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High-resolution mapping of a QTL for Fusarium Head Blight resistance on chromosome 2A in *Triticum monococcum*

Dissertationen aus dem Julius Kühn-Institut

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Bibliografische Information der Deutschen Nationalbibliothek
Die Deutsche Nationalbibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie: detaillierte bibliografische Daten sind im Internet über http://dnb.d-nb.de abrufbar.

Bibliographic information published by the Deutsche Nationalbibliothek
(German National Library)
The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available in the Internet at http://dnb.dnb.de.

ISBN 978-3-95547-100-2
ISSN (elektronisch) 2510-0602
ISSN (Druck) 2510-0599
DOI 10.5073/dissjki.2020.009

Herausgeber l Editor
Julius Kühn-Institut, Bundesforschungsinstitut für Kulturpflanzen, Quedlinburg, Deutschland
Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Quedlinburg, Germany

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Dissertation

"High-resolution mapping of a QTL for Fusarium Head Blight resistance on chromosome 2A in *Triticum monococcum*"

Submitted by

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born in **Bad Driburg**, Germany

for a Doctorate Degree in Agricultural Sciences

Doctor scientiarum agrarium (Dr. sc. agr.)

Göttingen 2020

Examining Committee:

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Examiner: Prof. Dr. Heiko Becker

Examiner: Prof. Dr. Frank Ordon

Examiner: Dr. Christian Möllers

Date of Defence: 21.09.2020

„It is easy to find a QTL, but much more difficult to find the same QTL twice“ (RC SCHOEMAKER 1995 in BERNARDO 2016)

For my parents, who have always supported me…

Content

Summary

Securing wheat production is of prime importance with regard to feeding the earth's growing population. Wheat is threatened by a lot of abiotic and biotic factors leading to severe yield losses. One important disease is Fusarium Head Blight (FHB), caused by different *Fusarium* spp. The disease leads to yield losses up to 40 %, a reduction in quality and a health risk for mankind due to toxic secondary metabolites that arise during the infection process. Therefore, FHB belongs to the most important wheat diseases and is extensively studied worldwide. To improve resistance of wheat to *Fusarium* spp., this study was conducted to get detailed information on the genetics of a new source of resistance, detcted in *Triticum monococcum*, which is a close relative of bread wheat. To achieve this, a DH-population based on a cross between *Triticum monococcum* accession *mon10-1*, which is moderately resistant to FHB and the FHB susceptible *Triticum monococcum* L. conv. *sinskayae* (*Sinskayae*) comprising of 94 DH-lines was analysed. The population was phenotyped in two years field trials and genotyped by DArT analyses resulting in a genetic map of 1987.55 cM. Based on these data, two neighbouring QTLs were mapped in an interval of 45.1 cM on the short arm of chromosome 2A. Further analyses aimed at shortening the QTL interval and the identification of closely linked markers and candidate genes by a map-based cloning approach. A high-resolution mapping population was developd out of 1991 F_2-plants, that traced back to crosses between three susceptible and two resistant DH-lines of the original population. 333 RILs were developed of which 268 were used for phenotypic evaluation with *F. culmorum* (Isolate: *Fc46*) in field and greenhouse trials. Marker saturation was conducted based on the 90K iSelect chip, genotyping-by-sequencing (GBS) and known genetic maps of *Triticum monococcum*. Out of these, 21 KASP markers were developed and mapped within the QTL interval. Assigning these markers to the physical map of *T. aestivum* resulted in an interval of 31.4 Mbp. However, by phenotyping respective segmental RILs, the resistance locus was not located within this interval. A new QTL analysis with a reduced marker set of the DH-mapping population using their physical postitions was conducted and resulted in a switch of the peak markers to a proximal region of chromosome 2A into an interval between 499.25 – 607.96 Mbp. This QTL mapped in the same region like the *soft glume (sog)*-gene, but it is unclear if the QTL effect is due to tight linkage between *sog*- and FHB resistance gene or pleiotropy.

Zusammenfassung

Die Sicherstellung und Erhöhung des Weizenertrags hat heutzutage weltweit große Bedeutung, um die Ernährung der stetig wachsenden Gesellschaft zu sichern. Eine sehr bedeutende Krankheit im Weizen ist die Ährenfusariose (engl. Fusarium Head Blight, FHB), die durch verschiede *Fusarium* spp.- Pilze hervorgerufen wird. Diese kann zu Ertragsverlusten bis zu 40% führen und durch die Bildung von Mykotoxinen während des Infektionszyklus, die Qualität mindern sowie die Gesundheit von Mensch und Tier gefährden. In der folgenden Studie wurde das Resistenzverhalten im Einkorn (*Triticum monococcum*) gegenüber *Fusarium* untersucht. Dazu wurde eine DH-population, bestehend aus 94 DH-Linien, erstellt und analysiert, die auf eine Kreuzung zwischen *Triticum monococcum* L. (*mon10-1:* moderates Resistenzverhalten) und *Triticum monococcum* L. conv. *sinskayae* (*Sinskayae*: anfällig) zurückgeht. Die DH-Population wurde in zweijährigen Feldversuchen phänotypisiert und mit DArT und SSR-Markern genotypisiert, was in einer genetischen Karte von 1987.55 cM resultierte. In einer anschließenden QTL-Analyse wurden zwei benachbarte QTL auf Chromosom 2A in einem Intervall von 45.1 cM (31.4 Mbp) kartiert. Mit der Methode der kartengestützten Genisolierung wurde das QTL Intervall verkleinert um eng gekoppelte Marker oder Kandidatengene zu identifizieren, die diese Variation bewirken. Dazu wurde eine hochauflösende Kartierungspopulation, bestehend aus 1991 F_2-Pflanzen erstellt, die auf eine Kreuzung zwischen zwei resistenten und drei anfälligen DH-Linien der ursprünglichen DH-Population zurückgeht. Es konnten 333 rekombinante Inzuchtlinien (RIL) identifiziert werden. Von diesen wurden 268 RILs in Gewächshaus- und Feldversuchen mit dem *Fusarium*-Isolat *Fc46* phänotypisiert und mit 21, durch genotyping-by-sequencing (GBS), den 90K iSelect Chip und der genetischen Karte von *Triticum monococcum*, neu entwickelten KASP-Markern genotypisiert. Dennoch war es nicht möglich den Resistenzlocus innerhalb des Intervalls zu kartieren. Eine neue QTL-Analyse mit den physikalischen Positionen eines reduzierten Markersets aus der ursprünglichen DH-Population zeigte, dass sich die Peak-Marker in eine Region zwischen 499.25 – 607.96 Mbp verschieben. Ebenfalls wird das *sog*-Gen in dieser Region vermutet, welches verantwortlich für die Ährenform von *Triticum sinskayae* ist. Es ist unklar, ob der beobachtete Effekt durch eine enge Kopplung beider Gene in dieser genomischen Region hervorgerufen wird oder durch Pleiotropie.

Chapter I | General introduction

1. Bread wheat (Triticum aestivum L.)

Bread wheat (*Triticum aestivum* L.) is one of the most important crops worldwide with an acreage of 214.8 million hectares and a global annual production of 735.2 million tons (FAOSTAT 2018). It is mainly used for human food and livestock feed. The largest wheat producers in the world are China and India with 131.4 and 99.7 million tons and in summary an acreage of 53 million hectares (FAOSTAT 2018). Germany is still under the top ten wheat producers with a total production of 20.2 million tons and a wheat acreage of 3.03 million hectares (FAOSTAT 2018). Next to maize (*Zea mays*) and rice (*Oryza sativa*), wheat serves as one of the key crops for food security in the world. Wheat covers about 17 % of the arable land worldwide, mainly in the temperate and sub-temperate zone, and about 35 % of the global population take wheat as staple food (IDRC 2010, FAOSTAT 2018). Wheat grain contains starch and protein, dietary fibre, minerals, vitamins, phytochemicals and antioxidants and it is processed into breads, biscuits, noodles, cakes, couscous and beer for human diet (CURTIS & HALFORD 2014, SHEWRY & HEY 2015). Moreover 85 % and 82 % of the human population use wheat as a fundamental calorie and protein source, respectively (CHAVES ET AL. 2013), in total wheat provide ~15% of the world's kcal intake (PELEG ET AL. 2011, TILMAN ET AL. 2011). So in the light of future challenges, i.e. a continuous growing of the human population in the background of climate change, stabilizing and improving wheat production is one of the major goals in agriculture. Since the green revolution in the mid 1960s, wheat yield per hectare increased steadily due to new disease-resistant and genetically improved wheat varieties as well as modern agronomic practices (CURTIS 2002, AHRENDS ET AL. 2018). But in the last two decades wheat yield is stagnating, because of e.g. climate change which is affecting cereal productivity. Especially rising temperatures during the reproductive phase and problems with drought and soil degradation in many regions as well as changes in cultivation management due to political restrictions have negative impact on yield (BRISSON ET AL. 2010, AHLEMEYER & FRIEDT 2011). However, annual wheat yield must rise from below 1 % to 1.6 % (LUCAS 2012) to nourish mankind in the future. There are two possible solutions dealing with this challenge. On the one hand arable land may be expanded and though the wheat growing area increased or the yield per unit area must be improved and pre- and post-harvest losses avoided (CURTIS 2002). Actually, in the last

50 years the portion of arable land increased by only 9 % globally, so the main focus is on the enhancement of grain yield and the implementation of modern agrotechnical practices, which get along with new environmental situations (GODFRAY ET AL. 2010). Crops and respective yields are threatened by lots of biotic and abiotic stresses, so important aims in wheat breeding today are improving the resistance against insects, viruses and fungi as well as the tolerance against heat, drought and soil salinity using conventional and new biotechnological methods. In this respect, genetic resources, e.g. other wheat species like close relatives such as einkorn wheat, wheat landraces as well as wild relatives represent a promising reservoir for the improvement of wheat (MWADZINGENI ET AL. 2017). Moreover, the recently published reference genome of wheat is of high importance for the improvement of wheat cultivars (APPELS ET AL. 2018).

Bread wheat (*Triticum aestivum* L., 2n=6x=42, AABBDD) has a genome size of 17.4 Gb and belongs to the family *Poacaea* and the genus *Triticum*. Present studies divide the genus *Triticum* in six species: two diploid species, *Triticum monococcum* L. and *Triticum urartu* Tum. Ex Grand., two tetraploid species, *Triticum turgidum* L. and *Triticum timopheevii* (Zhuk.) Zhuk., and two hexaploid species, *Triticum aestivum L*. and *Triticum zhukovskyi* Men. & Er. (FELDMANN & LEVY 2015). *Triticum aestivum* is the most widely grown species of the Triticae (95%) today, next to tetraploid durum wheat (*Triticum turgidum* ssp. *durum* (Desf.) Husn.*,* AABB) (5%) (SHEWRY 2009). First evidence of wheat is dated back to over 10.000 years ago in the 'Fertile crescent', a region spanning nowadays Turkey, Syria, Israel, Jordan, Iran and Iraq. Firstly, at this time only diploid and tetraploid wheat forms were detected in this area, but hexaploid species arise 2000 years later in the Caucasian region (CHARMET 2011, SHEWRY 2018, HAAS ET AL. 2019). Today's bread wheat (*Triticum aestivum* L.) originated from natural, independent hybridization of domesticated tetraploid wheat *Triticum turgidum* (AB-Genome) with the progenitor of the D-genome *Aegilops tauschii* (MATSUOKA 2011, MCFADDEN & SEARS 1946). This polyploidization event was very rare in the past and domestication and breeding led to a genetic narrowing of hexaploid wheat (PRZEWIESLIK-ALLEN ET AL. 2019). It is assumed that for bread wheat only a cultivated form and no wild form exist in contrast to einkorn or emmer wheat *(*MATSUOKA 2011).

2. Einkorn wheat (*Triticum monococcum* L.)

Einkorn wheat (*Triticum monococcum* L.) is a cultivated diploid wheat species (A^mA^m, 2n=14) with a genome size of 4.95 Gb (LING ET AL. 2018). The domestication took place 10.000 years ago in the northern 'Fertile crescent', mainly in the Karacadag mountains of South-Eastern Turkey from the wild ancestor *Titicum monococcum* ssp. b*oeticum* (ALLABY ET AL. 2017, HEUN ET AL. 1997). It was the time of the Neolithic revolution, resulting in a change from hunting and gathering to the settlement of humans and the beginning of agriculture (SHEWRY 2018). So, einkorn wheat was a founder crop and staple food in the past and it was used for bread and beer making as well as for animal feed (ZAHARIEVA & MONNEVEUX 2014). Beginning in the bronze age, the hulled einkorn wheat was replaced by higher yielding, free-threshing tetraploid and hexaploid wheat species (BÉKÉS ET AL. 2017). The growing area of einkorn wheat today is <1000 ha and it is cultivated mainly in the Balkans and the Mediterean regions, showing the minor importance of this crop for agricultural economy and human and livestock consumption (POURKHEIRANDISH ET AL. 2018, ZAHARIEVA & MONNEVEUX 2014).

However, in the last years there is increasing renewed interest in ancient wheat species like *Triticum monococcum* L. due to some positive qualities/traits, e.g. the possibility to grow on poor soils and in harsh environments, as well as the social demand for healthy food (WATANEBE 2017, DINU ET AL. 2018). Thus, einkorn wheat is lower in dietary fibre, but has a higher protein and lipid content, as well as high quantities of carotenoids and tocols (tocopherols and tocotrienols) in comparison to common wheat (LACHMAN ET AL. 2013, HIDALGO ET AL. 2014). Morevoer, einkorn wheat is high in important minerals like iron (Fe), zinc (Zn), phosphor (P), mangan (Mn) and copper (Cu) (CAKMAK ET AL. 2000, HLISNIKOVSKÝ ET AL. 2018). Possibly, *Triticum monococcum* can alter the gluten structure and is consequently well-tolerated by gluten-intolerant people (HLISNIKOVSKÝ ET AL. 2018). Nevertheless the benefit of einkorn wheat consumption in comparison to modern bread wheat varieties regarding nutrition affected diseases is still not proven until now (DINU ET AL. 2018). Next to these nutritional effects, einkorn wheat is as a valuable genetic resource for the improvement of bread wheat, especially with respect to resistance. Many resistances to different diseases like stem rust (ROUSE & JIN 2011, CHEN ET AL. 2018), stripe and leaf rust (ZAHARIEVA & MONNEVEUX 2014) as well as powdery mildew (YAO ET AL. 2007, SCHMOLKE ET AL. 2012) were already identified in *Triticum*

monococcum. Moreover, einkorn wheat possesses also interesting genes for abiotic stress tolerance, e.g. the salt tolerance gene *TmMnSOD*, which was recently isolated and gained much interest in wheat breeding (TOUNSI ET AL. 2019). Suitable genes can be introgressed into bread wheat via direct hybridization and homologous recombination with following repeated backcrossing and selection steps. In some cases tetraploid species as bridge species or 'embryo rescue' may be needed (KAUR ET AL. 2008, SCHMOLKE ET AL. 2012, WULFF & MOSCOU 2014, RATHER ET AL. 2017). *Triticum monococcum* is closely related to *Triticum urartu*, the A-genome donor of hexaploid bread wheat and thus often used for comparative wheat genomic studies (ZHAO ET AL. 2016). The genome sequence of *Triticum urartu* Tumanian ex Gandilyan, published in 2018 (LING ET AL. 2018), as well as the construction of high-resolution linkage einkorn wheat maps by advanced next-generation sequencing techniques (MARINO ET AL. 2018) may facilitate efficient map-based cloning of important genes as well as marker-assisted selection in *Triticum monococcum.* This opens the way for einkorn wheat to be an important source for future wheat breeding programs.

Figure 1 | Ear shape of *Triticum monoccocum* (left) and *Triticum monococcum* L. conv. sinskayae (right) (VALLEGA 1992)

Einkorn wheat is a hulled species, so the glume has to be removed during milling, which costs enormous effort and time. Thus, free-threshing was an important domestication trait and is controlled by two *Tg*-genes and the *Q*-locus on chromosome 5 of bread wheat and by the *sog*-gene in einkorn wheat (HAAS ET AL. 2019). The *sog*-gene is responsible for soft glumes and was mapped on the short arm of chromosome 2A (TAENZLER ET AL. 2002, SOOD ET AL. 2009). As first assumed, *sog* and *Tg* are no orthologs and the lack of the free-threshing trait in einkorn wheat may be due to the negative correlation between the ear length and the *sog*-gene, which can not be compensated by the presence of additional B and D-genomes (DUBCOVSKY & DVORAK 2007, HAAS ET AL. 2019). There is one variety among the diploid wheats possessing this free-threshing trait: *Triticum monococcum* L. conv. *sinskayae* A. Filat. et Kurkiev (MIELKE & RODEMANN 2007). It was detected in one accession K-20970 of *Triticum monococcum* in Turkey by Prof. Zhukovskii (FILATENKO

& KURKIEV 1975, AMAGAI ET AL. 2014). In comparison to *T. monoccocum* L., *Triticum monococcum* L. conv. *sinskayae* has a semi-compact ear shape and soft, longer and wider glumes and a lighter spike color (GONCHAROV ET AL. 2007). Moreover it is smaller in height and ear length. Both varieties were utilized in this study, analysing their reaction to Fusarium Head Blight.

3. Fusarium Head Blight (FHB)

Fusarium Head Blight is one of the most important diseases in wheat and other small grain cereals (e.g. barley, triticale, rye, oat) worldwide when considering yield and quality losses. The causal agents are different *Fusarium* species, particularly a complex of 17 species, which are more or less aggressive (MESTERHÁZY 1984, PARRY ET AL. 1995). They are ubiquitous soil-borne fungal pathogens and it is known that they infect cereals in a broad temperature range, so their appearence range from temperate to semi-tropical regions (ARSENIUK ET AL. 1999). The most prominent ones with high pathogenity in cereals are *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* [Schwein] Petch.) and *Fusarium culmorum* (W.G.Smith) Sacc. (no teleomorph) (WAALWIJK ET AL. 2003, FERNANDEZ & CHEN 2005). Their occurrence depends on the respective climate with *Fusarium graminearum* being more abundant in warmer regions and *Fusarium culmorum* in cooler environments. Thus in Germany, *F. culmorum* is predominant in North-Western Germany, while *F. graminearum* occurs in higher frequency in Southern Germany (MUTHOMI ET AL. 2000, AUFHAMMER ET AL. 2000). Nevertheless the presence of *Fusarium graminearum* in Central and Northern Europe is slowly increasing due to changing weather conditions and rising maize cultivation (PARIKKA ET AL. 2011).

Both are filamentous ascomycetes and hemibiotrophic, i.e. there is a short biotrophic interaction with the host, followed by a necrotrophic phase in which the fungus lives on dead tissue (GOSWAMI & KISTLER 2004). Furthermore, *Fusarium* spp. change between a saprophytic and a pathogenic way of living (SUTTON 1982, MIEDANER ET AL. 2001). In the saprophytic phase, particulary in winter times, the fungi live on crop debris for nutrient uptake, whereas in the pathogenic phase during the vegetation period, they live on living plant tissues (PARRY ET AL. 1995, AUDENAERT ET AL. 2013). *Fusarium graminearum* can reproduce in a sexual way with ascospores and in an asexual way with conidia, while for *Fusarium culmorum* no sexual stage is known (MIEDANER ET AL. 2008). The fungi can overwinter as mycelia or chlamydospores. During the growing

season, when optimal temperature und humidity conditions are present, growth and sporulation is promoted. They produce conidia (asexual stage) and ascospores (sexual stage), which are the main inoculum for the infection of plants (PARRY ET AL. 1995, OSBORNE & STEIN 2007). The favorable mycelia growth temperature ranges from 20 – 25 °C for *F. culmorum* and is about 25°C for *F. graminearum* (WAGACHA & MUTHOMI 2007, OSBORNE & STEIN 2007). Infection is in general supported by high humidity, whereas *F. culmorum* has the lowest need of humidity for successful infection (BEYER ET AL. 2005, KLIX ET AL. 2008, SCHERM ET AL. 2013). The ascospores or conidia are transferred via air and/or rain splash to the ears of host plants (JENKINSON & PARRY 1994). They enter the plant tissue through natural openings, like stomata or anthers during anthesis (BUSHNELL ET AL. 2003). After the initial contact, the spores begin to germinate and develop germ tubes and a dense hyphae network on the inner surface of the lemma (KANG & BUCHENAUER 2000, WAGACHA & MUTHOMI 2007, GUNUPURU ET AL. 2017). The fungi spread inside the plant internally through vascular bundles in the rachis and rachilla or via the exterior surface of the glumes and lemma (through stomata openings) (RIBICHICH ET AL. 2000, GOSWAMI & KISTLER 2004, GURUNUPU ET AL. 2017).

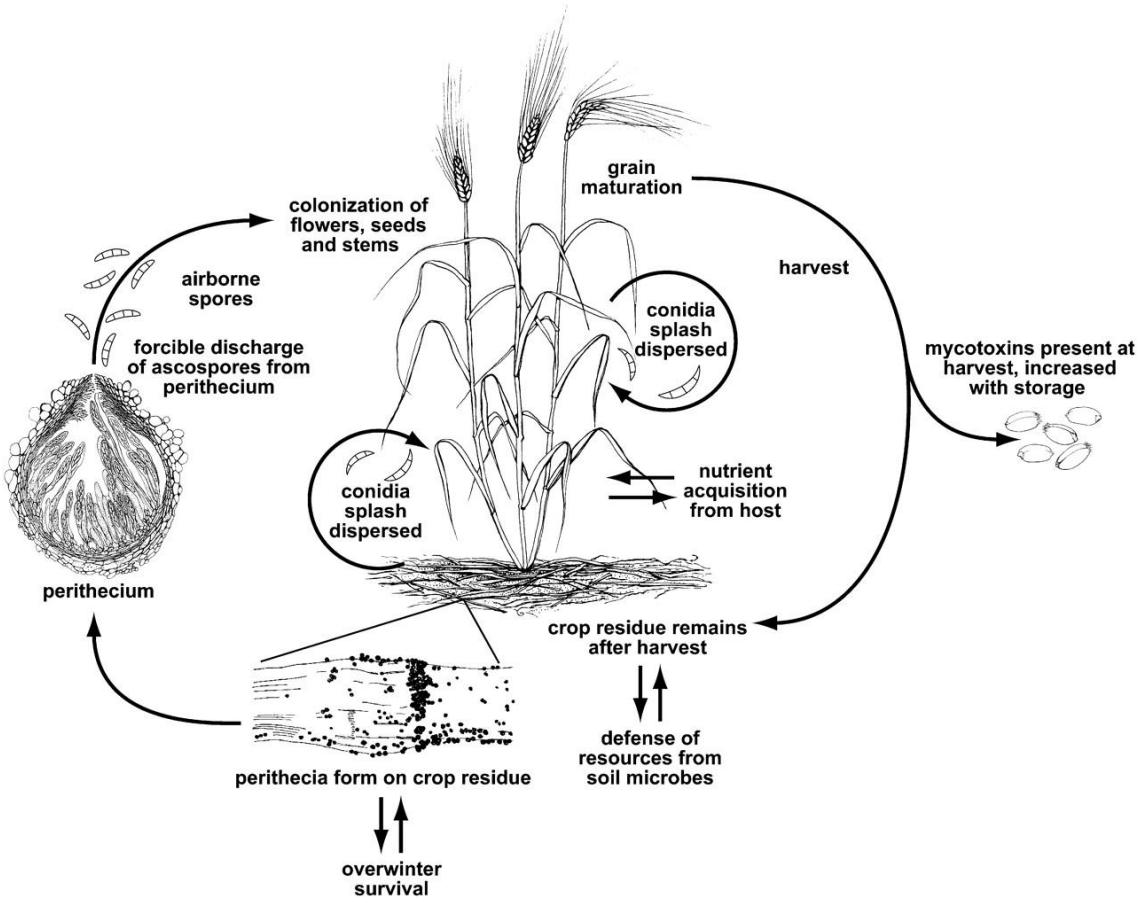

Figure 2 | Life cycle of *Fusarium* spp. (TRAIL 2009)

First symptoms of an infection arise during flowering (BBCH 65) in form of brown, nectrotic spots on the surface of the glume (KANG & BUCHENAUER 2000). Other typical FHB symptoms, i.e. bleaching of spikelets and bleached heads above the infection site, as well as shriveled grains are visible after milk ripeness (BBCH 71-75) and arise from the disruption of nutrient and water transport in the xylem and phloem tissue of the rachis (GOSWAMI & KISTLER 2004). In case of long periods with high humidity, salmon-pink-colored sporodochia on the edge of glumes or base of spikelets as a result of sporulating mycelium are observed (RUCKENBAUER ET AL. 2001, SCHERM ET AL. 2013).

Figure 3 | Symptoms of *F. culmorum* in *Triticum monococcum* (left) with bleached spiekelets and sporodochia and bleached spikes of wheat in the field (right)

FHB can lead to yiel losses up to 40 % in years of severe epidemcis (BAI & SHANER 1994). Next to economic losses, an infection with *Fusarium* spp. also affects the grain quality and contaminates food and feed due to the production of mycotoxins, harmful secondaray fungal metabolites, that arise during the infection process (MCMULLEN ET AL. 1997, AUDENAERT ET AL. 2013). Trichothecenes are an important group of mycotoxins and divided into four different types (A,B,C,D) (TALAS 2011). *F. graminearum* and *F. culmorum* are both main producers of trichothecene type B including the important Nivalenol (NIV), Deoxynivalenol (DON) and its acetylderivatives 3-acetyl-deoxynivalenol (3-ADON) and 15-acetyl-deoxynivalenol (15-ADON), whereas *F. culmorum* is only able to synthesize 3-ADON (JESTOI 2008, CASTIBLANCO ET AL. 2017). Nevertheless DON is economically most important and often detected with highest concentration in cereals (CANADY ET AL. 2001, PIACENTHENI ET AL. 2019). The legal

threshold in Europe for grain contamination with DON is 1.25 mg/kg in unprocessed wheat for human consumption to prevent accumulation of mycotoxins during food production (PIACENTINI ET AL. 2019). Trichothecenes suppress the eukaryotic protein synthesis and lead to mycotoxicosis in man and animal with severe health risks (PERAICA ET AL. 1999). An uptake of mycotoxins can cause liver cancer, convulsions or respiratory problems (DA ROCHA ET AL. 2014, FREIRE & DA ROCHA 2017). Animals, especially mice and pigs are also very sensitive towards mycotoxins and growth and weight gain suppression, anorexia (PESTKA 2010, PENG ET AL. 2018) and immunosuppression are observed (FINK-GREMMELS 1999). Yield reduction and DON contamination lead to economic losses and high risks for farmers when growing wheat, resulting in an observed shift towards other crops in the recent years instead of using costly management practices (DAHL & WILSON 2018). In summary, there is still an urgent need for an effective FHB control reducing the risks for farmers/growers and securing the worldwide wheat production.

Control measures in fighting FHB are an adapted crop management, fungicide treatment, biological control and resistance breeding. However, the best results will be achieved by an integration of two or more methods (WEGULO ET AL. 2015), whereas resistance breeding seems to be the most economic and ecological promising one to reduce losses (BAI & SHANER 2004). It is known that the *Fusarium* species need crop debris as an inoculum source, so conventional tillage is an effective agronomic method to decrease the frequency of Fusarium Head Blight infection as well as the inoculum level in the soil by reducing crop residues (CHAMPEIL ET AL. 2004), but in the last years conservation tillage methods are frequently used. Moreover, a short crop rotation interval with susceptible preceeding host plants like maize, which has risen in acreage considerably e.g. in Germany in the last decades, provide a continuous inoculum for the pathogens and lead to an increase in FHB infection in the following growing period (MCMULLEN 1997, LENC 2015). Thus, crops that are non-hosts for *Fusarium* spp. in the rotation can minimize spore production (FATIMA 2016). In summary, tillage and crop rotation are important factors, that should be taken into account in reducing FHB infection. Some attempts were done in identifying biological control agents, like *Trichoderma* spp.-isolates., that turned out to reduce the growth of *Fusarium graminearium* and *Fusarium culmorum* as well as the DON production (MATARESE ET AL. 2012). In addition, some bacterial *Pseudomonas*

strains reduce disease symptoms by more than 23 % and losses in 1000-grain weight by more than 16 % (KAHN & DOHAAN 2009, DWEBA ET AL. 2017). Another frequently used method in plant protection is the application of fungicides despite their moderate success in controlling FHB infection (DWEBA ET AL. 2017). There is a broad range of fungicides available, whereby most of these belong to the triazoles (WEGULO ET AL. 2013). Fungicides with tebuconazole as active ingriedient or in a mixture with prothioconazole seem to be very effective to reduce FHB (MESERHAZY ET AL. 2003, PAUL ET AL. 2008, WEGULO ET AL. 2013). However, metaconazoles appear to be more efficient for the reduction of DON (PAUL ET AL. 2008). Consequently, a combination of active agents is the best fungicide strategy to prevent yield losses as well as high toxin levels in the grain and to prevent that *Fusarium* spp. become insensitive or even resistant to specific active substances (SERFLING ET AL. 2014). There is high variation concerning the success of FHB suppression, e.g. MESTERHÁZY ET AL. (2003) reported that the successful application not only depends on the respective product, but also on the application date and sufficient fungicide coverage on the plants. Even variability between wheat species was observed, e.g. the efficiacy of fungicides was higher in spring wheat than in soft winter wheat (PAUL ET AL. 2008). Moreover, the interval for the application of fungicides is only a few days during anthesis (WEGULO ET AL. 2013) and should only be conducted when favorite infection conditions for the pathogens are given, which is before rain fall in the critical period to avoid potential environmental contamination (REIS ET AL. 2016). In summary, these results support that resistance breeding should be preferred for FHB control.

Genetic host resistance is the most effective mode of integrated wheat protection for FHB. But *Fusarium culmorum* and *Fusarium graminearum* are not host specific and the resistance is inherited in a quantitative manner, so the complex nature of resistance and genotype x environment (GxE) interactions render breeding challenging and it is diffictult to identify completely resistant genotypes (MESTERHÁZY ET AL 1999, BUERSTMAYR ET AL. 2013). Two types of resistance were recently described: morphological (passive) and physiological (active) resistance (RUDD ET AL. 2001). Morphological traits like plant height (SCHMOLKE ET AL. 2005), ear shape (RUDD ET AL. 2001) or flowering time as well as early grain filling (BAI ET AL. 2001) are often associated with FHB resistance. Thus shorter plants tend to be more susceptible for FHB due to microenvironmental effects, as taller plants dry faster resulting in a drier microclimate which may be considered as a

passive resistance mechanism (MESTERHÁZY 1995, BUERSTMAYR ET AL. 2000). *RhtD-1b* und *RhtB-1b* are reducing plant height alleles and widely used in wheat breeding programmes, although their use results in an increase of FHB susceptibility (BUERSTMAYR ET AL. 2012). However, there might be semi-dwarf genes like *Rht-24*, which reduce height without increasing FHB susceptibility (HERTER ET AL. 2018). Moreover, a negative relationship between heading date/flowering time and FHB severity was demonstrated, i.e. later heading genotypes are more successful in escaping an infection (PAILLARD ET AL. 2004, SCHMOLKE ET AL. 2005). Also traits like spike density and spike length are correlated with increased FHB resistance (BUERSTMAYR ET AL. 2011). In addition, compact spikes seems to be more susceptible against infection (PRAT ET AL. 2014). In contrast, physiological (active) resistance describes mechanisms on the cellular or molecular level during host-pathogen interaction to inhibit an infection or a distribution of infection. Five components of resistance were described (MESTERHÁZY 1995) for FHB, whereas Type I and II are the most commonly investigated mechanisms of FHB disease assesement in different studies (STEINER ET AL. 2004, BUERSTMAYR ET AL. 2013, ARRUDA ET AL. 2016). When using traditional spray inoculation methods for evaluation both types of resistances are assessed, whereas direct spikelet infection focuses on type II (PRAT ET AL. 2014). Type II resistance research is more common due to the difficult and complex evaluation of type I resistance (SUN ET AL. 2016). But, the most effective approach is combining multiple resistance types: initital infection by the pathogen, spread of fungal growth and inhibition of mycotoxin accumulation (YU ET AL. 2008).

Lots of efforts were done in the last decades to identify chromosomal regions being responsible for FHB resistance resulting in 250 QTLs with mostly small effects distributed over all 21 wheat chromosomes (BUERSTMAYR ET AL. 2009, JIA ET AL. 2018). Most prominent and designated QTLs are *Fhb1, Fhb2, Fhb3, Fhb4, Fhb5, Fhb6* and *Fhb7*. Main sources are wheat genotypes with Asian origin, but also European material and close and wild relatives (BUERSTMAYR ET AL. 2019).

4. Molecular markers

Moelcular markers are a powerful tool to detect and trace genes or QTLs of interest during the breeding process (JIANG 2013). Since the 1990s molecular markers are applied in plant breeding and reveal lots of advantages in comparison to morphological markers, like seed colour or yellow streak. They are available in unlimited numbers (JONES ET AL. 2009) and they can be used for screening at any develeopmental stage, especially in the seedling stage to shorten long phenotypic selection steps. They are divided into DNA markers, which differ due to variations in the DNA sequence, and protein markers, in which the variation between genotypes results from structural variants or forms of enzymes (HURKA 1993). The most common used marker type are the DNA markers, which are mainly applied in human genetics as well as in animal and plant breeding. For the implementation in plant breeding programmes the markers should be polymorphic to discriminate genotypic differences between individuals and co-dominant, to distinguish between the homozygous and heterozygous state. Moreover, they should be equally distributed across the whole genome, have no pleiotropic effects and a high reproducibility as well as low costs (WEISING ET AL. 2005).

First generation DNA markers were the RFLP markers (BOTSTEIN ET AL. 1980), which are based on the detection of different restriction fragments generated by mutations in restriction sites or deletions/insertions between those sites. Restriction enzymes cut specific sequences in the DNA resulting in fragments with varying length which are size-separated via gel electrophoresis (BECKMANN & SOLLER 1983). The fragmets are then analysed by southern blotting, so they are transferred to a filter membrane and visualized by hybridization of complementary sequences with fluorescence- or radioactive-labelled DNA probes. They are co-dominant, reliable and have a high reproducibility, but the application of these markers is very time and larbour intensive and a large amount of DNA is needed (WEISING ET AL. 2005). Nonetheless, they were predominantly used in the 1980s/1990s for the construction of genetic linkage maps or genetic fingerprinting, as well as for comparative and synteny studies (NAM ET AL. 1989, TANKSLEY ET AL. 1992, MOORE ET AL. 1995) before the development of PCR-based DNA markers.

PCR-based DNA markers like cleaved amplified polymorphic sequences (CAPS)-, randomly amplified polymorphic DNA (RAPD)-, amplified fragment length polymorphism (AFLP)-, and simple sequence repeats (SSR)-markers are the second

generation of DNA markers. CAPS technique also uses restriction enzymes, which cleave the PCR product on specific sites like RFLPs, and the polymorphism is shown through presence/absence of restriction sites (KONIECZNY & AUSUBEL 1993). In comparison to the RFLP method there is no need of radioactivity or blotting (JONES ET AL. 2009), so the results can be easily interpreted via gel electrophoresis and also shared between laboratories. RAPD markers were a common marker system, which is cheap, quick and simple. Important is here the use of single, arbitrary primers which amplify random genomic DNA segments in PCR (WILLIAMS ET AL. 1990). PCR products are seperated on agarose gels and stained with ethidium bromid for visualization (KARP ET AL. 1996). For the design of PCR primers, no further sequence information is necessary and only small amounts of DNA are needed. They were used for molecular ecology, population studies and resistance breeding, although they have some disadvantages like a low reproducibility and a dominant character (SCHACHERMAYR ET AL. 1994, KARP ET AL. 1996, KELLY & MIKLAS 1998, JONES ET AL. 2009). AFLP marker technique was developed by VOS ET AL. (1995) and its application results in the identification of variations in the resitriction sites of DNA sequences. That means, AFLPs produce restriction fragments with different lengths, that occur due to mutations in restriction sites. Suitable oligonucleotide adapters are ligated to restriction fragments, followed by a specific amplification with different primer combinations using PCR and subsequently visualization via gel electrophoresis (BECKER ET AL. 1995). The AFLP technique analyses a much larger number of loci for polymorphism than other PCR-based techniques and it reveals a higher number of sequenes amplified per reaction as well as a high reproducibility, some reasons why they were frequently used for high-density linkage maps and positional cloning of interesting genes in the past (THOMAS ET AL. 1995, KEIM ET AL. 1997, QI ET AL. 1998). However, they are dominant and purified, high molecular weight DNA is necessary and their application is time-consuming and larbourios (TOMAR 2015). SSR markers are widely used in plant genetic research for the construction of genetic linkage maps, QTL analysis or marker assisted selection since their discovery in the 1980s (VIEIRA ET AL. 2016, TAUTZ & RENZ 1984). Although they are costly in development, there are a lot of advantages like a high reproducibility, co-dominant nature, genome-wide distribution and a high reproducibility (JONES ET AL. 2009). Hence they are often applied in Fusarium Head Blight research (WEI ET AL. 2005) and high-density genetic maps for wheat were developed (SOMERS ET AL. 2004). SSRs are short repeats of nucleotides in coding and

mainly non-coding regions of the genome. The polymorphism is based on the varying number of repitive sequences between individuals in one population. These are amplified in a PCR using primers flanking those sequences and seperated by length with kapillar electrophoresis systems (ENGELMANN 2014).

Today's marker system of choice are the single nucleotide polymorphism (SNP) markers. They belong to the third generation of markers and are based on DNA seuqencing (JONES ET AL. 2009). Polymorphisms between individuals originate from single nucleotide alteration. These can be classified in two categories: transversions, purine-pyrimidine exchanges (C/G, T/A, C/A, G/T) and transitions, purine-purine or pyrimidine-pyrimidine exchanges (C/T, G/A) and are distinguished from insertions/deletions (indels) (VIGNAL ET AL. 2002, HAYWARD ET AL. 2012). SNPs occur in coding and non-coding regions of wheat and a frequency of one SNP per 540-569 bp in the entire genome and one SNP per 335-613 bp in different genes of interest was reported (SHAVRUKOV 2016). They are usually bi-allelic, because two allelic variants are segregating in the population (CASCI 2010) and have a co-dominant character. In addition, they have high genomic abundance, locus specifity and the potential for high-throughput analysis. Thus SNPs serve as a powerful tool for molecular breeding, especially for QTL mapping, genome-wide association studies (GWAS), marker assisted selection (MAS) and genomic selection (GS), where a large number of markers are needed to increase accuracy (SEMAGN ET AL. 2014, ALIPOUR ET AL. 2019). In the beginning, SNP detection methods in wheat research take resource on known nucleotide sequences of popular RFLPs/CAPs and EST-sequences (expressed sequence taqs), but this limited knowledge of the nucleotide sequence hindered the development of SNP markers in the past (KLESTHKINA & SALINA 2006).

Since the discovery of next-generation sequencing techniques, e.g. Hiseq/Miseq (Illumina, San Diego), Ion Torrent (Life Technologies,, Carlsbad) or Roche454 (Roche, Applied Science, Indianapolis) that produce up to millions of sequences simultaneously, SNP detection increases extremely and pave the way for high-throughput genotyping (HE ET AL. 2014, FATIMA 2016). Actually the costs of DNA sequencing are decreasing steadily due to new technologies whereas the number of genome-wide SNPs is increasing. Over 50 SNP arrays and 15 different GBS protocols have been established up to now in more than 25 crop species and perennial trees (RASHEED ET AL. 2017). For wheat the 90K

(WANG ET AL. 2014) and 9K Illumina SNP chip are available (CAVANAGH ET AL. 2013). A consensus map was constructed out of eight biparental populations and 40.267 SNP markers were mapped out of 81.587 SNPs, providing an essential resource for genomics based breeding (WANG ET AL. 2014, WEN ET AL. 2017). Today GBS is a low-cost, simple and powerful application to genotype and generate SNP markers in almost all species, not only for species with low genetic diverse and small genomes, but even for high genetic diverse and large genome species as wheat (ELSHIRE ET AL. 2011). For those species, target enrichment or reduction of genome complexity has to be conducted to ensure sufficient overlap in sequence coverage (ELSHIRE ET AL. 2011). In a first step, genome complexity has to be reduced by single (original: ApeKI) or two (PstI/Msp) restriction enzymes, dividing the genome into smaller fragments. Next, adapters with a bar-coding sequence in one of them are ligated to ends of DNA fragments for library preparation. Then the sequences are enriched by PCR and the resulting products are pooled for library preparation. The sequences of the library are processed with NGS sequencing systems and finally evaluated using bioinformatical pipelines (HE ET AL. 2014). GBS increases marker resolution for trait mapping through high density SNP marker detection and genome-wide marker coverage and is consequently used in genomic-assisted breeding efforts like GWAS, genomic diversity studies and genomic selection (GS), as well as ordering and anchoring physical maps (POLAND ET AL. 2012, HE ET AL. 2014). It is a cost-effective approach, but lots of missing data due to a low sequence coverage are produced, so complex bioinformatics analyses are required (BERNARDO ET AL. 2015, WICKLAND ET AL. 2017). Nevertheless, reference sequences can increase haplotype imputation of missing data and succesful physical mapping of interesting genes/traits (POLAND ET AL. 2012)

5. Genetic and physical maps

Genetic and physical maps are essential resources in molecular breeding research. Both maps display the marker arrangement and their distance to each other along chromosomes (COLLARD ET AL. 2005). Whereas genetic maps are constructed on the recombination frequency between two marker loci, the distance in a physical map is based on the number of nucleotides (O'ROURKE 2014). Hence, the genetic distance is based on the likelihood that a crossing-over occurs between two marker loci (genes) during meiosis. The distance

unit in genetic maps is called map units or centiMorgans (cM) with one mu/cM being equivalent to 1 % frequency of recombination. When two loci are far apart, a crossover is very likely and thus the recombination frequency is very high. If the recombination frequency is over 50 %, then the two genes/loci are unlinked (COLLARD ET AL. 2005). For conversion of recombination frequencies into map units, mapping functions like the Kosambi (KOSMABI 1944) or Haldane (HALDANE 1919) function are used (TAN & FORNAGE 2007). The ratio of genetic and physical distances is varying along chromosomes, because there are so called "hot spot" (proximal) and "cold spot" (centromeric) regions, where recombination events occur more or less and therefore distorting the real physical distance (PETES 2001, PETERS ET AL. 2003). For example, mean recombination rates in wheat range from 16.7 Mbp/cM in proximal/centromere regions to 1.1 Mbp/cM in distal regions of the chromosome (AKHUNOV ET AL. 2003). For bread and einkorn wheat high-density genetic maps are nowadays available (YU ET AL. 2017, WEN ET AL. 2017). While sequencing costs are decreasing, physical maps become more and more important in breeding research to order and join sequence data as well as marker positions on the genetic map and they serve as an efficient tool for cloning projects and candidate gene identification as well as for comparitve genomic studies (MEYERS ET AL. 2004). A physical map at highest resolution is a whole genome sequence of a species, which is nowadays available for lots of species, e.g. *Arabdiopsis* (AGI 2000), rice (IRGSP 2005) and even for species with large and complex genomes like barley (MASCHER ET AL. 2017), maize (SCHNABLE ET AL. 2009) and wheat (APPELS ET AL. 2018), due to the large progress in next generation sequencing technologies. Recently the genome sequence of wheat variety Chinese Spring was published and further wheat varieties like Robigus, Paragon, Claire and Cadenza and durum wheat Kronos are released, although they are not advanced to the quality of Chinese Spring (APPELS ET AL. 2018, UAUY 2017). Also for wheat relatives like *Triticum urartu* a fully seqenced genome was published in 2018 (LING ET AL. 2018).

6. QTL-analysis, map-based-cloning (MBC) and marker-assisted selection (MAS)

Marker-assisted selection (MAS) describes an indirect selection method and is nowadays a common procedure in wheat breeding programs due to many advantages in comparison to conventional breeding. For example, the accumulation of favorable alleles during the selection process through DNA-markers and the identification of suitable individuals

solely by genotypic data already at the seedling stage is time saving and cost effective in comparison to long traditional phenotypic assessments (FRANCIA ET AL. 2005, COLLARD & MACKILL 2008). Moreover, MAS gained increasing interest since the emergence of new high-troughput genoyping technologies, that cause a high marker density and increase the accuracy of marker-trait associations and thus the identification of markers tightly linked or even within the target gene (PÉREZ-DE-CASTRO ET AL. 2012), e.g. markers for broad-spectrum resistance gene *Lr34* (LAGUDAH ET AL. 2009, FANG ET AL. 2020). Thus, this facilitates the introgression of desired traits into breeding material by reducing undesired linkage drag. The use of MAS may be very easy for simply inherited monogenic traits, but is more complex for many agronomic traits being important for wheat improvement like yield, some disease resistances or abiotic stress tolerance, which are inherited in a quantitative manner. While qualitative traits are monogen and discontinious, quantitative traits show a continous phenotypic variation between two extreme characteristics of a given phenotype and are controlled by several genes (ASINS ET AL. 2009). Next to this polygenic nature, they are highly influenced by environmental factors and interacting epistatic effects (XU 2002). Moreover, they have a medium to low heritability and occasionally lots of small effect genetic loci are responsible for one phenotype. However, both traits are subjected to the same Mendelian laws of inheritance (BECKER 2011). A quantitative trait locus (QTL) defines genomic regions which significantly affect a quantitative trait (ALQUADH ET AL. 2019). QTLs are referred to major QTLs, when they explain a percentage of variation higher than 10 % and as minor QTLs, when explaining less (FATIMA 2016, COBB ET AL. 2019). The identification of closely linked markers to the gene of interest is a prerequisite for MAS to reduce linkage drag. A bulked segregant analysis (MICHELMORE ET AL. 1991) together with high troughput sequencing has great potential for the isolation of candidate genes for qualitative, simple traits, while linkage-based QTL analyses in bi-parental populations were traditionally performed for quantitative traits (LANGRIDGE ET AL. 2001, ZHANG ET AL. 2019).

Linkage-based QTL analyses are often used in molecular breeding research and result in the identification of genetic loci with their respective effects contributing to the phenotypic variation in a bi-parental population. Following fine-mapping and alignment of genetic marker with the physical map lead to identification and cloning of candidate

genes. The whole procedure is called map-based cloning (BETTGENHAEUSER & KRATTINGER 2019, JAGANATHAN ET AL. 2020). A perquisite for QTL mapping in bi-parental populations is the construction of genetic linkage maps, which was done in the past with AFLP- or SSR markers and is facilitated nowadays by high-throuhput genotyping technologies. Genotypic data (marker data) and phenotypic results, segregating for a specific trait within this population, are linked by statistical procedures like simple-interval-mapping SIM or composite-interval-mapping CIM (SALVI & TUBEROSA 2005/2007, FATIMA 2016). Traditionally bi-parental populations are used for the QTL mapping, i.e. F_2/F_3-populations, DH- (doubled haploid)-populations and RIL (recombinant inbred lines)-populations as well as BC (backcross)-populations (SHI ET AL. 2019). The required size of the population in classical mapping approaches is usually between 100-250 genotypes, but nowadays larger mapping populations are preferred, especially to detect QTLs with small effects on a target trait (COLLARD ET AL. 2005, TOMAR 2015). In bi-parental populations, QTLs are generally mapped at low resolution in a confidence interval between 10 – 30 cM, including hundreds of genes, which makes it difficult to identify the functional one (SALVI & TUBEROSA 2005). Thus the limitations of the bi-parental QTL mapping are a small allele richness and a low mapping resolution, that results from the respective population size. The small population size leads to a lower number of recombination events that originate during the construction of the mapping population (ALQUADH ET AL. 2019). Nevertheless, the QTL interval can be narrowed and the genetic resolution increased by a higher number of recombination events in a second step.

To achieve this, a high-reolution mapping population (HRM-population) comprising several thousands of F_2-plants (500-10.000 progenies), that are traced back to crosses between parental lines varying in their allelic constitution at a QTL position, is constructed (SALVI & TUBEROSA 2007). Ideally, the HRM-population consists of near-isogenic lines (NIL), because of their isogen/similar genetic background except of the variations within the target interval that lead to an accurate control of the QTL effect and allow to see the QTL as a single Mendalian factor (RAM 2014, JAGANATHAN ET AL. 2020). Another suitable population are RILs, but they always have a small amount of heterozygosity and segregate at different loci in the genetic background and not only at the QTL site. The HRM-population is screened with markers flanking the QTL interval

of interest to identify plants that have a recombination event within this interval. Heterozygous recombinant plants are further selfed to identify homozygous, fixed recombinant plants. After one additional selfing step, selected plants can be used for phenotypic and genotypic analysis. It is important that phenotypic analyses are conducted in multiple environments to proof the stability and repeatability of this QTL and to obtain robust assesments of phenotypic data (BOREVITZ & CHORY 2004, JAMANN ET AL. 2015). Additionally the interval is saturated with markers to delimit recombinational breakpoints. Suitable marker systems for fine mapping in the past were also AFLP- and SSR markers, but to date e.g. flourescencse-based PCR KASP assays, which are developed from SNP markers, are well suited for high-throughput recombinant screening (JAMANN ET AL. 2015, ALQUADH ET AL. 2019). In a next step, the high-resolution genetic map is anchored to the physical map (SILVA & TUBEROSA 2007). In the past, BAC contigs covering the target interval were used, but nowadays genome sequences are available for many species and facilitate the anchoring process. To date, well annotated reference sequences of many crop species, even for the large and complex wheat genome (APPELS ET AL. 2018) facilitate the selection of candidate genes due to predicted functions of the genes and gene ontologies (BOREVITZ & CHORY 2004). Especially genes with polymorphisms should be regarded as potential candidate genes, causing the phenotypic variation (JAMANN ET AL. 2015). Then the function of chosen candidate genes is validated using e.g. virus-induced gene silencing (VIGS) (BAULCOMBE 1999, DINESH-KUMAR ET AL. 2003), gene knockdown through RNAinterference (KUSABA 2004) or mutagenesis/tilling studies (MCCULLUM ET AL. 2000). Commonly used methods to date are rather zinc finger nucleases (ZFNs) (OSAKABE ET AL. 2010) or the Crispr/Cas9 system (KUMAR & JAIN 2015, BAO ET AL. 2019) mediating a gene knockout. Today's improvements in biotechnological and genomic methodologies, like NGS-based genotyping platforms contribute to rapid and inexpensive genotyping of large mapping populations, resulting in a higher genetic resolution as well as to the construction of high-density or consensus genetic maps (LI ET AL. 2015, WEN ET AL. 2017), that facilitate, together with complete reference sequences, cloning of genes/QTLs and reduce the time-span for gene cloning by half (JAGANATHAN ET AL. 2020).

Another possibility for successful marker-trait identification are genome-wide association studies (GWAS), based on linakge-disequlibrium (LD) mapping. GWAS

provides a higher allelic diversity and a higher number of recombination events and thus a higher mapping resolution compared to classical bi-parental QTL mapping. Moreover there is no need to construct a segregating mapping population or a linkage map (ALQUADH ET AL. 2019). Nevertheless, GWAS also has some drawbacks like the requirement of a large population size or spurious associations due to the population structure (ALQUADH ET AL. 2019). Moreover, todays multi-parent approaches like NAM- or MAGIC-populations try to combine both advantages of linkage- and LD mapping while overcoming their drawbacks and therefore were employed in several studies (BAJGAIN ET AL. 2016, SANNEMANN ET AL. 2018, STADLMEIER ET AL. 2018, KIDANE ET AL. 2019).

Finally, it can be stated, that bi-parental QTL mapping studies with following fine-mapping procedure as well as LD-based association mapping are powerful tools to identify candidate genes, which are responsible for phenotypic trait variation, and consequently to develop functional markers for MAS that accelerate and support wheat breeding research. However, a shift is observed in practical breeding from MAS to genomic selection (GS), which seems to be quite more efficient in improving complex quantitative traits with small effects (HEFFNER ET AL. 2009, CROSSA ET AL. 2017).

7. Aim of this study

The aim of this study was the investigation of FHB resistance in a *Triticum monococcum* DH-population, derived from a cross between *Triticum monococcum* L. accession *mon10-1* and *Triticum monococcum* L. conv. *sinskayae* (*Sinskayae*) up to fine mapping and identification of suitable markers and/or candidate genes. Genetic resources like *Triticum monococcum* provide a powerful gene reservoir for the improvement of modern wheat varieties. Therefore two neighbouring QTLs on chromosome 2A, which explain 81.8 % and 34.7 % phenotypic variance, respectively for FHB resistance act as basis for further map-based cloning. In more detail goals of this study were 1) to map QTL for FHB resistance in a *Triticum monococcum* DH-population with a genetic map comprising 1987.55 cM and two-years-field trials 2) to construct a high-resolution mapping population out of segregating F_2-plants that were developed by crossing three susceptible (A37, A39, B22) and two resisant DH-lines (C35, C42) to enhance genetic resolution, 3) to generate new molecular markers within the target interval for the genotyping of the HRM-population and to saturate this interval, 4) to phenotype recombinant inbred lines (RILs) in the F_4-generation of the HRM-population to locate the resistance loci in the interval and finally 5) to identify closely linked markers and/or candidate genes for MAS.

Chapter II |

Mapping of QTL for Fusarium Head Blight resistance in a *Triticum monococcum* doubled haploid population and development of a high-resolution mapping population

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1. Abstract

The causal agents of Fusarium Head Blight (FHB), a devastating disease in wheat and other small grain cereals worldwide are different *Fusarium* species, predominantly *Fusarium graminearum* and *Fusarium culmorum*. The infection leads to high yield losses up to 40 % and a decreased seed quality due to the production of mycotoxins during the infection cycle. In this study, a *Triticum monococcum* doubled haploid (DH)-population comprising 94 lines based on a cross between *Triticum monococcum* L. (*mon10-1*) and *Triticum monococcum* L. conv. *sinskayae* (*Sinskayae*) was analysed for resistance to FHB. To achieve this, the DH-population was phenotyped in two-years field trials (2008 and 2009) by an artificial infection with *Fusarium culmorum Fc46* and genotyped with DArT-, SNP-, and SSR markers resulting in a genetic map of 1987.55 cM. Two neighbouring QTLs were mapped on chromosome 2A in an interval of 45.1 cM. The first QTL interval comprises 29.8 cM and explains 81.8 % (LOD = 19.7) of the phenotypic variance on average of both years (2008/2009). The second QTL interval is 15.3 cM and explains 34.7 % (LOD=7.45) of the phenotypic variance on average of both years (2008/2009). Both QTLs are used for a following map based cloning approach, including the development of a high-resolution mapping population with 1991 F_2-plants and a genetic resolution of 0.025 % recombination.

2. Introduction

Cultivated einkorn wheat, *Triticum monococcum* L., an ancient diploid wheat species (2n = 2x = 14, A^mA^m) with hulled grains and a genome size of 4.94 Gb was one of the first domesticated crops and widely used in the beginning of agriculture for bread making and feed until the bronze age (ABBASOV ET AL. 2018). The domestication process started about

10.000 years ago in the Fertile Crescent, especially in South-Eastern Turkey from the wild species *Titicum monococcum* ssp. b*oeticum (*HARLAN 1980, DUBKOVSKY & DOVARK 2007). Today, there are only small regions in Turkey, Southern Europe and the Balkans where einkorn wheat is grown (SUCHOWILSKA ET AL. 2009, ZAHARIEVA & MONNEVEUX 2014). Nevertheless, the importance of einkorn wheat as a genetic resource in wheat breeding should not be neglected due to the adaptation to low-input conditions and a high level of resistance as well as the high amount of beneficial compounds for man's health and nutrition (CAKMAK ET AL. 2000, MIELKE AND RODEMANN 2007, TIWARI ET AL. 2009, SAHIN ET AL. 2017). In this respect, resistance to Fusarium Head Blight was detected in einkorn wheat (KOPAHNKE ET AL. 2008, LUTHARD 2012, KONVALINA ET AL. 2016, GÓRAL ET AL. 2017), while in other studies it turned out to be susceptible to FHB (MIELKE AND RODEMANN 2007). Fusarium Head Blight was first described by W. SMITH in 1884 and is one of the most damaging diseases in wheat (*Triticum aestivum* L.) and other small grain cereals worldwide (WEGULO ET AL. 2015). *Fusarium culmorum* (W.G. Smith) is one of the causal agents of Fusarium Head Blight (FHB). The occurrence was mainly reported in Northern, Western and Central Europe in the past, but recently there are already cases of infestation with *F. culmorum* in the Mediterranean region. In Germany, *F. culmorum* accounts for 20 % of all isolated *Fusarium* species from wheat next to *F. graminearum* with 67 % (LIENEMANN 2002, TALAS 2011, MIEDANER ET AL. 2013). Infected plants show brownish spots on the glumes, prematurely bleached spikelets and orange-salmon coloured sporodochia at the base of the spikelets resulting in shriveled kernels and high yield losses (SCHERM ET AL. 2013). The infection intensity depends on environmental conditions, so humidity and warm temperature higher 25°C during flowering are advantageous, although *F. culmorum* is the one with the lowest demand for humidity within the *Fusarium* species (WAGACHA & MUTHOMI 2007). It is no real sexual stage known for this pathogen, but two mating types (TÓTH ET AL. 2004) indicating a cryptic sexual stage or a loss of this stage in recent time (MIEDANER ET AL. 2013). Two chemotpypes are known for *F. culmorum*, chemotype I (DON and its derivative 3-ADON) and chemotype II (NIV and/or FusarenoneX (FUS)) (SCHERM ET AL. 2013). Both are toxic, secondary metabolites with fatal consequences for man and animals after consumption.

Control measures are fungicide application during early flowering or wide crop rotations, but the most cost effective and environment friendly approach is breeding of resistant cultivars, although this is difficult due to the polygenic inheritance and the strong influence of environmental conditions (SNIJDERS & PERKOWSKI 1990, SHANER 1995, KONVALINA ET AL. 2016, MESTERHÁZY ET AL. 2018). In addition to some passive resistance mechanisms like plant height, spike compactness or time of flowering (MESTERHÁZY 1995, SCHMOLKE ET AL. 2005, EMRICH ET AL. 2008), five active resistance responses to FHB are described: type I: resistance against initial infection (SCHROEDER & CHRISTENSEN 1963), type II: resistance against pathogen spreading (SCHROEDER & CHRISTENSEN 1963), type III: resistance to mycotoxin accumulation (MILLER ET AL. 1985), type IV: resistance to kernel infection (MESTERHÁZY 1995, MESTERHÁZY ET AL. 1999), type V: tolerance (MESTERHÁZY 1995, MESTERHÁZY ET AL. 1999). Although a combined resistance to DON accumulation and decreased FHB severity is advantageous and preferred by breeders, most QTL studies focused on resistance type I or II (BAI ET AL. 2001, MESTERHÁZY ET AL. 2017). At the moment, more than 250 QTLs are known for FHB resistance in wheat with *Fhb1* being the most stable and prominent one for resistance type II (JIA ET AL. 2018, STEINER ET AL. 2017). *Fhb1*, which was discovered in Sumai-3 and other Chinese landraces is located on chromosome 3BS and explains 6-60% of the phenotypic variance (BAI ET AL. 1999, WALDRON ET AL. 1999, CUTHBERT ET AL. 2006, LIN ET AL. 2006). It is widely used in western wheat breeding programmes, even though the usage of non-adapted material is still in discussion due to undesirable linkage-drag effects (BUERSTMAYR 2014, BAI ET AL. 2018). Some other important QTLs for resistance type II are *Fhb2* (YANG ET AL. 2003, CUTHBERT ET AL. 2007) and *Fhb3* (QI ET AL. 2008). *Fhb2* was also detected in Sumai-3, mapped on chromosome 6BS and accounts for 21 % of the phenotypic variance, whereas *Fhb3* originated from *Leymus racemosus*, a wild grass species, and was successfully transferred into wheat chromosome 7A (ANDERSON ET AL. 2001, CUTHBERT ET AL. 2007, QI ET AL. 2008). *Fhb4* and *Fhb5* both derived from Whangshuibai, a Chinese landrace unrelated to Sumai 3, and confer resistance type I, although they have also been associated with type II resistance in other populations (LIU ET AL. 2009). They are located on chromosome 4BL and 5AS (XUE ET AL. 2010, XUE ET AL. 2011). The two newest designated FHB-QTL are *Fhb6* (CAINONG ET AL. 2015) on chromosome 1A and *Fhb7* (GUO ET AL. 2015) on chromosome 7D, both from alien species *Elymus tsukushiensis* and *Thinopyrum ponticum*, respectively and they

were also successfully transferred into wheat. With regard to this study, there are also some minor QTLs detected on chromosome 2A in a tetraploid wheat population BGRC3487 x DT735 (RUAN ET AL. 2012) as well as in durum wheat (GHAVAMI ET AL. 2011), hexaploid wheat (ZHOU ET AL. 2002, MA ET AL. 2006) and in an interspecific wheat population (GIANCASPRO ET AL. 2016). Lots of research was also done with well-adapted European elite material to avoid linkage drag, when introducing QTL from non-adapted Asian germplasm (BUERSTMAYR ET AL. 2019). Although there was some research on *Fusarium* resistance in related wheat species like *Triticum spelta*, *Triticum timopheevii* and *Triticum dicoccum* (BUERSTMAYR ET AL. 2019), little is known on the genetics of resistance to FHB of *Triticum monococcum.* Thus, the main objectives of the study were i) to evaluate a doubled haploid *Triticum monococcum* population for FHB resistance ii) to identify QTL for FHB resistance in this population and iii) to construct a high-resolution mapping population for these QTL for fine-mapping thereby reducing linkage drag.

3. Material and Methods

QTL mapping

3.1. Plant material

For QTL analysis, a doubled haploid (DH)-population comprising 94 doubled haploid (DH)-lines derived from a cross between *Triticum monococcum* L. accession *mon10-1* and *Triticum monococcum* L. conv. *sinskayae* A. Filat. & Kurkiev (*Sinskayae*) providided by GEORGE FEDAK was used. *Mon10-1* is moderately resistant to Fusarium Head Blight while *Sinskayae* is highly susceptible (LUTHARD 2012).

3.2. Phenotyping: Field trials

All DH-lines and parental lines were sown in plots of 1 m^2 size on the field of the Julius Kuehn-Institute in Quedlinburg, Saxony-Anhalt (51.7694 N, 11.147 E, 140 m altitude) in 2008 and 2009 for evaluating Fusarium Head Blight resistance type I. Each genotype was inoculated by artificial infection with the highly aggressive *Fusarium culmorum*- Isolate *Fc46*, kindly provided by THOMAS MIEDANER, UNIVERSITY OF HOHENHEIM using a conidia suspension of 1.000.000 C/ml. The inoculum of *Fusarium culmorum Fc46* was

produced with *Fusarium*-infected wheat grains. In a first step, wheat grains were washed with water and soaked in water over night. Surplus water was removed and 850 ml of grains were filled in 1000 ml glass flasks. These were sealed with a plug and aluminium foil and autoclaved three times for 1h at 120°C within 24h. After 3-4 days in the brood chamber (20°C), grains were infected by dropping a half potato-dextrose agar (PDA)-plate of *Fusarium culmorum* isolate *Fc46* into the grains. The flasks were incubated at 20°C in the dark until the grains were fully covered with *Fusarium* mycelia. Then, wheat grains were placed into a plastic bowl and dried for 7-14 days. To determine the concentration of the spore suspension, 0.1 g of dried wheat grains of each plastic bowl were dissolved in water and the number of spores was counted with a Fuchs-Rosenthal-chamber. The average values of three samples of each plastic bowl as well as of all plastic bowls were calculated and the amount of infected wheat grains for a 10 l suspension with a concentration of 1.000.000 C/ml was calculated. Before inoculation, the wheat grains were dissolved in water with one drop of Tween 20 to produce the conidia suspension. The conidia suspension was sprayed directly on the ears with a backsprayer, when 50% of each plot were flowering (BBCH65). The inoculation was replicated once in an interval of three to four days to compensate for different flowering times and to guarantee optimal infection conditions for the pathogen. Fusarium Head Blight severity was visually recorded as percentage infestation of each plot according to MOLL ET AL. (2010) at four to five different timepoints. The screening started ten days after the second infection (10 dpi) and was repeated every four days until the yellow ripening state was reached, usually after 22 dpi or 26 dpi. The scoring data were used to calculate the Area under disease progress curve (AUDPC) and out of these the Average Ordinate (AO) as described by VATTER ET AL. (2017) with following formula:

$$\mathbf{AO} = \frac{\boxed{\sum_{i=1}^{N_{i-1}} \frac{(y_i + y_{i+1})}{2} * (t_{i+1} - t_i)}}{tp} \longleftarrow \textbf{AUDPC}$$

where (N) is the total number of observations, (y_i) the disease level at the ith observation, (t_i) the time at the ith observation and (tp) the trial period in days.

3.3. Statistical analyses

Statistical analyses of phenotypic data were performed using the software SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Frequency distribution of phenotypic data was tested for a Gaussian distribution employing the Kolomogorov-Smirnow- and the Saphiro-Wilk-tests using *proc univariate*. Analysis of variance (ANOVA) was carried out using *proc GLM* to examine significant differences between AO-values. Broad sense heritability across years was calculated using the following formula (in detail described by VATTER ET AL. 2017):

$$h^2 = \frac{V_G}{V_G + \frac{V_{GY}}{y} + \frac{V_R}{yr}}$$

Where V_G is genotypic variance, V_{GY} is genotype x year variance, V_R is residual variance and y and r indicate the number of years and replicates, respectively.

3.4. DNA Extraction

For genotyping, DNA was isolated from all 94 DH-lines of the mapping population as well as from parental lines with the CTAB-extraction method according to STEIN ET AL (2001). DNA concentration was measured with a NanoDrop ND 1000 Spectrophotometer (PEQLAB Biotechnology GmbH, Erlangen) and adjusted to 50 ng/µl for PCR analyses.

3.5. Genotyping: Marker analyses

SSR markers for the genotyping of the DH-population derived from already published genetic maps of chromosomes 1A,2A,3A,4A,5A,6A and 7A of bread wheat (*Triticum aestivum* L.) (RÖDER ET AL. 1998, SOURDILLE ET AL. 2001, SHI ET AL. 2003, SOMERS ET AL. 2004, JING ET AL. 2009) were selected and tested for polymorphism between the resistant and susceptible parent. Selected polymorphic marker were screened on the whole DH-population. PCR analyses were performed using a thermal cycler GeneAmp PCR System 9700 or 2700 (Applied Biosystems by Life Technologies Corporation, Carlsbad, California). PCR reaction volumes comprised 15 µl consisting of 1.5 µl 50 ng DNA and a Mastermix containing PCR reaction buffer, $MgCl_2$, dNTPs, polymerase,

forward Primer (Primer F), reverse Primer (Primer R) and fluorescence labeled M13-tailed Primer in different quantities (Table 1).

Table 1 | Composition of PCR mixtures used for SSR analyses

(1.)	conc.	approach	(2.)	conc.	approach	(3.)	conc.	approach
tdW		9.33 µl	tdW		8.73 µl	tdW		9.07 µl
BufferB without $MgCl_2$	10x	1.50 µl	BufferB without $MgCl_2$	10x	1.50 µl	BufferB without $MgCl_2$	10x	1.50 µl
dNTPs	10 mM	0.30 µl	dNTPs	10 mM	0.60 µl	dNTPs	10 mM	0.30 µl
Primer F	10 µM	0.23 µl	Primer F	10 µM	0.30 µl	Primer F	10 µM	0.40 µl
Primer R	10 µM	0.38 µl	Primer R	10 µM	0.30 µl	Primer R	10 µM	0.40 µl
M13-Tail	10 µM	0.15 µl	M13-Tail	10 µM	0.27 µl	M13-Tail	10 µM	0.40 µl
Polymerase FIRE	5U	0.12 µl	Polymerase FIRE	5U	0.30 µl	Polymerase HOTFIRE	5U	0.12 µl
$MgCl_2$	25 mM	1.50 µl	$MgCl_2$	25 mM	1.50 µl	$MgCl_2$	25 mM	1.31 µl
DNA		1.50 µl	DNA		1.50 µl	DNA		1.50 µl

PCR reactions were conducted with four different programs (Table 2) and subsequently visualized by gel electrophoreses. For checking the PCR-products 1.5 % agarose gel was produced and loaded with a 10 µl-mix (5 µl PCR-product/5 µl DNA loading Dye). The imaging was done by gel documentation system GEL DOC XR (Bio-Rad Laboratories GmbH, München) and the „Quantity One“- software v4.6.3 (Bio-Rad Laboratories GmbH, München). The fragment size of PCR products was estimated by loading D2, D3 and Cy5 flourescence labeled fragments on a CEQ 8000 Genetic Analysis System (Beckmann Coulter GmbH, Krefeld). For the detection and evaluation of fragments the software CEQ Systems (v9.0.25/v10.2.3) was used. Fragment analyses with 3130xL Genetic Analyzer system (Applied Biosystems by Life Technologies Corporation; Carlsbad, California) were performed for FAM, HEX and NED labeled PCR products and evaluated with the software GeneMapper (v4.0). In addition, the DH mapping population and the parental lines were genotyped using the DArT array and DArT seq

technologies resulting in 4912 DArT- and 3821 SNP markers by Triticarte (Triticarte P/L,Yarralumla, Australia, http://www.triticarte.com.au).

Table 2 | PCR programs for SSR marker analyses

td 62 - 56_30''		**td 62 - 56_30''_1**		**wms 50 hs**		**wms 50**	
94°C	5'	95°C	10'	96°C	10'	94°C	5'
94°C	30"	94°C	30"	94°C	1'	94°C	1'
62°C	30" (12x -0.5°C)	62°C	30" (12x -0.5°C)	50°C	1' (45x)	50°C	1' (45x)
72°C	30"	72°C	30"	72°C	2'	72°C	2'
94°C	30"	94°C	30"	72°C	7'	72°C	10'
56°C	30" (35x)	56°C	30" (35x)	4°C	20'	4°C	20'
72°C	30"	72°C	30"	15°C	∞	15°C	∞
72°C	7'	72°C	7'				
4°C	20'	4°C	20'				
15°C	∞	15°C	∞				

3.6. Genetic map construction

Based on the resulting A/B matrix, genetic map construction was performed with the software JoinMap v4.0 (VAN OOIJEN 2006) using the Kosambi function with a minimum LOD (logarithm of odds) threshold of 3.0. Mapped SSR markers from already published wheat genetic maps served as anchor markers for each linkage group. Monomorphic markers and markers with more than two missing values were excluded from mapping.

3.7. QTL mapping

QTL mapping was conducted for Fusarium Head Blight resistance using MapQTL v5.0 (VAN OOIJEN 2004). For a clear distinction of neighboured QTLs and improved localization, the multiple QTL mapping (MQM) procedure was used with prior identified significant markers for FHB resistance acting as co-factors. The permutation test was conducted to determine the logarithm of the odds (LOD)-score threshold ($p>0.05$) for the detection of significant QTLs. QTL analysis was done with 10 cM, 5 cM and 2 cM marker distances to get an exact position of the QTL interval.

Construction of a high-resolution mapping population

3.8. Plant material

A segregating F_2-population, consisting of 1991 F_2-plants, based on crosses between selected resistant (C35, C42) and susceptible DH-lines (A37, A39, B22), derived from the recent DH mapping population (see above), was used for the construction of the high-resolution mapping population (Figure 4).

Figure 4 | Resistant (left: C35, C42) and susceptible (right: A37, A39, B22) parental lines used for the construction of the high resolution mapping population

3.9. Marker development

For genotyping of the F_2-population three polymorphic SNP markers, flanking both QTL_mon1 and QTL_mon2 (*SNP_1216*, *SNP_0667*, *SNP_0833*, Figure 5) on chromosome 2A were selected from the genetic map of the DH-population and were converted into co-dominant competitive allele-specific PCR markers. KASP assays were generated by LGC-Customer-Technologies (http://www.lgcgenomics.com).

3.10. Construction of a high-resolution mapping population

The F_2-plants were sown in 96 quick-pot-trays in the greenhouse and plant material was harvested in the two leaf stage for DNA extraction according to DOROKHOV & KLOCKE (1997). For the identification of plants, showing a recombination event within the interval, the F_2-plants were analysed with the developed KASP markers using the real-time PCR system BIO-RAD CFX-96 with the following protocol: 15 min at 94°C, followed by 20 min at 94°C and 1 min at 65°C for annealing (annealing temperature was reduced during 10 cycles to 57°C), then 20 min at 94°C and 1 min at 57°C for 26 cycles and a finishing post-PCR-step with 1 min at 30°C. The PCR reaction was set up in 10 µl volumes with 5 µl 50ng DNA and 5 µl Mastermix, composed of 5 µl KASP-ReactionMix and 0.14 µl SNP-PrimerMix (LGC GENOMICS). Identified heterozygous recombinant F_2-plants were selfed and 12 F_3-plants per genotype screened again with the same flanking KASP markers to detect homozygous recombinant plants, which were used as segmental homozygous recombinant inbred lines (RILs) for subsequent phenotypic and genotypic studies. Homoyzgous recombinant plants, already identified in the F_2-generation were directly used for further analyses. The flanking SNP markers were subsequently compared to the physical positions in the reference maps of *Triticum aestivum* (APPELS ET AL. 2018) Therefore the SNP sequences were blasted as fasta files against the *T. aestivum* reference sequence: IWGSC RefSeq CS v1.0 (URGI 2018) The best hit (expected threshold = 0.0001) was chosen to determine physical marker positions. However, the SNP markers derive from *T. monococcum* and if there

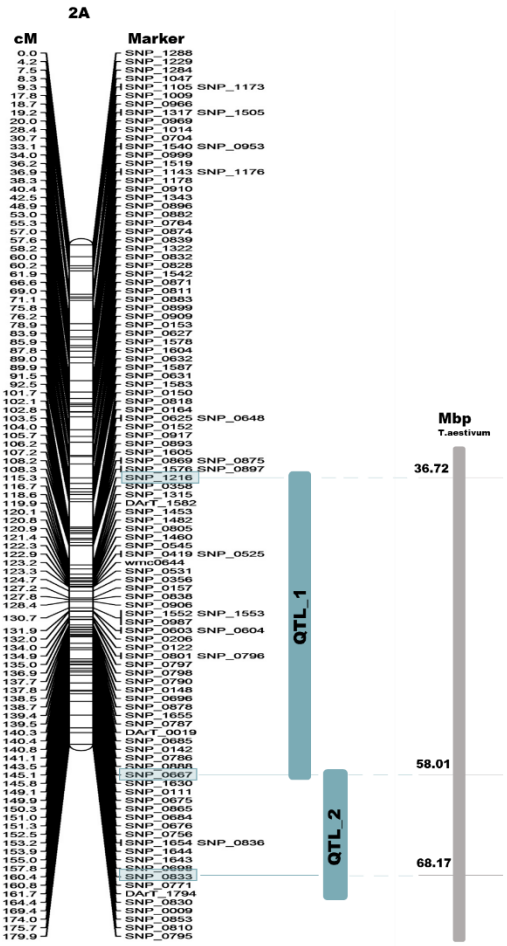

Figure 5 | Genetic map of chromosome 2A, QTL interval with selected SNP markers, genetic (left, cM) and physical (right, Mpb) map positions of selected SNP markers

was no match for one SNP marker on the wheat reference map (URGI 2018) the expected threshold was continously increased up to 1.

4. Results

4.1. Phenotypic evaluation

Fusarium inoculation resulted in AO-values ranging from 5 – 50 %. The population shows a bimodal distribution of the phenotypic traits with a peak at 15 % and 35 % infestation in 2008 and at 10 % and 40 % infestation in 2009, respectively (Figure 6, Table 3)

Table 3 | Performance of parents and DH-population for FHB-trait AO-value for both years and mean

AO-Values (FHB severity)				
Source	DF	MS	*F-value*	*P*
Genotypes	93	207,59336	5,58	<.0001
Years	1	206,03525	5,54	0,0209
Error	85	37,20236		
Total	179			

A normal distribution of phenotypic data is opposed for both years by Saphiro-Wilk- and Kolmogorow-Smirnow-tests at α=0.05 (Figure 6). The general linear model indicates 86 % variation of the AO-values between DH-lines (R^2=0.8605, GLM) with highly significant differences between genotypes ($p<0.001$) and significant differences between years ($p<0.0209$) (Table 4). Two year broad-sense heritability was estimated at $h^2 = 0.83$.

Table 4 | Analysis of variance for AO-values in DH-population

				DH-population			
Trait	Year	mon10-1 [R]	Sinskayae [S]	Min	Max	Mean	SE
AO-value	2008	13.25	40	5.75	48.75	27.09	10.70
	2009	10.5	40	4.67	41.67	24.92	11.90
	Mean	11.88	40	5.21	45.21	26.01	11.30

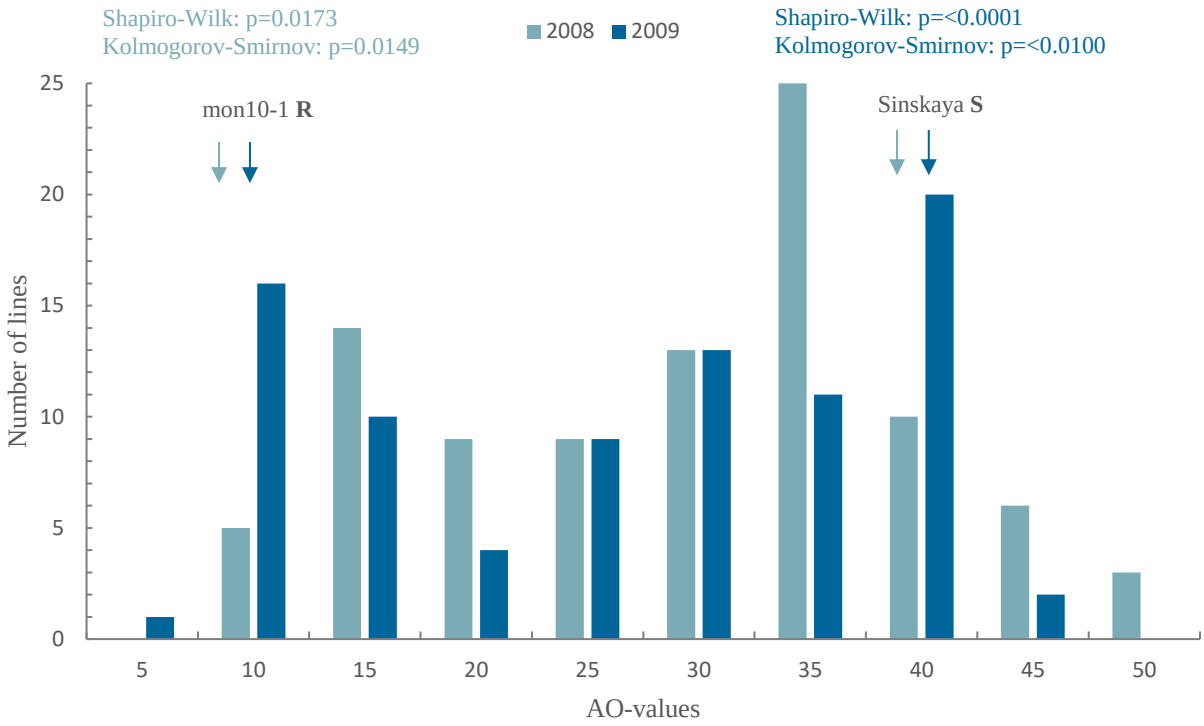

Figure 6 | Distribution of AO-values of parents and DH population in 2008/2009, normal distribution is opposed by Saphiro-Wilk and Kolmogorov-Smirnov Tests for both years

Table 5 | Characteristics of genetic mapping

Linkage Groups	Markers mapped	Map length	Density (N/cM)
1A	112	228.87	0.49
2A_1	143	138.34	1.03
2A_2	117	179.95	0.65
3A_1	31	263.54	0.12
3A_2	28	159.67	0.18
4A	131	251.45	0.52
5A	104	303.62	0.34
6A	127	221.18	0.57
7A	231	240.95	0.96
Total	1.024	1.987.554	

4.2. Genetic linkage maps

A number of 180 SSR-, 4912 DArT- and 3821 SNP markers were screened for polymorphisms between the parental lines, resulting in 38 (21.1 %) polymorphic SSR markers, 1803 (36.7 %) DArT markers and 1665 (43.5 %) SNP markers. Out of these, 1248 polymorphic SSR, DArT and SNP markers were used as input for map construction. Based on these data a genetic map of *Triticum monococcum* with a length of 1987.55 cM comprising 1024 markers on nine linkage groups was constructed. They were assigned to all seven chromosomes based on common markers with previously published maps. Chromosome 2A and 3A are divided into two parts. The respective linkage groups consists of numbers of mapped loci from n = 28 (3A_2) to n = 231 (7A) (Table 5).

4.3. QTL analysis

QTL analysis was performed for Fusarium Head Blight resistance type I using phenotypic field data and genotypic data from the marker screening of the DH-population. Maps were constructed with on average 10 cM, 5 cM and 2 cM marker distances to get a better indication of the exact QTL positions. For each calculation, the average AO-values of both years (2008/2009) were used. Moreover, the best position of the QTL was achieved using *SNP_0797* as co-factor. All QTLs reached the level of significance with α=0.05 estimated by permutation tests (LOD threshold = 2.4). In addition, the resistance carrying allele derived from the moderately resistant parent *Triticum monococcum* 10-1.

10 cM

Two neighbouring QTLs were mapped in an interval of 45.1 cM on chromosome 2A. The first QTL, named QTL_mon1, accounts for 81.8 % of phenotypic variance (LOD=19.7) and comprises a marker interval from 115.3 cM to 145.1 cM (29.8 cM) between flanking markers *SNP_1216* and *SNP_0667*. The peak marker is SSR *wmc0644* at position 123.1 cM. The second QTL interval, named QTL_mon2, ranges between 145.1cM – 160.1 cM (15.2 cM) and is flanked by markers *SNP_0667* and *SNP_0833* with *SNP_0865* as the peak marker at position 150.2 cM. QTL_mon2 explains 34.7 % of phenotypic variance (LOD=7.45) (Figure 7, Table 6). Both QTLs together cause a 20 % infestation reduction of Fusarium Head Blight in the DH-population and on average of both years.

5 cM

QTL analysis with 5 cM map distance shows a separation of the main QTL_mon1 in two QTLs (QTLmon1.1 and QTLmon1.2). QTLmon1.1 is located in a marker interval between 115.3 cM – 127.2 cM with flanking markers *SNP_1216 – SNP_0157*. The QTL comprises 11.9 cM and explains 24.4 % of phenotypic variance (LOD=2.85) with *SNP_0805* as closest marker at posisiton 120.9 cM. Whereas the highest QTL peak (QTLmon1.2) is flanked by markes *SNP_0603 – SNP_0142* and ranges between 131.9 cM – 140.8 cM with the peak marker *SNP_0797* at position 135.0 cM. It explains 78.2 % of the phenotypic variance (LOD=13.76). The second QTLmon2.1 was mapped in the same genetic region like QTL_mon2, but accounts for a higher phenotypic variance of 35.9 % (LOD=6.25) with peak marker *SNP_0865* (150.2 cM) (Figure 8, Table 6).

Table 6 | Locations and estimates of QTLs for FHB severity on chromosome 2A in *T. monococcum*

Marker distance	QTL	Map Interval	Genetic map location (cM)	LOD	R^2	Additive effect	Closest Markers
10 cM	QTL_mon1	*SNP_1216 - SNP_0667*	115.3 - 145.1	19.7	81.8	10.06	*wmc0644/SNP_0797*
10 cM	QTL_mon2	*SNP_0667 – SNP_0833*	145.1 – 160.4	7.45	34.7	10.04	*SNP_0865*
5 cM	QTL_mon1.1	*SNP_1216 - SNP_0157*	115.3 - 127.2	2.85	24.4	6.53	*SNP_0805/wmc0644*
5 cM	QTL_mon1.2	*SNP_0603 - SNP_0142*	131.9 - 140.8	13.76	78.2	9.93	*SNP_0797*
5 cM	QTL_mon2.1	*SNP_0667 – SNP_0833*	145.1 – 160.4	6.25	35.9	8.70	*SNP_0865*
2 cM	QTL_mon1.3	*SNP_1315 - SNP_0356*	118.6 - 124.7	3.29	27.9	7.29	*SNP_0805/wmc_0644*
2 cM	QTL_mon1.4	*SNP_0122 - SNP_0790*	134.0 - 137.6	10.67	44.8	7.86	SNP_0797
2 cM	QTL_mon2.2	*SNP_0111 - SNP_0756*	149.1 - 152.5	6.31	35.0	9.98	*SNP_0865*

2 cM

Three QTLs were mapped with a genetic map of 2 cM marker distances. As before, the main QTL_mon1 was divided into two single QTLs (QTL_mon1.3 and QTL_mon1.4). QTL_mon1.3 spans an interval of 6.1 cM between flanking markers *SNP_1315* – *SNP_0356* (118.6 cM – 124.7 cM) and explains 27.9 % of phenotypic variance (LOD=3.29) with *SNP_0805* (120.9 cM) as peak marker. QTL_mon1.4 accounts for 44.8 % of phenotypic variance (LOD=10.67). It is located between *SNP_0122* and *SNP_0790* in a smaller interval of 134.0 cM – 137.6 cM. The peak marker is *SNP_0797* (135.0 cM). The second QTL_mon2 was further delimited to an interval of 3.4 cM (QTL_mon2.2) between *SNP_0111* and *SNP_0756* and explains 35.0 % phenotypic variance (LOD = 6.31) with peak marker *SNP_0865* at position 150.2 cM (Figure 9, Table 6).

In summary, all QTLs were mapped in the same region between 115.3 cM – 160.7 cM on chromosome 2A in larger or smaller intervals. For further analyses, the QTL analysis with 10 cM map distance and wider intervals was chosen to ensure that no important genes, which are responsible for the trait variation, may get lost by fine-mapping procedure.

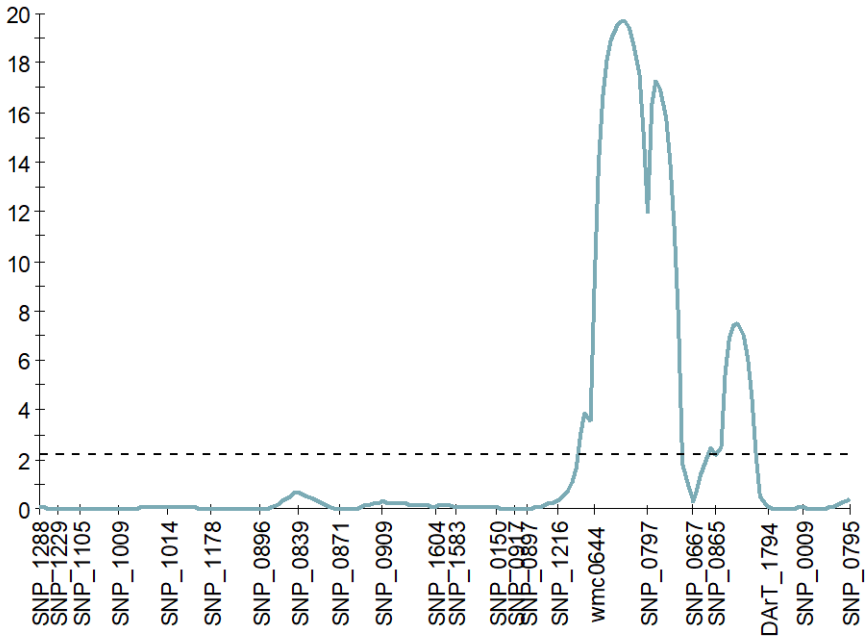

Figure 7 | QTL for FHB resistance on chromosome 2A using phenotypic data of two years (2008/2009) and a genetic map with an average distance of 10 cM

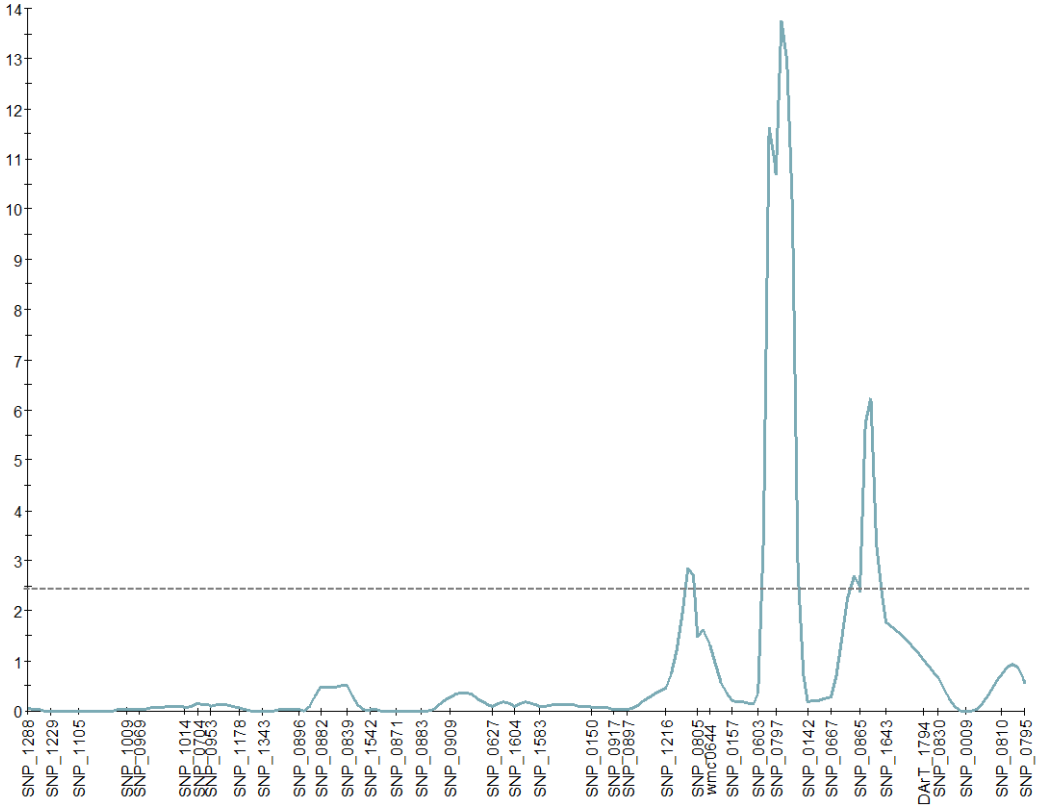

Figure 8 | QTL for FHB resistance on chromosome 2A using phenotypic data of two years (2008/2009) and a genetic map with an average distance of 5 cM

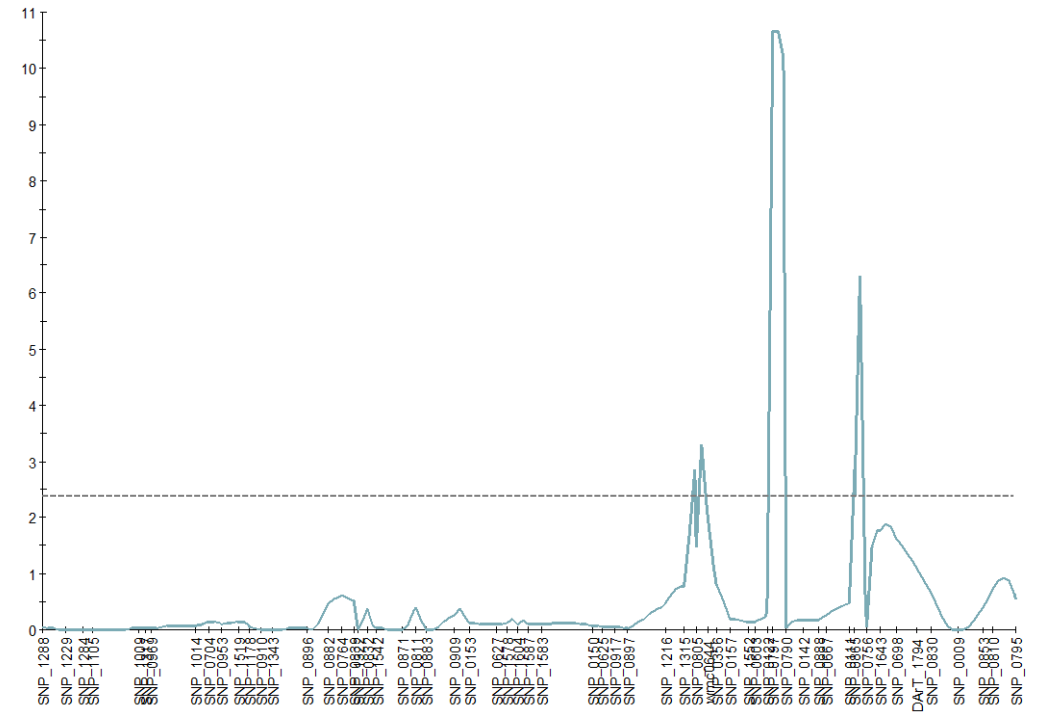

Figure 9 | QTL for FHB resistance on chromosome 2A using phenotypic data of two years (2008/2009) and a genetic map with an average distance of 2 cM

4.4. Construction of a high-resolution mapping population

QTL analysis with 10 cM maker distances was chosen as the basis for the construction of a high-resolution mapping population. Altogether 1991 F_2-plants, resulting in a resolution of 0.025 % recombination, were screened with the three flanking KASP markers (*SNP_1216, SNP_0667, SNP_0833*) for recombination events in the target intervals. A genetic distance of 11.4 cM was determined for the first QTL interval, 6.4 cM for the second QTL interval and 17.8 cM for both intervals (Figure 10). The physical distance is shown in Figure 5. In a first step, 23 F_2-plants were detected for the whole QTL region to be homozygous recombinant and 663 F_2-plants to be heterozygous recombinants. 333 recombinant inbred lines were finally developed out of the recombinant F_2-plants.

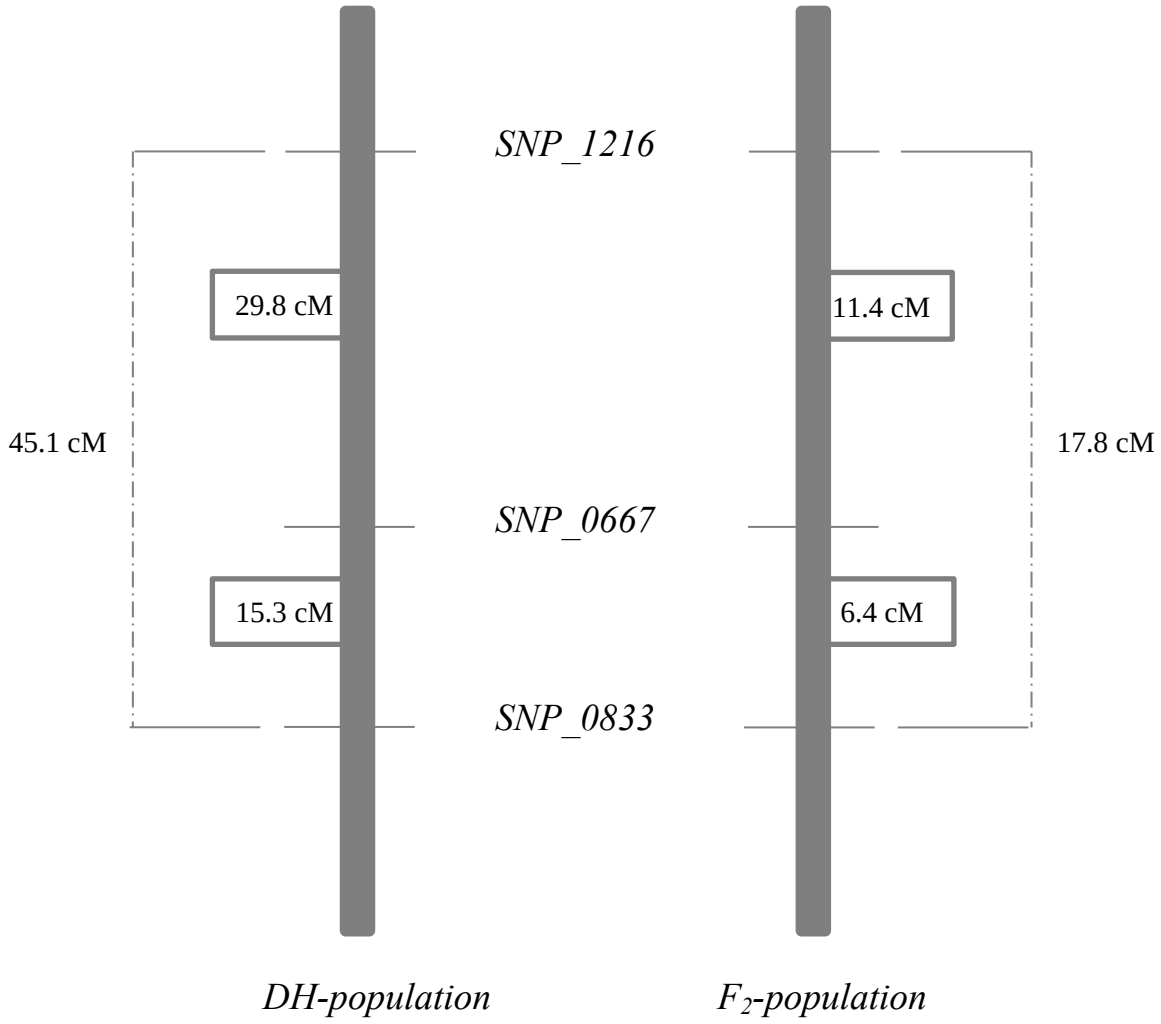

Figure 10 | Genetic distances between flanking markers (SNP_1216, SNP_0667, SNP_0833) in the DH- and the F_2-population

5. Discussion

This study was conducted to get information on the genetics of resistance of einkorn wheat to FHB. Toachieve this, the resistance of a *Triticum monococcum* DH-population to Fusarium Head Blight was assessed and QTLs identified. FHB still remains an important disease in wheat, because of high economic losses for farmers due to yield reduction and contamination with mycotoxins. Resistance breeding is a very promising and efficient way to control FHB and reduce mycotoxin production during the infection cycle (MCMULLEN ET AL. 2012, SHAH ET AL. 2018). Due to limited genetic variation occurring in cultivated hexaploid wheat, the identification of new resistance sources in wild relatives is of prime importance (KUMAR & SHUKLA 2014). In this study, two neighbouring QTLs were mapped between SNP marker *1216* and SNP marker *0833* on the short arm of chromosome 2A. Both derive from resistant parent *Triticum monococcum*-accession 10-1 and explain 81.8 % and 34.7 % of the mean phenotypic variance, respectively. This explained phenotypic variance is quite high compared to other studies, e.g. 16 % of the explained phenotypic variance for a major effect QTL for FHB resistance on chromosome 3A was reported (STEINER ET AL. 2004) or 22.1 % explained phenotypic variance for a QTL on chromosome 6DL (PAILLARD ET AL. 2004). Moreover, for fungal spread a lower phenotypic variance of 16 % and 36 % was reported on chromosome 3BS in two DH-populations (YANG ET AL. 2003). Even the major QTL *Fhb1* on chromosome 3BS accounts for a lower phenotypic variance of 60 % in comparison to our study. Heritability was estimated in this study at $h^2 = 0.83$, which is in accordance to other studies in which h^2 for FHB resistance was also calculated in a range between $h^2 = 0.79$ (MIEDANER ET AL. 2011) to $h^2 = 0.92$ (SCHMOLKE ET AL. 2005), although other studies estimated heritability for field severity lower at $h^2=0.55$ (RUAN ET AL. 2012) or $h^2 = 0.23$ (SUN ET AL. 2016). In general, heritability for FHB is moderate to high in dependence of the population (OLLIER ET AL. 2020). A population size of 94 DH-lines was investigated comparable to previously published studies (GARVIN ET AL. 2009, NISHIO ET AL. 2016). Nevertheless larger populations are needed for a more precise QTL detection, e.g. ZHANG ET AL. (2014) used 200 RILs for assessement of FHB resistance in field and greenhouse trials, MCCARTNEY ET AL. (2016) took 125 RILs for field evaluation of FHB severity and SUN ET AL. (2016) tested 198 RILs for FHB reaction in field and greenhouse experiments. FHB is quantitavely inherited and highly influenced by

environmental effects like temperature (JUROSZEK & TIEDEMANN 2015), humidity (COWGER 2005) or nitrogen availability (CHAMPEIL ET AL. 2004). Moreover, associations between a favorable allele and a respective marker genotype, that solely depend on a specific genetic background are also limitiations for the use of these markers in breeding (YANG ET AL. 2020). Thus, to counteract these effects, investigated QTLs have to be validated with respect to stability in different environmental conditions and genetic background. Therefore, the QTL detected in this study should be evaluated again in different environments as well as in different genetic backgrounds in future research. Moreover several morphological traits, which are considered having an influence on FHB severity like plant height, spike compactness or date of anthesis (BUERSTMAYR ET AL. 2019) should be taken into account in further studies to check for correlation in this population.

In the recently published reference sequence Chinese Spring v1.0 of wheat (APPELS ET AL. 2018, URGI 2018), our QTL region is located on the short arm of chromosome 2A in an interval between approximately 36 Mbp and 69 Mbp (Figure 5). Some other QTLs associated with FHB resistance located on chromosome 2A were already published in the last decades. In a tetraploid recombinant inbred chromosome line (RICL)-population, a QTL region between SSR markers *gwm558* and *gwm445* was identified for variation in FHB resistance, but both markers mapped on chromosome 2AL according to the wheat deletion bin map and are consequently not located in our QTL interval (SOURDILLE ET AL. 2004, GARVIN ET AL. 2009). In addition, another minor QTL *QFhb.rwg-2A* for FHB resistance deriving from the durum wheat cultivar Ben, with a phenotypic variance explained of 8 % was reported in an interval of 3.6 cM between flanking SNP markers *IWA111* and *IWA1103* on chromosome 2A. But this QTL is physically located between 262.58 Mbp - 341.92 Mbp and is thus positioned in another genomic region than our QTL (ZHANG ET AL. 2014). BUERSTMAYR ET AL. (2011) reported a QTL for FHB resistance on chromosome 2A in a *Triticum macha* x *Triticum aestivum*-population with a peak AFLP marker *Xs11m24_10,* 38 cM distal to SSR marker *Xgwm614*, a marker also associated with the spread of FHB in the hexaploid wheat population Ning 7840 x Clark (ZHOU ET AL. 2002). Furthermore, 40 cM proximal to SSR marker *gwm425*, another marker for FHB resistance and lower DON accumulation was detected in the hexaploid Chinese landrace Wangshuibai (MA ET AL. 2006). The exact position of *gwm614* is not clear, but

gwm 425 is located at 113.45 Mbp and is therefore proximal to our QTL interval. Closer flanking markers to *Xs11m24_10* are SSR markers *gwm296a* and *wmc177*, which are located at 19.81 Mbp and 33.03 Mbp. This indicates that the QTL of BUERSTMAYR ET AL. (2011) and our QTL are not the same. GIANSCAPRO ET AL. (2016) identified a QTL in an interspecific wheat population with the closest marker *IWB63138* (31.96 Mbp), which mapped 5 Mbp distal to our QTL interval. They proposed a *wheatPME1* gene being responsible for the observed resistance, which encodes for a pectin methylesterase enzyme. This could play an important role in defending fungal attacks due to the protection effect of the cell walls. In a newly published study by ZHAO ET AL. (2018) a minor effect QTL was mapped based on field tests on chromosome 2A with peak marker *SNP_79083*. This marker is located at 33.04 Mbp in the physical map of wheat and thus also mapped 3 Mbp distal to our QTL interval. Both QTLs mapped very close to our QTL interval, showing the major importance of this genomic region for FHB resistance.

In next steps, the large QTL region will be fine mapped following a map-based cloning approach (KRATTINGER ET AL. 2009a) to provide reliable markers for marker assisted selection and to identify possible genes underlying the FHB resistance. A successful identification is based on the genetic – physical resolution, so the genetic resolution in our QTL region has to be increased having a chance to identify markers in close proximity to our resistance locus. This means, the higher the genetic resolution provided by the number of analysed gametes, which is increased by a larger population size, the smaller is the relation between genetic and physical distance between two loci (PETERS ET AL. 2003). Ideally, 5000 plants are analysed, resulting in 10.000 analysed gametes and a genetic distance between two loci of 0.01 % recombination (0.01 cM). Some studies needed less or more plants for the identification of candidate genes, depending on the respective species genome size and the chromosomal region where the gene is located (PELLIO ET AL. 2004). Telomeric regions on the distal end of a chromosome are so called hot spots of recombinations, in comparison to centromeric regions (cold spot regions), where recombination rates are 5-10x lower (AKHUNOV ET AL. 2003, THIND 2018). For example, only 520 F_2-plants (1040 gamtes) were needed for map-based cloning of leaf rust resistance gene *Lr21*, because this gene is located in a highly recombinogenic region (HUANG ET AL. 2003), while higher mapping populations of 3120 F_2-plants (6240 gametes) were necessary for the isolation of *Lr10* (STEIN ET AL. 2000). Our QTL is located

in the telomeric, recombination-rich region of chromosome 2A, so it could be possible that the population size of approximately 2000 F_2-plants (~4000 ganetes) is sufficient to identify candidate genes.

Through the development of the high-resolution mapping population, the interval of 45.1 cM in the DH-population was decreased to 17.8 cM in the F_2-population. This phenomenon is even well known for genetic distanes in DH- and RIL-populations. Here, the difference may be due to differences in recombination rates between male and female gametes, that arise during meiosis by population development (HE ET AL. 2001). Moreover, CUTHBERT ET AL. (2006) stated, that different genetic marker distances in two varying populations come from differences in the population size and structure. Although map-based cloning is a challenging task in wheat due to the large genome size and a high Mbp/cM ratio of physical/genetic distance (4.4 Mbp/cM in wheat) (FARIS & GILL 2002), there are still some fine mapped and isolated/cloned resistance genes, e.g. leaf rust resistance genes *Lr10* (FEUILLET ET AL. 2003), *Lr34* (KRATTINGER ET AL. 2009b) and *Lr42* (GILL ET AL. 2019), powdery mildew resistance genes *Pm3* (YAHIAOUI ET AL. 2004) and *Pm52* (WU ET AL. 2019) as well as stripe rust resistance gene *Yr10* (LIU ET AL. 2014) and Septoria tritici blotch gene *Stb6* (SAINTENAC ET AL. 2018). In *Triticum monococcum* it is quite easier to perform positional cloning of candidate genes due to the diploid genome, but there are only few publications known about fine mapping approaches or positional cloning of genes, e.g. stem rust resistance gene *Sr35* (SAINTENAC ET AL. 2013) and powdery mildew resistance gene *TmMla1* (JORDAN ET AL. 2011). With respect to Fusarium QTLs, e.g. *Fhb1, Fhb4, Qfhs.ifa-5A* or *Fhb7* were recently fine mapped in wheat (CUTHBERT ET AL. 2006, XUE ET AL. 2010, BUERSTMAYER ET AL. 2017, WANG ET AL. 2020) and with the help of genome sequencing and gene anntotations in wheat, possible candidate genes are identified for *Fhb1* and *Fhb7* (RAWAT ET AL. 2016, SCHWEIGER ET AL. 2016, SU ET AL. 2019, WANG ET AL. 2020). Moreover, during the process of map-based cloning, tightly-linked or even functional markers for disease resistances can be developed and used for marker assisted selection (MAS). As an example, STS marker *Xumn10,* which was recently converted in an user-friendly KASP marker (*Umn10*) and has been widely applied in western wheat breeding programs for the prediction of *Fusarium* resistance QTL *Fhb1* in genetic resources or cultivars (LIU ET AL. 2008, SCHWEIGER ET AL. 2016, STEINER ET AL. 2017). Next to this, BERNARDO ET

AL. (2012) discovered alternative SNP markers, such as *SNP3BS-11* or *SNP3BS-8* for *Fhb1*, but with *Umn10* there might be problems with false-positives, limiting the diagnostic value of these markers (BAI ET AL. 2018) Therefore, SU ET AL. (2018) developed two highly diagnostic markers from one putative candidate gene of *Fhb1*, that are either gel-based or non gel-based (KASP) for using it in various laboratories with different equipment and research questions. Also for other important wheat diseases the development of accurate and diagnostic markers for the employment in breeding programs is an ongoing task, when candidate genes are not yet available, e.g. the recently developed SNP markers *IWA6121* and *IWA4096*, that flank stripe rust resistance gene *Yr5* and may be used for resistance improvement in wheat (NARUOKA ET AL. 2016). By cloning resistance genes even functional markers can be developed, that derive from the functionally characterized sequence motifs (ANDERSEN & LÜBBERSTEDT 2003) and increase the selection process efficiently and support existing breeding strategies (GOUTAM ET AL. 2015).

Finally, the use of new genomic resources like SNP derived KASP assays or the complete genome sequencing of *Triticum aestivum* (APPELS ET AL. 2018*)* allow a clear physical positioning of our QTL interval and may facilitate a rapid identification of possible candidate genes. In summary, this study provides a valuable foundation towards the cloning of a FHB resistance locus in *T. monococcum*. Therefore, future work aims at the marker saturation of the target interval and anchoring to the physical map of wheat and the identification of candidate genes.

Contribution to the manuscript

Lisa Luthard, Doris Kopahnke, Edgar Schliephake conducted fied phenotyping and following statistical analyses. Dragan Perovich constructed the genetic linkage map of *Triticum monococcum*. Ilona Krämer and Caroline Breidenbach did QTL analyses with 10cM, 5cM, 2cM marker distances. Caroline Breidenbach contribute to statistical analyses and wrote the manuscript. Frank Ordon managed the project.

Chapter III |

Fine mapping of a FHB resistance QTL on chromosome 2A in *Triticum monococcum*

Breidenbach C., Krämer I., Ordon F.

1. Abstract

Diploid wheat like *Triticum monococcum* L. is a source for new resistance genes or quality traits, which may be used for the improvement of bread wheat (*Triticum aestivum* L.). In a recently conducted QTL study, two neighbouring QTLs for resistance to Fusarium Head Blight (FHB) were mapped in an interval of 45.1 cM on chromosome 2A in a *Triticum monococcum* DH-population. For this resistance QTL, a high resolution mapping population comprising 1991 F_2-plants corresponding to a resolution of 0.025 % recombination was constructed and used in the present study for marker saturation of the interval and phenotyping in field trials and in the greenhouse with *Fusarium culmorum* isolate *Fc46*. For marker saturation newly available genomic resources, like the recently published wheat genome sequence, the 90K iSelect assay as well as genotyping-by-sequencing (GBS) were applied. By using these resources, 21 markers were developed and mapped in a segmental RIL-population. All markers were in perfect collinearity with the wheat reference sequence Chinese Spring v1.0. In field trials as well as in additional greenhouse trials, phenotypic data revealed a quantitative distribution of FHB resistance. However, the localization of the resistance locus within the marker saturated target interval was not possible. A new QTL analysis with a reduced marker set conducted on the original DH mapping population and the original phenotypic data revealed a switch of the peak markers *SNP_0797* and *wmc0644*, causing the major QTL effect, to the long arm of chromosome 2A.

2. Introduction

Long domestication and breeding processes of crop plants gradually decreased the genetic diversity of cultivated crops like wheat (PRZEWIESLIK-ALLEN ET AL. 2019). Nevertheless, continuous varying environmental conditions warrant high genetic potential of plants to react to new circumstances. Plant genetic resources are a valuable source for the improvement of resistance or quality traits and therefore to obtain the nowadays yield level of cultivated species (HALEWOOD ET AL. 2018). Especially ancient wheat like *Triticum dicoccum* (Schrank) Schuebl., *Triticum spelta* L. or *Triticum monococcum* L. offer lots of opportunities for the identification of useful genes for important agronomic traits, e.g. resistance genes for FHB (WIWART 2004, GARVIN ET AL. 2009, KONVALINA ET AL. 2016), which have been lost during the evolutionary process of bread wheat. *Triticum monococcum*, a diploid wheat species (A^mA^m genome) with a set of 2n=2x=14 chromosomes was domesticated in the Karacadag mountains more than 10.000 years ago (HEUN ET AL. 1997). It was wide important for thousands of years for human food and livestock feed but was replaced during the Bronze Age by higher yielding and free-threshing wheat (BÉKÉS ET AL. 2017). Today the cultivation of *Triticum monococcum* is restricted to some small regions in Europe, India or the Balkans, but the advantages of einkorn wheat as a source of resistance or improvement of bread wheat quality is still important (ZAHRAIVA & MONNEVEUX 2014). Especially in times with rising demand for organic and ecological food and with governmental restricitions of insecticide and fungicide applications, *Triticum monococcum* may play again an important role in wheat breeding. Thus, genes from wheat relatives may be transferred into bread wheat cultivars to improve resistance and/or quality. The *Triticeae* group consists of 150 species, that can be used for screening of desired genes and used for introgressions into wheat (ORTIZ ET AL. 2008). These include accessions from the hexaploid, tetraploid and diploid wheat group, as well as from *Aegolopsis* spp. and accessions from *Thinopyrum, Hordeum, Secale* etc., that can be transmitted into bread wheat via direct or bridge crossing and hybridization events followed by embryo rescue (RASHEED ET AL. 2018). In recent years several successful transfers or hybridization events of chrosmosomal regions from relative wheat species into bread wheat have been reported (SHI ET AL. 1998, MIRANDA ET AL. 2006, KAUR ET AL. 2008, HUANG ET AL. 2019). Even three QTLs for FHB resistance, *Fhb3*, *Fhb6* and *Fhb7* were recently introgressed into hexaploid bread wheat from alien species *Leymus racemosus* (QI ET AL. 2008), 1Ets#1S of *Elymus tsukushiensis*

(CAINONG ET AL. 2015) and *Thinopyrum ponticum* (GUO ET AL. 2015) using chromosome engineering and translocation lines.

Lots of attempts were conducted in the last decades to map FHB resistance in various sources, e.g. chinese landraces or breeding lines, CIMMYT material or wheat germplasm from gene banks sources. Moreover, map-based cloning procedures were conducted which comprise a fine-mapping step and an adjustment with physical maps to identify chromosomal regions of importance with responsible candidate genes (KRATTINGER ET AL. 2009a). Positional cloning in wheat is a challenging task, because of its high genome complexity as well as large amount of repetitive DNA sequences (>85%) (HUANG ET AL. 2003, PAUX ET AL. 2006, KELLER ET AL. 2018), while this process is easier in *T. monoccocum* due to its smaller genome size. Nevertheless, some resistance genes were fine-mapped and acutally cloned in wheat, like septoria tritici blotch resistance gene *Stb6* (SAINTENAC ET AL. 2018), stem rust resistance genes *Sr21* (CHEN ET AL. 2018), *Sr33* (PERIYANNAN ET AL. 2013) and *Sr35* (SAINTENAC ET AL. 2013), as well as leaf rust resistance genes *Lr67* (MOORE ET AL. 2015) and *Lr1* (CLOUTIER ET AL. 2007, QIU ET AL. 2007). In *T. monococcum* powdery mildew resistance gene *TmPm3* (ZHAO ET AL. 2016) was recently isolated. Even the major *Fhb1* QTL on chromosome 3BS conferring type II was fine-mapped and 28 putative candidate genes characterized (SCHWEIGER ET AL. 2016). A pore-forming toxin-like domain and a gene encoding a chimeric lectin with agglutinin domains are being regarded as the potential genes after map-based cloning (RAWAT ET AL. 2016), whereas SU ET AL.(2019) reported a potein *TaHR*C as responsible candidate gene.

An important requirement for map-based cloning is the availability of informative markers, which was limited in the past. Dominant AFLP or locus specific co-dominant SSR markers were widely used in plant genetic projects in the past, but with the emergence of next generation sequencing techniques the marker systems of choice shifted towards SNP markers (BERNARDO ET AL. 2015). The 90K iSelect Infinium wheat chip (WANG ET AL. 2014) or genotyping-by-sequencing (GBS) (ELSHIRE ET AL. 2011, POLAND ET AL. 2012) are often used high-throughput marker systems in the last years, which is shown through the increasing number of SNP genotyping arrays and GBS protocols in over 25 crop species (RASHEED ET AL. 2017) and were also applied in this study for marker saturation. GBS offers a powerful tool for marker discovery in targeted regions or the

whole genome in every species, even in species with high genetic diverse and large genomes (HE ET AL. 2014). It is a simple, specific and highly reproducible system (ELSHIRE ET AL. 2011, CHUNG ET AL. 2017). For wheat and barley, a two-restriction-enzyme system was developed and usually applied in plant genetic studies, providing appropriate genome complexity reduction and uniform library preparation (POLAND ET AL. 2012, HE ET AL. 2014). These multiplex marker technologies are very suitable for genome-wide SNP genotyping for genetic mapping or whole genome selection methods, whereas for gene identification or MAS flexible, high-throuput, uniplex genotyping platforms, like kompetitive allele specific PCR (KASP) (LGC-Genomics) or TaqMan® (Applied Biosystems, Forster City, CA) are more appropriate (SEMAGN ET AL. 2014, RASHEED ET AL. 2016). KASP is a homogenous, fluorescence-based genotyping technology using two competing allele-specific forward primers, one reverse primer and a master mix with a FRET cassette and Taq polymerase (SEMAGN ET AL. 2014, THOMAS 2014). KASP-assays can be done in-house or outsourced by LGC (THOMAS 2014). Thus, SNP markers detected by mapping studies can be easily converted into KASP markers to facilitate MAS, e.g. for *Lr23* (CHHETRI ET AL. 2017), *Yr26* (WU ET AL. 2018) or *Septoria tritici* blotch- QTL (DREISIGACKER ET AL. 2015). Moreover, the current version of the wheat reference genome Chinese spring v1.0 (APPELS ET AL. 2018) as well of wheat relatives, e.g. *Triticum urartu* (LING ET AL. 2018), *Triticum turgidum* spp. *diccocoides* (AVNI ET AL. 2017) *and Aegilops tauschii* (LUO ET AL. 2017, ZHAO ET AL. 2017) plus the development of public open databases like *Urgi* (http://wheat-urgi.versailles.inra.fr/), *GrainGenes* (https://wheat.pw.usda.gov), *Triticeae tool box* (https://triticeaetoolbox.org/wheat/) pave the way to a precise and fast identification of informative markers for MAS via association mapping or map-based cloning (RASHEED & XIA 2019).

This study focuses on research towards the isolation of a QTL for FHB resistance by saturating the QTL interval with SNP markers and integrating the resistance locus by phenotypic evaluation of a population of segmental RILs.

3. Material and Methods

3.1. Plant material & DNA extraction

A recombinant inbred line (RIL-) population, comprising 333 lines, was subjected to marker saturation and phenotyping. The development of this population was traced back to a cross between two resistant (C35, C42) and three susceptible (A37, A39, B22) doubled haploid lines from the recent DH mapping population (BREIDENBACH ET AL. 2020, in prep.). DNA of all developed segmental RILs was extracted according to STEIN ET AL. (2001) from plants in the two to three leaf stage and used for genotyping of the population. The RILs, at this time in the F_3-generation, are then further selfed in the greenhouse to get F_4 -kernels for the phenotypic analyses.

3.2. Marker development for marker saturation

a. Genetic map

The first SNP markers for the saturation of the QTL intervals were selected from the genetic map of *Triticum monococcum* (BREIDENBACH ET AL. 2020, in prep.). The prerequisite was the correct location within the target region, polymorphism between parental lines and available sequence information of at least one hundred nucleotides around the SNP site. Then, SNP sequences were send to LGC genomics for allele-specific KASP assay design (http://www.lgcgenomics.com).

b. 90K iSelect Chip

Next genotyping of parental lines as well as of susceptible and resistant bulks of DH-lines of the original mapping population with the 90K iSelect SNP Chip was conducted at TraitGenetics (Gatersleben). Extracted DNA was diluted to 50ng/µl using a NanoDrop ND 1000 Spectrophotometer (PEQLAB Biotechnology GmbH, Erlangen). Genotypic data were filtered to polymorphism between susceptible and resistant lines and bulks within the QTLregion (36 Mbp – 69 Mbp) on chromosome 2A. KASP primers of all polymorphic SNP markers were ordered by Mycrosynth AG in Switzerland and used for genotyping of the RIL-population.

c. Genotyping-by-sequencing

For the creation of the required genomic libraries, DNA samples consisting of 10 resistant and 10 susceptible DH-lines and the parental lines of the original mapping population, were compiled. The samples were prepared for sequencing at MiSeq (Illumina Inc.) following the protocol of WENDLER ET AL. (2014). The genomic library was then sequenced using the MiSeq Reagent Kit v3 (Illumina Inc., San Diego, USA) according to the instructions of the manufacturer. Obtained results were evaluated using Galaxy implemented bioinformatical pipelines. Adapter and quality trimming was done with trim galore: https://github.com/FelixKrueger/TrimGalore, version 0.4.0, non default parameter: quality > 30, read length > 50. Then, sequence data of every individual line were aligned with the sequence of chromosome 2A of *Triticum aestivum* v1.0 Chinese spring using BWA-mem (v0.7.15-r1140) (LI 2013). The results were further filtered to get informative SNP markers for the target region using SAMtools Mpileup: (v1.2 using htslib 1.2.1) (LI ET AL. 2009), SNPSift filter (CINGOLANI ET AL. 2012) and BCFtools_call:https://samtools.github.io/bcftools/bcftools.html. With these, bi-allelic SNPs were detected, that mapped within the QTL inverval of 36 Mbp to 69 Mbp (BREIDENBACH ET AL. 2020, in prep.), show a polymorphism between susceptible and resistant lines and have a minimum coverage of five reads per SNP.

3.3. KASP analyses

The GBS markers and the SNP markers from the genetic map of *Triticum monococcum* in this study were converted into KASP assays by LGCgenomics und run on a real-time-PCR-system (Bio-Rad Laboratories, München) with a protocol already described by BREIDENBACH ET AL. (2020, in prep.). The SNP markers from the 90K iSelect Chip were also converted into KASP-markers, but they were applied by Mycrosynth AG (Schweiz) and run on the same system.

3.4. Construction high-resolution map/ linkage analyses

All 333 RILs were genotyped with the newly developed KASP markers and results transferred in an A/B matrix, where A indicates the allele of the resistant parents and B

of the susceptible one. Linkage analyses for the development of a high-resolution map was performed by counting the recombinations between two marker loci and multiplicate them with the genetic resolution provided by the high-resolution mapping population. The genetic resolution was corrected due to losses of plants or seed during cultivation in field and greenhouse, by dividing the % recombination by the number of remaining RILs. A precise procedure for the implementation of linkage analyses for high-resolution maps was described by LÜPKEN ET AL. (2013). The genetic marker loci of the high-resolution map were subsequently compared to the physical positions in the reference maps of *Triticum aestivum* (APPELS ET AL. 2018). SNP sequences were blasted as fasta files against the *T. aestivum* reference sequence: IWGSC RefSeq CS v1.0 (URGI 2018). The best hit (expected threshold = 0.0001) was chosen to determine physical marker positions. However, the SNP-markers derive from *T. monococcum* and if there was no match for one SNP marker on the wheat reference map (URGI 2018), the expected threshold was continously increased up to 1.

3.5. Phenotyping field

a. Field 2017

In 2017, 37 RILs plus 17 sister lines, the parental lines (*mon10-1*, A37, A39, B22, C35, C42, *Sinskayae*) and wheat controls (Alsen, Bobwhite, Remus, CM82036) were sown in the beginning of April in double-rows with 60 seeds per genotype in the field at Quedlinburg (51.7694 N, 11.147 E, 140 m altitude) (Figure 11) and Leopoldshöhe (52.0160 N, 8.700 E, 113 m altitude). Infection of spikes started at 50% anthesis with a conidia suspension of 300.000 C/ml. The inoculum was produced as described by BREIDENBACH ET AL. (2020, in prep.) The suspension was applied with a backsprayer for

Figure 11 | Field trial in Quedlinburg 2017

two times (Figure 12). The second application was done three to four days after the first infection to compensate different flowering times of each genotype. The infected plant were irrigated shortly before inoculation in the early morning using a field syringe and the days between the infection period, depending on the respective weather situation to ensure humid conditions. The scoring started 10 days after the infection (dpi) and was repeated 14 dpi, 18 dpi and 22 dpi. FHB resistance was recorded by measuring percentage infestation of each double-row/genotype as described by MOLL ET AL. (2010). AUDPC- and AO-values were calculated following VATTER ET AL. (2017).

Figure 12 | Artificial inoculation of *T. monococcum* with *F. culmorum* Fc46 using a backsprayer

b. Field 2018

In 2018, 165 RILs plus 61 sister lines, the crossing parents (A37, A39, B22, C35, C42, *mon10-1*, *Sinskayae*) and wheat controls (Alsen, Remus, Bobwhite, CM80362) were sown in the beginning of April in single-rows with one replication following a complete randomized design in Quedlinburg (51.7694 N, 11.147 E, 140 m altitude) and in Leopoldshöhe (52.0160 N, 8.700 E, 113 m altitude). Further infection and scoring procedure are described above.

3.6. Phenotyping greenhouse

The RILs were evaluated for type I and type II resistance in the greenhouse from July 2017 to March 2018. Six plants per genotype were potted in two 5l clay pots, three plants in one pot for an infection using the spray inoculation method (Type I, Figure 13 (left)) method and three plants in another pot for the point inoculation (Type II, Figure 13 (right)) method. Until flowering time, plants were grown in the greenhouse with 16 h period of light, 20°C day temperature and 16°C night temperature. At BBCH65, at least three spikes per plant were inoculated with *Fusarium culmorum* isolate *Fc46* by applying a conidia suspension of 150.000 C/ml. Inoculum was produced according to BREIDENBACH ET AL. (2020, in prep.) To generate equal infection conditions, an approach of 10 ml suspension for each infection time point was used. For spray inoculation, the conidia suspension was sprayed on two sides of the ear and from above, while for point inoculation, the conidia suspension was injected directly with a syringe into two faced central spikelets. Inoculated heads were bagged with a plastic bag for two days to ensure high moisture for pathogens growths. The pots were then placed in a growth chamber with 22°C during the day and 17°C during night and a light period of 16 h, to get optimal infection conditions. The scoring started 7 days after the inoculation (dpi) and was repeated at 10 dpi and 14 dpi. The number of all spikelets per ear and those infected was counted to calculate the percentage infestation and out of this the area under the disease progress curve (AUDPC) followed by the calculation of the average ordinate (AO) as described by VATTER ET AL. (2017).

Figure 13 | Spray inoculation method (left) and point inoculation method (right)

3.7. Statistical analyses

Analysis of Variance (ANOVA) was performed using the software program SAS 9.4 (SAS Institute Inc. Cary, NC, USA) and the procedure *proc mixed*. RILs were set as fixed and replications as random effects. Pearson's correlation coefficient for the inoculation methods was calculated with *proc corr*.

3.8. QTL analysis

QTL analysis was performed with MapQTL v.5 (IM/MQM mapping procedure), using phenotypic greenhouse data 2017/2018 (spray inoculation) and genotypic data of the high-resolution mapping population. For verification of the QTL interval from the original DH mapping population (BREIDENBACH ET AL. 2020, in prep.), a new QTL analysis (MQM mapping procedure) was performed with physical positions of a reduced marker set and phenotypic data from fieldtrials 2008/2009. When selecting the markers, care was taken to ensure that they are evenly distributed over chromosome 2A and that they could be assigned to the wheat reference sequence CS v1.0 (APPELS ET AL. 2018), which resulted in 46 markers.

3.9. Correlation of morphological traits with FHB resistance

Mon10-1 and *Sinskayae* as well as the parental lines from the HRM-population (A37, A39, B22, C45, C42) show different spike morphology (BREIDENBACH ET AL. 2020, in prep.). 94 DH-lines were grown in the greenhouse until ear emergence to record their ear shape. They were scored in the *m-group*, when they have the ear shape of the parental line *mon10-1* (elongated spike, tenacious glumes, glaucouness) while they were classified in the *s-group,* when they show the ear shape of the susceptible *Sinskayae* (compact spike, soft glumes). FHB severity (mean AO-values from field trials 2008/2009) for each ear shape-group (*m/s*) was then tested for significant differences using software program SAS 9.4 (SAS Institute Inc. Cary, NC, USA) with the procedure *proc ttest.*

4. Results

4.1. Phenotypic evaluation

Sprouting difficulties at Leopoldshöhe in 2017 and unfavorable climatic conditions at both sites in 2018 resulted in a low FHB infection. Therefore, only the greenhouse data were considered for analyses. Due to losses of plants during cultivation in the greenhouse and growth chamber or because of an unsufficient infection, a different number of genotypes was scored for both inoculation methods. In total, 101 RILs were scored for FHB resistance after point inoculation (resistance type II) and 124 RILs plus three resistant (*mon10-1*, C35, C42) and three susceptible (*Sinskayae,* A37, B22) parental lines were assessed after spray inoculation (resistance type I). ANOVA revealed highly significant effects of the genotype for both inoculation methods (Table 7). A continuous variation was observed for spray and point inoulation (Figure 14, Figure 15).

Table 7 | Analysis of variance (ANOVA) for FHB severity for RILs evaluated with two inoculation methods (spray incoulation/point inoculation)

Inoculation	Effect	DF	F-value	P
Spray	Genotype	129	4.85	<0.0001
Point	Genotype	100	5.33	<0.0001

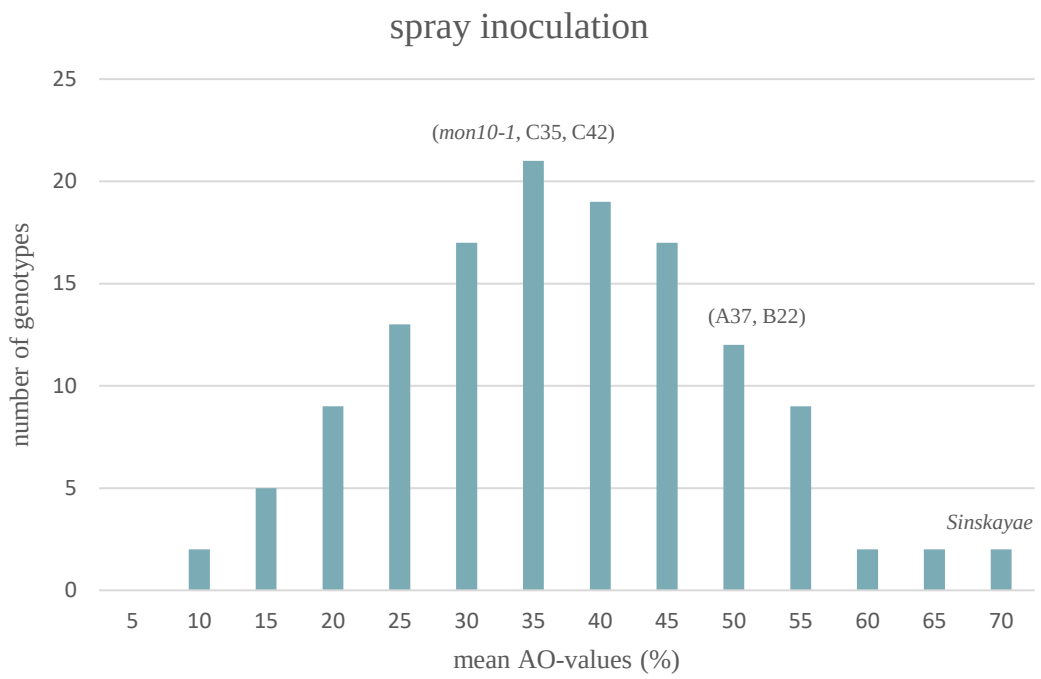

Figure 14 | Frequency of distribution for mean AO-values of 124 RILs and the six parental lines (res.: mon10-1, C35, C42; susc.: Sinskayae, A37, B22) using spray inoculation method and *F. culmorum Fc46*- isolate

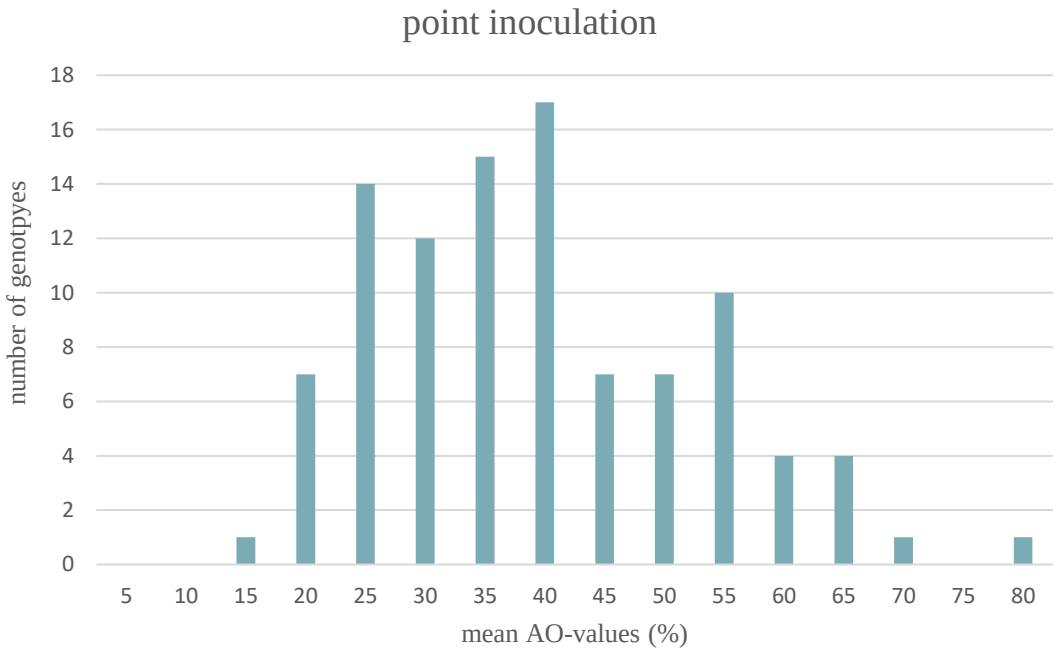

Figure 15 | Frequency of distribution for mean AO-values of 100 RILs using point inoculation method and *F.culmorum Fc46*- isolate

Disease severity for spray inoculation ranged between 9.07 % and 69.14 % with an average of 35 %. Resistant parents (*mon10-1*, C35, C42) show a mean level of infection between 31 % - 35 %, whereas the susceptible parents (*Sinskayae*, A37, B22) are highly susceptible with AO-values between approximately 50 % and 70 % disease severity. Due to losses of plants during cultivation, parental lines were not tested by point inoculation. For point inoculation, disease severity ranged between 15.38 % and 79.48 % and is on average 37 %, which indicates that RILs are a bit more susceptible when the *Fusarium*-isolate is injected directly into the ear. To check for a correlation between both inoculation methods, Pearson correlation coefficient was calculated and resulted in r=0.61 ($p<0.0001$) (Figure 16).

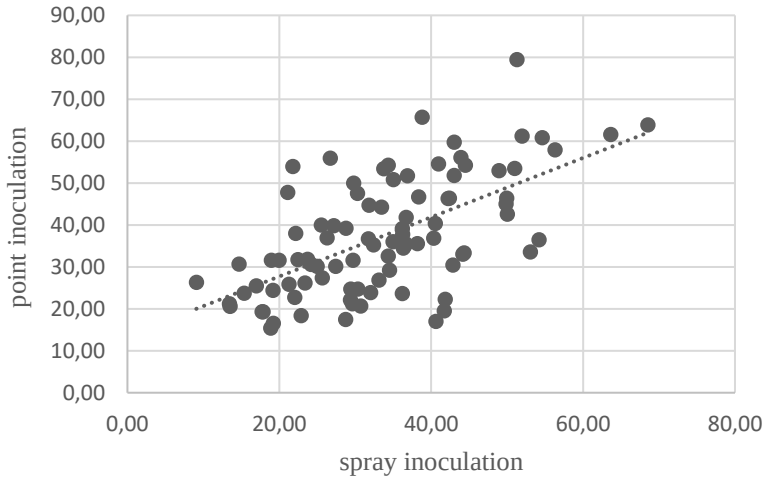

Figure 16 | Correlation between AO-values of 91 RILs inoculated in one case using point inoculation method and in the other case with spray inoculation method

4.2. Marker development for marker saturation

Nine polymorphic SNPs were selected from the original genetic map of *Triticum monococcum* (BREIDENBACH ET AL. 2020, in prep.) and converted into co-dominant PCR-based KASP markers. The other markers in the interval were either not polymorphic on the parental lines chosen for the construction of the high-resolution mapping population, could not be converted into KASP assays or the KASP primers did not work well in the RIL-population. The marker order of two markers *SNP_1315* and *SNP_1453* is inverted on the genetic and physical map. The other markers are in collinearity (Figure 17). The

genotyping of *Triticum monoccoum* resistant and susceptible lines with the 90K iSelect Chip resulted in only 27 polymorphic markers between or parental lines as well as susceptible and resistant bulks, that were mapped outside the target interval and were therefore excluded from further analyses (data not shown).

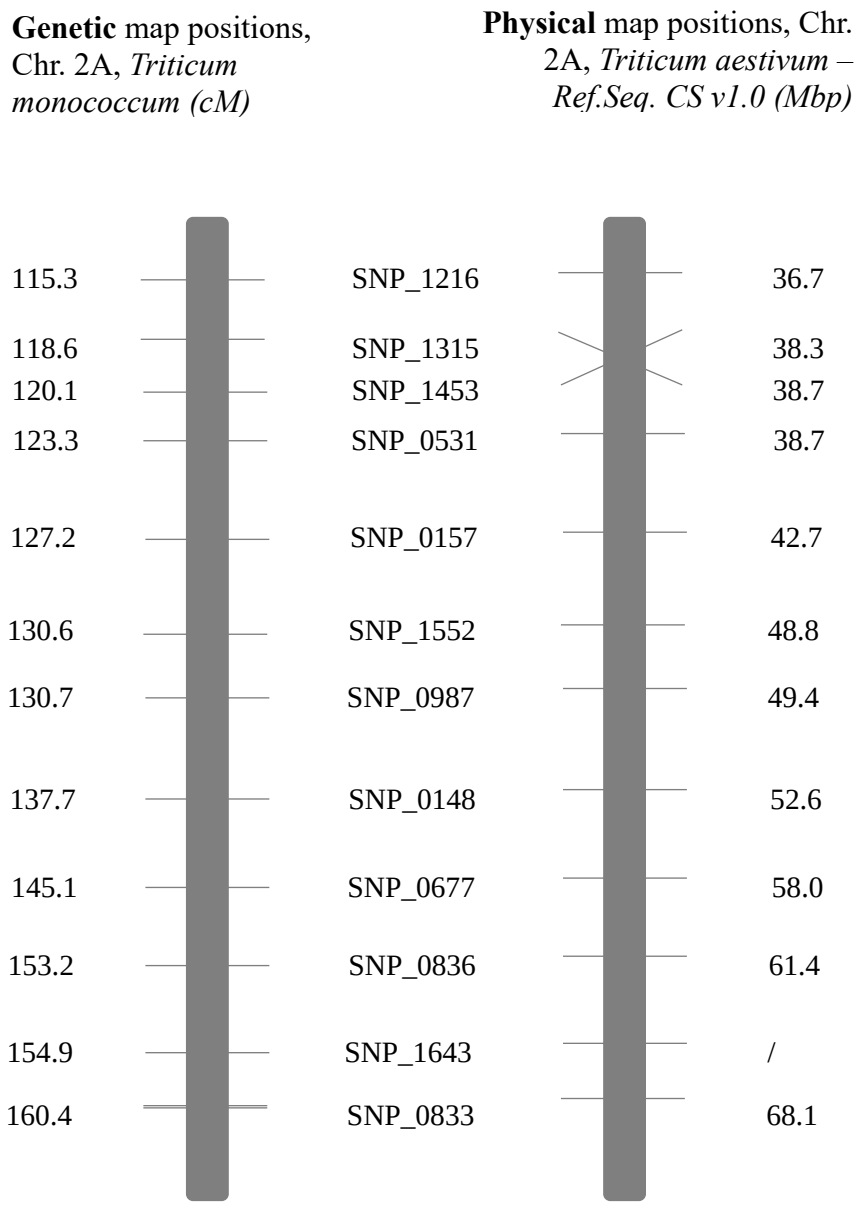

Figure 17 | Genetic and physical map positions of flanking markers (SNP_1216, SNP_0667, SNP_0833) plus selected markers from the original genetic map of chromosome 2A and their collinearity

GBS was also applied to generate new saturating markers for the respective QTL interval. With this approach, 52 markers, that were polymorphic between the parental lines and susceptible and resistant bulks and mapped within the target interval were developd. Out of these, 13 informative markers were selected for genotypic analyses, that were well distributed over the interval to achieve sufficient coverage of the high-resolution map between the flanking markers SNP_1216 and SNP_0833 (Table 8). GBS_9 did not perform well in PCR analyses and was therefore excluded from further analyses.

Table 8 | Selected GBS-marker, their physical positions and respective polymorphism

Chromosome	Marker	Position *T.aestivum* (Mbp)	Reference	Alternative
2A	GBS_1	36.8	A	C
2A	GBS_2	38.2	G	A
2A	GBS_3	38.7	A	G
2A	GBS_4	38.9	A	G
2A	GBS_5	38.9	T	A
2A	GBS_6	39.3	A	G
2A	GBS_7	42.1	G	C
2A	GBS_8	58.9	G	A
2A	GBS_10	61.4	A	G
2A	GBS_11	61.4	A	G
2A	GBS_12	62.2	C	T
2A	GBS_13	62.2	C	T

4.3. Construction high-resolution mapping population/high-resolution map

The RIL-population (BREIDENBACH ET AL. 2020, in prep.) initially comprised 333 lines, but has to be corrected to 268 lines due to losses of plants during cultivation in the greenhouse or removal of RILs showing heterozygosity for the saturating markers. This led to a corrected genetic resolution of 0.066 % rec.. The genotyping of the population with the newly 21 markers for the target interval (Figure 17, Table 8) resulted in the following high-resolution map (Figure 18).

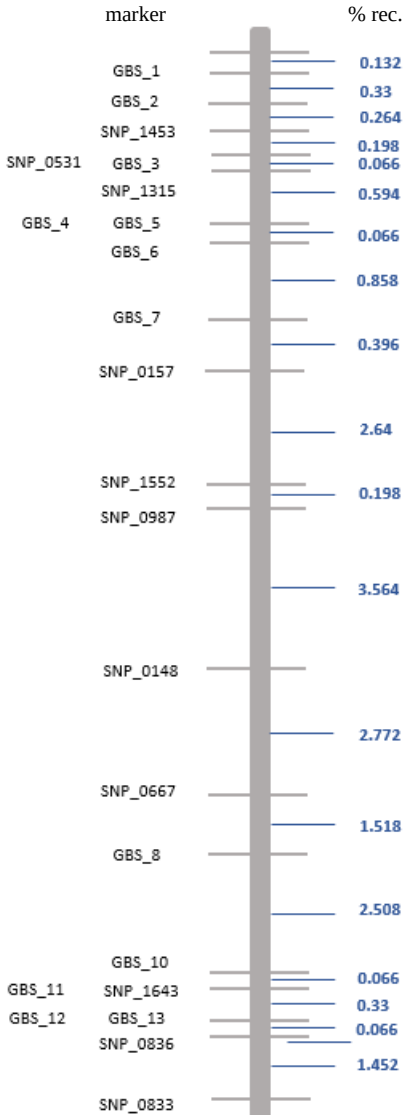

Figure 18 | High-resolution map (HR-map) with a genetic resolution of 0.066 % based on 268 RILs

4.4. QTL analysis for localization of the resistance locus

The phenotypic data from the greenhouse trial did not allow a classification into an A/B-matrix, making it difficult to map the resistance locus within the high-resolution map. A QTL analysis with phenotypic data from the spray inoculation method in the greenhouse and the high-resolution genetic map (Figure 18) was conducted to map the locus. Either with IM nor with MQM-mapping, a significant peak with LOD > 3 was observed (Figure 19). From these results it was concluded that the resistance to FHB is not located within the previously detected interval.

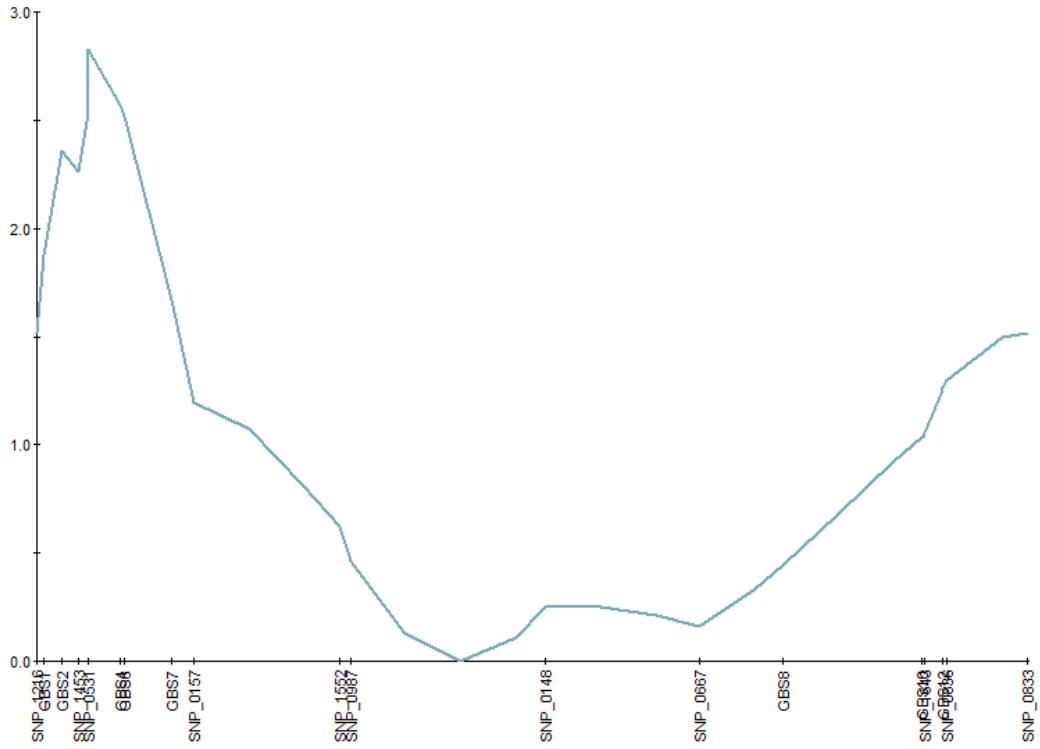

Figure 19 | QTL analysis for FHB resistance in the RIL-population comprising 124 genotypes and phenotypic greenhouse data for spray inoculation method

4.5. QTL analysis for verification of the interval

To get more detailed information on this unexpected result, a new QTL analysis was conducted with a reduced set of markers and their physical marker positions instead of genetic positions as well as original phenotypic data. One QTL was mapped after IM/MQM-mapping between marker *SNP_0796* (499.58 Mbp) and *SNP_0123* (607.96 Mbp) on chromsome 2AL and explains 86 % of phenotypic variance (Figure 20). The closest markers are *SNP_0797* and *SSR wmc0644*, which also have been the peak markers in the original QTL analysis (BREIDENBACH ET AL. 2020, in prep.) This result shows that the peak markers, causing the major QTL effect, switched from the former location, the short arm of Chromosome 2A (36.72 Mbp – 68.17 Mbp) towards the centromeric region on the long arm of the chromosome, which may be an explanation for the fact that the phenotypic data of the RILs did not allow mapping of the resistance locus within the target interval.

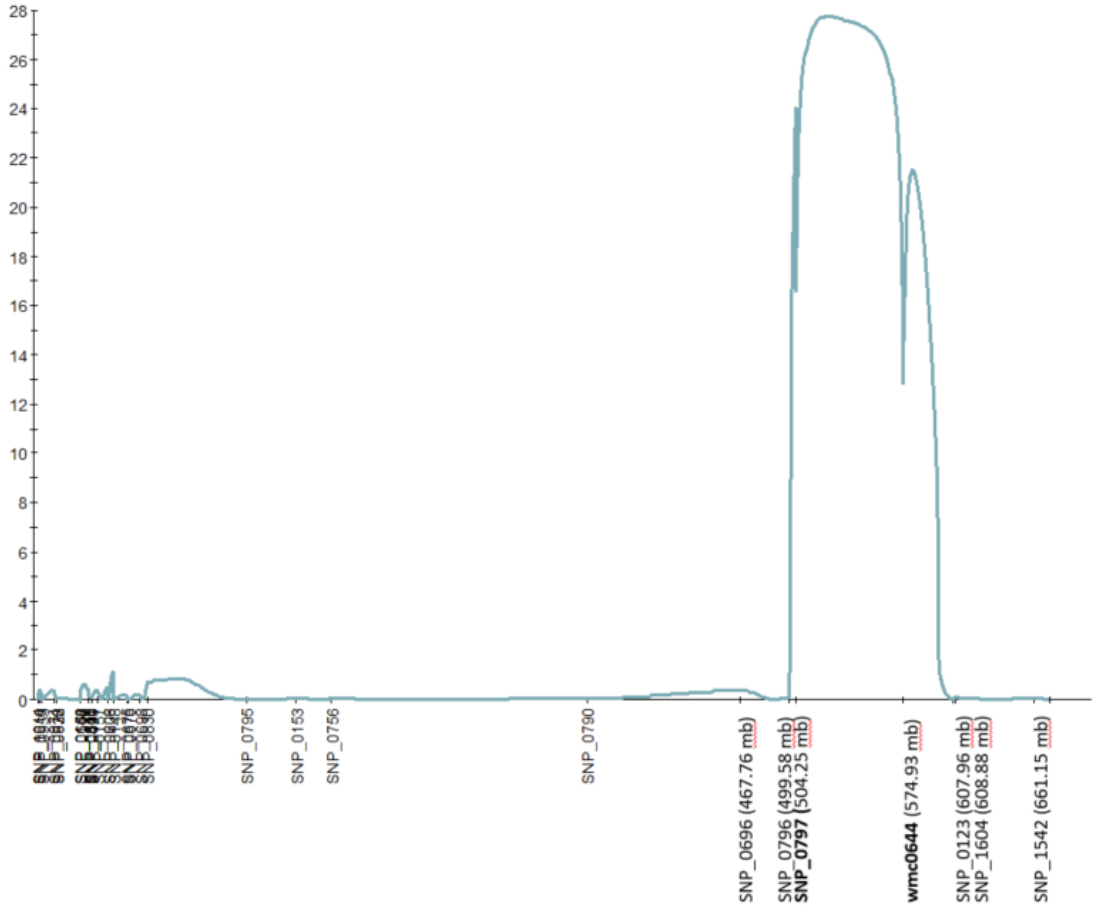

Figure 20 | QTL analysis for the DH-mapping population with a reduced marker set (physical positions) and phenotypic field data from 2008/2009

4.6. Correlation of ear shape with FHB resistance

94 DH-lines from the original mapping population as well as the parental lines, *mon10-1* and *Sinskayae*, were grown in the greenhouse for the characterisation of the ear. Out of these, 40 plants germinated and were examined for the ear type at BBCH51-59. 22 DH-lines show the ear shape of the resistant parent *mon10-1* and were sorted in the *m*-group. All of these lines, except of three (monA42, mon A50, monC5) were resistant in the field trials 2008/2009 and show mean AO-values below 20.00 %. The other 18 lines show the ear shape *s* of the susceptible parent *Sinskayae* and were also very suscepible in the field with mean AO-values higher 25.00 % (Suppl. Data 3). A significant difference ($p<0.0001$) concerning the AO-values with respect to the ear shape was observed (Figure 21).

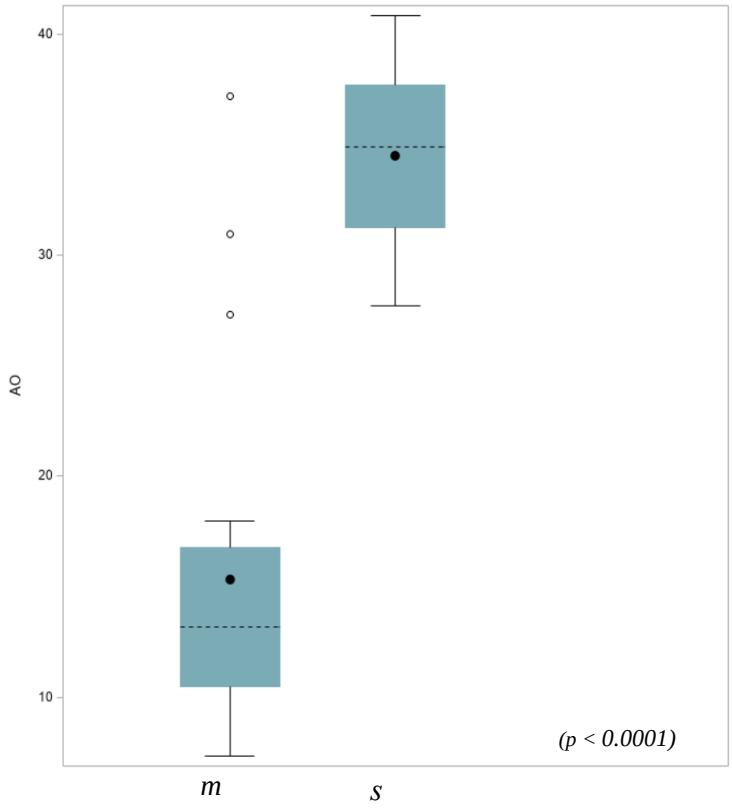

Figure 21 | Results of the t-test of grouped AO-values with respect to the ear shape

5. Discussion

This study was conducted to reduce the large QTL interval and to identify candidate genes for FHB resistance on chromsome 2A in a *Triticum monococcum* DH-population using a map-based cloning approach. The study made use of new genomic tools like the 90K iSelect Chip and genotyping-by-sequencing (GBS) for the development of new markers, which saturate the target interval. In the last years NGS-technologies enabled the construction of high-density genetic maps for fine mapping, elucidating their importance for genomics based breeding research (JAGANATHAN ET AL. 2020). Especially GBS offers a high SNP coverage throughout the whole genome, even in telomeric and some centromeric regions in a highly cost-effective manner. Nevertheless a higher SNP density in gene-rich regions was reported (DEDONATO ET AL. 2013, SONAH ET AL. 2013). In this

study, GBS was successfully applied using the two-restriction-enzyme procedure of POLAND ET AL. (2012) and 52 markers were detected, that are polymorphic between the parental lines of the high-resolution mapping population and mapped within the target interval. Out of these, 12 informative markers were used for further analyses (Table 8). For the generation of new markers, the GBS results were aligned with the reference sequence of *T. aestivum* Chinese spring v1.0 (APPELS ET AL. 2018). Nevertheless, at the end of the project, the reference sequence of *T. urartu* (LING ET AL. 2018) was even published and may be used in new studies for further marker development. In comparison, the analysis with the 90K iSelect chip was not so efficient, because only a small number of polymorphic SNPs was detected between the susceptible and resistant *T. monococcum* lines. That is because the 90K iSelect chip has main representation from bread wheat and is thus not an efficient tool to find polymorphisms in wild relatives like einkorn wheat (RASHEED & XIA 2019). It would have been better to screen *T. monococcum* lines with the recently developed 820K array or with the smaller subset array, the Axiom 35K SNP, which are based on the characterisation of 475 wheat accessions and wild relatives and therefore may serve as a powerful substitution to the 90 K array in case of the analyses of genetic resources of wheat (WINFIELD ET AL. 2016, ALLEN ET AL. 2017, RASHEED & XIA 2019).

All 21 SNP markers obtained from GBS and the genetic map of *T. monococcum* were converted into KASP markers for genotpying of the high-resolution mapping population. KASP markers are commonly used nowadays for high-throuput genotyping approaches, because KASP technology is very efficient and fast (RASHEED ET AL. 2018). KASP analyses consists of two forward primers with each being specific for a particular allele that is associated with one flourophore and one reserve primer and discrimination is achieved by real-time PCR or a flourescence microplate reader (JAMANN ET AL. 2015). In comparison to e.g. TaqMan®, KASP achieve higher success in assay design rates and conversion into working assays (SEGMAN ET AL. 2014). They are developed from SNP sequences and serve as an effective tool in genomics breeding research, as shown in our study, and may also be easy implemented in wheat breeding for MAS of specific traits. (CHHETRI ET AL. 2017, WU ET AL. 2017, VAGNDORF 2018, YANG ET AL. 2019).

Reliable phenotypic data are a prerequisite for developing closely linked markers. Greenhouse data show a continous distiribution and thus underline the polygenic,

quantitative behavior of FHB reistance (MA ET AL. 2006). In 2017 and 2018 field trials were conducted in Quedlinburg und Leopoldshöhe to test the resistance of the RILs. Both trials were artificially inoculated, which is necessary to get reliable resistance data (BUERSTMAYR ET AL. 2003). While too little seed was available in 2017, there were problems with low infection pressure in 2018 due to extreme weather conditions. Especially Central and North-Eastern Germany experienced severe drought due to abnormally high temperatures and low precipitation events (MEINERT & SCHUBE 2018), resulting in early ripening and a restricted development of *Fusarium culmorum*. The dependence of Fusarium growth on climatic conditions was also observed in other studies (SIMÓN ET AL. 2005, OSBORNE & STEIN 2007, GORZCZYCA ET AL. 2017). The weather conditions were so exceptional and disadvantageous for *F.culmorum* so that even artificial irrigation could not improve the infection rate in Quedlinburg und Leopoldshöhe. This observation supports the common view that optimale infection conditions for the pathogen are always of prime importance to get informative phenotypic results in field trials. Because of difficult conditions in the field, additional greenhouse trials were conducted in 2017/2018. Greenhouse trials provide resistance evalutation under highly controlled conditions and are therefore not dependend on environemental interactions, which lead to an advanced reproducibility of results. The infection rate in our experiment was assessed until the beginning of ripening, which is usually 21 days after infection in wheat (MIEDANER ET AL. 1996, YANG ET AL. 2003). Our assessment finished after 14 dpi, because the ears of *T. monococcum* are smaller than wheat ears and lost chlorophyll already after this time point. For *T. monococcum*, we try to implement the spray inoculation method in the greenhouse to get comparable results with field trials and natural epidemic conditions. Spray inoculation method display all possible resistance mechanisms contributing to the respective genotype, although no clear separation in respective mechanisms is possible when using spray incoulation (BUERSTMAYR ET AL. 2000, BUERSTMAYR ET AL. 2003). However, the handling is easier compared to point inoculation, especially when a large amount of ears has to be infected. In accordance with STEINER ET AL. (2004), the variation of FHB symptoms was quantitative for both inoculation techniques, but more phenotypic variation was observed after spray inoculation.

Although phenotypic greenhouse data showed a broad variation, the FHB resistance locus could not be integrated in the genetic map. So, the high phenotypic variance of 81.8 % and 34.7 % respectively, which was explained from the original QTL study of the *T. monococcum* DH-population (BREIDENBACH ET AL. 2020, in prep.) could not be recovered in the HRM-population. A new QTL analysis with genotypic data from the HRM-population and respective phenotypic data from greenhouse trials does not result in a reliable mapping. It is not an unkown phenomenon that cloning projects are unsuccesful on the basis of a so-called QTL fractionation, i.e. that the QTL effect is based on linked genes that co-segregate, but break-down with the identification of recombinant plants resulting in a loss of the respective effect (ASINS ET AL. 2009, JAMANN ET AL. 2015). Other possibiltes are an unsufficient selection of suitable recombinants or a loss of the QTL effect due to a different genetic background (JAMANN ET AL. 2015, SALVI & TUBEROSA 2007). In our case, however, it is likely that the error can be attributed to an incorrect construction of the genetic linkage map due to a false marker order. The first QTL interval ranged on the physical map of *T. aestivum* in an interval between 36.72 – 68.17 Mbp, but the responsible peak marker for the QTL, *SNP_0797* and *wmc0644*, were located at 504.25 and 574.93 Mbp, respectively in the physical map and were thus incorrectly mapped in the original gentic map of chromosome 2A. Genotyping errors or a limited number of informative meioses were regarded as possible causes for inaccuricies in genetic maps that can affect marker-trait linkage studies (DEWAN ET AL. 2002). Moreover, considerable discrepancies between the genetic map and the acutal marker positions on the physical map even to the extent of an incorrect ordering of genes were already demonstrated in yeast, a model organism, in the early 1990s (BROWN 2002). Genetic map distances are based on recombination events between two loci, while physical maps are constructed on the actual nucleotide number. And although genetic maps clarify mutual relationships between marker loci or genes, they do not reflect the real physical distance and position of marker loci (AZHAGUVEL ET AL. 2010), which may lead to an incorrect mapping of QTL. Nowadays, the availabilty of fully sequenced reference genomes of cereal crop species, e.g. barley (MASCHER ET AL. 2017) or wheat (APPELS ET AL. 2018) lead to a more reliable and accurate QTL mapping, because they can be mapped not soley by genetic positions but also by phsyical positions (WARD ET AL. 2019). Therefore today's studies made use of physical marker positions to get more convincing results (NOVAKAZI ET AL. 2019, LI F ET AL. 2019). In our study, the

verification of the QTL interval was also conducted with physical marker positions and demonstrate the switch of the peak markers contributing to the major QTL effect, to a more proximal region of chromosome 2A (Figure 20). As the construction of the HRM-population was based on an incorrect selection of recombinant plants due to a wrong interval, the main QTL effect was probably not considered. This may explain why phenotypic and genotypic data could not be linked in this study.

The parental lines of the DH-population are not only segregating in FHB resistance, but also in their spike morphology. *Mon10-1* is non free-threshing and has an elongated, glaucousness spike with tight glumes, whereas *Sinskayae* has a semi-compact spike, reduced length, softer glumes and is free-threshing. A significant difference in AO-values concerning the ear shape was determined in the DH-population (Figure 21). Lines with ear type *m*, like the resistant parent *mon10-1*, are more resistant to FHB than lines, that m have the same ear type *s* from *Sinskayae.* Responsible for the *Sinskayae* phenotype is a *soft glume*-gene (*sog)*, which was mapped on chromosome 2A near the centromere. *Sog* is responsible for soft glumes and threshability and it is assumed that soft glume, free-threshing and compact ear type are closely linked (GONCHAROV ET AL. 2007, KONOPATSKAIA ET AL. 2016). SOOD ET AL. (2009) mapped the *sog*-gene between SSR marker *gwm71* and RFLP marker *xbcd120* in an interval of 6.8 cM, while AMAGAI ET AL. (2017) located *sog* also on chromosome 2A in the same region between SSR markers *gwm558* and *wmc644* in an interval of 13.6 cM. These flanking markers were anchored to the wheat reference sequence CS v1.0 to identify the exact pysical position of *sog.* It was not possible to characterize the precise interval of *sog*, as indicated by SOOD ET AL. (2009). The distal position of marker *gwm71* is at 230.8 Mbp, while physical position of proximal RFLP-marker *xbcd20* could not be determined. By AMAGAI ET AL. (2017), the *sog*-gene was located between 361.16 Mbp and 574.93 Mbp on the physical map, while our QTL is also located in the same region, between 499.58 Mbp and 607.96 Mbp. Contrary to authors primary assumptions, that the *sog*-gene is located on the short arm of the chromosome, physical positions indicate that the *sog*-gene is rather placed on the long arm of chromosome 2A (URGI 2018, APPELS ET AL. 2018). This fact and the demonstration of the significant correlation between FHB severity and ear shape of *T. monococcum* DH-lines give hint that the large QTL effect may probably no active resistance factor, but resulted rather from passive resistance mechanism, caused by the

different spike morphology. Nevertheless, it remains unclear, if the *sog*-gene has a pleiotropic effect on FHB resistance or if both genes are in close linkage in the same genetic block. Further analyses are necessary to identify the responsible genes causing the major QTL effect detected in this study.

Chapter IV | General discussion

Active resistance response vs. passive resistance factors

Genetic resources are commonly used in wheat breeding to broaden genetic diversity and to improve resistance. Next to the primary genepool of hexaploid wheat, comprising landraces, breeding lines and cultivars, the secondary and tertiary genepools are used for improving FHB resistance (BUERSTMAYR ET AL. 2019). *Triticum monoccoum* belongs to the secondary genepool of wheat and was already subject of resistance studies for FHB (WIWART ET AL. 2016, GÓRAL & OCHODZKI 2017). This project also tried to make use of the genetic variation present in einkorn wheat concerning FHB resistance. In this respect, a *Triticum monoccoum* DH-population was analysed for FHB resistance and two neighbouring QTLs were detected on the short arm of chromosome 2A, which were fine mapped in a next step following a map-based cloning approach. QTL analysis was based on two years field trials and a genetic linkage map with a length of 1987.55 cM. The QTLs explain 81.8 % and 34.7 % of the phenotypic variance, respectively and spannend an interval on the genetic map of 45.1 cM and 31.45 Mbp on the physical maps of *T. aestivum*. There are quite more QTL mapping studies published than studies about successful cloning of QTL, indicating that map-based cloning of quantitave traits remains a challenging task (SALVI & TUBEROSA 2005). Especially minor QTLs and QTLs, in which multiple genes affect the trait are key limitations in the cloning procedure (REMINGTON ET AL. 2001).

Only nine QTLs for FHB were despite many efforts successfully fine-mapped in the last years: *Fhb1, Fhb2, Fhb4, Fhb5, Fhb7, Qfhs.ifa-5A, Qfhs.ndsu-3AS, Qfhb.nau-2B, Qfhb.mgb-2A*, of which only *Fhb1, Fhb7* and *Qfhb.mgb-2A* are cloned (BUERSTMAYR ET AL. 2019, WANG ET AL. 2020). Nevertheless, possible candidate genes are described for *Fhb2* (DHOKANE ET AL. 2016), for which six candidate genes involved in structural resistance by cell wall reinforcement and DON detoxification are mentioned. The same holds true for *Qfhs.ifa-5A* (SCHWEIGER ET AL. 2013), for which a lipid transfer protein and an uridine diphosphate (UDP)-glycosyltransferase gene was obtained. Moreover, it is still not unequivocally known which is the causative candidate gene for *Fhb1*. RAWAT ET AL. (2016) reported a pore-forming toxin-like (*PFT*) gene, that encodes a chimeric lectin with two agglutinin domains and an ETX/MTX2 toxin domain. Plant lectins are a

heterogenous group of proteins, that are able to bind to carbohydrates and play a role in pathogen defence mechanisms, although the biochemical mechanism for *PFT*-mediated FHB resistance remains currently unknown (RAWAT ET AL. 2016). However, *PFT* is present in highly susceptible accessions without mediating FHB resistance, therefore SU ET AL. (2019) and LI ET AL. (2019) claimed a histidine-rich calcium-binding protein (*TaHRC*) as responsible for FHB resistance encoded by *Fhb1*. *TaHRC* is a nuclear protein, but the detailed biological functions and resistance mechanisms has to be characterizd in the future (SU ET AL. 2019). For *Fhb7*, a glutathione S-transferase (GST) is described as the encoding gene, that confers broad resistance by detoxifying trichothecenes via de-epoxidation (WANG ET AL. 2020). In addition, *Qfhb.mgb-2A* encodes for a wall-associated receptor-like kinase gene (*WAK2*) that is involved in FHB resistance (GADALETA ET AL. 2019).

WAKs detect damage-associated molecular patterns (DAMPs) that emerge through cell damage during the infection process by necrotropic pathogens. Thus this proteinfamily is responsible for pathogen detection together with pattern recognition receptores (PRRs), that detect pathogen-associated molecular patterns (PAMPs) for hemibiotrophs and necrotrophs and receptores with nucleotide-binding (NB) domains and leucin-rich repeats (LLR) (NBS-LRR), which identify pathogens effectors from biotrophic pathogens (ANDERSEN ET AL. 2018, KUSHALAPPA ET AL. 2016). This is the first step in the defense cascade that is activated in plants by pathogen attack. Next step is the signal transduction, where resistance responses are regulated by mitogen-activated protein kinases (MAPKs), G-proteins, calcium, ubiquitin, transcription factors or hormones like giberellin, salicylic acid or jasmonic acid followed by a plant response like hypersensitive response (HR, programmed cell death), cell wall modification, closure of stomata or production of reactive oxygen compounds (ROS), that are toxic for pathogens (ANDERSEN ET AL. 2018). A PAMP/pattern triggered immunity (PTI) leads to e.g. activation of phytohormones as well as the generation of Ca^{2+} and oxidative burst and contribute to a non-specific, broad resistance, whereas an effector triggered immunity (ETI) recognizes pathogen effectors (avirulence genes) through specific plant resistance proteins e.g. nucleotide-binding site leucin rich repeats (NBS-LRR), which are encoded by disease resistance genes (R-genes), and often lead to a hypersensitive response (HR, cell death) (CHEVAL ET AL. 2013). The former one describes an unspecific horizontal resistance (quantitative), while the latter

one correspond to a vertical, race-specific resistance (JONES & DANGL 2006), which is considered to be monogenic and follows the gene-for-gene hypothesis (FLOR 1971, KUSHALAPPA ET AL. 2016). The resistance response switches depending on which pathogen attacks. Biotrophic and hemibiotrophic pathogens need living material, therefore the ETI pathway is activated, that usually leads to HR. While necrotrophic organisms live from dead tissue and need an alternative strategy, the PTI pathway is activated, because ETI leads to HR (hypersensitive cell death) and would therefore rather promote pathogen growth (VLEESHOUWERS & OLIVER 2014, ANDERSEN ET AL. 2018).

Lots of genes and molecular pathways are activated during an infection not only in the plants but also in the fungal pathogens. Understanding the mechanisms involved in plant-pathogen-interactions provides important knowledge to develop broad resistant wheat cultivars by pyramiding all types of resistance effects that may lead to a more durable and efficient resistance. Therefore a lot of transcriptomic analyses were conducted in the last years to study wheat-*F. graminearum* pathosystem, with main focus on the analyses of transcriptome profiles from FHB resistant and susceptible wheat lines (KAZAN & GARDINER 2018, SARI ET AL. 2019). Thus, GOTTWALD ET AL. (2012) reported, that especially ATP binding cassette (ABC)-transporters, UDP-glucosyltransferasen and protease inhibitor genes are activated by defense response of the resistant wheat cultivar *Dream* and *Sumai 3* after an infection with *F. graminearum*. ABC-transporter genes, bind and hydrolyse ATP and use the energy released from this process to transport substances across cells and next to this, they are associated with e.g. detoxification processes and ion channel regulation in plants (SÁNCHEZ-FERNÁNDEZ ET AL. 2001, VERRIER ET AL. 2008) They are divided into different subfamilies (A-G) while ABC subfamily C transporters are mainly involved in plant-pathogen interactions (DASSA & BOUIGE 2001, KANG ET AL. 2011). This was already reported in previous studies by WALTHER ET AL. (2008,2015), who showed that a *TaABCC*-gene is up-regulated by DON and linked with FHB resistance, mediated by the *Fhb1*-QTL on chromosome 3BS. The same was stated by HANDA ET AL. (2008), who identified a wheat *TaABCC*-gene as a responsible one for the enhancement of FHB resistance by a QTL on chromosome 2D of wheat. On the other hand, an ABC-C transporter in *F. graminearum* accounts for fungal mycelial growth, response to tebucoanzole and pathogenicity towards wheat (QI ET AL. 2018). In addition, members of ABC-transporter genes, here subfamily ABC-G, are even associated with

broad-spectrum disease resistance to multiple fungal pathogens (KRATTINGER ET AL. 2009b). In general, UDP-glucosyltransferases glycosylate different phytohormone and metabolites as response to biotic and abiotic stresses (REHMAN ET AL. 2018) They contribute to DON tolerance, a component of FHB resistance, by conversion of DON into less toxic DON-3-O-glucoside (D3G) (POPPENBERGER ET AL. 2003, GATTI ET AL. 2018). Next to these, cytochrom P450 has also shown to enhance host resistance to DON in wheat (GUNUPURU ET AL. 2018). Lots of more genes are expressed in wheat after *F. graminearum* infection, involved in e.g. Ca^{2+} -signaling, antioxidative stress, salicylate (SA)/ jasmonic acid (JA)/ ethylene (ET) biosynthesis or signaling pathways (JIA ET AL. 2018). SA/JA/ET are phytohormones, that play a criticial role in regulating signaling networks in pathogen defense. In general, SA is inolved in the activation of defense responses against biotrophic and hemibiotrophic pathogens, while JA and ET are more responsible for defence aginst necrotrophic pathogens (BARI & JONES 2009). Transcriptome analyses show that SA and JA play an important role in defense response and antifungal activity to *Fusarium graminearum*, because *Fusarium graminearum* is a hemibiotrophic species, that switch from the biotrophic phase in the initial infection phase to a necrotrophic interaction afterwards (STEINER ET AL. 2009, QI ET AL. 2012, 2016). At the early infection state, where *F. graminearum* is in the biotrophic growth, defense reaction starts with Ca^{2+} and SA signaling followed by JA signaling, when *F. graminearum* switches to the necrotrophic growth stage (STEINER ET AL. 2009, BUHROW ET AL. 2016). RLK-LLR (receptor-like kinase containing leucin rich repeats) are involved in the detection of elicitors, like chitin, mainly produced by hemobiotrophs and necrotrophs and contribute also to an early infection response in FHB resistance (THAPA ET AL. 2018, KUSHALAPPA ET AL. 2016)

In summary, plant lack adaptive immune systems, but possess a lot of components to protect themselves against pathogens (LEE ET AL. 2017). Active resistance underlies a complex regulatory system of pathogen detection, signal transduction and defense responses like hypersensitive response, oxidative burst induction or fortification of cell walls depending on the respective pathogens (JIA ET AL. 2018, ANDERSEN ET AL. 2018). Whereas passive resistances represent rather structural defenses of the plants and are not induced by pathogens stimuli. Structural barriers are e.g. cell wall composition or a waxy

cuticula hindering the pathogen to penetrate (PARRY 1990). FHB infection is also influenced by e.g. plant height or spike morpology (BUERSTMAYR & BUERSTMAYR 2015).

FHB resistance vs. sog-gene

Although the QTL in this study seems to be appropiate for map-based cloning due to the major effect it explains, a break down of the effect was observed in the HRM-population. The shift of the peak markers, causing the QTL effect, from the original location on the short arm of chromosome 2A to the long arm of chromosome 2A as well as the significant correlation of the ear shape with FHB resistance of the original DH-population give hint, that either the major effect originates from the *sog*-gene and is therefore rather a structural barrier or the effect results from pleiotropy or tight linkage of a *Fusarium* resistance gene and the *sog*-gene. It is possible that spike related traits modify FHB infection and severity, as FHB is a floral infection disease (BUERSTMAYR ET AL.2019). In general, numerous QTL studies have shown that FHB QTLs are coincident with QTLs linked to various agronomic and morphological traits (ZHU ET AL. 1999, MESFIN ET AL. 2003, HORSLEY ET AL. 2006). Moreover, the identification of FHB QTLs can be confounded by agronomic traits, like plant height, heading date or spike morphology (OGRODOWICZ ET AL. 2020). Some studies have shown that the ear shape or the compactness of the spike correlate with FHB resistance. A study of BUERSTMAYR ET AL. (2011) investigate the correlation between FHB resistance and ear traits, like compactness, threshability and glaucousness in a *Tiritcum macha* x *Tricium aestivum* population. Both are segregating concerning ear morphology. *T. macha* is non free-threshing, has waxy glumes and a non-compact phenotype. The results gave hint that plants with elongated, lax and more glaucousness spikes as well as non free-threshing ability also have an enhanced FHB resistance (BUERSTMAYR ET AL. 2011). This reaction is generated through the wild allele *q* of the major domestication *Q*-locus, which is located on chromosome 5A. *Q* is responsible for the free-threshing character and a square head spike genotype and pleiotropically effect glume shape and tenacity, spike length and rachis fragility. The wild *q*-allele, which was also found in *T. monococcum*, cause non-freethreshing seed and tenacious glumes, as well as fragile rachis and thus probably a higher FHB resistance (SIMONS ET AL. 2006, BUERSTMAYR ET AL. 2011). Other studies also suggest that genotypes with compact spikes tend to have an increased FHB susceptibiltiy and could therefore be a passive resistance factor (STEINER ET AL. 2004, GIANCASPRO ET AL. 2016). Already STEFFENSON

ET AL. (1996) reported a higher *Fusarium* resistance of lax spike types in barley NILs than dense spike types. This result was supported by MA ET AL. (2000), who also found strong associations between lax spikes and FHB resistance. Thus spike compactness may serve as one factor for enhanced FHB susceptibilty, as lax spikes are less moist than dense spikes, which make it difficult for *Fusarium* spp. to spread up-and downwards on the spike resulting in less infected plants. This was also observed by OGRODOWICZ ET AL. (2020), who identified a negative correlation between spike density and FHB resistance, too, indicating that spike compactness may enhance FHB susceptibility. The same strong correlation between spike compactness and FHB susceptibilty was also observed in our study (Figure 21, Suppl. Data 3).

WOLDE ET AL. (2019) investigated spike morphometric traits, like spike length (SL), internode length (IL) or node density (ND) in a tetraploid wheat (*Triticum turgidum* L.) RIL-population and mapped one QTL *QND.ipk-2AL* for IL and ND on chromosome 2AL near the centromere, which roughly resides within 617.7 – 674.0 Mbp (WOLDE G., pers. com.) on Chinese Spring. This could probably be a homoeolocus of the hexaploid wheat *compactum*-gene *C*, that was also mapped on chromosome 2D near the centromere, although no compact spike type for *T. turgidum* was previously described (JOHNSON ET AL. 2008, FELDMANN 2001). The *compactum C*-gene causes a more compact spike type in club wheat (*Triticum aestivum* ssp. *compactum*). Thus, it is a good indication that the *sog*-gene of diploid wheat and *QND.ipk-2AL* of tetraploid wheat cause the same spike morphology and are probably identical. The QTL position in our study and *QND.ipk-2AL* are not overlapping, but this may be due to the reduced amount of markers in both regions and could be further refined with new analyses and a higher marker density, so that the locations of the QTLs are better represented on the map. That could next serve as a good starting point for further investigation in spike morphology genes and their comparison between diploid, tetraploid and hexaploid wheat as well as their influence on FHB resistance. Next to spike related traits, this region also contains QTLs for FHB resistance. Thus, a QTL for FHB susceptibility was located on the long arm of chromosome 2A in a wild emmer wheat population between SSR marker *gwm558* (pysical position: 361.1 Mbp) and *gwm445* (physical position: 682.6 Mbp) (GARVIN ET AL. 2009). This is exactly in the same region as the *sog*-gene (AMAGAI ET AL. 2017) and the QTL detected in this study. In addition, even a newer study detected a QTL for FHB resistance on chromosome

2AL in close linkage to marker *BS00022896_51,* which is located at 612 Mbp and thus is also very close to the QTL detected in our study (YI ET AL. 2018). They also show with a multivariate conditional QTL mapping approach that spike compactness contributes to FHB resistance, but the QTL on chromosome 2AL is apparently independent from this trait. Also in barley (*Hordeum vulgare* L.) a large number of FHB QTLs were described on the long arm of chromosome 2H (OGRODOWICZ ET AL. 2020).

In summary, it is probable, that the variation in resistance to FHB detected in this study may be due to the *sog*-gene that have a pleiotropic effect on FHB resistance as assumed in the study concerning the *Q/q*-alleles (BUERSTMAYR ET AL. 2011) or a FHB-resistance gene is closely linked. It is known, that mapping and identification of traits, that are characterized by strong phenotypic correlations are very challenging due to pleiotropy or linkage (OGRODOWICZ ET AL. 2020). To identify the responsible gene for the phenotypic variance observed in this study, it is necessary to shorten the large QTL interval, as our QTL region spans an interval of 108.3 Mbp resulting in 619 annotated genes of *T. aestivum* CS v1.0 (URGI 2018), which is quite large to identify the responsible ones.

Future gene cloning

To meet problems and challenges of climate change, lots of effort was done in the past to identify genes in wheat and other cereal crop species, that may enhance resistance against biotic stresses, tolerance against abiotic factors as well as quality parameters for improvement of wheat. The method of choice in the past was map-based cloning (KRATTINGER ET AL. 2009a), although it was very challenging in wheat to clone genes and lasted up to a decade, due to the large and complex genome with highly repetitive sequences (WICKER ET AL. 2018) and the limitations of a low number of molecular markers. There are only ~25 cloned resistance genes in wheat until now (THIND 2018). Today, map-based cloning is facilitated through high-througput NGS technologies, providing thousands of SNP markers, that were used for rapid and cost-efficient genotyping of large mapping populations and association panels and result in the construction of highly saturated genetic maps (JAGANATHAN ET AL. 2020). Moreover, available high-quality reference sequences for many crop species simplify the anchoring of markers linked to a specific trait, on the genetic map with physical maps and accelerate the straightforward search for candidate genes. In addition, they help to understand the genetic and molecular control of important traits (BETTGENHAEUSER & KRATTINGER

2019). But next to the traditional map-based cloning approach, lots of novel technologies have been developed, e.g. MutMap, RenSeq/MutRenSeq, MutChromSeq and TACCA that lead to a faster cloning of genes.

Mutational mapping (MutMap) identifies gene sequences by gene mapping, mutagenesis and whole-genome sequencing and is therefore suitable mainly in crops with small genomes like rice, because there is no reduction of genome complexity, which is a need for inexpensive and fast cloning in large genome and polyploid species ((PERIYANNAN 2018). In this method, a cultivar with a reference genome sequence, is mutagenized with EMS (ethyl methane sulfonate) and subsequently crossed to a wild-type plant of the same cultivar, used for mutagensis. Bulked DNA of all mutant F_2 phenotypes is than subjected for whole genome sequencing and in the end, sequences of mutants and reference cultivar are aligned for SNP identification (ABE ET AL. 2012). This method was used to identify loss-of-function mutations in genes conferring semi-dwarfism and pale green leaves (ABE ET AL. 2012), as well as high salt tolerance in rice (TAKAGI ET AL. 2015).

Another novel technology to idenify and annotate resistance gene family members of nucleotide binding leucin rich repeat (NB-LRR)-class is Resistance gene enrichment and Sequencing (RenSeq). Hereby the genome complexity is reduced by enrichment of target sequences of this specific gene family from the whole genome followed by sequencing (JUPE ET AL. 2013, PERIYANNAN 2018). RNA-probes are designed for hybridization to all nucleotide binding site leucin rich repeat (NLR)-genes and thus to capture and enrich homologous DNA sequences from the resistant genotye (PERIYANNAN 2018). It was first applied in combination with bulked segregant analysis (BSA) in two biparental potato populations to identify SNPs linked to resistance genes for *Phytophtera infestans* (JUPE ET AL. 2013, HATTA ET AL. 2019). Another possibility is a combination with EMS mutagenesis, called Mutagenesis Resistance gene enrichment and Sequencing (MutRenSeq) that was recently deployed to clone stem rust resistance genes *Sr22* and *Sr45*, that encode for resistance NLR-genes (NOD-like receptors), from hexaploid bread wheat (STEUERNAGEL ET AL. 2016). To achieve this, sequences of six loss-of-function mutant lines (susceptible), that derived from EMS mutagenesis, were compared with the wild-type sequence from the resistant parent and looked up for polymorphisms/mutations (BETTGENHAEUSER & KRATTINGER 2019, HATTA ET AL. 2019). MutRenSeq can be applied in cereal crops, like e.g. wheat, barley, maize and rice and especially in wild

relatives. Thus this method seems even appropriate for gene cloning in *T. monococcum*. This method is fast (<24 months) and cheap, because the genome complexity is reduced resulting in reduced sequencing costs, e.g. the gene complement, sequenced by STERUNAGEL ET AL. (2016) consisted of 8235 NLR-contigs that span an overall interval of 14.5 Mbp that correspond to 00.9 % of the hexaploid wheat genome (BETTGENHAEUSER & KRATTINGER 2019). Moreover, it is independent of fine-mapping and is used to rescue genes from alien introgression, that are not involved in breeding before due to linkage drag (STEUERNAGEL ET AL. 2016).

In addition, another option to reduce genome complexity is to purify individual chromosomes by flourescent labelling of its DNA repeat sequences and though to isolate genes using flow cytometry for chromosome sorting in combination with mutagenesis (GIORGI ET AL. 2013, PERIYANNAN 2018). This technology is called mutant chromosome sequencing (MutChromSeq) and was proofed by SÁNCHEZ-MARTÍN ET AL. (2016) by cloning powdery mildew resistance gene *Pm2* in wheat. Before chromosome sorting the target gene has to be assigned to the respective chromosome, which is usually done with genetic and physical mapping, although recombination-per-se and fine-mapping is not required. Moreover the influence of the target gene on the phenotype should be determined in advance (SÁNCHEZ-MARTÍN ET AL. 2016). Mutants are genereated using EMS and their chromosomal sequences mapped to the refernce wild-type, e.g. for the isolation of *Pm2*, six *Pm2* loss-of-function mutants were chosen, sequenced on the Illumina platform and then aligned to the resistance wild-type parent. The technology is suitable for crops, where chromosome isolation is possible and it is easy and inexpensive, but bioinformatic skills and laboratory equipment is needed (PERIYANNAN 2018).

One bottleneck in gene cloning may be the production of high-quality genome sequence information from the region containing the gene of interest for every studied cultivar, which may be overcome by a newly developed method called TACCA (targeted chromosome-based cloning via long-range assembly). With this method broad-spectrum leaf rust resistance gene *Lr22a* was recently cloned (THIND ET AL. 2017). Advantages are on the one hand a rapid cloning of genes even from large genome and polyploid species as well as from species that show partial resistant phenotypes. On the other hand, this technology seems to be even suitable for gene cloning in low recombination rate regions and without the requirement of loss-of-mutants identification, except in gene-dense and

low-recombination rate regions (THIND 2018, HATTA ET AL. 2019). But it is only suitable for crops where chromosome isolation is possible and preliminary map informations of the genes are available and furthermore, the technique requires much expertise and costly equipment (PERIYANNAN 2018, THIND 2018, HATTA ET AL. 2019).

Nevertheless, all these mentioned technologies are mainly usable for qualitative traits or major effect QTLs. For most of the quantitative trait studies, map based cloning remins the best method of choice, even for the QTL in this study (BETTGENHAEUSER & KRATTINGER 2019). RenSeq or MutRenSeq are no appropriate methods although they are concentrating on the identification of R-genes. But resistance to FHB is usually not R-gene mediated as this often reults in a hypersensitive response (HR), which is not the best defense strategy for the hemibiotrophic *Fusarium* spp.. TACCA and MutChromSeq may be good alternatives, because our QTL is already mapped to a specific region on chromosome 2A, which is a prerequisite for e.g. MutChromSeq. Moreover both methods are not restricted on the identification of resistance genes, as it is not yet known where our QTL effect is based on. But specific lab material is needed to isolate particular chromosomes, which may be an obstacle for many research institutions.

Next to these new sequence-based technologies, also new *omics*-procedures like transcriptome, proteome, epigenome and metabolome studies are used nowadays to dissect loci, that are associated with complex traits and to understand their regulatory network (MOCHIDA & SHINOZAKI 2011). Those omics technologies can support positional cloning attempts and recognize candidate genes by explaining biological processes that determine the genetic effect (LANGRIDGE & FLEURY 2011). Even process in reverse genetic approaches lead to a faster and efficient determination of candidate genes like site-directed mutagenesis, e.g. CRISPR/Cas9 (BAO ET AL. 2019). Another important step to date in determining candidate genes is the international ongoing effort in developing a pan-genome for wheat. A pan-genome displays a collection of all DNA-sequences occuring in one species and not only from one cultivar and thus enhance the identification of genes or phenotypically consequential variants (SHERMAN & SALZBERG 2020). Moreover, progress is done in phenotyping technologies, that are very essential especially for the detection and cloning of quantitative traits, from e.g. small-scale applications in the greenhose (CZEDIG-EYSENBERG ET AL. 2018) up to fully automated robotic phenotyping platforms in the field (VIRLET ET AL. 2017).

Finally, gene cloning nowadays is no obstacle anylonger due to the high progress in NGS-technologies, whole-genome sequencing, genome complexity reduction, marker systems and genomic analyses as well as phenotyping technologies. Therefore cloning and cataloguing of many agronomically important traits should be an international effort in the future to support genomics-assisted breeding (BETTGENHAEUSER & KRATTINGER 2019).

Outlook

This study was conducted to get information on the genetics of FHB resistance in *Triticum monococcum*. A DH-population was constructed based on crosses between two parents *Triticum monococcum* (*mon10-1*) and *Triticum monococcum* L. conv. *sinskayae (Sinskayae*) that differ not only in their resistance to FHB but also in their spike morphology. In a first step, QTL mapping was performed with two years field data and a genetic map of 1987.55 cM. Two neighbouring QTLs were mapped on chromosome 2A in the distal region of the chromosome in a genetic interval of 45.1 cM corresponding to a physical location between 36 Mbp and 69 Mbp. Further analyses aimed at saturating this interval with markers and in the identification of the resistance locus within this interval. Nevertheless, analyses reveal a switch of the peak markers *SNP_0797* and *wmc0644*, causing the major QTL effect, to the long arm of chromosome 2A. Future studies may deal with the question, wether this effect derives from the *sog*-gene, which is also located in the same region as our QTL and may pleitropically influence FHB resistance, or from *Fusarium* resistance genes, that are closely linked to *sog*. The DH-population seems to be very suitable for further investigation in spike related traits in *T. monococcum* as well as for the mapping of the *sog*-gene and the probably linked FHB resistance, because of the highly contrasting crossing parents. It is well known that mapping populations, constructed from genetic diverse crossing parents allow high performance QTL analysis (OGRODOWICZ ET AL. 2020). The QTL analysis for FHB resistance should be replicated with the whole original DH-population and a higher marker density as well as an additional assessment of spike architecture to identify the exact location of the *sog*-gene. Moreover, the recently published sequence of *T. urartu* (LING ET AL. 2018) could be used in further studies for candidate gene identification on chromosme 2A for spike related traits or FHB resistance genes.

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Abbreviations

AFLP, Amplified fragment length polymorphism
ANOVA, Analysis of variance
AO, Average ordinate
AUDPC, Area under the disease progress curve
CAPS, cleaved amplified polymorphic sequences
CTAB, cetyl trimethylammonium bromide
CS, Chinese spring
cM, Centimorgan
DAMPs, damage-associated molecular patterns
DArT, Diversity Array Technologies
DH, Doubled haploid
DNA, Deoxyribonucleic acid
DON, Deoxynivalenol
DPI, Days past infection
ETI, Effector triggered immunity
FAO, Food and Agriculture Organization of the United Nations
FHB, Fusarium Head Blight
GB, Giga base
GBS, Genotyping by sequencing
GS, Genomic selection
GWAS, Genom-wide association study
Ha, Hectare
HR, hypersensitive response
HRM, High-resolution mapping
IWGSC, International Wheat Genome Sequencing Consortium
KASP, Kompetitive Allele Specific PCR
LOD, Logarithm of odds ratio
MAGIC, Multi-parent advanced generation inter-cross
MAPK, Mitogen-activated protein kinase
Mbp, Mega base pairs
MAS, Marker assisted selection
MTA, Marker trait association
NAM, Nested association mapping
NGS, Next generation sequencing
NIV, Nivalenol
PAMPs, Pathogen-associated molecular patterns
PCR, Polymerase chain reaction
PDA, Potato dextrose agar
PTI, Pattern triggered immunity
QTL, Quantitative trait locus/loci
R-Gene, Resistance gene
RAPD, Randomly amplified polymorphic DNA
RFLP, Restriction fragment length polymorphism
RIL, Recombinant inbred line
RICL, Recombinant inbred chromsome line
ROS, Reactive oxygen species
SNP, Single nucleotide polymorphism
SSR, Simple sequence repeats

Appendix

Supplemental data 1: Sequence information of flanking markers

SNP	Sequence
SNP_1216	ATGGAATCTCTGGGCTGCAGTAGCAAAAAGCACAAAGCCCAGAAGCTAAACAAAAAAGGTCCTCTT GTTTCTACAGGCTC[G/A]TAAAACCACCCGGCCCTCTTACCAGCGCAGCGACAAAAAGGCCAGAA GTTCGAGCCAGCCTACACATGCACACGAATCTT
SNP_0667	CTCTCCTCTCCTCCCATTTTAACCACGTCGGCAATAGAAAAATGCACAAG[T/G]GAAGGTCACTG CTGATTGGCATGTGCGCCCATGACTGCAGCGGCGACGTC
SNP_0833	CAAGGAGAAGAGGAAGAACAGGATGTACGTGAGATATAATCAGAGACCAA[C/T]GCCGCAACACC ACCAGAGATTCTCTTTAGCTCGTAGTGCTGATTACTGGG

Supplemental data 2: Sequence information of newly developed markers for marker saturation

Markers: genetic map of *Triticum monococcum*

SNP	Sequence
SNP_1315	ACTGCTGCGCATGTGGATGCTTGACCTGCAGGGAGCTGCTGTGTTTGCCT[T/C]GCATGGTGATG AGTCAGTGAAGCAGAGAGATCTTGCCCGGTCGGTTGGTT
SNP_1453	ATTTACATACAGTTGCAGTTCAGTTTACTGAACCACTATCTCTTTCTTGA[T/A]AATCAAATGAC AAATTATAGCGTACTGCAGTGTTGAGCGCGTACATTGGC
SNP_0531	AGTGTGTGTTTTTGGAAAGCAATGAAAGGAAGCAACACATGGATCATCCA[C/T]GGACCTCATGG ATCTGATTAGTTAACTACTCAAAGCTGATGACAATTAGA
SNP_0157	CAGAACACATATGAGGAGTGCCTGGAGCTCTTCAGCTCGCTCCGGGAGAG[T/C]GGCGTTCTTGT GCATCAGTGGTTTTGATTGCTGCCATACACATTCAGAGT
SNP_1552	GTCTGCAGGCTCTTCCTGCGGTCATCCGAGCTGCTCCCCATCATCGTGCA[T/C]CTCAGGCAGTC GCCTGACCAAACGATCTCCGAGTACGCCTCCGCCATCGC
SNP_0987	TCATAAGAAAAACATGTGTTAATGCGTGCGCGCCGCGTCAATTGGGAAGA[T/C]GACAAAGAACG CAAGACTGACCTGCAGAAATAAATGGCAGGCATGTAGAT
SNP_0148	GAGGAGGAGAGCGAGGAGGAGGAGGAGGACGACGACGTGGACGGCATCGA[G/A]GAGCTGGAGCG CCGCATGTGGCGCGACCGCATGAGGCTCAAGCGCCTCAA
SNP_0836	GCTGGCTGGCTGAAAGTTCCATGCACGCGTGACAAGAAACTGCAGCCAAC[C/T]TTTTCCTTGCC GTAATCGTGCCATTTTGTTTGACAAAAAGCTGTCGTTCC
SNP_1643	CGGTGTCAGCTAGGCGTACAGTTCAGAGGAAGACGAGGAGGAGGGAGCGG[A/G]ATGAGAGATTA CCATGGTTGCGCGGTGGTGGCTGCAGCAGCGGCGAGGGT

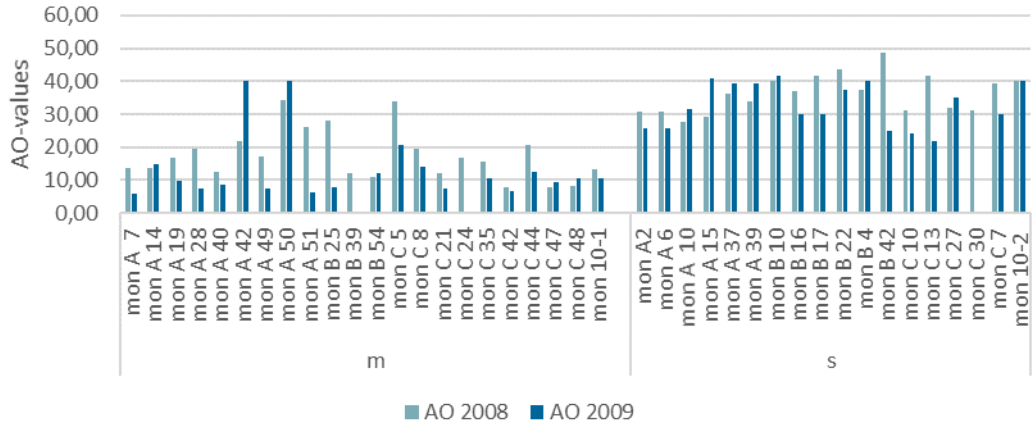
Appendix

Markers: Genotyping-by-Sequencing (GBS)

SNP	Sequence
GBS_1	CTATAATTGTGTTTGCTTCCCTTCTTTCCGCACTTGATGGTTTATAATTGGTATCTGATGACCCTA TGGACTGCCAATTTTTTCGTTTATGCATAGGATA[A/C]CTAGGCAAGAGACAAATGCCAAAATTA TCATTCCAGAATTGCTGCAATGCATTTTATCACATGATACCTACATACTCGAGCGGCTGTTTATTA TCCCATC
GBS_2	GCAGCTTTGAAAATGTTTTGTTAACATGTGTGGTGAACTGGTCATTGGATCTTCGCCCTGCTGCAG CAGTGTCACGGATCAGCCAAAGCCTCATTTCTTT[G/A]GACCCAACTATATCCATCGTTCATTTA CGTGACCTGGCCTCAACATTATTTGATTCTAAGAGCATCTCTAGCATATCCATCAACCGGCCGTCC CGCAAAA
GBS_3	GTTGTATTGTAGTGTCTCTCAGTCGCCATGTGCCTGCAGAAATGAAAAGCTCTAATTGTCATCAGC TTTGAGTAGTTAACTAATCAGATCCATGAGGTCC[A/G]TGGATGATCCATGTGTTGCTTCCTTTC ATTGCTTTCCAAAAACACACACTTTATCATCCTGTCACCCAACCATTCCTTGCGGCTCAAAGCAAT GTCCCCT
GBS_4	AGGCAGACAGCCACAGCCTGTGGTCAGCAATGAACCAACATTGCATTCTGAAGAGGACAACTGACC AACCACTAGGACAGATGCAACAAAATTCACGACA[A/G]CGACAACACGCGTAAATTCGGTTTCAC ATGTTAGTGCAAATATCTGGAGTTAAGCAGAGATCTTCACAGCAGTAGTATATGCATATGGACCGG CTGACAT
GBS_5	GTGATGATGAATGAACGCATTGGTTTAGTAGGTTTTTGCCGAGAGATATGGTAATGATGATGGAGT GGCAATTTAATTTGCCTGCAGATATCTAGTTTCA[T/A]CTGCTTCCTGTGGTATGCGCCTGTATT TTCGTCATATTGGAAGTTCTCACCTCAGTGAGTATGTCTTCGAGTTTTTCTTATAGATAAGTATAT CTATATA
GBS_6	GGCTCCTAAAACCGTAAAAAAGAAACCGGCTAGACACACCATAGAAGGTTCCAAAAACGGGATCGC TGAAACGCTAAACAGACCGGCCCACGCCTCGAAC[A/G]CTGGGTTGATCGCCCAAACAAAATCCA CAGACAACATCTGCAACAGATATGCAAATTTATTCAGTTTTCAACCTCATCTCACAAACACATTAC CCTTTCC
GBS_7	CAGACTAGCGGAAGTAAACCGACACGAGGGGGATGTCGACGTCGTTCTCGTCCTCGTCCTCGCAGG CCACGCCGATGTCCAGGTGCCTCCGGTACTCGGG[G/C]ACGTCCACCTTGGCCACCTCCCTGGCG ATGTCGACCACCTTCTTGGTCAGCCGGTCCTTGTGCCTCGCAAACATGTTGTTGTACAGCAGGGAG GTGCCGC
GBS_8	GCTTAGGCTAAACATGGAATATGACTACGACTACGAACGCAAGATCGGGAATATGGGCGGTGGACC CTTCGCTCAATCCATGGAAAGGTTCAATAGGCTG[G/A]CCCCCATGTCCTCGGCACATCGCGACA TGGTGCAGCTCGGCGGCGTCTCAGAGCTCCCGGGTTCTTTGACCAACAACAACTGCGCGTTCAGCT GCCTGCA
GBS_10	CCGATCGGTCGAGCTGGTCCCCCTGGATCGGCAACCTCGATCTTCTGCACGCACCGACACATAGAT TCGTCTGAGTACTGTAGGTTGCCAAACAGTGAGC[A/G]CTCGTCCAGTGGAGGGCAACACCTAGC ACATGCATGCACGCCTTTCACACCAGAAAGCAAGTCCTTATCGTTGGCAATACTCATGTTAAGCAA GAGGAGC
GBS_11	GTTGCGGCCGCCGGCGACGAGGTCGATACCCTGGGTGCCACGGGAACGGACGGTGTCAGCTAGGCG TACAGTTCAGAGGAAGACGAGGAGGAGGGAGCGG[A/G]ATGAGAGATTACCATGGTTGCGCGGTG GAGGCTGCAGCAGCGGCGAGGGTCAGGGCGGCAGCGGCGGCGGCGGGACTGGTGCTAGGGTTTTGG ATGGAGT
GBS_12	GCACTTGTCGGCCTGGCACACCAGGTTGGGCCCGTTGCTGCAGGAGAATGACAACGGCACGTTGAA GCCGTCGATGACAGAGATGTCGTAAAAGTCCTGG[C/T]CGTTGCCGATGGTGGACTCGGCCAGGG TCAGCGGCGGCTGCCCGGACAGCGTGCACGTCAGCGCACCGCCGCAGTCGGCAGTCTGGCAGCTCC CGGAGTT
GBS_13	TGACCGTGGGCGTACCATAGTCTACGCCAAGCCTGGCGACCAGCGGTGGGCTGTGATTGAGCATGA CGAGATCGGGAGACCCAATCGCTACGCATCATAT[C/T]GGCTATCGTCGGCATCAACCCTGCAGG GGCGCTTCTACTTTGCGACTCTTGAGGGGAATATAATGCATGTGAGGCTCTGCCCTGAGCCTCAGT TGGTGCC

Appendix

Supplemental data 3: 40 DH-lines plus parental lines (mon10-1/Sinskayae (mon10-2)) with mean AO-values from 2008/2009 and their classification in the respective group according to their ear shape (m/s)



Acknowledgement

First of all, I want to thank my supervisor Prof. Dr. Frank Ordon for giving me the opportunity to conduct my PhD on this project. Thank you for your trust and great support and sorry for giving you some grey hairs ;)

Lots of thanks to Prof. Heiko Becker, who has not hesitated to supervise this project and to accept me as an external PhD-student. I am also very grateful to Prof. Wolfgang Link and Dr. Christian Möller for their lively discussions during my presentations in Göttingen and that both immediately agreed to be reviewer and examiner.

I can't thank enough my lovely Ingrid Dubsky for her excellent technical assistance in this project. Although it was not always easy, she never gave up and lost her laugh and i think we did a great job together!! Thank you for your work and your support!

I also want to thank Dr. Ilona Krämer for her patience and help, her support, especially in times when lots of problems have arisen.

Thanks to all the other colleagues at the institute, especially to Dragan and Mathieu for their help in genomic questions, as well as to Heike and Thomas for their excellent bioinformatical support.

Britta, Sarah, Sandra and Fluture – you made the PhD-time perfect, without you it would not be the same. Thank you very much for your friendship, for your help, for always having an ear for me!

Lots of thanks to Jan and his family, especially Betty and Harald – you have all supported me during my PhD-time, i will never forget it!

I also want to thank my own family for their support and their trust in me, you always stand by my side and have allowed me to go my way.

Thank you Captain for being there for me and for your support by writing this thesis.

I can‘t finish to thank my wonderful dogs, they are always by my side and turned every bad day during this time into a good one!

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