Acta Universitatis Agriculturae Sueciae

SLU

Doctoral Thesis No. 2025:21
Faculty of Landscape Architecture, Horticulture and Crop Production Science

Harnessing wheat resistance to stem rust through wheat-rye introgression strategies

Mahboobeh Yazdani

Harnessing wheat resistance to stem rust through wheat-rye introgression strategies

Mahboobeh Yazdani
Faculty of Landscape Architecture, Horticulture and Crop Production Sciences
Department of Plant Breeding
Alnarp

SLU
SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

DOCTORAL THESIS
Alnarp 2025

Acta Universitatis Agriculturae Sueciae
2025:21

Cover: Wheat-Rye genome sharing (Illustrated by Mahboobeh Yazdani using Inkscape software)

ISSN 1652-6880
ISBN (print version) 978-91-8046-456-7
ISBN (electronic version) 978-91-8046-506-9
DOI: https://doi.org/10.54612/a.7sg5ufn9ft

Swedish University of Agricultural Sciences, Department of Plant Breeding, Alnarp, Sweden

Print: SLU Grafisk service, Alnarp 2025

Harnessing wheat resistance to stem rust through wheat-rye introgression strategies

Abstract

Wheat (*Triticum aestivum* L.) is one of the most important cereals worldwide that provides necessary daily protein and nutrition for humans. Unfortunately, this crop yield capacity is affected by various challenges. One of the key challenges is the emergence of new races of stem and stripe rust, which can overcome previously effective resistance genes. This emphasizes the critical need for new sources of resistance in wheat. This thesis explores the potential of wheat-rye introgression lines for improving wheat's resistance to these devastating pathogens. Using genotyping-by-sequencing (GBS) data and Kompetitive Allele-Specific PCR (KASP) markers, we developed a robust methodology to accurately track *Sr59*, a stem rust resistance gene, during breeding cycles and develop new wheat varieties with acceptable agronomic performance and resistance to stem rust. Additionally, we developed a new wheat-rye translocation line (#284) having *Sr59* as a small translocation, 2BS.2BL-2RL, located at the distal part of chromosome 2RL. This chromosomal segment is particularly advantageous for breeding programs due to its small size and simplicity of introgression into adapted wheat varieties. Further, GBS alignment with annotated rye nucleotide-binding leucine-rich repeat (NLR) genes identified two candidate NLRs on chromosome 2RL, further enriching our understanding of the genetic basis of resistance. Through phenotypic screening and molecular validation, we characterized a second stem rust resistance gene, *SrSLU*, present in line #C295, which exhibits broad-spectrum resistance to multiple stem rust races. To enhance durability and broaden resistance, we successfully pyramided *Sr59* with *YrSLU*, a stripe rust resistance gene, into a single wheat line. In this approach, we used marker-assisted selection (MAS) and speed breeding technologies to accelerate breeding cycles while maintaining high agronomic performance through top-crossing with elite commercial varieties. The resulting lines combine robust resistance against both stem and stripe rust with improved yield potential. This research provides crucial insights into the use of wheat-rye as a source of novel resistance genes and advances methodologies for their precise characterization and development. These findings are important steps towards food security and the fight against hunger.

Keywords: Breeding, Durable disease resistance, Marker assisted selection, Resistance gene, Rye, Seedling resistance test, Stem rust, Stripe rust, wheat, Wheat-rye introgression.

Author's Address: Mahboobeh Yazdani, Swedish University of Agricultural Sciences, Department of Plant Breeding, Box 190, 234 22, Lomma, Sweden

Utnyttjande av vetets resistens mot svartrost genom vete–råg-introgressionsstrategier

Sammanfattning

Vete (*Triticum aestivum* L.) är en av världens viktigaste spannmålsgrödor och utgör en daglig källa till protein och näring för människor. Tyvärr påverkas skördepotentialen av flera utmaningar, däribland uppkomsten av nya varianter av svart- och gulrost, som är motståndskraftiga mot tidigare effektiva resistensgener. Därför finns det nu ett akut behov av nya resistenskällor i veteförädling. Denna avhandling undersöker potentialen hos introgressionslinjer mellan vete och råg för att förbättra vetets resistens mot dessa skadegörar Med hjälp av genotypning via sekvensering (GBS) och Kompetitiv-allelespecifik PCR (KASP)-markörer utvecklades en robust metod för att exakt spåra resistensgenen *Sr59* under förädlingscyklerna och utveckla nya vetesorter med både god agronomisk prestanda och resistens mot svartrost. Vidare utvecklades en ny vete–råg-translokationslinje (#284) som bär *Sr59* i form av en liten translokation, 2BS.2BL–2RL, belägen i den yttre delen av kromosom 2RL. Detta kromosom-segment är särskilt attraktivt för förädlingsprogram på grund av sin begränsade storlek och enkelhet att integrera i anpassade vetelinjer. Vidare identifierades två nukleotidbindande, leucin-rika, repetitiva gener (NLR) på kromosom 2RL, med hjälp av en GBS-analys i kombination med annoterade rågsekvenser av NLR-gener - vilket fördjupar vår förståelse för resistensens genetiska grund. Genom fenotypisk screening och molekylär validering karaktäriserades ytterligare en resistensgen mot svartrost, *SrSLU*, i linje #C295, som uppvisade bredspektrumsresistens mot flera svartrostvarianter. För att förstärka och bredda resistensen pyramiderades *Sr59* med *YrSLU*—en resistensgen mot gulrost—i en och samma vetelinje. För detta användes markörbaserat urval (MAS) och så kallad speed breeding-teknik för att påskynda förädlingscyklerna. Samtidigt kunde hög agronomisk prestanda bibehållas genom toppkorsningar med elitlinjer. De resulterande linjerna kombinerar robust resistens mot både svart-och gulrost med ökad skördepotential.
Denna forskning bidrar med viktiga insikter om användningen av råg som källa till nya resistensgener och vidareutvecklar metodiken för exakt identifiering och införande av dessa gener i veteförädlingen. Resultaten utgör ett viktigt steg mot ökad livsmedelssäkerhet och kampen mot hunger.

Nyckelord: Veteförädling, Hållbar sjukdomsresistens, markörbaserat urval, Resistensgen, Råg, Resistenstest för plantor, svartrost, gulrost, vete, vete–råg-introgressionsstrategier.

بهره‌گیری از مقاومت گندم در برابر زنگ سیاه گندم از طریق استراتژی‌های واردسازی صفات چاودار به گندم (In Persian)

چکیده

گندم (*Triticum aestivum*) یکی از سه غله مهم در سطح جهانی است که منبع اصلی تأمین پروتئین و مواد مغذی لازم روزانه برای انسان به‌شمار می‌آید. متأسفانه، عملکرد این محصول به‌واسطه چالش‌های مختلفی تحت تأثیر قرار گرفته است. یکی از مهم‌ترین این چالش‌ها، ظهور نژادهای جدید زنگ سیاه و زرد است که قادر به غلبه بر ژن‌های مقاومت مؤثر پیشین می‌باشند. این موضوع نیاز فوری به یافتن منابع جدید مقاومت در گندم را برجسته می‌کند. این پایان‌نامه پتانسیل واردسازی صفات چاودار به گندم را برای بهبود مقاومت گندم در برابر این پاتوژن‌های مخرب مورد بررسی قرار می‌دهد.

با استفاده از داده‌های تعیین توالی ژنوتایپی (GBS) و نشانگرهای PCR اختصاصی آلل رقابتی(KASP) ، ما روشی دقیق برای ردیابی ژن *Sr59* و توسعه ارقام جدید گندم با عملکرد زراعی مناسب و مقاومت در برابر زنگ سیاه گندم ایجاد کردیم. علاوه بر این، یک لاین جدید از تلاقی گندم و چاودار (لاین شماره 284) تولید کردیم که در آن ژن *Sr59* به‌صورت یک قطعه کروموزومی کوچک رابرتسونی (2BS.2BL-2RL) در بخش انتهایی کروموزوم 2RL قرار دارد. این بخش کروموزومی به دلیل اندازه کوچک و سهولت واردسازی به ارقام تجاری گندم، برای برنامه‌های اصلاح نباتات بسیار مناسب است.

علاوه بر این، ترازکردن داده‌های GBS با ژن‌های NLR (ژن‌های دارای تکرار غنی از لوسین که قادر به اتصال نوکلئوتید هستند) شناسایی دو کاندیدای ژن NLR در کروموزوم 2RL را ممکن ساخت که به درک بهتر مبنای ژنتیکی مقاومت کمک کرد. از طریق غربالگری فنوتیپی و تأیید مولکولی، ژن مقاومت دیگری در برابر زنگ سیاه گندم به نام *SrSLU* را در لاین C295 شناسایی کردیم که مقاومت گسترده‌ای در برابر چندین نژاد زنگ سیاه از خود نشان می‌دهد. به‌منظور افزایش پایداری و گستردگی مقاومت، ما با موفقیت ژن *Sr59* را با ژن مقاومت زنگ زرد گندم (*YrSLU*) در یک لاین گندم ادغام کردیم. در این رویکرد از انتخاب با کمک نشانگر (MAS) و فناوری‌های اصلاح سریع نباتات (speed breeding) برای تسریع چرخه‌های اصلاح، همراه با تلاقی با ارقام تجاری برتر، استفاده کردیم. ارقام به‌دست آمده علاوه بر داشتن مقاومت به هر دو زنگ دارای عملکرد بالا نیز می باشند.

این تحقیق دیدگاه‌های مهمی در خصوص استفاده از تلاقی صفات چاودار به گندم به عنوان منبعی از ژن‌های مقاومت نوین ارائه می‌دهد. یافته‌های این مطالعه گام مهمی در راستای امنیت غذایی و مبارزه با گرسنگی در جهان محسوب می‌شوند.

واژگان کلیدی: گندم، مقاومت پایدار در برابر بیماری‌ها، انتخاب با کمک نشانگر، ژن مقاومت، چاودار، آزمون مقاومت نهال، زنگ سیاه گندم، زنگ زرد گندم، واردسازی صفات چاودار به گندم.

استغلال مقاومة القمح للصدأ الجذعى من خلال استراتيجيات تهجين القمح والجاودار (In Arabic)

الملخص

يعد القمح (*Triticum aestivum*) أحد أهم ثلاثة أنواع من الحبوب على مستوى العالم التى توفر البروتين والتغذية اليومية الضرورية للإنسان. ولسوء الحظ، تتأثر قدرة هذا المحصول على الإنتاجية بتحديات مختلفة. ومن أهم التحديات التى تواجهها ظهور أنواع جديدة من الصدأ الأسود والصدأ أصفر، وهى قادرة على التغلب على جينات المقاومة الفعالة سابقاً. وهذا يؤكد الحاجة الضرورية لمصادر جديدة للمقاومة فى القمح. تستكشف هذه الأطروحة إمكانات سلالات تهجين القمح (introgression lines) والصدأ لتحسين مقاومة القمح لهذه الممرضات المدمرة وباستخدام بيانات التنميط genotyping-by-sequencin (GBS) و Kompetitive Allele-Specific PCR (KASP)، قمنا بتطوير منهجية قوية لتتبع سلالة *Sr59* بدقة خلال دورات التربية وتطوير أصناف جديدة من القمح ذات أداء زراعى لائق ومقاومة لصدأ الساق/ الصدأ الأسود. بالإضافة إلى ذلك، قمنا بتطوير سلالة جديدة منتقلة من القمح و الجاودار (رقم 284) تحتوى على سلالة *Sr59* على شكل انتقال صغير من صبغى Robertsonian translocation, 2BS.2BL-2RL، وتقع فى الجزء البعيد من الكروموسوم 2RL تعتبر هذه القطعة الكروموسومية مفيدة بشكل خاص لبرامج التربية نظراً لصغر حجمها وبساطة دمجها فى أصناف القمح المتكيفة. بالإضافة إلى ذلك، حددت محاذاة GBS مع الجاودار nucleotide-binding leucine-rich repeat (NLR) المشروحة اثنين من NLRs المرشحة على الكروموسوم 2RL مما زاد من إثراء فهمنا للأساس الجينى للمقاومة. من خلال فحص النمط الظاهرى والتحقق من صحة الجزيئات، قمنا بتمييز جين ثانٍ لمقاومة صدأ الساق، وهو*SrSLU*، الموجود فى السلالة رقم C295، والذى يُظهر مقاومة واسعة الطيف لأجناس متعددة من صدأ الساق. ولتعزيز المتانة وتوسيع نطاق المقاومة، نجحنا فى دمج الجين *Sr59* مع الجين*YrSLU*، وهو جين مقاوم للصدأ أصفر، فى سلالة قمح واحدة. فى هذا النهج، نستخدم الانتقاء بمساعدة الواسمات (MAS) وتقنيات التربية السريعة لتسريع دورات التربية مع الحفاظ على الأداء الزراعى العالى من خلال التهجين العلوى مع نخبة الأصناف التجارية. وتجمع السلالات الناتجة بين المقاومة القوية ضد كل من صدأ الساق والصدأ المخطط مع تحسين إمكانات المحصول. ويوفر هذا البحث رؤى مهمة حول استخدام القمح-الجاودار كمصدر لجينات المقاومة الجديدة ويطور منهجيات توصيفها وتطويرها بدقة. وتمثل هذه النتائج خطوات مهمة نحو تحقيق الأمن الغذائى ومكافحة الجوع.

الكلمات الدليلية: التناسل، المقاومة الدائمة للأمراض، الانتقاء بمساعدة العلامات، الجين المقاوم، الجاودار، اختبار مقاومة الشتلات، صدأ أسود، الصدأ أصفر، القمح، تداخل القمح مع الجاودار.

Dedication

To My family and their endless supports,

To:

My father (Rahim Yazdani)

My mother (Zahra Moormeh)

My sisters (Zohreh and Mahdieh)

and to my brother (Abbas)

Contents

List of publications

This thesis is based on the following publications, indicated by Roman numbers in text:

I. **Yazdani, M**., Rouse, M.N., Steffenson, B.J., Bajgain, P., Patpour, M., Johansson, E. and Rahmatov, M., 2023. Developing adapted wheat lines with broad-spectrum resistance to stem rust: Introgression of *Sr59* through backcrossing and selections based on genotyping-by-sequencing data. *Plos one*, *18*(10), p.e0292724. https://doi.org/10.1371/journal.pone.0292724

II. **Yazdani, M.**, Rouse, MN., Bajgain, P., Danilova, T., Motsnyi, I., Steffenson, BJ., Patpour, M., Rahmatov, M. 2025. Identification and characterization of *Sr59*-mediated stem rust resistance in a novel wheat-rye translocation T2BL.2BS·2RL (Published in The Crop Journal). https://doi.org/10.1016/j.cj.2025.02.012

III. **Yazdani, M**., Patpour, M., Bajgain, P., Houben, A., and Rahmatov, M., 2024. Physical mapping and identification of a 2RL translocation with new stem rust resistance (*SrSLU*) in wheat. In the form of Manuscript.

IV. **Yazdani, M**.*, Ashraf, R.*, Johansson, E., Vallenback, P., Hovmøller, M.S., Patpour, M. and Rahmatov, M. 2024. Marker-assisted selection to harness rye genes for wheat improvement: Opportunities and challenges with combining novel stem and stripe rusts resistance genes in the same genotype (Accepted in Crop Science).
*Shared first authorship

V. Patpour, M., Rahmatov, M., **Yazdani, M**. and Justesen, A.F., 2023. First report of race TTRTF of the Wheat Stem Rust pathogen *Puccinia graminis* f. sp. *tritici* in Sweden. *Plant Disease*, *107*(6), p.1945. https://doi.org/10.1094/PDIS-06-22-1398-PDN

The contribution of Mahboobeh Yazdani to the papers included in this thesis was as follows:

I. Description of contribution to Paper I.

Planned and conducted the laboratory and greenhouse, including crossing (together with MR), KASP markers to detect rye chromatin in wheat-rye introgression lines, testing the material at the seedling stage against *Pgt* races, editing the final manuscript together with the other authors.

II. Description of contribution to Paper II.
Contributed to the conception and designed the study together with MR and MP. Performed the lab and greenhouse experiments including crossing, KASP marker and seedling resistance test together with MR and MP. Analyzed the data and wrote the first draft. All co-authors contributed to editing and revising the manuscript.

III. Description of contribution to Paper III.
Contributed to the conception and scheme of the study together with MR and MP. Performed the experiments and collected data including crossing, seedling resistance test, and cytology study. Wrote the first draft with the input of the co-authors. All authors contributed to the final version of the article.

IV. Description of contribution to Paper IV.
Planned the experiments together with other authors. Performed lab and greenhouse experiments including crossing, KASP marker, and seedling resistance test for both stem and stripe rust together with RA. Wrote the first draft of manuscript. All authors contributed to the final version of the article.

V. Description of contribution to Paper V.
Participated in *Pgt* survey in the south of Sweden. Performed race-typing protocol of *Pgt* together with MP. Wrote the first draft with the input from MP and MR. All authors contributed to the final version of the article.

List of tables

List of figures

Abbreviations

BGRI	Borlaug Global Rust Initiative
DT	Double Top cross
EST	Expressed Sequence Tag
FISH	Fluorescence in-situ hybridization
GBS	Genotyping-by-sequencing
GP	Gene pool
GRRC	Global Rust Reference Centre
IT	Infection Type
IWGSC	International Wheat Genome Sequencing Consortium
KASP	Kompetitive allele-specific polymerase
MAF	Minor Allele Frequency
MAS	Marker-assisted selection
NLR	Nucleotide-binding and leucine-rich repeat
PCR	Polymerase chain reaction
Pgt	*Puccinia graminis* f.sp. *tritici*
Pst	*Puccinia striiformis* f.sp. *tritici*
RGSCR	Rye genome sequencing consortium reference
SIM	Structured Illumination Microscopy
SLU	Swedish University of Agricultural Sciences
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat
TT	Triple Top cross

1. Introduction

Wheat (*Triticum aestivum* L., 2*n* = 6*x* = 42, ~17 Gb, AABBDD) is an important staple crop that supplies essential calories to the majority of the global population (FAO 2024). This crop has been grown for more than 10,000 years and was preliminarily domesticated in the Fertile Crescent and Mediterranean regions (Feldman & Levy 2015). Since then, farmers have constantly selected “the best” genotypes, starting with emmer and einkorn, for their “favorable traits” such as grain yield and easy threshing (Feldman & Millet 2001). Such domestication has led to intense cultivation of this crop worldwide (Hafeez *et al.* 2021). Indeed, wheat provides over 20% of the nutritional calories and protein consumed by humans globally (Shiferaw *et al.* 2013; D'Odorico *et al.* 2014). The global production of bread wheat in 2024 was 791 million tonnes, with an average yield of 3 t/ha (FAO 2024). Many important food products such as bread, pasta and noodles come from wheat. These products face challenges such as the pressure of biotic and abiotic stresses. While wheat's worldwide importance is undeniable, producing “better” varieties via breeding is vital to both address novel challenges and fulfil the expanding demands.

The primary goal of plant breeding is to enhance global food security for human civilization, thereby serving the demands of both producers and consumers (Fedoroff 2015). However, breeding faces several challenges. One of the major obstacles often discussed within the literature is the potential of narrowing the genetic gene pool as a result of years of intensive breeding and selection. While certain studies argue that this process has led to decreased genetic diversity in progenies (Hafeez *et al.* 2021), others suggest that the impact on diversity may not be as significant or uniform across all breeding programs. This ongoing debate highlights the complexity of assessing genetic diversity in modern crops and its implications for breeding efforts. Regardless of the differing perspectives, the genetic

limitation remains a valid concern, as it could render it increasingly difficult to achieve new genetic improvements and breed varieties with higher yields and stress tolerance (Voss-Fels *et al.* 2019). Moreover, the polyploid nature of wheat and its large and complex genome present considerable challenges for implementing certain biotechnology methods (Hafeez *et al.* 2021). On the other hand, climate change poses a major threat to wheat production, with forecasts predicting yield decreases of 4.1-6.4% for every 1°C increase in world temperature (Zhao *et al.* 2017). Further, the emergence of novel diseases and the persistence of "old enemies" such as rusts, continue to challenge breeders in developing resistant varieties (Borlaug 2008).

In addition to these various biotic and abiotic challenges, the global population continues to expand, with forecasts reaching 10 billion by 2050. The demand for wheat is predicted to increase by 60%, which will require considerable increases in yield and productivity (FAO 2009). To address these challenges, researchers are considering a variety of strategies, including the introduction of new genetic diversity from plant genetic resources and wild relatives, as well as the use of modern breeding technologies such as genomic selection and gene editing.

Cereal rusts, caused by fungus from the genus *Puccinia*, are among the most economically important plant diseases, posing a serious threat to world food security (Chaves *et al.* 2008). These obligate parasites have co-evolved with their cereal hosts such as wheat, barley, oats, and rye (Chaves *et al.* 2008). The three primary types of wheat rusts are stem rust (*Puccinia graminis* f.sp. *tritici*), stripe rust (*P. striiformis* f.sp. *tritici*), and leaf rust (*P. triticina*) which are each capable of causing considerable yield losses in susceptible varieties (Chaves *et al.* 2008). Historically, cereal rusts have caused widespread crop failures and famines, with records extending back to ancient civilizations. For example, Aristotle (384-322 B.C.) described rust outbreaks and their catastrophic consequences (Carefoot & Sprott 1967). Indeed, according to current estimates, cereal rusts cause almost $5 billion in global losses each year (Pardey *et al.* 2013; Beddow *et al.* 2015; Newbery *et al.* 2016). Throughout history, several rust outbreaks have occurred, demonstrating the devastating potential of these diseases. One of the most notable outbreaks involved stem rust in a global epidemic in North America in 1950, which destroyed up to 40% of the spring wheat production (Roelfs

1985a). Additionally, the Ug99 stem rust race, which emerged in Uganda in 1998, posed an extreme threat to world wheat products because it overcame resistance genes that had been previously effective for several decades (Singh *et al.* 2011). Climate change is expected to impact the prevalence and severity of rust outbreaks, possibly shifting their geographical distribution and increasing epidemic frequency (Hovmoller *et al.* 2016; Patpour *et al.* 2022).

To combat these challenges, research is currently aimed at developing resistant cultivars, enhancing fungicide effectiveness, and applying integrated disease management. Among these, developing resistant cultivars is the most cost-effective and ecologically environmentally friendly strategy for reducing wheat rust disease losses (Burdon *et al.* 2014; Singh *et al.* 2016). Through this approach, wheat wild relatives and wheat-rye introgression lines have played an essential role in expanding wheat's genetic background and transferring desirable rye characteristics into wheat cultivars (Kole 2011; Molnár-Láng *et al.* 2015). Moreover, Marker-assisted selection (MAS) has been recognized as a valuable technique to enhance disease resistance cultivars (Collins *et al.* 2018).

This thesis focuses on integrating stem rust resistance genes into adapted wheat cultivars and discovering possible new sources of resistance from wheat-rye introgression lines using MAS and cytogenetics approaches. Furthermore, we attempt to pyramid resistance genes from stem and stripe rust together in one cultivar.

2. Background

2.1. Stem rust

Stem rust fungus belongs to the genus *Puccinia*, which includes around 4000 species, in the family Pucciniaceae, order Pucciniales, class Pucciniomycete, and division Basidiomycota of the fungi kingdom (Kirk *et al.* 2008). This fungal population has several host-specific forms that can infect rye, barley, wheat, and oats (Eriksson & Henning 1896).

Puccinia graminis Pers f. sp. *tritici* Eriks. and E. Henn. (*Pgt*) is the scientific name of stem rust (Figure 1), which is the most dangerous form special (f. sp.) of *Puccinia graminis* in wheat (Kirk *et al.* 2008; Singh *et al.* 2011).

Figure 1-*Puccinia graminis* - Photo: Author.

2.1.1. Life cycle of stem rust

Puccinia gramins is a heteroecious and macrocyclic fungus with five spore stages including uredinial, telial, basidial, pycinial, and aecial (Roelfs 1985b). This pathogen requires a cereal or grass plant and an alternate host plant such as *Berberis* spp., *Mahonia* spp. to complete its sexual lifecycle (Leonard & Szabo 2005). Among all spores, urediniospore is the most important stage for the pathogen's survival and, in some cases, for initiating the disease, as it occurs in numerous cycles (Asexual) on host plants such as cereals or grasses.

The life cycle begins with the germination of overwintered teliospores in suitable conditions (Table 1) and the formation of basidiospores (Figure 2). These spores attack young leaves of the common barberry (*Berberis vulgaris*) and the other alternate host such as *Mahonia* spp., or *Mahoberberis* spp. (Wang *et al.* 2015). The ensuing infections on the barberry produce specialized infection structures termed pycnia, which are necessary for the fungus' reproductive stage (Figure 2).

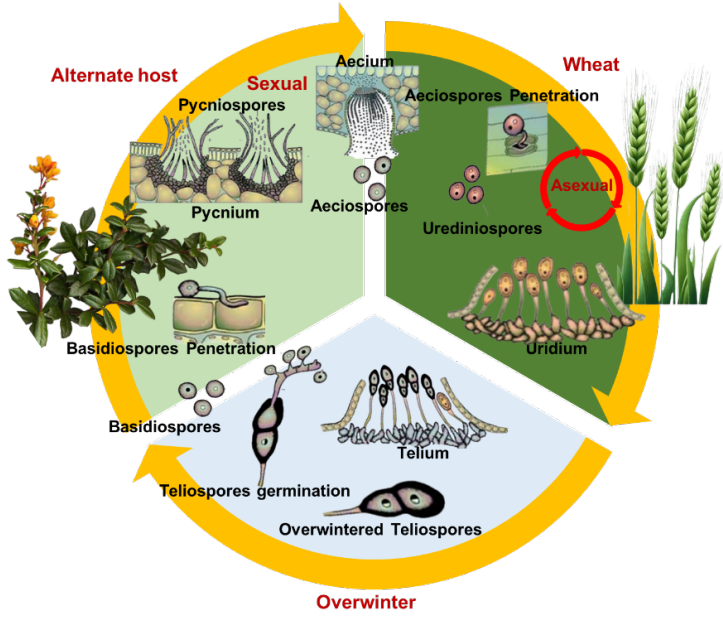


Figure 2-Life cycle of *Puccinia graminis* f. sp. *tritici* (Agrios 2005) with modification.

On barberry, *Pgt* completes its sexual cycle (Anikster *et al.* 1999), through one pycnium hyphae mating with the spores of another pycnium and the fertilized structure subsequently develops into an aecium (Craigie 1927). Aeciospores from the aecium infect wheat, and the asexual or repetitive phase of *Pgt* begins with the formation of uridium and urediniospores, which can infect other surrounding wheat plants or even proceed to another continent and cause infection there. At the end of the season, when the conditions are no longer suitable for pathogen establishment, urediniospores transform into black teliospores which can persist in the soil for up to 14 years (Leonard & Szabo 2005).

Table 1-Environmental conditions necessary for *Puccinia graminis* f. sp. *tritici*. (Roelfs *et al.* 1992).

Stage	**Temperature (°C)**			**Light**	**water**
	Minimum	Optimum	Maximum		
Germination	2	15-24	30	Low	Necessary
Sprout	-	20	-	Low	Necessary
Appressorium formation	-	16-27	-	None	Necessary
Penetration	15	29	35	High	Necessary
Growth	5	30	40	High	None
Sporulation	15	30	40	High	None

When the weather is warm and humid, wheat serves as a green bridge or primary inoculum source, triggering a fresh cycle of stem rust wheat disease the following season. However, in regions with cold temperatures, aeciospores are the most common source of primary inoculum for wheat stem rust infection (Leonard & Szabo 2005).

2.1.2. The importance of stem rust worldwide

Stem rust, also referred to as black rust, has been a serious threat to wheat production since ancient times. Evidence suggests that the Romans sacrificed red animals to the rust god Robigus as early as 700 B.C. to preserve their

grain fields from the reddish-brown rust spores (Zadoks 1985). In the United States, outbreaks of the disease have been documented in 1904, 1916, 1954, 1965, 2015, 2016, and 2017. The largest epidemic of stem rust in the United States occurred in 1935 when half of North Dakota and Minnesota's wheat yield was destroyed by *Pgt* (Roelfs 1985a; Leonard & Szabo 2005). In Australia, remarkable epidemics took place in 1973 on susceptible varieties to *Pgt* which resulted in a 40% total grain failure (Roelfs 1985a).

Despite the pathogen virulence, an international community of plant pathologists and wheat breeders significantly prevented stem rust epidemics globally in the second half of the twentieth century by introducing new resistance varieties and eradicating the barberry bushes (Peterson 2001). However, the emergence of the highly virulent Ug99 (TTKSK) race in Uganda in 1998 (Pretorius *et al.* 2000), which carries virulence to stem rust resistance gene *Sr31,* marked a turning point, causing more than 80% of the world's wheat varieties to be susceptible to stem rust (Singh *et al.* 2011). This important race and its variations have now spread throughout East and Southern Africa and into the Middle East and South Asia (Hovmøller *et al.* 2023; Patpour *et al.* 2024). Due to the presence of Ug99 cultivation of wheat has been prohibited or restricted in certain parts of the world such as Ethiopia, Uganda, and Rwanda (Singh *et al.* 2011).

To date, Ug99 has not been reported in Europe. However, the first re-emergence of stem rust in Europe was in 2013 with the regional epidemic in Germany followed by a series of sporadic infections in Denmark, Sweden, and the UK (Hovmøller *et al.* 2018). Following these outbreaks, a much larger wheat stem rust epidemic occurred in Sicily in 2016 which impacted thousands of hectares of durum and bread wheat (Bhattacharya 2017). The new race was assigned as TTRTF (Bhattacharya 2017), which contained the virulence gene to *Sr13b*, the durum wheat resistance gene (Patpour *et al.* 2020) . Since then the Sicily race has been reported in many other countries including Austria, Croatia, Czech Republic, Hungary, Italy, Slovak Republic, Slovenia, Spain, and Switzerland (Patpour *et al.* 2022). In a global effort to control an early-warning system for wheat rust diseases organizations such as RustWatch, Borlaug Global Rust Initiative (BGRI), and Global Rust Reference Center (GRRC) have been established (McIntosh & Pretorius 2011). So far, these international efforts have played a critical

role in monitoring the spread of stem rust and identifying new virulent strains. However, the continued emergence of new races such as TTRTF demonstrates the pathogen's ability to adapt and overcome existing resistance mechanisms. This underscores the importance of not only tracking rust pathogens but also developing durable resistance strategies to protect wheat crops. Although no Ug99 lineage has been detected in Europe yet, the presence of other aggressive races emphasizes the need for active monitoring of the pathogen movement. Understanding the genetic basis of resistance and susceptibility is key to stay ahead of the evolving threat that stem rust poses.

The first outbreak of stem rust in Sweden was in 2017 and this was characterized by an adaptation of race to cold temperatures and extremely moist conditions. Since then, stem rust has been continuously reported on wheat, barley, and rye in Sweden (Kjellström 2021; Patpour *et al.* 2022). Highly prevalent races identified in Sweden are TKTTF and TKKTF (Patpour *et al.* 2022). The evolving nature of the pathogen emphasizes the need for continually monitoring the pathogen and exploring new breeding programs to identify possible resistance sources.

2.2. Stripe rust

Stripe rust, also referred to as yellow rust, is a serious fungal disease in wheat caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*). This disease has been a constant threat to wheat production. Stripe rust is especially damaging in cold, humid environments and can result in significant production losses of up to 70% in susceptible wheat cultivars under ideal conditions (Chen 2005; Wellings 2011). The disease's common name is derived from the yellow-orange pustules that grow in stripes along leaf veins.

Figure 3-*Puccinia striiformis* - Photo: Author

2.2.1. The importance of stripe rust worldwide

Yellow rust has been a significant threat to wheat production throughout history, with its impact on agriculture dating back to ancient times. The disease was first characterized in 1777 by Gadd, but its effects on wheat yields were acknowledged much earlier than this (Stubbs 1985). Throughout the nineteenth and twentieth centuries, yellow rust remained a serious threat to wheat producers worldwide. Severe outbreaks were reported in several parts of the world, causing substantial crop losses and economic devastation. For example, an outbreak of stripe rust in China resulted in significant crop losses and economic devastation. This epidemic affected over 66 million acres (26.7 million hectares) of wheat, causing yield losses of up to 14 million tons. The economic impact was estimated to be around $1 billion in that year alone (Beddow *et al.* 2015). Over the years, the disease has exhibited considerable adaptability, with new races emerging that can overcome previously resistant genes (Hovmoller *et al.* 2011). In 2011, the emergence of two new stripe rust races, Warrior (*PstS7*) and Kranich (*PstS8*) caused outbreaks in many wheat varieties (Hovmoller *et al.* 2011; Hovmoller *et al.* 2016). In 2020, a new yellow rust race named *PstS16* was discovered

in numerous European countries, including Denmark, Sweden, and the United Kingdom, raising concerns about its possible impact on previously resistant wheat cultivars (Hovmøller *et al.* 2021). Furthermore, the spreading of races belonging to the *PstS2* lineage in Central and West Asia has caused concerns in wheat production in countries such as Afghanistan, Iran, and Pakistan (Ali *et al.* 2017). These rapid developments of disease emphasize the importance of the continuous need for careful monitoring and breeding efforts for long-term resistance to reduce the impact of yellow rust on worldwide wheat production.

3. Genetic resources of wheat

Wheat has a rather limited gene pool due to extensive breeding efforts and the widespread adoption of high-yielding and uniform cultivars. The limitations of the gene pool has generated considerable threats to wheat production by restricting the opportunity for breeding new varieties that can respond to future problems such as climate change and new emerging diseases (Feldman & Levy 2015; Hafeez *et al.* 2021). To broaden the gene pool, wheat genetic resources are critical for the long-term enhancement and sustainability of global wheat agriculture. These resources include a wide range of genetic materials such as wild relatives of wheat, landraces, traditional cultivars, advanced cultivars, and breeding lines (Reynolds & Braun 2022).

3.1. Gene pool

Harlan and de Wet (1971) first proposed the concept of a gene pool. Species were classified based on their crossover potential, taxonomy, and genetic relatedness (Harlan & de Wet 1971). Jiang et al. (1993) broadened the idea of gene pools to include primary, secondary, and tertiary groups based on evolutionary divergence and genomic makeup (Jiang *et al.* 1993).

The primary gene pool (GP-1) of wheat is an important resource for wheat improvement, consisting of species that can easily cross with bread wheat (*T. aestivum*, $2n = 6x = 42$, AABBDD) and produce fertile offspring through simple breeding techniques such as crossing, selection, and backcrossing. This gene pool includes modern wheat cultivars, wheat landraces, and closely related species that share homologous genomes with bread wheat. GP-1 includes *T. spelta* ($2n = 6x = 42$, AABBDD), tetraploid durum wheat

(*T. turgidum*, 2*n* = 4*x* = 28, AABB), and diploid wheat species such as *T. urartu* (2*n* = 2*x* = 14, AA) and *Aegilops tauschii* (2*n* = 2*x* = 14, DD) (Laugerotte *et al.* 2022). In wheat breeding projects, the GP-1is the major source of genetic diversity for improving traits including yield, disease resistance, and quality traits. The GP-1 species can easily hybridize with bread wheat, producing fertile hybrids with normal chromosomal pairing and gene segregation. This characteristic enables breeders to successfully transfer beneficial features from these species into new wheat varieties (Laugerotte *et al.* 2022).

The secondary gene pool (GP-2) of wheat includes species that share certain homology with the bread wheat genome but are not as closely related as those in the GP-1 (Anderson 1949; Schoen *et al.* 2024). These species typically share at least one homologous genome with wheat. These species can still be crossed with bread wheat, although the resulting hybrids are often less fertile due to unbalanced chromosome compositions (Schoen *et al.* 2024). Important examples in the GP-2 include *T. timopheevii* (2*n* = 4*x* =28, AAGG) and *Aegilops speltoides* (2*n* = 2*x* = 14, SS). Despite the challenges related to low fertility in hybrids, introgression from GP-2 species into bread wheat is fairly simple due to homologous recombination.

The tertiary gene pool (GP-3) of wheat is comprised of species that are more distantly related to bread wheat and have non-homologous genomes. These species can be crossed with wheat; however, the procedure often requires complex methods such as embryo rescue, chromosomal manipulation, or somatic hybridization to generate fertile hybrids (Hao *et al.* 2020). Some of the examples of this gene pool include (King *et al.* 2022): *Agropyron cristatum* (crested wheatgrass 2*n* = 2*x* =14, PP), *Pseudoroegneria spicata* (blue bunch wheatgrass 2*n* = 2*x* =14, StSt), *Psathyrostachys huashanica* (2*n* = 2*x* =14, NsNs), *Thinopyrum elongatum* (2*n* = 2*x* =14, EE), *Elymus scaber* (2*n* = 4*x* = 28 or 2*n* = 6*x* =42, StStHH or StStHHYY), *Hordeum vulgare* (Barley 2*n* = 2*x* =14, HH), *Leymus racemosus* (2*n* = 4*x* = 28, NsNsXmXm), and *Secale cereale* (Rye 2*n* = 2*x* = 14, RR). The GP-3 is a neglected yet extremely promising source of genetic variation for wheat improvement. Successful introgression of genes from the GP-3 species can considerably improve wheat's tolerance and production in the face of global concerns such as climate change and new emerging pathogens.

3.2. Rye for wheat resistance breeding

Rye has significant potential for enhancing wheat resistance through breeding programs. The similar order and arrangement of genes (collinearity) in rye and wheat chromosomes facilitates the transfer of genetic material between them (Saulescu *et al.* 2011). Wheat chromosomes 1, 2, 3, 5, and 6 are generally homologous with the rye chromosomes 1R, 2R, 3R, 5R, and 6R, and wheat chromosomes 4 and 7 show partial reciprocal homology with groups 4R and 7R (Bauer *et al.* 2017).

This genomic similarity enables effective interspecies chromosomal translocations and replacements, making rye an appropriate genetic resource for wheat improvement. Thus, rye chromatin has been widely utilized to transfer genes that confer resistance to numerous biotic and abiotic threats into the wheat genome (Table 2). A notable example is the widely used 1RS.1BL translocation, which contains disease-resistance genes (e.g., *Pm8*, *Sr31*, *Lr26*, and *Yr9*) and has been introduced into many wheat cultivars worldwide (Rabinovich 1998; Ren *et al.* 2012).

Table 2-Resistance genes against *Pgt* and *Pst* pathogens coming from rye

Gene Symbol	Rust Type	Chr.	Rye Cultivar	Translocation	Reference
Sr27	*Pgt*	3RS	Imperial	3AS.3RS, 3AL.3RS, 3BL.3RS	(McIntosh et al. 1995)
Sr1RS^{Amigo}	*Pgt*	1RS	Insave	1AL.1RS	(Zeller & Fuchs 1983)
Yr31	*Pst*	1RS	Petkus	1BL.1RS	(McIntosh et al. 1995)
Yr9	*Pst*	1RS	Petkus	1BL.1RS	(Friebe et al. 1996)
Sr50	*Pgt*	1RS	Imperial	1DL.1RS	(Mago et al. 2004)
YrCn17	*Pst*	1RS	Petkus-L155	1BL.1RS	(Ren et al. 2009)
Yr	*Pst*	1RS	Chinese rye R12	1BL.1RS	(Fu et al. 2010)
Sr59	*Pgt*	2RL	Triticale VT828041	2BL.2RL	(Rahmatov et al. 2016a)
Yr	*Pst*	1RS	Aigan rye	1RS.1BL	(Li et al. 2016)
Yr	*Pst*	5RL	Kustro	5R(5B)	(Xi et al. 2019)
Yr83	*Pst*	6RL	T-701a	6R(6D)	(Li et al. 2020)
Sr	*Pgt*	7RL	Baili	7BS.7RL	(Ren et al. 2020)
YrSLU	*Pst*	6RL	Triticale VT828041	6DS.6DL.6RL.6DL	(Ashraf et al. 2023)

3.3. Harnessing rye chromosome and role of *ph1b*

The introduction of rye chromatin into wheat, notably the 1RS chromosomal arm, has resulted in the development of significant disease-resistance genes such as *Sr31*, *Lr26*, *Yr9*, and *Pm8* (Powdery mildew, *Blumeria graminis* f. sp. *tritici*). These genes, originating from Petkus rye, have been widely used in wheat cultivars internationally since the 1960s and have been transferred to wheat as 1BL.1RS translocation (Schlegel & Meinel 1994; Rabinovich 1998). The usage of rye genetic material extends across disease resistance, contributing to increased yield, biomass production, and tolerance to abiotic stresses (Crespo-Herrera *et al.* 2017).

Harnessing rye chromosomes is possible through several strategies; one way is the usage of the *Ph* mutant, which has been instrumental in overcoming the reproductive barriers between wheat and rye. In normal hexaploid wheat, the *Ph* genes (*Ph1b* on chromosome 5B, *ph2a* on chromosome 3D) suppresses pairing between non-homologous chromosomes during meiosis, ensuring genome stability but limiting interspecific recombination (Riley & Chapman 1958; Sears 1976). However, the *Ph1b* mutant, which carries a ~51 Mb deletion in the *Ph1* locus, relaxes this restriction, enabling increased homoeologous pairing and facilitating the transfer of rye chromatin into wheat backgrounds. This approach allows for the developing of wheat-rye addition, substitution, and translocation lines, providing breeders with tools to introgress desirable traits such as stem rust resistance genes (*Sr59*) or stripe rust resistance genes (*YrSLU*) into wheat cultivars (Rahmatov *et al.* 2016a; Ashraf *et al.* 2023).

On the other hand, harnessing rye chromosomes for wheat development involves multiple challenges. One of the greatest issues is the possibility of negative impacts on wheat quality, including bread-making quality. For example, the 1RS.1BL translocation, while providing useful resistance genes such as *Sr31*, *Lr26*, *Yr9*, and *Pm8*, has been linked to poor dough characteristics, resulting in weak and sticky dough that is inappropriate for high-quality bread manufacture (Dhaliwal *et al.* 1987; Martin & Stewart 1990).

4. Aims and objectives of the research

The primary goal of this thesis was to enhance wheat resistance to major fungal diseases, particularly stem and stripe rust, by integrating resistance genes from rye, developing precise molecular markers, and utilizing advanced genomic and cytogenetic tools. This research aims to provide critical insights into disease management and breeding strategies, ultimately contributing to the development of resilient wheat cultivars and ensuring sustainable wheat production against ever-evolving rust pathogens.

Specific objectives of the study in Papers I- IV were to:

- Introgression the *Sr59* resistance gene against stem rust into adopted wheat cultivars, to develop new wheat varieties with a broad spectrum of resistance to stem rust (Paper I).
- Identify and characterize new wheat-rye translocation lines with stem rust resistance genes using conventional and new genomic technologies (Papers II and III).
- Develop KASP markers using genotype-by-sequencing for these resistance genes, to enable their usage in future breeding projects (Papers II and III).
- Pyramid stem and stripe rust resistance genes into adapted wheat cultivars to develop varieties with broad-spectrum resistance to both major wheat diseases (Paper IV).
- Use a marker-assisted gene approach to precisely identify the position of genes in parental lines and track the presence of genes in progenies (Papers I-IV).
- Validate of the presence of genes using cytogenetic approaches (Papers II and IV).
- Monitor *Pgt* variability and virulence (Paper V).

5. Materials and Methods

5.1. Plant material resources

The materials used in this thesis involve wheat-rye introgression lines developed through a series of strategic crosses. The process began with the initial hybridization of a hexaploid triticale line, VT828041, and the wheat cultivar Beagle (Merker 1984). From this cross, line SLU238 (2R/2D) was derived. To facilitate the transfer of rye chromatin into wheat, SLU238 was crossed with CS M*ph1b*, which promotes homologous chromosome pairing restrictions during meiosis. This resulted in the development of line TA5094, carrying a 2DS·2RL translocation and the stem rust resistance gene *Sr59* (Rahmatov *et al.* 2016a). In the first study (Paper I), TA5094 was crossed with three adapted wheat cultivars, BAJ#1, KACHU#1, and REEDLING#1, to introgression *Sr59* into commercial wheat backgrounds.

Another notable outcome of the SLU238 x CS M*ph1b* cross was line #284, which showed a different IT in multiple *Pgt* race tests compared to TA5094. This line was further investigated in Paper II.

Additionally, crossing TA5094 with Chinese Spring resulted in a population from which line #C295 was selected for its broad-spectrum stem rust resistance. This was studied in Paper III.

Furthermore, to combine resistance against both stem and stripe rust, pyramided lines were developed by crossing TA5094 with line #392 (*YrSLU*), followed by top-crossing these lines with commercial varieties (Linkert and Navruz) and an elite breeding line (SLU-Elite). These efforts are detailed in Paper IV (Figure 4).

To accelerate the breeding cycles, all experiments were conducted using the speed breeding method in a greenhouse, where plants were grown in small pots under a Day/Night temperature of 24/18°C and an 18/6-hour light cycle. The plants were harvested before full maturity and dried in an oven for three days.

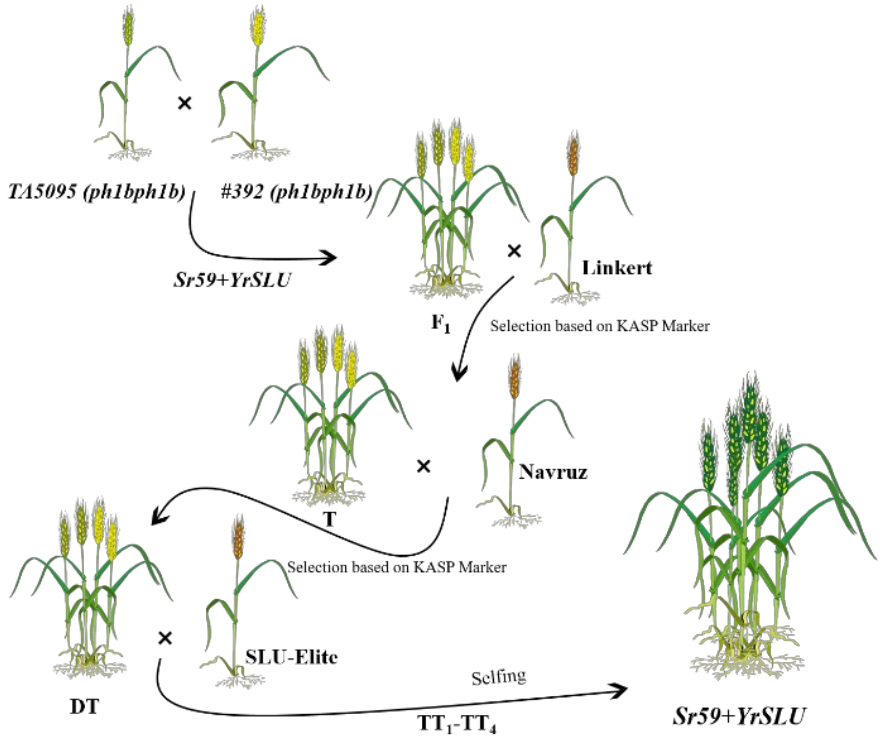


Figure 4-Scheme of gene pyramiding used in Paper IV, DT: Double Top cross, TT: Triple Top cross

5.2. Genotyping-by-sequencing (GBS)

Genotyping-by-sequencing (GBS) was performed at the Genomic Center at the University of Minnesota on parental lines CS M*ph1b*, CSA, SLU238, TA5094, SLU392, BAJ#1, KACHU#1, REEDLING#1, Linkert, Navruz and SLU-Elite using the method described by Poland *et al.* (2012). These GBS data were aligned with the wheat reference genome from the International Wheat Genome Sequencing Consortium- IWGSC (2018) and rye reference genome (Rye genome sequencing consortium reference- RGSCR- Rabanus-Wallace *et al.* (2021) to map the putative SNPs (single nucleotide

polymorphism) for the 2B and 2R chromosomes. After filtering SNP markers using the Burrow-Wheelers Alignment tool (BWA) v0.7.15 (Li & Durbin 2009), a total of 4,067 SNPs for 2B (11,067 bp to 800,998,610 bp) and 15,116 SNPs for 2R (347,694 bp to 945,773,747 bp) were identified (Papers II and III). This method enabled us to detect the small translocation which was not possible to discover by Cytogenetic fluorescence *in situ* hybridization (FISH) analysis (Paper III).

5.3. Identifying NLR genes and development of KASP marker

By aligning GBS data to rye nucleotide-binding and leucine-rich repeat (NLR) genes, using the BWA tool, the physical positions of SNPs linked with NLR genes on the 2R chromosomes in parental lines were identified. KASP markers were constructed using a 120-base pair flanking sequence (60 upstream and 60 downstream) around the NLR-GBS site. This technique converted chromosome-specific NLR-GBS markers into KASP primers. Using the Polymarker (www.polymarker.info) website, 14 (2RL) KASP primers with two allele-specific forward primers (A1 and A2) with FAM (5′GAAGGTGACCAAGTTCATGCT3′) and HEX (5′GAAGGTCGGAGTCAACGGATT3′) compatible tails and one common reverse primer (C1/C2) were developed. These KASP markers were then validated and used for further analysis. KASP markers for genes *Sr59* (Rahmatov *et al.* 2016a) and *YrSLU* (Ashraf *et al.* 2023) were used for gene pyramiding in Paper IV. Furthermore, the Physical position of the gene was drawn using MapChart (https://www.wur.nl/en/show/mapchart.htm).

5.4. Marker-assisted selection

Designed KASP Markers for genes *Sr59*, *YrSLU*, and *SrSLU* were used to select plants for the presence of genes in each generation (Paper I-IV). Additionally, Expressed Sequence Tag (EST) derived Simple sequence repeat (SSR) markers *Xrems1251* (Khlestkina *et al.* 2004) and *F3/R3* (Katto

et al. 2004) were used to detect the presence of 2R rye chromosome in progenies. These markers enabled us to associate traits, which were presumably transferred small segments of rye chromatin located in 2R to wheat (Paper I-III).

5.5. Seedling resistance evaluation to stem rust and stripe rust

Seedling resistance test to *Pgt* race TTTTF, QTHJC, TPMKC, RKQQC, RCRSC, TTRTF, TKTTF, TTKTT, TTKSK, TTKST, TTTSK, TRTTF, and JRCQC were conducted at the USDA-ARS Cereal Disease Laboratory and the University of Minnesota using a method described by (Rouse *et al.* 2011) (Papers I and II). The seedling assay to *Pgt* races TTKSK, TTRTF, and TTTTF and *Pst* races *Psts10*, *Psts16*, *Psts7*, and *Psts13* were carried out at the Global Rust Reference Center (GRRC), Aarhus University, Denmark following the method described by Patpour et al. (2022) (Papers I and IV). Seedling infections with *Pgt* were evaluated 16 days after inoculation using Stakman *et al.* (1962) scale of 0 to 4 with modification (Figure 5).

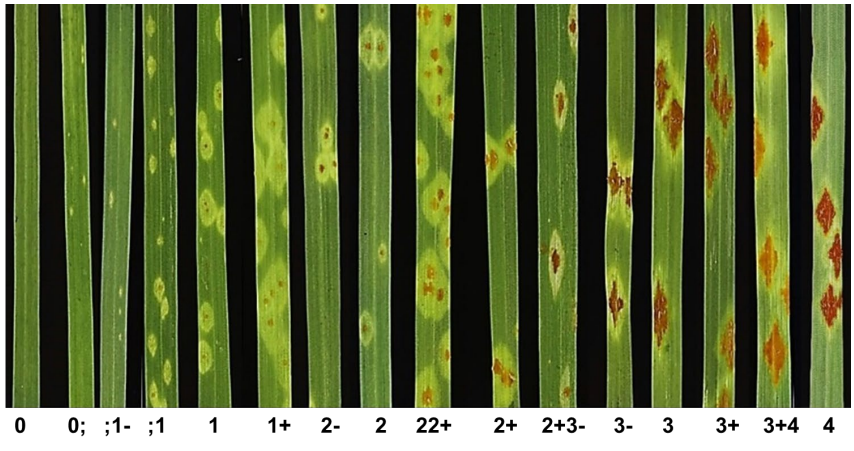


Figure 5-*Puccinia graminis f. sp. tritici* scoring - Photo: Mehran Patpour

To evaluate the severity of seedling infection with *Pgt*, a scale of 0 to 9 described by McNeal (1971) was used 14 days after inoculation (Figure 6)

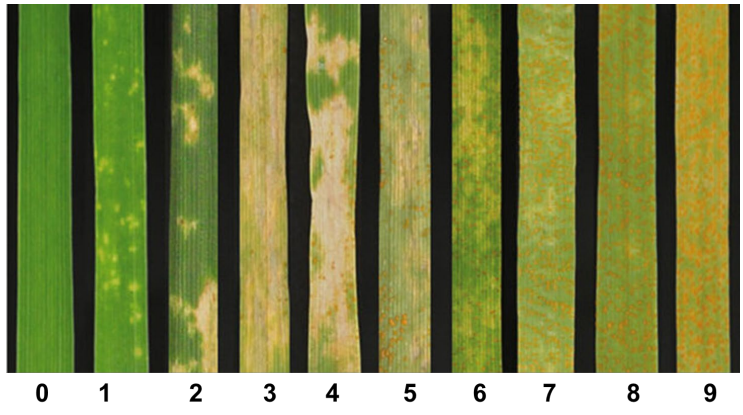


Figure 6-*Puccinia striiformis f. sp. tritici* scoring - Photo: McNeal 1971

5.6. Cytogenetic study and Fluorescent *in situ* hybridization (FISH)

To identify and visualize specific genetic sequences of rye in wheat chromosomes the Fluorescent *in situ* hybridization (FISH) approach was performed at Kansas State University-USA and Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)-Germany. In this experiment, parental lines and selected progenies were investigated using probes specific to rye and wheat repetitive DNA sequences to visualize wheat-rye introgressions (Papers II and III). Rye chromosomes were labelled with a mix of probes and dispersed repeatedly in a method described by (González-García *et al.* 2011). Wheat chromosomes were painted using the oligonucleotide probes Cy5-(GAA)9 and TEX615-pAs1-2 (Danilova *et al.* 2012). Additional visualization was performed using Structured Illumination Microscopy (SIM) with an Elyra PS.1 system, a 63×/1.4 Oil Plan-Apochromat objective, and ZENBlack software (Carl Zeiss GmbH), following the method described by Weisshart *et al.* (2016).

5.7. Monitoring stem rust variability and virulence

To monitor *Pgt* variability, samples of infected wheat from organic farms in the south of Sweden, particularly from Lomma, Svalöv, Eslöv, Kävlinge, and Sjöbo were collected, and the race of the pathogen was identified using 20 differential sets and molecular genotyping using seventeen SSR marker according to Patpour et al. (2022). The experiment was conducted at the Global Rust Reference Center (GRRC), Aarhus University, Denmark. The plants were scored according to a Stakman scale of 0 to 4 with modification (Stakman et al. 1962) (Figure 5).

6. Results and discussion

6.1. Introgression of rye gene into adopted wheat cultivars

Wheat-rye introgression lines have proven valuable genetic resource for enhancing disease resistance in wheat. This study successfully introgression the *Sr59* resistance gene from line TA5094 into elite wheat cultivars (BAJ#1, KACHU#1, and REEDLING#1) using marker-assisted backcrossing, GBS, and phenotypic selection. The newly developed lines not only exhibited broad-spectrum resistance against multiple stem rust races and were confirmed through KASP markers (Paper I) but also maintained high acceptable agronomic performances and protein concentration (unpublished data). Further introgression involved pyramiding *Sr59* with *YrSLU*, conferring dual resistance to both stem and stripe rust in commercial wheat varieties Linkert, Navruz, and the elite breeding line SLU-Elite, thereby improving agronomic performance and disease resilience (Paper IV). These findings underscore the value of wheat-rye introgression lines as a source for resistance breeding, as evidenced by previously identified genes such as *Sr31* (1RS), *Sr27* (3RS), and *Yr9* (1RS) (Rabinovich 1998; Marais 2001; Rahmatov *et al.* 2016b). The successful integration and pyramiding of resistance genes (*Sr5*9 and *YrSLU*) into elite wheat backgrounds illustrates the effectiveness of wheat-rye introgression lines as a genetic resource for crop improvement. This approach allows breeders to combine multiple resistances into a single variety, thus reducing the risk of pathogen adaptation and extending the durability of resistance. Furthermore, the use of molecular markers (e.g., KASP) ensures a precise tracking of target genes, accelerating breeding cycles, and minimizing linkage drag. This directly contributes to food security, notably in regions that are particularly vulnerable to climate change and emerging pathogens.

6.2. Stem rust seedling analysis

Stem rust seedling analyses were widely used in the present thesis work. Thus, in Paper I, seventeen *Pgt* races were used to assess the seedling response to stem rust. Results showed that SLU238 and TA5094 were resistant, exhibiting ITs ranging from ;1 to 1+2- against all tested races. The recurrent parents (BAJ#1, KACHU#1, REEDLING#1, Navruz and SLU-Elite) were all susceptible to the races TTTTF, TTKTT, TTRTF, TTKSK, TTTSK, and TRTTF with ITs of 3+4.

In Paper II, twelve *Pgt* races (TTTTF, TPMKC, RKQQC, RCRSC, TTRTF, TKTTF, TTKTT, TTKSK, TTKST, TTTSK, TRTTF, and JRCQC) were used to evaluate the resistance of the #284 family in the F_7 generation. The ITs of this family were ;1- to 1+2- for all the tested races (Paper II-Figure 7), thereby indicating the presence of resistance gene in #284 family F_7 generation.

In Paper III, multiple *Pgt* race tests (TTKTT, TTKSK, TKKTF, TKTTF, TTRTF, and TKGLK) showed that line #284, which carries *Sr59*, have an ITs of 11+ and 2- against races TTKSK, TKKTF and TKTTF, and TTRTF. Line #C295 showed ITs of 1+2- and 1+2 against races TTKSK and TTRTF but higher ITs (3 to 33+) to races TKKTF and TKTTF (Paper III). These results indicated the possibility of new resistance genes that are different from *Sr59* (Table 3).

Paper IV evaluated the Top-cross 6 and 7 ($TT_{6\text{-}7}$) family was using three *Pgt* races such as TTKSK, TTRTF, and TTTTF. The results revealed that SLU238 and TA5094 showed broad-spectrum resistance to all races examined in this experiment, with ITs ;1 and 11+, respectively. $TT_{6\text{-}7}$ families also displayed consistent resistance to all three *Pgt* races with ITs ranging from 11+ to 1+2-, which was related to the existence of the *Sr59* gene (Paper IV). The commercial variety Linkert showed IT ;1 against race TTTTF, likely due to the *Sr7a* resistance gene (Edae *et al.* 2024). Seedling resistance tests are an important step in the early identification of plants that exhibit resistance to diseases such as stem rust under controlled conditions. This approach, combined with MAS, facilitated the identification of resistance genes like *Sr59* and *YrSLU* (Rahmatov *et al.* 2016a; Ashraf *et al.* 2023).

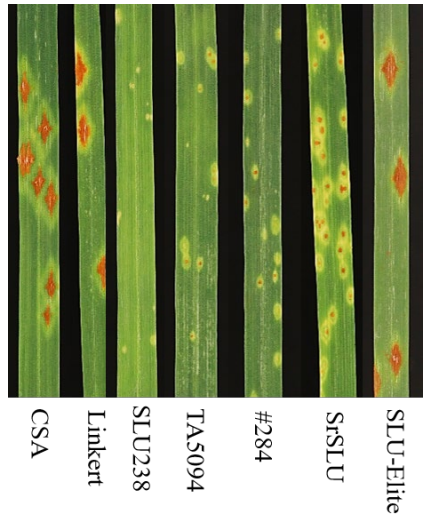


Figure 7-Stem rust reaction of lines CSA, Linkert, SLU238, TA5094, #284, *SrSLU*, and SLU-Elite to TTKSK.

6.3. Stripe rust seedling analysis

The stripe rust seedling test was conducted on $TT_{6\text{-}7}$ families using four *Pst* races (*Psts10*, *Psts16*, *Psts7*, and *Psts13*). The wheat-rye introgression parental line SLU126, which carries chromosomes 4R (4D), 5R (5D), and 6R (7D), along with its derivative #392, which carries a small translocation from chromosome 6RL, exhibited resistance to all four *Pst* races, showing an IT of 1/2. All commercial wheat varieties tested were highly susceptible compared to Linkert, Navruz, and SLU-Elite, with an IT of 7, indicating effective resistance was lacking. However, the $TT_{6\text{-}7}$ families demonstrated a consistent resistance response to all four *Pst* races, exhibiting ITs of 1/2, which confirms their resistance potential. These findings indicate that the *YrSLU* resistance gene was successfully transferred into new genotypes, and its resistance remains stable and effective across different *Pst* races. This suggests that $TT_{6\text{-}7}$ families can be promising genetic resources for stripe rust resistance breeding (Figure 8, Paper IV).

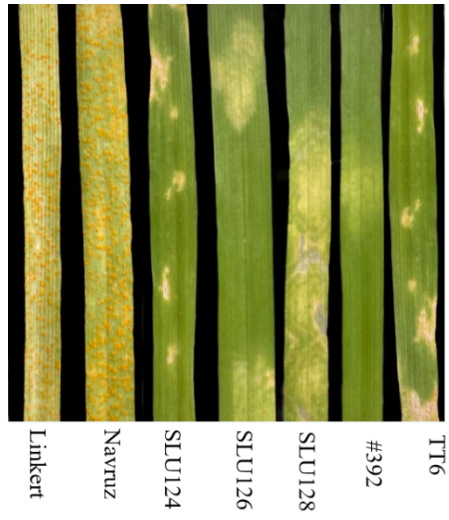


Figure 8-Stripe rust reaction of lines Linkert, Navruz, SLU124, SLU126, SLU128, #392 and TT_6 to *PstS10*.

6.4. FISH analysis

FISH analysis was used to identify *S. cereale* chromosomes in the wheat genomes of parental lines SLU238, TA5094, and #284, and their resistant progenies. The results confirmed the presence of rye chromosomal segments in distinct wheat genomic regions. In SLU238, FISH analysis detected a 2R/2D rye-wheat substitution, indicating successful rye chromatin integration. In TA5094, a 2DS.2RL Robertsonian translocation was identified, confirming rye chromatin introgression into the wheat genome. Similarly, in #284, a 2BS.2BL-2RL translocation was observed, with the rye segment located in the distal part of chromosome 2B. These findings confirm that rye chromosomal segments have been successfully transferred into the wheat genome, contributing to enhanced disease resistance (Paper II) (Figure 9).

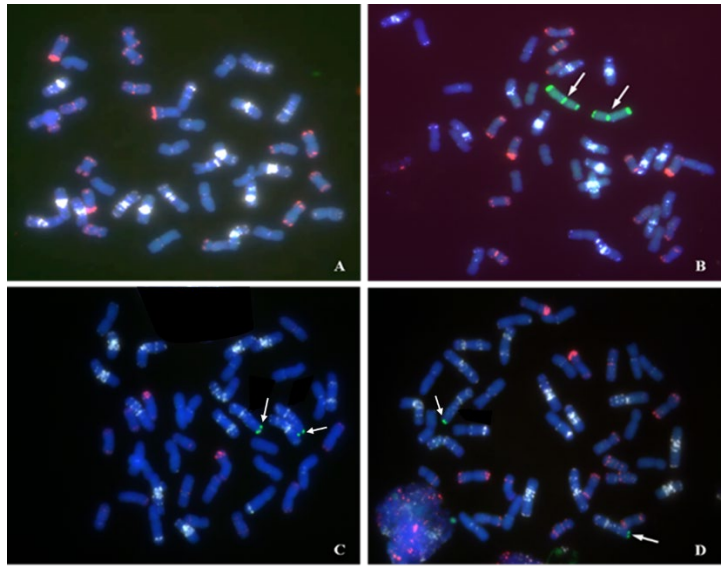
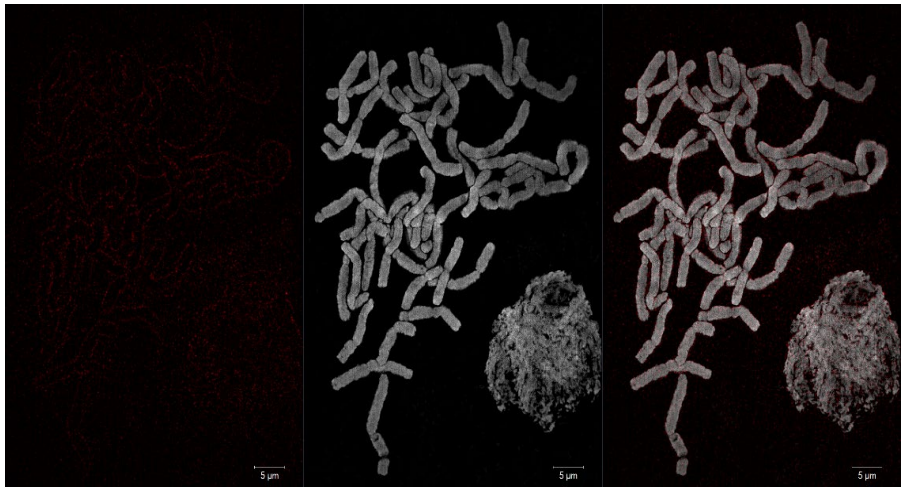


Figure 9-FISH results- A) CS ph1b; B) SLU238 2R (2D); C) #284 2BS.2BL-2RL; D) Cross from #284 in elite background with 2BS.2BL-2RL

However, it was not possible to detect the rye chromosome in *SrSLU* due to the small size of the rye chromosome translocation (Paper III) (Figure 10). Given this limitation, molecular marker validation was required to confirm the presence of these small translocations. Previous studies have shown that cytogenetic analyses alone were insufficient for detecting small translocations, highlighting the necessity of using molecular markers validation (Fu *et al.* 2013; Ashraf *et al.* 2023). The results demonstrate the importance of combining cytogenetic and molecular approaches to accurately identify small rye-wheat translocations, which are important for breeding disease resistance.



A B C

Figure 10- Structured Illumination Microscopy (SIM) from *SrSLU*, A) No signal observed from the rye detecting probes, B)Wheat chromosome painted using the oligonucleotide probes Cy5-(GAA)9 and TEX615-pAs1-2, C) Merging of A and B photo.

6.5. GBS analysis and NLR

A total of 15,116 SNPs were physically mapped to chromosome 2R, spanning positions 347,694 bp to 945,773,747 bp. Further annotation of NLR genes in rye revealed the presence of four NLR genes within chromosome 2R in SLU238. Two of these were located on the long arm of chromosome 2R, suggesting their potential role in disease resistance. One of these NLR genes was identified at position 945,483,852 bp, overlapping with the *Sr59* resistance gene and its associated KASP markers previously described by Rahmatov et al. (2016a). The second NLR gene, positioned at 843,226,528 bp, was validated through seedling resistance testing and MAS, indicating the potential identification of a new resistance gene, *SrSLU* (Paper III).

NLR genes encode a critical family of immune receptors in plants that play a fundamental role in pathogen recognition and disease resistance. Several well-characterized NLR genes in wheat have been linked to

resistance against fungal pathogens. For example, *Sr50*, which confers resistance to the stem rust race Ug99, was introgressed into wheat as a 1RS translocation from the rye cultivar Imperial (Mago *et al.* 2015; Cesari *et al.* 2016). Similarly, *YrSLU*, a gene providing resistance to stripe rust and derived from chromosome 6RL, was identified using GBS and the NLR approach (Ashraf *et al.* 2023).

GBS technology has been extensively used for gene mapping and cloning in wild wheat relatives, such as *Triticum turgidum* (2n = 4x = 28, AABB) and *Aegilops tauschii* (2n = 2x = 14, DD). This high-throughput approach, utilizing SNP markers, has facilitated the precise introgression and tracking of alien chromosomal segments in modern plant breeding programs (Tiwari *et al.* 2014). These findings underscore the potential of GBS and NLR-based approaches in identifying new resistance genes and enhancing wheat's defense against rust diseases.

6.6. Development of KASP marker and Molecular marker validation

KASP markers were designed by utilizing a 120-base pair flanking sequence (60 upstream and 60 downstream) surrounding the NLR regions. Among 14 (2RL) KASP primers, two successfully amplified *SrSLU* regions. Subsequently, resistant plants in the later generation were selected by these KASP markers along with other methods. In the CS *ph1b* mutant and susceptible plants, these KASP markers were absent (Paper III). The presence of *Sr59* (Papers I and IV) and *YrSLU* were validated using the KASP marker described by Rahmatov *et al.,* (2016a) and Ashraf *et al.,* (2023). The 1RS and 2RL specific KASP marker has previously been used to detect 1RS translocations in a population of 161 wheat cultivars/lines (Han *et al.* 2020).

6.7. Development and characterization of a new resistance gene

The derived lines from the initial cross between TA5094 and CSA were screened against the TTKSK (Ug99) race (isolate KE126a/23). Plants that exhibited resistant IT ranging from ;1 to 1+2- were further analyzed using SSR and KASP markers. The plants that tested positive for *Sr59* using KASP markers were discarded in order to ensure the identification of a novel resistance gene. Among the tested lines, plant #C295, derived from the TA5094 × CSA cross, showed a negative reaction to all *Sr59*-specific markers but tested positive for both F3/R3 and two KASP markers KASP_2RL_chr2R_nlr_79_16 and KASP_2RL_chr2R_nlr_79_19 (Figure 11). GBS data further mapped this new resistance gene to position 843,226,528 bp on chromosome 2R, with an estimated size of 102,257,324 bp. Further evaluation against multiple *Pgt* races confirmed that plant #C295 exhibited ITs of 1+2- and 1+2 against TTKSK and TTRTF, respectively, while showing ITs of 33+ and 3 against TKKTF and TKTTF. Although line #284, which carries *Sr59*, also displayed resistance, its reaction was slightly higher, with an IT of 1+ against these races (Table 3). All tested plants, except for LK, exhibited susceptibility to TKGLK, a newly emerging *Pgt* race known for overcoming multiple rye-derived resistance genes, including *Sr31* and *Sr59* (Patpour et al., 2022). This suggests that the newly identified gene originates from rye and is distinct from *Sr59*, representing a promising new source of stem rust resistance.

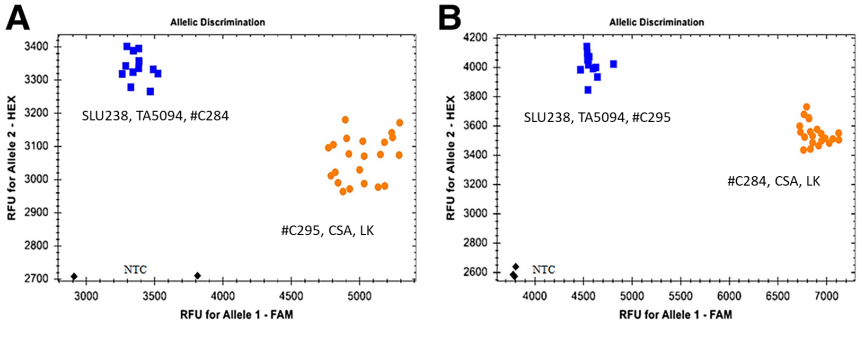


Figure 11-Allele discrimination plots of the kompetitive allele-specific PCR markers used for A, KASP_2RL_c2019 and B, KASP_2RL_chr2R_nlr_79_16.

Table 3-Mulitple seedling resistance stem rust response in parental lines and BC_1F_3 #C295

	SrSLU* Multi test to *Pgt		**01MN84A**	**KE126a/23**	**UG244a/19**	**ES64/23**	**SE271/21**	**IT16a/18**	**ES177e/19**
	Line	Gens	TTTTF	TTKTT	TTKSK	TKKTF	TKTTF	TTRTF	TKGLK
A	TA9054	*Sr59*+*SrSLU*	11+	11+	1-	1	1-	1+2-	3+
B	SLU238	*2R (2D)*	11+	11+	1-	11+	1+2	11+	3+
C	BC_1F_3 #C295	*SrSLU*	1+2-	1+2-	1+2-	33+	3	1+2	3
D	#284	*Sr59*	2-	2-	2-	1+	1+	11+	2+3-
E	LK	*Sr7a*	1+2-	3+	3	3	1+2-	1+2+	;1-
F	CSA	None	33+	33+	33+	33+	33+	33+	33+

6.8. Monitoring stem rust in the south of Sweden

Pathotyping and genotyping analysis of the *Pgt* collection in the south of Sweden revealed that three wheat samples were classified as TTRTF, belonging to Clade III-B. This was the first report of race TTRTF in Northern Europe. TTRTF race poses a significant threat to wheat productivity, and resistance testing of commercial European wheat varieties revealed that 70% of cultivars were susceptible to this race (Patpour et al. 2022). This result indicated that, if the conditions are favourable for the establishment and development of stem rust, the disease has the potential to severely damage the wheat harvest in these countries. Overall, the susceptibility of European wheat cultivars highlights the urgent need for new breeding efforts to develop effective sources of resistance to wheat stem rust within breeding programs.

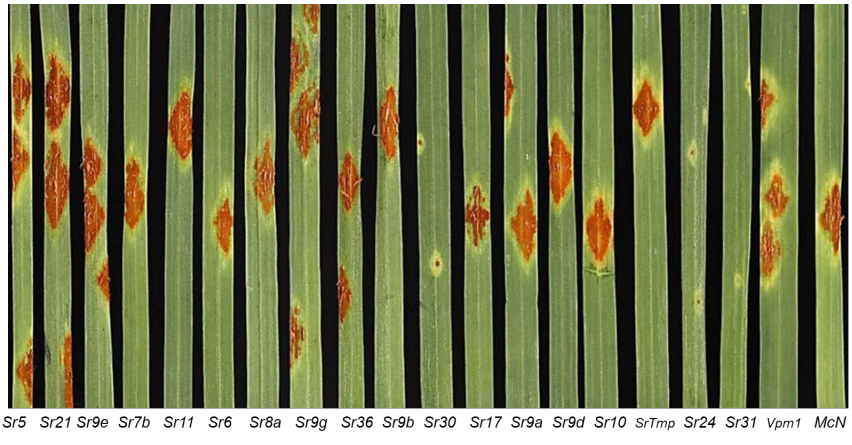


Figure 12-Infection types (IT) conferred by race TTRTF on standard Differential sets - Photo: Mehran Patpour

7. Conclusion

The emergence of new races of stem and stripe rust that overcome previously resistant genes increases the need to uncover new sources of resistance. This study precisely introgression a broad-spectrum resistance gene, *Sr59*, into adapted wheat cultivars, discovered the new stem rust resistance gene in wheat-rye introgression lines, and pyramided two resistance genes *Sr59* (stem rust resistance) and *YrSLU* (stripe rust resistance) into one cultivar. These findings highlight the significant potential of rye as a genetic resource for improving wheat traits and enhancing its resilience to evolving pathogens. This approach enables the development of varieties with more durable and effective resistance against multiple pathogens. Furthermore, the introgression of these genes was accelerated using advanced techniques such as speed breeding and MAS, significantly reducing the time required compared to traditional methods.

We also precisely mapped *Sr59* to a small translocation, 2BS.2BL-2RL, located at the distal part of chromosome 2RL. This small translocation facilitates the efficient integration of *Sr59* into breeding programs, making it an ideal option for breeders to enhance disease resistance without introducing significant linkage drag.

Moreover, this thesis demonstrates the effectiveness of GBS data and KASP markers to accurately trace and introgression of resistance genes into adapted wheat cultivars. Using GBS data and NLR-based methods, we identified a second resistance gene on 2RL, *SrSLU*, and characterized it using seedling resistance tests and marker-assisted selection.

Key achievements of this research obtained from Papers I to IV include:

- The successful introgression of *Sr59* into adapted wheat cultivars and tracking the presence of this gene into progenies using a specific KASP marker.
- The identification of two NLR genes on chromosome 2RL, providing valuable insights into the molecular basis of resistance.
- The development of line #284, carrying the cryptic 2BS.2BL-2RL translocation, which simplifies the introgression of *Sr59* into breeding programs.

- Comprehensive characterization of the introgression of wheat-rye chromatin using cytogenetic and molecular studies.
- The identification of line #C295, which harbors *SrSLU* and exhibits broad-spectrum resistance to multiple stem rust races, confirmed through phenotypic and genotypic analyses.
- The discovery and validation of new KASP markers linked to the *SrSLU* locus, enabling precise tracking of this resistance gene.
- The development of pyramided lines combining *Sr59* and *YrSLU*, top-crossed with high-yielding commercial cultivars to ensure superior agronomic performance.

This study emphasizes the importance of rye as a source of novel resistance genes and demonstrates the effectiveness of modern breeding technologies in accelerating the development of resilient wheat varieties. Using technologies such as GBS, KASP markers, and speed breeding, we have demonstrated the possibility of developing wheat varieties with enhanced and durable resistance to stem and stripe rust, contributing to global food security in the face of emerging pathogen threats.

8. Further perspectives

This research establishes a strong platform for advancing wheat breeding and disease resistance. By building on these findings, we can continue to develop innovative solutions to protect wheat from ever-evolving threats. Below are some of the potential future directions:

- **Explore other wheat-rye introgression lines for stem rust resistance genes.**

While *Sr59* and *YrSLU* represent significant advancements, the continuous evolution of rust pathogens demands ongoing efforts to identify new resistance genes. Wheat-rye introgression at SLU has been shown to have significant benefits, and exploring them could lead to the identification of new sources of resistance to stem rust.

- **Introgression of *SrSLU* into adapted wheat cultivars**

SrSLU should be introgression into adapted wheat cultivars and their agronomic performance should be assessed comprehensively.

- **Development of Multi-gene Pyramids**

The successful pyramiding of *Sr59* and *YrSLU* demonstrates the effectiveness of combining multiple resistance genes within a single plant. There is potential to incorporate even more genes targeting different pathogens or providing broader-spectrum resistance. For example, integrating genes against leaf rust, and fusarium head blight to *SrSLU* and *YrSLU* could enhance overall crop resilience. This approach aligns with the growing emphasis on durable, multi-pathogen resistance in modern breeding programs.

- **Integration of CRISPR/Cas9 Technology**

Advances in gene-editing technologies such as CRISPR/Cas9 offer exciting opportunities to directly introduce or enhance resistance traits

in wheat without relying solely on traditional breeding methods. Future studies could explore the use of CRISPR/Cas9 to precisely edit wheat genomes and potentially accelerate the development of resistant varieties.

- **Understanding Gene Function and Mechanisms**

Although the genes identified and characterized in this research are known to be NLR, further research into its molecular function and mode of action could provide valuable insights. Investigating how *Sr59* and *SrSLU* interact with the stem rust and stripe rust pathogen at the cellular level could reveal novel mechanisms of resistance and could uncover their specific roles in disease defense.

References

Agrios, G.N. (2005). *Plant pathology*. Elsevier

Ali, S., Rodriguez-Algaba, J., Thach, T., Sorensen, C.K., Hansen, J.G., Lassen, P., Nazari, K., Hodson, D.P., Justesen, A.F. & Hovmoller, M.S. (2017). Yellow Rust Epidemics Worldwide Were Caused by Pathogen Races from Divergent Genetic Lineages. *Frontiers in Plant Science,* 8. https://doi.org/10.3389/fpls.2017.01057

Anderson, E. (1949). *Introgressive Hybridization*. NY: Wiley & Sons.

Anikster, Y., Eilam, T., Mittelman, L., Szabo, L.J. & Bushnell, W.R. (1999). Pycnial nectar of rust fungi induces cap formation on pycniospores of opposite mating type. *Mycologia,* 91(5), 858-870. https://doi.org/10.2307/3761539

Ashraf, R., Johansson, E., Vallenback, P., Steffenson, B.J., Bajgain, P. & Rahmatov, M. (2023). Identification of a Small Translocation from 6R Possessing Stripe Rust Resistance to Wheat. *Plant Disease,* 107(3), 720-729. https://doi.org/10.1094/Pdis-07-22-1666-Re

Bauer, E., Schmutzer, T., Barilar, I., Mascher, M., Gundlach, H., Martis, M.M., Twardziok, S.O., Hackauf, B., Gordillo, A., Wilde, P., Schmidt, M., Korzun, V., Mayer, K.F.X., Schmid, K., Schön, C.C. & Scholz, U. (2017). Towards a whole-genome sequence for rye (*Secale cereale* L.). *Plant Journal,* 89(5), 853-869. https://doi.org/10.1111/tpj.13436

Beddow, J.M., Pardey, P.G., Chai, Y., Hurley, T.M., Kriticos, D.J., Braun, H.J., Park, R.F., Cuddy, W.S. & Yonow, T. (2015). Research investment implications of shifts in the global geography of wheat stripe rust. *Nature Plants,* 1(10). https://doi.org/10.1038/Nplants.2015.132

Bhattacharya, S. (2017). Deadly new wheat disease threatens Europe's crops. *Nature,* 542(7640), 145-160. https://doi.org/10.1038/nature.2017.21424

Borlaug, N.E. (2008). Stem Rust Never Sleeps. *The New York Times*.

Burdon, J.J., Barrett, L.G., Rebetzke, G. & Thrall, P.H. (2014). Guiding deployment of resistance in cereals using evolutionary principles. *Evolutionary Applications,* 7(6), 609-624. https://doi.org/10.1111/eva.12175

Carefoot, G. & Sprott, E. (1967). *Famine on the wind: man's battle against plant disease.* . Chicago, Ill: Rand McNally.

Cesari, S., Moore, J., Chen, C.H., Webb, D., Periyannan, S., Mago, R., Bernoux, M., Lagudah, E.S. & Dodds, P.N. (2016). Cytosolic activation of cell death and stem rust resistance by cereal MLA-family CC-NLR proteins. *Proceedings of the National Academy of Sciences of the United States of America,* 113(36), 10204-10209. https://doi.org/10.1073/pnas.1605483113

Chaves, M.S., Martinelli, J.A., Wesp, C.d.L. & Graichen, F.d. (2008). The cereal rusts: an overview. *Pest Technology,* 2(2), 38-55.

Chen, X.M. (2005). Epidemiology and control of stripe rust [*Puccinia striiformis* f. sp. *tritici*] on wheat. *Canadian Journal of Plant Pathology,* 27(3), 314-337. https://doi.org/10.1080/07060660509507230

Collins, P.J., Wen, Z. & Zhang, S. (2018). Marker-assisted breeding for disease resistance in crop plants. *Biotechnologies of Crop Improvement, Volume 3: Genomic Approaches*, 41-57.

Craigie, J.H. (1927). Discovery of the function of the pycnia of the rust fungi. *Nature,* 120, 765–767. https://doi.org/10.1038/120765a0

Crespo-Herrera, L.A., Garkava-Gustavsson, L. & Åhman, I. (2017). A systematic review of rye (*Secale cereale* L.) as a source of resistance to pathogens and pests in wheat (*Triticum aestivum* L.). *Hereditas,* 154, 1-9. https://doi.org/10.1186/s41065-017-0033-5

D'Odorico, P., Carr, J.A., Laio, F., Ridolfi, L. & Vandoni, S. (2014). Feeding humanity through global food trade. *Earths Future,* 2(9), 458-469. https://doi.org/10.1002/2014ef000250

Danilova, T.V., Friebe, B. & Gill, B.S. (2012). Single-copy gene fluorescence in situ hybridization and genome analysis: Acc-2 loci mark evolutionary chromosomal rearrangements in wheat. *Chromosoma,* 121(6), 597-611. https://doi.org/10.1007/s00412-012-0384-7

Dhaliwal, A.S., Mares, D.J. & Marshall, D.R. (1987). Effect of 1b/1r Chromosome-Translocation on Milling and Quality Characteristics of Bread Wheats. *Cereal Chemistry,* 64(2), 72-76. https://doi.org/10.5555/19871663173

Edae, E.A., Kosgey, Z., Bajgain, P., Ndung'u, K.C., Gemechu, A., Bhavani, S., Anderson, J.A. & Rouse, M.N. (2024). The genetics of Ug99 stem rust resistance in spring wheat variety 'Linkert'. *Frontiers in Plant Science,* 15. https://doi.org/10.3389/fpls.2024.1343148

Eriksson, J. & Henning, F. (1896). Die Getreideroste und Maaszegelen gegen dieselben. *Stock-holm (Ref. N.J. Vavilov)*.

FAO (2009). Global agriculture towards 2050. (Available at https://www.fao.org).

FAO (2024). FAOSTAT. Online statistical database: Production (Available at http://faostat3.fao.org/download/Q/QC/E).

Fedoroff, N. (2015). Food in a future of 10 billion. *Agriculture & Food Security,* 4, 1-10.

Feldman, M. & Levy, A.A. (2015). Origin and Evolution of Wheat and Related Triticeae Species. In: Molnár-Láng, M., Ceoloni, C. & Doležel, J. (eds) *Alien Introgression in Wheat: Cytogenetics, Molecular Biology, and Genomics*. Springer International https://doi.org/10.1007/978-3-319-23494-6

Feldman, M. & Millet, E. (2001). The contribution of the discovery of wild emmer to an understanding of wheat evolution and domestication and to wheat improvement. *Israel Journal of Plant Sciences,* 49, S25-S35. https://doi.org/10.1092/Jcmx-Wgxm-D40g-Bg4p

Friebe, B., Jiang, J., Raupp, W.J., McIntosh, R.A. & Gill, B.S. (1996). Characterization of wheat-alien translocations conferring resistance to diseases and pests: Current status. *Euphytica*, 91(1), 59-87. https://doi.org/10.1007/Bf00035277

Fu, S., Tang, Z., Ren, Z. & Zhang, H. (2010). Transfer to wheat (*Triticum aestivum*) of small chromosome segments from rye (*Secale cereale*) carrying disease resistance. *Journal of Applied Genetics*, 51(2), 115-121. https://doi.org/10.1007/Bf03195719

Fu, S.L., Sun, C.F., Yang, M.Y., Fei, Y.Y., Tan, F.Q., Yan, B.J., Ren, Z.L. & Tang, Z.X. (2013). Genetic and Epigenetic Variations Induced by Wheat-Rye 2R and 5R Monosomic Addition Lines. *Plos One,* 8(1). https://doi.org/10.1371/journal.pone.0054057

González-García, M., Cuacos, M., González-Sánchez, M., Puertas, M.J. & Vega, J.M. (2011). Painting the rye genome with genome-specific sequences. *Genome,* 54(7), 555-564. https://doi.org/10.1139/G11-003

Hafeez, A.N., Arora, S., Ghosh, S., Gilbert, D., Bowden, R.L. & Wulff, B.B.H. (2021). Creation and judicious application of a wheat resistance gene atlas. *Molecular Plant,* 14(7), 1053-1070. https://doi.org/10.1016/j.molp.2021.05.014

Han, G.H., Liu, S.Y., Jin, Y.L., Jia, M.S., Ma, P.T., Liu, H., Wang, J. & An, D.G. (2020). Scale development and utilization of universal PCR based and high-throughput KASP markers specific for chromosome arms of rye (*Secale cereale* L.). *Bmc Genomics,* 21(1). https://doi.org/10.1186/s12864-020-6624-y

Hao, M., Zhang, L.Q., Ning, S.Z., Huang, L., Yuan, Z.W., Wu, B.H., Yan, Z.H., Dai, S.F., Jiang, B., Zheng, Y.L. & Liu, D.C. (2020). The Resurgence of Introgression Breeding, as Exemplified in Wheat

Improvement. *Frontiers in Plant Science,* 11. https://doi.org/10.3389/fpls.2020.00252
Harlan, J. & de Wet, J. (1971). Toward a rational classification of cultivated plants. *Taxon,* 20, 509-517.
Hovmøller, M., Patpour, M., Rodriguez-Algaba, J., Thach, T., Justesen, A. & Hansen. JG. (2021). *GRRC report of yellow and stem rust genotyping and race analyses 2020*. (www.wheatrust.org).
Hovmøller, M., Patpour, M., Rodriguez-Algaba, J., Thach, T., Justesen, A. & Hansen. JG. (2023). *GRRC report of yellow and stem rust genotyping and race analyses 2022*. (www.wheatrust.org).
Hovmøller, M., Patpour, M., Rodriguez-Algaba, J., Thach, T., Justesen, A. & JG., H. (2018). *GRRC report of yellow and stem rust genotyping and race analyses 2017*. (www.wheatrust.org).
Hovmoller, M.S., Sorensen, C.K., Walter, S. & Justesen, A.F. (2011). Diversity of *Puccinia striiformis* on cereals and grasses. *Annual Review of Phytopathology, Vol 49,* 49, 197-217. https://doi.org/10.1146/annurev-phyto-072910-095230
Hovmoller, M.S., Walter, S., Bayles, R.A., Hubbard, A., Flath, K., Sommerfeldt, N., Leconte, M., Czembor, P., Rodriguez-Algaba, J., Thach, T., Hansen, J.G., Lassen, P., Justesen, A.F., Ali, S. & de Vallavieille-Pope, C. (2016). Replacement of the European wheat yellow rust population by new races from the centre of diversity in the near-Himalayan region. *Plant Pathology,* 65(3), 402-411. https://doi.org/10.1111/ppa.12433
IWGSC (2018). Shifting the limits in wheat research and breeding using a fully annotated reference genome. *Science,* 361(6403), 661-690. https://doi.org/10.1126/science.aar7191
Jiang, J., Friebe, B. & Gill, B. (1993). Recent advances in alien gene transfer in wheat. *Euphytica,* 73, 199-212.
Katto, C.M., Endo, T.R. & Nasuda, S. (2004). A PCR-based marker for targeting small rye segments in wheat background. *Genes Genet Syst,* 79(4), 245-50. https://doi.org/10.1266/ggs.79.245
Khlestkina, E.K., Ma, H.M.T., Pestsova, E.G., Röder, M.S., Malyshev, S.V., Korzun, V. & Börner, A. (2004). Mapping of 99 new microsatellite-derived loci in rye (*Secale cereale* L.) including 39 expressed sequence tags. *Theoretical and Applied Genetics,* 109(4), 725-732. https://doi.org/10.1007/s00122-004-1659-z
King, J., Grewal, S., Fellers, J.P. & King, I.P. (2022). Exploring untapped wheat genetic resources to boost food security. In: *Wheat improvement: food security in a changing climate*. Springer International Publishing Cham. 319-340.

Kirk, P.M., Cannon, P.F., Minter, D.W. & Stalpers, J.A. (2008). *Ainsworth & Bisby's Dictionary of the Fungi*.

Kjellström, C. (2021). *Population structure of Puccinia graminis, the cause of stem rust on wheat, barley, and rye in Sweden.* Dept. of Forest Mycology and Plant Pathology. The Swedish University of Agricultural Sciences.

Kole, C. (2011). *Wild crop relatives: genomic and breeding resources*.

Laugerotte, J., Baumann, U. & Sourdille, P. (2022). Genetic control of compatibility in crosses between wheat and its wild or cultivated relatives. *Plant Biotechnology Journal,* 20(5), 812-832. https://doi.org/10.1111/pbi.13784

Leonard, K.J. & Szabo, L.J. (2005). Stem rust of small grains and grasses caused by *Puccinia graminis*. *Molecular Plant Pathology,* 6(4), 489-489. https://doi.org/10.1111/J.1364-3703.2005.00299.X

Li, H. & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics,* 25(14), 1754-1760. https://doi.org/10.1093/bioinformatics/btp324

Li, J.B., Dundas, I., Dong, C.M., Li, G.R., Trethowan, R., Yang, Z.J., Hoxha, S. & Zhang, P. (2020). Identification and characterization of a new stripe rust resistance gene *Yr83* on rye chromosome 6R in wheat. *Theoretical and Applied Genetics*, 133(4), 1095-1107. https://doi.org/10.1007/s00122-020-03534-y

Li, Z., Ren, Z.L., Tan, F.Q., Tang, Z.X., Fu, S.L., Yan, B.J. & Ren, T.H. (2016). Molecular cytogenetic characterization of New Wheat-Rye 1R(1B) Substitution and Translocation Lines from a Chinese *Secale cereal* L. Plos One, 11(9). https://doi.org/10.1371/journal.pone.0163642

Mago, R., Spielmeyer, W., Lawrence, G.J., Ellis, J.G. & Pryor, A.J. (2004). Resistance genes for rye stem rust and barley powdery mildew are located in syntenic regions on short arm of chromosome. *Genome*, 47(1), 112-121. https://doi.org/10.1139/G03-096

Mago, R., Zhang, P., Vautrin, S., Simkova, H., Bansal, U., Luo, M.C., Rouse, M., Karaoglu, H., Periyannan, S., Kolmer, J., Jin, Y., Ayliffe, M.A., Bariana, H., Park, R.F., McIntosh, R., Dolezel, J., Berges, H., Spielmeyer, W., Lagudah, E.S., Ellis, J.G. & Dodds, P.N. (2015). The wheat *Sr50* gene reveals rich diversity at a cereal disease resistance locus. *Nature Plants,* 1(12). https://doi.org/10.1038/Nplants.2015.186

Marais, G. (2001). An evaluation of three *Sr27*-carrying wheat x rye translocations. *South African Journal of Plant and Soil,* 18(3), 135-136. https://doi.org/10.10520/AJA02571862_946

Martin, D.J. & Stewart, B.G. (1990). Dough Stickiness in Rye-Derived Wheat Cultivars. *Euphytica,* 51(1), 77-86. https://doi.org/10.1007/Bf00022895

McIntosh, R.A. & Pretorius, Z.A. (2011). Borlaug Global Rust Initiative provides momentum for wheat rust research. *Euphytica,* 179(1), 1-2. https://doi.org/10.1007/s10681-011-0389-y

McIntosh, R.A., Wellings, C.R. & Park, R.F. (1995). *Wheat rusts: an atlas of resistance genes*. CSIRO publishing.

McNeal, F. (1971). *A Uniform System for Recordin and Processing Cereal Research Data*. USDA-ARS.

Merker, A. (1984). The Rye Genome in Wheat Breeding. *Hereditas,* 100(2), 183-191. https://doi.org/10.1111/j.1601-5223.1984.tb00118.x

Molnár-Láng, M., Ceoloni, C. & Doležel, J. (2015). Alien introgression in wheat. *Cham: Springer*.

Newbery, F., Qi, A.M. & Fitt, B.D.L. (2016). Modelling impacts of climate change on arable crop diseases: progress, challenges and applications. *Current Opinion in Plant Biology,* 32, 101-109. https://doi.org/10.1016/j.pbi.2016.07.002

Pardey, P.G., Beddow, J.M., Kriticos, D.J., Hurley, T.M., Park, R.F., Duveiller, E., Sutherst, R.W., Burdon, J.J. & Hodson, D. (2013). Agriculture. Right-sizing stem-rust research. *Science,* 340(6129), 147-8. https://doi.org/10.1126/science.122970

Patpour, M., Baidya, S., Basnet, R., Justesen, A.F., Hodson, D., Thapa, D. & Hovmoller, M.S. (2024). First report of Ug99 Wheat Stem Rust caused by Puccinia graminis f. sp. tritici in South Asia. *Plant Dis*. https://doi.org/10.1094/PDIS-03-24-0644-PDN

Patpour, M., Hovmoller, M.S., Rodriguez-Algaba, J., Randazzo, B., Villegas, D., Shamanin, V.P., Berlin, A., Flath, K., Czembor, P., Hanzalova, A., Slikova, S., Skolotneva, E.S., Jin, Y., Szabo, L., Meyer, K.J.G., Valade, R., Thach, T., Hansen, J.G. & Justesen, A.F. (2022). Wheat Stem Rust Back in Europe: Diversity, Prevalence and Impact on Host Resistance. *Frontiers in Plant Science,* 13. https://doi.org/10.3389/fpls.2022.882440

Patpour, M., Justesen, A.F., Tecle, A.W., Yazdani, M., Yasaie, M. & Hovmoller, M.S. (2020). First Report of Race TTRTF of Wheat Stem Rust (*Puccinia graminis* f. sp. *tritici*) in Eritrea. *Plant Disease,* 104(3), 973-974. https://doi.org/10.1094/Pdis-10-19-2133-Pdn

Peterson, P.D. (2001). Stem Rust of Wheat: From Ancient Enemy to Modern Foe. *Amer Phytopathological Society*. https://doi.org/10.5555/20013088372

Poland, J., Endelman, J., Dawson, J., Rutkoski, J., Wu, S.Y., Manes, Y., Dreisigacker, S., Crossa, J., Sánchez-Villeda, H., Sorrells, M. & Jannink, J.L. (2012). Genomic Selection in Wheat Breeding using Genotyping-by-Sequencing. *Plant Genome,* 5(3), 103-113. https://doi.org/10.3835/plantgenome2012.06.0006

Pretorius, Z.A., Singh, R.P., Wagoire, W.W. & Payne, T.S. (2000). Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis* f. sp. *tritici* in Uganda. *Plant Disease,* 84, 203.

Rabanus-Wallace, M.T., Hackauf, B., Mascher, M., Lux, T., Wicker, T., Gundlach, H. & et-al. (2021). Chromosome-scale genome assembly provides insights into rye biology, evolution and agronomic potential. *Nature Genetics,* 53(4), 564-589. https://doi.org/10.1038/s41588-021-00807-0

Rabinovich, S.V. (1998). Importance of wheat-rye translocations for breeding modern cultivars of *Triticum aestivum* L. *Euphytica,* 100(1-3), 323-340. https://doi.org/10.1023/A:1018361819215

Rahmatov, M., Rouse, M.N., Nirmala, J., Danilova, T., Friebe, B., Steffenson, B.J. & Johansson, E. (2016a). A new 2DS·2RL Robertsonian translocation transfers stem rust resistance gene *Sr59* into wheat. *Theoretical and Applied Genetics,* 129(7), 1383-1392. https://doi.org/10.1007/s00122-016-2710-6

Rahmatov, M., Rouse, M.N., Steffenson, B.J., Andersson, S.C., Wanyera, R., Pretorius, Z.A., Houben, A., Kumarse, N., Bhavani, S. & Johansson, E. (2016b). Sources of Stem Rust Resistance in Wheat-Alien Introgression Lines. *Plant Disease,* 100(6), 1101-1109. https://doi.org/10.1094/Pdis-12-15-1448-Re

Ren, T.H., Chen, F., Yan, B.J., Zhang, H.Q. & Ren, Z.L. (2012). Genetic diversity of wheat-rye 1BL.1RS translocation lines derived from different wheat and rye sources. *Euphytica,* 183(2), 133-146. https://doi.org/10.1007/s10681-011-0412-3

Ren, T.H., Sun, Z.X., Ren, Z.L., Tan, F.Q., Luo, P.G., Tang, Z.X., Fu, S.L. & Li, Z. (2020). Molecular and cytogenetic characterization of a wheat-rye 7BS. 7RL translocation line with resistance to stripe rust, powdery mildew, and Fusarium head blight. *Phytopathology*, 110(10), 1713-1720. https://doi.org/10.1094/Phyto-02-20-0061-R

Ren, T.H., Yang, Z.J., Yan, B.J., Zhang, H.Q., Fu, S.L. & Ren, Z.L. (2009). Development and characterization of a new 1BL.1RS translocation line with resistance to stripe rust and powdery mildew of wheat. *Euphytica*, 169(2), 207-213. https://doi.org/10.1007/s10681-009-9924-5

Reynolds, M.P. & Braun, H.J. (2022). Wheat Improvement. In: Reynolds, M.P. & Braun, H.J. (eds) *Wheat Improvement: Food Security in a Changing Climate*. Springer Cham. https://doi.org/https://doi.org/10.1007/978-3-030-90673-3

Riley, R. & Chapman, V. (1958). Genetic Control of the Cytologically Diploid Behaviour of Hexaploid Wheat. *Nature,* 182(4637), 713-715. https://doi.org/10.1038/182713a0

Roelfs, A. (1985a). Epidemiology in North America. In: *Diseases, Distribution, Epidemiology, and Control*. Elsevier. 403-434. https://doi.org/10.1016/B978-0-12-148402-6.50021-3

Roelfs, A.P. (1985b). The cereal rusts: Diseases, distribution, epidemiology, and control. In: Roelfs, A.P. & Bushnel, W.R. (eds) *vol. 2,*. Academic Press. 337.

Roelfs, A.P., Singh, R.P. & Saari, E.E. (1992). *Rust diseases of wheat: concepts and methods of disease management*.

Rouse, M.N., Wanyera, R., Njau, P. & Jin, Y. (2011). Sources of Resistance to Stem Rust Race Ug99 in Spring Wheat Germplasm. *Plant Disease,* 95(6), 762-766. https://doi.org/10.1094/Pdis-12-10-0940

Saulescu, N.N., Ittu, G., Ciuca, M., Ittu, M., Serban, G. & Mustatea, P. (2011). Transferring Useful Rye Genes to Wheat, Using Triticale as a Bridge. *Czech Journal of Genetics and Plant Breeding,* 47, S56-S62. https://doi.org/10.17221/3255-Cjgpb

Schlegel, R. & Meinel, A. (1994). A Quantitative Trait Locus (Qtl) on Chromosome Arm 1rs of Rye and Its Effect on Yield Performance of Hexaploid Wheat. *Cereal Research Communications,* 22(1-2), 7-13. <Go to ISI>://WOS:A1994NN67000001

Schoen, A., Saripalli, G., Hosseinirad, S., Sharma, P., Kajla, A., Yadav, I. & Tiwari, V. (2024). Genome Sequences from Diploids and Wild Relatives of Wheat for Comparative Genomics and Alien Introgressions. In: Appels, R., Eversole, K., Feuillet, C. & Gallagher, D. (eds) *The Wheat Genome*. Springer Cham. https://doi.org/https://doi.org/10.1007/978-3-031-38294-9

Sears, E.R. (1976). Genetic-Control of Chromosome-Pairing in Wheat. *Annual Review of Genetics,* 10, 31-51. https://doi.org/10.1146/annurev.ge.10.120176.000335

Shiferaw, B., Smale, M., Braun, H.J., Duveiller, E., Reynolds, M. & Muricho, G. (2013). Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. *Food Security,* 5(3), 291-317. https://doi.org/10.1007/s12571-013-0263-y

Singh, R.P., Hodson, D.P., Huerta-Espino, J., Jin, Y., Bhavani, S., Njau, P., Herrera-Foessel, S., Singh, P.K., Singh, S. & Govindan, V. (2011). The Emergence of Ug99 Races of the Stem Rust Fungus is a Threat to World Wheat Production. *Annual Review of Phytopathology, Vol 49,* 49, 465-481. https://doi.org/10.1146/annurev-phyto-072910-095423

Singh, R.P., Singh, P.K., Rutkoski, J., Hodson, D.P., He, X.Y., Jorgensen, L.N., Hovmoller, M.S. & Huerta-Espino, J. (2016). Disease Impact on Wheat Yield Potential and Prospects of Genetic Control. *Annual Review of Phytopathology, Vol 54,* 54, 303-322. https://doi.org/10.1146/annurev-phyto-080615-095835

Stakman, E.C., Stewart, D. & Loegering, W.Q. (1962). Identification of physiologic races of *Puccinia graminis* var. *tritici*. 53 pp. https://doi.org/10.5555/19631101079

Stubbs, R.W. (1985). Stripe rust. In: Roelfs, A.P. & Bushnell, W.R. (eds) *The Cereal Rusts*. (ll) Academic Press. 61-101.

Tiwari, V.K., Wang, S., Sehgal, S., Vrána, J., Friebe, B., Kubaláková, M., Chhuneja, P., Doležel, J., Akhunov, E. & Kalia, B. (2014). SNP discovery for mapping alien introgressions in wheat. *Bmc Genomics,* 15(1), 1-11. https://doi.org/10.1186/1471-2164-15-273

Voss-Fels, K.P., Stahl, A. & Hickey, L.T. (2019). Q&A: modern crop breeding for future food security. *Bmc Biology,* 17. https://doi.org/10.1186/s12915-019-0638-4

Wang, M.N., Wan, A.M. & Chen, X.M. (2015). Barberry as Alternate Host Is Important for *Puccinia graminis* f. sp. *tritic*i But Not for *Puccinia striiformis* f. sp. *tritici* in the U.S. Pacific Northwest. *Plant Disease,* 99(11), 1507-1516. https://doi.org/10.1094/Pdis-12-14-1279-Re

Weisshart, K., Fuchs, J. & Schubert, V. (2016). Structured illumination microscopy (SIM) and photoactivated localization microscopy (PALM) to analyze the abundance and distribution of RNA polymerase II molecules on flow-sorted Arabidopsis nuclei. *Bio-protocol,* 6(3), e1725-e1725. https://doi.org/10.21769/BioProtoc.1725

Wellings, C.R. (2011). Global status of stripe rust: a review of historical and current threats. *Euphytica,* 179(1), 129-141. https://doi.org/10.1007/s10681-011-0360-y

Xi, W., Tang, Z.X., Luo, J. & Fu, S.L. (2019). Physical Location of New Stripe Rust Resistance Gene(s) and PCR-Based Markers on Rye (*Secale cereale* L.) Chromosome 5 Using 5R Dissection Lines. *Agronomy-Base*l, 9(9). https://doi.org/10.3390/agronomy9090498

Zadoks, J.C. (1985). Cereal rust, dogs and stars in antiquity. *Cereal Rusts Bullen,* 13, 1-10.

Zeller, F.J. & Fuchs, E. (1983). Cytology and disease resistance of a 1A/1R and some 1B/1R wheat-rye translocation cultivars. *Zeitschrift Fur Pflanzenzuchtung-Journal of Plant Breeding*, 90(4), 285-296. https://doi.org/10.5555/19831623619

Zhao, C., Liu, B., Piao, S.L., Wang, X.H., Lobell, D.B., Huang, Y., Huang, M.T., Yao, Y.T., Bassu, S., Ciais, P., Durand, J.L., Elliott, J., Ewert, F., Janssens, I.A., Li, T., Lin, E., Liu, Q., Martre, P., Müller, C., Peng, S.S., Peñuelas, J., Ruane, A.C., Wallach, D., Wang, T., Wu, D.H., Liu, Z., Zhu, Y., Zhu, Z.C. & Asseng, S. (2017). Temperature increase reduces global yields of major crops in four independent estimates. *Proceedings of the National Academy of Sciences of the United States of America,* 114(35), 9326-9331. https://doi.org/10.1073/pnas.1701762114

Popular science summary

Combat against forgotten enemy

Wheat is one of the three most important cereals for human utilization. In 2024, this crop was the second most produced and consumed cereal in the world. Unfortunately, wheat faces a growing threat from biotic and abiotic stress. One of the most important wheat diseases is stem rust, which is capable of transforming a healthy crop of wheat, only a few days before harvest, into a logging mess of stems, covered by rectangular pustules and at the end, the farmer will harvest only shriveled seeds or experience no grain yield at all. The fungus is spread rapidly by the wind, meaning it is capable of infecting other wheat farms. Historically, large-scale famines caused by this pathogen have been reported such as significant grain losses in North America (1903, 1905, and 1954), Australia (1973), Ethiopia (1993 and 1994) and several other countries.

For over three decades, a global network of plant pathologists and wheat breeders successfully prevented significant stem rust outbreaks in Western Europe and other major wheat-growing regions. This achievement was largely due to two key strategies: the widespread adoption of resistant wheat varieties and the systematic elimination of barberry bushes, which serve as an alternate host for the pathogen. These efforts effectively contained the virulent pathogen until 1999. In this year, a new race of pathogens called Ug99 was first detected in Uganda and was subsequently reported across both East and Southern Africa and in the Middle East. This race generates huge concern for wheat growing areas because studies on wheat commercial varieties show that more than 80% of them are susceptible to this race of pathogen.

In 2013 a regional outbreak of stem rust was reported in countries such as Germany, Denmark, Sweden, and the UK, which caught the attention of European scientists to the forgotten enemy. In 2016, a much larger wheat stem rust outbreak was reported in Sicily which affected thousands of hectares of both durum and bread wheat, causing average yield losses of 30–40% on a regional scale. The race was detected as new and it is estimated that more than 70% of wheat varieties in Europe are susceptible to this race.

Historically, due to the cold weather stem rust was considered a low-risk enemy of Swedish wheats. However, with the rise in improper distribution of rain during the growing season and increase in temperature, especially at

the end of the wheat maturation stage, one should never underestimate the potential of this enemy in Sweden. In 2021, in our survey of disease in the South of Sweden, we reported the first stem rust Sicily race in Sweden. We tested 200 Swedish cultivars and none of them showed resistance to this race.

In this thesis, we have attempted to cut the hand of this enemy from our tables by introducing new resistance genes with a broad-spectrum resistance to all known stem rust races and introgression them into adapted wheat cultivars. One way to achieve this is through wheat-rye introgression lines. Here, we combined different innovative technologies including marker-assisted selection (MAS) such as KASP, genotyping-by-sequencing (GBS), seedling resistance tests, and cytogenetic analysis to find new resistance genes and transfer them to progenies. Our work led to the development of a new wheat-rye translocation line, #284 having 2BS.2BL-2RL translocation with *Sr59* which has demonstrated broad resistance to various stem rust races. We further develop new sources of resistance to stem rust in #C295 with *SrSLU* resistance gene, showing a broad spectrum resistance to various stem rust races. In a key step toward durable disease resistance, we also pyramided two resistance genes—*Sr59* for stem rust and *YrSLU* for stripe rust into a single wheat line. This line was then top-crossed with high-yielding commercial varieties to increase its agronomic performance.

This result is a clear path toward the fight against stem rust. By using advanced genetic tools and innovative breeding techniques, we have shown that valuable resistance genes can be efficiently transferred into wheat varieties. With more resilient wheat, crop losses are reduced, ensuring stable yields and increasing food security for millions of people who consume wheat in their daily lives.

Populärvetenskaplig sammanfattning

Striden mot en bortglömd fiende

Vete är en av världens tre viktigaste grödor för människan. År 2024 var vete det näst mest producerade och konsumerade spannmålen globalt. Men grödan står inför växande hot från både sjukdomar och klimatrelaterade påfrestningar. En av de farligaste sjukdomarna är svartrost, som på bara några dagar kan förvandla ett friskt, skördefärdigt vetefält till en katastrof – stjälkarna knäcks, täcks av rostbruna blåsor, och kvar blir bara skrumpna korn eller ingen skörd alls. Sjukdomen sprids snabbt med vinden och kan därmed angripa hela regioner. Genom historien har den orsakat allvarliga svältkatastrofer i bland annat Nordamerika, Australien och Etiopien.

I över 30 år lyckades forskare och växtförädlare hålla svartrosten borta från stora delar av Europa och andra viktiga vete-regioner. Framgången byggde på två viktiga insatser: att odla motståndskraftiga vetesorter och att ta bort berberisbuskar, som fungerar som mellanvärd för svampen. Men 1999 upptäcktes en ny variant i Uganda – den så kallade Ug99 – och den har sedan dess spridit sig över östra och södra Afrika samt Mellanöstern. Mer än 80 % av dagens vetesorter är mottagliga för denna variant, vilket gör den särskilt oroande.

År 2013 rapporterades utbrott i länder som Tyskland, Danmark, Sverige och Storbritannien. Tre år senare, 2016, drabbades Sicilien av ett ännu större utbrott som angrep tusentals hektar och orsakade skördeförluster på 30–40 %. En ny variant av svartrost identifierades – och över 70 % av Europas vetesorter är mottagliga.

Tidigare har kallt klimat skyddat svenskt vete från svartrost, men med förändrat regnmönster och stigande temperaturer, särskilt under vetets mognadsfas, har risken för angrepp ökat. År 2021 rapporterades den sicilianska varianten av svartrost i södra Sverige för första gången. Vi testade 200 svenska vetesorter – och ingen visade sig vara resistent.

I den här avhandlingen har vi tagit upp kampen mot svartrost – med målet att förhindra att denna allvarliga sjukdom hotar vår livsmedelsförsörjning. Genom att introducera nya gener med bred och effektiv resistens mot alla kända svartrostraser har vi stärkt motståndskraften hos anpassade vetesorter. Ett angreppssätt har varit att använda så kallade vete–råg-introgressionslinjer, där vi har kombinerat flera innovativa tekniker: markörbaserat urval (MAS), DNA-sekvensering, resistenstester tidigt i

plantans untveckling, samt cytogenetisk analys. Tillsammans har dessa metoder gjort det möjligt att spåra, verifiera och föra över nya resistensgener till nästa generation av vete – med sikte på ökad sjukdomsresistens och hållbara skördar.

Vårt arbete har lett till utvecklingen av en ny translokationslinje för vete och råg, #284 med 2BS.2BL-2RL-translokation som innehåller genen *Sr59*, som bidrar med en bred resistens mot flera svartrostvarianter. Dessutom har vi identifierat en linje (#C295) med genen *SrSLU*, som också visar ett brett spektrum av resistens. I ett viktigt steg för hållbar sjukdomsresistens har vi även kombinerat två gener – *Sr59* för svartrost och *YrSLU* för gulrost – i en och samma vetesort, som sedan har korsats med högavkastande sorter.

Våra resultat visar tydligt vägen framåt i kampen mot svartrost. Med hjälp av avancerad bioteknologi och smart växtförädling har vi visat att det går att föra in värdefulla resistensgener i nya vetesorter. Mer motståndskraftigt vete betyder färre skördeförluster, säkrare livsmedelsförsörjning och tryggare tillgång till en av våra mest grundläggande baslivsmedel.

Acknowledgements

This PhD thesis would not have been possible without the help of several important people. I would not have got this far without the assistance of my supervisory team and encouragement from my family, friends and other colleagues who offered me their helping hand throughout this path. I would like to express my heartful gratitude, appreciation, and special thanks to the different institutes and individuals who provided outstanding guidance, endless support, unending patience, and amazing teamwork during this research.

I would like to take this opportunity to express my heartfelt appreciation to:

Eva Johansson, I would like to thank you for being my main supervisor, and for your never-ending guidance. Thank you for allowing me to work and learn from you and for all the fun moments we had together, especially on our trip to Budapest, where I understand you are a better swimmer than me!

Mahbubjon Rahmatov, before meeting you in person, I saw your picture at GRRC, where you were focusing on the leaf infected by stripe rust in the field (I think the poster is still there!) and I had your thesis in my office and read from it time to time. When you offered me this position, I was one of the luckiest people in the world. Thank you for being a good supervisor and a good friend. Thank you for all the good explanations and times that you spent with me. Thank you that my life and health in Sweden were as important to you as my academic life.

Mehran Patpour, I have known you since 2019 and I remember how interestingly you described stem rust and its importance to the world. Before knowing you, my main interest was in stripe rust and in flowers (!) but now I changed to stem rust lover! You always give me good bits of advice, calm me down when I am about to explode and show a new path in front of me. Thank you for getting me interested in this subject.

Mogens Støvring Hovmøller, although you are not officially my supervisor, you always help me and give me valuable advice. I always think that I am part of the GRRC team. We had lots of wonderful times together. Thank you for supporting me throughout these years.

Beyond the academic contributions, I got support from so many kind and generous individuals who helped me along the way. Special thanks to:

Beata Dedicova, I am especially thankful to you. Our chats and laughter were a much-needed source of support during this journey. Thank you for the surprise chocolates and spontaneous Fikas. Your friendship and discussions keep me motivated. Thanks for sharing your achievements with me and cherishing my achievements. Thanks for all the wonderful talks and moments together!

William Newson (Bill), and **Mulatu Geleta Dida** our hallway conversations provided a welcome break and often sparked new ideas. Thanks for your positive talks.

Ramesh Vetukuri, Thanks for your valuable advice and support during my PhD. Thanks for giving me the feeling that I am part of your group too.

Anders Ekholm, I am grateful for all your friendly and endless technical support. Thanks for helping me whenever I need you even at weekends!

I would like to express my heartfelt gratitude to my friends, whose presence and joyful moments made this journey truly memorable. Whether during our lunch breaks filled with laughter or the excursions that provided a much-needed break from our studies, their encouragement and friendship helped me navigate the challenges of my research.

Special thanks to **Farideh**, **Poorva**, **Azadeh**, **Nikwan, Sbatie**, **Mohammed, Lan**, **Olawale**, **Rimsha, Marvan**, **Maya**, **Sanjit**, **Kostas**, **Chinnu**, **Hanna, Johanna Osterman**, **Vaishnavi, Johanna Åsrand**, **Vera**, **Fantaye**, **Violet**, **Louise**, **Catja**, **Vishnu**, **Murillo**, **Kinga**, **Dushan**, **Manhal**, **Ferdi**, **Jojo, Jenifer, Reemana**, and **Lyazzat** for sharing in these experiences, reminding me of the importance of having good friends. Your support has been invaluable, and I cherish the memories we created together.

To the PPQ group,

I am happy to be part of the Plant Product Quality Group. Thank you for your support and help, and thanks for making work interesting and fun. To:

Emilia, Faraz, **Anna Lovisa**, **Jonas**, **Ramune**, **Waleed**, **Marina**, **Larisa**, **Gun**, **Oksana**, **Kateryna**, **Helena Persson**, **Julia**, **Beatrice**, **Karl**, **Senaite**, **Monalisa**, **Dessie**, **Erik**, and **Marie**.

You have all in different ways shaped my journey and without your help, I would not be here. I am deeply grateful for your support and encouragement throughout this research journey. Your friendship and positive energy were very valuable to me. I truly appreciate your presence in my life. Also many thanks to Emilia and Hanna for helping me with translating Swedish abstracts.

Special thanks to friends who make this place a happier one, Thanks for all of your positive friendship, I feel so blessed to be among you. Special thanks to all friends, researchers, PhD colleagues at the department and faculty, present and past:

Thanks to **Maria**, **Isabela**, **Alejandro**, **Milla**, **Max**, **Ronja**, **Frederik**, **Dennis**, **Ying**, **Mustafa**, **Martin**, **Oliver, Dewashi, Naga**, **Lara**, **Evelyn**, **Kamil**, **Bindu**, **katja**, **Awis**, **Sajeevan**, **Furkan**, **Viktor**, **Srisailam**, **Ivan**, **Admas**, **Kibrum**, **Haftom**, **Oliyad**, **Nehe**, **Su**, **Niklas**, **Kiros**, **Delnia**, **Jenny**, **Bekele, Federico, Dylan Jagadeesh, Jenifer**, **Behailu**, **Abera**, **Mulukan**, **Noelia**, **Aditi**, **Cecile, Anna**, **Dylan**, **Rolando**, **Pruthvi**, **Emelie, Zerihun**, **Laurène**, **Mirela**, **Matías**, **Sarala, Luboš**, **Bjørn**, **Bharathi**, **Khitma**, **Alaa**, **Wala**, **Tilal**, **Amro**, **Samrat**, and **Sara**.

And to: **Laura Vetukuri**, **Åsa Grimberg**, **Ida Lager**, **Cecilia Hammenhag**, **Per Hofvander**, **Aakash Chawade**, **Selvaraju Kanagarajan**, **Therese Bengtsson** and **Fredrik Reslow**.

And to everyone whose presence touched my heart, especially those I may have forgotten to explicitly name: I am grateful for every chance encounter – every chat on the bus, every conversation in the corridor, every shared lunchtime. Your words and smiles were a source of light and inspiration. These moments, both big and small, were invaluable, thank you.

Special thanks to: **Anders Carlsson**, **Camilla Stjärnäng**, **Annette Svensson**, **Malin Olsson**, **Desirée Lindqvist**, **Helene Larsson**, **Åsa Lankinen**, **Lotta Malmborg** and **Li-Hua Zhu**. Your assistance with administrative, financial, and education matters helped me to focus on my research with minimal disruption. Thanks for everything.

I would like to thank **Rodomiro Ortiz** for all of his suggestions and support during my studies. I would also like to thank **Firuz Odilbekov** from

Lantmännen for his support and suggestions, especially during my internship in Lantmännen.

I would like to express my sincere appreciation to all of my co-authors for their contributions to this research. Their expertise and collaboration were invaluable to the success of this project.

To GRRC team:

I am very grateful and feel lucky to work with GRRC team. From my first visit at GRRC I always receive your valuable feedback. Thank you very much for all of your support and positive energy throughout these years. I am deeply grateful to **Janne Holm**, **Ellen Frederiksen**, **Annemarie Fejer Justesen**, **Julian Rodriguez Algaba**, **Tine Thach**, **Chris Sørensen**, **Shideh Mojerlou** and **Jakob**.

To IPK team:

I am very thankful to all the wonderful people in the 'Chromosome Structure and Function" group at the Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany specifically to **Andreas Houben**, **Katrin Kumke**, **Solmaz Khosravi** and **Veit Schubert** for their collaboration and assistance during cytogenetic analysis. I am so happy to receive the IPK Walk of Fame "the rye chromatin detector of rust-bitten wheat" from you!

To my friends, **both near and far**, thank you for your friendship and for providing a much-needed sense of community in these years. Thanks to **Morteza**, **Jasi**, **Sina**, **Bahreh**, **Sahar**, **Ashkan**, **Anna**, **Ivan**, **Rosa**, **Dori**, **Raziyeh**, **Mohandes Yazdani**, **Mohandes Sedaghat**, **Mohandes khosrofard**, and all my Iranian friends in Sweden who make a wonderful community.

I would like to thank the Khalil family for welcoming me into their home and for their ongoing support. Their presence in my life has been a constant source of joy and happiness. Thanks for all the great moments we had together and thanks for treating me as one of your family members. Thanks to **Tayssir**, **Itidal**, **Rani**, **Atusa**, **Diana**, **Azar**, **Adrian** and **Amitis**.

I am deeply grateful to **Dani**: Thank you for your unwavering love, support, and understanding. Thank you for being my constant source of strength and joy. I am so grateful to have you by my side. Thanks for making my days in Sweden full of joy and happiness.

Last but not least, to my dear family, my deepest gratitude for your unwavering love, support, and belief in me. This accomplishment would not have been possible without you. Thanks to **my father (Rahim Yazdani)**, **my mother (Zahra Mormeh)**, **my sisters (Zohre and Mahdieh)** and **my brother (Abbas)**. Without your support, I was lost and sat in the dark. Thanks for taking my hand and bringing me to happiness, thanks for every wisdom words you give me. I would be nobody without you. Thanks for being there whenever I need help. Thanks for loving me without any condition.

فضل خدای را که تواند شمار کرد؟ یا کیست آنکه شکر یکی از هزار کرد؟

آن صانع قدیم که بر فرش کائنات چندین هزار صورت الوان نگار کرد

ترکیب آسمان و طلوع ستارگان از بهر عبرت نظر هوشیار کرد

بحر آفرید و بر و درختان و آدمی خورشید و ماه و انجم و لیل و نهار کرد

الوان نعمتی که نشاید سپاس گفت اسباب راحتی که نشاید شمار کرد

آثار رحمتی که جهان سر به سر گرفت حمال منتی که فلک زیر بار کرد

اجزای خاک مرده، به تأثیر آفتاب بستان میوه و چمن و لاله زار کرد

این آب داد بیخ درختان تشنه را شاخ برهنه پیرهن نوبهار کرد

چندین هزار منظر زیبا بیافرید تا کیست کو نظر ز سر اعتبار کرد

توحید گوی اوست بنی آدم و بس هر بلبلی که زمزمه بر شاخسار کرد

لالست در دهان بلاغت زبان وصف از غایت کرم که نهان و آشکار کرد

بخشنده‌ای که سابقه فضل و رحمتش ما را به حسن عاقبت امیدوار کرد

پرهیزگار باش که دادار آسمان فردوس جای مردم پرهیزگار کرد

نابرده رنج گنج میسر نمی‌شود مزد آن گرفت جان برادر که کار کرد

هر کو عمل نکرد و عنایت امید داشت دانه نکاشت ابله و دخل انتظار کرد

بعد از خدای هر چه پرستند هیچ نیست بی‌دولت آنکه بر همه هیچ اختیار کرد

شاید که التماس کند خلعت مزید سعدی که شکر نعمت پروردگار کرد

I

PLOS ONE

RESEARCH ARTICLE

Developing adapted wheat lines with broad-spectrum resistance to stem rust: Introgression of *Sr59* through backcrossing and selections based on genotyping-by-sequencing data

Mahboobeh Yazdani[1], Matthew N. Rouse[2,3], Brian J. Steffenson[3], Prabin Bajgain[4], Mehran Patpour[5], Eva Johansson[1], Mahbubjon Rahmatov[1]*

1 Department of Plant Breeding, Swedish University of Agricultural Sciences, Alnarp, Sweden, **2** United States Department of Agriculture, Agricultural Research Service, Cereal Disease Laboratory, St. Paul, MN, United States of America, **3** Department of Plant Pathology, University of Minnesota, St. Paul, MN, United States of America, **4** Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN, United States of America, **5** Department of Agroecology, Aarhus University, Slagelse, Denmark

* Mahbubjon.Rahmatov@slu.se

Citation: Yazdani M, Rouse MN, Steffenson BJ, Bajgain P, Patpour M, Johansson E, et al. (2023) Developing adapted wheat lines with broad-spectrum resistance to stem rust: Introgression of *Sr59* through backcrossing and selections based on genotyping-by-sequencing data. PLoS ONE 18(10): e0292724. https://doi.org/10.1371/journal.pone.0292724

Editor: Pramod Prasad, ICAR - Indian Institute of Wheat and Barley Research, INDIA

Received: July 6, 2023

Accepted: September 26, 2023

Published: October 12, 2023

Peer Review History: PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: https://doi.org/10.1371/journal.pone.0292724

Data Availability Statement: All relevant data are within the paper.

Abstract

Control of stem rust, caused by *Puccinia graminis* f.sp. *tritici*, a highly destructive fungal disease of wheat, faces continuous challenges from emergence of new virulent races across wheat-growing continents. Using combinations of broad-spectrum resistance genes could impart durable stem rust resistance. This study attempted transfer of *Sr59* resistance gene from line TA5094 (developed through CSph1bM-induced T2DS·2RL Robertsonian translocation conferring broad-spectrum resistance). Poor agronomic performance of line TA5094 necessitates *Sr59* transfer to adapted genetic backgrounds and utility evaluations for wheat improvement. Based on combined stem rust seedling and molecular analyses, 2070 BC_1F_1 and 1230 BC_2F_1 plants were derived from backcrossing BAJ#1, KACHU#1, and REEDLING#1 with TA5094. Genotyping-by-sequencing (GBS) results revealed the physical positions of 15,116 SNPs on chromosome 2R. The adapted genotypes used for backcrossing were found not to possess broad-spectrum resistance to selected stem rust races, whereas *Sr59*-containing line TA5094 showed resistance to all races tested. Stem rust seedling assays combined with kompetitive allele-specific PCR (KASP) marker analysis successfully selected and generated the BC_2F_2 population, which contained the *Sr59* gene, as confirmed by GBS. Early-generation data from backcrossing suggested deviations from the 3:1 segregation, suggesting that multiple genes may contribute to *Sr59* resistance reactions. Using GBS marker data (40,584 SNPs in wheat chromosomes) to transfer the recurrent parent background to later-generation populations resulted in average genome recovery of 71.2% in BAJ#1*2/TA5094, 69.8% in KACHU#1*2/TA5094, and 70.5% in REEDLING#1*2/TA5094 populations. GBS data verified stable *Sr59* introgression in BC_2F_2 populations, as evidenced by presence of the *Ph1* locus and absence of the 50,936,209 bp deletion in CSph1bM. Combining phenotypic selections, stem rust seedling assays, KASP markers,

Funding: For this research financial support obtained from the Swedish Research Council Vetenskapsrådet and FORMAS. There was no involvement of the funding agencies in the study's design, the collection and analysis of data, the decision to publish, or the preparation of the manuscript.

Competing interests: There are no competing interests among the author.

and GBS data substantially accelerated transfer of broad-spectrum resistance into adapted genotypes. Thus, this study demonstrated that the *Sr59* resistance gene can be introduced into elite genetic backgrounds to mitigate stem rust-related yield losses.

Introduction

Wheat (*Triticum aestivum* L.) is an important source of calories and protein in the daily human diet world-wide [1]. Due to the current rapid growth in the global population, a 60% increase in wheat production will be necessary in order to maintain its current share of the human diet by 2050 [2]. Wheat yield will need to be increased by at least 2% each year to meet this demand, a target that is currently not being attained [3]. The major constraints to achieving the necessary yield increase are biotic and abiotic stresses that impair crop performance, with rust diseases in particular having the potential to cause yield losses in severe outbreaks. Among these diseases, stem rust (caused by the fungus *Puccinia graminis* f. sp. *tritici* (*Pgt*)) is a major threat to wheat production across many regions of the world, because it is capable of causing severe yield loss [4]. Although fungicide application can effectively manage stem rust, it is associated with drawbacks such as high costs, significant environmental impact, and negative effects on human health [5]. Hence, genetic resistance is the most economical and environmentally sustainable control measure to protect wheat yields from the threat of stem rust. The frequent emergence of new *Pgt* races is a major challenge to success in breeding resistance to this pathogen in wheat. An example of this is emergence of the Ug99 race group, which is capable of overcoming all known and extensively deployed stem rust (*Sr*) resistance genes, including *Sr24*, *Sr31*, *Sr36*, and *SrTmp*. This constant adaptation of the pathogen has increased concerns about global epidemics [6, 7].

Other widely virulent *Pgt* races, such as TRTTF, TKTTF, TTRTF, TTKST, PRCTM, and TTKTT, have been found to possess additional virulence combinations, including virulence to *Sr22+Sr24*, *Sr24+Sr31*, *Sr13b+Sr35+Sr37*, and *Sr24+Sr31+SrTmp* genes [8–10]. The emergence of these novel races and their spread into Europe is alarming, since stem rust disease has largely been absent for nearly 60 years [11–13]. Moreover, a high proportion of cultivars grown in Europe are susceptible to these emerging races, e.g., ~80% of wheat cultivars currently grown in the United Kingdom are susceptible to race TKTTF [11], while resistance genes such as *Sr24*, *Sr31*, and *Sr38*, present in German wheat cultivars are limited in their effectiveness against these novel races of *Pgt* [14]. Of the currently known and described wheat *Sr* genes, 35 out of 73 derive from the primary gene pool of wheat and the majority of these do not confer broad-spectrum resistance [4]. Until recently, the *Sr31* resistance gene was considered highly effective in conferring broad-spectrum resistance against all *Pgt* races, and was introduced into many wheat cultivars over the past 30 years [7].

Rye (*Secale cereale* L., 2n = 14), belonging to the tertiary gene pool of wheat, is an important source of genes that can be used for increasing bread wheat resistance to both abiotic and biotic stresses [15]. For instance, *Sr27*, *Sr31*, $Sr1RS^{Amigo}$, *SrSatu*, *Sr50*, and *Sr59* are important stem rust resistance genes that have been introduced into wheat from rye, and several of these genes have been proven to confer broad-spectrum resistance [16–19]. However, introgression of genes from wild relatives into wheat relies on meiotic recombination, which is complicated between rye and bread wheat. Hexaploid bread wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD) was derived from *T. urartu* (2n = 2x = 14, AA), *Aegilops sp.* (2n = 2x = 14, BB), and *Ae. tauschii* (2n = 2x = 14, DD) through spontaneous interspecific crosses. Allohexaploid wheat behaves as a diploid during meiosis [20], due to the presence of *pairing homoeologous*

(*Ph*) loci that strictly control pairing homology during meiosis. Two major *Ph* loci (*Ph1* and *Ph2*, residing on chromosome 5BL and 3DS, respectively) control homoeologous recombination in wheat [20, 21]. Deletion of the *Ph1* locus (*ph1b* in hexaploid and *ph1c* in tetraploid wheat) results in homoeologous recombination [22, 23]. As a result, a Chinese Spring line mutated at the *ph1b* locus (CSph1bM) has been used effectively to induce recombination between wheat and alien chromosomes [24]. Many broad-spectrum resistance genes have been transferred using CSph1bM, including *Sr32* from *Ae. speltoides* [24], *Sr39* from *Ae. speltoides* [25], *Sr47* from *Ae. speltoides* [26], *Sr53* from *Ae. geniculata* [27], *Sr43* from *Thinopyrum ponticum* [28], *Sr47* from *Ae. speltoides* [26], *Sr53* from *Ae. geniculata* [27], *Sr59* from *S. cereale* [16], and *Yr83* from *S. cereale* [29]. Line CSph1bM has also been used to transfer genes other than those for wheat resistance, e.g., end-use quality has been improved by recombining the *Sec-1* (secalin) allele on the 1RS chromosome arm in wheat lines [30].

A large number of wheat-rye introgression lines were developed in the 1980s-2000s by the late Professor Arnulf Merker at the Swedish University of Agricultural Sciences [31, 32]. Some of these lines were used in field and greenhouse screenings to identify the line 'SLU238' [2R (2D) wheat-rye disomic substitution], which was found to confer broad-spectrum resistance to all *Pgt* races tested [33]. TA5094, a line derived from 'SLU238', has since been shown to possess a T2DS·2RL Robertsonian translocation with a stem rust resistance gene designated *Sr59* [16]. Due to the lack of acceptable agronomic performance in TA5094 based on the CSph1bM background, there is an urgent need to transfer this gene to a more suitable genetic background and evaluate its potential use in wheat resistance breeding. This paper describes transfer and subsequent evaluation of *Sr59* to agronomically suitable genetic background derived lines through: 1) marker-assisted backcross breeding; 2) stem rust seedling assessment; 3) background selection; and 4) physical mapping of the *Sr59* resistance gene on chromosome 2RL.

Materials and method

Plant materials and stem rust seedling evaluations in parental lines

TA5094 was derived from a cross between CSph1bM and line SLU238 [a 2R (2D) wheat-rye disomic substitution], and has been defined as a T2DS·2RL translocation containing the *Sr59* resistance gene [16]. In the present study, three spring bread wheat cultivars (BAJ#1, KACHU#1, and REEDLING#1), kindly provided by Dr. Ravi Singh (International Maize and Wheat Improvement Center (CIMMYT), El Batan, Mexico), were used as recurrent parents and crossed with TA5094. The resulting progeny were evaluated by seedling tests for stem rust reaction, molecular marker analysis, kompetitive allele specific PCR [KASP] markers, and genotyping by sequencing [GBS], with the four lines TA5094, CSph1bM, SLU238, and Chinese Spring (CSA) used as controls. The selected parental lines and controls were initially tested with the *Pgt* races TTTTF (isolate 01MN84A-1-2), TTTTF (isolate RU118b/16), QTHJC (C25; isolate 1541), TPMKC (C53; isolate 1373), RKQQC (C35; isolate 1312), RCRSC (isolate 77ND82A), TTRTF (isolate IT14a/16), TKTTF (isolate 13ETH60), TKTTF (isolate IQ115a/14 and isolate SE27121), TTKTT (isolate 14KEN58-1), TTKSK (isolate 04KEN156/04), TTKST (isolate 06KEN19v3), TTTSK (isolate 07KEN24-4), JRCQC (08ETH03-1), TRTTF (isolate 06YEM34-1), and LTBDC (Australian *Pgt* race 98–1,2,3,5,6).

Population development, stem rust seedling evaluations, and molecular marker analysis

The F_1 plants obtained from crosses between line TA5094 and the recurrent parents (BAJ#1, KACHU#1, and REEDLING#1) were backcrossed to each of the corresponding recurrent

parents, generating BC_1F_1 seeds. A total of 2,070 BC_1F_1 plants were assessed for their seedling responses to *Pgt* race TTTTF (isolate 01MN84A-1-2), in trials at the USDA-ARS Cereal Disease Laboratory and University of Minnesota using a previously described stem rust seedling assay [34, 35]. For each recurrent parent, 94 resistant BC_1F_1 plants were selected (i.e., in total 282 plants) and analyzed for the presence of *Sr59* by use of three KASP markers: KASP_2RL_c25837C1, KASP_2RL_c21825C1, and KASP_2RL_c20194C2 [16]. Based on the results of KASP marker analysis, BC_1F_1 plants with *Sr59* were selected and used for backcrossing to produce BC_2F_1 plants. The backcross generated 1,230 BC_2F_1 plants, which were assessed against *Pgt* race TTTTF. Resistant plants were selected and tested for the presence of *Sr59* with the three KASP markers. Plants carrying *Sr59* were selfed to produce the BC_2F_2 generation, which resulted in a total of 846 families (from all recurrent parents). These families were again evaluated against *Pgt* race TTTTF and resistant plants were selected and checked with the three KASP markers. The BC_2F_2 generation was also evaluated using race TTKSK, and the pattern of segregation was analyzed. From the BC_2F_2, additional generations (BC_2F_3, BC_2F_4, and BC_2F_5) were created through selfing, 20 plants from each generation were selected based on the seedling response to race TTTTF, and presence of *Sr59* was validated by KASP markers. In addition, 10–15 BC_2F_4 and BC_2F_5 plants from each family were tested for their seedling response to races TTKSK, TTTSK, and TRTTF. The BC_2F_5 families were also assessed against races TPMKC, QTHJC, RKQQC, and RCRSC. The segregation pattern data were assessed using chi-square (χ^2) analysis.

Genotyping and data analysis

The population was genotyped using GBS as described previously [36]. Tissue sampling and DNA extraction were carried as described previously [37]. Approximately 10 cm of young leaf tissue from each of the donor parents (TA5094 and CSph1bM), recurrent parents, and a total of 128 selected BC_2F_2 plants (from all three recurrent parents) were collected in a 96-well tissue collection plate. Genomic DNA was isolated using the Qiagen BioSprint 96 instrument and the associated Qiagen BioSprint DNA Plant kit (https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/biosprint-96-dna-plant-kit/#orderinginformation). DNA sequencing libraries were prepared and sequenced at the University of Minnesota Genomics Center. In brief, the isolated DNA was quantified with PicoGreen for GBS genotyping and normalized to 20 ng/μL. The GBS libraries were prepared in 96-plex using two restriction enzymes: a rare cutter *Pst*I (5′–CTGCAG–3′) and a frequent cutter *Msp*I (5′–CCGG–3′) with a common reverse adapter ligated [36, 38]. Libraries were sequenced on Illumina HiSeq2500 (Illumina, San Diego, CA, USA). Sequences obtained in the FASTQ files were passed through a quality filter of Q >30 and then de-multiplexed to obtain reads for each individual. Thereafter, the GBS reads were aligned to the International Wheat Genome Sequencing Consortium (IWGSC) Reference Sequence v1.0 (RefSeq v1.0) assembly and Rye Genome Sequencing Consortium Reference Sequence, using the Burrow-Wheelers Alignment tool (BWA) v0.7.4 [39]. Marker discovery, i.e., identification of SNPs, was accomplished using Samtools+Bcftools [40]. SNPs with minor allele frequency (MAF) <5% and more than 20% missing data were removed. After processing, 40,584 SNP markers for wheat and 15,116 SNP markers for chromosome 2R were retained for further analyses. Allele frequencies and genetic relationship between donor and recurrent parental lines were calculated using TASSEL v5.2.65 [41]. Principal component analysis (PCA) was performed using the function 'prcomp' in R 4.0.2. MapChart 2.2 (https://www.wur.nl/en/show/mapchart.htm) was used to draw physical maps.

Selection of plants for recovery of recurrent parents

Progeny lines were selected based on their phenotypic and genomic similarity to the recurrent parents and used in the next backcross generation. The phenotypic parameters of each backcross generation (BC_1F_1 and BC_2F_1) were evaluated in the greenhouse, to determine whether the plants were similar to the recurring parents based on their height, tillering, heading date, flowering, spike characteristics (with or without awns), seed fertility, and maturity day. Background selection for alleles similar to those of the recurrent parents was then performed on the BC_2F_2 generation, using GBS markers distributed across all 42 wheat and the 2R rye chromosomes. Next, individual plants from each generation (BC_2F_3 to BC_2F_6) were carefully selected based on highest phenotypic and genotypic similarities to the recurrent parents, ensuring consistent inheritance of desired traits across generations.

BC_2F_7 and BC_2F_8 lines resistant to races TTTTF and TTKSK were sown on the field of Lantmännen Research Station in Svalöv (55.925621˚N, 13.096742˚E) for phenotyping evaluations. In one replicate field evaluation, these lines were sown in small plots to assess phenotypic traits compared with the recurrent parents. Data were collected on characteristics such as number of days to 50% flowering and maturity, plant height, tillering, lodging, susceptibility to diseases (e.g., rusts, powdery mildew, septoria, FHB, etc.), and grain color.

Results

Stem rust seedling response in the parental lines

Seventeen *Pgt* races were used to evaluate the seedling response of parental lines to stem rust. The results revealed that SLU238 and TA5094 were broadly resistant, exhibiting infection types (ITs) of; 1 to 1+2- to all races in this experiment (Table 1). The recurrent parents (BAJ#1, KACHU#1, and REEDLING#1) were found to be susceptible to several of the *Pgt* races and exhibited ITs of 3+4 to races TTTTF (USA and Russia), TTKTT, TTRTF, TTKSK, TTTSK, and TRTTF (Table 1). For races RKQQC, RCRSC, TKTTF (Sweden, Iraq, Ethiopia), LTBDC (Australian *Pgt* race 98–1,2,3,5,6), QTHJC, and TPMKC, recurrent parents had ITs ranging from 0 to 11+ (Table 1). CSph1bM and CSA were highly susceptible to all 17 *Pgt* races tested, indicating that no resistance genes were present in these two lines (Table 1). Based on the virulence profile of the different *Pgt* races, REEDLING#1, BAJ#1, and KACHU#1 were postulated to carry resistance genes *Sr11* and *Sr38* (Table 1).

Stem rust seedling evaluations and marker-assisted backcrossing

Evaluation of the BC_1F_1 and BC_2F_1 populations using race TTTTF suggested presence of a major resistance gene following crossbreeding with BAJ#1 (Table 2). The P-values for all other BC_1F_1 and BC_2F_1 populations resulting from these crosses were <0.05, indicating a segregation pattern deviating from the expected 1:1 ratio. Such deviation suggests potential segregation distortion or the involvement of more than one major gene in resistance. However, the segregation ratio was close to 3:1 ($P>0.05$) for the BC_2F_2 populations from the backcrosses to BAJ#1 and REEDLING#1 (Table 2), indicating a single dominant major resistance gene. To verify presence of *Sr59* in plants that were selected for further generations, a number of resistant plants from each of the BC_1F_1, BC_2F_1, and BC_2F_2 populations were selected and genotyped with three KASP markers (Table 2). Plants found to contain the gene were transplanted for backcrossing and selfing (Table 2). The BC_2F_2 populations were also phenotyped with race TTKSK, which resulted in a significant deviation from the expected 3:1 segregation ratio ($P<0.01$). Instead, the segregation ratio was closer to 14:2 ($P>0.05$), suggesting presence of a major gene plus one or more additional genes co-acting with the major gene. The BC_2F_4 and

Table 1. Response to stem rust of seedlings from the parental lines used in this study.

Parental line	TTTTF USA	TTRTF Italy	TTTTF Russia	TKTTF +*Sr25* Iraq	TTKTT Rwanda	QTHJC USA	TPMKC USA	RKQQC USA	RCRSC USA	TKTTF Sweden	TKTTF Ethiopia	TTKSK Kenya	TTTSK Kenya	TTKST Kenya	JRCQC Ethiopia	TRTTF Yemen	LTBDC Australia	Gene postulation	Spike phenotype
CSph1bM	4	4	4	4	4	4	3+	4	4	4	4	4	4	4	4	4	4	None	Awnless
CSA	4	4	4	4	4	4	3+	4	4	4	4	4	4	4	4	4	4	None	Awnless
SLU238	1+2-	;1	11+	11+	;1	11-	11+	11+	;11-	11+	11+	;1-	;1-	;1-	11+	;11+	11+	*Sr59*	Awnless
TA5094	1+2-	;1	11+	1+	;1	11-	11+	11+	11+	11+	11+	;1-	;1-	;1-	11+	;11+	11+	*Sr59*	Awnless
BAJ#1	4	4	4	;1-	4	;0	11+	;0	11-	;1-	11+	4	4	4	N.A.	4	22-	*Sr11+Sr38*	Awn
KACHU#1	4	4	4	;1-	3+	11+	11-	;0	;1-	;1-	;1-	4	4	4	N.A.	4	11+	*Sr11+Sr38*	Awn
REEDLING#1	4	4	4	;1-	4	;0	11-	;0	;1-	;1-	11+	4	4	4	N.A.	4	11+	*Sr11+Sr38*	Awn

N.A.–Not available. Infection types observed based on 0–4 scale [35]. Plants with infection types; 0 to 22- were considered resistant, while plants with infection types 3–4 were considered susceptible.

https://doi.org/10.1371/journal.pone.0292724.t001

Table 2. Crossing, backcrossing, and selection procedures with *Pgt* race TTTTF, KASP markers, and phenotyping selection in BC_1F_1 and BC_2F_1 populations.

Cross	Generation	*Pgt* race TTTTF		χ2	P-value	No. of plants for KASP analysis	No. of transplanted plants	Selected plants for GBS	Expected phenotypes for selection
		Resistant	Susceptible						
BAJ#1*1/TA5094	BC_1F_1	265	195	10.65	0.001	94	40	-	Awn and short height*
BAJ#1*2/TA5094	BC_2F_1	135	155	1.37	0.24	94	40	-	Awn and short height
BAJ#1*2/TA5094	BC_2F_2	180	66	0.65	>0.1	40	32	32	Awn and short height
KACHU#1*1/ TA5094	BC_1F_1	380	310	7.1	0.007	94	40	-	Awn and short height
KACHU#1*2/ TA5094	BC_2F_1	235	195	4.35	0.036	94	40	-	Awn and short height
KACHU#1*2/ TA5094	BC_2F_2	215	35	24.2	<0.001	40	30	30	Awn and short height
REEDLING#1*1/ TA5094	BC_1F_1	520	400	15.65	<0.001	94	40	-	Awn and short height
REEDLING#1*2/ TA5094	BC_2F_1	278	232	4.14	0.041	94	40	-	Awn and short height
REEDLING#1*2/ TA5094	BC_2F_2	432	127	2.32	>0.1	80	66	66	Awn and short height

*Selection based on height ranging from 95 to 100 cm. Infection types observed based on 0–4 scale [35]. KASP markers were used to validate the presence of *Sr59* [16].

https://doi.org/10.1371/journal.pone.0292724.t002

BC_2F_5 populations, obtained through assessments against race TTTTF and subjecting selected resistant plants to KASP genotyping, showed a similar high level of resistance (IT; 1- to; 11+) against races TTTSK, TTKST, and TRTTF as seen in their resistant parental lines (Table 1), indicating successful transfer of *Sr59* to the later generations. The BC_2F_5 families also displayed similar responses (IT; 01- to; 11+) as SLU238 and TA5094 when evaluated against races TPMKC, QTHJC, RKQQC, and RCRSC.

GBS genotyping and physical location of *Sr59* on the 2R chromosome

Through alignment of raw GBS reads against the rye line 'Lo7' (International Rye Genome Sequencing Consortium (IRGSC)) reference genome), followed by filtering to remove SNPs with missing values ≤20% and minor allele frequency (MAF) ≥ 5%, the physical positions of the 15,116 SNPs obtained were mapped to chromosome 2R at 347,694 bp to 945,773,747 bp (Fig 1). In Fig 2B, the physical positions of the 15,116 SNPs from GBS (rye alleles) are shown in red color, whereas blue color indicates the presence of wheat alleles when mapping the BC_2F_2 populations. The physical positions of three KASP markers (c20194_115, c25837_157, and c21825_230) and the GBS results showed that *Sr59* was located in the 2RL segment (Fig 1). The three KASP markers were also used to track the *Sr59* introgression into the recurrent parent's background through the crossing scheme utilized in the present study. BLASTN searches of the three KASP markers against IRGSC positioned the *Sr59* resistance gene between 914,812,226 bp and 943,109,279 bp on chromosome 2RL (Fig 1). Presence of 2RL was also clearly verified in lines SLU238, SLU239, and TA5094, as demonstrated by the red color in Fig 2B, whereas 2D (wheat) was verified in BAJ#1, KACHU#1, REEDLING#1, CSA, CSph1bM, and susceptible lines (6-BAJ-S, 23-KACHU-S, 33-Reed-S, 35-Reed-S, and 45-Reed-S), as demonstrated by the blue color in Fig 2B. A strong association was observed between presence of the rye/wheat alleles determined by the GBS dataset and resistance (red color)/susceptibility (green color) reactions to race TTTTF and TTKSK (Fig 2A and 2C). Most of the BC_2F_3 lines showed homozygosity (100% red color) for resistance to the TTTTF race, although some lines segregated (40% red and 60% green; Fig 2A). The reaction to race TTKSK was

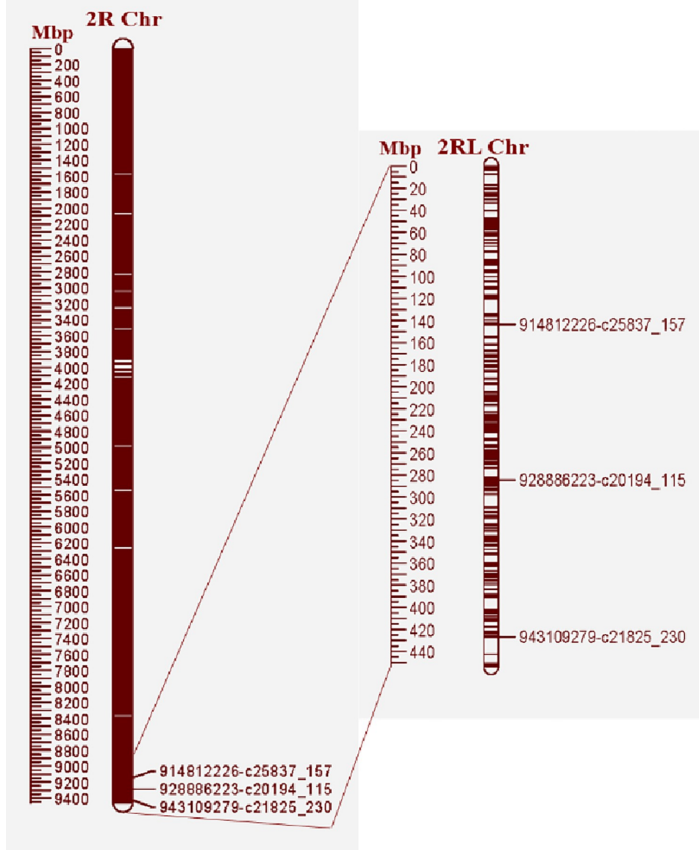


Fig 1. Location of the *Sr59* resistance gene on rye chromosome 2R, determined using GBS reads aligned to the rye line 'Lo7'. Dark red denotes presence of physical SNPs, while white denotes absence of SNPs throughout the 2R chromosome. The trio of SNPs on 2RL correspond to the three KASP markers identified previously.

https://doi.org/10.1371/journal.pone.0292724.g001

tested in the BC_2F_3 lines, where the homozygous lines showed IT; 1 (100% red color), while the segregating lines showed; 1 to 3+4 (varying percentages of green and red color) (Fig 2C). The BC_2F_3 lines are shown as 100% green color for both races (TTTTF and TTKSK) in Fig 2A and 2C. PCA analysis based on the results of the GBS data (15,116 SNPs on chromosome 2R) clustered the genotypes evaluated into five distinct clusters: A) SLU238, SLU239, and TA5094; B) The BC_2F_2 population consists of recurrent parents harboring the 2RL chromosome proximate to SLU238 and TA5094; C) BC_2F_2 population derived from recurrent parents carrying the 2RL chromosome; D) Recurrent parents (BAJ #1, KACHU #1, and REEDLING #1) and the susceptible BC_2F_2 population without 2RL; and E) CSph1bM and CSA (Fig 3).

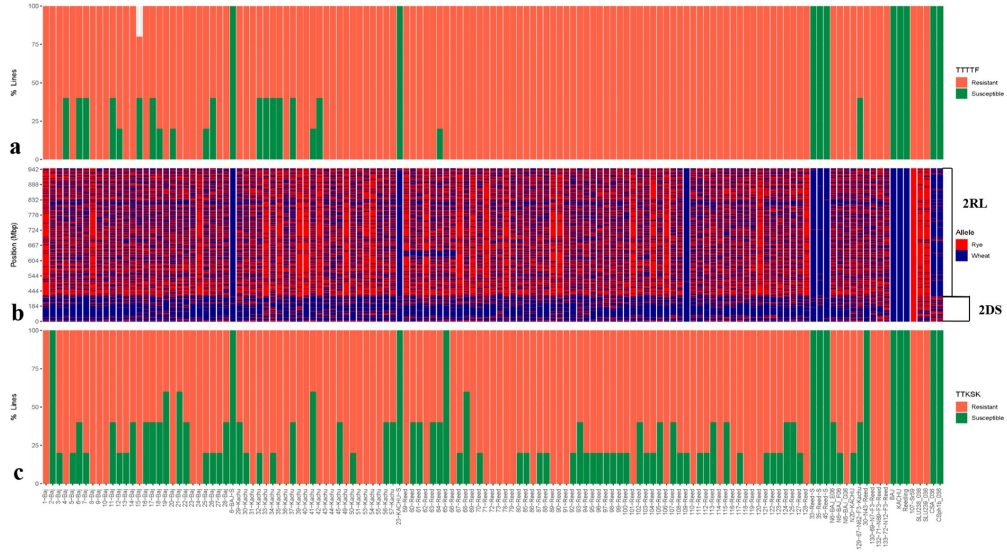


Fig 2. Physical location of rye chromosome 2R based on GBS data and seedling responses to stem rust races TTTTF and TTKSK. a) Seedling assay for race TTTTF in the BC_2F_3 population, where red denotes resistance and green susceptibility; b) physical positions of the 15,116 SNPs from GBS reads in the BC_2F_2 population, where red denotes the rye allele and dark blue the wheat allele; c) seedling assay for race TTKSK in the BC_2F_3 population, where red denotes resistance and green susceptibility.

https://doi.org/10.1371/journal.pone.0292724.g002

Recovery of the recurrent parent (background selection)

To select lines that resembled their recurrent parents as much as possible (with the exception of the addition of *Sr59*), a total of 40,584 SNPs across all 21 chromosomes were used to identify the most suitable lines in the BC_2F_2 population. Based on polymorphic SNPs, whole-wheat genome PCA identified five distinct clusters: 1) CSph1bM and CSA; 2) SLU238 and TA5094; 3) BC_2F_2 BAJ#1 population; 4) BC_2F_2 KACHU#1 population; and 5) BC_2F_2 REEDLING#1 population (Fig 4). The PCA results showed that most of the BC_2F_2 of a recurrent parent clustered at the same plot, indicating genomic recovery of the recurrent parent genome. As shown in Table 3, percentage genome recovery for the recurrent parents ranged from 66% to 75% across the three BC_2F_2 populations. The plants with the highest recurrent parent genome recovery and carrying *Sr59* were selected to generate homozygous lines through selfing.

Phenotypic selection in greenhouse and field conditions

Besides using SNPs to produce lines resembling recurrent parental lines, selection was carried out in greenhouse and field evaluations with plant height and awns/awnless spikes being key phenotypic characters considered during crossing and backcrossing, as these characters differed between SLU238 and TA5094 compared with the recurrent parents, BAJ#1, KACHU#1, and REEDLING#1 (Table 2). Thus, 40 BC_1F_1 plants with maximum phenotypic similarity to the recurrent parents were used for developing the BC_2F_1 population. In BC_2F_2 to BC_2F_6 populations, plants were selected based on four characteristics: plant height, awns/awnless, days to maturity, and seed fertility. BC_2F_7 lines showing a homozygous resistance reaction to *Pgt* race

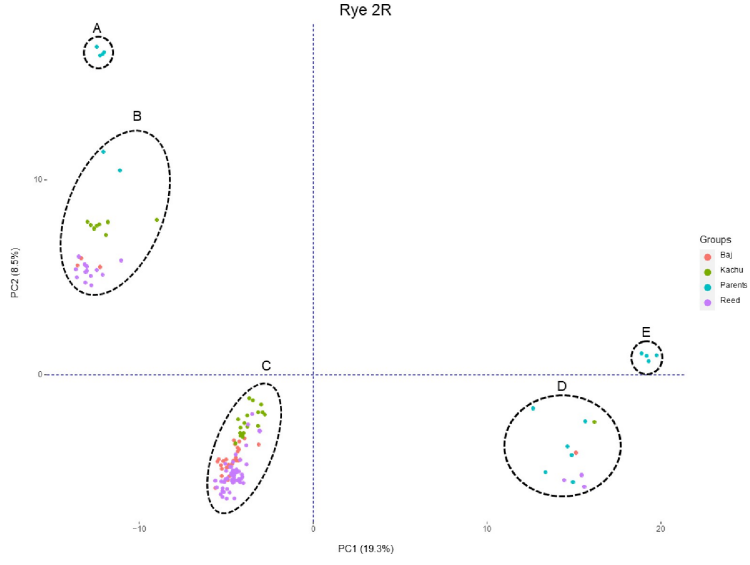


Fig 3. PCA plot of rye chromosome 2R using 15,116 SNPs from GBS reads. A) Resistant parental lines (SLU238, SLU239, and TA5094) with chromosome 2R; B) BC_2F_2 population comprising recurrent parents carrying chromosome 2RL close to SLU238 and TA5094; C) BC_2F_2 population derived from recurrent parents carrying the chromosome 2RL segment; D) recurrent parents (BAJ #1, KACHU #1, and REEDLING #1) and the susceptible BC_2F_2 population without chromosome 2RL; E) lines CSph1bM and CSA.

https://doi.org/10.1371/journal.pone.0292724.g003

TTTTF and positive KASP marker data were sown in the field in 2020. The following phenotypic parameters were considered when selecting single plants in the field in 2020: plant stand, tillering, plant height, awns/awnless spikes, lodging, days to maturity, and seed fertility. Following the phenotypic analysis described above, BC_2F_8 populations were sown in the greenhouse and selfed to produce another generation, and BC_2F_9 populations were planted in the field in spring 2021 to select plants whose phenotypic similarity to the recurrent parents was greatest. The three KASP markers (c20194_115, c25837_157, and c21825_230) were used again to verify presence of the *Sr59* resistance gene in the individual BC_2F_7 to BC_2F_9 plants.

Ph1 allele status

A BLAST search against the IWGSC reference sequence v1.0 resulted in 2,050 GBS SNPs annotated in the range 16,637–712,890,017 bp on chromosome 5B (Fig 5). Furthermore, a deletion breakpoint of 50,936,209 bp (51 Mb), located from 396,630,846 bp to 447,567,055 bp, was detected in line CSph1bM, indicating the position of the *Ph1* locus on the 5B chromosome (Fig 5). The GBS data also revealed presence of the *Ph1b* deletion in TA5094 and two BC_2F_2 populations (31-Kachu and 34-Kachu), whereas it was not detected in the other BC_2F_2 populations, SLU238, CSA, or the recurrent parents. These results show that most of the BC_2F_2 populations carry the *Ph1* allele, and that the status of 2DS.2RL (and thus the introgression of *Sr59* into the wheat genetic background of the recurrent parents) is stable.

Fig 4. PCA plot of wheat chromosomes using 40,584 SNPs from GBS reads. Relationship between recurrent parents (BAJ#1, KACHU#1, and REEDLING#1) and lines SLU238, TA5094, CSph1bM, and CSA, based on polymorphic sites in the entire wheat genome as determined by GBS.

https://doi.org/10.1371/journal.pone.0292724.g004

Discussion

In this study, the *Sr59* stem rust resistance gene was transferred, using TA5094 as a donor of rye chromatin, into three elite wheat lines, through marker-assisted backcrossing selection and stem rust seedling screening (BC_1F_1 to BC_2F_5). Seedling screening and KASP marker analysis allowed us to trace presence/absence of *Sr59* from the parental lines in all progeny through all generations. The use of high-throughput genomic tools, such as GBS, facilitated the application of a strong selection pressure that increased the probability of recovering the elite recurrent parent background genome, while at the same time preserving the translocation fragment 2DS.2RL containing *Sr59*. The GBS background selection accurately identified both the translocated 2DS.2RL and the deletion region on 5BL (*ph1b* deletion). *Sr59* contributed stable resistance, as demonstrated by seedling screening against multiple *Pgt* races and KASP marker analysis across all populations developed.

Thus our novel GBS- and marker-assisted method was able to eliminate the *ph1b* deletion while transferring *Sr59* into the genetic background of three widely adapted wheat cultivars (BAJ#1, KACHU#1, and REEDLING#1) from CIMMYT through backcrossing, and can ultimately produce wheat lines suitable for breeders. Initially, the *Sr59* resistance gene was selected

Table 3. Recurrent parent genome recovery in the BC_2F_2 generation using 40,584 genome-wide SNPs.

Cross	Generation	Genome recovered			Genome expected
		Minimum	Maximum	Average	
BAJ#1*2/TA5094	BC_2F_2	66.00%	74.60%	71.20%	87.50%
KACHU#1*2/TA5094	BC_2F_2	63.40%	74.20%	69.80%	87.50%
REEDLING#1*2/TA5094	BC_2F_2	66.2%	73.40%	70.50%	87.50%

https://doi.org/10.1371/journal.pone.0292724.t003

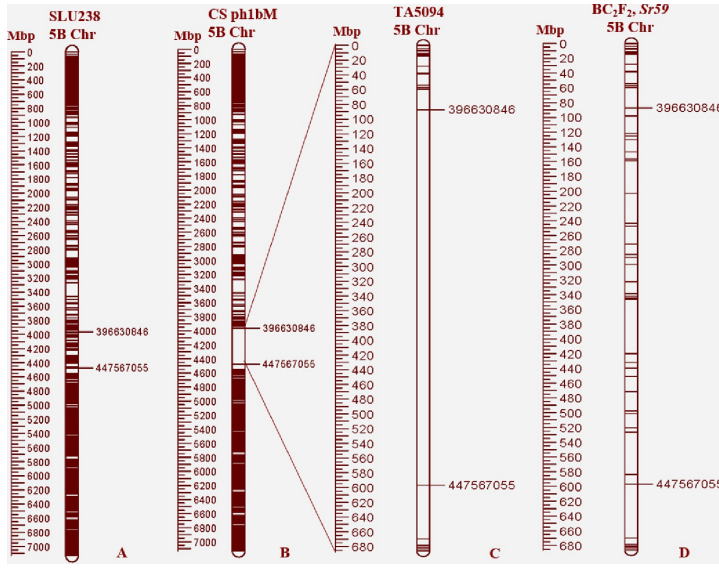


Fig 5. Status of the *Ph1* allele (*Ph1* deletion), determined using GBS reads aligned to the IWGSC wheat reference sequence v1.0. A) Line SLU238 depicting presence of the *Ph1* allele (SNPs) in chromosome 5B; B) deletion breakpoint in CSph1bM spans 50,936,209 bp, ranging from 396,630,846 bp to 447,567,055 bp; C) presence of the *Ph1b* deletion in TA5094 as revealed by GBS data; D) BC_2F_2 populations demonstrating stable presence of the *Ph1* allele.

https://doi.org/10.1371/journal.pone.0292724.g005

based on the stem rust seedling response and marker-assisted backcrossing selection. Previous studies have shown that two backcrossing generations can recover approximately 87.5% of the recurrent parent genome [41]. Use of a large backcrossing population is common practice to introgress resistance genes from an alien genome into an elite background [25]. Aside from marker-assisted backcrossing, we used GBS genotyping to select plants with the greatest amount of the recurrent parent genome. Average recurrent parent genome recovery of 71.2%, 69.8%, and 70.5% was observed in the BC_2F_2 populations (Table 3). This fairly low recovery might be because of a less-than-optimal representation of 2DL in the GBS dataset, due to the fact that we started off with a 2DS.2RL translocation in the donor parent (Table 3). In GBS genotyping, the A and B genomes are reported to have the highest number of SNPs, while the D genome has the lowest number [42]. Evolutionary history and gene flow may be the reason for the poor D genome representation [43]. In previous studies, GBS has been found to be an inexpensive and robust approach for genotyping crop genomes, as it enables discovery of a high number of genome-wide markers, often SNPs [38]. Several studies have demonstrated that GBS detects small introgressions in wheat and barley [44, 45]. In the present, we study aligned GBS reads and located the physical positions of SNPs in wheat and 2R rye chromosomes based on the reference genome RefSeq v1.0 and the International Rye Genome Sequencing Consortium. In this alignment, GBS demonstrated the physical positions of 40,584 SNPs across the wheat genome and 15,116 SNPs for the 2R chromosome. Through this high-throughput genotyping procedure, it was possible to detect both translocation lines and non-translocation lines in the BC_2F_2 populations.

We also used PCA to visualize the grouping of lines based on their genetic relationship, i.e., based on the differences in 2RL segments transferred to the BC_2F_2 progeny (Fig 3). Basically, the susceptible BC_2F_2 plants and parental lines without chromosome 2R (i.e., CSph1bM, BAJ#1, KACHU#1, REEDLING#1) clustered with a positive PCA1 (Fig 3). Likewise, 24 BC_2F_2 resistant plants grouped closely to the lines TA5094 and SLU238, indicating presence of the whole 2RL chromosome segment (Fig 3). Additionally, a total of 28 BC_2F_2 resistant plants clustered differently, indicating that these lines most likely resemble each other as regards their genomic composition, for both wheat and rye genome segments (Fig 3). Some factors, such as chromosomal segment rearrangements, segmental duplications, and differences in recombination frequencies caused by genomic structural variations, may explain the marker orders observed in this short segment. The introgression of rye chromosomes into wheat genomes can result in structural changes and rearrangements, as the heterochromatin DNA of rye chromosomes can interfere with chromosome synapsis [2, 46]. There may have been chromosome rearrangements in the BC_2F_2 population that resulted in a shorter 2RL segment than in the other 24 BC_2F_2 lines. This study showed that the *Sr59* resistance gene in the 2RL chromosome segment is stable for normal transmission through the male gamete, preventing segregation distortion in cultivar development. No segregation distortion was observed in any of the three populations evaluated, either for race TTTTF or for race TTKSK. However, previous studies have shown that segregation distortion is a common feature when alien chromosomes are introgressed in the wheat genome [25]. Development of chromosome-specific SNP markers covering target chromosomes and facilitating homologous recombination on chromosomes containing resistance genes can assist in tracking rye resistance genes within wheat more effectively by minimizing chromosome transfer and reducing the likelihood of linkage to undesirable alleles. Line SLU238 wheat-rye disomic substitution carrying 2R (2D) chromosome exhibits effective resistance to several virulent races of stem rust and has been used to develop 2DS.2RL wheat-rye translocation lines [16]. Substitution lines serve as bridging materials in the development of wheat-alien translocation lines [25]. By incorporating distinct alien chromosome segments with desired traits through chromosome translocations, linkage drag can be reduced [25, 28].

For the three KASP markers (c20194_115, c25837_157, and c21825_230), BLASTN was used and their positions were mapped at 914,812,229 bp to 943,109,279 (28 Mb), as physically mapped in a previous study [16]. Based on the GBS reads, we observed a deletion spanning from 396,630,846 Mb to 447,567,055 Mb on chromosome 5B in TA5094 and two BC_2F_2 plants (31-Kachu and 34-Kachu), indicating deletion of the CSph1bM allele. A deletion on the 5B chromosome was not detected in the other BC_2F_2 plants, all carrying the *Ph1* allele, preventing true homology in pairing. New *ph1b* deletion-specific markers based on 90K SNPs have been developed to accurately identify the *ph1b* deletion region [47]. Successful cross-over between the wheat chromosome and its wild relatives is challenging, which is why CSph1bM mutants are recommended for inducing meiotic homoeologous recombination [23]. Several resistance genes, such as *Sr32*, *Sr47*, *Sr39*, *Sr59*, and *Yr83* have been successfully transferred using CSph1bM mutants [16, 24, 25, 29]. This approach provides an effective means of introducing beneficial traits from wild relatives for wheat improvement. The *Sr11* and *Sr38* resistance genes were postulated to be present in three recurrent parents from CIMMYT (Table 1). The *Sr11* and *Sr38* resistance genes have previously been reported to be widely prevalent in wheat cultivars worldwide [48, 49], and the recurrent parents may carry both genes. Due to the limited agronomic performance of the CSph1bM mutant and the elimination of the *ph1b* allele in line TA5094, it is necessary to transfer *Sr59* into adapted cultivars. Here, we successfully transferred *Sr59* from TA5094 to the genetic background of the three cultivars BAJ#1, KACHU#1, and REEDLING#1. The progeny showed proven resistance to TTTTF (USA), TKTTF

(Ethiopia), QTHJC, TPMKC, RKQQC, RCRSC, TTKST, TTKSK, TTTSK, TRTTF, and LTBDC (Australian *Pgt* race 98–1,2,3,5,6). Following backcrossing, morphological and agronomic characteristics with the greatest similarity to the recurrent parents were considered. Other resistance genes such as *SrTA1662*, *Yr15*, and *Sr39* have been transferred in previous work using backcrossing to recover the recurrent parent phenotype [25, 50, 51]. In conclusion, *Sr59* offers broad-spectrum resistance to stem rust races, making it a valuable gene for wheat improvement, while the reliability of KASP markers for *Sr59* makes them suitable for marker-assisted selection of stem rust resistance in wheat breeding. These findings can facilitate further production of stem rust-resistant wheat cultivars, developed with *Sr59* resistance in their elite background, which can act as additional assets for improving wheat yields and preventing stem rust losses.

Acknowledgments

We thank the University of Minnesota Genomics Center for its genotyping services and the University of Minnesota Supercomputing Institute for providing computational resources. We also acknowledge Lantmännen for hosting the field trial at their Research Station in Svalöv.

Author Contributions

Conceptualization: Matthew N. Rouse, Mahbubjon Rahmatov.

Data curation: Prabin Bajgain, Eva Johansson, Mahbubjon Rahmatov.

Formal analysis: Prabin Bajgain, Mahbubjon Rahmatov.

Funding acquisition: Eva Johansson, Mahbubjon Rahmatov.

Investigation: Mahboobeh Yazdani.

Methodology: Mahboobeh Yazdani, Mehran Patpour.

Resources: Mahboobeh Yazdani, Brian J. Steffenson, Mehran Patpour.

Supervision: Matthew N. Rouse, Brian J. Steffenson, Mahbubjon Rahmatov.

Validation: Matthew N. Rouse, Brian J. Steffenson, Prabin Bajgain, Eva Johansson, Mahbubjon Rahmatov.

Visualization: Matthew N. Rouse, Eva Johansson.

Writing – original draft: Mahbubjon Rahmatov.

Writing – review & editing: Mahboobeh Yazdani, Matthew N. Rouse, Brian J. Steffenson, Prabin Bajgain, Mehran Patpour, Eva Johansson, Mahbubjon Rahmatov.

References

1. Shewry PR, Hey SJ. The contribution of wheat to human diet and health. Food Energy Secur. 2015; 4 (3):178–202. https://doi.org/10.1002/fes3.64 WOS:000367088200002. PMID: 27610232
2. Appels R, Eversole K, Stein N, Feuillet C, Keller B, Rogers J, et al. Shifting the limits in wheat research and breeding using a fully annotated reference genome. Science. 2018; 361(6403):eaar7191. https://doi.org/10.1126/science.aar7191 PMID: 30115783
3. Cassman KG, Grassini P. A global perspective on sustainable intensification research. Nat Sustain. 2020; 3(4):262–8. https://doi.org/10.1038/s41893-020-0507-8 WOS:000526392900005.
4. Singh RP, Hodson DP, Jin Y, Lagudah ES, Ayliffe MA, Bhavani S, et al. Emergence and Spread of New Races of Wheat Stem Rust Fungus: Continued Threat to Food Security and Prospects of Genetic

Control. Phytopathology. 2015; 105(7):872–84. https://doi.org/10.1094/PHYTO-01-15-0030-FI WOS:000360927100004. PMID: 26120730

5. Oliver RP. A reassessment of the risk of rust fungi developing resistance to fungicides. Pest Manag Sci. 2014; 70(11):1641–5. https://doi.org/10.1002/ps.3767 WOS:000342850800001. PMID: 24616024
6. Patpour M, Hovmoller MS, Justesen AF, Newcomb M, Olivera P, Jin Y, et al. Emergence of Virulence to *SrTmp* in the Ug99 Race Group of Wheat Stem Rust, *Puccinia graminis* f. sp *tritici*, in Africa. Plant Dis. 2016; 100(2):522–. https://doi.org/10.1094/Pdis-06-15-0668-Pdn WOS:000370305300054.
7. Pretorius ZA, Singh RP, Wagoire WW, Payne TS. Detection of Virulence to Wheat Stem Rust Resistance Gene *Sr31* in *Puccinia graminis*. f. sp. *tritici* in Uganda. Plant Dis. 2000; 84(2):203. https://doi.org/10.1094/PDIS.2000.84.2.203B PMID: 30841334.
8. Olivera P, Newcomb M, Szabo LJ, Rouse M, Johnson J, Gale S, et al. Phenotypic and Genotypic Characterization of Race TKTTF of *Puccinia graminis* f. sp *tritici* that Caused a Wheat Stem Rust Epidemic in Southern Ethiopia in 2013–14. Phytopathology. 2015; 105(7):917–28. https://doi.org/10.1094/Phyto-11-14-0302-Fi WOS:000360927100008. PMID: 25775107
9. Olivera PD, Sikharulidze Z, Dumbadze R, Szabo LJ, Newcomb M, Natsarishvili K, et al. Presence of a Sexual Population of *Puccinia graminis* f. sp. *tritici* in Georgia Provides a Hotspot for Genotypic and Phenotypic Diversity. Phytopathology. 2019; 109(12):2152–60. https://doi.org/10.1094/Phyto-06-19-0186-R WOS:000502011800017. PMID: 31339468
10. Hovmøller MS, Patpour M., Rodriguez-Algaba J., Thach T., Justesen A.F., and Hansen J.G. GRRC report of yellow and stem rust genotyping and race analyses 2020. Aarhus University, Flakkebjerg, DK-4200 Slagelse, Denmark. https://agro.au.dk/fileadmin/www.grcc.au.dk/International_Services/Pathotype_YR_results/GRRC_annual_report_2020.pdf, 2021.
11. Lewis CM, Persoons A, Bebber DP, Kigathi RN, Maintz J, Findlay K, et al. Potential for re-emergence of wheat stem rust in the United Kingdom. Commun Biol. 2018; 1. ARTN 13 https://doi.org/10.1038/s42003-018-0013-y WOS:000461126500013. PMID: 30271900
12. Firpo PDO, Newcomb M, Flath K, Sommerfeldt-Impe N, Szabo LJ, Carter M, et al. Characterization of *Puccinia graminis* f. sp *tritici* isolates derived from an unusual wheat stem rust outbreak in Germany in 2013. Plant Pathol. 2017; 66(8):1258–66. https://doi.org/10.1111/ppa.12674 WOS:000410746600005.
13. Bhattacharya S. Deadly new wheat disease threatens Europe's crops. Nature. 2017; 542(7640):145–+. https://doi.org/10.1038/nature.2017.21424 WOS:000393737500005. PMID: 28179687
14. Flath K, Miedaner T, Olivera PD, Rouse MN, Jin Y. Genes for wheat stem rust resistance postulated in German cultivars and their efficacy in seedling and adult-plant field tests. Plant Breeding. 2018; 137(3):301–12. https://doi.org/10.1111/pbr.12591 WOS:000434231800007.
15. Johansson E, Henriksson T, Prieto-Linde ML, Andersson S, Ashraf R, Rahmatov M. Diverse Wheat-Alien Introgression Lines as a Basis for Durable Resistance and Quality Characteristics in Bread Wheat. Frontiers in Plant Science. 2020; 11. ARTN 1067 https://doi.org/10.3389/fpls.2020.01067 WOS:000560098900001. PMID: 32765555
16. Rahmatov M, Rouse MN, Nirmala J, Danilova T, Friebe B, Steffenson BJ, et al. A new 2DS.2RL Robertsonian translocation transfers stem rust resistance gene *Sr59* into wheat. Theor Appl Genet. 2016; 129(7):1383–92. Epub 2016/03/31. https://doi.org/10.1007/s00122-016-2710-6 PMID: 27025509.
17. The TT, Gupta RB, Dyck PL, Appels R, Hohmann U, Mcintosh RA. Characterization of Stem Rust Resistant Derivatives of Wheat Cultivar Amigo. Euphytica. 1991; 58(3):245–52. https://doi.org/10.1007/Bf00025256 WOS:A1991HZ63400007.
18. Marais GF, Marais AS. The Derivation of Compensating Translocations Involving Homoeologous Group-3 Chromosomes of Wheat and Rye. Euphytica. 1994; 79(1–2):75–80. https://doi.org/10.1007/Bf00023578 WOS:A1994PZ62500010.
19. Mago R, Zhang P, Vautrin S, Simkova H, Bansal U, Luo MC, et al. The wheat *Sr50* gene reveals rich diversity at a cereal disease resistance locus. Nat Plants. 2015; 1(12). Artn 15186 https://doi.org/10.1038/Nplants.2015.186 WOS:000365704700005. PMID: 27251721
20. Riley R, Chapman V. Genetic Control of the Cytologically Diploid Behaviour of Hexaploid Wheat. Nature. 1958; 182(4637):713–5. https://doi.org/10.1038/182713a0 WOS:A1958ZQ50000023.
21. Mellosampayo T. Genetic Regulation of Meiotic Chromosome Pairing by Chromosome-3d of *Triticum-Aestivum*. Nature-New Biol. 1971; 230(9):22–+. WOS:A1971I753500015.
22. Giorgi B, Barbera F. Increase of homoeologous pairing in hybrids between a ph mutant of *T. turgidum* L. var. *durum* and two tetraploid species of Aegilops: *Aegilops kotschyi* and *Ae. cylindrica*. Cereal Res Commun. 1981; 9(205–2).
23. Sears ER. An induced mutant with homoeologous pairing in wheat. Can J Genet Cytol. 1977; 19:585–93.

24. Mago R, Verlin D, Zhang P, Bansal U, Bariana H, Jin Y, et al. Development of wheat-*Aegilops speltoides* recombinants and simple PCR-based markers for *Sr32* and a new stem rust resistance gene on the 2S#1 chromosome. Theor Appl Genet. 2013; 126(12):2943–55. Epub 2013/08/31. https://doi.org/10.1007/s00122-013-2184-8 PMID: 23989672.

25. Niu Z, Klindworth DL, Friesen TL, Chao S, Jin Y, Cai X, et al. Targeted introgression of a wheat stem rust resistance gene by DNA marker-assisted chromosome engineering. Genetics. 2011; 187(4):1011–21. Epub 2011/01/19. https://doi.org/10.1534/genetics.110.123588 PMID: 21242535; PubMed Central PMCID: PMC3070511.

26. Klindworth DL, Niu Z, Chao S, Friesen TL, Jin Y, Faris JD, et al. Introgression and characterization of a goatgrass gene for a high level of resistance to Ug99 stem rust in tetraploid wheat. G3 (Bethesda). 2012; 2(6):665–73. Epub 2012/06/13. https://doi.org/10.1534/g3.112.002386 PMID: 22690376; PubMed Central PMCID: PMC3362296.

27. Liu W, Rouse M, Friebe B, Jin Y, Gill B, Pumphrey MO. Discovery and molecular mapping of a new gene conferring resistance to stem rust, *Sr53*, derived from *Aegilops geniculata* and characterization of spontaneous translocation stocks with reduced alien chromatin. Chromosome Res. 2011; 19(5):669–82. Epub 2011/07/06. https://doi.org/10.1007/s10577-011-9226-3 PMID: 21728140.

28. Niu Z, Klindworth DL, Yu G, T LF, Chao S, Jin Y, et al. Development and characterization of wheat lines carrying stem rust resistance gene *Sr43* derived from *Thinopyrum ponticum*. Theor Appl Genet. 2014; 127(4):969–80. Epub 2014/02/08. https://doi.org/10.1007/s00122-014-2272-4 PMID: 24504553.

29. Li J, Dundas I, Dong C, Li G, Trethowan R, Yang Z, et al. Identification and characterization of a new stripe rust resistance gene *Yr83* on rye chromosome 6R in wheat. Theor Appl Genet. 2020; 133 (4):1095–107. Epub 2020/01/20. https://doi.org/10.1007/s00122-020-03534-y PMID: 31955232.

30. Lukaszewski AJ. Manipulation of the 1RS.1BL translocation in wheat by induced homoeologous recombination. Crop Sci. 2000; 40(1):216–25. https://doi.org/10.2135/cropsci2000.401216x WOS:000085505300035.

31. Merker A. The Rye Genome in Wheat Breeding. Hereditas. 1984; 100(2):183–91. https://doi.org/10.1111/j.1601-5223.1984.tb00118.x WOS:A1984SV71000040.

32. Forsstrom PO, Merker A. Sources of wheat powdery mildew resistance from wheat-rye and wheat-Leymus hybrids. Hereditas. 2001; 134(2):115–9. https://doi.org/10.1111/j.1601-5223.2001.00115.x WOS:000172203900003. PMID: 11732846

33. Rahmatov M, Rouse MN, Steffenson BJ, Andersson SC, Wanyera R, Pretorius ZA, et al. Sources of Stem Rust Resistance in Wheat-Alien Introgression Lines. Plant Dis. 2016; 100(6):1101–9. https://doi.org/10.1094/PDIS-12-15-1448-RE WOS:000375634200009. PMID: 30682285

34. Rouse MN, Wanyera R, Njau P, Jin Y. Sources of Resistance to Stem Rust Race Ug99 in Spring Wheat Germplasm. Plant Dis. 2011; 95(6):762–6. https://doi.org/10.1094/PDIS-12-10-0940 WOS:000290879700020. PMID: 30731910

35. Stakman EC, Stewart D.M., and Loegering W.Q. Identification of physiologic races of *Puccinia graminis* var. *tritici*. United States Department of Agriculture, Agricultural Research Service, Beltsville, MD E-617. 1962.

36. Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, et al. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PloS one. 2011; 6(5):e19379. https://doi.org/10.1371/journal.pone.0019379 PMID: 21573248

37. Ashraf R, Johansson E, Vallenback P, Steffenson BJ, Bajgain P, Rahmatov M. Identification of a Small Translocation from 6R Possessing Stripe Rust Resistance to Wheat. Plant Dis. 2023; 107(3):720–9. https://doi.org/10.1094/PDIS-07-22-1666-RE WOS:000951786700001. PMID: 35900348

38. Poland JA, Brown PJ, Sorrells ME, Jannink J-L. Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. PloS one. 2012; 7(2): e32253. https://doi.org/10.1371/journal.pone.0032253 PMID: 22389690

39. Li H, Durbin R. Fast and accurate short read alignment with Burrows–Wheeler transform. bioinformatics. 2009; 25(14):1754–60. https://doi.org/10.1093/bioinformatics/btp324 PMID: 19451168

40. Li H. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. Bioinformatics. 2011; 27(21):2987–93. https://doi.org/10.1093/bioinformatics/btr509 PMID: 21903627

41. Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES. TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics. 2007; 23(19):2633–5. https://doi.org/10.1093/bioinformatics/btm308 PMID: 17586829

42. Bajgain P, Rouse MN, Anderson JA. Comparing Genotyping-by-Sequencing and Single Nucleotide Polymorphism Chip Genotyping for Quantitative Trait Loci Mapping in Wheat. Crop Sci. 2016; 56 (1):232–48. https://doi.org/10.2135/cropsci2015.06.0389 WOS:000368266100024.

43. Przewieslik-Allen AM, Wilkinson PA, Burridge AJ, Winfield MO, Dai XY, Beaumont M, et al. The role of gene flow and chromosomal instability in shaping the bread wheat genome. Nat Plants. 2021;7(2). https://doi.org/10.1038/s41477-020-00845-2 WOS:000613614100001. PMID: 33526912

44. Tiwari VK, Wang S, Sehgal S, Vrána J, Friebe B, Kubaláková M, et al. SNP discovery for mapping alien introgressions in wheat. BMC genomics. 2014; 15(1):1–11. https://doi.org/10.1186/1471-2164-15-273 PMID: 24716476

45. Keilwagen J, Lehnert H, Berner T, Beier S, Scholz U, Himmelbach A, et al. Detecting Large Chromosomal Modifications Using Short Read Data From Genotyping-by-Sequencing. Frontiers in Plant Science. 2019; 10(1133). https://doi.org/10.3389/fpls.2019.01133 PMID: 31608087

46. Thomas JB, Kaltsikes PJ. A possible effect of heterochromatin on chromosome pairing. Proc Natl Acad Sci U S A. 1974; 71(7):2787–90. https://doi.org/10.1073/pnas.71.7.2787 PMID: 4527611; PubMed Central PMCID: PMC388556.

47. Gyawali Y, Zhang W, Chao S, Xu S, Cai X. Delimitation of wheat ph1b deletion and development of ph1b-specific DNA markers. Theoretical and Applied Genetics. 2019; 132(1):195–204. https://doi.org/10.1007/s00122-018-3207-2 PMID: 30343385

48. Bajgain P, Rouse MN, Bulli P, Bhavani S, Gordon T, Wanyera R, et al. Association mapping of North American spring wheat breeding germplasm reveals loci conferring resistance to Ug99 and other African stem rust races. BMC Plant Biol. 2015; 15:249. Epub 20151014. https://doi.org/10.1186/s12870-015-0628-9 PMID: 26467989; PubMed Central PMCID: PMC4606553.

49. Helguera M, Khan IA, Kolmer J, Lijavetzky D, Zhong-qi L, Dubcovsky J. PCR assays for the *Lr37-Yr17-Sr38* cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. Crop Sci. 2003; 43(5):1839–47. https://doi.org/10.2135/cropsci2003.1839 WOS:000185174300036.

50. Randhawa HS, Mutti JS, Kidwell K, Morris CF, Chen X, Gill KS. Rapid and targeted introgression of genes into popular wheat cultivars using marker-assisted background selection. PloS ONE. 2009; 4(6): e5752. https://doi.org/10.1371/journal.pone.0005752 PMID: 19484121

51. Olson EL, Rouse MN, Pumphrey MO, Bowden RL, Gill BS, Poland JA. Simultaneous transfer, introgression, and genomic localization of genes for resistance to stem rust race TTKSK (Ug99) from *Aegilops tauschii* to wheat. Theoretical and Applied Genetics. 2013; 126(5):1179–88.

V

Disease Note

Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Race TTRTF of the Wheat Stem Rust Pathogen *Puccinia graminis* f. sp. *tritici* in Sweden

Mehran Patpour,[1,†] Mahbubjon Rahmatov,[2] Mahboobeh Yazdani,[2] and Annemarie Fejer Justesen[1]

[1] Department of Agroecology, Aarhus University, Slagelse 4200, Denmark
[2] Swedish University of Agricultural Sciences, Department of Plant Breeding, Alnarp, Sweden

Funding: The work is funded by the European Union's Horizon 2020 Program research and innovation program under grant agreement no. 773311. Plant Dis. 107:1945, 2023; published online as https://doi.org/10.1094/PDIS-06-22-1398-PDN. Accepted for publication 4 November 2022.

Wheat (*Triticum aestivum* L.) stem rust caused by *Puccinia graminis* f. sp. *tritici* (Pgt) was generally insignificant in Europe from the 1960s until 2016, when a new race (TTRTF) caused damage on huge areas of durum wheat and bread wheat in Sicily (Bhattacharya 2017). During the following 5 years, TTRTF was detected in eight additional countries in south and central Europe (Patpour et al. 2022). In July 2021, seven wheat stem rust samples collected from spring wheat and one from barley in Svalov (55°54′10.8″N, 13°6′54″E) and Alnarp (55°39′39.6″N, 13°4′40.8″E), Sweden. Both cereal fields had a total disease incidence of 50% or higher. The samples were sent to the Global Rust Reference Center (Denmark). Urediniospores of each sample were recovered on two susceptible cultivars, Line E and Morocco, which were used as susceptible controls in all experiments. Single pustular isolates were extracted, and race typing was generally repeated two to three times based on the method of Patpour et al. (2022) using 20 North American stem rust differential lines. Seedling infection types (IT) were scored on the first and second leaf 17 days post-inoculation using a 0 to 4 scale (McIntosh et al. 1995; Stakman et al. 1962). Isolates conferring "low" ITs (i.e., 0, 0, 1, 1+, 2, and 2+), or combinations thereof, were considered "avirulent" (incompatible), whereas ITs of 3–, 3, 3+, and 4 were considered "high" (i.e., compatible, "virulent"). Race nomenclature was based on a modified letter code proposed by Jin et al. (2008). We conducted DNA extraction and molecular genotyping using 17 simple sequence repeat (SSR) primer pairs derived from Stoxen (2012) and applied at large scale by Patpour et al. (2022). Based on the results from pathotyping and genotyping, two samples from wheat showed Pgt race TKTTF (clade IV-B), three samples from wheat showed TKKTF (clade IV-F), and three samples from wheat and barley were identified as TTRTF (clade III-B). This is the first report of race TTRTF in northern Europe, specifically Sweden, which significantly extends the known distribution of this race. The TTRTF race is a serious threat to wheat productivity, and evaluation of resistance of commercial European wheat varieties to the TTRTF race confirmed that 70% of the cultivars were susceptible (Patpour et al. 2022). Therefore, if the conditions are suitable for the establishment and development of stem rust, the disease can cause significant damage to the wheat crop in these countries. Susceptibility of European wheat varieties stress an urgent need to initiate new breeding efforts to identify effective sources of resistance to wheat stem rust in breeding programs.

References:

Bhattacharya, S. 2017. Nature 542:145.
Jin, Y., et al. 2008. Plant Dis. 92:923.
McIntosh, R. A., et al. 1995. Page 1333 in: Proceedings of the 8th International Wheat Genetics Symposium, Beijing, 20–25 July 1993. China Agriculture Sciencetech, Beijing, China.
Patpour, M., et al. 2022. Front. Plant Sci. 13:882440.
Stakman, E. C., et al. 1962. ARS E-617. USDA, Washington, DC.
Stoxen, S. 2012. Population structure of *Puccinia graminis* f. sp. *tritici* in the United States. Master's thesis. University of Minnesota, Minneapolis, MN.

The author(s) declare no conflict of interest.

Keywords: black rust, clade III-B, race TTRTF, Sicily race, wheat commercial varieties

[†]Indicates the corresponding author.
M. Patpour; Mehran.patpour@agro.au.dk

Acta Universitatis agriculturae Sueciae
Doctoral Thesis No. 2025:21

This thesis identified wheat-rye introgression lines with novel stem rust resistance genes and highlighted rye's potential as a valuable genetic resource for wheat improvement. Using GBS data and KASP markers, we precisely integrated *Sr59* into adapted wheat cultivars, enabling efficient tracking of the gene in breeding programs. A second resistance gene, *SrSLU*, was discovered and characterized using NLR-based approaches. In addition, we successfully pyramided *Sr59* and *YrSLU* into a single wheat line providing broader-spectrum and more durable resistance against multiple pathogens. Finally, the integration of advanced technologies such as speed breeding accelerated the development of resilient wheat varieties.

Mahboobeh Yazdani received her doctoral education at the Department of Plant Breeding, Plant Production Quality Division, Swedish University of Agricultural Sciences (SLU), Alnarp. She received her Master of Science from Chamran University, Ahwaz, Iran and her Bachelor of Science degree from Zanjan University, Zanjan, Iran.

Acta Universitatis agriculturae Sueciae presents doctoral theses from the Swedish University of Agricultural Sciences (SLU).

SLU generates knowledge for the sustainable use of biological natural resources. Research, education, extension, as well as environmental monitoring and assessment are used to achieve this goal.

ISSN 1652-6880
ISBN (print version) 978-91-8046-456-7
ISBN (electronic version) 978-91-8046-506-9