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Master Thesis

Evaluation of FHB Resistance in related Spring Wheat Populations descending from a highly resistant semi-dwarf Breeding Line

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Herewith I affirm that I produced this Master thesis myself. No resources than those cited were used. Phrasings and thoughts which have been adopted directly or indirectly from foreign resources are marked. This thesis has never been submitted anywhere before.

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Abstract

Fusarium Head Blight (FHB) is one of the most common diseases in wheat with devastating consequences for yield and quality. Disease management occurs to be quite difficult and depends on several factors. Although no completely resistant variety is known, resistance breeding offers a way to support farmers. At the moment, breeders primarily use Asian resistance sources in their programs. Furthermore many studies observed a negative correlation between FHB severity and plant height. However, the spring wheat genotype number 6408-1 with the pedigree BAU/MILAN/CBRD attracted positive attention in the 9th *Fusarium* head blight screening nursery distributed by CIMMYT. Although this line contains the dwarfing allele *Rht-B1b* it showed high resistance levels. The underlying resistance could originate from South America and provides a new source for breeders; ultimately leading to higher genetic variability in wheat varieties.

To learn more about the genetic makeup of the FHB resistance line, 6408-1 was crossed with the two susceptible German spring wheat cultivars Remus and Michael by Prof. Hermann Buerstmayr. Another cross was done with the Chinese landrace Wang Shui Bai (WSB), which is also resistant. However, due to undesirable economic traits, it was not used in breeding programs. Data from 2019 to 2020 was analyzed. In both years, inoculation with *Fusarium culmorum* was performed to induce infection. FHB severity, anther retention, plant height, awn character and flowering date were rated for each plot. In 2019 molecular marker analysis was done by Jakob Seereiter, including the *Rht-B1* locus.

Statistical analysis of 2019 and 2020 showed following results:

FHB severity showed all nuances of disease level, from susceptible to resistant. WSB x 6408-1 showed rather resistant genotypes compared to the other populations (AUDPC=221). All populations included genotypes with a lower infestation rate than 6408-1. Regarding anther retention and FHB severity, results were inconsistent but showed rather positive correlations. A third year might clarify discrepancies. In general plant height was negatively correlated to the infestation rate ($-0.40 \leq r \leq -0.18$) across both years. However, some short stem genotypes, carrying the *Rht-B1b* allele, showed a high level of resistance. Furthermore, reduced plant height and the occurrence of the *Rht-B1b* allele showed negative correlation with anther retention. In these experiments, flowering date and FHB symptoms were clearly positively correlated. Moreover, later flowering populations had more retained anthers and taller plants on average.

Plants expressed four different types of awn character: genotypes without awns (awnless), with awns (awned), a mixture of plants with and without awns (heterogeneous) and awnletted plants, meaning only very short awns visible. Looking at the relation with FHB severity, only population Remus x 6408-1 had significant results and awned plants showed least disease symptoms. Awnletted plants not only had highest infestation rates in Remus x 6408-1, but also highest anther retention and shortest stems.

Based on these results, 6408-1 can be regarded as a promising genotype for further research and breeding programs, possibly providing a non-Asian resistance source alternative.

Keywords: *Fusarium* Head Blight, Wheat, Resistance Breeding, Plant Height, Anther Retention, Flowering Date, Awns, *Rht-B1;*

Zusammenfassung

Ährenfusariose ist eine der bedeutendsten Krankheiten, die im Weizen auftreten kann. Sie führt zu hohen Ernteeinbußen und senkt die Qualität des Getreides. Das Management dieser Krankheit hat sich als kompliziert herausgestellt und wird durch unterschiedliche Faktoren beeinflusst. Bis heute ist keine Weizensorte bekannt, die vollständige Resistenzen aufweist. Momentan werden in der Pflanzenzüchtung vor allem Resistenzen asiatischen Ursprungs verwendet. Zusätzlich haben viele Studien einen negativen Zusammenhang zwischen Wuchshöhe und Fusariumbefall ergeben.
Im 9. Ährenfusariose-Resistenz Prüfset des CIMMYT, Mexiko, ist allerdings der Genotyp 6408-1 mit dem Stammbaum BAU/MILAN/CBRD aufgefallen. Besonders ist, dass der Stammbaum auf einen südamerikanischen Ursprung der Resistenz hindeutet und obwohl 6408-1 das Zwergallel *Rht-B1b* hat, wies es eine hohe Resistenz auf. Somit stellt diese Linie eine neue potentielle Resistenzquelle für Züchter dar, die dazu beitragen könnte, die genetische Variabilität im Weizen zu erhöhen. Um mehr über das züchterische Potential des Genotyps 6408-1 herauszufinden kreuzte Prof. Hermann Bürstmayr ihn mit den zwei deutschen Sommerweizensorten Remus und Michael und mit der chinesischen Landrasse Wang Shui Bai (WSB). In diese Masterarbeit sind Daten von den Versuchsjahren 2019 und 2020 ausgewertet worden. In beiden Jahren wurden die Genotypen mit Konidien von *Fusarium culmorum* inokuliert. Für jede Parzelle sind Daten bezüglich Fusariumbefall, Antherenretention, Wuchshöhe, Grannentyp und Blühdatum erfasst worden. Im Jahr 2019 wurde zusätzlich eine molekulare Markeranalyse, unter anderem mit dem *Rht-B1* Lokus, durchgeführt. Statistische Auswertungen haben folgende Ergebnisse gebracht: Der Befall der Pflanzen mit Ährenfusariose war in allen Nuancen vorhanden, von anfällig bis resistent. WSB x 6408-1 wies im Vergleich zu den anderen Populationen eher resistente Genotypen auf (AUDPC=211). In allen Kreuzungspopulationen waren Genotypen zu finden, die einen geringeren Befall als 6408-1 zeigten. Bezüglich des Zusammenhangs von Antherenretention und Befall, waren die Ergebnisse nicht eindeutig. Allgemein wurde eine positive Korrelation sichtbar. Das dritte Versuchsjahr wird wahrscheinlich mehr Informationen zu dieser Eigenschaft liefern können. Über beide Jahre gerechnet, wiesen Wuchshöhe und Befall einen negativen Zusammenhang auf ($-0.40 \leq r \leq -0.18$). Allerdings waren auch Genotypen dabei, die sowohl das Kurzstrohallel *Rht-B1b* als auch einen niedrigen Befall hatten. Wuchshöhe und das Vorhandensein des *Rht-B1b* Allels korrelierten negativ mit Antherenretention. In diesen Versuchen waren Blühdatum und Fusariumbefall eindeutig positiv korreliert, später blühende Genotypen zeigten mehr Symptome. Des Weiteren zeigten später blühende Linien erhöhte Antherenretention und durchschnittlich höhere Pflanzen. In den Populationen wurden vier verschiedene Grannentypen festgestellt: unbegrannte (awnless), begrannte (awned), teilweise begrannt und unbegrannt (heterogeneous) und Ähren mit kleinen Grannenspitzen (awnletted). Dieses Merkmal hat allerdings nur in der Population Remus x 6408-1 signifikante Ergebnisse gebracht. Begrannte Linien zeigten die niedrigste Befallsrate. Pflanzen mit kleinen Grannenspitzen wiesen die höchsten Krankheitssymptome auf, hatten einen niedrigeren Antherenausstoß und waren kürzer. Aufgrund dieser Ergebnisse kann man schließen, dass der Genotyp 6408-1 eine vielversprechende Resistenzquelle, mit einem neuen genetischen Hintergrund, darstellt.

Schlüsselwörter: *Fusarium* Head Blight, Weizen, Resistenzzüchtung, Wuchshöhe, Antherenretention, Blühdatum, Grannen, *Rht-B1;*

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1 Introduction

1.1 Fusarium Head Blight

1.1.1 The Pathogen

Fusarium Head Blight (FHB) is an common disease with devastating consequences for yield and quality of wheat, maize and other small grain cereals caused by fungi of the group *Fusarium spp* (Buerstmayr et al. 2000; Buerstmayr et al. 2009). According to Taheri (2018) especially wheat and barley are affected. The genus *Fusarium* is an *Ascomycota* and consists of more than 16 species but according to Parry et al. (1995) *Fusarium graminearum* Schwabe and *Fusarium culmorum* are the most distributed ones. The variability of morphological and nonmorphological characteristics within the genus makes it difficult to separate between *Fusarium* species (Wagacha and Muthomi 2007). In general one can distinguish between primary and secondary characteristics. The former includes the shape of macroconidia, whether or not microconidia are present and are born in chains. Another primary character is the type of microconidiophores (Windels 1991). Windels (1991) further assigned the presence or absence of sclerotia or sporodochia, the existence of chlamydospores, their configuration and position to the secondary characteristics.

Fusarium culmorum and *Fusarium graminearum* both belong to section Discolour, which are also called cereal fusaria (Nelson et al. 1983; Booth 1971). *Fusarium culmorum* is the species which dominates in cool areas and *Fusarium graminearum* in warm and humid regions (Parry et al. 1995; Goswami and Kistler 2004). Both fungi are saprophyte and facultative, soil inhabiting species, which could be soilborne, airborne and seedborne (Goswami and Kistler 2004; Saremi et al. 2011; Lević et al. 2012).

1.1.1.1 Disease Cycle and Symptoms

Like the other group members of *Fusarium spp.*, *Fusarium culmorum* and *Fusarium graminearum* infest many plant species on different organs. The most critical one is certainly the infestation of the grain's ears, which leads to FHB (Parry et al. 1995). In Figure 1 the general life cycle of *Fusarium spp.* is shown.

In general FHB is a pre-harvest disease but *Fusarium spp.* is able to grow and overwinter in soil or debris for up to 16 months (Nyvall 1970). *Fusarium graminearum* has a sexual and asexual stage. In spring, the primary infection occurs via ascospores, which are dispersed by wind, rain splashes or insects and released by perithecia, which developed on crop residuals (Trail et al. 2003; Parry et al. 1995; Fernando et al. 2021). Macroconidia are produced in the asexual stage, serving an inoculum source for secondary infection. *Fusarium graminearum* is also able to form chlamydospores in the middle of the mycelia or in the macroconidia itself (Leplat et al. 2013). Sutton (1982)and Nyvall (1970) clarified that apart from perithecia, chlamydospores play an important role in surviving and overwintering. Macroconidia also infect roots and hypocotyls, causing crown and root rot (Lysøe et al. 2011).

Unlike other *Fusarium* species, no sexual stage of *Fusarium culmorum* is known, therefore no ascospores are produced. In this case the main mode of infection are the asexual conidia only (Fernando et al. 1997; Parry et al. 1995). They are transported to the flowering wheat mainly during anthesis, just like ascospores and conidia from *Fusarium graminearum* (Bai and & Shaner 2004; Sutton 1982).

Depending on factors like climate conditions, cultivar resistance level, humidity and nitrogen fertilization, the success of the infection varies, summarized by Wagacha and Muthomi (2007). According to Lacey et al. (1999) the most important factor is the presence of moisture. Ascospores or macroconidia germinate and form hyphae after landing on the host's spikes (Pritsch et al. 2000).The fungus then enters the plant through the floret mouth or the overlapping regions of lemma and palea (Lewandowski et al. 2006). Zange et al. (2005) found that conidia of *Fusarium culmorum* germinate on the inner surface of the lemma, palea and the ovary.

After a successful infection of the ears a short biotrophic phase follows, during which no harm occurs to the host. Afterwards, during a necrotrophic phase, the pathogen penetrates the thin epidermis of palea and lemma and starts to produce cell wall degrading enzymes. The hyphae differentiate into infection structures and conidia are produced (Pritsch et al. 2000; Bushnell et al. 2003). Beside enzymes, the production of deoxynivalenol (DON) increases, which is a virulence factor for wheat. In this way *Fusarium spp.* is able to break through the rachis and spread via vascular tissues (Jansen et al. 2005). Under high humidity, hyphae grow outside the spike and find their way to distant florets. Other possible entrances are provided by stomata or injuries (Imboden et al. 2018).

In the end the fungus develops inter- and intracellulary resulting in severe damage of the host cells (Zange et al. 2005).

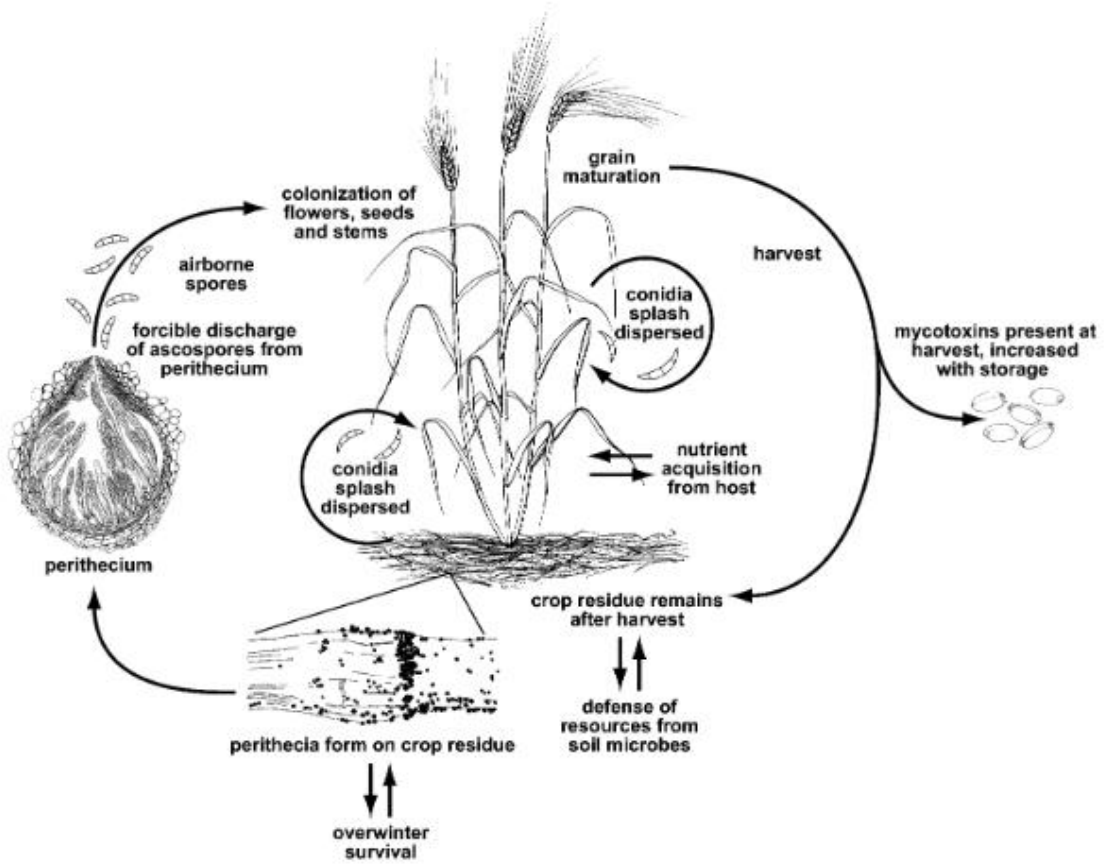

Figure 1: Life cycle of *Fusarium graminearum* (Trail 2009).

Under warm, humid conditions, first symptoms become visible after flowering. Infected wheat heads lose chlorophyll, appear bleached and prematurely whiten. Due to shriveled or non-developed kernels yield may be reduced up to 80% (Arthur 1891). Apart from low seed weight, the germination

of the seeds is reduced as well. Another problematic factor are mycotoxins (McMullen et al. 2012; Taheri 2018; Fernando et al. 2021).

1.1.2 Mycotoxins of *Fusarium spp.*

The important role of mycotoxins can be explained by the high toxicity for humans and animals. Fusarium spp. produces mycotoxins such as trichothecenes, zeralenone and butenolides (Khaledi et al. 2017; Ismaiel and Papenbrock 2015). The most important group represent trichothecenes, especially deoxynivalenol (DON). Fernando et al. (2021) determined the occurrence of DON and nivalenol (NIV). Both mycotoxins belong to trichothecenes type B and are the main mycotoxins produced by *Fusarium spp.* in wheat and barley. NIV and DON dominate in different environments and reports from Europe and China have shown that strains producing NIV are often more aggressive in maize. According to Taheri (2018) DON and NIV both are virulence factors, with DON being more phytotoxic on cereals (Desjardins 2006). It inhibits protein synthesis in eukaryotes (van de Walle et al. 2010).

In biosynthesis of trichothecenes several genes are involved. Tri5, for example, is the gene responsible for the first step, where it encodes trichidiene synthase. Required genes are often located in a gene cluster and the presence, absence and functionability of the genes result in the final toxin, produced by the *Fusarium* species (Mc Cormic 2003; Wagacha and Muthomi 2007; Hestbjerg et al. 2002).

To ensure no risks for humans and animals, a maximum level of toxins was set by the European Commission (Commission Regulation 2006/1881). The limit of DON in unprocessed wheat for human consumption is 1250 µg/kg for human and 8000 µg/kg for animal feed (Brodacz 2021).

1.2 Wheat

Wheat is the foremost crop, with 95% of the world's production being bread wheat and 5% durum wheat. With 17% of the total crop area, wheat provides more than 20% of total calories for humans (Peng et al. 2011).

The origin of wheat is located in the Fertile Cresent. 300.000 – 500.000 years before present the wild diploid *Triticum urartu,* providing the A genome, probably hybridized with the B genome providing goat grass *Aegilops speltoides.* The result was the wild tetraploid emmer wheat (*Triticum dicoccoides)*, having AABB as genome with 2n=4x=28 chromosomes (Huang et al. 2002; Dvorak and Akhunov 2005). Peng et al. (2011) summarized that approximately 10.000 years before present humans started cultivating emmer wheat, which hybridized with the goat grass *Aegilops tauschii* around 9000 years ago, providing the D genome. While most scientists agree that due to several mutations, the free threshing hexaploid wheat *Triticum aestivum* resulted from this hybridization, the origin of the hulled European spelt (*Triticum spelta*) is not clarified (Blatter et al. 2003). The assumption that *Triticum spelta* is an ancestor of *Triticum aestivum* is more and more doubted. Flaksberger already suggested in 1930 that *Triticum spelta* evolved from a hybridization of the cultivated emmer *Triticum dicoccum* and the free threshing *Triticum aestivum*. Dvorak et al. (2011) pursued a study on the relation of geographical center of diversity of crops and their geographical center of origin. They found out that not only gene flow from ancestors but also from wild species are sources of cultivated wheat. Therefore, pedigrees of wheat species are rather complex, and retracing them is complicated.

Despite a large genome size of 15.961 Mb for bread wheat and 11.660 for durum wheat (Bennett and Leitch 2010), domestication and modern breeding caused genetic erosion, resulting in increased vulnerability to environmental stress and susceptibility to pests and diseases(Nevo 2009, 2011; Fu and Somers 2009). Therefore, Peng et al. (2011) came to the conclusion, that the usage of adaptive genetic resources of wild progenies is a very beneficial method for wheat improvement.

1.2.1 Types of Resistance to Fusarium Head Blight

It is known that FHB resistance in wheat is controlled by several genes and therefore is a polygenetic resistance. The genes have small effects and are usually influenced by environmental factors with a significant genotype-environment-interaction (Snijders and Perkowski 1990; Ban and Suenaga 2000; Buerstmayr et al. 2009). Waldron et al. (1999) distinguished between five different types of resistance, whereby type I and II are the main and most excepted types (Steiner et al. 2017).

- Type I - Resistance to initial penetration
- Type II - Resistance to spreading to neighboring spikelets
- Type III - Resistance to kernel infection
- Type IV - Tolerance to trichothecenes
- Type V - Decomposition and therefore detoxification of DON

According to Bai and & Shaner (2004), type II resistance is the most stable one, and hence often used in breeding programs. Furthermore measurement of type II is easier than type I. Usually, single floret inoculation is used to evaluate type II. FHB severity is measured, rating the percentage of infected spikelets in a spike. For type I resistance, inoculation of spikes or grain-spawn is used, FHB severity or incidence are measured. Incidence is considered as the percentage of infected spikes in a plot (Bai and Shaner 1994).

According to Bai and Shaner (1994), every wheat genotype can be successfully infected by *Fusarium spp.* if spores are point-injected. The difference of resistant and susceptible genotypes becomes visible when the pathogen starts spreading. The fungus is not able to spread into neighboring spikelets in high resistant genotypes. The infection stays in the inoculated floret, whereas in susceptible genotypes, at worst *Fusarium spp.* infects the whole spike. Apart from the genotype, environmental factors also have great impact on spreading (Bai and Shaner 1994). It should be considered that in the field, infection may occur on several spikelets of one spike and measuring the FHB severity reflects type I as well as type II resistance (Burt et al. 2015).

1.2.2 Active Resistance

Wheat has several resistance mechanisms. A distinction is made between passive and active resistance. Chemical and physical barriers belong to the latter, whereby scientists have differing observations and opinions concerning the responsible mechanisms (Bai et al. 2018).

According to Zange et al. (2005) and Ribichich et al. (2000), some resistant lines have thicker cell walls. Additionally, many different biochemical compounds, like choline and betaine, have been associated with fungal growth and spreading (Browne and Brindle 2007). Zhang et al. (2013) and Ribichich et al. (2000), for example, observed the accumulation of toxic phenolic compounds and triticens (Zhang et al. 2013; Ribichich et al. 2000). Pritsch et al. (2000) described the presence of defense responsive genes. If an infection with *Fusarium graminearum* occurs, those genes are activated and defense-related proteins are produced. Zhang et al. (2013) were able observe that the

expression of some of those proteins varies between resistant and susceptible plants. On the contrary, Ding et al. (2011) and Li and Yen (2008) reported an increase of jasmonic acid, lipoxygenase and chalcone in resistant genotypes. Furthermore, they observed an increase of ethylene production. Ethylene is responsible for senescence and cell wall degradation, which in the end leads to cell death. All of these possibilities of active resistance have the target to prevent or delay the spreading of the fungus (Ribichich et al. 2000; Zhang et al. 2013; Bai et al. 2018).

1.2.3 Passive Resistance

Apart from active resistance, FHB-related traits also play an important role in FHB resistance. The so called passive resistance includes mainly morphological and developmental traits. In contrast to active resistance, passive resistance mechanisms help to prevent the initial infection (Bai et al. 2018). In the following chapters the most important aspects of plant height, anther retention, flowering date and awn character are discussed briefly.

1.2.3.1 Plant Height

Plant height is a morphological trait which is often associated with FHB resistance; especially under field conditions. Many studies and reports are known (Steiner et al. 2017). In general, higher plants are more resistant. One reason for this is the lower risk of infection from splash-dispersed spores. Those spores mainly come from the debris fungus uses to overwinter on (Jenkinson and Parry 1994). According to Hilton et al. (1999) and Yan et al. (2011), also microclimate conditions, like humidity and temperature, vary at different plant heights and affect the FHB severity. Therefore, higher plants also showed higher resistance after spray inoculation. However, almost all studies found a high correlation between plant height and FHB severity (Buerstmayr et al. 2019). These observations are confirmed by the discovery that several plant height QTL are overlapping with FHB resistance. Buerstmayr et al. (2019) reported a colocalization of plant height and FHB severity on 14 chromosomes, 40% of the QTLs were overlapping.

1.2.3.1.1 The role of *Rht-1* genes in FHB severity

Although not all plant height QTL are associated with FHB severity, the homologous *Rht-1* alleles play an important role (Buerstmayr et al. 2019). *Rht-B1b* and *Rht-D1b* are the most popular dwarfing alleles since the Green Revolution. Norman Borlaug managed to incorporate these alleles from the Japanese genotype “Norin 10” into modern wheat and barley cultivars, which are located on chromosomes 4BS and 4DS (Börner et al. 1996; Gale and Youssefian 1985; Borlaug 1968). *Rht-B1b* and *Rht-D1b* encode single-nucleotide polymorphism, located in the DELLA domain. Due to this mutation, premature stop codons are created, resulting in gibberellin-insensitive plants with reduced plant height (Peng et al. 1999). Plants having those alleles are short, with strong stalks and hence are highly resistant to lodging (Börner et al. 1996; Gale and Youssefian 1985). According to Evens (1998), nowadays more than 70% of the wheat varieties contain one of those alleles. Draeger et al. (2007) was the first one to show the negative impact of the *Rht-D1b* allele. Although both alleles have similar effects on plant height, several studies, including Miedaner and Voss (2008), Srinivasachary et al. (2009) and Lu et al. (2011), reported a much higher FHB severity in plants with the *Rht-D1b* allele compared to plants having the *Rht-B1b* genotype. While *Rht-D1b* is clearly associated with resistance type I, the function of *Rht-B1b* is not completely known (Draeger et al. 2007; Holzapfel et al. 2008; Srinivasachary et al. 2009; Miedaner et al. 2011). However, Miedaner et al. (2011) observed a significant epistatic interaction with the marker *Xbarc147* in European winter wheat. This marker is

closely linked to the major FHB resistance QTL *Fhb1*. The locus of the Rht-B1 gene is associated with plant height as well as anther retention (Lu et al. 2013).
Buerstmayr et al. (2019) summarized that the *Rht-1* alleles also affect flowering, spike related traits and compromise cell elongation. This leads to stiffer and more compact tissues, which modulates spike compactness, anther extrusion and filament elongation. Further, Lanning et al. (2012) reported an association of the semidwarf alleles and increased grain yield.

1.2.3.2 Association of Infection and Anther Retention

Parry et al. (1995) and Pugh et al. (1933) reported that wheat is particularly sensitive during anthesis. Especially warm and humid conditions support the infection with *Fusarium spp.* In general, degenerating tissues, such as pollen, retained anthers and stigma, support hyphal growth (Pugh et al. 1933). Many studies, like Buerstmayr and Buerstmayr (2015), Lu et al. (2013) and Graham and Browne (2009), observed a higher FHB infection in plants with retained anthers. Experiments of Buerstmayr et al. and Steiner et al. (2019) demonstrated that the success of the infection is not necessarily depending on the presence of retained anthers. They removed all anthers after pollination, using spray inoculation for artificial infection. The results showed that the initial infection and the early disease development were reduced but the spreading within the spikes was not affected. Regarding the role of endogenous compounds of anthers, reports of scientists are inconsistent. While Strange and Smith (1971) found a relation between the presence of choline and betaine with fungal growth, Engle et al. (2004) reported no correlation. Based on these results Buerstmayr et al. (2019) speculated that the reason for the association of retained anthers and infection with the fungus is due to the fragile and degenerating tissue of the anthers, which provides an easy target for initial infection.
Concerning the genetic background of anther extrusion and retention, the architecture turned out to be quite complex. Molecular-genetic analysis showed that this trait is under polygenetic control (Buerstmayr and Buerstmayr 2015). Compared to other passive resistance factors, only a few FHB mapping populations covered anther retention or extrusion. They found out that 60% of the QTL for anther retention overlap with FHB resistance QTL, leading to the suggestion that anther retention has a pleiotropic effect on FHB resistance (Buerstmayr and Buerstmayr 2015; Lu et al. 2013; He et al. 2016b). Buerstmayr and Buerstmayr (2015), Lu et al. (2013) and He et al. (2016b) further observed that only *Qfhs.ifa-5A* of Steiner et al. (2019) and the *Rht1* genes are overlapping for plant height and anther retention/extrusion. Both QTL are associated with increased amount of retained anthers and smaller plants. Despite this overlapping, reports by scientists in this case are inconsistent. Most studies confirmed that genotypes, carrying an *Rht1* allele, have a negative impact on anther extrusion (Buerstmayr and Buerstmayr 2016; Lu et al. 2013; Boeven et al. 2016). He et al. (2016a), on the other hand, found no effect and Okada et al. (2019) describes a dependency on the genotype, whether the *Rht-B1* allele affects anther retention extrusion or not. Regarding the *Rht-D1* allele, he found no association at all. However, due to the complexity of the genetic background and environment which affect the behavior of anthers, he concluded that there probably is a genetic interaction with the *Rht1* locus (Okada et al. 2019). In general, effects on FHB severity where reported for both, *Rht-B1* and *Rht-D1*, but several studies agree that especially *Rht-D1b* has a strong impact on anther retention (Buerstmayr and Buerstmayr 2016; Miedaner and Voss 2008; Liu et al. 2013).

As highly inherited trait, anther retention/extrusion offers a simple indirect selection and breeding strategy for FHB-resistance. If possible, the *Rht-B1* should be preferred over the *Rht-D1* locus (Steiner et al. 2017).

1.2.3.3 Association of Infection and Flowering Date

As already mentioned, anthesis is the most critical time for FHB infection. Not only whether anthers are retained or extruded is associated with FHB severity, but there are also some reports about flowering date and FHB resistance (Buerstmayr et al. 2019). The first one to observe a positive effect of early maturing plants was Arthur (1891). Looking at the flowering time of different wheat genotypes, scientists as Schmolke et al. (2005) and Gervais et al. (2003) found a positive correlation between early flowering plants and FHB resistance, where around 25% of the QTL overlapped. On the contrary, Buerstmayr et al. (2008) could not find a systematic association. 56 genotypes were tested under different environmental conditions. Positive correlations as well as negative and no correlations were found. Therefore, Buerstmayr et al. (2008) speculated that the distribution was rather due to differences in humidity and temperature than to the underlying genetic. Important to mention is also the experiment by He et al. (2016a). They found a highly significant effect of the *Vrn-A1* locus at first, which constitutes an essential gene for growth habit. After a correction for plant height and days to heading, nearly no significance was left, showing the importance of taking other FHB-associated traits into account (He et al. 2016a).

1.2.4 Association of Infection and Awn Character

Mesterházy (1995) suspected a negative influence of awns regarding FHB susceptibility. The higher humidity and the larger surface may create better conditions for airborne conidia. In his experiment under natural conditions, genotypes with awns had a higher FHB severity than plants without. However, this association could not be observed in artificial inoculation (Mesterházy 1995). Also Buerstmayr and Buerstmayr (2015) did not confirm this result.

1.2.5 Resistance Sources and QTLs in Wheat

As FHB resistance is controlled by several genes, it is classified as quantitative resistance, showing a continuous distribution of phenotypes (Corwin and Kliebenstein 2017). Although numerous resistance sources in wheat are known, its quantitative nature and the genotype-by-environment interaction complicates breeding (Buerstmayr et al. 2009; Niwa et al. 2014). Scientists have found around 50 individual resistance QTLs on all 21 chromosomes (Liu et al. 2009; Buerstmayr et al. 2009). In general, a lot of Asian sources are used in breeding (Buerstmayr et al. 2009). The first reported major QTL was *Fhb1* on the chromosome 3BS from the Chinese variety `Sumai3´ (Bai et al. 1999; Waldron et al. 1999). Further *Fhb2* and *Qfhs.ifa-5A* followed, deriving from `Sumai3´ as well. The QTLs *Fhb4* and *Fhb5* were found in the Chinese landrace `Wangshuibai´ (Xue et al. 2010; 2011) and *Qfhs.nau-2DL* in the breeding line CJ9306 (Jiang et al. 2007a; 2007b). However, resistance sources were not only found in common wheat, but also in alien species. *Fhb3* was derived from *Leymus racemosus* (Qi et al. 2008), *Fhb6* from $1E^{ts}$#1S of *Elymus tsukushiensis* (Cainong et al. 2015) and *Fhb7* from *Thinopyrum ponticum* (Guo et al. 2015).

Fhb1 is not only the first reported major QTL but also the one with the largest effect on type II resistance (Bai et al. 1999; Waldron et al. 1999). *Fhb1* and *Qfhs.nau-2DL* both show resistance to fungal spreading and the accumulation of toxins (Lemmens et al. 2005). Lemmens et al. (2005) described that *Fhb1* probably also plays a role in detoxification, by enhancing the ability to convert DON into DON-3-glucoside. *Fhb2* was mainly associated with resistance type II (Waldron et al. 1999),

although Szabó-Hevér et al. (2012) and Ágnes et al. (2014) also reported type I and III resistance. Those types were also reported for the QTLs *Fhb4* (Xue et al. 2010; Liu et al. 2007) and *Fhb5* (Liu et al. 2009; Lin. F. et al. 2006). *Zhang et al. (2012)*dealt with QTLs described some locally adapted cultivars having minor FHB resistance QTLs. Their effect may not be as large as that of *Fhb1* but the genotypes have got several desirable agronomic traits and are easy to breed with. A combination of these QTL can lead to additive effects (Li et al.; Cai 2016). Although the large amount of sources and types of resistance, no complete resistance to FHB is known yet and there are still an undefined number of unknown QTLs affecting the resistance (Khaledi et al. 2017; Buerstmayr et al. 2009).

1.3 Disease Management

Due to the missing genetic resistance to FHB, several strategies have been evolved to inhibit or mitigate an infestation. Cultural techniques mostly try to prevent spores landing on wheat heads during flowering (McMullen et al. 2012). Crop rotation plays an important role, as the fungus overwinters on crop debris. Choosing non-host cultivars of *Fusarium spp.* can substantially help to reduce the inoculum (Parry et al. 1995; Pereyra and Dill-Macky 2008). Pereyra et al. (2004) described tillage as another useful strategy. Turning the soil, crop residues are buried and do not function as inoculum source any more. Dill-Macky and Jones (2000) observed the influence of different tillage practices in spring wheat. The least FHB severity was found in wheat following soybean, followed by a wheat-wheat and wheat-corn rotation. Moldboard plowing turned out to be the most effective strategy when compared to chisel plowing or no treatment (Dill-Macky and Jones 2000). According to Bergstrom et al. (2011), FHB and DON may be reduced up to 30% in infected areas by implementing crop rotation and management strategies. Another important factor seems to be the content of spores of the surrounding environment, which is especially high in regions with predominant corn cultivation (Bergstrom et al. 2011). Apart from those strategies, fungicide application is also used to control FHB and DON. In an experiment in the US by Paul et al. (2008), a combination of prothioconazole and tebuconazole was able to control 52% of FHB; metconazole suppressed 45% of DON. Moreover they observed that fungicides were more effective in spring wheat than in winter wheat (Paul et al. 2008). Not only the choice of the active substances can be significant, but also the application method. Several studies took a closer look at different systems for field and greenhouse application. According to results by van Ee et al. (2004) for instance, dual flat fan spray nozzles provide the best coverage. The nozzles were angled in 30 degrees from the horizontal, spraying 140liters/ha. The droplet size was 300 to 350µm and they used a nonionic surfactant as an adjuvant. On the other hand, Halley et al. (2008) achieved similar results with a single flat fan nozzle.

The possibility of using antagonists for biological control could play an important role in organic production. Although a lot of research has been done, no popular method is used. McMullen et al. (2012) summarized that biological control mainly focuses on the initial infection, though it rather enhanced the protection of demethylation inhibitor (DMI) fungicides than showing consistent control for itself. A combination of biological control and fungicides could be useful for integrated management. As DMI fungicides are not allowed to be applied past full anthesis, antagonists provide the possibility to compete for nutrients, produce antifungal metabolites, or to induce localized resistance (Yuen et al. 2010).

Regarding the application of different mediums, the timing is not always easy to choose. Challenges such as wet plants, different heading dates and a short period of flowering, pose a problem in fungicide application as well as in biological control. Attention should also be paid to uneven terrain,

low spray volume and fast ground speed (McMullen et al. 2012). To support efficiency of disease management, prediction models for FHB are used. It not only helps farmers in evaluating the risk of infestation but also in proper fungicide application (Desjardins 2006).
The most cost-effective disease management strategy is the deployment of resistant genotypes (McMullen et al. 2012). To receive an ideally resistant genotype, different breeding strategies are used.

1.3.1 Breeding Strategies for Fusarium Head Blight Resistance

In general, resistance genes origin either from adapted or exotic wheat germplasm (McKendry 2008). However, for an acceptable resistance level of progenies, at least one parent should have moderate to high resistance (Steiner et al. 2017). McMullen et al. (2012) described that particularly in spring wheat, *Fhb1* is the most common resistance QTL. It was identified by Waldron et al. and Bai et al. (1999), and is one of the rare QTL which has been fine-mapped (Buerstmayr et al. 2019). Bai et al. (2018) reported a reduction of FHB severity by 20-50% in *Fhb1*- lines, depending on the genetic background. The high variability of effectiveness is probably due to unknown minor QTLs of the genetic background. Therefore, phenotypic selection is used to find the resistant genotypes (Jin et al. 2013; 2014). Like several QTLs for FHB resistance, *Fhb1* shows additive effects and gene pyramiding is often used in breeding programs to achieve high resistant cultivars. Major QTLs from different sources are taken and included into locally adapted genotypes (Jiang et al. 2007a; Jiang et al. 2007b; Bai et al. 1999). A direct usage of QTLs in breeding may lead to undesired agronomic traits. Marker-assisted backcross provides a way to eliminate those traits (Bai et al. 2018). Backcrosses and double haploids in combination with traditional forward breeding or marker assisted selection are common breeding strategies (McMullen et al. 2012).
As already mentioned, minor QTLs from adapted cultivars are often unknown and no molecular markers are available. Phenotypic selection is done several times with promising genotypes, which are tested in multiple environments. Afterwards, stable lines are crossed with other adapted genotypes, having desirable agronomic traits. Either single or three-way crosses are possible (McMullen et al. 2012) and they can further be used as recurrent parents for pyramiding of Asian genes (Bai et al. 2018).

1.3.1.1 Phenotypic Selection

Although it is a time-intensive method, this strategy prevails among breeders. Advanced generations of promising genotypes are have been tested in different locations (Steiner et al. 2017). According to Miedaner et al. (2001), phenotypic selection requires an artificial inoculation with *Fusarium spp.* for uniform infestation. Furthermore, the genotype-by-environment interaction must be taken into account. FHB symptoms are usually measured in incidence which represents the percentage of symptomatic spikes, or severity (percentage of diseased spikelets) (Steiner et al. 2017). After harvesting, visual scoring or digital image analysis of kernels for FHB symptoms and measuring of the DON content provide other possibilities, as described in Maloney et al. (2014) and Dill-Macky (2003).

1.3.1.2 Marker Assisted Selection

Marker assisted selection (MAS) is based on patterns of molecular markers, which are either morphological, biochemical or genetic markers (Fernando et al. 2021). This strategy is only possible with already known resistance QTL, which are normally identified via QTL mapping studies. Mostly MAS is used for the stable major QTLs *Fhb1, Fhb2, Fhb3, Fhb4, Fhb5, Fhb7, Qfhs.ifa-5a* and *Qfhs.nau-2DL*. Used are either closely linked diagnostic markers or markers flanking the QTL region (Steiner et

al. 2017). Due to its time efficiency and the fact that major as well as minor QTLs can be pyramided, MAS is often preferred over other strategies (Haber et al. 2008). Though Brar et al. (2019) reported difficulties with reduced grain protein content, using MAS of “Sumai 3” prevails.

1.3.1.3 Genomic Selection

The process of genomic selection (GS) often has a higher prediction accuracy than MAS and in contrast to MAS it is also suitable for minor QTL (Steiner et al. 2017; Buerstmayr and Lemmens 2015). Based on the estimation of genome wide marker effects, genomic estimated breeding values are predicted. This can be done for many markers at once and phenotyping of the population it is not necessary. Thus time and costs can be saved and the gain by selection is increased (Steiner et al. 2017).

2 Research Questions

This thesis is based on three biparental populations. They were artificially inoculated to investigate important traits, playing a role in FHB resistance under natural conditions. The parent of particularly interest was the highly resistant Mexican line 6408-1, which was used in any of the two-way crosses. Traits of interest were plant height, anther retention, flowering date, awn character and the stem length regulating gene *Rht-B1* on chromosome 4B. This study uses data from the years 2019 and 2020 individually, as well as seeks to combine results from both years.

The thesis set out to investigate the following research questions:

- How does FHB severity differ between the populations? Is WSB x 6408-1 less susceptible, taking into account that that both parents are considered as resistant?
- Do retained anthers increase FHB disease symptoms and is this trait also correlated with plant height and flowering date in populations Remus x 6408-1 and Michael x 6408-1?
- Plant height is known to have an influence on FHB resistance, but what impact does it have on the crossing populations even though 6408-1 has the dwarfing allele *Rht-B1b*? Which correlations can be found between plant height, FHB severity, anther retention and flowering date?
- Does a positive correlation exist between early flowering plants and FHB resistance in such field trials? What impact does the flowering date have on the other traits?
- What ratio of the phenotype for FHB severity, anther retention, plant height and flowering date can be explained by the genotype?
- Awn character is not often included into research. Those few existing observations conflict with each other. Does the awn character have a significant impact on FHB severity, anther retention and plant height in these trials?
- How are FHB severity, anther retention and plant height influenced by alternative alleles of the *Rht-B1* locus?

3 Materials and Methods

This chapter provides information about plant material, experimental set up, data collection and statistical analysis. Data for analysis of 2019 were provided by Jakob Seereiter *(Masterthesis in preparation)*. For both years, the same location, experimental design and genotypes were used.

3.1 Plant Material

For the experiments conducted in 2019 and 2020 recombinant inbred lines (RILs) were used. Parent of interest was the spring wheat genotype number 6408-1 with the pedigree BAU/MILAN/CBRD and the CIMMYT breeding code: CMSS97M01333S-030M-81Y-010M-2M-0Y-0FGR-0Y-0FGR. It attracted positive attention in the 9th SRSN international CIMMYT nursery. Most of the highly FHB resistance sources trace to Chinese germplasm, such as the variety Sumai 3, whereas the resistance genes in 6408-1 might originate from South America. Although it is a short straw genotype, 6408-1 is highly resistant to FHB. To learn more about the genetic makeup of the FHB resistance line, 6408-1 was crossed with the two susceptible German spring wheat cultivars Remus and Michael. Another cross was made with the Chinese landrace Wang Shui Bai, which is also resistant but according to Jia et al. 2018 breeders have not managed to use it in their programs successfully. Three populations of crossing were made by Prof. Hermann Bürstmayr, and RILs were generated. 6408-1 was used as mother as well as father plant. In 2019 and 2020, a number of 1064 RILs of $F_{4:6}$ generation was used to examine the symptoms of FHB. In addition, the parents and the highly resistant control lines Sumai 3 and CM 82036 were sown and compared to the phenotypes and FHB infestations of the RILs.

3.1.1 Experimental Set Up

2020 was the second year where the experiment took place in Tulln an der Donau in Lower Austria. The place is located at latitude 48°3’1’’, longitude 16°0’7’’ and an altitude of 177m (Google Maps). Climate data of the UFT in Tulln close to the field trials was used (https://dnw-web.boku.ac.at/dnw/wetter_form_uft.php).

The average temperature of the year 2019 was 11.55°C with an average precipitation of 537.8mm/m^2; and in 2020 it was 11.01°C with 572.05mm/m^2. Between May 20 and June 20, where the flowering, the *Fusarium culmorum* inoculation and the FHB infection took place, the precipitation was 111.7 mm/m^2 in 2019 and 234.3 mm/m^2 in 2020. It should be noticed that with 606 mm/m² the year 2020 had, in general and during the time of interest, a higher precipitation than 2019. Whereas in 2019 the average temperature between May 20 and June 20 was about 3.04°C warmer than in 2020.

Furthermore, especially year 2020 is discussed. Details of 2019 can be found in the theses by Jakob Seereiter (*Masterthesis in preparation*).

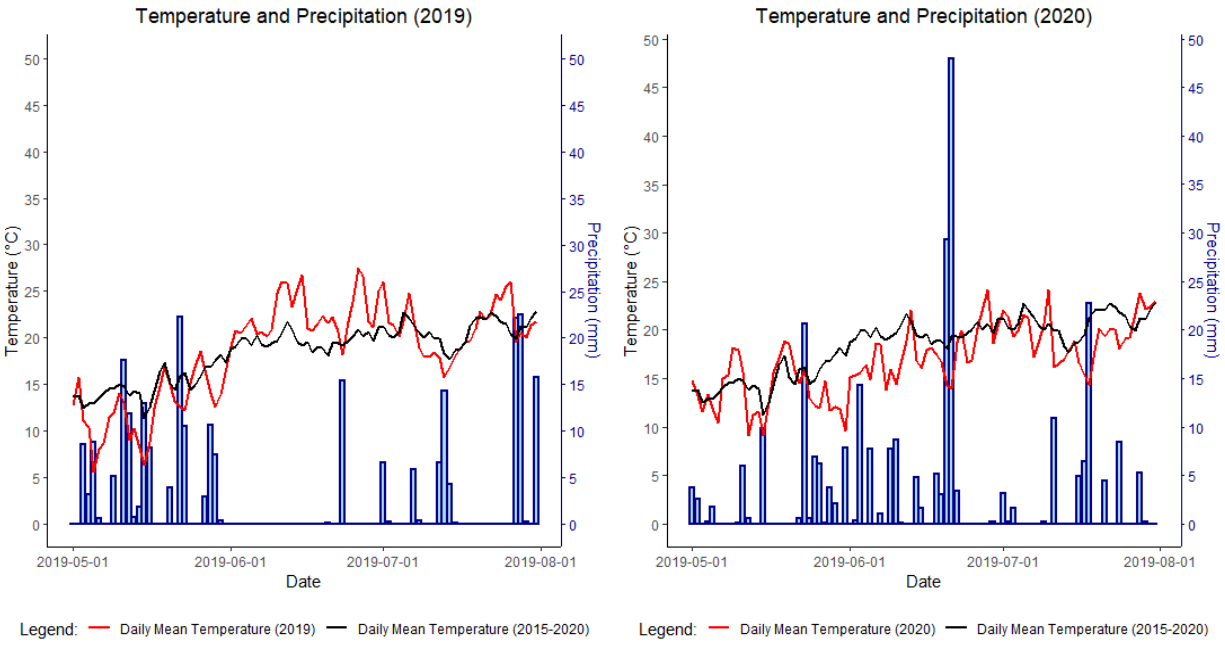

Figure 2: Daily mean temperature in °C from May 1 to July 31 2019 (left) and 2020 (right), represented by the red lines. The black line represents the daily mean temperature across six years (2015-2020). The histograms show the daily precipitation in mm for 2019 (left) and 2020 (right). Climate Data of the UFT in Tulln was used (https://dnw-web.boku.ac.at/dnw/wetter_form_uft.php).

The spring wheat was planted on the testing area of IFA Tulln as a randomized complete block design with two replications. In total, 1080 plots were sown. The preceding crop of the experiment in 2020 was soybean and the seeds were treated with Celest Trio (25 g/l Fludioxonil, 25 g/l Difenoconazol and 10 g/l Tebuconazol). For each genotype a double row plot was sown; 5 g seeds per plot were used. The first replication was sown on 27 February 2020 and the second on 5 March 2020. The plots had a length of 65 cm. The distance between the double rows was 17 cm. Within a plot, spacing between the rows was 33 cm. Between the different plots, spacing was 40 cm and 33cm.

Plant treatment started on 18 March 2020 with a nitrogen-phosphorus-potassium-fertilizer of Raiffeisen Ware Austria AG. 320 kg/ha containing 4.3 % of NO_3^-, 12.9 % of NH_4^+, 6% of P_2O_5 and 18 % of K_2O_5 where applied in addition with 7 % sulfur. On 30 April, the herbicide combination “Express SX + Pixxaro EX” of Kwizda Agro GmbH was applied in a concentration of 25 g/ha and 0.25 l/ha, on both replications. It contains the active ingredients Tribenuron-Methyl, Halauxifen-methyl and Fluroxpyr. 62 ml/ha of the insecticide “Decis forte” of Bayer Austria GmbH were also applied on the same day, containing Deltamethrin. A second fertilizer application took place on 8 May. 200 kg/ha of a calcium-ammonium-nitrate-fertilizer of Raiffeisen Ware Austria with an amount of 13.5 % NO_3^- and 13.5 % NH_4^- was applied. On 15 May 1 l/ha of the fungicide Sinstar of CERTIS EUROPE B. V. was applied, which contains Azoxystrobin.
On May 22 a mist irrigation system for constant humidity during the inoculation with *Fusarium culmorum* was established.

3.1.2 Inoculation with conidia of *Fusarium culmorum*

The inoculation with a suspension containing macroconidia of *F. culmorum* started on May 26 2020 when the first plot was recorded to flower. Spores were produced as described in Buerstmayr et al. 2000. The isolate of *Fusarium culmorum* 91015 for inoculum production was provided by Marc Lemmens. The inoculum had a conidia concentration of $2.5*10^4$ ml^{-1} and the treatment was carried out with backpack sprayers every second day in the late afternoon until the last plot finished flowering stage. For constant humidity and thereby resulting optimal conditions during the infection, the mist irrigation system was activated before spraying the inoculum. Duration of irrigation was 10 seconds, with a minimum break of 20 minutes. The mist irrigation was switched by leaf-wetness measurement and set to keep the wheat heads humid from 3pm of the inoculation day until midday of the following day. So on hot days, the system went on every 20 minutes, and once per hour during the night. In this way, 4-5 mm/m^2 water are administered per day.

Figure 3: Mist irrigation system in the spring wheat trial in 2020.

3.1.3 FHB Severity

Ten days after the flowering date, the recording of FHB severity started. For each plot the occurring symptoms were estimated in percentage according to the guideline of Mitt. Biol. Bundesanst. Land-Forstwirtsch. (2000), which is used at IFA-Tulln (see appendix). The assessment of the infection was done 10, 14, 18, 22, 26 and 30 days after the flowering date. Due to the ripening process, the score on day 30 was excluded from the analysis and only five scorings were taken into account for the statistical analysis.

3.1.4 Anther Retention

Anther retention was recorded 4-8 days after flowering. In 2020, from each plot 20 florets where opened and scored having retained anthers, when any anthers remained or were trapped between palea and lemma. In 2019, 21 florets were opened. The number of florets with at least one retained anther was counted and converted into percentage for statistical analysis.

Figure 4: A: Remained anther in a spring wheat floret in 2020. B: Counting system of retained anthers in 2020.

3.1.5 Plant Height

Plant height of each plot was measured in an interval of 5cm from the ground to the top of the heads (awns excluded).

3.1.6 Flowering Date

A plot was considered as flowering when 50% of its heads had started to flower. The data collection was carried out every second day. The first plot flowered on 26 May 2020, the last one on 15 June 2020. For statistical analysis, the date was afterwards converted into days after May 1.

3.1.7 Awn Character

To examine the correlation between awns FHB severity, anther retention and plant height respectively, the presence of awns was recorded for each plot. Rating was done for Remus x 6408-1 and Michael x 6408-1 in both years. Four different types of awn character were found in these populations. Beside the classical categories of awned and awnless spikes, genotypes having only very short awns were found and termed as “awnletted”. Some plots contained plants having awns as well as without awns and were therefore categorized as heterogeneous.

3.1.8 Molecular Marker Analysis

In 2019 molecular analysis with several markers were done for populations Remus x 6408-1 and Michael x 6408-1 by Jakob Seereiter (*Masterthesis in preparation)*. One locus of interest was *Rht-B1* on chromosome 4B. This gene is either expressed as the dwarfing allele *Rht-B1b* or the wild type allele *Rht-B1a*, having long straw. Parent 6408-1 is known to carry *Rht-b1b* and even though plant height is negatively correlated with FHB severity in many studies, 6408-1 is a highly resistant line. For the analysis markers for *Rht-B1a* and *Rht-B1b* of Ellis et al. (2002) were used.

Since *Rht-B1* was the only polymorphic marker which showed significant results in 2019, only this one is mentioned in the thesis further on.

3.2 Statistical analysis

For statistical analysis R Studio, Version 3.6.3, was used (R Core Team 2015).

3.2.1 Area Under the Disease Progress Curve (AUDPC)

The first five scorings of percentage of infected spikelets were used for the calculation of the area under the disease progressive curve (AUDPC). The account was done for the RILs, parents and control lines. For the calculation the formula by Buerstmayr et al. (2000) was used:

$$\text{AUDPC}= \sum_{i=1}^{n}\{[(y_i + y_{i-1})/2](x_i - x_{i-1})\} \quad (1)$$

y_i is the percentage of infected spikelets on day i, x_i presents the day of the i^{th} assessment (10, 14, 18, 22 and 26 days after the first inoculation) and n is the total number of recordings.

3.2.2 Best Linear Unbiased Estimates

For further analysis, Best Linear Unbiased Estimates (BLUES) were calculated for each recorded trait and genotype. This was done in R Studio with the breedR-package for the field data 2019 and 2020. Data from 2019 were contributed by Jakob Seereiter (*Masterthesis in preparation).* The calculation was done with the genotype and the variables of interest as fixed effects and the replication as random effect.

3.2.3 Repeatability, Heritability and Variance Components

The calculation of Repeatability and Heritability was done for 2019 and 2020 using the sommer-package of R (Covarrubias-Pazaran G. 2018). Calculation was done for FHB severity, plant height, anther retention and flowering date. For each trait following variance components were determined by using the restricted maximum likelihood (REML): V_G (Genotype), V_E (Environment = Year), V_{GE} (Genotype*Environment interaction), V_{error} (residual variance).

Variance components calculations for repeatability were done based on this model:

$$t_{ik} = \mu + g_i + b_k + e_{ik} \quad (2)$$

μ represents the overall mean and g_i is the i^{th} genotype. b_k refers to the k_{th} block and e_{ik} represents the error.

Repeatability for 2019 and 2020 was estimated with the formular:

$$H^2 = \frac{V_G}{V_G + \frac{V_E}{n_{rep}}} \quad (3)$$

For the variance components of the heritability both years were considered. Calculations were done based on the following model:

$$t_{ijk} = \mu + g_i + y_j + gy_{ij} + b_{jk} + e_{ijk} \quad (4)$$

with y_j for the j^{th} year, gy_{ij} for the genotype-environment-interaction and b_{jk} for the blocks in the individual years.

For the calculation of the broad sense heritability over both years the formular:

$$H^2 = \frac{V_G}{V_G + \frac{V_{GE}}{n_{year}} + \frac{V_{error}}{n_{rep} * n_{year}}} \quad (5)$$

was used, with n_{rep} being the number of replications in each year and n_{year} the number of years.

3.2.4 Least Significant Difference

The least significant difference was calculated with a significance level of α = 0.05, resulting in LSD5% values. They were calculated for all traits of interest for 2019, 2020 and across both years. T –values were calculated using the qt function in R (Becker et al. 1988; Johnson et al.). Following formular was used for the LSD5% values for individual years:

$$LSD5\% = t * \sqrt{2 * V_E / n_{rep}} \quad (6)$$

LSD5% across 2019 and 2020 was done with the formular:

$$LSD5\% = t * \sqrt{2 * (V_{GE}/n_{year} + V_{error}/(n_{year} * n_{rep}))} \quad (7)$$

3.2.5 Phenotypic Correlation

Phenotypic correlation was estimated, using the BLUEs for FHB severity (AUDPC), FHB severity of the 4th or 5th rating (%), anther retention, plant height and flowering date. To test for correlations, the rcorr function of the Hmisc package in R was used (Hollander and Wolfe 1973). Pearson's correlation was done, resulting in the correlation coefficient *r* and the p-value *p*. Analyses were done for 2019, 2020 and across both years.

3.2.6 Awn Character

To find out how much of the variation within FHB severity (AUDPC), anther retention and plant height can be explained by the awn character, multiple R-squared (R^2) were calculated. BLUEs across both years were used to run the lm function in R (Chambers 1992).

3.2.7 Marker-Trait Association

To find out how much of the variation within FHB severity (AUDPC), anther retention and plant height can be explained by the different alleles of *Rht-B1*, multiple R-squared (R^2) were calculated. BLUEs across both years were used to run the lm function in R (Chambers 1992).

4 Results

The following chapter contains results of 2019, 2020 and across both years. Only selected figures and tables are shown, while all additional results are available in the appendix.

4.1 General data structure

Population Remus x 6408-1 was the largest one with 198 genotypes. Michael x 6408-1 had 182 and Wang Shui Bai (WSB) 158 lines. FHB severity was measured, rating the percentage of infected spikelets in a spike.

Based on this visual scoring, AUDPC values were calculated. In 2019 score 1-4 and in 2020 1-5 was used for the calculation of AUDPC. Across both years, score 1-4 was used. Figure 5-7 show the data distribution of the BLUEs for Populations Remus x 6408-1, Michael x 6408-1 and WSB x 6408-1, including parents and control lines across both years. Histograms for individual years can be found in the appendix.

Table 1 and 2 show the values of the BLUEs for the parents Remus, Michael, WSB and 6408-1. They further give an overview of the raw data, showing minimum, maximum and mean for all populations for each individual year and across all years. In addition, the tables show the LSD5% values for each trait, year and population of the BLUEs.

With an AUDPC of 31 parent 6408-1 had the lowest disease level across both years, followed by WSB with 44. Remus and Michael had clearly higher infection scores with 599 and 488. In 2019 and 2020, 6408-1 had the lowest AUDPC (see table 1). The most infected parent in 2019 was Remus (940) and in 2020 it was Michael (668). Across 2019 and 2020 and in each individual year, population Michael x 6408-1 showed the highest infestation rate, population WSB x 6408-1 the lowest (see table 1 and 2). Assessing the histograms of FHB severity, plants showed all nuances from susceptible to resistant. Some genotypes even had lower disease levels than the resistant parents. Data for all populations are skewed to the right, especially for the population WSB x 6408-1. Across 2019 and 2020, 32 lines of population Remus x 6408-1 showed an ADUPC ≤ 100, having a LSD5% of 251.80. The histogram of population Michael x 6408-1 has its peak between 100 and 200 with 56 lines (LSD5% = 288.52). In population WSB x 6408-1 even 79 genotypes had an AUDPC ≤ 100, with a LSD5% of 194.19, suggesting rather resistant genotypes (see figure 5-7 and table 1 and 2).

Anther retention was only recorded for populations Remus x 6408-1 and Michael x 6408-1 in both years. Values ranged from zero retained anthers to 100%. In general in 2019, plants had more retained anthers than in 2020, except for parent 6408-1. Across both years, population Michael x 6408-1 showed with an average of 65% more retained anthers than Remus x 6408-1 with 53% (see table 1 and 2). LSD5% was 23.99 for Michael x 6408-1 and 29.66 for Remus x 6408-1.

Plant height ranged from 60cm to 125cm. Regarding both years, the tallest genotypes were found in population WSB x 6408-1 with a mean of 95cm, followed by Michael x 6408-1 with a mean of 93cm. The shortest lines contained Remus x 6408-1 with a mean of 85cm. In general, plants were higher in 2019 than in 2020 and LSD5% values ranged from 6.17 to 9.22 (see table 1 and 2). Data shows a nearly normal distribution for plant height (see figure 5-7). In 2019, 39 out of 198 lines of population Remus x 6408-1 had a height of 80cm to 85cm, whereas the peak in 2020 was between

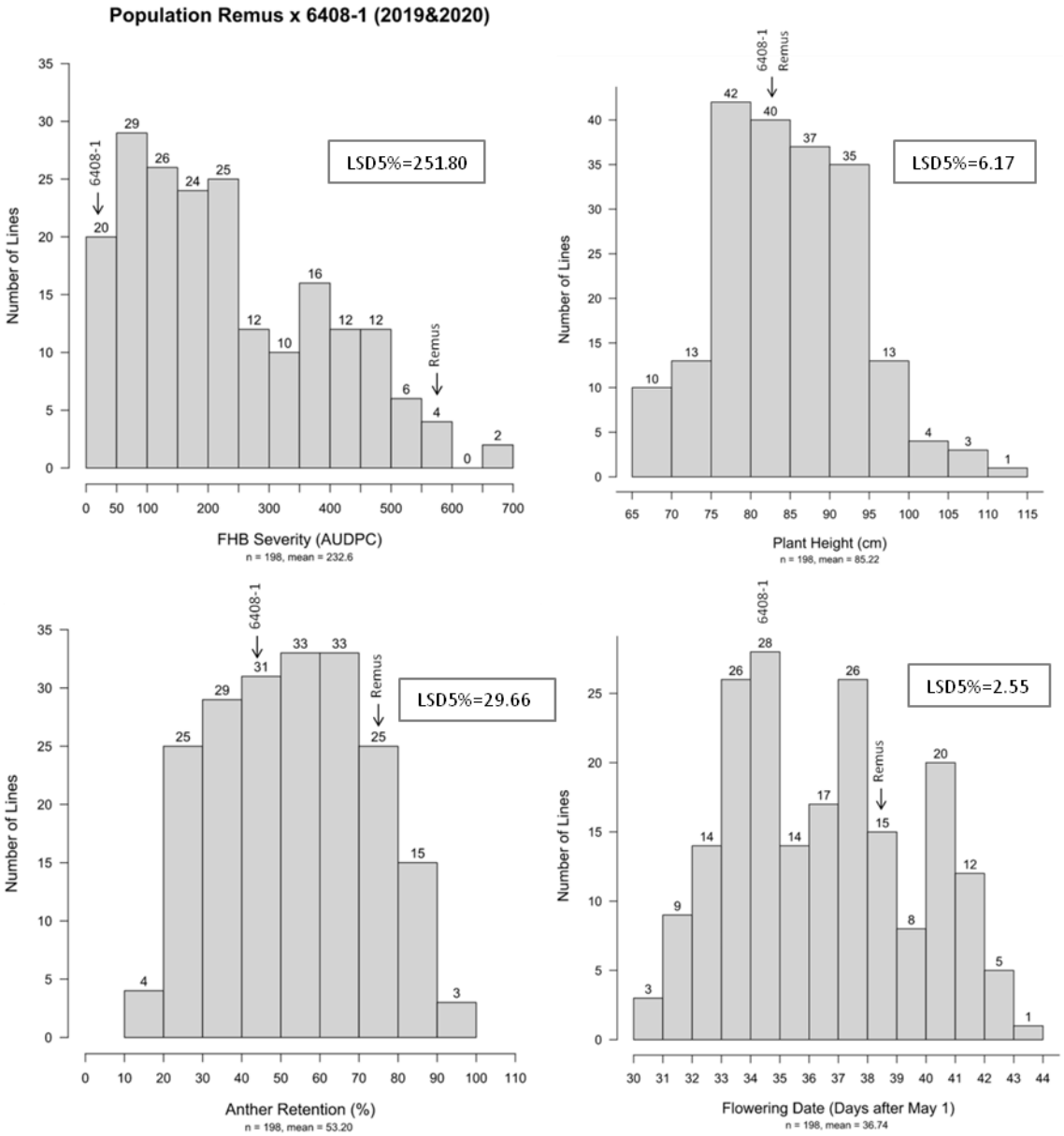

Figure 5: Histograms showing the data distribution of the BLUEs for Population Remus x 6408-1 for FHB Severity (AUDPC), Plant Height (cm), Anther Retention (%) and Flowering Date (Days after May 1) and the LSD5% value across 2019 and 2020.

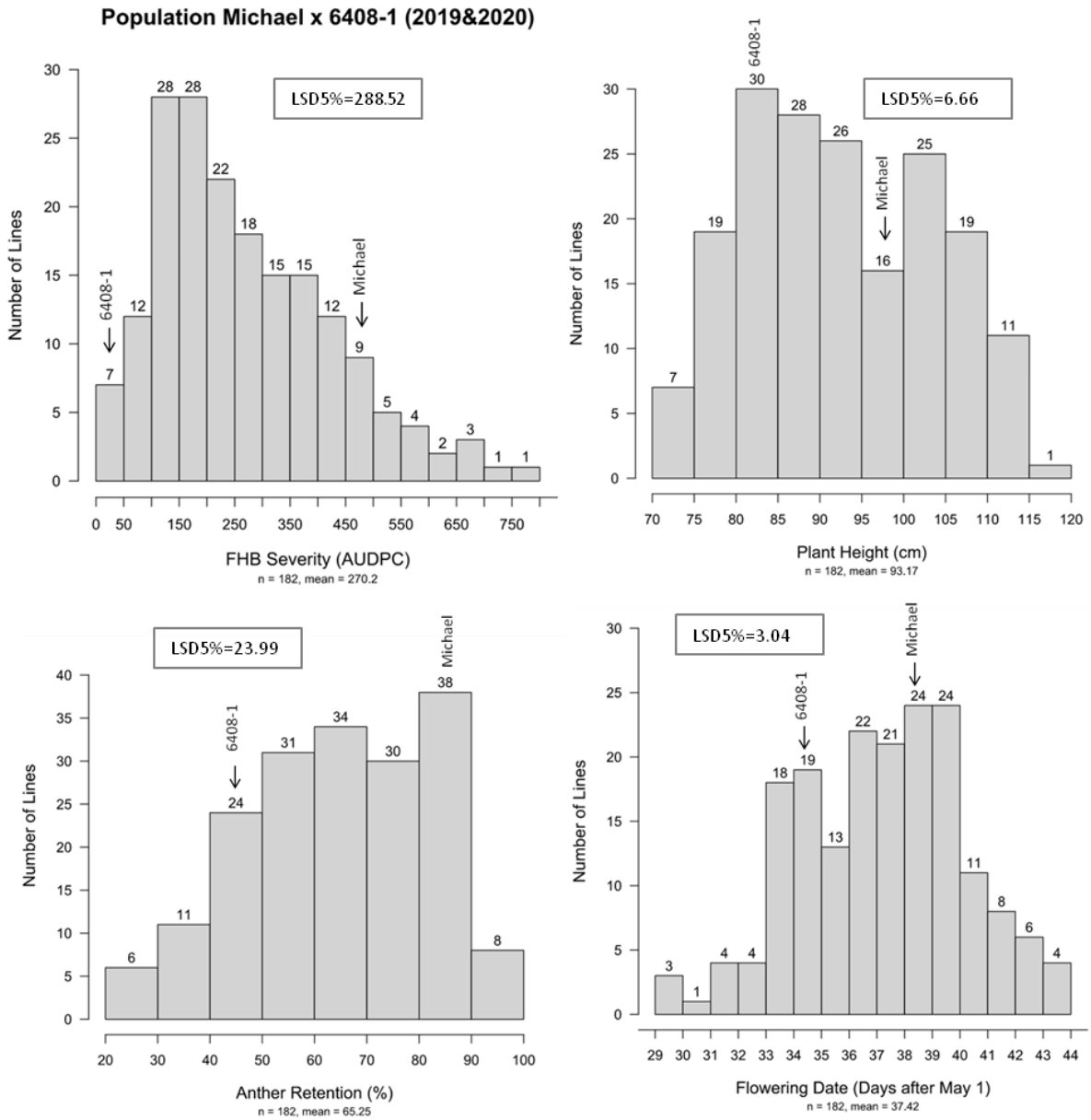

Figure 6: Histograms showing the data distribution of the BLUEs for Population Michael x 6408-1 for FHB Severity (AUDPC), Plant Height (cm), Anther Retention (%) and Flowering Date (Days after May 1) and the LSD5% value across 2019 and 2020.

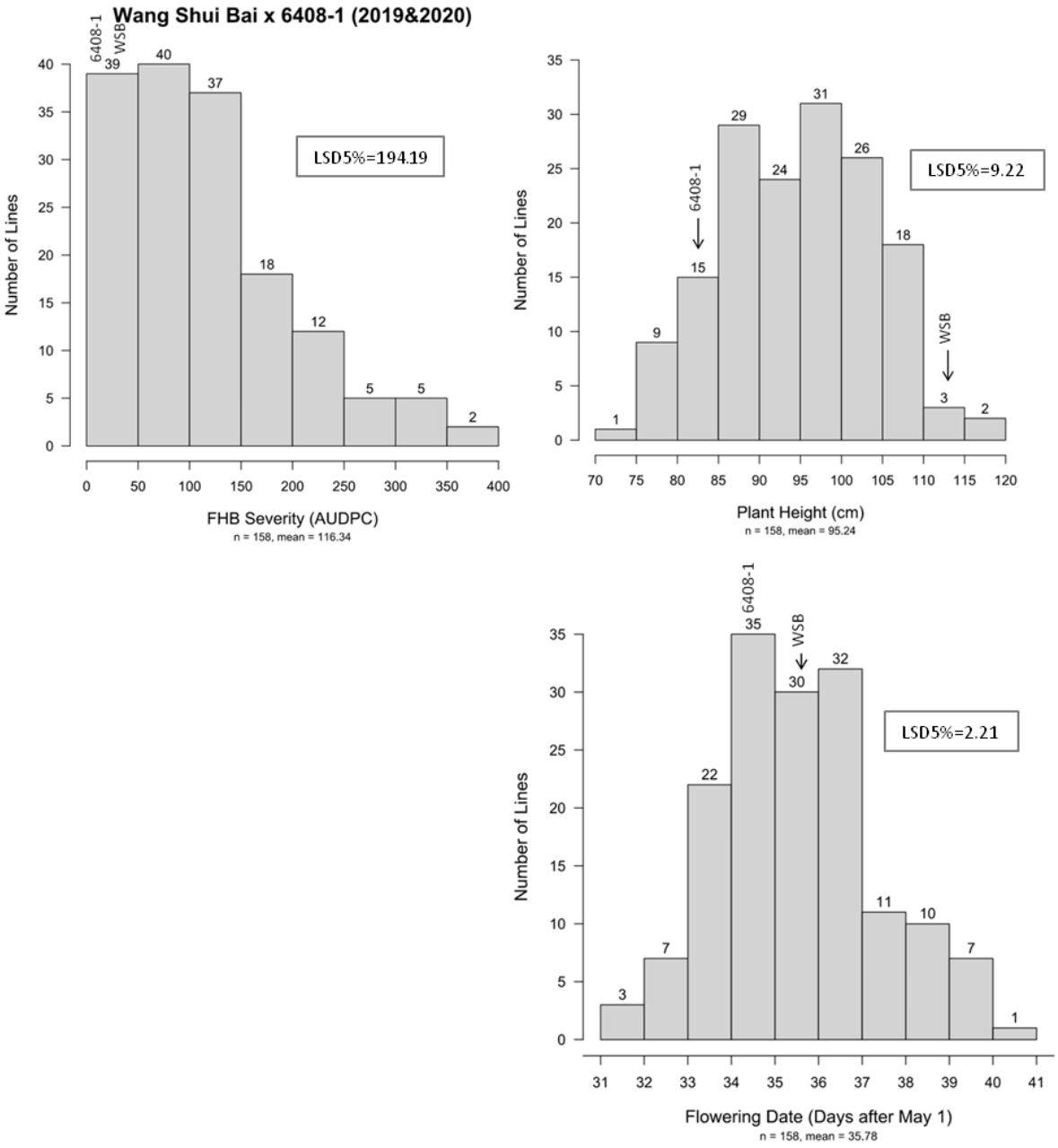

Figure 7:Histograms showing the data distribution of the BLUEs for Population Wang Shui Bai x 6408-1 for FHB Severity (AUDPC), Plant Height (cm) and Flowering Date (Days after May 1) and the LSD5% value across 2019 and 2020.

Table 1: Values for FHB Severity (Area under the Disease Progress Curve), Anther Retention (%), Plant Height (cm) and Flowering Date (days after May 1) of the parents Remus, Michael, Wang Shui Bai and 6408-1 in 2019, 2020 and across both years. Minimum, Maximum, Means (raw data) and LSD5% (Least Significant Difference) for individual years and across both years for the FHB Severity (Area under the Disease Progress Curve), Anther Retention (%), Plant Height (cm) and Flowering Date (days after May 1) of population Remus x 6408-1.

	Year	Parents				Remus x 6408-1			
		Remus	Michael	Wang Shui Bai	6408-1	Min	Max	Mean	LSD5%
FHB Severity (AUDPC)[1]	2019	940	598.00	29.7	24.55	6.8	1060.0	333.4	233.57
	2020	507.50	667.50	177.55	112.70	32.00	855.00	274.70	157.08
	Overall Mean	598.75	487.75	43.63	31.13	6.40	1060.00	232.30	251.80
Anther Retention (%)[2]	2019	90.50	97.50		37.00	0.00	100.00	58.83	25.98
	2020	62.50	72.50		51.25	0.00	100.00	47.51	27.46
	Overall Mean	76.50	85.00		44.13	0.00	100.00	53.24	29.66
Plant Height (cm)	2019	87.50	97.50	120.00	81.25	65.00	120.00	88.44	6.49
	2020	80.00	97.50	105.00	80.00	60.00	115.00	81.97	8.10
	Overall Mean	83.75	97.50	112.50	80.63	60.00	120.00	85.21	6.17
Flowering Date (Days after May 1st)	2019	39.00	38.00	38.00	36.00	31.00	45.00	38.41	1.77
	2020	37.00	39.00	32.00	33.00	27.00	45.00	35.37	2.72
	Overall Mean	38.00	38.50	35.00	34.50	27.00	45.00	36.76	2.55

[1]AUDPC was calculated with the 4th rating for 2019 and with the 5th rating for 2020. For the overall mean the 4th rating was taken.

[2]Data for anther retention was not assessed for the Wang Shui Bai Population.

Table 2: Minimum, Maximum, Means and LSD5% (Least Significant Difference) for individual years and across both years for the FHB Severity (Area under the Disease Progress Curve), Anther Retention (%), Plant Height (cm) and Flowering Date (days after May 1) of populations Michael x 6408-1 and Wang Shui Bai x 6408-1.

	Year	Michael x 6408-1				Wang Shui Bai x 6408-1			
		Min	**Max**	**Mean**	**LSD5%**	**Min**	**Max**	**Mean**	**LSD5%**
FHB Severity (AUDPC)[1]	2019	10.40	1170.00	357.10	238.24	0.60	695.00	144.39	238.22
	2020	42.40	1135.00	353.50	190.90	4.00	560.00	221.20	145.40
	Overall Mean	10.40	1170.00	271.00	288.52	0.60	695.00	116.68	194.19
Anther Retention (%)[2]	2019	10.00	100.00	72.49	21.79				
	2020	10.00	100.00	57.50	25.24				
	Overall Mean	10.00	100.00	65.11	23.99				
Plant Height (cm)	2019	70.00	125.00	95.00	7.44	70.00	125.00	99.35	13.81
	2020	65.00	120.00	91.54	7.22	60.00	120.00	90.91	9.39
	Overall Mean	65.00	125.00	93.14	6.66	60.00	125.00	95.13	9.22
Flowering Date (Days after May 1st)	2019	31.00	45.00	38.41	1.72	31.00	43.00	37.55	1.97
	2020	25.00	45.00	36.48	2.45	27.00	41.00	34.02	2.80
	Overall Mean	25.00	45.00	37.44	3.04	27.00	43.00	35.79	2.21

[1]AUDPC was calculated with the 4th rating for 2019 and with the 5th rating for 2020. For the overall mean the 4th rating was taken.
[2]Data for anther retention was not assessed for the Wang Shui Bai Population.

75cm and 80cm, with 51 genotypes. Plant height showed similar distribution in the other populations (see appendix).
Flowering date occurred between May 26 and June 15 and started earlier in 2020 than in 2019. Over all years, plants of WSB x 6408-1 flowered first (mean =36 days after May 1), followed by Remus x 6408-1 (mean = 37 days after May 1) and Michael x 6408-1 (mean = 37 days after May 1). This is confirmed, taking a closer look at the histograms across 2019 and 2020 (see figure 5-7). 89% of the genotypes of WSB x 5608-1 were recorded as flowering before June 8. At this time, only 69% of Remus x 6408-1 and 58% of Michael x 6408-1 were considered as flowering. Parents 6408-1 and WSB started before Remus and Michael. LSD5% vales were 2.21 for population WSB x 6408-1, 2.55 for Remus x 6408-1 and 3.04 for Michael x 6408-1.

4.2 Repeatability, Heritability and Variance Components

For population Remus x 6408-1, the genotype effect ($\sigma^2_{Genotype}$) accounted for the largest variation in AUDPC (see table 3). The year effect was the smallest with σ^2_{Year}=168, followed by the error variance σ^2_{error}=10219. Heritability was with a bit smaller than repeatability in individual years. In contrary the year had no effect at all in population Michael x 6408-1, with σ^2_{Year}=0. Nevertheless, the largest effect resulted from the genotype ($\sigma^2_{Genotype}$=22741). Repeatability and heritability showed similar values as in population Remus x 6408-1 (see table 3). For WSB x 6408-1 the year effect emerged relatively high with σ^2_{Year}=2777. Across 2019 and 2020, the genotype-environment interaction had the largest effect with $\sigma^2_{Genotype\ x\ Year}$=6914. Heritability in this population was lowest (H^2=0.46).
Performance of variance components for anther retention was similar to those of AUDPC. In every individual year and across all years, the genotype effect had the largest impact in both populations (see table 3). Across both years genotype effect in Remus x 6408-1 was with $\sigma^2_{Genotype}$=256 a bit larger than in Michael x 6408-1 ($\sigma^2_{Genotype}$=247). The effect of the year was the smallest. Data for anther retention was not assessed for WSB x 6408-1. Compared to results for heritability of the other traits, values were relatively low for both populations, with H^2= 0.70 for Remus x 6408-1 and H^2=0.77 for Michael x 6408-1.
For population Remus x 6408-1 and Michael x 6408-1 repeatability and heritability for plant height were high (≥0.89). Population WSB x 6408-1 showed smaller values (see table 4). In all three populations, genotype effect was the largest one, especially in Michael x 6408-1 with $\sigma^2_{Genotype}$=120. Smallest effect in all populations had the genotype x environment interaction (see table 3 and 4).
With $\sigma^2_{Genotype}$=9, the genotype effect for flowering date clearly had the largest effect in Remus x 6408-1 across 2019 and 2020. The smallest effect had the genotype x environment interaction ($\sigma^2_{Genotype\ x\ year}$=1). Variance components of population Michael x 6408-1 performed similar (see table 3) but in WSB x 6408-1 the year had the largest impact (σ^2_{Year}=6). Heritability was highest in Remus x 6408-1 and lowest in WSB x 6408-1 (H^2= 0.91 and H^2= 0.81).

Table 3: Estimates of the BLUEs for the variance components year (σ^2_{Year}), genotype ($\sigma^2_{Genotype}$), genotype-year interaction ($\sigma^2_{GenotypexYear}$), and the residual effects (σ^2_{year}) for individual years and across all years for FHB Severity (Area under the Disease Progress Curve), Anther Retention (%), Plant Height (cm) and Flowering Date (days after May 1) of populations Remus x 6408-1 and Michael x 6408-1.

	Year	Remus x 6408-1					Michael x 6408-1				
		H^2	σ'^2_{Year}	$\sigma'^2_{Genotype}$	$\sigma'^2_{GenotypexYear}$	σ'^2_{error}	H^2	σ'^2_{Year}	$\sigma'^2_{Genotype}$	$\sigma'^2_{GenotypexYear}$	σ'^2_{error}
FHB Severity (AUDPC)[1]	2019	0.88*		52400.70		14150.94	0.87*		48517.32		14725.19
	2020	0.86*		18947.63		6400.68	0.86*		28212.1		9454.13
	Overall Mean	0.75	168.18	24306.42	11366.89	10219.03	0.68	0	22740.81	15607.31	12050.96
Anther Retention (%)[2]	2019	0.87*		580.08		175.04	0.86*		366.15		123.22
	2020	0.66*		197.32		195.65	0.77*		282.87		165.23
	Overall Mean	0.69	51.00	252.76	135.70	185.83	0.77	93.76	247.08	77.23	144.65
Plant Height (cm)	2019	0.94*		86.56		10.92	0.95*		150.70		14.37
	2020	0.89*		66.79		17.04	0.94*		99.01		13.53
	Overall Mean	0.94	20.74	73.77	2.89	14.01	0.95	4.71	120.31	4.54	13.95
Flowering Date (Days after May 1st)	2019	0.94*		5.88		0.81	0.92*		4.43		0.77
	2020	0.94*		13.94		1.92	0.95*		14.80		1.56
	Overall Mean	0.91	2.98	8.90	1.01	1.36	0.87	1.37	7.79	1.82	1.17

[1]AUDPC was calculated with the 4th rating for 2019 and with the 5th rating for 2020. For the overall mean the 4th rating was taken.

[2]Data for anther retention was not assessed for the Wang Shui Bai Population.

*Repeatability

Table 4: Estimates of the BLUEs for the variance components year (σ^2_{Year}), genotype ($\sigma^2_{Genotype}$), genotype-year interaction ($\sigma^2_{GenotypexYear}$), and the residual effects (σ^2_{year}) for individual years and across all years for FHB Severity (Area under the Disease Progress Curve), Anther Retention (%), Plant Height (cm) and Flowering Date (days after May 1) of population Wang Shui Bai x 6408-1.

	Year	**Wang Shui Bai x 6408-1**				
		H^2	**σ^2_{Year}**	**$\sigma^2_{Genotype}$**	**$\sigma^2_{GenotypexYear}$**	**σ^2_{error}**
FHB Severity (AUDPC)[1]	2019	0.83*		15185.81		6069.03
	2020	0.72*		7149.81		5485.60
	Overall Mean	0.46	2777.22	4204.97	6913.59	5780.33
Anther Retention (%)[2]	2019					
	2020					
	Overall Mean					
Plant Height (cm)	2019	0.78*		89.34		49.46
	2020	0.85*		66.30		22.90
	Overall Mean	0.87	16.65	73.94	4.03	36.18
Flowering Date (Days after May 1st)	2019	0.78*		1.77		1.01
	2020	0.82*		4.65		2.03
	Overall Mean	0.81	5.69	2.70	0.51	1.52

[1]AUDPC was calculated with the 4th rating for 2019 and with the 5th rating for 2020. For the overall mean the 4th rating was taken.

[2]Data for anther retention was not assessed for the Wang Shui Bai Population.

*Repeatability

4.3 Phenotypic Correlations

Table 5 shows the pearson correlation coefficients (r) across 2019 and 2020 for all populations. Trait correlations for individual years can be found in the appendix. Scatterplots with histograms for each trait and population showing the relationship of FHB severity (ADUPC), anther retention, plant height and flowering date respectively of the BLUEs, is given in Figure 8, 9 and 10.

In general, phenotypic correlation varied a lot between 2019 and 2020. However AUDPC and FHB severity was well correlated in each individual and also across both years, for all populations. WSB x 6408-1 showed the highest correlation with r=0.94, over all years.

In 2019 anther retention was positively correlated to a relatively high degree with AUDPC in population Remus x 6408-1 and Michael x 6408-1 (see table 5). In 2020 values were significantly lower (see appendix). Across 2019 and 2020, trait correlation was similar in Remus x 6408-1 (r=0.66) and Michael x 6408-1

(r=0.63). The correlation of AUDPC and anther retention for both populations can also be seen in figure 8. Anther retention was not assessed for WSB x 6408-1.

Plant height was negatively correlated to all other traits in 2019 and in all populations (see appendix). Values ranged from -0.53 to -0.25. In 2020, significant correlation for plant height was only found with AUDPC and anther retention, respectively (see appendix). In population Remus x 6408-1 plant height and AUDPC were to 32%, in Micheal x 6408-1 and WSB x 6408-1 to 43% negatively correlated, across both years. Correlation between anther retention and plant height was not significant in population Michael x 6408-1 (see table 5). The correlation of AUDPC and plant height for all populations can be seen in figure 9.

Positive correlations for flowering date were higher in 2020 than in 2019, including some non significant values (see appendix). Across both years, flowering date was highest correlated with AUDPC in Remus x 6408-1 with r=0.63. In Michael x 6408-1 those traits correlated to 47% and in WSB x 6408-1 to 38%. Especially low was the value between flowering date and anther retention in population Michael x 6408-1 (r=0.16). In WSB x 6408-1 flowering date and plant height were not significantly correlated (see table 5). The correlation of AUDPC and flowering date for all populations can also be seen in figure 10.

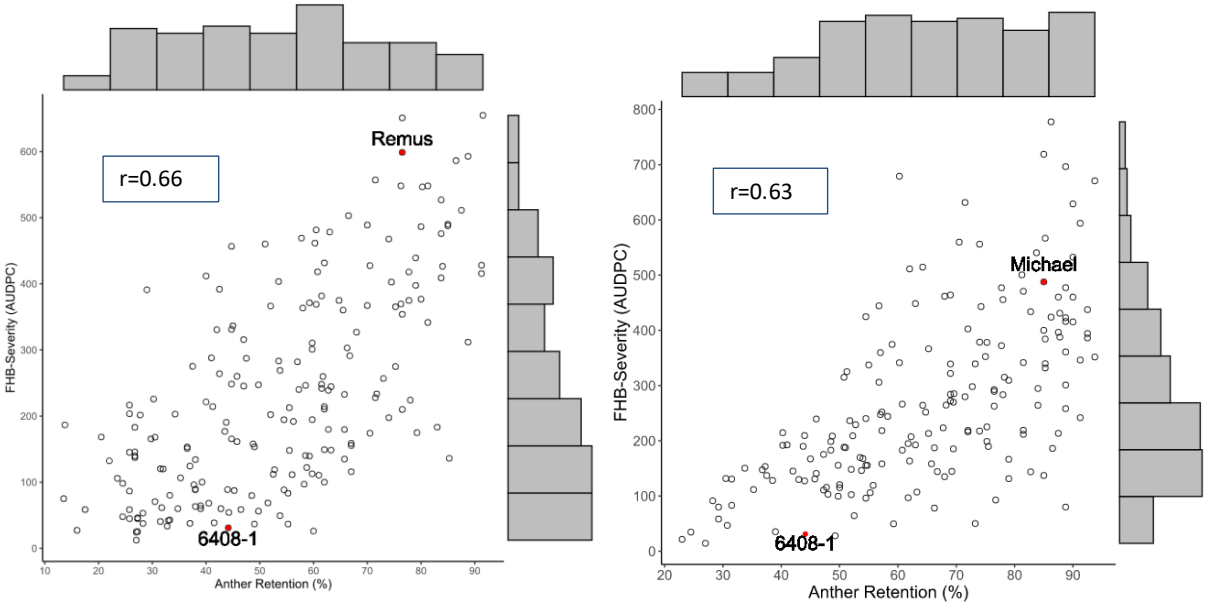

Figure 8: Scatterplots with histograms showing the relationship of the BLUEs across 2019 and 2020 between FHB Severity (AUDPC) and Anther Retention for population Remus x 6408-1 and Michael x 6408-1.
Pearson correlation coefficients are given with r.

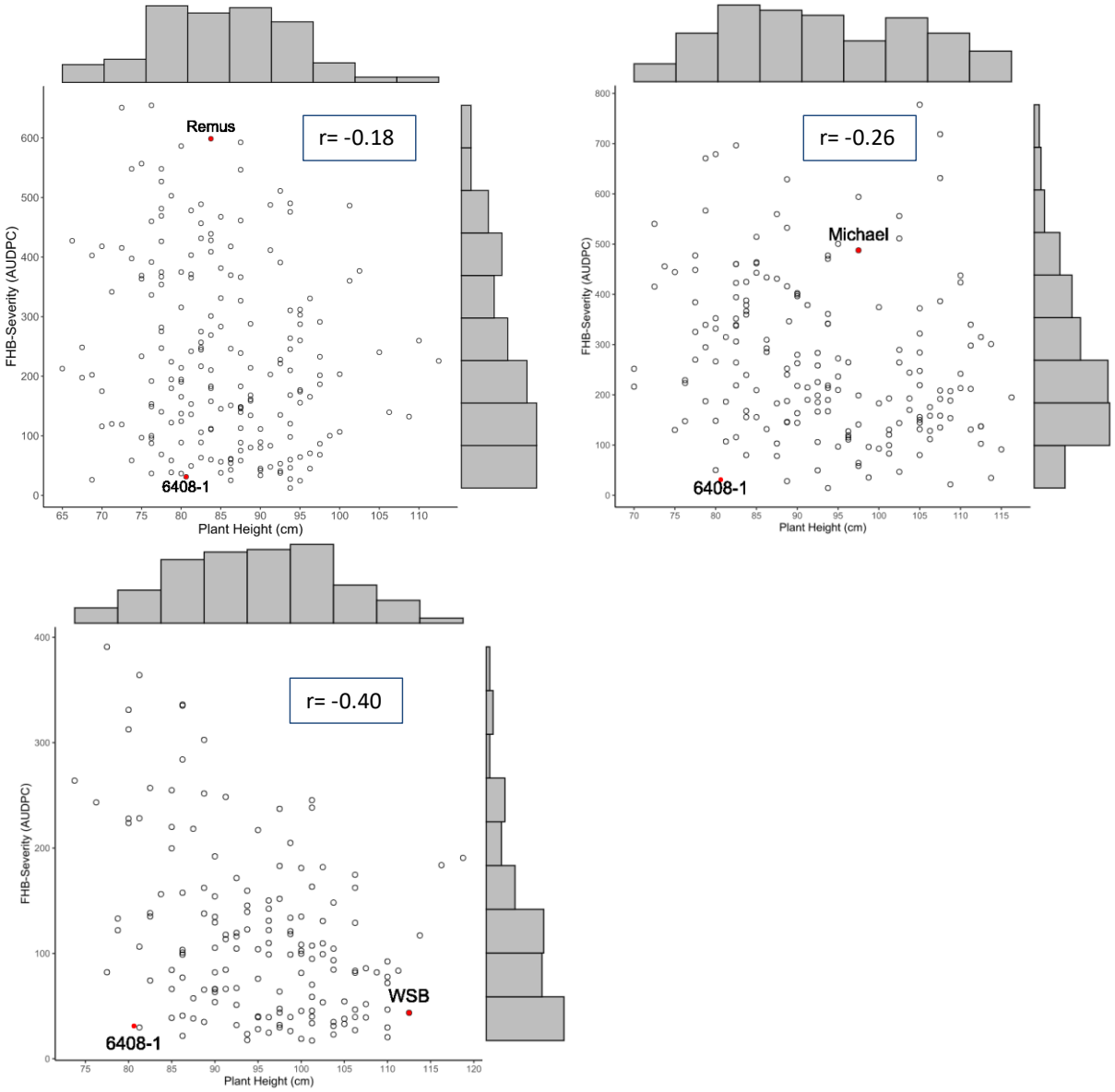

Figure 9: Scatterplots with histograms showing the relationship of the BLUEs across 2019 and 2020 between FHB Severity (AUDPC) and Plant Height for population Remus x 6408-1, Michael x 6408-1 and WSB x 6408-1. Pearson correlation coefficients are given with r.

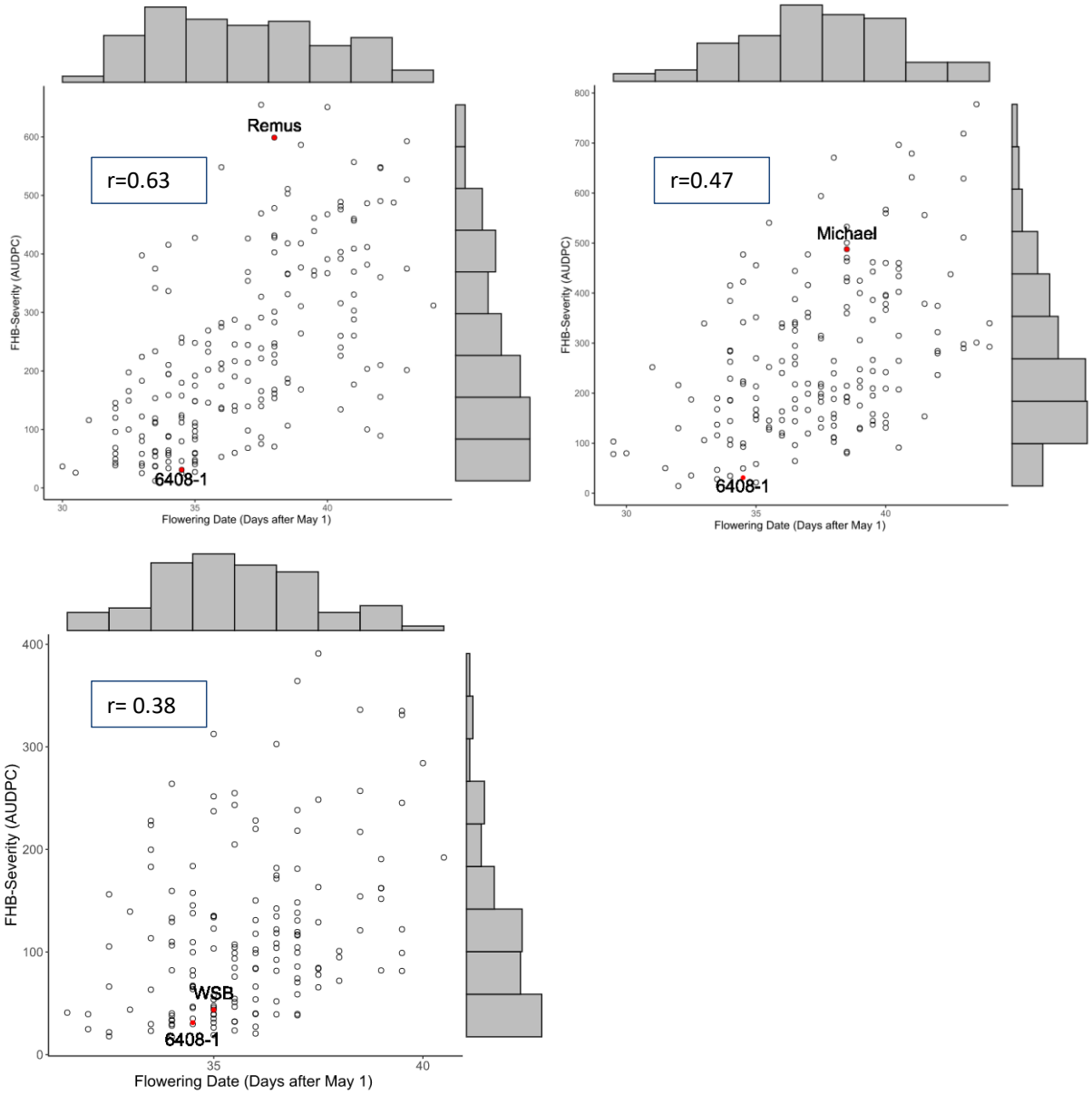

Figure 10: Scatterplots with histograms showing the relationship of the BLUEs across 2019 and 2020 between FHB Severity (AUDPC) and Plant Height and Flowering Date respectively for population WSB x 6408-1.
Pearson correlation coefficients are given with r.

Table 5: Trait correlations of the BLUEs for FHB Severity (AUDPC), FHB Severity of the 4th rating (FHB-22 in %), Anther Retention (%), Plant Height (cm) and Flowering Date (days after May 1) across 2019 and 2020 for population Remus x 6408-1, Michael x 6408-1 and WSB x 6408-1. P-values are given with * and **. "n.s." means that no significant correlation was found.

Correlation Coefficients (2019 and 2020)

Remus x 6408-1

	FHB-22 (%)	AUDPC	Anther Retention (%)	Plant Height (cm)
AUDPC	0.90**			
Anther Retention	0.63**	0.66**		
Plant Height (cm)	-0.21*	-0.18*	-0.28**	
Flowering Date[a]	0.53**	0.63**	0.24**	0.35**

Michael x 6408-1

	FHB-22 (%)	AUDPC	Anther Retention (%)	Plant Height (cm)
AUDPC	0.91**			
Anther Retention	0.66**	0.63**		
Plant Height (cm)	-0.35**	-0.26**	-0.21*	
Flowering Date[a]	0.36**	0.47**	0.16*	0.38**

Wang Shui Bai x 6408-1

	FHB-22 (%)	AUDPC	Plant Height (cm)
AUDPC	0.94**		
Plant Height (cm)	-0.40**	-0.40**	
Flowering Date[a]	0.25**	0.38**	n.s.

*p<0.05
**p<0.001
[a]Days after May 1

4.4 Awn Character

Figure 9 gives an overview of the influence of the awn character on FHB severity (A), anther retention (B) and plant height (C). Boxplots of the BLUEs across 2019 and 2020 show the distribution of plants with awns, awnless, heterogeneous and awnletted plants for population Remus x 6408-1 and Michael x 6408-1. For each population and trait, p-value and multiple R-squared (R^2) are given; "n" represents the number of genotypes of each boxplot.

Looking at FHB severity (figure 9A) for population Remus x 6408-1, awnletted plants certainly were more infected then the other groups. Values ranged from 275 to 548, with the median at an AUDPC of 403. However, only 11 genotypes were classified as awnletted in this population.

Awned genotypes showed the lowest infestation rate, including 126 lines with a few outliers. The least infested genotype had an AUDPC of 12. Most genotypes had an AUDPC < 279, the median was also the lowest at 157. Around 13% of the FHB severity could be explained by the awn character (R^2=0.1253). Although in general awnletted lines were also more infected in population Michael x 6408-1, differences were not as clear as in Remus x 6408-1 and with p=0.1463, results were not significant (see Figure 9A). Only 3% of the variation within the infestation rate could be explained by the awn character (R^2=0.02966). In both populations genotypes with awns predominated.

Regarding the anthers, awnletted plants had more retained anthers, with the median being at 73% in population Remus x 6408-1 (figure 9B). Values ranged from 14% to 91%. Plants with least retained anthers were heterogeneous. Half of the genotypes belonging to this group had < 40% retained anthers. Groups showed significant differences (p<0.001) and around 11% of the variation in anther retention could be explained by the awns character (R^2=0.1076). In population Michael x 6408-1 differences were again not as strong as in Remus x 6408-1. However, with p=0.02173 they were significant. In general, plants showed a higher percentage of retained anthers compared to Remus x 6408-1. Awnless and awnletted plants showed similar infestation rates. The median for awnletted genotypes was a bit higher (74% vs 73%). In comparison, awned and heterogeneous lines showed rather low values. The group of heterogeneous plants had the lowest median with 60% retained anthers. With R^2=0.05266, only 5% of the variation could be explained by the awn character.

Differences in plant height were not significant in population Remus x 6408-1 (p=0.1751) and barely 3% could be explained by the awn character (R^2=0.02516). However, awnletted plants seemed to be shortest and lines without awns highest (see figure 9C). Population Michael x 6408-1 showed significant results but only 8% of differences in plant height could be explained by awn character. Also in this population, awnletted plants were the smallest, with 50% being shorter than 88cm. Highest genotypes contained the group of heterogeneous plants with the median at 103cm.

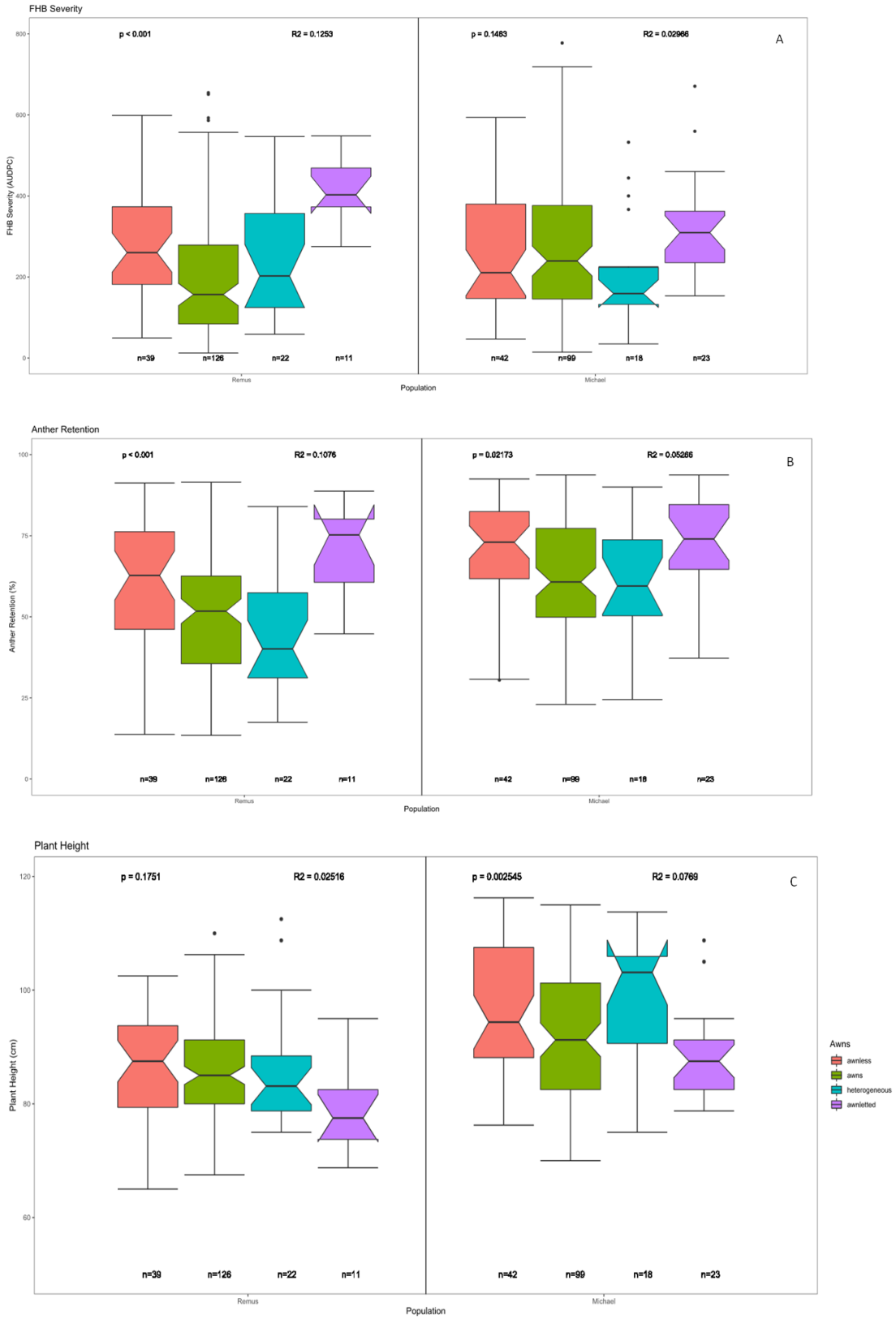

Figure 11: Boxplots showing the different awn characters (awnless, awns, heterogeneous and awnletted) of the BLUEs across 2019 and 2020. Relationships between awn character and FHB Severity (A), Anther Retention (B) and Plant Height (C) are given, with "n" being the number of genotypes of population Remus x 6408-1 and Michael x 6408-1. P-value and multiple R-squared (R2) are above the boxplots.

4.5 Marker-Trait Association

Figure 10 shows the influence of the *Rht-B1* gene on FHB severity (A), anther retention (B) and plant height (C). Boxplots of the BLUEs across 2019 and 2020 show the distribution of plants having the dwarfing allele *Rht-B1b* and the wild type allele *Rht-B1a* for population Remus x 6408-1 and Michael x 6408-1. For each population and trait, p-values and multiple R-squared (R^2) are given. "n" represents the number of genotypes of each boxplot. Results were significant for all traits and populations. Molecular marker analysis was not done for population WSB x 6408-1.

In both populations FHB severity was clearly lower in genotypes having the wild type allele, with some outliers (see figure 10A). 75% of the lines had an AUDPC < 231 in Remus x 6408-1. Lowest value was at 12. Genotypes carrying the *Rht-B1b* allele had the median at 275, R^2 was 0.1324. In population Michael x 6408-1 the median for plants with the wild type allele was at 192. For the dwarfing allele it was at 325. Only around 8% of the phenotypic variation were explained by this gene (R^2=0.08409).

Regarding anther retention, dwarfing allele carrying genotypes had more retained anthers in both populations (see figure 10B). With the median at 40% retained anthers, the group with *Rht-B1a* is clearly lower than the group with *Rht-B1b* (61%), in Remus x 6408-1. In population Michael x 6408-1, genotypes with the wild type allele had the median similar to the dwarfing type group of Remus x 6408-1 at 62%. Half of the dwarfing allele carrying lines had more than 72% retained anthers. Concerning the multiple R-squared, populations differed a lot. 20% of the variation were explained by the gene expression in population Remus x 6408-1, whereas only 3% in population Michael x 6408-1.

Genotypes having the wild type allele were taller in both populations. In general, population Michael x 6408-1 contained higher plants. Heights of lines with Rht-B1a were between 73cm and 113cm in Remus x 6408-1 and between 83cm and 116cm in Michael x 6408-1. 75% of genotypes with *Rht-B1b* were < 83cm in Remus x 6408-1 and <87cm in Michael x 6408-1. Both populations had some outliers. This relation can also be observed, by looking at the multiple R-squared. 43% of the phenotypic variation in population Remus x 6408-1 were explained by the alleles; in Michael x 6408-1 it was even 63%.

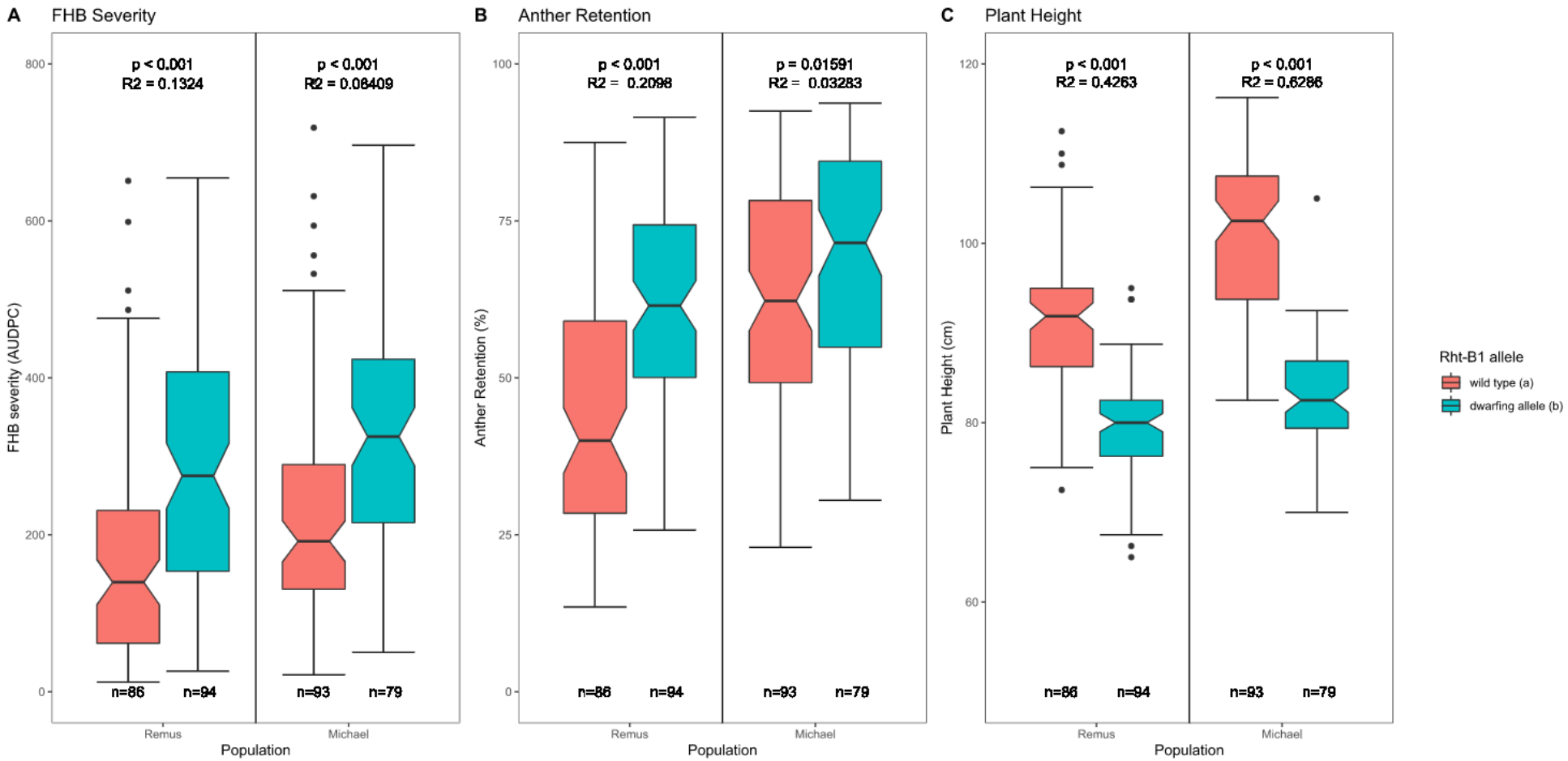

Figure 12: Boxplots Boxplots showing two different expressions of the *Rht-B1* gene (dwarfing and wild type allele) for the BLUEs across 2019 and 2020. Relationships between the allele and FHB Severity (A), Anther Retention (B) and Plant Height (C) are given, with "n" being the number of genotypes of population Remus x 6408-1 and Michael x 6408-1. P-value and multiple R-squared (R2) are above the boxplots.

5 Discussion

In this chapter, results will be discussed based on the questions posed in chapter 3.

5.1 FHB Severity

Based on the rating of the FHB severity (percentage of infected spikelets in a spike), the AUDPC was calculated for each genotype. All populations showed significant differences and variations of FHB symptoms were clearly quantitatively expressed. These results indicate that a polygenic mechanism is behind the resistance, which corresponds with results by Snijders and Perkowski (1990), Ban and Suenaga (2000) and Buerstmayr et al. (2009). Resistant lines 6408-1 and WSB showed significantly lower infestation rates compared to Remus and Michael across both years (see table 1 and 2). Crossing of these two resistant parents resulted in the population WSB x 6408-1 with rather resistant genotypes, but as expected FHB severity was still distributed. Population Michael x 6408-1 showed the highest disease levels (271) across 2019 and 2020, although parent Remus showed more symptoms than Michael in 2019. Some genotypes showed fewer symptoms than the resistant parents. In general, plants showed more disease symptoms in 2019. Higher temperatures in this year might be part of the reason. Due to the heat, the ripening process occurred faster in 2019 than in 2020. Rating was more difficult and disease symptoms on the spikes could easily be confused with ripened tissue. That is also the reason why not all scores were used for AUDPC calculation. However, it has to be considered that even though temperature varied across the years, artificial infection and mist irrigation were used, resulting in partway controlled environment conditions.

Heritability for Population Remus x 6408-1 (H^2=0.75) and Michael x 6408-1 (H^2=0.68) was relatively high. Variance components showed that the genotype had the biggest influence (see table 3). WSB x 6408-1 had a lower heritability with H^2=0.46 which matches with the result that the genotype-environment interaction had the greatest effect ($\sigma^2_{GenotypexYear}$=6914). The high genotypic results of at least two populations and the occurrence of genotypes with improved AUDPC provide the opportunity to select for those lines in further breeding programs.

5.2 Anther Retention

Anther retention was recorded for population Remus x 6408-1 and Michael x 6408-1. In 2019, 2020 and across both years, genotypes with more retained anthers also had a higher FHB severity (see table 1 and 2). This corresponds with reports by Buerstmayr and Buerstmayr (2015), Lu et al. (2013) and Graham and Browne (2009) who also observed this trend. However, concerning the correlation of those traits, results were not as clear. In 2019 the correlation coefficients of FHB severity (AUDPC) and anther retention were r=0.75 for Remus x 6408-1 and r=0.63 for Michael x 6408-1, whereas in 2020 it was only r=0.19 and r=0.25. Across both years, coefficients were relatively high (r=0.66 and r=0.63). Looking at figure 8, both populations showed similar tendencies. In Michael x 6408-1 plants were slightly more uniform. In each individual year, as well as across both years, genetic variance had the largest effect, the year the smallest. These inconsistent results lead to the assumption that correlation is weaker, as results of 2019 show, but climatic conditions were more favorable for a successful infection during flowering. This theory is supported by the fact that in general the infestation rate was higher in 2019 than in 2020 in all populations. Pugh et al. (1933) already mentioned the relation between FHB severity and climatic conditions. According to him and Parry et al. (1995) especially warm and humid conditions support the infection. Although precipitation was

higher during flowering in 2020, mist irrigation was used in both years. Temperature in contrary was 3.04°C higher than in 2019. These differences may have had a positive impact on the infection via spores. Furthermore in experiments of Steiner et al. and Buerstmayr et al. (2019) only initial infection and early disease development were associated with retained anthers, whereas spreading within the spikes was not affected. Recordings for FHB severity represent type I as well as type II resistance. This may lead to distorted results in correlation between FHB severity and anther retention.

Across both years anther retention and plant height were negatively correlated with r=-0.28 for Remus x 6408-1 and r=-0.21 for Michael x 6408-1, meaning that shorter plants tended to have more retained anthers. Considering that several alleles, including the *Rht-B1*, are overlapping for plant height and anther retention/extrusion (Buerstmayr and Buerstmayr 2015; Lu et al. 2013; He et al. 2016b), results are as expected.

Despite the discrepancies in correlation with FHB severity, anther retention is a promising trait to select for. Especially because no negative economic impact is known (Graham and Browne 2009).

5.3 The Influence of Plant Height on FHB Severity

Plant height was almost normally distributed with values from 60cm to 125cm. Tallest population was WSB 6408-1, which is not surprising since WSB was the tallest parent. Shortest genotypes were found in Remus x 6408-1. In 2019 plants were higher than in 2020 overall. It has to be considered that in both years different teams of two people measured plant height. Therefore, discrepancies are possible within and across years.

It is well known that most of the time plant height is negatively correlated with FHB symptoms and taller plants show higher resistance (Jenkinson and Parry 1994). Also in these experiments, significant negative correlations were found across both years. As Jenkinson and Parry (1994) and Buerstmayr et al. (2000) explained, this passive resistance mechanism is mostly associated with differences in the microenvironment. No matter if under natural circumstances or with mist irrigation, taller plants dry faster and have a lower risk to be infected by splash-dispersed spores. An unexpected result was the slightly positive correlation in 2020, though the only significant positive correlation was found in Remus x 6408-1 (r=0.20). Although parent 6408-1 was quite short with a mean of 80.63cm, it showed very low disease symptoms (31.13). Looking at the data, there were genotypes with short stems and low FHB severity in all populations. These relations are clearly visible in figure 9.

Heritability showed consistently high results, which agrees with Buerstmayr et al. (2000); and as expected, the genotype had the largest effect in all populations.

A high heritability and the presence of resistant lines with short straw provide a perfect base for further experiments and crossings.

5.4 The effect of the Flowering Date on FHB Severity, Anther Retention and Plant Height

Scientist have differing observations and opinions concerning the role of flowering date. Schmolke et al. (2005) and Gervais et al. (2003) observed that early flowering genotypes are less infected, whereas Buerstmayr et al. (2008) found positive, negative as well as no correlation at all.

Looking at the data of this experiment, FHB severity (AUDPC) and flowering date were clearly positively correlated with some not significant exceptions (see figure 10). Flowering date occurred between May 26 and June 15. Across both years, WSB x 6408-1 was the population whose genotypes flowered first (mean= 35.79 days after May 1) and had the lowest infection rate with a mean of 117.

Also parents WSB and 6408-1 tended to start earlier than Remus and Michael. Although Buerstmayr et al. (2008) speculated based on inconsistent results in his trials that distribution occurs rather due to environmental factors than due to the genetic background, genotypic variance was highest in populations Remus x 6408-1 ($\sigma^2_{Genotype}$=8.90) and Michael x 6408-1 ($\sigma^2_{Genotype}$=7.79). Only in population WSB x 6408-1 the year effect was highest (σ^2_{Year}= 5.69), suggesting the influence of humidity and temperature as described in Buerstmayr et al. (2008). 2020 was a cooler year with more precipitation and plots started flowering earlier than in 2019. However, heritability for this trait was high, meaning between 81% and 91% of the phenotype can be explained by the genotype. These results agree with Buerstmayr et al. (2000), who had a similar output. Flowering date and anther retention, as well as plant height, also showed positive correlations. Later flowering populations had more retained anthers and taller plants on average. A possible explanation would be a QTL overlapping of those traits.

As observations differ a lot between different studies, it remains unsure whether breeding for early flowering varieties is very promising. A possibility for farmers is to stag planting date or to use cultivars with different maturity time. This can lower the risk of total yield loss, as described in McMullen et al. (2012).

5.5 Awn Character

Awn character is a trait which is not mentioned in a lot of publications. In 1995, Mesterházy suspected that awned plants show a higher susceptibility. He assumed that the larger surface and higher humidity support the infection by conidia. However, his experiments and those of Buerstmayr and Buerstmayr (2015) did not confirm this presumption under artificial conditions.

In these experiments four different types of awn character were found (see figure 9). Distinction was done between genotypes without awns (awnless), with awns (awned), a mixture of plants with and without awns (heterogeneous) and awnletted plants, meaning only very short awns visible. Scoring was only done for populations Remus x 6408-1 and Michael x 6408-1. While parents Remus and Michael had no awns, 6408-1 was awned. A surprising and inexplicable high number of 126 genotypes in population Remus x 6408-1 and 99 in Michael x 6408-1 were classified as awned. Looking at the relation with FHB severity, only population Remus x 6408-1 had significant results. In contrast to Mesterházy's (1995) assumption, awned plants clearly showed least disease symptoms, while highest values were found in awnletted plants. Around 13% of the FHB severity were explained by the awn character (R^2=0.1253).

Also regarding the anthers, awnletted plants had the most retained ones inside the floret in both populations. Heterogeneous lines tended to have highest anther extrusion. Awnletted plants also had the shortest genotypes, which fits with the negative correlation between plant height and FHB severity mentioned above. Therefore, it is not surprising that plants with more symptoms also had higher anther retention and shorter plants in both populations, though results for plant height were only significant for population Michael x 6408-1. Multiple R-squared for anther retention and plant height were also very low, suggesting no great influence of awn character on those traits.

Awn character is a controversial trait, which sometimes seems to be correlated with FHB resistance. In this case, tendencies exist that awnletted plants had rather more disease symptoms, higher anther retention and shorter plants. Contrary to the assumption of Mesterházy (1995) that awned plants show a higher susceptibility, they had least infestation in Remus x 6408-1 and no significant results in

Michael x 6408-1. Awn character seems to have a negligible impact on FHB resistance mechanism and is hence an unreliable breeding trait for these populations.

5.6 Rht-B1

Results for *Rht-B1* locus on 4BS were significant in all populations and traits across 2019 and 2020. It explained 43% and 63% of the varieties of plant height in population Remus x 6408-1 (R^2=0.04263) and Michael x 6408-1 (R^2=0.6286). Parent 6408-1 was the only one carrying the dwarfing allele. With some outliers, plants carrying the *Rht-B1b* allele were significantly shorter. The median was at 80.00cm in population Remus x 6408-1 and at 82.50cm in Michael x 6408-1 (see figure 10C). Regarding the fact that *Rht-B1b* encodes a single-nucleotide polymorphism, leading to reduced plant height (Peng et al. 1999), results confirmed these findings.

Reduced plant height is also often correlated with higher anther retention. The negative correlation coefficients already approved this theory (see chapter 6.2). Looking at figure 10B, plants carrying the dwarfing allele also had more retained anthers. My results support the conclusion of Buerstmayr and Buerstmayr (2016) who also found an association between *Rht-B1b* and reduced anther extrusion in common wheat.

As expected, the *Rht-B1b* allele increased susceptibility FHB (figure 10A). Although more severe effects are accredited to the Rht-D1b allele (Miedaner and Voss 2008; Lu et al. 2011), results showed significant differences for plants with the *Rht-B1b* compared to the *Rht-B1a*. Even though shorter plants showed less disease symptoms, only 13% and 6% of the variation in Remus x 6408-1 and Michael x 6408-1 was explained by the *Rht-B1* locus.

6 Conclusion and Outlook

Results of Jakob Seereiter's and my experiments confirm the polygenic mechanism behind FHB resistance as several scientists such as Snijders and Perkowski (1990), Ban and Suenaga (2000) or Buerstmayr et al. (2009) observed. Plants with less infection than the resistant parent 6408-1 were found in each population and year. Calculations of the variance components showed that the genotype had a big influence on the improved resistant genotypes. As expected, the population WSB x 6408-1 showed significantly lower infestation rates than the others. However, due to undesired agronomic traits of WSB, further breeding with those lines is not advisable.

Despite inconsistent results regarding the correlation of FHB severity and anther retention in individual years, results across both years seemed promising, showing positive correlations. Maybe the third year of the experiment will enlighten the significance of anther retention in Remus x 6408-1 and Michael x 6408-1. As for anther retention no negative economic impact is known, it still is a promising trait to select for.

Plant height also showed a negative correlation with FHB severity across both years, as it was also observed in many studies (Jenkinson and Parry 1994; Buerstmayr et al. 2000). Selection for short plants is and will stay a very important breeding trait due to better adaption to modern practices. Although the dwarfing allele *Rht-B1b* increased susceptibility, all populations included genotypes, having short stems and low FHB severity. Further, reduced plant height and the occurrence of the *Rht-B1b* allele showed negative correlation with anther retention. Lanning et al. (2012) describe an association of the *Rht-B1b* allele with increased grain yield, which might be interesting for further research.

In these experiments, flowering date and FHB symptoms were clearly positively correlated. Furthermore, later flowering populations showed more retained anthers and taller plants. However this trait remains a controversial factor. It might be worthwhile to correct the statistical model for flowering date in further analysis. As He et al. (2016a) showed in their calculations, results can completely change their significance.

The awn turned out to be not very promising in these experiments. Awned plants had more FHB infestation but results do not coincide with observations by Mesterházy (1995). This trait has to be much more explored and understood in order to come to a resolution.

In conclusion parent 6408-1 seems to be a promising genetic source for further breeding programs. Although it carries the *Rht-B1b* dwarfing allele, it is a highly resistant genotype. The offspring included short genotypes with high level of resistance. The exact underlying genetic mechanism of the resistance is not known yet but provides possibilities for further research.

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8 Appendix

8.1 R-Code for Statistical Analysis

8.1.1 BLUEs for one year

```
#with breedR package
# remove any NA-values in the columns for heading date, plant height, severity, AUDPC, anther retention
# do not include cross 3 because anther retention was not observed in this cross and is all NA (WSB)
is.na(mydata[mydata$Cross != "3",c(5,6,10:15,17)]) <- FALSE
# initiate a dataframe for the results
BLUEs12.mydata <- NULL
# create a vector containing the relevant columns for which we want to calculate the BLUEs
cols <- c(5,6,10:15,17)
for (i in cols) {
temp <- droplevels(mydata[mydata$Cross != 3, c(2:4,i)])
# design the model to calculate the BLUEs: fixed effect the respective variable and the genotype, random effect the replication
model <- breedR::remlf90(fixed = temp[,4] ~ -1 + Name, random = ~ WH, method = "ai", data = temp)
# extract the estimates for the fixed effect from the model and write it into a temporary dataframe
frame.temp <- as.data.frame(model$fixed$Name)
BLUEs12.mydata <- as.data.frame(cbind(BLUEs12.mydata, frame.temp$value))
}
# include the genotype names
BLUEs12.mydata$Name <- rownames(frame.temp)
# change column order so that the genotypes are first
BLUEs12.mydata <- BLUEs12.mydata[,c(10,1:9)]
# define the colnames of the new BLUEs-dataframe so that they match the raw data
colnames(BLUEs12.mydata) <- c("Name",colnames(mydata)[cols])
# the same for Cross 3 (without anther retention)
is.na(mydata[mydata$Cross == "3",c(5,10:15,17)]) <- FALSE
BLUEs3.mydata <- NULL
cols2 <- c(5,10:15,17)
for (i in cols2) {
temp <- droplevels(mydata[mydata$Cross == 3, c(2:4,i)])
model <- breedR::remlf90(fixed = temp[,4] ~ -1 + Name, random = ~ WH, method = "ai", data = temp)
frame.temp <- as.data.frame(model$fixed$Name)
BLUEs3.mydata <- as.data.frame(cbind(BLUEs3.mydata, frame.temp$value))
}
BLUEs3.mydata$Name <- rownames(frame.temp)
BLUEs3.mydata <- BLUEs3.mydata[,c(9,1:8)]
# add the extra AR-column, fill with NAs
BLUEs3.mydata$AR <- rep("NA", times = nrow(BLUEs3.mydata))
# change column order in the new dataframe for cross 3 to match the order of BLUEs12
BLUEs3.mydata <- BLUEs3.mydata[,c(1,2,10,3:9)]
# define the colnames of the new BLUEs-dataframe so that they match the raw data
colnames(BLUEs3.mydata) <- c("Name",colnames(mydata)[cols])
```

```
# merge the two dataframes to have the BLUEs for all crosses in one frame
BLUES.mydata <- rbind(BLUEs12.mydata, BLUEs3.mydata)
# add a column with the cross number to the BLUEs-dataframe
# first initiate the column
BLUES.mydata$Cross <- NULL
# than add the cross name from the mydata-dataframe by matching the Name-columns
for(i in c(1:nrow(BLUES.mydata))){
BLUES.mydata$Cross[i] <- mydata[mydata$Name == BLUES.mydata$Name[i],4]
}
```

8.1.2 BLUEs across two years

```
#with breedR package
# remove any NA-values in the columns for heading date, plant height, severity, AUDPC, anther retention
# do not include cross 3 because anther retention was not observed in this cross and is all NA (WSB)
is.na(data1920[data1920$Cross != "3",c(8,9,13,16,20)]) <- FALSE
# initiate a dataframe for the results
BLUEs12_1920 <- NULL #NULL bedeutet es gibt den datensatz aber er ist leer
# create a vector containing the relevant columns for which we want to calculate the BLUEs
cols <- c(8,9,13,16,20)
for (i in cols) {
# create a temporary dataframe
#including the column with one of the interesting variables (i)
temp <- droplevels(data1920[data1920$Cross != 3, c(2:7,i)])
# design the model to calculate the BLUEs: fixed effect the respective variable and the genotype, random effect the replication
model <- breedR::remlf90(fixed = temp[,7] ~ -1 + Name, random = ~ WH, method = "ai", data = temp)
frame.temp <- as.data.frame(model$fixed$Name)
BLUEs12_1920 <- as.data.frame(cbind(BLUEs12_1920, frame.temp$value))
}
# include the genotype names
BLUEs12_1920$Name <- rownames(frame.temp)
# change column order so that the genotypes are first
BLUEs12_1920 <- BLUEs12_1920[,c(6,1:5)]
# define the colnames of the new BLUEs-dataframe so that they match the raw data
colnames(BLUEs12_1920) <- c("Name",colnames(data1920)[cols])
# the same for Cross 3 (without anther retention)
# add the extra AR-column, fill with NAs
is.na(data1920[data1920$Cross == "3",c(8,13,16,20)]) <- FALSE
# initiate a dataframe for the results
BLUEs3_1920<- NULL
# create a vector containing the relevant columns for which we want to calculate the BLUEs
cols2 <- c(8,13,16,20)
for (i in cols2) {
temp <- droplevels(data1920[data1920$Cross == 3, c(2:7,i)])
model <- breedR::remlf90(fixed = temp[,7] ~ -1 + Name, random = ~ WH, method = "ai", data = temp)
frame.temp <- as.data.frame(model$fixed$Name)
BLUEs3_1920<- as.data.frame(cbind(BLUEs3_1920, frame.temp$value))
}
BLUEs3_1920$Name <- rownames(frame.temp)
# add the extra AR-column
BLUEs3_1920$AR <- rep("NA", times = nrow(BLUEs3_1920))
```

```
# change column order in the new dataframe for cross 3 to match the order of BLUEs12
BLUEs3_1920 <- BLUEs3_1920[,c(5,1,6,2:4)]
# define the colnames of the new BLUEs-dataframe so that they match the raw data
colnames(BLUEs3_1920) <- c("Name",colnames(data1920)[cols])
#6408-1 entfernen (ist schon im BLUEs12 enthalten)
BLUEs3_1920 <- BLUEs3_1920 [-c(1),]
# merge the two dataframes to have the BLUEs for all crosses in one frame
BLUES_1920 <- rbind(BLUEs12_1920, BLUEs3_1920)
# add a column with the cross number to the BLUEs-dataframe:
BLUES_1920$Cross <- NULL
for(i in c(1:nrow(BLUES_1920))){
BLUES_1920$Cross[i] <- data1920[data1920$Name == BLUES_1920$Name[i],7]
}
```

8.1.3 Repeatability and Variance Components

```
#with sommer package
#repeatability of AUDPC for Cross 1
model_h2_C1<- mmer(fixed = AUDPC~1, random = ~Name+WH, data = mydata[mydata$Cross==1,])
#variance components
VG_1<- model_h2_C1$sigma$Name
VE_1<- model_h2_C1$sigma$units
#repeatability
h2_AUDPC_C1<- VG_1 / (VG_1 + VE_1/2)
h2_AUDPC_C1
```

8.1.4 Heritability and Variance Components

```
#with sommer package
#heritability of AUDPC for Cross 1
model_1<- mmer(fixed = AUDPC~1, random = ~Name + year + year:Name + year:WH , data = mydata_all_years[mydata_all_years$Cross==1,])
#variance components
VE_1 <- model_1$sigma$year
VGE_1 <- model_1$sigma$`year:Name`
VG_1 <- model_1$sigma$Name
Ve_1 <- model_1$sigma$units
#heritability
h2_AUDPC_2years_Cross1<- VG_1/(VG_1 + VGE_1/2 + Ve_1/ (2*2))
h2_AUDPC_2years_Cross1
```

8.1.5 LSD5% Values

```
#Cross 1, AUDPC, across both years
#t-value
df<- 2160 -1-(788-1)-(4-1)
qt(p=0.05/2, df=1369)
#LSD 5%
1.961698*sqrt(2*(VGE_AUDPC/2+Verror_AUDPC/4))
```

8.1.6 Phenotypic Correlation

```
#pearson correlation with Himsc package
#Cross1, year 2020
Cor_Cross1_20<- BLUES.Cross1[, c("B5", "AUDPC.B5.", "AR", "Dat", "WUH")]
str(Cor_Cross1_20)
rcorr(as.matrix(Cor_Cross1_20), type = c("pearson"))
#create csv file
x<- rcorr(as.matrix(Cor_Cross1_20), type = c("pearson"))
xr_c1<- x[["r"]]
write.csv( xr_c1, file = "cor_20.csv")
```

8.1.7 Awn Character

```
#Cross 1, AUDPC, across both years
#lm function
model_BG1_1920<-lm (AUDPC.B4.~ BG, data=data_BG_Cross1_1920)
summary (model_BG1_1920)
```

8.1.8 Marker-Trait Association

```
#Cross1, AUDPC, across both years
#lm function
model_1_1920<-lm (AUDPC.B4.~ Rht.B1_allele, data=Rht.B1.Cross1_1920)
summary (model_1_1920)
```

8.2 Scoring Guide for FHB disease symptoms

For visual estimation of FHB severity of each plot, scoring guide of table 6 and figure 11 was used.

Table 6: Scoring guide for FHB Severity (Mitt. Biol. Bundesanst. Land- Forstwirtsch. 2000).

Scoring giude for FHB severity scoring on a whole plot basis used at IFA-Tulln

% diseased spikelets per plot	visually estimated average per PLOT
0	no symptoms visible
0,1	traces of FHB visible
0,5	0.1 spikelets per ear infected (= one spikelet in 10 % of the heads)
1	0.2 spikelets per ear infected (= one spikelet in 20 % of the heads)
2	0.4 spikelets per ear infected
3	0.6 spikelets per ear infected
5	1 spikelet per ear infected
10	2 spikelets per ear infected
15	3 spikelets per ear infected
20	4 spikelets per ear infected
25	5 spikelets per ear infected
30	6 spikelets per ear infected
40	8 spikelets per ear infected
50	10 spikelets per ear infected
60	12 spikelets per ear infected
70	14 spikelets per ear infected
80	16 spikelets per ear infected
90	18 spikelets per ear infected
100	all spikelets per ear infected

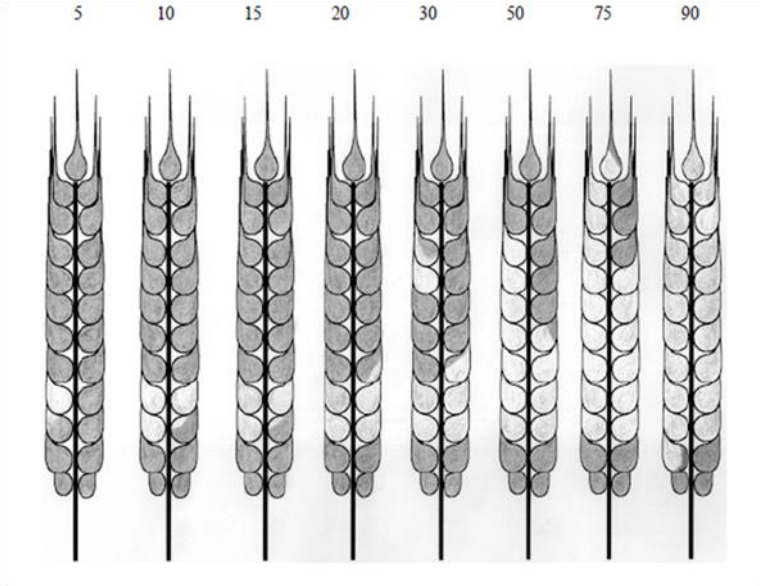

Figure 13: Visual scoring guide for FHB Severity (Mitt. Biol. Bundesanst. Land-Forstwirtsch. 2000).

8.3 General data structure

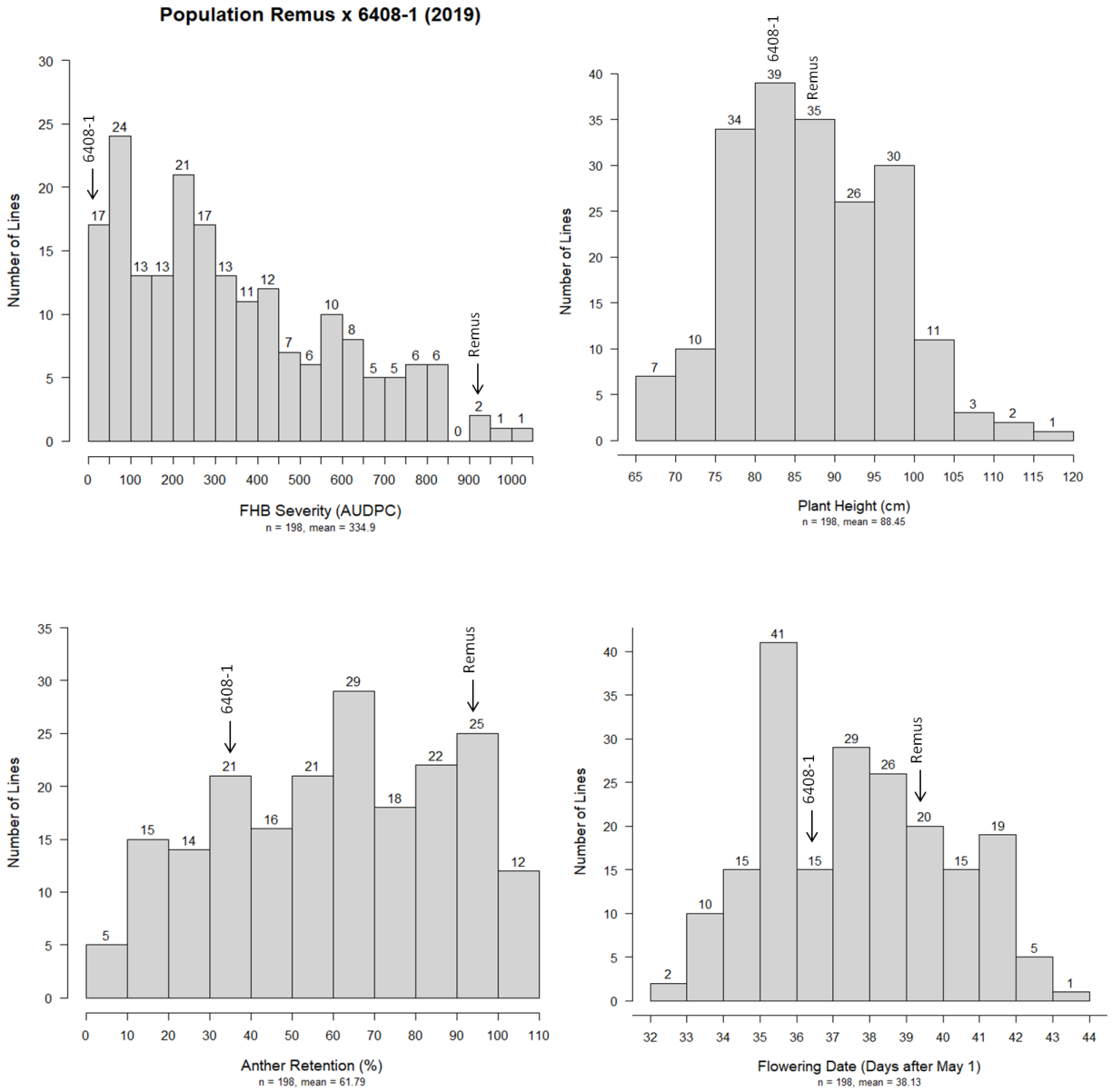

Figure 14: Histograms showing the data distribution of the BLUEs for Population Remus x 6408-1 for FHB Severity (AUDPC), Plant Height (cm), Anther Retention (%) and Flowering Date (Days after May 1) and the LSD5% value in 2019.

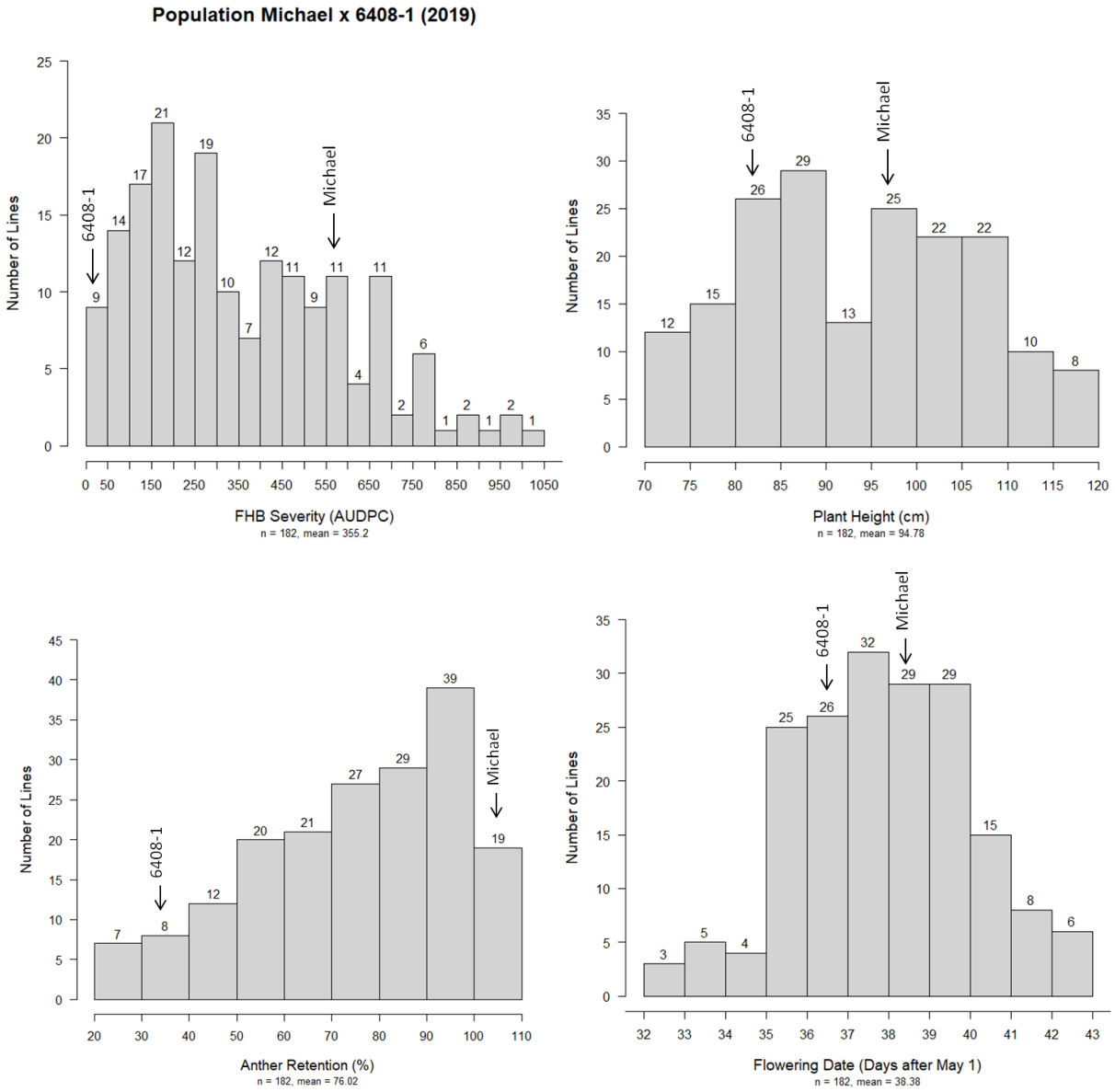

Figure 15: Histograms showing the data distribution of the BLUEs for Population Michael x 6408-1 for FHB Severity (AUDPC), Plant Height (cm), Anther Retention (%) and Flowering Date (Days after May 1) and the LSD5% value in 2019.

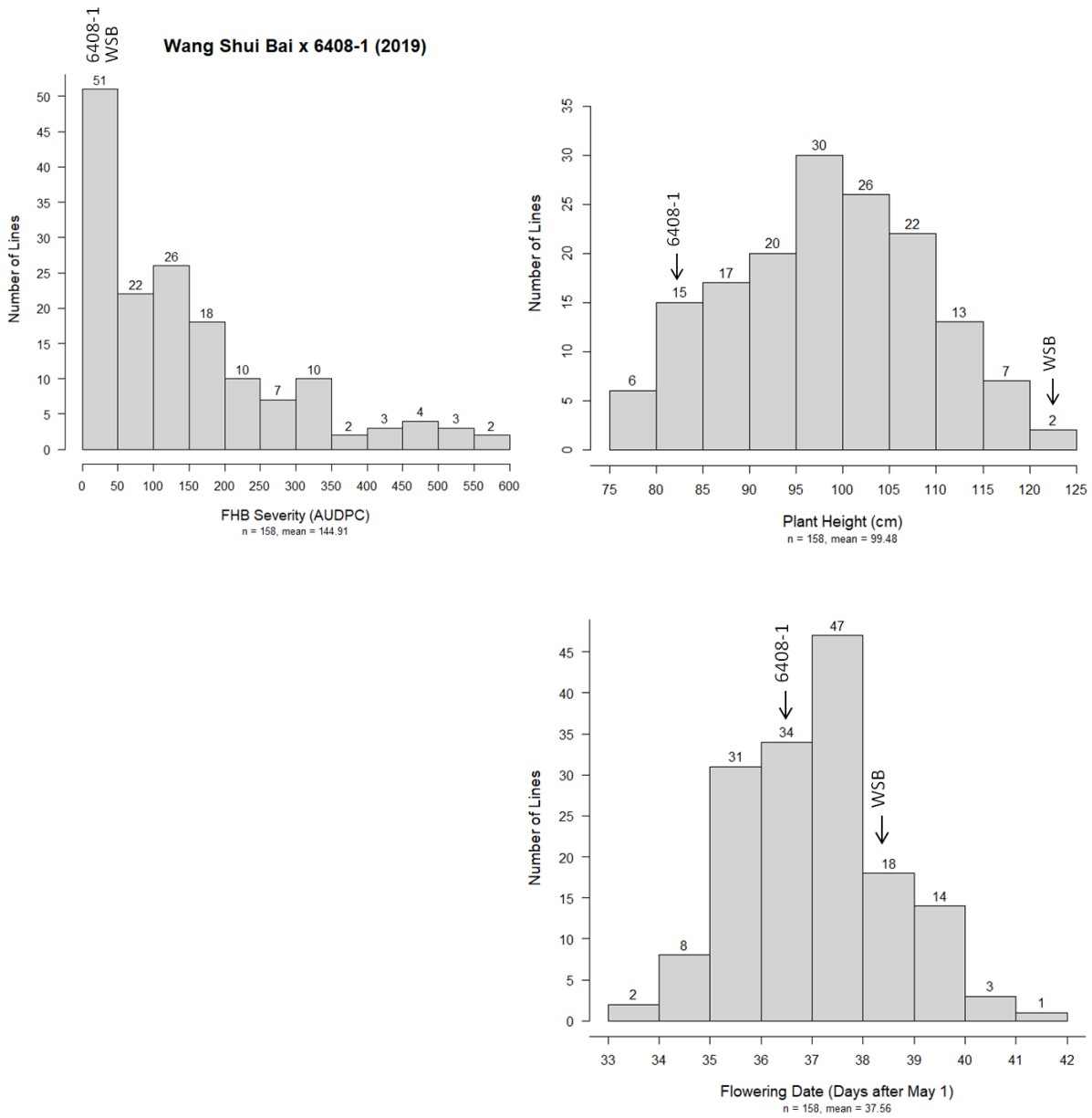

Figure 16: Histograms showing the data distribution of the BLUEs for Population WSB x 6408-1 for FHB Severity (AUDPC), Plant Height (cm) and Flowering Date (Days after May 1) and the LSD5% value in 2019.

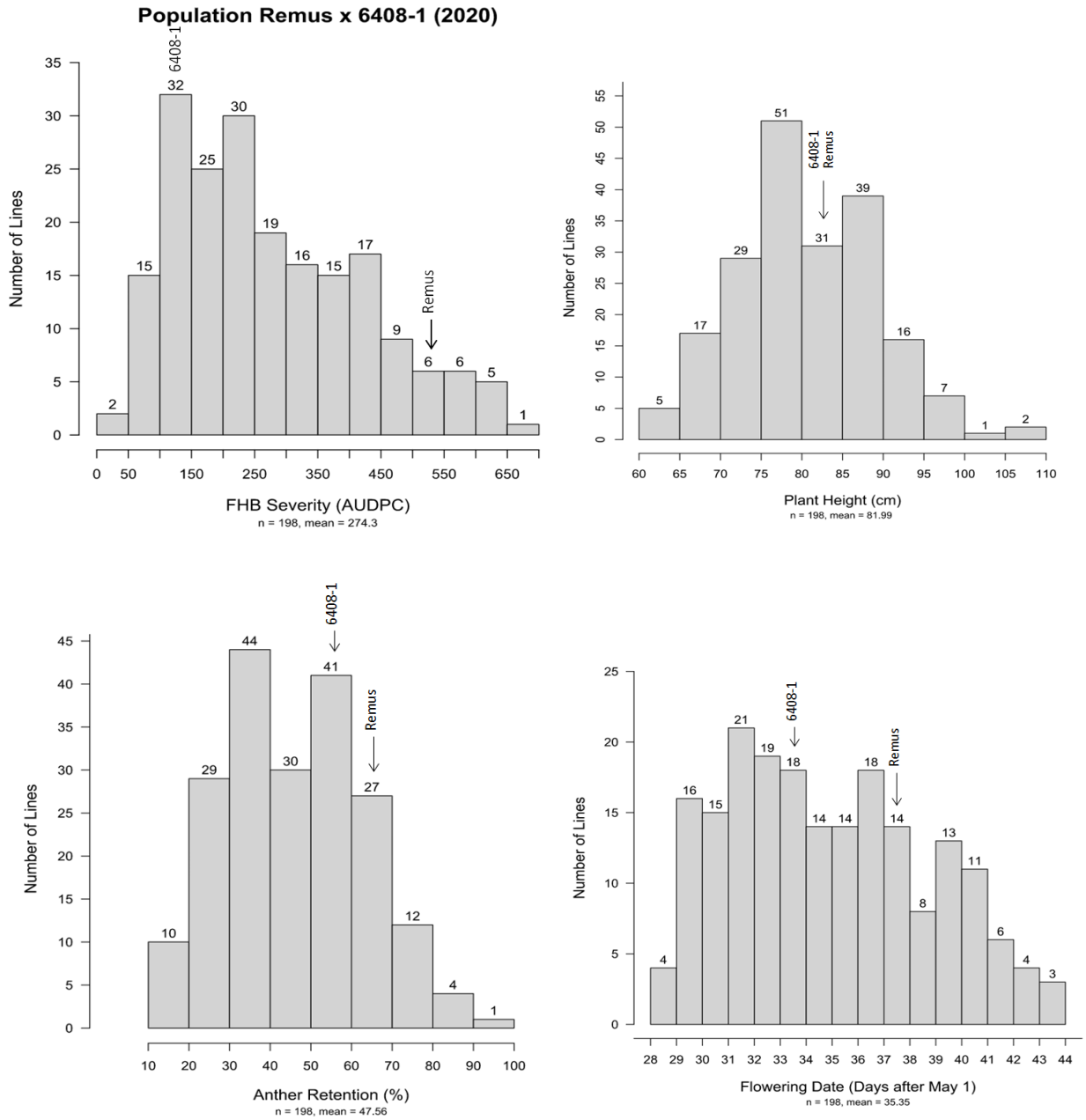

Figure 17: Histograms showing the data distribution of the BLUEs for Population Remus x 6408-1 for FHB Severity (AUDPC), Plant Height (cm), Anther Retention (%) and Flowering Date (Days after May 1) and the LSD5% value in 2020.

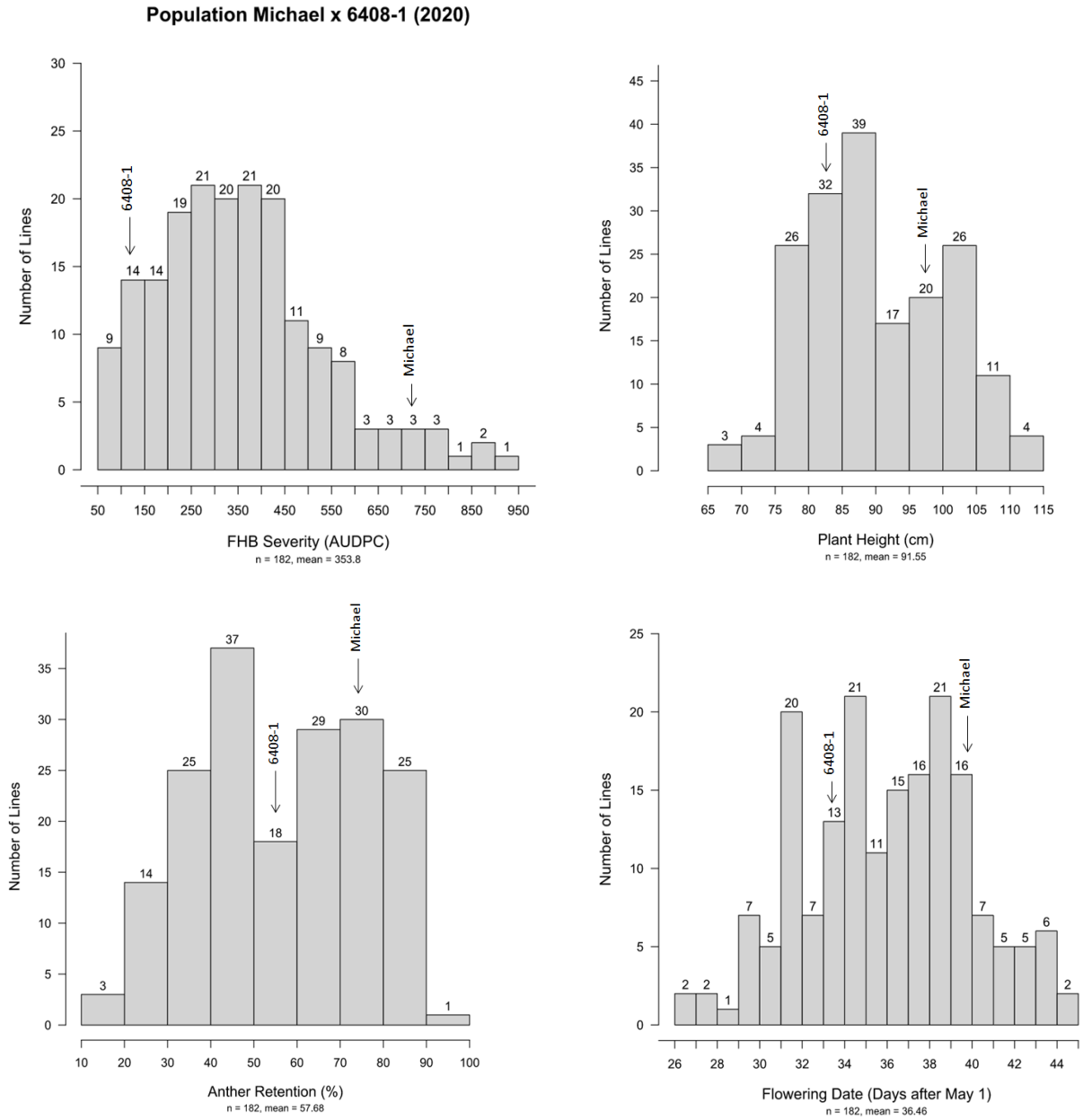

Figure 18: Histograms showing the data distribution of the BLUEs for Population Michael x 6408-1 for FHB Severity (AUDPC), Plant Height (cm), Anther Retention (%) and Flowering Date (Days after May 1) and the LSD5% value in 2020.

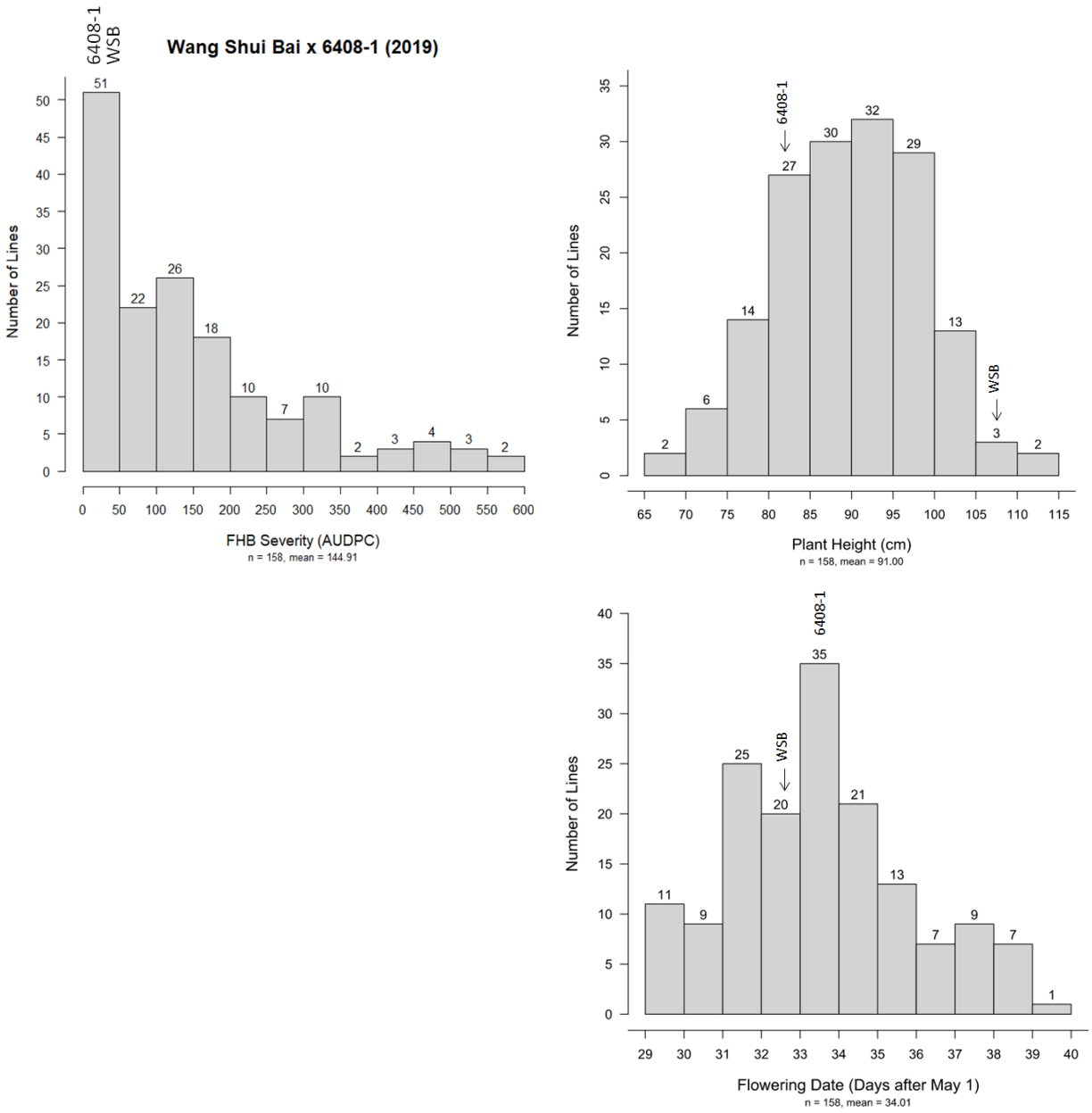

Figure 19: Histograms showing the data distribution of the BLUEs for Population Remus x 6408-1 for FHB Severity (AUDPC), Plant Height (cm), Anther Retention (%) and Flowering Date (Days after May 1) and the LSD5% value in 2020.

Table 7: Trait correlations of the BLUEs for FHB Severity (AUDPC), FHB Severity of the 4th rating (FHB-22 in %), Anther Retention (%), Plant Height (cm) and Flowering Date (days after May 1) in 2019 for population Remus x 6408-1, Michael x 6408-1 and WSB x 6408-1. P-values are given with * and **. "n.s." means that no significant correlation was found.

Correlation Coefficients (2019)

Remus x 6408-1

	FHB-22 (%)	AUDPC	Anther Retention (%)	Plant Height (cm)
AUDPC	0.86**			
Anther Retention	0.72**	0.75**		
Plant Height (cm)	-0.35**	-0.32**	-0.25**	
Flowering Date[a]	0.43**	0.55**	0.44**	0.27**

Michael x 6408-1

	FHB-22 (%)	AUDPC	Anther Retention (%)	Plant Height (cm)
AUDPC	0.87**			
Anther Retention	0.60**	0.63**		
Plant Height (cm)	-0.53**	-0.43**	n.s.	
Flowering Date[a]	0.18 *	0.32**	0.42**	0.31**

Wang Shui Bai x 6408-1

	FHB-22 (%)	AUDPC	Plant Height (cm)
AUDPC	0.93**		
Plant Height (cm)	-0.42**	-0.43**	
Flowering Date[a]	n.s.	0.24*	n.s.

*p<0.05
**p<0.001
[a]Days after May

Table 8: Trait correlations of the BLUEs for FHB Severity (AUDPC), FHB Severity of the 5th rating (FHB-26 in %), Anther Retention (%), Plant Height (cm) and Flowering Date (days after May 1) in 2020 for population Remus x 6408-1, Michael x 6408-1 and WSB x 6408-1. P-values are given with * and **. "n.s." means that no significant correlation was found.

Correlation Coefficients (2020)

Remus x 6408-1

	FHB-26 (%)	AUDPC	Anther Retention (%)	Plant Height (cm)
AUDPC	0.87**			
Anther Retention	0.20*	0.19*		
Plant Height (cm)	n.s.	0.20*	-0.28**	
Flowering Date[a]	0.57**	0.80**	n.s.	0.41**

Michael x 6408-1

	FHB-26 (%)	AUDPC	Anther Retention (%)	Plant Height (cm)
AUDPC	0.87**			
Anther Retention	0.28**	0.25**		
Plant Height (cm)	n.s.	0.14[b]	0.25**	
Flowering Date[a]	0.51**	0.76**	n.s.	0.42**

Wang Shui Bai x 6408-1

	FHB-26 (%)	AUDPC	Plant Height (cm)
AUDPC	0.86**		
Plant Height (cm)	n.s.	n.s.	
Flowering Date[a]	0.59**	0.81**	0.17*

*p<0.05
**p<0.001
[b]p=0.0584
[a]Days after May 1