</sup>
Environments	7	24129.2	202.6***
Replication	1	215.2	1.8
Lines x Environments	786	486.0	4.1***
Error	876	119.1	

<sup>a\*\*\*</sup> Significant at 0.001 probability level.

**Table 3.7.** Correlation coefficients among stem rust coefficient of infection (COI) values for 113 recombinant inbred lines (RILs) and the parents of the cross LMPG-6 /CI 14275 in eight environments (Kenya - 2016, 2017 and 2018; Ethiopia - 2016, 2017 and 2018; St. Paul - 2017 and 2018)<sup>a</sup>.

	KEN16	ETH16	STP17	KEN17	ETH17	STP18	KEN18	ETH18
KEN16	1.00							
ETH16	0.82	1.00						
STP17	0.57	0.63	1.00					
KEN17	0.73	0.73	0.59	1.00				
ETH17	0.25	0.21	0.30	0.32	1.00			
STP18	0.64	0.69	0.85	0.65	0.25	1.00		
KEN18	0.72	0.74	0.55	0.78	0.42	0.62	1.00	
ETH18	0.67	0.73	0.57	0.67	0.47	0.64	0.73	1.00

<sup>a</sup>KEN16 = Njoro, Kenya 2016; KEN17 = Njoro, Kenya 2017; KEN18 = Njoro, Kenya 2018; ETH16 = Debre Zeit, Ethiopia 2016; ETH17 = Debre Zeit, Ethiopia 2017; ETH18 = Debre Zeit, Ethiopia 2018; STP17 = St. Paul, MN 2017; STP18 = St. Paul, MN 2018.

**Table 3.8.** Quantitative trait loci (QTL) identified in the recombinant inbred lines (RILs) population derived from the cross LMPG-6/CI 14275 for seedling resistance to *Puccinia graminis* f. sp. *tritici* races RTQQC, TPMKC, and TTTTF and for field resistance in Njoro, Kenya (2016, 2017 and 2018); Debre-Zeit, Ethiopia (2016, 2017 and 2018) and St. Paul, Minnesota (2017 and 2018).

Race/ Environment <sup>a</sup>	QTL <sup>b</sup>	Chr. <sup>c</sup>	LOD <sup>d</sup>	Position (cM) <sup>e</sup>	Left flanking marker <sup>f</sup>	Right flanking marker <sup>g</sup>	Phenotypic variance (%) <sup>h</sup>
<b>Seedling stage</b>							
RTQQC	<i>QSr.cdl-6BL.1</i>	6BL	7.74	11	BS00109878_51	BobWhite_c47040_1 85	37.5
TPMKC	<i>QSr.cdl-6BL.1</i>	6BL	11.1	6	6 wsnp_Ex_rep_c68677	BobWhite_c27364_29 BS00109878_51	20.58
TTTTF	<i>QSr.cdl-4AL.1</i>	4AL	18.0	8	_67531081	Tdurum_contig42019 _1714	41.19
TTTTF	<i>QSr.cdl-5DL.1</i>	5DL	3.86	60	BS00062990_51	BS00021991_51	3.77
<b>Adult plant stage</b>							
KEN16	<i>QSr.cdl-2BS.2</i>	2BS	3.81	655	176	Tdurum_contig54704_ GENE-0592_352	12.8
	<i>QSr.cdl-3B.1</i>	3B	6.7	438	Excalibur_c57658_54	IAAV3838	21.4
KEN17	<i>QSr.cdl-6AS.1</i>	6AS	3.29	129	IAAV3806	BS00031178_51	9.8
KEN18	<i>QSr.cdl-3B.1</i>	3B	5.86	438	Excalibur_c57658_54	IAAV3838	8.3
ETH16	<i>QSr.cdl-6AS.1</i>	6AS	4.64	127	IAAV3806	BS00031178_51	10.38

Race/ Environment <sup>a</sup>	QTL <sup>b</sup>	Chr. <sup>c</sup>	LOD <sup>d</sup>	Position (cM) <sup>e</sup>	Left flanking marker <sup>f</sup>	Right flanking marker <sup>g</sup>	Phenotypic variance (%) <sup>h</sup>
					Tdurum_contig54704_		
	<i>Qsr.cdl-2BS.2</i>	2BS	5.71	654	176	GENE-0592_352	13.97
	<i>Qsr.cdl-6AS.1</i>	6AS	3.93	127	IAAV3806	BS00031178_51	9.9
					Tdurum_contig54704_		
ETH18	<i>Qsr.cdl-2BS.2</i>	2BS	4.35	652	176	GENE-0592_352	8.3
					Tdurum_contig32277_		
	<i>Qsr.cdl-3B.1</i>	3B	5.57	474	121	BS00057988_51	16.6
					BobWhite_c27364_29	BobWhite_c27364_2	
STP17	<i>Qsr.cdl-6BL.1</i>	6BL	5.94	0	6	96	17.8
						w SNP_Ra_c69_14951	
STP18	<i>Qsr.cdl-3B.1</i>	3B	4.18	454	RAC875_c10595_473	8	13.14
					BobWhite_c27364_29		
	<i>Qsr.cdl-6BL.1</i>	6BL	5.64	2	6	BS00109878_51	18.05

<sup>a</sup>Abbreviations of experimental locations: KEN16 = Njoro, Kenya 2016; KEN17 = Njoro, Kenya 2017; KEN18 = Njoro, Kenya 2018; ETH16 = Debre Zeit, Ethiopia 2016; ETH18 = Debre Zeit, Ethiopia 2018; STP17 = St. Paul, MN 2017; STP18 = St. Paul, MN 2018.

<sup>b</sup>Naming of QTL: *Q* = QTL; *Sr* = Stem rust; *cdl* = Cereal disease lab.

<sup>c</sup>Chr. = Wheat chromosomes

<sup>d</sup>LOD = Logarithm of odds value

<sup>e</sup>cM = centiMorgan, position of the peak LOD

<sup>f</sup>Left flanking marker = markers flanking the identified QTL, on the left side

<sup>g</sup>Right flanking marker = markers flanking the identified QTL, on the right side

<sup>h</sup>Percent of variance explained by the identified QTL.

**Table 3.9.** Coefficient of infection (COI) (%) for wheat lines with and without three adult plant resistance (APR) quantitative trait loci (QTL): *QSr.cdl-2BS.2*, *QSr.cdl-3B.1* and *QSr.cdl-6AS.1* detected in six environments tested in this study, excluding ETH17.

Environment <sup>a</sup>	KEN			ETH			STP			ETH & KEN	Overall	
	KEN16	KEN17	KEN18	average	ETH16	ETH18	average	STP17	STP18	average		environments
Average value for seven lines without QTL	60.2	36.6	35.5	43.5	54.5	51.6	52.3	65.3	92.9	79.1	47.1	56.7
Average value for 11 lines with QTL detected	6.7	3.7	6.9	5.9	8.8	14.7	12.4	23.8	19.1	21.4	8.2	12.3
<i>P</i> value <sup>b</sup>	**	**	***	***	***	***	***	***	***	***	***	***

<sup>a</sup>Abbreviations of experimental locations: KEN16 = Njoro, Kenya 2016; KEN17 = Njoro, Kenya 2017; KEN18 = Njoro, Kenya 2018; ETH16 = Debre Zeit, Ethiopia 2016; ETH18 = Debre Zeit, Ethiopia 2018; STP17 = St. Paul, MN 2017; STP18 = St. Paul, MN 2018.

<sup>b</sup>\*\*, Significant at  $p < 0.01$  probability level, \*\*\*, significant at  $p < 0.001$  probability level.

**Table 3.10.** Kompetitive allele-specific PCR (KASP) primer sequences derived from 90K single nucleotide polymorphism (SNP) markers linked to identified quantitative trait loci (QTL)<sup>a</sup>.

SNP	Sequence	A1	A2	C1	C2	SNP1	SNP2
	tcgctgtactctaccaatccaacaagacgaca				CAAATCCAA		
Tdurum_co	atggcaaaaacagagc[A/C]gaagtcatatca	GAAGGTGACCAAGTTC	GAAGGTCGGAGTCAAC	CGCGTGTAC	CAAGACGAC		
ntig32277_	ttcatatgccaccactagacagctccgagctacca	ATGCTGGTGGCATATG	GGATTGGTGGCATATG	TCTACCAAA	AATGGCAAA		
121	acg	AATGATATGACTTCT	AATGATATGACTTCG	TCCAACAA	A	A	C
	tcgctgtactctaccaatccaacaagacgaca				CAAATCCAA		
Excalibur_	atggcaaaaacagagc[A/C]gaagtcatatca	GAAGGTGACCAAGTTC	GAAGGTCGGAGTCAAC	CGCGTGTAC	CAAGACGAC		
c57658_54	ttcatatgccaccactagacagctccgagctacca	ATGCTGGTGGCATATG	GGATTGGTGGCATATG	TCTACCAAA	AATGGCAAA		
	acg	AATGATATGACTTCT	AATGATATGACTTCG	TCCAACAA	A	A	C
	tcgctgtactctaccaatccaacaagacgaca				CAAATCCAA		
IAAV3838	atggcaaaaacagagc[A/C]gaagtcatatca	GAAGGTGACCAAGTTC	GAAGGTCGGAGTCAAC	CGCGTGTAC	CAAGACGAC		
	ttcatatgccaccactagacagctccgagctacca	ATGCTGGTGGCATATG	GGATTGGTGGCATATG	TCTACCAAA	AATGGCAAA		
	acg	AATGATATGACTTCT	AATGATATGACTTCG	TCCAACAA	A	A	C
	tcgctgtactctaccaatccaacaagacgaca				CAAATCCAA		
BS0006646	atggcaaaaacagagc[A/C]gaagtcatatca	GAAGGTGACCAAGTTC	GAAGGTCGGAGTCAAC	CGCGTGTAC	CAAGACGAC		
6_51	ttcatatgccaccactagacagctccgagctacca	ATGCTGGTGGCATATG	GGATTGGTGGCATATG	TCTACCAAA	AATGGCAAA		
	acg	AATGATATGACTTCT	AATGATATGACTTCG	TCCAACAA	A	A	C
	tcgctgtactctaccaatccaacaagacgaca				CAAATCCAA		
GENE-	atggcaaaaacagagc[A/C]gaagtcatatca	GAAGGTGACCAAGTTC	GAAGGTCGGAGTCAAC	CGCGTGTAC	CAAGACGAC		
1805_135	ttcatatgccaccactagacagctccgagctacca	ATGCTGGTGGCATATG	GGATTGGTGGCATATG	TCTACCAAA	AATGGCAAA		
	acg	AATGATATGACTTCT	AATGATATGACTTCG	TCCAACAA	A	A	C

SNP	Sequence	A1	A2	C1	C2	SNP1	SNP2
	tcgctgtactctaccaaatccaacaagacgaca				CAAATCCAA		
	atggcaaaaacagagc[A/C]gaagtcatatca	GAAGGTGACCAAGTTC	GAAGGTCGGAGTCAAC	CGCGTGTAC	CAAGACGAC		
RAC875_c	ttcatatgccaccactagacagctccgagctacca	ATGCTGGTGGCATATG	GGATTGGTGGCATATG	TCTACCAA	AATGGCAA		
10595_473	acg	AATGATATGACTTCT	AATGATATGACTTCG	TCCAACAA	A	A	C
	tcgctgtactctaccaaatccaacaagacgaca				CAAATCCAA		
Excalibur_	atggcaaaaacagagc[A/C]gaagtcatatca	GAAGGTGACCAAGTTC	GAAGGTCGGAGTCAAC	CGCGTGTAC	CAAGACGAC		
c7963_172	ttcatatgccaccactagacagctccgagctacca	ATGCTGGTGGCATATG	GGATTGGTGGCATATG	TCTACCAA	AATGGCAA		
2	acg	AATGATATGACTTCT	AATGATATGACTTCG	TCCAACAA	A	A	C
	tcgctgtactctaccaaatccaacaagacgaca				CAAATCCAA		
	atggcaaaaacagagc[A/C]gaagtcatatca	GAAGGTGACCAAGTTC	GAAGGTCGGAGTCAAC	CGCGTGTAC	CAAGACGAC		
BS0003882	ttcatatgccaccactagacagctccgagctacca	ATGCTGGTGGCATATG	GGATTGGTGGCATATG	TCTACCAA	AATGGCAA		
0_51	acg	AATGATATGACTTCT	AATGATATGACTTCG	TCCAACAA	A	A	C
	tcgctgtactctaccaaatccaacaagacgaca				CAAATCCAA		
Tdurum_co	atggcaaaaacagagc[A/C]gaagtcatatca	GAAGGTGACCAAGTTC	GAAGGTCGGAGTCAAC	CGCGTGTAC	CAAGACGAC		
ntig54704_	ttcatatgccaccactagacagctccgagctacca	ATGCTGGTGGCATATG	GGATTGGTGGCATATG	TCTACCAA	AATGGCAA		
176	acg	AATGATATGACTTCT	AATGATATGACTTCG	TCCAACAA	A	A	C
	tcgctgtactctaccaaatccaacaagacgaca				CAAATCCAA		
	atggcaaaaacagagc[A/C]gaagtcatatca	GAAGGTGACCAAGTTC	GAAGGTCGGAGTCAAC	CGCGTGTAC	CAAGACGAC		
BobWhite_	ttcatatgccaccactagacagctccgagctacca	ATGCTGGTGGCATATG	GGATTGGTGGCATATG	TCTACCAA	AATGGCAA		
c2453_282	acg	AATGATATGACTTCT	AATGATATGACTTCG	TCCAACAA	A	A	C

SNP	Sequence	A1	A2	C1	C2	SNP1	SNP2
	tcgctgtactctaccaaaccaacaagacgaca				CAAATCCAA		
Tdurum_co	atggcaaaacagagc[A/C]gaagtcatatca	GAAGGTGACCAAGTTC	GAAGGTCGGAGTCAAC	CGCGTGTAC	CAAGACGAC		
ntig12899_347	ttcatatgccaccactagacagctccgagctacca acg	ATGCTGGTGGCATATG AATGATATGACTTCT	GGATTGGTGGCATATG AATGATATGACTTCG	TCTACCAAA TCCAACAA	AATGGCAAA A	A	C
	tcgctgtactctaccaaaccaacaagacgaca				CAAATCCAA		
Ra_c6672_1679	atggcaaaacagagc[A/C]gaagtcatatca ttcatatgccaccactagacagctccgagctacca acg	GAAGGTGACCAAGTTC ATGCTGGTGGCATATG AATGATATGACTTCT	GAAGGTCGGAGTCAAC GGATTGGTGGCATATG AATGATATGACTTCG	CGCGTGTAC TCTACCAAA TCCAACAA	CAAGACGAC AATGGCAAA A	A	C
	tcgctgtactctaccaaaccaacaagacgaca				CAAATCCAA		
Jagger_c83_10_70	atggcaaaacagagc[A/C]gaagtcatatca ttcatatgccaccactagacagctccgagctacca acg	GAAGGTGACCAAGTTC ATGCTGGTGGCATATG AATGATATGACTTCT	GAAGGTCGGAGTCAAC GGATTGGTGGCATATG AATGATATGACTTCG	CGCGTGTAC TCTACCAAA TCCAACAA	CAAGACGAC AATGGCAAA A	A	C
		GAAGGTGACCAAGTTC					
		ATGCTAGTTCAAGATA	GAAGGTCGGAGTCAAC	GTTTCTGGG	GTGGTGTTC		
		CCACAGCAACAACAA	GGATTGTTCAAGATAC	CGTGGTGTT	TTTGTAAGAG		
IAAV3806		A	CACAGCAACAACAAC	TCTTTGTA	ATCCCTTT		
		GAAGGTGACCAAGTTC	GAAGGTCGGAGTCAAC	TGAGGTTGA	GAACTAACTT		
		ATGCTAATAGTTTCTA	GGATTAGTTTCTAGAA	AGGAACTA	TTTAATGCTG		
BS0003117_8_51		GAAAATATGTTGTCTC CTTTT	AATATGTTGTCTCCTTT C	ACTTTTAA TGCT	GACATGACA A		

<sup>a</sup>SNP markers linked to the identified QTL; A1, A2, C1, and C2 are the primers.



**Table 3.11.** Kompetitive allele-specific PCR (KASP) marker data on the validation population derived from Kwale/Line #162<sup>a</sup>.

Lines/ Parents	Tduru m_cont	Tduru m_cont			Tduru m_cont	Tduru m_cont			Excalib	Excalib			BS000	BS000			
	ig5470 4_176	ig5470 4_176	BS000 38820_	BS000 38820_	ig3227 7_121	ig3227 7_121	Jagger _c8310	Jagger _c8310	ur_c79 63_172	ur_c79 63_172	Ra_c66 72_167	BS000 31178_	BS000 31178_	IAAV3 806 C1	IAAV3 806 C2		
	C1	C2	51 C1	51 C2	C1	C2	_70 C1	_70 C2	2 C1	2 C2 <sup>b</sup>	9 C2	51 C1	51 C2	806 C1	806 C2		
1	1	1	2	2	1	1	1	1	2	x	1	1	1	1	1		
2	2	2	1	1	1	1	2	2	2	x	2	1	1	1	1		
3	2	2	1	1	-	2	2	2	1	x	2	2	2	2	2		
4	H	1	1	2	1	1	1	1	-	x	1	H	H	1	1		
5	2	2	1	1	H	H	H	H	1	x	H	H	H	-	1		
6	2	2	1	-	1	1	1	1	-	x	1	N	N	N	2		
7	1	1	2	2	2	2	1	1	1	x	1	2	2	1	2		
8	1	1	2	2	2	2	1	1	2	x	1	2	2	1	N		
9	1	1	2	2	2	2	1	1	2	x	1	2	2	1	1		
10	H	1	2	2	1	1	2	2	2	x	2	1	1	-	1		
11	1	1	2	2	H	H	H	H	2	x	H	2	2	2	1		
12	1	1	2	2	H	H	2	2	1	x	2	1	1	1	1		
13	H	1	2	2	1	1	H	H	2	x	H	1	1	1	2		
14	2	2	1	1	2	2	1	1	1	x	1	2	2	2	2		
15	1	1	2	2	1	1	H	H	2	x	H	1	1	1	2		
16	1	1	2	2	H	H	1	1	2	x	1	2	2	2	2		
17	H	1	2	2	1	1	2	2	2	x	2	1	1	1	1		
18	2	2	1	1	1	1	1	1	1	x	1	2	2	2	1		

Lines/ Parents	Tduru				Tduru				Excalib		Excalib					
	m_cont	m_cont	BS000	BS000	m_cont	m_cont	Jagger	Jagger	ur_c79	ur_c79	Ra_c66	BS000	BS000	IAAV3	IAAV3	
	ig5470	ig5470	38820_	38820_	ig3227	ig3227	_c8310	_c8310	63_172	63_172	72_167	31178_	31178_	IAAV3	IAAV3	
	4_176	4_176	51 C1	51 C2	7_121	7_121	_70 C1	_70 C2	2 C1	2 C2 <sup>b</sup>	9 C2	51 C1	51 C2	806 C1	806 C2	
19	2	2	1	1	1	1	1	1	1	x	1	1	1	1	1	
20	H	1	2	2	1	1	H	H	1	x	H	1	1	1	1	
21	H	1	2	2	1	1	2	2	2	x	2	N	N	N	1	
22	H	1	2	2	1	1	2	2	2	x	2	1	1	1	1	
23	-	1	2	2	H	H	2	2	1	x	2	1	1	1	2	
24	2	2	1	1	2	2	2	2	1	x	2	1	1	1	N	
25	H	1	2	2	2	2	H	H	2	x	H	1	1	1	1	
26	1	1	2	2	H	H	2	2	2	x	2	1	1	1	1	
27	H	1	2	2	H	H	2	2	2	x	2	1	1	1	1	
28	2	2	1	1	H	H	1	1	1	x	1	2	2	2	1	
29	2	2	1	1	2	2	-	H	1	x	H	1	1	1	2	
30	H	1	2	2	1	1	H	-	1	x	-	-	-	-	2	
31	2	2	1	1	2	2	1	1	1	x	1	2	2	2	2	
32	1	1	2	2	2	2	2	2	2	x	2	2	2	2	-	
33	-	2	1	1	H	-	H	H	1	x	-	H	H	-	1	
34	-	1	-	2	H	-	1	1	2	x	1	-	-	-	1	
35	2	2	1	1	H	H	1	1	1	x	1	2	2	2	1	
36	H	1	2	2	2	2	2	2	2	x	2	1	1	1	2	
37	1	1	2	2	H	2	1	1	-	x	1	1	1	1	2	

Lines/ Parents	Tduru	Tduru			Tduru	Tduru			Excalib	Excalib			BS000	BS000		
	m_cont	m_cont	BS000	BS000	m_cont	m_cont	Jagger	Jagger	ur_c79	ur_c79	Ra_c66	BS000	BS000	IAAV3	IAAV3	
	ig5470	ig5470	38820_	38820_	ig3227	ig3227	_c8310	_c8310	63_172	63_172	72_167	31178_	31178_	806 C1	806 C2	
	4_176	4_176	51 C1	51 C2	7_121	7_121	_70 C1	_70 C2	2 C1	2 C2 <sup>b</sup>	9 C2	51 C1	51 C2			
38	1	1	2	2	1	1	2	2	2	x	2	2	2	2	2	
39	1	1	2	2	2	2	1	1	2	x	1	2	2	2	2	
40	1	1	2	2	2	2	H	H	2	x	H	2	2	2	-	
41	-	-	-	-	-	-	-	-	-	x	2	-	-	-	1	
42	H	1	2	2	1	1	1	1	2	x	1	2	2	2	1	
43	H	1	2	2	1	1	2	2	2	x	2	1	1	1	1	
44	2	2	1	1	H	H	2	2	1	x	2	2	2	2	1	
45	1	1	2	2	2	2	H	-	1	x	H	2	2	2	2	
46	H	1	2	2	1	1	1	1	2	x	1	H	H	1	2	
47	H	1	2	2	1	1	H	H	2	x	H	1	1	1	2	
48	2	2	-	1	1	1	2	2	1	x	2	1	1	1	-	
49	2	2	1	1	1	1	1	1	1	x	1	1	1	1	1	
50	2	2	1	1	H	H	1	1	1	x	1	H	H	1	1	
51	1	1	2	2	2	2	2	2	2	x	2	1	1	1	1	
52	2	2	-	-	2	2	1	1	1	x	1	2	2	2	1	
53	2	2	1	-	H	2	H	H	1	x	H	2	2	2	2	
54	-	-	1	1	-	2	-	-	1	x	2	2	2	2	2	
55	2	-	1	1	-	2	-	-	1	x	2	1	1	1	2	
56	1	1	2	2	2	2	-	-	2	x	H	2	2	2	2	

Lines/ Parents	Tduru	Tduru			Tduru	Tduru			Excalib	Excalib			BS000	BS000		
	m_cont	m_cont	BS000	BS000	m_cont	m_cont	Jagger	Jagger	ur_c79	ur_c79	Ra_c66	BS000	BS000	IAAV3	IAAV3	
	ig5470	ig5470	38820_	38820_	ig3227	ig3227	_c8310	_c8310	63_172	63_172	72_167	31178_	31178_	IAAV3	IAAV3	
	4_176	4_176	51 C1	51 C2	7_121	7_121	_70 C1	_70 C2	2 C1	2 C2 <sup>b</sup>	9 C2	51 C1	51 C2	806 C1	806 C2	
57	H	1	2	2	H	H	1	1	2	x	1	1	1	1	1	
58	2	2	1	-	1	1	1	1	1	x	1	2	2	2	1	
59	2	2	1	1	2	2	H	H	1	x	H	1	1	1	1	
60	2	1	-	2	H	H	2	2	1	x	2	2	2	2	1	
61	H	1	2	2	2	2	H	H	2	x	H	2	2	2	2	
62	2	-	-	1	H	H	1	1	1	x	1	1	1	1	2	
63	1	1	-	2	2	2	H	H	2	x	H	-	-	-	2	
64	H	1	-	2	1	1	2	-	2	x	2	H	H	1	-	
65	1	1	-	2	2	2	H	H	2	x	H	-	2	2	1	
66	2	2	1	1	2	2	H	H	1	x	H	-	1	1	1	
67	1	1	2	2	H	H	2	2	2	x	2	1	1	1	1	
68	H	1	-	2	1	1	H	H	2	x	H	2	2	2	2	
69	1	1	2	2	2	2	2	2	2	x	2	2	2	2	2	
70	H	1	2	2	H	H	2	2	2	x	2	1	1	1	2	
71	1	1	2	2	H	H	1	1	2	x	1	2	2	2	2	
72	2	2	1	1	H	H	2	2	1	x	2	2	2	2	2	
73	H	1	-	2	H	H	1	1	2	x	1	H	H	1	1	
74	-	2	-	1	1	1	1	1	1	x	1	-	-	-	1	
75	-	-	-	1	H	-	2	-	-	x	-	-	1	1	1	

Lines/ Parents	Tduru				Tduru				Excalib		Excalib					
	m_cont	m_cont	BS000	BS000	m_cont	m_cont	Jagger	Jagger	ur_c79	ur_c79	Ra_c66	BS000	BS000	IAAV3	IAAV3	
	ig5470	ig5470	38820_	38820_	ig3227	ig3227	_c8310	_c8310	63_172	63_172	72_167	31178_	31178_	IAAV3	IAAV3	
	4_176	4_176	51 C1	51 C2	7_121	7_121	_70 C1	_70 C2	2 C1	2 C2 <sup>b</sup>	9 C2	51 C1	51 C2	806 C1	806 C2	
76	-	1	2	2	1	1	2	2	2	x	H	2	2	2	2	
77	H	1	2	2	1	1	H	1	2	x	-	-	1	1	-	
78	1	1	2	2	2	2	1	1	2	x	1	-	-	-	-	
79	1	1	2	2	2	2	1	1	2	x	1	1	1	-	-	
80	2	2	1	1	H	H	2	2	-	x	2	1	1	-	-	
81	1	1	2	2	1	1	H	H	2	x	H	1	1	-	1	
82	2	2	1	1	1	1	1	1	2	x	H	2	2	2	1	
83	H	1	-	2	2	2	H	H	2	x	H	1	1	1	1	
84	1	1	2	2	-	2	H	H	2	x	H	2	2	2	1	
85	1	1	2	2	H	H	H	H	2	x	H	1	1	1	2	
86	1	1	-	2	1	1	2	2	2	x	2	2	2	2	2	
87	2	2	1	1	2	2	2	2	1	x	-	1	1	1	2	
88	2	1	1	1	2	2	1	1	1	x	1	2	2	-	2	
89	1	1	2	2	2	2	1	1	2	x	1	2	2	1	1	
90	2	2	-	1	H	H	1	1	2	x	1	2	2	1	1	
91	2	-	2	2	2	2	H	H	1	x	H	1	1	1	1	
92	H	2	2	2	2	2	1	1	2	x	1	1	1	-	1	
93	1	2	2	2	2	2	1	1	2	x	1	H	H	1	1	
94	H	-	2	2	1	1	H	H	2	x	H	H	H	-	1	

Lines/ Parents	Tduru	Tduru			Tduru	Tduru			Excalib	Excalib			BS000	BS000		
	m_cont	m_cont	BS000	BS000	ig3227	ig3227	Jagger	Jagger	ur_c79	ur_c79	Ra_c66	BS000	BS000	IAAV3	IAAV3	
	ig5470	ig5470	38820_	38820_	ig3227	ig3227	_c8310	_c8310	ur_c79	ur_c79	72_167	31178_	31178_	IAAV3	IAAV3	
	4_176	4_176	51 C1	51 C2	7_121	7_121	_70 C1	_70 C2	2 C1	2 C2 <sup>b</sup>	9 C2	51 C1	51 C2	806 C1	806 C2	
95	2	-	1	1	2	2	H	H	1	x	H	H	H	1	2	
96	2	2	2	-	-	2	H	H	2	x	H	H	H	1	2	
97	H	2	2	-	1	1	H	H	-	x	-	1	1	1	1	
98	2	-	1	-	H	-	-	-	-	x	2	H	H	-	1	
99	2	-	1	1	1	1	H	2	1	x	2	H	H	-	1	
100	-	-	-	1	-	-	-	-	-	x	H	1	1	-	1	
101	2	-	1	1	H	H	2	2	1	x	2	2	2	-	2	
102	-	-	2	2	1	-	-	H	-	x	H	1	1	-	1	
103	-	-	-	1	-	-	-	H	-	x	H	2	2	-	2	
104	1	2	2	2	1	1	H	H	2	x	H	1	1	-	1	
105	1	2	2	2	2	2	H	H	2	x	H	1	1	-	1	
106	-	2	-	2	H	-	-	H	2	x	H	-	2	1	-	
107	H	2	2	2	H	H	H	-	2	x	H	2	2	2	2	
108	H	2	2	2	2	2	1	1	2	x	1	2	2	2	2	
109	2	-	2	2	2	2	1	1	1	x	1	1	1	1	1	
110	1	2	2	2	2	-	2	2	2	x	2	2	2	2	2	
111	1	2	2	2	2	2	H	H	2	x	H	1	1	1	1	
112	2	-	1	1	2	2	1	1	2	x	1	1	1	-	1	
113	1	2	2	2	1	1	1	1	2	x	1	-	H	-	1	

Lines/ Parents	Tduru				Tduru				Excalib		Excalib					
	m_cont	m_cont	BS000	BS000	m_cont	m_cont	Jagger	Jagger	ur_c79	ur_c79	Ra_c66	BS000	BS000	IAAV3	IAAV3	
	ig5470	ig5470	38820_	38820_	ig3227	ig3227	_c8310	_c8310	63_172	63_172	72_167	31178_	31178_	IAAV3	IAAV3	
	4_176	4_176	51 C1	51 C2	7_121	7_121	_70 C1	_70 C2	2 C1	2 C2 <sup>b</sup>	9 C2	51 C1	51 C2	806 C1	806 C2	
114	2	-	1	1	2	2	2	2	1	x	2	2	2	2	2	
115	1	2	2	2	H	H	H	H	2	x	H	2	2	2	2	
116	H	2	2	2	H	H	2	2	2	x	2	1	1	1	1	
117	2	-	1	-	H	H	2	2	1	x	2	2	2	2	2	
118	H	2	2	2	H	H	2	2	2	x	2	2	2	2	2	
119	2	-	1	-	H	H	2	2	1	x	2	2	2	2	2	
120	2	-	1	-	2	2	H	H	1	x	H	1	1	1	1	
121	-	-	-	1	2	-	-	-	-	x	H	1	1	-	1	
122	-	-	-	-	-	-	-	-	-	x	H	-	-	-	-	
123	1	2	2	2	-	H	-	H	-	x	H	1	1	1	1	
124	1	2	2	2	H	H	2	2	2	x	2	-	-	-	-	
125	H	2	2	-	1	1	H	H	2	x	H	1	1	1	1	
126	1	2	2	-	2	2	1	1	2	x	1	1	1	1	1	
127	2	-	1	-	1	1	H	H	1	x	H	2	2	2	2	
128	H	2	2	2	2	2	1	1	2	x	1	2	2	-	2	
129	-	-	2	-	2	-	-	-	-	x	1	1	1	-	1	
130	-	-	-	-	2	-	-	-	2	x	H	2	2	1	2	
131	2	-	1	-	1	-	-	H	-	x	H	2	2	1	2	
132	2	-	1	-	2	2	2	2	1	x	2	2	2	1	2	

Lines/ Parents	Tduru				Tduru				Excalib		Excalib					
	m_cont	m_cont	BS000	BS000	m_cont	m_cont	Jagger	Jagger	ur_c79	ur_c79	Ra_c66	BS000	BS000	IAAV3	IAAV3	
	ig5470	ig5470	38820_	38820_	ig3227	ig3227	_c8310	_c8310	63_172	63_172	72_167	31178_	31178_	806 C1	806 C2	
	4_176	4_176	51 C1	51 C2	7_121	7_121	_70 C1	_70 C2	2 C1	2 C2 <sup>b</sup>	9 C2	51 C1	51 C2			
133	2	-	1	-	1	1	H	H	1	x	H	2	2	1	2	
134	H	2	1	1	H	H	2	2	2	x	2	2	2	1	2	
135	H	2	2	2	2	2	2	2	2	x	2	2	2	1	2	
136	H	2	2	2	2	2	H	-	2	x	H	1	1	1	1	
137	H	2	2	-	H	H	2	2	2	x	2	H	H	-	1	
138	1	2	2	-	2	2	H	H	2	x	H	1	1	1	1	
139	2	-	1	-	H	H	-	H	1	x	H	-	-	-	-	
140	-	-	1	-	2	2	1	1	1	x	1	2	2	2	2	
141	2	-	1	1	1	1	-	1	1	x	1	H	H	1	1	
142	1	2	2	2	1	1	2	2	2	x	2	1	1	1	1	
143	1	2	2	2	2	2	2	2	2	x	2	H	H	1	1	
144	1	2	2	-	H	H	1	1	2	x	1	-	-	-	-	
145	H	2	2	-	1	1	H	H	2	x	H	2	2	-	-	
146	1	2	2	2	H	H	2	2	2	x	2	H	H	1	1	
147	H	2	-	1	-	-	1	1	-	x	1	-	-	-	-	
148	1	2	2	2	2	2	1	1	2	x	1	1	1	1	1	
149	H	2	2	-	2	2	1	1	2	x	1	2	2	2	2	
150	2	-	1	1	2	2	1	1	1	x	1	2	2	2	2	
151	2	-	2	2	2	2	2	2	1	x	2	1	1	1	1	



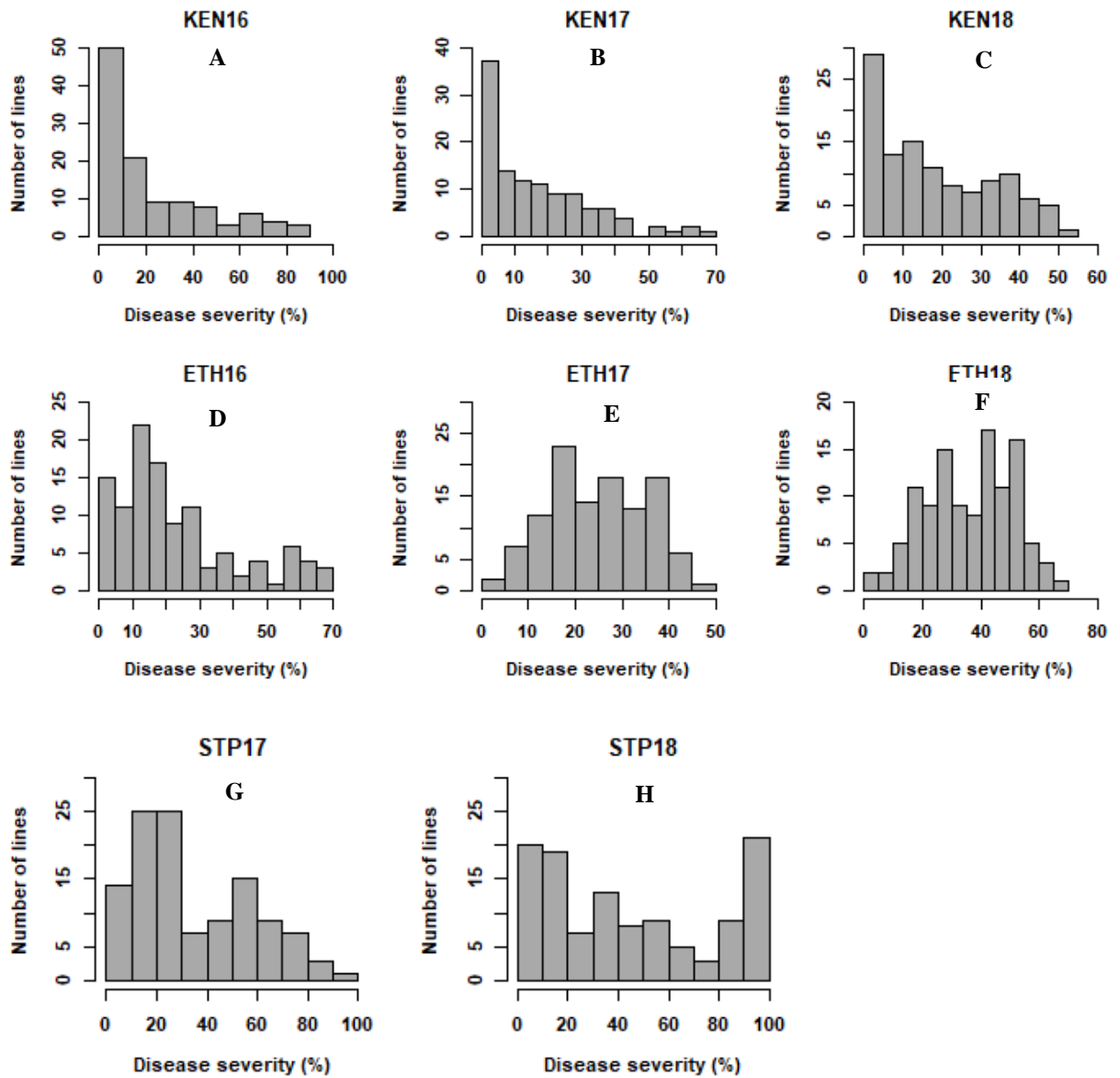
Lines/ Parents	Tduru	Tduru			Tduru	Tduru			Excalib	Excalib			BS000	BS000		
	m_cont	m_cont	BS000	BS000	m_cont	m_cont	Jagger	Jagger	ur_c79	ur_c79	Ra_c66	BS000	BS000	IAAV3	IAAV3	
	ig5470	ig5470	38820_	38820_	ig3227	ig3227	_c8310	_c8310	63_172	63_172	72_167	31178_	31178_	806 C1	806 C2	
	4_176	4_176	51 C1	51 C2	7_121	7_121	_70 C1	_70 C2	2 C1	2 C2 <sup>b</sup>	9 C2	51 C1	51 C2			
152	2	-	-	1	H	H	2	2	1	x	2	1	1	1	1	
153	2	-	-	1	1	1	1	1	1	x	1	H	H	-	1	
154	1	2	-	2	1	1	2	2	2	x	2	2	2	2	2	
155	-	-	-	2	-	-	-	1	-	x	1	2	2	2	2	
156	-	2	-	2	-	-	-	2	2	x	2	2	2	2	2	
157	2	-	-	1	-	1	2	2	1	x	2	2	2	2	2	
158	H	2	-	2	1	1	1	1	2	x	1	2	2	2	2	
159	H	2	-	2	-	1	H	2	-	x	2	1	1	1	1	
160	2	-	1	1	2	2	1	1	1	x	1	1	1	-	1	
161	1	2	2	2	H	H	2	2	2	x	2	2	2	-	2	
162	2	-	1	1	1	1	2	2	1	x	2	2	2	2	2	
163	1	2	2	2	H	H	H	H	2	x	H	2	2	2	2	
164	H	2	2	2	H	H	2	2	2	x	2	2	2	2	2	
165	H	2	2	2	1	1	H	H	2	x	H	-	-	-	-	
166	2	-	1	1	2	2	1	1	1	x	1	2	2	2	2	
167	1	2	-	-	-	-	1	-	1	x	-	2	2	2	2	
168	1	-	2	2	2	2	1	1	2	x	-	1	1	1	1	
169	2	-	-	1	2	2	2	2	1	x	2	2	2	-	2	
170	1	2	2	2	1	1	H	H	2	x	H	H	H	-	1	

Lines/ Parents	Tduru	Tduru			Tduru	Tduru			Excalib	Excalib			BS000	BS000		
	m_cont	m_cont	BS000	BS000	m_cont	m_cont	Jagger	Jagger	ur_c79	ur_c79	Ra_c66	BS000	BS000	IAAV3	IAAV3	
	ig5470	ig5470	38820_	38820_	ig3227	ig3227	_c8310	_c8310	63_172	63_172	72_167	31178_	31178_	IAAV3	IAAV3	
	4_176	4_176	51 C1	51 C2	7_121	7_121	_70 C1	_70 C2	2 C1	2 C2 <sup>b</sup>	9 C2	51 C1	51 C2	806 C1	806 C2	
171	H	2	2	2	1	1	2	2	2	x	2	1	-	1	1	
172	-	-	1	-	2	-	-	-	-	x	1	1	1	1	1	
173	H	2	2	-	2	2	1	1	2	x	1	1	1	1	1	
174	2	-	1	1	1	1	H	H	-	x	H	-	-	-	-	
175	H	2	2	2	2	-	-	2	2	x	2	1	1	-	-	
176	1	2	2	2	2	2	2	2	2	x	2	-	-	-	-	
177	H	2	2	2	2	2	1	1	2	x	1	2	2	-	2	
178	H	2	2	2	1	H	1	1	2	x	H	-	-	-	-	
179	H	2	2	2	1	1	1	1	2	x	1	2	2	2	2	
180	2	-	1	1	1	1	H	H	1	x	H	1	1	-	1	
Kwale	1	2	H	H	1	1	1	1	H	x	1	2	2	-	2	
Line																
#162	-	-	-	-	-	-	-	-	-	x	-	1	1	H	H	

<sup>a</sup>1 = Allele 1, 2 = Allele 2, H = Heterozygous, N = No template call, The dash (-) symbol indicates missing data

<sup>b</sup>X indicates that the marker is not polymorphic.

### 3.7 Figures



**Figure 3.1.** Frequency distribution for stem rust severity (%) in RILs developed from the LMPG-6/CI 14275 cross evaluated in Kenya (KEN), Ethiopia (ETH) and St. Paul (STP) in 2016, 2017 and 2018.

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## 5.0 Appendix

**Appendix 2.1.** Accession information for 52 wheat cultivars and one released breeding line originating from Kenya, Ethiopia or the USA and screened for reaction to Fusarium head blight in St. Paul and Crookston, MN in 2016 and 2017. Information for these accessions was obtained from the Genetic Resources Information System for Wheat and Triticale (GRIS) website (<http://www.wheatpedigree.net/>). The accessions are ranked alphabetically, based on the accession name.

Accession Name	Accession		Origin <sup>b</sup>	Identified Genes <sup>c</sup>	Status <sup>d</sup>	Habit <sup>e</sup>	Year of Registration
	Number <sup>a</sup>	Pedigree					
ALIDORO	-	-	ETH	-	CVR	S	2007
BAILEY	PI-299416	KENYA58/NEWTATCH//2*THATCHER/ 3/Frontana/4*THATCHER[2331];FRON TANA/4*THATCHER/3/THATCHE R//KENYA58/NEWTATCH/4/THATCHE R/5/Frontana/4*THATCHER	KEN	ne1, ne2, Ne2m, Ut1, Glu-A1b, B1c, D1a	CVR	S	1964
BEACON-KEN	PI-294566	Frontana / Kenya 58 // Newthatch /3/3* Bonza	KEN		CVR	S	1968
BIQA'A		PASTOR//HXL7573/2*BAU/3/WBLL1	ETH	-	CVR	S	-
BOLLO		VEERY/LIRA//BOBWHITE/3/BACANORA -88/4/KAUZ	ETH		CVR	S	2009
BONZA 63	PI-337705	RIO-NEGRO/2*BONZA-55	KEN	Sr6,Sr8a,Sr9b; GluA1b, B1c, D1d	CVR	S	1963
DANDAA	-	KIRITATI//2*PBW-65/2*SERI-82	ETH	Sr2, Sr2+; SrTm	CVR	S	2010

Accession Name	Accession		Origin <sup>b</sup>	Identified Genes <sup>c</sup>	Status <sup>d</sup>	Habit <sup>e</sup>	Year of Registration
	Number <sup>a</sup>	Pedigree					
DIGELU	-	SHANGHAI #7//KAUZ	ETH	Sr5, Sr21, Sr29, Sr37, Glu-A1c, B1c, D1d	CVR	S	2005
DURE	-	BOBWHITE/YDING//ZENZONTLI	ETH	Sr5, Sr9e, Glu- A1c, B1b, D1	CVR	S	2002
GALAMA	-	4777*2//FKN/GABO/3/PAVON-76	ETH	Rht2, Lr23, Lr37, Sr9e, Sr11	CVR	S	1995
GAMBO	-	AVRORA//KALYANSONA/BLUEBIRD/3/( SIB)WOODPECKER	ETH	-	CVR	S	2011
GASAY	-	PFAU/SERI-M-82//BOBWHITE	ETH	-	CVR	S	2007
HAWI	-	CHILERO/PARULA	ETH	Lr2c, Lr23, Lr27, Lr31, Yr4a, Yr9, Yr27, Glu-A1b, B1c, D1d	CVR	S	2000
HIDASE	-	YANAC/3/PARULA/ICTA-SARA 82//TESIA-79/VEERY-5/4/CROC 1/ (224) AE. SQ//OPATA-85	ETH	-	CVR	S	2012
HOGGANA	-	PAYNE/BAGULA//MILAN PAYNE/BAGULA//MILAN	ETH	-	CVR	S	2011



Accession Name	Accession		Origin <sup>b</sup>	Identified Genes <sup>c</sup>	Status <sup>d</sup>	Habit <sup>e</sup>	Year of
	Number <sup>a</sup>	Pedigree					Registration
HONQOLO	-	NJORO SD-7	ETH	-	CVR	-	-
HULLUKA	-	-	ETH	-	CVR	-	-
KBG-01	-	300-SM-501-M/HAR-1709	ETH	Yr9, Yr2, Yr27, Glu-Ala, Blc, D1d	CVR	S	2001
KENYA-CHEETAH	PI-323399	WARIGO/STERLINGLEE/Frontana//3* KENYA-354-P/3/CI 13632/3*KENYA-354-P	KEN	Glu-A1a, B1b, D1d	CVR	S	1967
KENYA-CIVET	PI-323390	CI 12632 /3* KENYA 354	KEN	Pm2, Pm6, Pm3c, Sr36, ne1, ne2, Glu-A1a, B1b, D1d	CVR	S	1966
KENYA-EAGLE 10	-	EMBRAPA16(EMB16)/(CBRD)CATBIRD/( CBRD)CATBIRD	KEN	SrCbrd	CVR	S	2011
KENYA-GABRINO	PI-290746	KENTANA/RIONEGRO//GABO-54	KEN	Glu-A1c, B1b, D1a	CVR	S; W	1963
KENYA-GOBLET	PI-320099	GABO54/LERMA52//GABO/3/KENYA/GE NERAL-URQUIZA	KEN	Glu-A1b, B1b, D1d	CVR	S	1965
KENYA-JAY	PI-307513	EQUATOR/KENYA-318	KEN	Glu-A1c, B1c, D1d	CVR	S	1962
KENYA-KINGBIRD	-	TAM-200/TUI/6/PAVON-76//CAR- 422/ANAHUAC75/5/BOBWHITE/CROW//B UCKBUCK/PAVON-76/3/YECORA- 70/4/TRAP-1	KEN	-	CVR	S	2012

Accession Name	Accession		Origin <sup>b</sup>	Identified Genes <sup>c</sup>	Status <sup>d</sup>	Habit <sup>e</sup>	Year of
	Number <sup>a</sup>	Pedigree					Registration
KENYA-KORONGO	-	BABAX/LR4//BABAX*2/4/SONOITA-81/TRAP1/3/KAUZ, MEX*2/TRAP//KAUZ, MEX	KEN	-	CVR	S	2012
KENYA-PAGE	PI-290747	MENTANA/KENYA 58//BAGE/3/KENYA-184-P	KEN	MENTANA/KENYA 58//BAGE/3/KENYA-184-P	CVR	S	1963
KENYA-PAKA	K-60148	WISCONSIN-245/II-50-17//CI 8154/2*TOBARI-66	KEN	Glu-A1a, B1b, D1d	CVR	S	1975
KENYA-PRIMEX	PI-324155	90875, MEX	KEN	Glu-A1b, B1b, D1a	CVR	S	1969
KENYA-TAMA	PI-290751	YAKTANA-54/LERMA-52	KEN	vrn1,vrn2,Vrn3; Sr6, Sr8+; Glu-A1c, B1c, D1d	CVR	S	1963
KENYA-WREN	-	THELIN-2/TUKURU	KEN	Sr2, SrTmp	CVR	S	2012
KENYA-1012-B-1-L	CI-14333	MENTANA/KENYA//BAGE/3/KENYA-184-P	KEN	Glu-Ala, B1c, D1d	CVR	S	1969
KINGBIRD	-	TAM-200/TUI/6/PAVON-F-76//CARIANCA-422/ANAHUAC-F-75/5/BOBWHITE/CROW//BUCKBUCK/PAVON-F-76/3/YECORA-F 70/4/TRAP-1	ETH		CVR	S	1999

Accession Name	Accession		Origin <sup>b</sup>	Identified Genes <sup>c</sup>	Status <sup>d</sup>	Habit <sup>e</sup>	Year of
	Number <sup>a</sup>	Pedigree					Registration
KUBSA	-	NORD-DESPREZ/VG 9144//KALYANSONA/BLUEBIRD/3/YACO /4/VEERY	ETH	Lr44; Yr2+; Glu- A1b, B1a, D1d	CVR	S	1994
LCS ALBANY	PI-658002	ALSEN, USA//BUCK-1021(B1021)/NORM	USA	Lr24	CVR	S	2008
LENANA	PI-290748	YAQUI- 48 / KENTANA- 48	KEN	Glu-A1c, B1b, D1a	CVR	S	1963
LINKERT	PI-672164	MN-97695-4/ADA	USA	-	CVR	S	2013
MADA- WALABU	-	TECOLOTE/3/FRONTANA(FN)/(TH)THAT CHER/2*NARINO-59/4/(SIB)BOLAN	ETH	Yr6,Yr7,Yr32; Sr8a; Glu-A1b, Bli, D1d	CVR	S	2000
WB- MAYVILLE	PI-661061	POLARIS/TROOPER	USA	-	CVR	S	2011
MENZE	-	MILAN/SHANGHAI-7	ETH	-	CVR	S	2007
MILLENIUM	-	-	ETH	-	CVR	S	2007
MORRIS	PI-297015	THATCHER//KENYA-117 A/MIDA/3/FRONTANA/4*THATCHER/4/T HATCHER/5/FRONTANA/4*THATCHER	KEN	Ne2ms; Glu-A1b, B1b, D1d	CVR	S	1964
NJORO-BW-II	-	IAS58/4/KALYANSONA/BLUEBIRD//CAJ AMA-F 71/3/ALONDRA/5/BOBWHITE	KEN	Glu-A1b, B1i, D1d	CVR	S	2007
P. WALKER- MUNRO	PI-320105	-	KEN	Glu-A1c, B1g, D1a	BL	S	1967
QULQULLU	-	PAYNE/BAGULA//MILAN	ETH	-	CVR	S	2009
RFN	-	(S)SABANERO	KEN	-	CVR	S	1949

Accession Name	Accession		Origin <sup>b</sup>	Identified Genes <sup>c</sup>	Status <sup>d</sup>	Habit <sup>e</sup>	Year of
	Number <sup>a</sup>	Pedigree					Registration
ROLLAG	PI-665250	MN-95229-40*2/RL-70-4	USA	Lr34, Fhb1	CVR	S	2011
SAMSON	PI-652923	EXPRESS/KNUDSON	USA	-	CVR	S	2007
SHORIMA	-	UTIQUE96/3/PAYNE/BAGULA//MILAN	ETH	-	CVR	S	2011
SOFUMAR	-	4777*2//FKN/GABO, AUS/3/PAVON-F-76	ETH	Glu-A1b, B1a, D1d	CVR	S	2000
TAY	-	ET-12-D-4/4/(HAR-604)4777 2//FKN/GB/3/PAVON-F-76	ETH	-	CVR	S	2005
TROPHY	PI-323398	TIMSTEIN/2*KENY A-RF 324//2*YAQUI-50	KEN	Glu-A1b,B1c, D1d	CVR	S	1968
WHEATON	PI-469271	CRIM(CI-13465)/2*(CI 13986) ERA//BUITRE/GALLO	USA	Yr18; Lr1, Lr2a, Lr3, Lr10, Lr13, Lr15+, Lr23, Lr34+; csLV34b, Ltn; Bdv1; Glu- A1b, B1b, D1d	CVR	S	1983

<sup>a</sup>Accession number prefixes were assigned by the wheat gene bank of origin; PI = Plant Introduction.

<sup>b</sup>Geographical origin; KEN = Kenya, ETH = Ethiopia, USA = United States of America, COL = Colombia, MEX = Mexico.

<sup>c</sup>Identified gene alleles; Yr = Stripe (Yellow) rust, Lr = Leaf rust, Sr = Stem rust, ne = Hybrid necrosis, Ut1= *Ustilago tritici*,

Glu = Glutenin, Vrn- Response to vernalization, Pin = Puroindolines and grain softness protein, Rht = Reduced height, Pm = Reaction to *Erysiphe graminis*, Ltn = Leaf tip necrosis.

<sup>d</sup>Genetic status; CVR = Cultivar, BL = Breeding Line.

<sup>e</sup>Growth habit; S = Spring, W = Winter.

**Appendix 2.2.** Pedigree information of the 167 advanced wheat lines originating from International Maize and Wheat Improvement Centre (CIMMYT) and Kenya Seed Company, screened for reaction to Fusarium head blight in St. Paul and Crookston, MN in 2016 and 2017. The accessions are ranked alphabetically.

Accession Number <sup>a</sup>	Pedigree <sup>b</sup>
ETBW 6109	SOKOLL/EXCALIBUR
ETBW 6114	SOKOLL//SUNCO/2*PASTOR
ETBW 6496	CROC-1/AE.SQUARROSA (205)//FCT/3/PASTOR
ETBW 6696	PBW343*2/KUKUNA//SRTU/3/PBW343*2/KPASTOR
ETBW 6832	CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/HAR311
ETBW 6850	FRNCLN/ROLF07
ETBW 6875	WAXWING/KIRITATI*2//YANAC
ETBW 6965	SAFI-3/ZEMAMRA-8
ETBW 7058	ROLF07//TAM200/TUI/6/WBLL1/4/HD2281/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/TACUPETO F2001
ETBW 7101	KAMB2/PANDION
ETBW 7213	CHAM-4/SHUHA'S'/6/2*SAKER/5/RBS/ANZA/3/KVZ/HYS//YMH/TOB
ETBW 7258	SABA/FLAG-1
ETBW 7364	ACSAD1115
ETBW 7698	FRNCLN/4/WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1
ETBW 7724	KIRITATI//PRL/2*PASTOR/3/FRANCOLIN #1
ETBW 7730	ONIX/KBIRD
ETBW 7872	QUAIU/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ
ETBW 8469	AMNA-4
Kenya Seed 1	-
Kenya Seed 2	-

Accession Number <sup>a</sup>	Pedigree <sup>b</sup>
Kenya Seed 3	-
Kenya Seed 4	-
Kenya Seed 5	-
Kenya Seed 6	-
Kenya Seed 7	-
Kenya Seed 8	-
Kenya Seed 9	-
Kenya Seed 10	-
R 1286	QUAIU/3/PGO/SERI/BAV92
R 1301	KSW/5/2*ALTAR 84/AE.SQUARROSA (221)//3*BORL95/3/URES/JUN/KAUZ/4/WBLL1
R 1317	KSW/7/CAL/NH/H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAUZ/6/PASTOR/8/CAL/NH//H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAUZ/6/PASTOR
R 1325	WBLLI*2/KURUKU/4/BABAX/LR42//BABAX*2/3/KURUKU
R 1331	KENYA NYANGUMI/3/2*KAUZ/PASTOR//PBW343
R 1351	WAXWING/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/KIRITATI//ATTILA*2/PASTOR
R 1352	ALTAR 84/AE.SQUARROSA (221)//3*BORL95/3/URES/JUN//KAUZ/4/WBLL1/5/KACHU/6/KIRITATI//PBW65/2*SERI.1B
R 1353	PFAU/SERI.1B//AMAD/3/WAXWING/4/TECUE #1/5/PFAU/SERI.1B//AMAD/3/WAXWING
R 1354	PBW65/2*PASTOR/3/KIRITATI//ATTILA*2/PASTOR/4/DANPHE #1
R 1357	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1/4/QUAIU
R 1360	BABAX/LR42//BABAX*2/3/TUKURU*2/4/HEILO
R 1361	TC870344/GUI//TEMPORALERA M 87/AGR/3/2*WBLL1/5/CROC-1/AE.SQUARROSA (205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2

Accession Number <sup>a</sup>	Pedigree <sup>b</sup>
R 1362	PFAU/MILAN/3/BABAX/LR42//BABAX/4/YANG87-142//SHA4/CHIL/3/TNMU
R 1363	PFAU/MILAN/3/BABAX/LR42//BABAX/4/ATTILA/BAV92//PASTOR
R 1364	MILAN/DUCULA/3/PSN/BOW//MILAN/4/PASTOR/3/BJY/COC//PRL/BOW
R 1365	NING MAI 96035/FINSI//HEILO/3/KA/NAC//TRCH
R 1366	ATTILA/HEILO/3/KA/NAC//TRCH
R 1367	ATTILA/HEILO//PAURAQ
R 1370	SUNCO.6/FRAME//PASTOR/3/2*BAVIS
R 1371	EGA BONNIE ROCK/6/CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (208)/5/2*WESTONIA
R 1372	M6SRRSN/011
R 1373	M6SRRSN/011
R 1374	LERKE/5/KAUZ/3/MYNA/VUL//BUC/FLK/4/MILAN/6/PROGRESO F2007/7/MUNAL
R 1376	PFAU/SERI.1B//AMAD*2/3/PBW343*2/KUKUNA/4/PRL/2*PASTOR/PBW343*2/KUKUNA/3/ROLF07
R 1378	PBW343/HUITES/4/YAR/AE.SQUARROSA (783)//MILAN/3/BAV92/5/FRET2*2/BRAMBLING
R 1379	TACUPETO F2001*2/BRAMBLING//ND643/2*WBLL1/3/TACUPETO F2001*2/BRAMBLING
R 1380	WBLL4/KUKUNA//WBLL1*2/3/KBIRD
R 1382	CROSBILL #1/DANPHE/7/CNDO/R143//ENTE/MEXI-2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*KAUZ/6/PRL/2*PASTOR
R 1388	PFAU/MILAN/5/CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/VEE#7/BOW/4/PASTOR/6/KINGBIRD #1
R 1389	PRL/2*PASTOR//SUNSTATE/3/KINGBIRD #1
R 1390	SHA7//PRL/VEE#6/3/FASAN/4/HAAS8446/2*FASAN/5/CBRD/KAUZ/6/MILAN/AMSEL/7/FRET2*2/KUKUNA/8/KIN GBIRD #1
R 1391	BRBT1*2/KIRITATI//KINGBIRD #1
R 1392	PICAFLO #1/KINGBIRD #1

Accession Number <sup>a</sup>	Pedigree <sup>b</sup>
R 1393	PAURAQ/KINGBIRD #1
R 1394	KAUZ//ALTAR 84/AOS/3/PASTOR/4/873.97/5/KINGBIRD #1
R 1395	SHA7/VEE#5/5/VEE#8//JUP/BJY/3/F3.71/TRM/4/2*WEAVER/6/SKAUZ/PARUS//PARUS/7/DANPHE #1
R 1396	WHEAR//2*PRL/2*PASTOR*2/8/NG8201/KAUZ/4/SHA7//PRL/VEE#6/3/FASAN/5/MILAN/KAUZ/6/ACHYUTA/7/PB W343*2/KUKUNA
R 1397	CROC-1/AE.SQUARROSA (205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2/5/WHEAR/SOKOLL
R 1398	PRL/2*PASTOR/5/HUITES/4/CS/TH.SC//3*PVN/3/MIRLO/BUC/6/KIRITATI/7/PRL/8/WAXWING/KIRITATI//FISCAL
R 1402	QUAIU/KINGBIRD #1
R 1403	BECARD/KINGBIRD #1
R 1404	ATTILA*2/PBW65/5/PRL/2*PASTOR/4/CHOIX/STAR/3/HE1/3*CNO79//2*SERI/6/PFAU/MILAN/3/SKAUZ/KS94U215 //SKAUZ
R 1406	SHA7//PRL/VEE#6/3/FASAN/4/HAAS8446/2*FASAN/5/CBRD/KAUZ/6/MILAN/AMSEL/7/FRET2*2/KUKUNA/8/2*W HEAR/SOKOLL
R 1407	MEX94.27.1.20/3/SOKOLL//ATTILA/3*BCN/4/HUW234+LR34/PRINIA//PBW343*2/KUKUNA/3/ROLF07
R 1408	CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92/4/HUW234+LR34/PRINIA//PBW343*2/KUKUNA/3/ROLF07
R 1409	BRBT1*2/KIRITATI*2//KINGBIRD #1
R 1410	PFAU/SERI.1B//AMAD/3/WAXWING*2/4/ND643/2*WAXWING
R 1411	PFAU/MILAN/3/BABAX/LR42//BABAX*2/4/NIINI #1
R 1412	NJBWII/IR 1081//NJBWII
R 1415	R1073/NJBW II
R 1416	R1076/NJBW II
R 1417	K8674/YOMBI
R 1418	DUMA/R1084



Accession Number <sup>a</sup>	Pedigree <sup>b</sup>
R 1420	CROC-1/AE.SQUARROSA (213)//PGO/10/ATTILA*2/9/KT/BAGE//FN/U/3/BZA/4/TRM/5/ALDAN/6/SERI/7/VEE#10/8/OPATA
R 1421	HAIEL-1*2/ABREG-4
R 1422	BERKUT/EXCALIBUR
R 1423	FRET2/KUKUNA//FRET2/3/YANAC/4/FRET2/KIRITATI
R 1424	PRL/2*PASTOR*2//FH6-1-7
R 1426	ROLF07*2/5/FCT/3/GOV/AZ//MUS/4/DOVE/BUC
R 1427	ATTILA*2/PBW65*2//MURGA
R 1429	MURGA
R 1430	WBLL1*2/VIVITSI//MESIA/3/KIRITATI/WBLL1
R 1431	BABAX/LR42//BABAX*2/3/KURUKU/4/KINGBIRD #1
R 1432	SAAR/WBLL1//VILLA JUAREZ F2009
R 1433	KAUZ*2/MNV//KAUZ/3/MILAN/4/BAV92*2/5/KIRITATI
R 1434	GRACK/VILLA JUAREZ F2009
R 1435	ND643//2*ATTILA*2/PASTOR/3/WBLL1*2/KURUKU/4/WBLL1*2/BRAMBLING
R 1436	SERI.1B*2/3/KAUZ*2/BOW//KAUZ*2/4/KINGBIRD #1
R 1439	SUNCO/2*PASTOR/3/SLVS/ATTILA//WBLL1/4/KA/NAC//TRCH
R 1440	CHOZI/16TH ASWSN#74
R 1441	K.YOMBI/K7892Y//K.YOMBI
R 1442	R1002/BOUNTY
R 1445	R960/R1091
R 1447	R1052/NJBW II
R 1448	R1065 /K. POPO

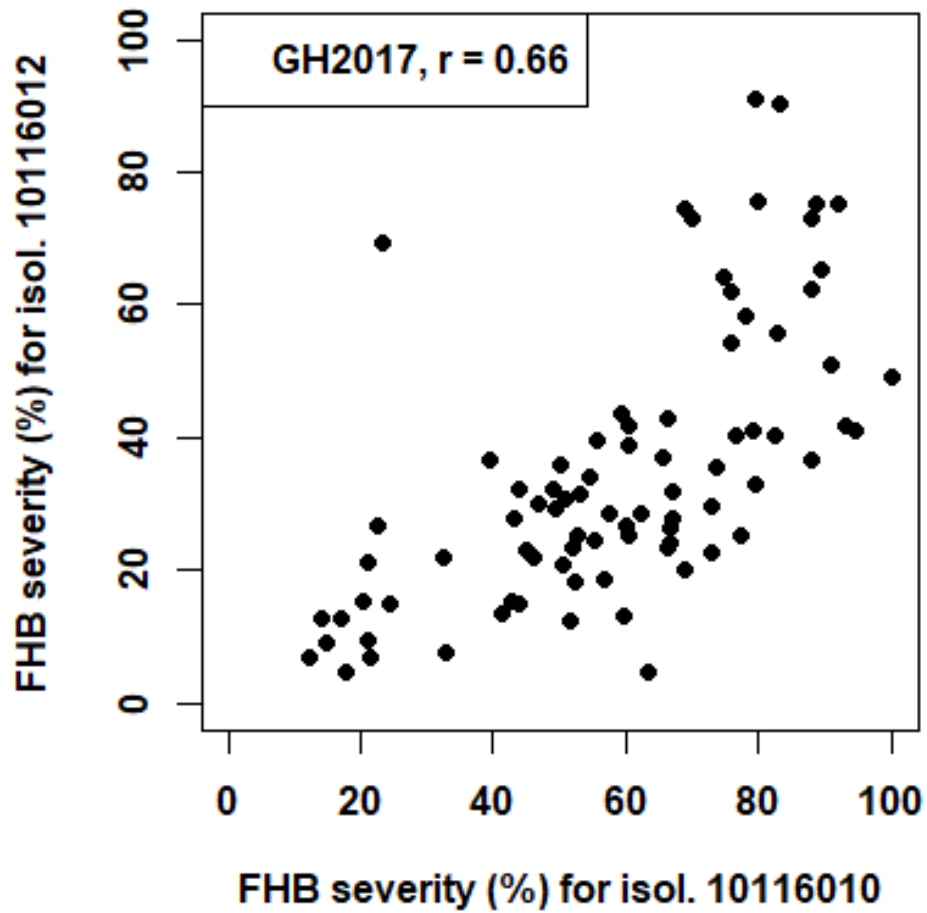
Accession Number <sup>a</sup>	Pedigree <sup>b</sup>
R 1449	R1087/NJBW II
R 1450	R1092/DH4
R 1451	NJBW II/R1096
R 1452	KWALE/K. ZABADI
R 1453	PAKA/K8665
R 1454	DH4/R1002
R 1455	KULUNGU/R1084
R 1456	KULUNGU/DH4
R 1459	KIRITAT//ATTILA*2/PASTOR/3/AKURI
R 1460	THELIN#2/TUKURU//KIRITATI
R 1462	MUU/KBIRD
R 1463	BECARD #1/4/KIRITATI/3/2*SERI.1B*2//KAUZ*3/BOW
R 1466	BECARD//ND643/2*WBLL1
R 1467	ATTILA/3*BCN*2//BAV92/3/KIRITATI/WBLL1/4/DANPHE
R 1468	ATTILA/3*BCN//BAV92/3/PASTOR/4/TACUPETO F2001*2//.....
R 1471	ND643//2*ATTILA*2/PASTOR/3/WBLL1*2/KURUKU/4/WBLL1*2//.....
Ravi 1	KACHU/KIRITATI
Ravi 2	SUPER152/BAJ #1
Ravi 3	BAJ #1/3/KIRITATI//ATTILA*2/PASTOR
Ravi 4	FRNCLN*2/TECUE #1
Ravi 5	SUPER152/AKURI//SUPER152
Ravi 6	MUTUS*2/TECUE #1
Ravi 7	BAJ #1*2/WHEAR

Accession Number <sup>a</sup>	Pedigree <sup>b</sup>
Ravi 8	WBLL1*2/BRAMBLING//CHYAK
Ravi 9	BECARD//ND643/2*WBLL1
Ravi 10	ATTILA/3*BCN*2//BAV92/3/KIRITATI/WBLL1/4/DANPHE
Ravi 11	KACHU/BECARD//WBLL1*2/BRAMBLING
Ravi 12	SUNCO.6/FRAME//PASTOR/3/PAURAQ
Ravi 13	METSO/ER2000/5/2*SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92
Ravi 14	WORRAKATTA/2*PASTOR//DANPHE #1
Ravi 15	KA/NAC//TRCH/3/DANPHE #1
Ravi 16	SNLG/3/EMB16/CBRD//CBRD/4/KA/NAC//TRCH
Ravi 17	KA/NAC//TRCH/3/VORB
Ravi 18	KA/NAC//TRCH/3/DANPHE #1
Ravi 20	ROLF07*2/5/REH/HARE//2*BCN/3/CROC-1/AE.SQUARROSA (213)//PGO/4/HUITES
Ravi 24	KIRITATI//ATTILA*2/PASTOR/3/AKURI
Ravi 25	KIRITATI//ATTILA*2/PASTOR/3/AKURI
Ravi 26	BAJ #1/3/KIRITATI//ATTILA*2/PASTOR
Ravi 27	WHEAR*2/3/FRET2/WBLL1//TACUPETO F2001
Ravi 29	MUTUS*2/MUU
Ravi 30	CROC-1/AE.SQUARROSA (205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2/5/KINDE
Ravi 33	FRNCLN/4/WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1
Ravi 34	CHIBIA//PRLII/CM65531/3/FISCAL/4/ND643/2*WBLL1
Ravi 35	PBW65/2*PASTOR/3/KIRITATI//PBW65/2*SERI.1B/4/DANPHE #1
Ravi 36	PCAFLR/KINGBIRD #1//KIRITATI/2*TRCH
Ravi 37	MUTUS/AKURI #1//MUTUS

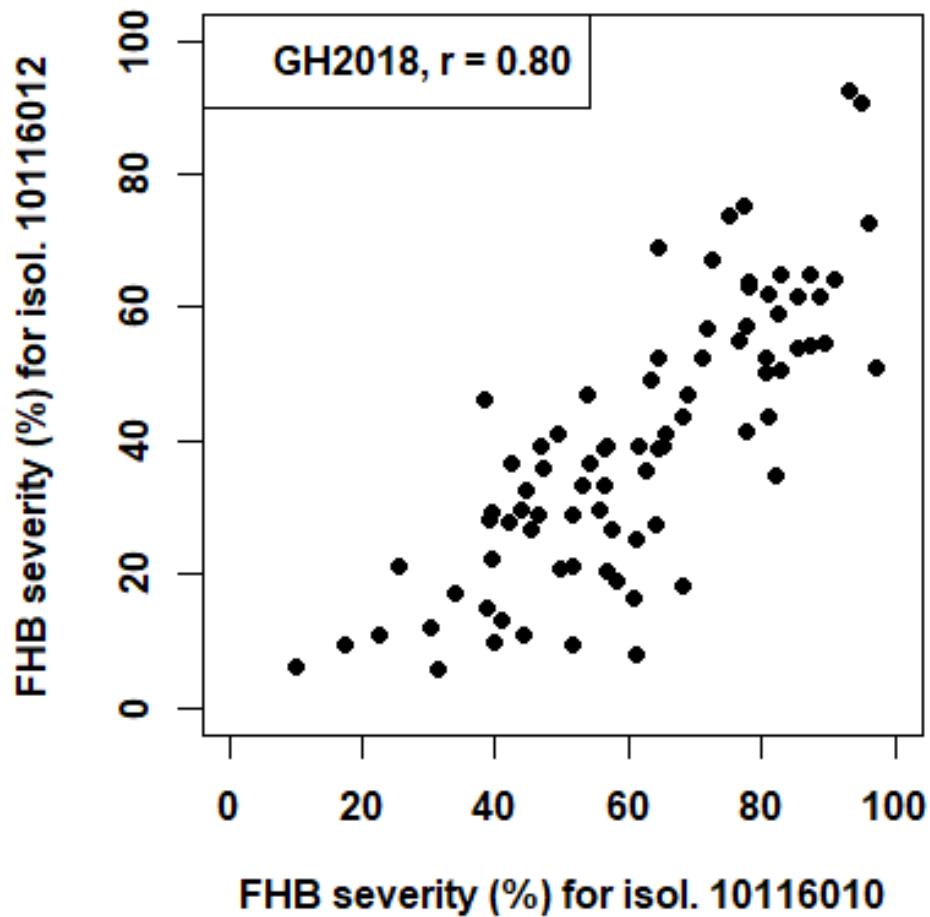
Accession Number <sup>a</sup>	Pedigree <sup>b</sup>
Ravi 39	WBLL1*2/KIRITATI//FRNCLN/3/BECARD
Ravi 40	ND643/2*WBLL1//ATTILA*2/PBW65/3/MUNAL
Ravi 41	ND643/2*WBLL1/3/KIRITATI//PRL/2*PASTOR/4/KIRITATI//PBW65/2*SERI.1B
Ravi 42	ND643/2*WBLL1//2*BAJ #1
Ravi 43	EMB16/CBRD//CBRD/4/BETTY/3/CHEN/AE.SQ//2*OPATA/5/KA/NAC//TRCH
Ravi 44	EMB16/CBRD//CBRD/4/BETTY/3/CHEN/AE.SQ//2*OPATA/5/KA/NAC//TRCH
Ravi 45	KA/NAC//TRCH/3/VORB
Ravi 46	KA/NAC//TRCH/3/DANPHE #1
Ravi 47	MILAN/KAUZ//DHARWAR DRY/3/BAV92/4/CHONTE
Ravi 48	BERKUT/MUU
Ravi 49	FRNCLN/ROLF07
Ravi 50	TRCH/HUIRIVIS #1
Ravi 51	NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR/5/T.DICOCCON PI94624/AE.SQUARROSA (409)//BCN/6/WBLL4//BABAX.1B.1B*2/PRL/3/PASTOR
Ravi 52	FRET2/TUKURU//FRET2/3/MUNAL #1
Ravi 53	KIRITATI//ATTILA*2/PASTOR/3/AKURI
Ravi 54	KAUZ*3/MNV//MILAN/3/BAV92/4/DANPHE #1
Ravi 55	KACHU/KINDE
Ravi 56	SUPER152*2/TECUE #1

<sup>a</sup>All accessions are from CIMMYT except 'Kenya Seed' lines which are from a Kenya Seed Company in Kenya.

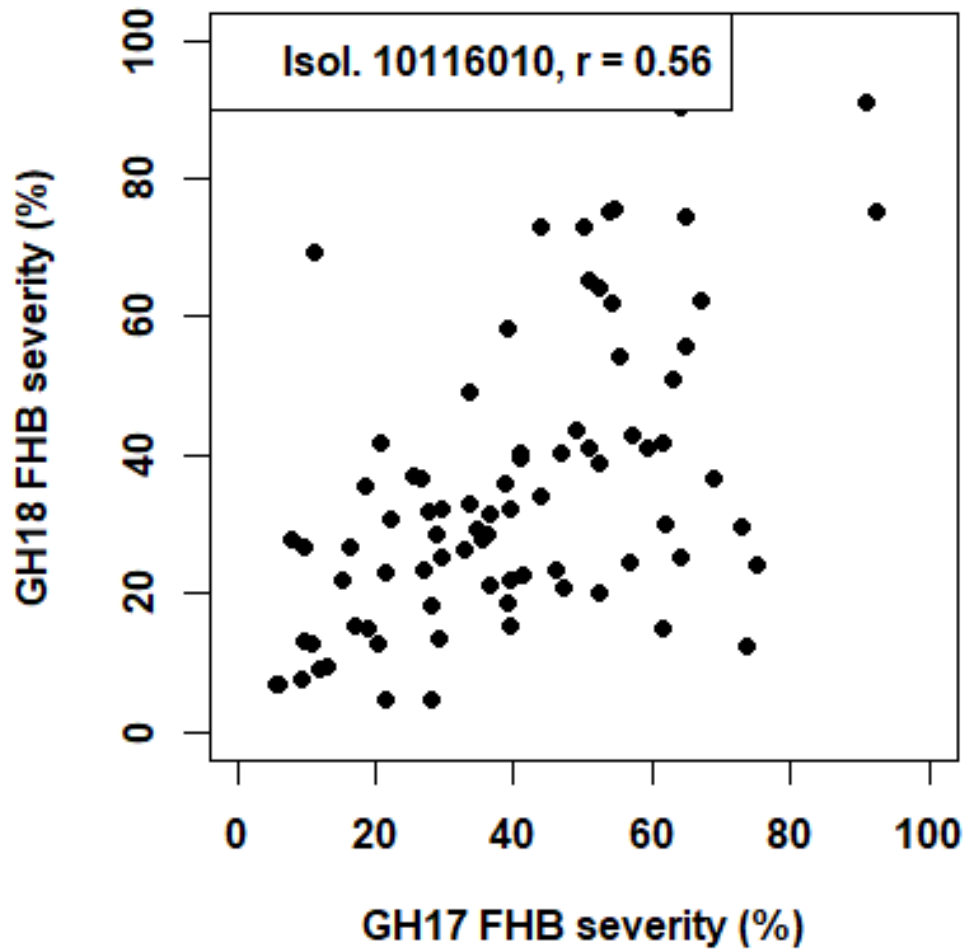
<sup>b</sup>-, denotes no pedigree information was available; b<sup>.....</sup>, denotes incomplete pedigree.



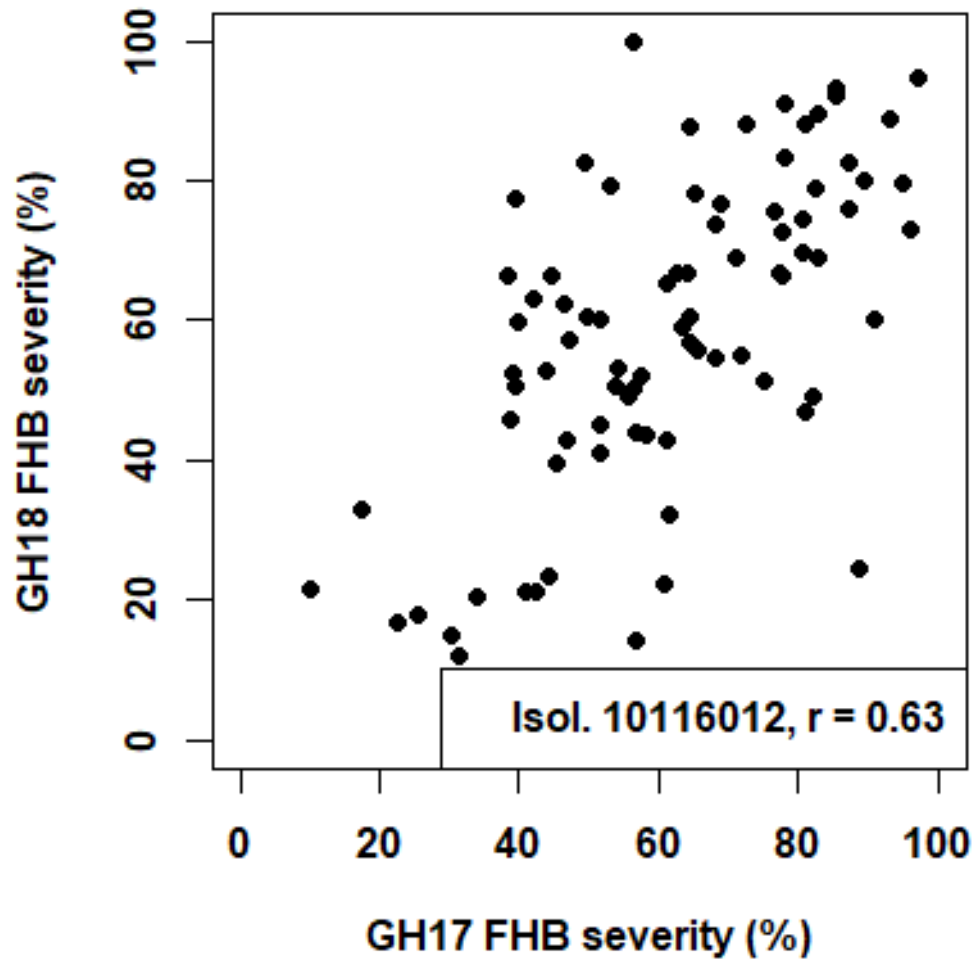
**Appendix 2.3.** Correlation between the FHB severities (%), assessed 21 days after inoculation, for 82 wheat genotypes inoculated with each of two *F. graminearum* isolates (isolate 10116010 and isolate10116012). The experiment was conducted in the greenhouse in 2017 (GH2017).



**Appendix 2.4.** Correlation between the FHB severities (%), assessed 21 days after inoculation, for 82 wheat genotypes inoculated with each of two *F. graminearum* isolates (isolate 10116010 and isolate 10116012). The experiment was conducted in the greenhouse in 2018 (GH2018).



**Appendix 2.5.** Correlation between the FHB severities (%), assessed 21 days after inoculation, for 82 wheat genotypes inoculated with *F. graminearum* isolate 10116010 in two experiments, conducted in the greenhouse in 2017 (GH17) and 2018 (GH18).



**Appendix 2.6.** Correlation between the FHB severities (%), assessed 21 days after inoculation, for 82 wheat genotypes inoculated with *F. graminearum* isolate 10116012 in two experiments, conducted in the greenhouse in 2017 (GH17) and 2018 (GH18).



**Appendix 3.1.** Conversion scale for seedling infection types from 0-4 scale to a linearized scale (0-9) by Gao *et al.* (2019)<sup>a</sup>.

Seedling Infection Types		Score (Linearized Values)		Weighted Average	Linearized and Rounded
First	Last	First	Last		
;	;	0	0	0.0	0
;	1-	0	1	0.3	0
;	1	0	2	0.7	1
;	1+	0	3	1.0	1
;	2-	0	4	1.3	1
;	2	0	5	1.7	2
;	2+	0	6	2.0	2
;	3-	0	7	2.3	2
;	3	0	8	2.7	3
;	3+	0	9	3.0	3
1-	;	1	0	0.7	1
1-	1-	1	1	1.0	1
1-	1	1	2	1.3	1
1-	1+	1	3	1.7	2
1-	2-	1	4	2.0	2
1-	2	1	5	2.3	2
1-	2+	1	6	2.7	3
1-	3-	1	7	3.0	3
1-	3	1	8	3.3	3
1-	3+	1	9	3.7	4
1	;	2	0	1.3	1
1	1-	2	1	1.7	2
1	1	2	2	2.0	2
1	1+	2	3	2.3	2
1	2-	2	4	2.7	3
1	2	2	5	3.0	3

Seedling Infection Types		Score (Linearized Values)		Weighted Average	Linearized and Rounded
First	Last	First	Last		
1	2+	2	6	3.3	3
1	3-	2	7	3.7	4
1	3	2	8	4.0	4
1	3+	2	9	4.3	4
1+	;	3	0	2.0	2
1+	1-	3	1	2.3	2
1+	1	3	2	2.7	3
1+	1+	3	3	3.0	3
1+	2-	3	4	3.3	3
1+	2	3	5	3.7	4
1+	2+	3	6	4.0	4
1+	3-	3	7	4.3	4
1+	3	3	8	4.7	5
1+	3+	3	9	5.0	5
2-	;	4	0	2.7	3
2-	1-	4	1	3.0	3
2-	1	4	2	3.3	3
2-	1+	4	3	3.7	4
2-	2-	4	4	4.0	4
2-	2	4	5	4.3	4
2-	2+	4	6	4.7	5
2-	3-	4	7	5.0	5
2-	3	4	8	5.3	5
2-	3+	4	9	5.7	6
2	;	5	0	3.3	3
2	1-	5	1	3.7	4
2	1	5	2	4.0	4

Seedling Infection Types		Score (Linearized Values)		Weighted Average	Linearized and Rounded
First	Last	First	Last		
2	1+	5	3	4.3	4
2	2-	5	4	4.7	5
2	2	5	5	5.0	5
2	2+	5	6	5.3	5
2	3-	5	7	5.7	6
2	3	5	8	6.0	6
2	3+	5	9	6.3	6
2+	;	6	0	4.0	4
2+	1-	6	1	4.3	4
2+	1	6	2	4.7	5
2+	1+	6	3	5.0	5
2+	2-	6	4	5.3	5
2+	2	6	5	5.7	6
2+	2+	6	6	6.0	6
2+	3-	6	7	6.3	6
2+	3	6	8	6.7	7
2+	3+	6	9	7.0	7
3-	;	7	0	4.7	5
3-	1-	7	1	5.0	5
3-	1	7	2	5.3	5
3-	1+	7	3	5.7	6
3-	2-	7	4	6.0	6
3-	2	7	5	6.3	6
3-	2+	7	6	6.7	7
3-	3-	7	7	7.0	7
3-	3	7	8	7.3	7
3-	3+	7	9	7.7	8
3	;	8	0	5.3	5

Seedling Infection Types		Score (Linearized Values)		Weighted Average	Linearized and Rounded
First	Last	First	Last		
3	1-	8	1	5.7	6
3	1	8	2	6.0	6
3	1+	8	3	6.3	6
3	2-	8	4	6.7	7
3	2	8	5	7.0	7
3	2+	8	6	7.3	7
3	3-	8	7	7.7	8
3	3	8	8	8.0	8
3	3+	8	9	8.3	8
3+	;	9	0	6.0	6
3+	1-	9	1	6.3	6
3+	1	9	2	6.7	7
3+	1+	9	3	7.0	7
3+	2-	9	4	7.3	7
3+	2	9	5	7.7	8
3+	2+	9	6	8.0	8
3+	3-	9	7	8.3	8
3+	3	9	8	8.7	9
3+	3+	9	9	9.0	9

<sup>a</sup>Infection types were score based on the 0-4 scale developed by Stakman *et al.* (1962).

The plus (+) and minus (-) symbols were used for the pustules that were relatively larger or smaller, respectively, than normal. The seedling infection types based on a 0 to 4 scale were then converted to a 0 to 9 linear scale.

**Appendix 3.2.** Linkage map of LMPG-6/CI 14275 cross constructed using 90K Infinium iSelect assay. The positions of significant QTLs at seedling stage against races RTQQC, TPMKC and TTTTF and at adult plant stage in Njoro, Kenya (KEN16, KEN17, KEN18); Debre-Zeit, Ethiopia (ETH16, ETH18) and St. Paul, MN (STP17, STP18) are shaded.

Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
BS00064204_51	1AS	0	
RAC875_rep_c110533_92	1AS	5.3402292	
Excalibur_c58555_110	1AS	8.5620722	
Tdurum_contig44888_837	1AS	11.696607	
IAAV3919	1AS	15.530188	
BS00056550_51	1AS	20.598651	
BS00105601_51	1AS	0	
CAP7_c4833_55	1AS	4.0960952	
GENE-0507_285	1AS	12.607313	
BS00029346_51	1AS	18.961458	
IAAV5931	1AS	26.344772	
BS00026917_51	1AS	30.856777	
Excalibur_c35312_109	1AS	33.904022	
wsnp_Ex_c24686_33942264	1AS	37.650146	
BS00048117_51	1AS	41.833796	
RAC875_rep_c76047_63	1AS	45.929891	
IAAV1463	1AS	50.025986	
wsnp_Ex_c11374_18361760	1AS	54.122081	
BS00076668_51	1AS	58.218177	
BS00080438_51	1AS	62.314272	
BobWhite_c18773_75	1AS	66.410367	
wsnp_Ex_c18662_27538313	1AS	70.418933	
BS00048118_51	1AS	74.690162	
Kukri_c18608_729	1AL	0	
wsnp_JD_c12333_12595897	1AL	4.3588347	
wsnp_Ex_rep_c72011_70562321	1AL	8.6300636	
CAP11_c5573_163	1AL	13.802807	
wsnp_Ex_c1255_2411550	1AL	19.579145	
IAAV3217	1AL	24.288675	
Excalibur_c46365_442	1AL	28.735142	

Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
GENE-0543_577	1AL	33.006371	
Excalibur_c46365_304	1AL	38.418717	
IAAV8446	1AL	42.514812	
wsnp_Ku_c330_683203	1AL	46.698461	
BS00070951_51	1AL	50.88211	
BS00022514_51	1AL	54.890677	
BobWhite_c1027_1127	1AL	59.337144	
Kukri_rep_c69829_735	1AL	63.871271	
CAP12_rep_c5332_341	1AL	71.34648	
BS00056823_51	1AL	75.968294	
CAP11_c1500_171	1AL	83.373315	
Kukri_c310_1953	1AL	88.981698	
wsnp_Ex_c271_521429	1AL	94.251018	
BS00106641_51	1AL	100.75087	
Tdurum_contig51167_534	1AL	112.8743	
Excalibur_c987_197	1AL	117.58383	
Tdurum_contig78830_348	1AL	137.95449	
BS00056547_51	1AL	142.66402	
BS00072408_51	1AL	147.762	
BS00066659_51	1AL	152.55928	
wsnp_BF474340A_Ta_2_1	1AL	158.24751	
BobWhite_c5242_400	1AL	162.25608	
Tdurum_contig11106_264	1AL	171.95624	
Ku_c4369_351	1AL	178.37035	
Ra_c5683_1762	1AL	183.79448	
Ra_c41164_730	1AL	187.71554	
wsnp_Ex_c1359_2604298	1AL	202.16543	
BS00078085_51	1AL	210.78666	
BS00095510_51	1AL	214.70772	
BS00109991_51	1AL	225.82289	
GENE-0262_431	1AL	229.13206	
wsnp_BG606986A_Ta_2_1	1AL	233.78548	
BS00022239_51	1AL	237.5316	
BS00081002_51	1AL	240.84077	
BS00012042_51	1AL	244.78172	

Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
w SNP_CAP7_c3472_1623955	1AL	248.1981	
Kukri_c45512_193	1AL	252.20666	
BS00028146_51	1AL	257.19128	
CAP7_c4879_249	1AL	260.41312	
BobWhite_c26122_129	1AL	263.54765	
w SNP_Ku_c33917_43336069	1AL	268.03396	
CAP7_c11581_78	1AL	274.81033	
BobWhite_c96_170	1AL	279.44389	
BobWhite_c27438_81	1AL	285.49908	
CAP7_c1891_230	1AL	288.45906	
w SNP_Ku_c5210_9289260	1AL	291.6809	
BS00039378_51	1AL	294.55362	
w SNP_BE517729A_Ta_2_1	1AL	297.60087	
Excalibur_c49946_169	1AL	301.25956	
Excalibur_c29605_100	1AL	306.80748	
Excalibur_c29605_535	1AL	311.61651	
BobWhite_c12977_65	1AL	315.2752	
IAAV2694	1AL	320.08423	
Excalibur_c44668_382	1AL	330.20792	
IAAV6234	1AL	334.67018	
BobWhite_c46007_582	1AL	340.70813	
Excalibur_c3475_903	1AL	344.90351	
BS00030036_51	1AL	349.34997	
BS00021728_51	1AL	355.57147	
BS00022916_51	1BS	0	
Excalibur_c12710_740	1BS	5.3021914	
BS00067507_51	1BS	12.309244	
Tdurum_contig82187_189	1BS	15.80233	
Excalibur_c95656_129	1BS	21.655001	
RFL_Contig2160_617	1BS	26.735657	
BS00076889_51	1BS	0	
BS00108057_51	1BS	10.62639	
Excalibur_c37693_203	1BS	15.687078	
IAAV3430	1BS	22.545506	
BS00003917_51	1BS	25.418228	

Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
BS00068429_51	1BS	28.913819	
BS00110900_51	1BS	32.22299	
BS00110896_51	1BS	35.444833	
Tdurum_contig62624_190	1BS	38.492079	
BS00032266_51	1BS	41.576378	
IAAV392	1BS	44.885549	
Ku_c36151_691	1BS	48.64743	
wsnp_Ex_c1085_2078686	1BS	60.447618	
wsnp_Ex_c58292_59652859	1BL	0	
IACX4461	1BL	3.9210618	
BS00021975_51	1BL	7.317581	
BobWhite_c35484_282	1BL	11.063705	
Ra_c698_1987	1BL	16.643691	
Excalibur_c8613_1266	1BL	19.881268	
BS00038643_51	1BL	22.492334	
BS00048610_51	1BL	26.25017	
Excalibur_c21451_352	1BL	31.226876	
Tdurum_contig31102_299	1BL	37.632815	
wsnp_Ex_c1495_2864718	1BL	42.621493	
CAP12_c424_402	1BL	47.873786	
wsnp_CAP11_c6264_2895652	1BL	52.691007	
wsnp_BE495786B_Ta_2_2	1BL	57.855397	
Tdurum_contig60509_232	1BL	61.883854	
BS00022878_51	1BL	65.212875	
BS00041355_51	1BL	70.170422	
BS00042197_51	1D	0	
D_F5XZDLF01A85DT_301	1D	3.65869	
RAC875_c27954_378	1D	9.1708192	
BS00104199_51	1D	16.334719	
CAP12_c46_333	1D	20.693553	
BS00023049_51	1D	25.052388	
CAP11_rep_c6465_98	1D	29.411223	
Ex_c6145_833	1D	34.764108	
RAC875_c7752_1223	1D	39.473638	
RAC875_c7752_145	1D	44.007765	



Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
CAP8_c2401_433	1D	49.180509	
BobWhite_c14032_277	1D	62.516267	
BobWhite_c1715_887	1D	69.528977	
Kukri_c837_436	1D	74.063104	
w SNP_Ex_c1085_2078944	1D	78.509571	
w SNP_Ex_c1358_2600929	1D	82.956038	
BobWhite_rep_c49356_237	1D	87.314873	
w SNP_Ex_c1358_2602235	1D	91.76134	
w SNP_Ra_c2633_5017265	1D	96.470871	
GENE-1792_762	2AL	0	
Excalibur_rep_c69014_112	2AL	4.7972752	
Excalibur_c3454_1674	2AL	16.414306	
BobWhite_c3833_815	2AL	20.172142	
Kukri_c29170_680	2AL	23.934022	
w SNP_Ex_c7829_13320760	2AL	26.981268	
BobWhite_c15867_215	2AL	30.906167	
GENE-1381_132	2AL	34.564857	
BobWhite_c17403_635	2AL	38.756255	
BS00089497_51	2AL	42.152775	
BS00035883_51	2AL	45.20002	
BS00022666_51	2AL	48.247265	
BS00039422_51	2AL	51.294511	
IAAV1698	2AL	54.341756	
Excalibur_c40617_983	2AL	58.708345	
BS00105171_51	2AL	69.137961	
BobWhite_c11081_1619	2AL	73.059023	
Ku_c9320_1234	2AL	79.015757	
w SNP_Ex_c10751_17505459	2AL	85.16166	
BS00110386_51	2AL	91.479879	
BobWhite_c16923_64	2AL	96.376686	
RAC875_c19904_308	2AL	111.51985	
BobWhite_c25764_348	2AL	119.11259	
BS00022813_51	2AL	123.03365	
BS00077768_51	2AL	127.66322	
BS00077769_51	2AL	130.97239	

Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
BS00022446_51	2AL	138.06052	
BS00071660_51	2AL	141.36969	
BobWhite_c39769_102	2B.3	0	
BS00074091_51	2B.1	10.814297	
BS00080318_51	2B.3	15.260764	
BS00076003_51	2B.3	23.569612	
BS00010988_51	2B.3	30.100574	
GENE-0862_110	2B.1	33.934155	
CAP8_c3234_216	2B.1	37.592845	
BS00043338_51	2B.3	41.700665	
BS00009060_51	2B.3	45.534246	
BobWhite_c7050_792	2B.1	50.156061	
RAC875_c35399_497	2B.3	54.42729	
BobWhite_c9690_94	2B.1	62.422846	
Tdurum_contig28795_219	2B.3	66.518942	
Excalibur_rep_c78626_295	2B.1	76.447582	
BS00036456_51	2B.1	82.022664	
JD_c9115_954	2B.1	86.995518	
JD_c3211_962	2B.1	93.659054	
CAP7_c12727_215	2B.1	98.643669	
BobWhite_c2521_117	2B.3	102.82732	
Excalibur_c47745_63	2B.1	106.57344	
BS00021682_51	2B.3	110.58201	
BS00046164_51	2B.1	114.50307	
RFL_Contig2577_584	2B.1	118.33665	
w SNP_Ex_c22271_31463467	2B.1	122.60788	
BobWhite_c2244_259	2B.1	126.44146	
Ku_c12447_2002	2B.3	130.27504	
BobWhite_c4831_490	2B.1	134.28361	
BS00046165_51	2B.3	137.85489	
Ex_c16948_754	2B.3	141.25141	
Excalibur_c65341_303	2B.1	144.64793	
w SNP_Ku_c9901_16493072	2B.3	148.04445	
IAAV2917	2B.3	151.44096	
Excalibur_c7449_587	2B.3	155.21116	

Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
Tdurum_contig96648_102	2B.3	158.433	
Excalibur_c76598_570	2B.1	164.43398	
BS00104667_51	2B.1	168.26756	
tplb0049b10_696	2B.3	173.62364	
BobWhite_c5543_492	2B.3	178.2572	
Tdurum_contig59522_262	2B.3	182.44085	
BS00011545_51	2B.3	186.27443	
BobWhite_c22106_204	2B.3	189.93312	
BS00011579_51	2B.1	194.46725	
BobWhite_c23054_1464	2B.1	198.6509	
BobWhite_c23046_293	2B.3	202.74699	
Kukri_c4294_371	2B.3	208.35538	
wsnp_Ex_c17845_26604587	2B.3	212.1015	
GENE-0910_153	2B.1	217.01071	
Excalibur_c73922_55	2B.3	222.26715	
CAP7_rep_c5636_213	2B.1	226.80127	
BS00012036_51	2B.3	231.71049	
GENE-0675_161	2B.3	235.89413	
Excalibur_c99477_90	2B.3	253.82973	
wsnp_BG608232B_Ta_2_2	2B.3	258.98606	
BobWhite_c40428_349	2B.3	262.90712	
BobWhite_c14686_86	2B.3	266.82818	
BobWhite_c31523_130	2B.3	269.96272	
wsnp_be499362B_Td_2_1	2B.3	272.57378	
wsnp_Ex_c25438_34703568	2B.1	276.08599	
BS00066545_51	2B.3	279.48251	
Kukri_c20819_497	2B.3	283.05379	
BobWhite_c12732_205	2B.3	287.93884	
wsnp_Ex_c28243_37383894	2B.3	292.91169	
wsnp_JD_c9251_10121113	2B.3	296.48297	
IAAV3255	2B.3	299.87949	
wsnp_Ex_rep_c104478_89183627	2B.3	303.27601	
Ex_c66545_1078	2B.3	307.37985	
wsnp_Ex_rep_c66545_64828225	2B.3	311.12597	
Kukri_c42289_582	2B.3	314.95956	

Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
wsnp_JD_c3314_4323825	2B.3	318.79314	
BobWhite_c3693_85	2B.3	322.53926	
wsnp_CAP11_c307_255609	2B.3	326.54783	
Kukri_c2507_962	2B.3	330.55639	
RAC875_c60230_122	2B.3	334.56496	
Kukri_c2507_382	2B.3	338.57353	
IAAV3295	2B.3	342.58209	
BobWhite_c13066_776	2B.1	346.15337	
Kukri_c2507_1183	2B.3	349.81206	
wsnp_Ex_c9805_16183499	2B.3	353.38334	
Excalibur_c3839_2483	2B.3	357.04203	
BobWhite_c3757_55	2B.3	361.32904	
wsnp_Ex_c36002_44045355	2B.3	364.90032	
Excalibur_rep_c69016_57	2B.3	368.99641	
Excalibur_c4984_162	2B.3	373.18006	
wsnp_Ex_c7947_13490279	2B.3	376.57658	
wsnp_Ex_c30037_39004913	2B.3	379.9731	
BS00062485_51	2B.3	383.28227	
Excalibur_c65830_82	2B.2	386.85355	
BobWhite_c10453_396	2B.3	390.07539	
wsnp_BE488220B_Ta_1_1	2B.3	393.20993	
BobWhite_c13455_112	2B.3	396.25717	
wsnp_Ex_c1541_2943791	2B.3	398.86824	
BS00076369_51	2B.3	401.82821	
Excalibur_c85610_72	2B.2	404.70094	
IAAV3148	2B.3	407.66091	
Excalibur_c58566_284	2B.1	411.95205	
BobWhite_c5852_103	2B.2	415.43594	
Tdurum_contig20589_247	2B.1	419.26952	
RAC875_c63112_460	2B.2	422.31676	
wsnp_BE445278B_Ta_2_1	2B.2	427.42494	
wsnp_CAP8_rep_c6230_2943068	2B.1	430.73411	
wsnp_BE445278B_Ta_2_3	2B.2	434.04329	
Excalibur_c44495_105	2B.1	440.74579	
Ku_c4777_2494	2B.2	444.31706	

Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
w SNP_ Ex_ rep_ c106085_90293854	2B.2	448.23813	
w SNP_ Ku_ c12517_20191465	2B.1	452.15919	
w SNP_ BF291736B_ Ta_ 1_ 1	2B.2	455.73047	
BobWhite_ c948_ 52	2B.2	459.30174	
RAC875_ c19210_ 348	2B.2	462.87302	
RAC875_ c97988_ 87	2B.1	466.7066	
w SNP_ Ex_ c22018_31193171	2B.2	472.13452	
GENE-1389_396	2B.1	477.04802	
Excalibur_ c9248_ 771	2B.2	481.05659	
BS00092275_51	2B.2	484.89017	
BS00099361_51	2B.1	491.12192	
Excalibur_ c60347_ 56	2B.2	495.83145	
RFL_ Contig1139_ 817	2B.2	501.46175	
IAAV4434	2B.2	504.94564	
Tdurum_ contig18197_ 86	2B.2	507.12092	
Tdurum_ contig92963_ 204	2B.2	509.81919	
w SNP_ Ku_ c13905_22034406	2B.2	513.47788	
Kukri_ c9387_ 112	2B.2	518.28292	
Ra_ c2110_ 1660	2B.2	521.2429	
BS00022417_51	2B.2	523.94117	
GENE-4359_102	2B.2	527.68729	
w SNP_ BF202681B_ Ta_ 2_ 2	2B.2	531.08381	
GENE-3867_1133	2B.1	534.39298	
BS00059315_51	2B.2	539.11832	
Kukri_ c13884_ 74	2B.2	543.93141	
BS00083078_51	2B.2	549.37546	
Ku_ c4042_ 576	2B.2	554.53179	
GENE-0767_ 62	2B.1	558.36537	
Kukri_ c2387_ 2011	2B.2	562.19895	
w SNP_ Ku_ c4042_ 7375053	2B.2	565.94508	
Ku_ c4042_ 1178	2B.2	569.6912	
RAC875_ c22552_ 380	2B.2	574.50429	
w SNP_ RFL_ Contig3060_ 2967139	2B.1	578.60038	
RAC875_ c35438_ 474	2B.2	582.69648	
BS00071690_51	2B.2	587.31829	

Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
Tdurum_contig68806_321	2B.2	592.03167	
RFL_Contig2231_1297	2B.2	594.81716	
Tdurum_contig94176_65	2B.2	602.93533	
Tdurum_contig5056_173	2B.2	606.79299	
Ku_c21663_1390	2B.1	609.66571	
Tdurum_contig74936_387	2B.2	614.31152	
Tdurum_contig74936_264	2B.2	617.35876	
Excalibur_c53027_302	2B.1	620.66794	
Tdurum_contig61293_131	2B.2	624.589	
Jagger_c8098_88	2B.2	628.33512	
BobWhite_c11739_325	2B.2	632.75947	
Excalibur_c6502_397	2B.1	636.19308	
BS00038820_51	2B.1	642.52714	
Tdurum_contig54704_176	2B.2	648.52956	
GENE-0592_352	2B.1	665.5764	<i>Qsr.cdl-2BS.2</i>
BS00076982_51	2B.1	672.31005	
Tdurum_contig30210_226	2B.1	680.3166	
BS00110814_51	2BL	0	
BS00083998_51	2BL	4.7253381	
BS00072839_51	2BL	8.9207153	
Jagger_c2989_134	2BL	13.104365	
Excalibur_c48871_625	2BL	17.025426	
Kukri_c365_93	2BL	20.859008	
Excalibur_c7051_1027	2BL	32.119559	
Excalibur_c7051_1115	2BL	35.865683	
Excalibur_c16679_215	2BL	40.224518	
BS00070301_51	2BL	43.883208	
wsnp_Ex_c10441_17078853	2BL	51.774162	
wsnp_Ex_c10193_16730348	3B	0	
wsnp_Ex_c45195_51056617	3B	6.2716125	
RAC875_c27548_417	3B	14.821473	
Tdurum_contig41998_1213	3B	19.451049	
CAP7_rep_c5216_143	3B	23.985176	
wsnp_Ex_c10550_17231294	3B	28.519303	
wsnp_Ex_c10550_17231658	3B	32.96577	

Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
BS00089942_51	3B	38.042288	
GENE-4796_696	3B	44.523696	
Tdurum_contig3914_153	3B	55.868608	
Excalibur_c6738_2072	3B	60.315076	
BobWhite_c3541_152	3B	65.991512	
Kukri_c10108_115	3B	73.359293	
w SNP_BQ171683B_Ta_2_1	3B	85.679729	
RAC875_c8890_148	3B	91.830654	
RAC875_c8890_244	3B	95.576778	
Excalibur_c5700_527	3B	105.30326	
Excalibur_c28715_447	3B	109.13684	
Tdurum_contig16275_277	3B	115.60751	
RAC875_c9790_116	3B	138.39822	
BS00027346_51	3B	146.5639	
Tdurum_contig11297_571	3B	151.62459	
Ra_c10565_1109	3B	157.44624	
w SNP_Ex_c1558_2976128	3B	160.84276	
IACX6214	3B	164.85133	
BS00009393_51	3B	179.01151	
BS00033209_51	3B	182.49539	
BS00111294_51	3B	186.85423	
BS00025838_51	3B	200.61516	
Kukri_c17082_519	3B	216.35678	
Kukri_c17082_378	3B	218.27076	
RAC875_c13385_1268	3B	220.53317	
BS00001335_51	3B	231.40396	
Tdurum_contig12899_347	3B	238.61714	
BobWhite_c2453_282	3B	242.07898	
BobWhite_c54480_387	3B	243.20961	
BS00058654_51	3B	248.27537	
BS00036089_51	3B	255.35333	
Kukri_rep_c107494_369	3B	274.35877	
BS00025792_51	3B	277.69624	
Excalibur_c24391_321	3B	280.74349	
Excalibur_c35645_587	3B	288.86143	

Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
Jagger_c4901_95	3B	292.79825	
BS00076248_51	3B	303.26687	
CAP8_c1799_237	3B	308.28807	
wsnp_CAP11_c323_263628	3B	312.12165	
RAC875_c20041_976	3B	318.60415	
GENE-1900_115	3B	322.52522	
GENE-4064_599	3B	326.3588	
BobWhite_rep_c63085_120	3B	333.16803	
Excalibur_c11246_659	3B	337.43926	
BS00022741_51	3B	341.71049	
BS00063036_51	3B	346.15696	
Ra_c965_2702	3B	353.55796	
Tdurum_contig43263_246	3B	364.12122	
Kukri_c3305_2048	3B	368.75479	
wsnp_JD_c9902_10674725	3B	372.76335	
wsnp_JD_c9902_10674626	3B	376.85945	
RFL_Contig738_557	3B	382.37979	
BS00086717_51	3B	387.08932	
BS00023074_51	3B	391.53579	
CAP8_c490_173	3B	399.88404	
Tdurum_contig28033_420	3B	404.59357	
BS00030581_51	3B	412.53348	
BS00066466_51	3B	417.71051	
GENE-1805_135	3B	422.79538	
BS00032695_51	3B	427.59266	
Excalibur_c5298_171	3B	432.30219	
Excalibur_c57658_54	3B	437.82675	
IAAV3838	3B	441.92285	
Ku_c47648_1403	3B	446.61914	
RAC875_c10595_473	3B	451.0656	
wsnp_Ra_c69_149518	3B	460.79528	
RAC875_c69_499	3B	463.84252	
RAC875_c69_392	3B	468.28899	
Tdurum_contig32277_121	3B	473.52544	
BS00057988_51	3B	479.07233	

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Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
BS00099738_51	3B	482.64361	
Tdurum_contig59953_282	3B	491.30768	
RFL_Contig4186_852	3B	494.87896	
wsnp_Ra_c10710_17570054	3B	499.34535	
BS00024499_51	3B	502.74187	
Ex_c303_3825	3BL	0	
Ra_c2553_1880	3BL	4.0085663	
BS00073411_51	3BL	9.4956231	
BS00091257_51	3BL	14.246933	
BS00079029_51	3BL	19.206531	
Excalibur_c45326_479	3BL	25.043837	
Excalibur_c766_705	3BL	28.98898	
RAC875_c66953_100	3BL	33.188408	
BS00064876_51	3BL	36.759686	
Kukri_c7860_911	3BL	40.593267	
BS00044955_51	3BL	44.077155	
BS00044942_51	3BL	47.998217	
BS00044944_51	3BL	52.269446	
BS00057746_51	3BL	56.540675	
Kukri_rep_c103205_101	3BL	60.199365	
BS00057343_51	3BL	63.421208	
Excalibur_c55096_613	3BL	67.955335	
RAC875_c13406_329	3BL	73.387676	
wsnp_Ex_c284_548711	3BL	78.94507	
Excalibur_c63009_102	3BL	82.428958	
BobWhite_c37848_381	3BL	86.645345	
Tdurum_contig9738_170	3BL	94.654908	
Tdurum_contig63460_144	3BL	98.401032	
CAP8_c1657_183	3BL	103.7454	
wsnp_Ku_c2249_4335279	3DL	0	
Excalibur_c42667_427	3DL	3.3965192	
Excalibur_c17654_1090	3DL	6.182006	
D_GDEEGVY01CO81T_81	3DL	9.403849	
BS00105800_51	3DL	12.014916	
RFL_Contig148_359	3DL	14.97489	

Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
RFL_Contig3495_261	3DL	18.721015	
wsnp_Ex_rep_c66706_65037564	4A	10.780432	
wsnp_Ex_rep_c66600_64897324	4A	13.740407	
IAAV7376	4A	25.344166	
wsnp_BE398523A_Ta_2_1	4A	30.596459	
tplb0035b22_184	4A	37.76315	
IAAV3906	4A	42.297277	
GENE-2491_309	4A	46.130858	
IAAV8784	4A	50.402087	
GENE-2354_155	4A	54.864347	
IAAV5596	4A	57.301057	
wsnp_Ex_c2617_4864441	4A	61.440517	
wsnp_JD_c19109_17348071	4A	62.484147	
Excalibur_c10390_104	4A	72.252714	
Tdurum_contig33628_129	4A	89.710932	
Tdurum_contig11919_360	4A	94.420462	
Ku_c766_2292	4A	98.166586	
wsnp_Ku_rep_c102527_89481571	4A	103.66693	
BS00101512_51	4AL	0	
Excalibur_c7034_234	4AL	3.65869	
CAP8_c1180_342	4AL	11.09292	
BobWhite_c22176_230	4AL	20.309789	
BS00059503_51	4AL	26.487275	
wsnp_Ex_rep_c106527_90571247	4AL	31.295213	
Excalibur_c74397_238	4AL	38.667577	
Excalibur_c5624_845	4AL	42.72748	
wsnp_Ex_c2288_4293430	4AL	45.554505	
RAC875_c56535_256	4AL	49.125783	
Ku_c61_917	4AL	0	
Ku_c61_424	4AL	6.2214919	
wsnp_Ex_rep_c68677_67531081	4AL	7.0198363	
Tdurum_contig42019_1714	4AL	8.3244801	<i>Qsr.cdl-4AL.1</i>
BS00009680_51	4AL	12.245542	
BS00110281_51	4AL	15.81682	
Jagger_c8310_70	4AL	19.737882	

Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
w SNP_Ex_c17294_25964947	4AL	22.785127	
Excalibur_c43822_370	4AL	26.813584	
Tdurum_contig82236_117	4AL	31.347711	
Excalibur_c41533_321	4AL	36.320565	
Tdurum_contig63050_181	4AL	40.154146	
w SNP_Ex_c8976_14964359	4AL	43.812836	
Tdurum_contig31218_208	4AL	48.454584	
Tdurum_contig71513_242	4AL	51.501829	
Tdurum_contig47476_441	4AL	55.617821	
RAC875_c82470_174	4AL	58.926992	
Excalibur_c33995_302	4AL	64.535375	
Ra_c6672_1679	4AL	69.94772	
w SNP_Ex_c16369_24860698	4AL	77.571474	
BS00070687_51	4AL	91.792751	
BS00039811_51	4AL	97.137122	
Tdurum_contig47783_895	4AL	101.84665	
BobWhite_c11327_248	4AL	106.20549	
Tdurum_contig10482_110	4AL	112.03592	
Ra_c60252_743	4AL	121.31137	
w SNP_BG313770B_Ta_1_1	4AL	138.16444	
BobWhite_c17731_56	4AL	148.4694	
Kukri_c17417_134	4AL	155.66894	
Kukri_c2706_1424	4AL	0	
TA005380-0966	4AL	5.5320601	
Kukri_c67793_237	4AL	9.509835	
BS00079389_51	4AL	12.731678	
w SNP_Ex_c21165_30292808	4AL	17.122089	
Ku_c12469_983	5AL	0	
tplb0044j06_689	5AL	4.0085663	
Ku_c12469_837	5AL	8.4619758	
Tdurum_contig50175_875	5AL	14.409118	
Excalibur_c41710_417	5AL	19.397516	
BobWhite_c21949_150	5AL	26.054036	
IAAV1650	5AL	29.014011	
w SNP_AJ612027A_Ta_2_1	5AL	33.488883	

Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
IAAV3043	5AL	37.235007	
GENE-3601_145	5AL	41.905102	
Tdurum_contig86202_145	5AL	44.516169	
BS00022311_51	5AL	48.087447	
BS00044408_51	5AL	51.30929	
BS00065481_51	5AL	54.705809	
Excalibur_c61241_109	5AL	0	
BS00076948_51	5AL	3.483888	
BS00090847_51	5AL	6.8804072	
BS00063793_51	5AL	18.054785	
BS00055102_51	5AL	20.404339	
BS00068435_51	5AL	24.603766	
BobWhite_rep_c63332_67	5AL	32.668143	
Ku_c19516_384	5AL	43.528987	
BobWhite_c8266_227	5AL	62.250827	
Tdurum_contig8348_831	5AL	68.5367	
GENE-2673_1010	5AL	76.782091	
BS00076190_51	5AL	0	
BS00109052_51	5AL	5.2482269	
BS00040916_51	5AL	10.065448	
BS00073404_51	5AL	13.287291	
BS00067453_51	5AL	16.945981	
BS00076221_51	5AL	20.954548	
BS00077533_51	5AL	24.875609	
BobWhite_c10901_578	5AL	31.563431	
Excalibur_rep_c74009_217	5AL	35.397012	
BS00069980_51	5AL	39.230593	
RAC875_rep_c110032_317	5AL	43.151655	
BS00097930_51	5AL	46.897779	
GENE-3440_199	5AL	50.731361	
BS00027465_51	5AL	54.12788	
BS00027466_51	5AL	57.78657	
BS00022509_51	5AL	61.183089	
BS00065386_51	5AL	64.666977	
BS00107192_51	5AL	72.662534	

Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
Excalibur_c49664_409	5BL	0	
CAP11_c506_72	5BL	10.468612	
BS00022520_51	5BL	16.256752	
BobWhite_c6633_288	5BL	20.830734	
Excalibur_c36377_615	5BL	25.14178	
BobWhite_c29279_307	5BL	29.724172	
Tdurum_contig31075_260	5BL	46.10574	
BS00020982_51	5BL	49.502259	
RAC875_c91986_410	5BL	53.248383	
BS00067841_51	5BL	58.153385	
w SNP_Ex_c8322_14030310	5BL	62.424614	
w SNP_Ex_c2582_4804223	5BL	66.871081	
RAC875_c985_387	5BL	71.317548	
BS00068710_51	5BL	75.588777	
Tdurum_contig97342_274	5BL	80.669433	
BS00036434_51	5BL	86.013804	
BS00008767_51	5BL	89.934866	
Excalibur_c29304_176	5BL	94.48479	
BS00093522_51	5BL	98.230914	
BS00023077_51	5BL	105.15954	
BS00099719_51	5BL	108.49702	
w SNP_Ra_c39562_47242455	5BL	111.54426	
BobWhite_c11495_120	5BL	115.76772	
RFL_Contig539_1789	5BL	119.33899	
BS00021735_51	5BL	127.66459	
w SNP_Ex_c44230_50338772	5BL	0	
w SNP_BE606403B-Ta_2_1	5BL	4.0960952	
BS00045446_51	5BL	7.9296765	
w SNP_Ex_c5632_9904112	5BL	13.842461	
w SNP_Ex_c2132_4004831	5BL	17.938556	
Kukri_c17783_58	5BL	22.385023	
Excalibur_c15207_994	5BL	26.831491	
CAP8_c1968_64	5BL	31.628766	
Excalibur_c60683_908	5BL	36.338296	
BS00022652_51	5BL	45.219909	

Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
BS00002208_51	5BL	48.441752	
IAAV850	5BL	55.077761	
IACX8091	5BL	60.266266	
BobWhite_c11365_273	5BL	64.999943	
Excalibur_c37146_747	5BL	71.020592	
BS00028183_51	5BL	73.718861	
BS00049213_51	5BL	0	
BS00022689_51	5BL	6.0926482	
BS00026679_51	5BL	11.584989	
BS00037487_51	5BL	14.196055	
BS00033487_51	5BL	16.807122	
BS00066039_51	5BL	19.418188	
BS00022065_51	5BL	22.814708	
IACX5792	5BL	26.648289	
Excalibur_c7035_353	5BL	31.57061	
BS00065029_51	5BL	36.455659	
BS00049997_51	5BL	40.551755	
CAP7_rep_c12715_390	5DL	0	
Excalibur_c42190_383	5DL	3.3965192	
CAP8_c145_89	5DL	6.7056902	
Excalibur_c687_886	5DL	9.7529357	
Excalibur_c28592_173	5DL	12.974779	
BS00022036_51	5DL	16.28395	
IACX10520	5DL	19.505793	
BS00022876_51	5DL	23.28343	
BS00064893_51	5DL	25.632984	
BS00074353_51	5DL	28.418471	
BS00022267_51	5DL	31.291193	
BS00075368_51	5DL	33.989462	
IAAV1744	5DL	37.123996	
Excalibur_c63110_92	5DL	40.607884	
BobWhite_c7604_254	5DL	43.567859	
BS00070751_51	5DL	56.419577	
BS00062990_51	5DL	59.466823	
BS00021991_51	5DL	62.252309	

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Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
BS00085191_51	5DL	64.863376	
BS00094333_51	5DL	68.911725	
BS00011935_51	5DL	71.784447	
RAC875_c87324_202	6A	0	
w SNP_Ku_c34036_43438136	6A	4.4464672	
BS00109919_51	6A	8.9805941	
RAC875_c13610_822	6A	12.726718	
BS00066555_51	6A	16.735285	
w SNP_Ku_c7471_12865307	6A	28.077451	
Excalibur_c2978_667	6A	31.299294	
Kukri_c49017_404	6A	33.823174	
BobWhite_c15802_72	6A	35.127818	
BS00011010_51	6A	58.246497	
RAC875_c30571_189	6A	72.088527	
BS00093964_51	6A	76.447362	
w SNP_JD_c3441_4455541	6A	80.806196	
Tdurum_contig64407_219	6A	85.252664	
w SNP_Ex_rep_c67468_66068960	6A	88.823942	
IAAV4117	6A	92.657523	
Tdurum_contig25770_308	6A	96.491104	
GENE-1530_263	6A	100.14979	
Tdurum_contig62141_496	6A	103.98338	
IAAV7418	6A	107.99194	
Excalibur_c60006_452	6A	111.21378	
BS00023627_51	6A	121.65427	
IAAV3806	6A	126.55928	
BS00031178_51	6A	131.7395	<i>Qsr.cdl-6AS.1</i>
CAP11_c989_113	6A	139.31003	
BobWhite_c11312_346	6A	142.18275	
Tdurum_contig55124_310	6A	144.79382	
w SNP_Ex_rep_c66433_64661643	6A	147.40488	
Excalibur_c48569_78	6A	150.53942	
CAP11_c6962_208	6A	153.58666	
Kukri_c22149_276	6A	156.80851	
Tdurum_contig83190_441	6A	160.03035	

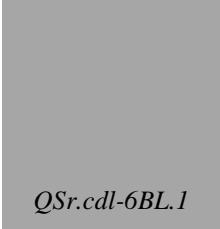
Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
wsnp_Ex_c14192_22135363	6A	163.07759	
wsnp_Ra_c16745_25482384	6A	172.02917	
wsnp_Ex_c17692_26437459	6A	177.7937	
wsnp_Ex_rep_c102011_87270703	6A	182.67875	
Excalibur_c49419_202	6A	187.47603	
Excalibur_c33017_141	6A	192.55668	
Ku_c6998_485	6A	196.74033	
Ku_c9204_918	6A	200.92398	
Ra_c90_3168	6A	204.93255	
Excalibur_c37358_676	6A	208.76613	
Ra_c77116_279	6A	212.42482	
IAAV1385	6A	216.52091	
BS00023089_51	6A	220.61701	
BS00033795_51	6A	224.45059	
BS00063977_51	6A	228.98472	
Ex_c9854_373	6A	233.69425	
BS00065079_51	6A	237.17813	
Ku_c71238_1537	6A	240.57465	
BS00022120_51	6A	244.67075	
Excalibur_c60816_99	6A	248.67932	
BS00064632_51	6A	252.5129	
BS00078715_51	6A	256.52146	
BS00024191_51	6A	260.53003	
IAAV6004	6A	264.5386	
BS00036211_51	6A	268.54716	
Ra_c77116_338	6A	272.55573	
Excalibur_c34574_452	6A	278.44847	
Excalibur_c56264_188	6A	285.04779	
BS00023092_51	6A	289.58192	
BS00066623_51	6A	295.38651	
BS00037006_51	6A	300.46303	
RAC875_c62614_191	6A	305.53549	
tplb0025i05_1836	6A	309.63158	
wsnp_Ex_c2350_4403690	6A	314.79597	
GENE-4154_365	6A	319.24244	



Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
w SNP_BE403818A_Ta_2_1	6A	324.86819	
BS00065082_51	6A	332.55409	
BobWhite_c10832_1131	6AL	0	
Kukri_c40994_61	6AL	3.1345347	
RAC875_c76675_372	6AL	6.3563778	
Jagger_c1423_102	6AL	10.922101	
GENE-4167_145	6AL	13.882075	
CAP8_c1237_68	6AL	18.48176	
RAC875_c6429_55	6AL	22.939966	
IAAV173	6AL	26.861028	
Ra_c2235_1339	6AL	29.12344	
BS00011899_51	6AL	31.472994	
Ku_c14920_502	6AL	34.52024	
Tdurum_contig82605_94	6AL	37.829411	
Jagger_c1134_353	6AL	41.051254	
Tdurum_contig82605_187	6AL	44.273097	
BS00078678_51	6AL	47.756985	
BS00083914_51	6AL	50.716959	
Kukri_c3570_1817	6AL	53.415228	
RAC875_c41438_223	6AL	56.637071	
Kukri_c21058_880	6AL	58.285701	
RAC875_c5893_368	6AL	59.266219	
BobWhite_c10343_320	6AL	61.232627	
Excalibur_rep_c90454_251	6AL	64.653047	
tp1b0031m24_341	6AL	67.089757	
Ra_c1709_839	6AL	69.613638	
BS00094893_51	6AL	72.835481	
RAC875_c23449_205	6AL	75.708203	
BobWhite_c5872_589	6AL	84.62728	
w SNP_JD_c5872_7032077	6AL	87.674526	
RAC875_c40653_612	6AL	90.372794	
w SNP_Ex_c7002_12063380	6AL	93.769313	
RAC875_c1998_1744	6AL	101.8259	
Tdurum_contig46670_911	6AL	106.09713	
BS00022372_51	6AL	111.46149	

Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
w SNP_ Ex_ rep_ c67436_66026057	6AL	115.37483	
w SNP_ Ex_ rep_ c101766_87073440	6AL	118.11922	
Kukri_ c57452_1040	6AL	121.34107	
w SNP_ Ex_ c20457_29526403	6AL	124.65024	
Ra_ c3533_880	6AL	127.95941	
BS00023050_51	6B	0	
BS00104265_51	6B	4.0960952	
IAAV6593	6B	13.76344	
IAAV1816	6B	18.405187	
GENE-4183_1109	6B	24.050634	
CAP8_ c7260_77	6B	28.421203	
Excalibur_ c7785_123	6B	32.616581	
Ku_ c19477_1206	6B	35.925752	
BS00073906_51	6B	40.196981	
BS00047615_51	6B	44.555815	
BS00046717_51	6B	48.127093	
CAP7_ rep_ c10311_217	6B	51.523612	
BS00087798_51	6B	54.570858	
BS00099498_51	6B	57.44358	
BS00048295_51	6B	60.316302	
BS00068659_51	6B	63.450837	
CAP8_ c1310_127	6B	66.149105	
BS00040380_51	6B	69.657043	
Kukri_ c54_306	6B	76.289206	
BS00022155_51	6B	81.286231	
Kukri_ c41694_285	6B	86.1234	
BobWhite_ c34920_228	6B	90.307049	
BobWhite_ c32911_243	6B	93.965739	
Ku_ c22026_1410	6B	98.061834	
BS00058305_51	6B	102.42067	
Kukri_ c25377_240	6B	106.6919	
w SNP_ Ex_ c1603_3056226	6B	110.96313	
BS00049565_51	6B	115.23436	
BS00067873_51	6B	119.68082	
CAP7_ c2536_65	6B	124.30264	

Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
Excalibur_c47738_334	6B	128.04876	
Kukri_c44940_117	6B	132.58289	
IAAV6218	6B	136.94172	
BS00071912_51	6B	140.60041	
RAC875_c90029_92	6B	144.43399	
w SNP_Ra_c11600_18784716	6B	148.79283	
w SNP_Ex_c3858_7011837	6B	153.15166	
RAC875_c7110_2706	6B	157.16023	
BS00056278_51	6B	161.1688	
BS00026203_51	6B	165.26489	
Kukri_c55096_140	6B	169.36099	
Excalibur_rep_c84475_162	6B	173.54464	
Excalibur_c7823_397	6B	177.4657	
BS00052478_51	6B	181.38676	
BS00038162_51	6B	185.39533	
IAAV6675	6B	188.9666	
BobWhite_c17750_568	6B	193.23783	
IACX8906	6B	197.2464	
RAC875_c5041_328	6B	201.25497	
BS00051998_51	6B	205.79294	
BS00073259_51	6B	209.8015	
BS00058774_51	6B	214.77821	
BS00111399_51	6B	218.52433	
BS00057297_51	6B	223.16195	
BS00014588_51	6B	227.60842	
BobWhite_c26504_163	6B	232.05489	
BS00107795_51	6B	236.93993	
IAAV1319	6B	241.03603	
BS00033008_51	6B	246.86209	
Excalibur_c45952_256	6B	250.71975	
BS00046963_51	6B	253.94159	
Ku_c25908_277	6B	257.60028	
BS00066437_51	6B	261.52134	
RAC875_c25902_594	6B	265.4424	
RAC875_rep_c104354_333	6B	269.01368	

Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
Tdurum_contig75750_432	6B	272.58496	
Tdurum_contig45714_546	6B	275.45768	
RAC875_rep_c71306_97	6B	278.67952	
Tdurum_contig62383_259	6B	282.42565	
wsnp_Ex_c12577_20022294	6B	285.82217	
RAC875_c54818_481	6B	290.00582	
BobWhite_c27364_296	6B	0	
BS00109878_51	6B	7.7011217	
BobWhite_c47040_185	6B	13.593867	
Tdurum_contig65998_258	6B	19.478307	
Tdurum_contig67619_532	6B	26.940187	
Tdurum_contig52364_941	6BS	0	
wsnp_Ex_c7191_12352173	6BS	2.9839924	
RAC875_c18689_1870	6BS	6.9448259	
Ku_c5002_1541	6BS	13.271961	
BS00063429_51	6BS	15.708671	
BobWhite_c12846_196	6BS	18.058225	
RAC875_c44002_81	6BS	21.542113	
BS00068735_51	6BS	25.638208	
wsnp_Ku_c2119_4098330	6BS	29.400089	
Excalibur_c96134_152	6BS	31.836799	
Excalibur_c96134_182	6BS	34.360679	
Tdurum_contig43538_1306	6BS	36.100512	
wsnp_Ku_c24391_34351602	6BS	39.060486	
BS00092845_51	6BS	42.457005	
BobWhite_c13400_229	7AL	0	
IAAV6961	7AL	6.0397709	
Kukri_c855_2107	7AL	9.5236589	
Kukri_rep_c98227_230	7AL	17.157681	
Excalibur_c61603_1138	7AL	23.564127	
Tdurum_contig54832_139	7AL	27.397708	
Excalibur_c61603_1052	7AL	31.581358	
Excalibur_c8066_791	7AL	36.971504	
BS00075425_51	7AL	44.377406	
Ku_c43151_811	7AL	49.270224	

Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
Kukri_c28968_130	7AL	53.453873	
BS00002510_51	7AL	57.90034	
Kukri_c13171_474	7AL	62.434467	
BS00076120_51	7AL	66.968594	
wsnp_Ex_c1159_2225557	7AL	71.502721	
wsnp_Ex_c1159_2224684	7AL	76.212252	
BS00065624_51	7BL	0	
BS00040283_51	7BL	3.1422603	
GENE-4710_573	7BL	8.3507419	
BobWhite_c12355_1548	7BL	11.485277	
BS00043554_51	7BL	14.532522	
Tdurum_contig49656_679	7BL	17.579767	
BS00021666_51	7BL	21.998349	
BS00047623_51	7BL	33.902876	
RAC875_c31851_342	7BL	37.649001	
RAC875_c31851_1298	7BL	41.482582	
BS00075569_51	7BL	46.287623	
BS00023069_51	7BL	49.946313	
BS00064933_51	7BL	53.605003	
BS00011767_51	7BL	57.263693	
Excalibur_c87219_179	7BL	61.097275	
Tdurum_contig46338_2305	7BL	64.231809	
RFL_Contig3005_1138	7BL	68.240376	
Tdurum_contig83564_600	7BL	72.248942	
CAP12_c4824_85	7BL	75.208917	
BobWhite_c23287_57	7BL	78.380514	
Excalibur_c26939_1225	7BL	80.294492	
RAC875_rep_c84729_410	7BL	82.905558	
Kukri_rep_c71636_533	7BL	86.276039	
BobWhite_c13098_526	7BL	0	
Tdurum_contig76013_766	7BL	3.8619289	
BobWhite_c36268_275	7BL	8.0455781	
Tdurum_contig76013_605	7BL	11.616856	
RAC875_rep_c70325_264	7BL	14.227923	
CAP8_rep_c3680_203	7BL	18.370317	

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Marker Name	Linkage Group	Genetic Position	Position of Identified QTL
IAAV4133	7D	0	
wsnp_Ex_rep_c66483_64738995	7D	4.8850495	
wsnp_CAP11_c2839_1425826	7D	10.60985	
CAP8_rep_c9420_186	7D	13.395337	
Excalibur_c55782_55	7D	21.960509	

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**Appendix 3.3.** Stem rust severity (0-100 scale), infection response (resistant (R)-susceptible (S)), and coefficient of infection for 181 validation population lines derived from Kwale/Line #162 cross (F3:4) in Njoro, Kenya in 2018. Average coefficient of infection for the two replicates were determined and used for analyses<sup>a</sup>.

Entry Name/ Number	Replicate I			Replicate II		
	Stem rust severity <sup>b</sup>	Infection response <sup>c</sup>	Coefficient of infection <sup>d</sup>	Stem rust severity <sup>b</sup>	Infection response <sup>c</sup>	Coefficient of infection <sup>d</sup>
Line #162	30	MS	24	15	M	9
Kwale	20	MS	16	25	M	15
1	15	M	9	20	M	12
2	1	RMR	1	5	RMR	1
3	40	M	24	40	M	24
4	5	M	3	10	M	6
5	25	M	15	30	M	18
6	30	M	18	30	M	18
7	70	S	70	30	M	18
8	60	S	60	15	M	9
9	15	M	9	15	M	9
10	40	M	24	15	M	9
11	40	M	24	30	M	18
12	40	M	24	25	M	15
13	30	MSS	26	30	M	18
14	25	M	15	15	M	9
15	25	MS	20	30	M	18
16	5	RMR	1	5	RMR	1
17	30	M	18	30	M	18
18	15	M	9	15	M	9
19	25	M	15	25	M	15
20	40	M	24	40	M	24
21	-	-	-	5	M	3
22	30	M	18	5	RMR	1
23	40	M	24	40	M	24
24	15	M	9	5	M	3
25	10	M	6	15	M	9
26	10	M	6	15	M	9
27	20	M	12	5	M	3

Entry Name/ Number	Replicate I			Replicate II		
	Stem rust severity <sup>b</sup>	Infection response <sup>c</sup>	Coefficient of infection <sup>d</sup>	Stem rust severity <sup>b</sup>	Infection response <sup>c</sup>	Coefficient of infection <sup>d</sup>
28	20	M	12	30	M	18
29	5	RMR	1	5	RMR	1
30	30	M	18	5	RMR	1
31	15	M	9	10	M	6
32	15	M	9	15	M	9
33	15	M	9	30	M	18
34	5	M	3	5	RMR	1
35	5	RMR	1	5	RMR	1
36	5	RMR	1	5	RMR	1
37	30	M	18	20	M	12
38	15	M	9	15	M	9
39	15	MS	12	15	M	9
40	30	MS	24	30	M	18
41	5	M	3	5	RMR/M	2
42	60	S	60	30	M	18
43	5	RMR	1	5	RMR/M	2
44	30	MSS	26	20	M	12
45	40	M	24	40	M	24
46	40	MS	32	40	M	24
47	30	M	18	25	M	15
48	40	M	24	30	M	18
49	40	M	24	40	M	24
50	30	M	18	40	M	24
51	10	M	6	5	RMR/M	2
52	15	MSS	13	13	MR/M	6
53	60	S	60	30	M	18
54	70	S	70	40	M	24
55	15	M	9	15	RMR	4
56	5	M	3	7	RMR/M	3
57	15	M	9	5	RMR	1
58	5	RMR	1	5	RMR	1
59	5	RMR	1	2	TR	1
60	20	M	12	30	M	18
61	10	M	6	5	M	3



Entry Name/ Number	Replicate I			Replicate II		
	Stem rust severity <sup>b</sup>	Infection response <sup>c</sup>	Coefficient of infection <sup>d</sup>	Stem rust severity <sup>b</sup>	Infection response <sup>c</sup>	Coefficient of infection <sup>d</sup>
62	40	M	24	40	M	24
63	50	S	50	30	MS	24
64	30	MS	24	15	M	9
65	5	M	3	10	M	6
66	10	M	6	25	M	15
67	3	TR/M	1	5	RMR	1
68	5	M	3	7	RMR/M	3
69	5	RMR	1	5	M	3
70	5	RMR	1	5	RMR	1
71	30	M	18	30	M	18
72	10	M	6	15	M	9
73	40	M	24	40	M	24
74	10	M	6	30	M	18
75	25	M	15	30	M	18
76	5	RMR	1	5	RMR	1
77	5	RMR	1	15	M	9
78	5	MSS	4	5	M	3
79	10	M	6	8	RMR/M	3
80	5	RMR	1	5	RMR	1
81	10	M	6	5	RMR	1
82	40	M	24	20	M	12
83	20	M	12	30	M	18
84	0	-	-	5	MS	4
85	30	M	18	30	M	18
86	5	RMR	1	10	RMR	3
87	50	M	30	30	M	18
88	15	M	9	15	M	9
89	25	M	15	25	M	15
90	5	RMR	1	5	RMR	1
91	40	M	24	30	M	18
92	30	M	18	25	M	15
93	2	TR	0.4	5	M	3
94	15	M	9	10	M	6
95	40	M	24	30	M	18

Entry Name/ Number	Replicate I			Replicate II		
	Stem rust severity <sup>b</sup>	Infection response <sup>c</sup>	Coefficient of infection <sup>d</sup>	Stem rust severity <sup>b</sup>	Infection response <sup>c</sup>	Coefficient of infection <sup>d</sup>
96	5	RMR	1	7	RMR/M	3
97	20	M	12	15	M	9
98	15	M	9	15	M	9
99	15	M	9	15	M	9
100	25	M	15	30	M	18
101	-	-	-	30	M	18
102	30	M	18	30	M	18
103	40	M	24	40	M	24
104	5	M	3	10	M	6
105	15	M	9	10	RMR/M	4
106	5	RMR	1	13	RMR/M	5
107	15	M	9	7	RMR/M	3
108	15	M	9	10	M	6
109	15	M	9	20	M	12
110	15	M	9	20	M	12
111	30	M	18	30	M	18
112	5	RMR	1	-	-	-
113	3	TR/M	1	5	RMR/M	1.9
114	20	M	12	30	M	18
115	12	RMR/M	4	15	M	9
116	5	RMR	1	5	RMR	1
117	30	MS	24	40	M	24
118	30	M	18	5	M	3
119	50	M	30	20	M	12
120	5	RMR	1	15	M	9
121	5	RMR	1	5	RMR	1
122	10	M	6	-	-	-
123	40	M	24	30	M	18
124	20	M	12	-	-	-
125	15	M	9	-	-	-
126	30	M	18	25	M	15
127	10	M	6	5	RMR	1
128	40	M	24	25	M	15
129	40	M	24	30	M	18

Entry Name/ Number	Replicate I			Replicate II		
	Stem rust severity <sup>b</sup>	Infection response <sup>c</sup>	Coefficient of infection <sup>d</sup>	Stem rust severity <sup>b</sup>	Infection response <sup>c</sup>	Coefficient of infection <sup>d</sup>
130	20	M	12	30	M	18
131	40	MS	32	25	M	15
132	30	M	18	25	M	15
133	30	M	18	20	M	12
134	30	M	18	20	M	12
135	15	M	9	10	M	6
136	40	M	24	40	M	24
137	5	RMR	1	5	RMR/M	2
138	10	M	6	30	M	18
139	5	RMR	1	5	RMR	1
140	10	M	6	20	M	12
141	40	M	24	30	M	18
142	15	M	9	8	M/RMR	4
143	5	RMR	1	5	RMR	1
144	5	RMR	1	-	-	-
145	5	RMR	1	30	M	18
146	30	M	18	20	M	12
147	20	M	12	-	-	-
148	40	M	24	40	M	24
149	5	RMR	1	5	M	3
150	25	M	15	25	M	15
151	15	M	9	10	M	6
152	13	MR/M	6	20	M	12
153	20	M	12	20	M	12
154	50	S	50	40	M	24
155	50	M	30	20	M	12
156	5	RMR	1	5	RMR	1
157	10	RMR	2.7	40	M	24
158	40	M	24	15	M	9
159	30	M	18	40	M	24
160	30	M	18	20	M	12
161	13	M/RMR	7	7	RMR/M	3
162	5	RMR	1	5	RMR	1
163	15	M	9	15	M	9

Entry Name/ Number	Replicate I			Replicate II		
	Stem rust severity <sup>b</sup>	Infection response <sup>c</sup>	Coefficient of infection <sup>d</sup>	Stem rust severity <sup>b</sup>	Infection response <sup>c</sup>	Coefficient of infection <sup>d</sup>
164	30	M	18	30	M	18
165	10	M	6	-	-	-
166	40	M	24	40	M	24
167	10	M	6	13	RMR/M	5
168	5	RMR	1	5	RMR	1
169	5	RMR	1	13	RMR/M	5
170	15	M	9	5	M	3
171	30	M	18	15	M	9
172	60	S	60	5	RMR	1
173	40	M	24	25	M	15
174	30	M	18	15	M	9
175	5	RMR	1	5	RMR	1
176	25	M	15	60	S	60
177	15	M	9	10	M	6
178	5	RMR/M	2	15	M	9
179	30	M	18	15	M	9
180	40	M	24	40	M	24
181	10	RMR	3	5	RMR	1

<sup>a</sup>-Indicates missing data;

<sup>b</sup>Stem rust severity was visually scored based on the modified Cobb scale of 0-100, where 0 = immune; no uredinia or any other sign of infection and 100% = completely susceptible (Peterson *et al.*, 1948);

<sup>c</sup>The infection responses were assigned as either; resistant (R), small uredinia surrounded by necrosis; moderately resistant (MR), medium-sized uredinia surrounded by necrosis or chlorosis; moderately susceptible (MS), medium-sized uredinia without necrosis; MRMS, infection response that included both the MR and MS categories; susceptible (S), large uredinia without necrosis; MSS infection responses that included both the MS and S (Roelfs *et al.*, 1992);

<sup>d</sup>Coefficient of infection (COI) values were generated by multiplying the stem rust severity value for each line by a constant value for each infection response: 0 = 0, R = 0.2, RMR = 0.3, MR = 0.4, M = 0.6, MS = 0.8, S = 1.0 (Knott, 1989).