Acta Universitatis Agriculturae Sueciae

SLU

Doctoral Thesis No. 2023:40
Faculty of Landscape Architecture, Horticulture and Crop Production Science

Novel Methods for Disease Resistance Breeding in Winter Wheat

Mustafa Zakieh

Novel Methods for Disease Resistance Breeding in Winter Wheat

Mustafa Zakieh

Faculty of Landscape Architecture, Horticulture and Crop Production Sciences

Department of Plant Breeding

Alnarp

SLU

SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

DOCTORAL THESIS

Alnarp 2023

Acta Universitatis Agriculturae Sueciae
2023:40

Cover: Description of photograph (Disease symptoms of Fusarium head blight (FHB) on wheat spikes. Seeds of winter wheat infected with FHB. Spores of the pathogen *Fusarium graminearum*. Septoria tritici blotch (STB) with pycnidial structures on wheat leaf surface)

ISSN 1652-6880
ISBN (print version) 978-91- 8046 -132-0
ISBN (electronic version) 978-91-7760- 132-0
https://doi.org/10.54612/a.7187o2ndne

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

Print: SLU Service/Repro, Alnarp 2023

Abstract

Breeding for disease resistance in winter wheat is a critical task in the agricultural industry, as plant diseases can significantly impact crop yield and quality. Traditional breeding methods are time-consuming, and disease resistance screenings are often cost and labour-demanding. Therefore, novel breeding tools are being developed to speed up winter wheat genetic gain and increase its genetic diversity.

A protocol to characterize winter wheat germplasm for resistance to Fusarium head blight (FHB) under accelerated growth conditions was carried out. The results showed that it is possible to reduce the time necessary to characterize germplasm for FHB resistance by growing up to three generations per year. In a genome-wide association study (GWAS), several markers were identified that were significantly associated with FHB resistance. These markers overlapped with previously known markers contributing to FHB resistance. Novel phenomic methods, the low throughput and affordable SmartGrain and the high throughput Cgrain Value™ were implemented to predict FHB severity in the tested germplasm. Both methods showed good correlation to visual scoring, suggesting a potential alternative for the traditional visual assessment methods with machine-based methods that offer higher throughput and lower cost. The study also investigated seedling resistance to Septoria tritici blotch (STB) using association mapping and genomic prediction (GP). The study identified 20 QTL for STB seedling resistance of which nine were potentially novel QTL for STB seedling resistance and four overlapped with previously identified genomic regions at the adult stage. The identified QTL could be exploited in winter wheat marker-assisted selection (MAS) against STB and promote the seedling stage for early selection instead of the adult stage. Furthermore, the study investigated the genotypic responses of winter wheat seedlings infected with STB to the fungal biocontrol agent *Clonostachys rosea*. SNP markers associated with *C. rosea* biocontrol efficacy and disease resistance were identified, laying the groundwork for further research in genotype-specific-biocontrol compatibility in disease resistance breeding. The thesis provides useful insights into developing novel breeding tools for disease resistance in winter wheat and emphasizes the importance of industry collaboration to transfer knowledge from research to application.

Keywords: Winter wheat, Fusarium head blight (FHB), Septoria tritici blotch (STB), accelerated growth conditions, quantitative trait loci (QTL), seedlings stage resistance, single nucleotide polymorphism (SNP), Genome wide association study (GWAS), genomic prediction (GP).

Sammanfattning

Att förädla för sjukdomsresistens i höstsådda spannmål är en central uppgift inom jordbruksindustrin, eftersom växtsjukdomar kan ha betydande negativ inverkan på skörd och kvalitet. Traditionella förädlingssmetoder kan vara tidskrävande och selektion av sjukdomsresistens kan kräva stora fältförsök. Därför behöver nya förädlingsverktyg utvecklas för att påskynda ökningen av den genetisk vinsten och biodiversiteten i höstvete.

Ett protokoll för att karaktärisera höstvete för resistens mot axfusarios under accelererade tillväxtvillkor genomfördes. Resultaten visade att det är möjligt att minska den tid som krävs för att karaktärisera genbanksmaterial för resistens mot axfusarios och att odla upp till fyra generationer per år. I en genome-wide association study (GWAS) identifierades flera genetiska markörer som var signifikant associerade med sjukdomsresistens. Dessa markörer överensstämde med tidigare kända markörer som bidrar till resistens mot axfusarios. Nya fenomiska metoder, den billigare SmartGrain med låg kapacitet och den mer kostsamma Cgrain Value™ med hög kapacitet implementerades för att förutsäga graden av axfusarios i det infekterade frömaterialet. Båda metoderna visade på en korrelation med den visuella graderingen, vilket tyder på att traditionella visuella bedömningsmetoder kan ersättas med maskinbaserade metoder som erbjuder högre kapacitet och lägre kostnad. Studien undersökte också resistens mot bladsjukdomen svartpricksjuka i tidigt plantstadium genom associationsanalys och genomisk prediktion (GP). Studien identifierade nio potentiellt nya genetiska loci (QTL) för resistens mot svartpricksjuka och ytterligare fyra QTL som överlappade med tidigare markörer identifierade i vuxna plantor. De identifierade QTL kan utnyttjas vid markör-assisterat urval för resistens mot svartpricksjuka i höstvete och utnyttja tidiga utvecklingsstadier i stället för vuxensstadier för urval. Dessutom undersöktes den genetiska bakgrunden hos höstvete för kompatibilitet med svampen *Clonostachys rosea*, som användes för biologisk bekämpning av svartpricksjuka. Genetiska markörer associerade med biologisk bekämpnings-effektivitet och sjukdomsresistens identifierades, vilket lägger grunden för ytterligare forskning om förädling av genotypspecifik kompatibilitet med biologisk bekämpning och sjukdomsresistens i höstvete. Sammanfattningsvis ger avhandlingen användbara insikter för utvecklingen av nya förädlingsverktyg för sjukdomsresistens i höstvete och betonar vikten av samarbete för att överföra kunskap från forskning till tillämpning.

Nyckelord: Höstvete, axfusarios, svartpricksjuka, genetiska loci, resistens vid plantstadiet, växtmaterial, fröbanksmaterial, associeringskartläggning, genetisk markör, accelererade tillväxtförhållanden, genetisk prediktion.

Preface

Together with persistence and belief, the key factor in success is "Speed"

"O son of Adam, you are only but a collection of days, so whenever a day passes away, a part of you has gone too"

/Al Hasan Al Basri

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. Zakieh, M., Gaikpa, D. S., Leiva Sandoval, F., Alamrani, M., Henriksson, T., Odilbekov, F., & Chawade, A. (2021). Characterizing Winter Wheat Germplasm for Fusarium Head Blight Resistance under Accelerated Growth Conditions. Frontiers in Plant Science, 12(August). https://doi.org/10.3389/fpls.2021.705006

II. Zakieh, M., Alemu, A., Henriksson, T., Pareek, N., Singh, P, K., Chawade, A. (2023). Genomic association and prediction studies for Septoria tritici blotch seedling resistance in wheat (submitted).

III. Leiva, F., Zakieh, M., Alamrani, M., Dhakal, R., Henriksson, T., Singh, P. K., & Chawade, A. (2022). Phenotyping fusarium head blight through seed morphology characteristics using RGB imaging. Frontiers in Plant Science, 13(October), 1–13. https://doi.org/10.3389/fpls.2022.1010249

IV. Chaudhary, S., Zakieh, M., Dubey, M., Jensen, D, F., Grenville-Briggs, L., Chawade, A., Karlsson., M. (2023). Plant genotype-specific modulation of *Clonostachys rosea*-mediated biocontrol of Septoria tritici blotch disease in wheat (manuscript).

Papers I and III are reproduced with the permission of the publishers.

The contribution of Mustafa Zakieh to the papers included in this thesis was as follows:

I. Contributed to experimental design, execution, data collection, analysis, and manuscript writing. These tasks involved developing a protocol for disease screening at adult wheat stage using speed breeding, as well as in the analysis of large-scale data and sequence information, which are critical proficiencies for conducting genome-wide association studies (GWAS).

II. Participated in developing and designing the experiments. Conducted the tests for isolates' virulence. Carried out field sampling of pathogen isolates and writing the manuscripts

III. Participated in developing the study, designing the experiments and writing the manuscript. Provided a better understanding of the project on how novel phenotyping methods can be implemented in disease resistance screening and prediction in relation to GWAS results.

IV. Participated in designing and executing the experiment. Performed data collection the experiment, guided the team to execute the protocol.

List of tables

List of figures

1. Background

Globally, wheat was cultivated on 220.8 million hectares with a total production of 770.9 million tons in 2021 (FAOSTAT, 2022). In Northern Western European countries (NWE), winter wheat is the leading crop in terms of arable land (Chawade et al., 2018). In Sweden, 15% of the arable land is cultivated with wheat making it the largest produced small grain crop in the country (Lantmännen, 2022). In Sweden, wheat crop has been growing in numbers in terms of yields and cultivated area in the past decades (FAOSTAT, 2022). This could be explained by the rise in global temperature (Olesen and Bindi, 2002), but also by the development of more resilient varieties and the advances in agronomic practices. Wheat is typically grown in the southern and central parts of Sweden, in a climate suitable for its growth, with an estimated production of approximately 1.5 million metric tons of wheat in 2019 (FAOSTAT, 2022). Even though Sweden is not the largest producer of wheat in northern Europe, a high rate of increase has been achieved in both production and growing areas in the past decades compared to other NWE countries. Between the years 1990 and 2020, the total harvested areas in Sweden increased by approximately 23% compared to Germany (16%), Denmark (0.4%) and the UK (10.08%) (FAOSTAT, 2022). However, the higher rate of expansion of wheat growing areas in Sweden may ultimately surpass other major producers in Northern Europe in upcoming decades, accompanied by the crop growing success towards the northern parts of the country due to climatic changes. A clear picture of the changes in growing the crop is the shift where traditional wheat growing areas in Southern European countries have been steadily decreasing in contrast to the increase in Northern European countries (FAOSTAT, 2022).

Most of the production shifts are driven by climate changes leading to drought in main wheat production areas and an increase in the thermal suitability for crop production in Northern Europe (Trnka et al., 2014). Farmers in northern climates favor winter wheat, where the crop thrives. Not only due to its ability to withstand long cold climate, but factors such as biotic and abiotic stress tolerance, technological properties, and yield, make winter wheat an excellent choice for farmers in these regions.

1.1 Importance of wheat as a crop

Wheat is an elemental crop being a key constituent in human and animal nutrition. Staple crop production is required to meet the requirements of feeding the increasing human population by the year 2050 (Hickey et al., 2019). The importance of wheat is that it contributes to 20% of the globally consumed calories (Shiferaw et al., 2013). However, fluctuations in production over seasons and due to supply from major producers may result in unstable wheat supply threatening the food security of populations dependent on wheat as a source of nutrition. To sustain the growing population by 2050, global food production will require a 60 percent increase from its current levels (Scott et al., 2021). Additionally, there has been a significant rise in the number of people suffering from malnutrition, which has increased from just under 800 million in 1996 to 925 million in 2010 (Bratspies, 2014). The escalating food insecurity is evident in the fluctuating food prices. Moreover, the changing consumption patterns, including the shift from traditional meals to high-energy and high-fat foods, are influenced by globalization and rapid urbanization (Logan and Jacka, 2014). While the genetic gain of wheat is approximated at 1% annually (Mackay et al., 2011), it is possible to attain future global nutritional demands provided by wheat through sustainable genetic improvement of wheat together with other cultural management strategies. This means that over the course of a few years, breeders can develop wheat varieties that are significantly better than their predecessors in terms of yield, disease resistance, and other important traits.

1.2 Challenges in winter wheat breeding

Wheat breeding requires significant resources, including time, money, and labour. Developing efficient breeding strategies are essential to overcome resource constraints and produce improved cultivars in a timely manner.

In greenhouse conditions, winter wheat breeding is challenging and even with the best novel methodologies, identifying factors that contribute to the traits is usually tedious due to its inability to flower and form the ears without being subjected to low temperatures for a long time (vernalization). Hence, winter wheat breeding programs are usually longer compared to the spring wheat.

Bread wheat (*Triticum aestivum* 2n=6x=42) is a hexaploid species that with its three subgenomes together make a size of approximately 17 Gb, which is quite large compared to many other plant genomes (Wang et al., 2015). The three subgenomes of wheat contribute to its complexity and genetic diversity. Each subgenome contains a set of chromosomes, resulting in a total of 21 chromosome pairs. Understanding the genomic factors influencing yield potential, therefore is a complex task due to the size and complexity of the genome. In order to understand these factors, it is vital to study the function of the thousands of genes that make up the bread wheat genome and their interaction with each other and with the environment that affect important traits such as yield. This approach requires extensive use of advanced breeding techniques, as well as extensive data analysis and computational modeling of the genetic factors. Despite the challenges, significant progress in identifying the key genomic factors that influence yield in bread wheat has been achieved, aiding in the development of varieties that are better adapted to different environments (Slafer, 2003, Rajaram, 2001). Depending on its growth habit, bread wheat can be either of winter or spring type. The difference between the two types is that winter wheat requires an extended period of growth in low temperatures (typically achieved in winter) prior to commencing active (reproductive) growth in spring and summer. It is essential for winter wheat to be subjected to lower temperatures during winter in order for the plants to flower and subsequently form the spikes. Due to this requirement, winter wheat has been traditionally grown in colder climates tolerating the freezing temperature of the winter.

1.3 Factors impacting wheat yield

Winter wheat yield can be significantly impacted by a range of abiotic and biotic factors. Even though being subject to a multitude of abiotic and biotic stresses, it is expected that wheat production in the southern part of Sweden to increase by up to 20% by the year 2050 (Eckersten et al., 2001).

1.3.1 Abiotic stresses

Abiotic stress factors caused by climatic changes drive significant yield losses in all wheat-growing areas (Pequeno et al., 2021). However, up to 50% of food crops globally are lost due to abiotic stresses (Gull et al., 2019). Drought stress is one of the leading causes of yield loss and is expected to cause further detrimental effects on the crop in the upcoming decades (Pequeno et al., 2021). Together with other cultural management strategies, winter wheat varieties with improved tolerance to drought stress can play a significant role in mitigating the impact of drought stress on wheat yield (Zia et al., 2021). Improved water use efficiency, increased root depth, and the ability to better withstand water stress during critical stages of growth are major breeding targets for improving drought stress tolerance (Blum, 2005). Fluctuations in annual yield may not only affect human wheat supply but can also impact sectors relying on the crop for energy and feed production. Abiotic factors, mainly drought, significantly contributed to reducing common wheat and spelt production in Europe in 2018 followed by price peaking between the second half of 2018 and the first half of 2019 (EUROSTATS, 2020). In addition to drought, heat stress negatively affects grain yield. The rising global temperatures caused by climate change are a significant risk to wheat production, especially during the reproductive and grain-filling phases (Farooq et al., 2011). Developing heat-resistant wheat varieties that possess traits such as heat shock proteins, high stability of chloroplastic membrane, high photosynthetic efficiency, and efficient grain filling are needed to combat the negative effects of the rising temperatures (Farooq et al., 2011). Accelerating variety development for abiotic stress tolerance, is therefore needed under changing environment. Combining next generation breeding methods with biotechnological tools, can enhance this process, leading to lower abiotic stress impact on grain yield in wheat crop.

1.3.2 Biotic stresses

Biotic stresses, including plant pests such as nematodes, insects and diseases caused by fungal, bacterial and viral agents, pose a significant challenge to wheat production globally (McIntosh, 1998). Wheat is susceptible to several major diseases such as rusts, powdery mildew, Fusarium head blight (FHB), and Septoria tritici blotch (STB). These biotic stressors can directly impact the host plant by depriving it of essential nutrients, leading to a decrease in plant vigor and potentially resulting in the death of the plant in severe epidemics (Singla and Krattinger, 2015). Additionally, many biotic stresses may continue to cause economic damage even at postharvest stages (Singla and Krattinger, 2015). This, for instance is known when mycotoxin accumulation as a result of *Fusarium* fungal species infect the ears of cereal crops causing deterioration to the technological properties of the produce.

Fungal diseases

Biotic stresses caused by fungi, insects, bacteria and viruses are among the factors that are currently lowering production of major crops by an estimated 20-40% globally (Velasquez et al., 2018). In wheat, global losses up to 21.47% of yield are caused by pests and pathogens, for which fungal diseases are likely taking the largest portion by and estimated 18.2% (Savary et al., 2019). However, in Northwest Europe (United Kingdom, Ireland, Belgium, the Netherlands, Luxembourg, Northern France, Germany, Denmark, Norway, Sweden, Iceland, Finland, Switzerland and Austria), loss estimates in wheat production due to pests and pathogens are higher than their global average at 24.91% out of which 19.84% are due to fungal diseases (Savary et al., 2019). This situation necessitate the need to develop efficient resistance breeding programs to lower disease impact on wheat production in NWE.

I. Fusarium head blight (FHB) (paper I and III)

FHB, also known as scab, is a fungal disease that affects winter wheat and other cereal crops. FHB disease has expanded globally over the past decades (Lenc, 2011, Zhang et al., 2012). The disease is devastating to global wheat production, particularly in some provinces of China (Zhang et al., 2012), Argentina (Malbran et al., 2014) and in some parts of Canada and the USA (Gilbert et al., 2009, Martinez-Espinoza et al., 2014, Wegulo et al., 2015). The incidence and severity of the disease vary, and the species of *Fusarium*

involved in its occurrence vary from one geographical area to another (Gilbert et al., 2009), depending on weather conditions and agricultural activities conducted (Klix et al., 2008, Windels, 2000). The two species, *Fusarium graminearum* and *Fusarium culmorum* are considered the most frequent and economically important *Fusarium* species that produce the Trichothecenes fungal toxins (Toth et al., 2005). Numerous studies have shown a variation in the pathogenesis of FHB-causing *Fusarium* isolates/species (Golinski et al., 2002), in particular *F. culmorum* and *F. graminearum* (Fernandez and Chen, 2005, Xu et al., 2008). The consequence of the disease-caused damage to wheat and barley, resulted in significantly reduced quantity and quality of production (Madden and Paul, 2009, Osborne and Stein, 2007), at an estimated global loss of more than one billion US dollars annually (Wegulo et al., 2015).

Spores of the pathogen *Fusarium*, spread through the air and can be carried by the wind from infected plants to healthy ones causing them to produce fewer seeds or none at all. Symptoms of FHB include discolored, shriveled kernels on the head of the plant and a whitish or pinkish mold on the infected tissue. Various strategies exist to manage FHB disease, including the use of chemical and biological treatments, crop rotations, and tillage operations (McMullen et al., 2012). However, the use of chemical pesticides poses a significant risk to both humans and the ecosystem. Additionally, the extensive cultivation of wheat in large areas renders the use of pesticides economically unfeasible. As such, recent studies have focused on investigating the resistance of wheat varieties to FHB disease (Laidig et al., 2021).

Wheat resistance to FHB disease encompasses various aspects related to phenotypic characteristics such as plant height, presence or absence of awns, the density of spikelets, length of the peduncle, narrowness of the floret opening, and short flowering time (Gilsinger et al., 2005, Rudd et al., 2001, Snijders, 2004, Somers et al., 2003). In addition, there are characteristics specific to FHB resistance that can be classified into five types (I-V). Type I refers to resistance to primary infection, Type II refers to resistance against the spread of the disease on the spike, Type III refers to the degradation of DON mycotoxins and prevention of accumulation, Type IV refers to resistance against grain infestation, and Type V refers to the plant's tolerance to infection, mycotoxin stress, and yield production (Mesterhazy et al., 1999).

II. Septoria tritici blotch (STB) (paper II and IV)

Blotch diseases of wheat are among the most damaging to wheat production. In the Baltic region that includes Sweden, the three top blotch diseases with significant economic loss are Stagonospora nodorum blotch (SNB), tan spot, and STB.

Relative to the other two blotch diseases, STB has the highest impact on wheat production and comes as the second leading cause of production losses in Northwest Europe after stripe rust (Savary et al., 2019). In fact, STB is ranked number one among wheat fungal diseases in terms of fungicides application, where it accounts for the use of up to 70% of all used fungicides in the EU (Fones and Gurr, 2015). The disease is caused by the fungal pathogen *Zymoseptoria tritici* resulting in losses cumulating to 50% of the yield when conditions are conducive for disease development (Ghaffary et al., 2018, Platel et al., 2020). Infected stubble provide the initial inoculum which can be windborne to be carried over a large area upscaling potential infections mainly if the favorable conditions of high relative humidity and cool weather are present. At conducive conditions, spores give germ tube penetrating leaf stomata, where mycelium will grow in leaf tissue for a period of 10-13 days which is known as the latent symptomless phase (LP). Gradually the infected areas turn into necrotic tissues where pycnidia form in the decaying tissues. These symptoms of the disease usually appear on the older lower leaves with lesions conferring dark spots of pycnidia typical for STB (Fig. 1a, 1b), with chlorotic marginal areas surrounding the lesions (Fig. 1a and 1b). Pycnidia are the asexual fruiting bodies of the anamorphs of spores that germinate after absorbing water and swell (Fig. 1c) followed by release of pycnidiospores by rain splashes. Due to this, the newly released pycnidiospores move upward the plant and to the nearby plants or carried over to further distances causing secondary infection to the new leaves. With this cycle being repeated in wet conditions, secondary infection therefore could occur multiple times during the growing season resulting in the highest impact on wheat production when the flag leaf is infected. With climate shift especially in regions that are expected to receive increased rates of precipitation in the higher latitudes and the increased spring temperature of the northern hemisphere (Gigliotti et al., 2005), secondary infection can cause STB to be potentially the predominant disease in wheat cultivated areas at early growing stage.

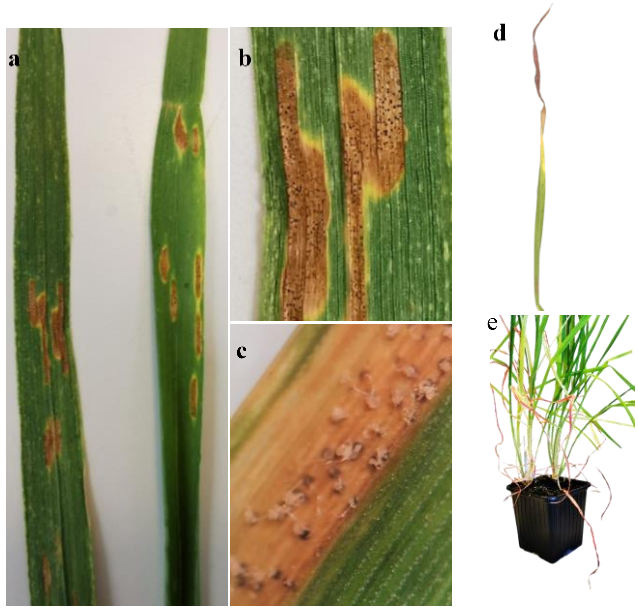


Figure 1. Symptoms of Septoria tritici blotch (STB) on wheat leaves infected in field conditions (a, b and c). (a) Dark spots of pycnidia on older lower leaves with chlorotic marginal areas surrounding the lesions. (b) Enlarged view of pycnidia showing dark spots with chlorotic margins. (c) Swollen pycnidia after absorbing water, indicating the presence of asexual fruiting bodies of the anamorphs of spores. (d and e) symptoms of STB in controlled environment.

In greenhouse artificial inoculation of wheat, the infected leaves develop STB symptoms that can differ to a large extent to those observed on plants naturally infected in the field (Fig. 1d and 1e). In contrast to natural infection, infection of plants in greenhouse conditions begins with general chlorosis and spreads across the entire leaf or partially from the leaf tip (Fig. 1d and 1e). Following that, reddish necrosis develops in place of chlorosis, resulting in tissue collapse in the infected area.

STB could be considered one of the most difficult diseases to control by farmers. The pathogen is able to infect the plant at the juvenile stage up to the adult stage on the flag leaf. The variability of *Z. tritici* aids the pathogen in overcoming varietal resistance (McDonald et al., 1999). The pathogen reproduces sexually several times within the growing season resulting in potentially more virulent strains that can evade fungicide effects and host plan resistance (Kema et al., 1996, Suffert et al., 2019). It is well documented

that certain varieties initially resistant to STB lose their resistance within a few years from their release (Cowger et al., 2000). The *Stb6*, a major resistance gene for STB resistance, found in nearly half of the European varieties, has been shown to be no longer effective against *Z. tritici* populations (McDonald and Mundt, 2016). Therefore, repeated fungicide application is one approach to manage the disease hence there is a high cost of fungicide use for STB control at 1.2 billion US dollars per annum (Torriani et al., 2015).

1.4 Significance of improving disease resistance breeding in winter wheat

Crop rotation, continuous fungicide applications, seed treatment, drainage systems, soil fertility schemes, multiline mixture cultivation, and biocontrol application are among the prevailing cultural practices used to lower the impact caused by diseases on wheat yields. Fungicide use is the major approach for managing several wheat diseases in Northern Europe and other parts of the world (Cook et al., 1999, Lynch et al., 2017). Over time fungicide efficacy is reduced due to the high selection of pathogen populations leading to the rise of fungicide-resistant strains of the disease-causing pathogens (de Chaves et al., 2022, Mae et al., 2020, Yerkovich et al., 2020). With the increased number of applications, fungicides may become an economic and environmental problem in wheat-growing areas. Taking into consideration the length of winter wheat breeding, an efficient, rapid and sustainable disease resistance breeding strategy becomes highly needed. Such a strategy will make use of the available resources and advances to be integrated into winter wheat breeding programs, ultimately aiming to quickly respond to epidemics caused by the rapidly evolving pathogens.

1.4.1 Sources of host resistance

Awareness of the rich diversity of exotic or wild germplasm has grown in recent decades which resulted in a greater reliance on germplasm in breeding (Kearsey, 1997). Therefore, a positive relationship has been noted between the number of accessions evaluated in genebanks and the number of varieties developed and released from the evaluated material (Hammer, 1993 as cited in Acquaah, 2012). Diverse germplasm utilization in breeding programs can accelerate the genetic gain of the crop that can be in turn reflected by

socioeconomic gains. An example of economic gains is the utilization of Turkish wheat varieties to enhance the resistance in some American wheat cultivars to diseases which resulted in an annual estimated value of US$50 million in 1995 (Acquaah, 2012). Increasing the levels of wheat resistance to fungal diseases requires the employment of novel tools in plant genetics for identifying novel resistance among collections of breeding lines and unadapted germplasms including old cultivars, landraces and wild cultivars. These will then be exploited to introgress resistance into elite cultivars in breeding programs followed by selection in greenhouse and testing under several field environments.

1.4.2 Major vs. minor genes in resistance breeding

Plants have evolved complex means of response and resistance mechanisms for recognition and defense against various environmental elements (de Wit, 2007). Such resistance responses are elicited through specific resistance (R) genes in the host and avirulence/virulence genes in the pathogen. Identifying and understanding R genes in the host and avirulence/virulence genes of the pathogen is an essential step in breeding success (Singh et al., 2021b). When the effectors encoded by the avirulence (AVR) genes are recognized by the host's R gene products, resistance can be conferred. Generally, R genes are limited in number, where for instance, 21 R genes to the STB pathosystem have been identified (Brown et al., 2015). Meanwhile, in other diseases such as FHB, resistance responses are largely quantitatively controlled (Ma et al., 2020, Ollier et al., 2020, Venske et al., 2019). Host resistance R genes may render wheat genotypes ineffective against the range of AVR genes due the ability of fungal species of sexual reproduction resulting in more virulent pathogens.

I. Quantitative host resistance to FHB

Multiple resistance types (mentioned earlier) are under the influence of a large number of genes, with studies suggesting that 37% of wheat genes have a susceptibility/resistance response to *F. graminearum* (Golkari et al., 2005). Earlier studies have shown that more than 100 genetic loci are associated with resistance to the disease (Buerstmayr et al., 2002). Upon that, breeding programs began utilizing FHB-resistance genes for the genetic improvement of wheat against FHB (Brar et al., 2019, Xu et al., 2020). The genes *Fhb1*,

Fhb2, *Fhb3*, *Fhb4*, *Fhb5*, *Fhb6* and *Fhb7* are the main genes discovered so far, which have a role in resistance against FHB disease in some wheat varieties (Zhu et al., 2019). To assess the variation of these genes and their expressions in different varieties, numerous genetic markers have been identified that are linked (to varying degrees) to these genes located on the different chromosomes. It has been found that the markers associated with the resistance gene *Fhb1*, which is located on chromosome 3BS, are specialized in Type II resistance such as the markers *gwm493* and *gwm533* (Cuthbert et al., 2007, Yu et al., 2008). An earlier study found the association of *Fhb1* gene with DON resistance through its conversion to the low-toxicity compound DON-3-O-glucoside (Lemmens et al., 2005). The two markers *gwm133* and *gwm644* from *Fhb2* were found associated with the same quantitative resistance type (Type II) (Cuthbert et al., 2007). It has been further shown that for this type of resistance, the markers *BE586744-STS*, *BE404728-STS*, and *BE586111-STS* were associated with *Fhb3* gene (Qi et al., 2008). As for the Type I resistance, the *Fhb4* gene is associated with both the *barc20* and *wmc349* markers (Xue et al., 2010), while the *Fhb5* gene encoded two markers *barc56* and *barc100* markers for this type of resistance (Xue et al., 2011).

The role of the *Fhb1* gene in enhancing resistance has been demonstrated in wheat varieties carrying this gene. Values of the DON mycotoxin production, the percentage of damaged grains, the disease index, and the incidence and severity of the disease were lower compared to the absence of the gene (Castro Aviles et al., 2020). Many resistance genes have been discovered that encode secondary metabolites that play an important role in disease resistance. For example, QTL-*Fhb2* region encodes several secondary metabolites such as CoA ligase (4CL), callose synthase (CS), basic Helix Loop Helix (bHLH041) transcription factor, glutathione S-transferase (GST), ABC transporter-4 (ABC4), cinnamyl alcohol dehydrogenase (CAD) (Dhokane et al., 2016), GDSL Lipase TaGDSL (Schweiger et al. 2016), Pore-forming toxin-like TaPFT (Rawat et al., 2016), Pectin methyl esterase inhibitor W*Fhb1*_c1 (aka W*Fhb1*-1) (Paudel et al., 2020, Su et al., 2019) and His-rich Ca-binding protein TaHRC or TaHis (Su et al., 2019). Furthermore, the presence of the wall-associated receptor-like kinase (WAK2) gene in resistant cultivars contributes to preserving the integrity of the plant cell walls by maintaining high levels of methyl-esterified pectin, which in turn protects the plant cell wall from fungal attack.

Cultivars containing this gene have been used in a series of breeding programs (Gadaleta et al., 2019).
Thus, exploring the resistance mechanisms of wheat varieties to FHB disease can pave the way for the development of more effective and sustainable management strategies for this devastating disease. Based on the above, it appears that screening for resistant cultivars or those carrying some resistance traits to this disease is promising for disease management. Therefore, the current study aimed to test sets of winter wheat lines and cultivars for FHB resistance under artificial infection conditions in the greenhouse.

II. Quantitative host resistance to STB

A sustainable strategy to mitigate the impact of STB disease can be realized by adopting an integrated approach that encompasses host resistance and other management practices. Developing wheat varieties with enhanced resistance through breeding is a key approach that can reduce the environmental impact of fungicides in the long run. Major gene resistance, while initially effective, is often overcome by emerging virulent strains due to sexual reproduction under field conditions. In contrast, minor genes confer cumulative and long-acting quantitative resistance against diverse and evolving *Z. tritici* strains. Biparental populations have helped identify many quantitative trail loci (QTL) associated with STB resistance, and association mapping has identified several regions linked to resistance (Brown et al., 2015, Naz et al., 2015, Riaz et al., 2020, Tamburic-Ilincic and Rosa 2019). Phenotypic evaluation of disease development can be assessed by the rate of disease symptom development on the leaf area before associating it with genotypes. STB LP from incubation to the appearance of disease symptoms can be used as a marker for the quantitative genotypic resistance, but factors such as sowing date, pycnidia concentration, environmental conditions, growth stage, and varietal responses contribute to varying length of STB LP under field conditions. Investigating genotypes with delayed symptom appearance of STB on seedling stage can be beneficial in restricting STB infection spread, as prolonged presymptomatic LP is associated with reduced blotch size and capacity for producing larger amounts of the pathogen. The delay in developing the necrotic phase during early growth stages of the plant also renders the polycyclic nature of infection less efficient towards infecting new upper leaves. Genotypes with extended LP at seedling stage can express

quantitative seedling stage resistance (SSR) or overlap with adult plant stage resistance (APR). Statistical analysis of variance for the spatio-temporal development of STB coupled with genome-wide association analysis can help identify genotypes with quantitative nature of resistance to the disease. In the past, a total of 89 meta-QTL were identified with STB resistance (Brown et al., 2015). Recently, several regions linked to STB resistance in the wheat genome have been discovered through association mapping (Ando et al., 2018, Gerard et al., 2017, Gurung et al., 2014, Kollers et al., 2013, Muqaddasi et al., 2019, Odilbekov et al., 2019, Vagndorf et al., 2017, Yates et al., 2019). However, earlier studies about stage specificity have suggested that certain genomic regions in wheat specialized in STB responses might be specific to particular growth stages and could go unnoticed due to environmental interactions with genotypes at the seedling stage (Brown et al., 2015). Therefore, it is crucial to eliminate environmental noise to uncover the genetic nature of resistance, which can be accomplished by infecting plants homogeneously under controlled conditions. Recent studies have shown that an increasing number of STB APR QTL contribute to resistance at all stages rather than being limited to the adult stage (Alemu et al., 2021, Odilbekov et al., 2019).

1.4.3 Speed breeding in winter wheat resistance breeding (paper I)

Speed breeding is a technique used in plant breeding to reduce the period for the plants to complete their life cycle (Cha et al., 2022, Liu et al., 2016, Song et al., 2022, Yao et al., 2016). It involves growing plants under conditions of extended daylight and controlled temperature, which allows for faster growth and development (Ghosh et al., 2018, Watson et al., 2018). In traditional winter wheat breeding, it typically takes several years to develop a new variety of wheat, as the plants must go through several generations of growth and reproduction before the desired traits can be selected and fixed in the new variety. In a modified backcross strategy, resistance to four diseases in barley were introgressed into 87 intorgession lines within two years while growing under speed breeding conditions (Hickey et al., 2017). In winter wheat, using similar approach, the generation cycle can be shortened significantly, which can allow breeders to grow up to three generations of winter wheat per year instead of one generation in field or two in greenhouse conditions while selection for resistance is carried out (Zakieh et al., 2021). However, without disease screening, up to four generations of winter wheat

per year can be achieved in speed breeding conditions given that vernalization infrastructure is available. Figure 2 illustrates time shortening in conventional greenhouse methods compared to speed breeding for winter wheat. Moreover, recent advances by modifying growth conditions of the plants by exploiting the lengthy vernalization period have demonstrated the ability to grow five generations of winter wheat per year (Cha et al., 2022).

Figure 2. Comparison of time required for winter wheat breeding using speed breeding (upper timeline) and conventional greenhouse methods (lower timeline). The figure illustrates the time shortening of winter wheat breeding using speed breeding compared to conventional greenhouse methods. The chart shows the time required for each generation in the conventional greenhouse method and speed breeding. Speed breeding can shorten the generation cycle, thereby reducing the total time required for winter wheat breeding.

The exact length of the generation cycle of winter wheat in speed breeding will depend on a variety of factors, including the specific breeding goals such as simply acquiring a few seeds for the next generation in single seed descend (SSD), disease phenotyping on flag leaf or ears or other traits. Other factors that play a role in the duration of the winter wheat generation cycle in speed breeding are related to the range of variation in the growth and development of the screening population and the conditions under which the plants are grown such as light sources, their intensities and growth temperature. Therefore, it is essential to carefully control these factors in order to optimize the speed breeding protocol to be integral in resistance breeding when disease selection is the goal.

Vernalization is critical in winter wheat. The phenology in natural conditions requires the plant to be growing at low extended temperatures for structural and developmental traits to be commenced, such as flowering and forming the ears. The extended photoperiod promotes the plants to flower in a shorter time (Gonzalez et al., 2002, Miralles and Richards, 2000, Watson et al.,

2018). After vernalization, the period until flowering is reduced due to the shortening of time between the sequential emergence of leaves on the stem at fixed growth temperature with prolonged photoperiods (Cao and Moss, 1989, Warrington and Kanemasu, 1983). However, while winter wheat is well-suited for northern climates due to its ability to tolerate long periods of cold, it is important to consider the effects of light intensity and duration, CO2 concentration and temperature on its growth and development under speed breeding conditions. Different wavelengths of light can affect the plant's growth and development in different ways, with some wavelengths promoting growth and others inhibiting it. Recent studies have shown the beneficial effect of light-enriched far-red wavelengths to the growth and development of different plant species growing under speed breeding conditions (Ghosh et al., 2018). By carefully controlling the light conditions, wavelength intensities, light source and light duration, it is possible to optimize the speed breeding protocol to be integrated into disease resistance evaluation of a large number of winter wheat genotypes.

1.4.4 Genomic selection in resistance breeding (paper II)

In crops, Genomic Selection (GS) is a valuable tool for enhancing the accuracy of quantitative disease resistance selection and accelerating genetic gains (Poland and Rutkoski, 2016). A group of individuals with both whole-genome marker data and phenotypic information is selected and used as a training population. The training population is used to develop prediction models and determine the effects of markers in the breeding population (Meuwissen et al., 2001). By relying solely on genotypic data, the breeding values of untested individuals, known as "selection candidates," can be predicted (Meuwissen et al., 2001). Using these estimated breeding values calculated using genomic prediction models (GP), GS of genotypes based on their genetic merit can be carried out in breeding programs. Several studies have demonstrated the promise of GS for various wheat diseases, including FHB and STB (Alemu et al., 2021, Juliana et al., 2017, Rutkoski et al., 2012), making it an appealing strategy for breeders looking to reduce time and resources spent on phenotyping. The potential of using GS is that it makes it possible to identify individuals with resistance suitable for crossing and intensify the selection process in the initial breeding phases.

1.4.5 Phenomics in disease resistance breeding (paper III)

Phenomic approaches offer a tool for the measurement of observable traits, or phenotypes, of organisms. It is becoming an increasingly important tool for crop breeding, as it allows for the capturing of the fine phenotypic differences more efficiently that can be later used in genotypic association studies (Harfouche et al., 2019). By measuring and analyzing various phenotypic traits, such as plant height, leaf area, chlorophyll content, and disease symptoms, it is possible to identify which traits are associated with resistance to specific diseases (Mohanty et al., 2016). For example, the use of high-throughput phenotyping platforms, such as unmanned aerial vehicles (UAVs), can provide detailed information on plant growth and development, including canopy cover, leaf angle, and plant height, which can be used to identify disease-resistant varieties (Singh et al., 2021a). Similarly, imaging technologies like hyperspectral imaging and thermal imaging can be used to identify disease symptoms, such as changes in leaf color and temperature, which can be used in selection for disease resistance (Simko et al., 2017).

FHB is a major disease affecting wheat grain yield and quality, leading to the formation of sterile and wizened florets and the build-up of mycotoxins such as deoxynivalenol (DON). Resistance types for FHB have been defined into five categories, and traditionally, studies on FHB resistance have relied on measuring the symptoms associated with the disease on spikes and kernels. However, visual screening for FHB resistance is a labour- and time-consuming process with low reproducibility, and possible subjectivity leading to investigate the use of image analysis methods to evaluate FHB-damaged kernels (FDK) (Maloney et al., 2014).
The morphological seed traits, such as colour, thickness, length, and width, can be functional for predicting FHB (Leiva et al., 2022). In this context, image-based methods could be developed to examine their consistency and to predict FHB with the assigned traits in relation to the phenotype-genotype association. This can potentially provide higher throughput data generation ultimately replacing traditional visual assessments.

1.4.6 Host/biocontrol compatibility breeding (paper IV)

Pathogen virulence, its genomic plasticity in changing environment, development of resistance to fungicides, host plant responses and mechanisms of plant defenses, plant genotype-pathogen and environment

interaction, are key players that need to be taken into consideration for the development of durable varietal resistance. Biological control utilizes living organisms to control pathogens (Stenberg et al., 2021), and is a promising strategy to replace chemical pesticides. Insights into biological control mechanisms such as competition, antibiosis, parasitism and elicitation of defense responses in host plants are important for increasing biocontrol efficacy of biological control agents (BCAs) (Jensen et al., 2021). *Clonostachys rosea* is a fungal species with biocontrol effect against several pathogenic fungi including several *Fusarium* species (Chatterton and Punja, 2009, Inglis and Kawchuk, 2002) and *Z. tritici* (Jensen et al., 2021). In a field trial, spraying a strain of *C. rosea* reduced FHB severity on host plants infected with *F. graminearum* and showed lower DON content in grains with an increase in yield compared to the fungicide tebuconazole (Xue et al., 2009). In a separate field trial, similar effects on yield with reduced FHB severity and DON content in grains were observed where three varying cultivars with regard to resistance to FHB, showed that the highest efficacy of the biocontrol was detected in the resistant cultivar (Xue et al., 2014). This insight into this relationship between host resistance and biocontrol efficacy is of paramount importance where possible host-BCA compatibility can enhance durable varietal resistance. Understanding the roles BCAs play in inducing resistance of plants in which host plant genetics are involved have the potential to lead resistance breeding efforts for screening for genotypes with enhanced BCAs compatibilities. Several studies have shown the ability of *Clonostachys* spp. strains, including *C. rosea*, to act as endophytes within host plants (Chatterton and Punja, 2010, Maillard et al., 2020, Mueller and Sinclair, 1986, Saraiva et al., 2015). This close relationship between *C. rosea* and plants can trigger the expression of defense genes in plants as been demonstrated on wheat by Jensen et al. (2021). In an earlier study, activation of pathogenesis-related proteins caused a notable inhibition of the growth of the pathogen *F. culmorum* in wheat seedlings colonized by *C. rosea* (Roberti et al., 2008). The findings of genotypic-biocontrol specific efficacy can potentially lay the groundwork to advance implications in breeding programs for disease resistance. This practice may open the way for a new field in breeding by which germplasm selection will be based on subset of genotypes that best perform against a disease when a biocontrol agent is applied compared to other treated genotypes.

1.5 The experimental set up (paper I to IV)

A well-designed experiment allows for the accurate measurement of the effect of a specific treatment, such as a new winter wheat variety or a disease control strategy. Which in turn allows for informed decisions about the treatment's efficacy and whether it warrants further research or adoption. In addition to addressing the design, replicates (i.e., repeating the experiment multiple times) are vital as they help to increase the statistical power of the experiment. This means that the results are more likely to be reliable and generalizable to other situations. Without replicates, it may be difficult to determine whether the observed effects are real or due to chance. Additionally, the focus on a single set of time points may not fully capture the dynamics of disease development in plants. Therefore, several time points are used in order to avoid potential biases in the studies, including the lack of replication or control groups, which may impact the accuracy and validity of the results (papers I, III, and IV). The final goal of experimental design and replication is to provide the possibility to isolate the effect of the treatment being studied from the effects of confounding variables of the environment.

Augmented randomized block design (ARBD) is a type of experimental design that is commonly used in agricultural trials, including trials involving winter wheat. It is a variant of the traditional randomized block design, which is a widely used method for designing experiments in which the effect of one or more factors is being studied. In an ARBD, the experimental units (e.g., plots of land) are arranged in blocks, with each block containing a random selection of the treatments being studied. The blocks are then randomly assigned to one of the treatments, and the experiment is carried out. The advantage of ARBD is that it can allow for the simultaneous comparison of multiple treatment within a single experiment. By randomly assigning treatment levels to blocks and controlling for these variables using control genotypes randomized repeatedly in each block of the replicate, which can more accurately determine the effect of the treatment being studied. However, the advantage of the ARBD design that it allows evaluting large screening population in limited space when conducted in controlled environment.

In this thesis, the number of replicates and individual genotypes per replicates necessitated the usage of ARBD to limit the need for large growing space. All genotypes studied for their response to FHB and STB were grown

in the biotron, the cultivation unit of the Swedish University of Agricultural Sciences in Alnarp. The biotron is a facility that allows growing plants in precisely climate-controlled chambers for different parameters such as light, temperature and humidity.

2. Objectives

The overall objective of the thesis is to implement modern breeding methods to accelerate selection of resistant breeding lines in winter wheat breeding programs. In this context, the adult stage FHB and seedling stage STB diseases can both be investigated for their genetic basis of resistance in winter wheat germplasm collections. Seedling stage evaluation for STB resistance is possibly a straightforward approach. However, it lacks the important information about the potential markers that can be detected at later developmental stages. Thus expanding the knowledge about these markers by increasing the number of studied genotypes can offer the possibility to reveal all stage markers early at seedling stage. This can be achieved by comparing the novel seedling stage markers that are possibly overlapping with previously described ones at the adult stage. This approach is not possible to explore the markers for FHB resistance. Therefore, embedding speed breeding into a protocol for winter wheat can shorten the time needed for evaluating FHB resistance due to the rapid development of the plants in comparison to traditional growth conditions in the greenhouse. With this major objective, the following specific objectives were investigated:

- To examine the suitability of deployment of speed breeding methodology for adult winter wheat resistance breeding by characterizing FHB resistance on panels of winter wheat germplasm (paper I).
- To identify markers for FHB resistance in the developed protocol integrating speed breeding, to eventually accelerate selection in winter wheat breeding programs (paper I).

- To develop a high throughput, cost-efficient and simple phenomic platform for the prediction of FHB-infected seeds. This objective is carried out in conjunction with the phenotypes resulted from the germplasm evaluated visually for FHB severity in the earlier objective (paper III).
- To expand the knowledge of the underlying quantitative genetic basis for STB resistance in winter wheat seedlings in controlled growth conditions (paper II).
- To develop genomic prediction models for STB resistance breeding (paper II).
- To investigate the possibility of enhancing STB resistance modulated by biocontrol (*C. rosea*)-genotype-specific interaction in winter wheat seedling stage (paper IV).
- Investigate the possibility of applying the methodologies and knowledge obtained from this academic research in industry.

3. Materials and Methods

3.1 Plant material

Winter wheat germplasm from two different sources were used in this thesis work. The first group included a germplasm of 181 diverse genotypes of landraces, old cultivars, and wild relatives (genebank set), obtained from the Nordic Genetic Resource Centre (Nordgen), Sweden. The other group consisted of 338 advanced breeding lines (breeding set) from a breeding program provided by the Swedish agricultural cooperative, Lantmännen Lantbruk, Svalöv, Sweden. The material from the two sources was used in the studies to develop a protocol implementing accelerated growth conditions for FHB characterization (paper I). The same material earlier subjected to FHB in paper I, was subsequently used to develop phenomic methods to predict FHB in wheat seeds (paper III). One set was used for GWAS and prediction of STB-seedling resistance (breeding set, paper II), and the genebank set for studying biocontrol-genotype compatibility for STB resistance (paper IV).

3.2 Protocol for FHB phenotyping integrating accelerated growth of winter wheat (paper I and III)

3.2.1 Germination

The 519 winter wheat genotypes from the genebank and the breeding program were arranged according to an augmented block design, as described in the experimental set up. On peat soil, the seeds were left to germinate for five days under adjusted day parameters of 8 hours with light intensity (LI) at 250 μmol m−2 s−1, at a temperature of 22°C and a relative humidity (RH) of 50%, followed by 16 hours of darkness at 20°C. After germination, only one plant was left per pot and the pots were watered as required.

3.2.2 Vernalization conditions

The seedlings were vernalized using short-day conditions, with an 8/16 h day/night regime, a temperature of 3°C, and a light intensity of 250 μmol

m−2 s−1. The type of light during vernalization, the composition of wavelengths and the intensities of individual wavelengths are detailed under accelerated growth conditions. Together with the previous conditions, the relative humidity was maintained at 80% for approximately 60 days.

3.2.3 Accelerated growth conditions

After vernalization, plants were acclimatized gradually over a period of 6 days. The temperature was increased by 3-4°C per day and day-length by 2-3 hours. LI was increased to 400 µmol m−2 s−1 on the next day and remained constant during acclimatization, while RH was gradually reduced until it reached 50% at the end of acclimatization with 22 hours of daylight.
Following the acclimatization period, plants were grown for 31-33 days under accelerated growth conditions, with the same light period and intensity, temperature, and humidity as the last day of acclimatization. The light source used was LED lights with nine individually wavelengths within a controlled range, starting from 380 nm to 735 nm, and white light. Wavelengths 380, 400, 420, and 450 were made to radiate at 480 µmol m−2 s−1 intensity, while the remaining wavelengths were adjusted to 960 µmol m−2 s−1. A sensor-feedback-based lighting system was used to control the LI at the plant canopy level to 400 µmol m−2 s−1 for 22 hours per day. The temperature was maintained at 22°C throughout the accelerated growth phase (Zakieh et al., 2021).

3.2.4 Inoculum preparation for FHB

Isolates of *Fusarium* species were provided by Lantmännen Lantbruk for the preparation of inoculum for inoculation of FHB. In order to identify germplasm with broad resistance, a total of nine isolates were included in the study, consisting of six isolates of *F. graminearum* and three isolates of *F. culmorum*. The isolates were cultured on agar media Spezieller Nahrstoffarmer (SNA) (Leslie and Summerell, 2008), then incubated at 24°C for 4 days. Afterward, the fungal cultures were exposed to near ultra-violet UV radiation for 10 hours to promote macroconidial formation. The cultures were incubated again at 24°C for 3-4 days prior to harvesting macroconidial spores for the inoculum preparation. The spores were suspended in water with a spore concentration of 5×10^5 spore/ml before adding a surfactant.

3.2.5 Inoculation conditions and optimization of FHB severity assessment

After the completion of ear emergence and anthers protrusion, the plants were moved to a long-day regime in glasshouse chamber, with a 16/8 hour day/night cycle. At this stage, the genotypes have reached at 75% heading. The temperature was maintained at 24°C, and the relative humidity was adjusted to 60%. These new growth conditions allowed the plants to continue growing without accelerated growth for 24 hours prior to inoculation. Wheat heads were spray-inoculated once with the isolate mix suspension and then incubated at a high relative humidity 90% for 48 hours, while other growth parameters were kept unchanged. After the incubation period, the relative humidity was lowered to 60%, and the plants were allowed to grow with this humidity level till the end of the following visual assessment of the FHB disease.

FHB disease severity on spikes was visually assessed at four-time points after inoculation. Visual symptoms, including withered, pale or discolored, and diminished spikes were used as a marker for the development of FHB in the wheat heads. Disease development was assessed as a percentage of infection, ranging from 5% (most resistant) to 100% (most susceptible). The scoring method was adjusted to determine the proportion of infected spikelets to the total number of spikelets per spike on the main tiller. Furthermore, the scoring method focused on assessing the overall extent of infection in relation to all infected spikelets on the ear, irrespective if the specific symptoms displayed connectivity between infection sites on the same ear.

Considerations in the scoring method (paper I)

The study faced challenges due to genotypic variation in heading and flowering, which could impact the uniformity of the disease development on the diverse genotypes. To minimize bias in the subsequent analysis of FHB resistance, germplasm genotypes that exhibited a 0% infection phenotype were discarded, as were genotypes that had not reached the heading of 75% at the time of inoculation. The genotypes that displayed varying FHB symptoms ranging between 5 and 100% were included in the analysis.

3.2.6 Evaluating agronomic traits and disease development

To assess the growth and development of different genotypes during the reproductive phase, various measurements were taken. For each genotype, the measurement of flag leaf area (FLA) was conducted using an LI-3000C Portable Leaf Area Meter, while spike length (SPL) and spike width (SPW) were measured using a digital Vernier caliper scale. To avoid bias, SPW measurements were taken at the third lower spikelet. The heading time (HT) was recorded for 75% heading of spikes from the flag leaf at three time points with three day interval. Based on this, were divided into early, medium, and late HT groups based on the three measuring time points. Anther extrusion was observed twice, with a 2-day interval, and was classified as early (AE1) and late (AE2).

3.2.7 Phenotypic evaluation of FHB severity

To analyze the phenotypic data, unadjusted means of cultivars with a percentage of 0% within the augmented design of each replicate were removed. The analysis was conducted in two steps. Firstly, the means for each trait per experiment/replicate were adjusted using the Agricolae R package (De Mendiburu, 2014) using the following model:

$$y\_il = u + G\_il + B\_l + \varepsilon\_il$$

where y_il represents the adjusted means of the ith wheat genotype in the lth block, u is the general mean value, G_il is the effect of the ith wheat genotype in the lth block, B_l is the lth block effect, and ε_il is the residual. Secondly, the best linear unbiased estimates (BLUEs) were calculated using the randomized complete block design option in META-R 6.04 (Alvarado et al., 2020) based on the model:

$$y\_ijm = u + S\_j + G\_ijm + R\_m + \varepsilon\_ijm$$

where y_ijm is the BLUE of the ith wheat genotype from the jth source/population in mth replicate, u is the general mean value, S_j is the effect of the jth source of material, G_ijm is the effect of the ith wheat genotype in the mth replicate, R_m is the mth replicate effect, and ε_ijm is the residual effect. The source of wheat genotypes, S_j, was treated as the grouping factor. Additionally, the area under disease progress curve (AUDPC) was estimated from the adjusted means of the four disease ratings for each experiment, specifically for FHB severity.

3.3 FHB-infected seed shape parameters obtained through automated imaging and software analysis (paper III)

In this study, two different methods were employed to phenotype grain seeds: the commercially available automated imaging instrument, Cgrain Value™, and the publically available SmartGrain software (Tanabata et al., 2012), on the Quantitative Plant website (Lobet, 2017). The two methods are described below.

3.3.1 The SmartGrain method

The method used a low-cost protocol to capture seed images with a Canon EOS 1300D camera mounted on a repro stand. Digital images were stored in JPEG format with a 3,456 × 2,304-pixel resolution. SmartGrain software was used for image analysis, which generated seven morphological characteristics of the seeds: PL (perimeter length), AS (area seed), W (width), L (length), LWR (length-to-width ratio), and CS (circularity of the seed). The software's output provided detailed seed shape parameters. The method followed the implementation described by Tanabata et al. (2012).

3.3.2 Cgrain Value™ imaging instrument

The Cgrain Value™ is an imaging instrument that provides single kernel analysis. The device utilizes a mirror design that captures over 90% of the kernel's surface in every image, allowing for a thorough inspection of each seed. To perform the analysis, a batch of seeds per genotype was poured into the metal bowl of the device, where they were rotated, photographed, and analyzed individually. The analysis generated three reports: a result file, a stat file, and an image file. The result file contains morphological features pertaining to seed batches, such as seed count and thousand kernels. The stat file provides data for individual seeds in a group, including length, width, and thickness, while the image file consists of single seed images acquired during the analysis. The Cgrain Value™ tool offers a range of morphological characteristics, encompassing length, width, thickness, average width, volume, weight, light, hue, and saturation. Measurements for length, width, and thickness were obtained by assessing the major axis, higher minor axis, and minor axis, respectively. Seed volume (V) was derived from the 3D image, while weight (WT) was determined using an internal balance

integrated into the device. Furthermore, the instrument facilitated the determination of color parameters such as hue, saturation, and light, providing information on the color base, saturation level, and brightness of each seed.

3.3.3 Statistical analysis of wheat genotypes for predicting FHB infected seeds using multiple regression models

For the execution of statistical analysis, the following steps described table 1 were followed.

Table 1. Steps taken to analyse scoring data of infected wheat spikes and predict Fusarium head blight (FHB) in seeds using multiple regression models.

Step	Description
1	Compilation of visual scoring data of infected wheat spikes from the final time-point, including cultivars without symptoms, with mean values per genotype obtained from Cgrain Value™ and SmartGrain methods.
2	Filtering out replicates with missing data and substituting those with presence in more than one replicate using FactoMineR (Lê et al., 2008) and missMDA (Josse and Husson, 2016) packages.
3	Adjusting the means for each trait per replicate using the Agricolae R package with the checks in each augmented block, following the model: yil = u + Gil + b1 + ϵil.
4	Calculating the best linear unbiased estimates (BLUEs) using the randomized complete block design option in META-R 6.04 (Alvarado et al., 2020) based on the following model: yijm = u + Sj + Gijm + Rm + ϵijm.
5	Using the previously centered BLUEs data to predict FHB using a multiple regression model.
6	Creating three models using the morphological characteristics generated by both Cgrain Value™ and SmartGrain methods as while the dependent variable is represented by visual scorings.
7	The data set was divided into training and test sets using the "*createDataPartition*" function from the caret package. (Kuhn et al., 2020). The training set comprises 70% of the data, while the remaining 30% is allocated for evaluating the model's performance.
8	Fitting the model to the training set and predicting the responses using the test set.
9	Executing cross-validation 100 times to improve quality of the predictions and lower the errors resulting from random data partitioning, and taking the mean of the criterion as the final result.

3.4 Set up for characterizing seedling stage resistance to STB and biocontrol efficacy (papers II and IV)

Over 316 winter wheat breeding lines (the breeding set used in the evaluation and of FHB resistance in paper I and III), provided by Lantmännen, Svalöv, Sweden, were used to assess their seedling stage resistance to STB under controlled growth conditions (paper II). While the genebank set consisting of 202 winter wheat genotypes was used to investigate the genotypic-biocontrol-specific efficacy of STB at the seedling stage (paper IV).

The randomized augmented block design was followed in the arrangement of test genotypes and the randomly replicated checks in each replicate using the R package agricolae (De Mendiburu, 2014). This resulted with total of 23 blocks of breeding lines (from the breeding set) and eight to nine blocks of test genotypes from the genebank set with four check genotypes in each block.

The pathogenic inoculum was prepared by growing two single-spore *Z. tritici* isolates, Alnarp and Svalöv, using the method described by Odilbekov et al. (2019). The inoculum concentration was adjusted to $1x10^6$ conidial spores/ml and surfactant Tween®20 was added at 0.002% v/v. Three-leaf-stage 19-day-old winter wheat seedlings were spray-inoculated three times, with leaves allowed to dry for 20-30 minutes between each spray. On the third spray, plants were moved into a high-humidity chamber with 90% RH at 23 ºC for 48 hours, then RH was lowered to 65% for the remainder of the experiment.

The foliar application of *C. rosea* and *Z. tritici* was detailed in table 2. In ZtCr treatment, plants were sprayed until run-off with *C. rosea* suspension at the concentration of $1e10^7$ cfu/ml, and with water only in Zt treatment. After 24 h, plants were sprayed until run-off with *Z. tritici* at the concentration of $1e10^6$ cfu/ml in both treatments and incubated at 90% relative humidity for 48 h. To maintain high humidity, plants were also sprinkled with water 4 to 5 times per day. Disease was visually assessed on two fully developed leaves marked at the base before the inoculation, using the percentage of leaf necrotic area with 5% step intervals. Disease scoring was conducted at three time-points in 2019 and four-time points in 2022 with three-day intervals, and the disease progress over time was summarized by estimating the relative area under the disease progress curve (rAUDPC).

Table 2. Foliar application of *C. rosea* and *Z. tritici* for genotype-specific *Cr. rosea* compatibility.

Treatment	Fungal Inoculum	Replicates	Evaluation Year
Zt	*Z. tritici*	2 (2019), 1 (2022)	2019, 2022
ZtCr	*Z. tritici* + *C. rosea*	3 (2022)	2022

Zt represents the treatment with *Z. tritici* alone, while ZtCr represents the treatment with both *Z. tritici* and *C. rosea*.

Considerations taken in the experimental set up (paper II and IV)

Paper II: To examine the virulence of the used isolates (paper II), four cultivars with known STB resistance backgrounds were inoculated at the seedling stage, including Stigg, Kranich, Julius, and Nimbus. The four cultivars were randomly replicated 19 times along with 25 advanced breeding lines and 41 official trial lines in an augmented randomized block design. Studies have identified Stigg as moderately resistant to STB, Kranich and Julius as resistant, and Nimbus as susceptible (Benbow et al., 2020, Brennan et al., 2020, Hehir et al., 2018). Cultivar Julius has high resistance to several wheat diseases, including STB (Kollers et al., 2013, Laidig et al., 2021).

Paper II and IV: In contrast to natural infection, greenhouse conditions result in initial chlorosis that spreads across the entire or partial leaf tip, followed by reddish necrosis and tissue collapse (Fig. 1d and 1e). To assess disease severity, all genotypes were visually evaluated for the second and third leaves 15 days post inoculation (dpi) under greenhouse conditions. Disease response was recorded every third day for four consecutive time points, and a visual scaling scheme was used to estimate the percentage of reddish necrotic areas relative to the total leaf area (Odilbekov et al., 2019).

Paper IV: To optimize concentrations of Zt and Cr and confirm the biocontrol efficacy of Cr on STB disease, four winter wheat genotypes with varying susceptibility to STB: Nimbus and Kask (susceptible), SW_150428 and Festival (resistant) were tested in Zt and Cr treatments. Four wheat seeds were sown per plastic pot in potting soil, and six treatments were used for each genotype, including a control treatment with no Cr and Zt. Disease progression was visually scored from 0 to 100% with 5% step interval, and the rAUDPC was estimated. This experiment aimed to observe the effect of Cr, Zt, and their various combinations, and five biological replicates were used for each treatment in each genotype. 20-day old plants were treated with *C. rosea* suspension and *Z. tritici* suspension after 24 hours, with disease

progression measured using necrotic leaf area scoring at various time points up to 30 dpi. The rAUDPC was then calculated.

3.5 Phenotypic analysis of STB resistance (paper II and IV)

3.5.1 Statistical analysis of phenotypic data (paper II)

To assess the resistance of winter wheat breeding lines to STB, area under the disease progression curve (AUDPC) approach was employed. Visual assessment of the disease severity ratio was conducted on the second and third leaves of seedlings 15 days post inoculation (dpi) in greenhouse conditions, and the ratio of necrotic leaf area was estimated as a percentage of disease severity. The AUDPC was calculated from the adjusted means recorded from four consecutive scoring time points using the Agricolae R package (De Mendiburu, 2014). The best linear unbiased prediction (BLUPs) was estimated across the two replicates from the adjusted mean values of genotypes using META-R 6.04 (Alvarado et al., 2020). The ANOVA and broad-sense heritability (H^2) were retrieved in this step. Finally, the frequency distribution of AUDPC BLUPs was performed in the Minitab software package.

3.5.2 Statistical analysis of phenotypic data (paper IV)

The statistical analysis was carried out on the infected winter wheat genotypes on the 202 genotypes from the genebank set. Initially, rAUDPC values were centred and scaled to correct for scoring on different days, and a linear mixed model analysis was performed using Kenward-Roger's approximation of the degrees of freedom to estimate best linear unbiased estimators (BLUEs). ANOVA was performed separately in each treatment, and a full mixed model with genotype and treatment interaction was applied to check for genotypic differences between treatments. Post-hoc Tukey's tests were performed for multiple comparisons among genotypes across treatments and to estimate differences between treatments for each genotype. All statistical analyses were performed using R software with various packages. Table 3 summarizes the corresponding phenotypic data analysis.

Table 3. Phenotypic data analysis for biocontrol-genotype specific efficacy against STB in seedling stage winter wheat germplasm.

Statistical methodology	**Large scale biocontrol efficacy screening**
Phenotypic data analysis	Phenotypic performance of genotype in block within replicate; Linear mixed model. using Kenward-Roger's approximation of degrees of freedom.
Statistical model	y_ijkl=μ+g_i+t_l+〖(gt)〗_il+ r_lj+ b_ljk+ ε_ijkl
Variables in model	Genotype, treatment, interaction, replicate, block nested within replicate
Random factor	Blocks nested within replicates
Post-hoc analysis	Tukey's test among genotypes at treatment level and inter-treatment genotype contrasts
Heritability estimation	H^2P (Piepho and Möhring, 2007) and H^2C i(Cullis et al., 2006)n treatments Zt and ZtCr
Statistical packages used	Imer package (Bates et al. 2021), lmertest (Kuznetsova et al. 2020), emmeans, (Lenth 2022), cld (Hothorn et al. 2021), Tidyverse suite (Wickham, 2021), (Bates et al. 2021), (Kuznetsova et al. 2020), Lenth 2022, (Hothorn et al. 2021), (Piepho & Möhring, 2007), (Cullis et al. 2006)

3.6 Genome-wide association analysis of wheat genotypes for STB resistance (paper II and IV)

The panel of 316 winter breeding lines was previously genotyped using a 25K SNP chip (Zakieh et al., 2021), resulting in 24 145 markers. 10 120 SNP markers were used after filtering for minor allele frequency (MAF) and missing values and were used in the downstream association analysis.
Meanwhile, the panel of the winter wheat genotypes from the genebank set was previously genotyped using a 20K SNP chip (Odilbekov et al., 2019). After similar treatment and quality checks, 7360 SNP markers were left for the GWAS.

Two single-locus and five seven multi-locus models for genome-wide association studies (GWAS) using the GAPIT 3.0 (Lipka et al., 2012) and mrMLM v4.0.2 (Zhang et al., 2020) were used for GWAS of the breeding set. Meanwhile, the same single-locus models and three multi-locus models using GAPIT 3.0 (Lipka et al., 2012) were applied to detect marker-trait associations (MTAs) in the genebank set for biocontrol compatibility. Bonferroni corrected threshold was used to evaluate the significance of identified marker-trait associations. SNP marker positions were mapped

against the *Triticum aestivum* IWGSC CS RefSeq v2.1 genome from 90K SNPs consensus map (Wang et al., 2014) using the BLAST algorithm.

3.7 Genomic prediction of STB resistance in winter wheat breeding lines

The ridge regression BLUP (RR-BLUP) model was used for genomic prediction analysis (Spindel et al., 2016) of STB resistance in the 316 winter wheat breeding lines from the genebank set. The weighted RR-BLUP (wRR-BLUP) model was tested by including the top five significant GWAS-SNP markers as fixed effects (Spindel et al., 2016). The prediction accuracy was evaluated through cross-validation analysis using the correlation coefficient between the observed adjusted AUDPC BLUPs of the genotypes and the genomic estimated breeding values (GEBVs). The accuracy of predictions was determined by dividing the ability to predict by the square root of the broad sense heritability of the traits being studied (Chen et al., 2011, Legarra et al., 2008).

4. Results and Discussion

4.1 Significant time reduction in plant generation cycle (paper I)

Not many studies have been conducted in which utilization of speed breeding was made to evaluate genetic resistance of wheat plants to diseases. Furthermore, winter wheat growth requirements had not been included in other studies investigating the resistance as they were conducted on spring genotypes (Hickey et al., 2017). A new speed breeding protocol for winter wheat with the inclusion of resistance selection has been optimized to include disease selection in winter wheat genotypes. Under the accelerated growth conditions, the plants exhibited a remarkable ability to transition to the reproductive phase in a short period of time within 30-33 days. Notably, these conditions facilitated the growth of plants without any evident signs of stress. It was at this stage that the measurements for HT, AE, and FLA were conducted to assess the respective traits in the plants. Subsequently, the FHB infection conditions were introduced to facilitate disease symptoms on the spikes. The plants were then allowed to mature until reaching their full growth before being harvested. The entire protocol, including the growth, infection, and maturation stages, spanned a period of approximately 120 to 130 days, with the duration varying slightly depending on the specific genotypes being studied.. Figure 3 shows the different stages of plant growth in the accelerated growth for FHB (AGFHB) characterization protocol.

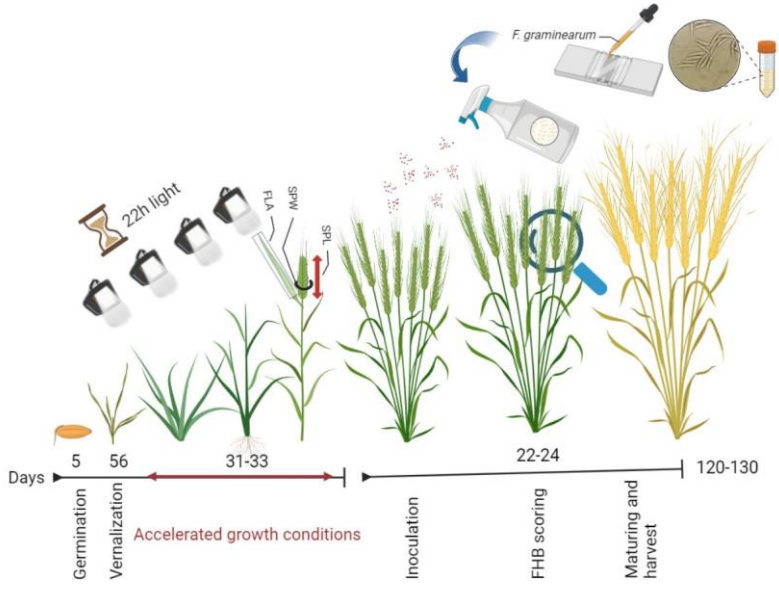


Figure 3. Schematic illustration of a timeline in a vertical, linear format for evaluating FHB resistance of winter wheat plants integrating accelerated growth conditions. The arrow indicates to the progression from one stage to the next. Seeds were germinated for 5 days. After vernalization, the plants were acclimatized for 6 days before they were moved to the next stage of accelerated growth period lasting for 31-33 days, under high-intensity and extended daily light. By the end of the latter stage, agronomic traits such as flag leaf area (FLA), spike length (SPL) and width (SPW) were measured. Plants were spray-inoculated with an inoculum containing a mixture of *fusarium* isolates, left to incubate at high humidity in greenhouse conditions, and followed by four-time points scoring of FHB severity after the development of disease symptoms. After scoring, the plants were left to mature and harvested by the end of the protocol. The last stage took a duration approximately 23 days. Illustration created with BioRender.com (2023).

4.1.1 Accelerated growth conditions can be utilized as a tool in resistance breeding and for agronomic trait selection

The AGFHB protocol was originally set to evaluate 519 genotypes, with 181 and 338 genotypes from the genebank and the breeding set respectively. However, not all genotypes were successfully employed in the downstream analysis as genotypic variations mainly in heading and anther extrusion dates made them unreliable for the subsequent inoculation and later disease development. During the FHB inoculation, the majority of plants in both sets had reached 75% heading of their spikes, with 88% in the breeding set and 90% in the genebank set (table 4). In terms of flowering, a higher percentage of plants had reached anthesis in the genebank set compared to the breeding

set, with 88% and 67%, respectively. It is important to note that genotypes that did not reach 75% heading at the time of inoculation were not included in the FHB severity scoring. Additionally, genotypes that did not exhibit any visible disease development on the ears were also discarded from further analysis. When comparing the percentages of missing genotypes at HT and AE, the breeding set had a higher percentage. Hence, a high number of genotypes from the breeding set were excluded from the subsequent inoculation and the later downstream phenotypic evaluation (table 4). This could be explained by the high genotypic variability in the genebank germplasm compared to the adapted background of the breeding set genotypes that may possess a narrower window for flowering. Overall, 272 breeding lines from the breeding set and 160 genebank genotypes were included in the study.

The best linear unbiased estimates of measured agronomic traits in both source populations showed similar heading means for both sets (Fig. 4a). In conclusion, both sets had a high percentage of plants that completed heading at the time of FHB inoculation, with a slightly higher percentage in the genebank set. The mean heading stage was similar in both sets (Fig. 4a), indicating that both source populations were relatively similar in their development under accelerated growth conditions.

Table 4. Summary table for the accumulative frequency of heading and anthesis extrusion incidences for winter wheat plants from both Lantmännen and Nordgen sets.

Trait	**Averages of frequency (%) of occurrence in the Germplasm**	
	Breeding set (n=338 lines)	Genebank set (n=181 lines)
	Heading	
HT1	20	22
HT2	57	63
HT3	88	90
	Anthesis	
AE1	35	55
AE2	67	88
No. of genotypes left	272	160

At inoculation spikes were still at heading and are partially enclosed with the flag leaf. Heading at 25-75% at three time points (HT1 to HT3). Anther extrusion at two time points (AE1 and AE2).

In the genebank set, the mean FLA was 17.15mm² (*s*=3.50; Figure 4b), whereas in the breeding set, it was 18.02mm² (*s*=3.87). This indicates that the genebank set exhibited a slightly smaller mean FLA compared to the breeding set. Similarly, the mean SPL in the genebank set was measured at 76.44mm (*s*=8.29), whereas in the breeding set, it was 87.82mm (*s*=9.47; Figure 4c). Consequently, the genebank set displayed a comparatively smaller mean SPL compared to the breeding set. Regarding SPW, the genebank set had a mean of 11.23mm (*s*=1.05), while the breeding set's mean was not specified in the provided text. Lastly, the mean SPW in the genebank set was 11.23 mm (s = 1.05), while in the breeding set, it was 11.10 mm (s = 1.25; Figure 4d). The means of FLA, SPL and SPW were larger in the breeding set compared to the genebank set, indicating the differences in agronomic traits between the breeding and genebank sets usefulness in selection for breeding programs.

The progression of FHB was evaluated in this study at four different time points. The assessment involved visually observing and quantifying the percentage of symptoms present on the main tiller spike of each plant. The AUDPC of FHB used for the genome-wide association study showed an overall mean of 213.10 (*s* = 130.80), with an average AUDPC of 225.13 (*s*= 129.98) for the breeding set and 195.53 (*s* = 130.44) for the genebank set (Fig. 5). In most cases, the correlation between FHB severity (measured as AUDPC) and the five agronomic traits was weak and non-significant. However, there was a moderately high and significant correlation observed between heading and anthesis ($r = 0.51$, $p < 0.001$).

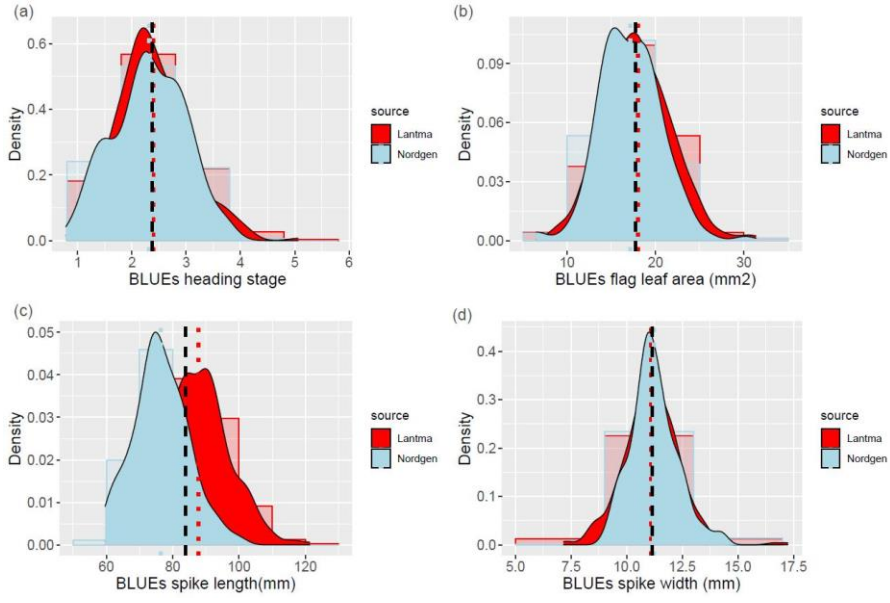


Figure 4. The phenotypic distribution of four traits, namely (a) heading stage, (b) flag leaf area, (c) spike length, and (d) spike width, were compared between the breeding set (represented by the colour red) and the genebank (represented by the color light blue). The overall mean for combined genotypes from both the breeding and genebank sets is represented by the black dashed line..

Significant genotypic variances ($p< 0.0001$) and moderate to high broad-sense heritabilities were observed in the study, with values varying depending on the specific trait and the source of genotypes. The broad-sense heritability for FHB was determined to be 0.55 in the combined set, 0.57 in the genebank set, and 0.53 in the breeding set, taking into account replication in time and space. In order to validate the FHB severity estimates, the study compared the scores obtained in the current research with those from a previous field trial conducted by Lantmännen Lantbruk in 2019. A Spearman correlation coefficient of 0.24 was identified when examining the FHB scores between the two datasets, indicates a weak positive relationship between the severity of FHB in the current study and that in the previous field trial. However, there was a statistically significant difference ($p< 0.0001$) between resistant and susceptible genotypes based on FHB scores of 1–3 (resistant phenotype) and 6–8 (susceptible phenotype), respectively, for mean FHB estimates obtained under controlled conditions.

The proposed protocol shows moderate to high heritability for FHB resistance and good phenotypic diversity in Nordic winter wheat germplasm.

Moderate to high heritability estimates were obtained for heading, FHB, FLA, spike length, and spike width. Highly resistant and susceptible genotypes were present in both breeding lines and genebank germplasm, with genebank germplasm generally less susceptible. This indicates to the potential for exploiting genetic variation to improve FHB resistance in Nordic winter wheat.

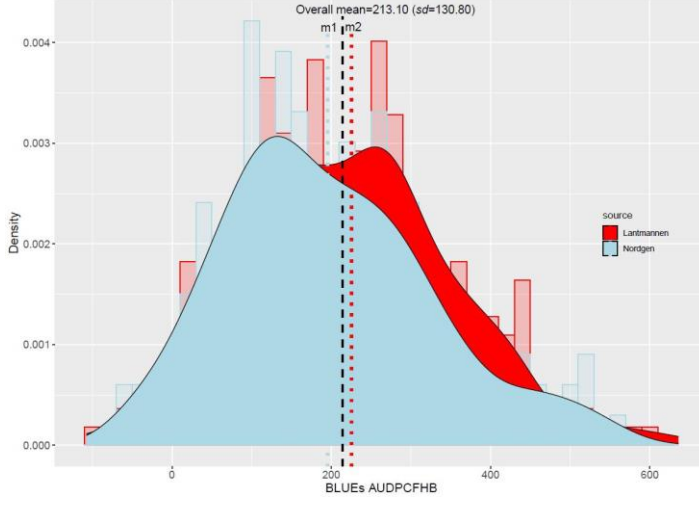


Figure 5. Histogram showing the area under the disease progress curve (AUDPC) for Fusarium head blight (FHB) in wheat genotypes collected from two sources. The genebank set and breeding set have mean values of m1 and m2, respectively.

High genetic variation was observed for the HT, AE, SPL, SPW, and FLA, consistent with previous studies on winter wheat (Bogard et al., 2011, Liu et al., 2018a, Liu et al., 2018b, Zanke et al., 2014, Zhai et al., 2016). These traits play important roles in agronomic adaptation and disease severity, but weak correlations were found between AUDPC (FHB) and these traits in this study. Although heading and anthesis are typically highly correlated, weak correlations were found in some cultivars due to variations in ear emergence from the flag leaf. The flag leaf plays a crucial role in photosynthesis and nutrient partitioning, particularly influencing spike length. Consistent with previous findings, the correlation between flag leaf area (FLA) and spike length, which are both important for yield-related traits, was found to be low and significant. (Liu et al., 2018a).

However, the phenotypic analysis showed that the genebank set may harbor more resistance genes for resistance against the disease, as represented by a

lower mean for disease severity. This illustrates and aligns with the concept that genebank germplasm is a rich source of resistance. While the breeding set had a wider range of phenotypic diversity compared to the genebank set, it may still retain more diversity for resistance. Therefore, in a different scenario where a similar number of genotypes from two different sources are simultaneously screened, it will then be possible to assume that the diversity in either germplasm exceeds that of the other.

4.1.2 Identification of QTL for FHB Resistance in Wheat and Their Co-localization with Previously Reported QTL

The results of the multi-model GWAS conducted on a panel of 432 wheat lines revealed significant associations between SNP markers and FHB severity (Zakieh et al, 2021). A total of 12 significant SNPs were identified, representing nine QTL. Notably, four QTL were co-detected by multiple GWAS models. Among the significant SNPs, three exceeded the Bonferroni corrected threshold (α=0.05). Of particular interest are the SNPs associated with the QTL located on chromosome 3B, which exhibited the largest marker effects. The analysis also identified significant SNPs associated with five agronomic traits and some SNPs were found to be associated with common QTL across these traits. Between FHB severity and heading stage, wo QTL were found common on chromosomes 3B and 6A. More QTLs were found using lines from the breeding and genebank sets combined than within each set alone.

Over 500 QTL for FHB resistance have been reported since 1999, with sub-genome B containing the largest number of QTLs (Venske et al., 2019). The current association analysis identified significant SNPs on chromosome 3BS (62.31-68.71 cM), which may be associated with a major QTL controlling FHB severity (p= 0.0001) and overlapped with previously reported meta-QTLs (Venske et al., 2019). The *Fhb1* QTL, originating from Chinese spring wheat Sumai 3, was located on the short arm of chromosome 3B (Bai et al., 1999, Venske et al., 2019, Waldron et al., 1999, Ma et al., 2020). Significant SNPs were also found within the breeding set in the region 9 cM to 14 cM on chromosome 3B (p= 0.001) and localized between the *Fhb1* QTL and meta-QTL 1/3B (Venske et al., 2019). Additionally, QTL for FHB resistance were discovered on other sub-genomes of bread wheat, including 3A (Venske et al., 2019). Using both breeding and genebank materials increased the power to detect QTL and incorporating QTL from the genebank set into

the breeding set could further improve marker-assisted selection for FHB resistance in wheat breeding programs.

The QTL identified in the current study overlaps with QTL from previous studies, specifically for the QTL located close to the *Fhb1* QTL on 3BS. This demonstrates the validation of the current protocol implementing accelerated growth conditions to evaluate FHB resistance in disease resistance breeding.

4.2 Comparison of Cgrain value™ and SmartGrain methods for measuring grain characteristics (paper III)

The Cgrain Value™ and SmartGrain methods were used to analyze various characteristics of non-infected and infected samples. The analysis showed that infected samples generally had lower values for most characteristics measured by both methods (Table 5). Infected samples also had higher coefficients of variation, indicating greater variability in the measurements (Table 5). However, infected samples had a higher value for circularity (CS) in the SmartGrain method (Table 5). The coefficients of variation for SmartGrain were generally higher than for Cgrain Value™, indicating greater variability in the measurements.

Table 5. Summary of a multiple linear regression model utilizing 16 morphological characteristics from Cgrain Value™ and SmartGrain (Leiva et al, 2022).

Morphological Traits	Sum of Squares	Mean Squares	*F*-Value	Pr (>*F*)
C_L	23,829	23,829	64.587	6.99E-15 ***
C_W	51,079	51,079	138.446	< 2e-16 ***
C_T.RAW	40,500	40,500	109.772	< 2e-16 ***
C_AVG.W	2,013	2,013	5.456	0.0199 *
C_V	2,603	2,603	7.055	0.00816 **
C_WT	680	680	1.843	0.17526
C_LIGHT	31,656	31,656	85.802	< 2e-16 ***
C_HUE	39,386	39,386	106.752	< 2e-16 ***
C_SATURATION	2,649	2,649	7.18	0.00762 **
S_AS	178	178	0.483	0.48734
S_PL	624	624	1.691	0.1941
S_L	3,027	3,027	8.204	0.00436 **
S_W	45	45	0.121	0.72828
S_LWR	0	0	0.001	0.9802
S_CS	1,651	1,651	4.476	0.03489 *
S_DS	539	539	1.461	0.22731

Note: The most significant characteristics concerning Fusarium head blight (FHB) disease infection according to the *P*-value has an *. (No significance $P>0.05$; *$P\leq0.05$; **$P\leq0.01$; ***$P\leq0.001$).

The results of a multiple linear regression model that combines all 16 morphological characteristics provided by Cgrain Value™ and SmartGrain , show how each characteristic contributes to predicting FHB infection. Several morphological traits were significantly associated with FHB infection, while others were not. The characteristics C_L, C_W, C_T.RAW, C_LIGHT, C_HUE were highly significant, and C_AVG.W, C_V, C_SATURATION, and S_CS were significant in predicting the FHB disease infection. The other characteristics, S_AS, S_PL, S_W, S_LWR, and S_DS, were not significant ($P> 0.05$) in predicting FHB disease infection.

4.2.1 Relevance of the phenomic methods for predicting FHB-infected seeds

The results indicate that both methods are effective in predicting FHB in different sets of genotypes evaluated, with statistical analysis showing significant correlations between certain morphological traits and visual scores of the FHB symptoms.

However, major differences are present in the applicability of the two methods in terms of cost, accuracy, and time efficiency. The Cgrain Value™ method could be a costly approach demanding costly operational machinery. Nonetheless, the method provides instantaneous image capture and processing. However, SmartGrain is a significantly cheaper approach with longer image acquisition time as its drawback. The morphological traits color traits in the HSL colour representation and thickness were identified as predictive of FHB. Giving this method an advantage over SmartGrain approach. At the same time, both approaches were efficient utilizing other morphological traits, specifically length and width to predict FHB infection. Notably, methods may the costly and labour-intensive visual assessment of disease severity, consequently leading to feasible assessment of the percentage of disease severity of spray-inoculated wheat spikes or other natural or artificial inoculated infected seed. This approach ultimately enables a precise evaluation of the extent of damage inflicted by the disease on all kernels of screening panels. The implementation of this method can potentially reduce the time required for disease resistance assessment, lower associated costs, and decrease labor intensity.

The study demonstrates the importance of morphological seed traits for predicting FHB in wheat revealing the accuracy and efficiency of two image-based methods. The findings may hold significant implications for wheat breeding programs and provide valuable insights into the use of image-based methods for disease prediction.

4.3 Paving the way for the seedling stage to be an all stage for the identification of novel QTL for STB (paper II)

In order to avoid any genotype-isolate/strain specific resistance, inoculation was first tested on four different cultivars with varying degrees of resistance with the two isolates. The inoculation elicited STB responses corresponding to the degree of resistance, with the most resistant cultivar showing the lowest AUDPC scores, and the most susceptible cultivar showing the highest AUDPC scores. Analysis of variance indicated significant differences among the tested 316 winter wheat lines for STB resistance, with the AUDPC-BLUPs scores ranging from 193.6 to 666.2 and average value of 434.2. The study recorded high broad-sense heritability (H^2=0.62) from the evaluated breeding lines, with the genotypic variance accounting for 44% of the total variation.

The multi-locus GWAS models identified 24 MTAs significantly linked to STB resistance. These MTAs were located on 20 different QTL across 14 chromosomes (paper II). Figure 6 shows the distribution of some markers identified in the study associated with STB resistance in the 316 breeding set genotypes. Specific SNP markers within certain QTL were highly significantly associated with STB resistance. Seven QTL were possibly novel for seedling stage resistance. Equally important is that the current study identified four potentially novel QTL markers associated with seedling stage STB resistance that overlapped with previously identified markers at adult stage where some of which were identified in multi-Baltic environments under natural infection conditions. 14 SNP variants associated with STB-seedling stage resistance were from six B-genome chromosomes, while seven and three SNPs were from A and D-genome chromosomes, respectively.

Two QTL (SLUSTB_2 and SLUSTB_3) overlapped with a previous study using similar growth conditions and isolates on 175 genebank genotypes that

had wide genotypic base (Odilbekov et al., 2019). The SNP marker AX_89326139 on chromosome 1B was found to overlap with the previously identified marker IAAV3905 as an APR QTL for STB (Muqaddasi et al., 2019), while Kidane et al. (2017) identified a QTL qSTB.04 physically located close to the marker AX_89326139. Therefore, the identified QTL is a potentially novel seedling stage marker, where harboring resistance to STB throughout the plant life cycle. The two closely located markers AX_158573239 and AX_158596603 in SLUSTB_4 overlapped with the recently identified QTL linked to seedling stage STB (Mahboubi et al., 2021). The two SNP markers of QTL SLUSTB_7 were found to be only 7.5 cM distant from the previously reported seedling stage QTL SRT_71-R3_2 on chromosome 2B (Louriki et al., 2021). Another nearby SNP marker, AX_94734086, was identified for APR to STB and is possibly part of the *Stb9* resistance gene involved in all-stage STB resistance in wheat (Muqaddasi et al., 2019).

SLUSTB_8, SLUSTB_16, and SLUSTB_20 were the three QTL identified from the D genome on chromosomes 2D, 6D, and 7D, respectively. The SNP marker AX_111036153 on chromosome 2D was identified in the current study and may be linked to a potential QTL identified for adult plant STB resistance (Riaz et al., 2020). The marker IAAV64 in the QTL SLUSTB_16 was discovered on chromosome 6D. The SLUSTB_9 QTL on chromosome arm 3AL was identified with the SNP marker wsnp_Ex_c8517_14315660 that was found to be nearby the marker wsnp_Ex_c5929_10402147 (Odilbekov et al., 2019). Four QTL situated between 80.4 cM and 87.1 cM peaking at an average of 83.7 cM on 3AL that were reported in a meta-QTL analysis for several biotic stresses (Soriano et al., 2021). Several previous investigations have identified this chromosome region as a source of MTAs for STB resistance within proximities of 71 cM (Eriksen et al., 2003, Ghaffary et al., 2011, Kidane et al., 2017, Radecka-Janusik and Czembor, 2014). These findings suggest the potential existence of a QTL on this chromosome region linked to all-stage STB resistance in wheat.

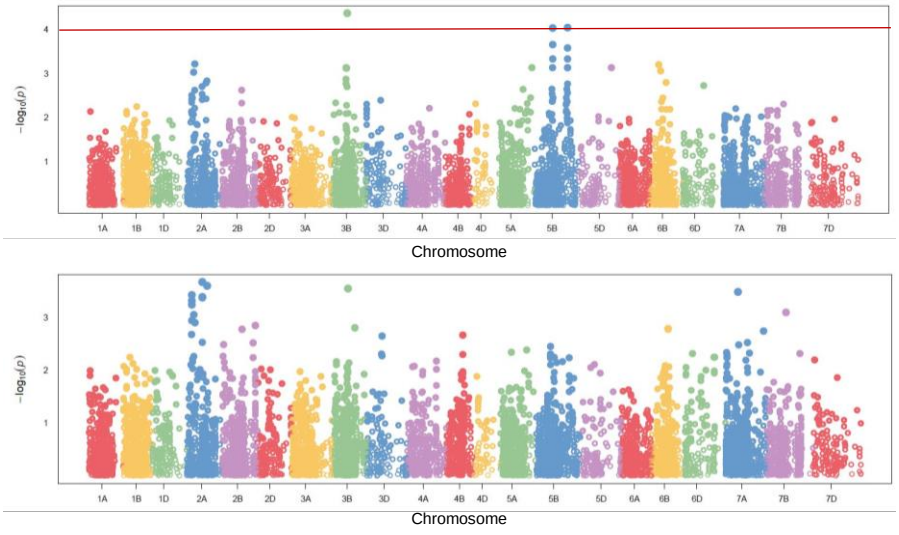


Figure 6. Manhattan plots for seedling stage resistance to STB in GWAS. (A) Manhattan plot from the multi-locus Blink model. (B) single-locus model MLM. Both models were used from the GAPIT package. The red solid line represents the exploratory threshold at p= 0.0001.

Excalibur_c4325_1150 (SLUSTB_11 QTL) was identified as the marker on chromosome arm 4AL located at 120.4 cM. Two nearby SNPs on chromosome 4A were previously reported to be significantly linked to STB resistance in adult and seedling stages (Alemu et al., 2021, Mahboubi et al., 2021). Muqaddasi et al. (2019) reported the SNP marker wsnp_JD_c27162_22206547 that exactly overlapped with the currently identified marker on chromosome arm 4AL at 120.4 cM. The marker wsnp_CAP12_c1101_569783 on 4B was highly significantly associated with STB resistance discovered by the multi-locus model FarmCPU with –log10 p> 9. Louriki et at. (2021) reported two SNP markers located on chromosome arm 4BL, associated with seedling stage STB resistance. The marker they identified RAC875_c24515_602 was found at 76.8 cM, potentially co-localized with a marker discovered in the current study. Riaz et al. (2020) found several QTL for adult-plant STB resistance on chromosome 4B. The region between 40 and 45 cM on chromosome 5A could contribute to STB resistance through all stages of the life cycle of the plant. Chromosome 7B has been identified as a source of several MTAs for both seedling stage and APR to STB, with two markers significantly associated with STB resistance in the current study. Three QTL were

previously detected at adult plant stage located between 60-65 cM on chromosome 7B linked to STB resistance in (Louriki et al., 2021).
This study's identification of potentially novel QTL for STB resistance is a significant step in expanding the Nordic winter wheat gene pool for quantitative resistance to STB. The discovery of novel resistance markers provides a potential tool for marker-assisted selection in breeding programs. This study contributes to the growing body of literature on the genetic basis of STB resistance. Previous GWA analyzes have addressed different stages of STB resistance and reported overlap among detected resistance markers between these stages. The validation of overlapping MTAs in breeding material with MTAs identified in previous studies at the adult stage further supports the robustness of the identified markers and their potential usefulness for MAS at either stage. Furthermore, the significant markers that overlapped with previously identified markers at the seedling stage in the genebank set are of major importance, indicating that breeding lines still retained a significant degree of diversity that can be considered a source in breeding programs.

4.3.1 GP for developing wheat varieties against STB

GEBVs for STB resistance in wheat were estimated using the RR-BLUP model and the wRR-BLUP model. The RR-BLUP model achieved a prediction accuracy of 0.49 with a range of 0.21-0.84, while the wRR-BLUP model estimated a mean genomic prediction accuracy of 0.58. The genomic prediction accuracy of both models was in the range of 0.26-0.85. The five top-most significantly linked SNP markers were fitted as fixed effects in the wRR-BLUP model, leading to improved prediction accuracy by 5.1% compared to the RR-BLUP model.
Various factors affect the accuracy of genomic prediction, such as the number and effect size of QTL, the choice of prediction model, and the size and diversity of the training population. Using fixed-effect markers, is a proposed strategy to overcome these challenges.
Unlike GWAS or linkage mapping methods, genomic prediction estimates the breeding values of individuals based on overall marker information accounting for all contributing QTL. The current study estimated GEBVs of 20% of genotypes using two GP models trained with the remaining 80% of the panel. The RR-BLUP model estimated GEBVs of STB resistance with prediction accuracy of 0.49 and 0.53 averaged from 500 and 20 iterations,

respectively. The wRR-BLUP model, which fitted the five most significantly linked SNPs identified via GWAS from training sets as fixed effects, showed an improved prediction accuracy of 5.1% compared to the RR-BLUP model. Previous studies have also reported genomic prediction accuracy improvements with the RR-BLUP model supplemented with QTL linked markers as fixed-effect (Alemu et al., 2021, Juliana et al., 2017, Muqaddasi et al., 2019).

4.4 Significant variation among winter wheat genotypes in their response to *C. rosea* for STB caused by *Z. tritici* (paper IV)

Variation among wheat genotypes for Septoria tritici blotch disease and *C. rosea* biocontrol efficacy was detected. Both treatments showed significant variation in disease severity ($P< 0.001$) and moderate to high heritability (H^2P = 0.67, H^2C = 0.59 for treatment Zt; H^2P = 0.74, H^2C = 0.62 for treatment ZtCr). There was a strong positive correlation between rAUDPC in treatment Zt and STB rAUDPC data reported in a previous study (R = 0.69, $P< 0.001$), and a moderate positive correlation between treatments Zt and ZtCr (R = 0.4, $P< 0.001$). These results indicate the potential for using biocontrol agents to manage STB disease in wheat genotypes. The results are summarized in Table 6.

For the same panel of winter wheat genotypes (genebank set), Alemu et al. (2021) reported a heritability of 0.39 in adult stage, naturally infected plants in a multi-location trial. It is therefore possible to show the genotypic control of the disease when carried in controlled artificial infection settings. Hence, the magnitude of the biocontrol efficacy on the genotypic background can be further revealed with lower impact of environmental cues on the interaction.

Table 6. Variation in rAUDPC values among wheat genotypes for *Z. tritici* alone (treatment Zt) and *C. rosea* formulation before *Z. tritici* application (treatment ZtCr)

Treatment	Heritability (H^2P)	Heritability (H^2C)	Significant Variation	Correlation with STB rAUDPC (Odilbekov et al., 2019)
Zt	0.67	0.59	Yes ($P<$ 0.001)	Strong positive correlation (R = 0.69, $P<$ 0.001)
ZtCr	0.74	0.62	Yes ($P<$ 0.001)	Moderate positive correlation (R = 0.4, $P<$ 0.001)

4.4.1 Identification of novel MTAs associated with *C. rosea*-wheat genotypic compatibility for enhanced STB resistance in juvenile plants

To determine the association between SNP markers and rAUDPC variation in different treatments of *Z. tritici* alone (Zt), *Z. tritici* with biocontrol agent *C. rosea* (ZtCr), and biocontrol efficacy estimator (Zt – ZtCr), genome-wide marker trait associations were performed. The results showed that eleven SNP markers were significantly associated with rAUDPC variation, and five of them were significantly associated with disease progress in treatment Zt. In treatment ZtCr, two SNP markers were significantly associated with rAUDPC estimates. For biocontrol efficacy, four significant SNP marker–trait associations were detected at two locations on chromosome 1D and 6B. Seven out of eleven SNP marker–trait associations were co-detected by more than one GWAS model. The genotypes carrying specific alleles for certain SNP markers exhibited significantly less rAUDPC or more biocontrol efficacy. Some of the results are highlighted in table 7 (paper IV for full description of the identified markers).

Table 7. Summary of significant SNP marker-trait associations for rAUDPC variation and biocontrol efficacy in different treatments.

Triat	SNP	Chr	Model	Alleles
Zt	Excalibur_c29625_222	3B	GLM	N/A
	Excalibur_c49875_479	2B	BLINK, FarmCPU, and MLMM	N/A
	IAAV4876	3B	GLM	AA
	Kukri_rep_c70198_1436	3B	BLINK	N/A
	RAC875_rep_c83245_239	3B	GLM	N/A
ZtCr	BS00022902_51	1B	BLINK, FarmCPU, and MLMM	GT, TT
	BS00070856_51	6D	BLINK and FarmCPU	N/A
Biocontrol Efficacy (Zt-ZtCr)	Kukri_c837_436	1D	BLINK, FarmCPU, and MLMM	GG, GT
	wsnp_Ex_c1358_2600929	1D	BLINK and FarmCPU	AA, AG
	wsnp_Ex_c1358_2602235	1D	BLINK and FarmCPU	CC, CT
	BS00027770_51	6B	BLINK and FarmCPU	N/A

In the analysis allele effect was estimated. This resulted in some effect for certain alleles, meanwhile other alleles did not have any reported effect and was referred to as NA. This means that the model did not estimate an effect for that SNP, or that the effect was not significant at the chosen level of significance. For example, for the biocontrol efficacy trait, the SNP BS00027770_51 has a *P*-value of 2.68E-05 and a negative allele effect of -

0.41, while the SNP Kukri_c837_436 has a *P*-value of 7.74E-06 and a positive allele effect of 0.75. For the Zt trait, the SNP Excalibur_c29625_222 has a P-value of 4.10E-05 and a negative allele effect of -0.32, while the SNP Excalibur_c49875_479 has a very small *P*-value of 6.55E-08 but a "NA" value for allele effect in the Blink model, indicating that there is evidence for association, but the effect size could not be estimated with confidence.

4.4.2 Exploring the impact of *C. rosea* on STB disease development: Implications for improved disease management

The study demonstrates that *C. rosea* strain IK726 can provide protection for wheat leaves against STB disease caused by *Z. tritici*, with its biocontrol efficacy being modulated by plant genotype. This finding is significant as it highlights the importance of considering plant host genotype-specific interactions with BCAs for efficient biocontrol and biostimulation. The moderate positive correlation observed between disease resistance and biocontrol efficacy suggests that susceptible plant genotypes benefit more from *C. rosea* application, however, it implies the presence of an additional level of genetic predisposition in the wheat material to benefit from the BCA treatment as well. Hence the study provides new insights into the potential of *C. rosea* as a BCA for controlling STB disease in wheat and highlights the need to account for plant genotype-specific effects when using BCAs.

The GWA analysis showed that several QTL associated with STB disease resistance were present throughout the wheat genome, including on chromosomes 1A, 1B, 2B, 3A, 3B, 5A, and 6D. The identification of two significant MTAs on chromosomes 2B and 3B, as well as significant QTL on chromosomes 1B and 6D, is consistent with previous studies (Alemu et al., 2021, Brown et al., 2015, Odilbekov et al., 2019). These QTL are known to contribute to disease resistance at either seedling or adult plant stages, or throughout the plant life cycle, reflecting the complexity of STB resistance genes. The presence of these resistance sources in Nordic wheat germplasm suggests a genetic potential that can be utilized in breeding to improve STB resistance in wheat. Additionally, the study identified significant associations between SNP markers and *C. rosea* biocontrol efficacy on chromosomes 1D and 6B that were reported in earlier studies (Alemu et al., 2021, Brown et al., 2015, Odilbekov et al., 2019, Vagndorf et al., 2017). This is distinct MTAs found for disease resistance. The results indicate that genes contributing to disease resistance and *C. rosea*-genotype compatibility are

located in different genomic regions, suggesting that it is possible to breed wheat genotypes with both high disease resistance and high biocontrol efficacy.

The current study shows that disease resistance and genotype-specific biocontrol efficacy are genetically distinct traits, emphasizing the possibility of breeding plants with a greater genetic potential to profit from beneficial microorganisms in sustainable agriculture. These finding show the importance of understanding the interactions between plant genotype, pathogens, and biocontrol agents to promote the advancement of more effective and sustainable disease management strategies.

The findings of this study have significant implications for the plant breeding industry. Identifying genotypes that are more susceptible to a pathogen when treated with a biocontrol agent allows for early elimination of those lines during breeding. At the same time, selection of genotypes that demonstrate higher resistance to the pathogen when treated with the biocontrol agent can be based on the markers of resistance identified in biocontrol-genotype compatibility studies. This approach can significantly improve breeding efficiency, leading to the development of more resilient and disease-resistant plant varieties. Overall, these findings demonstrate the importance of understanding the interaction between biocontrol agents and plant genotypes to enhance the effectiveness of plant breeding programs.

5. Transfer of Knowledge: From Research to Application in Industry

The collaboration with Swedish agricultural cooperative, Lantmännen Lantbruk, aims to exchange knowledge and establish connections primarily between students from the Swedish University of Agricultural Sciences (SLU) with strong academic backgrounds in order to support breeding programs for disease resistance in wheat. This approach will potentially provide opportunities for SLU students to gain practical experience in breeding through interactions with Lantmännen Lantbruk scientists and breeders.

In this context, a framework has been established to assess the feasibility of implementing the protocols used at SLU at Lantmännen Lantbruk, including the necessary infrastructure and trained personnel to conduct successful evaluations of resistance in plant collections grown in greenhouse conditions. The framework is planned to take into consideration implementing academic research in the upcoming years together with Lantmännen Lantbruk. The main activity at SLU, the screening of germplasms, will be moved to Lantmännen for larger wheat accessions.

5.1 Report: Speed breeding and its industrial implication in cereal crop breeding, SLU, Alnarp (2021)

5.1.1 Background

The experiment conducted at the Swedish University of Agricultural Sciences in Alnarp aimed to assess the applicability of speed breeding conditions, previously found to promote winter wheat development, on other

cereal plants. The study focused on spring wheat, spring barley, and spring oat, evaluating the effects of controlled conditions such as light duration, intensity, and other factors. Earlier studies found positive effect of speed breeding conditions on plants' development including the species under the current evaluation (Ghosh et al., 2018, Watson et al., 2018). The outcome of this research has the potential to be applied in industrial settings, particularly in the breeding programs of Lantmännen Lantbruk.

5.1.2 Experimental set up

The experiment was carried out between June 15th of 2021 and 30th of July of the same year in the cultivation unit (Biotron), SLU, Alnarp. A total of 15 blocks (trollies) comprised of nine spring oat trollies, five spring wheat and two barley trollies included in the experimental setup. Each trolley contained approximately 120 plants grown on 15x8 well trays. The plants were different genotypes adapted for Nordic environment and used in Lantmännen Lantbruk's breeding program. The experiment utilized controlled environment to create speed breeding conditions for the cereal plants previously tested in accelerated growth conditions for winter wheat (Zakieh et al., 2021). Heading was recorded with focus on recoding the complete heading (average) date.

5.1.3 Results:

The results of the experiment demonstrated that the speed breeding conditions, previously found to positively contribute to winter wheat development (Zakieh et al., 2021), also yielded favorable outcomes for the other cereal species. The optimized light duration, intensity, and other controlled factors stimulated accelerated growth and early heading in spring wheat, spring barley, and spring oat. Table 8 summarizes total days from sowing the seeds to complete (100%) heading of the respective plant species.

Table 8. Summary of the average days required for each plant species to reach full heading based on accelerated growth conditions described by Zakieh et al. (2021):

Plant Species	Total Days from Sowing to Heading
Spring barley	28-30 days
Spring oat	32-38 days
Spring wheat	36-38 days

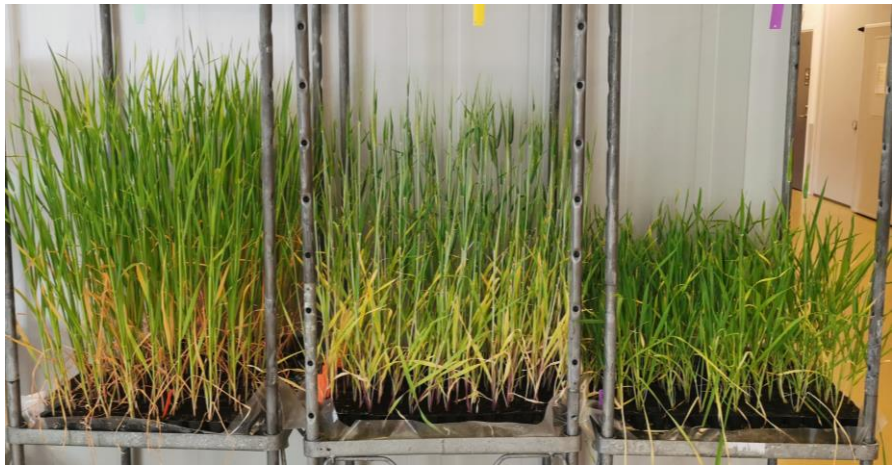


Figure 7. The development of reproductive structures on 33 days old spring barley to the left, spring oat in the middle and spring wheat to the right. The plants grew under accelerated growth conditions following Zakieh et al. (2021).

5.1.4 Application in Lantmännen Lantbruk

The results found earlier by Zakieh et al. (2021) and the current test on the three cereal species, suggest that speed breeding conditions can be successfully extended to other cereal plants used in Lantmännen Lantbruk breeding. By incorporating speed breeding techniques, Lantmännen Lantbruk breeders can significantly reduce generational cycle and speed up the process of developing improved cereal varieties. The positive outcomes observed in the development of spring wheat, spring barley, and spring oat under speed breeding conditions have significant implications for cereal breeding programs. The expedited growth cycle has the potential for faster genetic improvement and trait selection, intrgression line development and SSD in these cereal crops.

Furthermore, the infrastructure for speed breeding in cereal breeding is now fully operational at Lantmännen Lantbruk in Svalöv. The facility is equipped with upscaled growth rooms that provide a precisely controlled climate environment. These advanced growth rooms allow for the efficient cultivation of a large number of plants, ensuring consistent and accelerated growth rates. With the integration of speed breeding techniques and state-of-the-art facilities, Lantmännen Lantbruk is well-positioned to harness the full potential of speed breeding in cereal breeding. The combination of cutting-edge technology, expertise in plant breeding, and optimized growth

conditions opens up new avenues for collaboration for innovative research with SLU researchers and Lantmännen Lantbruk breeders.

5.2 Report: STB screening in Lantmännen Lantbruk, Svalöv (2023)

5.2.1 Background

The following report details an experiment conducted in Lantmännen Lantbruk agricultural cooperative facilities, Svalöv, Sweden for the artificial infection of STB on seedling stage on the winter wheat genotype Nimbus. Originally, the protocol have been optimized for the use in SLU, Alnarp, Sweden. Furthermore, the protocol has been successfully used to screen panels of winter wheat genotypes for STB resistance at juvenile growth stage in controlled environmental conditions. This has resulted in several publications where markers for STB resistance have been identified. Applying the protocol in industry of paramount importance where actual breeding programs such as that of Lantmännen Lantbruk can benefit from the advancements in the academic sector.

Nimbus is an STB susceptible cultivars that is used to identify and optimize STB symptom development in order to optimize greenhouse conditions for STB screening. The plants were planted on the 20th of February and inoculated on the 2nd/2023 of March on 10-day-old seedlings. This report describes the success of the artificial infection in greenhouse conditions, the experimental setup, the observed symptoms, and the possible ways of optimizing large scale STB screening on spring and winter wheat genotypes.

5.2.2 Experimental Setup:

- 360 Nimbus seeds were germinated in half-15x8 well trays and 1L plastic pots on peat soil supplemented with high nitrogen fertilizer. These were distributed within the chamber in six different location. This was made in order to test plants location and control variations that arise from the environment of the location (Fig. 8).
- Germinated seedlings were left to grow on 24°C, 16/8 h light/dark cycle and 60-70% RH conditions.

Figure 8. Distribution of plant around greenhouse chamber in six different locations.

- Two Faran HR15 humidifiers with a capacity of 1.5L per hour were installed in the chamber. Humidity was adjusted automatically using a digital Cornwall Hygrostat attached to each humidifier to control humidity level in different room locations.
- Three isolates were used to inoculate the winter wheat genotype Nimbus. The two isolates Svalöv and Alnarp that were collected in the 2016-2017 and a third isolate 42-3 collected in the 2021 were grown at campus Alnarp at SLU.
- Spore harvest and inoculum preparation, spore concentration adjustment were carried out in Lantmännen's microbiological laboratory. 10-day-old Nimbus seedlings were inoculated on the 2nd of March/2023 on the second leaf stage. The plants were left to incubate at high humidity (85-90% RH) for four days with a temperature ranging between 25-30°C in the room (Fig. 8). Following the incubation period, the humidity dropped to 60-70% RH.

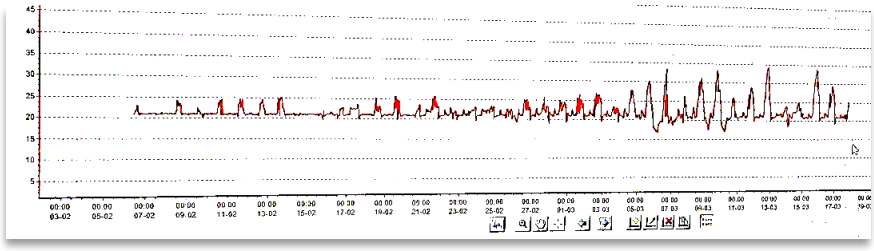


Figure 9. Fluctuations in atmospheric temperature in Lantmännen Lantbruk greenhouse during February and March. The fluctuations, specifically the rise in the temperature, are mainly attributed to sunny days during March.

- A second inoculation was carried out on the 6th of March, to ensure proper inoculation due to the rise in temperature in the greenhouse chamber. The temperature ranged between 22-35°C after the second inoculation (Fig. 9). The plants were left to incubate at high humidity at 85-90% RH for three days before lowering the humidity back to 65-70% RH.

5.2.3 Results

- On the 17th of March, 15 and 11 days from the first and second inoculations respectively, symptoms of STB on the first inoculated leaves were observed (Fig. 10).
- On the 24th of March, 22 and 18 days from the first and inoculations respectively, higher number of plants were observed to have symptoms of STB on the first, second and third leaves. Additionally, pycnidial structures where frequently observed (Fig. 11).
- Significant effect of growth location was observed on STB development. Plants placed in the distal areas of the growth close to walls facing the direction of the sun had significantly less developed symptoms of STB. Meanwhile the plants in the middle location and those that are close to the chamber entrance were frequently infected.

Figure 10. The earliest observed symptoms of STB development on the winter wheat plants 15 and 11 days from the first and inoculations respectively. Cultivar Nimbus.

5.2.4 Evaluation and optimization of the protocol

- The protocol carried out in Lantmännen Lantburuk need further optimization. Factors such as fluctuation in humidity and temperature, the symptoms may not develop in the expected speed).
- It is clear that certain areas of the room have effect on the development of the disease. Installing more humidifiers is possibly to solve the issue of humidity variation within the greenhouse chamber.
- The facility does not have a cooling system to regulate temperature, and instead relies on ventilation through chamber windows. This can result in a sharp decrease in humidity, which may take a significant amount of time to return to its optimal levels. If temperatures exceed 26°C, this could negatively impact on STB infection. To effectively implement the protocol for STB development, it would be advisable

to conduct the protocol between mid-November and mid-February, while minimizing exposure to sunny days.

- The greenhouse rooms allow for large-scale screening of thousands of wheat genotypes. Scaling up the inoculum is necessary to allow for artificial infection in the facility. Therefore using liquid or modified isolate culture methods are of paramount importance.

Figure 11. Winter wheat genotype Nimbus, 22 and 18 days from the first and second inoculations, respectively. A higher number of plants were observed to have symptoms of STB, and pycnidial structures were frequently observed.

5.2.5 Lantmännen Lantbruk, Svalöv capacity building for STB resistance breeding

Lantmännen Lantbruk personnel are able to perform various tasks related to STB protocol. These include tasks such as media plates, culture the pathogen on plates, identify spores, count the number of spores for final inoculum concentration, and set up humidity chambers.

Other personnel, have been trained to perform in microscopic examination to identify the differences in morphology between the different isolates used in inoculation, properly identify spore structures, adjust humidifiers to control room humidity, create inoculum, and inoculate plants. Moreover, the team in Lantmännen Lantbruk now have the ability to distinguish STB

symptoms from other symptoms. Chlorosis followed by reddish necrosis are markers for STB disease, and they have been trained to identify these symptoms and separate them from other plant issues.

References

Acquaah, G. (2012). Principles of plant genetics and breeding. Chapter 11. *Plant genetic resources, 2*.

Alemu, A., Brazauskas, G., Gaikpa, D. S., Henriksson, T., Islamov, B., Jorgensen, L. N., Chawade, A. (2021). Genome-Wide Association Analysis and Genomic Prediction for Adult-Plant Resistance to Septoria Tritici Blotch and Powdery Mildew in Winter Wheat. *Frontiers in Genetics, 12*. doi:ARTN 66174210.3389/fgene.2021.661742

Alvarado, G., Rodríguez, F. M., Pacheco, A., Burgueño, J., Crossa, J., Vargas, M., Lopez-Cruz, M. A. (2020). META-R: A software to analyze data from multi-environment plant breeding trials. *The Crop Journal, 8*(5), 745-756.

Ando, K., Rynearson, S., Muleta, K. T., Gedamu, J., Girma, B., Bosque-Perez, N. A., Pumphrey, M. O. (2018). Genome-wide associations for multiple pest resistances in a Northwestern United States elite spring wheat panel. *Plos One, 13*(2). doi:ARTN e019130510.1371/journal.pone.0191305

Bai, G., Kolb, F. L., Shaner, G., & Domier, L. L. (1999). Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. *Phytopathology, 89*(4), 343-348.

Benbow, H. R., Brennan, C. J., Zhou, B. B., Christodoulou, T., Berry, S., Uauy, C., Doohan, F. M. (2020). Insights into the resistance of a synthetically-derived wheat to Septoria tritici blotch disease: less is more. *Bmc Plant Biology, 20*(1). doi:ARTN 40710.1186/s12870-020-02612-z

Blum, A. (2005). Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? *Australian Journal of Agricultural Research, 56*(11), 1159-1168.

Bogard, M., Jourdan, M., Allard, V., Martre, P., Perretant, M. R., Ravel, C., Griffiths, S. (2011). Anthesis date mainly explained correlations between post-anthesis leaf senescence, grain yield, and grain protein concentration in a winter wheat population segregating for flowering time QTLs. *Journal of experimental botany, 62*(10), 3621-3636.

Brar, G. S., Brule-Babel, A. L., Ruan, Y. F., Henriquez, M. A., Pozniak, C. J., Kutcher, H. R., & Hucl, P. J. (2019). Genetic factors affecting Fusarium head blight resistance improvement from introgression of exotic Sumai 3 alleles (including Fhb1, Fhb2, and Fhb5) in hard red spring wheat. *Bmc Plant Biology, 19*. doi:ARTN 17910.1186/s12870-019-1782-2

Bratspies, R. M. (2014). Food, technology and hunger. *Law, Culture and the Humanities, 10*(2), 212-224.

Brennan, C. J., Zhou, B. B., Benbow, H. R., Ajaz, S., Karki, S. J., Hehir, J. G., Doohan, F. M. (2020). Taxonomically Restricted Wheat Genes Interact With Small Secreted Fungal Proteins and Enhance Resistance to Septoria

Tritici Blotch Disease. *Frontiers in Plant Science, 11*. doi:ARTN 43310.3389/fpls.2020.00433

Brown, J. K. M., Chartrain, L., Lasserre-Zuber, P., & Saintenac, C. (2015). Genetics of resistance to Zymoseptoria tritici and applications to wheat breeding. *Fungal Genetics and Biology, 79*, 33-41. doi:10.1016/j.fgb.2015.04.017

Buerstmayr, H., Lemmens, M., Hartl, L., Doldi, L., Steiner, B., Stierschneider, M., & Ruckenbauer, P. (2002). Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (Type II resistance). *Theoretical and Applied Genetics, 104*, 84-91.

Cao, W. X., & Moss, D. N. (1989). Daylength Effect on Leaf Emergence and Phyllochron in Wheat and Barley. *Crop Science, 29*(4), 1021-1025. doi:DOI 10.2135/cropsci1989.0011183X002900040039x

Castro Aviles, A., Alan Harrison, S., Joseph Arceneaux, K., Brown-Guidera, G., Esten Mason, R., & Baisakh, N. (2020). Identification of QTLs for resistance to Fusarium head blight using a doubled haploid population derived from southeastern United States soft red winter wheat varieties AGS 2060 and AGS 2035. *Genes, 11*(6), 699.

Cha, J. K., O'Connor, K., Alahmad, S., Lee, J. H., Dinglasan, E., Park, H., Dixon, L. E. (2022). Speed vernalization to accelerate generation advance in winter cereal crops. *Molecular Plant, 15*(8), 1300-1309. doi:10.1016/j.molp.2022.06.012

Chatterton, S., & Punja, Z. K. (2009). Chitinase and beta-1,3-glucanase enzyme production by the mycoparasite Clonostachys rosea f. catenulata against fungal plant pathogens. *Canadian Journal of Microbiology, 55*(4), 356-367. doi:10.1139/W08-156

Chatterton, S., & Punja, Z. K. (2010). Factors influencing colonization of cucumber roots by Clonostachys rosea f. catenulata, a biological disease control agent. *Biocontrol Science and Technology, 20*(1), 37-55. doi:10.1080/09583150903350253

Chawade, A., Armoniene, R., Berg, G., Brazauskas, G., Frostgard, G., Geleta, M., Weih, M. (2018). A transnational and holistic breeding approach is needed for sustainable wheat production in the Baltic Sea region. *Physiologia Plantarum, 164*(4), 442-451. doi:10.1111/ppl.12726

Chen, C. Y., Misztal, I., Aguilar, I., Tsuruta, S., Meuwissen, T. H. E., Aggrey, S. E., Muir, W. M. (2011). Genome-wide marker-assisted selection combining all pedigree phenotypic information with genotypic data in one step: An example using broiler chickens. *Journal of Animal Science, 89*(1), 23-28. doi:10.2527/jas.2010-3071

Cook, R. J., Hims, M. J., & Vaughan, T. B. (1999). Effects of fungicide spray timing on winter wheat disease control. *Plant Pathology, 48*(1), 33-50. Retrieved from <Go to ISI>://WOS:000079406400005

Cowger, C., Hoffer, M. E., & Mundt, C. C. (2000). Specific adaptation by Mycosphaerella graminicola to a resistant wheat cultivar. *Plant Pathology, 49*(4), 445-451. doi:DOI 10.1046/j.1365-3059.2000.00472.x

Cullis, B. R., Smith, A. B., & Coombes, N. E. (2006). On the design of early generation variety trials with correlated data. *Journal of agricultural, biological, and environmental statistics*, *11*, 381-393.

Cuthbert, P. A., Somers, D. J., & Brule-Babel, A. (2007). Mapping of Fhb2 on chromosome 6BS: a gene controlling Fusarium head blight field resistance in bread wheat (Triticum aestivum L.). *Theoretical and Applied Genetics, 114*(3), 429-437. doi:10.1007/s00122-006-0439-3

de Chaves, M. A., Reginatto, P., da Costa, B. S., de Paschoal, R. I., Teixeira, M. L., & Fuentefria, A. M. (2022). Fungicide Resistance in Fusarium graminearum Species Complex. *Current Microbiology, 79*(2). doi:ARTN 6210.1007/s00284-021-02759-4

De Mendiburu, F. (2014). Agricolae: statistical procedures for agricultural research. *R package version, 1*(1), 1-4.

de Wit, P. J. G. M. (2007). Visions & reflections (minireview) - How plants recognize pathogens and defend themselves. *Cellular and Molecular Life Sciences, 64*(21), 2726-2732. doi:10.1007/s00018-007-7284-7

Dhokane, D., Karre, S., Kushalappa, A. C., & McCartney, C. (2016). Integrated Metabolo-Transcriptomics Reveals Fusarium Head Blight Candidate Resistance Genes in Wheat QTL-Fhb2. *Plos One, 11*(5). doi:ARTN e015585110.1371/journal.pone.0155851

Eckersten, H., Blomback, K., Katterer, T., & Nyman, P. (2001). Modelling C, N, water and heat dynamics in winter wheat under climate change in southern Sweden. *Agriculture Ecosystems & Environment, 86*(3), 221-235. doi:Doi 10.1016/S0167-8809(00)00284-X

Eriksen, L., Borum, F., & Jahoor, A. (2003). Inheritance and localisation of resistance to Mycosphaerella graminicola causing septoria tritici blotch and plant height in the wheat (Triticum aestivum L.) genome with DNA markers. *Theoretical and Applied Genetics, 107*(3), 515-527. doi:10.1007/s00122-003-1276-2

EUROSTATS. (2020). Agricultural production - crops: Production of main cereals, EU, 2010-2020 (million tonnes). Retrieved from https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Agricultural_production_-_crops&oldid=550150#Cereals

FAOSTAT. (2022). Retrieved from https://www.fao.org/faostat/en/#data

Farooq, M., Bramley, H., Palta, J. A., & Siddique, K. H. M. (2011). Heat Stress in Wheat during Reproductive and Grain-Filling Phases. *Critical Reviews in Plant Sciences, 30*(6), 491-507. doi:10.1080/07352689.2011.615687

Fernandez, M. R., & Chen, Y. (2005). Pathogenicity of Fusarium species on different plant parts of spring wheat under controlled conditions. *Plant Disease, 89*(2), 164-169. doi:10.1094/Pd-89-0164

Fones, H., & Gurr, S. (2015). The impact of Septoria tritici Blotch disease on wheat: An EU perspective. *Fungal Genetics and Biology, 79*, 3-7. doi:10.1016/j.fgb.2015.04.004

Gadaleta, A., Colasuonno, P., Giove, S. L., Blanco, A., & Giancaspro, A. (2019). Map-based cloning of QFhb.mgb-2A identifies a WAK2 gene responsible for Fusarium Head Blight resistance in wheat. *Scientific Reports, 9*. doi:ARTN 692910.1038/s41598-019-43334-z

Gerard, G. S., Borner, A., Lohwasser, U., & Simon, M. R. (2017). Genome-wide association mapping of genetic factors controlling Septoria tritici blotch resistance and their associations with plant height and heading date in wheat. *Euphytica, 213*(1). doi:ARTN 2710.1007/s10681-016-1820-1

Ghaffary, S. M. T., Chawade, A., & Singh, P. K. (2018). Practical breeding strategies to improve resistance to Septoria tritici blotch of wheat. *Euphytica, 214*(7). doi:ARTN 12210.1007/s10681-018-2205-4

Ghaffary, S. M. T., Robert, O., Laurent, V., Lonnet, P., Margale, E., van der Lee, T. A. J., Kema, G. H. J. (2011). Genetic analysis of resistance to septoria tritici blotch in the French winter wheat cultivars Balance and Apache. *Theoretical and Applied Genetics, 123*(5), 741-754. doi:10.1007/s00122-011-1623-7

Ghosh, S., Watson, A., Gonzalez-Navarro, O. E., Ramirez-Gonzalez, R. H., Yanes, L., Mendoza-Suarez, M., Hickey, L. T. (2018). Speed breeding in growth chambers and glasshouses for crop breeding and model plant research. *Nat Protoc, 13*(12), 2944-2963. doi:10.1038/s41596-018-0072-z

Gigliotti, C. L., Totten, L. A., Offenberg, J. H., Dachs, J., Reinfelder, J. R., Nelson, E. D., Eisenreich, S. J. (2005). Atmospheric concentrations and deposition of polycyclic aromatic hydrocarbons to the Mid-Atlantic East Coast Region. *Environmental Science & Technology*, *39*(15), 5550-5559. doi:DOI 10.1021/es050401k

Gilbert, J., Tekauz, A., Kaethler, R., Slusarenko, K., Leclerc, C., Mueller, E., Beyene, M. (2009). Survey of fusarium head blight of spring wheat in Manitoba in 2008. *Canadian plant disease survey= Inventaire des maladies des plantes au Canada*.

Gilsinger, J., Kong, L., Shen, X., & Ohm, H. (2005). DNA markers associated with low Fusarium head blight incidence and narrow flower opening in wheat. *Theoretical and Applied Genetics, 110*(7), 1218-1225. doi:10.1007/s00122-005-1953-4

Golinski, P., Kacmarek, Z., Kiecana, I., Wisniewska, H., Kaptur, P., Kostecki, M., & Chelkowski, J. (2002). Fusarium head blight of common Polish winter wheat cultivars - Comparison of effects of Fusarium avenaceum and Fusarium culmorum on yield components. *Journal of Phytopathology-Phytopathologische Zeitschrift, 150*(3), 135-141. doi:DOI 10.1046/j.1439-0434.2002.00723.x

Golkari, S., Gilbert, J., Prashar, S., & Procunier, J. D. (2005). *High resolution profiling of wheat genes differentially expressed in response to Fusarium graminearum infection.* Paper presented at the 2005 National Fusarium Head Blight Forum.

Gonzalez, F. G., Slafer, G. A., & Miralles, D. J. (2002). Vernalization and photoperiod responses in wheat pre-flowering reproductive phases. *Field Crops Research, 74*(2-3), 183-195. doi:Pii S0378-4290(01)00210-6Doi 10.1016/S0378-4290(01)00210-6

Gull, A., Lone, A. A., & Wani, N. U. I. (2019). Biotic and abiotic stresses in plants. *Abiotic and biotic stress in plants*, 1-19.

Gurung, S., Mamidi, S., Bonman, J. M., Xiong, M., Brown-Guedira, G., & Adhikari, T. B. (2014). Genome-Wide Association Study Reveals Novel Quantitative Trait Loci Associated with Resistance to Multiple Leaf Spot Diseases of Spring Wheat. *Plos One, 9*(9). doi:ARTN e10817910.1371/journal.pone.0108179

Harfouche, A. L., Jacobson, D. A., Kainer, D., Romero, J. C., Harfouche, A. H., Mugnozza, G. S., Altman, A. (2019). Accelerating Climate Resilient Plant Breeding by Applying Next-Generation Artificial Intelligence. *Trends in Biotechnology, 37*(11), 1217-1235. doi:10.1016/j.tibtech.2019.05.007

Hehir, J. G., Connolly, C., O'Driscoll, A., Lynch, J. P., Spink, J., Brown, J. K. M., Mullins, E. (2018). Temporal and spatial field evaluations highlight the importance of the presymptomatic phase in supporting strong partial resistance in Triticum aestivum against Zymoseptoria tritici. *Plant Pathology, 67*(3), 573-583. doi:10.1111/ppa.12780

Hickey, L. T., Germán, S. E., Pereyra, S. A., Diaz, J. E., Ziems, L. A., Fowler, R. A., Dieters, M. J. (2017). Speed breeding for multiple disease resistance in barley. *Euphytica, 213*, 1-14.

Hickey, L. T., Hafeez, A. N., Robinson, H., Jackson, S. A., Leal-Bertioli, S. C. M., Tester, M., Wulff, B. B. H. (2019). Breeding crops to feed 10 billion. *Nature Biotechnology, 37*(7), 744-754. doi:10.1038/s41587-019-0152-9

Inglis, G. D., & Kawchuk, L. M. (2002). Comparative degradation of oomycete, ascomycete, and basidiomycete cell walls by mycoparasitic and biocontrol fungi. *Canadian Journal of Microbiology, 48*(1), 60-70. doi:10.1139/W01-130

Jensen, D., Dubey, M., Jensen, B., & Karlsson, M. (2021). Clonostachys rosea to control plant diseases. *Microbial Bioprotectants for Plant Disease Management; Köhl, J., Ravensberg, WJ, Eds*, 429-471.

Josse, J., & Husson, F. (2016). missMDA: a package for handling missing values in multivariate data analysis. *Journal of statistical software, 70*, 1-31.

Juliana, P., Singh, R. P., Singh, P. K., Crossa, J., Huerta-Espino, J., Lan, C. X., Sorrells, M. E. (2017). Genomic and pedigree-based prediction for leaf, stem, and stripe rust resistance in wheat. *Theoretical and Applied Genetics, 130*(7), 1415-1430. doi:10.1007/s00122-017-2897-1

Kearsey, M. (1997). Genetic resources and plant breeding. *Biotechnology and plant genetic resources*, 175.

Kema, G. H. J., Verstappen, E. C. P., Todorova, M., & Waalwijk, C. (1996). Successful crosses and molecular tetrad and progeny analyses demonstrate

heterothallism in Mycosphaerella graminicola. *Current Genetics, 30*(3), 251-258. doi:DOI 10.1007/s002940050129

Kidane, Y. G., Hailemariam, B. N., Mengistu, D. K., Fadda, C., Pe, M. E., & Dell'Acqua, M. (2017). Genome-Wide Association Study of Septoria tritici Blotch Resistance in Ethiopian Durum Wheat Landraces. *Frontiers in Plant Science, 8*. doi:ARTN 158610.3389/fpls.2017.01586

Klix, M. B., Beyer, M., & Verreet, J. A. (2008). Effects of cultivar, agronomic practices, geographic location, and meteorological conditions on the composition of selected Fusarium species on wheat heads. *Canadian Journal of Plant Pathology, 30*(1), 46-57. doi:Doi 10.1080/07060660809507495

Kollers, S., Rodemann, B., Ling, J., Korzun, V., Ebmeyer, E., Argillier, O., Roder, M. S. (2013). Genetic architecture of resistance to Septoria tritici blotch (Mycosphaerella graminicola) in European winter wheat. *Molecular Breeding, 32*(2), 411-423. doi:10.1007/s11032-013-9880-6

Kuhn, M., Wing, J., Weston, S., Williams, A., Keefer, C., Engelhardt, A., Team, R. C. (2020). Package 'caret'. *The R Journal, 223*(7).

Laidig, F., Feike, T., Hadasch, S., Rentel, D., Klocke, B., Miedaner, T., & Piepho, H. P. (2021). Breeding progress of disease resistance and impact of disease severity under natural infections in winter wheat variety trials. *Theoretical and Applied Genetics, 134*(5), 1281-1302. doi:10.1007/s00122-020-03728-4

Lê, S., Josse, J., & Husson, F. (2008). FactoMineR: an R package for multivariate analysis. *Journal of statistical software, 25*, 1-18.

Legarra, A., Robert-Granié, C., Manfredi, E., & Elsen, J.-M. (2008). Performance of genomic selection in mice. *Genetics, 180*(1), 611-618.

Leiva, F., Zakieh, M., Alamrani, M., Dhakal, R., Henriksson, T., Singh, P. K., & Chawade, A. (2022). Phenotyping Fusarium head blight through seed morphology characteristics using RGB imaging. *Innovative Use of Imaging Techniques within Plant Science, 16648714*, 142.

Lemmens, M., Scholz, U., Berthiller, F., Dall'Asta, C., Koutnik, A., Schuhmacher, R., Ruckenbauer, P. (2005). The ability to detoxify the mycotoxin deoxynivalenol colocalizes with a major quantitative trait locus for fusarium head blight resistance in wheat. *Molecular Plant-Microbe Interactions, 18*(12), 1318-1324. doi:10.1094/Mpmi-18-1318

Lenc, L. (2011). Fusarium head blight and Fusarium spp. occurring on grain of spring wheat in an organic farming system. *Phytopathologia, 62*, 31-39.

Leslie, J. F., & Summerell, B. A. (2008). *The Fusarium laboratory manual*: John Wiley & Sons.

Lipka, A. E., Tian, F., Wang, Q. S., Peiffer, J., Li, M., Bradbury, P. J., Zhang, Z. W. (2012). GAPIT: genome association and prediction integrated tool. *Bioinformatics, 28*(18), 2397-2399. doi:10.1093/bioinformatics/bts444

Liu, H., Zwer, P., Wang, H. B., Liu, C. J., Lu, Z. Y., Wang, Y. X., & Yan, G. J. (2016). A fast generation cycling system for spring oat and triticale breeding. *Plant Breeding, 135*(5), 574-579. doi:10.1111/pbr.12408

Liu, K., Xu, H., Liu, G., Guan, P., Zhou, X., Peng, H., Du, J. (2018). QTL mapping of flag leaf-related traits in wheat (Triticum aestivum L.). *Theoretical and Applied Genetics, 131*, 839-849.

Liu, Y., Tao, Y., Wang, Z., Guo, Q., Wu, F., Yang, X., Wei, Y. (2018). Identification of QTL for flag leaf length in common wheat and their pleiotropic effects. *Molecular Breeding, 38*, 1-11.

Lobet, G. (2017). Image Analysis in Plant Sciences: Publish Then Perish. *Trends in Plant Science, 22*(7), 559-566. doi:10.1016/j.tplants.2017.05.002

Logan, A. C., & Jacka, F. N. (2014). Nutritional psychiatry research: an emerging discipline and its intersection with global urbanization, environmental challenges and the evolutionary mismatch. *Journal of Physiological Anthropology, 33*, 1-16.

Louriki, S., Rehman, S., El Hanafi, S., Bouhouch, Y., Al-Jaboobi, M., Amri, A., Tadesse, W. (2021). Identification of Resistance Sources and Genome-Wide Association Mapping of Septoria Tritici Blotch Resistance in Spring Bread Wheat Germplasm of ICARDA. *Frontiers in Plant Science, 12*. doi:ARTN 60017610.3389/fpls.2021.600176

Lynch, J. P., Glynn, E., Kildea, S., & Spink, J. (2017). Yield and optimum fungicide dose rates for winter wheat (Triticum aestivum L.) varieties with contrasting ratings for resistance to septoria tritici blotch. *Field Crops Research, 204*, 89-100. doi:10.1016/j.fcr.2017.01.012

Ma, Z. Q., Xie, Q., Li, G. Q., Jia, H. Y., Zhou, J. Y., Kong, Z. X., Yuan, Y. (2020). Germplasms, genetics and genomics for better control of disastrous wheat Fusarium head blight. *Theoretical and Applied Genetics, 133*(5), 1541-1568. doi:10.1007/s00122-019-03525-8

Mackay, I., Horwell, A., Garner, J., White, J., McKee, J., & Philpott, H. (2011). Reanalyses of the historical series of UK variety trials to quantify the contributions of genetic and environmental factors to trends and variability in yield over time. *Theoretical and Applied Genetics, 122*(1), 225-238. doi:10.1007/s00122-010-1438-y

Madden, L. V., & Paul, P. A. (2009). Assessing Heterogeneity in the Relationship Between Wheat Yield and Fusarium Head Blight Intensity Using Random-Coefficient Mixed Models. *Phytopathology, 99*(7), 850-860. doi:10.1094/Phyto-99-7-0850

Mae, A., Fillinger, S., Soovali, P., & Heick, T. M. (2020). Fungicide Sensitivity Shifting of Zymoseptoria tritici in the Finnish-Baltic Region and a Novel Insertion in the MFS1 Promoter. *Frontiers in Plant Science, 11*. doi:ARTN 38510.3389/fpls.2020.00385

Mahboubi, M., Talebi, R., Mehrabi, R., Naji, A. M., Maccaferri, M., & Kema, G. (2021). Genome-wide association mapping in wheat reveals novel QTLs

and potential candidate genes involved in resistance to septoria tritici blotch.

Maillard, F., Andrews, E., Moran, M., Kennedy, P. G., Van Bloem, S. J., & Schilling, J. S. (2020). Stem-inhabiting fungal communities differ between intact and snapped trees after hurricane Maria in a Puerto Rican tropical dry forest. *Forest Ecology and Management, 475*. doi:ARTN 11835010.1016/j.foreco.2020.118350

Malbran, I., Mourelos, C. A., Girotti, J. R., Balatti, P. A., & Lori, G. A. (2014). Toxigenic Capacity and Trichothecene Production by Fusarium graminearum Isolates from Argentina and Their Relationship with Aggressiveness and Fungal Expansion in the Wheat Spike. *Phytopathology, 104*(4), 357-364. doi:10.1094/Phyto-06-13-0172-R

Maloney, P. V., Petersen, S., Navarro, R. A., Marshall, D., McKendry, A. L., Costa, J. M., & Murphy, J. P. (2014). Digital image analysis method for estimation of Fusarium-damaged kernels in wheat. *Crop Science, 54*(5), 2077-2083.

Martinez-Espinoza, A., Ethredge, R., Youmans, V., John, B., & Buck, J. (2014). Identification and control of Fusarium head blight (Scab) of wheat in Georgia. *University of Georgia Extension*, 1-8.

McDonald, B., Mundt, C., & Zhan, J. (1999). *Population genetics of Mycosphaerella graminicola and Phaeosphaeria nodorum.* Paper presented at the 5. Proceedings of the International Septoria Workshop, Mexico, DF (Mexico), 20-24 Sep 1999.

McDonald, B. A., & Mundt, C. C. (2016). How Knowledge of Pathogen Population Biology Informs Management of Septoria Tritici Blotch. *Phytopathology, 106*(9), 948-955. doi:10.1094/Phyto-03-16-0131-Rvw

McIntosh, R. (1998). Breeding wheat for resistance to biotic stresses. *Euphytica, 100*, 19-34.

McMullen, M., Bergstrom, G., De Wolf, E., Dill-Macky, R., Hershman, D., Shaner, G., & Van Sanford, D. (2012). A Unified Effort to Fight an Enemy of Wheat and Barley: Fusarium Head Blight. *Plant Disease, 96*(12), 1712-1728. doi:10.1094/Pdis-03-12-0291-Fe

Mesterhazy, A., Bartok, T., Mirocha, C. G., & Komoroczy, R. (1999). Nature of wheat resistance to Fusarium head blight and the role of deoxynivalenol for breeding. *Plant Breeding, 118*(2), 97-110. doi:DOI 10.1046/j.1439-0523.1999.118002097.x

Meuwissen, T. H. E., Hayes, B. J., & Goddard, M. E. (2001). Prediction of total genetic value using genome-wide dense marker maps. *Genetics, 157*(4), 1819-1829. Retrieved from <Go to ISI>://WOS:000168223400036

Miralles, D. J., & Richards, R. A. (2000). Responses of leaf and tiller emergence and primordium initiation in wheat and barley to interchanged photoperiod. *Annals of Botany, 85*(5), 655-663. doi:DOI 10.1006/anbo.2000.1121

Mohanty, S. P., Hughes, D. P., & Salathe, M. (2016). Using Deep Learning for Image-Based Plant Disease Detection. *Frontiers in Plant Science, 7*. doi:ARTN 141910.3389/fpls.2016.01419

Mueller, J. D., & Sinclair, J. B. (1986). Occurrence and Role of Gliocladium-Roseum in Field-Grown Soybeans in Illinois. *Transactions of the British Mycological Society, 86*, 677-680. doi:Doi 10.1016/S0007-1536(86)80078-X

Muqaddasi, Q. H., Zhao, Y. S., Rodemann, B., Plieske, J., Ganal, M. W., & Roder, M. S. (2019). Genome-wide Association Mapping and Prediction of Adult Stage Septoria tritici Blotch Infection in European Winter Wheat via High-Density Marker Arrays. *Plant Genome, 12*(1). doi:ARTN 18002910.3835/plantgenome2018.05.0029

Odilbekov, F., Armoniene, R., Koc, A., Svensson, J., & Chawade, A. (2019). GWAS-Assisted Genomic Prediction to Predict Resistance to Septoria Tritici Blotch in Nordic Winter Wheat at Seedling Stage. *Frontiers in Genetics, 10*. doi:ARTN 122410.3389/fgene.2019.01224

Olesen, J. E., & Bindi, M. (2002). Consequences of climate change for European agricultural productivity, land use and policy. *European journal of agronomy, 16*(4), 239-262. doi:Pii S1161-0301(02)00004-7Doi 10.1016/S1161-0301(02)00004-7

Ollier, M., Talle, V., Brisset, A. L., Le Bihan, Z., Duerr, S., Lemmens, M., Buerstmayr, H. (2020). QTL mapping and successful introgression of the spring wheat-derived QTL Fhb1 for Fusarium head blight resistance in three European triticale populations. *Theoretical and Applied Genetics, 133*(2), 457-477. doi:10.1007/s00122-019-03476-0

Osborne, L. E., & Stein, J. M. (2007). Epidemiology of Fusarium head blight on small-grain cereals. *International Journal of Food Microbiology, 119*(1-2), 103-108. doi:10.1016/j.ijfoodmicro.2007.07.032

Paudel, B., Zhuang, Y. B., Galla, A., Dahal, S., Qiu, Y. J., Ma, A. J., Yen, Y. (2020). WFhb1-1 plays an important role in resistance against Fusarium head blight in wheat. *Scientific Reports, 10*(1). doi:ARTN 779410.1038/s41598-020-64777-9

Pequeno, D. N. L., Hernandez-Ochoa, I. M., Reynolds, M., Sonder, K., MoleroMilan, A., Robertson, R. D., Asseng, S. (2021). Climate impact and adaptation to heat and drought stress of regional and global wheat production. *Environmental Research Letters, 16*(5). doi:ARTN 05407010.1088/1748-9326/abd970

Piepho, H.-P., & Möhring, J. (2007). Computing heritability and selection response from unbalanced plant breeding trials. *Genetics, 177*(3), 1881-1888.

Platel, R., Chaveriat, L., Le Guenic, S., Pipeleers, R., Magnin-Robert, M., Randoux, B., Halama, P. (2020). Importance of the C12 carbon chain in the biological activity of rhamnolipids conferring protection in wheat against Zymoseptoria tritici. *Molecules, 26*(1), 40.

Poland, J., & Rutkoski, J. (2016). Advances and Challenges in Genomic Selection for Disease Resistance. *Annual Review of Phytopathology, Vol 54, 54*, 79-98. doi:10.1146/annurev-phyto-080615-100056

Qi, L., Pumphrey, M., Friebe, B., Chen, P., & Gill, B. (2008). Molecular cytogenetic characterization of alien introgressions with gene Fhb3 for resistance to Fusarium head blight disease of wheat. *Theoretical and Applied Genetics, 117*, 1155-1166.

Radecka-Janusik, M., & Czembor, P. C. (2014). Genetic mapping of quantitative trait loci (QTL) for resistance to Septoria tritici blotch in a winter wheat cultivar Liwilla. *Euphytica, 200*(1), 109-125.

Rajaram, S. (2001). *Prospects and promise of wheat breeding in the 21 st century.* Paper presented at the Wheat in a Global Environment: Proceedings of the 6th International Wheat Conference, 5–9 June 2000, Budapest, Hungary.

Rawat, N., Pumphrey, M. O., Liu, S. X., Zhang, X. F., Tiwari, V. K., Ando, K., Gill, B. S. (2016). Wheat Fhb1 encodes a chimeric lectin with agglutinin domains and a pore-forming toxin-like domain conferring resistance to Fusarium head blight. *Nature Genetics, 48*(12), 1576-1580. doi:10.1038/ng.3706

Riaz, A., KockAppelgren, P., Hehir, J. G., Kang, J., Meade, F., Cockram, J., Byrne, S. (2020). Genetic Analysis Using a Multi-Parent Wheat Population Identifies Novel Sources of Septoria Tritici Blotch Resistance. *Genes, 11*(8). doi:ARTN 88710.3390/genes11080887

Roberti, R., Veronesi, A., Cesari, A., Cascone, A., Di Berardino, I., Bertini, L., & Caruso, C. (2008). Induction of PR proteins and resistance by the biocontrol agent Clonostachys rosea in wheat plants infected with Fusarium culmorum. *Plant Science, 175*(3), 339-347. doi:10.1016/j.plantsci.2008.05.003

Rudd, J. C., Horsley, R. D., McKendry, A. L., & Elias, E. M. (2001). Host plant resistance genes for fusarium head blight: Sources, mechanisms, and utility in conventional breeding systems. *Crop Science, 41*(3), 620-627. doi:DOI 10.2135/cropsci2001.413620x

Rutkoski, J., Benson, J., Jia, Y., Brown-Guedira, G., Jannink, J. L., & Sorrells, M. (2012). Evaluation of Genomic Prediction Methods for Fusarium Head Blight Resistance in Wheat. *Plant Genome, 5*(2), 51-61. doi:10.3835/plantgenome2012.02.0001

Saraiva, R. M., Czymmek, K. J., Borges, A. V., Caires, N. P., & Maffia, L. A. (2015). Confocal microscopy study to understand Clonostachys rosea and Botrytis cinerea interactions in tomato plants. *Biocontrol Science and Technology, 25*(1), 56-71. doi:10.1080/09583157.2014.948382

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., & Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution, 3*(3), 430-+. doi:10.1038/s41559-018-0793-y

Scott, M. F., Fradgley, N., Bentley, A. R., Brabbs, T., Corke, F., Gardner, K. A., Cockram, J. (2021). Limited haplotype diversity underlies polygenic trait architecture across 70 years of wheat breeding. *Genome Biol, 22*(1), 137. doi:10.1186/s13059-021-02354-7

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

Simko, I., Jimenez-Berni, J. A., & Sirault, X. R. (2017). Phenomic approaches and tools for phytopathologists. *Phytopathology, 107*(1), 6-17.

Singh, A., Jones, S., Ganapathysubramanian, B., Sarkar, S., Mueller, D., Sandhu, K., & Nagasubramanian, K. (2021). Challenges and opportunities in machine-augmented plant stress phenotyping. *Trends in Plant Science, 26*(1), 53-69.

Singh, P. K., Gahtyari, N. C., Roy, C., Roy, K. K., He, X. Y., Tembo, B., Chawade, A. (2021). Wheat Blast: A Disease Spreading by Intercontinental Jumps and Its Management Strategies. *Frontiers in Plant Science, 12*. doi:ARTN 71070710.3389/fpls.2021.710707

Singla, J., & Krattinger, S. (2015). Biotic stress resistance genes in wheat. In *The Production and Genetics of Food Grains* (pp. 388-392): Elsevier Inc.

Slafer, G. (2003). Genetic basis of yield as viewed from a crop physiologist's perspective. *Annals of Applied Biology, 142*(2), 117-128.

Snijders, C. H. A. (2004). Resistance in wheat to Fusarium infection and trichothecene formation. *Toxicology Letters, 153*(1), 37-46. doi:10.1016/j.toxlet.2004.04.044

Somers, D. J., Fedak, G., & Savard, M. (2003). Molecular mapping of novel genes controlling Fusarium head blight resistance and deoxynivalenol accumulation in spring wheat. *Genome, 46*(4), 555-564. doi:10.1139/G03-033

Song, Y. X., Duan, X. Y., Wang, P. F., Li, X., Yuan, X., Wang, Z. Y., Hong, D. F. (2022). Comprehensive speed breeding: a high-throughput and rapid generation system for long-day crops. *Plant biotechnology journal, 20*(1), 13-15. doi:10.1111/pbi.13726

Soriano, J. M., Colasuonno, P., Marcotuli, I., & Gadaleta, A. (2021). Meta-QTL analysis and identification of candidate genes for quality, abiotic and biotic stress in durum wheat. *Scientific Reports, 11*(1), 1-15.

Spindel, J. E., Begum, H., Akdemir, D., Collard, B., Redona, E., Jannink, J. L., & McCouch, S. (2016). Genome-wide prediction models that incorporate de novo GWAS are a powerful new tool for tropical rice improvement. *Heredity (Edinb), 116*(4), 395-408. doi:10.1038/hdy.2015.113

Stenberg, J. A., Sundh, I., Becher, P. G., Bjorkman, C., Dubey, M., Egan, P. A., Viketoft, M. (2021). When is it biological control? A framework of definitions, mechanisms, and classifications. *Journal of Pest Science, 94*(3), 665-676. doi:10.1007/s10340-021-01354-7

Su, Z. Q., Bernardo, A., Tian, B., Chen, H., Wang, S., Ma, H. X., Bai, G. H. (2019). A deletion mutation in TaHRC confers Fhb1 resistance to Fusarium head blight in wheat. *Nature Genetics, 51*(7), 1099-+. doi:10.1038/s41588-019-0425-8

Suffert, F., Delestre, G., & Gelisse, S. (2019). Sexual Reproduction in the Fungal Foliar Pathogen Zymoseptoria tritici Is Driven by Antagonistic Density Dependence Mechanisms. *Microbial Ecology*, *77*(1), 110-123. doi:10.1007/s00248-018-1211-3

Tanabata, T., Shibaya, T., Hori, K., Ebana, K., & Yano, M. (2012). SmartGrain : High-Throughput Phenotyping Software for Measuring Seed Shape through Image Analysis. *Plant Physiology*, *160*(4), 1871-1880. doi:10.1104/pp.112.205120

Torriani, S. F. F., Melichar, J. P. E., Mills, C., Pain, N., Sierotzki, H., & Courbot, M. (2015). Zymoseptoria tritici: A major threat to wheat production, integrated approaches to control. *Fungal Genetics and Biology*, *79*, 8-12. doi:10.1016/j.fgb.2015.04.010

Toth, B., Mesterhazy, A., Horvath, Z., Bartok, T., Varga, M., & Varga, J. (2005). Genetic variability of central European isolates of the Fusarium graminearum species complex. *European Journal of Plant Pathology*, *113*(1), 35-45. doi:10.1007/s10658-005-0296-y

Trnka, M., Rotter, R. P., Ruiz-Ramos, M., Kersebaum, K. C., Olesen, J. E., Zalud, Z., & Semenov, M. A. (2014). Adverse weather conditions for European wheat production will become more frequent with climate change. *Nature Climate Change*, *4*(7), 637-643. doi:10.1038/Nclimate2242

Vagndorf, N., Nielsen, N. H., Edriss, V., Andersen, J. R., Orabi, J., Jorgensen, L. N., & Jahoor, A. (2017). Genomewide association study reveals novel quantitative trait loci associated with resistance towards Septoria tritici blotch in North European winter wheat. *Plant Breeding*, *136*(4), 474-482. doi:10.1111/pbr.12490

Velasquez, A. C., Castroverde, C. D. M., & He, S. Y. (2018). Plant-Pathogen Warfare under Changing Climate Conditions. *Current Biology*, *28*(10), R619-R634. doi:10.1016/j.cub.2018.03.054

Venske, E., dos Santos, R. S., Farias, D. D., Rother, V., da Maia, L. C., Pegoraro, C., & de Oliveira, A. C. (2019). Meta-Analysis of the QTLome of Fusarium Head Blight Resistance in Bread Wheat: Refining the Current Puzzle. *Frontiers in Plant Science*, *10*. doi:ARTN 72710.3389/fpls.2019.00727

Waldron, B., Moreno-Sevilla, B., Anderson, J., Stack, R., & Frohberg, R. (1999). RFLP mapping of QTL for Fusarium head blight resistance in wheat. *Crop Science*, *39*(3), 805-811.

Wang, M., Wang, S., & Xia, G. (2015). From genome to gene: a new epoch for wheat research? *Trends in Plant Science*, *20*(6), 380-387.

Wang, S. C., Wong, D. B., Forrest, K., Allen, A., Chao, S. M., Huang, B. E., Sequencing, I. W. G. (2014). Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. *Plant biotechnology journal*, *12*(6), 787-796. doi:10.1111/pbi.12183

Warrington, I. J., & Kanemasu, E. T. (1983). Corn Growth-Response to Temperature and Photoperiod .2. Leaf-Initiation and Leaf-Appearance Rates. *Agronomy*

Journal, *75*(5), 755-761. doi:DOI 10.2134/agronj1983.00021962007500050009x

Watson, A., Ghosh, S., Williams, M. J., Cuddy, W. S., Simmonds, J., Rey, M. D., Hickey, L. T. (2018). Speed breeding is a powerful tool to accelerate crop research and breeding. *Nature Plants*, *4*(1), 23-29. doi:10.1038/s41477-017-0083-8

Wegulo, S. N., Baenziger, P. S., Nopsa, J. H., Bockus, W. W., & Hallen-Adams, H. (2015). Management of Fusarium head blight of wheat and barley. *Crop Protection*, *73*, 100-107. doi:10.1016/j.cropro.2015.02.025

Windels, C. E. (2000). Economic and social impacts of Fusarium head blight: Changing farms and rural communities in the Northern Great Plains. *Phytopathology*, *90*(1), 17-21. doi:Doi 10.1094/Phyto.2000.90.1.17

Xu, Q., Xu, F. C., Qin, D. D., Li, M. F., Fedak, G., Cao, W. G., Dong, J. (2020). Molecular Mapping of QTLs Conferring Fusarium Head Blight Resistance in Chinese Wheat Cultivar Jingzhou 66. *Plants-Basel*, *9*(8). doi:ARTN 102110.3390/plants9081021

Xu, X. M., Parry, D. W., Nicholson, P., Thomsett, M. A., Simpson, D., Edwards, S. G., Ritieni, A. (2008). Within-field variability of Fusarium head blight pathogens and their associated mycotoxins. *European Journal of Plant Pathology*, *120*(1), 21-34. doi:10.1007/s10658-007-9189-6

Xue, A. G., Chen, Y. H., Voldeng, H. D., Fedak, G., Savard, M. E., Langle, T., Harman, G. E. (2014). Concentration and cultivar effects on efficacy of CLO-1 biofungicide in controlling Fusarium head blight of wheat. *Biological Control*, *73*, 2-7. doi:10.1016/j.biocontrol.2014.02.010

Xue, A. G., Voldeng, H. D., Savard, M. E., Fedak, G., Tian, X., & Hsiang, T. (2009). Biological control of fusarium head blight of wheat with Clonostachys rosea strain ACM941. *Canadian Journal of Plant Pathology*, *31*(2), 169-179. Retrieved from <Go to ISI>://WOS:000279668700002

Xue, S., Li, G. Q., Jia, H. Y., Xu, F., Lin, F., Tang, M. Z., Ma, Z. Q. (2010). Fine mapping Fhb4, a major QTL conditioning resistance to Fusarium infection in bread wheat (Triticum aestivum L.). *Theoretical and Applied Genetics*, *121*(1), 147-156. doi:10.1007/s00122-010-1298-5

Xue, S. L., Xu, F., Tang, M. Z., Zhou, Y., Li, G. Q., An, X., Ma, Z. Q. (2011). Precise mapping Fhb5, a major QTL conditioning resistance to Fusarium infection in bread wheat (Triticum aestivum L.). *Theoretical and Applied Genetics*, *123*(6), 1055-1063. doi:10.1007/s00122-011-1647-z

Yao, Y., Zhang, P., Wang, H. B., Lu, Z. Y., Liu, C. J., Liu, H., & Yan, G. J. (2016). How to advance up to seven generations of canola (Brassica napus L.) per annum for the production of pure line populations? *Euphytica*, *209*(1), 113-119. doi:10.1007/s10681-016-1643-0

Yates, S., Mikaberidze, A., Krattinger, S. G., Abrouk, M., Hund, A., Yu, K., Pereira, D. (2019). Precision phenotyping reveals novel loci for quantitative resistance to septoria tritici blotch. *Plant Phenomics*.

Yerkovich, N., Cantoro, R., Palazzini, J. M., Torres, A., & Chulze, S. N. (2020). Fusarium head blight in Argentina: Pathogen aggressiveness, triazole tolerance and biocontrol-cultivar combined strategy to reduce disease and deoxynivalenol in wheat. *Crop Protection, 137*. doi:ARTN 10530010.1016/j.cropro.2020.105300

Yu, J. B., Bai, G. H., Zhou, W. C., Dong, Y. H., & Kolb, F. L. (2008). Quantitative trait loci for Fusarium head blight resistance in a recombinant inbred population of Wangshuibai/Wheaton. *Phytopathology, 98*(1), 87-94. doi:10.1094/Phyto-98-1-0087

Zakieh, M., Gaikpa, D. S., Sandoval, F. L., Alamrani, M., Henriksson, T., Odilbekov, F., & Chawade, A. (2021). Characterizing Winter Wheat Germplasm for Fusarium Head Blight Resistance Under Accelerated Growth Conditions. *Frontiers in Plant Science, 12*. doi:ARTN 70500610.3389/fpls.2021.705006

Zanke, C., Ling, J., Plieske, J., Kollers, S., Ebmeyer, E., Korzun, V., Beier, S. (2014). Genetic architecture of main effect QTL for heading date in European winter wheat. *Frontiers in Plant Science, 5*, 217.

Zhai, H., Feng, Z., Li, J., Liu, X., Xiao, S., Ni, Z., & Sun, Q. (2016). QTL analysis of spike morphological traits and plant height in winter wheat (Triticum aestivum L.) using a high-density SNP and SSR-based linkage map. *Frontiers in Plant Science, 7*, 1617.

Zhang, H., Van der Lee, T., Waalwijk, C., Chen, W. Q., Xu, J., Xu, J. S., Feng, J. (2012). Population Analysis of the Fusarium graminearum Species Complex from Wheat in China Show a Shift to More Aggressive Isolates. *Plos One, 7*(2). doi:ARTN e3172210.1371/journal.pone.0031722

Zhang, Y. W., Tamba, C. L., Wen, Y. J., Li, P., Ren, W. L., Ni, Y. L., Zhang, Y. M. (2020). mrMLM v4.0.2: An R Platform for Multi-locus Genome-wide Association Studies. *Genomics Proteomics & Bioinformatics, 18*(4), 481-487. doi:10.1016/j.gpb.2020.06.006

Zhu, Z. W., Hao, Y. F., Mergoum, M., Bai, G. H., Humphreys, G., Cloutier, S., He, Z. H. (2019). Breeding wheat for resistance to Fusarium head blight in the Global North: China, USA, and Canada. *Crop Journal, 7*(6), 730-738. doi:10.1016/j.cj.2019.06.003

Zia, R., Nawaz, M. S., Siddique, M. J., Hakim, S., & Imran, A. (2021). Plant survival under drought stress: Implications, adaptive responses, and integrated rhizosphere management strategy for stress mitigation. *Microbiological research, 242*, 126626.

Popular science summary

In Europe, including Sweden, wheat is a major crop, and most of Sweden's wheat is grown during winter. But there's a problem – fungal diseases can damage wheat and cause big losses in its production. Compared to other crops, it takes a long time to breed winter wheat because it requires long period to grow in low temperature in order to flower. To save time and money for the breeders, we created new methods for selection of plants resistant to diseases. These methods incorporate disease selection into rapid growth conditions and early stage of wheat plants in combination with modern genetic tools.

We focused on two diseases that largely damage wheat production: Fusarium head blight (FHB) and Septoria tritici blotch (STB). FHB reduces the productivity of wheat, lowers the quality of the grains, and creates toxins that can be dangerous for human and animal consumption. STB on the other hand is the second most damaging disease to wheat production in Europe. For this reason, farmers spend large sums of money on fungicides to control STB using chemicals, which can be an environmental problem.

To tackle these challenges, we made a special breeding plan called Speed breeding (SB) for winter wheat which we tested on hundreds of winter wheat breeding lines. With SB, wheat grew 30-50% faster in greenhouses compared to the traditional ways, and the plants remained healthy. This time-saving let us study three generations of wheat for FHB resistance in just one year. We also developed genetic markers for FHB resistance. These markers show that SB works well for breeding for FHB resistance. To make it easier to check for FHB, we developed new techniques to see how severe the

disease is on infected seeds. For that we used an RGB camera and a technique that can read and capture the qualities of the infected seeds with FHB. .
Unlike FHB, which needs the plant to be fully grown to be studied, we studied STB resistance when wheat was much younger at seedling stage. This way we can speed up the breeding process and find out which genes make it resist the disease. We discovered new genetic markers that help wheat resist STB at seedling stage and confirmed other markers that can have effect throughout its life. Also, we checked if a microorganism agent could help wheat seedlings resist STB disease. Although we only studied the interaction of the microorganism and wheat under controlled conditions, if it works in field conditions, it will prove to be an environmentally safe alternative strategy for STB control.

We took all these ideas and put them to use in the breeding industry. Breeders can now use our new breeding methods and make wheat varieties with improved resistance against diseases. This will help farmers keep wheat growing sustainably, ensures we have enough food, and avoid problems when diseases strike.

Populärvetenskaplig sammanfattning

I Europa, inklusive Sverige, är vete en betydande gröda och majoriteten av Sveriges vete odlas som höstvete. Men det finns ett problem – svampsjukdomar kan skada vetet och orsaka stora förluster i avkastning. Jämfört med andra grödor tar det lång tid att förädla fram höstvete eftersom det växer långsamt i de kalla vintertemperaturerna. Att få fart på saker och ting och hitta veteplantor som kan stå emot sjukdomar. För att spara tid och pengar för förädlarna skapade vi nya metoder för att hjälpa till att tidigt identifiera växter att bli av med tidigt i förädlingsprocessen som och behålla de som är resistenta mot sjukdomar.

Vi fokuserade på två sjukdomar som till stor del skadar veteproduktionen: axfusarios Fusarium huvudbryst (FHB) och Septoria tritici fläck (STB). FHB gör att vetet producerar mindre, sänker kvaliteten på spannmålen och skapar gifter som kan vara farliga om människor eller djur äter dem. Jordbrukare spenderar stora summor pengar på fungicider för att bekämpa STB med kemikalier, vilket kan vara ett miljöproblem.

För att tackla dessa utmaningar gjorde vi en speciell förädlingsavelsplan som heter Speed breeding (SB) för höstvete som. vi Vi testade denna plan på hundratals avelslinjer för av höstvete. Med SB växte vete 30-50% snabbare i växthus jämfört med de traditionella sätten, och plantorna förblev friska. Denna tidsbesparing låter oss studera tre generationer vete för FHB-resistens på bara ett år. Vi utvecklade också genetiska markörer för förädling för FHB-sjukdomsresistens. Dessa markörer som fungerar bra i visar att SB fungerar bra för avel för FHB-resistens. För att göra det lättare att kontrollera FHB utvecklade vi nya tekniker för att se hur allvarlig sjukdomen är på infekterade

frön. Till det använde vi en RGB-kamera och en teknik som kan läsa och fånga egenskaperna hos de infekterade fröna med FHB.

Till skillnad från FHB som kräver att plantan är fullvuxen för att kunna studeras, studerade vi STB-resistens när vete var mycket yngre. På så sätt kan vi påskynda förädlingsprocessen och ta reda på vilka gener som bidrar tillgör den stark resistens mot sjukdomen och. Vi hittade genetiska markörer som hjälper vetet att motstå STB under hela dess liv. Vi kollade också om ett mikroorganismmedel kunde hjälpa vetet att motstå STB. Även om vi endast studerade interaktionen mellan mikroorganismen och vete under kontrollerade förhållanden, om det fungerar under fältförhållanden, kommer det att visa sig vara en alternativ strategi för sjukdomsbekämpning och vara bättre för miljön.

Vi tog alla dessa idéer och använde dem i växtförädlingsavelsindustrin. Uppfödare Växtförädlare kan nu använda våra nya förädlingsmetoder och göra vetesorter som är starka mer resistenta mot sjukdomar. Detta kommer att hjälpa bönder att odlahålla vetet på ett mer växande hållbart sätt, säkerställa att vi har tillräckligt med mat och undvika problem när sjukdomar slår till.

I

frontiers in Plant Science

ORIGINAL RESEARCH
published: 25 August 2021
doi: 10.3389/fpls.2021.705006

Characterizing Winter Wheat Germplasm for Fusarium Head Blight Resistance Under Accelerated Growth Conditions

*Mustafa Zakieh[1], David S. Gaikpa[1], Fernanda Leiva Sandoval[1], Marwan Alamrani[1], Tina Henriksson[2], Firuz Odilbekov[1,2] and Aakash Chawade[1]**

[1]Department of Plant Breeding, Swedish University of Agricultural Sciences, Lomma, Sweden, [2]Lantmännen Lantbruk, Svalöv, Sweden

OPEN ACCESS

Edited by:
Morten Lillemo,
Norwegian University of Life Sciences, Norway

Reviewed by:
Yong Suk Chung,
Jeju National University, South Korea
Tomasz Góral,
Plant Breeding and Acclimatization Institute, Poland

***Correspondence:**
Aakash Chawade
aakash.chawade@slu.se

Specialty section:
This article was submitted to Plant Breeding, a section of the journal Frontiers in Plant Science

Received: 04 May 2021
Accepted: 02 August 2021
Published: 25 August 2021

Citation:
Zakieh M, Gaikpa DS, Leiva Sandoval F, Alamrani M, Henriksson T, Odilbekov F and Chawade A (2021) Characterizing Winter Wheat Germplasm for Fusarium Head Blight Resistance Under Accelerated Growth Conditions. Front. Plant Sci. 12:705006. doi: 10.3389/fpls.2021.705006

Fusarium head blight (FHB) is one of the economically important diseases of wheat as it causes severe yield loss and reduces grain quality. In winter wheat, due to its vernalization requirement, it takes an exceptionally long time for plants to reach the heading stage, thereby prolonging the time it takes for characterizing germplasm for FHB resistance. Therefore, in this work, we developed a protocol to evaluate winter wheat germplasm for FHB resistance under accelerated growth conditions. The protocol reduces the time required for plants to begin heading while avoiding any visible symptoms of stress on plants. The protocol was tested on 432 genotypes obtained from a breeding program and a genebank. The mean area under disease progress curve for FHB was 225.13 in the breeding set and 195.53 in the genebank set, indicating that the germplasm from the genebank set had higher resistance to FHB. In total, 10 quantitative trait loci (QTL) for FHB severity were identified by association mapping. Of these, nine QTL were identified in the combined set comprising both genebank and breeding sets, while two QTL each were identified in the breeding set and genebank set, respectively, when analyzed separately. Some QTLs overlapped between the three datasets. The results reveal that the protocol for FHB evaluation integrating accelerated growth conditions is an efficient approach for FHB resistance breeding in winter wheat and can be even applied to spring wheat after minor modifications.

Keywords: Fusarium head blight, winter wheat, speed breeding, accelerated growth conditions, genome-wide association study, disease resistance

INTRODUCTION

Hexaploid winter wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD) is an essential small-grain cereal crop grown for food and feed. In northern Europe, including Germany, wheat is the single most cultivated cereal crop where winter wheat is occupying the first place in production (Chawade et al., 2018). Studies examining global trends in wheat yield showed that with other major crops, wheat production must be doubled to meet the future demand to feed 10 billion people by the year 2050 (Ray et al., 2012; Hall and Richards, 2013;

Ray et al., 2013). Current wheat production in the world is impacted by environmental factors, such as abiotic and biotic stresses and climate change. Meeting the 2050 demand is becoming increasingly dependent on the genetic improvement of new cultivars and developing novel techniques for agricultural practices. The investment in the development of new breeding methodologies for cultivar improvement emerged as one of the recommended strategies to tackle the 2050 challenges that are aiming to alleviate poverty, feed the 10 billion, and reduce greenhouse gas emissions (Searchinger et al., 2019). In northern Europe, wheat farming areas and yield trends have been increasing in the past decades (FAOSTAT, 2020), possibly driven by climate change where wheat productivity was positively correlated with warmer climates (Olesen and Bindi, 2002). However, factors that affect yield negatively in wheat are diseases, such as Septoria tritici blotch and Fusarium head blight (FHB; Chawade et al., 2018). FHB is one of the major diseases affecting winter (bread) wheat (Miedaner et al., 2010; Buerstmayr et al., 2020). The disease leads to reduced grain yield globally and is the second most serious disease affecting the wheat yield after leaf rust (Buerstmayr et al., 2020). FHB infected grains have poor quality as they contain mycotoxins which are harmful to humans and animal consumption (Schmolke et al., 2008; Buerstmayr et al., 2009; Berthiller et al., 2013; Nakagawa et al., 2017). Under humid and semi-humid conditions, FHB can severely impact wheat production and can lead to further losses due to increased accumulations of mycotoxins. This is of critical importance when considering the European Union maximum levels of mycotoxins allowed for cereals sold for food and feed production (European Union, 2020). Therefore, additional losses to FHB can be predicted mainly in rainy years. Previous experiences with severe FHB pandemic impacted farmers planting decisions as it was in the 1990s in some parts of the world (Ali and Vocke, 2009). Resistance to FHB in wheat can be dissected into five types that can be either evaluated independently or in combination with each other (Mesterhazy, 1995; Mesterhazy et al., 1999; Gong et al., 2020; Kumar et al., 2020). During the growth of plants, type I (initial infection of the florets) and type II (spread of the disease along the spike) have long been used for FHB resistance testing. In contrast, type III resistance (the accumulation of mycotoxins) can be evaluated during the development of FHB on the spikes and post-harvest. Type IV (kernel damage) and type V (reduction in yield) can be evaluated at the post-harvest stage. FHB resistance is quantitatively inherited, influenced by both additive and non-additive genetic effects (Venske et al., 2019; Ma et al., 2020; Ollier et al., 2020). Quantitative trait loci (QTL) mapping and genome-wide association studies (GWAS) are used extensively to identify QTLs for FHB resistance in wheat, for possible application in marker-assisted selection (Miedaner et al., 2019; Venske et al., 2019; Buerstmayr et al., 2020; Hu et al., 2020; Ollier et al., 2020).

Efforts to address FHB resistance through QTL mapping revealed so far the presence of 556 QTL spread across wheat genome (Steiner et al., 2017; Venske et al., 2019). The majority of the FHB resistance associated QTLs has been shown to add minor resistance effects to FHB in wheat (Schweiger et al., 2016; Fabre et al., 2020). However, a small subset of genes has been identified in FHB-mediated resistance (Venske et al., 2019; Fabre et al., 2020). The locus *Fhb1* found on chromosome 3BS has been long identified as a key player in mediating FHB resistance in wheat (Bai et al., 1999). More recent studies of the *Fhb1* revealed its role in harboring resistance to FHB by transforming *Arabidopsis* and FHB susceptible wheat cultivars with *Fhb1* locus (Rawat et al., 2016; Li et al., 2019; Su et al., 2019). Despite the conflicting results in terms of the mechanisms on how *Fhb1* is mediating the resistance, cloning the locus validated its strong association in enhancing the resistance in the susceptible genotypes (Rawat et al., 2016; Li et al., 2019; Su et al., 2019). Driven by its role in FHB resistance, several studies were carried out to identify the presence of *Fhb1* locus in the germplasms adapted in breeding programs for many regions in the world (Liu and Anderson, 2003; Wang et al., 2017; Zhu et al., 2020). However, so far, studies have demonstrated a low frequency of *Fhb1* in their germplasms (Hao et al., 2020; Zhu et al., 2020). Interestingly, *Fhb1* is reportedly the only resistance QTL found in many new European wheat cultivars exhibiting high resistance levels (Hao et al., 2020).

The winter wheat growth cycle is relatively longer compared to the spring cereal crops, as winter wheat requires a vernalization period of up to 12 weeks to initiate the reproductive growth period (Ferrie and Polowick, 2020). Thus, up to two generations of winter wheat a year can be achieved in greenhouse growth conditions provided there is infrastructure available for vernalization (Ferrie and Polowick, 2020). Reducing the growth cycle is of paramount importance in increasing the genetic gain of the crops (Cobb et al., 2019). While the vernalization period of winter wheat is a limiting factor in shortening its life cycle (Voss-Fels et al., 2019), speeding up winter wheat life cycle can be achieved by optimizing post-vernalization growth conditions. The speed breeding (SB) technique in spring crops is shown to accelerate the growth and development of plants resulting in considerably shortening the time from sowing to harvest (Ghosh et al., 2018; Watson et al., 2018; Hickey et al., 2019). SB can be achieved by using an artificially prolonged light period, increased daylight intensity where light quality can be controlled (Ghosh et al., 2018; Watson et al., 2018). Under SB conditions, up to six generations of spring wheat and spring barley can be completed in 1 year (Hickey et al., 2019). SB protocols were also developed for other plant species, including peanuts, chickpea, oats, and quinoa (Hickey et al., 2019).

Growing plants in controlled environments can greatly reduce the environmental variation associated with field trials and allow the possibility of several screening per year without being limited to one season in the field (Riaz et al., 2016). Aspects plant development under continuous light conditions SB must be in the direction of enhancing the growth rate without negatively affecting the steps undertaken for the evolution of disease resistance. The phenotypic characterization of leaf rust resistance in spring wheat plants grown under artificial conditions has been shown to give similar results to those in field trials (Riaz et al., 2016). In winter wheat, and regardless of the photoperiodism and vernalization, the developmental rate of the plants has been shown to be positively promoted in

continuous light setting made with a light spectrum from combining different fluorescent light lamps grown constantly at 20°C (Sysoeva et al., 2010). Increased photosynthetic rate of several crops including wheat has been observed in long-day conditions leading to increased dry matter accumulation where the partitioning of the dry matter appears to be undisrupted by the continues light in wheat (Sysoeva et al., 2010). More recent studies have revealed the even though some physiological disorders in wheat plants have been observed when grown under continuous light (Sysoeva et al., 2010), other studies indicated suitability of SB for wheat (Ghosh et al., 2018). The light settings provided by LED light spots giving light spectrum of blue, red, and far-red with photosynthetic photon flux density between 540 and 500 $\mu mol\,m^{-2}\,s^{-1}$ for 22 h/day have been shown to be suitable in SB of spring wheat and barley plants (Ghosh et al., 2018). Winter wheat may slightly differ in its light responses compared to spring wheat. Therefore, light settings must be adjusted (photoperiod, composition, and intensities) so light injury reflected by symptoms, such as leaf chlorosis, are not visible.

This study aimed to develop a protocol to combine accelerated growth conditions under SB with the evaluation of FHB resistance in winter wheat plants. The developed protocol was tested using two different sets of germplasm obtained from the breeding program and the genebank. The germplasm phenotypic characterization was later used for GWAS to identify QTL in the studied germplasm. The developed protocol and the results from the germplasm characterization are presented.

MATERIALS AND METHODS

Plant Material

The plant material used in this work included winter wheat germplasm from two different sources. The first group of winter wheat genotypes was made up of 181 genotypes of highly diverse plant materials that included landraces and old cultivars (genebank set) obtained from the Nordic Genetic Resource Center (Nordgen). The second source of the plant material consisted of 338 genotypes (breeding set) provided by the Swedish agricultural cooperative (Lantmännen Lantbruk, Svalöv, Sweden).

Plant Growth Conditions

Germination

This work was conducted in the biotron, a facility with controlled-climate chambers at the Swedish University of Agricultural Sciences (SLU) in Alnarp, Sweden. Several seeds of each genotype were planted in 8 × 8 × 8 cm plastic pots filled with peat soil from Emmaljunga Torvmull AB, Sweden. The pots were arranged using the augmented block design described under the experimental design section. The pots were watered as required, and the seeds were left to germinate for 5 days. During the seed germination period, day-length parameters were adjusted at a light intensity (LI) of 250 $\mu mol\,m^{-2}\,s^{-1}$ for 8 h at °C 22, night 16 h of darkness with at 20°C while keeping relative humidity (RH) of 50%. After successful germination, plants were thinned and only one plant was allowed to grow in each pot.

Vernalization

Seedlings were vernalized by growing under short-day conditions of 8/16 h day/night regime with the temperature of 3°C and LI of 250 $\mu mol\,m^{-2}\,s^{-1}$. At this intensity, vernalization light source, wavelength composition, and individual wavelength intensities are described under accelerated growth conditions. RH was 80% for 8–9 weeks (approximately 60 days).

Acclimatization

After vernalization, plants were allowed to acclimatize to the upcoming vegetative growth period. This included a period of gradual change in growth conditions for 6 days (**Table 1**). The temperature was set to increase per day by 3–4°C and day-length by 2–3 h. LI was increased to 400 $\mu mol\,m^{-2}\,s^{-1}$ on the second day and was left unchanged throughout the acclimatization period. RH was gradually lowered to reach 50% at the end of the acclimatization (**Table 1**).

Accelerated Growth Conditions

At the end of the acclimatization period, the plants were allowed to grow for 32 days under the same conditions as on the last acclimatization day (**Table 1**). The lighting source was LED lights model RX30 grow lights (Heliospectra AB, Gothenburg, Sweden). The LED grow lights provided nine individually controlled wavelengths ranging from 380 nm (UVA) to 735 nm (far-red) and white light. Wavelengths 380, 400, 420, and 450 were set to radiate at 480 $\mu mol\,m^{-2}\,s^{-1}$ intensity. Meanwhile, the remaining wavelengths that included 530, 620, 660, 735, and the white light were adjusted with high intensity at 960 $\mu mol\,m^{-2}\,s^{-1}$. Sensor-feedback-based lighting continuously adjusted at the level of the plant canopy was set to give 400 $\mu mol\,m^{-2}\,s^{-1}$ intensity from the light source for 22 h. The temperature throughout the extended long day was constantly maintained at 22°C following the speed breeding protocol published earlier (Ghosh et al., 2018). Due to the rapid nature of plant growth under the extended long-day conditions, a schedule of daily watering and weekly fertilization was followed. Initially, a mix of high phosphate and high nitrogen soluble fertilizer SW-BOUYANT 7-1-5 + Mikro + KH_2PO_4 was added 3 days post-acclimatization (dpa). High nitrogen fertilizer was added at 10 dpa followed by high potassium soluble fertilizer

TABLE 1 | Growth conditions for acclimatization of vernalized winter wheat plants to the growth conditions of accelerated growth.

Days after vernalization	Temp °C	Day/Night (Hours)	Light intensity $\mu mol\,m^{-2}\,s^{-1}$	Relative humidity %
1	3	8/16	250	80
2	6	11/13	400	80
3	9	14/10	400	80
4	12	17/7	400	80
5	15	20/4	400	50
6	18	22/2	400	50
7	22	22/2	400	50

Yara Tera Kristalon NPK 12-5-30 with S, and micro was added twice at 15 and 20 dpa.

Inoculum Preparation for Fusarium Head Blight

Isolates belonging to *Fusarium* species *F. graminearum* and *F. culmorum* provided by the plant breeding company Lantmännen Lantbruk were used in the preparation of the inoculum. These included six isolates of *F. graminearum* and three isolates of *F. culmorum*. Using a large number of isolates was intended to identify germplasm with broad resistance to various *Fusarium* species. The isolates were cultured on the weak Spezieller Nahrstoffarmer agar media (Leslie and Summerell, 2006). The cultures were incubated at 24°C for 4 days, followed by near ultra-violet UV radiation for 10 h to promote macroconidial formation. Following the UV light treatment, the cultures were moved back to incubate for another 3–4 days at 24°C before collecting macroconidial spores for the inoculum preparation by pouring water on the surface of the cultures and scarping using a spatula. The surfactant Tween®20 0.002% (v/v) was added to the final suspension containing the spore concentration of 5×10^5 spore/ml.

FHB Infection Conditions

Upon completing ear emergence and the emergence of anthers, approximately 33 dpa plants were moved to grow under a long-day regime with 16/8 h day/night in the greenhouse chamber. RH was adjusted to 60%, and the temperature was maintained at 24°C. The new growth conditions were intended to allow the plants to continue growing for 24 days without accelerated growth until physiological maturity. Daily watering and weekly fertilization were carried out at this stage. Plants at 75% heading were spray-inoculated once, and inoculated plants were incubated at a high RH of 90% for 48 h while keeping other growth parameters unchanged. At the end of this incubation period, RH was lowered to 60%, and plants were allowed to grow until the end of the 24 day period.

The visual assessment of FHB disease severity on the spikes was carried out at 6, 8, 10, and 12-days post-inoculation (dpi). Generally, visual symptoms, such as bleached, yellowish or discolored, and stunted spikes, indicate the development of FHB on the ears. Disease spread was evaluated as percentage infection ranging between 5% (most resistant phenotypes) and 100% (most susceptible phenotype). The percentage rating scoring was based on the relative number of infected spikelets to the total number of spikelets per spike on the main tiller (Stack and McMullen, 1998) with an adjustment of the scoring method. Unlike the visual assessment of disease spread of FHB type II resistance, the current scoring method relied on assessing the disease severity in relation to all infected spikelets on the ear regardless of the symptom continuity. **Figure 1** shows the scale used for the visual assessment of FHB severity. Discontinued spread of the disease (symptoms are located distantly on the same spike and separated by spikelets that show no visual FHB infection symptoms) is taken together to represent the total severity on the spike (**Figures 1C,E**).

The genotypic variation in heading and flowering represents a challenge that may affect the uniformity of FHB development on a large and diverse number of artificially inoculated plants. Additionally, certain genotypes may require longer periods of vernalization to promote heading and subsequently flowering leading to the inoculated plants at earlier stage for those genotypes compared to the rest of the genotypes in the germplasm. In order to limit the bias in the downstream analysis of FHB resistance, germplasm genotypes that showed 0% infection phenotype (absence of infection symptoms) in the material were discarded together with genotypes that have not reached heading at the time of inoculation. Only genotypes that scored varying FHB symptoms that ranged between 5 and 100% were included in the analysis.

Harvest

Watering was discontinued 21 days after FHB infection conditions while keeping all other growing conditions unchanged. RH was lowered to 40% 24 days after reproductive growth in the greenhouse and the plants were left to mature. Spikes were harvested approximately 30 days after FHB infection conditions.

Flag Leaf Area, Spike Length, and Spike Width Measurements

During the reproductive growth period, flag leaf area (FLA) was measured for each genotype using LI-3000C Portable Leaf Area Meter. Spike length (SL) and spike width (SW) were estimated using a digital Vernier caliper scale. In order to avoid bias in SW (thickness of the spike), width measurement was always performed at the third lower spikelet.

Heading Time and Anther Extrusion

Heading time (HT) was taken depending on the emergence of 75% of the spikes out of the sheath of the flag leaf at three time points recorded every third day consecutively. Spikes were categorized according to the three HTs as early (HT1), medium (HT2), and late (HT3). Anther extrusion was recorded at two time points with 2 days difference and was recorded as early (AE1) and late (AE2).

Experimental Design

Four replicates each of genebank and breeding sets were arranged in an augmented block design developed using the package Agricolae in R (De Mendiburu, 2014). The design included four checks of winter wheat cultivars per block, namely, Nimbus, Stigg, Norin, and Julius. According to this design, 11 blocks per replicate were assigned for the breeding set and six blocks per replicate for the genebank set.

Phenotypic Analyses

Unadjusted means of cultivars within the augmented design of each replicate were filtered and removed for the cultivars that gave a percentage of 0%. Phenotypic data were analyzed in two steps. First, the checks in each augmented block were used to adjust the means for each trait per experiment/replicate using the Agricolae R package (De Mendiburu, 2014) based on the following model:

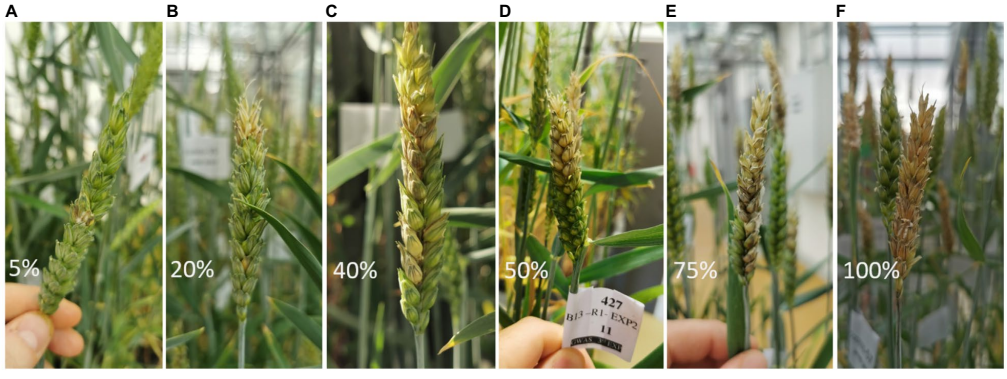


FIGURE 1 | Scale for Fusarium head blight (FHB) severity scoring on winter wheat spike. Rating of disease severity ranged from **(A)** 5 to **(F)** 100%. FHB infection can be continues **(B, D)** or disconnected **(C, E)** on a spike. Scoring was based on the proportion of total infected spikelets to the total numbers of spikelets.

$$y\_il = u + G\_il + B\_l + \varepsilon\_il,$$

where, y_il is the adjusted means of the ith wheat genotype in the lth block, u is the general mean value, G_il is the effect of the ith wheat genotype in the lth block, B_l is the lth block effect, and ε_il is the residual. For FHB severity, the area under disease progress curve (AUDPC) was estimated from the adjusted means of the four disease ratings for each experiment. In the second step, the adjusted means were used to calculate the best linear unbiased estimates (BLUEs) following the randomized complete block design option in META-R 6.04 (Alvarado et al., 2015) based on the model:

$$y\_ijm = u + S\_j + G\_ijm + R\_m + \varepsilon\_ijm,$$

where, y_ijm is the BLUE of the ith wheat genotype from the jth source/population in mth replicate, u is the general mean value, S_j is the effect of the jth source of material, G_ijm is effect of the ith wheat genotype in the mth replicate, R_m is the mth replicate effect, and ε_ijm is the residual effect. The source of wheat genotypes, S_j, was treated as the grouping factor.

Genotyping and Genome-Wide Association Studies

The genebank set was genotyped previously using a 20K SNP marker array as described by Odilbekov et al. (2019). While the breeding set was genotyped using the 25K SNP chip by TraitGenetics GmbH, Germany.[1] Markers with ≥20% missing values were removed. The remaining missing values were imputed by setting SNP.impute = "Major" in Genome Association and Integrated Prediction Tool (GAPIT) 3.0 R package (Lipka et al., 2012). After the quality check, 432 lines (breeding set: 272 and genebank set: 160) and 10,328 SNP markers were left for all genome-based analyses.

Seven models were used for the GWAS: general linear model (Pritchard et al., 2000), mixed linear model (Yu et al., 2005), compressed MLM (Zhang et al., 2010), settlement of MLM under progressively exclusive relationship (Wang et al., 2014), multiple locus linear mixed-model (Segura et al., 2012), fixed and random model circulating probability unification (Liu et al., 2016), and Bayesian-information and linkage-disequilibrium iteratively nested keyway (Huang et al., 2018) implemented in R package GAPIT version 3.0 (Lipka et al., 2012). GLM, MLM, CMLM, and SUPER are single locus GWAS models while MLMM, FarmCPU, and Blink are multiple loci GWAS models (described in detail by the respective authors cited above). The kinship (K) and top 5 to 10 principal components (PCs) were used depending on the model and trait, to control familial relatedness and possible population structure following the settings in GAPIT 3.0 (Lipka et al., 2012).

RESULTS

Accelerated Growth With FHB Protocol for Winter Wheat

The protocol for winter wheat using accelerated growth for the evaluation of FHB resistance (AGFHB) consisted of three major growth periods, namely, (a) the pre-accelerated growth period when the plants were allowed to germinate and vernalize under optimal growth conditions; (b) the accelerated growth period when the plant growth was fast-tracked; and (c) the FHB infection period when the plants were grown in conditions optimal for FHB infection and maturity (**Figure 2**). The pre-accelerated growth consisted of germination, vernalization, and acclimatization phases. Germination was promoted for 5 days followed by vernalization for 56 days. Thereafter, to acclimatize the plants for the upcoming stage, the growth conditions were gradually changed over a period of 6 days.

[1]http://www.traitgenetics.com/en/

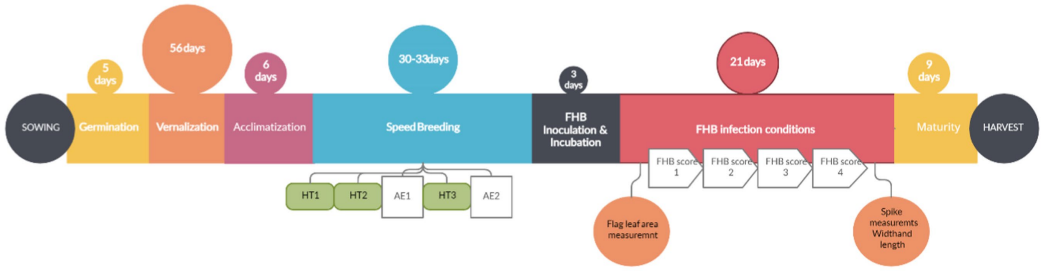


FIGURE 2 | Schematic overview of the AGFHB protocol for FHB evaluation in winter wheat. Time points for the three time points of heading, heading time HT1 to HT3. Anther extrusion times AE1 and AE2. FHB scoring time points FHB score 1 to 4.

During this time, the temperature was gradually increased from 3°C to 22°C, day-length was gradually increased from 8 h to 22 h, and light intensity was increased from 250 to 400 $\mu mol\,m^{-2}\ s^{-1}$ while RH was decreased from 80 to 50%. After that, accelerated growth conditions allowed plants to rapidly reach the reproductive phase within 30–33 days while limiting any visible symptoms of plant stress. At this stage, scoring for heading time, anthesis time, and FLA was performed. Thereafter, FHB infection conditions were introduced to promote FHB infection. Plants were thereafter allowed to mature before harvesting (**Figure 3**). The entire protocol took between 120 and 130 days depending on the genotype.

Evaluation of Agronomic Traits of Germplasm

The AGFHB protocol was used for the evaluation of a total of 519 genotypes consisting of 181 genotypes in the genebank set and 338 genotypes in the breeding set. At the time of the FHB inoculation, 88 and 90% of the plants completed 75% heading of their spikes intended for FHB resistance evaluation in the breeding and genebank sets, respectively. With regard to flowering, 67 and 88% of the plants reached anthesis in the breeding and genebank sets, respectively. As previously stated, genotypes that did not reach the stage of 75% heading at inoculation time were discarded from the following FHB severity scoring together with genotypes that exhibited no visual disease development on the ears.

Best linear unbiased estimates of measured agronomic traits of genebank and breeding sets showed that the mean heading stage was similar in both source populations (**Figure 4A**). The mean FLA of the breeding set was 18.02 mm^2 ($s = 3.87$), while for the genebank set, it was 17.15 mm^2 ($s = 3.50$; **Figure 4B**). Thus, the mean FLA of the genebank set was smaller compared to the breeding set. The mean SL in the genebank set was 76.44 mm ($s = 8.29$), while in the breeding set was 87.82 mm ($s = 9.47$; **Figure 4C**). SL was smaller in the genebank set compared to the breeding set. The mean SW in the genebank set was 11.23 mm ($s = 1.05$), while in the breeding set was 11.10 mm ($s = 1.25$; **Figure 4D**).

FHB Evaluation

Fusarium head blight progression was evaluated at four time points and recorded by visually assessing the percentage of FHB on the main tiller spike of each plant. The BLUEs of the area under the FHB progress curve used for our GWAS showed approximately normal phenotypic distribution with an overall mean of 213.10 ($s = 130.80$). The average AUDPC was 225.13 ($s = 129.98$) for breeding set and 195.53 ($s = 130.44$) for genebank set (**Figure 5**). The correlation between FHB severity (AUDPC) and the five agronomic traits was weak and non-significant in most instances (**Supplementary Figure 1**). The correlation between heading and anthesis was moderate and highly significant ($r = 0.51$, $p < 0.001$). We found highly significant genotypic variances ($p < 0.0001$) and moderate to high broad-sense heritabilities, depending on the trait and the source of genotypes (**Supplementary Table 1**). Broad-sense heritability for FHB based on replication in time and space was 0.55 in the combined set, 0.57 in the genebank set, and 0.53 in the breeding set.

To further evaluate the FHB severity estimates from this work, comparison was done with FHB scores from a previous field trial from 2019 conducted by the breeding company Lantmännen Lantbruk. The FHB scores from the field trial were collected in the scale of 1–8. From the breeding set, 275 genotypes were found to be common in the two datasets. A spearman correlation of 0.24 was observed in the FHB scores between the two datasets. When the genotypes were grouped as resistant (FHB scores 1–3) and susceptible (FHB scores 6–8) a statistically significant difference ($p < 0.0001$) was observed between the two groups for mean FHB estimates obtained under controlled conditions.

Genome-Wide Association Studies

The multi-model GWAS detected 12 significant SNPs associated with nine QTLs for FHB severity ($p \leq 0.0001$) in the combined dataset ($N = 432$). Four QTLs were co-detected by at least two GWAS models ($p \leq 0.0001$, **Table 2**). Three SNPs, wsnp_Ex_c34975_43204180, Kukri_c18009_398 (chromosome, chr. 3B), and RAC875_c12733_1509 (chr. 7A), were detected above the Bonferroni corrected threshold by SUPER and Blink models ($\alpha = 0.05$, **Figure 6**). The SNPs associated with the QTLs on chr. 3B (qtlfhb4) had the largest marker effects (**Table 2**). The majority of the SNPs detected in the combined dataset as well

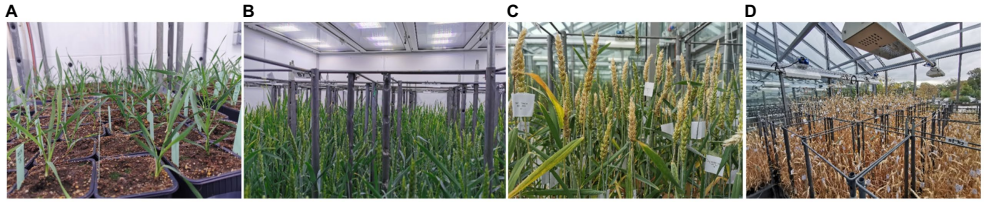


FIGURE 3 | The rapid development of winter wheat plants under accelerated growth conditions. **(A)** first day post-acclimatization (dpa); **(B)** 31 dpa end of accelerated growth; **(C)** winter wheat ears showing FHB symptoms; and **(D)** maturity.

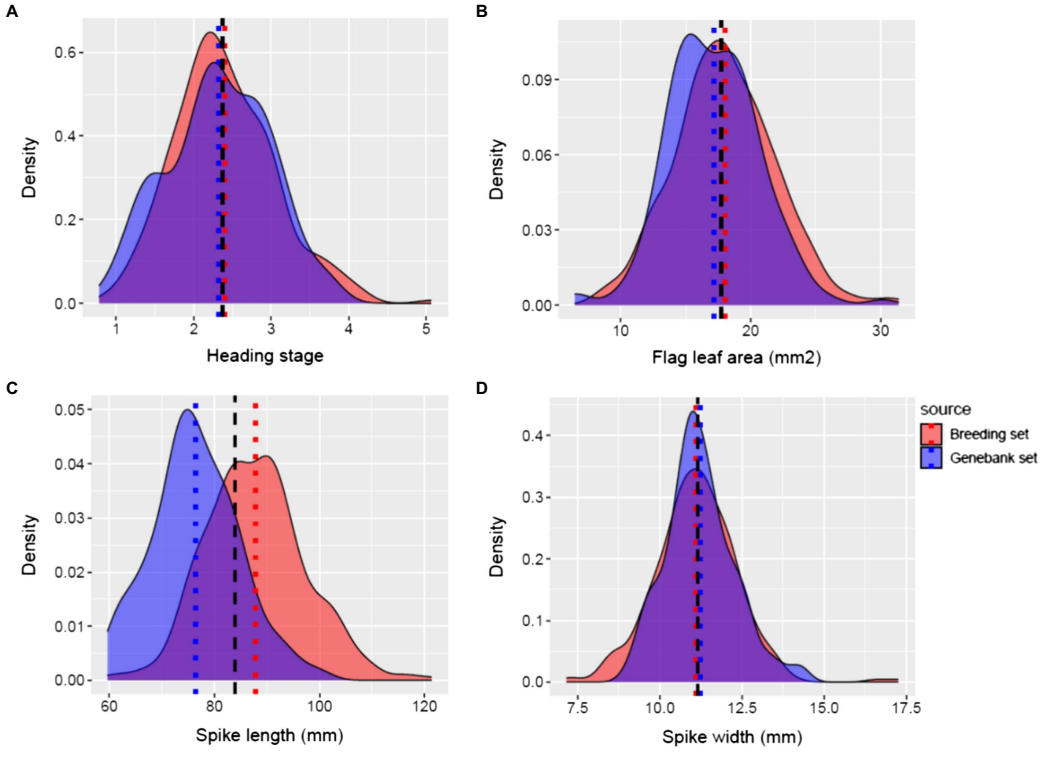


FIGURE 4 | Phenotypic distribution of **(A)** heading stage, **(B)** flag leaf area, **(C)** spike length, and **(D)** spike width. Breeding set (red) and genebank set (blue). The black dashed line represents the overall mean for combined genotypes from both breeding set and genebank set.

as within the breeding set ($N=272$) and genebank set ($N=160$) for the resistance against FHB severity was located on the sub-genome B (**Table 2**; **Figure 6**; **Supplementary Table 2**). Additionally, we found several significant SNPs for the five agronomic traits ($p=0.0001$, **Supplementary Tables 3 and 4**). At least two GWAS models simultaneously detected 21, 5, 3, 14, and 2 markers for heading, anthesis, SW, SL, and FLA, respectively, in the 432 wheat lines (**Supplementary Table 3**). A few SNPs were associated with common QTLs between these traits (**Supplementary Table 3**). Two QTLs on chr. 3B and 6A were common between FHB severity and heading stage (**Table 2; Supplementary Table 3**). At $p=0.0001$, for all traits, we found more QTLs using lines from breeding set and genebank set combined ($N=432$) than for lines from within each source

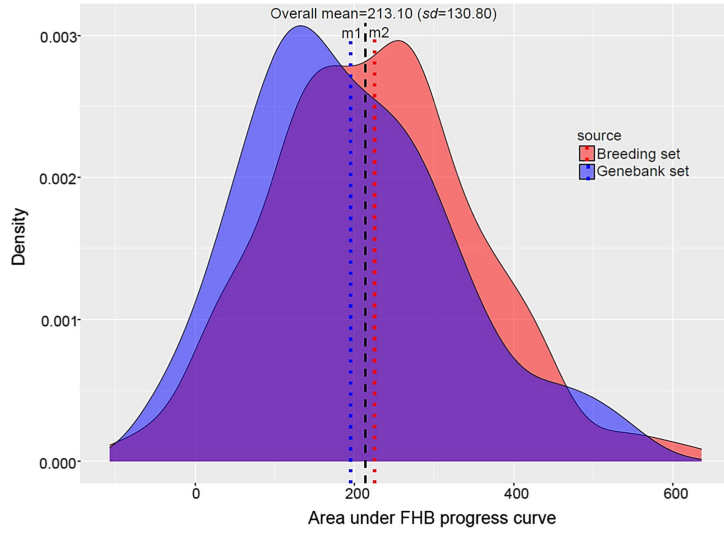


FIGURE 5 | Histogram of the area under disease progress curve (AUDPC) for FHB in the wheat genotypes collected from two sources. m1 and m2 represent the mean AUDPC for FHB in genebank set and breeding set, respectively.

population alone (**Table 2; Supplementary Tables 2–4**; **Figure 6**). As a result, we lowered the significant threshold to $p = 0.001$ [$-\log(P) = 3$] for the GWAS within each source population (**Supplementary Tables 2–4**).

DISCUSSION

Developing and implementing new techniques to accelerate wheat genetic gains are essential to achieve the goal of feeding 10 billion people by 2050. Crop genetic gain for disease resistance can be accelerated by reducing generation time and increasing selection intensity. Increasing the genetic gain will not only contribute to increasing the genetic diversity for resistance but will also enable faster introgressions and selection of resistance genes in wheat. It takes up to 10 years to develop a new winter wheat cultivar; thus, accelerating this process by increasing the number of generations per year can contribute to the genetic gain of wheat when breeding for yield, climate resilience, and biotic and abiotic stresses. SB is a technique that utilizes affordable growing equipment under greenhouse conditions to shorten generation time in plants. This technique was shown to be effective in several crops, including spring wheat, spring barley, chickpea, oat, quinoa, peanut, and amaranth (Watson et al., 2018; Hickey et al., 2019).

In this work, we developed a protocol to integrate accelerated growth with FHB resistance screening, followed by association mapping. Previously, SB was used to introgress resistance to four diseases in barley in a modified backcross strategy and plants were evaluated and selected based on disease resistance under accelerated growth conditions and later in field trials (Hickey et al., 2017). The protocol proposed in this work allows accelerated growth while avoiding any visible stress symptoms on plants, which is necessary to be able to screen for disease resistance. While the plants are grown under accelerated growth conditions until heading, the growth conditions are changed to regular growth conditions prior to inoculation for FHB which allows the plants to stabilize prior to FHB infection. This provides an advantage of reduced time to reach heading while obtaining disease resistance scores based on plants grown under regular growth conditions. It could be postulated though that there are certain molecular responses in plants activated due to the accelerated growth which continues to remain active even after plants receive regular growth conditions during FHB infection. Further research would be required to fully understand and unravel such responses. It was earlier shown that the most resistant wheat line consistently expressed highest resistance for FHB severity and deoxynivalenol under both greenhouse and field conditions (Kang et al., 2011) suggesting that evaluating plants for resistance to FHB under controlled conditions can accelerate resistance breeding for FHB. Previous studies on winter wheat grown under SB conditions reported 105.4 ± 1.7 days are needed to reach flowering of winter wheat (Ghosh et al., 2018). The current protocol shortens the period required from sowing to anthesis of the plants to 97–100 days. Moreover, while FHB resistance is screened for in a large number of genotypes, the whole period required from seed to seed is achieved within a time frame of 120–130 days. The current protocol enables the evaluation of FHB resistance in three consequent

TABLE 2 | Quantitative trait loci (QTLs) detected by seven GWAS models at $p = 0.0001$ (LOD ≥ 4) for FHB severity in winter wheat from combined (CS), breeding (BS), and genebank (GS) sets.

QTL	Marker	Chr.	Position (cM)	FAF	Effect[b]	Model(s)	Set
SLUfhbchr1B.1	BS00021877_51	1B	154.58	0.06	NA	Blink	Combined
SLUfhbchr2A.2	BobWhite_c16923_64	2A	125.33	0.06	NA	Blink; (SUPER)*	Combined
SLUfhbchr3A.3	Kukri_rep_c89183_282	3A	15.05	0.64	27.84 to 28.10	GLM, CMLM	Combined
SLUfhbchr3B.4	wsnp_Ex_c34975_43204180[a]	3B	67.45	0.95(CS), 0.94 (BS), 0.97(GS)	65.78 to 82.47	GLM, MLM, CMLM, SUPER, MLMM, FarmCPU, Blink	Combined, Breeding, Genebank
	Kukri_c18009_398[a]	3B	67.67	0.95	78.20 to 80.15	GLM, MLM, CMLM, SUPER	Combined
	wsnp_Ex_c5378_9505533	3B	68.71	0.94	NA	SUPER	Combined
SLUfhbchr3D.5a	RFL_Contig4591_1759	3D	0.00	0.94	51.94 to 54.69*	MLMM; (GLM, MLM, CLM, SUPER, Blink)*	Combined
	RAC875_rep_c115090_51	3D	0.00	0.02	NA	Blink	Breeding
SLUfhbchr3D.5b	JD_c7714_954	3D	143.01	0.04	NA	Blink, SUPER	Genebank
SLUfhbchr5A.6	RAC875_rep_c106118_339	5A	39.02	0.03	−31.55 to −29.40	GLM, MLM, SUPER, MLMM	Combined
SLUfhbch6A.7	Tdurum_contig46670_911	6A	128.26	0.96	NA	SUPER	Combined
SLUfhbchr7A.8	Kukri_c11530_92	7A	232.11	0.84	44.1	CMLM, SUPER, MLMM	Combined
	RAC875_c12733_1509[a]	7A	228.37	0.83	40.41 to 45.14	GLM, MLM, CMLM, SUPER, MLMM, FarmCPU, Blink	Combined
SLUfhbchr7B.9	wsnp_Ex_c351_689415	7B	143.23	0.02	NA	Blink, SUPER	Breeding
	RAC875_c8752_1079	7B	158.98	0.84	39.97*	SUPER; (CMLM)*	Combined

*Chr., chromosome; FAF, favorable allele frequencies; * also detected by these models at p = 0.0002.*

[a]detected above Bonferroni corrected threshold (α = 0.05).

[b]marker effects are estimated for only GLM, MLM, and CMLM; and FarmCPU in GAPIT (Lipka et al., 2012).

generations of winter wheat per year compared to two generations under regular growth conditions in a greenhouse.

Previous work on comparing measurements taken to evaluate leaf rust resistance in spring wheat grown in controlled environment with continuous light and field conditions showed that the source of variation for the resistance was greatly genotypic (Riaz et al., 2016). The evaluated resistance to leaf rust under continuous light was correlated to that in the field in a panel of diverse cultivars of spring wheat (Riaz et al., 2016). Despite the dissimilarities in terms of growth conditions between the current protocol and field trials, the variations in FHB resistance in winter wheat grown in controlled environment integrating SB are reduced largely to genotypic variations without ignoring the possibilities for physiological disorders, developmental errors, and environmental internal factors of the plants. Hence, when applying the protocol, the phenotypic evaluation results for instance of FHB results are repeatable once the standardized controlled environment of plant growth is met.

Over 500 genotypes from a breeding program and genebank were evaluated using the proposed protocol, and a good phenotypic diversity was observed in the studied germplasm. Moderate to high broad-sense heritability estimates were obtained based on replication in time for heading (0.69–0.79), FHB (0.53–0.57), FLA (0.41–0.53), spike length (0.70–0.77), and spike width (0.44–0.64). In previous studies, the average broad-sense heritabilities for FHB resistance traits were 0.54–0.73 ($sd = 0.15$–0.18) based on field trials (Ma et al., 2020). The heritabilities in this work compared to previously published work indicate that FHB resistance is a moderately to highly heritable trait.

Fusarium head blight resistance is quantitatively inherited and controlled by a plethora of genes (Mesterhazy, 1995; Mesterhazy et al., 1999; Miedaner et al., 2010, 2019; Venske et al., 2019). In this work, the AUDPC showed that both highly resistant and susceptible genotypes were present in Nordic winter wheat. On average, the genebank germplasm was less susceptible to FHB than the breeding lines (**Figure 1**). This can be explained by the presence of some highly resistant germplasm in the genebank collection. Previous studies indicated that genetic resources, such as landraces, might harbor more resistance genes than elite lines (Kidane et al., 2017; Buerstmayr et al., 2020). The genetic variation for FHB resistance in the materials evaluated can be exploited to improve FHB resistance in the Nordic winter wheat.

In addition, we found high genetic variation for the heading stage, anthesis, spike length, spike width, and FLA. Similarly, high genetic variation for heading (Zanke et al., 2014), anthesis (Bogard et al., 2011), spike length (Zhai et al., 2016), and FLA (Liu et al., 2017, 2018) has been reported in winter wheat. These traits are important for agronomic adaptation and can have pleiotropic effects on disease severity, which may delay the use of resistance alleles in commercial cultivars (Gervais et al., 2002; Buerstmayr et al., 2020; Ogrodowicz et al., 2020). However, in this present study, we found very weak correlations between AUDPC (FHB) and all five agronomic traits measured. A high correlation between heading and anthesis is expected (Langer et al., 2014), since wheat ears usually emerge from the flag leaf before anthesis. However, in some cultivars, the ears may not fully emerge from the flag leaf before shedding

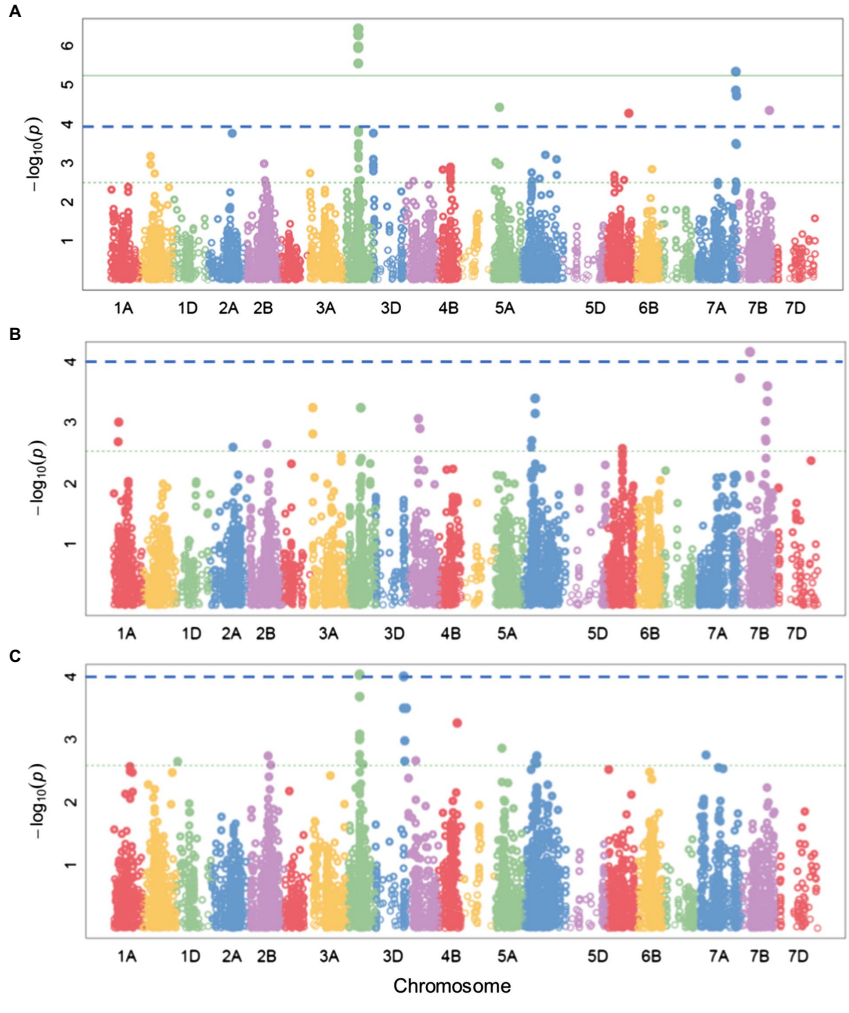


FIGURE 6 | Manhattan plots of AUDPC (FHB severity) identified with SUPER **(A)** combined set; **(B)** breeding set; and **(C)** genebank set. Green continuous and blue dashed horizontal lines represent Bonferroni corrected threshold at $\alpha = 0.05$ and exploratory threshold at $p = 0.0001$, respectively.

pollens. Flag leaf is an important organ that influences yield-related traits, such as spike length, because of its role in photosynthesis and nutrient partitioning. The correlation between FLA and the two spike traits was low, only significant for spike length (**Supplementary Figure 1**). In earlier studies, Liu et al. (2018) also found a significant and positive correlation between spike length and flag leaf length.

Fusarium head blight resistance is quantitative, being controlled by many loci. The significant SNPs detected on chr. 3BS (62.31–68.71 cM) might be associated with a major QTL (SLUfhbchr3B.4) that regulates FHB severity in the material analyzed ($p = 0.0001$, **Table 2; Supplementary Table 2**). Within ±20 cM, SLUfhbchr3B.4 overlapped with QTLs projected into meta-QTL 3/3B and 4/3B in the previous studies (Venske et al., 2019). The high impact *Fhb1* QTL originating from the Chinese spring wheat, Sumai 3, is located on the short arm of chr. 3B between 1 cM and 7 cM (Bai et al., 1999; Waldron et al., 1999; Venske et al., 2019; Ma et al., 2020). At $p = 0.001$, the significant SNPs found between 9 cM and 14 cM on chr. 3B within the breeding set was localized between the *Fhb*1 QTL and meta-QTL 1/3B (16.02–16.84 cM) reported by Venske et al. (2019). Similar to the outcome of this study, previous studies found QTLs for FHB resistance

on the other sub-genomes of bread wheat (Miedaner et al., 2010, 2019; Kollers et al., 2013; Venske et al., 2019). For example, the QTL on chr. 3A (SLUfhbchr3A.3; **Table 2**) colocalized with the meta-QTL1/3A located at 14.01–26.18 cM (Venske et al., 2019). The average effect of the favorable QTL alleles for six SNPs detected by at least two GWAS models simultaneously could reduce FHB severity below the overall mean (**Supplementary Figure 2**; **Table 2**). Since 1999, over 500 QTLs scattered across all wheat sub-genomes and chromosomes have been reported for FHB resistance, the sub-genome B containing the largest number of the QTLs followed by A (Venske et al., 2019). Chromosome 3B can be described as a hot spot for FHB resistance because the majority of the FHB QTLs found in our study and literature was localized on this sub-genome (Liu et al., 2009; Venske et al., 2019; Ma et al., 2020; **Table 1**; **Figure 1**; **Supplementary Table 2**). Colocalization of two QTLs between heading and FHB severity might partly explain the significant negative correlation between FHB and heading ($r = -0.16$, $p = 0.001$). FHB resistance QTLs may be population specific and QTLs with minor effects control FHB resistance and are difficult to detect in smaller populations. In this study, the presence of common FHB resistance QTL regions in both breeding and genebank sets increased the power to detect more QTLs in the combined set and even at a higher significance threshold (e.g., Bonferroni corrected threshold at $\alpha = 0.05$; **Figure 6**). Thus, higher gains should be expected from MAS for FHB resistance in wheat breeding programs when lines from both breeding and genebank materials are used.

The genetic architecture of heading, anthesis, SW, SL, and FLA is complex, being influenced by several QTLs (**Supplementary Tables 3 and 4**). Similar to our results, Langer et al. (2014) and Zanke et al. (2014) found many QTLs for heading time, majority was located on chromosome 5B. Also, QTLs were reported for anthesis (Bogard et al., 2011), spike characteristics (Zhou et al., 2017), and FLA (Liu et al., 2018). The presence of QTLs in similar genomic regions might explain the positive and moderate phenotypic correlations observed between the heading stage and anthesis ($r = 0.51$, $p < 0.001$) as well as FLA and SL ($r = 0.23$, $p = 0.001$).

In GWAS, large population sizes are required to detect QTLs with small effects and to reduce the Beavis effect (Beavis and Paterson, 1998; Xu, 2003). Consequently, at $p = 0.0001$, we found more QTLs for GWAS incorporating lines from both genebank and breeding sets than GWAS within each source population separately. However, within genebank set or breeding set, several QTLs could be detected at a lower significant threshold (e.g., $p = 0.001$), only a few were present at $p < 0.0001$, depending on the trait (**Supplementary Tables 2 and 4**). For example, the FHB QTLs on chr. 3B (SLUfhbchr3B.4) and 3D (SLUfhbchr3D.5a and SLUfhbchr3D.5b) in breeding set and genebank set (**Supplementary Table 2**). The results found for analyses within individual sets showed that both common QTLs and partially different QTLs might regulate FHB resistance in the two populations. The presence of some common resistance QTLs in both breeding and genebank sets might have increased the power to detect more QTLs in the combined set and even at a higher significance threshold (e.g., Bonferroni corrected threshold at $\alpha = 0.05$; **Figure 6**). Higher gains should be expected from MAS for FHB resistance breeding when lines from both breeding and genebank populations are used. A strategy to incorporate QTL from the genebank set to the breeding set will lead to improved resistance to FHB in the germplasm of the breeding program.

CONCLUSION

Speeding up of the generation cycle was achieved by integrating SB protocol in diverse winter wheat genotypes used in the improvement for Nordic winter wheat cultivars. Within this work frame, screening for disease resistance among the genotypes for FHB was evaluated in the assigned Nordic germplasm. A significant genetic variation could be found for FHB resistance and agronomic traits in Nordic wheat germplasm. The molecular mechanism of FHB resistance is very complex, governed by multiple loci. Resistant alleles were present in both LM and NG materials and can be harnessed to improve FHB resistance in winter wheat by genomics-assisted speed breeding.

Due to the prolonged nature of winter wheat growth requiring vernalization at every generation, conventional breeding programs have the potential to release new cultivars in 15 years. Taking into account the period required for vernalization, the current protocol for disease resistance in wheat provides the potential for reducing the growth by 55 to 110 days per generation. Therefore, a significant time saving up to 2–3 years can be expected in trait introgression breeding programs using several generations of backcrossing and 1 year in conventional SSD programs.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

AC and TH conceived the study. AC, MZ, and FO planned the greenhouse experiments. TH developed the breeding population set. MZ, FL, MA, and FO performed the greenhouse experiments. MZ and DG analyzed the data and wrote the first draft. All authors contributed to the data interpretation and approved the final version of this manuscript.

FUNDING

This study was supported by funding from the SLU Grogrund (SLU.ltv.2019.1.1.1-623).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2021.705006/full#supplementary-material

REFERENCES

Ali, M., and Vocke, G. (2009). Consequences of higher input costs and wheat prices for U S wheat producers. Electronic Outlook Report from the Economic Research Service (WHS-09c-01), 11.

Alvarado, G., López, M., Vargas, M., Pacheco, Á., Rodríguez, F., Burgueño, J., et al. (2015). META-R (Multi Environment Trail Analysis with R for Windows) Version 6.04.

Bai, G., Kolb, F. L., Shaner, G., and Domier, L. L. (1999). Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. *Phytopathology* 89, 343–348. doi: 10.1094/PHYTO.1999.89.4.343

Beavis, W. D., and Paterson, A. (1998). "QTL analyses: power, precision, and accuracy," in *Molecular Dissection of Complex Traits*. ed. Andrew H. Paterson (Florida, USA: CRC Press), 145–162.

Berthiller, F., Crews, C., Dall'Asta, C., De Saeger, S., Haesaert, G., Karlovsky, P., et al. (2013). Masked mycotoxins: a review. *Mol. Nutr. Food Res.* 57, 165–186. doi: 10.1002/mnfr.201100764

Bogard, M., Jourdan, M., Allard, V., Martre, P., Perretant, M. R., Ravel, C., et al. (2011). Anthesis date mainly explained correlations between post-anthesis leaf senescence, grain yield, and grain protein concentration in a winter wheat population segregating for flowering time QTLs. *J. Exp. Bot.* 62, 3621–3636. doi: 10.1093/jxb/err061

Buerstmayr, H., Ban, T., and Anderson, J. A. (2009). QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: a review. *Plant Breed.* 128, 1–26. doi: 10.1111/j.1439-0523.2008.01550.x

Buerstmayr, M., Steiner, B., and Buerstmayr, H. (2020). Breeding for Fusarium head blight resistance in wheat-progress and challenges. *Plant Breed.* 139, 429–454. doi: 10.1111/pbr.12797

Chawade, A., Armoniené, R., Berg, G., Brazauskas, G., Frostgård, G., Geleta, M., et al. (2018). A transnational and holistic breeding approach is needed for sustainable wheat production in the Baltic Sea region. *Physiol. Plant.* 164, 442–451. doi: 10.1111/ppl.12726

Cobb, J. N., Juma, R. U., Biswas, P. S., Arbelaez, J. D., Rutkoski, J., Atlin, G., et al. (2019). Enhancing the rate of genetic gain in public-sector plant breeding programs: lessons from the breeder's equation. *Theor. Appl. Genet.* 132, 627–645. doi: 10.1007/s00122-019-03317-0

De Mendiburu, F. (2014). Agricolae: statistical procedures for agricultural research. R package version 1(1).

European Union (2020). Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (Text with EEA relevance). Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02006R1881-20201014 (Accessed February 28, 2021).

Fabre, F., Rocher, F., Alouane, T., Langin, T., and Bonhomme, L. (2020). Searching for FHB resistances in bread wheat: susceptibility at the crossroad. *Front. Plant Sci.* 11:731. doi: 10.3389/fpls.2020.00731

FAOSTAT (2020). F.a.A.O.o.t.U.N. Available at: http://www.fao.org/faostat/en/#data/QC (Accessed February 28, 2021).

Ferrie, A. M. R., and Polowick, P. L. (2020). "Acceleration of the breeding program for winter wheat," in *Accelerated Plant Breeding, Volume 1: Cereal Crops*. eds. S. S. Gosal and S. H. Wani (Cham: Springer International Publishing), 191–215.

Gervais, L., Dedryver, F., Morlais, J. Y., Bodusseau, V., Negre, S., Bilous, M., et al. (2002). Mapping of quantitative trait loci for field resistance to Fusarium head blight in an European winter wheat. *Theor. Appl. Genet.* 106, 961–970. doi: 10.1007/s00122-002-1160-5

Ghosh, S., Watson, A., Gonzalez-Navarro, O. E., Ramirez-Gonzalez, R. H., Yanes, L., Mendoza-Suárez, M., et al. (2018). Speed breeding in growth chambers and glasshouses for crop breeding and model plant research. *Nat. Protoc.* 13, 2944–2963. doi: 10.1038/s41596-018-0072-z

Gong, X., He, X. Y., Zhang, Y. H., Li, L., Sun, Z. X., Bai, G. H., et al. (2020). Development of an evaluation system for Fusarium resistance in wheat grains and its application in assessment of the corresponding effects of Fhb1. *Plant Dis.* 104, 2210–2216. doi: 10.1094/PDIS-12-19-2584-RE

Hall, A. J., and Richards, R. A. (2013). Prognosis for genetic improvement of yield potential and water-limited yield of major grain crops. *Field Crop Res.* 143, 18–33. doi: 10.1016/j.fcr.2012.05.014

Hao, Y. F., Rasheed, A., Zhu, Z. W., Wulff, B. B. H., and He, Z. H. (2020). Harnessing wheat Fhb1 for Fusarium resistance. *Trends Plant Sci.* 25, 1–3. doi: 10.1016/j.tplants.2019.10.006

Hickey, L. T., Germán, S. E., Pereyra, S. A., Diaz, J. E., Ziems, L. A., Fowler, R. A., et al. (2017). Speed breeding for multiple disease resistance in barley. *Euphytica* 213:64. doi: 10.1007/s10681-016-1803-2

Hickey, L. T., Hafeez, N. A., Robinson, H., Jackson, S. A., Leal-Bertioli, S. C. M., Tester, M., et al. (2019). Breeding crops to feed 10 billion. *Nat. Biotechnol.* 37, 744–754. doi: 10.1038/s41587-019-0152-9

Hu, W., Gao, D., Wu, H., Liu, J., Zhang, C., Wang, J., et al. (2020). Genome-wide association mapping revealed syntenic loci QFhb-4AL and QFhb-5DL for Fusarium head blight resistance in common wheat (*Triticum aestivum* L.). *BMC Plant Biol.* 20:29. doi: 10.1186/s12870-019-2177-0

Huang, M., Liu, X., Zhou, Y., Summers, R. M., and Zhang, Z. (2018). BLINK: a package for the next level of genome-wide association studies with both individuals and markers in the millions. *GigaScience* 8:giy154. doi: 10.1093/gigascience/giy154

Kang, J., Clark, A., Van Sanford, D., Griffey, C., Brown-Guedira, G., Dong, Y., et al. (2011). Exotic scab resistance quantitative trait loci effects on soft red winter wheat. *Crop Sci.* 51, 924–933. doi: 10.2135/cropsci2010.06.0313

Kidane, Y. G., Hailemariam, B. N., Mengistu, D. K., Fadda, C., Pè, M. E., and Dell'Acqua, M. (2017). Genome-wide association study of *Septoria tritici* blotch resistance in Ethiopian durum wheat landraces. *Front. Plant Sci.* 8:1586. doi: 10.3389/fpls.2017.01586

Kollers, S., Rodemann, B., Ling, J., Korzun, V., Ebmeyer, E., Argillier, O., et al. (2013). Whole genome association mapping of Fusarium head blight resistance in European winter wheat (*Triticum aestivum* L.). *PLoS One* 8:e57500. doi: 10.1371/journal.pone.0057500

Kumar, J., Rai, K. M., Pirseyedi, S., Elias, E. M., Xu, S., Dill-Macky, R., et al. (2020). Epigenetic regulation of gene expression improves Fusarium head blight resistance in durum wheat. *Sci. Rep.* 10:17610. doi: 10.1038/s41598-020-73521-2

Langer, S. M., Longin, C. F. H., and Würschum, T. (2014). Flowering time control in European winter wheat. *Front. Plant Sci.* 5:537. doi: 10.3389/fpls.2014.00537

Leslie, J., and Summerell, B. (2006). *Fusarium Laboratory Manual*. Oxford, U.K.: Blackwell Publishing, doi: 10.1002/9780470278376

Li, G. Q., Zhou, J. Y., Jia, H. Y., Gao, Z. X., Fan, M., Luo, Y. J., et al. (2019). Mutation of a histidine-rich calcium-binding-protein gene in wheat confers resistance to Fusarium head blight. *Nat. Genet.* 51, 1106–1112. doi: 10.1038/s41588-019-0426-7

Lipka, A. E., Tian, F., Wang, Q., Peiffer, J., Li, M., Bradbury, P. J., et al. (2012). GAPIT: genome association and prediction integrated tool. *Bioinformatics* 28, 2397–2399. doi: 10.1093/bioinformatics/bts444

Liu, S. X., and Anderson, J. A. (2003). Marker assisted evaluation of Fusarium head blight resistant wheat germplasm. *Crop Sci.* 43, 760–766. doi: 10.2135/cropsci2003.7600

Liu, S., Hall, M. D., Griffey, C. A., and McKendry, A. L. (2009). Meta-analysis of QTL associated with Fusarium head blight resistance in wheat. *Crop Sci.* 49, 1955–1968. doi: 10.2135/cropsci2009.03.0115

Liu, X., Huang, M., Fan, B., Buckler, E. S., and Zhang, Z. (2016). Iterative usage of fixed and random effect models for powerful and efficient genome-wide association studies. *PLoS Genet.* 12:e1005767. doi: 10.1371/journal.pgen.1005767

Liu, Y., Tao, Y., Wang, Z., Guo, Q., Wu, F., Yang, X., et al. (2017). Identification of QTL for flag leaf length in common wheat and their pleiotropic effects. *Mol. Breed.* 38:11. doi: 10.1007/s11032-017-0766-x

Liu, K., Xu, H., Liu, G., Guan, P., Zhou, X., Peng, H., et al. (2018). QTL mapping of flag leaf-related traits in wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 131, 839–849. doi: 10.1007/s00122-017-3040-z

Ma, Z., Xie, Q., Li, G., Jia, H., Zhou, J., Kong, Z., et al. (2020). Germplasms, genetics and genomics for better control of disastrous wheat Fusarium head blight. *Theor. Appl. Genet.* 133, 1541–1568. doi: 10.1007/s00122-019-03525-8

Mesterhazy, A. (1995). Types and components of resistance to Fusarium head blight of wheat. *Plant Breed.* 114, 377–386. doi: 10.1111/j.1439-0523.1995.tb00816.x

Mesterhazy, A., Bartok, T., Mirocha, C. G., and Komoroczy, R. (1999). Nature of wheat resistance to Fusarium head blight and the role of deoxynivalenol

for breeding. *Plant Breed.* 118, 97–110. doi: 10.1046/j.1439-0523.1999.118002097.x

Miedaner, T., Akel, W., Flath, K., Jacobi, A., Taylor, M., Longin, F., et al. (2019). Molecular tracking of multiple disease resistance in a winter wheat diversity panel. *Theor. Appl. Genet.* 133, 419–431. doi: 10.1007/s00122-019-03472-4

Miedaner, T., Würschum, T., Maurer, H. P., Korzun, V., Ebmeyer, E., and Reif, J. C. (2010). Association mapping for Fusarium head blight resistance in European soft winter wheat. *Mol. Breed.* 28, 647–655. doi: 10.1007/s11032-010-9516-z

Nakagawa, H., He, X. Y., Matsuo, Y., Singh, P. K., and Kushiro, M. (2017). Analysis of the masked metabolite of deoxynivalenol and fusarium resistance in CIMMYT wheat germplasm. *Toxins* 9:238. doi: 10.3390/toxins9080238

Odilbekov, F., Armonieńe, R., Koc, A., Svensson, J., and Chawade, A. (2019). GWAS-assisted genomic prediction to predict resistance to *Septoria tritici* blotch in Nordic winter wheat at seedling stage. *Front. Genet.* 10:1224. doi: 10.3389/fgene.2019.01224

Ogrodowicz, P., Kuczynska, A., Mikolajczak, K., Adamski, T., Surma, M., Krajewski, P., et al. (2020). Mapping of quantitative trait loci for traits linked to Fusarium head blight in barley. *PLoS One* 15:e0222375. doi: 10.1371/journal.pone.0222375

Olesen, J. E., and Bindi, M. (2002). Consequences of climate change for European agricultural productivity, land use and policy. *Eur. J. Agron.* 16, 239–262. doi: 10.1016/S1161-0301(02)00004-7

Ollier, M., Talle, V., Brisset, A.-L., Le Bihan, Z., Duerr, S., Lemmens, M., et al. (2020). QTL mapping and successful introgression of the spring wheat-derived QTL Fhb1 for Fusarium head blight resistance in three European triticale populations. *Theor. Appl. Genet.* 133, 457–477. doi: 10.1007/s00122-019-03476-0

Pritchard, J. K., Stephens, M., and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* 155, 945–959. doi: 10.1093/genetics/155.2.945

Rawat, N., Pumphrey, M. O., Liu, S. X., Zhang, X. F., Tiwari, V. K., Ando, K., et al. (2016). Wheat Fhb1 encodes a chimeric lectin with agglutinin domains and a pore-forming toxin-like domain conferring resistance to Fusarium head blight. *Nat. Genet.* 48, 1576–1580. doi: 10.1038/ng.3706

Ray, D. K., Mueller, N. D., West, P. C., and Foley, J. A. (2013). Yield trends are insufficient to double global crop production by 2050. *PLoS One* 8:e66428. doi: 10.1371/journal.pone.0066428

Ray, D. K., Ramankutty, N., Mueller, N. D., West, P. C., and Foley, J. A. (2012). Recent patterns of crop yield growth and stagnation. *Nat. Commun.* 3:1293. doi: 10.1038/ncomms2296

Riaz, A., Periyannan, S., Aitken, E., and Hickey, L. (2016). A rapid phenotyping method for adult plant resistance to leaf rust in wheat. *Plant Methods* 12:17. doi: 10.1186/s13007-016-0117-7

Schmolke, M., Zimmermann, G., Schweizer, G., Miedaner, T., Korzun, V., Ebmeyer, E., et al. (2008). Molecular mapping of quantitative trait loci for field resistance to Fusarium head blight in a European winter wheat population. *Plant Breed.* 127, 459–464. doi: 10.1111/j.1439-0523.2007.01486.x

Schweiger, W., Steiner, B., Vautrin, S., Nussbaumer, T., Siegwart, G., Zamini, M., et al. (2016). Suppressed recombination and unique candidate genes in the divergent haplotype encoding Fhb1, a major Fusarium head blight resistance locus in wheat. *Theor. Appl. Genet.* 129, 1607–1623. doi: 10.1007/s00122-016-2727-x

Searchinger, T., Waite, R., Hanson, C., Ranganathan, J., Dumas, P., Matthews, E., et al. (2019). Creating a sustainable food future: a menu of solutions to feed nearly 10 billion people by 2050. Final report. WRI.

Segura, V., Vilhjálmsson, B. J., Platt, A., Korte, A., Seren, Ü., Long, Q., et al. (2012). An efficient multi-locus mixed-model approach for genome-wide association studies in structured populations. *Nat. Genet.* 44, 825–830. doi: 10.1038/ng.2314

Stack, R. W., and McMullen, M. P. (1998). *A Visual Scale to Estimate Severity of Fusarium Head Blight in Wheat*. USA: NDSU Extension Service.

Steiner, B., Buerstmayr, M., Michel, S., Schweiger, W., Lemmens, M., and Buerstmayr, H. (2017). Breeding strategies and advances in line selection for Fusarium head blight resistance in wheat. *Trop. Plant Pathol.* 42, 165–174. doi: 10.1007/s40858-017-0127-7

Su, Z., Bernardo, A., Tian, B., Chen, H., Wang, S., Ma, H., et al. (2019). A deletion mutation in TaHRC confers Fhb1 resistance to Fusarium head blight in wheat. *Nat. Genet.* 51, 1099–1105. doi: 10.1038/s41588-019-0425-8

Sysoeva, M. I., Markovskaya, E. F., and Shibaeva, T. G. (2010). Plants under continuous light: a review. *Plant Stress* 4, 5–17.

Venske, E., dos Santos, R. S., Farias, D. D., Rother, V., da Maia, L. C., Pegoraro, C., et al. (2019). Meta-analysis of the QTLome of Fusarium head blight resistance in bread wheat: refining the current puzzle. *Front. Plant Sci.* 10:727. doi: 10.3389/fpls.2019.00727

Voss-Fels, K. P., Herzog, E., Dreisigacker, S., Sukumaran, S., Watson, A., Frisch, M., et al. (2019). ""SpeedGS" to accelerate genetic gain in spring wheat," in *Applications of Genetic and Genomic Research in Cereals* (Elsevier), 303–327.

Waldron, B. L., Moreno-Sevilla, B., Anderson, J. A., Stack, R. W., and Frohberg, R. C. (1999). RFLP mapping of QTL for Fusarium head blight resistance in wheat. *Crop Sci.* 39, 805–811. doi: 10.2135/cropsci1999.0011183X003900030032x

Wang, R., Chen, J., Anderson, J. A., Zhang, J., Zhao, W., Wheeler, J., et al. (2017). Genome-wide association mapping of Fusarium head blight resistance in spring wheat lines developed in the Pacific northwest and CIMMYT. *Phytopathology* 107, 1486–1495. doi: 10.1094/PHYTO-02-17-0073-R

Wang, Q. S., Tian, F., Pan, Y. C., Buckler, E. S., and Zhang, Z. W. (2014). A SUPER powerful method for genome wide association study. *PLoS One* 9:e107684. doi: 10.1371/journal.pone.0107684

Watson, A., Ghosh, S., Williams, M. J., Cuddy, W. S., Simmonds, J., Rey, M. D., et al. (2018). Speed breeding is a powerful tool to accelerate crop research and breeding. *Nat. Plants* 4, 23–29. doi: 10.1038/s41477-017-0083-8

Xu, S. (2003). Theoretical basis of the Beavis effect. *Genetics* 165, 2259–2268. doi: 10.1093/genetics/165.4.2259

Yu, J., Pressoir, G., Briggs, W. H., Vroh Bi, I., Yamasaki, M., Doebley, J. F., et al. (2005). A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nat. Genet.* 38, 203–208. doi: 10.1038/ng1702

Zanke, C., Ling, J., Plieske, J., Kollers, S., Ebmeyer, E., Korzun, V., et al. (2014). Genetic architecture of main effect QTL for heading date in European winter wheat. *Front. Plant Sci.* 5:217. doi: 10.3389/fpls.2014.00217

Zhai, H., Feng, Z., Li, J., Liu, X., Xiao, S., Ni, Z., et al. (2016). QTL analysis of spike morphological traits and plant height in winter wheat (*Triticum aestivum* L.) using a high-density SNP and SSR-based linkage map. *Front. Plant Sci.* 7:1617. doi: 10.3389/fpls.2016.01617

Zhang, Z., Ersoz, E., Lai, C.-Q., Todhunter, R. J., Tiwari, H. K., Gore, M. A., et al. (2010). Mixed linear model approach adapted for genome-wide association studies. *Nat. Genet.* 42, 355–360. doi: 10.1038/ng.546

Zhou, Y., Conway, B., Miller, D., Marshall, D., Cooper, A., Murphy, P., et al. (2017). Quantitative trait loci mapping for spike characteristics in hexaploid wheat. *Plant Genome* 10, 1–15. doi: 10.3835/plantgenome2016.10.0101

Zhu, Z. W., Chen, L., Zhang, W., Yang, L. J., Zhu, W. W., Li, J. H., et al. (2020). Genome-wide association analysis of Fusarium head blight resistance in Chinese elite wheat lines. *Front. Plant Sci.* 11:206. doi: 10.3389/fpls.2020.00206

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary Table 1 Broad-sense heritability (H^2), genetic variance, coefficient of variation and grand mean of Fusarium head blight (FHB) severity and five agronomic traits in wheat

Statistic	AUDPCFHB	Heading stage	Anthesis	Spike length	Spike width	FLA
Lantmannen						
H^2	0.53	0.69	0.67	0.77	0.44	0.41
Genotype Variance	7129.3	0.26	0.25	66.94	0.64	5.66
Residual Variance	24952.84	0.45	0.48	79.37	3.23	32.77
Grand Mean	225.13	2.39	2.09	87.82	11.1	18.02
CV	70.17	28.08	33.32	10.14	16.18	31.78
Genotype significance	***	***	***	***	***	***
Nordgen						
H^2	0.57	0.79	0.41	0.7	0.64	0.53
Genotype Variance	8424.66	0.34	0.08	47.48	0.7	5.97
Residual Variance	25817.1	0.37	0.47	81.4	1.54	21.12
Grand Mean	195.53	2.32	1.62	76.44	11.23	17.15
CV	82.17	26.14	42.22	11.8	11.05	26.79
Genotype significance	***	***	***	***	***	***

AUDPC, area under disease progress curve; FLA, flag leaf area, ***significant at P<0.0001; CV, coefficient of variation

Supplementary Table 2: QTLs detected by Blink and SUPER for FHB severity within breeding and genebank wheat materials at P<0.001 (-log(P)=3)

QTL	SNP	Chromosome	Position (cM)	Minor allele frequency	Model
Breeding set (N=272)					
	D_GBUVHFX01API9H_416	1B	102.92	0.14	Blink, SUPER
	BS00023068_51	2B	20.61	0.37	Blink, SUPER
	BS00081231_51	2B	109.24	0.18	SUPER
	BobWhite_c41676_137	2B	109.53	0.36	SUPER
	Tdurum_contig12008_803	3B	9.7	0.16	Blink, SUPER
	wsnp_Ex_rep_c66331_64502558	3B	11.56	0.17	Blink
	wsnp_Ex_c2723_5047696	3B	13.79	0.37	SUPER
SLUfhbchr3B.4	wsnp_Ex_c34975_43204180*	3B	67.45	0.05	Blink, SUPER
	Kukri_c18009_398	3B	67.67	0.05	Blink, SUPER
SLUfhbchr3D.5a	RAC875_rep_c115090_51*	3D	0	0.19	Blink, SUPER
	BS00021873_51	5A	38.74	0.43	Blink, SUPER
	RFL_Contig5616_1779	5B	183.93	0.39	SUPER
	Kukri_rep_c104648_439	6A	54.65	0.06	Blink, SUPER
	Kukri_rep_c104648_106	6A	54.91	0.05	Blink, SUPER
	wsnp_Ex_c351_689415*	7B	143.23	0.32	SUPER
Genebank set (N=160)					
SLUfhbchr3B.4	Tdurum_contig43263_243[a]	3B	62.31	0.05	Blink, SUPER
	BS00025114_51	3B	62.57	0.04	SUPER
	BS00022512_51	3B	80.13	0.37	Blink
SLUfhbchr3D.5b	JD_c7714_954*	3D	143.01	0.04	Blink, SUPER
	BobWhite_c332_340	3D	156.06	0.03	SUPER

	Kukri_rep_c78644_408	4B	95.64	0.33	SUPER
	RAC875_c34971_137	7A	50.49	0.43	Blink
	Kukri_c66626_288	7A	97	0.35	Blink

*detected at $P<0.0001$ (-log10(P)>4), ᵃdetected at Bonferroni threshold at 0.05
Naming of QTLs detected at $P\leq0.0001$ continued from combined data set (Table 1)

Supplementary Table 3: Significant SNPs detected by seven GWAS models at $P<0.0001$ (-log10(P)>4) for heading stage, anthesis, spike width, spike length and flag area in 432 wheat lines from both breeding and genebank set

QTL	SNP	Chromosome	Position (cM)	Minor allele frequency	Effect	Model(s)
HEADING STAGE						
SLUhschr1A.1	wsnp_Ex_rep_c81556_76277906	1A	111.55	0.09	0.14	FarmCPU
SLUhschr1B.2	RAC875_c400_1363	1B	60.62	0.03	-0.32	GLM, SUPER
SLUhschr1B.3	BobWhite_c14304_687	1B	85.57	0.17	-0.15	GLM,SUPER
SLUhschr1B.4	Kukri_c18109_331	1B	110.16	0.03	NA	SUPER
SLUhschr1D.5	CAP7_c9557_164	1D	105.88	0.04	NA	SUPER
SLUhschr2A.6	wsnp_Ku_c16358_25225060	2A	120.18	0.14	0.16	GLM, FarmCPU, Blink
SLUhschr2B.7	RAC875_rep_c72435_90	2B	88.44	0.22	0.19	FarmCPU, Blink
SLUhschr2B.8	RAC875_c29937_325	2B	114.91	0.22	NA	SUPER
SLUhschr3A.9	wsnp_JD_c2722_3653988	3A	35.55	0.06	-0.24	GLM, SUPER
SLUhschr3A.10	Tdurum_contig22253_104	3A	86.93	0.33	-0.19	FarmCPU, Blink
SLUhschr3A.11	Kukri_rep_c69970_717	3A	110.55	0.37	0.16	CMLM
SLUhschr3A.12	BS00097939_51	3A	173.15	0.16	-0.16	GLM,CMLM
SLUhschr3B.13	Ku_c27771_508	3B	66.78	0.05	-0.23 to -0.32	GLM,SUPER, FarmCPU, Blink
"	Kukri_c30535_58	3B	62.31	0.12	-0.17	GLM, SUPER
SLUhschr4A.14	Ex_c17894_1159	4A	48.52	0.04	-0.47	GLM,SUPER
SLUhschr4B.15	Tdurum_contig76213_958	4B	38.07	0.04	-0.30	GLM,SUPER
SLUhschr5A.16	Tdurum_contig10128_593	5A	41.88	0.09	NA	SUPER
"	wsnp_Ex_c2718_5038582	5A	43.38	0.09	NA	SUPER
"	wsnp_CAP11_c2100_1109583	5A	53.47	0.06	NA	SUPER
SLUhschr5A.17	JD_c5000_410	5A	67	0.27	-0.12	GLM, FarmCPU, Blink
"	Kukri_c57965_109	5A	70.3	0.41	-0.16	GLM,CMLM
SLUhschr5A.18	BobWhite_c11539_336	5A	148.3	0.31	-0.13	CMLM
SLUhschr5B.19	wsnp_Ex_c831_1625061	5B	11.23	0.12	-0.16	SUPER, FarmCPU
SLUhschr5B.20	Excalibur_c4031_1227	5B	68.36	0.43	-0.15	GLM
"	IAAV6818	5B	69.19	0.42	-0.16	GLM,CMLM
"	wsnp_JD_c9613_10432955	5B	71.12	0.43	-0.14	GLM
"	RFL_Contig5739_1542	5B	71.24	0.06	0.29	CMLM, FarmCPU
"	Kukri_c23752_659	5B	71.64	0.38	-0.14	GLM
SLUhschr5B.21	wsnp_Ku_c25613_35580381	5B	128.64	0.08	-0.23	MLM, SUPER, MLMM
SLUhschr6A.22	Tdurum_contig44853_1083	6A	3.79	0.40	0.14	GLM,SUPER
"	BS00083630_51	6A	13.45	0.15	NA	SUPER
SLUhschr6A.23	RAC875_c28637_1004	6A	71.24	0.48	NA	Blink
"	IACX5813	6A	85.07	0.08	NA	SUPER

SLUhschr6A.24	wsnp_BE403154A_Ta_2_9	6A	100.12	0.20	NA	SUPER
"	Tdurum_contig46670_911	6A	128.26	0.03	-0.23	FarmCPU
SLUhschr6B.25	Kukri_c3292_670	6B	76.2	0.04	-0.23 to -0.33	GLM,SUPER, FarmCPU
SLUhschr7B.26	Kukri_c52496_434	7B	86.39	0.13	-0.17	CMLM
"	Excalibur_c5700_244	7B	86.68	0.14	-0.17	CMLM
SLUhschr7B.27	BS00101364_51	7B	120.11	0.31	0.14	GLM
"	wsnp_Ex_c56425_58548596	7B	120.42	0.33	0.16	GLM,SUPER
"	RAC875_c41903_122	7B	121.6	0.09	-0.19	GLM,SUPER

ANTHESIS

SLUanthchr1A.1	IACX5994	1A	105.74	0.34	0.16	GLM,SUPER, FarmCPU, Blink
"	Ku_c8992_405	1A	106.63	0.33		SUPER
SLUanthchr2B.2	Ex_c55735_1012	2B	105.91	0.35	NA	Blink
SLUanthchr4A.3	BS00107069_51	4A	61.91	0.26	-0.14	GLM, SUPER, Blink
SLUanthchr4A.4	RAC875_c40654_206	4A	120.11	0.49		SUPER
SLUanthchr4D.5	IAAV6015	4D	86.08	0.06	-0.23	GLM, Blink
SLUanthchr5A.6	Kukri_c57965_109	5A	70.3	0.41	NA	Blink
SLUanthchr5B.7	Kukri_c48575_470	5B	176.61	0.16	NA	Blink
SLUanthchr6B.8	RAC875_c6649_642	6B	75.75	0.17	NA	Blink
SLUanthchr7A.9	Tdurum_contig54832_139	7A	201.78	0.30	-0.13	GLM, SUPER
SLUanthchr7B.10	RFL_Contig5898_807	7B	143.23	0.14	0.15	GLM, Blink
"	Kukri_rep_c79716_389	7B	150.6	0.23		SUPER

SPIKE LENGTH

SLUsplchr1A.1	Kukri_c27785_400	1A	79.46	0.05	-5.10 to '-3.10	GLM,MLM,CMLM, SUPER, MLMM, FarmCPU, Blink
SLUsplchr1D.2	RFL_Contig1338_2062	1D	32.98	0.33		Blink
SLUsplchr1D.3	Kukri_c44738_477	1D	67.72	0.16		Blink
SLUsplchr2A.4	BS00066186_51	2A	82.77	0.39	-1.82 to '-1.34	GLM,CMLM, SUPER, FarmCPU
SLUsplchr2A.5	RFL_Contig5277_888	2A	177.65	0.08	NA	SUPER
SLUsplchr2B.6	Ra_c105904_1191	2B	159.66	0.36	´ -2.6, '-0.30	GLM, SUPER, FarmCPU
SLUsplchr3A.7	Kukri_c54729_181	3A	85.39	0.16	2.79	GLM, CMLM, SUPER, Blink
"	Tdurum_contig22253_104	3A	86.93	0.33	-3.79 to '-2.63	GLM,MLM,CMLM, SUPER, MLMM, FarmCPU, Blink
"	wsnp_Ex_rep_c69577_68526990	3A	89.47	0.19	2.54	GLM, CMLM, SUPER
SLUsplchr3B.8	Tdurum_contig47635_876	3B	34.2	0.20	-2.36	GLM, CMLM, SUPER
SLUsplchr5A.9	Excalibur_c45894_552	5A	76.81	0.04	4.61	GLM

SLUsplchr5B.10	BS00039874_51	5B	147.85	0.08	-3.69	GLM,CMLM, SUPER, Blink
SLUsplchr6A.11	wsnp_Ex_c1104_2118684	6A	79.08	0.45	2.67	CMLM, SUPER
"	IAAV7384	6A	80.1	0.48	-2.23 to -0.20	GLM,MLM,CMLM, SUPER, MLMM, FarmCPU
"	BS00036878_51	6A	80.71	0.45	-2.21	CMLM
"	IACX2250	6A	81.17	0.45	2.32	GLM,CMLM, SUPER
"	RFL_Contig3687_972	6A	81.64	0.29	2.10	GLM, CMLM, SUPER, Blink
"	IAAV622	6A	82.38	0.30	NA	SUPER
"	RAC875_c62614_191	6A	82.79	0.29	NA	SUPER
SLUsplchr6A.12	Tdurum_contig46670_911	6A	128.26	0.03		Blink
SLUsplchr7B.13	wsnp_JD_c9040_9947841	7B	68.84	0.30	2.56	CMLM, SUPER
SLUsplchr7B.14	wsnp_RFL_Contig3854_4205716	7B	69.93	0.29	2.79	GLM, CMLM, SUPER, Blink
SLUsplchr7B.15	Excalibur_rep_c116920_300	7B	71.66	0.10	3.66	GLM
SPIKE WIDTH						
SLUspwchr1A.1	wsnp_Ex_c1427_2736441	1A	70.79	0.08	NA	Blink
SLUspwchr2A.2	Excalibur_rep_c112367_293	2A	25.97	0.29	NA	Blink
SLUspwchr3A.3	Ra_c38505_555	3A	88.02	0.04	-0.73	GLM
SLUspwchr4A.4	Jagger_rep_c10288_53	4A	54.83	0.03	0.74	GLM, SUPER, Blink
SLUspwchr5A.5	BS00099534_51	5A	16.62	0.25	-0.29	GLM, MLM,CMLM, SUPER,MLMM, Blink
SLUspwchr7A.6	Tdurum_contig12326_232	7A	133.99	0.21	-0.32	FarmCPU
SLUspwchr7B.7	Ku_c28853_1518	7B	76.17	0.21	-0.34	GLM, FarmCPU, Blink
FLAG LEAF AREA						
SLUflachr3A.1	wsnp_Ex_c1538_2937905	3A	85.39	0.03	NA	SUPER
"	Tdurum_contig22253_104	3A	86.93	0.32		Blink
SLUflachr3B.2	Kukri_c32803_150	3B	8.59	0.33	NA	SUPER
SLUflachr4A.3	BS00039641_51	4A	125.87	0.38	0.96	GLM, SUPER, Blink
SLUflachr5A.4	wsnp_Ku_c15816_24541162	5A	62.72	0.24		Blink
SLUflachr5B.5	CAP7_c1155_57	5B	69.19	0.36	-1.19	FarmCPU, Blink
SLUflachr5B.6	RAC875_c8927_1434	5B	127.96	0.07		Blink
SLUflachr5B.7	BS00029720_51	5B	176.18	0.17	NA	SUPER
"	BobWhite_c16916_658	5B	176.61	0.17	NA	SUPER

Supplementary Table 4: QTLs detected by Blink for heading, anthesis, spike length, spike width and Flag leaf area in Breeding set and Genebank set lines at P<0.001

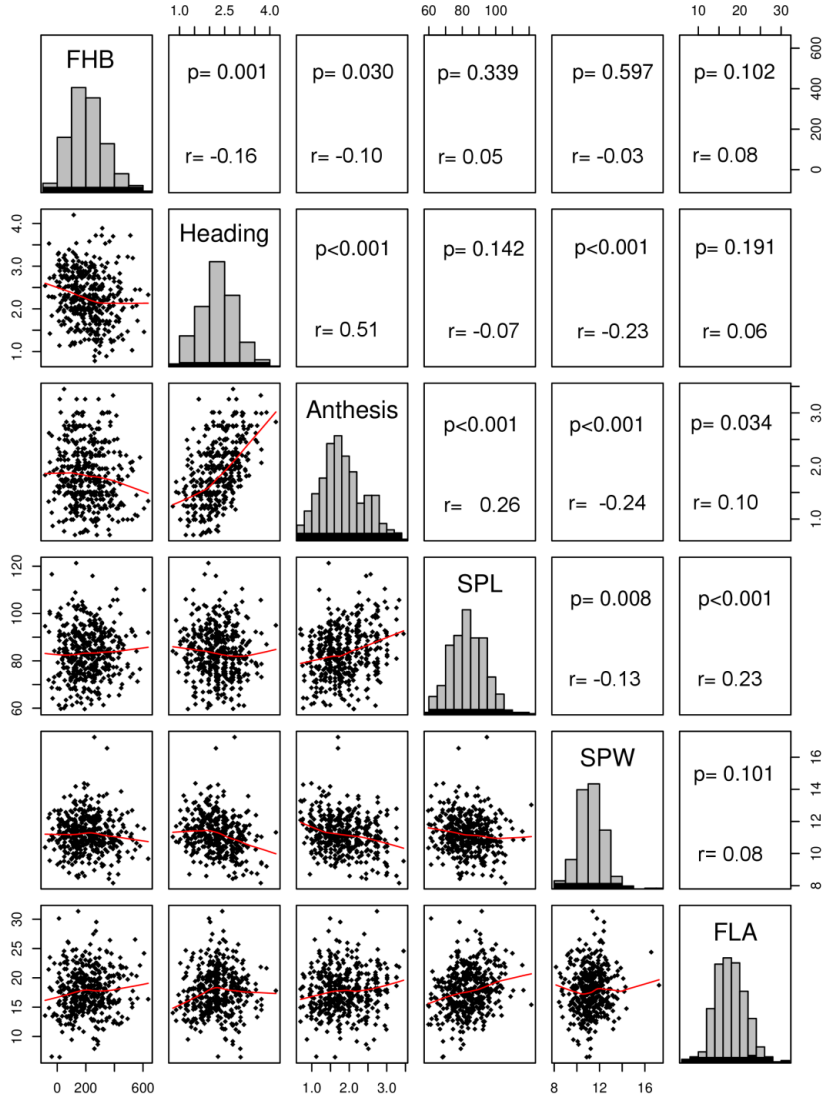
QTL	SNP	Chromosome	Position (cM)	P.value	Minor allele frequency
HEADING					
Breeding set					
	Ra_c68984_1882a	1B	70.08	3.45146E-06	0.11
	tplb0048b10_1365	1B	79.77	0.000870953	0.04
	RFL_Contig5277_888	2A	177.65	0.000565524	0.10
	IAAV2065	2A	183.77	0.00027176	0.08
	wsnp_Ex_c3964_7181151	2D	98.59	0.00027176	0.08
SLUhschr3B.13	wsnp_Ex_c1934_3648624[a]	3B	75.19	1.93192E-06	0.09
	Kukri_rep_c68263_453	5A	53.47	0.000945627	0.20
	BS00023152_51	5A	137.98	0.000713841	0.41
SLUhschr5B.19	wsnp_Ex_c831_1625061[a]	5B	11.23	9.35834E-09	0.15
	IAAV1706	5B	71.64	0.00075825	0.22
	Tdurum_contig62941_85	6B	39.24	0.000167721	0.42
Genebank set					
	BS00100774_51	1A	13.73	0.000163616	0.25
	wsnp_JD_c13903_13781269	1A	77.78	0.000184196	0.15
	Excalibur_c23473_451	1D	65.89	0.000234488	0.29
SLUhschr3B.13	Ku_c27771_508*	3B	66.78	4.94855E-05	0.07
	Kukri_rep_c104877_2166	5A	59.11	0.000258787	0.19
	Tdurum_contig12540_72	5B	47.27	0.000757006	0.04
	IACX6496	5D	70.11	0.001009767	0.12
SLUhschr6A.23	tplb0052b07_577[a]	6A	71.24	3.25593E-06	0.33
SLUhschr7B.28	Kukri_c18055_1740[a]	7A	230.4	7.73214E-11	0.20
	Ku_c32426_324	7D	148.35	0.000631205	0.03
ANTHESIS					
Breeding set					
	IACX5994	1A	105.74	0.000213204	0.47
	Excalibur_c47013_1503	1A	104.82	0.000900644	0.48
	Nordgen				
	BS00100774_51	1A	13.73	0.000510576	0.25
	Ex_c17894_1159	4A	48.52	0.000368571	0.10
	Excalibur_rep_c103261_161	4A	154.3	0.000738659	0.04
	Ku_c6319_201	5A	53.25	0.000587669	0.17
SPIKE LENGTH					
Breeding set					
	BS00026037_51	2B	159.66	0.000235666	0.36
	wsnp_BE444144D_Ta_1_1	2D	44.3	0.000191132	0.32
	Kukri_c49670_857	2D	43.37	0.0008884	0.26
	BS00080239_51	2D	44.96	0.000934737	0.25
	BS00083504_51	2D	45.29	0.000954195	0.25
	Kukri_c48750_1416	3B	61.89	0.0005334	0.06
	BS00072151_51	3B	66.63	0.000811265	0.08
	BS00062823_51	6A	79.08	0.000668199	0.25

	Excalibur_c34574_452	6A	79.39	0.000559748	0.24
	IAAV7384	6A	80.1	0.000879879	0.32
	BS00082460_51	6A	136.85	0.000570987	0.27
	Tdurum_contig70819_393	6A	140.87	0.000187124	0.28
	wsnp_Ex_c2325_4355706	6A	141.08	0.000331167	0.27
SLUsplchr7A.16	IAAV2865*	7A	126.8	4.22602E-05	0.25
Genebank set					
SLUsplchr1A.17	JG_c936_115[a]	1A	29.11	4.30133E-09	0.29
	Excalibur_c7026_2635	1A	52.55	0.000402607	0.08
	BS00067339_51	1A	65.44	0.000835674	0.10
	BS00087600_51	1A	77.78	0.000249869	0.24
SLUsplchr1A.18	RAC875_c25814_361[a]	1A	102.92	3.57445E-08	0.23
	IACX1240	1B	96.16	0.001021289	0.30
	wsnp_Ex_c1969_3705930	1D	21.8	0.000750778	0.14
SLUsplchr5A.19	BS00109052_51[a]	5A	49.73	4.17867E-07	0.08
	wsnp_Ex_c48257_53217539	5B	43.31	0.000218213	0.12
SLUsplchr6A.11	BS00036878_51*	6A	80.71	9.81603E-05	0.15
	IAAV7384	6A	80.1	0.000282726	0.15
	RAC875_c39200_260	6A	71.73	0.000727323	0.34
	wsnp_Ex_c30264_39202224	6A	85.07	0.000895452	0.13
SLUsplchr7D.20	D_F5XZDLF02FKJFM_220*	7D	197.58	7.09856E-06	0.05
SPIKE WIDTH					
Breeding set					
	Tdurum_contig12326_232	7A	133.99	0.000665455	0.27
	Ku_c28853_1518	7B	76.17	0.000254486	0.27
	RAC875_c30453_292	7B	76.31	0.001032865	0.16
	BobWhite_c3269_141	7B	77.13	0.000331115	0.34
	Kukri_c99107_143	7B	77.59	0.00023796	0.34
	wsnp_Ku_rep_c103690_90365438	7B	120.81	0.000698672	0.08
Genebank set					
	Excalibur_c23473_451	1D	65.89	0.000246614	0.29
	wsnp_Ex_rep_c70299_69243401	2A	140.94	0.000549162	0.29
SLUspwchr2A.8	BS00110128_51*	2A	141.66	7.00388E-05	0.37
	Ex_c525_1401	2B	16.88	0.000871578	0.33
	Kukri_rep_c103261_918	2B	28.5	0.000396616	0.15
	RAC875_c62831_255	2B	32.16	0.000130283	0.14
	BS00011612_51	3A	88.02	0.000839406	0.11
	BS00063946_51	3B	95.1	0.000946505	0.40
	RAC875_c19303_228	4B	56.19	0.000306769	0.17
SLUspwchr5A.9	Tdurum_contig12967_831*	5A	49.73	8.73845E-06	0.45
SLUspwchr6B.10	Kukri_c85856_60[a]	6B	106.13	2.14726E-13	0.40
	wsnp_Ex_c662_1301994	7A	33.45	0.000408767	0.12
SLUspwchr7B.7	RFL_Contig5098_1248*	7B	68.84	3.00671E-05	0.25
	Kukri_c67810_105	7B	76.31	0.000553247	0.18
FLAG LEAF AREA					

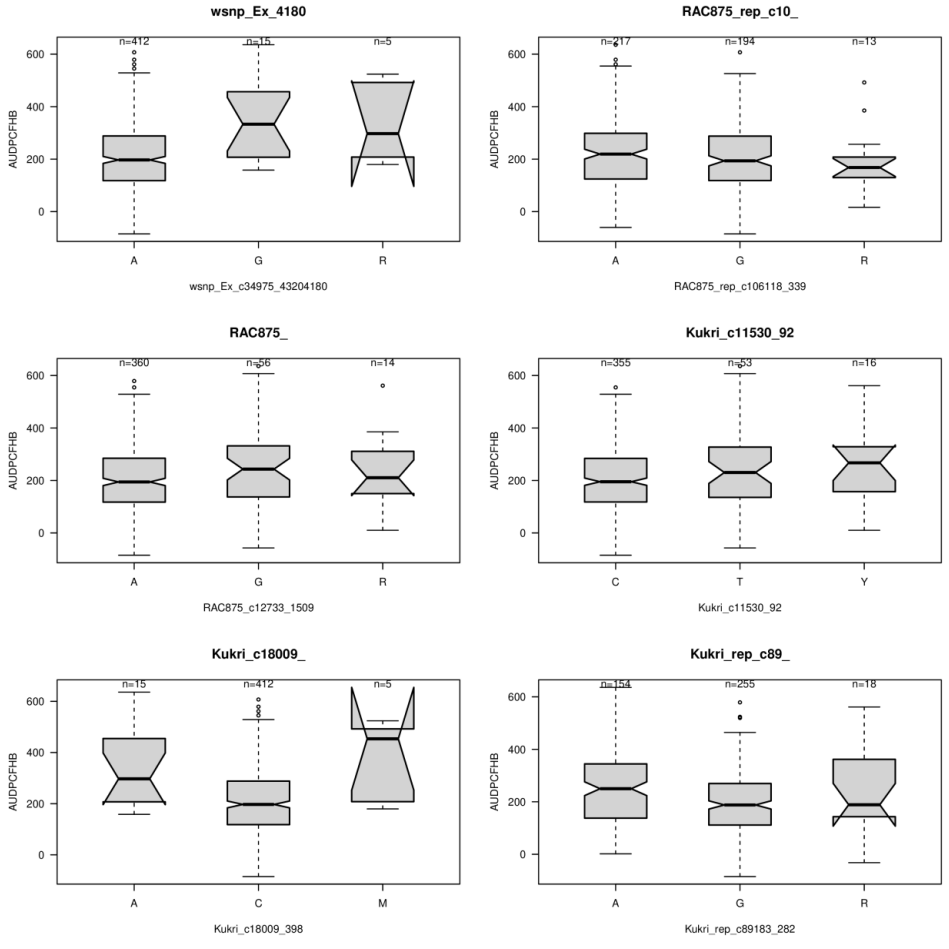
Breeding set					
	Excalibur_c1936_1072	1A	113.19	0.000840634	0.22
Genebank set					
SLUflachr1A.8	Tdurum_contig83113_134*	1A	38.11	4.48391E-05	0.30
	Excalibur_c5892_1129	1D	32.98	0.000813006	0.14
	BS00063365_51	2B	114.82	0.000916765	0.21
	RAC875_c69_499	3B	85.66	0.000265746	0.03
	Excalibur_rep_c71645_94	3B	9.7	0.000532727	0.46
SLUflachr4B.9	BS00022090_51*	4B	61.84	9.03818E-05	0.14
	RAC875_c35152_372	4B	62.92	0.000418691	0.13
	BS00066143_51	5A	67	0.000115899	0.43
SLUflachr5B.10	Excalibur_c92555_283[a]	5B	161.32	2.60293E-08	0.32

*detected at $P<0.0001$ (-log10(P)>4), [a]detected at Bonferroni threshold at 0.05
Naming of QTLs detected at $P\leq0.0001$ continued from combined data set (Suppl. Table 3)

Supplementary Figure 1: Pearson correlation among various phenotypic traits



Supplementary Figure 2: Average effect of the favorable QTL alleles for six SNPs detected by at least two GWAS models



III

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 18 October 2022
DOI 10.3389/fpls.2022.1010249

OPEN ACCESS

EDITED BY
Gregorio Egea,
University of Seville, Spain

REVIEWED BY
Giorgio Mariano Balestra,
University of Tuscia, Italy
Sara Francesconi,
University of Tuscia, Italy

*CORRESPONDENCE
Fernanda Leiva
Fernanda.leiva@slu.se

SPECIALTY SECTION
This article was submitted to Technical Advances in Plant Science, a section of the journal Frontiers in Plant Science

RECEIVED 02 August 2022
ACCEPTED 22 September 2022
PUBLISHED 18 October 2022

CITATION
Leiva F, Zakieh M, Alamrani M, Dhakal R, Henriksson T, Singh PK and Chawade A (2022) Phenotyping Fusarium head blight through seed morphology characteristics using RGB imaging. *Front. Plant Sci.* 13:1010249. doi: 10.3389/fpls.2022.1010249

Phenotyping Fusarium head blight through seed morphology characteristics using RGB imaging

Fernanda Leiva[1]*, Mustafa Zakieh[1], Marwan Alamrani[1], Rishap Dhakal[1], Tina Henriksson[2], Pawan Kumar Singh[3] and Aakash Chawade[1]

[1]Department of Plant Breeding, Swedish University of Agricultural Sciences, Lomma, Sweden, [2]Lantmännen Lantbruk, Svalöv, Sweden, [3]International Maize and Wheat Improvement Center (CIMMYT), Texcoco, Mexico

Fusarium head blight (FHB) is an economically important disease affecting wheat and thus poses a major threat to wheat production. Several studies have evaluated the effectiveness of image analysis methods to predict FHB using disease-infected grains; however, few have looked at the final application, considering the relationship between cost and benefit, resolution, and accuracy. The conventional screening of FHB resistance of large-scale samples is still dependent on low-throughput visual inspections. This study aims to compare the performance of two cost–benefit seed image analysis methods, the free software "SmartGrain" and the fully automated commercially available instrument "Cgrain Value™" by assessing 16 seed morphological traits of winter wheat to predict FHB. The analysis was carried out on a seed set of FHB which was visually assessed as to the severity. The dataset is composed of 432 winter wheat genotypes that were greenhouse-inoculated. The predictions from each method, in addition to the predictions combined from the results of both methods, were compared with the disease visual scores. The results showed that Cgrain Value™ had a higher prediction accuracy of R^2 = 0.52 compared with SmartGrain for which R^2 = 0.30 for all morphological traits. However, the results combined from both methods showed the greatest prediction performance of R^2 = 0.58. Additionally, a subpart of the morphological traits, namely, width, length, thickness, and color features, showed a higher correlation with the visual scores compared with the other traits. Overall, both methods were related to the visual scores. This study shows that these affordable imaging methods could be effective to predict FHB in seeds and enable us to distinguish minor differences in seed morphology, which could lead to a precise performance selection of disease-free seeds/grains.

KEYWORDS

Fusarium head blight, seed phenotyping, seed morphological characters, wheat, visual scores, SmartGrain, Cgrain Value™

Introduction

In the countries of the Baltic Sea region, the most widely cultivated crop is winter wheat (*Triticum aestivum L.*), (Shiferaw et al., 2013; Chawade et al., 2018). While efforts are made to achieve sustainable intensification of high grain yields in wheat production, the emergence and increase in the virulence of plant pathogens conversely leave the nutritional integrity and production of wheat grains at risk (Castro Aviles et al., 2020). The decrease in grain quality and protein content negatively impacts the use of the grains and therefore affects food security and safety (Asseng et al., 2019). Fusarium head blight (FHB), mainly caused by the fungus *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* (Schwein) Petch], is one of the wheat diseases with a major impact on wheat grain yield and quality. FHB can dramatically reduce grain quality and yield through the formation of sterile and wizened florets. FHB-infected grains suffer from major marketing, consumption, and processing constraints, which is the buildup of mycotoxins—mainly deoxynivalenol (DON) (Del Ponte et al., 2022). DON inhibits protein synthesis, cutting off normal cell function, which is hazardous for the consumption of humans and animals (Polak-Śliwińska and Paszczyk, 2021). FHB disease management strategies rely on integrating several cultural practices such as fungicide treatment, crop rotation, mixed culture, and tillage (Gilbert and Haber, 2013). However, growing FHB-resistant cultivars is seen as a more sustainable and durable strategy for mitigating disease epidemics, thus avoiding large economic losses. Hence, identifying sources of novel resistance is a key component in pre-breeding activities that can be introgressed to develop commercial FHB-resistant cultivars.

The resistance components for FHB, commonly known as resistance types, have been defined into type I to type V (Mesterhazy, 2020): type I is resistance to initial infection, type II is resistance to disease spread (Schroeder and Christensen, 1963), type III is resistance to damage of Fusarium-damaged kernels (FDK), type IV is resistance to the buildup of DON toxins, and type V is tolerance. Traditionally, studies on FHB resistance have relied on measuring the symptoms in spikes and kernels (resistance types II and III). Type II is assessed by rating the visual symptoms on the spikes, which appear as bleached, yellowish or discolored, and stunted (Zakieh et al., 2021; Steed et al., 2022). FDK is quantified traditionally by estimating the amount of visibly damaged kernels, which appear smaller, shriveled, and in a range of colors from pale pink to brown (Delwiche et al., 2010), according to a predetermined scale for visual assessments or by employing manual tools (Ackerman et al., 2022). Comparisons between both types of resistance (resistance types II and III) have revealed that it would be more efficient and consistent to estimate FHB than the degree of colonization on the spike (Agostinelli, 2009; Balut et al., 2013; Khaeim et al., 2019; Ackerman et al., 2022). However, screening by either manual or visual assessments is a labor- and time-consuming process for rating genotypes, is biased due to the subjectivity of visual assessments, and has low reproducibility among experiments (Barbedo et al., 2015; Khaeim et al., 2019). As a result of the previously cited limitations, the use of image analysis approaches has been investigated to evaluate FDK, particularly in estimating morphological characteristics. However, the existing different imaging approaches have their disadvantages and trade-off in terms of costs, time expenses, resolution, and precision when considering an application (Saccon et al., 2017).

Among the investigated methods, Iwata and Ukai (2002) and Iwata et al. (2010) investigated changes in grain shape using elliptic Fourier descriptors of two- and three-dimensional features from vertically and horizontally located seed images. Despite the accuracy reached, there are limitations in terms of image resolution and regarding the manual handling of samples during the procedure. Menesatti et al. (2009) presented a method to classify FHB in wheat-infected kernels—according to the shape criteria—into the following groups: chalky, shriveled, or healthy. The method proved to be functional to categorize kernels as chalky or healthy, but not for shriveled or gravely affected samples. Jirsa and Polišenská (2011) developed a model for the identification of Fusarium-damaged wheat kernels using image analysis. The characterization of healthy or damaged kernels based on color parameters revealed a high accuracy compared with the shape and DON content parameters. However, image processing was done with manual selections and comparing only 40 kernels—either heavily damaged or healthy—without considering any halfway stage. Similarly, the use of hyperspectral imaging for detecting Fusarium sp. in seeds has been previously investigated (Delwiche et al., 2010; Shahin and Symons, 2011; Bauriegel and Herppich, 2014; Barbedo et al., 2015; Femenias et al., 2022; Rangarajan et al., 2022; Yipeng et al., 2022). The methods have been shown to be accurate and have identified more factors involved in FDK. A more advanced technique based on X-ray computed tomography has been implemented for evaluating seed shape in finer detail (Gomes and Duijn, 2017; Liu et al., 2020). Nevertheless, inconsistencies because of specular reflection, correct wavelength selection, kernel orientation, selection of reference parameter, costs of acquisition devices, and the storage requirement for highly dimensional and massive data sets may be limiting the application of these methods (Dissing et al., 2013; Lu et al., 2020).

In the face of the constraints cited earlier, automated and light-weight free software for grain image analysis have been developed (Wang et al., 2009; Komyshev et al., 2017; Colmer et al., 2020; Zhu et al., 2021); some examples of them are GrainScan (Whan et al., 2014), which analyzes size and color features, and SmartGrain (Tanabata et al., 2012), which analyzes size and shape features. Both software are instantaneous in image recognition despite the position, overlapping, or the

number of seeds. Alternatively, commercially available imaging instruments for grain image analysis combine hardware and software, including WinSEEDLE (Regent Instruments Inc.), Seed Count (Next Instrument Pty Ltd.), Vibe QM3 Grain Analyzer (VIBE), and Cgrain Value™ (Cgrain AB). The instruments use optical or flatbed scanners to extract features such as size, shape, and color in the color representation hue, saturation, and light (HSL). However, SeedCount and Vibe QM3 Grain Analyzer only scan the top surface of the samples, thus omitting morphological characteristics that are not in the viewing area. A more advanced instrument is Videometer Lab (Videometer A/S, Denmark), which provides rapid color, shape, and texture measurements. Videometer Lab is ideal to use in analyzing kernel surfaces, but it requires certain expertise and allows the analysis of only a few samples at once.

In this context, this paper has three objectives; first is to investigate the applicability of low-cost digital image analysis to predict FHB infection in harvested grains through morphological traits. This will offer more insight into the traits that are correlated to the degree of FDK. The second objective is to compare the applicability of the two methods used for grain image analysis—SmartGrain, and Cgrain Value™—in terms of consistency and throughput. The third one is to illustrate the processing chain and result interpretation with a descriptive data analysis.

Materials and methods

Plant material

Wheat kernel samples were collected from an experiment under accelerated indoor growth conditions (Zakieh et al., 2021) using winter wheat genotypes from two different sources. The first source consisted of 338 genotypes (breeding set) provided by the Swedish agricultural cooperative (Lantmännen Lantbruk, Svalöv, Sweden). The second source consisted of 181 germplasm genotypes (genebank set) provided by the Nordic Genetic Resource Center (Nordgen), with highly diverse plant materials including landraces and old cultivars.

Experimental design/growth and inoculation protocol

Plants were grown following an augmented block design in a climate-controlled chamber. After germination, the plants were subjected to a vernalization period of 57 days at 3°C with 8 h of daily light at medium–high light intensity (LI) of 250 µmol m^{-2} s^{-1}. At the end of the vernalization period, the climatic conditions were adjusted with a gradual increase in temperature and LI for the acclimatization of the plants to the next phase of accelerated growth conditions. Once the acclimatization period was concluded, the plants were left to grow at a constant temperature of 22°C. The accelerated growth conditions were adapted by exposing the plants to a prolonged daily light duration of 22 h, with LI at 400 µmol m^{-2} s^{-1} of uniform light intensity from LED light plates. Under these accelerated growth conditions, the plants were watered daily and fertilized weekly using first a combination of a high-phosphate and high-nitrogen soluble fertilizer SW-BOUYANT 7-1-5 + Mikro + KH_2PO_4, then only with a high-nitrogen fertilizer, and finally with a high-potassium soluble fertilizer Yara Tera Kristalon NPK 12-5-30 with S and Mikro.

After completing the anthesis stage, at 33 days post-acclimatization, the plants were moved to a glasshouse chamber with relative humidity (rh) of 60% and a constant temperature of 24°C for 24 h to allow their adaptation to the new growth conditions prior to inoculation. Thereafter, the winter wheat spikes were spray-inoculated with an inoculum suspension prepared from the harvested spore of *F. graminearum* and *F. culmorum*, with a concentration of 5×10^5 spore/ml. Subsequently, the plants were left to incubate at 90% rh with 16/8 h dark/light cycle at a constant temperature of 24°C for 48 h before adjusting the climatic conditions back to 60% rh. The plants were eventually left to grow under the latter conditions for 24 days before harvesting the seeds. Eight isolates from *F. graminearum* and *F. culmorum* species were used in inoculating the plants provided by the Swedish agricultural cooperative Lantmännen Lantbruk. An inoculum preparation was carried out by incubating the fungal spores at 24°C for 4 days in dark conditions to allow for mycelial growth on SNA media plates. Later, the fungal plates were exposed to near ultra-violet UV radiation for 10 h to induce macroconidia formation. Afterward, the fungal plates were incubated for 4 days at 24°C in dark conditions. Finally, macroconidia spores were collected to make the inoculation suspension with the provided concentration after adding the surfactant Tween®20 0.002% (v/v) final volume of the inoculum. A more detailed protocol is described in Zakieh et al. (2021).

FHB visual assessment

In order to evaluate FHB resistance on a large number of genotypes, a modified visual scoring of the FHB disease severity method was adopted. The method took into account the incidence of all FHB symptoms across the main tiller spike of each genotype. Therefore, disease severity was assessed as the percentage score of infected spikelets relative to all spikes, regardless of symptom continuity on the same spike. FHB development was scored at 6, 8, 10, and 12-days post-inoculation (dpi) (Stack and McMullen, 1998). The FHB disease severity scores varied between 100 to 5% for the most susceptible phenotypes and the most resistant ones, respectively. Finally, the results of the visual scores were validated by association mapping, thus identifying the quantitative trait loci of FHB resistance (Appendix 1).

Seed shape parameters

Two different grain phenotyping methods were employed in this study: an automated imaging instrument with software and hardware named Cgrain Value™ which is commercially available (Cgrain AB) and the free software named SmartGrain developed by Tanabata et al. (2012) and can be downloaded from the Quantitative Plant website (Lobet, 2017). The implementation of both methods is described in the following sections.

SmartGrain

For image acquisition, the seeds were captured with a low-cost image protocol acquisition from a top-view angle of 55 cm above the seeds and placed manually on a flat surface using a digital single-lens reflex camera Canon EOS 1300D (Canon U.S.A. Inc., Huntington, NY, USA), which has a resolution of 18 megapixels, mounted on a Kaiser RS-1 repro stand. The camera was tethered to the software digiCamControl (Istvan, 2014) with optimal exposure settings based on the best seed view, F-Stop 1/160, exposure time 1/10, and ISO 800. The seeds were placed manually per genotype uniformly on a blue cardboard that was used as a background on a stand aside from a 15-cm ruler for further analysis. Digital images were stored with 3,456 × 2,304-pixel resolution in JPEG format (Figure 1, top images).

The image analysis was thereafter carried out using SmartGrain software following its default protocol (Tanabata et al., 2012). Briefly, the image scale was set up by taking a known sample from the ruler and registering it on the software. Then, the segmentation method by color was chosen, the precision sensibility was set at the minimum value of "1", and the seed detection intensity was at a maximum value of "4" to obtain all possible shape details; the rest of the parameters were set to default. Finally, all the processed images were saved as TIFF files, and the results were saved in a CSV format. The software provides seven morphological characteristics: area seed (AS), perimeter length (PL), length (L), width (W), length-to-width ratio (LWR), circularity of the seed (CS), distance between the intersection of length and width, and the center of gravity (DS). **AS** corresponds to the total number of pixels of the segmented seed, this parameter estimates the seed size. **PL** refers to the length measurement of the seed outline. **L** corresponds to the major length measurement in the axis and **W** to the minor length axis measurement. **CS** estimates how round the region of interest is (seed), and it is calculated as $\frac{4\times\pi\times AS}{PL^2}$. **LWR** is calculated by $\frac{L}{W}$, and it provides an idea of the seed shape between rectangular and circular depending on the value. The distance between the transverse axis from the outline of the seed (IS) and the center of gravity (CG) is used to estimate **DS** [described in detail by Tanabata et al. (2012)].

Cgrain Value™

For single kernel analysis, seeds were scanned with Cgrain Value™, which is an analytical imaging instrument. The device

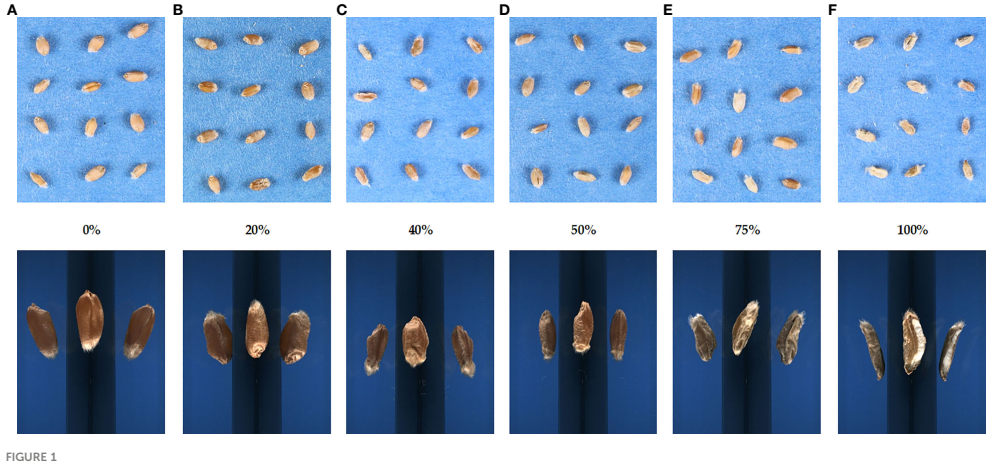


FIGURE 1
Images of the different levels of Fusarium head blight severity on winter wheat seeds. The rating of disease severity ranged from (**A**) 0 to (**F**) 100%. Scoring was based on the proportion of total infected spikes to the total amount of spikes. The top images were obtained for the SmartGrain analysis, and the bottom images were acquired using the Cgrain Value™ instrument.

inspects each kernel through a unique mirror design covering more than 90% of the grains' surfaces in every image. The analysis starts by pouring into the metal bowl of the Cgrain Value™ a batch of seeds per line and per genotype. The seeds rotate into the bowl and then, one by one, are photographed and analyzed simultaneously. After the analysis is completed, three different reports are created (result file, stat file, and image file). The result file consists of the morphological characteristics for each batch of seeds (seed count, thousand kernels, *etc.*), the stat file provides data per individual seed of a group (length, width, *etc.*), and the image file corresponds to the single seed images acquired (Figure 1, bottom images).

The instrument provides nine morphological attributes: length (L), width (W), thickness (T), average width (AVG.W), volume (V), weight (WT), light, hue, and saturation. Parameters such as L, W, and T are estimated by taking the longitudinal measurement of the axis major, higher minor, and minor, respectively. In the case of AVG.W, as the seed is received as a three-dimensional image, the measurement is referring to the mean of the average curvature. V corresponds to the seed volume obtained from the 3D image. For WT, the device has an internal balance, so while acquiring the image, it also weighs the grain. Color parameters, hue, saturation, and light are also determined by the instrument; it specifies the color base of a sample, how saturated it is, and how bright it is, respectively.

Statistical analysis

Statistical analyses were conducted using R (Team, R. C, 2013). The visual scorings of the last time-point on infected spikes, including cultivars with zero symptoms, were included in a file together with the mean values per genotype of the results given by Cgrain Value™ and SmartGrain. Each replicate of the data set was filtered by missing data (NA). Those with NA along the four replicates were removed and those with presence in more than one replicate were substituted using FactoMineR (Lê et al., 2008) and missMDA (Josse and Husson, 2016) packages. Then, using the Agricolae R package (De Mendiburu, 2014), the checks in each augmented block were used to adjust the means for each trait per replicate, the model of which is as follows:

$$y_{il} = u + G_{il} + \beta_1 + \epsilon_{il}$$

where y_{il} corresponds to the adjusted means of the i^{th} wheat cultivar in the l^{th} block, u is the general mean value, G_{il} is the effect of the i^{th} wheat genotype in the l^{th} block, β_1 is the l^{th} block effect, and ϵ_{il} is the residual. Subsequently, using the adjusted means, the best linear unbiased estimates (BLUEs) was calculated using the randomized complete block design option in META-R 6.04 (Alvarado et al., 2015) based on the following model:

$$y_{ijm} = u + S_j + G_{ijm} + R_m + \epsilon_{ijm}$$

where y_{ijm} corresponds to the BLUE of the i^{th} genotype from the j^{th} population in the m^{th} replicate, u is the general mean value, S_j is the effect of the j^{th} source of material, G_{ijm} is the effect of the i^{th} genotype in the m^{th} replicate, R_m is the m^{th} replicate of the effect, and ϵ_{ijm} is the residual effect. The source of wheat genotypes S_j was considered the grouping factor.

The BLUEs data previously centered were used to predict FHB using a multiple regression model:

$$y_i = \beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + \ldots + \beta_p x_{ip} + \epsilon$$

Where for i=n observations: y_i corresponds to the dependent variable, x_i to the explanatory variables, β_0 corresponds to y-intercept (constant term), β_p corresponds to the slope coefficients for each explanatory variable, and ϵ corresponds to the error of the model (also known as the residuals). Three models were created using the morphological traits provided by both methods (Cgrain Value™ and SmartGrain) as independent variables and visual scorings as the dependent variable. One model combines all the traits, and two others use the traits provided by each method. To build each model, the data set was partitioned employing the function "createDataPartition" of the caret package (Kuhn et al., 2020) into 70% for model training (training set) and the remaining 30% for evaluating model performance (test set). Subsequently, the model was fitted to the training set, and it predicted the responses using the test set. To evaluate the quality of the predictions and mitigate the possibility of errors due to the random data partitioning, the cross-validation was executed 100 times, which means resampling the data set, and the mean of the criterion was taken as the final result.

Results

This study examined a total of 16 morphological traits, including size, color, and shape of winter wheat grains from the genebank and breeding sets with different levels of FHB infection. Nine traits were obtained with the instrument Cgrain Value™ and seven traits with the software SmarGrain. The distribution of all the morphological traits measured by the two methods showed a Gaussian distribution (Figure 2). In order to understand the association between these traits and FHB resistance, a comparison with the traits measured of 80 FHB susceptible and resistant genotypes was performed. For this purpose, five genotypes per replicate (four replicates) from both sets, breeding and genebank, were selected based on the FHB severity scores on the spikes, genotypes scored as 0% (visually non-infected or resistant), and ones scored as 100% (visually infected or susceptible). Among the infected and non-infected selected groups, there was a 22.61% reduction in V and 11.32% in AS. Other parameters also showed a reduction, such as T_RAW at 10.60%, W at 8.30% in both methods, and WT at

22.63%. Additionally, L was reduced according to the results by 1.96% in Cgrain Value™ and 2.26% in SmartGrain. Similarly, CS and PL showed a decrease, but in less proportions with 4.60 and 3.25%, respectively. The minimum seed L measured was 4.59 mm for non-infected and 4.50 mm for infected genotypes. On the other hand, color parameters expressed major changes compared with all the other morphological traits. Hue and the light increased with the infection by 19.91 and 8.28%, respectively, while saturation decreased at about 15.52% (Table 1). According to the analysis of variance (two-way ANOVA), the morphological traits L, W, T_RAW, light, and hue were highly significant ($P< 0.001$), likewise with V, CS, and saturation ($P< 0.01$), indicating a clear association with FHB disease severity level. Meanwhile, the parameters WT, AS, LWR, PL, and DS did not indicate any significance but still showed slight differences between infected and non-infected grains.

Additionally, a principal component analysis (Figure 3) was performed to show the response of all the seed traits studied regarding the disease infection and how they correlate to each other. The proportion of total variance on the two first principal

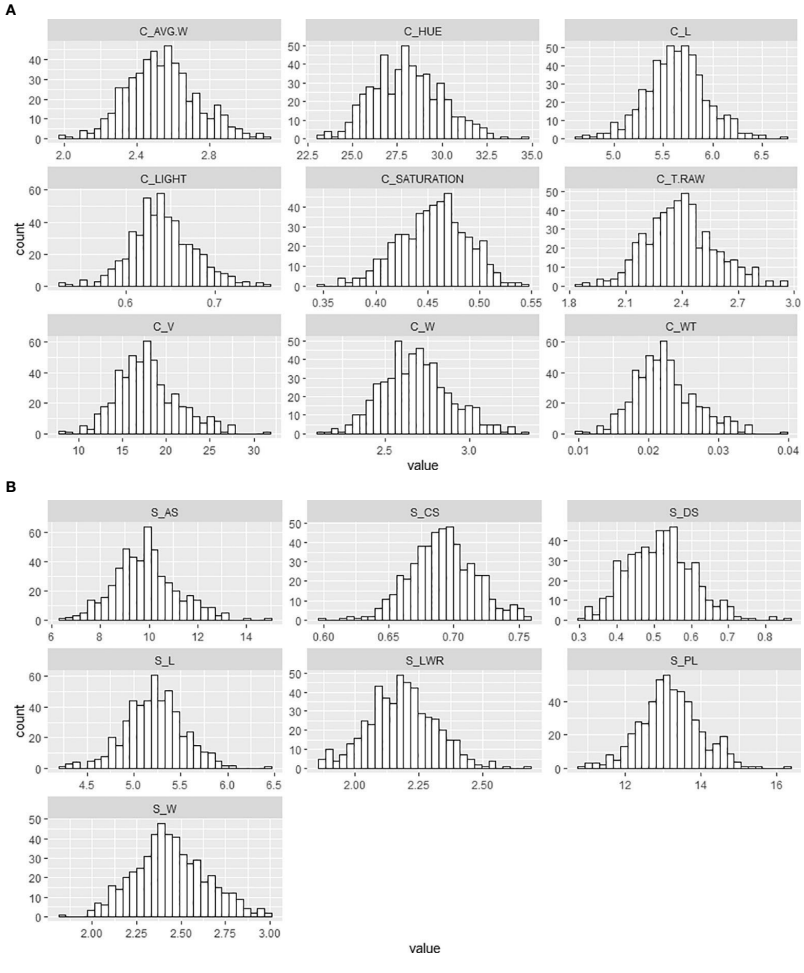


FIGURE 2
Frequency distribution of the different morphological traits of wheat genotypes seeds from the breeding and genebank sets collected with **(A)** the Cgrain Value™ instrument and **(B)** the SmartGrain software.

TABLE 1 Descriptive statistics showing differences between the seed shape characters of 80 genotypes from genebank and breeding set under non-infection (0%) and full infection (100%) FHB symptoms, with five genotypes of each one per replicate.

		a) CGRAIN VALUE™								
Description	**Level**	**L**	**W**	**T.RAW**	**AVG.W**	**V**	**WT**	**HUE**	**SAT**	**LIGHT**
Mean	**Non_Infected**	5.6	2.76	2.47	2.61	19.18	0.02	25.78	0.48	0.62
	Infected	5.49	2.53	2.2	2.36	14.84	0.01	30.81	0.4	0.68
% Reduction		1.96	8.29	10.6	9.41	22.61	25	-19.51	16.52	-9.67
Max	**Non_Infected**	6.88	3.7	3.245	3.41	38.9	0.04	30.46	0.55	0.715
	Infected	6.46	3.13	2.93	3.03	26.6	0.03	38.99	0.51	0.81
Min	**Non_Infected**	4.59	2.18	1.98	2.08	10.66	0.01	23.45	0.43	0.55
	Infected	4.5	2.05	1.88	1.96	7.1	0.008	24.88	0.3	0.58
SD	**Non_Infected**	0.52	0.36	0.3	0.32	6.74	0.008	1.32	0.02	0.04
	Infected	0.45	0.23	0.22	0.23	4.02	0.005	3.01	0.05	0.05
SE	**Non_Infected**	0.08	0.05	0.048	0.05	1.06	0.001	0.21	0.004	0.006
	Infected	0.07	0.04	0.036	0.04	0.63	0.0007	0.47	0.008	0.007
CV (%)		9.44	13.02	12.26	12.53	35.15	35.15	5.14	5.8	6.79

		b) SMARTGRAIN					
Description	**Level**	**AS**	**PL**	**L**	**W**	**LWR**	**CS**	**DS**
Mean	**Non_Infected**	9.77	12.91	5.08	2.44	2.13	0.7	0.48
	Infected	8.66	12.49	4.97	2.23	2.25	0.67	0.51
% Reduction		11.32	3.25	2.26	8.27	-5.64	4.6	-6.9
Max	**Non_Infected**	17.36	17.15	6.57	3.71	2.53	0.8	0.85
	Infected	13.63	15.54	6.25	2.95	2.65	0.73	1.01
Min	**Non_Infected**	3.41	7.91	3.2	1.39	1.53	0.63	0.24
	Infected	3.01	7.31	2.88	1.36	1.88	0.61	0.23
SD	**Non_Infected**	3.21	2.16	0.81	0.48	0.17	0.03	0.13
	Infected	2.55	1.95	0.79	0.38	0.15	0.02	0.18
SE	**Non_Infected**	0.5	0.34	0.12	0.07	0.02	0.005	0.02
	Infected	0.4	0.3	0.12	0.06	0.02	0.004	0.02
CV (%)		32.85	16.72	16.11	19.72	8.41	4.76	28.27

a) Cgrain Value™ size, shape and color characteristics, (L) [mm], Width (W) [mm], Raw Thickness (T.RAW) [mm], Mean Width (AVG.W) [mm], Weight (WT) [g], Hue, Saturation, and Light; b) SmartGrain size and shape characteristics, Area size (AS) [mm2], Perimeter length (PL) [mm], Length (L) [mm], Width (W) [mm], Length to width ratio (LWR), Circularity (CS) Distance between IS and CG (DS) [mm].

components and correlations represents 60.50 and 19.90%, respectively, of the total variance. The LWR trait was shown to be the higher positive in the first principal component; similarly, hue was shown to be positive but in a lesser proportion. In the same component but with negative loading, we found CS as the variable with the highest contribution; the traits W from both methods, AVG.W, and T_RAW were also projected onto this component with a loading of a slightly lesser norm. Although saturation was also projected onto this component, it was shown to be the smallest loading. On the other hand, in the second principal component, the traits DS and L from both methods, PL, AS, V, and WT showed a high positive loading with similar proportions, whereas the trait light was the only one with a negative loading into the second principal component and the one with less projection among all the traits. In general, all the seed morphological traits assessed expressed variability and influence in the two principal components. In addition, as can be observed in the graph, the variation of LWR has an opposite projection to the CS trait, expressing a good indicator to study the deformation of the grains caused by the disease infection.

Considering Table 1, the mean values for the same morphological traits measured by both methods (L and W) across the two sets, genebank and breeding, were similar. The difference between infected and non-infected seeds was 0.11 mm in L in both methods and between 0.21 and 0.25 mm in W and AVG_W. Both methods provide important parameters for seed morphology studies. Cgrain Value™ provides V and WT values and color information. Although these are important characteristics for different study purposes, mainly for identifying FHB-infected kernels, SmartGrain, in turn, provides information such as PL, AS, and CS that can show variabilities between infected and non-infected seeds. Here the BLUES for all the measured parameters were correlated with each other and in association with the visual scorings on the spikes (Figure 4). A moderate to high positive correlation was found with the color parameter hue, and a low positive

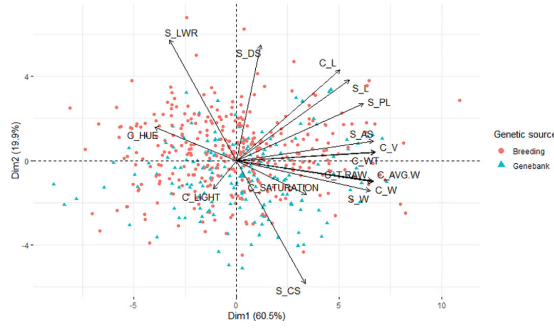


FIGURE 3
Principal component analysis biplot of the morphological traits collected with Cgrain Value™ and SmartGrain of the breeding and genebank seeds infected with different levels of Fusarium head blight.

correlation with light was given by Cgrain Value™ and LWR as well as given by SmartGrain (r = 0.65, r = 0.36, and r = 0.27, respectively). Negative correlations were also found between the visual evaluations of symptoms and the other characteristics in different levels of strength of association. There was no correlation between FHB visual scoring and DS (r = 0.01).

The multiple linear regression model developed to identify the contributions of the 16 different morphological traits provided by Cgrain Value™ and SmartGrain expressed a high moderate prediction (R^2 = 0.58), (Figure 5A). Aiming to identify which of both methods used in this study provides a higher prediction and also to identify the best morphological traits to predict FHB, two more models were constructed: one for the results given by Cgrain Value™ and another one for the results of SmartGrain. The model of Cgrain Value™ traits showed a moderate prediction (R^2 = 0.52), (Figure 5B). On the other hand, the model of SmartGrain traits showed medium–low prediction (R^2 = 0.30), (Figure 5C), clearly showing that the first model had

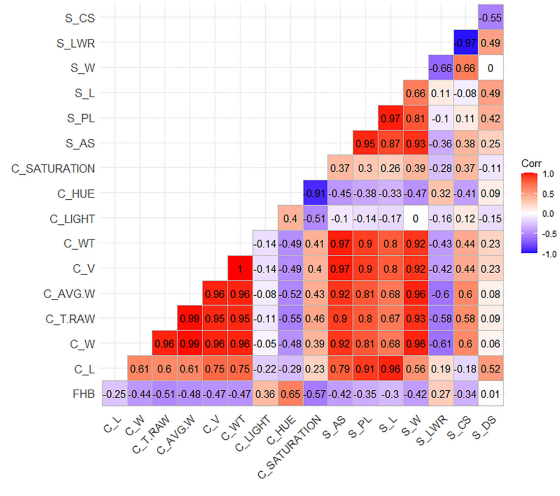


FIGURE 4
Sorted upper triangle correlation matrix among the morphological attributes of the wheat genotype seeds from the breeding and genebank sets collected with the Cgrain Value™ and the SmartGrain software.

10.3389/fpls.2022.1010249

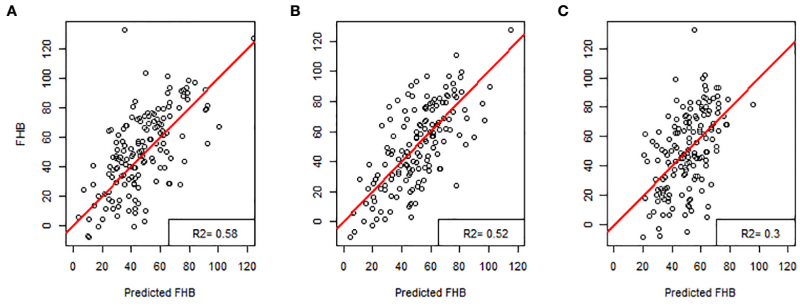


FIGURE 5
Regression models for predicting Fusarium head blight in wheat: **(A)** all the characteristics obtained with Cgrain Value™ and SmartGrain, **(B)** Cgrain Value™ morphological traits, and **(C)** SmartGrain morphological traits.

a higher prediction than separately. In addition, the morphological parameters that are the most suitable to assess FHB in grains above all the 16 evaluated were identified. According to the regression model and the ANOVA analysis, the parameters that provided more information about the disease are the length, width, thickness, average width, circularity, and the color parameters in the color representation HSL (Table 2). The sensitivity test showed that these variables provide the highest value of *R*-square, ($R^2 = 0.52$). These morphological traits are enumerated from most significant to least significant in Figure 6.

Discussion

This study compared the potential performances of two different image-based methods to predict FHB. The results of both indicated that morphological seed traits are functional for predicting FHB among two different sets of genotypes evaluated. Furthermore, a comparison of the applicability of the two methods was properly addressed by evaluating the cost, accuracy, and time efficiency—for instance, to extract dimension, shape, and color parameters, Cgrain Value™ utilizes a unique mirror design to inspect all possible angles of

TABLE 2 Summary of the multiple linear regression model combining all the 16 morphological characteristics provided by Cgrain Value™ and SmartGrain.

	Model summary				
Morphological traits	**Sum sq**	**Mean sq**	***F*-value**	**Pr (>*F*)**	
C_L	23,829	23,829	64.587	6.99E-15	***
C_W	51,079	51,079	138.446	< 2e-16	***
C_T.RAW	40,500	40,500	109.772	< 2e-16	***
C_AVG.W	2,013	2,013	5.456	0.0199	*
C_V	2,603	2,603	7.055	0.00816	**
C_WT	680	680	1.843	0.17526	
C_LIGHT	31,656	31,656	85.802	< 2e-16	***
C_HUE	39,386	39,386	106.752	< 2e-16	***
C_SATURATION	2,649	2,649	7.18	0.00762	**
S_AS	178	178	0.483	0.48734	
S_PL	624	624	1.691	0.1941	
S_L	3,027	3,027	8.204	0.00436	**
S_W	45	45	0.121	0.72828	
S_LWR	0	0	0.001	0.9802	
S_CS	1,651	1,651	4.476	0.03489	*
S_DS	539	539	1.461	0.22731	

The most significant characteristics concerning the Fusarium head blight disease infection according to the P-value has an *. (No significance P>0.05; *P≤0.05; **P≤0.01; ***P≤0.001).

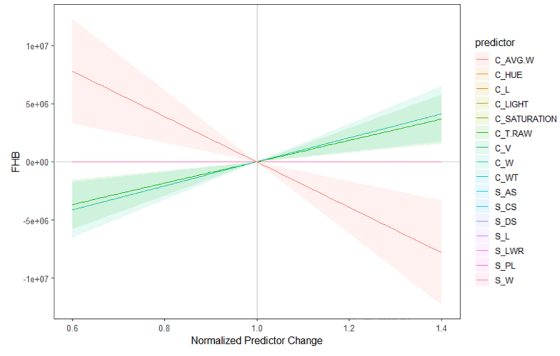


FIGURE 6
Sensitivity plot of the morphological characteristics to predict Fusarium head blight in wheat. The parameters are organized from the best predictors to the less significant to predict the disease. Color lines indicate the significance, considering red as the most important predictor and pink as the less important one. The highlighted regions reflect the correlation of the parameters among each other.

individual kernels in the sample. Additionally, image capture and processing are instantaneous, thanks to the hardware and software combination. Conversely, image acquisition using the SmartGrain system was carried out over a relatively long period, yet image processing was done relatively fast. However, compared with Cgrain Value™, the earlier approach is cheaper considering the cost of the tools used in image capture, requiring a simple RGB camera, a static frame, and the free software.

On the other hand, the morphological traits, based on the statistical analysis results, that showed significant correlations to the visual scores were color traits in the HSL color representation and thickness from Cgrain Value™, length and width, from both methods (Figures 5, 6). Although the other measured morphological traits were not significantly correlated to the visual scores, infected grains still expressed differences in these traits that may be ultimately informative about seed health and refine the prediction (Table 1). Nevertheless, DS was not correlated and did not express significant differences in infected seeds of FHB, but it could prove useful in other applications.

The evaluated visual scores of the symptoms associated with FHB—bleached, yellowish or discolored, and stunted spikes—were previously validated by the identification of several loci by genome-wide association studies (GWAS) (Appendix 1), in a previous study with the same plants and visual scorings (Zakieh et al., 2021). The proposed methods aim to replace costly and labor-intensive genetic analysis.

Therefore, the prediction of both methods studied here appears to be consistent for FHB with the assigned traits concerning the phenotype–genotype association. Previous investigations showed a high correlation between symptoms that are present on wheat heads and the rate of kernel damage (Góral et al., 2018). Therefore, it is feasible to reference the estimated visual scores of disease severity to establish similar results of association/disassociation with the corresponding assessments of grain traits following the methodology in this study.

An important aspect to highlight is that the percentage of disease severity can be assessed, where, in contrast to disease spread from the point of inoculation, it offers less intensive labor by spray inoculation of a larger number of wheat genotypes. Additionally, unlike point-inoculated wheat spikelets, spray-inoculated spikes allow for evaluating the degree of damage caused by the disease to all kernels of the infected spike. Within this work frame, whole spike kernels are investigated for their characteristics rather than the damage to a limited number of kernels caused by Fusarium colonization from the point of inoculation. This, in turn, is expected to shorten the period for disease resistance assessment, lower its cost, and be less labor demanding.

Conclusion

The results indicated that the traits with a higher correlation to FHB were length, width, thickness, and especially color values in HSL color representation. Moreover, Cgrain Value™ was advantageous to SmartGrain in terms of the time required for image capture and outperformed the latter when applied to a large number of samples, yet SmartGrain processes samples fast and is cheaper in comparison to Cgrain Value™. Although the disease prediction showed a low–moderate accuracy for SmartGrain and a high–moderate accuracy for Cgrain Value™ and the results of both methods combined, this is attributed to the prediction reference,

which corresponds to FHB disease severity scorings done on the spikes. However, the novelty of this study resides in the accuracy reached even with a different reference source, but which is directly related. Additionally, as the plant material genotypes and visual scores were validated by GWAS analysis, then the results presented here are phenotype–genotype-associated.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

AC conceived the study. TH developed the breeding population set. MZ provided the material and the scores of the disease severity. MA performed the image and data acquisition with SmartGrain. RD performed the image and data acquisition with Cgrain Value™. FL analyzed the data and wrote the draft. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by funding from the SLU Grogrund (SLU.ltv.2019.1.1.1-623), Nordic Council of Ministers (PPP #6P3), and NordForsk (#84597). Formas (#2020-01828).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Ackerman, A. J., Holmes, R., Gaskins, E., Jordan, K. E., Hicks, D. S., Fitzgerald, J., et al. (2022). Evaluation of methods for measuring fusarium-damaged kernels of wheat. *Agronomy* 12 (2), 532. doi: 10.3390/agronomy12020532

Agostinelli, A. M. (2009). *Phenotypic and genotypic selection for head scab resistance in wheat*. University of Kentucky Master's Theses. 582. Available at: https://uknowledge.uky.edu/gradschool_theses/582

Alvarado, G., López, M., Vargas, M., Pacheco, Á., Rodriguez, F., Burgueño, J., et al. (2015). META-R (Multi Environment Trail Analysis with R for Windows) Version 5.0. CIMMYT Research Data & Software Repository Network 23, 2015.

Asseng, S., Martre, P., Maiorano, A., Rötter, R. P., O'Leary, G. J., Fitzgerald, G. J., et al. (2019). Climate change impact and adaptation for wheat protein. *Global Change Biol.* 25 (1), 155–173. doi: 10.1111/gcb.14481

Balut, A. L., Clark, A. J., Brown-Guedira, G., Souza, E., and Van Sanford, D. A. (2013). Validation of Fhb1 and QFhs. nau-2DL in several soft red winter wheat populations. *Crop Sci.* 53 (3), 934–945. doi: 10.2135/cropsci2012.09.0550

Barbedo, J. G., Tibola, C. S., and Fernandes, J. M. (2015). Detecting fusarium head blight in wheat kernels using hyperspectral imaging. *Biosyst. Eng.* 131, 65–76. doi: 10.1016/j.biosystemseng.2015.01.003

Bauriegel, E., and Herppich, W. B. (2014). Hyperspectral and chlorophyll fluorescence imaging for early detection of plant diseases, with special reference to fusarium spec. infections on wheat. *Agriculture* 4 (1), 32–57. doi: 10.3390/agriculture4010032

Castro Aviles, A., Alan Harrison, S., Joseph Arceneaux, K., Brown-Guidera, G., Esten Mason, R., and Baisakh, N. (2020). Identification of qtls for resistance to fusarium head blight using a doubled haploid population derived from southeastern united states soft red winter wheat varieties ags 2060 and ags 2035. *Genes* 11 (6), 699. doi: 10.3390/genes11060699

Cgrain AB *Cgrain value TM, the new standard for analysis grain quality*. Available at: www.cgrain.se.

Chawade, A., Armoniené, R., Berg, G., Brazauskas, G., Frostgård, G., Geleta, M., et al. (2018). A transnational and holistic breeding approach is needed for sustainable wheat production in the Baltic Sea region. *Physiol. Plantarum* 164 (4), 442–451. doi: 10.1111/ppl.12726

Colmer, J., O'Neill, C. M., Wells, R., Bostrom, A., Reynolds, D., Websdale, D., et al. (2020). SeedGerm: a cost-effective phenotyping platform for automated seed imaging and machine-learning based phenotypic analysis of crop seed germination. *New Phytol.* 228 (2), 778–793. doi: 10.1111/nph.16736

Del Ponte, E. M., Moreira, G. M., Ward, T. J., O'Donnell, K., Nicolli, C. P., Machado, F. J., et al. (2022). Fusarium graminearum species complex: A bibliographic analysis and web-accessible database for global mapping of species and trichothecene toxin chemotypes. *Phytopathology®* 112 (4), 741–751. doi: 10.1094/PHYTO-06-21-0277-RVW

Delwiche, S. R., Kim, M. S., and Dong, Y. (2010). "Damage and quality assessment in wheat by NIR hyperspectral imaging," in *Sensing for agriculture and food quality and safety II: SPIE*, Vol.7676, 45–52.

De Mendiburu, F. (2014). Agricolae: statistical procedures for agricultural research. *R. Package Version* 1 (1), 1–4. doi: 10.7287/peerj.preprints.1404v1

Dissing, B. S., Papadopoulou, O. S., Tassou, C., Ersbøll, B. K., Carstensen, J. M., Panagou, E. Z., et al. (2013). Using multispectral imaging for spoilage detection of pork meat. *Food Bioprocess Technol.* 6 (9), 2268–2279. doi: 10.1007/s11947-012-0886-6

Femenias, A., Llorens-Serentill, E., Ramos, A. J., Sanchis, V., and Marín, S. (2022). Near-infrared hyperspectral imaging evaluation of fusarium damage and DON in single wheat kernels. *Food Control* 142, 109239. doi: 10.1016/j.foodcont.2022.109239

Gilbert, J., and Haber, S. (2013). Overview of some recent research developments in fusarium head blight of wheat. *Can. J. Plant Pathol.* 35 (2), 149–174. doi: 10.1080/07060661.2013.772921

Gomes, F. G. Jr, and Duijn, B. V. (2017). Three-dimensional (3-d) X-ray imaging for seed analysis. *Seed Testing Int.* 154), 48–52. Available at: https://www.scopus.com/inward/record.uri?eid=2-s2.0-85084478678&partnerID=40&md5=f95af32d6e967fe8630289cf7b49a4f0

Góral, T., Wiśniewska, H., Ochodzki, P., Nielsen, L. K., Walentyn-Góral, D., and Stępień, Ł. (2018). Relationship between fusarium head blight, kernel damage, concentration of fusarium biomass, and fusarium toxins in grain of winter wheat inoculated with fusarium culmorum. *Toxins* 11 (1), 2. doi: 10.3390/toxins11010002

Istvan, D. (2014). *"DigiCamControl software". 2.1.2. ed.*

Iwata, H., Ebana, K., Uga, Y., Hayashi, T., and Jannink, J.-L. (2010). Genome-wide association study of grain shape variation among oryza sativa l. germplasms based on elliptic Fourier analysis. *Mol. Breed.* 25 (2), 203–215. doi: 10.1007/s11032-009-9319-2

Iwata, H., and Ukai, Y. (2002). SHAPE: a computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. *J. Heredity* 93 (5), 384–385. doi: 10.1093/jhered/93.5.384

Jirsa, O., and Polišenská, I. (2011). Identification of fusarium damaged wheat kernels using image analysis. *Acta Universitatis Agricult. Silvicult. Mendelianae Brunensis* 59 (5), 125–130. doi: 10.11118/actaun201159050125

Josse, J., and Husson, F. (2016). missMDA: a package for handling missing values in multivariate data analysis. *J. Stat. Software* 70, 1–31. doi: 10.18637/jss.v070.i01

Khaeim, H. M., Clark, A., Pearson, T., and Van Sanford, D. (2019). Methods of assessing fusarium damage to wheat kernels. *Al-Qadisiyah J. For Agric. Sci. (QJAS) (P-ISSN: 2077-5822 E-ISSN: 2617-1479)* 9 (2), 297–308. doi: 10.33794/qjas.Vol9.Iss2.91

Komyshev, E., Genaev, M., and Afonnikov, D. (2017). Evaluation of the SeedCounter, a mobile application for grain phenotyping. *Front. Plant Sci.* 7, 1990. doi: 10.3389/fpls.2016.01990

Kuhn, M., Wing, J., Weston, S., Williams, A., Keefer, C., Engelhardt, A., et al. (2020). Package 'caret'. *R. J.* 223, 7. doi: 10.18637/jss.v028.i05

Lê, S., Josse, J., and Husson, F. (2008). FactoMineR: an r package for multivariate analysis. *J. Stat. Software* 25, 1–18. doi: 10.18637/jss.v025.i01

Lipka, A. E., Tian, F., Wang, Q., Peiffer, J., Li, M., Bradbury, P. J., et al. (2012). GAPIT: genome association and prediction integrated tool. *Bioinformatics* 28 (18), 2397–2399. doi: 10.1093/bioinformatics/bts444

Liu, W., Liu, C., Jin, J., Li, D., Fu, Y., and Yuan, X. (2020). High-throughput phenotyping of morphological seed and fruit characteristics using X-ray computed tomography. *Front. Plant Sci.* 11, 601475. doi: 10.3389/fpls.2020.601475

Lobet, G. (2017). Image analysis in plant sciences: publish then perish. *Trends Plant Sci.* 22 (7), 559–566. doi: 10.1016/j.tplants.2017.05.002

Lu, B., Dao, P. D., Liu, J., He, Y., and Shang, J. (2020). Recent advances of hyperspectral imaging technology and applications in agriculture. *Remote Sens.* 12 (16), 26–59. doi: 10.3390/rs12162659

Menesatti, P., Antonucci, F., Costa, C., Santori, A., Niciarelli, I., and Infantino, A. (2009). Application of morphometric image analysis system to evaluate the incidence of fusarium head blight wheat infected kernels. *Proc. Bornimer Agrartechnische Berichte* 69), 157–161. Available at: https://www.scopus.com/inward/record.uri?eid=2-s2.0-84876834788&partnerID=40&md5=44c21b369e2d5fdc1b165442fd8dbb7d

Mesterhazy, A. (2020). Updating the breeding philosophy of wheat to fusarium head blight (FHB): Resistance components, QTL identification, and phenotyping–a review. *Plants* 9 (12), 1702. doi: 10.3390/plants9121702

Polak-Śliwińska, M., and Paszczyk, B. (2021). Trichothecenes in food and feed, relevance to human and animal health and methods of detection: A systematic review. *Molecules* 26 (2), 454. doi: 10.3390/molecules26020454

Rangarajan, A. K., Whetton, R. L., and Mouazen, A. M. (2022). Detection of fusarium head blight in wheat using hyperspectral data and deep learning. *Expert Syst. Appl.* 208, 118240. doi: 10.1016/j.eswa.2022.118240

Saccon, F. A., Parcey, D., Paliwal, J., and Sherif, S. S. (2017). Assessment of fusarium and deoxynivalenol using optical methods. *Food Bioprocess Technol.* 10 (1), 34–50. doi: 10.1007/s11947-016-1788-9

Schroeder, H., and Christensen, J. (1963). Factors affecting resistance of wheat to scab caused by gibberella zeae. *Phytopathology* 53 (1), 831–838.

Shahin, M. A., and Symons, S. J. (2011). Detection of fusarium damaged kernels in Canada Western red spring wheat using visible/near-infrared hyperspectral imaging and principal component analysis. *Comput. Electron. Agric.* 75 (1), 107–112. doi: 10.1016/j.compag.2010.10.004

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

Stack, R. W., and McMullen, M. P. (1998). *A visual scale to estimate severity of fusarium head blight in wheat.* NDSu Extension Circular: n. pag

Steed, A., King, J., Grewal, S., Yang, C.-Y., Clarke, M., Devi, U., et al. (2022). Identification of fusarium head blight resistance in triticum timopheevii accessions and characterization of wheat-t. timopheevii introgression lines for enhanced resistance. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.943211

Tanabata, T., Shibaya, T., Hori, K., Ebana, K., and Yano, M. (2012). SmartGrain: High-throughput phenotyping software for measuring seed shape through image analysis. *Plant Physiol.* 160 (4), 1871–1880. doi: 10.1104/pp.112.205120

Team, R. C. (2013). *R: A language and environment for statistical computing.*

Wang, L., Uilecan, I. V., Assadi, A. H., Kozmik, C. A., and Spalding, E. P. (2009). HYPOTrace: image analysis software for measuring hypocotyl growth and shape demonstrated on arabidopsis seedlings undergoing photomorphogenesis. *Plant Physiol.* 149 (4), 1632–1637. doi: 10.1104/pp.108.134072

Whan, A. P., Smith, A. B., Cavanagh, C. R., Ral, J.-P. F., Shaw, L. M., Howitt, C. A., et al. (2014). GrainScan: a low cost, fast method for grain size and colour measurements. *Plant Methods* 10 (1), 1–10. doi: 10.1186/1746-4811-10-23

Yipeng, L., Wenbing, L., Kaixuan, H., Wentao, T., Ling, Z., Shizhuang, W., et al. (2022). Determination of wheat kernels damaged by fusarium head blight using monochromatic images of effective wavelengths from hyperspectral imaging coupled with an architecture self-search deep network. *Food Control* 135, 108819. doi: 10.1016/j.foodcont.2022.108819

Zakieh, M., Gaikpa, D. S., Leiva Sandoval, F., Alamrani, M., Henriksson, T., Odilbekov, F., et al. (2021). Characterizing winter wheat germplasm for fusarium head blight resistance under accelerated growth conditions. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.705006

Zhu, F., Paul, P., Hussain, W., Wallman, K., Dhatt, B. K., Sandhu, J., et al. (2021). SeedExtractor: an open-source GUI for seed image analysis. *Front. Plant Sci.* 11, 581546. doi: 10.3389/fpls.2020.581546

10.3389/fpls.2022.1010249

Appendix 1

Quantitative trait loci (QTL) detected in genome-wide association studies employing seven models at $p = 0.0001$ (LOD $\geq$ 4) for Fusarium head blight severity in winter wheat from the breeding, genebank, and combined sets (**Zakieh et al., 2021**). Chr., chromosome; FAF, favorable allele frequencies. The asterisk means also detected by these models at $p = 0.0002$. A, detected above Bonferroni corrected threshold ($\alpha = 0.05$). *B*, the marker effects are estimated for only GLM, MLM, and CMLM and FarmCPU in GAPIT (**Lipka et al., 2012**).

QTL	Marker	Chr.	Position (cM)	FAF	Effect	Model (s)	Set
SLUfhbchr1B.1	BS00021877_51	1B	154.58	0.06	NA	Blink	Combined
SLUfhbchr2A.2	BobWhite_c16923_64	2A	125.33	0.06	NA	Blink; (SUPER)*	Combined
SLUfhbchr3A.3	Kukri_rep_c89183_282	3A	15.05	0.64	27.84 to 28.10	GLM, CMLM	Combined
SLUfhbchr3B.4	wsnp_Ex_c34975_43204180	3B	67.45	0.95 (CS), 0.94 (BS), 0.97 (GS)	65.78 to 82.47	GLM, MLM, CMLM, SUPER, MLMM, FarmCPU, Blink	All
	Kukri_c18009_398a	3B	67.67	0.95	78.20 to 80.15	GLM, MLM, CMLM, SUPER	Combined
	wsnp_Ex_c5378_9505533	3B	68.71	0.94	NA	SUPER	Combined
SLUfhbchr3D.5a	RFL_Contig4591_1759	3D	0.00	0.94	51.94 to 54.69*	MLMM; (GLM, MLM, CLM, SUPER, Blink)*	Combined
	RAC875_rep_c115090_5	3D	0.00	0.02	NA	Blink	Breeding
SLUfhbchr3D.5b	JD_c7714_954	3D	143.01	0.04	NA	Blink, SUPER	Genebank
SLUfhbchr5A.6	RAC875_rep_c106118_339	5A	39.02	0.03	-31.55 to -29.40	GLM, MLM, SUPER, MLMM	Combined
SLUfhbch6A.7	Tdurum_contig46670_911	6A	128.26	0.96	NA	SUPER	Combined
SLUfhbchr7A.8	Kukri_c11530_92	7A	232.11	0.84	44.1	CMLM, SUPER, MLMM	Combined
	RAC875_c12733_1509a	7A	228.37	0.83	40.41 to 45.14	GLM, MLM, CMLM, SUPER, MLMM, FarmCPU, Blink	Combined
SLUfhbchr7B.9	wsnp_Ex_c351_689415	7B	143.23	0.02	NA	Blink, SUPER	Breeding
	RAC875_c8752_1079	7B	158.98	0.84	39.97*	SUPER; (CMLM)*	Combined

Acta Universitatis agriculturae Sueciae
Doctoral Thesis No. 2023:40

This thesis investigated innovative tools to accelerate resistance breeding in winter wheat against Fusarium head blight (FHB) and Septoria tritici blotch (STB). A modified speed breeding protocol shortened the time required to evaluate FHB resistance and enabled the development of genetic markers for FHB resistance. Affordable seed phenotyping method for FHB was also developed. STB resistance was evaluated at the seedling stage in both genebank and breeding germplasm and genetic markers were developed for STB seedling stage resistance and biocontrol-compatibility. The study also emphasizes academia-industry collaboration for practical wheat breeding applications.

Mustafa Zakieh received his graduate education at the Department of Plant Breeding, SLU, Alnarp, Sweden. He holds an MSc in Horticultural Genetics and Biotechnology from the Mediterranean Agronomic Institute of Chania (MAICh), Greece, and another MSc in Biology from Uppsala University, Sweden. He received his BSc in Agricultural Engineering from Damascus University, Syria.

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

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

ISSN 1652-6880
ISBN (print version) 978-91- 8046 -132-0
ISBN (electronic version) 978-91-7760- 132-0