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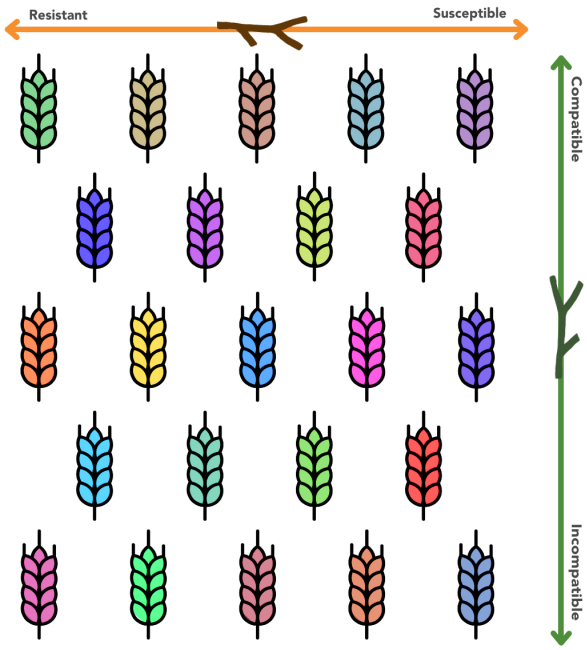
Plant genotype-dependent biocontrol of wheat diseases

Sidhant Chaudhary



Plant genotype-dependent biocontrol of wheat diseases

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Abstract

Sustainable crop production requires the reduction of chemical pesticides, and the use of beneficial microorganisms such as biological control agents (BCAs) is recommended as a sustainable alternative for disease management. However, the interaction between a host plant and a BCA can influence its biocontrol efficacy, which is currently not well understood. To better understand the role of plant genetic variation in influencing biocontrol efficacy, in this thesis, a winter wheat germplasm of approximately 200 genotypes was explored under controlled conditions for biocontrol efficacy of the BCA *Clonostachys rosea* during interactions with pathogens – *Zymoseptoria tritici* causing septoria tritici blotch (STB) and *Fusarium graminearum* causing fusarium foot rot (FFR). In both pathosystems, significant phenotypic variation was observed for disease susceptibility and *C. rosea* biocontrol efficacy. However, *C. rosea* efficacy varied in managing STB (positive effect: 7 genotypes, negative effect: 11 genotypes) and FFR (positive effect: 180 genotypes), suggesting that biocontrol efficacy can be specific not only to plant genotype but also to pathogen and/or plant tissue. Moreover, disease susceptibility and biocontrol efficacy were positively correlated, but distinct marker-trait associations were identified using genome-wide association studies (GWAS). The independent inheritance of disease susceptibility and *C. rosea* biocontrol efficacy offers the potential for simultaneous selection of these traits in future breeding programs. A few plant defence-related genes were co-localised in GWAS-identified regions for *C. rosea* biocontrol efficacy. To gain a deeper understanding, two genotypes with varying *C. rosea* biocontrol efficacy towards STB were used in a transcriptomic study, where differences in gene expression at early hours of inoculation were investigated in direct interaction with *Z. tritici*, *C. rosea* and their co-inoculation. The results showed a temporal difference between the genotypes, where the genotype with higher biological control efficacy showed a delayed but strong induction of the immune system by *C. rosea*. Overall, this thesis contributes towards advancing the knowledge of plants–BCA interaction in affecting biocontrol efficacy, which can aid future disease management and plant breeding efforts.

Keywords: Biological control, *Clonostachys rosea*, *Fusarium graminearum*, fusarium foot rot, genome-wide association study, septoria tritici blotch, transcriptomics, wheat, *Zymoseptoria tritici*

Växtgenotypens betydelse för biologisk bekämpning av sjukdomar på vete

Sammanfattning

Hållbar växtproduktion kräver en minskad användning av kemiska växtskyddsmedel, och användningen av nyttiga mikroorganismer för biologisk bekämpning (BCA) kan utgöra ett hållbart alternativ för att uppnå ett effektivt växtskydd. Växtens genetik kan dock påverka effektiviteten av den biologiska bekämpningen, vilket dock för närvarande inte är väl undersökt. För att bättre förstå den roll som den genetiska variationen hos växten spelar för att påverka den biologiska bekämpningens effektivitet användes i denna avhandling en samling av ca. 200 genotyper av höstvete. Dessa växter inokulerades under kontrollerade förhållanden med BCA svampen *Clonostachys rosea* för att testa effektiviteten av bekämpningen av patogenerna *Zymoseptoria tritici* som orsakar svartpricksjuka och *Fusarium graminearum* som orsakar stråbasröta. I båda fallen observerades betydande fenotypisk variation för sjukdomsmottaglighet hos växterna, men också för effektiviteten av den biologiska bekämpningen. Effektiviteten av bekämpningen av svartpricksjuka varierade stort (positiv effekt: 7 genotyper, negativ effekt: 11 genotyper) och men också för stråbasröta (positiv effekt: 180 genotyper), vilket tyder på att effekten av biologisk bekämpning kan vara specifik inte bara för växtgenotypen utan också för patogenen och/eller växtvävnaden. Dessutom var sjukdomsmottaglighet och effektivitet av biologisk bekämpning positivt korrelerade, men distinkta kopplingar mellan genetiska markörer och egenskaper identifierades med hjälp av storskaliga associationsstudier (GWAS). Den oberoende nedärvningen av sjukdomsmottaglighet och effektiviteten av biologisk bekämpning möjliggör för oberoende urval av dessa två egenskaper i framtida förädlingsprogram. Ett antal gener relaterade till växtens immunförsvar var lokaliserade i GWAS-identifierade regioner i arvsmassan hos vete. För att få en djupare förståelse för den mekanistiska bakgrunden till skillnader i effektivitet av den biologiska bekämpningen användes två genotyper med olika respons mot *C. rosea* vid bekämpningen av svartpricksjuka i en transkriptomstudie. Här undersöktes skillnader i geners aktivitet över tid efter inokulering med *C. rosea*, *Z. tritici*, eller båda svamparna samtidigt. Resultatet visade på en tidsmässig skillnad mellan genotyperna, där effektiv biologisk bekämpning var kopplad till en långsam men kraftig inducering av immunförsvaret. Sammantaget bidrar den här avhandlingen till att öka kunskapen om hur växters genetiska variation påverkar deras interaktion med nyttiga mikroorganismer och effektiviteten av biologisk bekämpning av sjukdomar, vilket kan bidra till ett effektivt och miljövänligt växtskydd i framtidens växtproduktion.

Nyckelord: Biologisk bekämpning, *Clonostachys rosea*, *Fusarium graminearum*, stråbasröta, storskalig associationsanalys, svartpricksjuka, transkriptomanalys, vete, *Zymoseptoria tritici*

Dedication

To my late father and my mother

Contents

List of publications

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

I. Chaudhary, S., Zakieh, M., Dubey, M., Jensen, D. F., Grenville-Briggs, L., Chawade, A., & Karlsson, M. (2024). Plant genotype-specific modulation of *Clonostachys rosea*-mediated biocontrol of septoria tritici blotch disease on wheat. (Submitted)

II. Chaudhary, S., Ricardo, R. M. N., Dubey, M., Jensen, D. F., Grenville-Briggs, L., & Karlsson, M. (2024). Genotypic variation in winter wheat for fusarium foot rot and its biocontrol using *Clonostachys rosea*. G3 Genes|Genomes|Genetics. https://doi.org/10.1093/g3journal/jkae240

III. Chaudhary, S., Piombo, E., Dubey, M., Jensen, D. F., Grenville-Briggs, L., & Karlsson, M. Transcriptomic analysis of two wheat genotypes in the presence of the pathogen *Zymoseptoria tritici* and the biocontrol agent *Clonostachys rosea*. (Manuscript)

The contribution of Sidhant Chaudhary to the papers included in this thesis was as follows:

I. Contributed to the design of the study and bioassay data collection. Organised data and conducted all the data analysis. Interpreted results with support from co-authors. Wrote the first manuscript draft and made revisions with support from co-authors. Responsible for journal correspondence.

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III. Designed the study with support from co-authors. Conducted all the bioassay sampling, laboratory work and data analysis. Interpreted results with support from co-authors. Wrote the first manuscript draft and made revisions with support from co-authors.

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Some icons used in Figures 6 and 11 are adopted from Flaticon.com

Abbreviations

AUDPC	Area Under Disease Progress Curve
BCA	Biological Control Agent
BLUE	Best Linear Unbiased Estimator
Cr	*Clonostachys rosea*
DAMP	Damage Associated Molecular Pattern
DEG	Differentially Expressed Genes
ETI	Effector Triggered Immunity
FAO	Food and Agriculture Organization of the United Nations
FFR	Fusarium Foot Rot
Fg	*Fusarium graminearum*
FHB	Fusarium Head Blight
GO	Gene Ontology
GWAS	Genome Wide Association Study
IPM	Integrated Pest Management
MAMP	Microbe Associated Molecular Pattern
MAS	Marker Assisted Selection
NLR / NB-LRR	Nucleotide-Binding Leucine-Rich Repeat
PAMP	Pathogen Associated Molecular Pattern
PRR	Pattern Recognition Receptor
PTI	Pattern Triggered Immunity

QTL	Quantitative Trait Locus
RLK	Receptor like kinase
SNP	Single Nucleotide Polymorphism
STB	Septoria Tritici Blotch
Zt	*Zymoseptoria tritici*

1. Background and context

> "Food is the moral right of all who are born into this world."
>
> – Norman Borlaug (Nobel Peace Prize)

The Green Revolution of the mid-20th century played a pivotal role in ensuring global food security via a dramatic increase in agricultural yields. This progress is generally credited to simultaneous advances in plant breeding, improved agronomic practices, the use of inorganic fertilisers and chemical pesticides, and controlled irrigation systems. This led to exceptional socioeconomic improvements in the livelihoods of the masses, particularly in the developing world, by reducing poverty, malnutrition, and infant mortality while improving incomes and life expectancy. However, the intensive agricultural practices of the Green Revolution are also attributed to long-term impacts on soil, water and the environment. Large-scale monoculturing and excessive use of chemical pesticides have led to a decline in the biodiversity of crop plants and other flora and fauna, as well as increased soil and water pollution. Irrigation practices have accelerated the depletion of freshwater resources. To overcome these challenges, sustainable use of resources for crop production has been extensively advocated and adopted.

Towards this goal, this work explores the combination of sustainable practices of crop improvement via plant breeding as well as the use of biological control to manage diseases. As proof of concept, a winter wheat breeding population was utilised to explore the genetic basis of compatibility with the fungal biological control agent *Clonostachys rosea* against the fungal pathogens *Zymoseptoria tritici* and *Fusarium graminearum*.

1.1 Plant breeding for genetic crop improvement

Plant breeding over time

Plant breeding, albeit initially unknowingly, has been practised by humans since the dawn of agriculture, where primitive farmers identified and saved seeds with desirable traits, such as larger fruits, higher yields, or improved flavour, for the next growing season. From early domestication of edible plants by selecting seeds with preferred characteristics to current high-yielding varieties and the hybrids of modern agriculture, plant breeding has become a vastly organised and interdisciplinary science. Plant breeding is often described as the art and science of improving plants for human benefit. Acquaah (2007) described plant breeding as "… a deliberate effort by humans to nudge nature, with respect to the heredity of plants, to an advantage." Mass selection by early farmers led to the gradual domestication of many crop species and the development of diverse landraces – locally adapted varieties that serve as a crucial source of genetic diversity for current and future breeding efforts. Through generations of selection, humans shaped the genetic makeup of crops, adapting them to various environments and fulfilling diverse needs.

Plant breeding has undergone various milestones, as discussed in the literature (Schlegel 2018; Ramstein et al. 2019). The 20th century marked a turning point in plant breeding with the rediscovery of Mendel's laws of inheritance. This newfound understanding of genetics revolutionised plant breeding, moving it from an art based on observation to a science grounded in the principles of heredity, enabling breeders to combine desirable traits from different parent plants. Moreover, the traits for which selection and breeding were targeted became increasingly specific. Beyond selecting for larger fruits or higher yields, breeders began to focus on traits like disease resistance, nutritional content, and adaptation to specific environmental conditions. Simultaneously, the development of other powerful techniques further expanded the possibilities for crop improvement. The establishment of systematic agricultural experiments through well-structured experimental design and effective control of variation facilitated the segregation of genetic variation from environmental variation, leading to more efficient selection (Fisher 1935; Cochran & Cox 1950). Controlled hybridisation, which involves crossing genetically distinct individuals to create new trait combinations, and induced mutations, using radiation or chemicals to generate novel genetic variation, became cornerstones of breeding programs, further expanding the pool of exploitable genetic variation (Acquaah 2007). The gene-for-gene hypothesis provided a theoretical framework for understanding the genetic basis of plant-pathogen interactions, paving the

way for early resistance breeding strategies against virulent pathogens (Flor 1971). These advancements led to the development of high-yielding, disease-resistant, and input-responsive varieties, which played a crucial role in achieving dramatic crop yield increases for global food security.

Marker-assisted breeding

Advances in biotechnology and genomics further revolutionised plant breeding in the late 20th and early 21st centuries. As many essential breeding traits are quantitative (controlled by multiple genes with small and cumulative effects, i.e., polygenic or complex), inheritance and selection of these traits can be challenging and time-consuming when using only traditional breeding approaches. The development of molecular DNA markers enabled marker-assisted selection (MAS) for both qualitative (controlled by one to a few genes) and quantitative traits (genomic regions containing genes associated with quantitative traits are termed quantitative trait loci or QTLs), allowing breeders to select genes underlying desirable traits by tracking the inheritance of linked DNA markers (Collard & Mackill 2008). MAS accelerates breeding by utilising DNA markers to complement field trials, improving selection accuracy, enabling early selection and gene pyramiding, mitigating the linkage drag of undesirable genes, and facilitating selection for complex quantitative traits (Collard et al. 2005).

MAS utilises linkage disequilibrium (i.e. the non-random association of loci) between a gene of interest and closely linked markers to facilitate trait selection (Van Inghelandt et al. 2011). Two common approaches for dissecting the genetic basis of complex traits are linkage analysis and association mapping (Lander & Schork 1994). Linkage analysis, often implemented as QTL mapping, relies on segregating populations (typically derived from crosses between contrasting parents), a process that can be costly, time-consuming and labour-intensive (Collard & Mackill 2008). Until a couple of decades ago, linkage analysis was the predominant approach due to the limited markers available to plant breeders. Association mapping, on the other hand, leverages naturally occurring genetic variation within diverse populations (Lander & Schork 1994; Yu et al. 2008). Therefore, association mapping, particularly the genome-wide association studies (GWAS) approach, offers a powerful and efficient alternative for identifying QTLs and candidate genes underlying complex traits. Genome-wide association studies rely on the availability of dense sets of markers, typically single nucleotide polymorphism (SNPs) markers, and large sample sizes to detect subtle associations between genetic variation and phenotypic variation (Korte & Farlow 2013; Uffelmann et al. 2021). The rapid decrease in sequencing costs has enabled the generation of a vastly greater number of

SNPs and facilitated the high-throughput genotyping of numerous individuals (Varshney et al. 2009; Edwards & Batley 2010). Consequently, GWAS has become a widely adopted approach.

Utilising Transcriptomics

Complementing GWAS, transcriptomics has become an increasingly important approach, providing insights into gene expression patterns and revealing how plants respond to various stimuli, including pathogen attacks. The genome provides the complete genetic information of an organism, whereas the transcriptome reveals the dynamic patterns of gene expression using the transcript information. Transcriptome analysis provides valuable insights into gene structure and how gene expression changes in response to specific conditions. The primary method of transcriptome analysis is RNA-seq, where cDNA libraries prepared from RNA profiles are sequenced deeply (Wang et al. 2009). A comparative transcriptomics approach using RNA-seq allows for the identification of differentially expressed genes across different treatments, genotypes, or time points. Comparative transcriptomics using RNA-seq is a valuable tool in plant breeding, enabling the identification of candidate genes involved in traits of interest by comparing gene expression levels across different samples (Rossi 2023).

Whilst GWAS identifies regions of the genome that are statistically linked to the trait, transcriptomic analysis reveals the dynamic gene expression patterns. Therefore, integrating GWAS and transcriptomics provides a powerful approach to connect genetic variation with gene function, enabling the identification of key genes and pathways that contribute to desirable phenotypes and ultimately accelerate the development of improved crop varieties.

1.2 Plant–pathogen interactions

What is a pathogen and a diseased plant?

Plants constantly interact with their environment and are challenged by various abiotic and biotic stresses. Terms and definitions in this section are adopted after Agrios (2005) unless otherwise specified. A plant is considered healthy when its physiological functions operate without interference and diseased when a pathogenic organism or adverse environmental factor disrupts this state. Diseases caused by organisms are infectious, whereas abiotic factor-caused diseases are non-infectious. Plants engage in a wide range of biotic interactions, including those with beneficial and pathogenic microorganisms, insects, arachnids, nematodes, and viruses. Pathogenic microorganisms, or pathogens, are transmissible living agents that induce disease by disrupting plant cell metabolism through enzymes, toxins, and other secreted substances while utilising host resources. Depending on the feeding strategy employed, pathogens can be biotrophs, which extract nutrients from living tissue; hemibiotrophs, which initially establish on living tissue and eventually kill to feed on dead tissue; and necrotrophs, which kill host cells for nutrients. Parasitism, the act of an organism living on or in another to obtain food, is often associated with pathogenicity; however, not all parasitic interactions are pathogenic and disease-causing. Pathogenicity, in itself, is a complex phenomenon determined by a combination of factors, including pathogen and host genotypes, abiotic and other environmental stresses, and microbial interactions (Agrios 2005; Brader et al. 2017).

Plant resistance

From a plant's perspective, several defence strategies are employed to achieve resistance. Resistance to microorganisms in plants is the rule, while susceptibility is the exception (Yarwood, 1967). The ability of all genotypes of a plant species to confer resistance to all genotypes of a pathogen species is termed non-host resistance (Panstruga & Moscou 2020). Non-host resistance is still poorly understood and is suggested to function as a complex and layered resistance mechanism (Jones & Dangl 2006; Harris et al. 2020; Panstruga & Moscou 2020). Even as a suitable host, plants may impose resistance and interfere with the pathogen's completion of its sexual or asexual life cycle (Agrios, 2005; Panstruga & Moscou, 2020). Plants can resist disease development through different genetic mechanisms. Qualitative resistance, often conferred by one or a few major genes, can be cultivar-specific or race-specific. Qualitative resistance is often less durable due to strong selection pressure on the pathogen. In contrast, quantitative

resistance is controlled by many genes (QTLs) and is often broader and more durable. Different combinations of these genes determine the level of compatibility between a pathogen and the plant, ranging from more compatible (more disease, less resistance) to less compatible (less disease, more resistance), thereby influencing the extent of disease development. In addition to genetic resistance, plants can also exhibit disease escape, avoiding infection or disease tolerance and minimising the impact of infection on yield or fitness.

Mechanisms underlying plant defences

In recent decades, significant progress has been made in understanding the molecular basis underlying plant-pathogen interactions. The 'gene-for-gene' concept, developed through Flor's research on linseed and its rust fungus *Melampsora lini* (Flor, 1971), provided a theoretical framework for understanding the genetic basis of plant-pathogen interactions. It was proposed that a single resistance *R* gene in the plant can recognise an avirulence *Avr* gene in the pathogen, leading to an incompatible interaction and disease resistance. The absence of the *R* gene and/or the *Avr* gene will result in a compatible interaction and a susceptible plant. However, attempts to demonstrate these direct interactions often failed, leading to the formulation of the guard hypothesis, which proposes that *R* proteins don't directly detect *Avr* proteins but instead 'guard' host proteins targeted by pathogen molecules (Thomma et al. 2011). Pathogen molecules originally called avirulence factors are instead suggested to be virulence factors and are now commonly referred to as effectors (Thomma et al. 2011).

Jones and Dangl's (2006) expository 'zigzag' model further illustrated the dynamic interplay between plants and pathogens through two main branches of plant immunity. Under this model, the first line of defence is formed by detecting the microorganisms as threats using pattern recognition receptors (PRRs) to detect microbial- or pathogen-associated molecular patterns (MAMPs or PAMPs), resulting in PAMP-triggered immunity (PTI). Plants can detect signatures of non-self as infection by membrane-localized receptor-like kinases and proteins (RLKs and RLPs) (Bentham et al. 2020). Successful pathogens suppress the PTI response of plants using effectors, resulting in effector-triggered susceptibility. Depending on the genetic makeup of the plant, certain *R* genes can recognise the pathogen effectors and induce effector-triggered immunity (ETI). ETI can trigger a hypersensitive response by causing programmed cell death at the infection site. This immunity in plants is achieved by nucleotide-binding leucine-rich repeats (NB-LRRs or NLRs), which perceive the presence and/or activities of effectors (Bentham et al. 2020). Pathogens evade plant defences by

inhibiting chitinase and protease activity, detoxifying the apoplast from various anti-microbial compounds, and manipulating targets for reactive oxygen species burst, salicylic acid, jasmonic acid and other host immune receptors and defence signalling pathways (Ökmen & Doehlemann 2014; Lo Presti et al. 2015). Moreover, biotrophic, hemibiotrophic and necrotrophic pathogens vary in their pathogenicity repertoire to accommodate differences in feeding strategies and plant targets (Lo Presti et al. 2015). The interaction between effectors and *R* genes drives selection pressure on plants and pathogens, leading to the evolution of new pathogen isolates that can evade ETI and new plant genotypes that can induce ETI (Jones & Dangl 2006; Lo Presti et al. 2015).

It should be noted that the 'zigzag' model is also considered too simplistic, as it is chronological and does not fully capture the complexity of plant-pathogen interactions (Thomma et al. 2011; Pritchard & Birch 2014; Harris et al. 2020). Instead, it is observed that plant defences are a continuum between PTI and ETI instead of two distinct layers (Thomma et al. 2011). Moreover, the distinction between PAMPs and effectors, as well as between PRRs and R proteins, cannot be strictly maintained. A synergistic interaction and mutual potentiation of PTI and ETI to activate strong defences is demonstrated in *Arabidopsis thaliana*, conceptually uniting hitherto dichotomous layers of plant defences (Ngou et al. 2021; Yuan et al. 2021).

1.3 Wheat production and its challenges

Wheat (*Triticum aestivum L.*) is one of the most important crops for global food security, contributing approximately 20 % to global caloric consumption (Shiferaw et al. 2013). Wheat is the most widely cultivated crop globally, covering a total area of 220.4 million hectares, producing 798.98 million tonnes in 2023 (FAO 2025b). Wheat yield has more than doubled worldwide since the Green Revolution of the 1960s (Figure 1). Wheat production in Europe accounts for a third of the global output (269.26 million tonnes) and is one of the most significant cereal crops with the largest arable land (FAO 2025b). Wheat is also Sweden's most widely grown crop, with winter wheat accounting for nearly 90 % of wheat production (Statistikmyndigheten SCB 2008). According to the Swedish Statistical Database, it occupies the country's largest cultivated area (323,182 hectares), covering approximately 12 % of the total farmland. Wheat, alongside most cereals, is mainly grown in southern Sweden. In 2023, winter wheat production in Sweden reached 2.6 million tonnes, with an average yield of 5.7 tonnes per hectare (Statistikmyndigheten SCB 2024).

Advances in wheat productivity over the past century are primarily due to improvements in agronomic practices, technological innovations, and, most notably, plant breeding. The Green Revolution marked a turning point

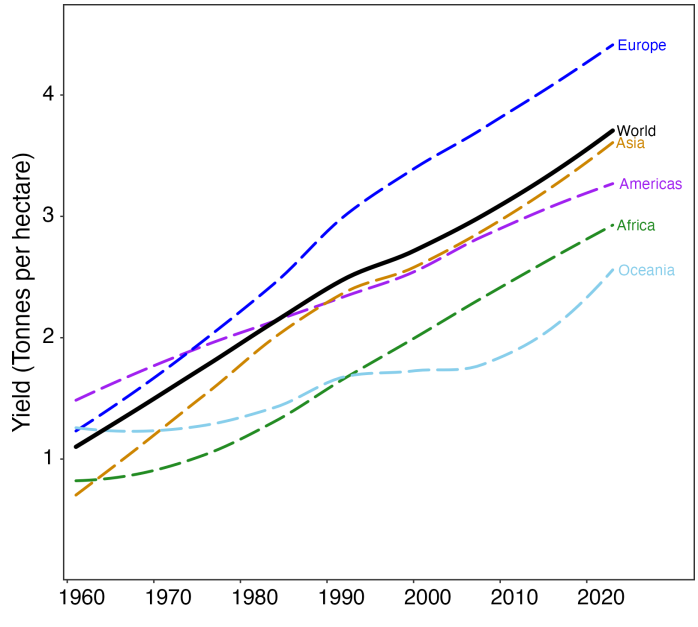

Figure 1. Global wheat yield by region, 1961 – 2023. Source: FAO (2025b), accessed on 27.01.2025.

in wheat cultivation, with the development of high-yielding, semi-dwarf and fertilizer-responsive varieties that dramatically increased global wheat production (Shiferaw et al. 2013). In Europe, yield increase in the last decades is suggested to be principally due to plant breeding (Brancourt-Hulmel et al. 2003; Mackay et al. 2011; Laidig et al. 2021). Selection through plant breeding played a fundamental role in improving wheat yield attributing traits, such as kernels per spike, spikes per unit area, and kernels per square meter.

Wheat production, however, is significantly impacted by various abiotic and biotic factors. Climate change, by exacerbating abiotic stresses such as soil degradation, drought, and heat stress, is estimated to cause a 2 % decline in global wheat production by 2050 (Pequeno et al. 2021). Drought, a key driver of yield loss expected to worsen in coming decades, coupled with heat stress from rising global temperatures—projected to cause a 6 % yield decline for every 1°C increase—poses significant threats to future wheat production (Shiferaw et al. 2013; Asseng et al. 2015; Zhao et al. 2017; Pequeno et al. 2021). Developing cultivars for improved tolerance to abiotic stresses is among the most effective adaptation strategies (Ceccarelli et al. 2010; Lopes et al. 2015; Pequeno et al. 2021).

Alongside abiotic stresses, biotic stresses caused by insects, nematodes, viruses, bacteria, and fungi are major agents of quantity and quality losses in wheat production. An estimated one-fifth of total wheat production losses are attributed to pests and pathogens (Oerke 2006; Savary et al. 2019). Globally, the major pests and pathogens impacting wheat production are leaf rust, fusarium head blight (FHB), septoria tritici blotch (STB), stripe rust, spot blotch, tan spot, aphids, and powdery mildew (Savary et al. 2019). In Europe, major fungal diseases of concern are STB caused by *Zymoseptoria tritici*, septoria nodurum blotch caused by *Parastagonospora nodorum*, stem rust caused by *Puccinia graminis* f. sp. *tritici*, leaf rust caused by *Puccinia triticina*, yellow rust caused by *Puccinia striiformis*, powdery mildew caused by *Blumeria graminis*, FHB caused by *Fusarium* spp. complex (Figueroa et al. 2018; Willocquet et al. 2021).

In the context of this work, two major fungal pathogens of wheat are detailed below.

1.3.1 *Zymoseptoria tritici*

Zymoseptoria tritici (teleomorph: *Mycosphaerella graminicola*) is a fungal pathogen that causes the foliar disease STB (Figure 2). Septoria tritici blotch is ranked as the third leading cause of global yield losses after leaf rust and FHB; it is also the second most significant wheat pathogen in northwestern Europe, following stripe rust (Savary et al. 2019). Yield losses due to STB disease are twice the global average (2.44 %) in north-western Europe (5.51 %), with losses reaching up to 50 % in Europe during severe outbreaks (Ghaffary et al. 2012; Fones & Gurr 2015; Savary et al. 2019). In Sweden, leaf blotch caused by *Z. tritici*, in combination with septoria nodurum blotch and tan spot, is chronic and causes crop losses annually across regions (Willocquet et al. 2021). STB is particularly concerning due to its widespread occurrence and the pathogen's ability to evolve rapidly, making it a persistent challenge for wheat growers.

Zymoseptoria tritici is an ascomycete fungus with a heterothallic lifestyle with both asexual and sexual reproduction stages (Waalwijk et al. 2002). The infection cycle is initiated by wind-dispersed sexual ascospores and rain splash-dispersed asexual pycnidiospores present on crop debris of the previous season (Eyal et al. 1987; Ponomarenko et al. 2011; Suffert et al. 2011). These ascospores and pycnidiospores act as primary inoculum sources, infecting wheat seedlings in cool and high-humidity conditions (Eyal et al. 1987). Spores germinate on wheat leaves and penetrate through the stomata (Eyal et al. 1987; Kema et al. 1996). The lifestyle of the fungus is considered hemibiotrophic with a long symptomless latent phase of 8 to 11 days, during which mycelial growth in the apoplast and invasion of host mesophyll occurs (Duncan & Howard 2000). As shown in Figure 2a, necrotic lesions begin to appear on leaves after 10 to 14 days, accompanied by the initiation of the formation of asexual fruiting bodies called pycnidia (Kema et al. 1996; Duncan & Howard 2000). The onset of symptoms and pycnidia formation can vary depending on pathogen strain, wheat cultivar and environmental factors (Eyal et al. 1987). During the growing season, asexual pycnidiospores are released from pycnidia in the presence of moisture, further spreading the disease through rain splash. In the dead host tissue, *Z. tritici* grows saprotrophically, and the sexual fruiting structures called pseudothecia are formed between 25 to 30 days post-initial infection (Sánchez-Vallet et al. 2015). Within a season, *Z. tritici* can complete five to six asexual infection cycles through rain-dispersed pycnidiospores and one to two sexual infection cycles through air-borne ascospores (Karisto et al.

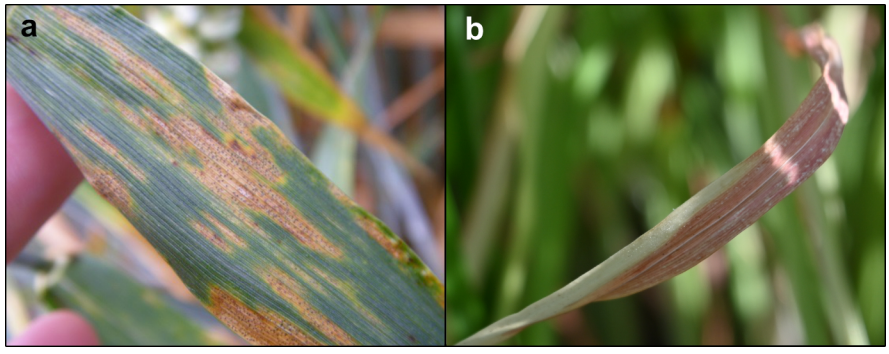

Figure 2. Septoria tritici blotch symptoms on wheat leaves (a) in the field and (b) in the greenhouse. Photo by: (a) Magnus Karlsson and (b) Sidhant Chaudhary

2018). It should also be noted that symptoms in artificial inoculation under greenhouse conditions can differ from the field symptoms. Zakieh et al. (2023) highlighted that leaves under controlled conditions show general chlorosis that spreads across the entire leaf, which eventually turns necrotic with a reddish colour. In this work, similar symptoms were observed, as shown in Figure 2b.

STB poses a significant challenge for disease management due to the pathogen's high genetic variability and its capacity to infect wheat at all growth stages. Cultural practices of removing crop residue are recommended to reduce inoculum for the next season (Suffert et al. 2011; McDonald & Mundt 2016). Moreover, crop rotation with non-host plants is also suggested; however, its effectiveness may be limited due to the ability of ascospores to disperse over large distances (Ponomarenko et al. 2011). The most common management strategies against STB are resistant cultivars and chemical pesticides (Willocquet et al. 2021).

Breeding of resistant wheat cultivars has been an active strategy for managing STB since the 1970s in Western Europe, driven by the disease's increasing prevalence and significance (Brown et al. 2015; Torriani et al. 2015). Similar to disease resistance genetics in other crop–pathogen systems, wheat germplasm possesses major qualitative resistance genes, denoted as *Stb* genes, as well as many minor quantitative resistance genes against STB. To date, over 20 major resistance genes and hundreds of QTLs associated with STB resistance have been identified and mapped across the wheat genome (Brown et al. 2015; Yang et al. 2018). However, regular sexual reproduction and standing genetic variation in the field drive the selection for more aggressive *Z. tritici* strains that can overcome host resistance (Kema et al. 1996; Suffert et al. 2019; McDonald et al. 2022). Therefore, the effectiveness of major resistance genes for disease control is typically short-

lived, as demonstrated by the case of the *Stb6* major *R* gene (Cowger et al. 2000; McDonald & Mundt 2016). To this extent, even stacking multiple major resistance genes may not provide durable disease resistance, as most known *Z. tritici* isolates are already virulent against the majority of *Stb* genes (Brown et al. 2015). Therefore, for effective and durable STB resistance against diverse and rapidly evolving *Z. tritici* field populations, the integration of major and minor genes is recommended for developing cultivars (Brown et al. 2015).

Given the complexity and variability in the effectiveness of genetic resistance, STB management is still highly reliant on fungicide applications in intensive wheat production areas. Management of STB represents the largest fungicide market in Europe, with an estimated 70 % of all fungicide use and costing approximately $1.2 billion annually (Torriani et al. 2015). The overuse and misuse of fungicides pose a significant risk of developing pesticide resistance in pathogens, threatening the effectiveness of disease control and the long-term security of crop production (Gould et al. 2018; Karlsson Green et al. 2020). Most fungicides used to manage STB are 14α-demethylase inhibitors (DMIs or azoles), quinone-outside inhibitors (QoIs or strobilurins), and succinate dehydrogenase inhibitors (SDHIs or carboxamides) (Jørgensen et al. 2018). With its high evolutionary potential, *Z. tritici* can rapidly develop resistance to single-target fungicides (Torriani et al. 2015; Hellin et al. 2021; Klink et al. 2021). In European *Z. tritici* populations, azole and SDHI resistance-conferring alterations were widespread at key target genomic sites such as CYP51 and SDH (Hellin et al. 2021). Furthermore, in Danish and Swedish field populations of *Z. tritici*, reduced efficacy of DMIs or azoles was observed (Heick et al. 2020).

Hence, incorporating additional management strategies, to complement resistant cultivars and fungicide applications to reduce selection pressure, is essential for effective STB disease management.

1.3.2 *Fusarium graminearum*

Fusarium species are a devastating and economically important group of pathogens affecting wheat and other cereals worldwide. *Fusarium* spp. are often present as a species complex of closely related species, infecting various plant tissues at different growth stages, causing fusarium foot and root rot (FFR), fusarium root rot, fusarium seedling blight, fusarium crown rot, and FHB (Dean et al. 2012; Karlsson et al. 2021). Across the globe, fusarium diseases cause severe yield losses as well as quality losses through the production of mycotoxins (Dean et al. 2012; Savary et al. 2019; Karlsson et al. 2023). The predominant *Fusarium* spp. responsible for various fusarium diseases vary across different geographical regions (Backhouse &

Burgess 2002; Becher et al. 2013). FHB is a major disease of wheat, and in Europe, it is primarily caused by *F. graminearum*, *F. culmorum*, *F. avenaceum*, and *F. poae* (Becher et al. 2013). Beyond FHB, *Fusarium* spp. also causes significant economic damage through various ground-level and below-ground diseases globally in dry climates (Kazan & Gardiner 2018). Moreover, rising temperatures and earlier summers in northern Europe pose an increased risk of *F. graminearum* infections, causing FFR and FHB (Strandberg et al. 2024). The significant economic impact of *Fusarium* spp. has driven extensive research and breeding efforts to manage these diseases and mitigate their effects on global wheat production.

This project focused on *F. graminearum* (teleomorph *Gibberella zeae*), an ascomycete fungus that can cause diseases in various crops. The primary sources of *F. graminearum* inoculum include contaminated crop residues, gramineous and broad-leaf weeds, and seeds (Becher et al. 2013; Karlsson et al. 2021). *Fusarium graminearum* disperses through both asexual conidia, spread by wind or rain, and sexual ascospores, released from perithecia (Karlsson et al. 2021). *Fusarium graminearum* can infect all plant parts from the seedling stage until maturity. *Fusarium graminearum* can infect germinating seeds, reducing germination rate, emergence and vigour of germinated seedlings, and cause root rot and seedling blight (Jones 1999; Wang et al. 2006). Later in the season, *F. graminearum* can cause crown rot and infect plants at anthesis (Becher et al. 2013; Karlsson et al. 2021). FHB infection initiates when airborne spores deposit on flowering spikelets, subsequently germinating and penetrating the plant through natural openings, such as the base of the lemma and palea, or via degenerating anthers (Trail 2009). Upon floret infection, *F. graminearum* produces deoxynivalenol, which facilitates fungal spread within the wheat head. Bleached heads and shrivelled, underdeveloped kernels are the main symptoms of FHB.

This project focused on infection at the seedling stage, termed FFR, characterised by browning of the root system and stem base (Figure 3). These symptoms are consistent with seedling blight and root rot reported in the literature.

Various strategies exist to manage the diseases caused by *Fusarium* spp. To reduce the inoculum load from the field, a well-planned crop rotation and crop residue management are recommended (Becher et al. 2013; Karlsson et al. 2021). Using uninfected and fungicide-treated seeds has been shown to improve seed germination and reduce seedling blight incidence (Jones 1999). The use of triazole fungicides to manage FHB is a common practice; however, successful application varies with the choice and dose of fungicide

Figure 3. Symptoms of fusarium foot rot. Photo by Mukesh Dubey

and the timing of application (Becher et al. 2013). The use of bacterial and fungal biocontrol agents to reduce the reliance on chemical fungicides has also shown some potential, albeit not yet at scale commercially.

Harnessing host-plant resistance by breeding for resistant cultivars is by far the most effective and sustainable approach to disease management, but achieving durable host-plant resistance is complex. Over the past few decades, significant efforts have been invested in identifying QTLs associated with FHB resistance, aiming to develop wheat varieties with improved resistance to this devastating disease (Buerstmayr et al. 2020). However, resistance to FHB is shown to not always correlate with fusarium diseases of vegetative tissues, suggesting differences in host plant resistance (Li et al. 2010; Wang et al. 2015). Therefore, understanding the genetic architecture of resistance to *F. graminearum* causing ground-level and below-ground diseases is also crucial.

1.4 Biological control in the context of integrated pest management

Current agricultural production relies heavily on chemical pesticides to achieve optimal yields and quality. According to the latest FAO report (FAO 2025a), pesticide usage has increased by approximately 50 %, rising from 1.2 kg/ha in 1990 to 2.37 kg/ha in 2020, reaching a total pesticide use of 3.7 million tonnes. A similar trend is observed in fungicide and bactericide use specifically, with increasing global use since 1990 in most parts of the world (Figure 4). The dependence of agricultural systems on chemical pesticides has led to significant adverse environmental effects, such as the contamination of soil and water resources, detrimental impacts on non-target flora and fauna, and a reduction in biodiversity (Tudi et al. 2021). Furthermore, the development of resistance to pesticide applications in pathogens presents a significant challenge that affects both pesticide efficacy and long-term crop security (Gould et al. 2018; Karlsson Green et al. 2020). To reduce reliance on pesticides, integrated pest management (IPM) strategies can be employed as a holistic approach to managing pests and pathogens effectively (Karlsson Green et al. 2020).

FAO defines IPM as "… careful consideration of all available pest control techniques and subsequent integration of appropriate measures that

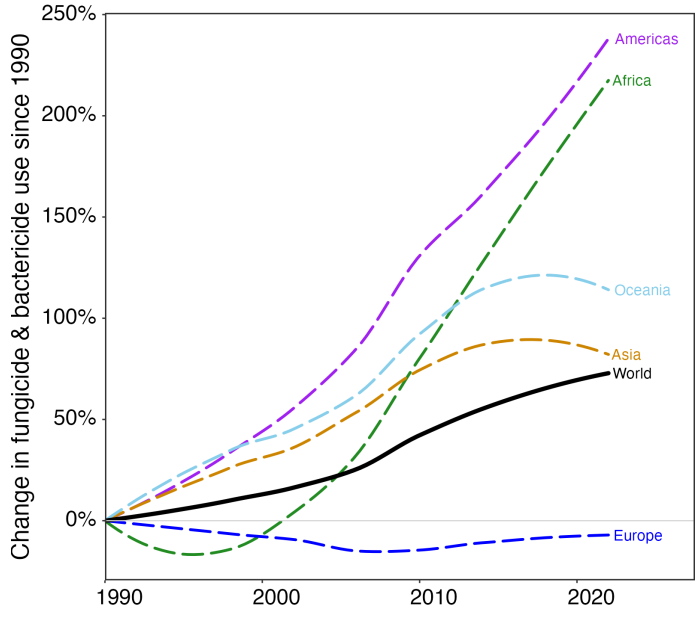

Figure 4. Region-specific fungicide and bactericide use change since 1990. Source: FAO (2025a), accessed on 27.01.2025.

discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimise risks to human health and the environment. IPM promotes the growth of a healthy crop with the least possible disruption to agroecosystems and encourages natural pest control mechanisms" (Deguine et al. 2021). To emphasise the significance of IPM, the European Union (EU) Directive 2009/128/EC mandates all professionals involved in plant production to comply with IPM principles (European Union 2009).

IPM employs a comprehensive range of solutions and interventions, including agronomic, mechanical, physical, and biological methods, with chemical intervention utilised only as a last resort for the effective management of pests and diseases. IPM principles follow a hierarchy from exclusion and avoidance to protection (Barzman et al. 2015; Tronsmo et al. 2020). IPM emphasises prevention, suppression, and avoidance of pests and diseases through the implementation of appropriate crop rotations, sowing time, judicious irrigation and fertilisation regimes and other field management practices. Additionally, the utilisation of resistant and tolerant cultivars plays a crucial role. Following the establishment of crops, continuous monitoring of levels of pests and diseases is vital to inform critical management decisions. When pest or pathogen populations rise above the economic threshold, intervention is required. Initially, management of pests or pathogens through non-chemical approaches is emphasised, which may include physical methods such as the removal of diseased plants, weeds and/or insects, as well as mechanical and biological control measures. When non-chemical methods are insufficient, careful consideration is given to pesticide selection and application, as well as strategies to prevent or delay pesticide resistance development. Finally, ongoing evaluation of the implemented strategies is essential to assess their effectiveness and adapt management plans as needed.

Biological control, the focus of this work, is further detailed below.

Biological control

Disease development, and ultimately its management, is influenced by various factors. Traditionally, the disease triangle, comprising a susceptible host, a virulent pathogen, and favourable environmental conditions, is used to conceptualise disease establishment. However, with our understanding of the role of host-associated microbiome and other engaged organisms, an extension of the disease triangle in the form of a disease tetrahedron has been proposed (Brader et al. 2017). Under this concept, particular importance is given to biotic factors other than the host and the pathogen in affecting disease development. Expanding on this, it can be emphasised here that among various biotic factors, beneficial organisms used to manage pests and diseases, i.e., biological control agents or biocontrol agents (BCAs), can play an important role in the disease outcome (Figure 5).

Within the context of IPM, biological control is a key strategy to control pests and diseases, both in conventional and organic agriculture. Biological control measures are often considered a more sustainable alternative to chemical pesticides, offering the potential to minimise the social costs linked to chemical pesticides, such as environmental pollution and potential impacts on human health (Jensen et al. 2016b). Biological control is further promoted and incentivised by the European Green Deal, and its use is specifically recommended in the European Commission's proposal for a new regulation

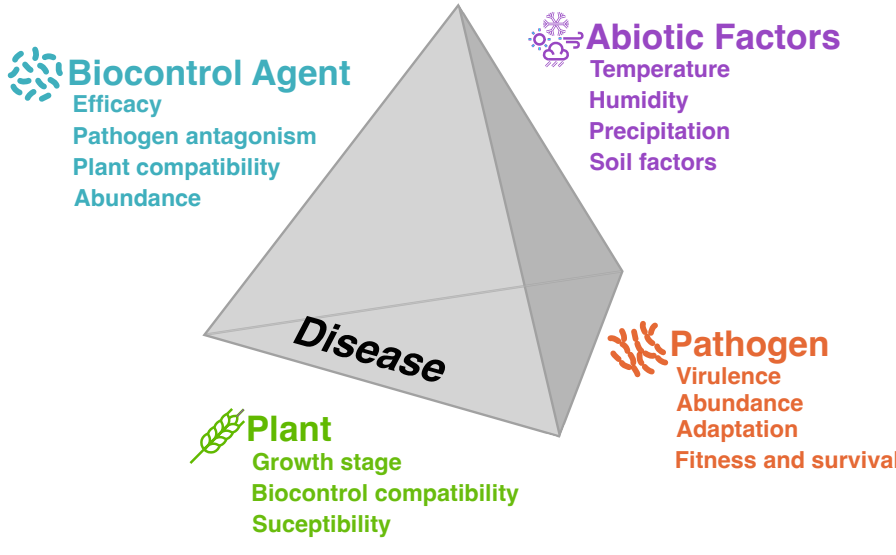

Figure 5. Disease tetrahedron which considers role of biological control agents separately. Modified after Brader et al. (2017)

on the sustainable use of plant protection products, which aims to reduce synthetic chemical pesticide use by 50% by 2030 (European Commission 2022). The use of biological products, including living organisms and natural substances, is a rapidly growing industry, with its global market value reaching USD 7.54 billion in 2023 and projected to reach USD 28.61 billion by 2032 (Fortune Business Insights 2025). The successful commercialisation of numerous biological control agents (BCAs), including bacterial, fungal, oomycete, and viral types, has already been achieved (Collinge et al. 2022).

Stenberg et al. (2021) defines biological control as "… the exploitation of living agents (including viruses) to combat pestilential organisms (pests and pathogens), directly or indirectly, for human good". It should be noted that the above definition is anthropocentric. Additionally, an organism is considered a BCA owing to its ecological function; however, it must be emphasised that the ecological function assigned to a micro-organism can be fluid and context-dependent (Stengel et al. 2022). The beneficial–antagonistic continuum exploited by microorganisms is recognised in the literature, with host factors and various spatial and temporal factors determining their position on this continuum. *Fusarium oxysporum* is a known plant pathogen, but certain strains also exhibit biocontrol properties (Brader et al. 2017). Similarly, *Pseudomonas* spp. can be highly opportunistic plant antagonists as well as offer beneficial plant-growth promoting effects under different environmental conditions (Stengel et al. 2022). Furthermore, ectomycorrhizal fungi are also shown to transition between saprotrophic and biotrophic lifestyles, exhibiting antagonistic, commensal, and beneficial effects towards the plant host (Smith et al. 2017). As such, context-specific variability should always be taken into consideration in the case of BCAs of plant pathogens.

Biological control is categorised into natural, conservation, classical, and augmentative approaches (Stenberg et al. 2021). "Natural biological control" refers to the pest control activities of indigenous species independent of human intervention. In contrast, "conservation biological control" involves targeted human intervention to enhance the pest control potential of these natural enemies. "Classical biological control" aims for the intentional permanent establishment of non-indigenous organisms, whereas "augmentative biological control" involves the temporary establishment of released, mass-reared BCAs into target regions. Certain BCAs also exhibit fungicide tolerance, which allows for simultaneous or rotational use with fungicides for disease management (Chaparro et al. 2011; Dubey et al. 2014a, 2016; Ons et al. 2020; Piombo et al. 2024a). As such, BCAs can be effectively incorporated into IPM strategies as standalone solutions and in conjunction with chemical pesticides through various applications, including

seed and soil treatments, spraying during the growth phase of crops, post-harvest treatments, and applications between cropping seasons (Jensen et al. 2016b). BCAs can exhibit one or more modes of action depending on the host, the pathogen and environmental factors (Jensen et al. 2022). These modes of action can be classified into four categories (Jensen et al. 2017; Collinge et al. 2022):

1. exploitation competition for resources such as oxygen, carbon, nitrogen, and other vital nutrients,
2. interference competition for space, achieved through antibiosis, where the BCA inhibits the pathogen by producing specialised secondary toxic metabolites,
3. hyperparasitism, where the BCA acts as a predator, preying on the pathogen,
4. induced resistance, involving the indirect interaction of a BCA by triggering plant defence mechanisms against invading pathogens.

1.4.1 *Clonostachys rosea*

In this work, *Clonostachys rosea* strain IK726 was used as a BCA, which was originally isolated from barley roots in Denmark (Knudsen et al. 1995) and genome sequenced in 2015 (Karlsson et al. 2015). *Clonostachys rosea* is an ascomycete fungus (order Hypocreales) and is regarded as an ecological generalist capable of exhibiting saprotrophism, plant endophytism and mycoparasitism (Schroers et al. 1999; Jensen et al. 2022). Various strains of *C. rosea* are reported to be successful BCAs against more than 30 common fungal and oomycete plant pathogens (Jensen et al. 2022), including *Alternaria* spp. (Koch et al. 2010), *Botrytis cinerea* (Peng et al. 1992), *Fusarium* spp. (Xue et al. 2009), *Bipolaris sorokiniana* (Jensen et al. 2016a), *Plasmodiophora brassicae, Phytophthora* spp., *Puccinia* spp., *Pythium tracheiphilum* (Møller et al. 2003), and *Z. tritici* on a range of crops, including fruits, vegetables, pulses, cereals, oil crops and forest trees. Some strains of *C. rosea* can also positively and negatively influence the populations of soil microorganisms, including bacteria, protozoa, and fungi (Ravnskov et al. 2006; Fournier et al. 2020). Moreover, *C. rosea* is also reported to be antagonistic against plant-parasitic nematodes (Iqbal et al. 2018, 2020; Iqbal 2019). A few *C. rosea* strains are successfully commercialised in the EU and the rest of the world (Jensen et al. 2022). Recently, *C. rosea* was also patented in Europe, the USA, and Australia for its biocontrol of STB under field conditions (Jensen et al. 2024).

Owing to its generalist lifestyle, the literature reports various strategies employed by *C. rosea* in its interactions with other microorganisms. *Clonostachys rosea* can directly parasitise fungal and oomycete plant pathogens (Barnett & Lilly 1962; Jensen et al. 2022). It can also compete for nutrients and space by prioritising colonisation over that of the pathogen (Sutton et al. 1997; Jensen et al. 2017). Furthermore, the production of fungal cell-wall degrading enzymes such as chitinases, glucanases, and proteases, as well as antibiosis through the secretion of secondary metabolites, are considered essential components of the biocontrol potential of *C. rosea* (Han et al. 2020; Saraiva et al. 2020).

Clonostachys rosea has also been reported to engage with plants. *Clonostachys* strains are reported to colonise roots as well as above-ground plant tissues in *Arabidopsis thaliana,* tomatoes, cucumber and barley (Chatterton & Punja 2010; Dubey et al. 2014b; Saraiva et al. 2015; Jensen et al. 2016a). While interacting with plants, *C. rosea* has also been reported to induce plant defence responses. Wang et al. (2019) reported that *C. rosea*-mediated biocontrol of *B. cinerea* in tomatoes involved the induction of protective enzymes, accumulation of reactive oxygen species (ROS), and the regulation of stress response genes such as mitogen-activated protein kinase

(MAPK), WRKY transcription factor, β-xylanase, and ATP synthase. Similarly, Kamou et al. (2020) reported induction defence-related genes in tomatoes after treatment with *C. rosea* IK726. Lysøe et al. (2017) reported that *C. rosea* IK726 modulated the expression of defence-related genes in potatoes, both directly and in the presence of the pathogen *Helminthosporium solani*. Roberti et al. (2008) reported the induction of peroxidase, chitinase, and pathogenesis-related proteins in wheat by *C. rosea* directly and in the presence of the pathogen *F. culmorum*. Recently, Piombo et al. (2024b) showed transcriptional reprogramming of wheat genes associated with stress response and growth during root colonisation by *C. rosea* IK726. These findings suggest that plants recognise *C. rosea* via MAMPs, potentially leading to subsequent PTI induction.

In a few studies, *C. rosea* has also been reported to be pathogenic towards certain plants. Certain strains of *C. rosea* have been reported to cause dry rot of potatoes (Theron & Holz 1991), wilt and crown rot of faba bean (Afshari & Hemmati 2017) and root rot of *Astragalus membranaceus* (Qi et al. 2022), *Angelica sinensis* (Ma et al. 2022), garlic (Díaz et al. 2022), *Morchella sextelata* (Fu et al. 2023), soybean (Bienapfl et al. 2012), and *Gastrodia elata* (Lee et al. 2020). The above reports used morphological identification and the ITS region to identify *C. rosea*; however, it has been suggested that these methods may lack sufficient resolution for accurate identification (Jensen et al. 2022). Nevertheless, these reports of occasional exploitation and damage of plants by *C. rosea* underscore the significance of considering context-dependent plasticity in ecological functions within plant-microbial interactions (Stengel et al. 2022). It is suggested that poor plant physiology, high *C. rosea* inoculum, and particular genotype-by-genotype interaction between *C. rosea* and the plant may disrupt the equilibrium, shifting the role of *C. rosea* from a commensal or beneficial one to an antagonistic one (Jensen et al. 2022). Therefore, in future studies, it is essential to conduct precise identification and thorough evaluation within the specific context to gain a comprehensive understanding of the intricate relationship between *C. rosea* and plants.

1.5 Variation in host plant responses to biological control

The inherent natural variation within a species is fundamental to its overall fitness at the population level. Within the agricultural context, this variation has been successfully exploited by plant breeders to improve yield and quality and to reduce stress resistance in crops. As stated above in section §1.1, new traits have been targeted by plant breeders during the last century to improve crop production. Furthermore, as stated in section §1.2, plants and pathogens exhibit variation, enabling them to outcompete each other. Similarly, it can be envisaged that plants possess variation in their ability to benefit from beneficial microorganisms. As illustrated in Figure 5, genotype-by-genotype interactions may occur between beneficial microorganisms and plants, making certain strains more suitable for plants and enabling specific plant genotypes to benefit more regarding growth promotion and/or BCA-assisted disease management in the presence of pathogens.

The role of plant genotype variation in modulating the benefits derived from beneficial microorganisms in general and in disease control efficacy of BCAs, in particular, has been discussed previously (Collinge et al. 2022), although, as yet, no extensive research has been carried out. Here, biocontrol efficacy broadly refers to the ability of a BCA to reduce the negative impact of a target pest or pathogen. Smith and Goodman (1999) motivated the idea of crop improvement for optimising interactions with beneficial microorganisms by highlighting the potential for untapped variation in interactions with rhizobia, mycorrhizal fungi, BCAs, and the microbial community as a whole. Similarly, Stenberg et al. (2015) have suggested breeding crops for optimised biocontrol of herbivores. However, until now, the exploration of plant genotype variation for BCA efficacy in tripartite interactions involving plant genotypes, pathogens, and BCAs has largely been confined to a limited number of plant genotypes.

The few known examples of augmentative biological control exploring the role of host plant genotype in modulating biocontrol efficacy are summarised in Table 1. Moraga-Suazo et al. (2016) reported a differential response in two contrasting *Pinus radiata* genotypes to *C. rosea*-mediated biocontrol of *Fusarium circinatum*. This research further indicated that the activation of induced systemic resistance (ISR) by *C. rosea* was genotype-specific. Meyer et al. (2010) reported cultivar–specific differences among three tested Swiss winter wheat genotypes in their ability to benefit from *Pseudomonas flourescens* strain CHA0 in the biocontrol of *Pythium ultimum* and suggested optimised cultivar-soil combinations as well as breeding efforts for improved plant–bacterial interactions. Tucci et al. (2011) similarly reported differences among five tomato genotypes for enhanced ISR against

Table 1. Previous work on exploring plant genotype variation in biocontrol efficacy

Beneficial microorganism	**Pathogen**	**Plant**	**# Plant genotypes**	**Reference**
Clonostachys rosea	*Fusarium circinatum*	*Pinus radiata*	2	(Moraga-Suazo et al. 2016)
Pseudomonas flourescens	*Pythium ultimum*	Wheat	3	(Meyer et al. 2010)
Trichoderma atroviride, T. harzianum	*Botrytis cinerea*	Tomato	5	(Tucci et al. 2011)
Streptomyces spp.	*Streptomyces scabies*	Potato	5	(Ryan et al. 2004)
Bacisllus velezensis, Pseudomonas azotoformans	*Phytophthora capsici*	Tomato	6	(Arkhipov et al. 2023)
T. harzianum. T. virens	*Aphanomyces euteiches*	Lentils	23	(Prashar & Vandenberg 2017)
Fusarium oxysporum	*Striga hermonthica*	Sorghum	50	(Rebeka et al. 2013)
Bacillus cerus	*Pythium torulosum*	Tomato	61	(Smith et al. 1999)
T. asperellum T34	*Puccinia striiformis f. tritici*	Wheat	198	(Esmail et al. 2023)

the grey mold pathogen *B. cinerea* using *Trichoderma atroviride* and *T. harzianum*. Field trials conducted by Ryan et al. (2004) revealed variation in BCA effectiveness against potato scab, influenced by factors including potato cultivar, pathogen isolate, and growing season. Arkhipov et al. (2023) further showed that six tomato genotypes varied in their response to *Phytophthora capsici* biocontrol by *Pseudomonas azotoformans*, with the mechanism involving the induction of ISR and a hypersensitive response. Prashar and Vandenberg (2017) tested commercial biocontrol formulations of *Trichoderma* spp. for aphanomyces root rot biocontrol. While no genotype-specific differences were observed for disease severity, genotype-specific differences in response to the *Trichoderma* formulations were observed for biomass-related traits under both infected and uninfected conditions. Schmidt et al. (2020) further highlighted that growth promotion by *T. afroharzianum* varied among seven sugar beet genotypes. Furthermore, Rebeka et al. (2013) demonstrated significant variation among 50 sorghum genotypes in *Fusarium oxysporum* compatibility in controlling the root hemiparasitic weed *Striga hermonthica*. Smith et al. (1999a) demonstrated

variation among 61 tomato genotypes in their interaction with the disease-suppressive bacterium *Bacillus cereus* in suppressing the pathogen *Pythium torulosum* and identified its genetic basis through QTL mapping. Lastly, a large-scale study by Esmail et al. (2023) used a GWAS approach to explore spring wheat genotype variation for yellow rust resistance directly and in the presence of the BCA *T. asperellum*.

Taken together, it is evident that plant genetic variation modulates BCA efficacy. Therefore, plant genetic variation should be considered for effective utilisation of BCAs. Understanding the genetic basis of host plant interactions with BCAs offers potential improvement in efficient plant protection within an IPM context. Current genotyping-by-sequencing methodologies allow for the characterisation of large and complex genomes such as *T. aestivum* (Lukaszewski et al. 2014) that can be leveraged in GWAS to investigate traits such as BCA compatibility. This thesis attempts to further the knowledge in this area of study.

2. Aims and objectives

This work aimed to investigate the wheat genotype-specific effects on the BCA *C. rosea* in the biocontrol of wheat diseases. The thesis focused on two fungal diseases in wheat, i.e. septoria tritici blotch (STB) caused by *Z. tritici* and fusarium foot rot (FFR) caused by *F. graminearum*. The variation in disease susceptibility and biocontrol efficacy for the diseases was explored in controlled experiments on a winter wheat germplasm panel consisting of approximately 200 genotypes. Using GWAS and transcriptomics approaches, potential underlying candidate genes associated with diseases and biocontrol by *C. rosea* were also identified. The thesis findings are divided into three papers.

In **paper I**, it was hypothesised that winter wheat genotypes possess genotypic variation for STB and its biocontrol using *C. rosea*. The objectives were to:

i. assess the genotypic variation among winter wheat genotypes for resistance to *Z. tritici* causing STB and their response to *C. rosea*-mediated biocontrol
ii. conduct a GWAS to identify marker-trait associations linked to STB resistance and *C. rosea*-mediated biocontrol efficacy and to determine whether these traits are inherited together or independently.

Similarly, in **paper II**, it was hypothesised that winter wheat genotypes possess genotypic variation for susceptibility for FFR and its biocontrol using *C. rosea*. The objectives were to:

i. assess genotype variation among winter wheat genotypes for resistance to *F. graminearum* causing FFR and their response to *C. rosea*-mediated biocontrol
ii. conduct a GWAS to identify marker-trait associations linked to FFR resistance and *C. rosea*-mediated biocontrol efficacy and to determine whether these traits are inherited together or independently.

In **paper III**, a transcriptomic analysis was performed on two winter wheat genotypes, selected from **paper I**, differing in biocontrol compatibility with *C. rosea* against STB at early time points. It was hypothesised that the transcriptome of the two wheat genotypes differ in response to *C. rosea* and *Z. tritici*. The objectives were to:

i. identify differentially expressed genes (DEGs) among wheat genotypes during the early phase of infection with *Z. tritici*
ii. identify DEGs among wheat genotypes in direct inoculation with *C. rosea* and in presence of *Z. tritici*
iii. identify plant genotype-specific defence-related genes induced by *C. rosea* directly and in the presence of *Z. tritici*.

3. Variation in wheat genotypes for biocontrol efficacy of *Clonostachys rosea* towards *Zymoseptoria tritici* and *Fusarium graminearum*

To investigate the genetic variation in winter wheat for the biocontrol efficacy of *C. rosea*, two distinct studies were conducted. **Paper I** focussed on the biocontrol of STB caused by *Z. tritici*, while **paper II** assessed the biocontrol of FFR caused by *F. graminearum*. This chapter provides a concise summary of the methodology and findings presented in these two papers.

Plant material

In these studies, a panel of winter wheat genotypes initially sourced from the Nordic Genetic Resource Centre in Alnarp, Sweden, was utilised. These genotypes primarily included landraces and cultivars cultivated in the Scandinavian countries between 1900 and 2012. In **paper I**, a total of 202 genotypes were used, whereas in **paper II**, 190 genotypes were used.

This genotypic panel has been previously investigated for genetic variability for abiotic stressors, including freezing and winter hardiness (Vaitkevičiūtė et al. 2023) and drought tolerance (Kumar et al. 2020). Additionally, it has been evaluated for its response to various biotic stresses, such as powdery mildew (Hysing et al. 2007; Alemu et al. 2021), leaf rust (Hysing et al. 2006), yellow rust (Koc et al. 2022), fusarium head blight (Zakieh et al. 2021), and septoria tritici blotch (Odilbekov et al. 2019).

In these studies, we demonstrated that this genotypic panel also represents a valuable resource for assessing the biocontrol efficacy of *C. rosea* in controlling STB and FFR.

3.1 Methodological notes

The experimental methodologies used in **paper I** and **paper II** were similar, as illustrated in Figure 6. To evaluate the hypothesis concerning variations in biocontrol efficacy among wheat genotypes, two distinct treatment conditions were used:

i. pathogen exclusively
ii. pathogen + BCA (*C. rosea*)

In **paper I**, the treatments consisted of foliar spray inoculation of *Z. tritici* (1×10^7 cfu[1]/ml) alone (Zt) and spray inoculation of *Z. tritici* on plants sprayed with *C. rosea* (1×10^7 cfu/ml) 24 h earlier (ZtCr). Likewise, in **paper II**, the treatments were *F. graminearum* in isolation (Fg) and *F. graminearum* on *C. rosea*-treated seeds (FgCr).

Visual scoring of disease development was performed in both studies, as shown in Figure 7. For STB, disease severity was assessed using necrotic leaf area as a proxy over multiple days, and the relative area under the disease progress curve (rAUDPC[2]) was calculated to represent overall disease

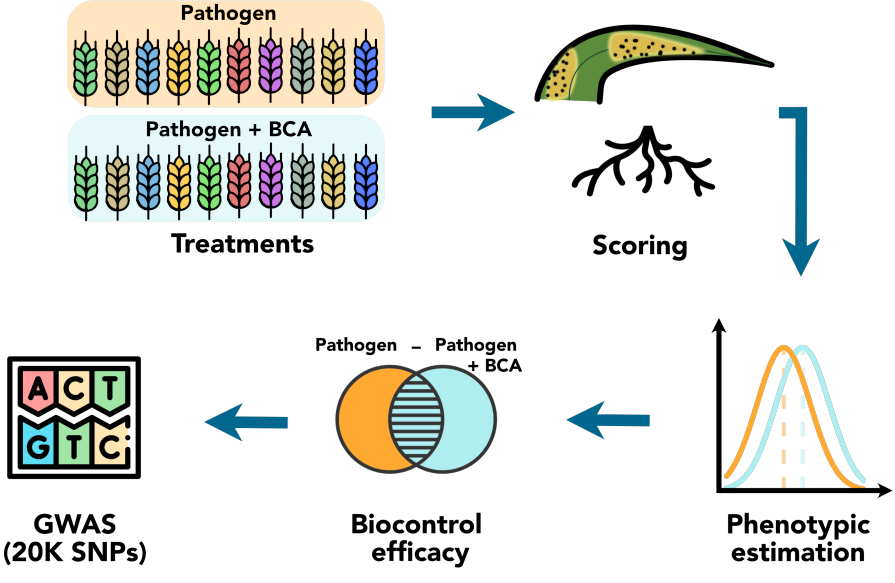

Figure 6. Summary of the experimental setup used in **paper I** and **paper II**

[1] cfu: colony forming unit

[2] Prior to the analysis in **paper I**, the rAUDPC values were standardised through centring and scaling to account for scoring on different days. Consequently, the mean estimates at both the replicate and treatment levels were adjusted to a baseline of zero.

development. For FFR, the browning of roots and stems was used as a disease proxy. Additionally, in **paper II**, shoot and root length (and combined plant length) were measured as additional proxies for disease stress on growth.

Phenotypic data analysis was conducted using linear mixed models[3] to estimate the best linear unbiased estimators (BLUEs). The models accounted for experimental design variables such as replicates and blocks as well as biological variables for treatment, genotype and their interaction. This

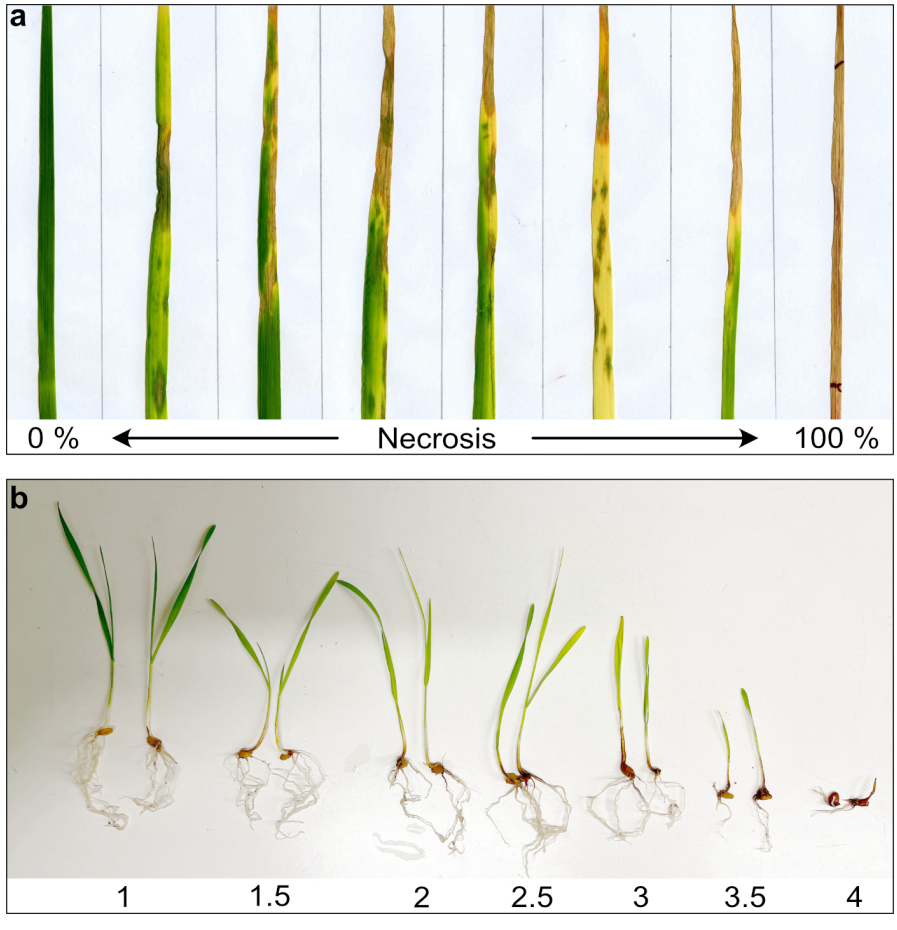

Figure 7. Disease severity scoring of (a) septoria tritici blotch caused by *Zymoseptoria tritici* and (b) fusarium foot rot caused by *Fusarium graminearum*. Photos by Sidhant Chaudhary

[3] Kenward-Roger's approximation of degrees of freedom was used (Kenward & Roger 1997)

allowed for the estimation of inter-treatment contrasts (pathogen – pathogen and BCA) for each genotype, which was used as an estimator for biocontrol efficacy. BLUEs from both treatments and biocontrol efficacy estimates were used as traits for conducting GWAS, as most of the genotypes used were previously genotyped using a 20K SNP array (Odilbekov et al. 2019). For phenotypic estimates in both treatments (pathogen exclusively and pathogen + *C. rosea*) as well as for *C. rosea* biocontrol efficacy estimates, GWAS analysis[4] was performed using GAPIT (Wang & Zhang 2021) on a filtered SNP marker set (n = 7,360) after filtering for minor allele frequency and missing allele information. Genes co-localised in the significant marker–trait associated regions were identified. All statistical analysis was conducted in R (R Core Team 2023, 2024).

3.2 Differential responses of wheat genotypes to pathogens exclusively and *C. rosea* co-inoculation

In **paper I**, phenotypic evaluation of STB susceptibility was performed on 202 genotypes in the treatment Zt, and 183 of these genotypes overlapped in the treatment ZtCr. Significant variation ($P < 0.001$) was observed among genotypes for the STB disease severity estimator scaled rAUDPC in both treatments. STB rAUDPC estimates showed a strong positive correlation

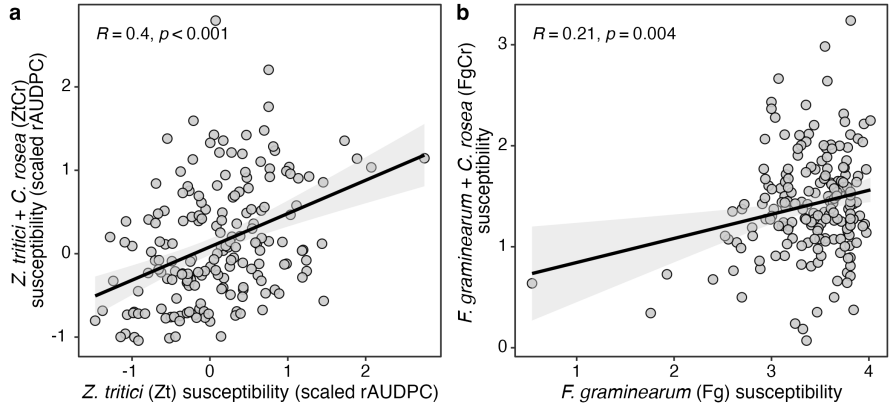

Figure 8. Relationship between two treatments. (a) Pearson's correlation between scaled rAUDPC in treatment Zt (*Z. tritici*) and treatment ZtCr (*Z. tritici* + *C. rosea*) **(paper I)**, and (b) Pearson's correlation between disease score in treatment Fg (*F. graminearum*) and treatment FgCr (*F. graminearum* + *C. rosea*) **(paper II)**

[4] For GWAS analysis, a total of 5 different models were used as follows: GLM (Price et al. 2006), MLM (Yu et al. 2006), MLMM (Segura et al. 2012), FarmCPU (Liu et al. 2016), and BLINK (Huang et al. 2019).

($R = 0.69$, $P < 0.001$) with STB rAUDPC data reported by Odilbekov et al. (2019), where the same plant material was used. Moreover, significant ($P < 0.001$) genotype × treatment interaction, as well as a moderate positive correlation ($R = 0.4$, $P < 0.001$) between Zt and ZtCr treatments, was observed, indicating changes in disease development in wheat genotypes due to the presence of *C. rosea* (Figure 8a).

Similarly, in **paper II**, 190 winter wheat genotypes were evaluated for FFR and its biocontrol by *C. rosea*. Additionally, shoot length, root length and plant length were measured to understand disease-associated stress. Significant differences ($P < 0.001$) between treatments Fg and FgCr were observed for all traits. The four traits showed strong correlations ($R > |0.85|$, $P < 0.001$) with each other. Shoot, root, and plant length were strongly positively correlated, and all three traits were strongly negatively correlated with disease susceptibility, highlighting the impact of disease severity on growth. Overall, high susceptibility to *F. graminearum* was observed, consistent with other findings (Voss-Fels et al. 2018; Shi et al. 2020). This may indicate that the tested germplasm may only possess partial resistance to FFR. Disease severity to FFR in **paper II** was not significantly correlated ($R = 0.11$, $P = 0.16$) with FHB disease severity previously assessed on the same panel (Zakieh et al. 2021). However, part of the variation could be attributed to differences in the *Fusarium* spp. strains used between the two studies. This may be attributed to the independent inheritance of FHB and FFR, underlining the necessity to have separate screening programs for various fusarium diseases, as previously suggested (Li et al. 2010; Liu et al. 2021). Moreover, a significant ($P < 0.0001$) genotype × treatment interaction was observed for all the traits, indicating that genotype performance varied significantly across treatments. This is further highlighted by a weak correlation between treatments for disease score (Figure 8b, $R = 0.21$, $P = 0.004$) and root length ($R = -0.18$, $P = 0.016$) and a non-significant correlation for shoot length and plant length.

3.3 Positive correlation of biocontrol efficacy with disease susceptibility

Clonostachys rosea biocontrol efficacy was estimated using pairwise contrasts between treatments (Zt – ZtCr or Fg – FgCr or FgCr – Fg[5]) for each genotype, with a higher difference in genotype performance between treatments indicates a greater effect of *C. rosea*. For the 183 genotypes overlapping between treatments in **paper I**, post-hoc comparisons revealed variation in *C. rosea* biocontrol efficacy, ranging from significant negative effects (n = 11, $P < 0.05$) to significant positive effects (n = 7, $P < 0.05$). The negative response of certain wheat genotypes to *C. rosea* application in the presence of *Z. tritici* highlights the delicate balance between BCAs, pathogens, and plants (Jensen et al. 2022). In **paper II**, *C. rosea* biocontrol efficacy was much stronger and positive. Post-hoc comparison of 190 genotypes revealed that *C. rosea* seed treatment resulted in most genotypes having significant reductions ($P < 0.05$) in disease score (n = 180), as well as significant increases ($P < 0.05$) in shoot length (n = 166), root length (n = 135), and plant length (n = 163). Moreover, *C. rosea* biocontrol efficacy estimates were positively correlated with disease susceptibility in both

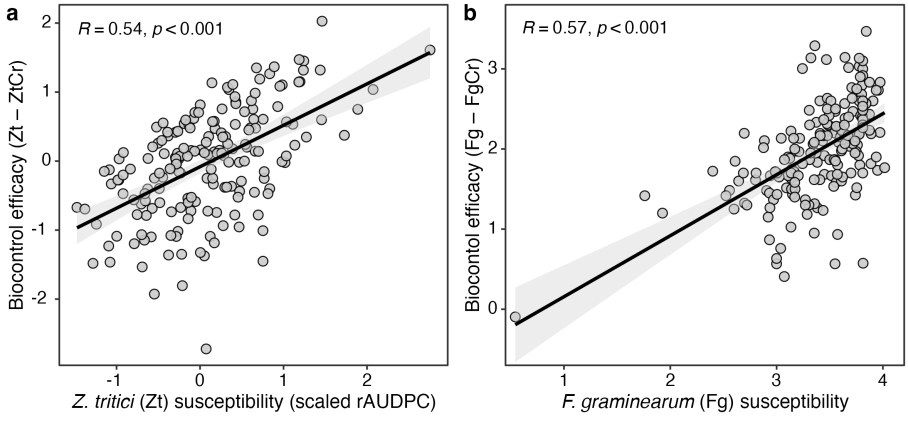

Figure 9. Relationship between susceptibility and biocontrol efficacy. (a) Pearson's correlation between scaled rAUDPC in treatment Zt (*Z. tritici*) and biocontrol efficacy estimates (Zt – ZtCr) of *C. rosea* in controlling septoria tritici blotch **(paper I)**, and (b) Pearson's correlation between disease score in treatment Fg (*F. graminearum*) and biocontrol efficacy estimate (Fg – FgCr) of *C. rosea* in controlling fusarium foot rot **(paper II)**

[5] FgCr – Fg was used to invert the scale for shoot length, root length, and plant length, such that positive values indicated a positive effect from *C. rosea*.

studies. In **paper I**, *C. rosea* biocontrol efficacy estimates were in significant moderate positive correlation ($R = 0.54$, $P < 0.001$) with rAUDPC estimates from treatment Zt (Figure 9a). Similarly, in **paper II**, *C. rosea* biocontrol efficacy estimates for disease score were in a significant moderate positive correlation ($R = 0.57$, $P < 0.001$) with disease susceptibility in the treatment Fg (Figure 9b). A similar but inverted pattern was observed for the traits plant length ($R = -0.7$, $P < 0.001$), shoot length ($R = -0.63$, $P < 0.001$), and root length ($R = -0.75$, $P < 0.001$). The positive correlation between disease susceptibility and *C. rosea* biocontrol efficacy suggests that susceptible genotypes benefit more from *C. rosea* application. Similarly, negative correlations with growth traits indicate that plants with poor growth in the *F. graminearum* infected treatment benefited more from *C. rosea* seed treatment. Smith et al. (1999b) also reported a similar trend, finding that less resistant tomato genotypes exhibited better disease suppression of *P. torulosum* by the BCA *B. cereus*. The positive relationship between increased disease susceptibility and higher biocontrol efficacy can be attributed to the greater potential for disease reduction with higher pathogen loads.

3.4 GWAS reveals significant marker-trait associations for disease susceptibility and biocontrol efficacy

In **paper I**, SNP marker information was available for 188 genotypes in the Zt treatment as well as for 173 genotypes in the ZtCr treatment and biocontrol efficacy. In **paper II**, SNP marker information was available for 181 genotypes in both treatments and 180 genotypes for biocontrol efficacy. Owing to the low sample size and over-stringency of the Bonferroni test (Yang et al. 2011; Wang et al. 2012), a negative log threshold ($P = 0.00014$, after $P = 1/n$, where n = 7,360 is the number of SNP markers) was used.

In **paper I**, GWAS analysis detected five SNP markers at three locations for the rAUDPC estimated in the Zt treatment, two SNP markers at two locations in the ZtCr treatment and four SNP markers at two locations for *C. rosea* biocontrol efficacy (Figure 10a). In the Zt treatment-associated regions, previous studies have identified QTLs for STB resistance (Riaz et al. 2020; Thauvin et al. 2024; Kumar et al. 2025). In **paper II**, for disease score, six SNP markers at three locations were detected in the Fg treatment, no SNP marker-trait associations were found in the FgCr treatment, and six SNP markers at one location were detected for biocontrol efficacy (Figure 10b). For plant length, only biocontrol efficacy showed a significant marker-trait association. For root length, two SNP markers at one location were associated with the Fg treatment and one SNP marker in the FgCr treatment.

Finally, for shoot length, one SNP marker was associated with the Fg treatment, and one was associated with the biocontrol efficacy.

The two studies identified distinct regions segregating with *C. rosea* biocontrol efficacy. This suggests that plant genotype-mediated biocontrol efficacy may vary depending on the pathogen and/or the plant organs (leaves or roots). In both[6] studies, genes co-localised within ± 100 Kbp flanking the significant markers were identified, of which selected genes co-localised with *C. rosea* biocontrol efficacy are summarised in Table 2. Interestingly, various genes predicted to encode disease resistance proteins, proteins involved in detoxification, receptor kinases, proteases, transporters,

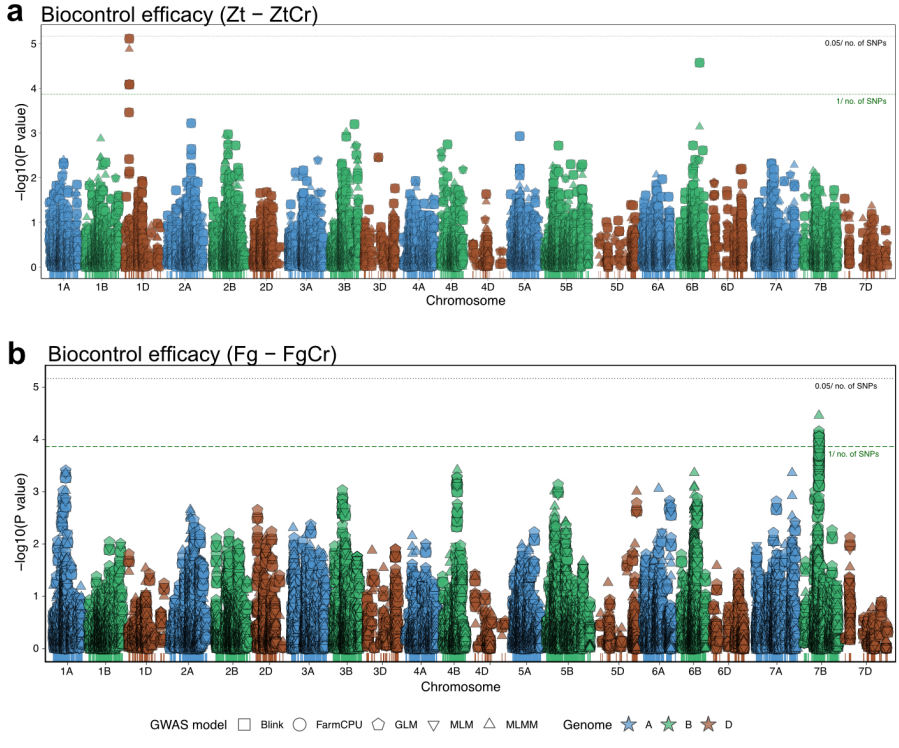

Figure 10. GWAS manhattan plot for marker–trait association for biocontrol efficacy of *Clonostachys rosea* against (a) septoria tritici blotch **(paper I)** and (b) fusarium foot rot **(paper II)**. Dotted line depicts the Bonferroni significance threshold (P = 0.00000679, after P = 0.05/n, where n = 7,360 is the number of SNP markers), dashed line depicts negative log threshold (P = 0.00014, after p = 1/n, where n = 7,360 is the number of SNP markers).

[6] In **paper I**, gene nomenclature was based on Ensembl Traes IDs, however, for cross-referencing in Table 2 and paper III, the genomic regions were re-scanned using the NCBI database.

transcriptional regulators, and biosynthesis of secondary metabolites were identified. In **paper I**, RGA-like disease-resistance proteins were identified, which are known NLR immunoreceptors involved in pathogen recognition (César et al. 2014). In **Paper II**, a detoxification protein associated with *C. rosea* biocontrol efficacy was also identified, which is predicted to belong to the multidrug and toxic compound extrusion (MATE) transporter family, a large plant family involved in detoxification, heavy metal transport, and disease resistance (Sun et al. 2011; Takanashi et al. 2014; Watanabe et al. 2022). Moreover, in **paper II,** three different cytochrome P450 (CYP) encoding genes were also identified, which are putatively involved in flavonoid biosynthesis, protein folding, cell signalling, and immunosuppression in vertebrates and yeast (He et al. 2004; Wang & Heitman 2005). In particular, genes predicted to encode Pik-like (specifically Pik-2-like) disease resistance proteins were found in both studies. Pik proteins are classified as R-type proteins that play a crucial role in triggering a hypersensitive response in plants, helping to limit pathogen growth (Ashikawa et al. 2008). Segregation of Pik-2-like disease resistance protein with *C. rosea* biocontrol efficacy may indicate the differential ability of wheat genotypes to recognise *C. rosea*, potentially triggering early MAMP-triggered PTI or later ETI (Jones & Dangl 2006; Köhl et al. 2019; Jensen et al. 2022). This pattern has been observed previously, where *C. rosea* was shown to differentially induce systemic resistance in *P. radiata* during biocontrol of pitch canker pathogen *F. circinatum* (Moraga-Suazo et al. 2016).

Table 2. Selected genes co-localised with *C. rosea* biocontrol efficacy in **paper I** and **paper II**

Paper	Trait[1]	Ch[2]	Gene ID	Putative function or description
I	D	6B	LOC123139532	DNA (cytosine-5)-methyltransferase 1-like
I	D	6B	LOC123139534	putative disease resistance protein RGA1
I	D	1D	LOC123156846	disease resistance protein RGA5-like
I	D	1D	LOC123162502	thionin-like
I	D	1D	LOC123179874	disease resistance protein PIK6-NP-like
I	D	1D	LOC123179875	PH, RCC1 and FYVE domains-containing protein
I	D	1D	LOC123179876	protein Brevis radix-like 2
II	D	7B	LOC123157439	ATP-citrate synthase beta chain protein
II	D	7B	LOC100136970	cadmium/zinc-transporting ATPase HMA2
II	D	7B	LOC123162411	flavonoid 3'-monooxygenase CYP75B4-like
II	D	7B	LOC123161993	pentatricopeptide repeat-containing protein
II	D	7B	LOC123156907	peptidyl-prolyl cis-trans isomerase CYP19-4-like
II	D	7B	LOC123158901	peptidyl-prolyl cis-trans isomerase CYP28
II	D	7B	LOC123160319	isoprenylcysteine alpha-carbonyl methylesterase
II	D	7B	LOC123162359	detoxification 16-like protein
II	D	7B	LOC123162566	methylene blue sensitivity protein
II	D	7B	LOC123156565	RER1A-like protein
II	D	7B	LOC123162360	putative UPF0496 protein 2
II	D	7B	LOC123161995	thylakoid lumenal 16.5 kDa protein, chloroplastic-like
II	D	7B	LOC123156564	type 2 DNA topoisomerase 6 subunit B-like
II	D	7B	LOC123158178	tyrosine N-monooxygenase-like
II	D	7B	LOC123162565	WAT1-related protein
II	PL, SL	7A	LOC123149896	coiled-coil domain-containing protein
II	PL, SL	7A	LOC123149892	disease resistance protein Pik-2-like
II	PL, SL	7A	LOC123149893	protease 2-like
II	PL, SL	7A	LOC123149895	disease resistance protein
II	PL, SL	7A	LOC123147305	wall-associated receptor kinase-like
II	PL, SL	7A	LOC123147308	tricetin 3',4',5'-O-trimethyltransferase-like
II	PL, SL	7A	LOC123147306	wall-associated receptor kinase 2-like

[1] *C. rosea* biocontrol efficacy for trait D: Disease score, PL: Plant length, SL: Shoot length
[2] Ch: Chromosome

3.5 Concluding remarks

These studies explored genetic variation in wheat germplasm for resistance towards, and biocontrol of, STB caused by *Z. tritici* and FFR caused by *F. graminearum*. The primary objective was to evaluate the variation in biocontrol efficacy of *C. rosea* in both systems. The results show that this wheat germplasm offers significant genetic variation that can be utilised in resistance breeding against STB and FFR. Furthermore, the germplasm exhibited significant variation for *C. rosea*-mediated biocontrol efficacy against both diseases. The results further show that susceptibility and *C. rosea* biocontrol efficacy are positively correlated, indicating that susceptible genotypes may benefit more from BCA application. However, the correlation is moderate, with distinct marker-trait associations at the genome level. Therefore, selecting genotypes with lower susceptibility and higher biocontrol efficacy within this population is feasible. Moreover, marker-assisted selection techniques, such as GWAS, can facilitate concurrent selection by dissecting these traits and breaking negative linkages. Furthermore, these studies identified distinct regions within the wheat genome associated with biocontrol efficacy for each of the diseases, indicating that distinct host genes may modulate specific plant genotype-pathogen-BCA interactions; which may be similar to specific plant *R* genes modulating resistance to particular pathogens. The identification of independent associations for disease resistance and *C. rosea* biocontrol efficacy indicates that simultaneous breeding for these traits is achievable, thereby enhancing the management of STB and FFR through the integration of plant breeding and biocontrol strategies.

4. Gene expression in wheat in response to *Clonostachys rosea* and *Zymoseptoria tritici*

To gain a deeper understanding of differences among wheat genotypes in response to *C. rosea* biocontrol efficacy of STB, two genotypes varying for *C. rosea* biocontrol efficacy were investigated for their transcriptional response at early time points. This chapter provides a concise summary of the findings in **paper III**.

4.1 Methodological notes

Winter wheat genotype NGB6704, showing high *C. rosea* biocontrol efficacy and NGB348, showing low *C. rosea* biocontrol efficacy in the **paper I**, were subjected to the following four treatments (Figure 11):

i. Control (water sprayed)
ii. Zt (*Z. tritici* at 1×10^6 cfu/ml)
iii. Cr (*C. rosea* at 1×10^7 cfu/ml)
iv. ZtCr (*C. rosea* at 1×10^7 cfu/ml + *Z. tritici* at 1×10^6 cfu/ml)

C. rosea application was performed 24h prior to *Z. tritici* application. Leaf samples were collected for RNA extraction at two time points (8h and 16h) after *C. rosea* application and two more time points (32h and 40h) after *Z. tritici* application. These treatments allowed for the exploration of gene expression (compared to control) in the exclusive presence of the BCA *C. rosea* and *Z. tritici*, as well as during the co-inoculation. RNA-seq data was processed using the Nextflow nf-core workflow (Di Tommaso et al. 2017; Ewels et al. 2020) of the pipeline nf-core/rnaseq v3.14.0 (Patel et al. 2024). Reads were aligned to a combined reference genome of wheat, *C. rosea* and *Z. tritici*; however, reads that mapped only to wheat were used for downstream differential gene expression analysis. Differential gene

expression analysis was performed using DESeq2 v1.44.0 (Love et al. 2014) in R (R Core Team 2024). Differentially expressed genes (DEGs) were identified for various relevant contrasts with specific genotypes, treatments and time points compared to their respective control. Genes were considered differentially expressed with absolute log2 fold change of > 1 at a false discovery rate (FDR) adjusted significance of $P < 0.05$ (Benjamini & Hochberg 1995). Gene ontology (GO) enrichment analysis was also performed for DEGs using the parent-child Fisher's test with FDR-adjusted $P < 0.05$. Moreover, DEGs were further compared with genes identified in wheat genomic regions segregating with STB disease susceptibility and *C. rosea*-mediated biocontrol efficacy in the **paper I**.

4.2 Phenotyping confirms similar disease susceptibility but varying *C. rosea* biocontrol efficacy

Leaves remaining after sampling for RNA extraction were kept for disease development until 28 days post-*C. rosea* application. Phenotypic assessment of disease severity[7] of the two wheat genotypes showed similar susceptibility to *Z. tritici* infection, confirming the results of **paper I**. Moreover, NGB6704 showed reduced disease following *C. rosea* application in the ZtCr treatment, indicating biocontrol efficacy consistent with **paper I**, whereas NGB348 showed no significant disease reduction in this study and a negative effect in **paper I**.

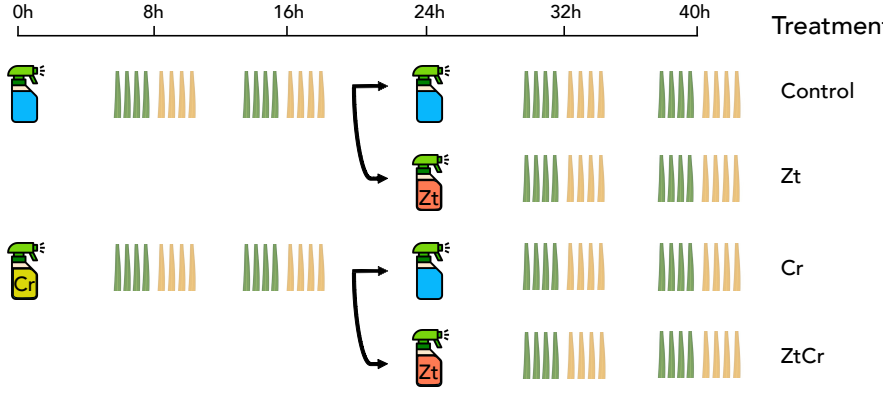

Figure 11. Experimental setup for RNA-seq. Reproduced from **paper III**

[7] Disease severity was scored similar to **paper I**, as shown in Figure 7a.

4.3 Differential read counts between two genotypes

Percent reads mapping only to *C. rosea* and *Z. tritici* were filtered and used as a proxy for biomass (Figure 12). Neither *C. rosea* nor *Z. tritici* showed significant differences ($P < 0.05$) between genotypes at any time point when applied separately. Read counts of *Z. tritici* were comparable to previously reported levels during the initial 24 hours (Rudd et al. 2015). Using microscopy, the foliar survival, germination, growth and sporulation of *C. rosea* has been previously reported on barley leaves (Jensen et al. 2016a). However, in the co-inoculation treatment ZtCr, after 40h, a significant increase in the biomass of both *Z. tritici* and *C. rosea* was detected in NGB6704 but not in NGB348. This genotype-specific increase in reads is further correlated with higher biocontrol efficacy during phenotypic assessment, as well as with a greater transcriptional response and a higher number of DEGs in NGB6704 compared to NGB348 at 40h. It may be hypothesised that stronger immune suppression, resembling induced susceptibility (Seybold et al. 2020), by *C. rosea,* was exhibited in NGB6704, rendering the plant more susceptible, leading to increased *Z. tritici* growth followed by *C. rosea* mycoparasitism. Alternatively, increased production of enzymes and nutrient release to support fungal growth may have been

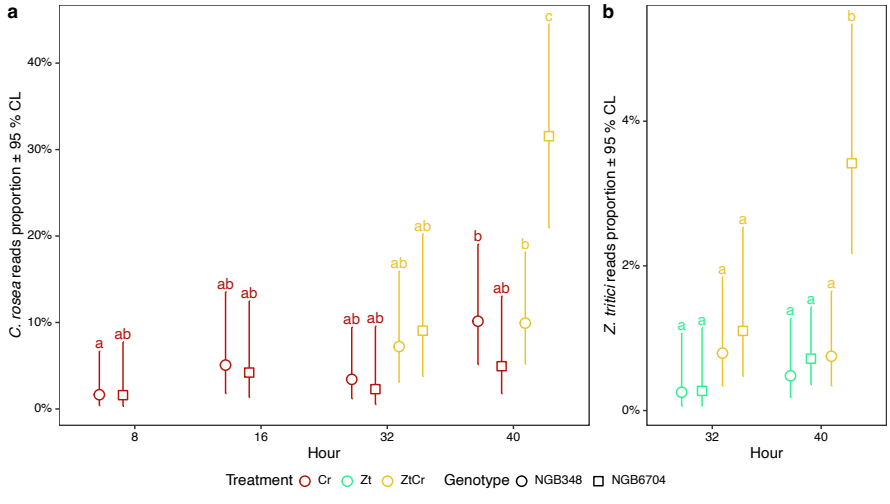

Figure 12. Percent reads mapping to (a) *Clonostachys rosea* IK726 in treatments Cr (*C. rosea*) and ZtCr (*Zymoseptoria tritici* and *C. rosea*) at 8h, 16h, 32h and 40h and (b) *Zymoseptoria tritici* in treatments Zt (*Z. tritici*) and ZtCr (*Z. tritici* and *C. rosea*) at 32h and 40h in two genotypes. Model estimates were back-transformed for interpretation where points show mean estimates and error bars show 95 % confidence intervals. Treatments sharing the same letters indicate non-significant difference ($P > 0.05$) as determined by Tukey's post-hoc comparisons test. Reproduced from **paper III.**

triggered by *C. rosea* in NGB6704. Microscopic investigation of *C. rosea* and *Z. tritici* on these genotypes can aid in gaining further insights.

4.4 Plant genotype-specific gene expression in the presence of *Z. tritici*, *C. rosea*, and their co-inoculation

The primary source of variation in the overall wheat transcriptomic response was due to plant genotype (Figure 13), suggesting that differences between NGB6704 and NGB348 influence the interactions with *C. rosea* and/or *Z. tritici*. Genotype-specific transcriptome response has been reported in previous studies (van Leeuwen et al. 2007; Kälin et al. 2024; Rossi et al. 2024). This further complements the genotype specificity observed in **paper I** and **paper II** in response to *Z. tritici* and *C. rosea* application.

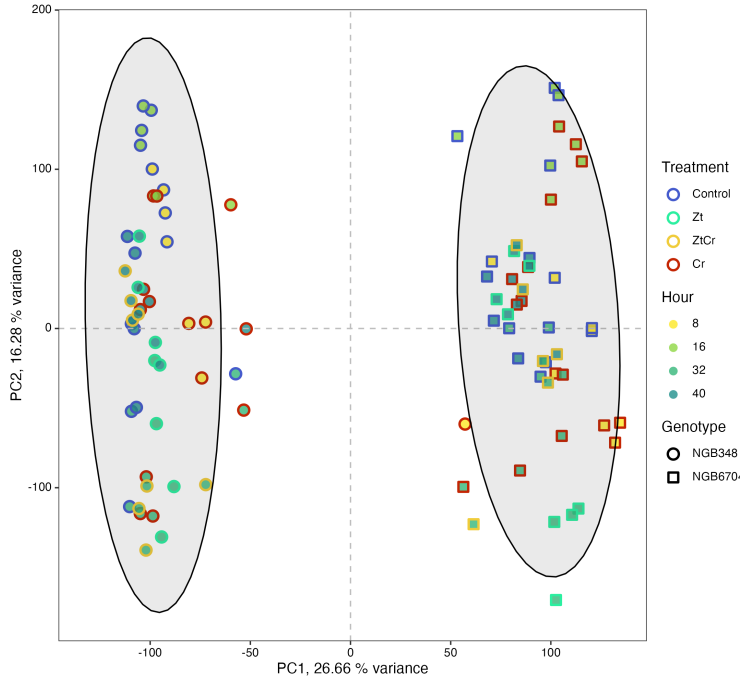

Figure 13. Principal component analysis (PCA) of variance stabilised wheat transcriptome data set showing sample distribution in PC1 and PC2. Point shape represents two genotypes (NGB6704 and NGB348), border colour represents four treatments (Control, Zt: *Zymoseptoria tritici*, Cr: *Clonostachys rosea*, ZtCr: *Z. tritici* + *C. rosea*) and fill colour represents four time points (8h, 16h, 32h and 40h). Ellipses cluster genotypes.

In response to *C. rosea*, *Z. tritici* and their co-application, the two genotypes exhibited distinct gene expression patterns, differing in the specific genes regulated and the extent of regulation of shared genes (

Table 3). In all three treatments, Various defence-related genes were differentially expressed in both genotypes. Defence signalling in plants is complex, involving various genes, some of which are described here. Pathogenesis-related (PR) proteins include chitinases, β-1,3-glucanases, peroxidases, oxalate oxidases, and endoproteinases, which are expressed by plants only in pathological or related conditions (Jain & Khurana 2018). Receptor-like kinases play an essential role in cell-surface immunity by detecting non-self signatures as a sign of infection (Bentham et al. 2020). WRKY transcription factors also play an important role in plant growth, stress response and disease resistance; as activators and/or suppressors of PTI and ETI (Javed & Gao 2023). Similarly, germin-like proteins play roles in developmental processes and plant defence against biotic and abiotic stresses (Bernier & Berna 2001; Barman & Banerjee 2015).

Despite exhibiting similar susceptibility to STB disease, the two genotypes differed in their transcriptomic responses to *Z. tritici*, where NGB6704 showed a high number of DEGs compared to NGB348. The lower *Z. tritici*-induced DEG response in NGB348 may suggest successful suppression of defences by *Z. tritici* or a slower response of NGB348 in response to *Z. tritici*, which might not have been observed until 40h (16 hours post-*Z. tritici*). In NGB6704, many upregulated genes are associated with immune defence responses, including serine/threonine-protein kinases, cysteine-rich receptor-like kinases, ABC transporters, chitinases, germin-like proteins, PR-proteins, WRKY transcription factors, and MYB transcription factors. This may suggest a strong immune response involving a direct attack on *Z. tritici*, synthesis and efflux of specialised metabolites, and cross-linking of plant cell wall components.

Plant genotype-specific transcriptional responses were also observed with direct *C. rosea* application. Specifically, NGB348 displayed a strong induction of defence-related genes at 8h post-*C. rosea* application, including serine/threonine protein kinases, lectin domain-containing kinases, cysteine-rich receptor-like kinases, wall-associated receptor-like kinases, receptor-like protein kinases, WRKY transcription factors, auxin-responsive proteins, disease resistance proteins, PR-proteins, E3 ubiquitin-protein ligases, germin-like proteins, and chitinases, suggesting a rapid and robust defence response to *C. rosea* exposure. In contrast, NGB6704 showed induction of similar genes at 40h post-*C. rosea* application, although less intense, indicates a more modulated or controlled defence activation. In both genotypes, upregulation of E3 ubiquitin-protein ligase PUB23-like genes

Table 3. Number of differentially expressed genes (DEGs) specific to NGB6704, NGB348 and shared between the two genotypes.

		Total no. of DEG (Upregulated, Downregulated)		
Treatment	Hour	NGB6704	Shared	NGB348
Cr v Control	8 h	120 (97, 23)	170 (167, 3)	957 (899, 58)
	16 h	45 (34, 11)	28 (27, 1)	119 (75, 44)
	32 h	53 (47, 6)	30 (29, 1)	118 (111, 7)
	40 h	676 (476, 200)	31 (27, 4)	28 (24, 4)
Zt v Control	32 h	1444 (1295, 150)	72 (71, 1)	63 (61, 3)
	40 h	543 (341, 202)	–	6 (3, 3)
ZtCr v Control	32 h	62 (52, 10)	27 (26, 1)	266 (247, 19)
	40 h	155 (116, 39)	24 (22, 2)	58 (46, 12)

(3 genes in NGB348 and 2 genes in NGB6704) was observed at 8h post-*C. rosea* application was observed. Ubiquitin E3 ligases can positively or negatively regulate plant immunity by controlling the degradation of diverse protein substrates (McLellan et al. 2020). These genes are also localised in the *C rosea* biocontrol efficacy-associated region on chromosome 6B, suggesting that there may be structural differences between alleles, contributing to the plant genotype-mediated response to *C. rosea*. The E3 ubiquitin ligase has also been demonstrated to play an important role in plant immunity, where its downregulation during the interaction with a small secreted protein from *Z. tritici* was found to increase disease susceptibility (Karki et al. 2021).

Notably, *C. rosea*-induced genes in NGB348 at early inoculation were similar to genes upregulated upon *Z. tritici* application at 32h and 40h in NGB6704. Cross-referencing DEGs with genes located in genomic regions segregating with exposure to *Z. tritici* exclusively and in combination with *C. rosea* in the **paper I** further highlighted this pattern. Specifically, upregulation of several receptor-like protein kinase-like genes, ABC transporter G family member 32-like protein, an oxalate oxidase, and a chitin elicitor receptor kinase 1-like genes was observed in NGB348 upon *C. rosea* inoculation and in NGB6704 upon *Z. tritici* inoculation. Taken together, it may be suggested that *C. rosea* may have initially activated defence genes in NGB348, which returned to normal expression levels later. On the other hand, in NGB6704, C. rosea may have initially evaded plant immune responses and is subsequently followed by a stronger PTI response. Co-inoculation of *C. rosea* and *Z. tritici* showed a strong and similar defence

response in both genotypes; however, a temporal difference was observed. Stronger gene expression was observed for NGB348 at 32h and NGB6704 at 40h. As NGB348 did not respond strongly to *Z. tritici* exclusive application, the response can potentially be driven by *C. rosea* stimulating a more dominant defence response in NGB348, even in the presence of *Z. tritici*. In NGB6704, a stronger response at 40h may be speculated to be related to the higher biomass of both fungi.

4.5 Concluding remarks

In conclusion, this study highlights the genotype-specific responses in gene expression to both *C. rosea* and *Z. tritici*, which may affect biocontrol efficacy. Specifically, genotype-specific induction of defence-related genes by *C. rosea* was demonstrated, highlighting the delicate and intricate nature of these interactions. Strong upregulation of defence-related genes in NGB348 with low biocontrol efficacy may suggest an exaggerated defence response to *C. rosea*, potentially hindering the effectiveness of *C. rosea*-mediated biocontrol. However, further studies are required to better understand the varying molecular responses between plant genotypes due to *C. rosea*, ultimately bettering its biocontrol efficacy.

5. Synthesis and future perspectives

The aim of this thesis was to gain a deeper understanding of the role that plant genetic variation plays in interactions among plants, pathogens and biological control agents. A combined transcriptomics and population genetics approach was utilised in an attempt to dissect the complex mechanisms underlying these tripartite interactions.

Summary

Using winter wheat germplasm, variation in plant genotype-specific biocontrol efficacy of *Clonostachys rosea* IK726 was explored in controlling the pathogens *Zymoseptoria tritici* causing septoria tritici blotch and *Fusarium graminearum* causing fusarium foot rot. The two pathogens were chosen for their economic and future importance in a north European context (Savary et al. 2019; Strandberg et al. 2024) and utilising already established bioassay systems (Jensen et al. 2000; Odilbekov et al. 2019). The biocontrol agent *C. rosea* was used, which is reported to control diseases caused by more than 30 plant pathogens, including *Z. tritici* and *F. graminearum* (Jensen et al. 2022). Using genome-wide association studies, independent regions associated with disease susceptibility and *C. rosea*-mediated biocontrol efficacy were identified. Biocontrol efficacy between the two systems also varied, with a very good overall disease control of fusarium foot rot and only a few genotypes responding positively and negatively in controlling septoria tritici blotch. For septoria tritici blotch, by exploring the gene expression changes between genotypes varying for biocontrol efficacy, genotype-specific and time-specific induction of defence-related genes by *C. rosea* was demonstrated. Taken together, the results illustrate the complexities and sensitivities at the cellular and physiological levels in plants during interactions with pathogens and biological control agents. The data generated in this work can be directly utilised by various stakeholders in plant breeding and plant protection. Moreover, *C. rosea* biocontrol efficacy variation shows potential for further biocontrol optimisation with

future breeding efforts by selecting plant genotypes with favourable agronomic traits, disease resistance and improved biocontrol efficacy.

Simplifying the complexities, pros and cons

Plant-microbial interactions are very complex. In the last decades, the gain in understanding of the molecular dialogue in plant-pathogen interactions has been exciting, sometimes surprising, and indeed humbling. More importantly, it is incomplete, and there is still much more to understand. Matters are further complicated by adding another layer of complexity in tripartite interactions involving plants, pathogens and beneficial microorganisms.

The scientific method often advocates simple and controlled experiments to detect small differences from tested factors while controlling for environmental variation (Montesinos 2024). In this work, pot experiments in greenhouses and growth chambers were conducted under controlled conditions. This allowed for backwards and potentially forward compatibility of experiments and, more importantly, controlled for environmental variation to focus on variation inherent to winter wheat genotypes in response to pathogens and *C. rosea*. Controlling variation in transcriptomics experiments is a necessity; however, exploring plant genetic variation in controlled conditions for quantitative traits such as disease susceptibility and biocontrol efficacy has its advantages and disadvantages. Natural disease occurrence for pathogens, including *Z. tritici* and *F. graminearum,* can vary spatiotemporally, and this can be overcome with controlled and consistent inoculations. In some cases, disease assessment between field trials and controlled experiments can correlate well, as previously reported, when comparing field results with the above-used sand-based bioassay ($R = 0.94$, $P < 0.001$) for fusarium foot rot severity caused by *F. culmorum* in wheat and barley (Jensen et al. 2000). Nevertheless, field testing is warranted, as it offers more realistic conditions and takes environmental variables into account. Biocontrol of STB using *C. rosea* IK726 has been previously reported from multi-year field trials in Denmark (Jensen et al. 2024). In this work, field testing of *C. rosea* biocontrol efficacy variation involving natural infection of *Z. tritici* on wheat varieties under development was planned; however, due to the absence of disease occurrence[8], it was not feasible.

In this work, single strains of *Z. tritici, F. graminearum* and *C. rosea* were used. This was motivated by the aim of exploring genetic variation on the plant side. However, to generalise and broaden these findings, future

[8] Tina Henriksson, Lantmännen, personal communication

research should examine the impact of diverse pathogens, biological control agents and their strains. Moreover, to determine the underlying mechanisms of genotype-specificity, bioinformatic and biometrics approaches utilised in this study should be further complemented with microscopy and functional studies. As these pathogens can also cause disease in mature plants, future studies assessing disease susceptibility and biocontrol efficacy at the adult plant stage can aid in exploring the temporal variation for these traits. Additionally, in this thesis, the role of the microbiome is not taken into consideration. The plant-associated microbiome can significantly influence plant performance and disease outcome, and it has also been utilised to identify novel BCAs (Brader et al. 2017; French et al. 2021; Collinge et al. 2022). The impact of BCA application on the composition of the plant-associated microbiome and its subsequent effect on BCA performance is another important area of investigation.

Knowledge transfer and potential industrial application

This thesis is developed under the Grogrund[9] initiative, a collaborative initiative at the Swedish University of Agricultural Sciences working with academia, society and the business sector to build competencies within plant breeding. Within the Grogrund project "Breeding for Biologicals[10]", the role of wheat and sugar beet plant genotypes during interactions with beneficial microorganisms is explored to optimise and exploit the interaction in agricultural systems using plant breeding. Taken together, the two work packages explore the role of plant genotypes in modulating the effects of beneficial microorganisms for biostimulation and biocontrol efficacy.

In this work, the data generated by phenotyping the two pathogens and their disease control by *C. rosea* can be utilised in the plant breeding industry. Genotypes tested in this study are used as pre-breeding material in Swedish wheat breeding programs. Moreover, the identification of genetic regions and localised genes can be directly screened in the breeding material under development. Notably, a positive correlation between disease susceptibility and *C. rosea* biocontrol efficacy was observed, indicating that susceptible genotypes benefitted more from *C. rosea* application. However, the correlation was moderate, and marker-assisted selection can aid in breaking the negative linkage and, ultimately, simultaneous selection of both traits. Plant disease resistance should act as the first line of defence, and

[9] https://www.slu.se/en/Collaborative-Centres-and-Projects/grogrund/

[10] Short for: "Plant breeding for optimised interactions between crops and microorganisms to enhance disease management and production with reduced agrochemical use". https://www.slu.se/en/Collaborative-Centres-and-Projects/grogrund/projekt/plant-breeding-for-optimised-interactions-between-crops-and-microorganisms-to-enhance-disease-management-and-production-with-reduced-agrochemical-use/

therefore, it must be emphasised that any further manipulation in cultivar development, such as biocontrol compatibility breeding, should not come at the cost of undermining disease resistance. Furthermore, various beneficial microorganisms also promote growth in plants; therefore, the relationship with other growth, development and agronomic traits should also be taken into consideration. Moreover, the plant protection industry is heavily investing in and diversifying into biological solutions for disease management. Formulations of *C. rosea* are licensed and commercially available in Europe. However, most commercial products are generically recommended, and exploring specificity in biocontrol agent performance will be crucial to optimise further and improve their efficacy.

Looking forward

Biological systems and their ecological interactions are complex. In the last 50 years, since the establishment of the gene-for-gene concept, significant progress has been made by continuous selection for disease resistance in crops. In the tripartite interactions among plants, pathogens, and beneficial microorganisms, underlying gene-gene-gene interactions may be hypothesised. In this thesis, only plant variation was explored; however, future studies can be motivated to investigate variation in all three interacting organisms.

We are living in troubling times with the climate crisis dominating our day-to-day decisions and outcomes. Food production is very resource intensive, with large negative impacts on biodiversity and environmental health. At this point, sustainable food production is not only desirable but also necessary. Integrated pest management to reduce reliance on chemical pesticides by incorporating various cultural, mechanical, and biological strategies for pest and disease management has the potential to be further optimised. Towards this goal, this thesis suggests further improvement of biocontrol application by optimising its interactions with plants. The transition to food production systems with lower chemical pesticide input can be facilitated by breeding plants with a high genetic potential to benefit from the application of beneficial microorganisms. Already established methods in plant pathology and plant breeding can be exploited in this new context. Overall, further research is recommended, and it may be hoped that conscious collaborative efforts by research institutions, government agencies, private stakeholders and farmers can further advance our knowledge in this field. There is great potential for more sustainable agriculture, in which effective biocontrol solutions can play a significant role.

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Popular science summary

The agricultural sector is one of the biggest contributors to climate change. To maintain high production, current agricultural practices rely heavily on fertilisers and chemical pesticides to protect against pests and pathogens. Unsustainable use of chemical pesticides can pollute soil and water and harm non-target organisms such as plants, animals, insects and microorganisms. To reduce reliance on pesticides, a holistic approach combining various agronomic, mechanical and biological practices is recommended within the integrated pest management (IPM) framework. Within the European Union, Directive 2009/128/EC even mandates all plant production professionals to comply with IPM principles. Biological control, i.e. exploiting beneficial organisms to manage pests and pathogens, is a sustainable alternative to chemical pesticides, and the European Commission also recommends its use for sustainable plant protection. Many biological control agents (BCAs) are microorganisms, and they can vary in performance and efficacy due to various biological and environmental factors. One of the factors that can influence BCA efficacy is their interaction with plants. Plant-microbe interactions involve complex molecular mechanisms, which aid plants in recognising pathogens and other microorganisms, while microbes utilise their molecular strategies to overcome or suppress plant defences. It has been established that plants exhibit genetic variation, which makes some individuals (genotypes) more or less susceptible to pathogens than others; however, how plant genetic variation can influence BCA efficacy is still not well understood.

This thesis aimed to better understand the influence of plant genetic variation on BCA efficacy using approximately 200 winter wheat genotypes. Two commercially important fungal pathogens, *Zymoseptoria tritici,* causing septoria tritici blotch (STB) and *Fusarium graminearum,* causing fusarium foot rot (FFR), were used. Disease management of these two pathogens contributes towards a large proportion of overall fungicide use globally. *Clonostachys rosea* was used as a BCA, which is reported to

successfully control more than 30 pathogens, including *Z. tritici* and *F. graminearum*. Wheat genetic variation was explored by subjecting plants directly to the pathogen inoculation and on plants initially treated with *C. rosea,* which allowed disease susceptibility to be differentiated from biocontrol efficacy. The degree to which a particular genotype benefitted from *C. rosea* application in disease reduction was used as a measure for biocontrol efficacy. Disease susceptibility and biocontrol efficacy were estimated at the phenotypic level by visually assessing the disease, and underlying genetic regions associated with the phenotypic variation were identified using genetic markers in the genome-wide association studies. The results showed differences among winter wheat genotypes in susceptibility to both pathogens and genetic regions associated with disease resistance were identified. In addition, *C. rosea* biocontrol efficacy also varied among winter wheat genotypes, and genetic regions associated with biocontrol efficacy were distinct from disease susceptibility. Biocontrol efficacy by *C. rosea* showed better control of FFR compared to STB. Disease susceptibility and biocontrol efficacy also showed a positive correlation, indicating that susceptible plants benefitted more from *C. rosea* application. Furthermore, changes in gene expression of underlying molecular mechanisms in wheat genotypes varying for biocontrol efficacy were investigated in direct interactions with *Z. tritici* and *C. rosea* exclusively and during their co-inoculation. The results show that *C. rosea* can induce distinct sets of defence-related genes directly and in the presence of *Z. tritici*, which can vary between genotypes with high and low biocontrol efficacy.

In summary, these findings demonstrate that winter wheat germplasm exhibits genetic variation for disease susceptibility caused by pathogens *Z. tritici* and *F. graminearum* and for *C. rosea* biocontrol efficacy of these diseases. Plant breeders consistently exploit plant genetic diversity for disease resistance to develop resistant cultivars. Similarly, genetic variation can potentially be utilised to optimise the biocontrol efficacy of *C. rosea*. Using molecular markers, the selection of genotypes with lower susceptibility and higher biocontrol efficacy may be possible, making the simultaneous selection of traits feasible in future breeding programs. However, further research is recommended to expand these findings in other systems using diverse pathogens, BCAs and plant populations to better understand the breeding potential of biocontrol efficacy. The insights gained in this thesis contribute towards optimising biological control applications and offer knowledge that will support future disease management strategies and plant breeding initiatives, with the ultimate aim of minimising reliance on chemical pesticides.

Populärvetenskaplig sammanfattning

Jordbrukssektorn är en av de sektorer som bidrar mest till klimatförändringarna. För att upprätthålla en hög produktion är nuvarande jordbruksmetoder starkt beroende av konstgödningsmedel och kemiska växtskyddsmedel för att skydda grödan mot skadedjur och sjukdomar. Användning av kemiska växtskyddsmedel kan dock förorena mark och vatten och skada andra växter, djur, insekter och mikroorganismer. För att minska beroendet av kemiska växtskyddsmedel rekommenderas ett holistiskt synsätt som kombinerar olika agronomiska, mekaniska och biologiska metoder inom ramen för integrerat växtskydd (IPM). Inom EU föreskriver direktiv 2009/128/EG att alla som arbetar med växtproduktion ska följa principerna för IPM eller ekologisk odling. Biologisk bekämpning, dvs. utnyttjande av nyttoorganismer för att hantera skadedjur och patogener, är ett hållbart alternativ till kemiska växtskyddsmedel, och EU-kommissionen rekommenderar också att det används för hållbart växtskydd. Många biologiska bekämpningsorganismer (BCA) är mikroorganismer, och de kan variera i effektivitet på grund av olika biologiska och miljömässiga faktorer. En av de faktorer som kan påverka effektivitet hos biologisk bekämpning är interaktionen med växten. Växter och mikroorganismer samverkar genom komplexa molekylära mekanismer som hjälper växterna att känna igen sjukdomsalstrande patogener och andra mikroorganismer, medan mikroberna använder sina molekylära strategier för att övervinna eller undertrycka växternas försvar. Det är etablerat att växter bär på genetisk variation vilket gör vissa individer (genotyper) mer eller mindre mottagliga för patogener än andra. Hur växters genetisk variation kan påverka effektiviteten av biologisk bekämpning är dock fortfarande inte väl studerat.

Syftet med denna avhandling är att bättre förstå hur växters genetisk variation påverkar effektiviteten av biologisk bekämpning. För detta ändamål användes cirka 200 genotyper av höstvete, BCA svampen *Clonostachys rosea*, samt två viktiga svampsjukdomar; svartpricksjuka orsakad av *Zymoseptoria tritici* och stråbasröta som orsakas av *Fusarium*

graminearum. Bekämpning av sjukdomar orsakade av dessa två patogener står för en stor del av den globala användningen av fungicider. *Clonostachys rosea* har framgångsrikt använts för biologisk bekämpning av mer än trettio sjukdomar/patogener, inklusive *Z. tritici* och *F. graminearum*. Vetets fenotypiska variation undersöktes genom att plantor inokulerades med patogenen, med eller utan en förbehandling med *C. rosea*, vilket gjorde att mottagligheten för sjukdomarna kunde särskiljas från effekten av den biologiska bekämpningen. Som mått på den biologiska bekämpningens effektivitet användes i vilken grad en viss genotyp gynnades av *C. rosea*-behandlingen när det gällde minskningen av sjukdomen. Sjukdomsmottagligheten och effektiviteten av den biologisk bekämpning uppskattades genom visuell bedömning av sjukdomen, och regioner i vetets arvsmassa som var associerade med den fenotypiska variationen identifierades med hjälp av genetiska markörer i storskaliga associationsstudierna.

Resultaten visade att det fanns skillnader mellan olika genotyper av höstvete när det gäller mottaglighet för båda sjukdomarna, och genetiska regioner som var kopplade till mottaglighet/resistens identifierades. Dessutom varierade även effektiviteten av den biologiska bekämpningen mellan genotyperna, och de genetiska regioner som var associerade med denna egenskap skilde sig från de regioner som var kopplade till mottaglighet för sjukdomen. Den biologiska bekämpningen av stråbasröta var betydligt mer effektiv än bekämpningen av svartpricksjuka. Hög mottaglighet för sjukdomen och hög effektivitet av biologisk bekämpning var positivt korrelerade, vilket tyder på att mottagliga växter hade större nytta av *C. rosea*. Dessutom undersöktes förändringar i geners aktivitet över tid efter inokulering med *C. rosea*, *Z. tritici*, eller båda svamparna samtidigt. Resultaten visar att *C. rosea* kan inducera specifika gener kopplade till växtens immunförsvar, med eller utan *Z. tritici*. Resultatet visade dock på en tidsmässig skillnad mellan genotyperna, där effektiv biologisk bekämpning var kopplad till en långsam men kraftig inducering av immunförsvaret.

Sammanfattningsvis visar dessa resultat att genotyper av höstvete uppvisar genetisk variation för mottaglighet/resistens mot sjukdomarna svartpricksjuka och stråbasröta, samt effektiviteten av biologisk bekämpning av nämnda sjukdomar med *C. rosea*. Växtförädlare utnyttjar växternas genetiska variation för att utveckla sjukdomsresistenta sorter. På samma sätt kan genetisk variation potentiellt utnyttjas för att förädla fram växter med hög förmåga att dra fördel av nyttiga mikroorganismer, såsom *C. rosea* för biologisk bekämpning av sjukdomar. Molekylära markörer kan underlätta växtförädlarnas arbete med urvalet av genotyper med hög resistens och hög kompatibilitet med nyttiga mikroorganismer i framtida förädlingsprogram.

Ytterligare forskning behövs dock för att förstå hur dessa resultat kan överföras till andra grödor, patogener och BCAs. Den kunskap som genererats i detta arbete bidrar till att optimera tillämpningar av biologisk bekämpning och växtförädling, som bidrar till framtidens effektiva och miljövänliga växtskyddstrategier och en minskad användning av kemiska växtskyddsmedel.

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अंत में, भारत में मेरे बड़े परिवार को भरपूर श्रेय जाता है। मम्मी और पापा, मैं आज जो भी हूँ, वह आपकी वजह से हूँ। अगर पापा आज यहाँ होते तो उन्हें बहुत खुशी होती। दीपांशी, हम अलग-अलग देशों में रहते हुए भी एक-दूसरे के करीब हैं। परिवार के बाकी सदस्यों का नाम लिए बिना, आप सभी ने बहुत बड़ा सहयोग दिया। परिवार का मूल्य मेरे लिये अतुलनीय है।

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Plant Genetics and Genomics

Genotypic variation in winter wheat for fusarium foot rot and its biocontrol using *Clonostachys rosea*

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Biological control to manage plant diseases is an environmentally friendly alternative to using chemical pesticides. However, little is known about the role of genetic variation in plants affecting the efficacy of biological control agents (BCAs). The aim of this study was to explore the genetic variation in winter wheat for disease susceptibility to fusarium foot rot caused by *Fusarium graminearum* and variation in biocontrol efficacy of the fungal BCA *Clonostachys rosea* to control the disease. In total, 190 winter wheat genotypes were evaluated under controlled conditions in 2 treatments, i.e. (1) *F. graminearum* (Fg) and (2) *F. graminearum* infection on *C. rosea*–treated seeds (FgCr). Alongside disease severity, plant growth-related traits such as shoot length and root length were also measured. Comparison of genotypes between the 2 treatments enabled the dissection of genotypic variation for disease resistance and *C. rosea* efficacy. The study revealed significant variation among plant genotypes for fusarium foot rot susceptibility and other growth traits in treatment Fg. Moreover, significant variation in *C. rosea* efficacy was also observed in genotype contrasts between the 2 treatments for all traits. Using a 20K marker array, a genome-wide association study was also performed. We identified a total of 18 significant marker–trait associations for disease resistance and *C. rosea* efficacy for all the traits. Moreover, the markers associated with disease resistance and *C. rosea* efficacy were not co-localized, highlighting the independent inheritance of these traits, which can facilitate simultaneous selection for cultivar improvement.

Keywords: biological control; *Clonostachys rosea*; disease resistance; *Fusarium graminearum*; GWAS; wheat

Introduction

Agricultural production relies heavily on the use of chemical pesticides to achieve optimal yields and quality. According to the latest report from the Food and Agriculture Organization of the United Nations (FAO 2022), pesticide usage has increased by about 50% from 1.2 kg/ha in 1990 to 1.8 kg/ha in 2020 with a total amount of active ingredients at 2.7 million tons. The overreliance of agricultural systems on chemical pesticides has led to negative environmental impacts such as soil and water contamination, impacting nontargeted plants and animals, and biodiversity losses (Tudi *et al.* 2021). Moreover, resistance evolution to pesticide application in pathogens is a severe problem affecting efficacy and future crop security (Gould *et al.* 2018; Karlsson Green *et al.* 2020). Integrated pest management (IPM) approaches to managing pests and pathogens below economic injury levels using a combination of sustainable methods offers considerable potential to reduce the dependence on chemical pesticides in agricultural systems. Furthermore, the European Union Framework Directive 2009/128/EC asks all plant production professionals to comply with IPM principles (European Union 2009; Barzman *et al.* 2015; Karlsson Green *et al.* 2020). One such potential IPM approach is using biological control methods for pest and pathogen management. The use of biological control is specifically recommended in the European Commission's proposal for a new regulation on the sustainable use of plant protection products to reduce the use of synthetic chemical pesticides by 50% by 2030 as per the European Green Deal (European Commission 2022).

Biological control, or biocontrol, is defined as the exploitation of living organisms (biological control agents, BCA) to combat pests and pathogens, directly or indirectly, to provide human benefits (Stenberg *et al.* 2021). There are already numerous bacterial, fungal, oomycete, and viral BCAs that have been isolated, tested, and successfully commercialized (Collinge *et al.* 2022). The global market for BCAs is continuously growing, with a market value of 5.61 billion USD in 2021 and with a projected market value in 2029 of 18.15 billion USD in 2029, reflecting the demand from various players involved in plant protection (Fortune Business Insights 2022). The modes of action of BCAs can be classified into 4 categories: (1) exploitative competition for resources such as oxygen, carbon, nitrogen, and other vital nutrients, (2) interference competition for space, achieved through antibiosis, where the BCA inhibits the pathogen by producing toxic specialized metabolites or enzymes, (3) hyperparasitism, where the BCA acts as a predator, preying on the pathogen, (4) induced resistance, involving the indirect interaction of a BCA by triggering plant defense mechanisms against invading pathogens (Jensen *et al.* 2017; Collinge

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et al. 2022). It is possible for a BCA to exhibit more than one mode of action against a pathogen and it can vary depending on the pathogen, plant, and other environmental factors (Jensen et al. 2021).

Clonostachys rosea is one such BCA, which is an ascomycete fungus with a generalist lifestyle including saprotrophism, plant endophytism, and mycoparasitism (Schroers et al. 1999; Jensen et al. 2021). Using *C. rosea* in augmentative biological control strategies, where it is released into target areas after mass-rearing, it has been reported to exhibit biocontrol properties against a multitude of fungal and oomycete pathogens. Different strategies employed by *C. rosea* in interactions with other microorganisms, such as competition for nutrients and space (Sutton et al. 1997), antibiosis (Han et al. 2020; Saraiva et al. 2020), induction of plant defense responses (Wang et al. 2019; Kamou et al. 2020), and direct parasitism (Barnett and Lilly 1962; Jensen et al. 2021), are reported in the literature. *C. rosea* strain IK726 was isolated from barley roots in 1992 (Knudsen et al. 1995), the genome was sequenced in 2015 (Karlsson et al. 2015), and it has been explored in detail for its mycoparasitism and modes of action. As summarized in Jensen et al. (2021), *C. rosea*–mediated biocontrol is observed against a multitude of pathogens, such as *Botrytis cinerea* in strawberry, raspberry, rose, and tomato; *Fusarium* spp. in tomato, pine, cereals, and pulses; *Plasmodiophora brassicae* in Brassicaceae crops; *Puccinia* spp. in cereals; *Zymoseptoria tritici* in wheat; *Alternaria* spp. in tomato, carrot, and pulses; *Pythium* spp.; and *Phytophthora* spp. in various crops.

Plant breeding is another integral part of sustainable agriculture and IPM, offering a sustainable and cost-effective approach to pest control by enhancing resistance to biotic and abiotic stresses and increasing yield. Breeding efforts for winter wheat in Europe in the last decades have led to a steady increase in yield potential and improved resistance to diseases and abiotic stresses (Voss-Fels et al. 2019; Leišová-Svobodová et al. 2020; Zetzsche et al. 2020; Laidig et al. 2021). Among the pathogens in wheat cultivation, *Fusarium* spp., which are often present as a species complex, are one of the most devastating and economically important groups of pathogens infecting various plant parts at different growth stages, causing fusarium foot rot, fusarium root rot, fusarium seedling blight, fusarium crown rot, and fusarium head blight (Dean et al. 2012; Karlsson et al. 2021). *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium avenaceum*, and *Fusarium poae* are the species with the highest incidence of fusarium head blight in Europe (Becher et al. 2013). In the last decades, a lot of breeding efforts have been made to identify quantitative trait loci (QTL) for the management of fusarium head blight across the globe (Buerstmayr et al. 2020). In addition to causing fusarium head blight, *Fusarium* spp. are also economically important pathogens causing ground-level and below-ground diseases in dry climates across continents (Kazan and Gardiner 2018). Moreover, with the changing climate and increasing temperatures in northern Europe, *F. graminearum* is also predicted to become more important in the future (Strandberg et al. 2024). While the understanding of *F. graminearum* causing head blight is well-developed, knowledge about its infestation at early stages, leading to blights, foot rot, and root rot, remains limited (Voss-Fels et al. 2018). Furthermore, resistance to fusarium head blight does not always correlate with resistance to fusarium crown rot and fusarium root rot, which is suggested to be due to differences in host plant resistance (Li et al. 2010; Wang et al. 2015). Therefore, it is essential to explore the genetic architecture for resistance to *F. graminearum* causing ground-level and below-ground diseases.

Alongside resistance genotypes, chemical seed treatment is used to manage seed-borne and seedling-stage diseases. Seed treatment with BCAs, instead of chemical pesticides, can be an environmentally friendly alternative (Jensen et al. 2000). However, it has been frequently proposed that the disease control efficacy of BCAs can be modulated by plant genotype variation (Smith and Goodman 1999; Stenberg et al. 2015; Köhl et al. 2019; Collinge et al. 2022). However, these tri-partite interactions among plant genotypes–pathogen–BCA have mostly been explored with a limited number of plant genotypes. Moraga-Suazo et al. (2016) reported a differential response of 2 contrasting *Pinus radiata* genotypes toward *C. rosea*–mediated biocontrol of the pitch canker pathogen *Fusarium circinatum*. The study demonstrated the ability of *C. rosea* to produce plant genotype-specific induced systemic resistance (ISR). Tucci et al. (2011) also reported differences among 5 tomato genotypes for enhanced ISR against the gray mold pathogen *B. cinerea* using *Trichoderma atroviride* and *Trichoderma harzianum*. Furthermore, Arkhipov et al. (2023) showed variation among 6 tomato genotypes toward *Phytophthora capsici* biocontrol by *Pseudomonas azotoformans*, which involved induction of ISR involving a hypersensitive response. Ryan et al. (2004) reported differences in the effectiveness of BCAs for potato scab among 5 cultivars in field trials. Biocontrol efficacy of *Pseudomonas fluorescens* against *Pythium ultimum* was also observed to differ among 3 wheat cultivars (Meyer et al. 2010). Furthermore, Rebeka et al. (2013) revealed significant differences among 50 genotypes for *Fusarium oxysporum* compatibility in controlling *Striga hermonthica*. In a study by Smith et al. (1999), variation among 61 tomato genotypes in interacting with disease suppressive bacteria *Bacillus cereus* is shown against the pathogen *Pythium torulosum*. Moreover, differences among plant genotypes were also observed for biostimulation by *Trichoderma* spp. as shown in sugar beet for plant dry weight and shoot dry weight (Schmidt et al. 2020) and lentils for root and shoot development parameters (Prashar and Vandenberg 2017). These examples show that plant genotypes impact the compatibility between plants and beneficial microorganisms. Therefore, considering plant genetic variation is crucial for the effective deployment of BCAs. Understanding the genetic basis of host plant interactions with BCAs offers opportunities to augment traditional plant breeding for yield and resistance traits with enhanced compatibility with beneficial microorganisms.

In this study, we hypothesized that wheat genotypes vary in their susceptibility to *F. graminearum* causing foot and root rot and *C. rosea*–mediated biocontrol efficacy to control the disease. Specifically, the objectives were to (1) test for plant genotype variation in 190 winter wheat genotypes for resistance to *F. graminearum* causing foot and root rot; (2) test for plant genotype variation for *C. rosea*–induced biocontrol efficacy against fusarium foot and root rot; and (3) conduct a genome-wide association study (GWAS) to identify marker–trait associations of fusarium foot and root rot disease resistance and *C. rosea*–mediated biocontrol efficacy, and to determine whether these traits are inherited together or independently.

Materials and methods

Plant and fungal material

In this study, a total of 190 winter wheat genotypes were used, which included landraces and cultivars initially obtained from the Nordic Genetic Resource Center and later multiplied (Supplementary Table 1). For foot and root rot disease, *F. graminearum* strain PH1 was used as the pathogen in this study (Trail and Common 2000). The strain was revived from −80°C glycerol stock and grown on potato dextrose agar (PDA) media (BD Difco Laboratories, France) at 20°C in dark conditions. BCA *C. rosea*

strain IK726, initially isolated from barley roots in Denmark, was used (Knudsen *et al.* 1995). The strain was revived from a glycerol conidial stock stored at −80°C and grown on PDA media petri plates at 20°C in dark conditions.

Bioassay setup

Bioassays for *F. graminearum* foot and root rot and *C. rosea* biocontrol efficacy were conducted in the sand seedling test modified from the test described previously (Knudsen *et al.* 1995). In total, surface sterilized seeds of 190 genotypes were tested for FRR disease resistance and *C. rosea* biocontrol efficacy under 2 treatments: (1) Fg (pathogen only) and (2) FgCr (pathogen and BCA *C. rosea*). Three seeds were sown per pot (5 × 5 × 5 cm) in trays of 40 pots. Pathogen inoculation was carried out in both treatments by placing a 5 mm diameter *F. graminearum* agar plug equidistant from seeds in the pot. For the BCA seed coating in the treatment FgCr, a conidial suspension of *C. rosea* was made by flooding the PDA plates with sterile water, followed by filtration through Miracloth (Merck KGaA, Darmstadt, Germany) to remove mycelia and growth media. Seed surface coating with *C. rosea* conidia at the concentration of 1×10^6 cfu/mL (colony forming units per mL) was performed by shaking the seeds in *C. rosea* suspension on a rotary shaker at 120 rpm for 30 min. For treatment Fg, seeds were shaken as above in sterile water.

To accommodate 190 winter wheat genotypes, the experiment was conducted in 6 batches, each testing a subset of genotypes. Within each batch, a randomized complete block design was used with 5 trays randomly assigned to each treatment (Fg and FgCr), making 5 biological replicates per genotype. To account for batch-to-batch variation, 3 check genotypes (Kranich, Stava, and Festival) were used in all trays of each batch. Trays were kept in a growth chamber with a photoperiod of 16 h light (200 μmol/m^2 s) at 20°C and 8 h dark at 16°C. Plants were grown for 19 days and the germinated seedlings were harvested and evaluated for disease symptom scoring on a 0–4 scale with 0.5 increments, 0 = healthy plants with no symptoms and 4 = dead plants, as previously described (Knudsen *et al.* 1995). Moreover, shoot and root length (±0.5 cm) were measured and combined to make plant length (±1 cm).

Phenotypic data analysis

Unadjusted arithmetic means from each pot were used for the analysis. To estimate the best linear unbiased estimators (BLUEs) of genotypes in treatments Fg and FgCr, a mixed model approach using Kenward–Roger's approximation of the degrees of freedom was used (Kenward and Roger 1997). The model is as follows:

$$y_{ijkl} = \mu + r_i + b_{ij} + g_k + t_l + (gt)_{kl} + \varepsilon_{ijkl}$$

where y_{ijkl} is the BLUE estimate for the y-th trait of the k-th genotype in the l-th treatment, μ denotes the overall mean; r_i is the effect of the i-th batch, b_{ij} the effect of the j-th block nested within the i-th batch, g_k the effect of the k-th genotype, t_l the effect of l-th treatment, $(gt)_{kl}$ the interaction effect of the k-th genotype with the l-th treatment, and ε_{ijkl} the residual term. Batches and blocks nested within batches were treated as random factors.

Analysis of variance (ANOVA) was performed on the model to evaluate the significance of various model terms. BLUEs were estimated for genotypes in each treatment. Inter–treatment contrasts for each genotype were used as estimators for the biocontrol efficacy effect. To facilitate interpretation, the contrast direction was Fg–FgCr for disease score, where a positive value indicated disease reduction in *C. rosea* seed treatment; while the contrast direction was FgCr–Fg for shoot length, root length, and plant length, where a positive value indicated length increase with *C. rosea* seed treatment. A post–hoc Tukey's test was performed to test the significance of inter–treatment contrasts, and false discovery rate–adjusted *P*-values were used to correct for multiple testing (Benjamini and Hochberg 1995). Broad-sense heritability of traits as H_P^2 after Piepho and Möhring (2007) and H_C^2 after Cullis *et al.* (2006) was also estimated separately in each treatment following a reduced version of the above-described model without any treatment effect and genotype × treatment interaction effect.

Genome-wide association analysis

A total of 181 out of 190 winter wheat genotypes used in the current panel were previously genotyped using a 20K single nucleotide polymorphism (SNP) marker array at TraitGenetics GmbH, Germany (Odilbekov *et al.* 2019). A total of 7,360 SNP markers were retained for the GWAS after filtering out the markers with >20% missing alleles and <5% minor allele frequency. The remaining missing alleles were imputed to the major allele. For GWAS, a total of 5 different models were used as follows: GLM (Price *et al.* 2006), MLM (Yu *et al.* 2006), MLMM (Segura *et al.* 2012), FarmCPU (Liu *et al.* 2016), and BLINK (Huang *et al.* 2019). GLM and MLM are single-locus GWAS models, whereas MLMM, FarmCPU, and BLINK are multiple loci models. To correct for relatedness and population structure, the kinship matrix and the first 17 principal components (explaining 50% variation) were used as covariates in the analyses. For significant marker–trait association, a threshold of negative log (1/number of SNP markers) was used to overcome the over stringency of the Bonferroni test threshold (0.05/number of SNP markers) and low sample size (Yang *et al.* 2011; Wang *et al.* 2012). For each significant marker at the negative log threshold, an allelic level comparison was made for the phenotypic distribution of the trait using 1-way ANOVA, followed by a Tukey's post–hoc test. Heterozygous alleles with a frequency <5 were dropped prior to the comparisons.

All statistical analyses were performed using the statistical software R version 4.3.1 "Beagle Scouts" (R Core Team 2023). The linear mixed model analysis was performed using the package "lme4" version 1.1-35.3 (Bates *et al.* 2015) and its extension "lmerTest" version 3.1-3 (Kuznetsova *et al.* 2017). In addition, the estimation of BLUEs and post–hoc comparisons of individual genotypes between treatments were performed using packages "emmeans" version 1.10.1 (Lenth 2023), "multcomp" version 1.4-25 (Hothorn *et al.* 2008), and "multcompView" version 0.1-10 (Graves *et al.* 2023). Genome-wide association analysis was performed using the genome association and prediction integrated tool (GAPIT) version 3 (Wang and Zhang 2021). "Tidyverse" suite version 2.0.0 was used for most data processing and visualization alongside other dependency packages (Wickham *et al.* 2019).

Candidate gene identification

To search for genes localized at significant SNP marker–trait associations, a stringent window of ± 100 kb was explored. Firstly, the physical positions of SNP markers were identified by mapping SNP marker sequences against the *Triticum aestivum* IWGSC CS RefSeq v2.1 genome (GCF_018294505.1) using the BLAST algorithm (Altschul *et al.* 1990). Genes localized within ± 100 kb surrounding significant SNP markers were filtered using the gene annotation data available at the National Center for Biotechnology Information (NCBI) *T. aestivum* release 100 (2021 October 27th).

Further description of the genes was performed by searching the filtered genes in the gene library at NCBI.

Results

Performance of wheat genotypes across treatments

The performance of 190 winter wheat genotypes for fusarium foot rot and its biocontrol by *C. rosea* was evaluated in the absence (Fg) and presence (FgCr) of *C. rosea* seed treatment. Significant differences ($P < 0.001$) between treatments were observed for disease score, plant length, shoot length, and root length (Table 1). On average, the disease score was reduced by approximately half in treatment FgCr (1.42 ± 0.5) in comparison to treatment Fg where the disease was very high (3.4 ± 0.44) (Fig. 1a, Table 2, Supplementary Table 2). Similarly, estimates for root length, shoot length, and total plant length were almost doubled in treatment FgCr with *C. rosea* seed treatment (Fig. 1c, e, and g, Table 2, Supplementary Table 2). Heritability estimates for all traits ranged from low to moderate ranging from 0.14 to 0.6 for H^2_p and from 0.11 to 0.51 for H^2_C. Heritability estimates were lower in treatment Fg than in FgCr for disease score and shoot length, similar across treatments for plant height, and higher in treatment Fg than in FgCr for root length (Table 2). Overall, the 4 traits used in this study were found in highly significant correlation ($R > |0.85|$, $P < 0.001$) among each other (Supplementary Fig. 1). The 3 growth-related traits plant length, shoot length, and root length were in strong positive correlation with each other. Disease score was in the overall strong negative correlation with plant length ($R = -0.92$, $P < 0.001$), shoot length ($R = -0.91$, $P < 0.001$), and root length ($R = -0.87$, $P < 0.001$), emphasizing the impact of disease severity on growth. Particularly, a negative correlation of disease score was weaker in treatment FgCr for plant length ($R = -0.44$, $P < 0.001$), shoot length ($R = -0.43$, $P < 0.001$), and root length ($R = -0.31$, $P < 0.001$), suggesting a variable effect of *C. rosea* in reducing fusarium foot rot among wheat genotypes along with a variable impact on plant growth (Supplementary Fig. 1).

Intertreatment contrasts for *C. rosea* efficacy

Significant ($P < 0.0001$) genotype-by-treatment (G × T) interaction was observed for all the traits, suggesting that the performance of different genotypes varied significantly across the treatments (Table 1). Correlations between treatments showed a weak positive correlation for disease score ($R = 0.21$, $P = 0.004$), a weak negative correlation for root length ($R = -0.18$, $P = 0.016$), and no significant correlation for plant length and shoot length, further highlighting variability in genotype-to-genotype performance across treatments (Fig. 1b, d, f and h).

Pairwise contrasts between treatments (Fg–FgCr or FgCr–Fg) for each genotype were used as estimators for *C. rosea* efficacy, i.e. a higher difference in genotype performance between treatments reflects a greater effect of *C. rosea* seed treatment. For disease score, 180 genotypes had a significant ($P < 0.05$) reduction in disease score in the treatment FgCr ranging from 0.93 to 3.47 with an average reduction of 2.05 ± 0.52 (Fig. 2a, Supplementary Table 3). Similarly, most genotypes had a significant ($P < 0.05$) increase in plant length ($n = 163$), shoot length ($n = 166$), and root length ($n = 135$), reflecting the overall treatment effect of *C. rosea* (Fig. 2b–d, Supplementary Table 3). In treatment FgCr, in the presence of *C. rosea*, an average plant length increase of 13.6 ± 3.72 cm (6.68–23.9 cm), an average shoot length increase of 9.00 ± 2.56 cm (4.37–16.1 cm), and an average root length increase of 5.03 ± 1.30 cm (2.83–9.4 cm) was observed. Moreover, the above-described *C. rosea* efficacy estimates from pairwise contrasts were found in significant ($P < 0.001$) correlations with the estimates in the treatment Fg for each trait (Fig. 3). For disease score, a significant moderate positive correlation ($R = 0.57$, $P < 0.001$) was observed between *C. rosea*–mediated biocontrol efficacy to reduce disease and disease susceptibility in the treatment Fg, showing an overall increase in biocontrol efficacy among susceptible genotypes (Fig. 3a). Similarly, negative correlations between treatment Fg estimates and pairwise contrasts for *C. rosea* efficacy for plant length ($R = -0.7$, $P < 0.001$), shoot length ($R = -0.63$, $P < 0.001$), and root length ($R = -0.75$, $P < 0.001$) show that plants with poor growth in treatment Fg had a bigger benefit from *C. rosea* seed treatment (Fig. 3b–d).

Genome-wide marker–trait associations

Phenotypic estimates for genotypes from both treatments, Fg and FgCr, and pairwise contrasts for *C. rosea* efficacy in each trait were assessed for significant ($P \leq 0.00014$, after $P \leq 1/n$, where $n = 7{,}360$ is the number of SNP markers retained after filtering) genome-wide marker–trait associations. A total of 181 genotypes for treatment-level associations and 180 genotypes for contrasts had SNP data and phenotypic data and were retained in the analysis. For disease score, significant marker–trait associations were observed in treatment Fg on chromosome 1A at 53 cM, 2A at 115–116 cM, and 4B at 71–73 cM (Fig. 4a). Allele level comparisons at chromosome 1A show no differences in disease scores, significant reduction ($P < 0.05$) in disease scores in genotypes with minor alleles GG and AA for SNP markers BS00089497_51 and Kukri_c40121_373, respectively, at chromosome 2A, and also significant ($P < 0.05$) reduction in disease scores in genotypes with

Table 1. ANOVA results from linear mixed model analysis.

Trait	Term	Sum of squares	Mean squares	NumDF	DenDF	*F*-value	*P*-value	$P < 0.05$
Disease score (0–4)	G	169.5	0.9	189	1,288.6	1.7	5.8044E−07	*
	T	1,707.8	1,707.8	1	1,523.5	3,156.4	0	*
	G × T	142.7	0.8	187	1,518.1	1.4	0.00047086	*
Plant length (cm)	G	8,845.3	46.8	189	1,448.2	1.7	9.2194E−08	*
	T	6,5763.5	65,763.5	1	1,523.9	2,389.3	0	*
	G × T	9,729.5	52.0	187	1,518.2	1.9	1.2054E−10	*
Shoot length (cm)	G	4,975.7	26.3	189	1,327.0	2.2	1.8173E−15	*
	T	29,451.1	29,451.1	1	1,525.0	2,456.3	0	*
	G × T	4,333.1	23.2	187	1,517.7	1.9	2.519E−11	*
Root length (cm)	G	1,109.8	5.9	189	1,493.6	1.1	0.12458869	
	T	7,213.0	7,213.0	1	1,521.6	1,386.3	3.008E−216	*
	G×T	1,665.3	8.9	187	1,516.9	1.7	6.4025E−08	*

G, genotype; T, treatment; G × T, genotype × treatment interaction; NumDF, numerator degrees of freedom; DenDF, denominator degrees of freedom; * significance at $P < 0.05$.

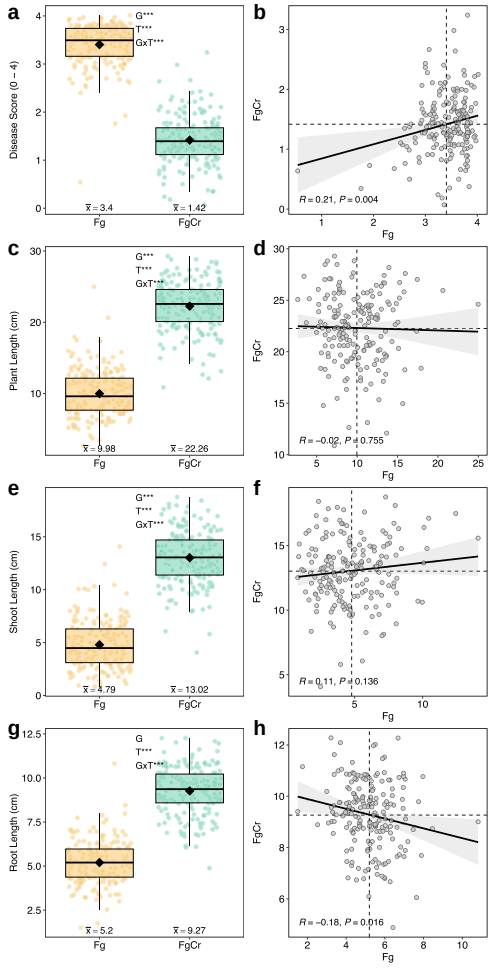

Fig. 1. Comparisons and correlations between 2 treatments Fg (*Fusarium graminearum*) and FgCr (*F. graminearum* and *Clonostachys rosea* seed treatment). Box plots show comparison of BLUEs of genotypes in treatments Fg and FgCr for disease score (a), plant length (c), shoot length (e), and root length (g). Thick horizontal line in the box represents the median and black diamond represents the mean estimate of each treatment. G, T, and G × T annotation summarize the ANOVA results for genotype effect, treatment effect, and genotype-by-treatment interaction effect, respectively. ***Significance at $P < 0.001$. Pearson's correlation coefficient between treatment Fg and FgCr are shown for disease score (b), plant length (d), shoot length (f), and root length (h). Dashed vertical and horizontal lines indicate the mean estimate of the trait in treatment Fg and FgCr, respectively.

minor alleles TT, GG, and CC for SNP markers BS00096604_51, RFL_Contig2459_2314, and Ku_c33858_325, respectively, at chromosome 4B (Supplementary Fig. 2a–f). No significant SNP marker–trait associations were detected for disease score in treatment FgCr (Fig. 4b), while a significantly associated region was detected for disease score contrast on chromosome 7B at 77–78 cM (Fig. 4c). Allele level comparisons of all 6 associated SNP markers (BobWhite_c3564_81, BS00021972_51, Excalibur_rep_c111629_239, wsnp_Ex_rep_c109138_92064554, BS00010557_51, and wsnp_Ku_rep_c68953_68153061) for disease score contrast showed a significant ($P < 0.05$) increase in *C. rosea* efficacy in genotypes with minor alleles (Supplementary Fig. 2g–l).

For trait plant length, only one significant SNP marker (Ra_c956_2318 on chromosome 7A at 228 cM) was significantly associated with plant length contrast with minor nucleotide T contributing to an increase in plant length due to *C. rosea* seed treatment (Supplementary Figs. 2m and 3). However, the same SNP marker, i.e. Ra_c956_2318 on chromosome 7A at 228 cM, was significantly associated with shoot length contrast but with a nonsignificant effect in allelic comparison (Supplementary Figs. 2o and 4). SNP marker wsnp_Ex_c17914_26681837 on Chr 7D at 139 cM was associated with shoot length in treatment Fg where allele CC was significantly associated with less shoot length (Supplementary Figs. 2n and 4). For root length, one significantly associated region was detected at chromosome 6B at 65 cM in treatment Fg, one significantly associated region at chromosome 7A at 114 cM in treatment FgCr, and no significant association for root length contrast was observed (Supplementary Figs. 2p–r and 5).

Candidate gene content in SNP-associated genomic regions

Within a stringent interval of ±100 kb surrounding significant SNP marker–trait associations, localized genes were browsed. Supplementary Table 4 contains all the gene IDs and descriptions for the localized genes. Briefly, for disease score in treatment Fg, 3 genes were found localized with SNP marker BS00089497_51 at chromosome 2A, 2 genes were found localized with SNP marker BS00096604_51 at chromosome 4D, 6 genes were found localized with SNP marker Excalibur_c7026_2635 at chromosome 1A, 3 genes were found localized with SNP marker Ku_c33858_325 at chromosome 4B, 10 genes were found localized with SNP marker Kukri_c40121_373 at chromosome 2A, and 1 gene was found localized with SNP marker RFL_Contig2459_2314 at chromosome 4B. Besides several genes annotated as encoding uncharacterized proteins, 2 genes were predicted to encode kinases, 1 gene was predicted to encode a kinase regulator and 1 gene was predicted to encode an ethylene-responsive transcription factor (Supplementary Table 4).

For disease score contrast (Fg–FgCr), 6 SNP markers were in significant association at chromosome 7B at 77–78 cM. In total, 6 genes were found localized with SNP marker BobWhite_c3564_81, 4 genes were found localized with SNP marker BS00010557_51, 3 genes were found localized with SNP marker BS00021972_51, 1 gene was found localized with SNP marker Excalibur_rep_c111629_239, 4 genes were found localized with SNP marker wsnp_Ex_rep_c109138_92064554, and 3 genes were found localized with SNP marker wsnp_Ku_rep_c68953_68153061. Predicted functions of these gene products included several monooxygenases, transporters, and biosynthesis of secondary metabolites (Supplementary Table 4).

For plant length contrast and shoot length contrast, 9 genes were found localized with SNP marker Ra_c956_2318 at chromosome 7A. These included 2 genes predicted to encode disease resistance proteins, including a Pik-2-like disease resistance protein, and 2 genes predicted to encode receptor kinases (Supplementary Table 4). For shoot length in treatment Fg, 11 genes were found localized with SNP marker wsnp_Ex_c17914_26681837 at chromosome 7D. For root length in treatment Fg, 8 genes were found localized with SNP marker Kukri_c41694_285 at

Table 2. Summary statistics of traits across treatments.

Trait	Treatment	Min	Mean	SD	Median	Max	H^2_P	H^2_C
Disease score (0–4)	Fg	0.54	3.4	0.44	3.49	4.02	0.3	0.22
	FgCr	0.07	1.42	0.5	1.39	3.24	0.41	0.32
Plant length (cm)	Fg	2.66	9.98	3.3	9.61	24.97	0.45	0.36
	FgCr	10.86	22.26	3.46	22.57	29.29	0.44	0.36
Shoot length (cm)	Fg	0.83	4.79	2.28	4.48	14.07	0.47	0.37
	FgCr	4.07	13.02	2.52	13.05	18.76	0.6	0.51
Root length (cm)	Fg	1.51	5.2	1.23	5.2	10.82	0.36	0.28
	FgCr	4.88	9.27	1.35	9.37	12.27	0.14	0.11

SD, standard deviation; H^2_P, heritability (Piepho and Möhring 2007); H^2_C, heritability (Cullis *et al.* 2006).

chromosome 6B, and 3 genes were found localized with SNP marker Tdurum_contig15235_951 at chromosome 6B. Moreover, for root length in treatment FgCr, 5 genes were found localized with SNP marker Excalibur_rep_c101407_222 at chromosome 7A (Supplementary Table 4).

Discussion

In this study, we report genome-wide association analyses of 190 winter wheat genotypes from northern Europe for fusarium foot rot susceptibility and its biocontrol efficacy using *C. rosea*. The same panel of genotypes has previously been explored for genetic variation for resistance to abiotic stress, such as freezing and winter hardiness (Vaitkevičiūtė *et al.* 2023) and drought tolerance (Kumar *et al.* 2020); and to biotic stress, including powdery mildew (Hysing *et al.* 2007; Alemu *et al.* 2021), leaf rust (Hysing *et al.* 2007), yellow rust (Koc *et al.* 2022), fusarium head blight (Zakieh *et al.* 2021), and septoria tritici blotch (Odilbekov *et al.* 2019). Moreover, this panel has been screened for biocontrol efficacy of septoria tritici blotch by *C. rosea* (Chaudhary *et al.* 2024). Here, we show that this panel also serves as a resource for resistance to fusarium foot rot and biocontrol efficacy with *C. rosea*.

We observed significant variation among 190 wheat genotypes for susceptibility to fusarium foot rot caused by *F. graminearum* in the only pathogen treatment. The sand-based bioassay used in this study offers a cost-effective and efficient alternative to field testing for exploring disease severity to fusarium foot rot, as a high correlation ($R = 0.94$, $P < 0.001$) between growth chamber sand bioassay and field conditions were observed for *F. culmorum* disease severity in wheat and barley genotypes (Knudsen *et al.* 1995; Jensen *et al.* 2000). Overall, the genotypes showed a high susceptibility to *F. graminearum* which has been observed in some other works too. Shi *et al.* (2020) observed more than 80% of tested genotypes grouped in susceptible and highly susceptible categories for seedling stage rotting caused by *Fusarium pseudograminearum*. Voss-Fels *et al.* (2018) also observed a high stem discoloration, a metric used to evaluate disease severity caused by *F. graminearum*, in half of 215 tested wheat genotypes. This suggests that the current tested material might not offer full resistance to *F. graminearum* foot rot and might only possess partial resistance with the ability to have reduced symptom development. Kazan and Gardiner (2018) also highlighted the lack of full resistance to fusarium crown rot caused by *F. pseudograminearum*. Disease severity was also found to have a strong negative correlation with other growth-related traits in the study, showing a direct impact on stunting of plant growth and development.

Only a handful of studies have been conducted for *Fusarium* spp.–related ground-level and below-ground diseases in wheat (Li *et al.* 2010; Voss-Fels *et al.* 2018; Liu *et al.* 2021; Malosetti *et al.* 2021). In this study, genome-wide associations revealed significant marker–trait associations for disease score, shoot length, and root length. The SNP marker associations identified at chromosomes 1A, 2A, and 4B for disease score are different from previously identified SNPs in the above-mentioned studies, indicating different genes segregating in the current winter wheat population. Moreover, a significant marker–trait association at chromosome 7D for shoot length and 6B for root length in the presence of pathogen captures segregation at additional locations in the wheat genome. The allelic differences at these markers reveal a significant improvement for growth-related traits and a significant reduction in disease severity, showing the potential for improvement in future breeding programs.

The correlation between resistance to ground-level and below-ground diseases caused by *Fusarium* spp. and resistance to fusarium head blight has been explored previously. Wang *et al.* (2015, 2018) demonstrated a lack of correlation between resistance to fusarium root rot and fusarium head blight and suggested different resistance genes. Similarly, Li *et al.* (2010) observed a very weak correlation ($R = -0.06$–0.27) between fusarium head blight and crown rot severities. Interestingly, Liu *et al.* (2021) observed a significant negative correlation ($R = -0.263$, $P < 0.01$) between fusarium head blight and fusarium seedling blight lesion length. Comparing the results of disease scores from this study to previously conducted FHB using the same panel of winter wheat genotypes (Zakieh *et al.* 2021), we observed no significant correlation ($R = 0.11$, $P = 0.16$, not shown), indicating a different set of resistance genes segregating for *Fusarium* spp.–related disease at seedling stage and flowering stage. This is also further highlighted at the genome-wide level with different regions segregating for disease severity for fusarium foot rot and fusarium head blight between the 2 studies. We note that Zakieh *et al.* (2021) used a mix of 6 *F. graminearum* and 3 *F. culmorum* strains for head infection, while we employed a single *F. graminearum* strain in the current study, which may account for some of the variation. However, as suggested before (Li *et al.* 2010; Liu *et al.* 2021), it is important to have separate screening programs to select for resistance to various *Fusarium* spp. diseases.

One of the main aims of this study was to explore the genetic variation in winter wheat genotypes for the biocontrol efficacy of *C. rosea* in controlling fusarium foot rot. Several previous studies have demonstrated plant-genotype-specific modulation of biocontrol efficacy in various BCA–pathogen interactions, although these studies typically involved limited number of plant genotypes (Smith *et al.* 1999; Ryan *et al.* 2004; Meyer *et al.* 2010; Tucci *et al.* 2011; Rebeka *et al.* 2013; Moraga-Suazo *et al.* 2016; Arkhipov *et al.* 2023). This report, alongside our previous work studying plant genotype effects for biocontrol efficacy of *C. rosea* against septoria tritici blotch (Chaudhary *et al.* 2024), is the exploration of the largest number of plant genotypes for these 3-way interactions among plant, pathogen, and BCA. We observed significant

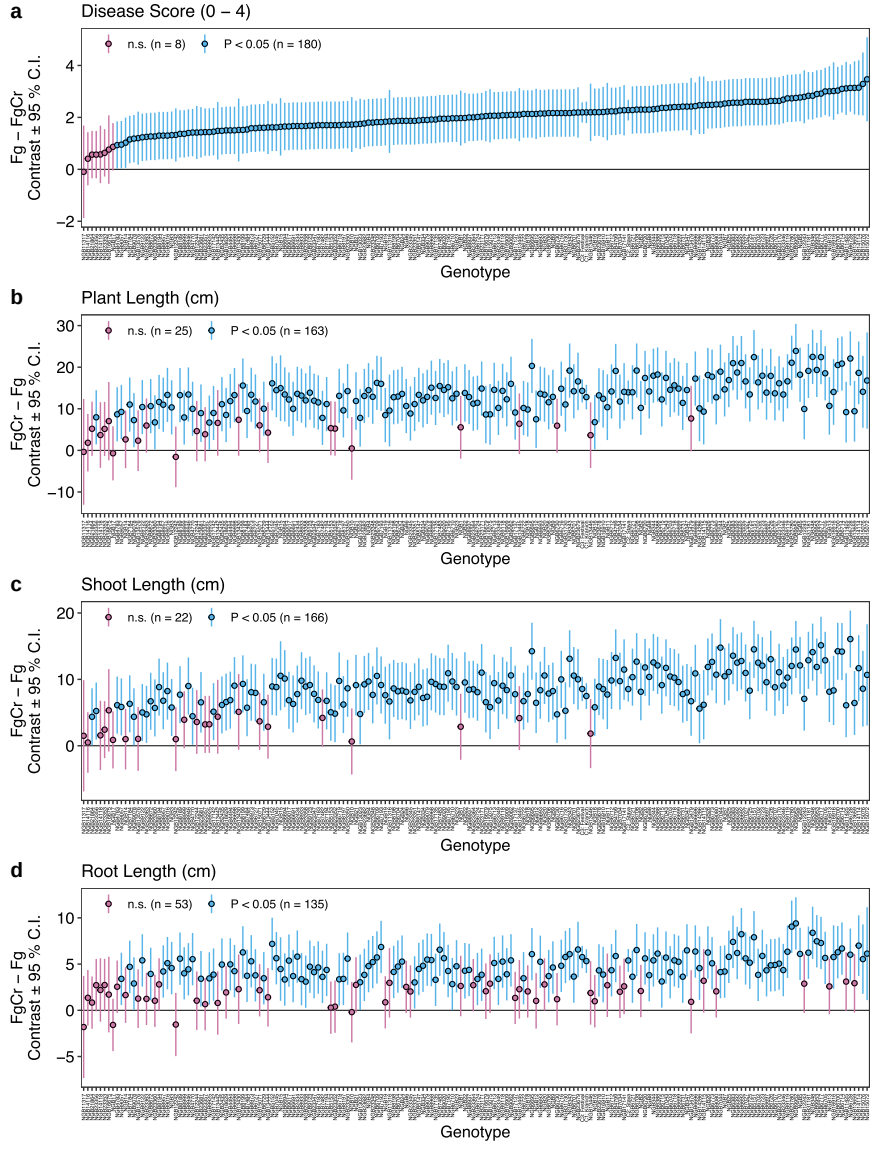

Fig. 2. Inter–treatment pairwise contrasts estimates for traits disease score (a), plant length (b), shoot length (c), and root length (d). Inter–treatment pairwise contrasts were estimated for each genotype using post–hoc Tukey tests. Points represent the estimated mean difference between the treatments Fg and FgCr and error bars represent 95% confidence intervals for each genotype. Points with 95% confidence interval overlapping the horizontal line at 0 represent non–significant inter–treatment pairwise contrast.

variation among plant genotypes for the biocontrol efficacy of *C. rosea* to control fusarium foot rot. *Clonostachys rosea* is very successful in controlling *Fusarium* spp. diseases at various plant stages in wheat (Knudsen *et al.* 1995; Jensen *et al.* 2000; Roberti *et al.* 2008; Xue *et al.* 2009; Gimeno *et al.* 2021; Abaya *et al.* 2023). However, by identifying the genetic basis in plants for interactions with beneficial microorganisms, the efficacy to reduce the disease can be further enhanced. Due to the large-scale screening of plant genotypes, it was possible to explore the genomic-level segregation among wheat genotypes for biocontrol efficacy. We identified a region at chromosome 7B which is significantly associated with segregation for *C. rosea* biocontrol efficacy and another region on chromosome 7A segregating with *C. rosea* efficacy for shoot length and plant length, suggesting different underlying mechanisms for

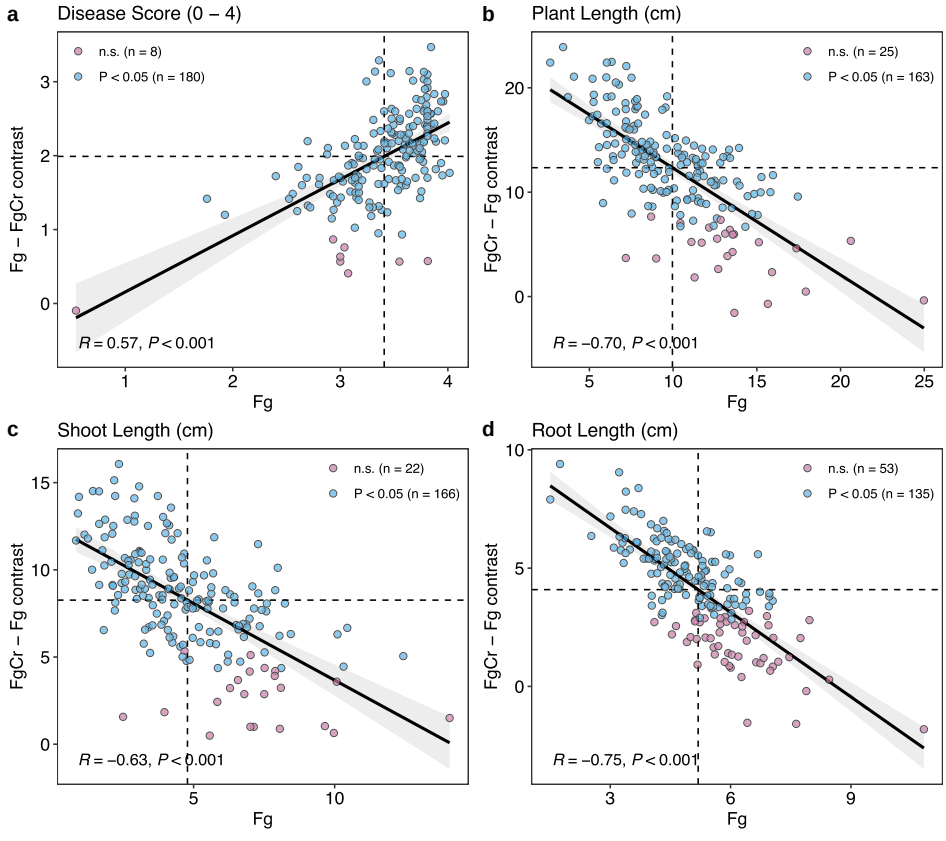

Fig. 3. Pearson's correlation between disease score in treatment Fg and *C. rosea* efficacy estimate from inter–treatment pairwise contrast (Fg–FgCr or FgCr–Fg) for traits disease score (a), plant length (b), shoot length (c), and root length (d). Dashed vertical and horizontal lines indicate the mean estimate of the trait in treatment Fg and inter–treatment pairwise contrast (Fg–FgCr or FgCr–Fg) for *C. rosea* efficacy, respectively. Points with pink and blue color represent genotypes with non–significant and significant ($P < 0.05$) inter–treatment pairwise contrast, respectively.

these traits. Interestingly, association mapping of *C. rosea*–mediated biocontrol efficacy of septoria leaf blotch disease in the same winter wheat collection identified 2 distinct segregating regions on chromosomes 1D and 6B (Chaudhary *et al.* 2024). This shows that plant genotype-mediated biocontrol efficacy can be specific to different pathogens (*F. graminearum* or *Z. tritici*) and/or different plant organs (head or roots).

No overlapping *Fusarium* disease trait associations on chromosome 7B at 77–78 cM are reported in the literature. However, 2 studies using linkage maps reported FHB-related QTLs upstream at 53–66 cM (Eckard *et al.* 2015) and downstream at 92 cM (Wang *et al.* 2023) of the region identified in this study. It should be noted that linkage maps are population-specific, and thus, it is uncertain whether these QTLs are localized within the genomic region identified in this study. The genomic region associated with *C. rosea* biocontrol efficacy on chromosome 7B contained genes predicted to encode various monooxygenases, transporters, and biosynthesis of secondary metabolites. Specifically, a detoxification protein, Detoxification 16-like, belonging to the multidrug and toxic compound extrusion (MATE) transporter family was located in the region. The MATE family is a large multigene family in plants, where the proteins are involved in detoxification of toxic compounds, heavy metals, and disease resistance (Sun *et al.* 2011; Takanashi *et al.* 2014; Watanabe *et al.* 2022). Moreover, 3 different cytochrome P450 (CYPs) encoding genes were located in this region. CYP75B4-like is putatively involved in flavonoid biosynthesis, whereas CYP19-4-like and CYP28 encode for cyclophilin which are involved in protein folding, cell signaling, and also plays a role in immunosuppression in vertebrates and yeast (He *et al.* 2004; Wang and Heitman 2005).

The region on chromosome 7A contained a gene predicted to encode a Pik-2-like disease resistance protein. Pik-2-like disease resistance proteins belong to a known R protein type demonstrated to induce a hypersensitive response in plants to restrict pathogen growth (Ashikawa *et al.* 2008). Interestingly, 2 different Pik-2-like disease resistance protein paralogs are present in a genomic region on chromosome 1D in the same wheat collection, segregating with *C. rosea*–mediated biocontrol efficacy of septoria leaf blotch (Chaudhary *et al.* 2024). The presence of Pik-2-like disease resistance protein genes in different regions segregating with

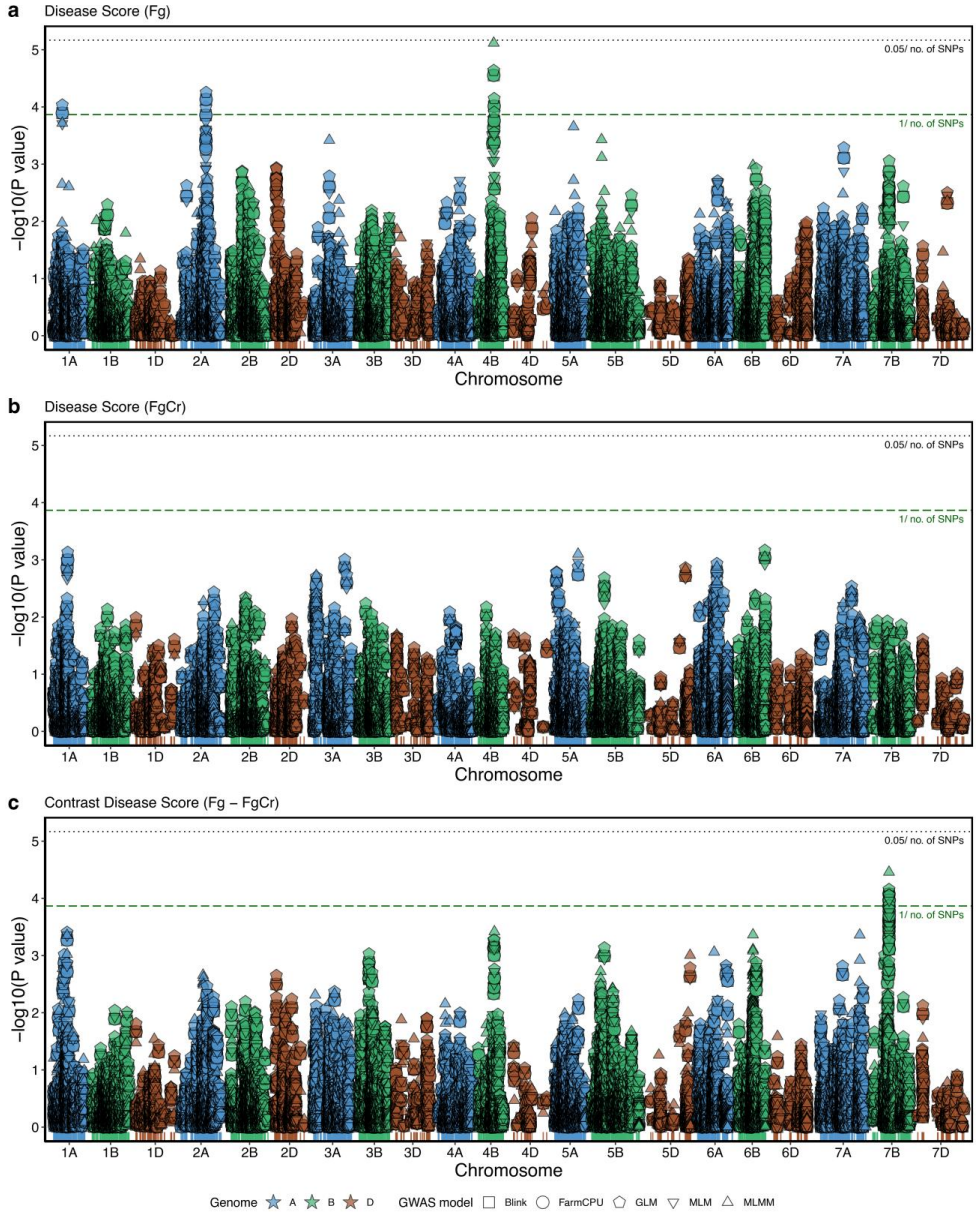

Fig. 4. Manhattan plot for marker–trait association for disease score in (a) treatment Fg (*F. graminearum* alone), (b) treatment FgCr (*F. graminearum* on seed treated with *C. rosea*), and (c) disease score contrast (Fg–FgCr) for *C. rosea* efficacy from 5 GWAS models. Dotted line depicts the Bonferroni significance threshold ($P = 0.00000679$, after $P = 0.05/n$, where $n = 7{,}360$ is the number of SNP markers), dashed line depicts negative log threshold ($P = 0.00014$, after $P = 1/n$, where $n = 7{,}360$ is the number of SNP markers).

biocontrol efficacy may suggest the ability of wheat genotypes to recognize microbe-associated molecular patterns or microbial effectors and subsequently induce pattern-triggered immunity or effector-triggered immunity to partially contribute to the BCA compatibility trait (Jones and Dangl 2006; Köhl *et al.* 2019; Jensen *et al.* 2021).

It must be emphasized that plant disease resistance must act as the first line of defense in an integrated disease management

approach. Therefore, any further manipulation in cultivar development, such as BCA compatibility breeding, should not come at the cost of undermining disease resistance. In our study, we observed a significant positive correlation between disease susceptibility and plant genotype-dependent *C. rosea* biocontrol efficacy, highlighting the better performance of *C. rosea* as a BCA in more susceptible genotypes. Smith *et al.* (1999) also observed a similar trend where better disease suppression by the BCA *B. cereus* was found in less resistant tomato genotypes toward *P. torulosum*. The positive relationship observed between increased disease susceptibility and improved biocontrol efficacy can be attributed to the greater opportunity for disease reduction when higher pathogen loads are present. The correlation is also rather moderate and, therefore, it is possible to select genotypes with lower susceptibility and higher biocontrol efficacy from the population. Moreover, techniques such as GWAS can help in dissecting the traits and break negative linkages, if any, and aid in more precise selection of traits for cultivar improvement. We identified independent associations for disease resistance and *C. rosea* biocontrol efficacy, highlighting the potential for simultaneous breeding for resistance to fusarium foot rot and biocontrol efficacy of *C. rosea* in managing the disease.

Data availability

Phenotypic and genotypic raw data are available at figshare: https://doi.org/10.25387/g3.26064079. The authors affirm that all data necessary for confirming the conclusions of the article are present within the article, figures, and tables.

Supplemental material available at G3 online.

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Conflicts of interest

The author(s) declare no conflicts of interest.

Author contributions

SC, MK, MD, DFJ, and LG-B conceived the study. All authors contributed to designing the experiments. SC and RMNR performed the experiments. SC performed the analyses and wrote the first draft of the manuscript. All authors read, provided input, and approved the submitted version of the manuscript.

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The thesis aimed to study the winter wheat genotype-specific modulation of *Clonstachys rosea* biocontrol efficacy in controlling the pathogens *Zymoseptoria tritici* and *Fusarium graminearum*. Using genome-wide association and transcriptomic studies, it was determined that *C. rosea*-mediated biocontrol efficacy varied among plant genotypes and pathosystems, with differential induction of defence-related genes. These findings advance the knowledge of interactions between plants and biological control agents, showing potential for optimisation in the context of disease management and plant breeding.

Sidhant Chaudhary received his doctoral education at the Department of Forest Mycology and Plant Pathology, SLU, Uppsala, Sweden. He received his M.Sc. at the University of Hohenheim, Stuttgart, Germany, and his B.Sc. at the CCS Haryana Agricultural University, Hisar, India.

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