Norwegian University
of Life Sciences

Master's Thesis 2022 60 ECTS
Faculty of Biosciences

Fine-mapping and Characterization of Fusarium Head Blight Resistance QTL on Chromosome 2D in Wheat.

Oda Otilie Holltrø Spongsveen
Genome Science

Acknowledgements

Firstly, I would like to thank my supervisor Morten Lillemo for all the guidance throughout my thesis. Thank you for all the help and for allowing me to work in the field the summer before my thesis, to learn about the practical aspects and enjoy a summer in the field with such interesting work. Your encouragement and excitement was what made this project so fun.

I also want to thank my second supervisor, Min Lin, for helping me with the work along the way, and for always answering my stressed emails and taking the time to guide and teach me so much.

Thank you to the team at Vollebekk for showing me the practical aspects of my thesis project during the field season. I want to especially thank Yalew Tarkegne for making the inoculums used in my experiments and showing me how everything is done step by step.

Thank you to the laboratory technicians Anne Guri Marøy and Sylvia Sagen Johnsen for helping me with my experiments and for doing the genotyping for this research, and to NIBIO for providing me with *Fusarium graminearum* isolates.

I also want to thank UMBs forskningsfond for giving me a travel scholarship, which enabled me to travel to Austria once the pandemic allowed so. I also want to thank Prof. Hermann Buerstmayr and Prof. Barbara Steiner and the rest of their team, for welcoming me and making it possible to visit BOKU University in Tulln, despite all the covid complications and a few scares during my stay. I learned so much from seeing your work and discussing my project, and your advice was very useful for my experiments the second time around.

Lastly, I want to thank my family for encouraging and supporting me from start to finish, and always lending me an ear when I need it.

Abstract

Fusarium head blight (FHB) is a destructive disease in cereals (and other plants) caused by several plant pathogenic species of *Fusarium* spp. In bread wheat (*Triticum aestivum*) it infects the kernels, which greatly impacts the yield and grain quality. Additionally, certain species cause the production of mycotoxins after infections, which are detrimental to the health of humans and livestock. In this project, the focus was on resistance to *Fusarium graminearum*, one of the most common *Fusarium* pathogens in Norwegian wheat production.

The objective of this master project was to fine-map and characterize a resistance quantitative trait locus (QTL) on the long arm of chromosome 2D to further locate resistance gene(s) involved in FHB resistance. A BC_1F_7 mapping population was used, and the search for markers around the QTL of interest was narrowed down based on a literature study on previous QTL mapping studies. Our fine-mapping population was genotyped by these markers, and we investigated recombinations between these to further pin down the region of interest and get more knowledge on the markers. The second part of the project was a point inoculation experiment performed in greenhouse, with the goal of studying the phenotypic effect of the QTL and follow the disease development. Point inoculations allowed us to isolate the phenotypic effects of Type II resistance (resistance to spread within the spike) to FHB. From the QTL mapping we found three genetic markers (*gwm539*, *WGRB3803,* and *wsnp_Ex_c8303_14001708*) in our mapping population which were linked to the QTL. Based on field data from the years 2019, 2020, and 2021, the two markers *gwm539* and *WGRB3803* showed the most significant effect on phenotypic scores. From physical and linkage maps these two markers also appeared to be closest to each other, separated by an area of approximately 6 Mbp, a highly conserved distance among the sequenced pangenome varieties. Comparison of marker alleles in the published wheat pangenome indicated that Norin 61 could be used as a reference genome for this resistance QTL. The point inoculation experiment was also successful after optimizing the *F. graminearum* strains used for inoculum production, finding that aggressive isolates were essential for clear results. Additionally, the point inoculations revealed that there was a clear phenotypic difference between the two near isogenic lines (NILs) with and without the resistance QTL from our mapping population. However, the experiment on NILs with different recombinations between the three markers did not reveal any further details. Further experiments are needed to locate the resistance QTL more closely on chromosome 2DL; however, we have shown that point inoculation experiments can be a useful method for Type II resistance investigation of this QTL.

Sammendrag

Aksfusariose (Fusarium head blight, FHB) er en ødeleggende sykdom på korn og andre planter som forekommer etter infeksjon av ulike typer *Fusarium* spp. Når hvete blir infisert av denne soppen, har det en stor påvirkning på avling og kornkvalitet. Noen typer *Fusarium* produserer i tillegg mykotoksiner i kornet, som er skadelige for både menneskers og dyrs helse. I denne oppgaven var søkelyset på resistens mot *Fusarium graminearum*, som er en av de viktigste *Fusarium*-soppene i norsk hveteproduksjon.

Formålet med denne masteroppgaven var å finkartlegge og karakterisere et resistens «quantitative trait locus» (QTL) på den lange armen av kromosom 2D for å nærmere lokalisere resistensgen(er) involvert i Fusariumresistens. Kartleggingspopulasjonen som ble brukt var en BC_1F_7 populasjon, og leting etter markører i QTL-området ble avgrenset av et litteratursøk på tidligere QTL-kartleggingsforsøk på kromosom 2D. Markørene ble genotypet i kartleggingspopulasjonen, og deretter ble rekombinasjoner mellom markørene undersøkt for å videre plassere QTLet og finne ut mer om markørene. Den andre delen av masterprosjektet var å gjennomføre et punktinokuleringsforsøk i veksthus, der formålet var å undersøke den fenotypiske effekten av QTLet, samt å følge sykdomsforløpet. Ved å gjennomføre punktinokuleringsforsøk var det mulig å isolere kun Type II resistens (resistens mot spredning i akset). Fra QTL kartleggingen ble det funnet tre genetiske markører (*gwm539, WGRB3803* og *wsnp_Ex_c8303_14001708*) i kartleggingspopulasjonen som var koblet til QTLet. Basert på feltdata fra 2019, 2020 og 2021, hadde markørene *gwm539* og *WGRB3803* mest signifikant effekt på fenotype-målingene. Utfra fysiske kart og koblingskart var det tydelig at disse to markørene er plassert nærmest hverandre, med en avstand på omtrent 6 Mb. Denne avstanden var konservert i alle de sekvenserte pangenomsortene. En sammenligning av markøralleler i det publiserte hvete-pangenomet tyder på at genomet til den japanske sorten Norin 61 kan bli brukt som et referansegenom for videre studier av QTLet på kromosom 2D.

Punktinokuleringsforsøket ble en suksess etter optimalisering av *F. graminearum* isolat, der vi oppdaget at et aggressivt isolat var essensielt for tydelige resultater. Punktinokuleringene viste at det var en tydelig effekt av resistens-QTLet i de to nærisogene linjene (NILs) med og uten resistens QTL fra kartleggingspopulasjonen vår. NILs med rekombinasjoner mellom de tre markørene, derimot, viste ingen tydelige resultater. Videre eksperimenter kreves for å plassere QTLet nærmere på kromosomet, men vi har funnet ut at punktinokuleringsforsøk kan være en nyttig metode for å undersøke Type II resistens på dette QTLet.

Table of contents

1 Introduction 1
1.1 Research objectives 2
2 Literature review 3
2.1 Bread wheat and its genome 3
2.2 Genetic markers 4
2.3 Quantitative trait loci and QTL mapping 7
2.4 Marker-assisted selection 8
2.5 Haplotypes 11
2.6 Genomics-based breeding 12
2.7 Fusarium head blight 12
2.7.1 Factors for FHB infection 14
2.7.2 Types of FHB resistance 15
2.7.3 Measures to control FHB 15
2.8 Previous QTL mapping studies 17
2.8.1 Previous studies on chromosome 2D 17
3 Methods 21
3.1 Plant material 21
3.1.1 QTL mapping 21
3.1.2 Point inoculation 22
3.2 Phenotype evaluation in the field 24
3.3 Genotyping and looking for markers 25
3.4 Inoculum preparations 27
3.5 Point inoculation 28
3.4.1 Calculations 30
3.4.2 Analysis of phenotypic data 30
4 Results 31
4.1 QTL fine-mapping for FHB traits 31
4.1.1 Marker identification 31
4.1.2 Marker alleles in the pangenome 36
4.1.3 Physical position of markers in the pangenome 37
4.1.4 Haplotype blocks 37
4.1.5 Fine-mapping status 40
4.2 Phenotypic analysis 41
4.2.1 First point inoculation experiment 41
4.2.2 Second point inoculation experiment 43
5 Discussion 52

5.1 Fine-mapping QTL 52
5.2 Point inoculation and phenotyping 54
5.3 Recommendations 59
6 Future perspectives 61
7 Conclusion 63
Reference list 64
Appendix 1 - Field data for boxplot analysis 77
Appendix 2 - Recombination marker data for linkage map 84
Appendix 3 - Physical position of markers in the pangenome 89
Appendix 4 - Haplotype data 90
Appendix 5 - Physical map marker positions 96
Appendix 6 - Phenotypic scores from first point inoculation experiment 98
Appendix 7 - Phenotypic scores from second point inoculation experiment 103
Appendix 8 - Percentage of diseased spikelets after 18 days post inoculation 116
Appendix 9 - AUDPC values 117

List of abbreviations

Abbreviation	Definition
AE	Anther extrusion
AGE	Agarose gel electrophoresis
ANOVA	Analysis of variance
AUDPC	Area under the disease progress curve
BLAST	Basic local alignment search tool
CAPS	Cleaved amplified polymorphic sequences
CIM	Composite interval mapping
dCAPS	Derived cleaved amplified polymorphic sequences
DH	Double haploid
DNA	Deoxyribonucleic acid
DON	Deoxynivalenol
DPI	Days post inoculation
FHB	Fusarium head blight
FRET	Fluorescent resonance energy transfer
GBS	Genotyping by sequencing
GS	Genomic selection
GWAS	Genome wide association mapping
InDel	Insertion/Deletion
KASP	Kompetitive allele specific PCR
LD	Linkage disequilibrium
LOD	Logarithm of the odds
MAS	Marker-assisted selection
MBA	Mung bean agar
NGS	Next-generation sequencing
NIL	Near-isogenic line
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PDS	Percentage of diseased spikelets
PH	Plant height
QTL	Quantitative trait locus
RF	Recombination frequency
RFLP	Restriction fragment length polymorphism
RIL	Recombinant inbred line
RRS	Reduced representation sequencing
SIM	Simple interval mapping
SNP	Single nucleotide polymorphism
SSR	Single sequence repeat
TR	Tandem repeat
Tukey's HSD	Tukey’s honest significant difference
WGR	Whole genome resequencing
WGS	Whole genome sequencing

1 Introduction

Fusarium head blight (FHB) is a floral disease in crops, caused by several species of the fungus *Fusarium* spp (M. Buerstmayr et al., 2020). The two most common *Fusarium* species to infect wheat in Norway, are *Fusarium graminearum* and *Fusarium columorum* (Hofgaard et al., 2016). The fungus infects the kernels in wheat, making them shrivelled and bleached, affecting yield and grain quality (McMullen et al., 2012). Some species also cause the production of mycotoxins, which are harmful to humans and livestock, and crops with a mycotoxin content above a certain threshold will be disregarded and thrown away (Wegulo et al., 2008). This has a negative impact on the sustainability of food production as well as for the economy, particularly for the farmers.

Fusarium spp. thrives in warm, humid climates. FHB has long been an issue in certain regions of China, particularly the Middle and Lower Valleys of Yangtze River, with the first report in China in 1936. There have been numerous FHB epidemics in these regions since then which has created a high disease pressure. Since the 1990s, it has become a serious issue in European agriculture (Qu et al., 2008; Zhu et al., 2020). Bread wheat (*Triticum aestivum*) is one of the most important crops in the world and is a major source of food and feed worldwide (McMullen et al., 2012; Shude et al., 2020). In wheat breeding, resistance to FHB is an important field of research, especially considering that there are no completely effective fungicides or any fully resistant wheat cultivars available today (H. Buerstmayr et al., 2009).

One method for studying resistance genes and quantitative trait loci (QTL) is through QTL mapping and fine-mapping. This method utilizes a mapping population, which must segregate for the trait of interest. Through phenotyping and genotyping, it is possible to find information about genetic markers linked to the phenotypic expression of the trait of interest (Collard et al., 2005). A magnitude of different genetic markers can be found and further analysed in the population, depending on the techniques available. Fine-mapping a QTL is a more thorough or detailed mapping of a QTL that has already been identified and shown to influence the phenotype. With detailed information about resistance QTL and the genome of wheat cultivars, marker assisted selection (MAS) can be utilized to improve the cultivars used in farmers' fields more rapidly compared to traditional methods (Tester & Langridge, 2010).

1.1 Research objectives

The goal of this project was to fine-map and characterize the FHB resistance QTL on chromosome 2D in bread wheat. Several studies (Chen et al., 2021; Dhariwal et al., 2020; X. He et al., 2016; Hu et al., 2019; Jiang, Dong, et al., 2007; Jiang, Shi, et al., 2007; Long et al., 2015; Lu et al., 2013; Yan et al., 2021; Zhang et al., 2021) have found that this chromosome contains a region with resistance to FHB, but it is not well characterized yet. Therefore, investigating the area for new genetic markers and looking into the properties of this region was the main goal. With previous studies (Jiang, Dong, et al., 2007; Jiang, Shi, et al., 2007) revealing that the QTL is available in the Chinese wheat cultivar CJ9306, the mapping population for fine-mapping was created as a BC_1F_7 population with resistance source from CJ9306 and backcrossing to Zebra, with the goal of finding more genetic markers and further locate the QTL on chromosome 2DL. Moreover, another goal for the project was to perform a point inoculation experiment in greenhouse to closely follow the disease development and determine if there was a clear phenotypic effect of this QTL when isolated from other resistance genes and QTL. This experiment was performed on a subset of the fine-mapping population constructed specifically for this 2D QTL, and could in turn potentially aid in further locating the genes responsible for the FHB resistance at this QTL.

2 Literature review

Crop breeding is important for food and feed production, considering that crops such as wheat, rice and maize are the main food sources for humans and livestock. Plants make up 90% of the energy intake of the human population, where rice, maize and wheat make up two thirds of this (National Geographic Society, 2011). However, there are challenges constantly affecting crop breeding, namely diseases or abiotic factors such as drought, heat, flooding, as a few examples. In addition to dealing with these challenges, we constantly want to improve the yield and productivity of crops due to limited space to grow plants, a growing population and increasing demand for food and feed. Not only is crop breeding necessary for producing enough food and feed, how crops are produced are also a matter of sustainability. With global warming and climate change, the need for a more sustainable agriculture has never been more essential. The Food and Agricultural Organization of the United Nations has set 17 sustainable developmental goals known as the 2030 Agenda for Sustainable Development to work towards this (FAO, 2022). Climate change also causes environmental factors to change faster, requiring breeders to adapt more efficiently.

2.1 Bread wheat and its genome

Bread wheat is the most widely cultivated crop in the world, with a cultivation range spanning from 64° North in Scandinavia and Russia to 45° South in Argentina (Shewry & Hey, 2015). Wheat is also one of the major sources of food for much of the world's population, contributing with nutrients such as protein and B vitamins in addition to carbohydrates and starch (IWGSC et al., 2018; Shewry & Hey, 2015). It contributes to about a fifth of the total calories consumed by humans, meaning that major yield losses affect the world's population both socially and economically.

Agriculture and wheat domestication started about 10,000 years ago. To begin with, only wild diploid wheat species, such as *Aegilops* and *Triticum* species, were used in early farming. However, with evolving agricultural practices, these crops were gradually substituted with domesticated diploid and polyploid wheat varieties (Marcussen et al., 2014). Bread wheat is hexaploid, composed of three related genomes (A, B, and D) from naturally occurring hybridization events, each haploid genome containing 7 chromosomes (IWGSC et al., 2018; Sorrells et al., 2003). The subgenome A was originally derived from *Triticum urartu,* B was derived from an unknown close relative of *Aegilos speltoides* and the D subgenome comes from *Ae. tauschii* (Marcussen et al., 2014). This means that hexaploid bread wheat has three

homoeologous copies of 2x=14 chromosomes in each cell, making a total of 2n=6x=42 chromosomes. According to Marcussen et al. (2014), the A and B genomes diverged from a common ancestor ~7 million years ago, and these genomes gave rise to the D genome through homoploid hybrid speciation 1 to 2 million years later (Marcussen et al., 2014). Wheat is estimated to have a genome size of ~17 Gb and consists of approximately 85% repetitive DNA (IWGSC et al., 2018; Shi & Ling, 2018).

2.2 Genetic markers

Genetic markers are used to detect genes or QTL in the genome. They are close to the gene of interest and the tighter linked, the better the marker will perform (Collard et al., 2005). In fine-mapping studies, there are usually several types of markers being used.

A widely used marker system is simple sequence repeat (SSR) markers, also known as microsatellites, which is a sub-category of tandem repeats (TRs) (Mason, 2015; Vieira et al., 2016). SSR markers are stretches of DNA where the same short nucleotide sequence is repeated multiple times. Polymorphisms in SSR markers are determined by the number of times the sequence is repeated (Mason, 2015). For instance, the two sequences AGTTAGTT vs. AGTTAGTTAGTTAGTT are two polymorphisms of the same marker, where the core sequence is AGTT. They can vary in the number of repetitions at a given locus and are therefore considered highly polymorphic. Some advantages of SSR markers are their abundancy and how widely they are spread across the genome, as well as being multi-allelic and easy to score. They are relatively cheap, and can be genotyped using instruments common in most molecular laboratories (Mason, 2015). Genotyping of SSR markers requires the design of DNA-based primers to amplify the SSR sequences from extracted genomic DNA. These primers are specific to the flanking sequence of the SSR region and can be available in public databases for some of the major crops. For the genotyping, polymerase chain reaction (PCR)-based amplification is most common, as this is the simplest method. However, it is also possible to use next-generation sequencing (NGS), which is a more expensive method. For amplification using PCR, oligonucleotide primers are specific to each side of the SSR region, one forward primer specific to the sequence in the 5`-3` direction, and a reverse primer specific to the sequence in the 3`-5`direction. Lastly, the DNA products are visualized, typically using agarose gel electrophoresis (AGE), in which the DNA products are loaded onto an agarose gel and the fragments are separated by size over time after applying electrical current through the solid gel (Mason, 2015). Smaller fragments travel faster through the gel compared to larger fragments,

creating bands based on size. Alternative visualization methods are polyacrylamide gel electrophoresis (PAGE) and capillary gel electrophoresis. PAGE has higher resolution than AGE but is technically more difficult to perform. Capillary gel electrophoresis uses fluorescent labelling, and the DNA fragments are loaded onto capillary tubes for electrophoresis. Afterwards, the fluorescent dyes are detected using a Sanger sequencing machine (Mason, 2015). Which visualization method to choose depends on the available lab equipment and the needs for the specific experiment, as there are trade-off between costs, specificity, and simplicity.

Another type of genetic marker is cleaved amplified polymorphic sequences (CAPS). Markers are developed based on genetic changes in the restriction enzyme recognition sites of amplification fragments (amplicons), which is typically caused by single nucleotide polymorphisms (SNPs) or insertions/deletions (InDels) (Shavrukov, 2016; U.S. National Library of Medicine, 2017). It is based on three main steps: 1) PCR with specific primers, 2) digestion of amplicons, and 3) using agarose gels to separate digestion products. If the recognition site of an endonuclease is not modified, it will be cleaved and result in two fragments on an agarose gel. Conversely, if the recognition site is modified, the endonuclease will not cut, and this results in only one band on the gel. These markers resemble the restriction fragment length polymorphism analysis (RFLP), except that CAPS use small fragments for amplification, not entire genomes. However, not all markers have mutations occurring in the recognition sites of restriction enzymes. Therefore, a modified method called derived CAPS (dCAPS) was developed, which eliminates the need for the SNP to fall within a recognition site of a restriction enzyme (Neff et al., 1998; Shavrukov, 2016). The modified dCAPS utilizes a restriction enzyme recognition site containing the SNP, which is introduced into the PCR product by a primer containing one or more mismatches to template DNA. The resulting PCR product is then subjected to digestion and the presence or absence of SNP is determined by the restriction pattern on an agarose gel.

Advantages of using CAPS, is firstly the codominant inheritance, which allows for identification of both homozygotes and heterozygotes during genotyping. Secondly is the simple and relatively cheap equipment needed, which is typically available in most molecular biology laboratories. Lastly, CAPS has simple identification of results on an agarose gel. One of the main limitations is that this method is less adaptable for high-throughput systems, as well as needing mutations in the recognition site of endonucleases unless utilizing dCAPS (Neff et al., 1998; Shavrukov, 2016).

Several of the genetic markers mentioned are based on SNPs, but we can also mention SNP genotyping as its own method of detecting genetic markers. SNPs are the most common type of genetic variation in a genome and is therefore a popular genetic marker system. SNP arrays are useful for studying small variations between genomes, and the method consists of three main steps: allele discrimination, amplification using PCR, and lastly allele detection (Kim & Misra, 2007). There are several technologies available, two of these being the Affymetrix and Illumina SNP arrays (LaFramboise, 2009). Both technologies rely on the biochemistry causing complementary base pairs to bind to each other, and the hybridization of hundreds of thousands of unique nucleotide probe sequences. Each probe is designed to bind to a target DNA sequence, which allows for discrimination of alleles. The underlying principle of SNP array genotyping is that the signal intensity from the arrays depends on the amount of target DNA in the sample, in addition to the affinity between target and probe (LaFramboise, 2009). Among the most used SNP arrays in wheat are the Illumina iSelect 90K wheat array (S. Wang et al., 2014), the 35K Axiom wheat breeders array (Allen et al., 2017), and the custom 25K Illumina array from TraitGenetics (currently not published).

Kompetitive Allele Specific PCR (KASP) is a novel SNP genotyping method based on dual fluorescent resonance energy transfer (FRET) (Zhao et al., 2017). The main components of this methodology are amplification of DNA using allele-specific primers, adding fluorometric dyes HEX and FAM to the primers, and then hybridizing the DNA to the FRET cassette. The hybridization causes fluorometric dye and quencher to be separated, which in turn leads to the corresponding fluorescence being emitted, allowing for easy detection of genotypes based on fluorometric signals (Zhao et al., 2017). KASP has low costs, is a high throughput method and gives high specificity and sensitivity, which is the reason for its popularity in large SNP genotyping studies with few markers. It uses a single-plex method where one marker is genotyped at a time. Consequently, KASP is not the most cost-efficient method for genotyping a large quantity of markers, in this case SNP arrays are cheaper.

Lastly, a method which is becoming increasingly popular with decreasing sequencing costs, is genotyping by sequencing (GBS). Although there are numerous different molecular markers being used routinely in plant breeding, limitations such as availability and the high cost for large scale analyses, opens a need for different methods (J. He et al., 2014). NGS has revolutionized sequencing technologies to become cheaper and more accessible. There are two main strategies for NGS, 1) whole genome resequencing (WGR) and 2) reduced representation sequencing (RRS) (N. Wang et al., 2020). Whereas WGR sequences the entire genome, the

RRS library consists of only a subset of the genome which can be sequenced more in depth, this subset usually being the transcriptome (Van Tassell et al., 2008). The main difference between the two, is that WGR avoids the biases that comes with RRS, while RRS is a much cheaper method (N. Wang et al., 2020). Another reason to choose only the transcriptome instead of the entire genome, is that there is a larger probability of the genes being actively expressed (transcriptome) having an association to the phenotype, compared to DNA polymorphisms in regions without expressed genes. An example of this method being used in crops, is the study by Barbazuk et al. (2007), where they used 454 transcriptome sequencing to look for SNPs in maize (Barbazuk et al., 2007).

GBS is one of the most widely used types of RRS. It has an improved barcoding system that allows for multiplexing the sequencing reactions and detection of SNPs at low cost with a low error rate (N. Wang et al., 2020). Two different strategies have been developed for GBS, restriction enzyme digestion and multiplex enrichment PCR. Restriction enzyme digestion is not based on specified SNPs and is mainly used for detection of new markers for MAS. Particularly methylation-sensitive restriction enzymes are used, as this leads to amplification of DNA containing transcribed genes (J. He et al., 2014; Pootakham et al., 2016). Multiplex enrichment PCR, on the other hand, is used when SNPs have been identified for the region of interest and uses PCR primers to amplify this area. Some advantages of GBS are low costs, reduced sample handling, and fewer PCR and purification steps. Additionally, there is no need for size fractioning, no reference sequence limits, while allowing efficient barcoding and an easiness to scale up (J. He et al., 2014). It can also be applied to crop species with a poorly characterized genome (Pootakham et al., 2016).

2.3 Quantitative trait loci and QTL mapping

A quantitative trait locus (QTL) is a segment of a chromosome that correlates with the variation of a quantitative trait in the phenotype of a population. It has been described by Geldermann (1975) as “a region of the genome associated with an effect on a continuous trait” (Arrones et al., 2020; Geldermann, 1975). A QTL can span large regions and include one gene or a cluster of genes and can be detected by looking for polymorphisms between markers, investigating segregation and linkage.

QTL mapping starts with a segregating population, also known as a mapping population, which usually consists of random progenies from a cross between two parent genotypes with

contrasting phenotypes (Collard et al., 2005). This population is both phenotyped and genotyped, and the results can be used to construct a linkage map. A linkage map is based on the recombination rates between the markers and will show the relative distances between them. Additionally, you can perform a QTL analysis using either single-marker analysis, simple interval mapping (SIM), or composite interval mapping (CIM) (Collard et al., 2005). Single-marker analysis tests the statistical association of a marker with the phenotype without using linkage map information, usually using statistical tests such as t-test, analysis of variance (ANOVA) and linear regression. This is a simple analysis, but not the most accurate. SIM and CIM are improvements that include modelling of recombinations between marker and QTL and calculate a logarithm of the odds (LOD) score which tells us the likelihood of the QTL being located on different positions between the markers. The difference between the two is that CIM combines interval mapping with regression analysis, giving a more accurate estimate by reducing the background "noise" as it considers genetic variation across the genome (Collard et al., 2005).

The advantages of performing a conventional QTL mapping with bi-parental mapping populations is that it is useful for discovering rare alleles, and these often have a major effect on the trait. However, there are limited recombinations, considering that the mapping populations usually have few crossings (Pascual et al., 2016). It is also difficult to discover closely linked markers or genes, and you need to perform additional steps to narrow down a QTL, i.e. fine-mapping. Fine-mapping of a QTL typically involves a much larger mapping population than for a normal QTL mapping study (>1000 progenies) in order to sample many recombination events, and use of a homogeneous genetic background to mendelize the QTL (Collard et al., 2005).

2.4 Marker-assisted selection

Marker-assisted selection (MAS) is an indirect method of selection based on closely linked markers to the gene or QTL of interest. It uses genetic variation to track regions of the genomes during crossing and selection (Tester & Langridge, 2010). MAS is dependent on knowledge of available genetic markers for desired traits (Collard & Mackill, 2008). Therefore, QTL mapping is usually the basis for MAS. By utilizing MAS, it is possible to select individual plants based on genotype. This is useful for different breeding strategies because it is not possible to distinguish between homozygote and heterozygote for most traits purely based on the phenotype. Moreover, MAS allows for selection of traits without phenotyping and can also

accelerate the creation of backcross mapping populations. Another great advantage of using MAS is when target traits have low heritability, are recessive, involve complex phenotyping, and where pyramiding is desired (Tester & Langridge, 2010). In these instances, MAS is cheaper compared to phenotyping-based methods. A representation of a typical pipeline for MAS is shown in **Figure 1**.

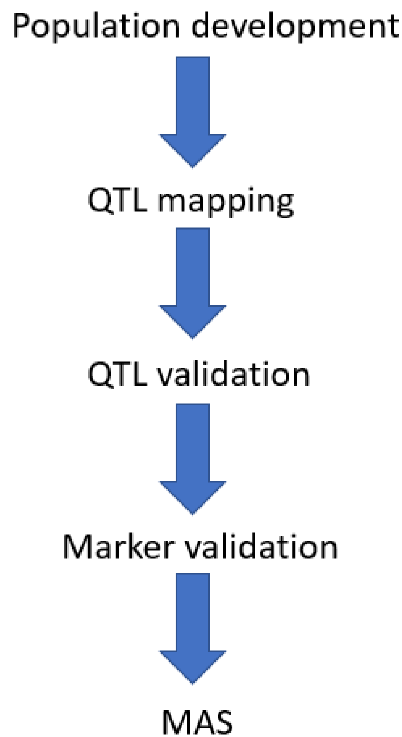

Figure 1: Pipeline for a typical marker-assisted selection (MAS), interpreted from Collard & Mackill (2008).

When choosing markers to use for MAS, there are five considerations to take into account (Collard & Mackill, 2008). The first is the reliability of the markers, meaning how tightly linked are the markers to the target loci, preferably with a genetic distance less than 5 cM. The more tightly linked, the more likely it is that the marker will follow the target allele during meiosis. Second is DNA quality and quantity, as some methods require high amounts and/or high quality of DNA. Technical procedure is also important to consider. High-throughput and quick methods are often preferable to save time and give fast results. Then, the level of polymorphisms needs to be considered. It is desirable to have markers with many polymorphisms between them because this makes it easier to distinguish genotypes. The last factor is the cost of the markers (Collard & Mackill, 2008). When working with genomics in breeding, technologies can quickly become expensive, and it must always be a factor of consideration.

An alternative method for QTL mapping is genome-wide association studies (GWAS). This is a method for studying the genetic basis of desired phenotypic traits using the naturally occurring diversity on a genome-wide scale (Gali et al., 2019). GWAS has been used in several wheat disease resistance studies (Crossa et al., 2007; Edae et al., 2014; Lopes et al., 2015; Sukumaran et al., 2015; Yu et al., 2012), and have certain advantages over the standard bi-parental QTL mapping. The main advantages of GWAS are higher resolution for common alleles, due to the diverse germplasm being used. QTL for many traits can be detected with a high resolution in the same study, making GWAS more efficient and less expensive compared to bi-parental QTL mapping (Edae et al., 2014; Pascual et al., 2016). However, GWAS is less precise when it comes to rare alleles, so QTL mapping would be preferable in this case. Many resistance genes used for MAS are typically rare alleles, which is a reason why bi-parental QTL mapping is still commonly used (Pascual et al., 2016). Additionally, for breeding purposes it is often just one QTL being investigated at once, making GWAS too complex for the study in question. Due to the large scale of GWAS and usually diverse breeding history of the lines being analysed, there is a risk of population structure, which can lead to false associations. Therefore, it is necessary to determine the genetic relatedness in the diversity panel, which makes this method more complex. QTL mapping populations might take longer to create, as these are typically recombinant inbred lines (RILs) which have been selfed for several generations to become homozygous, which is not necessary for GWAS. However, QTL mapping is a faster and often cheaper method, at least when focusing on only one or a few QTL at a time. Another important factor determining which of these methods would be best fitting to the study in question, is the linkage disequilibrium (LD) in the population. LD greatly effects the population structure within a GWAS, so a population with high LD in the region of interest might not be easy to investigate using GWAS (Edae et al., 2014; Pascual et al., 2016). In summary, bi-parental populations are typically formed for specific traits, whereas GWAS is used to phenotype different traits and genotypes at once, according to the genetic diversity of the traits in the population (Sukumaran et al., 2015).

Once a QTL has been detected from RIL population studies, there is still much work needed to further locate and characterize a resistance gene. One alternative is fine-mapping using near-isogenic lines (NILs). The mapping population used for QTL mapping, or typically a much larger population of >1000 progenies is screened or genotyped for recombinations between markers flanking the QTL. The individuals with recombinations in the regions of interest will typically be backcrossed to create a NIL population, which is used to see if there are significant

differences in phenotype between the different recombinations (Xue et al., 2011). This type of population is usually derived from a RIL population, and consists of lines which are identical, except for the QTL of interest. Using NILs allow for more detailed detection, as it can measure allelic variation at one locus only. Due to the lines being identical outside this region, they eliminate any background genetic variation. One of the fine-mapped FHB resistance QTL in wheat is *Fhb5* on chromosome 5A. A few examples of studies using NILs for fine-mapping this QTL are Steiner et al. (2019), Jia et al. (2018), and Xue et al. (2011).

In contrast to the regular MAS, a method called genomic selection (GS) has potential to be better suited for quantitative traits governed by many small-effect loci. GS is a type of MAS which utilizes genetic markers across the entire genome, resulting in models that capture all QTL that are in LD with at least one marker (Bhat et al., 2016). It is a method that can increase genetic gain of complex traits considering the time and cost. Combining GS with whole genome sequencing (WGS) could be an ultimate approach to finding genetic markers across a genome, but this is still rather expensive. Therefore, targeted sequencing or SNP array genotyping are mainly used as genotyping methods for GS (Bhat et al., 2016).

2.5 Haplotypes

Marker selection is often based on allelic variations in the germplasm, and breeders will often make targeted crossings to exploit the recombination that occurs during meiosis to obtain different allelic combinations for genes of interest. However, alleles are not necessarily inherited independently, but rather as a set of genes (Walkowiak et al., 2020). Haplotypes are these combinations of genetic polymorphisms which are co-inherited from one generation to the next (Lesk, 2017). Investigating haplotypes makes it easier to look for genes responsible for phenotype-genotype relations in the genome. Lesk (2017) compares haplotypes with a magnifying glass, if you can find the haplotype correlated with a phenotype, you only need to study this region of the genome sequence to find your gene, making the search much easier (Lesk, 2017). An example of how haplotypes can be used is from Walkowiak et al. (2020), who created a haplotype database for the published wheat pangenome in order to study and characterize the locus with resistance to the orange wheat blossom midge (Walkowiak et al., 2020).

2.6 Genomics-based breeding

Genomics-based breeding is the use of genomic tools to assist in breeding. Following the Green Revolution in the 1960s, technology has been an integral part of modern breeding (Arrones et al., 2020). With new genomic technologies advancing rapidly over the last few decades, breeding has become more efficient and specialized. There have been many previous technologies aiding the crop production and breeding, but these have mainly focused on monogenic traits. However, many of the major agronomic traits of interest for crop breeding are quantitative, controlled by many loci, and heavily affected by the environment (Arrones et al., 2020). Newer methods are continuously being developed to solve issues in breeding.

2.7 Fusarium head blight

Fusarium head blight (FHB) is a floral disease that affects cereal crops in many areas in the world. It is a result of an infection of the genus of fungi known as *Fusarium* spp. The most common species to infect wheat are *Fusarium graminearum* and *Fusarium culmorum,* but other *Fusarium* species are also responsible for FHB. Infection occurs when the fungus reaches the kernels of cereal crops, such as wheat, barley, and oats. Once infected, the grains become toxic for humans and animals, resulting in major yield loss due to shrivelled grains for farmers with infected crops (M. Buerstmayr et al., 2020; X. He et al., 2016; Lu et al., 2013).

The *Fusarium* pathogen is opportunistic, as it lives as a saprophyte on plant debris in the field, and then infects the heads during the limited time window around flowering (M. Buerstmayr et al., 2020). It infects cereal crops by attacking the kernels, either stopping the development, or making them shrivelled and bleached. This results in lower yield for the farmers and a lower quality of the wheat (McMullen et al., 2012). FHB is a monocyclic disease, meaning that it does not spread to other plants in the field within a season (Wegulo et al., 2008). This is because *Fusarium* infects the plant during flowering, and the life cycle of the fungus is longer than the flowering period. It will therefore not have enough time to develop spores from new infections before the infection window is over.

In addition to lower yield, certain *Fusarium* species that are the most common in farmers' fields, such as *F. graminearum*, also produce mycotoxins in the kernels, the most prominent being deoxynivalenol (DON). DON is a mycotoxin harmful to humans and animals. It can lead to feed refusal and poor weight gain in farm animals, as well as immunological and teratogenic problems in humans (McMullen et al., 2012). This means that even the kernels without visible infection could be discarded, as wheat with a DON level above a certain threshold is not

approved for human or animal feed (Wegulo et al., 2008). Therefore, FHB in crops is an important problem in food production and food safety. Considering the harmful nature of *Fusarium* induced mycotoxins, regulations have been put in place for the maximum level of DON accepted in wheat. In the EU, this limit for food production is 1250 µg/kg for unprocessed wheat, which is the regulations used in Norway as well (*EØS-tillegget til Den europeiske unions tidende*, 2007). In a study spanning the six years from 2004 to 2009 in Norway, it was found that the main producer of DON in spring wheat was *F. graminearum*, taking over after *F. culmorum* which was the most prevalent *Fusarium* fungus in wheat in Norway the previous years (Hofgaard et al., 2016).

FHB has been a well-known disease in cereal crops since the end of the 19th century (M. Buerstmayr et al., 2020). It has typically been an issue in warm, humid climates, and found therefore in many areas in Asia and some states in the USA. However, it has become an increasingly large issue in food production across the world, including many European countries in later years (McMullen et al., 2012). FHB ranks as number two on the list of most damaging wheat diseases on a global scale and is a major disease in all crops (Savary et al., 2019). According to a study by McMullen et al. (2012), which is mostly based on the USA, billions of dollars of wheat and barley yield and quality was lost due to FHB in the 1990s and 2000s. The economic impacts for the farmers themselves can be rather large if *Fusarium* is detected in their crops. In these instances, there are price reductions for the wheat with different DON levels (Felleskjøpet, 2022). As mentioned, the threshold for wheat for food production in the EU and Norway is 1250 µg/kg, and any yield with a DON content above this will be used for feed and have a price reduction. Additionally, any cereals with visible *Fusarium* infection will automatically be used for feed instead of food production. Yield downgraded from food to feed results in a price reduction of 0.60-0.80 NOK/kg depending on which price class the cultivar belongs to (Felleskjøpet, 2022). High levels of DON (>1999 Mg/kg) causes additional price reductions. For DON levels between 2000-4999 µg/kg, there will be a reduction of 0.10 NOK/kg (Norwegian currency), while DON levels ≥15000 µg/kg causes a price reduction of 1.00 NOK/kg.

Cereal crops make up most of the world's food intake and are among the most important crops we grow. Wheat is the number one food crop consumed each year, with 65 kg consumed per person per year. Additionally, if we are to meet the needs of the increasing world population estimated to be 9.6 billion in 2050, wheat production needs to be increased with 60 % (IWGSC, 2018). Animal feed is also dependent on cereals, particularly for farm animals such as pigs,

poultry, and cattle. With an increase in the world population as well as global warming causing more countries to move towards warmer climates, FHB remains a significant problem. However, there are no easy solutions to this issue, as there are no fully effective fungicides, nor any fully resistant cultivars on the market (H. Buerstmayr et al., 2009). Finding solutions is essential if we want to grow crops more sustainably as well as ensuring food safety for humans as well as animals.

2.7.1 Factors for FHB infection

Fusarium infection usually varies from year to year due to environmental variations (McMullen et al., 2012). FHB is an airborne disease, meaning that the ascospores are windblown or splashed with the rain onto the spikes of wheat. The fungus can survive as saprophytes on crop residue, such as small grains and maize or other plant surfaces, without causing disease (M. Buerstmayr et al., 2020; McMullen et al., 2012; Wegulo et al., 2008). *Fusarium* fungi surviving on crop residues are able to grow and sporulate well if the growing season coincides with long periods of moist weather (McMullen et al., 2012).

There are three main parts central to FHB infection and development. The first is the abundance and aggressiveness of inoculum around anthesis, as wheat heads are susceptible from anthesis until the soft dough stage (Wegulo et al., 2008). The environmental condition during this critical stage is also a central factor. Favorable conditions for fungal growth are prolonged periods, 48-72 hours of high moisture or relative humidity up to 90%. Additionally, moderately warm temperature, between 15-30°C, frequent rainfall and air currents favor *Fusarium* growth (Shude et al., 2020). The last central part is the susceptibility or resistance status of the plant (M. Buerstmayr et al., 2020). Resistant varieties are an essential alternative to using large quantities of fungicides, especially from a sustainable perspective. Furthermore, it has been shown that the use of fungicides is more effective when used on moderately resistant cultivars, compared to susceptible cultivars (M. Buerstmayr et al., 2020).

Morphological traits that contribute to resistance in addition to the genetic resistance, are plant height (PH) and anther extrusion (AE) (M. Buerstmayr et al., 2020; Lu et al., 2013). PH can affect the level of infection due to the spores surviving on plant debris on the ground. In this case, taller plants are preferred as this longer distance becomes a larger barrier for infection. Shorter plants are also exposed to a different microclimate. Closer to the ground, there is higher humidity which adds to favourable FHB conditions (M. Buerstmayr et al., 2020). It has been

shown that certain dwarfing alleles, more specifically the two gibberellin-insensitive semi-dwarfing alleles *Rht-B1b* and *Rht-Db1* located on chromosomes 4B and 4D respectively, also lead to increased FHB in plants while reducing plant height (M. Buerstmayr et al., 2020). However, shorter plants are agronomically desirable, so choosing plants with dwarfing alleles neutral to FHB is advisable. An alternative dwarfing gene is located on chromosome 6A, called *Rht24* (Würschum et al., 2017). This is a common dwarfing gene found in European winter wheat and causes a considerable reduction in plant height without increasing FHB susceptibility (Herter et al., 2018).

AE is also an important factor involved in FHB infection, as *Fusarium* spores are spread through the air currents and through water splashing. Anthers left in the opening between palea and lemma create a window for the spores to enter and infect. Additionally, the anthers become a source of food for the fungus to grow on. Therefore, high anther extrusion is preferred for resistance (M. Buerstmayr et al., 2020; Lu et al., 2013).

2.7.2 Types of FHB resistance

FHB resistance in wheat is a polygenic trait, meaning that there are more than one gene controlling resistance to FHB. The resistance is a complex trait itself, as it is both influenced heavily by inheritance and genotype-by-environment interactions. Several types of resistance have been suggested, however Schroeder and Christensen (1963) first suggested the two initial types of resistance, Type I and Type II resistance (M. Buerstmayr et al., 2020; X. He et al., 2016; Schroeder & Christensen, 1963). Type I is the resistance to initial infection. This can include morphological traits, such as height or tight spikelets, and is typically affected by environmental factors. The second type of resistance, Type II, is resistance to the spread of the disease after initial infection, which can typically be controlled by underlying genetics rather than morphological traits. Additionally, three more types of resistances have been suggested. Type III resistance is to toxin accumulation, Type IV to kernel infection, and Type V to yield reduction (Mesterházy et al., 1999; Miller & Arnison, 1986).

2.7.3 Measures to control FHB

Considering that there are no completely resistant cultivars, nor any fully effective fungicides against FHB, it is necessary to combine several control measures to reduce the FHB infection in the field (Buerstmayr et al., 2009). An important control measure is the amount of inoculum

in the field, which can be limited by rotating crops (Wegulo et al., 2008). *Fusarium* can survive on plant debris on the soil, so cultivating crops less affected by FHB between growing FHB susceptible crops can reduce the infection from year to year. Another option is to plough the soil to remove most of the plant debris remaining on the ground. This is effective for FHB, but can have negative agricultural effects, such as reducing soil structure quality. In addition to these agricultural control measures, fungicides can reduce the FHB infection in the years with heavy infections if used in years with humid conditions around flowering. Wegulo et al. (2008) mention two fungicides available for FHB control, prothioconazole and propiconazole. In Norway, only prothioconazole based fungicides are used, and they have shown to give an average decrease in FHB severity by 50% if sprayed during flowering (Edwards & Godley, 2010; Elen et al., 2009). These are used to suppress FHB, as they are unable to completely eradicate the fungus (Wegulo et al., 2008). Lastly, the choice of cultivar is essential to reduce FHB, and is the most sustainable measure to take. Some cultivars are already available and have been important to reduce FHB infections so far. However, FHB is still a large issue, and during disease heavy years, these cultivars are still not completely resistant.

Resistance levels in wheat cultivars in Norway have improved over the last ~15 years, due to consistent phenotyping of elite breeding lines and newer cultivars. **Figure 2** is a representation of the improvement of resistance levels in Norwegian cultivars, based on resistance testing data from 2007 to 2020. The experiments were performed using spawn inoculation in the field, using the same method as a screening of oat accessions by Tekle et al. (2018). Sumai 3 is the cultivar with the currently highest FHB resistance but is agronomically poor. It is clear to see from **Figure 2** that the Norwegian breeding programs have improved FHB resistance, with Mirakel and Caress having 40% reduction in mycotoxin levels compared to Zebra.

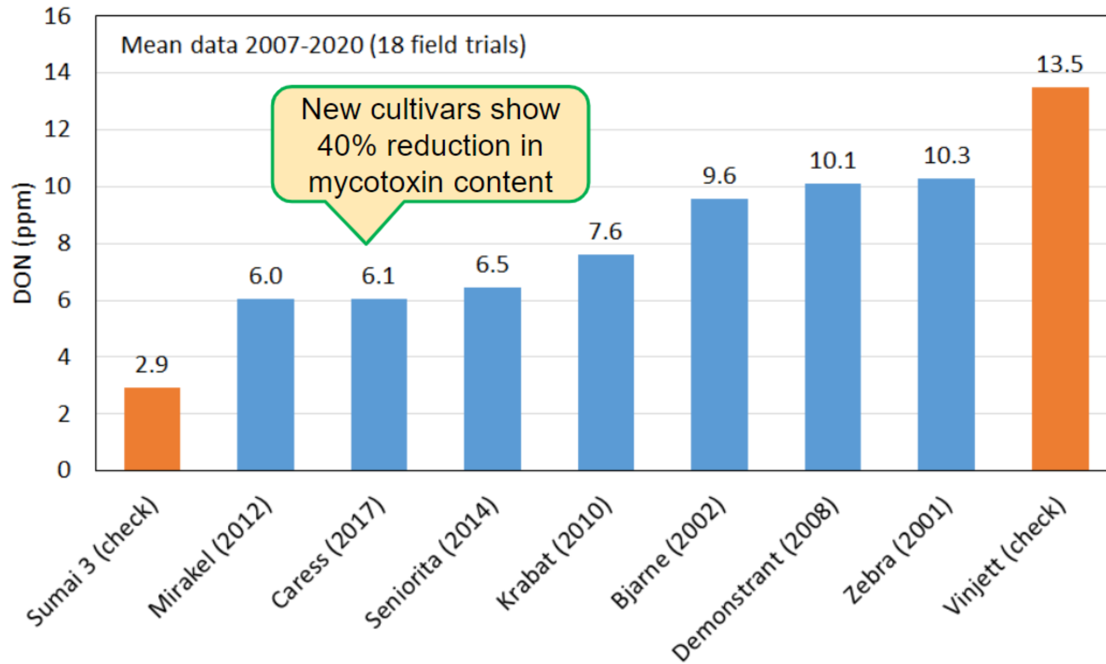

Figure 2: Resistance level of wheat cultivars being used in Norway based on data from 2007 to 2020. The resistance level is evaluated based on DON levels in ppm and the release years of cultivars are shown in parentheses (Lillemo, unpublished).

2.8 Previous QTL mapping studies

FHB resistance in wheat is a highly complex trait to map, partly due to the complexity of the resistance itself, but also due to the complexity of the wheat genome. A magnitude of genetic studies have been reported on FHB resistance in wheat. For FHB in general, approximately 500 QTL have been reported, where roughly estimated 20% are major QTL and 80% are minor QTL (M. Buerstmayr et al., 2020). Only 20 of these 500 QTL have been validated. Fine-mapping has been done on 8 well-established QTL, these being *Fhb1*, *Fhb2*, *Fhb4*, *Fhb5*, *Qfhs.ifa-5A*, *Qfhs.ndsu-3AS*, *Qfhb.nau-2B*, and *Qfhb.mgb-2A* (M. Buerstmayr et al., 2020). These are the most well-known QTL for FHB resistance currently and have been proven valuable for resistance-breeding. However, FHB resistance is affected by many smaller QTL as 80% of those reported are minor QTL. Therefore, there is much need to continue mapping and fine-mapping QTL for FHB resistance.

2.8.1 Previous studies on chromosome 2D

To summarize the most important studies in recent years surrounding the QTL on wheat chromosome 2DL, an overview is presented in **Table 1**. These studies are the ones which appear to have localized FHB resistance QTL in the same area on chromosome arm 2DL studied in this project.

Table 1: Overview of discovered resistance QTL and their closest markers found on chromosome 2DL in previous papers. The overview contains resistance types, resistance source, the populations' parental lines, QTL name, marker name with start and stop position in bp, and lastly which paper reported the QTL. Marker positions for *AX-110955068* and *AX-109419238* are based on their positions in cM from the linkage map, calculating the physical position based on marker *WGRB3803* which was also included in Chen et al. (2021) region of interest.

Resistance type	Resistance source	Population	QTL name	Markers	Start position (bp)	End position (bp)	Paper
Type II and III	CJ9306	Veery x CJ9306	*QFhs.nau-2DL*	*Xgwm539*	513098578	513098599	Jiang, Dong, et al. (2007)
Type II	CJ9306	Veery x CJ9306	*QFhs.nau-2DL*	*Xgwm539*	513098578	513098599	Jiang, Shi, et al. (2007)
Type I and II	Soru#1	Soru#1 x Naxos	-	*Kukri_c36639_186*	574351948	574352048	He et al. (2016)
Type I and II				*Excalibur_c7282_512*	571217662	571217681	
Type I and II				*gwm539*	513098578	513098599	
Type II	Wuhan-1	Wuhan1 x Nyubai	*Traes_2DL_179570792, UN25696*	*Ku_c19185_1569*	461301312	461301212	Hu et al. (2019)
Type II				*cfd233*	561157752	561157470	
Type I and II	ACC Tenacious	AAC Innova x ACC Tenacious	*QFhb.lrdc-2D.2**	*BobWhite_c17782_194*	555098707	555098805	Dhariwal et al. (2020)
Type III	ACC Tenacious	AAC Innova x ACC Tenacious	*Qdon.lrdc-2D.2*	*BobWhite_c17782_194*	555098707	555098805	
Type I and II	ACC Tenacious	AAC Innova x ACC Tenacious	*QFhs.lrdc-2D.2*	*BobWhite_c17782_194*	555098707	555098805	
Type I	Yangmai 158	Annong 8455 x Veery	*Qfhi.nau-2D*	*WGRB3753*	516638960	516638979	Yan et al. (2021)
Type I				*WGRB3803*	519126074	519126093	
Type I and II	Wuhan-1	HC374 x BW301	*Ta.25696.1*	*gwm539*	513098578	513098599	Long et al. (2015)
Type I and II				*gpw8003*	478111878	478111860	
Type I and II				*cfd73*	553728845	553728826	
Type I and II				*cfd233*	561157752	561157733	
Type II	Yangmai 13	N553 x Yangmai 13	*QFhbp-hnau.2DL, QFhbs.hnau.2DL, QFhbn-hnau.2DL*	*AX-110955068*	517469774*	517469793*	Chen et al. (2021)
Type II				*AX-109419238*	519771174*	519771193*	
Type I	SHA3/CBRD	SHA3/CBRD x Naxos	-	*Xgwm539*	513098578	513098599	Lu et al. (2013)

The first QTL detected on chromosome arm 2DL was in Wuhan-1, a moderately resistant wheat variety with Chinese origins (Somers et al., 2003; Zhu et al., 2020). Since then, many other studies have published resistance QTL in this region. The primary background for the fine-mapping population in this project were the two papers by Jiang, Dong, et al. (2007) and Jiang, Shi et al. (2007) which showed that there was a significant FHB resistance QTL on chromosome 2DL in the CJ9306 germplasm, this being the basis for creating a mapping population with a resistance source from CJ9306 in our experiments. They found the QTL *QFhs.nau-2DL* in both studies, which was significant for both Type II resistance and DON accumulation (Type III resistance) (Jiang, Dong, et al., 2007; Jiang, Shi, et al., 2007). They did find a stronger Type III resistance, but it was not independent of Type II resistance (Jiang, Dong, et al., 2007). Additionally, another important background for the fine-mapping population was the results from the two papers by Lu et al. (2013) and He et al. (2016), which found a strong and consistent QTL on 2DL in the Chinese-derived resistance sources SHA3/CBRD and Soru#1 that coincided with the previously published 2DL QTL from CJ9306 (Jiang, Dong, et al., 2007; Jiang, Shi, et al., 2007). Both papers found that the QTL for FHB resistance must be located around the SSR marker *gwm539*, with a physical position around 519 Mbp. Given this, these are presumably the same QTL.

More FHB resistance QTL and associated markers have been found on chromosome 2D in wheat in the last decade. Long et al. (2015) found several candidate genes with correlations to the QTL, but there was only one located on chromosome 2DL, which was *Ta.25696.1* (Long et al., 2015). This candidate gene also showed consistent expression profile and higher expression level in four additional breeding lines to Wuhan-1. The markers used by Long et al. (2015) vary in physical position, some located near the area we are interested in (such as *gwm539* already reported by He et al. (2016) and Lu et al. (2013)), and some far away. Hence, the candidate gene can be in the QTL of interest on 2DL. Moreover, Hu et al. (2019) found one gene (*Traes_2DL_179570792*) with complete overlap of the mapping interval for the 2DL QTL. Additionally, *UN25696* mapped near the mapping interval for 2DL, but did not overlap and is therefore less likely an important gene (Hu et al., 2019). Followingly, Dhariwal et al. (2020) found several major QTL (*Qfhi.lrdc-2D*, *Qdon.lrdc-2D.2, Qfhb-lrdc-2D.1*, and *Qfhb.lrdc-2D.2*), all located near the marker *BobWhite_c17782_194*. This marker is slightly further away from *gwm539* but could still be linked to the same QTL. They remark that the QTL on chromosome 2DL has been detected repeatedly across different backgrounds and with high

levels of expression, indicating that it might be a very important QTL for FHB resistance breeding.

In 2021, two papers reported finds on similar positions on chromosome 2DL. Yan et al. (2021) used two Yangmai 158 derived RIL populations from crosses with the susceptible cultivars Annong 8455 and Veery for QTL detection. None of the cultivars contained the well-characterized QTL *Fhb1*, *Fhb2*, *Fhb4*, and *Fhb5*. In their populations, they found a QTL on 2DL (*Qfhi.nau-2D*) flanked by the two markers *AX-110423675* and *AX-1115380*. Furthermore, Chen et al. (2021) reported a QTL (*QFhb-hnau.2DL*) on chromosome 2DL in the region flanked by the two markers *AX-110955068*, and *AX-109419238*, a region which includes *AX-11151380* (also known as *WGRB3803*), which is the same marker as found in Yan et al. (2021). This QTL was derived from Yangmai 13, and Chen et al. (2021) report that this could be a novel QTL stable for both Type I and Type II FHB resistance. *QFhb-hnau.2DL* was not the QTL they found with the strongest effect, but it seemed to be consistent across three different experiments. Chen et al. (2021) note that Yangmai 13 is from Italian pedigree and does not share lineage with Wuhan-1 or CJ9306, both of Chinese origins, indicating that this QTL could be novel. On the other hand, Zhu et al. (2020) also mention mapping the QTL to cultivars with Italian resistance sources, meaning these could report the same QTL (Zhu et al., 2020).

Yangmai 158 is a widely used Chinese cultivar with good agronomic traits as well as a moderate FHB resistance in the field (Yan et al., 2021; Zhang et al., 2021). As mentioned, FHB resistance in the same area on 2DL has also been found in the Chinese cultivar Wuhan-1, but this is known to have poor agronomic traits, making it difficult to incorporate in modern cultivars. If the same QTL can be found in Yangmai 158, this could be a solution for breeders to create more FHB resistant cultivars. Furthermore, several of the markers and QTL reported in this section have their source in Asian cultivars. The Middle and Lower Valleys of Yangtze River in China, is an area with severe *Fusarium* epidemics and an area where FHB resistance is particularly important for breeders (Zhang et al., 2021). Therefore, Asian cultivars are more likely to contain FHB resistance QTL and germplasm of interest for QTL mapping.

3 Methods

3.1 Plant material

3.1.1 QTL mapping

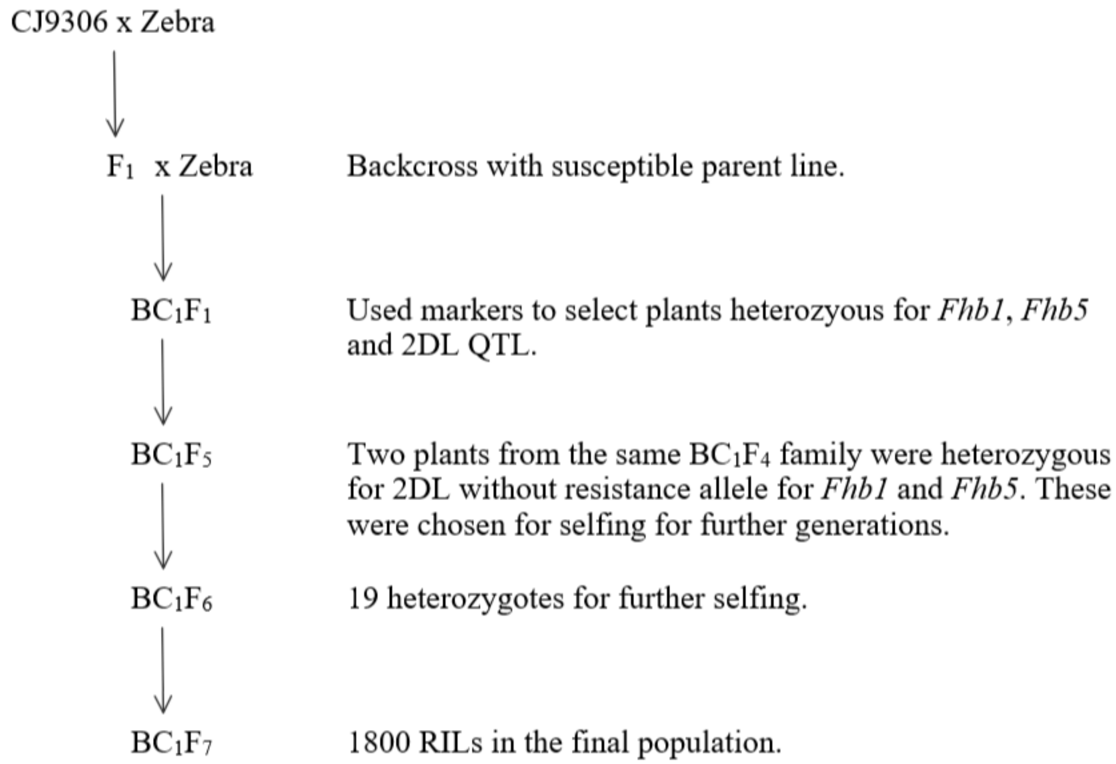

Figure 3: Crossing scheme for the BC_1F_7 mapping population used for fine-mapping, based on the parental lines CJ9306 (resistance source) and Zebra (susceptible).

The mapping population used for field trials was derived from a cross between CJ9306 and Zebra, see crossing scheme in **Figure 3**. This population was chosen based on previous QTL mapping studies on chromosome 2D, which found QTL linked to FHB resistance on this chromosome (X. He et al., 2016; Jiang, Dong, et al., 2007; Jiang, Shi, et al., 2007; Lu et al., 2013). Limited backcrosses of CJ9306 to Zebra were developed as part of ongoing FHB resistance introgression work in Norwegian spring wheat breeding. Briefly, heterozygous plants for major FHB resistance loci were identified in BC_1F_1 populations using the following SSR markers: *UMN10* (Liu et al. 2008) for *Fhb1*, *gwm539* for 2DLc, and *gwm304* and *gwm293* for *Fhb5*. Progenies from these heterozygous plants were advanced in the breeding program allowing for selection of agronomic traits like plant height, earliness and overall appearance. In BC_1F_5, a new round of selection was performed with the same markers. Within one BC_1F_4 family, two BC_1F_5 plants were identified that were heterozygous for *gwm539* but lacking the resistant alleles of *Fhb1* and *Fhb5*. These two plants were selfed, and from the next generation,

a total of 19 heterozygous BC_1F_6 plants based on *gwm539* were selfed to produce a population of 1800 recombinant inbred BC_1F_7 lines. Approximately 10% of these lines were randomly chosen for field trials in 2019, 2020 and 2021 at Vollebekk, Ås, Norway, and genotyping as part of this work.

3.1.2 Point inoculation

The plant material used for the first round of a point inoculation experiment consisted of 10 different genotypes of wheat, see **Table 2**. Of these lines, the Zebra/CJ9306//Zebra NILs (hereafter referred to as NIL 6A5 and NIL 6B5) are homozygous segregants from the same BC_1F_7 family used for making the fine-mapping population used in this work. The parental lines Zebra and CJ9306 were also included, as well as the susceptible control lines Naxos, Ocoroni F86, and Gamenya, and resistant control lines SHA3/CBRD, Soru#1, and Wuhan-1.

Table 2: Information about the plant material used for greenhouse point inoculation experiment, including line name, allele for the NILs and resistance information, source of the seeds, and ID number. Under resistance information, +2D and -2D indicate resistant and susceptible alleles, respectively.

Name	Allele	Resistance information	Source	ID number
Zebra-2/CJ9306//Zebra-2	6A5	+2D (*gwm539*)	18EMLOPF	1908
Zebra-2/CJ9306//Zebra-2	6B5	-2D (*gwm539*)	18EMLOPF	1909
CJ9306		Resistant parent	MASBASIS	1079
Zebra		Susceptible parent	MASBASIS	1011
SHA3/CBRD		+2D	MASBASIS	1086
SORU#1		+2D	MASBASIS	1087
Naxos		-2D	MASBASIS	1041
Wuhan-1		+2D	CIMMYT	BW 11778
Ocoroni F86		Susceptible control	CIMMYT	BW18095
Gamenya		Susceptible control	MASBASIS	1634

The plants were sown in eight rounds with a five-day interval. Each round consisted of two repetitions of each line, meaning 20 pots per round. To make sure that enough plant material would be available, six seeds were sown in each pot, accounting for some failed seeds during germination. Then, the plants were grown in a greenhouse, with a day/night temperature of

25/20°C for 14 days, and later decreased the temperature to 20/16°C until the plants were used for point inoculation, to give time for growth of larger spikes.

A second round of point inoculation was done, to test out different isolates of *F. graminearum* with higher aggressivity. The plant material sown out can be seen in **Table 3**. New inoculums were made with two new isolates using the same method as previously. Six different lines were sown out in three rounds with a five-day interval, using the NILs and parent lines, as well as Gamenya and Ocoroni F86 as control lines. Additionally, ten extra pots of Gamenya were sown out the last round. This was to test whether the new *F. graminearum* isolates were aggressive enough.

Table 3: Cultivars sown out for the second round of inoculation to test new *F. graminearum* isolates. The table contains cultivar name, allele for the NILs, resistance information, source and ID number.

Name	Allele	Resistance information	Source	ID number
Zebra-2/CJ9306//Zebra-2	6A5	+2D	18EMLOPF	1908
Zebra-2/CJ9306//Zebra-2	6B5	-2D	18EMLOPF	1909
CJ9306		Resistant parent	MASBASIS	1079
Zebra		Susceptible parent	MASBASIS	1011
Ocoroni F86		Susceptible control	CIMMYT	BW18095
Gamenya		Susceptible control	MASBASIS	1634

Three weeks after the first round of sowing the second point inoculation material, new plant material of the NILs with different combinations of genotypes for markers *gwm539*, *WGRB3803* and *wsnp_Ex_c8303_14001708* were sown out, see **Table 4**. These NILs were sown out for point inoculation with the purpose of investigating which of the markers are closer to the QTL.

Table 4: NILs for point inoculation experiment, with the family history and entry, and genotype information showing recombinations between the markers *gwm539*, *WGRB3803,* and *wsnp_Ex_c8303_14001708*. The alleles are Z for susceptible allele derived from Zebra (red) and CJ for resistant allele derived from CJ9306 (green).

Family	Entry	*gwm539*	*WGRB3803*	*wsnp_Ex_c8303_14001708*
6C5_01_E01	5	CJ	CJ	CJ
6C5_01_E02	13	Z	Z	CJ
6C5_09_A08	441	CJ	CJ	CJ
6C5_09_C04	411	Z	Z	CJ
6D5_07_C02	971	CJ	CJ	Z
6D5_07_C07	1011	CJ	CJ	CJ
6D5_07_E03	981	Z	Z	Z
6D5_09_A04	1081	Z	Z	Z
6D5_09_E06	1101	CJ	CJ	Z
6D5_19_E08	1501	CJ	CJ	Z
6D5_19_G09	1511	CJ	CJ	CJ

3.2 Phenotype evaluation in the field

The field trials were done at Vollebekk Research Farm in the summer season. The mapping population in 2021 was planted as a hill plot with two repetitions, in total 122 plants (51 lines) including parents and different check cultivars. These trials were inoculated using grain spawn inoculation. Briefly, oat kernels are inoculated with *F. graminearum* in the lab, then dried and spread out on the soil surface in the hill plot when plants were at the stem elongation stage. To make sure the climate around the plants was good for *Fusarium* disease infection, a mist irrigation system was set up to ensure moist conditions at night. The field season in 2021 was very warm, so to make sure it was not too dry for the *F. graminearum* to infect the plants, additional watering was important. The amount of watering used was 15 minutes each hour between 19:00 and 22:00. The hill plot was sown in May and harvested in late August.

Phenotypical data collected from the mapping population was done by scoring the infection before harvesting. The scoring was done by taking 10 random plants, count and average the number of spikelets per head, and then count the number of infected spikelets. The FHB severity score is the fraction of number of infected spikelets / total number of spikelets. All the plots were scored on the same day by one person to avoid differences in length of disease development and to reduce any subjective bias.

Field data was also available for the testing of a limited number of lines from the fine-mapping population in the previous field seasons 2019 (24 lines) and 2020 (12 lines). These trials were conducted in the same way as described above. In addition, these samples were analysed for DON content at the University of Minnesota, Department of Plant Pathology. A representative

set of samples were taken from each plot using a Rationel Sample Divider Vario and ground with a Stein Laboratories mill. The mycotoxin analysis was done for four types of mycotoxins, DON, 3A-DON, 15A-DON, and NIV. This was done using gas chromatography coupled with mass spectroscopy, following the protocol from Mirocha et al. (1998) and modified in Fuentes et al. (2005) (Tekle et al., 2018).

3.3 Genotyping and looking for markers

Fine-mapping was based on the previous work that has been published to date, particularly Lu et al. (2013) and He et al. (2016). Based on previous genotyping results, a physical map was constructed using MapChart (Voorrips, R.E., 2002), and the fine-mapping study was focused around the marker *gwm539*, a well-established marker on chromosome 2D. The region 400-570 Mbp was chosen for genotyping the fine-mapping population, based on previous genotyping rounds that revealed the area downstream of 570 Mbp to be monomorphic in the fine-mapping population.

To find more markers to genotype, a literature study was done to identify published FHB resistance markers on 2DL and check whether they could be in the same QTL area. The markers found in the literature study were subsequently genotyped in the mapping population. As already mentioned, the genotyping was done on approximately 10% of the initial mapping population, using SSR markers and KASP for SNPs (90K and 35K). Genotyping revealed which markers that were useful for further analyses, and boxplots were created based on field data in R using ggplot2 (Wickham, 2016), ggpubr (Kassambara, 2020), and ggrepel (Slowikowski, 2021), doing a mean comparison of p-values to compare groups (stat_compare_means() function from ggplot2 package). A one-way ANOVA was also performed on the marker data using a standard ANOVA function in R. For the analyses in R, R version 4.1.2 was used and RStudio version 2021.9.1.372.

After obtaining a few markers, these were researched in the wheat pangenome, looking into the alleles of the markers in each of the genomes. This was done in a few different ways due to the markers being of different types, i.e., SSR, SNP (KASP), and dCAPS markers. For *gwm539*, which is an SSR marker, the length of the amplicon was found in the pangenome using the primer sequence in BLAST (WheatOmics: http://202.194.139.32/) and the different genomes were compared based on this. Two of the markers found in literature were dCAPS markers which were designed to have a cut site for the susceptible version of the marker allele. The

markers were searched in the genomes using BLAST on the primer sequence and checking whether the sequence found in the genome contained the cut site or not. Lastly, the rest of the candidate markers were SNPs. The sequences of each SNP allele were blasted against the pangenome to check if it was a 100% match or not. If that allele of the SNP was present in the genome, it would get a 100% match.

Based on the recombination data from the genotyping, a linkage map was constructed using JoinMap4 (Van Ooijen, 2006). The recombination frequencies (RF) were also calculated by hand to compare with the linkage map. This was done using equation 1:

$$RF = \frac{RCO}{TO} * 100 \quad (1)$$

Here RF is recombination frequency, RCO is total number of recombinants, and TO is the total number of offspring. The frequency is given as a percentage, and 1% is roughly 1 cM. Considering that the fine-mapping population constitutes progenies of a selfed heterozygote at the QTL region (similar to an F_2 population), it is important to note that the calculations use gametes, not individuals.

After retrieving a linkage map for the markers, we wanted to find the physical position of the markers in the pangenome, which was done by running the primer sequences of the markers for each of the genomes in the pangenome through BLAST. With the physical positions, it was possible to compare the haplotypes of different wheat cultivars which were sequenced by the pangenome project, using the database already created by Walkowiak et al. (2020), available at http://www.crop-haplotypes.com/Wheat/haplotype/2D (Walkowiak et al., 2020).

As for the haplotype data, haplotypes containing all three markers were in focus. We also wanted to compare the genomes with different alleles for the three markers, which were grouped according to the alleles. The lengths of *gwm539* were grouped into two main groups, where only Zang1817 was excluded. This was done for the purpose of simplicity and considering that Zang1817 was not included in the haplotype map. Therefore, to compare with the haplotype information, groups were created based on the lengths of *gwm539* and compared this with the other allele information, see **Table 5**.

Table 5: Grouping of different lengths of *gwm539* marker in the pangenome. The two main groups are from 130-139 bp and 140-149 bp, with Zang1817 placed in its own group.

gwm539		
130-139 (bp)	**140-149 (bp)**	**150 < (bp)**
ArinaLrFor	Chinese Spring	Zang1817
Jagger	Fielder	
Julius	CDC Landmark	
LongReach Lancer	MACE	
Norin61	*Triticum spelta*	
CDC Stanley		
SY Mattis		

3.4 Inoculum preparations

Preparing the inoculum for the point inoculation experiments was done with the help of Yalew Tarkegne at Vollebekk Research Farm. *F. graminearum* was grown on potato dextrose agar (PDA) and mung bean agar (MBA) plates under UV-light. Four different isolates (Fg. 77, Fg. 23, Fg. 200838, and Fg. 200726) were used. The desired concentration of each isolate was obtained by counting the conidia in a counting chamber glass and dilute with sterilised water accordingly. The final concentration we used was 1 x 10^5 spores/mL. Later, all four isolates were mixed to create the inoculum. This was distributed in smaller doses of 1 mL to retrieve just enough for each experiment at once. The inoculum was kept in a freezer until it was used for point inoculation.

The same procedure was used for the second round of inoculations, but with new *F. graminearum* isolates curtesy of NIBIO, Ås, Norway (Fg. 200630 and Fg. 200646). After creating the last inoculums from the isolates from NIBIO, a germination test was performed to see the percentage of germinating spores. This was done by pipetting 1 mL of inoculum on water agar plates, which are clear and nutrient poor, making it easy to observe spores under the microscope (**Figure 4**). These were left in room temperature for 24 hours. Then, the number of germinating spores were counted out of a 100 in three different directions, and the germination percentage was calculated as the average of these three scores. A percentage over 80-90% was considered good (Barbara Steiner, pers. comm).

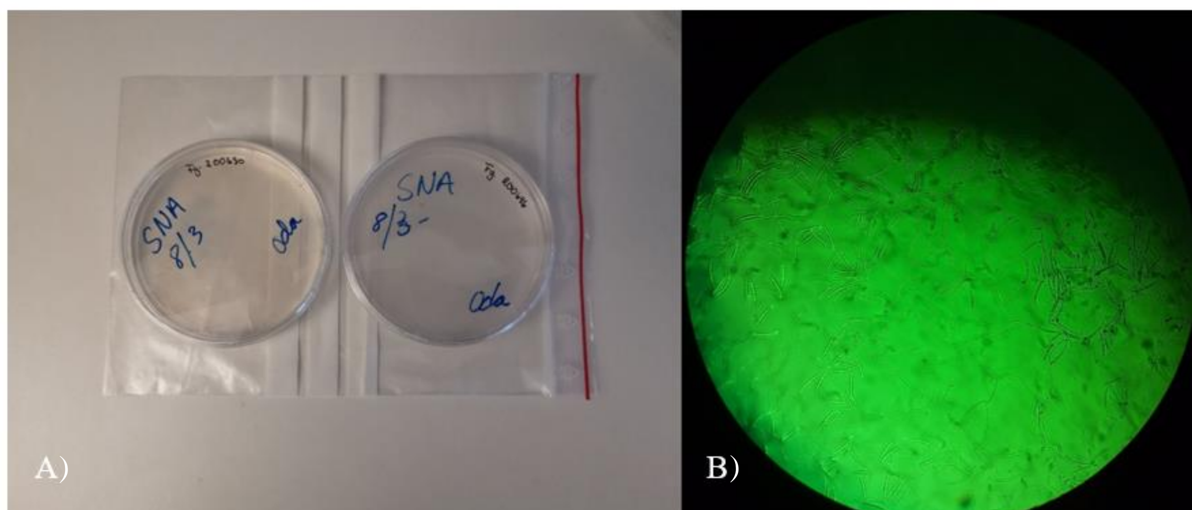

Figure 4: A) Water agar plates with the two *F. graminearum* isolates for germination test. Agar plates were left in room temperature for 24h. B) Example of how the water agar plate with inoculum looks after 24 hours under a microscope. This picture is of isolate Fg. 200630.

3.5 Point inoculation

The point inoculation was performed in two different experiments, were the second experiment consisted of two parts, one with a mapping population and one with a NIL population chosen based on recombinations between markers, see **Table 4**. In preparations of the first round of inoculations, the greenhouse room containing the plants was regulated to day/night temperatures of 25°C/18°C and a humidity of 90%, to give an optimal climate for *F. graminearum* growth. For the second round of inoculations, the conditions were altered to 22°C/18°C and with normal humidity. To replace the humidity in the greenhouse, plastic bags sprayed with water on the inside were placed over the inoculated heads and kept on for 48h after inoculation.

Point inoculation was done by inoculating 10 µL in one spikelet between the palea and lemma in the middle of the spike right after flowering, marking each inoculated spikelet after inoculation to easily recognise later (**Figure 5** and **Figure 6**). For the first experiment, hanging tags with the date were added to keep track of each spike, while for the second experiment, colour coded tape was used instead to recognise the plants inoculated on the same day. The number of infected spikelets were recorded every three days for 15 days the first experiment, and every three days for 21 days in the second experiment. Some deviations occurred for the time of the scoring. For the second round of inoculations, two inoculums were used, one with isolate Fg. 200630 and one with isolate Fg. 200646. The two inoculums were used in approximately equal number of inoculations.

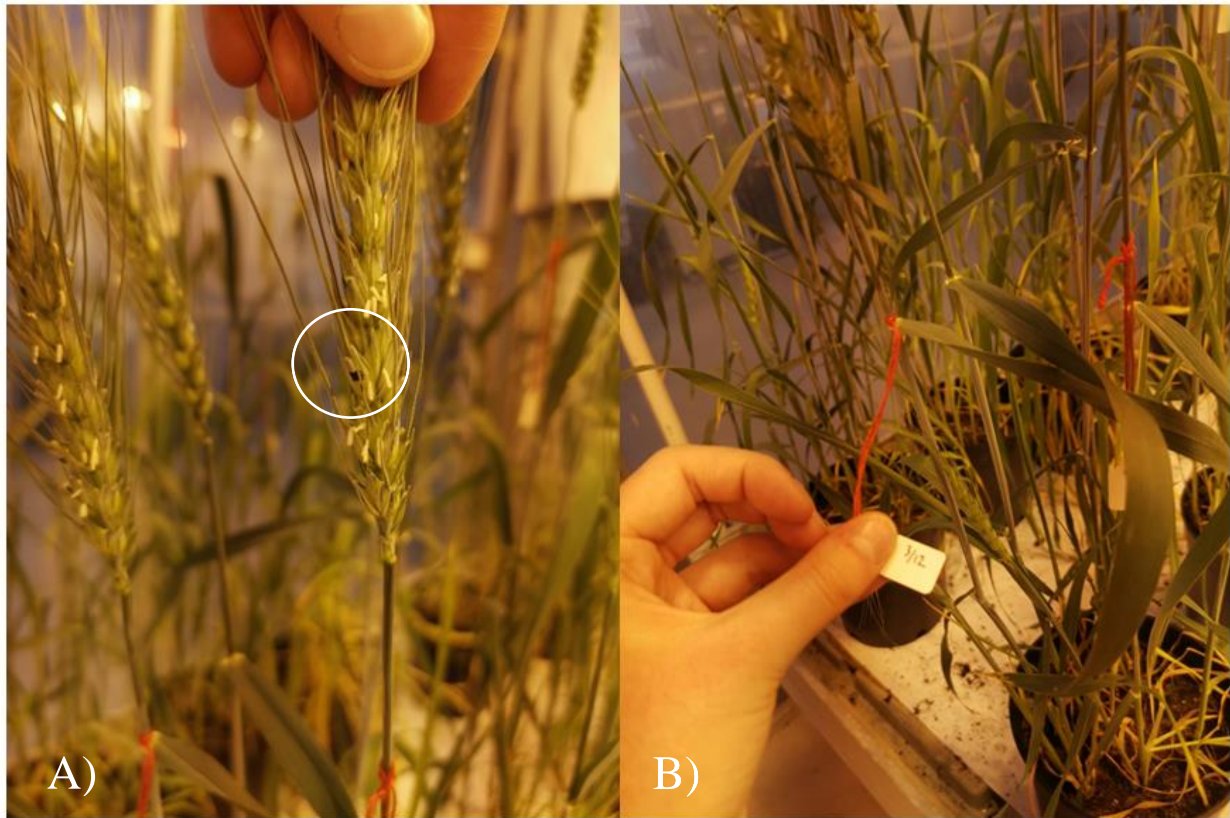

Figure 5: A) An inoculated spikelet marked with a black marker to be recognised for phenotyping later. B) Hanging tag on the inoculated plant to show which date the spike was inoculated.

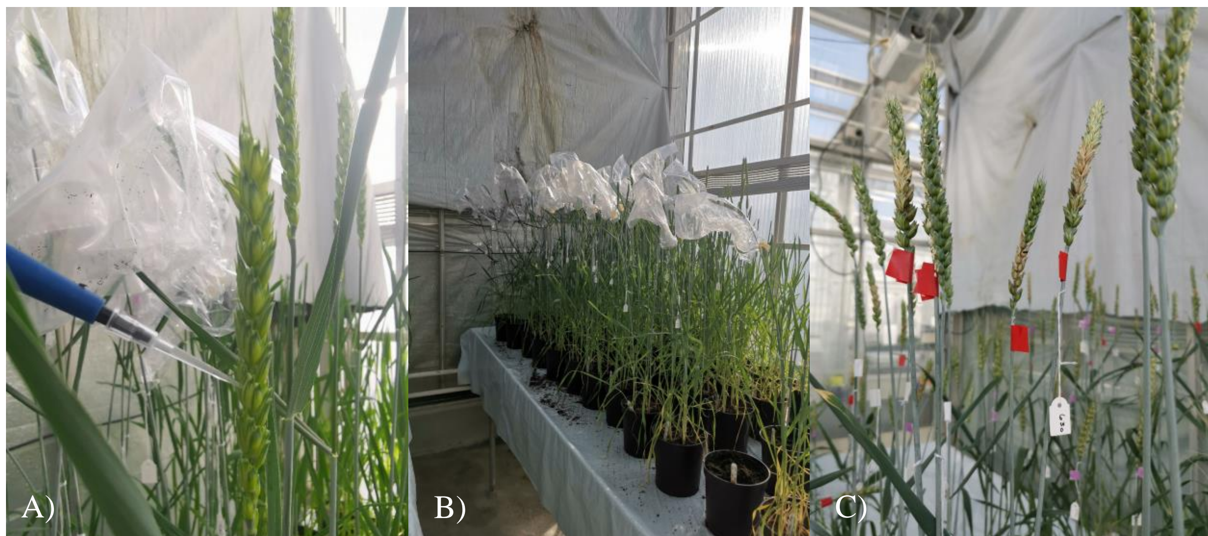

Figure 6: Point inoculation in wheat spikelet using *F. graminearum* inoculum. A) Shows the pipette being placed between the palea and lemma, 10 µL inoculated in one spikelet. B) Bagging method on inoculated spikes, a plastic bag sprayed with water placed over the head for 48 hours. C) Colour coded marking system on inoculated spikes used for the second point inoculation experiment, one colour was used for all spikes inoculated in the same day.

3.4.1 Calculations

The phenotyping results, i.e., number of diseased spikelets observed were noted down every three days for 15 days for the first experiment, and then for 21 days for the second experiment. Followingly, the proportion of diseased spikelets (PDS) was calculated based on the last date noted, according to equation 2:

$$PSD = \frac{no.of\ diseased\ spikelets\ identified}{tot.no.of\ spikelets} \quad (2)$$

3.4.2 Analysis of phenotypic data

In the first point inoculation experiment, only calculations of PDS were done, as the results obtained from this were too poor to continue further experiment with. The second experiment, however, was more promising and led to point inoculation of NILs afterwards. The analysis done for the second experiment was a study of the disease development, separating the data for the two isolates. This was again based on PDS calculations. Furthermore, the area under the disease progress curve (AUDPC) was calculated for each of the replicates and then averaged for each line using the functions lsmeans (Lenth, 2016) and lme4 (Bates et al., 2015) and a basic AUDPC function in R, adapted from Sparks et al. (2008). The error bars were added as the confidence intervals of the means of each line from lsmeans. Then, the mean was taken for each line, and the average AUDPC values were illustrated as bar plots using ggplot2 (Wickham, 2016) in R. A one-way ANOVA was performed based on PDS data, using a standard function (aov) in R. Lastly, based on the ANOVA results, a Tukey's honestly significant difference (HSD) test was performed also in R using function TukeyHSD. The Tukey's HSD test was only performed on the data from the mapping population, not the NIL population.

4 Results

4.1 QTL fine-mapping for FHB traits

4.1.1 Marker identification

This project was based on previous research on the 2D chromosome of bread wheat. We had some data already available of markers in this region, which can be seen in the physical map in **Figure 7**.

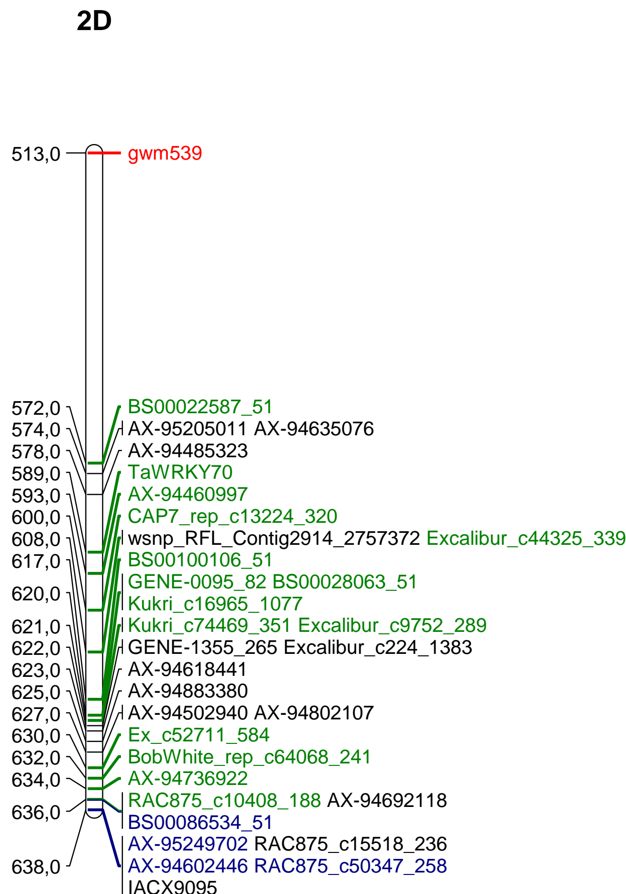

Figure 7: Physical map of the markers investigated before the start of the project. Red indicates a segregating marker, black is monomorphic markers between the parents, blue represents the markers that are monomorphic in the fine-mapping population and show the same allele as CJ9306, and lastly, green represents the markers that are monomorphic and show the same allele as Zebra. The physical positions (Mbp) are on the left and marker name on the right.

The marker *gwm539* at position 513 Mbp was already well known and was used as the starting point for fine-mapping of the resistance QTL on chromosome 2D. The first round of genotyping in the region 400-570 Mbp, resulted in only one additional marker that was polymorphic and segregated in the mapping population. This was marker *wsnp_Ex_c8303_14001708* at position 482 Mbp.

From the papers mentioned under previous work on 2D in the introduction, there were several potentially relevant markers in our region of interest (see **Table 1**). We selected markers from more recent publications (2020 and 2021), because older markers had already been genotyped for the population previously, see **Table 6** for the markers selected.

Table 6: Markers selected for genotyping based on the literature study in our region of interest. Marker name, SNP name, start and end positions (bp), and the paper referencing the markers are included in the table.

Marker name	SNP marker name	Start position	End position	Paper
BobWhite_c17782_194		555098707	555098805	Dhariwal et al. (2020)
WGRB3753	*AX-110423675*	516638960	516638979	Yan et al. (2021)
WGRB3803	*AX-11151380*	519126074	519126093	Yan et al. (2021)

The resistant allele of the dCAPS markers was determined by cutting in the marker region, and *WGRB3753* was successfully amplified but no cutting occurred, and we were unable to map this marker in the population. The other marker *WGRB3803* was also amplified and did cut successfully, and the marker segregated in the mapping population. Therefore, we investigated this marker further. It was close to *gwm539* in physical position (*WGRB3803* positioned at 519 Mbp and *gwm539* at 513 Mbp), and there was little recombination between them, see linkage map in **Figure 10**. The last marker that was genotyped was *BobWhite_c17782_194*, which is slightly outside of the area previously defined. This marker did not segregate in the population, so it was discarded for further mapping.

After these two rounds of genotyping, we had three segregating markers in our region of interest. This area was then investigated by comparing the genotype and phenotype data from the mapping population, and we found that recombination had occurred between the markers. The phenotype data in focus was FHB severity and DON levels for the three years 2019, 2020, and 2021. Only 2019 and 2020 had data on DON levels, see **Appendix 1** for additional information.

Figure 8 and **Figure 9** show boxplots for the allelic effects of all three markers (*gwm539*, *WGRB3803*, and *wsnp_Ex_c8303_14001708*) on FHB and DON data from the field trials. There were no significant differences in most of the boxplots, except for the markers *gwm539* and *WGRB3803* in 2019, as the datasets were very limited. However, there were some trends

to note in addition to the two significant boxplots. For FHB, it was clear across years and markers that the susceptible alleles had higher levels of FHB compared to resistant alleles. Furthermore, the difference appeared to be larger for marker *gwm539* than for *wsnp_Ex_c8303_14001708*, whereas *WGRB3803* had very similar results to *gwm539*. In **Figure 9**, data was only available for the two years 2019 and 2020. Again, even though there were no significant differences, the DON plots also showed trends of susceptible alleles having higher DON levels. There were some small differences between *wsnp_Ex_c8303_14001708* and the other two markers in the 2019 data, mainly that the median is more similar between alleles for *wsnp_Ex_c8303_14001708*.

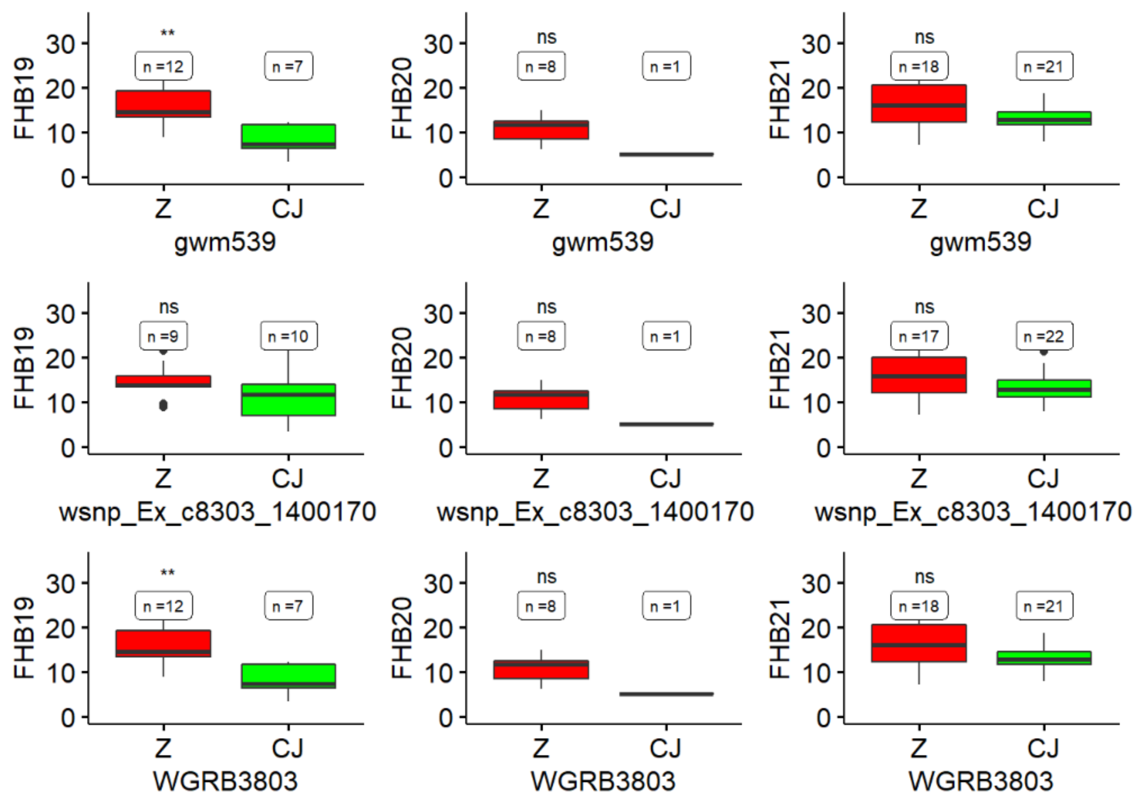

Figure 8: Boxplots of the allelic effect for markers *gwm539*, *WGRB3803*, and *wsnp_Ex_c8303_14001708* on FHB severity for the three years 2019, 2020, and 2021. Red boxes indicate susceptible allele, while green box indicates resistant allele.

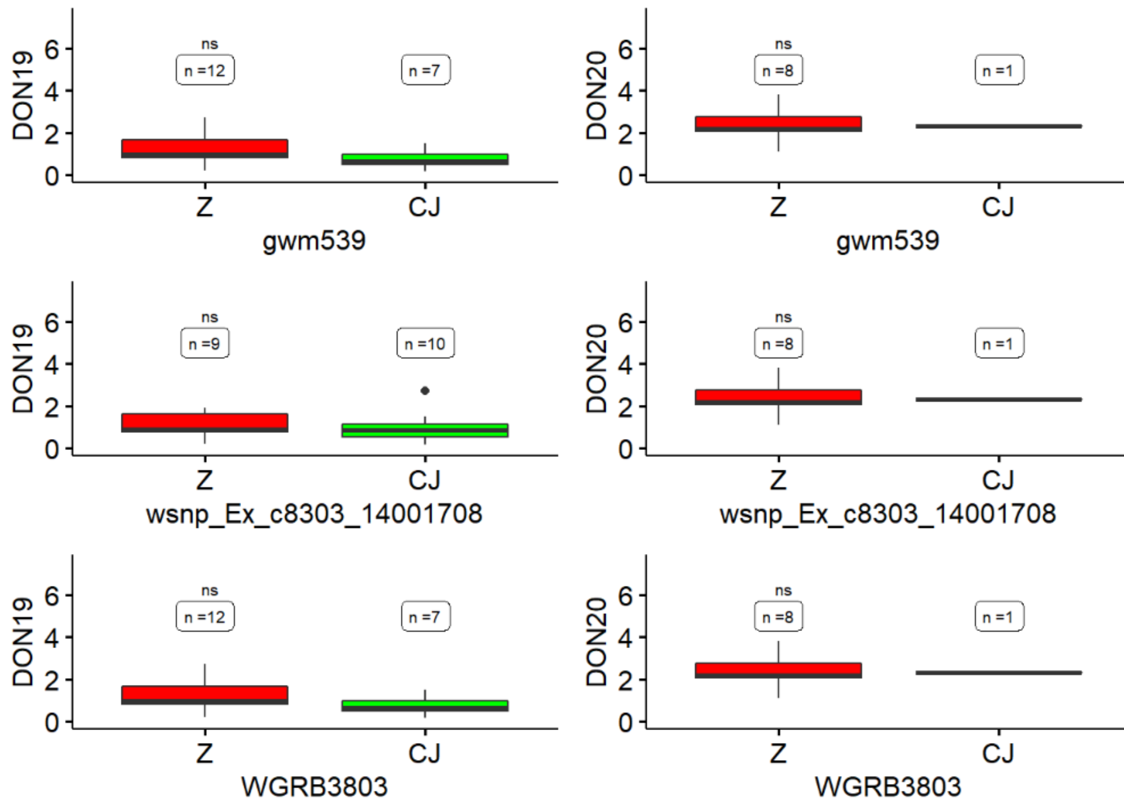

Figure 9: Boxplots revealing the allelic effect on DON levels in the years 2019 and 2020 for markers *gwm539*, *WGRB3803*, and *wsnp_Ex_c8303_14001708*. Red boxes indicate susceptible allele, while green box indicates resistant allele.

In addition to boxplots, one-way ANOVAs were also performed for testing alleleic effects of the markers on FHB and DON separately for the years 2019, 2020, and 2021. Firstly looking at FHB severity in **Figure 8**, the ANOVA based on 2019 data revealed a significant p-value for *gwm539* (p-value of 0.0017) and *WGRB3803* (p-value of 0.0017), but not for *wsnp_Ex_c8303_14001708* (p-value 0.222). In 2020, the p-values for *gwm539*, *wsnp_Ex_c8303_14001708* and *WGRB3803* were all 0.0998, meaning none were singificant, which was the case in 2021 as well, with p-values of 0.624 for both *gwm539* and *WGRB3803*, and 0.128 for *wsnp_Ex_c8303_14001708*. Over to DON accumulation in **Figure 9**, the dataset was even more reduced. Based on the 2019 data, the p-values were 0.191, 0.191, and 0.92 (*gwm539, WGRB3803*, and *wsnp_Ex_c8303_14001708,* respectively), whereas the p-values from 2020 were 0.947 for all three markers, meaning no markers had a significant effect on DON.

A linkage map was created (**Figure 10**) based on the recombination frequencies between the markers, which are available in **Appendix 2**. The calculated recombination frequencies are shown in **Table 7**. Here, the two markers *WGRB3803* and *gwm539* are closest and had fewer recombinations occuring between them. *Wsnp_Ex_c8303_14001708* showed more recombinations between the two other markers and is relatively far away from the other two, being 13.1 and 10.9 cM away, respectively.

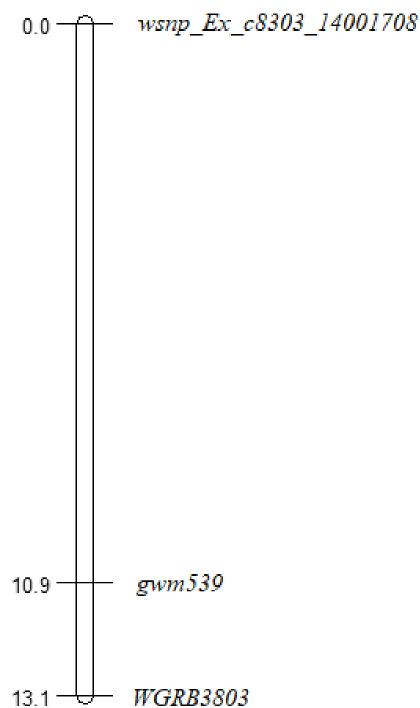

Figure 10: Linkage map of the three markers *WGRB3803*, *gwm539,* and *wsnp_Ex_c8303_14001708*. The genetic distance is shown on the left in cM, with marker names on the right.

Table 7: The recombination frequencies (RF) (in %) between the combinations of the three markers in linkage map, calculated based on equation 1.

Marker 1	Marker 2	RF (%)
gwm539	*WGRB3803*	2.8
gwm539	*wsnp_Ex_c8303_14001708*	14.6
WGRB3803	*wsnp_Ex_c8303_14001708*	14.7

4.1.2 Marker alleles in the pangenome

The pangenome alleles of each marker is summarised in **Table 8**. None of the genomes contained the resistant allele of *wsnp_Ex_c8303_14001708,* except CJ9306 which is the resistant parent of our mapping population. For the SSR marker *gwm539*, the genomes were differentiated by comparing the amplicon size of the markers. It was already known that a length of 136 is susceptible and 128 is resistant (see **Table 8**), therefore the longer marker allele was considered susceptible whilst the shorter marker allele was considered resistant. Chinese Spring is typically used as a reference and had a length of 144 bp for the *gwm539* marker. The two other genomes that deviate the most from this, are Zang1817 with 158 bp and Norin61 with 130 bp. The markers from Yan et al. (2021) are dCAPS markers and use restriction enzymes to separate different alleles. For *WGRB3803*, the alleles are cut/no cut with restriction enzyme RsaI, meaning susceptible/resistant respectively. Chinese Spring, Fielder, Zang1817 and SY Mattis were the only genomes with the resistant allele of this marker, as can be seen in **Table 8**.

Table 8: Alleles for the three markers in the pangenome and the two parent lines CJ9306 and Zebra. As *gwm539* is an SSR marker, the differences were measured by the length of the marker in the genome. *Wsnp_Ex_c8303_14001708* is a SNP marker with the alleles C and T, where T is the resistant allele, and C the susceptible. *WGRB3803* is a dCAPS marker where a cut site is susceptible, and no cut site means resistant. *The *gwm539* alleles for CJ9306 and Zebra are estimations based on genotyping data, while the rest of the genomes' alleles for this marker are from BLAST. For *WGRB3803*, the alleles are based in genotype data while the other genomes are again based on BLAST searches.

Genome	*wsnp_Ex_c8303_14001708*	*gwm539*	*WGRB3803*
	SNP (C/T)	Length (bp)	RsaI cut site
Chinese spring	C	144	no
Fielder	C	148	no
Zang1817	C	158	no
ArinaLrFor	C	132	yes
Jagger	C	138	yes
Julius	C	138	yes
LongReach Lancer	C	134	yes
CDC Landmark	C	140	yes
MACE	C	140	yes
Norin61	C	130	yes
CDC Stanley	C	134	yes
SY Mattis	C	138	no
CJ9306	T	128*	no*
Zebra	C	136*	yes*

4.1.3 Physical position of markers in the pangenome

Table 9 shows the approximate physical positions in Mbp of the three markers *gwm539*, *WGRB3803*, and *wsnp_Ex_c8303_14001708* in the pangenome. For the detailed position in bp, see **Appendix 3**.

Table 9: Physical positions in Mbp for each of the three markers *gwm539*, *WGRB3803*, and *wsnp_Ex_c8303_14001708* in the genomes included in the wheat pangenome.

Genome	*wsnp_Ex_c8303_14001708*	*gwm539*	*WGRB3803*
Chinese Spring	482	513	519
Norin61	480	512	518
Fielder	488	519	525
Zang1817	477	509	515
ArinaLrFor	483	514	520
Jagger	500	531	537
Julius	488	521	527
LongReach	479	510	516
CDC Landmark	485	516	522
MACE	479	510	516
CDC Stanley	486	517	524
SY Mattis	480	512	518
Spelta	481	513	519

4.1.4 Haplotype blocks

The location of each of the three markers was marked in the pangenome in the **Figure 11**, **Figure 12**, and **Figure 13**. This gives an overview of which haplotype blocks the different genomes share, as well as which haplotype blocks include all three markers. The three different figures are similar, but in different resolutions (5 Mbp, 2.5 Mbp, and 1 Mbp). For detailed information on haplotype blocks, see **Appendix 4**.

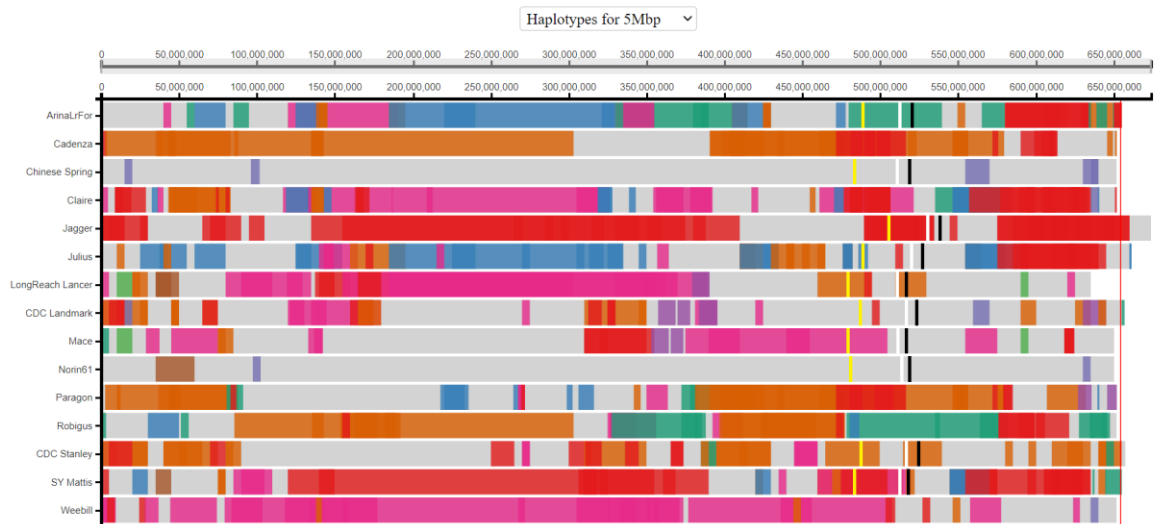

Figure 11: Haplotype groups in the pangenome at 5 Mbp resolution. Coloured lines corresponding to physical position of markers, yellow for *wsnp_Ex_c8303_14001708*, white for *gwm539*, and black for *WGRB3803*.

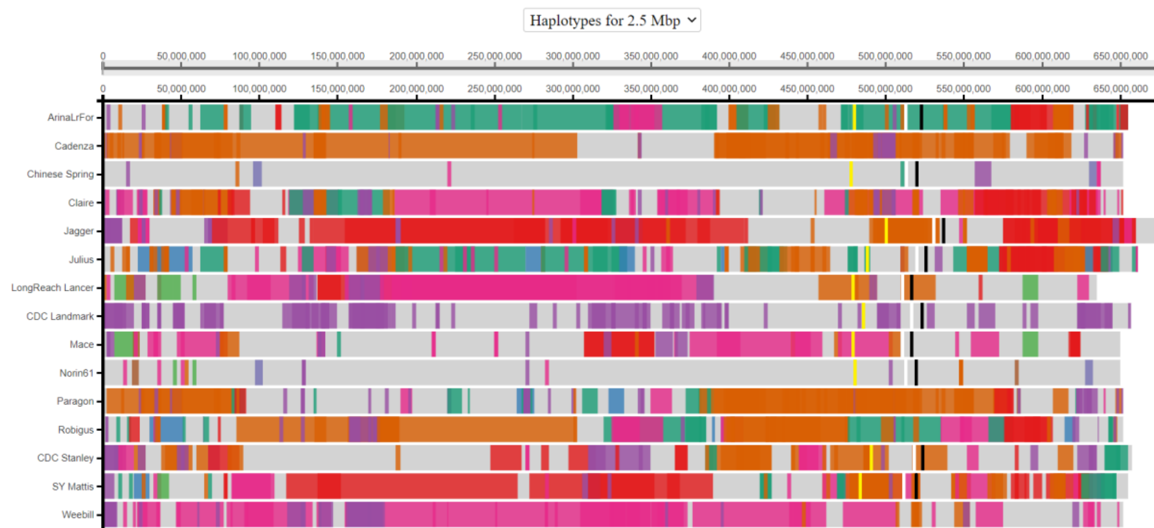

Figure 12: Haplotype groups in the pangenome at 2.5 Mbp resolution. Coloured lines corresponding to physical position of markers, yellow for *wsnp_Ex_c8303_14001708*, white for *gwm539*, and black for *WGRB3803*.

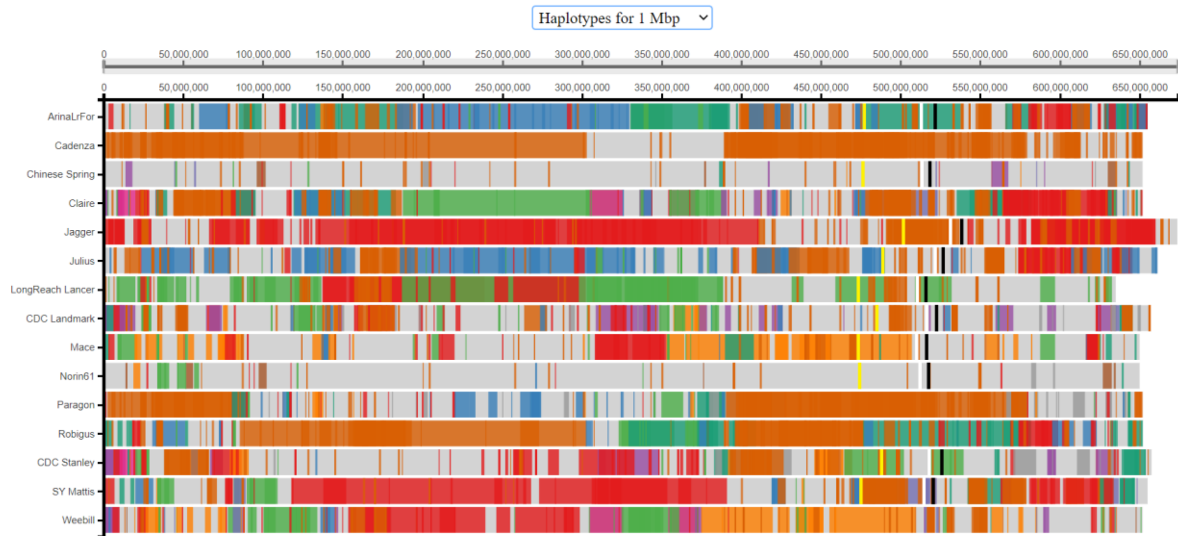

Figure 13: Haplotype groups in the pangenome at 1 Mbp resolution. Coloured lines corresponding to physical position of markers, yellow for *wsnp_Ex_c8303_14001708*, white for *gwm539*, and black for *WGRB3803*.

The general trend to note is that both Chinese Spring and Norin 61 shared almost no haplotype blocks with the other pangenome lines, only a few very small at the highest resolution. There were many haplotype blocks for the different markers, but not all blocks contained all three. For the 1 Mbp resolution (**Figure 13**) for example, only three haplotype blocks contained all three markers, and these can be found in the genomes ArinaLrFor, Robigus, Jagger, Paragon, Claire, and SY Mattis.

Based on the allele data of the genetic markers, the alleles were compared with the pangenome haplotype data. Only the haplotype map for 1 Mbp was used, as this included more information than 2.5 Mbp and 5 Mbp. As already reported in **Table 8**, all genomes had the same allele for *wsnp_Ex_c8303_14001708*, so this was not considered for the comparison to haplotype blocks. *Gwm539* was divided into groups as seen in **Table 5**, while *WGRB3803* alleles were dependent on the presence or absence of a cut site. Comparing the genomes with the resistance/susceptible alleles for these two markers with the haplotype groups they contained resulted in no clear correspondence. The only two genomes with similar alleles for both markers that also had the same haplotype blocks were LongReach Lancer and CDC Stanley, while the rest did not share haplotype blocks.

4.1.5 Fine-mapping status

At the end of mapping, there were three segregating markers detected close to the QTL of interest on chromosome 2D. **Figure 14** shows an updated physical map compared to the map in **Figure 7**. This figure also includes all the markers tested along the way, the data behind this figure is available in **Appendix 5**. With the new markers, the segregating region with polymorphic markers in the fine-mapping population is narrowed down to the area between 468 and 523 Mbp on the physical map.

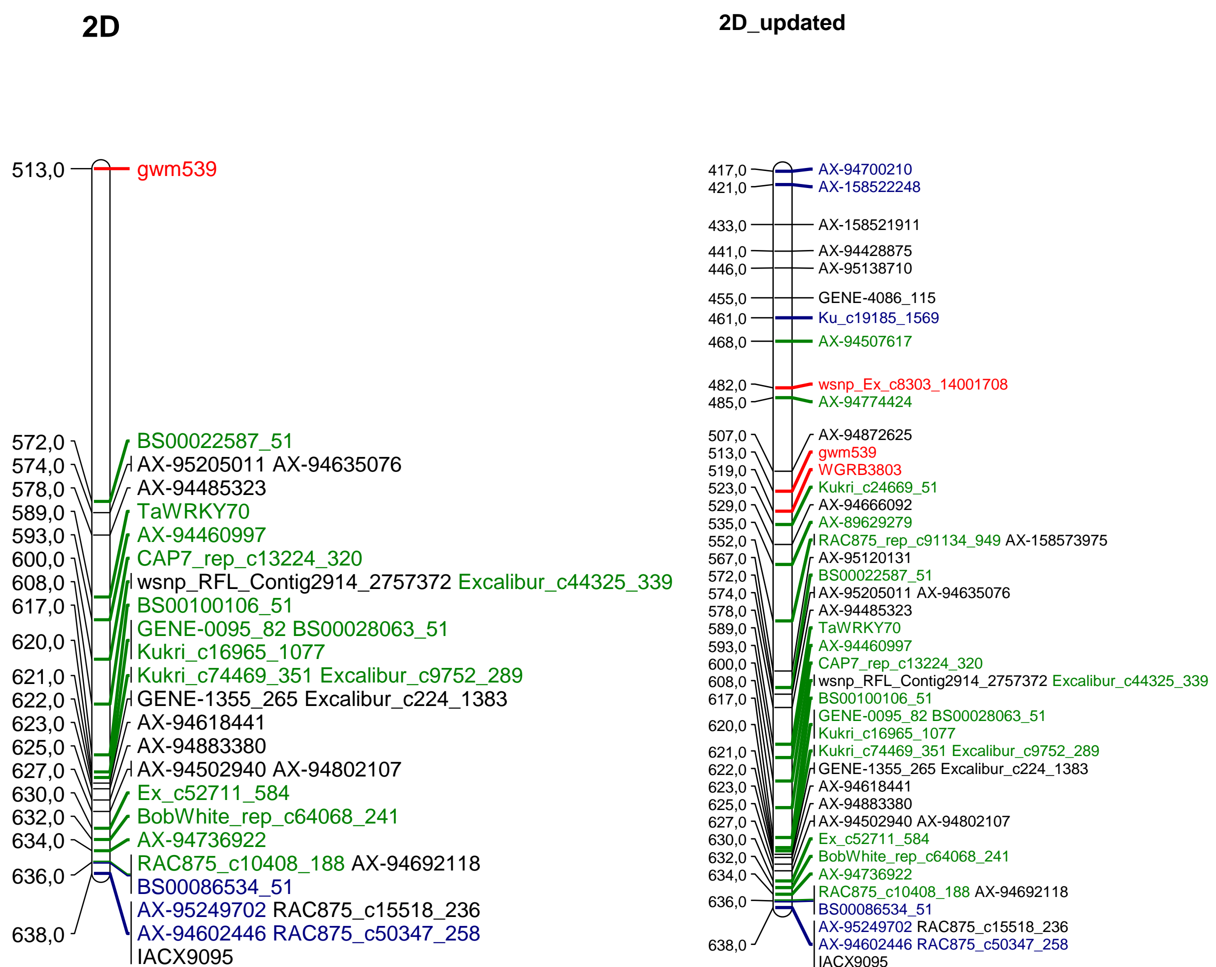

Figure 14: Physical maps of markers on chromosome 2D. The left map is the same as in **Figure 7**, which shows the markers we knew about before starting this project. The map to the right is an updated map with the marker data we found. Red is segregating markers, black is monomorphic, green is for monomorphic markers with alleles that are the same as Zebra, and blue for CJ9306.

4.2 Phenotypic analysis

For the phenotypic analysis, the goal was to investigate the effect of the resistance QTL being fine-mapped in the mapping population, to see if it would show a significant effect on Type II FHB resistance. The first part was to test out a point inoculation experiment in greenhouse, using the varieties mentioned in **Table 2**. If there was a clear difference between the resistant and susceptible NILs for the 2DL QTL, we could further investigate the specific plants with different recombinations between the genotypes for the three markers we had found by this time.

4.2.1 First point inoculation experiment

The first part of this investigation was to do a point inoculation experiment in greenhouse during the winter of 2021/2022. To create the inoculum, a mixture of the isolates Fg. 77, Fg. 23, Fg. 200838, and Fg. 200726 were used. For the phenotypical scores of the first point inoculation experiment, see **Appendix 6**. The results showed generally very low spread within the inoculated heads, see average PDS values in **Table 10**. Several controls that were already known to be highly susceptible or resistant were included, but the results did not correlate with prior knowledge. Gamenya is a highly susceptible variety, but only a few of the inoculated heads showed full spread, while some had no spread at all. Zebra is the susceptible parent of the NILs, yet this variety had little spread within the head after successful inoculation as well. There was little difference between the two NILs. The resistant cultivars SHA3/CBRD, CJ9306, and Wuhan-1 showed most resistance, but Naxos and Zebra exhibited a similar level of resistance to these three cultivars, even though both are susceptible. Ocoroni F86, a susceptible cultivar, had the highest average PDS, but Soru#1 is a resistant cultivar and expressed similar results. Examples of some of the phenotypes from this experiment are shown in **Figure 15**.

Table 10: Average proportion of diseased spikelets (PDS) for each variety tested in the first point inoculation experiment conducted with a mix of the four isolates Fg. 77, Fg. 23, Fg. 200838, and Fg. 200726. Failed inoculations are excluded from the dataset.

Variety	Avg. PDS
CJ9306	0.08 (n=10)
Gamenya	0.18 (n=17)
Naxos	0.09 (n=9)
NIL 6A5	0.14 (n=23)
NIL 6B5	0.11 (n=28)
Ocoroni F86	0.23 (n=9)
SHA3/CBRD	0.04 (n=3)
Soru#1	0.21 (n=10)
Wuhan-1	0.08 (n=5)
Zebra	0.10 (n=12)

A one-way ANOVA was performed on the phenotypic data, to compare the effect of variety on PDS. It revealed that there was no statistically significant effect, with a p-value of 0.556.

Figure 15: Examples of successful point inoculations in the first experiment. White rings are placed around infected spikelets. A) and B) are the lines CJ9306 and Gamenya and are only infected in the spikelet which has been point inoculated, whereas C) also the line Gamenya, exhibits spread within the spike to the lower half.

4.2.2 Second point inoculation experiment

Before continuing with a second round of inoculations with the new inoculums, a germination test was performed to see if the spores were germinating well in the inoculums. The germination test revealed that isolate Fg. 200630 had a germination rate of 96%, while isolate Fg. 200646 had 92% germination. A few examples of the phenotypes that were observed during the second point inoculation experiment can be seen in **Figure 16**.

Figure 16: Phenotypes of different wheat lines after point inoculation second experiment. A) NIL 6A5, only the inoculated spikelet is infected. B) Line CJ9306, also only one spikelet infected. C) Line Gamenya after infection, shows several infected spikelets as well as bleached spikelets due to infection in the top of the spike. Lastly, D) also of Gamenya shows a fully infected spike, all spikelets are infected and dried out.

4.1.2.1 Mapping and control population

The start of the second point inoculation experiment was to use the two NILs and some control lines to test whether the resistance QTL resulted in an observable phenotypic response in the plants, see **Appendix 7** for data from the phenotypic scoring. Considering two different inoculums were used, the average PDS was calculated for each inoculum separately for each cultivar, which is reported in **Table 11**. The resistant cultivar CJ9306 deviated the most from the other lines and was identical for both isolates. The NIL containing the resistant allele of the QTL, 6A5, also had a much lower PDS than all the susceptible lines for both isolates. One exception, which is also revealed in the Tukey's HSD test, is the difference between 6A5 and 6B5 for isolate Fg. 200646. The NIL 6B5 had a high PDS (0.75) for isolate Fg. 200630, but a much lower PDS for isolate Fg. 200646 (0.44). This line also has the largest difference in the number of values behind the PDS (11 and 9 individuals). The susceptible cultivars Gamenya, Ocoroni F86 and Zebra expressed similar PDS values for both isolates. Gamenya had PDS values of 0.87 and 0.65 (Fg. 200630 and Fg. 200646 respectively), while Ocoroni F86 had values of 0.79 and 0.63, and Zebra of 0.82 and 0.69. For all of these, the isolate Fg. 200646 resulted in a much lower PDS, but still on the higher end of the scale, in contrast to NIL 6B5.

A one-way ANOVA was done for each isolate, comparing the effect of line on PDS, and resulted in a p-value of $6.53e^{-14}$ (Fg. 200630) and $1.74e^{-08}$ (Fg. 200646) both of which are highly significant. The Tukey's HSD test revealed that the NILs 6A5 and 6B5 were only significantly

different in the plants inoculated with isolate Fg. 200630. Otherwise, the susceptible and resistant lines were significantly different from each other.

Table 11: Average percentage of diseased spikelets (PDS) after 21 days post inoculation for the six different lines CJ9306, Gamenya, NIL 6A5 and 6B5, Ocoroni F86, and Zebra with the number of values behind the calculated PDS. The Tukey's honest significant difference (HSD) test is also added for each of the inoculums, revealing the lines with significant differences. If two lines are not significantly different, they will have the same letter. Any lines with no common letters are significantly different from each other.

Line	Avg. PDS (Fg. 200630)	Tukey's HSD	Avg. PDS (Fg. 200646)	Tukey's HSD
CJ9306	0.05 (n=9)	A	0.05 (n=9)	A
Gamenya	0.87 (n=16)	B	0.65 (n=15)	C
NIL 6A5	0.22 (n=11)	A	0.18 (n=11)	AB
NIL 6B5	0.75 (n=12)	B	0.44 (n=9)	BC
Ocoroni F86	0.79 (n=8)	B	0.63 (n=7)	C
Zebra	0.82 (n=12)	B	0.69 (n=11)	C

The disease development for the wheat lines for each of the inoculums are visualized in **Figure 17** and **Figure 18**. The spreading appears to be stronger in the plants inoculated with isolate Fg. 200630 based on the two figures. Both figures also indicate that there was a clear divide between the susceptible and resistant lines around 9 DPI, and the biggest difference seems to be around 18 DPI, see **Appendix 8** for PDS calculations after 18 DPI. After this, the susceptible lines also started to accumulate infected spikelets more rapidly. Both figures reveal that CJ9306 and NIL 6A5 was the most resistant lines, as these had the least amount of spread within the heads of infected plants. NIL 6B5 was the line with the least spread out of the susceptible lines for both inoculums.

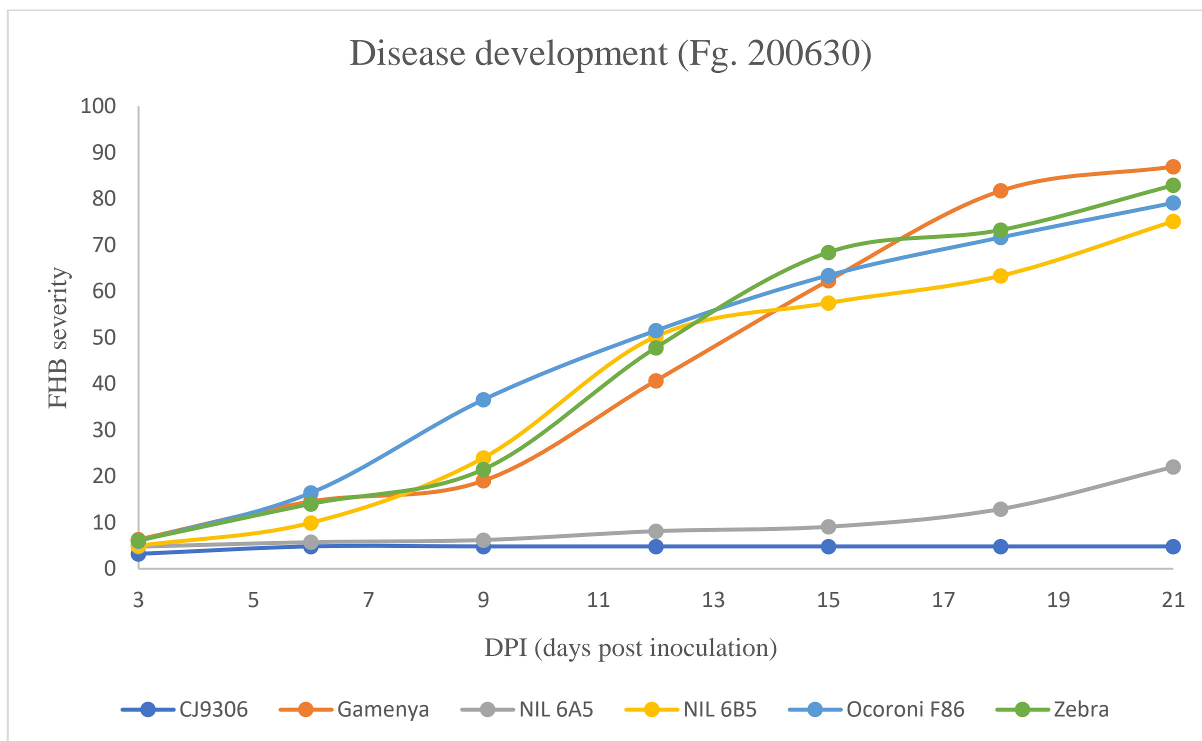

Figure 17: Disease development after point inoculation using the *F. graminearum* isolate Fg. 200630 from NIBIO. Scoring was done every 3 days for 21 days, starting at 3 days post inoculation (DPI). Six wheat lines (CJ9306, Gamenya, NIL 6A5 and 6B5, Ocoroni F86 and Zebra) were used. The FHB disease severity was measured in percentage of diseased spikelets (PDS).

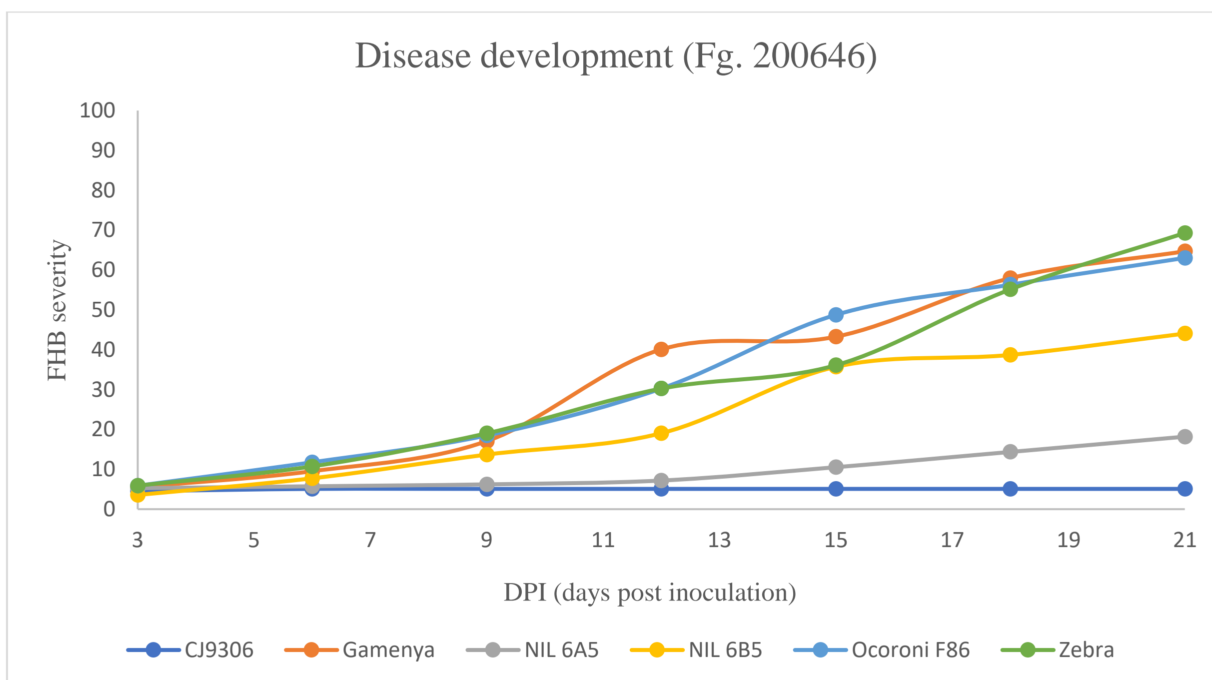

Figure 18: Disease development after point inoculation using the *F. graminearum* isolate Fg. 200646 from NIBIO. Scoring was done every 3 days for 21 days, starting at 3 days post inoculation (DPI). Six wheat lines (CJ9306, Gamenya, NIL 6A5 and 6B5, Ocoroni F86 and Zebra) were used. The FHB disease severity was measured in percentage of diseased spikelets (PDS).

In addition to studying the disease development over 21 days of phenotyping, the phenotypic data was also used to calculate the AUDPC. See **Appendix 9** for full tables of AUDPC values. AUDPC was calculated for each inoculated head and the mean for each line is represented in **Figure 19** with error bars based on the confidence intervals.

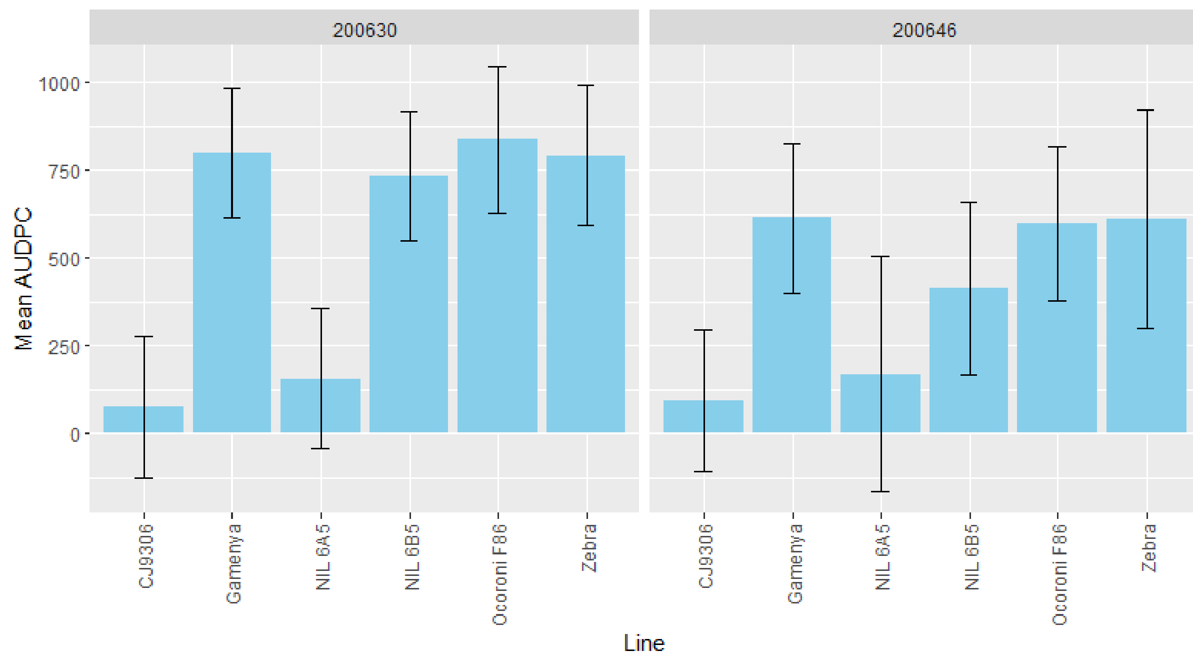

Figure 19: Mean average under the disease progress curve (AUDPC) values for each of the lines with confidence intervals, grouped by each of the two isolates (Fg. 200630 and Fg. 200646) used in the first part of the second point inoculation experiment. Error bars indicate confidence intervals for the means of each line.

As seen in **Figure 19** the two lines CJ9306 and NIL 6A5 had much lower AUDPC compared to the rest of the lines, and NIL 6B5 had the lowest AUDPC out of the four susceptible lines. This corresponds well with the other analyses of the disease development and PDS values. However, it is worth noting that the error bars are rather large, particularly for isolate Fg. 200646.

4.1.2.2 Near isogenic lines with recombinations

For the second part of the experiment, NILs with different recombinations between the three markers were chosen, as described in the methods section. All lines were inoculated with the same isolates and the FHB severity was measured by the same method as the previous experiment. During the experiment, the temperature seemed to increase due to more sunlight, so a few measurements were done to monitor this. Within the same week, three measurements

were done on temperature and humidity in the greenhouse. The results were temperatures of 30.9℃, 26.5℃, and 28.5℃, and humidity of 32.2%, 54.5%, and 48.9%. It should be noted that these measurements were taken at varying times of the day, spanning from around 9 AM to 4 PM.

The average PDS of each line for each of the isolates, as well as the allele of each marker for that line is shown in **Table 12**. The lines with all three resistance alleles are 5, 441, 1011, and 1511, while the lines with all susceptible alleles are 981 and 1081. Lines 13 and 411 contain the resistance allele for *wsnp_Ex_c8303_14001708* but not for the two other markers, while lines 971, 1101, and 1501 have the resistance allele for *gwm539* and *WGRB3803* but not *wsnp_Ex_c8303_14001708*. The average PDS varied between isolates for all lines, but most for the two lines 1011 (0.87 and 0.58 for Fg. 200630 and Fg. 200646 respectively) and 1501 (0.67 and 0.38). Out of the fully resistant lines, 5 and 441 were in the lower end of the scale with PDS around 30% (0.21 and 0.28 for line 5, 0.36 and 0.24 for line 441). However, lines 1011 and 1511 had much higher PDS averages (0.87 and 0.58 for 1011 and 0.45 and 0.59 for 1511). The fully susceptible lines were also around the same PDS values, 981 having average PDS of 0.43 and 0.54, and 1081 with 0.70 and 0.50. The rest of the lines showed PDS values around the same levels with no clear differences based on marker genotypes.

Lastly, one-way ANOVA was done to compare the effect of line on PDS for each isolate and resulted in p-values of 2.38^{-11} (Fg. 200630) and 0.000283 (Fg. 200646), which are both highly significant, though there is a large difference between them.

Table 12: The average percentage of diseased spikelets (PDS) after 21 days post inoculation (DPI) for both isolates in the NIL population with parental lines and Gamenya as a control line. The marker genotypes are also shown for each line with available information, where CJ indicates the resistant allele from CJ9306 and Z indicates the susceptible allele from Zebra. The number of replicates are shown in parenthesis behind each PDS value, and the allele genotype for each marker is also shown with the control lines as exceptions.

Line	*gwm539*	*WGRB3803*	*wsnp_Ex_c8303_14001708*	Avg. PDS (Fg. 200630)	Avg. PDS (Fg. 200646)
5	CJ	CJ	CJ	0.21 (n=10)	0.28 (n=11)
13	Z	Z	CJ	0.41 (n=11)	0.23 (n=10)
411	Z	Z	CJ	0.65 (n=10)	0.46 (n=8)
441	CJ	CJ	CJ	0.36 (n=8)	0.24 (n=7)
971	CJ	CJ	Z	0.45 (n=7)	0.30 (n=8)
981	Z	Z	Z	0.43 (n=6)	0.54 (n=8)
1011	CJ	CJ	CJ	0.87 (n=10)	0.58 (n=7)
1081	Z	Z	Z	0.70 (n=5)	0.50 (n=5)
1101	CJ	CJ	Z	0.29 (n=5)	0.44 (n=7)
1501	CJ	CJ	Z	0.67 (n=8)	0.38 (n=7)
1511	CJ	CJ	CJ	0.45 (n=8)	0.59 (n=8)
CJ9306	NA	CJ	NA	0.05 (n=7)	0.07 (n=7)
Gamenya	NA	NA	NA	0.91 (n=5)	0.68 (n=4)
Zebra	NA	Z	NA	0.67 (n=6)	0.60 (n=4)

The disease development curves for the NILs are shown in **Figure 20** and **Figure 21** for isolates Fg. 200630 and Fg. 200646 respectively. These lines were not as clearly divided into susceptible and resistant lines, and not all lines showed the same spreading for both inoculums. Nevertheless, line 971 had the strongest spread for both inoculums, while lines 5, 441 and 13 were in the lower end of FHB severity throughout both curves.

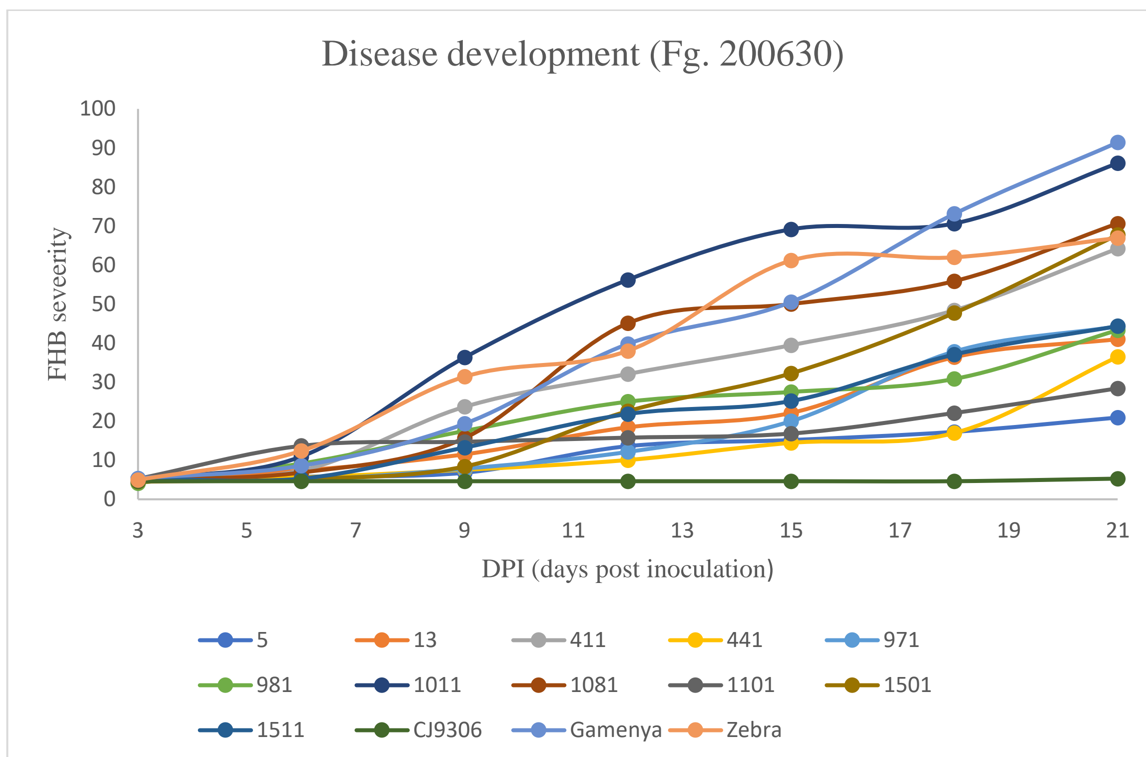

Figure 20: Disease development of the NILs, parent lines and Gamenya as a susceptible control, using isolate Fg. 200630. FHB severity was measured from 3 to 21 days post inoculation every 3 days. The FHB disease severity was measured in percentage of diseased spikelets (PDS).

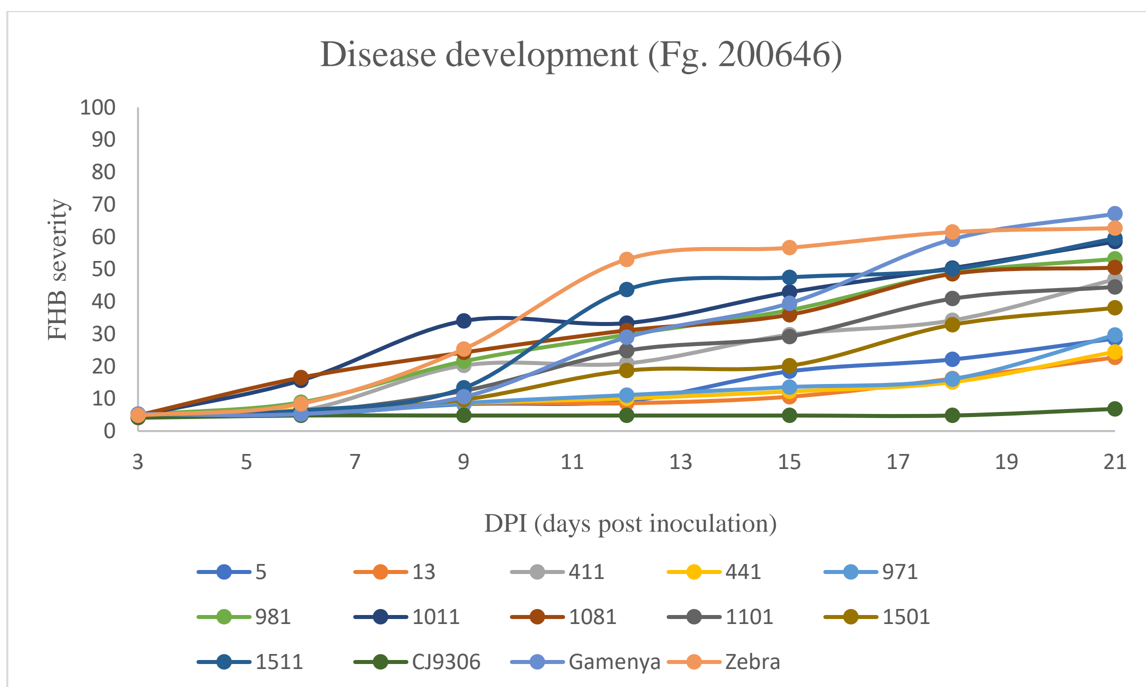

Figure 21: Disease development of the NILs, parent lines and Gamenya as a susceptible control, using isolate Fg. 200646. FHB severity was measured from 3 to 21 days post inoculation every 3 days. The FHB disease severity was measured in percentage of diseased spikelets (PDS).

AUDPC was measured for these lines as well, an overview plotted in **Figure 22**. The average AUDPC seems to be correlated with spike-spread, with CJ9306, 5, 13,441, and 971 at the lower spectrum and Gamenya, Zebra, 1011, and 1081 at the upper end of the spectrum.

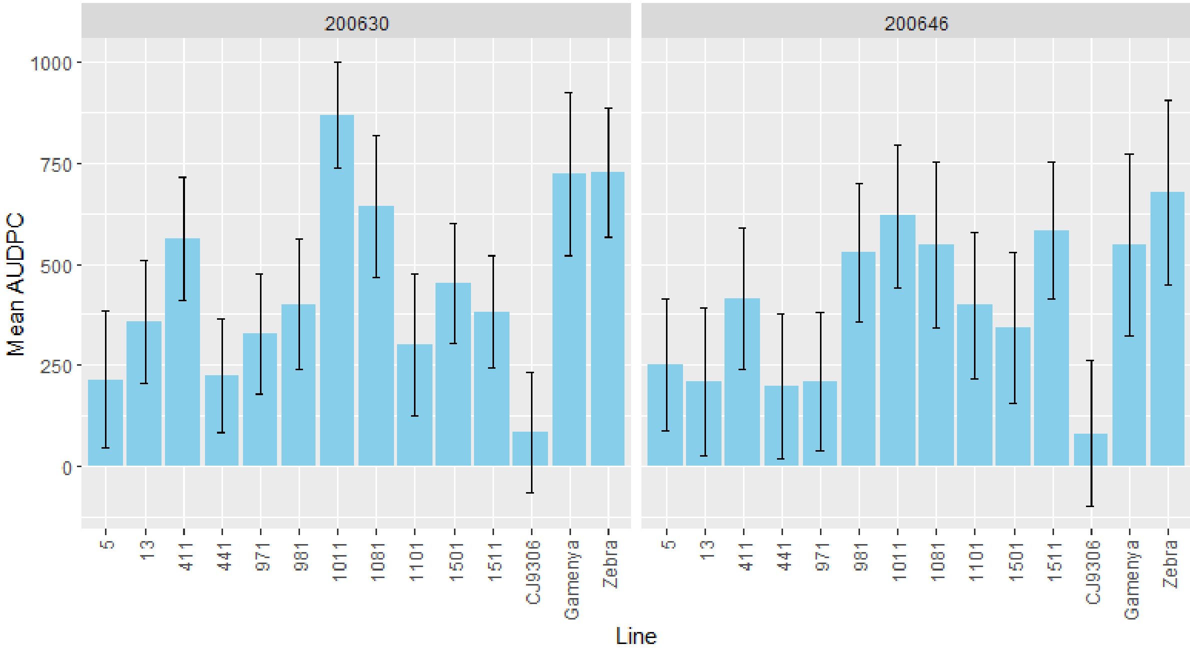

Figure 22: Mean area under the disease progress curve (AUDPC) for each of the NILs and control lines with confidence intervals, grouped for each isolate (Fg. 200630 and Fg. 200646). Gamenya and Zebra are susceptible controls (Zebra susceptible parent), while CJ9306 is the resistant parent. Error bars indicate confidence intervals for the means of each line.

5 Discussion

5.1 Fine-mapping QTL

Looking into the effect of marker alleles on FHB severity and DON accumulation, the results appear to be similar in several years. There were few statistically significant differences between the marker alleles, but the boxplots reveal that the two markers *gwm539* and *WGRB3803* appear to have a stronger effect on both FHB and DON values compared to *wsnp_Ex_c8303_14001708*. There were no clear differences between the *gwm539* and *WGRB3803* for FHB and DON, which is likely because there were few recombinations between these two markers and very few lines were tested in the field. The limitation in the number of lines tested in the field was also reflected in the results from *wsnp_Ex_c8303_14001708*, only a few lines tested in the field contained recombinations between this and the other two markers. Therefore, in some years, the same lines were used for the statistical analysis of all or some of the markers.

Moreover, when considering the linkage and physical map, *gwm539* and *WGRB3803* are also closer to each other than to *wsnp_Ex_c8303_14001708*. The physical distance between these markers is approximately 6 Mbp for all genomes, which should contain around an estimate of 40 genes, if we assume this area has the same gene density as the average of the genome. Taking all this into account, it is likely that the region between these two markers is well conserved and therefore probably lacking larger restructurings such as InDels, leading us to think that the genetic content in this region is likely very similar. More work is needed, but our finds suggest that this region could be very interesting to study, since it is likely to contain the gene responsible for the FHB resistance.

Followingly, the haplotype study revealed no clear correlations between haplotype blocks and marker genotypes, the only result to note was that Chinese Spring and Norin 61 did not share haplotype blocks with any genomes, meaning both are very different from the rest of the genomes in the pangenome. Furthermore, based on the marker data, it can be concluded that none of the pangenome lines show the same resistant haplotype for the 2DL QTL interval as CJ9306. CJ9306 had a 128 bp allele for *gwm539*, smaller than all the other genomes in the pangenome. However, the allele for Norin 61 genome had a size of 130 bp, which is the closest out of all the lines in the pangenome. Given the nature of SSR markers, it would be possible that Norin 61 has the same origin for this marker and QTL or is at least closer to a similar origin. Both CJ9306 and Norin 61 are of Asian origin (Chinese and Japanese respectively) (X. He et al., 2016; Shimizu et al., 2021), which makes it more likely that they share the resistance source.

On the other hand, Chinese Spring is also of Chinese pedigree, and had an allele for *gwm539* with the size 144 bp, much larger than both CJ9306 and Norin 61. Chinese Spring did, however, have the resistance allele for *WGRB3803*, which Norin 61 did not have. As *gwm539* and *WGRB3803* are very close, it is interesting that Norin 61 and Chinese Spring deviate in genotypes for these markers. Then again, *WGRB3803* was reported by Yan et al. (2021) in their RIL population with a resistance source from Yangmai 158, which is widely cultivated in China. This could lead us to believe that Norin 61 contains the resistance QTL. Additionally, Shimizu et al. (2021) reported that Norin 61 has shown FHB resistance, and that the cultivar contains a different variant from Chinese Spring of the resistance gene *Fhb1* (Shimizu et al., 2021). The resistance published from Norin 61 germplasm was not in our region, but considering the polygenic nature of FHB resistance, it does not exclude the possibility of containing the 2DL resistance QTL. With the knowledge that Norin 61 has established FHB resistance, in addition to it being the only cultivar with a close SSR allele to *gwm539*, one could argue for the use of this genome as a reference genome when studying this QTL on chromosome 2D. The last marker was *wsnp_Ex_c8303_14001708*, and none of the genomes investigated shared the same allele as CJ9306 for this marker.

FHB resistance is an additive trait, meaning that for agricultural purposes, breeding for more than one resistance QTL is essential to develop a cultivar with a wide resistance in terms of types of FHB resistance. To be able to study the 2DL QTL isolated from other resistance QTL, the germplasm used in this study segregates only for resistance on chromosome 2DL. Therefore, it is advisable to introduce other resistance QTL in addition to this when breeding new varieties. The currently well-established FHB resistance QTL are *Fhb1, Fhb2, Fhb4, Fhb5, Qfhs-ifa-5A, Qfhs.ndsu-3AS, Qfhb.nau-2B,* and *Qfhb.mgb-2A* (M. Buerstmayr et al., 2020). Only a limited selection of cultivars have *Fhb1*, some of these being Sumai 3 and Ningmai 9 with its derivatives. However, Zhu et al (2020) recommended using Ningmai 9 derivatives as a source of *Fhb1* for breeding, to avoid the linkage drag seen from Sumai 3 (Zhu et al., 2020). Even though only Type II resistance was detected in this mapping population, the QTL on 2D has shown a clear effect on both Type I and Type II resistance (Schroeder & Christensen, 1963) as well as strong DON resistance (Dhariwal et al., 2020), also evident from the literature study (**Table 1**). Which resistance types detected depends on germplasm, environment, and experimental design. We have not found any Type I experiments on this QTL from CJ9306 germplasm (X. He et al., 2016), so making sure to include other QTL with Type I resistance is necessary. Pyramiding FHB resistance QTL is therefore essential for breeding new cultivars

with a strong resistance. Chen et al. (2021) reported that pyramiding of *Fhb1*, the QTL they found on 2DL (*QFhb.hnau.2DL*) and 5AL (*QFhbn.hnau.5AL*) have significant additive effects, and that the additive effect was much more significant than their isolated effect (Chen et al., 2021).

5.2 Point inoculation and phenotyping

The first round of point inoculation experiments was not successful, as was apparent in the ANOVA of the Type II resistance after phenotyping. There was no clear difference between the resistant and susceptible controls. A likely reason was that the *F. graminearum* isolates used for creating the inoculum were not aggressive enough. All inoculated spikelets included in the analysis were successfully infected after inoculation, as the few plants with unsuccessful inoculation were removed from the analyses. Therefore, it was not the initial infection that caused the issue, but the failure of disease spread within the spike. Certain varieties such as the highly susceptible variety Gamenya, should have the entire spike infected for all plants, but this only happened for one or two heads. We were also not able to separate the two NILs, even though one harboured the resistance QTL. This was confirmed after checking with the aggressiveness scale of isolates in NIBIO's database, where two of our isolates were among the least aggressive available, which supports our hypothesis. The other two isolates used in the inoculum mixture were not from NIBIO, so there was no available information about the aggressiveness.

Based on the observations from the first round, we decided to change certain aspects of the experiment to optimize the environment. The obvious factor to change first was the inoculum, so we obtained the isolates Fg. 200646 and Fg. 200630 from NIBIO which were highly aggressive strains of *F. graminearum.* Contrasting to the first inoculum production, we did not mix the isolates this time, but rather used two different inoculums, one for each isolate. This was also advised from Prof. Hermann Buerstmayr's group, as mixing the isolates could have a counterproductive effect where the isolates cancel each other out, while it is unlikely for them to have an additive effect. It should also be noted that the phenotyping was not the most consistent in this first experiment, due to poor timing. However, we still expected to see clearer results despite this.

Additionally, instead of maintaining a high humidity of 90% and 25°C during the entire course of the experiment, we chose to lower both the temperature and humidity, and put plastic bags

sprayed with water on the spikes for the first 48 hours to ensure infection after inoculation. This was also advised from Prof. Barbara Steiner and Prof. Hermann Buerstmayr and is a method that has been mentioned in the literature (M. Buerstmayr et al., 2021; Kage et al., 2017). A possible downfall of using the plastic bags is that if there is a lot of direct light on the plastic bags, this could increase the temperature out of our control. However, the same can be said for any greenhouse, as the sun hitting the greenhouse on sunny days increase the temperature in the room. During the second round of inoculations, we observed that the greenhouse room was very warm, so I recorded the temperature and humidity on a few different days during the same week. The measurements showed around 4°C span in temperature measurements throughout the week, meaning that the temperature was not constantly too high. Due to variations in weather, there were, naturally, also variations in the greenhouse. These types of factors are out of our control, and if the temperature increases too much, this could be problematic. However, maintaining the high humidity is important for infection to occur, and the short period of uncontrolled high temperature is likely better than having an excessively high temperature during the entire course of the experiment. In a way, these uncontrolled environmental variations contribute to simulate more realistic conditions, as the temperature and humidity varies constantly in the field.

The germination rate of both inoculums was checked for the second point inoculation experiment, after advice from Prof. Barbara Steiner, who mentioned that if the germination rate is above 80-90% it is considered good. Both new isolates surpassed this threshold. Considering that no germination test was performed for the first inoculum, it is impossible to determine whether this was the issue the first time around. However, as the initial infections were successful the first time, this was probably not the problem, there were clearly enough spores to cause infection. Even so, testing the germination rate of the inoculum is a good routine to implement, to be certain that any issues that might occur later are not due to poorly germinating inoculums. It is also not very time consuming, nor does it add much extra cost.

Looking at the point inoculation results from the mapping population and control lines, there were clear differences between resistant and susceptible lines with the new, more aggressive isolates. Not as many cultivars were sown out this second time but considering that the issue the first time was a lack of spread within the spike, we decided to prioritize susceptible controls to make sure that we could detect spreading. There was a large difference between the susceptible and resistant parents, and all susceptible lines had similar average PDS values. As for the NILs 6A5 and 6B5, there was also a large difference (6A5 average PDS 0.22 and 0.18,

6B5 0.75 and 0.44 for inoculums Fg. 200630 and Fg. 200646 respectively). NIL 6A5 carries the resistant allele of the QTL (specifically for the marker *gwm539*) on chromosome 2DL, while 6B5 does not carry this allele. The Tukey's HSD test revealed that the difference between the two NILs was only significant for isolate Fg. 200630. This was surprising results, but further support that the choice of isolate is very important for the experimental design. It is also worth noting that neither of the NILs were as extreme as the parental lines or the controls. This is expected since CJ9306 carries the resistant alleles of all three QTL *Fhb1*, *Fhb5* and 2DL while the NIL 6A5 only carries the resistant allele of the 2DL QTL.

The disease development was measured and illustrated for each of the inoculums, showing that the susceptible and resistant lines typically start to diverge around 9 DPI, with the biggest difference seemingly around 18 DPI. After 18 DPI, the resistant lines started to increase in FHB severity. As shrivelled spikelets as well as spikelets with mycelium were considered while scoring, the scores towards the end of the disease progress are perhaps not as trustworthy as at the earlier stages due to the plants starting to mature and dry out which could be mistaken as shrivelled spikelets. The inoculation was done at the start of flowering, while the anthers were still yellow, and after 21 days they were mostly all matured. The AUDPC was also calculated for the different lines and showed results corresponding to the PDS results. However, the confidence intervals are very large, meaning that no clear conclusions can be made based purely on mean AUDPC. An improvement which could limit the confidence intervals would be to increase the sample size by inoculating more spikes of each line and for each inoculum.

The two isolates used were Fg. 200630 and Fg. 200646 from NIBIO, chosen based on aggressiveness. The hypothesis after the first round of inoculations during the winter was that the isolates used were not aggressive enough, causing us to choose more aggressive isolates this time. Regarding the results, this hypothesis appears to be correct. Based on the disease development diagrams in **Figure 17** and **Figure 18**, isolate Fg. 200630 appears to be more aggressive, as the FHB severity for the susceptible lines was much higher compared to individuals inoculated with isolate Fg. 200646. However, Fg. 200630 had a slightly higher germination rate (4% higher), which could potentially contribute, though it is unlikely to be a large contributor as both isolates were well over the threshold. The disease development for isolate Fg. 200646 showed a smaller difference in FHB severity between the two NILs (6A5 PDS value 0.18 and 6B5 with a value of 0.44, compared to 0.22 and 0.75 respectively for isolate Fg. 200630).

Due to the much clearer difference between the NILs in the second point inoculation, it was possible to continue investigating the QTL though point inoculation experiments. The lines chosen for this part of the experiment were a combination of different genotypes for the three markers found from the fine-mapping, as well as the parental lines and Gamenya as a susceptible control. The NILs had four different genotypes: either susceptible/resistant alleles for all three markers, susceptible alleles for *gwm539* and *WGRB3803* and resistant allele for *wsnp_Ex_c8303_14001708,* or resistant alleles for *gwm539* and *WGRB3803* and susceptive allele for *wsnp_Ex_c8303_14001708*. We were unable to find complete recombination between the two markers *gwm539* and *WGRB3803* changing from one homozygote genotype to the other. Therefore, these two markers have the same allele in all selected NILs. The expectation based on genotypes would be to see a clear difference between the NILs with complete resistance and susceptibility, and hopefully see a difference between *wsnp_Ex_c8303_14001708* and the two other markers. That would potentially be able to narrow down the location of the QTL, giving an idea of which marker is closer to the QTL.

For this part of the experiment, the average PDS, the disease development over time, ANOVA and AUDPC for all lines were analysed. Throughout these sections, the controls performed as expected, with CJ9306 being the most resistant of all lines tested, and Gamenya and Zebra being highly susceptible. The NILs with resistant alleles for all markers were entries 5, 441, 1011, and 1511. The first two were within the lower part of the scale when it comes to avg. PDS values, while the other two show more surprising results. Entry 1511 should be fully resistant but had an average PDS around 0.50 (0.45 for Fg. 200630 and 0.59 for Fg. 200646), meaning approximately 50% diseased spikelets. In comparison, entry 981 contained all three susceptible alleles and had an average PDS of 0.43 (Fg. 200630) and 0.54 (Fg. 200646), both lower than 1511. Additionally, entry 1011 contains all three resistance alleles and had average PDS values of 0.87 (Fg. 200630) and 0.58 (Fg. 200646), which is higher on average than Zebra at 0.67 (Fg. 200630) and 0.60 (Fg. 200646). This is very odd, and there is a possibility of errors occurring during the experiment that causes this. One possibility is that there were mix-ups of line 1011 with line 1101 during either genotyping or sowing. The plants were sowed in two different rounds without any notice of sowing errors, so it is more likely to have occurred during genotyping. It would therefore be natural to genotype these lines again using the same seed source as was used in the point inoculation experiment to investigate this. There was not enough time during this project, but this will be the next step. A Tukey's HSD test was not performed

on this data, as the PDS and AUDPC results did not show the clear differences we were expecting and would therefore not give us any more information.

These results raise the question of whether isolate Fg. 200630 might be too aggressive, causing unrealistic spreading. As can be seen in **Table 11** and **Table 12**, isolate Fg. 200630 is the one with highest average PDS in most of the cases, and the instances where isolate Fg. 200646 had the highest value, the difference is not equally drastic as for Fg. 200630. However, there is probably a different reason for entry 1011 to have such surprising results, as the resistant and susceptible checks were as expected for the mapping population and the NILs. Considering that the NILs homozygous for either CJ0306 or Zebra alleles at all three markers presented unclear phenotypic results, it was not possible to compare the lines with recombination between the markers to further locate the QTL.

Looking into the disease development graphs, isolate Fg. 200630 seemed to be more aggressive here as well. Apart from the controls, the lines with highest and lowest FHB severity were not the same in both plots, which can be a coincidence considering that the datasets for each of the inoculums were somewhat limited. There was also variation between the different lines regarding how quickly they increased in FHB severity, it was not as uniform as for the mapping population. The AUDPC values diverged more in this second part however, with CJ9306 deviating from the rest, and the only NIL with a similar AUDPC to the susceptible controls was 1011, which is one of the lines which should be completely resistant as it has all three resistant alleles. As surprising as these results are, it correlates well with the PDS values.

AE influences FHB, and could be a part of the FHB resistance for this QTL on chromosome 2DL. As mentioned, AE is one of the morphological traits with an established effect on FHB in wheat (M. Buerstmayr et al., 2020). A QTL for AE has been shown to be close to the QTL in He et al. (2016), as well as noted in Milan Sapkota's master's thesis (Sapkota, 2018). This could contribute to the resistance shown, as AE contributes to FHB resistance. To test this, I checked if there was any clear difference in AE in the two NILs 6A5 and 6B5 but could not find any clear difference. This was not a part of the phenotyping experiment, but rather something that was checked once. However, most of the NILs in the latter part of the point inoculation experiment, the plants containing different recombinations of the three markers all showed high levels of AE, just from quick observations while performing the main part of the experiment. Given the close location of genes involved in AE as shown by Lu et al. (2013), there is a possibility of these genes being in the QTL of interest on chromosome 2DL. Conversely, another study showed that AE genes were located further away from the 2DL QTL

(X. He et al., 2016), contrasting previous results. This indicates that the difference in AE is likely due to a different gene in the same chromosomal region and which is connected to the resistance QTL in some resistance sources, but not all. It seems to not be connected to the QTL in our fine-mapping population, but a more thorough analysis is needed to conclude on this.

5.3 Recommendations

For further studies on the FHB resistance QTL on chromosome 2D in wheat, there are several points to consider improving. As for the use of genetic markers, fine-mapping revealed that *gwm539* and *WGRB3803* appeared to be the most interesting for further studies based on our results. Based on the literature study of previous work on this QTL, several studies used *gwm539* (X. He et al., 2016; Jiang, Dong, et al., 2007; Jiang, Shi, et al., 2007; Long et al., 2015; Lu et al., 2013) for QTL mapping, while only two studies mentioned *WGRB3803* (Chen et al., 2021; Yan et al., 2021). However, the two studies that mentioned this last marker, were from different pedigrees, Chinese and Italian, whereas the origins of our resistance source is also Chinese. *WGRB3803* appears to be a robust marker to use for further analyses on this QTL, though I would recommend using *gwm539* too, as this study was not able to find clear differences between these two with regards to their effect on FHB severity. Considering that several other studies have already used *gwm539,* it might be interesting to choose a different marker, which is why *WGRB3803* could be preferred. Then again, *gwm539* was the marker used to distinguish the resistant QTL in our mapping population, which was successful.

Additionally, pyramiding of FHB resistance QTL is essential to develop new wheat lines with a good enough resistance to be a useful, new cultivar. As mentioned, there are several well-characterized resistance QTL available, but I would recommend making sure that they cover different types of resistance to FHB, considering that the QTL on 2D from our germplasm (CJ9306) has only shown Type II resistance so far. *Fhb5* has also been shown to be good for pyramiding with this QTL on 2D.

The recommendations I would suggest for a point inoculation experiment of this type, is to create a good routine for inoculations, scoring of phenotypes and germination testing. After discussing it with several people, I concluded that scoring every three days was best, but it was also suggested to do scoring every 5 days. This depends on what you want to investigate and what analyses you want to perform on the phenotypic data. Considering one of my interests was to study the disease development, I thought a more detailed scoring was preferable.

However, this is very time consuming and requires constant attention to the plants. I would also advise to perform the scoring approximately at the same time every day for consistency's sake. Light conditions can affect the scoring, and the differences are minimized if it is performed at the same time of day.

The inoculations were most successful when performed at the start of flowering, and after some trial and error, I found inoculating one spikelet in the middle of the spike to work best. A change made in the method for scoring from the first experiment was that instead of adding tags with the date, I added coloured tape to the stem of the plant, using the same colour on all the heads inoculated on the same day, based on recommendations from Prof. Barbara Steiner. This made it much easier to recognise which heads to score at the same day later. The use of bags also worked well for the second experiment, and I would advise this method over very high humidity in the greenhouse room the entire length of the experiment. If there is too much humidity, the mycelium can start to grow on the outside of the spikelets, making it harder to recognise the spread within the spike.

Due to a limited timeframe for the second experiment, only two repetitions with two pots were sowed out for the NILs, which consequently limited the number of inoculated spikes per line. An improvement would be to sow out more material, either more per repetition, or more rounds of sowing. A larger dataset would potentially give clearer results than what this study obtained. We also used two inoculums, which meant that the amount of data per inoculum was quite limited. Doing a test experiment to determine inoculum beforehand would be advised, as well as making sure to choose aggressive isolates for testing.

6 Future perspectives

An interesting point for future work on this resistance QTL is comparing some of the genomes available in the pangenome to look for specific genes in the region between the two makers *gwm539* and *WGRB3803*. As mentioned, we estimate that there could be around 40 genes in this region, so looking into these could reveal valuable information. The only marker we found with lines containing the resistance allele from CJ9306 in the pangenome, was *WGRB3803*. However, even though none of the lines in the pangenome had the same allele for *gwm539* as CJ9306, Norin 61 had the most similar length and could be closer to the resistance source. Norin 61 is a Japanese cultivar, and as a lot of resistant germplasm have their sources from East Asia, this cultivar could have an FHB resistance from the same source. Chinese Spring is also an Asian cultivar and is likely more genetically related to Norin 61 than the other pangenome varieties, and given the results from the haplotype blocks, it was clear that Chinese Spring is at least very different from the other lines. One future experiment could be to compare this region with Chinese Spring and see if there are specific genes that differ and investigate the function of these genes. Moreover, the region is now much narrower though still relatively large for studying specific genes. Investigating the genes in this region could still be an efficient method for fine-mapping considering the point inoculation experiment was unsuccessful in narrowing down which marker is closer.

The data we have on different combinations of genotypes for the three markers *gwm539*, *WGRB3803,* and *wsnp_Ex_c8303_14001708* was used to create a NIL population for point inoculation experiment but could also be used for further fine-mapping. In this experiment, we only used homozygotes as these were the only ones we can know the genotype of without further genotyping. However, by genotyping selfed offspring of the heterozygotes, this could result in useful information about which of the markers are closer to the QTL. Furthermore, additional genotyping using high density SNP arrays can be done to identify more markers in this region.

Other potential experiments could be to sequence the chromosomal region on chromosome 2DL in the NILs, which would give more information on the genetic diversity between the region of interest and elucidate which markers are closer to the position of the QTL. Another possibility is to perform transcription analyses based on point inoculation experiments. In this case, sequencing the RNA from infected spikelets of resistant and susceptible NILs would enable identification of differentially expressed genes, and comparisons with mock-inoculated spikelets would help compare which genes are up/down regulated or completely turned on/off.

Exome capture sequencing is also a possibility, a cheaper method which does not require costly whole genome sequencing, while still finding information on expressed genes.

As mentioned, the 6 Mbp region between the markers *gwm539* and *WGRB3803* would be very interesting for further studies on this QTL. Genotyping this region would hopefully limit the number of candidate genes, which would in turn be interesting to further investigate. One alternative after this is to create a TILLING population. In contrast to the typical forward approach of QTL mapping which links mutations to phenotypic changes, TILLING utilizes a reverse approach to genomics (Kurowska et al., 2011). This includes mutagenesis and high throughput sequencing and would in this case mainly be used to find mutations and new alleles in a QTL. It is therefore best to have a few candidate genes before creating a TILLING population. Similarly, another possibility for further characterization after finding some candidate genes would be to design a CRISPR experiment combined with pooled screening. Then, it would be possible to do several edits in candidate genes and when the pathogen is introduced to a pool, it will result in a ranked list of how the edited genes were affected by the pathogen (Bock et al., 2022). This should reveal if the edits made resulted in stronger or weaker resistance.

7 Conclusion

In conclusion, the fine-mapping of resistance QTL on chromosome 2DL in wheat resulted in three genetic markers that can be useful for further studies, in particular the two markers *gwm539* and *WGRB3803* appeared to have the strongest effect on FHB resistance and have been reported in previous studies. An updated physical map containing all the markers tested in this and previous projects, can also contribute to further fine-mapping studies on this QTL. As for the phenotypic characterization, the changes made for the second attempt at point inoculations improved the experiment significantly. The key elements discovered through trial and error was the importance of using an aggressive isolate for point inoculations with *Fusarium graminearum* and creating a good routine for the execution of the inoculations and phenotypic scoring. The point inoculations revealed a clear phenotypic effect of the resistance QTL in the mapping population but was not successful in differentiating different genotypes of the three genetic markers in the NILs.

Reference list

Allen, A. M., Winfield, M. O., Burridge, A. J., Downie, R. C., Benbow, H. R., Barker, G. L. A., Wilkinson, P. A., Coghill, J., Waterfall, C., Davassi, A., Scopes, G., Pirani, A., Webster, T., Brew, F., Bloor, C., Griffiths, S., Bentley, A. R., Alda, M., Jack, P., … Edwards, K. J. (2017). Characterization of a Wheat Breeders' Array suitable for high-throughput SNP genotyping of global accessions of hexaploid bread wheat (Triticum aestivum). *Plant Biotechnology Journal*, *15*(3), 390–401. https://doi.org/10.1111/pbi.12635

Arrones, A., Vilanova, S., Plazas, M., Mangino, G., Pascual, L., Díez, M. J., Prohens, J., & Gramazio, P. (2020). The Dawn of the Age of Multi-Parent MAGIC Populations in Plant Breeding: Novel Powerful Next-Generation Resources for Genetic Analysis and Selection of Recombinant Elite Material. *Biology*, *9*(8), 229. https://doi.org/10.3390/biology9080229

Barbazuk, W. B., Emrich, S. J., Chen, H. D., Li, L., & Schnable, P. S. (2007). SNP discovery via 454 transcriptome sequencing. *The Plant Journal*, *51*(5), 910–918. https://doi.org/10.1111/j.1365-313X.2007.03193.x

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Bhat, J. A., Ali, S., Salgotra, R. K., Mir, Z. A., Dutta, S., Jadon, V., Tyagi, A., Mushtaq, M., Jain, N., Singh, P. K., Singh, G. P., & Prabhu, K. V. (2016). Genomic Selection in the Era of Next Generation Sequencing for Complex Traits in Plant Breeding. *Frontiers in Genetics*, *7*. https://www.frontiersin.org/article/10.3389/fgene.2016.00221

Bock, C., Datlinger, P., Chardon, F., Coelho, M. A., Dong, M. B., Lawson, K. A., Lu, T., Maroc, L., Norman, T. M., Song, B., Stanley, G., Chen, S., Garnett, M., Li, W.,

Moffat, J., Qi, L. S., Shapiro, R. S., Shendure, J., Weissman, J. S., & Zhuang, X. (2022). High-content CRISPR screening. *Nature Reviews Methods Primers*, *2*(1), 1–23. https://doi.org/10.1038/s43586-021-00093-4

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

Buerstmayr, M., Steiner, B., & Buerstmayr, H. (2020). Breeding for Fusarium head blight resistance in wheat—Progress and challenges. *Plant Breeding*, *139*(3), 429–454. https://doi.org/10.1111/pbr.12797

Buerstmayr, M., Wagner, C., Nosenko, T., Omony, J., Steiner, B., Nussbaumer, T., Mayer, K. F. X., & Buerstmayr, H. (2021). Fusarium head blight resistance in European winter wheat: Insights from genome-wide transcriptome analysis. *BMC Genomics*, *22*(1), 470. https://doi.org/10.1186/s12864-021-07800-1

Chen, S., Zhang, Z., Sun, Y., Li, D., Gao, D., Zhan, K., & Cheng, S. (2021). Identification of quantitative trait loci for Fusarium head blight (FHB) resistance in the cross between wheat landrace N553 and elite cultivar Yangmai 13. *Molecular Breeding*, *41*(3), 24. https://doi.org/10.1007/s11032-021-01220-5

Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B., & Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica*, *142*(1), 169–196. https://doi.org/10.1007/s10681-005-1681-5

Collard, B. C. Y., & Mackill, D. J. (2008). Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1491), 557–572. https://doi.org/10.1098/rstb.2007.2170

Crossa, J., Burgueño, J., Dreisigacker, S., Vargas, M., Herrera-Foessel, S. A., Lillemo, M., Singh, R. P., Trethowan, R., Warburton, M., Franco, J., Reynolds, M., Crouch, J. H., & Ortiz, R. (2007). Association Analysis of Historical Bread Wheat Germplasm Using Additive Genetic Covariance of Relatives and Population Structure. *Genetics*, *177*(3), 1889–1913. https://doi.org/10.1534/genetics.107.078659

Dhariwal, R., Henriquez, M. A., Hiebert, C., McCartney, C. A., & Randhawa, H. S. (2020). Mapping of Major Fusarium Head Blight Resistance from Canadian Wheat cv. AAC Tenacious. *International Journal of Molecular Sciences*, *21*(12), 4497. https://doi.org/10.3390/ijms21124497

Edae, E. A., Byrne, P. F., Haley, S. D., Lopes, M. S., & Reynolds, M. P. (2014). Genome-wide association mapping of yield and yield components of spring wheat under contrasting moisture regimes. *Theoretical and Applied Genetics*, *127*(4), 791–807. https://doi.org/10.1007/s00122-013-2257-8

Edwards, S. G., & Godley, N. P. (2010). Reduction of Fusarium head blight and deoxynivalenol in wheat with early fungicide applications of prothioconazole. *Food Additives & Contaminants: Part A*, *27*(5), 629–635. https://doi.org/10.1080/19440040903515942

Elen, O., Hofgaard, I. S., Brodal, G., Klemsdal, S., & Aamot, H. U. (2009). Kan vi redusere mykotoksinmengden i korn ved å sprøyte med fungicider? *Bioforsk FOKUS*, *4*(2), 198–199.

EØS-tillegget til Den europeiske unions tidende (Kommisjonsforordning No. 56/717). (2007). Lovdata.no. https://lovdata.no/static/NLX3/32007r1126.pdf

FAO. (2022). *Overview | Sustainable Development Goals*. Food and Agriculture Organization of the United Nations. https://www.fao.org/sustainable-development-goals/overview/en/

Felleskjøpet. (2022). *Kornguiden 2021-2022* (pp. 8–13). https://ipaper.ipapercms.dk/TidRom/felleskjopet/kornguiden/

Gali, K. K., Sackville, A., Tafesse, E. G., Lachagari, V. B. R., McPhee, K., Hybl, M., Mikić, A., Smýkal, P., McGee, R., Burstin, J., Domoney, C., Ellis, T. H. N., Tar'an, B., & Warkentin, T. D. (2019). Genome-Wide Association Mapping for Agronomic and Seed Quality Traits of Field Pea (Pisum sativum L.). *Frontiers in Plant Science*, *10*. https://www.frontiersin.org/article/10.3389/fpls.2019.01538

Geldermann, H. (1975). Investigations on inheritance of quantitative characters in animals by gene markers I. Methods. *TAG. Theoretical and Applied Genetics. Theoretische Und Angewandte Genetik*, *46*(7), 319–330. https://doi.org/10.1007/BF00281673

He, J., Zhao, X., Laroche, A., Lu, Z.-X., Liu, H., & Li, Z. (2014). Genotyping-by-sequencing (GBS), an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding. *Frontiers in Plant Science*, *5*. https://www.frontiersin.org/article/10.3389/fpls.2014.00484

He, X., Lillemo, M., Shi, J., Wu, J., Bjørnstad, Å., Belova, T., Dreisigacker, S., Duveiller, E., & Singh, P. (2016). QTL Characterization of Fusarium Head Blight Resistance in CIMMYT Bread Wheat Line Soru#1. *PLOS ONE*, *11*(6), e0158052. https://doi.org/10.1371/journal.pone.0158052

Herter, C. P., Ebmeyer, E., Kollers, S., Korzun, V., Leiser, W. L., Würschum, T., & Miedaner, T. (2018). Rht24 reduces height in the winter wheat population 'Solitär × Bussard' without adverse effects on Fusarium head blight infection. *Theoretical and Applied Genetics*, *131*(6), 1263–1272. https://doi.org/10.1007/s00122-018-3076-8

Hofgaard, I. S., Aamot, H. U., Torp, T., Jestoi, M., Lattanzio, V. M. T., Klemsdal, S. S., Waalwijk, C., Lee, T. van der, & Brodal, G. (2016). Associations between Fusarium

species and mycotoxins in oats and spring wheat from farmers fields in Norway over a six-year period. *World Mycotoxin Journal*, *9*(3), 365–378. https://doi.org/10.3920/WMJ2015.2003

Hu, X., Rocheleau, H., McCartney, C., Biselli, C., Bagnaresi, P., Balcerzak, M., Fedak, G., Yan, Z., Valè, G., Khanizadeh, S., & Ouellet, T. (2019). Identification and mapping of expressed genes associated with the 2DL QTL for fusarium head blight resistance in the wheat line Wuhan 1. *BMC Genetics*, *20*(1), 47. https://doi.org/10.1186/s12863-019-0748-6

IWGSC. (2018). *Wheat—IWGSC*. http://www.wheatgenome.org/News/Media-resources/Fact-Sheets-Infographics/Wheat-A-Key-Crop-for-Food-Security

IWGSC, Appels, R., Eversole, K., Stein, N., Feuillet, C., Keller, B., Rogers, J., Pozniak, C. J., Choulet, F., Distelfeld, A., Poland, J., Ronen, G., Sharpe, A. G., Barad, O., Baruch, K., Keeble-Gagnère, G., Mascher, M., Ben-Zvi, G., Josselin, A.-A., … Wang, L. (2018). Shifting the limits in wheat research and breeding using a fully annotated reference genome. *Science*, *361*(6403), eaar7191. https://doi.org/10.1126/science.aar7191

Jia, H., Zhou, J., Xue, S., Li, G., Yan, H., Ran, C., Zhang, Y., Shi, J., Jia, L., Wang, X., Luo, J., & Ma, Z. (2018). A journey to understand wheat Fusarium head blight resistance in the Chinese wheat landrace Wangshuibai. *The Crop Journal*, *6*(1), 48–59. https://doi.org/10.1016/j.cj.2017.09.006

Jiang, G.-L., Dong, Y., Shi, J., & Ward, R. W. (2007). QTL analysis of resistance to Fusarium head blight in the novel wheat germplasm CJ 9306. II. Resistance to deoxynivalenol accumulation and grain yield loss. *Theoretical and Applied Genetics*, *115*(8), 1043–1052. https://doi.org/10.1007/s00122-007-0630-1

Jiang, G.-L., Shi, J., & Ward, R. W. (2007). QTL analysis of resistance to Fusarium head blight in the novel wheat germplasm CJ 9306. I. Resistance to fungal spread. *Theoretical and Applied Genetics*, *116*(1), 3–13. https://doi.org/10.1007/s00122-007-0641-y

Kage, U., Yogendra, K. N., & Kushalappa, A. C. (2017). TaWRKY70 transcription factor in wheat QTL-2DL regulates downstream metabolite biosynthetic genes to resist Fusarium graminearum infection spread within spike. *Scientific Reports*, *7*(1), 42596. https://doi.org/10.1038/srep42596

Kassambara, A. (2020). *Ggpubr: 'ggplot2' Based Publication Ready Plots. R package version 0.4.0.* https://CRAN.R-project.org/package=ggpubr

Kim, S., & Misra, A. (2007). SNP Genotyping: Technologies and Biomedical Applications. *Annual Review of Biomedical Engineering*, *9*(1), 289–320. https://doi.org/10.1146/annurev.bioeng.9.060906.152037

Kurowska, M., Daszkowska-Golec, A., Gruszka, D., Marzec, M., Szurman, M., Szarejko, I., & Maluszynski, M. (2011). TILLING - a shortcut in functional genomics. *Journal of Applied Genetics*, *52*(4), 371–390. https://doi.org/10.1007/s13353-011-0061-1

LaFramboise, T. (2009). Single nucleotide polymorphism arrays: A decade of biological, computational and technological advances. *Nucleic Acids Research*, *37*(13), 4181–4193. https://doi.org/10.1093/nar/gkp552

Lenth, R. V. (2016). Least-Squares Means: The R Package lsmeans. *Journal of Statistical Software*, *69*(1), 1–33. https://doi.org/10.18637/jss.v069.i01

Lesk, A. M. (2017). *Introduction to Genomics* (Third). Oxford University Press.

Long, X., Balcerzak, M., Gulden, S., Cao, W., Fedak, G., Wei, Y.-M., Zheng, Y.-L., Somers, D., & Ouellet, T. (2015). Expression profiling identifies differentially expressed genes associated with the fusarium head blight resistance QTL 2DL from the wheat variety

Wuhan-1. *Physiological and Molecular Plant Pathology*, *90*, 1–11. https://doi.org/10.1016/j.pmpp.2015.02.002

Lopes, M. S., Dreisigacker, S., Peña, R. J., Sukumaran, S., & Reynolds, M. P. (2015). Genetic characterization of the wheat association mapping initiative (WAMI) panel for dissection of complex traits in spring wheat. *Theoretical and Applied Genetics*, *128*(3), 453–464. https://doi.org/10.1007/s00122-014-2444-2

Lu, Q., Lillemo, M., Skinnes, H., He, X., Shi, J., Ji, F., Dong, Y., & Bjørnstad, Å. (2013). Anther extrusion and plant height are associated with Type I resistance to Fusarium head blight in bread wheat line ‘Shanghai-3/Catbird’. *Theoretical and Applied Genetics*, *126*(2), 317–334. https://doi.org/10.1007/s00122-012-1981-9

Marcussen, T., Sandve, S. R., Heier, L., Spannagl, M., Pfeifer, M., THE INTERNATIONAL WHEAT GENOME SEQUENCING CONSORTIUM, Jakobsen, K. S., Wulff, B. B. H., Steuernagel, B., Mayer, K. F. X., & Olsen, O.-A. (2014). Ancient hybridizations among the ancestral genomes of bread wheat. *Science*, *345*(6194), 1250092. https://doi.org/10.1126/science.1250092

Mason, A. S. (2015). SSR Genotyping. In J. Batley (Ed.), *Plant Genotyping: Methods and Protocols* (pp. 77–89). Springer. https://doi.org/10.1007/978-1-4939-1966-6_6

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. https://doi.org/10.1094/PDIS-03-12-0291-FE

Mesterházy, Á., Bartók, T., Mirocha, C. G., & Komoróczy, R. (1999). Nature of wheat resistance to Fusarium head blight and the role of deoxynivalenol for breeding. *Plant Breeding*, *118*(2), 97–110. https://doi.org/10.1046/j.1439-0523.1999.118002097.x

Miller, D. J., & Arnison, P. G. (1986). Degradation of deoxynivalenol by suspension cultures of the fusarium head blight resistant wheat cultivar Frontana. *Canadian Journal of Plant Pathology*, *8*(2), 147–150.

Neff, M. M., Neff, J. D., Chory, J., & Pepper, A. E. (1998). dCAPS, a simple technique for the genetic analysis of single nucleotide polymorphisms: Experimental applications in Arabidopsis thaliana genetics. *The Plant Journal*, *14*(3), 387–392. https://doi.org/10.1046/j.1365-313X.1998.00124.x

Pascual, L., Albert, E., Sauvage, C., Duangjit, J., Bouchet, J.-P., Bitton, F., Desplat, N., Brunel, D., Le Paslier, M.-C., Ranc, N., Bruguier, L., Chauchard, B., Verschave, P., & Causse, M. (2016). Dissecting quantitative trait variation in the resequencing era: Complementarity of bi-parental, multi-parental and association panels. *Plant Science*, *242*, 120–130. https://doi.org/10.1016/j.plantsci.2015.06.017

Pootakham, W., Sonthirod, C., Naktang, C., Jomchai, N., Sangsrakru, D., & Tangphatsornruang, S. (2016). Effects of methylation-sensitive enzymes on the enrichment of genic SNPs and the degree of genome complexity reduction in a two-enzyme genotyping-by-sequencing (GBS) approach: A case study in oil palm (Elaeis guineensis). *Molecular Breeding*, *36*(11), 154. https://doi.org/10.1007/s11032-016-0572-x

Qu, B., Li, H. P., Zhang, J. B., Huang, T., Carter, J., Liao, Y. C., & Nicholson, P. (2008). Comparison of genetic diversity and pathogenicity of fusarium head blight pathogens from China and Europe by SSCP and seedling assays on wheat. *Plant Pathology*, *57*(4), 642–651. https://doi.org/10.1111/j.1365-3059.2008.01824.x

Sapkota, M. (2018). *Field testing and molecular marker characterization of near-isogenic lines with Fusarium Head Blight resistance QTL from CJ9306 in Zebra and Berserk spring wheat backgrounds* [Master thesis]. Brage.

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), 430–439. https://doi.org/10.1038/s41559-018-0793-y

Schroeder, H. W., & Christensen, J. J. (1963). Factors affecting resistance of Wheat to scab caused by Gibberella zeae. *Phytopathology*, *53*(7, 1), 831–838.

Shavrukov, Y. N. (2016). CAPS markers in plant biology. *Russian Journal of Genetics: Applied Research*, *6*(3), 279–287. https://doi.org/10.1134/S2079059716030114

Shewry, P. R., & Hey, S. J. (2015). The contribution of wheat to human diet and health. *Food and Energy Security*, *4*(3), 178–202. https://doi.org/10.1002/fes3.64

Shi, X., & Ling, H.-Q. (2018). Current advances in genome sequencing of common wheat and its ancestral species. *The Crop Journal*, *6*(1), 15–21. https://doi.org/10.1016/j.cj.2017.11.001

Shimizu, K. K., Copetti, D., Okada, M., Wicker, T., Tameshige, T., Hatakeyama, M., Shimizu-Inatsugi, R., Aquino, C., Nishimura, K., Kobayashi, F., Murata, K., Kuo, T., Delorean, E., Poland, J., Haberer, G., Spannagl, M., Mayer, K. F. X., Gutierrez-Gonzalez, J., Muehlbauer, G. J., … Handa, H. (2021). De Novo Genome Assembly of the Japanese Wheat Cultivar Norin 61 Highlights Functional Variation in Flowering Time and Fusarium-Resistant Genes in East Asian Genotypes. *Plant and Cell Physiology*, *62*(1), 8–27. https://doi.org/10.1093/pcp/pcaa152

Shude, S. P. N., Yobo, K. S., & Mbili, N. C. (2020). Progress in the management of Fusarium head blight of wheat: An overview. *South African Journal of Science*, *116*(11/12). https://doi.org/10.17159/sajs.2020/7854

Slowikowski, K. (2021). *Ggrepel: Automatically Position Non-Overlapping Text Labels with 'ggplot2'. R package version 0.9.1.* https://CRAN.R-project.org/package=ggrepel

Society, N. G. (2011, February 22). *Food Staple*. National Geographic Society. http://www.nationalgeographic.org/encyclopedia/food-staple/

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. https://doi.org/10.1139/g03-033

Sorrells, M. E., La Rota, M., Bermudez-Kandianis, C. E., Greene, R. A., Kantety, R., Munkvold, J. D., Miftahudin, Mahmoud, A., Ma, X., Gustafson, P. J., Qi, L. L., Echalier, B., Gill, B. S., Matthews, D. E., Lazo, G. R., Chao, S., Anderson, O. D., Edwards, H., Linkiewicz, A. M., … Qualset, C. O. (2003). Comparative DNA Sequence Analysis of Wheat and Rice Genomes. *Genome Research*, *13*(8), 1818–1827. https://doi.org/10.1101/gr.1113003

Sparks, A. H., Esker, P. D., Bates, M., Dall'Aqua, W., Guo, Z., Segovia, V., Silwal, S. D., Tolos, S., & Garrett, K. A. (2008). Ecology and Epidemilogy in R: Disease progress over time. *The Plant Health Instructor*. https://doi.org/10.1094/PHI-A-2008-0129-02

Steiner, B., Buerstmayr, M., Wagner, C., Danler, A., Eshonkulov, B., Ehn, M., & Buerstmayr, H. (2019). Fine-mapping of the Fusarium head blight resistance QTL Qfhs.ifa-5A identifies two resistance QTL associated with anther extrusion. *Theoretical and Applied Genetics*, *132*(7), 2039–2053. https://doi.org/10.1007/s00122-019-03336-x

Sukumaran, S., Dreisigacker, S., Lopes, M., Chavez, P., & Reynolds, M. P. (2015). Genome-wide association study for grain yield and related traits in an elite spring wheat population grown in temperate irrigated environments. *Theoretical and Applied Genetics*, *128*(2), 353–363. https://doi.org/10.1007/s00122-014-2435-3

Tekle, S., Lillemo, M., Skinnes, H., Reitan, L., Buraas, T., & Bjørnstad, Å. (2018). Screening of Oat Accessions for Fusarium Head Blight Resistance Using Spawn-Inoculated

Field Experiments. *Crop Science*, *58*(1), 143–151. https://doi.org/10.2135/cropsci2017.04.0264

Tester, M., & Langridge, P. (2010). Breeding Technologies to Increase Crop Production in a Changing World. *Science*. https://doi.org/10.1126/science.1183700

U.S. National Library of Medicine. (2017). *Cleaved Amplified Polymorphic Sequences (CAPS)*. https://www.ncbi.nlm.nih.gov/probe/docs/techcaps/

Van Ooijen, J. W. (2006). JoinMap 4: Software for the Calculation of Genetic Linkage Maps in Experimental Populations. *Kyazma B.V., Wageningen.*

Van Tassell, C. P., Smith, T. P. L., Matukumalli, L. K., Taylor, J. F., Schnabel, R. D., Lawley, C. T., Haudenschild, C. D., Moore, S. S., Warren, W. C., & Sonstegard, T. S. (2008). SNP discovery and allele frequency estimation by deep sequencing of reduced representation libraries. *Nature Methods*, *5*(3), 247–252. https://doi.org/10.1038/nmeth.1185

Vieira, M. L. C., Santini, L., Diniz, A. L., & Munhoz, C. de F. (2016). Microsatellite markers: What they mean and why they are so useful. *Genetics and Molecular Biology*, *39*(3), 312–328. https://doi.org/10.1590/1678-4685-GMB-2016-0027

Voorrips, R.E. (2002). MapChart: Software for the graphical presentation of linkage maps and QTLs. *Journal of Heredity*, *93*(1), 77–78. https://doi.org/10.1093/jhered/93.1.77

Walkowiak, S., Gao, L., Monat, C., Haberer, G., Kassa, M. T., Brinton, J., Ramirez-Gonzalez, R. H., Kolodziej, M. C., Delorean, E., Thambugala, D., Klymiuk, V., Byrns, B., Gundlach, H., Bandi, V., Siri, J. N., Nilsen, K., Aquino, C., Himmelbach, A., Copetti, D., … Pozniak, C. J. (2020). Multiple wheat genomes reveal global variation in modern breeding. *Nature*, *588*(7837), 277–283. https://doi.org/10.1038/s41586-020-2961-x

Wang, N., Yuan, Y., Wang, H., Yu, D., Liu, Y., Zhang, A., Gowda, M., Nair, S. K., Hao, Z., Lu, Y., San Vicente, F., Prasanna, B. M., Li, X., & Zhang, X. (2020). Applications of genotyping-by-sequencing (GBS) in maize genetics and breeding. *Scientific Reports*, *10*(1), 16308. https://doi.org/10.1038/s41598-020-73321-8

Wang, S., Wong, D., Forrest, K., Allen, A., Chao, S., Huang, B. E., Maccaferri, M., Salvi, S., Milner, S. G., Cattivelli, L., Mastrangelo, A. M., Whan, A., Stephen, S., Barker, G., Wieseke, R., Plieske, J., International Wheat Genome Sequencing Consortium, Lillemo, M., Mather, D., … Akhunov, E. (2014). Characterization of polyploid wheat genomic diversity using a high-density 90,000 single nucleotide polymorphism array. *Plant Biotechnology Journal*, *12*(6), 787–796. https://doi.org/10.1111/pbi.12183

Wegulo, S. N., Jackson, T. A., Baenziger, P. S., Carlson, M. P., & Nopsa, J. H. (2008). Fusarium Head Blight of Wheat. *Extension*, *EC1896*. https://extensionpublications.unl.edu/assets/pdf/ec1896.pdf

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. *Springer-Verlag New York*.

Würschum, T., Langer, S. M., Longin, C. F. H., Tucker, M. R., & Leiser, W. L. (2017). A modern Green Revolution gene for reduced height in wheat. *The Plant Journal*, *92*(5), 892–903. https://doi.org/10.1111/tpj.13726

Xue, S., Xu, F., Tang, M., Zhou, Y., Li, G., An, X., Lin, F., Xu, H., Jia, H., Zhang, L., Kong, Z., & Ma, Z. (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. https://doi.org/10.1007/s00122-011-1647-z

Yan, H., Li, G., Shi, J., Tian, S., Zhang, X., Cheng, R., Wang, X., Yuan, Y., Cao, S., Zhou, J., Kong, Z., Jia, H., & Ma, Z. (2021). Genetic control of Fusarium head blight resistance

in two Yangmai 158-derived recombinant inbred line populations. *Theoretical and Applied Genetics*, *134*(9), 3037–3049. https://doi.org/10.1007/s00122-021-03876-1

Yu, L.-X., Morgounov, A., Wanyera, R., Keser, M., Singh, S. K., & Sorrells, M. (2012). Identification of Ug99 stem rust resistance loci in winter wheat germplasm using genome-wide association analysis. *Theoretical and Applied Genetics*, *125*(4), 749–758. https://doi.org/10.1007/s00122-012-1867-x

Zhang, P., Guo, C., Liu, Z., Bernardo, A., Ma, H., Jiang, P., Song, G., & Bai, G. (2021). Quantitative trait loci for Fusarium head blight resistance in wheat cultivars Yangmai 158 and Zhengmai 9023. *The Crop Journal*, *9*(1), 143–153. https://doi.org/10.1016/j.cj.2020.05.007

Zhao, S., Li, A., Li, C., Xia, H., Zhao, C., Zhang, Y., Hou, L., & Wang, X. (2017). Development and application of KASP marker for high throughput detection of AhFAD2 mutation in peanut. *Electronic Journal of Biotechnology*, *25*, 9–12. https://doi.org/10.1016/j.ejbt.2016.10.010

Zhu, Z., Chen, L., Zhang, W., Yang, L., Zhu, W., Li, J., Liu, Y., Tong, H., Fu, L., Liu, J., Rasheed, A., Xia, X., He, Z., Hao, Y., & Gao, C. (2020). Genome-Wide Association Analysis of Fusarium Head Blight Resistance in Chinese Elite Wheat Lines. *Frontiers in Plant Science*, *11*. https://www.frontiersin.org/article/10.3389/fpls.2020.00206

Appendix 1 - Field data for boxplot analysis

Table S1.1: Data used to create the boxplots for the three markers *gwm539*, *WGRB3803,* and *wsnp_Ex_c8303_14001708* with FHB severity scores for 2019, 2020, and 2021, and DON scores for 2019 and 2020. FHB is measured as described under chapter 3.2 and DON is measured in ppm (parts per million). The marker alleles are Z for susceptible from Zebra and CJ for resistant from CJ9306.

Entry	FHB19	FHB20	FHB21	*gwm539*	*wsnp_Ex_c8303_14001708*	*WGRB3803*	DON19	DON20
1361	13.46	8.50	NA	Z	Z	Z	0.87	1.60
891	19.23	NA	NA	Z	Z	Z	0.27	NA
1591	21.54	NA	NA	Z	Z	Z	0.97	NA
1311	14.62	8.50	NA	Z	Z	Z	1.80	2.20
8310	12.31	NA	NA	NA	NA	NA	1.70	NA
8309	8.08	NA	NA	NA	NA	NA	0.47	NA
301	13.46	15.00	NA	Z	Z	Z	0.80	2.20
711	8.85	NA	NA	Z	Z	Z	0.18	NA
511	9.62	NA	NA	Z	Z	Z	1.60	NA
191	13.85	NA	NA	Z	Z	Z	0.75	NA
551	11.15	NA	NA	CJ	CJ	CJ	0.71	NA
571	11.92	NA	NA	Z	Z	NA	0.99	NA
15	4.62	NA	NA	Z	NA	Z	0.36	NA
311	6.15	6.00	NA	CJ	NA	CJ	1.90	0.53
21	12.31	NA	NA	CJ	CJ	CJ	0.64	NA
411	14.62	NA	NA	Z	CJ	Z	1.00	NA
111	5.77	NA	NA	CJ	CJ	CJ	1.20	NA
161	7.31	NA	NA	CJ	CJ	CJ	1.50	NA
1	21.92	NA	NA	Z	CJ	Z	0.99	NA
441	3.46	NA	NA	CJ	CJ	CJ	0.47	NA
8	12.31	NA	NA	CJ	CJ	CJ	0.46	NA
81	19.23	NA	NA	Z	CJ	Z	2.70	NA
731	6.92	NA	NA	CJ	CJ	CJ	0.17	NA
241	15.77	NA	NA	Z	Z	Z	1.90	NA

1081	NA	13.75	NA	Z	Z	Z	NA	3.20
1131	NA	11.75	NA	Z	Z	Z	NA	2.60
1161	NA	6.25	NA	Z	Z	Z	NA	1.10
1211	NA	12.00	NA	Z	Z	Z	NA	3.80
1261	NA	11.50	NA	Z	Z	Z	NA	2.20
1371	NA	5.00	NA	CJ	CJ	CJ	NA	2.30
1908	NA	6.00	9.52	NA	NA	NA	NA	4.00
1909	NA	14.00	13.10	NA	NA	NA	NA	2.50
2	NA	NA	14.17	Z	CJ	NA	NA	NA
7	NA	NA	12.50	CJ	CJ	CJ	NA	NA
31	NA	NA	9.17	CJ	CJ	CJ	NA	NA
71	NA	NA	10.83	Z	CJ	Z	NA	NA
101	NA	NA	14.58	CJ	CJ	CJ	NA	NA
181	NA	NA	20.83	Z	Z	Z	NA	NA
231	NA	NA	15.00	CJ	CJ	CJ	NA	NA
281	NA	NA	19.17	Z	Z	Z	NA	NA
331	NA	NA	15.83	Z	Z	Z	NA	NA
361	NA	NA	14.17	CJ	CJ	CJ	NA	NA
421	NA	NA	21.25	Z	CJ	Z	NA	NA
461	NA	NA	11.67	CJ	CJ	CJ	NA	NA
491	NA	NA	10.83	Z	Z	Z	NA	NA
501	NA	NA	7.92	CJ	CJ	CJ	NA	NA
591	NA	NA	25.00	Z	Z	Z	NA	NA
601	NA	NA	17.08	CJ	NA	CJ	NA	NA
611	NA	NA	20.00	Z	Z	Z	NA	NA
621	NA	NA	12.08	CJ	CJ	CJ	NA	NA
751	NA	NA	14.17	CJ	CJ	CJ	NA	NA
761	NA	NA	5.83	Z	NA	Z	NA	NA
781	NA	NA	15.00	Z	Z	Z	NA	NA
801	NA	NA	12.92	CJ	CJ	CJ	NA	NA

811	NA	NA	12.92	CJ	CJ	CJ	NA	NA
851	NA	NA	11.67	Z	Z	Z	NA	NA
871	NA	NA	8.33	Z	NA	Z	NA	NA
881	NA	NA	9.58	CJ	CJ	CJ	NA	NA
901	NA	NA	15.83	Z	Z	Z	NA	NA
931	NA	NA	11.67	CJ	CJ	CJ	NA	NA
961	NA	NA	9.62	CJ	CJ	CJ	NA	NA
1011	NA	NA	9.62	CJ	CJ	CJ	NA	NA
1031	NA	NA	7.12	Z	Z	Z	NA	NA
1051	NA	NA	7.12	Z	Z	Z	NA	NA
1111	NA	NA	13.78	Z	Z	Z	NA	NA
1117	NA	NA	13.37	NA	NA	NA	NA	NA
1121	NA	NA	12.12	CJ	Z	CJ	NA	NA
1291	NA	NA	16.28	Z	Z	Z	NA	NA
1301	NA	NA	27.12	CJ	CJ	CJ	NA	NA
1351	NA	NA	12.95	CJ	CJ	CJ	NA	NA
1381	NA	NA	22.95	Z	Z	Z	NA	NA
1491	NA	NA	24.62	Z	Z	Z	NA	NA
1521	NA	NA	18.78	CJ	CJ	CJ	NA	NA
1741	NA	NA	17.12	CJ	CJ	CJ	NA	NA
1771	NA	NA	16.28	Z	Z	Z	NA	NA
1791	NA	NA	24.62	Z	Z	NA	NA	NA
1808	NA	NA	22.99	NA	NA	NA	NA	NA
1811	NA	NA	15.45	CJ	CJ	CJ	NA	NA
1901	NA	NA	13.33	NA	NA	NA	NA	NA
1902	NA	NA	7.76	NA	NA	NA	NA	NA
2708	NA	NA	2.56	NA	NA	NA	NA	NA
11	NA	NA	NA	NA	CJ	NA	NA	NA
51	NA	NA	NA	NA	CJ	NA	NA	NA
61	NA	NA	NA	NA	CJ	NA	NA	NA

91	NA	NA	NA	Z	CJ	Z	NA	NA
121	NA	NA	NA	NA	NA	NA	NA	NA
5	NA	NA	NA	CJ	CJ	CJ	NA	NA
141	NA	NA	NA	CJ	CJ	CJ	NA	NA
151	NA	NA	NA	NA	CJ	CJ	NA	NA
171	NA	NA	NA	NA	NA	NA	NA	NA
201	NA	NA	NA	Z	Z	Z	NA	NA
211	NA	NA	NA	Z	Z	NA	NA	NA
221	NA	NA	NA	NA	CJ	NA	NA	NA
251	NA	NA	NA	NA	NA	NA	NA	NA
261	NA	NA	NA	NA	CJ	NA	NA	NA
271	NA	NA	NA	Z	Z	Z	NA	NA
321	NA	NA	NA	NA	NA	NA	NA	NA
341	NA	NA	NA	NA	NA	NA	NA	NA
351	NA	NA	NA	NA	NA	NA	NA	NA
371	NA	NA	NA	Z	Z	Z	NA	NA
16	NA	NA	NA	NA	CJ	NA	NA	NA
391	NA	NA	NA	NA	CJ	CJ	NA	NA
401	NA	NA	NA	NA	CJ	NA	NA	NA
431	NA	NA	NA	NA	CJ	NA	NA	NA
451	NA	NA	NA	NA	CJ	NA	NA	NA
471	NA	NA	NA	NA	CJ	NA	NA	NA
481	NA	NA	NA	NA	NA	NA	NA	NA
521	NA	NA	NA	NA	NA	NA	NA	NA
531	NA	NA	NA	Z	Z	Z	NA	NA
541	NA	NA	NA	Z	Z	Z	NA	NA
561	NA	NA	NA	156	Z	Z	NA	NA
581	NA	NA	NA	NA	NA	NA	NA	NA
651	NA	NA	NA	Z	Z	Z	NA	NA
661	NA	NA	NA	CJ	CJ	CJ	NA	NA

671	NA	NA	NA	Z	Z	Z	NA	NA
681	NA	NA	NA	CJ	CJ	CJ	NA	NA
691	NA	NA	NA	NA	NA	NA	NA	NA
701	NA	NA	NA	NA	NA	NA	NA	NA
721	NA	NA	NA	NA	NA	NA	NA	NA
741	NA	NA	NA	CJ	CJ	CJ	NA	NA
771	NA	NA	NA	CJ	CJ	CJ	NA	NA
791	NA	NA	NA	NA	NA	NA	NA	NA
821	NA	NA	NA	NA	NA	NA	NA	NA
831	NA	NA	NA	Z	NA	Z	NA	NA
841	NA	NA	NA	CJ	NA	CJ	NA	NA
861	NA	NA	NA	NA	NA	Z	NA	NA
10	NA	NA	NA	Z	CJ	Z	NA	NA
921	NA	NA	NA	NA	CJ	NA	NA	NA
941	NA	NA	NA	CJ	CJ	CJ	NA	NA
951	NA	NA	NA	NA	NA	NA	NA	NA
971	NA	NA	NA	CJ	Z	CJ	NA	NA
981	NA	NA	NA	Z	Z	Z	NA	NA
991	NA	NA	NA	CJ	CJ	CJ	NA	NA
1001	NA	NA	NA	CJ	CJ	CJ	NA	NA
1021	NA	NA	NA	CJ	CJ	CJ	NA	NA
1041	NA	NA	NA	141	Z	NA	NA	NA
12	NA	NA	NA	NA	CJ	NA	NA	NA
1071	NA	NA	NA	NA	Z	NA	NA	NA
1091	NA	NA	NA	Z	Z	Z	NA	NA
1101	NA	NA	NA	CJ	Z	CJ	NA	NA
1141	NA	NA	NA	Z	Z	Z	NA	NA
1151	NA	NA	NA	Z	Z	NA	NA	NA
1171	NA	NA	NA	Z	Z	Z	NA	NA
1181	NA	NA	NA	NA	NA	NA	NA	NA

1191	NA	NA	NA	Z	Z	Z	NA	NA
1201	NA	NA	NA	NA	NA	NA	NA	NA
1221	NA	NA	NA	NA	NA	NA	NA	NA
1231	NA	NA	NA	Z	Z	Z	NA	NA
1241	NA	NA	NA	CJ	CJ	CJ	NA	NA
1251	NA	NA	NA	Z	Z	Z	NA	NA
1271	NA	NA	NA	NA	NA	NA	NA	NA
1281	NA	NA	NA	Z	Z	Z	NA	NA
1321	NA	NA	NA	NA	NA	NA	NA	NA
1331	NA	NA	NA	Z	Z	NA	NA	NA
13	NA	NA	NA	Z	CJ	Z	NA	NA
1391	NA	NA	NA	NA	CJ	CJ	NA	NA
1401	NA	NA	NA	Z	Z	Z	NA	NA
1411	NA	NA	NA	NA	NA	NA	NA	NA
1421	NA	NA	NA	CJ	CJ	CJ	NA	NA
1431	NA	NA	NA	CJ	CJ	CJ	NA	NA
1441	NA	NA	NA	NA	Z	NA	NA	NA
1451	NA	NA	NA	NA	NA	NA	NA	NA
1461	NA	NA	NA	NA	NA	NA	NA	NA
1471	NA	NA	NA	NA	NA	NA	NA	NA
1481	NA	NA	NA	Z	Z	NA	NA	NA
1501	NA	NA	NA	CJ	Z	CJ	NA	NA
1511	NA	NA	NA	CJ	CJ	CJ	NA	NA
1531	NA	NA	NA	NA	NA	NA	NA	NA
1541	NA	NA	NA	Z	Z	Z	NA	NA
1551	NA	NA	NA	CJ	CJ	CJ	NA	NA
1561	NA	NA	NA	NA	Z	NA	NA	NA
1571	NA	NA	NA	CJ	CJ	CJ	NA	NA
1581	NA	NA	NA	CJ	CJ	CJ	NA	NA
1601	NA	NA	NA	CJ	CJ	CJ	NA	NA

1611	NA	NA	NA	Z	Z	Z	NA	NA
1621	NA	NA	NA	NA	NA	NA	NA	NA
1631	NA	NA	NA	Z	Z	Z	NA	NA
1641	NA	NA	NA	CJ	CJ	CJ	NA	NA
1651	NA	NA	NA	CJ	CJ	CJ	NA	NA
1661	NA	NA	NA	CJ	CJ	CJ	NA	NA
1671	NA	NA	NA	CJ	CJ	CJ	NA	NA
14	NA	NA	NA	NA	CJ	NA	NA	NA
1691	NA	NA	NA	CJ	CJ	CJ	NA	NA
1701	NA	NA	NA	NA	NA	NA	NA	NA
1711	NA	NA	NA	Z	NA	NA	NA	NA
1721	NA	NA	NA	CJ	NA	CJ	NA	NA
1731	NA	NA	NA	CJ	CJ	CJ	NA	NA
1751	NA	NA	NA	NA	NA	NA	NA	NA
1761	NA	NA	NA	NA	NA	NA	NA	NA
1781	NA	NA	NA	CJ	CJ	CJ	NA	NA
1801	NA	NA	NA	NA	NA	NA	NA	NA
1821	NA	NA	NA	CJ	CJ	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA

Appendix 2 - Recombination marker data for linkage map

Table S2.1: Overview of recombinations between markers *gwm539*, *WGRB3803*, and *wsnp_Ex_c8303_14001708*. The alleles are Z for susceptible allele derived from Zebra, CJ for resistant allele derived from CJ9306, and H for heterozygote. Family history and entry number are also included.

Family	Entry	*gwm539*	*WGRB3803*	*wsnp_Ex_c8303_14001708*
6C5_07_G03	311	CJ	CJ	H
6D5_23_A12	1721	CJ	CJ	H
6C5_01_G02	15	Z	Z	H
6D5_01_G08	831	Z	Z	H
6D5_23_G10	1711	Z	NA	H
6C5_01_C02	11	H	H	CJ
6C5_02_G07	151	H	CJ	CJ
6C5_06_E04	221	H	H	CJ
6C5_09_G01	391	H	CJ	CJ
6C5_09_G06	431	H	H	CJ
6D5_05_A08	921	H	H	CJ
6D5_09_G02	1071	H	NA	Z
6D5_19_A01	1441	H	H	Z
6D5_22_A04	1561	H	H	Z
6C5_01_E01	5	CJ	CJ	CJ
6C5_09_A08	441	CJ	CJ	CJ
6D5_07_C02	971	CJ	CJ	Z
6D5_07_C07	1011	CJ	CJ	CJ
6D5_09_E06	1101	CJ	CJ	Z
6D5_19_E08	1501	CJ	CJ	Z
6D5_19_G09	1511	CJ	CJ	CJ
6C5_01_E02	13	Z	Z	CJ
6C5_09_C04	411	Z	Z	CJ
6D5_07_E03	981	Z	Z	Z
6D5_09_A04	1081	Z	Z	Z
6D5_19_A06	1481	Z	H	Z
6D5_09_A09	1121	CJ	CJ	Z
6D5_19_A11	1521	CJ	CJ	CJ
6C5_18_A04	601	CJ	CJ	H
6D5_07_C12	1051	Z	Z	Z
6D5_19_C07	1491	Z	Z	Z
6C5_01_B01	2	Z	H	CJ
6C5_22_A12	761	Z	Z	H
6D5_05_G01	871	Z	Z	H
6C5_01_A01	1	Z	Z	CJ
6C5_01_A11	81	Z	Z	CJ
6C5_01_B02	10	Z	Z	CJ
6C5_01_C07	51	H	H	CJ
6C5_01_C12	91	Z	Z	CJ
6C5_01_D02	12	H	H	CJ
6C5_01_E03	21	CJ	CJ	CJ

6C5_01_E08	61	H	H	CJ
6C5_01_F02	14	H	H	CJ
6C5_01_G01	7	CJ	CJ	CJ
6C5_01_G04	31	CJ	CJ	CJ
6C5_01_G09	71	Z	Z	CJ
6C5_01_H01	8	CJ	CJ	CJ
6C5_01_H02	16	H	H	CJ
6C5_02_A04	121	H	H	H
6C5_02_A09	161	CJ	CJ	CJ
6C5_02_C10	171	H	H	H
6C5_02_E01	101	CJ	CJ	CJ
6C5_02_E06	141	CJ	CJ	CJ
6C5_02_E11	181	Z	Z	Z
6C5_02_G02	111	CJ	CJ	CJ
6C5_02_G12	191	Z	Z	Z
6C5_06_A02	201	Z	Z	Z
6C5_06_A07	241	Z	Z	Z
6C5_06_A12	281	Z	Z	Z
6C5_06_C03	211	Z	H	Z
6C5_06_C08	251	H	H	H
6C5_06_E09	261	H	H	CJ
6C5_06_G05	231	CJ	CJ	CJ
6C5_06_G10	271	Z	Z	Z
6C5_07_A05	321	H	H	H
6C5_07_A10	361	CJ	CJ	CJ
6C5_07_C06	331	Z	Z	Z
6C5_07_C11	371	Z	Z	Z
6C5_07_E02	301	Z	Z	Z
6C5_07_E07	341	H	H	H
6C5_07_G08	351	H	H	H
6C5_09_A03	401	H	H	CJ
6C5_09_C09	451	H	H	CJ
6C5_09_E05	421	Z	Z	CJ
6C5_09_E10	461	CJ	CJ	CJ
6C5_09_G11	471	H	H	CJ
6C5_14_A01	481	H	H	H
6C5_14_A06	521	H	H	H
6C5_14_A11	561	NA	Z	Z
6C5_14_C02	491	Z	Z	Z
6C5_14_C07	531	Z	Z	Z
6C5_14_C12	571	Z	H	Z
6C5_14_E03	501	CJ	CJ	CJ
6C5_14_E08	541	Z	Z	Z
6C5_14_G04	511	Z	Z	Z
6C5_14_G09	551	CJ	CJ	CJ
6C5_18_C05	611	Z	Z	Z

6C5_18_C10	651	Z	Z	Z
6C5_18_E01	581	H	H	H
6C5_18_E06	621	CJ	CJ	CJ
6C5_18_E11	661	CJ	CJ	CJ
6C5_18_G02	591	Z	Z	Z
6C5_18_G12	671	Z	Z	Z
6C5_22_A02	681	CJ	CJ	CJ
6C5_22_A07	721	H	H	H
6C5_22_C03	691	H	H	H
6C5_22_C08	731	CJ	CJ	CJ
6C5_22_E04	701	H	H	H
6C5_22_E09	741	CJ	CJ	CJ
6C5_22_G05	711	Z	Z	Z
6C5_22_G10	751	CJ	CJ	CJ
6D5_01_A05	801	CJ	CJ	CJ
6D5_01_A10	841	CJ	CJ	NA
6D5_01_C01	771	CJ	CJ	CJ
6D5_01_C06	811	CJ	CJ	CJ
6D5_01_C11	851	Z	Z	Z
6D5_01_E02	781	Z	Z	Z
6D5_01_E07	821	H	H	H
6D5_01_E12	861	H	Z	H
6D5_01_G03	791	H	H	H
6D5_05_A03	881	CJ	CJ	CJ
6D5_05_C04	891	Z	Z	Z
6D5_05_C09	931	CJ	CJ	CJ
6D5_05_E05	901	Z	Z	Z
6D5_05_E10	941	CJ	CJ	CJ
6D5_05_G11	951	H	H	H
6D5_07_A01	961	CJ	CJ	CJ
6D5_07_A06	1001	CJ	CJ	CJ
6D5_07_E08	1021	CJ	CJ	CJ
6D5_07_G04	991	CJ	CJ	CJ
6D5_07_G09	1031	Z	Z	Z
6D5_09_C05	1091	Z	Z	Z
6D5_09_C10	1131	Z	Z	Z
6D5_09_E11	1141	Z	Z	Z
6D5_09_G07	1111	Z	Z	Z
6D5_09_G12	1151	Z	H	Z
6D5_10_A02	1161	Z	Z	Z
6D5_10_A07	1201	H	H	H
6D5_10_A12	1241	CJ	CJ	CJ
6D5_10_C03	1171	Z	Z	Z
6D5_10_C08	1211	Z	Z	Z
6D5_10_E04	1181	H	H	H
6D5_10_E09	1221	H	H	H

6D5_10_G05	1191	Z	Z	Z
6D5_10_G10	1231	Z	Z	Z
6D5_12_A05	1281	Z	Z	Z
6D5_12_A10	1321	H	H	H
6D5_12_C01	1251	Z	Z	Z
6D5_12_C06	1291	Z	Z	Z
6D5_12_C11	1331	Z	H	Z
6D5_12_E02	1261	Z	Z	Z
6D5_12_E07	1301	CJ	CJ	CJ
6D5_12_G03	1271	H	H	H
6D5_12_G08	1311	Z	Z	Z
6D5_15_A03	1361	Z	Z	Z
6D5_15_A08	1401	Z	Z	Z
6D5_15_C04	1371	CJ	CJ	CJ
6D5_15_C09	1411	H	H	H
6D5_15_E05	1381	Z	Z	Z
6D5_15_E10	1421	CJ	CJ	CJ
6D5_15_G01	1351	CJ	CJ	CJ
6D5_15_G06	1391	NA	CJ	CJ
6D5_15_G11	1431	CJ	CJ	CJ
6D5_19_C02	1451	H	H	H
6D5_19_C12	1531	H	H	H
6D5_19_E03	1461	H	H	H
6D5_19_G04	1471	H	H	H
6D5_22_A09	1601	CJ	CJ	CJ
6D5_22_C05	1571	CJ	CJ	CJ
6D5_22_C10	1611	Z	Z	Z
6D5_22_E01	1541	Z	Z	Z
6D5_22_E06	1581	CJ	CJ	CJ
6D5_22_E11	1621	H	H	H
6D5_22_G02	1551	CJ	CJ	CJ
6D5_22_G07	1591	Z	Z	Z
6D5_22_G12	1631	Z	Z	Z
6D5_23_A02	1641	CJ	CJ	CJ
6D5_23_C03	1651	CJ	CJ	CJ
6D5_23_C08	1691	CJ	CJ	CJ
6D5_23_E04	1661	CJ	CJ	CJ
6D5_23_E09	1701	H	H	H
6D5_23_G05	1671	CJ	CJ	CJ
6D5_24_A05	1761	H	H	H
6D5_24_A10	1801	H	H	H
6D5_24_C01	1731	CJ	CJ	CJ
6D5_24_C06	1771	Z	Z	Z
6D5_24_C11	1811	CJ	CJ	CJ
6D5_24_E02	1741	CJ	CJ	CJ
6D5_24_E07	1781	CJ	CJ	CJ

6D5_24_E12	1821	CJ	H	CJ
6D5_24_G03	1751	H	H	H
6D5_24_G08	1791	Z	NA	Z
CJ9306	1079	CJ	NA	CJ
NA	NA	NA	CJ	NA
Naxos	1041	NA	H	Z
Sh3-CBRD	1086	CJ	NA	CJ
Soru#1	1087	CJ	NA	CJ

Appendix 3 - Physical position of markers in the pangenome

Table S3.1: Physical position of markers *gwm539*, *WGRB3803*, and *wsnp_Ex_c8303_14001708* in the pangenome, in bp. The UK cultivars are not published fully at this time.

Genome	*gwm539*	*WGRB3803*	*wsnp_Ex_c8303_14001708*
Chinese Spring	513098578-513098722	519126074-519126285	481601586-481601603
Norin61	511693371-511693501	517673324-517673535	480428091-480428108
Fielder	519336524-519336672	525315709-525315920	487860788-487860805
Zang1817	508798991-508799149	514797855-514798066	477485491-477485508
ArinaLrFor	514335256-514335388	520328228-520328439	482947306-482947322
Jagger	531242374-531242512	537269859-537270070	499828569-499828586
Julius	521240226-521240364	527236547-527236758	487525302-487525319
LongReach	510193184-510193318	516177564-516177775	478951054-478951071
CDC Landmark	516174627-516174767	522206919-522207130	484597614-484597631
MACE	510264987-510265127	516248080-516248291	479014629-479014646
CDC Stanley	517471884-517472018	523512119-523512330	485856883-485856900
SY Mattis	511596159-511596297	517577570-517577781	480250332-480250349
Spelta	512689568-512689710	518669048-518669259	481319424-481319441
Robigus	18116-24971	40663-40874	18031-18048
Paragon	21941-22075	37221-37432	39761-39778
Claire	38776-38912	31526-31737	22849-22832
Cadenza	15606-15740	159293-159504	8808-8791
Weebill	114171-114150	31416-31627	14211-14228

Appendix 4 - Haplotype data

Table S4.1: Overview of haplotype blocks in 5 Mbp, where the start, stop and length values are sorted after alphabetical order of the lines sharing the same haplotype block. As an example, in the first row with data, this means that the start, stop and length of the haplotype block in line 1 (in this case ArinaLrFor) are the first values (in this case start = 480 Mbp, stop = 540 Mbp, and length = 60 Mbp).

Blocks	Start (line 1, line 2)	Stop (line 1, line 2)	Length (line 1, line 2)	Lines with same haplotype block (line 1, line 2)	Block number
			gwm539		
1	480 Mbp, 478.7 Mbp	540 Mbp, 538.6 Mbp	60 Mbp, 59.9 Mbp	ArinaLrFor, Robigus	1973
2	471.9 Mbp, 490 Mbp	516.8 Mbp, 535 Mbp	44.9 Mbp, 45 Mbp	Cadenza, Jagger	1980
3	490 Mbp, 471.9 Mbp	535 Mbp, 516.8 Mbp	45 Mbp, 44.9 Mbp	Jagger, Paragon	1982
4	510 Mbp, 515 Mbp	530 Mbp, 540 Mbp	20 Mbp, 25 Mbp	LongReach Lancer, CDC Stanley	2011
5	461.3 Mbp, 460 Mbp	521.6 Mbp, 520 Mbp	60.3 Mbp, 60 Mbp	Claire, SY Mattis	1975
6	461.3 Mbp, 460 Mbp	516.4 Mbp, 515 Mbp	55.1 Mbp, 55 Mbp	Paragon, SY Mattis	1978
			WGRB3803		
1	480 Mbp, 478.7 Mbp	540 Mbp, 538.6 Mbp	60 Mbp, 59.9 Mbp	ArinaLrFor, Robigus	1973
2	510 Mbp, 515 Mbp	530 Mbp, 540 Mbp	20 Mbp, 25 Mbp	LongReach Lancer, CDC Stanley	2011
3	461.3 Mbp, 460 Mbp	521.6 Mbp, 520 Mbp	60.3 Mbp, 60 Mbp	Claire, SY Mattis	1975
4	518.2 Mbp, 516.6 Mbp	523.6 Mbp, 522 Mbp	5.5 Mbp, 5.4 Mbp	Cadenza, SY Mattis	2205
			wsnp_Ex_c8303_14001708		
1	480 Mbp, 478.7 Mbp	540 Mbp, 538.6 Mbp	60 Mbp, 59.9 Mbp	ArinaLrFor, Robigus	1973
2	471.9 Mbp, 490 Mbp	516.8 Mbp, 535 Mbp	44.9 Mbp, 45 Mbp	Cadenza, Jagger	1980
3	490 Mbp, 471.9 Mbp	535 Mbp, 516.8 Mbp	45 Mbp, 44.9 Mbp	Jagger, Paragon	1982
4	476.8 Mbp, 495 Mbp	506.8 Mbp, 525 Mbp	30 Mbp, 30 Mbp	Claire, Jagger	1995
5	495 Mbp, 475 Mbp	525 Mbp, 505 Mbp	30 Mbp, 30 Mbp	Jagger, SY Mattis	1997
6	486.3 Mbp, 480.3 Mbp	492.3 Mbp, 486.4 Mbp	6 Mbp, 6.1 Mbp	Julius, Robigus	2257
7	460 Mbp, 465 Mbp	495 Mbp, 500 Mbp	35 Mbp, 35 Mbp	LongReach Lancer, CDC Stanley	1993
8	375 Mbp, 376.8 Mbp	505 Mbp, 507.8 Mbp	130 Mbp, 131 Mbp	Mace,Weebill	1966
9	461.3 Mbp, 460 Mbp	521.6 Mbp, 520 Mbp	60.3 Mbp, 60 Mbp	Claire, SY Mattis	1975
10	461.3 Mbp, 460 Mbp	516.4 Mbp, 515 Mbp	55.1 Mbp, 55 Mbp	Paragon, SY Mattis	1978

11	461.3 Mbp, 460 Mbp	506.5 Mbp, 505 Mbp	45.2 Mbp, 45 Mbp	Cadenza, SY Mattis	1984

Table S4.2: Overview of haplotype blocks in 5 Mbp, where the start, stop and length values are sorted after alphabetical order of the lines sharing the same haplotype block. As an example, in the first row with data, this means that the start, stop and length of the haplotype block in line 1 (in this case ArinaLrFor) are the first values (in this case start = 477.5 Mbp, stop = 537.5 Mbp, and length = 60 Mbp).

Blocks	Start (line 1, line 2)	Stop (line 1, line 2)	Length (line 1, line 2)	Lines with same haplotype block (line 1, line 2)	Block number
			gwm539		
1	477.5 Mbp, 476.3 Mbp	537.5 Mbp, 536.1 Mbp	60 Mbp, 59.8 Mbp	ArinaLrFor, Robigus	2647
2	490 Mbp, 471.9 Mbp	535 Mbp, 516.8 Mbp	45 Mbp, 44.9 Mbp	Jagger, Paragon	2651
3	474.5 Mbp, 492.5 Mbp	516.8 Mbp, 535 Mbp	42.3 Mbp, 42.5 Mbp	Cadenza, Jagger	2653
4	510 Mbp, 517.5 Mbp	532.5 Mbp, 540 Mbp	22.5 Mbp, 22.5 Mbp	LongReach Lancer, CDC Stanley	2687
5	461.3 Mbp, 460 Mbp	524.1 Mbp, 522.5 Mbp	62.7 Mbp, 62.5 Mbp	Claire, SY Mattis	2646
6	476.4 Mbp, 475 Mbp	513.7 Mbp, 512.5 Mbp	37.4 Mbp, 37.5 Mbp	Paragon, SY Mattis	2660
			WGRB3803		
1	477.5 Mbp, 476.3 Mbp	537.5 Mbp, 536.1 Mbp	60 Mbp, 59.8 Mbp	ArinaLrFor, Robigus	2647
2	510 Mbp, 517.5 Mbp	532.5 Mbp, 540 Mbp	22.5 Mbp, 22.5 Mbp	LongReach Lancer, CDC Stanley	2687
3	461.3 Mbp, 460 Mbp	521.6 Mbp, 520 Mbp	60.3 Mbp, 60 Mbp	Claire, SY Mattis	1975
4	518.2 Mbp, 516.6 Mbp	523.6 Mbp, 522 Mbp	5.5 Mbp, 5.4 Mbp	Cadenza, SY Mattis	2205
5	461.3 Mbp, 460 Mbp	524.1 Mbp, 522.5 Mbp	62.7 Mbp, 62.5 Mbp	Claire, SY Mattis	2646
6	518.2 Mbp, 516.6 Mbp	523.6 Mbp, 522 Mbp	5.5 Mbp, 5.4 Mbp	Cadenza, SY Mattis	3098
7	519.1 Mbp, 517.6 Mbp	523.6 Mbp, 522 Mbp	4.5 Mbp, 4.4 Mbp	Paragon, SY Mattis	3226
8	517.6 Mbp, 519.1 Mbp	522 Mbp, 523.6 Mbp	4.4 Mbp, 4.5 Mbp	SY Mattis, Weebill	3245
			wsnp_Ex_c8303_14001708		
1	477.5 Mbp, 476.3 Mbp	537.5 Mbp, 536.1 Mbp	60 Mbp, 59.8 Mbp	ArinaLrFor, Robigus	2647
2	481.9 Mbp, 486.5 Mbp	486.2 Mbp, 490.7 Mbp	4.2 Mbp, 4.2 Mbp	ArinaLrFor, Julius	3048
3	490 Mbp, 471.9 Mbp	535 Mbp, 516.8 Mbp	45 Mbp, 44.9 Mbp	Jagger, Paragon	2651
4	474.5 Mbp, 492.5 Mbp	516.8 Mbp, 535 Mbp	42.3 Mbp, 42.5 Mbp	Cadenza, Jagger	2653
5	476.8 Mbp, 495 Mbp	506.8 Mbp, 525 Mbp	30 Mbp, 30 Mbp	Claire, Jagger	2670
6	495 Mbp, 477.5 Mbp	522.5 Mbp, 505 Mbp	27.5 Mbp, 27.5 Mbp	Jagger, SY Mattis	2677
7	499.8 Mbp, 485.8 Mbp	503.1 Mbp, 489 Mbp	3.3 Mbp, 3.3 Mbp	Jagger, CDC Stanley	3122
8	487.5 Mbp, 481.6 Mbp	490 Mbp, 484.1 Mbp	2.5 Mbp, 2.5 Mbp	Julius, Robigus	2921

9	457.5 Mbp, 465 Mbp	495 Mbp, 502.5 Mbp	37.5 Mbp, 37.5 Mbp	LongReach Lancer, CDC Stanley	2659
10	482.5 Mbp, 480.1 Mbp	485 Mbp, 482 Mbp	2.5 Mbp, 1.9 Mbp	CDC Landmark, Paragon	2964
11	470 Mbp, 472.9 Mbp	505 Mbp, 507.8 Mbp	35 Mbp, 35 Mbp	Mace, Weebill	2663
12	461.3 Mbp, 460 Mbp	524.1 Mbp, 522.5 Mbp	62.7 Mbp, 62.5 Mbp	Claire, SY Mattis	2646
13	476.4 Mbp, 475 Mbp	513.8 Mbp, 512.5 Mbp	37.4 Mbp, 37.5 Mbp	Paragon, SY Mattis	2660
14	478.9 Mbp, 477.5 Mbp	503.9 Mbp, 502.5 Mbp	25 Mbp, 25 Mbp	Cadenza, SY Mattis	2683
15	485.8 Mbp, 480.2 Mbp	489.1 Mbp, 483.5 Mbp	3.3 Mbp, 3.3 Mbp	CDC Stanley, SY Mattis	3240

Table S4.3: Overview of haplotype blocks in 5 Mbp, where the start, stop and length values are sorted after alphabetical order of the lines sharing the same haplotype block. As an example, in the first row with data, this means that the start, stop and length of the haplotype block in line 1 (in this case ArinaLrFor) are the first values (in this case start = 478 Mbp, stop = 538 Mbp, and length = 60 Mbp).

Blocks	Start (line 1, line 2)	Stop (line 1, line 2)	Length (line 1, line 2)	Lines with same haplotype block (line 1, line 2)	Block number
			gwm539		
1	478 Mbp, 476.7 Mbp	538 Mbp, 536.5 Mbp	60 Mbp, 59.9 Mbp	ArinaLrFor, Robigus	4957
2	491 Mbp, 472.9 Mbp	537 Mbp, 518.8 Mbp	46 Mbp, 46 Mbp	Jagger, Paragon	4962
3	506.9 Mbp, 525 Mbp	516.8 Mbp, 535 Mbp	9.9 Mbp, 10 Mbp	Cadenza, Jagger	5053
4	529 Mbp, 511.4 Mbp	532 Mbp, 513.8 Mbp	3 Mbp, 2.3 Mbp	Jagger, Robigus	5277
5	521 Mbp, 512.9 Mbp	524 Mbp, 515.8 Mbp	3 Mbp, 2.9 Mbp	Julius, Paragon	5285
6	512.9 Mbp, 521 Mbp	513.8 Mbp, 522 Mbp	844780 bp, 1 Mbp	Claire, Julius	5709
7	510 Mbp, 512.9 Mbp	513 Mbp, 515.8 Mbp	3 Mbp, 2.9 Mbp	LongReach Lancer, Paragon	5294
8	509 Mbp, 517 Mbp	532 Mbp, 540 Mbp	23 Mbp, 23 Mbp	LongReach Lancer, CDC Stanley	4988
9	513 Mbp, 516 Mbp	513.8 Mbp, 517 Mbp	763750 bp, 1 Mbp	Claire, CDC Landmark	5866
10	470.4 Mbp, 469 Mbp	523.4 Mbp, 522 Mbp	53 Mbp, 53 Mbp	Claire, SY Mattis	4960
11	512.7 Mbp, 511 Mbp	516.4 Mbp, 515 Mbp	3.7 Mbp, 4 Mbp	Paragon, SY Mattis	5258
			WGRB3803		
1	478 Mbp, 476.7 Mbp	538 Mbp, 536.5 Mbp	60 Mbp, 59.9 Mbp	ArinaLrFor, Robigus	4957
2	491 Mbp, 472.9 Mbp	537 Mbp, 518.8 Mbp	46 Mbp, 46 Mbp	Jagger, Paragon	4962
3	509 Mbp, 517 Mbp	532 Mbp, 540 Mbp	23 Mbp, 23 Mbp	LongReach Lancer, CDC Stanley	4988
4	470.4 Mbp, 469 Mbp	523.4 Mbp, 522 Mbp	53 Mbp, 53 Mbp	Claire, SY Mattis	4960
5	514 Mbp, 515.6 Mbp	518 Mbp, 519.3 Mbp	4 Mbp, 3.8 Mbp	SY Mattis, Weebill	5259
			wsnp_Ex_c8303_14001708		
1	478 Mbp, 476.7 Mbp	538 Mbp, 536.5 Mbp	60 Mbp, 59.9 Mbp	ArinaLrFor, Robigus	4957
2	481.9 Mbp, 486.5 Mbp	486.2 Mbp, 490.7 Mbp	4.2 Mb, 4.2 Mb	ArinaLrFor, Julius	6217
3	491 Mb, 472.9 Mb	537 Mb, 518.8 Mb	46 Mbp, 46 Mbp	Jagger, Paragon	4962
4	472.9 Mbp, 491 Mbp	502.6 Mbp, 521 Mbp	29.7 Mbp, 30 Mbp	Cadenza, Jagger	4977
5	477.8 Mbp, 496 Mbp	523 Mbp, 503 Mbp	29 Mbp, 29 Mbp	Claire, Jagger	4979
6	496 Mbp, 476 Mbp	523 Mbp, 503 Mbp	27 Mbp, 27 Mbp	Jagger, SY Mattis	4984

7	499.8 Mbp, 485.8 Mbp	503.1 Mbp, 489.1 Mbp	3.3 Mbp, 3.3 Mbp	Jagger, CDC Stanley	6291
8	459 Mbp, 465 Mbp	495 Mbp, 502 Mbp	36 Mbp, 37 Mbp	LongReach Lancer, CDC Stanley	4969
9	453 Mbp, 455.5 Mbp	507 Mbp, 509.8 Mbp	54 Mbp, 54.3 Mbp	Mace, Weebill	4959
10	470.4 Mbp, 469 Mbp	523.4 Mbp, 522 Mbp	53 Mbp, 53 Mbp	Claire, SY Mattis	4960
11	477.4 Mbp, 476 Mbp	506.5 Mbp, 505 Mbp	29.1 Mbp, 20 Mbp	Paragon, SY Mattis	4980
12	477.4 Mbp, 476 Mbp	498.5 Mbp, 497 Mbp	21.1 Mbp, 21 Mbp	Cadenza, SY Mattis	4993
13	485.8 Mbp, 480.2 Mbp	489.1 Mbp, 483.5 Mbp	3.3 Mbp, 3.3 Mbp	CDC Stanley, SY Mattis	6409

Appendix 5 - Physical map marker positions

Table S5.1: Physical positions in Mbp of available marker data used to create the updated physical map in **Figure 14**. All markers shown in the first physical map (**Figure 7**) are also included in this table.

Marker	2D position (Mbp)
AX-94700210	417
AX-158522248	421
AX-158521911	433
AX-94428875	441
AX-95138710	446
GENE-4086_115	455
Ku_c19185_1569	461
AX-94507617	468
wsnp_Ex_c8303_14001708	482
AX-94774424	485
AX-94872625	507
gwm539	513
WGRB3803	519
Kukri_c24669_51	523
AX-94666092	529
AX-89629279	535
RAC875_rep_c91134_949	552
AX-158573975	552
AX-95120131	567
BS00022587_51	572
AX-95205011	574
AX-94635076	574
AX-94485323	578
TaWRKY70	589
AX-94460997	593
CAP7_rep_c13224_320	600
wsnp_RFL_Contig2914_2757372	608
Excalibur_c44325_339	608
BS00100106_51	617
GENE-0095_82	620
BS00028063_51	620
Kukri_c16965_1077	620
Kukri_c74469_351	621
Excalibur_c9752_289	621
GENE-1355_265	622
Excalibur_c224_1383	622
AX-94618441	623
AX-94883380	625
AX-94502940	627
AX-94802107	627
Ex_c52711_584	630

BobWhite_rep_c64068_241	632
AX-94736922	634
RAC875_c10408_188	636
AX-94692118	636
BS00086534_51	636
AX-95249702	638
RAC875_c15518_236	638
AX-94602446	638
RAC875_c50347_258	638
IACX9095	638

Appendix 6 - Phenotypic scores from first point inoculation experiment

Table S6.1: Phenotypic scores from the first point inoculation experiment, including the lines tested, number of infected spikelets (NIS) after 3, 6-, 9-, 12-, and 15-days post inoculation (DPI), total number of infected spikelets (TNS) and the calculated percentage of diseased spikelets (PDS) after 15 DPI. PDS is coloured in a gradient from red to green, where red is high PDS values and green for low PDS values.

Line	NIS (DPI = 3)	NIS (DPI = 6)	NIS (DPI = 9)	NIS (DPI = 12)	NIS (DPI = 15)	TNS	PDS
CJ9306	1	3	4	4	4	18	0.222
CJ9306	0	1	1	2	5	18	0.278
CJ9306	1	1	1	NA	NA	26	0.038
CJ9306	0	0	1	NA	1	24	0.042
CJ9306	1	1	1	NA	1	20	0.050
CJ9306	1	1	1	NA	1	20	0.050
CJ9306	1	1	1	NA	1	24	0.042
CJ9306	1	1	NA	NA	1	24	0.042
CJ9306	1	1	NA	NA	1	26	0.038
CJ9306	0	1	1	1	1	24	0.042
Gamenya	0	0	1	1	1	15	0.067
Gamenya	1	1	1	1	1	17	0.059
Gamenya	1	1	1	1	2	15	0.133
Gamenya	0	1	1	1	1	17	0.059
Gamenya	0	1	1	2	2	16	0.125
Gamenya	1	1	1	2	6	18	0.333
Gamenya	1	1	1	1	4	16	0.250
Gamenya	1	2	1	NA	NA	17	0.059
Gamenya	7	NA	NA	NA	NA	17	0.412
Gamenya	1	NA	NA	NA	NA	19	0.053
Gamenya	1	NA	NA	NA	NA	17	0.059
Gamenya	18	NA	NA	NA	NA	18	1.000
Gamenya	1	NA	NA	NA	NA	18	0.056
Gamenya	1	NA	NA	NA	NA	19	0.053

Gamenya	2	NA	NA	NA	NA	20	0.100
Gamenya	1	1	1	1	1	10	0.100
Gamenya	0	0	1	1	1	10	0.100
Naxos	1	1	2	2	3	15	0.200
Naxos	0	1	1	2	2	16	0.125
Naxos	1	1	1	2	2	17	0.118
Naxos	1	1	1	NA	NA	20	0.050
Naxos	1	1	1	NA	NA	20	0.050
Naxos	1	1	2	NA	NA	20	0.100
Naxos	1	1	1	NA	NA	17	0.059
Naxos	1	1	1	NA	NA	19	0.053
Naxos	1	1	2	NA	NA	20	0.100
NIL 6A5	1	1	2	2	2	14	0.143
NIL 6A5	0	1	1	1	1	12	0.083
NIL 6A5	0	1	1	1	1	13	0.077
NIL 6A5	0	1	1	1	1	14	0.071
NIL 6A5	1	1	1	2	2	15	0.133
NIL 6A5	1	1	1	1	1	17	0.059
NIL 6A5	1	1	5	8	17	17	1.000
NIL 6A5	1	1	1	1	1	19	0.053
NIL 6A5	1	1	1	1	1	17	0.059
NIL 6A5	1	1	5	5	8	16	0.500
NIL 6A5	1	1	1	1	NA	16	0.063
NIL 6A5	1	1	1	1	NA	16	0.063
NIL 6A5	0	1	1	1	NA	18	0.056
NIL 6A5	0	1	1	NA	NA	21	0.048
NIL 6A5	1	1	NA	NA	1	21	0.048
NIL 6A5	1	1	NA	NA	1	22	0.045
NIL 6A5	1	1	NA	NA	1	21	0.048
NIL 6A5	1	1	NA	NA	1	22	0.045

NIL 6A5	3	NA	NA	NA	NA	21	0.143
NIL 6A5	3	NA	NA	NA	NA	20	0.150
NIL 6A5	1	NA	NA	NA	NA	19	0.053
NIL 6A5	0	1	1	2	2	18	0.111
NIL 6A5	0	1	1	1	1	16	0.063
NIL 6B5	0	1	1	1	2	11	0.182
NIL 6B5	1	1	2	2	3	14	0.214
NIL 6B5	1	1	1	1	1	14	0.071
NIL 6B5	1	1	1	1	1	15	0.067
NIL 6B5	1	1	1	1	1	15	0.067
NIL 6B5	1	1	1	1	1	18	0.056
NIL 6B5	0	1	1	3	NA	15	0.200
NIL 6B5	1	1	1	2	NA	17	0.118
NIL 6B5	1	1	1	1	NA	17	0.059
NIL 6B5	1	1	1	1	NA	16	0.063
NIL 6B5	1	1	1	1	NA	20	0.050
NIL 6B5	1	1	4	4	NA	18	0.222
NIL 6B5	1	1	1	3	NA	19	0.158
NIL 6B5	1	1	1	NA	1	20	0.050
NIL 6B5	1	1	1	NA	1	22	0.045
NIL 6B5	1	1	2	NA	4	20	0.200
NIL 6B5	1	1	NA	NA	1	20	0.050
NIL 6B5	1	1	NA	NA	1	20	0.050
NIL 6B5	1	1	NA	NA	1	19	0.053
NIL 6B5	0	1	NA	NA	1	19	0.053
NIL 6B5	1	1	NA	NA	2	18	0.111
NIL 6B5	1	1	NA	NA	1	18	0.056
NIL 6B5	1	NA	NA	NA	NA	20	0.050
NIL 6B5	1	NA	NA	NA	NA	21	0.048
NIL 6B5	1	1	2	10	10	16	0.625

NIL 6B5	1	1	1	1	1	16	0.063
NIL 6B5	1	1	1	3	3	19	0.158
NIL 6B5	0	1	1	1	1	13	0.077
Ocoroni	0	1	1	1	1	15	0.067
Ocoroni	0	0	4	4	4	14	0.286
Ocoroni	1	6	16	NA	NA	16	1.000
Ocoroni	1	1	1	NA	NA	17	0.059
Ocoroni	1	1	2	NA	NA	19	0.105
Ocoroni	1	2	4	NA	NA	18	0.222
Ocoroni	1	2	1	NA	NA	20	0.050
Ocoroni	1	1	3	NA	NA	20	0.150
Ocoroni	3	NA	NA	NA	NA	18	0.167
SHA3/CBRD	1	1	1	NA	NA	25	0.040
SHA3/CBRD	1	1	1	NA	NA	25	0.040
SHA3/CBRD	1	1	1	NA	NA	23	0.043
Soru#1	0	1	1	1	1	17	0.059
Soru#1	0	1	1	1	1	18	0.056
Soru#1	1	1	1	1	1	20	0.050
Soru#1	1	1	2	2	NA	20	0.100
Soru#1	1	1	1	NA	NA	19	0.053
Soru#1	1	1	1	NA	NA	17	0.059
Soru#1	1	1	1	NA	1	20	0.050
Soru#1	18	NA	NA	NA	NA	18	1.000
Soru#1	9	NA	NA	NA	NA	17	0.529
Soru#1	2	NA	NA	NA	NA	18	0.111
Wuhan-1	0	1	1	1	1	18	0.056
Wuhan-1	1	2	2	1	NA	23	0.043
Wuhan-1	1	1	1	5	NA	22	0.227
Wuhan-1	1	1	NA	NA	1	25	0.040
Wuhan-1	1	NA	NA	NA	NA	22	0.045

Zebra	0	1	1	1	1	14	0.071
Zebra	1	1	1	1	1	15	0.067
Zebra	1	1	2	3	3	15	0.200
Zebra	1	2	2	3	6	17	0.353
Zebra	0	1	1	1	1	16	0.063
Zebra	1	1	1	1	1	15	0.067
Zebra	1	1	1	1	1	18	0.056
Zebra	1	1	1	1	1	17	0.059
Zebra	2	NA	NA	NA	NA	20	0.100
Zebra	1	NA	NA	NA	NA	20	0.050
Zebra	1	NA	NA	NA	NA	20	0.050
Zebra	0	1	1	2	2	16	0.125

Appendix 7 - Phenotypic scores from second point inoculation experiment

Table S7.1: Phenotypic scores from the second round of point inoculations, mapping population with control lines. The table shows the number of infected spikelets (NIS) for each of the timepoints of scoring, which started at 3 days post inoculation (DPI) to 21 DPI. The total number of spikelets (TNS) are included, as well as the calculated percentage of diseased spikelets (PDS) after 21 DPI. PDS is coloured in a gradient from red to green, where red is high PDS values and green for low PDS values. Lastly, the inoculum with which isolate used is also specified.

Line	NIS (DPI = 3)	NIS (DPI = 6)	NIS (DPI = 9)	NIS (DPI = 12)	NIS (DPI = 15)	NIS (DPI = 18)	NIS (DPI = 21)	TNS	PDS	Inoculum
CJ9306	1	1	1	1	1	1	1	22	0.045	200630
CJ9306	1	1	1	1	1	1	1	20	0.050	200630
CJ9306	1	1	1	1	1	1	1	20	0.050	200630
CJ9306	1	1	1	1	1	1	1	22	0.045	200630
CJ9306	0	1	1	1	1	1	1	21	0.048	200630
CJ9306	0	1	1	1	1	1	1	20	0.050	200630
CJ9306	0	1	1	1	1	1	1	19	0.053	200630
CJ9306	1	1	1	1	1	1	1	22	0.045	200630
CJ9306	1	1	1	1	1	1	1	20	0.050	200630
CJ9306	1	1	1	1	1	1	1	23	0.043	200646
CJ9306	1	1	1	1	1	1	1	18	0.056	200646
CJ9306	1	1	1	1	1	1	1	19	0.053	200646
CJ9306	1	1	1	1	1	1	1	18	0.056	200646
CJ9306	1	1	1	1	1	1	1	17	0.059	200646
CJ9306	1	1	1	1	1	1	1	21	0.048	200646
CJ9306	0	1	1	1	1	1	1	20	0.050	200646
CJ9306	1	1	1	1	1	1	1	20	0.050	200646
CJ9306	1	1	1	1	1	1	1	21	0.048	200646
Gamenya	1	4	4	6	13	17	17	17	1.000	200630
Gamenya	1	3	3	9	11	11	18	18	1.000	200630
Gamenya	1	1	1	3	10	16	16	16	1.000	200630
Gamenya	1	4	6	9	12	18	18	18	1.000	200630
Gamenya	1	3	6	18	18	18	18	18	1.000	200630

Gamenya	1	2	2	5	6	12	13	17	0.765	200630
Gamenya	2	4	6	8	10	17	17	17	1.000	200630
Gamenya	1	4	4	14	15	16	16	16	1.000	200630
Gamenya	1	1	1	10	16	16	16	16	1.000	200630
Gamenya	1	1	1	2	11	15	15	15	1.000	200630
Gamenya	1	4	5	8	14	16	16	16	1.000	200630
Gamenya	1	2	2	5	5	14	17	17	1.000	200630
Gamenya	1	2	2	3	11	12	14	17	0.824	200630
Gamenya	1	2	3	4	5	7	7	18	0.389	200630
Gamenya	1	1	2	2	2	5	6	18	0.333	200630
Gamenya	1	1	3	3	8	9	9	14	0.643	200630
Gamenya	1	3	6	12	13	15	16	16	1.000	200646
Gamenya	1	1	8	17	17	17	17	17	1.000	200646
Gamenya	1	1	1	4	4	5	12	16	0.750	200646
Gamenya	1	1	1	3	3	9	13	17	0.765	200646
Gamenya	1	2	3	10	10	11	11	17	0.647	200646
Gamenya	1	2	2	8	9	11	11	17	0.647	200646
Gamenya	1	2	3	12	13	15	15	15	1.000	200646
Gamenya	1	1	2	2	4	6	6	16	0.375	200646
Gamenya	1	2	3	8	9	18	18	18	1.000	200646
Gamenya	1	2	2	3	5	7	10	16	0.625	200646
Gamenya	1	1	1	2	2	3	3	18	0.167	200646
Gamenya	1	2	5	9	9	12	12	18	0.667	200646
Gamenya	0	2	3	7	7	10	11	17	0.647	200646
Gamenya	1	1	1	2	2	2	2	17	0.118	200646
Gamenya	1	1	2	2	2	5	6	17	0.353	200646
NIL 6A5	1	1	1	2	3	7	9	19	0.474	200630
NIL 6A5	0	1	1	1	1	2	2	18	0.111	200630
NIL 6A5	1	2	2	2	2	2	3	18	0.167	200630
NIL 6A5	1	1	1	1	1	1	3	19	0.158	200630

NIL 6A5	1	1	1	3	3	3	6	20	0.300	200630
NIL 6A5	1	1	1	1	1	1	1	19	0.053	200630
NIL 6A5	1	1	1	1	1	1	1	21	0.048	200630
NIL 6A5	1	1	1	1	1	2	2	17	0.118	200630
NIL 6A5	1	1	1	1	1	2	4	19	0.211	200630
NIL 6A5	1	1	2	2	2	3	4	19	0.211	200630
NIL 6A5	1	1	1	2	3	3	11	20	0.550	200630
NIL 6A5	1	1	1	1	2	2	2	18	0.111	200646
NIL 6A5	1	1	1	1	2	2	3	20	0.150	200646
NIL 6A5	1	1	1	2	2	2	3	19	0.158	200646
NIL 6A5	1	1	1	1	1	1	3	19	0.158	200646
NIL 6A5	1	2	2	2	4	4	4	20	0.200	200646
NIL 6A5	1	1	1	1	2	3	5	19	0.263	200646
NIL 6A5	1	1	1	1	1	3	4	22	0.182	200646
NIL 6A5	1	1	1	1	3	3	4	17	0.235	200646
NIL 6A5	1	1	2	3	3	5	5	18	0.278	200646
NIL 6A5	1	1	1	1	1	2	2	18	0.111	200646
NIL 6A5	1	1	1	1	1	3	3	19	0.158	200646
NIL 6B5	1	1	3	4	5	5	9	20	0.450	200630
NIL 6B5	1	1	2	12	13	13	16	19	0.842	200630
NIL 6B5	1	2	3	5	6	6	10	16	0.625	200630
NIL 6B5	1	2	6	10	15	15	17	18	0.944	200630
NIL 6B5	0	1	3	5	8	9	18	18	1.000	200630
NIL 6B5	1	2	6	19	19	19	19	19	1.000	200630
NIL 6B5	1	3	5	6	7	8	8	17	0.471	200630
NIL 6B5	1	4	14	19	19	19	19	19	1.000	200630
NIL 6B5	1	2	2	15	18	18	18	18	1.000	200630
NIL 6B5	1	1	2	4	4	4	6	19	0.316	200630
NIL 6B5	1	2	5	8	9	18	18	18	1.000	200630
NIL 6B5	1	1	2	4	4	6	8	20	0.400	200630

NIL 6B5	0	1	4	5	19	19	19	19	1.000	200646
NIL 6B5	1	1	1	1	3	3	4	18	0.222	200646
NIL 6B5	0	2	3	4	4	4	8	19	0.421	200646
NIL 6B5	1	2	3	3	3	4	6	19	0.316	200646
NIL 6B5	0	1	3	5	7	8	9	18	0.500	200646
NIL 6B5	1	1	1	1	2	3	4	18	0.222	200646
NIL 6B5	1	1	1	1	1	2	2	19	0.105	200646
NIL 6B5	1	3	3	7	8	9	9	19	0.474	200646
NIL 6B5	1	1	4	5	13	13	13	19	0.684	200646
Ocoroni	1	2	2	2	10	10	11	17	0.647	200630
Ocoroni	1	1	2	2	8	9	9	16	0.563	200630
Ocoroni	1	3	11	11	12	12	16	16	1.000	200630
Ocoroni	1	1	4	13	13	13	13	18	0.722	200630
Ocoroni	1	3	5	5	5	5	6	16	0.375	200630
Ocoroni	1	4	14	15	15	17	17	17	1.000	200630
Ocoroni	1	4	6	7	8	16	16	16	1.000	200630
Ocoroni	1	4	5	14	14	14	18	18	1.000	200630
Ocoroni	1	1	3	5	10	17	17	17	1.000	200646
Ocoroni	1	1	3	3	3	3	3	17	0.176	200646
Ocoroni	1	1	2	2	3	3	3	16	0.188	200646
Ocoroni	1	2	2	3	7	7	7	17	0.412	200646
Ocoroni	1	2	4	5	17	17	17	17	1.000	200646
Ocoroni	1	4	5	6	6	8	13	18	0.722	200646
Ocoroni	1	3	3	12	12	12	15	17	0.882	200646
Zebra	1	1	1	1	1	1	1	17	0.059	200630
Zebra	1	1	1	4	10	10	13	17	0.765	200630
Zebra	1	1	1	6	13	17	17	17	1.000	200630
Zebra	1	3	4	8	20	20	20	20	1.000	200630
Zebra	1	4	5	17	17	18	21	21	1.000	200630

Zebra	1	1	3	5	5	6	21	21	1.000	200630
Zebra	1	1	5	7	19	21	21	21	1.000	200630
Zebra	3	6	8	10	10	10	10	19	0.526	200630
Zebra	1	3	5	14	14	14	14	20	0.700	200630
Zebra	1	4	5	17	17	17	17	17	1.000	200630
Zebra	1	3	5	6	15	17	18	18	1.000	200630
Zebra	1	4	6	14	15	16	16	20	0.800	200630
Zebra	1	1	1	1	1	2	11	17	0.647	200646
Zebra	1	1	1	1	5	10	12	18	0.667	200646
Zebra	1	1	1	1	2	5	11	18	0.611	200646
Zebra	1	2	4	4	7	9	11	19	0.579	200646
Zebra	1	2	3	15	15	15	15	15	1.000	200646
Zebra	1	3	5	5	6	6	10	19	0.526	200646
Zebra	-	1	1	1	1	1	6	19	0.316	200646
Zebra	2	2	2	3	4	14	15	21	0.714	200646
Zebra	1	4	15	21	21	21	21	21	1.000	200646
Zebra	1	4	5	8	8	9	9	17	0.529	200646
Zebra	1	1	1	2	4	21	21	21	1.000	200646

Table S7.2: Phenotypic scores from the second round of point inoculations, NILs and parental lines, with the control line Gamenya. The table shows the number of infected spikelets (NIS) for each of the timepoints of scoring, which started at 3 days post inoculation (DPI) to 21 DPI. The total number of spikelets (TNS) are included, as well as the calculated percentage of diseased spikelets (PDS) after 21 DPI. PDS is coloured in a gradient from red to green, where red is high PDS values and green for low PDS values. Lastly, the inoculum with which isolate used is also specified.

Line	NIS (DPI = 3)	NIS (DPI = 6)	NIS (DPI = 9)	NIS (DPI = 12)	NIS (DPI = 15)	NIS (DPI = 18)	NIS (DPI = 21)	TNS	PDS	Inoculum
5	1	1	1	1	2	2	3	18	0.167	200630
5	1	1	1	10	10	10	11	19	0.579	200630
5	1	1	1	2	2	3	3	17	0.176	200630
5	0	1	2	2	2	3	4	19	0.211	200630
5	1	1	1	1	1	1	1	18	0.056	200630
5	1	1	1	3	3	4	5	19	0.263	200630
5	1	1	1	1	1	1	1	20	0.050	200630
5	1	1	2	3	3	4	4	21	0.190	200630
5	1	2	2	2	2	2	3	19	0.158	200630
5	1	1	1	1	3	3	5	21	0.238	200630
5	1	1	2	2	10	10	10	19	0.526	200646
5	1	1	1	1	2	3	5	18	0.278	200646
5	1	1	2	2	2	2	4	19	0.211	200646
5	1	1	1	1	1	3	4	19	0.211	200646
5	1	1	1	1	2	2	2	19	0.105	200646
5	1	1	1	1	1	1	2	19	0.105	200646
5	1	1	3	3	11	12	12	21	0.571	200646
5	1	1	2	2	2	2	2	22	0.091	200646
5	1	1	2	3	4	6	14	22	0.636	200646
5	1	1	2	3	4	6	6	20	0.300	200646
5	1	1	1	1	1	1	1	19	0.053	200646
13	1	1	4	4	4	14	14	19	0.737	200630
13	1	1	2	2	2	10	11	19	0.579	200630
13	1	1	2	10	10	11	11	19	0.579	200630
13	1	1	1	2	2	2	3	19	0.158	200630

13	1	1	1	1	2	9	10	19	0.526	200630
13	1	2	3	4	4	6	6	18	0.333	200630
13	1	1	3	3	3	4	4	21	0.190	200630
13	1	1	1	2	4	4	5	20	0.250	200630
13	1	4	4	6	8	8	11	22	0.500	200630
13	1	1	2	3	4	5	7	21	0.333	200630
13	1	2	2	3	5	6	7	20	0.350	200630
13	1	1	3	3	4	5	8	19	0.421	200646
13	1	1	2	2	2	4	6	19	0.316	200646
13	1	1	2	2	2	4	4	19	0.211	200646
13	1	1	1	1	2	3	8	19	0.421	200646
13	1	1	1	1	1	1	1	18	0.056	200646
13	1	1	2	2	2	2	2	21	0.095	200646
13	1	1	1	1	1	2	3	20	0.150	200646
13	1	1	1	1	1	1	1	21	0.048	200646
13	1	1	2	2	3	3	4	21	0.190	200646
13	1	1	2	2	3	7	8	21	0.381	200646
411	1	1	2	4	4	4	18	18	1.000	200630
411	1	3	10	10	10	10	10	18	0.556	200630
411	1	1	2	3	9	11	14	18	0.778	200630
411	1	1	3	4	4	10	11	18	0.611	200630
411	1	1	3	3	3	4	7	18	0.389	200630
411	1	2	4	12	12	12	13	20	0.650	200630
411	1	1	11	11	11	12	12	21	0.571	200630
411	1	1	2	4	4	4	12	21	0.571	200630
411	1	1	3	4	6	8	8	21	0.381	200630
411	1	2	5	6	12	17	17	17	1.000	200630
411	1	1	3	3	3	3	4	18	0.222	200646
411	1	1	3	3	4	4	4	19	0.211	200646
411	1	1	1	2	3	5	5	19	0.263	200646

411	1	1	4	4	4	5	19	19	1.000	200646
411	1	3	13	13	13	13	15	21	0.714	200646
411	1	1	3	3	12	16	16	21	0.762	200646
411	1	1	1	1	3	3	4	21	0.190	200646
411	1	1	4	4	5	5	7	20	0.350	200646
441	1	1	2	3	7	11	20	20	1.000	200630
441	1	1	1	1	1	1	2	20	0.100	200630
441	1	1	1	2	2	2	5	20	0.250	200630
441	1	1	1	1	1	1	1	19	0.053	200630
441	1	1	1	1	1	1	4	20	0.200	200630
441	1	2	2	4	5	5	17	21	0.810	200630
441	1	1	2	2	3	3	5	21	0.238	200630
441	1	1	2	2	3	3	4	18	0.222	200630
441	1	1	1	1	1	1	3	18	0.167	200646
441	1	1	2	2	2	3	6	19	0.316	200646
441	1	1	1	1	3	3	4	20	0.200	200646
441	1	2	2	2	2	3	5	20	0.250	200646
441	1	1	2	2	2	3	4	20	0.200	200646
441	1	1	2	2	2	3	5	22	0.227	200646
441	1	2	2	4	5	5	7	20	0.350	200646
971	1	1	1	3	3	11	11	20	0.550	200630
971	1	1	1	2	3	3	10	20	0.500	200630
971	1	1	2	3	3	9	9	19	0.474	200630
971	1	1	1	1	2	11	11	20	0.550	200630
971	1	1	1	2	3	3	3	21	0.143	200630
971	1	1	2	2	4	6	7	21	0.333	200630
971	1	1	3	4	10	10	11	19	0.579	200630
971	1	1	3	3	3	4	13	20	0.650	200646
971	1	1	1	1	2	2	3	19	0.158	200646

971	1	1	1	2	3	4	5	20	0.250	200646
971	1	1	2	2	2	2	3	19	0.158	200646
971	1	1	1	2	3	4	4	22	0.182	200646
971	1	1	3	3	3	4	4	21	0.190	200646
971	1	1	2	3	3	3	3	22	0.136	200646
971	1	1	1	2	3	3	13	19	0.684	200646
981	1	1	4	4	4	4	5	19	0.263	200630
981	0	1	2	3	3	4	6	19	0.316	200630
981	1	2	3	5	5	5	6	21	0.286	200630
981	1	3	4	5	6	9	10	20	0.500	200630
981	1	1	3	4	5	5	5	21	0.238	200630
981	1	3	5	9	10	10	20	20	1.000	200630
981	1	1	2	2	10	10	10	19	0.526	200646
981	1	1	4	4	5	12	13	19	0.684	200646
981	1	1	3	4	5	14	18	19	0.947	200646
981	1	1	3	4	4	4	5	19	0.263	200646
981	1	3	4	13	15	16	17	21	0.810	200646
981	1	1	4	5	5	5	5	22	0.227	200646
981	1	1	2	3	3	4	4	21	0.190	200646
981	1	5	12	12	12	12	12	18	0.667	200646
1011	1	2	5	6	13	13	20	20	1.000	200630
1011	1	1	4	18	18	18	18	18	1.000	200630
1011	1	4	4	12	13	13	15	20	0.750	200630
1011	1	1	4	13	13	14	19	19	1.000	200630
1011	1	3	5	8	9	9	19	19	1.000	200630
1011	1	2	10	13	16	18	20	20	1.000	200630
1011	1	1	4	5	16	16	17	20	0.850	200630
1011	1	2	13	13	13	13	14	22	0.636	200630
1011	1	3	12	13	15	15	15	22	0.682	200630

1011	1	3	12	12	13	13	16	21	0.762	200630
1011	1	1	11	12	14	16	20	20	1.000	200646
1011	1	2	2	4	4	4	6	20	0.300	200646
1011	1	1	4	7	7	16	16	21	0.762	200646
1011	1	1	12	4	4	4	5	20	0.250	200646
1011	1	12	13	13	14	14	14	22	0.636	200646
1011	1	3	5	5	6	6	7	22	0.318	200646
1011	1	3	3	4	14	14	18	22	0.818	200646
1081	1	1	4	13	15	16	18	18	1.000	200630
1081	1	3	3	13	15	17	21	21	1.000	200630
1081	1	1	3	5	5	6	6	21	0.286	200630
1081	1	1	3	12	12	12	12	21	0.571	200630
1081	1	1	3	3	4	6	14	21	0.667	200630
1081	1	12	14	15	17	21	21	21	1.000	200646
1081	1	1	1	3	4	4	4	20	0.200	200646
1081	1	2	5	7	8	16	16	22	0.727	200646
1081	1	1	3	4	4	5	7	20	0.350	200646
1081	1	1	2	3	4	4	4	20	0.200	200646
1101	1	9	9	9	9	9	9	19	0.474	200630
1101	1	1	1	2	2	4	6	17	0.353	200630
1101	1	1	2	2	3	4	5	20	0.250	200630
1101	1	1	1	1	1	2	4	20	0.200	200630
1101	1	1	1	1	1	2	3	19	0.158	200630
1101	1	1	1	2	3	4	4	19	0.211	200646
1101	1	1	2	3	3	4	4	19	0.211	200646
1101	1	1	1	1	1	2	4	18	0.222	200646
1101	1	1	4	12	12	12	12	21	0.571	200646
1101	1	2	3	3	7	17	20	20	1.000	200646
1101	1	1	2	9	9	9	9	19	0.474	200646

1101	1	1	4	4	5	8	8	21	0.381	200646
1501	1	1	1	2	4	5	6	18	0.333	200630
1501	1	1	3	10	10	12	19	19	1.000	200630
1501	1	1	2	12	13	13	21	21	1.000	200630
1501	1	1	1	2	2	5	9	20	0.450	200630
1501	1	1	1	2	2	4	15	19	0.789	200630
1501	1	1	2	3	10	10	10	20	0.500	200630
1501	1	1	2	2	4	20	20	20	1.000	200630
1501	0	1	1	2	5	5	5	18	0.278	200630
1501	0	1	2	2	2	2	5	19	0.263	200646
1501	1	1	1	2	2	2	2	19	0.105	200646
1501	1	1	2	2	2	3	7	20	0.350	200646
1501	1	1	2	4	4	13	13	19	0.684	200646
1501	1	1	2	9	9	9	9	18	0.500	200646
1501	1	2	2	3	4	4	4	19	0.211	200646
1501	1	1	2	3	4	11	11	20	0.550	200646
1511	1	1	10	11	11	11	11	19	0.579	200630
1511	1	1	1	1	2	3	8	16	0.500	200630
1511	1	1	3	4	4	11	11	18	0.611	200630
1511	1	1	1	1	1	3	4	20	0.200	200630
1511	1	1	2	10	10	12	14	20	0.700	200630
1511	1	1	1	3	4	6	9	20	0.450	200630
1511	1	1	1	1	4	8	8	19	0.421	200630
1511	1	1	1	2	2	2	2	19	0.105	200630
1511	1	2	2	9	9	10	10	19	0.526	200646
1511	1	2	10	10	10	10	17	19	0.895	200646
1511	1	1	2	12	12	12	12	21	0.571	200646
1511	1	1	2	6	9	11	19	21	0.905	200646
1511	1	1	1	9	10	10	10	20	0.500	200646

1511	1	1	2	11	11	11	11	20	0.550	200646
1511	1	1	1	1	2	2	2	19	0.105	200646
1511	1	1	1	11	12	13	13	19	0.684	200646
CJ9306	1	1	1	1	1	1	1	23	0.043	200630
CJ9306	1	1	1	1	1	1	1	19	0.053	200630
CJ9306	1	1	1	1	1	1	1	22	0.045	200630
CJ9306	1	1	1	1	1	1	1	21	0.048	200630
CJ9306	1	1	1	1	1	1	1	20	0.050	200630
CJ9306	1	1	1	1	1	1	1	23	0.043	200630
CJ9306	1	1	1	1	1	1	2	23	0.087	200630
CJ9306	1	1	1	1	1	1	1	21	0.048	200646
CJ9306	1	1	1	1	1	1	1	18	0.056	200646
CJ9306	1	1	1	1	1	1	4	21	0.190	200646
CJ9306	1	1	1	1	1	1	1	24	0.042	200646
CJ9306	1	1	1	1	1	1	1	22	0.045	200646
CJ9306	1	1	1	1	1	1	1	20	0.050	200646
CJ9306	0	1	1	1	1	1	1	20	0.050	200646
Gamenya	1	1	2	5	5	10	19	19	1.000	200630
Gamenya	1	2	5	10	10	11	19	19	1.000	200630
Gamenya	1	1	1	3	5	19	19	19	1.000	200630
Gamenya	1	1	2	7	9	10	10	18	0.556	200630
Gamenya	1	3	8	12	18	18	18	18	1.000	200630
Gamenya	1	1	3	15	18	18	18	18	1.000	200646
Gamenya	1	1	2	2	2	12	16	19	0.842	200646
Gamenya	1	1	1	3	5	10	12	20	0.600	200646
Gamenya	1	1	2	2	5	5	5	19	0.263	200646
Zebra	1	3	11	12	20	20	20	20	1.000	200630
Zebra	1	3	6	12	12	12	13	18	0.722	200630
Zebra	1	2	3	4	15	16	20	20	1.000	200630

Zebra	1	2	2	2	2	2	2	20	0.100	200630
Zebra	1	1	11	11	12	12	12	21	0.571	200630
Zebra	1	4	5	5	13	13	14	22	0.636	200630
Zebra	1	1	11	11	11	12	13	21	0.619	200646
Zebra	1	2	4	14	15	15	15	21	0.714	200646
Zebra	1	1	1	1	1	1	1	18	0.056	200646
Zebra	1	3	5	18	20	23	23	23	1.000	200646

Appendix 8 - Percentage of diseased spikelets after 18 days post inoculation

Table S8.1: The average PDS after 18 days post inoculation (DPI) for both isolates in the NIL population with parental lines and Gamenya as a control line. The marker genotypes are also shown for each line with available information, where CJ indicates the resistant allele from CJ9306, and Z indicates the susceptible allele from Zebra.

Line	*gwm539*	*WGRB3803*	*wsnp_Ex_c8303_14001708*	Avg. PDS (Fg. 200630)	Avg. PDS (Fg. 200646)
5	CJ	CJ	CJ	0.17 (n=10)	0.22 (n=11)
13	Z	Z	CJ	0.37 (n=11)	0.16 (n=10)
411	Z	Z	CJ	0.49 (n=10)	0.33 (n=8)
441	CJ	CJ	CJ	0.17 (n=8)	0.15 (n=7)
971	CJ	CJ	Z	0.38 (n=7)	0.16 (n=8)
981	Z	Z	Z	0.31 (n=6)	0.49 (n=8)
1011	CJ	CJ	CJ	0.71 (n=10)	0.50 (n=7)
1081	Z	Z	Z	0.57 (n=5)	0.48 (n=5)
1101	CJ	CJ	Z	0.22 (n=5)	0.40 (n=7)
1501	CJ	CJ	Z	0.47 (n=8)	0.33 (n=7)
1511	CJ	CJ	CJ	0.37 (n=8)	0.50 (n=8)
CJ9306	NA	CJ	NA	0.05 (n=7)	0.05 (n=7)
Gamenya	NA	NA	NA	0.73 (n=5)	0.60 (n=4)
Zebra	NA	Z	NA	0.62 (n=6)	0.59 (n=4)

Appendix 9 - AUDPC values

Table S9.1: Area under the disease progress curve (AUDPC) values for the first part of the second point inoculation experiment, for the spikes inoculated with isolate Fg. 200630. Line, replicate, inoculum, and percentage of diseased spikelets (PDS) for each time point (3-, 6-, 9-, 12-,15-, 18-, and 21-days post inoculation (DPI)) are included in addition to the AUDPC value for each line.

Line	Rep	Inoculum	PDS (3 DPI)	PDS (6 DPI)	PDS (9 DPI)	PDS (12 DPI)	PDS (15 DPI)	PDS (18 DPI)	PDS (21 DPI)	AUDPC
CJ9306	1	200630	4.545	4.545	4.545	4.545	4.545	4.545	4.545	81.818
CJ9306	1	200630	5.000	5.000	5.000	5.000	5.000	5.000	5.000	90.000
CJ9306	2	200630	5.000	5.000	5.000	5.000	5.000	5.000	5.000	90.000
CJ9306	2	200630	4.545	4.545	4.545	4.545	4.545	4.545	4.545	81.818
CJ9306	2	200630	0.000	4.762	4.762	4.762	4.762	4.762	4.762	78.571
CJ9306	2	200630	0.000	5.000	5.000	5.000	5.000	5.000	5.000	82.500
CJ9306	2	200630	0.000	5.263	5.263	5.263	5.263	5.263	5.263	86.842
CJ9306	2	200630	4.545	4.545	4.545	4.545	4.545	4.545	4.545	81.818
CJ9306	1	200630	5.000	5.000	5.000	5.000	5.000	5.000	5.000	90.000
Gamenya	2	200630	5.882	23.529	23.529	35.294	76.471	100.000	100.000	935.294
Gamenya	2	200630	5.556	16.667	16.667	50.000	61.111	61.111	100.000	775.000
Gamenya	test	200630	6.250	6.250	6.250	18.750	62.500	100.000	100.000	740.625
Gamenya	test	200630	5.556	22.222	33.333	50.000	66.667	100.000	100.000	975.000
Gamenya	test	200630	5.556	16.667	33.333	100.000	100.000	100.000	100.000	1208.333
Gamenya	test	200630	5.882	11.765	11.765	29.412	35.294	70.588	76.471	600.000
Gamenya	test	200630	11.765	23.529	35.294	47.059	58.824	100.000	100.000	961.765
Gamenya	test	200630	6.250	25.000	25.000	87.500	93.750	100.000	100.000	1153.125
Gamenya	test	200630	6.250	6.250	6.250	62.500	100.000	100.000	100.000	984.375
Gamenya	test	200630	6.667	6.667	6.667	13.333	73.333	100.000	100.000	760.000
Gamenya	1	200630	6.250	25.000	31.250	50.000	87.500	100.000	100.000	1040.625
Gamenya	1	200630	5.882	11.765	11.765	29.412	29.412	82.353	100.000	652.941
Gamenya	1	200630	5.882	11.765	11.765	17.647	64.706	70.588	82.353	661.765
Gamenya	test	200630	5.556	11.111	16.667	22.222	27.778	38.889	38.889	416.667
Gamenya	test	200630	5.556	5.556	11.111	11.111	11.111	27.778	33.333	258.333

Gamenya	test	200630	7.143	7.143	21.429	21.429	57.143	64.286	64.286	621.429
NIL 6A5	2	200630	5.263	5.263	5.263	10.526	15.789	36.842	47.368	300.000
NIL 6A5	2	200630	0.000	5.556	5.556	5.556	5.556	11.111	11.111	116.667
NIL 6A5	1	200630	5.556	11.111	11.111	11.111	11.111	11.111	16.667	200.000
NIL 6A5	1	200630	5.263	5.263	5.263	5.263	5.263	5.263	15.789	110.526
NIL 6A5	2	200630	5.000	5.000	5.000	15.000	15.000	15.000	30.000	217.500
NIL 6A5	2	200630	5.263	5.263	5.263	5.263	5.263	5.263	5.263	94.737
NIL 6A5	1	200630	4.762	4.762	4.762	4.762	4.762	4.762	4.762	85.714
NIL 6A5	2	200630	5.882	5.882	5.882	5.882	5.882	11.765	11.765	132.353
NIL 6A5	2	200630	5.263	5.263	5.263	5.263	5.263	10.526	21.053	134.211
NIL 6A5	2	200630	5.263	5.263	10.526	10.526	10.526	15.789	21.053	197.368
NIL 6A5	2	200630	5.000	5.000	5.000	10.000	15.000	15.000	55.000	240.000
NIL 6B5	2	200630	5.000	5.000	15.000	20.000	25.000	25.000	45.000	345.000
NIL 6B5	2	200630	5.263	5.263	10.526	63.158	68.421	68.421	84.211	781.579
NIL 6B5	1	200630	6.250	12.500	18.750	31.250	37.500	37.500	62.500	515.625
NIL 6B5	1	200630	5.556	11.111	33.333	55.556	83.333	83.333	94.444	950.000
NIL 6B5	1	200630	0.000	5.556	16.667	27.778	44.444	50.000	100.000	583.333
NIL 6B5	2	200630	5.263	10.526	31.579	100.000	100.000	100.000	100.000	1184.211
NIL 6B5	2	200630	5.882	17.647	29.412	35.294	41.176	47.059	47.059	591.176
NIL 6B5	2	200630	5.263	21.053	73.684	100.000	100.000	100.000	100.000	1342.105
NIL 6B5	2	200630	5.556	11.111	11.111	83.333	100.000	100.000	100.000	1075.000
NIL 6B5	1	200630	5.263	5.263	10.526	21.053	21.053	21.053	31.579	292.105
NIL 6B5	1	200630	5.556	11.111	27.778	44.444	50.000	100.000	100.000	858.333
NIL 6B5	2	200630	5.000	5.000	10.000	20.000	20.000	30.000	40.000	322.500
Ocoroni F86	1	200630	5.882	11.765	11.765	11.765	58.824	58.824	64.706	564.706
Ocoroni F86	2	200630	6.250	6.250	12.500	12.500	50.000	56.250	56.250	506.250
Ocoroni F86	2	200630	6.250	18.750	68.750	68.750	75.000	75.000	100.000	1078.125
Ocoroni F86	2	200630	5.556	5.556	22.222	72.222	72.222	72.222	72.222	850.000
Ocoroni F86	2	200630	6.250	18.750	31.250	31.250	31.250	31.250	37.500	496.875
Ocoroni F86	1	200630	5.882	23.529	82.353	88.235	88.235	100.000	100.000	1305.882

Ocoroni F86	1	200630	6.250	25.000	37.500	43.750	50.000	100.000	100.000	928.125
Ocoroni F86	2	200630	5.556	22.222	27.778	77.778	77.778	77.778	100.000	1008.333
Zebra	1	200630	5.882	5.882	5.882	5.882	5.882	5.882	5.882	105.882
Zebra	1	200630	5.882	5.882	5.882	23.529	58.824	58.824	76.471	582.353
Zebra	2	200630	5.882	5.882	5.882	35.294	76.471	100.000	100.000	829.412
Zebra	2	200630	5.000	15.000	20.000	40.000	100.000	100.000	100.000	982.500
Zebra	2	200630	4.762	19.048	23.810	80.952	80.952	85.714	100.000	1028.571
Zebra	2	200630	4.762	4.762	14.286	23.810	23.810	28.571	100.000	442.857
Zebra	2	200630	4.762	4.762	23.810	33.333	90.476	100.000	100.000	914.286
Zebra	1	200630	15.789	31.579	42.105	52.632	52.632	52.632	52.632	797.368
Zebra	2	200630	5.000	15.000	25.000	70.000	70.000	70.000	70.000	862.500
Zebra	2	200630	5.882	23.529	29.412	100.000	100.000	100.000	100.000	1217.647
Zebra	2	200630	5.556	16.667	27.778	33.333	83.333	94.444	100.000	925.000
Zebra	2	200630	5.000	20.000	30.000	70.000	75.000	80.000	80.000	952.500

Table S9.2: Area under the disease progress curve (AUDPC) values for the first part of the second point inoculation experiment, for the spikes inoculated with isolate Fg. 200646. Line, replicate, inoculum, and percentage of diseased spikelets (PDS) for each time point (3-, 6-, 9-, 12-,15-, 18-, and 21-days post inoculation (DPI)) are included in addition to the AUDPC value for each line.

Line	Rep	Inoculum	PDS (3 DPI)	PDS (6 DPI)	PDS (9 DPI)	PDS (12 DPI)	PDS (15 DPI)	PDS (18 DPI)	PDS (21 DPI)	AUDPC
CJ9306	1	200646	4.348	4.348	4.348	4.348	4.348	4.348	4.348	78.261
CJ9306	1	200646	5.556	5.556	5.556	5.556	5.556	5.556	5.556	100.000
CJ9306	2	200646	5.263	5.263	5.263	5.263	5.263	5.263	5.263	94.737
CJ9306	2	200646	5.556	5.556	5.556	5.556	5.556	5.556	5.556	100.000
CJ9306	2	200646	5.882	5.882	5.882	5.882	5.882	5.882	5.882	105.882
CJ9306	1	200646	4.762	4.762	4.762	4.762	4.762	4.762	4.762	85.714
CJ9306	1	200646	0.000	5.000	5.000	5.000	5.000	5.000	5.000	82.500
CJ9306	1	200646	5.000	5.000	5.000	5.000	5.000	5.000	5.000	90.000
CJ9306	2	200646	4.762	4.762	4.762	4.762	4.762	4.762	4.762	85.714
Gamenya	1	200646	6.250	18.750	37.500	75.000	81.250	93.750	100.000	1078.125
Gamenya	1	200646	5.882	5.882	47.059	100.000	100.000	100.000	100.000	1217.647
Gamenya	2	200646	6.250	6.250	6.250	25.000	25.000	31.250	75.000	403.125
Gamenya	test	200646	5.882	5.882	5.882	17.647	17.647	52.941	76.471	423.529
Gamenya	test	200646	5.882	11.765	17.647	58.824	58.824	64.706	64.706	741.176
Gamenya	test	200646	5.882	11.765	11.765	47.059	52.941	64.706	64.706	670.588
Gamenya	test	200646	6.667	13.333	20.000	80.000	86.667	100.000	100.000	1060.000
Gamenya	test	200646	6.250	6.250	12.500	12.500	25.000	37.500	37.500	346.875
Gamenya	test	200646	5.556	11.111	16.667	44.444	50.000	100.000	100.000	825.000
Gamenya	test	200646	6.250	12.500	12.500	18.750	31.250	43.750	62.500	459.375
Gamenya	1	200646	5.556	5.556	5.556	11.111	11.111	16.667	16.667	183.333
Gamenya	test	200646	5.556	11.111	27.778	50.000	50.000	66.667	66.667	725.000
Gamenya	test	200646	0.000	11.765	17.647	41.176	41.176	58.824	64.706	608.824
Gamenya	test	200646	5.882	5.882	5.882	11.765	11.765	11.765	11.765	167.647
Gamenya	test	200646	5.882	5.882	11.765	11.765	11.765	29.412	35.294	273.529
NIL 6A5	1	200646	5.556	5.556	5.556	5.556	11.111	11.111	11.111	141.667
NIL 6A5	1	200646	5.000	5.000	5.000	5.000	10.000	10.000	15.000	135.000

NIL 6A5	1	200646	5.263	5.263	5.263	10.526	10.526	10.526	15.789	157.895
NIL 6A5	1	200646	5.263	5.263	5.263	5.263	5.263	5.263	15.789	110.526
NIL 6A5	1	200646	5.000	10.000	10.000	10.000	20.000	20.000	20.000	247.500
NIL 6A5	1	200646	5.263	5.263	5.263	5.263	10.526	15.789	26.316	173.684
NIL 6A5	1	200646	4.545	4.545	4.545	4.545	4.545	13.636	18.182	129.545
NIL 6A5	2	200646	5.882	5.882	5.882	5.882	17.647	17.647	23.529	202.941
NIL 6A5	1	200646	5.556	5.556	11.111	16.667	16.667	27.778	27.778	283.333
NIL 6A5	1	200646	5.556	5.556	5.556	5.556	5.556	11.111	11.111	125.000
NIL 6A5	1	200646	5.263	5.263	5.263	5.263	5.263	15.789	15.789	142.105
NIL 6B5	1	200646	0.000	5.263	21.053	26.316	100.000	100.000	100.000	907.895
NIL 6B5	2	200646	5.556	5.556	5.556	5.556	16.667	16.667	22.222	191.667
NIL 6B5	1	200646	0.000	10.526	15.789	21.053	21.053	21.053	42.105	331.579
NIL 6B5	1	200646	5.263	10.526	15.789	15.789	15.789	21.053	31.579	292.105
NIL 6B5	1	200646	0.000	5.556	16.667	27.778	38.889	44.444	50.000	475.000
NIL 6B5	1	200646	5.556	5.556	5.556	5.556	11.111	16.667	22.222	175.000
NIL 6B5	1	200646	5.263	5.263	5.263	5.263	5.263	10.526	10.526	118.421
NIL 6B5	1	200646	5.263	15.789	15.789	36.842	42.105	47.368	47.368	552.632
NIL 6B5	2	200646	5.263	5.263	21.053	26.316	68.421	68.421	68.421	678.947
Ocoroni F86	1	200646	5.882	5.882	17.647	29.412	58.824	100.000	100.000	794.118
Ocoroni F86	1	200646	5.882	5.882	17.647	17.647	17.647	17.647	17.647	264.706
Ocoroni F86	2	200646	6.250	6.250	12.500	12.500	18.750	18.750	18.750	243.750
Ocoroni F86	2	200646	5.882	11.765	11.765	17.647	41.176	41.176	41.176	441.176
Ocoroni F86	2	200646	5.882	11.765	23.529	29.412	100.000	100.000	100.000	952.941
Ocoroni F86	1	200646	5.556	22.222	27.778	33.333	33.333	44.444	72.222	600.000
Ocoroni F86	1	200646	5.882	17.647	17.647	70.588	70.588	70.588	88.235	882.353
Zebra	1	200646	5.882	5.882	5.882	5.882	5.882	11.765	64.706	211.765
Zebra	1	200646	5.556	5.556	5.556	5.556	27.778	55.556	66.667	408.333
Zebra	1	200646	5.556	5.556	5.556	5.556	11.111	27.778	61.111	266.667
Zebra	1	200646	5.263	10.526	21.053	21.053	36.842	47.368	57.895	505.263
Zebra	1	200646	6.667	13.333	20.000	100.000	100.000	100.000	100.000	1160.000

Zebra	2	200646	5.263	15.789	26.316	26.316	31.579	31.579	52.632	481.579
Zebra	1	200646	NA	5.263	5.263	5.263	5.263	5.263	31.579	NA
Zebra	1	200646	9.524	9.524	9.524	14.286	19.048	66.667	71.429	478.571
Zebra	1	200646	4.762	19.048	71.429	100.000	100.000	100.000	100.000	1328.571
Zebra	1	200646	5.882	23.529	29.412	47.059	47.059	52.941	52.941	688.235
Zebra	1	200646	4.762	4.762	4.762	9.524	19.048	100.000	100.000	571.429

Table S9.3: Area under the disease progress curve (AUDPC) values for the second part of the second point inoculation experiment, for the spikes inoculated with isolate Fg. 200630. Line, replicate, inoculum, and percentage of diseased spikelets (PDS) for each time point (3-, 6-, 9-, 12-,15-, 18-, and 21-days post inoculation (DPI)) are included in addition to the AUDPC value for each line.

Line	Rep	Inoculum	PDS (3 DPI)	PDS (6 DPI)	PDS (9 DPI)	PDS (12 DPI)	PDS (15 DPI)	PDS (18 DPI)	PDS (21 DPI)	AUDPC
5	2	200630	5.556	5.556	5.556	5.556	11.111	11.111	16.667	150.000
5	2	200630	5.263	5.263	5.263	52.632	52.632	52.632	57.895	600.000
5	2	200630	5.882	5.882	5.882	11.765	11.765	17.647	17.647	194.118
5	2	200630	0.000	5.263	10.526	10.526	10.526	15.789	21.053	189.474
5	2	200630	5.556	5.556	5.556	5.556	5.556	5.556	5.556	100.000
5	2	200630	5.263	5.263	5.263	15.789	15.789	21.053	26.316	236.842
5	2	200630	5.000	5.000	5.000	5.000	5.000	5.000	5.000	90.000
5	2	200630	4.762	4.762	9.524	14.286	14.286	19.048	19.048	221.429
5	2	200630	5.263	10.526	10.526	10.526	10.526	10.526	15.789	189.474
5	2	200630	4.762	4.762	4.762	4.762	14.286	14.286	23.810	171.429
13	2	200630	5.263	5.263	21.053	21.053	21.053	73.684	73.684	544.737
13	2	200630	5.263	5.263	10.526	10.526	10.526	52.632	57.895	363.158
13	2	200630	5.263	5.263	10.526	52.632	52.632	57.895	57.895	631.579
13	2	200630	5.263	5.263	5.263	10.526	10.526	10.526	15.789	157.895
13	2	200630	5.263	5.263	5.263	5.263	10.526	47.368	52.632	307.895
13	2	200630	5.556	11.111	16.667	22.222	22.222	33.333	33.333	375.000
13	1	200630	4.762	4.762	14.286	14.286	14.286	19.048	19.048	235.714
13	2	200630	5.000	5.000	5.000	10.000	20.000	20.000	25.000	225.000
13	2	200630	4.545	18.182	18.182	27.273	36.364	36.364	50.000	490.909
13	2	200630	4.762	4.762	9.524	14.286	19.048	23.810	33.333	271.429
13	2	200630	5.000	10.000	10.000	15.000	25.000	30.000	35.000	330.000
411	1	200630	5.556	5.556	11.111	22.222	22.222	22.222	100.000	408.333
411	2	200630	5.556	16.667	55.556	55.556	55.556	55.556	55.556	808.333
411	2	200630	5.556	5.556	11.111	16.667	50.000	61.111	77.778	558.333
411	2	200630	5.556	5.556	16.667	22.222	22.222	55.556	61.111	466.667
411	2	200630	5.556	5.556	16.667	16.667	16.667	22.222	38.889	300.000

411	2	200630	5.000	10.000	20.000	60.000	60.000	60.000	65.000	735.000
411	2	200630	4.762	4.762	52.381	52.381	52.381	57.143	57.143	750.000
411	2	200630	4.762	4.762	9.524	19.048	19.048	19.048	57.143	307.143
411	2	200630	4.762	4.762	14.286	19.048	28.571	38.095	38.095	378.571
411	2	200630	5.882	11.765	29.412	35.294	70.588	100.000	100.000	900.000
441	1	200630	5.000	5.000	10.000	15.000	35.000	55.000	100.000	517.500
441	2	200630	5.000	5.000	5.000	5.000	5.000	5.000	10.000	97.500
441	2	200630	5.000	5.000	5.000	10.000	10.000	10.000	25.000	165.000
441	2	200630	5.263	5.263	5.263	5.263	5.263	5.263	5.263	94.737
441	2	200630	5.000	5.000	5.000	5.000	5.000	5.000	20.000	112.500
441	1	200630	4.762	9.524	9.524	19.048	23.810	23.810	80.952	385.714
441	1	200630	4.762	4.762	9.524	9.524	14.286	14.286	23.810	200.000
441	2	200630	5.556	5.556	11.111	11.111	16.667	16.667	22.222	225.000
971	2	200630	5.000	5.000	5.000	15.000	15.000	55.000	55.000	375.000
971	2	200630	5.000	5.000	5.000	10.000	15.000	15.000	50.000	232.500
971	2	200630	5.263	5.263	10.526	15.789	15.789	47.368	47.368	363.158
971	2	200630	5.000	5.000	5.000	5.000	10.000	55.000	55.000	330.000
971	1	200630	4.762	4.762	4.762	9.524	14.286	14.286	14.286	171.429
971	1	200630	4.762	4.762	9.524	9.524	19.048	28.571	33.333	271.429
971	1	200630	5.263	5.263	15.789	21.053	52.632	52.632	57.895	536.842
981	2	200630	5.263	5.263	21.053	21.053	21.053	21.053	26.316	315.789
981	2	200630	0.000	5.263	10.526	15.789	15.789	21.053	31.579	252.632
981	1	200630	4.762	9.524	14.286	23.810	23.810	23.810	28.571	335.714
981	1	200630	5.000	15.000	20.000	25.000	30.000	45.000	50.000	487.500
981	1	200630	4.762	4.762	14.286	19.048	23.810	23.810	23.810	300.000
981	1	200630	5.000	15.000	25.000	45.000	50.000	50.000	100.000	712.500
1011	1	200630	5.000	10.000	25.000	30.000	65.000	65.000	100.000	742.500
1011	1	200630	5.556	5.556	22.222	100.000	100.000	100.000	100.000	1141.667
1011	1	200630	5.000	20.000	20.000	60.000	65.000	65.000	75.000	810.000
1011	2	200630	5.263	5.263	21.053	68.421	68.421	73.684	100.000	868.421

1011	2	200630	5.263	15.789	26.316	42.105	47.368	47.368	100.000	694.737
1011	2	200630	5.000	10.000	50.000	65.000	80.000	90.000	100.000	1042.500
1011	1	200630	5.000	5.000	20.000	25.000	80.000	80.000	85.000	765.000
1011	1	200630	4.545	9.091	59.091	59.091	59.091	59.091	63.636	838.636
1011	1	200630	4.545	13.636	54.545	59.091	68.182	68.182	68.182	900.000
1011	1	200630	4.762	14.286	57.143	57.143	61.905	61.905	76.190	878.571
1081	1	200630	5.556	5.556	22.222	72.222	83.333	88.889	100.000	975.000
1081	1	200630	4.762	14.286	14.286	61.905	71.429	80.952	100.000	885.714
1081	2	200630	4.762	4.762	14.286	23.810	23.810	28.571	28.571	335.714
1081	2	200630	4.762	4.762	14.286	57.143	57.143	57.143	57.143	664.286
1081	1	200630	4.762	4.762	14.286	14.286	19.048	28.571	66.667	350.000
1101	2	200630	5.263	47.368	47.368	47.368	47.368	47.368	47.368	789.474
1101	2	200630	5.882	5.882	5.882	11.765	11.765	23.529	35.294	238.235
1101	1	200630	5.000	5.000	10.000	10.000	15.000	20.000	25.000	225.000
1101	1	200630	5.000	5.000	5.000	5.000	5.000	10.000	20.000	127.500
1101	2	200630	5.263	5.263	5.263	5.263	5.263	10.526	15.789	126.316
1501	1	200630	5.556	5.556	5.556	11.111	22.222	27.778	33.333	275.000
1501	2	200630	5.263	5.263	15.789	52.632	52.632	63.158	100.000	726.316
1501	2	200630	4.762	4.762	9.524	57.143	61.905	61.905	100.000	742.857
1501	2	200630	5.000	5.000	5.000	10.000	10.000	25.000	45.000	240.000
1501	2	200630	5.263	5.263	5.263	10.526	10.526	21.053	78.947	284.211
1501	2	200630	5.000	5.000	10.000	15.000	50.000	50.000	50.000	472.500
1501	1	200630	5.000	5.000	10.000	10.000	20.000	100.000	100.000	592.500
1501	2	200630	0.000	5.556	5.556	11.111	27.778	27.778	27.778	275.000
1511	2	200630	5.263	5.263	52.632	57.895	57.895	57.895	57.895	789.474
1511	1	200630	6.250	6.250	6.250	6.250	12.500	18.750	50.000	234.375
1511	1	200630	5.556	5.556	16.667	22.222	22.222	61.111	61.111	483.333
1511	2	200630	5.000	5.000	5.000	5.000	5.000	15.000	20.000	142.500
1511	2	200630	5.000	5.000	10.000	50.000	50.000	60.000	70.000	637.500
1511	2	200630	5.000	5.000	5.000	15.000	20.000	30.000	45.000	300.000

1511	1	200630	5.263	5.263	5.263	5.263	21.053	42.105	42.105	307.895
1511	1	200630	5.263	5.263	5.263	10.526	10.526	10.526	10.526	150.000
CJ9306	2	200630	4.348	4.348	4.348	4.348	4.348	4.348	4.348	78.261
CJ9306	1	200630	5.263	5.263	5.263	5.263	5.263	5.263	5.263	94.737
CJ9306	1	200630	4.545	4.545	4.545	4.545	4.545	4.545	4.545	81.818
CJ9306	1	200630	4.762	4.762	4.762	4.762	4.762	4.762	4.762	85.714
CJ9306	2	200630	5.000	5.000	5.000	5.000	5.000	5.000	5.000	90.000
CJ9306	1	200630	4.348	4.348	4.348	4.348	4.348	4.348	4.348	78.261
CJ9306	2	200630	4.348	4.348	4.348	4.348	4.348	4.348	8.696	84.783
Gamenya	1	200630	5.263	5.263	10.526	26.316	26.316	52.632	100.000	521.053
Gamenya	1	200630	5.263	10.526	26.316	52.632	52.632	57.895	100.000	757.895
Gamenya	1	200630	5.263	5.263	5.263	15.789	26.316	100.000	100.000	615.789
Gamenya	1	200630	5.556	5.556	11.111	38.889	50.000	55.556	55.556	575.000
Gamenya	1	200630	5.556	16.667	44.444	66.667	100.000	100.000	100.000	1141.667
Zebra	1	200630	5.000	15.000	55.000	60.000	100.000	100.000	100.000	1147.500
Zebra	1	200630	5.556	16.667	33.333	66.667	66.667	66.667	72.222	866.667
Zebra	1	200630	5.000	10.000	15.000	20.000	75.000	80.000	100.000	757.500
Zebra	2	200630	5.000	10.000	10.000	10.000	10.000	10.000	10.000	172.500
Zebra	2	200630	4.762	4.762	52.381	52.381	57.143	57.143	57.143	764.286
Zebra	2	200630	4.545	18.182	22.727	22.727	59.091	59.091	63.636	647.727

Table S9.4: Area under the disease progress curve (AUDPC) values for the second part of the second point inoculation experiment, for the spikes inoculated with isolate Fg. 200646. Line, replicate, inoculum, and percentage of diseased spikelets (PDS) for each time point (3-, 6-, 9-, 12-,15-, 18-, and 21-days post inoculation (DPI)) are included in addition to the AUDPC value for each line.

Line	Rep	Inoculum	PDS (3 DPI)	PDS (6 DPI)	PDS (9 DPI)	PDS (12 DPI)	PDS (15 DPI)	PDS (18 DPI)	PDS (21 DPI)	AUDPC
5	1	200646	5.263	5.263	10.526	10.526	52.632	52.632	52.632	481.579
5	1	200646	5.556	5.556	5.556	5.556	11.111	16.667	27.778	183.333
5	1	200646	5.263	5.263	10.526	10.526	10.526	10.526	21.053	181.579
5	1	200646	5.263	5.263	5.263	5.263	5.263	15.789	21.053	150.000
5	1	200646	5.263	5.263	5.263	5.263	10.526	10.526	10.526	134.211
5	1	200646	5.263	5.263	5.263	5.263	5.263	5.263	10.526	102.632
5	2	200646	4.762	4.762	14.286	14.286	52.381	57.143	57.143	521.429
5	1	200646	4.545	4.545	9.091	9.091	9.091	9.091	9.091	143.182
5	1	200646	4.545	4.545	9.091	13.636	18.182	27.273	63.636	320.455
5	2	200646	5.000	5.000	10.000	15.000	20.000	30.000	30.000	292.500
5	1	200646	5.263	5.263	5.263	5.263	5.263	5.263	5.263	94.737
13	1	200646	5.263	5.263	15.789	15.789	21.053	26.316	42.105	323.684
13	1	200646	5.263	5.263	10.526	10.526	10.526	21.053	31.579	228.947
13	1	200646	5.263	5.263	10.526	10.526	10.526	21.053	21.053	213.158
13	1	200646	5.263	5.263	5.263	5.263	10.526	15.789	42.105	197.368
13	1	200646	5.556	5.556	5.556	5.556	5.556	5.556	5.556	100.000
13	1	200646	4.762	4.762	9.524	9.524	9.524	9.524	9.524	150.000
13	1	200646	5.000	5.000	5.000	5.000	5.000	10.000	15.000	120.000
13	1	200646	4.762	4.762	4.762	4.762	4.762	4.762	4.762	85.714
13	1	200646	4.762	4.762	9.524	9.524	14.286	14.286	19.048	192.857
13	1	200646	4.762	4.762	9.524	9.524	14.286	33.333	38.095	278.571
411	1	200646	5.556	5.556	16.667	16.667	16.667	16.667	22.222	258.333
411	1	200646	5.263	5.263	15.789	15.789	21.053	21.053	21.053	276.316
411	1	200646	5.263	5.263	5.263	10.526	15.789	26.316	26.316	236.842
411	1	200646	5.263	5.263	21.053	21.053	21.053	26.316	100.000	442.105
411	1	200646	4.762	14.286	61.905	61.905	61.905	61.905	71.429	900.000

411	1	200646	4.762	4.762	14.286	14.286	57.143	76.190	76.190	621.429
411	2	200646	4.762	4.762	4.762	4.762	14.286	14.286	19.048	164.286
411	2	200646	5.000	5.000	20.000	20.000	25.000	25.000	35.000	345.000
441	1	200646	5.556	5.556	5.556	5.556	5.556	5.556	16.667	116.667
441	1	200646	5.263	5.263	10.526	10.526	10.526	15.789	31.579	213.158
441	1	200646	5.000	5.000	5.000	5.000	15.000	15.000	20.000	172.500
441	2	200646	5.000	10.000	10.000	10.000	10.000	15.000	25.000	210.000
441	2	200646	5.000	5.000	10.000	10.000	10.000	15.000	20.000	187.500
441	2	200646	4.545	4.545	9.091	9.091	9.091	13.636	22.727	177.273
441	2	200646	5.000	10.000	10.000	20.000	25.000	25.000	35.000	330.000
971	1	200646	5.000	5.000	15.000	15.000	15.000	20.000	65.000	315.000
971	1	200646	5.263	5.263	5.263	5.263	10.526	10.526	15.789	142.105
971	2	200646	5.000	5.000	5.000	10.000	15.000	20.000	25.000	210.000
971	2	200646	5.263	5.263	10.526	10.526	10.526	10.526	15.789	173.684
971	2	200646	4.545	4.545	4.545	9.091	13.636	18.182	18.182	184.091
971	2	200646	4.762	4.762	14.286	14.286	14.286	19.048	19.048	235.714
971	2	200646	4.545	4.545	9.091	13.636	13.636	13.636	13.636	190.909
971	1	200646	5.263	5.263	5.263	10.526	15.789	15.789	68.421	268.421
981	1	200646	5.263	5.263	10.526	10.526	52.632	52.632	52.632	481.579
981	1	200646	5.263	5.263	21.053	21.053	26.316	63.158	68.421	521.053
981	1	200646	5.263	5.263	15.789	21.053	26.316	73.684	94.737	576.316
981	2	200646	5.263	5.263	15.789	21.053	21.053	21.053	26.316	300.000
981	2	200646	4.762	14.286	19.048	61.905	71.429	76.190	80.952	857.143
981	2	200646	4.545	4.545	18.182	22.727	22.727	22.727	22.727	313.636
981	2	200646	4.762	4.762	9.524	14.286	14.286	19.048	19.048	221.429
981	2	200646	5.556	27.778	66.667	66.667	66.667	66.667	66.667	991.667
1011	2	200646	5.000	5.000	55.000	60.000	70.000	80.000	100.000	967.500
1011	1	200646	5.000	10.000	10.000	20.000	20.000	20.000	30.000	292.500
1011	1	200646	4.762	4.762	19.048	33.333	33.333	76.190	76.190	621.429
1011	2	200646	5.000	5.000	60.000	20.000	20.000	20.000	25.000	420.000

1011	1	200646	4.545	54.545	59.091	59.091	63.636	63.636	63.636	1002.273
1011	2	200646	4.545	13.636	22.727	22.727	27.273	27.273	31.818	395.455
1011	2	200646	4.545	13.636	13.636	18.182	63.636	63.636	81.818	647.727
1081	1	200646	4.762	57.143	66.667	71.429	80.952	100.000	100.000	1285.714
1081	2	200646	5.000	5.000	5.000	15.000	20.000	20.000	20.000	232.500
1081	2	200646	4.545	9.091	22.727	31.818	36.364	72.727	72.727	634.091
1081	1	200646	5.000	5.000	15.000	20.000	20.000	25.000	35.000	315.000
1081	1	200646	5.000	5.000	10.000	15.000	20.000	20.000	20.000	247.500
1101	1	200646	5.263	5.263	5.263	10.526	15.789	21.053	21.053	213.158
1101	1	200646	5.263	5.263	10.526	15.789	15.789	21.053	21.053	244.737
1101	2	200646	5.556	5.556	5.556	5.556	5.556	11.111	22.222	141.667
1101	2	200646	4.762	4.762	19.048	57.143	57.143	57.143	57.143	678.571
1101	2	200646	5.000	10.000	15.000	15.000	35.000	85.000	100.000	637.500
1101	2	200646	5.263	5.263	10.526	47.368	47.368	47.368	47.368	552.632
1101	2	200646	4.762	4.762	19.048	19.048	23.810	38.095	38.095	378.571
1501	1	200646	0.000	5.263	10.526	10.526	10.526	10.526	26.316	181.579
1501	1	200646	5.263	5.263	5.263	10.526	10.526	10.526	10.526	150.000
1501	1	200646	5.000	5.000	10.000	10.000	10.000	15.000	35.000	210.000
1501	1	200646	5.263	5.263	10.526	21.053	21.053	68.421	68.421	489.474
1501	2	200646	5.556	5.556	11.111	50.000	50.000	50.000	50.000	583.333
1501	1	200646	5.263	10.526	10.526	15.789	21.053	21.053	21.053	276.316
1501	1	200646	5.000	5.000	10.000	15.000	20.000	55.000	55.000	405.000
1511	1	200646	5.263	10.526	10.526	47.368	47.368	52.632	52.632	592.105
1511	1	200646	5.263	10.526	52.632	52.632	52.632	52.632	89.474	805.263
1511	1	200646	4.762	4.762	9.524	57.143	57.143	57.143	57.143	650.000
1511	2	200646	4.762	4.762	9.524	28.571	42.857	52.381	90.476	557.143
1511	2	200646	5.000	5.000	5.000	45.000	50.000	50.000	50.000	547.500
1511	2	200646	5.000	5.000	10.000	55.000	55.000	55.000	55.000	630.000
1511	1	200646	5.263	5.263	5.263	5.263	10.526	10.526	10.526	134.211
1511	1	200646	5.263	5.263	5.263	57.895	63.158	68.421	68.421	710.526

CJ9306	1	200646	4.762	4.762	4.762	4.762	4.762	4.762	4.762	85.714
CJ9306	2	200646	5.556	5.556	5.556	5.556	5.556	5.556	5.556	100.000
CJ9306	2	200646	4.762	4.762	4.762	4.762	4.762	4.762	19.048	107.143
CJ9306	2	200646	4.167	4.167	4.167	4.167	4.167	4.167	4.167	75.000
CJ9306	2	200646	4.545	4.545	4.545	4.545	4.545	4.545	4.545	81.818
CJ9306	1	200646	5.000	5.000	5.000	5.000	5.000	5.000	5.000	90.000
CJ9306	2	200646	0.000	5.000	5.000	5.000	5.000	5.000	5.000	82.500
Gamenya	2	200646	5.556	5.556	16.667	83.333	100.000	100.000	100.000	1075.000
Gamenya	1	200646	5.263	5.263	10.526	10.526	10.526	63.158	84.211	434.211
Gamenya	2	200646	5.000	5.000	5.000	15.000	25.000	50.000	60.000	397.500
Gamenya	1	200646	5.263	5.263	10.526	10.526	26.316	26.316	26.316	284.211
Zebra	2	200646	4.762	4.762	52.381	52.381	52.381	57.143	61.905	757.143
Zebra	2	200646	4.762	9.524	19.048	66.667	71.429	71.429	71.429	828.571
Zebra	1	200646	5.556	5.556	5.556	5.556	5.556	5.556	5.556	100.000
Zebra	2	200646	4.348	13.043	21.739	78.261	86.957	100.000	100.000	1056.522

Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway