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SCREENING GAMMA IRRADIATED WHEAT LINES FOR RADIATION-INDUCED DELETIONS ON CHROMOSOME ARM 5AS AND FINE-MAPPING OF THE FHB RESISTANCE QTL *Qfhs.ifa-5A* INTERVAL

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Eidesstattliche Erklärung

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Abstract

Fusarium head blight (FHB) is a widespread and serious fungal disease in common wheat (*Triticum aestivum*) and other small grains caused by *Fusarium graminearum* and related species. A major concern is the production of mycotoxins harmful to humans and livestock. Exploitation of host resistance is the single most effective control measure. Major FHB resistance quantitative trait locus (QTL) *Qfhs.ifa-5A* resides in a recombination-poor pericentromeric region of chromosome 5A, making fine-mapping the QTL interval by linkage mapping unfeasible. Therefore, radiation selfing (RS) mapping, a recombination-independent approach relying on radiation-induced deletions, was used for high-resolution fine-mapping of this region. M_2 plants from gamma irradiation of pollen (subpanel DP) and M_2 plants from gamma irradiation of heads (subpanel DPS) of experimental line NIL3 (C3) that harbours the resistance-conferring *Qfhs.ifa-5A* allele were screened for deletions. Pre-screening 1503 plants of subpanel DP and 1085 plants of subpanel DPS with 18 5AS-specific genetic markers yielded 26 informative lines with unique deletions distributed over the chromosome arm. These lines represent fertile mutants with homozygous deletions and can be used for developing inbred deletion lines for phenotyping experiments. The 26 selected informative lines were genotyped with 96 5AS-specific markers and the generated marker data was used for map construction resulting in a map with 400.1 cR length, an overall resolution of 0.62 Mbp/cR, and a fairly consistent resolution across different chromosomal bins. A 384-fold increase in map resolution in comparison to a linkage map was obtained in the recombination-poor pericentromeric 5AS segment. Between subpanel DP and subpanel DPS, no differences in the proportion of informative lines could be detected. The ratio of interstitial to terminal deletions was higher in subpanel DPS, suggesting a possible advantage of head irradiation over (sole) pollen irradiation. However, when compared to the results of a previous study, neither pollen nor head irradiation seem to outperform the simpler approach of seed irradiation in an RS mapping context.

Deutsche Zusammenfassung

Die Ährenfusariose ist eine weitverbreitete und folgenschwere Pilzerkrankung bei Weich-Weizen (*Triticum aestivum*) und anderen kleinkörnigen Getreidearten, ausgelöst durch *Fusarium graminearum* und verwandte Arten. Ein Hauptbedenken ist dabei die Produktion von Mykotoxinen, die eine Gefahr für Mensch und Nutztier darstellen. Die Züchtung resistenter Sorten stellt die effektivste Kontrollstrategie dar. Der bedeutende Quantitative Trait Locus (QTL) *Qfhs.ifa-5A* befindet sich in einer rekombinationsarmen centromernahen Region des Weizenchromosoms 5A, wodurch eine Feinkartierung des QTL-Intervalls nicht durch Kopplungsanalyse realisierbar ist. Es wurde daher für die hochauflösende Feinkartierung der Region radiation selfing (RS) mapping, eine rekombinationsunabhängige Methode, die auf der Induktion von Deletionen durch Bestrahlung beruht, angewandt. 1503 M_2-Pflanzen aus Gamma-Bestrahlung von Pollen (Unterpanel DP) und 1085 M_2-Pflanzen aus Gamma-Bestrahlung von Ähren (Unterpanel DPS) der Versuchslinie NIL3 (C3), die das resistenzbedingende *Qfhs.ifa-5A*-Allel trägt, wurden auf Deletionen hin untersucht. Eine Voruntersuchung mit 18 5AS-spezifischen genetischen Markern resultierte in 26 informativen Linien mit einmaligen, über den Chromosomenarm 5AS verteilten Deletionen. Diese Linien stellen fertile Mutanten mit homozygoten Deletionen dar, die für die Entwicklung von deletionstragenden Inzuchtlinien für Phänotypisierungsexperimente verwendet werden können. Die 26 selektierten informativen Linien wurden mit 96 5AS-spezifischen Markern genotypisiert. Die dabei generierten Markerdaten wurden für die Erstellung einer Karte verwendet, für die sich eine Länge von 400,1 cR, eine Gesamtauflösung von 0,62 Mbp/cR und eine einigermaßen konsistente Auflösung über chromosomale Bins hinweg ergaben. Es konnte eine im Vergleich zu einer Kopplungskarte 384-fach verbesserte Auflösung im rekombinationsarmen centromernahen 5AS-Abschnitt erreicht werden. Zwischen Unterpanel DP und Unterpanel DPS konnte kein Unterschied im Anteil informativer Linien festgestellt werden. Das Verhältnis zwischen interstitiellen und terminalen Deletionen war in Unterpanel DPS höher, was auf einen möglichen Vorteil von Ährenbestrahlung gegenüber (reiner) Pollenbestrahlung hindeutet. Allerdings scheinen im Kontext von RS mapping weder Pollen- noch Ährenbestrahlung bessere Ausbeuten als die in einer früheren Studie angewandte, simplere Samenbestrahlung zu bringen.

List of abbreviations

15-Ac-DON	15-acetyl-deoxynivalenol	**FHB**	*Fusarium* head blight
3-Ac-DON	3-acetyl-deoxynivalenol	**FISH**	fluorescent in situ hybridisation
3BS	short arm of chromosome 3B	**Gb, Gbp**	gigabases, gigabase pairs
5AL	long arm of chromosome 5A	**GS**	genomic selection
5AS	short arm of chromosome 5A	**Gy**	gray (unit)
A/T	adenine/thymine	**IAEA**	International Atomic Energy Agency
AFLP	amplified fragment-length polymorphism	**ISBP**	insertion site-based polymorphism
AGES	Österreichische Agentur für Gesundheit und Ernährungssicherheit (Austrian Agency for Health and Food Safety)	**IWGSC**	International Wheat Genome Sequencing Consortium
APHA	Animal and Plant Health Agency (United Kingdom)	**JECFA**	Joint FAO/WHO Expert Committee on Food Additives
ARfD	acute reference dose	**kb, kbp**	kilobases, kilobase pairs
BAC	bacterial artificial chromosome	**krd**	kilorad
BC	backcross	**M_1, M_2**	first and second generation after mutagenesis, respectively
BME	β-mercaptoethanol	**MAS**	marker assisted selection
bp	base pair(s)	**Mb, Mbp**	megabases, megabase pairs
bw	body weight	**NHEJ**	nonhomologous end joining
C/G	cytosine/guanine	**NIL**	near isogenic line
cDNA	complementary DNA	**PCR**	polymerase chain reaction
cM	Centimorgan	**PMTDI**	provisional maximum tolerable daily intake
'CM-82036'	wheat line 'CM-82036-1TP10Y-OST-10Y-OM-OFC'	**PPMCC**	Pearson product-moment correlation coefficient
COS	conserved ortholog set (Fulton et al., 2002)	**QTL, QTLs**	quantitative trait locus, quantitative trait loci
cR	centiRay	**RCF**	relative centrifugal force
CSAR	Commissie Samenstelling Aanbevelende Rassenlijst (Recommended List Committee)	**RFLP**	restriction fragment length polymorphism
CTAB	cetyltrimethylammonium bromide	**RH**	radiation hybrid
DEB	diepoxybutane	**RJM**	repeat junction marker
DH	double haploid	**ROS**	reactive oxygen species
dH_2O	distilled water	**RS**	radiation selfing
DNA	deoxyribonucleic acid	**Rvp**	Raad voor plantenrassen (Plant Variety Board)
dNTPs	deoxyribonucleoside triphosphates	**SNP**	single nucleotide polymorphism
DON	deoxynivalenol	**SRCC**	Spearman's rank correlation coefficient
DON-3-Glc	deoxynivalenol-3-glucoside	**SSR**	simple sequence repeat/microsatellite
DSB	double strand break	**T/A**	thymine/adenine
EDTA	ethylenediaminetetraacetic acid	**TBE**	Tris-borate-EDTA
EFSA	European Food Safety Authority	**TPS**	travelling salesman problem
eSSR	EST-derived simple sequence repeat	**Tris**	tris(hydroxymethyl)aminomethane
EST	expressed sequence tag	**UDP**	uridine 5'-diphosphate
EtOh	ethanol	**US**	United States of America
FAO	Food and Agriculture Organization of the United Nations	**UV/TMP**	photo-activated trimethylpsoralen
FDK	*Fusarium* damaged kernel content	**VCU**	value for cultivation and use
FEB	*Fusarium* ear blight	**WHO**	World Health Organization
FGSC	*Fusarium graminearum* species complex	**ZEN**	zearalenone

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1. Introduction

1.1 *Fusarium* head blight (FHB) – a serious wheat disease

Fusarium head blight (FHB), also known as *Fusarium* ear blight (FEB) (Parry et al., 1995), scab, pink mold, and white head (Martin & Johnston, 1982), is an important disease of small grain cereals that has been reported throughout the world (Pirgozliev et al., 2003). It has become one of the most serious cereal grain diseases in temperate regions (Somers et al., 2003).

FHB is caused by members of the genus *Fusarium,* where the *Fusarium graminearum* species complex (FGSC), comprising of several phylogenetically distinct species (O'Donnell et al., 2004), belongs to the most dangerous and widespread mycotoxin producing representatives (van der Lee et al., 2015). *F. graminearum* (teleomorph *Gibberella zeae*), *F. culmorum,* and *F. avenaceum* (*G. avenacea*) are the *Fusarium* species predominantly found associated with FHB in wheat all over Europe (Bottalico & Perrone, 2002) with *F. graminearum* of various chemotypes currently being the dominant FGSC component in Europe (Przemieniecki et al., 2014; van der Lee et al., 2015).

Abundant natural inoculum during warm and humid weather at flowering imply a high risk of an FHB epidemic (H. Buerstmayr et al., 2002) and probably greater resistance is needed in warm and humid climates (Mesterházy, 2003). In extreme cases, FHB can cause yield losses as high as or higher than 50% (Martin & Johnston, 1982; Mihuta-Grimm & Forster, 1989), but it mainly reduces grain quality (Dean et al., 2012). For example, baking quality is reduced due to proteolytic enzymes (Nightingale et al., 1999), but the most serious concern is mycotoxin contamination of the crop (H. Buerstmayr et al., 2002). In addition, many FHB causing species can also cause seedling blight and foot rot (Parry et al., 1995) and FHB affected seeds give rise to low-vigour seedlings (Martin & Johnston, 1982).

1.1.1 Mycotoxin contamination as a major concern

A wide range of mycotoxins is produced by Fusaria with the most important *Fusarium* toxins regarding human health being trichothecenes including deoxynivalenol (DON), zearalenone (ZEN) and its derivatives, fumonisins, moniliformin, fusarochromanones, fusaric acid, fusarins, cyclic peptides, and amino acid esters (beauvericin type) (D'Mello et al., 1997). DON and its derivatives are the most frequently encountered mycotoxins in FHB in European wheat and zearalenone (ZEN) is commonly associated with DON and its derivatives (Bottalico & Perrone, 2002).

In the EU, maximum levels for several *Fusarium* toxins in foodstuffs exist – for DON e.g. 1250 µg/kg for unprocessed wheat, 500 µg/kg for bread, and 200 µg/kg for baby food (European Commission, 2006b). For products intended for animal feed, guidance values exist, e.g. for wheat 8 mg/kg (ppm) DON relative to a feeding stuff with a moisture content of 12% (European Commission, 2006a).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) defined a group provisional maximum tolerable daily intake (PMTDI) of 1 µg/kg body weight (bw) and a group acute reference dose (ARfD) of 8 µg/kg bw for DON and its acetylated derivatives 3-acetyl-deoxynivalenol (3-Ac-DON) and 15-acetyl-deoxynivalenol (15-Ac-DON) in 2010 (WHO, 2011). A survey including three Austrian isolates found Central European *F. graminearum* isolates to belong to chemotype I (DON producing) with most isolates producing more 15-Ac-DON than 3-Ac-DON (suggesting they belong to chemotype Ib) (Tóth et al., 2005). The DON conjugate deoxynivalenol-3-glucoside (DON-3-Glc) formed in plants was not included into the group PMTDI due to insufficient information at that time (WHO, 2011) and not taken into account in the European Food Safety Authority's 2013 exposure assessment due to a lack of occurrence data (EFSA, 2013). This "masked" metabolite is of a special interest for breeders since FHB resistance factors that significantly reduce DON have been shown to increase the ratio of DON-3-Glc to DON (Lemmens et al., 2016). A point stressed by D'Mello et al. (1997) is that risk assessments should not be based solely on pure compounds but should consider synergistic effects of co-occurring mycotoxins.

1.1.2 Mycotoxins and mycoflora

F. graminearum is the single most dominant, widespread and destructive FHB causing pathogen worldwide (Miedaner, Cumagun, et al., 2008). In 2012, *F. graminearum* was voted fourth by fungal pathologists in a Top 10 list of fungal plant pathogens based on scientific/economic importance (Dean et al., 2012). However, species opportunistic or less pathogenic than *F. graminearum* can contribute considerably to the mycotoxin content in wheat, while high FHB severity does not necessarily mean high mycotoxin or DON concentrations if certain species including *Microdochium nivale* (syn: *F. nivale*) predominate in the FHB complex (Bottalico & Perrone, 2002). In short, members of the complex differ in their virulence as well as the quantity and type of mycotoxins produced (Przemieniecki et al., 2014). They do not only interact with one other, but may also interact with saprophytic species (Liggitt et al., 1997; Pirgozliev, 2002). Fungicide applications may alter the balance within the mycoflora and the spectrum and amount of mycotoxins (Müllenborn et al., 2008). Moreover, Mathis et al. (1986) presumed that powdery mildew (*Erysiphe graminis*) pustules allow *F. culmorum* to infect wheat leaves. Apparent shifts in pathogen populations (Miedaner, Cumagun, et al., 2008) may be influenced by climate change whose impact could also generally result in a dramatic increase of FHB and in increased DON contamination (Moretti et al., 2019).

1.1.3 FHB control strategies

Control measures against FHB and mycotoxins include cultural, chemical, and perhaps biological strategies, as well as the exploitation of host plant resistance (Pirgozliev et al., 2003).

Cultural control includes crop-rotation and tillage (Dill-Macky & Jones, 2000). Even non-grass species like soybean (Broders et al., 2007; Pioli et al., 2004) and potato and sugar beet (Burlakoti et al., 2007) can be infected by *F. graminearum* and thus be a potential source of inoculum.

An azole fungicide application at heading is the most common chemical FHB and DON control practice in temperate regions and was also proven to be effective in reducing various other mycotoxins (Scarpino et al., 2015). For organic wheat production, tannic acid and botanicals show potential in reducing FHB severity and mycotoxin content (Forrer et al., 2014). According to

Mesterházy (1995), wheat genotypes with a higher resistance level have a higher resistance stability while highly susceptible genotypes cannot even be protected by chemicals when heavy epidemics occur.

Several promising biocontrol microorganisms have been proposed. For example, under field conditions, CLO-1, a formulated product of fungus *Clonostachys rosea* strain ACM941, reduced FHB index and DON by 30–46% and 22%–33%, respectively (A. G. Xue et al., 2014), while bacterium *Bacillus subtilis* strain SG6 was able to reduce FHB index by 77.5% and DON by 69.1% compared with an untreated control (Zhao et al., 2014). FHB index was defined as $incidence \times severity \div 100$, with incidence being the proportion of heads infected and severity being the proportion of spikelets infected on infected heads (Groth et al., 1999).

However – and while it is highly desirable to aim for an integrated, multiple-strategy control approach (Blandino et al., 2012; Shah et al., 2018) – the most reliable and consistent one of the single strategies seems to be the exploitation of genetic resistance (Pirgozliev et al., 2003).

1.2 Exploitation of genetic FHB resistance in wheat

Research in FHB resistance breeding has a tradition of about 100 years (Mesterházy et al., 2018). Smith (1884) gave an early description of FHB symptoms and attributed the disease to *Fusisporium culmorum*, a synonym of *Fusarium culmorum* (MycoBank, n.d.). Arthur (1891) observed that different wheat varieties were unequally affected by scab in a variety screening, but attributed differences mainly to differences in plant vigour due to a prior infection by another pathogen. He concluded that the variety of wheat holds no very important relation to the disease and found that late-sown plants were more diseased. In the early 20th century, it was found that large differences in resistance to FHB exist between varieties (Atanasoff, 1920; Christensen et al., 1929; MacInnes & Fogelman, 1923; Scott, 1927) and several State experiment stations and Government field stations in the US were working on the development of resistant wheat varieties (Dickson & Mains, 1929). However, it

was the case that breeding programs that were started many times in the 20th century would cease after several years because of lack of interest or resources (Mesterházy, 2003).

Today, however, many breeders have included selection for *Fusarium* resistance as a routine trait in their bread wheat breeding schemes (H. Buerstmayr et al., 2014). In different European countries FHB resistance is part of the assessments to be made in growing trials for the examination of value for cultivation and use (VCU) (AGES, 2019; APHA, 2019; Bundessortenamt, 2019; Rvp & CSAR, 2017). In Germany, in the early 1980s the first targeted FHB resistance breeding programs were integrated into commercial wheat breeding (Spanakakis, 2003).

The ultimate goal is productive cultivars with low disease symptoms and low mycotoxin contamination despite high infection pressure (H. Buerstmayr et al., 2009). While it is relatively simple to breed genotypes highly resistant to FHB, it is a great challenge to combine FHB resistance with all other desirable traits (Mesterházy, 2003).

1.2.1 Fundamentals of pathogen virulence

FHB resistance is horizontal and species- and race-nonspecific (Mesterházy, 2003; van Eeuwijk et al., 1995). Highly resistant wheat genotypes have excellent resistance all over the world, thus resistance is independent of the population structure of the *Fusarium* species/races (no highly specialized races exist) (Mesterházy, 2003). However, isolates differ significantly in aggressiveness (Mesterházy et al., 1999). Aggressiveness of *F. culmorum* isolates has been shown to have a quantitative-genetic basis (Miedaner et al., 1996) and Carter et al. (2002) found differences in aggressiveness between geographical groups of *F. graminearum*. According to van der Lee et al. (2015), *F. graminearum* should be considered as a meta-population rather than isolates around the world belonging to one and the same population.

Given the fact that resistance does not seem to depend on the *Fusarium* species and there are no vertical races, selection against a highly aggressive isolate of one important species should be satisfactory for screening purposes in breeding (Mesterházy, 2003; van Eeuwijk et al., 1995). However, more than one genetically unrelated resistance source must be used in breeding to

minimize the risk of developing *Fusarium* pathogen populations showing increased aggressiveness and mycotoxin production (Miedaner, Cumagun, et al., 2008) and wheat varieties should in any case have a complex resistance to the different species, especially the leading species *F. graminearum* and *F. culmorum* (Mesterházy, 2003).

Summerell & Leslie (2011) remarked that the amount of genetic variation within the genome of *F. graminearum* is quite large at around 3% – enough to separate some plants and animals into different genera – and outcrossing occurs frequently. The authors stressed the possible trouble for resistance breeding via partial resistance when facing populations of *F. graminearum* carrying enough additional multi-locus potential for aggressiveness.

1.2.2 Fundamentals of host resistance

Two major types of FHB resistance were described by Schroeder & Christensen (1963): Resistance to initial infection and resistance to hyphal spread. This division into type I and type II, respectively, is widely accepted (M. Buerstmayr et al., 2012). Three additional types of active resistance were listed by Mesterházy (1995) and Mesterházy et al. (1999): Resistance to toxin accumulation, resistance to kernel infection, and tolerance. Morphological traits may have an influence on FHB resistance, too. For example, plant height and anther extrusion have been found to be associated with type I FHB resistance (M. Buerstmayr & Buerstmayr, 2015; Lu et al., 2013; Yan et al., 2011). Indirect selection for FHB resistance through selection for rapid and complete anther extrusion appears promising and combining this trait with type II resistance quantitative trait loci (QTLs) seems advisable (M. Buerstmayr & Buerstmayr, 2015).

According to Mesterházy (2003), different resistance components are under different genetic control but may be highly correlated because of pseudo-pleiotropic effects or linkage of genes. According to Dweba et al. (2017), resistance QTLs generally confer responses to two or more resistance types, albeit at varying levels.

According to Mesterházy et al. (1999), low kernel infection, yield loss, and low DON contamination seem to be more or less correlated. However, since the genetics of resistance traits against FHB,

FDK (*Fusarium* damaged kernel content), and DON accumulation can differ, all these traits should be analysed during resistance tests (Szabó-Hevér et al., 2014).

In FHB resistance breeding, crosses with at least one moderately to highly resistant parent are necessary to obtain progeny with acceptable resistance levels (Steiner et al., 2017), but ideally both crossing partners have considerable resistance and transgressive segregation can be hoped for (Mesterházy, 2003).

1.2.3 Sources of FHB resistance

Mostly spring wheats from China, Japan, and Brazil have been existing sources of high FHB resistance for decades (Mesterhazy, 1996). The Chinese spring wheat cultivar 'Sumai 3' and its derived lines is arguably the most widely used and certainly the best characterized resistance source (Rudd et al., 2001). H. Buerstmayr et al. (1996) performed an extensive investigation of international wheat germplasm and found considerable variation for FHB resistance among both the winter wheat and the spring wheat germplasm, with the most resistant genotypes being spring types. However, they found good resistance also in some winter wheat germplasm, including some old Austrian land races.

European breeders strongly prefer resistance donors from their own programs or at least from released European varieties over non-adapted germplasm because of the high grain yield and quality demands (Miedaner et al., 2011). Usually, breeders have found genetic variability for FHB resistance in their existing germplasm (Rudd et al., 2001) and at a low rate, even good or excellent resistance can be found in breeding material without exotic FHB resistance sources (Mesterházy et al., 2008).

Notably, von der Ohe et al. (2010) and Salameh et al (2011) demonstrated that major “exotic” resistance QTLs can be introgressed into European winter wheat backgrounds to greatly increase FHB resistance without substantially compromising agronomic performance and quality parameters. With 'Jaceo', Syngenta Seeds (France) released a European winter wheat cultivar harbouring QTL *Fhb1* in 2013 (H. Buerstmayr et al., 2014).

H. Buerstmayr et al. (2009) stated that while “complete” FHB resistance has not yet been found in cultivated wheat, some alien species probably possess resistance genes that lead to an almost immune phenotype. The authors pointed out that such genes would, on the one hand, facilitate breeding for FHB resistance in wheat but that, on the other hand, relying on major gene resistance bears a high risk of sudden resistance breakdown as the experience with other wheat diseases shows. Expanding the wheat gene pool using other species, conventionally either by amphiploidization or by direct backcrossing, appears in any case promising, since common wheat has a rather narrow genetic base (Cox, 1997). However, this stands in contrast to the demand of practical breeders for well-adapted resistant germplasm rather than “exotic” material.

Increasing FHB resistance through transgenic strategies has excellent long-term prospects, but constraints including regulatory issues, proprietary rights, and low public acceptance have to be overcome (Muehlbauer & Bushnell, 2003). Recent transgenic approaches include host-induced silencing of *Fusarium* genes (Chen et al., 2016) and DON detoxification via *in planta* glycosylation (Gatti et al., 2019; Li et al., 2015; Mandalà et al., 2019).

1.2.4 Quantitative trait loci (QTLs) and marker-assisted selection (MAS)

Breeding for FHB resistance is difficult because the most resistant germplasm has poor agronomic traits, resistance is inherited oligogenically or polygenically, and screening for resistance is environmentally biased, tedious, and expensive (H. Buerstmayr et al., 2002). To augment conventional methods, molecular breeding methods, including marker-assisted selection (MAS) and in a more general approach genomic selection (GS) have the great potential to support and accelerate FHB resistance breeding (H. Buerstmayr et al., 2009; Poland & Rutkoski, 2016). GS appears especially promising for breeding programs deploying “native” resistance sources with many small-effect QTLs (Steiner et al., 2017) and may outperform MAS in an FHB breeding program (Arruda et al., 2016).

The usefulness of molecular markers for FHB resistance breeding was already stressed by Van Sanford et al. (2001) almost two decades ago. Marker-based selection may greatly speed up the

breeding for FHB resistance, which cannot be estimated phenotypically on a single-plant basis sufficiently, and marker-based backcrossing can minimize the non-adapted genome proportion from a donor species (Wilde et al., 2007). Markers are particularly useful in resistance breeding, because selection in absence of the pathogen and resistance gene pyramiding is possible (Langridge et al., 2001).

Requirements for effective MAS for FHB resistance are QTLs with relatively large and stable effects as well as the availability of tightly linked markers (Steiner et al., 2017). In breeding programmes, MAS for the major QTLs in early generations combined with phenotypic selection is favourable (Salameh et al., 2011; Wilde et al., 2007). Agostinelli et al. (2012) proposed an initial round of phenotypic selection at moderate selection intensity, enriching the population with major QTL resistance alleles while maintaining variation at other loci, followed by genotyping for the major QTLs in lines of phenotypic value.

More than 100 published QTLs for FHB resistance were reviewed by H. Buerstmayr et al. (2009), with 22 QTL regions having been detected in more than one mapping population. Venske et al. (2019) found 556 QTL in a recent review of 76 scientific papers, distributed on all sub-genomes and chromosomes, and generated 65 meta-QTLs.

The two most effective QTLs identified are *Qfhs.ndsu-3BS* (*Fhb1*) and *Qfhs.ifa-5A* from Chinese spring wheat variety 'Sumai 3' and derivatives (Schweiger et al., 2013). The 3BS QTL was identified by Waldron et al. (1999) and assigned the designation *QFhs.ndsu-3B*. Later on it was named *Qfhs.ndsu-3BS* (Anderson et al., 2001) and redesignated as *Fhb1* by Liu et al. (2006). Recently, *Fhb1* was introgressed into and validated in durum wheat (*Triticum durum*), representing a significant step forward in enhancing FHB resistance in this species (Prat et al., 2017). In North America, both *Fhb1* and *Qfhs.ifa-5A* are used in practical wheat breeding (Steiner et al., 2017).

1.2.5 Chromosome 5A harbouring QTL *Qfhs.ifa-5A*

As early as 1982, Yu (1982) found in a monosomic analysis that spikelet infection in 'Chinese Spring' × 'Soo-Mo 3' F_2 populations was reduced when chromosome 5A of 'Chinese Spring' was missing.

Another study using monosomic analysis found a positive effect on chromosome 5A in resistant breeding line 'U.-136.1' with 'Sumai 3' and 'Nobeokabozu' in its background (H. Buerstmayr et al., 1999). Ban & Suenaga (1997, 2000) suggested that the resistance of 'Sumai 3' was controlled by two major genes with additive effects and assumed that one of the resistance genes may be linked in repulsion to the dominant suppressor *B1* for awnedness on the long arm of chromosome 5A (chromosome 5AL). The work of Xu et al. (2001) supported the result of a resistance-conferring genomic region on 5AL in 'Sumai 3' by finding a significant association of 5AL specific markers with Type I resistance.

H. Buerstmayr et al. (2002) started a QTL mapping approach using a population with resistance from spring wheat line 'CM-82036-1TP10Y-OST-10Y-OM-OFC' (abbreviated 'CM-82036'), a 'Sumai 3' derivative, in order to identify and map markers associated with type II FHB resistance. They found QTLs residing on chromosomes 3B, 5A, and 1B and concluded that type II FHB resistance in this material was under control of a few major QTLs with additive effects and an unknown number of minor genes. The *Xgwm293–Xgwm304* interval was found to be the most-likely position of the 5A QTL. In an analysis for combined type I and type II resistance in the same population, H. Buerstmayr et al. (2003) found a QTL on chromosome 5A that explained 20% of the phenotypic variance for visual phenotypic FHB severity, and seemed to be primarily associated with resistance to fungal penetration. The allele conferring resistance originated from 'CM-82036'; the most likely position of the QTL was found to be in the *Xgwm293– Xgwm156* interval, a region close to the centromere. The QTL was designated *Qfhs.ifa-5A*. Somers et al. (2003) found a QTL controlling DON accumulation on the short arm of chromosome 5A (chromosome 5AS) with the resistance allele likely inherited from 'Frontana'. QTL *Qfhi.nau-5A* from 'Wangshuibai' associated with type I resistance (F. Lin et al., 2006) was mapped to chromosome 5AS as *Fhb5* by S. Xue et al. (2011).

Qfhs.ifa-5A and the advantage of stacking *Fhb1* and *Qfhs.ifa-5A* were validated by Miedaner et al. (2006). Also, an *a posteriori* approach following phenotypic selection showed the large effect of *Qfhs.ifa-5A* (Miedaner, Wilde, et al., 2008). Taken together, after the original report this QTL was found significant in at least eight independent mapping studies based on FHB resistant sources from

East Asia (Salameh et al., 2011). Interestingly, QTLs in this region have also been found in germplasm originating from South and North America and Europe (H. Buerstmayr et al., 2009). The non-species specificity of *Qfhs.ifa-5A* was proven by Lemmens et al. (2004). Wilde et al. (2007) showed that marker-based selection for *Qfhs.ifa-5A* from 'CM82036' was effective.

Von der Ohe et al. (2010) evaluated *Fhb1* and *Qfhs.ifa-5A* in two European Winter Wheat backcross populations and found only small negative effects on agronomic traits that should be possible to be further decreased. The authors additionally showed that also high-quality performance is possible with the non-adapted QTLs. In a similar approach, Salameh et al. (2011) found an average increase in plant height associated with *Qfhs.ifa-5A*, but no systematic yield penalty or quality reduction (Salameh et al., 2011).

Steiner et al. (2009) analysed gene expression in wheat after *Fusarium* attack through the cDNA-AFLP method and found five transcript-derived fragments associated with the presence or absence of resistance alleles at QTLs *Fhb1* and *Qfhs.ifa-5A* (Steiner et al., 2009). Homologies to a UDP-glucosyltransferase, phenylalanine ammonia-lyase, DnaJ-like protein, pathogenesis-related family protein and to one gene with unknown function were revealed. Schweiger et al. (2013) found a transcript corresponding to a lipid transfer protein constitutively more abundant in lines with the *Qfhs.ifa-5A* resistant allele and proposed Ta.1282.4.S1_at as a promising candidate gene.

Research and breeding programs have often concentrated on Type II resistance and the 3BS QTL, but Type I as conferred by the 5A QTL has been shown to be an equally important component of host resistance (Mesterházy et al., 2008; Mesterházy et al., 2007). Von der Ohe et al. (2010) even concluded that for the European winter wheat pool *Qfhs.ifa-5A* should be sufficient, especially when introgressed into an already moderately resistant genotype.

However, further efforts are required to resolve the *Qfhs.ifa-5A* locus in the centromeric region of chromosome 5A (Schweiger et al., 2013). In the study of Malla et al. (2012), the percentage of lines with resistant alleles for *Qfhs.ifa-5A* exhibiting susceptibility was rather high when primers Xgwm293, Xgwm304, and Xbarc186 were used. As H. Buerstmayr et al. (2009) pointed out, there is a need for diagnostic markers for other QTLs than *Fhb1* and such markers with close linkage to the QTL can

be found by fine-mapping. Moreover, mapping of the locus is central in the cloning of a resistance gene (Langridge et al., 2001).

1.3 Fine-mapping

The size and structure of the wheat genome make genetic analysis particularly challenging (Langridge et al., 2001). Hexaploid wheat (*Triticum aestivum*) has a very large genome that contains more than 85% repetitive DNA (IWGSC et al., 2018). The nuclear DNA content is around 16 Gb/1C, 6 times higher than the one of maize and almost 40 times higher than the one of rice (Arumuganathan & Earle, 1991). The minimal average size of a wheat centromere is 4.9 Mb (6.7 Mb when unassigned scaffolds are included) (IWGSC et al., 2018).

Sakamura (1918) was the first to report common wheat to have 42 chromosomes. These constitute seven groups, each containing a set of three homoeologous chromosomes belonging to the A, B, and D genomes (B. S. Gill et al., 2004). This genomic formula, BBAADD, originated as a result of two natural amphiploidization events (Cox, 1997). The 3 sub-genomes probably were derived from *Aegilops tauschii* (DD), *Triticum urartu* (A^uA^u), and *Aegilops speltoides* (BB) (Petersen et al., 2006). Because probably *Aegilops speltoides* is the plasmon donor in the cultivated wheats (G.-Z. Wang et al., 1997), the correct designation for common wheat is BBAADD, although generally AABBDD is used (Jauhar, 2007). Since homoeologous chromosomes normally do not pair with one another during meiosis, common wheat behaves much like a diploid organism (B. S. Gill et al., 2004). Consequently, the 3 genomes translate to 21 linkage groups (Langridge et al., 2001).

1.3.1 Linkage mapping

Linkage mapping, or genetic mapping, is based on genetic recombination during meiosis. Sturtevant (1913) was the first to use the number of cross-overs between two genes per 100 events to measure genetic distances and create a chromosome map. Numerous linkage maps of wheat have been

published (Langridge et al., 2001). A more recent one for chromosome 5A was obtained by Gadaleta et al (2014).

However, uneven recombination across chromosomes results in inaccurate estimates of genetic to physical distances (Kumar, Bassi, et al., 2012). It further has been shown in wheat that recombination is very low in proximal regions of the chromosome, increasing with distance from the centromere (Akhunov et al., 2003). Thus, it is not possible to determine the order of loci within centromeric regions merely through recombination mapping (Balcárková et al., 2017). More than 30% of the wheat genes are in recombination-poor regions (Erayman et al., 2004). To fine-map such regions including the *Qfhs.ifa-5A* interval, consequently a recombination-independent mapping approach is needed.

A possible solution to this problem is deletion mapping, i.e., using lines with deleted chromosomes or chromosome segments to assign markers to the deleted regions. A further benefit of deletion mapping is the fact that it does not require allelic variation (Endo, 1990), while linkage maps depend on recombination between polymorphic molecular markers (Balcárková et al., 2017).

1.3.2 Deletion mapping

Due to the buffering effect of polyploidy on gene deletions in young polyploid species (arising from functionally redundant homoeoloci), wheat shows a relatively high tolerance to high mutation densities (Dubcovsky & Dvorak, 2007; Fitzgerald et al., 2012). The work of Sears (1939, 1944, 1954, 1966) on aneuploids resulted in 'Chinese Spring' wheat lines with full chromosomes missing (nullisomes), often with tetrasomes (chromosome present in four doses) of the same homoeologous group compensating for the loss. These lines have proved extremely useful in assigning markers to linkage groups and by using ditelosomic lines, a marker can even be assigned to a chromosome arm (Langridge et al., 2001).

By the use of “aneuploids that have sub-arm aneuploidy” (Endo, 2015), an even higher resolution is possible. Such plants, carrying deletions induced by a gametocidal *Aegilops* chromosome, were obtained by Endo (1988). The author stressed the possible usefulness of aneuploids with deletions

of different lengths for physical mapping. Such deletion stocks were then used by Werner et al. (1992) to successfully construct cytogenetically based physical maps of RFLP loci. Physical and genetic maps have been compared and composite maps have been constructed (K. S. Gill & Gill, 1994; K. S. Gill et al., 1996). Endo & Gill (1996) identified 436 deletions and produced homozygous lines for 347 of them, including 11 short-arm deletions on chromosome 5A. However, the authors found hot spots for breakage, making the distribution of breakpoints obtained by the gametocidal system non-random. Moreover, such lines usually have deletions only from the telomere (Langridge et al., 2001). The number of deletion breaks is limited and the isolation of new stocks is time consuming (Tiwari et al., 2016). Finally, mapping to chromosomal bins cannot provide accurate ordering of markers within these segments (Kalavacharla et al., 2006).

A most elegant way of overcoming such problems is radiation hybrid (RH) mapping. RH mapping relies on the random formation of deletions for mapping after high dosages of radiation have been applied (Kumar, Bassi, et al., 2012). Independently derived RHs are assayed for the presence or absence of marker loci and the patterns and frequencies of marker co-retention are used to calculate physical proximities and to develop a map (Balcárková et al., 2017). Distances are expressed in the unit centiRay (cR) and depend on the radiation dosage. For example, 1 centiRay (cR_{5000}) is equal to 1% probability of breakage between two markers after 5000 rads of gamma rays (Womack et al., 1997). Riera-Lizarazu et al. (2010) described a method to produce RHs for the simultaneous mapping of the D-genome chromosomes of wheat: Seeds of hexaploid wheat are irradiated and surviving plants are crossed to tetraploid (AABB) wheat. Resulting quasi-pentaploid plants are monosomic for the D-genome chromosomes, allowing RH mapping of these chromosomes. For a higher deletion rate, Tiwari et al. (2016) used irradiated pollen from a hexaploid plant to pollinate tetraploids. Again, D-genome markers could be mapped by their presence or absence in the resulting AABBD plants. Since A- and B-chromosomes were also present from the tetraploid parent, dominant or co-dominant markers were needed to detect deletions in the other sub-genomes. A different approach that has already been followed at IFA Tulln before this thesis (Schwarz, 2017; Wagner, 2017) is selfing hexaploid wheat plants after irradiation in order for induced deletions to become

homozygous and thus again be easily scorable by simple presence or absence of markers. This way, also stable inbred deletion lines e.g. for thorough phenotyping can be obtained. This particular method is referred to as radiation selfing (RS) mapping.

1.3.3 Special applications of RH mapping

Since Goss & Harris (1975) had first published the RH approach as a new method for mapping human genes, RH mapping has been used for numerous studies in both humans and animal species (Faraut et al., 2009; International Human Genome Sequencing Consortium, 2001). RH studies in plants were pioneered by Riera-Lizarazu et al. (2000), using oat-maize chromosome addition lines (Oscar Riera-Lizarazu et al., 1996). While in animals the process is restricted to *in vitro* panels where a radiated genome is rescued by fusion with a recipient cell line, in plants *in vivo* panels following an artificial cross can be generated (Kumar et al., 2014). Somatic plant hybrids from protoplast fusion expand the scope of plant RH mapping to cross-incompatible species combinations with a high level of polymorphism between the species. For example, asymmetric somatic hybrids between wheat and *Bupleurum scorzonerifolium*, an Apiaceae species, proved feasible for mapping purposes in wheat (Zhou et al., 2012, 2006). In this thesis, however, only *in vivo* panels are further discussed.

The general advantages of RH mapping over linkage mapping for fine-mapping low-recombinogenic regions have been discussed in the chapters 1.3.1 and 1.3.2. Hereafter, RH mapping in the context of selected applications is described.

Positional cloning and forward/reverse genetics

Conventional map-based cloning requires the ability to generate thousands of progeny in which to screen for recombinants in small genetic intervals (Remington et al., 2001). Apart from its recombination-dependence, positional cloning using a genetic mapping approach is time consuming: Developing a population from NILs that only segregate for the region of interest takes four to six generations (Kumar et al., 2014).

Kumar et al. (2014) list several advantages of RH panels in this context: A high map resolution is possible and can be influenced through radiation dosage, a homogeneous population can be

obtained in only one generation, and monomorphic genes may be mapped or cloned, too. Furthermore, distances obtained from RH mapping provide a better estimate of physical distances (Kumar, Bassi, et al., 2012).

In the study of Faris et al. (2003), radiation-induced deletion mutants of wheat aided in narrowing down the region for *Q* gene candidates. The species cytoplasm-specific gene scs^{ae} was the first gene in wheat to be localised using RH mapping (Hossain et al., 2004).

Generally, plant RH populations are good candidates in forward/reverse genetic studies to associate changes in gene sequence with phenotypes and vice versa (Kumar et al., 2014). RS mapping seems to be particularly suited for forward/reverse genetics, since any genotype can be used for developing RS panels.

Sequencing

Physical maps provide a framework to anchor sequence data (Meyers et al., 2004). RH mapping is suitable to obtain sufficient resolution of such maps proximal to the centromere (Kumar et al., 2014). In wheat, the RH approach was used, for example, to help the assembly of BAC contigs from the wheat D-genome sequencing project (Kumar, Simons, et al., 2012). With the publication of the whole genome sequence of wheat (IWGSC et al., 2018), in this particular species the use of RH mapping to support genome sequencing has become obsolete.

Comparative genomics

For studying syntenic relationships among species by comparing gene-based maps of a species of interest with a sequence of a model species, RH maps are of great value because of their high resolution and the possibility of mapping monomorphic markers (Kumar et al., 2014). Michalak et al. (2009) used RHs to physically map rice-derived genes on chromosome 1D of wheat. In a later study, RH mapping combined with chromosome-wise synteny analysis led to conclusions about the evolution of chromosome 1D and its centromere (Michalak de Jimenez et al., 2013).

1.4 Deletion induction by artificial mutation

Deletions have been induced in wheat by various types of irradiation, including X-rays (Sears, 1977) and ion beams (Fitzgerald et al., 2015; Yang et al., 2014), a mutagen that has gained popularity in plant science and breeding only rather recently (Tanaka et al., 2010). Xiao et al., (2011) described a deletion on chromosome arm 3BS induced by fast neutron irradiation that increased FHB susceptibility in Chinese landrace 'Wangshuibai'. Gamma rays are an irradiation type that has been widely used for deletion induction in wheat (e.g. Cheng et al., 2015; Hossain et al., 2004; Spielmeyer et al., 2008; Tiwari et al., 2016). In principle, deletions can also be induced chemically, e.g. by DNA crosslinking agents (Anai, 2015). Van Schendel et al. (2016) demonstrated that in nematode *Caenorhabditis elegans* treated with photo-activated trimethylpsoralen (UV/TMP), deletions result from end joining of double strand breaks (DSBs) mediated by polymerase Theta. Regarding cereals, Wang et al. (2004) used another chemical “deletogen”, diepoxybutane (DEB), in rice with results indicating deletions of only small size as opposed to large deletions induced by fast neutrons in the same study. DSBs can also be induced by radiomimetic substances like bleomycin (Povirk, 1996), an antitumor antibiotic that induces breaks at specific sites (D’Andrea & Haseltine, 1978). In plants, bleomycin resistance has been proposed as a selectable marker for plant cell transformation (Hille et al., 1986).

An induced deletion is hemizygous in M_1 plants and is expected to become homozygous in the M_2 generation in the Mendelian ratio 1 (homozygous mutant) to 3 (hemizygous or heterozygous wild-type) (Murai et al., 2013). Homozygous mutations in the M_1 generation are not expected to occur in a considerable frequency, but have been described for certain genes in tomato (Jain et al., 1968) and pepper (Honda et al., 2006) after hydrazine treatments and heavy-ion bombardment, respectively. M_1 plants from irradiated seeds are chimaeric (Bado et al., 2015). In contrast, when seeds are obtained by pollination with irradiated pollen, each mutation will effect an entire plant that thus will be non-chimaeric for a given induced deletion (Sears, 1977; Vizir et al., 1994). The same can be expected after irradiation of the haploid female gamete (egg cell). In this thesis, for practical reasons, seeds from irradiated heads or seeds from pollination with irradiated pollen are denoted

"M_1", and progeny of those seeds, after one round of selfing, "M_2". Another possibility would have been to refer to the irradiated pollen as M_1 generation (Naito et al., 2005).

In nature, spontaneous deletions might act as a counterbalance against genome size increasements and genomic obesity over evolutionary time scales (Petrov, 1997).

1.4.1 Mutations induced by gamma irradiation

First mutation experiments with gamma irradiation where described in the 1920s by Stadler (1928) in barley. Gamma irradiation induces DNA single- and double-strand breaks. When there are not enough nucleotide pairs between two independently induced single-strand breaks in opposite strands, a DSB cannot be prevented (van der Schans, 1978). Achey & Duryea (1974) showed that hydroxyl radicals, which are produced by irradiation of water, may produce strand breaks as a secondary radiation damage.

There are two basic mechanisms working together in DSB repair: homologous recombination and nonhomologous end joining (NHEJ), where any end can join with any end (Britt, 1999; Gorbunova & Levy, 1999). In higher eukaryotes, such illegitimate recombination is more frequent than homologous recombination (Gorbunova & Levy, 1997). Due to the unspecificity of NHEJ, chromosomal inversions, deletions, translocations, and partial duplications can be produced (Britt, 1999). DSBs may differ in their complexity: For example, Pastwa et al. (2003) showed that the repair of DSBs produced by gamma rays was less efficient than the repair of restriction enzyme-induced DSBs.

Apart from inducing DSBs, by generating reactive oxygen species (ROS) (Tuteja et al., 2001), gamma irradiation may induce base substitution mutations, too. For example, the oxidative DNA damage adduct 8-hydroxy-2'-deoxyguanosine (Kasai & Nishimura, 1984; Shigenaga et al., 1989) has been shown to induce transversions both in vitro (Shibutani et al., 1991) and in vivo (Moriya et al., 1991). Morita et al. (2009) found transversions both from C/G to A/T and from T/A to A/T in gamma irradiated rice.

1.4.2 Deletion sizes

In rice (*Oryza sativa*), a popular model system for grasses (Chang et al., 2016; Izawa & Shimamoto, 1996), Morita et al. (2009) analysed mutations induced by gamma irradiation and found that deletions, particularly small ones (1-16 bp), were the most frequent mutations. Cecchini et al. (1998) had shown that conditions capable of producing deletions of at least 5 kbp without apparently inducing small deletions or rearrangements can be established in *Arabidopsis*, M_2 generation. Naito et al. (2005) gamma irradiated *Arabidopsis* pollen and detected large deletions up to >60 kbp, most of which were not transmitted to progeny. They hypothesized that large deletions not even transmittable heterozygously contained a gene or genes required for the formation or viability of pollen and egg cells. Nontransmissibility of deletions is expected to be less of a problem in common wheat due to its aforementioned genomic redundancy. Functional redundancy may play a role for mutations in *Arabidopsis*, too, a plant with an estimated 17% of non-tandem duplicated loci, most of which are non-linked (McGrath et al., 1993). However, the genome size and structure of allohexaploid wheat is exceptional. Furthermore, it has been shown that large deletions that were eliminated in diploid *Arabidopsis* progeny due to dominant lethality could be rescued in triploid progeny (Vizir & Mulligan, 1999).

Kirik et al. (2000) showed differences in deletion length between *Arabidopsis thaliana* and tobacco following an endonuclease-induced single DSB, indicating a putative inverse correlation between genome size and average length of deletions. However, only deletions of up to 2.5 kb were subject to this study and differences were hypothesised to be due to different exo- or endonuclease activities attacking the break ends or a better protection against end degradation. For the occurrence of interstitial deletions spanning at least several Mb, such as are required for the mapping approach of this thesis, a different process has to be considered. As postulated by Naito et al. (2005), large deletions may occur by NHEJ when two DSBs in one chromosome occur, resulting in three fragments, and both terminal DNA fragments are joined while the middle one is omitted (Naito et al., 2005). Terminal deletions on a chromosome, of course, only require one break and the loss of a

distal fragment (Qi et al., 2003). A telomere sequence is synthesized *de novo* at the breakpoint (Tsujimoto et al., 1999).

1.4.3 Randomness of induced deletions

Sparsely ionizing radiations like gamma irradiation induce DSBs more or less uniformly, although stochastically, throughout the genome (Sachs et al., 2000). Complete randomness, however, cannot be assumed. Khush & Rick (1968) found surviving breaks after X-ray and fast neutron irradiation of tomato pollen to be more frequent in heterochromatin with the deviation from randomness being more pronounced in the X-ray treatment. Similarly, working with gamma irradiated tomato pollen, Liharska et al. (1997) found deletion breakpoints in M_1 plants to be predominantly located in the pericentromeric heterochromatic region. Quite contrarily, using immunoFISH to directly visualise DSBs, Falk et al. (2008) showed that in human cells decondensed, open, genetically active chromatin is more sensitive to DSB induction by gamma rays. In plants, Hase et al. (2010) showed in an ion beam irradiation experiment with petunia (*Petunia hybrida*) that highly expressed genes might be more radiation sensitive, leading to a higher mutation frequency in those genes. However, ion beam irradiation causes a different mutation spectrum than gamma irradiation, as shown in *Arabidopsis* (Tanaka, 1999) and carnation (Okamura et al., 2003). Working with gamma irradiation, Kumar et al. (2012) observed a significant interaction between the DNA repair mechanism and the distribution of crossing over events in durum wheat, showing that the hypothesis of RH mapping being independent of meiotic recombination patterns might not be entirely true. However, the RH map obtained by the authors still offered a fairly consistent physical to cR conversion and a much higher uniformity than a comparable genetic map.

1.5 Aim of thesis and research questions

The general aim of this thesis is to screen mutant wheat lines for irradiation-induced deletions in the pericentromeric region of chromosome 5AS presumably harbouring QTL *Qfhs.ifa-5A* and to use both the generated data and other available map data to construct a highly resolved map of this region.

Moreover, genotyped informative lines were to be maintained (generation advancement/ backcrossing to eliminate background mutations) by the Institute of Biotechnology in Plant Production, IFA Tulln, and used for phenotyping studies.

Two subpanels of M_2 plants derived from experimental line NIL3 (C3), a line that carries the resistance-conferring *Qfhs.ifa-5A* allele, were screened. For one subpanel, M_1 plants were generated by pollinating non-irradiated plants with irradiated pollen. For the other subpanel, seeds from irradiated heads gave rise to M_1 plants.

Research questions were the following:

- Can any deletions in the *Qfhs.ifa-5A* interval be detected and characterized in the two M_2 subpanels where irradiation was used to induce deletions randomly in the genome?
- Does the chosen “radiation selfing” approach prove fit to lead to a highly resolved map of the low-recombining region harbouring *Qfhs.ifa-5A*?
- Can any differences regarding marker retention be detected between the two subpanels?

2. Material and methods

2.1 Plant material

2.1.1 Deletion panel

QTL *Qfhs.ifa-5A* was first detected in the 'Sumai 3' derivative 'CM-82036-1TP10Y-OST-10Y-OM-OFC', abbreviated 'CM-82036' (H. Buerstmayr et al., 2002, 2003). As described by Schweiger *et al.* (2013), near-isogenic lines (NILs) were developed by crossing susceptible 'Remus' and resistant 'CM-82036': During backcrossing, marker assisted selection was applied to confirm the transfer of *Fhb1* and *Qfhs.ifa-5A* alleles to the next generation. BC_5F_1 plants were selfed and BC_5F_2 were screened for the genotypic status at *Fhb1* and *Qfhs-ifa-5A*. Selected line NIL3 harbours the *Qfhs.ifa-5A* resistance allele in a 'Remus' background and carries the susceptible allele at the *Fhb1* QTL. 'Remus' is a spring wheat cultivar developed at the Bavarian State Institute for Agronomy in Freising, Germany, that is highly susceptible to *Fusarium* ear infection but otherwise well-adapted for cultivation in central Europe (H. Buerstmayr et al., 2002).

Prior to this thesis, NIL3 (= C3) had been used for the generation of a radiation-induced deletion panel: At anthesis, heads were detached and gamma irradiated at a dose of 1 Kilorad [krd] (10 Gray [Gy]) at the IAEA laboratories at Seibersdorf, Austria. Radiation doses must ensure a sufficient number of deletions without causing damage that is not tolerated by the plant during its development or reproduction (Kumar, Simons, et al., 2012). Moreover, radiation dose/mean fragment size must reflect the desired map resolution/number of markers to be scored (Jones, 1996). Obtained irradiated pollen was used for pollination of non-irradiated plants, giving rise to the first of two subpanels (subpanel DP). The detached heads were kept for maturing of their seeds, giving rise to the second subpanel (subpanel DPS). The subsequent generation (M_1) was self-pollinated for induced deletions to become homozygous. Following a Mendelian inheritance pattern, a hemizygous deletion is generally expected to become homozygous in every forth plant after one selfing step. This of course only applies when a deletion is transmittable homozygously and viable seeds are produced.

For subpanel DP, 1536 M_2 (= RS_1) seeds (for 16 96-well extraction plates) were sown, numbered consecutively from 1 to 384 with sister lines a to d. For subpanel DPS, 1152 M_2 seeds (for 12 96-well extraction plates) were sown, numbered consecutively from 1 to 240 with sister lines a to d, and from 241 to 336 with sister lines a and b. Sister lines in this context are M_2 plants descending from the same M_1 plant: DP and DPS M_2 plants traced back to 384 and 336 M_1 plants, respectively.

Since not all seeds had produced sufficient leaf material for genotyping in time (seeds not germinated, plants dead or too small), eventually 1503 M_2 lines from subpanel DP (tracing back to all 384 M_1 plants) and 1085 M_2 lines from subpanel DPS (tracing back to 334 of the 336 M_1 plants) were screened.

2.1.2 Control lines

“Pre-screening” for deletions on subpanels DP and DPS with few markers was done with control lines C3 and d393 for comparison. d393 misses the whole 5AS chromosome, but not the 5AL chromosome (Wagner, 2017), while C3 would yield PCR amplicons for all markers.

For further screening of selected deletion lines, nullisomic5A-tetrasomic5B line CS-N5AT5B derived from ‘Chinese Spring’ with chromosome pair 5A replaced by an additional 5B pair (Sears, 1966) and 'Remus' were used as controls in addition to C3 and d393.

2.2 Experimental methods

2.2.1 Leaf sampling and DNA extraction

DNA was extracted following a modified Saghai-Maroof *et al.* (1984) protocol.

Disposable 8-tube extraction strips (1.2 ml wells) were labelled by hand with permanent markers and filled with three glass grinding beads per tube. Henceforth, the strips were kept in labelled 96-well racks.

In the greenhouse, leaf pieces of each plant were directly cut into the tubes: Material of one plant per tube. The scissors used were wiped down with paper towels to avoid contamination between genotypes. Per plant, three fresh leaf pieces short enough to not impede the closing of the tube were used as a rough guide for the suitable amount per tube. The DNA extraction protocol had been elaborated for up to 40 µg dry leaf material per tube.

The leaf samples were put in a drying oven at 36 °C for up to three days. When leaves were completely dry, i.e. had become crisp, the tubes were closed tightly with plug strips, the plug strips were labelled, and the samples were moved to a cold storage room at 4 °C until further use.

A buffer containing CTAB (cetyltrimethylammonium bromide) was prepared in the morning of each day of DNA extraction. Since 700 µl CTAB extraction buffer were needed for each well, 280 ml were prepared for four 96-well racks. Amounts of all ingredients are listed in Table 1.

Table 1: CTAB extraction buffer mixture

Reagent stock	Final concentration	Amount to add for 280 ml
dH_2O		182 ml
Tris 1 M, pH 7.5	100 mM	28 ml
NaCl 5 M	700 mM	39.2 ml
EDTA 0.5 M, pH 8.0	50 mM	28 ml
CTAB powder	1 %	2.8 g
BME 14 M	140 mM	2.8 g

After having mixed Tris (tris(hydroxymethyl)aminomethane), NaCl, EDTA (ethylenediaminetetraacetic acid), and dH_2O in a glass bottle, the liquid was warmed in a water bath to 60–65 °C. The bottle then was transferred to a heated magnetic stirrer hotplate under a fume hood and loaded with a magnetic stir bar. Only after that, CTAB powder was added and dissolved by stirring. Then, BME (β-mercaptoethanol) was added and the now complete buffer was mixed by stirring.

Already during the heating of the liquid for the CTAB extraction buffer, the leaf samples were homogenized, i.e. the leaf material was physically disrupted for a high DNA yield. A *Retsch* ball mill in combination with the glass beads already inside the tubes was used for grinding. Always two 96-well racks at a time were worked with. Milling took place for 10 min at a vibrational frequency of 300 min^{-1} with a change of rack orientation after 5 min.

The tube strips were centrifuged at the low relative centrifugal force (RCF) of 330 for approximately one minute. This was done in order to carefully get leaf powder off the plugs without compressing it at the bottom of the tubes.

Under the fume hood, the plug strips were removed carefully to not contaminate any tubes with leaf powder of a different genotype. Open tube strips were kept covered by a paper sheet during the process and used plug strips were discarded. Then, 700 µl of 65°C warm CTAB extraction buffer were added to each tube. The tube strips were closed again tightly with new, labelled plug strips. The racks were put into fasteners, shaken by inversion and placed into a gently swirling water bath outside the fume hood at 65 °C for 60–90 min. After a few minutes in the water bath, the screws of the fasteners were checked and tightened as necessary.

After the heating step, cooling down to room temperature was accelerated by a cool water bath in a sink. The racks were removed from the fasteners, followed by a short centrifugation with tissues underneath the racks to remove adhesive water.

Again under the fume hood, the tube strips were opened and 350 µl – half the amount of CTAB extraction buffer – of chloroform:isoamyl alcohol 25:1 were added to each tube. The tube strips were

closed again using the same plug strips and the racks were put into the fasteners one more time. For 15 min, they were gently shaken by inversion mechanically.

After centrifuging for 10 min at an RCF of 4000, 300 µl of each tube's top aqueous phase (supernatant) that had formed were pipetted into new labelled tube strips with new labelled plug strips. The new strips usually had been prepared and labelled in the 60–90 min span during which the samples were in the water bath after the adding of the CTAB extraction buffer. The order of the samples within one 96-well rack was kept. No RNase was applied.

300 µl of isopropyl alcohol (propan-2-ol) were added to each well under the fume hood and the tubes were closed with new labelled plug strips. The liquids were mixed by gently shaking and inverting the tubes and DNA precipitation could be observed.

After centrifuging for 8 min at an RCF of 1000, a DNA pellet would stick to the bottom of each tube. The plug strips were removed and one rack at a time was inverted quickly over the sink. That way, the liquid would be poured off while the DNA pellet would still stick to the bottom of the tube. In order to not lose a tube strip, the strips were held back in the rack against gravity using a laboratory spatula.

100 µl of Wash 1 (see Table 2) were added to each tube. Where the pellet would still stick to the tube after having added the wash, the tube was flicked until the pellet was loose within the wash. The racks then were transferred to a shaker where they were gently shaken for 30 min.

Centrifuging and pouring off the liquid were performed again as described above. 100 µl of Wash 2 (see Table 2) were added to each well. The strips were inverted gently for 5 min and flicked where necessary to ensure that pellets were loose within the wash.

Again, centrifuging and pouring off the liquid was performed as described above. The tubes were kept open at room temperature overnight in order to let the pellets dry. In the next morning, 100 µl 0.05× TE buffer (see Table 2) were added to each well. To ensure good dissolution, the racks were again put on a shaker and gently rocked for several hours and finally stored at 4 °C for further use.

Table 2: Composition of wash 1, wash 2, and TE buffer

Wash 1	Wash 2	TE buffer
76% EtOH	76% EtOH	10 mM Tris-8.0
25 mM NaOAc	10 mM NH_4OAc	1 mM EDTA-8.0

2.2.2 DNA quality and concentration measurement

Concentration and purity of at least four samples per rack were measured on a micro-volume spectrophotometer (*Shimadzu* BioSpec-nano micro-volume UV-Vis Spectrophotometer) after shaking the plates two times for 30 sec at 1250 rpm with an *Eppendorf* MixMate®. With concentrations below 500 ng/µl, samples were not further diluted at this step. Purity was measured by using the ratio of absorbance at 260 and 280 nm ($A_{260/280}$; desired value 1.8–2.0) and at 260 and 230 nm ($A_{260/230}$; desired value 2.0–2.2).

Concentration of each sample was then measured with a *TECAN* Multichannel Photometer: Extraction racks were shaken (10–15 sec at 1000-1400 rpm) and centrifuged (some seconds at 3000 rpm). Photometer plates were loaded with 90 µl DNA solution per well (90 µl 0.05× TE buffer for blanks) and again shaken and spun down before the measurement. The plates were sealed with a foil until and after the measurement was performed and their bottom was cleaned with a *Kimtech* wipe just prior to measuring.

2.2.3 DNA normalisation, dilution, and aliquoting for PCR

DNA content was equilibrated to 200 µl/ng by adding 0.05× TE buffer. Normalisation was done either manually or with a *TECAN* liquid handling robot.

The samples were transferred from the photometer plates into stock plates (master plates) which were labelled with date, concentration and a bar code.

Working plates with 120 µl DNA solution of 50 ng/µl concentration were prepared. For diluting, dH_2O was used. Pre-defined wells were filled with DNA solution of control lines.

384-well PCR plates were prepared with 2 µl (50 ng/µl) DNA solution per well. Pipetting was done either manually or with a *TECAN* liquid handling robot.

2.2.4 Multiplex PCR

Eighteen 5AS-specific primer pairs were used for pre-screening the 2588 mutant lines and overall 101 5AS-specific primer pairs were used for screening thirty-three lines with deletions or possible deletions that were selected during pre-screening (see Appendix 1 for the list of primers). Two additional 5AL-specific primer pairs did not yield positive results and so are not discussed in this thesis.

A multiplex PCR, where different sequences are amplified simultaneously (Chamberlain et al., 1988), was used for time and cost efficiency reasons. An optimized multiplex PCR (Henegariu et al., 1997) protocol with maximum three primer pairs per well was followed. Good primer combinations (different size of PCR products and preferably at least one additional, 5AS-nonspecfic amplicon to recognise false positives due to PCR failures) had already been established at the institute (Schwarz, 2017; Wagner, 2017).

Master mix was prepared on ice and 8 µl were added to each well of the 384-well-plates already containing 2 µl DNA solution (see 2.2.3). Composition of the master mix for a PCR with three primer pairs is given in Table 3. For an economic way of fluorescently labelling PCR products, sequence-specific forward primers 5′-tailed with an M13 sequence (CCAGTCACGACGTT) were used in combination with a fluorescently labelled (FAM or Cy5 dye) universal M13 primer (Schuelke, 2000).

After aliquoting the master mix, the plates were immediately subjected to a hot-start touchdown PCR (cycler pre-heated to 95 °C). The exact PCR program is shown in Table 4.

Table 3: PCR master mix composition (three primer pairs)

	volume for one plate*	volume per reaction	stock concentration	final concentration
DNA solution	840 µl	2 µl	50 ng/µl	10 ng/µl
PCR buffer (incl. 1,5 mM $MgCl_2$)	420 µl	1 µl	10 ×	1 ×
dNTP mix (equal amounts of dNTPs)	420 µl	1 µl	2 mM	0.2 nmol/µl
1st reverse primer	84 µl	0.2 µl	10 µM	0.2 pmol/µl
1st forward primer	8.4 µl	0.02 µl	10 µM	0.02 pmol/µl
2nd reverse primer	84 µl	0.2 µl	10 µM	0.2 pmol/µl
2nd forward primer	8.4 µl	0.02 µl	10 µM	0.02 pmol/µl
3rd reverse primer	84 µl	0.2 µl	10 µM	0.2 pmol/µl
3rd forward primer	8.4 µl	0.02 µl	10 µM	0.02 pmol/µl
M13 primer	126 µl	0.3 µl	10 µM	0.3 pmol/µl
H_2O (PCR grade)	2074.8 µl	4.94 µl	-	-
TAQ	42 µl	0.1 µl	5 U/µl	0.05 U/µl
Sum	**4200 µl**	**10 µl**	**calculated for 420 reactions*	

Table 4: Steps during hot-start touchdown PCR

Step	Temperature [°C]	Time [min:sec]	Cycles
Pre-denaturation	95	04:00	-
Denaturation	95	00:50	7
Annealing with touch-down (-2 °C/cycle)	starting at 65	01:00	
Elongation	72	01:00	
Denaturation	95	00:30	25
Annealing	51	00:30	
Elongation	72	00:30	
Final post-elongation step	72	05:00	-
Storage	14	Infinite	-

2.2.5 Gel electrophoresis

For separation of the PCR amplicons by electrophoresis, 12% polyacrylamide gels were casted. 2.5 µl loading dye (Glycerol + dH_20, 3:10) containing bromophenol blue were added to each well of the PCR plates and gels were loaded with 2 µl aliquots of PCR product/loading dye mixture. Wells of the first or last lanes were loaded with molecular-weight size markers (DNA ladders): Primer-specific amplicons that had been obtained by PCRs of non-irradiated C3 (Schwarz, 2017). Gels were run in a vertical electrophoresis unit (*C.B.S. Scientific Co. Inc.*) for 2 hours at 400 V and 10 °C with 1× TBE as running buffer. After a few minutes of running, the program could be paused and PCR products with different fluorescent labelling could be loaded.

Gels were scanned on a Typhoon Trio imager (*GE Healthcare*) in fluorescence mode at 520 nm and 670 nm, visualising FAM-labelled and Cy5-labelled products, respectively. After scanning, gels were run unloaded and then re-used, if gel and image quality were still suitable for doing so.

2.2.6 Image analysis

Images were checked for deletions (scored) in Adobe Photoshop (Figure 1). The following characters were used in scoring:

1 – band/marker present (no deletion)
d – band/marker absent (deletion)
? – band faint (possible deletion)
s – band faint (probably no deletion)
e – empty (negative control)
m – band missing probably due to PCR failure
x – not scorable for other reasons

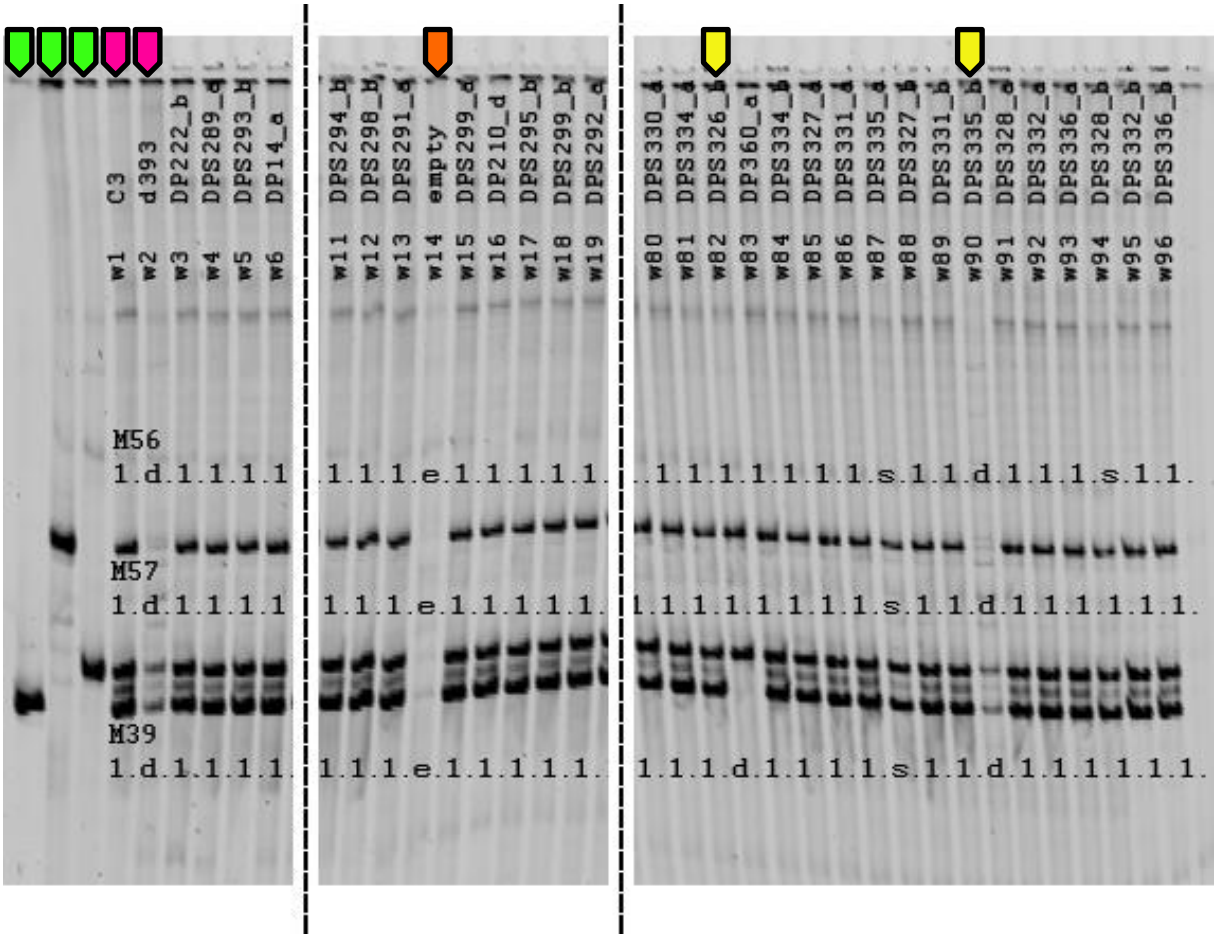

Figure 1: Example of an image scan (cropped) after scoring deletions

Green ( ) marks lanes of DNA ladders for markers gpg1294 (M39), gpg2123 (M56), and gpg2126 (M57)

Pink ( ) marks lanes of control lines C3 (non-irradiated) and d393 (5AS missing)

Orange ( ) marks a lane where no PCR products were loaded (negative control)

Yellow ( ) marks lanes where deletions (missing bands) could be detected

2.2.7 Map calculation and visualisation

Using the generated genotypic data of both subpanels, an optimal map with estimated marker distances (in cR) was built by CarthaGène (de Givry et al., 2005), version 1.2, a free program for genetic and radiation hybrid mapping, available for download at http://www7.inra.fr/mia/T/CarthaGene/.

The data was pre-formatted in Microsoft Excel and loaded in CarthaGène. A diploid radiation hybrid model was selected. Since all markers were 5A-specific, they were taken to represent a single linkage group.

The problem of ordering markers in RH mapping is connected to the travelling salesman problem (TPS) (Ben-Dor et al., 2000) and CarthaGène relies on this connection (de Givry et al., 2005). A correct solution to the TPS would have a salesman visit each given city exactly once and return to his starting point while using the overall shortest route (Larrañaga et al., 1999). Similarly, for mapping, an optimal order of markers (minimal map length and distances between markers) has to be found. This was computed using the Lin-Kernighan heuristic (Helsgaun, 2000; S. Lin & Kernighan, 1973) by running the commands `lkh`, `lkhd`, `lkhl`, and `lkhn`. To improve the best map, the commands `polish` ("polishing") and `flips` ("flipping") were run.

For the graphical presentation of the map, MapChart (Voorrips, 2002), version 2.32, was used. This program and a free license can be downloaded at https://www.wur.nl/en/show/Mapchart.htm.

2.2.8 Characterisation of the panels

Using genotypic data and the output of CarthaGène, several characteristics of the analysed panel were calculated. Most values were calculated for the whole panel, while some values were calculated separately for the two subpanels.

The unit of the distances calculated by CarthaGène is **cR**. Physical **distances in Mbp** were taken from the reference sequence IWGSC RefSeq v1.0 (IWGSC et al., 2018). To calculate map resolution [Mbp/cR] across the chromosome arm, 5AS was separated into bins C-5AS1-0.40, 5AS1-0.40-0.75, and 5AS3-0.75-0.97 representing 40%, 35%, and 22% of the chromosome arm length, respectively.

Based on a length of 255 Mbp for the whole chromosome arm (IWGSC et al., 2018), bin sizes in Mbp and an estimated map resolution [Mbp/cR] for each bin were calculated. The attribution of markers to bins obtained by this simplified approach is not necessarily in agreement with bin attribution according to available literature (see Appendix 1 for marker/primer publications).

The number of **informative lines** was counted as the number of lines with at least one detected deletion. While deletions of only a single marker or spanning all markers do no give information on marker order, every break influences map length. Furthermore, also lines with such deletions might prove useful in further research. They were thus included in the number of informative lines. Sister lines (descending from the same M_1 seed) with the same deletion/retention pattern were treated as one informative line. All further calculations were carried out using only informative lines.

For the number of **deletions** (interstitial and terminal), a row of deleted adjacent markers was counted as one deletion. A deletion was assumed to be terminal when the most distal marker (gpg2 or IWB11440) had been deleted.

The number of **obligate breaks** is the number of breaks observable between a retained marker and a lost one (Jones, 1997). Informative obligate breaks and mapped loci were counted.

Deletion lengths were calculated as minimum and maximum deletion lengths and the means thereof. The minimum length of a deletion is the distance between the most proximal and the most distal deleted marker and the maximum length of a deletion is the distance between the two flanking retained markers. For deletions not spanning more than one locus or concerning all markers, only one deletion length could be calculated. For the calculation of mean deletion lengths, the minimum deletion length of one-locus deletions was set to be 0. Even though a deletion including the most distal markers on the chromosome arm (gpg2 and IWB11440) is assumed to be terminal, the remaining 3% of chromosome arm length were not considered in the calculations.

Retention per line was calculated both as proportion of **retained markers per line** and proportion of **retained Mbp per line**. For the calculation of retained Mbp per line, mean deletion lengths (see

above) were used. **Retention frequency per marker** was calculated as the proportion of lines having retained a certain marker.

Mapped loci and **markers per locus** were counted.

2.2.9 Correlation analysis with reference sequence

Correlation between the generated data (in cR) and the Mbp according to reference sequence IWGSC RefSeq v1.0 (IWGSC et al., 2018) was calculated. Correlation coefficients were computed by R (version 3.5.1) via RStudio (version 1.1.463).

Both Pearson's product-moment correlation (R function `cor.test(X, Y,method="pearson")`) and Spearman's rank correlation (R function `cor.test (X,Y,method="spearman")`) were used (`X` and `Y` being variables for the data formatted as numeric vectors of the same length; therefore, only shared markers were used).

3. Results

3.1 Panel and map characteristics

During pre-screening 2588 mutant lines (1503 from subpanel DP and 1085 from subpanel DPS) with 18 primer pairs, 32 lines with clear or possible deletions on chromosome arm 5AS were selected for fine-mapping with 101 primer pairs. Six primer pairs did not yield reasonably scorable bands while one primer pair (wmc150) yielded two distinct 5AS-specific banding patterns, denoted wmc150a and wmc150b. Therefore, effectively 96 markers could be used for map construction (see Table 7). On two of the 32 selected lines, DP336_c and DPS24_b, no deletion could be confirmed. Eight selected lines were “sister” to another selected line (descending from four M_1 plants). Individual sister pairs had the same markers deleted, and were thus further treated as four informative lines. Thus, fine-mapping the 32 preselected lines resulted in 26 informative lines.

A map length of 400.1 cR was calculated by CarthaGène. The 255 Mbp physical length of chromosome arm 5AS was partitioned into segments representing 40% (bin C-5AS1-0.40), 35% (bin 5AS1-0.40-0.75) and 22% (bin 5AS3-0.75-0.97) of the chromosome arm, respectively. Calculated map resolution was 0.54 Mbp/cR in the most centromere-proximal bin and 1.00 Mbp/cR in the most distal bin. Table 5 gives a summary of bin sizes and map resolution.

Several markers had identical retention/deletion patterns and were mapped to the same loci (up to 8 markers per locus). 34 informative obligate breaks could be detected, translating to 35 mapped loci. Within loci, marker order could not be determined. In Table 7, marker order and distances as well as deletion patterns are shown. Figure 2 is a graphical presentation of the map created in MapChart, including marker order and distances.

Of 28 deletions, 20 were interstitial and 8 were terminal. One line, DP250_a, showed 3 deletions, while all other informative lines only had one single deletion. (Although, when markers were ordered according to the reference sequence, DP361_c|F3/DP361_d did show two deletions).

Distances between loci ranged from 5.7 cR to 48.7 cR. The mean deletion length was 105.7 cR (65.1 Mbp). The lowest calculable deletion length was 6.4 cR (3.3 Mbp). Minimum lengths of deletions not spanning more than one marker or locus (and, of course, deletions too small to be detected by the used markers) remain unknown. The largest deletion spanned all markers and therefore 400.1 cR (246.7 Mbp). Possibly, the whole chromosome arm was deleted in this line. Accordingly, no Mbp were retained in this line, while the line with the smallest deletion had at least 98.3% of Mbp retained. Table 8 gives a summary of deletions lengths, obligate breaks, retained markers, and retained Mbp per line. A compact summary of panel and map characteristics is given in Table 6. Marker retention and distribution of obligate breaks across the chromosome arm are shown in Figure 3 and Figure 4, respectively.

In Table 9, selected characteristics of pollen-irradiation subpanel DP and head-irradiation subpanel DPS are compared. Eight of 17 deletions in subpanel DP were terminal while all eleven deletions in subpanel DPS were interstitial. In subpanel DP, one line had all 96 markers deleted. The largest deletion in subpanel DPS spanned 77 markers. Two lines in subpanel DPS had only one marker (gpg2326 and IWB62899, respectively) deleted. In subpanel DP, the smallest deletions spanned five markers. Mean deletion length was 105.8 cR (70.6 Mbp) in subpanel DP and 105.5 cR (55.9 Mbp) in subpanel DPS. Median deletion length in subpanels DP and DPS, calculated from mean deletion lengths of lines (see Table 8), was 71.3 cR (51.9 Mbp) and 59.5 cR (32.8 Mbp), respectively. The proportion of informative lines (based on seeds sown) was 1.0% in both subpanels.

Table 5: Summary of bin characteristics

Bin	Length [Mbp]	Length [cR]	Map resolution [Mbp/cR]	Markers
5AS3-0.75-0.97	54.4	54.6	1.00	16
5AS1-0.40-0.75	86.6	162.8	0.53	28
C-5AS1-0.40	98.9	182.7	0.54	52
All 3 bins	247.4	400.1	0.62	96

Table 6: Compact summary of panel and map characteristics

Lines with deletions	30	Deletions		
Informative lines	26	Interstitial	20	
Markers	96	Terminal	8	
Mapped loci	35	Sum	28	
Distances between loci	[cR]	Deletion lengths	[cR]	[Mbp]
Minimum	5.7	Minimum[1]	6.4	3.3
Maximum	48.7	Maximum	400.1	246.7
Mean	11.8	Mean	105.7	65.1
Markers per locus		Retention frequency per marker		
Minimum	1	Minimum	53.8%	
Maximum	8	Maximum	96.2%	
Mean	2.7	Mean	73.0%	
Retained markers per line		Retained Mbp per line		
Minimum	0.0%	Minimum	0.0%	
Maximum	99.0%	Maximum	≥98.3%[2]	
Mean	73.0%	Mean	70.6%	
Obligate breaks		Map length		
Overall	48	[cR]	400.1	
Informative	34	[Mbp]	246.7	

[1]where at least two neighbouring loci are deleted
[2]calculated from maximum deletion length of smallest deletion

Table 7: Marker order and deletion pattern of lines

A / - *Marker deleted / Missing value, marker presumably deleted*

H / - *Marker retained / Missing value, marker presumably retained*

Obligate break (in at least one line)

Bin	marker	between-marker distance [cR]	cumulative distance [cR]	[Mbp]	DP199_b|G12	DP156_b	DP267_a	DP267_c|G5	DP260_a	DP260_d	DP55_a	DP316_d|H11	DP129_d|A7	DP361_c|F3	DP361_d	DP250_a	DP264_b	DP346_a	DP293_a	DP81_c|F5	DP69_b	DP369_c	DPS41_c	DPS335_b	DPS220_c	DPS326_b	DPS315_b	DPS214_d	DPS209_a	DPS209_b	DPS180_c	DPS147_b	DPS215_a	DPS217_b	DP336_c	DPS24_b
5AS3-0.75-0.97	IWB11440	-	0	9.8	A	H	H	H	H	H	H	A	A	H	H	A	A	H	H	A	A	A	H	H	H	-	H	H	H	H	H	H	H	H	H	H
5AS3-0.75-0.97	gpg2		0	10.2	A	H	H	H	H	H	H	-	A	H	H	A	A	H	H	A	A	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H
5AS3-0.75-0.97	gwm443	6.3	6.3	11.3	A	H	H	H	H	H	H	A	A	H	H	A	A	H	A	A	A	A	H	H	H	H	-	H	H	H	H	H	H	H	H	H
5AS3-0.75-0.97	cwem44c		6.3	18.9	A	H	H	H	H	H	H	A	A	H	H	A	A	H	A	A	A	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H
5AS3-0.75-0.97	wmc654		6.3	27.8	A	H	H	H	H	H	H	A	A	H	H	A	A	H	A	A	A	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H
5AS3-0.75-0.97	gpg537	6.1	12.4	29.2	A	H	H	H	H	H	H	A	A	H	H	A	A	A	A	A	A	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H
5AS3-0.75-0.97	IWB4146	5.9	18.3	36.5	-	H	H	H	H	H	H	-	-	H	H	A	A	A	A	A	A	A	H	H	H	H	H	H	H	H	H	A	H	H	H	H
5AS3-0.75-0.97	gpg2328		18.3	37.2	A	H	H	H	H	H	H	A	A	H	H	A	A	A	A	A	A	A	H	H	H	H	H	H	H	H	H	A	H	H	H	H
5AS3-0.75-0.97	IWB29780		18.3	41.5	A	H	H	H	H	H	H	A	A	H	H	A	A	A	A	A	A	A	H	H	H	H	H	H	H	H	H	A	H	H	H	H
5AS3-0.75-0.97	gpg2326	5.7	24	37.9	A	H	H	H	H	H	H	A	A	H	H	A	A	A	A	A	A	A	H	H	H	H	H	H	H	H	H	A	H	A	H	H
5AS3-0.75-0.97	IWB62899	11.7	35.7	33.0	A	H	H	H	H	H	H	A	A	H	H	A	A	A	A	A	A	A	H	H	H	H	H	H	H	H	H	A	A	H	H	H
5AS3-0.75-0.97	IWB68241	18.9	54.6	46.0	A	H	H	H	H	H	-	A	A	H	H	A	A	A	A	A	A	H	A	H	H	H	H	H	H	-	H	A	H	H	H	H
5AS3-0.75-0.97	barc186		54.6	46.6	A	H	H	H	H	H	H	A	A	H	H	A	A	A	A	A	A	H	A	H	H	H	H	H	H	H	H	A	H	H	H	H
5AS3-0.75-0.97	ldk243		54.6	47.5	A	H	H	H	H	H	H	A	A	H	H	A	A	A	A	A	A	H	A	H	H	H	H	H	H	H	H	A	H	H	H	H
5AS3-0.75-0.97	IWB51518		54.6	48.2	A	H	H	H	H	H	H	A	A	H	H	A	A	A	A	A	A	H	A	H	H	H	H	H	H	H	H	A	H	H	H	H
5AS3-0.75-0.97	ldk267		54.6	48.7	A	H	H	H	H	H	H	A	A	H	H	A	A	A	A	A	A	H	A	H	H	H	H	H	H	H	H	A	H	H	H	H
5AS1-0.40-0.75	barc56	6.1	60.7	70.7	A	H	H	H	H	H	H	A	A	H	H	A	A	A	A	A	H	H	A	H	H	H	H	H	H	H	H	A	H	H	H	H
5AS1-0.40-0.75	ldk284	21.2	81.9	77.1	A	H	H	H	H	H	H	A	A	H	H	A	A	-	H	H	H	H	A	A	H	H	H	H	H	H	H	A	H	H	H	H
5AS1-0.40-0.75	gpg2162	6.7	88.6	79.2	A	H	H	H	H	H	H	A	A	H	H	H	A	A	H	H	H	H	A	A	H	H	-	H	H	H	H	A	H	H	H	H
5AS1-0.40-0.75	gpg2163		88.6	79.1	A	H	H	H	H	H	H	A	A	H	H	H	A	A	H	H	H	H	A	A	H	H	H	H	H	H	H	A	H	H	H	H
5AS1-0.40-0.75	gpg1438	13.3	101.9	82.2	A	H	H	H	H	H	H	A	A	A	A	H	A	A	H	H	H	H	A	A	H	H	H	H	H	H	H	A	H	H	H	H
5AS1-0.40-0.75	barc117	6.2	108.1	85.3	A	H	H	H	H	H	H	A	A	A	A	H	A	H	H	H	H	H	A	A	H	H	H	H	H	H	H	A	H	H	H	H
5AS1-0.40-0.75	gpg2038	14.1	122.2	96.6	-	H	H	H	H	H	H	-	-	A	A	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	H	H	H
5AS1-0.40-0.75	IWB75561		122.2	98.0	A	H	H	H	H	H	H	A	A	A	A	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	H	H	H
5AS1-0.40-0.75	IWB8393		122.2	109.3	A	H	H	H	H	H	H	A	-	A	A	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	H	H	H
5AS1-0.40-0.75	gpg2049	6.9	129.1	115.5	A	H	H	H	H	H	H	A	A	A	A	A	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	H	H	H
5AS1-0.40-0.75	gpg2060		129.1	117.2	A	H	H	H	H	H	H	A	A	A	A	A	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	H	H	H
5AS1-0.40-0.75	IWB33435	6.8	135.9	133.0	A	H	H	H	H	H	H	A	A	A	A	A	H	H	H	H	H	H	A	A	H	H	H	H	H	H	A	H	H	H	H	H
5AS1-0.40-0.75	IWB58275	6.6	142.5	128.4	A	H	H	H	H	H	H	A	A	A	A	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H	A	H	H	H	H	H
5AS1-0.40-0.75	ldk217		142.5	130.2	A	H	H	H	H	H	H	A	A	A	A	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H	A	H	H	H	H	H
C-5AS1-0.40	gpg574		142.5	141.3	A	H	H	H	H	H	H	A	A	A	A	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H	A	H	H	H	H	H
C-5AS1-0.40	gpg2126		142.5	144.7	A	H	H	H	H	H	H	A	-	A	A	H	H	H	H	H	H	H	-	A	H	H	H	H	H	H	A	H	H	H	H	H
C-5AS1-0.40	gpg277		142.5	147.4	A	H	H	H	H	H	H	A	A	A	A	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H	A	H	H	H	H	H
C-5AS1-0.40	ldk218		142.5	147.7	A	H	H	H	H	H	H	A	A	A	A	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H	A	H	H	H	H	H
5AS1-0.40-0.75	gpg1763	13.3	155.8	124.0	-	H	H	H	H	H	H	-	-	A	A	H	H	H	H	H	H	H	A	A	H	H	H	H	A	A	A	H	H	H	H	H
5AS1-0.40-0.75	gpg2092		155.8	125.1	A	H	H	H	H	H	H	A	A	A	A	H	H	H	H	H	H	H	A	A	H	H	H	H	A	A	A	H	H	H	H	H
5AS1-0.40-0.75	gpg2072		155.8	125.4	-	H	H	H	H	H	H	A	A	A	A	H	H	H	H	H	H	H	-	A	H	H	H	H	A	A	A	H	H	H	H	H
5AS1-0.40-0.75	IWB10809	12.9 – 13.0	168.8	118.7	A	H	H	H	H	H	H	A	A	A	A	A	H	H	H	H	H	H	A	A	H	H	H	H	A	A	H	H	H	H	H	H
5AS1-0.40-0.75	BE498768		168.8	118.8	A	H	H	H	H	H	H	A	A	A	A	A	H	H	H	H	H	H	A	A	H	H	H	H	A	A	H	H	H	H	H	H
5AS1-0.40-0.75	ldk2		168.7	119.9	A	H	H	H	H	H	H	A	A	A	A	A	H	H	H	-	H	H	A	A	H	H	H	H	A	A	H	H	H	H	H	H
5AS1-0.40-0.75	ldk49		168.7	119.9	A	H	H	H	H	H	H	A	A	A	A	A	H	H	H	H	H	H	A	A	H	H	H	H	A	A	H	H	H	H	H	H
5AS1-0.40-0.75	gpg2233		168.7	120.1	A	H	H	H	H	H	H	A	A	A	A	A	H	H	H	H	H	H	A	A	H	H	H	H	A	A	H	H	H	H	H	H
5AS1-0.40-0.75	wmc150b		168.7	NA	A	H	H	H	H	H	H	A	A	A	A	A	H	H	H	H	H	H	A	A	H	H	H	H	A	A	H	H	H	H	H	H
5AS1-0.40-0.75	wmc150a	48.5 – 48.7	217.3	NA	A	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	H	H	H
5AS1-0.40-0.75	gpg1440		217.4	NA	A	H	H	H	H	H	H	A	-	H	H	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	H	H	H
5AS1-0.40-0.75	jfio7		217.4	101.0	A	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	A	A	H	-	H	H	H	H	H	H	H	H	H	H
5AS1-0.40-0.75	gwm293		217.4	104.2	A	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	-	H	H	H	H
5AS1-0.40-0.75	gwm304		217.4	105.4	A	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	-	H	H	H	H

Table 7: continued

C-5AS1-0.40	gpg1139		217.4	161.9	A	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg1789		217.4	168.4	A	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	jfio4	16.6	234	173.5	A	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	A	A	A	H	A	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg1994	14.7	248.7	177.3	A	H	H	H	A	A	H	A	A	H	H	H	H	H	H	H	H	H	A	A	A	H	A	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg2250		248.7	179.2	A	H	H	H	A	A	H	A	A	H	H	H	H	H	H	H	H	H	A	A	A	H	A	H	H	H	H	H	H	H	H
C-5AS1-0.40	ldk50		248.7	182.2	A	H	H	-	A	A	H	-	-	H	H	H	H	H	H	-	H	H	A	-	A	H	A	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg2244	6.4	255.1	188.0	A	H	H	H	A	A	H	A	A	H	H	H	H	H	H	H	H	H	A	A	A	H	A	A	H	H	H	H	H	H	H
C-5AS1-0.40	gpg2313	6.4 – 6.5	261.5	193.2	A	H	H	H	A	A	H	A	H	H	H	H	H	H	H	H	H	H	A	A	A	H	A	A	H	H	H	H	H	H	H
C-5AS1-0.40	ldk16		261.6	193.3	A	H	H	H	A	A	H	A	H	H	H	H	H	H	H	H	H	H	A	A	A	H	A	A	H	H	H	H	H	H	H
C-5AS1-0.40	ldk14		261.5	195.3	A	H	H	H	A	A	H	A	H	H	H	H	H	H	H	H	H	H	A	A	-	H	A	-	H	H	H	H	H	H	H
C-5AS1-0.40	gpg2019		261.6	200.6	A	H	H	H	A	A	H	A	H	H	H	H	H	H	H	H	H	H	A	A	A	H	A	A	H	H	H	H	H	H	H
C-5AS1-0.40	gpg2108		261.6	200.6	A	H	H	H	A	A	H	A	H	H	H	H	H	H	H	H	H	H	A	A	A	H	A	A	H	H	H	H	H	H	H
C-5AS1-0.40	gpg2231		261.6	209.5	A	H	H	-	A	A	H	-	-	H	H	H	H	H	H	-	H	H	A	-	A	H	A	A	H	H	H	H	H	H	H
C-5AS1-0.40	gpg1383	6.4	268	204.0	A	H	H	H	A	A	A	A	H	H	H	H	H	H	H	H	H	H	A	A	A	H	A	A	H	H	H	H	H	H	H
C-5AS1-0.40	ldk242		268	204.3	A	H	H	H	A	A	A	A	-	H	H	H	H	H	H	H	H	H	A	A	A	H	A	A	H	H	H	H	H	H	H
C-5AS1-0.40	gwm129		268	205.2	A	H	H	H	A	A	A	A	H	H	H	H	H	H	H	H	H	H	A	A	A	H	A	A	H	H	H	-	H	H	H
C-5AS1-0.40	jfio2	6.4	274.4	207.7	A	H	H	H	A	A	A	H	H	H	H	H	H	H	H	H	H	H	A	A	A	H	A	A	H	-	H	H	H	H	H
C-5AS1-0.40	ldk289		274.4	208.1	A	H	H	H	A	A	A	H	H	H	H	H	H	H	H	H	H	H	A	A	-	H	A	A	H	H	H	H	H	H	H
C-5AS1-0.40	gpg2232	6.8	281.2	209.5	A	H	H	H	A	A	H	H	-	H	H	H	H	H	H	H	H	H	A	A	A	H	A	A	H	-	H	H	H	H	H
C-5AS1-0.40	gpg2075	6.7	287.9	211.8	A	H	H	H	A	A	H	H	H	H	H	H	H	H	H	H	H	H	A	A	A	A	A	A	H	H	H	H	H	H	H
C-5AS1-0.40	gpg2083	6.7	294.6	212.1	A	H	H	H	A	A	H	H	H	H	H	H	H	H	H	H	H	H	A	A	A	A	A	H	H	H	H	H	H	-	H
C-5AS1-0.40	gpg2097		294.6	212.4	A	H	H	H	A	A	H	H	H	H	H	H	H	H	H	H	H	H	A	A	A	A	A	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg2121	22.5	317.1	215.1	-	H	A	A	A	A	H	H	H	H	H	H	H	H	H	H	H	H	-	A	A	A	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg119		317.1	215.3	A	H	A	A	A	A	H	H	H	H	H	H	H	H	H	H	H	H	A	A	A	A	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg35		317.1	216.3	A	H	A	A	A	A	H	H	H	H	H	H	H	H	H	H	H	H	A	A	A	A	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg2336		317.1	218.0	A	H	A	A	A	A	H	H	H	H	H	H	H	H	H	H	H	H	A	A	A	A	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg743		317.1	218.1	A	H	A	A	A	A	H	H	-	H	H	H	H	H	H	H	H	H	A	A	A	A	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg1294		317.1	218.3	A	H	A	A	A	A	H	H	-	H	H	H	H	H	H	H	H	H	-	A	A	A	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg2117		317.1	219.7	A	H	A	A	A	A	H	H	H	H	H	H	H	H	H	H	H	H	A	A	-	A	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	ldk113		317.1	221.7	A	H	A	A	A	A	H	H	H	H	H	H	H	H	H	H	H	H	A	A	A	A	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg214	14.4	331.5	226.1	A	H	A	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	A	A	A	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg2020		331.5	226.5	A	H	A	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	A	A	A	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg2147	25	356.5	231.1	A	A	A	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg2123		356.5	232.2	A	A	A	A	H	H	H	H	-	H	H	H	H	H	H	H	H	H	-	A	H	H	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg2102		356.5	232.2	A	A	A	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg1395		356.5	233.0	A	A	A	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg2158	16.8	373.3	234.4	A	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg1321	8.5	381.8	NA	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	ldk215		381.8	236.3	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	A	H	H	H	H	H	H	H	-	H	H	H
C-5AS1-0.40	gpg1777	8.9	390.7	240.5	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg158	9.4	400.1	242.0	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg2034		400.1	242.3	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg2255		400.1	242.9	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	gpg2011		400.1	244.2	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	BE425161		400.1	244.5	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	-	H	H	H	H
C-5AS1-0.40	gpg542		400.1	244.5	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
C-5AS1-0.40	cfa2250		400.1	245.9	A	H	H	H	H	H	H	H	-	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	-	H	H	H	H	H
C-5AS1-0.40	jfio6		400.1	246.7	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H

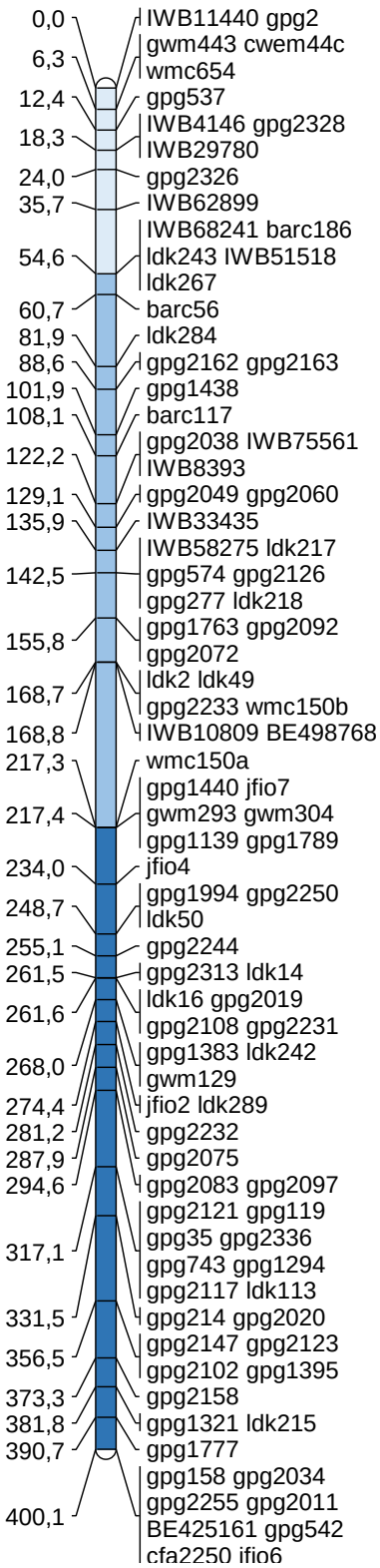

Figure 2: Map visualisation

Numbers on the left are marker positions in cR. Different colours correspond to different bins (markers gpg574, gpg2126, gpg277, ldk218 not considered).

Table 8: Summary of deletion characteristics of lines

line	Deletion length [cR]			Deletion length [Mbp]			Obligate breaks	Retained markers	Retained Mbp
	Min	Max	Mean	Min	Max	Mean			
DP199_b|G12	400.1	-	(400.1)	246.7	-	(246.7)	1	0.0%	0.0%
DP156_b	16.8	50.3	33.6	3.3	9.8	6.6	2	94.8%	97.3%
DP267_a+c|G5	39.4	78.7	59.1	17.9	22.0	20.0	2	85.4%	91.9%
DP260_a+d	68.4	97.5	83	44.5	52.6	48.5	2	71.9%	80.3%
DP55_a	6.4	19.7	13.1	4.1	8.9	6.5	2	94.8%	97.4%
DP316_d|H11	268	274.4	271.2	205.2	207.7	206.4	1	33.3%	16.3%
DP129_d|A7	255.1	261.6	258.4	188.0	193.2	190.6	1	42.7%	22.7%
DP361_c|F3+d	66.9	128.8	97.9	38.4	56.5	47.5	2	76.0%	73.1%
				15.8	21.8	18.8			
DP250_a	-	61.6	(30.8)	-	11.0	(5.5)	5	71.9%	62.2%
	6.8	20.3	13.6	4.5	14.6	9.6			
	81.9	88.6	85.3	77.1	79.1	78.1			
DP264_b	108.1	122.2	115.2	85.3	96.6	90.9	1	77.1%	63.1%
DP346_a	89.5	101.8	95.7	53.0	57.5	55.3	2	83.3%	77.6%
DP293_a	54.4	81.9	68.2	59.4	66.9	63.2	2	84.4%	74.4%
DP81_c|F5	60.7	81.9	71.3	70.7	77.1	73.9	1	82.3%	70.0%
DP69_b	54.6	60.7	57.7	48.7	70.7	59.7	1	83.3%	75.8%
DP369_c	35.7	54.6	45.2	41.5	46.0	43.7	1	88.5%	82.3%
DPS41_c	336.1	364.4	350.3	194.5	200.5	197.5	2	19.8%	20.0%
DPS335_b	299.9	330	315	159.2	169.8	164.5	2	27.1%	33.3%
DPS220_c	97.5	139.2	118.4	53.0	62.7	57.8	2	68.8%	76.6%
DPS326_b	43.6	75.3	59.5	14.8	21.6	18.2	2	85.4%	92.6%
DPS315_b	60.6	99.8	80.2	38.9	46.7	42.8	2	79.2%	82.7%
DPS214_d	32.8	45.9	39.4	23.8	29.9	26.8	2	85.4%	89.1%
DPS209_a+b	13	74.9	44	6.7	11.3	9.0	2	90.6%	96.4%
DPS180_c	19.9	39.7	29.8	23.8	41.9	32.8	2	89.6%	86.7%
DPS147_b	89.8	109.8	99.8	52.3	67.4	59.8	2	83.3%	75.8%
DPS215_a	-	30.6	(15.3)	-	7.3	(3.6)	2	99.0%	98.5%
DPS217_b	-	17.4	(8.7)	-	4.3	(2.1)	2	99.0%	99.1%

Table 9: Comparison of selected characteristics of panels DP and DPS

	Subpanel DP			Subpanel DPS		
Informative lines	15			11		
relative to seeds sown	1.0%			1.0%		
relative to plants analysed	1.0%			1.0%		
relative to all M_1 plants	3.9%			3.3%		
Deletions	17			11		
relative to seeds sown	1.1%			1.0%		
relative to plants analysed	1.1%			1.0%		
relative to all M_1 plants	4.4%			3.3%		
Interstitial deletions	9			11		
Terminal deletions	8			0		
Obligate breaks	26			22		
relative to seeds sown	1.7%			1.9%		
relative to plants analysed	1.7%			2.0%		
relative to all M_1 plants	6.8%			6.5%		
	Min	Max	Mean	Min	Max	Mean
Number of deleted markers per line	5	96	28	1	77	24
	[cR]	[Mbp]		[cR]	[Mbp]	
Minimum deletion length[1]	6.4	3.3		13	9.5	
Maximum deletion length	400.1	246.7		364.4	264.7	
Mean deletion length	105.8	70.6		105.5	55.9	
Median deletion length[2]	71.3	51.9		59.5	32.8	

[1]where at least two neighbouring loci are deleted
[2]calculated from mean deletion lengths of lines

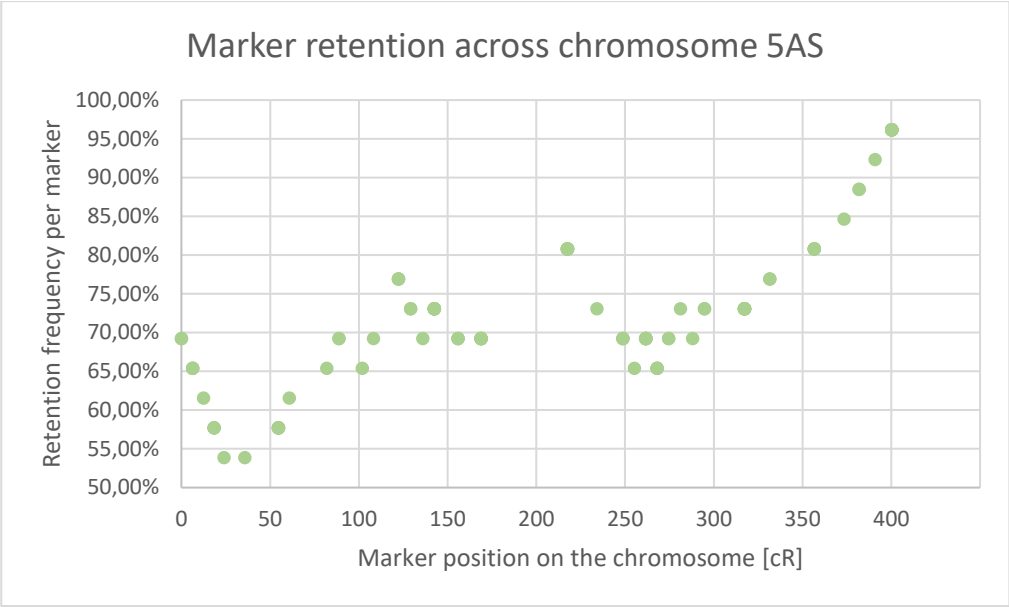

Figure 3: Retention frequency per marker (%) across the chromosome arm

Telomeric region is on the left, centromere towards the right.

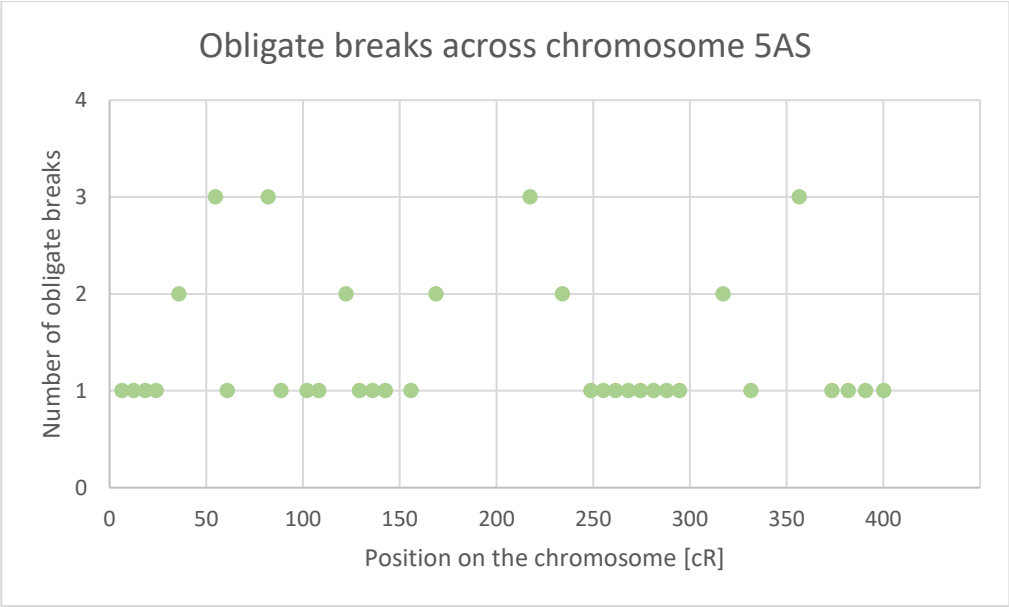

Figure 4: Distribution of obligate breaks across the chromosome arm

Telomeric region is on the left, centromere towards the right.

3.2 Correlation with reference sequence

For the correlation analysis between cR calculated in this thesis and Mbp according to reference sequence IWGSC RefSeq v1.0 (IWGSC et al., 2018), 92 shared markers were used.

A Pearson product-moment correlation coefficient (PPMCC) of 0.973 and a Spearman's rank correlation coefficient (SRCC) of 0.981 were computed. Outputs of R/RStudio including p-values are shown in Table 10. In Figure 5, cR and Mbp are plotted against each other.

Table 10: R output for correlation analysis

PPMCC (cR of this thesis and Mbp of reference sequence)	SRCC (cR of this thesis and Mbp of reference sequence)

`> cor.test(thesis_cR,Mbp,method="pearson")`
 	
`Pearson's product-moment correlation`
 	
`data:  thesis_cR and Mbp`
`t = 40.244, df = 90, p-value < 2.2e-16`
`alternative hypothesis: true correlation is not equal to 0`
`95 percent confidence interval:`
`0.9598544 0.9823121`
`sample estimates:`
`cor`
`0.9733218`
	`> cor.test(thesis_cR,Mbp,method="spearman")`
	
	`Spearman's rank correlation rho`
	
	`data:  thesis_cR and Mbp`
	`S = 2478.3, p-value < 2.2e-16`
	`alternative hypothesis: true rho is not equal to 0`
	`sample estimates:`
	`rho`
	`0.9809018`

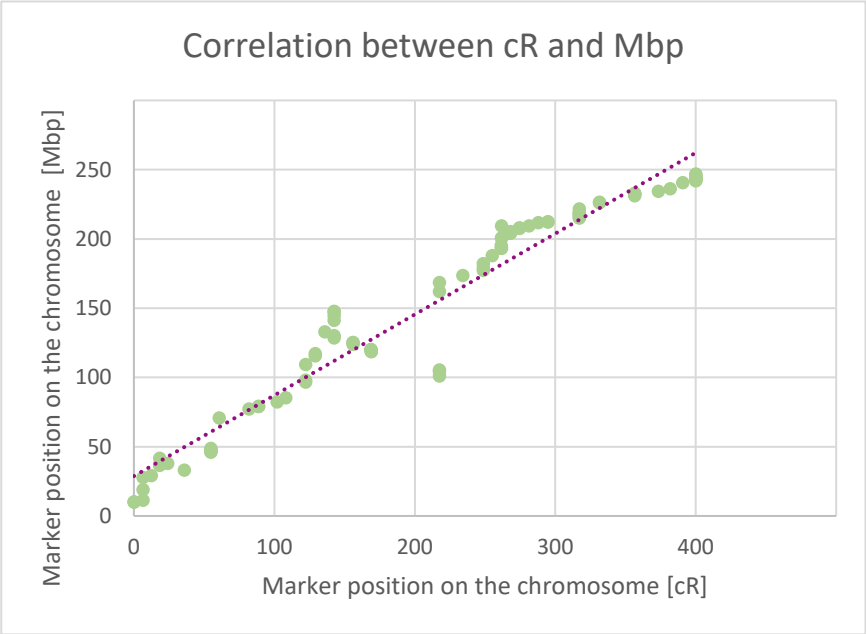

Figure 5: Correlation between cR and Mbp

Telomeric region is on the left, centromere towards the right.

Linear trendline (purple) calculated by Microsoft Excel 2016.

4. Discussion

4.1 Map resolution

Overall map resolution – a function of the number of chromosome breaks, marker distribution, retention patterns, and number of lines used (Tiwari et al., 2016) – was 0.62 Mbp/cR. Resolution was consistent across centromeric and interstitial bin (0.54 and 0.53 Mbp/cR, respectively) and only considerably lower in the telomeric bin (1.00 Mbp/cR) where only seven loci could be mapped. This is in some agreement with Wagner (2017) who, similarly to this thesis, used 102 5A-specific markers on 28 informative lines and found map resolution across bins to increase towards the centromere, with an overall map resolution of 0.72 Mbp/cR. Looking at the distribution of obligate breaks (Figure 4) and marker retention patterns (Figure 3), one can see that while breaks were relatively evenly distributed across the chromosome arm, marker retention increased with proximity to the centromere. This general trend was also observed by Wagner (2017), Schwarz (2017), and Blažek (2019) at the institute. A possible explanation to this is that (i) markers closer to the telomere are more likely to be affected by terminal deletions and (ii) centromere-proximal deletions are more likely to span across the centromere and become lethal.

A preferential loss of markers due to unevenly distributed DSBs cannot be concluded from this data. On the contrary, the radiation mapping approach proved useful for achieving a comparatively high map resolution in the centromeric region. A linkage mapping approach with 3650 F_2 plants resulted in a distance of 0.9 cM between markers barc186 and cfa2250 (M. Buerstmayr et al., 2018). Comparing this to the 345.5 cR obtained in the same interval in this thesis, a 384-fold increase of map resolution was reached with the radiation mapping approach used. Twenty-nine loci mapped to this region in the radiation map. Figure 6 visualises the radiation map and the linkage map with markers shared between both maps (maps drawn in MapChart, see 2.2.7).

Still, the 96 used markers only allowed for a separation of chromosome 5AS into 35 loci, with the majority of markers showing identical retention/deletion patterns to at least one other marker.

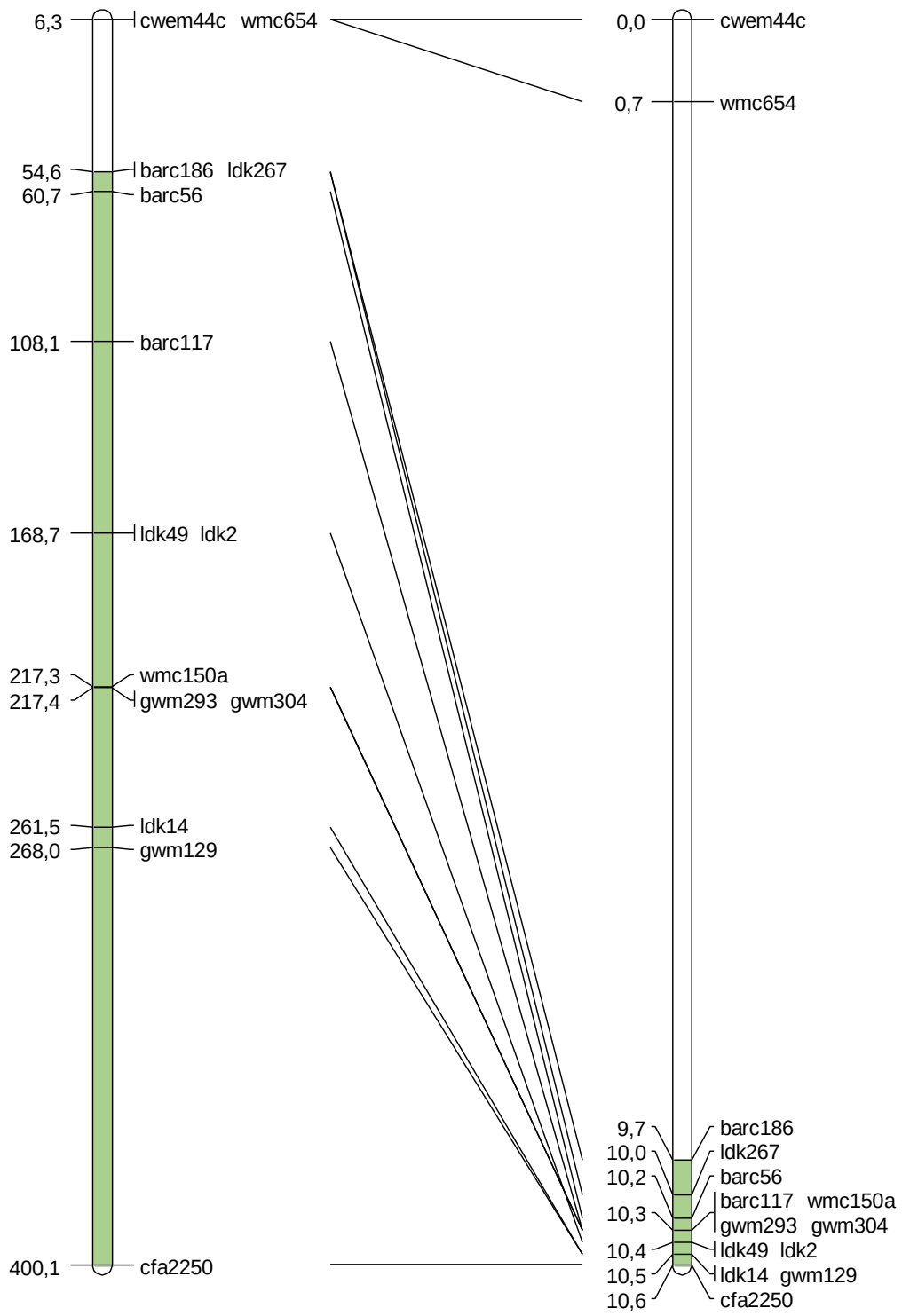

Figure 6: RS map vs. linkage map

Numbers on the radiation map (left) are marker positions in cR. Numbers on the linkage map (right) are marker positions in cM. Only shared markers are shown. Segments between markers barc186 and cfa2250 are coloured in green.

Therefore, for an even better separation of genetically linked markers, a higher number of informative lines would be needed, assuming that inducing more obligate breaks per line by increasing radiation dosage is not feasible. This is especially true for the marker clusters IWB10809/BE498768/ldk2/ldk49/gpg2233/wmc150b and wmc150a/gpg1440/jfio7/gwm293/ gwm304/gpg1139/gpg1789 that constitute two neighbouring loci separated by 48.7 cR.

According to Jones (1996), the optimal marker retention frequency is a function of the distribution of the size of fragments and density of markers to be mapped. In this thesis, an average of 73% retained markers per line was observed. This is comparable to or slightly lower than average retention frequencies in plant RH panels (Kalavacharla et al., 2006; Kumar, Simons, et al., 2012; O Riera-Lizarazu et al., 2010, 2000; Tiwari et al., 2016). Marker retention in an in vivo plant RH panel cannot be as low as in human or animal RH panels developed by cell fusion (Kumar, Simons, et al., 2012), and this is even truer for a plant RS panel that has undergone selfing steps like in the approach of this thesis: Nuclei with highly fragmented chromosomes that do not survive subsequent cell divisions will be eliminated (Kumar et al., 2014). It has to be stressed that these 73% are the average retention frequency of only the informative panel. An even much higher percentage would result if one was to calculate the average retention frequency for the whole panel, adjusted to take account of sister lines.

4.2 Marker order and correlation with reference sequence

The created map contains up to eight markers per locus and marker order within loci cannot be resolved. However, even considering this factor, the calculated marker order is not in perfect agreement with the reference sequence IWGSC RefSeq v1.0 (IWGSC et al., 2018). The correlation coefficient was 0.973 or 0.981, depending on the measure (PPMCC or SRCC). While this does prove a very high positive correlation, discrepancies can easily be seen in Table 11: Marker order of the reference sequence is compared with positions in cR calculated in this thesis that have been coloured according to their value. Lower numbers are more red/orange while higher numbers are more yellow/green. This was done by applying “conditional formatting” in Microsoft Excel 2016.

Especially noticeable are markers jfio7, gwm293, and gwm304 that cluster together in both maps but do so at a much more distal position according to the reference sequence.

According to Jones (1996), mapping power in RH studies appears to be a function of radiation dose/fragment size, retention frequency, distribution of the loci to be mapped, and the number of hybrids. It can be expected that a higher number of lines would increase the probability of finding the correct marker order just as it would increase map resolution. When the dataset of this thesis is merged with the data of 120 lines from subpanels RS-NIL3 and RH-CS from the publication of M. Buerstmayr et al. (2018), the resulting consensus map is in almost perfect correlation with the reference sequence (SRCC ρ=0.9998; analysis performed as described in 2.2.9). This can also be seen in Table 11. Figure 7 shows visualisations of both the map from data created in this thesis and the consensus map (with shared markers only), making marker rearrangements easily visible. Map calculation and map visualisation were performed as described in 2.2.7.

Table 11: Comparison of marker order in different maps

Only shared markers. Different varieties of blue correspond to different bins (see 2.2.8).

Marker order according to **this thesis** with distances calculated in this thesis [cR]		Marker order according to **consensus map** with distances calculated in this thesis [cR]		Marker order according to **reference sequence**[1] with distances calculated in this thesis [cR]		Marker order according to **reference sequence**[1] with distances from **reference sequence**[1] [Mbp]	
IWB11440	0	IWB11440	0	IWB11440	0	IWB11440	9.8
gpg2	0	gpg2	0	gpg2	0	gpg2	10.2
gwm443	6.3	gwm443	6.3	gwm443	6.3	gwm443	11.3
cwem44c	6.3	cwem44c	6.3	cwem44c	6.3	cwem44c	18.9
wmc654	6.3	wmc654	6.3	wmc654	6.3	wmc654	27.8
gpg537	12.4	gpg537	12.4	gpg537	12.4	gpg537	29.2
IWB4146	18.3	IWB62899	35.7	IWB62899	35.7	IWB62899	33.0
gpg2328	18.3	IWB4146	18.3	IWB4146	18.3	IWB4146	36.5
IWB29780	18.3	gpg2328	18.3	gpg2328	18.3	gpg2328	37.2
gpg2326	24	gpg2326	24	gpg2326	24	gpg2326	37.9
IWB62899	35.7	IWB29780	18.3	IWB29780	18.3	IWB29780	41.5
IWB68241	54.6	IWB68241	54.6	IWB68241	54.6	IWB68241	46.0
barc186	54.6	barc186	54.6	barc186	54.6	barc186	46.6
ldk243	54.6	ldk243	54.6	ldk243	54.6	ldk243	47.5
IWB51518	54.6	IWB51518	54.6	IWB51518	54.6	IWB51518	48.2
ldk267	54.6	ldk267	54.6	ldk267	54.6	ldk267	48.7
barc56	60.7	barc56	60.7	barc56	60.7	barc56	70.7
ldk284	81.9	ldk284	81.9	ldk284	81.9	ldk284	77.1
gpg2162	88.6	gpg2162	88.6	gpg2162	88.6	gpg2162	79.1
gpg2163	88.6	gpg2163	88.6	gpg2163	88.6	gpg2163	79.2
gpg1438	101.9	gpg1438	101.9	gpg1438	101.9	gpg1438	82.2
barc117	108.1	barc117	108.1	barc117	108.1	barc117	85.3
gpg2038	122.2	gpg2038	122.2	gpg2038	122.2	gpg2038	96.6
IWB75561	122.2	IWB75561	122.2	IWB75561	122.2	IWB75561	98.0
IWB8393	122.2	jfio7	217.4	jfio7	217.4	jfio7	101.0
gpg2049	129.1	gwm293	217.4	gwm293	217.4	gwm293	104.2
gpg2060	129.1	gwm304	217.4	gwm304	217.4	gwm304	105.4
IWB33435	135.9	IWB8393	122.2	IWB8393	122.2	IWB8393	109.3
IWB58275	142.5	gpg2049	129.1	gpg2049	129.1	gpg2049	115.5
ldk217	142.5	gpg2060	129.1	gpg2060	129.1	gpg2060	117.2
gpg574	142.5	IWB10809	168.8	IWB10809	168.8	IWB10809	118.7
gpg2126	142.5	BE498768	168.8	BE498768	168.8	BE498768	118.8
gpg277	142.5	ldk2	168.7	ldk2	168.7	ldk2	119.9
ldk218	142.5	ldk49	168.7	ldk49	168.7	ldk49	119.9
gpg1763	155.8	gpg2233	168.7	gpg2233	168.7	gpg2233	120.1
gpg2092	155.8	gpg1763	155.8	gpg1763	155.8	gpg1763	124.0
gpg2072	155.8	gpg2092	155.8	gpg2092	155.8	gpg2092	125.1
IWB10809	168.8	gpg2072	155.8	gpg2072	155.8	gpg2072	125.4
BE498768	168.8	IWB58275	142.5	IWB58275	142.5	IWB58275	128.4
ldk2	168.7	gpg2126	142.5	ldk217	142.5	ldk217	130.2
ldk49	168.7	ldk217	142.5	IWB33435	135.9	IWB33435	133.0
gpg2233	168.7	IWB33435	135.9	gpg574	142.5	gpg574	141.3
jfio7	217.4	gpg574	142.5	gpg2126	142.5	gpg2126	144.7
gwm293	217.4	gpg277	142.5	gpg277	142.5	gpg277	147.4
gwm304	217.4	ldk218	142.5	ldk218	142.5	ldk218	147.7
gpg1139	217.4	gpg1139	217.4	gpg1139	217.4	gpg1139	161.9
gpg1789	217.4	gpg1789	217.4	gpg1789	217.4	gpg1789	168.4
jfio4	234	jfio4	234	jfio4	234	jfio4	173.5
gpg1994	248.7	gpg1994	248.7	gpg1994	248.7	gpg1994	177.3
gpg2250	248.7	gpg2250	248.7	gpg2250	248.7	gpg2250	179.2
ldk50	248.7	ldk50	248.7	ldk50	248.7	ldk50	182.2
gpg2244	255.1	gpg2244	255.1	gpg2244	255.1	gpg2244	188.0
gpg2313	261.5	gpg2313	261.5	gpg2313	261.5	gpg2313	193.2
ldk16	261.6	ldk16	261.6	ldk16	261.6	ldk16	193.3
ldk14	261.5	ldk14	261.5	ldk14	261.5	ldk14	195.3
gpg2019	261.6	gpg2019	261.6	gpg2019	261.6	gpg2019	200.6
gpg2108	261.6	gpg2108	261.6	gpg2108	261.6	gpg2108	200.6
gpg2231	261.6	gpg1383	268	gpg1383	268	gpg1383	204.0
gpg1383	268	ldk242	268	ldk242	268	ldk242	204.3
ldk242	268	gwm129	268	gwm129	268	gwm129	205.2
gwm129	268	jfio2	274.4	jfio2	274.4	jfio2	207.7
jfio2	274.4	ldk289	274.4	ldk289	274.4	ldk289	208.1
ldk289	274.4	gpg2231	261.6	gpg2231	261.6	gpg2231	209.5
gpg2232	281.2	gpg2232	281.2	gpg2232	281.2	gpg2232	209.5
gpg2075	287.9	gpg2075	287.9	gpg2075	287.9	gpg2075	211.8
gpg2083	294.6	gpg2083	294.6	gpg2083	294.6	gpg2083	212.1
gpg2097	294.6	gpg2097	294.6	gpg2097	294.6	gpg2097	212.4
gpg2121	317.1	gpg2121	317.1	gpg2121	317.1	gpg2121	215.1
gpg119	317.1	gpg119	317.1	gpg119	317.1	gpg119	215.3
gpg35	317.1	gpg35	317.1	gpg35	317.1	gpg35	216.3
gpg2336	317.1	gpg2336	317.1	gpg2336	317.1	gpg2336	218.0
gpg743	317.1	gpg743	317.1	gpg743	317.1	gpg743	218.1
gpg1294	317.1	gpg1294	317.1	gpg1294	317.1	gpg1294	218.3
gpg2117	317.1	gpg2117	317.1	gpg2117	317.1	gpg2117	219.7
ldk113	317.1	ldk113	317.1	ldk113	317.1	ldk113	221.7
gpg214	331.5	gpg214	331.5	gpg214	331.5	gpg214	226.1
gpg2020	331.5	gpg2020	331.5	gpg2020	331.5	gpg2020	226.5
gpg2147	356.5	gpg2147	356.5	gpg2147	356.5	gpg2147	231.1
gpg2123	356.5	gpg2123	356.5	gpg2123	356.5	gpg2123	232.2
gpg2102	356.5	gpg2102	356.5	gpg2102	356.5	gpg2102	232.2
gpg1395	356.5	gpg1395	356.5	gpg1395	356.5	gpg1395	233.0
gpg2158	373.3	gpg2158	373.3	gpg2158	373.3	gpg2158	234.4
ldk215	381.8	ldk215	381.8	ldk215	381.8	ldk215	236.3
gpg1777	390.7	gpg1777	390.7	gpg1777	390.7	gpg1777	240.5
gpg158	400.1	gpg158	400.1	gpg158	400.1	gpg158	242.0
gpg2034	400.1	gpg2034	400.1	gpg2034	400.1	gpg2034	242.3
gpg2255	400.1	gpg2255	400.1	gpg2255	400.1	gpg2255	242.9
gpg2011	400.1	gpg2011	400.1	gpg2011	400.1	gpg2011	244.2
BE425161	400.1	BE425161	400.1	BE425161	400.1	BE425161	244.5
gpg542	400.1	gpg542	400.1	gpg542	400.1	gpg542	244.5
cfa2250	400.1	cfa2250	400.1	cfa2250	400.1	cfa2250	245.9
jfio6	400.1	jfio6	400.1	jfio6	400.1	jfio6	246.7

[1]**IWGSC RefSeq v1.0 (IWGSC et al., 2018)**

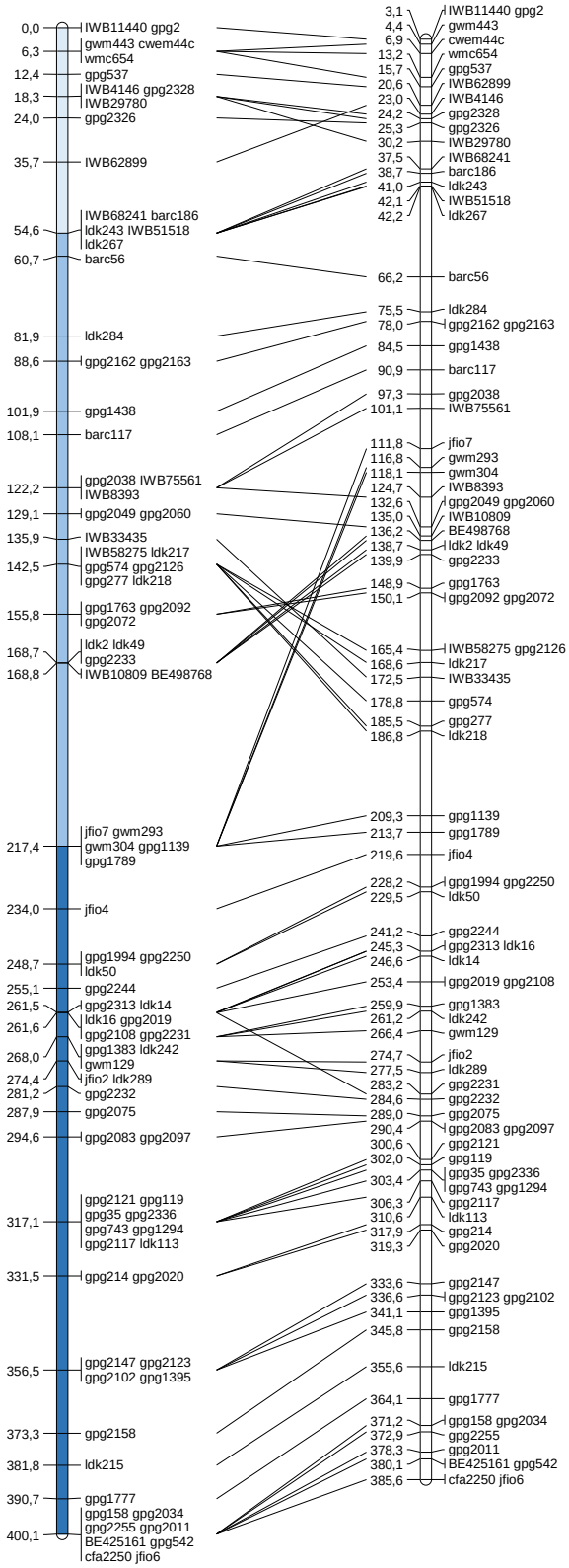

Figure 7: Comparison of RS map and consensus map visualisations

Map from data created in this thesis is on the left. Consensus map is on the right. Numbers are marker positions in cR.

4.3 Comparison of subpanels DP and DPS

1503 M_2 lines from pollen-irradiation subpanel DP and 1085 M_2 lines from head-irradiation subpanel DPS were screened. In ca. 1% of lines of each subpanel (15 and 11 lines, respectively), deletions could be detected (sister lines with the same deletion/retention pattern were treated as one informative line). The ratio of obligate breaks to M_2 plants analysed was 0.017 in subpanel DP and 0.020 in subpanel DPS. Based on M_1 plants, the proportion of informative M_2 plants was 3.9% and 3.3%, respectively. However, in subpanel DP, slightly more M_2 plants per M_1 plant were analysed than in subpanel DPS.

Tiwari et al. (2012) noted the advantage of radiation-induced chromosome damage post-meiosis when irradiating pollen, minimizing selection against chromosomal aberrations and allowing for a maximum recovery of deletions. Since all three nuclei mutate independently, quite deleterious mutants can be transmitted by one sperm, if the function of the other sperm and the tube nucleus are not affected (Coe et al., 1988). In the “radiation selfing” approach of this thesis, however, this advantage is sacrificed in favour of establishing stable or “immortal” inbred deletion lines. A possible disadvantage of irradiating pollen rather than zygotes or seeds could be a reduced competitiveness of pollen with deletions, resulting in a reduced proportion of M_1 plants with deletions in the region of interest. However, a competition disadvantage of irradiated pollen is expected to be due to zygotic lethality after fertilization rather than fertilization as such being affected by irradiation (Pfahler, 1967). Moreover, chimaeric M_1 plants from irradiated seeds cannot be screened and have to undergo gametogenesis after all. In any case, the data generated in this thesis does not suggest an advantage of pollen irradiation over irradiation of entire wheat heads or vice versa regarding the number of lines with deletions in a radiation selfing approach. It shall be noted here that irradiating heads at anthesis is not be equated with irradiating mature seeds. It further cannot be determined if chromosome damage during head irradiation took place *pre* or *post* fertilization.

Subpanel DP included one line where all markers (and presumably the whole 5AS chromosome) were missing, while subpanel DPS had two lines where only one marker was deleted. Mean deletion

length (71.8 Mbp in subpanel DP and 77.0 Mbp in subpanel DPS) and median deletion length (46.0 Mbp in subpanel DP and 46.3 in subpanel DPS), however, were similar between the two subpanels.

An interesting difference between the subpanels was the ratio between interstitial and terminal deletions. In subpanel DP, 8 of 17 deletions were terminal. Thus, more than half of the 15 informative lines had a terminal deletion. In contrast, all deletions in subpanel DPS were interstitial, with no line showing a terminal deletion. This is in some disagreement with Vizir & Mulligan (1999) and Tiwari et al. (2012), who suggested that interstitial deletions may predominate in their pollen-irradiation experiments in *Arabidopsis* and wheat, respectively. Schwarz (2017) found a higher proportion of interstitial deletions in an RS panel from seed irradiation than in an RH panel from pollen irradiation. While Schwarz' result is in general agreement to the findings of this thesis, the complete absence of terminal deletions in subpanel DPS is difficult to explain since results from subpanel DP show that such deletions are generally transmittable. One can speculate that the DSB repair mechanism in haploid cells works differently, leading to the different deletion pattern observed after pollen irradiation. Since a higher proportion of interstitial deletions is a consequence of a higher number of obligate breaks, a higher map resolution can be expected. This could point towards a possible advantage of head irradiation over (sole) pollen irradiation.

More importantly, however, neither subpanel DP nor subpanel DPS showed a higher proportion of obligate breaks or a higher proportion of informative lines than a comparable RS panel derived from irradiated mature seeds: Wagner (2017) obtained 51 breaks on 1764 lines (0.029 breaks per line) with 28 lines being informative (1.6%). The RH mapping approach by Schwarz (2017) using irradiated pollen resulted in a proportion of informative lines one order of magnitude higher than obtained in this thesis. Deletions in the hemizygous RH_1 (from pollinating line CS-N5AT5B with irradiated pollen) could immediately be screened. In RS mapping, however, deletions are lost by natural selection and genetic drift. Furthermore, hemizygous deletions remain undetected. From the results of the present thesis, it can be concluded that pollen irradiation offers no advantage over irradiation of mature seeds in a RS mapping context.

5. Conclusion and outlook

Fusarium head blight (FHB) is a widespread and serious disease in wheat with mycotoxin contamination of the crop being a major concern. Exploitation of host resistance is the single most effective control measure. Major resistance QTL *Qfhs.ifa-5A* resides in a recombination-poor pericentromeric region of chromosome 5A, making fine-mapping the QTL interval by linkage mapping unfeasible. In this thesis, radiation selfing (RS) mapping, a recombination-independent approach, was used to fine-map this region: Mutant lines with gamma-irradiation-induced deletions were genotypically characterized and a map was built.

Out of 2588 M_2 plants, 26 informative lines with unique deletions distributed over chromosome arm 5AS could be genotyped. Thus, detection and characterization of deletions in the *Qfhs.ifa-5A* interval in this thesis was successful. An advantage of RS over traditional RH mapping is the fact that fertile mutant lines with homozygous deletions can be established: The selected lines have been used for further research at the Institute of Biotechnology in Plant Production, Department of Agrobiotechnology, IFA-Tulln.

The map constructed, using the genotypic data of the informative lines, has a fairly consistent resolution across the chromosome arm and a 384 times higher resolution in a recombination-poor segment than a comparable linkage map. Thus, the RS approach chosen in this thesis proved fit to fine-map a low-recombining region not accessible by linkage mapping. More informative lines would help to further improve map resolution and marker order. By merging the data generated in this thesis with data from 120 other mutant lines that has been generated at the institute, a very highly resolved deletion map of the *Qfhs.ifa-5A* interval could be obtained. Tightly linked markers will aid the utilization of *Qfhs.ifa-5A* in wheat breeding programs.

No differences in the proportion of informative lines could be detected between the pollen-irradiation subpanel and the head-irradiation subpanel. However, the proportion of interstitial deletions was higher after head irradiation, suggesting a possible advantage of head irradiation over (sole) pollen irradiation. Neither of the subpanels showed an advantage of the respective irradiation mode over

the irradiation of mature seeds that was examined in a previous study. Since it is much easier to perform, seed irradiation thus appears to be the most advisable method for RS mapping.

Very recently, Steiner et al. (2019) were able to separate *Qfhs.ifa-5A* into a major QTL, *Qfhs.ifa-5Ac*, mapping across the centromere, and a minor effect QTL, *Qfhs.ifa-5AS*, positioned at the distal half of 5AS. The authors explained that for practical breeding, still introgression of the complete resistance locus is reasonable. In the same study, anther extrusion was shown to be a major resistance component of *Qfhs.ifa-5A*. This knowledge will help in future attempts to identify candidate genes underlying FHB resistance QTL *Qfhs.ifa-5A*.

6. References

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7. Appendix

Appendix 1: List of primers used

COS	…	conserved ortholog set (Fulton et al., 2002)
eSSR	…	EST-derived microsatellite/simple sequence repeat
EST	…	expressed sequence tag
ISBP	…	insertion site-based polymorphism
SNP	…	single nucleotide polymorphism
SSR	…	microsatellite/simple sequence repeat
RJM	…	repeat junction marker

marker also used in pre-screening

Marker	Marker type	Left/forward primer (5'-3')	Right/reverse primer (5'-3')	Reference	Bin
barc117	SSR	TCATGCGTGCTAAGTGCTAA	GAGGGCAGGAAAAAGTGACT	Song et al. (2005) (Supplementary material 3)	5AS3-0.75-0.98 (https://wheat.pw.usda.gov; Somers et al., 2004)
barc186	SSR	GGAGTGTCGAGATGATGTGGAAAC	CGCAGACGTCAGCAGCTCGAGAGG	Song et al. (2005) (Supplementary material 3)	5AS3-0.75-0.97 (Schwarz, 2017)
barc56	SSR	GCGGGAATTTACGGGAAGTCAAGAA	GCGAGTGGTTCAAATTTATGTCTGT	Song et al. (2005) (Supplementary material 3)	5AS3-0.75-0.98 (https://wheat.pw.usda.gov; Somers et al., 2004)
BE425161	SNP/COS	GGATGGTTCTGACCCAATATG (BE425161A_F1)	ATCATGCCGACAAACAGCTT (BE425161_cpR1)	Akhunov et al. (2010); http://probes.pw.usda.gov:8080/snpworld/Search	C-5AS1-0.40
BE498768	SNP/COS	CTGCCCCTAGAAGTTTCTCGT (BE498768A_F1)	CAGCGAGTGACAATTCCAGA (BE498768_cpR1)	Akhunov et al. (2010); http://probes.pw.usda.gov:8080/snpworld/Search	5AS1-0.40-0.75
cfa2250	SSR	AGCCATAGATGGCCCTACCT	CACTCAATGGCAGGTCCTTT	Sourdille et al. (2001) (as cited on https://wheat.pw.usda.gov); Somers et al. (2004) (Supplementary material)	C-5AS1-0.40
cwem44c	eSSR	AGTGCACTGCAAACACAGAG	AGCCGTACACCTTCATAGGC	Peng & Lapitan (2005) (Appendix 1)	5AS3-0.75-0.97 (Barabaschi et al., 2015, Supplemental Table 3)
gpg1139	ISBP	ACCCGTAAGTTGCCGTTATG	CACTCATGTTGAACACACCCA	Barabaschi et al. (2015) (Supplemental Table 3)	5AS1-0.40-0.75
gpg119	ISBP	CACGTCACTGTCAAGTGGCT	CACACATGTATTACGGTTTCCG	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg1293	ISBP	GCAGCAGGAAAAATCAGCAT	GGTTCGGCCTGAGATCATT	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg1294	ISBP	CCTCGAGAGTTTTGGTCGAG	GCACCAACCAGGAGTAAAGG	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg1321	ISBP	CCATCGATCTTAGACGCACA	ATTGCTCTACGTGGTGCATG	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg1383	ISBP	CCTCTAAGTCGTGCCTCGAC	AGTCCATCCGAGGTGAATTG	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg1395	ISBP	CTTCGGCCAATCAGAATTGT	GGGCGACCAAGGATTCTATT	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg1438	ISBP	GCGGTTGGATGAAGATCCTA	TCCGTATTGCCTAGCTTGCT	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg1440	ISBP	ACAGGCCTGATCTGGTATGG	TGCTTGCTACGTCTCCAATG	Barabaschi et al. (2015) (Supplemental Table 3)	5AS1-0.40-0.75
gpg158	ISBP	ACGCACACCAACTTTTACCC	GTGGTGCATGAAGGAACAGA	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg1763	ISBP	CCAACACAACATGAGCAACC	AATTTTTCCTGCATTGGTCG	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg1777	ISBP	TTCCTCAAGGAGCGTAGCAT	ACCAATCCATTGCCTACGAG	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg1789	ISBP	GGATGAGATCCACCTCCTGA	CCATCTCTTCGCCGAACTAG	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg1994	ISBP	GGTGGAGGAATGTTCACAGG	CACCGTTTGCGATTATTGTG	Barabaschi et al. (2015) (Supplemental Table 3)	5AS1-0.40-0.75 (Schwarz, 2017)
gpg2	ISBP	CGGTTGTGCCATTATTTGTG	CACCGGTCCTTCGATAAAAA	Barabaschi et al. (2015) (Supplemental Table 3)	
gpg2011	ISBP	GTCTATCCACCCATCCATGG	GAACGCCGACAGTCATCAC	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg2019	ISBP	TCCCCACTTGCAACTAAACC	AAGTGGCATCAGCTGAAGGT	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg2020	ISBP	GAGATGACCGACGGATTCAT	AACAGAACCATATGCCCTGC	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg2034	ISBP	CCTCCTGGCGAGCAGATAT	TTATCCACCATTGGTCCGTT	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg2038	ISBP	GAGTCCAAAACATGGGCAAT	TGGTGTGCTCACGTCAGATT	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg2049	ISBP	GGCCAAAGAAAGCTTATCCC	CCAGTGAACCGTCTGCTGTA	Barabaschi et al. (2015) (Supplemental Table 3)	5AS1-0.40-0.75
gpg2060	ISBP	CCGACAGGAACTTCCACTGT	CTCAATTCGGTTCTTCCCAA	Barabaschi et al. (2015) (Supplemental Table 3)	5AS1-0.40-0.75
gpg2072	ISBP	TCCGAGTGACCTGTATGCTG	AATCCATGCTTCCCTCTGTG	Barabaschi et al. (2015) (Supplemental Table 3)	5AS1-0.40-0.75
gpg2075	ISBP	ATAAGGCGCACTACCAGTGG	CCCTAGCCCATTATGCTCAA	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40

Appendix 1: continued

gpg2083	ISBP	TTAGTTCAATGGCAGGTCGA	CCATCTCTTCGCCGAACTAG	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg2092	ISBP	GGTCCGCATTGTTAACAGGT	TTGGCTTGAAGCTATGCATG	Barabaschi et al. (2015) (Supplemental Table 3)	5AS1-0.40-0.75
gpg2097	ISBP	TTGTGATTGCTGCTCACCTC	TTCCTCCAAAGGCACTGTCT	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg2102	ISBP	TCCTTTGAAGTCCTCGCACT	TGTACCTGTGAACGGAACCA	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg2108	ISBP	GCAACCGAAGAGATCCTAAGG	TTCCCAAGATGGGAGAGTTG	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg2117	ISBP	GCAAGGTGTACGTCCTTCGT	CATGCTTGAACTTGCTCCAA	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg2121	ISBP	TGCTTGTTCTTGCTCCAATG	GGCCACCTTGCTACACATCT	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg2123	ISBP	CTCTCGGAGTTGGTTTAGCG	GGAAGTTCCTTGGACATAACC	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg2126	ISBP	TGACCAAGTGATGGGAATCA	CCGAAGAAGGACGAGAGATG	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg214	ISBP	TAGCCCATCACAAGCATTCA	TCCCTTGTGGATTCAAGACC	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg2147	ISBP	TTGACATGCTTGTGGTGGTT	ACCTTAGCAATGCAGCCAGT	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg2158	ISBP	GGCTGTCATTAATCGTCCGT	CGTGCATCACAGAAGTGCTT	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg2162	ISBP	AAGATCAAATGGCCCTTCCT	GGCTATGCATGGTCCAATCT	Barabaschi et al. (2015) (Supplemental Table 3)	5AS3-0.75-0.97
gpg2163	ISBP	AGGTCGCGCACTGTTAGATT	CATGTACTCGGCGTTCACAT	Barabaschi et al. (2015) (Supplemental Table 3)	5AS3-0.75-0.97
gpg2168	ISBP	TGTCCCCTGCCTTCTGTTAC	GTCCACCGTCAGGTCATCTT	Barabaschi et al. (2015) (Supplemental Table 3)	5AS1-0.40-0.75
gpg2231	ISBP	CCTATCGGCCACACTCACTT	TTGGCTGCTCTTGACCATTA	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg2232	ISBP	CGATTAAGAGCGATAATCAACCA	TAAGAGACCGTTTTGGCCTG	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg2233	ISBP	GTCGACGTTCACATGACACC	TGGTCTTCCACCACTTGTCC	Barabaschi et al. (2015) (Supplemental Table 3)	5AS1-0.40-0.75
gpg2244	ISBP	GCCTGGATCATGCGATAACT	GGTACGAGGGACTTGCATGT	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg2250	ISBP	AGCATCAGTGTTGTTGCAATG	GTATGAAACCCGTTTGGGTG	Barabaschi et al. (2015) (Supplemental Table 3)	5AS1-0.40-0.75
gpg2255	ISBP	CGACCAGATAGGCTGGTAGC	GTTTCCATTAGGACCCCGTT	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg2309	ISBP	GACCACCTTCGGATTAGTGC	CACCGTCAATAGGTCACGAA	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg2313	ISBP	CTCACCGCCATGAGTGAGTA	TCCAACTGCCAGAATTCTCC	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg2326	ISBP	CAGCGTCAGTCCGGATTAGT	TCTAATTCTTCGGCGACGAT	Barabaschi et al. (2015) (Supplemental Table 3)	5AS3-0.75-0.97 (Schwarz, 2017)
gpg2328	ISBP	GACGACACAAGTGCCATGTT	CGTTTGTTCCACAAATCACG	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg2336	ISBP	TGAAAGAGACACGACGCAAC	TCTTCCTCTGTGGTCCAACC	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg277	ISBP	AGGAGCTGTCAGACGTCCAT	TCGTCCTCGAGATTTCTGCT	Barabaschi et al. (2015) (Supplemental Table 3)	5AS1-0.40-0.75
gpg35	ISBP	TTAACACGTCAGGTTGCGAG	GAGCCGACTGAACTGTCTCC	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg5	ISBP	TGATTGGGTAATCCTCACCAA	CCGTGTAAGGAACGCAAAAT	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg537	ISBP	ATCTCGTCGCGAGAAACCTA	CGGCTACACGTAAGGGGTAA	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg542	ISBP	CAAATACCGAGGGGTTGCTA	TGACACTGAGGACATCTGCC	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gpg574	ISBP	TGCTCCAAAACTCTCAACCA	ACACCAAACTTGCCTTCCAC	Barabaschi et al. (2015) (Supplemental Table 3)	5AS1-0.40-0.75
gpg743	ISBP	CTATGTACGCACACAATGCG	GAACGTAAGAAGGCAGGCAC	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
gwm129	SSR	TCAGTGGGCAAGCTACACAG	AAAACTTAGTAGCCGCGT	Röder et al. (1998) (Appendix)	C-5AS1-0.40 (https://wheat.pw.usda.gov; Somers et al., 2004)
gwm293	SSR	TACTGGTTCACATTGGTGCG	TCGCCATCACTCGTTCAAG	Röder et al. (1998) (Appendix)	5AS1-0.40-0.75 (https://wheat.pw.usda.gov; Somers et al., 2004)
gwm304	SSR	AGGAAACAGAAATATCGCGG	AGGACTGTGGGGAATGAATG	Röder et al. (1998) (Appendix)	5AS1-0.40-0.75 (Schwarz, 2017)
gwm415	SSR	GATCTCCCATGTCCGCC	CGACAGTCGTCACTTGCCTA	Röder et al. (1998) (Appendix)	C-5AS1-0.40 (https://wheat.pw.usda.gov; Somers et al., 2004)
gwm443	SSR	GGGTCTTCATCCGGAACTCT	CCATGATTTATAAATTCCACC	Röder et al. (1998) (Appendix); Paillard et al. (2003)	5AS3-0.75-0.97 (Barabaschi et al., 2015, Supplemental Table 3)
IWB10809	SNP			Wang et al. (2014) (Supplementary Table S6); Ramirez-Gonzalez et al. (2015)	
IWB11440	SNP			Wang et al. (2014) (Supplementary Table S6); Ramirez-Gonzalez et al. (2015)	5AS3-0.75-0.97 (Schwarz, 2017)
IWB29780	SNP			Wang et al. (2014) (Supplementary Table S6); Ramirez-Gonzalez et al. (2015)	
IWB33435	SNP			Wang et al. (2014) (Supplementary Table S6); Ramirez-Gonzalez et al. (2015)	
IWB4146	SNP			Wang et al. (2014) (Supplementary Table S6); Ramirez-Gonzalez et al. (2015)	

Appendix 1: continued

IWB51518	SNP			Wang et al. (2014) (Supplementary Table S6); Ramirez-Gonzalez et al. (2015)	
IWB58275	SNP			Wang et al. (2014) (Supplementary Table S6); Ramirez-Gonzalez et al. (2015)	5AS1-0.40-0.75 (Schwarz, 2017)
IWB62899	SNP			Wang et al. (2014) (Supplementary Table S6); Ramirez-Gonzalez et al. (2015)	5AS3-0.75-0.97 (Schwarz, 2017)
IWB68241	SNP			Wang et al. (2014) (Supplementary Table S6); Ramirez-Gonzalez et al. (2015)	
IWB75561	SNP			Ramirez-Gonzalez et al. (2015)	5AS1-0.40-0.75 (Schwarz, 2017)
IWB8393	SNP			Wang et al. (2014) (Supplementary Table S6); Ramirez-Gonzalez et al. (2015)	5AS1-0.40-0.75 (Schwarz, 2017)
jfio2	RJM	ACGCTGGAGACGTATCACTGT	GGTGTCCTTCCTGATCTCCA	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
jfio4	RJM	CGCAAGGTGATATGAGGTGTT	TACGTACATACGGGCGGGT	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
jfio6	RJM	CAGTCCCTTATTCAGCACCG	TGCGTCGGTAACATCATCAT	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
jfio7	RJM	CTCCTGTGGCAGAACAGAGG	ATCGTGGGCGTCACACTATA	Barabaschi et al. (2015) (Supplemental Table 3)	5AS1-0.40-0.75
ldk113	SSR	CACTGCTCCACCACAGC	GCGAAGGGTTAAACCGTAAAC	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
ldk14	SSR	TTTCTGTTTTGCCTCTGGAAA	GGGCCTTTCCCTTTTGTTTT	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40 (Schwarz, 2017)
ldk16	SSR	CTCTTGGGCTGATGGTGATG	ATCGAATCAGTGGGTGATCG	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
ldk2	SSR	ATCAGGTCCACACACCACAC	AATCCACGAAGACGCTATCC	Barabaschi et al. (2015) (Supplemental Table 3)	5AS1-0.40-0.75
ldk215	SSR	CTGAGCTGAAGCAAGACAcg	CGGGCATCTTCTCTACATCG	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
ldk217	SSR	TGGACTCCGAATAGGACTGG	ACCAACTTCATCGCTGtTGC	Barabaschi et al. (2015) (Supplemental Table 3)	5AS1-0.40-0.75
ldk218	SSR	GTTGAAGATGTCGCTCATGG	CTTcACAAGGtCCGcTTcC	Barabaschi et al. (2015) (Supplemental Table 3)	
ldk241	SSR	AATCAGTCTTGATGAAGCAACG	CATGAAGCGTCAGCAGTAGG	Barabaschi et al. (2015) (Supplemental Table 3)	
ldk242	SSR	CCTACAAACCTCTGCACTTGG	CGGAGGGAATATTGAACACG	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
ldk243	SSR	GGTTTCACCTCTAGCCTACCC	CACCTTGTGTGGGAGTTTCC	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
ldk267	SSR	AATTAGCAGACCGCATGTACG	TCCAAGTTGAGAGCTGATGG	Barabaschi et al. (2015) (Supplemental Table 3)	5AS3-0.75-0.97 (Schwarz, 2017)
ldk284	SSR	TCTCATTGGTCAGGGTCAGG	TTCTCCTCCAGGTAGCTCTCC	Barabaschi et al. (2015) (Supplemental Table 3)	5AS3-0.75-0.97
ldk289	SSR	GCACATACCTTCATAGTGG	TGATGATGTGGCAAAGAAGC	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
ldk49	SSR	TCCACACACCACACACACAC	AGACGCTATCCGATCCTCTG	Barabaschi et al. (2015) (Supplemental Table 3)	5AS1-0.40-0.75
ldk50	SSR	ACCGTGTGTGATGCTTCTTG	GGTGCATGTGTGTGTGCTC	Barabaschi et al. (2015) (Supplemental Table 3)	C-5AS1-0.40
wmc150	SSR	CATTGATTGAACAGTTGAAGAA	CTCAAAGCAACAGAAAAGTAAA	Somers et al. (2003) Somers et al. (2004) (Supplementary data)	5AS1-0.40-0.75 (Schwarz, 2017)
wmc654	SSR	CTGTGATGAACTGAAATAACCA	TATTCTACTTTTCTCTTCCCCC	Somers et al. (2004) (Supplementary data) https://wheat.pw.usda.gov	5AS3-0.75-0.97 (Barabaschi et al., 2015, Supplemental Table 3)