# GENETIC RELATIONSHIP OF ADULT PLANT RESISTANCE TO WHEAT RUSTS AND VALIDATION OF STEM RUST QTL

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## Abstract

Cereal rusts are among the most important fungal diseases worldwide and pose a major threat to global food security. Wheat is attacked by three rusts known as stripe rust, leaf rust, and stem rust caused by fungal pathogens, *Puccinia striiformis* f. sp. *tritici* (*Pst*), *Puccinia triticina* (*Pt*), and *Puccinia graminis* f. sp. *tritici* (*Pgt*), respectively. These pathogens are widely distributed across the world, produce spores with the ability to travel long distances, rapidly multiply under favorable environmental conditions, and evolve new races that overcome the resistant genes in cultivated varieties. The rapid appearance of new races of rust pathogens with virulence for the major seedling resistance genes in wheat has intensified the focus to breeding for durable resistance. Durable rust resistance is more likely to be of adult plant resistance (APR) rather than seedling resistance, and not associated with the genes conferring hypersensitive reaction. Two projects were developed to identify and map the genetic sources and dissect the mechanism of APR to rusts. The first project utilized a bi-parental mapping population derived from a cross of COPIO x Apav#1. It consisted of 178, $F_4$:$F_5$ recombinant inbred lines (RIL) developed at the International Maize and Wheat Improvement Centre (CIMMYT) in Mexico. The objectives of this study were to map the APR genes in 'COPIO' and to understand the genetic relationship of APR genes conferring resistance to all three rusts. The parents of the RIL mapping population were tested against selected *Pgt*, *Pst*, and *Pt* races at the seedling stage and were also assayed for the known APR genes and 2NS/2AS translocation using molecular markers. The RIL population was also evaluated under field conditions in six environments for leaf and stem rust and nine environments for stripe rust. Genotyping of the population and parents was carried out

through genotyping-by-sequencing (GBS) and 762 resulting polymorphic markers each representing a unique locus, were used for the downstream QTL mapping analysis.

Molecular characterization and quantitative/qualitative analysis revealed that COPIO harbors some important pleiotropic and APR genes along with qualitative genes for *Pgt*, *Pst*, and *Pt*. Pleiotropic gene *Lr46/Yr29/Sr58* on chromosome 1BL reduced disease severities of all three rusts with $R^2$ values ranging from 10 to 42%. The APR genes *Sr2/Yr30* for stem and stripe rust along with either a new gene for leaf rust resistance or due to pleiotropic effects of *Sr2/Yr30* resulted in reduced severities of all three rusts. The 2NS/2AS translocation segment on chromosome 2AS containing the race-specific resistance genes *Sr38, Lr37, and Yr17* is present in COPIO. A new putative stripe rust APR QTL (*QYr.umn.2A*) in the same region, along with partial effects of *Yr17*, significantly reduced stripe rust severities in all nine environments. We also postulated the presence of *Lr13* on chromosome 2B and *Yr31*, *Yr45*, and *Yr60* on chromosomes 2B, 3D, and 4B respectively in COPIO. This study also detected minor effect QTL, both previously reported (for stem rust; *QSr.umn.3B.2; QSr.umn.4B*, for stripe rust; *QYr.umn.1A; QYr.umn.3B.3*) and potentially new sources of resistance (for stem rust; *QSr.umn.2A.3*; *QSr.umn.7A*, for leaf rust; *QLr.umn.2A.1, QLr.umn.3B*, for stripe rust; *QYr.umn.1B.1; QYr.umn.3A.2*) in COPIO. These QTLs are also contributing quantitative resistance to all three rusts in COPIO. Our findings show that wheat line COPIO contains pleiotropic, APR and seedling genes along with small to medium effect QTL that are working in combination to enhance genetic resistance against rust pathogens. Broad spectrum resistance against wheat rust diseases in COPIO makes it a valuable source of resistance and its utilization in recombination breeding can potentially enhance durable

resistance. Development of diagnostic markers, particularly for the 2A QTL (*QYr.umn.2A*), will be useful for marker assisted selection in breeding programs.

The second project involved a bi-parental mapping population developed by crossing a wheat line 'MN06113-8' and cultivated wheat variety 'Sabin'. The wheat breeding program at the University of Minnesota previously mapped a large effect stem rust APR QTL (*QSr.umn-2B.2*) on chromosome 2B in wheat line 'MN06113-8'. This QTL is effective against the North American, Kenyan and Ethiopian stem rust pathogen races. The objectives of this study were to:1) understand the genetics of APR to wheat stem rust in the breeding line 'MN06113-8' and cultivated wheat variety 'Sabin'; and 2) validate 2B QTL in MN06113-8. A total of 184 recombinant inbred lines (RILs) from the cross Sabin/MN06113-8 were tested in stem rust nurseries in Kenya, Ethiopia and Saint Paul. Both parental lines were highly susceptible to Ug99 races TTKSK, TTKST, and TTKTT at the seedling stage but MN06113-8 exhibited adult plant resistance (APR) in Kenya and Ethiopia under field testing conditions. Genotyping by sequencing (GBS) was used to genotype the population and both parents. A total of 4,100 polymorphic GBS markers were assigned to 21 wheat chromosomes to develop the linkage maps. The GBS single nucleotide polymorphism (SNP) markers covered 2,931 cM of the genome with an average of 0.71 markers $cM^{-1}$. Composite interval mapping detected six quantitative trait loci (QTL) on chromosomes 2A, 3B, 4A, 4B, and 6B associated with stem rust resistance. Among these seven QTL, three were detected in African environments and four were detected in Saint Paul against the North American stem rust pathogen races. We could not validate *QSr.umn-2B.2*, discovered in a previous study involving MN06113-8 because the QTL region was monomorphic in our population. A large effect

QTL (*QSr.umn.3B*) was mapped on chromosome 3B conferring resistance to Ug99 and North American *Pgt* races. The QTL (*QSr.umn.4A.1*) detected in Kenya on chromosome 4A has not been previously reported. Development of diagnostic markers and pyramiding of these genes through marker assisted selection will accelerate the development of durable rust resistance.

## Table of Contents

## List of Tables

### Chapter 1



### Chapter 2

**Chapter 3**



**Chapter 4**

**Chapter 5**

## List of Figures

### Chapter 1



### Chapter 2

**Chapter 3**



**Chapter 4**



**Chapter 5**

# Chapter 1

## Molecular Characterization of Genomic Regions for Adult Plant Resistance to Stem Rust in a Spring Wheat Mapping Population

### Introduction

Wheat (*Triticum aestivum* L.) is an important and widely cultivated cereal crop since the ancient times (Zohary and Hopf, 2000). This crop alone provides almost 20% of the calories and protein to 9.4 billion people in the developing countries (Braun et al. 2010). Multiple biotic and abiotic factors are influencing the global food system and ultimately food security, but wheat production needs to be increased up to 60% to feed the estimated world population of 9.5 billion by 2050 (Borlaug 2009, UN-DESA 2017). Among biotic factors, wheat stem rust caused by *Puccinia graminis* f. sp. *tritici* is a very devastating disease and threat to wheat production in all the continents (Singh et al. 2011). Continuous rust monitoring is essential due to two main characteristics of the stem rust pathogen. Firstly, rust spores can be rapidly transmitted to the adjacent areas in the same region or even across the continents through wind movements and quarantine is impossible (Hodson et al. 2011). Secondly, this pathogen can use sexual recombination and mutation to evolve new races (Knott 1989; Park 2007; Watson 1981). Wheat yield losses due to stem rust have averaged 2.5 % annually in the US from 1918 to 1960. Later on, yield losses were significantly decreased (0.3% annually) and since a half century are very minor (Pardey et al. 2013). Stem rust control in the US was an integrated and coordinated research effort through the development and deployment of resistant varieties, effective rust monitoring and early warning system, and most importantly barberry (*Berberis vulgaris* L.) eradication, which is an alternate host for the stem rust pathogen (Campbell and long 2001; Roelfs 1982; Kolmer 1996; Jin and Singh 2006;

Peterson 2018). The developed world has successfully mitigated stem rust impact on yield but in the developing countries of South Asia, Africa, and the Middle East, the economic losses are still up to $3 billion US (BGRI 2012). The evolution of a very devastating stem rust pathogen race found in Uganda and named as Ug99 based on its origin and the year it was characterized (Pretorius et al. 2000). Since the time of Ug99 emergence, the pathogen has rapidly evolved and thirteen different variants from the same lineage have been reported so far in thirteen different countries (Rust tracker 2019). The wide dispersal of aggressive pathogen strains in East Africa and Middle East are of great concern for the global food security, because the pathogen can be easily transmitted to other parts of the world where a majority of commercially cultivated wheat varieties are susceptible (Hovmøller et al. 2010; Stokstad 2007; Bhattacharya 2017; Singh et al. 2015; Nagarajan 2012). Ug99 and its variants are virulent to very important stem rust resistant genes (*Sr31*, *Sr24*, *Sr36*, *Sr9h*, *SrTmp*) and now a total of 34 genes are ineffective to Ug99 (Singh et al. 2015). Gene *Sr31* along with *Lr26*, *Yr9*, and *Pm8* on chromosome 1B was a spontaneous exchange of rye (*Secale cereale* L.) and commonly known as T1BL.1RS translocation (Mettin et al. 1973; Zeller 1973). This gene was deployed across large wheat growing areas around the world and was very effective against stem rust before the emergence of Ug99 (Hartsman et al. 2015).

In addition to Ug99, some other non-Ug99 races are getting more attention due to their wide geographic distribution and unique virulence. Two stem rust races (RRTTF and TKTTF) which do not belong to the Ug99 lineage are widely distributed in East Africa, the Middle East and South Asia (Singh 2015). RRTTF has virulence to *Sr38* and has been reported in Iran (1997) Ethiopia (2007), Yemen (2007), and Pakistan (2009).

RRTTF was reported in Ecuador in 2016. The origin of the Ecuador isolate is not known, but it has similarities with the RRTTF isolates from Pakistan, Yemen and Ethiopia (Barnes et al. 2018). Stem rust isolates of RRTTF from Pakistan were virulent to 55% of Canadian hard red spring wheat varieties during the seedling test (Fetch et al. 2012). So, the race RRTTF is a potential threat to wheat production in North and South America.

In southern Ethiopia, severe stem rust epidemics were recorded during 2013 and 2014 due to race TKTTF that is virulent to gene *SrTmp*, causing up to 100% yield losses to the widely cultivated wheat variety 'Digalu'. TKTTF is also commonly known as the Digalu race. Molecular studies revealed that isolates of the TKTTF race do not belong to the Ug99 race group (Olivera et al. 2015). Recently, stem rust isolate UK-01 collected from Southern England was found to have close genetic relationship to the Ethiopian TKTTF isolates based on molecular diagnostics (Lewis et al, 2018). Later, the virulence profiling at the seedling stage using the North American differential lines confirmed that UK-01 is identical to TKTTF. TKTTF has already been in the Middle East (Olivera et al. 2015) and recently in Europe, where it was the predominant race of an unusual rust outbreak in Germany during 2013 (Olivera et al. 2017). Stem rust was almost eradicated in the Western Europe but in recent years few isolated outbreaks occurred. In Western Europe, *Pgt* race TKTTF (UK-01) is the first occurrence in several decades and based on the seedling data on 57 old and new cultivated wheat varieties in the United Kingdom, 80% are susceptible to race TKTTF (Lewis et al. 2018).

Evolution of new stem rust pathogen races belonging to the Ug99 race group and the recent spread of highly virulent non Ug99 races (especially RRTTF and TKTTF) are narrowing down the list of available genetic sources of resistance in bread wheat and its

related wild relatives. In the past, wheat resistance to stem rust was conferred by selection for race-specific resistance, but genes controlling this kind of resistance often break down within 5 years of their deployment (Singh and Huerta- Spino 2001). In contrast, race-nonspecific resistance genes usually have partial and additive effects with a susceptible infection response, but reduced disease progression. During the last two decades, wheat breeding for rust resistance had mainly focused on the development of wheat cultivars with adult plant resistance (APR). Genes conferring the APR can be either race-specific or non-race-specific and are effective only at the adult plant stage (Ellis et al. 2014; McIntosh et al. 1995). Some APR genes like *Sr2* and *Sr57* (pleiotropic with *Lr34*) have been deployed for almost a century and are good examples of genes conferring durable resistance. Only a few APR genes are well characterized along with their DNA markers (Lagudah et al. 2006; Moore et al. 2015). New genetic sources of resistance are required to timely and effectively cope with the potential threat of stem rust, due to the pathogen's unique ability of rapid emergence and wide dispersal across continents. The International Maize and Wheat Improvement Center (CIMMYT) is actively engaged in finding new sources of resistance to stem rust in the Triticeae family to utilize for wheat improvement in collaboration with public and private sector institutions. The mapping population (Apav x COPIO) used in this study was developed by CIMMYT with the motivation for its development being that 'COPIO' has displayed high levels of APR to different stem rust races including Ug99. The targeted objective of our study was to investigate the genetic basis of resistance and better understand the mechanism of APR in the breeding line COPIO.

## Materials and methods

### Mapping population

This study used a set of 176 $F_4$ derived recombinant inbred lines (RILs) from a cross of Apav#1 and COPIO. Apav#1 (CIMMYT GID 1854090) is a RIL from a cross of Avocet S and Pavon 76. Apav#1 is highly susceptible to all three rusts at the seedling and adult plant stages. Moreover, it shows higher disease response than Avocet S under field conditions. For simplicity Apav#1 hereafter referred as Apav. COPIO (CIMMYT GID 6178401; pedigree; CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/FH6-1-7) is an experimental breeding line developed by the International Maize and Wheat Improvement Centre (CIMMYT) global wheat breeding program in Mexico and has shown resistance to wheat rusts including the Ug99 race group at the adult plant stage. The RILs were developed using the modified bulk approach from three single $F_1$ plants ($1F_1$, $2F_1$, $3F_1$) About 500 seeds of each $F_2$ population were sown at 4-5 cm apart at 10 m long paired rows. One spike every 5 cm of each $F_2$ row was harvested and bulk threshed to advance the next generation. The same procedure was used in generation $F_3$ to obtain generation $F_4$ plants. In $F_4$, a population of about 200 plants was space planted (10 cm plant to plant distance) and individual plants (from two $F_4$ populations derived from 1 $F_1$ and 1 $F_3$) were selected, harvested and threshed. The $F_4$:$F_5$ RILs were used for phenotyping during 2016, seed was multiplied, and $F_4$ derived $F_6$ lines were used in 2017 for field phenotyping and genotyping using genotyping-by-sequencing (GBS).

**Seedling stage stem rust evaluation**

Apav and COPIO were tested for seedling resistance to stem rust using a panel of US common races (QFCSC, QTHJC, RKQQC, TPMKC, TTTTF) and a US race SCCSC with unique virulence (avirulence to *Sr12* and virulence to *Sr9e*). Parental lines were also screened against three Ug99 lineage races (TTKSK, TTKST, TTKTT), and some other important races from Ethiopia (TKTTF), Yemen (TRTTF), Pakistan (RRTTF), and Mexico (RTR). The RIL population was also tested at the seedling stage using the predominant Pakistani stem rust race RRTTF and Chi-squared test was performed to check the goodness-of-fit for the Mendelian segregation of expected ratios with the observed ratios for 1 and 2 genes. Seedling tests were carried out at the Cereals Disease Laboratory (CDL), United States Department of Agriculture (USDA) facility in Saint Paul. Foreign races were tested in a Biosafety Level-3 containment facility (BSL-3) at the University of Minnesota. Stem rust seedling evaluations were performed following the protocols described by Rouse and Jin (2011a). Disease infection was recorded 12 to 13 d post inoculation using the 0-4 scale described by Stakman et al. (1962). Infection types (ITs) 0, ;, 1, 2, and combinations were classified as low infection types considering avirulent pathogen and resistant host, while 3 and 4 were considered as high ITs. Heterogeneous infection types were denoted as X, which describes variable uredinia which sometimes produce all infection types on the same leaf. For Chi-squared test, phenotype data of 0-4 scale was converted into a 0-9 linearized scale using the custom Perl script described by Gao et al. (2016). The perl script is mainly based on the seedling data conversion scale proposed by Zhang et al. (2014) with some modifications. For simple scores like 0, 2+, 3 it only used Zhang's 0-9 scale but if the scored values were

more complex (for example ;13+) then the first value was weighted double, and final score was obtained based on the arithmetic means. Lines with a score of 0 to 7 were considered resistant, while lines scored 8 and 9 were counted as susceptible. All the races and their isolates used for seedling evaluation are outlined in Table 1.

**Field based stem rust evaluation**

The RILs and both parents of Apav x COPIO mapping population were tested for *P. graminis* f. sp. *tritici* (*Pgt*) reaction under the field conditions in St. Paul for two seasons during April through August in 2016 and 2017 (hereafter referred as Stp16, Stp17). The population was also tested during the main and off-seasons of 2016 in Kenya for Ug99 response (hereafter referred as Ken16_MS, Ken16_OS), and for two seasons in Pakistan against the predominant stem rust pathogen race during 2016 and 2017 (hereafter referred as Pak16, Pak17). For each trial the abbreviations represent a location where the experiments were conducted (Stp for Saint Paul, Ken for Kenya and Pak for Pakistan) followed by a number that represents when (16, 17 for years 2016 and 2017) the trial was conducted. For Kenya, MS and OS represents main-season (June through October) and off-season (December through April).

In Saint Paul, the mapping population was included in the stem and leaf rust nurseries planted for screening U.S. spring wheat germplasm. Two-meter single rows were planted, 20 cm apart with 2-meter alleys in an augmented design. Five check varieties; Faller (Mergoum et al. 2008), Prosper (Mergoum et al. 2012), Forefront (Glover et al. 2013), Thatcher (Hayes et al. 1936), and Linkert (Anderson et al. 2018) were planted after every 45 lines. Spreader rows of susceptible cultivars ‘Morocco’ and ‘LMPG-6’ were planted surrounding the nursery plots and a continuous row on the

alternate alleys. Spreaders rows were sown perpendicular to the nursery plots, 7 to 14 days earlier than the experimental lines. A mixture of six North American *Pgt* races (QTHJC, MCCFC, TPMKC, QFCSC, RCRSC, RKRQC) was used for the inoculation with an Ulva+ sprayer (Micron Sprayers Ltd., Bromyard U.K.) (Rouse at al. 2011b). The *Pgt* races and their virulence pattern is given in Table 2. To develop the rust epidemics, *Pgt* races were suspended in the light mineral oil (1:3 v/v) and sprayed on the spreader rows after the heading stage; Feekes growth stage 10.3 (Feekes W. 1941).

For Ug99 field testing, the population was included in the CIMMYT shuttle breeding nurseries for Africa. RILs and parents were planted in paired rows of about 0.7 m long on flat beds with 0.3 m space at the experimental station of Kenya Agricultural & Livestock Research Organization (KARLO) in Njoro, Kenya. Spreader rows consisting on a mixture of susceptible lines were planted around the experimental lines and on one side of the alley. Wheat cultivars Cacuke and Robin were used as spreader lines. Spreaders were inoculated with two Ug99 lineage races (TTKST; virulent to *Sr31+Sr24* and TTKTT; virulent to *Sr31+Sr24 + SrTmp*). The urediniospores of both races were suspended in Soltrol 170 (1g $L^{-1}$) and sprayed on spreader rows. One liter of mixture was applied on approximately 400 ft of spreader rows (8 mg $m^{-1}$). The same mixture was used for needle inoculation at the jointing stage (Njau et al. 2012).

In Pakistan, lines were screened as part of National Uniform Wheat Yield Trials (NUWYT) and nurseries were planted at Crop Diseases Research Institute (CDRI), Karachi. During the 2016 phenotyping season, 19 RILs were missing from the mapping population, while in 2017 the complete RIL population was tested with both parents. Lines were sown as 1 m single rows with 20 cm between rows. Wheat variety NARC-

2011 (Rehman et al. 2018) and Morocco were planted as resistant and susceptible checks every 20 lines. Spreader rows of Morocco were planted surrounding the experimental lines. Plants infected with the stem rust pathogen race RRTTF (virulent to *Sr38*) were transplanted at approximately every 2 m in the spreader rows to initiate the rust infection. Spreader rows were also inoculated using urediniospores of RRTTF suspended in light mineral oil (1g $L^{-1}$) and two drops of tween 20, about 7-8 weeks after planting with the same concentration as described above.

**Phenotyping and data analysis**

Lines were visually assessed for stem rust infection response. Based on pustule size and associated chlorosis and necrosis, infection response was classified into four categories; S = susceptible, MS = moderately susceptible, MR = moderately resistant, and R = resistant (Roelfs et al. 1992). Infection responses overlapping two categories were denoted with a dash (-) for example MR-MS. Stem rust severity was recorded on a scale of 0-100 % according to the modified Cobb Scale method (Peterson et al. 1948). In all environments, rust severity was recorded on a whole plot basis, considering the percent area of plant stems covered with disease. Stem rust severities and infection types were recorded on the flag leaf at least twice in all trials between heading and maturity stage, but the last score was considered the final disease severity (FDS). A custom perl script was used (Gao et al. 2016) to convert the field data to three categories (severity, linearized infection response and coefficient of infection). Linearized infection response (LIR) is infection response converted into a 0-1 scale and coefficient of infection (COI) is the product of severity and LIR. COI values were used for all the phenotyping and QTL mapping analyses. Histograms and Pearson coefficient correlations were developed

among all the phenotyping environments using the COI values in the R program and IBM SPSS 1.0.0.1174 (IBM Corp.).

**DNA extraction, genotyping and SNP calling**

For genomic DNA extraction, 5 seeds of each $F_4$:$F_6$ RIL were planted in a 126-well plastic tray filled with cotton balls soaked with water. Leaf tissue of approximately 2 cm was harvested in microtubes at the two-leaf stage and lyophilized for 3 d. Plant TissueLyzer II (QIAGEN) was used for grinding the leaf tissue and a BioSprint 96 DNA plant kit was used to extract the DNA on a BioSprint workstation (QIAGEN). Eighteen lines that did not germinate on cotton balls had their genomic DNA extracted from ground seeds following the cetyl trimethylammonium bromide (CTAB) protocol described by Kidwell and Osborn (1992). DNA extracted from both protocols was dispensed in 1X TE buffer, labeled as stock DNA and preserved. All genomic DNA samples were quantified by PicoGreen dsDNA assay kit (Ahn et al. 1996). Marker screening for the known APR genes (*Sr2*, *Sr55*, *Sr57*, *Sr58*) was conducted on both parent lines following the MAS Wheat protocols (https://maswheat.ucdavis.edu/protocols/StemRust/). Ten GBS markers that cover the 2NS/2AS translocation were tested to check the presence or absence of this translocation from *Triticum ventricosum* carrying *Lr37/Sr38/Yr19* genes (Suppl. Table 1). Genotyping of the population and parents was carried out through genotyping-by-sequencing (GBS) according to the protocols describes by Poland at al. (2012). Two genomic libraries were developed, where the 1$^{st}$ library was 96-plexed while the 2$^{nd}$ library was 82-plex with both parents repeated seven times. All inbred lines had same plex level and parents were repeated to get a greater number of reads for possible future imputation in the

downstream SNP calling process. For next generation sequencing (NGS), both GBS libraries were submitted to University of Minnesota Genomic Center (UMGC). Quality control (QC) analysis was performed on both libraries using the Bioanalyzer on pre and post size selection. Size selection was performed on the PippinHT @ 160-240bp (3% agarose) and validated by Picogreen, Agilent bioanalyzer and Kapa qPCR. Illumina Hi-Seq 2500 was used for sequencing the genomic libraries. The 100 bp single ended sequences were generated by loading each library on one lane of an Illumina flow cell using v4 chemistry.

For SNP calling, the sequences from both libraries were aligned with the IWGSC Chinese Spring wheat genome assembly v1.0 (IWGSC 2018). Alignment of our sequences with the reference genome was performed using ALN function in Burrows-Wheeler Aligner (bwa) under the default parameters. Samtools was used to further process the aligned sequences and 'mpileup' procedure was used for SNP calling. Finally, SNP filtration was performed at 30/70% minor allele frequency (MAF) and major allele frequency respectively. SNP calls were accepted at a minimum criteria of ≥ 3 alignment read depth and ≥ 25 for the read mapping quality.

**Linkage map construction and QTL mapping**

Nucleotide base calls were changed into numbers with Apav alleles coded as '2' and COPIO coded as '0' while the missing and heterozygous SNP calls were coded as '-1'. Linkage maps were developed using 2,575 polymorphic SNP markers using the software QTL ICiMapping version 4.1 (Meng et al. 2015). Linkage groups were created representing all 21 wheat chromosomes and the 'nnTwoOpt' algorithm was used for marker ordering. A total of 33 redundant and poor-fitting markers were removed from the

linkage maps and finally 2,542 markers were retained. To further refine the linkage maps, rippling was performed at a window size of 8, using the sum of adjacent recombination fractions (SARF) criteria. The Kosambi mapping function was used to convert the recombination frequency into genetic distances (Kosambi 1943) between markers. Windows QTL cartographer 2.5 was used to identify marker trait associations and map stem rust QTL (Wang et al. 2012). The composite interval mapping (CIM) procedure was used for QTL detection through forward and backward regression at a walk speed of 1.0 cM across all linkage groups. A LOD (logarithm of the odds) threshold of 2.5 was considered significant to declare a QTL. QTL names were adopted from the gene nomenclature described by McIntosh et al. (2013). MapChart 2.3 (Voorrpis, 2002) was used to visualize the detected QTLs on wheat chromosomes.

## Results

### Rust evaluations at seedling and in the field

Infection response of RIL parents to the Ug99 group races (TTKSK, TTKST, TTKTT; virulent to *Sr31, Sr24* and *SrTmp* respectively) was high at the seedling stage. Both parent lines were scored with an IT of 3 and 3+ in two repeated experiments. For North American stem rust races, Apav was susceptible to 4 out of 6 tested races, with an IT range of 3 to 3+, while COPIO was resistant with an IT range of 0; to 1. Both parents were susceptible to race TTTTF (IT = 3+) and resistant to race SCCSC (IT = 0; to ;1). Among non Ug99 races, TKTTF from Ethiopia was highly virulent (IT = 3+) to both parent lines, TRTTF and RRTTF were avirulent to Apav and highly virulent (IT = 3 to 33+) to COPIO, while the Mexican stem rust race RTR was highly virulent (IT = 3+) to Apav and avirulent to COPIO (IT = X). Infection types of all the races used in the

seedling assay are given in Table 1 and seedling reactions of *Pgt* races are shown in Figure 1. Based on the seedling assays we postulated that COPIO has *Sr38* and Apav has *Sr8a*. Seedling tests were also conducted on the RIL population using the predominant race (RRTTF) from Pakistan. Among 176 population lines, 120 were resistant while 56 were susceptible and Chi-squared tests ($\chi^2 = 3.36$; $p$ value = 0.03) indicated that two genes for resistance to this race are segregating in this population.

Excellent disease development was observed in all six field environments. During the off-season of 2016 in Kenya (Ken16_OS), disease pressure was very high and resistant parent COPIO had the FDS of 24% but in the main season (Ken16_MS) it was 18%. Susceptible parent Apav had 100% FDS during both seasons in Kenya. The FDS of COPIO was 1 to 5% during the both seasons (Stp16, Stp17) in Saint Paul, while Apav showed 70% and 60.9% FDS, respectively. In Pakistan, resistant parent COPIO had an FDS 4% to 18.4%, while 43.5% and 60.9% FDS was recorded on the susceptible parent Apav during 2016 and 2017, respectively. The mean FDS on the RIL population was in the range of 26.6-30.5%, 30.3-69.8%, and 31.8-52.5% in Saint Paul, Kenya, and Pakistan respectively during both seasons (Table 3). Disease severity distribution was more skewed towards susceptibility in Kenya during the off-season due to high disease pressure. The disease distribution spectrum was continuous in all the environments (Fig. 2) and almost represented all severity classes. Pearson correlation coefficients (r) among the stem rust severities were significant at either 1% and 5% ($\alpha = 0.01, 0.05$) significance levels across all six environments (Table 4). Both environments in Saint Paul (Stp16, Stp17) were highly correlated ($r = 0.79$) followed by the environments (Pak16, Pak17) in Pakistan ($r = 0.64$) for the stem rust severities. Pak16 had lowest correlation with

Ken16_MS ($r$ = 0.18), while Pak17 had lowest correlation with Stp16 ($r$ = 0.21) but still significant.

**GBS libraries, linkage maps construction, and QTL mapping**

Both GBS libraries selected at a size of 200bp (±20%) DNA fragments were subjected to sequencing on Illumina HiSeq 2500 and generated >220 million reads. Genomic libraries passed the pre and post size selection QC analysis for both size and mass (Agilent size = 235bp; Pooled sample concentration = 7-11nM). To pass the QC test, library size should be between 200-700 bp and it must quantitate >2nM. Illumina sequencing generated 241 and 250 million single ended 100 bp raw reads for the 1$^{st}$ and 2$^{nd}$ GBS library, respectively. Approximately 95% of the generated reads passed the Q30 quality criteria with a mean read quality score of 36.

The SNP calling pipeline identified 3,880 polymorphic markers with less than 20% missing data. SNP filtration was performed at 30% minor allele frequency (MAF) and 70% major allele. Through this filtration process, 2,575 polymorphic markers were identified which were used to develop the linkage maps. These markers were assigned to chromosomes based on the anchoring information and 21 linkage groups were developed. After removing poor fitting markers, a total of 2,542 markers were retained for the final linkage maps. The total genetic distance covered by the linkage map was 2,232 centimorgans (cM). Among the three wheat genomes, 1,061 polymorphic SNPs were from the A-genome, 1,231 from B-genome, and 50 from the D-genome. Chromosome 4D represented the smallest linkage group with a total genetic distance of 5.5 cM, while chromosome 4A was the largest with 190.9 cM. After the linkage groups were developed, markers were removed manually which co-located at the same genetic positions and only

the marker with the least missing data was retained in the map. This resulted in a total of 762 markers across the genome each at a unique locus that were used for the downstream QTL mapping analysis (S Fig.1A and 1B). Chromosome 5A had highest number of markers (75) at unique loci, while 5D had the fewest number of markers (6). The average chromosomal length across the whole genome was 106 cM with a distance of 2.9 cM between two adjacent markers. Twenty-two gaps of more than 20 cM between the two adjacent markers were detected. Chromosomes 2B, 3B,4D, 5A, 6B, and 7A had no gaps greater than 20 cM while, chromosomes 2D, 3D, and 7D had 2 gaps and 4A, and 6D had three gaps. Molecular marker assay results indicated that COPIO is positive for *Sr2* and *Lr46/Sr58* genes, while Apav is negative for all tested known genes. GBS markers for 2NS/2AS translocation were positive for COPIO meaning that it carries *Sr38/Lr37/Yr17* linked genes (Table 7).

The QTL mapping procedure identified 18 genomic regions conferring field resistance to stem rust in all six testing environments. The parent line COPIO contributed 11 QTL associated with stem rust resistance in Kenya, Pakistan, and Saint Paul in a range of 2.5 to 24.6 LOD (logarithm of odds) values. Apav contributed 7 small effect QTL in Kenya and Saint Paul with a LOD values ranging from 2.7 to 5.1 (Table 6). A large effect QTL (*QSr.umn.2A.2*) mapped on chromosome 2A was contributed by COPIO and was detected in two environments in Saint Paul. This QTL explained the maximum phenotypic variation ($R^2$) ranging from 34 to 37 %. The LOD curve for this QTL is shown in Suppl. Figure 2. Three QTL were mapped on each chromosome 2A and 2B, while chromosomes 3A, 3B, and 6A had two QTL. Only one QTL was detected on

chromosomes 1B, 2D, 4B, 5D, 6D, and 7A. Detected QTL on each chromosome along with their respective environments are presented in Figure 3.

**Discussion**

Both parental lines were highly susceptible to the Ug99 race group (races TTKSK, TTKST, and TTKTT) at the seedling stage and we were confident that the field data from Kenya will allow us to map adult plant resistance to Ug99 in COPIO. Disease severity on COPIO was low (18 – 24%) during both seasons of field testing in Kenya under high disease pressure. COPIO was resistant at the seedling stage to the North American *Pgt* races except TTTTF, while susceptible to other important races except the Mexican race (RTR). Based on the stem rust seedling assays we postulated that COPIO has *Sr38* and Apav has *Sr8a* with possibly additional genes providing resistance against the race SCCSC. To confirm the postulation results *Pgt* races TMPKC and SCCSC were used for seedling assays. TPMKC is avirulent to *Sr38* and virulent to *Sr8a* and had a low infection type (0;) to COPIO but a high infection type (3+) to Apav. The *Pgt* race SCCSC is avirulent to both *Sr38* and *Sr8a* and showed low infection types (0; and ;1) against both parental lines (Table 1). Further confirmation of the presence of *Sr38* in COPIO was its high IT (3) when challenged against the *Pgt* races from Pakistan RRTTF (Virulent to *Sr38* + *Sr13*) and Yemen (TRTTF). Both races (RRTTF and TRTTF) are avirulent to *Sr8a* and had low infection types (2) on Apav. During the field evaluations in six environments rust development was uniform and a continuous disease distribution spectrum was observed across all six environments representing all phenotypic classes which indicated the resistance is likely quantitative. The Pearson correlation coefficients (*r*) among the final disease severities on Apav x COPIO RILs were significant in all

environments. Some environments were comparatively less correlated and some of the reasons for low correlations among the phenotyping environments can be due to the genotype by environment interactions (G X E). Multiple factors, like differences in environmental conditions, inoculum pressure and different rust pathogen races can contribute to G X E.

A total of 762 markers were used for QTL mapping analysis. Chromosome 5A had highest number of markers (75) at unique loci, while 5D had the fewest number of markers (6). The D genome had least representation in terms of polymorphic markers and unique independent segregating loci as compared to A and B genome (Table 5), which was not surprising because D genome is less represented in all genotyping platforms (Allen et al. 2011). Chromosome group 1 had only one QTL on 1B detected in Kenya 2016 main-season and Saint Paul 2016 at approximately 80 cM, which contributed to the phenotype up to 13 %. For Kenya 2016 off-season we observed an LOD curve in the same region, but it did not reach our minimum threshold of LOD value 2.5 to be declared a QTL. Quantitative gene *Sr58* has been mapped distally to the long arm of chromosome 1B (Suenega et al. 2003). *Sr58* confers quantitative APR to stem rust and is linked with other genes (*Lr46/Yr29/Pm39*) providing resistance to multiple fungal pathogens. Parent line COPIO which contributed this QTL (*QSr.umn.1B*) was positive for *Sr58* allele based on the marker screening (Table 7), so most likely the detected genomic region in our study is *Sr58*.

Chromosome group 2 possessed 7 QTL; three each on 2A, 2B, and one on 2D. All three QTL of chromosome 2A were distally mapped on the short arm and were detected in Pak17, Stp16, Stp17, and Ken16_OS environments. The QTL *QSr.umn.2A.1* was

detected only in one environment (Pak17) and mapped at the distal end of the short arm at 4.8 cM. Based on the genetic positions, it is believed to be a different QTL from the ones that were mapped at 11.04 cM (Suppl. Figure 2). The QTL *QSr.umn.2A.2* explained the maximum phenotypic variation ($R^2$ = 34 – 37%) in both Saint Paul environments and is believed to be the same QTL. The short arm of chromosome 2A in some wheat cultivars is known to carry a chromosomal translocation (2NS/2AS) which was initially introgressed into winter wheat 'VPM1' from *Triticum ventricosum* ((Helguera et al., 2003; Maia, 1967). The 2NS chromosomal segment has been reported to carry race specific rust resistance linked genes *Lr37*, *Yr17*, and *Sr38* (Helguera et al., 2003). Although the virulent races to this gene cluster have been identified, these genes are still being used in many parts of the world in combination with other effective genes (Robert et al. 1999). Based on GBS markers COPIO is positive for 2NS/2AS translocation which carries *Sr38*, *Lr37*, and *Yr17* genes, while Apav is negative. In our study, we also postulated *Sr38* gene in COPIO based on the seedling assays using North American stem rust races (Tables 1 and 2). QTL mapping procedure using the genome wide GBS markers mapped *Sr38* in COPIO and confirmed our hypothesis. *Sr38* gene was not detected in both environments in Pakistan because the inoculated race, RRTTF, is virulent to *Sr38*. We observed a QTL peak in the same region of *Sr38* in Kenya 16 off-season (Suppl. Figure 2). The African races (TTKSK, TTKTT) which were used for inoculation in Kenya are virulent to *Sr38*, so the detected QTL (*QSr.umn.2A.3*) in Ken16_OS is probably different from *Sr38*. In two Saint Paul environments, eight significant markers were associated with stem rust resistance, while six and two markers were associated with Ken16_OS and Pak17, respectively. In the QTL region two makers

were common in all four environments. In figure 3, QTLs are overlapping each other due to the association of common markers. CIMMYT data suggest that the 2NS translocation segment has slow rusting genes for leaf rust in addition to *Lr37*, so it is also possible that this segment carries some other QTL effective to Ug99 and other races in addition to *Sr38* gene (R. P Singh; Personal communication). Moreover, previous and recent studies also revealed that the 2NS segment carries genes for root-knot nematode resistance (Williamson et al. 2013), cereal nematode resistance (Jahier et al., 2001), and wheat blast resistance (Cruz et al. 2016). At this stage we were unable to differentiate *Sr38* gene from the other small effect QTL in the same region conferring resistance to stem rust in Pakistan and Kenya. It might be only *Sr38* and its residual effects are providing small effects in the environments (Pakistan, Kenya) where virulent pathotypes exist. This hypothesis would need to be tested by further investigations.

Chromosome 2B has 3 QTL detected in Kenya and Pakistan and were contributed by COPIO. The QTL *QSr.umn.2B.1* was detected in both seasons (Pak16, Pak17) in Pakistan. It mapped distally to the short arm and explained 7% of the phenotypic variation. QTL *QSr.umn.2B.2* (66.34 cM) was detected in one environment (Ken16_OS) with 5.6 LOD and explained 9% phenotypic variance. Previous studies have reported at least 15 QTL on chromosome 2B. Among these QTL, two were located on the short arm, seven were found on the long arm, and the arm location of six QTL was unknown (Yu et al. 2014). Additionally, 11 designated stem rust genes have also been reported on chromosome 2B. Stem rust genes *Sr39*, and *Sr36* have been mapped on the short arm. We rule out *Sr39* gene because it confers major seedling effects for Ug99 resistance but both parents in our study are susceptible to Ug99 at the seedling stage. We also eliminate the

possibility of *Sr36* gene because the Pakistani race RRTTF is virulent to *Sr36*. The QTL (*QSr.umn.2B.2*) identified on the long arm in Ken16_OS is in the region of *Sr9h* gene. The stem rust gene *Sr9h* is susceptible against the Ug99 race TTKSF+ but remains resistant to the original Ug99 race TTKSK (Rouse at al. 2014). The seedling susceptibility of both parents against the Ug99 clearly rule out *Sr9h* possibility. QTL *QSr.umn.2B.3* was mapped on the distal part of the long arm with a range of 05 – 14% phenotypic variation. This genomic region was detected in two Pakistani and one Kenyan environments. The seedling gene *Sr28* has been mapped distally on the long arm. This gene has been reported to confer field resistance to Ug99 and other stem rust races (Jin et al. 2007; Rouse et al. 2012). We eliminated *Sr28* possibility because both parents of the mapping population are susceptible to Ug99 at the seedling stage.

Only one genomic region was identified on chromosome 2D which explained 5% of the phenotypic variation in stem rust severity during the off-season of Kenya 2016. This QTL was contributed by Apav and mapped towards the end of the short arm (0.1 cM). This region of chromosome 2D contains *Sr32* which is effective for the Ug 99 races. *Sr32* was identified in *Aegilops speltoides* and introgressed into bread wheat (McIntosh et al. 1995; Friebe et al. 1996). Apav is highly susceptible to Ug99 *Pgt* races at seedling and under field conditions and is not known to carry *Aegilops speltoides* in the pedigree, therefore we eliminate *Sr32* possibility. *QSr.umn.2D* might be a new small effect QTL in Apav which has not been reported previously.

The chromosome homeologs of group 3 carried four QTL, with two each on 3A and 3B. Both QTL of 3A were contributed by Apav and identified only in Saint Paul environments. The QTL *QSr.umn.3A.1* was mapped distal to the short arm (3.9 cM) in

Stp16 and explained 5% phenotypic variation. This same QTL peak also appeared for Stp17 environment but did not reach the significant LOD threshold. The other QTL (*QSr.umn.3A.2*) was mapped at 50 cM and identified in both Saint Paul environments with $R^2$ value ranging from 4-5 %. The LOD peak was also observed below the threshold level for Kenya 2016 main-season in the same region. The stem rust gene *Sr35* has been mapped on the long arm of chromosome 3A which is also effective for the Ug99 group races (Zhang et al. 2010). This gene was transferred to hexaploid wheat from *Triticum monococcum* (McIntosh et al. 1984). *Sr35* was reported to be mapped by Haile et al. 2012 in durum wheat and significant markers linked with *Sr35* gene were spanning 43.8 to 134 cM region on the genetic map. However, it is unlikely that *Sr35* has been introgressed from *T. monococcum* into the parents of this durum population. *Sr35* is a recent introgression from *Triticum monococcum* and definitely the susceptible parent Apav does not carry this gene. Moreover, *Sr35* gene is effective for Ug99 races, while Apav is highly susceptible both at seedling and adult plant stage. It is more likely that *QSr.umn.3A.2* is a unique small effect race specific QTL in Apav.

On chromosome 3B, the QTL mapped on the short arm was detected in both Saint Paul environments. It was contributed by COPIO and $R^2$ value ranged from 4-7 %. The distal end of short arm of chromosome 3B is known to harbor *Sr2* gene. The marker assay of *Sr2* was positive for COPIO (Table 7), so we are confident that the identified genomic region in our study is *Sr2*. The other QTL on 3B (*QSr.umn.3B.2*) was mapped on the long arm (53 cM) and was only detected in Kenya main season. This QTL was contributed by Apav and explained 8% of the phenotypic variation. Apav is a derived line from a cross of Avocet S and Pavon76. Njau et al. (2012) used a subset of 76 RILs from

Avocet S x Pavon76 mapping population to identify the APR for stem rust Ug99. For three years field testing a QTL (*QSr.cim-3B*) contributed by Pavon76 was mapped between 52- 57 cM which explained 4.8 – 10.8 % phenotypic variation. It is likely that the QTL (*QSr.umn.3B.2*) identified in our study is the same previously reported by Njau et al. 2012.

A small effect QTL (*QSr.umn.4B*) was identified on the distal end (4.3cM) of chromosome 4B. This QTL was contributed by Apav and explained 4% of the phenotypic variation in Saint Paul 2017. LOD curves were observed in Saint Paul 2016 and Kenyan environments in the same region but were non-significant. No designated stem rust gene is known to be located distally on the short arm of chromosome 4B. A genomic region was identified using an association panel of 170 wheat lines from CIMMYT elite spring wheat yield trials (ESWYT) (Crossa et al. 2007). DArT marker *wPt8650* at 7.8 cM was significantly associated with stem rust resistance and explained 9% phenotypic variance. The 4B QTL detected in our study is likely the same region previously reported in CIMMYT association mapping study. A consensus map was developed for wheat stem rust Ug99 using the information on loci associated with stem rust from 21 mapping studies (Yu et al. 2014). This consensus map revealed that the marker trait association described by Crossa et al. (2007) on the distal end of chromosome 4B is potentially a new putative source of small effect APR gene for stem rust.

The QTL *QSr.umn.5D* was detected in only one environment (Pak16) on the long arm (61.4 cM) of chromosome 5D. This QTL was contributed by COPIO and explained 11% of the phenotypic variance. The *Sr53* gene effective against stem rust including the Ug99 race TTKSK is located at approximately 57 cM on the long arm of chromosome

5D. This gene was introgressed into bread wheat from *Aegilops geniculata* (Liu et al.2011). Another QTL (*QSr.sun-5DL*) was identified in Indian wheat cultivar HD2009 which corresponds to the location of *Sr30* and conferred adult plant resistance (Kaur et al.2009). The significant markers for this QTL spanned on 60-85 cM and explained 20-44% phenotypic variation in three environments. We eliminate the possibility of both genes (*Sr53* and *Sr30*) because COPIO do not have *Aegilops geniculata* in the pedigree and *Pgt* race RRTTF inoculated in Pakistan nurseries is virulent to *Sr30*. It is more likely that 5D genomic region in COPIO represents a new medium effect race specific QTL.

The chromosome group 6 has three QTL; two on 6A and one on 6D. One QTL of 6A (*QSr.umn.6A.1*) was identified in Ken16_OS and mapped on the distal end (14 cM). It was contributed by COPIO and explained 12% of the phenotypic variance. No known designated stem rust gene is in this region except *Sr8*, of which *Sr8115B1* may be an allele of *Sr8* locus and effective to some variants of Ug99 (Nirmala et al. 2017). Yu et al. 2011, reported a QTL conferring APR in CIMMYT lines that were screened for three years in Kenya. Association mapping using DArT markers indicated that markers *wPt-6520* (2.53 cM) and *wPt -4016* (3.25 cM) on chromosome 6A are associated with stem rust Ug99 resistance at adult plant stage. The other QTL (*QSr.umn.6A.2*) on 6A was contributed by Apav and was identified in Ken16_MS. This QTL is located on the long arm proximal to the centromere (58 cM). The long arm of chromosome 6A carries three qualitative stem rust genes (*Sr13, Sr26, Sr52*) which are effective against the stem rust Ug99 race TTKSK. Among these three genes, *Sr52* displays temperature sensitive resistance. It is effective at low temperature while ineffective at higher temperature (Qi et al. 2011). We eliminate the possibility of these genes because Apav is highly susceptible

to Ug99 race group at the seedling stage (Table 1). It is more likely that this QTL is a new source of resistance to stem rust Ug99. The 6D QTL (*QSr.umn.6D*) was identified in only one environment (Pak17), but LOD peaks were also observed in the same region for Kenyan environments which were below the threshold to declare a QTL. This QTL was mapped distal to the short arm (7.5 cM) and was contributed by COPIO. Stem rust gene *SrTA10187* is already known to be located on 6DL distal region and effective to Ug99 and many North American races (Wiersma et al. 2016). Other genes in this region are *SrCad* and *Sr42*. *SrCad* provided moderate resistance to stem rust Ug99 when tested in Kenya and the closest maker (*CFD49*) for this gene is at 7.5 cM on chromosome 6DS (Hiebert et al. 2010). We rule out the possibility of *SrTA10187* and *SrCad* because both genes confer resistance to Ug99, while COPIO is susceptible to Ug99 at the seedling stage.

Chromosome 7A harbors one QTL (*QSr.umn.7A*) on the short arm and it was detected in both environments in Pakistan (Pak16, Pak17). It was contributed by Apav and explained 3-7% of the phenotypic variation with an average additive effect of 5.5%. Stem rust gene *Sr22* and a major QTL (*QSr.abr- 7AL*) have been reported on the long arm of chromosome 7A, so we eliminate these two possibilities for the QTL identified in our study because it mapped on the short arm. No known qualitative gene for Sr resistance has been reported on the short arm of chromosome 7A. Few QTL conferring resistance to Ug99 have been identified in bread wheat mapping populations (PBW343/Kenya Nyangumi; PBW343/Muu) and located at the distal end (Sing et al. 2013; Yu et al. 2014). *QSr.umn.7A* is closer to the centromere and only detected during two seasons in Pakistan, so might be a new race specific APR QTL.

## Conclusions

The wheat line COPIO has a consistent adult plant resistance to stem rust including the Ug99 races in almost all testing environments. It has known quantitative resistance genes *Sr58* and *Sr2* on chromosomes 1BL and 3BS, respectively. We also postulated stem rust gene *Sr38* in COPIO which comes from 2NS/2AS translocation and provides qualitative resistance to some *Pgt* races. Genome wide markers provided a confirmation of *Sr38* gene in COPIO on chromosome 2A. It is more likely that 2NS/2AS translocation region has some new APR gene providing resistance to Ug99 and other *Pgt* races which needs further investigation. Some small to medium effect QTL which have been either previously reported (*QSr.umn.3B.2; QSr.umn.4B*) or potentially a new source of resistance (*QSr.umn.2A.1; QSr.umn.2A.3*; *QSr.umn.5D*; *QSr.umn.7A*) are also contributing quantitative stem rust resistance in the population. The results of this study show that COPIO has a handful of genes that provide both qualitative and quantitative resistance. This line can be used in wheat breeding programs for recombining with other effective genes to develop more durable resistance in wheat cultivars.

Table 1: Seedling infection types of both parents to North American, Ug99 and other important stem rust races

| Races | Isolates | Apav | COPIO |
|---|---|---|---|
| **North American races** | | | |
| QFCSC | 06ND76C | 3 | 0; |
| QTHJC | 75ND717C | 3+ | 0;1 |
| RKQQC | 99KS76A-1 | 3+ | 0; |
| TPMKC | 74MN1409 | 3+ | 0; |
| TTTTF | 01MN84A-1-2 | 3+ | 3+ |
| SCCSC | 09ID73-2 | ;1 | 0; |
| **Ug 99 races** | | | |
| TTKSK | 04KEN156/04 | 3 | 3+ |
| TTKST | 06KEN19-V-3 | 33+ | 3+ |
| TTKTT | 14KEN58-1 | 33+ | 3+ |
| **Other important races** | | | |
| TRTTF | 06YEM34-1 | 2 | 33+ |
| TKTTF | 13ETH18-1 | 3+ | 3+ |
| RRTTF | 10PAK05-1 | 2 | 3 |
| RTR | - | 3+ | X |

Table 2: Races of *Puccinia graminis f. sp. tritici* used to screen both the parents at seedling stage and RIL population in the field

| Races | Isolate | Virulence/avirulence formula |
|---|---|---|
| QFCSC | 06ND76C | 5,8a,9a,9d,9g,10,17,21, McN/6,7b,9e,9b,11,24,31,30,36,38, Tmp |
| QTHJC | 75ND717C | 5,6,8a,9b,9d,9g,10,11,17,21, McN/7b,9a,9e,24,30,31,36,38, Tmp |
| MCCFC | 59KS19 | 5,7b,9g,10,17, McN,Tmp/6,8a,9a,9d,9e,9b,11,21,24,31,30,36,38 |
| RCRSC | 77ND82A | 5,7b,9a,9b,9d,9g,10,17,21,36, McN/6,8a,9e,11,24,30,31,38, Tmp |
| RKQQC | 99KS76A | 5,6,7b,8a,9a,9b,9d,9g,21,36, McN/9e,10,11,17,24,30,31,38, Tmp |
| TPMKC | 74MN1409 | 5,7b,8a,9d,9e,9g,10,11,17,21,36, Tmp,McN/6,9a,9b,24,30,31,38 |
| TTTTF | 01MN84A-1-2 | 5,6,7b,8a,9a,9b,9d,9e,9g,10,11,17,21,30,36,38, McN, Tmp/24,31 |
| SCCSC | 09ID73-2 | 5,9a,9d,9e,9g,10,17,21, McN/6,7b,8a,9b,11,24,30,31,36,38, Tmp |
| $TTKSK_a$ | 04KEN156/04 | 5,6,7b,8a,9a,9b,9d,9e,9g,10,11,17,30,31,38, McN/24,36, Tmp |
| TTKST | 06KEN19-V-3 | 5,6,7b,8a,9a,9b,9d,9e,9g,10,11,17,24,30,31,38, McN/36, Tmp |
| TTKTT | 14KEN58-1 | 5,6,7b,8a,9a,9b,9d,9e,9g,10,11,17,21,24,30,31,38, McN,Tmp/36 |
| TRTTF | 06YEM34-1 | 5,6,7b,9a,9b,9d,9e,9g,10,11,17,21,30,36,38, McN,Tmp/8a,22,24,31,35 |
| RRTTF | 10PAK05-1 | 5 6 7b 9a 9b 9d 9g 10 11 17 21 30 36 38 McN + 1A.1R/8a 9e 24 31 |
| RTR | | 5,6,7b,8a,8b,9a,9b,9d,9f,9g,11,15,17,21, 28,34,36/7a,9e,10,12,13,14,22,23,24,25,26,27,29,30,31,32,33,35, Dp2, H |
| TKTTF | 13ETH18-1 | 5,6,7b,8a,9a,9b,9d,9e,9g,10,11,17,21,30,36,38, McN,Tmp/11,24,31 |

Avirulence for *Sr21* is not included in the formula for races TTKSK or TTKST because the reaction of these races is not certain

Table 3: Final stem rust severity (%) based on COI on both parents, population mean, standard deviation and range of stem rust coefficient of infection in all environments

| | FDS (%) on parents | | | | | | |
|---|---|---|---|---|---|---|---|
| Environments | Apav | COPIO | Pop. Size | Pop. mean | Std. Deviation | Low range | High range |
| Stp16 | 70 | 5 | 176 | 30.5 | ±21.8 | 1.0 | 85 |
| Stp17 | 60.9 | 1 | 176 | 27.6 | ±21.5 | 1.0 | 70 |
| Ken16_OS | 100 | 24 | 176 | 69.8 | ±22.3 | 13.5 | 100 |
| Ken16_MS | 100 | 18 | 176 | 30.3 | ±17.3 | 3 | 100 |
| Pak16 | 43.5 | 4 | 157 | 31.8 | ±20.8 | 2 | 90 |
| Pak17 | 60.9 | 18.4 | 176 | 52.5 | ±15.8 | 12 | 90 |

Stp = Saint Paul, Ken = Kenya, Pak = Pakistan

16 and 17 are the years 2016 & 2017 during which nurseries were evaluated for stem rust in field

For Kenya ‘OS’ and ‘MS’ represents off-season and main season respectively.

Table 4: Pearson correlation (*r*) among coefficient of infection values based on final disease severities on Apav x COPIO RILs in six environments for stem rust.

| | Stp16 | Stp17 | Ken16_OS | Ken16_MS | Pak16 | Pak17 |
|---|---|---|---|---|---|---|
| Stp16 | 1 | | | | | |
| Stp17 | 0.79** | 1 | | | | |
| Ken16_OS | 0.42** | 0.46** | 1 | | | |
| Ken16_MS | 0.30** | 0.27** | 0.44** | 1 | | |
| Pak16 | 0.29** | 0.33** | 0.41** | 0.18* | 1 | |
| Pak17 | 0.21** | 0.24** | 0.46** | 0.31** | 0.64** | 1 |

*, ** Correlation is significant at the 0.05 and 0.01 level, respectively

Stp = Saint Paul, Ken = Kenya, Pak = Pakistan

16 and 17 are the years 2016 & 2017 during which nurseries were evaluated for stem rust in field

For Kenya ‘OS’ and ‘MS’ represents off-season and main season respectively.

Table 5: Density and marker distribution across all 21 chromosomes of the Apav x COPIO mapping population.

| Chromosome | Total SNP | Unique loci | Length (cM) | Marker density | No. of gaps (>20cM) | cM Intervals |
|---|---|---|---|---|---|---|
| 1A | 129 | 42 | 67 | 1.6 | 1 | 5.12 - 28.82 |
| 1B | 208 | 42 | 88 | 2.1 | 1 | 36.84-60.28 |
| 1D | 29 | 16 | 102 | 6.4 | 1 | 70.40-101.75 |
| 2A | 113 | 34 | 132 | 3.9 | 1 | 98.5-132.4 |
| 2B | 280 | 62 | 91 | 1.5 | 0 | |
| 2D | 39 | 13 | 69 | 5.3 | 2 | 10.17-33.5, 42.38-69.39 |
| 3A | 111 | 45 | 122 | 2.7 | 1 | 18.54-49.34 |
| 3B | 214 | 57 | 138 | 2.4 | 0 | |
| 3D | 71 | 23 | 171 | 7.4 | 2 | 13.5-45.36, 83.66-110.12 |
| 4A | 202 | 44 | 191 | 4.3 | 3 | 0.93-32.25, 48.59-72.48, 147.0-190.4 |
| 4B | 69 | 23 | 73 | 3.2 | 1 | 42.69-70.15 |
| 4D | 13 | 6 | 6 | 0.9 | 0 | |
| 5A | 205 | 75 | 155 | 2.1 | 0 | |
| 5B | 153 | 53 | 150 | 2.8 | 1 | 69.09-100.17 |
| 5D | 7 | 6 | 79 | 13.1 | 1 | 52.43-78.83 |
| 6A | 80 | 28 | 76 | 2.7 | 1 | 13.48-37.37 |
| 6B | 178 | 51 | 58 | 1.1 | 0 | |
| 6D | 50 | 23 | 118 | 5.1 | 3 | 7.46-30.59, 30.59-53.84, 89.89-103.02 |
| 7A | 221 | 62 | 118 | 1.9 | 0 | |
| 7B | 129 | 29 | 65 | 2.2 | 1 | 15.38-49.5 |
| 7D | 41 | 28 | 163 | 5.8 | 2 | 40.93-64.07, 116.17-140.48 |
| Mean | 121.0 | 36.3 | 106 | 2.9 | 1 | |
| Total | 2542 | 762 | 2233 | | 22 | |

Table 6: Quantitative trait loci (QTL) detected in six environments and associated with stem rust resistance in Apav x COPIO RIL mapping population

| Chr. [a] | Environment [b] | QTL | Peak marker | Position (cM) | LOD | $R^2$(%) [c] | Add [d] |
|---|---|---|---|---|---|---|---|
| 1B | Ken16_MS | QSr.umn.1B | chr1B_670401542 | 79.91 | 7.32 | 0.13 | 6.45 |
| | Stp16 | QSr.umn.1B | chr1B_673699294 | 80.86 | 2.51 | 0.03 | 3.63 |
| 2A | Pak17 | QSr.umn.2A.1 | chr2A_21005775 | 4.81 | 3.53 | 0.07 | 4.89 |
| | Stp16 | QSr.umn.2A.2 | chr2A_21273919 | 11.04 | 24.67 | 0.37 | 14.29 |
| | Stp17 | QSr.umn.2A.2 | chr2A_21273919 | 11.04 | 21.61 | 0.34 | 13.56 |
| | Ken16_OS | QSr.umn.2A.3 | chr2A_17002870 | 10.97 | 5.37 | 0.09 | 7.45 |
| 2B | Pak16 | QSr.umn.2B.1 | chr2B_16009483 | 2.2 | 3.32 | 0.07 | 5.95 |
| | Pak17 | QSr.umn.2B.1 | chr2B_16009483 | 3.2 | 3.44 | 0.07 | 4.13 |
| | Ken16_OS | QSr.umn.2B.2 | chr2B_615353135 | 66.34 | 5.61 | 0.09 | 7.63 |
| | Ken16_MS | QSr.umn.2B.3 | chr2B_767379363 | 77.86 | 3.07 | 0.05 | 4.23 |
| | Pak16 | QSr.umn.2B.3 | chr2B_769717318 | 85.57 | 4.85 | 0.12 | 7.95 |
| | Pak17 | QSr.umn.2B.3 | chr2B_769717318 | 85.57 | 6.73 | 0.14 | 6.44 |
| 2D | Ken16_OS | QSr.umn.2D | chr2D_9262372 | 0.01 | 2.76 | 0.05 | -5.04 |
| 3A | Stp16 | QSr.umn.3A.1 | chr3A_13035037 | 3.95 | 5.06 | 0.06 | -5.52 |
| | Stp16 | QSr.umn.3A.2 | chr3A_69503729 | 50.25 | 3.04 | 0.04 | -4.59 |
| | Stp17 | QSr.umn.3A.2 | chr3A_69503729 | 50.25 | 4.11 | 0.05 | -5.57 |
| 3B | Stp16 | QSr.umn.3B.1 | chr3B_6071517 | 25.61 | 3.75 | 0.04 | 4.58 |
| | Stp17 | QSr.umn.3B.1 | chr3B_8634715 | 28 | 5.02 | 0.07 | 5.79 |
| | Ken16_MS | QSr.umn.3B.2 | chr3B_225453676 | 53.57 | 4.29 | 0.08 | -4.92 |
| 4B | Stp17 | QSr.umn.4B | chr4B_4852517 | 4.26 | 3.00 | 0.04 | -4.43 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 5D | Pak16 | QSr.umn.5D | chr5D_422348015 | 61.44 | 2.62 | 0.11 | 7.45 |
| 6A | Ken16_OS | QSr.umn.6A.1 | chr6A_2408343 | 14.49 | 6.29 | 0.12 | 7.91 |
| | Ken16_MS | QSr.umn.6A.2 | chr6A_581457113 | 58.13 | 3.98 | 0.08 | -5.18 |
| 6D | Pak17 | QSr.umn.6D | chr6D_7504260 | 7.47 | 2.55 | 0.05 | 3.58 |
| 7A | Stp16 | QSr.umn.7A | chr7A_47772026 | 24.05 | 6.27 | 0.07 | -6.08 |
| | Stp17 | QSr.umn.7A | chr7A_47772026 | 24.05 | 2.75 | 0.03 | -4.01 |

[a] Chromosomes on which QTL was detected

[b] Phenotyping environments for stem rust (Stp16 = Saint Paul 2106, Stp17 = Saint Paul 2107, Ken16_OS = Kenya 2016 off-season, Ken16_MS = Kenya 2016 main-season, Pak16 = Pakistan 2016, Pak17 = Pakistan 2017)

[c] Maximum phenotypic variance explained by the QTL

[d] Maximum additive effects of the QTL, values with negative sing (-) indicate that the QTL is contributed by Apav

Table 7: Molecular marker assays on both parents for the known APR and seedling stem rust genes

| Genes | Chromosome | Marker | Apav | COPIO |
|---|---|---|---|---|
| *Sr57/Lr34/Yr18* | 7DS | *csLV34* | – [a] | –[a] |
| *Sr58/Lr46/Yr29* | 1BL | *csLV46G22* | – | + |
| *Sr2/Yr30* | 3BS | *gwm533* | – | + |
| *Sr55/Lr67/Yr46* | 4DL | *Lr67SNP* | – | – |
| *Sr38/Lr37/Yr17* | 2AS | *GBS* | – | + |

[a] + and – represent presence or absence of the gene, respectively

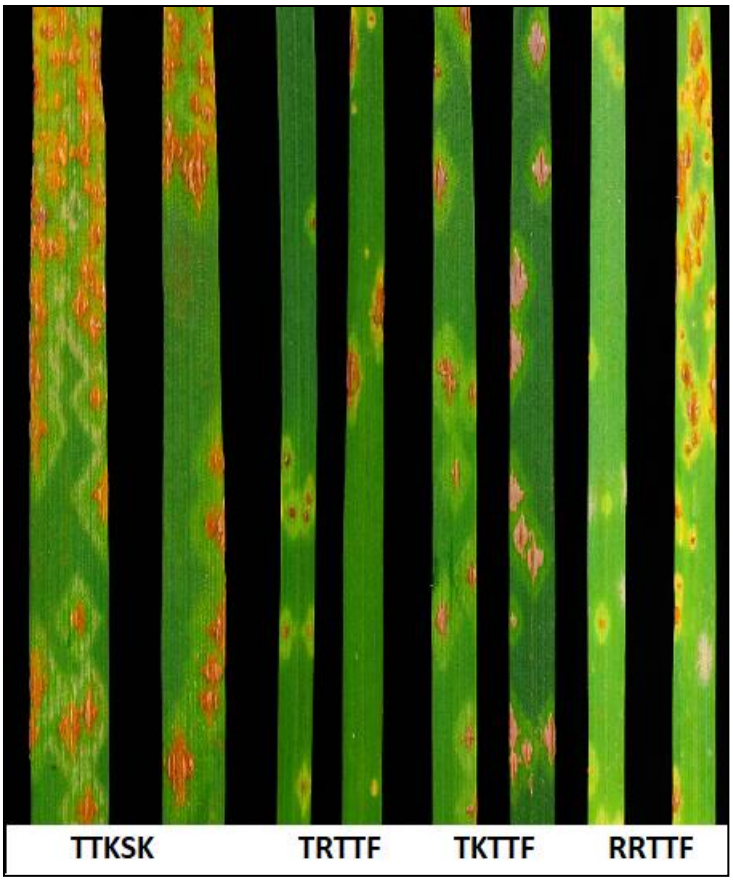



Figure 1: Seedling reaction of *Pgt* races to both parents of the RIL mapping population. Left side of each panel is 'Apva' and right side is 'COPIO'. TTKSK (Ug99, Virulent to *Sr31*), TRTTF (from Yemen), TKTTF (from Ethiopia) and RRTTF (only predominant race from Pakistan).

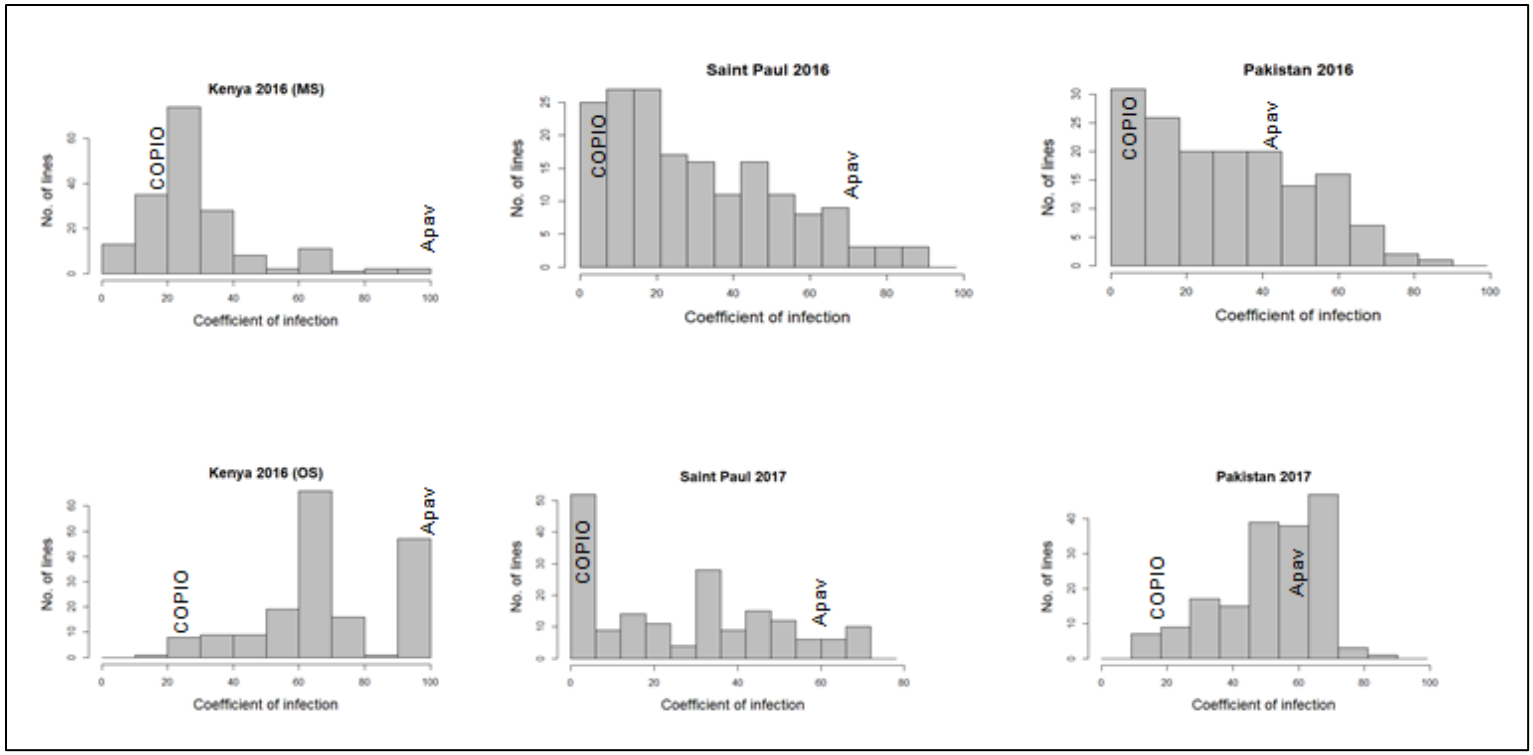



Figure 2: Frequency distribution of stem rust coefficient of infection (COI) in the Apav/COPIO RIL mapping population in six field environments. Parents (Apav, COPIO) shown on the bars represent the COI values in each environment

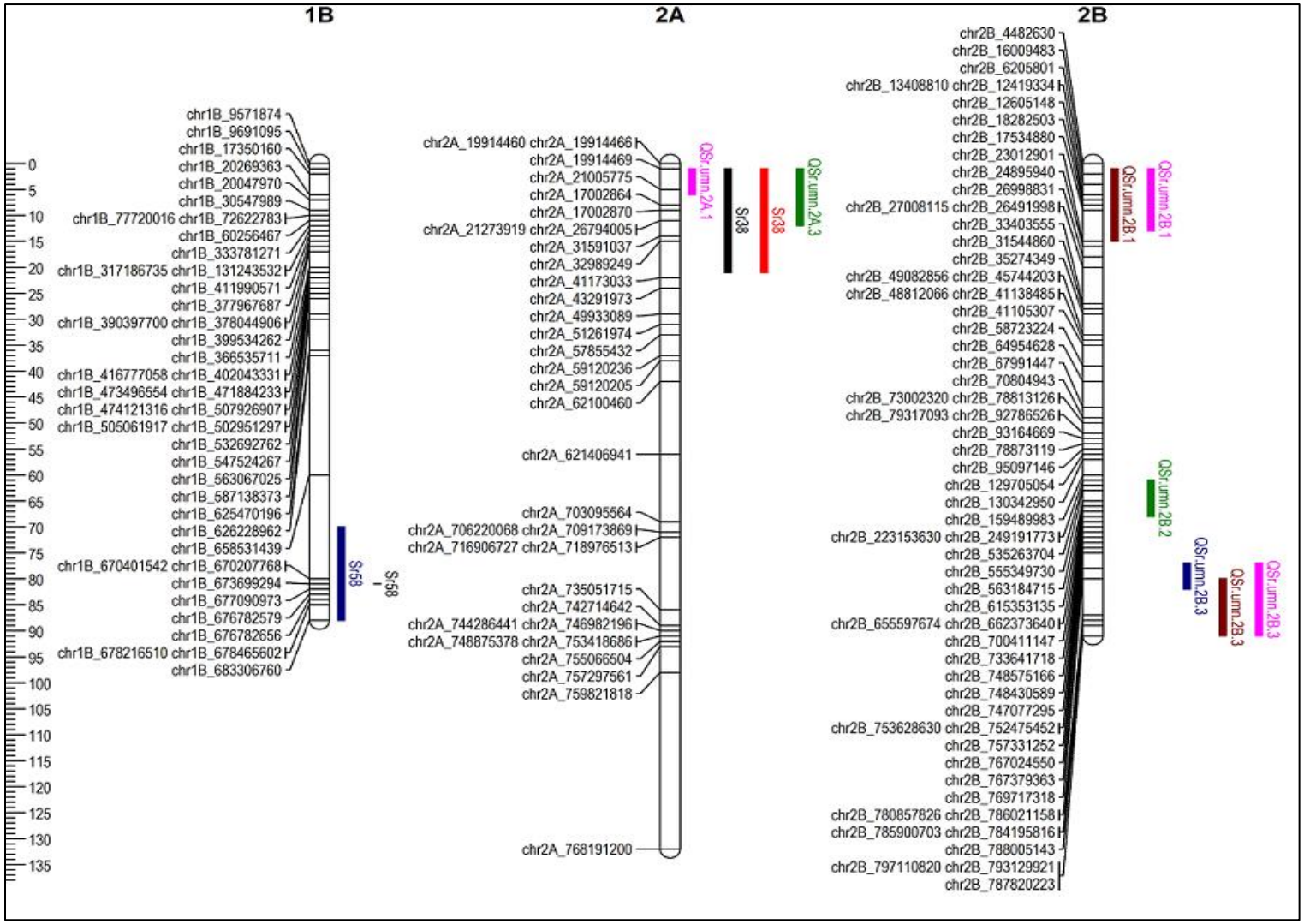



Figure 3 (continued)

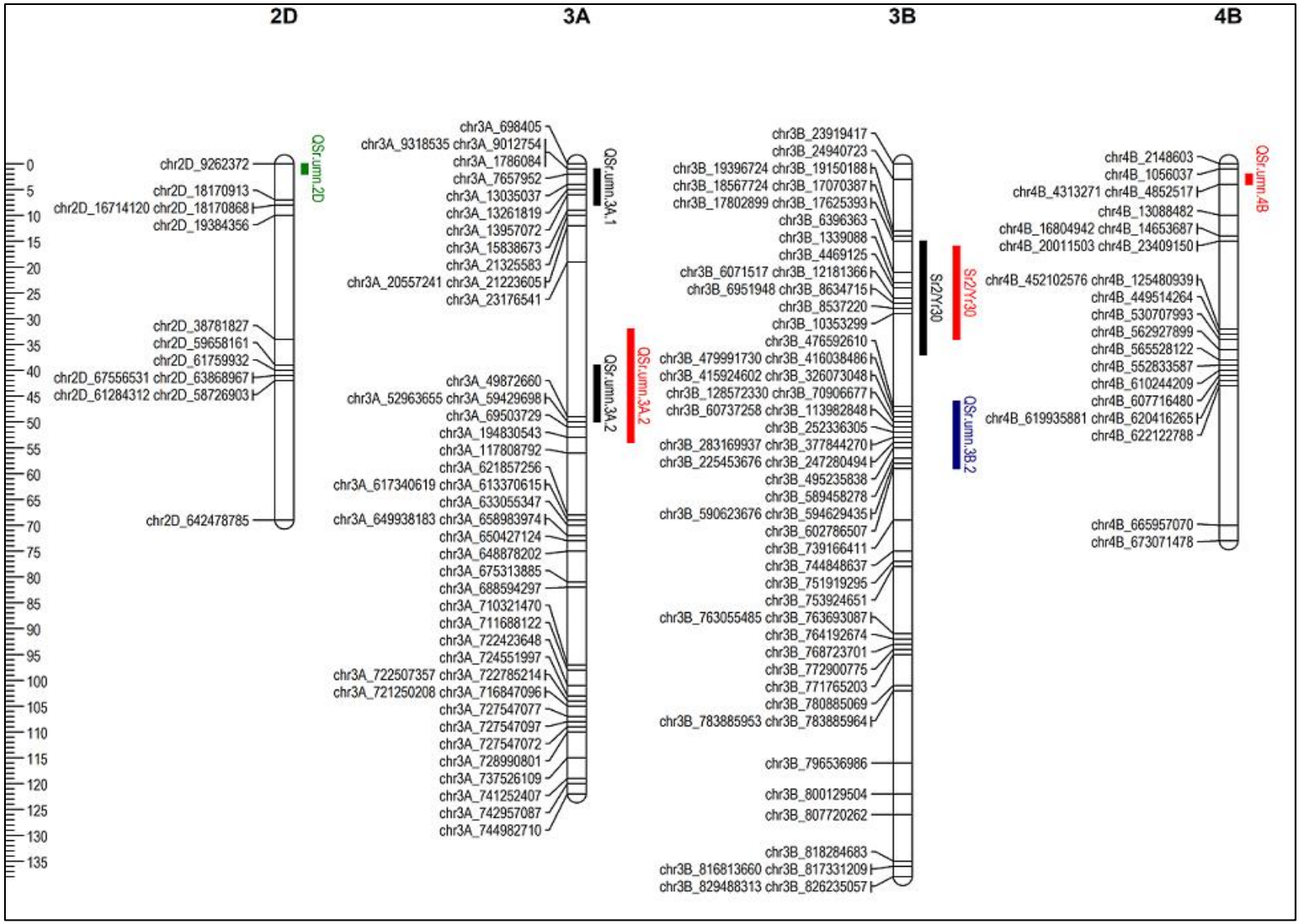



Figure 3 (continued)

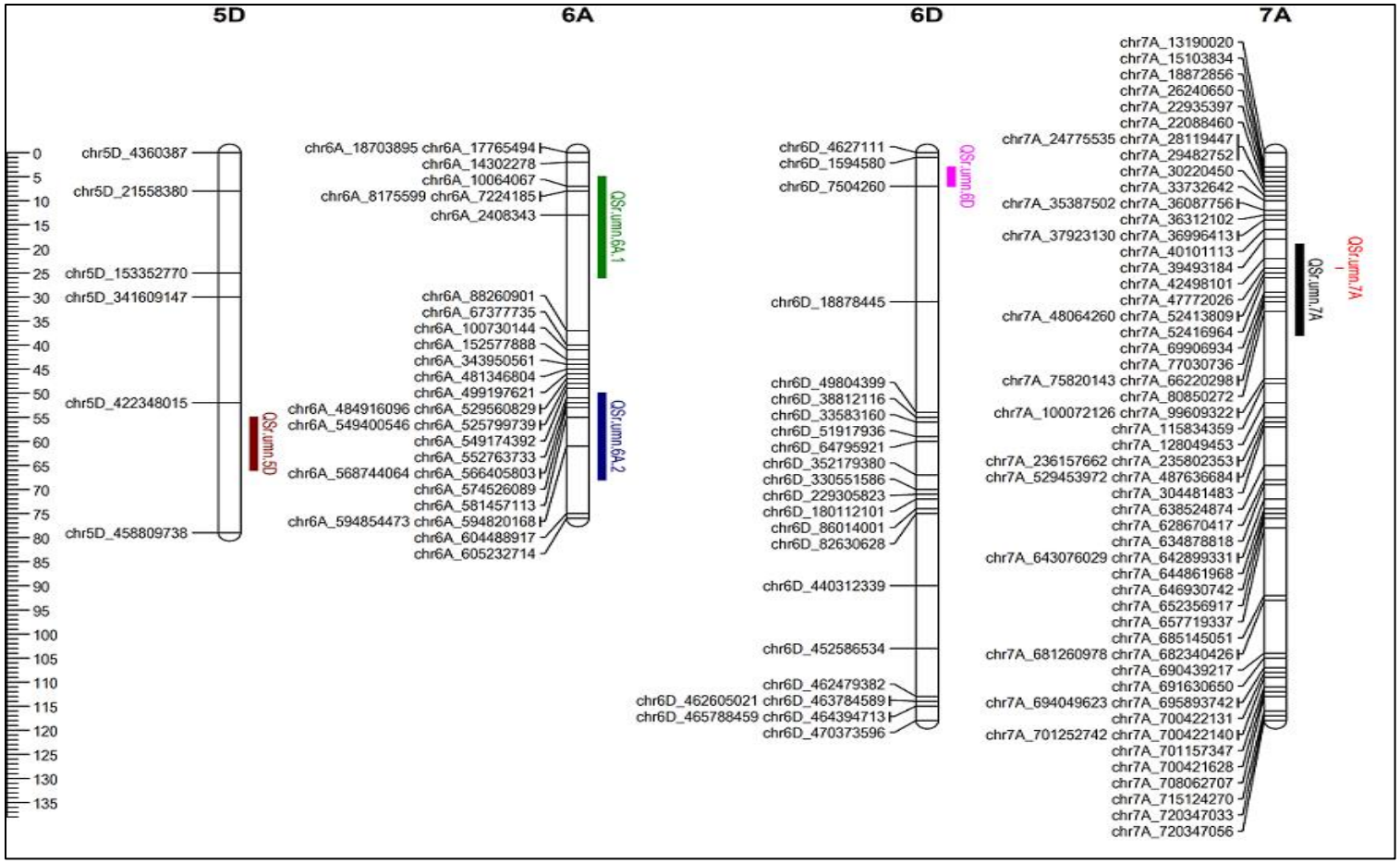


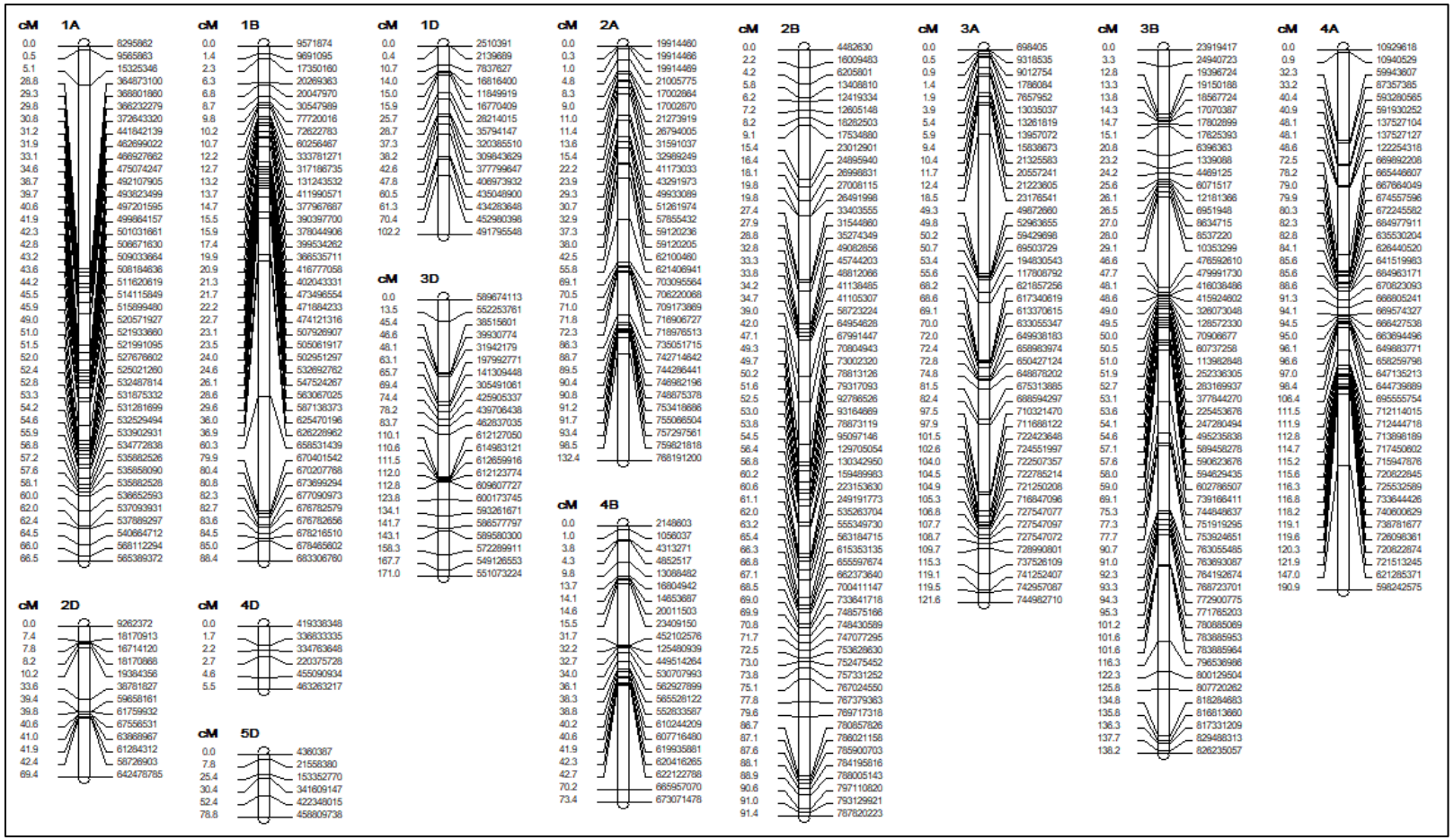

Figure 3: Chromosomes on which stem rust QTL were detected across six environments. GBS SNP markers are on the left side of each chromosome and the detected QTL is on the right side shown in colored bars perpendicular to the chromosomes. Black, red, dark green, blue, brown, and pink colors represent that QTLs were detected in Saint Paul 2016, Saint Paul 2017, Kenya 2016 off-season, Kenya 2016 main-season, Pakistan 2016, and Pakistan 2017, respectively. Known genes were detected/predicted on chromosomes 1B (*Sr58*), 2A (*Sr38*), and 3B (*Sr2/Yr30*). QTL on chromosomes 3B (*QSr.umn.3B.2*) and 4B (*QSr.umn.4B*) have been reported in previous studies, while QTL on chromosomes 2A (*QSr.umn.2A.1, QSr.umn.2A.3*), 5D (*QSr.umn.5D*), 6A (*QSr.umn.6A.2*) and 7A (*QSr.umn.7A*) are potentially new sources of resistance.

S. Table 1: Genotyping-by-sequencing (GBS) markers used to assay 2NS/2AS translocation in Apav and COPIO

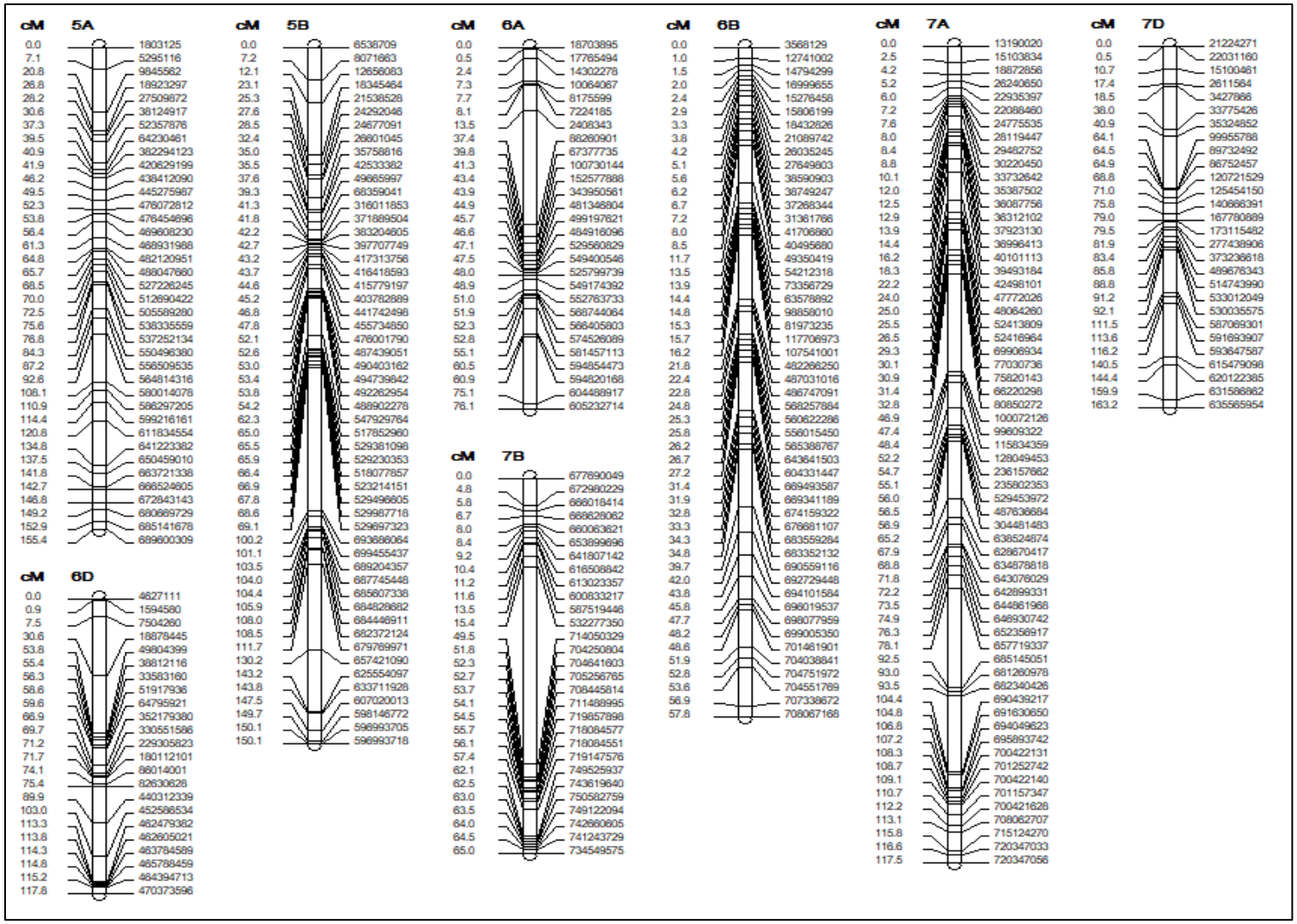
| Marker [a] | Chromosome | Gene | Apav | COPIO |
|---|---|---|---|---|
| *2A_1872142* | 2AS | *Lr37/Sr38/Yr19* | –[b] | +[b] |
| *2A_2800711* | 2AS | *Lr37/Sr38/Yr19* | – | + |
| *2A_8142744* | 2AS | *Lr37/Sr38/Yr19* | – | + |
| *2A_14418760* | 2AS | *Lr37/Sr38/Yr19* | – | + |
| *2A_15449240* | 2AS | *Lr37/Sr38/Yr19* | – | + |
| *2A_15755581* | 2AS | *Lr37/Sr38/Yr19* | – | + |
| *2A_15962301* | 2AS | *Lr37/Sr38/Yr19* | – | + |
| *2A_18468495* | 2AS | *Lr37/Sr38/Yr19* | – | + |
| *2A_18495181* | 2AS | *Lr37/Sr38/Yr19* | – | + |
| *2A_19902461* | 2AS | *Lr37/Sr38/Yr19* | – | + |

[a] = The numbers succeeding 2A_ refers to the physical position of the marker in the reference genome (RefSeq v1.0)

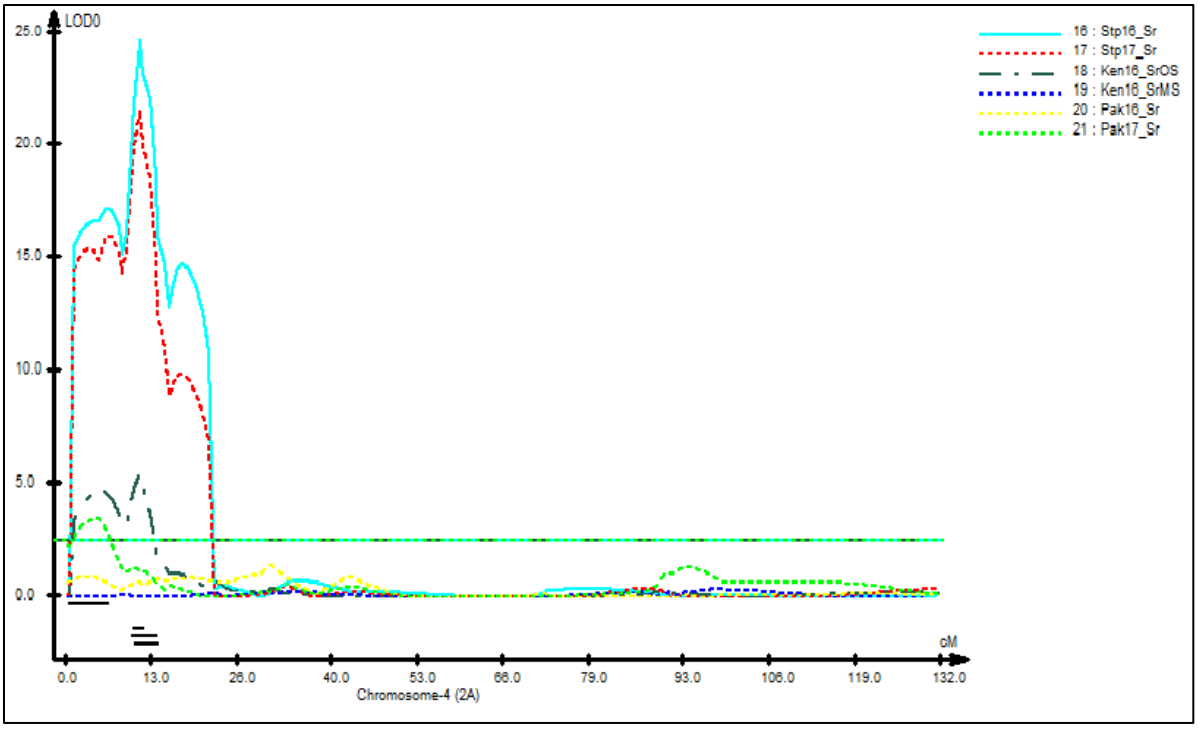
[b] = + and – represent presence or absence of the gene, respectively




S Figure 1A: Linkage maps of the Apav x COPIO population with unique loci; GBS SNP index numbers on the right and cM distance on the left. Chromosomal identities are shown above each linkage group.




S Figure 1B: Linkage maps of the Apav x COPIO population with unique loci; GBS SNP index numbers on the right and cM distance on the left. Chromosomal identities are shown above each linkage group.




S Figure 2: Large effect QTL mapped on chromosome 2A, associated with resistance to North American and African stem rust races in field conditions. X-axis represent the logarithm of odds (LOD) values and Y-axis indicates genetic distance in centimorgans (cM) on chromosome 2A. Horizontal dotted line along the X-axis is the threshold level of LOD value on which a QTL was declared significant.

# Chapter 2

## Molecular Characterization of Genomic Regions for Adult Plant Resistance to Leaf Rust in Spring Wheat Mapping Population

### Introduction

Leaf rust is a common disease of hexaploid wheat (*Triticum aestivum*), tetraploid wheat (*Triticum turgidum*) and triticale – a cross of wheat and rye (Roelfs et al. 1992). It is caused by the fungal pathogen *Puccinia triticina* (hereafter abbreviated as *Pt*) formerly known as *Puccinia recondita* f. sp. *tritici*. Leaf rust is less destructive than stem rust (*Puccinia graminis* f. sp. *tritici*) and stripe rust (*Puccinia striiformis* f. sp. *tritici*) but is responsible for greater losses due to its frequent occurrence and widespread distribution worldwide (Huerta-Espino et al. 2011; Kolmer 2005, Roelf et al. 1992). Leaf rust losses are severe when susceptible varieties are infected at early growth stages (Marasas et al. 2004). In North America, the *Pt* pathogen overwinters in Mexico and Southern parts of the US and spores move towards the North through the Central and Northern great plains during the spring or early summer (Fetch et al. 2011). This rust movement route in the US is commonly known as *Puccinia* pathway. Leaf rust epidemics in the North American great plains during 2007 caused yield losses of 14% across the entire state of Kansas (Kolmer et al. 2010). Other than causing yield losses, leaf rust infection also affects grain quality and reduces protein content (Bolton et al. 2008). Timely application of fungicides can control leaf rust and other rusts, but it is still a limitation in the developing countries with less resources and limited availability of appropriate fungicides. Moreover, fungicides are an environmental concern worldwide adding cost to wheat production (Kumar et al. 2013). Development and deployment of genetically resistant wheat

varieties is a more sustainable and eco-friendly process to control leaf rust (Oelke and Kolmer, 2004; Pink, 2002; Singh et al. 2000; Singh and Rajaram, 1991). Genetic resistance to wheat rusts is mainly characterized as race-specific seedling resistance also known as all stage resistance (ASR), race-specific adult plant resistance (APR), and race-non-specific adult plant resistance also described as partial resistance or slow rusting (Chen, 2013; Das et al. 1992; Johnson and Law 1973). So far, 15 leaf rust APR genes have been designated, among them 7 genes (*Lr12, Lr13, Lr22a/b, Lr35, Lr37, Lr48,* and *Lr49*) are confirmed to be race specific while 8 are race-non-specific (McIntosh et al. 1995; 2008, and 2016). Among race-specific APR genes, three genes (*Lr12, Lr13, Lr22b*) are qualitative which provide hypersensitive reactions but are functional only at the adult plant stage (McIntosh et al. 1995; Singh and Bowden, 2011). Race specific resistances that are controlled by seedling genes are less durable and the pathogen can easily overcome these by evolving new races (Lowe et al. 2011; Jones and Dangl, 2006). Many ASR genes became ineffective after a few years of deployment. For example, two genes, *Lr10* and *Lr16,* from the Canadian wheat variety Selkirk became ineffective within 2-8 years of deployment (McCallum et al. 2016). Leaf rust seedling gene *Lr9* was deployed in the eastern United States during 1970s and within few years it was overcome by virulent *Pt* races (Kolmer et al. 2009). Moreover, in the United States, race-specific genes *Lr24*, *Lr26*, *Lr41*, and *Lr50* were also overcome by the pathogen (Kolmer et al.2009). More recently, *Lr21*, which was common in many spring wheat varieties grown in Minnesota, was defeated in 2010 by a new virulent race (Kolmer and Anderson, 2011).

Race-non-specific resistance genes are generally long lasting but do not provide high levels of resistance when used alone but provide adequate resistance when used in combination with other race-specific or race-non-specific genes (Singh et al. 2000). Race-non-specific APR gene *Lr34* has been used in many wheat cultivars arounds the world and has proven to be a durable gene for leaf rust resistance (Singh et al. 2000; Lagudah et al. 2009, Krattinger, et al. 2009). Combining *Lr34* gene with other APR genes (*Lr46, Lr67, Lr68*) has significantly reduced damage from leaf rust (Silva et al. 2015). Currently, eight leaf rust genes: *Lr34* (Dyck 1977; 1987; Lagudah et al. 2009), *Lr46* (Singh et al. 1998), *Lr67* (Dyck and Samborski, 1979), *Lr68* (Herrera-Foessel et al. 2012), *Lr74* (Chhetri et al. 2016), *Lr75* (Singla et al. 2017), *Lr77* (Kolmer et al. 2018a), and *Lr78* (Kolmer et al. 2018b) are considered as race-non-specific APR genes. Among these race-non-specific APR genes, three have been demonstrated to be pleiotropic; *Lr34/Yr18/Pm38/Sr57* (Singh et al.,2012*), Lr46/Yr29/Pm39/Sr58* (Singh et al., 2013), and *Lr67/Yr46/Pm46/Sr55* (Herrera-Foessel et al., 2014) and confer partial resistance to all three rust pathogens including powdery mildew caused by the fungal pathogen *Blumeria graminis* f. sp. *tritici*. ((Lillemo et al. 2008; William et al. 2003).

Evolution of new *Pt* races and rapid ineffectiveness of race-specific seedling genes has diverted the attention of rust pathologists and the wheat breeding community to identify and utilize race-non-specific APR genes for sustainable resistance. For leaf rust QTL mapping, different types of populations have been used which include: $F_2$, Recombinant inbred lines (RIL), doubled haploids (DH), backcrosses (BC), near-isogenic lines (NIL), association mapping (AM), and multiparent advanced generation intercrosses (MAGIC) (Pinto de Silva et al. 2018). These mapping populations have advantages and

disadvantages regarding number of alleles assessed, degree of recombination, mapping resolution, population structure, and ease of QTL validation (Xu et al. 2017; Prins et al. 2016; Korte and Farlow, 2013). For leaf rust, most of the reported QTLs have been mapped using bi-parental populations (Pinto de Silva et al. 2018).

Advancements in DNA technologies and reduced genotyping costs has revolutionized QTL mapping and resulted in greatly improved marker coverage. Next generation sequencing (NGS) (Ponce-Molina et al. 2018) and release of the wheat reference genome (IWGSC 2018) has facilitated more precise QTL mapping and QTL position comparisons with other genotypes. This study utilized a bi-parental RIL mapping population developed by the Global Wheat Program of International Maize and Wheat Improvement Centre (CIMMYT) in Mexico. The specific objective of this study was to identify QTL which are associated with leaf rust APR in US, Mexico, and Pakistan using genotyping-by-sequencing (GBS) – a next generation sequencing approach.

## Materials and Methods

### Plant material and seedling evaluations

This study involved the same 176 $F_4$ derived recombinant inbred lines (RILs) from a cross of Apav x COPIO as described in chapter 1 of this dissertation. Both parental lines were tested for Mexican leaf rust races MBJ/SP and BBG/BP at the seedling stage. The avirulence/virulence formula of race MBJ/SP is *Lr2a, 2b, 2c, 3ka, 9 , 16, 18, 19, 21, 24, 25, (26), 28, 29, 30, 32, 33, 36/1, 3, 3bg, 10, 11, 13, 15, 17, 20, 23, 27+31*. Race MBJ/SP has partial virulence for *Lr26* (Herrera-Foessel et al. 2012) and presented inside parenthesis. The other race BBG/BP is a variant of BBG/BN which is virulent on race-specific gene *Lr12* and qualitative complimentary genes *Lr27+Lr31*

(Huerta-Espino et al. 2009). The avirulence/virulence formula of race BBG/BP is *Lr1, 2a, 2b, 2c, 3, 3ka, 3bg, 9, 13, 14a, 15, 16, 17, 18, 19, 21, 22a, 24, 25, 26, 28, 29, 30, 32, 35, 37/Lr10, 11, 12, 14b, 20, 23, 27 + 31, 33, 72* (Huerta-Espino et al. 2011). The RIL mapping population and both parental lines were also screened for two US *Pt* races BBBDS (virulent to *Lr14a*) and MCDSB (virulent to *Lr1, 3, 10, 17, 14a, 26* and *B*). Race BBBDS is widely avirulent while race MCDSB is virulent to many leaf rust genes. In seedling assays, differential lines carrying known genes mostly in the Thatcher background were also included. Apav and COPIO along with differential lines were planted using 5-7 seeds per line. For inoculation, stored urediniospores were heat-shocked in a 45 °C water bath for 15 minutes and then rehydrated overnight at 80% relative humidity inside a humidity chamber. Plants were inoculated at 2 leaf stage with urediniospores suspended in light mineral oil (approximately 12-15 mg of urediniospores per 0.8 ml of Soltrol 170 oil) using an atomizer. Inoculum concentration was approximately 0.15 mg on each plant. Inoculated plants were placed on a bench for about 60 minutes to allow the oil to evaporate because it may cause phytotoxicity. After oil evaporation, plants were placed in the dew chamber overnight to provide enough moisture for urediniospore germination and then transferred to the greenhouse. At 12 to 14 d after inoculation, infection types (ITs) were assessed on plants using the 0-4 scale described by Long and Kolmer (1989). According to this scale infection type "0" = no visible disease symptoms, ";" = only flecks and no uredinia, "1" = small sized uredinia which are encircled by necrosis, "2" = small to medium sized uredinia surrounded by chlorosis or necrosis, "3" = medium-sized uredinia with no chlorosis or necrosis, and "4" = large-sized uredinia without chlorosis or necrosis. Infection types 0 - 2 and their

variations (0;, ;1, ;2, and 12) were considered resistant host and avirulent pathogen, while ITs 3 and 4 were considered as susceptible host and virulent pathogen. The 0-4 seedling score on the RIL mapping population was converted into a linearized 0-9 scale using the modified perl script as described by Gao et al (2016). The perl script is mainly based on the seedling data conversion scale proposed by Zhang et al. (2014) with some modifications. For simple scores like 0, 2+, 3 it only used Zhang's 0-9 scale but if the scored values were more complex (for example ;13+) then the first value was weighted double, and final score was obtained based on the arithmetic means. Based on the linear scale, lines with a 1-6 score were considered resistant, while 7-9 were susceptible. Chi-squared tests were performed to check the assumptions of expected fitting-ratios for seedling gene segregation in the RIL population.

**Field evaluations for leaf rust**

The RIL mapping population from the cross of Apav x COPIO along with both parents was evaluated for leaf rust (*Puccinia triticina*) in six environments at the adult plant stage. The phenotyping environments include Mexico 2016 & 2017 (hereafter referred as Mex16 and Mex17), Saint Paul 2016 & 2017 (hereafter referred as Stp16 and Stp17), and Pakistan 2016 & 2017 (hereafter referred as Pak16 and Pak17).

For adult plant resistance evaluations, both parents and the mapping population (176 RIL) were planted at CIMMYT experimental station at Ciudad Obregon in the Yaqui Valley during the 2015-2016 and 2016-2017 growing seasons (Mex16 and Mex17). Experimental lines of 0.7 m long were sown in paired rows with 0.3 m between rows. An 'Avocet' near isogenic line (*Yr24*/*Yr26*) was used as a spreader. The experimental lines were surrounded by the spreader lines and along one side of the trials

spreader hill plots were sown in the alley. To initiate rust infection and develop epidemic conditions, spreader rows were inoculated using an equal proportion of MBJ/SP and MCJ/SP *Pt* races. The only difference between both races is MBJ/SP has partial while MCJ/SP has complete virulence on *Lr26* (Lan et al. 2014). The urediniospores of both races were suspended in Soltrol 170 (1g $L^{-1}$) and sprayed on spreader rows. One liter of mixture was applied on approximately 400 ft of spreader rows (8 mg $m^{-1}$).

In Saint Paul, the mapping population (176 RILs) and parental lines were included in nursery inoculated with both leaf and stem rust during 2016 and 2017 growing seasons. The planting procedure and check varieties used for these trials are described in chapter 1 (field-based stem rust evaluation) of this dissertation. A mixture of 6 *Pt* races (MHDSB, MFPSB, MLDSD, TFBGQ, TBBGS, and MJBJG) was suspended in Soltrol 170 oil (Chevron-Phillips Petroleum, Bartlesville, OK) and was inoculated on the spreader rows following the same procedures described above. These races are virulent to the seedling (*Lr12, Lr13*) and race-specific APR genes (*Lr37*) in US wheat cultivars (Kolmer et al. 2018).

The Pakistan nurseries were planted at the Wheat Research Institute (WRI), Faisalabad during 2015-2016 and 2016-2017 growing seasons (November to April). During 2016, twenty-one lines were missing from the mapping population, while in 2017 a complete set of RILs comprised of 176 lines was planted. Lines were planted as 1 m long rows in an augmented design with repeated check varieties after every 20 lines. The susceptible check ‘Morocco’ was also planted surrounding the experimental lines and the wheat varieties Millat-11, Pb-11, and AARI-11 were planted as resistant checks. Nurseries were inoculated using a mixture of *Pt* isolates collected from the wheat fields

from the same area. The *Pt* isolates are not characterized, and virulence/avirulence is not known. Spreader rows were sprayed with the bulk inoculum (1g) suspended in light mineral oil (1L) and tween 20 (2 drops). Inoculum application procedures and concentration per m of spreader rows was the same as described above.

**Leaf rust phenotyping and data analysis**

Nurseries were evaluated in all six environments for leaf rust severity on a scale of 0-100 % using the modified Cobb Scale method (Peterson et al. 1948). In all environments, at least two readings were taken after the anthesis [10.51 Feekes growth stage (Feekes, W. 1941)] when disease severity was about 80% on the spreader rows. Infection response was also recorded on the RILs and both parents at the same time and classified into four categories: R = Resistant (necrosis surrounded by small uredinia); MR = moderately resistant (necrosis surrounded by moderate sized uredinia); MS = moderately susceptible (chlorosis surrounded by moderate to large sized uredinia); S = susceptible (large sized uredinia without necrosis or chlorosis). For all leaf rust trials, disease severity was recorded on a whole plot basis, considering the percent of flag leaf area covered with disease. Terminal rust score was considered as the final disease severity (FDS) and was used for all downstream analysis. Field scores were converted into coefficient of infection (COI) values as we did for stem rust, described in chapter 1. COI values were used for all the phenotyping and QTL mapping analysis. Histograms and Pearson coefficient correlations were developed among all the phenotyping environments using the COI values in the R program and IBM SPSS 1.0.0.1174 (IBM Corp.).

**Genotyping and QTL mapping**

Both parent lines were also genotyped for known seedling and APR leaf rust resistance genes. Closely linked polymerase chain reaction (PCR) markers to *Sr2/Yr30/Lr27* (*gwm533*), *Lr34/Yr18/Sr57* (*csLV34*), *Lr46/Yr29/Sr58* (*csLV46G22*), *Lr68* (*csGS*), *Lr14a/Lr68* (ubw14), and *Lr67/Yr46/Sr55* (*Lr67SNP*) were used to determine the presence or absence of these genes in parental lines. For each marker a 10 µl PCR reaction mix was prepared that contained 120 ng genomic DNA, 0.3 U Taq DNA polymerase, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.25 µM each of two primers (reverse and forward). The denaturation was performed at 94 °C for 5 minutes, followed by 35-40 cycles of amplification (94 °C for 45 S, 55 to 65 °C for 60 S; depending on primer pair, and 72 °C for 60 S) and finally the extension step at 72 °C for 7 minutes. PCR products were visualized following the MAS Wheat protocols (https://maswheat.ucdavis.edu/protocols/index.htm). We were unable to test other known APR genes which have been recently designated, namely *Lr74* (Chhetri et al. 2016), *Lr75* (Singla et al. 2017), *Lr77* (Kolmer et al. 2018a), and *Lr78* (Kolmer et al. 2018b) due to the lack of diagnostic markers. Both parents were also tested for 2NS/2AS translocation using 10 GBS markers associated with translocation segment (Chapter 1, Suppl. Table 1) For this study we used the same genotyping data set (as described in chapter 1) generated through sequencing two GBS libraries on Illumina HiSeq 2500 and obtained more than 220 million reads for each genomic library. Polymorphic markers were assigned to chromosomes which represented 21 linkage groups. Poor fitting and co-locating markers which mapped to the same genetic position were removed and a total 762 markers

representing unique loci were used for QTL mapping. For complete procedures, refer to the material and methods section of chapter 1.

## Results

### Seedling and adult plant phenotyping

The parents of our RIL population, Apav and COPIO, had high infection responses against the Mexican *Pt* races at the seedling stage (Table 1). Infection type (IT) was recorded as 4 and 3 for MBJ/SP, and 3+ and 4 for BBG/SP on Apav and COPIO, respectively. The US race BBBDB was avirulent on both parents (IT =; and ;12) while race MCDSB had high infection on both Apav (3+) and COPIO (3) (Table 1). Seedling assays were performed on the RIL population using both US *Pt* races. For race BBBDB, 129 lines were resistant, 32 were susceptible, and 15 lines were heterogeneous. Chi-squared tests fit a 2-gene model ($\chi^2 = 2.65$; *p* value = 0.11) for resistance to race BBBDB in the RIL population. To check gene segregation assumptions, heterogenous lines were dropped from the analysis. All lines in the population were highly susceptible (IT = 3 and 4) for the race MCDSB and no seedling resistant gene was segregating for resistance to this race. Seedling evaluations of differential lines showed that the race BBBDB is virulent on *Lr14a*, *Lr14b*, and *Lr20*, while race MCDSB is virulent on *Lr1*, *Lr3*, *Lr26*, *Lr17*, *LrB*, *Lr10*, *Lr14a*, *Lr3bg*, *Lr14b*, *Lr20*, and *Lr23* genes (Table 2).

In field evaluations, disease development was excellent in all six phenotyping environments. Apav had high disease severity in Mexican (FDS = 90-100%) and US (FDS = 70-90%) environments, while it was low (0-5%) in Pakistan during both seasons. COPIO was highly resistant (FDS = 0-10%) in all testing environments except Pak16 (FDS = 50%), where it was moderately susceptible (Table 3). The mean disease severity

on the RIL mapping population was 33.6% (Mex16) and 41.5 % (Mex17) in Mexico, 73.1% (Stp16) and 33.0% (Stp17) in Saint Paul, and 31.4% (Pak16) and 29.6% (Pak17) in Pakistan. High disease pressure was observed during 2016 in Saint Paul nurseries and disease frequency distribution was more skewed towards susceptibility. Histograms reflected the continuous disease distribution spectrum across all the environments, which also represented all severity classes in the RIL mapping population (Figure 1). Pearson coefficient of correlation (*r*) among leaf rust disease severities was high and significant in most of the phenotyping environments at 1% and 5% significance levels (Table 4). High correlation was observed between Mex16 and Mex17 environments ($r$ = 0.80), followed by Mex17 and Stp17 ($r$ = 0.57). Disease severities were least correlated ($r$ = 0.03-0.15) and non-significant with all other environments in Pakistan during 2016 but were significantly correlated ($r$ = 0.18-0.40) during 2017 except with Stp17 ($r$ = 0.06).

**Genotyping and QTL mapping**

Marker assays on the parents of RIL population revealed that Apav did not carry any known APR genes (*Lr34*, *Lr46*, *Lr67* and *Lr68*), while COPIO likely contains APR gene *Lr46* and seedling genes *Lr27* and *Lr14a* that are closely linked with APR genes *Sr2/Yr30* and *Lr68*, respectively. Moreover, COPIO was positive for 2NS/2AS translocation and carries qualitative gene *Lr37* (Table 5). Ten QTL associated with leaf rust resistance were identified, 9 of which were contributed by COPIO. The logarithm of odds (LOD) values of these QTL ranged from 2.77 to 11.25 and phenotypic variation for leaf rust severities explained ranged from 6-18%. Only one QTL was contributed by Apav in both Pakistan (Pak16, Pak17) environments with LOD values ranging from 6.1 to 10.6, explaining 13-22% of the phenotypic variance. Chromosome 2A and 2B

contained two QTL each, and one QTL was found on chromosomes 1A, 1B, 3A, 3B, 3D, and 5D (Table 6). Detected QTL on each chromosome along with their respective environments are presented in Figure 2.

**Discussion**

Both parents of RIL mapping population were susceptible at the seedling stage (IT ≥ 3) against the Mexican *Pt* races MBJ/SP and BBG/BP which are virulent on race-specific genes**;** *1, 3, 3bg, 10, 11, 13, 15, 17, 20, 23, (26), 27+31* and *Lr10, 11, 12, 14b, 20, 23, 27+31, 33, 72*, respectively (Table 1). So, we are confident that these genes are not associated with field resistance during our adult plant evaluations in Mexican environments. In addition to race MBJ/SP we also used race MCJ/SP for field inoculations in Mexico and the difference between both races is that the former race has partial virulence on *Lr26* while the latter race has complete *Lr26* virulence (Herrera-Foessel et al. 2012). *Lr26* was a spontaneous introgression from rye (*Secale cereale* L.) into wheat along with *Sr31, Yr9*, and *Pm8* and commonly known as T1BL:1RS translocation (Zeller et al. 1973). Based on the seedling data against the MBJ/SP race we also eliminate the possibility of *Lr26* being present in either parent. Both parents were resistant (IT < 3) against the most avirulent US *Pt* race BBBDB and the Chi-squared tests on RILs data showed that two seedling genes are segregating in our population for this race. The other US *Pt* race MCDSB which is widely virulent on many leaf rust genes had high infection (IT > 3) on both parents and the RIL population. Leaf rust field evaluations showed that COPIO had very low disease severity in all environments except Pak16, where it was moderately susceptible. We hypothesize that COPIO might have race-specific seedling genes and multiple QTL with additive effects. Apav was highly

susceptible under field conditions in all environments except Pak16 and Pak17 (FDS = 0-5%), so most likely it has some seedling gene providing strong resistance against *Pt* races in Pakistan.

A continuous disease distribution spectrum was observed in the RIL population, representing all phenotypic classes across all the environments which indicates that adult plant field-based resistance in this population is quantitative and polygenic (Fig. 1). Overall, disease severities were significantly correlated in all phenotyping environments except Pak16, which had low and non-significant correlation with all other environments (Table 4). Overall, both Pakistan environments had low correlation with Mexico and Saint Paul, which can be expected due to entirely different weather conditions and *Pt* races. Genotype by environment interactions (G X E) can also be the reason for low correlations which can arise from environmental conditions, inoculum pressure and different rust races in different environments.

Medium to large effect QTL that contributed substantial phenotypic variance in at least two or more environments were detected on chromosomes 1B, 2A, 2B, and 3B. Other small effect QTL were mapped on 1A, 3A, 3D, and 5D and were detected only in one environment (Table 6; Fig. 2). Here we provide some insights on the QTLs that were detected in more than one environment and contributed substantial variation for leaf rust.

The QTL *QLr.umn.1B* was contributed by COPIO and was detected in two environments (Mex16, Pak17) on the long arm (80-87 cM) of chromosome 1B. This QTL explained 5-8% of the phenotypic variation for leaf rust with a range of 2.8 to 5.4 LOD values. This region of chromosome 1B is known to carry a non-race-specific APR gene *Lr46* (Singh et al. 2013) that is pleiotropic with *Sr58* and *Yr29* and confer resistance to

other wheat rusts as well. The molecular marker assay for this gene was positive for COPIO, so we are confident that the QTL *QLr.umn.1B* in our population represents *Lr46*. Although this gene was detected in only two environments, LOD values were near our significance threshold (LOD ≥ 2.5) to declare a QTL in other environments (Suppl. Fig.1).

Two QTL (*QLr.umn.2A.1* and *QLr.umn.2A.2*) were mapped on the short arm of chromosome 2A and were contributed by COPIO. The QTL *QLr.umn.2A.1* is located on the distal end (1.02-4.8 cM) while the other QTL *QLr.umn.2A2* (22.2 cM) was located proximal to the short arm and was detected in only one environment (Pak16). The short arm of chromosome 2A is known to carry a chromosomal translocation (2NS/2AS) from *Triticum ventricosum* ((Helguera et al., 2003; Maia, 1967) and this translocated segment harbors three linked genes *Lr37, Yr17,* and *Sr38* (Helguera et al., 2003). *Sr38* gene was mapped in COPIO at 11.2 cM (Chapter 1, Table 6) and based on its proximity with the leaf rust QTL (*QLr.umn.2A.2*) it is likely that *QLr.umn.2A.2* represents *Lr37* gene. Helguera et al., (2003) has mapped *Lr37, Yr17, Sr38* linked genes at a genetic distance of 10cM on the short arm of chromosome 2A using the RFLP marker *cmwg682*. The other explanation is that *QLr.umn.2A2* (*Lr37*) was only detected in Pakistan where the most common races (FHPSQ and KHPQQ) are only virulent to *Lr16, Lr17,* and *Lr26* (Kolmer et al. 2017). This was not detected in US and Mexico environments because *Lr37* virulence is present in both environments. Seedling leaf rust gene *Lr17* is also located on the short arm of chromosome 2A but we eliminate this possibility because the *Pt* races (MBJ/SP, MCJ/SP, MCDSB, MHDSB, MFPSD, MLSDS) used for seedling assay and field evaluation are virulent to *Lr17*. Moreover, the most frequent and common races in

Pakistan (FHPSQ, KHPQQ) are also virulent to *Lr17* (Kolmer et al. 2017). The QTL *QLr.umn.2A.1* was detected in three environments (Mex16, Mex17, Stp17) but LOD peaks were also observed for other environments which did not reach the threshold level to be declared significant (Fig. 3). This QTL explained 6-11% phenotypic variation for leaf rust severities with an additive effect of 7.5 to 13.8 (Table 6). Some other studies have also reported leaf rust QTLs co-located with *Lr37* (Wang et al. 2015; Azzimonti et al. 2014). CIMMYT-Mexico has also enough data (unpublished) to support that the *Lr37/Yr17/Sr38* region harbors a slow rusting gene other than *Lr37* (R. P Singh; Personal communication). Therefore, it is more likely that the QTL *QLr.umn.2A.1* does not represent *Lr37* and it is a new source of resistance to leaf rust.

The chromosome 2B harbored a large effect QTL (*QLr.umn.2B.1*) that was mapped distally (2-3 cM) to the short arm during both seasons (Pak16, Pak17) in Pakistan. The closest and most significant marker is physically located at the 16 Mb position (Table 6). The short arm of chromosome 2A is known to carry *Lr13, Lr16, Lr23, Lr48*, and *Lr73* genes and the linked markers *Xwmc764* (*Lr16*), *wPt-4453* (*Lr73*), *Xwmc154* (*Lr13* and *Lr23*), and *Xbarc7* (*Lr48*) are physically located at 8.07, 13.48, 36.44, and 117.25 Mb positions, respectively (Sapkota et al. 2019). This QTL was only detected in Pakistan where the most frequent and common races (FHPSQ, KHPQQ) are virulent to *Lr16* (Kolmer et al. 2017) which eliminates *Lr16* as a possibility. The parental line Apav is derived from Pavon76 which is known to carry *Lr13* (Singh and Rajaram, 1991). Based on the pedigree information and physical position, we are confident that *QLr.umn.2B.1* locus reflects *Lr13* gene in Apav. Additionally, *QLr.umn.2B.1* was not detected in Mexico environments because the *Pt* races used for field inoculation

(MBJ/SP, MCJ/SP) are virulent to *Lr13* (Herrera-Foessel et al. 2012). The other QTL *QLr.umn.2B.2* mapped distally to the long arm of chromosome 2B and was detected in one environment (Mex16). Terminal chromosomal region of 2B carries two seedling genes *Lr50* and *Lr58* which are derived from *T. timopheevii* subsp. *armeniacum* (Brown-Guedira et al, 2003) and *Aegilops triuncialis* (Kuraparthy et al, 2007), respectively. COPIO is not known to carry these sources of resistance in its pedigree. Buerstmayr et al. (2014) reporetd a large effect QTL (*QLr.ifa-2BL* ) in wheat genotype Capo on 2BL which was consistlently detected in all testing environmnets. In our study *QLr.umn.2B.2* is a small effect QTL detected in one environment and may represent a new source of resistance for leaf rust.

A large effect QTL (*QLr.umn.3B*) for leaf rust contributed by COPIO was mapped on the short arm of chromosome 3B. This genomic region is known to affect the development of many fungal diseases with *Lr27* (Nelson et al. 1997), *Sr2* (Kota et al. 2006), *Yr30* (Singh et al. 2001), *Fhb1* (Liu et al. 2008) and *Pm* (Mango et al. 2001) genes. The qualitative gene *Lr27* and APR genes *Sr2*, *Yr30* are closely linked (Singh and McIntosh 1984). In our study, the QTL *QLr.umn.3B* was detected in four environments (Mex16, Mex17, Stp16, Stp17) out of six (Fig. 4) and explained 10-17% phenotypic variance in Mexico and 15-18% in Saint Paul for leaf rust severities (Table 6). The 3BS seedling gene *Lr27* requires a complementary gene *Lr31* on chromosome 4B for its function (Singh and McIntosh, 1984). In our study, genome wide markers did not detect *Lr31* in either parental line. The pathotypes (MBJ/SP, MCJ/SP, BBG/BP) used for the seedling assays and field inoculation in Mexico are virulent on *Lr27* + *Lr31* (Herrera-Foessel et al. 2012; Huerta-Espino et al. 2011). Both parents of RIL population had high

infection types (IT > 3) when challenged against these races at the seedling stage (Table 1). 'Gatcher', which carries *Lr10* and *Lr27+Lr31* genes had high disease severity in Saint Paul nurseries in 2016 (Kolmer et al. 2018c). Hence the presence of *Lr27* virulent pathotypes supports our hypothesis that leaf rust resistance in Saint Paul is not associated with *Lr27* in COPIO; however, COPIO was positive for the closely linked stem rust APR gene *Sr2* (Table 5). Some studies have reported that few wheat lines show *Lr27* specificity and do not confer resistance to stem rust (Singh and McIntosh, 1984), which suggests that *Lr27* and *Sr2* are encoded by separate genes (Mango et al. 2011). However, no wheat genotypes have been reported that carry *Sr2* gene but lack *Lr27* (Mango et al. 2011). Buerstmayr et al. (2014) reported a QTL (*QLr.ifa-3BS*) on 3BS in Austrian wheat cultivar 'Capo' that does not carry either *Lr27* or *Sr2*. This QTL confers resistance to both leaf and stripe rust. Our finding needs further investigation to reveal the relationship of *Sr2* with leaf rust QTL. It might be a possibility that *Sr2* allele is conferring resistance to leaf rust without *Lr27* or *QLr.umn.3B* is a different race specific APR QTL providing resistance in Mexico and Saint Paul.

**Conclusions**

In this study we detected nine genomic regions in COPIO that are associated with leaf rust resistance in six environments. The non-race-specific pleiotropic gene *Lr46* was mapped on chromosome 1B and race-specific gene *Lr37* which comes from the 2NS/2AS translocation was mapped on chromosome 2A in COPIO. Furthermore, two large effect QTLs (*QLr.umn.2A.1*) on chromosome 2A and (*QLr.umn.3B*) 3B were also detected in COPIO and are potentially new sources of resistance to *Pt* pathotypes. One QTL was detected in Apav on chromosome 2B that most likely represents the race-specific gene

*Lr13*. Many wheat growing countries are known to have *Lr13* virulent pathotypes, but still this gene can be used with other resistant genes and can be deployed in areas where virulent pathotypes do not exist. Some other small effect QTLs were also contributed by COPIO that were detected in only one environment and mapped on chromosomes 1A, 3A, 3D, and 5D. The findings of this study suggest that COPIO harbors some important quantitative and qualitative genes which can be used as a source resistance in recombination wheat breeding programs to develop leaf rust resistant cultivars.

Table 1: Seedling infection types of both parents to *Puccinia triticina* (*Pt*) races

| | Mexican *Pt* races | | US *Pt* races | |
|---|---|---|---|---|
| Parent lines | MBJ/SP | BBG/BP | BBBDB | MCDSB |
| Apav | 4 | 3+ | ; | 3+ |
| COPIO | 3 | 3 | ;12 | 3 |

Table 2: Infection types of two US leaf rust races on North American differential lines

| | *Pt* races [a] | |
|---|---|---|
| Genes [b] | BBBDB [c] | MCDSB [d] |
| *Lr1* | 0; | 3+ |
| *Lr2a* | 0; | 0; |
| *Lr2c* | ; | ; |
| *Lr3* | ; | 3+ |
| *Lr9* | 0; | ; |
| *Lr16* | ;1- | 22+ |
| *Lr24* | ; | ; |
| *Lr26* | ;12 | 3+ |
| *Lr3ka* | ;12- | 22- |
| *Lr11* | ;12- | ;22- |
| *Lr17* | ; | 3+ |
| *Lr30* | ;2- | ;1 |
| *LrB* | 2+ | 3+ |
| *Lr10* | ;1 | 3+ |
| *Lr14a* | 3+ | 3+ |
| *Lr18* | 22+ | ;1 |
| *Lr21* | ;1 | 22+ |
| *Lr28* | 0; | ; |
| *Lr41* | 0; | ; |
| *Lr42* | ;1 | ;1 |
| *Lr3bg* | ; | 3+ |
| *Lr14b* | 32+ | 3+ |
| *Lr20* | 3+ | 3+ |
| *Lr23* | ;1 | 3+ |

[a] = *Puccinia triticina* (leaf rust) races, [b] = North American differential lines carrying single leaf rust genes, [c] = Widely avirulent *Pt* race, [d] = Virulent race to many leaf rust genes

Table 3: Final disease severity (FDS) based on COI on both parents, population mean, standard deviation and range of leaf rust coefficient of infection in six environments

| Environments | FDS (%) on parents | | Pop. Size | Pop. mean | Std. Deviation | Low range | High range |
|---|---|---|---|---|---|---|---|
| | Apav | COPIO | | | | | |
| Mex16 | 90 | 0 | 176 | 33.6 | ±24.9 | 0.0 | 100 |
| Mex17 | 100 | 5 | 176 | 41.5 | ±32.4 | 0.4 | 100 |
| Stp16 | 90 | 10 | 176 | 73.1 | ±16.1 | 11.7 | 95 |
| Stp17 | 70 | 5 | 176 | 33.0 | ±19.8 | 1.35 | 80 |
| Pak16 | 5 | 50 | 155 | 31.4 | ±25.6 | 0 | 80 |
| Pak17 | 0 | 5 | 174 | 29.6 | ±30.9 | 0 | 100 |

Table 4: Pearson correlation (*r*) among coefficient of infection values based on final disease severities on Apav x COPIO RILs in six environments for leaf rust.

| | Mex16 | Mex17 | Stp16 | Stp17 | Pak16 | Pak17 |
|---|---|---|---|---|---|---|
| Mex16 | 1 | | | | | |
| Mex17 | 0.80** | 1 | | | | |
| Stp16 | 0.47** | 0.40** | 1 | | | |
| Stp17 | 0.56** | 0.57** | 0.46** | 1 | | |
| Pak16 | 0.10 | 0.1 | 0.15 | 0.03 | 1 | |
| Pak17 | 0.19* | 0.29** | 0.18* | 0.06 | 0.40** | 1 |

*, ** Correlation is significant at the 0.05 and 0.01 level, respectively

Table 5: Molecular marker assays for known leaf rust APR and seedling genes

| Gene | Chromosome | Marker | Apav | COPIO |
|---|---|---|---|---|
| *Lr34* | 7DS | *csLV34* | – | – |
| *Lr46* | 1BL | *csLV46G22* | – | + |
| *Sr2/Yr30/Lr27* | 3BS | *gwm533* | – | + |
| *Lr67* | 4DL | *Lr67SNP* | – | – |
| *Lr14a/Lr68* | 7BL | *ubw14* | – | + |
| *Lr68* | 7BL | *csGS* | – | – |
| *Lr34/Sr38/Yr17* | 2AS | *GBS* | – | + |

(–) represents that the gene is absent
(+) represents that the gene is present

Table 6: Quantitative trait loci (QTL) associated with leaf rust resistance in Apav x COPIO RIL mapping population across six environments

| Chr | Environments | QTL | Peak marker | Position(cM) | LOD | $R^2$ | Additive |
|---|---|---|---|---|---|---|---|
| 1A | Mex17 | QLr.umn.1A | chr1A_568112294 | 65.99 | 3.49 | 0.06 | 8.19 |
| 1B | Mex16 | QLr.umn.1B | chr1B_670207768 | 80.38 | 5.44 | 0.08 | 7.05 |
| | Pak17 | QLr.umn.1B | chr1B_678465602 | 87 | 2.81 | 0.05 | 6.88 |
| 2A | Mex16 | QLr.umn.2A.1 | chr2A_19914469 | 1.02 | 11.25 | 0.17 | 11.95 |
| | Mex17 | QLr.umn.2A.1 | chr2A_19914469 | 1.02 | 7.43 | 0.13 | 13.88 |
| | Stp17 | QLr.umn.2A.1 | chr2A_21005775 | 4.81 | 5.66 | 0.11 | 7.55 |
| | Pak16 | QLr.umn.2A.2 | chr2A_41173033 | 22.21 | 2.77 | 0.06 | 6.29 |
| 2B | Pak16 | QLr.umn.2B.1 | chr2B_6205801 | 3.2 | 6.15 | 0.13 | -9.74 |
| | Pak17 | QLr.umn.2B.1 | chr2B_16009483 | 2.01 | 10.59 | 0.22 | -14.66 |
| | Mex16 | QLr.umn.2B.2 | chr2B_769717318 | 82.57 | 4.43 | 0.08 | 7.61 |
| 3A | Mex17 | QLr.umn.3A | chr3A_688594297 | 95.37 | 3.11 | 0.07 | 8.91 |
| 3B | Mex16 | QLr.umn.3B | chr3B_6071517 | 25.61 | 7.00 | 0.10 | 8.18 |
| | Mex17 | QLr.umn.3B | chr3B_6071517 | 25.61 | 9.29 | 0.17 | 13.62 |
| | Stp16 | QLr.umn.3B | chr3B_6396363 | 20.77 | 7.21 | 0.15 | 6.41 |
| | Stp17 | QLr.umn.3B | chr3B_4469125 | 25.19 | 7.93 | 0.18 | 8.45 |
| 3D | Stp16 | QLr.umn.3D | chr3D_305491061 | 69.4 | 3.20 | 0.06 | 3.92 |
| 5D | Mex16 | QLr.umn.5D | chr5D_21558380 | 15.85 | 4.64 | 0.13 | 9.72 |

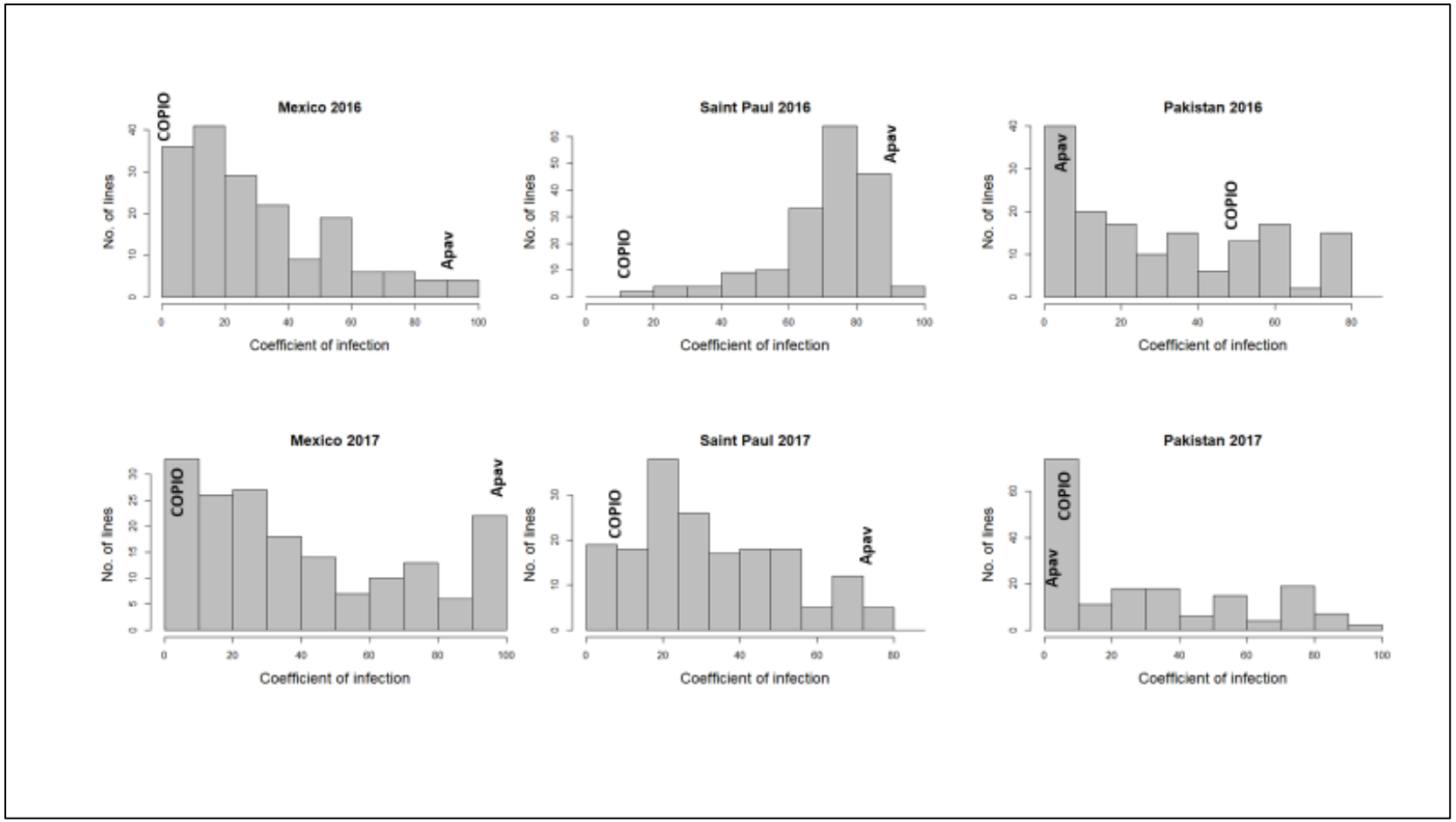



Figure 1: Frequency distribution of leaf rust coefficient of infection (COI) in the Apav/COPIO RIL mapping population in six field environments. Parents (Apav, COPIO) shown on the bars represent the COI values in each environment.

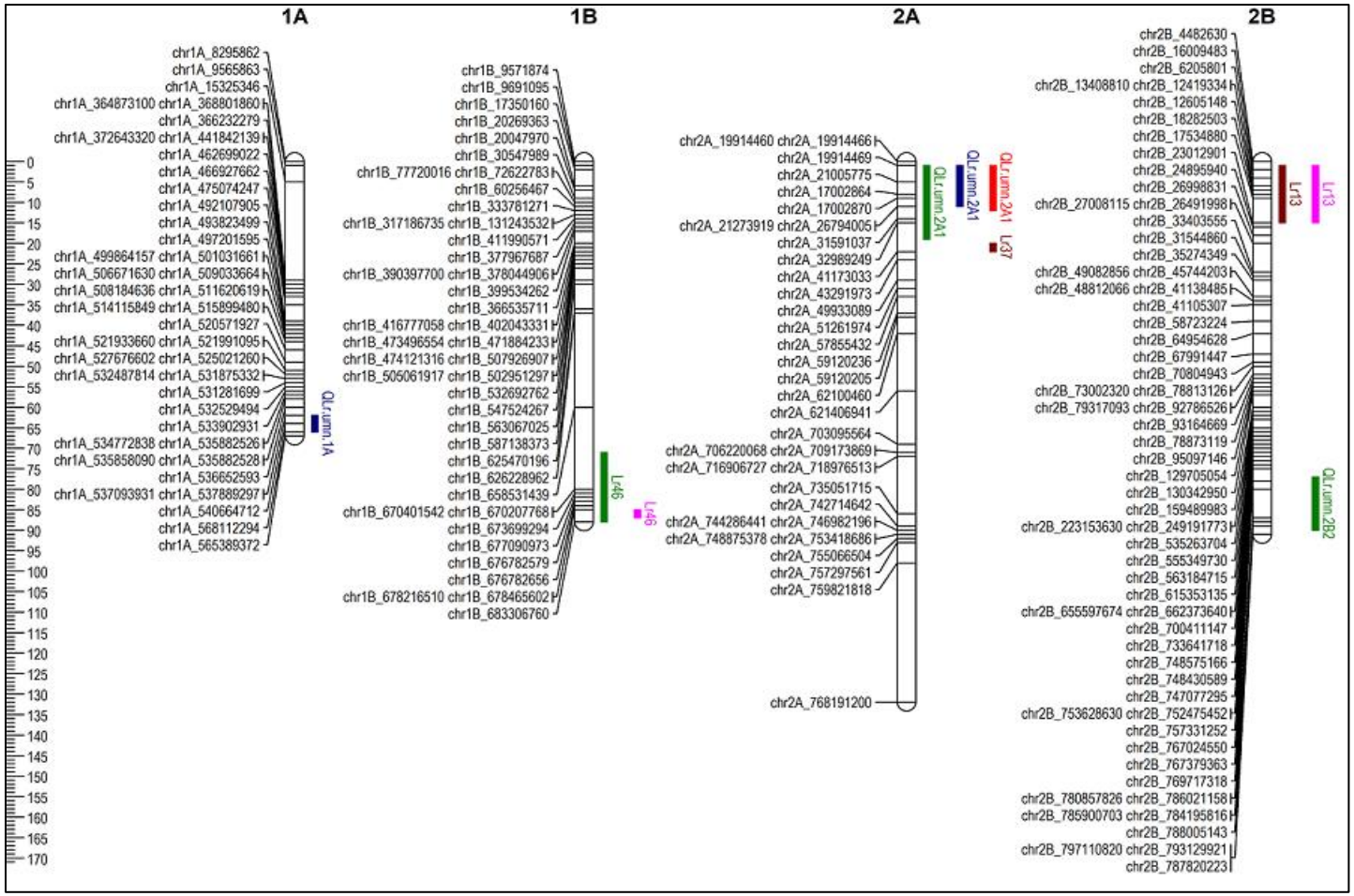


Figure 2: (continued)

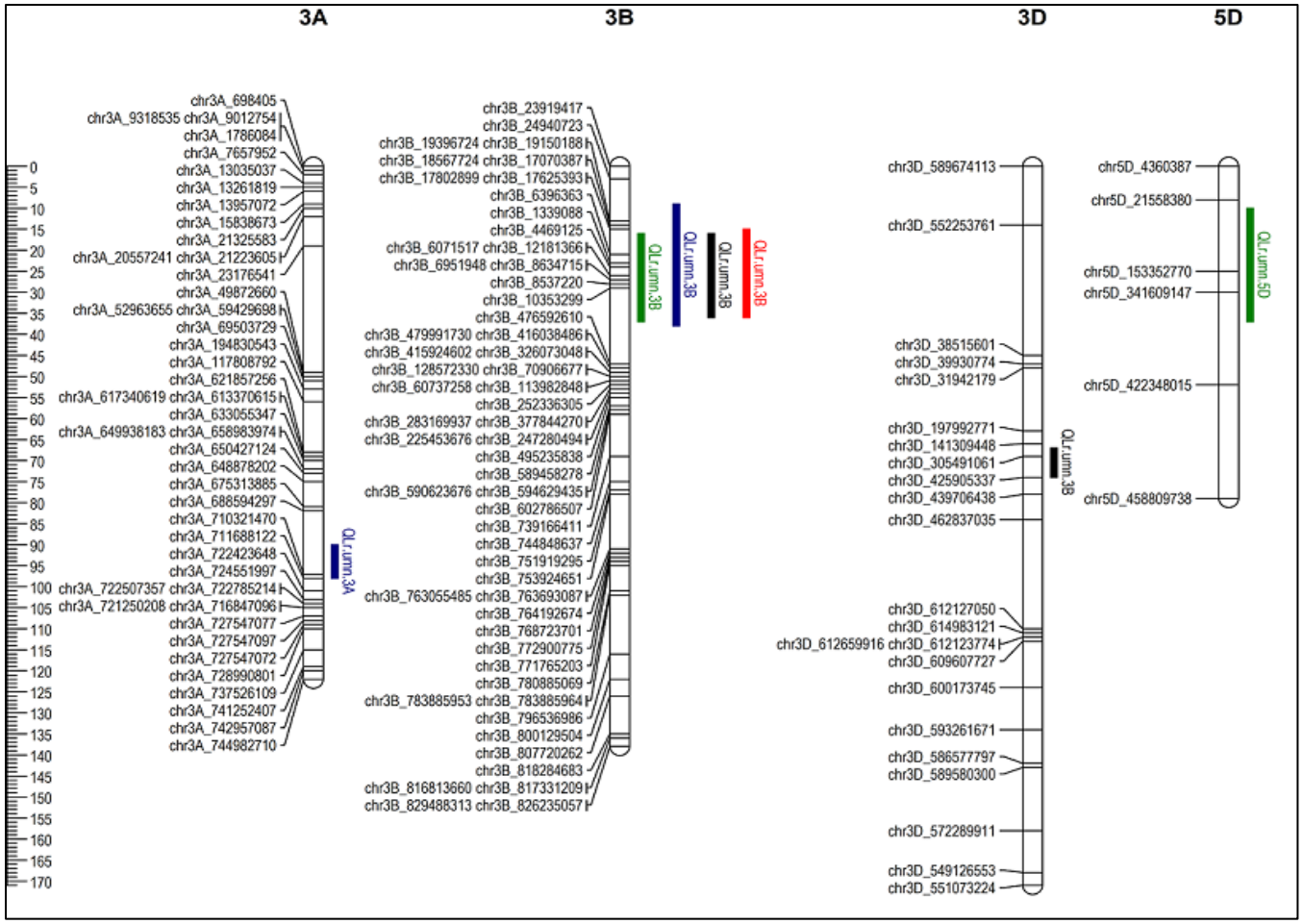



Figure 2: Chromosomes on which leaf rust QTL were detected across six environments. GBS SNP markers are on the left side of each chromosome and the detected QTL is on the right side shown in colored bars perpendicular to the chromosomes. Black, red, dark green, blue, brown, and pink colors represent that QTLs were detected in Saint Paul 2016, Saint Paul 2017, Mexico 2016, Mexico 17, Pakistan 2016, and Pakistan 2017, respectively. Already known genes were detected on 1B (*Lr46*), 2A (*Lr37*) and 2B (*Lr13*). QTLs stacking on each other at chromosomes 2A (*QLr.umn.2A1*) and 3B (*QLr.umn.3B*) were detected in different environments, represent same QTL and are potentially new sources of resistance.

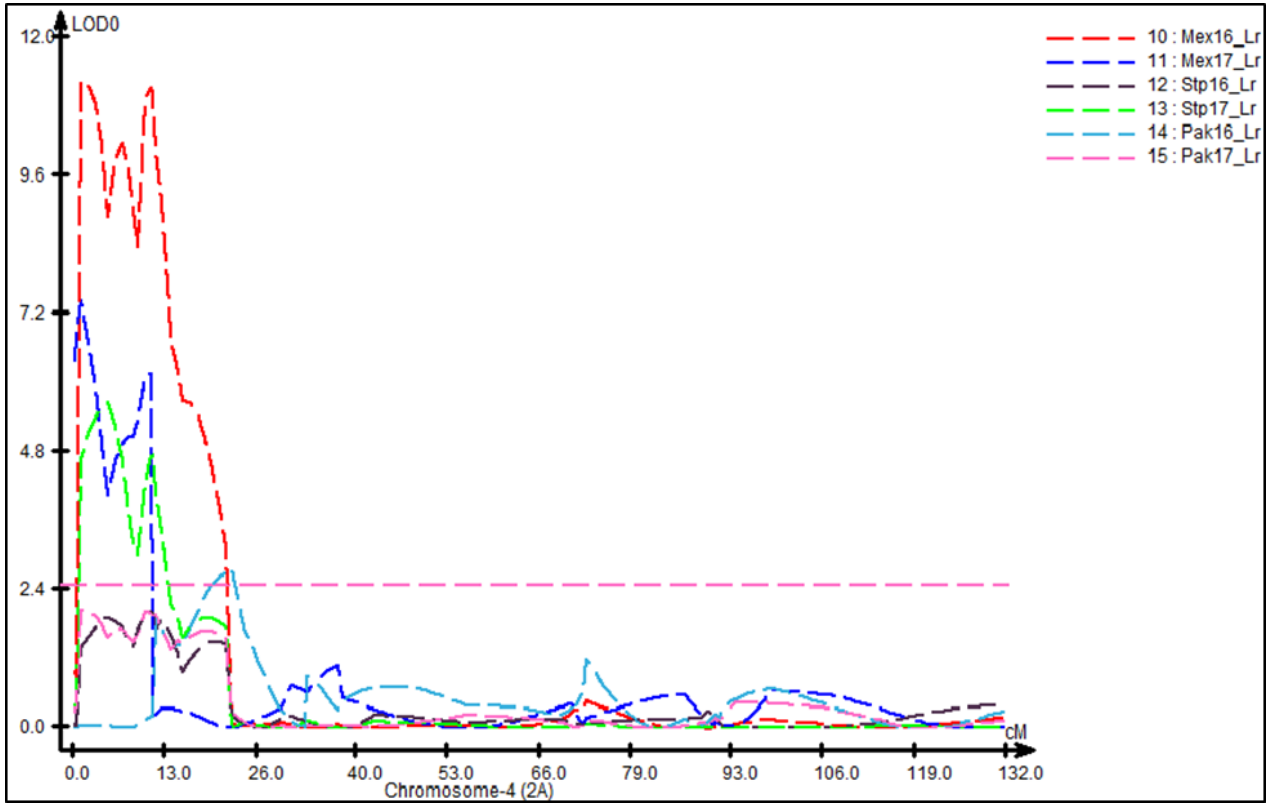



Figure 3: Large effect QTL mapped on chromosome 2A, associated with resistance to leaf rust races in field conditions. X-axis represent the logarithm of odds (LOD) values and Y-axis indicates genetic distance in centimorgans (cM) on chromosome 2A. Horizontal dotted line along the X-axis is the threshold level of LOD value on which a QTL was declared significant.

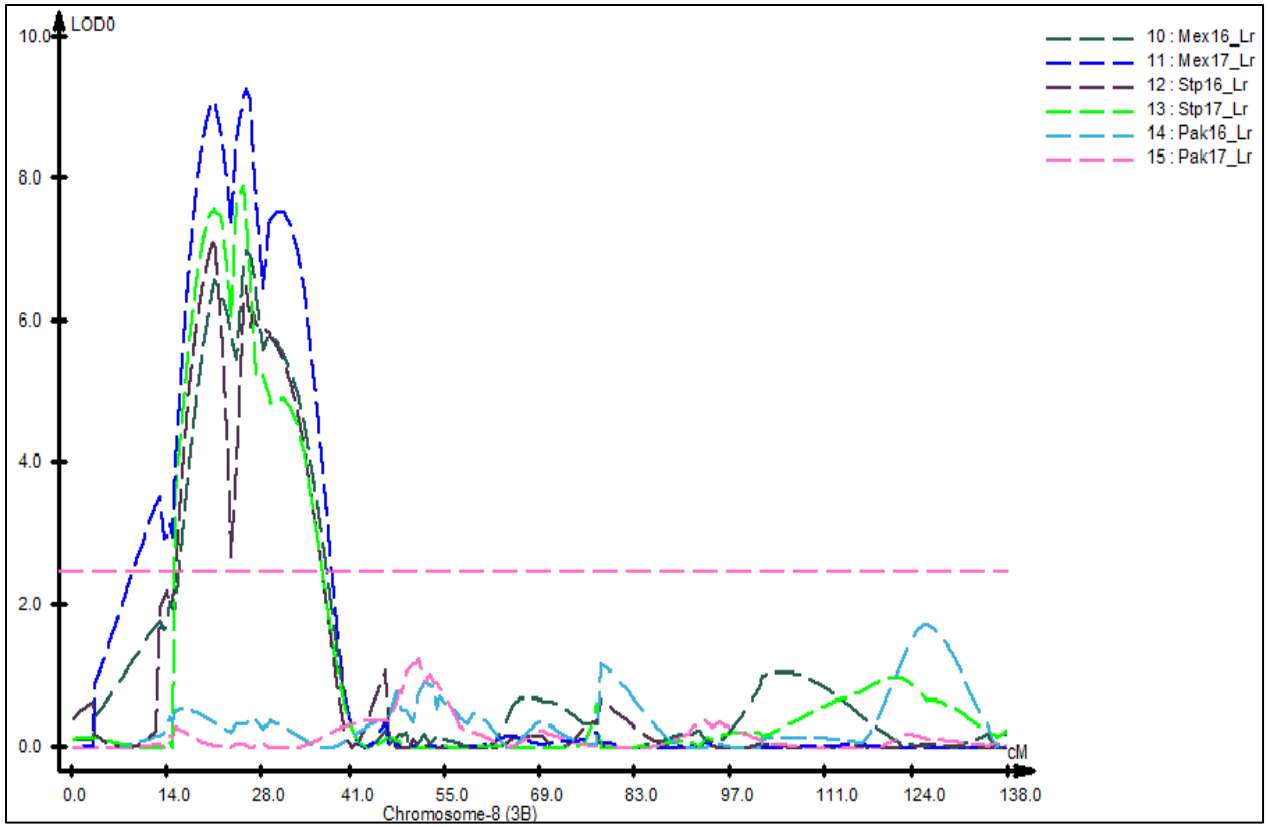
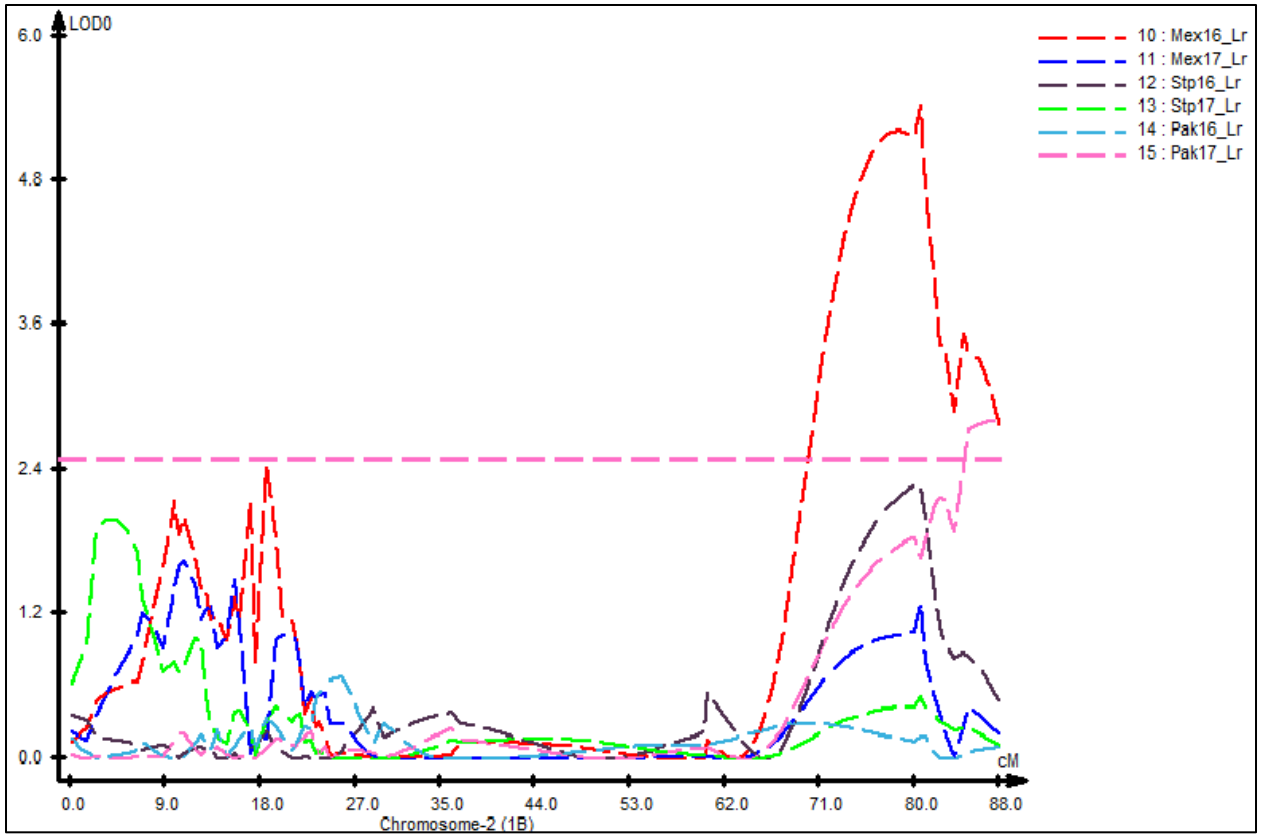



Figure 4: A large effect QTL on chromosome 3BS in the region of *Sr2/Yr30/Lr27* detected in four environments where nurseries had *Lr27* virulent pathotypes. This QTL either represent *Sr2* pleiotropic effects or a new race specific QTL for leaf rust. X-axis represent the logarithm of odds (LOD) values and Y-axis indicates genetic distance in centimorgans (cM). Horizontal dotted line along the X-axis is the threshold level of LOD value on which a QTL was declared significant.




S. Figure 1: Leaf rust QTL mapped on the long arm of chromosome 1B which represents race-non-specific adult plant resistant (APR) gene *Lr46*. X-axis represent the logarithm of odds (LOD) values and Y-axis indicates genetic distance in centimorgans (cM) on chromosome 1B. Horizontal dotted line along the X-axis is the threshold level of LOD value on which a QTL was declared significant. QTL peaks appeared for other environments but did not hit the threshold LOD level.

# Chapter 3

## Molecular Characterization of Genomic Regions for Adult Plant Resistance to Stripe Rust in a Spring Wheat Mapping Population

### Introduction

Wheat stripe rust is caused by the air-borne fungal pathogen *Puccinia striiformis* f.sp. *tritici* (Liu and Hambleton, 2010); hereafter abbreviated as *Pst*. Among wheat biotic stresses, all three rusts, *Pst*, leaf rust (*Puccinia triticina*), and stem rust (*Puccinia graminis* f. sp. *tritici*) have caused huge economic losses in different wheat growing areas around the world (Singh et al, 2016; Beddow et al, 2015; Chen, 2014a). Recently, stripe rust has developed an alarming situation (Hovmøller et al, 2010) with frequent disease epidemics (Hovmøller et al, 2016; Walter et al, 2016, Ali et al, 2014) probably due to unique pathogen characteristics; to travel long distances (Brown and Hovmøller, 2002), adoptability to a wide range of climatic conditions (Milus et al, 2009; Marker and Milus, 2008), recombination through its sexual life cycle (Thach et al, 2016; Rodriguez-Algaba et al, 2014; Jin et al, 2010), and high frequency of natural mutation ((Hovmøller and Justesen, 2007) to evolve new variants. Historically, stripe rust was known to occur in cooler environments (Saari and Prescott, 1985), but recently evolved highly aggressive races “Warrior” and “Kranich” that originated from the *Pst* center of genetic diversity closer to the Himalayan region and these new races are adopted to warmer temperatures (Hovmøller et al, 2016). In the United States, stripe rust mainly causes significant yield losses traditionally in the Pacific Northwest region (Chen, 2005), but now more aggressive and high temperature adapted virulent pathotypes have been reported to affect

wheat in the Southern and Central Great Plains (Chen 2007; Milus et al, 2015). A variant of aggressive *Pst* races from North America with a combined virulence on *Yr3, Yr27,* and *Yr31* caused a widespread epidemic in Mexico during 2014 (Huerta-Espino et al., 2015). Since 2011, the predominant *Pst* populations in Europe have been mainly replaced by a new divergent group of pathotypes and have caused more frequent rust epidemics (Rahmatov et al, 2016). During the last decade, numerous regional stripe rust epidemics have also been reported in Asia and Africa (Singh et al, 2016; El Amil, 2015; Rahmatov et al., 2012). The *Pst* pathogen is causing an estimated worldwide yield loss of 5.47 million metric tons every year with an economic loss of $979 million (Beedow et al, 2015).

Although stripe rust can be controlled by foliar fungicide application, this costs growers millions of dollars per annum (Chen, 2014b). Alternatively, genetic control through developing resistant varieties to *Pst* is most preferred and is a sustainable process (Chen, 2013). Different types of stripe rust resistance have been described depending upon the criteria, but the two main categories are 1) all stage resistance; and 2) adult plant resistance (Chen, 2013). All stage or seedling resistance is usually race-specific, qualitatively inherited and is expressed at the seedling stage and remains effective throughout the plant's life span. On the other hand, adult plant resistance is expressed during the late growth stages and can be either race-specific or non-race-specific. In recent years, high temperature adult plant (HTAP) resistance and its molecular basis has also been described, which is expressed and become more effective when plants get older and temperature is increased (Chen, 2013).

Including recently designated genes *Yr78* (Dong et al, 2017) and *Yr79* (Feng et al, 2018) a total of 80 genes for stripe rust have been designated and numerous provisionally named genes and quantitative trait loci (QTL) have been reported (Wang and Chen, 2017). Most of these genes were reported from *Triticum aestivum*, while some are from other species of *Triticum* (*spelt, turgidum, comosa, dicoccoides, ventricosum*), species of *Aegilops* (*tauschii, kotschyi, sharonensis, geniculata, neglecta*), *Thinopyrum intermedium*, and *Scale cereale* (Park, 2016). Most of the catalogued genes are race-specific, conferring seedling resistance (McIntosh et al, 2016) usually by encoding NBS-LRR resistant proteins (Lowe et al, 2011) and many of them have been overcome by virulent races (Wang and Chen, 2017). To date, twenty catalogued genes (*Yr11, Yr12, Yr13, Yr14, Yr16, Yr18, Yr29, Yr30, Yr36, Yr39, Yr46, Yr48, Yr49, Yr52, Yr54, Yr56, Yr59, Yr62, Yr78,* and *Yr79*) are known to confer APR to *Pst* races (Park, 2016; Dong et al, 2017; Feng et al, 2018) and among them *Yr18, Yr29, Yr46* confer pleiotropic APR to multiple fungal pathogens (Singh et al. 2012, 2013; Herrera-Foessel et al., 2014). APR genes usually encode very diverse types of proteins (Lowe et al, 2011) and two APR pleiotropic genes (*Yr18* and *Yr46*) have unique molecular structures and their resistance mechanisms are controlled by a specific transporter (Krattinger et al., 2009; Moore et al., 2015).

Wheat cultivars with APR genes have demonstrated durable resistance (Hou et al, 2015; Paillard et al, 2012; Lin and Chen, 2008). In recent years, several QTL have been identified through mapping studies (Liu et al., 2019; Ma et al., 2019; Yao et al., 2019; Zhou et al, 2019; Zeng et al., 2018; Yuan et al., 2018; Feng et al., 2018; Dong et al., 2017; Lan et al, 2017). Identified QTL associated with stripe rust resistance have been

mapped using a wide range of molecular markers, which mainly include diversity array technology (DArT), Simple Sequence Repeats (SSR) and Single Nucleotides Polymorphic (SNP) markers (Yao et al, 2019; Mu et al, 2019; Maccaferri et al, 2015).

Although quite few stripe rust genes and QTL have been identified and are being used in wheat breeding programs around the world (Wang and Chen, 2017), additional genes and QTL are required to genetically diversify wheat cultivars for durable resistance. The wheat line COPIO demonstrated high levels of APR under field conditions during the routine germplasm screening at the International Maize and Wheat Improvement Centre (CIMMYT) in Mexico. This study involved 176 recombinant inbred line (RIL) population developed by crossing COPIO with a highly susceptible line Apav with the objectives to dissect the underlying genetic mechanism of APR in COPIO.

## Materials and Methods

### Seedling evaluations

Both parents of the RIL mapping population were screened against *Pst* races from Mexico (Mex14.191) and United States (PSTv-14, PSTv-37, & PSTv-40) at the seedling stage. PSTv-37 was the most widely distributed and predominant race in the United States in 2017 (https://striperust.wsu.edu/races/data/). The virulence and avirulence patterns of races used in the seedling and field assays are described in Table 1. Experiments were conducted at the plant growth facility, University of Minnesota and CIMMYT headquarters at El-Batan near Mexico City in Mexico. The procedures for *Pst* seedling assays were the same as described for leaf rust in chapter 2 with some modifications. For the infection development, inoculated plants were placed in a dew

chamber for 24 h at 10 °C. After this time period the dew chamber door was opened to let the plants dry and plants were moved to a growth chamber at 19/15 °C day and night temperatures with 16 h light and 8 h dark photoperiod by using 400 W sodium vapor lamps that produce 300 µmol photons $s^{-1}$ $m^{-2}$. The *Pst* pathogen has slower development in wheat, therefore the infection types (ITs) were recorded at 18-19 d after inoculation. Plants were rated for the *Pst* ITs using the 0 to 9 scale described by Wan and Chen (2014). According to this scale, IT '0' represents no visible disease infection; IT '1' is assigned to plants on which the pathogen produces necrotic or chlorotic flecks; IT '2' represents chlorotic or necrotic stripes but no sporulation; and ITs '3' to '6' represent chlorotic and necrotic stripes with trace to moderate level of uredinial spores. Plants having necrotic or chlorotic stripes with abundant uredinial spores were assigned IT '7'. Plants having abundant uredinial sporulation associated with underlying chlorosis were assigned IT '8' and IT '9' was assigned to plants with abundant uredinial sporulation and no chlorosis or necrosis. Seedling assays were performed at least twice on both parents against each *Pst* race. Plants exhibiting 0-6 ITs were considered resistant host and avirulent pathotypes, while plants with 7-9 ITs were considered susceptible host and virulent pathotypes.

**Field evaluations for stripe rust**

For the characterization of stripe rust APR in COPIO, the RIL mapping population along with both parents was evaluated in nine environments. The phenotyping environments included Mexico 2015, 2016, and 2017 (hereafter referred as Mex15, Mex16, & Mex17), Kenya 2016 off-season and main-season (hereafter referred as Ken16_OS, & Ken16_MS), Pakistan 2016 and 2017 (hereafter referred as Pak16 &

Pak17), Pullman 2017 in Eastern Washington (hereafter referred as Pum17), and Mount Vernon 2017 in Western Washington (hereafter referred as Mtv17). In Mexico and Pakistan, nurseries were artificially inoculated while in Washington and Kenya, nurseries were evaluated under natural infection.

In Mexico, trials were conducted at the CIMMYT research station in Toluca during the crop seasons of 2015, 2016, and 2017. Paired rows of 0.7 m long with 0.3 m alley were planted in all three crop seasons. Mixture of susceptible lines comprised of cultivar Morocco, Avocet near isogenic line for *Yr31*, and six lines derived from a cross of Avocet/Atilla were used as spreader rows in all field trials. The spreader rows were planted surrounding the nurseries and along one side of the experimental plots in the alleys. In 2015 and 2016, nurseries were inoculated with a mixture (1:1) of two *Pst* races (Mex08.13 & Mex96.11). Mex08.13 is virulent to *Yr31* and avirulent to *Yr27* while Mex96.11 is the opposite. The *Pst* race Mex08.13 belongs to the same lineage of aggressive races detected in California (Chen 2005). In 2017, nurseries in Toluca were inoculated with a different *Pst* race named as 'Borlaug race' which is virulent to *Yr17,* but avirulent to *Yr27* and *Yr31*.

In Pakistan, nurseries were planted at the Cereal Crops Research Institute (CCRI) Pirsabak, Peshawar during the crop season of 2016 and 2017. Experimental lines were planted as 1m long rows in an augmented design with a repeated susceptible check variety 'Morocco' after every 30 lines. Morocco was also planted around the experimental lines. Artificial inoculation was performed using a bulk inoculum collected from wheat lines infected by early natural infection. In the RIL population, 19 lines were

missing during the 2016 phenotyping season, while a complete set of 176 lines was available in 2017.

In Kenya, lines were included as part of the CIMMYT shuttle wheat breeding nurseries in Africa and were tested at the experimental station of Kenya Agricultural & Livestock Research Organization (KARLO) in Njoro, Kenya. The planting procedure and check varieties used for these trials are described in chapter 1 (field-based stem rust evaluation) of this dissertation. No artificial inoculation was performed, and disease was recorded based on the natural infection. In 2016 main and off-season crop cycles, PstS2 (virulent to *Yr27*) was the most frequent race in Kenyan nurseries with a low frequency of PstS11 (virulent to *Yr17* and *Yr27*). *Yr17* virulence (PstS11) was first detected in Afghanistan in 2012 and was detected in 2017-18 in Kenya and is now the most prevalent lineage in East Africa (S. Bhavani; Personal communication).

In Washington State the RIL population and both parents were evaluated at two locations during 2017: Pullman and Mount Vernon (500 Km apart). Both locations have different weather and *Pst* races almost every year but PSTv-37 was the predominant race with 41.3% frequency (https://striperust.wsu.edu/races/data/). A single 1 m row with 20 cm distance was planted at both locations with a repeated check 'Avocet susceptible' after every twenty lines.

Spreader rows of nurseries in Mexico and Pakistan were inoculated by spraying a suspension of urediniospores in Soltrol 170 (1g $L^{-1}$). One liter of mixture was applied on approximately 122 m of spreader rows (8 mg $m^{-1}$). Nurseries in Kenya and Washington were naturally infected, and no artificial inoculation was performed.

**Disease scoring and data analysis**

Nurseries were visually evaluated for disease severity (DS) and ITs. In all nine environments disease severities were recorded based on the 0-100 modified Cobb's scale method (Peterson et al. 1948). The host response to infection was assessed based on Roelfs et al (1992) in Kenya, Mexico and Pakistan environments (scale description in chapter 2). In Washington state at both locations (Pum17 & Mtv17) host response was recorded based on a 0-9 scale (Line and Qayoum, 1992). ITs were categorized into three classes; ITs 0-3 were considered resistant (R), 4-6 intermediate (MR-MS), and 7-9 susceptible (S). In all the environments disease was scored at least twice between 10.5 and 11.3 Feekes growth stages (Feekes, W. 1941) and terminal score was used as final disease severity (FDS) for downstream analysis. For quantitative analysis, field scores were converted into coefficient of infection (COI) values using the Perl script described by Gao et al, (2016). Conversion procedures are described in chapter 1. COI values were used for all the phenotyping and QTL mapping analysis. Histograms and Pearson coefficient correlations were developed among all the phenotyping environments using the COI values in the R program and IBM SPSS 1.0.0.1174 (IBM Corp.).

**Genotyping and QTL mapping**

Both parents of the RIL mapping population were genotyped for the known APR stripe rust genes. Closely linked polymerase chain reaction (PCR) markers for *Yr30/Sr2* (*gwm533*), *Yr18/Sr57/Lr34* (*csLV34*), *Yr29/Sr58/Lr46* (*csLV46G22*), and *Yr46/Sr55/Lr67* (*Lr67SNP*) were used to determine the presence or absence of these genes in parental lines. Detailed protocols for these markers are described in chapter 2. Ten GBS markers associated with 2NS/2AS translocation were also tested to check the presence or absence

of translocation from *Triticum ventricosum* carrying *Lr37/Sr38/Yr19* genes (Chapter 1, Suppl. Table 1). This study involved the genotyping data set generated through sequencing two GBS libraries on Illumina HiSeq 2500 and obtained more than 220 million reads for each genomic library. Genomic library preparation, sequencing and SNP calling procedures are described in chapter 1. A total 762 polymorphic SNP markers representing unique loci were used for the QTL mapping. QTL mapping procedures and assigning the names to identified QTL are described in chapter 1.

## Results

### Seedling and adult plant evaluations

The Mexican *Pst* race Mex14.191 had high infection (ITs > 7) on both parental lines Apav and COPIO. For all three US *Pst* races (PSTv-14, PSTv-37, PSTv-40), Apav was highly susceptible (IT ≥7) while COPIO was highly resistant with an IT range from 0-3 (Table 2). During the field evaluations, excellent disease development was observed in all environments. Usually the *Pst* natural infection remains low during the off-season in Kenya therefore only 20% FDS was recorded on the susceptible parent Apav (Table 3). In all other environments the FDS on Apav ranged from 80 to 100%. COIPO was highly resistant to *Pst* in the filed across all environments and the FDS ranged from 0 to 4.7%. The mean FDS on the RIL mapping population ranged from 35 to 48 % over the three seasons in Mexico, while it was 47% (Pum17) and 41% (Mtv17) at two locations in Washington. The population means were in the range of 41-44% and 36-40% during both seasons in Kenya and Pakistan, respectively (Table 3). In all nine environments, the disease distribution spectrum was continuous, and the mapping population lines were well represented in all severity classes (Fig. 1). The coefficient of infection values for

stripe rust had significant Pearson correlations (*r*) at either 1% or 5% (α = 0.01, 0.05) significance levels across all nine environments. Correlations ranged from 0.74 to 0.84 over three environments (Mex15, Mex16, & Mex17) in Mexico, 0.57 to 0.78 over two seasons (Ken16_OS & Ken16_MS) in Kenya, 0.45 to 0.72 across two locations (Pum17 & Mtv17) in Washington, and 0.42 to 0.68 over two environments (Pak16 & Pak17) in Pakistan (Table 4). The Mexican environments (Mex15 & Mex16) were the most highly correlated (*r* =0.82), followed by Mex15 and Ken16_OM (*r* = 0.78). Pum17 and Pak17 were the least correlated environments (*r* = 0.42) for stripe rust severities, but this level of correlation was still highly significant.

**Marker assays and QTL mapping**

Molecular marker assays on both parents revealed that Apav was negative for the known APR stripe rust genes (*Yr18, Yr29, Yr30,* and *Yr46*), while COPIO might have positive alleles for *Yr29* and *Yr30* on chromosomes 1BL and 3BS respectively. GBS markers were tested for 2NS/2AS translocation (segment carries *Sr38/Lr37/Yr17* genes) and COPIO was positive while Apav was negative (Table 5). The composite interval mapping (CIM) procedure detected 14 QTL conferring stripe rust resistance under field conditions in 9 tested environments. The resistant parent COPIO contributed 10 QTL, while 4 QTL were contributed by the susceptible parent Apav (Table 6). A large effect QTL (*QYr.umn.2A*) contributed by COPIO was detected in all nine environments and mapped on the short arm of chromosome 2A. This QTL explained phenotypic variance ($R^2$) ranging from 10-42% with logarithm of odds (LOD) values in the range of 5.8 to 26. Two other QTL contributed by COPIO were mapped on chromosomes 1B (*QYr.umn.1B.2*) and 3B (*QYr.umn.3B.1*) and were detected in five and seven

environments and the phenotypic variance was explained 4-22% and 6-14%, respectively. Chromosome 3B had three QTL, while chromosomes 1B and 3A had two QTL. Only one QTL was mapped on chromosomes 1A, 2A, 2B, 3D, 4A, 4B, and 5A (Figure 3).

**Discussion**

The results of seedling assays on the parental lines against the *Pst* race Mex14.191 showed that Apav and COPIO are highly susceptible, so mostly likely both lines do not carry qualitative genes *Yr5, Yr10, Yr15* and *Yr17* (based on virulence/avirulence formulae, Table 1). During field evaluations, COPIO was highly resistant across all environments, while Apav was highly susceptible except in the Kenya 2016 off-season because of usually low natural infection (Table 3). A continuous disease severity spectrum was observed in the RIL population across all environments, indicating that resistance is polygenic with additive effects (Fig. 1). Overall high coefficient of correlations among disease severities indicate that a similar response among genotypes was observed across most environments. Some environments have moderately low correlations (Table 4) that were expected because the experimental locations have different weather conditions and *Pst* races.

Molecular marker assays suggested that COPIO carry known APR genes, *Yr29* and *Yr30* on chromosomes 1BL and 3BS respectively, and genome wide markers also detected these genes. Susceptible parent Apav did not carry any known genes for stripe rust. Markers were also positive in COPIO for 2NS/2AS translocation segment which harbors *Yr17* gene. A total 14 QTL were detected in nine tested environments. The resistant parent COPIO contributed 10 QTL, while 4 QTL were contributed by the susceptible parent Apav (Table 6). Quantitative analysis detected one small effect QTL

(*QYr.umn.1A*) located on the long arm of chromosome 1A. This QTL was mapped at 62 cM and was detected in only one environment (Mex16) and accounted for 4% of the phenotypic variation for stripe rust. LOD peaks were observed for Mex15 and Mex17 as well but were non-significant. Therefore, this is most likely a race-specific QTL. Rosewarne et al. (2012) reported a small effect QTL in CIMMYT line 'Pastor' flanked by *wPT-6005* and *wPT-4709*, Diversity Array Technologies (DArT) markers. This QTL was detected in only two environments out of four and explained 3.6-4.1% of the phenotypic variation for stripe rust in Mexico. Later, in the consensus map for stripe rust QTL in wheat, Rosewarne et al. (2013) described Pastor QTL genetic positions at about 57 to 70 cM on chromosome 1AL. The QTL *QYr.umn.1A* in our study was contributed by COPIO which contains 'Pastor' as one of the parents in its pedigree. Based on the genetic position and the pedigree information, *QYr.umn.1A* is the same QTL previously reported by Rosewarne et al. (2012).

There were two QTL identified on chromosome 1B (*QYr.umn.1B.1* & *QYr.umn.1B.2*). The small effect QTL *QYr.umn.1B.1* was mapped distally on the short arm where two seedling genes, *Yr9* and *Yr10,* have been previously mapped. *QYr.umn.1B.1* was detected in only one environment (Mex16) and explained 3% of the phenotypic variation. Two races used for field inoculation in Mexico during the 2016 crop season were virulent on both *Yr9* and *Yr10* genes (Table 1), so most likely *QYr.umn.1B.1* represent a new small effect QTL for stripe rust resistance. The other QTL, *QYr.umn.1B.2,* was mapped on the long arm and detected in five environments (Suppl. Fig. 1). This QTL explained 4-22% of the phenotypic variance (Table 6). Chromosome 1BL is known to carry a very important locus, *Lr46/Yr29*; hereafter

referred as *Yr29*. In molecular maker assays for the known APR genes, *Yr29* (*csLV46G22*) allele was positive in COPIO. Furthermore, wheat cultivar 'Pastor' in the pedigree of COPIO carries *Yr29* allele (Rosewarne et al. 2012); therefore, we are confident that *QYr.umn.1B.2* represents the *Yr29* locus in our study. Many previous studies have reported presence of the *Yr29* locus (Lan et al, 2015; Rosewarne et al, 2012; Jagger et al, 2011; Bariana et al, 2010; Zwart et al, 2010) with a wide range of LOD values (2.7-23) and phenotypic variance (4.5-65%) explained by this allele.

A large effect QTL (*QYr.umn.2A*) located distally on the short arm of chromosome 2A was consistently detected in all nine environments. This QTL was contributed by COPIO with LOD values ranging from 5.8-26 and explained 10-42% of the stripe rust phenotypic variation (Fig. 2). The distal end of chromosome 2AS is known to carry a translocation (2NS/2AS) from *Triticum ventricosum* (Helguera et al., 2003) which harbors three qualitative genes *Lr37*, *Yr17*, and *Sr38* (Helguera et al., 2003). We postulated *Sr38* gene conferring resistance to stem rust (Chapter 1) and *Lr37* for leaf rust (Chapter 2) in COPIO in some of the tested environments along with possibly new genes in the same region. Molecular assays also revealed that COPIO is positive for 2NS/2AS translocation (Table 5). Although COPIO was positive for the *Yr17* gene, but we had unique observations on COPIO, when it was tested against *Yr17* virulent pathotypes in field. Most of the phenotyping environments had *Yr17* virulence (either natural infection or artificial inoculations). At two locations (Pum17 & Mtv17) in Washington state during 2017 *Pst* race PSTv-37 was predominant and is virulent on *Yr17*; in Mexico during 2017 crop season the 'Borlaug race' which is highly virulent on *Yr17*, was used as inoculum. In 2016 phenotyping environments of Kenya (Ken16_MS & Ken16_OS), PstS2 (virulent

to *Yr27*) was the most frequent race and a low frequency of PstS11 (virulent to *Yr17*). In seedling assays, *Pst* race Mex14.191 (avirulent on *Yr17*) had high infection (IT = 8) on COPIO and Apav (Table 2). Moreover, COPIO had resistant reaction against a *Pst* race from India which is completely virulent on *Yr17* (R. P. Singh; Personal communication). Another QTL *QYr.sun-2A* (*Yr56*) has been previously mapped in the same region in the Australian durum cultivar Wollario (Bansal et al, 2014). This QTL was detected in three environments with LOD values 2-3.40 and mean phenotypic variance 9.5%. COPIO is not known to have durum (*Triticum turgidum*) parents in the pedigree, so we eliminate the possibility of *Yr56*. Based on the above-mentioned observations, we are confident that an additional APR gene on or tightly linked with the 2NS/2AS translocation is responsible for the results we have observed. It is also possible that *Yr17* has some residual effects in the environments where virulent pathotypes had low frequency.

The QTL *QYr.umn.2B* was mapped proximal to the centromere of chromosome 2B. This QTL was contributed by COPIO ($R^2$ = 8-18%) and was detected in three environments (Pak16, Pak17, & Ken16_OS). The proximal region of chromosome 2B has seedling genes *Yr27* (McDonald et al, 2004) and *Yr31* (Singh et al, 2003). All phenotyping environments had virulent pathotypes for *Yr27*; PSTv37, Mex96.11 and PstS2 at Washington state, Mexico and Kenya respectively. *Yr27* virulence is also frequent in Pakistan (Bux et al, 2012). Therefore, it is not likely that *QYr.umn.2B* represents the *Yr27* gene. The seedling gene *Yr31* was found in the CIMMYT cultivar Pastor (Singh et al, 2003). It is more convincing that *QYr.umn.2B* QTL represents *Yr31* because Pastor is one of the parents in the pedigree of COPIO. *QYr.umn.2B* (*Yr31*) was detected during both seasons in Pakistan, where a bulk of unknown races was inoculated

on the nurseries. Although *Yr31* virulent pathotype is present in some parts of the country, frequency is very low compare to other virulences (Bux et al, 2011).

Two QTL, *QYr.umn.3A.1* and *QYr.umn.3A.2,* were mapped on chromosome 3A in the vicinity of the centromere on the short arm and distal end of the long arm, respectively. The QTL *QYr.umn.3A.1* was contributed by Apav and detected in two environments (Mex16 & Pum17). Rosewarne et al. (2012) reported an inconsistent small effect QTL ($R^2$ = 2.4-3.2%) flanked by DArT markers *wPT-6422* and *wPT-7890* in susceptible Avocet. This QTL was mapped on the short arm, close to the centromere, and was detected in two out of four Mexican environments. Apav carries susceptible Avocet in the pedigree so it is more likely that *QYr.umn.3A.1* is the same QTL previously reported by Rosewarne et al. (2012). The other QTL, *QYr.umn.3A.2,* was mapped at 107 cM and detected in only one environment (Pum17). This QTL was derived from COPIO and had very small effect with LOD value 2.7 and $R^2$ 3.0%. A minor effect QTL ($R^2$ = 4.7 %) was reported on 3BL in a slow rusting winter wheat line AQ2478883. This QTL mapped close to the centromere (49 cM) flanked by SSR markers *wmc627c* and *wmc624* on 3BL (Quan et al, 2013). According to the high-density microsatellite wheat consensus map, the centromere in chromosome 3A is at approximately 46 cM (Somers et al, 2004). Another QTL (*QYrst.orr-3AL*) was reported in wheat cultivar 'Stephens' which was released in 1978 in the Pacific Northwest (Vazquez et al, 2012). This QTL mapped at about 30 cM on the genetic map and was detected only in one environment (Mount Vernon) with LOD value 3.1 and $R^2$ of 10%. No any known seedling or APR gene/QTL has been reported distally on the long arm of chromosome 3A, hence *QYr.umn.3A.2* could be a small effect new QTL for stripe rust.

Chromosome 3B harbored three QTL; *QYr.umn.3B.1*, *QYr.umn.3B.2*, and *QYr.umn.3B.3*. Two QTL *QYr.umn.3B.1*, and *QYr.umn.3B.3* were derived from COPIO while *QYr.umn.3B.2* was contributed by Apav (Table 6). The QTL *QYr.umn.3B.1* was detected in seven out of nine environments and non-significant LOD peaks were observed in other two environments as well (Suppl. Fig. 2). The short arm of chromosome 3B carries an APR gene *Yr30* which is linked or pleiotropic to stem rust APR gene *Sr2* and Pseudo-black chaff (PBC) (Singh and McIntosh, 1984). Wheat cultivar Pastor displays PBC (Rosewarne et al, 2012). Molecular assays for *Yr30*/*Sr2* (*gwm533*) were positive in COPIO (Table 5); therefore, it provides strong evidence that the genomic region *QYr.umn.3B.1* represents *Yr30*. The second small effect QTL *QYr.umn.3B.3* was located close to the centromere on the short arm of chromosome 3B and was detected in one environment (Pak16). In the same chromosomal region, two previous studies have reported small to medium effect, inconsistently detected QTL in wheat cultivars Renan (Dedryver et al, 2009) and Pastor (Rosewarne et al, 2012). The QTL described by Dedryver et al. (2009) is closely linked with SSR marker *Xgwm533* which represents *Sr2*/*Yr30* locus. Most likely the QTL *QYr.umn.3B.3* contributed by COPIO is the same previously described by Rosewarne et al, (2012) because 'Pastor' is present in the pedigree of COPIO. The third small effect QTL, *QYr.umn.3B.2,* was centrally located on the short arm of chromosome 3B and was also detected in one environment (Ken16_OS). This QTL was contributed by the susceptible parent Apav and explained 4% of the phenotypic variation for stripe rust. At this stage we could not find any comparison of this small effect genomic region. Many mapping studies may not detect small effect QTL.

We identified four genomic regions for stripe rust resistance on chromosomes 3D, 4A, 4B, and 5A. All these genomic regions represented small to medium effect QTL that were detected in single environments. The QTLs on chromosome 3D and 4A were contributed by COPIO, while on 4B and 5A QTLs were contributed by Apav (Table 6). The QTL (*QYr.umn.3D*) on chromosome 3D was mapped distally on the long arm. This chromosomal region harbors seedling resistant gene *Yr45*. This gene was identified in spring wheat genotype PI 181434 from Afghanistan and was highly resistant to the US *Pst* races (PST-17, PST-37, PST-43, PST-45) at the seedling stage (IT = 2) and also during the field evaluations in Pullman, Washington State (Li et al, 2011). The *Yr45* gene map spanned 50 cM on 3DL, flanked by eight resistance gene analog polymorphism (RGAP) and two SSR markers. The genomic region in our study was only detected in one environment (Mtv17) in Washington. It is likely that QTL *QYr.umn.3D* represents *Yr45*. Some non-significant LOD peaks were also observed in the same region which reflects the possibility of *Yr45* detection in other environments as well where its virulence was not present in the prevalent races. Moreover, the D genome of wheat is less represented by the GBS genotyping platform with less marker coverage and low mapping resolution. For the validation of *Yr45* gene in COPIO, the closely linked RGAP markers *Xwpg115* and *Xwgp118* needs to be assayed.

The QTL (*QYr.umn.4A*) on chromosome 4A was mapped distally to the long arm and detected in one environment (Mex15). The LOD peak was observed in the same region for Mex17 environment as well but it was not significant. A seedling gene *Yr60* has been mapped on the distal end of chromosome 4AL. This gene was derived from spring wheat line Almop (pedigree; Avocet*3//Lalbmonol*4/Pavon) and most likely this

resistance came from ‘Lalbahadur’ which was transferred to Pavon 76 (Herrera-Foessel et al, 2015). Many CIMMYT lines have Pavon 76 as a common parent in the pedigree. Apav is also derived from a cross of Pavon 76 and Avocet S. Furthermore, *Yr60* tightly linked SSR markers *wmc313* and *wmc219* mapped to the distal end of 4AL on Somers et al. (2004) microsatellite consensus map of bread wheat. This is the same region where the QTL *QYr.umn.4A* was detected in our study. Therefore, it is likely that *Yr60* allele is segregating in our population and QTL *QYr.umn.4A* represents this allele.

A small effect QTL (*QYr.umn.4B*) on chromosome 4B was contributed by Apav and detected in a single environment (Mex15). This QTL mapped close to the centromere on 4BL (36 cM) and explained 3% of the phenotypic variance for stripe rust (Table 6). Seedling resistant gene *Yr50* which was derived from *Thinopyrum intermedium* has been mapped to the centromeric region on 4BL. Our parental lines are not known to carry *Thinopyrum intermedium* in their pedigree, so we eliminated *Yr50* possibility in Apav. William et al. (2006) reported a QTL in a biparental mapping population contributed by ‘Avocet S’ and closely linked with SSR marker ‘*Xgwm495*’. *Xgwm495* is located at 36 cM on the Somers et al. (2004) microsatellite consensus map of bread wheat. This is a similar genetic position to the QTL identified in our study. Additionally, Avocet S is one of the parents in ‘Apav’ pedigree, so it is more convincing that QTL *QYr.umn.4B* is the same previously described by William et al. (2006).

Another small effect QTL (*QYr.umn.5A*) was contributed by Apav and mapped distally to the short arm of chromosome 5A. This QTL was only detected in one environment (Mex17). No known genes have been previously mapped on the short arm of chromosome 5A. Two small effect QTL; *Qyrsicau-5AS* (Ye et al, 2019) and a QTL

linked with marker '*wsnp_Ex_c807_1586396-5AS'* (Zegeye et al, 2014) have been reported on the distal end of chromosome 5A. The QTL *Qyrsicau-5AS* (101 Mb) is ~80 Mb from the QTL linked with *wsnp_Ex_c807_1586396-5AS* and ~99 Mb from *QYr.umn.5A* (1.8 Mb). We suggest that *QYr.umn.5A* is a different QTL from other two reported and most likely represents a unique locus for stripe rust in Apav.

**Conclusions**

The wheat line COPIO displayed a high level of resistance against *Pst* in all phenotyping environments under field conditions. This line carries quantitative genes *Yr29* and *Yr30* on chromosomes 1BL and 3BS, respectively. A large effect QTL (*QYr.umn.2A*) mapped distally on the short arm of chromosome 2A was consistently detected in all nine environments and represents a new locus for stripe rust resistance. Moreover, three seedling genes *Yr31*, *Yr45*, and *Yr60* on chromosomes 2B, 3D and 4B respectively, were also mapped in COPIO. Some small to medium effect QTL which have been either previously reported (*QYr.umn.1A; QYr.umn.3B.3*) or potentially new genes of resistance (*QYr.umn.1B.1; QYr.umn.3A.2*) are also contributing quantitative resistance to stripe rust in COPIO. The findings of this study revealed that COPIO harbors a couple of qualitative and quantitative genes for stripe rust and they are in combination providing significant resistance against *Pst* pathotypes. Although virulent pathotypes are known for the seedling resistance genes *Yr31*, *Yr45*, and *Yr60* in many wheat growing areas across the world, these genes can be deployed in combination with other resistant genes. COPIO can be a potential source of resistance for stripe rust and

can be used in recombination breeding with other effective genes to enhance durable resistance in wheat.

Table 1: Virulence and avirulence patterns of *Puccinia striiformis* f. sp. *tritici* (*Pst*) races involved in field and seedling assays

| *Pst* races | Virulence | Avirulence |
|---|---|---|
| PSTv-14 | *Yr1,6,7,8,9,17,27,43,44, Tr1, Exp2, Tye* | *Yr5,10,15,24,32, SP* |
| PSTv-37 | *Yr6,7,8,9,17,27,43,44, Tr1, Exp2* | *Yr1,5,10,15,24,32, SP,Tye* |
| PSTv-40 | *Yr6,7,8,9,10,24,27,32,43,44, Tr1, Exp2* | *Yr1,5,15,17, SP, Tye* |
| Mex96.11 | *Yr2,6,7,9,10,27, A* | *Yr1,3,4,5,8,15, 17,24,26,31, Sp, Poll* |
| Mex08.13 | *Yr2,6,7,8,9,10,31, A* | *Yr1,3,4,5,15, (17),24,26,27, Sp, Pol* |
| Mex14.191 | *Yr2,3,6,7,8,9,27,31, A* | *Yr1,4,5,10,15, (17),24,26, Sp, Poll* |

The virulence/avirulence formula is based on the reactions of the *Yr* near isogenic lines in the 'Avocet S' background to *Pst* races.

Table 2: Seedling infection types of both parents to *Puccinia striiformis* f. sp. *tritici* (*Pst*) races

| | Mexican *Pst* race | US *Pst* races | | |
|---|---|---|---|---|
| Parent lines | Mex14.191 | PSTv-14 | PSTv-37 | PSTv-40 |
| Apav | 8 | 8 | 7 | 7 |
| COPIO | 8 | 2 | 0 | 3 |

Lines with infection type (IT) 0-6 were considered resistant and 7-9 were susceptible

Table 3: Final disease severity (FDS %) based on coefficient of infection (COI) on both parents, population mean, standard deviation and range of stripe rust in nine environments

| | FDS (%) on parents | | | | | |
|---|---|---|---|---|---|---|
| Environments [a] | Apav | COPIO | Pop. Mean | Std. Deviation | Low range | High range |
| Mex15 | 100 | 0 | 35.3 | 30.7 | 0 | 100 |
| Mex16 | 100 | 0.27 | 38.4 | 31.3 | 0.6 | 100 |
| Mex17 | 100 | 0.4 | 48.6 | 37.5 | 0 | 100 |
| Ken16_OS | 20 | 0 | 44.5 | 25.8 | 0 | 100 |
| Ken16_MS | 90 | 2 | 41.7 | 29.5 | 0 | 100 |
| Pum17 | 80 | 1 | 47 | 39.6 | 0.2 | 100 |
| Mtv17 | 100 | 1 | 41.8 | 35.9 | 1 | 100 |
| Pak16 [b] | 90 | 4.7 | 40.9 | 30.1 | 0 | 100 |
| Pak17 | 80 | 4 | 36.9 | 26.1 | 0 | 90 |

[a] = Mex = Mexico, Ken = Kenya, Pum = Pullman, Mtv = Mount Vernon, Pak = Pakistan; 15, 16 and 17 are the years 2015, 2016 & 2017 during which nurseries were evaluated for stripe rust in field. For Kenya 'OS' and 'MS' represents off-season and main season respectively.

[b] = In Pakistan 2016 environment 19 RILs were missing while all other environments had 176 lines.

Table 4: Pearson correlation coefficients (r) among coefficient of infection values based on final disease severities on Apav x COPIO RILs in nine phenotyping environments for stripe rust.

| | Mex15 | Mex16 | Mex17 | Ken16_OS | Ken16_MS | Pum17 | Mtv17 | Pak16 | Pak17 |
|---|---|---|---|---|---|---|---|---|---|
| Mex15 | 1 | | | | | | | | |
| Mex16 | 0.82** | 1 | | | | | | | |
| Mex17 | 0.80** | 0.74** | 1 | | | | | | |
| Ken16_OS | 0.62** | 0.57** | 0.58** | 1 | | | | | |
| Ken16_MS | 0.78** | 0.69** | 0.69** | 0.61** | 1 | | | | |
| Pum17 | 0.67** | 0.68** | 0.61** | 0.45** | 0.64** | 1 | | | |
| Mtv17 | 0.63** | 0.54** | 0.52** | 0.45** | 0.65** | 0.72** | 1 | | |
| Pak16 | 0.68** | 0.58** | 0.56** | 0.50** | 0.58** | 0.46** | 0.53** | 1 | |
| Pak17 | 0.59** | 0.55** | 0.53** | 0.50** | 0.62** | 0.42** | 0.50** | 0.60** | 1 |

*, ** Correlation is significant at the 0.05 and 0.01 level, respectively

Table 5: Molecular marker assays on both parents of the RIL mapping population for known stripe rust APR genes

| Genes | Chromosome | Marker | Apav | COPIO |
|---|---|---|---|---|
| *Yr18/Sr57/Lr34* | 7DS | *csLV34* | –[a] | –[a] |
| *Yr29/Sr58/Lr46* | 1BL | *csLV46G22* | – | + |
| *Yr30/Sr2* | 3BS | *gwm533* | – | + |
| *Yr46/Sr55/Lr67* | 4DL | *Lr67SNP* | – | – |
| *Yr17/Sr38/Lr37* | 2AS | *GBS* | – | + |

[a] + and – represent presence or absence of the gene, respectively

Table 6: Quantitative trait loci (QTL) detected in nine environments and associated with stripe rust resistance in Apav x COPIO RIL mapping population

| Chr [a] | Environment [b] | QTL | Peak Marker | Position(cM) | LOD | $R^2$ (%) [c] | Add [d] |
|---|---|---|---|---|---|---|---|
| 1A | Mex16 | QYr.umn.1A | chr1A_537093931 | 62.0 | 3.5 | 0.04 | 6.5 |
| 1B | Mex16 | QYr.umn.1B.1 | chr1B_390397700 | 15.5 | 2.7 | 0.03 | 5.6 |
| | Mex15 | QYr.umn.1B.2 | chr1B_670401542 | 79.9 | 3.1 | 0.04 | 6.2 |
| | Mex17 | QYr.umn.1B.2 | chr1B_658531439 | 74.3 | 6.6 | 0.22 | 17.8 |
| | Ken16_OS | QYr.umn.1B.2 | chr1B_678465602 | 87.0 | 12.8 | 0.18 | 11.2 |
| | Ken16_MS | QYr.umn.1B.2 | chr1B_658531439 | 77.3 | 5.0 | 0.09 | 9.2 |
| | Pak17 | QYr.umn.1B.2 | chr1B_670401542 | 79.9 | 3.5 | 0.05 | 5.8 |
| 2A | Mex15 | QYr.umn.2A | chr2A_21273919 | 11.0 | 20.5 | 0.31 | 18.4 |
| | Mex16 | QYr.umn.2A | chr2A_17002870 | 11.0 | 26.0 | 0.42 | 21.2 |
| | Mex17 | QYr.umn.2A | chr2A_17002870 | 11.0 | 22.7 | 0.38 | 24.7 |
| | Ken16_OS | QYr.umn.2A | chr2A_17002870 | 10.0 | 16.2 | 0.24 | 13.9 |
| | Ken16_MS | QYr.umn.2A | chr2A_21273919 | 11.0 | 19.7 | 0.30 | 17.2 |
| | Pum17 | QYr.umn.2A | chr2A_21273919 | 11.0 | 21.3 | 0.32 | 23.8 |
| | Mtv17 | QYr.umn.2A | chr2A_17002870 | 11.0 | 12.3 | 0.20 | 17.2 |
| | Pak16 | QYr.umn.2A | chr2A_17002870 | 11.0 | 5.8 | 0.10 | 10.5 |
| | Pak17 | QYr.umn.2A | chr2A_21273919 | 11.0 | 10.4 | 0.15 | 10.9 |
| 2B | Ken16_OS | QYr.umn.2B | chr2B_535263704 | 62.0 | 6.7 | 0.08 | 8.1 |
| | Pak16 | QYr.umn.2B | chr2B_159489983 | 60.2 | 6.3 | 0.11 | 11.1 |
| | Pak17 | QYr.umn.2B | chr2B_130342950 | 59.8 | 12.0 | 0.18 | 12.2 |
| 3A | Mex16 | QYr.umn.3A.1 | chr3A_23176541 | 30.6 | 2.6 | 0.09 | -9.7 |
| | Pum17 | QYr.umn.3A.1 | chr3A_23176541 | 40.6 | 3.6 | 0.09 | -12.6 |
| | Pum17 | QYr.umn.3A.2 | chr3A_727547097 | 107.7 | 2.7 | 0.03 | 7.3 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 3B | Mex15 | QYr.umn.3B.1 | chr3B_6071517 | 25.6 | 7.1 | 0.09 | 9.5 |
| | Mex16 | QYr.umn.3B.1 | chr3B_1339088 | 23.2 | 7.9 | 0.10 | 10.3 |
| | Mex17 | QYr.umn.3B.1 | chr3B_12181366 | 26.1 | 5.4 | 0.07 | 10.3 |
| | Ken16_MS | QYr.umn.3B.1 | chr3B_17625393 | 20.8 | 9.0 | 0.13 | 10.7 |
| | Pum17 | QYr.umn.3B.1 | chr3B_4469125 | 25.2 | 10.2 | 0.14 | 15.0 |
| | Mtv17 | QYr.umn.3B.1 | chr3B_6071517 | 25.6 | 7.6 | 0.11 | 12.5 |
| | Pak17 | QYr.umn.3B.1 | chr3B_18567724 | 13.8 | 4.3 | 0.06 | 6.3 |
| | Ken16_OS | QYr.umn.3B.2 | chr3B_753924651 | 77.7 | 3.6 | 0.04 | -5.5 |
| | Pak16 | QYr.umn.3B.3 | chr3B_768723701 | 93.3 | 4.3 | 0.07 | 8.5 |
| 3D | Mtv17 | QYr.umn.3D | chr3D_549126553 | 170.7 | 2.6 | 0.04 | 7.3 |
| 4A | Mex15 | QYr.umn.4A | chr4A_621285371 | 173.0 | 2.6 | 0.11 | 10.2 |
| 4B | Mex15 | QYr.umn.4B | chr4B_530707993 | 36.0 | 2.7 | 0.03 | -6.8 |
| 5A | Mex17 | QYr.umn.5A | chr5A_1803125 | 0.1 | 2.9 | 0.04 | -7.9 |

[a] Chromosome on which QTL was detected

[b] Phenotyping environments for stripe rust (Mex15 = Mexico 2015, Mex16 = Mexico 2016, Mex17 = Mexico 2017, Ken16_OS = Kenya 2016 off-season, Ken16_MS = Kenya 2016 main-season, Pak16 = Pakistan 2016, Pak17 = Pakistan 2017, Pmv17 = Pullman 20107, and Mtv17 = Mount Vernon 2017)

[c] Maximum phenotypic variance explained by the QTL

[d] Maximum additive effects of the QTL, values with negative sing (-) indicate that the QTL is contributed by Apav

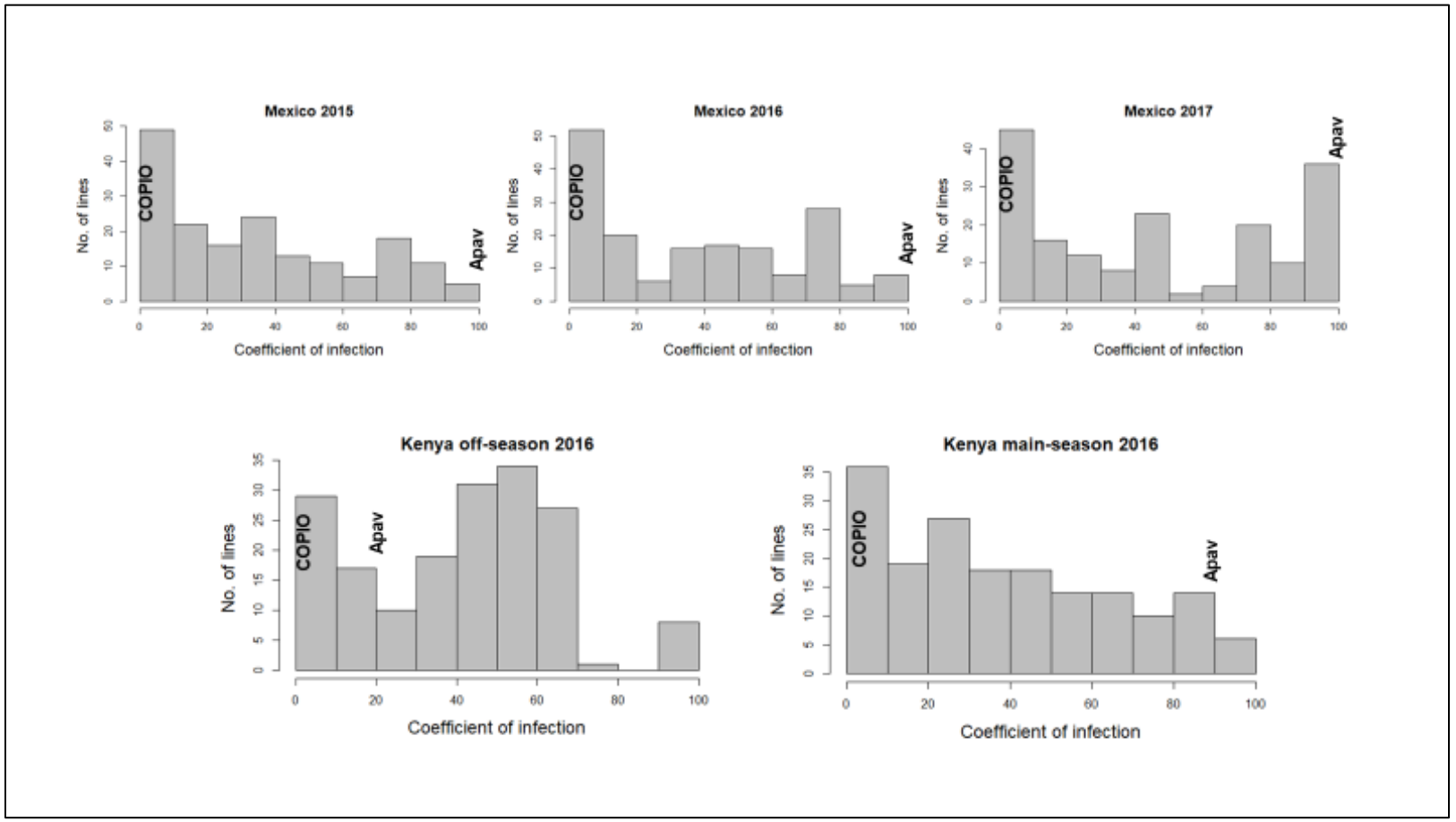



Figure 1 (continued)

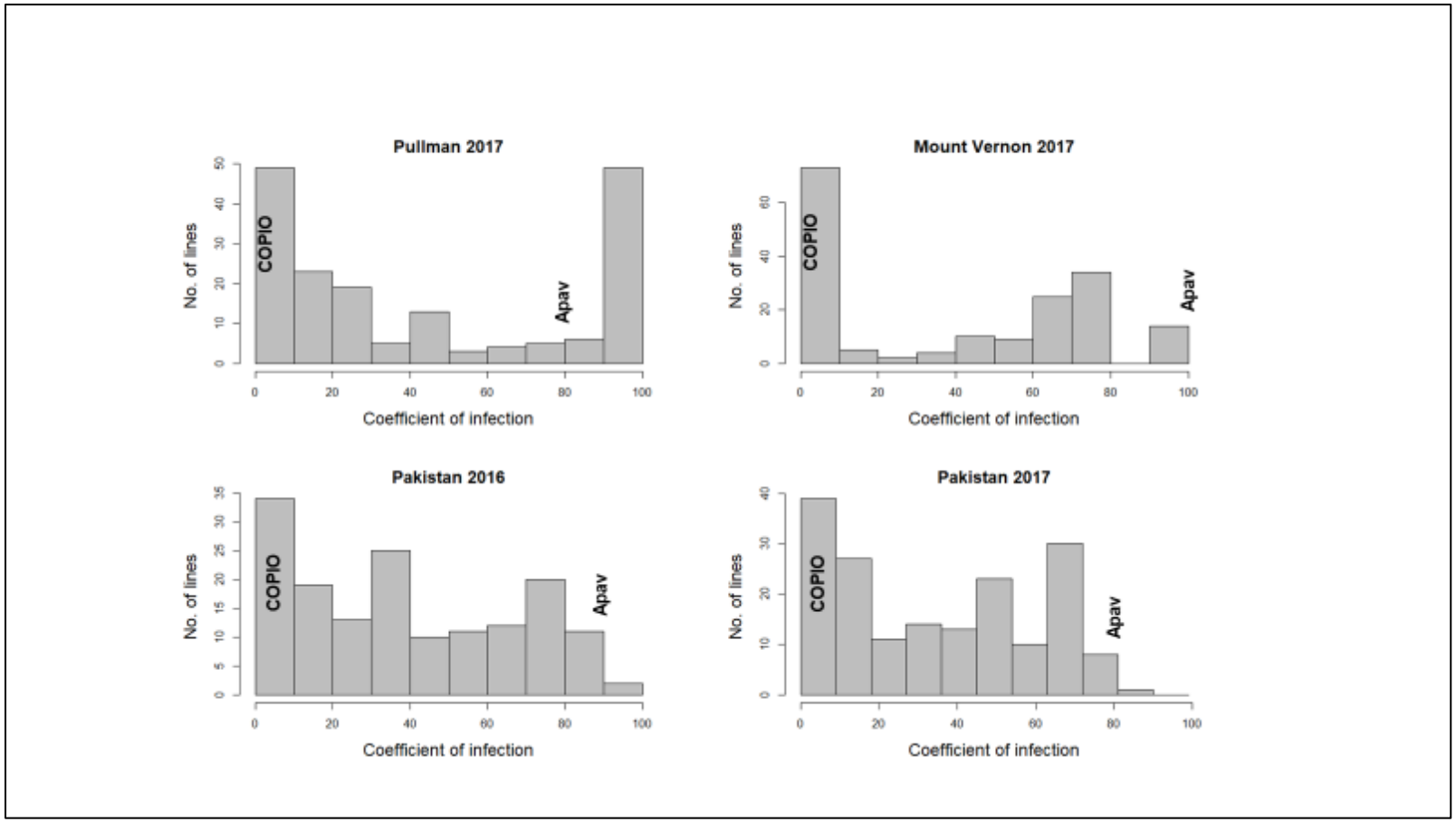



Figure 1: Frequency distribution of stripe rust coefficient of infection (COI) in the Apav/COPIO RIL mapping population in nine field environments. Parents (Apav & COPIO) shown on the bars represent the COI values in each environment.

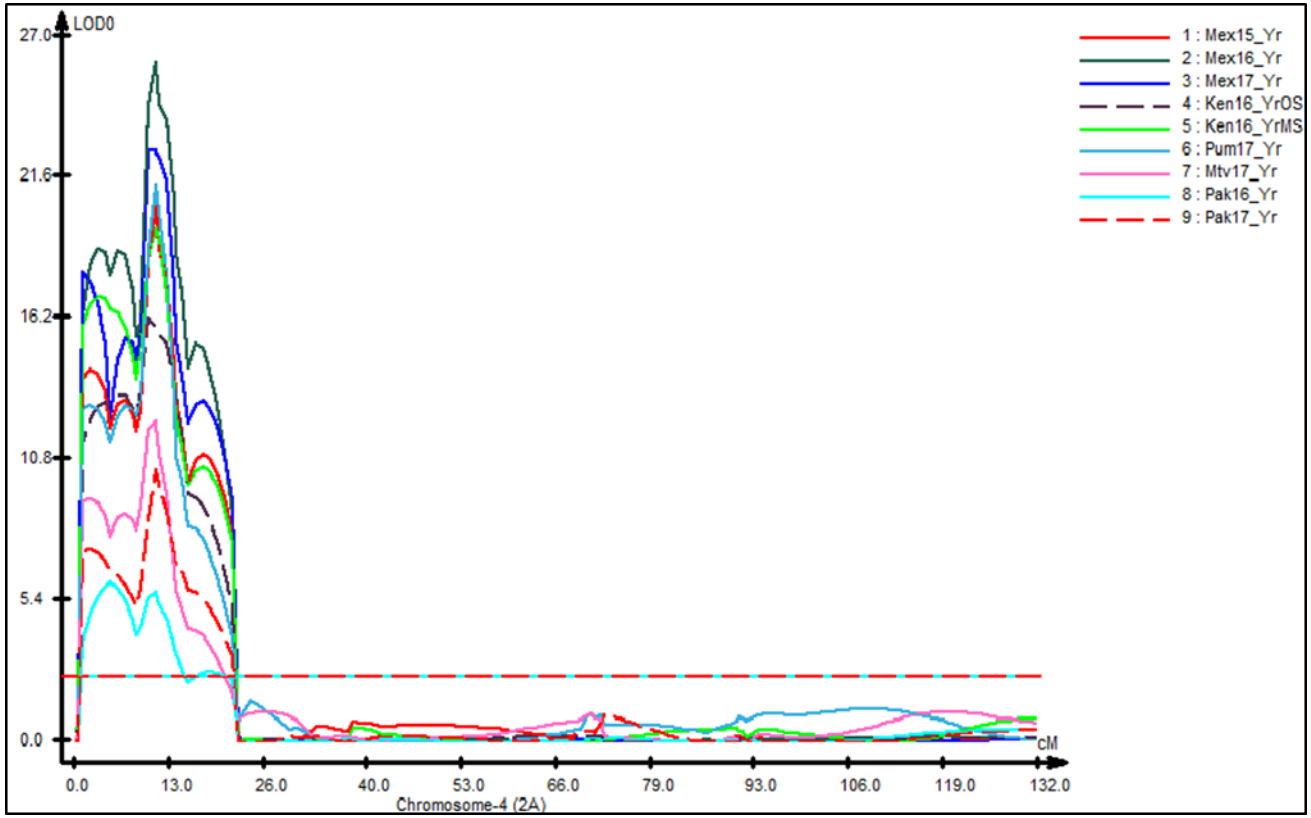



Figure 2: The large effect QTL (*QYr.umn.2A*) mapped distally on the short arm of chromosome 2A in all nine environments. X-axis represent the logarithm of odds (LOD) values and Y-axis indicates genetic distance in centimorgans (cM) on chromosome 2A. Horizontal dotted line along the X-axis is the threshold level of LOD value at which a QTL was declared significant.

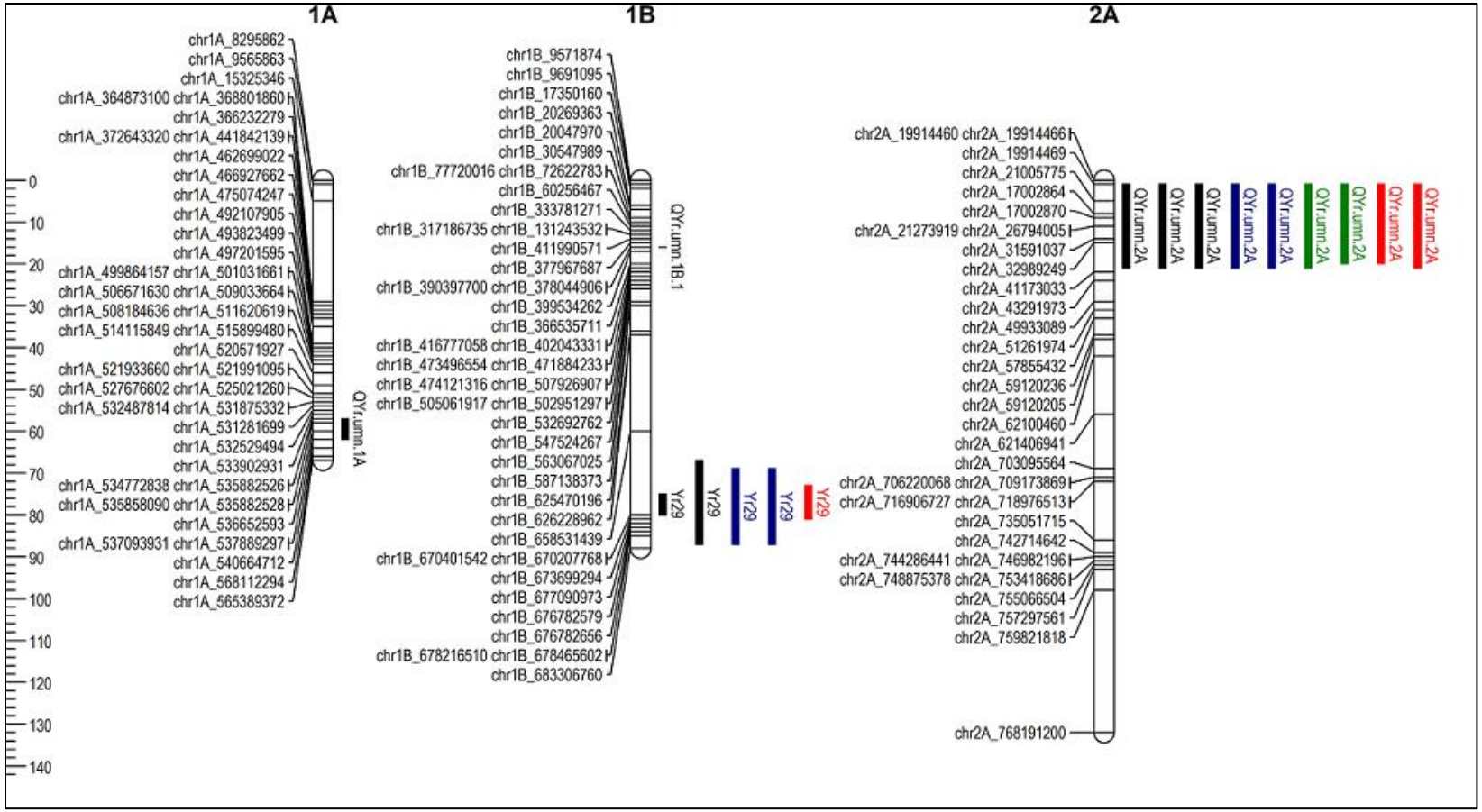



Figure 3 (continued)

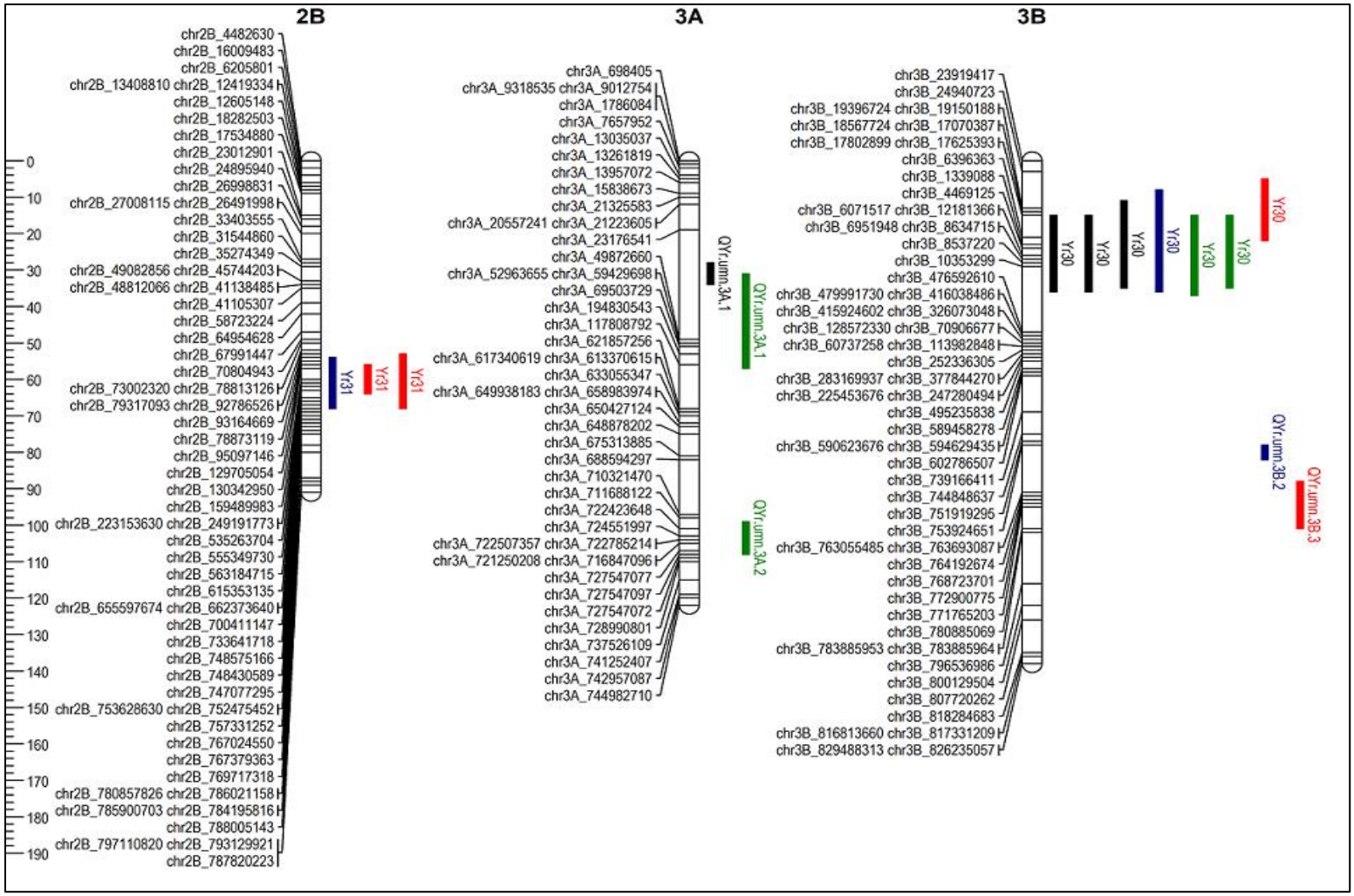



Figure 3 (continued)

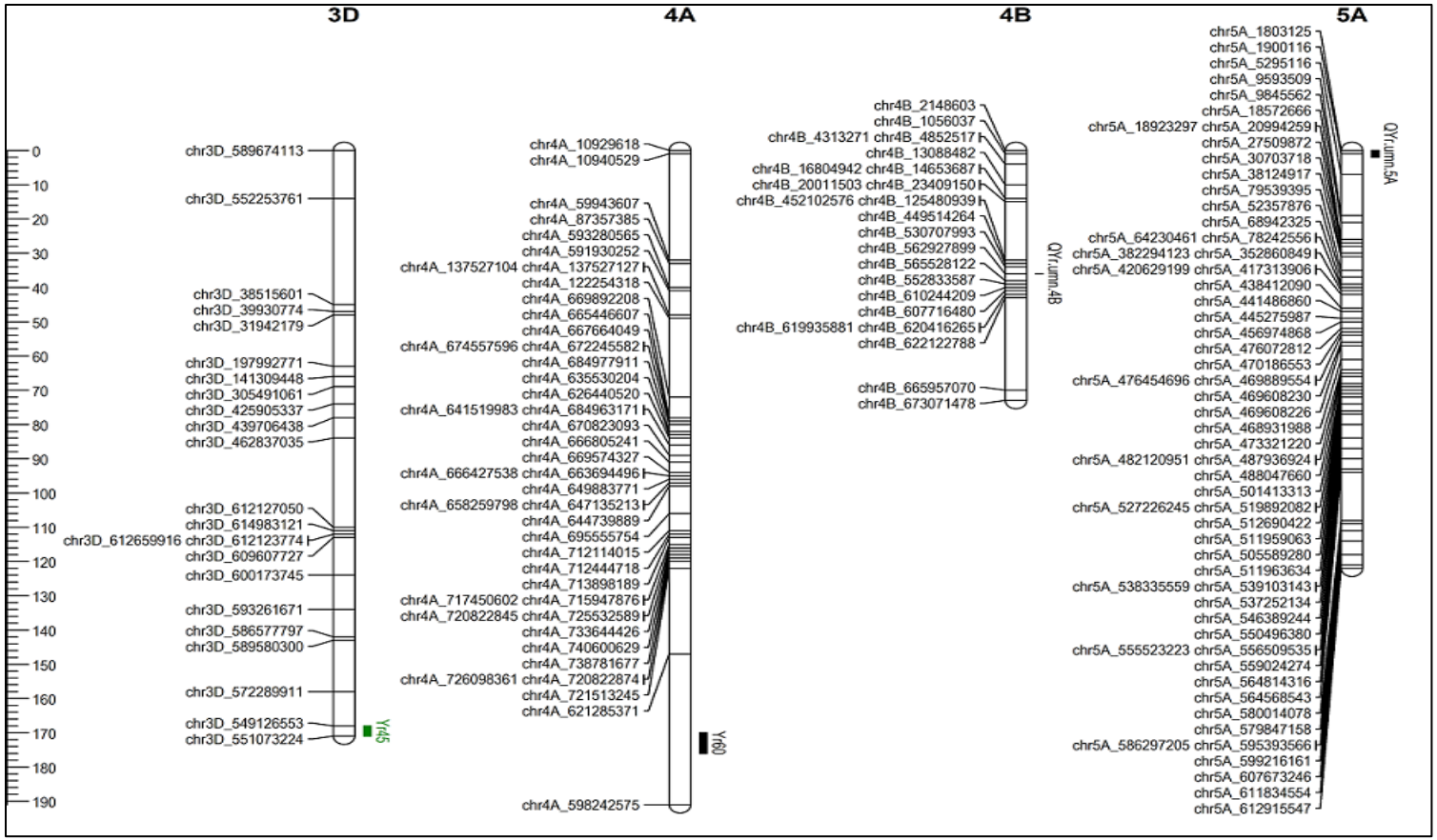
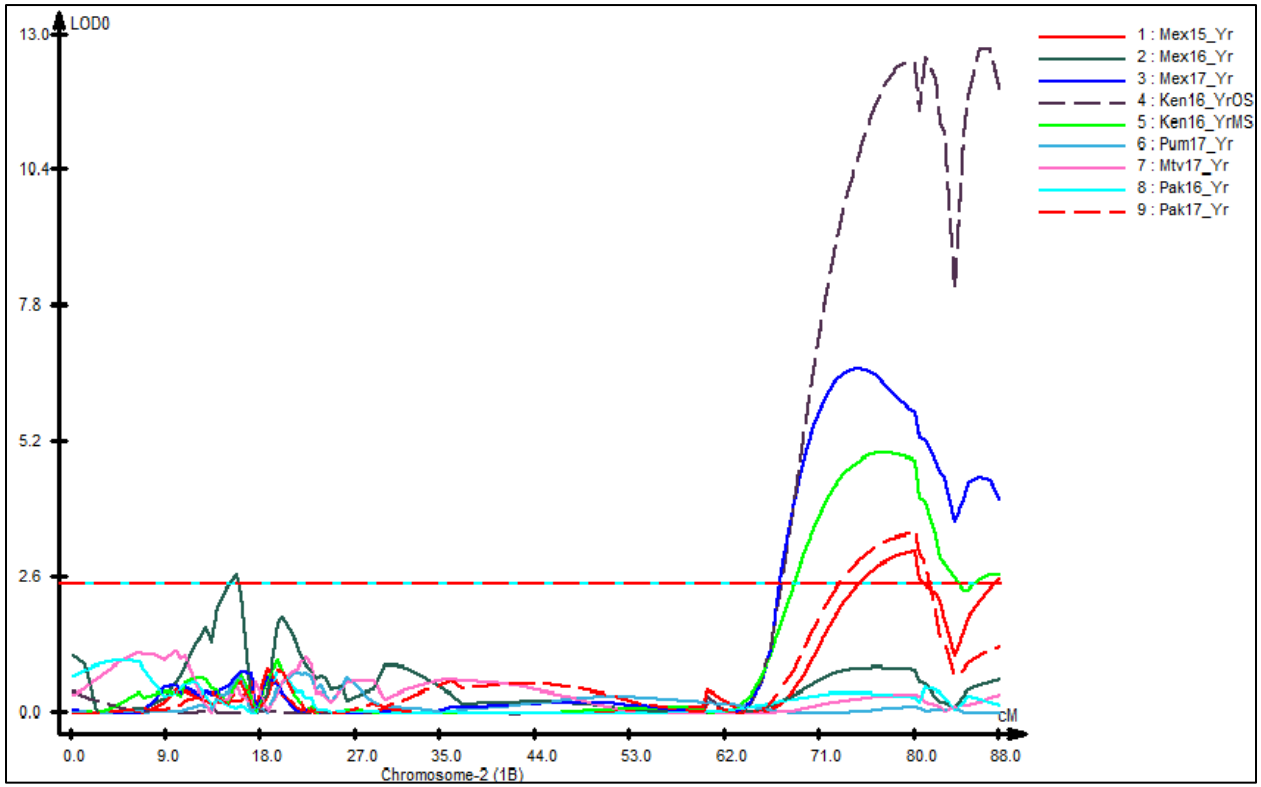
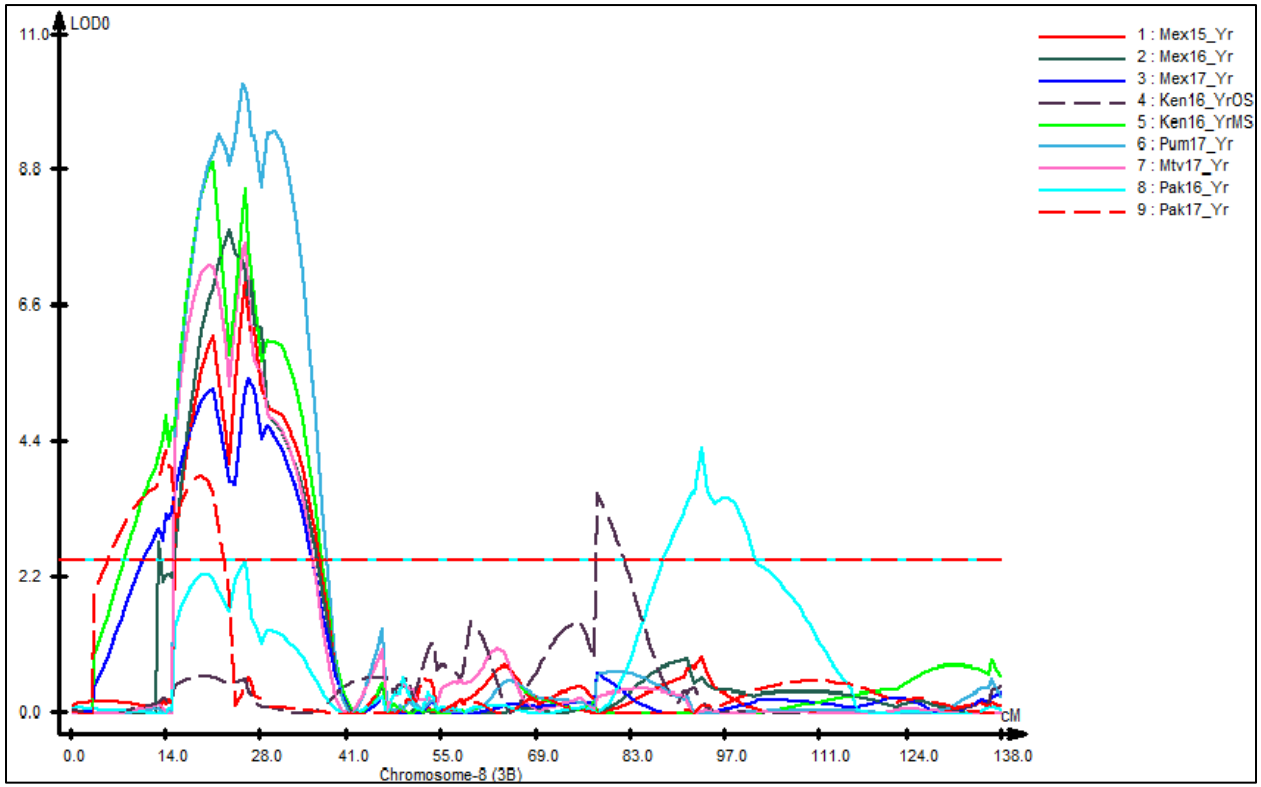



Figure 3: Chromosomes on which stripe rust QTL were detected across environments. GBS SNP markers are on the left side of each chromosome and the detected QTLs are on the right side shown in colored bars perpendicular to the chromosomes. Black, red, dark green, and blue colors represent that QTLs were detected in Mexico, Pakistan, Washington state, and Kenya, respectively. Known genes were detected on 1B (*Yr29*), 2A (*Lr37*), 2B (*Yr31*), 3B (*Yr30*), 3D (*Yr45*), and 4A (*Yr60*). QTLs stacking on each other at chromosomes 2A (*QYr.umn.2A*) and 3A (*QYr.umn.3A.1*) were detected in different environments and represent the same QTL. The QTL *QYr.umn.2A* was detected in all environments and most likely is a new resistance gene.




S. Figure. 1: Stripe rust QTL mapped on the long arm of chromosome 1B which represents non-race-specific adult plant resistant (APR) gene *Yr29*. A small effect QTL detected in Mexico 2016 is located on the short arm. X-axis represent the logarithm of odds (LOD) values and Y-axis indicates genetic distance in centimorgans (cM) on chromosome 1B. Horizontal dotted line along the X-axis is the threshold level of LOD value on which a QTL was declared significant. QTL peaks appeared for other environments but did not hit the threshold LOD level.




S. Fig. 2: Stripe rust QTL mapped on the long arm of chromosome 3B which represents adult plant resistant (APR) gene *Yr30*. Two relatively small effect QTL mapped at 77 and 93 cM on the long arm detected in Ken16_OS and Pak16, respectively. X-axis represent the logarithm of odds (LOD) values and Y-axis indicates genetic distance in centimorgans (cM) on chromosome 3B. Horizontal dotted line along the X-axis is the threshold level of LOD value on which a QTL was declared significant. QTL peaks appeared for other two environments but did not hit the threshold LOD level.

# Chapter 4

## Genetic Relationship of Adult Plant Resistance to Wheat Rusts in Spring Wheat 'COPIO'

### Introduction

Fungal pathogens are mainly described as being either biotrophic or necrotrophic. Biotrophic fungi are highly specialized and require living plants for their nutrition to survive. These fungi typically grow between the host cells and invade only a few plant cells to develop the nutrient absorbing structures called haustoria. In wheat the most important disease caused by the biotrophic pathogens are; stem rust (caused by *Puccinia graminis* f. sp. *tritici*, hereafter referred as *Pgt*), leaf rust (caused by *Puccinia triticina*, hereafter referred as *Pt*), stripe rust (caused by *Puccinia striiformis* f. sp. *tritici*, hereafter referred as *Pst*), and powdery mildew (caused by *Blumeria graminis* f. sp. *tritici*, hereafter referred as *Bgt*). Three wheat rusts are grouped into a sub-class of biotrophic fungi 'Basidiomycota' while powdery mildew belongs to 'Ascomycota'. These fungal pathogens have various distinct physiological races (Stakman, and Piemeisal, 1917) that are catalogued by assaying the host response on lines carrying single genes also known as differential lines (Stakman et al, 1962). Wheat rusts and the mildews have been a concentrated area for research and harnessing breeding efforts due to the pathogen nature to overcome deployed race-specific resistance in a short period of time and cause yield losses due the 'Boom and Bust' cycle (Kilpatrick, 1975). Conversely, the necrotrophic fungi survive on the dead plant tissues and do not necessarily require living plant cells. Some of the more important necrotrophic fungi affecting wheat are: Fusarium head blight (FHB, caused by *Fusarium graminearum*), Septoria tritici blotch (ST, caused by *Zymoseptoria tritici* syn. anamorph *Septoria tritici*; teleomorph *Mycosphaerella*

*graminicola*), tan spot (TS, caused *by Pyrenophora tritici-repentis*, spot blotch (SB, caused by *Cochliobolus sativus*), and Stagonospora nodorum blotch (SNB, caused by *Parastagonospora nodorum*), and wheat blast (WB, caused by *Magnaporthe oryzae*). In wheat, annual yield losses due to pests and pathogens have been estimated at 21.5% globally, with a range of 10.1 to 28.1% (Savary et al, 2019). The three rust diseases (*Pgt*, *Pst*, *Pt*) under epidemic conditions can cause 60-100% yield losses in wheat (Park et al, 2007).

In wheat, yield losses due to the fungal pathogens can be mitigated by proper and timely fungicide applications (Kumar et al. 2013), but utilizing resistant cultivars is the most cost-effective and eco-friendly practice for sustainable production (Jørgensen et al, 2014; Loyce et al, 2008; Oelke and Kolmer, 2004; Pink, 2002; Singh et al. 2000). More than 200 genes for rusts have been mapped and catalogued in bread wheat (*Triticum aestivum* L.), durum wheat (*Triticum turgidum*) and their wild relatives by using different molecular approaches (McIntosh et al, 2016). Most of these genes are race-specific and historically it is proven that they are easily overcome by the virulent pathotypes (McCallum et al. 2016; Lowe et al. 2011; Kolmer and Anderson, 2011; Kolmer et al.2009; Jones and Dangl, 2006). On the other hand, race-non-specific genes are long lasting and provide more durable resistance against pathogens (Johnson, 1988). Various QTL have been reported to confer resistance to wheat rust and powdery mildew (Li et al, 2014; Yu et al, 2014; Rosewarne et al, 2013) but only three genes *Lr34/Yr18/Pm38/Sr57/Ltn1* (Singh et al.,2012*), Lr46/Yr29/Pm39/Sr58/Ltn2* (Singh et al., 2013), and *Lr67/Yr46/Pm46/Sr55/Ltn3* (Herrera-Foessel et al., 2014) are known to be pleiotropic and confer partial resistance to all three rusts and powdery mildew (Lillemo et

al. 2008; William et al. 2003). These genes are also associated with leaf tip necrosis (*Ltn*), used as a phenotypic marker for their presence. In wheat, the APR genes like *Sr2/Yr30* and *Lr34/Yr18/Pm38/Sr57/Ltn1* have been deployed for almost a century and are good examples of genes conferring durable resistance.

Apart from the pleiotropic genes, some chromosomal translocations into bread wheat have a substantial role in providing genetic resistance to many wheat pathogens. Translocated chromosomal fragments carry multiple genes that are closely linked and inherited together due to lack of recombination between the translocated and non-translocated chromosome segments. The most widely used translocation in the global wheat breeding programs is T1BL.1RS translocation, a spontaneous exchange of rye (*Secale cereale* L.) chromosome to wheat (Mettin et al. 1973; Zeller 1973). This chromosomal translocation carries genes for *Pgt* (*Sr31*), *Pt* (*Lr26*), *Pst* (*Yr9*) and *Bmg* (*Pm8*) on chromosome 1B. Stem rust gene *Sr31* was deployed across large wheat growing areas around the world and was very effective against *Pgt* races before the emergence of Ug99 (Hartsman et al. 2015). The other translocation, T1AL.1RS originating from 'Amigo' wheat is probably the second most frequently used translocation in wheat (Sebesta et al, 1994). Globally, this translocation is being used in wheat breeding programs due to its resistance to greenbug (Porter et al, 1994) and Ug99 effective gene *Sr50* (Mago et al, 1994). The short arm of chromosome 2A in some wheat cultivars is known to carry a chromosomal translocation (2NS/2AS) that was initially introgressed into winter wheat 'VPM1' from *Triticum ventricosum* (Helguera et al., 2003; Maia, 1967). During the last few years the 2NS/2AS translocation frequency has rapidly increased in wheat and now >80% of CIMMYT lines carry this translocation (R. P.

Singh; Personal communication). The 2NS chromosomal segment has been reported to carry race specific rust resistance linked genes *Lr37, Yr17,* and *Sr38* (Helguera et al., 2003). Moreover, previous and recent studies also revealed that the 2NS segment carries genes for root-knot nematode resistance (Williamson et al. 2013), cereal nematode resistance (Jahier et al., 2001), and wheat blast resistance (Cruz et al. 2016).

The spring wheat line COPIO from CIMMYT displayed a high level of APR to three wheat rusts (*Pgt, Pst, Pt*) during field evaluations in Mexico and Kenya. To investigate the APR genetic mechanism, COPIO was crossed with a highly susceptible line Apav to develop a recombinant inbred line (RIL) mapping population. The specific objective of this study was to understand the genetic relationship of APR to all three wheat rusts in COPIO.

**Materials and methods**

The experimental wheat line COPIO is a cross involving six lines having the pedigree (CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/FH6-1-7). Four lines: CIANO79, Musala, Pastor, and Baviacora 92 are from the CIMMYT global wheat breeding program and PF70354 and FH61-7 are introductions from Brazil and Ethiopia, respectively. This study utilized a RIL mapping population of 176 individuals developed by crossing COPIO with a susceptible line Apav. Details regarding development of the population are described in chapter 1.

**Seedling and adult plant evaluations**

Both parental lines were tested against selected *Pgt*, *Pt*, and *Pst* races at the seedling stage. The RIL mapping population was also assayed for *Pgt* (RRTTF) and *Pt*

(BBBDS, MCDSB) races from Pakistan and Mexico respectively at the seedling stage. The detailed seedling evaluation procedures for stem rust (hereafter referred as Sr), leaf rust (hereafter referred as Lr), and stripe rust (hereafter referred as Yr) are described in chapter 1, 2, and 3 of this dissertation, respectively. For field evaluations, experiments were performed in 21 environments which includes six environments for each Sr and Lr and nine for Yr in Kenya, Mexico, US and Pakistan. For each trial the abbreviations represent a location where the experiments were conducted (Mex for Mexico, Stp for Saint Paul, Ken for Kenya, Pak for Pakistan, Mtv for Mount Vernon, and Pum for Pullman) followed by a number represents when (15, 16, 17 for years 2015, 2016 and 2017) this trial was conducted. For Kenya, MS and OS represents main-season (June through October) and off-season (December through April). Each trial has a suffix (Sr, Lr, and Yr) that represents the specific rust for which the trial was conducted. For example, the abbreviation 'Stp16_Sr' represents that this experiment was conducted in Saint Paul during 2016 for stem rust. In Saint Paul, same trial was scored for Sr and Lr, while in Kenya for Sr and Yr during both seasons. The experimental designs, planting procedures, artificial inoculations/natural infections and data collection are described in chapter 1 (Sr), chapter 2 (Lr), and chapter 3 (Yr) of this dissertation. For all field trials, disease severity was recorded on a whole plot basis. Terminal rust scores were considered as the final disease severity (FDS) and were used for all downstream analysis. Field scores were converted into coefficient of infection (COI) values (details described in chapter 1). COI values were used for all the phenotyping and QTL mapping analysis. Pearson coefficient correlations (*r*) were calculated among 21 phenotyping environments using the COI values of all three rusts in the IBM SPSS 1.0.0.1174 (IBM Corp.).

**Genotyping and QTL mapping**

To investigate the presence or absence of three pleiotropic known genes *Lr34/Yr18/Sr57/Pm38/Ltn1* (*csLV34*), *Lr46/Yr29/Sr58/Pm39/Ltn2* (*csLV46G22*), and *Lr67/Yr46/Sr55/Pm46/Ltn3* (*Lr67SNP*) along with other APR and seedling genes, *Sr2/Yr30/Lr27* (*gwm533*), *Lr68* (*csGS*), *Lr14a/Lr68* (*ubw14*) closely linked PCR markers were assayed on both parents. Detailed molecular marker assay protocols are described in chapter 2. Parents were also assayed for the 2NS/2AS translocation from *Triticum ventricosum* that harbors closely linked genes for *Pt* (*Lr37*), *Pgt* (*Sr38*), and *Pst* (*Yr17*) using ten GBS markers (Chapter 1, Suppl. Table 1). Both parents and the RIL mapping population were genotyped using the genotyping-by-sequencing (GBS) described by Poland at al. (2012). The procedures for developing genomic libraries, next generation sequencing (NGS) on Illumina Hi-Seq 2500, SNP calling, and constructing linkage maps are described in chapter 1. For QTL mapping, 762 polymorphic SNP markers (each representing a unique locus) were used for QTL mapping analysis. Windows QTL cartographer 2.5 was used to identify marker trait associations and map QTL for all three rusts (Wang et al. 2012). The composite interval mapping (CIM) procedure was used for QTL detection through forward and backward regression at a walk speed of 1.0 cM across all linkage groups. A LOD (logarithm of the odds) threshold of 2.5 was considered significant to declare a QTL. QTL names were adopted from the gene nomenclature described by McIntosh et al. (2016).

## Results and Discussion

### Seedling and field evaluations

During seedling assays against the *Pgt* Ug99 races (TTKSK, TTKST, TTKTT) both parents of the RIL mapping population were highly susceptible with infection types (ITs) more than 3 and we were confident that the field data from Kenya will allow us to map adult plant resistance to Ug99 in COPIO. COPIO was resistant at the seedling stage against the North American *Pgt* races except TTTTF, while susceptible to other important races except the Mexican race (RTR). Based on the stem rust seedling assays we postulated that COPIO has *Sr38* that is located on chromosome 2AS (Chapter1, Table 1). Seedling tests on the RIL mapping population against predominant *Pgt* race (RRTTF) from Pakistan suggested that two genes are segregating in the population for this race.

For leaf rust, both parents of the RIL mapping population were susceptible at the seedling stage (IT $\geq$ 3) against the Mexican *Pt* races MBJ/SP and BBG/BP. Two *Pt* races MBJ/SP and MCJ/SP were used for field inoculations in Mexico and the difference between both races is that the former race has partial virulence on *Lr26* while the latter race has complete *Lr26* virulence (Herrera-Foessel et al. 2012). The leaf rust gene *Lr26* comes from T1BL:1RS translocation from rye (*Secale cereale* L.) with other important genes for *Pgt* (*Sr31*), *Pst* (*Yr9*), and *Bmg* (*Pm8*), (Zeller et al. 1973). Based on the seedling data against the MBJ/SP race we eliminated the possibility of *Lr26* being present in either parent. Both parents were resistant (IT < 3) against the most avirulent US *Pt* race BBBDB and the Chi-squared tests on seedling data from the RIL population showed that two seedling genes are segregating in our population for this race (chapter 2). The other

US *Pt* race, MCDSB, is widely virulent on many leaf rust genes and had high infection (IT > 3) on both parents and the RIL population (Chapter 2, Table 1).

The seedling assays against the Mexican *Pst* race (Mex14.191) revealed that both parents are susceptible (Chapter 3, Table 2) and most likely they do not carry highly important stripe rust genes *Yr5*, *Yr10*, *Yr15* and *Yr17* (based on virulence/avirulence formulae). No virulent races have been identified for *Yr15* (https://maswheat.ucdavis.edu/protocols/Yr15/index.htm) but virulent pathotypes for *Yr5*, *Yr10* and *Yr17* have been detected in many wheat growing areas around the world. In the United States, *Yr5* is resistant against all known *Pst* races (X. M. Chen, personal communication; https://maswheat.ucdavis.edu/protocols/Yr5/index.htm).

During the field evaluations, the experimental line COPIO showed consistent resistance at adult plant stage against all three rusts in 21 phenotyping environments. For all three rusts, the disease distribution spectrums were continuous (Sr, Chapter 1, Fig.2; Lr, Chapter 2, Fig. 1; Yr, Chapter 3, Fig. 1) and RILs represented almost all severity classes. These phenotypic distributions indicate that resistance to all three rusts in this RIL population is quantitative and polygenic. Pearson correlation coefficients (*r*) ranged from 0.66 to -0.04 between leaf and stem rust disease severities in six environments. Correlations were moderately low to high (0.26 to 0.66) among four Sr (Stp16_Sr, Stp17_Sr, Ken16_SrMS, Ken16_SrOS) and Lr environments (Mex16_Lr, Mex17_Lr, Stp16_Lr, Stp_17). Two Lr environments in Pakistan (Pak16_Lr, Pak17_Lr) had very low correlation (0.0 to 0.19) with all Sr environments in Saint Paul and Kenya and were negatively correlated (-0.04 to -0.25) with Sr environments in Pakistan (Pak16_Sr, Pak17_Sr). In previous studies, negative correlation between field resistance to various

*Pgt* races has been reported. Edae et al. (2018) conducted a genome wide association study in North American Spring wheat panel using individual *Pgt* races in field and at the seedling stage to test race specific resistance in wheat. They found negative correlation in field resistance between North American *Pgt* races QFCSC and QTHJC. Lr (six environments) and Yr (nine environments) correlation ranging from 0.06 to 0.70. All Lr environments had high and significant correlations with all nine Yr environments except two Lr environments (Pak16_Lr, Pak17_Lr) in Pakistan (Table 1). Correlation between Sr (six environments) and Yr (nine environments) disease severities ranged from 0.15 to 0.73. Most of the correlations were high and all environments were significant (Table 1). In our study, the moderate correlations between all three rusts are likely due to the pleiotropic genes along with other QTLs providing resistance to Sr, Lr, and Yr with varying magnitude of additive effects. Similar to our results, some previous studies have reported moderately high to moderately low correlations between Sr and Lr, Sr and Yr, and Lr and Yr. Randhawa et al. (2018) reported moderately high to low correlations between Sr and Yr (0.31-0.78) and between Lr and Sr (0.23-0.58) in a RIL mapping population derived from a cross of Apav and Arula. This population had known pleiotropic (*Lr34/Yr18/Sr57*) and APR genes (*Sr2/Yr30 & Lr68*). Lan et al. (2015) reported moderate correlations between Lr and Yr ($r$ = 0.47-0.56) in a biparental mapping population in which two pleiotropic APR genes *Lr46/Yr29/Pm39/Sr58/Ltn2* and *Lr67/Yr46/Pm46/Sr55/Ltn3* were mapped. Moreover, Basnet et al. (2013) reported relatively low correlations ($r$ = 0.21–0.32) between Lr and Yr in a mapping population in which three co-located QTL for both diseases were detected.

**Known pleiotropic/APR genes**

Marker assays on the parents of RIL population revealed that Apav did not carry any known pleiotropic/APR genes (*Lr34/Yr18/Sr57*, *Lr46/Yr29/Sr58*, *Lr67/Yr46/Pm46/Sr55*, and *Lr68*), while COPIO likely contains APR gene *Lr46/Yr29/Sr58* pleiotropic to all three wheat rusts on chromosome 1BL and APR genes *Sr2/Yr30* on chromosomes 3BS (Table 2). Our QTL mapping procedure using GBS markers also detected these genomic regions. The *Lr46/Yr29/Sr58* locus had significant LOD peaks in two environments for Lr (Mex16_Lr, Pak17_Lr) and Sr (Ken16_SrOS, Stp16_Sr), and in five environments for Yr (Mex15_Yr, Mex17_Yr, Ken16_YrOS, Ken16_YrMS, Pak17_Yr). The phenotypic variance ($R^2$) ranged from 5-13%, 5-8%, and 4-22% for Sr, Lr, and Yr, respectively. LOD peaks were observed for all three rusts in most of the other environments but were below the significance threshold of 2.5 (Fig. 1). Previous studies have reported similar results as ours for the *Lr46/Yr29/Sr58* gene showing a wide range of LOD and $R^2$ values for three rusts (Lan et al, 2015; Rosewarne et al, 2012; Jagger et al, 2011; Bariana et al, 2010). The APR genes *Sr2/Yr30* are closely linked with seedling gene *Lr27* and are located on the short arm of chromosome 3B. This genomic region is known to confer resistance to many fungal diseases in wheat with *Sr2*, *Yr30*, *Lr27*, *Fhb1* (most commonly linked in repulsion), and *Pm* genes. In our study, genomic regions were detected in two Sr (Chapter1, Table 6) and seven Yr (Chapter 3, Table 6) environments which represent *Sr2* and *Yr30* genes (Fig. 2a). In the same region on 3BS, a large effect QTL (*QLr.umn.3B*) with $R^2$ value ranging from 10-18% was detected in four Lr (Chapter 2, Table 6) environments out of six (Fig. 2b). The seedling gene *Lr27* on 3BS requires a complementary gene, *Lr31,* on chromosome 4B for its

function (Singh and McIntosh, 1984). In our study, genome wide markers did not detect *Lr31* in either parental line. The pathotypes (MBJ/SP, MCJ/SP, BBG/BP) used for the seedling assays and field inoculation in Mexico are virulent on *Lr27* + *Lr31* (Herrera-Foessel et al. 2012; Huerta-Espino et al. 2011). Both parents of the RIL population had high infection types (IT > 3) when challenged against these races at the seedling stage (Chapter 2, Table 1). 'Gatcher', which carries *Lr10* and *Lr27+Lr31* genes had high disease severity in Saint Paul nurseries in 2016 (Kolmer et al. 2018). Hence the presence of *Lr27* virulent pathotypes supports our hypothesis that leaf rust resistance in Saint Paul is not associated with *Lr27* in COPIO; however, COPIO was positive for the closely linked stem rust APR gene *Sr2* (Table 2). Some studies have reported that few wheat lines show *Lr27* specificity and do not confer resistance to stem rust (Singh and McIntosh, 1984), which suggests that *Lr27* and *Sr2* are encoded by separate genes (Mango et al. 2011). However, no wheat genotypes have been reported that carry *Sr2* gene but lack *Lr27* (Mango et al. 2011). We speculate that perhaps the *Sr2* allele is conferring resistance to leaf rust without *Lr27* or *QLr.umn.3B* is a different race specific APR QTL providing resistance in Mexico and Saint Paul.

**2NS/2AS translocation segment with potentially new QTL**

Wheat lines carrying QTL for multiple disease resistance, either closely linked or a single gene with pleotropic effects are more important for breeding programs because these types of resistances are inherited together. The short arm of chromosome 2A is known to carry a chromosomal translocation commonly called 2NS/2AS that was initially introgressed into winter wheat 'VPM1' from *Triticum ventricosum* (Helguera et al., 2003; Maia, 1967). The translocated segment harbors closely linked genes for *Pt*

(*Lr37*), *Pgt* (*Sr38*), and *Pst* (*Yr17*). In our study, molecular assays using ten GBS markers revealed that COPIO is positive for the 2NS translocation while Apav is negative (Table 2). For Sr, we postulated *Sr38* gene in COPIO based on the seedling tests against the North American *Pgt* races. The quantitative analysis detected three QTL (*QSr.umn.2A.1, QSr.umn.2A.2, QSr.umn.2A.3*) for Sr on chromosome 2AS (Chapter 1, Table 6). The QTL *QSr.umn.2A.1* was detected in one environment (Pak17) and mapped at the distal end of the short arm at 4.8 cM. The QTL *QSr.umn.2A.2* explained the maximum phenotypic variation ($R^2$ = 34 – 37%) in both Saint Paul environments and mapped at 11.04 cM. The QTL *QSr.umn.2A.3* was detected in one environment in Kenya with $R^2$ value 9%. In Pakistan and Kenya, Sr nurseries were inoculated with *Pgt* races virulent to *Sr38*. So, the detected QTLs (*QSr.umn.2A.1,* & *QSr.umn.2A.3*) apparently do not represent *Sr38*. The QTL *QSr.umn.2A.2* has pronounced effects ($R^2$ = 34-37%) in Saint Paul environments where *Pgt* races were avirulent to *Sr38*. In this study, the QTL *QSr.umn.2A.*2 represents *Sr38* in COPIO. The small to medium effect QTLs detected in Pakistan and Kenya in the 2NS/2AS genomic region are either new sources of resistance or the residual effects of *Sr38* seedling gene (Chapter 1, Suppl. Fig. 2). CIMMYT data suggests that the 2NS translocation segment has slow rusting genes for leaf rust in addition to *Lr37*, so it is also possible that this segment carries some other QTL effective to Ug99 and other races in addition to *Sr38* (R. P Singh; Personal communication).

For Lr, two QTLs (*QLr.umn.2A.1* and *QLr.umn.2A.2*) were mapped in the same region on chromosome 2AS and contributed by COPIO (Chapter 2, Table 6). The QTL *QLr.umn.2A.1* is located on the distal end (1.02-4.8 cM) and was detected in three environments (Mex16, Mex17, Stp17), while the other QTL, *QLr.umn.2A.2* (22.2 cM)

was located proximal to the short arm and was detected in one environment (Pak16). Although QTL *QLr.umn.2A.1* was detected in three environments (Mex16, Mex17, Stp17), LOD peaks were also observed for other environments which did not reach the threshold level to be declared significant (Chapter 2, Suppl. Fig. 2). This QTL explained 6-11% of the phenotypic variation for leaf rust with an additive effect of 7.5 to 13.8% (Chapter 2, Table 6). Based on our conclusion in chapter 2, the QTL *QLr.umn.2A.2* represents *Lr37* in COPIO from the 2NS/2AS translocation, while the QTL *QLr.umn.2A1* is most likely a new source of resistance to *Pst* races that resides in the same region. In accordance to our findings, some other studies have also reported leaf rust QTLs co-located with *Lr37* (Wang et al. 2015; Azzimonti et al. 2014). CIMMYT-Mexico also has data (unpublished) to support that the hypothesis that *Lr37/Yr17/Sr38* region harbors a Lr slow rusting gene other than *Lr37* (R. P Singh; Personal communication).

A large effect QTL (*QYr.umn.2A*) for stripe rust resistance was consistently detected in all nine environments in the 2NS/2AS chromosomal region. The 2NS/2AS translocation segment carries seedling gene *Yr17* for *Pst* races. Based on the marker assays, COPIO was positive for the *Yr17* gene, but we had unique observations on COPIO when it was tested against *Yr17* virulent pathotypes in field. Most of the field phenotyping environments had *Yr17* virulence but COPIO showed a highly resistant response. In seedling assays, *Pst* race Mex14.191 (avirulent on *Yr17*) from Mexico had high infection (IT = 8) on COPIO and Apav (Chapter 3, Table 2). Moreover, COPIO had a resistant reaction against a *Pst* race from India that is completely virulent on *Yr17* (R. P. Singh; Personal communication). Although COPIO is positive for *Yr17*, we are confident that an additional APR gene on or tightly linked with the 2NS/2AS translocation is

responsible for the results we have observed (Fig. 3). It is possible that *Yr17* has some residual effects in the environments where virulent pathotypes had low frequency. Furthermore, the new QTLs ( *QSr.umn.2A.1, QSr.umn.2A.3, QLr.umn.2A.1*, *QYr.umn.2A*) for all three rusts in the 2NS/2AS translocation region share some common GBS markers (data not shown), so it is likely that these genomic regions represent a single QTL which is pleiotropic to all three rusts, but it needs further investigations.

**Conclusion**

Molecular characterization and quantitative/qualitative analysis revealed that COPIO harbors some important pleiotropic and APR genes along with qualitative genes for *Pgt*, *Pst*, and *Pt*. Although most of the detected qualitative genes have virulent pathotypes, they are still contributing resistance to rust pathogens in combination with other effective APR and pleiotropic genes. Pleiotropic gene *Lr46/Yr29/Sr58* on chromosome 1BL is affecting disease severities of all three rusts with medium to high $R^2$ values. APR genes *Sr2/Yr30* for stem and stripe rust along with either a new gene for leaf rust or due to the pleiotropic effects of *Sr2/Yr30* are contributing in disease reduction. The 2NS/2AS translocation segment on chromosome 2AS in COPIO with *Sr38* and *Lr37* genes conferring resistance against *Pgt* and *Pt* races. Potentially a new putative QTL (*QYr.umn.2A*) in the same region along with partial effects of *Yr17* gene (depending on inoculum race composition), has high effects on stripe rust disease severities in all testing environments. Our findings show that wheat line COPIO contains pleiotropic, APR and seedling genes along with small to medium effect QTL that are working in combination to enhance genetic resistance against rust pathogens. Broad spectrum resistance against

wheat rust diseases in COPIO makes it valuable source of resistance and its utilization in recombination breeding can potentially enhance durable resistance.

Table 1: Pearson correlation (*r*) among coefficient of infection values based on final disease severities on Apav x COPIO RILs in six environments each for stem rust and leaf rust and nine environments for stripe rust.

| | Stp16_Sr | Stp17_Sr | Ken16_SrOS | Ken16_SrMS | Pak16_Sr | Pak17_Sr | Mex16_Lr | Mex17_Lr | Stp16_Lr | Stp17_Lr | **Pak16_Lr** | Pak17_Lr | Mex15_Yr | Mex16_Yr | Mex17_Yr | Ken16_YrOS | Ken16_YrMS | Pum17_Yr | Mtv17_Yr | Pak16_Yr | Pak17_Yr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Stp16_Sr | 1 | | | | | | | | | | | | | | | | | | | | |
| Stp17_Sr | 0.79** | 1 | | | | | | | | | | | | | | | | | | | |
| Ken16_SrOS | 0.42** | 0.46** | 1 | | | | | | | | | | | | | | | | | | |
| Ken16_SrMS | 0.31** | 0.27** | 0.44** | 1 | | | | | | | | | | | | | | | | | |
| Pak16_Sr | 0.29** | 0.33** | 0.41** | 0.18* | 1 | | | | | | | | | | | | | | | | |
| Pak17_Sr | 0.21** | 0.24** | 0.46** | 0.31** | 0.64** | 1 | | | | | | | | | | | | | | | |
| Mex16_Lr | 0.66** | 0.58** | 0.39** | 0.39** | 0.26** | 0.28** | 1 | | | | | | | | | | | | | | |
| Mex17_Lr | 0.55** | 0.52** | 0.33** | 0.35** | 0.22** | 0.22** | 0.80** | 1 | | | | | | | | | | | | | |
| Stp16_Lr | 0.36** | 0.32** | 0.24** | 0.29** | 0.13 | 0.11 | 0.47** | 0.40** | 1 | | | | | | | | | | | | |
| Stp17_Lr | 0.35** | 0.42** | 0.26** | 0.26** | 0.20* | 0.23** | 0.56** | 0.57** | 0.46** | 1 | | | | | | | | | | | |
| **Pak16_Lr** | 0.08 | 0.04 | 0.04 | 0.00 | -0.24** | -0.25** | 0.10 | 0.09 | 0.15 | 0.02 | 1 | | | | | | | | | | |
| **Pak17_Lr** | 0.15 | 0.18* | 0.19* | 0.16* | -0.04 | -0.05 | 0.19* | 0.29** | 0.18* | 0.06 | 0.40** | 1 | | | | | | | | | |
| Mex15_Yr | 0.73** | 0.70** | 0.44** | 0.34** | 0.27** | 0.24** | 0.68** | 0.70** | 0.36** | 0.45** | 0.13 | 0.29** | 1 | | | | | | | | |
| Mex16_Yr | 0.65** | 0.66** | 0.39** | 0.28** | 0.26** | 0.23** | 0.63** | 0.63** | 0.41** | 0.38** | 0.11 | 0.24** | 0.82** | 1 | | | | | | | |
| Mex17_Yr | 0.66** | 0.62** | 0.42** | 0.24** | 0.15 | 0.14 | 0.67** | 0.61** | 0.36** | 0.34** | 0.19* | 0.31** | 0.80** | 0.74** | 1 | | | | | | |
| Ken16_YrOS | 0.57** | 0.56** | 0.69** | 0.48** | 0.30** | 0.37** | 0.54** | 0.52** | 0.30** | 0.34** | 0.06 | 0.34** | 0.62** | 0.57** | 0.59** | 1 | | | | | |
| Ken16_YrMS | 0.69** | 0.66** | 0.44** | 0.41** | 0.18* | 0.22** | 0.69** | 0.64** | 0.42** | 0.46** | 0.17* | 0.24** | 0.78** | 0.69** | 0.69** | 0.61** | 1 | | | | |
| Pum17_Yr | 0.60** | 0.60** | 0.38** | 0.15* | 0.20* | 0.19* | 0.47** | 0.47** | 0.32** | 0.34** | 0.13 | 0.24** | 0.69** | 0.68** | 0.62** | 0.45** | 0.64** | 1 | | | |
| Mtv17_Yr | 0.56** | 0.55** | 0.33** | 0.25** | 0.13 | 0.14 | 0.47** | 0.47** | 0.28** | 0.44** | 0.12 | 0.20** | 0.63** | 0.54** | 0.53** | 0.45** | 0.65** | 0.72** | 1 | | |
| Pak16_Yr | 0.64** | 0.55** | 0.33** | 0.25** | 0.26** | 0.26** | 0.52** | 0.44** | 0.25** | 0.39** | 0.04 | 0.21** | 0.68** | 0.58** | 0.57** | 0.50** | 0.58** | 0.46** | 0.53** | 1 | |
| Pak17_Yr | 0.55** | 0.51** | 0.37** | 0.27** | 0.20* | 0.23** | 0.55** | 0.44** | 0.24** | 0.40** | 0.08 | 0.11 | 0.59** | 0.55** | 0.52** | 0.50** | 0.62** | 0.42** | 0.50** | 0.60** | 1 |

*, ** Correlation is significant at the 0.05 and 0.01 level, respectively. Highlighted values in yellow and red represents low and non-significant correlations.

Table 2: Molecular marker assays on both parents of the RIL mapping population for known pleiotropic/APR and seedling genes

| Genes | Chromosome | Marker | Apav | COPIO |
|---|---|---|---|---|
| *Lr34/Yr18/Sr57* | 7DS | *csLV34* | –[a] | –[a] |
| *Lr46/Yr29/Sr58* | 1BL | *csLV46G22* | – | + |
| *Sr2/Yr30* | 3BS | *gwm533* | – | + |
| *Lr67/Yr46/Sr55* | 4DL | *Lr67SNP* | – | – |
| *Lr68* | 7BL | *csGS* | – | – |
| *Lr37/Sr38/Yr19* | 2AS | *GBS* | – | + |

[a] + and – represent presence or absence of the gene, respectively

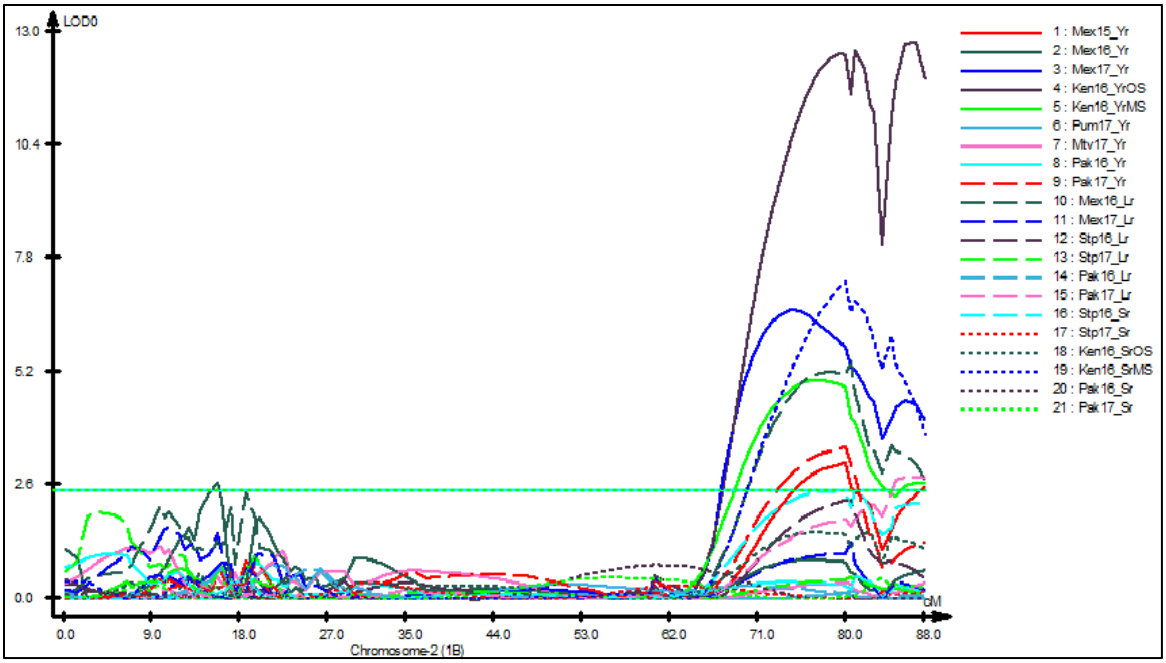



Figure 1: Pleiotropic gene *Lr46/Yr29/Sr58* on chromosome 1BL conferring resistance to all three rusts. X-axis represent the logarithm of odds (LOD) values and Y-axis indicates genetic distance in centimorgans (cM). Horizontal dotted line along the X-axis is the threshold level of LOD value on which a QTL was declared significant.

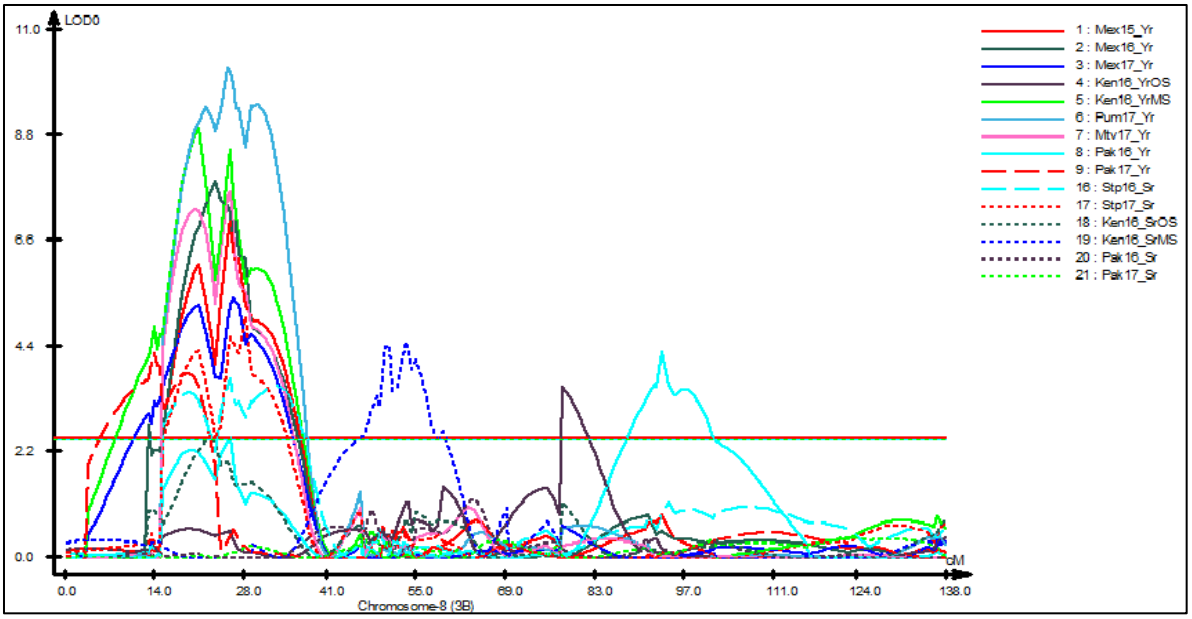



Figure 2a: APR genes *Sr2/Yr30* distally located on the short arm of chromosome 3B. These genes were detected in two stem rust and seven stripe rust environments. X-axis represent the logarithm of odds (LOD) values and Y-axis indicates genetic distance in centimorgans (cM). Horizontal dotted line along the X-axis is the threshold level of LOD value on which a QTL was declared significant.

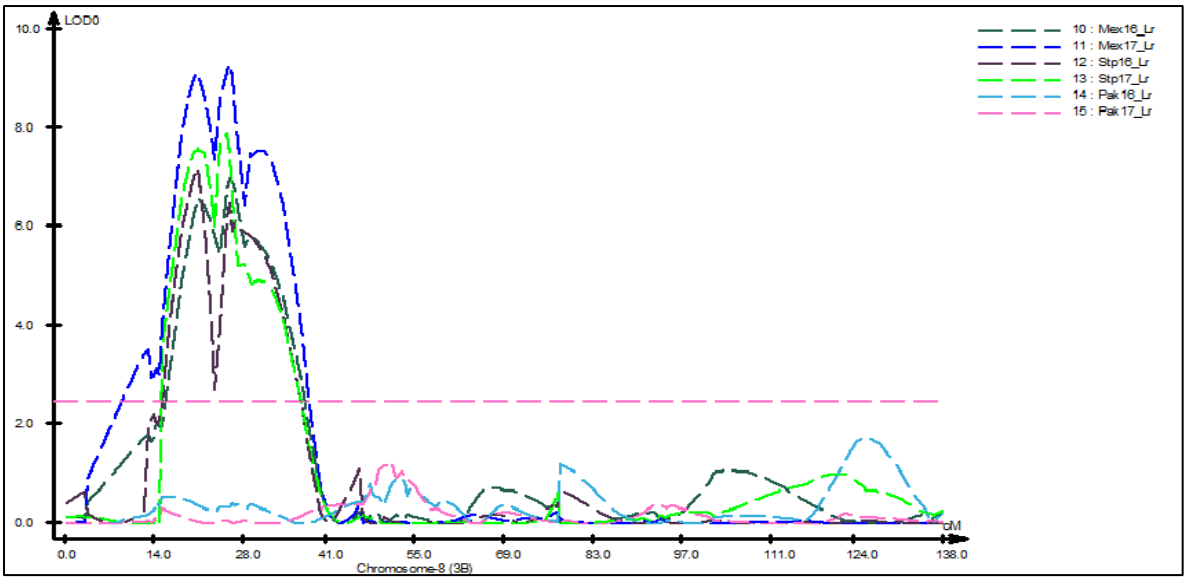



Figure 2b: A large effect potentially new race-specific QTL in the same region of *Sr2/Yr30* conferring resistance to leaf rust in four environments out of six. X-axis represent the logarithm of odds (LOD) values and Y-axis indicates genetic distance in centimorgans (cM). Horizontal dotted line along the X-axis is the threshold level of LOD value on which a QTL was declared significant.

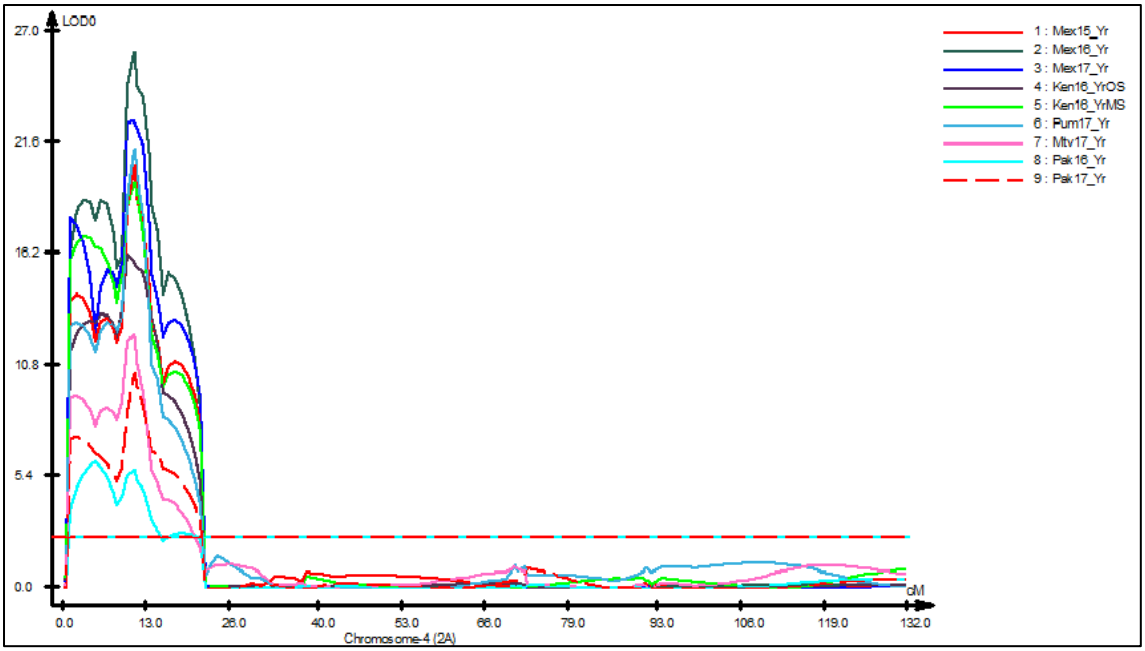



Figure 3: A large effect potentially new QTL in or closely linked to the 2NS/2AS region conferring resistance to stripe rust in all nine environments. X-axis represent the logarithm of odds (LOD) values and Y-axis indicates genetic distance in centimorgans (cM). Horizontal dotted line along the X-axis is the threshold level of LOD value on which QTL was declared significant.

# Chapter 5

## Quantitative Trait Loci Associated with Adult Plant Resistance to Stem Rust in the Spring Wheat Mapping Population Sabin/MN06113-8

### Introduction

Wheat stem rust, also known as black rust, is caused by the fungal pathogen *Puccinia graminis* f. sp. *tritici* Eriks. E. Henn (*Pgt*) and is distributed worldwide in wheat growing areas. This disease can be very devastating due to its production of spores with the ability to travel long distances, rapidly multiply under favorable environmental conditions, and evolve new races that overcome the resistant genes (Dean et al. 2012). Stem rust epidemics have been very severe in the North Central States due to the presence of the alternate host barberry (*Berberis vulgaris*) and virulent races. In 1935, virulent race 56 caused yield losses of 51.6% in Minnesota and 56.1% in North Dakota. Later, 1953 and 1954 stem rust epidemics were caused by race 15B due to the predominance of susceptible cultivars (Roelfs 1978). Since 1954 rust epidemics in the United States have been infrequent and mild as a result of effective gene deployment strategies and the eradication of barberry (Peterson 2018).

In recent decades stem rust has again posed a threat to global wheat production due to the emergence of the highly virulent stem rust isolate named Pgt-Ug99 and found in Uganda in 1998. The Pgt-Ug99 isolate was typed as race TTKS that is highly virulent on wheat lines with stem rust resistance gene *Sr31* (Pretorius et al. 2000). Genes *Sr31*, *Lr26*, and *Yr9* reside on a 1BL.IRS chromosome arm translocation from rye that has been used extensively around the world as a source of rust resistance for many decades. Race

TTKS was later named TTKSK after adding and characterizing an additional set of differentials (Jin et al. 2008). Since the detection of TTKSK, thirteen other variants from the Ug99 lineage group have been reported in thirteen countries (Patpour et al. 2015; Fetch et al. 2016). The most recent race identified in the Ug99 lineage group is TTKTT (Newcomb et al. 2016). Within a short period of time, this group of *Pgt* races has overcome many important resistance genes from bread wheat and its wild relatives. Including the *Sr9h* virulence (Rouse at al. 2014a), there are 34 genes which are ineffective, and 39 genes that are effective against the Ug99 lineage group at seedling and adult plant growth stages (Singh et al. 2015). Many of the effective genes to Ug99 originate from wild relatives of wheat (Jin et al. 2007). For example, *Sr32* and *Sr39* are derived from *Aegilops speltoides* (McIntosh et al. 1995; Kerber and Dyck 1990), *Sr37* from *Triticum timopheevi* (McIntosh and Gyarfas 1971), *Sr40* from *Triticum araticum* (Dyck 1992), *Sr44* from *Thinopyrum intermedium* (Friebe et al. 1996), and *Sr53* from *Aegilops geniculata* (Liu et al. 2011).

The rapid appearance of new races of rust pathogens with virulence for the major seedling resistance genes in wheat has intensified the focus to breeding for durable resistance (Singh and Rajaram 1992; Singh et. al. 2000). The term durable resistance is defined as a resistance which has remained effective for a long period of time in a cultivar that has been cultivated over a widespread area in a disease favorable environment (Johnson, R. 1988). Durable rust resistance is more likely to be of adult plant type rather than seedling type and not associated with the genes conferring hypersensitive reaction (McIntosh 1992, Bariana et. al. 2001). Host genetic investigations conducted by the International Maize and Wheat Improvement Centre (CIMMYT) in

Mexico reveals that a minimum of 10-12 genes are involved in adult plant resistance (APR) in CIMMYT germplasm (Singh et. al. 2005), and resistance close to immunity can be achieved by combining 4-5 minor genes in a single line. If 2-3 genes are combined in a line it could provide a moderate level of resistance (Singh et. al. 2005, 2009). Most of these APR genes are undesignated except *Sr55, Sr56, Sr57, Sr58,* and *Sr2/Yr30*. International collaborative efforts are in place to identify new sources of APR to ensure more durable resistance in cultivated varieties and sustainable wheat production.

Wild relatives of bread wheat represent a rich source of resistance to wheat stem rust, but utilization of these sources remains a great challenge for the wheat breeding community due to linkage drag. Moreover, it is also tedious for the breeders to improve breeding lines for agronomic traits while using wild relatives in recombination breeding. International breeding efforts have helped to identify resistance sources in common wheat lines that facilitate their introgression into elite lines. The wheat breeding program at the University of Minnesota is also involved in identifying the potential sources of seedling and APR genes in wheat lines and further utilize them in the variety developmental process. Breeding lines have been tested in Kenya and Ethiopia through the USDA-ARS coordinated stem rust nurseries since 2005. During the routine screening of UMN lines, the UMN wheat variety ‘Sabin’ was moderately susceptible whereas the advanced breeding line MN06113-8 was found to exhibit APR in the African rust nurseries yet both are susceptible to Ug99 race TTKSK at the seedling stage. To investigate the genetic mechanism of resistance to stem rust, these lines were crossed to develop a RIL population and map QTLs segregating for APR to stem rust races. The main motivation to pursue this population was to validate the large effect QTL in

MN06113-8 identified by Bajgain et al (2015) that conferred APR in all environments in Africa and Saint Paul. The specific objective of this study was to understand the genetics of adult plant resistance to wheat stem rust in the wheat breeding line 'MN06113-8' and cultivated wheat variety 'Sabin' against the Ug99 and North American *Pgt* races.

## Materials and Methods

### Plant material

A biparental RIL mapping population was developed from the cross of the hard-red spring wheat cultivar 'Sabin' and an experimental line 'MN06113-8'. The population consisted of 184 $F_6$:$F_8$ recombinant inbred lines (RILs) developed by single seed descent. Sabin is an $F_6$-derived selection from the cross of experimental lines MN98389 and MN97518. The University of Minnesota Agricultural Experiment Station developed Sabin for release in 2009 based on its high grain yield, moderate level of resistance to fusarium head blight (FHB, caused primarily by *Fusarium graminearum* Schwabe), high level of adult plant resistance against leaf rust caused by *Puccinia triticina* Eriks., and acceptable range of end-use quality (Anderson et al. 2012). MN06113-8 is an $F_6$ derived breeding line from the University of Minnesota Wheat Breeding program and was not considered as a candidate wheat variety after the second year of its yield trials.

### Seedling stem rust evaluation

Both parents of the RIL population were screened for resistance to Ug99 group races (TTKSK, TTKST, TTKTT) and other important races from Ethiopia (TKTTF), Yemen (TRTTF), and USA (QFCSC, QTHJC, MCCFC, RCRSC, RKRQC, TPMKC, TTTTF, GFMNC, QCCSM) at the seedling stage (Table 1). Seedling tests were performed

according to the protocols outlined by Rouse and Jin (2011a), at the Cereal Disease Laboratory, United States Department of Agriculture, Agricultural Research Service (USDA-ARS) facility, Saint Paul Minnesota. Disease reactions were scored 12 to 14 d after inoculation based on the 0-4 scale as described by Stakman et al. (1962). Infection types (ITs) 0, ;, 1, 2 were considered as low ITs characteristic of host resistance and pathogen avirulence, while ITs 3 and 4 were considered high.

**Field adult plant stem rust evaluation**

The RIL mapping population along with both parents were evaluated for APR to Kenyan, Ethiopian and domestic (U.S.) isolates of *P. graminis* f. sp. *tritici* (*Pgt*). The population was grown for one season in St. Paul from April through August 2015 (hereafter referred as Stp15) and in Njoro, Kenya during the off-season from January through April of 2017 (hereafter referred as Ken17). The population was grown for two off-seasons from January through June of 2016 and 2017 (hereafter referred as Eth16 and Eth17, respectively) in Debre Zeit, Ethiopia.

In Saint Paul, the population was included in the stem and leaf rust nursery plots planted for screening the U.S. spring wheat germplasm using a mixture of domestic isolates. The lines were planted as 2-meter-long single rows with 20 cm separation and 2-meter alleys in a randomized fashion. Wheat lines ‘Max’ and ‘Line E’ were planted as susceptible checks while wheat variety ‘Linkert’ (Anderson et al. 2018) was planted as resistant check after every 45 entries. The alternate alleys and the surrounding borders of the nursery plots were planted with a mixture of susceptible cultivars ‘Morocco’ and ‘LMPG-6’ as spreader rows. The spreaders were planted 1 to 2 weeks earlier than the experimental nursery plots. The spreader rows were planted perpendicular to the

experimental plots. To initiate stem rust disease epidemics, urediniospores of North American *Pgt* isolates corresponding to races QTHJC, MCCFC, TPMKC, QFCSC, RCRSC, and RKRQC (Table-2) were suspended in distilled water with one drop of Tween 20 per 0.5 L and inoculated on the spreader rows by syringe injection at the jointing stage (Feekes 7 growth stage), (Feekes W. 1941). Approximately 0.5 ml of rust suspension was delivered every 2 m of spreader row using the hypodermic syringe (Rouse et al. 2011b). Another round of spray inoculation was performed on the spreader rows after heading (Feekes 10.3 growth stage) using a bulk of the same six *Pgt* isolates. The *Pgt* isolates were suspended in the light mineral oil (1:3 v/v) and sprayed on spreaders (Njau et al. 2013) in the evening on a night when dew was expected using an Ulva+ sprayer (Micron Sprayers Ltd., Bromyard U.K.).

In Debre Zeit, Ethiopia the mapping population was included as part of the USDA stem rust spring wheat screening nursery (Eth16 and Eth17). Lines were planted as one meter long paired rows with perpendicular spreader rows on all sides. The spreader rows were comprised of a mixture of the susceptible varieties ‘Morocco’, ‘Local Red’, and ‘PBW343’. The mapping population lines were planted in an augmented design with a susceptible check ‘Red Bobs’ every 50 entries. To initiate and develop the epidemics, inoculation was performed using freshly collected *Pgt* urediniospores from PBW343 and mixed with talc powder (one volume of fresh urediniospores, 20 volumes of talcum powder) and dusted on the spreader rows. (Sobhy et al.2015; Jin et al. 2007). PBW343 is an Indian common wheat variety that carries the 1BL-1RS translocation from rye and has the *Sr31* gene. Several Ug99 lineage races are virulent to *Sr31* gene and all other *Pgt* races are avirulent (Li et al. 2016).

In Njoro, Kenya the population was also included as part of the USDA stem rust screening nursery (Ken17). The lines were planted as 1 m long single rows using an augmented design with the repeated susceptible checks after every 50 entries. The Ug99 susceptible lines 'Duma' and 'Chozi' were planted perpendicular to the experimental lines as the spreader rows and were artificially inoculated with the urediniospores collected from Njoro site.

**Phenotyping and data analysis**

Each line of the RIL mapping population was scored for infection response based on the pustule size and abundance of chlorosis and necrosis (Roelf et al., 1992). Infection response was classified as resistant (R), moderately resistant (MR), moderately susceptible (MS), or susceptible (S). Multiple infection response of lines against stem rust was categorized as MR-MS. Disease severity was recorded based on the 0-to-100 Modified Cobb scale method (Peterson et al., 1948). Stem rust phenotyping data was converted into coefficient of infection (COI) values using the custom perl script as described by Gao et al. (2016). COI values were used for all the downstream phenotyping and molecular mapping data analysis. Histograms and Pearson coefficient correlations were developed among all the phenotyping environments using the COI values in the R program and JMP Pro 14 (SAS Institute, Inc.).

**Molecular marker assay**

Leaf tissues of both the parents and $F_6$:$F_8$ lines were harvested from 2-3 plants at the two-leaf stage and lyophilized before further processing. The leaf tissues were ground using the plant TissueLyser II (QIAGEN) and genomic DNA was extracted using the

BioSprint 96 DNA plant kit on the BioSprint workstation (QIAGEN). Extracted DNA was dispensed in 1X TE buffer and preserved as stock DNA. The stock DNA of all samples was quantified using the PicoGreen dsDNA assay kit methods (Ahn et al. 1996) and normalized to 10ng/µL with a total volume of 50 µL. The RIL population and parents were genotyped using the SNP markers from genotyping-by-sequencing (GBS; Elshire et al. 2001) platform, following the protocols developed by Poland et al. (2012) with some modifications. GBS libraries were prepared using 200 ng DNA of each sample and double-digested with *Pst*I/*Msp*I restriction enzymes. Digested DNA fragments were ligated with the barcoded forward adapter and common reverse adapter. A total of 96 ligated samples were pooled into a single tube to develop 96-plex libraries and two clean ups were performed on Qiagen columns using the QIAquick PCR purification kit. Each library was amplified through six polymerase chain reactions (PCR) using Illumina primers having the complementary sequences to adapters used for library preparation. PCR reactions for each library were pooled and another round of clean up was performed on Qiagen columns, following the manufacturer's directions. Two 96-plex GBS libraries were submitted to the University of Minnesota Genomics Center (UMGC) for next generation sequencing (NGS). Libraries were sequenced on Illumina Hi-Seq 2500 by loading each library on one lane of an Illumina flow cell to generate 100 bp single-end sequences.

The sequences of both GBS libraries containing the RILs and two parents were processed by aligning with the IWGSC Chinses Spring wheat genome assembly v1.0 (IWGSC 2018). Sequence alignment to the reference genome was carried out using ALN in 'bwa' with default parameters. The aligned sequences were further processed using the

'Samtools' software and SNP calling was performed using the mpileup method. SNPs were filtered and accepted at a minimum criterion of ≥ 3 and ≥ 25 for the alignment read depth and mapping quality respectively.

**Linkage map construction and QTL mapping**

To develop chromosomal linkage maps, nucleotide base calls were converted into numbers with Sabin alleles coded as '2', and MN06113-8 alleles coded as '0' and heterozygous and missing SNP calls coded as missing ('-1'). A total of 4180 polymorphic SNP markers were used to develop the linkage maps using the QTL ICiMapping version 4.1 (Meng et al. 2015). Markers were assigned to 21 linkage groups using the marker anchoring information and marker ordering was performed using the 'nnTwoOpt' algorithm. Removal of 80 erroneous and poor-fitting markers helped to refine the linkage map that contained 4100 SNP markers. For each linkage group rippling was also performed using the SARF (sum of adjacent recombination fractions) criterion with a window size of 8. The Kosambi mapping function was used to calculate the genetic distances between the markers (Kosambi 1943).

Composite interval mapping (CIM) was performed using Windows QTL cartographer 2.5 to find marker-trait associations (Wang et al. 2012). QTL were identified and considered to be significant at a LOD threshold of 2.5 by using forward and backward regression for all traits and environments at a walk speed of 1.0 cM across the linkage groups. Significant QTL with their genetic position, LOD score values, phenotypic variation explained by each QTL ($R^2$), and the allelic effects were calculated using the same software. QTL names were assigned using the nomenclature described by McIntosh et al. (2013) in the 'Catalogue of gene symbols of wheat'. For physical position

comparison of mapped QTL in our study with previously reported QTL, a local BLAST database was developed using the maker sequences from reported studies and was searched against our GBS sequences using the BLASTN program in ViroBLAST (Deng et al. 2007). The filtering criteria used for the blast results was: percentage of identical matches, alignment length, number of mismatches, number of gap openings, and expected values (E-values). Small E value means a smaller number of hits but of high quality, while large E value means a greater number of hits but of low quality. In this study we used at least an E-value of 1.0e $^{-}$25 for the query.

## Results

### Seedling and field rust evaluations

Both parents of the RIL mapping population were resistant at the seedling stage to the tested North American *Pgt* races with infection types of ;, 0, 1, or 2, but were susceptible to the Ug99 races TTKSK, TTKST, and TTKTT with infection type (IT) 33+. Both parents were also susceptible (IT >3) to TRTTF (a race from Yemen) but resistant to TKTTF (a non-Ug99 race from Ethiopia) with an IT of ;, 0, and 1 (Table 1, Fig. 1). The virulence and avirulence pattern of all the races used for seedling and field evaluations is given in Table 2. During the field evaluation, disease pressure was adequate in all environments except Ethiopia 17, to discriminate among RIL stem rust phenotypes. In Ethiopia during 2017, population was planted late and disease was escaped. We removed Eth17 environment for the downstream qualitative and quantitative analysis. Both parents showed adult plant resistance against Ug99. The high range of disease score (coefficient of infection) in the RIL population was up to 60% in Ethiopia during 2016, while the low range of disease was up to 20% in St. Paul during 2015

(Table 3). Across all three environments the stem rust response of both parents was not significantly different (*t* test *p* value 0.19 at α 0.05) which can be expected because both parents have almost the same level of APR. The frequency distribution of stem rust coefficient of infection was more skewed toward resistance, but the overall distribution spectrum was continuous which is suggestive that resistance in the RILs is quantitative and polygenic (Fig. 2). Pearson correlation of stem rust severity in the RILs were significant ($\alpha = 0.001$) and highly correlated in some environments. Ethiopia 16 was highly correlated with St. Paul 15 and Kenya 17. Saint Paul 15 was moderately correlated with Kenya 17, but still all environments were significant (Table 4).

**GBS libraries, linkage map construction, and QTL mapping**

Two GBS libraries of the Sabin/MN06113-8 RIL mapping population representing 184 lines and two parents were constructed and sequenced on Illumina HiSeq 2500 using one lane per library. On average, 250 million reads of 100 bp were generated on each lane. Approximately 95% of the generated reads passed the Q30 quality criteria with a mean quality score of 36.

A total of 4,100 polymorphic SNP markers were used to develop linkage maps corresponding to 21 wheat chromosomes. These markers covered 2,931 cM of the genome with an average interval of 0.71 cM. Marker distribution was unequal across all three genomes. The A and B genomes were assigned 1,484 and 2,231 SNPs respectively, while the D genome had only 385 SNPs (Table 5). Chromosome 2B had the highest number of markers (461) and 6D chromosome had the lowest number of marker (26). In wheat, the D genome is less represented in all genotyping platforms and results in a smaller number of polymorphic markers (Allen et al. 2011). Chromosomal arms were

determined based on mapping of significant SNP sequences to the wheat reference genome sequences and assigning the centromere and chromosomal arms to individual chromosomes using QTL ICiMapping version 4.1. Correlations between the physical and genetic positions were also calculated to test if markers are properly ordered on each chromosome and linkage maps are not inverted. The lowest correlation was observed for chromosome 4A (0.763), while the highest correlation was observed for chromosome 6D (0.954). Physical positions and their correlation with genetic positions, number of markers, and the marker density for each chromosome and the whole genome are described in Table 5. Overall, correlations were high for all chromosomes.

The CIM method in WinQTL Cartographer detected seven significant loci associated with stem rust APR in Kenya and Ethiopia with a range of 2.5 to 14.8 LOD values, while only three QTLs were detected in St. Paul with a range of 2.8 to 6.7 LOD values (Table 6). One QTL (*QSr.umn.3B*) on the short arm of 3B chromosome was detected in three environments; Stp15, Eth16, and Ken17. This QTL was contributed by MN06113-8 and explained a maximum phenotypic variation ($R^2$) of 26.7% in Eth16 and lowest of 6.5% in Ken17. All other QTLs explained 4.7-11.9% of the phenotypic variation but were detected only in one environment. Two chromosome 4A had two QTL detected in Stp15 and Ken17. Six out of seven QTL were contributed by MN06113-8, and one QTLs *QSr.umn.6B* was contributed by Sabin. Disease severity reduction was estimated using two QTL combinations detected in a same environment (Table 7) and results suggested that disease can be reduced up to 46% by pyramiding at least two QTL regions.

## Discussion

Sabin and MN06113-8 were highly susceptible to the Ug-99 races at the seedling stage but displayed moderate to high level of resistance in the field, which shows that their resistance is controlled by APR loci. Both parents were resistant to the North American *Pgt* races at the seedling stage, so both seedling and APR genes contribute to resistance to these races. In our study, we detected several APR loci associated with resistance to Ug99 and North American *Pgt* races. A total seven QTL were identified in the RIL population on chromosomes 2A, 3B, 4A, 4B, and 6B. A large effect QTL on chromosome 3B (*QSr.umn.3B*) was detected in all three environments and is believed to be the same QTL. We used the sequences of several genetic maps from previous studies (Li et al. 2015; Kandel et al. 2017; Wen et al. 2015; Maccaferi et al. 2015; Bajgain et al. 2015; Bajgain et al. 2016) to compare the positions of QTLs identified in our study using a local BLAST database. For the filtering criteria BLASTn output format 6 was followed to develop a query (http://www.metagenomics.wiki/tools/blast/blastn-output-format-6).

Chromosome group 2 had only one QTL on the short arm of 2A (Fig. 3), which explained 4.7% phenotypic variation. The QTL *QSr.umn.*2A was detected only in one environment, Eth16, and was contributed by MN06113-8. This QTL is in the same region where stem rust resistant gene *Sr21* is located. The source of *Sr21* gene is *Triticum monococcum* (The 1973), so most likely *QTL QSr.umn.*2A is not the same because the parents of our population do not have *Triticum monococcum* in their pedigrees (Suppl. Fig.1). Moreover, *Sr21* is a seedling effective gene to Ug99 at high temperature (Chen et al. 2018), while both parents of our population are highly susceptible to Ug99 group races at the seedling stage (Table 1). Haile et al. (2012) reported a QTL (*QSr.ipk.2A*) on 2AS

flanked by *Xgwm448* (66cM) and *Xgwm1198* (79.5cM) that explained 3.9% phenotypic variation for stem rust in Ethiopia in the Kristal/Sebatel durum wheat mapping population. This QTL was contributed by durum wheat variety 'Sebatel', which was released in 1989. Letta et al. (2013) also reported a QTL in an association mapping panel of durum wheats on 2AS in the same region with most associated simple sequence repeats (SSR) marker *Xgwm1045* (87.7cM) with an average $R^2$ value of 3.9 in three phenotyping environments. These results indicate that *QTL QSr.umn.*2A in MN06113-8 is most likely a different QTL from the previously reported QTLs in the above-mentioned studies.

The QTL *QSr.umn.3B* is located distally on the short arm of 3B chromosome (Fig. 3) and was detected in all three environments (Eth16, Ken17, Stp15). This is a large effect QTL compared to other QTL with a range of 6.5 to 27.6 $R^2$ value and was contributed by MN06113-8. Yu et al. (2011) reported the *Sr2* gene in the same region as ours in an association mapping study of CIMMYT spring wheat germplasm. A consensus map of Ug99 resistant loci was developed by Yu et al. (2014) involving 21 different studies to compile the information available on stem rust loci conferring resistance. This consensus map revealed that 19 QTL are located on the short arm of 3B chromosome and 11 of them coexist with *Sr2*. *Sr2* is known as a pleiotropic gene and effective against multiple pathogens (Singh et al. 2006). In our mapping, *3B_3582933* and *3B_6395759* were the peak makers linked with the resistance conferring loci in three environments. We used the integrated genetic map developed by Maccaferri et al. (2015) for the comparison of 3B QTL position with previously significant reported markers. The peak marker *3B_6395759* (6.56 cM) of our 3B QTL had a perfect sequence similarity with

*3B_IWB62488* and it was located at 16.1cM from *Sr2*, previously reported in biparental mapping studies. The significant marker IWA5202 linked with *Sr2*/*Yr30* did not show any sequence similarity with the 3B QTL significant markers in our study. MN06113-8 was negative for the *Sr2* allele based on the marker screening (*csSr2*). *Sr2* is in repulsion linkage phase with *Fhb1* being about 3 cM apart (Zhang et al. 2016). The parental line MN06113-8 is negative for the *Fhb1* allele and Sabin is positive. Pseudo-black chaff (PBC) is a phenotypic marker for the *Sr2* gene (Juliana et al. 2015) and during our field trials neither parent showed any pigmentation on the stem inter-nodes and glumes. Further studies are needed to determine, either *QSr.umn.3B* is *Sr2* or not.

Two QTL (*QSr.umn.4A.1, QSr.umn.4A.2*) were found on the short arm of chromosome 4A (Fig. 3). Both QTL were detected in single environments (*QSr.umn.4A.1*; Ken17 and *QSr.umn.4A.2*; Stp15) and loci associated with resistance were contributed by MN06113-8. Numerous studies have reported QTL and genes on chromosome 4A responsible for stem rust resistance in wheat. Most of these genes are located on the long arm including the qualitative gene *SrND643* which is distally located on 4AL (Basnet at al. 2015) and association mapping studies conducted at CIMMYT reported QTL on chromosome 4AL associated with stem rust resistance (Crossa et al. 2007; Yu et al. 2011). Bajgain et al. (2015) reported a QTL flanked by GBS and 9K markers (*TP10795*; *wsnp_Ex_c2352_ 4405961)* in wheat line 'MN06113-8' on chromosome 4A for quantitative resistance to stem rust in St. Paul. *QSr.umn.4A.2* is a small effect QTL detected in the St. Paul environment and it is most probably the same QTL reported in Bajgain et al. (2015). The other medium effect QTL (*QSr.umn.4A.1*) was associated with quantitative resistance to Ug99. This QTL mapped to the short arm

(16 cM) of chromosome 4A. The low infection types (IT = 0; and ;1) of both parents to the *Pgt* race TKTTF (Fig.1c, Table 1) suggests *Sr7a* gene in MN06113-8, but *Sr7a* is located on the long arm of chromosome 4A. An association mapping panel of elite spring wheat yield trials of CIMMYT reported a DArT maker (*wPt2788*) associated with stem rust resistance (Crossa et al. 2007). We could not make an accurate comparison of DArT marker *wPt2788* with our closely associated GBS marker (*4A_28074311*) to the 4AS QTL but comparing with the consensus map (Maccaferi et al. 2015) the genetic positions of both markers are 48 cM apart. Li et al. (2015) also reported a QTL (*QSr.cim-4AS*) flanked by GBS markers located distally on 4AS in a PBW343/Kingbird mapping population. We blast-searched the sequences of our GBS markers with the Li et al. (2015) consensus map based on GBS markers. We did not find any similarity between the significant markers from the 4AS QTL region. No known genes have been mapped on 4AS and neither of the previously reported markers have been closely linked or flanked the region of the QTL reported in our study. So, QTL *QSr.umn.4A.1* most likely represent a new small effect genomic region for stem rust Ug99.

A minor effect QTL *QSr.umn.4B* was observed in the St. Paul 2015 environment, contributed by MN06113-8. Bajgain et al. (2015) also reported a 4BL QTL in MN06113-8, effective in their St. Paul environment and flanked by 9K (*wsnp_Ku_c8075_13785546*) and GBS (TP4428) markers. Some other studies have also reported small effect QTL on the 4B chromosome associated with stem rust resistance (Bajgain et al. 2016; Bhavani et al. 2011).

*QSr.umn.5B* is another minor effect QTL on the long arm of 5B chromosome contributed by MN06113-8 and was detected in Eth17. QTL on the long arm of 5B have

been frequently described in CIMMYT lines Kingbird, Hurivis#1, and PBW343 linked with DArT and SSR markers *XwPt-2607, XwPt-1733, XwPt-0750, XwPt-5896, XwPt-6014, XwPt-3661, Xbarc109* and conferring quantitative resistance to Ug99 (Bhavani et al. 2011). Stem rust APR gene *Sr56* mapped in the winter wheat cultivar 'Arina' is also located in the same region (Bansal et al.2014), but diagnostic markers are not available to validate this gene.

*QSr.umn.6B* was found in the St. Paul 2015 environment and the resistant allele was contributed by Sabin, explaining 11.9% of the phenotypic variance. Chromosome 6BL also carries seedling resistance gene *Sr11* that is effective to many North American stem rust races including the predominant race QFCSC. Sabin was highly resistant against the QFCSC race at the seedling stage (Table 1), which supports our hypothesis that resistance in Sabin might be due to *Sr11*.

We could not validate the large effect QTL (*QSr.umn.2B.2*), which was previously mapped in MN06113-8 using a biparental mapping population (RB07/MN06113-8) from the University of Minnesota wheat breeding program (Bajgain et al. 2015). This QTL was detected in all four environments in Africa and St. Paul where the RIL population was screened. The 2B genetic map of RB07/MN06113-8 (Bajgain et al. 2015) was inverted and when it was flipped the significant markers mapped to the region between 15-37 cM. In our Sabin/MN06113-8 population the 20 to 43 cM region of chromosome 2B is monomorphic which is the most compelling reason for not detecting this QTL. The high monomorphic regions in the populations is, because both parents (Sabin and MN06113-8) share common lines in their pedigree (S Fig.1). The co-ancestry between the two parents of RIL population was found to be (*Sabin, MN06113-8*) = 1/16.

The genetic maps also reflect that there are many monomorphic regions with no marker coverage across all 21 chromosomes (Suppl. Fig. 2).

In conclusion, due to the technical issues with the mapping population the 2B large effect QTL previously mapped in MN06113-8 was not validated. The large effect 3B QTL (*QSr.umn.3B*) detected in three environments along with other minor effect QTLs can be used in the wheat breeding programs to breed resistant lines for APR stem rust resistance. According to this study, the disease severity can be reduced 28 to 46 % by combining two QTL detected in the same environment. Further research efforts are required to develop diagnostic markers especially for the 3B QTL, that can be used to accelerate the gene pyramiding process through the marker assisted selection.

Table 1: Seedling infection types of RIL parents Sabin and MN06113-8 to North American, Ug99 and other important *Puccinia graminis* f. sp. *tritici* races

| Races | Isolate | MN06113-8 | Sabin |
|---|---|---|---|
| **North American races** | | | |
| QFCSC | 06ND76C | 0 | 0 |
| QTHJC | 75ND717C | ;2- | 2 |
| MCCFC | 59KS19 | 0 | 0 |
| RCRSC | 77ND82A | 0 | 0 |
| RKRQC | 99KS76A-1 | 2- | ;1- |
| TPMKC | 74MN1409 | ;2- | 0 |
| TTTTF | 01MN84A-1-2 | 0; | 0 |
| QCCSM | 75WA165-2A | ; | 0 |
| **Ug99 race group** | | | |
| TTKSK | 04KEN156/04 | 3+ | 33+ |
| TTKST | 06KEN19-V-3 | 3+ | 3+ |
| TTKTT | 14KEN58-1 | 3+ | 33+ |
| **Other important races** | | | |
| TRTTF | 06YEM34-1 | 33+ | 33+ |
| TKTTF | 13ETH18-1 | ;1 | 0; |

Table 2: Virulence/avirulence formulae of *Puccinia graminis* f. sp. *tritici* races used to screen both the parents at the seedling stage and the RIL population in the field

| Races | Isolate | Virulence/avirulence formula |
|---|---|---|
| QFCSC | 06ND76C | 5,8a,9a,9d,9g,10,17,21,McN/6,7b,9e,9b,11,24,31,30,36,38,Tmp |
| QTHJC | 75ND717C | 5,6,8a,9b,9d,9g,10,11,17,21,McN/7b,9a,9e,24,30,31,36,38,Tmp |
| MCCFC | 59KS19 | 5,7b,9g,10,17,McN,Tmp/6,8a,9a,9d,9e,9b,11,21,24,31,30,36,38 |
| RCRSC | 77ND82A | 5,7b,9a,9b,9d,9g,10,17,21,36,McN/6,8a,9e,11,24,30,31,38,Tmp |
| RKRQC | 99KS76A | 5,6,7b,8a,9a,9b,9d,9g,21,36,McN/9e,10,11,17,24,30,31,38,Tmp |
| TPMKC | 74MN1409 | 5,7b,8a,9d,9e,9g,10,11,17,21,36,Tmp,McN/6,9a,9b,24,30,31,38 |
| TTTTF | 01MN84A-1-2 | 5,6,7b,8a,9a,9b,9d,9e,9g,10,11,17,21,30,36,38,McN,Tmp/24,31 |
| QCCSM | 75WA165-2A | 6 7b 8a 9b 9e 11 30 31 36 38 Tmp 1A.1R/5 9a 9d 9g 10 17 21 24 McN |
| TTKSK[a] | 04KEN156/04 | 5,6,7b,8a,9a,9b,9d,9e,9g,10,11,17,30,31,38,McN/24,36,Tmp |
| TTKST[a] | 06KEN19-V-3 | 5,6,7b,8a,9a,9b,9d,9e,9g,10,11,17,24,30,31,38,McN/36,Tmp |
| TTKTT[a] | 14KEN58-1 | 5,6,7b,8a,9a,9b,9d,9e,9g,10,11,17,24,30,31,38,McN,Tmp/36 |
| TRTTF | 06YEM34-1 | 5,6,7b,9a,9b,9d,9e,9g,10,11,17,21,30,36,38,McN,Tmp/8a,22,24,31,35 |
| TKTTF | 13ETH18-1 | 5,6,7b,8a,9a,9b,9d,9e,9g,10,11,17,21,30,36,38,McN,Tmp/11,24,31 |

[a]*Sr21* is not included in the formula for races TTKSK, TTKST, or TTKTT because the reaction of these races to *Sr21* is inconsistent

Table 3: Mean values, standard deviation and range of stem rust coefficient of infection of the Sabin/MN06113-8 RIL mapping population in three environments

| Environments | Mean | ±SD | Range |
|---|---|---|---|
| Eth16 | 23.03 | ±12.8 | 2.0-60.0 |
| Ken17 | 13.16 | ±9.3 | 2.0-40.0 |
| Stp15 | 5.73 | ±4.6 | 0.0-20.0 |

Table 4: Pearson correlation of stem rust coefficient of infection in the RIL mapping population of Sabin/MN06113-8 in three field environments.

| | Stp15 | Eth16 | Ken17 |
|---|---|---|---|
| Stp15 | 1 | | |
| Eth16 | 0.41*** | 1 | |
| Ken17 | 0.29*** | 0.49*** | 1 |

***: Correlations are significant at $\alpha < 0.001$

Table 5: Range of physical position, correlation with genetic position, total number of markers, chromosomal length, and marker density for all 21 chromosomes in the Sabin/MN06113-3 RIL population.

| | Range | | | | |
|---|---|---|---|---|---|
| Chromosome | Physical position (bp) | Correlation | No. markers | Length cM | Marker density Markers $cM^{-1}$ |
| 1A | 2289043-593790090 | 0.82 | 264 | 170 | 0.65 |
| 1B | 8798713-683589393 | 0.82 | 296 | 110 | 0.37 |
| 1D | 15381192-433364049 | 0.92 | 46 | 62 | 1.37 |
| 2A | 1529370-768191163 | 0.90 | 223 | 184 | 0.83 |
| 2B | 10993544-789673174 | 0.90 | 461 | 155 | 0.34 |
| 2D | 12032405-605914354 | 0.95 | 139 | 116 | 0.84 |
| 3A | 607725-747256726 | 0.92 | 224 | 186 | 0.83 |
| 3B | 3582945-810656139 | 0.90 | 393 | 134 | 0.34 |
| 3D | 41035072-609607733 | 0.83 | 59 | 111 | 1.89 |
| 4A | 13149233-666806800 | 0.76 | 141 | 127 | 0.91 |
| 4B | 1706615-672116501 | 0.82 | 135 | 121 | 0.90 |
| 4D | 24948254-481580244 | 0.82 | 32 | 36 | 1.14 |
| 5A | 773465-572043726 | 0.91 | 123 | 128 | 1.05 |
| 5B | 218949-711738934 | 0.88 | 355 | 211 | 0.60 |
| 5D | 153816630-491660343 | 0.89 | 38 | 146 | 3.86 |
| 6A | 3206675-616823662 | 0.85 | 175 | 170 | 0.97 |
| 6B | 1328402-715070291 | 0.89 | 229 | 120 | 0.53 |
| 6D | 18528715-451938160 | 0.95 | 26 | 127 | 4.89 |
| 7A | 1858861-731888427 | 0.91 | 334 | 186 | 0.56 |
| 7B | 24171814-735978708 | 0.88 | 362 | 103 | 0.29 |
| 7D | 4424074-562060989 | 0.81 | 45 | 216 | 4.82 |
| Whole genome | | 0.76 | 4100 | 2931 | 0.71 |

Table 6: Quantitative trait loci (QTL) associated with adult plant resistance to stem rust in Sabin/MN06113-8 RIL mapping population under three environments

| Environment [a] | QTL | Chr | Flanking markers | | Position(cM) | LOD | $R^2$(%) [b] | Add [c] |
|---|---|---|---|---|---|---|---|---|
| Eth16 | QSr.umn.2A | 2A | 2A_622390852 | 2A_617823868 | 84.08 | 2.93 | 4.7 | 2.8 |
| | QSr.umn.3B | 3B | 3B_3582933 | 3B_15808856 | 0.01 | 14.81 | 27.6 | 6.7 |
| Ken17 | QSr.umn.3B | 3B | 3B_3582933 | 3B_5857925 | 0.01 | 3.09 | 6.5 | 2.3 |
| | QSr.umn.4A.1 | 4A | 4A_28074311 | 4A_262617724 | 16.12 | 3.69 | 9.2 | 2.7 |
| Stp15 | QSr.umn.3B | 3B | 3B_3582933 | 3B_15808856 | 6.56 | 5.21 | 8.9 | 0.9 |
| | QSr.umn.4A.2 | 4A | 4A_596975878 | 4A_598153809 | 43.4 | 2.8 | 5.1 | 0.7 |
| | QSr.umn.4B | 4B | 4B_645297937 | 4B_660497001 | 101.25 | 2.96 | 5.3 | 0.7 |
| | QSr.umn.6B | 6B | 6B_700650707 | 6B_714491581 | 118.71 | 6.68 | 11.9 | -1.0 |

[a] Eth16 = Debre Zeit Ethiopia 2016, Ken17 = Njoro Kenya 17, Stp15 = Saint Paul Minnesota

[b] Maximum phenotypic variation explained by the QTL

[c] Values indicate the estimated additive effects of the QTL, negative values indicate that the allele was contributed by Sabin

Table 7: Reduction of stem rust severity in the Sabin/MN06113-8 RIL mapping population combining 2 QTLs in each environment

| Environments | QTL combinations | Stem rust severity (%) | | Difference in severity | Disease reduction (%) |
|---|---|---|---|---|---|
| | | No QTL | 2QTL combination | | |
| Eth16 | 2A+3B | 28.1 | 18.8 | 9.3 | 33.0 |
| Ken17 | 3B+4A.1 | 15.7 | 10.6 | 5.0 | 32.1 |
| Stp15 | 3B+4A.2 | 7.3 | 4.3 | 2.9 | 40.4 |
| | 3B+4B | 7.2 | 4.4 | 2.8 | 39.1 |
| | 3B+6B | 7.6 | 4.1 | 3.5 | 46.1 |
| | 4A.2+4B | 6.8 | 4.8 | 2.0 | 28.7 |
| | 4A.2+6B | 7.1 | 4.5 | 2.6 | 36.7 |
| | 4B+6B | 7.1 | 4.6 | 2.5 | 35.3 |

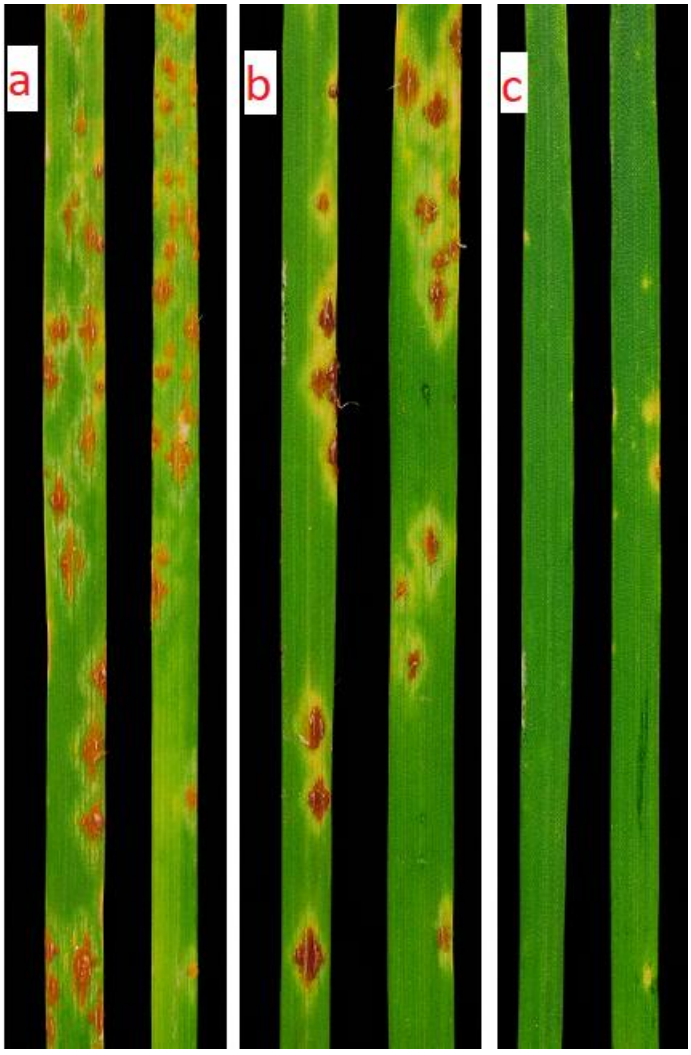



Figure 1: Seedling reaction of *Pgt* races on parents Sabin (left side of each panel) and MN06113-8 (right). (a) Ug99 race TTKSK; (b) race TRTTF from Yemen; and (c) race TKTTF from Ethiopia.

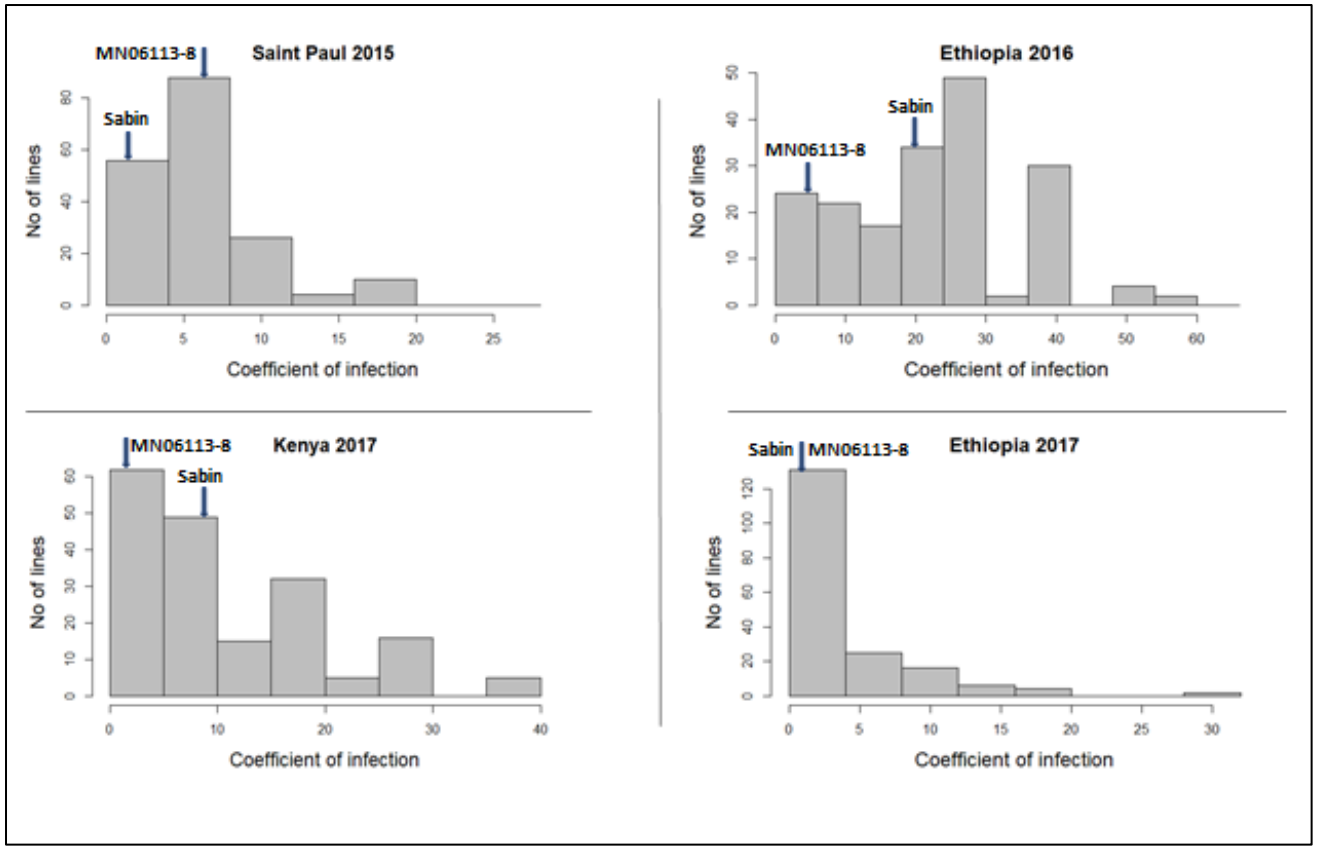



Figure 2: Frequency distribution of stem rust coefficient of infections in the Sabin/MN06113-8 RIL mapping population in three field environments. Disease infection values for both parents in each environment are indicated by arrows.

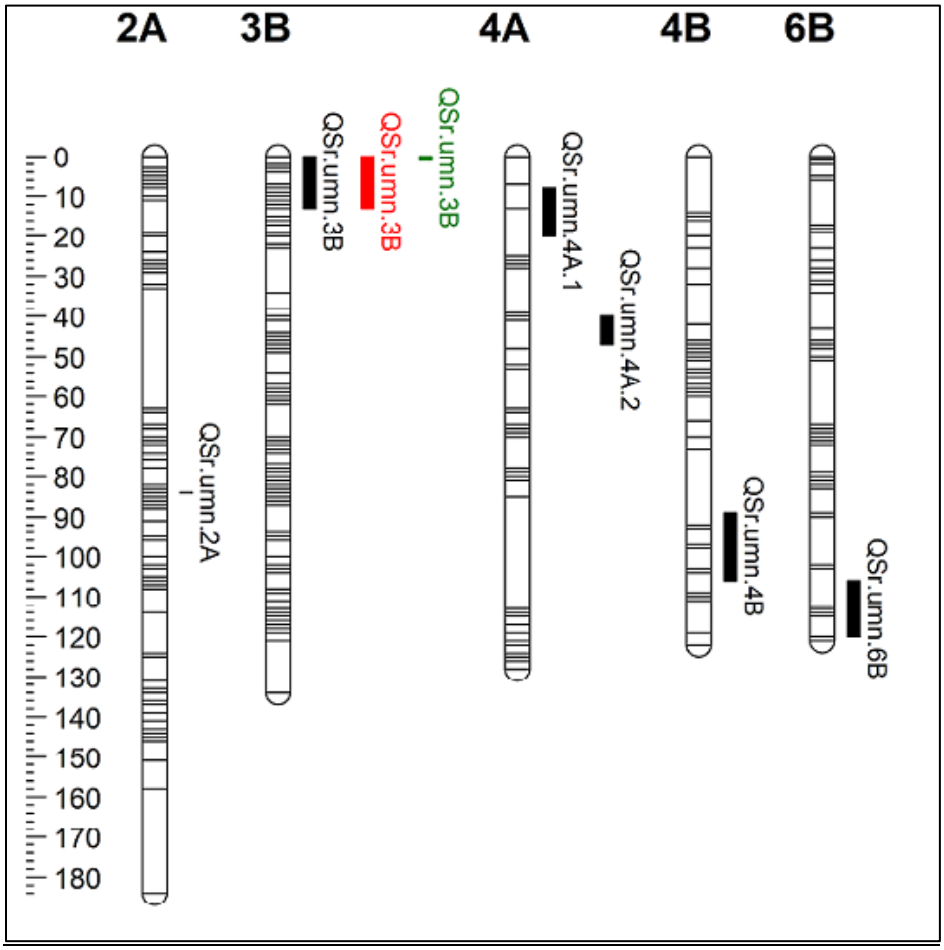
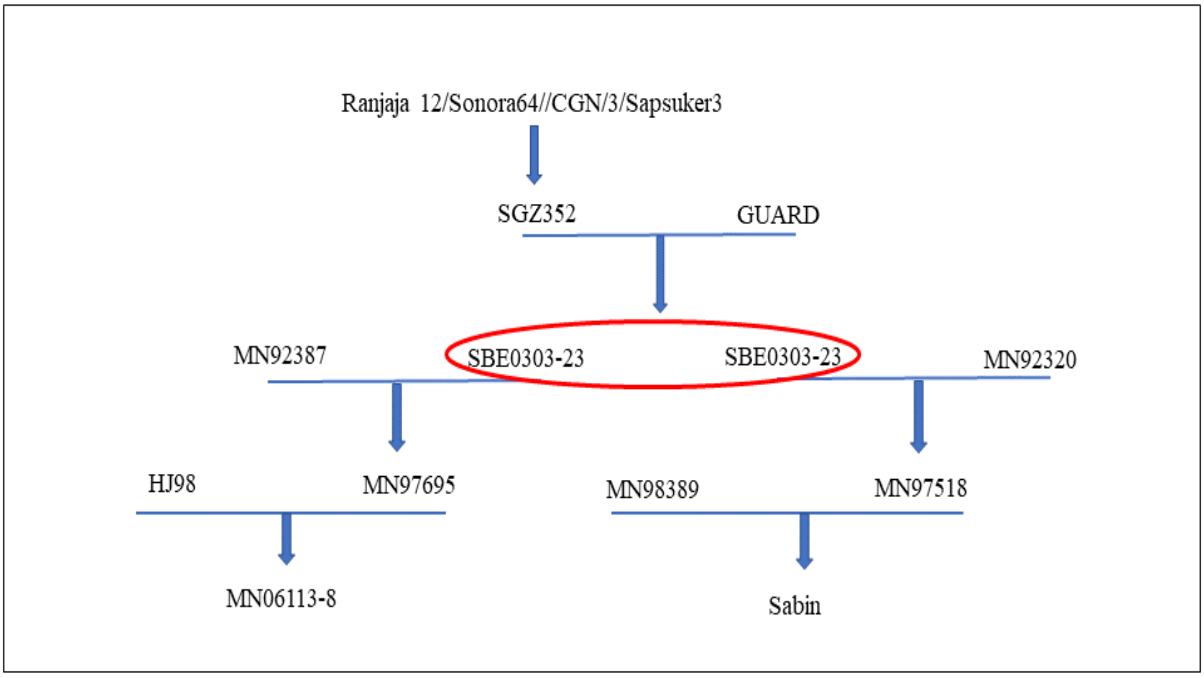
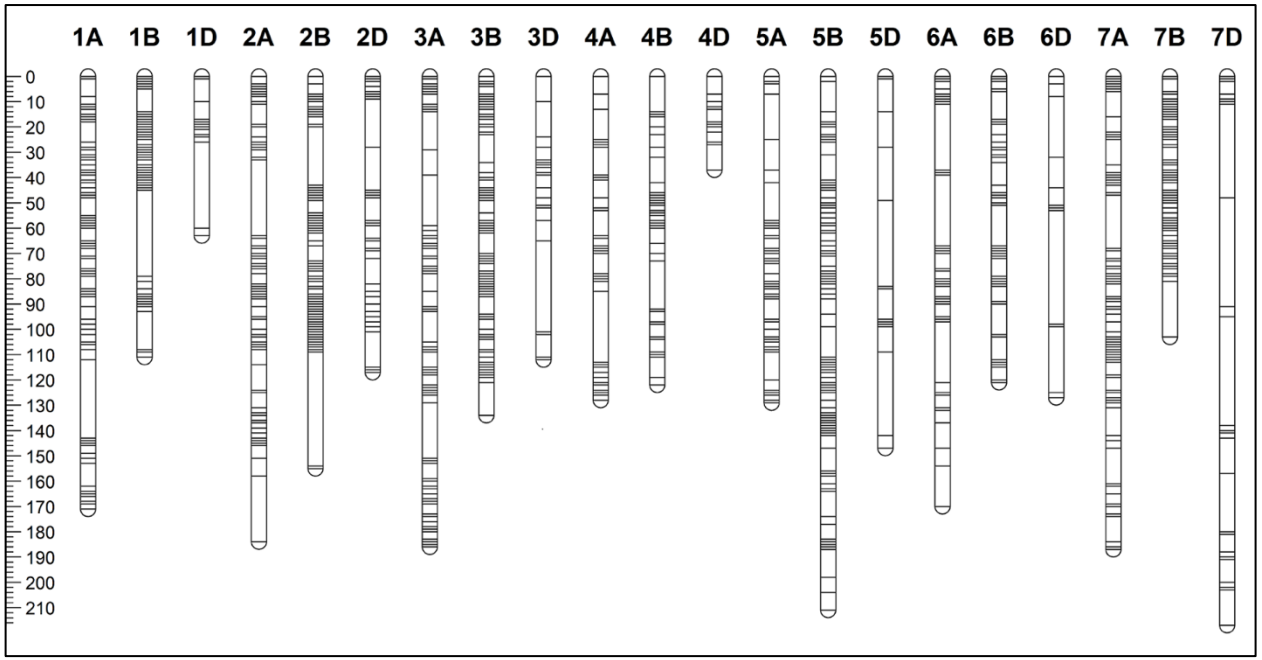



Figure 3: Chromosomes with the stem rust APR QTLs detected by composite interval mapping. The QTL on 3BS was detected in 3 environments: Stp15 (black), Eth16 (red), and Ken17 (green).




S. Figure1: Pedigree of parental lines ‘Sabin’ and ‘MN06113-8’ used for the development of RIL mapping population. The red circled line ‘SBE0330-23, is a common line shared by both parents.




S. Figure 2: Genetic maps of Sabin x MN06113-8 across all 21 chromosomes. Bars on each chromosome reflects the unique loci. Large gaps between loci represent monomorphic regions with no marker coverage.

## References

### Chapter 1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