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The wheat Fusarium head blight resistance QTL *Qfhs.ifa-5A:* its association with anther extrusion and fine-mapping using irradiation-induced deletion lines

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Abstract

Fusarium head blight (FHB or scab) is a severe fungal disease of small grain cereals with global concern, causing yield and quality losses. FHB resistance is a quantitative trait and one of the strongest QTL, namely *Qfhs.ifa-5A,* protects wheat plants against initial fungal infection.

In the first part of the master thesis, the influence of anther extrusion (AE) on FHB resistance and its association with *Qfhs.ifa-5A* were studied in a greenhouse experiment. At anthesis heads from the cultivar Remus (susceptible, retaining anthers) and its near isogenic line NIL-C3 (C3; moderately resistant, harbouring *Qfhs.ifa-5A,* extruding anthers) were shortened to 16 spikelets and anthers were either compressed into florets or removed and a group without anther manipulation was implemented as control. One day after the manipulation heads were spray-inoculated with *Fusarium graminearum* and disease severity and incidence were evaluated multiple times. Following results were obtained: (i) C3 and Remus differed significantly for FHB resistance and AE with C3 being more resistant and having fewer anthers retained; (ii) for both genotypes, heads with removed anthers had significantly less symptoms than heads with compressed anthers or control heads, whereas C3 with removed anthers was significantly more resistant than Remus with removed anthers; (iii) Remus heads with compressed anthers were about as susceptible as Remus control heads while C3 heads with compressed anthers were significantly more diseased than C3 control heads but significantly less diseased than Remus heads with compressed anthers. This experiment confirmed that AE has an influence on FHB resistance controlled by *Qfhs.ifa-5A*; furthermore, the results suggest that – in addition to AE – other unknown factor(s) in this QTL region may also contribute to FHB resistance of C3.

In the second part the *Qfhs.ifa-5A* region on the 5AS chromosome was fine-mapped. Maps based on recombination could not give a sufficient resolution due to the centromeric location of *Qfhs.ifa-5A* and therefore a deletion mapping approach was performed. Seeds of C3 were gamma-irradiated and in the M_2/M_3 generation 1764 C3 plants were pre-screened for deletions in the QTL interval employing 15 5AS specific markers, resulting in 42 potentially interesting mutant lines. In this master thesis, these lines were further characterised with 102 5AS specific markers. Genotyping the 42 preselected lines yielded 28 lines with different deletion patterns. The calculated map had a size of 399.8 cR and separated the 5AS chromosome into 38 loci. The deletion mapping approach strongly increased the resolution of the *Qfhs.ifa-5A* locus compared to a recombination-depending map where only seven loci on the 5AS chromosome could be mapped within a distance of 1.2 cM.

Lines with deletions in the *Qfhs.ifa-5A* interval were backcrossed with C3 to reduce unwanted background deletions. It is expected that phenotyping these lines for FHB resistance and AE will narrow down the most likely QTL region.

Understanding the resistance mechanism(s) of *Qfhs.ifa-5A* can help to breed highly resistant cultivars, which is the most effective method to control FHB and to reduce mycotoxin levels in grain.

Keywords: Fusarium head blight, *Fusarium graminearum*, Fusarium *Type 1* resistance in wheat, *Qfhs.ifa-5A*, FHB resistance QTL, anther extrusion, deletion mapping

Deutsche Zusammenfassung

Ährenfusariose (engl. Fusarium Head Blight, FHB oder scab) ist eine weltweit bedeutende Getreidekrankheit, die durch Pilze der Gattung *Fusarium* verursacht wird und Ertrags- bzw. Qualitätseinbußen (Mykotoxinbildung) zur Folge hat. Die Resistenz gegenüber Ährenfusariosen wird quantitativ vererbt. Ein wichtiger Resistenz-QTL ist *Qfhs.ifa-5A*, dieses schützt Weizenähren vor Fusariuminfektionen.

Im ersten Teil dieser Masterarbeit wurde der Einfluss des Antherenausstoßes bzw. der Antherenzurückhaltung auf die Fusarioseresistenz in einem Glashausexperiment untersucht. Die Sorte Remus (anfällig, hält Antheren zurück) und die davon abgeleitete nah-isogene Linie Remus NIL-C3 (C3, resistent, stößt Antheren aus, trägt *Qfhs.ifa-5A*) wurden in diesem Versuch verwendet. Während der Blüte wurden die Ähren auf 16 Blütchen eingekürzt und die Weizenblüten auf drei unterschiedliche Weisen manipuliert: keine Manipulation (Kontrollgruppe), Antheren wurden in Blüten hineingepresst (simuliert Antherenzurückhaltung) und Antheren wurden entfernt (simuliert Antherenausstoß). Anschließend wurden die Weizenähren mit *Fusarium graminearum* sprühinokuliert. Zu verschieden Zeitpunkten wurde der Fusariumbefall ermittelt. Folgendes wurde beobachtet: 1) Der Genotyp C3 war resistenter als Remus und hatte einen höheren Antherenausstoß, 2) bei beiden Genotypen zeigten Ähren mit entfernten Antheren die höchste Resistenz, wobei C3-Ähren mit entfernten Antheren resistenter waren als Remus-Ähren mit entfernten Antheren, 3) Remus-Ähren mit hineingepressten Antheren zeigten ein ähnliches Resistenzniveau wie die Remus-Kontrollgruppe, während C3-Ähren mit hineingepressten Antheren signifikant anfälliger waren als die C3-Kontrollgruppe, jedoch resistenter als Remus-Ähren mit simulierter Antherenzurückhaltung.

Die Ergebnisse zeigen, dass der Antherenausstoß einen Einfluss auf Ährenfusariosenresistenz hat, möglicherweise tragen aber auch noch andere Faktoren/Gene zur Resistenz der Linie C3 bei.

Im zweiten Teil der Arbeit wurde die *Qfhs.ifa-5A*-Region auf dem kurzen Arm des Weizenchromosoms 5A (5AS) feinkartiert. Bisher verfügbare Karten, die auf Rekombinationskartierungen basieren, erzielten nur eine geringe Auflösung, da *Qfhs.ifa-5A* im zentromernahen Bereich lokalisiert ist und die Rekombinationsrate in diesem Bereich sehr niedrig ist. Es wurde daher eine gammastrahlungsinduzierte Deletionskartierung durchgeführt. 31 vorselektierte Genotypen (von einer abgeleiteten gammastrahlungsinduzierten Mutantenpopulation von 1764 M_2- bzw. M_3-C3-Pflanzen) wurden mit 102 5AS-spezifischen Markern genotypisiert. Aus 28 Deletionslinien mit unterschiedlichen Deletionsmustern konnte eine Karte mit 38 Loci und einer Länge von 399.8 cR erstellt werden. Es konnte bestätigt werden, dass die strahlungsinduzierte Deletionskartierung eine geeignete Methode für die Feinkartierung der *Qfhs.ifa-5A*-Region ist.

Nach der Elimination ungewollter Hintergrundmutationen durch Rückkreuzung mit C3-Pflanzen können die Deletionslinien für die Merkmale Antherenausstoß und Fusarioseresistenz bonitiert werden und so die Genidentifizierung unterstützen.

Der Einsatz von resistenten Sorten ist die einfachste Methode, um Ährenfusariosen und die damit verbundenen Mykotoxingehalte zu reduzieren. Das Wissen über den Resistenzmechanismus von *Qfhs.ifa-5A* ist essentiell für die Züchtung von Sorten mit verbesserter Ährenfusarioseresistenz.

Schlüsselwörter: Ährenfusariose, *Fusarium graminearum*, Antherenausstoß, Fusarium-Typ 1-Resistenz in Weizen, *Qfhs.ifa-5A*, Fusariumresistenz-QTL, Deletion Mapping

List of abbreviations

AE	Anther extrusion
AR	Anther retention
ANOVA	Analysis of variance
AUDPC	Area under disease progress curve
BBCH	Plant development stages scale Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie, Germany
BC	Backcross
Bp	Base pairs
C3	NIL-C3, Remus NIL C3
cM	Centimorgan
cR	Centi-Ray
CTAB	Cetyl trimethylammonium bromide
Cy5	Cyanin 5
dai	Days after inoculation
df	Degrees of freedom
DNA	Deoxyribonucleic acid
DON	Deoxynivalenol
EST	Expressed-sequence-tag
FAM	6-carboxy fluorescein
FDK	Fusarium damaged kernels
FHB	Fusarium head blight
Gy	Gray, unit of radiation dosage
h^2	Heritability
IFA BP	Department of Agrobiotechnology Tulln, Institute for Biotechnology in Plant Production
IWGSC	International Wheat Genome Sequencing Consortium
MAS	Marker assisted selection
NIL	Near isogenic line
$MgCl_2$	Magnesium chloride
PCR	Polymerase chain reaction
QTL	Quantitative trait loci
RIL	Recombinant inbreed line
sd	Standard deviation
SSR	Single sequence repeats, microsatellite marker
Taq	*Thermophilus aquaticus* polymerase
ZON	Zearalenone

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1 Introduction

1.1 Wheat – an important crop

Wheat (*Triticum aestivum*) is one of the most important crops for human nutrition. Worldwide wheat production was 730 million metric tons in 2014, cultivated on 220 million hectares (FAOSTAT, 2016). Wheat is not only used for food, also for livestock feed, industrial biomaterials (like starch, wheat protein, ethanol) and bioenergy.

To satisfy the increasing worldwide wheat demand, research and breeding is done continuously to improve wheat. Besides the most important trait, which is yield, other traits such as quality parameters, disease resistance, and resistance to abiotic stress (droughts) are important breeding aims (Fernandes et al., 2000). A very devastating disease is Fusarium head blight (FHB, or scab) which causes substantial yield and quality losses in wheat production. The resistance aspect against FHB in wheat will be the focus of this master thesis.

1.2 Fusarium head blight in wheat – a fungal disease with global concern

FHB is a fungal disease with global concern (Gilbert and Tekauz, 2011; McMullen et al., 1997). FHB occurs in most parts of the world, epidemics were reported from Europe, North America, South America, Asia and Australia (Buerstmayr et al., 2012; McMullen et al., 1997; Nganje et al., 2004).

FHB is a fungal disease with a huge economic impact (Gilbert and Tekauz, 2011; Nganje et al., 2004; Salgado, 2014; Schmale and Bergstrom, 2003). Nganje et al. (2004) estimates the FHB-related financial loss of the main wheat and barley growing regions in the United States in the time period from 1993-2001 with 2.49 billion US Dollars. Farmers and cereal processors can have substantial losses or costs in context to FHB:

- Losses in yield, quality losses
- Costs for fungicide application
- Costs for increased soil tillage (under plow crop residues)
- Costs for mycotoxin contaminated feed (if allowed: blend feed with uncontaminated feed, use feed additives to bind/detoxify mycotoxins)
- Loss in livestock production due to mycotoxin contaminated feed (reduced feed conversation rate, aborts)
- Sorting cost of kernels (e.g. optical sorters like Sortex (Buhler Group, 2016))

1.2.1 Causal pathogens and disease cycle

FHB can be caused by different *Fusarium* species, of these *Fusarium graminearum* (teleomorph *Gibberella zea*) and *F. culmorum* are the most aggressive species (Buerstmayr et al., 2012; Mesterházy, 1977; Mesterházy et al., 2003). *Table 1* gives an overview of *Fusarium* species identified on wheat and the corresponding mycotoxins. For some *Fusarium* species, only the asexual form (anamorph) is known (*Table 1*), while other *Fusarium* species can produce sexual ascospores in perithecial (teleomorph form). *Fusarium* species belong into the genus of *Gibberella*, family of *Nectriaceae* in the phylum of *Ascomycota*. *Fusarium* species are facultative parasitic and/or saprophytic fungi, that means they can live on live plants and/or on dead plant material like crop debris (Summerell, 2001).

Table 1: Toxigenic *Fusarium* species identified in Europe on wheat heads and kernels modified after Stepien and Chelkowski (2010, further references therein)

Fusarium species	Teleomorph	Metabolites produced on solid media[1]
F. graminearum	*Gibberella zeae*	DON, 3AcDON, 15AcDON, NIV, ZEA, ZEA-sulphate, 4.7DeDON
F. culmorum		DON, 3AcDON, NIV, ZEA
F. avenaceum	*Gibberella avenacea*	MON, ENN-A, ENN-A_1, ENN-B, ENN-B_1, Aven Y
F. cerealis (F. crookwellense)		NIV, FUS-X, ZEA
F. poae		NIV, FUS-X, FU-C, DAS, MAS, NEO, T-2, HT-2, BEA, ENN-A, ENN-A_1, ENN-B, ENN-B_1
F. sporotrichioides		T-2, HT-2, NEO, DAS, SCI, AcT-2, 8Ac T-2, 3-hydroxy T-2, T-2 triol, 4Actetraol, 15Actetraol
F. langsethiae		T-2, HT-2, MAS, DAS, SCI, NEO, T-2 tetraol, T-2 triol, ENN-B
F. tricinctum	*Gibberella tricincta*	MON
F. chlamydosporum		MON
F. semitectum (F. pallidoroseum)		BEA
F. equiseti	*Gibberella intricans*	FUS-X, SCI, FCH, ZEA, DAS, NIV, T-2 tetraol
F. acuminatum var. armeniacum	*Gibberella acuminata*	T-2, MON, ENN-B
F. verticillioides (= moniliforme)	*Gibberella moniliformis*	FB_1, FB_2, FB_3, FU-C, BIK
F. subglutinans		MON, BEA, FUSA, BIK
F. proliferatum	*Gibberella intermedia*	FB_1, FB_2, MON, BEA, FUSA, BIK
F. anthophilum		MON, FB_1, FB_2, BIK
F. solani	*Haematonectria haematococca*	MON, BIK
F. oxysporum		MON, BIK

[1] Used metabolite abbreviations: Aven Y – avenacein Y, yellow pigment with strong antibiotic characteristics; BEA – beauvericin; BIK – bikaverin, pigment with antibiotic characteristics; DAS – diacetoxyscirpenol; DON – deoxynivalenol and its derivatives 3-AcDON, 15-AcDON and 4.7DeDON; ENN – enniatin (A, A_1, B, B_1); FB_{1-3} – fumonisin B_{1-3}; FCH – fusarochromanone; FU-C – fusarin C; FUSA – fusaproliferin; FUS-X – fusarenone; HT-2 – HT-2 toxin; MAS – monoacetoxyscirpenol; MON – moniliformin; NEO – neosolaniol; NIV – nivalenol; SCI – scirpentriol; T-2 – T-2 toxin, and derivatives T-2 tetraol, T-2 triol, 8Ac T-2 and 3-hydroxy T-2; ZEA – zearalenone and derivative ZEA-sulphate.

Some authors number *Microdochium nivale* (snow mold) among the *Fusarium* complex, this pathogen can also cause head blight but it does not produce toxins (Bai and Shaner, 1994).

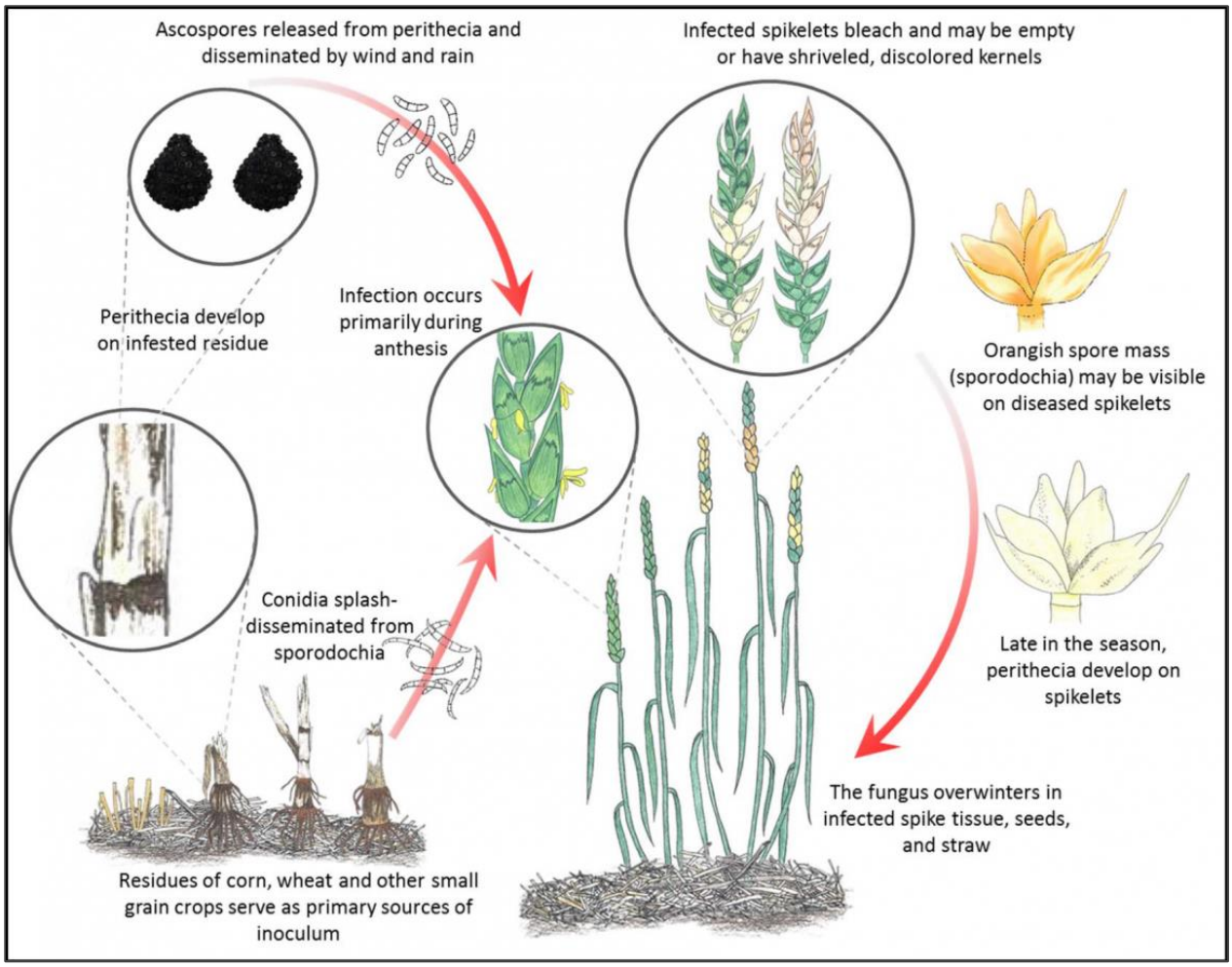

Figure 1: FHB disease cycle (Mills et al., 2016)

Disease cycle

Figure 1 presents an overview on the disease cycle of *F. graminearum* in wheat. The pathogen overwinters as mycelium, asexual conidia or in perithecia on infected crop residues (like straw, corn stalks, stubble) on soil surface (Mills et al., 2016). Here the fungi live in a saprophytic manner on dead plant material. It is relevant for the disease cycle that crop residues are on the soil surface, because *Fusarium* fungi need oxygen for growth and spores can only be released and transmitted to plants if perithecia have contact to air. In spring, when the temperature increases, conidia (or ascospores) can be transmitted via raindrop-splashes and wind from the crop residues directly to flowering wheat heads. Spores can also be indirectly transmitted by ´jumping´ stepwise from leaf levels to the florets of the head. The critical time point for infection is wheat flowering (BBCH 60-69) (Buerstmayr and Buerstmayr, 2015; Parry et al., 1995). Rainy weather during anthesis is favorable for FHB transmission and spore germination (as they need free water) and increases the risk of FHB infection (McMullen et al., 1997; Mills et al., 2016). *Fusarium* infection starts with penetration of spores into the florets and germination on anthers or on soft tissue (Bushnell et al.,

2003; Kang et al., 2005). From this initial infection, the fungi can grow to other spikelets of the head. Depending on the weather/temperature, first symptoms can be observed on the spikelets about four days after infection: first infected spikelets bleach and become necrotic due to death of tissue. Then the fungi spread within the head and more and more spikelets or even the whole head get bleached (which is the reason for the name head blight). Spikelets above infected spikelets can die or dry out even if they are not colonized, because the fungi can also affect the stem/rachis and block water/nutrient transport (Mills et al., 2016). Some days after symptoms are visible (depending on the weather situation), salmon to pinkish mycelia and sporodochia (asexual spore bodies where conidia are formed) can occur. Through early occurring sporodochia, conidia can be blown to late tillers or neighboring (later flowering, e.g. spring wheat) fields and secondary infections can appear (Mills et al., 2016).

After harvest the fungi live saprophytically on infected crop residues and the infection can start again in the following spring. Also perithecial (sexual spore bodies) can be observed on dead plant material in the case of *Gibberella zeae*.

Figure 2: Fusarium damaged kernels are shrunk and brighter than unaffected kernels (Mills et al., 2016).

Infected spikelets fail to develop kernels or produce shrunk *Fusarium* damaged kernels (FDK) (*Figure 2)*. These kernels are brighter (sometimes a little pinkish), have a lower thousand kernel weight and can be contaminated with mycotoxins. Very small FDK are not the problem, they are normally sorted out during the threshing process on the combine harvester. Problems are caused by nearly normal sized FDK, as they cannot be separated easily and contaminate food and feed with mycotoxins. There are some optical sorters (Buhler Group, 2016) which can eliminate FDK by color difference; but this process does not always work properly and only big cereal processers can afford such machines.

Host plants

All small grain cereals (wheat, durum wheat, triticale, rye, barley oats, rice, corn) can act as a host for *Fusarium* species. *Fusarium* species can live also on other plant species (dicotyledons), in some cases without showing symptoms or they live saprophytically on various kinds of dead plant material. Goswami and Kistler (2004) report following species as host plants for *Fusarium* ssp: *Agropyron, Agrostis, Bromus, Calamagrostis, Cenchrus, Cortaderia, Cucumis, Echinochloa, Glycine, Hierochloe, Lolium, Lycopersicon, Medicago, Phleum, Poa, Schizachyrium, Secale, Setaria, Sorghum, Spartina* and *Trifolium*.

1.2.2 Losses due to FHB and mycotoxins

FHB reduces grain yield and quality. Yield loss results because infected florets fail to develop kernels or shrunk kernels are formed. The most important consequence of FHB is the quality loss due to the contamination with mycotoxins. These mycotoxins are secondary fungal metabolites and have negative effects (i.e. toxicity) for humans, livestock, and plants. Mycotoxins can cause severe illness even in the ppm or ppb range. Furthermore, FHB infected kernels have negative influence on quality parameters:

- Poor baking properties: low dough stability and volumes (Lancova et al., 2008)
- Poor malting/brewing properties: gushing of beer (Nielsen et al., 2014)
- Low germination rate and seedling blight (Bai and Shaner, 1994)

Many different mycotoxins (and also derivatives, masked mycotoxins) are known. Deoxynivalenol (DON) and Zearalenone (ZON) are the most important derivatives in case of FHB in wheat.

Deoxynivalenol (belonging to the trichothecenes) inhibits the protein biosynthesis (Goswami and Kistler, 2004; Schmeitzl et al., 2015). Especially in pig husbandry this toxin causes problems. Pigs being fed on DON contaminated feed show a lower feed conversion rate (lower feed uptake) and have a lower immune defense. If the feed is highly contaminated, vomiting and diarrhea can occur (thus, DON is also known as vomitoxin) (Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, 2016).

Zearalenone has a high estrogenic activity, it binds on estrogen receptors. Especially in mother sow husbandry, ZON causes severe problems. If female pigs are feed with ZON contaminated feed, aborts and infertility can occur (Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, 2016).

Mycotoxins are chemically and thermally very stable compounds, procedures like heating or long storage do not degrade or reduce the toxins (Bullerman and Bianchini, 2007). As a result, mycotoxins stay in the whole food chain from the field to the ultimate consumer.

Because of the negative consequences of the mycotoxins, there are strict regulations concerning the mycotoxin contents in food. In Europe the EU regulation EC 1881/2006 specifies mycotoxin levels for processed and unprocessed food (Commission Regulation (EC) No 1881/2006). For feed, there are guidelines from the European Commission (Commission Recommendation No 576/2006).

1.2.3 FHB and mycotoxin management

There are numerous approaches for managing FHB. Some factors like weather or pathogen aggressiveness cannot be influenced by farmers. Thus, the application of farm management practices is an important factor to reduce FHB and consequently also mycotoxin contaminations. The following section presents the most important farm management practices to reduce FHB and mycotoxin contaminations.

Crop rotation

Fusarium species have a wide host plant range; therefore, crop rotation has a big impact on FHB. The risk of FHB is increased if a very susceptible crop is grown after a crop with a high *Fusarium* inoculum potential; in particular, the cultivation of wheat after maize is a critical combination (Miedaner, 2012). Crop rotations with mainly *Fusarium* host plants like maize and small grain cereals increase the risk of FHB (Miedaner, 2012), whereas dicotyledonous plants can significantly decrease the FHB risk.

Tillage

Non- or minimal tillage systems are getting more common in agriculture. Due to minimal tillage, a lot of crop residues remain on the soil surface, representing a massive inoculum source for the following crop. After pre-crops with a high *Fusarium* inoculum potential, the best way is to plow under the potential inoculum source; so no crop residues remain on the surface (Osborne and Stein, 2007). For soils where ploughing is not possible, all methods which lead to a fast rotting process are beneficial to reduce *Fusarium* inoculum sources.

Fungicide application

Chemical control of FHB is difficult: Many factors like application time, weather/environmental conditions, active ingredient of fungicides, and application technique have an influence on the effectiveness of a chemical treatment. The most effective fungicides contain a triazole, especially tebuconazole, as active ingredient (Bayer CropScience AG, 2016; Paul et al., 2008, 2010). In Austria several fungicides against FHB are registered, mostly with a triazole as active ingredient (AGES, 2016): Ampera® (prochloraz + tebuconazole), Caramba® (metconazole), Diapazon® (fenpropidin + propiconazole + tebuconazole), Folicur® (tebuconazole), Gladio® (fenpropidin + prothioconazole + tebuconazole), Magnello® (difenoconazole + tebuconazole), Prosaro® (prothioconazole + tebuconazole), Pronto Plus® (spiroxamine

+ tebuconazole), Osiris® (epoxiconazole + metconazole), Soleil® (bromuconazole + tebuconazole) and Zantara® (bixafen + tebuconazole). Fungicides must be distributed evenly on the whole head to get a good fungicide effectiveness. For this kind of treatment, twin flat spray nozzles show the best application results: heads are covered from two sides with the fungicide solution (Bayer CropScience AG, 2016). Timing of fungicide application is very difficult, particularly in inhomogeneous fields (lot of later tillers) or for long anthesis duration. Only a small time frame is available to obtain a satisfying FHB reduction: early anthesis to mid anthesis (BBCH 61-65) represents the optimal application time (Paul et al., 2007). Several studies about fungicide effectiveness were published (Mesterházy et al., 2003; Paul et al., 2008, 2010); furthermore, numerous field trials for fungicide effectiveness were performed by plant protection companies and agricultural advisory bodies (Balz, 2016). These studies conclude that fungicide application can only reduce about 80% of FHB/mycotoxins in the best cases; therefore, fungicide application is not sufficiently effective as a single FHB management method. Mesterházy et al. (2003) reported that the resistance of the wheat cultivar has a significant impact on the fungicide effectiveness. The fungicide effectiveness is higher for relatively resistant genotypes and lower for very susceptible genotypes.

The application of biological agents, e.g. microorganism, might be another possible treatment option. Biological agents could be applied on crop residues for reducing inoculum amount, on grain kernels as seed treatment or as biological fungicide on flowering cereals (Palazzini et al., 2007). So far, no biological product is available in Austria or Germany.

Resistant cultivars

Resistant cultivars are the best FHB management tool to reduce FHB/mycotoxin contamination (Buerstmayr et al., 2012). Resistant cultivars are the least expensive and easiest FHB control measure for famers, both conventional and organic. Further details on FHB resistance breeding are presented in section 1.3.

Beside these main approaches, a lot of other plant cultivation practices also have an influence on FHB, for example irrigation management, stem shortening, and plant density.

The combination of different FHB management strategies is the best way to reduce FHB.

1.3 FHB resistance breeding in wheat

The use of resistant cultivars is the most effective and the most cost-efficient way to control FHB. Resistant cultivars also protect succeeding plants in the crop rotation (e.g. maize), as a smaller amount of inoculum source remains on the field when compared to susceptible cultivars.

FHB resistance is an important trait for farmers as well as for breeders. FHB resistance becomes a trait in value for cultivation and use (VCU) testing at official testing authorities (AGES, 2016; Bundessortenamt, 2016). For example, in Germany, cultivars with a FHB rating of 7 or worse will not pass the VCU testing and therefore will not be registered (Bundessortenamt, 2016; Miedaner, 2012).

Breeding for FHB resistance is challenging: FHB resistance is a quantitative (polygenic) trait, meaning several genes/quantitative trait loci (QTL) control the resistance (Buerstmayr et al., 2012; Miedaner, 2012; Salameh et al., 2011). Combining FHB resistance with other important traits increases the difficulty for breeders even more. In most cases, a compromise between FHB resistance and other traits has to be made.

Breeding programs in Europe use different strategies for resistance breeding (Mesterházy, 2003; Miedaner, 2012): i) combing resistance from well adapted, high yielding cultivars with moderate resistance; ii) repeated backcrossing of very resistant germplasm to well adapted lines. Salameh et al. (2011) and Miedaner (2012) reported that resistance from the highly resistant cultivar Sumai-3 was transferred into European winter wheat without negative linkages (yield, etc.). FHB resistance breeding is also performed for low input farming systems: in North America, resistance QTL from Sumai-3 was introgressed into adapted spring wheat cultivars without negative linkage drags (Anderson et al., 2007; U.S. Wheat and Barley Scab Initiative, 2016).

Pyramiding FHB resistance QTL in breeding programs can increase resistance levels (Anderson et al., 2007).

1.3.1 Types of resistance

Different types of active and passive resistance were reported. Mesterházy (2003) summarized the five most important types of active resistance:

1) Resistance to initial infection/penetration – *Type 1* resistance (Schroeder and Christensen, 1963)
2) Resistance to fungal spread – *Type 2* resistance (Schroeder and Christensen, 1963)
3) Resistance to kernel infection (Mesterházy, 1995; Mesterházy, 1997)
4) Tolerance to FHB (Mesterházy, 1995)
5) Resistance to toxins (Mesterházy, 1995; Mesterházy, 1997)

Beside active resistance mechanisms, also passive resistance mechanisms (morphological traits) were reported, which can have a distinct influence on FHB resistance/susceptibility (Jones, 2015; Rudd et al., 2001; Schmolke et al., 2005):

Anther extrusion (AE): Genotypes with a high level of AE show a higher FHB *Type 1* resistance compared to cultivars which partially extrude their anthers. Further details on the connection between AE and FHB resistance are given in section 1.4.

Plant height**:** Many studies show that taller plants are more resistant (*Type 1*) than dwarf cultivars (with *Rht*-genes) (He et al., 2016; Miedaner and Voss, 2008; Schmolke et al., 2005; Teutschl, 2016). Shorter cultivars are more susceptible: due to the smaller distance of heads to soil surface inoculum from crop debris on the soil surface can easier reach heads and also the humidity in the head region is higher.

Heading/Flowering date: The effect of flowering date on FHB resistance is strongly discussed (Liu et al., 2007; Jones, 2015 and references therein): positive, negative and no correlation between flowering date and FHB resistance were reported. Schmolke et al. (2005) found a negative correlation between FHB severity and flowering date, meaning that later flowering lines show less FHB severity. Other authors did not observe a correlation between flowering date and FHB resistance (Liu et al., 2007). Potentially, weather conditions at anthesis (in terms of temperature and humidity) influence infection and disease development.

Tillering numbers: Jones (2015) reported that tillers can act as a barrier for spore movement so that in a dense field it is more difficult for the *Fusarium* spores to reach heads.

It has also been reported that head characteristics such as presence/absence of awns and head compactness /spikelet density can have an influence on FHB resistance (Schmolke et al., 2005).

1.3.2 Source of FHB resistance - FHB resistance QTL

Many QTL mapping studies for FHB resistance have been performed and more than 100 FHB resistance QTL have been reported (Buerstmayr et al., 2009, 2012). An overview is given in *Table 2.* Very resistant cultivars are Frontana (Brazil), Arina (Switzerland), Chokwang (Korea), Sumai-3 and Wangshiubai (both China). In addition to the primary wheat gene pool, resistance against FHB can also be found in wild emmer wheat or in relatives of wheat, like *Aegilops* (Cai et al., 2005, 2008). Resistance from these sources can be introgressed in modern cultivars more easily when markers from QTL studies are available.

Table 2: Mostly reviewed QTL for FHB resistance, summarized after Buerstmayr et al. (2012; references therein)

Chromosome	Proposed QTL	Source of resistance allele	FHB resistance component
3BS	*Qfhs.ndsu-3BS, Fhb1*	Sumai-3 and related lines (Ning 7840, CM-82036)	FHB spread within spikes, FHB field severity
3BS	*Qfhs.nau-3BS, Qfhs.ksu-3BS1*	Wangshiubai, Nyu bai and Chokwang	FHB spread within spikes
5AS	*Qfhs.ifa-5A, Qfhi.nau5A*	Sumai-3 and related lines, landraces of Wangshiubai, Nyu bai	FHB incidence, FHB field severity
6BS	*Qfhs.nau-6B, Qfhs.lfl-6BS, Fhb2*	Sumai-3 and related lines, Ning 894037, Wangshiubai, Blackbird (*T. carthhlicum*)	FHB spread within spikes
4B	*Qfhi.nau-4B, Qfhs.ksu-4BL1, Fhb4*	Wuhan #1, Wangshiubai, Chokwang	FHB incidence, FHB spread within spikes (in Chokwang)
3BSc		Wangshiubai, Nyu-Bai, DH 181	FHB spread within spikes
1BL	*Qfhs.lfl-1BL*	Cansas, Pirat, History, Biscay	FHB field severity
6AL	*Qfhs.lfl-6AL*	Dream	FHB field severity
7BS	*Qfhs.lfl-7BS*	Dream	FHB field severity
4DS	*Rht-D1b*	Lines with *Rht-D1a*	FHB field severity
3AS	*Qfhs.ndsu-3AS*	FA-15-3 (*T. dicoccoides*)	FHB spread within spikes

1.3.3 Testing FHB resistance: inoculation and phenotyping

For resistance breeding and research it is necessary that FHB resistance can be evaluated in field trials. Testing cultivars (breeding lines) for FHB resistance is costly (Mesterházy, 2003; Rudd et al., 2001). Natural infection is inexpensive, but may not always be successful due to (i) yearly changes of inoculum quantity, (ii) unfavorable weather conditions at infection, (iii) potential origin of natural infection from a low aggressive *Fusarium* species (thus pretending a good resistance) and (iii) uneven infection pressure across nursery/testing area.

Due to these reasons, artificial inoculation for FHB resistance evaluations is frequently applied for estimating genetically determined resistance as precisely as possible (Mesterházy, 2003). For testing *Type 1* resistance, artificial spray inoculation and/or grain spawn inoculation are most commonly used. A high rate of conidia of an aggressive *Fusarium* species and optimal microclimate (humid and warm) for conidia germination promote successful infection. High humidity can be ensured by mist irrigation or covering wheat heads with polyethylene bags after inoculation (Mesterházy, 2003). Testing *Type 2* resistance is even more difficult; in this case, point inoculation of spikelets is often done to measure spreading of the fungus within the head (Jungreithmeier, 2016).

Different parameters can be used to express FHB susceptibility/resistance:

- Incidence (percentage of infected heads per plot)
- Severity (percentage of infected spikelets)
- Area under disease progress curve (AUDPC) – an integrated measurement of infection level and progress
- Fusarium damaged kernels (%FDK)
- Mycotoxin concentrations in grain

In most cases, phenotyping of incidence and severity is used, because it is a cost-effective method and can be done easily in the field or greenhouse. If phenotyping is done several times, also AUDPC can be calculated. For evaluation of FDK, sampling is important (combine harvester settings) in order to obtain the real amount of very small FDK in the sample (Blöch, 2014). Mycotoxin measurements are performed via high performance liquid chromatography (HPLC) or enzyme-linked immunosorbent assay (ELISA). These tests are expensive, but necessary for testing resistance against mycotoxins.

1.3.4 Marker assisted selection and genomic selection for FHB resistance

Marker assisted selection (MAS) becomes a very important tool in plant breeding. The main advantages of MAS compared to phenotypic selection are low costs and fast execution (Arruda et al., 2016). The presence of resistance genes/QTL can be predicted even at the seedling stage. Through a lot of mapping studies, markers for many agronomically important traits and also FHB resistance are known (Sørensen, 2016). MAS is also very valuable in FHB resistance breeding: (i) phenotyping for FHB resistance is very costly (many plants for FHB resistance testing are needed) – due to MAS selection can be done without FHB infection; (ii) in marker assisted backcrossing often a resistant, exotic genotype is many times backcrossed with an adapted cultivar – here selection for the resistance QTL can be done even at seedling stage; (iii) if QTL are pyramided, a single QTL may not be evaluated at phenotypic level, because other QTL may overlay the effect.

Due to inexpensive and whole genome genotyping also genomic selection (GS) can be done for FHB resistance (Rutkoski et al., 2012). By intensive phenotyping and genome wide genotyping of the training population also minor QTL can be exploited. GS can be done for many traits simultaneously and can shortening the breeding cycle which lead into increasing selection gain per unit time (Steiner et al., 2017).

1.4 The effect of anther extrusion on FHB resistance

In the following section, an overview of the wheat flower morphology, the flowering process, the fungal growth in the floret and the relationship of AE with the *Type 1* resistance QTL *Qfhs.ifa-5A* is given.

1.4.1 The wheat flower morphology and flowering process

The wheat head consists of several spikelets which are located on the rachis (main axis). The spikelets in the middle of the head are developed best, while spikelets on the head top and bottom are smaller, later in development and can also be infertile. A spikelet is surrounded with two hull glumes and can have two to five florets; the basal florets of a spikelet are bigger and earlier in development compared to the florets in the middle.

A floret is surrounded by lemma and palea. The sexual reproductive organs are located inside the floret (*Figure 3, 4*): anthers on filaments and stigmas on the ovary. On the bottom of the floret there are two lodicules which control flower opening (Bushnell et al., 2003).

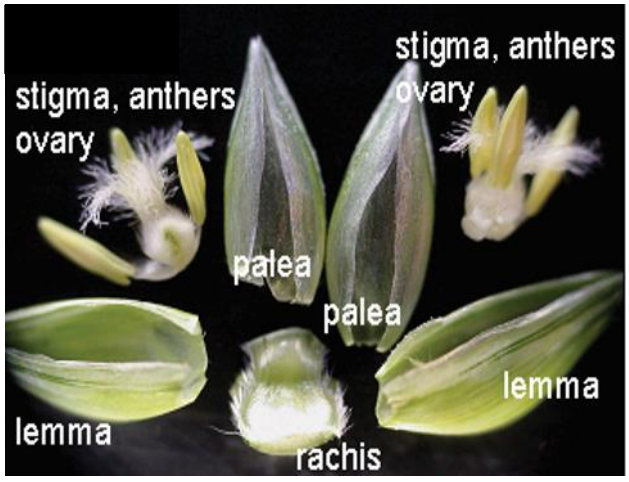

Figure 3 (above)*:* Wheat flower morphology: floral tissues, separated in the reproductive part (ovary, stigma, anthers), the lemma palea and the subtending section of the rachis (from Steiner et al., 2009)

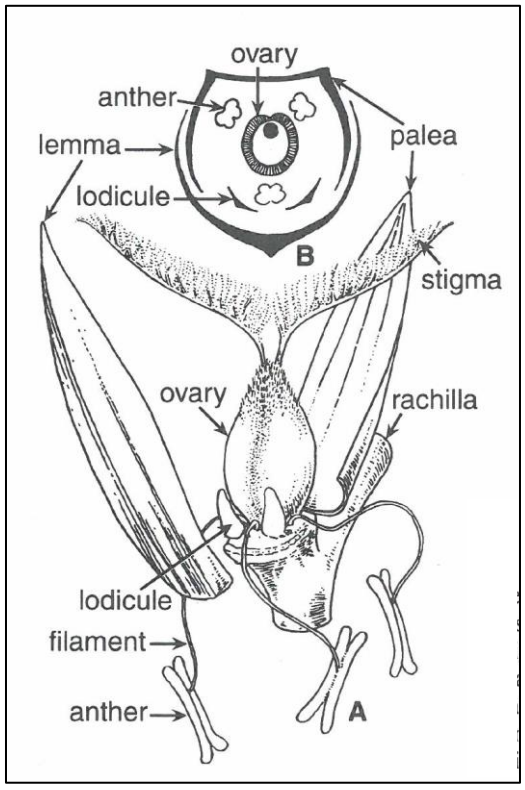

Figure 4 (right): The grass floret. A: partly dissected at anthesis. The ovary and two stigmas comprise the pistil. B: Transverse diagram showing overlap of lemma and palea. (Bushnell et al., 2003; adapted after Esau, K. 1977, Anatomy of Seed Plants 2nd ed.)

The flowering process

In the following paragraph the flowering process is summarized after Bushnell et al. (2003; more references therein). Before flowering, the immature green anthers are on short filaments in the closed floret. The stigmas are close together. Flowering starts when the lodicules swell and push lemma and palea apart. The filaments elongate to about three times of their original length and push the yellow anthers with mature pollen outside. At filament elongation, which only takes three to four minutes, the pollen is released and falls on the diverge, feathery stigmas. At flowering lemma and palea are spread apart and the florets open. Flower opening only takes approximately 20–35 minutes and during this time, the floret interior is very vulnerable to airborne spores.

Usually, flowering starts at spikelets which are located a little bit above the middle of head and then continues up- and downwards. Normally, anthesis for a whole head is finished in three to four days; wet and cold weather can extend this process. Depending on the genotype and environmental conditions, anthers can be

- Fully extruded outside the floret
- Partially extruded - caught between lemma and palea
- Completely remain inside the flower and dehisce

1.4.2 *Fusarium* development in florets

A lot of studies have been performed on the role of AE on FHB resistance, since it has been shown that initial FHB infection of wheat preferentially occurs via anthers by Pugh et al. (1933). The infection process of *Fusarium* in wheat was investigated by several studies using different methods. Kang and Buchenauer (2000, 2005) investigated the fungal growth of different *Fusarium* species by light and electron microscopy. Miller et al. (2004) used fluorescent microscopy of a green fluorescent transformed *F. graminearum* strain. Kang and Buchenauer (1999) used immunogold labeling for DON movement. They showed that DON can be transported to (more highly situated) plant parts which are not colonized from the fungus.

Fusarium species cannot directly penetrate the thick-walled epidermal cells of glumes, palea and lemma (Bushnell et al., 2003). At the beginning, fungal germ tubes branch, hyphal networks are formed on the inner surfaces of the glume, lemma, palea, ovary (also on pollen grain on brush hair) and retained anthers 36–48 hours after inoculation (Kang and Buchenauer, 2000; Kang et al., 2005). On soft tissues (thin-walled cells) like anthers, stigma and ovary (also pollen grains covered with brush hair of ovary) penetration starts first and germ tubes grow into the plant tissue. After the fungus moves down to the ovary, the node is

infected. From there, the fungus grows into rachis (especially in the vascular bundles) and then up-/downwards the head colonizing other spikelets. Fungal hyphae were detected in xylem vessels, phloem sieves and paratracheal parenchyma cells (Kang and Buchenauer, 2000). If vascular bundles get occluded by fungal growth, spikelets above can bleach without directly fungal infection due to water/nutrient shortage (Miller et al., 2004). Furthermore, also uninfected spikelets can bleach due to transported mycotoxins (Kang and Buchenauer, 1999).

1.4.3 Anther extrusion and FHB resistance

As mentioned above, *Fusarium* spores cannot directly penetrate thick-walled cells of glumes, palea and lemma; they can only penetrate soft tissues. Of these soft tissues anthers are the only connection from the closed flower to the environment; and the *Fusarium* spores, especially when placed on anthers caught between lemma and palea, can germinate and grow into the floret (Bushnell et al., 2003; Kubo et al., 2010).

Several studies showed a negative correlation between AE and FHB severity (Graham and Browne, 2009; Kubo et al., 2010, 2013, Skinnes et al., 2008, 2010), meaning that genotypes which extrude anthers completely are more resistant to FHB. Kubo et al. (2013) report that wheat genotypes with partially extruded anthers showed significantly higher FHB susceptibility than genotypes with cleistogamous flowering and genotypes which extrude their anthers fully and rapidly. Similar results have been reported for barley: cleistogamous flowering cultivars show higher FHB resistance than chasmogamous (open) flowering cultivars (Yoshida et al., 2005).

Different floral traits, for instance openness of florets, duration of flower opening, size of anthers and length of filaments, tenacity and form of glumes may influence the extent of AE (Buerstmayr and Buerstmayr, 2015; Gilsinger et al., 2005).

1.4.4 Genetics of AE

Several studies for AE/AR genetics were made, indicating that AE has a high heritability and AE is controlled by few genes in a polygenic manner. Associations between AE and FHB *Type 1* resistance were found as well. Skinnes et al. (2010) reported that AE has a high heritability (h^2 = 0.91) and has minimal genotype × year interactions. In a doubled haploid (DH) population derived from the cross Arina × NK93604, QTL for AE were located on chromosomes 1AL, 1BL, 4DL and 6AS; explaining a total phenotypic variance of about 54%.

Lu et al. (2013) reported a high heritability (h^2 = 0.80) for AE. In a Shanghai-3/Catbird × Naxos recombinant inbreed line (RIL) population, QTL for AE were found on 4BS, 5BL, 2DL, 3DL and 7AL. A correlation of plant height and AE was found, a QTL for low AE overlapped with the dwarfing gene *Rht-B1b*. The QTL for AE coincided with QTL for FHB resistance.

At the IFA BP Tulln, research has been performed on the *Qfhs.ifa-5A* and its association to AE and FHB *Type 1* resistance. The wheat near isogenic line (NIL) C3 (Schweiger et al., 2013) harbors the *Qfhs.ifa-5A* FHB resistance QTL in a susceptible background and shows increased FHB resistance and also a higher level of AE compared to the recurrent parent, suggesting that a gene controlling AR is located in the *Qfhs.ifa-5A* interval (Buerstmayr, unpublished results).

Buerstmayr and Buerstmayr (2015) found three QTL for anther retention on chromosomes 4AL, 5AS and 6BL in a RIL mapping population of Capo × Arina, explaining 40% of the total variance of AR. The heritability coefficient of AR was 0.87. Anther retention was positively correlated with FHB severity (r = 0.63) reflecting the lower FHB severity on plants with high anther extrusion.

Danler (2016) reports a high heritability (h^2 = 0.77) for AE in European winter wheat breeding lines. A correlation (r^2 = 0.50) between AR and FHB severity was evaluated: lines with high AE were more resistant. An association of the *Qfhs.ifa-5A* and AE was observed, as genotypes with *Qfhs.ifa-5A* had a higher level of AE. Danler (2016) simulated also a high level of AE by the removing of anthers out of the floret and showed that heads with simulated AE, independent from the genotype, were more resistant.

Due to the correlation of AE and FHB resistance and the high heritability, screening for high AE can therefore also be an indirect selection criteria for screening for FHB resistance (Buerstmayr and Buerstmayr, 2015).

1.5 Mapping the *Qfhs.ifa-5A* FHB resistance QTL

Aims of QTL mapping studies

QTL mapping studies have been performed for many agricultural traits and crops. The aim of the QTL mapping studies is to localize the genes and to find good linked markers for traits of interest. QTL mapping is usually done in biparental populations. Multi-parent advanced generation inter-cross (MAGIC) populations or association mapping studies are also possible methods for QTL detection. Markers linked to the QTL can then be used for MAS in plant breeding to support selection.

Subsequent fine-mapping of identified QTL can narrow down the QTL interval in order to obtain better linked or perfect markers and perform gene cloning or forward/reverse genetic studies.

Genetic and physical maps

In general, two kinds of maps can be distinguished: genetic (or linkage) maps, which are relying on recombination rates, distances are given in centi Morgan (cM- which is a unit for recombination probability); and physical maps, which are relying on physical position on chromosomes, distances are given in kilo or mega base pairs.

1.5.1 Overview of the wheat genome

Wheat is one of the oldest crops, its cultivation and domestication started together with the beginning of agriculture about 10,000 years ago in the fertile descent (Bell, 1987; Salamini et al., 2002). In the fertile descent, which hosts the biggest diversification of wheat and wheat relatives (Riaz et al., 2016), also the hybridization of hexaploid wheat took place. The hybridization of *Triticum urartu* (genome AA, 2n = 14) with an unknown species of the *Sitopsis* section, with *Aegilops speltoides* as closest relative (genome BB, 2n = 14), led to the formation of *Triticum dicoccum* (emmer wheat) with a tetraploid genome (AABB, 2n = 28) (Salse et al., 2008). Hybridization of *Triticum dicoccum* with *Aegilops tauschii* (genome DD, 2n = 14) caused the genesis of hexaploid wheat (genome AABBDD, 2n = 6x = 42).

The hexaploid wheat genome consists of six homoeologous chromosome sets. Also the big genome size of 16,937 giga base pairs and its gene number of 90,000 illustrate the complexity of the wheat genome (Vitulo et al., 2011). The big genome size and the homoeologous chromosomes – with about 80% repetitive sequences – were a challenge for the sequencing of the wheat genome (IWGSC et al., 2014). Sequencing of the model wheat cultivar Chinese Spring by the international wheat genome sequencing

consortium (IWGSC) is still under progress and for most chromosomes draft references are available (International Wheat Genome Sequence Consortium, 2016).

Wheat cytogenic stocks

For genetic research in wheat a lot of Chinese Spring aneuploid stocks are available, such as nullisomic-tetrasomic and ditelosomic lines (Endo and Gill, 1996; Gustafson, 2004; Sears, 1954). These stocks have been very useful for locating genes on chromosomes. Endo and Gill (1996) developed 436 deletion stocks for the cultivar Chinese Spring by using the gametocidal chromosome of *Aegilops* species (Endo, 2007). These deletion stocks have been extensively used in molecular mapping of the wheat genome and are, next to fluorescence in situ hybridization, a very valuable tool for physical mapping (Lehmensiek et al., 2009; Qi et al., 2003).

1.5.2 Mapping approaches for *Qfhs.ifa-5A*

Numerous FHB resistance QTL were mapped (Buerstmayr et al., 2009). An important resistance QTL against fungal penetration is *Qfhs.ifa-5A* which is found in the Chinese cultivars Sumai-3 and related lines (Buerstmayr et al., 2012). For the *Qfhs.ifa-5A* following linkage mapping approaches have been done at IFA Tulln.

QTL mapping analysis using 293 DH lines generating from the cross CM-82036 × Remus identified the two major resistance QTL *Qfhs.ndsu-3BS* (syn. *Fhb1)* and *Qfhs.ifa-5A. Qfhs.ifa-5A*. They were mapped on the 5A chromosome between flanking markers gwm293 and gwm156 and explained 20% of the phenotypic variance (Buerstmayr et al., 2002, 2003).

A fine-mapping approach was initiated using a near isogenic recombinant inbred line (NI-RIL) population from crossings of different NILs: NIL1 (Remus background, harbor *Fhb1* and *Qfhs.ifa-5A* from CM-82036), NIL2 (Remus background, harbor *Fhb1* from CM-82036), NIL3 (Remus background, harbor *Qfhs.ifa-5A* from CM-82036) and NIL4 (Remus background). About 4000 NIL-RIL plants were screened for recombination within the QTL intervals. NIL-RILs having a recombination in the target interval were propagated and phenotyped for AE and FHB resistance. The marker cfa2250, located in the centromeric region 5AS, was found to be the closest linked marker with FHB T*ype 1* resistance. The same locus is also associated with higher AE (*Figure 5*) (IFA BP research, unpublished).

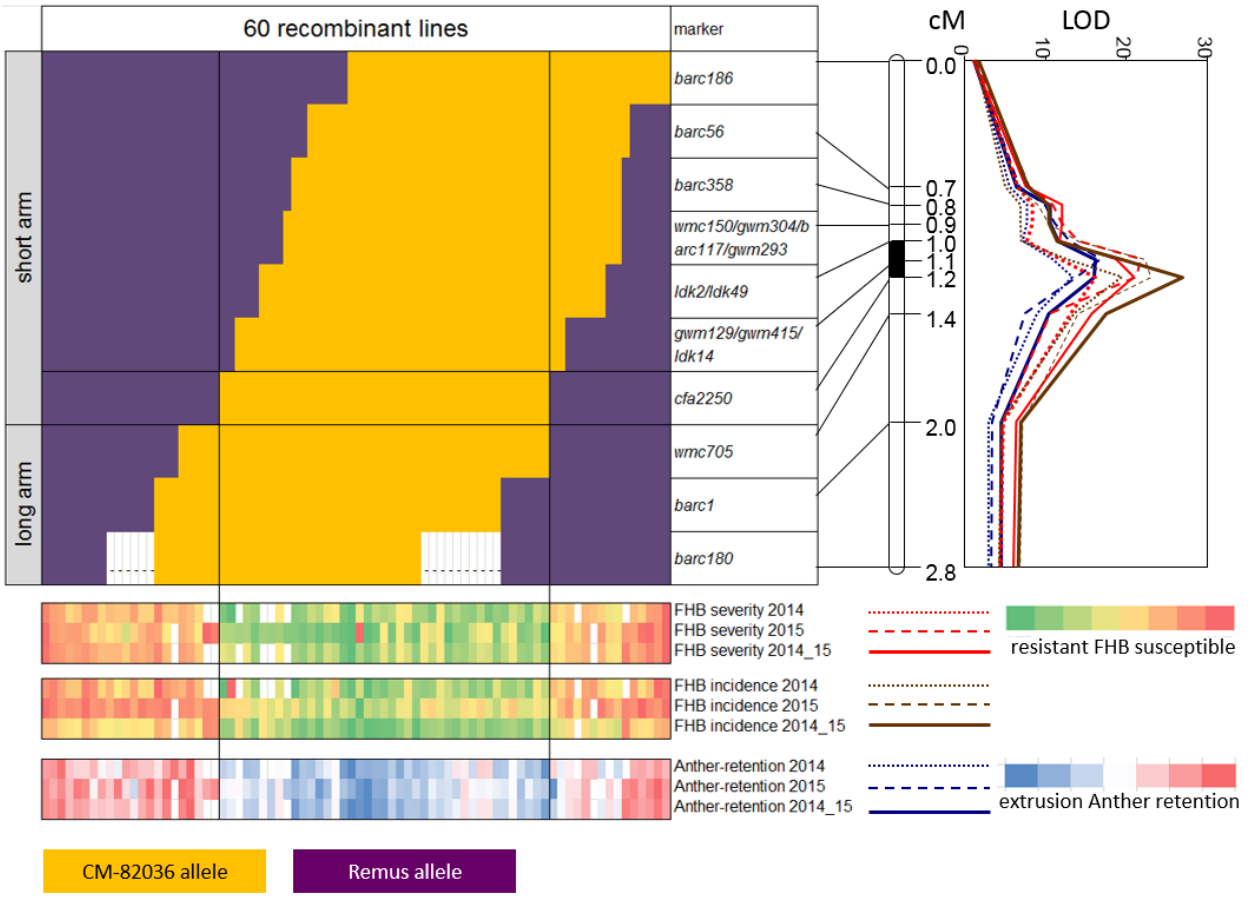

Figure 5: Genotype for the *Qfhs.ifa-5A* map interval and the phenotype for FHB severity and incidence and anther retention of 60 lines with recombination in the QTL region. Lines with the CM-82036 allele at the marker cfa2250 show increased FHB resistance and also a higher level of anther extrusion illustrated by a heat map (IFA BP research, unpublished).

These linkage mapping approaches are based on recombination. The recombination rate is unevenly distributed along the chromosome: regions of high recombination were located closer to the telomers, while regions near the centromere have low recombination rates (Gill, 2005; Lukaszewski, 2016; Stein, 2009). *Qfhs.ifa-5A* resides in the low recombinogenic centromeric interval that strongly impeded fine-mapping.

Therefore, several deletion mapping approaches were performed:

- Deletion mapping approach with C3, started 2014 (Vukelic, unpublished; this master thesis)
- Radiation hybrid mapping with 274 Chinese Spring lines (Mayer, 2016; Vukelic, unpublished)

1.5.3 Radiation-induced deletion mapping as alternative to linkage mapping

Radiation-induced deletion mapping is a powerful tool in genetic research, especially if linkage mapping is not possible. Advantages of radiation-induced deletion mapping are: radiation-induced breaks are independent of recombination events, radiation-induced breaks are randomly distributed across the genome, radiation dosage can be adjusted to provide varied resolution without greatly affecting the population (deletion panel) size, and polymorphic markers are not necessary (marker is retained or absent) (Kumar et al., 2014; Lehmensiek et al., 2009).

Radiation induce deletion mutations

For deletion mapping, plants have to be mutagenized first in order to induce deletions. Gamma radiation, fast neutrons, and X-rays are sources that are frequently used to create deletions. Radiation causes double-strand breaks of the DNA helix, which can lead to deletions. The deletion frequency is depending on radiation dosage: too high radiation dosage leads to plant/cell dead or less vigorous/sterile plants in following generations, while lower radiation dosage leads to lower deletion frequency (Kumar et al., 2014). For wheat seeds, a dosage of about 300- 350 gray (Gy) (Kalavacharla et al., 2009; Matijevic Mirta IAEA/FAO Joint program Seibersdorf, 2016: personal communication) is a good compromise between deletion frequency and plant survival. Due to its hexaploid character, wheat can bear more and also relatively large deletions compared to diploid plants (Endo and Gill, 1996).

Deletions occur randomly at chromosome, genome, and also cell level. After deletion induction, the plant material is an irradiation chimera (M_1), containing different deletions in different cells at heterozygous stage (if it is not haploid). Thus, plants have to be selfed (M_2) to make deletions homozygous and detectable. After deletions are homozygous, the deletion lines can be scored with any type of PCR markers for the absence of the deleted fragment in the genetic region of interest.

The use of radiation hybrids (RH) (cross between irradiated pollen source and a chromosome specific nullisomic line) can speed up the process, here M_1 are hemizygous for the specific chromosome. The disadvantage of this method is that most plants may not be viable and can only be used for mapping, not for phenotyping.

1.5.4 The wheat 5A chromosome

Wheat has a big genome of about 17,000 Mbp, the 5A chromosome has an estimated size of 827 Mbp and harbors 4.9% of the wheat genome (IWGSC et al., 2014; Vitulo et al., 2011). It can be divided into:

- 5A short chromosome arm (5AS): estimated size 295 Mbp
- 5A long chromosome arm (5AL): estimated size 532 Mbp

Several 5A specific maps (genetic and physical, different marker types) have been published and are summarized at GrainGenes homepage (GrainGenes, 2016a). Sequencing the wheat genome (5A) is still under progress, the IWGSC is looking forward to publish soon the 5A sequence (International Wheat Genome Sequence Consortium, 2016).

Use of aneuploid and deletion stocks in (5A chromosome) deletion mapping

Wheat aneuploid and deletion stocks are very important for physical/deletion mapping (Randhawa, 2004; Stein, 2009). For example, the Wheat Genetics Resource Center of Kansas State University (WGRC) stores and provides 2,200 cytogenetic wheat stocks (WGRC, 2016). *Figure 6* illustrates the use of deletion bins for physical mapping (6B chromosome as example).

For the 5A chromosome (Chinese Spring) aneuploid stocks are available:

- CS-N5AT5B, CS-N5AT5D: nullisomic for 5A, tetrasomic for 5B/D (chromosome pair 5A is replaced by an extra pair of 5B or 5D chromosomes)
- CS-DT5AL: ditelosomic line for long arm of 5A chromosome

Endo and Gill produced eleven and 23 deletion stock lines for the 5AS and 5AL chromosome, respectively (Endo and Gill, 1996). They can be used for 5A-intrachromosomal mapping. For the 5AS chromosome, the following three deletion stocks are of interest: C-5AS1-0.40, 5AS1-0.40-0.75, and 5AS3-0.75-0.97. With these deletion stocks, markers can be allocated to specific bins within the 5AS chromosome.

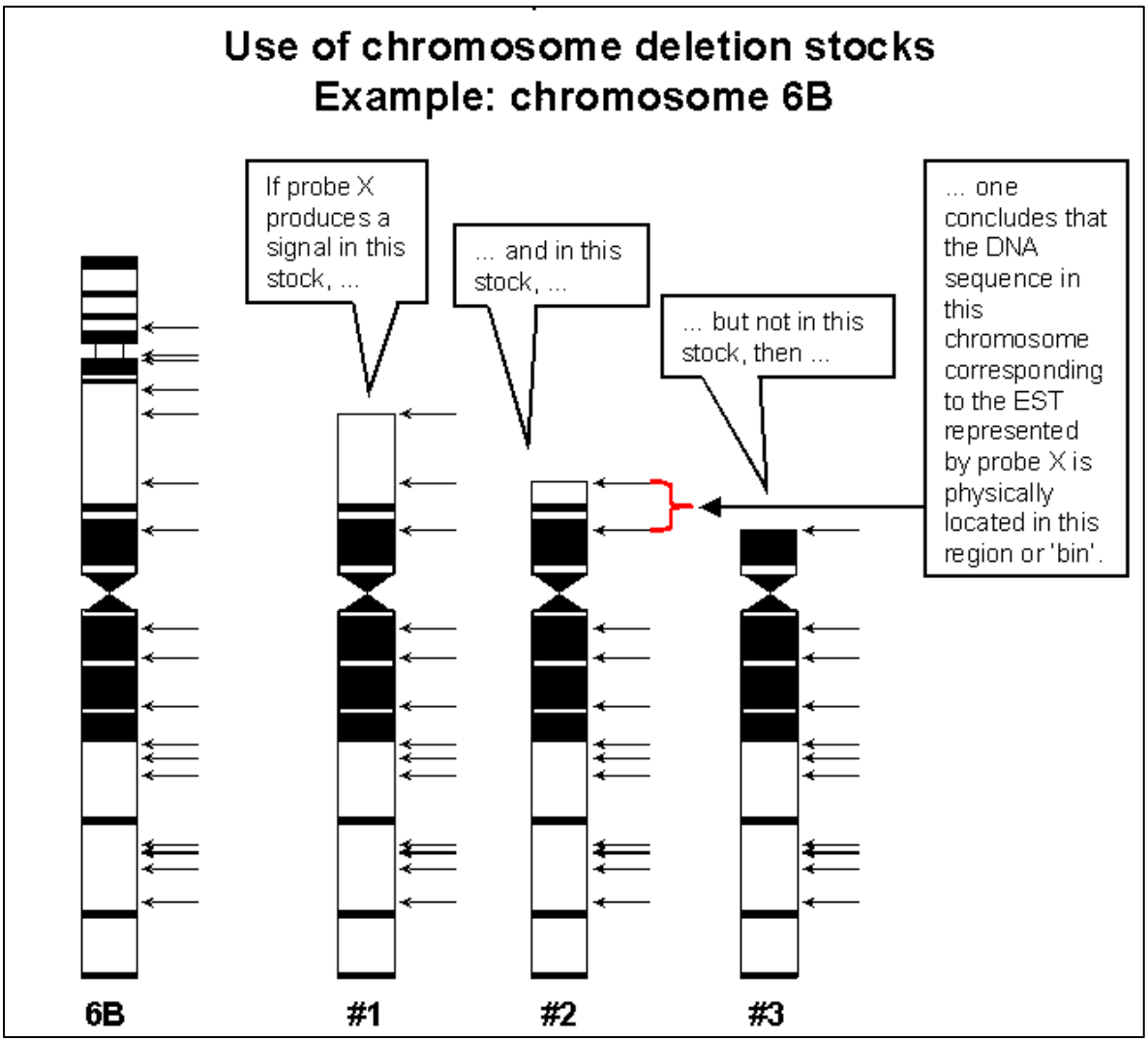

Figure 6: Example for the use of deletion stocks for mapping, demonstrated on chromosome 6B (Figure simplified, from NSF Wheat EST Genomics Project at: https://wheat.pw.usda.gov/NSF/deletionuse.gif (10.01.2017)

1.6 Research questions and aims of the master thesis

The master thesis is divided into two parts:

1) Influence of retained anthers after flowering on FHB resistance conferred by *Qfhs.ifa-5A*
2) Fine-mapping of the *Qfhs.ifa-5A* region using deletions lines

In the first part, the effect of anther extrusion/retention on FHB resistance controlled by the resistance QTL *Qfhs.ifa-5A* was evaluated. In a greenhouse trial, anthers of two genotypes differing in the possession of *Qfhs.ifa-5A* were manipulated. At anthesis anthers were either removed from or compressed into the florets followed by spray inoculation with *F. graminearum* and evaluations of the disease severity and incidence.

Following research questions were addressed:

- Is there a difference in the AE level between genotypes which differ in the possession of *Qfhs.ifa-5A*?
- Does fast and complete extrusion of anthers improve the FHB resistance? Is it possible to increase the resistance of a susceptible cultivar by removing the anthers?
- Does retention of anthers reduce FHB resistance? Does the moderately resistant line harboring the resistance QTL *Qfhs.ifa-5A* become susceptible if anthers are manually inserted/compressed into the floret?
- Will both lines show the same levels of resistance and susceptibility, if anthers are removed or compressed?

In the second part of the work, which deals with fine-mapping the *Qfhs.ifa-5A* on the 5AS chromosome, the map resolution of the 5AS chromosome, especially in the centromeric region, was increased.

Research questions/objectives were:

- Is it possible to increase the map resolution on 5AS chromosome by radiation-induced deletion mapping? Can the map resolution also be increased in the centromeric region?
- Is it possible to identify lines with small deletions in the expected *Qfhs.ifa-5A* support interval?

The lines with deletions in the *Qfhs.ifa-5A* interval will be backcrossed with C3 to eliminate unwanted background deletions. Phenotyping these lines for FHB resistance and AE will assist in the detection of the genetic basis for the phenotypic trait correlations.

2 Materials and methods

The materials and methods chapter is dived into two parts:

- Greenhouse trial for evaluating the effect of AE on FHB resistance (section 2.1.)
- Mapping approach to refine the *Qfhs.ifa-5A* map region (section 2.2.)

2.1 Materials and methods: association of *Qfhs.ifa-5A* with AE and FHB resistance

In December 2015, a greenhouse trial was performed to simulate the effect of AE/AR on FHB resistance similar to experiments performed by Danler (2016).

2.1.1 Plant material

In this trial the two genotypes Remus and C3 (*Figure 7*) were used:

- Remus ('Sappo'/'Mex'/'Famos') is a German cultivar that has been bred by the Bavarian State Research Center for Agriculture Freising (Bayerische Landesanstalt für Landwirtschaft, Freising) in the 1990s (Buerstmayr et al., 2002). Remus is very susceptible to FHB and has a low level of AE.
- C3 (synonym Remus NIL3) is a near isogenic line (NIL) of Remus: the highly resistant breeding line CM-82036 (CIMMYT full name: CM-82036-1TP-10Y-OST-10Y-OM-OFC; Sumai-3/Thornbird-S) was crossed with Remus and then backcrossed five times (BC_5) to Remus (Schweiger et al., 2013). In the BC_5F_2, the C3 line harboring *Qfhs.ifa-5A* in homozygous state was selected. C3 is moderately resistant against FHB and shows a high level of AE (Danler, 2016).

Figure 7: Comparison of genotypes regarding AE: Remus (left) has a low level of anther extrusion, C3 (right) has a high level of anther extrusion

2.1.2 Plant cultivation and experimental design

The wheat plants were grown as described in Danler (2016): Remus and C3 kernels were sown in multiplates. At about BBCH 12-16, ten wheat plants of the same genotype were transplanted into pots. Pots with a diameter of 20 cm were filled with 6 l of a substrate mixture consisting of 500 l heat-sterilized compost, 250 l peat, 10 kg sand, and 250 g rock flour. Pots were placed in double rows to facilitate plant maintenance and phenotyping (*Figure 22*, appendix page 75) in two replications with 45 pots per genotype. At about BBCH 31, plants were fertilized with about two grams of low in chlorine NPK fertilizer ($12N + 8P_2O_5 + 16K_2O + 3MgO + 10S$ – Blaukorn classic). Temperature and illumination were set as in former trials (*Table 3*) (Danler, 2016).

Table 3: Temperatures and illumination times at the different growth stages for anther experiment (modified after Danler, 2016)

Growth stage (BBCH stages)	Day temperature [°C]	Night temperature [°C]	Illumination time [h]
Germination (00) - end of tillering (29)	12	10	12
Stem elongation (30-39)	14	10	14
Booting (40-49)	16	14	14
Heading (50-59)	18	14	14
Flowering (60) - end of experiment	22	18	16

As plant protection measurement, sulfur was evaporated (sulfur evaporator, Nivola®) twice a week until BBCH 60 to prevent mildew (*Erysiphe graminis f.sp. tritici*).

2.1.3 Evaluating anther retention (AR)

To determine the differences in the trait AR between the two genotypes C3 and Remus, retained anthers were counted similar to Danler (2016). From each block (double row), five heads (about BBCH 69) were chosen randomly and five basal florets in the central part of each head were manually opened and inspected for retained or trapped anthers. For each block 25 florets were investigated. Florets were counted as retained if at least one anther remained inside the floret, or if anthers were trapped between palea and lemma.

Statistical analysis was performed by using R-Studio under R version 3.3.0 (R Development Core Team, 2016). A two sample t-test was performed to compare the two genotypes.

2.1.4 Anther manipulation

Three different anther manipulations were implemented:

(i) No manipulation – control (color-code blue - B)

(ii) Removal of all anthers (color-code yellow - Y)

(iii) Compression of anthers into florets (color-code red - R)

Right after pollination, heads were labeled with a colored sticker with date codes (*Table 4*) and trimmed to 16 spikelets per head in order to obtain an equal spikelet number for comparison. Very small spikelets (stunted, delayed flowering) on the basis and the top of the heads were removed to ensure that only well-developed spikelets at nearly the same flowering stage were examined. In the ´control´ group, heads were only shortened to 16 spikelets and labeled with a blue sticker with a date code. In the ´anthers removed´ group, all anthers were removed with forceps and heads were labeled with yellow stickers with a time code. In the ´anthers compressed´ group, anthers of the 16 basal florets were separated from the filaments (by shortly pulling with the forceps) and then directly placed back into the florets. Heads with ´anthers compressed´ treatments were labeled with red stickers with a date code. Spikelets that were straddled due to manipulation were gently pushed together to their normal position.

As not all plants flowered at the same time, anther manipulation was done every second day. The manipulations were performed between Dec 3rd (date code 1) and Dec 15th (date code 7). From each genotype and treatment group, at least 60 heads were manipulated. Anther manipulation was done randomly, so that in each pot and at each date, approximately the same number of the different treatments was conducted.

Table 4: Color code for anther manipulation

Manipulation	Code	Sticker color
Control - no manipulation	B	blue
Anthers removed	Y	yellow
Anthers compressed into florets	R	red

2.1.5 Spray inoculation with *F. graminearum*

One day after anther manipulation, heads were inoculated with *F. graminearum* isolate IFA 65 (*Figure 8*). The *F. graminearum* isolate IFA 65 is a very aggressive isolate and has already been used many times at IFA trials (Blöch, 2014). The inoculation concentrate was produced by Marc Lemmens (IFA Tulln) according to the IFA protocol ´Production of Inoculum of *Fusarium ssp*. with Bubble Breeding´ (Blöch, 2014; Danler, 2016; IFA BP, 2007).

Directly before inoculation, the deep frozen inoculation concentrate (stored at –80°C) was defrosted in lukewarm water. The inoculation concentrate was diluted to 1 l with deionized water, yielding a concentration of 20,000 macroconidia/ml. Furthermore, 1 ml of Tween 20 was added per liter of inoculation suspension for better surface wetting of spikelets. For every inoculation suspension, conidia germination rate was tested on media by counting germinated conidia under the microscope. For every inoculation date, the macroconidia germination rate was ≥ 96%. Manipulated heads were sprayed from each side with approx. 2 ml of conidia suspension with a spray flask. To ensure high humidity for better conidia germination, plastic bags were put over inoculated heads for about 24 h.

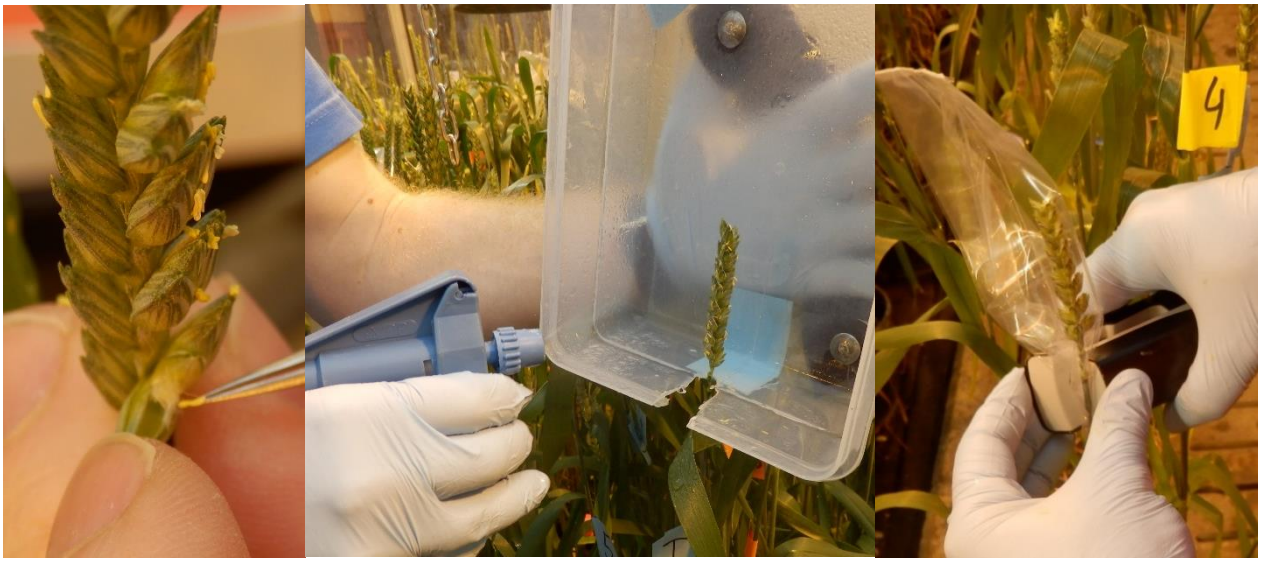

Figure 8: Anther manipulation, inoculation and covering with plastic bags

2.1.6 Phenotyping of FHB resistance

Heads were scored for FHB symptoms 6, 10, 14, 18 and 22 days after inoculation (dai). Phenotyping was performed for disease incidence by counting the number of infection sites (IS: IS 6 dai, IS 10 dai, IS 14 dai) per spike and for disease severity by counting the total number of symptomatic spikelets per head (S: S 6 dai, S 10 dai, S 14 dai, S 18 dai, S 22 dai). Each spikelet that showed FHB symptoms was counted, scores ranged from 0 (no visible symptoms) to 16 (all 16 spikelets per head showed symptoms).

2.1.7 Statistical analysis of FHB resistance

In addition to the point-in-time specific scorings, the area under disease progress curve (AUDPC) was calculated for the time interval from inoculation to 18 dai and 22 dai (*Formula 1*). Statistical analysis was performed by using R-Studio under R version 3.3.0 (R Development Core Team, 2016). Linear models were set up with replication, genotype, anther treatment, and genotype-anther treatment interactions as fixed effects (*Formula 2*). Analysis of variance (ANOVA) with Tukey post-hoc test was done separately for each parameter (IS 6 dai, IS 10 dai, IS 14 dai, S 18 dai, S 22 dai, AUDPC 18 dai, AUDPC 22 dai).
To obtain an overview, boxplots of different test groups were made with R.

Formula 1: Calculation of area under disease progress curve (AUDPC) (Buerstmayr et al., 2000)

$$\mathbf{AUDPC} = \sum_{i=1}^{n} [(\frac{y_i + y_{i-1}}{2}) * (x_i - x_{i-1})]$$

y_i score of total infected spikelets per head on the i^{th} day
x_i day of observation

Formula 2: ANOVA model

$$y_{ijkn} = \mu + a_i + b_j + c_k + w_{ij} + e_{ijkn}$$

y_{ijkn} phenotypic observed value
μ overall mean
a_i effect of genotype i
b_j effect of anther treatment j
c_k effect of replication k
w_{ij} interactions of genotype i and anther treatment j
e_{ijkn} residual error

2.2 Materials and methods: radiation-induced deletion mapping of the *Qfhs.ifa-5A* region

In the second part of this master thesis, the *Qfhs.ifa-5A* interval on the 5AS chromosome was fine-mapped through radiation-induced deletion mapping and a 5AS specific C3 radiation-induced deletion map (C3) was created. Prescreened lines from Vukelic (unpublished) were used.

2.2.1 Plant material, mutagenesis of C3 seeds and preselection of lines with deletions on 5AS

For this mapping approach, C3 (pedigree see section 2.1.1), harboring the *Qfhs.ifa-5A* OTL from Sumai-3 in the susceptible Remus background, was used. C3 seeds (M_0) were irradiated at the Department of Plant Science, North Dakota State University, Fargo USA (Shahrayr Kian laboratory) with a dosage of 250 Gy. Furthermore, additional seeds of C3 were irradiated at the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture in Seibersdorf, Austria with a dosage of 240, 270, 300 and 330 Gy, respectively. Seeds were gamma-irradiated with Cobalt-60 as radiation source. The radiated seeds (M_1) were selfed to M_2 or M_3 generation to obtain possible deletions in a homozygote form. Prescreening of 1764 mutant plants with 14 5AS specific markers (*Table 5*) by Petra Vukelic (unpublished) identified 31 lines with deletions.

Table 5: Deletion subpanels: radiation dosage and number of prescreened plants

Mutant generation	Dosage	Prescreened plants	Plants with deletions (%)
M_3	250 Gy	800	20 (2.50)
M_2	240 Gy	383	4 (1.04)
M_2	270 Gy	115	2 (1.74)
M_2	300 Gy	367	4 (1.09)
M_2	330 Gy	99	1 (1.01)
Total		1764	31

5AS Control Lines

To control the specificity of markers for 5AS, different cytogenetic and deletion stocks were used: CS-N5AT5B, CS-DT5AL, C-5AS1-0.40, 5AS1-0.40-0.75 and 5AS3-0.75-0.97. The lines Chinese Spring, C3, and Remus were used to check if markers were monomorphic/polymorphic between the deletion panel and the deletion stocks.

2.2.2 Genotyping of the 5AS deletion lines

Analysis has been performed on 48 samples (divisible number for PCR-plates):

- Thirty-one preselected lines with clear deletions 5AS
- Eleven additional lines with unsure scorings or a deletion of one single marker (D_179, D_345, D_643, D_646, D_1562, D_1571, D_1592, D_1675, D_1763-3, D_1763-4, D_1809)
- Non-irradiated control lines (C3- two times, Remus, Chinese Spring, CS-N5AT5B, CS-DT5AL)

DNA extraction

Selected plants being either in M_3 and M_4 generation were grown in the greenhouse. Around BBCH 13, two to three leaves were cut and dried in paper bags at 36°C in a compartment dryer and then in a desiccator. Dried leaf material was cut and put in 1.5 ml Eppendorf tubes, containing approx. five glass beads. Plant material was then ground in a Retsch mill (MM301) to fine powder (two times for five minutes). DNA was then extracted via the CTAB (cetyltrimethylammonium-bromide) method, according to the IFA DNA extraction protocol (appendix page 76). The CTAB method is a cost-efficient and simple way for DNA extraction, in particular for the processing of large sample numbers. This procedure is a standard method and has already been used many times at BP IFA (Gratl, 2015; Jungreithmeier, 2016).

After extraction, DNA pellets were dissolved in 100 µl TE-8 buffer. DNA concentration and quality was measured with a spectrophotometer (Shimadzu BioSpec-nano Micro-volume UV-Vis Spectrophotometer). DNA concentration was adjusted to 100 ng/µl in a master plate and to 50 ng/µl in a working plate to simplify the preparation of PCR plates. DNA was stored at –20°C in a freezer.

Chromosome 5AS-specific markers

5AS specific markers were chosen according to published maps and public databases (Akhunov et al., 2010; Barabaschi et al., 2015; Gadaleta et al., 2014; GrainGenes, 2016b; Röder et al., 1998; Somers et al., 2004; Song et al., 2005; Sourdille et al., 2004). Details on used markers with primer information are given in *Table 22* at page 80 in the appendix (more information at GrainGenes, at: http://wheat.pw.usda.gov/cgi-bin/GG3/browse.cgi?class=marker).

Different types of 5AS specific markers were used: single sequence repeat (SSR), single nucleotide polymorphism (SNP), insertion site-based polymorphism (ISBP), conserved ortholog set (COS), and repeat DNA junction markers (RJM). Prior to the screening of the deletion lines, the markers have been tested on the parental lines and control lines Remus, C3, Chinese Spring, CS-N5AT5B line, CS-DT5AL, C-5AS1-0.40, 5AS1-0.40-0.75, and 5AS3-0.75-0.97 for (i) specificity for 5A chromosome (determined via CS-N5AT5B line

and CS-DT5AL line for 5AS specificity), (ii) physical location on 5AS chromosome (determined via cytogenetic Chinese Spring deletion stocks C-5AS1-0.40, 5AS1-0.40-0.7, and 5AS3-0.75-0.97) and (iii) scoring and multiplexing ability (length of fragment(s) and position(s) of ´deleted´ band(s)).

Multiplex PCR

Multiplex PCR (by combing primer pairs) has been applied in order to reduce costs and save time. One to three primer pairs have been chosen in a way that fragments were not overlapping and at least one additional band (amplification of a non–5AS specific band) was produced to distinguish between deletions and PCR-failures. Information on number of fragments, fragment sizes, and presence of 5A specific and unspecific amplicons of individual markers has been obtained by testing markers for their allocation on chromosome 5AS (see above). For visualization a fluorescently labeled M13 primer was used. This M13 primer consists of a short sequence (5' CCCAGTCACGACGTTG 3') and a FAM (6-carboxy fluorescein, fluorescence at 520 nm) or a Cy5 (cyanin 5, fluorescence at 670 nm) fluorescence tail on the 5´ end (Schuelke, 2000). Gels were then scanned with a Typhon Trio gel fluorescence image scanner (GE Healthcare, Freiburg, Germany).

The master mix was prepared in following order: PCR-water, PCR-buffer, dNTPs, M13 primer, forward and reverse primer (combinations), and finally Taq-polymerase (components and amounts can be seen in *Table 6*). The master mix was usually prepared for 55 reactions (48 samples + pipetting rest for the use of stepping pipets). Master mix was added to 384 PCR-plates already containing 2 µl of DNA (50 ng/µl). PCR parameters for the adapted M13 hot-start touchdown PCR are displayed in *Table 7.*

Table 6: Components and amount of a master mix for two primer pairs: each master mix has been prepared for 55 reactions. DNA was already pre-pipetted into PCR plates.

	Stock conc.	Final concentration	Per reaction	Total (55 reactions)
PCR-H_2O	-	-	5.16 µl	284.5 µl
Reaction buffer ($MgCl_2$)	10x	1x	1 µl	55 µl
dNTPs	2 mM	0.2 mM/µl	1 µl	55 µl
M13 primer	10 µM	0.27 µM/µl	0.27 µl	15 µl
Reverse primer 1	10 µM	0.2 µM/µl	0.2 µl	11 µl
Reverse primer 2	10 µM	0.2 µM/µl	0.2 µl	11 µl
Forward primer 1	10 µM	0.027 µM/µl	0.027 µl	1,5 µl
Forward primer 2	10 µM	0.027 µM/µl	0.027 µl	1,5 µl
Taq polymerase	5 U/µl	0.05 U/µl	0.1 µl	5,5 µl
DNA	50 ng/µl	10 ng/µl	2 µl	110 µl
Sum			10 µl	550 µl

Table 7: PCR conditions for M13 hot-start touchdown PCR

Steps	Temperature [°C]	Time [min]	Runs
Pre-denaturation	94	04:00	×1
Denaturation	94	00:50	×7
Annealing	65-53	01:00	
Elongation	72	01:00	
Denaturation	94	00:30	×25
Annealing	51	00:30	
Elongation	72	00:30	
Final	72	05:00	×1
Storage	14	∞	×1

Gel electrophoresis screening PCR results

PCR products were separated on a 12% polyacrylamide gel using the CBS vertical gel electrophoresis system. Depending on fragment sizes, primer combinations, and gel conditions, a voltage of 400–550 V for 1:20 to 2:30 h was chosen. The exact procedure (chemicals and buffers, gel production and gel loading) is summarized in Mayer (2016). Gels were scanned in a Typhon Trio gel fluorescence image scanner and saved as .tif files for scoring via graphics software. FAM fragments were detected at a wavelength of 520 nm, Cy5 fragments were detected at a wavelength of 670 nm.

Primer specific size markers (C3 PCR product) were loaded next to the PCR samples in order to allocate the bands to the respective markers (PCR was mostly done with two primer pairs). C3, CS-N5AT5B and CS-DT5AL were used in every PCR assay as controls: C3 acted as a positive control (amplification of all bands), CS-N5AT5B and CS-DT5AL as a deletion control (no amplification of 5A specific fragments, in the case of CS-N5AT5B; or of 5AS, in the case of CS-DT5AL).

Gel pictures were scored by using Adobe Photoshop Version 10. A scored gel is shown in *Figure 9*. Following scores were given:

- ´1´ for present marker (bands) - undeleted like in C3,
- ´w´ for weak amplification,
- ´d´ for deleted/absent marker (bands), like in CS-N5AT5B CS-DT5AL,
- ´m´ for missing values (PCR fails),
- ´x´ for not scorable, or
- ´?´ for unsure scores.

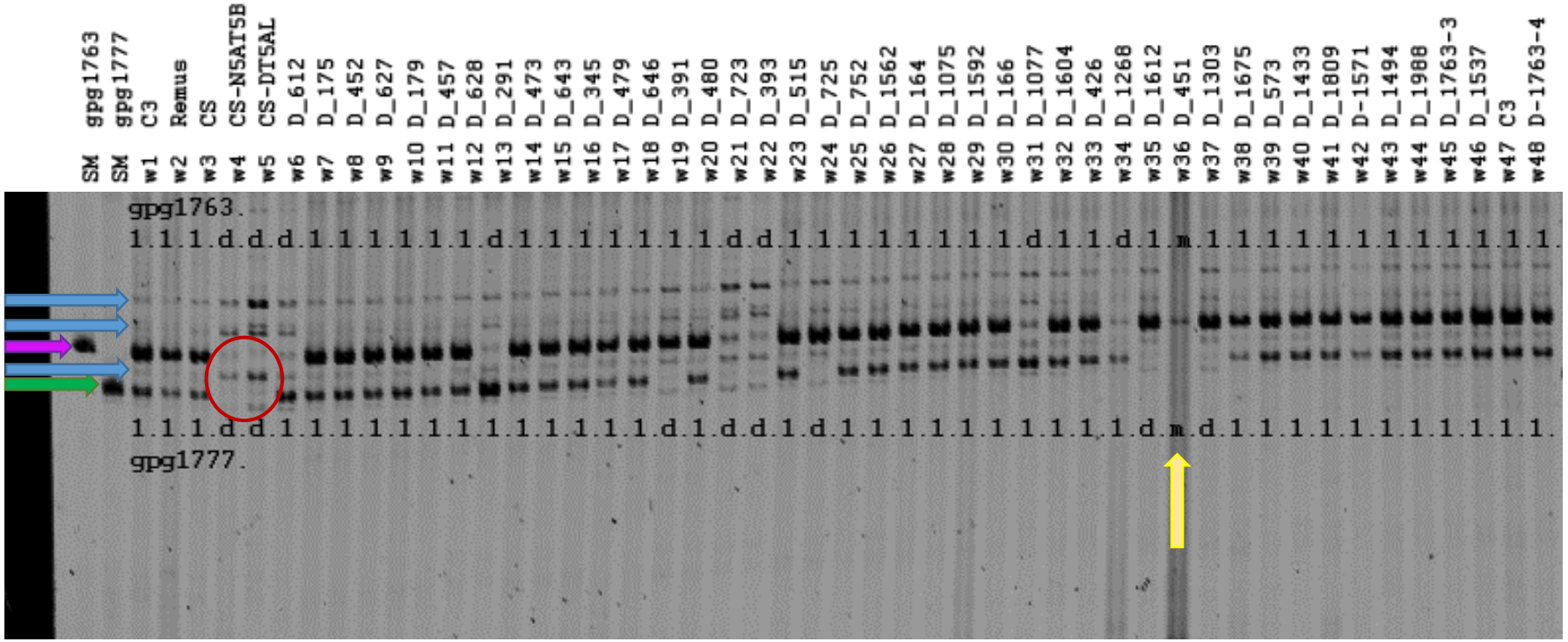

Figure 9: Scorings of markers gpg1763 and gpg1777: multiplex PCR has been prepared with two 5AS specific markers and was separated on a polyacrylamide gel. The used markers had additional bands on other chromosomes than 5AS (blue arrows indicate the very fine, non 5AS specific bands). On the left side gpg1763 and gpg1777 size markers (C3 PCR product of respective marker) were loaded to allocate the correct bands to the marker (purple arrow: gpg1763 5AS band, green arrow: gpg1777 5AS band). Next to the size markers, the control lines C3, Remus, Chinese Spring (CS), CS-N5AT5B, and CS-DT5AL were loaded. A deletion score (d) was given if the band pattern looks like the band pattern in the CS-N5AT5B and CS-DT5AL line (red ring). Retained marker bands got a ´1´ score, PCR failures got a ´m´ (missing, no band amplified, yellow arrow) score.

2.2.3 Establishment of the C3 radiation-induced deletion map

Calculating the C3 radiation-induced deletion map with CarthaGène

The general problem in mapping is to find marker orders and distances that explain the dataset in the best way (Schiex et al., 2016). For n markers there are n!/2 possibilities for the right marker order (e.g. for 50 markers are 1.52 * 10^64 possibilities to order the markers) (De Givry et al., 2005). The markers should be ordered in a way, that the distances between the markers (and total map length) are minimized; this is similar to the traveling salesman problem.

CarthaGène 1.2-LKH is an integrated genetic and radiation hybrid mapping software which has sophisticated algorithms for marker ordering (De Givry et al., 2005). The program is freely available at http://www7.inra.fr/mia/T/CarthaGene/. CarthaGène can also handle multiple populations, including mixtures of genetic and radiation hybrid mapping data.

After presorting the markers in Microsoft Excel and adapting the markers matrix (deleted marker got a ´A´-score, retained markers a ´H´-score, and ´-´ for unknown scoring), the matrix was loaded into CarthaGène.

For the C3 radiation-induced deletion map (C3 map) calculation all markers were taken together to form a single linkage group, because only 5AS specific markers were used. Parameters were set for a haploid model (allows faster ordering). Initially, markers were ordered using two-point log-likelihood by running the *lkh* commands (*lkh, lkhn, lkhl, lkhd*). Commands *polish* and *flips* were run to find a map with an improved log-likelihood compared to the initial map. The *polish* algorithm removes one marker of the initial map and tries to insert it in all possible intervals. For the *flips* command a sliding window of five was chosen to improve the map by iteratively testing all possible marker orders within this window size (Buerstmayr, unpublished; Vukelic, unpublished).

The mapping distances calculated from CarthaGène are in centi-Rays (cR): a distance of one cR is equal to a 1% probability of a breakage occurring between two loci after exposure to a specific radiation dose (Rédei, 2008).

5AS deletion map visualization

To get a graphical overview of the linkage groups on the 5AS chromosome also a chart was drawn. The distances calculated with CarthaGène were used to create a map of the 5AS chromosome. The maps were drawn with MapChart v.2.20, which is a software for the graphical presentation of linkage maps and QTL (Voorrips, 2002).

2.2.4 Characterization of the deletion panel

After the map calculation, the following deletion panel characteristics were calculated:

Number of informative lines: Number of lines, where at least two neighboring markers are deleted. Sister lines (developed from same M_1 seed) which show the same deletion pattern are merged to one informative line.

Number of obligate breaks: An obligate break occurs, when one of two consecutive markers is deleted and the other one is retained. Lines with terminal deletions have one obligate break, lines with interstitial deletions have two obligate breaks (Kalavacharla, 2006). Obligate breaks were calculated for each informative line and across the whole deletion panel. Also, the distribution of the obligate breaks along the 5AS chromosome is visualized.

Number and frequency of deletions: The total number of deletions was summarized for the informative lines. Deletions can be either terminal (loss of telomere) or interstitial (telomere present). Also, the deletion frequency for each irradiation dosage panel was calculated (*Table 5*).

Deletions sizes in centi-Ray: By using the calculated distances from CarthaGène the deletion sizes in cR were estimated. The true deletion sizes cannot be calculated directly, the deletion starts anywhere between the deleted locus (marker) and the retained locus (marker). For this reason, three different deletion sizes were calculated:

- Minimum deletion size: distance between deleted markers flanking the deletion
- Maximum deletion size: distance between retained markers flanking the deletion
- Mean deletion size: mean between minimum and maximum deletion size

Physical deletion sizes in Mbp: For the 5AS chromosome a physical size of 295 Mbp has been reported (IWGSC et al., 2014), this size was used for the approximate calculations of physical deletion sizes. These 295 Mbp were apportioned to the three used physical bins: 118 Mbp for C-5AS1-0.40 bin, 103.25 Mbp for 5AS1-0.40-0.75 bin and 64.9 Mbp for 5AS3-0.75-0.97 bin (*Table 19*).

With these parameters, the cR distances calculated in CarthaGène were transferred to Mbp. Minimal deletion sizes, maximum deletion sizes, and average deletion sizes in Mbp were calculated just as the deletion sizes in cR. For this approximate calculation, it was assumed that deletion breaks occur evenly distributed within the bins. These bin specific deletion map parameters are shown in *Table 19*.

Marker retention frequency was calculated as the proportion of marker being retained on the total number of analyzed markers. Individual retention frequency was calculated for each line and each marker and across the deletion panel (*Figure 19, Table 20*).

2.2.5 Creating 5AS consensus map

To increase the resolution on the 5AS chromosome, the C3 map was combined with an existing 5AS specific Chinese Spring radiation hybrid (CSRH) map.

This 5AS specific CSRH map was constructed in previous IFA BP research (Buerstmayr unpublished; Mayer, 2016; Vukelic, unpublished). In this radiation hybrid mapping approach, Chinese Spring heads were radiated at anthesis to pollinate CS-N5AT5B plants. By this way, deletions on the 5A chromosome were hemizygous and scorable in the RH_1 generation. In this mapping approach 40 informative lines (out of 276 plants) with deletions on the 5AS chromosome were identified. Thus obtained map of the 5AS chromosome was 273.1 cR in size and had 39 loci.

CarthaGène has been designed to create consensus maps from multiple populations/panels allowing to merge C3 panel with the CSRH panel using the command *dsmergen*. *Dsmergen* command assumes that lines of the C3 and CSRH panels are members of a single unique panel.

For visualization, the three maps were graphically compared with MapChart v.2.20 (Figure 21).

3 Results

For the association of *Qfhs.ifa-5A* to AE and FHB resistance, results are shown in section 3.1. Results of the *Qfhs.ifa-5A* deletion mapping approach are given in section 3.2.

3.1 Results of the greenhouse trial: associations of *Qfhs.ifa-5A* with AE and FHB

The differences between the genotypes C3 and Remus for AR is shown in section 3.1.1. In section 3.1.2, the effect of different anther manipulation strategies on FHB resistance is presented.

3.1.1 Anther retention – differences between genotypes

The trait ´anther retention´ (AR) was measured via counting florets with retained anthers out of 25 florets as described in section 2.6. Significant differences between the genotypes were evaluated by a t-test: Remus retained more anthers than C3 (mean 22.70 retained anthers out of 25 florets versus mean 11.82 retained anthers out of 25 florets, respectively). Scoring values of AR are presented in *Table 23* (appendix on page 83), results of anther retention comparison of C3 and Remus are shown in *Table 8* and *Figure* 10.

Table 8: Result of t-test comparison of anther retention between C3 and Remus

Df	t-value	p-value
15.782	-8.5602	2.53e-07***

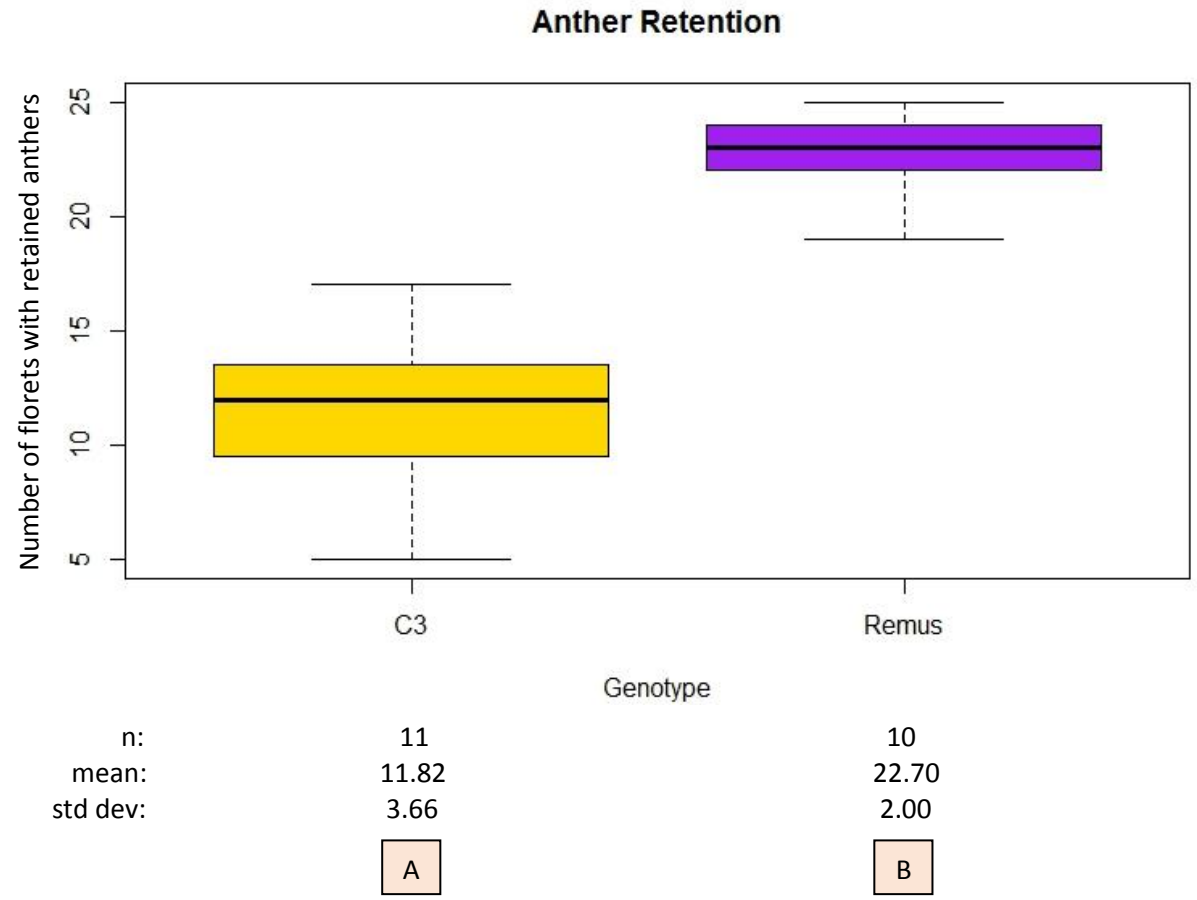

Figure 10: Boxplot comparison of the trait anther retention (AR) of the genotypes C3 and Remus. 25 florets per entry were inspected for evaluating AR. Different letters indicate significant differences between groups (α = 0.01). Detailed results of t-test are presented in the appendix page 84.

3.1.2 Effect of genotype and anther manipulation on FHB resistance

For testing the influence of AE/AR on FHB resistance, 804 heads were manipulated to simulate AE and AR. Pretreated heads were spray-inoculated and phenotyped for FHB resistance at 6, 10, 14, 18 and 22 dai. *Table 9* gives an overview of the number of genotypes and their treatments.

Inoculation of the wheat heads with the aggressive *F. graminearum* isolate IFA 65 has been identified as suitable method. First FHB symptoms were observed four dai. No significant differences between the replications were found (*Figure 24* in the appendix page 86). At early scoring dates (6 dai, 10 dai) heads showed less symptomatic spikelets, the number of infection sites (IS) was mostly equal to the total

number of symptomatic spikelets (S). Very small discolorations (< 3 mm, or unclear abnormalities which could also occur due to anther manipulation injuries) were not scored at early scoring dates – if these discolorations were caused by FHB, they were scored at later scoring dates if they could be attributed doubtlessly to FHB symptoms. At late scoring dates (only counting symptomatic spikelets, not infection sites) heads showed a high number of FHB diseased symptoms, due to disease spreading nearly all spikelets showed symptoms (*Figure 23,* appendix page 85).

Effect of the genotype on FHB resistance level

As expected, the genotype C3 was significantly more resistant than the genotype Remus regardless of scoring date and anther manipulation treatment (*Figure 11 -17*; ANOVA with Tukey post hoc tests in the appendix page 87 and following).

Effects of the anther manipulation treatments on FHB resistance

In this trial, the anther manipulation treatments showed following significant influence on FHB resistance:

Removed anthers:

Heads with removed anthers were significantly ($\alpha = 0.01$) more resistant than the control heads or heads with compressed anthers. At later scoring dates the difference in resistance became smaller due to disease spreading within the head.

Compressed anthers:

For heads with compressed anthers, no general statement can be made. On the first scoring date heads with compressed anthers showed less symptoms than the control heads (*Figure 11*). For the second scoring date (IS 10 dai) no significant difference between heads with compressed anthers and control heads was observed (*Figure 12*). On the third scoring date (IS 14 dai) heads with compressed anthers were slightly more susceptible than control heads (p value 0.015). For the fourth and fifth scorning dates (S 18 dai and S 22 dai) heads with compressed anthers were significantly more susceptible compared to control heads (*Figure 14-15*).

Details on the influence of the anther manipulation treatments for each parameter date are presented below (in *Figures 11-17*). Interactions of genotype × treatment are given as well.

Table 9: Overview of scoring parameters: IS – incidence measured as infection sites per head, S – severity measured as number of symptomatic spikelets per head.

		C3 control	C3 compressed	C3 removed	Remus control	Remus compressed	Remus removed
n replication 1:		**70**	**64**	**67**	**64**	**65**	**65**
n replication 2:		**71**	**68**	**69**	**73**	**66**	**64**
n total:		**141**	**132**	**136**	**137**	**131**	**129**
IS 6 dai	**mean**	4.09	3.47	0.29	8.39	5.37	0.40
	range	0-13	0-13	0-3	0-16	0-13	0-3
	sd	3.15	2.68	0.57	3.61	3.20	0.68
IS 10 dai	**mean**	6.36	7.15	2.40	10.65	9.57	3.69
	range	0-16	0-14	0-14	2-16	1-16	0-12
	sd	3.78	3.13	2.23	3.36	3.01	2.44
IS 14 dai	**mean**	8.46	9.98	6.07	12.04	12.01	8.42
	range	0-16	0-16	0-14	2-16	3-16	1-16
	sd	3.85	3.19	3.05	2.94	2.79	2.94
S 6 dai	**mean**	4.09	3.47	0.29	8.39	5.37	0.40
	range	0-13	0-13	0-3	0-16	0-13	0-3
	sd	3.15	2.68	0.57	3.61	3.20	0.68
S 10 dai	**mean**	6.46	7.31	2.41	10.74	9.67	3.70
	range	0-16	0-16	0-14	2-16	1-16	0-12
	sd	3.87	3.27	2.24	3.40	3.06	2.44
S 14 dai	**mean**	8.65	10.27	6.13	12.31	12.29	8.51
	range	0-16	0-16	0-15	2-16	3-16	1-16
	sd	3.99	3.37	3.10	3.01	2.86	3.01
S 18 dai	**mean**	10.11	12.14	9.28	13.51	14.02	11.54
	range	1-16	3-16	1-16	3-16	6-16	4-16
	sd	3.89	3.13	3.67	2.81	2.22	2.86
S 22 dai	**mean**	11.79	13.52	11.46	14.29	15.11	13.45
	range	1-16	3-16	2-16	3-16	7-16	6-16
	sd	4.01	2.77	3.76	2.54	1.61	2.66
AUDPC 18	**mean**	101.10	111.95	54.17	161.13	142.76	73.90
	range	2-225	6-225	2-146	30-240	46-217	12-145
	sd	51.35	40.05	26.91	45.62	37.55	25.24
AUDPC 22	**mean**	144.90	163.27	95.65	216.74	201.02	123.88
	range	14-289	22-289	12-206	48-304	76-281	34-209
	sd	64.66	48.74	39.51	53.99	42.63	33.66

FHB disease incidence as number of infection sites (IS) 6 dai

Genotype, treatment, and genotype-treatment interactions had a significant influence on the number of FHB infection sites per spike 6 dai (*Table 10*). Heads with removed anthers were significantly more resistant than control heads or heads with compressed anthers (*Figure 11*). There was no significant difference for FHB resistance between genotypes when anthers had been removed. Unexpectedly, heads with compressed anthers had a resistance level between heads with removed anthers and control heads (for both genotypes).

Table 10: ANOVA-table IS 6 dai

ANOVA 6 dai	Df	Sum of squares	Mean squares	F value	p value
Genotype	1	945.8	945.85	136.3863	< 2.2e-16 ***
Treatment	2	4838.3	2419.14	348.8268	< 2.2e-16 ***
Replication	1	1.0	1.02	0.1466	0.7019
Genotype:Treatment	2	599.2	299.58	43.1974	< 2.2e-16 ***
Residuals	797	5527.3	6.94		

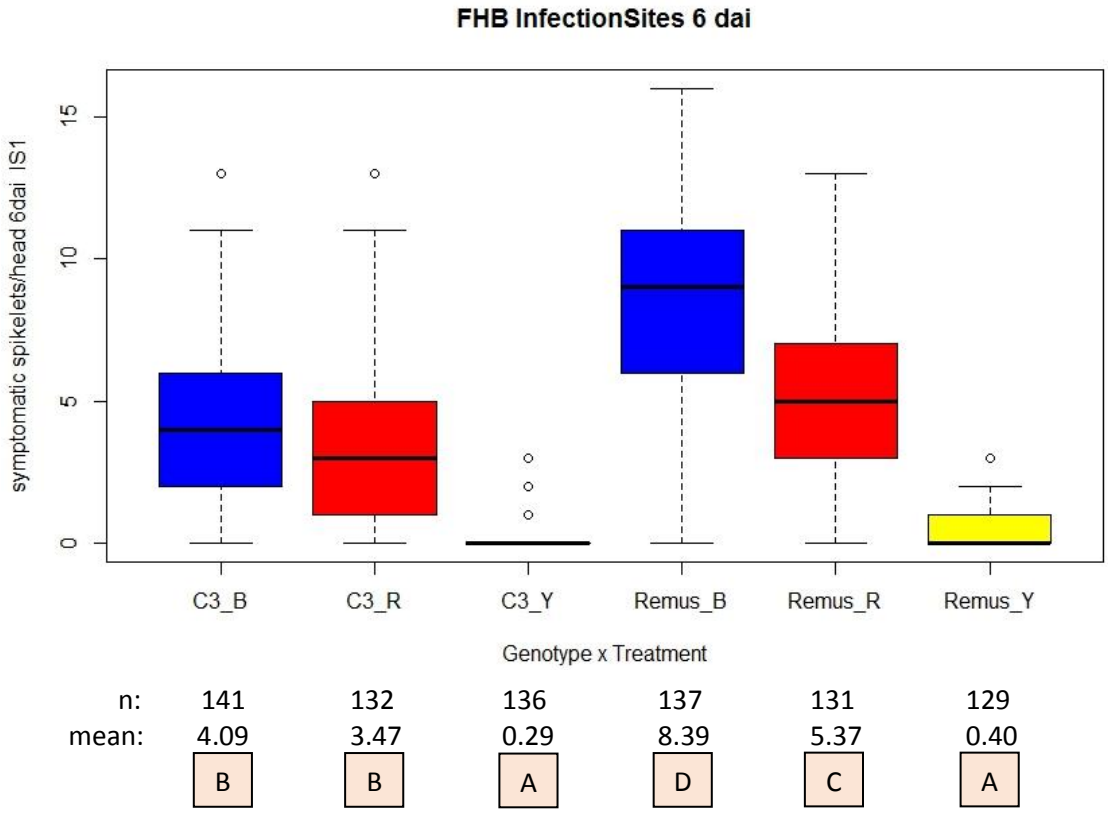

Figure 11: FHB infection sites 6 dai grouped by genotype (C3, Remus) and treatment (B(lue) = control, R(ed) = anthers compressed, Y(ellow) = anthers removed). Different letters indicate significant differences between groups (genotype × treatment, α = 0.01). Detailed results of the ANOVA with Tukey post hoc test are in the appendix page 87 and following.

FHB disease incidence as number of infection sites (IS) 10 dai

Genotype, treatment and genotype-treatment interactions had a significant influence on FHB infection sites 10 dai (*Table 11*). Heads with removed anthers were significantly more resistant than control heads or heads with compressed anthers (*Figure 12*). C3 heads with removed anthers were only slightly more resistant than Remus heads with removed anthers (p-value C3_Y – Remus_Y: 0.008). Resistance levels for heads with compressed anthers changed in comparison to IS 6 dai: C3 heads with compressed anthers showed more symptoms than C3 control heads, while Remus heads with compressed anthers were about as susceptible as Remus control heads.

Table 11: ANOVA-table IS 10 dai

ANOVA IS 10 dai	Df	Sum of squares	Mean squares	F value	p value
Genotype	1	1495.7	1495.69	161.0687	< 2.2e-16 ***
Treatment	2	5117.9	2558.93	275.5676	< 2.2e-16 ***
Replication	1	9.6	9.57	1.0303	0.3104
Genotype:Treatment	2	312.4	156.19	16.8197	7.003e-08 ***
Residuals	797	7401.0	9.29		

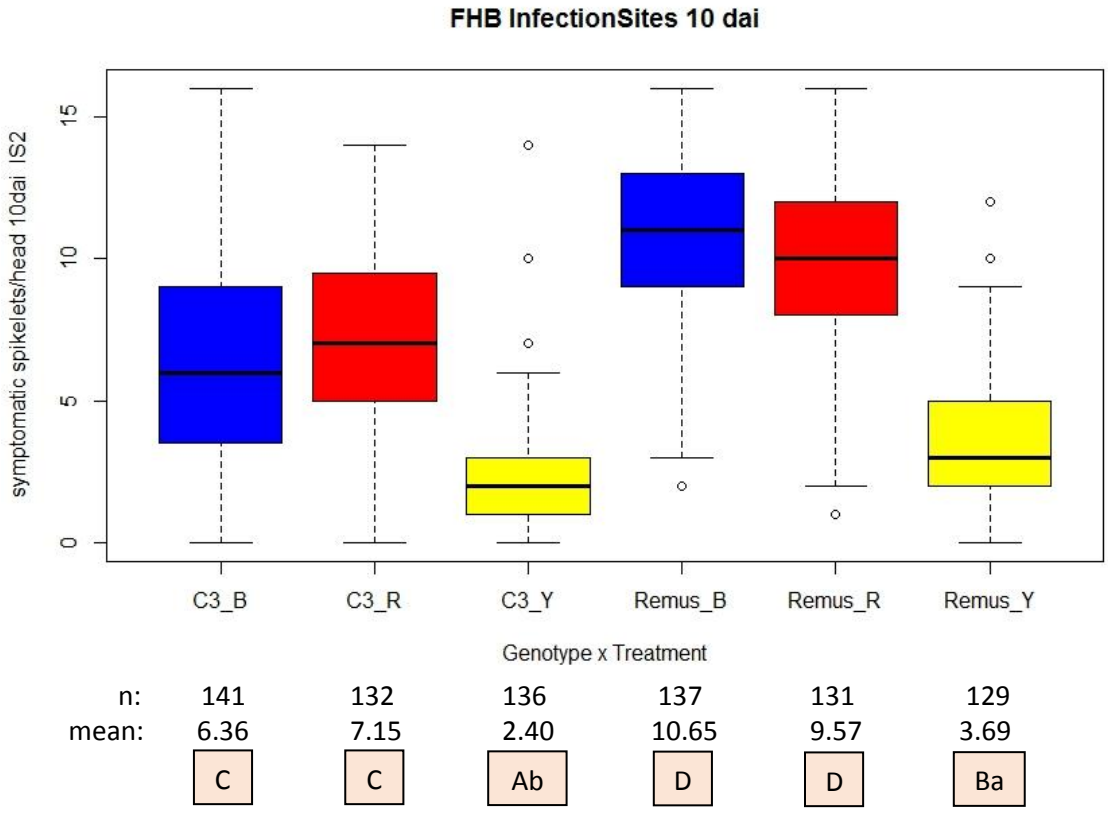

Figure 12: FHB infection sites 10 dai grouped by genotype (C3, Remus) and treatment (B(lue) = control, R(ed) = anthers compressed, Y(ellow) = anthers removed). Different letters indicate significant differences between groups (genotype × treatment, α = 0.01). Detailed results of the ANOVA with Tukey post hoc test are in the appendix page 87 and following.

FHB disease incidence as number of infection sites (IS) 14 dai

Genotype, treatment, genotype-treatment interaction, and the replication had a significant influence on the number of FHB infection sites 14 dai (*Table 12*). All C3 heads with removed anthers showed the highest resistance, followed by Remus heads with removed anthers and C3 control heads, which had about the same resistance level (*Figure 13*). C3 heads with compressed anthers were more susceptible than control heads, while Remus heads with compressed anthers were as susceptible as control heads.

Table 12: ANOVA-table IS 14 dai

ANOVA IS 14 dai	Df	Sum of squares	Mean squares	F value	p value
Genotype	1	1456.0	1456.0	147.465	< 2e-16 ***
Treatment	2	2076	1037.8	105.104	< 2e-16 ***
Replication	1	66	65.5	6.637	0.01017 *
Genotype:Treatment	2	94	47.2	4.776	0.00868 **
Residuals	797	7869	9.9		

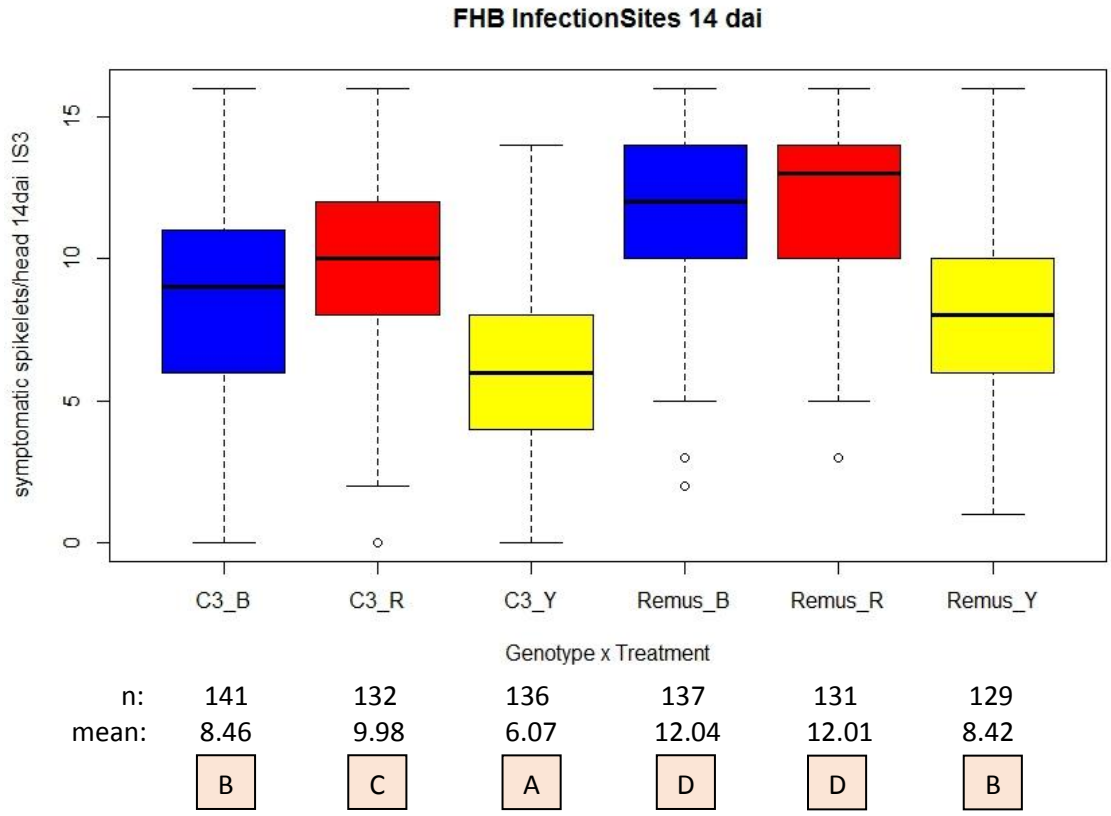

Figure 13: FHB infection sites 14 dai grouped by genotype (C3, Remus) and (B(lue) = control, R(ed) = anthers compressed, Y(ellow) = anthers removed). Different letters indicate significant differences between groups (genotype × treatment, α = 0.01). Detailed results of the ANOVA with Tukey post hoc test are in the appendix page 87 and following.

FHB disease severity as number of infected spikelets (S) 18 dai

Genotype, treatment, and genotype-treatment interaction had a significant influence on number of FHB symptomatic spikelets 18 dai (*Table 13*). Due to disease spreading, heads with removed anthers showed also a lot of FHB symptomatic spikelets on this scoring date (*Figure 14*). Each anther treatment is significantly different compared to other treatments. The group with removed anthers was significantly more resistant than control heads or heads with compressed anthers. Heads with compressed anthers were significantly more susceptible than control heads at this scoring date.

Table 13: ANOVA-table S 18 dai

ANOVA S 18 dai	Df	Sum of squares	Mean squares	F value	p value
Genotype	1	1305.7	1305.67	130.9217	<2e-16 ***
Treatment	2	936.8	468.40	46.9668	<2e-16 ***
Replication	1	6.0	6.05	0.6063	0.43641
Genotype:Treatment	2	85.8	42.90	4.3015	0.01386 *
Residuals	797	7948.4	9.97		

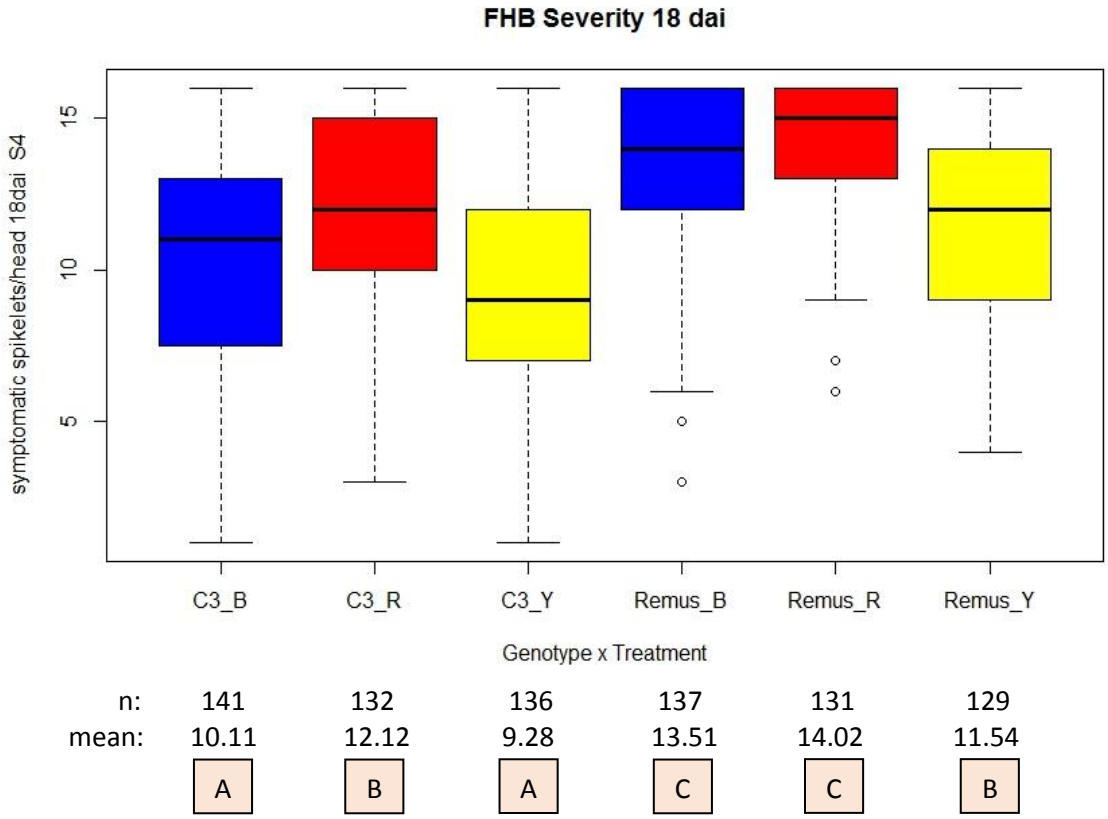

Figure 14: FHB diseased spikelets 18 dai grouped by genotype (C3, Remus) and treatment (B(lue) = control, R(ed) = anthers compressed, Y(ellow) = anthers removed). Different letters indicate significant differences between groups (genotype × treatment, α = 0.01). Detailed results of the ANOVA with Tukey post hoc test are in the appendix page 87 and following.

FHB disease severity as number of infected spikelets (S) 22 dai

Genotype and treatment had a significant influence on number of FHB symptomatic spikelets 22 dai (*Table 14*). At this scoring date, numerous heads were already completely diseased (all 16 spikelets showed symptoms, *Figure 15*). Heads with removed anthers did not show an increased resistance compared to control heads, but a higher resistance compared to heads with compressed anthers. Heads with compressed anthers were significantly more susceptible than control heads on this scoring date: Remus heads with compressed anthers showed symptoms most frequently.

Table 14: ANOVA-table S 22 dai

ANOVA S 22 dai	Df	Sum of squares	Mean squares	F value	p value
Genotype	1	840	839.6	92.395	< 2.2e-16 ***
Treatment	2	478	238.8	26.279	8.87e-12 ***
Replication	1	8	7.6	0.833	0.362
Genotype:Treatment	2	29	14.5	1.594	0.204
Residuals	797	7242	9.1		

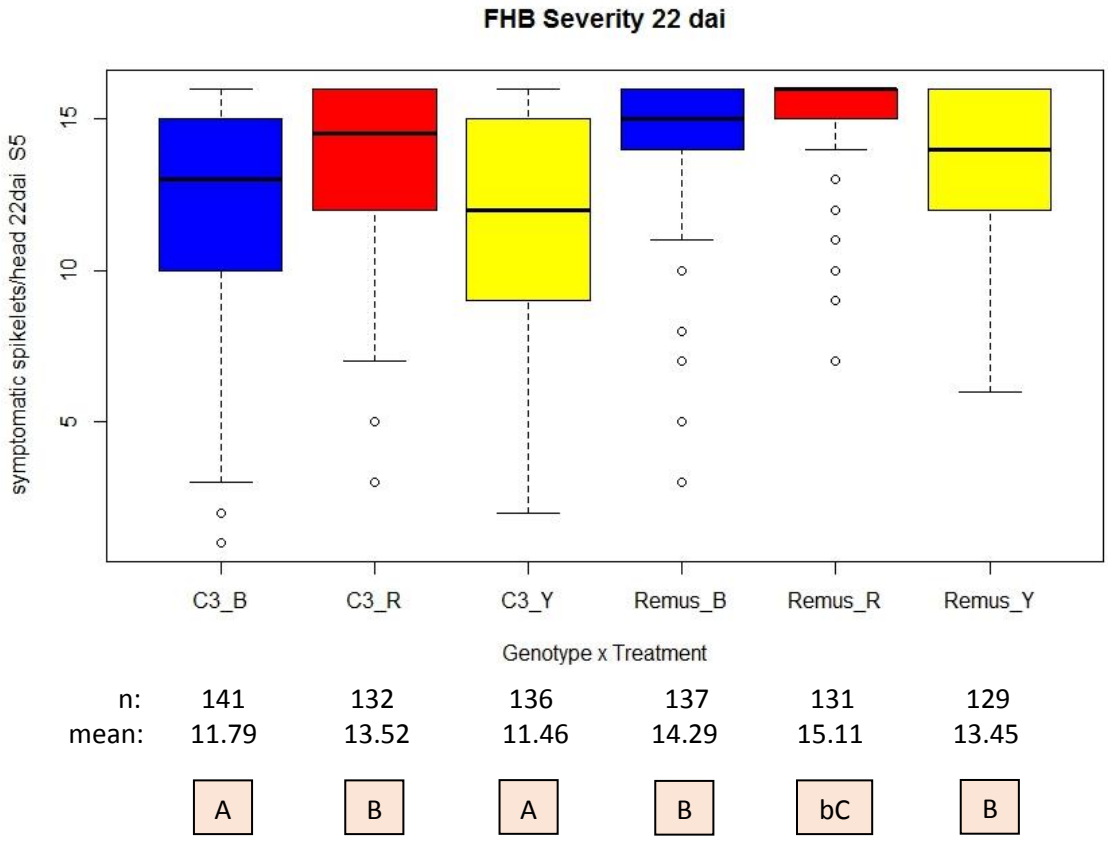

Figure 15: FHB diseased spikelets 22 dai grouped by genotype (C3, Remus) and treatment (B(lue) = control, R(ed) = anthers compressed, Y(ellow) = anthers removed). Different letters indicate significant differences between groups (genotype × treatment, α = 0.01). Detailed results of the ANOVA with Tukey post hoc test are in the appendix page 87 and following.

FHB severity as area under diseased progress curve (AUDPC) 18 dai

Genotype, treatment, and genotype-treatment interaction had a significant influence on the FHB AUDPC 18 dai (*Table 15*). For the parameter AUDPC 18 dai, heads with removed anthers were more resistant: C3 heads with removed anthers were most resistant, followed by Remus heads with removed anthers (*Figure 16*). C3 heads with compressed anthers and C3 control heads showed the same resistance level. Remus control heads were most susceptible followed by Remus heads with compressed anthers.

Table 15: ANOVA-table AUDPC 18 dai

ANOVA AUDPC 18 dai	Df	Sum of squares	Mean squares	F value	p value
Genotype	1	285202	285202	186.40	< 2.2e-16 ***
Treatment	2	750763	375381	245.34	< 2.2e-16 ***
Replication	1	1760	1760	1.15	0.284
Genotype:Treatment	2	59324	29662	19.39	6.01e-09 ***
Residuals	797	1219433	1530		

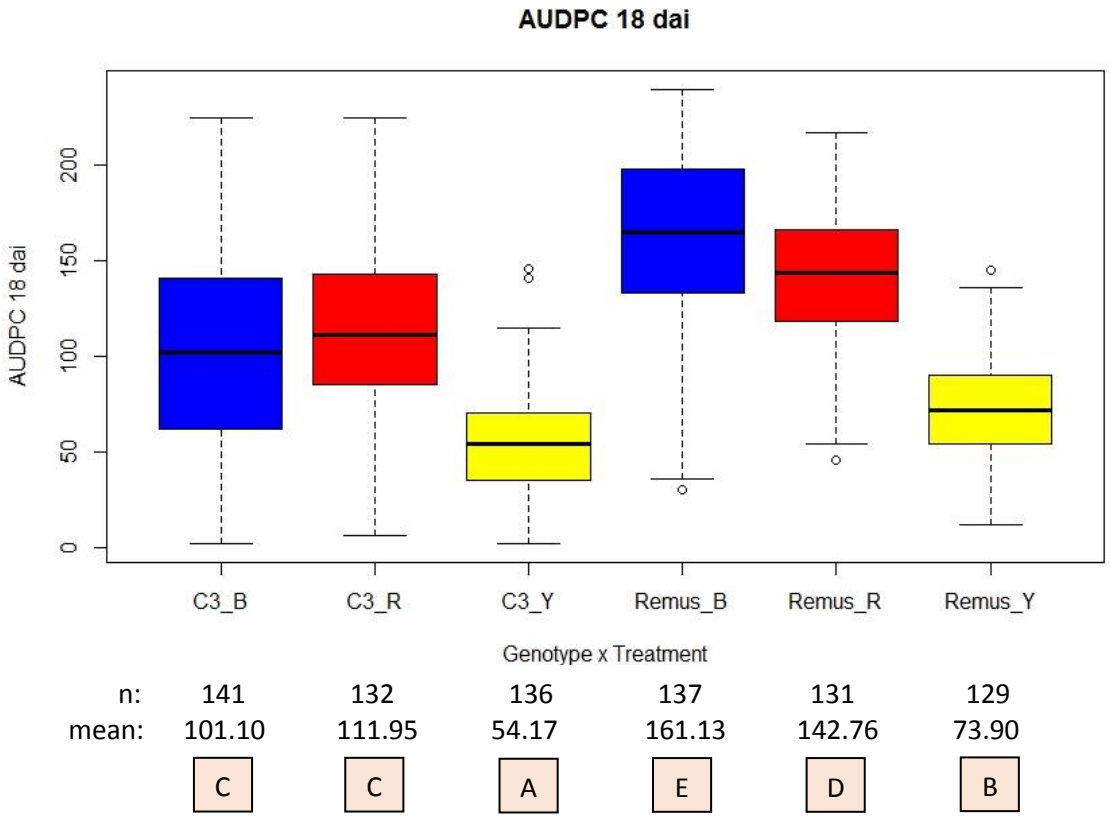

Figure 16: AUDPC 18 dai grouped by genotype (C3, Remus) and treatment (B(lue) = control, R(ed) = anthers compressed, Y(ellow) = anthers removed). Different letters indicate significant differences between groups (genotype × treatment, α = 0.01). Detailed results of the ANOVA with Tukey post hoc test are in the appendix page 87 and following.

FHB severity as area under diseased progress curve (AUDPC) 22 dai

Genotype, treatment, and genotype-treatment interaction had a significant influence on the FHB AUDPC 22 dai (*Table 16*). The parameter AUDPC 22 dai is similar to AUDPC 18 dai, but it shows an increased severity (*Figure 17*). Again, the treatments with anthers removed were most resistant (C3 heads with removed anthers were more resistant than Remus heads with removed anthers). The treatment control group and the group with compressed anthers did not show significant differences within the genotype.

Table 16: ANOVA-table AUDPC 22 dai

ANOVA AUDPC 22 dai	Df	Sum of squares	Mean squares	F value	p value
Genotype	1	441243	441243	187.448	< 2.2e-16 ***
Treatment	2	904929	452464	192.215	< 2.2e-16 ***
Replication	1	2743	2743	1.165	0.281
Genotype:Treatment	2	72067	36034	15.308	3e-07 ***
Residuals	797	1876098	2354		

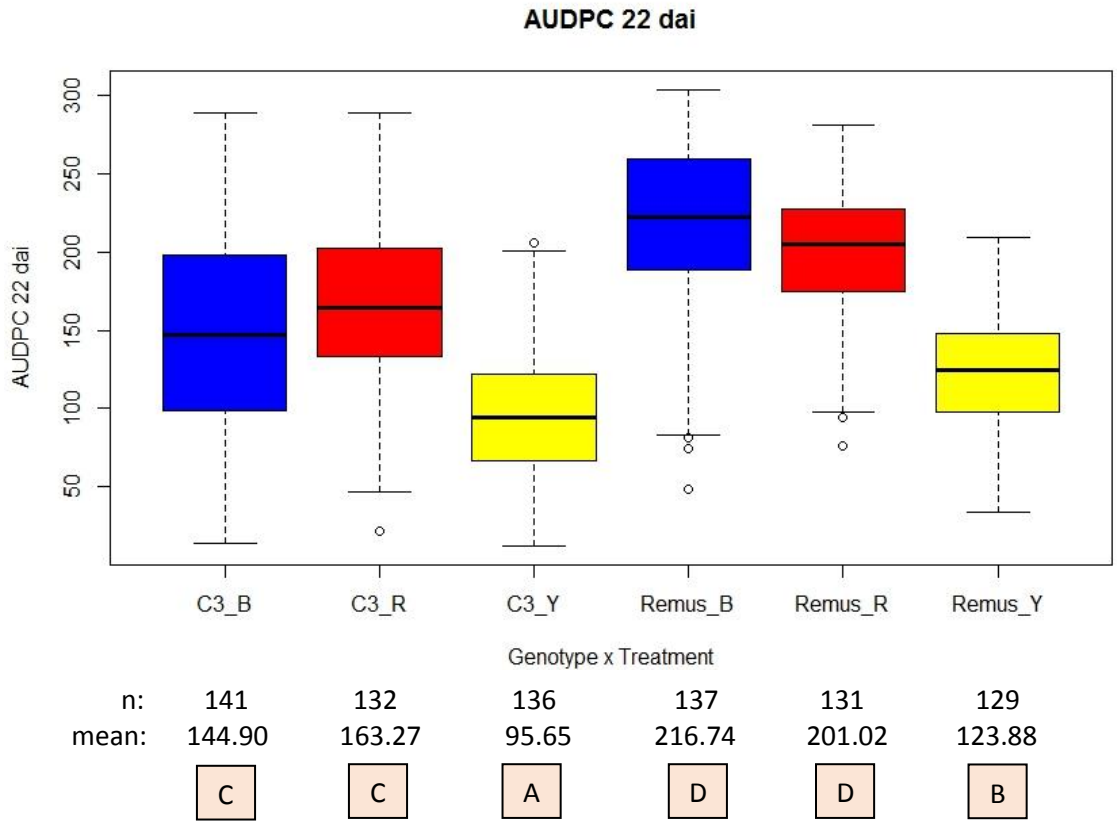

Figure 17: AUDPC 22 dai grouped by genotype (C3, Remus) and treatment (B(lue) = control, R(ed) = anthers compressed, Y(ellow) = anthers removed). Different letters indicate significant differences between groups (genotype × treatment, α = 0.01). Detailed results of the ANOVA with Tukey post hoc test are in the appendix page 87 and following.

3.2 Fine-mapping results of the *Qfhs.ifa-5A* resistance QTL

Based on pre-screening, 1764 lines with 15 markers a subset of 48 lines were selected for fine-mapping. This subset was composed of 31 lines with clear deletions, 11 with unsure marker scores, and 6 control lines. Selected lines were genotyped with 102 5AS specific markers (101 markers, SSR marker wmc150 has two 5AS specific bands). None of the eleven lines with unsure marker scores showed deletions. Analysis confirmed deletions in 31 lines. Of these lines, there are three pairs of sister lines with identical deletion patterns descending from the same M_1 seed: D_451 with D_452, D_479 with D_480 and D_164 with D_166. Identical sister lines were merged to represent a single genotype resulting in 28 informative lines.

These 28 informative lines were used for further calculations. Line D_627 showed two separate deletion blocks, resulting in a total of 29 deletions. The deletion map (calculated with CarthaGène) had a size of 399.8 cR and is shown in *Table 18 and Figure 18*. Sixty-four of the 102 tested markers had duplicated retention patterns, resulting in 38 unique loci. In average 2.68 markers were present within a locus (range: one to eight markers per locus). Within one locus, markers cannot be ranked. *Table 18* summarizes the marker presence/retention of the 28 informative lines. *Table 17* gives an overview on the deletion panel characteristics.

Table 17: Summary of deletion panel and map characteristics

Deletion panel and map characteristics			
Lines with deletions	31	Map distance	
Informative lines	28	in cR	399.8
Markers	102	in Mbp	286.2
Deletion number	29	Retention frequency per marker (%)	
Breaks	51	Minimum	64.3
Informative breaks	37	Maximum	92.9
Mapped loci	38	Average	75
Markers per locus		Retention frequency per line (%)	
Minimum	1	Minimum	0
Maximum	8	Maximum	98
Average	2.68	Average	75
Distances between loci (cR/ Mbp)		Deletion length (cR/Mbp)	
Minimum	6.7/4.28	Minimum	3.8/6.5
Maximum	26.2/21.0	Maximum	399.8/286.2
Average	10.3/7.7	Average	91.8/74.1

Table 18: Marker retention/deletion part 1: BIN 5AS3-0.75-0.97

BIN	Marker	Distance (cumulative) in cR	Distance (cumulative) in Mbp	Distance (between loci) in cR	Distance (between loci) in Mbp	Lines D_723	D_393	D_1612	D_752	D_1303	D_725	D_391	D_175	D_451+452	D_573	D_291	D_457	D_515	D_479+480	D_1077	D_1268	D_1075	D_1988	D_1604	D_612	D_1494	D_1433	D_627	D_628	D_1537	D_473	D_164+166	D_426
5AS3 0.75 - 0.97 (64.9Mbp)	gwm443	0,0	0,0			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	-	1	1	1	D	1	D	1	1	1
	gpg1293	0,0	0,0			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	D	1	D	1	1	1
	IWB11440	0,0	0,0			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	-	1	1	1	1	1	1	D	1	D	1	1	1
	gpg2	0,0	0,0			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	-	1	1	D	1	D	1	1	1
	cwem44c	7,5	6,5	7,5	6,5	D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	-	1	1	1	1	1	1	1	1	D	1	1	1
	wmc654	7,5	6,5			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	1	1	D	1	1	1
	gpg537	7,5	6,5			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	1	1	D	1	1	1
	IWB62899	14,8	12,7	7,3	6,3	D	D	1	1	-	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	1	1	D	1	D	1
	IWB4146	14,8	12,7			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	1	1	D	1	D	1
	gpg2328	21,9	18,9	7,1	6,1	D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	1	1	D	1	D	D
	gpg2326	21,9	18,9			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	1	1	D	1	D	D
	IWB29780	21,9	18,9			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	1	1	D	1	D	D
	IWB68241	21,9	18,9			D	D	1	-	1	1	1	1	1	1	D	1	-	1	D	D	1	1	1	1	1	1	1	1	-	1	D	D
	barc186	21,9	18,9			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	1	1	D	1	D	D
	Idk243	21,9	18,9			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	1	1	D	1	D	D
	IWB51518	21,9	18,9			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	1	1	D	1	D	D
	Idk267	21,9	18,9			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	-	1	1	1	1	1	1	1	1	D	1	D	D
	barc56	46,3	39,9	24,4	21,0	D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	D	1	D	1	1	1
	Idk284	46,3	39,9			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	D	1	D	1	1	1
	gpg2162	46,3	39,9			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	D	1	D	1	1	1
	gpg2163	46,3	39,9			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	D	1	D	1	1	1
	gpg1438	53,7	46,2	7,4	6,4	D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	D	D	D	1	1	1
	gpg5	60,8	52,3	7,1	6,1	D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	D	D	D	D	1	1
	barc117	60,8	52,3			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	D	D	D	D	1	1
	gpg2168	67,9	58,4	7,1	6,1	D	D	-	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	-	1	1	1	D	D	D	1	1	1
	gpg2038	75,4	64,9	7,5	6,5	D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	D	D	1	1	1	1

Legend

- D Deletion
- - Missing value, deletion estimated
- 1 Marker present
- - Missing value, marker estimated to be retained
- __ Break

Table 18 continued: Marker retention/deletion part 2: BIN 5AS1-0.40-0.75

BIN	Marker	Distance (cumulative)		Distance (between loci)		Lines																											
		in cR	in Mbp	in cR	in Mbp	D_723	D_393	D_1612	D_752	D_1303	D_725	D_391	D_175	D_451+452	D_573	D_291	D_457	D_515	D_479+480	D_1077	D_1268	D_1075	D_1988	D_1604	D_612	D_1494	D_1433	D_627	D_628	D_1537	D_473	D_164+166	D_426
5AS1 0.40 - 0.75 (103.25 Mbp)	IWB75561	75,4	64,9	7,5	6,5	D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	D	D	1	1	1	1
	jfio7	83,3	70,7	7,9	5,8	D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	D	1	1	1	1	1
	gpg1440	83,3	70,7			D	D	1	1	1	1	1	1	1	-	D	1	1	1	D	D	1	1	1	1	1	1	D	1	1	1	1	1
	wmc150a	83,3	70,7			D	D	-	1	-	1	1	1	1	-	D	1	1	1	-	D	1	1	1	1	1	-	D	1	1	1	1	1
	gwm293	83,3	70,7			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	D	1	1	1	1	1
	gwm304	83,3	70,7			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	D	1	1	1	1	1
	IWB8393	83,3	70,7			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	D	1	1	1	1	1
	wmc150b	99,9	83,0	16,6	12,3	D	D	1	1	1	1	1	1	1	-	D	1	1	1	D	D	1	1	1	D	D	1	D	1	1	1	1	1
	gpg2049	107,1	88,4	7,2	5,3	D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	D	D	D	D	1	1	1	1	1
	gpg2060	107,1	88,4			D	D	1	1	1	1	1	1	1	1	-	1	-	1	D	D	1	-	1	D	D	D	D	1	1	1	1	1
	IWB10809	107,1	88,4			D	D	1	1	1	1	1	1	1	1	D	-	1	1	D	D	1	1	1	D	D	D	D	1	1	1	1	1
	ldk49	114,3	93,7	7,2	5,3	D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	D	D	D	1	1	1	1	1	1
	BE498768	114,3	93,7			D	D	-	1	1	1	1	1	1	-	D	1	1	1	-	-	1	1	1	D	D	D	1	-	1	1	1	1
	ldk2	114,3	93,7			D	D	1	1	-	1	1	1	1	1	D	1	1	1	D	-	1	1	1	-	D	D	1	1	-	1	1	1
	gpg2233	121,9	99,3	7,6	5,6	D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	D	D	1	1	1	1	1	1	1
	gpg2092	129,9	105,2	8,0	5,9	D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	D	1	1	1	1	1	1	1	1
	gpg2072	129,9	105,2			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	D	1	1	1	1	1	1	1	1
	gpg1763	129,9	105,2			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	D	1	1	1	1	1	1	1	1
	IWB58275	129,9	105,2			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	-	1	1	1	D	-	-	1	1	1	1	1	1
	ldk217	129,9	105,2			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	D	1	1	1	1	-	1	1	1
	IWB33435	129,9	105,2			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	-	1	D	1	1	1	1	1	1	1	1
	gpg2126	138,5	111,6	8,6	6,4	D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	1	1	1	1	1	1
	gpg574	156,5	124,9	18,0	13,3	D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	D	D	1	1	1	1	1	1	1	1	1	1
	gpg277	164,1	130,6	7,6	5,6	D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	D	D	D	1	1	1	1	1	1	1	1	1
	ldk218	164,1	130,6			D	D	1	1	1	1	1	1	1	1	D	1	1	1	-	D	D	D	D	1	1	1	1	1	1	1	1	1
	ldk241	180,9	143,0	16,8	12,4	D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	D	1	1	1	1	1	1	1	1	1	1	1
	gpg1139	189,5	149,4	8,6	6,4	D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	1	1	1	1	1	1
	gpg1789	189,5	149,4			D	D	1	1	1	1	1	1	1	1	-	1	1	1	D	D	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2309	189,5	149,4			D	D	1	1	1	1	1	1	1	1	D	1	1	1	D	D	1	1	1	1	1	1	1	1	1	1	1	1
	jfio4	198,1	155,7	8,6	6,4	D	D	1	1	1	1	1	1	1	1	D	1	D	1	D	D	1	1	1	1	1	1	1	1	1	1	1	1
	gpg1994	214,9	168,2	16,8	12,4	D	D	1	1	1	1	1	D	D	1	D	1	D	1	D	D	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2250	214,9	168,2			D	D	1	1	1	1	1	D	D	1	D	1	D	1	D	D	1	1	1	1	1	1	1	1	1	1	1	1

Table 18 continued: Marker retention/deletion part 3: BIN C-5AS1-0.40

BIN	Marker	Distance (cumulative)		Distance (between loci)		Lines																											
		in cR	in Mbp	in cR	in Mbp	D_723	D_393	D_1612	D_752	D_1303	D_725	D_391	D_175	D_451+452	D_573	D_291	D_457	D_515	D_479+480	D_1077	D_1268	D_1075	D_1988	D_1604	D_612	D_1494	D_1433	D_627	D_628	D_1537	D_473	D_164+166	D_426
5AS1 -0.40 (118 Mbp)	Idk50	214,9	168,2	16,8	12,4	D	D	1	1	1	1	1	D	D	1	D	1	D	1	D	D	1	1	1	1	1	1	1	1	1	1	1	-
	gpg2244	241,1	184,9	26,2	16,7	D	D	1	1	D	1	1	D	D	1	D	1	D	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Idk16	241,1	184,9			D	D	1	1	D	1	1	D	D	1	-	1	D	1	1	1	1	-	-	1	1	1	1	1	1	1	1	1
	gpg2313	241,1	184,9			D	D	1	1	D	1	1	D	D	1	D	1	D	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Idk14	241,1	184,9			D	D	1	1	D	1	1	D	D	1	D	1	D	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2019	248,7	189,7	7,6	4,9	D	D	1	1	D	1	1	D	D	1	D	1	D	D	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2108	248,7	189,7			D	D	1	1	D	1	1	D	D	1	D	1	D	D	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg1383	248,7	189,7			D	D	1	1	D	1	1	D	D	-	D	1	D	D	1	1	1	1	1	1	1	1	1	1	1	-	1	1
	Idk242	248,7	189,7			D	D	1	1	D	1	1	D	D	1	D	1	D	D	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gwm129	248,7	189,7			D	D	1	1	D	1	1	D	D	1	D	1	D	D	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	jfio2	256,3	194,6	7,6	4,9	D	D	1	1	D	1	1	D	D	1	D	1	D	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Idk289	264,4	199,7	8,1	5,2	D	D	1	1	D	1	1	D	D	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2231	272,5	204,9	8,1	5,2	D	D	1	1	D	1	-	D	D	1	D	D	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2232	272,5	204,9			D	D	1	1	D	1	1	D	D	1	D	D	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2075	280,1	209,8	7,6	4,9	D	D	1	1	D	1	1	-	D	D	-	D	1	1	1	1	1	1	-	1	1	1	-	1	1	1	1	1
	gpg2083	280,1	209,8			D	D	-	1	-	1	1	D	D	D	D	D	1	1	-	-	-	1	-	1	1	-	1	1	1	1	1	1
	gpg2097	280,1	209,8			D	D	1	1	D	1	1	D	D	D	D	D	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gwm415	280,1	209,8			D	D	1	1	D	1	1	D	D	D	D	D	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg2336	294,8	219,1	14,7	9,4	D	D	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
	gpg2121	294,8	219,1			D	D	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
	gpg119	294,8	219,1			D	D	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
	gpg35	294,8	219,1			D	D	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
	gpg743	294,8	219,1			D	D	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
	gpg1294	294,9	219,2			D	D	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
	gpg2117	301,6	223,5	6,7	4,3	D	D	1	1	D	D	D	D	D	D	D	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-	1	1
	Idk113	301,6	223,5			D	D	1	1	D	D	D	D	D	D	D	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	gpg214	308,8	228,1	7,2	4,6	D	D	1	1	D	D	D	D	D	D	1	1	1	1	1	1	1	1	1	1	1	-	1	1	1	1	1	1
	gpg2020	308,8	228,1			D	D	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
	gpg2147	316,3	232,9	7,5	4,8	D	D	1	1	D	D	D	D	D	1	1	1	1	1	1	1	1	1	1	-	1	1	-	-	1	1	1	1
	gpg2123	316,3	232,9			D	D	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
	gpg2102	316,3	232,9			D	D	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
	gpg1395	333,7	244,0	17,4	11,1	D	D	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
	gpg2158	333,7	244,0			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
	gpg1321	333,7	244,0			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
	Idk215	342,0	249,3	8,3	5,3	D	D	D	1	-	D	D	1	1	1	1	1	1	1	-	-	1	1	1	1	1	1	1	1	1	1	1	1
	gpg1777	342,1	249,3			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
	gpg158	360,5	261,1	18,4	11,7	D	D	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
	gpg2034	360,5	261,1			D	D	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
	gpg2255	360,5	261,1			D	D	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
	gpg2011	369,8	267,0	9,3	5,9	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
	BE425161	379,1	272,9	9,3	5,9	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
	gpg542	379,1	272,9			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
	cfa2250	399,8	286,2	20,7	13,2	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
	jfio6	399,8	286,2			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

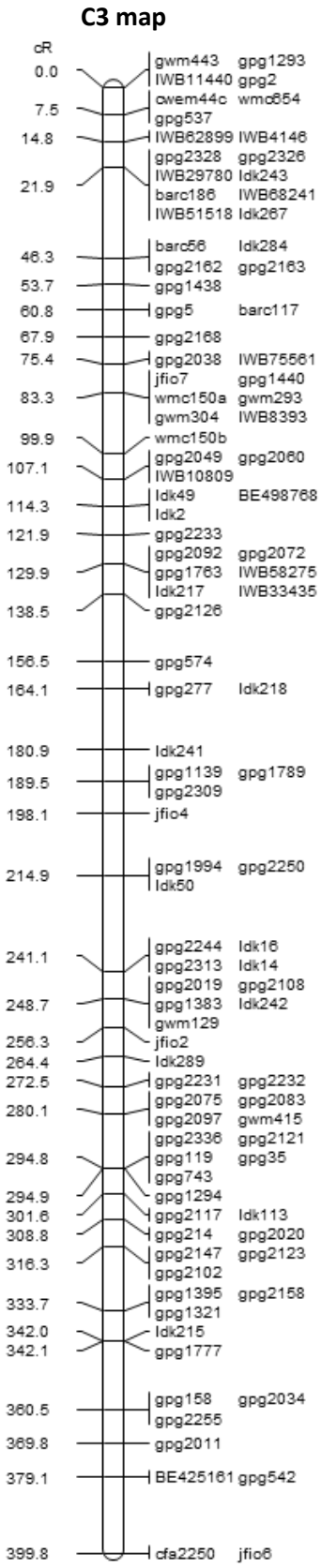

Figure 18: C3 map of 5AS chromosome

Deletion sizes

For all lines the deletion sizes in cR and Mbp were calculated, except for line D_627 where the size of two deletion blocks was calculated separately (*Table 20*). Seven lines have all terminal markers deleted, assuming that the entire telomeric region is deleted. *Table 19* gives an overview about the estimated bin sizes. However, this telomeric region with an estimated size of 8.85 Mbp was not considered for deletion sizes. The lines D_393 and D_723 showed the largest deletion, all 102 used markers were absent. For these two lines the whole 5AS chromosome was deleted; markers on the 5AL chromosome were retained, indicating that the 5AL chromosome is undeleted.

Table 19: 5AS chromosome bin sizes and map resolution

Bin	Size in Mbp[1)]	Size in cR[2)]	Markers	Loci	Map resolution cR/Mbp
5AS3-0.75-0.97	64.90	75.4	26	8	0.86
5AS1-0.40-0.75	103.25	139.5	32	13	0.74
C-5AS1-0.40	118.00	184.9	44	17	0.64
3 Bins together	286.15	399.8	102	38	0.72

1) Whole 5AS chromosome size of 295 Mbp (IWGSC, 2014) separated for bins
2) Whole calculated map size 399,8 cR, separated for bins

Table 20: Deletion characteristics of screened lines

Deletion lines	Retention frequency (%)	Obligate breaks	Deletion size (cR)			Deletion size (Mbp)		
			Minimum	Average	Maximum	Minimum	Average	Maximum
D_393	0	1	399,8	399,8	399,8	286,2	286,2	286,2
D_723	0	1	399,8	399,8	399,8	286,2	286,2	286,2
D_291	18	1	301,6	305,2	308,8	223,5	225,8	228,1
D_1077	42	1	214,9	228,0	241,1	168,2	176,5	184,9
D_1268	42	1	214,9	228,0	241,1	168,2	176,5	184,9
D_1303	63	2	119,4	137,2	154,9	76,2	87,5	98,9
D_175	68	2	101,4	118,5	135,6	64,7	76,5	88,3
D_451+452	68	2	101,4	118,5	135,6	64,7	76,5	88,3
D_1537	75	1	67,9	71,7	75,4	58,4	61,7	64,9
D_627	76	3	60,8	76,6	92,4	48,5	61,7	74,8
			-	3,8	7,5	-	3,2	6,5
D_391	82	2	47,3	63,9	80,4	30,2	40,7	51,3
D_725	82	2	47,3	63,9	80,4	30,2	40,7	51,3
D_515	86	2	58,2	66,6	74,9	38,9	44,6	50,4
D_573	86	2	28,7	36,3	43,8	18,3	23,1	28,0
D_612	86	2	30,0	42,6	55,2	22,2	31,5	40,9
D_457	88	2	22,4	29,8	37,2	14,3	19,0	23,7
D_164+166	90	2	7,1	23,0	38,8	6,1	19,8	33,4
D_426	92	2	-	15,8	31,5	-	13,6	27,1
D_1494	92	2	22,0	34,3	46,6	16,3	25,4	34,5
D_1612	92	2	37,1	51,6	66,1	23,7	32,9	42,2
D_628	94	2	21,7	29,4	37,0	18,7	24,8	30,9
D_1433	94	2	7,2	14,6	22,0	5,3	10,8	16,3
D_479+480	95	2	-	7,6	15,2	-	4,9	9,7
D_1075	96	2	24,4	37,7	51,0	18,1	27,9	37,7
D_1988	97	2	7,6	25,0	42,4	5,6	18,5	31,4
D_473	98	2	-	7,1	14,2	-	6,1	12,2
D_752	98	2	-	15,0	30,0	-	9,6	19,1
D_1604	98	2	-	12,2	24,4	-	9,0	18,1
Average:	**75,0**	**1,8**	**80,8**	**91,8**	**102,9**	**58,4**	**66,2**	**74,1**

For calculating minimum deletion size, the distance between positions of the deleted marker loci flanking the deletion were taken, for maximum deletion size the distance between retained makers flanking the deletion were taken. Minimum deletion sizes ´-´ mean that just one locus was deleted and a minimum deletion size cannot be calculated (would be zero). Line D_627 has two deletion blocks. Note: decimal is ´,´.

Marker retention frequency along the 5AS chromosome

Marker retention (per marker) was in average 75%. The highest marker retention (92.9%) was observed in the centromeric region for the markers cfa2250 and jfio6 (*Figure 19*).

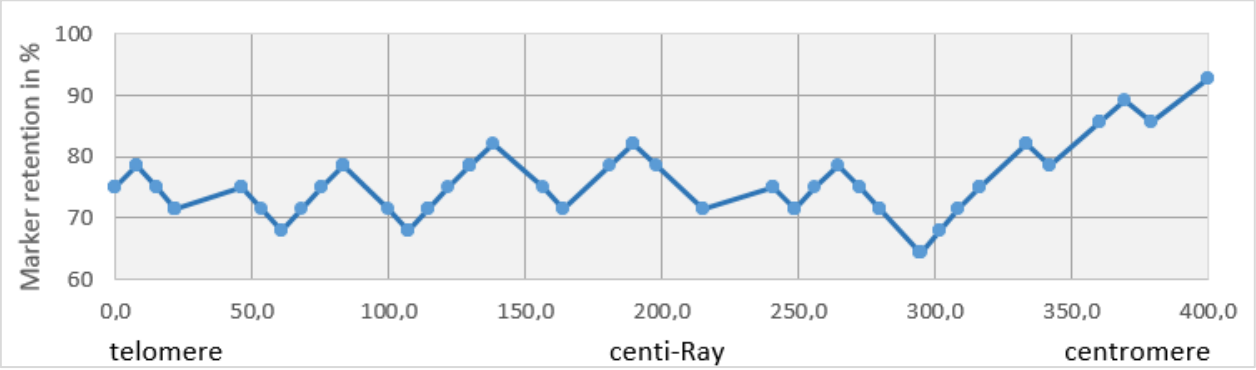

Figure 19: Marker retention along the 5AS chromosome

Distribution of obligate breaks along the 5AS chromosome

Figure 20 illustrates the distribution of the obligate breaks. Altogether 51 obligate breaks were observed. These breaks separated the 5AS chromosome by 38 loci. The mean distance between loci was 10.3 cR (minimal distance 6.8 cR, maximal distance 26.2 cR).

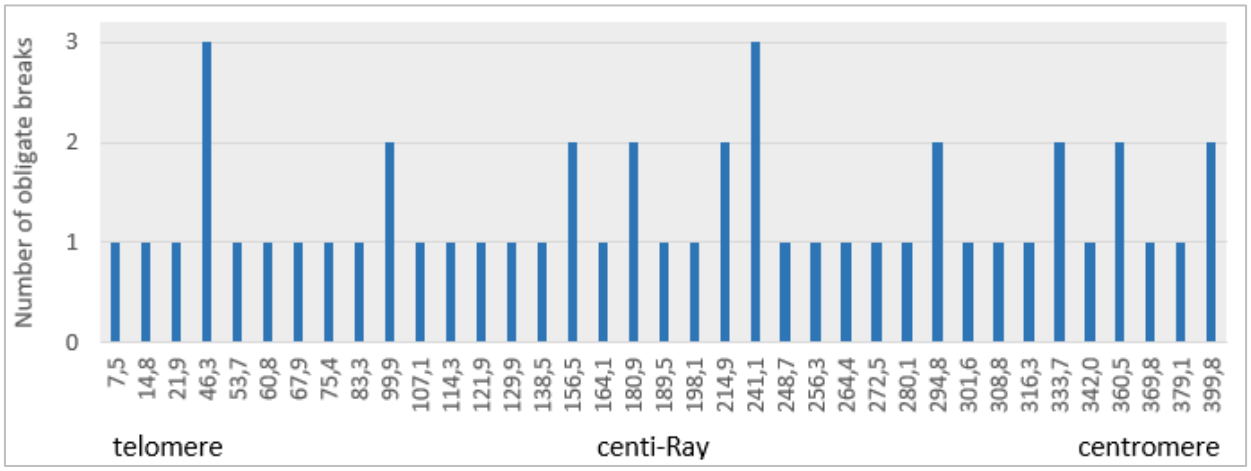

Figure 20: Distribution of obligate breaks along the 5AS chromosome

Map comparison

The C3 map was merged with the CSRH map to a consensus map, using the software CarthaGène. In both maps the 5AS chromosome was screened with the same 102 5AS specific markers. Genotypic data of both panels calculated a map length of 300.3 cR and the markers mapped to 60 loci. *Table 21* gives an overview of the consensus map and the two single maps. *Figure 21* presents all maps.

Table 21: Summary statistics of C3 map, CSRH map and consensus map (simplified after Buerstmayr, unpublished)

	C3 map mean (range)	**CSRH map mean (range)**	**Consensus map mean (range)**
number of informative lines	28	40	68
number of markers	102	102	102
number of mapped loci	38	39	60
total map size (cR)	399.8	273.1	300.3
retention frequency /line	0.75 (0-0.98)	0.55 (0.09-0.97)	0.64 (0-0.97)
retention frequency /marker	0.75 (0.64-0.93)	0.55 (0.33-0.96)	0.64 (0.50-0.96)
markers per locus	2.6 (1-3)	2.6 (1-5)	1.7 (1-7)
distances between loci (cR)	10.3 (6.8-26.2)	7.0 (3.9-24.8)	5.0 (2.3-18.4)
total number of breakpoints	51	61	112
total number of deletions	29	42	71

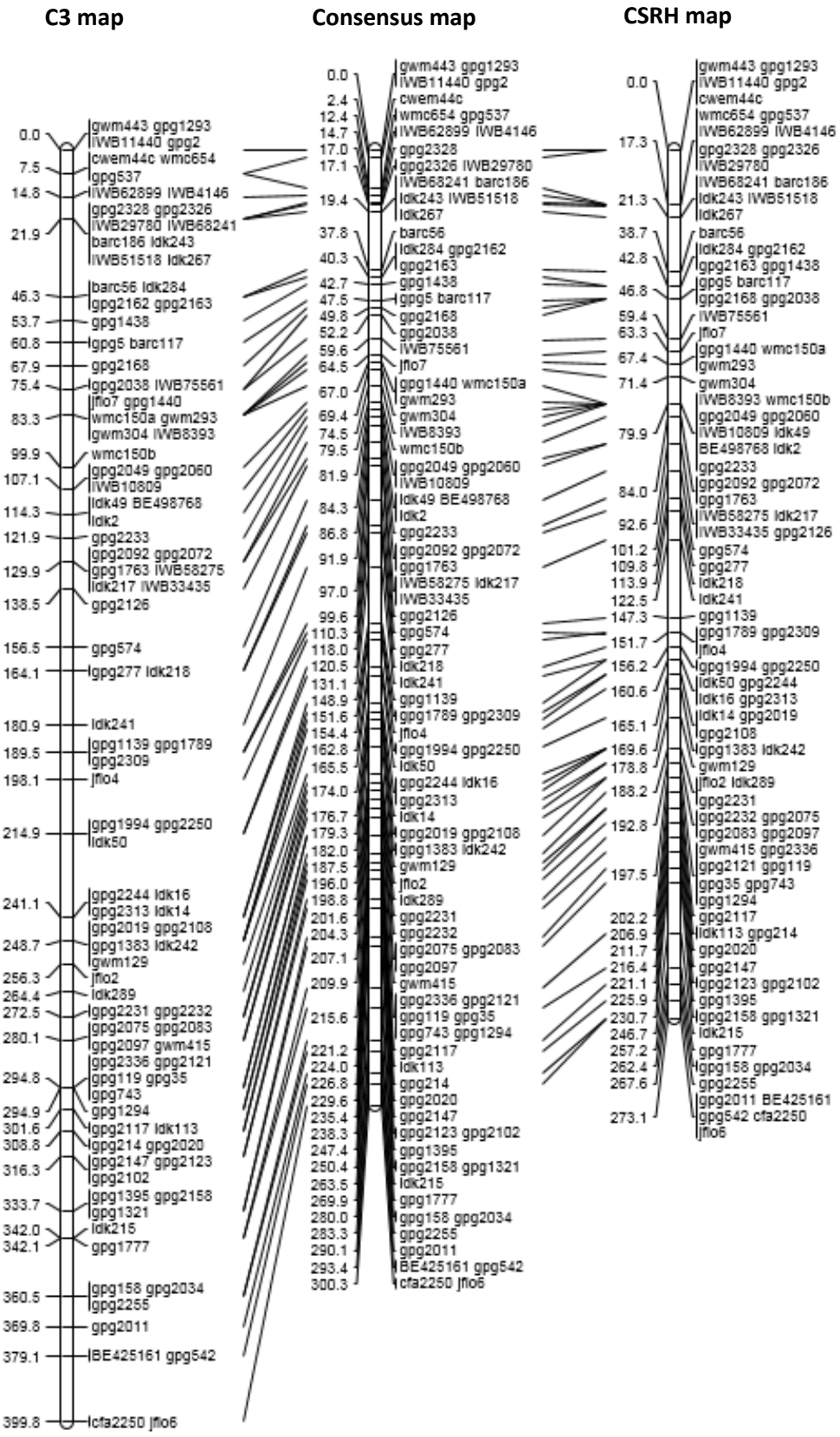

Figure 21: Map overview of the 5AS chromosome (created with MapChart): C3 map (left), 5AS consensus map (middle) and Chinese Spring radiation hybrid (CSRH) map (right) (Buerstmayr, unpublished).

4. Discussion

In section 4.1 the association of *Qfhs.ifa-5A* with AE and FHB resistance in wheat is discussed. The discussion of the results of the *Qfhs.ifa-5A* radiation-induced deletion mapping approach is given in section 4.2.

4.1 Association of *Qfhs.ifa-5A* with AE and FHB resistance

The aim of this study was to increase the knowledge about *Qfhs.ifa-5A*, AE and FHB resistance. In the ´associations of *Qfhs.ifa-5A* with AE and FHB´ part it was confirmed that *Qfhs.ifa-5A* has an influence on both on AE and FHB resistance. In the greenhouse experiment it was also shown that simulated AE/AR, independent whether the genotype harbors *Qfhs.ifa-5A* or not, had an influence on FHB resistance; suggesting that resistance of *Qfhs.ifa-5A* can partially be explained by a higher proportion of extruded anthers.

4.1.1 AE/AR in wheat

Associations of AE with FHB resistance were reported already hundred years ago (Percival, 1921; Pugh et al., 1933). Several studies about AE in wheat have been performed with different genotypes and several QTL for AE were mapped. Generally, AE had a high heritability and genotypes with high degree of extruded anthers had improved FHB resistance (Blöch, 2014; Buerstmayr and Buerstmayr, 2015; Danler, 2016; Lu et al., 2013; Skinnes et al., 2010).

Lu et al. (2013) found associations for AE, plant height, and FHB severity in a Shanghai-3/Catbird × Naxos RIL population. Two loci (4BS and 2DLc) were found to influence AE, plant height, and FHB resistance simultaneously. In a Capo × Arina RIL population, three QTL for AR located on chromosomes 4A, 5AS, and 6B were identified and QTL on 4A and 6B coincided with QTL for FHB resistance (Buerstmayr and Buerstmayr 2015).

Skinnes et al. (2008) reported for a set of 68 winter and 116 spring wheat varieties/lines a negative correlation of AE with FHB severity as well as a negative correlation of AE with DON concentration. In general varieties/lines with higher AE had lower FHB severity and lower DON concentration. Blöch (2014) reported a negative correlation of AE with DON and ZON content in a field trial with 96 wheat genotypes. Danler (2016) investigated the impact of AE on FHB severity in a set of 403 winter wheat genotypes and reported a significant correlation of r = 0.5 between AR and FHB severity.

In addition, the trait AE can be useful in hybrid wheat production: parental lines with good pollen shedding properties are needed – higher AE promotes cross fertilization for more efficient hybrid seed production (Langer, 2014; Langer et al., 2014; Muqaddasi et al., 2016). Pollen mass and plant height are also positively correlated with AE (Langer, 2014).

Due to the correlation of AE and FHB resistance and the high heritability of AE, plant breeders can use AE (easily and inexpensively appraisable) as an indirect indicator for FHB resistance (Buerstmayr and Buerstmayr, 2015; Skinnes et al., 2008).

***Qfhs.ifa-5A* and AE**

In this thesis the specific association of *Qfhs.ifa-5A* with AE was investigated by using the cultivar Remus (susceptible for FHB, low level of AE) and C3, a near isogenic line of Remus (FHB resistant, high level of AE) which harbors *Qfhs.ifa-5A* from CM-82036. For C3 (harboring *Qfhs.ifa-5A*) a higher level of AE (53%) has been evaluated than for Remus (9.2% AE). Remus retained anthers twice as often (22.7 florets out of 25) as C3 (11.8 florets out of 25). Most anthers, especially for the genotype Remus, were not retained completely inside the floret, but trapped between lemma and palea.

C3 has a higher AE level than Remus, this result is in agreement with Danler (2016). Danler (2016) compared C3 and Remus and observed for C3 that 21% of florets showed retained anthers, while for Remus 57.7% of florets showed retained anthers. The study of Danler (2016) used the same evaluation procedure for AE/AR; the differences are the number of florets (Danler (2016): 20 florets; this study: 25 florets) and that AR was observed by Danler (2016) in the field and not in the greenhouse.

In the current study, the level of AR was much higher than in the study of Danler (2016). The ratio of AR between genotypes was similar: in both studies Remus had more than two times more retained anthers than C3. It might be possible that some growing factors in the greenhouse had an influence on AE/AR, leading to a generally higher level of AR in this study. A very important factor for AE is light: if there is not enough light at flowering, e.g. cloudy weather, the amount of AR is higher. The artificial illumination in the greenhouse was maybe not strong enough, so that the amount of AR was higher compared to the study of Danler (2016), where AE was observed in the field. Although AE has a high heritability, some environmental conditions (or greenhouse growing conditions) can also influence AE (Muqaddasi et al., 2016).

4.1.2 Influence of simulated AE/AR on FHB resistance of lines differing in *Qfhs.ifa-5A*

A very high infection rate was achieved, compared to natural infection. Due to high conidia amount and optimal conditions for FHB development, a very high FHB infection rate was observed.

From the results it can be concluded that *Qfhs.ifa-5A* influences AE and therefore indirectly also FHB *Type 1* resistance. In the results section, FHB symptoms for several scoring dates were presented and compared, the following observations were made: (i) C3 and Remus control heads differed significantly for FHB resistance and AE with C3 being more resistant and having fewer anthers retained; (ii) heads of both genotypes with removed anthers had significantly less symptoms than heads with compressed anthers or control heads; (iii) C3 heads with compressed anthers were significantly more susceptible than C3 control heads genotype at the phenotyping dates IS 14 dai, S 18 dai and S 22 dai, while Remus heads with compressed anthers were only slightly (not significant at $\alpha = 0.01$) more susceptible than Remus control heads at phenotyping dates S 18 dai and S 22 dai; (iv) C3 heads with removed anthers were significantly more resistant than Remus heads with removed anthers for the phenotyping dates IS 14 dai, S 18 dai and S 22 dai; (v) heads with compressed anthers of C3 were significantly less diseased than heads with compressed anthers of Remus for the phenotyping dates IS 6 dai, IS 10 dai, IS 14 dai and S 18 dai.

Due to the removal of anthers it was shown that FHB infection can also occur without the presence of anthers. Nevertheless, FHB infection rates were much lower when anthers were removed.

If anthers were compressed, which simulates the effect of retained anthers, no uniform resistance pattern was observed: C3 heads with compressed anthers were more resistant than C3 control heads at IS 6 dai, but more susceptible at the other phenotyping dates. Remus heads with compressed anthers were more resistant than Remus control heads at the phenotyping dates IS 6 dai, IS 10 dai and showed an equal resistance level at IS 14 dai. At late phenotyping dates (S 18 dai, S 22 dai) Remus heads with compressed anthers were more susceptible than Remus control heads.

Due to the compression of anthers into the florets, both genotypes were more susceptible at late phenotyping dates. The difference in susceptibility between heads with compressed anthers and control heads was more pronounced and significant for the C3 genotype.

AE has only an influence on FHB *Type 1* resistance – initial infection, but not directly on disease spreading. All different test groups, independently from genotype and anther treatment, were highly diseased at late FHB scorings, e.g. S 18 dai and S 22 dai (*Figures 14 and 15*). The use of a *Type 2* resistance QTL, like *Fhb1* which protects against disease spreading, would have led to less infestation at later scorings in this experiment. For the farmers, the yield and the mycotoxin concentration at harvest, not a resistance

scoring between anthesis and harvest, are of interest. Therefore, the combination (or pyramiding) of FHB *Type 1* and FHB *Type 2* QTL can give a better protection against FHB and mycotoxins.

For estimating *Type 1* resistance, the earlier dates IS 10 dai and IS 14 dai are the best fitting parameters, because at the first scoring date (IS 6 dai), not all initially infected spikelets showed already symptoms and on the fourth (S 18 dai) or fifth (S 22 dai) scoring date, FHB severity was phenotyped and *Type 1* resistance would be faked by fungal spread to other spikelets within the head. The scoring parameters S 18 dai, S 22 dai, and also AUDPC 18 dai and AUDPC 22 dai were better suited for evaluating FHB severity.

Danler (2016) suggested in her study the scoring ´FHB severity 10 dai´ (in that study only FHB symptomatic spikelets were scored, no infection sites) as best parameter for evaluating FHB *Type 1* resistance and considered the same statements: at too early scoring dates FHB symptoms may not be developed, while at late scoring dates the influence of disease spreading is too high.

General observations of this study match with results of the study of Danler (2016), except some observations concerning the resistance of heads with compressed anthers: in Danler (2016), C3 heads with compressed anthers were at every scoring date more susceptible than the control heads for C3, while Remus heads with compressed anthers were always more resistant than Remus control heads.

It might be surprising that at the first scoring date heads with compressed anthers were more resistant than their control heads. A possible explanation might be that C3 and Remus control heads had high levels of trapped anthers between lemma and palea. The anthers of the ´anthers compressed treatment´ group were fully stuck into the floret so that no parts of the anthers could be seen visually. This was somehow simulating a cleistogamous flowering with no connection between anthers and the environment. These observations are well in agreement with studies of Kubo et al. (2010; 2013): heads with trapped anthers between lemma and palea are more susceptible for FHB than cleistogamous flowering heads.
An interesting anther manipulation technique for further experiments would therefore be to fake trapped anthers between lemma and palea in addition to the anther manipulation treatments in this study.

Taken together, this study revealed that in the QTL region of *Qfhs.ifa-5A* between barc186 (5AS) and barc1 (5AL) a locus affecting AE is present, which indirectly contributes to FHB *Type 1* resistance. But the results also suggest that an additional locus has an effect on FHB resistance, because C3 heads were always more resistant than Remus heads when comparing same anther treatments. However, further

research has to be done to clarify the actions/genes which are responsible for AE and FHB *Type 1* resistance.

4.2 Discussion of fine-mapping *Qfhs.ifa-5A*

Deletions give a present-absent information and thus need to be homozygous to be detectable. Therefore irradiated seeds and plants have to pass at least one selfing generation. Selfing will reduce number of deletions due to genetic drift and natural selection. Moreover, some deletions might have been missed at the pre-screening, either because deletions were not homozygous or too small. Nevertheless, 28 informative lines out of 1764 mutant plants could be identified (1.58%). In this study 28 informative lines were genotyped with 102 markers and with the software CarthaGène, a 5AS specific map with 38 loci and a distance of 399.8 cR was created.

Radiation dosage

Different gamma radiation dosages and also mutation generations were used for establishing the deletion lines (*Table 5*). Generally, proportion of lines containing deletions was low and ranged from 1.01 to 2.5% among the subpanels irradiated at 240, 250, 270, 300 or 330 Gy. The highest deletion rate was observed in the M_3 of the seeds irradiated with 250 Gy. Normally, the deletion rate increases with radiation dosage (Kumar et al., 2014; Lehmensiek et al., 2009), this was not observed in our experiment. This might be explained by the facts that i) the subpanels were too small and ii) the subpanel irradiated with 250 Gy was in the M_3 generation so that more deletions were homozygous and detectable compared to the other M_2 subpanels.

Distribution of deletions and marker retention along the 5AS chromosome

Deletions were evenly distributed over the 5AS chromosome; marker retention was on average 75% (*Figure 19*). Markers near the centromere had a notable higher marker retention >85% (markers with largest retention rate are cfa2250 and jfio6 with 93%). Potentially, deletions close to the centromere stretch across the centromere that lead into total 5A chromosome loss and/or infertility of plants.

Consensus map

A 5AS consensus map was created by combining genotypic information of the C3 map with the CSRH map (Buerstmayr, unpublished; Mayer, 2016; Vukelic, unpublished). Due to merging the data from the C3 and CSRH panel, more loci could be identified: the consensus map had a length of 300.3 cR and divided the 5AS chromosome by 60 loci.

Comparison of C3 linkage map vs C3 radiation-induced deletion map

In the genetic linkage map (*Figure 5*; IFA BP research, unpublished), markers were tightly linked despite being physically located at distant chromosomal regions. In the *Qfhs.ifa-5A* region, the C3 map had a much higher resolution (especially in the centromeric region). In addition, much more loci could be mapped: in the C3 deletion mapping approach, 1764 plants were prescreened, resulting in 28 informative lines (1.6%) with 38 mapped loci on the 5AS chromosome. In the linkage mapping approach, about 4000 RIL plants were prescreened, resulting in about 30 lines (0.75%) with recombination on 5AS (interval barc186 to cfa2250), but only seven loci were mapped on 5AS within a distance of 1.2 cM.

In the C3 radiation-induced deletion mapping approach, the 5AS was genotyped with 102 markers and the interval barc186–cfa2250 was separated by 35 loci and had a length of 377.9 cR. The number of loci increased 5-fold and map resolution given by the ratio cM to cR increased by 315-fold.

Radiation-induced deletion mapping is therefore an adequate method to increase map resolution and also decrease the size of mapping population (Kumar et al., 2014; Lehmensiek et al., 2009).

5AS CSRH map

For wheat, cytogenetic stocks are available, allowing a creation of radiation hybrid panels (Kalavacharla, 2006; Kalavacharla et al., 2009). RH mapping has been used extensively to support high-resolution mapping of individual wheat chromosomes: 1D (Kalavacharla et al., 2006), 3B (Kumar et al., 2012; Paux et al., 2008), 5A (Zhou et al., 2012), 6B (Kobayashi et al., 2015), and 4A (Balcárková et al., 2017). The main benefit of radiation hybrid panels is that the RH plants are hemizygous and thus can be directly used for genotyping. Most RH panels are used for mapping only; therefore, deletions need not be transmitted to the next generation, avoiding losses of deletions due to selfing. A disadvantage is that plants derived from radiation hybrids are confounded by the genome of the aneuploid parent that may interfere with phenotyping. In the 5AS CSRH panel (Buerstmayr, unpublished; Mayer, 2016; Vukelic, unpublished), 40 informative lines (14.5%) out of a panel of 276 plants were identified and a map with 39 loci within a distance of 273.1 cR was created.

5. Outlook

Phenotypic evaluation of deletion lines

To reduce background mutations, deletion lines were backcrossed with C3. After one selfing step, offspring plants will be genotyped for deletions and plants being homozygous for the deletions will be propagated to obtain seeds for multiple testing. These derived lines will be phenotyped for FHB resistance and AE to link genotypic data with phenotypic data and will hopefully allow precisely mapping *Qfhs.ifa-5A.*

Development of further deletion lines

To date only a limited number of lines containing deletions could be selected. Deletions occur randomly and scoring data confirmed deletions of varying size and location. Deleted fragments that partially overlap divide the chromosome into small bins. To increase both, number of lines having deletions and number of bins across the QTL interval, additional deletion panels have been developed that are currently analysed: i) a deletion mapping panel derived from irradiated seeds where C3 seeds were irradiated with a higher dosage (300, 330, and 350 Gy) and propagated to M_2 generation; prescreening of these lines with at least 18 markers resulted in 90 genotypes with deletions; and ii) a deletion mapping panel derived from irradiated pollen (Brugger, 2017): at anthesis, C3 heads were gamma-irradiated for the production of two deletion panels: irradiated C3 heads pollinated unirradiated C3 plants and irradiated C3 heads were kept for producing seeds. Seeds were propagated to M_2 generation to obtain homozygous deletion, screening is still under progress.

6. Conclusion

FHB is a devastating fungal disease with global concern and causes substantial losses in wheat production, especially in years with high humidity at anthesis. Until now, no cultivar/genotype with absolute resistance to FHB is known, but highly resistant germplasm (like Sumai-3) have been already used in breeding programs. Deployment of cultivars with good FHB resistance is the most effective and most economic way for farmers to reduce FHB. Pyramiding resistance genes/QTL in one cultivar increases the level of FHB resistance. Next to cultivar resistance, also other farm practices (crop rotation, tillage, fungicide application, etc.) are and will be important to reduce FHB and mycotoxins.

AE has a significant influence on FHB *Type* 1 resistance: cultivars with fast and a high level of AE are more resistant. Due to the correlation of AE and FHB resistance as well as due the high heritability of AE, plant breeders can use AE (easily and inexpensively appraisable) as an indirect indicator for FHB resistance (Buerstmayr and Buerstmayr, 2015). Genotypes with high AE are of interest for hybrid wheat production; parental lines with good pollen shedding properties are needed.
The major FHB resistance QTL *Qfhs.ifa-5A* protects against fungal infection and is also associated with a higher level of AE. This study showed that AE has a strong influence on FHB *Type 1* resistance conferred by *Qfhs.ifa-5A*. Results suggest that *Qfhs.ifa-5A* acts as a passive resistance mechanism controlling AE. In addition, other gene(s) in this QTL interval may also affect FHB resistance.

The *Qfhs.ifa-5A* fine-mapping approach confirmed that deletion mapping is a suitable alternative to linkage mapping and can increase the map resolution; especially in centromeric regions, where the recombination rate is small. The map resolution of the 5AS chromosome could be strongly increased. Deletion lines will be backcrossed to reduce unwanted background mutations and will then be phenotyped for AE and FHB resistance to assist gene identification.

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8. Appendix

A: Experimental design in greenhouse:

Replication 1						Replication 2					
C	4	3	2	1		180	179	178	177	C
C	5							176	175	C
R	6	7	8	9		171	172	173	174	R
R	13	12	11	10		170	169	168	167	R
R	14								166	R
C	15	16	17	18		162	163	164	165	C
C	22	21	20	19		161	160	159	158	C
C	23								157	C
R	24	25	26	27		153	154	155	156	R
R	31	30	29	28		152	151	150	149	R
R	32								148	R
C	33	34	35	36		144	145	146	147	C
C	40	39	38	37		143	142	141	140	C
C	41				Runway				139	C
R	42	43	44	45		135	136	137	138	R
R	49	48	47	46		134	133	132	131	R
R	50								130	R
C	51	52	53	54		126	127	128	129	C
C	58	57	56	55		125	124	123	122	C
C	59								121	C
R	60	61	62	63		117	118	119	120	R
R	67	66	65	64		116	115	114	113	R
R	68								112	R
C	69	70	71	72		108	109	110	111	C
C	76	75	74	73		107	106	105	104	C
C	77								103	C
R	78	79	80	81		99	100	101	102	R
R	85	84	83	82		98	97	96	95	R
R	86					91	92	93	94	
	87	88	89	90						

Figure 22: Plan of greenhouse trail. Genotypes were placed in blocks (double rows) for easier manipulation (watering and scoring). Remus pots are labeled in purple, C3 pots in yellow.

B: IFA CTAB DNA extraction protocol

DNA-Extraction on 96plate with

8-strips

Slightly adjusted to the procedure described by Eric

Harvest young leaves

Dry leaves:

Dry them either at a temperature of ca 35°C (one to two days, according to leave amount) or lyophilize tissue. When you dry leaves at 35°C, don't freeze leaves before drying.

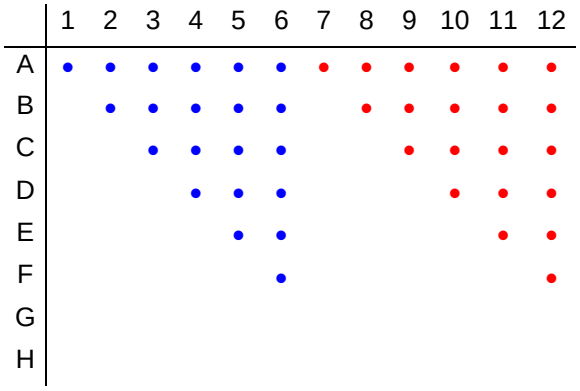
NOTE: Leaf samples may be frozen and stored at –80°C until ready to be lyophilized. If leaves are frozen before drying you can only lyophilize them (72 hours). Frozen plant material must not thaw before lyophilizing. Make sure the lyophilizer is down to temperature (the chamber is ≤ -50°C) and pulling a good vacuum (≤ 10 microns Hg) before loading samples. Do not overload lyophilizer: make sure the vacuum is always ≤ 100 microns and condenser temperature is ≤ -50°C. Samples should be dry in 72 hours. Typically, fresh weight ≈ 10× dry weight. Dried leaf samples may be stored in sealed plastic bags at room temperature for a few days or at –20°C for several years.

1) **fill each tube with 5 – 7 small glass beads**
2) **cut leaf-material into each tube, avoid contamination**

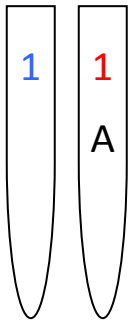
   Bring dried leaves into1. 2 ml stripes (8), cut leaves when you fill the tubes. A small glass funnel makes filling easier. When you put your samples into a desiccator overnight they are perfectly dry for grinding in the Retsch-mill. If leaves are not fully dried grinding will be poor. But as finer the powder as better the amount of extracted DNA!!!!

3) **prepare one set of 1.2ml 8-stripe-tubes and 2 sets of caps and label them**

   labelling of caps (8-strips):



labelling of tubes (8-strips, side-view):



row number top (A) / row number bottom (H)

plate name

4) **grind leafs in Retsch-mill for 10 minutes (change orientation of plates after 5 minutes)**

ensure that both arms of the mill are encumbered equally

alternatively use the shaker in BP/E/17 for ~20 minutes.

Leaf powder can be stored tightly capped in a cool place for several weeks or at -20°C (Samples are stable for several years), or DNA extraction can begin immediately in the same tubes.

5) **prepare CTAB-Buffer[1]: per sample 0.6 ml**

Stock	final	10 ml	50 ml	100 ml
dH20		6.5 ml	32.5 ml	65 ml
1M Tris-7.5	100 mM	1 ml	5 ml	10 ml
5M NaCl	700 mM	1.4 ml	7 ml	14 ml
0.5 M EDTA-8.0	50mM	1 ml	5 ml	10 ml
CTAB[2]	1%	0.1g	0.5g	1g
14 M BME[3]	140 mM	0.1 ml	0.5 ml	1 ml

1 Use freshly made; warm buffer to 60-65°C before adding the CTAB and BME

2 CTAB = Mixed alkyltrimethyl-ammonium bromide (Sigma M-7635).

3 Add BME (β-mercaptoethanol) just prior to use, **under a fume hood**.

6) **If necessary, centrifuge** stripes **at very low rpm** to get the powder down from the covers (not too strong because then the powder sticks on the bottom and cannot be mixed properly to the buffer)

7) **Keep stripes at an appropriate distance** when opening them, open stripes carefully, so that leave powder does not scatter and contaminate nearby tubes.

8) **add 700 µL of CTAB-Buffer to each well under the fume hood** using the Brand-Handystep for pipetting

   If leave powder sticks to the bottom you can stir it with a tooth stick or a yellow tip, powder must get in contact with CTAB.

   Close stripes tightly! You can vortex if necessary

9) **shake short by inversion and place the rack (with tubes) into a water bath (with gentle rocking) at 65°C for 60-90 minutes**

   cover racks with a tray and put heavy stones into the tray, so that the tubes cannot open or float. Be careful with the water level (not to high, but sufficient) and check after 5/10min the fasteners (screw again if it is possible).

10) **Let stripes cool down to room temperature**

11) **add 350 µL chloroform:isoamylalcohol (24:1) to each tube under the fume hood,** using the Brand-Handystep for pipetting,

12) **close stripes and put them into plate fastener, screw tightly, shake by gentle inversion for 10 minutes**

13) **All centrifugation-steps are at room temperature**

14) **centrifuge for 10 minutes, 3800 rcf** Sigma 4K15 BP/E/21

15) **pipette off 300µL (top aqueous layer) into a new stripes containing RNase 5 µl,**

16) mix and incubate at room temperature 30 min

17) **add 300µL of isopropyl alcohol, mix well by gentle inversion**

    using the Brand-Handystep for pipetting, you can put stripes into freezer to support precipitation of DNA

18) **centrifuge 8 minutes at low rpm**

    using the , Sigma 4K15 BP/E/21 at ~1000 rcf

19) **pour off liquid (DNA-pellet must stick to the bottom of the tube)**

    if the pellet does not stick to bottom in some tubes, pipette off the liquid carefully

20) **add 100µL of Wash 1 and for 20 minutes**

    using a Brand Transferpette 8-chanel pipette for 30-300µL, pellets should be loose within the wash now

21) centrifuge 8 minutes at low rpm 1000

using the , Sigma 4K15 BP/E/21 at ~600 rcf

22) pour off liquid (DNA-pellet must stick to the bottom of the tube)

if the pellet does not stick to bottom in some tubes, pipette off the liquid carefully

23) add 100µL Wash 2 and mix gently for 5 minutes

using a Brand Transferpette 8-chanel pipette for 30-300µL, pellets should be loose within the wash now

24) centrifuge 8 minutes at low 1000rpm

using the Beckman , Sigma 4K15 BP/E/21 at ~600 rcf

25) pour off liquid (DNA-pellet must stick to the bottom of the tube)

if the pellet does not stick to bottom in some tubes, pipette off the liquid carefully

26) let dry over night

put a tissue atop your open tubes

27) dissolve DNA-pellet in 100 µL 0,5 or 0,1 x TE buffer

using a Brand Transferpette 8-chanel pipette for 30-300µL

28) mix for some hours at room-temperature, then store plate at 4°C

wait for at least 1 day before continuing with an agarose-gel or a photometer scan to allow proper dissolving

C: Used markers

Table 22: Used markers (ordered from distal to proximal the centromere) with primer sequences. On the forward primer the M13 sequence (5' CCCAGTCACGACGTTG 3') was attached. References are: 1) Akhunov et al., 2010; 2) Barabaschi et al., 2015; 3) Polygenes/Grain-Genes; 4) Röder et al., 1998; 5) Somers et al., 2004; 6) Song et al., 2005; 7) Sourdille et al., 2004.

Marker	Marker IFA code	Marker type	Bin location	Forward primer sequence (5´to 3´)	Reverse primer sequence (5´to 3´)	Ref.
IWB11440	IWB11440	SNP	5AS3-0.75-0.97	CCGATTATTTGCCTTGCGTTTTTAC	AGCGTCGTGAAATCTGTC	3
gpg1293	M38	ISBP	5AS3-0.75-0.97	GCAGCAGGAAAAATCAGCAT	GGTTCGGCCTGAGATCATT	2
gpg2	gpg2	ISBP	5AS3-0.75-0.97	CGGTTGTGCCATTATTTGTG	CACCGGTCCTTCGATAAAAA	2
cwen44c	cwen44c	-	5AS3-0.75-0.97	AGTGCACTGCAAACACAGAG	AGCCGTACACCTTCATAGGC	-
gpg537	M73	ISBP	5AS3-0.75-0.97	ATCTCGTCGCGAGAAACCTA	CGGCTACACGTAAGGGGTAA	2
wmc654	wmc654	SSR	5AS3-0.75-0.97	CTGTGATGAACTGAAATAACCA	TATTCTACTTTTCTCTTCCCCC	5
ldk267	ldk267	SSR	5AS3-0.75-0.97	AATTAGCAGACCGCATGTACG	TCCAAGTTGAGAGCTGATGG	2
IWB51518	M15	SNP	5AS3-0.75-0.97	AAGCGCATCCAAGAACCTGA	TCCAAAAGGAGGAACCCGAT	3
ldk243	ldk243	SSR	5AS3-0.75-0.97	GGTTTCACCTCTAGCCTACCC	CACCTTGTGTGGGAGTTTCC	2
IWB68241	IWB68241	SNP	5AS3-0.75-0.97	AAGTTCAGGATCATTCACTATTTC	AGCATTCCTTTCCTTTCACTACA	3
barc186	barc186	SSR	5AS3-0.75-0.97	GGAGTGTCGAGATGATGTGGAAAC	CGCAGACGTCAGCAGCTCGAGAGG	6
gpg2326	gpg2326	ISBP	5AS3-0.75-0.97	CAGCGTCAGTCCGGATTAGT	TCTAATTCTTCGGCGACGAT	2
gpg2328	M66	ISBP	5AS3-0.75-0.97	GACGACACAAGTGCCATGTT	CGTTTGTTCCACAAATCACG	2
IWB4146	M9	SNP	5AS3-0.75-0.97	GGCTGGGAAACTCAAGGATC	AAACCGTTCTCATTAGCCTC	3
IWB62899	IWB62899	SNP	5AS3-0.75-0.97	TGCTATGGCTATACTACGGC	GCGCCGAAGCCATTGACT	3
BE444720	BE444720	COS	5AS3-0.75-0.97	GCCCTCGAGAAGATGTTCAG	GAGCATTAACAGTAACTCGGG	1
barc56	barc56	SSR	5AS3-0.75-0.97	GCGGGAATTTACGGGAAGTCAAGAA	GCGAGTGGTTCAAATTTATGTCTGT	6
gpg2162	gpg2162	ISBP	5AS3-0.75-0.97	AAGATCAAATGGCCCTTCCT	GGCTATGCATGGTCCAATCT	2
ldk284	ldk284	SSR	5AS3-0.75-0.97	TCTCATTGGTCAGGGTCAGG	TTCTCCTCCAGGTAGCTCTCC	2
gpg2163	gpg2163	ISBP	5AS3-0.75-0.97	AGGTCGCGCACTGTTAGATT	CATGTACTCGGCGTTCACAT	2
gpg1438	M44	ISBP	5AS3-0.75-0.97	GCGGTTGGATGAAGATCCTA	TCCGTATTGCCTAGCTTGCT	2
gpg2168	M33b	ISBP	5AS3-0.75-0.97	TGTCCCCTGCCTTCTGTTAC	GTCCACCGTCAGGTCATCTT	2
gpg5	M72	ISBP	5AS3-0.75-0.97	TGATTGGGTAATCCTCACCAA	CCGTGTAAGGAACGCAAAAT	2
barc117	barc117	SSR	5AS3-0.75-0.97	TCATGCGTGCTAAGTGCTAA	GAGGGCAGGAAAAAGTGACT	6
gpg2038	M51	ISBP	5AS1-0.40-0.75	GAGTCCAAAACATGGGCAAT	TGGTGTGCTCACGTCAGATT	2
IWB75561	IWB75561	SNP	5AS1-0.40-0.75	TGGCATTCCTTACCTATTTGCG	CTAGTGGATGGGTGTTCACAT	3
jfio7	jfio7	RJM	5AS1-0.40-0.75	CTCCTGTGGCAGAACAGAGG	ATCGTGGGCGTCACACTATA	2
gpg1440	gpg1440	ISBP	5AS1-0.40-0.75	ACAGGCCTGATCTGGTATGG	TGCTTGCTACGTCTCCAATG	2
wmc150a	wmc150a	SSR	5AS1-0.40-0.75	CATTGATTGAACAGTTGAAGAA	CTCAAAGCAACAGAAAAGTAAA	5
gwm293	gwm293	SSR	5AS1-0.40-0.75	TACTGGTTCACATTGGTGCG	TCGCCATCACTCGTTCAAG	4
gwm304	gwm304	SSR	5AS1-0.40-0.75	AGGAAACAGAAATATCGCGG	AGGACTGTGGGGAATGAATG	4
IWB8393	IWB8393	SNP	5AS1-0.40-0.75	ACCGAAATAGGATTTGCCTCAT	TGCTTATCTTGATGGCCACA	3
wmc150b	wmc150b	SSR	5AS1-0.40-0.75	CATTGATTGAACAGTTGAAGAA	CTCAAAGCAACAGAAAAGTAAA	5
gpg2049	gpg2049	ISBP	5AS1-0.40-0.75	GGCCAAAGAAAGCTTATCCC	CCAGTGAACCGTCTGCTGTA	2
IWB10809	M1	SNP	5AS1-0.40-0.75	TGGTACCACGCCAAAGTATACT	TTGCTCCAATGAGATGTGGA	3
gpg2060	gpg2060	ISBP	5AS1-0.40-0.75	CCGACAGGAACTTCCACTGT	CTCAATTCGGTTCTTCCCAA	2
ldk49	ldk49	SSR	5AS1-0.40-0.75	TCCACACACCACACACACAC	AGACGCTATCCGATCCTCTG	2

Marker	Marker IFA code	Marker type	Bin location	Forward primer sequence (5´to 3´)	Reverse primer sequence (5´to 3´)	Ref.
ldk2	ldk2	SSR	5AS1-0.40-0.75	ATCAGGTCCACACACCACAC	AATCCACGAAGACGCTATCC	2
BE498768	BE498768	COS	5AS1-0.40-0.75	CTGCCCCTAGAAGTTTCTCGT	CAGCGAGTGACAATTCCAGA	1
gpg2233	gpg2233	ISBP	5AS1-0.40-0.75	GTCGACGTTCACATGACACC	TGGTCTTCCACCACTTGTCC	2
gpg2092	gpg2092	ISBP	5AS1-0.40-0.75	GGTCCGCATTGTTAACAGGT	TTGGCTTGAAGCTATGCATG	2
gpg1763	M45	ISBP	5AS1-0.40-0.75	CCAACACAACATGAGCAACC	AATTTTTCCTGCATTGGTCG	2
IWB33435	M7	SNP	5AS1-0.40-0.75	AGATAAGTGGCCTTGTCCTCT	CGGAAGTTGTGATGGGCTTATATA	3
IWB58275	IWB58275	SNP	5AS1-0.40-0.75	ATATAGTGAGTTGGAAGGGCAG	GTGAAGCTGATGGGAAGAAG	3
gpg2126	M57	ISBP	5AS1-0.40-0.75	TGACCAAGTGATGGGAATCA	CCGAAGAAGGACGAGAGATG	2
ldk217	ldk217	SSR	5AS1-0.40-0.75	TGGACTCCGAATAGGACTGG	ACCAACTTCATCGCTGTTGC	2
gpg574	M86	ISBP	5AS1-0.40-0.75	TGCTCCAAAACTCTCAACCA	ACACCAAACTTGCCTTCCAC	2
gpg277	gpg277	ISBP	5AS1-0.40-0.75	TCCATGTTGTCTTCAACCCA	TCCAAGTAGAGACCCATCCG	2
ldk218	ldk218	SSR	5AS1-0.40-0.75	GTTGAAGATGTCGCTCATGG	CTTCACAAGGTCCGCTTCC	2
ldk241	ldk241	SSR	5AS3-0.75-0.97	AATCAGTCTTGATGAAGCAACG	CATGAAGCGTCAGCAGTAGG	2
gpg2309	M63	ISBP	5AS1-0.40-0.75	GACCACCTTCGGATTAGTGC	CACCGTCAATAGGTCACGAA	2
gpg1139	gpg1139	ISBP	5AS1-0.40-0.75	ACCCGTAAGTTGCCGTTATG	CACTCATGTTGAACACACCCA	2
gpg1789	M47	ISBP	5AS1-0.40-0.75	GGATGAGATCCACCTCCTGA	CCATCTCTTCGCCGAACTAG	2
jfio4	M77	RJM	5AS1-0.40-0.75	CGCAAGGTGATATGAGGTGTT	TACGTACATACGGGCGGGT	2
gpg1994	gpg1994	ISBP	5AS1-0.40-0.75	GGTGGAGGAATGTTCACAGG	CACCGTTTGCGATTATTGTG	2
gpg2250	gpg2250	ISBP	5AS1-0.40-0.75	AGCATCAGTGTTGTTGCAATG	GTATGAAACCCGTTTGGGTG	2
ldk50	M81	SSR	C-5AS1-0.40	ACCGTGTGTGATGCTTCTTG	GGTGCATGTGTGTGTGCTC	2
ldk16	ldk16	SSR	C-5AS1-0.40	CTCTTGGGCTGATGGTGATG	ATCGAATCAGTGGGTGATCG	2
gpg2313	M64	ISBP	C-5AS1-0.40	CTCACCGCCATGAGTGAGTA	TCCAACTGCCAGAATTCTCC	2
gpg2244	M61	ISBP	C-5AS1-0.40	GCCTGGATCATGCGATAACT	GGTACGAGGGACTTGCATGT	2
gpg2108	gpg2108	ISBP	C-5AS1-0.40	GCAACCGAAGAGATCCTAAGG	TTCCCAAGATGGGAGAGTTG	2
gpg2019	M49	ISBP	C-5AS1-0.40	TCCCCACTTGCAACTAAACC	AAGTGGCATCAGCTGAAGGT	2
gpg1383	M41	ISBP	C-5AS1-0.40	CCTCTAAGTCGTGCCTCGAC	AGTCCATCCGAGGTGAATTG	2
ldk242	M79	SSR	C-5AS1-0.40	CCTACAAACCTCTGCACTTGG	CGGAGGGAATATTGAACACG	2
gwm129	gwm129	SSR	C-5AS1-0.40	TCAGTGGGCAAGCTACACAG	AAAACTTAGTAGCCGCGT	5
ldk14	ldk14	SSR	C-5AS1-0.40	TTTCTGTTTTGCCTCTGGAAA	GGGCCTTTCCCTTTTGTTTT	2
jfio2	jfio2	RJM	C-5AS1-0.40	ACGCTGGAGACGTATCACTGT	GGTGTCCTTCCTGATCTCCA	2
ldk289	M80	SSR	C-5AS1-0.40	GCACATACCTTCATAGTGG	TGATGATGTGGCAAAGAAGC	2
gpg2231	gpg2231	ISBP	C-5AS1-0.40	CCTATCGGCCACACTCACTT	TTGGCTGCTCTTGACCATTA	2
gpg2232	gpg2232	ISBP	C-5AS1-0.40	CGATTAAGAGCGATAATCAACCA	TAAGAGACCGTTTTGGCCTG	2
gpg2075	gpg2075	ISBP	C-5AS1-0.40	ATAAGGCGCACTACCAGTGG	CCCTAGCCCATTATGCTCAA	2
gpg2097	M84	ISBP	C-5AS1-0.40	TTGTGATTGCTGCTCACCTC	TTCCTCCAAAGGCACTGTCT	2
gpg2083	gpg2083	ISBP	C-5AS1-0.40	TTAGTTCAATGGCAGGTCGA	CCATCTCTTCGCCGAACTAG	2
gwm415	gwm415	SSR	C-5AS1-0.40	GATCTCCCATGTCCGCC	CGACAGTCGTCACTTGCCTA	4
gpg2336	gpg2336	ISBP	C-5AS1-0.40	TGAAAGAGACACGACGCAAC	TCTTCCTCTGTGGTCCAACC	2
gpg35	M71	ISBP	C-5AS1-0.40	TTAACACGTCAGGTTGCGAG	GAGCCGACTGAACTGTCTCC	2
gpg743	M75	ISBP	C-5AS1-0.40	CTATGTACGCACACAATGCG	GAACGTAAGAAGGCAGGCAC	2
gpg119	M37	ISBP	C-5AS1-0.40	CACGTCACTGTCAAGTGGCT	CACACATGTATTACGGTTTCCG	2
gpg1294	M39	ISBP	C-5AS1-0.40	CCTCGAGAGTTTTGGTCGAG	GCACCAACCAGGAGTAAAGG	2

Marker	Marker IFA code	Marker type	Bin location	Forward primer sequence (5´to 3´)	Reverse primer sequence (5´to 3´)	Ref.
gpg2121	gpg2121	ISBP	C-5AS1-0.40	TGCTTGTTCTTGCTCCAATG	GGCCACCTTGCTACACATCT	2
ldk113	ldk113	SSR	C-5AS1-0.40	CACTGCTCCACCACAGC	GCGAAGGGTTAAACCGTAAAC	2
gpg2117	M55	ISBP	C-5AS1-0.40	GCAAGGTGTACGTCCTTCGT	CATGCTTGAACTTGCTCCAA	2
gpg214	M58	ISBP	C-5AS1-0.40	TAGCCCATCACAAGCATTCA	TCCCTTGTGGATTCAAGACC	2
gpg2020	M50	ISBP	C-5AS1-0.40	GAGATGACCGACGGATTCAT	AACAGAACCATATGCCCTGC	2
gpg2147	M59	ISBP	C-5AS1-0.40	TTGACATGCTTGTGGTGGTT	ACCTTAGCAATGCAGCCAGT	2
gpg2102	M52	ISBP	C-5AS1-0.40	TCCTTTGAAGTCCTCGCACT	TGTACCTGTGAACGGAACCA	2
gpg2123	M56	ISBP	C-5AS1-0.40	CTCTCGGAGTTGGTTTAGCG	GGAAGTTCCTTGGACATAACC	2
gpg1395	M42	ISBP	C-5AS1-0.40	CTTCGGCCAATCAGAATTGT	GGGCGACCAAGGATTCTATT	2
gpg2158	M60	ISBP	C-5AS1-0.40	GGCTGTCATTAATCGTCCGT	CGTGCATCACAGAAGTGCTT	2
gpg1321	gpg1321	ISBP	C-5AS1-0.40	CCATCGATCTTAGACGCACA	ATTGCTCTACGTGGTGCATG	2
ldk215	ldk215	SSR	C-5AS1-0.40	CTGAGCTGAAGCAAGACACG	CGGGCATCTTCTCTACATCG	2
gpg1777	M46	ISBP	C-5AS1-0.40	TTCCTCAAGGAGCGTAGCAT	ACCAATCCATTGCCTACGAG	2
gpg158	gpg158	ISBP	C-5AS1-0.40	ACGCACACCAACTTTTACCC	GTGGTGCATGAAGGAACAGA	2
gpg2034	gpg2034	ISBP	C-5AS1-0.40	CCTCCTGGCGAGCAGATAT	TTATCCACCATTGGTCCGTT	2
gpg2255	M62	ISBP	C-5AS1-0.40	CGACCAGATAGGCTGGTAGC	GTTTCCATTAGGACCCCGTT	2
BE425161	BE425161	COS	C-5AS1-0.40	GGATGGTTCTGACCCAATATG	ATCATGCCGACAAACAGCTT	1
gpg542	M74	ISBP	C-5AS1-0.40	CAAATACCGAGGGGTTGCTA	TGACACTGAGGACATCTGCC	2
gpg2011	M48	ISBP	C-5AS1-0.40	GTCTATCCACCCATCCATGG	GAACGCCGACAGTCATCAC	2
cfa2250	cfa2250	SSR	C-5AS1-0.40	AGCCATAGATGGCCCTACCT	CACTCAATGGCAGGTCCTTT	7
jfio6	M78	RJM	C-5AS1-0.40	CAGTCCCTTATTCAGCACCG	TGCGTCGGTAACATCATCAT	2

D: Differences in anther extrusion between genotypes C3 and Remus

Table 23: Scoring values of Anther Retention (AR) of the genotypes C3 and Remus. AR represents the number of florets with retained or trapped anthers out of 25 florets (five florets of five heads per block).

Genotype	Replication	Block	AR of 25 florets	AR relation
C3	Replication1	Block 1-5	14	0,56
C3	Replication1	Block 15-23	12	0,48
C3	Replication1	Block 33-41	17	0,68
C3	Replication1	Block 51-59	10	0,40
C3	Replication1	Block 69-77	8	0,32
C3	Replication2	Block 91-93	9	0,36
C3	Replication2	Block 103-111	13	0,52
C3	Replication2	Block 121-129	17	0,68
C3	Replication2	Block 139-147	5	0,20
C3	Replication2	Block 157-165	13	0,52
C3	Replication2	Block 175-179	12	0,48
C3 average			11,82	0,47
Remus	Replication1	Block 6-14	24	0,96
Remus	Replication1	Block 24-32	24	0,96
Remus	Replication1	Block 42-50	20	0,80
Remus	Replication1	Block 60-68	25	1,00
Remus	Replication1	Block 78-90	22	0,88
Remus	Replication2	Block 94-102	23	0,92
Remus	Replication2	Block 112-120	25	1,00
Remus	Replication2	Block 130-138	22	0,88
Remus	Replication2	Block 148-156	19	0,76
Remus	Replication2	Block 166-174	23	0,92
Remus average			22,70	0,91

E: R script and R output of AE/AR

R-script:

R-script:

```
#Tabelle als Excel importieren
AR <- read_excel("C:/Users/Wagner/Desktop/Masterarbeit/Statistik/AR.xlsx")

View(AR)
names(AR) # shows the names of the columns from your dataframe
summary(AR$ARof25Florets ~ AR$Genotype)

attach(AR)

#########################
#boxplot for visualization
boxplot(AR$ARof25Florets~Genotype, data=AR, main="Anther Retention", xlab="Genotype",
ylab="Retained anthers of 25 florets", col= c("gold1", "purple"))       # creates a boxplot of C2 over C1
with exemple of title of boxplot and title of axis

#########################
# independent 2-group t-test
t.test(AR$ARof25Florets ~ AR$Genotype)
```

R-output:

```
> t.test(AR$ARof25Florets ~ AR$Genotype)

        Welch Two Sample t-test

data:  AR$ARof25Florets by AR$Genotype
t = -8.5602, df = 15.782, p-value = 2.53e-07
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -13.579703  -8.183933
sample estimates:
   mean in group C3 mean in group Remus
           11.81818             22.70000
```

F: Boxplots for FHB symptoms

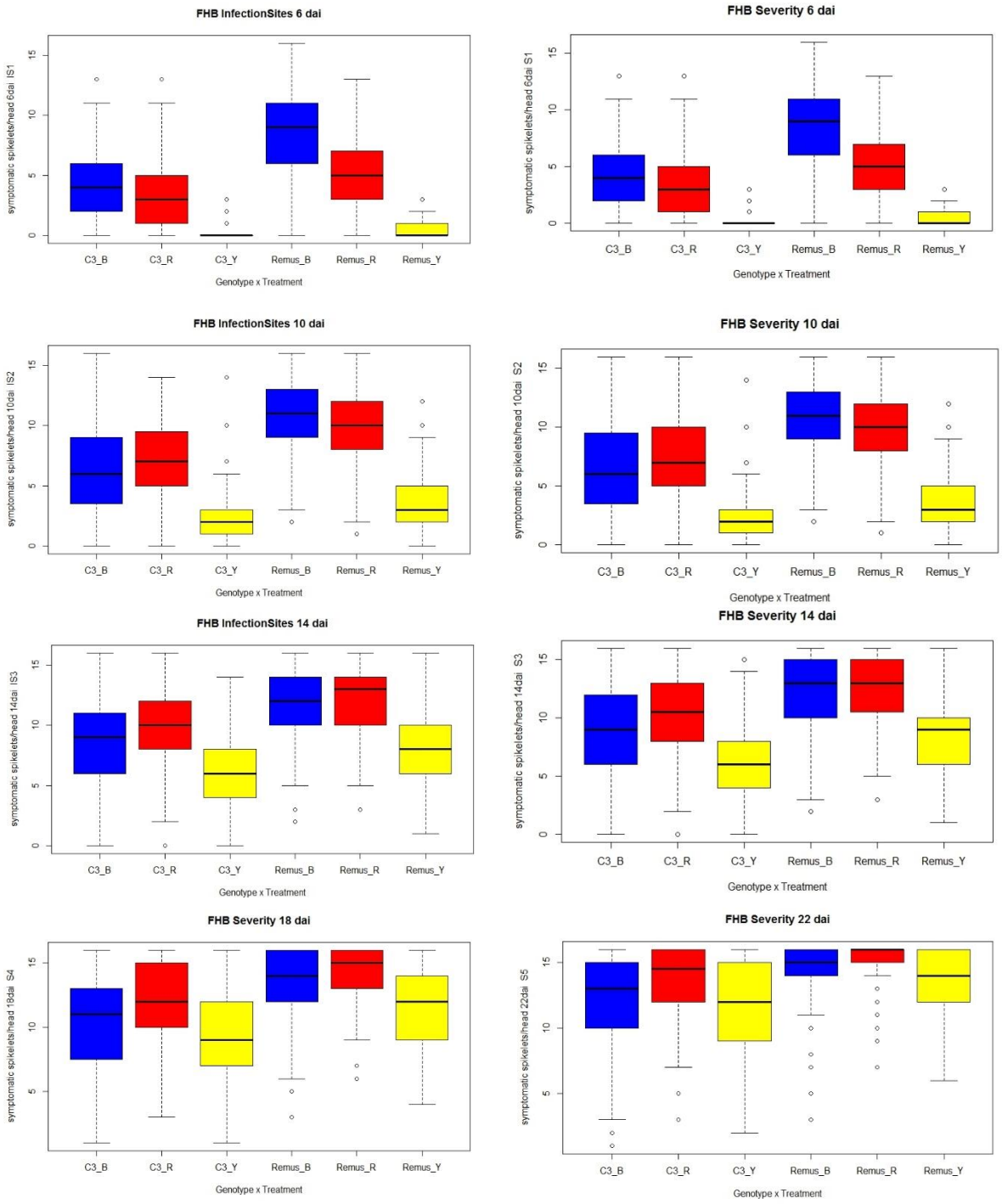


Figure 23: Boxplot overview for FHB symptomatic spikelets grouped for genotype and anther treatment (B(lue) = control, R(ed) = anthers compressed, Y(ellow) = anthers removed) at several time points: IS 6 dai, IS 10 dai, IS 14 dai, S 6 dai, S 10 dai, S 14 dai, S 18 dai and S 22 dai.

G: Boxplots for FHB symptoms, separated after replication

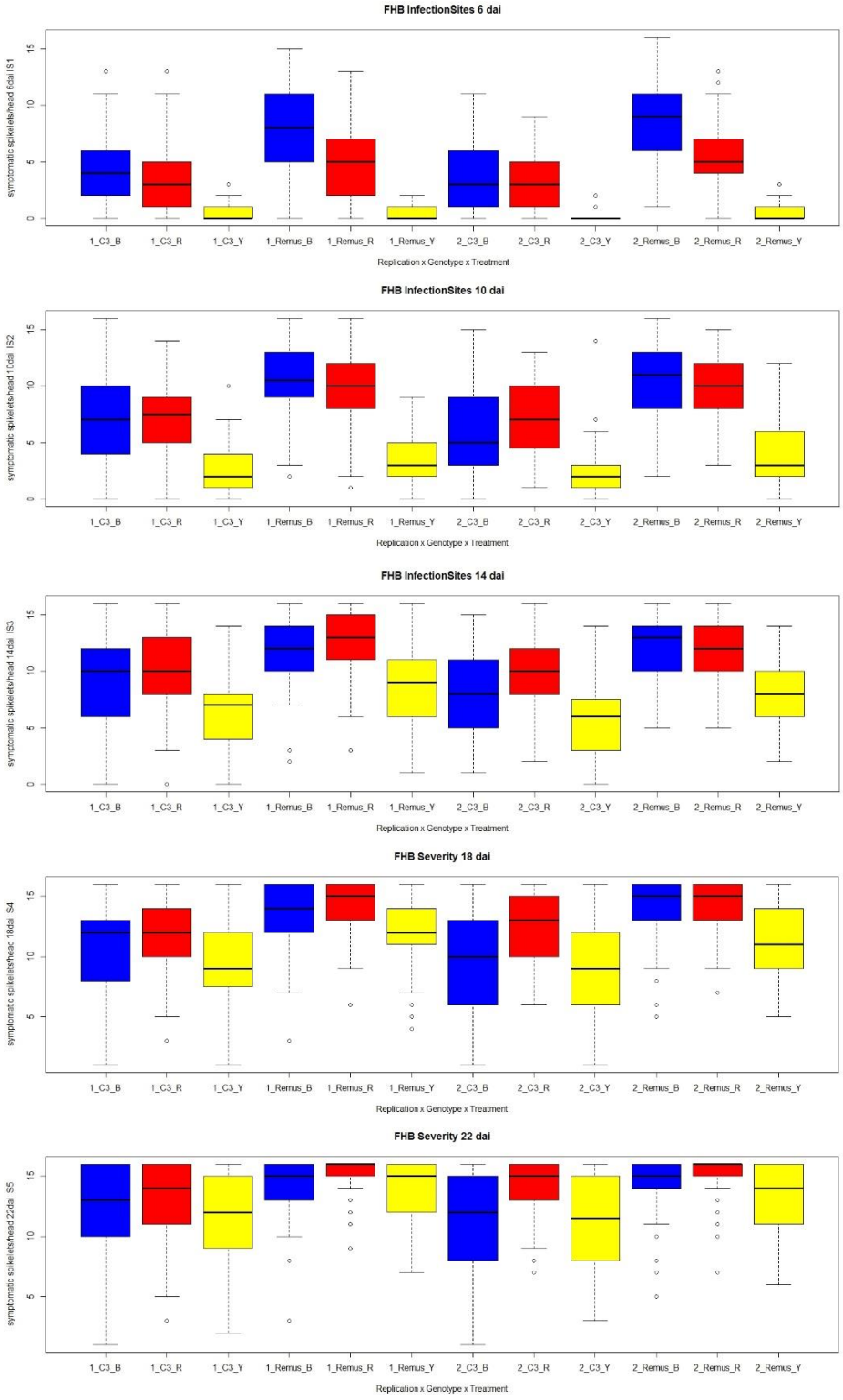


Figure 24: Boxplot overview for FHB symptomatic spikelets grouped for replication, genotype and anther treatment (B(lue) = control, R(ed) = anthers compressed, Y(ellow) = anthers removed) at several time points: IS 6 dai, IS 10 dai, IS 14 dai, S 18 dai and S 22 dai.

H: R script and R output for anther treatment experiment

R-script:

```
setwd("C:/Users/Wagner/Desktop/Masterarbeit/Statistik") # Sets work directory
getwd()

#install.packages("lme4")
library(lme4)
install.packages("multcompView")
library(multcompView)
#install.packages("ggplot2")
library(ggplot2)
library(readxl)
library(plyr)
install.packages("lsmeans")
library(lsmeans)

#Tabelle als Excel importieren
mydata <- read_excel("C:/Users/Wagner/Desktop/Masterarbeit/Statistik/AntherExpforR.xlsx")

View(mydata)
names(mydata) # returns the names of the columns from your dataframe
summary(mydata$geno_treat)

mydata$genotyp<-as.factor(as.character(mydata$genotyp))   # example : sets the values of the column name as factors
mydata$treatment<-as.factor(as.character(mydata$treatment))
mydata$geno_treat<-as.factor(as.character(mydata$geno_treat))
mydata$name<-as.factor(as.character(mydata$name))
mydata$experiment<-as.factor(as.character(mydata$experiment))
mydata$pot<-as.factor(as.character(mydata$pot))

mydata$IS1<-as.numeric(as.character(mydata$IS1))
mydata$S1<-as.numeric(as.character(mydata$S1))
mydata$IS2<-as.numeric(as.character(mydata$IS2))
mydata$S2<-as.numeric(as.character(mydata$S2))
mydata$S3<-as.numeric(as.character(mydata$S3))
mydata$S4<-as.numeric(as.character(mydata$S4))
mydata$S5<-as.numeric(as.character(mydata$S5))
mydata$AU4<-as.numeric(as.character(mydata$AU4))
mydata$AU5<-as.numeric(as.character(mydata$AU5))


#Boxplots for overview: insgesamt ohne Replication aufgetrennt
#IS1-IS3
boxplot(IS1~geno_treat, data=mydata, main="FHB InfectionSites 6 dai", xlab="Genotype x Treatment", ylab="symptomatic
spikelets/head 6dai  IS1", col= c("blue", "red", "yellow", "blue", "red", "yellow"))       # creates a boxplot of C2 over C1 with
exemple of title of boxplot and title of axis
boxplot(IS2~geno_treat, data=mydata, main="FHB InfectionSites 10 dai", xlab="Genotype x Treatment", ylab="symptomatic
spikelets/head 10dai  IS2", col= c("blue", "red", "yellow", "blue", "red", "yellow"))          # creates a boxplot of C2 over C1 with
exemple of title of boxplot and title of axis
boxplot(IS3~geno_treat, data=mydata, main="FHB InfectionSites 14 dai", xlab="Genotype x Treatment", ylab="symptomatic
spikelets/head 14dai  IS3", col= c("blue", "red", "yellow", "blue", "red", "yellow"))          # creates a boxplot of C2 over C1 with
exemple of title of boxplot and title of axis
```

```
#S1-S5
boxplot(S1~geno_treat, data=mydata, main="FHB Severity 6 dai", xlab="Genotype x Treatment", ylab="symptomatic
spikelets/head 6dai S1", col= c("blue", "red", "yellow", "blue", "red", "yelloW"))       # creates a boxplot of C2 over C1 with
exemple of title of boxplot and title of axis
boxplot(S2~geno_treat, data=mydata, main="FHB Severity 10 dai", xlab="Genotype x Treatment", ylab="symptomatic
spikelets/head 10dai  S2", col= c("blue", "red", "yellow", "blue", "red", "yelloW"))          # creates a boxplot of C2 over C1 with
exemple of title of boxplot and title of axis
boxplot(S3~geno_treat, data=mydata, main="FHB Severity 14 dai", xlab="Genotype x Treatment", ylab="symptomatic
spikelets/head 14dai  S3", col= c("blue", "red", "yellow", "blue", "red", "yelloW"))          # creates a boxplot of C2 over C1 with
exemple of title of boxplot and title of axis
boxplot(S4~geno_treat, data=mydata, main="FHB Severity 18 dai", xlab="Genotype x Treatment", ylab="symptomatic
spikelets/head 18dai  S4", col= c("blue", "red", "yellow", "blue", "red", "yelloW"))          # creates a boxplot of C2 over C1 with
exemple of title of boxplot and title of axis
boxplot(S5~geno_treat, data=mydata, main="FHB Severity 22 dai", xlab="Genotype x Treatment", ylab="symptomatic
spikelets/head 22dai  S5", col= c("blue", "red", "yellow", "blue", "red", "yelloW"))          # creates a boxplot of C2 over C1 with
exemple of title of boxplot and title of axis

#AUDPC
boxplot(AU4~geno_treat, data=mydata, main="AUDPC 18 dai", xlab="Genotype x Treatment", ylab="AUDPC 18 dai", col=
c("blue", "red", "yellow", "blue", "red", "yelloW"))          # creates a boxplot of C2 over C1 with exemple of title of boxplot and
title of axis
boxplot(AU5~geno_treat, data=mydata, main="AUDPC 22 dai", xlab="Genotype x Treatment", ylab="AUDPC 22 dai", col=
c("blue", "red", "yellow", "blue", "red", "yelloW"))          # creates a boxplot of C2 over C1 with exemple of title of boxplot and
title of axis

#Boxplots: Replication=Experiment aufgetrennt
boxplot(IS1~name, data=mydata, main="FHB InfectionSites 6 dai", xlab="Replication x Genotype x Treatment",
ylab="symptomatic spikelets/head 6dai IS1",col=c("blue", "red", "yellow", "blue", "red", "yelloW","blue", "red", "yellow", "blue",
"red", "yelloW") )          # creates a boxplot of C2 over C1 with exemple of title of boxplot and title of axis
boxplot(IS2~name, data=mydata, main="FHB InfectionSites 10 dai", xlab="Replication x Genotype x Treatment",
ylab="symptomatic spikelets/head 10dai  IS2", col=c("blue", "red", "yellow", "blue", "red", "yelloW","blue", "red", "yellow",
"blue", "red", "yelloW"))          # creates a boxplot of C2 over C1 with exemple of title of boxplot and title of axis
boxplot(IS3~name, data=mydata, main="FHB InfectionSites 14 dai", xlab="Replication x Genotype x Treatment",
ylab="symptomatic spikelets/head 14dai  IS3", col=c("blue", "red", "yellow", "blue", "red", "yelloW","blue", "red", "yellow",
"blue", "red", "yelloW"))          # creates a boxplot of C2 over C1 with exemple of title of boxplot and title of axis
boxplot(S4~name, data=mydata, main="FHB Severity 18 dai", xlab="Replication x Genotype x Treatment", ylab="symptomatic
spikelets/head 18dai  S4", col=c("blue", "red", "yellow", "blue", "red", "yelloW","blue", "red", "yellow", "blue", "red", "yelloW"))
# creates a boxplot of C2 over C1 with exemple of title of boxplot and title of axis
boxplot(S5~name, data=mydata, main="FHB Severity 22 dai", xlab="Replication x Genotype x Treatment", ylab="symptomatic
spikelets/head 22dai  S5" , col=c("blue", "red", "yellow", "blue", "red", "yelloW","blue", "red", "yellow", "blue", "red", "yelloW"))
# creates a boxplot of C2 over C1 with exemple of title of boxplot and title of axis

#########################################################################
#ANOVA + Tukey post hoc test

#IS1
modelIS1 <- aov(mydata$IS1 ~  mydata$genotyp + mydata$treatment +mydata$genotyp:mydata$treatment
+mydata$experiment , data =mydata)
anova(modelIS1)
summary(modelIS1)
TukeyHSD(modelIS1)
tapply(X = mydata$IS1, INDEX = list(mydata$geno_treat),  FUN=mean)
tapply(X = mydata$IS1, INDEX = list(mydata$geno_treat),  FUN=sd)
tapply(X = mydata$IS1, INDEX = list(mydata$geno_treat),  FUN=min)
tapply(X = mydata$IS1, INDEX = list(mydata$geno_treat),  FUN=max)
```

```
#IS2
modelIS2 <- aov(mydata$IS2 ~  mydata$genotyp + mydata$treatment +mydata$genotyp:mydata$treatment +
mydata$experiment, data =mydata)
anova(modelIS2)
summary(modelIS2)
TukeyHSD(modelIS2)
tapply(X = mydata$IS2, INDEX = list(mydata$geno_treat),  FUN=mean)
tapply(X = mydata$IS2, INDEX = list(mydata$geno_treat),  FUN=sd)
tapply(X = mydata$IS2, INDEX = list(mydata$geno_treat),  FUN=min)
tapply(X = mydata$IS2, INDEX = list(mydata$geno_treat),  FUN=max)

#IS3
modelIS3 <- aov(mydata$IS3 ~  mydata$genotyp + mydata$treatment +mydata$genotyp:mydata$treatment+
mydata$experiment, data =mydata)
anova(modelIS3)
summary(modelIS3)
TukeyHSD(modelIS3)
tapply(X = mydata$IS3, INDEX = list(mydata$geno_treat),  FUN=mean)
tapply(X = mydata$IS3, INDEX = list(mydata$geno_treat),  FUN=sd)
tapply(X = mydata$IS3, INDEX = list(mydata$geno_treat),  FUN=min)
tapply(X = mydata$IS3, INDEX = list(mydata$geno_treat),  FUN=max)

############
#S1
modelS1 <- aov(mydata$S1 ~  mydata$genotyp + mydata$treatment +mydata$genotyp:mydata$treatment+
mydata$experiment, data =mydata)
anova(modelS1)
summary(modelS1)
TukeyHSD(modelS1)
tapply(X = mydata$S1, INDEX = list(mydata$geno_treat),  FUN=mean)
tapply(X = mydata$S1, INDEX = list(mydata$geno_treat),  FUN=sd)
tapply(X = mydata$S1, INDEX = list(mydata$geno_treat),  FUN=min)
tapply(X = mydata$S1, INDEX = list(mydata$geno_treat),  FUN=max)


#S2
modelS2 <- aov(mydata$S2 ~  mydata$genotyp + mydata$treatment +mydata$genotyp:mydata$treatment +
mydata$experiment, data =mydata)
anova(modelS2)
summary(modelS2)
TukeyHSD(modelS2)
tapply(X = mydata$S2, INDEX = list(mydata$geno_treat),  FUN=mean)
tapply(X = mydata$S2, INDEX = list(mydata$geno_treat),  FUN=sd)
tapply(X = mydata$S2, INDEX = list(mydata$geno_treat),  FUN=min)
tapply(X = mydata$S2, INDEX = list(mydata$geno_treat),  FUN=max)

#S3
modelS3 <- aov(mydata$S3 ~  mydata$genotyp + mydata$treatment +mydata$genotyp:mydata$treatment+
mydata$experiment, data =mydata)
anova(modelS3)
summary(modelS3)
TukeyHSD(modelS3)
tapply(X = mydata$S3, INDEX = list(mydata$geno_treat),  FUN=mean)
tapply(X = mydata$S3, INDEX = list(mydata$geno_treat),  FUN=sd)
tapply(X = mydata$S3, INDEX = list(mydata$geno_treat),  FUN=min)
tapply(X = mydata$S3, INDEX = list(mydata$geno_treat),  FUN=max)
```

```
#S4
modelS4 <- aov(mydata$S4 ~  mydata$genotyp + mydata$treatment +mydata$genotyp:mydata$treatment +
mydata$experiment, data =mydata)
anova(modelS4)
summary(modelS4)
TukeyHSD(modelS4)
tapply(X = mydata$S4, INDEX = list(mydata$geno_treat),  FUN=mean)
tapply(X = mydata$S4, INDEX = list(mydata$geno_treat),  FUN=sd)
tapply(X = mydata$S4, INDEX = list(mydata$geno_treat),  FUN=min)
tapply(X = mydata$S4, INDEX = list(mydata$geno_treat),  FUN=max)

#S5
modelS5 <- aov(mydata$S5 ~ mydata$genotyp + mydata$treatment +mydata$genotyp:mydata$treatment +
mydata$experiment, data =mydata)
anova(modelS5)
summary(modelS5)
TukeyHSD(modelS5)

tapply(X = mydata$S5, INDEX = list(mydata$geno_treat),  FUN=mean)
tapply(X = mydata$S5, INDEX = list(mydata$geno_treat),  FUN=sd)
tapply(X = mydata$S5, INDEX = list(mydata$geno_treat),  FUN=min)
tapply(X = mydata$S5, INDEX = list(mydata$geno_treat),  FUN=max)

################
#AUDPC
#AU4
modelAU4 <- aov(mydata$AU4 ~ mydata$genotyp + mydata$treatment +mydata$genotyp:mydata$treatment +
mydata$experiment, data =mydata)
anova(modelAU4)
summary(modelAU4)
TukeyHSD(modelAU4)
tapply(X = mydata$AU4, INDEX = list(mydata$geno_treat),  FUN=mean)
tapply(X = mydata$AU4, INDEX = list(mydata$geno_treat),  FUN=sd)
tapply(X = mydata$AU4, INDEX = list(mydata$geno_treat),  FUN=min)
tapply(X = mydata$AU4, INDEX = list(mydata$geno_treat),  FUN=max)

#AU5
modelAU5 <- aov(mydata$AU5 ~  mydata$genotyp + mydata$treatment +mydata$genotyp:mydata$treatment
+mydata$experiment , data =mydata)
anova(modelAU5)
summary(modelAU5)
TukeyHSD(modelAU5)
tapply(X = mydata$AU5, INDEX = list(mydata$geno_treat),  FUN=mean)
tapply(X = mydata$AU5, INDEX = list(mydata$geno_treat),  FUN=sd)
tapply(X = mydata$AU5, INDEX = list(mydata$geno_treat),  FUN=min)
tapply(X = mydata$AU5, INDEX = list(mydata$geno_treat),  FUN=max)

################################
```

R-output: ANOVA with Tukey post hoc tests

```
> modelIS1 <- aov(mydata$IS1 ~  mydata$genotyp + mydata$treatment +mydata$genotyp:myda
ta$treatment +mydata$experiment , data =mydata)
> anova(modelIS1)
Analysis of Variance Table

Response: mydata$IS1
                               Df Sum Sq Mean Sq  F value Pr(>F)
mydata$genotyp                  1  945.8  945.85 136.3863 <2e-16 ***
mydata$treatment                2 4838.3 2419.14 348.8268 <2e-16 ***
mydata$experiment               1    1.0    1.02   0.1466 0.7019
mydata$genotyp:mydata$treatment   2  599.2  299.58  43.1974 <2e-16 ***
Residuals                       797 5527.3    6.94
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> TukeyHSD(modelIS1)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = mydata$IS1 ~ mydata$genotyp + mydata$treatment +
mydata$genotyp:mydata$treatment + mydata$experiment, data = mydata)

$`mydata$genotyp`
             diff     lwr      upr p adj
Remus-C3 2.169434 1.80479 2.534078     0

$`mydata$treatment`
         diff       lwr       upr p adj
R-B -1.802369 -2.334747 -1.269990     0
Y-B -5.859182 -6.391043 -5.327322     0
Y-R -4.056814 -4.595538 -3.518089     0

$`mydata$experiment`
          diff        lwr      upr    p adj
2-1 0.07113376 -0.2935375 0.435805 0.701898

$`mydata$genotyp:mydata$treatment`
                       diff        lwr        upr     p adj
Remus:B-C3:B      4.2991744  3.3951081  5.2032407 0.0000000
C3:R-C3:B        -0.6175761 -1.5302554  0.2951031 0.3826647
Remus:R-C3:B      1.2879435  0.3734731  2.2024140 0.0008872
C3:Y-C3:B        -3.7982426 -4.7056872 -2.8907979 0.0000000
Remus:Y-C3:B     -3.6908790 -4.6090043 -2.7727537 0.0000000
C3:R-Remus:B     -4.9167505 -5.8342664 -3.9992346 0.0000000
Remus:R-Remus:B  -3.0112309 -3.9305286 -2.0919332 0.0000000
C3:Y-Remus:B     -8.0974170 -9.0097260 -7.1851079 0.0000000
Remus:Y-Remus:B  -7.9900534 -8.9129868 -7.0671200 0.0000000
Remus:R-C3:R      1.9055196  0.9777504  2.8332889 0.0000001
C3:Y-C3:R        -3.1806665 -4.1015113 -2.2598216 0.0000000
Remus:Y-C3:R     -3.0733029 -4.0046748 -2.1419310 0.0000000
C3:Y-Remus:R     -5.0861861 -6.0088063 -4.1635658 0.0000000
Remus:Y-Remus:R  -4.9788225 -5.9119498 -4.0456953 0.0000000
Remus:Y-C3:Y      0.1073636 -0.8188794  1.0336066 0.9994732

> tapply(X = mydata$IS1, INDEX = list(mydata$geno_treat),  FUN=mean)
     C3_B      C3_R      C3_Y   Remus_B   Remus_R   Remus_Y
4.0857143 3.4696970 0.2888889 8.3868613 5.3740458 0.3953488
```

```
> modelIS2 <- aov(mydata$IS2 ~  mydata$genotyp + mydata$treatment +
mydata$genotyp:mydata$treatment + mydata$experiment, data =mydata)
> anova(modelIS2)
Analysis of Variance Table

Response: mydata$IS2
                               Df Sum Sq Mean Sq  F value    Pr(>F)
mydata$genotyp                  1 1495.7 1495.69 161.0687 < 2.2e-16 ***
mydata$treatment                2 5117.9 2558.93 275.5676 < 2.2e-16 ***
mydata$experiment               1    9.6    9.57   1.0303    0.3104
mydata$genotyp:mydata$treatment   2  312.4  156.19  16.8197 7.003e-08 ***
Residuals                       797 7401.0    9.29
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> TukeyHSD(modelIS2)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = mydata$IS2 ~ mydata$genotyp + mydata$treatment +
mydata$genotyp:mydata$treatment + mydata$experiment, data = mydata)

$`mydata$genotyp`
             diff      lwr      upr p adj
Remus-C3 2.728077 2.306129 3.150026     0

$`mydata$treatment`
           diff        lwr        upr     p adj
R-B -0.1359266 -0.7519686  0.4801154 0.8625363
Y-B -5.4372236 -6.0526668 -4.8217804 0.0000000
Y-R -5.3012970 -5.9246825 -4.6779115 0.0000000

$`mydata$experiment`
           diff        lwr       upr     p adj
2-1 -0.2181802 -0.6401598 0.2037994 0.3104517

$`mydata$genotyp:mydata$treatment`
                      diff        lwr         upr     p adj
Remus:B-C3:B     4.2913997  3.2452588  5.33754068 0.0000000
C3:R-C3:B        0.7920106 -0.2640968  1.84811799 0.2666353
Remus:R-C3:B     3.2094235  2.1512433  4.26760356 0.0000000
C3:Y-C3:B       -3.9599390 -5.0099891 -2.90988876 0.0000000
Remus:Y-C3:B    -2.6727881 -3.7351974 -1.61037875 0.0000000
C3:R-Remus:B    -3.4993892 -4.5610933 -2.43768502 0.0000000
Remus:R-Remus:B -1.0819763 -2.1457422 -0.01821035 0.0435483
C3:Y-Remus:B    -8.2513387 -9.3070177 -7.19565965 0.0000000
Remus:Y-Remus:B -6.9641878 -8.0321609 -5.89621479 0.0000000
Remus:R-C3:R     2.4174129  1.3438441  3.49098165 0.0000000
C3:Y-C3:R       -4.7519495 -5.8175058 -3.68639326 0.0000000
Remus:Y-C3:R    -3.4647987 -4.5425363 -2.38706106 0.0000000
C3:Y-Remus:R    -7.1693624 -8.2369731 -6.10175174 0.0000000
Remus:Y-Remus:R -5.8822115 -6.9619804 -4.80244272 0.0000000
Remus:Y-C3:Y     1.2871509  0.2153482  2.35895353 0.0082884

> tapply(X = mydata$IS2, INDEX = list(mydata$geno_treat),  FUN=mean)
     C3_B      C3_R      C3_Y   Remus_B   Remus_R   Remus_Y
 6.364286  7.151515  2.400000 10.649635  9.572519  3.689922
```

```
modelIS3 <- aov(mydata$IS3 ~  mydata$genotyp + mydata$treatment +
mydata$genotyp:mydata$treatment+ mydata$experiment, data =mydata)
> anova(modelIS3)
Analysis of Variance Table

Response: mydata$IS3
                                Df Sum Sq Mean Sq  F value    Pr(>F)
mydata$genotyp                   1 1456.0 1456.05 147.4653 < 2.2e-16 ***
mydata$treatment                 2 2075.6 1037.78 105.1044 < 2.2e-16 ***
mydata$experiment                1   65.5   65.53   6.6369  0.010168 *
mydata$genotyp:mydata$treatment  2   94.3   47.15   4.7755  0.008677 **
Residuals                      797 7869.4    9.87
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> TukeyHSD(modelIS3)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = mydata$IS3 ~ mydata$genotyp + mydata$treatment +
mydata$genotyp:mydata$treatment + mydata$experiment, data = mydata)

$`mydata$genotyp`
            diff      lwr      upr p adj
Remus-C3 2.69168 2.256583 3.126778     0

$`mydata$treatment`
          diff        lwr       upr     p adj
R-B  0.7516977  0.1164578  1.386938 0.0154136
Y-B -2.9917285 -3.6263509 -2.357106 0.0000000
Y-R -3.7434262 -4.3862384 -3.100614 0.0000000

$`mydata$experiment`
          diff       lwr        upr     p adj
2-1 -0.5710181 -1.006148 -0.1358882 0.0101752

$`mydata$genotyp:mydata$treatment`
                       diff        lwr        upr     p adj
Remus:B-C3:B     3.59518856  2.5164465  4.6739306 0.0000000
C3:R-C3:B        1.53264304  0.4436239  2.6216622 0.0008980
Remus:R-C3:B     3.55360540  2.4624490  4.6447618 0.0000000
C3:Y-C3:B       -2.37169250 -3.4544657 -1.2889193 0.0000000
Remus:Y-C3:B    -0.03441578 -1.1299332  1.0611017 0.9999992
C3:R-Remus:B    -2.06254553 -3.1573358 -0.9677553 0.0000014
Remus:R-Remus:B -0.04158317 -1.1384995  1.0553332 0.9999979
C3:Y-Remus:B    -5.96688106 -7.0554585 -4.8783036 0.0000000
Remus:Y-Remus:B -3.62960434 -4.7308589 -2.5283498 0.0000000
Remus:R-C3:R     2.02096236  0.9139377  3.1279870 0.0000035
C3:Y-C3:R       -3.90433554 -5.0030980 -2.8055731 0.0000000
Remus:Y-C3:R    -1.56705882 -2.6783823 -0.4557354 0.0008700
C3:Y-Remus:R    -5.92529790 -7.0261788 -4.8244170 0.0000000
Remus:Y-Remus:R -3.58802118 -4.7014391 -2.4746033 0.0000000
Remus:Y-C3:Y     2.33727672  1.2320732  3.4424802 0.0000000

> tapply(X = mydata$IS3, INDEX = list(mydata$geno_treat),  FUN=mean)
     C3_B      C3_R      C3_Y   Remus_B   Remus_R   Remus_Y
 8.457143  9.977273  6.074074 12.036496 12.007634  8.418605
```

```
> modelS1 <- aov(mydata$S1 ~  mydata$genotyp + mydata$treatment +
mydata$genotyp:mydata$treatment+ mydata$experiment, data =mydata)
> anova(modelS1)
Analysis of Variance Table

Response: mydata$S1
                                Df Sum Sq Mean Sq  F value Pr(>F)
mydata$genotyp                   1  945.8  945.85 136.3863 <2e-16 ***
mydata$treatment                 2 4838.3 2419.14 348.8268 <2e-16 ***
mydata$experiment                1    1.0    1.02   0.1466 0.7019
mydata$genotyp:mydata$treatment  2  599.2  299.58  43.1974 <2e-16 ***
Residuals                      797 5527.3    6.94
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> TukeyHSD(modelS1)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = mydata$S1 ~ mydata$genotyp + mydata$treatment +
mydata$genotyp:mydata$treatment + mydata$experiment, data = mydata)

$`mydata$genotyp`
             diff     lwr      upr p adj
Remus-C3 2.169434 1.80479 2.534078     0

$`mydata$treatment`
         diff       lwr       upr p adj
R-B -1.802369 -2.334747 -1.269990     0
Y-B -5.859182 -6.391043 -5.327322     0
Y-R -4.056814 -4.595538 -3.518089     0

$`mydata$experiment`
          diff        lwr      upr    p adj
2-1 0.07113376 -0.2935375 0.435805 0.701898

$`mydata$genotyp:mydata$treatment`
                      diff        lwr        upr     p adj
Remus:B-C3:B     4.2991744  3.3951081  5.2032407 0.0000000
C3:R-C3:B       -0.6175761 -1.5302554  0.2951031 0.3826647
Remus:R-C3:B     1.2879435  0.3734731  2.2024140 0.0008872
C3:Y-C3:B       -3.7982426 -4.7056872 -2.8907979 0.0000000
Remus:Y-C3:B    -3.6908790 -4.6090043 -2.7727537 0.0000000
C3:R-Remus:B    -4.9167505 -5.8342664 -3.9992346 0.0000000
Remus:R-Remus:B -3.0112309 -3.9305286 -2.0919332 0.0000000
C3:Y-Remus:B    -8.0974170 -9.0097260 -7.1851079 0.0000000
Remus:Y-Remus:B -7.9900534 -8.9129868 -7.0671200 0.0000000
Remus:R-C3:R     1.9055196  0.9777504  2.8332889 0.0000001
C3:Y-C3:R       -3.1806665 -4.1015113 -2.2598216 0.0000000
Remus:Y-C3:R    -3.0733029 -4.0046748 -2.1419310 0.0000000
C3:Y-Remus:R    -5.0861861 -6.0088063 -4.1635658 0.0000000
Remus:Y-Remus:R -4.9788225 -5.9119498 -4.0456953 0.0000000
Remus:Y-C3:Y     0.1073636 -0.8188794  1.0336066 0.9994732

> tapply(X = mydata$S1, INDEX = list(mydata$geno_treat),  FUN=mean)
     C3_B      C3_R      C3_Y   Remus_B   Remus_R   Remus_Y
4.0857143 3.4696970 0.2888889 8.3868613 5.3740458 0.3953488
```

```
> modelS2 <- aov(mydata$S2 ~  mydata$genotyp + mydata$treatment +
mydata$genotyp:mydata$treatment + mydata$experiment, data =mydata)
> anova(modelS2)
Analysis of Variance Table

Response: mydata$S2
                                Df Sum Sq Mean Sq  F value    Pr(>F)
mydata$genotyp                   1 1470.6 1470.62 152.3317 < 2.2e-16 ***
mydata$treatment                 2 5316.2 2658.10 275.3344 < 2.2e-16 ***
mydata$experiment                1    9.9    9.88   1.0239    0.3119
mydata$genotyp:mydata$treatment  2  311.9  155.95  16.1535 1.328e-07 ***
Residuals                      797 7694.3    9.65
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> TukeyHSD(modelS2)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = mydata$S2 ~ mydata$genotyp + mydata$treatment +
mydata$genotyp:mydata$treatment + mydata$experiment, data = mydata)

$`mydata$genotyp`
             diff      lwr      upr p adj
Remus-C3 2.705116 2.274888 3.135345     0

$`mydata$treatment`
          diff        lwr        upr     p adj
R-B -0.1004312 -0.7285619  0.5276996 0.9252608
Y-B -5.5236472 -6.1511674 -4.8961270 0.0000000
Y-R -5.4232161 -6.0588344 -4.7875977 0.0000000

$`mydata$experiment`
          diff        lwr       upr     p adj
2-1 -0.2217691 -0.6520293 0.2084911 0.3119595

$`mydata$genotyp:mydata$treatment`
                      diff        lwr         upr     p adj
Remus:B-C3:B     4.2790905  3.2124209  5.34576014 0.0000000
C3:R-C3:B        0.8511801 -0.2256515  1.92801181 0.2127622
Remus:R-C3:B     3.2086797  2.1297346  4.28762472 0.0000000
C3:Y-C3:B       -4.0524600 -5.1231156 -2.98180444 0.0000000
Remus:Y-C3:B    -2.7650102 -3.8482675 -1.68175297 0.0000000
C3:R-Remus:B    -3.4279104 -4.5104486 -2.34537214 0.0000000
Remus:R-Remus:B -1.0704108 -2.1550513  0.01422966 0.0554754
C3:Y-Remus:B    -8.3315505 -9.4079455 -7.25515565 0.0000000
Remus:Y-Remus:B -7.0441008 -8.1330309 -5.95517059 0.0000000
Remus:R-C3:R     2.3574995  1.2628638  3.45213523 0.0000000
C3:Y-C3:R       -4.9036402 -5.9901062 -3.81717422 0.0000000
Remus:Y-C3:R    -3.6161904 -4.7150768 -2.51730404 0.0000000
C3:Y-Remus:R    -7.2611397 -8.3497004 -6.17257905 0.0000000
Remus:Y-Remus:R -5.9736899 -7.0746473 -4.87273251 0.0000000
Remus:Y-C3:Y     1.2874498  0.1946149  2.38028474 0.0103602

> tapply(X = mydata$S2, INDEX = list(mydata$geno_treat),  FUN=mean)
     C3_B      C3_R      C3_Y   Remus_B   Remus_R   Remus_Y
 6.464286  7.310606  2.407407 10.737226  9.671756  3.697674
```

```
> modelS3 <- aov(mydata$S3 ~  mydata$genotyp + mydata$treatment +
mydata$genotyp:mydata$treatment+ mydata$experiment, data =mydata)
> anova(modelS3)
Analysis of Variance Table

Response: mydata$S3
                                Df Sum Sq Mean Sq  F value    Pr(>F)
mydata$genotyp                   1 1493.9 1493.95 142.2120 < 2.2e-16 ***
mydata$treatment                 2 2311.9 1155.95 110.0373 < 2.2e-16 ***
mydata$experiment                1   54.4   54.40   5.1780  0.023140 *
mydata$genotyp:mydata$treatment  2  103.8   51.92   4.9428  0.007355 **
Residuals                      797 8372.5   10.51
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> TukeyHSD(modelS3)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = mydata$S3 ~ mydata$genotyp + mydata$treatment +
mydata$genotyp:mydata$treatment + mydata$experiment, data = mydata)

$`mydata$genotyp`
             diff      lwr      upr p adj
Remus-C3 2.726487 2.277697 3.175277     0

$`mydata$treatment`
          diff        lwr       upr     p adj
R-B  0.8095019  0.1542708  1.464733 0.0106654
Y-B -3.1468108 -3.8014049 -2.492217 0.0000000
Y-R -3.9563126 -4.6193542 -3.293271 0.0000000

$`mydata$experiment`
          diff        lwr         upr     p adj
2-1 -0.5202392 -0.9690627 -0.07141579 0.0231536

$`mydata$genotyp:mydata$treatment`
                       diff        lwr        upr     p adj
Remus:B-C3:B     3.67099625  2.5583059  4.7836866 0.0000000
C3:R-C3:B        1.63412768  0.5108369  2.7574185 0.0005126
Remus:R-C3:B     3.64291403  2.5174187  4.7684094 0.0000000
C3:Y-C3:B       -2.50630204 -3.6231503 -1.3894538 0.0000000
Remus:Y-C3:B    -0.13461626 -1.2646099  0.9953774 0.9993979
C3:R-Remus:B    -2.03686857 -3.1661121 -0.9076250 0.0000048
Remus:R-Remus:B -0.02808223 -1.1595187  1.1033543 0.9999997
C3:Y-Remus:B    -6.17729829 -7.3001335 -5.0544631 0.0000000
Remus:Y-Remus:B -3.80561251 -4.9415238 -2.6697012 0.0000000
Remus:R-C3:R     2.00878634  0.8669234  3.1506493 0.0000092
C3:Y-C3:R       -4.14042972 -5.2737705 -3.0070890 0.0000000
Remus:Y-C3:R    -1.76874395 -2.9150410 -0.6224469 0.0001720
C3:Y-Remus:R    -6.14921606 -7.2847419 -5.0136902 0.0000000
Remus:Y-Remus:R -3.77753029 -4.9259877 -2.6290729 0.0000000
Remus:Y-C3:Y     2.37168578  1.2317013  3.5116703 0.0000001

> tapply(X = mydata$S3, INDEX = list(mydata$geno_treat),  FUN=mean)
     C3_B      C3_R      C3_Y   Remus_B   Remus_R   Remus_Y
 8.650000 10.272727  6.133333 12.306569 12.290076  8.511628
```

```
> modelS4 <- aov(mydata$S4 ~  mydata$genotyp + mydata$treatment +
mydata$genotyp:mydata$treatment + mydata$experiment, data =mydata)
> anova(modelS4)
Analysis of Variance Table

Response: mydata$S4
                                Df Sum Sq Mean Sq  F value  Pr(>F)
mydata$genotyp                   1 1305.7 1305.67 130.9217 < 2e-16 ***
mydata$treatment                 2  936.8  468.40  46.9668 < 2e-16 ***
mydata$experiment                1    6.0    6.05   0.6063 0.43641
mydata$genotyp:mydata$treatment  2   85.8   42.90   4.3015 0.01386 *
Residuals                      797 7948.4    9.97
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> TukeyHSD(modelS4)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = mydata$S4 ~ mydata$genotyp + mydata$treatment +
mydata$genotyp:mydata$treatment + mydata$experiment, data = mydata)

$`mydata$genotyp`
             diff      lwr      upr p adj
Remus-C3 2.548902 2.111626 2.986178     0

$`mydata$treatment`
         diff        lwr        upr   p adj
R-B  1.276475  0.6380549  1.9148951 9.4e-06
Y-B -1.389088 -2.0268875 -0.7512885 1.2e-06
Y-R -2.665563 -3.3115933 -2.0195327 0.0e+00

$`mydata$experiment`
          diff        lwr      upr     p adj
2-1 -0.1734552 -0.6107634 0.263853 0.4364535

$`mydata$genotyp:mydata$treatment`
                       diff        lwr        upr     p adj
Remus:B-C3:B      3.4086162  2.3244736  4.4927588 0.0000000
C3:R-C3:B         2.0330218  0.9385508  3.1274929 0.0000022
Remus:R-C3:B      3.9167040  2.8200850  5.0133231 0.0000000
C3:Y-C3:B        -0.8222057 -1.9103995  0.2659882 0.2586776
Remus:Y-C3:B      1.4367450  0.3357431  2.5377470 0.0028306
C3:R-Remus:B     -1.3755943 -2.4758655 -0.2753232 0.0050396
Remus:R-Remus:B   0.5080878 -0.5943200  1.6104957 0.7758674
C3:Y-Remus:B     -4.2308218 -5.3248490 -3.1367947 0.0000000
Remus:Y-Remus:B  -1.9718711 -3.0786389 -0.8651034 0.0000066
Remus:R-C3:R      1.8836822  0.7711154  2.9962490 0.0000234
C3:Y-C3:R        -2.8552275 -3.9594907 -1.7509643 0.0000000
Remus:Y-C3:R     -0.5962768 -1.7131638  0.5206103 0.6481290
C3:Y-Remus:R     -4.7389097 -5.8453019 -3.6325175 0.0000000
Remus:Y-Remus:R  -2.4799590 -3.5989510 -1.3609670 0.0000000
Remus:Y-C3:Y      2.2589507  1.1482142  3.3696872 0.0000001

> tapply(X = mydata$S4, INDEX = list(mydata$geno_treat),  FUN=mean)
     C3_B      C3_R      C3_Y   Remus_B   Remus_R   Remus_Y
10.107143 12.136364  9.281481 13.510949 14.022901 11.542636
```

```
> modelS5 <- aov(mydata$S5 ~ mydata$genotyp + mydata$treatment +
mydata$genotyp:mydata$treatment + mydata$experiment, data =mydata)
> anova(modelS5)
Analysis of Variance Table

Response: mydata$S5
                                Df Sum Sq Mean Sq F value    Pr(>F)
mydata$genotyp                   1  839.6  839.56 92.3950 < 2.2e-16 ***
mydata$treatment                 2  477.6  238.79 26.2789 8.866e-12 ***
mydata$experiment                1    7.6    7.57  0.8333    0.3616
mydata$genotyp:mydata$treatment  2   29.0   14.49  1.5943    0.2037
Residuals                      797 7242.1    9.09
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> TukeyHSD(modelS5)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = mydata$S5 ~ mydata$genotyp + mydata$treatment +
mydata$genotyp:mydata$treatment + mydata$experiment, data = mydata)

$`mydata$genotyp`
            diff      lwr      upr p adj
Remus-C3 2.04391 1.626517 2.461304     0

$`mydata$treatment`
          diff        lwr         upr     p adj
R-B  1.2757239  0.6663318  1.88511595 0.0000032
Y-B -0.5849045 -1.1937043  0.02389523 0.0627803
Y-R -1.8606284 -2.4772847 -1.24397211 0.0000000

$`mydata$experiment`
          diff      lwr       upr     p adj
2-1 -0.1940955 -0.61152 0.2233289 0.3616568

$`mydata$genotyp:mydata$treatment`
                       diff        lwr        upr     p adj
Remus:B-C3:B     2.50449618  1.4696479  3.5393445 0.0000000
C3:R-C3:B        1.73412350  0.6894163  2.7788307 0.0000369
Remus:R-C3:B     3.31507180  2.2683143  4.3618293 0.0000000
C3:Y-C3:B       -0.32973095 -1.3684463  0.7089844 0.9447864
Remus:Y-C3:B     1.65815652  0.6072154  2.7090976 0.0001100
C3:R-Remus:B    -0.77037268 -1.8206162  0.2798708 0.2906474
Remus:R-Remus:B  0.81057562 -0.2417074  1.8628586 0.2385772
C3:Y-Remus:B    -2.83422714 -3.8785106 -1.7899437 0.0000000
Remus:Y-Remus:B -0.84633966 -1.9027844  0.2101051 0.1998765
Remus:R-C3:R     1.58094830  0.5189682  2.6429284 0.0003394
C3:Y-C3:R       -2.06385445 -3.1179085 -1.0098004 0.0000004
Remus:Y-C3:R    -0.07596698 -1.1420709  0.9901369 0.9999520
C3:Y-Remus:R    -3.64480275 -4.7008890 -2.5887165 0.0000000
Remus:Y-Remus:R -1.65691528 -2.7250285 -0.5888021 0.0001549
Remus:Y-C3:Y     1.98788747  0.9276545  3.0481205 0.0000017

> tapply(X = mydata$S5, INDEX = list(mydata$geno_treat),  FUN=mean)
    C3_B     C3_R     C3_Y  Remus_B  Remus_R  Remus_Y
11.79286 13.52273 11.45926 14.29197 15.10687 13.44961
```

```
> modelAU4 <- aov(mydata$AU4 ~ mydata$genotyp + mydata$treatment +
mydata$genotyp:mydata$treatment + mydata$experiment, data =mydata)
> anova(modelAU4)
Analysis of Variance Table

Response: mydata$AU4
                               Df  Sum Sq Mean Sq  F value    Pr(>F)
mydata$genotyp                  1  285202  285202 186.4028 < 2.2e-16 ***
mydata$treatment                2  750763  375381 245.3427 < 2.2e-16 ***
mydata$experiment               1    1760    1760   1.1503    0.2838
mydata$genotyp:mydata$treatment   2   59324   29662  19.3865 6.011e-09 ***
Residuals                       797 1219433    1530
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> TukeyHSD(modelAU4)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = mydata$AU4 ~ mydata$genotyp + mydata$treatment +
mydata$genotyp:mydata$treatment + mydata$experiment, data = mydata)

$`mydata$genotyp`
             diff     lwr      upr p adj
Remus-C3 37.67139 32.2552 43.08757     0

$`mydata$treatment`
          diff       lwr        upr     p adj
R-B  -3.622612 -11.53021   4.284988 0.5294493
Y-B -66.755920 -74.65583 -58.856007 0.0000000
Y-R -63.133309 -71.13517 -55.131448 0.0000000

$`mydata$experiment`
         diff       lwr      upr    p adj
2-1 -2.959275 -8.375862 2.457313 0.283853

$`mydata$genotyp:mydata$treatment`
                      diff         lwr        upr     p adj
Remus:B-C3:B      60.11345   46.685044  73.541859 0.0000000
C3:R-C3:B         10.91939   -2.636944  24.475733 0.1947670
Remus:R-C3:B      41.67950   28.096556  55.262445 0.0000000
C3:Y-C3:B        -46.87067  -60.349260 -33.392085 0.0000000
Remus:Y-C3:B     -27.17941  -40.816642 -13.542180 0.0000003
C3:R-Remus:B     -49.19406  -62.822236 -35.565878 0.0000000
Remus:R-Remus:B  -18.43395  -32.088596  -4.779306 0.0017265
C3:Y-Remus:B    -106.98412 -120.534964 -93.433284 0.0000000
Remus:Y-Remus:B  -87.29286 -101.001510 -73.584215 0.0000000
Remus:R-C3:R      30.76011   16.979631  44.540581 0.0000000
C3:Y-C3:R        -57.79007  -71.467693 -44.112441 0.0000000
Remus:Y-C3:R     -38.09881  -51.932793 -24.264818 0.0000000
C3:Y-Remus:R     -88.55017 -102.254169 -74.846177 0.0000000
Remus:Y-Remus:R  -68.85891  -82.718971 -54.998851 0.0000000
Remus:Y-C3:Y      19.69126    5.933456  33.449067 0.0006783

> tapply(X = mydata$AU4, INDEX = list(mydata$geno_treat),  FUN=mean)
     C3_B      C3_R      C3_Y   Remus_B   Remus_R   Remus_Y
101.10000 111.95455  54.17037 161.13139 142.76336  73.89922
```

```
> modelAU5 <- aov(mydata$AU5 ~  mydata$genotyp + mydata$treatment +
mydata$genotyp:mydata$treatment +mydata$experiment , data =mydata)
> anova(modelAU5)
Analysis of Variance Table

Response: mydata$AU5
                                Df  Sum Sq Mean Sq  F value    Pr(>F)
mydata$genotyp                   1  441243  441243 187.4480 < 2.2e-16 ***
mydata$treatment                 2  904929  452464 192.2149 < 2.2e-16 ***
mydata$experiment                1    2743    2743   1.1653    0.2807
mydata$genotyp:mydata$treatment  2   72067   36034  15.3077 2.995e-07 ***
Residuals                      797 1876098    2354
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> TukeyHSD(modelAU5)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = mydata$AU5 ~ mydata$genotyp + mydata$treatment +
mydata$genotyp:mydata$treatment + mydata$experiment, data = mydata)

$`mydata$genotyp`
             diff      lwr      upr p adj
Remus-C3 46.85701 40.13898 53.57505     0

$`mydata$treatment`
          diff        lwr       upr     p adj
R-B   1.481786  -8.326507  11.29008 0.9329961
Y-B -70.703905 -80.502665 -60.90515 0.0000000
Y-R -72.185692 -82.110904 -62.26048 0.0000000

$`mydata$experiment`
         diff       lwr      upr     p adj
2-1 -3.694376 -10.41291 3.024159 0.2807446

$`mydata$genotyp:mydata$treatment`
                       diff         lwr         upr     p adj
Remus:B-C3:B      71.93968   55.283576   88.595776 0.0000000
C3:R-C3:B         18.45369    1.638904   35.268466 0.0219227
Remus:R-C3:B      56.14305   39.295270   72.990834 0.0000000
C3:Y-C3:B        -49.17455  -65.892887  -32.456204 0.0000000
Remus:Y-C3:B     -20.98961  -37.904725   -4.074491 0.0055475
C3:R-Remus:B     -53.48599  -70.389880  -36.582102 0.0000000
Remus:R-Remus:B  -15.79662  -32.733340    1.140092 0.0836742
C3:Y-Remus:B    -121.11422 -137.922183 -104.306261 0.0000000
Remus:Y-Remus:B  -92.92928 -109.932983  -75.925584 0.0000000
Remus:R-C3:R      37.68937   20.596575   54.782159 0.0000000
C3:Y-C3:R        -67.62823  -84.593452  -50.663010 0.0000000
Remus:Y-C3:R     -39.44329  -56.602459  -22.284127 0.0000000
C3:Y-Remus:R    -105.31760 -122.315528  -88.319668 0.0000000
Remus:Y-Remus:R  -77.13266  -94.324166  -59.941154 0.0000000
Remus:Y-C3:Y      28.18494   11.120265   45.249611 0.0000413

> tapply(X = mydata$AU5, INDEX = list(mydata$geno_treat),  FUN=mean)
     C3_B      C3_R      C3_Y   Remus_B   Remus_R   Remus_Y
144.90000 163.27273  95.65185 216.73723 201.02290 123.88372
```