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High-resolution mapping of the *Qfhs.ifa-5A* QTL support interval on the short arm of chromosome 5A in wheat, using radiation hybrid mapping

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Eidesstattliche Erklärung

Hiermit erkläre ich ehrenwörtlich, diese Arbeit selbständig angefertigt zu haben, ohne andere als die angegebenen Hilfsmittel benutzt zu haben. Übernommene Formulierungen und Gedanken, direkt oder indirekt, aus fremden Quellen sind als solche kenntlich gemacht worden. Diese Arbeit wurde in dieser oder ähnlicher Form noch an keiner anderen Stelle vorgelegt.

Abstract

Fusarium head blight (FHB) caused by different *Fusarium* species, leads to severe yield losses in wheat and small grain cereals. Additionally, the production of mycotoxins and its accumulation in the seeds presents a serious health risk for food safety. Breeding for resistant wheat cultivars is one of the most effective ways to control FHB outbreaks. Previous work reported the quantitative trait locus (QTL) *Qfhs.ifa-5A*. It mapped to the centromeric region on chromosome 5A. *Qfhs.ifa-5A* primarily contributes FHB Type 1 resistance, which confers resistance to fungal entry into the plant. Infection usually occurs during anthesis, when moist and warm weather supports fungal infection. Genetic linkage, for fine-mapping *Qfhs.ifa-5A,* showed to be insufficient, as it strongly depends on recombination frequencies, which are not uniform along the chromosome and strongly repressed around the centromere. Radiation hybrid mapping (RH) is a recombination independent approach, utilizing deletions, produced by gamma irradiation. Seeds of a near isogenic line NIL3, that carries the *Qfhs.ifa-5A* resistance alleles, were irradiated and underwent one selfing steps to produce homozygous deletions. Evenly and randomly distributed deletions provided a uniform mapping resolution and produced a map with a 612-fold higher resolution size compared to the genetic map. Screening of 91 preselected lines with 104 markers resulted in 51 informative lines with unique deletion patterns. The resulting map has a distance of 639.4 cR and a resolution of 0.3988 Mbp/cR. The approach of RH showed to be a suitable solution for fine mapping the *Qfhs.ifa-5A* region.

Key words: Wheat, Fusarium head blight, FHB, *Fusarium graminearum*, Quantitative trait loci, QTL, *Qfhs.ifa-5A*, Radiation hybrid mapping, fine mapping

Zusammenfassung

Ährenfusariose (FHB), welche von verschiedenen *Fusarium*-Arten verursacht wird, führt zu erheblichen Ertragseinbußen bei Weizen und anderen Getreidearten. Darüber hinaus stellt die Produktion von Mykotoxinen und deren Ansammlung in den Samen ein ernstes Risiko für die Lebensmittelsicherheit dar. Die Züchtung von resistenten Weizensorten ist einer die effektivsten Wege zur Bekämpfung von FHB-Ausbrüchen. In früheren Arbeiten wurde der quantitative Trail-Locus (QTL) *Qfhs.ifa-5A* beschrieben, welcher sich über den Zentromer erstreckt. Dieser QTL ist verantwortlich für die Resistenz gegen den Eintritt von FHB in die Pflanze. Der Befall geschieht normalerweise während der Blüte, wenn feuchtes und warmes Wetter eine Pilzinfektion unterstützt. Eine genetische Kartierung erwies sich als unzureichend, da diese stark von Rekombinationsevents abhängt ist. Rekombination ist jedoch nicht einheitlich entlang des Chromosoms und vor allem stark unterdrückt in Bereich des Zentromers. Radiation Hybrid Mapping (RH) ist eine rekombinationsunabhängige Kartierungsmethode. RH verwendet ionisierende Gammastrahlung um Doppelstrangbrüche (DBS) in der DNS zu verursachen, die zu verschieden langen Deletionen führen können. Diese Deletionen sind zufällig und gleichmäßig verteilt und werden zur Kartierung verwendet. Samen der near isogeneic line NIL3 (enthält *Qfhs.ifa-5A)* wurden bestrahlt und durchliefen eine Selbstungen, um homozygote Deletionen zu erhalten. Das Screening von 91 Linien mit 104 Markern führte zu 51 informativen Linien. Die resultierende Karte hat eine Länge von 639.4 cR und eine Auflösung von 0.3988 Mbp/cR. Die gleichmäßig und zufällig verteilten Deletionen konnten eine 612-mal höhere Karten-Auflösung, im Vergleich zur genetischen Karte produzieren. Der Ansatz von RH erwies sich als eine geeignete Methode für die Feinkartierung der Region *Qfhs.ifa-5A*.

Schlüsselwörter: Weizen, Ährenfusariose, FHB, *Fusarium graminearum*, Quantitative Trait Loci, QTL, *Qfhs.ifa-5A*, Radiation Hybrid Mapping, Feinkartierung

List of abbreviations

ATM - Ataxia Telangiectasia Mutated signalling pathway
BC - Backcross
BEA - Beauvericin
cM - Centi Morgan
COS - Conserved Ortholog Set
cR - Centi Ray
CS - Chinese Spring
CS DT5AL - Chinese Spring ditelosomic-5AL aneuploid line
CS N5AT5B - Chinese Spring nullisomic-5A-tetrasomic-5B aneuploid line
CTAB - Cetyl trimethylammonium bromide
Cy5 - Cyanin 5
DH - Double haploid
DNA - Deoxyribonucleic acid
DON - Deoxynivalenol
DSB - Double strand breaks
ENNs - Enniatins
FAM - 6-carboxy fluorescein
FEB - Fusarium ear blight
FHB - Fusarium head blight
Gy - Gray
HR - Homologous recombination
IFA BP - Department of Agrobiotechnology Tulln, Institute for Biotechnology in Plant Production
ISBP - Insertion site-based polymorphism
LOD - Logarithm of odds
Mbp - Megabase pair
MON - Moniliformin
NHEJ - Non-homologous end-joining
NIL - Near isogenic lines
NI-RIL - Near isogenic recombinant inbred lines
NIV - Nivalenol
PCR - Polymerase chain reaction
QTL - Quantitative trait locus/loci
rcf - Relative centrifugal force
RH - Radiation hybrid
RJM - Repeat Junction Marker
RNA - Ribonucleic acid
SNP - Single nucleotide polymorphism
SSR - Simple sequence repeats
Taq - Thermophilus aquaticus polymerase
TE buffer - Tris-EDTA buffer
TE - Transposable elements
WGRH - Whole genome radiation hybrid
ZEA - Zearalenone

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1. Introduction

1.1 Fusarium head blight

Fusarium head blight (FHB) also known as Fusarium ear blight (FEB) or scab, is a devastating fungal disease, caused by different *Fusarium* species. Stepien and Chelkowski (2010) identified 18 different *Fusarium* species in Europe, infecting wheat heads and kernels. *Fusarium graminearum* (telemorph *Gibberella zeae*) is the predominant species in warm and temperate regions (McMullen et al., 1997; Osborne and Stein, 2007).

1.1.1 Conditions for infection and disease cycle

Like other fungal diseases, a wet weather favours the infection. The Fusarium spores are either transported by wind or splashing water onto the developing wheat heads. During flowering (anthesis) and until the soft dough stage of the kernel, the plants are most susceptible to FHB infection. Most infections happen during anthesis, especially under humid and warm weather conditions (Buerstmayr et al., 2009; McMullen et al., 1997; Osborne and Stein, 2007).

An infection either happens through asexual spores, the conidia, harboured by sporodochia or sexual spores, the ascospores, which are formed in the perithecia, the fruit bearing bodies of this fungus. Both forms may be found in previously infected plant residues left on the field or in contaminated kernels, used as seeds. The spores are then transported by wind, rain or are water dispersed to the spikes and if the weather conditions are favourable, leading to an infection. After this primary infection, the pathogen forms conidia on the infected spikelets. The conidia, also called mitospores, may be blown to other plants on the same field or on neighbouring fields, which may lead to a secondary infection. The fungus overwinters on the field and infects the kernels, which starts the infection circle again (McMullen et al., 1997, 2012; Mills et al., 2016). An infection can lead to severe yield losses in wheat and other small grain cereals and a reduction in the kernel quality (McMullen et al., 1997; Buerstmayr et al., 2009).

1.1.2 Symptoms

If it stays humid after flowering, the first symptoms may appear before 18 to 21 days. The infected spikelets turn from green into a prematurely tan colour, starting with single infected spikelets and gradually affecting the whole head. The kernels show discoloration from pink (spores) to black (perithecia) and might shrivel and turn into so called "tombstones". The reduced weight of the shrivelled kernels causes a reduction in yield. If the main axis of the head is infected, the development of the head may get disturbed and may lead to no kernel formation at all (McMullen et al., 1997; Mills et al., 2016).

1.1.3 Mycotoxins

Apart from yield losses the production of mycotoxins and the accumulation in the kernels, presents a serious risk for human and animal health. The pathogens are able to produce a multitude of different mycotoxins. Bryla et al. (2016) found in their study 26 different mycotoxins in grain samples.

One of the most important mycotoxins is deoxynivalenol (DON), produced by *F. graminearum* and *F. culmorum.* DON, also called vomitoxin, causes vomiting and feed refusal by animals (McMullen et al., 1997; Buerstmayr et al., 2009). Other important mycotoxins like nivalenol (NIV), zearalenone (ZEA), moniliformin (MON), beauvericin (BEA) and enniatins (ENNs), also have toxic properties to humans and animals (Lindblad et al., 2013; Osborne and Stein, 2007; Stepien and Chelkowsk, 2010). The European Commission (Commission Regulation 2006/1881) set a maximum level of mycotoxins in cereal to prevent serious health issues. For human consumption in unprocessed wheat (Lindblad et al., 2013):

- DON is 1250 µg/kg (durum wheat 1750 µg/kg)
- ZEA is 100 µg/kg.

For animal feed:

- DON is 8000 µg/kg
- ZEA is 2000 µg/kg.

Like previously mentioned, DON may cause vomiting, abdominal pain, nausea and reduces the weight gain in animals. ZEA lead to fertility problems and hormonal problems, because of its oestrogenic activity (Lindblad et al., 2013).

1.1.4 Disease management and control

Environment and weather conditions are major factors, contributing to an FHB infection. The high demand of wheat and other cereals leads to cultivating wheat in many parts of the world, even if it is known that the environmental conditions of some areas may favour fungal infection. An important factor to minimize or even avoid FHB infections are agronomic practices on the farm (McMullen et al., 1997, 2012; Mills et al., 2016).

Following agronomic practices, after McMullen et al. (1997, 2012) and Mills et al. (2016) can contribute to minimize scab infection and its severity:

- Conventional tillage, with infection free machines. Deep ploughing and residue coverage on the field.
- Abstaining from using manure, containing infected straw.
- Strict crop rotation, especially after infection. Unsusceptible crops between susceptible species, like wheat and maize.
- Applying fungicides near/during anthesis.
- Usage of resistant cultivars.

Studies showed that the resistance to FHB is not ruled by a single gene, infection and symptoms are rather quantitative, therefore the FHB resistance is governed by quantitative trait loci (QTL) (Buerstmayr et al., 2009).

The practice of one single agronomic strategy often failed, especially in environments highly favourable for infection. The combination of resistant cultivars and the usage of different agronomic strategies is the best approach to reduce the FHB infection (McMullen et al., 2012).

1.1.5 Financial impact of FHB

Cereals infected with FHB show vast yield and quality loss. Reduced yield and damaged kernels and the contamination with fungal toxins seriously reduces the income of the producers, in addition to the severe health risks caused by infected kernels. Between the 1990s and 2000s primary (direct losses like yield reduction) and secondary (indirect losses through tax revenue or unemployment and others) economic losses, in the United States were estimated about $7.67 billion (McMullen et al., 2012).

1.2 Wheat

Bread wheat *Triticum aestivum* L. is one of the most important crop plants. The worldwide production in the year 2017 was 771.7 million tons at 218.5 million hectares. The yield increased since the 60s 3.5-fold although the harvested area only increased by 7% (FAOstat, 2019). The development of high yield varieties and changes in agronomic practises led to this drastic yield increase (Berkman et al., 2012).

The domestication of *Triticum spp*. started 10 000 years ago in the so-called fertile crescent, specially pointing to today's south-eastern Turkey and northern Syria. The further selection and distribution of wheat outside of its origin started the Neolithic revolution, leading to the uprising of bread wheat, approximately 8000 years ago. The hybridization between tetraploid emmer wheat (AABB, *Triticum dicoccoides*) and the wild diploid goat grass (DD, *Aegilops tauschii*), led to the now hexaploid bread wheat (2n=6x=42; AABBDD, *Triticum aestivum*). The subgenomes (A, B and D) are highly similar and consists of 7 chromosomes each. (IWGSC, 2018; Nesbitt and Samuel, 1996; Peleg et al., 2011).

A recent wheat genome assembly (IWGSC, 2018), presented a 13.8 to 14.5 Gbps long assembly, in which up to 107891 high-confidence gene models were found. Based on this assembly, the wheat genome is believed to consists of up to 85.7% transposable (repeated) elements (TE), which are one mayor problem for genome assembly and estimating the exact genome size.

1.2.1 Types of resistance to Fusarium head blight

One of the earliest mentions of FHB was in 1891, even back then the severity of an infection was feared (Arthur, 1891). Since the 80s of last century, scientists have been working on identifying the resistance mechanism behind *Fusarium* infections (Mesterházy, 1995). It is known that the resistance to FHB is not only ruled by one gene, but by several quantitative trait loci (Kosová et al., 2009). Kosová et al. (2009) listed seven types of resistance found in wheat, based on Mesterházy (1995, 2002) and Schroeder and Christensen (1963):

- Type I – Resistance to infection
- Type II – Resistance to spreading
- Type III – Resistance to infection of the seeds
- Type IV – Tolerance to infection
- Type V – Resistance to mycotoxin accumulation
- Type VI – Resistance to secondary infection (late blithing)

- Type VII – Resistance to head death above infection site

Type I resistance is mainly associated with genes determining morphological and physiological traits, like plant height, inflorescence structure and anther extrusion. These types of resistances can be categorised as unspecific, and are also called passive resistance (Mesterházy, 1995). Type V resistance can be further split up into degradation or detoxification of mycotoxins and prevention of mycotoxin production (Kosová et al., 2009).

1.2.2 QTL *Qfhs.ifa-5A*

Two major FHB resistance QTLs, *Qfhs.ndsu-3BS* (syn. *Fhb1*) by Anderson et al. (2001) and *Qfhs.ifa-5A* by Buerstmayr et al. (2002, 2003), were identified.

Former QTL research (Buerstmayr et al., 2018) at IFA BP Tulln used near isogenic recombinant inbred lines (NI-RIL) to further locate *Qfhs.ifa-5A,* associated with Type 1 resistance.

Buerstmayr et al. (2002, 2003) mapped *Qfhs.ifa-5A* on chromosome 5A, to a genetically small (1.6 cM), but physically large interval (316 Mbp), that stretched across the centromere (Buerstmayr et al. 2018). The SSR marker barc186 (5AS; bin 5AS3-0.75-0.97) and wmc805 (5AL, bin 5AL5-0.46-0.55) flanking this putative QTL (Buerstmayr et al., 2003, 2018).

In addition to *Qfhs.ifa-5A*, Lin et al. (2006) and Xue et al. (2011) detected *Qfhi.nau-5A* (*Fhb5*), also associated with Type 1 resistance. Fine-mapping of *Fhb5* located the QTL on the short arm of 5A, near to the pericentromeric region, which covers 75% of the short arm (Xue et al., 2011). Based on Xue et al. (2011), the QTL is believed to be on the short arm of 5A, therefore marker on 5AS, close to the centromere were searched and used for fine-mapping. Cfa2250 was found to be closest linked to FHB Type 1 resistance and was at the same time the most proximal marker on 5AS (Buerstmayr et al., 2018).

1.3 Radiation hybrid mapping

Due to the fact, that the *Qfhs.ifa-5A* is near the centromeric region, genetic mapping showed to be insufficient. Classic genetic mapping strongly depends on recombination frequencies, which are not uniform along the chromosome and strongly repressed around the centromere, which makes mapping this region extremely difficult (Erayman et al., 2004; Kumar et al., 2014). Erayman et al. (2004) stated that 30% of wheat genes are in recombination poor zones, which makes them inaccessible to genetic mapping. Due to this information, other alternative mapping approaches are needed.

1.3.1 Generating radiation hybrid panel

The first radiation hybrid (RH) mapping approach by Goss and Harris (1975) were done in vitro with the human X chromosome. Cells, taken from a tissue culture were treated with a lethal dose of radiation, which caused random distributed breaks of the DNA. The generated fragments were fused with a suitable receiver cell line of a different species. The "donor" fragments are getting incorporated into the recipient genome, so called "hybrids", which are revered as RH lines. For a mapping panel, about 100 RH lines are needed, to characterize the entire donor genome. Every line gets examined for the presence of markers specific to the donor species. It is assumed that the breaks, caused by the radiation, are random. Markers further apart, have a higher chance to be separated onto different genome fragments. To make assumptions about the distance between marker and their order to each other, the frequencies of co-retentions were calculated, similar to meiotic mapping. Centi Rays (cR), the map distance unit, resembles one break between two markers in every 100 lines, given a specific radiation dose. RH panels, in animals, can only be generated with in vitro cell cultures (Goss and Harris, 1975; Kumar et al., 2014).

In plants, either in vitro or in vivo strategies can be employed (Kumar et al., 2014). In vitro approaches like, protoplast fusion, showed to have a higher deletion frequency and can be used for developing a physical map or study marker scaffolds. One of its main disadvantages is the incapability to generate fully developed plants (Wardrop et al., 2002, 2004; Zhou et al., 2006).

In vivo panels are obtained by crosses between irradiated donors and a different "rescuer" species (Riera-Lizarazu et al., 2000). These panels can either be a single chromosome panel or a whole genome panel (WGRH panels) (Kumar et al., 2014). Another in vivo strategy can be the development of RH populations either through pollen or seed irradiation (Gao et al., 2004, 2006; Kumar et al., 2012b). This method can be used, besides other possibilities, for WGRH studies (by using irradiated pollen for

wide crosses) and the production of cytogenetic stocks (Gao et al., 2004, 2006). In vivo RH panels are stable, viable and fertile, due to the reason that cells with a highly fragmented chromosome do not survive cell division (Kumar et al., 2014).

1.3.2 The principles of radiation hybrid mapping

Radiation hybrid mapping is a recombination independent mapping approach. The high radiation causes randomly distributed double strand breaks (DSB) in the genome leading to deletions (Kumar et al., 2012a). These obtained radiation induced deletions, produced by gamma irradiation, can be used for map construction in a similar way as recombination in genetic linkage maps.

After breakage, two different repair mechanisms are responsible to repair the DSBs damage. These mechanisms play a crucial role for the survival of the genome and hence for all organisms. The first repair pathway is via homologous recombination (HR), the second via non-homologous end-joining (NHEJ), also called illegitimate recombination (Puchta, 2005; Bernstein and Rothstein, 2009). Although both mechanisms seem to be highly conserved in eukaryotes, NHEJ showed to be the major repairing pathway in higher eukaryotes (Bleuyard et al., 2006; Puchta, 2005).

The Ataxia Telangiectasia Mutated signalling pathway (ATM) is sensing the broken DNA ends. The *Ku* protein complex then prevents further degradation of the break. It is anchored at the DSB site and acts as starting point for the DNA phosphokinases. Via protein bridges, the split ends are pulled together and then re-joined by DNA ligase. During this re-joining event, parts within the adjacent DSBs location are lost. These deletions may be small (only a few nucleotides) or larger in size. For mapping, the absence or presence of a deletion can be used as a binary polymorphism (Bleuyard et al., 2006; Bernstein and Rothstein, 2009; Kumar et al., 2012a).

The RH lines can be scanned for the presence or absence of known marker loci and then used to calculate the hybrid-map. Radiation-induced breaks and co-retention frequency of deleted/retained markers can be used for mapping and provide a uniform mapping resolution and produce a higher resolution size compared to genetic maps (Kumar et al., 2014). Therefore, RH was chosen as a suitable approach for fine mapping the *Qfhs.ifa-5A* region (Buerstmayr et al., 2018).

1.3.3 Advantages of radiation hybrid mapping

Kumar et al. (2012a, 2014) listed the following points as the main advantages of radiation hybrid mapping compared to genetic linkage mapping:

- The ability of gaining a higher map resolution in comparison to the classical genetic map. The map resolution depends on DSB and can be influenced with the applied radiation dose.
- Allelic polymorphism is not a requirement. Only the presence or absence of a marker loci will be evaluated. This enables the usage of all kinds of marker.
- Uniform map resolution across the chromosome. RH mapping is independent of crossing-over events. Therefore, crossing-over poor regions have no influence on the map resolution.
- Rather fast development of viable panels. Plant of irradiated seeds can be crossed with nullitetra plants and can be immediately scanned for deletions. Viable panels can be used for phenotyping and for associating phenotype to genotype.

1.4 Research questions and aims of the master thesis

Previous studies at IFA (Wagner, 2017; Schwarz, 2018) showed that RH mapping is a suitable way to get a higher map resolution, compared to genetic mapping.

Aim of this work was to produce a high-resolution map of the short arm of wheat chromosome 5A.

In this study, 5AS specific genetic markers were used to fine-map the putative QTL *Qfhs.ifa-5A* interval and further increase the map resolution. The resulting RS-NIL3 panel were compared to a formerly produced RS-NIL3 panel (Schwarz, 2018).

In addition, the linear relationship between cR of the RS-NIL3 panel of this study and cM of the NI-RIL panel (Buerstmayr et al., 2018) were investigated.

The resulting map and the RS-NIL3 panel will be subject for additional investigation in the future. To delete unwanted background deletions, the lines with deletions at the *Qfhs.ifa-5A* interval will be backcrossed with NIL3. The F_2 will be screened, in order to select for plants with deletions in homozygous state, and these plants will be propagated and used for further field trials.

2. Material and Methods

2.1 Plant material

CM-82036, short for CM-82036-1TP-10Y-OST-10Y-OM-OFC is a highly resistant line to FHB, resulting of a cross between Sumai-3 (FHB resistance) and Thornbird-s (good agronomic adaption), from a breeding program between CIMMYT Mexico and South-America (Buerstmayr et al., 1996). Sumai-3 is the donor of *Qfhs.ifa-5A*.

Remus is a spring wheat cultivar, from the Bavarian State Institute for Agronomy in Freising, Germany. The cultivar was created by crossing of the cultivars 'Sappo', 'Mex' and 'Famos', which resulted in a high FHB susceptibility.

Both cultivars were crossed and a DH population was created (Buerstmayr et al., 2002). To create the NIL, the chosen DH line (with present *Fhb1* and *Qfhs.ifa-5A*) was backcrossed five times with Remus (BC_5). After each backcross, the presence of *Fhb1* and the *Qfhs.ifa-5A* were checked. The BC_5 were selfed (BC_5F_2), to generate plants with a homozygous QTL (Schweiger et al., 2013).

The goal was to not only create a RH panel, but also produce viable plants with deletions across the *Qfhs.ifa-5A* interval for testing in the field. NIL3, a line with Remus background and fixed *Qfhs.ifa-5A* was chosen.

The NIL3 seeds were gamma-irradiated with a Cobalt-60 source, at the International Atomic Energy Agency (IAEA) Division of Nuclear Techniques in Food and Agriculture in Seibersdorf, Austria. Approximately 1000 seeds per dosage, of 300, 330 and 350 Gy were irradiated.

The plants (M_1) of the irradiated seeds were than selfed, in order create M_2 generation and for possible deletions to get homozygous. This selfing step is rather crucial for this study, because only deletion in a homozygous state can be detected with molecule markers. Deletions in heterozygous state would be covered by the presence of the marker locus of the other copy of that chromosome. Out of the kernels irradiated with a dosage of 300, 330 and 350 Gy, 355, 324 and 325 plants were viable and produced M2 seeds, respectively. Supposedly, due to the deletion damage, many of the M1 seeds seemed to be sterile and did not produce a M2 generation.

Per fertile M_1 plant, in average four M_2 seeds were used for screening, considering Gregor Mendel's segregation law, at least one out of four off-springs should have the deletion in homozygous state.

In total 3418 M_2 plants descending from 1004 irradiated M_1 seeds could be obtained and were further pre-screened with a subset of 25- 30 5AS markers.

In this study 91 out of the pre-screened lines were used to create the RH panel, for fine mapping. This panel consisted of plants with confirmed deletions and plants with uncertain marker scores. The generated RH panel will be referred as RS-NIL3 panel.

2.2 DNA preparation

The DNA from the RS-NIL3 panel (91 plants) was extracted, according to the IFA CTAB DNA extraction protocol, based on Saghai-Maroof et al. (1984). Samples were taken from young leaves and dried at 35-36°C for one to two days. The dried samples were cut into 1.2ml 8-strip tubes, approximate five to seven glass beads were added, to grind the leaves into a fine powder using a Retsch MM301 mill.

700 µL of pre-heated CTAB-Buffer (cetyltrimethylammonium-bromid) were added to each tube, thoroughly mixed and immediately placed into a 65°C water bath for 60 to 90 minutes. When cooled down to room temperature, 350 µL chloroform:isoamylalcohol (24:1) were added. Then the tubes were inverted for 10 minutes, after that centrifuged in a Sigma Laboratory Centrifuge 4K15 for 10 minutes, at 3800 to 4000 rcf.

These steps separated the plant/buffer mixture into two phases, the supernatant fluid containing the DNA and RNA. 300 µL of this aqueous layer were carefully pipetted into new tubes and 5µL RNase were added. It was gently mixed and incubated for 30 minutes at room temperature and 300 µL isopropyl alcohol were added to precipitate the DNA. The tubes were centrifuged at 1000 rcf for eight minutes so that the DNA pellets adhered to the bottom of the tubes.

The formed DNA pellets were separated by either pouring or pipetting off the supernatant. Two washing steps with additional centrifuging after each step (8 minutes at 1000rcf each) purified the DNA (see Table 1). The DNA pellets were dried overnight and dissolved in 100 µL 0.5x TE buffer, gently rocked for several hours and then stored at 4°C. For a long-term storage the DNA was kept at -20°C in a freezer.

Table 1: Washing solution for DNA purification

For 100ml		Wash 1	Wash 2
8ml	1ml	200 mM sodium acetate	10 mM ammonium acetate
76ml		Ethanol	Ethanol
16ml	23ml	Distilled water	Distilled water

2.3 Quantity control of DNA

To estimate the DNA concentrations a TECAN Plate reader was used. Quantification was done at 230, 260 and 280 nm (Gallagher and Desjardins, 2007). DNA were manually diluted to a concentration of 200 ng/µl by adding the desired volume of 0.1 M TE-8 buffer. This stock solution was kept at -20°C in a freezer. The work solution, used for analysis, was further diluted to a concentration of 50 ng/µl.

2.4 Marker

5AS specific marker were chosen, based on previously published maps, by Buerstmayr et al. (2018). The markers were previously tested for their location on 5AS and their fragment size. The location of the markers was tested on CS-N5AT5B (Sears, 1966), CS-DT5AL (Sears and Sears, 1978), and cytogenetic CS deletion lines C5AS1-0.40, 5AS1-0.40-0.75, and 5AS3-0.75-0.97 (Endo and Gill, 1996). The CS-N5AT5B was used to locate the marker to the 5A chromosome, CS-DT5AL to the short arm and the CS deletion lines to allocate the markers to the bins, covering 40 %, 75%, and 97% of 5AS. Formerly produced amplicons were used as size marker for electrophoresis. All markers are listed (see Table 9, appendix) with further details, their sequence, their bin position and their origin according to information from previously published maps. For genotyping, different types of markers were used. The majority, with 56 were insertion site-based polymorphism (ISBP) markers. The exact numbers of markers used are listed in Table 2.

Table 2: Used marker types

	Marker Type	Count
SSR	Simple Sequence Repeat	26
SNP	Single Nucleotide Polymorphism	10
RJM	Repeat Junction Marker	4
COS	Conserved Ortholog Set	2
ISBP	Insertion Site-based Polymorphism	56
NV	No information on marker type	6

2.5 Multiplex PCR

Genotyping with 104 markers, specific for 5AS, were performed by multiplex polymerase chain reaction (PCR). In previous studies at IFA (Buerstmayr et al., 2018) multiplex PCR was performed by combining 5AS specific and 5A unspecific marker in a single reaction. This ensured, that true deletions could be differentiated from PCR failure. If the PCR worked correctly, deletion on the 5AS chromosome would lack bands, but the 5A unspecific marker still would be present.

In this study multiplex PCR with only 5AS specific markers were performed, with the side effect of saving working time and reducing the used resources for PCR and gel electrophoresis. For multiplex PCR, amplification with more than one (in this case up to three) marker combinations were performed. To distinguish between more than one marker, the PCR fragments should not overlap in the screening process. To make useful combinations, the results of previous marker tests (marker specific number of bands and fragment sizes) were used. In a 384 well PCR plate 2 µl of the DNA working solution (50 ng/µl) were mixed with 8 µl of master mix solution (see Table 3), containing purified water, reaction buffer ($MgCl_2$), dNTPs, reverse and forward primer, Taq-polymerase and M13 primer. The M13 primer (5′ CCCAGTCACGACGTTG 3′) has either fluorescently labelled FAM (6-carboxy fluorescein) or Cy5 (cyanin 5) tail (Schuelke, 2000) and is used for visualisation.

Table 3: Master mix preparation

	Stock concentration	Final concentration	Per Reaction for two markers
PCR-H20	-	-	5.16 µl
PCR Reaction buffer (MgCl2)	10x	1x	1 µl
dNTPs	2mM	0.2mM/µl	1 µl
M13 primer	10 µM	0.27 µM/µl	0.27 µl
Forward primer 1	10 µM	0.027 µM/µl	0.027 µl
Forward primer 2	10 µM	0.027 µM/µl	0.027 µl
Reverse primer 1	10 µM	0.2 µM/µl	0.2 µl
Reverse primer 2	10 µM	0.2 µM/µl	0.2 µl
Taq polymerase	5 U/µl	0.05 U/µl	0.1 µl
DNA	50 ng/µl	10 ng/µl	2 µl

To avoid the possible occurrence of various unwanted bands in electrophoresis and making the marker scoring difficult, a hot-start touchdown PCR, after Don et al. (1991) were performed. Table 4 shows the steps for this PCR protocol in detail.

Table 4: Hot-start touchdown PCR protocol

Pre-denaturation at 94°C for 4 minutes;
7x touchdown cycles:
1. DNA denaturation at 94°C for 50 seconds;
2. Annealing at 65°C decreasing by 2°C every further cycle for 1 minute;
3. Elongation at 72°C for 1 minute;
25x cycles:
1. DNA denaturation at 94°C for 30 seconds;
2. Annealing at 51°C for 30 seconds;
3. Elongation at 72°C for 30 seconds;
Post-extension at 72°C for 5 minutes with final cooling to 14°C;

2.6 Marker analysis and scoring

The PCR products were separated via gel electrophoresis on polyacrylamide gels, submerged in TBE buffer. After PCR, gel electrophoresis on a CBS vertical gel electrophoresis system was performed. Loading dye was added to the PCR products, in order to make the loading onto the gels easier.

Per lane, 2µl of the PCR product/loading dye mixture were transferred onto a 12% polyacrylamide gel and separated on the gel in the electrophoresis chamber for one to two hours, at a voltage of 400V. In addition to the PCR product, the size markers, (formerly produced amplicons) matching the used marker, were loaded on the first positions on the gel, to show at which position a present marker would produce a band.

The presence or absence of markers was visually determined. Right after electrophoresis the gels were scanned with a Typhoon Trio gel fluorescence image scanner. To detect FAM labelled fragments, a wavelength of 520nm was used, for Cy5 670nm. The images were saved (tif-files) and via a graphic software (Adobe Photoshop

Version 10) visually scored (example see Figure 1). The images were valuated, with following non-numeric values:

- **1** for a present marker, visualized as a band (or in some cases multiple bands)
- **d** for absent marker, which indicates a deletion at this marker position
- **m** for missing values, due to possible loading error or PCR failure
- **x** for not scorable bands or unclear results

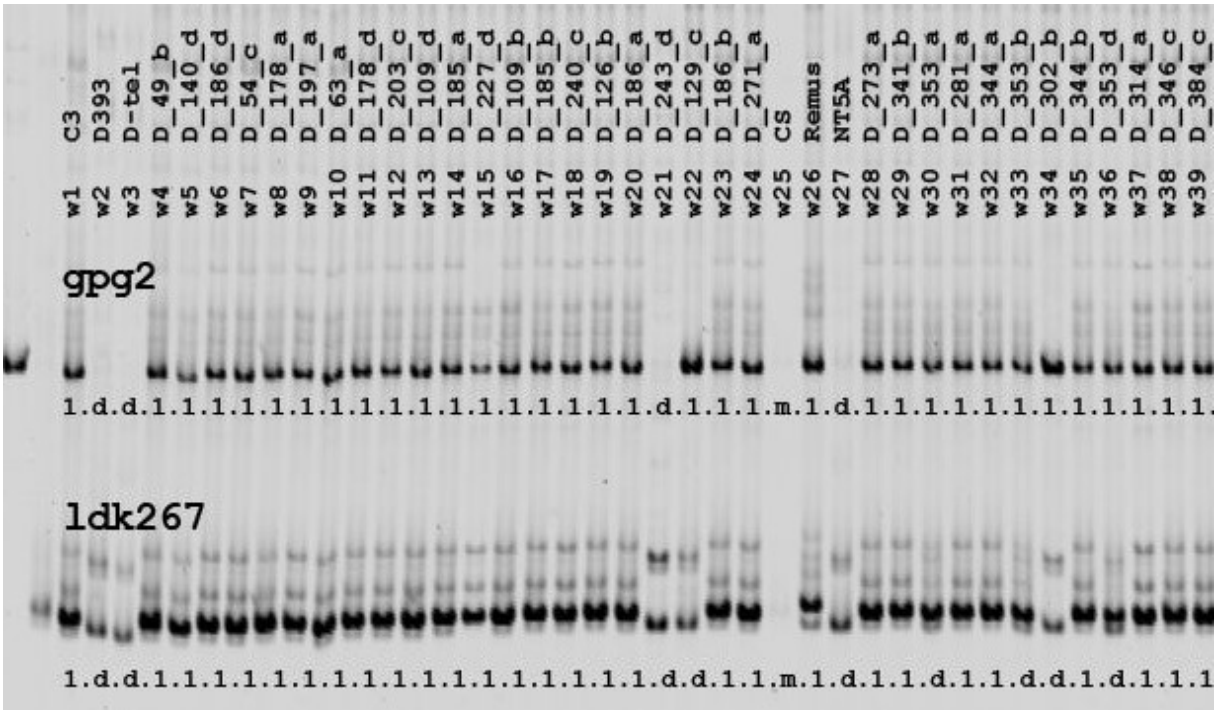

Figure 1: Multiplex PCR of the marker combination gpg2 and ldk267 on polyacrylamide gel. The first two columns are the size markers. The absence of a black band (PCR product) indicates a deletion on the chromosome and is scored as a **d**, the presence as a **1**.

2.7 Map construction

The results of the marker scoring were recorded and pre-sorted in Microsoft Excel. CarthaGène 1.2-LKH (de Givry et al., 2005) a program for mapping, was used to create the RH map. Since all markers were 5AS specific, they were taken together in a single linkage group. The algorithm parameters were set for a haploid model, which allowed faster ordering of the marker (Lange et al., 1995). Markers with the same retention/deletion pattern were merged together. For an initial ordering step of the markers, the two-point log-likelihood (LOD) was used by running the "lkh" (lkh, lkhn, lkhl, lkhd) commands. The marker data got converted into a Traveling Salesman Problem. The problem of finding an order of markers with the shortest distance

between them, is solved by the Lin–Kernighan heuristic algorithm of this program (Lin and Kernighan, 1973).

The commands "polish" and "flips" were used to improve the LOD based on the initial map. The "polish" command tries to insert single marker, form the initial map, in all possible intervals. "Flips" tests iteratively, in a sliding window of five marker, all possible marker orders. To determine the distances between the marker (in cR) the command "bestprintd" were used.

For visualization MapChart, (version 2.3), a software for visual representation of linkage maps and QTLs, was used (Voorrips, 2002).

2.8 Characterization of the RS-NIL3 panel

To further interpret the results gained from the mapping, different parameters were calculated, to further characterize the RS-NIL3 panel.

INFORMATIVE LINES

Informative lines are plants that have two or more neighbouring markers deleted. Plants arising from the same M_1 seed and having the same markers deleted were merged together to present a single informative plant.

OBLIGATE BREAKS

Kalavacharla (2006) describes an obligate break as the change of deletion and retention between two adjacent markers. Terminal deletions results in one obligate break, interstitial deletion into two obligate breaks.

MARKER RETENTION/DELETION FREQUENCY

The marker retention frequency was calculated as the marker present (retained) across the RS-NIL3 panel. The deletion frequency equals one minus retention frequency.

LINE RETENTION/DELETION FREQUENCY

Mazaheri et al. (2015) describes the line retention frequency as a value that indicates the proportion of a chromosome that is retained (not deleted) after irradiation. A

frequency of 1 indicates no deletions, a frequency of 0 the complete loss of the chromosome. The deletion frequency equals one minus retention frequency.

DELETETION SIZE

The obtained distances by CarthaGene were used to calculate the deletion size in cR. The real deletion size can only be estimated, because the deletion starts and ends between a retained and deleted marker. The minimum deletion size was calculated as the distance between deleted marker bordering the deletion, the maximum deletion size was calculated as the distance between retained marker bordering the deletion. The mean of these two sizes were calculated, to get the estimated deletion size.

MAP RESOLUTION

The marker positions in Mbp based on the physical map IWGSC RefSeq v1.0 (IWGSC et al., 2018) were used to compare cR with the physical Mbp positions. Based on the IWGSC RefSeq v1.0 the physical length of the 5A short arm is 255 Mbp. Map resolution was calculated as Mbp divided by cR.

CORRELATION AND COMPARISON OF RS-NIL3 AND NI-RIL MAP

The map unit of the RH map obtained from the RS-NIL3 panel is cR, the map unit of the genetic NI-RIL map is centi Morgan (cM). Rédei (2008), defined:

- 1 cR as, the distance between two loci, which there is a 1% probability of breakage, induced by a specified radiation dose
- 1 cM as, the unit of eukaryotic recombination, where the distance of 1 cM between to loci, refers to the probability of getting separated by recombination, by one of 100 meiosis events

For the correlation calculations, besides the data of the RS-NIL3 map in cR, the physical data in Mbp (IWGSC RefSeq v1.0), and the data, of the genetic linkage map of Buerstmayr et al. (2018) NI-RIL population in cM were used.

Calculations were performed in R-Studio Version 3.3.0 (R Development Core Team, 2018). Pearson's test for correlation between paired samples were calculated.

3. Results

3.1 Characterization of RS-NIL3 panel

The genotyping with 104 5AS specific markers, verified deletions on 80 out of the 91 preselected RS-NIL3 plants. Uninformative lines were removed and sister lines, derived from the same irradiated seed were merged to present a single genotype. This resulted in 51 informative lines, which were further used for calculations.

The 104 markers mapped to 63 different loci (number of unique positions). The generated map of 5AS covers the distance of 639.4 cR (see Table 5 and Figure 3) with a resolution of 0.3988 Mbp/cR. The map resolution for bin CS-5AS3-0.75-1, 5AS1-0.40-0.75 and C-5AS1-0.40, are 0.6183 Mbp/cR, 0.3571 Mbp/cR and 0.3561 Mbp/cR, respectively.

In total 103 obligate breaks were found. The smallest number of breaks, 18, were found at bin 5AS3-0.75-1 furthers away of the centromeric region. The number goes up to 24 at bin 5AS1-0.40-0.75 and peaks with 29 breaks at the bin C-5AS1-0.40.

Table 5: Summary map resolution per 5AS bins

Bin	Markers	Obligate breaks	Retention frequency	Mapped Loci	Size in cR	Size in Mbp	Map resolution
5AS3-0.75-1	24	18	0.862	17	215.3	63.75	0.6183
5AS1-0.40-0.75	37	38	0.832	20	185.9	89.25	0.3571
C-5AS1-0.40	43	47	0.874	26	238.2	102	0.3561
Sum	104	103	0.856	63	639.4	255	0.3988

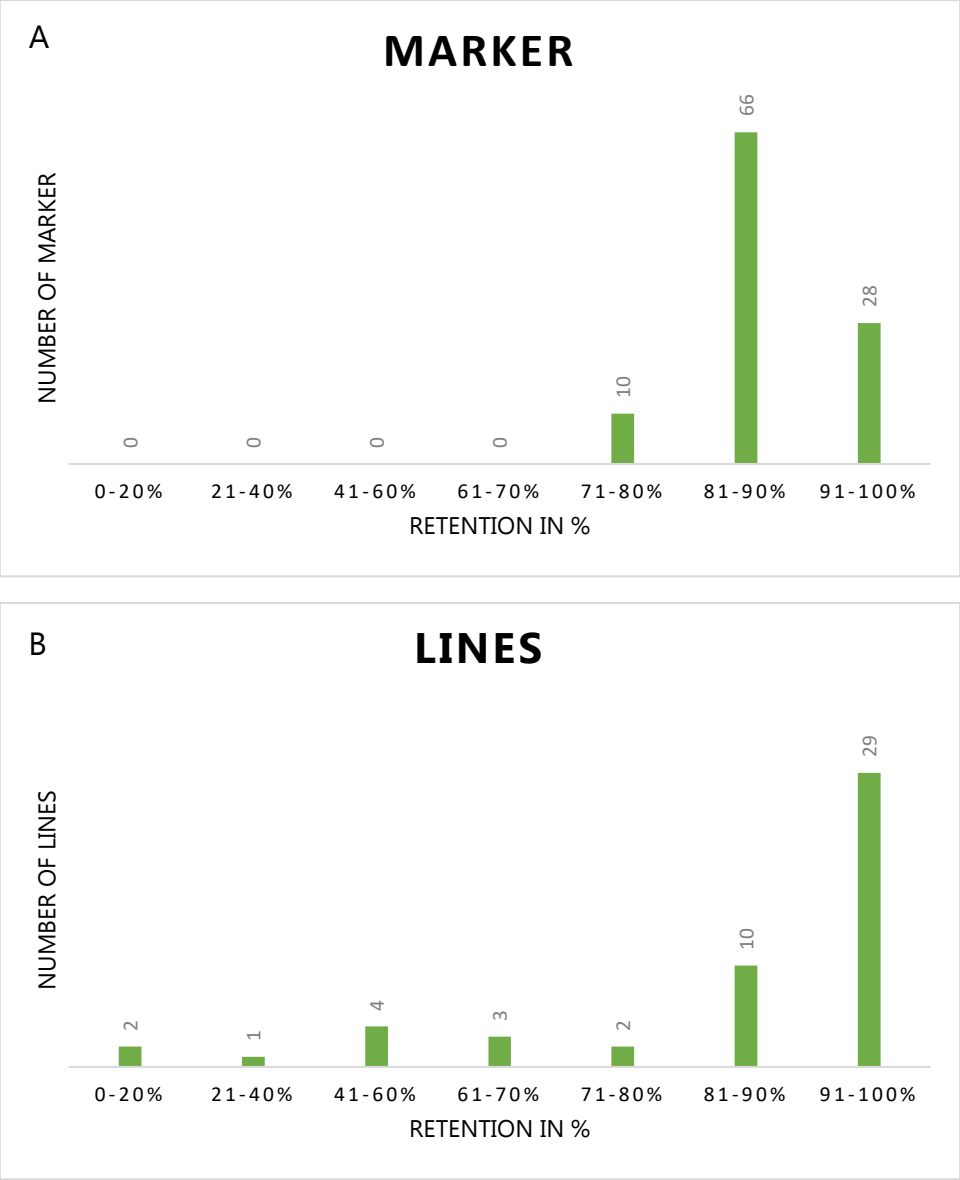

Figure 2: Retentions frequencies, of (A) marker and (B) lines.

Out of the 104 markers, 24 mapped to the bin 5AS3-0.75-1, 37 to 5AS1-0.40-0.75 and 43 to C-5AS1-0.40. The mean retention frequency per marker is 83.69%, with the lowest of 11.5% and the highest of 98.0%. The majority of marker has a retention frequency between 81 and 90% (see Figure 2A).

The mean retention frequency per line was 85.63%, ranging from 11.54% to 99.04%. Most lines have a retention frequency between 81 and 100% (see Figure 2B). Only 2 lines lost more than 80% of the 5AS chromosome due to deletions.

The scoring results of all 51 informative lines can be seen at Table 6.

Table 6: Scoring results of 51 informative lines genotyped with 104 markers. Marker data shows arrangement and deletion position for each line. Marker order is calculated in cR, physical Mbp positions according to IWGSC RefSeq v1.0 are included. Deletions on the chromosome are scored as a blue **d**, the presence as a yellow **1**. Resulting deletion map covering the distance of 639.4 cR, with a resolution of 0.3988 Mbp/cR. Part 1

		Distance (cumulative)		Distance (between marker)		Informative Lines																																																		
Bin	**Marker**	**cR**	**Mbp**	**cR**	**Mbp**	D_240_c	D_665_c	D_353_b	D_773_d	D_1763	D_203_c	D_126_b	D_197_a	D_314_a	D_178_a	D_271_a	D_631_a	D_185_a	D_350_a	D_716_b	D_938_a	D_54_c	D_227_d	D_893_b	D_384_c	D_823_a	D_63_a	D_742_d	D_818_b	D_140_d	D_339_d	D_467_d	D_729_a	D_281_a	D_273_a	D_346_c	D_386_a	D_508_a	D_560_c	D_662_c	D_937_a	D_353_a	D_575_b	D_714_b	D_915_b	D_243_d	D_737_a	D_129_c	D_657_d	D_585_b	D_302_b	D_109_b	D_49_b	D_867_c	D_186_d	D_775_b
5AS3-0.75-0.97	gpg1293	0,0	2,8	0,0	2,8	1	1	d	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	d	1	1	1	1	d	1	1	1	d	d	1	1	1	1	1	1	1	1	1
	IWB1144	13,6	9,8	13,6	7,0	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	1	d	d	1	1	1	1	1	1	1	1	1
	gpg2	13,6	10,2	0,0	0,4	1	1	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	1	d	d	1	1	1	1	1	1	1	1	1
	gwm443	13,6	11,3	0,0	1,1	1	1	1	d	1	-	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	x	1	1	1	1	1	1	1	1	d	d	1	1	1	1	1	1	1	1	1
	cwem44	19,7	19,0	6,1	7,6	1	1	1	d	1	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	d	1	-	1	1	1	1	1	d	1	1	1	1	1	1	1	-	d	d	1	1	1	1	1	1	1	1	d
	gpg537	32,4	29,2	12,7	10,3	1	1	1	x	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	1	d	d	d	1	1	1	1	1	1	1	1
	IWB6289	38,2	33,0	5,8	3,8	1	1	-	x	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	1	d	d	d	1	1	1	1	1	d	1	1
	IWB4146	43,8	36,5	5,6	3,5	1	1	1	x	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	x	1	1	1	1	1	1	1	1	d	d	d	1	1	1	1	1	d	d	1
	gpg2328	43,8	37,2	0,0	0,7	1	1	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	1	d	d	d	1	1	1	1	1	d	d	1
	gpg2326	43,8	37,9	0,0	0,7	1	1	1	d	1	1	1	1	1	1	1	1	1	1	1	-	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	1	d	d	d	1	1	1	1	1	d	d	1
	IWB6824	83,6	46,0	39,8	8,0	1	1	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	1	d	d	d	d	d	1	d	d	1	1	1
	barc186	88,5	46,6	4,9	0,6	-	d	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	1	d	d	d	d	d	1	d	d	1	1	1
	ldk243	98,3	47,5	9,8	0,9	1	d	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	1	d	d	d	d	d	d	d	1	1	1	1
	IWB5151	103,1	48,2	4,8	0,7	1	d	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	1	d	d	d	d	d	d	1	1	1	1	1
	ldk267	103,1	48,7	0,0	0,6	1	d	-	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	1	d	d	d	d	d	d	1	1	1	1	1
5AS1-0.40-0.75	barc56	176,4	70,7	73,3	22,0	1	d	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	d	d	1	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	ldk284	196,7	77,1	20,3	6,4	1	d	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	d	1	1	d	1	d	1	d	1	1	1	1	1	1	1	1	1	1	1
	gpg2162	196,8	79,2	0,1	2,1	1	d	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	d	1	1	d	1	d	1	d	1	1	1	1	1	1	1	1	1	1	1
	gpg2163	196,8	79,2	0,0	0,0	1	d	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	d	1	1	d	1	d	1	d	1	1	1	1	1	1	1	1	1	1	1
	gpg1438	202,8	82,2	6,0	3,1	1	d	1	d	1	1	1	1	1	1	1	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	d	1	1	d	1	d	1	1	1	1	1	1	1	1	1	-	1	1	1
	barc117	209,0	85,3	6,2	3,1	1	d	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	d	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2168	209,1	91,6	0,1	6,3	1	d	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	d	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg1778	209,1	94,0	0,0	2,4	1	x	1	d	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-	1	-	1	1	d	1	1	1	d	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2038	215,3	96,6	6,2	2,6	1	d	1	x	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	x	1	1	1	d	1	d	d	1	1	1	1	1	1	1	1	1	1	1	1

Table 6 continued: Scoring results Part 2

5AS1-0.40-0.75	IWB7556	215,3	98,0	0,0	1,4	1	d	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	d	1	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg1358	227,3	99,1	12,0	1,1	1	d	d	d	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	d	1	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1
	jfio7	238,7	101,0	11,4	1,8	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	d	1	1	d	d	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	wmc150	244,0	NA	5,3	NA	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	d	1	1	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gwm293	254,8	104,2	10,8	NA	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	d	1	d	1	1	d	d	d	1	1	1	1	-	1	1	1	1	1	1	1	1	1
	gpg1440	254,8	105,2	0,0	1,0	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-	1	1	d	1	1	1	1	1	1	1	d	1	d	1	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gwm304	254,8	105,4	0,0	0,2	1	d	d	d	1	1	1	1	1	1	-	-	1	1	-	1	1	1	1	1	1	d	1	-	1	1	1	1	1	d	1	d	1	-	d	d	d	1	1	1	1	-	1	1	1	1	1	1	1	1	1
	IWB8393	260,1	109,3	5,3	3,9	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-	1	1	d	1	1	1	1	1	1	1	d	1	d	1	1	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2186	260,1	110,2	0,0	0,8	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	1	1	1	1	d	1	d	1	1	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	wmc150	270,9	NA	10,8	NA	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	1	1	d	1	d	d	d	1	1	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2049	270,9	115,5	0,0	NA	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	1	1	d	1	d	d	d	1	1	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2060	270,9	117,2	0,0	1,6	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	1	1	d	1	d	d	d	1	1	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	IWB1080	270,9	118,7	0,0	1,5	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	1	1	d	1	d	d	d	1	1	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	BE49876	270,9	118,8	0,0	0,1	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	1	1	1	d	1	d	d	d	1	1	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	ldk49	281,2	119,9	10,3	1,1	1	d	d	d	-	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	1	1	1	1	1	d	1	d	d	d	1	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	ldk2	281,2	119,9	0,0	0,0	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	1	1	1	1	1	d	1	d	d	d	1	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2233	281,2	120,1	0,0	0,2	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	1	1	1	1	1	d	1	d	d	d	1	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg1763	291,0	124,0	9,8	3,9	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	1	1	1	1	1	d	1	d	d	x	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2092	295,5	125,1	4,5	1,1	1	d	x	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	1	1	1	1	1	d	d	d	d	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2072	295,5	125,4	0,0	0,3	1	d	x	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	1	1	1	1	1	d	d	d	d	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2126	334,3	128,3	38,8	3,0	1	d	d	x	1	1	1	1	1	1	1	1	1	1	1	1	-	1	d	1	1	d	1	1	-	1	1	d	d	1	1	1	1	1	1	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1
	IWB5827	334,3	128,4	0,0	0,1	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	1	1	1	1	1	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	ldk217	346,8	130,2	12,5	1,8	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	1	1	1	1	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	IWB3343	352,9	133,0	6,1	2,7	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	1	1	1	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg1342	352,9	133,6	0,0	0,6	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	-	1	1	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg574	353,0	141,3	0,1	7,7	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	1	1	1	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
C-5AS1-0.40	gpg277	365,6	147,4	12,6	6,2	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	1	d	1	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	ldk218	365,6	147,7	0,0	0,3	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	1	d	1	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg1323	371,4	156,2	5,8	8,4	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	1	d	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	ldk241	371,4	155,5	0,0	-0,7	1	d	d	x	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	1	d	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg1139	377,6	161,9	6,2	6,5	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg1789	390,3	168,4	12,7	6,4	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2309	390,3	171,2	0,0	2,8	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg1575	395,7	172,9	5,4	1,7	1	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	d	d	d	1	1	1	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	jfio4	395,8	173,5	0,1	0,6	1	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg1994	401,2	177,3	5,4	3,8	1	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2250	401,2	179,2	0,0	2,0	1	d	d	d	d	1	1	1	-	1	1	1	1	1	1	1	1	1	d	1	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table 6 continued: Scoring results Part 3

C-5AS1-0.40	ldk50	401,3	182,2	0,1	2,9	1	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	d	1	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2244	428,9	188,0	27,6	5,8	1	x	d	x	d	1	1	1	1	-	1	1	1	1	1	1	1	1	x	d	d	x	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2313	440,5	193,2	11,6	5,2	1	d	d	d	d	1	1	1	1	1	1	1	1	d	d	1	1	1	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	ldk16	440,5	193,3	0,0	0,1	1	d	d	d	d	1	1	1	1	1	-	-	-	d	d	1	1	1	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	ldk14	440,5	195,3	0,0	2,0	1	d	d	d	d	1	1	1	1	1	1	1	1	d	d	1	1	1	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2019	461,1	200,6	20,6	5,3	1	d	d	d	d	1	1	1	1	1	d	d	1	d	d	d	1	d	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2108	461,1	200,6	0,0	0,0	1	x	d	d	d	1	1	1	1	1	d	d	1	d	d	d	1	d	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg1383	474,2	204,0	13,1	3,4	1	d	d	d	d	1	1	1	1	1	d	d	d	d	d	d	d	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	ldk242	478,3	204,3	4,1	0,3	1	d	d	d	d	1	1	1	1	1	d	d	d	d	d	d	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gwm129	487,4	205,2	9,1	0,9	1	d	d	d	d	1	1	1	1	1	d	d	d	d	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	jfio2	497,3	207,7	9,9	2,5	1	d	d	d	d	1	1	1	1	1	x	d	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	ldk289	502,5	208,1	5,2	0,4	1	d	d	d	d	1	1	1	-	1	d	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-	1	1	1	-	1	-	1	1	1	1	1	1
	gpg2231	514,3	209,5	11,8	1,4	1	d	d	d	d	1	1	1	1	1	x	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2232	514,3	209,5	0,0	0,0	1	d	d	d	d	1	1	1	1	1	x	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2075	520,4	211,8	6,1	2,3	1	d	d	d	d	1	1	1	1	1	x	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2097	520,4	212,4	0,0	0,6	1	d	d	d	d	1	1	1	1	1	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2121	526,9	215,1	6,5	2,7	1	d	d	d	d	1	1	1	1	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg119	533,8	215,3	6,9	0,2	1	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg35	540,7	216,3	6,9	1,1	1	d	d	d	d	1	1	1	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2336	540,7	218,0	0,0	1,7	1	d	d	d	d	1	1	1	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg743	540,7	218,1	0,0	0,1	1	d	d	d	d	1	1	1	1	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg1294	540,7	218,3	0,0	0,2	1	d	d	x	d	1	1	1	1	d	1	1	1	1	1	1	-	1	1	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1
	gpg2117	540,7	219,7	0,0	1,4	1	d	d	d	d	1	1	1	-	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-	1	-	1	1	1	1	1	1
	ldk113	554,0	221,7	13,3	2,0	1	d	d	d	d	1	1	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1
	gpg214	566,3	226,1	12,3	4,4	1	d	d	d	d	1	d	d	x	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2020	566,4	226,5	0,1	0,4	1	d	d	d	d	1	d	d	d	1	1	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-	1	1	1
	gpg2147	596,4	231,1	30,0	4,6	1	d	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2123	602,7	232,2	6,3	1,1	d	d	d	x	d	d	1	1	1	1	1	1	1	1	1	1	-	1	1	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1
	gpg2102	602,7	232,2	0,0	0,1	d	d	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg1395	602,7	233,0	0,0	0,8	d	d	x	x	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2158	602,7	234,4	0,0	1,4	d	d	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg1321	602,8	NA	0,1	NA	d	d	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	ldk215	609,0	236,3	6,2	NA	d	d	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg1777	623,7	240,5	14,7	4,2	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg158	623,7	242,0	0,0	1,5	x	d	d	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2034	623,8	242,3	0,1	0,4	d	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2255	623,8	242,9	0,0	0,6	d	d	d	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2011	631,3	244,2	7,5	1,3	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	BE42516	631,3	244,5	0,0	0,2	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg542	631,3	244,5	0,0	0,0	d	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	cfa2250	639,3	245,9	8,0	1,4	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	jfio6	639,3	246,7	0,0	0,9	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg503	639,4	246,9	0,1	0,2	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

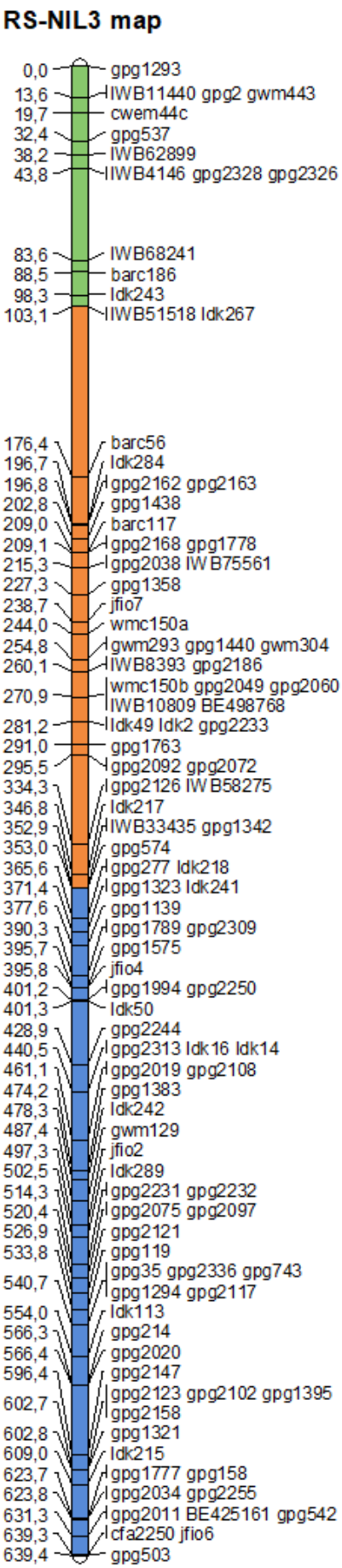

Figure 3: 5AS map, bin C-5AS1-0.40 is coloured blue, 5AS1-0.40-0.75 orange, and 5AS3-0.75-1 green.

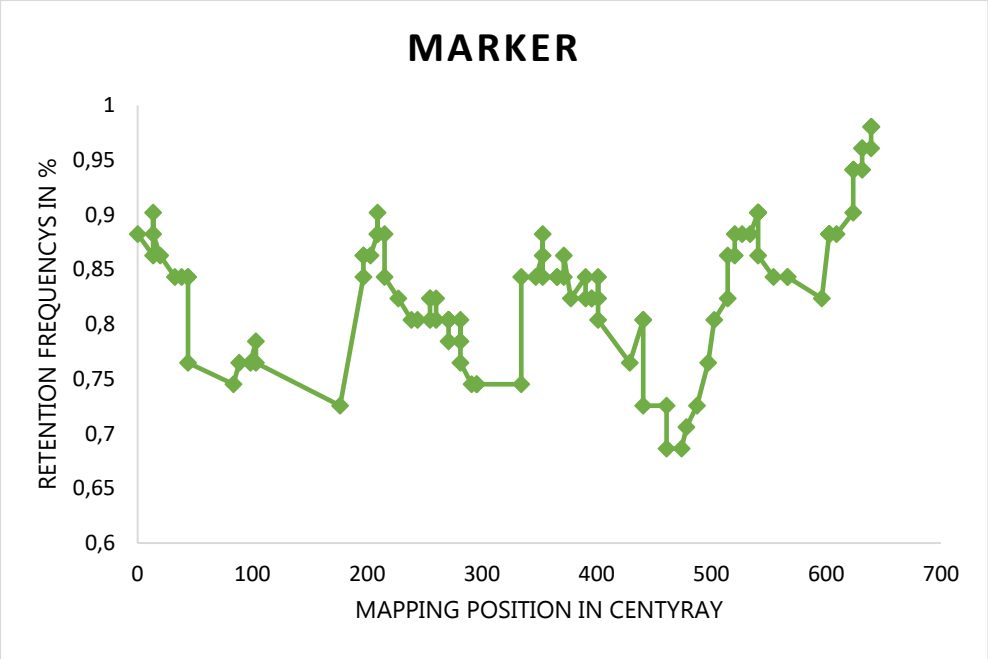

Figure 4: Retention frequencies per marker at each locus. Ranging from the telomeric region (0 cR) to the centromere (700 cR).

The mean retention frequency per marker is 0.84. The lowest is 0.69 and the highest 0.98 (see Figure 4).

Table 7: Deletion statistics, Part 1

			Deletion size in cR			Deletion size in Mbp		
Line	Retention frequency	Obligate breaks	Maximum	Minimum	Average	Maximum	Minimum	Average
D_773_d	11,54%	2	610,1	595,4	602,8	230,7	226,1	228,4
D_665_c	13,46%	2	555,7	542,8	549,3	199,9	197,9	198,9
D_353_b	28,85%	3	13,6	0,0	6,8	7,0	2,8	4,9
			416,0	396,5	406,3	146,2	143,8	145,0
D_386_a	57,69%	2	320,7	295,5	308,1	125,5	115,5	120,5
D_63_a	61,54%	2	230,2	206,3	218,3	99,8	96,4	98,1
D_1763	64,42%	2	233,4	213,3	223,4	69,3	63,4	66,3
D_893_b	69,23%	2	216,5	197,1	206,8	86,5	84,5	85,5
D_818_b	73,08%	3	197,7	176,4	187,1	74,3	67,9	71,1
			75,9	37,7	56,8	46,7	34,7	40,7
D_937_a	77,88%	2	178,1	94,5	136,3	71,1	48,0	59,6
D_662_c	81,73%	2	119,0	68,2	93,6	30,3	26,2	28,2
D_273_a	82,69%	2	107,0	56,8	81,9	29,2	24,4	26,8
D_240_c	84,62%	1	43,0	36,7	39,9	15,9	14,8	15,3
D_243_d	85,58%	1	162,8	103,1	133,0	67,9	38,9	53,4
D_737_a	85,58%	1	162,8	103,1	133,0	67,9	38,9	53,4
D_729_a	86,54%	2	92,8	75,9	84,4	22,8	14,7	18,8
D_271_a	88,46%	2	93,3	65,8	79,6	20,0	14,5	17,3
D_575_b	88,46%	2	78,4	47,3	62,9	33,4	23,9	28,7
D_631_a	89,42%	2	86,4	59,3	72,9	19,8	11,8	15,8
D_346_c	89,42%	2	74,2	24,6	49,4	18,2	9,8	14,0
D_350_a	90,38%	2	85,4	62,0	73,7	21,5	14,9	18,2
D_716_b	90,38%	2	85,4	62,0	73,7	21,5	14,9	18,2
D_129_c	90,38%	2	156,7	70,7	113,7	51,8	19,5	35,7
D_384_c	92,31%	2	86,1	49,4	67,8	23,0	16,3	19,7
D_508_a	92,31%	3	105,9	26,4	66,2	36,5	11,5	24,0
			53,1	5,5	29,3	8,2	1,4	4,8
D_185_a	93,27%	2	59,3	40,1	49,7	11,2	5,5	8,3
D_823_a	93,27%	2	77,0	45,3	61,2	22,2	16,0	19,1
D_742_d	93,27%	2	51,3	11,0	31,2	26,1	13,8	19,9
D_203_c	94,23%	2	42,6	6,4	24,5	9,8	3,3	6,6
D_178_a	94,23%	2	32,5	13,3	22,9	10,9	5,4	8,1

Table 7 continued: Deletion statistics, Part 2

			Deletion size in cR			Deletion size in Mbp		
Line	Retention frequency	Obligate breaks	Maximum	Minimum	Average	Maximum	Minimum	Average
D_938_a	94,23%	2	62,0	36,2	49,1	12,8	7,1	10,0
D_467_d	94,23%	2	37,1	18,8	28,0	27,8	17,5	22,6
D_227_d	95,19%	2	56,8	26,3	41,6	12,4	4,6	8,5
D_353_a	95,19%	3	13,6	0,0	6,8	7,0	2,8	4,9
			21,4	10,8	16,1	8,4	2,9	5,6
D_657_d	95,19%	2	132,6	19,5	76,1	32,8	2,8	17,8
D_585_b	95,19%	2	132,6	19,5	76,1	32,8	2,8	17,8
D_140_d	96,15%	2	23,6	5,5	14,6	15,3	5,1	10,2
D_339_d	96,15%	2	24,6	5,8	15,2	20,7	8,7	14,7
D_281_a	96,15%	2	55,8	38,8	47,3	6,3	3,4	4,8
D_867_c	96,15%	2	51,2	5,6	28,4	16,8	4,9	10,9
D_197_a	97,12%	2	55,7	12,4	34,1	11,3	4,8	8,1
D_314_a	97,12%	2	55,7	12,4	34,1	11,3	4,8	8,1
D_54_c	97,12%	2	36,2	13,2	24,7	7,1	1,2	4,1
D_560_c	97,12%	2	53,1	4,5	28,8	8,2	1,4	4,8
D_714_b	97,12%	2	29,6	12,0	20,8	7,0	2,5	4,8
D_915_b	97,12%	2	26,4	0,1	13,3	11,5	2,1	6,8
D_302_b	97,12%	2	87,9	4,8	46,4	24,2	1,3	12,7
D_109_b	97,12%	2	59,3	14,7	37,0	10,2	1,5	5,8
D_186_d	97,12%	2	45,4	0,0	22,7	13,0	1,4	7,2
D_126_b	98,08%	2	42,4	0,1	21,3	9,3	0,4	4,9
D_49_b	98,08%	2	54,5	4,9	29,7	9,5	0,6	5,0
D_775_b	99,04%	2	18,8	0,0	9,4	17,9	0,0	8,9
Mean	85,63%	2,02	110,5	73,7	92,1	37,4	27,4	32,4

The marker analysis found a total number of 103 obligate breaks, which produced six terminal and 49 interstitial deletions. A further characterisation of the deletions can be found at Table 7. The longest deletion is 602.8 cR and shortest 6.8 cR, the average deletion size is 92.1 cR. The average physical deletion size is 32.4 Mbp, the longest is 240.4 Mbp long, the shortest 2.7 Mbp. 4 lines have more than one separate fragment deleted, the remaining 47 had only one segment missing. Only one line, D_775_b, had one single marker missing (cwem44c).

3.2 Comparison of two different RS-NIL3 panels

Prior to this study, Schwarz (2018), generated a hybrid deletion map, based on 1764 analysed M_2 and M_3 plants. This resulted in 28 informative lines. 35 different markers were used and generated a map size of 433.3 cR (see Figure 5). In this study pre-screening of 3418 M_2 plants with a subset of markers led to the selection of 91 plants for further analysis. Selected plants were fine-mapped using 104 markers and based on these analyses all M_2 plants which were derived from the same M_1 and had the same co-retention pattern were merged to represent a single genotype and plants where deletions could not be verified were excluding from map construction. This resulted in a total number of 51 informative lines. Using markers data of these 51 informative plants calculated a map size of 639.4 cR.

With the increase of used marker and the higher number of informative plants, more loci could be mapped, increasing from 27 (Schwarz, 2018) to 63 in this study. The mean distance between two loci decreased from 16.04 cR (Schwarz, 2018) to 6.15 cR. In total Schwarz (2018) could detect 29 deletions, in this study 55 deletions were found. The mean deletion size for the RS-NIL3 (Schwarz, 2018) had a mean of 105.31 cR, in this study 92.1 cR. The map size between the marker barc186 and cfa2250 was 406.9 cR with a resolution of 0.4898 Mbp/cR, for the RS-NIL3 panel of Schwarz (2018). Compared to the RH panel of this study, a distance of 550.8 cR could be mapped, with a resolution of 0.3618 Mbp/cR. See Table 8 for a more detailed comparisons between the two deletion panels.

Table 8: Comparison of the RS-NIL3 panels (Schwarz, 2018) and of this study

	RS-NIL3	
	Blazek (2019)	Schwarz (2018)
Markers	104	35
Informative lines	51	28
Map size (cR)	639,4	433,3
Mapped loci	63	27
Markers per locus	1,65 (1 - 3)	1,29 (1 - 4)
Mean distance between two loci (cR)	6,15 (0,1 - 73,3)	16,04 (6,8 - 63,9)
Mean distance between two loci (Mbp)	2,84	10,58
Obligate breaks	103	49
Deletions	55	29
Terminal deletions	6	7
Interstitial deletions	49	22
Retention frequency per line	85,6 (11,54 - 99,0)	74,9 (0,00 – 97,22)
Retention frequency per marker	85,6 (68,6 - 98,0)	74,9 (64,29 – 92,86)
Mean deletion size (cR)	92,1 (6,8 - 602,75)	105,31 (13,20 – 433,3)
Mean deletion size (Mbp)	42,5 (3,14 - 278,11)	69,55 (8,72 – 286,15)
Map resolution (Mbp/cR)	0,399	0,66

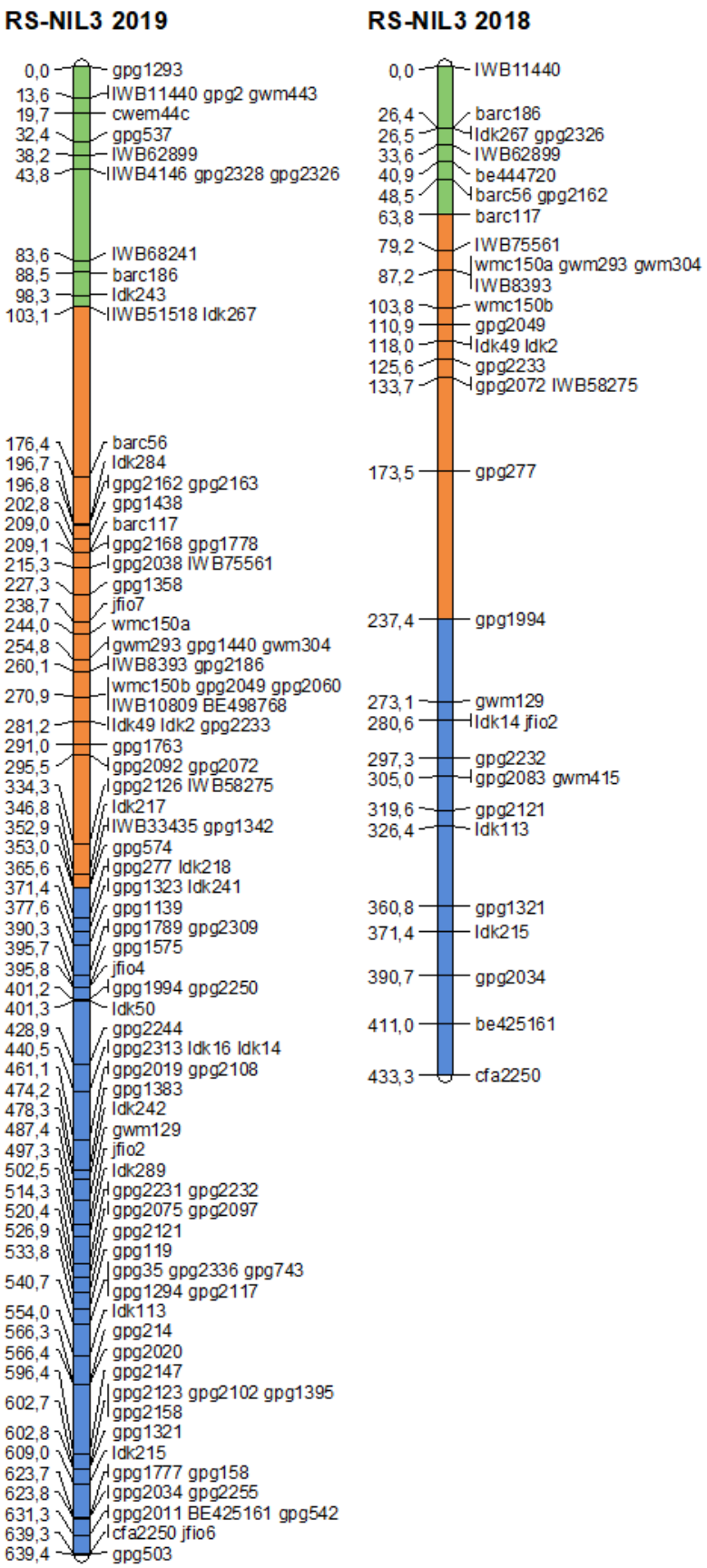

Figure 5: Comparison of the map size, influenced by the number of used markers and informative lines. Left the 2019 RS-NIL3 map, right the RS-NIL3 map by Schwarz (2018). C-5AS1-0.40 is coloured blue, 5AS1-0.40-0.75 orange, and 5AS3-0.75-1 green.

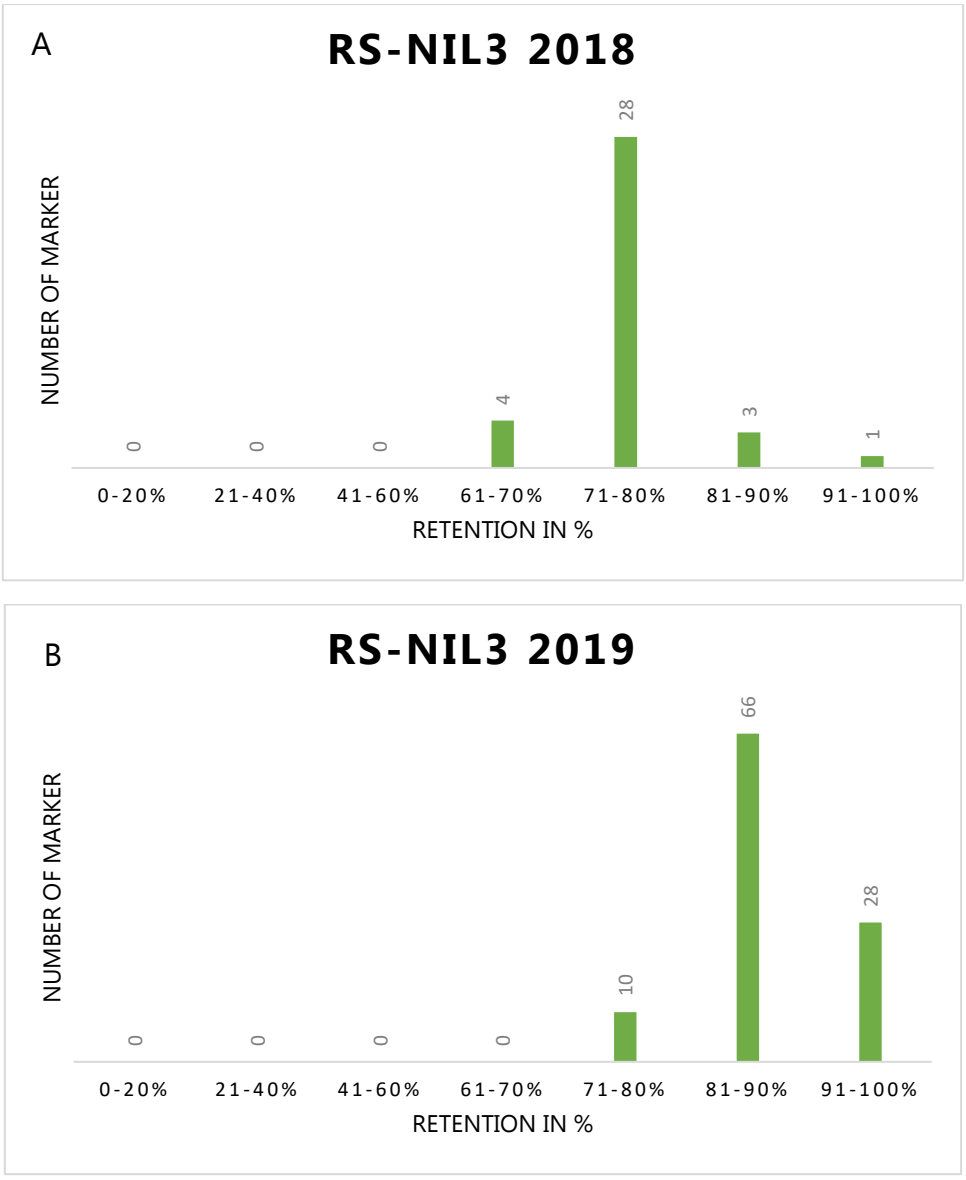

Figure 6: Marker retention of RS-NIL3 panels of (A) Schwarz (2018) and of the (B) this study (2019)

The distribution of the marker retention frequencies is clearly different between the RS-NIL3 panel of 2018 and 2019. For the 2018 panel, the majority of marker had a retention frequency of 71-80% (see Figure 6A), with a mean retention frequency of 74.9%. For the 2019 panel, the majority was between 81-90% (see Figure 6B), with a mean frequency of 85.6%.

The markers, which were used in both panels had the same order, only marker gpg2326, which were found in the 2018 map under the marker barc186 and in the 2019 map above.

3.3 Correlation of RI-NIL3 map with a physical map and NI-RIL map

Pearson's correlation coefficient between cR postion of the RS-NIL3 panel of this study (see Figure 7A) with the physical distance in Mbp (IWGSC RefSeq v1.0) (101 shared marker) was 0.9946, which indicates a strong positive correlation.

A moderate correlation, r=0.5311 could be observed between the NI-RIL panel (Buerstmayr et al., 2018) in cM and the RS-NIL3 panel of this study (13 shared marker) (see Figure 7B).

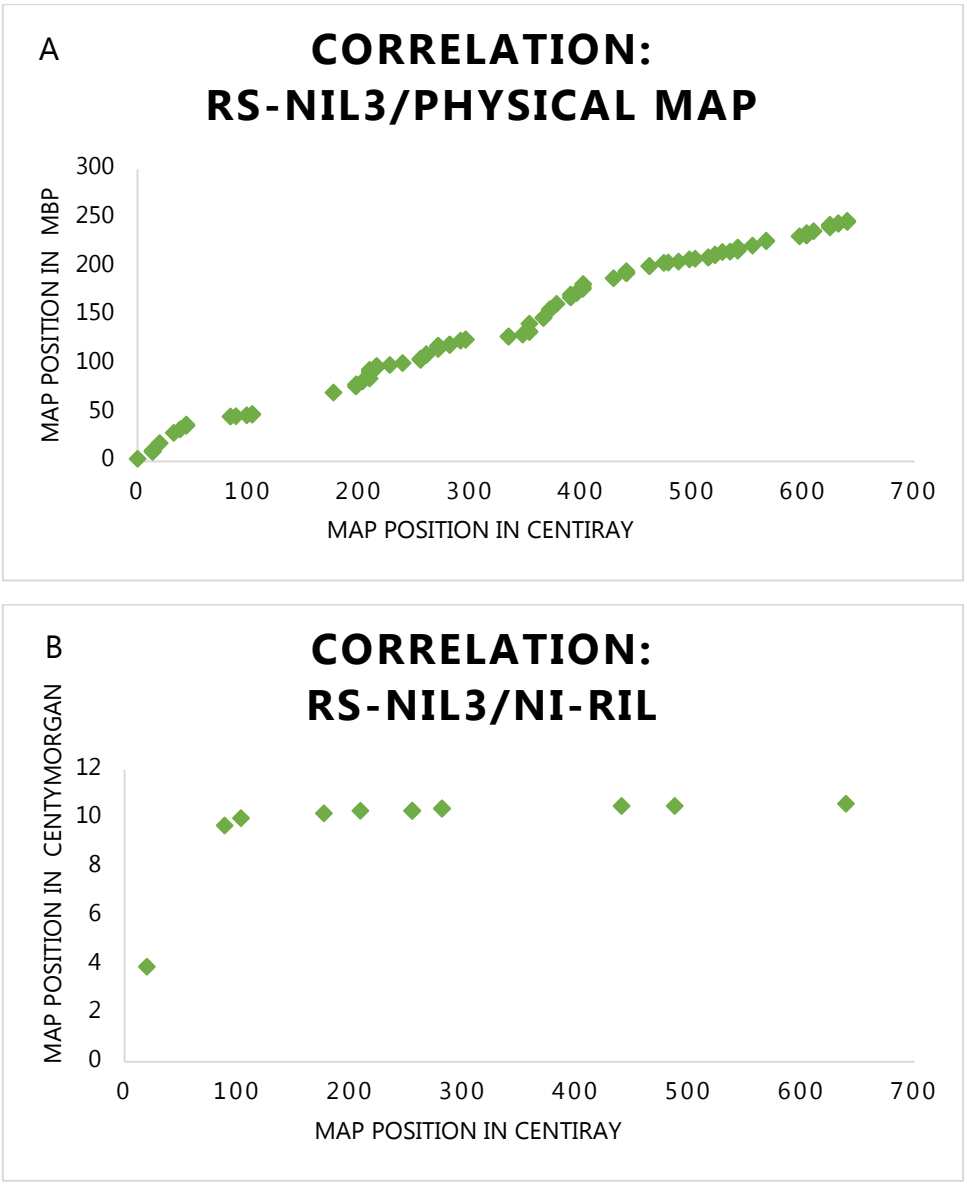

Figure 7: Comparison of correlation between cR postion of the RS-NIL3 panel with (A) the physical distance in Mbp (IWGSC RefSeq v1.0) and (B) the genetic distance, of the NI-RIL panel (Buerstmayr et al., 2018) in cM

Spearman's rank order between RS-NIL3 and the pysical map was r=0.9998, between the RS-NIL3 and the NI-RIL map r=0.9861.

The map size of RS-NIL3 map of this study, between the marker barc186 and cfa2250, is 550.8 cR. Compared to the NI-RIL map (Buerstmayr et al., 2018) a 612-fold increased map resolution could be calculated in this interval. The marker order was the same in all three maps (see Figure 8).

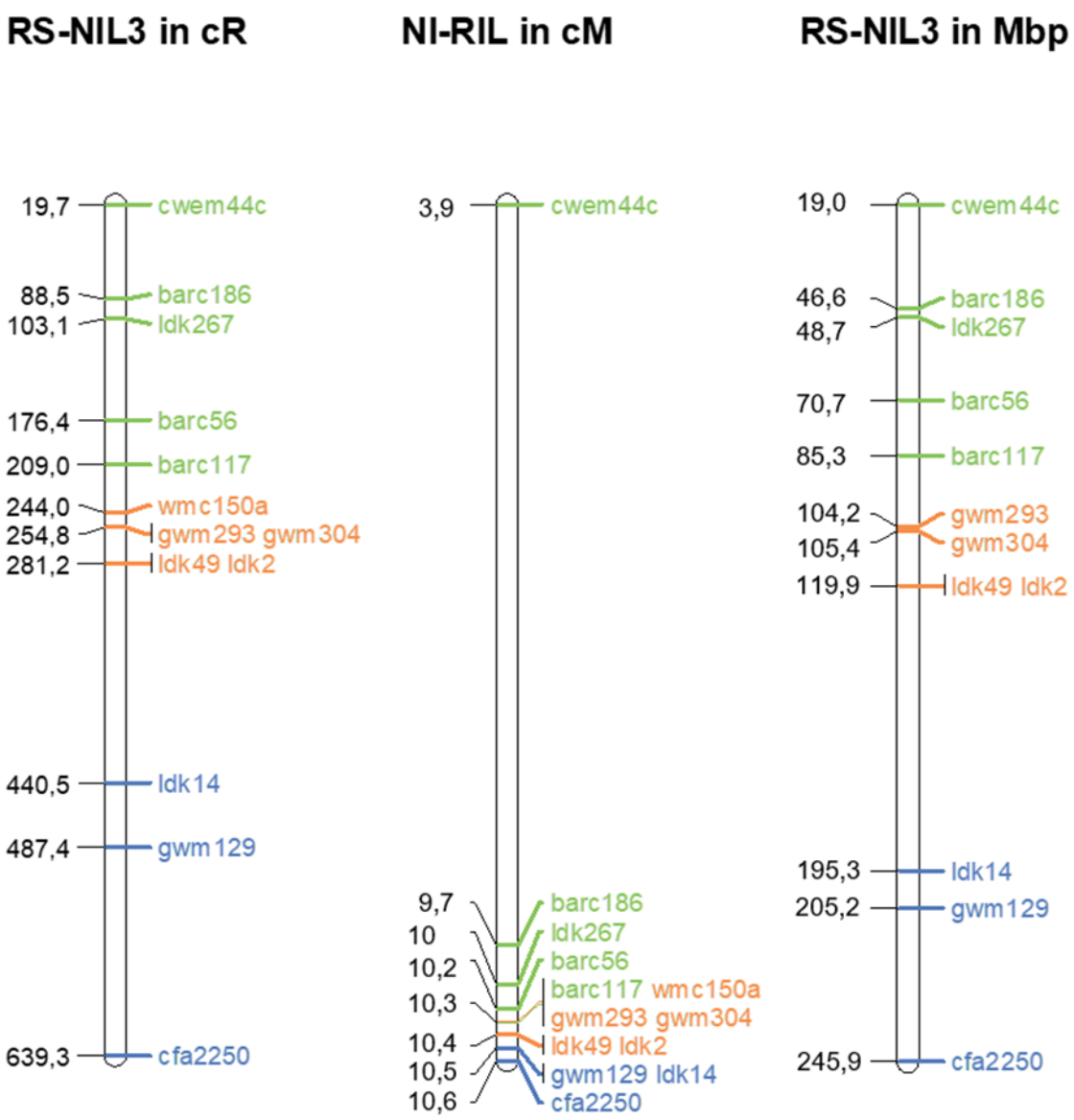

Figure 8: Comparison of RS-NIL3 map with the physical map (based on IWGSC RefSeq v1.0) and genetic map (Buerstmayr et al., 2018) of 5AS, with shared marker, present in all three maps. Markers are coloured according to their bin affiliation: C-5AS1-0.40 are coloured blue, 5AS1-0.40-0.75 orange, and 5AS3-0.75-1 green.

4. Discussion

RADIATION DOSE

Kumar et al. (2014) stated that the resolution in RH mapping, is highly dependent on DSB and therefore highly influence by the radiation dosage. Producing more deletions with a higher dose of irradiation may increase the mapping resolution, but in plants the rearrangement of chromosomal parts might hinder the map analysis. The study of Hossain et al. (2004b) found out that a radiation dose higher than 400 Gy led to a decrease in plant survival and vigour. A dosage smaller than 300 Gy produced not enough deletions and couldn't contribute information for mapping. Choosing radiation dosage between 400 Gy and 300 Gy seem to be a good guideline, but the optimum dosage with maximum breakage, with enough plant survival should be determined for each deletion panel individually (Kumar et al., 2014).

MARKER RETENTION

The mean marker retention frequency was 0.836, the highest 0.9803 and the lowest 0.682. Jones (1996) stated, that an optimal marker retention frequency should be around 50%. Although this is rarely achieved in plant-based studies. The marker retention frequency in plants is with 75-97% rather high, compared to animal studies (20-30%). In studies of GAO et al. (2004, 2006) and Kumar et al. (2012a, 2012b) viable RH panels showed a marker retention frequency over 90%. The problem lies here in the method of RH mapping itself. The DSBs cause difficulties in meiosis and mitosis. Plants with highly fragmented chromosomes lead to a decrease of germination and plant survival. This has the effect, that only panels with high marker retention frequencies can be obtained (Kumar et al., 2014).

Kumar et al. (2012a) found, that the DBS are independent of the chromosomal region. This is in agreement with our study, where the marker retention frequencies per bin are quite similar, 85.63%, 81.15% and 83.57%, for the bins 5AS3-0.75-0.97, 5AS1-0.40-0.75 and C-5AS1-0.40, respectively. However, Kumar et al. (2012a) found that the DSB repairing system is influenced by the state of condensation of the DNA. Areas with an open chromatin region are favored by the repairing system, compared to more condensed regions. These favored regions are supposed to get faster and better repaired, therefore breaks in these areas may have smaller deletions. Kumar et al. (2012b), in wheat chromosome 1D and 7D and Mazaheri et al. (2015), in barley chromosome 3H reported similar findings of uneven deletion frequencies along the

chromosome. In this study, uneven deletion frequencies could be not observed. The deletions are evenly and randomly distributed. The only expectation were markers near the centromere. These had a very high retention frequency (up to 0.98). But this seems rather unsurprisingly, considering deletions across the centromere would mean a whole chromosome loss and would lead to unfertile plants and could be therefore not observed in the next generation.

COMPARISON OF THE RS-NIL3 PANELS AND NI-RIL PANEL

Compared to Schwarz (2018), nearly three times more markers (104 compared to 35) and 1.8 times more informative lines (51 compared to 28) were analyzed in this study, leading to a 1.43-fold increase of mapping resolution. Interestingly, the RS-NIL3 panel from Schwarz (2018) shows a lower marker retention frequency than this study, 74.9% compared to 85.6%, respectively. This could be the case since, Schwarz (2018) only used 15 markers for pre-screening, where in this study 25 to 30 markers were used for pre-screening, therefore the chance of finding small deletions were higher.

Referring to the interval between the marker barc186 and cfa2250, Schwarz (2018) could calculate a distance of 406.9 cR, Buerstmayr et al. (2018) obtained 350.3 cR for their RSH-consensus map and the RS-NIL3 map of this study calculated a distance of 550.8 cR. Schwarz (2018) and Buerstmayr et al. (2018) reported a 452.1- and 389.2-fold map improvement in comparison to the genetic NI-RIL map. The RS-NIL3 map received in this study revealed a 612-fold improved map. These high map increasements compared to genetic maps agrees with Kumar et al. (2012a, 2014), who found that RH-mapping could produce higher map resolution compared to genetic maps.

CORRELATION BETWEEN cR, Mbp and cM

Spearsman's rank order (r=0.9998) and Pearson's correlation coefficient (r=0.9946) between cR (RS-NIL3) and Mbp (IWGSC RefSeq v1.0) indicated an almost perfect agreement of marker order and marker distance between Mbp and cR.

Spearman's rank order (r=0.9861) between cR and cM (NI-RIL, Buerstmayr et al., 2018) was also quite high and indicates an agreement of marker order. However, Person's correlation coefficient (r=0.5311), between the NIR-RIL and the RS-NIL3 panel, only showed a moderate linear relationship and the p-value of 0.62 (threshold 0.05) indicates a strong discrepancy in markers distances between genetic and radiation hybrid map. Buerstmayr et al. (2018) also found a moderate linear relationship between their RHS-consensus map (cR) and their NI-RIL map. Boehnke et al. (1991) and Newell et al. (1998), stated that cR were better suited to provide an estimate for physical distance, because of the evenly and randomly distribution of DBS along the chromosome, compared to cM, which seems to be strongly influenced by recombination poor regions.

5. Conclusion and Outlook

Fusarium head blight is a devastating fungal disease, which threatens the cereal production around the world. Although agronomic practice, like tillage or crop rotation, might lead to a reduced *Fusarium* infection, these methods are not always enough. Environmental factors, like precipitation during anthesis can't be influenced and a combination of agronomic methods and resistant cultivars is the best approach to reduce or even avoid a *Fusarium* infection.

Former studies identified the QTL *Qfhs.ifa-5A* as promising candidate for FHB resistance. Classical genetic linkage maps could not be used, as they strongly depend on recombination frequencies, which are not uniform along the chromosome. To avoid this problem, radiation hybrid mapping (RH), a recombination independent approach was used. RH generated promising results and showed to be a good approach for fine-mapping recombination-poor regions on the genome. This study showed, that the map resolutions could be strongly increased compared to genetic maps. It also showed, that the RH map unit cR is a better estimator for real physical distance than the map unit cM of genetic maps.

In future, the generated informative lines, with known deletions can be used in field trials, in order to find relations between deleted regions and their contribution to susceptibly to FHB. If plants with a deletion on a specific area are more susceptible to the disease, than this area becomes a point of interest to further search for the underlying resistant genes. These field trials will help to link the underlying genotype to phenotype.

Eventually, more precisely mapping the position of *Qfhs.ifa-5A* on the genome will help identifying genes involved in the resistance mechanism.

Getting more information about the genetic properties of resistant cultivars is a big step for gaining fundamental knowledge and can be a useful tool for breeding programs and one further step to prevent devastating FHB outbreaks.

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7. Appendix

Table 9: List of all used marker, with type, putative bin position and reference.

Marker	Primer 1	Primer 2	Autor
gpg1293	GCAGCAGGAAAAATCAGCAT	GGTTCGGCCTGAGATCATT	2
IWB11440	CCGATTATTTGCCTTGCGTTTTTAC	AGCGTCGTGAAATCTGTC	3
gpg2	CGGTTGTGCCATTATTTGTG	CACCGGTCCTTCGATAAAAA	2
gwm443	GGGTCTTCATCCGGAACTCT	CCATGATTTATAAATTCCACC	4
cwem44c	AGTGCACTGCAAACACAGAG	AGCCGTACACCTTCATAGGC	8
gpg537	ATCTCGTCGCGAGAAACCTA	CGGCTACACGTAAGGGGTAA	2
IWB62899	TGCTATGGCTATACTACGGC	GCGCCGAAGCCATTGACT	3
IWB4146	GGCTGGGAAACTCAAGGATC	AAACCGTTCTCATTAGCCTC	3
gpg2328	GACGACACAAGTGCCATGTT	CGTTTGTTCCACAAATCACG	2
gpg2326	CAGCGTCAGTCCGGATTAGT	TCTAATTCTTCGGCGACGAT	2
IWB68241	AAGTTCAGGATCATTCACTATTTC	AGCATTCCTTTCCTTTCACTACA	3
barc186	GGAGTGTCGAGATGATGTGGAAAC	CGCAGACGTCAGCAGCTCGAGAGG	5
ldk243	GGTTTCACCTCTAGCCTACCC	CACCTTGTGTGGGAGTTTCC	2
IWB51518	AAGCGCATCCAAGAACCTGA	TCCAAAAGGAGGAACCCGAT	3
ldk267	AATTAGCAGACCGCATGTACG	TCCAAGTTGAGAGCTGATGG	2
barc56	GCGGGAATTTACGGGAAGTCAAGAA	GCGAGTGGTTCAAATTTATGTCTGT	5
ldk284	TCTCATTGGTCAGGGTCAGG	TTCTCCTCCAGGTAGCTCTCC	2
gpg2162	AAGATCAAATGGCCCTTCCT	GGCTATGCATGGTCCAATCT	2
gpg2163	AGGTCGCGCACTGTTAGATT	CATGTACTCGGCGTTCACAT	2
gpg1438	GCGGTTGGATGAAGATCCTA	TCCGTATTGCCTAGCTTGCT	2
barc117	TCATGCGTGCTAAGTGCTAA	GAGGGCAGGAAAAAGTGACT	5
gpg2168	TGTCCCCTGCCTTCTGTTAC	GTCCACCGTCAGGTCATCTT	2
gpg1778	-	-	-
gpg2038	GAGTCCAAAACATGGGCAAT	TGGTGTGCTCACGTCAGATT	2
IWB75561	TGGCATTCCTTACCTATTTGCG	CTAGTGGATGGGTGTTCACAT	3
gpg1358	-	-	-
jfio7	CTCCTGTGGCAGAACAGAGG	ATCGTGGGCGTCACACTATA	2
wmc150a	CATTGATTGAACAGTTGAAGAA	CTCAAAGCAACAGAAAAGTAAA	6
gwm293	TACTGGTTCACATTGGTGCG	TCGCCATCACTCGTTCAAG	4
gpg1440	ACAGGCCTGATCTGGTATGG	TGCTTGCTACGTCTCCAATG	2
gwm304	AGGAAACAGAAATATCGCGG	AGGACTGTGGGGAATGAATG	4
IWB8393	ACCGAAATAGGATTTGCCTCAT	TGCTTATCTTGATGGCCACA	3
gpg2186	-	-	-
wmc150b	CATTGATTGAACAGTTGAAGAA	CTCAAAGCAACAGAAAAGTAAA	6
gpg2049	GGCCAAAGAAAGCTTATCCC	CCAGTGAACCGTCTGCTGTA	2
gpg2060	CCGACAGGAACTTCCACTGT	CTCAATTCGGTTCTTCCCAA	2
IWB10809	TGGTACCACGCCAAAGTATACT	TTGCTCCAATGAGATGTGGA	3
BE498768	CTGCCCCTAGAAGTTTCTCGT	CAGCGAGTGACAATTCCAGA	1
ldk49	TCCACACACCACACACACAC	AGACGCTATCCGATCCTCTG	2

ldk2	ATCAGGTCCACACACCACAC	AATCCACGAAGACGCTATCC	2
gpg2233	GTCGACGTTCACATGACACC	TGGTCTTCCACCACTTGTCC	2
gpg1763	CCAACACAACATGAGCAACC	AATTTTTCCTGCATTGGTCG	2
gpg2092	GGTCCGCATTGTTAACAGGT	TTGGCTTGAAGCTATGCATG	2
gpg2072	TCCGAGTGACCTGTATGCTG	AATCCATGCTTCCCTCTGTG	2
gpg2126	TGACCAAGTGATGGGAATCA	CCGAAGAAGGACGAGAGATG	2
IWB58275	ATATAGTGAGTTGGAAGGGCAG	GTGAAGCTGATGGGAAGAAG	3
ldk217	TGGACTCCGAATAGGACTGG	ACCAACTTCATCGCTGTTGC	2
IWB33435	AGATAAGTGGCCTTGTCCTCT	CGGAAGTTGTGATGGGCTTATATA	3
gpg1342	-	-	-
gpg574	TGCTCCAAAACTCTCAACCA	ACACCAAACTTGCCTTCCAC	2
gpg277	TCCATGTTGTCTTCAACCCA	TCCAAGTAGAGACCCATCCG	2
ldk218	GTTGAAGATGTCGCTCATGG	CTTCACAAGGTCCGCTTCC	2
gpg1323	-	-	-
ldk241	AATCAGTCTTGATGAAGCAACG	CATGAAGCGTCAGCAGTAGG	2
gpg1139	ACCCGTAAGTTGCCGTTATG	CACTCATGTTGAACACACCCA	2
gpg1789	GGATGAGATCCACCTCCTGA	CCATCTCTTCGCCGAACTAG	2
gpg2309	GACCACCTTCGGATTAGTGC	CACCGTCAATAGGTCACGAA	2
gpg1575	-	-	-
jfio4	CGCAAGGTGATATGAGGTGTT	TACGTACATACGGGCGGGT	2
gpg1994	GGTGGAGGAATGTTCACAGG	CACCGTTTGCGATTATTGTG	2
gpg2250	AGCATCAGTGTTGTTGCAATG	GTATGAAACCCGTTTGGGTG	2
ldk50	ACCGTGTGTGATGCTTCTTG	GGTGCATGTGTGTGTGCTC	2
gpg2244	GCCTGGATCATGCGATAACT	GGTACGAGGGACTTGCATGT	2
gpg2313	CTCACCGCCATGAGTGAGTA	TCCAACTGCCAGAATTCTCC	2
ldk16	CTCTTGGGCTGATGGTGATG	ATCGAATCAGTGGGTGATCG	2
ldk14	TTTCTGTTTTGCCTCTGGAAA	GGGCCTTTCCCTTTTGTTTT	2
gpg2019	TCCCCACTTGCAACTAAACC	AAGTGGCATCAGCTGAAGGT	2
gpg2108	GCAACCGAAGAGATCCTAAGG	TTCCCAAGATGGGAGAGTTG	2
gpg1383	CCTCTAAGTCGTGCCTCGAC	AGTCCATCCGAGGTGAATTG	2
ldk242	CCTACAAACCTCTGCACTTGG	CGGAGGGAATATTGAACACG	2
gwm129	TCAGTGGGCAAGCTACACAG	AAAACTTAGTAGCCGCGT	6
jfio2	ACGCTGGAGACGTATCACTGT	GGTGTCCTTCCTGATCTCCA	2
ldk289	GCACATACCTTCATAGTGG	TGATGATGTGGCAAAGAAGC	2
gpg2231	CCTATCGGCCACACTCACTT	TTGGCTGCTCTTGACCATTA	2
gpg2232	CGATTAAGAGCGATAATCAACCA	TAAGAGACCGTTTTGGCCTG	2
gpg2075	ATAAGGCGCACTACCAGTGG	CCCTAGCCCATTATGCTCAA	2
gpg2097	TTGTGATTGCTGCTCACCTC	TTCCTCCAAAGGCACTGTCT	2
gpg2121	TGCTTGTTCTTGCTCCAATG	GGCCACCTTGCTACACATCT	2
gpg119	CACGTCACTGTCAAGTGGCT	CACACATGTATTACGGTTTCCG	2
gpg35	TTAACACGTCAGGTTGCGAG	GAGCCGACTGAACTGTCTCC	2
gpg2336	TGAAAGAGACACGACGCAAC	TCTTCCTCTGTGGTCCAACC	2
gpg743	CTATGTACGCACACAATGCG	GAACGTAAGAAGGCAGGCAC	2
gpg1294	CCTCGAGAGTTTTGGTCGAG	GCACCAACCAGGAGTAAAGG	2

gpg2117	GCAAGGTGTACGTCCTTCGT	CATGCTTGAACTTGCTCCAA	2
ldk113	CACTGCTCCACCACAGC	GCGAAGGGTTAAACCGTAAAC	2
gpg214	TAGCCCATCACAAGCATTCA	TCCCTTGTGGATTCAAGACC	2
gpg2020	GAGATGACCGACGGATTCAT	AACAGAACCATATGCCCTGC	2
gpg2147	TTGACATGCTTGTGGTGGTT	ACCTTAGCAATGCAGCCAGT	2
gpg2123	CTCTCGGAGTTGGTTTAGCG	GGAAGTTCCTTGGACATAACC	2
gpg2102	TCCTTTGAAGTCCTCGCACT	TGTACCTGTGAACGGAACCA	2
gpg1395	CTTCGGCCAATCAGAATTGT	GGGCGACCAAGGATTCTATT	2
gpg2158	GGCTGTCATTAATCGTCCGT	CGTGCATCACAGAAGTGCTT	2
gpg1321	CCATCGATCTTAGACGCACA	ATTGCTCTACGTGGTGCATG	2
ldk215	CTGAGCTGAAGCAAGACACG	CGGGCATCTTCTCTACATCG	2
gpg1777	TTCCTCAAGGAGCGTAGCAT	ACCAATCCATTGCCTACGAG	2
gpg158	ACGCACACCAACTTTTACCC	GTGGTGCATGAAGGAACAGA	2
gpg2034	CCTCCTGGCGAGCAGATAT	TTATCCACCATTGGTCCGTT	2
gpg2255	CGACCAGATAGGCTGGTAGC	GTTTCCATTAGGACCCCGTT	2
gpg2011	GTCTATCCACCCATCCATGG	GAACGCCGACAGTCATCAC	2
BE425161	GGATGGTTCTGACCCAATATG	ATCATGCCGACAAACAGCTT	1
gpg542	CAAATACCGAGGGGTTGCTA	TGACACTGAGGACATCTGCC	2
cfa2250	AGCCATAGATGGCCCTACCT	CACTCAATGGCAGGTCCTTT	7
jfio6	CAGTCCCTTATTCAGCACCG	TGCGTCGGTAACATCATCAT	2
gpg503	AGCACCGGGACTTTAACCTT	AATTTCAGGATGGATGACCG	2

Autor legend:

1	Akhunov et al. (2010)
2	Barabaschi et al. (2015)
3	Ramirez-Gonzalez et al. (2015)
4	Roder et al. (1998)
5	Song et al. (2005)
6	Somers et al. (2004)
7	Sourdille et al. (2001)
8	http://wheat.pw.usda.gov/GG2/index.shtml

R script and output: Correlation

```
setwd("C:/Users/gries/Desktop")

corr <- read.table(file ="corr.txt", header = TRUE, stringsAsFactors=FALSE)
class(corr$cr) # numeric

cor.test(corr$cr, corr$mbp,m="spearman")
#p 2.2e-16
#r 0.9997699

cor.test(corr$cr, corr$mbp,m="pearson")
#p 2.2e-16
#r 0.9945828

cr_2019 <- c(19.7,
        88.5,
        103.1,
        176.4,
        209,
        244,
        254.8,
        254.8,
        281.2,
        281.2,
        440.5,
        487.4,
        639.3
)

cr_2019_markerred <- c(19.7,
                       88.5,
                       103.1,
                       176.4,
                       209,
                       254.8,
                       254.8,
                       281.2,
                       281.2,
                       440.5,
                       487.4,
                       639.3
)
```

```
mbp_ref <- c(19,
             46.6,
             48.7,
             70.7,
             85.3,
             104.2,
             105.4,
             119.9,
             119.9,
             195.3,
             205.2,
             245.9
)

cm_2018 <-c(3.9,
       9.7,
       10,
       10.2,
       10.3,
       10.3,
       10.3,
       10.3,
       10.4,
       10.4,
       10.5,
       10.5,
       10.6
       )

cor.test(cr_2019, cm_2018, m="spearman")
#p 6.363e-10
#r 0.9860911

cor.test(cr_2019, cm_2018, m="pearson")
#p 0.06184 > als 0.05
#r 0.5310666

cor.test(cr_2019_markerred, mbp_ref, m="spearman")
#p 1.311e-13
#r 0.9982441

cor.test(cr_2019_markerred, mbp_ref, m="pearson")
#p 2.256e-11
#r 0.9950783
```