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Fine-mapping of the major Fusarium head blight resistance QTL *Fhb1*

Master thesis

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Abstract

Fusarium head blight (FHB, or scab) is a severe fungal disease in small grain cereals and can lead to high yield and quality losses. The biggest concern is the health risk to humans and animals because of mycotoxin contamination of cereal food and feed. Breeding of resistant varieties and its cultivation helps to control this fungal disease. Resistance to FHB in wheat is quantitative in nature and on the wheat chromosome 3BS a Quantitative trait locus (QTL) for FHB resistance is located. This resistance QTL, named *Fhb1*, is mostly operating against spreading of the pathogen within the host (so called type-II resistance) and explains about 25% of the phenotypic variation for FHB resistance.

There is a high international interest in identifying/ cloning the actual underlying *Fhb1* gene(s). The main aim of this thesis was to narrow down the most likely region of the gene(s) to facilitate gene cloning and breeding FHB resistant wheat lines.

In a previous study over 2000 near isogenic lines (NILs), segregating for the *Fhb1* QTL in the homogenous background of the highly resistant line CM-82036, were analyzed and over 100 lines with recombinations in the putative *Fhb1* region were identified using molecular markers flanking the QTL. In the course of this master thesis these NILs with different introgressions of susceptible alleles in the putative *Fhb1* region were further genotyped with additional molecular markers and phenotyped in the greenhouse to determine the resistance level of the lines. Thus the seeds of the recombinant lines were planted in a temperature controlled greenhouse in two replications. At anthesis stage the wheat plants were point-inoculated with a conidia suspension. From each spike, two spikelets with two florets were inoculated by pipetting the conidia suspension between the palea and lemma. The spikes were sprayed with distilled water and then covered with plastic bags for 48 hours to obtain a better infection rate due to higher humidity. Twenty six days after inoculation the inoculated heads were visually evaluated by counting infected spikelets. In addition, the recombinant lines were genotyped with seven molecular markers, flanking *Fhb1*. A new SNP marker for gel-free detection was developed for the *Fhb1* region. With these markers the lines could be grouped into six haplotypes. The combined analysis of the genotypic and phenotypic data narrowed down the most likely region of *Fhb1* to a 860 kb interval with the proximal border at the marker IFA-FM958 and the distal border at the marker snp3BS-8, but also suggests an additional resistance gene between the markers IFA-FM155 and GWM493. Further investigations are needed to finally decode the *Fhb1* region and its gene(s). Increasing our knowledge about FHB resistance mechanisms in wheat to be able to breed good quality and high yielding lines is an important food security issue in a constant growing world.

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Key words: Fusarium head blight, Fusarium graminearum, Quantitative trait loci, *Fhb1*, fine mapping

Zusammenfassung

Die Ährenfusariose (*Fusarium head blight, FHB*) ist eine der bedeutendsten Pilzkrankheiten, die bei vielen wichtigen Getreidearten vorkommt, u.a. in Weizen. Ertrags- und Qualitätsverluste sind ein schwerwiegendes Problem, eine noch größere Gefahr stellen aber die bei der Krankheit gebildeten Mykotoxine dar, die sowohl bei Menschen als auch bei Tieren ein Gesundheitsrisiko darstellen können. Die Züchtung von resistenten Sorten ist eine der wichtigsten Herausforderungen, um dem Erreger entgegenzutreten.
Die Resistenz gegen Ährenfusariose in Weizen ist von quantitativer Natur. Einer der wichtigsten QTL (*Quantitative Trait Locus*) ist am Chromosom 3BS zu finden und trägt den Namen *Fhb1*. Dieser QTL verhindert vor allem die Ausbreitung des Pathogens in der Pflanze (Typ-II Resistenz) und erklärt in etwa 25% der phänotypischen Variation der FHB Resistenz. Die Identifizierung und dadurch mögliche Klonierung des *Fhb1* Gens (oder Genen) ist von internationalem Interesse.

Das Hauptziel der Masterarbeit besteht darin, die wahrscheinlichste Lage des Gens (der Gene) im Weizengenom zu kartieren und somit die Genklonierung zu ermöglichen und die Züchtung von FHB resistenten Weizensorten zu unterstützen.
Im Vorhinein wurden im Zuge einer Masterarbeit 2000 nah-isogene Linien (NILs), die mit homogenem Hintergrund der hoch-resistenten CM-82036 Linie in der *Fhb1* Region spalten, untersucht und mit Hilfe von flankierenden molekularen Markern über 100 Linien mit Rekombinationen in der vermeintlichen *Fhb1* Region identifiziert.
Im Zuge der vorliegenden Masterarbeit wurden diese NILs, mit unterschiedlichen Introgressionen von anfälligen Allelen in der mutmaßlichen *Fhb1* Region mit zusätzlichen Markern genotypisiert und im Glashaus phenotypisiert, um das Resistenzniveau der ausgewählten Linien zu bestimmen.
Dazu wurde das Saatgut der rekombinanten Linien in zwei Wiederholungen in einem temperierten Glashaus angebaut. Zum Zeitpunkt der Anthese wurden die Weizenpflanzen mit einer *F. graminearum* Konidiensuspension punkt-inokuliert. Von jeder Ähre wurden je zwei Ährchen mit zwei Blütchen ausgewählt und die Suspension zwischen Deckspelze und Vorspelze pipettiert. Danach wurden die Ähren mit destilliertem Wasser besprüht und mit einer Plastiktüte für 48 Stunden verschlossen, um für eine bessere Etablierung der Krankheit durch ausreichende Luftfeuchtigkeit zu sorgen. Sechsundzwanzig Tage nach der

Inokulation wurde der Befall der Ähren ausgewertet, indem die befallenen Ährchen der einzelnen Ähren gezählt wurden.
Zusätzlich wurden die Linien mit sieben weiteren molekularen Markern in der *Fhb1* Region genotypisiert. Neue SNP (single nucleotide polymorphism) Marker wurden für die *Fhb1* QTL Region entwickelt, darunter Marker für eine Agarosegel-freie Anwendung.
Mit den entwickelten Markern konnten die Linien in sechs Haplotypen unterteilt werden. Beim Vergleich der genotypischen und phenotypischen Daten konnte die vermeintliche Region des *Fhb1* QTLs auf ein 860 kb Intervall reduziert werden, mit dem Marker IFA-FM958 an der proximalen Grenze und dem Marker snp3BS-8 an der distalen Grenze. Weiters kann die Möglichkeit angenommen werden, dass ein weiteres Resistenzgen zwischen den Markern IFA-FM155 und GWM493 liegt.
Weitere Untersuchungen sind nötig, um die *Fhb1* Region und ihr(e) Gen(e) endgültig zu entschlüsseln. Es ist nach wie vor von großer Bedeutung, das Wissen über FHB Resistenz Mechanismen in Weizen zu erforschen, um hoch qualitative und ertragreiche Linien zu züchten, die zur Nahrungsversorgung einer ständig wachsenden Weltbevölkerung beitragen.

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Schlüsselwörter: Ährenfusariose, Fusarium graminearum, Quantitative Trait Loci, *Fhb1*, Feinkartierung

List of abbreviations

APS – ammonium persulfate

BAC – bacterial artificial chromosome

BC5Fn – Backcross five filial generation n

bp – base pairs

CIMMYT – International Maize and Wheat Improvement Center

cM – centimorgan

DH – double haploid

dh2O – distilled water

DMSO – dimethyl sulfoxide

EDTA – ethylenediaminetetraacetic acid

FAM – 6-carboxy-fluorescine

GADPH - Glycerinaldehyd-3-phosphat-Dehydrogenase

Gbp – giga base pairs

HEX – hexachlorocyclopentadiene

KASP – Kompetitive Allel Specific PCR (polymerase chain reaction)

kb – kilo base pairs

MAS – marker-assisted selection

MgCl2 – magnesium chloride

mL – millilitre

NaOAc – sodium acetate

NH4OAc – ammonium acetate

NILs – near-isogenic lines

nm – nanometer

PAA – polyacrylamide

PCR – polymerase chain reaction

QTL – quantitative trait loci

rcf – relative centrifugal force

RFU – relative fluorescence unit

ROX - 5-carboxy-X-rhodamine, succinimidyl ester

TAE buffer – Tris-Acetat-EDTA buffer

TEF - Thyrotroph embryonic factor

TEMED – tetramethylethylenediamine

Ubi - Ubiquition

USD – United States Dollar µL – microliter

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1. Introduction

1.1. Background

Bread wheat (*Triticum aestivum* L.) is the global leading source of vegetable protein in human food and mainly grown in temperate and sub tropical regions of the world on about 218 million hectares, with annual production of more than 713 million metric tons in 2013 (FAO, 2015). Due to the maintenance of a continuous wheat production for global food supply, crop improvement, regarding yield as well as resistance to pathogens, is of major importance. Global wheat production should increase by 2% annually until 2020 (Reynolds, Borlaug, 2006) and should reach around 840 million tonnes of wheat by 2050 (Sharma I. et al., 2015) in order to meet future food demand of the growing population of the world. Productivity of wheat is at risk due to the incidence of pests, especially weeds, pathogens and animal pests and the total global potential loss due to pests varied from about 50% in wheat production (Oerke, 2006). Furthermore the production and productivity of wheat is highly constrained by reduced water availability, a scenario of global warming, stricter end-use quality characteristics, and evolving pathogen and pest populations associated with climate change (Trethowan, Mujeeb-Kazi, 2008).

On the one hand better agronomic and soil management practices are equally important to increase the desired wheat production (Reynolds, Borlaug, 2006; Trethowan, Mujeeb-Kazi, 2008). Contrariwise breeding wheat cultivars with high grain yielding, enhanced water use efficiency, drought and heat tolerance, improving end-use quality, and durable resistance to important diseases and pests have to contribute to food security.

Fusarium head blight (FHB), also referred as scab, is a severe fungal disease affecting wheat and barley worldwide and is mainly caused by the plant pathogen fungi *F. graminearum* (Parry et al., 1995). The disease causes negative effects on field quantity and quality, but the most-serious concern is mycotoxin contamination of the crop, making it a hazard to both human and animals by the consumption of contaminated food and feed.

The implementation of research and development activities is important to develop strategies to prevent the loss of wheat due to FHB, such as crop rotation, fungicide application and development of resistant cultivars. Therefore, the improvement of

resistant wheat varieties without harming other agronomically important crop traits, is a main aim of several plant breeders and scientists, either by conventional breeding or transgenic approaches.

1.2. Wheat – genomic characteristics

Bread wheat (*Triticum aestivum; Poaceae*) continues to be one of the most important food sources for human consumption (FAO, 2015). It provides nearly 20% of the world's daily food supply measured by calorie intake and the yield of wheat has doubled over the last 40 decades because of improved agronomic practice and advanced germplasm through selective breeding (Berkman et al., 2012).

Multiple polyploidization events occured when wheat was domesticated about 10,000 years ago between several species of the Triticum and Aegilops genera, leading to diversification during the evolution of Triticum species. The genome evolution of wheat also generated a change in agronomic traits, adaptation and productivity over their ancestors and shows a positive correlation between ploidy and success as a crop. (Dubcovsky and Dvorak, 2007; Matsuoka, 2011).

Two sequential allopolyploidization events involved the origin of allohexaploid bread wheat (2n=6X=42, AABBDD genome), beginning with allotetraploidization between two diploid species, represented by modern *Triticum urartu* (genome AA) and a yet unknown or extinct goatgrass, but which may be the section of Sitopsis (belonging to *Aegilops speltoides*), gave rise to the allotetraploid wheat *T. dicoccum* (AABB; Dvořák, 1976; Huang et al., 2002; Dvorak and Akhunov, 2005).

Second, a spontaneous hybridization event between, *T. dicoccum* with the wild wheat species *Aegilops tauschii* Coss (DD genome) led to the formation of the wild progenitor of bread wheat (Feldman et al., 1995; Salamini et al., 2002). With a size of 17 Gbp (80% of the genome are repetitive elements) the three wheat genomes are large and complex in comparison to many other cereal crops. This complexibility causes major difficulties to determine the genetic basis of phenotypic traits, making it a big challenge for wheat genome assembly because of length limited DNA sequencing technologies that are not able to span the long repetitive wheat genome (Dubcovsky, Dvorak, 2007; Matsuoka, 2011; Berkman et al., 2012; Brenchley et al., 2012).

1.3. Fusarium Head Blight (FHB)

1.3.1. The pathogen

Fusarium spp. are filamentous and septate fungi that belong to phylum Ascomycota and genus Gibberella. Fusarium head blight is mainly caused by the plant pathogen fungi *F. graminearum* and *F. culmorum*, while *F. graminearum* is the predominant causal agent of FHB in most areas of the world (Walter et al., 2010; Windels, 1999; Yang et al., 2003; Goswami, Kistler, 2004, Osborne, Stein, 2007; Leplat et al., 2013).

The fungi are distributed on plants and soil. It has strictly asexual life cycle (*F. graminearum*) or homothallic sexual (*Gibberella zeae*) and asexual life cycle that occur together (Goswami, Kistler, 2004). However *F. graminearum* is the most predominant causal agent of FHB in most areas of the world, mainly in temperate and sub tropical environments (Yang et al., 2003; Goswami, Kistler, 2004, Osborne, Stein, 2007; Leplat et al., 2013). FHB was first discovered in England in the year 1884. Because of the chalky, lifeless appearance of the infected kernels, it was called wheat scab, and later tombstone disease (Clear, Patrick, 2010; Parry et al., 1995).

1.3.2. Distribution and impact

The prevalence of FHB increases in almost every cereal-growing region and is strongly influenced by local and regional climate and environment, physiological status of hosts, genetic make-up and pathogen related factors including adaptation and virulence (Walter et al., 2010; McMullen et al., 1997; Osborne, Stein, 2007). So FHB has become a worldwide problem with economic importance (Windels, 1999). FHB is able to completely devastate a potentially high-yielding crop within a few weeks before harvest and causes about 10% - 70% yield loss during epidemic years (Matthies et al., 2000). In Canada, FHB epidemics occurred about once every 9 years from 1927 to 1980 (Bai, Shaner, 1994). In China, FHB has been found with more than 7 million hectares of wheat-growing areas affected and yield losses of more than 1 million tons in epidemic years (Wang et al., 1982). In the U.S. devastating FHB outbreaks during the 1990s resulted in a total economic loss of about USD $3 billion (Windels, 2000). Scientists presume that the impact of the disease may even increase further because of indirect effects of climate change (West et al., 2012).

1.3.3. Infection and life cycle

F. graminearum spores, returning to the soil from infected kernels and head debris, are pervasive in the soil and overwinter as saprophytic mycelia on infected crop residues of previous years (straws of wheat, corn stalk, and other host plants). The mycelium on crop residues allows the production of both macroconidia (asexual spores) and mostly ascospores (sexual spores produced in perithecia), which constitutes the primary inoculum that causes primary infection of wheat heads (Osborne, Stein, 2007; Leplat et al., 2013). The spores can be carried by wind or water onto the heads of flowering crops (McMullen et al., 1997) and the pathogen penetrates the floret actively or is carried through natural openings (Walter et al., 2010), often infecting sterile spikelets (Kelly, 2013). Frequent rainfalls, prolonged moist or heavy dews during the flowering (anthesis) and the kernel-fill period (soft dough stage) of the crop increases sporulation of the fungus and cause high infection rates (Windels, 1999). The spores may land on the exposed anthers of the flower and then grow into the kernels, glumes, or other head parts (Goswami, Kistler, 2004; Kugler et al., 2013; McMullen et al., 1997, 2012).

1.3.4. Symptoms and negative effects

The symptoms caused by FHB are generally similar in all small grain cereal crops and appear about 15 to 20 days after flowering (Kelly, 2013). In case of wheat, the tissue of single spikelets or whole heads get blighted and appear bleached and tan (Kelly, 2013; McMullen et al., 1997) The surface of florets and glumes can form brown, dark purple to black necrotic lesions and the peduncles below the inflorescence may become discoloured brown or purple immediately. The symptoms will extend to the entire inoculated spike after a few days depending on the type of resistance mechanism going both up and down direction of the spike. The disease can reduce kernel set and grains appear discoloured and shrivelled (tombstone kernels), wherefore the seed weight is decreased and grain yield loss is the consequence. Quality is reduced due to endosperm storage protein losses (baking quality is affected). Also, the awns often become deformed or twisted (Buerstmayr et al., 2009; Goswami, Kistler, 2004; Parry et al., 1995; Windels, 1999).

But the major problem related to *F. graminearum* infected crop is the contamination with toxic fungal secondary metabolites known as mycotoxins (Buerstmayr et al., 2009). Different kind of mycotoxins can be produced, such as deoxynivalenol (DON) nivalenol

(NIV), zearalenone (ZEA) and moniliformin (MON), all of which have a range of toxicity to humans and animals, inhibiting protein synthesis, counteracting defense activities and inducing apoptosis (Terzi et al., 2013; Desjardins, 2006; Leslie, Summerell, 2006; Rotter et al., 1996). The most abundant toxin produced by *Fusarium graminearum* associated with FHB in wheat is the type B trichothecene DON, also called vomitoxin (Pestka, 2010; Snijders, 1990; Windels, 1999). Vomiting and feed refusal can be causes of contaminated feed in animals, especially nonruminant animals, as well as absence of appetite, growth retardation, immunotoxicity and impaired reproduction and development resulting from maternal toxicity. Higher levels of DON also can harm humans, demonstrating symptoms of nausea, fever, headaches, and vomiting when flour made from contaminated wheat is consumed (Schmale et al., 2003). That is why norms were established regulating the maximum DON levels in food for human consumption (McMullen et al., 1997; Snijders, 1990). The European Commission has proposed limits of 750µg/kg (750 ppb) in cereals and 500µg/kg in cereal-based products such as flour (Champeil et al., 2004).

1.4. Control of FHB

1.4.1. Agricultural practices and chemical control

A lot of studies and development activities have been implemented to develop strategies to prevent the loss of wheat due to FHB. Agricultural practices play a very important role and different approaches can be found to minimize infection levels by crop residues and soil, although long distance spread of inocula and extended period of life cycle under suitable conditions can hinder these practices a lot. A main agronomy based method to be mentioned is crop rotation. Potential hosts of FHB as a preceding crop and the frequency of rotation have to be considered, leading to higher frequency of FHB the shorter the rotation of the susceptible crop gets (Champeil et al., 2004; McMullen et al., 1997). Tillage and burning excess residues also help to reduce accumulation of potential infection sources on the fields (Dill-Macky, Jones, 2000).

Fungicide application technology can contribute mainly to sustain high yield and grain weight increase, but no product is found to ensure a full extend protection yet. The most promising and widely used fungicides to date are Triazole fungicides (tebuconazole,

metaconazole, or prothioconazole), on their own and in combination (Homdork et al., 2000; Mesterhazy et al., 1996; Paul et al., 2010; Terzi et al., 2013). Fungicide application for FHB is most challenging because of the fact that it depends mostly on perfect timing during flowering period. Humid conditions, uneven fields, low spray volume and fast ground speeds in addition are making the application a risky and costly procedure. In addition agrochemical applications mean serious problems for environmental concerns, soil health and development of pest resistance. Though experiences with biological control approaches have to deal with quite the same difficulties as mentioned before (McMullen et al., 2012).

1.4.2. Breeding for resistance against FHB

The most important method for FHB control can be found in cultivation and breeding of resistant varieties, which is an economical, environmental friendly and more or less easy approach for FHB control. As mentioned, the wheat genome is quite big and thankfully harbours a large genetic variation. Despite of this advantage, genotypes differ in their susceptibility (Pugh et al., 1933) and regionally adapted and highly productive cultivars are often susceptible to FHB. So the main aim for plant breeders is to combine resistance and high agronomical performance by creating new wheat varieties. The greatest challenge for breeding new resistant varieties lies in the long effort that is needed to produce such plants. Typically, it takes up to ten years from when a cross is first made until release (Anderson, 2007; Buerstmayr et al., 2002, 2009; Ruckenbauer et al., 2001).
Finding new resistance sources was the main objective when FHB resistance breeding started and shifted to incorporate resistance genes into adapted cultivars (McMullen et al., 2012). Measurably input has been made in conventional selection, through repeated testing of wheat lines under induced and natural epidemic conditions (Buerstmayr et al., 2009). The prominent sources of resistance, mostly lacking agronomic traits needed in modern wheat varieties, are spring wheat genotypes from Asia (e.g. Chinese 'Sumai-3' and 'Wangshuibai', Korean 'Chokwang' and Japanese 'Nobeokabouza'), Latin America (e.g. 'Frontana'), USA and European winter wheat germplasm and related species of wheat. So far the Chinese Sumai-3 is the best known source of resistance and found to be more heritable, stable and consistent across environments than other sources (Liu et al., 2013; Buerstmayr et al., 1999, Rudd et al., 2001, Kollers et al., 2013).

1.5. Resistance mechanisms in wheat

On principle, several resistance types can be subdivided. First of all, this is the case for morphological or physiological types (Rudd et al., 2001). Morphological traits, usually playing minor role in disease manifestation and acting as passive resistance mechanism (Rudd et al., 2001), can be plant height or head forms. Tall types of wheat are less susceptible to FHB infection than dwarf cultivars (Mesterhazy, 1995). This can be explained due to the fact that inoculum from crop debris takes a larger distance to the heads in taller plants than in dwarf ones and can be tested with artificially spray-inoculated plants that often do not differ in their susceptibility related to their height. (Buerstmayr et al., 2009; Couture, 1982; Hilton et al., 1999; Ransom, 2008). Plant height has also been associated with type I resistance (resistance against initial infection, explained in 1.5.1.; Lu et al., 2013). Awned and compact spike genotypes are more susceptible than awnless genotypes with a lax spike. Flowering date (Gilsinger et al., 2005; Kubo et al., 2010; Schuster, Ellner, 2008), waxy surface, anther extrusion and narrow flower openings also influence the resistance to FHB (Kollers et al., 2013). The amount of anther extrusion might be negatively correlated with the incidence and severity of FHB (Kubo et al., 2013; Skinnes et al., 2010; Skinnes et al., 2008) and narrow flower opening is also shown to have an impact on lower levels of FHB (Gilsinger et al., 2005; Kubo et al., 2010; Schuster, Ellner, 2008).

1.5.1. Resistance types

The most important physiological and active resistance types, as described by Schroeder, Christensen (1963), are type I and type II and were extended by Mesterhazy (1995) and Wang and Miller (1988), adding four more resistance types :

I. Resistance against initial infection (Schroeder, Christensen, 1963; Buerstmayr et al., 2012; Mesterházy, 2003a; Bai, Shaner, 1994; Mesterházy et al., 1999; Stack, 2003).
II. Resistance against spread of the pathogen within the head (Schroeder and Christensen, 1963; Buerstmayr et al., 2012; Mesterházy, 2003a; Bai, Shaner 1994; Mesterházy et al., 1999; Stack, 2003).

III. Resistance to kernel infections (Mesterházy, 1995).
IV. Ability to degrade or detoxify deoxynivalenol (Boutigny et al., 2008; Lemmens et al., 2005; Miller, Arnison, 1986).
V. Limited accumulation of trichothecenes in infected kernels (Boutigny et al., 2008; Miller et al., 1985; Snijders, Krechting, 1992).
VI. Tolerance of high trichothecene levels in kernels (Wang, Miller, 1988).

The complexity of plant resistance to FHB complicates varietal resistance studies (Champeil et al., 2004) and early infection events are still little understood (Steiner et al., 2009). Different genotypes can react against initial infection (type I resistance), meaning the penetration of the pathogen is inhibited. This resistance arises both from morphological (e.g. plant height, anther extrusion) and physiological traits and remains largely exclusive, because of challenging screening and due to the fact that type I resistance is hindered by any differences in type II resistance (Gosman et al., 2010; McMullen et al., 2012; Steiner et al., 2004). Most breeding programs are focusing on type II resistance (McMullen et al., 2012), which intervenes after the pathogen has already entered the floret and prevents *F. graminearum* to spread within the head (Walter et al., 2010). It can be measured by observing symptoms due to disease spread after some type of point inoculation as done in the thesis at hand (Rudd et al., 2001). A numerous used source of type II resistance in bread wheat is 'Sumai-3' (Osborne, Stein, 2007). Food safety concerns associated with mycotoxins in cereal grains (especially DON) led to a growing interest in resistance traits. Worldwide breeding programs focused on FHB resistance, mostly for type II resistance (McMullen et al., 2012).

1.5.2. Resistance genetics

Resistance to FHB is quantitatively inherited and of oligo- to polygenic nature (Bai, Shaner, 1994; Buerstmayr et al., 2012; Mesterházy, 2003a; Ruckenbauer et al., 2001; Snijders, 1990c). The resistance can be classified as horizontal type across probably all FHB pathogens, because no specific resistances have been found against distinct Fusarium species (Mesterházy, 2003a; Mesterházy et al., 2005; Miedaner, 1997; Miedaner and Schilling, 1996; Snijders, 1990a; Stack and McMullen, 1985; Tóth et al., 2008; van Eeuwijk et al., 1995). At the short arm of chromosome 3BS and 5AS, the two most important QTLs

(quantitative trait locus/loci) can be found. The *Qfhs.ifa-5A* QTL at the 5AS chromosome seems to influence type I resistance (Buerstmayr et al., 2009), while *Fhb1* QTL (former designation *Qfhs.ndsu-3BS*) is found on the 3BS and is primarily associated with type II resistance and used for the study at hand.

1.6. QTL mapping studies and marker-assisted breeding (MAS)

The accurate method to examine resistance to FHB is a QTL mapping approach, involving genomic regions in resistance against FHB (Buerstmayr et al., 2009). In wheat several QTLs have been found on almost all chromosomes, at least in wheat (Anderson et al., 2007; Buerstmayr et al., 2012; Buerstmayr et al., 2009; Buerstmayr et al., 1999; Grausgruber et al., 1999; Liu et al., 2009; Löffler et al., 2009). QTLs are unknown loci of genes on chromosomes, which govern a certain trait (Jansen, 1996). Those loci can be identified via statistical procedures that integrate genotypic and phenotypic data (Miedaner, Korzun, 2012). In plant breeding QTL analysis is typically performed in two steps: firstly QTLs have to be discovered. This can be done by hybridizing parental lines, which differ for one or more quantitative traits, and a segregating population is made. To identify QTL regions, molecular markers are used. Secondly identified QTL map locations are used to breed resistant plant varieties (Anderson et al., 2007; Anderson et al., 2001; Buerstmayr et al., 2009; Buerstmayr et al., 2002; Buerstmayr et al., 2003; Liu et al., 2009; Löffler et al., 2009; Tanksley, Nelson, 1996). Two types of QTL are used for this purpose. QTL from exotic resistance sources and QTL from locally adapted sources (Buerstmayr et al., 2012; Holzapfel et al., 2008; McMullen et al., 2012). For providing resistance stability the stacking or pyramiding of resistance QTL is highly recommended (Miedaner et al., 2001; Miedaner et al., 2006; Colin et al., 2010).

1.6.1. Molecular Markers

Molecular markers are specific molecules of DNA with a particular location on the chromosome that can be identified within the whole genome and give information about neutral sites of variation (Miedaner, Korzun, 2012). A DNA marker closely linked to a disease resistance locus can be used to predict whether a plant is likely to be resistant to that disease. There are basic requirements for the use of molecular markers: reliability,

quality and quantity of DNA, technical procedure of marker assay, level of polymorphism and cost of marker assay (Collard, Mackill, 2008). Co-dominant markers are in favour because they are able to distinguish between homo- and heterozygote genotypes (Ben-Ari, Lavi, 2012). Perfect markers are markers without recombination to the gene of interest (in an optimal way drawn directly from the gene sequence). The availability of such a marker is the most critical point for successful marker-assisted selection (Miedaner, Korzun, 2012). Molecular markers are helpful tools in plant breeding approaches since the 1980´s to reveal certain characteristics about the respective source. Detecting the presence of different alleles, mapping genes, QTL and confirming the identity between parents, progeny and evolutionary relationships is a very important possibility to assist the breeders increasing FHB resistance (Bagge et al., 2007). There are various types of markers detecting variation at the DNA level such as nucleotide changes (deletion, duplication, inversion and/ or insertion). In the master thesis at hand differences were detected by a single base-pair change in the sequence (single nucleotide polymorphism, SNP), and by repeated or long units of a sequence (microsatellite or simple sequence repeats; SSR).

SSR markers are highly reliable, co-dominant in inheritance, relatively simple and cheap to use and generally highly polymorphic. SSRs are short (< 13 bp) repeating DNA sequences and different number of repeats can be found within a population (Watson et al., 2011) as well as within the alleles of a single separated according to different sizes (Davidson, 2013). The only disadvantage of SSRs is that typically polyacrylamide gel electrophoresis is required for SSRs. Information of only a single locus per assay can be achieved (Ben-Ari, Lavi, 2012; Collard, Mackill, 2008).

SNPs are co-dominant single locus markers and occur when a single nucleotide (A, T, C, or G) in the genome or other shared sequence differs between members of a species or between paired chromosomes in an individual. SNPs normally have a low level of polymorphism and most important is the fact that they are highly abundant and high-throughput technologies are available (Ben-Ari, Lavi, 2012).

An approach for detection of SNPs is **Kompetitive Allele Specific PCR (KASP™)** and was also used for the master thesis at hand. KASP™ initially developed by KBioscience and a

proprietary genotyping technology from LGC genomics (Middlesex, UK; www.lgcgenomics.com) has evolved to be a global benchmark technology (Semagn K. et al., 2014; Rasheed A., et al., 2015). The system uses a combination of two molecular techniques: allele- specific polymerase chain reaction (PCR) and fluorescence resonance energy transfer (FRET): It is a homogenous and fluorescence endpoint genotyping technology that enables accurate bi-allelic discrimination of known SNPs (and also insertions and deletions – InDels) at specific loci via PCR and universal fluorescent reporting system based on fluorescence resonance energy transfer (FRET) for signal generation (Kumpatla et al. 2012), eliminating the need for dual-labelled probes. The KASP™ genotyping technology is available as both a product (non-validated and validated reagent kits) and a genotyping service through LGC Genomics service labs in North America and Europe. KASP™ manages low error rates, low cost and high efficiency per reaction (Cortés et al., 2011; Rosso et al., 2011; Semagn et al., 2014). That is why KASP™ markers have been intensly used at the International Maize and Wheat Improvement Center (CIMMYT) amongst others for crop improvement purposes, quality control analysis, QTL mapping, and recurrent selection (Semagn et al., 2014). KASP™ technology is optimal for plant breeding applications when it comes to automation analyzing hundreds of samples. KASP™ markers have been widely used for MAS and were prosperous especially in soybean (Rosso et al., 2011), faba bean (Cottage et al., 2012), oat (Gnanesh et al., 2013), and wheat (Liu et al., 2014).

For KASP™ genotyping technology the follwing components are needed:

- Sample DNA
- The KASP™ universal Master Mix 2xReaction V.4.0 containing:

    - universal FRET cassettes (FAM™ and HEX™ dye specific)
    - ROX™ passive reference dye, a 5-carboxy-X-rhodamine, succinimidyl ester (ROX)
    - special Taq polymerase
    - free nucleotides
    - MgCl2 (50 mM; for particularly A/T-rich DNA regions)
    - optimised buffer solution DMSO (for particularly G/C-rich DNA regions)

- The KASP™ Assay Mix (Primer Mix) containing:

    - two allele-specific forward primers (one for each SNP allele) with the complementary SNP allele at its 3' end and each with a unique unlabelled tail sequence at the 5' complementary to one of the fluorescent-dyed oligonucleotides
    - one common (reverse) primer

The KASP™ Assay Mix can be purchased in two formats:

- as KASP™ by Design (KBD) consisting of three custom-designed KASP™ primers for SNP detection. Assay design (primer selection) is performed using the LGC proprietary Kraken™ software system (validated in-silico and not functionally validated prior to shipment). Two allele-specific forward primers and the common reverse primer are combined to create the SNP-specific custom KASP™ assay.
- as KASP™ on Demand (KOD) including design and full validation and optimisation of assays by LGC laboratory scientists prior to delivery. Assay design (primer selection) is performed either using LGC proprietary Kraken™ software, or manually by LGC scientists. Two allele-specific forward primers and the common reverse primer are combined to create the SNP-specific custom assay. The assay aliquot is then validated in a LGC laboratory using LGC validation panel (human assays only) or customer DNA samples.

KASP™ methodology:

In the first round of thermal cycling, the relevant allele-specific forward primer matches to the target DNA region and the polymerase lays down the rest of the complementary nucleotides and elongates the strand attaching the tail sequence. During this time, also the common reverse primer amplifies the other part of the DNA strand. In proximate PCR rounds the complement of the allele-specific tail sequence is then generated when the DNA strand from the common reverse primer binds to the strand with the specific forward primer and its tail sequence. This enables the complementary FRET cassette to bind specifically to the DNA. Depending on the SNP, either the FAM™- or the HEX™-

fluorescently labeled oligo binds and therefor the FRET cassette is no longer quenched and is able to emit a fluorescence signal. This procedure occurs multiple times throughout PCR continues and the competitive binding and specific allele discrimination ensures that the fluorescent signalling becomes stronger in the amplification process.
With a FRET-capable plate reader the fluorophores FAM™ and HEX™ can be detected and distinguished measuring RFU. The quantitative PCR instrument can record the fluorescence readings immediately after the reaction is complete. Due to differences in well-to-well liquid volume the passive reference dye ROX is used to normalise variations of detected signals for each dye for result interpretation. Relevant excitation and emission wavelengths are shown in Table 1.

Tabelle 1: Excitation and Emission values for the fluors used in KASP™

Fluorophore	Excitation (nm)	Emission (nm)
FAM	485	520
HEX	535	556
ROX	575	610

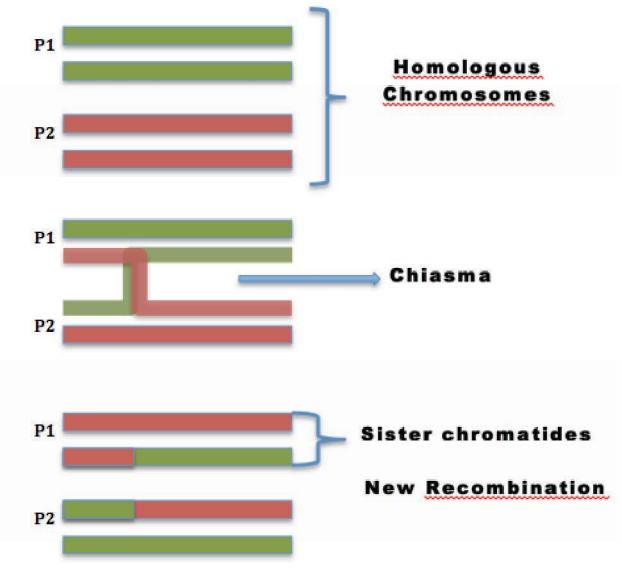
With a software the data results can be plotted on a cartesian coordinate system to be able to determine genotypes according to sample clusters. A genotype at a given SNP is homozygous when only one of the two feasible fluorescent signals will be generated. Heterozygous genotypes generate a mixed or equal fluorescence signal from each dye. This is why those genotypes can be observed roughly in between the other two homozygous groups. To be able to distinguish the clusters it is important to always add known control lines and also NTC (non template controls) to each KASP™ PCR.

In general KASP™ uses a set of thermal cycling conditions comprised of two temperature steps, rather than the more traditional three steps. Protocols created for KASP™ normally denature DNA at a higher temperature and anneal and extend at the more or less same (lower) temperature. Also touchdown PCR protocols can be run with the elongation and annealing steps incorporated into a single step. The high annealing stage temperature determines the specificity of the reaction and consquently the capability of the primers to anneal to the DNA template. Only very specific base pairing will occur between the DNA

□y□□i □favt a□dvli□i□l□vni□l t f a□lRa□ubaulsSl□dlv□i□□uuRa u□li □□t fsvUv□□lvoy□oU□li □ u NR y□ □oU□yl a ulM□ i vs□y□a t yl□ssS□□ □a□uvyn□li □□yy□swyn□l t f a□lRa□Uba□□□i □ □□□□□S□s□lo□y□a□u□Uv□v y□S□oU□i□a□□lvoyl□yoyJuf□vUv□□□t fsvUv□□lvoyu□li□l□o□□Ra□□l□ li□sod a□l t f a□lRa u□dvss□□□oRl□ot f l□□□S□a nvoyu□□t fsvUv□□□Rawyn□li□i vnisS□ uf□vUv□□□asS□loR□i□od y□□S□s uM0□oa□URali a□□□l□vsu□□□oRl□li□□□□□) □n yolSfwyn□ l□i yosonS□Ru□li□□□□□□n yolSfwyn□□i t vulaS□□u a□nRv□□□y□□t□yR□s□lo□□□UoRy□□□l□ d d d M□n yot v□uM□ot M□

5kTlRk□□o□ce□□eoy□□□□w□dS□□o□□□□□w□ddeo□□

□□□□□ l□lvoy□□□y□□□□□i v c□□tv□□n y lv□□t□fu□li□l□□a□Ll yuvc sS□Rlvsv,□□□u□□□□la□l nS□ lo□v□ ylvUS□n yot v□□a nvoyu□wy□dli□l M□□i ou□□□y□MM□□□uoRa□u□oU□□wu□u□a uwul□y□□ba□ oli a□n y lv□□ssS□□ot f s L□la□vluM□□n y lv□□t□f□wut□□□Rf□□S□li□□aa□yn t yl□oU□□□haoRf□ oU□t□ar aul□□dlv□i□la□□r□li□wyi avl□y□□oU□□□y□a□S□n y□li□l□i□u□ba□i□u□yol□S l□□ y□ v□ ylvUv□□□Rl□dl ou□□ffaoLvt□l□so□□lvoy□vu□ryod yM□□ot osonoRu□a□ot□vy□lvoy□c ylu□ n y a□l□□□S□□aouuwyn□□c au□0□□1□□Rawyn□t vouvuf s□S□□yl□t foal□yl□aos□wy□□□□□□ l□lvoy□ □y□□□a□wy cvl□□s□lo□□a□l□n y lv□□t□fuM□□aouuwyn□□c au□0□□iv□ut□□oU□□iaot□lv□ ul□□uu□u y□ wy□□vnRa□q1□n y a□ssS□o□□Ra□a□y□ot sS□oy□□iaot ouot u□0□vR□□qÄÄ41M□□□a yl□sa□la□wyu□dlvli□ □vUU a y□u□□ ld y□li t□wy□li□□ss s u□li□l□□UU□l□c□av□lvoy□wy□□□la□vl□y□□yol□□□□vUU a ylM□ □ y lv□□t□fu□□ssod□lo□□□□vulwynRwui□li□ld o□u□la□wyu□n y lv□□ssS□0□□Rav□vo□□□M□t55q1M□□



□e□h: □□5a□□□K□w□ce□□□e□□: □w□r□□□: rSS□rl□: □F□Ke□Sw□H□□□eo□□□r□o□ü□□□rw□eo□□cero□r□□□K: rwrSrw□S□□h: eo□w□erSeSi□□□wroSc: □ceo□□□K: rwrSrw□S□r□□d□: □ocr□o□□eo□: □□o□□o□□d□: □ocr□ür□eo□□□□F□e□h: □□□t□□□□□h oH□

This information provides a number of possibilities to study evolutionary relationships and genetic distance and to build physical and genetic maps for localization of genes/QTLs or genomic regions accountably for trait of interests (Dekkers,Hospital, 2002; Collard, Mackill, 2008). In genetic maps the distance between two genes shows how often CO events occur between them. The unit of measure for this distance is Centimorgan (cM). One cM means, that the likelihood for separation by a Crossing Over of two neighbouring genes is 1 %, which means that fewer recombination events between two genes equal to a closer position of the gene loci. On the other hand physical maps show real distances usually in Kilo base pair (Kbp) as measuring unit (Alberts et al., 2004). A method to create a physical map is e.g. based on deletions, especially for organisms with large genome sizes, such as wheat (Sourdille et al., 2004). It is important to say that there is no specific relationship between the recombination distance and the physical distance because the rate of recombination varies along the length of the chromosome (Kumar, 1999). Higher condensed chromation called heterochromatin can be found in regions like the centromere and telomere and usually shows less recombination rates then on other locations of the chromosome (Choo, 1998; Xue et al., 2011). At all times when a marker is tightly linked to the target gene the genotypic importance is increased due to this association. Association of marker and QTL alleles can be tested statistically and the likelihood for the presence of a putative QTL can be plotted at the marker positions along the chromosome (Jansen, 1996).

1.6.3. Marker Assisted Selection (MAS)

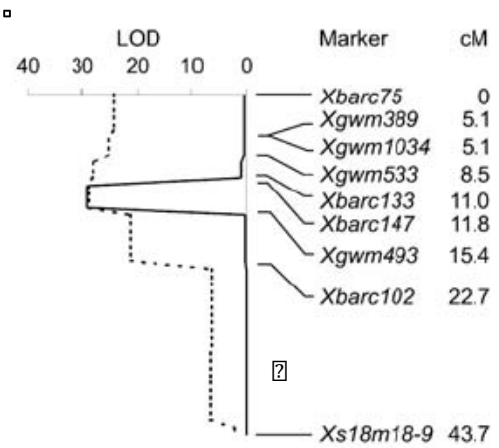
Marker-assisted selection (MAS) is a technique that uses genetic variation at the DNA level to track and monitor specific regions of the genomes during selection and crossing. Specific genotypes (normally a particular DNA) are used that associate with the desirable traits. E.g. a marker closely linked to a disease resistance locus can be used to predict whether a genotype is likely to be resistant or susceptible to that disease. MAS provides opportunities for enhancing the response from selection because molecular markers can be applied at the seedling stage with high precision and reductions in costs (Farokhzadeh et al., 2014). It enables indirect selection method and not only reduces the costs but more importantly the time and difficulties in phenotyping. It is also useful in pyramiding or stacking of desirable genes by backcrossing results such as disease resistance (Dekkers, Hospital, 2002; Collard, Mackill, 2008; Tester and Langridge, 2010; Von der Ohe et al.,

2010; Miedaner et al., 2011; Miedaner, Korzun, 2012). One drawback of MAS is that the use of perfect markers is limited by the restricted availability of genes that control agronomic characters. Due to the fact that different subsets of QTL are polymorphic in each population, QTL mapping can only be done for each individual mapping population. Even among closely related genotypes the linkage phases between the marker and QTL alleles can differ (Bagge et al., 2007) and because of this reason, QTL detection and MAS are often carried out independently (Lv et al., 2014). MAS is ineffective when many genes of small effects are segregating and therefore the technique is mainly used on major genes. The genome-wide prediction approach termed genomic selection could complement MAS for polygenic traits and strongly enhance breeding efficiency by increasing the accuracy of breeding value estimates (Muranty et al, 2015).

1.6.4. QTL mapping studies for FHB resistance in wheat

QTL mapping can be seen as one of the most important approaches to discover complex traits in plants in general. Several analysis for FHB resistance in cereal crops have been done so far (Anderson et al., 2001; Buerstmayr et al., 2002, 2003; Otto et al; 2002; Shen et al., 2003; Somers et al., 2003; Hartel et al., 2004; Paillard et al., 2004; Gilsinger et al., 2005; Mardi et al., 2005; Liu et al., 2006; Steiner et al., 2009; Miedaner et al, 2006; Buerstmayr et al., 2012). Independent studies revealed over 100 QTLs but only 22 QTLs were detected in more than one mapping population, indicating real QTL effects. These QTL are on following chromosomes: 1B (two regions), 1D, 2A (2), 2B (2), 2D (2), 3A, 3BS (2), 3D, 4B, 4D, 5A, 5B, 6A, 6B, 7A and 7B (2). The B genome revealed the most repeatable QTL distribution across the wheat genome with 11 QTLs, followed by A genome with 5 and D genome with 4 QTL (Buerstmayr et al., 2009), among 3BS (namely *Fhb1*) and 5A do have the highest breeding purposes (Anderson, 2007).

A major FHB resistance QTL was detected by Waldron et al. (1999) on chromosome 3BS, originally called *Qfhs.ndsu-3BS* and was later renamed *Fhb1* by Liu et al. (2006). Anderson et al. (2001) confirmed this QTL just as well as Buerstmayr et al. (2002) using large double haploid (DH) population resulting from a cross between CM-82036 (a highly resistant CIMMYT line derived from the cross Sumai-3 x Thornbird) and Remus. A lot of other studies revealed that *Fhb1* is the best validated gene for FHB resistance (Anderson, 2007;


LOD
Marker
cM
40
30
20
10
0
Xbarc75 0
Xgwm389 5.1
Xgwm1034 5.1
Xgwm533 8.5
Xbarc133 11.0
Xbarc147 11.8
Xgwm493 15.4
Xbarc102 22.7
Xs18m18-9 43.7

Cuthbert et al. (2006) could successfully fine map *Fhb1* on chromosome 3BS within a 6.5 cM interval. A nearly diagnostic marker could be created, named XUMN10 (Liu et al., 2008). Also Bernardo et al. (2012) proposed two new SNP markers for fine mapping, named xsnp11 (for Chinese and Japanese accessions carrying *Fhb1*) and snp3BS-8 (for Sumai-3-related accessions with *Fhb1*).

1.8. Aims of the master thesis

This master thesis is a continuation of previous work with near isogenic lines (NILs) and was only possible due to a lot of efforts from the IFA institute and colleagues.
The experiments that were carried out for the master thesis at hand contributed directly to the fine mapping of the QTL *Fhb1* on chromosome 3BS. Therefore collecting and linking genotypic and phenotypic data is necessary to be able to identify the region of interest.

I. The main aim was to narrow down the most likely region of the *Fhb1* gene(s) with molecular markers by comparing the genotypic and phenotypic data of 100 NILs with recombinations in the *Fhb1* region.

II. The development of user-friendly high-throughput KASP™ markers contributing to the research for optimized genotyping. The simplicity of the gel-free KASP™ genotyping assay guarantees a rapid workflow, higher flexibility and ideal accuracy and performance. For the long term the open format PCR system is an appropriate tool for fine mapping and ensure clear end-user advantages.

xi b aAk bs A

Rl5k oc c: e

oa Uy t f f wyn ulR v u y f f aof av l f s yl t f f wyn f of Rs lvoy i u lo ul swui lo vsvl l au na n lvoy oU i y dL n y lv rnaoRy Ma st vy aS d oar ba t oa i y l y S au l us lo i ou f s yl sw y u i v i d a Ru ba i t ul a li uvu l i y M

Rl5l5k oc c: e 3KeScr: t r gSr oe eoS

 aJvuon yv sw u0 u1Ru ba i vu t ul a i uvu a uRsl aot i vyvlv s aouu oU d o f a yl s sw u J9x5%8 y t Ru0u u y vy vnRa %y /1M J9x5%8 oavnvy l u Uaot i aouu Rt vJ%W i oay va J d u c sof vy ui Rlls a vyn f aona t ld y Lv o y oRli t av y vu1 vni sSa uvul yl lo M t Ru oy i oli a l y Pvu f avyn d i l RslvcaPd i v i d u c sof l i c av y l l yyulvlRl ba naoyot S vy a uvynP at yS M l i a oRau d ssJ f l naoyot v i a l au ba Rslvc lvoy vy yla s Raof Rl vl vu1 vni sSuRu f lv s lo yU lvoy 0R ault Sa l sM x55x1M u t f f wyn f of Rs lvoy q avc oR s Ji f sov 0 1 sw u aot i vyvlv s aouu d a Ru y i d o t eoa a uvul y u 1 y U uM JC d a v ylvUv 0R ault Sa l sM x55x x55%1M

eh: 7a: oc eo wh S hS r: cKeSw Sc: cK SeS

eh: 6a: oc eo gpRf 7T hS r: cKeSw Sc: cK SeS

Two different DH lines (E2-106-U and E2-62-T) with the genetic background of Remus in the *Fhb1* region were chosen for the development of NILs. Those lines were five times backcrossed with CM-82036 and after each backcross cycle plants were genotyped with SSR markers flanking *Fhb1* and plants heterozygous in the *Fhb1* region were selected and further used for continuous backcross events. In the end, the plants of this BC5 population had a 98% genetic background with CM-82036 genotype.

Additional four different NILs were created as control lines for phenotyping tests, relating to the QTL region on chromosome 3BS (*Fhb1*) and 5A (Qfhs.ifa-5A). The first control line CM-NIL38 harbours the alleles from CM-83036 at QTL *Fhb1* and *Qfhs.ifa-5A*. The second control line CM-NIL43 harbours the alleles from CM-83036 at QTL *Fhb1* and alleles from Remus at QTL *Qfhs.ifa-5A*. Thirdly the control line CM-NIL47 harbours the alleles of Remus at QTL *Fhb1* and CM-83036 at QTL *Qfhs.ifa-5A*. The last control line CM-NIL51 exhibit the alleles from Remus at both QTL regions.

In the fine mapping population of the BC5 generation, four plants (line number 8 and 34 with E2-62-T and line number 16 and 26 with E2-106-U as donor) heterozygous for the Remus alleles in the *Fhb1* region were selected. Further multiplication of those four lines was achieved to obtain a larger fine mapping population. This was done by planting twenty seeds from each of the four lines. Those have been genotyped with markers flanking *Fhb1* and again plants heterozygous in the *Fhb1* region were selected. Seeds of the selected lines were harvested and bulked for the four different subpopulations. This plant material was used for the previous master thesis performed by Verena Gratl (2015) starting in February 2014, representing a BC5 population with four subpopulations in a 98% CM-82036 background and for the *Fhb1* region on chromosome 3BS in the F2 generation. Initially 2080 F2 lines were planted and screened with the two flanking SSR markers GWM493 and BARC133 for the *Fhb1* region. (Already seven homozygous recombinant lines were found in this first screening but unfortunately, only three could be used for further analyses, since no seeds were available for the other identified lines due to several reasons, e.g. unfertile heads, mice, birds. Those homozygous lines were 957.1, 1469.1 and 1546.1, as found in Table 2. The rest of the BC5F2 lines identified as heterozygous recombinant were brought to the next generation, representing a BC5F3 population, for further selection of homozygous recombinants. Since one allele was already fixed in the F2 generation, the heterozygous allele would segregate according to Mendelian rules, resulting in

homozygous recombinant F3 lines. All F3 lines were screened again with the two flanking SSR markers GWM493 and BARC133. Additionally and for the purpose of genetic fine mapping, two more markers were applied, namely UMN10 and snp3BS-8.

At the end of all marker screenings, 98 homozygous recombinant lines and three lines with still heterozygous state for some alleles could be identified and formed five haplotypes, which were used for the master thesis at hand and can be seen in Table 2. The three lines still being heterozygous for some alleles, named 31.6, 1022.7, and 1789.3 were also planted to bring them to a homozygous state for all alleles. This was done for lines 1022.7 (further named 1077.7(3), because other sister lines still showed heterozygous states and were kept as potential plant material) and 1789.3, which are labeled with a grey background in Table 27. Line 31.6 did not show clear results and was excluded from the trial, marked with an orange background in Table 2. In the case of sister lines 1121.1 and 1121.3, 1177.1 and 1177.6 as well as for 1250.6 and 1250.8, seeds got mixed together because not enough material could be harvested for each line (as those lines carry same genotype they were mixed togehter on account of plant material quantity for each line). The plant material used in this thesis, (including control lines CM-82036, Remus, CM-NIL38, CM-NIL43, CM-NIL47 and CM-NIL51), can be seen in Table 2, showing the genotype of each line with the four used markers in the previous master thesis. A detailed scheme about the development of the plant material used for this master thesis can be seen in Figure 5.

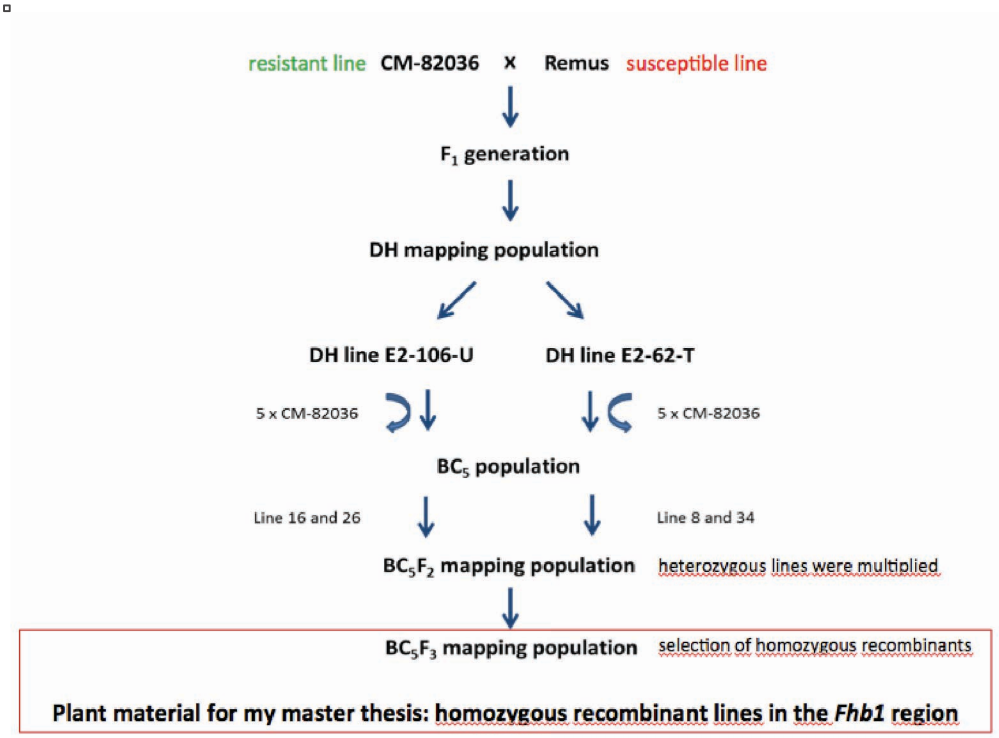
Table 2: List of wheat lines and control lines used for the master thesis at hand. Lines are recombinant within the *Fhb1* QTL region shown for four different markers and their haplotype. The green colour indicates the parental CM-82036 allele, the red colour the parental Remus allele, the blue colour heterozygous allelic state and yellow colour signifies undetermined allelic state. 1022,7 and 1789,3 showed heterozygous states and are marked with a grey background. 31.6 was excluded from the trial posterior, marked with an orange background

	CM-82036 allele
	Remus allele

	heterozygous
	undetermined

No.	haplotype	lines	GWM493	Snp3 BS-8	UMN10	BARC133
1	1	9,4				
2	1	12,3				
3	1	13,2				
4	?	31,6				
5	5	73,2				
6	5	73,5				
7	1	82,2				
8	1	116,3				
9	4	151,3				
10	1	181,4				
11	1	248,1				
12	4	265,5				
13	4	273,2				
14	5	296,5				
15	1	365,3				
16	4	381,5				
17	4	447,5				
18	4	528,3				
19	4	535,6				
20	1	592,2				
21	1	678,5				
22	1	694,8				
23	4	731,2				
24	1	749,2				
25	1	773,8				
26	1	789,6				
27	5	809,1				
28	3	827,6				
29	2	837,4				
30	2	837,5				
31	2	837,7				
32	3	857,3				
33	1	869,7				
34	1	890,8				
35	4	942,8				
36	1	957,1				
37	1	988,1				
38	5	990,4				
39	?	1022,7				
40	5	1026,2				
41	5	1026,6				
42	3	1058,3				
43	1	1067,3				
44	1	1121.1 /.3				
45	4	1137,8				
46	4	1177.1 /.6				
47	4	1247,7				
48	1	1250.6 /.8				
49	1	1259,4				
50	4	1261,4				
51	1	1265,6				
52	1	1301,1				
53	5	1330,3				
54	4	1344,3				

No.	haplotype	lines	GWM493	Snp3 BS-8	UMN10	BARC133
55	4	1372,2				
56	3	1386,1				
57	3	1386,4				
58	3	1386,6				
59	4	1441,4				
60	5	1443,4				
61	5	1443,5				
62	4	1469,1				
63	1	1516,1				
64	3	1546,1				
65	4	1558,8				
66	4	1566,4				
67	4	1606,2				
68	3	1609,3				
69	3	1609,4				
70	3	1609,7				
71	1	1627,4				
72	4	1633,1				
73	3	1633,6				
74	3	1635,1				
75	3	1635,4				
76	3	1659,3				
77	4	1659,4				
78	3	1659,6				
79	4	1663,6				
80	4	1682,1				
81	3	1695,1				
82	3	1695,5				
83	1	1711,7				
84	1	1726,1				
85	1	1737,8				
86	1	1757,3				
87	4	1769,6				
88	1	1785,6				
89	1	1786,3				
90	?	1789,3				
91	1	1808,5				
92	3	1835,3				
93	1	1850,1				
94	1	1873,1				
95	1	1883,1				
96	5	1957,7				
97	5	1963,3				
98	5	1963,6				
99	1	2016,3				
100	2	2073,2				
101	2	2073,6				
I.		CM				
II.		Remus				
III.		NIL 38				
IV.		NIL 43				
VI.		NIL 47				
VII.		NIL 51				



resistant line CM-82036 × Remus susceptible line
F1 generation
DH mapping population
DH line E2-106-U
DH line E2-62-T
5 x CM-82036
5 x CM-82036
BC5 population
Line 16 and 26
Line 8 and 34
BC5F2 mapping population
heterozygous lines were multiplied
BC5F3 mapping population
selection of homozygous recombinants
Plant material for my master thesis: homozygous recombinant lines in the Fhb1 region

2.2.2. DNA extraction

Leaf samples were taken when two-leave stadium was reached. A length of about 10 cm from the second leaf was cut into paper bags from each line and dried in an oven at 34°C for two days. Afterwards leaf material was put in a desiccator for at least one day to assure complete drying. The dried leaf samples were then stored in a cooling chamber before DNA extraction.

The DNA extraction was performed in 1,2 mL tubes with 8 stripes fixed in 96 well racks. In every tube, five to seven small glass beads were filled. Dried leave material was cut into each tube avoiding contamination. Two 96 racks at a time were spanned into a Retsch-mill and shaken for ten minutes, in order to break down the cell wall. 600 mL of freshly made CTAB-Buffer (details can be seen in Table 3) were added to the powdered samples under a fume hood. The racks were placed in a water bath at 65°C for 60- 90 minutes. After the stripes had reached room temperature, 350 µL chloroform:isoamylalcohol (24:1) was added to each tube under the fume hood. For five minutes they had to be shaken by inversion, afterwards the tubes were centrifuged for ten minutes at 3500 rcf (with Sigma 4K15 centrifuge). 300 µL of the top aqueous layer were pipetted off and transferred into new tubes. 300 µL of isopropyl alcohol were added and the tubes were mixed by gentle inversion. A centrifugation step followed, eight minutes at about 600 rcf. Since the DNA-pellet sticks to the bottom of the tube, liquid can be poured off easily. 100 µL of Wash 1 solution were added to the pellets and mixed for five minutes, then the tubes were centrifuged for further eight minutes at about 600 rcf. The liquid was poured off and the washing step was repeated with Wash solution 2. The pellet dried overnight and dissolved the next day with 0.1 M TE buffer. The racks were shaken at room temperature for one day, and then they were stored at 4°C for further uses (Buffer and Wash 1 and 2 components can be seen in Table 26, 27 and 28 in the Appendix)

7a r w d r o ocS o w r h ocS r h : F5 f f w Hk

bs D	k a s k kb bs k	k a s a n s Ehhn
I	R	HV5
/ 71ROLV	/ . . 5	/ . 5
V 8	O . . 5	/ L5
. zV RnTz 3 d	V. 5	/ . 5
D	/ %	/
/ L A	/ L. 5	/ 8
E 1 7 1 8h 5 y 75 - 7M0 H. RHV 9 07 2 M 2 D U e 84h8V5 M h8R5 5 02 5 705 n 5 ROHNVd A CR5 7 3M0 M 208 - 1M8 7 07M0 1 z 2 7 - 5 00 u		

RkRk7 k s h ect K y o e hcer o

y ba a lo h l ul ul t lvoy oRl i a uRslwyn by ylalvoyu oU i Lla l ut fs uP i f laofiolot l a vo f Jy yo d u Ru M t fs u d vi c aS vni by ylalvoy 0t oa li y q55 ynW Gs 1 d a vsRl d vi J$_x$ M oa L l s Rs lvoyu oU i by ylalvoy y i NR svl S oU i u t fs uP vaofs l a a 0wyUyvl x55fao uf la U Roa fsRu1 d u Ru M uoaflvoyu l x%5 yt Px85 yt y x95 yt d a t uRa bal ss u t fs u y i NR svl S oU i d u uu uu Ruwyn ss d c synli uM i Lla l u t fs u d a vsRl lo Uy s d oarwyn by ylalvoy oU q55 ynW S wyn i baa ufoy wyn t oRylu oU J$_x$ M l a vUUa yl d oarwyn lo ru d vi od a by ylalvoyu d a t lo u U i M

Rk7 k r o e: w cer o r cK or ct d r: cK oyeo w : y : Sk

o c avS li n yolSf oU i fs yl C % swy uP y URali a n s s laofioa uwu d vi f avt au d u ff sv by ss sy u Ruwyn i favoa Lla l M y i u bU i t ul a i uwu l y d vi i Us yrwyn t ar a q% l i faoLvt s oa a y t ar a q5 y i 1 a nvoy d a ff sv M a nt ylu hol uf a l S osS aSs t v h s s laofioa uwu Ruwyn 1 d o Roa u yl S uP y SCP oa URali a cwuR sv, lvoy cv t n u yy a Sfiooy avoM i u yy a t uRa l /99 yt lo l l ss a nt ylu y l 8% yt lo l l SC s ss a nt ylu a uf lvc SM i fao R lu d vi f avt au d a u f a l by h s y u oa

d vli □iqH□ba□□ □ss s u□ix H□ba□ t Ru□ss s u□i%H□ba□ l ao, SnoRu□ss s u□i□H□ba□ vuuyn□ c□sR u□y□□iH□ba□Ry□ l at w □a uRsluM□

Rk7k5 □□□ □: ew □: S□o □□□□ d: r cr □r S□

□i □u NR y□ □oU□i □s□yr wyn□□□□t □ar au□ba□i □□□1□a nvoy□□□ □/Ä%□y□□□□□q%□u□ uRt t □av, □□y□□□s □/M□i □□□f aolo□bs□u□vc y□y□□□s □CM□

□

□□□ □6a□□□ d: ew □: S□r: □□□□ □er o□

□□□	□ □ D□ □	g □n □ E□	g □n □ x□	□s n n □k b□
□□□	□□ □ □□□	□□□□□□□□□□□□□□□□□□□	□□□□□□□□□□□□□□□□□□□	□s □□ □b□a□EOOR1□
□□□	□□ □□□	□□□□□□□□□□□□□□	□□□□□□□□□□□□□□	□t □b□a□xhhR1□

□

□□□ □Qa□□:)KS)57): □w d)T7)Q5□□□ d: r cr □r □

□□□g s bs □s a□□n□ak□□s k□□bs k A□□ T□ATE(T □n gTH(TME□			
□b□g A□	□n g□ □bt □	□n □n □k1□	□t k A□
/□	[1] V9□	. l g . □	/ e□
l □	[1] V9□	. . gV. □	He□□□ n7□3□□M1M3 1□□ l RL d□
N□	HN9□	. / g/ V□	
L□	Ol 9□	. / g/ V□	
V□	[1] V9□	. . gV. □	l Ve□□□ n7□3□□M1M3 1□□ VROd□
H□	□V/ 9□□□	. . gV. □	
O□	Ol 9□	. / g . □	
T□	Ol 9□	. Vg . □	/ e□
[1] □	/ L9□	; □	; □

□

Rk6k□r w d□ceed □□ □ □d □e□e□□ □F□ □ □4 H□r o□ □r w □eo □oc□eo □S□

□ vli □i □□□J□f □s □f s□l □a □ a□□J□□□%9/□oR□i) □ □sJ□vt □□□ l □voy□ □Sul t □i □sRoaof i oa u□□) □y□□) □d a □ l □l □□y□wulwynRwi □t □uRawyn□ □□□ba□ □i □t f s M□□J□□□□ □y□n a□%□q□d □uRu □lo□f sol□□) □y□□) □l □oy□ □al uv□y□boa□wy□l □uSul t □□ba□wyn□lo□i □u□t f s □sRul au□o□ □s □o□ l at w □ d i li a□i □u□t f s u□aaS□i □J9x5%8□oa□i □ t Ru□ss s u□l □ al□y□a nvoyM□

□

2.4.1. Preparation of KASP™ mix assembly and PCR run

The SNP-specific KASP™ Assay mix and the universal KASP™ Master mix were added to the recombinant wheat DNA samples in 384-well format BIO-RAD Hard-Shell® PCR plates (using BIO-RAD Microseal® `B´seals during PCR).

Total reaction volumes of 5 µl for each well (per sample) were used containing:

- 2,43 – 2,50 µl KASP™ universal Master Mix 2xReaction V.4.0
- 0,07 µl KASP™ Assay Mix (Primer Mix)
- DNA:
  1 – 2,5 µl of DNA were added, depending on concentration (LGC recommends concentrations between 5-50 ng per sample) and PCR protocol
- in case and depending on amount of DNA, the missing volume was filled up with PCR-H_2O to a obtain a total reaction volume of 5µl for each well

E.g. 2,43 µl Master mix, 0,07 µl Primer mix, 2 µl DNA (concentration of 25 ng/µl) and 0,5 µl H_2O.

2.4.2 KASP™ Assay Mix primers and PCR protocols

KASP™ assay Mix primers used for this thesis are SNP primers distinguishing different alleles according to changes of one base pair in the DNA sequence. All primers have been developed before the start of master thesis at hand accept from primer IFA-FM958, which was designed in the course of this thesis. (Development of the primer can be seen in chapter „2.5 Development and testing new markers for the *Fhb1* region"). An additional KASP assay was constructed based on resequencing the PCR product of UMN10 from the susceptible and highly polymorphic locus of Remus, named IFA-FM426_umn10. More detailed information about the primers is presented in Table 6.

KASP™ Assay PCR protocols used for this thesis can be seen in Tables 7 – 11.
Primers used for which KASP™ Assay PCR protocol are given in Table 12.

□□□□ □□ a□r h□K□r ü o F□□ H□T5gQQ9□□d: r cr □r □□

□□□□g s bs □s a□□m□a□k□□□s k□□bs k A□□□s t □□□s c k□HEvMM□□			□□
□b□g A□	□□n g□ □bt □□	□□h □□n □k1□	□t kA□
/□	[1] L9□□	/Vg . □	/e□
I □	[1] L9□□	. . g . □	/. e□
N□	H/9□□R VV9□□□□□ n□703□0□□R uH9□□3□7□□h□8□d□	. /g . □	n7□3□□N□1N□3 1□ RNd□
L□	[1] L9□□	. . g . □	I He□
V□	VV9□□	. /g . □	n7□3□□N□1N□3 1□1 RVd□
H□	NO9□□	. /g . □□	/e□
□8□N□1□□□□□	R□	R□	/e□

□

□□□□□ □□ □5f a□r h□K□r ü o F□□ H□TpgTR9□□□□d: r cr □r □□

□□□□g s bs □s a□□m□a□k□□□s k□□bs k A□□□s t □□□s c k□HRvHx□□			□□
□b□g A□	□□n g□ □bt □□	□□h □□n □k1□	□t kA□
/□	[1] L9□□	/Vg . □	/e□
I □	[1] L9□□	. . g . □	/. e□
N□	HT9□□R HI 9□□□□□ n□703□0□□R uH9□□3□7□□h□8□d□	. /g . □	n7□3□□N□1N□3 1□ RNd□
L□	[1] L9□□	. . g . □	I He□
V□	HI 9□□	. /g . □	n7□3□□N□1N□3 1□1 RVd□
H□	NO9□□	. /g . □□	/e□
□8□N□1□□□□□	R□	R□	/e□

□

□□□ □□55a□□ □□t □ eo □□r o □ecer o S□□□□d: r cr □r k□o □□S□□□c□□K□S□or c□r: w □□□e□Kc□□ hSc□: S□□c□: □cK□□ eoece□ □cK□: w □ □□t □ eo □□d: r cr □r i□: □□t □ eo □□r □□cK□□d □c□□□o □□□□□□Ke□l □□□3□□w r hoc□r □□□□ecer o□ □ □t □ □S□□d□o □S□w □eo t □ro □□ □□o □□d: ew □: Sk□

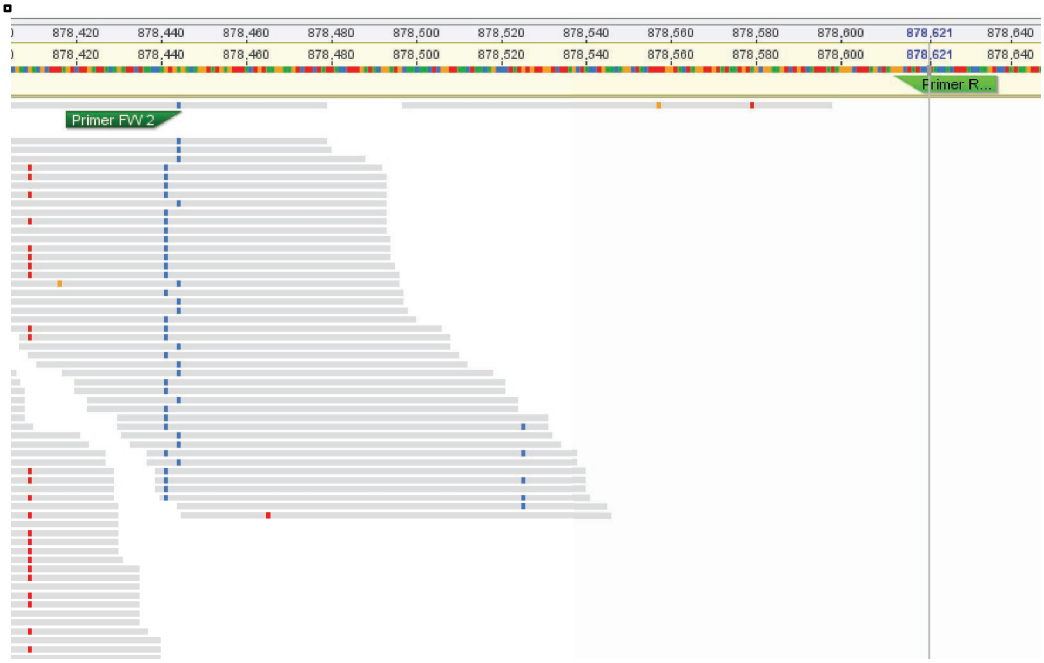
□□□□g s bs □s a□□m□a□k□□□s k□□bs k A□□□□m□a□k□□□s k□□bs k□			□□
□b□g A□	□□n g□ □bt □□	□□h □□n □k1□	□t kA□
/□	[1] L9□□	. . g . □	2075 □8□h□/ R/. e□□□□ n□□3□2□□2□□02□ 38 0N□2□□7□1- 8N1d□
/□	[1] L9□□	. . g . □	
□8□N□1□□□□□	R□	R□	/e□

RlQ l r dw oc o c Sceo o ü w: y: S r: cK er o

d fav t au wy li 1a nvoy d a ul swui dvli li uoUd a f r n y voRu 9MqM4 au l sMx5qx1y l ul cv s lvt dvli li J %9/ oR i) sJ vt l lvoy Sul t y dvli fosS aSs t v h s s laofi oa uvuM

RlQl5k Se o r o ü w: y: eo cK r o

vli oylvnu NR y uf yywyn li 1a nvoy0 i d vn a lsMx5q81y d t ar au0d iv i y uR u NR ylsS oyc al ylo) i ar au1 oRs c sof dvli li uoUd a f r n y voRul l lwyn fosSt oafi wut uRlvsv,wyn t f f Ju N l1 aot li uRu f lv s t Ru1o li 1a nvoy oU J9x5%8 u y y vnRa 8 0t oa l vs vnRa uy y li f f y vLHy y t vnRa qÄ y vnRa x51M



eh: Ta roce S sh o r cK r di S s Sr
wh S: w dd r cK roce S sh o k K r: ü: o l : S d: ew : dr Secero Sr cK
o ü l r d w : y : S : SKrü o o : o o cK Sr cü : o erh S

2.5.2. New marker IFA-FM958 tested with Real Time PCR

The new marker IFA-FM958 (former marker sequence details in Table 14) was at first tested with CM-82036 and Remus DNA samples. The first step was to find the adequate temperature for amplification with the Real time PCR detection system, attempting a gradient PCR with four different annealing temperatures shown in Table 15, starting from 60°C to 68°C and using the PCR protocol shown in Table 16, DNA was put in 384-well format BIO-RAD Hard-Shell® PCR plates (using BIO-RAD Microseal® `B´seals during PCR). A total reaction volume of 5 µl for each well (per sample) was used containing PCR components as seen in Table 13.

Tabelle 13: Real time PCR components and amounts of PCR reaction

PCR components	Stock	Final concentration	Reaction volume
DNA	100 ng/µL	25 ng	1 µL
KAPA SYBR qPCR (Code KM4100)	2 x	-	2,5 µL
PrimerFW2	100 ng/µL	2,5 ng/µL	0,75 µL
PrimerRV2	100 ng/µL	2,5 ng/µL	0,75 µL

Tabelle 14: Sequence of the forward and reverse primers for the marker IFA-FM958

Marker	Sequence (5'-> 3')
PrimerFW2	GACAAGTAATCTGTAACATTCCTGGGC
PrimerRV2	TCAAGGATTTGAGGATGGTATGCC

Tabelle 15: PCR annealing temperatures

Temperature in [°C]	60	62	64,5	66,1	68

[illegible] 5pa w : y : S: oeo d: r cr r to h eo w c h: l o t SeS: r w TQ9 g- Q9 r : c Sceo cK o ü w : y :

g sbs s ao m a k s k bs k A n D A k k			
b g A	n g bt	n n k 1	t k A
/	^{1} V9	. l g .	/ e
l	^{1} V9	. . g/ V	L . e n7 3 M M 3 1 l R d
N	HL 9	. . gN.	
L	Ol 9	. . g/ V	
V	^{1} V9	. . g/ .	
H	8M - 7a 7 0 5 HV9 R^{1} V9 n 2 7 5 2 M 0 zV9 0 7 . g Vd	[illegible]	/ e
O	/ l 9 ^{3} 8 M	. . g/ .	/ e

[illegible] 5- a r h S y de o o S s h o S r w o ceo

s t A D g k k	ut k e M 2 (d
m	[illegible]
m	[illegible]
M 2 m	[illegible]
M 2 m	[illegible]

RlQl7 k ü w : y : c Sc i eKdr t : t w e c: r dKr: SeS F H

[illegible] ot a ot y yl d i l y u d u f Rl y l y a 9/Jd s at l f s l u y d u oy d vi f f y oa Uc f oMaol l) ul a S s a(f ao 9/ t i y M d u Ry d vi f aolo os f aocv y s xqM ol s a lvoy cosRt bUq 5 s f a u t f s d u Ru y y u y y s 5 M

[illegible]

[illegible] Rf a r w dr o ocS o w r hocS r cer o

s n gs k kb A	bs D	k a s k kb bs k	bs k rs a n
[illegible]	/ . . 2 sw	l V2	/ w
6	V w	. z V sw	. z w
7 5 7 l	/ . . 2 sw	l zV 2 sw	/ w
7 5 7 l	/ . . 2 sw	l zV 2 sw	/ w
m	/ . . 2 sw	l zV 2 sw	/ w
m	/ . . 2 sw	l zV 2 sw	/ w
- 7	/ . e	/ zV 5	/ w
1	l 2	. z 5	/ w
R l	R	R	l zT w

From each line five plants were replanted from the seedling trays into one pot (of an approximate diameter of 25 cm). This was done for both replications using the same substrate as for the seedling trays).
As some seeds did not germinate and some seedlings did not develop, the number of plants per pot/replication was evenly distributed. Odd-numbered survived seedlings were divided by their stage of growth: higher developed plants were divided in favor of planting more plants in the first replication. (For plant line 248.1, only one plant survived and was planted in the first replication, but multiple wheat heads were formed due to tillering enabling enough heads at least for one replication and prospective plant material).
The aim was to obtain at least ten wheat heads per pot/replication for inoculation.

2.6.1. Glasshouse conditions

Temperature in the greenhouse was set on average 18/12°C (day/night) from tillering to heading with 12–14 h daylight. At anthesis stage temperature was set on 22°C during day and 17°C during night with a 16-h photoperiod at 15,000 lux, supplied by two different light sources - sodium-vapor and metal-halide lamps - which were distributed homogeneiously in the glasshouse. Diseases and pests were monitored during growing season.

2.6.2. Inoculation and evaluation of the FHB symptoms

The lines were inoculated at anthesis stage starting in mid of December. Because not all lines were in anthesis stage at the same time it was necessary to permanently observe the wheat plants for inoculation, which was done preferably every two days. A spore suspension of *Fusarium graminearum* produced with bubble breeding method as described by Buerstmayr et al. (2000) with a concentration of 50.000 spores/mL by single floret inoculation method was used for inoculation. To rule out the possibility of non germinable FHB spores, the germination of the used spores was analysed visually under the microscope at random. Therefore, 10 µl of conidia (500 conidia per floret) suspension was pipetted between the palea and lemma of the two basal florets of two central spikelets per spike (as seen in Figures 7 and 8). After inoculation the heads were sprayed with distilled water and got covered with plastic bags for 48 hours to

faocv i vni i Rt v vlS Uba li wyU lvoyM yo Rs lvoyu d a f alobat l boyuwl yl boy vlvoyu y fa Ua sS wy i t oaywynM i ufa obUi wu u uSt flot ulaot li wyo Rs lvoyuwl soyn i d ua a oba q/Pq9Pxx y x8 Su U auwyns Usoa l wyo Rs lvoy 0 l S oRylwyn li yRt a oU cwuR ssS wyU l oa s i ufvr s lu y lo lossod y o u ac i wyU lvoyfao uuM vli i u l i lossod wyn f a t l ad a s Rs l Uba i f ol 0X sw y a fsv lvoy1Q yRt a oU wyo Rs l i ul yRt a oU uSt lot lv ufvr s luf a yl n oU wyU l i u 0wy v y 1P f a yl n oUi u d vli t oa wyU l ufvr s lu li y li ld o wyo Rs l ufvr s lu 0ufa wyn wy v y 1P c a n yRt a bUuSt flot lv ufvr s luf a ufvr 0u c avlS1 y li ufa wyn wy L t Rslvfsv lvoy bUufa wyn wy v y t Rslvfsv S ufa wyn u c avlSM iRulPli wy v y bUufa wyn d u c sRl S li f a yl n bUwyU l ufvr s luf a i d vli t oa uSt flot lv ufvr s luli y li ld o wyo Rs l P Rl suo c sRlvoyu by ss wyU l ufvr s lu wy n y a s y wyU l ufvr s lud a oyuv a M

e h: La wroSc: cero r cK hS ao r h cero w cKr

e h: pa or h c d ocS ü ecK d Sce rl : Sd: rl e eo sh c ro ecero S r: eS S Sd:

RkTk7k o tSeSr cK dK or ct de c

l lwulv s ot fRlwyn y ha f iv u l c y oy dlvli li la uoUd a ycvaoyt yl P uu uuwyn y sSuwubUc av y y lot on y vlSbUc av y M L s x5q5 d u suoRu ba s Rs lvoyud vli l y cwuR sv, lvoyM

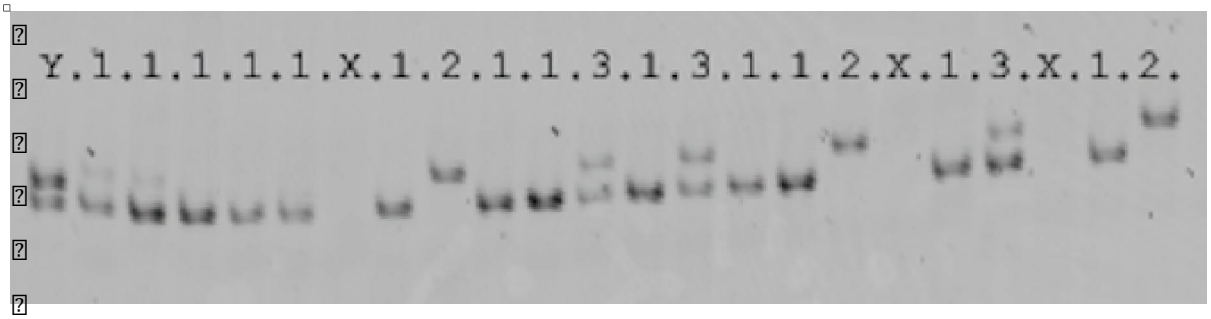
(i AtabA

yli vu i fl a a uRslu y by s oRl ot u a f a u yl y ssRula l Pul alwyn dvli li n yolSfv l y uR u NR yl fi yolSfv l M uv u li t wy a uRslu y u s lvoy oU a ot wy yl swy u wy li 1 a nvoyP y sSuwu vt wyn t li o vt f aoc t yl d a oyuv a ul ssM

7l5k orctdeo r dK SuecK rw eo cero Sdo dK . er o

7l5l5k : oeo r : w ocSr o

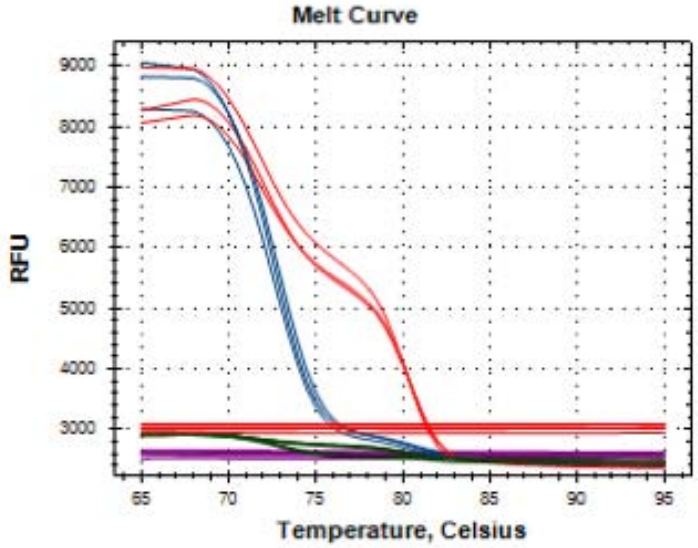
i f ao R luoU f avt aud a u f a l oy h su y a uiod y L t fs aS y vnRa ÄM i wyvlv s c avU lvoy oU n yolSf u Uaot C%sswy u dvli f avt au oyUat li n yolSf u ba li ld o s yrwynt ar au /Ä%y q%%ua c s wyli C x y y u y wyli s xÄwyli f f y vLM

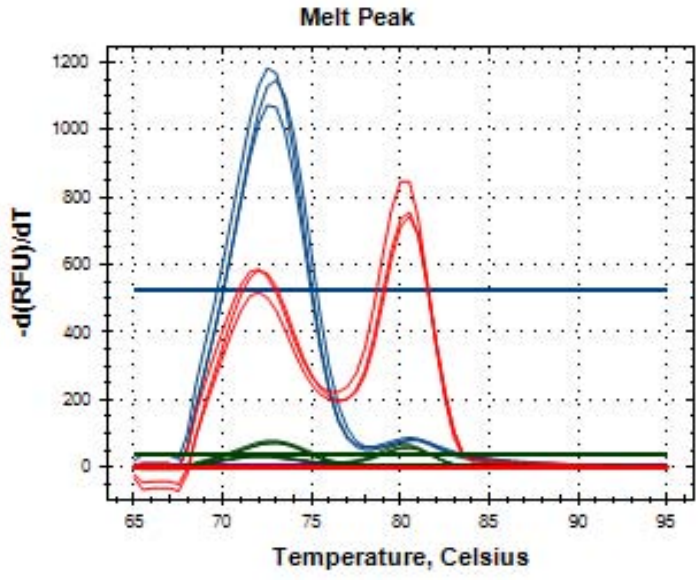


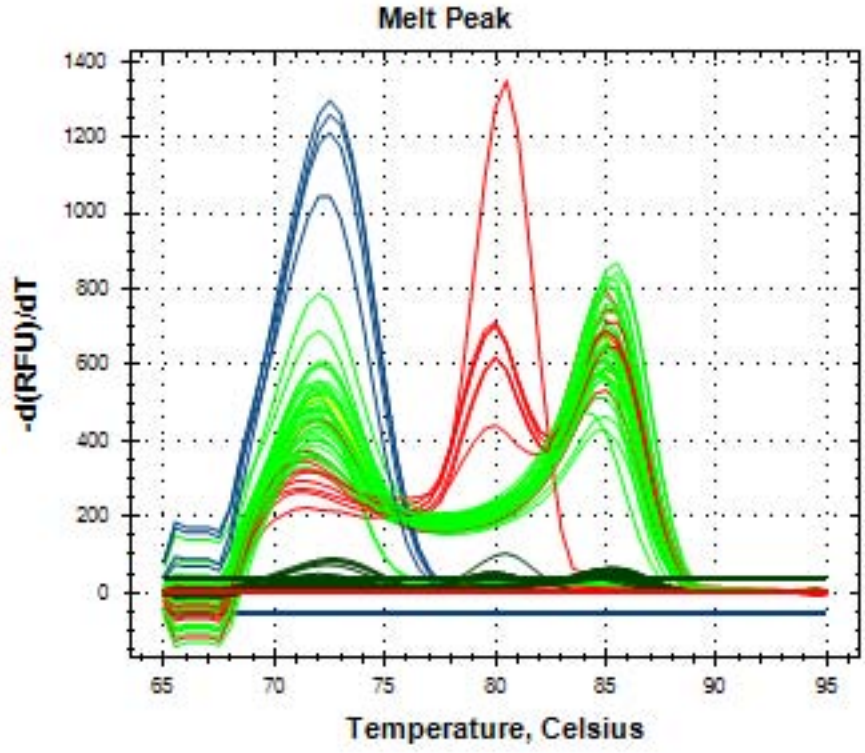
e h: a S: oeo öwd r: 5f k K d: r h cS: S r: S r rü a-52 r: Si –R2 r: whS Si-72 r: K c: r1t rhS Si-2 r: weSSeo l h S o-2 r: ho c: weo Sh cS

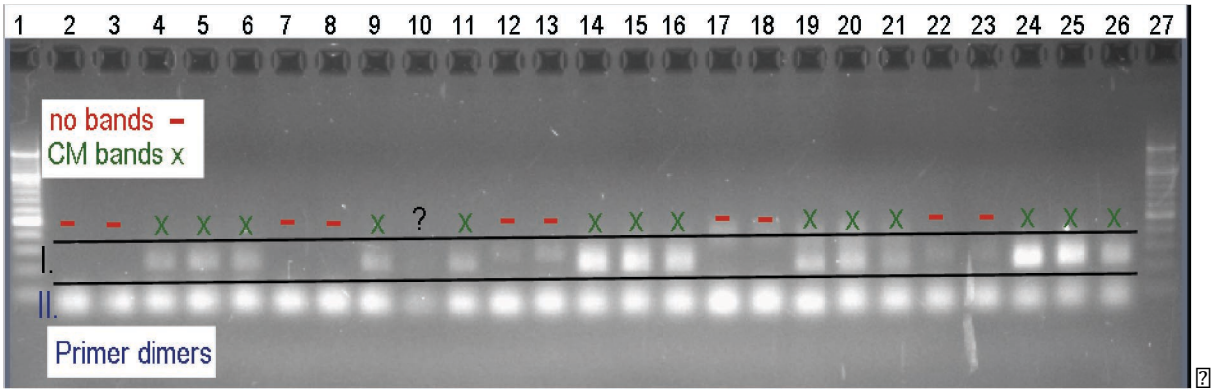
7lRlRk Sh cSr o ü t l rd w: y: g - Qp

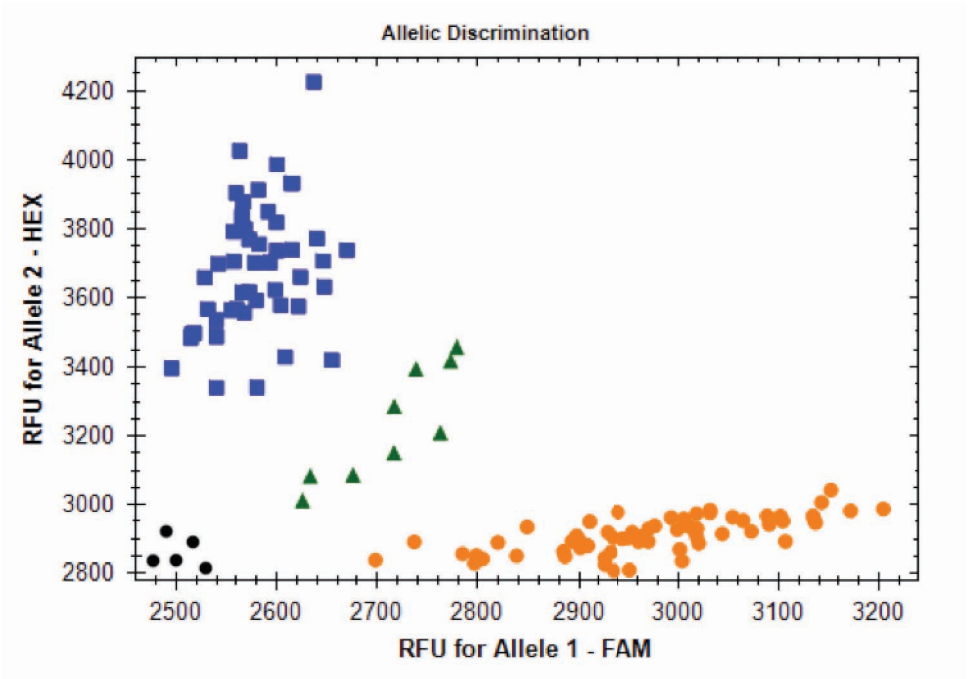
i y d t ar a J ÄC9d ud ul dvli li s vt f aolo os u u av wy i fl a xMMMs q41f alobat wynli ul t fsvU lvoy l y yy swynl t f a lRa oU 8/; M a c s lo li t oul Ry lvoy s a Ua y h y dvli s vt y d u URali a f f sv Mv J x5%8 y t RuoRs s asSso l Mi vu vuoy Sh y a lwyn t slwyn Rac u u y wy vnRa q5P y lobat oa oyc yv yl tvuR sv, lvoy lobay sSuvu li va y n lvc avc lvc u a ot fRl uiod wynli fowylu oU wyUs lvoy dvli vU a yl f rul u y wy vnRa qqMvU a yl f r u l vU a yl t f a lRa u a a l R lo


Melt Curve
RFU
9000
8000
7000
6000
5000
4000
3000
65
70
75
80
85
90
95
Temperature, Celsius


Melt Peak
-d(RFU)/dT
1200
1000
800
600
400
200
0
65
70
75
80
85
90
95
Temperature, Celsius


Melt Peak
-d(RFU)/dT
1400
1200
1000
800
600
400
200
0
65
70
75
80
85
90
95
Temperature, Celsius


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
no bands –
CM bands x
– – x x x – – x ? x – – x x x – – x x x – – x x x
I.
II.
Primer dimers


Allelic Discrimination
RFU for Allele 2 - HEX
RFU for Allele 1 - FAM
4200
4000
3800
3600
3400
3200
3000
2800
2500
2600
2700
2800
2900
3000
3100
3200

The markers IFA-FM155 and IFA-FM277, positioned distal of marker IFA-FM426_umn10, and IFA-FM836, positioned proximal of marker IFA-FM426_umn10, did not reveal new haplotypes and hence demonstrate the same alleles as marker IFA-FM426_umn10. (Due to the fact that marker IFA-FM155 is the most distal marker of markers IFA-FM277, IFA-FM426_umn10 and IFA-FM836 and for reasons of simplification, only IFA-FM155 is named in the thesis at hand, also refering to the markers demonstrating the same alleles).

3.3.1. Identifiction of new haplotypes in the *Fhb1* region

Three recombinant lines, named 1957,7, 1026,2 and 1026,6 (the last two beeing sister lines), presented different alleles for the new marker IFA-FM958, positioned proximal to marker IFA-FM836 and distal to the flanking marker BARC133. Compared to the other markers in the *Fhb1* region, the lines 1957,7, 1026,2 and 1026,6 carry Remus alleles for the markers GWM493, snp3BS-8 and IFA-FM155 and CM-82036 alleles for the new marker IFA-FM958 and the BARC113 and therefore constitutes a new haplotype - haplotype 5.
In summary, six haplotypes can be defined with the KASP™ marker analysis of the recombinant wheat lines for *Fhb1* region.

Table 22: Haplotypes and molecular markers used for the thesis at hand. Green fields indicate CM-82036 alleles and red fields Remus alleles

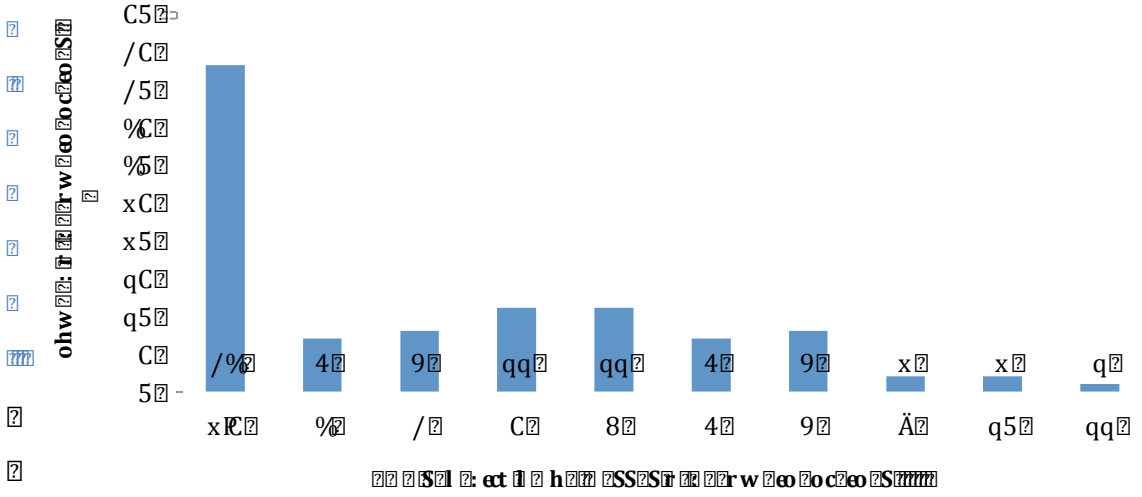
haplotype nr.	samples no. / haplotype	GWM493	snp3BS-8	IFA-FM155 IFA-FM227 IFA-FM426_UMN10 IFA-FM836	IFA-FM958	BARC133
1	38					
2	5					
3	19					
4	25					
5	3					
6	10					

3.4. Phenotyping results for FHB resistance of the recombinant lines

In general, the inoculation trial was successful. 99.4% of the inoculated heads developed disease symptoms (Random spore analysis under the microscope revealed germination to a very high extend). The spreading of the disease was estimated by different parameters. On the one hand the total number of symptomatic spikelets of all inoculated heads was counted for each line and the average number of symptomatic spikelets/spike was calculated presenting the FHB severity in general. Of capital importance for further calculations was the number of spikes with more than the two inoculated spikelets, indicating unartificial symptoms and therefore spreading of the disease, termed as FHB spreading incidence (Table 30). Few plants did not show any symptoms at all (although all plants got inoculated the same way). Those plants were among lines number 731.2, 1566.4, 1635.4 and 1659.4 in replication one and number 1386.4, 1785.6 and 2016.3 in replication two. The control lines CM-82036, NIL38, and NIL43 exhibited disease symptoms on the two inoculated spikelets only resulting in low FHB severity ranging from 1,9 to 2,1 symptomatic spikelets/spike. Control lines NIL47 and NIL51 showed a high FHB severity of 4,6 and 6,8 symptomatic spikelets/spike, respectively, and Remus exhibited a very high FHB severity of 17,9 symptomatic spikelets/spike, as seen in Table 23. These results reflected our expectations and gave evidence that the point inoculation worked very well for the disease spreading assessment.

Table 23: Graphical illustration of the control lines in the *Fhb1* interval for 8 markers and their phenotypes for FHB resistance. Resistant CM-82036 alleles for the markers are illustrated in green, whereas susceptible Remus alleles are shown in red. FHB severity values in number of diseased spikelets per head 26 dai with F. graminearum and their standard deviations are shown next to the marker data

control lines	GWM493	snp3BS-8	IFA-FM155	FA-FM227	IFA-FM426_umn10	IFA-FM836	IFA-FM958	BARC133	FHB severity		
CM-82036									**1,9**	(±0,2)	**R**
CM-NIL38									**1,9**	(±0,2)	**R**
CM-NIL43									**2,1**	(±0,2)	**R**
CM-NIL47									**4,6**	(±0,3)	**S**
CM-NIL51									**6,8**	(±0,2)	**S**
Remus									**17,9**	(±0,2)	**S**


C5
/C
/5
%C
%5
xC
x5
qC
q5
C
5
/%
4
9
qq
qq
4
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%
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C
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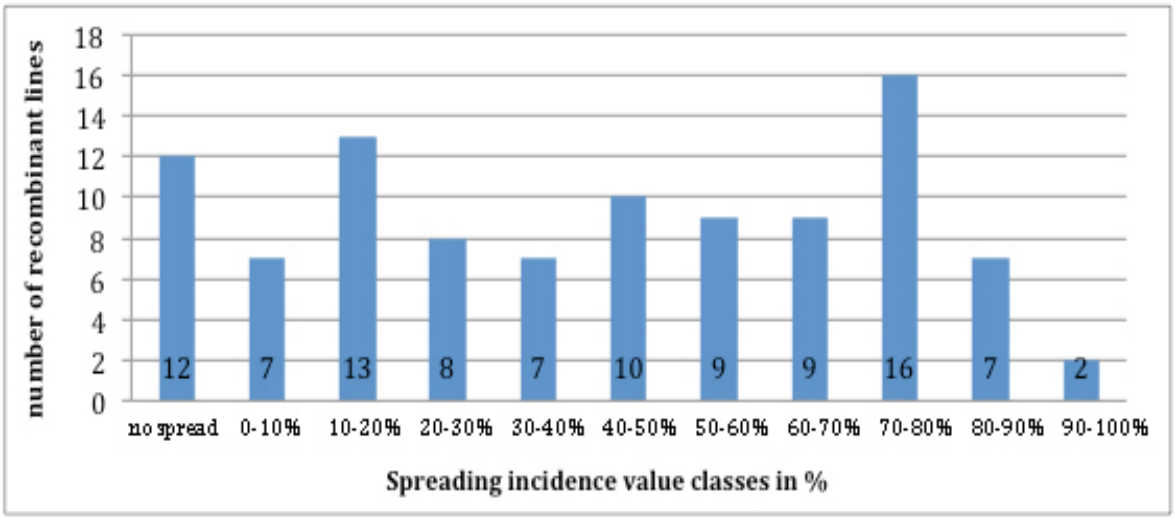
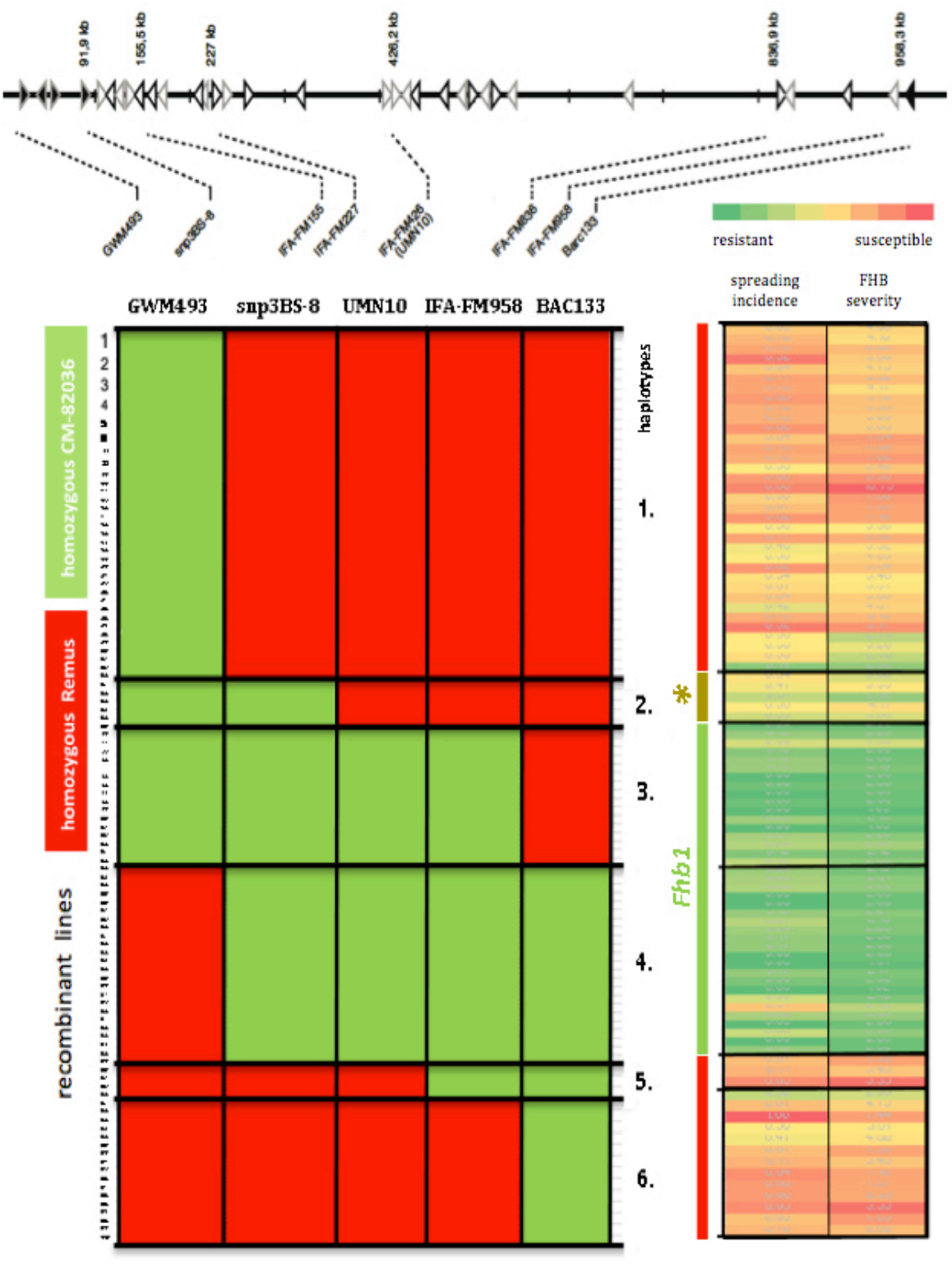
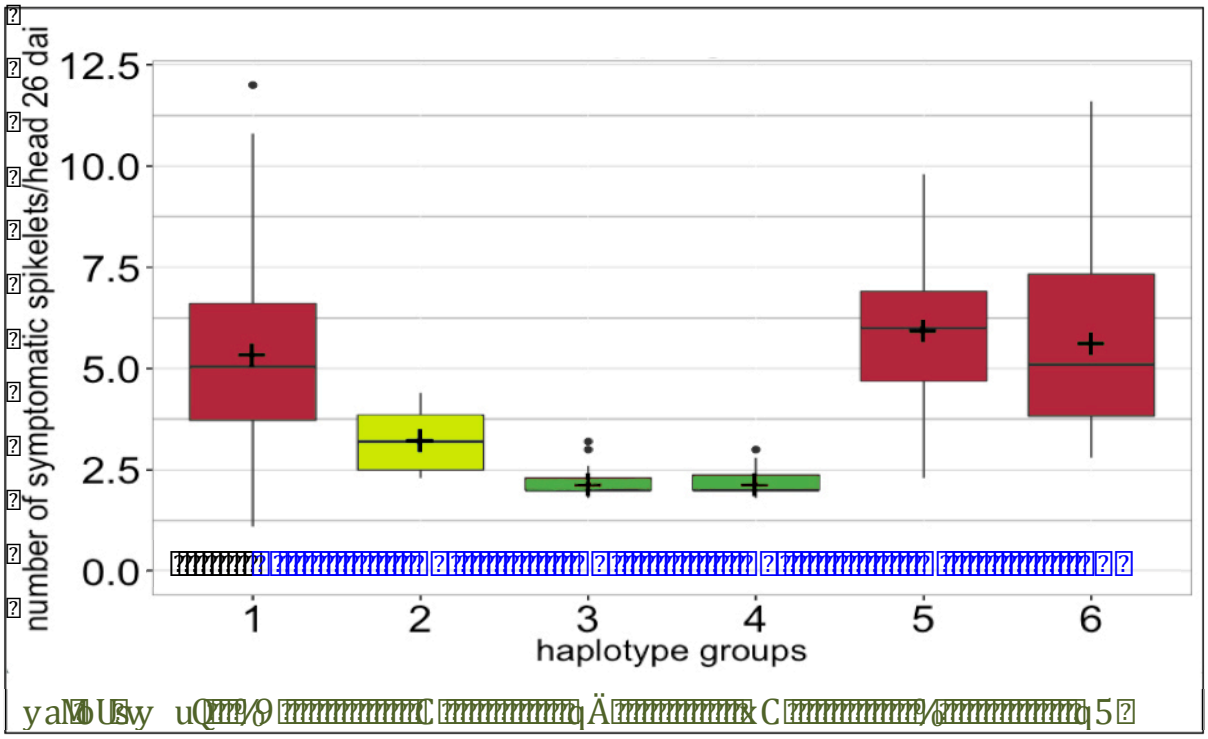
q5
qq


number of recombinant lines
18
16
14
12
10
8
6
4
2
0
12
7
13
8
7
10
9
9
16
7
2
no spread
0-10%
10-20%
20-30%
30-40%
40-50%
50-60%
60-70%
70-80%
80-90%
90-100%
Spreading incidence value classes in %


91,9 kb
155,5 kb
227 kb
426,2 kb
836,9 kb
956,3 kb
GWM493
snp3BS-8
IFA-FM155
IFA-FM227
IFA-FM426 (UMN10)
IFA-FM836
IFA-FM958
Barc133
resistant
susceptible
spreading incidence
FHB severity
GWM493
snp3BS-8
UMN10
IFA-FM958
BAC133
homozygous CM-82036
homozygous Remus
recombinant lines
haplotypes
1.
2.
3.
4.
5.
6.
*
Fhb1

Haplotype groups 1, 5 and 6 showed significant results for beeing susceptilbe to FHB due to high valuations of severity and incidence in the mean values (Table 31). Exceptions could be made upon line number 2016.3 in haplotype group 1, showing low spreading symptoms with a spread incidence of only 16% and FHB severity of 2,5 symptomatic spikelets/spike. Haplotype 3 and 4 represented very resistant lines with exceptions for the spreading incidence parameter of line number 1606.2 with 63%, line number 1058.3 with 42% and line number 1566.4 with 35%, but all mentioned lines showing severity results not exceeding 2,8 symptomatic spikelets/spike. Haplotype 2, only consisting five lines (two and three sister lines respectivly) revealed intermediate phenotypic data with spreading incidence values from 31% to 58% of the inoculated heads and severity ranging from 2,3 to 4,1 symptomatic spikelets/spike. This intermediate haplotype suggests the presence of an additional resistance gene contributing to FHB resistance (Figure 17).

Analysis of variance evaluating FHB severity measured in the average number of infected spikelets/spike for each haplotype revealed significant ($p<0.05$) differences between haplotypes, as summarized in Figure 17 and Table 25. Haplotype 1 does show significant differences to all haplotypes except haplotype 6 and haplotype 6 does not show significant differences to haplotype 5. The expectation, that haplotype 1 and haplotype 5 do also not differ from each other, could not be confirmed. But as haplotype 1 does already show high susceptibility on average, and haplotype 5 and 6 are not significantly different and show almost the same or even higher susceptibility, those three haplotypes can be composited into one susceptibility class. The upper whisker from box plot haplotype 1, including 25% of the results, is heading almost to the same amount of infected spikelets as the box plot upper whisker from boxplot haplotype 5, demonstrating that also lines from haplotype 1 get as susceptible as lines from haplotpye 5. Haplotype 2 does show significant differences to all haplotypes, except of haplotype 3 and 4, both of them showing significant differences to the rest of the haplotypes. Haplotype 3 and 4 show very clear results, with very compact box plots pointing out a significant picture of resistance. Haplotype 2 is statistically not different from haplotpye 3 and 4, the box plot exhibits an intermediate class, ranging phenotypes between resistant and susceptible, with a compact box plot in between the other haplotype box plots. Thereupon and summarized three different phenotypic classes can be distinguished grouped in a highly resistant class (green box plots, representing haplotypes 3 and 4), a highly susceptible class (red box plots, representing haplotype 1, 5


number of symptomatic spikelets/head 26 dai
12.5
10.0
7.5
5.0
2.5
0.0
1
2
3
4
5
6
haplotype groups

To put it in a nutshell, the newly designed marker IFA-FM958 could replace the marker BARC133 as the new flanking marker on the distal border of the *Fhb1* region, due to the fact that lines with CM-82036 alleles for the marker IFA-FM958 and BARC133 and Remus alleles for the rest of the markers did show high FHB susceptibility, revealing no possible resistance gene in the region between markers IFA-FM958 and BARC133.

The comparison of genotypic and phenotypic data makes it clear that the causal gene(s) behind QTL *Fhb1* is/are located between markers snp3BS-8 and IFA-FM958 with contig positions 91.9 kb and 958.3 kb respectively. Haplotype 2 representing lines with recombinations between snp3BS-8 (CM-82036 alleles) and IFA-FM155 (Remus alleles) exhibited an intermediate FHB resistance phenotype, not revealing a significant difference between resistant and susceptible classes with a mean of 3.2 symptomatic spikelets/spike. This leads to the hypothesis that not only one gene is responsible for resistance in the QTL *Fhb1*, but also a different second resistance gene between markers IFA-FM155 and GWM493 can be suspected.

4. Discussion

The analysis with the genotypic and phenotypic data could narrow down the possible region of *Fhb1* with the proximal border at the marker IFA-FM958 and the distal border at the marker snp3BS-8. After inoculation and evaluation process the distribution of FHB severity pointed out that the susceptibility range is rather heterogeniously and did not show a clear picture of susceptibility. Beside the resistant haplotypes 3 and 4 and the susceptible haplotypes 1, 5 and 6, haplotype 2 constituted an intermediate value class between resistant and susceptible phenotypes, leading to the suggestion for an additional resistance gene contributing to the QTL *Fhb1*.

4.1. Genotyping of the *Fhb1* region with different molecular markers

Fine-mapping of the *Fhb1* region with molecular markers is one of the most important approaches to identify the harbouring resistance gene or genes for FHB resistance. In general, the genotyping with the SSR markers GWM493 and BARC133 and KASP™ markers snp3BS-8, IFA-FM155, IFA-FM277, IFA-FM426_umn10, IFA-FM836 and IFA-FM958 worked out well. A difficulty for the design of the new marker IFA-FM958 was the fact that it was challenging to find the right primer positions in the BAC contig sequence, because a lot of design considerations had to be made in the relatively small sequence areas, such as primer length, melting and annealing temperature, oligonucleotide content and proportion, primer secondary structures as well as repeats. Good primer design is essential for a succesfull PCR (Young et al. 2006). Three potential primer positions could be found, but only one was finally operating, achieving the new KASP™ marker IFA-FM958. The genotypic data from the previous thesis (Gratl, 2015) were checked again via SSR and KASP™ marker analysis with the existing SSR markers GWM493 and BARC133 and KASP™ SNP markers snp3BS-8 and IFA-FM155, IFA-FM277, IFA-FM426_umn10 and IFA-FM836. Multiple measurements of lines ensured high confidence in the identified alleles. Both SSR and SNP marker approaches feature some major advantages. SSR markers are very reliable, but they need substantial investment of time and money to develop, and adequate numbers for high-density mapping are not available in some crops (Collard, Mackill, 2008). SNP markers, on the other hand, are highly abundant and most importantly the genotyping process is very fast and a lot less elaborate (Ben-Ari, Lavi, 2012), especially because of gel

free systems. That is also the fact why I personally consider SNP markers as the more adequate markers for fine-mapping processes, though the development and application is (still) relatively expensive (Berkman et al., 2012).
Although only assumptions upon the few lines with divergent results compared to their haplotype group mean values could be made, such as genotyping errors, inoculation mistakes during the treatment, insufficient suspension concentration due to loading errors or genotype x environment interactions (Miedaner et al., 2001), the mean values of the results show a reliable picture.

4.2. Phenotyping of the recombinant lines for FHB resistance

Fusarium head blight is mainly caused by the plant pathogen fungi *F. graminearum* and *F. culmorum*, while *F. graminearum* is the predominant causal agent of FHB in most areas of the world and therefore only *F. graminearum* inoculum was used for the master thesis at hand (Walter et al., 2010; Windels, 1999; Yang et al., 2003; Goswami, Kistler, 2004, Osborne, Stein, 2007; Leplat et al., 2013).

The point inoculation method as described in the master thesis at hand was selected among other inoculation methods (Sanghyun et al., 2014) to test for resistance against fungal spread within the spike, as conferred by *Fhb1* (Miedaner et al., 2003). The aim of the point inoculation method was not to harm the wheat spikelets by pipetting the spore suspension between palea and lemma of the flower. The assumption was that the suspension itself promotes the fungal growth and infection when placed at flowering stage with emerging anthers on wheat spikes serving as a disease entrance. Accordingly FHB spreads into other spike parts increasing the severity. It is assumed that too cold or too hot temperatures can interfere with the inoculation succeess.

As expected, the flowering periode of all wheat lines lasted for a longer period of more than one month. Plants were checked almost every day to ensure the optimal inoculation date for each individual spike. Though the inoculation could be consistently performed every two days during the whole experiment.
In general, at least two independent biological experiments (locations or years) are required to estimate the repeatability of the resistance evaluation (Buerstmayr et al.,

2009). The high number of recombinant lines inoculated in the randomized complete block design provided reliable and reproducible FHB infection data.
When comparing the phenotypic parameters, spreading incidence and FHB severity, the conclusion can be made that severity gives a more eligible picture for disease symptoms, because of the fact that all inoculated spikes/line are considered in the calculation. The spreading incidence parameter can lead to biased results when most of the inoculated spikes/line only show marginal disease symptoms with disease spread of e.g. one spikelet (a total of three symptomatic spikelets - two point-inoculated and one spikelet due to disease spread). As seen in Figure 16, the distribution of disease spread incidence counts only one third of the recombinant lines as highly resistant. The severity parameter is preferable for evaluating disease symptoms.

4.3. Fine-mapping approaches - supposed candidate genes and assumptions for the QTL *Fhb1*

More than a decade has passed since the major FHB resistance QTL *Fhb1* was detected on chromosome 3BS by Waldron et al. in 1999 (originally named *Qfhs.ndsu-3BS*), and plenty of effort has been put in fine-mapping approaches for this important resistance trait, leading to different theories about candidate genes for FHB resistance (Schweiger et al., 2013).

Research groups are continuously working on several candidate genes assumed to be responsible for the QTL. Since today no gene(s) could be validated. Although multiple markers have been used for MAS, they still define a quite large QTL region. More closely linked markers would be an enormous help in MAS and enhance selection efficiency (Bernardo et al., 2012). Reproducible data are mandatory to create a reliable fine map of QTL candidate genes. Anderson et al. (2001) positioned *Fhb1* between the markers GWM493 and GWM533. The approximate genetic distance of 7 cM could almost be stated by Buerstmayr et al. (2002) with 8 cM. Cuthbert et al. (2006) indicated a major effect of *Fhb1* on infection using flanking markers GWM493 and GWM533 in two independent and large mapping populations (Sumai-3*5/Thatcher and HC374/3*98B69-L47) calculating a genetic distance of 10.63 and 11.05 cM, respectively. The *Fhb1* region could be narrowed down on the proximal boarder with the new flanking marker BARC133 (instead of GWM533) and a genetic distance of 6.5 cM was stated with the markers GWM493 and

BARC133 in the Sumai-3*5/Thatcher mapping population. Gratl (2015) calculated a genetic distance of 3.3 cM, using the flanking markers GWM493 and BARC133, indicating possible reasons for the deviations of the genetic distances due to ambiguous data values. Possible reasons for genetic distances could be population differences in size and structure, genetics of different FHB resistance sources of *Fhb1* and differences between the generations used in the studies (Cuthbert et al., 2006).

In the master thesis at hand the new flanking marker IFA-FM958 could successfully replace the marker BARC133 and furthermore narrow down the proximal boarder of the *Fhb1* region, attaining a more precise map position of the QTL and aiding to reduce linkage drag associated with MAS (Cuthbert et al., 2006). The flanking marker GWM493 on the distal boarder remained and could be assumed as the distal boarder for an additional resistance gene. The major causal gene behind *Fhb1* is located between markers snp3BS-8 and IFA-FM958, surrounding the marker IFA-FM426_umn10, emphasizing the function as nearly diagnostic marker (Liu et al., 2008). Bernardo et al. (2012) suggests the marker snp3BS-8 for Sumai-3 related accessions to be useful for MAS for *Fhb1* in breeding programs. Future research activities should concentrate on the discovery and development of more diagnostic markers to improve FHB resistence breeding (Buerstmayr, 2009).

Several studies were conducted to unravel the mechanism underlying *Fhb1* and a lot of assumptions for the the causal gene(s) and its biological mode of action exist. Lemmens et al. (2005) suggest that resistance to DON is important in the FHB resistance mechanism and hypothesized that *Fhb1* either encodes a DON-glucosyltransferase or regulates the expression of such an enzyme. In contrast Gunnaiah et al. (2012) disagreed with Lemmens et al. (2005) suggestions and claims the involvement of hydroxycinnamic acid amides, flavonoids and lignin monomers in the formation of cell wall appositions, playing a prominent role in interfering the movement of *F. graminearum* in the rachises of plants (Gunnaiah et al., 2012). It is believed, based on transcriptome data, that the activation of the jasmonic acid (JA) defence pathway is mainly responsible that wheat restrains *F. graminearum* spread in the infected wheat parts (Xiao et al., 2013). Zhuang et al. (2013) have associated the downregulation of a pectin methyl esterase inhibitor gene in strains lacking *Fhb1* with the QTL. Whole genome mapping data based on the recently released full wheat gene models of Chinese Spring (Mayer et al. 2014) were used in large-scale

RNA-seq studies (Nussbaumer et al. 2015; Hofstad et al. 2016) for candidate genes research in the *Fhb1* region, presenting stress dependent and QTL associated expression.

Hofstad et al. (2016) found six interesting genes, compared to transcripts from the Jia et al. (2009) study, encoding a Bowman–Birk trypsin inhibitor, a family of protease inhibitors playing a major role in the plant defense response that can be induced by wounding (Eckelkamp et al., 1993; Qi et al., 2005), a 3-isopropylmalate dehydrogenase (He et al., 2011) and a gene with an NB-ARC domain, which is a resistance gene that is involved in pathogen detection and plant resistance (DeYoung, Innes, 2006; Ooijen et al., 2008). These genes represent genes in the resistant genotype that exhibit an *Fhb1* specific response.

Results by Schweiger et al. (2016) highlight DON resistance (bleaching resistance after application of DON toxin via point inoculation) association with *Fhb1*, but still not giving evidence if DON resistance leads to FHB resistance or the DON resistance causing gene is closely linked to the FHB related gene by a different mechanism. Although transgenic wheat expression results by Li et al. (2015) revealed that a barley-derived gene confers high FHB resistance when brought into susceptible lines, no gene could be associated with *Fhb1* yet. But it is supposed that genes conferring to toxin resistance are potential prime candidate *Fhb1* genes (Schweiger et al., 2016).

The release of parts of chromosome 3BS covering the *Fhb1* locus, has not led to new insights into the genetic determinants of *Fhb1* (Choulet et al., 2010). Hao et al. (2012) identified multiple marker associations within an association mapping study and haplotype analysis of a region involving FHB traits, still not able to connect the genetic map to the physical sequence. Schweiger et al. (2016) could generate and screen a BAC library of the highly resistant line CM-82036, a donor for *Fhb1*, establish a genomic contig of 1Mbp in chromosome 3BS with several putative resistance genes and achieving a connection of the physical map to the genetic map of the *Fhb1* region. A relevant resource for candidate gene(s) could be provided for the identification of the highly resistant line CM-82036 harbouring *Fhb1*. The transformation of candidate genes into susceptible lines did not trigger resistance yet and a higher resolved map is needed, but still more putative candidate genes remain for functional testing (Schweiger et al. 2016).

4.3. Methods and approaches for candidate gene validation

The identification of candidate genes in charge of FHB resistance is facilitated with fine-mapping approaches. As soon as candidate genes are detected, their contributions to the resistance trait have to be verified and various methods are available.

- Target- induced local lesions in genomes (TILLING) is a flexible reverse genetic, nontransgenic method, to detect induced or natural DNA polymorphisms (Ben-Ari, Lavi, 2012) and to improve a quality trait in a polyploid crop plant (Slade et al., 2005). It combines random chemical mutagenesis with PCR-based screening of genes of interest (Varshney et al., 2006).

- Virus-induced gene silencing (VIGS) vector technology exploits the plant defense system against virus RNA. The dsRNA replication intermediate is processed into small interfering RNAs (siRNA) in the infected cell that correspond to parts of the viral vector genome, including any nonviral insert (Pang et al., 2013). Those are then recruited to host RISC complexes, which target and inhibit gene expression and protein translation (Zhang et al., 2013). Thus, VIGS can be used to investigate gene function as an alternative to plant transformation.

- Zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) are site-spedific nucleases and allow scientists to directly manipulate virtually any gene in a diverse range of cell types and organisms (Gaj et al., 2013).

- Plant transformation approaches are aiming to introduce DNA from virtually any source into plant cells or tissues and have become part of an essential technology for dissecting the mechanism(s) of plant gene regulation and identifying agriculturally useful genes and their traits (Dandekar, 2004). A number of methods for DNA transfer are available, most importantly among these should be named: Agrobacterium tumefaciens and viral mediated transformation as vector-mediated and particle bombardment (gene gun) and electroporation as vectorless methods (Riveraa, 2012; Peyret et al., 2015).

- CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats – memory system) sequences and Cas (CRISPR-associated - effectors) proteins are the two elements of an ancient defence prokaryotic adaptive restriction system conserved in bacterial genomes and can be seen as an alternative to ZFNs and TALENs for inducing targeted genetic alterations (Gaj et al., 2013).

4.4. Difficulties associated with QTL analyses and MAS

The isolation of relevant genes is influenced by the large genome size of wheat, comprising three genomes (Bagge et al., 2007). Moreover FHB resistance is a very complex quantitative trait (Terzi et al., 2013). There are several causes for the low impact of marker-assisted breeding in general, thereunder the difficult prediction of QTL x environment interactions, the expensive development and use of molecular markers, the retention of studies using molecular markers by companies or research groups or the application gap between research laboratories and plant breeding institutes (Collard, Mackill, 2008). Nevertheless, respectable improvements in resistance breeding have been realized due to great efforts like repeated field and greenhouse-based screenings (Anderson, 2007). FHB resistance in European elite winter wheat is contributed from marker-based backcrossing of the QTL *Fhb1* and a lot of time and effort was placed from the beginning of QTL mapping to the final introgression of this QTL into practical breeding programs. This was possible since *Fhb1* is one of those QTL for disease resistance with the highest explained phenotypic variance found to date, because the marker alleles linked to the resistance allele are not present in European and North American elite wheat, and last but not least due to the fact that near-perfect markers are available (Miedaner, Korzun, 2012). Genes with little effects supporting quantitative traits are more or less ignored by QTL analyses and therefore impede important information for resistance breeding. A new approach with high prospects in the world of plant breeders and scientists is Genomic Selection. This method combines marker data with phenotypic and pedigree data (if available) trying to improve the accurateness of the outlook of breeding and genotypic values (Desta, Ortiz, 2014). Genomic Selection may also enhance a better comprehension of complex resistance traits and facilitate breeding for resistant crop varieties.

5. Conclusion and Outlook

Fusarium head blight is a devastating disease worldwide, affecting quantity and quality of wheat. The cultivation of resistant cultivars is a possibility to avoid infections with the pathogenic fungi. For breeding such cultivars, it is essential to acquire knowledge on the molecular level and identify genes that are responsible for resistance. Numerous studies have been conducted to localize QTL and to estimate their phenotypic effects on FHB resistance in wheat and the *Fhb1* region has been resolved for the susceptible wheat reference genotype Chinese Spring. This emphasizing the complexity of FHB resistance and the importance of a worldwide collaboration of many research groups and breeding stations for a prosper advancement in this challenging field of research, to finally identify the responsible gene(s) for QTL *Fhb1.*

Fine-mapping is an important approach and helps to narrow down QTL regions to facilitate map-based cloning and potentially helps to identify candidate genes. The outcomes gained by this master thesis are part of a fine-mapping approach of the QTL *Fhb1*.

Additional recombinant lines and a broader range of markers are necessary for a further saturation of the genetic map to narrow down the QTL region of *Fhb1*. The sequence of the *Fhb1* region and the near-diagnostic KASP™ markers for *Fhb1* represent an important resource for breeding and further studies aiming to identify the gene(s) responsible for Fusarium resistance.

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7. Appendix

7.1. Formulations

Table 26: Components and amounts of TE-8 buffer

Stock solutions	Final concentration)	Amounts for end volume (100 mL)
1 M Tris, pH: 8	10 mM	1
0,5 M EDTA, pH: 8	1mM	0,2
dH2O		98,8

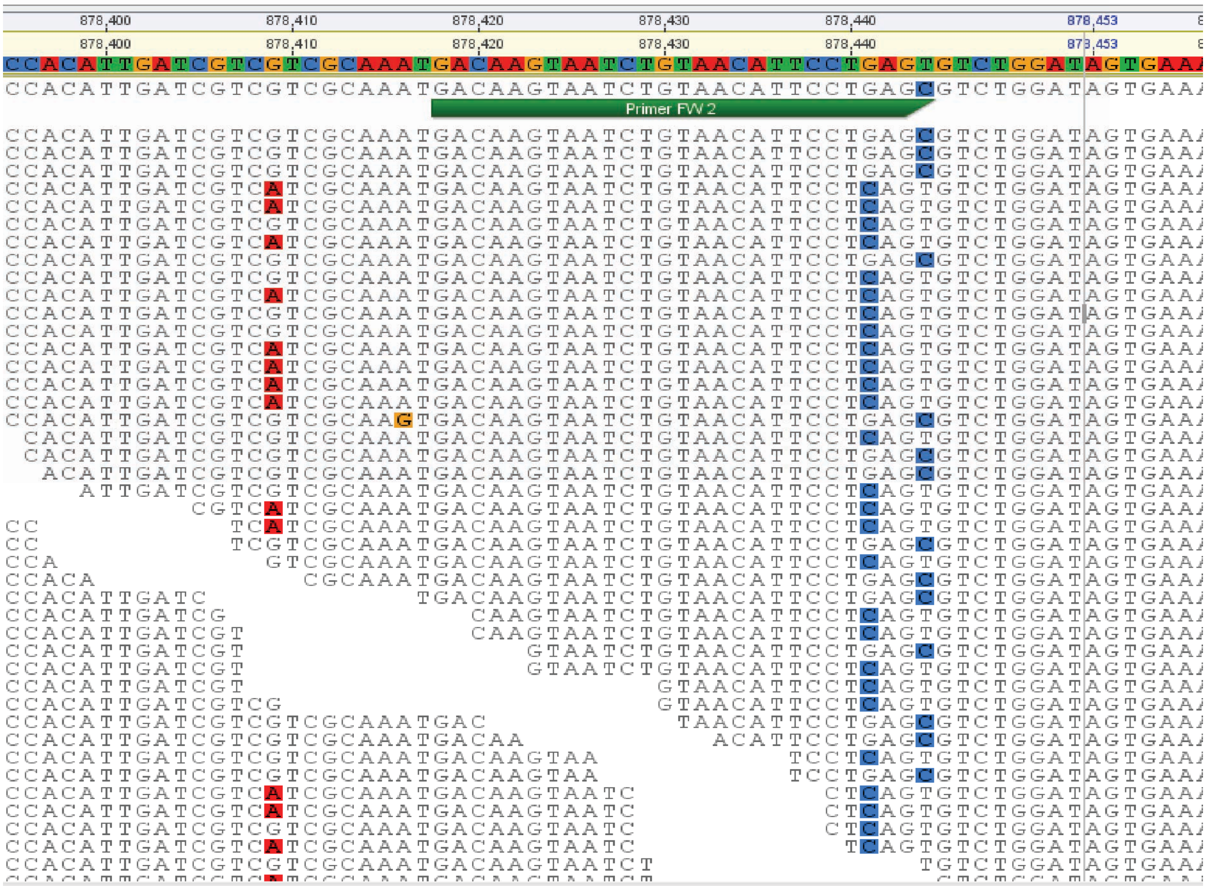
Table 27: Components and amounts of Wash 1

Stock solutions	Final concentration)	Amounts for end volume (100 mL)
dH2O		16
2,5 M NaOA	200mM	8
Ethanol absolute	76%	76

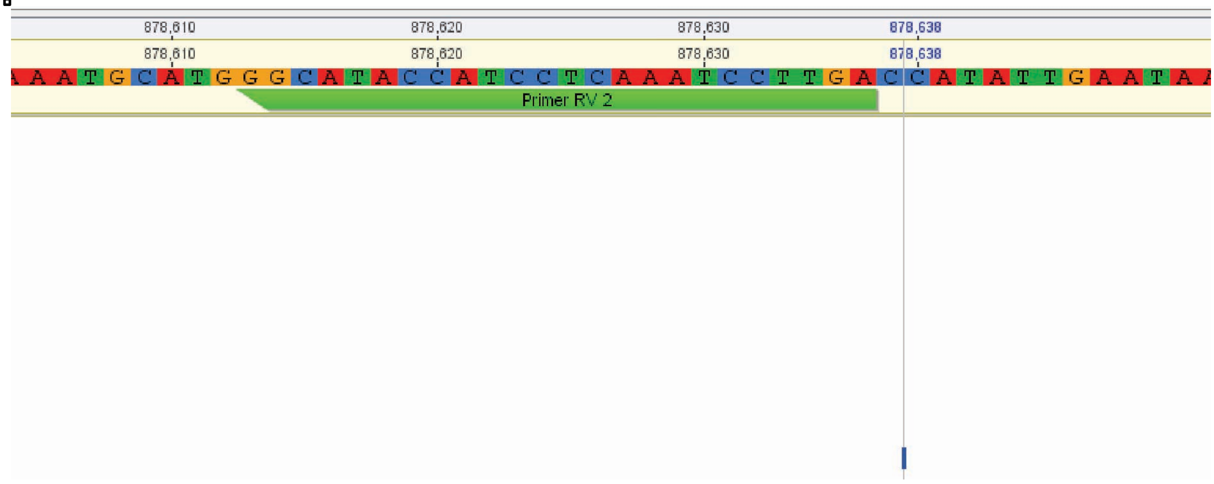
Table 28: Components and amounts of Wash 2

Stock solutions	Final concentration)	Amounts for end volume (100 mL)
dH2O		23
1 M NH4OAc	10mM	1
Ethanol absolute	76%	76

LlRlk? ?ü ?w ?: y ?: g???? g?? - Qp ?? ?Se?o ?



?e?h: ?5- a?? ? ??r oce?S?s h?o ???r ?????? ??er o??o ?cK??r : ü ?: ??d: ew ?: ?dr Secer o?r ?cK??o ?ü ??l ? r d ??w ?: y ?: eo ?cK??Sr ?cü ?: ??? ?o ?er hS?



?e?h: ?Rf a?? ? ??r oce?S?s h?o ???r ?????? ??er o??o ?cK??l ?: S??d: ew ?: ?dr Secer o?r ?cK??o ?ü ??l ? r d ??w ?: y ?: eo ?cK??Sr ?cü ?: ??? ?o ?er hS?

7.3. Genotypic results

Table 29: Recombinant wheat lines and control lines with *Fhb1* marker alleles and haplotype groups. The green colour indicates the parental CM-82036 allele, the red colour the parental Remus allele, the yellow colour undetermined allelic state and empty fields (-) signify no result. Lines with grey background had heterozygous alleles before and were brought to a homozygous state

CM 82036 allele	REMUS allele	heterozygot	undetermined	-	no result

lines	GWM493	Snp3 BS-8	IFA-FM155	IFA-FM227	IFA-FM42 (UMN10)	IFA-FM836	IFA-FM958	BARC133	sample no. / haplotype	haplotype nr.	lines	GWM493	Snp3 BS-8	IFA-FM155	IFA-FM227	IFA-FM42 (UMN10)	IFA-FM836	IFA-FM958	BARC133	sample no. / haplotype	haplotype nr.
9,4									1	1	1546,1									8	3
12,3	-								2	1	1609,3									9	3
13,2	-								3	1	1609,4									10	3
82,2									4	1	1609,7									11	3
116,3									5	1	1633,6									12	3
181,4									6	1	1635,1									13	3
248,1									7	1	1635,4									14	3
365,3									8	1	1659,3									15	3
592,2									9	1	1659,6									16	3
678,5									10	1	1695,1	-								17	3
694,8									11	1	1695,5									18	3
749,2									12	1	1835,3	-								19	3
773,8									13	1	151,3									1	4
789,6									14	1	265,5									2	4
869,7									15	1	273,2									3	4
890,8									16	1	381,5									4	4
957,1									17	1	447,5									5	4
988,1									18	1	528,3									6	4
1067,3									19	1	535,6	-								7	4
1121,1/3									20	1	731,2									8	4
1250.6/8									21	1	942,8									9	4
1259,4									22	1	1137,8									10	4
1265,6									23	1	1177,1/6									11	4
1301,1									24	1	1247,7									12	4
1516,1									25	1	1261,4									13	4
1627,4									26	1	1344,3									14	4
1711,7									27	1	1372,2									15	4
1726,1									28	1	1441,4									16	4
1737,8									29	1	1469,1									17	4
1757,3									30	1	1558,8									18	4
1785,6									31	1	1566,4									19	4
1786,3									32	1	1606,2									20	4
1789,3									33	1	1633,1									21	4
1808,5									34	1	1659,4									22	4
1850,1									35	1	1663,6									23	4
1873,1									36	1	1682,1									24	4
1883,1									37	1	1769,6									25	4
2016,3									38	1	1026,2									1	5
837,4									1	2	1026,6									2	5
837,5									2	2	1957,7									3	5
837,7									3	2	73,2									1	6
2073,2									4	2	73,5									2	6
2073,6									5	2	296,5									3	6
827,6									1	3	809,1									4	6
857,3									2	3	990,4									5	6
1022,7(3)									3	3	1330,3									6	6
1058,3									4	3	1443,4									7	6
1386,1									5	3	1443,5									8	6
1386,4									6	3	1963,3									9	6
1386,6									7	3	1963,6									10	6

7.4. R commands

 # Calculations of spreading severity and spreading incidence

```
mydata <- read.table("CSV file with phenotypic glasshouse data", header = TRUE, sep = ";", quote = "\"'", dec = ".", na.strings="NA")

names(mydata) # returns the names of the columns from your dataframe

mydata$name<-as.factor(as.character(mydata$name))
mydata$WH<-as.factor(as.character(mydata$WH))
mydata$B4<-as.numeric(as.character(mydata$B4))

boxplot(B4~name, data=mydata, main="control lines", xlab="title of x axis", ylab="number of symptomatic spikelets 26dai")          # creates a boxplot of C2 over C1 with exemple of title of boxplot and title of axis

mydata <- read.table("Fhb1_control_linies.csv",header=T,sep=";"
            ,dec=",",blank.lines.skip = TRUE)

str(mydata)
mydata$ID <- as.factor(paste(mydata$name,mydata$WH,sep="/"))

#no.ino.heads
no.ino.heads <- tapply(mydata$B4,mydata$ID,length)
no.ino.heads <- data.frame(ID=names(no.ino.heads),no.ino.heads=no.ino.heads)

#no.spread
zero.spread <- function(x){sum(x==0)}
no.spread <- tapply(mydata$B4,mydata$ID,zero.spread)
no.spread <- data.frame(ID=names(no.spread),no.spread=no.spread)

#no of infected spikelets
no.inf.spikelets <- tapply(mydata$B4,mydata$ID,sum)
no.inf.spikelets <- data.frame(ID=names(no.inf.spikelets),no.inf.spikelets=no.inf.spikelets)

#more than two infected spikelets
larger.than.two <- function(x){sum(x>2)}
more.than.two <- tapply(mydata$B4,mydata$ID,larger.than.two)
more.than.two <- data.frame(ID=names(more.than.two),more.than.two=more.than.two)

#spreading severity
SPREADED.EARS <- mydata[which(mydata$B4>2),]
spreading.severity <- tapply(SPREADED.EARS$B4,SPREADED.EARS$ID,sum)
spreading.severity <- data.frame(ID=names(spreading.severity),spreading.severity=spreading.severity)

########################################
#collect all the results in one dataframe
RESULTS <- merge(no.ino.heads,no.spread,by.x="ID",by.y="ID",all.x=T,all.y=T)
RESULTS <- merge(RESULTS,no.inf.spikelets,by.x="ID",by.y="ID",all.x=T,all.y=T)
RESULTS <- merge(RESULTS,more.than.two,by.x="ID",by.y="ID",all.x=T,all.y=T)
RESULTS <- merge(RESULTS,spreading.severity,by.x="ID",by.y="ID",all.x=T,all.y=T)

RESULTS$spread.incidence <- RESULTS$more.than.two/RESULTS$no.ino.heads
```

```
RESULTS$spreading.severityB <- RESULTS$spreading.severity/RESULTS$more.than.two
RESULTS$severity <- RESULTS$no.inf.spikelets/RESULTS$no.ino.heads

write.table(RESULTS,"RESULTS.txt",col.names=TRUE, row.names=FALSE, quote=TRUE)
```

 # Box plots comparing haplotypes and their number of symptomatic spikelets/spike 26 dai

```
DATA <- read.table("CSV file with prior calculations of spreading severity and
incidence",header=T,sep=";",dec=",")
str(DATA)
DATA$LINE <- as.factor(paste(DATA$recombgr,sep="."))
levels(DATA$LINE)

boxplot(DATA$spreading.severity~DATA$LINE,cex.axis=0.5)

DATA$LINE <- as.character(DATA$LINE)
DATA$LINE[which(DATA$LINE=="1")] <- "A"
DATA$LINE[which(DATA$LINE=="2")] <- "B"
DATA$LINE[which(DATA$LINE=="3")] <- "C"
DATA$LINE[which(DATA$LINE=="4")] <- "D"
DATA$LINE[which(DATA$LINE=="5")] <- "E"
DATA$LINE[which(DATA$LINE=="6")] <- "F"

NAMES <- c("1","2","3","4","5",“6")

DATA$GROUP <- "Z"
DATA$GENO <- as.factor(paste(DATA$rec,DATA$donor,DATA$Rht,sep="."))
DATA$GROUP[which(DATA$GENO %in% c("1"))] <- "C"
DATA$GROUP[which(DATA$GENO %in% c("2","3"))] <- "B"
DATA$GROUP[which(DATA$GENO %in% c("4","5","6"))] <- "A"

COLORS.PLOT <- brewer.pal(5,"Set2")
COLORS.PLOT <- COLORS.PLOT[-4]

BREAKS <- seq(60,120,10)

#-->pooled for all the events

DATA$LINE <- as.factor(paste(DATA$recombgr,sep="."))
levels(DATA$LINE)

boxplot(DATA$spreading.severity~DATA$LINE,cex.axis=0.5)

DATA$LINE <- as.character(DATA$LINE)
DATA$LINE[which(DATA$LINE=="1")] <- "A"
DATA$LINE[which(DATA$LINE=="2")] <- "B"
DATA$LINE[which(DATA$LINE=="3")] <- "C"
DATA$LINE[which(DATA$LINE=="4")] <- "D"
DATA$LINE[which(DATA$LINE=="5")] <- "E"
DATA$LINE[which(DATA$LINE=="6")] <- "F"


DATA <- droplevels(DATA[which(DATA$LINE %in% c("A","B","C","D","E","F")),])
NAMES <- c("1","2","3","4","5","6")

DATA$GROUP <- "Z"
```

```
DATA$GENO <- as.factor(paste(DATA$recombgr,sep="."))
DATA$GROUP[which(DATA$GENO %in% c("1","5","6"))] <- "C"
DATA$GROUP[which(DATA$GENO %in% c("2"))] <- "B"
DATA$GROUP[which(DATA$GENO %in% c("3","4"))] <- "A"
DATA$GROUP
COLORS.PLOT <- brewer.pal(4,"Set1")
COLORS.PLOT <- COLORS.PLOT[-4]
COLORS.PLOT
BREAKS <- seq(60,120,10)

ggplot(data=DATA,aes(x=factor(DATA$LINE),y=DATA$severity)) +
 geom_boxplot(aes(fill = factor(DATA$GROUP)),fatten=2) +
 stat_summary(fun.y=mean, colour="black", geom="point",
         shape="+", size=8,show_guide = FALSE) +
 theme_bw() +
 ggtitle("severity of recombination lines / QTL Fhb1") +
 #scale_fill_brewer(palette="Set1") +
 scale_fill_manual(values=COLORS.PLOT) +
 scale_y_continuous(limits=c(0,12),name="mean number of symptomatic spikelets/spike 26dai") +
 scale_x_discrete(name="",labels=NAMES) +
 theme(title = element_text(size=23,color="black"),
     axis.title.x = element_text(size=18,color="black"),
     axis.title.y = element_text(size=18,color="black"),
     axis.text = element_text(size=22,color="black"),
     #axis.text.x= element_text(angle=0,hjust=0,vjust=0),
     panel.grid.major.y = element_line(color = "grey"),
     panel.grid.major.x = element_line(color = "white"),
     panel.grid.minor = element_line(color = "grey"),
     legend.title = element_blank(),
     legend.justification=c(1,0),
     legend.position="none",
     legend.title = element_text(size=16),
     legend.text = element_text(size=16),
     legend.key = element_rect(fill="white",linetype=0))
```

 # ANOVA – pair-wise differences among haplotype groups

```
DATA$LINE <- as.factor(paste(DATA$recombgr,sep="."))
DATA$WH <- as.factor(DATA$WH)
str(DATA)
DATA$LINE <- as.character(DATA$LINE)
DATA$LINE <- as.factor(DATA$LINE)
levels(DATA$LINE)
#-->now come the severity
model <- lmer(severity ~ LINE + (1|WH),DATA)
anova(model)
qqnorm(resid(model))
plot(resid(model)~fitted(model),xlab="Fitted value",ylab="Studendized residuals")
differences.list <- difflsmeans(model)
LSMEANS.DIFF <- differences.list$diffs.lsmeans.table
LSMEANS.DIFF$COMPARISON <- row.names(LSMEANS.DIFF)
LSMEANS <- lsmeans(model,"LINE")
LSMEANS <- LSMEANS[[1]]
```

C Sh cS 3d e gü eS e: o S w r o K d r ct d : r hdS

	Estimate	Standard Error	DF	t-value	Lower CI	Upper CI	p-value	COMPARISON
LINE 1 - 2	2.1086	0.5975	174	3.53	0.9294	3.2878	0.0005	LINE 1 - 2
LINE 1 - 3	3.1950	0.3903	174	8.19	2.4247	3.9654	0.0000	LINE 1 - 3
LINE 1 - 4	3.1864	0.3354	174	9.50	2.5243	3.8485	0.0000	LINE 1 - 4
LINE 1 - 5	-1.7781	0.7518	174	-2.37	-3.2619	-0.2943	0.0191	LINE 1 - 5
LINE 1 - 6	-0.3039	0.4481	174	-0.68	-1.1883	0.5805	0.4985	LINE 1 - 6
LINE 2 - 3	1.0865	0.6481	174	1.68	-0.1927	2.3656	0.0955	LINE 2 - 3
LINE 2 - 4	1.0778	0.6166	174	1.75	-0.1392	2.2949	0.0822	LINE 2 - 4
LINE 2 - 5	-3.8867	0.9126	174	-4.26	-5.6879	-2.0854	0.0000	LINE 2 - 5
LINE 2 - 6	-2.4125	0.6845	174	-3.52	-3.7635	-1.0615	0.0005	LINE 2 - 6
LINE 3 - 4	-0.0086	0.4191	174	-0.02	-0.8357	0.8185	0.9836	LINE 3 - 4
LINE 3 - 5	-4.9731	0.7926	174	-6.27	-6.5376	-3.4087	0.0000	LINE 3 - 5
LINE 3 - 6	-3.4990	0.5137	174	-6.81	-4.5128	-2.4851	0.0000	LINE 3 - 6
LINE 4 - 5	-4.9645	0.7671	174	-6.47	-6.4786	-3.4504	0.0000	LINE 4 - 5
LINE 4 - 6	-3.4903	0.4734	174	-7.37	-4.4246	-2.5560	0.0000	LINE 4 - 6
LINE 5 - 6	1.4742	0.8226	174	1.79	-0.1495	3.0978	0.0749	LINE 5 - 6

e h: R5a 3d e gü eS e: o S w r o K d r cdt : r hdS

7.5. Phenotypic results & calculations

Table 30 Phenotypic data and calculations showing from left to right: rep. = number of replication (two replications); name = sample names (sample names varying only in numbers after comma are sister lines); haplotype = haplotype group coloured for a better distinction (six different haplotypes can be found in the *Fhb1* region); no.ino.heads. = number of heads that got inoculated per replication; no.inf.spikelets. = total number of infected spikelets per replication; > 2 spikelets/spike infected = number of spikes where more than two spikelets got infected; spread.incidence = (spreading incidence) Percentage of spikes with more infected spikelets than the two inoculated spikelets; severity = division of no.inf.spikelets divided by no.ino.heads. The table cells from the last two columns – spread.incidence and severity – are coloured for a better visualization, whereupon a colour range from green to red signalizes infection rate. Green colour tones (from dark green to bright green and yellow) are signalizeing no or low infection rate and colour tones from bright orange to dark orange and red tones are signalizing higher infection rates

rep.	name	haplotype	no.ino.heads	no.inf.spikeletes	> 2 spikelets/spike infected	spread. incidence	severity
1	9,4	1	4	10	1	0,25	2,5
1	12,3	1	4	16	2	0,5	4
1	13,2	1	3	28	3	1	9,33
1	82,2	1	4	27	3	0,75	6,75
1	116,3	1	4	16	1	0,25	4
1	181,4	1	7	45	4	0,57	6,43
1	248,1	1	2	17	1	0,5	8,5
1	365,3	1	7	31	5	0,71	4,43
1	592,2	1	5	33	3	0,6	6,6
1	678,5	1	6	41	5	0,83	6,83
1	694,8	1	5	28	3	0,6	5,6
1	749,2	1	5	23	3	0,6	4,6
1	773,8	1	6	56	4	0,67	9,33
1	789,6	1	8	59	6	0,75	7,38
1	869,7	1	5	38	3	0,6	7,6
1	890,8	1	5	21	2	0,4	4,2
1	957,1	1	5	23	3	0,6	4,6
1	988,1	1	5	54	4	0,8	10,8
1	1067,3	1	5	53	4	0,8	10,6
1	1121,1/,3	1	7	79	7	1	11,29
1	1250,6/,8	1	5	30	5	1	6
1	1259,4	1	6	49	5	0,83	8,17
1	1265,6	1	6	44	5	0,83	7,33
1	1301,1	1	5	12	2	0,4	2,4
1	1516,1	1	6	50	6	1	8,33
1	1627,4	1	6	32	3	0,5	5,33
1	1711,7	1	5	17	1	0,2	3,4
1	1726,1	1	6	39	4	0,67	6,5
1	1737,8	1	6	19	4	0,67	3,17
1	1757,3	1	6	22	4	0,67	3,67
1	1785,6	1	5	21	5	1	4,2
1	1786,3	1	5	10	0	0	2
1	1789,3	1	5	21	4	0,8	4,2
1	1808,5	1	6	26	3	0,5	4,33
1	1850,1	1	5	14	2	0,4	2,8
1	1873,1	1	5	15	3	0,6	3
1	1883,1	1	5	12	2	0,4	2,4
1	2016,3	1	5	16	1	0,2	3,2
1	837,4	2	3	11	2	0,67	3,67
1	837,5	2	6	15	2	0,33	2,5
1	837,7	2	6	15	2	0,33	2,5
1	2073,2	2	8	35	5	0,63	4,38
1	2073,6	2	10	24	4	0,4	2,4
1	827,6	3	6	13	1	0,17	2,17
1	857,3	3	6	13	1	0,17	2,17
1	1022,7(3)	3	4	8	0	0	2

rep.	name	haplotype	no.ino.heads	no.inf.spikeletes	> 2 spikelets/spike infected	spread. incidence	severity
1	1058,3	3	2	5	1	0,5	2,5
1	1386,1	3	6	14	2	0,33	2,33
1	1386,4	3	6	14	2	0,33	2,33
1	1386,6	3	6	15	2	0,33	2,5
1	1546,1	3	2	4	0	0	2
1	1609,3	3	8	17	1	0,13	2,13
1	1609,4	3	8	16	0	0	2
1	1609,7	3	10	20	0	0	2
1	1633,6	3	9	20	2	0,22	2,22
1	1635,1	3	9	18	0	0	2
1	1635,4	3	10	18	0	0	1,8
1	1659,3	3	9	21	3	0,33	2,33
1	1659,6	3	9	16	0	0	1,78
1	1695,1	3	7	18	3	0,43	2,57
1	1695,5	3	9	18	0	0	2
1	1835,3	3	10	22	2	0,2	2,2
1	151,3	4	5	12	2	0,4	2,4
1	265,5	4	5	9	0	0	1,8
1	273,2	4	5	10	0	0	2
1	381,5	4	6	12	0	0	2
1	447,5	4	6	12	0	0	2
1	528,3	4	5	10	0	0	2
1	535,6	4	5	13	2	0,4	2,6
1	731,2	4	6	11	1	0,17	1,83
1	942,8	4	5	9	0	0	1,8
1	1137,8	4	3	6	1	0,33	2
1	1177,1/,6	4	6	12	1	0,17	2
1	1247,7	4	6	12	0	0	2
1	1261,4	4	5	10	0	0	2
1	1344,3	4	5	9	0	0	1,8
1	1372,2	4	6	13	1	0,17	2,17
1	1441,4	4	6	12	0	0	2
1	1469,1	4	6	13	1	0,17	2,17
1	1558,8	4	6	12	0	0	2
1	1566,4	4	5	9	1	0,2	1,8
1	1606,2	4	6	17	4	0,67	2,83
1	1633,1	4	5	15	3	0,6	3
1	1659,4	4	6	10	0	0	1,67
1	1663,6	4	5	10	0	0	2
1	1682,1	4	5	11	2	0,4	2,2
1	1769,6	4	6	12	0	0	2
1	1026,2	5	3	7	1	0,33	2,33
1	1026,6	5	6	28	4	0,67	4,67
1	1957,7	5	10	69	7	0,7	6,9
1	73,2	6	10	31	4	0,4	3,1
1	73,5	6	5	15	3	0,6	3
1	296,5	6	8	40	8	1	5
1	809,1	6	8	34	6	0,75	4,25
1	990,4	6	10	46	6	0,6	4,6
1	1026,2	6	3	7	1	0,33	2,33
1	1026,6	6	6	28	4	0,67	4,67
1	1330,3	6	9	67	7	0,78	7,44
1	1443,4	6	5	26	3	0,6	5,2
1	1443,5	6	5	31	3	0,6	6,2
1	1957,7	6	10	69	7	0,7	6,9
1	1963,3	6	10	41	6	0,6	4,1
1	1963,6	6	10	40	7	0,7	4

rep.	name	haplotype	no.ino.heads	no.inf.spikeletes	> 2 spikelets/spike infected	spread. incidence	severity
2	9,4	1	5	29	4	0,8	5,8
2	12,3	1	6	25	5	0,83	4,17
2	13,2	1	5	20	3	0,6	4
2	82,2	1	8	34	8	1	4,25
2	116,3	1	7	36	6	0,86	5,14
2	181,4	1	6	32	6	1	5,33
~~2~~	~~248,1~~	~~1~~	-	-	-	-	-
2	365,3	1	5	19	4	0,8	3,8
2	592,2	1	5	24	5	1	4,8
2	678,5	1	5	20	3	0,6	4
2	694,8	1	6	28	5	0,83	4,67
2	749,2	1	6	33	6	1	5,5
2	773,8	1	5	28	3	0,6	5,6
2	789,6	1	8	53	6	0,75	6,63
2	869,7	1	6	48	5	0,83	8
2	890,8	1	5	33	3	0,6	6,6
2	957,1	1	5	60	5	1	12
2	988,1	1	3	32	3	1	10,67
2	1067,3	1	6	36	3	0,5	6
2	1121,1/,3	1	8	57	6	0,75	7,13
2	1250,6/,8	1	7	68	6	0,86	9,71
2	1259,4	1	6	39	3	0,5	6,5
2	1265,6	1	5	35	4	0,8	7
2	1301,1	1	3	15	2	0,67	5
2	1516,1	1	7	37	4	0,57	5,29
2	1627,4	1	7	19	3	0,43	2,71
2	1711,7	1	5	31	4	0,8	6,2
2	1726,1	1	5	23	5	1	4,6
2	1737,8	1	7	26	3	0,43	3,71
2	1757,3	1	8	28	4	0,5	3,5
2	1785,6	1	9	49	4	0,44	5,44
2	1786,3	1	7	46	5	0,71	6,57
2	1789,3	1	5	43	4	0,8	8,6
2	1808,5	1	9	66	8	0,89	7,33
2	1850,1	1	7	19	4	0,57	2,71
2	1873,1	1	5	17	2	0,4	3,4
2	1883,1	1	5	15	3	0,6	3
2	2016,3	1	9	17	1	0,11	1,89
2	837,4	2	10	28	5	0,5	2,8
2	837,5	2	9	38	5	0,56	4,22
2	837,7	2	7	16	2	0,29	2,29
2	2073,2	2	10	39	4	0,4	3,9
2	2073,6	2	10	36	3	0,3	3,6
2	827,6	3	10	19	0	0	1,9
2	857,3	3	10	23	2	0,2	2,3
2	1022,7(3)	3	6	13	1	0,17	2,17
2	1058,3	3	6	19	2	0,33	3,17
2	1386,1	3	10	20	0	0	2
2	1386,4	3	10	19	1	0,1	1,9
2	1386,6	3	10	20	1	0,1	2
2	1546,1	3	2	4	0	0	2
2	1609,3	3	10	20	0	0	2
2	1609,4	3	10	20	0	0	2
2	1609,7	3	10	20	1	0,1	2
2	1633,6	3	10	20	0	0	2
2	1635,1	3	10	29	2	0,2	2,9
2	1635,4	3	10	19	0	0	1,9
2	1659,3	3	6	11	0	0	1,83
2	1659,6	3	10	20	0	0	2
2	1695,1	3	10	21	1	0,1	2,1
2	1695,5	3	10	30	5	0,5	3
2	1835,3	3	7	14	0	0	2
2	151,3	4	5	11	1	0,2	2,2
2	265,5	4	5	12	2	0,4	2,4
2	273,2	4	8	20	3	0,38	2,5

rep.	name	haplotype	no.ino.heads	no.inf.spikeletes	> 2 spikelets/spike infected	spread. incidence	severity
2	381,5	4	6	16	3	0,5	2,67
2	447,5	4	7	14	0	0	2
2	528,3	4	5	10	0	0	2
2	535,6	4	6	12	0	0	2
2	731,2	4	5	13	2	0,4	2,6
2	942,8	4	5	13	2	0,4	2,6
2	1137,8	4	3	6	0	0	2
2	1177,1/,6	4	5	10	1	0,2	2
2	1247,7	4	6	13	2	0,33	2,17
2	1261,4	4	6	12	0	0	2
2	1344,3	4	6	12	0	0	2
2	1372,2	4	7	16	2	0,29	2,29
2	1441,4	4	6	14	2	0,33	2,33
2	1469,1	4	5	10	0	0	2
2	1558,8	4	6	11	0	0	1,83
2	1566,4	4	6	15	3	0,5	2,5
2	1606,2	4	5	13	3	0,6	2,6
2	1633,1	4	8	16	0	0	2
2	1659,4	4	5	10	0	0	2
2	1663,6	4	5	10	0	0	2
2	1682,1	4	7	17	2	0,29	2,43
2	1769,6	4	5	10	0	0	2
2	1026,2	5	6	59	5	0,83	9,83
2	1026,6	5	8	48	6	0,75	6
2	1957,7	5	10	130	10	1	13
2	73,2	6	9	25	2	0,22	2,78
2	73,5	6	10	56	7	0,7	5,6
2	296,5	6	10	94	10	1	9,4
2	809,1	6	10	31	3	0,3	3,1
2	990,4	6	9	30	3	0,33	3,33
2	1330,3	6	10	69	9	0,9	6,9
2	1443,4	6	10	80	9	0,9	8
2	1443,5	6	10	73	9	0,9	7,3
2	1963,3	6	10	116	7	0,7	11,6
2	1963,6	6	10	80	7	0,7	8

Table 31: Phenotypic data and mean calculations of both replications showing (from left to right): name = sample names (sample names varying only in numbers after comma are sister lines); haplotype = haplotype group (six different haplotypes can be found in the *Fhb1* region); no.ino.heads. = number of heads that got inoculated from both replications; mean spread. incidence = (mean of division of > 2 spikelets/spike divided by no.ino.heads from both replications); mean. severity = (mean of division of no.inf.spikelets divided by no.ino.heads from both replications). The table cells from the last calculations – mean spread. incidence and mean severity – are coloured for a better visualization, whereupon a colour range from green to red signalizes infection rate. Green colour tones (from dark green to bright green and yellow) are signalizeing no or low infection rate and colour tones from bright orange to dark orange and red tones are signalizing higher infection rates

name	haplotype	no.ino.heads	mean spread. incidence	mean. severity
9,4	1	9	0,53	4,15
12,3	1	10	0,67	4,08
13,2	1	8	0,8	6,67
82,2	1	12	0,88	5,5
116,3	1	11	0,55	4,57
181,4	1	13	0,79	5,88
248,1	1	2	0,5	4,25
365,3	1	12	0,76	4,11
592,2	1	10	0,8	5,7
678,5	1	11	0,72	5,42
694,8	1	11	0,72	5,13
749,2	1	11	0,8	5,05
773,8	1	11	0,63	7,47
789,6	1	16	0,75	7
869,7	1	11	0,72	7,8
890,8	1	10	0,5	5,4
957,1	1	10	0,8	8,3
988,1	1	8	0,9	10,73
1067,3	1	11	0,65	8,3
1121,1/,3	1	15	0,88	9,21
1250,6/,8	1	12	0,93	7,86
1259,4	1	12	0,67	7,33
1265,6	1	11	0,82	7,17
1301,1	1	8	0,53	3,7
1516,1	1	13	0,79	6,81
1627,4	1	13	0,46	4,02
1711,7	1	10	0,5	4,8
1726,1	1	11	0,83	5,55
1737,8	1	13	0,55	3,44
1757,3	1	14	0,58	3,58
1785,6	1	14	0,72	4,82
1786,3	1	12	0,36	4,29
1789,3	1	10	0,8	6,4
1808,5	1	15	0,69	5,83
1850,1	1	12	0,49	2,76
1873,1	1	10	0,5	3,2
1883,1	1	10	0,5	2,7
2016,3	1	14	0,16	2,54
837,4	2	13	0,58	3,23
837,5	2	15	0,44	3,36
837,7	2	13	0,31	2,39
2073,2	2	18	0,51	4,14
2073,6	2	20	0,35	3
827,6	3	16	0,08	2,03
857,3	3	16	0,18	2,23
1022,7(3)	3	10	0,08	2,08
1058,3	3	8	0,42	2,83
1386,1	3	16	0,17	2,17
1386,4	3	16	0,22	2,12
1386,6	3	16	0,22	2,25
1546,1	3	4	0	2

name	hap.group	no.ino.heads	mean.spread.incidence	mean.severity
1609,3	3	18	0,06	2,06
1609,4	3	18	0	2
1609,7	3	20	0,05	2
1633,6	3	19	0,11	2,11
1635,1	3	19	0,1	2,45
1635,4	3	20	0	1,85
1659,3	3	15	0,17	2,08
1659,6	3	19	0	1,89
1695,1	3	17	0,26	2,34
1695,5	3	19	0,25	2,5
1835,3	3	17	0,1	2,1
151,3	4	10	0,3	2,3
265,5	4	10	0,2	2,1
273,2	4	13	0,19	2,25
381,5	4	12	0,25	2,33
447,5	4	13	0	2
528,3	4	10	0	2
535,6	4	11	0,2	2,3
731,2	4	11	0,28	2,22
942,8	4	10	0,2	2,2
1137,8	4	6	0,17	2
1177,1/,6	4	11	0,18	2
1247,7	4	12	0,17	2,08
1261,4	4	11	0	2
1344,3	4	11	0	1,9
1372,2	4	13	0,23	2,23
1441,4	4	12	0,17	2,17
1469,1	4	11	0,08	2,08
1558,8	4	12	0	1,92
1566,4	4	11	0,35	2,15
1606,2	4	11	0,63	2,72
1633,1	4	13	0,3	2,5
1659,4	4	11	0	1,83
1663,6	4	10	0	2
1682,1	4	12	0,34	2,31
1769,6	4	11	0	2
1026,2	5	9	0,58	6,08
1026,6	5	14	0,71	5,33
1957,7	5	20	0,85	9,95
73,2	6	19	0,31	2,94
73,5	6	15	0,65	4,3
296,5	6	18	1	7,2
809,1	6	18	0,53	3,68
990,4	6	19	0,47	3,97
1330,3	6	19	0,84	7,17
1443,4	6	15	0,75	6,6
1443,5	6	15	0,75	6,75
1963,3	6	20	0,65	7,85
1963,6	6	20	0,7	6