Genetic Characterisation of Novel Resistance Alleles to Stem Rust and Stripe Rust in Wheat-Alien Introgression Lines

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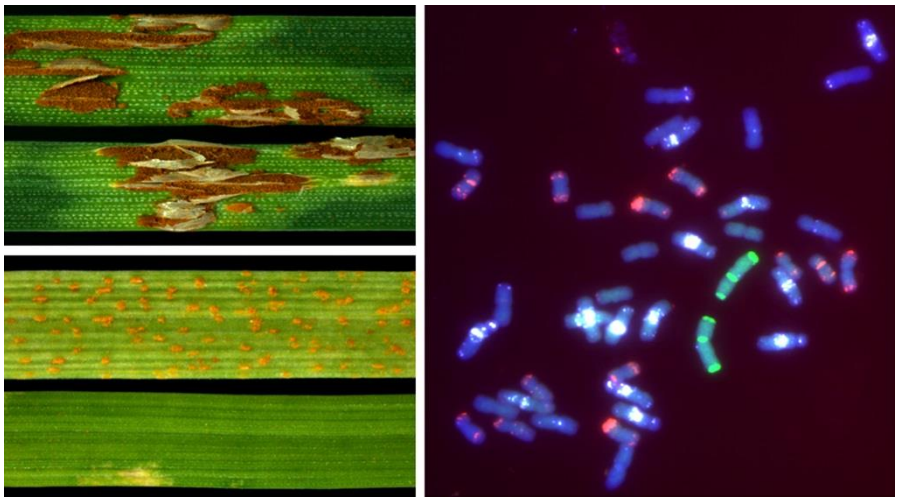

Doctoral Thesis
Swedish University of Agricultural Sciences
Alnarp 2016

Acta Universitatis agriculturae Sueciae
2016:78

Cover: Stem rust and stripe rust seedling reactions, and Fluorescent *in situ* hybridization method that detected 2R (2D) wheat-rye substitution

ISSN 1652-6880
ISBN (print version) 978-91-576-8658-9
ISBN (electronic version) 978-91-576-8659-6

Print: SLU Service/Repro, Alnarp 2016

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Abstract

Bread wheat (*Triticum aestivum* L., 2*n* = 6*x* = 42, AABBDD) is one of the most important food crops world-wide, but is attacked by many diseases and pests that cause significant yield losses. Globally, stem rust (*Sr*) (*Puccinia graminis* f. sp. *tritici* Erikss & E. Henning), stripe rust (*Yr*) (*Puccinia striiformis* Westend. f. sp. *tritici* Eriks) and leaf rust (*Lr*) (*Puccinia triticina* Eriks) are a great threat to wheat production. The majority of the *Sr*, *Yr* and *Lr* resistance genes are already defeated by numerous virulent races, so enhanced genetic resistance against these devastating diseases are essential. Wheat-alien introgressions from derivatives of *Secale cereale* L. (2*n* = 2*x* = 14, RR), *Leymus mollis* (2*n* = 4*x* = 28, NsNsXmXm), *Leymus racemosus* (2*n* = 4*x* = 28, NsXm) and *Thinopyrum junceiforme* (2*n* = 4*x* = 28; $J_1J_1J_2J_2$) are important genetic resources for new sources of resistance genes. To identify new sources of resistance, this thesis evaluated seedling and adult plant resistance to a wide array of stem rust and stripe rust races. Three wheat-rye disomic substitution lines 2R (2D) were found to carry new resistance gene/s to stem rust races and six multiple wheat-rye introgression lines with 5RS·5AL+4R+6R carried new resistance gene/s to stripe rust races. At adult plant stage, the wheat-rye translocation line with 1BL·1RS and 2RL·2BS exhibited low susceptibility to race TTKSK under field conditions.

The wheat-rye T2DS·2RL Robertsonian translocation line (TA5094) with a new stem rust resistance gene was developed through the breakage-fusion mechanism and verified using seedling resistance assays and molecular and cytogenetic analyses. Three kompetitive allele-specific PCR (KASP) markers located on rye chromosome 2RL were identified as being closely associated with the new stem rust resistance gene. Fluorescence *in situ* hybridisation (FISH) analysis confirmed the resistance gene in $F_{3:4}$ homozygous lines. The stem rust resistance gene in TA5094 line on chromosome 2RL arm was designated *Sr59*.

Wheat cultivars, advanced lines and landraces from Tajikistan were assessed at seedling and adult plant stages against *Sr*, *Yr* and *Lr* races. Based on multipathotype assessment and molecular markers, the presence of *Sr6*, *Sr31*/*Yr9*/*Lr26*, *Sr38*/*Yr17*/*Lr37*, *Yr2* and *Yr27* and pleiotropic resistance genes *Sr57/Lr34/Yr18/* and *Sr2/Yr30*/*Lr27* was postulated.

Overall, this thesis identified novel genetic resistance resources against stem rust, stripe rust and leaf rust in Tajik wheat and in wheat-alien introgressions. This resistance gene/s will be useful in diversifying the current set of resistance genes deployed to control these devastating diseases.

Keywords: Chromosome engineering, gene postulation, KASP, *ph1b*, *Secale cereale*, *Triticum aestivum*, translocation, Ug99

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Dedication

To my family

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Rahmatov, M., M.N. Rouse, B.J. Steffenson, S.C. Andersson, R. Wanyera, Z.A. Pretorius, A. Houben, K. Nazari, S. Bhavani, and E. Johansson. 2016a. Sources of stem rust resistance in wheat-alien introgression lines. *Plant Disease* 100: 1101-1109. doi:10.1094/PDIS-12-15-1448-RE.

II Rahmatov, M., M.N. Rouse, J. Nirmala, T. Danilova, B. Friebe, B.J. Steffenson, and E. Johansson. 2016b. A new 2DS·2RL Robertsonian translocation transfers stem rust resistance gene *Sr59* into wheat. *Theoretical and Applied Genetics* 129: 1383-1392. doi:10.1007/s00122-016-2710-6.

III Rahmatov, M., L. Garkava-Gustavsson, R. Wanyera, B. Steffenson, M.N. Rouse and E. Johansson. 2015. Stem rust resistance in 1BL·1RS and 2RL·2BS double wheat-rye translocation lines. *Czech Journal of Genetics and Plant Breeding* 51: 148-154.

IV Rahmatov, M., M. Hovmøller, K. Nazari, S.C. Andersson, M.N. Rouse, B.J. Steffenson and E. Johansson. Seedling and adult plant stripe rust resistances in diverse wheat-alien introgression lines. (Submitted)

V Rahmatov, M., M. Otambekova, H. Muminjanov, M.N. Rouse, M. Hovmøller, K. Nazari, B.J. Steffenson, J.A. Kolmer and E. Johansson. Stem, stripe and leaf rust seedling and adult plant resistances in Tajik bread wheat. (Manuscript)

The contribution of Mahbubjon Rahmatov to the papers included in this thesis was as follows:

I Planned and conducted the experiments, analysed the data and wrote the manuscript with the input of the co-authors.

II Planned the experiment with the supervisors, conducted most of the experiments and wrote the first draft of the manuscript. All the co-authors contributed to writing and editing the final manuscript.

III Planned and conducted the experiments, analysed the data and wrote the manuscript with the input of the co-authors.

IV Planned and conducted the experiments, analysed the data and wrote the manuscript with the input of the co-authors.

V Planned and conducted the experiments, analysed the data and wrote the manuscript with the input of the co-authors.

Abbreviations

APR	Adult Plant Resistance
CC-NB-LRR	Coiled Coil-Nucleotide Binding-Leucine Rich Repeat Protein
CIMMYT	International Maize and Wheat Improvement Centre
FISH	Fluorescence *in situ* Hybridisation
GBS	Genotyping-by-Sequencing
GISH	Genomic *in situ* Hybridisation
GRRC	Global Rust Reference Center
GS	Genomic Selection
GWAS	Genome-Wide Association Mapping
ICARDA	International Center for Agricultural Research in Dry Areas
IWGSC	International Wheat Genome Sequencing Consortium
LRR-TrD-PEST-ECS	Leucine Rich Repeat-Transmembrane Domain-Proline Glycine Serine Threonine-Endocytosis Cell Signalling Domain
MAS	Marker Assisted Selection
NBS-LRR	Nucleotide Binding Site-Leucine Rich Repeat
NBS-LRR-TIR	Nucleotide Binding Site-Leucine Rich Repeat-Toll Interleukin-1 Receptor
NLRs NOD	Nucleotide Binding and Oligomerisation Domain-Like Receptors
QTL	Quantitative Trait Locus
RCRRC	Regional Cereal Rust Research Center
SLU	Swedish University of Agricultural Sciences
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
TIR-NBS-LRR-NLS-WRKY	Toll Interleukin-1 Receptor-Nucleotide Binding Site-Leucine Rich Repeats-Nuclear Localisation Signal-Amino Acid Domain
TrD-CC	Transmembrane Domain-Coiled Coil
UM	University of Minnesota
USDA-ARS, CDL	United States Department of Agriculture-Agricultural Research Service, Cereal Disease Laboratory

1 Introduction

The primary goal of plant breeding is to improve global food security for human civilisation, fulfilling the needs of both producers and consumers (Fedoroff 2015). However, growing threats from climate change, including global warming, places pressure on breeders to increase the genetic gain and adaptability of new crop cultivars (Steenwerth et al. 2014). Furthermore, world-wide demand for food is increasing rapidly due to a rising global population and, as a consequence, the competition for arable land for food production is also increasing (Ray et al. 2013; Zabel et al. 2014). The ability to meet these demands requires the development of modern crop cultivars that are adapted to a range of adverse environmental conditions, including production in marginal crop production areas. Therefore, breeding will play an essential role in developing modern cultivars that are adapted to current and future adverse environments.

Wheat (*Triticum aestivum* L., $2n = 6x = 42$, ~17 Gb, AABBDD genome) is a major cereal crop cultivated world-wide and contributes substantially to human daily calories and food security (Braun et al. 2010). Wheat was already cultivated about ~10 000 years ago and became domesticated in the Fertile Crescent and Mediterranean regions (Feldman and Levy 2015). From that time onwards, farmers have continually made selections of the best genotypes, starting with emmer and einkorn grasses, for favourable traits such as ease of threshing and grain yield (Nevo et al. 2002). Since then, wheat has become the worldôs largest and most important food crop for direct human consumption. A total of 95% of the daily breads, cakes and pastries that humans consume come from bread wheat, while the remaining 5% come from tetraploid durum wheat ($2n = 4x = 28$; ~12 Gb, AABB genomes) (Dubcovsky and Dvorak 2007). Global production of bread wheat in 2014 was 725 million tonnes, with an average yield of 3 t/ha (FAO 2015).

The global human population will be more than 9 billion by 2050 (McKenzie and Williams 2015), and agriculture will be required to meet the food security needs of this growing population. The demand for wheat is continually increasing, with estimates indicating a requirement for a 60% increase in wheat production by 2050 (Ray et al. 2013). To reach that goal, wheat breeding needs to focus heavily on genetic improvements to increase grain yield (Valluru et al. 2014). A

tremendous improvement in wheat productivity has been achieved over the past decades, a development to which the Green Revolution technologies have contributed (Pingali 2012). For future wheat improvement, the focus has turned to exploitation of the genetic diversity within Triticeae species, which have the ability to contribute resistance to diverse biotic and abiotic stresses (Kole 2011; Mujeeb-Kazi et al. 2013). The exploitation of wheat diversity resulted in the Green Revolution, which was primarily due to widespread use of genetically improved cultivars with high yields (Khush 2001). To further enhance the breeding efficiency, techniques such as genomic selection, sequencing, phenomics and other omics methodologies could make a substantial contribution. Wheat production is currently facing several challenges, such as the emergence of novel pathogens and pests, as well as abiotic stresses.

The fungal diseases stem rust (caused by *Puccinia graminis* f. sp. *tritici* Erikss & E. Henning), stripe rust (caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks) and leaf rust (caused by *Puccinia triticina* Eriks) result in significant yield losses to wheat production world-wide (Kolmer 2005; Hovmøller et al. 2011; Szabo et al. 2014). Stem rust can cause up to 100% yield losses, stripe rust up to 100% losses and leaf rust up to 70% losses in susceptible wheat cultivars (Chen 2005; Huerta-Espino et al. 2011; Singh et al. 2015). Several epidemics and outbreaks of stem, stripe and leaf rust have significantly threatened the food security and livelihoods of poor farmers in many wheat growing regions world-wide (Wellings 2011; Solh et al. 2012). Moreover, the emergence and spread of novel stem, stripe and leaf rust races have led to the breakdown of most of the widely deployed resistance genes used in wheat production (Huerta-Espino et al. 2011; Wellings 2011; Singh et al. 2015). However, deployment of host-plant genetic resistance is still seen as the most economically and environmentally safe approach to reduce losses due to rust diseases in wheat (Burdon et al. 2014; Singh et al. 2016). Marker-assisted selection (MAS) is seen as a promising tool to enhance the efficiency of the wheat breeding process.

This thesis examined the usefulness of wheat-alien introgression derivatives from *Secale cereale*, *Leymus mollis*, *Leymus racemosus* and *Thinopyrum junceiforme* as novel sources of resistance to stem and stripe rust diseases in wheat, and then their corresponding resistance genes were revealed with molecular and cytogenetics approaches. Furthermore, Tajik wheat breeding lines, landraces and cultivars were evaluated for presence of stem, stripe and leaf rust resistances.

2 Background

2.1 Stem rust

Wheat stem rust (also known as black rust) is caused by the fungus *Puccinia graminis* f. sp. *tritici* Erikss. & E. Henning, which belongs to the phylum Basidiomycota, class Urediniomycetes, order Uredinales and family Pucciniaceae (Szabo et al. 2014). This family contains 17 genera and approximately 4121 species, of which the majority belong to the genus *Puccinia* (Leonard and Szabo 2005). Stem rust is one of the most devastating diseases of wheat, oats, barley, rye and wild cereal grasses, resulting in 80-100% yield losses (Singh et al. 2011; Szabo et al. 2014). Details of wheat stem rust were first reported in 1767 (Fontana 1932; Tozzetti 1952). *Puccinia graminis* f. sp. *tritici* (*Pgt*) is a heteroecious fungus and the requirement for a complete life cycle is the presence of wheat as a primary host and *Berberis* spp. (barberry) as an alternate host (Jin 2010). The life cycle of *Pgt* mostly consists of asexual, cyclical uredinial generations. As uredinia mature, teliospores form in order to produce basidiospores (Szabo et al. 2014). In regions with cold winters, *Berberis* spp. serve as a source of primary inoculum to infect wheat via aeciospores in spring (Leonard and Szabo 2005). In warm regions, the weather creates ideal conditions for the stem rust cycle, including a green bridge that can carry the rust pathogen into the next season (Park et al. 2011). Several major wheat stem rust epidemics occurred in the 20th century, resulting in significant yield losses (Roelfs 1985; Hodson 2011; Dean et al. 2012; Singh et al. 2015). Recent epidemics (2013-2014) have also occurred caused by the race TKTTF in Ethiopia, on the variety ôDigaluô carrying wheat resistance gene *SrTmp*. Such epidemics have led to severe yield reductions, thereby highlighting the dynamic challenges for breeders in breeding for stem rust resistance (Olivera et al. 2015). Stem rust epidemics were one driver behind the breeding programmes that initiated the Green Revolution in 1960-1970. Stem rust resistance genes have been incorporated successfully into high yielding semi-dwarf wheat cultivars, with significant reduction of stem rust incidence globally (Hodson 2011). This has

played a great role for the global reduction of stem rust to near insignificant levels in the last 20-30 years (Hodson 2011). However, in 1999 a new strain of *Puccinia graminis* f. sp. *tritici*, Ug99, also called race TTKSK, was reported in central Africa, and emergence of this race is suggested to pose a major threat to global wheat production (Pretorius et al. 2000; Singh et al. 2011). The subsequent emergence of a widely virulent group of stem rust races in the Ug99 lineage, such as TTKST, TTTSK, TTKSF+, TKTTF, TTKTK and TTKTT, has rendered the *Sr9h*, *Sr24*, *Sr31*, *Sr36* and *SrTmp* resistance genes ineffective (Rouse et al. 2014a; Patpour et al. 2015a; Patpour et al. 2015b; Singh et al. 2015). The resistance genes *Sr13* and *Sr1RS*^{Amigo} are effective against race TTKSK, but not race TRTTF (Olivera et al. 2012). Furthermore, another race, TKTTF (not a member of the Ug99 lineage), has recently spread widely in several countries and caused severe epidemics (Olivera et al. 2015). All these stem rust pathogen races are currently spreading in the major wheat production regions, starting from Africa, through the Middle East and are expected to progress farther (Singh et al. 2015).

2.2 Stripe rust

The basidiomycete fungus *Puccinia striiformis* Westend f. sp. *tritici* Eriksson is the causal agent of stripe rust (also known as yellow rust) in cereal crops and grasses, and is considered to be the most economically important disease of wheat production world-wide (Hovmøller et al. 2011). Stripe rust was first documented by Gadd and Bjerkander in 1777 and there was an epidemic on rye in 1794 in Sweden (Eriksson and Henning 1896). The fungus has previously been characterised as *Uredo glumarum*, *Puccinia striaeformis*, *Puccinia straminis* and *Puccinia glumarum*, until it was given its present name, *Puccinia striiformis* (Schmidt 1827; Westendorp 1854; Fuckel 1860; Eriksson and Henning 1894; Hylander et al. 1953). *Puccinia striiformis* f. sp. *tritici* belongs to the Pucciniaceae family, order Uredinales, phylum Basidiomycota and class Basidiomycetes (Chen et al. 2014). Until recently, the alternate host of stripe rust was unknown and urediniospores were considered the only source of inoculum. However, in recent studies *Berberis* spp. have been shown to serve as the alternate host for stripe rust populations (Jin et al. 2010). The teliospores germinate into aerial basidiospores and then infect the alternate *Berberis* host (Hovmøller et al. 2011; Rodriguez-Algaba et al. 2014). It is thought that the centre of origin for the stripe rust pathogen is South-East Asia, the Middle East, East Africa, Transcaucasia, Himalayan, Mediterranean and Central Asia (Ali et al. 2014). However, stripe rust is widespread globally and causes significant yield losses every year (Chen 2005; Wellings 2011). Destructive stripe rust epidemics in wheat have often proven difficult to control even with fungicide application, and in recent epidemics in Central Asia, West Asia and Africa up to 80% yield losses have been reported. One

reason is that the pathogen has overcome the major resistance gene/s in widely cultivated wheat cultivars (Wellings 2011; Solh et al. 2012; Beddow et al. 2015; Jighly et al. 2015). With the emergence of new stripe rust races, several important resistance genes, such as *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17* and *Yr27*, are no longer effective (Zegeye et al. 2014; Jighly et al. 2015; Maccaferri et al. 2015). Therefore, there is a need to utilise different genetic stocks in order to obtain an effective disease management strategy and to broaden the genetic base of stripe rust resistance in wheat.

2.3 Leaf rust

Leaf rust (also known as brown rust), caused by *Puccinia triticina* Eriks, is a serious, widespread and damaging disease in wheat (Kolmer 2005). *Puccinia triticina* also belongs to the Pucciniaceae family, order Uredinales, phylum Basidiomycota and class Basidiomycetes (Kolmer 2013). It is an obligate biotrophic pathogen that is macrocyclic. Leaf rust also causes large crop losses every year (Huerta-Espino et al. 2011). The severity of leaf rust disease varies in cultivated wheat depending on environmental conditions, inoculum levels and susceptible host cultivars. A highly favourable environment together with a high proportion of leaf rust-susceptible or moderately susceptible wheat cultivars grown in an area allow severe epidemics (Huerta-Espino et al. 2011). Historical epidemics of leaf rust have occurred in many wheat growing regions of the world and have caused severe economic losses (Bolton et al. 2008; Huerta-Espino et al. 2011). Leaf rust has been controlled by deployment of genetic resistance from bread wheat or from gene pools in related species to wheat (Kolmer 2013). Similarly to the other rusts, emergence and evolution of new leaf rust races have contributed to breakdown of many resistance genes and these races are threatening wheat productions world-wide. The *Lr9*, *Lr14a*, *Lr16*, *Lr17a*, *Lr24*, *Lr26* and *Lr41* resistance genes for leaf rust have been individually defeated by the new races (Huerta-Espino et al. 2011). Therefore, use of novel genetic resistance genes is the primary strategy available for preventing yield losses caused by leaf rust disease.

3 Types of host resistance

All kinds of host resistance, including those for the three major rusts discussed above, can be characterised as qualitative or quantitative resistance. Qualitative resistance is often race-specific, monogenic (consists of major genes), hypersensitive and expressed at the seedling stage, which usually means that the resistance is expressed in all plant stages. Quantitative resistance is often race-nonspecific, slowing rust progression, polygenic (minor genes), durable and may be only expressed at the adult plant stage (adult plant resistance; APR).

3.1 Seedling resistance

At present, a total of approximately 70 each of stem rust resistance (*Sr*), stripe rust resistance (*Yr*) and leaf rust resistance (*Lr*) seedling genes have been identified in wheat (McIntosh et al. 1995b; Huerta-Espino et al. 2011; Maccaferri et al. 2015; Singh et al. 2015). In general, the seedling resistance approach is an effective way to identify genes contributing effective resistance during the entire stages of plant growth. Genetically, the major genes within the rust pathosystems usually display gene-for-gene interaction, i.e. an avirulence gene in the pathogen is matched to a resistance gene in the host (Flor 1955). The recognition of the pathogen molecule by the host is currently described as effector-triggered immunity (ETI) (Jones and Dangl 2006). In short, the host resistance genes encode receptors that are only capable of recognising specific pathogen effector molecules, and thereafter the effector molecules are encoded by a corresponding avirulence gene in the pathogen (Bent and Mackey 2007). Seedling resistance genes mostly encode immune receptors of the nucleotide binding site-leucine rich repeat (NBS-LRR) (Periyannan et al. 2013; Saintenac et al. 2013; Liu et al. 2014), whereas APR genes have been found to encode a kinase-START and ABC transporter (Fu et al. 2009; Krattinger et al. 2009) and non-ABC transporter (Moore et al. 2015). Cloned resistance genes of some *Sr*, *Yr* and *Lr* resistance genes in wheat have been shown to encode proteins with NBS-LRR domains. The NBS-LRR proteins from wheat

interact functionally and physically to mediate resistance to the rust pathogens and accomplish different functions in avirulence recognition (Jones and Dangl 2006; Bent and Mackey 2007). Upon detection of pathogen molecule activity, disease resistance proteins signal to downstream factors, resulting in induction of the defence response (Jones and Dangl 2006). The seedling resistance type often confers major-effect defence responses that involve chlorosis or necrosis, in order to limit the formation and spread of fungal hyphae and uredinia in the host cell. This type of resistance is highly effective and easily manipulated in breeding programmes to improve crop resistance. However, the seedling resistance gene often leads to a boom and bust cycle, thereby resulting in large-scale epidemics.

3.2 Adult plant resistance

Adult plant resistance is only expressed at the adult stage of the plant, i.e. when the plant matures into its reproductive phase. The general function of APR is to extend the latent period and reduce sporulation. Several genes that confer APR to all three rusts have been identified (Rosewarne et al. 2013; Yu et al. 2014; Gao et al. 2016). Adult plant resistance can be pleiotropic, as exemplified by the *Sr2*/*Yr30*/*Lr27*, *Sr55/Yr46*/*Lr67*, *Sr57/Yr18*/*Lr34* and *Sr58/Yr29*/*Lr46* APR genes (McFadden 1930; Fu et al. 2009; Herrera-Foessel et al. 2010; Yang et al. 2013; Lan et al. 2014). Moreover, *Sr12* and *Sr57* have been found to act in concert in conferring APR (Rouse et al. 2014b). To fully elucidate the genetic architecture of APR, molecular mapping studies are a highly effective approach. Adult plant resistance often provides resistance against a wide range of pathogen races (Krattinger et al. 2009; Herrera-Foessel et al. 2010; Moore et al. 2015). Another feature of the APR genes is that they confer a slow-rusting form of resistance that delays disease progression. A further type of resistance, to stripe rust in particular, is high-temperature adult-plant resistance (HTAP), which is often associated with race non-specific resistance and only expressed during the adult plant stage at higher temperatures (Chen and Line 1995).

3.3 Durable resistance

The concept of durable resistance was first introduced and described by Dr. Roy Johnson as ñresistance that remains effective during its prolonged and widespread use in an environment favorable to the diseaseò (Johnson 1984). However, the durable resistance definition does not provide any statement or indication about the genetic control of the resistance or its race specificity. Nevertheless, APR genes are in general more durable than seedling genes and they can interact in an additive and/or epistatic manner when pyramided, leading to very high levels of resistance that can remain effective for a long period (Ayliffe et al. 2008; Rouse et al. 2014b;

Brown 2015). Classic examples of durable resistance are the *Sr2*/*Yr30/Lr27* and *Sr57/Yr18*/*Lr34* pleiotropic genes, which have provided long-lasting and widely-used durable partial resistance. Furthermore, these genes have been used in conjunction with additional major and minor resistance genes in order to obtain adequate levels of rust resistance (Singh et al. 2011; Ellis et al. 2014). The *Sr31* major-effect, race-specific gene demonstrated durable resistance and was therefore deployed widely and provided stable resistance against stem rust races world-wide for over 30 years in commercial wheat cultivars (Singh et al. 2008). However, emergence of the rust race TTKSK (Ug99) led to breakdown of *Sr31* in Uganda in 1998 (Pretorius et al. 2000), although *Sr31* still contributes resistance to all other stem rust races except the TTKSK race group. Wheat breeding strategies are striving to accumulate sources of durable resistance, incorporating both seedling and APR genes into breeding lines, to achieve modern wheat cultivars with durable resistance to the three major rust diseases.

4 Wheat breeding for rust resistance

Traditional wheat breeding for wheat improvement includes crossing and backcrossing between cultivars for transfer of a few genes through recombination and selection events. Therefore wheat breeding relies on sources of genetic variation in order to adapt wheat to particular environments for effective improvement. Through wheat breeding efforts, numerous agronomic traits, bread quality and sources of disease resistance have been deployed in wheat cultivars world-wide. For example, the 1BL·1RS wheat-rye translocation with multiple disease resistance *Sr31*/*Yr9*/*Lr26*/*Pm8* and other useful traits has been widely distributed by the International Maize and Wheat Improvement Centre (CIMMYT) and other breeding programmes world-wide. The use of resistance is still seen as the most economically and environmentally friendly strategy for wheat breeding against destructive fungal diseases such as stem, stripe and leaf rust (Ellis et al. 2014). Marker-assisted selection approaches provide tools that help wheat breeding to become more efficient and accurate and have made a great contribution to wheat improvement and gene pyramiding. Furthermore, genome-wide association mapping (GWAS) and genomic selection (GS) promise to advance future wheat breeding programmes. Moreover, synthetic wheat has mediated the introgression of valuable sources of resistance to diseases and pests, as well as tolerance to abiotic stresses (Mondal et al. 2016).

4.1 Wheat gene pools as a source of rust resistance in wheat

Hexaploid wheat carries the AA genome from *Triticum urartu*, the BB genome from *Aegilops speltoides* and the DD genome from *Aegilops tauschii* (Faris 2014). According to their crossability with hexaploid wheat, the relatives of wheat can be divided into three major gene pools (the primary, secondary and tertiary gene pools) (Mujeeb-Kazi et al. 2013). Gene pool classification has also been based on evolutionary and cytogenetically relationships and on homologous and homoeologous chromosome pairing (Chaudhary et al. 2014). The primary gene

pool consists of species that can be crossed through direct hybridisation, homologous recombination and relatively simple breeding strategies. Genetic transfer in the secondary gene pool is also possible through direct crosses and backcrosses to utilise the homologous pairing between common genomes, but some of the species (*Aegilops* spp.) in this gene pool require manipulative methods. The tertiary gene pool includes the diploid and polyploid *Triticeae* species that are extremely difficult to cross with wheat through direct hybridisation and homologous recombination (Molnár-Láng et al. 2014; Molnár-Láng 2015). Introgressions into wheat from the tertiary gene pool have most often been facilitated by irradiation, tissue culture, use of the *ph1b* mutant and embryo rescue techniques. Several intergeneric hybridisations have been made between wheat and *Aegilops*, *Leymus*, *Haynaldia*, *Secale*, *Dasypyrum*, *Hordeum*, *Thinopyrum* and *Agropyron* species, to produce wheat-alien introgressions for wheat breeding, using the wheat-alien species as new sources of genetic diversity (Merker 1984; Merker and Rogalska 1984; Merker and Lantai 1997; Kole 2011; Mujeeb-Kazi et al. 2013; Schneider et al. 2016).

Utilisation of wild species is one of the best strategies in wheat breeding in order to bring new genetic variation into the hexaploid wheat gene pool. The rust resistance genes in the hexaploid wheat gene pool are often broken down through the constant evolution and mutation of stem, stripe and leaf rust pathogen races. Therefore, relatives of wheat have been used to contribute rust resistance genes, while genes conferring numerous other traits have been transferred from these species into bread wheat (Kole 2011; Molnár-Láng et al. 2015). Rye (*Secale cereale* L., $2n = 2x = 14$, ~8 Gb, RR genome), a temperate cereal crop, is part of the valuable gene pool for wheat improvement, especially as a source of broad tolerance to biotic and abiotic stresses (Martis et al. 2013; Schlegel 2014). The *Sr27*, *Sr31*/*Yr9*/*Lr26*/*Pm8*, *Sr50*, *Sr1RS*^{Amigo} and *SrSatu* resistance genes have all originated from the 1R and 3R rye chromosomes and these genes have contributed to the control of wheat rust and powdery mildew diseases (Marais and Marais 1994; Friebe et al. 1996; Mago et al. 2002; Singh et al. 2011; Olivera et al. 2013). Furthermore, the chromosome 2R from different rye genotypes has been described as a source of resistance to various wheat diseases and insects and this chromosome has also contributed to various agronomic traits (Hysing et al. 2007; Lei et al. 2013). The chromosome arm 2RL is the source of resistance to Hessian fly mediated by the resistance gene *H21* present in the form of a T2BS·2RL Robertsonian translocation (Friebe et al. 1990; Cainong et al. 2010). The 2R chromosome is also the source of the leaf rust resistance gene *Lr45* that is present on a T2AS-2RS·2RL terminal translocation chromosome (McIntosh et al. 1995a). Furthermore, the wheat-rye introgressions have been characterised as sources of resistance to Russian wheat aphid and cereal aphids (Crespo-Herrera et al. 2013; Andersson et al. 2015). Other important resistance genes, such as *Sr24*, *Sr25*, *Sr26*

and *Sr43*, originate from wheat-*Thinopyrum ponticum* introgressions, while *Sr44* originates from wheat-*Thinopyrum intermedium* introgression and *Sr52* originates from wheat-*Dasypyrum villosum* introgression (Mago et al. 2005a; Liu et al. 2010; Niu et al. 2014). These genes have thus been transferred from the tertiary gene pool and are now being used in wheat breeding. The leaf rust resistance genes *Lr19*, *Lr24*, *Lr25*, *Lr29* and *Lr38* are also derived from the tertiary gene pool (McIntosh et al. 1995b; Dedryver et al. 1996; Zhang et al. 2005; Qi et al. 2011). Moreover, *Yr5* is derived from *Triticum spelta album* (Yan et al. 2003) and *Yr15*, *YrH52* and *Yr36* comes from *Triticum turgidum* ssp. *dicoccoides* (Peng et al. 2000; Fu et al. 2009). Furthermore, *Secale cereale*, *Leymus mollis*, *Leymus racemosus* and *Thinopyrum junceiforme* have all proven to be useful as genetic resources for wheat breeding against rust diseases (Merker 1984; Merker and Lantai 1997; Ellneskog-Staam and Merker 2002; Kole 2011). Thus, the secondary and tertiary gene pools are extremely valuable sources of novel alleles suitable for introgression into the hexaploid genome. The majority of the *Sr* resistance genes are derived from the primary and secondary gene pools, but more than 70 resistance genes are from the tertiary gene pool (McIntosh et al. 1995b; Singh et al. 2015). The majority of the *Yr* and *Lr* genes originate from the primary gene pool, although several of the *Yr* and *Lr* genes are also derived from the secondary and tertiary gene pools (e.g. *Aegilops* spp., *Brachypodium*, *Secale cereale*, *Thinopyrum* spp. etc.) (McIntosh et al. 1995b; McCallum et al. 2012; Chen et al. 2014; Maccaferri et al. 2015; Gao et al. 2016). Moreover, a number of the *Sr*, *Yr* and *Lr* APR (quantitative trait locus, QTL) genes have been identified as originating from all three gene pools (Rosewarne et al. 2013; Yu et al. 2014; Gao et al. 2016).

4.2 Functional analysis and gene cloning of rust resistance genes

Plant resistance genes are divided into: nucleotide binding site-leucine rich repeats-toll interleukin-1 receptor (NBS-LRR-TIR), nucleotide binding site-leucine rich repeats-coiled coil (NBS-LRR-CC), leucine rich repeats-transmembrane domain (LRR-TrD), transmembrane domain-coiled coil (TrD-CC), toll interleukin-1 receptor-nucleotide binding site-leucine rich repeats-nuclear-localization signal-amino acid domain (TIR-NBS-LRR-NLS-WRKY), leucine rich repeats-transmembrane domain-proline glycine serine threonine-endocytosis cell signalling domain (LRR-TrD-PEST-ECS) and enzymatic R-genes, based on their amino acid motif and membrane spanning domains (Gururani et al. 2012). The nucleotide-binding oligomerisation domain receptors (NLRs) have been identified as an important part of the cell death and pathogen immunity pathways in the crop (Franchi et al. 2006). Plant disease resistance can also be triggered by plant immune receptors encoding nucleotide binding-leucine rich repeats (NB-LRR),

which thereby recognise microbial effectors (Jones and Dangl 2006). To better understand the functionality of rust resistance genes in wheat, a number of *Sr*, *Yr* and *Lr* genes have been cloned. Through this method, the wheat stem rust resistance genes *Sr33* and *Sr35* (Periyannan et al. 2013; Saintenac et al. 2013), the stripe rust resistance genes *Yr10*, *Yr18* and *Yr36* (Fu et al. 2009; Krattinger et al. 2009; Liu et al. 2014) and the leaf rust resistance genes *Lr1*, *Lr10*, *Lr21* and *Lr34* have been found encoding NBS-LRR proteins, a kinase-START and ABC transporter proteins (Feuillet et al. 2003; Huang et al. 2003; Cloutier et al. 2007; Krattinger et al. 2009). The functional resistance mechanisms of *Sr33*, *Sr35*, *Yr10*, *Lr1*, *Lr10* and *Lr21* have been found to be connected to the encoding of NBS-LRR proteins, whereas the *Sr57*/*Yr18*/*Lr34* and *Yr36* genes have not been found to encode such proteins. Recent cloning of the *Lr67* resistance gene, which contributes partial resistance to all three wheat rusts and to powdery mildew, shows that this is explained by encoding of an orthologous hexose transporter. The only difference between this hexose transporter and those found in susceptible forms of wheat is the presence of two amino acids that have been conserved in the orthologous hexose transporter (Moore et al. 2015). The stem rust resistance gene *Sr50* has been identified as being homologous to the barley *Mla* gene encoding coiled coil-nucleotide binding-leucine rich repeat (CC-NB-LRR) proteins (Mago et al. 2015). In a recent study, a new technology called MutRenSeq was able to accurately pinpoint the location of different resistance genes. Cloning of the stem rust resistance genes *Sr22* and *Sr45* has been carried out using the MutRenSeq technique (Steuernagel et al. 2016).

4.3 Chromosome rearrangements - aneuploid, substitution and translocation lines

Basic types of chromosome rearrangements are known as segmental chromosome interchanges, duplications, deletions, inversions, translocations and substitutions, and all of these rearrangements have been successfully reported for rearrangements of alien species into the wheat genome. Thus, *Aegilops* spp., *Hordeum* spp., *Leymus* spp., *Thinopyrum* spp. and *Secale cereale* have been introgressed into the wheat genome for improvement of resistance to biotic and abiotic stresses. Sears (1954), was the first to report the presence of a complete set of aneuploids in the common hexaploid wheat cultivar ‘Chinese Spring’. This set comprises series of monosomics, nullisomic, nullisomic-tetrasomics and ditelosomics and has since been widely utilised for genetic studies in wheat (Sears 1966). The first spontaneous 5R (5A) wheat-rye chromosome substitution was identified by Kattermann (1937) and O'Mara (1940). Substitutions of the wheat chromosomes 1D, 3A, 3B and 3D with the rye chromosome 3R have also been described (Driscoll and Anderson 1967), and 1R (1D) and 1R (1A) wheat-rye substitutions

have been produced to improve resistance to biotic and abiotic stresses in wheat (Koebner and Singn 1984; Müller et al. 1989). Other studies have demonstrated the option of applying centric misdivision followed by the breakage-fusion mechanism of broken arms, leading to the formation of Robertsonian translocations (Robertson 1916), which could be of great importance in resistance breeding. Accordingly, Robertsonian translocations have been used in a number of studies to develop wheat-rye translocations (Merker 1982; Zhang et al. 2001). In nature, spontaneous wheat-rye translocation events are routinely found in wheat-rye substitution lines, and this fact has been used to develop 1BL·1RS wheat-rye translocations (Mettin et al. 1973; Zeller 1973). In this way, the 1BL·1RS wheat-rye translocation, containing the resistance genes *Sr31*/*Yr9*/*Lr26*/*Pm8* originating from rye cultivar ‗Petkus', was developed and spread to wheat breeding programmes world-wide through the wheat cultivars ‗Kavkaz' and ‗Aurora' (Rajaram et al. 1983). Other wheat-rye translocations such as 1AL·1RS (*Sr1RS*^{Amigo}) from rye cultivar ‗Insave' and 1DL·1RS (*Sr50*) from rye cultivar ‗Imperial' have been identified and spread as sources of resistance to stem rust (The et al. 1991; Mago et al. 2015). Furthermore, the Danish wheat cultivar ‗Viking' with 4B-5R interchange has been found to carry a high content of iron, copper and zinc (Schlegel et al. 1993). Wheat-rye introgressions have also been produced with the rye chromosomes 2R, 3R and 6R incorporated into the wheat genome (Merker 1984; Mujeeb-Kazi et al. 2013). Homoeologous recombination between chromosomes 2B and 2R has been identified (Lukaszewski et al. 2004), as have the wheat-barley 2DS·2DL-1HS, 3HS·3BL, 6BS·6BL-4HL, 4D-5HS and 7DL·7DS-5HS translocations, which have been used for wheat improvement (Nagy et al. 2002). Furthermore, wheat-*D. villosum* 6AL·6VS and 6AL·6AS translocations have been produced and contain resistance genes to stem rust (*Sr52*), stripe rust and powdery mildew (*Pm21*) (Qi et al. 2011; He et al. 2016a). Although a range of different wheat lines with alien introgressions have been produced, however translocated lines are preferred for the development of commercial disease-resistant cultivars, due to the fact that smaller introgressions of alien chromatin lead to less linkage drag and regular meiotic behaviour (Friebe et al. 1996; Liu et al. 2011b; Niu et al. 2011; Niu et al. 2014; Tiwari et al. 2014). The main conventional techniques used to detect chromosome rearrangement events in the wheat genome are genomic *in situ* hybridisation (GISH), fluorescence *in situ* hybridisation (FISH) and C-banding.

4.4 Chromosome engineering and molecular cytogenetics

Induced homologous recombination and irradiation are the most commonly applied methods to introgress alien chromosome material into the wheat genome (Niu et al. 2011; Wang et al. 2012). For example, the leaf rust resistance gene *Lr9* from *Ae. umbellulata* (2*n* = 2*x* =14, UU genome) was introgressed through ionising

irradiation (Sears 1956). In general, regular and homologous pairing of genes from the tertiary gene pool with those of hexaploid wheat happens only rarely, due to the presence of the *Ph1* allele on chromosome 5B hindering this event (Riley and Chapman 1958). However, the *Ph1* locus can be deleted by the use of X-ray radiation in order to allow homoeologous pairing and recombination (Sears 1977). Therefore the *ph1b* mutants, promoting meiotic pairing between homoeologous chromosomes, have been used extensively to induce recombination between wheat and alien chromosomes (Qi et al. 2007; Niu et al. 2011). The use of the *ph1b* mutant has resulted in translocations harbouring resistance genes such as *Sr32* (Mago et al. 2013), *Sr39* (Niu et al. 2011), *Sr43* (Niu et al. 2014), *Sr47* (Klindworth et al. 2012), *Sr51* (Liu et al. 2011a) and *Sr53* (Liu et al. 2011b). Moreover, the *ph1b* mutant has successfully been used to improve the end-use quality of wheat containing the 1RS translocation. For this purpose, the *Sec-1* (secalin) allele on 1RS has been replaced by genes encoding wheat storage proteins (Lukaszewski 2000). Gametocidal chromosomes are also known to induce chromosomal mutation by random breakage, and these chromosomes have therefore been introduced into common wheat as a tool of chromosome manipulation for genetic improvement (Endo and Gill 1996; Endo 2007). Furthermore, the Ph^I (inhibitor) gene has been transferred from *Aegilops speltoides* to the ócChinese Springô cultivar, in order to induce homoeologous recombination between wheat and alien species (Chen et al. 1994). The breakage-fusion mechanism contributes to the production of wheat-alien Robertsonian translocations (Friebe et al. 2005). Thus, wheat lines containing Robertsonian translocations with disease resistance genes originating from *Dasypyrum villosum* and *Thinopyrum intermedium* have been produced applying the breakage-fusion mechanism (Qi et al. 2011; Zhan et al. 2015). Wheat-rye translocation lines have also been obtained by applying crossing and backcrossing with triticale for both winter and spring wheat and here also the breakage-fusion mechanism may have played a role (Merker 1984; Forsström and Merker 2001). Desirable genes have also been introgressed into wheat from wild species through distant hybridisation aided by tissue culture-based embryo rescue techniques (Wňdzony et al. 2014), e.g. *Leymus mollis*, *Leymus racemosus* and *Thinopyrum junceiforme* hybrids were developed by the embryo culture technique. The hybrids from these crosses were thereafter backcrossed once with the Hpph 5RL·5BS wheat-rye translocation line and *Triticum turgidum* var. *carthlicum*, which have the ability to form unreduced gametes in the hybrids (Merker and Lantai 1997). The first techniques used to identify alien chromosomes in the wheat genome were C-banding and N-banding (Gill and Kimber 1974; Gerlach 1977). The GISH and FISH approaches are two more recent microscopy-based techniques that can be used to detect the translocation breakpoint and these methods thereby effectively complement classical diagnostic and selection tools for more efficient and precise detection and

characterisation of the alien chromosome segment. Thus, GISH and FISH have been widely used to determine the chromosomal constitution of the wheat-alien introgressions in the mitotic metaphase cell stage (Zhang et al. 2001; Danilova et al. 2014). Furthermore, the GISH and FISH methods can be complemented with exome-captured sequence methods (Winfield et al. 2012). Chromosome engineering methodologies based on the manipulation of pairing control mechanisms and of induced introgressions from different wild species have thus been employed to transfer specific genes conferring biotic and abiotic stress resistance and wheat material containing these genes has been deployed for the benefit of farmers world-wide (Gill et al. 2006). The ultimate contribution of the above-mentioned methods is to enhance the diversity of alien alleles in wheat breeding and improvement. Wheat-alien introgression lines have a proven track record and great potential to enhance resistance to stem, stripe and leaf rust. All these methods have also been utilised to reduce the size of the alien chromatin in the wheat genome, in order to avoid linkage drag.

4.5 Marker-assisted selection

The use of molecular markers is a powerful technique to identify genetic diversity, allow complex trait dissection, map qualitative and quantitative traits and link these genetic traits to phenotypic variation (Hayward et al. 2015). Marker-assisted selection has been used successfully in diverse crop species to reveal genome wide polymorphisms at scales ranging from a single base pair up to duplications and translocations of entire chromosomal regions. The first and second molecular marker generations, restriction fragment length polymorphisms (RFLPs), randomly amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSR), have progressively been used to determine the genetic relationship between individuals in plant breeding. The SSR molecular markers have been widely used to tag and map resistance genes in wheat, rye and other wild relatives due to their high level of polymorphism compared with other markers (Röder et al. 1998; Saal and Wricke 1999; Khlestkina et al. 2004; Somers et al. 2004; Rey et al. 2015). Thus, a range of diagnostic molecular markers for the *Sr*, *Yr* and *Lr* resistance genes are available, e.g. for *Sr2*, *Sr6*, *Sr13*, *Sr21*, *Sr22*, *Sr31*, *Sr32*, *Sr33*, *Sr35*, *Sr38*, *Sr39*, *Sr45*, *Sr46*, *Sr47*, *Sr49*, *Sr52*, *Sr55*, *Sr56*, *Yr5*, *Yr9*, *Yr17*, *Yr36*, *Yr45*, *Yr48*, *Yr51*, *Yr59*, *Yr60*, *Lr9*, *Lr18*, *Lr25*, *Lr26*, *Lr34*, *Lr37*, *Lr38*, *Lr39*, *Lr47*, *Lr50*, *Lr51* and *Lr68*, and these have been used for MAS in wheat breeding (Goutam et al. 2015; Chhuneja et al. 2016; MASWheat 2016). Later generations of molecular markers such as single nucleotide polymorphisms (SNP), genotype-by-sequencing (GBS), diversity array technology (DArT) and kompetitive allele-specific PCR (KASP) markers, specifically used for high density genetic mapping, have revolutionised the options

for marker work. These markers are used in order to precisely locate the monogenic (single gene) or polygenic traits (conferred by more than one gene) of relevance in wheat breeding. High density genetic maps have robustly improved the precision and efficiency of QTL mapping in wheat breeding (Tian et al. 2015a; Tian et al. 2015b). Genome-wide association mapping (GWAS) is an approach that uses phenotyping and genotyping to identify chromosomal regions having a significant association with traits in wheat (Bajgain et al. 2015; Maccaferri et al. 2015; Gao et al. 2016). In addition, the implementation of genomic selection (GS) in wheat breeding is a promising approach to accelerate selection gain and improve complex traits (Daetwyler et al. 2014; He et al. 2016b). Thus, SNP, GBS and DArT markers are currently being widely used for GWAS and GS applications in wheat breeding. High-throughput sequencing technologies including chips of 9,000 SNPs (9K SNP chip genotyping) and 90,000 SNPs (90K SNP chip genotyping), DArT-seq and GBS (~41,000 SNPs) have been developed for use in wheat (Allen et al. 2011; Poland et al. 2012; Wang et al. 2014; Li et al. 2015). Furthermore, reduced-representation sequencing, restriction site-associated DNA sequencing (RAD-seq) and low coverage genotyping approaches, including multiplexed shotgun genotyping, have been made available (Davey et al. 2011). The RAD-seq approach for high-density mapping has contributed to mapping of stem rust resistance on chromosome 7AL of wheat (Pujol et al. 2015), while GBS- and array-derived SNP markers have been used to map the stem rust resistance gene *Sr42* on chromosome arm 6DS, with the GBS- and array-derived SNP markers then converted to KASP assays (Gao et al. 2015). The KASP assay is a robust molecular marker tool for detection of short introgressed segments in the wheat genome, thereby facilitating MAS applications (Semagn et al. 2014; Tiwari et al. 2014; Bernardo et al. 2015). A resistance gene effective against the Ug99 race group has been mapped on chromosome 7A in wheat and thereafter recombinant inbred lines have been screened with the KASP assay. Moreover, similar mapping results have been obtained while using the GBS and 9,000 SNP chip genotyping technologies to compare their mapping utilities in wheat (Bajgain et al. 2016). The GWAS approach has also been used to map the major and minor resistance genes of stem, stripe and leaf rust, by applying DArT and SNP markers closely linked to the resistance genes (Bajgain et al. 2015; Jighly et al. 2015; Maccaferri et al. 2015; Gao et al. 2016). The GS method has been found to be particularly important for the improvement of quantitative traits by the use of genome-wide marker coverage (like GBS and SNP markers) in order to predict breeding values (Rutkoski et al. 2010; Ornella et al. 2012; Daetwyler et al. 2014; Rutkoski et al. 2014). To produce small alien transfers without linkage drag in wheat, high-density molecular markers such as SNP and GBS should be used in order to detect the smallest translocation/introgression breakpoints possible in the wheat relatives. Bioinformatic pipelines for the SNP and GBS datasets are also useful instruments

that make it possible to analyse and interpret the GWAS and GS implementations. Prediction models have been used in wheat breeding to understand the accuracy of various resistance genes and options for using these genes without too much linkage drag (Ornella et al. 2012; Daetwyler et al. 2014; Rutkoski et al. 2014; He et al. 2016b). In such studies, wheat lines containing the *Yr40*/*Lr57* resistance genes and showing reduced linkage drag have been obtained (Tiwari et al. 2014). However, the resistance genes *Sr27*, *Sr39*, *Sr40 Sr43*, *Sr44* etc. have not been widely used due to concerns about linkage drag (Singh et al. 2015).

4.6 Phenomics and omics in wheat breeding

Plant breeding is a major driving force for improving and providing enough food and nutrition for the increasing human population world-wide. The use of conventional methods for genetic improvement in wheat through phenotypic selection at the breeding level is challenging and sometimes impractical due to complexity in phenotyping traits (Furbank and Tester 2011). However, prediction of phenotypes from genotypes is generally also challenging due to the large number of genes that contribute to most phenotypes. Therefore, recent advances in modern methods, such as next-generation sequencing, phenomics and omics technologies, are facilitating current plant breeding strategies. For example, many genetic dissections of complex traits and discovery of associated genes and their deployment have been facilitated through phenomics and omics technologies (Barh et al. 2015; Fritsche-Neto and Borém 2015). In the context of phenomics, identification of a candidate genotype that carries genes for targeted traits is only possible when a precise and accurate phenotyping profile of the genotype is available (Comar et al. 2012; Kumar et al. 2015). The bottlenecks with field phenotyping have resulted in intense interest in the development of new high-throughput phenotyping tools and techniques such as spectroscopy, imaging and image analysis and robotics, as well as high-performance computing (Singh and Singh 2015). In wheat, high-throughput phenotyping technologies have revealed variations in shoot relative growth rate, salinity tolerance, biomass, transpiration, water use efficiency and leaf area (Munns et al. 2010; White et al. 2012; Parent et al. 2015; Takahashi et al. 2015). The ñField Phenomicsò project (http://www.fieldphenomics.org/research/vehicles) has developed several technologies within a current field-based high-throughput phenotyping platform for wheat. In general, phenomics approaches have the potential to provide insights into the physiological mechanisms underlying disease symptoms, and these can be useful for the development of methods for rust disease phenotyping in wheat (Giglioti et al. 2015; Mutka and Bart 2015).

The rise and continuous improvement of high-throughput genome-scale genotyping platforms has allowed scientists to focus on omics technologies, e.g.

whole-genome re-sequencing, genomics, proteomics, transcriptomics and metabolomics, in order to provide greater opportunities to dissect the molecular basis of the responses of plants and the discovery of key genes in developing ideal genotypes in the changing climate scenario. Genetics and omics tools have revolutionised plant breeding; more than 40 crop species have been sequenced and the sequencing results have been made publicly available (Barabaschi et al. 2011; Michael and Jackson 2013). Moreover, whole-genome shotgun sequencing and a chromosome-based draft sequence of hexaploid wheat have been reported (Brenchley et al. 2012; Consortium 2014).

5 Objectives of the research

The overall aims of the work described in this thesis were to: 1) identify novel sources of resistance to stem and stripe rust in different wheat-alien introgression lines; 2) identify presence of resistance genes towards stem, stripe and leaf rust in Tajik wheat; 3) characterise the genetic basis of the resistance from these sources; and 4) incorporate specific alien genes conferring resistance to stem rust into wheat cultivars adapted to the environment of interest, in order to lead to greater sustainability of wheat production.

The specific objectives of the studies described in Papers I-V were to:

- Evaluate the wheat-alien introgression derivatives from *Secale cereale*, *Leymus mollis*, *Leymus racemosus* and *Thinopyrum junceiforme* against a wide array of stem and stripe rust pathogen races for the purpose of identifying new sources of resistance.

- Evaluate Tajik wheat cultivars, breeding lines and landraces for presence of specific stem, stripe and leaf rust resistances by the use of an array of rust pathogen races.

- Identify seedling and adult plant *Sr* and *Yr* resistance genes present in the wheat-alien introgression lines.

- Develop a new wheat-rye Robertsonian translocation containing a new resistance gene.

- Perform molecular and cytogenetic validation of wheat-alien introgression lines.

6 Materials and methods

6.1 Plant materials

The Swedish winter triticale lines SV856003, SV876012 and SV876032 and a AD99 wheat-*Leymus* hybrid were crossed with winter hexaploid wheat cultivars (óGoerzenô, óHolmeô and óKrakaô) adapted to Swedish conditions to develop a large number of winter wheat-alien introgression lines (Forsström and Merker 2001). The Mexican spring hexaploid triticale cultivars óBeagleô and óDriraô were crossed with the popular Swedish spring hexaploid wheat cultivars óDrabantô, óPrinsô, óSonettô and line SV77328, and from these crosses a number of spring wheat-rye introgression lines were developed (Merker 1984). Moreover, wheat-*Leymus mollis*, wheat-*Leymus racemosus* and wheat-*Thinoporym junceiforme* introgression hybrids were developed by embryo culture techniques and the hybrids were then backcrossed once with a Hpph 5RL·5BS wheat-rye translocation line and *Triticum turgidum* var. *carthlicum* (Merker and Lantai 1997). The lines derived from these crosses contain rye chromosomes 1R, 2R, 3R, 4R, 5R and 6R in the form of a single disomic substitution. Lines with wheat-rye translocations such as 1DL·1RS, 1BL·1RS, 2RL·2BS, 3DL·3RS and 5AL·5RS, and lines with multiple combinations of rye chromosome substitutions such as 1R+2R, 1R+3R, 1R+6R, 5R+4R+7R and 1R+6R+4R+7R were also present (Merker 1979; Merker and Rogalska 1984). The materials also included wheat lines with introgressed chromatin from *Leymus mollis*, *Leymus racemosus* and *Thinopyrum junceiforme* (Ellneskog-Staam and Merker 2001, 2002). All of these lines were used in stem rust and stripe rust seedling and adult plant resistance tests (Papers I and IV).

The spring wheat line óSLU238ô, containing *Sr59* resistance gene on the 2R (2D) wheat-rye disomic substitution, has its origin from the hexaploid triticale line VT828041. Thereafter, óSLU238ô was crossed with the óChinese Springô *ph1b* mutant in order to induce meiotic recombination between 2R and 2D homoeologous regions (Paper II).

The line KR99-139 (double wheat-rye translocation line with 1BL·1RS and 2RL·2BS) and bread wheat variety ‘Topper’ were crossed. The F_1s from this cross were then backcrossed to both parents to produce BC_1F_1 populations. Thereafter, the selfed BC_1F_3 and BC_1F_4 populations were evaluated for stem rust seedling and adult plant resistance (Paper III).

Widely cultivated wheat cultivars, landraces and advanced breeding lines of hexaploid wheat from Tajikistan were evaluated for their seedling reaction to stem, stripe and leaf rust and also their adult plant reaction to stem and stripe rust (Paper V). In addition, for all seedling resistance assays the differential genotypes with known *Sr*, *Yr* and *Lr* resistance genes were included.

6.2 Seedling resistance tests to stem rust, stripe rust and leaf rust

The stem rust seedling resistance tests were conducted at the United States Department of Agriculture Agricultural Research Service Cereal Disease Laboratory (USDA-ARS-CDL) and the Biosafety Level-3 containment facility at the University of Minnesota (UM) in St. Paul, USA. Stem rust seedling resistance tests were also conducted at the Regional Cereal Rust Research Center (RCRRC), located at the Aegean Agricultural Research Institute, International Center for Agricultural Research in Dry Areas (ICARDA) in Izmir, Turkey, and the University of the Free State, Bloemfontein, South Africa. The African and North American races TTKSK (Ug99), TTKST, TTTSK, TRTTF, TPMKC, TTTTF, QTHJC, MCCFC, RHQQC and BCCBC were used at USDA-ARS-CDL and UM, according to Rouse et al. (2011). Stem rust seedling resistance tests at the University of the Free State (BPGSC, BPGSC+*SrKiewiet* and BPGSC+*SrSatu* races) and RCRRC, Izmir (TKTTF race) were carried out following similar protocols to that used at USDA-ARS-CDL and UM. To characterise seedling infection of *Puccinia graminis*, a scale of 0 to 4 was used, with scoring performed 14 days after inoculation as described by Stakman et al. (1962) (Papers I, II, III and V).

Stripe rust seedling resistance tests were conducted at the Global Rust Reference Center (GRRC), Aarhus University, Flakkebjerg, Denmark, and RCRRC. All wheat genotypes were evaluated with 12 stripe rust races from six different countries. The seedling assays at GRRC were carried out according to Sørensen et al. (2016) and those at RCRRC according to Jighly et al. (2015). Infection of *Puccinia striiformis* on wheat seedlings was scored on a scale of 0 to 9, with scoring performed 16 days after inoculation as described by McNeal et al. (1971) (Papers IV and V).

Seedling evaluation assays for leaf rust in wheat cultivars, landraces and advanced lines from Tajikistan were conducted at USDA-ARS-CDL. The leaf rust

seedling assays were according to Oelke and Kolmer (2004), and a 0 to 4 scale was used for scoring (Long and Kolmer 1989) (Paper V).

6.3 Assessment of adult plant resistance to stem rust and stripe rust

Stem rust APR was evaluated at the Kenyan Agricultural and Livestock Research Organization (KALRO) in Njoro, RCRRC and UM. Wheat genotypes with winter habitat were vernalised for six weeks at +4°C and then transplanted to the field in KALRO. To establish uniform disease development within plants and plots, a mix of susceptible wheat cultivars was planted as spreader rows surrounding nurseries in all locations. Initially, the spreader rows were needle-injected at the booting and heading stages, with races TTKSK+TTKST at KALRO and race MCCFC at UM. Direct foliar inoculation was also carried out with urediniospore/oil suspension on the spreader plants. At RCRRC, the race TKTTF was inoculated by dusting with a mixture of fresh urediniospores and talcum powder. Due to the dry environment at the KALRO and RCRRC nurseries, mist-irrigated conditions were applied (Papers I, III and V).

Stripe rust field experiments were conducted at Lönnstorp Field Station, Lomma, Sweden, and at RCRRC. The lines were exposed to natural epidemics during the growing season in 2012 at Lönnstorp Station. In the RCRRC field, the highly susceptible ‘Morocco’ cultivar and an additional mixture of susceptible wheat cultivars were used as spreader rows in all pathways perpendicularly between the tests plots along the main wind direction. On several occasions (at least 4-5, e.g. at tillering, booting, heading and flowering stages), the spreader rows at Izmir were artificially inoculated using talcum powder, while the nurseries at RCRRC were mist-irrigated. The APR to stripe rust of the wheat genotypes from Tajikistan was evaluated based on natural epidemics during the growing season in 2010 in Tajikistan. Stripe rust-infected leaves were collected from Sweden and Tajikistan and sent to GRRC for race analysis. The recovered races (SE205/12 from Sweden and Taj01a/10 from Tajikistan) were used for seedling resistance tests at GRRC (Papers IV and V).

In all locations, the adult plant response to stem rust and stripe rust was assessed between growth stages 50-90 based on the Zadoks scale (Zadoks et al. 1974). Disease severity assessments (0-100%) were scored visually based on the modified Cobb scale (Peterson et al. 1948) and the host response according to Roelfs et al. (1992).

6.4 Molecular marker validations of the *Sr*, *Yr* and *Lr* resistance genes

In addition to gene postulation, some known *Sr*, *Yr* and *Lr* diagnostic markers, such *XcsSr2* and *Xgwm533* for *Sr2*/*Yr30*/*Lr27* (Mago et al. 2011), *Xcfd43* for *Sr6* (Tsilo et al. 2009), *Xscm9* and *Xiag95* for *Sr31*/*Yr9*/*Lr26* (Saal and Wricke 1999; Mago et al. 2002), *Xstm773* for *Sr36* (Tsilo et al. 2008), *VENTRIUP-LN2* for *Sr38*/*Yr17*/*Lr37* (Helguera et al. 2003), *Xwmc364* for *Yr2* (Lin et al. 2005), *csLV34* for *Sr57*/*Yr18*/*Lr34* (Lagudah et al. 2006) and *Xwmc198* for *Yr32* (Eriksen et al. 2004) were assayed. The KASP markers for *Sr2*/*Yr30*/*Lr27* (*wMAS000005*) (http://maswheat.ucdavis.edu/protocols/Sr2/index.htm), *Sr36* (*wMAS000015*) (http://maswheat.ucdavis.edu/protocols/Sr36/index.htm) and *Sr57/Yr18/Lr34* (*wMAS000003*) (http://maswheat.ucdavis.edu/protocols/Lr34/index.htm) were also applied (Papers I, IV and V).

6.5 Developing T2DS·2RL wheat-rye Robertsonian translocation

The line ‘SLU238’ was crossed with the ‘CS’ *ph1b* mutant to induce meiotic recombination between the 2R and 2D homoeologous regions. The F_2 population obtained (selfed from F_1 plants) was phenotyped at the seedling stage with the TTKSK and TTTTF races. Resistant F_2 plants were evaluated with the *PSR128*, *PSR574* and *AWJL3* touchdown molecular markers to select plants homozygous for the *ph1b* allele (Roberts et al. 1999; Niu et al. 2011). Resistant F_2 plants homozygous for the *ph1b* allele were selected for production of F_3 populations. The $F_{3:4}$ populations were phenotyped with race TTTTF, and were then genotyped with molecular markers for the presence of 2R and 2D chromosomes. Seven F_4 population recombinants with reduced rye chromatin were identified, and then tested at the seedling stage with additional stem rust races (Paper II).

6.6 Development of kompetitive allele-specific PCR markers

The Rye5K Illumina iSelect high-throughput SNP array containing 5234 markers, developed by Haseneyer et al. (2011), has been used for high-throughput genotyping in four individual rye mapping populations (Martis et al. 2013). Next, 34 KASP primers with two allele-specific forward primers with FAM: 5´GAAGGTGACCAAGTTCATGCT3´ and VIC: 5´GAAGGTCGGAGTCAACGGATT3´ compatible tails and one common reverse primer were developed for chromosome 2R. All the KASP markers were used to screen for polymorphisms between two parents, the SLU238 line and the CS *ph1b* mutant, and thereafter the KASP markers were used to analyse the F_4-resistant plants (Paper II).

6.7 Genomic *in situ* hybridisation (GISH) and fluorescence *in situ* hybridisation (FISH)

The GISH and FISH approaches are valuable and powerful methods for studying genomic composition and interactions in interspecific and intergeneric hybrids of Triticeae species. To characterise the wheat-alien introgression chromosome constitutions, GISH was performed on metaphase cells fixed onto a glass slide. Genomic DNA from *Secale cereale*, *Leymus mollis* and *Leymus racemosus* was used as a probe, labelled with fluorescein 12-dUTP green or Texas Red 12-dUTP by nick translation (Paper I).

To visualise wheat-rye Robertsonian translocation, the parents and resistant F_4 lines were analysed by FISH with probes specific to rye and wheat repetitive DNA sequences. The probes used were a UCM600 (González-García et al. 2011) synthesised by SGI DNA, La Jolla, CA, centromere-specific pAWRC.1 (Francki 2001) and subtelomeric repeat pSc74 (Bedbrook et al. 1980; Lapitan et al. 1986), all labelled with fluorescein-12-dUTP (PerkinElmer, cat. no. NEL413001EA). Wheat chromosomes were identified using Cy5-$(GAA)_9$ and TEX615-pAs1-2 oligonucleotide probes (Danilova et al. 2012) synthesised by IDT, Coralville, IA (Paper II).

7 Results and discussion

7.1 Evaluation of seedling resistance to stem, stripe and leaf rust

7.1.1 Stem rust

Stem rust seedling tests showed presence of resistance in a number of the wheat-alien introgression lines evaluated to a range of the stem rust races considered (TTKSK, TTKST, TTTSK, TRTTF, TTTTF, TPMKC, QTHJC, RKQQC, TKTTF, MCCFC, BPGSC, BPGSC+*SrKiewiet* and BPGSC+*SrSatu*) (Paper I). A number of the lines considered being resistant, the infection types 0 to 2+ were observed. However, a relatively high proportion of the lines were scored as susceptible with infection types 3 to 4 for each of the stem rust races evaluated (Paper I). Postulation of presence of various stem rust resistance genes was carried out based on the virulence profile of the different races of stem rust. Thus, some of the wheat-alien introgression lines were postulated to carry *Sr7b*, *Sr8a*, *Sr9d*, *Sr10*, *Sr31*, *Sr36* and *SrSatu* resistance genes. Exploitation of genetic variability, especially for stem rust resistance genes, is essential for the development of new wheat cultivars. Thus, the seedling resistance tests revealed important sources of novel stem rust resistance gene/s in the wheat-alien introgression lines. Three wheat-rye substitution lines (originating from the triticale line VT828041 and the cultivar ôBeagleô) exhibited high level of resistance to all stem rust races tested. In these lines (SLU210, SLU238 and SLU239), the 2R chromosome of *Secale cereale* was substituted to chromosome 2D in wheat (Paper I). Thus, the results demonstrate higher effectiveness of the chromosome 2R compared with the chromosome 1R (1D), 3R (3D) and 3DL·3RS introgressions from the same triticale line (Tables 1 and 2). The SLU210, SLU238 and SLU239 lines were demonstrated to be a new source of stem rust resistance that is cytogenetically stable and would be useful in wheat improvement (Paper I). Several effective resistance genes to stem rust have been transferred from the secondary and tertiary gene pools and are now being used in wheat breeding (Singh et al. 2015). The most

well-known and widely used rye chromosome, 1R, is translocated as 1AL·1RS, 1BL·1RS and 1DL·1RS, and these variants are all known as sources of stem rust resistance genes and other improvements in tolerance to biotic and abiotic stresses (Friebe et al. 1996; Yediay et al. 2010). Globally, *Sr31* from 1R is the most widely deployed resistance gene and this gene has provided a high level of resistance to all known stem rust races except the Ug99 race group (Pretorius et al. 2000; Singh et al. 2008).

The wheat-rye translocation lines with 1BL·1RS and 2RL·2BS, also including their parents (Topper and KR99-139), were found to be susceptible to all stem rust races at the seedling stage, but some lines exhibited resistance at the adult plant stage in the Njoro stem rust nursery in Kenya (Paper III). Thus, based on the seedling resistance tests, no highly effective stem rust resistance genes were present. Therefore, the basis for resistance in these lines must be due to some minor genes located on chromosomes 1RS, 2RL and 2BL, and possibly also to additional genes on other chromosomes not identified in this thesis. This result is in agreement with previous findings by Zhang et al. (2016) of seedling susceptibility, but resistance at the adult plant stage. Similar performance has been reported for the *Sr2* gene, a race-specific APR gene often displaying seedling susceptibility but effective at the adult plant stage (Mago et al. 2011; Singh et al. 2015).

To examine the presence of stem rust resistance genes in Tajik genotypes, a seedling resistance test was conducted in widely cultivated Tajik wheat cultivars, landraces and advanced lines. The seedling resistance test successfully detected three known resistance genes (*Sr6*, *Sr31* and *Sr38*). However, some genes were also classified as uncharacterised, indicating that some genotypes might possess novel sources of stem rust resistance gene/s (Paper V). Virulence to the *Sr31* gene was first detected in Africa (Pretorius et al. 2000), and this virulence type has spread to new wheat production areas. Moreover, new races of stem rust with additional virulence to important resistance genes have been detected and are spreading across eastern, southern and northern Africa and the Middle East (Olivera et al. 2015; Patpour et al. 2015a; Patpour et al. 2015b; Singh et al. 2015). Approximately 90% of the wheat cultivars grown world-wide are susceptible to Ug99 and related races, which poses a severe threat to world food security (Singh et al. 2016). Therefore, discovery of new resistance genes effective in the seedling and adult plant stages of wheat is important to prevent major stem rust epidemics.

Table 1. *Seedling infection type and comparison of resistance level in 1R (1D) and 2R (2D) wheat-rye substitution lines from triticale cultivar ‘Beagle’ and line VT828041*

#	Pedigree/Cross	Chromosome	Type	Seedling Resistance Test												Adult Plant Resistance			Postulated *Sr* genes
				TTKSK, 1 Rep.	TTKSK, 2 Rep.	TPMKC	TTTTF	QTHJC	RKQQC	TTKST	TRTTF	TTTSK	TKTTF	MCCFC	BPGSC	BPGSC+*Sr Kiewiet*	BPGSC+*Sr Satu*	TTKSK	TKTTF	MCCFC	
SLU183	Triticale VT 82 8041	1R replaced 1D	Spring	3+	3+	4	4	33+	4	33+	3+	3+	3+	4				100S	20MRMS	50S	*None*
SLU184	Triticale VT 82 8041	1R replaced 1D	Spring	3+	3+	4	4	33+	3+4	3+4	3+	3+	33+	4				100S	20MS	50S	*None*
SLU185	Triticale VT 82 8041	1R replaced 1D	Spring	3+	4	4	4	33+	3+	4	3+	3+	3+	4				100S	20MRMS	60S	*None*
SLU186	Triticale VT 82 8041	1R replaced 1D	Spring	3+	4	4 LIF	4	33+/2+	3+	3+	3+	3+	33+	4				100S	20MRMS	60S	*Unknown*
SLU189	Triticale Beagle	1R replaced 1D	Spring	3+	3+	4	4	3//2	;01	3+	3+	3+	3+	4				100S	60MSS	40S	*Unknown*
SLU190	Triticale Beagle	1R replaced 1D	Spring	0;	0;	0;	3+	0;	3+/0;	;0/2	22+	3+	3+	0;	;	;	;	10R	20MR	TR	*Sr36*
SLU209	Triticale Beagle	2R replaced 2D	Spring	0;	0;	32+1	32+	0;	0;	0;	;12	32+	3	0;				10RMR	5R	TR	*36,+*
SLU210	Triticale Beagle	2R replaced 2D	Spring	0;	0;	0;	1+	0;	;1	0;	;12	;1	1	0;	;	;	;	20RMR	5RMR	TR	*36,+*
SLU211	Triticale VT 82 8039	2R replaced 2D	Spring	3	3	33+	3-	1+2+	0;	2+	;11+	2+3	1	3+				10R	5R	TR	*Unknown*
SLU212	Triticale VT 82 8039	2R replaced 2D	Spring	3	3	32+	32+	0;-1	0;	2+	;11+/2+	22+	1	4				30S	5RMR	5MR	*Unknown*
SLU223	Triticale VT83 591	2R replaced 2D	Spring	3	3	3+	33+	1+/3-	1+3-	1+3-	2+3	3	3+	3+4				10RMR	50MSS	TR	*Unknown*
SLU224	Triticale VT83 591	2R replaced 2D	Spring	2	2	3+	33+	1+/3	2+3	0;	2+3	32+	1-	3+				10RMR	5MR	TR	*Unknown*
SLU238	Triticale VT 82 8041	2R replaced 2D	Spring	1	1	2	22-	12-	;1	;11+	;01	;1	1-	;01-	1	2	2	10R	10RMR	TR	*Unknown*
SLU239	Triticale VT 82 8041	2R replaced 2D	Spring	1	1-	2	22-	12-	;1	;01	;01	;1	1-	;01-	1	2	2	20RMR	5RMR	TR	*Unknown*

Table 2. *Infection type responses and postulated SrSatu resistance gene in 3R (3D) wheat-rye substitution lines*

#	Pedigree/Cross	Chromosome	Type	Seedling Resistance Test														Adult Plant Resistance			Postulated *Sr* genes
				TTKSK, 1 Rep.	TTKSK, 2 Rep.	TPMKC	TTTTF	QTHJC	RKQQC	TTKST	TRTTF	TTTSK	TKTTF	MCCFC	BPGSC	BPGSC+*Sr Kiewiet*	BPGSC+*Sr Satu*	TTKSK	TKTTF	MCCFC	
SLU213	Triticale Beagle	3R replaced 3D	Spring	3	3	3+1C	4	2+/1C	3+/0;	33+C	1+2	3+	2	4	X	;1	;3+	20MSS	20MR	50S	*Unknown*
SLU214	Triticale Beagle	3R replaced 3D	Spring	0;	0;	11+	4	0;	0;	0;	;11+	3+	3+	0;	0;	0;	0;	20R	10MRMS	TR	*Unknown*
SLU215	Triticale Beagle	3R replaced 3D	Spring	3+	3+	3+4	4	;11+/3	3+	3+	2+3	3+	3	4	3+	2	2	100S	10MR	30S	*Unknown*
SLU216	Triticale Drira	3R replaced 3D	Spring	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	;	;	4	5TRR	5R	TR	*SrSatu*
SLU217	Triticale Drira	3R replaced 3D	Spring	0;	0;	0;	0;	0;	0;	0;	0;	0;	;	0;	;	;	4	10R	5R	TR	*SrSatu*
SLU218	Triticale Drira	3R replaced 3D	Spring	0;	0;	0;	0;	0;	0;	0;	0;	0;	;	0;	;	;	4	5TRR	5R	TR	*SrSatu*
SLU219	Triticale Drira	3R replaced 3D	Spring	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	;	;	2	5TR	5R	TR	*SrSatu,+*
SLU220	Cimmyt 1974	3R replaced 3D	Spring	0;	0;	0;	0; 1	0;	0;	0;	0;	0;	;	0;	X-,;	;1	;3+	10R	5RMR	TR	*SrSatu*
SLU221	Cimmyt 1974	3R replaced 3D	Spring	3+	3+	3	3+	1+2C	;3	3-	33+	3+	;1=	0;	;12	;1	;3+	10R	5RMR	TR	*Unknown*
SLU222	Cimmyt 1974	3R replaced 3D	Spring	0;	0;	33+	4	1+2/3-	3	0;	22+	;	33+	11+	X+	;1	;3+	10RMR	70S	10R	*Unknown*
SLU231	Triticale Beagle	3RS.3DL	Spring	4	3+	3+	4	1+3C	4	3+4	33+	3+	3+	4	X	;1c	;3+	40MSS	80S	30S	*Sr7b*
SLU232	Triticale Beagle	3RS.3DL	Spring	4	4	1+3+	4	11+C/3+	4	3+4	33+	3+	3+	4	X+	;1c	;3+	30MRMS	80S	30S	*Sr7b*
SLU233	Triticale Beagle	3RS.3DL	Spring	4	3+	3+1+	4	3+	4	3+4	33+/4	3+	3+	4	X	;1c	;3+	50MSS	70S	30S	*Unknown*

7.1.2 Stripe rust

All wheat-alien introgression lines and their parental lines were postulated for possible presence or absence of the commonly found stripe rust seedling resistance genes *Yr1*, *Yr2*, *Yr9* and *Yr32*. Presence of additional unknown resistance gene/s in the material was also postulated. The majority of the wheat-alien introgression lines were postulated to have similar stripe rust resistance genes as their parental lines, i.e. in total *Yr1*,+ in 18 (7%) of the lines, *Yr2*,+ in 50 (20%) of the lines, *Yr9*,+ in 31 (12%) of the lines, *Yr1*,*Yr32*,+ in six (2%) of the lines, *Yr2*,*Yr9*,+ in 14 (5%) of the lines and *Yr1*,*Yr2*,*Yr32*,+ in 28 (11%) of the lines were postulated. However, the postulation also resulted in 98 (37%) of the lines being classified as possessing unknown resistance genes and in nine (4%) lines being classified as having no resistance genes at all. Furthermore, six (2%) of the lines were classified as uncharacterised, meaning that they were resistant to all stripe rust races at the seedling stage (Paper IV). Presence of seedling stripe rust resistance genes in these wheat-alien introgression lines was postulated based on comparison of the seedling infection types of each line with differential genotypes carrying known resistance gene/s. Six lines with 5RS·5AL+4R+6R wheat-rye introgressions exhibited a high level of resistance to all stripe rust races used in this study and most likely represent a new source of resistance to stripe rust. However, in previous studies the wheat-*Secale cereanum* addition lines with chromosome 6R have been suggested to carry a novel resistance gene/s to stripe rust (Schneider et al. 2016). In this thesis, most of the wheat-alien introgression lines with chromosome 1R were found to carry the *Yr9* resistance gene alone with additional unknown resistance gene/s. As an example, a combination of *Yr9/Sr31* resistance genes was postulated in the SLU168 and SLU173 lines with 1BL·1RS wheat-rye translocation, but not in the 1RS·1DL, 1R (1D), 1R+6R and 1R+4R+6R substitution and translocation lines (Papers I and IV). Genetically, *Yr9/Sr31/Lr26/Pm8* located on the 1BL·1RS wheat-rye translocation was associated with the rye cultivar ‘Petkus’ (Friebe et al. 1996). The *Yr9* resistance gene, found only in triticale, has not been genetically associated with the *Yr9/Sr31/Lr26/Pm8* resistance genes (Zhang et al. 2010).

According to the results in this thesis, four known *Yr* genes (*Yr2*, *Yr9*, *Yr17* and *Yr27*) and some unidentified resistance genes are present in Tajik wheat genotypes (Paper V). The *Yr9* (*Sr31*/*Lr26*/*Pm8*) gene in varieties ‘Alex’, ‘Sadokat’ and ‘Ziroat-70’ is probably derived from the widely used rye cultivar ‘Petkus’ for wheat improvement, suggested to originate from a 1BL·1RS wheat-rye translocation. However, several new varieties (‘Sarvar’, ‘Yusufi’, ‘Alex’, ‘Oryon’ etc.) that are widely cultivated in Tajikistan were highly resistant during the epidemic years of 2010 and 2016. The evolution and spread of new virulent races has had a significant effect for epidemics of stripe rust world-wide (Wellings 2011; Ali et al. 2014; Hovmøller et al. 2015). In Tajikistan, several stripe rust epidemics have threatened food security and farmers’ livelihoods (Eshonova et al. 2005;

Rahmatov et al. 2011; Rahmatov et al. 2012). Therefore, there is an urgent need for identification of new stripe rust resistance genes that can be utilised in the national wheat breeding programme as a low-cost and environment-friendly strategy (Paper V).

7.1.3 Leaf rust

Widely cultivated wheat cultivars, landraces and advanced lines from Tajikistan were assessed with nine races of leaf rust at the seedling stage, and only *Lr26* was postulated to be present. *Lr26* is a widely deployed resistance gene, located on the 1BL·1RS translocation (Friebe et al. 1996; Mago et al. 2002). In terms of seedling resistance, more than 20% of the genotypes tested were highly resistant to the leaf rust races used in this study. This suggests the presence of new leaf rust resistance genes in Tajik genotypes. However, to fully elucidate the number of genes carried in each genotype, genetic analyses must be performed (Paper V).

7.2 Field responses to stem and stripe rust

7.2.1 Stem rust

Field experiments to evaluate the presence of stem rust APR were carried out at KALRO, RCRRC and UM and showed high disease severity (~90%) in susceptible controls. The resistance identified with the seedling tests in the wheat-alien introgression lines remained highly effective during field evaluations (Figure 1). However, additional lines possessed a high level of resistance in the field compared with the number of lines that were resistant at the seedling stage (Paper I). Lines determined as containing stem rust APR gene/s showed severity ranging from 0 to 30 RMR, 5 to 40 MR-MS and 5 to 30 MS-MSS (Paper I). The genetic basis of the APR in this material has still not been evaluated. A number of 1BL·1RS and 2RL·2BS double wheat-rye translocation lines were revealed as showing APR to the rust race TTKSK. This APR was most likely due to the presence of multiple minor genes on at least three chromosomes leading to APR resistance (Paper III). Several previous studies of APR to stem rust in wheat have revealed that the high level of resistance in the adult plant is due to the presence of four or five minor genes (Singh et al. 2014). However to date, only the *Sr2*, *Sr55*, *Sr56*, *Sr57* and *Sr58* pleiotropic APR genes have been characterised and these genes are conferring slow rusting resistance (Knott 1968; Lagudah et al. 2006; Herrera-Foessel et al. 2010; Bansal et al. 2014). Therefore, pyramiding of major and minor gene combinations could be an effective strategy contributing to durable stem rust resistance in wheat (Singh et al. 2011; Singh et al. 2015). To fully elucidate the genetic architecture of APR, molecular mapping studies are a highly effective approach (Yu et al. 2014).

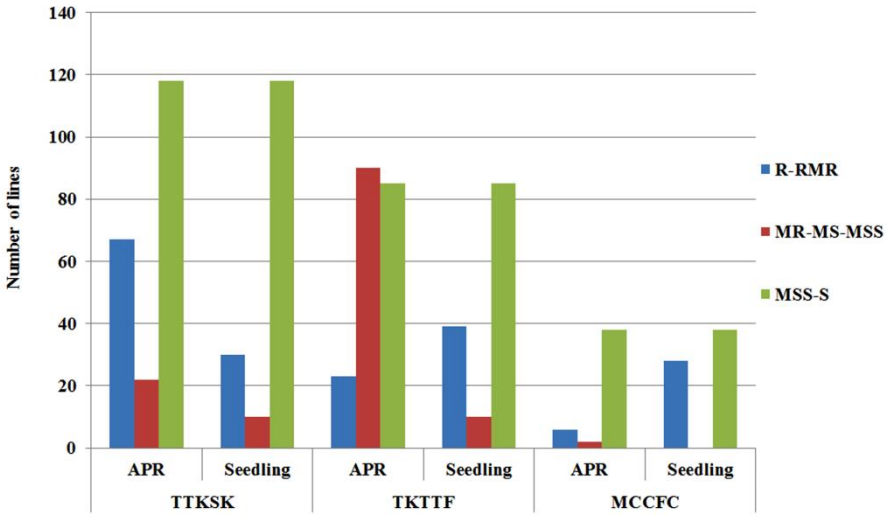

Figure 1. Correlation between seedling and APR genes to stem rust in wheat-alien introgression lines.

The APR of Tajik genotypes to stem rust was evaluated at three locations (KALRO, RCRRC and UM). In the field, three, nine and ten of the lines tested showed APR to TTKSK, MCCFC and TKTTF respectively, with reduced stem rust severity (5RMR to 60MR), although all genotypes were susceptible at the seedling stage (Paper V) (Figure 2A-F). The pseudo black chaff (*PBC*) phenotype, which is known to be linked with *Sr2*/*Yr30*/*Lr27* genes under field conditions (Juliana et al. 2015), was found in several genotypes. The APR genes *Sr2*/*Yr30*/*Lr27* have been widely and effectively used in conjunction with other R and APR genes and have provided durable resistance against all virulent races of stem rust (Li et al. 2015). Again, a combination of major and minor resistance genes is the most promising solution to provide durable resistance in wheat cultivars (Paper V). Recently, several QTLs for APR to stem rust have been mapped in different wheat chromosomes and this may contribute to APR (Yu et al. 2014).

7.2.2 Stripe rust

High levels of stripe rust disease severity were found for susceptible controls at Lönnstorp (80%) and RCRRC (100%) during APR tests. All lines identified as resistant to stripe rust at the seedling stage also remained resistant at the adult plant stage under field conditions. A large number of genes, including *Yr16*, *Yr18*, *Yr29*, *Yr30*, *Yr31*, *Yr36*, *Yr39*, *Yr46*, *Yr48* and *Yr52*, have been characterised as stripe rust APR genes (Herrera-Foessel et al. 2010; Rosewarne et al. 2012; Chen 2013). The majority of the APR effect noted at Lönnstorp could be due to the presence of resistance in the lines to rust race SE2015/12 (Paper IV). The stripe rust race

TK34/11 was the dominant race used and present at RCRRC. Resistance towards this race is based on a seedling resistance effect and many of the wheat-alien introgression lines showed seedling resistance to race TK34/11 (Paper IV). The genetic basis of APR in the lines is still unknown.

Five and two of the Tajik genotypes were susceptible at the seedling stage to the stripe rust races TK34/11 and Taj01a/10, respectively, although showing APR at the adult plant stage (Paper V). The slow rusting resistance genes *Yr18* (*Sr57*/*Lr34*), *Yr29* (*Sr58*/*Lr46*), *Yr30* (*Sr2*/*Lr27*) and *Yr46* (*Sr55*/*Lr67*) and the high-temperature APR genes *Yr36*, *Yr39* and *Yr52* have been shown to be major sources of durable resistance to stripe rust (Chen 2013; Singh et al. 2014). However, the leaf tip necrosis (*LTN*) phenotype, known to be associated with the *Yr18/Lr34/Sr57* APR genes under field conditions, was found in several of the genotypes (Paper V). Thus, it was concluded that the majority of the genotypes displayed a high level of stripe rust resistance in the field, most likely due to the presence of seedling resistance genes in the genotypes (Figure 2G-L).

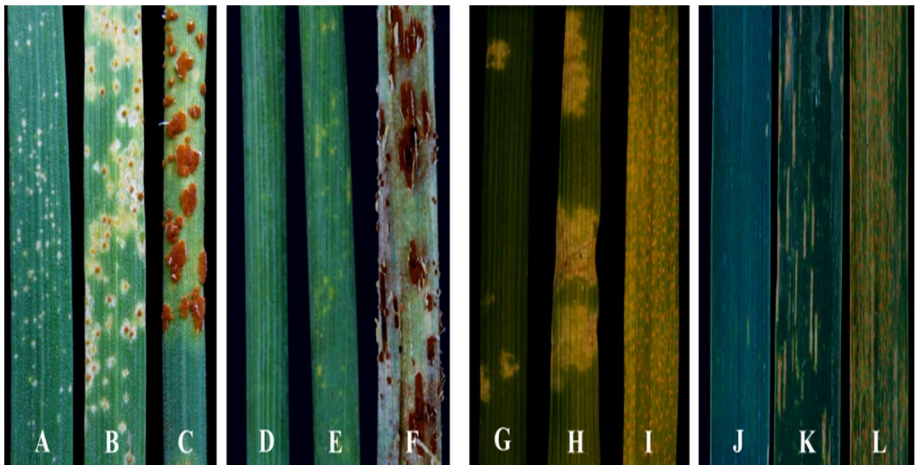

Figure 2. Stem rust seedling infection types in wheat cultivars, A) ‘Sarvar’, B) ‘Alex’, and C) ‘Navruz’ and adult plant stem rust responses in wheat cultivars D) ‘Sarvar’, E) ‘Alex’, and F) ‘Navruz’ to race TKTTF. Stripe rust seedling infection types in wheat cultivars, G) ‘Sarvar’, H) ‘Ormon’, and I) ‘Ziroat-70’ and adult plant stripe rust responses in wheat cultivars J) ‘Sarvar’, K) ‘Ormon’, and L) ‘Ziroat-70’ to race Taj01a/10.

7.3 Molecular marker validation

In addition to seedling and adult plant resistance tests, CAPS, KASP, SSR and STS molecular markers were used to verify presence/absence of stem, stripe and leaf rust resistance genes. Lines suspected to carry the *Sr31* gene were assessed with the *Xscm9* and *Xiag95* markers, while those suspected to carry the *Sr36* gene were assessed with markers *wMAS0000015* and *Xstm773*. These markers were able to

verify the presence of the suspected genes for stem rust in the lines investigated (Paper I). The molecular markers for the stripe rust resistance genes, *Yr2*, *Yr9* and *Yr32*, were also able to verify presence of these genes (Paper IV). However, the resistance spectra of some wheat-alien introgression lines phenotypically indicated the presence of the *Sr36* and *Sr38* stem rust resistance genes, although the molecular markers (*wMAS0000015, Xstm773* and *VENTRIUP/LN2*) were not able to identify the presence of these genes (Paper I). Molecular markers were complemented with GISH analysis to verify presence of rye and *Leymus racemosus* chromatin in the wheat background (Paper I). The results showed that molecular markers can be used to identify the resistance genes rapidly and accurately. Wheat stem rust and stripe rust resistance genes *Sr31*, *Sr36*, *Yr2*, *Yr9* and *Yr32* have been mapped on the wheat chromosomes and perfect markers are available to detect the presence of these genes (Eriksen et al. 2004; Lin et al. 2005; Mago et al. 2005b; Tsilo et al. 2008; MASWheat 2016). The efficacy of the *Sr*, *Yr* and *Lr* gene postulation and molecular marker validation has been demonstrated in recent studies in Nordic wheat genotypes (Randhawa et al. 2016). Cytological methods, such as GISH and FISH, have been used extensively to identify introgression lines in wheat, but these approaches are not suitable for small chromosome segments (Tiwari et al. 2014). Thus, usage of molecular markers can detect small alien chromosome segments in the wheat genome.

The presence of seedling resistance genes such as *Yr2*, *Sr6*, *Sr31*/*Yr9*/*Lr26* and *Sr38*/*Yr17*/*Lr37* in the Tajik genotypes was assessed with available molecular markers. Presence of APR genes *Sr2*/*Yr30*/*Lr27* and *Sr57*/*Yr18/Lr34* was also assessed with molecular markers (Paper V). However, due to phenotyping challenges in the seedling and adult plant stages, the available molecular markers were used for accurate gene postulation. These molecular markers were suitable for identifying lines with multiple resistance genes. Several lines showed resistance to all three rusts due to the presence of multiple resistance genes (Paper V). The presence of pleiotropic APR genes *Sr2*/*Yr30*/*Lr27* and *Sr57*/*Yr18/Lr34* in combination with other seedling resistance genes has been demonstrated to provide adequate rust resistance (Singh et al. 2014; Singh et al. 2015; Singh et al. 2016).

7.4 GISH analysis

A GISH analysis was performed to detect *Secale cereale*, *Leymus racemosus* and *Leymus mollis* chromosomes in the wheat genome (Paper I). In most of the lines, 42 wheat chromosomes were counted, but in some lines the presence of one, two and five alien chromosome pairs was detected. In the wheat-rye translocations and substitutions, the 1B, 1D, 2B, 2D and 3D wheat chromosomes were replaced with the 1R, 2R and 3R chromosomes. Thus, it was possible to identify wheat-alien substitution and translocation lines based on the GISH analyses of these lines

(Paper I) (Figure 3). Cytogenetic analysis is widely used and has become an efficient tool for identifying the presence of alien chromosomes in the wheat genome (Danilova et al. 2014; Kielsmeier-Cook et al. 2015). In previous studies, the alien chromosome introgressions were found primarily in the B and D chromosomes of wheat (Merker 1975, 1979, 1984).

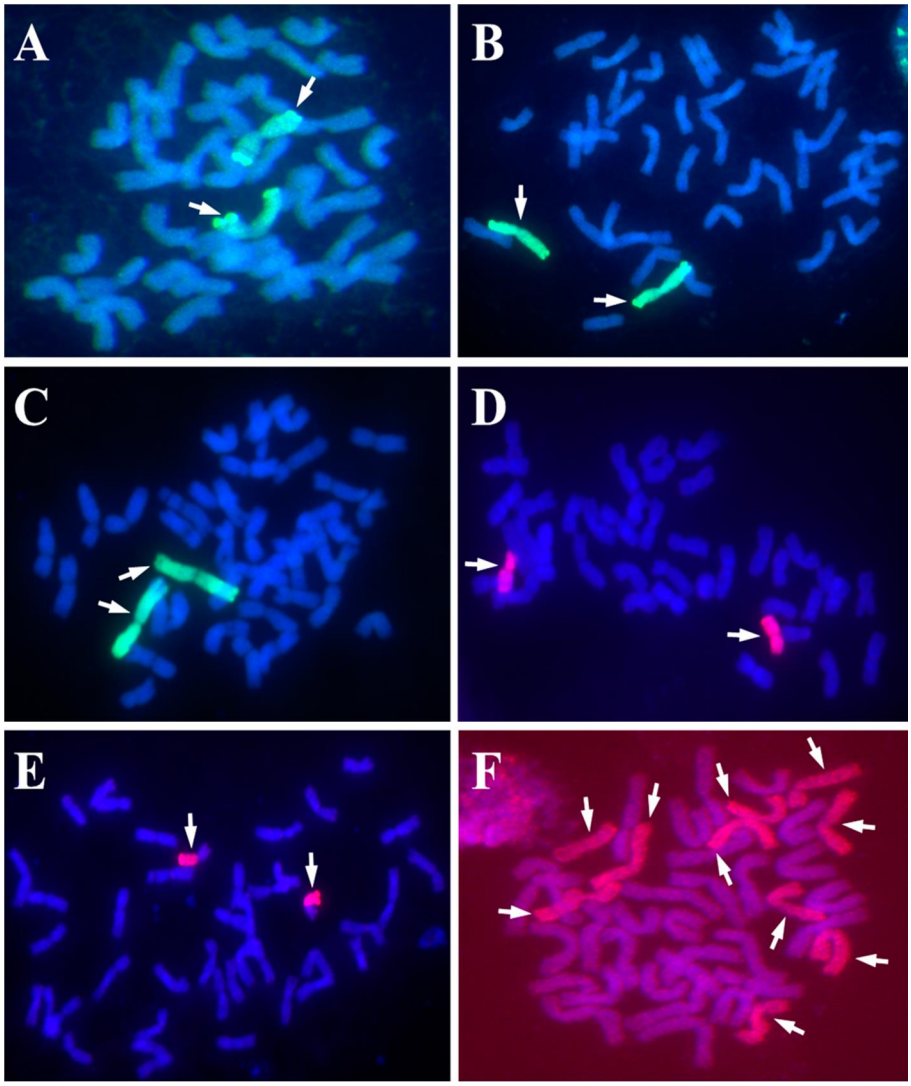

Figure 3. Genomic *in situ* hybridisation (GISH) patterns of wheat-rye, wheat-*L. racemosus* and wheat-*L. mollis* substitutions and translocation lines. A) SLU190, 1R (1D) 2*n* = 40+2; B) SLU238, 2R (2D) 2*n* = 40+2; C) SLU219, 3R (3D) 2*n* = 40+2; D) SLU235, wheat-*L. racemosus* 2*n* = 40+2; E) SLU237, wheat-*L. racemosus* 2*n* = 42; and F) SLU176, wheat-*L. mollis* 2*n* = 32+10.

7.5 Development and characterisation of a new T2DS·2RL wheat-rye Robertsonian translocation with *Sr59* resistance to stem rust

Wheat-alien introgressions provide rich genetic resources for wheat improvement (Mujeeb-Kazi et al. 2013). Seedling and adult plant resistance screenings showed that lines with the 2R (2D) wheat-rye disomic substitution exhibited a highly resistant response to all diverse African and North American stem rust races tested (Paper I). Homologous chromosome pairing in wheat is strictly controlled by the *Ph1* gene, which prevents meiotic pairing and recombination between homoeologous chromosomes (Riley and Chapman 1958). Therefore, the ócChinese Springô *ph1b* mutant was crossed with the 2R (2D) wheat-rye substitution line (SLU238) (Paper II). To identify the induced homoeologous recombinants, stem rust seedling resistance tests and molecular markers were applied at multiple generations. The stem rust resistance present in óSLU238ô was confirmed as being the result of gene/s detected from the rye chromosome arm 2RL (Papers I and II). A homozygous T2DS·2RL wheat-rye Robertsonian translocation line was obtained through a breakage-fusion mechanism (Paper II). Presence of the resistance gene at the distal part of 2RL was verified and the gene was designated *Sr59* (Paper II). The introgression on a part of a chromosome arm of an alien species in wheat has also in previous studies been developed through breakage-fusion, resulting in a Robertsonian translocations (Friebe et al. 2005). For example, the production of wheat-rye, wheat-*Dasypyrum villosum* (*Sr44*), wheat-*Thinopyrum intermedium* (*Sr52*) and wheat-*Agropyron cristatum* Robertsonian translocations have occurred through the breakage-fusion mechanism (Zhang et al. 2001; Qi et al. 2011; Liu et al. 2013; Li et al. 2016). The FISH investigation showed that the line contained a pair of *Secale cereale* as 2*n* = 41 and 2*n* = 42 T2DS·2RL wheat-rye Robertsonian translocations (Paper II) (Figure 4).

The T2DS·2RL wheat-rye Robertsonian translocation lines were highly resistant to the races TTTTF and TTKSK (Figure 5). This T2DS·2RL wheat-rye Robertsonian translocation source of *Sr59* resistance gene will provide valuable resources for stem rust resistance breeding. Stem rust resistance in wheat has greatly relied on the tertiary gene pool for new resistance sources. Among the stem rust resistance genes, *Sr27*, *Sr31*, *Sr50*, *Sr1RS*^{Amigo} and *SrSatu* were introduced from *Secale cereale* (Olivera et al. 2013; Singh et al. 2015). Rye chromosome 2R can also be used as a source of other biotic and abiotic stresses with improved agronomic performance (Paper II). This thesis revealed the presence of a new resistance gene *Sr59* that is effective against a wide array of stem rust races.

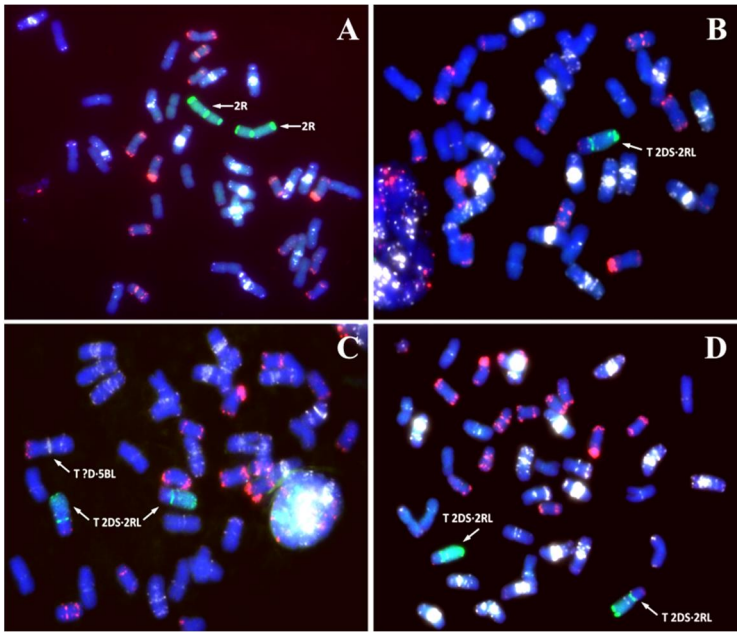

Figure 4. Fluorescence *in situ* hybridisation visualisation of A) SLU238 2R (2D), 2*n* = 42; B) #99 T2DS·2RL, 2*n* = 41; C) #100 T2DS·2RL, 2*n* = 41; and D) #101 (TA5094) T2DS·2RL, 2*n* = 42.

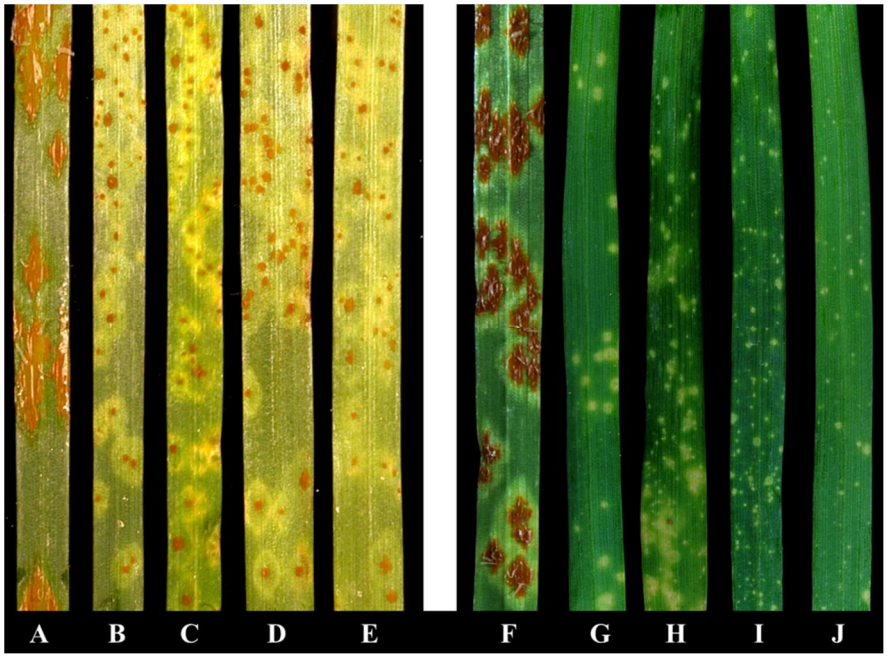

Figure 5. Infection types of A) CS *ph1b* mutant; B) SLU238; C) #99; D) #100; and E) #101 (TA5094) to race TTTTF, and of F) CS *ph1b* mutant; G) SLU238; H) #99; I) #100; and J) #101 (TA5094) to race TTKSK.

7.6 Development of kompetitive-allele specific PCR markers to stem rust resistance gene *Sr59*

The KASP assays were designed using the SNP sequences from the rye 5K iSelect (Haseneyer et al. 2011; Martis et al. 2013). The KASP assays were initially validated in ‘SLU238’ and ‘Chinese Spring’ *ph1b* mutant. Of the 34 markers used in KASP assays, three were able to clearly distinguish the ‘Chinese Spring’ *ph1b* mutant from ‘SLU238’ (Paper II). Thereafter, resistant F_4 plants were validated by the three KASP markers, and the ‘Chinese Spring’ *ph1b* mutant and susceptible plants were not detected. The putatively distal segment of 2RL chromosome was detected by three KASP markers in #284 and #409 families, but not by the SSR and PLUG markers (Paper II). Thus, a KASP marker is thereby available for MAS in wheat breeding applying the *Sr59* resistance gene. Several KASP markers are reported to be associated with *Sr*, *Yr* and *Lr* resistance genes (MASWheat 2016). The KASP markers associated with *Sr11* (Jayaveeramuthu et al. 2016), *Sr12* (Hiebert et al. 2016), *Sr42* (Gao et al. 2015) and *Sr49* (Bansal et al. 2015) resistance genes have been developed and are now available for MAS in wheat breeding. KASP markers have been observed to be most accurate and robust assay for locating disease resistance genes in wheat (Allen et al. 2011; Neelam et al. 2013; Babiker et al. 2015; Thapa et al. 2016).

8 Conclusions

This thesis clearly identified new sources of stem rust and stripe rust resistance in wheat-alien introgression derivatives from *Secale cereale*, *Leymus mollis*, *Leymus racemosus* and *Thinopyrum junceiforme*. These findings show that the wheat-alien introgressions are a potentially useful genetic resource for wheat improvement. Identification of novel sources of stem rust and stripe rust resistance for use in wheat breeding is essential because of the rapid evolution of the pathogens.

The following conclusions were made:

- The 2R (2D) disomic wheat-rye substitution lines SLU210, SLU238 and SLU239 possess a novel stem rust resistance gene/s introgressed from *Secale cereale*.
- Stem rust seedling screening demonstrated the presence of *Sr7b*, *Sr8a*, *Sr9d*, *Sr10*, *Sr31*, *Sr36* and *SrSatu* resistance genes in various wheat-alien introgression lines.
- A new T2DS·2RL wheat-rye Robertsonian translocation was developed through the breakage-fusion mechanism and seems promising for wheat improvement.
- The T2DS·2RL wheat-rye Robertsonian translocation carries a new stem rust resistance gene *Sr59* that confers a high level of resistance against stem rust races.
- A robust KASP marker assay was developed for marker-assisted selection for the *Sr59* gene.
- The combination of cytogenetic and molecular analysis used in this thesis was effective in characterising wheat-alien chromatin introgressed into wheat.
- The presence of adult plant resistance to race TTKSK in the 1BL·1RS and 2RL·2BS double rye translocation lines is most likely due to presence of several minor genes.

- Six of the lines with 5RS·5AL+4R+6R rye chromosomes demonstrated high resistance to all stripe rust races. This suggests that lines with 5RS·5AL+4R+6R introgressions are a potential source of new stripe rust resistance gene/s.
- The stripe rust resistance genes *Yr1*, *Yr2*, *Yr9* and *Yr32* and additional unknown resistance genes/s were postulated in different lines of the wheat-alien introgression.
- Using available molecular markers combined with gene postulation provided reliable results in validating seedling and adult plant resistance genes of stem, stripe and leaf rust.
- To fully elucidate the genetic architecture of unknown stem rust and stripe rust seedling and adult plant resistance in wheat-alien introgressions, molecular mapping studies are a highly effective approach.
- Screening of Tajik wheat for presence of stem, stripe and leaf rust resistance genes, resulted in a broad knowledge as to which known and possible novel genes are present in this material.
- Knowledge of available genes in the Tajik wheat can be combined with introductions of novel genes from the evaluated introgression lines to secure resistant wheat to be grown in Tajikistan for future generations.

9 Future perspectives

- Further chromosome engineering of 5RS·5AL+4R+6R wheat-rye introgression lines is needed to understand the background for the new source of stripe rust resistance gene.
- The *Sr59* resistance gene needs to be transferred into adapted wheat cultivars, and the agronomic performance assessed.
- The line with the T2DS·2RL wheat-rye Robertsonian translocation should be tested against other diseases and pests, as well as drought tolerance.
- The wheat-alien introgression lines should be tested against leaf rust, fusarium head blight, powdery mildew and tan spot disease.
- The MutRenSeq method should be used for cloning of R gene in these wheat-alien introgression lines.
- Wheat-alien introgression lines as potential donors of genes for high micronutrient (zinc and iron) content should be evaluated.
- The novel resistance genes should be pyramided into the Tajik wheat for durable and long-term resistance.

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Acknowledgements

In the name of Allah, the most Compassionate, the most Merciful. I am very thankful to Allah Almighty who enabled me to carry out this study.

I would like to express my sincere gratitude, appreciation and special thanks to various institutes and individuals for excellent guidance, unreserved support, endless patience and great collaboration during this research project.

Eva Johansson: I am eternally grateful to my main supervisor, Eva Johansson, for inspiring guidance and valuable assistance in all aspects of the research process. I am very thankful to her for accepting me as her licentiate and PhD student. I am also very grateful to her and her family for several unforgettable invitations to social events. This project would not have been possible without her constant support and encouragement.

Brian Steffenson: I would like to express my sincere and deepest gratitude to my co-supervisor Brian Steffenson. I also gratefully acknowledge his invitation to the University of Minnesota and for providing me with all support. Thanks for his constant help and willingness during all the time in Minnesota. I also thank Brian and Winnie for their wonderful hospitality.

Matthew Rouse: My sincere appreciation goes to my co-supervisor Matthew Rouse. I am highly grateful for all of his insightful discussions and valuable supervision of all experiments on stem rust. I am also very grateful to him for giving me this amazing opportunity and providing me with research support to conduct my experiments at USDA-ARS-CDL in St. Paul, Minnesota.

Mogens Hovmøller: My deepest gratitude goes to my co-supervisor Mogens Hovmøller for his supervision and hosting at GRRC in Flakkebjerg, Denmark,

when conducting my research experiments on stripe rust. I am also very grateful to him for providing me with research supplies and accommodation at GRRC.

Kumarse Nazari: I am also very thankful to my co-supervisor Kumarse Nazari, for providing me with research support and accommodation at RCRRC in Izmir, Turkey. I also very appreciate all the support and valuable suggestions on stem rust and stripe rust experiments at RCRRC.

Financial support from the Monsantoôs Beachell-Borlaug International Scholars Program and a UD40 grant from the Swedish Government are gratefully acknowledged. Thanks to Dr. Ed Runge for the invitation and arrangements for attending the World Food Prize ceremony and Leadership training in Des-Moines, Iowa.

I am very thankful to all of my colleagues at the University of Minnesota: Tamas Szinyei, Matthew Martin, Austin Case, Matthew Haas, Jeness Scott, Jamie Kaufman, Ahmad Sallam and Stephanie Dahl, and at USDA-ARS-CDL: Sam Stoxen, Yue Jin, Jayaveeramuthu Nirmala, Jordan Briggs, Same Gale and Pablo Olivera, for their indescribable help and collaboration in lab, greenhouse and field experiments. I am also thankful to James Kolmer and Amy Fox for the leaf rust seedling experiment at USDA-ARS-CDL. I am grateful to Shahryar Kianian and Mark Hughes for administrative support at USDA-ARS-CDL. I also would like to thank Carol Anderson and James Bradeen at the University of Minnesota for the invitation and for administrative help. Thanks also to Bernd Friebe and Tatiana Danilova from Kansas State University for their collaboration in the FISH experiment.

Thanks for the technical support from the staff at GRRC, Flakkebjerg, Denmark, and at RCRRC, Aegean Agricultural Research Institute, Izmir, Turkey. I am deeply grateful to Ellen Frederiksen, Annemarie Fejer Justesen, Julian Rodriguez Algaba, Tine Thach, Chris Sørensen, Anne Pia, Stephanie Walter, Steen Meier and Sarah Adams at GRRC and to Maha-al-Almad, Ezgi Kurtuluĸ and Nergis Çelik at RCRRC for their valuable assistance and collaboration during stripe rust phenotyping.

I am very thankful to Rolf Schlegel for his valuable suggestions and guidance in starting my PhD research. Thanks to Andreas Houben and Katrin Kumke from the Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany, for their collaboration and assistance during cytogenetic analysis.

I am very grateful to Ruth Wanyera from the Kenyan Agricultural and Livestock Research Organization Food Crops Research Center and Sridhar Bhavani from the International Maize and Wheat Improvement Center in Kenya for their kind assistance in conducting field experiments in Njoro, Kenya. Thanks to Zacharias Pretorius from the University of Free State, Bloemfontein, South Africa, for his help on seedling screening with South African races of stem rust.

I would like to express my sincerest appreciation to my Swedish ómotherô Ann-Sofie Fält for her kind hospitality, encouragement and constant support during my stay in Sweden. I am also thankful to Kjell-Åke Fält for several memorable meetings and events.

Many thanks to Maria Luisa Prieto-Linde for kind assistance and several invitations to her home. I am deeply grateful to Helen Lindgren for her kind technical assistance. I am grateful to Camilla Stjärnäng for her kind assistance in financial affairs. Thanks also to Tomas Bryngelsson, Anders Carlsson and Mariette Andersson for administrative assistance. I would like to thank Rodomiro Ortiz, Inger Åhman, Mualtu Geleta, Larisa Garkava, Waheeb Heneen, Erland Liljeroth, Sten Stymne and Li-Hua for their suggestions and support during my studies.

Special thanks to Bahrom Husenov, Maruf Makhkamov, Firuz Odilbekov, Kibrom Abreha, Abel Teshome and Mohammed Elsafi for their kind friendship and help in Alnarp and Arlöv. I would particularly like to thank Hafiz Mumnjanov and the Sub-regional Office for Central Asia, Food and Agriculture Organization of the United Nations (FAO-SEC) for giving me the opportunity to be part of the FAO-SEC family in order to develop my international career. Thanks also to Anna Berlin for fruitful collaboration. Many thanks to Borlaug Global Rust Initiative (BGRI) and the Durable Rust Resistance in Wheat Project for their constant support and invitations to technical workshops and training. Thanks also to Robert McIntosh and Robert Park from Sydney University for their fruitful discussions and advice during BGRI meetings. I would like to thank my colleagues in Tajik Agrarian University and National Wheat Breeding Program in Tajikistan: Usmon Mahmadyorov, Zubunisso Eshonova, Munira Otambekova, Ahad Ibrahimov, Bahriddin Soliev, Ulugbek Hotamov, Mirzoali Karimov and Anvar Jalilov, for their fruitful collaboration. Thanks to Tina Henriksson from Lantmännen for providing some requested seed.

I am happy to be part of the Plant Product Quality Group, and thanks to Marie Olsson, Helena Persson, Faraz Muneer, Alphonsine Mukamuhirwa, Lijie Zhong, Staffan Andersson, Hans Larsson, Faiza Rasheed, Karl-Erik Gustavsson, Joel Markgren, Ramune Kuktaite, Sven-Erik Svensson, Antonio Capezza, Bill Newson,

Elaine Ceresino, Evelyne Villanueva and Yanrong Lv for discussing research progress and sharing information.

At SLU Alnarp, I acknowledge my present and past colleagues Ida, Nadejda, Pia, Jenny, Beata, Åsa, Zeratsion, Karina-Ustariz, Therese, Svetlana, Helle, Marjan, Ali, Ania, Mehboob, Dickson, Sonja, Masoud, Tiny, Mira, Emelie, Elnura, Busie, Vehbo, Sandeep, Birjan, Mbaki, Malin, Sewalem, Aakash, Nils-Ove, Fredrik, Abrar, Michael, Mirela, Leonardo, Maksat, Thuy, Rui and Dharani. Thanks also to Mary McAfee for her language editing, and Repro Service in Alnarp for printing.

Finally, my sincerest gratitude to my family for their constant support and prayers.