Genetic Decryption of Stripe Rust Resistant Gene (Yr24) In Bread Wheat

By

Sadam Hussain

Reg. No. 02041713036

Department of Plant Sciences

Quaid-I-Azam University

Islamabad, Pakistan

2019

Genetic Decryption of Stripe Rust Resistant Gene (Yr24) In Bread Wheat

This work is submitted as a dissertation in partial fulfillment for the award of the degree of

Master of Philosophy
In
Plant Sciences

By

Sadam Hussain
Reg. No. 02041713036

Department of Plant Sciences
Quaid-I-Azam University
Islamabad, Pakistan
2019

APPROVAL CERTIFICATE

This is to certify that the dissertation entitled **"Genetic Decryption of Stripe Rust Resistance Gene (Yr24) in Bread Wheat"** submitted by **Sadam Hussain (Registration No. : 02041713036)** is accepted in its present form by the Department of Plant Sciences, Quaid-I- Azam University, Islamabad as to satisfy the partial fulfilment for the degree of Master of Philosophy in Plant Sciences.

Supervisor: _____

Dr. Umar Masood Quraishi
Assistant Professor
Department of Plant Sciences,
Quaid-I-Azam University,
Islamabad, Pakistan**.**

External Examiner_____

Dr. Fayyaz- ul – Hassan
Department of Agronomy,
Pir Mehr Ali Shah Arid Agriculture
University, Rawalpindi.

Chairman: _____

Dr. Abdul Samad Mumtaz
Associate Professor
Department of Plant Sciences,
Quaid-I-Azam University,
Islamabad, Pakistan.

PLAGIARISM CERTIFICATE

This is to certify that, **Sadam Hussain** (**Reg. No. 02041713036**) has completed Master of Philosophy in Plant Sciences from Department of Plant Sciences, Quaid –I-Azam University Islamabad. The title of thesis is **"Genetic Decryption of Stripe Rust Resistance Gene (Yr24) in Bread Wheat"**. This thesis has been checked on Turnitin for similarity index and found that it lies within the limit provided by HEC.

Overall plagiarism =18%, that lies in the limit provided by HEC (19%).

.

Supervisor: _____

Dr. Umar Masood Quraishi
Assistant Professor
Department of Plant Sciences,
Quaid-I-Azam University,
Islamabad, Pakistan**.**

DECLARATION

The research work presented in this thesis was carried out by me in the Plant Physiology laboratory, Department of Plant Sciences, Quaid-i-Azam University Islamabad. The results, findings and conclusions were of my own investigation with the discussion of my supervisor **Dr.Umar Masood Quraishi**. No part of this work has been presented for any other degree.

Sadam Hussain

DEDICATION

I dedicate this effort to my three teachers who inspired me a lot

Dr. Zakir Abdul Karim Naik

Dr. Israr Ahmad (late) and

Dr. Umar Masood Quraishi

Table of Contents

List Of Figures

List of Tables

List of Abbreviations

AFLP	Amplified Fragment Length Polymorphism
AIC	Akaik Information Criterion
BAC	Bacterial Artificial Chromosome
cDNA	Complementary DNA
CI	Confidence Interval
CIMMYT	International Maize and Wheat improvement Center
cM	centiMorgan
DarTS	Diversity Array Technology
DNA	Deoxyribose Nucleic Acid
RNA	Ribose Nucleic Acid
EST	Expressed Sequence Tags
Gb	Giga Base
GBS	Genotyping-By-Sequencing
GDP	Gross Domestic Products
Gr no	Grain Number
Gr wgt	Grain weight
GS	Genomic Selection
GWAS	Genome-Wide-Association Studies
GY	Grain Yeild
INDEL	Insertion/Deletion
INRA	Institute national de la recherché agronomique
ITEC	International Triticeae EST Cooperative
ITMI	International Triticeae Mapping Initiative
K	Relative Kinship Matrix
Kb	Kilo base
LD	Linkage Disequilibrium
Lr	Leaf Rust
MAS	Marker-Assisted Selection
MQTL	Meta-QTL
MTA	Marker Trait Association
NGS	Next Generation Sequencing
NIL	Near Isogenic Line
Pm	Powdery Mildew
QTL	Quantitative Trait loci
RAPD	Random amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SNP	Single Nucleotide Polymorphism
Sr	Stem Rust
SSR	Single sequence Repeats
Yr	Yellow Rust
90K array	90,000k SNP Array

ACKNOWLEDGMENTS

All praise to almighty Allah, who created the universe, I would like to be grateful for the unconditional love and care of the Almighty Allah and for blessing me with the patience and health to prepare this thesis. I am obliged to show my affection and respect to **Prophet Muhammad (SAW);** a blessing for mankind.

To my loving and respected parents, **Mr. Iltaf Hussain** and **Ms. Mumliqat Naz**: Because I have no words to thank and acknowledge the sacrifices, efforts, lot of prayers, guidance, support, encouragement and appreciation of my loving parents. Their endless prayers contributed to the successful completion of this research work. I would like to thank my sisters Salma and Sumbal and brothers Abbas khan and Wasim khan for their love and support.

My supervisor, **Dr. Umar Masood Quraishi:** The man of patience. Your sincerity and humbleness inspired me to join your prestigious research group. Your thought provoking guidance, valuable suggestions and constant encouragement enabled me to complete this challenging task. His endurance and support in carrying out this project always created in me the sense of patronization and humbleness. God bless you with unending happiness and success in your future life.

Also, I would like to thank **Dr. Javed Iqbal Mirza** (Crop disease research institute Murree) for his sincere support and providing me a laboratory facilities for my research experiment.

I am grateful to **Prof. Dr. Abdul Samad Mumtaz**, Chairman Department of Plant sciences for providing me the research facilities. I am also thankful to all my teachers, who motivated me a lot in my curricular and extracurricular achievements.

I feel highly privileged to take this opportunity to express my sincerest and deep sense of indebtedness from the core of my heart to **Dr. Sadia Latif** (aapi) for her precious, constant moral support and encouragement throughout my research work. You were so generous, polite and helping throughout these two years that I can hardly remember if I ever saw you in anger.

Special thanks to the two most important people in my university life, **Ayesha** and **Fazaria**. My sincere gratitude to you for believing in me and making the university life exciting and memorable.

The past two years with the Plant breeding group of Quaid-i- Azam University was a great experience. I am grateful to my most respectable and honorable senior's laboratory fellows, Sirajuddin, Luqman bin Safdar, Tayyaba Andaleeb (aapi) Saeed Ur Rahman,You were always amazing, Thank you very much for your moral support and valuable suggestions.

The other members of my research group, Uncle Amjad, Arif Ali, Said Akbar, Rizwana, Nazia, Sidra, Saeed Anwar, Javaria and Ambrine. All of you were wonderful and the time spent with you was memorable.

Last but by no means least, I would like to thank to all my friends at Quaid-i-Azam University Islamabad. The two years at this University were memorable and there were so many amazing people and memories to cheer about. Thank you very much for all your encouragement.

Sadam Hussain

Abstract

Wheat is the most significant cereal crop across the world, which means that it has to deal with a wide range of diseases as well. Wheat breeders have worked over the years to collect genetic and genomic resources to reduce the losses to wheat diseases. To demonstrate this translational approach, we used reverse genetic method to clone and validate stripe rust loci in bread wheat. Integration of recently available wheat genome sequence, its annotation and genetic mapping of resistance loci in bread wheat now enables us to use transitional reverse genetics in bread wheat. 147 genetic loci were positioned on WCGM2019. Markers of WCGM2019 were aligned to Wheat genome sequence to identify the regions and putative candidate genes in bread wheat. To validate this reverse genetic approach, we used near isogenic lines of stripe rust from CIMMYT Mexico. 23 Nils for stripe rust along with susceptible check (cultivar Morocco) were screened under controlled conditions in glass house at Crop Disease Research Institute (CDRI) Murree with races **574232**, **574207**, **574210** and **574203** to find genes avirulent to these races. Yr24 (uncloned), Yr10 (cloned) and Yr15 (cloned) gave avirulent results against the inoculated pathotypes. QRT-PCR was used to identify the candidate genes for Yr24 and to verify the genes resistant to Yr10 and Yr15. Overall, this study illustrates a translational research approach in transferring information gained from wheat genome sequence to dissect the genomic regions for disease resistance, development of diagnostic chip base markers and associated candidate genes to be now considered as a key resource for breeding programs

1. INTRODUCTION

1.1 Wheat

Modern wheat has been categorized in two cultivated forms i.e. common wheat "*Triticum aestivum L*., with 2n=6x=42, AABBDD genome", and macaroni wheat "*Triticum turgidum L*., with 2n=4x=28, AABB genome" (Huang, Brooks *et* al. 2003). About 95% of overall wheat is hexaploid i.e. common wheat. It is mostly consumed by the world population in the form of breads, cookies, cakes and noodles. The remaining 5% is Durum wheat, used in the formation of pasta and other products (Pena, 2002). Wheat is further categorized into two major groups i.e. winter wheat and spring wheat.

1.2 Socio- economic importance of wheat

Wheat is an important cereal crop that fulfills the dietary needs of one-third population across the world (Dhanda, Sethi *et* al. 2004).Importance of wheat can be realized in agricultural organization's symbol that shows a wheat spike with its description ''Let there be bread''. Being widely cultivated crop, it is also one of the youngest polyploidy species along with first domesticated plant (Hanson *et al.* 1982). The modern wheat is of two types, the hexaploid wheat (*Triticum aestivum*) which is 95% of world wheat production and the tetraploid durum wheat *(T.durum*) accounting for 5% (peng et al., 2011). Wheat is also considered an excellent model organism for the study of evolution of allopolyploid species, adaptation and domestication in plants (Gustafson *et* al., 2009). The world leading wheat producing countries are the United States, China, and Russia; extensive wheat growing is carried on also in India, W Europe, Canada, Argentina, and Australia.

1.3 Historical perspective of wheat

Wheat belongs to Poaceae family, was first cultivated about ten thousand years ago near the fertile crescent in Pre-Pottery Neolithic period (Harlan and Zohary 1966).The tetraploid emmer wheat was reported to be cultivated about 8500 B.C By 6500 B.C in the Fertile Crescent. It extended to Cyprus, Greece and India and then to Egypt after 6000 B.C and Spain and Germany after 5000 B.C. Wheat was first grown in England And Scandinavia by 3000 B.C, and after 1000 year later, it extended to china (Cooper 2015).Landraces, also known as former's varieties have been developed

through years of humans and natural selection and that's why they are better adopted to local environment (Zeven 1998).They are conferring better adoptability to stress conditions because of their genetic structure and some physiological traits. Apart from that, Landraces also play a key role in making of new verities with stable yield under varying environmental conditions (Jaradat 2013).

1.4 Wheat as a staple food

Wheat is the most favored staple food across the world because it provides more nourishment to humans than any other food (Johnson, Wilhelmi *et* al. 1978).and (Šramková, Gregová et al. 2009).wheat is ranked among the most prominent food crop because it fulfills the dietary requirements of one- third population across the world (Almas, Hassan *et* al. 2018).An incredible quantity of 660 million tons of wheat is harvested annually by the wheat producing regions (Carver, 2009).Modern wheat as a staple food is of great economic importance because it has high nutritional value and large amount of nutrients. It contains vitamins A, B, E, proteins and carbohydrates (Ortiz-Monasterio, Palacios-Rojas *et* al. 2007).Apart from its main role in food industry, wheat is also used in making biofuel (Kaparaju, Serrano *et* al. 2009).there is an endless increase in the demand and need of bread wheat in developed countries(Faridi and Faubion 1995). The reason of increasing demand is that a number of people exchange the consumption of simple carbohydrates with complex carbohydrates and proteins. An annual of 2% wheat production is needed to fulfill the increase dietary demand (Reynolds, Rajaram *et* al. 1999).

1.5. Wheat genomics, evolution and genetic resources

1.5.1. Wheat genomics

Bread wheat possess the largest genome of approximately 17 GB which is 40x larger than rice, 52x larger than Brachypodium, 20x larger than Sorghum and 7x larger than maize. Wheat has three sub genomes which makes it a heterogeneous polyploidy (Salse et al. 2009, Bolot*et* al. 2009) having more than 80 percent of high proportion of repetitive sequences (Wheat genome sequence 2017). Therefore, to understand the evolutionary history the available genomic, cytogenetic and genetic resources can be better utilized as illustrated in next section.

1.5.2 Evolution of wheat

The evolution of grass family has occurred about 50 to 70 million years ago (Huang *et* al 2002). The bread wheat arose about 6000-8000 years ago by the cross between *Triticum turgidum* and *Aegilops tauschii,* through which the whole genome was created (Qi, Friebe *et* al. 2007).The wild emmer wheat was produced by cross between *T. urartu* (A genome) and *Aegilopsspeltoides* (B genome) about 3 to 5 million years ago. The *Triticum spelta* (AuAuBBDD) was formed by allopolyploidization event between *Aegilops tauschii* and *T. dicoccoides* about 9000 million years ago (Matsuoka and Nasuda 2004).

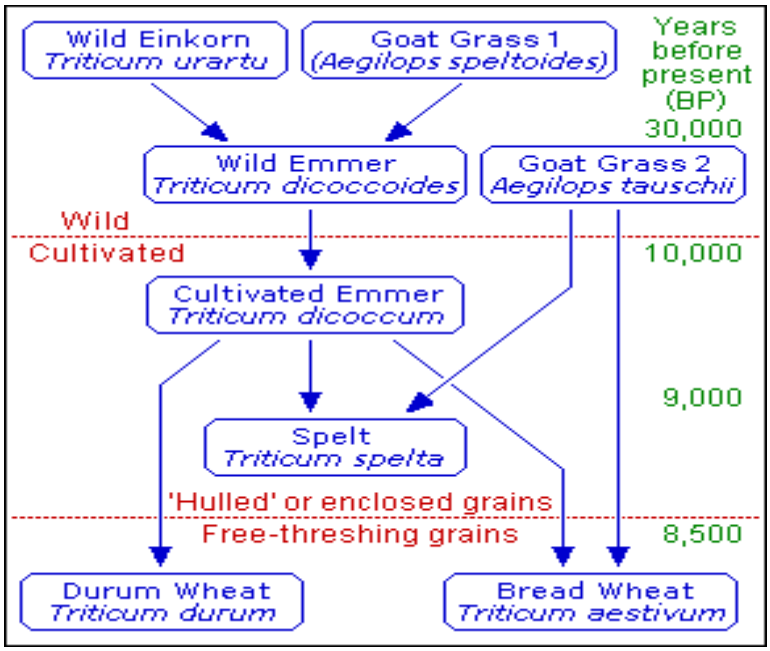


FIGURE 1: EVOLUTION OF WHEAT FROM THE PREHISTORIC STONE AGE GRASSES TO MODERN MACARONI WHEAT AND BREAD WHEAT (REPRODUCED FROM HTTP://WWW.NEWHALLMILL.ORG.UK/WHT-EVOL.HTM)

The diploid einkorn and Tetraploid emmer wheat were started for cultivation about 10000 years ago (Dubcovsky and Dvorak 2007).The modern wheat genome is created from closely related sub genomes A,B and D. The sub genomes A and B arises from an ancestor about 7 million years ago while sub genome D arise by homoploidhybride speciation about 1 to 2 million years later. The modern genome of bread wheat is the result of several rounds of hybrid speciation (Marcussen, Sandve et al. 2014). So, seven

chromosomes of each sub genome is structured in 21 pairs holding 17 billion base pairs (Sears, 1954; Okamoto, 1962).

1.5.3 Cytogenic resources

Being a polyploidy genome, wheat can endure the loss of whole arm or segment of chromosome. Keeping this specific phenomenon in mind, various deletion stocks have been developed (Sears 1954) The cytogenic materials can be distinguished into (1) monosomic (missing an entire chromosome); (2) nullisomic (missing pair of homologous chromosome); (3) trisomic (presence of an extra chromosome); (4) nulli-tetrasomic (one missing chromosome pair is replaced by another pair of chromosome); (5) ditelosomic (absence of an arm of chromosome,); (6) Introgression lines (integration of a small part of chromosome); and (7) deletion lines (removal of a chromosome segments,). These cytogenic materials can be widely used in order to study the chromosomal location of major traits as well as for comprehensive mapping of molecular markers into chromosomal "bins" regions described by neighboring deletion breakpoints (Hohmann, Endo *et* al. 1994).

1.5.4 Genetic resources

Bread wheat (*Triticum aestivum)* consists of one of the complex genome known to science carrying 6 copies of each chromosome. The genome has large number of near identical sequences with estimated 15 billion bases. Past efferts have produced assemblies in order to assemble the genome that were short the the genome size. First near complete assembly of Bread Wheat have been reported using deep sequencing coverage with the grouping of short IIIumina reads and long Pacific Biosciences reads. The concluding assembly comprise of 15 344 693 583 bases and contig size of 232 659 bases which exhibits the most complex assembly of the wheat genome up to date. The genome of *Aegilops tauschii* have also been used in order to recognize 4 179 762 575 bp of *T. aestivum* that correspond to the component of D-genome.

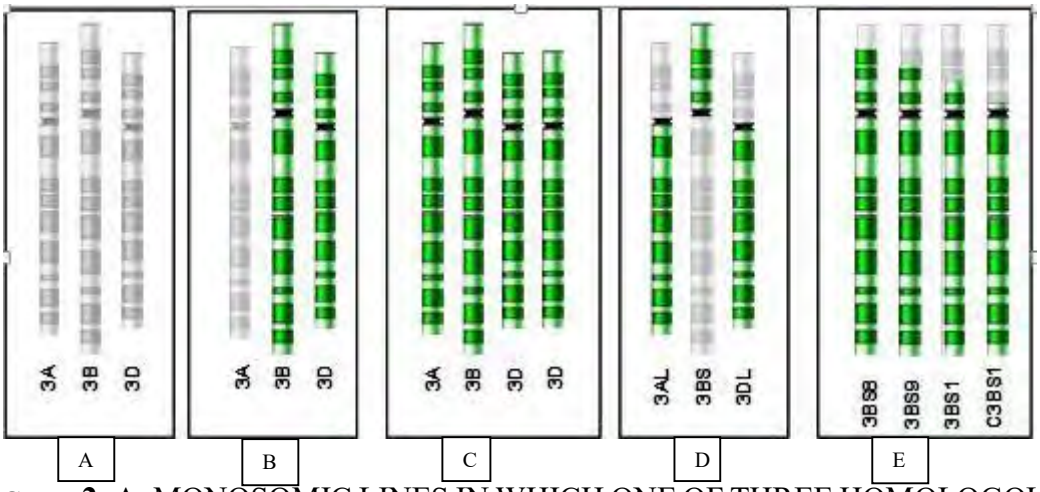


FIGURE 2: A: MONOSOMIC LINES IN WHICH ONE OF THREE HOMOLOGOUS CHROMOSOME IS MISSING, B: NULLISOMIC LINES IN WHICH ONE PAIR OF CHROMOSOME IS MISSING, C: TRISOMIC LINE IN WHICH ONE EXTRA CHROMOSOME IS PRESENT, D: DITELOSOMIC LINES IN WHICH ARM OF CHROMOSOME IS MIS

1.6 Wheat in the world

Wheat being a basic cereal crop consider as an important staple food across the world (CIMMYT, 1996). It is included in the 'big three' cereal crops, with more than 600 million ton being harvested per annum (http://faostat.fao.org/).

In temperate regions wheat is the most dominant cereal crop (Shewry 2009).which is cultivated on more than 240 million hectares, with an estimated production of 564.6 million tons and an avg production of 2500 kg grains per hec (Abid, Maqbool *et* al. 2014).

In the world trade wheat contributes more than all other cereal crops. The four leading wheat producing countries are the European Union, China, India and United States having cultivated area are 25500, 24300, 29690, and 19826 thousand hec respectively with anavg production of 5.17, 4.86, 3.16 and 3.11 ton/hec respectively (USDA, 2012).

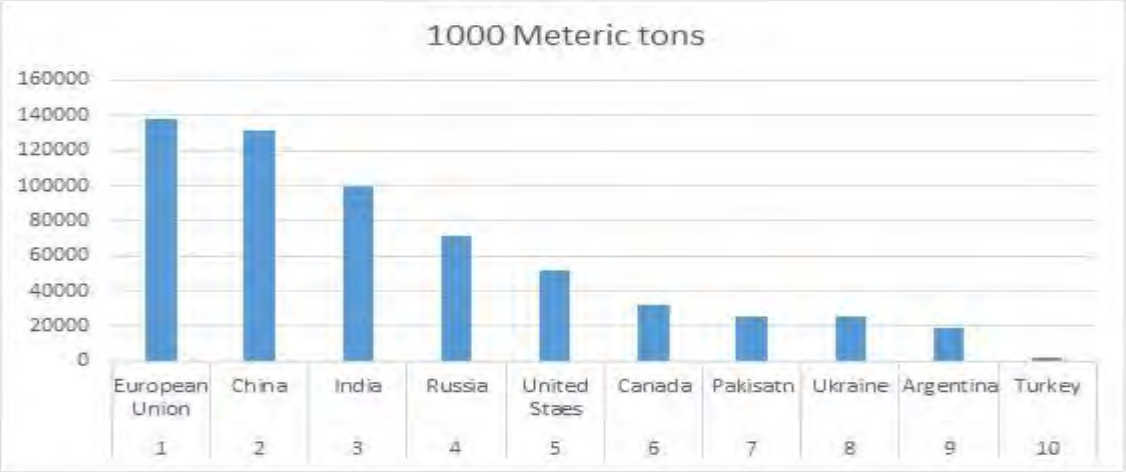


FIGURE 3: MAJOR WHEAT PRODUCING COUNTRIES IN 2018/2019 (IN 1000 MT) HTTPS://WWW.INDEXMUNDI.COM/ AGRICULTURE /? COMMODITY=WHEAT

1.7 Wheat in Pakistan

In Pakistan, wheat is the major staple food (Godfray, Beddington *et* al. 2010). It has an important place in agriculture and economy by contributing 8.9% to the agriculture sector and about 1.6% to the GDP of Pakistan (Pakistan Economic Survey 2018-19).In Pakistan, being a major cereal crop wheat shares 75% to the total grain production (Pinckney 1989).Wheat crop showed minimal increase of 0.5% to the 25.195 million tons as compared to the last year production of 25.076 million tons missed the target by 4.9%. The total harvested area of wheat for year 2019 was 8,740 thousand hectares which was 0.6% less than the previous year's 8,797 thousand hectares (Pakistan Economic Survey 2018-19). Domestic consumption of 2019 increased to 25.4 million tons as compared to the last year 25.3 million tons (https://www.indexmundi.com). The annual production, consumption, export and import by Pakistan for the last decade is shown in figure (Source; USDA- United States Department of agriculture).

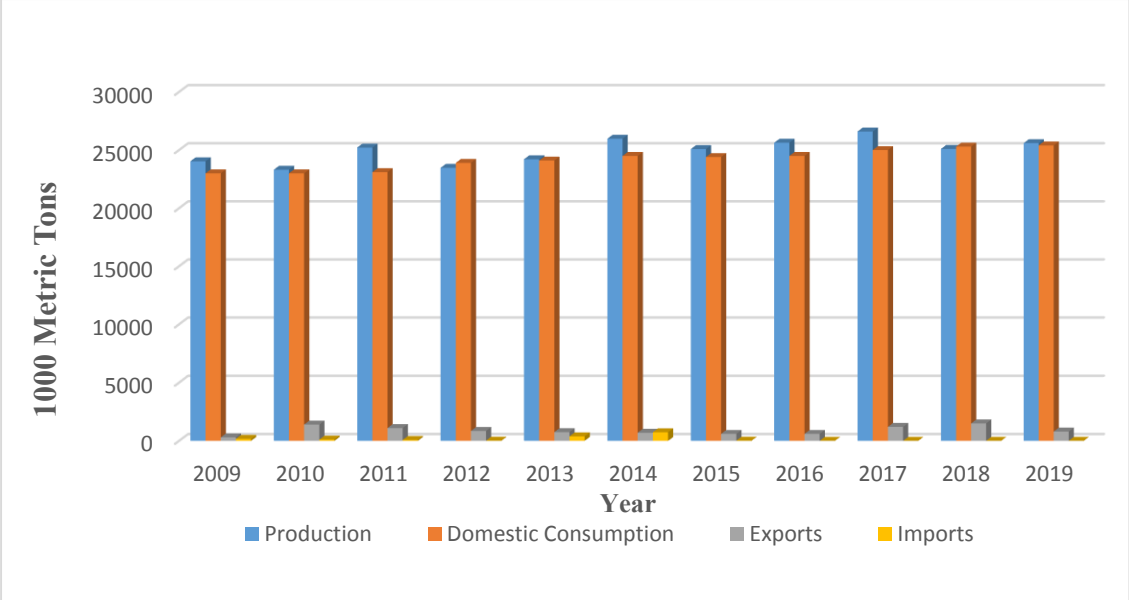


FIGURE 4: ANNUAL PRODUCTION, DOMESTIC CONSUMPTION, EXPORT AND IMPORT HTTPS://WWW.INDEXMUNDI.COM/AGRICULTURE /? COMMODITY=WHEAT

1.8 Aims and Objectives

The aims and objective of the present study were

- Development of wheat genetic map for projection of yield and its related QTLs, resistant loci, resistance proteins and cloned agronomic genes.
- Projection of genetic map on physical map of bread wheat
- Identification and development of new diagnostic markers for resistance loci for breading program.
- Insilco mapping of resistance protein on physical map of bread wheat.
- Identification of candidate genes for resistance loci in bread wheat.
- Screening of stripe rust differentials against Pakistani stripe races.
- Genetic decryption of Yr24 using the Insilco method.
- Resistance proteins in Yr24 differential line.

2. CONSTRUCTION OF NEW BREAD WHEAT COMPREHENSIVE GENETIC MAP

2.1 Introduction

The importance of genetic map has increasingly improved in crop species after their first introduction. Earlier genetic maps composed of RFLP markers (Chao, Sharp *et* al. 1989) was replaced by the PCR based markers i.e. SSR's, AFLP and RAPD's ((Williams, Kubelik et al. 1990).The relocation towards PCR based markers is actually based on the reason to use these maps in plant breeding (Gupta and Varshney 2000). These significant platforms assist marker-assisted-selection (MAS) in plant breeding programs. Moreover, wheat genomic research depends upon the map based cloning of gens which needs an accurate and fine mapping in order to correctly position the gene of interest between close flanking markers(Peters, Cnudde *et* al. 2003) for better understanding of genetic development of complex traits and its deployment in breeding programs for crop improvement through whole genome association studies and genomic selection(Elshire, Glaubitz *et* al. 2011).

Due to the complexity of wheat genome, particularly the development of molecular markers has always been an intimidating task (Paux E, Faure S, Choulet F, Roger D, Gauthier V, *et* al. (2010). Polymorphism markers based on insertion site has opened new outlook for genome saturation and marker-assisted selection in wheat. Plant Biotechnology Journal 8: 196–210. 2. Akhunov E, Nicolet C, Dvorak J (2009) Single nucleotide polymorphism genotyping in polyploid wheat with the Illumina Golden Gate assay. TAG Theoretical and Applied Genetics 119: 507–517. 3. Paux E, Sourdille P, Salse J, Saintenac C, Choulet F, et al. (2008) A Physical Map of the 1-Gigabase Bread Wheat Chromosome 3B. Science 322: 101–104.). However, the dawn of new sequencing technologies assisted the discovery of SNP's in many species i.e. rice, maize, soybean, common bean and sorghum. SNPs discoveries in the D- genome of *Aegilops tauschii*, marked a step forward for SNPs markers in large and repetitive genomes like wheat. The emergence of next generation technology has also facilitated the variant analysis of complex and large genomes. At the beginning, discovery of SNPs was the major tool for next generation sequencing but in last few decades' concentration has been moved to the analysis of complex reduced fraction of wheat genome. SNP discovery in datasets are

successfully used to develop genotyping assays based on Golden Gate(Akhunov, Nicolet *et* al. 2009; Chao, Dubcovsky *et* al. 2010), Bead Express(Trebbi, Maccaferri et al. 2011), KASPar (Allen *et* al. 2011), and Infinium platforms (Cavanagh *et* al. 2013). There are various applications of SNP's including exploration of diversity within species, development of haplotypes maps and carrying out genome wide association studies [13](Elshire, Glaubitz et al. 2011). The SNP's with wide range of application, detect the markers trait association in QTL mapping and genome wide association studies) (Cook, McMullen et al. 2012); Jia*et* al., 2013; Tian*et* al., 2011; Zhao *et* al., 2011). New approaches in next generation sequencing have outstandingly facilitated the discovery of SNPs by whole genome sequencing (Berkman, Lai *et* al. 2012), transcriptome (Allen, Barker et al. 2011)or reduced-representation sequencing in diverse populations of individuals (Elshire, Glaubitz *et* al. 2011)In order to explore common alleles for crop improvement requires further investigation, crop breeding will probably to benefit from the introduction of new allelic variation from distant relatives (Cavanagh, Chao *et* al. 2013).

Using high density SNP array for economically important crops and animals (Ganal*et* al., 2011; Sim*et* al., 2012; Song *et* al., 2013; Wiedmann*et* al., 2008; Zhao *et* al., 2011) and for genetic studies is found successful in different studies. 44K SNP array has been used for the recognition of alleles controlling 34 morphological, development and agronomic traits for the genome wide association of 413 different rice accessions (Zhao *et* al., 2011). 50k maize SNP chip was used in order to study the genetic control of maize kernel composition in association mapping panel (Cook *et* al., 2012). Cavange et al., 2013 has recently used 9k SNP wheat chip in order to detect genomic regions targeted by breeding selection in wheat (Cavanagh *et* al., 2013).

90K array has been developed, genotype calling algorithms and high density genetic maps which will provide a constructive resource for analyzing genome wide variation in wheat. The low fraction of missing haplotypes and high data quality will create an opportunity to develop a future framework for genome analysis and diversity studies. These developments in the field of wheat genetics and genomics will help in explaining the complex relationship between phenotype and genotype (Wang, Wong *et* al. 2014)

On contrary, with the advent of new sequencing technologies, the genome dissecting strategies are moving to the direct and robust sequencing of population (Hamilton and Buell 2012). The discovery of SNPs has led to the reduction of ascertainment bias by direct approach approach (Gan et al. 2011). Re-sequencing is carried out depending upon the genomic DNA (Huang *et* al. 2009). Digestion of genomic DNA by restriction enzymes for complexity reduction is called RAD-seq, CroPs, or genotyping-by-sequencing (GBS) (Davey *et* al. 2011). Genotyping-by-sequencing is a simple and robust techniques for complex reduction in large genomes. Genotyping-by-sequencing is less time consuming approach. In the absence of reference map, genotyping-by-sequencing approach assists in making high density genetic map (Poland, Brown *et* al. 2012). The above reported information for mapping is a constructive asset for the development of a comprehensive map. Keeping these in account effort is made to contribute to the wheat genomics resources.

2.2 Materials and Methods

2.2.1 Selection of public reference genetic maps

For the development of comprehensive genetic map, we required the projection of one map onto another. But restrictions to this approach is the absence of common markers between two genetic maps that results in doubling of chromosome in size compared to the original chromosome. As a result, the interest was to project all the different genetic maps and three possible approaches were found for a reference genetic map in wheat.

The first is the International Triticeae Mapping Initiative, (Nelson et al., 1995a, 1995b, 1995c; Rder et al., 1998a, 1998b). Total number of 2293 molecular markers which cover 3980.4 cM resulting in a high marker density of one every 1.735 cM. Moreover, this map can be used as the deletion bins of Chinese spring (Qi et al., 2004) are assigned (Sourdille et al., 2004),http://wheat.pw.usda.gov/- ggpages/SSRclub/-GeneticPhysical/).

The second approach is the development of genetic map developed by R Appels i.e. Wheat Composite map 2004 which can be found at http://wheat.pw.usda.gov/. This is

A consensus map of various mapping populations such as Synthetic-W7984 x Opata85 (four studies), Arina x Forno, CD87 x Katepwa, CS x DH, Cranbrook x Halberd, Egret x Sunstar, and Sunco x Tasman. Chromosomes of homologous group 1 and 2 were covered with an average of 209 and 190 markers respectively while markers on homologous group 4 was lower with 134 markers on the chromosomes. There are about 3660 various markers on the genetic map cover the distance of 3121 cM, with average distance of 0.85 cM between two markers.

The third and last possible approach that has been developed by D.Somers's group (Somers et al., 2004). In this study four different mapping population have been assembled into a single consensus map. There are total 1235 microsatellites loci were mapped that covering the distance of 2569 cM, with an average distance of 2.2 cM between two markers. The composition of genetic maps and number of markers are given below:

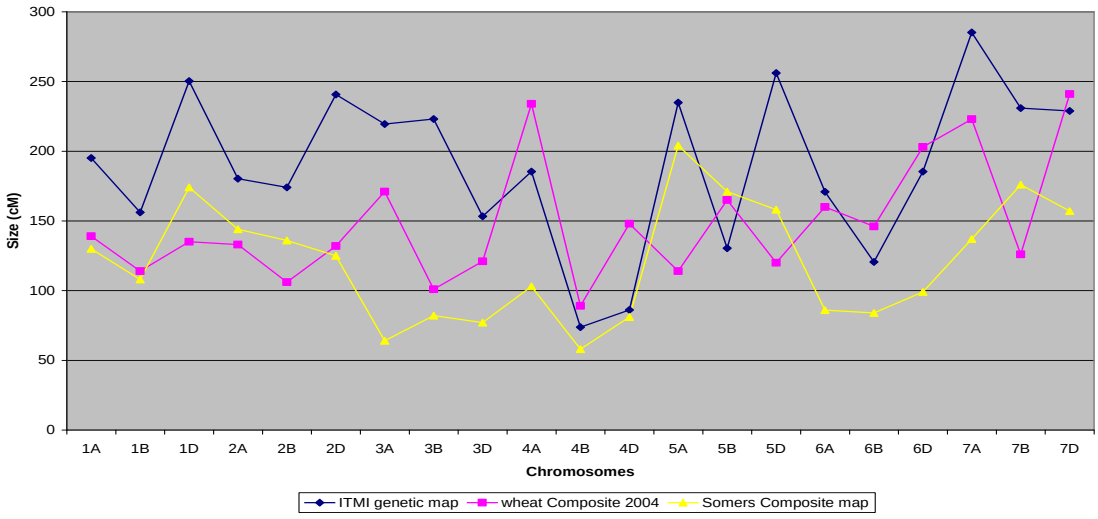


FIGURE 5: COMPARISON OF THE SIZES OF DIFFERENT GENETIC MAP (ITMI, WCGM 2004, AND SOMER'S.

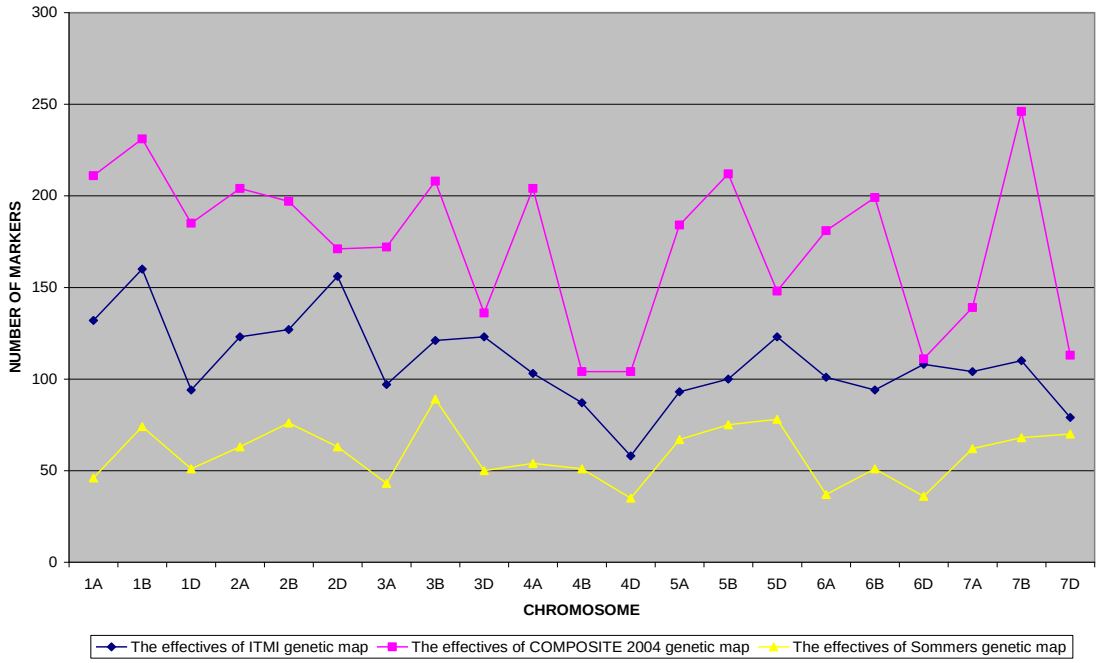


FIGURE 6: COMPARISON OF THE GENETIC MAP MARKER EFFECTIVES (ITMI, WCGM 2004, AND SOMER'S MAP).

With the advent of next generation technology i.e. single nucleotide polymorphism and genotyping arrays are powerful genetic resources in order to study genomic motifs of diversity, interpretation of ancestral relationship of an individual within a population and studying association of markers and traits in mapping experiment. That's why, genotyping array that include about 90,000 gene-associated SNPs of which 46,977 from wheat 90K array mapped using a composite of double haploid eight mapping populations. This is consensus map of various mapping populations including BT-Schomburgk 9 AUS33384 (CIGM92.1712), Young 9 AUS33414 (CIGM93.238), Chara 9 Glenlea, W7984 9 Opata M85, Sundor 9 AUS30604, Westonia 9 Kauz, Avalon 9 Cadenza and Savannah 9 Rialto. There are total 40267 markers are available to onto the all available maps.

An estimated 416,856 markers of 9K SNP iselect assay have been developed on the synthetic-W7984 x Opata85 population were also available to produce high density map (Saintenac, Jiang et al. 2013). This is a consensus map that comprising of 104804 markers that have been assembled into a highly dense map. Additional, diversity arrays which is actually a hybridization based approach used to observe the presence or absence of a genomic segment have been provided by the Triticarte's wheat DArT service. Approximately 2,134 molecular markers are available for the wheat genome that can be used for the evaluation of genetic diversity and for the development of genetic linkage map. The comparison of all the maps used for projections are given below.

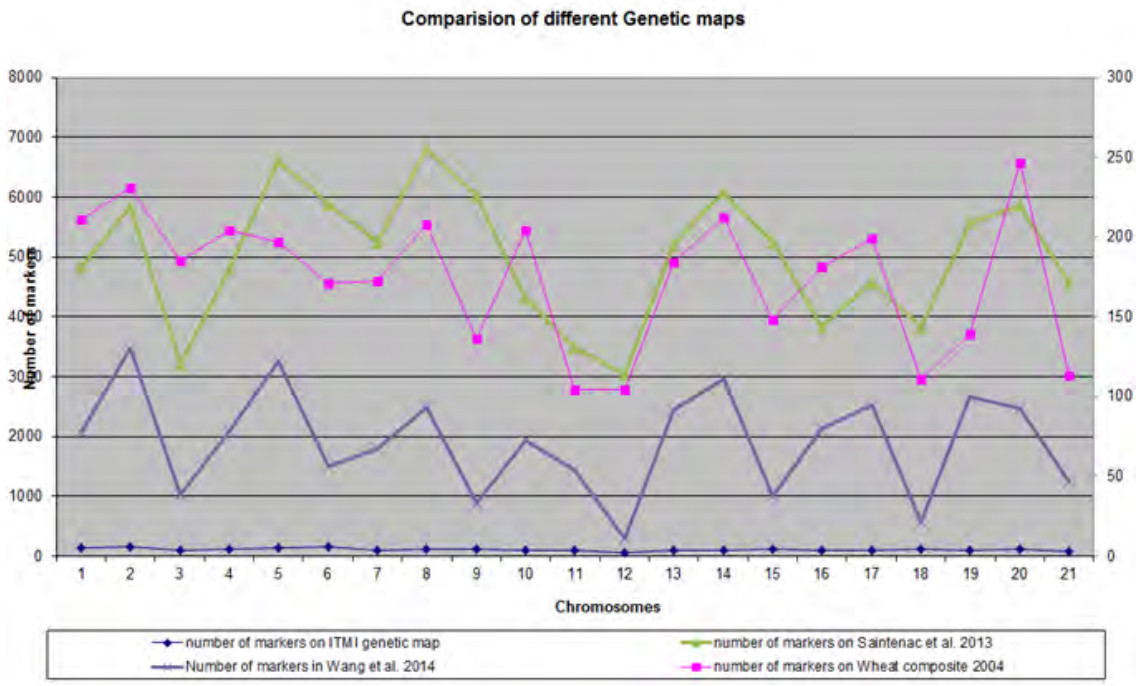


FIGURE 7: COMPARISON OF THE SIZES OF DIFFERENT GENETIC MAP (ITMI, WCGM 2004, AND SOMER'S).

2.2.2 Construction of the wheat Composite Genetic Map (WCGM 2019)

The graph 2b exhibit efficacy of the maps and can be clearly concluded that wheat Composite map 2004 is more preferable than ITMI and Somer's maps. But the presence of deletion bins on ITMI map takes an advantage on wheat Composite genetic Map 2004 (Appels R. 2004). To realize the usefulness of both maps, the best technique

is to assemble both the maps for developing a new reference map including ITMI with deletion bins and WCGM 2004 with molecular markers distribution.

Till now, five map integration techniques have been described in literature. The first technique is the visual alignment of maps which is based on common markers that has already been used in the development of consensus map of homologous groups of wheat (NELSON *et al.* 1995A, NELSON *et al.* 1995B, NELSON *et al.* 1995C; VAN DEYNZE *et al.* 1995; MARINO *et al.* 1996). The second technique suggests the development of pooled map using MAPMAKER software (LANDER *et al.* 1987; LINCOLN *et al.* 1993). The third techniques implies on the population on the population structure and size by JoinMap (STAM 1993; STAM and OOIJEN 1995). The forth approach is the graphical representation of that is based on visual alignment and combination. The fifth technique was use for the Human Genome Database, RTI international, and North Carolina USA for the construction of comprehensive mapping of human genome. This is the most allowable technique that depends on selection of a specific map as standard map onto which other maps are projected in order to get a single comprehensive map.

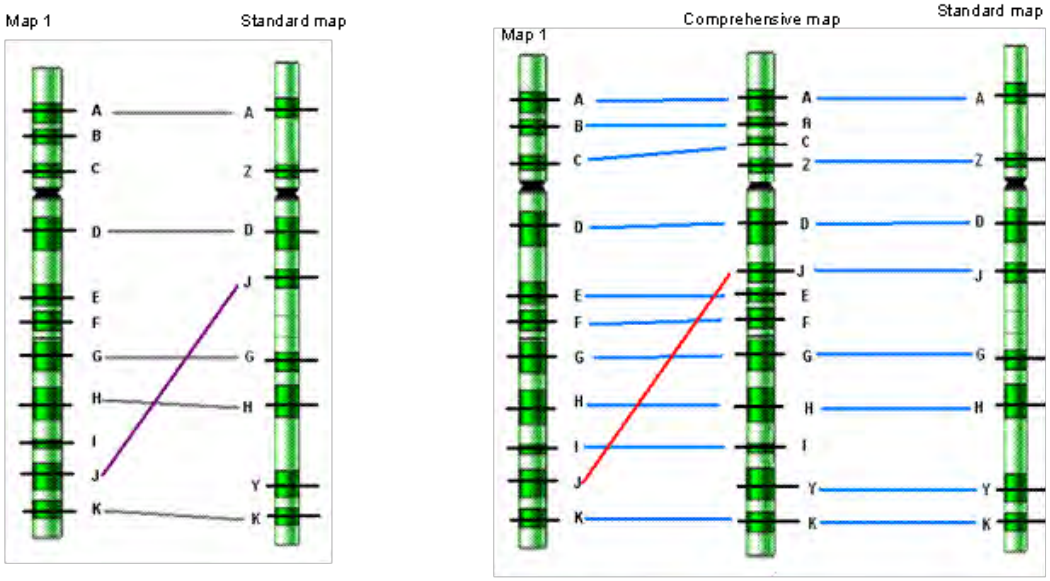


FIGURE 8: STRATEGY FOR COMPREHENSIVE MAP CONSTRUCTION

Marker Common Markers removed Markers removed from projected markers

The above figure exhibit the fifth approach which was used for the construction of consensus map. For advance studies as discussed earlier, a highly dense comprehensive map was needed. To chase this, all the maps i.e. WCGM 2004 (Appels R. 2004), DArT's map, Somer's Map, GBS SynOpata Map with 9K SNP array and 90K maps were projected on the reference ITMI map. As a result, a new wheat Composite map was produced.

The projections of all the maps were carried out in Biomercator version 2.0 for the development of Wheat Composite Genetic Map 2019 (Arcade et al., 2004). The Biomercator version 2.0 have a graphical interface that permits the visual projections of different maps. A text file with markers and inter-maker distance is needed. Biomercator version 2.0 uses the algorithm of map projection in order to incorporate the independent maps. Using this specific feature, al the maps were incorporated in the ITMI map (reference map) and an integrated version was created. The unreliable markers were removed which were actually non- collinear using mapinspect software (Van Berloo, 1999). All the 21 chromosomes were manually studied in mapinspect software. Only small versions were modified by the specific feature of Biomercator version 2.0.

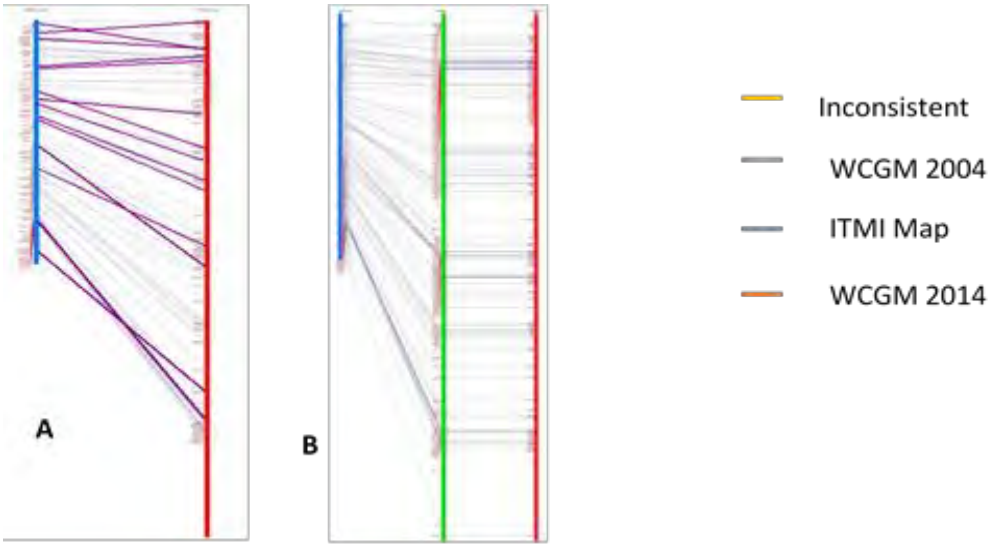


FIGURE 9: CONSTRUCTION OF WCGM2014 CHROMOSOME 5B:A. COMPARISON OF ITMI AND WHEAT COMPOSITE B. COMPREHENSIVE MAP OF CHROMOSOME 5

2.3 RESULTS

The incorporation of all the maps on ITMI as reference map from the co-dominant markers and markers from WCGM 2019 to 90k array markers produced a comprehensive, polymorphic and with the deletion bin mapping is an efficient approach for the development of a cytogenetically physical map of wheat chromosomes. On these deletion bins, Expressed Sequence Tags have been recognized by an American program (NSF, Qi et al 2004).

The new map was created having 140316 markers on distance of 4828.3 cM. The highest number of markers have been originated on chromosome 2B with 9368 markers, on homologous group 2 with 23313 and on B-genome with 55526 markers, while the lowest number were located on chromosome 4D with 3306 markers, and on homologous group 4 with 14175 markers and on D-sub genome with 38058 markers. The genetic length for genome A was 1684.13 cM, for genome B was 1476.74 cM and for genome D was 1667.41 cM. The longest genetic distance was found for chromosome 7A which was 334.85 cM and lowest distance was found for chromosome 4D which was 141.82 cM. The longest genetic distance was found for homologous group 7 which was 876.5 cM and lowest distance of 499.25 was found for homologous group 4. The high number of markers is shown as WCGM 2019 in the graph:

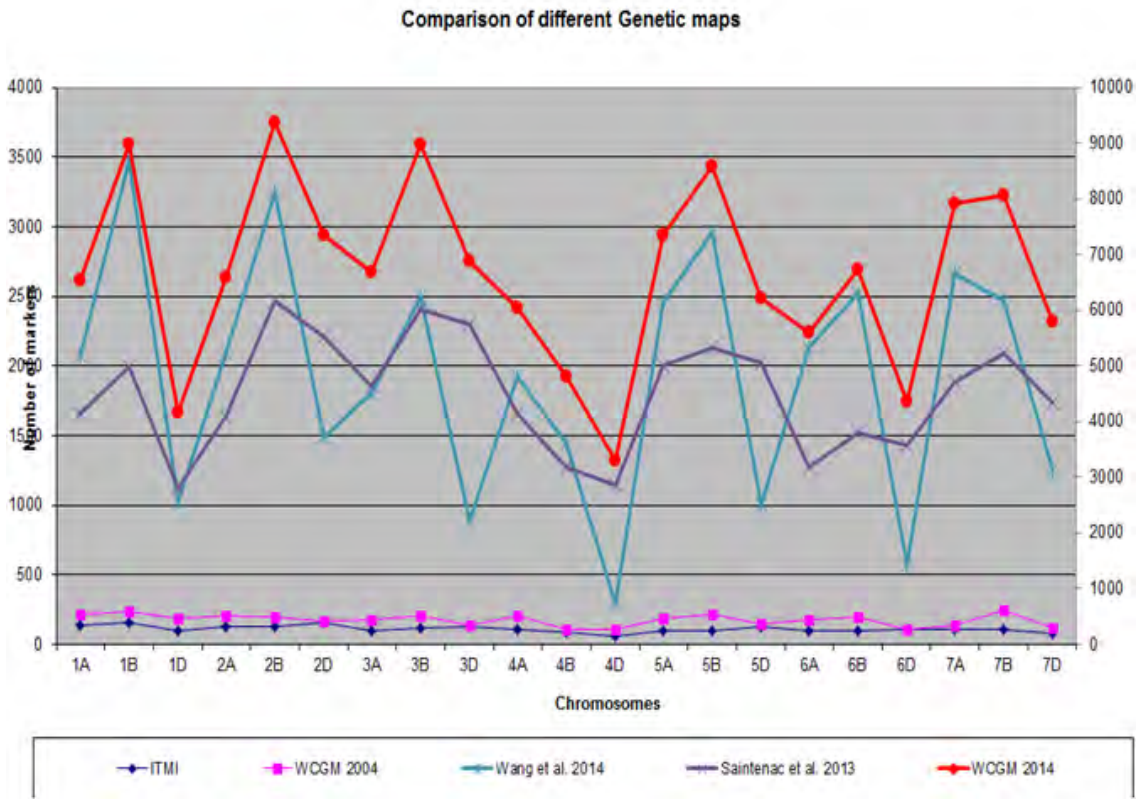


FIGURE 10: COMPARISON OF THE SIZES OF DIFFERENT GENETIC MAP (ITMI, WCGM 2004, SOMER'S, SAINTENAC ET AL. 2013, WANGET AL. 2014, AND WCGM 2014)

The chromosomes having marker density of 0.036 cM is significant. The highly dense markers were found to 7A with 0.042 cM, 5D with 0.045 cM and 6D with 0.055 cM. Chromosomes with lowest coverage of marker density were 1B with 0.021 cM and 2B with 0.019 cM. Homologous group 7 shared a maximum genetic distance of 0.121 cM and homologous group 2 shared the least genetic distance with 0.08 cM. Markers density of B-genome was found to 0.19 cM and that of D-genome was 0.323 cM. The marker density was found homogenous among homologous groups. As the marker order and genetic length were conserved between the WCGM 2009 and ITMI maps, we projected the deletion bins information available from the ITMI genetic map provided by Sourdille et al., (2004) (available at http://wheat.pw.usda.gov/ggpages /SSRclub/Genetic Physical/), onto the WCGM2014 map. Moreover, the important information in the WCGM 2014 map was the information regarding marker's sequence that facilitate the users in analysis, mapping and identification of wheat genome. Total of 132638

Sequences are supplemented to WCGM 2019 map that had been gathered from all the data available. The sequence information of A-genome was 44006, B-genome was 52314 and D-genome was 36318. High number of was shared by homologous group 2 with 22086 sequences and least number was shared by homologous group 4 with 13375 sequences.

2.4 WHAET DISEASES AND PATHOGENS RESISTNCE

There are several biotic factors that cause wheat diseases such as fungi, bacteria, viruses, birds, insects and mammals etc. Favorable conditions, host susceptibility and pathogen results in wheat diseases. Cropping system have been modified due to adaptation of agricultural conservation and have been employed in various ways. But genetic resistance along with crop rotation, timely sowing, proper irrigation and use of fungicide can reduce the crop losses. Some of the important diseases are given

2.4.1 Stripe rust

The Fungus *Puccinia striformis*is the causal agent of this disease. It is also known as yellow rust because of its color of urediniospores. Almost 50 resistant genes have been identified on different chromosomal locations (McIntosh *et* al. 1998).

2.4.2 Leaf rust

The Fungus *Puccinia triticina* is the causal pathogen of this disease. It is also known as Brown rust because of its spores color. It is one of destructive disease of wheat across the world (Roelfs 1992).

2.4.3 Stem rust

The fungus Puccinia graminis is the causal pathogen of stem rust. It is also called as Black rust due to its spores color. It is most devastating disease of wheat. More than 60 resistant genes have been identified against this disease (Peterson 2001).

2.4.4 Fusarium Head Blight

Fusarium species like *Fusarium graminearum, Fusarium culmorum*and *Fusarium avena* are most important species that cause the disease. Fusarium fungus

Produces mycotoxin which is deoxynivalenol (DON). More than 24 resistant genes have been identified against that disease (Parry, Jenkinson *et* al. 1995)

2.4.5 Septoria tritici blotch

*Mycosphaerella graminicola*is the main pathogen of this disease. This disease has become a major disease across the world. More than 15 resistant genes have been identified on different chromosomal locations (Eyal, Scharen *et* al. 1985)

2.4.6 Powdery mildew

This disease is caused a fungus named *Blumeria graminis.* Almost 38 resistant genes have been identified against this disease in bread wheat (Miranda, Murphy *et* al. 2006)

2.4.7 Stagnospora nodorum blotch

It is fungal disease caused by *Phaeosphaeria nodorum.* The disease has the ability of 50% loss in wheat production (King, Cook *et* al. 1983).

2.4.8 Tan spot

Pyrenophora trirtici repentis is the main causal agent of tan spot of wheat. More than 15 resistant genes have been identified. The best way to control this disease is the cultivation of resistant varieties of wheat (Lamari and Bernier 1989).

2.4.9 Eyespot

Tapesia yallundae is the causal agent of eyespot. It is the major disease of winter wheat. More than 4 resistant genes have been identified.

2.4.10 Cereal yellow dwarf

It is a major limiting factor of wheat across the world. It is caused by various viruses that belongs to family Luteovirus. Aphids are the carrier of these virus. More than 3 resistant genes have been identified (Singh, Burnett *et* al. 1993)

2.4.11 Kernal bunt

This disease is caused by a fungus known as *TilletiaindicaMitra.*12 resistant genes have been identified (Mitra 1931).

2.4.12 Loose smut

This is a fungal disease caused by *Ustilago tritici.* More than 6 resistant genes have been identified against this disease.

2.4.13 Wheat curl mite

Wheat streak mosaic virus is the main source of this disease. This disease is caused losses to wheat mainly in North America, Europe and Asia (Cooper 1993).

2.4.14 Root knot

It is another disease of wheat caused by pathogen belongs to the genus *Meloidogyne*. This disease also losses in many other crops (Sasser 1980).

2.4.15 Hessian fly

It is destructive pest of wheat, also called as *Mayetiola destructor*. This majorly found in various parts of North America, Europe, North Africa and Asia. More than 30 resistant bgenes have been identified for this disease (Delibes, Del Moral *et* al. 1997).

2.4.16 Cereal root eelworm

Heterodera avenae is an important parasite on wheat. In barley first resistant was found against cereal root eelworm (Nilsson-Ehle 1920).

2.4.17 Diuraphis noxia

It is also called as Russian wheat aphid. It is also one of the major wheat pest. The pest was first recognized in US in 1986 (Webster, Starks *et* al. 1987).

2.4.18 Green bug

Green bug caused by *Schizaphis graminum* is known as one of the destructive disease across the world. Seven resistant genes have been found for resistant against green bug (Rohrich, Kenkel *et* al. 1999).

2.5 Disease Resistance

In powdery mildew of wheat, 18 genes have been mapped out of total 51 resistance genes on the consensus map WCGM2019. In Cereal dwarf only 1 gene have been mapped out of 3 resistance genes. In *Diuraphis noxia*, 7 genes have been mapped out of total 12 genes. In Cereal root eelworm, 2 genes have been mapped out of 11 genes. In Hessian fly, 8 genes have been mapped out of 34 genes. In Root knot nematode, no one gene have been mapped out 2 resistance genes. In *Septoria tritici* blotch 10 genes have been mapped out 15 resistance genes. In *Stagnospora nodorum blotch* only 2 gene is mapped out of 9 genes. In Stem rust 25 genes have been mapped out of 53 genes. In leaf rust, 32 genes have been mapped out of 57 genes. In Yellow rust, 18 genes have been mapped out 50 genes. In Tan spot disease, 9 genes have been mapped out 15 genes. In Green bug, 11 genes have been mapped out 15 resistance genes. In Kernal bunt disease, no one gene have been mapped out of total 12 genes. In Loose smut, 1 gene is mapped out of total 6 genes. In Wheat curl mite, only one genes have been mapped out of total 4 genes. In *Eriophyes tulipae* no one gene is mapped out of 1 resistance gene. In Fusarium head blight, only one gene is mapped out of 24 resistance genes. I Bacterial leaf strike, no gene is mapped out of 5 genes. Out of 394 resistance genes, 147 resistance genes have been projected and mapped on the consensus map WCGM 2019. Information is given about each disease and pathogen in the table 1.

TABLE 1: Wheat disease, Pathogen symbols and loci number

S.No	Diseases	Pathogens	Resistance genes	Mapped resistance genes
1	Leaf rust	*Puccina triticina*	57	32
2	Stem rust	*Puccinia graminis*	53	25
3	Powdery mildew	*Blumeria graminis*	51	18
4	Yellow rust	*Puccinia striformis*	50	18
5	Fusarium head blight	*Fusariumssp*	24	1
6	Septoriatritici blotch	*Mycosphaerella graminicola*	15	10
7	Tanspot	*Phyrenophora tritici*	15	9
8	Septorianodorum blotch	*Phaeosphaeia nodorum*	9	2
9	Bunt dwarf	*Tilletia*	10	0
10	Loose smut	*Ustilogotritici*	6	1
11	Bacterial leaf treak	*Xanthomonose campestris*	5	0
12	Eyespot	*Pseudocercosporella*	4	1
13	Cereal yellow dwarf	*Monogemini virus*	3	1
14	Wheat curlmite	*Eriophyestulipae*	4	1
15	Cereal root eelworm	*Heteroderaavena*	11	2
16	Rissain wheat aphid	*Diuraphisnoxia*	12	7
17	Green bug	*Schizaphisaraminum*	15	11
18	Heassian fly	*Myetiola destructor*	34	8
19	Keranl bunt	*Telletia indica*	12	0

20	Root knot nematode	*Meloodogyne spp*	2	0
21	Seedling leaf chlorosis	*Pseudo black chaff*	1	0
22	Wheat streak mosaic	*Eriophyes tulipae*	1	0
	Total		**394**	**147**

TABLE 2. PUTATIVE MAPPED R-GENES

Disease	Gene	Publications	Chromosome	Position on Ref genetic	SSR Markers	RFLPs Markers	Darts Markers
Tanspot	Toxe	R.J. Effertz *et al.* 2002	1A	24.9	Xgpw2186 (23.1)	Xfba165 (23.1)	Wpt-7872 (24.5)
					Xgpw7052 (25.6)	Xcdo431 (28.4)	Wpt-11723 (25.4)
Powdery mildew	Pm3g	Christina Neu *et al.* 2002	1A	26.1	Xgwm136 (26)	Xfba165 (23.1)	Wpt-4886 (25.5)
					XgwcES (28)	Xcdo431 (28.4)	Wpt-1167 (32.6)
Hessian fly	Hdie	Tao Wang *et al.* 2006	1A	34.6	Xgpw2246 (31.2)	XKsuD14_d2 (29.3)	Wpt-6290 (32.8)
					Xgpw2276 (34.6)	Xcdo426 (41.5)	Wpt-6358 (35.3)
Powdery mildew	Pm17	Yehia Mater *et al.* 2004	1A	45.2	Xgpw4071 (43.5)	Xfbb160 (44.2)	Wpt-4666 (42.9)
					Xgpw2032 (45.4)	Xfba285 (48.2)	Wpt-4735 (45.8)
Hessian fly	H9	L. Kong *et al.* 2005	1A	48.9	Xbarc263 (48.2)	Xfba286 (48.2)	Wpt-2150 (48.3)
					Xwmc33 (51.2)	XKsuE18 (52.2)	Wpt-6122 (50.2)
Green bug	Gb2	Yehia Mater *et al.* 2004	1A	53.4	Xwmc329 (53.1)	XKsuE18 (52.2)	Wpt-3560 (53.1)
					Xcfa2226 (55.5)	XKsuE19 (55.6)	Wpt-0164 (53.8)
Stagonospora nodorum blotch	Snn1	Z. H. Liu *et al.* 2004	1B	28.3	Xgpw13 (25.4)	XKsuD14 (28.3)	Wpt-4200 (27.2)
					Xefd15 (31.9)	Xbcd1434 (31.2)	Wpt-1568 (29.6)
Stem rust	Sr24-50	RF Park *et al.*2002	1B	58.3	Xefd20 (58.1)	Xbcd1124 (58.1)	Wpt-5678 (58.1)
					Xefd70 (58.6)	Xcdo1188 (59.7)	Wpt-3266 (58.7)
Yellwo rust	Yr26	Chunmei Wang *et al.* 2007	1B	67.1	Xgdm28 (67)	Xfba28 (66.9)	Wpt-3566 (65.2)
					Xwmc611 (67.3)	Xcdo127 (67.1)	Wpt-1374 (70)

Yellwo rust	YrCH42	G. Q. Li *et al*. 2006	1B	69.1	Xgwm498 (69)	Xbcd (69)	Wpt-3566 (65.2)
					Xgwm134 (69.2)	Xksu951 (69.2)	Wpt-1374 (70)
Diuraphis anoxia	Dn2414	Junhua Peng *et al*. 2007	1B	69.2	Xgwm789 (69.2)	Xbcd (69)	Wpt-3566 (65.2)
					Xbarc148 (69.2)	Xksu951 (69.2)	Wpt-1374 (70)
Leaf rust	Lr40	Li Huang et al. 2003	1D	45.1	Xgdm33 (45)	Xfba199 (45.1)	
					Xbarc149 (47)	XksuD14_d1 (45.5)	Wpt-9181 (46.7)
Leaf rust	Lr21	Li Huang *et al*. 2003	1D	45.5	Xgdm33 (45)	Xksu937 (46)	
					Xgpw2067 (46.6)	Xfbb250 (46)	Wpt-9181 (46.7)
Hessian fly	H22	Tao Wang et al. 2006	1D	49.2	Xwme147 (47.1)	XksuD14_d3 (47.3)	Wpt-3738 (47.1)
					Xwme85 (51.4)	Xfbb260 (52.1)	Wpt-1387 (67.5)
Stem rust	Sr33	W. Spielmeyer et al. 2000	1D	61.5	Xefd15 (57.7)	Xfba329 (52.1)	Wpt-3738 (47.1)
					Xbarc 152 (65)	Xfbb160 (63)	Wpt-1387 (67.5)
Diuraphis anoxia	Dn4	A. Arzani et al. 2004	1D	76	Xgpw5253 (75.1)	Xksug9 (73.7)	Wpt-8960 (69.9)
					Xgwm848 (82.4)	Xfbb237 (79.1)	Wpt-7953 (78)
Powdery mildew	Pm24	X.Q. Huang et al. 1999	1D	90.4			
					Xwme 169 (92.1)	Xfbb377 (91.6)	Wpt-8854 (111.9)
Diuraphis anoxia	Din9	X.M. Liu et al. 2000	1D	13.1	Xgwm642 (131.1)	XksuG2 (130.1)	Wpt-3743 (123)
					Xfbb 12(134.5)	Xfbb12 (138.2)	Wpt-7980 (134.8)
Leaf rust	Lr17	Julia X. Zhang et al. 2008	2A	88.8	Xgpw5177 (86.7)	Xcdo456_d1 (70.2)	Wpt-8328 (50.4)
					Xgdm5 (89.3)	Xcdo456_d2 (90.7)	Wpt-3611 (93.2)
Leaf rust	Lr38	B Friebe et al. 1996	2A	88.8	Xgpw5177 (86.7)	Xcdo456_d1 (70.2)	Wpt-8328 (50.4)
					Xgdm5 (89.3)	Xcdo456_d2 (90.7)	Wpt-3611 (93.2)
Yellow rust	Yr17	Alena Hanzal Ova et al. 2007	2A	88.8	Xgpw5177 (86.7)	Xcdo456_d1 (70.2)	Wpt-8328 (50.4)
					Xgdm5 (89.3)	Xcdo456_d2 (90.7)	Wpt-3611 (93.2)
Stem rust	Sr38	M. Helgueral et al. 2003	2A	88.8	Xgpw5177 (86.7)	Xcdo456_d1 (70.2)	Wpt-8328 (50.4)
Leaf rust	Lr37	M. Helgueral et al. 2003	2A	108	Xgpw512 (116.4)	XksuD18 (107.5)	Wpt-0003 (105.8)
					Xgwm2127 (110.3)	Xfba3 (108.8)	Wpt-7285 (116.7)
Yellow rust	Yr17	Alena Hanzal Ova et al. 2007	2A	109.4	Xgdm005 (109.1)	Xfba3 (108.8)	Wpt-0003 (105.8)

					Xgpw359 (110.1)	Xfba8 (112.4)	Wpt-7285 (116.7)
Stem rust	SrAN1	U. K. Bansal et al. 2009	2A	112.4	Xgwm2127 (110.3)	Xfba8 (112.4)	Wpt-0005 (105.8)
					Xgpw71_d1 (115)	Xfba178 (116.7)	Wpt-7285 (116.7)
Yellow rust	Yr32	M. J. Christiansen et al. 2006	2A	174.3	Xgwm47_d2 (172.2)	XksuE16 (172.5)	Wpt-7056 (173.1)
					Xwmc35 (178.3)	Xbcd1095 (175.1)	Wpt-7024 (177.7)
Powdery mildew	Pm4	L. W Briggal 1996	2A	177.2	Xgwm47_d2 (172.2)	Xbcd1970_d2 (175.1)	Wpt-7056 (173.1)
					XwmcD6 (181.6)	Xbcd1086 (186.3)	Wpt-7024 (177.7)
Leaf rust	Lr16	C.A. McCarteny et al. 2006	2B	8.1	Xwmc764 (7)		
					Xwmc382 (8.3)	Xfbb274 (9)	Wpt-5587 (8.4)
Tanspot	Tsr6	P. K Sing et al. 2009	2B	13.1	Xwmc243 (13)	Xfba280 (12.4)	Wpt-4527 (12.5)
					Xwmc357 (15.4)	Xcfd11 (24.3)	Wpt-8404 (16.2)
Leaf rust	Lr48	U. K. Bansal et al. 2009	2B	46.7	Xgwm429 (46.7)	Xbed18 (39.6)	Wpt-647 (45.9)
					Xwmc442 (48.2)	Xfba29_d2 (48.2)	Wpt-1920 (47.8)
Stem rust	Sr40	B Friebi et al.1996	2B	55.8	Xgwm148 (54.3)	Xfba106 (55)	Wpt-2120 (53.2)
					Xwmc35 (57.1)	Xfbb353 (56.3)	Wpt-6477 (56)
Leaf rust	Lr35	R. Seyfarth et al.1999	2B	69.9	Xgpw3215 (69.6)	Xbcd260 (69.9)	Wpt-2110 (68.7)
					Xbarc128 (70)	XksuD22 (70)	Wpt-6278 (76.4)
Leaf rust	Lr13	U. K. Bansal et al. 2009	2B	71.5	Xgwm129 (71.4)	Xfbb226 (70.7)	Wpt-2110 (68.7)
					Xgpw5062 (72.5)	Xfbb21 (72.4)	Wpt-6278 (76.4)
Stem rust	Sr36	Tori J. Tslio et al. 2008	2B	85.5	Xwmc477 (85.5)	Xcdo684 (84.1)	Wpt-5502 (85.4)
					Xwmc360 (86.3)	Xksuf11 (85.8)	Wpt-1294 (85.6)
Stem rust	Srweb		2B	95.9	Xgpw4165 (95.5)	Xfbb284_d1 (95.6)	Wpt-1650 (91.6)
					Xwmc332 (104.1)	Xfba345 (99.9)	Wpt-0047 (99.5)
Yello rust	Yr5	P. H. SmithSTS et al.2007	2B	96	Xgpw4165 (95.5)	Xfbb284_d1 (95.6)	Wpt-1650 (91.6)
					Xgpw4103 (98.7)	Xfba61 (97.8)	Wpt-3632 (108.8)
Powder mildew	Pm6	W. Tao et al. 2000	2B	116.8	Xgpw4043 (114.4)	Xfba16 (110.2)	Wpt-3632 (108.8)
					Xgpw7506 (137.4)	Xfbb171 (127.8)	Wpt-2135 (145.1)

Cereal root eelworm	Cre1	John de Majnik et al. 2003	2B	170.2	Xgdm93 (168.4)	Xbcd1231 (170.2)	Wpt-7360 (145.1)
					Xwmc149 (176.6)	Xfbb113_d3 (185)	Wpt-4917 (181.7)
Steam rust	Sr6	Tori J. Tslio et al. 2008	2D	6.2	Xgwm358 (0)	Xfbb89 (3.2)	
					Xcfd43 (7.7)	Xfbb274 (15.4)	Wpt-8004 (45.6)
Leaf rust	Lr39		2D	50	Xgpw294 (49.8)	Xcdo456_d1 (47.7)	Wpt-9070 (45.7)
					Xgpw5155 (53.6)	Xfba83_d1 (50.8)	Wpt-6179 (150.4)
Cereal root eelworm	Cre3	John de Majnik et al. 2003	2D	193.5	Xbarc159 (191.4)	XksuH16 (191.4)	Wpt-2544 (183)
					Xcfd239 (206.2)	Xfba116_d2 (194.6)	
Yellow rust	YrRub	U.K Bansal et al., 2009	3B	0.7		Xbarc907(0)	
					Xgwm389(1.7)	Xfbb147-d18.6	Wpt-0325(9.6)
Stem rust	Sr2	Jagdeep Kaur et al., 2009	3B	6.3	Xgwm389(1.7)	Xfba311(0)	
					Xgwm533 di(8.9)	Xfbb147-d1(8.6)	Wpt-0325(9.6)
Septoria tritici blotch	Stb2	Tika B.Adhikar et al., 2004	3B	15.4	Xbarc147(12.10	Xcfb6059(14.7)	Wpt-4739(64.9)
					Xgwm493(19.7)	Xcfa2226(15.8)	Wpt-2720(17.7)
Powdery mildew	Pml3	A.Cenci et al., 1999	3B	24.7	Xgwm161(22.3)	Xcdo583-d2(24.7)	Wpt-1741(20.2)
					Xgwm32489(26.6)	Xfbb185(26.4)	Wpt-0267(25.7)
Tan spot	tsn2	P.K. Singh et al., 2006	3B	99.9	Xgwm802(94.7)	Xbcd147(99.8)	Wpt-0280(99.3)
					Xbarc8849(103.4)	Xfbb378(105.1)	Wpt-8412(101.1)
Tan spot	Tsn5	P.K.singh et al., 2007	3B	115.7	Xwgm134(112.7)	Xbbb147-d2(114.8)	Wpt-9161(108.9)
					Xgpw5016(117.7)	Xcfa2170(119)	Wpt-4364(117.4)
Tan spot	Tsn3b	W.Tadesse et al., 2007	3D	57.7	Xpgw4047(55.1)	Xfba241(56.6)	Wpt-4220(20)
					Xbarc52(59.6)	Xbcd1532(58.2)	
Tan spot	Tsn3c	W.Tadesse et al., 2007	3D	69.4	Xwmc443968.3)	XksuA6-d2(67.2)	Wpt-4220(20)
					Xwmc375(69.4)	Xfba213(76.8)	
Hesssian fly	H26	Tao wang et al., 2006	3D	122.7	Xgwm4(121.29)	Xcfd9(118.1)	Wpt-4220(20)
					Xgwm3(128.6)	XksuE14(123.6)	
Hessian fly	H32	N.sardesai et al., 2005	3D	128.9	Xbarc270(128.8)	Xfbb316(128)	Wpt-4220(20)
					Xcfd223(129.2)	Xfbb269(129.2)	
Hessian fly	H24	Tao Wang et al., 2006	3D	140.6	Xmwg11(139.4)	Xcdo482(139.4)	Wpt-4220(20)
						Xbcd451(141.7)	
Leaf rust	Lr49	U.K. Bansal et al., 2008	4B	107.8	Xgwmd1(106.3)	Xbcd446-d1(104.3)	Xbcd446-d1(104.3)
					Xgpw4175(108.5)	Xfbb7-d1(112.5)	Xfbb7-d1(112.5)
					Xgwm234(63.1)	Xbcd873-d1(62.1)	Xbcd873-d1(62.1)
Septoria tritici blotch	Stb1	T.B.Adhikari et al.,2004	5B	91.4	Xgpw4460(91.3)	Xcfa2070(89.6)	Xcfa2070(89.6)
					Xgpw110299(91.5)	XksuA1(91.9)	XjsuA1(91.9)
Powdery mildew	Pml34		5D	216	Xbarc177(205)	Xbcd1670(205)	Xbcd1670(205)

					Xwmc161(216.1)	Xbcd1421(221.3)	Xbcd1421(221.3)
Stem rust	Sr13	Daryl L. Klindowrth et al.,	6A	133.9	Xgwm1699(133.7)	Xcfa2114(133.7)	Xcfa2114(133.7)
					Xgwm617(140.3)	XksuD12(139.9)	XksuD12(139.9)
Fusarium head fly	Fhb2	Patricia A. Cuthbert et al	6B	71.8	Xgpw5212(71.8)	Xfba357(71.4)	Xfba357(71.4)
					Xgwm191(72)	Xcfd13(72)	Xcfd13(72)
Powdery mildew	Pml2	Wei Song et al., 2007	6B	77.2	Xwmc388(74.4)	XksuH14-d1(76.4)	XksuH14-d1(76.4)
					Xwmc127(77.2)	Xfba7-d1(78)	Xfba67-d1(78)
Wheat curlmite	Cmc4	Malik et al., 2002	7A	36.9	Xgdm141(37.3)	XksuG48(36)	Wpt-1695(18.6)
					Xgpw3087(40.7)	Xfba187(37.8)	Wpt-7394(43.2)
Hessian fly	H13	Tao Wang et	7A	45.5	Xcfd132(45.5)	Xcfd132(45.5)	Wpt-7394(43.2)
					Xmwg916(61.8)	Xmwg916(61.8)	
Stem rust	Sr22	R.R. Khan et al., 2005	7A	168.5	Xwmc83-d2(166.4)	Xwmc83-d2(166.4)	Wpt-4553(166.6)
					Xwmc405(169)	Xwmc405(169)	Wpt-1128(173.5)

2.6 Discussion

In the above study, an integrated reference genetic map comprising of 140316 markers and 132638 sequences was constructed. The efficacy of map permits the gene mapping for trait of interest i.e. agronomic and resistance traits in bread wheat as well as provide the facility for contributing toward physical and genetic mapping. The present constructed map is an amazing source for evaluating the wheat genome comprising of maximum types of markers i.e. DArT's, SNP's, SSR's, RFLP, and GBS. Consensus map play a significant role in marker assisted selection as well as in plant breeding. Because it provides a rich source of markers with their length of chromosomes for recombination detection and fixing loci genetic background for desired crosses and can also be used for genome scanning and mapping approaches (Michelmore et al. 1991; McCartney et al. 2003) (Somers et al. 2003a). Markers such as SSr's and SNP's decreases the muddle of allele scoring from paralogous and homologous loci which is actually a major disquiet of wheat species. With the assistance of SNP based markers, the position to the physical map and its employment in haplotypes studies is aided for wheat. Information regarding deletion bins to which markers are labelled, creates an opportunity for gene cloning of agronomic interest.

Consensus map can also be used for various purposes including meta-analysis, functional validation and trait dissection. Though, the identification of candidate genes is limited by the inadequate information regarding physiology and permit co-segregation. Therefore, to restrict such unauthentic identification, large population is required (Leister et al. 1996; Pflieger et al. 1999). For QTL and meta-analysis purpose, high and dense genetic map is required in order to validate and carrying out of positional cloning of candidate gene. The comprehensive genetic map also plays an important role in the improvement of wheat genomics for all the analysis tools. Because identification and validation of candidate genes requires common markers as much as possible. The available information regarding marker's sequences can contribute in new markers development. Integration of all these information may lead to do the genomic selection of a species or germplasm without to construct any prier molecular tools as well as conservation biologist may fix a population structure without having knowledge about genome diversity within species. The new approaches for applying GBS and SNP's to

breeding, conservation and global species and population are now balanced to become a crucial component of future biology.

2.6.1 Map Based Putative Genes Projection

Overall, 258 were mapped, including 147 disease resistance genes and 111 genes related to agronomic traits. The comprehensive genetic map consists of 25 resistance genes of stem rust, 18 resistance genes of yellow rust and 32 resistance genes of leaf rust. The homologous group 2 contributed 9 resistance genes in stem rust, while group 4 has no contribution. Homologous group 7 has most influence regarding yellow rust while group 4 has no genes for resistance. Maximum resistance genes in leaf rust have been found on homologous group 2, whereas homologous group 3 and 4 has minimum share with 1 and 1 resistance genes on both groups.

In total, the resistance genes shared by A- genome, B- genome and D- genome are 32, 67 and 48 respectively. The resistance genes owned by homologous group 1, 2, 3, 4, 5, 6 and 7 are 30, 37, 17, 5. 13. 12 and 33 respectively. Other diseases such as Cereal yellow dwarf, Wheat curlmite, Cereal root eelworm, *Diuraphis noxia*, Fusarium head blight, Green bug, Hessian fly, leaf rust, Eye spot, powdery mildew, *Septoria nodorum blotch*, Stem rust, *Septoria tritici blotch*, *Tanspot,* Loose smut and Yellow rust possess resistance genes are 1,1, 2, 7, 1, 11, 8, 32, 1, 18, 2, 25, 10, 9, 1 and 18 respectively.

Mapping of resistance genes on consensus mapping allow the differential expression of these resistance genes during disease arrival. The identification of candidate genes can be used in order to coincide the QTL analysis and cloning of genes involved in plant defense system. Identification of candidate gene plays a crucial role in QTL analysis because regions controlled by polygenes have been identified (Yu et al., 1991). There are two types of plant disease resistance i.e. Resistance genes and Defense response genes. Resistance genes play a key role in defense strategy and being cloned (Bent 1996).

The stripe rust resistance genes Yr26, YrCH42, Yr17, Yr32, Yr5 and YrRub had been mapped on the consensus map. Stripe rust is increasingly spreading in regions from Syria, Turkey to Uzbekistan (Abdullah et al., 2010). Using resistance genes is the robust way of creating resistant varieties in wheat production.

3. STRIPE RUST DISEASE IN BREAD WHEAT

3.1 Introduction

Fungi are the major foliar pathogens of wheat, particularly rust (*Puccinia sp*,) *Fusarium graminearum, Blumeria graminis, Zymoseptoria tritici and Stagnospora nodorum* (Shiferaw, Smale et al. 2013). Rust is considered one of the major threat to food security with an estimated loss of $UDA 1billion per annum (Beddow, Pardey et al. 2015).Rust pathogens are capable of producing millions of asexual urediniospores in a single infection. The huge production of uridiniospores causes mutation in the pathogens which make them to overcome the resistant crop (Wellings 2007; Ali, Gladieux et al. 2014).Asexual uridiniospores has the capability to travel long distances; a specific stripe rust isolate travelled 24 Km distance in a period of six months in North America (Chen 2014).The recent strategies to overcome fungal diseases include deployment of host resistant genes, fungicides and agriculture practices, but these are not fully effective and infection remain substantial (Ali, Gladieux et al. 2014; Beddow, Pardey et al. 2015).

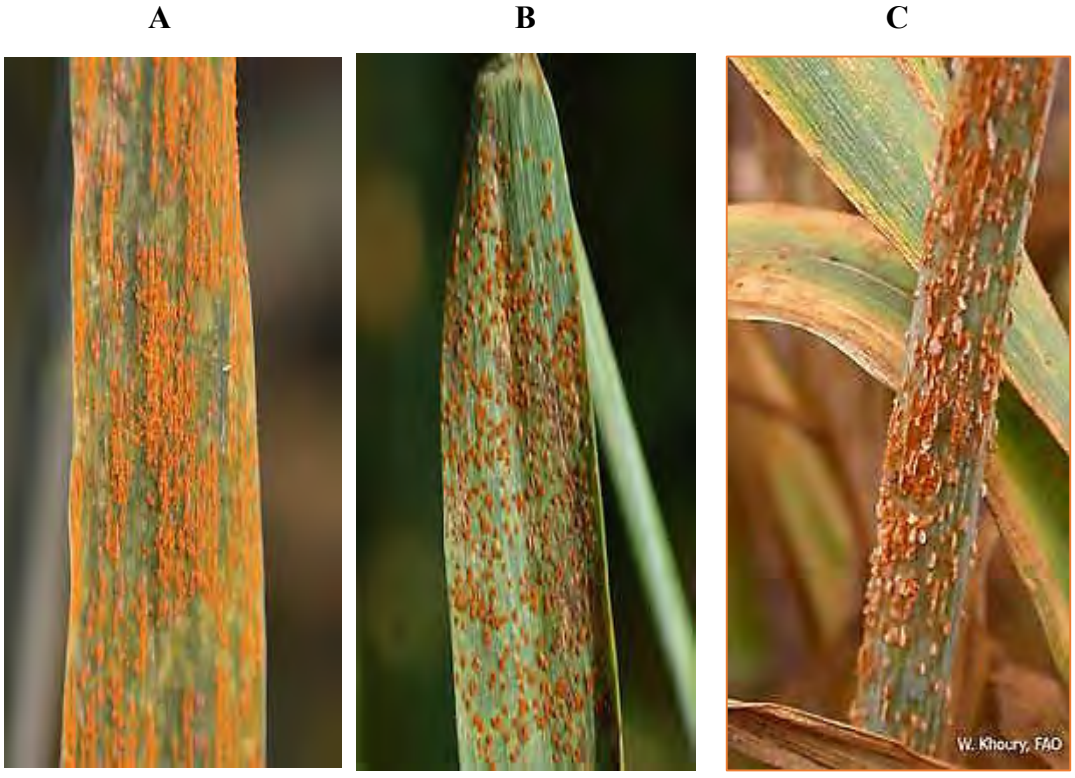


FIGURE 11: THE THREE WHEAT RUSTS. A) *P. STRIFORMIS* PUSTULES ON FLAG LEAF OF WHE

3.2. Types of pathogen

3.2.1 Stem rust

Stem rust or black rust caused by *P. graminisf.sp. Tritici.* Temperature ranges from 15°C to 35°C along with humid and warm conditions play an important role in developing and spreading of spores. Stem rust can cause 100% destruction of the susceptible crop into dead mass of damaged stem and shriveled grains (Sanders 2011).

3.2.2 Leaf rust

Leaf rust or brown rust is caused by *P. triticina*. Temperature ranges from 10°C to 30°C is favorable for spores development and spreading. Leaf rust can cause up to 30% destruction of the susceptible crop (Sanders 2011).

3.2.3 Stripe Rust

Stripe rust also known as yellow rust of wheat caused by *P. striformis*. It can also infect more than 50 grass species such as barley and rye. Favorable conditions include 10°C to 18°C, and 100% humidity (Line, 2002). It has the ability of 100% destruction if cultivars are susceptible (Begum, Iqbal et al. 2014).

3.3 Stripe Rust

Stripe rust also known as yellow rust of wheat is caused by an obligate biotrophic fungus *Puccinia Striformis f.sp. Tritici.* The pathogen can also infect other grass species such as barley and rye (Line 2002). The threat of the pathogen to agriculture is due to its genetic diversity. The pathogens can move across continents and rapid adaptation through evolution, overcoming a single gene at a time (Hovmøller, Sørensen et al. 2011)**.** The pathogen has the capability to loss 100% of crop if prone cultivars are infected. Losses of the prone cultivars depends upon the favorable environmental conditions, advent of new races of pathogen, vulnerability of a cultivar, degree of disease progress and length of disease (Begum, Iqbal et al. 2014)**.** More than 70 Yellow rust resistant gens have been identified till now by the constant struggle of plant breeders and plant pathologist in the last 100 years (McIntosh, Dubcovsky et al. 2013**).** Historically, pathologist tried to isolate Pst from the infected field to determine

the capability of these isolates to infect a specific set of wheat lines carrying Yellow rust resistant genes. Infected phenotype determine the virulence pattern and Pst nomenclature , enable to compare between spatial and temporal distinct collection events (Chen, Wellings et al. 2014). Modern DNA base techniques strengthened our ability to focus on the fungus. These techniques have led to the cloning of yellow rust resistant genes, identification of full life cycle of Pst, genetic diversity and global migration pattern (Hovmøller, Sørensen et al. 2011; Wellings 2011; Saunders 2015).

3.3.1 Back ground of stripe rust

Stripe rust exist before humans began to grow wheat as a crop. The first report on the disease was published in 1777 by Gadd in Europe.in 1794, the disease spread on rye in Sweden (Curtis, Rajaram et al. 2002)**.** Epidemics of stripe rust with massive yield loss make it as a famous disease across the world (Roelfs 1992)**.** The presence of stripe rust is reported in more than 60 countries (Stubbs 1985; Curtis, Rajaram et al. 2002)**.** More than 46% losses occurred in Asia due to stripe rust (Singh, William et al. 2004)**.** It is therefore important to cultivate resistant varieties which is the most effective and environmental friendly way to control the disease (Line and Chen 1995**;** Zhang, McIntosh et al. 2009).

3.3.2 Stripe rust in the world

Stripe rust is found in all wheat producing regions except Antarctica. Stripe rust is the major yield constraint in United States and Canada. In south America, periodic losses is caused by stripe rust in Chile (Germán, Barcellos et al. 2007)**.** In Europe, stripe rust is the major wheat constraint to wheat production in France, Germany, Netherland and United Kingdom. Western Asia and North Africa have faced three epidemics of stripe rust since 1970s. In Pakistan, India and china, stripe rust is a major and serious disease of wheat (Solh, Nazari et al. 2012).Stripe rust was first reported in Australia in 1979 (O'Brien, Brown et al. 1980) and then was introduced to New Zealand in 1980 (Wellings and McIntosh 1990; Viljanen-Rollinson and Cromey 2002).

3.3.3 Stripe rust in Pakistan

Stipe rust is the main yield constraint than stem rust and leaf rust in Asia. The pathogen has the potential of 60% (43m ha) destruction of cultivated land if susceptible

varieties are grown. Countries like Pakistan, India and China contribute 139.3 m ha for wheat production of which 17% (24.8%) is prone to stripe rust of wheat. In Pakistan, more than 70% of the cultivated land is prone to stripe rust (Singh et al. 2004). Most of the high yield and resistant varieties are reported prone to stripe rust of wheat. New strains of stripe rust have been reported against resistant genes. This dreadful conditions needs regular monitoring of the pathogen virulence and to build breeding programs in order to ensure the food security in the country.

3.3.4 Losses

Stripe rust is the major yield limiting factor. It severely affect the quality of grain. Seeds affected with stripe rust have low vigor and poor emergence after germination, reduced growth, dry matter production, plant height, size and even number of seeds in the spike. Losses due to stripe rust have been estimated from 10% to 70% across the world (Chen 2005; Hakro and Khan 2012). In Pakistan, an average losses due to stripe rust have been estimated at Rs. 1500 million per annum (Hakro and Khan 2012). Yield losses of 10.1% (US$86 million) have occurred in 1977-1978 (Hassan et al. 1979). A revenue loss of US$ 8 million have occurred in Baluchistan (Ahmad et al., 1991). Stripe rust epidemic in 1995 on Pak 8 and, Pirsabak 85 and during 2003 on Inquilab 91 have also been reported (Khan and Mumtaz 2004). Hussain et al. (2004) reported a loss of price 2 billion in years 1997 and 1998 due to successive increase in stripe rust virulence on pathotypes attacked cultivars having YrA (Bahawalpur 79, Chenab 79 and Nuri 70), YrA and Yr6 (LU26, Lyallpur 73, Pari 73, Sandal 73, Yecora, etc.) and Yr22 (Blue Silver, Sonalika and WL 711). Yield losses of 5.77%, 663% and 14.90% caused by stripe rust in Pakistani varieties Inqlab-91, Wafaq-2001 and Bakhtawar (Khan and Mumtaz 2004).

3.3.5 Epidemiology

Stripe rust disease has been a major yield constraint in Khyber Pakhtunkhwa, Punjab and Baluchistan (Khan, Naseer et al. 2005). In Peshawar valley, Abbottabad, Naoshera and Mingora stripe rust occurs regularly due to persistent favorable conditions. In year 2007, stripe rust severely attacked the wheat crop in Quetta, Pishin, Mustang, Lorelai and Qilasaifullah (Bux, Ashraf et al. 2012). Stripe rust pathogen have

potential of adaptability to different environmental conditions. Stripe rust mainly affect areas of cool climates of in Khyber Pakhtunkhwa, Northern Punjab and Baluchistan.

Historically, rust epidemics have occurred across subcontinent since 1786 (Kumar, Verma et al. 1977)Severe epidemics have been reported in 1948 and 1954. Severe outbreaks occurred in years 1973, 1976 and 1977 consecutively with considerable yield loss. Severe epidemic of 1977 and 1978 causes yield losses of 10.1% (0.83 million tons) valuing US$86 million (Hassan et al. 1979). Stripe rust epidemic in year 1991-1992 on ‘’Local white’’ in Baluchistan caused considerable yield losses (Ahmad, Rodriguez et al. 1991).Severe outbreak of stripe rust 1994-1995 in Khyber Pakhtunkhwa and Northern Punjab caused yield loss of Rs. 2.0 billion and same losses occurred in years 1995-1996 (Bux, Rasheed et al. 2012).In the years 2002-2003 and 2003-2004, in Khyber Pakhtunkhwa yellow rust epidemics attacked the grain severely. In recent past, yellow rust have also been found attacking grains in hotter and dry climates of Southern Punjab and Sindh (Anonymous 2008). In 2005, late sowing material have been severely attacked by stripe rust epidemics. Total cost of US$100 million based on a rapid average total losses estimate of 3–4% (Duveiller, Singh et al. 2007)

3.3.6 Classification of rust fungus (*Puccinia* sp).

Rust pathogen have been classified and placed in kingdom -Fungi, phylum-Basidiomycota, class- *Urediniomycetes,* Order-*Uredinales*, Family- *Pucciniacae* and Genus-*Puccinia.*

TABLE 3. CLASSIFICATION OF RUST PATHOGEN

Kingdome	Fungi
phylum	*Basidiomycota*
Class	*Uridiniomycetes*
Order	*Uridinales*
Family	*Pucciniacae*
Genus	*Puccinia*

3.3.7 Host Crops and Other Plants

The main host plant of stripe rust include wheat (*Triticum* spp.), Barley (*Hordeumvulgare*), and triticale (X *Tritocosecale*), Berberis species have also been discovered as an alternate host of stripe rust (Jin, Szabo et al. 2010).

3.3.8 Nomenclature of stripe rust

A series of changes have been occurred in the nomenclature of stripe rust before finally being named as *Puccinia striformisTritici*(Chen 2013).

- In 1827, Schmidt named the pathogen as *Uredoglumerum,* capable of infecting barley.
- Later on in 1854, Westend named the pathogen as *Puccinia striiaeformis* that infect rye.
- In 1860, Fuckel given the name *Puccinia striforminis.*
- In 1894, Eriksson and Henning recognized the stripe rust as a prominent pathogen and given the name *Puccinia glumarum.*
- Finally the term was studied again and was modified to spacialis of *Puccinia striformis.*
- *Puccinia striformis* has the potential of infecting other members of grass family such as barley and rye and more than 60 grass species **(Line 2002)**. Chen and

his colleagues categorized *Puccinia striformis* into five formea speciales (Chen 2013). Later, three more formae spciales were reported.

Table 4. LIST OF P. STRIFORMIS WITH THEIR HOST PLANTS

S.No	Species	Host Plant
1	*P. striformisf. sp. Tritici*	Wheat (Yellow/Stripe rust)
2	*P.striformisf.sp. Hordei*	Barley (Chen et al., 2014)
3	*P.striformisf.sp. Secalis*	Rye (Chen et al., 2014)
4	*P.striformisf.sp. Elymi*	Elymusspp (Chen et al., 2014)
5	*P.striformisf.sp. Agropyron*	Agropyronspp (Chen et al., 2014)
6	*P.striformisf.sp. Dactylidis*	Orchard grass (Manners, 1960; Tollenear 1967)
7	*P.stroformisf.sp. Poae*	Kentucky blue grass (Tollenear 1967)
8	*P.striformisf.sp. Leym*	Leymussecalinus (Georgi) Tzvel (Niu et al.,1991)

3.3.9 Economic importance

The major economic impact of stripe rust is the reduction in grain yield. Yield reduction depends upon the time of infection, duration of infection and severity of the disease (Murray et al. 1994; Line 2002; Chen 2005).Reduction in yield ranges from 10% to 70% (Chen2005) and early infection may cause 50% loss (Batts 1957; Doodson et al.1964; Murray et al. 1994). Chen (2005) reported 100% yield loss in susceptible varieties. Yield losses of 0.1% to 5% and rare losses of 5-25% are common in major wheat producing regions of the world (Welling's 2011).

3.4 Life Cycle

The life cycle of *Puccinia striformis* comprises of five different spores on two host plants (Chen, Wellings et al. 2014).Asexual reproduction occures on primary host plant and sexual reproduction occures on on berberis which is the secondary host plant **(**Jin, Szabo et al. 2010**).** Asexual dicaryotic(n+n) urediniospores are produced on primary host in small pustules called uredinia. These urediniospores are airborn and can travell long distances (Stubbs, 1985). Uridiniospores produces striping pattern on wheat that become obvious when uredinia break the leaf epidermis.

Telia, two-celled teliospores (2n) replace urediniospores which may over-summer on the senescing leaves of wheat (Primary host plant). Four-celled basidia produces haploid basidiospore by meiosis on barberis (secondary host). **(**Jin, Szabo et al. 2010)revealed that teliospores produces uninucleatepycniosores (n). Haploid receptive hyphae is fertilized by pycniospores. Dikaryotic (n+n) aeciospores are produced by the haploid hyphae on the leaves of secondary host plant. The life cycle completes when the aeciospores from the berberis (secondary host) transfer and infect the leaves of wheat (primary host) to produce dikaryaotic urediniospores**(**Jin, Szabo et al. 2010).

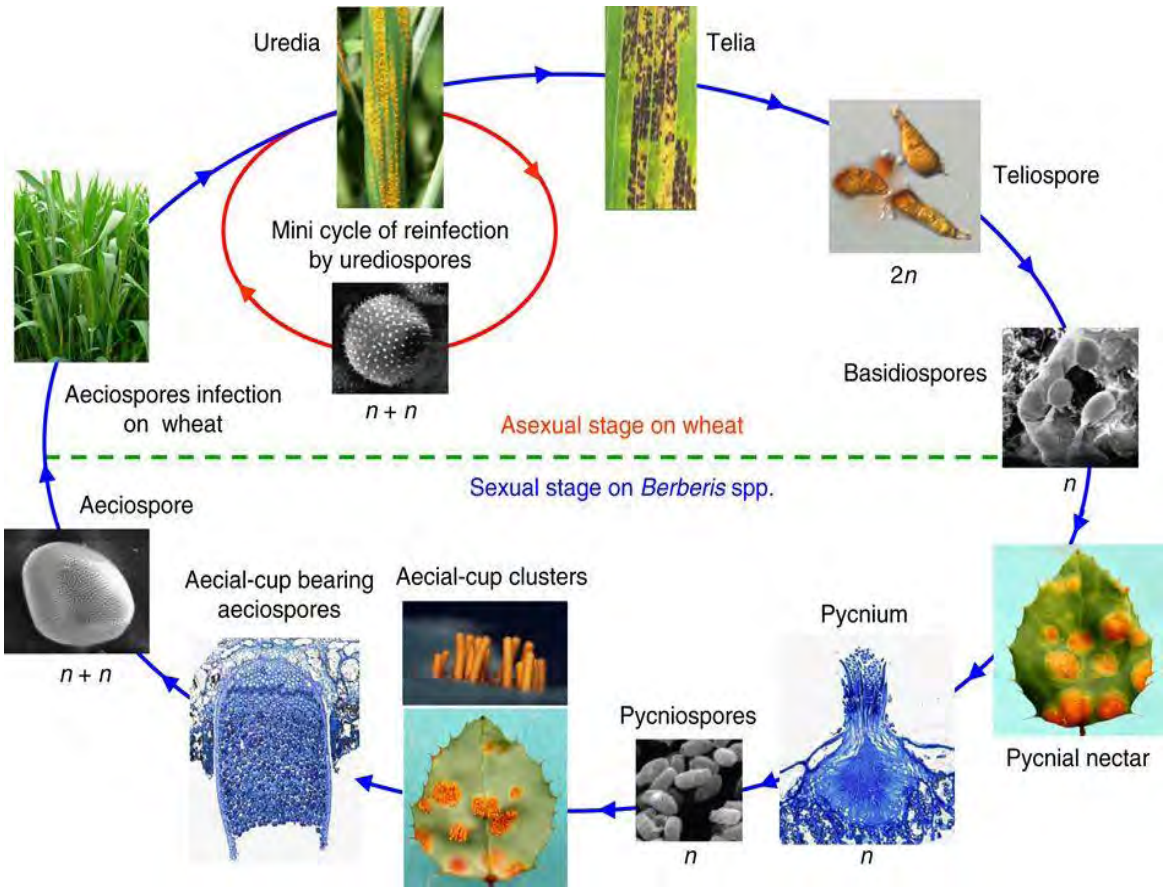


FIGURE 12: LIFE CYCLE OF RUST DISEASE OF WHEAT.WWW.GOOGLE.COM

3.5 Symptoms and signs

The stripe rust pathogen can infect the green tissues of wheat and many other grass species. Infection on green leaves can take place from one leaf stage to plant maturity. The symptoms and signs generally appear on leaves, however; in severe conditions, the symptoms and sign may appear on glumes, sheaths and awns (Mehrotra and Aggarwal 2003).Initial symptoms appear after 1 week of infection and sporulation develops after 2 week of infection under optimum conditions (Chen 2005).In a susceptible host, the fungus form yellow to orange colored stripes on green leaves, sheaths, glums and awns. The pathogen continue to grow parallel to the leaf axis producing long stripes. Stripes formation is the important and distinguishable characteristics of stripe rust. Stripes are composed of rust pustules called uredia which contains thousands of urediniospores(Chen 2005).Urediniospores are ovate in form and the size may vary from 23-35 x 20-35 µm.The spores are colorless, echinulate with 6-16 germ spores (Mehrotra and Aggarwal 2003).

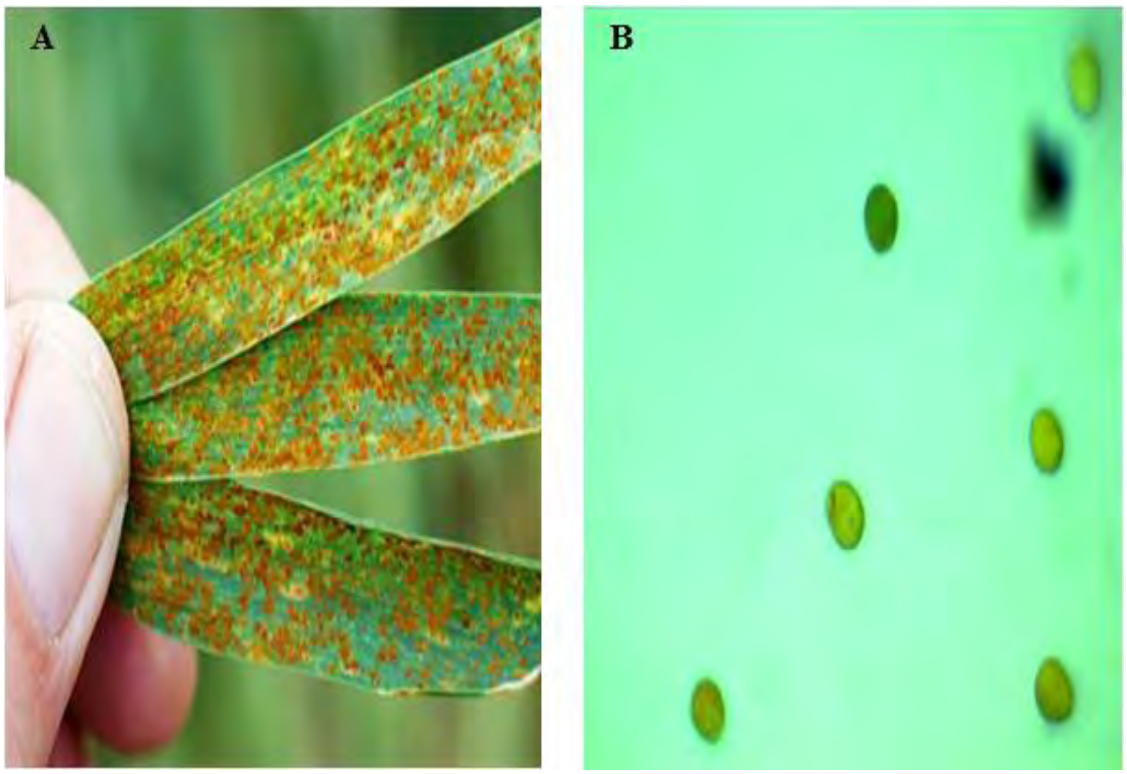


FIGURE 13: A) YELLOW RUST UREDINIOSPORE ON WHEAT FLAG LEAVES. B) 40X MICROSCOPY OF UREDINIOSPORES

3.6 Conditions favoring the disease

Stripe rust is frequently found in high altitudes and cooler regions. The pathogen is best persistent when the night temperature <60ºF (15ºC). Urediniospores can germinate best at 44-59ºF (7-15ºC). Infection and development of disease is most fast between 50-60ºF (10-16ºC).Urediniospores can spread by wind to healthy plants where they infect new plants. Heavy dew can facilitate the disease. Infection tends to reduce when the temperature consistently exceed 71-73ºF (21-23ºC) (Martínez-Espinoza, Youmans et al. 2009**)**.

3.7 Virulence Variation

The ability of a pathogen to overcome a specific resistant gene is called virulence (Flor, 1971; Brown 2003). A race with a specific virulence pattern only attack specific cultivars. Differentials (A set of wheat genotype) is used to recognize the virulence variation of stripe rust isolates. Mutation, somatic recombination or sexual recombination cause changes in the virulence pattern of Pst. Migration also brings changes in the pathogen virulence where they become established (Brown and Hovmøller 2002).

3.8 Dynamics of pathogenic variability

New races and strains in the stripe rust pathogen results due to mutation and somatic recombination **(**Chen 2005**).** The sexual stage of Stripe rust pathogen and alternate host remained a mystery for a long time and have recently been resolved by identifying berberis as an alternate host **(**Jin, Szabo et al. 2010**).** Diversity in the stripe rust pathotypes are the result of sexual recombination in an areas wherever berberisspp exist **(**Jin, Szabo et al. 2010**).** *B. luceum* and *B. vulgaris* are commonly found in Pakistan (Anjum and Muhammad 2010**).** These species are known as an alternate host to stripe rust pathogen. Being an air borne pathogen, new races can also be migrate from new areas. In past, Yr9 gene evolved in East Africa and migrated to North Africa, West Asia, and South Asia over a nine year of period **(**Singh and Huerta Espino 2001)causing major epidemics in Ethiopia, Turkey, Iran, Afghanistan and Pakistan.

3.9 Resistance through conventional breeding

Genetic resistant in wheat against *P.striformis* was resolved for the first time by Biffen in 1905. He designated multiple resistant types for stripe rust. Chen (2013) grouped the types of resistance based on:

- Growth stage (All stage [seedling] resistance, Adult plant resistance).
- Testing condition (green house, field).
- Specificity (Race specific, race non-specific).
- Degree of resistance (Absolute, relative).
- Sensitivity to pathogen infection (Hypersensitive, non-hypersensitive).
- Speed of symptom/sign development (Fast rusting, slow rusting).
- Response to temperature (Temperature sensitive, temperature non sensitive).
- Inheritance (qualitative, quantitative).
- Effect of genes (Major, minor).
- Number of genes (Monogenic, Polygenic).
- Molecular basis (NBS-LRR type resistance, non NBS-LRR type resistance).
- Durability (Non-durable, durable).
- Race specificity, Growth stage and temperature sensitivity (Race-specific all-stage resistance, non-race specific high-temperature adult-plant (HTAP) resistance).

We can control stripe rust through genetic resistance in wheat cultivars. Resistant variety can be develop for race specific or for broad spectrum multiple race resistant. Qualitative and quantitative resistant can also be develop against stripe rust. Cultivation of resistant varieties against stripe rust is the best approach to control the disease (Chen 2005; St. Clair 2010;Lowe, Cantu et al. 2011**).**

3.10 Genetic Resistance

Resistant to stripe rust pathogen can be classified into all stage resistance (ASR) and adult plant resistance (APR). All stage resistance (Seedling Resistance) is race specific resistance and can be qualitatively inherited as monogenic resistance which is actually not as durable. Plants with all stage resistance show high resistant to pathogen and are used in many elite cultivars but they become susceptible with the emergence of new pathogen races. On the other hand, plants having adult plants resistance are

susceptible at seedling stage and become resistant at adult stage. This kind of resistance is quantitative which is controlled by multiple genes (Rosewarne, Herrera-Foessel et al. 2013). APR genes provide low level of resistance(Bariana and McIntosh 1993), (Singh, Nelson et al. 2000).Adult plant resistance is short lived because pathogen has the ability to infect plants with new virulent strains (Singh 2001).Till now, more than 80 resistant genes have been identified at different loci and mapped to different wheat chromosomes. Most of the identified genes are race specific and play an important role in wheat breeding.

Among the identified genes, 18 are adult plant resistant genes namely, Yr11,Yr14,Yr16,Yr18,Yr29,Yr30,Yr34,Yr36,Yr39,yr46,Yr48,Yr49,Yr52,Yr54,Yr59a ndYr62 (McIntosh, Wellings et al. 1995).The most commonly used genes for the development of resistance in wheat varieties are Yr5, Yr10, Yr15, Yr24, Yr36, Yr57, Yr40/Lr57, and Lr34/Yr18 etc(McDonald, McIntosh et al. 2004). Majority of the rust resistant genes have been originated from hexaploid wheat except for few genes that originated from related species (Riley, Chapman et al. 1968).

TABLE 5.GENE FOR RESISTANCE TO STRIPE RUST [PUCCINIA STRIIFORMIS F. SP. TRITICI], EXAMPLES OF WHEAT GENOTYPES CONTAINING THE GENES, THEIR CHROMOSOMAL LOCATIONS, TYPES OF RESISTANCE, AND REFERENCES

Yr genes	Wheat genotype	Chromosomal location	Resistance type	Reference
Yr1	Chinese 166	2AL	RS,AS	Lupten and Macer 1962
Yr2	Heines VII	7B	RS,AS	Lupten and Macer 1962
Yr3a	Cappelle 46	1B	RS,AS	Lupten and macer 1962
Yr3b	Minister		RSAS	Lupten and Macer 1962
Yr3c	Cappelle-Desprez	1B	Rs,AS	Lupten and Macer 1962
Yr4a	Hybrid	6B	RS,AS	Lupten and Macer 1962
Yr5	T.eastivumsubsp.Spelt, Album	6B	RS,AS	Macer 1966
Yr6	HeinesKolben	2BL	RS,AS	Riley et al. 1968
Yr7	Lee	7BS	RS,AS	Macer 1975
Yr8	Compare	2BL	RS,AS	Macer 1975
Yr9	Clement	2D(2A,3D)	RS,AS	McIntosh 1988
Yr10	Moro	1BS	RS,AS	McIntosh 1988
Yr11	Joss- Cambier		RS.AP	McIntosh 1988
Yr12	Fronteir		RS,AP	McIntosh 1088
Yr13	Hustler		RS,AP	Mcintosh 1988
Yr14	Kador		RS,AP	Mcintosh 1988

Yr15	T.Turgidum var. Dicoccoides G-25	1BS	RS,AS	Gerechter-Amitai et al.1989
Yr16	Bersee	2D	NRS,AP	Warland& Law 1986
Yr17	VPMI	2AS	RS,AS	Bariana&Mcintosh 1993
Y18	Jupateco 73R	7DS	NRS,HTAP	Singh 1992
Yr19	Compare	5B	RS,AS	Chen et al 1995b
Yr20	Fielder	6D	RS,AS	Chen et al 1995b
Yr21	Lemhi	1B	RS,AS	Chen et al. 1995b
Yr22	Lee	4D	RS,AS	Chen et al. 1995b
Yr23	Lee	6D	RS,AS	Chen et al. 1995b
Yr24	Yr24/6*AVS	1BS	RS,AS	McIntosh et al. 1998
Yr25	StrubesDickkopf	1D	RS,AS	McIntosh et al. 1998
Yr26	R55	1BS	RS,AS	McIntosh et al.1998
Yr27	Ciano 79	2BS	RS.AS	McDonald et al. 2004
Yr28	Synthetic	4DS	RS,AS	Singh et al. 2000
Yr29	Pavon F76	1BL	NRS,AP	McINtosh et al.2001
Yr30	OPata 85	3BS	NRS,AP	McIntosh et al. 2001

3.11. Materials and Methods

3.11.1 Preliminary experiment

Experiment was conducted under controlled conditions in glass house at Crop Disease Research Institute (CDRI) Murree. Differential lines of wheat yellow rust along with morocco were planted in black pots and inoculated with strains 18YKPK-29, 18YSPK-04A and 18YSPK-03B in order to find out genes avirulent to these strains. All the differential lines and strains were provided by Crop Disease Research Institute (CDRI) Murree. Twenty six yellow rust differential lines along with Morocco were planted in black pots for screening purpose. All the differential lines were inoculated on 2nd leaf stage with 18YKPK-29, 18Ykpk04A and 18YSPK-03B strains. Inoculation was done in inoculation chamber. On the development of full-fledged symptoms, 0-9 scale was used to screen out all the Yr differential lines against each inoculated strain. The result is given as under.

Table 6. NEAR ISOGENIC LINES STRIPE RUST STRAINS

S.No	Genes	18YKPK-18	18YSPK-04A	18YSPK-03B
1	Yr1	9	9	9
2	Yr5	2	3	3
3	Yr6	9	8	8
4	Yr7	8	8	9
5	Yr8	8	8	9
6	Yr9	8	9	8
7	Yr10	**0**	**0**	**0**
8	Yr15	**0**	**0**	**0**
9	Yr17	8	7	8
10	Yr24	**3**	**3**	**3**
11	Yr27	8	8	8
12	Yr32	5	3	3
13	Yr43	4	8	6
14	Yr44	0	5	3
15	YrSP	4	0	0

16	YrTr1	7	6	8
17	YrExp2	7	-	8
18	YrTye	7	3	0
19	Yr2	8	8	9
20	Yr48	7	5	6
21	Yr4B	8	7	9
22	Yr25	8	6	8
23	Yr28	8	8	9
24	Yr31	7	6	4
25	YrA	8	9	9
26	YrNG	8	9	9
27	Morocco	9	9	9

3.11.2 Sowing of A-virulent genes

Five seeds of each avirulent differential line were planted in black pots in two sets. Two weeks old seedlings of Avocet-Yr24, Avocet-Yr10, Avocet-Yr15 and Morocco in one set was infected with 18YSPK-04a inoculum using freshly generated spores. The other set of Avocet-Yr24, avocet-Yr10, Avocet-Yr15 and Morocco was mock infected in inoculation chamber. The infected seedlings were then incubated at 18^0C at extremely high humidity (100%) for 24 hours in the dark. Following the incubation period, the normal growth conditions were set to 18 ˚C. For expression analysis seedling leaves were sampled at 0, 7, 14 and 20 Dai. Samples were then flash frozen in liquid nitrogen and stored at -80 before the isolation of total RNA.

3.11.3. RNA Isolation (Trizol Reagent)

Total RNA was isolated using TRIzol Reagent (15596). 50 mg of leaf samples was used for RNA isolation. Following protocol was followed.

3.11.4. Homogenization

Leaf samples were powdered using mortar and pestle in liquid nitrogen. Powdered was then homogenized in 1 ml TRlzol® Reagent per 50 mg of leaf sample in 2 ml sterile tube.

- The homogenized sample was incubated for 5 minutes at room temperature to permit the complete dissociation of nucleoprotein complex.
- Chilled chloroform (0.2mL) per 1 mL of TRIzol Reagent was added to the homogenate and was vigorously shacked for 15 seconds.
- The sample was again incubated at room temperature for 2-3 minutes followed by centrifugation at 12,000 x g for 15 minutes at 4^0C.
- The sample was separated into three phases.
  a. Lower phase which was red phenol chloroform layer.
  b. An interphase which was DNA layer.
  c. A colorless upper aqueous phase which was RNA layer.
- Colorless upper aqueous phase was removed by angling the tube at 45º and the solution was pipette out.
- The removed aqueous phase was placed into a new Eppendorf and was proceeded to RNA isolation procedure.

3.11.5. RNA precipitation

- 0.5 mL of chilled isopropanol (100%) was added to the upper aqueous phase, per 1 mL of TRIzol Reagent and vertex for 10 seconds.
- The sample was then incubated for 10 seconds and centrifuge for 12,000 x g for 10 minutes at 4^0C.
- The supernatant was removed leaving only the RNA pellet.

3.11.6. RNA wash

- The RNA pellet was washed with 1 mL of 75% chilled ethanol per 1 mL of TRIzol Reagent used in homogenization.
- The sample was briefly vertex and centrifuge the tube at 7500 x g for 5 minutes at 4^0C.
- Finally the RNA pellet was air dried.

3.11.7. RNA resuspension

- The RNA pellet was resuspended in RNase-free water (20µl) and stored at -80^0C for further use.

3.11.8. RNA quantification

Nano drop was used to check the purity at A260/A280 and concentration of total RNA.

The intactness of RNA was checked by running on 1% agarose gel electrophoresis.

3.12. Synthesis of first strand cDNA

Following components were combined in a 200 µL sterile PCR tube: 1 µL oligo (dT) 18, total RNA (0.1 ng – 5 µg) and nuclease free water up to 12 µL. Mixture was then incubated at 65^0 C for 5 min, chilled on ice, spin down and the following components were added in the indicated order: 5X Reaction Buffer (4µL), RiboLockRNase inhibitor (1 µL), 10mM dNTP Mix (2 µL) and Revert Aid M-MuLV RT 200 U/ µL (1 µL). The components of the tube mixed briefly and incubated for 60 min at 42C and stopped by incubating at 700C for 5 min.

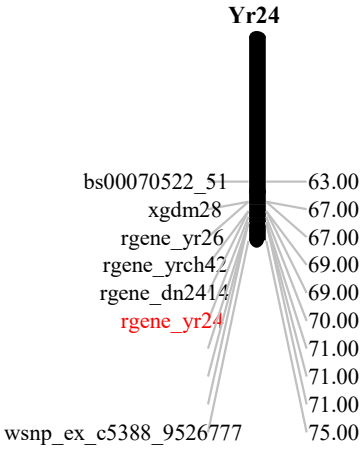
3.13. Gene Expression Analysis

For RT-qPCR primers reference and target genes were designed using software Primer3 (V.0.4.0). After designing, primers were manufactured from Humanizing Genomics Macrogen. The primer's sequence and optimum annealing temperatures are given in (Table).

TABLE 7. LIST OF TARGET GENES AND PRIMERS

Target genes	**Primer's Sequence 5' to 3'**	**TM**	**GC %**	**Amplicon Size (bp)**
KIN1 EX1	F<CTCGGACAAGGTGGTTTC R<CAAGATGCTTGTGATGAACG	64.0 64.0	55% 45%	154
KINASE EX7	F<GGAGGATCTTAACGAAGGTGC R<TGGCTGCTCGTGTAGTCG	64.0 64.0	50% 61%	147
NBSLRR1B	F<CCTAAACTACGGGAGCTCAAG R<GAAAGCCAACCCGTTCCAG	63.0 63.0	54% 57%	213
KIN2 EX1	F<GTCAAGCAGCTGAGGGATG R<CGTGAAGGTGATAATGCAGAT	60.0 60.0	57% 45%	181
KIN3 EX1	F<TTGGTGTGGGAATTGCATGG R<CCTGGTCCACAACTTTGAGC	62.0 62.0	50% 55%	106

GENE5 EX2	F<GGCAAACTCTTCTGTCGACC R<CTGCTCATTCCTCAAGTCGC	64.0 64.0	55% 55%	235
G5 EX1	F<GAGGGGGTACTACTACGCCA R<GCGTGGAGTACCTGACGAC	62.0 62.0	60% 63%	172
G5 EX7	F<CACTACAACGCCGGCACA R<TGTCACCACTTCCCAGCATCT	64.0 64.0	61% 52%	212
KIA EX2	F<TTGGGAACAACACTGATGGC R<TGTTGTTTGACAGATCCAGCG	63.0 63.0	52% 50%	169
KIA EX3	F<AATTGGTATTGGTGCAGCG R<CCTAGCAAGCCCAAAATCC	54.0 54.0	50% 47%	171



chromoso	locus.id	Position	sequence2	sequence3	sequence4	
ch1B	BS00070522_51	63	Resistance	*Sorghum bi*	TKL_IRAK_	DUF26-lf.3-DUF2
ch1B	Xgdm28	67.00	no			
ch1B	RGene_Yr26	67	Resistance loci			
ch1B	RGene_YrCH42	69	Resistance loci			
ch1B	RGene_Dn2414	69	Resistance loci			
ch1B	RGene_Yr24	70	Resistance loci			
ch1B	RGene_Yr9	71	Resistance loci			
ch1B	RGene_Lr26	71	Resistance loci			
ch1B	RGene_Sr31	71	Resistance loci			
ch1B	wsnp_Ex_c5388_9526777	75	Resistance	Brachypodiu	PF00560	PF00069 PF07714

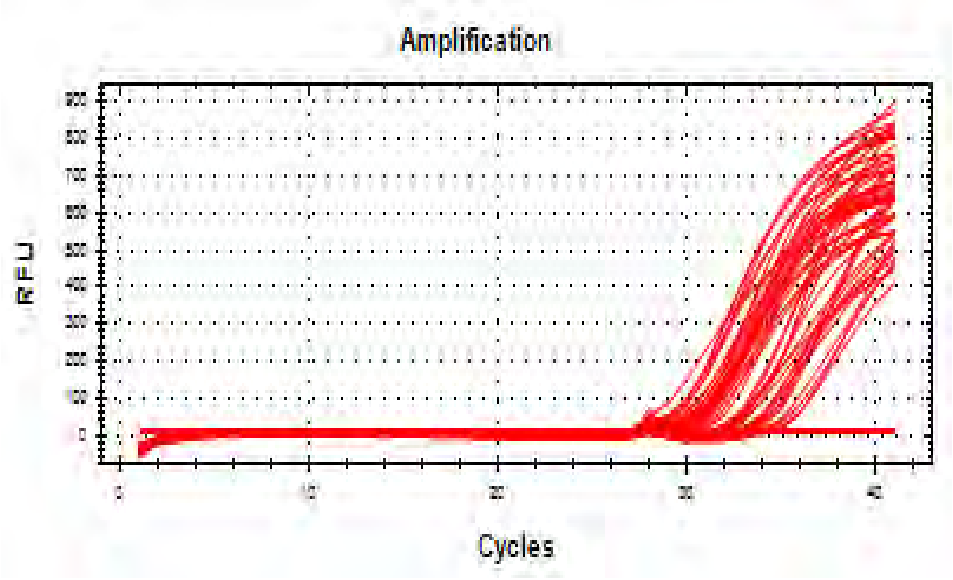
Status of wheat rust research and control in China(Kang, Zhao et al. 2010) Cloned gene Yr24

3.14. Results

3.14.1. QRT-PCR analysis

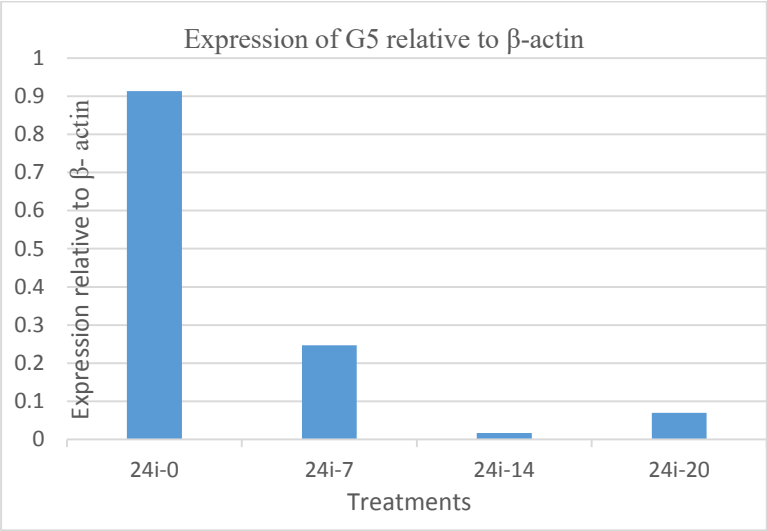
Currently, some housekeeping genes are described for the normalization of expression signals. The most common ones are actin, glyceraldehyde-3-phosphate 52 dehydrogenase, ribosomal RNA genes, ubiquitin, cyclophilin, and elongation factor 1-α (ef1α) (Stürzenbaum et.al. 2001; Bezier et.al. 2002). We used β-actin for

normalization. All QRT PCR analysis was performed for avocet Yr24 at day-0, day-7, day-14 and day-20.



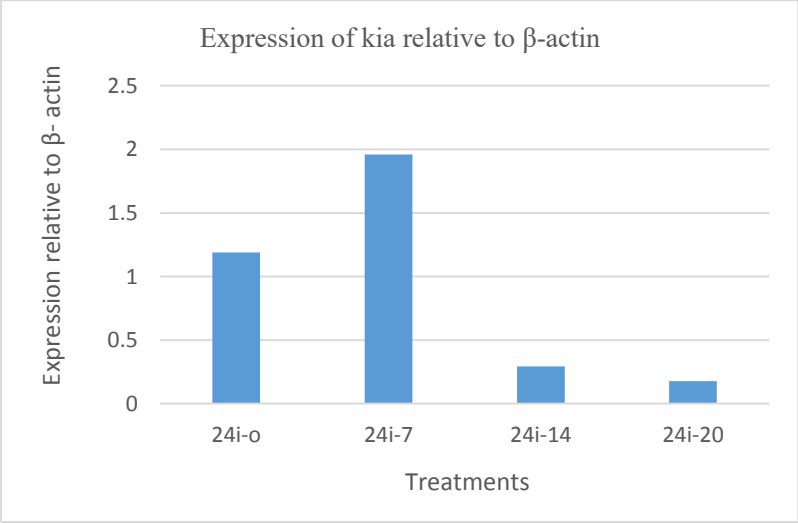
Real-time PCR (QRT-PCR) profile. The infections were performed using 18YSPK-04a pathogen race on Avocet-Yr24 seedlings. RNA samples are from 48th hour of post infection. A: Normalization of mRNA levels using β-actin gene expression for infected samples.

3.14.2. Relative expression



Comparison of G5 gene expression relative to β-actin

Analysis was carried out by qRT-PCR at 24i-0, 24i-7, 24i-14 and 24i-20. Data was normalized using wheat β- actin gene.



Comparison of G5 gene expression relative to β-actin

Analysis was carried out by qRT-PCR at 24i-0, 24i-7, 24i-14 and 24i-20. Data was normalized using wheat β- actin gene.

3.15. Discussion

Breeding of resistance genes have been reported the most effective techniques in controlling crop diseases especially rust diseases of wheat. Under biotic stress, the up and down regulation of G5 and KIA genes were observed on stripe rust differential line Yr24. The expression analysis was done at dai-0, dai-7, dai-14 and dai-20. G5 gene showed up regulation at dai-7 as compared to dai-0. Low expression was observed at dai-14 and again relatively high expression was observed at dai-20. The upregulatio of G5 gene at dai-7 show positive expression in comparison with day-0. Low expression was found at dai-14 but again high expression was found at dai-20 which shows that localized resistance is involved. Similarly, expression analysis of KIA gene was also observed at dai-0, dai-7, dai-14 and dai-20. High expression of KIA gene at dai-7 against stripe rust race 18YSPK-04a was observed as compared to dai-0. The upregulation of KIA at dai-7 indicates the expression of KIA gene against 18YSPK-04a race. Low expression was observed at dai-14 in comparison with dai-0 which indicates that KIA gene was not expressed against the inoculated race. Expression analysis was again observed at dai-20 in comparison with dai-0. There was low expression of KIA gene at dai-20 in comparison with dai-14 and dai-0. The pattern of findings suggests the role of resistance proteins against the stripe rust race. The Yr24 against 18YSPK-04a shows up and down regulation against stripe rust race at different days.

4. CONCLUSION AND FUTURE PERSPECTIVES

Probably, wheat has been put through among all the crops, over 100 years. In last two or three decades, an extensive genomic research admitted solicitous study in particulars of structure, evolution and function.

Bread wheat is a model organism to study the influence of whole genome duplication on the sub- genome dominance. The ancestry of the wheat genome consists of seven sub genomes that correlate to the shared paleo-tetraploidization event that has already been recognized in other cereals besides to its two current neo-polyploidization events due to which *Triticum aestivum* and *Triticum turgidum* originated. Not only the wheat genome is influenced by neo-polyploidisation event but has also been exposed to diplodization of sub genomes A, B and D. The transcriptome analysis of whole genome revealed that approximately 46% of the homologous genes are neo or sub functionalized (Pont et al., 2013). Shortly, the grass family is the result of duplication of whole genome and fusion of ancestral chromosomes. This structural and functional imbalance has muddled the diploid mode of expression in wheat (Edger and Pires, 2009). The study in these thesis is a contribution to the genomic resources of wheat in order to encourage the evolutionary studies along with the genes validation among all the cereals. The comprehensive map generated in this study will not only be source of trait development but will also be the source of to create more functional markers.

- The projected and mapped resistance genes on the consensus map are required to be integrated and assembled into the breeding programs minimize the yield crises.
- In addition, control of chemical use through genes breeding.
- Screening of other differential lines should also be done against Pakistani stripe rust races.
- Construction of resistance gene bank against stripe rust races.

REFERENCES

Akhunov, E., C. Nicolet, et al. (2009). "Single nucleotide polymorphism genotyping in polyploid wheat with the Illumina GoldenGate assay." Theoretical and Applied Genetics 119 (3): 507-517.

Ali, S., P. Gladieux, et al. (2014). "Origin, migration routes and worldwide population genetic structure of the wheat yellow rust pathogen Puccinia striiformis f. sp. tritici." PLoS Pathogens 10 (1): e1003903.

Allen, A. M., G. L. Barker, et al. (2011). "Transcript-specific, single-nucleotide polymorphism discovery and linkage analysis in hexaploid bread wheat (Triticum aestivum L.)." Plant Biotechnology Journal 9 (9): 1086-1099.

Almas, F., A. Hassan, et al. (2018). "Identification of genome-wide single-nucleotide polymorphisms (SNPs) associated with tolerance to chromium toxicity in spring wheat (Triticum aestivum L.)." Plant and Soil 422 (1-2): 371-384.

Anjum, P. and Q. Muhammad (2010). "Pollen flora of Pakistan-LXV. Berberidaceae." Pakistan Journal of Botany 42 (1): 1-6.

Bariana, H. and R. McIntosh (1993). "Cytogenetic studies in wheat. XV. Location of rust resistance genes in VPM1 and their genetic linkage with other disease resistance genes in chromosome 2A." Genome 36 (3): 476-482.

Beddow, J. M., P. G. Pardey, et al. (2015). "Research investment implications of shifts in the global geography of wheat stripe rust." Nature Plants 1 (10): 15132.

Begum, S., M. Iqbal, et al. (2014). "Allelic variation at loci controlling stripe rust resistance in spring wheat." Journal Of Genetics 93 (2): 579-586.

Berkman, P. J., K. Lai, et al. (2012). "Next-generation sequencing applications for wheat crop improvement." American Journal Of Botany 99 (2): 365-371.

Brown, J. K. and M. S. Hovmøller (2002). "Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease." Science 297 (5581): 537-541.

Bux, H., M. Ashraf, et al. (2012). "Expression of high-temperature adult-plant (HTAP) resistance against stripe rust (Puccinia striiformis f. sp. tritici) in Pakistan wheat landraces." Canadian Journal Of Plant Pathology 34 (1): 68-74.

Bux, H., A. Rasheed, et al. (2012). "An overview of stripe rust of wheat (Puccinia striiformis f. sp. tritici) in Pakistan." Archives of Phytopathology and Plant Protection 45 (19): 2278-2289.

Cavanagh, C. R., S. Chao, et al. (2013). "Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars." Proceedings of the National Academy of Sciences 110 (20): 8057-8062.

Chao, S., J. Dubcovsky, et al. (2010). "Population-and genome-specific patterns of linkage disequilibrium and SNP variation in spring and winter wheat (Triticum aestivum L.)." BMC genomics 11 (1): 727.

Chao, S., P. Sharp, et al. (1989). "RFLP-based genetic maps of wheat homoeologous group 7 chromosomes." Theoretical and Applied Genetics 78 (4): 495-504.

Chen, L. Q. (2014). "SWEET sugar transporters for phloem transport and pathogen nutrition." New Phytologist 201(4): 1150-1155.

Chen, W., C. Wellings, et al. (2014). "Wheat stripe (yellow) rust caused by P uccinia striiformis f. sp. tritici." Molecular Plant Pathology 15 (5): 433-446.

Chen, X. (2005). "Epidemiology and control of stripe rust [Puccinia striiformis f. sp. tritici] on wheat." Canadian Journal of Plant Pathology 27 (3): 314-337.

Chen, X. (2013). "High-temperature adult-plant resistance, key for sustainable control of stripe rust." American Journal of Plant Sciences 4 (03): 608.

Cook, J. P., M. D. McMullen, et al. (2012). "Genetic architecture of maize kernel composition in the nested association mapping and inbred association panels." Plant physiology 158 (2): 824-834.

Cooper, J. I. (1993). Virus diseases of trees and shrubs, Springer Science & Business Media.

Cooper, R. (2015). "Re-discovering ancient wheat varieties as functional foods." Journal of Ttraditional and Complementary Medicine 5 (3): 138-143.

Curtis, B. C., S. Rajaram, et al. (2002). Bread wheat: improvement and production, Food and Agriculture Organization of the United Nations (FAO).

Delibes, A., J. Del Moral, et al. (1997). "Hessian fly-resistance gene transferred from chromosome 4Mv of Aegilops ventricosa to Triticum aestivum." Theoretical and Applied Genetics 94 (6-7): 858-864.

Dhanda, S., G. Sethi, et al. (2004). "Indices of drought tolerance in wheat genotypes at early stages of plant growth." Journal of Agronomy and Crop Science 190 (1): 6-12.

Dubcovsky, J. and J. Dvorak (2007). "Genome plasticity a key factor in the success of polyploid wheat under domestication." Science 316 (5833): 1862-1866.

Duveiller, E., R. P. Singh, et al. (2007). "The challenges of maintaining wheat productivity: pests, diseases, and potential epidemics." Euphytica 157 (3): 417-430.

Elshire, R. J., J. C. Glaubitz, et al. (2011). "A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species." PloS one 6 (5): e19379.

Elshire, R. J., J. C. Glaubitz, et al. (2011). "A Robust, Simple Genotyping-by-Sequencing (GBS) Approach for High Diversity Species." Plos One 6 (5).

Eyal, Z., A. Scharen, et al. (1985). "Global insights into virulence frequencies of Mycosphaerella graminicola." Phytopathology 75 (12): 1456-1462.

Faridi, H. and J. M. Faubion (1995). Wheat end uses around the world, American Association of Cereal Chemists.

Germán, S., A. Barcellos, et al. (2007). "The situation of common wheat rusts in the Southern Cone of America and perspectives for control." Australian Journal of Agricultural Research 58 (6): 620-630.

Godfray, H. C. J., J. R. Beddington, et al. (2010). "Food security: the challenge of feeding 9 billion people." Science 327 (5967): 812-818.

Gupta, P. K. and R. Varshney (2000). "The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat." Euphytica 113 (3): 163-185.

Hakro, A. and A. Khan (2012). Role of yellow rust-resistance genes of wheat in Pakistan. Meeting the Challenge of Yellow Rust in Cereal Crops.

Harlan, J. R. and D. Zohary (1966). "Distribution of wild wheats and barley." Science 153 (3740): 1074-1080.

Hohmann, U., T. R. Endo, et al. (1994). "Comparison of genetic and physical maps of group 7 chromosomes from Triticum aestivum L." Molecular and General Genetics 245 (5): 644-653.

Hovmøller, M. S., C. K. Sørensen, et al. (2011). "Diversity of Puccinia striiformis on cereals and grasses." Annual Review Of Phytopathology 49: 197-217.

Huang, L., S. A. Brooks, et al. (2003). "Map-based cloning of leaf rust resistance gene Lr21 from the large and polyploid genome of bread wheat." Genetics 164 (2): 655-664.

Jaradat, A. A. (2013). "Wheat landraces: A mini review." Emirates Journal of Food and Agriculture: 20-29.

Jin, Y., L. J. Szabo, et al. (2010). "Century-old mystery of Puccinia striiformis life history solved with the identification of Berberis as an alternate host." Phytopathology 100 (5): 432-435.

Johnson, V., K. Wilhelmi, et al. (1978). Breeding progress for protein and lysine in wheat. Proc. V. International Wheat Genet. Symp.

Kaparaju, P., M. Serrano, et al. (2009). "Bioethanol, biohydrogen and biogas production from wheat straw in a biorefinery concept." Bioresource Technology 100 (9): 2562-2568.

Khan, J., N. Naseer, et al. (2005). "Occurrence of major diseases of wheat under different agro climatic zones of Pakistan." Pak. J. Biol. Sci 8 (2): 356-360.

Khan, M. and H. Mumtaz (2004). Combining yellow rust resistance with high yield in grain wheat. Abstracts, Second Regional Yellow Rust Conference for Central & West Asia and North Africa.

King, J., R. Cook, et al. (1983). "A review of Septoria diseases of wheat and barley." Annals of Applied Biology 103 (2): 345-373.

Kumar, J., M. Verma, et al. (1977). "Breeding for disease resistance–a review." Crop Improve 4: 123-144.

Lamari, L. and C. Bernier (1989). "Virulence of isolates of Pyrenophora tritici-repentis on 11 wheat cultivars and cytology of the differential host reactions." Canadian Journal of Plant Pathology 11 (3): 284-290.

Line, R. F. (2002). "Stripe rust of wheat and barley in North America: a retrospective historical review." Annual Review Of Phytopathology 40 (1): 75-118.

Line, R. F. and X. Chen (1995). "Successes in breeding for and managing durable resistance to wheat rusts." Plant Disease 79 (12): 1254-1255.

Lowe, I., D. Cantu, et al. (2011). "Durable resistance to the wheat rusts: integrating systems biology and traditional phenotype-based research methods to guide the deployment of resistance genes." Euphytica 179 (1): 69-79.

Marcussen, T., S. R. Sandve, et al. (2014). "Ancient hybridizations among the ancestral genomes of bread wheat." Science 345 (6194): 1250092.

Martínez-Espinoza, A. D., J. D. Youmans, et al. (2009). "Stripe rust (yellow rust) of wheat."

Matsuoka, Y. and S. Nasuda (2004). "Durum wheat as a candidate for the unknown female progenitor of bread wheat: an empirical study with a highly fertile F 1

hybrid with Aegilops tauschii Coss." Theoretical and Applied Genetics 109 (8): 1710-1717.

McDonald, D., R. McIntosh, et al. (2004). "Cytogenetical studies in wheat XIX. Location and linkage studies on gene Yr27 for resistance to stripe (yellow) rust." Euphytica 136 (3): 239-248.

McIntosh, R., J. Dubcovsky, et al. (2013). CATALOGUE OF GENE SYMBOLS FOR WHEAT: 2013-2014. Proceedings of the 12th International Wheat Genetics Symposium, Yokohama, Japan.

McIntosh, R. A., C. R. Wellings, et al. (1995). Wheat rusts: an atlas of resistance genes, Csiro Publishing.

Mehrotra, R. and A. Aggarwal (2003). "Rots, Damping offs, Downy mildews and White Rusts." Plant Pathology, 2nd Edn. Tata McGraw-Hill Publishing Company Limited, New Delhi: 313-368.

Miranda, L. M., J. P. Murphy, et al. (2006). "Pm34: a new powdery mildew resistance gene transferred from Aegilops tauschii Coss. to common wheat (Triticum aestivum L.)." Theoretical and Applied Genetics 113 (8): 1497-1504.

Mitra, M. (1931). "A new bunt on wheat in India." Annals of Applied Biology 18 (2): 178-179.

Nilsson-Ehle, H. (1920). "Über Resistenz gegen Heterodera Schachti bei gewissen Gerstensorten, ihre Vererbungsweise und Bedeutung für die Praxis." Hereditas 1 (1): 1-34.

O'Brien, L., J. Brown, et al. (1980). "Occurrence and distribution of wheat stripe rust in Victoria and susceptibility of commercial wheat cultivars." Australasian Plant Pathology 9 (1): 14-14.

Ortiz-Monasterio, J. I., N. Palacios-Rojas, et al. (2007). "Enhancing the mineral and vitamin content of wheat and maize through plant breeding." Journal of cereal science 46 (3): 293-307.

Parry, D., P. Jenkinson, et al. (1995). "Fusarium ear blight (scab) in small grain cereals—a review." Plant Pathology 44 (2): 207-238.

Peters, J. L., F. Cnudde, et al. (2003). "Forward genetics and map-based cloning approaches." Trends in Plant Science 8 (10): 484-491.

Peterson, P. D. (2001). Stem rust of wheat: from ancient enemy to modern foe, American Phytopathological Society (APS Press).

Pinckney, T. C. (1989). The demand for public storage of wheat in Pakistan, Intl Food Policy Res Inst.

Poland, J. A., P. J. Brown, et al. (2012). "Development of High-Density Genetic Maps for Barley and Wheat Using a Novel Two-Enzyme Genotyping-by-Sequencing Approach." PLoS ONE 7 (2): e32253.

Qi, L., B. Friebe, et al. (2007). "Homoeologous recombination, chromosome engineering and crop improvement." Chromosome Research 15 (1): 3-19.

Reynolds, M., S. Rajaram, et al. (1999). "Physiological and genetic changes of irrigated wheat in the post–green revolution period and approaches for meeting projected global demand." Crop Science 39 (6): 1611-1621.

Riley, R., V. Chapman, et al. (1968). "Introduction of yellow rust resistance of Aegilops comosa into wheat by genetically induced homoeologous recombination." Nature 217 (5126): 383.

Roelfs, A. P. (1992). Rust diseases of wheat: concepts and methods of disease management, Cimmyt.

Rohrich, R. J., J. M. Kenkel, et al. (1999). "Preventing capsular contracture in breast augmentation: in search of the Holy Grail." Plastic and Reconstructive Surgery 103 (6): 1759-1760.

Rosewarne, G., S. Herrera-Foessel, et al. (2013). "Quantitative trait loci of stripe rust resistance in wheat." Theoretical and Applied Genetics 126 (10): 2427-2449.

Saintenac, C., D. Jiang, et al. (2013). "Sequence-based mapping of the polyploid wheat genome." G3 (Bethesda) 3 (7): 1105-1114.

Sanders, R. (2011). "Strategies to reduce the emerging wheat stripe rust disease."

Sasser, J. (1980). "Root-knot nematodes: a global menace to crop production." Plant Disease 64 (1): 36-41.

Saunders, D. G. (2015). "Hitchhiker's guide to multi-dimensional plant pathology." New Phytologist 205 (3): 1028-1033.

Sears, E. R. (1954). The aneuploids of common wheat, University of Missouri, College of Agriculture, Agricultural Experiment Station.

Shewry, P. R. (2009). "Wheat." Journal of Experimental Botany 60(6): 1537-1553.

Shiferaw, B., M. Smale, et al. (2013). "Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security." Food Security 5 (3): 291-317.

Singh, R. (2001). "Slow rusting genes based resistance to leaf and yellow rusts in wheat: genetics and breeding at CIMMYT." Proceedings of the 10th Assembly of the Wheat Breeding Society of Australia, Mildura, Australia, 16-21 September 2001: 103-108.

Singh, R. and J. Huerta Espino (2001). Global monitoring of wheat rusts and assessment of genetic diversity and vulnerability of popular cultivars. Research highlights of the CIMMYT Wheat Program 1999-2000.

Singh, R., J. Nelson, et al. (2000). "Mapping Yr28 and other genes for resistance to stripe rust in wheat." Crop Science 40 (4): 1148-1155.

Singh, R. P., P. A. Burnett, et al. (1993). "Bdv1: a gene for tolerance to barley yellow dwarf virus in bread wheats." Crop Science 33 (2): 231-234.

Singh, R. P., H. M. William, et al. (2004). Wheat rust in Asia: meeting the challenges with old and new technologies. Proceedings of the 4th International Crop Science Congress, The Regional Institute Ltd Gosford, Australia.

Solh, M., K. Nazari, et al. (2012). The growing threat of stripe rust worldwide. Borlaug Global Rust Initiative (BGRI) conference, Beijing, China.

Šramková, Z., E. Gregová, et al. (2009). "Chemical composition and nutritional quality of wheat grain." Acta Chimica Slovaca 2 (1): 115-138.

St. Clair, D. A. (2010). "Quantitative disease resistance and quantitative resistance loci in breeding." Annual Review Of Phytopathology 48: 247-268.

Stubbs, R. (1985). Stripe rust. Diseases, Distribution, Epidemiology, and Control, Elsevier: 61-101.

Trebbi, D., M. Maccaferri, et al. (2011). "High-throughput SNP discovery and genotyping in durum wheat (Triticum durum Desf.)." Theoretical and Applied Genetics 123 (4): 555-569.

Viljanen-Rollinson, S. and M. Cromey (2002). "Pathways of entry and spread of rust pathogens: Implications for New Zealand's biosecurity." New Zealand Plant Protection: 42-48.

Wang, S., D. Wong, et al. (2014). "Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array." Plant Biotechnol J.

Webster, J. A., K. J. Starks, et al. (1987). "Plant resistance studies with Diuraphis noxia (Homoptera: Aphididae), a new United States wheat pest." Journal of Economic Entomology 80 (4): 944-949.

Wellings, C. (2007). "Puccinia striiformis in Australia: a review of the incursion, evolution, and adaptation of stripe rust in the period 1979–2006." Australian Journal of Agricultural Research 58 (6): 567-575.

Wellings, C. and R. McIntosh (1990). "Puccinia striiformis f. sp. tritici in Australasia: pathogenic changes during the first 10 years." Plant Pathology 39 (2): 316-325.

Wellings, C. R. (2011). "Global status of stripe rust: a review of historical and current threats." Euphytica 179 (1): 129-141.

Williams, J. G., A. R. Kubelik, et al. (1990). "DNA polymorphisms amplified by arbitrary primers are useful as genetic markers." Nucleic Acids Research 18 (22): 6531-6535.

Zeven, A. C. (1998). "Landraces: a review of definitions and classifications." Euphytica 104 (2): 127-139.

Zhang, P., R. A. McIntosh, et al. (2009). "Wheat stripe rust resistance genes Yr5 and Yr7 are allelic." Theoretical and Applied Genetics 120 (1): 25-29.