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Master thesis

Field testing and molecular marker characterization of near-isogenic lines with Fusarium Head Blight resistance QTL from CJ9306 in Zebra and Berserk spring wheat backgrounds

Feltundersøkelser og molekylære markøranalyser for karakterisering av nær-isogene linjer med Fusariumresistens-QTL fra CJ9306 i Zebra og Berserk vårhvete bakgrunner.

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English summary (abstract)

Fusarium head blight (FHB or head scab) is a devastating disease of wheat (*Triticum aestivum* L.) and other small grain cereals induced by *Fusarium* spp. all around globe, causing higher yield loss and increased mycotoxin contamination like Deoxynivalenol (DON) which is a serious risk for human and livestock health. The sustainable way to reduce the problems of *Fusarium* infection is to increase resistance by stacking Quantitative Trait Loci (QTL) on our cultivars. Resistance breeding depends heavily on Chinese resistance cultivar Sumai 3 and its derivatives with focused on the *Fhb1* QTL. The purpose of our study were to (i) confirm the allelic states of FHB resistance QTL *Fhb1*, *QFs.nau-2DL* and *Qfhi.nau-5AS* in the NILs derived from the cross between CJ9306 (derivative of Sumai 3) and Zebra, Berserk spring wheat cultivars of Norway by marker genotyping, (ii) investigate the effect of three QTL and their combination on FHB resistance traits (visual symptom, DON content and anther excretion (AE)), and (iii) to evaluate the potential side-effects of the QTL on the important agronomic traits like plant height (PH), days to heading (DH), grain yield, and grain quality parameters. To determine the association between FHB severity and passive resistance related traits like DON, DH, PH, and AE; grain spawn inoculation in one field trial out of three was performed in Norway, where FHB scoring was done. Strong significant correlation between these traits were confirmed except for DH. Highly significant correlation (0.483***) between FHB severity and DON was obtained. PH was associated most with DON content (-0.353*, -0.464*, -0.413*) followed by FHB severity (-0.241, -0.357*, -0.341) in all three experimental fields. Similarly, AE had higher correlation with DON (-0.371*, -0.356*) than with FHB severity (-0.298*, -0.201). Genotyping of 57 BC_1F_5 NILs with Kompetitive allele specific PCR (KASP), Sequence tagged sites (STS) and Simple sequence repeat (SSR) markers confirmed the allelic states of the NILs. The resistant allele at QTL were found to be introgressed in NILs successfully. *Fhb1* and 5AS were found introgressed in 26 lines and 2DL on 19 lines out of 57 NILs. QTL *Fhb1* and 2DL were found significant with DON content reduction while 5AS did not have any measurable contribution. Besides, effects of these three QTL on FHB severity were not significant. *Fhb1* was found delaying DH by one day in NILs. However, the QTL were not found significant with PH and AE. The stacking of QTL with resistance allele showed stronger effect on the traits than acting alone. Effect of all three QTL combination for grain quality parameters like test weight, protein content, starch content and yield in both the

agronomic field trial were not in measurable amount. However, 2-3 gm reduced 1000 kernels weight was seen for QTL combination in both agronomic field trail. Therefore, promotion of Marker – assisted selection based on QTL introgression of *Fhb1*, 2DL and phenotypic selection method of high AE and optimal PH are recommended to the companies and research institution working on resistance breeding for wheat FHB in Norway.

Norwegian summary

Aksfusariose er en ødeleggende plantesykdom på hvete (*Triticum aestivum* L.) og andre kornslag verden over som forårsakes av ulike *Fusarium*-arter og fører til avlingstap og produksjon av mykotoksiner som for eksempel deoxynivalenol (DON) som kan føre til helseplager både for mennesker og husdyr. Den mest bærekraftige måten å redusere problemene med *Fusarium*-infeksjoner er resistensforedling ved å kombinere ulike QTL (Quantitative Trait Loci) for resistens i sortene. Resistensforedling er i stor grad basert på den kinesiske resistenskilden Sumai 3 og foredlingslinjer og sorter med resistens fra denne, med fokus på *Fhb1*. Målsettingen med vår studie var å (i) bekrefte hvilke resistensallel av de tre QTL-ene *Fhb1*, *QFs.nau-2DL* og *Qfhi.nau-5AS* som ble krysset inn i nær-isogene linjer (NILs) fra krysninger mellom CJ9306 (avkom fra Sumai 3) og vårhvetesortene Zebra og Berserk som dyrkes i Norge, (ii) Å undersøke effekten av de tre QTL-ene og kombinasjoner av disse på viktige parametere for *Fusarium*-resistens som aksfusariose, DON-innhold og støvknappfelling, og (iii) undersøke mulige effekter av disse QTL-ene på andre viktige agronomiske egenskaper som dager til aksskyting, strålengde, avling og kvalitetsegenskaper. Tre feltforsøk ble gjennomført, og ett av dem ble inokulert med *Fusarium*-smittede havrekorn for å evaluere *Fusarium*-resistens. En meget signifikant korrelasjon ble funnet mellom aksfusariose og DON (0.483***). Strålengde var sterkest assosiert med DON-innhold (-0.353*, -0.464*, -0.413*) fulgt av aksfusariose (-0.241, -0.357*, -0.341) i alle de tre feltforsøkene. På samme måte hadde støvknappfelling sterkere korrelasjon med DON (-0.371*, -0.356*) enn aksfusariose (-0.298*, -0.201). Genotyping av 57 BC_1F_5 NILs med KASP (Kompetitive Allele-Specific PCR), STS (Sequence Tagged Sites) og SSR (Simple Sequence Repeat) markører verifiserte de ulike linjenes resistensallel, og viste at introduksjonen av resistens i de nær-isogene linjene var vellykket. *Fhb1* og 5AS ble funnet i 26 av de 57 linjene mens 19 linjer hadde 2DL-QTLet. *Fhb1* og 2DL viste signifikant effekt på reduksjon av DON-innhold mens 5AS ikke viste noen målbar effekt. Disse tre QTL-ene viste til gjengjeld ingen signifikant effekt på aksfusariose. *Fhb1* ble funnet å forsinke aksskytingen med én dag, men hadde ingen effekt på strålengde og støvknappfelling. Kombinering av flere resistens-QTL viste større effekt på egenskapene enn bare ett QTL. Det ble ikke funnet noen effekt av QTL-kombinasjoner på kornkvalitetsegenskaper som hektolitervekt, proteininnhold, stivelsesinnhold og avling. Men kombinasjonen av tre QTL viste seg å redusere 1000-kornvekta med 2-3 gram i begge feltforsøkene hvor agronomiske egenskaper ble målt. På

bakgrunn av disse resultatene, kan markørbasert innkryssing av *Fhb1*, 2DL og 5AS resistens-QTL og fenotypisk seleksjon for høy støvknappfelling og optimal strålengde anbefales overfor planteforedlingsfirma og forskningsinstitusjoner som driver med foredling av resistens mot *Fusarium* i Norge.

Abbreviations

NIL: Near Isogenic Lines

AE: Anther extrusion

DH: Days to heading

PH: Plant height

FHB: Fusarium head blight

DON: Deoxynivalenol

QTL: Quantitative trait locus

MAS: Marker assisted selection

PCR: Polymerase Chain Reaction

SNP: Single nucleotide polymorphism

KASP: Kompetitive Allele Specific PCR

SSR: Simple Sequence Repeat

STS: Sequence Tagged Sites

MAS: Marker Assisted Selection

1. Introduction

Fusarium head blight (FHB), also called head scab, is a serious disease in wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and other cereals. The disease is well established and can be seen in most countries and epidemic outbreaks have been reported from Asia, Europe, North and South America (Bai and Shaner 1994). The International Maize and Wheat Improvement Center (CIMMYT) has recognized FHB as one of the major factors reducing the production of wheat around the world (Dubin 1997). The predominantly found *Fusarium* species causing FHB in wheat and different small-grain cereals around Europe are *F. graminearum, F. avenaceum, and F.culmorum* (Bottalico and Perrone 2002). In Norway, *Fusarium* is a problem mainly in spring wheat and oat (*Avena sativa* L.). Here, the most important species involved in the infection of spring wheat are *F. graminearum* and *F. avenaceum* but *F. culmorum, F. poae* and *F. langsethiae* may also contribute (Hofgaard, Aamot et al. 2016). Over years, there has been a shift in relative occurrence towards more *F. graminearum* in Norwegian spring wheat (Hofgaard, Aamot et al. 2016). The pathogen *F. graminearum* is able to infect wheat, triticale, barley, oat, rye and maize (Becher, Miedaner et al. 2013, Miedaner, Gwiazdowska et al. 2017). The damage is especially clear in humid and semi-humid regions of the world where FHB reduces both grain yield and quality (Bai and Shaner 1994). To illustrate the yield loss, an occurrence of around 20% FHB can cause a reduced yield in the range of 1 Mg/ha (Salgado, Madden et al. 2015, Miedaner, Gwiazdowska et al. 2017). Yield losses in the range of 10 to 30% in wheat and barley have been reported in Europe due to *Fusarium* infections on grains (Bottalico and Perrone 2002). Quality loss is an even bigger concern in wheat production as highly toxic substances are accumulated in the infected grains. Mycotoxin contamination, such as high contents of deoxynivalenol (DON), moniliformin, 3-Acetyldeoxynivalenol, zearalenone, nivalenol are well known (Hofgaard, Aamot et al. 2016). These infected grains and mycotoxins are dangerous to human health and livestock feeding. The maxmium DON level range from 0.5 to 2 ppm in wheat used for human consumption (Cai 2012). For livestock feed the research has shown a reduction in feed uptake and a reduced weight gain at DON levels of around 1 ppm in the feedstock, and with vomiting problems at around 10 ppm (De Wolf, Madden et al. 2003). Due to incomplete understanding of factors influencing FHB disease development and constraints in efficient application, use of FHB controlling fungicide is left behind (Goswami and Kistler 2004). Also the application efficiency of fungicides when evaluated under different application methodology shows on an

average 60% reduction on disease severity as described by Lechoczki-Krsjak, Tóth et al. (2008).

The current thesis is focusing on FHB resistance in wheat. Therefore, the following sections are referring to research on wheat. However, the mechanisms and principles may also count for other cereals.

1.1 Norwegian wheat production and its challenges

Having 1 million ha arable land for cultivation, wheat occupied 65-85,000 ha in area for the last four years in Norway (SSB, 2018). Spring wheat is the dominant type, covering 70-80% of the in the Norwegian wheat production, while winter wheat stands for the remaining 20-30% (SSB, 2018). Some of the adapted and popular spring wheat varieties of Norway are Mirakel, Zebra, Bjarne, Rabagast, Krabat and Berserk, however, Berserk was withdrawn after just a few years of productionin (2009-2012) due to unstable yield as reported by Strand (2017). Most wheat is produced for bread and human consumption, which also drags researchers to produce high quality flour in Norway. The production is increasing slightly with an average of 300,000 MT from 2011 to 2016 (SSB, 2018). The production is subsidized from the Norwegian government and thus needs to always address aspects of quality and environment. The perception of Norwegian wheat farmers is that a short growing season combined with pre-harvest sprouting and diseases are major challenges in the production. For winter wheat sever long winter is a limiting factor for the production which shows forced movement for the increment of spring wheat production. *F. graminearum, F. avenaceum, and F.culmorum, F. tricinctum* are found to reduce grain quality below acceptable level when Norwegian wheat samples from producing sites all around Norway are tested (Kosiak, Torp et al. 2004). Examination of 169 wheat samples of Norway showed contamination of 1.2% (20 µg/kg) for HT-2, 0.6% (20 µg/kg) for T-2, 14% (53 µg/kg) for DON and 0% for nivalenol (Langseth and Rundberget 1999). Overall, diseases are challenging and FHB, powdery mildew, stripe rust and leaf blotch disease are the most concerned diseases in the wheat producing areas of the country. Graminor is a plant breeding company in Hedmark district of Norway. The main aim for Graminor is to develop varieties (field crops and horticultural crops) that are suitable to Norwegian or Nordic growing condition and to disseminate them to farmer's fields. Collaboration between the national plant breeding company Graminor and the Norwegian University of Life Sciences (NMBU) has been established for breeding research

of suitable and resistant varieties. Zebra and Berserk are two of the spring wheat varieties that have been adopted in Norway. Graminor is also carrying out research on introgressing FHB resistance into these and other adapted spring wheat backgrounds.

Breeding FHB resistanct spring wheat is of major importance in Norway as the loss and toxin content was seen on wheat and barley (Langseth and Rundberget 1999). Researchers are focused on developing varieties with higher resistance to *Fusarium* every year. Thus the release of new cultivars in recent years are seen more resistance to DON (Figure 1.1). Wheat market of Norway is in favour of high yielding, disease resistant and more biotic and abiotic stress tolerant cultivars (Figure 1.1, 1.2). Berserk which was popular in between 2009 - 2012 was stopped due to its low production capacity. Vinjett and Demonstrant with high DON content have been replaced, and Bjarne and Zebra on the way due to high susceptibility to stripe rust. They are all being replaced by varieties with improved FHB resistance. Mirakel is increasing its market share in recent years due to its excellent baking quality. Fortunately, it also has very low Don content compared to other varieties (Fig. 1.1 and Fig. 1.2).

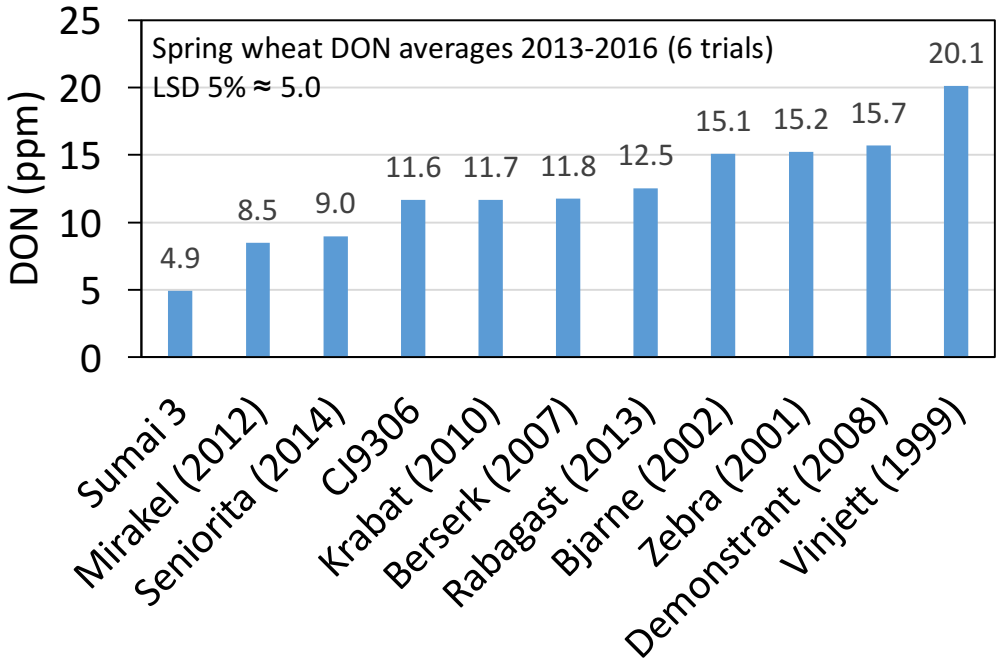


Figure 1. 1: Bar graph for DON content of Norwegian spring wheat, Sumai 3 and CJ9306. Graph prepared from the six field trial datas of Norwegian spring season from 2013 to 2016 by the researcher of NMBU and Graminor in Norway.

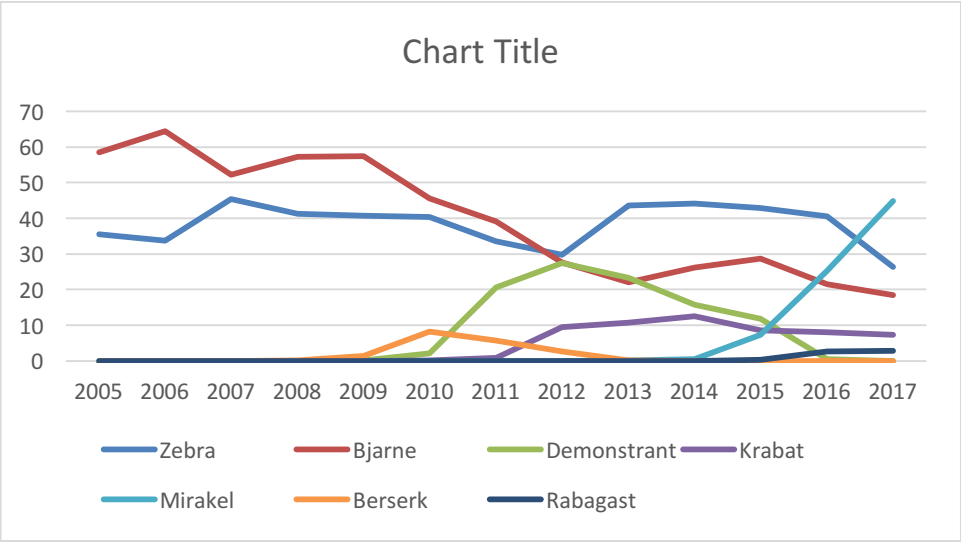


Figure 1.2: Line graph showing the market share (%) of Norwegian spring varieties from 2005 to 2017 (Strand 2017). Note the trend of market growth or release of cultivars with less toxin content.

1.2 Fusarium head blight (FHB)

1.2.1 FHB environment

For FHB to develop, several factors must work together. Firstly, the pathogen must be present. Thereafter, climatic conditions such as rainfall and temperature during the flowering stage are of importance for infection and further disease development along with agronomic factors (reduced tillage, no crop rotation). In general, wet and warm conditions during the flowering stage will accelerate the fungal activity and the risk of developing serious FHB damages (Xu 2003, Cai 2012). Rain during flowering stage helps *Fusarium* in germination and enter inside plant tissue.

As mentioned, the *Fusarium* toxicity is encountered with the production of DON, nivalenol, zearelenone and other mycotoxins. Mycotoxins are mainly due to *F. graminearum* and *F. culmorum*. Looking at Europe, *F.graminearum* was previously most common in the southern parts, while *F. culmorum* was more common in the north, including the Scandinavian countries (Bottalico and Perrone 2002). However, the picture has changed and *F.graminearum* is now also causing infection in the north (Hofgaard, Aamot et al. 2016). Use of susceptible cultivars, inadequate crop rotation, and reduced tillage combined with moist and warm weather may cause serious epidemics (Dill-Macky and Jones 2000, Champeil, Doré et al. 2004, Edwards 2004, Beyer, Klix et al. 2006).

1.2.2 Life cycle and infection path

The life cycle of the *F. graminearum* pathogen has both a sexual and an asexual stage. The asexual stage produces spore microconidia, and this stage is termed *F. graminearum*. The sexual stage is termed *Gibberella zeae* and in this stage, ascospores are produced. Crop debrises are the main source, holding spores from past FHB incidence. Ascospores can be transported over long distances by wind. In addition, rain splash can carry these ascospores from crop debris on the ground up to spikelets (Fig. 1) (Gregory, Guthrie et al. 1959, Trail 2009). Brown discoloration at the base of the spikelets is the first visible symptom of FHB infection (De Wolf, Madden et al. 2003). After some days, the discoloration continues towards the adjacent florets and infected spikes get orangish or pinkish discoloration. Infected florets get infertile and the kernels bleached, shriveled and chalky (tombstone), if at all developed (Bai and Shaner 1994). *F. graminearum* is more agressive than *F.culmorum* and other species and has regular and abundant sexual stage (*Gibberella zeae*) therefore produces ascospores from colonized residue of perithecia which are then dispersed in air for infection (Fernando, Paulitz et al. 1997).

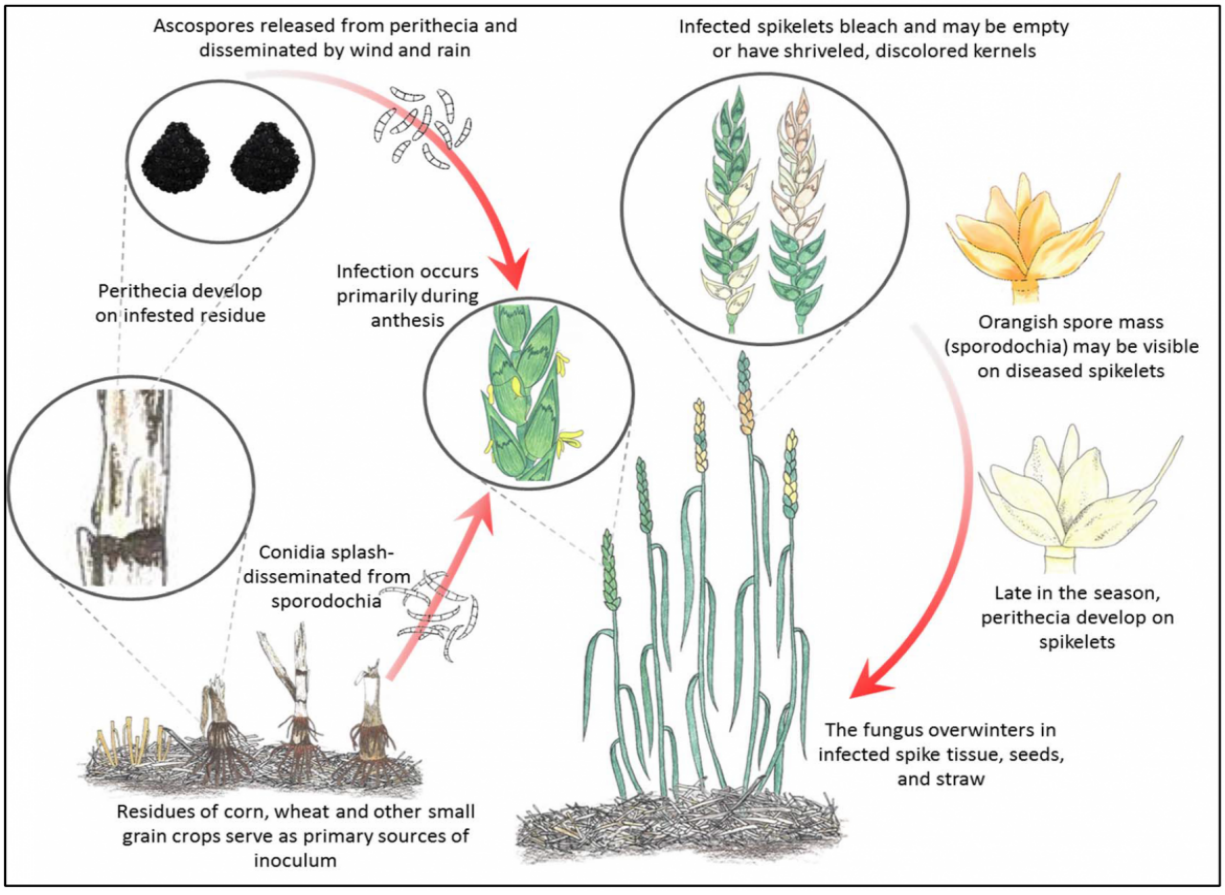


Figure 1.3: Life cycle of FHB (Karasi Mills et al.2016).

Figure 1.4: FHB affected head with pinkish or orangish discoloration (left) and Shriveled FHB affected kernel (right) (Karasi Mills et al.2016)

1.2.3 Types of FHB resistance

FHB resistance is a quantitative trait that is made up of the sum of five active resistance types, hereafter termed as Type I-V (Mesterházy, Bartók et al. 1999). Type I is resistance to initial infection, Type II is resistance to spread of fungus, Type III is resistance to toxin accumulation, Type IV is resistance to infection in kernel, Type V is resistance to tolerance. Along with these, passive resistance types of *Fusarium* are also reported (Mesterházy 1995) Type I: plant height, Type II: presence/ absence of awn, Type III: Spike density in individual head, Type IV: Flowering in boot stage and anther extrusion (AE).

Our research was focused on Type I, II and III active resistance along with resistance manipulated by plant height, days to heading and anther extrusion.

1.3 Breeding for FHB resistance - Marker Assisted Selection (MAS)

The most sustainable way to reduse disease problem, yield loss caused by disease, and increased mycotoxin level, is to work on resistance breeding. The ultimate aim of such a breeding is to develop resistant varieties against *fusarium*. Resistance breeding for FHB was initiated in China in the 1980s (Wu, Shen et al. 1984) and in Europe and North America in the 1990s (Miedaner 1997, Rudd, Horsley et al. 2001, Jiang, Shi et al. 2007). Access to germplasm and good knowledge and understanding of the resistance mechanisms and the genetics of the resistance is needed to move forward (Jiang, Shi et al. 2007). Detection of Quantitative trait loci (QTL) and association mapping is becoming an efficient way to detect favorable resistance QTL that support breeders to understand the genetic basis of the complex resistance mechanisms.

1.3.1 Molecular Markers

In recent decades, breeding programs are being performed based on a combination of using genomic tools and phenotypic characterization. Types of genetic markers are of morphological types (visible), biochemical types (variation of isoenzymes) and molecular (DNA) types, the latter exploring variation sites in DNA (Jones, Ougham et al. 1997). Today, genetic markes often refer to molecular markers.

Phenotypic breeding, with selection based on visual observations or measurements alone, is often time consuming and costly as compared to breeding using molecular markers. MAS is the selection of progenies based on the presence of molecular markers. When successful, such a method is a highly cost efficient and reliable supplement to conventional methods (Collard, Jahufer et al. 2005). The markers (often termed signs of flags) by themselves do not control the trait, as they are only located near the controlling gene, but these markers can show the position of the gene of interest (Collard, Jahufer et al. 2005). These markers are used for construction of linkage maps or for QTL mapping. Polymorphic markers are those which differentiate between the population genotypes and can be dominant (presence/absence allele) or co-dominant (difference in allele size). However, those which do not discriminate between genotypes are monomorphic markers.

1.3.2 Polymerase chain reaction (PCR)

PCR in is the most commonly used technique in molecular biology. It helps to replicate the genomic DNA exponentially from very few numbers of template strand. Kary Mullis (1983) developed the PCR method which is now used in almost every medical and biological research lab around the world. It involves the denaturation of tempelate genomic DNA, annealing of forward and reverse primers by the help of Taq polymerase and elongation of new formed DNA template by adding nucleotides. The general working mechanism of PCR is explained in Fig. 1.6 in KASP genotyping process.

There are different types of molecular marker systems. The marker systems used in this research are PCR based, and are used in conforming the presence or absence of QTL. These marker types are described in more in detail below.

Sequence Tagged Sites (STS)

STS are DNA sequences 100 to 500 bp long co-dominant marker which are easily detectable and usually occur only once in the genome being studied. STS are short repeated nucleotide sequence in genome region which are easily confirmed by PCR (Saiki, Gelfand et al. 1988). For this reason, they are being used in constructing genetic and physical maps from the available sequence data in many laboratories. STS DNA marker contains huge information showing around 70 % to 90% heterozygosity within individual cultivar along with variation in gene level between cultivars; this made this marker potential for mapping and typing genome (Thomas and Scott 1993). STS is also known as an alternative to Random Amplified Polymorphic DNA, where primer designing is done from low mapped copy of repeated sequences (Talbert, Blake et al. 1994). This STR based PCR is found to be useful in getting essential molecular markers in hexaploid wheat.

Single Sequence Repeat (SSR):

SSR or microsatellite is a class of STS that is highly polymorphic in nature. These are a tandem repeats of 1-10 nucleotides in the genomic region of DNA along with interspersed repeates. SSRs or microsatellites are codominant markers that are technically simple, robust and transferable between populations (Powell, Morgante et al. 1996). SSR having 10 times high mutation rate than point mutation (10^3 to 10^6 per cell generation) were extensively used in breeding these days (Gemayel, Cho et al. 2012). Due to the fact that SSR have higher mutation rate they are less available in gene region and are randomly distributed (Vieira, Santini et al. 2016). However, large amount of time is required for the production of the primers and the analysis generally need polyacrylamide gel or capillary electrophoresis which may create time and economic constraints in genotyping.

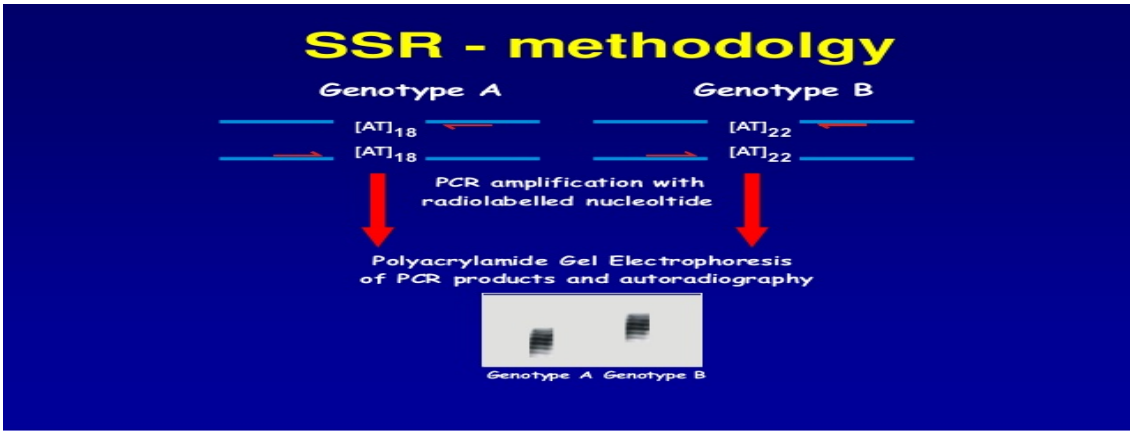


Figure 1. 5*: STS/SSR working process (Bahauddin Zakariya University, Lahore 2017)*

Kompetitive Allele-Specific PCR for Single Nucleotide Polymorphism

SNP is a change in a single nucleotide base (Adenine, Thiamin, Cytocin, Guanine) in a DNA sequence with the complementary base along the same position (Vignal, Milan et al. 2002). KASP genotyping is a unique type of competitive allele-specific PCR along with homogenous and novel fluorescence based visualization technique. KASP technique was developed by KBioscience for in-house genotyping which later turned into global benchmark in genotyping technology (Semagn, Babu et al. 2014). KASP system uses a technique based on allele specific oligo extension and fluorescence resonance energy transfer (FRET) for one generation (Kumpatla, Buyyarapu et al. 2012). This system helps in measuring genetic variation at nucleotide level to detect SNP (He, Holme et al. 2014). The KASP technique has been used across the field of animal, human and plant genetics, and both in 96-, 384-, and 1,536- well plates (He, Holme et al. 2014).

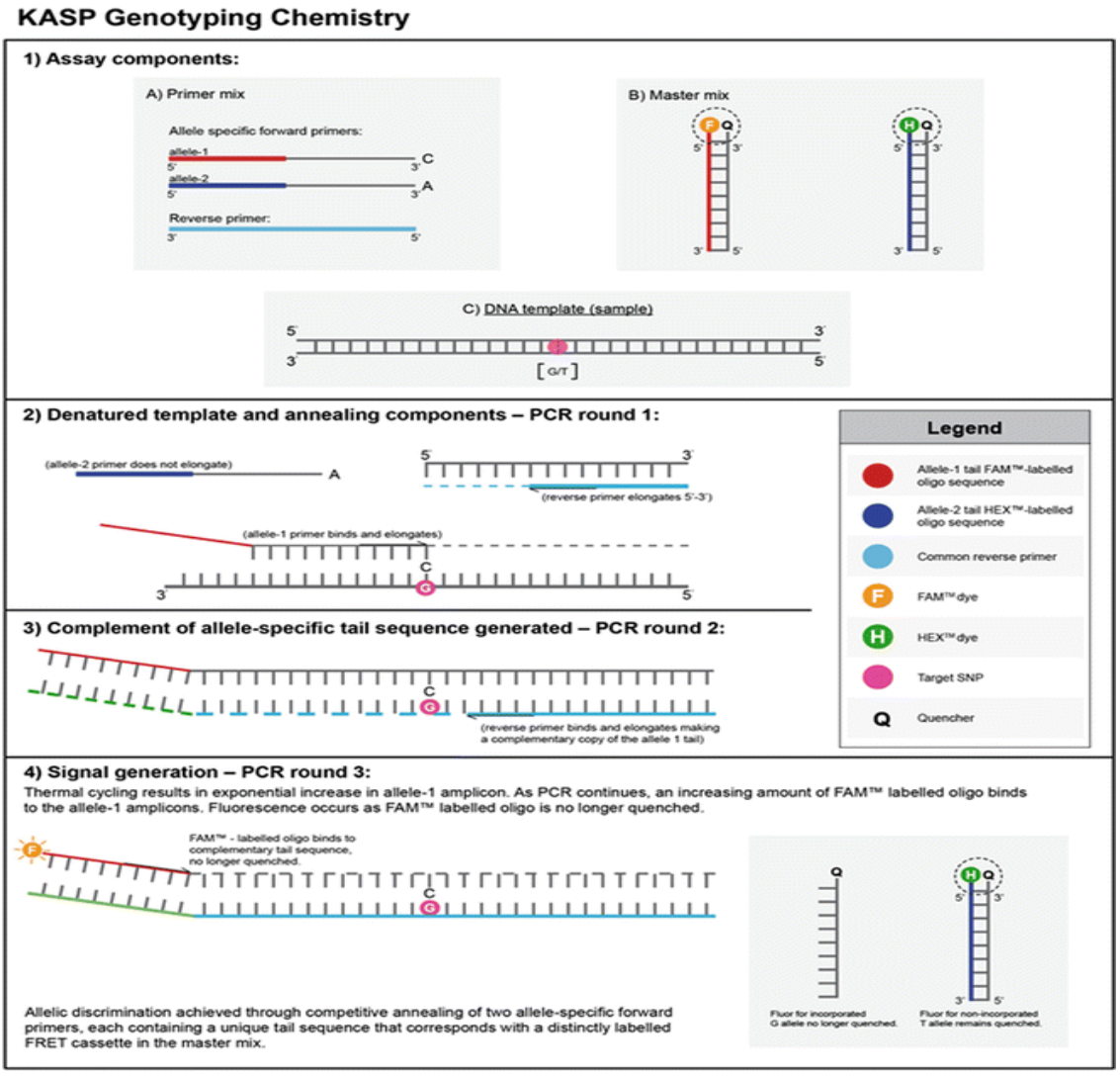


Figure 1.6: KASP Genotyping Methodology explained (He, Holme et al. 2014)

The availability of DNA sequence in recent years have facilitated the recognition and development of KASP/SNP markers and also in replacing SSR and other genetic markers in genotyping many crop species (Semagn, Babu et al. 2014). Low assay cost in labrotary, abundant number of SNP in DNA, specificity of locus, co-dominant inheritance, lowgenotyping error (0.7-1.6%) and simple documentation (Rafalski 2002, Schlötterer 2004), it turned out a strong tool in genetic applications like quality control analysis, linkage mapping and QTL mapping. The figure 1.6 explains the detailed working of KASP genotyping.

1.3.3 QTL mapping and Association mapping

Linkage map construction involves formation of a mapping population, identification of the existence of polymorphism, and a linkage analysis of the markers using software like Map maker/EXP (Lincoln, Daly et al. 1993) or MapManager QTX (Manly, Cudmore Jr et al. 2001). These softwares are available for free, however considered old. In recent QTL mapping process commonly used free software is QTL ICImapping (Meng, Li et al. 2015). The important traits of agriculture production system like yield, disease resistance, tolerance to biotic and abiotic stress and quality are known as quantitative trait and are governed by many genes. The genomic regions which include genes governing the certain trait are called Quantative Trait Locus (QTL) (Collard, Jahufer et al. 2005). The procedure of linkage map construction and QTL analysis for identification of genomic region governing the trait is QTL mapping (McCough and Doerge 1995).

Genomic-wide-association mapping(GWAS)/Association Mapping in recent years helped in detecting QTL that are resistant to various fungi, which facilitates capturing recombination events and processes wide genetic parameters collected. GWAS is conducted on collection of breeding line where association between marker and traits are identified without linkage map construction. It helps plant breeders in collection of important QTL for specific resistance and introgress them in breeding program. However, care should be taken to correct for false positive associations due to kinship and population structure as described by Gupta, Kulwal et al. (2014).

1.4 QTL for FHB resistance

In recent years, many QTL have been reported having association with FHB resistance and these have been linked to different chromosomes or chromosome sites. For Type I resistance, QTL have been reported on chromosome arms 3AS, 3BS, 4B, 5AS and 5DL (Yu, Bai et al. 2008). 'Sumai-3' and its derivatives have been used as the main source for FHB resistance in breeding programmes around the world. The novel germplasm 'CJ9306' has the potential of being superior to 'Sumai 3' in both FHB resistance and agronomic performance (Jiang and Ward 2006, Jiang, Shi et al. 2007). 'CJ9306' was developed by crossing multiple-parent and recurrent selection combining modified pedigree with the aid of male sterile gene (Jiang, Shi et al. 2007). This line is believed to catch interest for resistance breeding and production. It is also being used as an important exotic source of FHB resistance in Norwegian spring wheat breeding. Studies explored the inheritance trait of 'CJ9306' with major and minor QTL (Jiang and Ward 2006). Due to the presence of markers for *Fhb1*, this QTL is used worldwide, like in Alsen (an U.S variety)(Mergoum, Frohberg et al. 2007). There is also a 2DL QTL in CJ9306 which was validated by Jiang, Shi et al. (2007). Besides having better field resistance similar to Sumai 3, CJ9306 harbors excellent resistance to mycotoxin accumulation and high Type II resistance (Jiang and Ward 2006). Three QTL are used for this research and they are described briefly below.

QTL *Fhb1* on 3BS

Fhb1 (syn. *Qfhs.ndsu-3BS*) is the most researched FHB resistance gene till date and was fine mapped to the 3BS chromosome segment (Cuthbert, Somers et al. 2006). Sumai 3 (Chinese variety) and Nyubai (Japnese variety) were used as major parents for this gene harboring on same locus (Cuthbert, Somers et al. 2006). The genotype carrying *Fhb1* resistance allele is found significant in disease severity reduction by 23% and decreased kernel infection by 27% (Pumphrey, Bernardo et al. 2007). This QTL contributes for Type I, II and III active resistance and explains 14% phenotypic variance on severity after point inoculation in China and below 7% after inoculation made by spray in Norway and Hungary (Lu, Szabo-Hever et al. 2011) . *Fhb1* is also considered as additive gene compared to other QTL and specialized in Type II active resistance (Cuthbert, Somers et al. 2006). The resistance mechanism for Nyubai with *Fhb1* was due to thickening of cell walls caused by deposition of hydroxycinnamic acid amides, flavonoids and glucosides, however, not due to transformation of DON to reduced toxic DON 3-O-glucoside **(**Gunnaiah**,** Kushalappa et al. 2012**).** This major QTL *Fhb1* was

also validated in CJ9306 and showed phenotypic variation of 18.2-27.8% on individual experiment and 30.7% when all three replicated experiment were combimed (Jiang, Shi et al. 2007). This is the only gene for *Fusarium* resistance that has been successfully cloned (Rawat, Pumphrey et al. 2016) and shown to encode a chimeric lectin.

QTL on 2DL

The next major QTL, on chromosome 2DL (*Qfhs.nau-2DL*) is responsible for Type II active resistance and the collection of Chinese germplasm have shown to possess this QTL. This QTL was validated in CJ9306 with 9.9-28.4% phenotypic variance and with a greater QTL-environment interaction (Jiang, Shi et al. 2007). Within the cluster 2DL; QTL 2DLc for FHB infection and DON was mapped in wheat lines SHA3/CBRD (Lu, Lillemo et al. 2013) and Soru#1 (He, Lillemo et al. 2016) and was a major QTL for disease severity and DON in both studies. Genotypes with both QTL 2DL and QTL 3BS showed a 32% reduction in spread of disease on single floret injections (Somers, Fedak et al. 2003).

QTL *Fhb5* on 5AS

QTL 5AS (*Qfhi.nau-5A*) is a important Type I resistance QTL of FHB on germplasm Wangshuibai (Xue, Xu et al. 2011). This gene was fine mapped to an interval of 0.3cM (Lu, Szabo-Hever et al. 2011). 5A showed 16% phenotypic variance in Recombinant Inbreed Lines for Type I resistance (Lin, Xue et al. 2006) which is supported by 17% phenotypic variance after spray inoculation in 2005 (Lu, Szabo-Hever et al. 2011). This QTL was validated by joint trial/experiment Interval Mapping/Composite Interval Mapping and simple marker analysis (Jiang, Shi et al. 2007). QTL 5A with 3BS showed a 17 % reduction in DON accumulation (Somers, Fedak et al. 2003).

1.5 Association between Anther extrusion and Plant height with FHB

The role of Anther extrusion (AE) and Plant height (PH) on FHB biology was first considered by Percival in 1921 (Lu, Lillemo et al. 2013). QTL were observed associated with PH and AE, tall alleles and high AE contributed by SHA3/CBRD along with reduced PH and low AE always linked with increased susceptibility after spray and spawn inoculation in a cross between SHA3/CBRD and Naxos; a German spring wheat (Lu, Lillemo et al. 2013).

Previous research results described no significant correlation between hyphal growth of *F. graminearum* and substrate in floret (anther, palea, or lemma) (Engle, Lipps et al. 2004). However, AE showed strong negative correlations with both FHB (-0.53 to -0.69, P=0.0001) and DON content (r= -0.39 to -0.46, P= 0.0001) in double haploid population Arina x NK93604 (Skinnes, Semagn et al. 2010). QTL for AE and association of FHB and DON with AE were first explained by this report. QTL are detected on the same region for AE and FHB, on chromosome 7AL (Skinnes, Semagn et al. 2010) and on chromosome 2DLc, 4BS, 7AL (Lu, Lillemo et al. 2013). Minor QTL of FHB coinciding with AE are also seen (He, Lillemo et al. 2016) Furthermore, the high choline and betaine content present in anther enhance *Fusarium* growth and infect spike tissue (Bai and Shaner 1994) which also showed negative correlation between AE and FHB. Massive growth of *F. graminearum* occurred on extruded anther than other parts of wheat after 48 hour of artificial inoculation (Strange and Smith 1971).

Negative association between PH and FHB severity was shown by meta-analysis of QTL for reported *Rht* genes and more PH QTL (Mao, Wei et al. 2010). *Rht* gene of NILs showed higher resistance level for tall plants than their dwarf counterparts (Yan, Li et al. 2011). However, these resistance level showed no difference when the dwarf isolines were raised physically so that the spikes of all NILs were on same height. Fusarium spores harboring on debris in the ground are found to be the prominent source of infection, the reason why shorter plants tend to be susceptible to FHB (Miedaner and Voss 2008). 50% of wheat grown in Britain and Germany carry dwarfing allele *Rht-D1b* or *Rht-B1b* which increases the susceptibility by up to 30% (Gosman et al., 2007; Knopf et al., 2008). QTL mapping of gene *Rht-D1b* and *Rht-B1b* from Norin 10 also coincide with major QTL for susceptibility in spray inoculation (Srinivasachary et al 2009, Holzapfel). Study conducted on two double haploid population for evaluating impact of two dwarfing allele *Rht-D1b or Rht-B1b* showed 0-41% disease suscebtibility and 13-23% reduction in AE (He, Singh et al. 2016). They also proposed the high FHB incidence might be due to reducing AE ability of the two dwarfing alleles.
However, recent research on *Rht24* dwarfing gene on chromosome 6A in winter wheat population ‘Solitaire X Bussard’, showed plant height reduction without increasing FHB severity (Herter, Ebmeyer et al. 2018).

1.6 Objectives

The research is focussed on the FHB infection caused by *F. graminearum* on NILs developed from exotic source of FHB resistance CJ9306 and spring wheat cultivars of Norway (Zebra & Berserk). Scoring of FHB severity from Vollebekk research station and plant agronomic traits from Bjørke & Staur was made. Then the genotyping of all NILs were carried out in the CICENE genotyping lab of NMBU.

The objectives were to:

I. Confirm the allelic states of Fusarium head blight resistance QTL in the NILs by marker genotyping.
II. Assess the effect of the three QTL and their combination on FHB resistance traits (visual symptom, DON content, anther extrusion).
III. Evaluate potential side-effects of the QTL on important agronomic traits like plant height, grain yield, and grain quality parameters.

2. Materials and Methods

2.1 Plant Material

For developing the working NILs; FHB resistant exotic Chinese germplasm CJ9306 and adapted spring cultivar Zebra (moderately susceptible) and Berserk (moderately resistance) were used. To develop Near-isogenic lines (NILs); BC_1F_5 populations of CJ9306 were used that were back-crossed to the adapted spring wheat cvs.'Zebra' and 'Berserk' as a section of ongoing work of Graminor to introgress exotic resistance to Norwegian spring wheat (Figure 2.1).

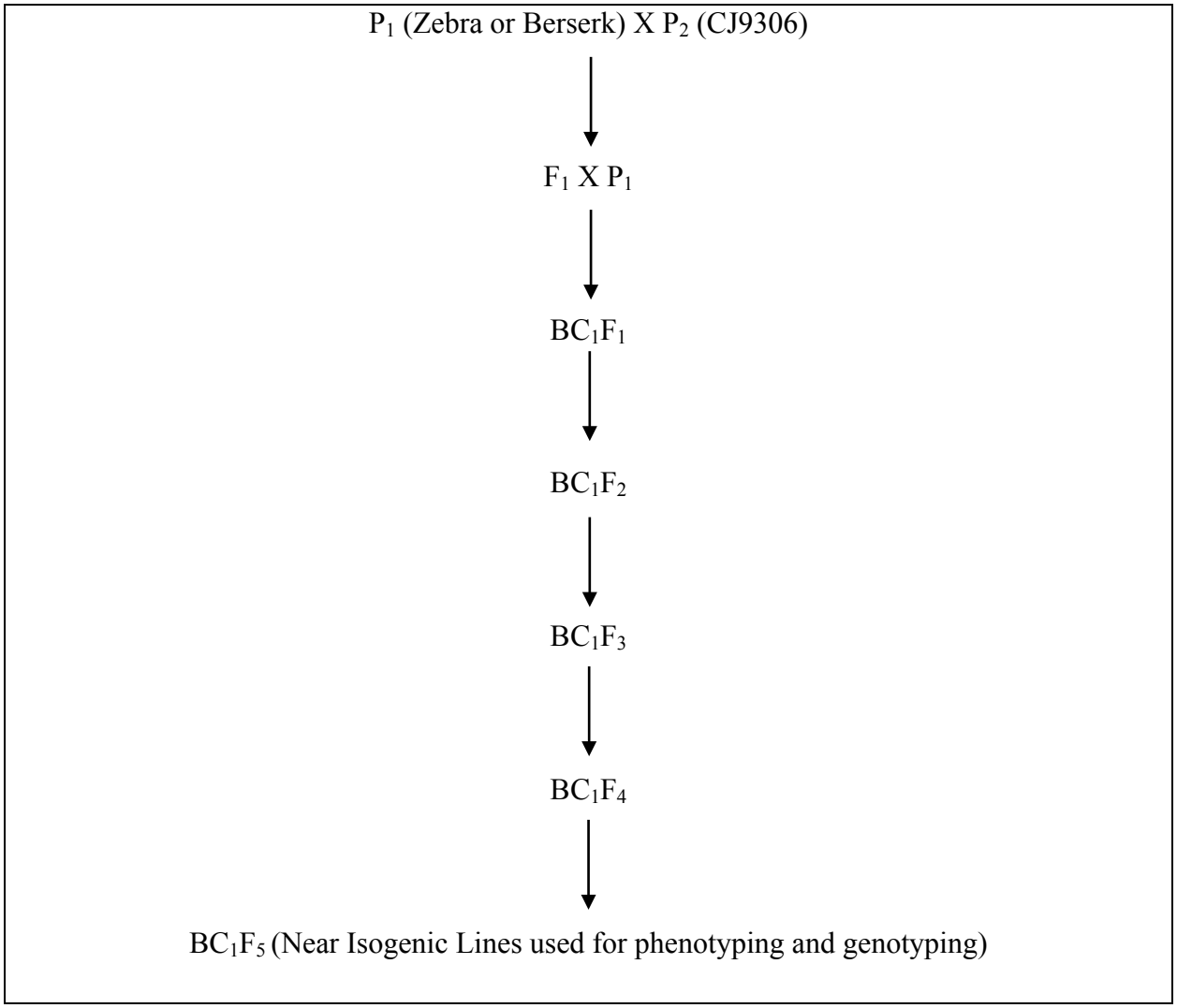


Figure 2. 1. Development of NILs from single backcross and continued selfing of CJ9306 and Zebra or Berserk background.

The pre-selection of population in BC_1F_1 was done for heterozygosity at the three different FHB resistance QTL contributed by CJ9306: *Fhb1* on 3BS and major QTL on 2DL and 5AS. The population was genotyped in Feb 2015 with flanking marker for respective QTL in order to select breeding lines combining the three QTL. This genotyping was done on seed from BC_1F_5 population selected from Graminor breeding nursery of 2014. Heads that still segregated for any of these three QTL were identified and individual porogenies were selected from such heads with contrasting homozygous marker genotypes for the respective QTL. The resulting NILs were used for seed increment at Vollebekk research farm in spring of 2015. In 2016, these lines were tested for FHB resistance through grain spawn inoculation and mist irrigation in FHB Nursery at Vollebekk and seed increment at fungicided trial was preformed at the same location. Data on anther extrusion, days to heading and plant height from infection scoring trial were also recorded. The seed increment of field trial 2016 were then used for performing two agronomic field trial in Bjørke and Staur; and one FHB scoring trial in Vollebekk in 2017.

CJ9306 is a derivative of Sumai 3 and have QTL responsible for FHB resistance as described in the introduction. The three important QTL were introgressed into Zebra and Berserk using SSR markers. These were:

- *Fhb1* on 3BS: UMN10
- QTL on 2DL: gwm539
- QTL on 5AS: gwm304, gwm293, gwm415

In total, 57 Near Isogenic Lines (NILs) population were developed from CJ9306 (high FHB resistance) and Berserk and Zebra in generation BC_1F_5. The NILs and the check varieties Berserk and Zebra along with the FHB resistance donor CJ9306 made up the material of my study.

2.2 Agronomic field trials

Field trials were carried out at Graminor's experimental fields at Staur (60^{o} N, 153 masl) and Bjørke (62^{o}N, 68 masl). Sowing was done in 12th May in Staur and 8th May in Bjørke. The experimental design we used was modified Randomized Complete Block Design (RCBD) where replication and family were random. The experiment included two replications with 65 individual plots in each which included NILs and 2-3 replicated parents. Sister lines of same

family were odered differently between the replication but were always planted next to eachother to provide almost similar field growing condition for the whole family. The plot size was 1.5x2 m. Traits like days to heading (DH), anther extrusion (AE), and plant height (PH) were assessed during the season. Parameters like grain yield and moisture, protein and starch contents, test weight, 1000 kernels weight were measured in Graminor laboratory after harvest, in December 2017.

Figure 2. 2: The agronomic field trial at Staur in the bank of lake Mjøsa

2.3 Fusarium field design, inoculation and scoring trial

A Fusarium scoring trial was conducted in 2017 at Vollebek research station at Ås/NMBU (59^{o} N, 90 masl) applying a modified RCBD experimental design with three replicates. Sowing was done in May 26 and an arrangement with mist irrigation was set up over the entire experimental field before head emergence.

Figure 2. 3: FHB field inspection experimental plot of Vollebekk/Ås with mist irrigation setup.

Spawn inoculum (*F. graminearum*-inoculated oat kernels) was prepared in a greenhouse at Vollebekk. This inoculum was prepared based on the protocol of Dr. Bernd Rodemann (Julius Kuhn-Institut, Germany) as disceribed by Tekle, Lillemo et al. (2018). The isolates were provided by Norwegian Veterinary Institute which are collected either from wheat or oat field in growing season in Norway. Thus obtained isolate were grown on potato dextrose agar (PDA) in ambient temperature and light for 7 days. By the end of incubation period, five to six pieces of PDA containing mycelia of isolate were transferred into flasks containing sterile deionized 100 ml water and 1 g oat flour. These mixtures were then placed at shaker set at 90 rpm for 7 days at ambient light and temperature to make liquid *F. graminearum* culture. The liquid culture is then being used to inoculate 2 kg of sterile and cooked oat kernels which were soaked in water overnight, autoclaved for 3 hours at 121°C in a heat stable polyethylene bag. Then prepared culture product were left for 3 weeks in upright position in ambient light and temperature to colonize the kernels. Then the product was left to dry in trolleys with supplemental mist irrigation (sterile distilled water) to facilitate perithecial development. Lastly, after 3 weeks prepared inoculum were stored in 15-25°C until the date of inoculation. Grain spawn inoculum was then spread in FHB scoring field trial at Zadoks stage 32-33 with the density of 10 g/m^2. To induce disease pressure (optimal germination of ascospore), mist irrigation (10-15 min/hr.) was applied in evening from 17:00- 23:00 every day from booting to flowering stage.

2.4 FHB experimental trial 2016

The trial was conducted in Vollebekk research station in the spring growing season by Morten Lillemo. Two replications of NILs for *Fusarium* disease scoring and one for seed increament were done. The mode of inoculum, disease scoring and agronomic data collection were similar to the research of 2017. DH, AE and PH were measured from the field and DON content later after harvest. The results from this experiment are also included here to compare the result with 2017 field trials.

2.5 Phenotype scoring

FHB disease assessment

Disease assessment was performed at Vollebekk experimental farm at Ås/NMBU. The assesments were carried out when the peduncles were starting to turn yellow but heads still were green. The scoring was done three times in 12th, 14th and 18th of August 2017. The evaluation was performed visually by counting *Fusarium* –infected spikelets and dividing this by total number of spikelets, which gave the percentage of infected spikelets for individual plots. Twenty heads per plot (from both ends of plot) were counted for scoring each time. Mean of three recordings were used as FHB severity for data analysis.

Figure 2. 4: FHB infected spikelets in the Vollebekk field; infection starting from top (right) and infection starting from middle (leftt).

DON measurement

DON content was measured for all samples of FHB scoring field of Vollebekk with spawn inoculation. After harvest, grain samples were sent for analysis at University of Minnesota that applied Gas Chromatography-Mass Spectrometry (Mirocha, Kolaczkowski et al. 1998, Fuentes, Mickelson et al. 2005) for DON measurement.

Days to heading

Days to heading (DH) were scored in the two experimental locations at Staur and Bjørke. DH is defined as the number of days from seeding to the time when more than 50% of heads had emerged. One scoring was made for each plot. DH was also scored in the disease scoring field of Vollebekk/Ås.

Plant height and Anther extrusion

Plant height (PH) was measured on all three field trials taking height of plants at 3 places from each plot and computing mean from them. PH was also measured in the disease scoring field at Vollebekk. Anther extrusion (AE) was scored in the two field trials at Staur and Bjørke and was scored visually using a 0 to 9 scale where 0 represents no anther extrusion and 9 full anther extrusion as described by Skinnes, Semagn et al. (2010).

Figure 2. 5: Flowering stage of wheat at Staur. Note the Anther extrusion (Anthers coming out) on left and anther residing inside the spike on right

2.6 Genotyping

DNA extraction

To perform genotyping of the NILs (same used in field trial), their seed were grown in greenhouse at SKP at NMBU, Ås. The genotyping included 57 NILs, 1 no-template control, 2 replications of parents (Zebra, Berserk and CJ9306) and 35 MASBASIS l to fill the tray and to run 96 well plate for convinience. MASBASIS include the adapted cultivars of Norway and some exotic resistant lines and cultivars. The MASBASIS are not analysed in this thesis but are listed in Appendix Table 7. The material was planted with four seeds of each line in a 96 well tray in greenhouse. Then the prepared tray with seeds were kept in a cold room for a day to enhance germination. The plants were grown in greenhouse for 10 days to get optimum seedling size.

A small leaf sample (50-70 g) was cut from each line and kept in a tube with tungsten carbide beads to improve sample grinding. The 96 well plate with leaf samples were stored at minus 80°C freezer for a day before DNA extraction. DNeasy Plant DNA extraction kit (QIAGEN) was used for the extraction of Genomic DNA from NILs and parents. Buffer AP1 (preheated to 80°C), RNase A (100 mg/ml), and Reagent DX, Buffer AW1, Buffer AW2 are used for DNA extraction. Agarose gel (1%) was run to see the bands of DNA, which were viewed in a Molecular imager "GelDoc ^TM XR+" from BIORAD. Nanodrop analysis was used to find the concentration of genomic DNA of all samples.

KASP Genotyping

DNA samples were first diluted to a concentration of 10 ng/µl. LGC Genomics protocol was used for the KASP genotyping and forward and reverse primers were used. All samples were fitted in a 384 well plate. The KASP markers UMN10_SNP, wMAS000033/Vrn-A1_9K0001, IAAV5302 and Tdur_contig4633 were used from laboratory of NMBU which was ordered from LGC Group. The four KASP markers used to genotype the samples (NILs and parents) are presented in Table 2.1.

Table 2. 1: KASP Markers used in genotyping the NILs.

KASP Marker	**Characterization**
UMN10_SNP (*fhb1*)	KASP marker for *Fhb1* normally used for MAS GG: Resistance Allele(CJ9306) AA: Susceptible Allele (Zebra, Berserk)
*Vrn-A1*_9K0001 (wMAS0033)	Marker for the *Vrn-A1* vernalization gene X:X = *vrn-A1* (winter) (CJ9306) Y:Y = *Vrn-A1* (spring) (Zebra, Berserk)
IAAV5302	90K SNP on 3B validated for FHB resistance (Sørensen 2016) X:X = high FHB (Zebra) Y:Y = low FHB (Berserk, CJ9306)
Tdurum_contig 46334_832	90K SNP on 3B validated for FHB resistance (Sørensen 2016) X:X = low FHB (CJ9306) Y:Y = high FHB (Zebra, Berserk)

PCR- Condition:

Denaturation step (unwinding of DNA): 94°C for 15 min

Touch down step 1 (10 cycles, 0.8°C down on each cycle):

- 94°C for 20 s
- 63°C for 1 min

Touchdown step 2 (28 cycles):

- 94°C for 20 s
- 55°C for 1 min

Plate reading:

	Excitation (nm)	Emission (nm)
FAM:	485	528
HEX:	528	560
ROX:	575	620

KASP final mix was run with PCR on KASP-55 with condition mentioned above. The plate sample reading was done by FLUOstar Omega F – SNP Microplate Reader in the laboratory at NMBU. To determine the marker alleles, the allele specific primers were with two different

fluorescent dyes were used. One allele is labeled with a dye Hex and another allele is labeled with the dye FEM. Klusterkaller was used to analyze the result.

SSR genotyping

SSR markers used for genotyping were:

- On QTL 2DL: gwm539
- On QTL 5A: gwm304, gwm293, gwm415

96 well PCR plates were used in SSR Genotyping. CIGENE protocols were used for genotyping. Based on the protocol PCR products were multiplaced and analyzed on ABI3730 sequencer for fragment analysis.

STS genotyping

96 well PCR plates were used in STS Genotyping. CIGENE protocols were used for genotyping. Based on the protocol PCR products were mixed together and analyzed on ABI3730 sequencer for fragment analysis.

Table 2. 2: STS markers for genotyping the NILs. These STS markers were provided by Nidhi Rawat in December 2016.

Marker	Forward Primer	Reverse Primer	PCR condition	Polymorphism expected	Product size
Tryp_Syn	AGTACCAACTACAG GCGGAATAC	TCCACCATATAGCA CTCCACGA	TD 67-60	Susceptible allele ~15bp short than Sumai 3 allele	428 bp
HCBT	TGGGGAGGGTGTGA GCATCTCCGCT	AGCACCTCCGGGG CAAGGCACA	TD 67-60	Susceptible allele ~60bp short than Sumai 3 allele	982 bp
SGNH	ATAGAGCGTCACCA TATGCAG	TCGCTCATCAGCCT GTACAG	TD 67-60	Presence/Absence	494 bp

2.7 Statistical analysis

For analyzing all the field trials with both fixed and random effects, linear mixed model were used. Least square means were calculated using the PROC MIXED procedure in SAS software (SAS Institute Inc., Version 9.1). The lsmeans calculation reduces chances of error that may occur due to varying micro climate and field condition. Cross and QTL allele were considered as fixed effect and Replication and family as random variable. Then this calculated LSmeans for all traits were used in further stastistical analysis. Histograms for frequency distributions, Pearson correlation matrixes, boxplots for QTL effects were conducted in R software (R core team 2017). Type 3 test of fixed effect for FHB and traits were calculated from PROC MIXED COVTEST in SAS.

3. Results

3.1 Marker Genotyping

To confIrm the allelic states of QTL, marker genotyping was conducted. The presence/absence or different sized allele were detected in the NILs of CJ9306 X Berserk and Zebra crosses. Through the use of KASP, SSR and STS markers the presence of resistance or susceptible allele on the loci 3BS, 2DL and 5AS were figured out and is also compared to the genotyping results of same NILs done in 2015. All three resistance alleles were present in CJ9306, while Zebra and Berserk carried the susceptible allele at all three respective loci. The introgressing effect were seen on NILs as we found the presence of resistance alleles on the trait locus.
Among the types of marker we used, KASP marker were found to easily confIrm the allelic states in lines. The amplification of all KASP markers UMN10, IAAV5302 and Tdurum_contig4634_832 in 384 well plate were good. However, the genotyping of one KASP marker for vernalization gene Vrn-A1_9K0001(Wmas0033) went wrong due to some experimental error. These amplified marker result are visualized through Fluorescence resonant energy transfer(FRET) plate reader. For STS genotyping we used two co-dominant and one dominant markers which we run through ABI3730 sequencer after PCR DNA amplification. The co-dominant STS markers were able to detect the heterozygosity in our experiment. The STS marker HCBT and Tryp_Syn showed almost ten heterozygous resistance allele out of 57 NILs. These markers were provided by Nidhi Rawat, Kansas State University, in December 2016. The product size of resistance and susceptible allele showed slight variation as expected. Genotyping of SSR marker is done based on CIGENE protocols and was analyzed on ABI3730 sequencer after PCR. The resistance allele for most of the SSR marker were homozygous, however, UMN10 showed heterozygosity for some NILs. Despite, the result of SSR markers for 5AS showed same pattern of presence of resistant or susceptible allele for each individual NILs.

Genotyping result of NILs with all marker details are presented in Table 1 in the Appendix.

Table 3. 1: Presence and absence of resistance allele on three QTL of NILs from marker genotyping. 0 represence presence of susceptible allele and 1 represent presence of resistance allele.

Line	Entry	Name	Fhb1	2DL	5AS
4	**1501**	Zebra	0	0	0
72	**1079**	CJ9306	1	1	1
83	**2204**	Berserk	0	0	0
8304	**1503**	Zebra-2/CJ9306//Zebra-2	0	0	0
8305	**1504**	Zebra-2/CJ9306//Zebra-2	0	0	0
8306	**1505**	Zebra-2/CJ9306//Zebra-2	0	1	0
8307	**1506**	Zebra-2/CJ9306//Zebra-2	1	0	0
8308	**1507**	Zebra-2/CJ9306//Zebra-2	0	0	0
8309	**1508**	Zebra-2/CJ9306//Zebra-2	1	0	0
8310	**1509**	Zebra-2/CJ9306//Zebra-2	0	0	1
8311	**1510**	Zebra-2/CJ9306//Zebra-2	0	0	0
8312	**1601**	Zebra-2/CJ9306//Zebra-2	0	0	0
8313	**1602**	Zebra-2/CJ9306//Zebra-2	1	0	0
8314	**1603**	Zebra-2/CJ9306//Zebra-2	1	1	0
8316	**1605**	Zebra-2/CJ9306//Zebra-2	0	1	0
8317	**1606**	Zebra-2/CJ9306//Zebra-2	1	1	0
8318	**1607**	Zebra-2/CJ9306//Zebra-2	0	0	0
8319	**1608**	Zebra-2/CJ9306//Zebra-2	0	0	0
8320	**1609**	Zebra-2/CJ9306//Zebra-2	0	0	0
8405	**1704**	Zebra-2/CJ9306//Zebra-2	1	1	0
8406	**1705**	Zebra-2/CJ9306//Zebra-2	1	1	0
8407	**1706**	Zebra-2/CJ9306//Zebra-2	1	0	0
8408	**1707**	Zebra-2/CJ9306//Zebra-2	0	1	0
8409	**1708**	Zebra-2/CJ9306//Zebra-2	1	1	0
8410	**1709**	Zebra-2/CJ9306//Zebra-2	1	1	0
8411	**1710**	Zebra-2/CJ9306//Zebra-2	0	1	0
8501	**1807**	Zebra-2/CJ9306//Zebra-2	1	0	1
8502	**1808**	Zebra-2/CJ9306//Zebra-2	0	0	1
8503	**1809**	Zebra-2/CJ9306//Zebra-2	1	0	1
8504	**1810**	Zebra-2/CJ9306//Zebra-2	0	0	1
8505	**1901**	Zebra-2/CJ9306//Zebra-2	1	0	1
8506	**1902**	Zebra-2/CJ9306//Zebra-2	0	0	1
8507	**1903**	Zebra-2/CJ9306//Zebra-2	0	0	1
8510	**1905**	Zebra-2/CJ9306//Zebra-2	1	1	1
8511	**1906**	Zebra-2/CJ9306//Zebra-2	1	0	1
8512	**1907**	Zebra-2/CJ9306//Zebra-2	1	1	1
8513	**1908**	Zebra-2/CJ9306//Zebra-2	1	0	1
8514	**1909**	Zebra-2/CJ9306//Zebra-2	1	1	1
8515	**1910**	Zebra-2/CJ9306//Zebra-2	1	0	1
8516	**2001**	Zebra-2/CJ9306//Zebra-2	1	1	1
8517	**2002**	Zebra-2/CJ9306//Zebra-2	1	1	1
8609	**2102**	Berserk-4/CJ9306//Berserk-4	0	1	0
8610	**2103**	Berserk-4/CJ9306//Berserk-4	0	1	0
8611	**2104**	Berserk-4/CJ9306//Berserk-4	0	0	0
8612	**2105**	Berserk-4/CJ9306//Berserk-4	0	0	0
8613	**2106**	Berserk-4/CJ9306//Berserk-4	0	0	0
8614	**2107**	Berserk-4/CJ9306//Berserk-4	0	0	0
8615	**2108**	Berserk-4/CJ9306//Berserk-4	0	1	0
8616	**2109**	Berserk-4/CJ9306//Berserk-4	0	1	0
8617	**2110**	Berserk-4/CJ9306//Berserk-4	0	0	1
8618	**2201**	Berserk-4/CJ9306//Berserk-4	0	0	0
8619	**2202**	Berserk-4/CJ9306//Berserk-4	0	0	1

8706	**2208**	Berserk-4/CJ9306//Berserk-4	0	0	1
8707	**2209**	Berserk-4/CJ9306//Berserk-4	1	0	1
8708	**2210**	Berserk-4/CJ9306//Berserk-4	1	0	1
8709	**2301**	Berserk-4/CJ9306//Berserk-4	1	0	1
8710	**2302**	Berserk-4/CJ9306//Berserk-4	1	0	1
8711	**2303**	Berserk-4/CJ9306//Berserk-4	0	0	1
8713	**2304**	Berserk-4/CJ9306//Berserk-4	1	0	1
8714	**2305**	Berserk-4/CJ9306//Berserk-4	0	0	1

3.2 Field and weather condition

Sowing in the Bjørke field was three days later than for the Staur field but both Graminor fields were sown in early May 2017. Sowing in the disease assessment field at Vollebekk, Ås was done in late May, 2017. In all the field trials seeds germinated well. A few off-type plants were seen and removed during the monitoring. A few errors were detected in the sowing on some rows, and these errors were taken into account during data collection.

The warmest temperature recorded on Ås (based on meterologigal data of Ås station) was on June & July 2017 with max of 24° C and 25° C respectively and the month of August was a bit cooler ranging from 3°C to 23°C. July 23 recorded highest temperature 25.9°C; hottest day in 2017. The average temperature recorded on August was 16.7°C and 15.6° C in year 2016 and 2017 respectively as reported by Meterologisk Institutt on Ås station. The warmer temperature in 2016 facilitated higher degree infection compared to infection on 2017.
The average temperature recorded on Hamar field areas (Bjørke and Staur) in August 2017 was 14.1°C. In general, wet and cool weather during the 2017 growing season reduced the overall FHB infection compared to year before.

Mode of infection seen

The NILs were in good condition till the beginning of heading stage. However, two weeks after the inoculation of grain spawn, brown discoloration was seen in the spikelets. Thereafter, infection started to spread up and down and changed its color to pinkish, orangish discoloration that were easily detected on visual scoring. The infection was found slightly increased in the later readings than the earlier ones. The infection was seen starting from all section of wheat head (top, middle and bottom) (Fig.2.4).

3.3 Histogram of frequency for LSmean

After grain spawn inoculaton, the FHB severity measured greatly varied in two consequtive years 2016 and 2017. Trial carried out in same location for FHB scoring showed very high FHB average severity of 31.57% in 2016. However, the average FHB severity on 2017 field trial was 0.84%. DON content also greatly varied due to big difference in FHB. In 2016 DON content measured was 16.46 ppm on average and was 5.45 ppm in 2017.

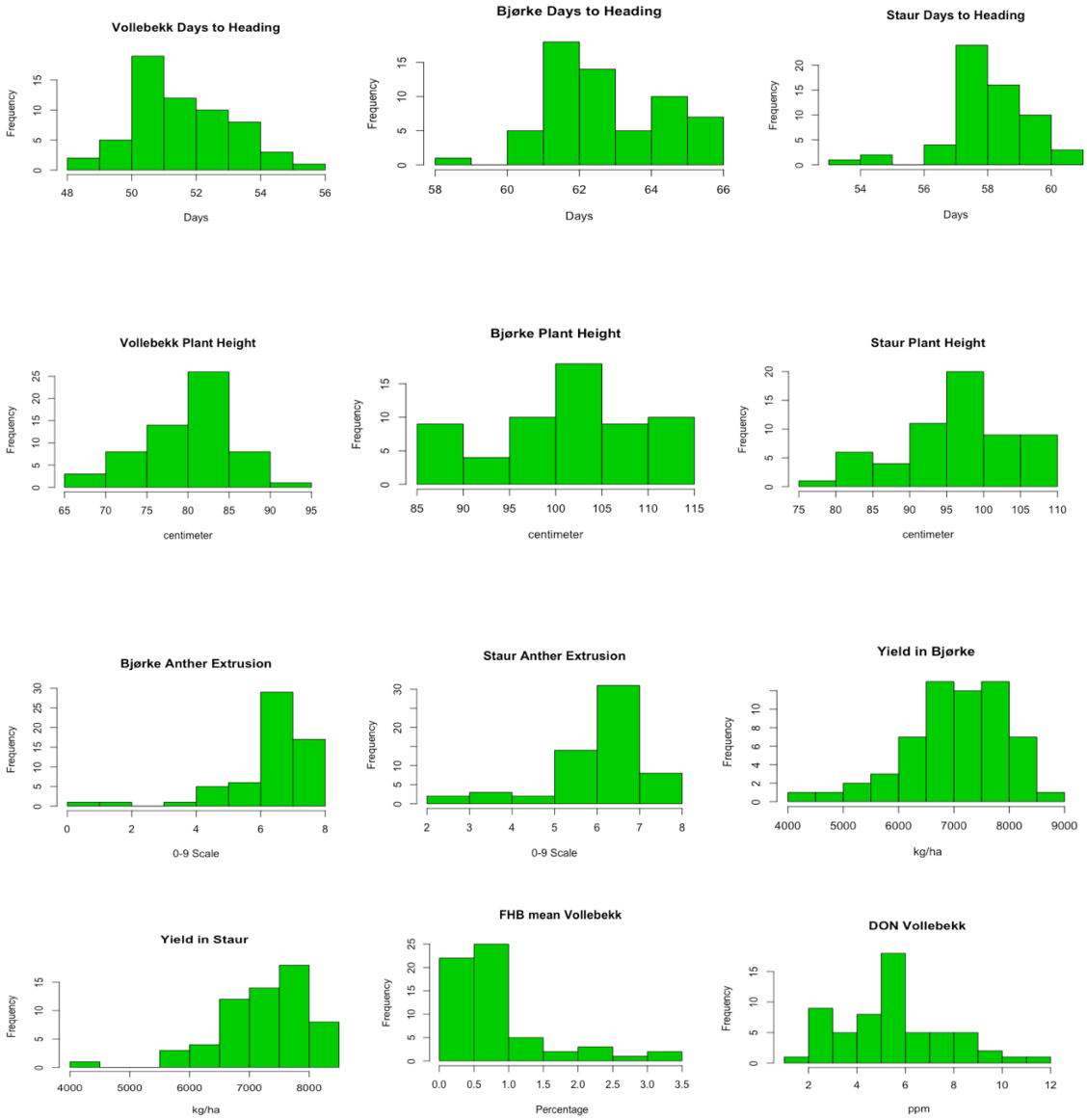


Figure 3. 1: Histograms showing the frequency distribution of FHB associated traits in NILs in 2017 field trials. Histogram of days to heading and plant height in three experimental field, histogram of anther extrusion and yield in two experimental field trial and FHB Mean and DON for fusarium scoring field of Vollebekk.

DH was shorter in Vollebekk field trial than in Bjørke and Staur. The shape of histogram for PH was nearly uniform for all three field trials. AE and Yield were right skewed. FHB severity was right skewed but DON as nearly uniform in the range 2-12 ppm.

*Table 3.2: Pearson correlation coefficient between FHB Mean/DON and quantative traits in the NILs of field trial of 2017 (**p<0.05, ** p<0.001, *** p<0.0001)

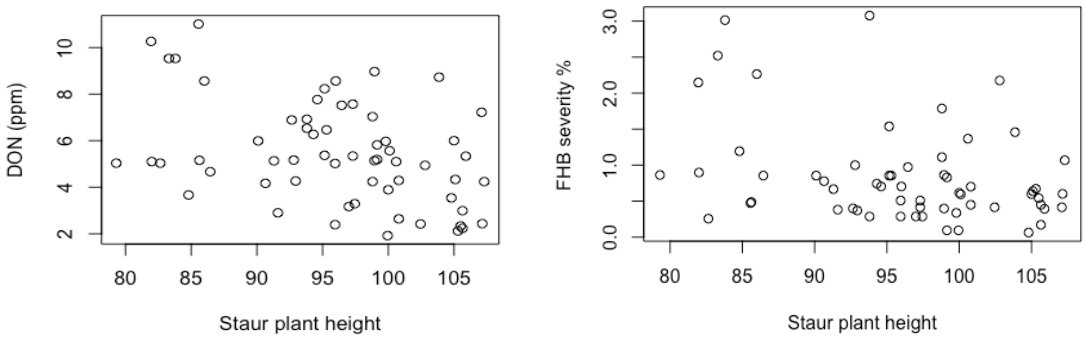
	Vollebekk days to heading	Vollebekk plant height	Bjørke days to heading	Bjørke plant height	Bjørke anther extrusion	Staur days to heading	Staur plant height	Staur anther extrusion	DON
FHB Mean	0.128	-0.241	0.238	-0.357*	-0.298 *	-0.0027	-0.341*	-0.201	0.483 ***
DON Content	-0.121	-0.353*	-0.128	-0.464**	-0.356*	-0.247	-0.413*	-0.371*	

Table 3.3: Pearson correlation coefficient between FHB Mean and DON and quantative traits in the NILs trial of field trial 2016. (*p<0.05, ** p<0.001, *** p<0.0001)

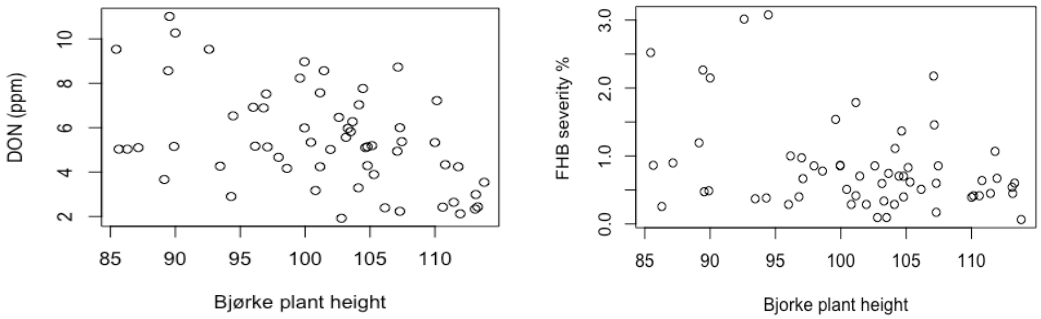
	Vollebekk days to heading	Vollebekk plant height	Vollebekk anther extrusion	DON
DON	-0.027	-0.370*	-0.275	

3.4 DON/FHB and Plant height

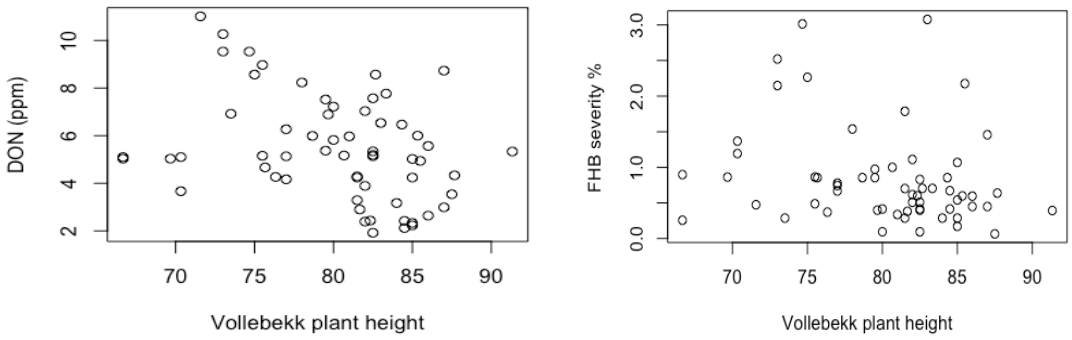
As DON being the consequences of FHB severity the association between DON and PH were seen highly significant (-0.413*, -0.464**, -0.353*) for Staur, Bjørke & Vollebekk respectively and stronger than with FHB (Table.3.2, Figure 3.3). Generally, FHB severity and PH for all trials were seen associated more than other FHB traits. FHB showed high significant negative correlation (-0.357*) with PH in Bjørke. But have slight lower correlation in other two fields of Vollebekk (-0.241) and Staur (-0.341*) (Table 3.2).



(a) Staur field experiment (PH and DON/FHB)



(b)Bjørke field experiment (PH and DON/FHB)

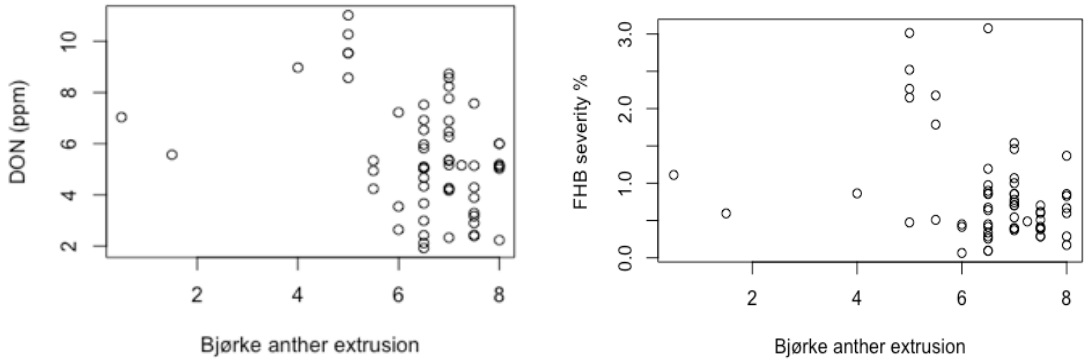


(c)Vollebekk FHB scoring field (PH and DON/FHB)

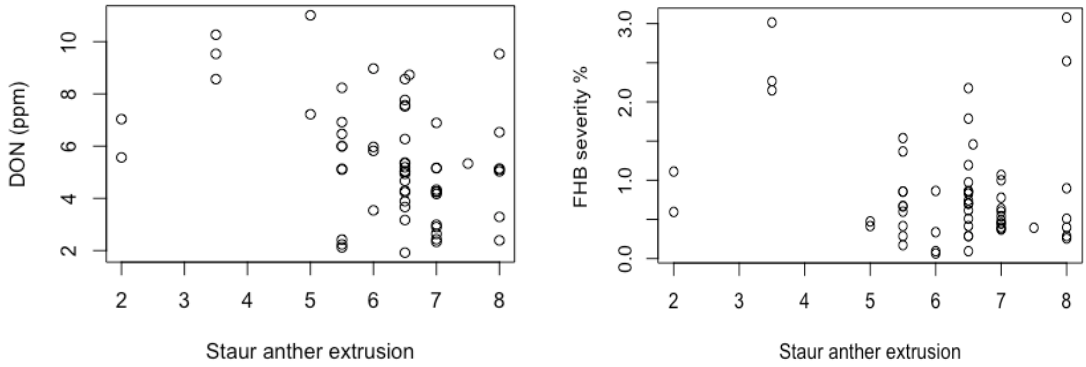
Figure 3. 2: The relationship between PH of three field trial and DON/FHB Mean of Vollebekk field trial after spawn inoculation (a) Staur, (b)Bjørke and (c)Vollebekk

3.6 DON/FHB and Anther extrusion

Association between DON and AE was seen highly significant with correlation of -0.356* and -0.371* on Bjørke and Staur field trial (Table.3.2). AE was only scored in two fields of Bjorke and Staur and got -0.2985* correlation coefficient with FHB severity in Bjorke which was significant. The correlation on Staur field was -0.201, but was not significant (Table 3.2).



(a) Bjørke field experiment (AE and DON/FHB)



(b) Staur field experiment (AE and DON/FHB)

Figure 3. 3: The relationship between AE in fields at (a) Bjørke and (b) Staur, and DON/FHB Mean after inoculation at Vollebekk/Ås field trial.

3.7 FHB and DON

The correlation of FHB and DON showed very high significant positive correlation of 0.483***(Table 3.2). The increased FHB incidence in field highly increased the toxin content in infected seed after laboratory testing of DON.

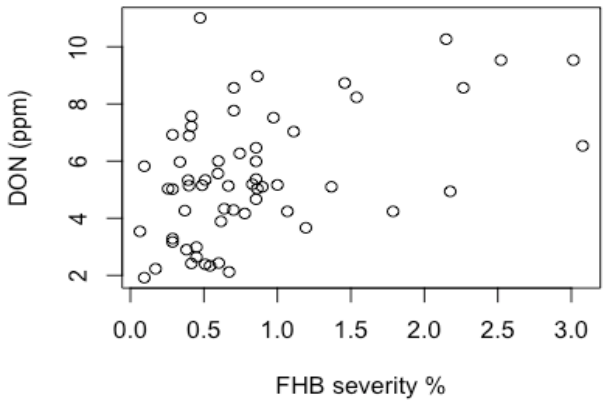


Figure 3. 4: Scatter plot representation of FHB and DON relationship in the year 2017 in Vollebekk field trial.

3.8 QTL effect on phenotypes (Type 3 tests of Fixed effect) 2017

QTL combination was found to have significant effect (p value 0.0027) on DON content and confirms having almost 2 ppm less DON content when three QTL were acts together compared to no QTL at all. *Fhb1* was seen significant in reducing DON by 1.5 ppm compared to NILs without resistance allele on 3BS. QTL 2DL was also seen significant to DON. However, another QTL 5AS showed no significant effect on DON and neither any QTL have measurable significant effect on FHB severity. Piled QTL showed effect on lowering FHB severity and DON content (Table 3.4), however, significant for DON content only. QTL combination showed a significant effect on delaying days to heading. NILs carrying QTL *Fhb1* showed delayed heading by a day compred to the lines with no QTL in Vollebekk field (Table 3.6). QTL had no significant effect on AE and PH for all three field trials.

Table 3.4: Test of fixed effects of QTL and QTL COmbination against FHB severity and DON.

Type 3 Tests of Fixed Effects (FHB severity %)				
Effect	Num DF	Den DF	F Value	Pr > F
Fhb1	1	35	0.82	0.3725
QTL_2DL	1	35	2.58	0.1171
QTL_5AS	1	35	0.02	0.8995

Type 3 Tests of Fixed Effects (DON ppm)				
Effect	Num DF	Den DF	F Value	Pr > F
Fhb1	1	35	10.78	**0.0023**
QTL_2DL	1	35	7.99	**0.0077**
QTL_5AS	1	35	1.07	0.3084

Type 3 Tests of Fixed Effects(DON)				
Effect	Num DF	Den DF	F Value	Pr > F
QTL_Combination17	6	32	4.32	0.0027

Table 3.5: Test of fixed effect for QTL against plant height at all three field trials.

Type 3 Tests of Fixed Effects (PH Vollebekk)				
Effect	Num DF	Den DF	F Value	Pr > F
Fhb1	1	35	0.13	0.7201
QTL_2DL	1	35	0.05	0.8317
QTL_5AS	1	35	0.02	0.8894

Type 3 Tests of Fixed Effects (PH Bjørke)				
Effect	Num DF	Den DF	F Value	Pr > F
Fhb1	1	35	1.94	0.1728
QTL_2DL	1	35	3.85	0.0577
QTL_5AS	1	35	1.76	0.1931

Type 3 Tests of Fixed Effects (PH Staur)				
Effect	Num DF	Den DF	F Value	Pr > F
Fhb1	1	35	0.14	0.709
QTL_2DL	1	35	0.26	0.6101
QTL_5AS	1	35	0.68	0.4169

Table 3.6: Test of fixed effect for QTL against days to heading at all three field trials.

Type 3 Tests of Fixed Effects (DH Vollebekk)				
Effect	Num DF	Den DF	F Value	Pr > F
Fhb1	1	35	6.81	**0.0133**
QTL_2DL	1	35	0.52	0.4756
QTL_5AS	1	35	2.35	0.1343

Type 3 Tests of Fixed Effects (DH Bjørke)				
Effect	Num DF	Den DF	F Value	Pr > F
Fhb1	1	35	4.58	**0.0395**
QTL_2DL	1	35	1.04	0.3153
QTL_5AS	1	35	2.15	0.1512

Type 3 Tests of Fixed Effects (DH Staur)				
Effect	Num DF	Den DF	F Value	Pr > F
Fhb1	1	35	2.79	0.1041
QTL_2DL	1	35	1	0.3235
QTL_5AS	1	35	1.16	0.2889

Table 3.7: Test of fixed effect for QTL against anther extrusion in two agronomic trial.

Type 3 Tests of Fixed Effects (Bjørke AE)				
Effect	Num DF	Den DF	F Value	Pr > F
Fhb1	1	35	3.61	0.0657
QTL_2DL	1	35	0.33	0.5682
QTL_5AS	1	35	0.43	0.5158

Type 3 Tests of Fixed Effects (Staur AE)				
Effect	Num DF	Den DF	F Value	Pr > F
Fhb1	1	35	0.05	0.8306
QTL_2DL	1	35	2.22	0.145
QTL_5AS	1	35	0	0.9654

3.7 Type 3 Test of Fixed Effect- 2016

For FHB severity and DON, *Fhb1* was the only significant QTL. No QTL were found significant for DH and AE; however, QTL 5AS was significant for PH (Table 3.8)

Table 3.8: Type 3 tests for fixed effects on FHB Severity, DON, Days to heading, Anther extrusion and Plant height, respectively. Significant results are marked in bold.

Type 3 Tests of Fixed Effects (FHB severity %)

Effect	Num DF	Den DF	F Value	Pr > F
Fhb1	1	32	4.89	**0.0343**
QTL_2DL	1	32	3.76	0.0614
QTL_5AS	1	32	0.29	0.5962

Type 3 Tests of Fixed Effects(DON)

Effect	Num DF	Den DF	F Value	Pr > F
Fhb1	1	32	4.79	**0.0361**
QTL_2DL	1	32	0.8	0.3764
QTL_5AS	1	32	0.05	0.8213

Type 3 Tests of Fixed Effects(DH)

Effect	Num DF	Den DF	F Value	Pr > F
Fhb1	1	32	1.61	0.2141
QTL_2DL	1	32	0.71	0.4072
QTL_5AS	1	32	0.17	0.6803

Type 3 Tests of Fixed Effects(AE)

Effect	Num DF	Den DF	F Value	Pr > F
Fhb1	1	32	1.1	0.3029
QTL_2DL	1	32	1.1	0.3015
QTL_5AS	1	32	0.84	0.365

Type 3 Tests of Fixed Effects(PH)

Effect	Num DF	Den DF	F Value	Pr > F
Fhb1	1	32	0.06	0.8018
QTL_2DL	1	32	0.01	0.9173
QTL_5AS	1	32	5.05	**0.0317**

3.9 Allelic effect of the three QTL on FHB visual score

Separate analysis of each QTL on FHB Mean shows that QTL on 2DL and *Fhb1* are responsible for FHB resistance. However, QTL on 5A had no measurable effect on FHB visual symptom in Vollebekk 2017 trial. Similar pattern of result was seen when boxplot was drawn for the QTL against FHB visual scoring in 2016 field trial. The boxplot results for 2016 were presented in Fig. 1 in Appendix.

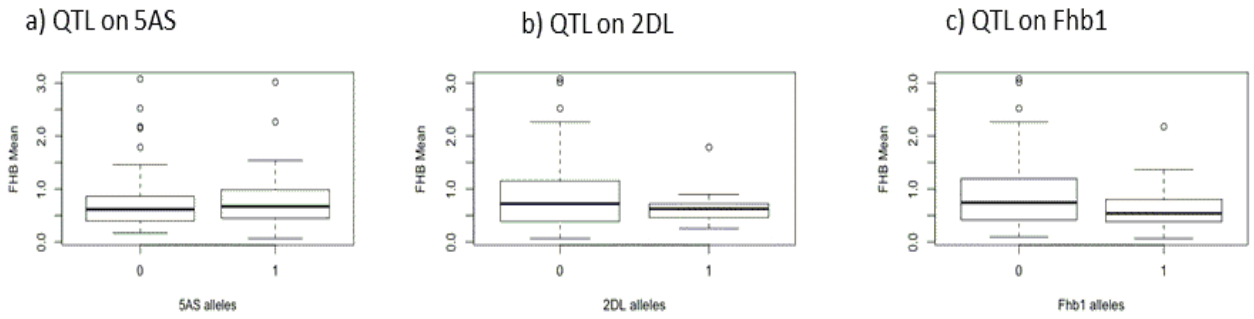


Figure 3.5: Phenotypic variation in FHB index between genotypes with and without resistance and susceptible alleles on QTL in 2017 field trial, where 1 represents the resistance allele and 0 the susceptible allele for the respective QTL.

3.10 Allelic effect of 3 QTL on DON

All three QTL showed positive response on DON accumulation. The genotypes with QTL showed less DON content. Among three, QTL on *Fhb1* is found to reduce DON (ppm) highly on its presence. In a similar way these QTL had same pattern of effect on the FHB severity and DON for 2016 field trial, except for 2DL QTL decreasing more DON content on its presence. The box plot results for 2016 field trial are presented in Fig. 1 in Appendix.

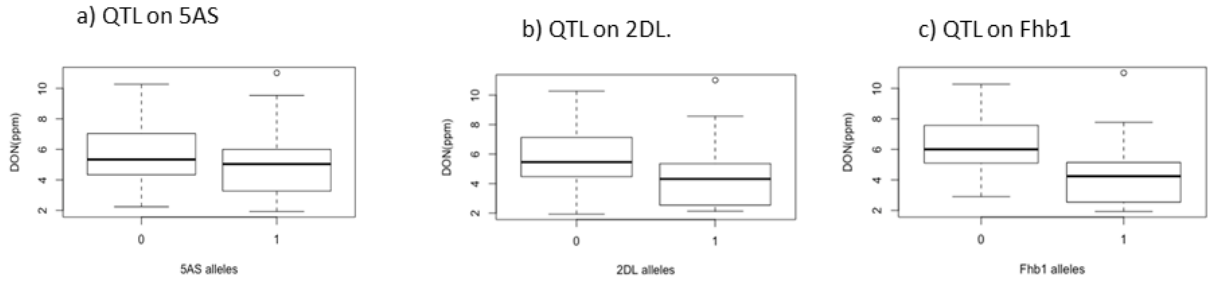
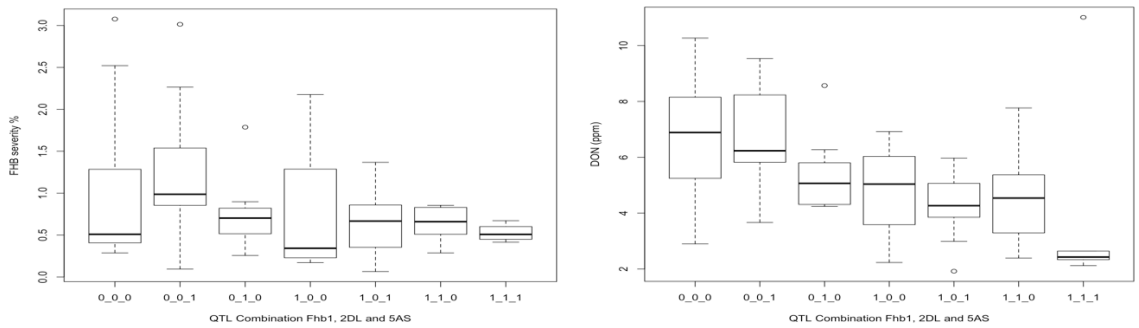


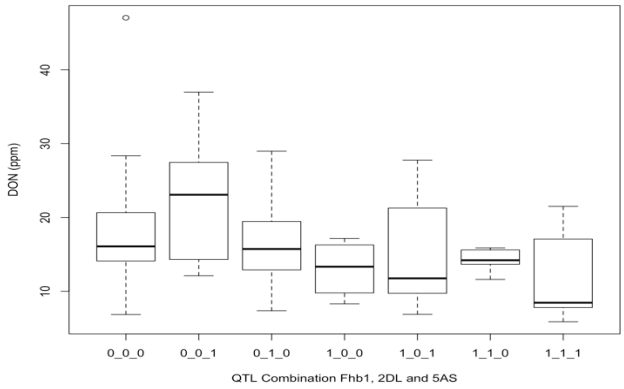
Figure 3. 6: Phenotypic variation in DON content between genotypes with resistance or susceptible allele on locus in 2017 field trial. 1 represents the resistance allele and 0 represents the susceptible allele of respective QTL.

3.11 QTL Combination on FHB severity and DON

QTL combination showed positive impact on lowering FHB severity but was not distinct and measurable. But in case of DON, QTL combination showed significant positive impact on lowering DON content. The frequency of genotypes with presence of all three resistance allele on QTL were low in comparison.



a. b.



c.

Figure 3. 7: QTL combination effect on (a) FHB severity upper (left), (b) DON content upper (right) for 2017 trial and (c) DON content for 2016; where 0 represent presence of susceptible and 1 represent presence of resistance allele on trait locus.

3.12 QTL effect on grain quality

Effect of QTL Combination was not seen on test weight of field trials. However, significant effect was seen for 1000 kernels weight, yield, protein content and starch content on either one or both fields. Though, their effect was not very high to measurable amount.

Table 3.9: QTL effect on Grain quality parameter from Staur and Bjørke field trials (yield, test weight, protein content, starch content, and 1000 kernels weight respectively).

Type 3 Tests of Fixed Effects (Yield Staur)				
Effect	Num DF	Den DF	F Value	Pr > F
QTL_Combination17	6	32	6.17	**0.0002**

Type 3 Tests of Fixed Effects (Yield Bjørke)				
Effect	Num DF	Den DF	F Value	Pr > F
QTL_Combination17	6	32	0.87	0.5286

Type 3 Tests of Fixed Effects (Test weight Staur)				
Effect	Num DF	Den DF	F Value	Pr > F
QTL_Combination17	6	32	2.02	0.0913

Type 3 Tests of Fixed Effects (Test weight Bjørke)				
Effect	Num DF	Den DF	F Value	Pr > F
QTL_Combination17	6	32	0.56	0.7589

Type 3 Tests of Fixed Effects (ProtDM Staur)				
Effect	Num DF	Den DF	F Value	Pr > F
QTL_Combination17	6	32	2.8	**0.0266**

Type 3 Tests of Fixed Effects (ProtDM Bjørke)				
Effect	Num DF	Den DF	F Value	Pr > F
QTL_Combination17	6	32	5.08	**0.0009**

Type 3 Tests of Fixed Effects (StarchDM Staur)				
Effect	Num DF	Den DF	F Value	Pr > F
QTL_Combination17	6	32	1.83	0.1236

Type 3 Tests of Fixed Effects (StarchDM Bjørke)				
Effect	Num DF	Den DF	F Value	Pr > F
QTL_Combination17	6	32	5.21	**0.0008**

Type 3 Tests of Fixed Effects (1000 grain wt. Staur)				
Effect	Num DF	Den DF	F Value	Pr > F
QTL_Combination17	6	32	3.43	**0.01**

Type 3 Tests of Fixed Effects (1000 grain wt. Bjørke)				
Effect	Num DF	Den DF	F Value	Pr > F
QTL_Combination17	6	32	5.16	**0.0008**

4. Discussion

The spawn inoculation for fungal infection copy the same condition as natural infection process for FHB (Buerstmayr, Ban et al. 2009). In our research, inoculation was done through grain spawn spreading on ground surface, which acted as if the fungus is residing on ground debris naturally. This reflects both Type I & II resistance (Schroeder and Christensen 1963). Field testing in multiple location is crucial for measuring all agronomic traits since they might be affected by the varying environmental condition where crops are grown. To address this trait our trials were conducted in three different locations - Vollebekk (59^{o} N, 90 masl), Staur (60^{o} N, 153 masl) and Bjørke (62^{o}N, 68 masl). This helped us in comparing results from different environments which is of high importance in resistance breeding.

The disease severity was very high in the field experiment of 2016 as compared to consequtive year. This might be due to the weather variability in different years, scoring error and the infection capacity of inoculum. The need of warm and moist environment was seen as a major favorable environment for *Fusarium* infection (Xu 2003), which was not much favorable in the year 2017. The high disease incidence in 1994 during warm weather and low disease incidence in 1995 and 1996 due to cool weather was also recorded by (Zinkernagel, ADOLF et al. 1997)

4.1 Allelic States in genotyping

Allelic states were confirmed using using PCR based markers which were available in NMBU laboratory from different external sources. The use of KASP markers in marker genotyping was found to be time saving. They were found to have high level of DNA amplification in our genotyping process. As the results were easily visualized in FRET plate reader through cluster plot, it was easy to confirm the type of allele present. KASP is leading in genotyping industry with >99.8% accuracy, flexible primer design and minimal (10ng) DNA requirement per sample per SNP (LGC Group). It also reduces the cost of dye as it uses the universal reporting system, the labeled component present in mastermix. The effectiveness of KASP (with respect to cost and scalable flexibility) that uses moderate number of markers, like QTL mapping, MAS, QTL fine mapping, quality control analysis are put forwarded by Semagn, Babu et al. (2014).

Almost all three STS markers showed similar way for resistance in NILs except few of them. Means, for any specific NIL all three STS marker either possess resistance allele or susceptible

allele. The capacity of detecting heterozygosity by STS marker is seen in two of the STS markers (Tryp_Syn, HCBT). However, detection of heterozygosity is not seen in SGNH STS marker which may be due to its dominant nature. Many researchers have been using STS markers for genome mapping of different disease resistance (Thomas and Scott 1993, 2003, 2006).

The results from SSR markers for the loci 3BS, 2DL and 5AS were convincing as it showed similar pattern to presence/absence allele for all five SSR markers. Markers of specific locus for any one NIL; all showed either resistance or susceptible allele which helped in confirming allelic states. However, analyzing the result obtained from ABI sequencer was critical in the sence that it required intense experience for resolving some of the unknown allele size nearby the main allele. SSR markers are widely used in genotyping due to their high mutation rate forwarded by Gemayel, Cho et al. (2012) and simplicity and robustness (Powell, Morgante et al. 1996). Chromosome region for FHB resistance was well documented by the use of SSR marker in Ning,7840 wheat cultivar by Zhou, Kolb et al. (2002).

For the genotyping of *Fhb1,* KASP and SSR marker UMN10 was found to be better choice than IAAV5302 and Tdurum_contig46334_832 in our genotyping. This is because this two UMN10 marker show same trend in presence of resistance and susceptible allele while not by other two. Besides, Tryp_Syn was seen more reliable among three STS marker for *Fhb1*. Also the use of marker gwm493 and gwm533 for *Fhb1* on 3BS showed disease severity reduction by 41.6% and 24.8% in two wheat population (Anderson, Stack et al. 2001) could be the reliable source as well.

All three SSR markers showed similar level of reliability for QTL 5AS. However, for QTL 2DL we used one SSR marker gwm539 which gave moderate satisfying result with some unknown different sized allele other than resistant and susceptible alleles.

For the genotyping of QTL numerous sensitive procedures like DNA extraction, dilution, adding buffer to prepare PCR product and handling of all the laboratory equipments should be done precisely. At any point result might be wrong if correct protocols were not followed. Thus, during our genotyping procedure we followed well accepted protocols more presicely.

The polymorphic QTL were found to be introgressed successfully in the NILs (Table3.1). 26 NILs got *Fhb1* and 5AS QTL while 19 got 2DL QTL introgressed out of 57 NILs. Similar genotyping result from 2015 were used to compare the allelic state presence in NILs. The genotyping result from 2015 was almost similar except the unknown QTL for some NILs which were resolved by our finding. This validation of QTL with molecular marker might increase the FHB resistance in lines when selecting the lines with these QTL which is also

supported by Pumphrey, Bernardo et al. (2007). Also, since IAAV5302 and Tdurum_contig46334_832 contains 90K SNP on 3B validated for FHB by Sørensen (2016), it is also recommended to use these marker in resistance breeding further. Allelic state of three QTL were confirmed and this information was used for phenotypic trait evaluation.

4.2 QTL effect on traits FHB & DON

Genotypes carrying *Fhb1* gene showed less FHB infection than those not carrying this gene in our experiment of 2016 (Table 3.6) but not in the experiment of 2017. Similar result of lowering disease severity was seen in NILs when evaluating this gene (Anderson, Stack et al. 2001, Somers, Fedak et al. 2003, Pumphrey, Bernardo et al. 2007). Thus the lines without *Fhb1* gene are more prone to FHB infection severity. Effect of *Fhb1* is higher on DON reduction than on FHB infection as per our experiment in both year (Table 3.2, Table 3.6). And the experimental error might have some role in low FHB effect seen than DON. *Fhb1* on 3BS highly reduced DON content on its presence which is also supported by Somers, Fedak et al. (2003) with 17% reduction in DON development when alleles of 3BS and 5A act together. The preliminary experiment by Lemmens, Koutnik et al. (2008) showed that the gene *Fhb1* protects wheat against nivalenol and DON. The resistance mechanism of this gene to DON content due to cell wall thickening was put forward by Gunnaiah, Kushalappa et al. (2012). The reduced Fusarium damaged kernel (FDK) and DON was also reported by Balut, Clark et al. (2013). Our finding shows the higher capacity of this gene in DON reduction and FHB visual symptoms.

Our experiment result showed low DON content for those genotype with resistance allele on the locus 2DL (Table 3.2). The reduced FHB infection and DON content was also seen in double haploid wheat from the cross Wuhan-1 x Maringa (Somers, Fedak et al. 2003) also supported by Long, Balcerzak et al. (2015). The QTL 2DL shows more significant in reducing DON content than FHB severity. Similar finding of reduced DON content by 24% in four out of five population compared to FDK by 29% in two out of five was shown by Balut, Clark et al. (2013). As per our experimental result, it is concluded that the lines carrying QTL 2DL only acts on reducing DON toxicity content.

The effect of QTL on 5AS showed no measurable reduction in DON concentration and FHB infection in our experiment. This is also supported by Lu, Lillemo et al. (2013) showing no

FHB severity relation but having significant association with DON. In contrast the FHB severity reduction was seen higher for 5A greater than *Fhb1* by Von der Ohe, Ebmeyer et al. (2010). By these we came to a point that further introgressing research should be conducted to see the effect of QTL 5AS on FHB/DON.

When considering the individual effect of these three QTL on FHB severity and DON in this experiment we can not see high level of resistance to FHB severity and DON, however combination of QTL showed stronger effects (Figure 3.3, Table 3.2) which is supported by McCartney, Somers et al. (2007). Meaning the piling of QTL with resistance alleles have combined effect and are stronger in reducing disease severity and toxin level.

4.3 QTL effect on DH, PH & AE

The presence of QTL *Fhb1* showed delayed days to heading by one day in our experiment and was significant in one field trial (Vollebekk) while not in another two (Bjørke and Staur) (Table 3.4). So by choosing the lines with the gene *Fhb1* we will have higher DH by one day. However, other QTL on 2DL and 5AS showed no effect on DH. Research of Fedak, Cao et al. (2008) showed the tendency of delay in DH and increased PH when increasing resistant capacity of Sumai 3 offspring. In contrast, no any delay on DH by Von der Ohe, Ebmeyer et al. (2010). There was not any measurable effect of QTL on plant height in our experiment which contradicts Fedak, Cao et al. (2008). Similarly, effect of these QTL on AE was also not seen in our experiment. No effect of QTL on PH and AE says we will not have problems of increased or decreased in PH, AE when introgressing these QTL on our adapted cultivars.

Therefore, introgressing of this QTL on adapted lines is suggested further as it will not affect traits like PH, DH and AE. We will not have to face problems like high delay in heading date, increased plant height, and reduced AE by introgressing these QTL.

4.4 FHB and AE

Our result showed negative correlation between AE and FHB of -0.2985* & -0.201 in two experimental fields with former being significant (Table 3.8). Which depicts that choosing the lines with higher AE we will be able to get good resistance level (reduced FHB severity) in our production units. The similar correlation is also shown but are highly significant in Skinnes, Semagn et al. (2010) and Lu, Lillemo et al. (2013). forwarded that the trapped anther

acts as substrate for saprophytes such as *Fusarium* and under favorable condition infection may occur. The correlation between AE and FHB infection intensity (-0.45 to -0.64) within years shows no difference at all, showing full genetic control for AE (Lu, Lillemo et al. 2013). Also the QTL analysis have showed that most of the QTL for AE being coincided with FHB severity (Lu, Lillemo et al. 2013). Study done by Kubo, Fujita et al. (2013) showed closed head or full extrusion of anthers are giving higher resistance to FHB severity than partial extrusion of anthers. This tendency of correlation between AE and FHB shows AE as governing factor for FHB severity. Therefore, breeding of varieties having high AE characteristics should be focused in resistance breeding.

4.5 FHB and PH

Increased PH showed significant negative correlation -0.357* & -0.341* in both the experimental fields of Bjørke and Staur, respectively. Hence, taller plants are less prone to disease severity, however, should keep in mind as taller plants are more prone to logging problems. However, the correlation was -0.241 for one disease scoring field of Vollebekk and was not significant which may be due to less difference in PH among the NILs due to environmental condition and measurement error of PH in the field (Table 3.7). This clarifies the increased susceptibility with reduced plant height. Similar negative relationship between dwarfing gene and FHB were also put forward by many researchers (Draeger, Gosman et al. 2007, Lu, Lillemo et al. 2013). Further, the semi dwarfing alleles *Rht-B1b and Rht-D1b* together had 0 to 41% disease susceptibility along with reduced 13-23% AE (He, Singh et al. 2016). Though having same height reducing capacity of genes *Rht-B1b and Rht-D1b,* it is seen that former have less negative relation with FHB resistance (Miedaner and Voss 2008), which could be the choice for resistance breeding. Taller plant showed significant reduction on FHB severity with correlation of -0.65 (Somers, Fedak et al. 2003).

Plant with dwarf genotypes were seen more susceptible than tall genotypes in normal natural condition but have not shown any significant difference in artificial inoculation (Mesterhazy 1995). In contrast, the dwarfing gene *Rht24* on 6BS chromosome acting on winter wheat population ‘Solitår x Bussard’, have highly reducing plant height character but have no rise in FHB severity and delaying in heading date (Herter, Ebmeyer et al. 2018). Thefore, the seletion of genotype in resisatnce breeding should not underestimate the PH parameter.

4.6 AE and PH

Our experiment showed very low positive correlation between AE and PH. However, significant correlation between AE and PH was seen with correlation coefficient 0.43, for which two shared QTL were also detected (Lu, Lillemo et al. 2013).

4.7 FHB and DON

This experiment showed significant positive correlation (0.483***) between FHB infection and DON content which was quite typical (Table 3.7). Thus the increase in FHB infection obviously increases the toxic DON content in infected grains and cause human and animal health problems when they are used as feed. Resistance breeding effort in decreasing FHB will ultimately decrease toxin DON level in wheat. It is also researched that DON resistance phenotype is also governed by FHB resistant QTL on 3BS by Lemmens, Scholz et al. (2005) However, the findings of Arseniuk, Foremska et al. (1999) suggested that the process of DON accumulatiom might be different and may not be dependend on FHB reaction. Therefore, further research on the field of biological mechanism of DON and FHB development on wheat should be focused.

4.8 QTL effect on grain quality parameter

Test weight, starch content and protein content in both the agronomic field trials showed not much difference due to presence and absence of QTL combination (Table 3.9). This shows that the presence of three FHB resistance QTL on the lines will not reduce the content of protein, starch and test weight quantity in any case. However, 1000 grain weight for both the field have lowered 2-3 gm of weight in the presence of all three QTL compared to no QTL at all (Table3.9) as proposed (McCartney, Somers et al.) due to presence of QTL 5AS from Sumai 3. In this case after introgressing the resistant QTL in adapted wheat cultivars, 1000 kernel weight should be calculated carefully as it may reduce the chance of variety release and farmer's acceptance.

The yield reduction was seen very small but significant ($p<0.05$) in Staur (Table 3.9). While in Bjørke the yield reduction was not significant and was not of comparable amount between

lines with and without QTL. The unmeasurable yield reduction on NILs on agronomic field trials might be due to fungicidal application on Staur and Bjørke which played role in FHB severity reduction. Similarly, the non significant decrease in yield measured in the presence of QTL *Fhb1* and 2DL was seen (Fedak, Cao et al. 2008, Von der Ohe, Ebmeyer et al. 2010). Accordingly the presence of leaf rust resistance gene *Lr47* showed the decrease in yield by just 3.8% (Brevis, Chicaiza et al. 2008). This small decrease in yield parameter on the presence of FHB resistance QTL can be overcomed by the introgressing of high yielding responsible gene. Besides, lowering the FHB and DON level are significant complementary benefit against small yield reduction. In the current experiment done grain yield and FHB infection showed not significant but slight positive correlation. In contrast, screening of Nebraska winter wheat through KASP showed significant phenotypic correlation of -0.66** between FHB infection and grain yield when the yield of FHB infected experimental plots were measured (Sallam A, Sidiqi J, Baenzier S. 2017).

However, these small fluctuations on grain quality parameter is based on combination of all three QTL; which when acted alone have almost no effects on quality. So introgressing theses QTL on breeding line will not compete on important grain quality parameters.

5. Conclusion and Recommendation

Resistance breeding is the heart of many plant breeding programs. Developing *Fusarium* resistance cultivars by introgressing exotic germplasm genes is a huge effort that requires extensive knowledge, experience and experiments. *Fusarium* infection varies highly on a very small change in weather and environmental condition. Developing FHB resistant cultivars should address different environmental condition and different year of field trials. Testing NILs from original mapping population is most common method of validating QTL presence/absence. After the characterization of molecular markers, we seek to validate and see the QTL relationship with traits like FHB, DON, PH, AE, DH and grain quality parameters. Although the disease severity was seen low, the association between introgressed QTL *Fhb1* with FHB & DON showing significant positive effect. The QTL in combination had more effect than when acting alone on FHB and DON shows that the breeding program should be focused on piling the resistance QTL. Passive resistance mechanisms like increased PH and higher AE, which are favourable for FHB and DON reductiona were put forward by our findings. The field trials carried out in three different locations in our experiment helped to address environmental variability effect on agronomic traits. We also concluded strong significant correlation between DON/FHB with FHB traits (PH & AE). This significant correlation should be addressed by breeding companies while working on developing resistant cultivars. The resistant cultivar also should not affect the grain quality parameter in any case which may lead to unacceptance at farmer level. Parameters like grain yield, test weight, protein & starch content and 1000 grain weight should be considered while breeding resistance line. Further, the small decrease in 1000 kernels weight in the current research should not suppress their use in breeding program. Selecting the current NILs with 2-3 QTL and performing further research in multi location to see their effect on FHB traits are recommended. Furthermore, yield measurement of disease scoring field which was lacking on our experiment would help to quantify the actual yield loss due to disease severity.

Complete FHB resistance is not achieved by one or two QTL with resistant allele. Many major and many more minor QTL piling for higher resistant is accepted and is also seen in our research. Therefore, GWAS, QTL mapping and introgressing QTL research should be continued more presiesly. Company like Graminor should work dense on selecting the NILs with maximum QTL with resistance allele and follow further breeding program. According to Gunnaiah, Kushalappa et al. (2012) more than hundered resistance QTL for FHB have been reported for wheat which would not proceed well unless the host resistance mechanism is well

known. Thus the companies working on resistance breeding should also focus on how a host plant behave on the certain QTL presence.

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8. APPENDIX

Figure 1: Box plot of QTL versus FHB severity and DON in 2016:

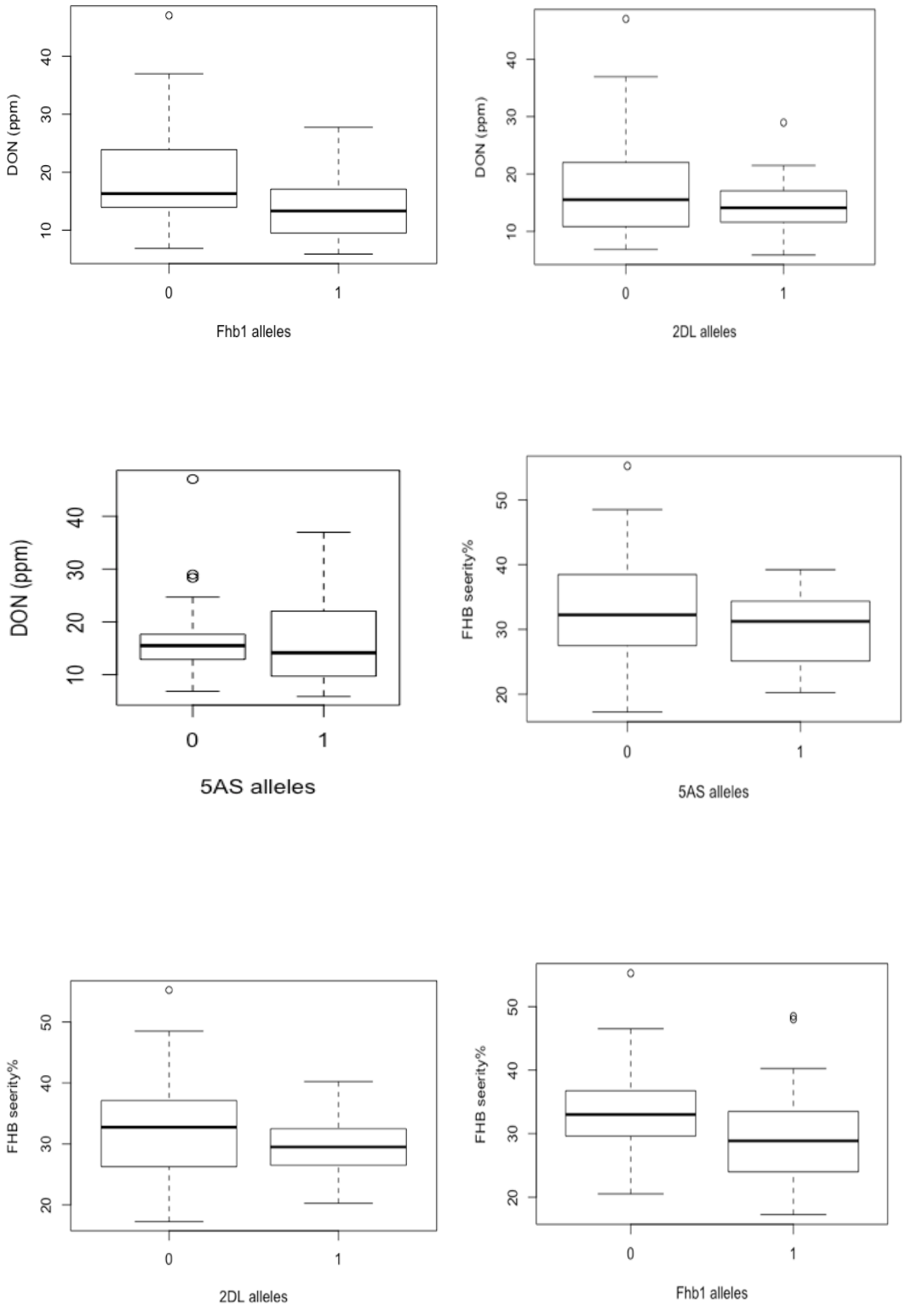

Table 1: Marker characterization detail for NILs 2017.

Line	Entry	Name	IAAV5302	Tdur_contig4633	wMAS000033	UMN10-kasp	SGNH	HCBT	Terp-Syn	UMN10-ssr	5A.gwm293	5A.gwm304	5A.gwm415	2DL.gwm539
4	1501	Zebra	X:X	Y:Y	G:G	0al.el.ia	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ
72	1079	CJ9306	Y:Y	X:X	A:A	G:G	CC	CC	CC	CC	CC	CC	CC	CC
83	2204	Berserk	Y:Y	Y:Y	G:G	0al.el.ia	BB	BB	BB	BB	BB	BB	BB	BB
8304	1503	Zebra-2/CJ9306//Zebra-2	X:X	Y:Y	G:G	A:A	ZZ	CC	ZZ	M	ZZ	ZZ	ZZ	ZZ
8305	1504	Zebra-2/CJ9306//Zebra-2	X:X	Y:Y	G:G	0al.el.ia	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ
8306	1505	Zebra-2/CJ9306//Zebra-2	X:X	Y:Y	G:G	0al.el.ia	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ	CC
8307	1506	Zebra-2/CJ9306//Zebra-2	Y:Y	Y:Y	G:G	G:G	CC	ZC	ZC	ZC	ZZ	ZZ	ZZ	ZZ
8308	1507	Zebra-2/CJ9306//Zebra-2	ProbX	Y:Y	G:G	A:A	ZZ	ZC	ZZ	M	ZZ	ZZ	ZZ	ZZ
8309	1508	Zebra-2/CJ9306//Zebra-2	Y:Y	Y:Y	G:G	G:G	CC	ZZ	ZC	ZC	ZZ	ZZ	ZZ	ZZ
8310	1509	Zebra-2/CJ9306//Zebra-2	X:X	Y:Y	G:G	0al.el.ia	ZZ	ZZ	ZZ	ZZ	CC	CC	CC	ZZ
8311	1510	Zebra-2/CJ9306//Zebra-2	X:X	Y:Y	G:G	0al.el.ia	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ
8312	1601	Zebra-2/CJ9306//Zebra-2	X:X	Y:Y	G:G	A:A	ZZ	CC	ZZ	M	ZZ	ZZ	ZZ	ZZ
8313	1602	Zebra-2/CJ9306//Zebra-2	X:X	Y:Y	G:G	G:G	CC	CC	CC	CC	ZZ	ZZ	ZZ	ZZ
8314	1603	Zebra-2/CJ9306//Zebra-2	Y:Y	Y:Y	G:G	G:G	CC	ZC	ZC	ZC	ZZ	ZZ	ZZ	CC
8316	1605	Zebra-2/CJ9306//Zebra-2	Y:Y	Y:Y	G:G	0al.el.ia	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ	CC
8317	1606	Zebra-2/CJ9306//Zebra-2	Y:Y	Y:Y	G:G	G:G	CC	ZC	ZC	ZC	ZZ	ZZ	ZZ	CC
8318	1607	Zebra-2/CJ9306//Zebra-2	X:X	Y:Y	G:G	0al.el.ia	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ
8319	1608	Zebra-2/CJ9306//Zebra-2	X:X	Y:Y	G:G	0al.el.ia	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ
8320	1609	Zebra-2/CJ9306//Zebra-2	X:X	Y:Y	G:G	0al.el.ia	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ
8405	1704	Zebra-2/CJ9306//Zebra-2	Y:Y	Y:Y	G:G	G:G	CC	CC	CC	CC	ZZ	ZZ	ZZ	CC
8406	1705	Zebra-2/CJ9306//Zebra-2	Y:Y	Y:Y	G:G	G:G	CC	CC	CC	CC	ZZ	ZZ	ZZ	CC
8407	1706	Zebra-2/CJ9306//Zebra-2	Y:Y	Y:Y	G:G	G:G	CC	CC	CC	CC	ZZ	ZZ	ZZ	ZZ
8408	1707	Zebra-2/CJ9306//Zebra-2	Y:Y	Y:Y	G:G	0al.el.ia	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ	ZZ	CC
8409	1708	Zebra-2/CJ9306//Zebra-2	Y:Y	Y:Y	G:G	G:G	ZZ	ZC	ZC	ZC	ZZ	ZZ	ZZ	CC
8410	1709	Zebra-2/CJ9306//Zebra-2	Y:Y	Y:Y	G:G	G:G	CC	CC	CC	CC	ZZ	ZZ	ZZ	CC
8411	1710	Zebra-2/CJ9306//Zebra-2	Y:Y	not ampli	G:G	A:A	ZZ	ZZ	ZZ	ZM	ZZ	ZZ	ZZ	ZC
8501	1807	Zebra-2/CJ9306//Zebra-2	X:X	Prob.Y	G:G	G:G	CC	ZC	ZC	CC	CC	CC	CC	ZZ
8502	1808	Zebra-2/CJ9306//Zebra-2	X:X	DNA	G:G	0al.el.ia	ZZ	ZZ	ZZ	ZC	CC	CC	CC	ZZ
8503	1809	Zebra-2/CJ9306//Zebra-2	X:X	DNA	G:G	G:G	CC	ZC	CC	CC	CC	CC	CC	ZZ
8504	1810	Zebra-2/CJ9306//Zebra-2	X:X	X:X	G:G	0al.el.ia	ZZ	ZZ	ZZ	ZZ	CC	CC	CC	ZZ
8505	1901	Zebra-2/CJ9306//Zebra-2	X:X	Y:Y	G:G	G:G	ZZ	ZC	ZC	ZC	CC	CC	CC	ZZ
8506	1902	Zebra-2/CJ9306//Zebra-2	X:X	X:X	G:G	0al.el.ia	ZZ	ZZ	ZZ	ZZ	CC	CC	CC	ZZ
8507	1903	Zebra-2/CJ9306//Zebra-2	X:X	Y:Y	G:G	0al.el.ia	ZZ	ZZ	ZZ	ZZ	CC	CC	CC	ZZ
8510	1905	Zebra-2/CJ9306//Zebra-2	Y:Y	Y:Y	G:G	G:G	CC	CC	CC	CC	CC	CC	CC	CC
8511	1906	Zebra-2/CJ9306//Zebra-2	Y:Y	Y:Y	G:G	G:G	CC	ZC	ZC	ZC	ZZ	ZC	ZC	ZZ
8512	1907	Zebra-2/CJ9306//Zebra-2	Y:Y	Y:Y	G:G	G:G	CC	CC	CC	CC	CC	CC	CC	CC
8513	1908	Zebra-2/CJ9306//Zebra-2	Y:Y	Y:Y	G:G	G:G	CC	CC	CC	CC	CC	CC	CC	ZZ
8514	1909	Zebra-2/CJ9306//Zebra-2	Y:Y	Y:Y	G:G	G:G	CC	CC	CC	CC	CC	CC	CC	CC
8515	1910	Zebra-2/CJ9306//Zebra-2	Y:Y	Y:Y	G:G	G:G	CC	CC	CC	CC	CC	CC	CC	ZZ
8516	2001	Zebra-2/CJ9306//Zebra-2	Y:Y	Y:Y	G:G	G:G	CC	CC	CC	CC	CC	CC	CC	CC
8517	2002	Zebra-2/CJ9306//Zebra-2	Y:Y	Y:Y	G:G	G:G	CC	CC	CC	CC	CC	CC	CC	CC
8609	2102	Berserk-4/CJ9306//Berserk-4	Y:Y	X:X	G:G	0al.el.ia	BB	BB	BB	BB	BB	BB	BB	CC
8610	2103	Berserk-4/CJ9306//Berserk-4	Y:Y	DNA	G:G	0al.el.ia	BB	BB	BB	BB	BB	BB	BB	CC
8611	2104	Berserk-4/CJ9306//Berserk-4	X:X	X:X	G:G	A:A	BB	CC	BB	MM	BB	BB	BB	BB
8612	2105	Berserk-4/CJ9306//Berserk-4	Y:Y	X:X	G:G	A:A	BB	CC	BB	MM	BB	BB	BB	BB
8613	2106	Berserk-4/CJ9306//Berserk-4	Y:Y	Y:Y	G:G	0al.el.ia	CC	BB	BB	BB	BB	BB	BB	BB
8614	2107	Berserk-4/CJ9306//Berserk-4	Y:Y	Y:Y	G:G	0al.el.ia	BB	BB	BB	BB	BB	BB	BB	BB
8615	2108	Berserk-4/CJ9306//Berserk-4	X:X	Y:Y	G:G	0al.el.ia	BB	BB	BB	BB	BB	BB	BB	CC
8616	2109	Berserk-4/CJ9306//Berserk-4	X:X	Y:Y	G:G	0al.el.ia	BB	BB	BB	BB	BB	BB	BB	CC
8617	2110	Berserk-4/CJ9306//Berserk-4	Y:Y	Y:Y	G:G	0al.el.ia	BB	BB	BB	BB	CC	CC	CC	BB
8618	2201	Berserk-4/CJ9306//Berserk-4	Y:Y	Y:Y	G:G	0al.el.ia	BB	BB	BB	BB	BB	BB	BB	BB
8619	2202	Berserk-4/CJ9306//Berserk-4	Y:Y	Y:Y	G:G	0al.el.ia	BB	BB	BB	BB	CC	CC	CC	BB
8706	2208	Berserk-4/CJ9306//Berserk-4	Y:Y	Y:Y	G:G	A:A	BB	CC	BB	MM	CC	CC	CC	BB
8707	2209	Berserk-4/CJ9306//Berserk-4	Y:Y	Y:Y	G:G	G:G	CC	CC	CC	CC	CC	CC	CC	BB
8708	2210	Berserk-4/CJ9306//Berserk-4	Y:Y	Y:Y	G:G	G:G	CC	CC	CC	CC	CC	CC	CC	BB
8709	2301	Berserk-4/CJ9306//Berserk-4	Y:Y	Y:Y	G:G	G:G	CC	BC	BC	BC	CC	CC	CC	BB
8710	2302	Berserk-4/CJ9306//Berserk-4	Y:Y	Y:Y	G:G	G:G	CC	CC	CC	CC	CC	CC	CC	BB
8711	2303	Berserk-4/CJ9306//Berserk-4	Y:Y	Y:Y	G:G	0al.el.ia	BB	BB	BB	BB	CC	CC	CC	BB
8713	2304	Berserk-4/CJ9306//Berserk-4	Y:Y	X:X	G:G	G:A	CC	BC	BC	BMC	CC	CC	CC	BB
8714	2305	Berserk-4/CJ9306//Berserk-4	Y:Y	X:X	G:G	0al.el.ia	BB	BB	BB	BB	CC	CC	CC	BB

G:G resistant allele, A:A susceptible allele

ZZ: susceptible allele of Zebra, BB: susceptible allele of Berserk, CC: resistant allele of CJ9306

MM: allele of unknown size than resistant and susceptible allele size.

Table 2: Lsmeans of traits in Vollebekk (disease scoring field trial 2017).

Line	Family	Navn	Cross	FHB_Mean	A_DH	A_PH	DON_AS
4		Zebra		1.4578	50.9857	87	8.7302
72		CJ9306		0.4743	49.9772	71.5714	11.0117
83		Berserk		0.4878	52.9857	75.5	5.1552
8304	7359003	Zebra-2/CJ93	Z	1.111	51.6667	82	7.0333
8305	7359003	Zebra-2/CJ93	Z	0.5953	51.6667	86	5.5667
8306	7359010	Zebra-2/CJ93	Z	1.7869	49.994	81.5	4.24
8307	7359010	Zebra-2/CJ93	Z	2.1759	49.494	85.5	4.94
8308	7359010	Zebra-2/CJ93	Z	0.509	50.494	82.5	5.34
8309	7360012	Zebra-2/CJ93	Z	0.1711	48	85	2.2333
8310	7360012	Zebra-2/CJ93	Z	0.5984	48	85.3333	6
8311	7360017	Zebra-2/CJ93	Z	0.4149	50.4774	80	7.2203
8312	7360017	Zebra-2/CJ93	Z	0.8637	50.4774	75.5	8.9703
8313	7360017	Zebra-2/CJ93	Z	0.2867	52.9774	73.5	6.9203
8314	7361003	Zebra-2/CJ93	Z	0.616	50.994	82	3.89
8316	7361003	Zebra-2/CJ93	Z	0.7015	50.494	81.5	4.29
8317	7361003	Zebra-2/CJ93	Z	0.8297	50.994	82.5	5.19
8318	7361010	Zebra-2/CJ93	Z	0.4152	51.9774	82.5	7.5703
8319	7361010	Zebra-2/CJ93	Z	0.2869	51.4774	84	3.1703
8320	7361010	Zebra-2/CJ93	Z	0.2869	50.4774	85	5.0203
8405	7361016	Zebra-2/CJ93	Z	0.2869	50.994	81.5	3.29
8406	7361016	Zebra-2/CJ93	Z	0.5092	50.994	82	2.39
8407	7361016	Zebra-2/CJ93	Z	0.3979	50.994	82.5	5.14
8408	7361020	Zebra-2/CJ93	Z	0.744	50.9774	77	6.2703
8409	7361020	Zebra-2/CJ93	Z	0.855	51.9774	79.5	5.3703
8410	7361020	Zebra-2/CJ93	Z	0.7037	52.6667	83.3333	7.7667
8411	7361020	Zebra-2/CJ93	Z	0.7036	52	82.6667	8.5667
8501	7390001	Zebra-2/CJ93	Z	0.09436	49.9774	82.5	1.9206
8502	7390001	Zebra-2/CJ93	Z	0.09452	50.4774	80	5.8203
8503	7390001	Zebra-2/CJ93	Z	0.337	49.9774	81	5.9703
8504	7390001	Zebra-2/CJ93	Z	0.9734	50.4774	79.5	7.5203
8505	7390009	Zebra-2/CJ93	Z	1.3676	51.6667	70.3333	5.1
8506	7390009	Zebra-2/CJ93	Z	1.5384	51.3333	78	8.2333
8507	7390009	Zebra-2/CJ93	Z	0.8548	51	84.3333	6.4667
8510	7398002	Zebra-2/CJ93	Z	0.6011	54	82.3333	2.4333
8511	7398002	Zebra-2/CJ93	Z	1.0682	52.6667	85	4.24
8512	7398002	Zebra-2/CJ93	Z	0.5414	53.3333	85	2.3333
8513	7398005	Zebra-2/CJ93	Z	0.4492	53.994	87	2.99
8514	7398005	Zebra-2/CJ93	Z	0.4492	53.494	86	2.64
8515	7398005	Zebra-2/CJ93	Z	0.06469	53.994	87.5	3.54
8516	7398007	Zebra-2/CJ93	Z	0.415	52.9774	84.5	2.4203
8517	7398007	Zebra-2/CJ93	Z	0.6714	52.4774	84.5	2.1203
8609	7385021	Berserk-4/CJ	B	0.8976	51	66.6667	5.1
8610	7385021	Berserk-4/CJ	B	0.2566	50.6667	66.6667	5.0333
8611	7385021	Berserk-4/CJ	B	2.5212	52.6667	73	9.5333
8612	7385021	Berserk-4/CJ	B	3.077	54.6667	83	6.5333
8613	7399021	Berserk-4/CJ	B	0.3818	51	81.6667	2.9
8614	7399021	Berserk-4/CJ	B	0.3989	51	79.6667	6.89
8615	7399021	Berserk-4/CJ	B	0.6381	51.3333	87.6667	4.3333
8616	7399021	Berserk-4/CJ	B	0.3931	51.6667	91.3333	5.3333
8617	7401002	Berserk-4/CJ	B	2.265	51.6667	75	8.5667
8618	7401002	Berserk-4/CJ	B	2.148	52	73	10.2667
8619	7401002	Berserk-4/CJ	B	3.0142	52.3333	74.6667	9.5333
8706	7410013	Berserk-4/CJ	B	1.1937	53	70.3333	3.6667
8707	7410013	Berserk-4/CJ	B	0.8548	53.6667	75.6667	4.6667
8708	7410013	Berserk-4/CJ	B	0.8632	53.6667	69.6667	5.0333
8709	7410014	Berserk-4/CJ	B	0.3702	54.6667	76.3333	4.2667
8710	7410014	Berserk-4/CJ	B	0.7777	53.6667	77	4.1667
8711	7410014	Berserk-4/CJ	B	1	53	80.6667	5.1667
8713	7410019	Berserk-4/CJ	B	0.6666	55.6667	77	5.1333
8714	7410019	Berserk-4/CJ	B	0.8549	54.6667	78.6667	5.99

Table 3: Lsmeans of traits in Bjørke (agronomic field trial 2017).

Line	Family	Navn	Cross	B_PH	B_DH	B_AE
4		Zebra		107.15	61.5	7
72		CJ9306		89.55	58	5
83		Berserk		89.9	64.5	7.25
8304	7359003	Zebra-2/CJ93	Z	104.15	62.5	0.5
8305	7359003	Zebra-2/CJ93	Z	103.15	62.5	1.5
8306	7359010	Zebra-2/CJ93	Z	101.15	61	5.5
8307	7359010	Zebra-2/CJ93	Z	107.1	61	5.5
8308	7359010	Zebra-2/CJ93	Z	100.45	61	5.5
8309	7360012	Zebra-2/CJ93	Z	107.3	60.5	8
8310	7360012	Zebra-2/CJ93	Z	107.3	60.5	8
8311	7360017	Zebra-2/CJ93	Z	110.15	61.5	6
8312	7360017	Zebra-2/CJ93	Z	99.95	62	4
8313	7360017	Zebra-2/CJ93	Z	96	64	6.5
8314	7361003	Zebra-2/CJ93	Z	105.3	61.5	7.5
8316	7361003	Zebra-2/CJ93	Z	104.8	61.5	7.5
8317	7361003	Zebra-2/CJ93	Z	105.15	61.5	8
8318	7361010	Zebra-2/CJ93	Z	101.15	62	7.5
8319	7361010	Zebra-2/CJ93	Z	100.8	62	7.5
8320	7361010	Zebra-2/CJ93	Z	101.95	62	8
8405	7361016	Zebra-2/CJ93	Z	104.1	62.5	7.5
8406	7361016	Zebra-2/CJ93	Z	106.15	62.5	7.5
8407	7361016	Zebra-2/CJ93	Z	104.8	62.5	7.5
8408	7361020	Zebra-2/CJ93	Z	103.65	62	7
8409	7361020	Zebra-2/CJ93	Z	107.45	62	7
8410	7361020	Zebra-2/CJ93	Z	104.45	62.5	7
8411	7361020	Zebra-2/CJ93	Z	101.45	62.5	7
8501	7390001	Zebra-2/CJ93	Z	102.8	61.5	6.5
8502	7390001	Zebra-2/CJ93	Z	103.5	61.5	6.5
8503	7390001	Zebra-2/CJ93	Z	103.3	61.5	6.5
8504	7390001	Zebra-2/CJ93	Z	97	61.5	6.5
8505	7390009	Zebra-2/CJ93	Z	104.65	62.5	8
8506	7390009	Zebra-2/CJ93	Z	99.6	61.5	7
8507	7390009	Zebra-2/CJ93	Z	102.6	62	7
8510	7398002	Zebra-2/CJ93	Z	113.3	64.5	7.5
8511	7398002	Zebra-2/CJ93	Z	111.8	64.5	7
8512	7398002	Zebra-2/CJ93	Z	113.1	64.5	7
8513	7398005	Zebra-2/CJ93	Z	113.15	62.5	6.5
8514	7398005	Zebra-2/CJ93	Z	111.45	63	6
8515	7398005	Zebra-2/CJ93	Z	113.8	63	6
8516	7398007	Zebra-2/CJ93	Z	110.6	63	6.5
8517	7398007	Zebra-2/CJ93	Z	111.95	63	6.5
8609	7385021	Berserk-4/CJ	B	87.15	63	6.5
8610	7385021	Berserk-4/CJ	B	86.3	62	6.5
8611	7385021	Berserk-4/CJ	B	85.45	65	5
8612	7385021	Berserk-4/CJ	B	94.45	65	6.5
8613	7399021	Berserk-4/CJ	B	94.3	64	7.5
8614	7399021	Berserk-4/CJ	B	96.8	63.5	7
8615	7399021	Berserk-4/CJ	B	110.8	64	6.5
8616	7399021	Berserk-4/CJ	B	110	64.5	7
8617	7401002	Berserk-4/CJ	B	89.45	64	5
8618	7401002	Berserk-4/CJ	B	90	64.5	5
8619	7401002	Berserk-4/CJ	B	92.6	64.5	5
8706	7410013	Berserk-4/CJ	B	89.15	65.5	6.5
8707	7410013	Berserk-4/CJ	B	97.95	65.5	6.5
8708	7410013	Berserk-4/CJ	B	85.65	65	6.5
8709	7410014	Berserk-4/CJ	B	93.45	65.5	7
8710	7410014	Berserk-4/CJ	B	98.6	66	7
8711	7410014	Berserk-4/CJ	B	96.15	65.5	7
8713	7410019	Berserk-4/CJ	B	97.1	66	8
8714	7410019	Berserk-4/CJ	B	99.95	66	8

Table 4: Lsmeans of traits in Staur (agronomic field trial 2017).

Line	Family	Navn	Cross	S_PH	S_DH	S_AE
4		Zebra		103.86	57.3101	6.5714
72		CJ9306		85.56	53.9659	5
83		Berserk		85.625	59	7
8304	7359003	Zebra-2/CJ93	Z	98.8	58.5	2
8305	7359003	Zebra-2/CJ93	Z	100.1	58.5	2
8306	7359010	Zebra-2/CJ93	Z	98.8	57	6.5
8307	7359010	Zebra-2/CJ93	Z	102.8	56.5	6.5
8308	7359010	Zebra-2/CJ93	Z	97.3	56.5	6.5
8309	7360012	Zebra-2/CJ93	Z	105.65	55	5.5
8310	7360012	Zebra-2/CJ93	Z	105	55	5.5
8311	7360017	Zebra-2/CJ93	Z	107.1	57.5	5
8312	7360017	Zebra-2/CJ93	Z	98.95	58.5	6
8313	7360017	Zebra-2/CJ93	Z	93.8	60.5	5.5
8314	7361003	Zebra-2/CJ93	Z	100	57.5	6.5
8316	7361003	Zebra-2/CJ93	Z	100.8	57.5	6.5
8317	7361003	Zebra-2/CJ93	Z	99.15	57.5	6.5
8318	7361010	Zebra-2/CJ93	Z	97.3	57.5	6.5
8319	7361010	Zebra-2/CJ93	Z	97	57	6.5
8320	7361010	Zebra-2/CJ93	Z	95.95	58	6.5
8405	7361016	Zebra-2/CJ93	Z	97.45	57.5	8
8406	7361016	Zebra-2/CJ93	Z	95.95	57.5	8
8407	7361016	Zebra-2/CJ93	Z	98.95	57.5	8
8408	7361020	Zebra-2/CJ93	Z	94.3	58	6.5
8409	7361020	Zebra-2/CJ93	Z	95.15	58.5	6.5
8410	7361020	Zebra-2/CJ93	Z	94.6	58	6.5
8411	7361020	Zebra-2/CJ93	Z	96	58.5	6.5
8501	7390001	Zebra-2/CJ93	Z	99.95	58	6.5
8502	7390001	Zebra-2/CJ93	Z	99.15	58	6
8503	7390001	Zebra-2/CJ93	Z	99.8	58	6
8504	7390001	Zebra-2/CJ93	Z	96.45	58	6.5
8505	7390009	Zebra-2/CJ93	Z	100.6	57.5	5.5
8506	7390009	Zebra-2/CJ93	Z	95.15	57.5	5.5
8507	7390009	Zebra-2/CJ93	Z	95.3	57.5	5.5
8510	7398002	Zebra-2/CJ93	Z	107.15	59.5	7
8511	7398002	Zebra-2/CJ93	Z	107.3	59.5	7
8512	7398002	Zebra-2/CJ93	Z	105.5	59.5	7
8513	7398005	Zebra-2/CJ93	Z	105.65	59	7
8514	7398005	Zebra-2/CJ93	Z	100.8	59	7
8515	7398005	Zebra-2/CJ93	Z	104.8	59	6
8516	7398007	Zebra-2/CJ93	Z	102.45	59.5	5.5
8517	7398007	Zebra-2/CJ93	Z	105.3	59.5	5.5
8609	7385021	Berserk-4/CJ	B	82	57.5	8
8610	7385021	Berserk-4/CJ	B	82.65	57.5	8
8611	7385021	Berserk-4/CJ	B	83.3	59	8
8612	7385021	Berserk-4/CJ	B	93.8	60	8
8613	7399021	Berserk-4/CJ	B	91.6	59	7
8614	7399021	Berserk-4/CJ	B	92.65	58.5	7
8615	7399021	Berserk-4/CJ	B	105.1	58.5	7
8616	7399021	Berserk-4/CJ	B	105.9	59	7.5
8617	7401002	Berserk-4/CJ	B	86	57.5	3.5
8618	7401002	Berserk-4/CJ	B	81.95	57.5	3.5
8619	7401002	Berserk-4/CJ	B	83.8	57.5	3.5
8706	7410013	Berserk-4/CJ	B	84.8	59	6.5
8707	7410013	Berserk-4/CJ	B	86.45	59.5	6.5
8708	7410013	Berserk-4/CJ	B	79.3	59	6.5
8709	7410014	Berserk-4/CJ	B	92.95	60	7
8710	7410014	Berserk-4/CJ	B	90.65	60	7
8711	7410014	Berserk-4/CJ	B	92.8	60	7
8713	7410019	Berserk-4/CJ	B	91.3	60.5	5.5
8714	7410019	Berserk-4/CJ	B	90.1	60.5	5.5

Table 5: Lsmeans of traits of grain quality parameter in Bjørke after harvest 2017.

Line	Family	Navn	Cross	B_Moisture	B_TestWeigh	B_ProtDM	B_StarchDM	B_Yield_kg_H	B_Grain1000
4		Zebra		11.8	77.75	12.6	68.075	7860.83	42.399
72		CJ9306		11.8875	70.175	15.0125	65.6875	4443.33	38.6179
83		Berserk		12.075	78.225	14.15	66.7	6585	44.0195
8304	7359003	Zebra-2/CJ93	Z	12.75	78.4	11.5	69.05	8443.33	39.509
8305	7359003	Zebra-2/CJ93	Z	12.75	78.25	11.35	69.05	7186.67	39.913
8306	7359010	Zebra-2/CJ93	Z	12.75	78.2	11.5	69.1	7955	43.5305
8307	7359010	Zebra-2/CJ93	Z	12.7	77.8	12.75	67.95	7025	42.56
8308	7359010	Zebra-2/CJ93	Z	12.75	77.95	11.35	69.15	8046.67	43.1555
8309	7360012	Zebra-2/CJ93	Z	12.2	76	13.35	67.05	6233.33	41.0805
8310	7360012	Zebra-2/CJ93	Z	11.65	76.25	13.25	67.4	6491.67	45.3895
8311	7360017	Zebra-2/CJ93	Z	11.75	74.55	13.25	68.85	6045	42.874
8312	7360017	Zebra-2/CJ93	Z	11.7	74.65	12.1	68.4	6723.33	39.658
8313	7360017	Zebra-2/CJ93	Z	12.25	76.5	12.75	68.75	6403.33	42.225
8314	7361003	Zebra-2/CJ93	Z	12.4	77.9	14.1	66.55	7476.67	40.503
8316	7361003	Zebra-2/CJ93	Z	12.35	78.05	13.9	65.35	7353.33	40.8415
8317	7361003	Zebra-2/CJ93	Z	12.25	77.8	14	65.85	6868.33	40.0995
8318	7361010	Zebra-2/CJ93	Z	12.3	78.45	12.9	66.9	7738.33	47.292
8319	7361010	Zebra-2/CJ93	Z	12.5	78.15	12.9	67.6	7620	46.8025
8320	7361010	Zebra-2/CJ93	Z	12.5	79.05	13.2	67.15	7410	44.954
8405	7361016	Zebra-2/CJ93	Z	12.25	75.4	13	68	7250	40.7685
8406	7361016	Zebra-2/CJ93	Z	12	74.8	13.05	68.1	6751.67	42.5915
8407	7361016	Zebra-2/CJ93	Z	11.6	75.7	12.95	67.55	7451.67	44.713
8408	7361020	Zebra-2/CJ93	Z	12	77	13.35	67.45	8201.67	40.5505
8409	7361020	Zebra-2/CJ93	Z	11.6	76.65	13.1	67.2	7715	39.6385
8410	7361020	Zebra-2/CJ93	Z	11.7	75.75	13.4	66.5	6988.33	38.0225
8411	7361020	Zebra-2/CJ93	Z	11.45	75.4	13.55	66.35	6833.33	40.315
8501	7390001	Zebra-2/CJ93	Z	12.45	79.15	12.8	68.85	7805	44.6625
8502	7390001	Zebra-2/CJ93	Z	12.65	78.4	12.55	68	7808.33	44.214
8503	7390001	Zebra-2/CJ93	Z	12.35	78.25	12.15	66.25	7758.33	43.335
8504	7390001	Zebra-2/CJ93	Z	12.7	79.2	12.55	68.95	8150	44.6485
8505	7390009	Zebra-2/CJ93	Z	11.85	77.95	12.8	68.25	7571.67	43.7445
8506	7390009	Zebra-2/CJ93	Z	11.85	78.65	12.6	68.8	7526.67	45.076
8507	7390009	Zebra-2/CJ93	Z	11.25	78.3	12.9	68.55	7173.33	45.1825
8510	7398002	Zebra-2/CJ93	Z	11.75	77.9	13.95	66.6	7121.67	39.585
8511	7398002	Zebra-2/CJ93	Z	11.9	79.1	13.6	66.85	7018.33	41.2335
8512	7398002	Zebra-2/CJ93	Z	11.7	78.55	13.5	65.8	6993.33	39.1485
8513	7398005	Zebra-2/CJ93	Z	12.3	79.25	13.85	66.4	8251.67	41.6775
8514	7398005	Zebra-2/CJ93	Z	11.95	80.05	13.7	65.6	7680	41.276
8515	7398005	Zebra-2/CJ93	Z	12	79.9	13.7	65.5	8106.67	42.259
8516	7398007	Zebra-2/CJ93	Z	11.9	79.6	14.2	66.05	8040	40.365
8517	7398007	Zebra-2/CJ93	Z	11.75	77.95	14.1	66.1	7855	39.0535
8609	7385021	Berserk-4/CJ	B	12.15	79.55	14.7	65.8	6790	35.885
8610	7385021	Berserk-4/CJ	B	12	79.35	15.1	66	7196.67	39.0025
8611	7385021	Berserk-4/CJ	B	11.75	78.7	12.2	69.3	7688.33	43.239
8612	7385021	Berserk-4/CJ	B	12.2	79.25	12.3	68.5	8586.67	47.3935
8613	7399021	Berserk-4/CJ	B	11.55	78.45	14.3	67.1	6480	48.6395
8614	7399021	Berserk-4/CJ	B	11.65	77.5	14.4	66.65	6585	48.607
8615	7399021	Berserk-4/CJ	B	11.45	74.15	15.4	65.4	5803.33	43.9415
8616	7399021	Berserk-4/CJ	B	11.45	77.4	15.25	65.6	7151.67	44.6845
8617	7401002	Berserk-4/CJ	B	12.55	79.85	14.8	66.25	6848.33	44.9505
8618	7401002	Berserk-4/CJ	B	12.3	78.9	14.6	66.6	6745	45.3985
8619	7401002	Berserk-4/CJ	B	12.5	79.55	15.05	66	6995	45.3655
8706	7410013	Berserk-4/CJ	B	12.45	76	12.4	68.45	6448.33	34.761
8707	7410013	Berserk-4/CJ	B	12	77.15	12.4	67.75	6671.67	36.763
8708	7410013	Berserk-4/CJ	B	11.75	75.45	13.5	66.9	5396.67	35.9585
8709	7410014	Berserk-4/CJ	B	11.75	77	13	67.5	5651.67	39.568
8710	7410014	Berserk-4/CJ	B	12.25	75.05	13.75	65.45	6070	38.7045
8711	7410014	Berserk-4/CJ	B	11.5	75.2	12.85	68	5848.33	40.283
8713	7410019	Berserk-4/CJ	B	12.4	76.15	12.85	67.25	4816.67	37.957
8714	7410019	Berserk-4/CJ	B	12.3	76.95	12.9	67.5	5420	38.2525

Table 6: Lsmeans of traits of grain quality parameter in Staur after harvest 2017.

Line	Family	Navn	Cross	S_Moisture	S_TestWeigh	S_ProtDM	S_StarchDM	S_Yield_kg_H	S_Grain1000
4		Zebra		11.9333	78.1167	12.6167	68.75	8223.33	46.081
72		CJ9306		11.8588	75.6951	15.76	65.82	4450.42	40.6823
83		Berserk		11.8012	78.8449	13	67.42	7518.24	46.5273
8304	7359003	Zebra-2/CJ93	Z	11.8	78.2	12.15	69.15	7196.67	43.8095
8305	7359003	Zebra-2/CJ93	Z	11.45	78.4	11.95	68.9	7206.67	45.0665
8306	7359010	Zebra-2/CJ93	Z	11.75	79.45	12.8	69.6	7861.67	49.1
8307	7359010	Zebra-2/CJ93	Z	11.45	79.65	13.45	67.75	6896.67	46.5265
8308	7359010	Zebra-2/CJ93	Z	11.75	78.95	12.4	67.9	7603.33	47.342
8309	7360012	Zebra-2/CJ93	Z	11.65	77.8	14.65	62.75	6678.33	43.8835
8310	7360012	Zebra-2/CJ93	Z	10.9	78.25	14.2	66.75	7816.67	50.05
8311	7360017	Zebra-2/CJ93	Z	11.1	76.3	13.8	66.2	8186.67	45.822
8312	7360017	Zebra-2/CJ93	Z	11.3	76.5	12.75	68.05	7971.67	43.3835
8313	7360017	Zebra-2/CJ93	Z	11.15	76.9	12.55	68.3	7456.67	42.508
8314	7361003	Zebra-2/CJ93	Z	11.6	77.95	14.6	65.05	6236.67	39.817
8316	7361003	Zebra-2/CJ93	Z	11.3	78.4	14.4	67.05	6671.67	42.33
8317	7361003	Zebra-2/CJ93	Z	11.4	78.15	13.9	65.85	6633.33	40.432
8318	7361010	Zebra-2/CJ93	Z	11.4	78.2	13.15	65	6641.67	49.435
8319	7361010	Zebra-2/CJ93	Z	11.4	77.95	13.3	67.25	7005	48.15
8320	7361010	Zebra-2/CJ93	Z	11.2	78.45	13	67.55	6811.67	48.555
8405	7361016	Zebra-2/CJ93	Z	11.9	78.1	13.35	66.5	6480	42.904
8406	7361016	Zebra-2/CJ93	Z	11.65	76.8	13.35	68.25	6551.67	45.95
8407	7361016	Zebra-2/CJ93	Z	11.7	77	13.2	68.25	7253.33	49.32
8408	7361020	Zebra-2/CJ93	Z	11.35	77.4	14.25	66.8	6926.67	41.4615
8409	7361020	Zebra-2/CJ93	Z	11.85	77.45	14.3	66.45	6838.33	41.939
8410	7361020	Zebra-2/CJ93	Z	12.05	77.6	14.1	65.8	7175	42.1115
8411	7361020	Zebra-2/CJ93	Z	11.8	77.5	14.25	65.45	6741.67	41.5315
8501	7390001	Zebra-2/CJ93	Z	11.6	78.35	11.75	69.55	7845	47.473
8502	7390001	Zebra-2/CJ93	Z	11.75	78.1	12.05	69.5	7086.67	49.1
8503	7390001	Zebra-2/CJ93	Z	11.8	78.4	12.55	68.8	7850	48.9155
8504	7390001	Zebra-2/CJ93	Z	11.6	78.3	12.45	69.95	7685	48.07
8505	7390009	Zebra-2/CJ93	Z	11.45	78.7	12.4	69.15	7006.67	47.492
8506	7390009	Zebra-2/CJ93	Z	11.65	78.15	13	68.05	7141.67	46.8
8507	7390009	Zebra-2/CJ93	Z	11.7	78.25	12.2	68.7	7031.67	60.425
8510	7398002	Zebra-2/CJ93	Z	11.35	79.4	13.6	67.6	7895	43.895
8511	7398002	Zebra-2/CJ93	Z	11.5	79.45	13.55	67.4	7666.67	45.474
8512	7398002	Zebra-2/CJ93	Z	11.45	79.2	13.65	66.9	7761.67	43.92
8513	7398005	Zebra-2/CJ93	Z	12.1	79.15	12.75	68.15	6876.67	45.5
8514	7398005	Zebra-2/CJ93	Z	12	78.75	12.85	67.6	6596.67	45.249
8515	7398005	Zebra-2/CJ93	Z	11.95	79.85	13.95	66.2	7053.33	45.773
8516	7398007	Zebra-2/CJ93	Z	11.75	79.3	12.9	66.75	7655	46.3455
8517	7398007	Zebra-2/CJ93	Z	11.65	79.35	13.4	67.25	7903.33	44.6235
8609	7385021	Berserk-4/CJ	B	11.9	78.75	15.25	64.5	5711.67	36.7
8610	7385021	Berserk-4/CJ	B	12.05	79.1	15.2	65.7	5845	37.205
8611	7385021	Berserk-4/CJ	B	12.25	79.35	11.7	70.25	8025	47.371
8612	7385021	Berserk-4/CJ	B	12.05	80.05	11.4	68.75	8341.67	51.6445
8613	7399021	Berserk-4/CJ	B	11.85	79.4	14.7	66	8023.33	50.7345
8614	7399021	Berserk-4/CJ	B	11.75	78	14.35	66.65	7710	47.8335
8615	7399021	Berserk-4/CJ	B	11.4	76.65	14.7	65.2	7111.67	45.1795
8616	7399021	Berserk-4/CJ	B	11.7	78.75	14.05	66.3	7208.33	47.093
8617	7401002	Berserk-4/CJ	B	11.4	80.05	15.45	65.7	6486.67	50.1155
8618	7401002	Berserk-4/CJ	B	11.4	79.5	14.8	66.15	6358.33	49.564
8619	7401002	Berserk-4/CJ	B	11.7	79.35	14.5	66.4	5978.33	45.1755
8706	7410013	Berserk-4/CJ	B	12.2	79.5	12.45	69.15	8500	42.359
8707	7410013	Berserk-4/CJ	B	12.35	80.45	12.4	67.95	8421.67	42.2
8708	7410013	Berserk-4/CJ	B	12.05	79.4	12.75	66.85	8351.67	41.2125
8709	7410014	Berserk-4/CJ	B	12.3	79.3	12.85	67.15	7895	43.4
8710	7410014	Berserk-4/CJ	B	12.2	78.35	13.45	66.4	7530	42.05
8711	7410014	Berserk-4/CJ	B	11.95	78.25	12.85	67.25	7951.67	42.2165
8713	7410019	Berserk-4/CJ	B	12.3	79.45	12.55	68.25	7788.33	42.214
8714	7410019	Berserk-4/CJ	B	12.25	79.2	12.65	67.4	7426.67	39.5475

Table 7: MASBASIS used in genotyping process 2017

Entry	MASBASIS
1003	Bastian
1005	Bjarne
1011	Zebra
1016	Berserk
1041	Naxos
1048	Saar
1073	Avocet YrA
1079	CJ9306
1080	CJ9403
1081	512-21
1082	512-50
1083	512-54
1084	512-70
1085	512-87
1086	SHA3/CBRD
1087	SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)
1091	Sumai 3 (18.)
1102	Nobeokabouzu (Mhazy)
1106	Frontana (95)
1111	Nanjing 7840 - Pl.4
1114	Ning 8343 - Pl.4
1116	Vinjett
1124	MILAN/SHA7
1137	NG8675/CBRD
1142	BCN*2//CROC_1/AE.SQUARROSA (886)
1173	Demonstrant
1174	Krabat
1401	Mirakel
1402	Rabagast
1403	Seniorita
1413	Berlock
1414	Arabella
1627	N894037
1633	IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)
1634	GAMENYA

Table 8: Lsmeans of traits for 2016 field trials in Vollebekk.

Line	Family	Cross	A_DH	A_PH	A_AE	FHB_Mean	DON
8304	7359003	Z	53.6667	75.5	3.6667	46.5	24.7
8305	7359003	Z	53	75	4.6667	32.5	16.3
8306	7359010	Z	52.0927	74	6	29.2434	28.9737
8307	7359010	Z	51.3333	75.5	7.6667	33.5	11.25
8308	7359010	Z	51.3333	65	6.3333	34.5	8.65
8309	7360012	Z	49.3333	75	8.6667	17.25	8.3
8310	7360012	Z	49.3333	73	7.3333	36.5	14.45
8311	7360017	Z	51.6667	80	6.6667	44	16.3
8312	7360017	Z	53.0927	79	6.5	55.2434	20.4737
8313	7360017	Z	54.6667	75	7.3333	48	15.4
8314	7361003	Z	53	74.5	8.3333	30	13.65
8315	7361003	Z	53.3333	77	8	24	12.9
8316	7361003	Z	53.3333	76.5	7.3333	31.5	17.65
8317	7361003	Z	54	75.5	7.6667	24	14.1
8318	7361010	Z	53.3333	75	8	37	20.65
8319	7361010	Z	53	82	7.3333	36.5	15.4
8320	7361010	Z	52.3333	82.5	8	40.75	15.65
8405	7361016	Z	52.6667	80	8.6667	27.5	11.6
8406	7361016	Z	53.3333	72.5	9	32.5	15.6
8407	7361016	Z	52.6667	75.5	7	48.5	17.15
8408	7361020	Z	53.3333	77	7	35	13.8
8409	7361020	Z	53.6667	76.5	7	29.5	14.3
8410	7361020	Z	53.5927	71	6.5	40.2434	15.8737
8411	7361020	Z	53.6667	74.5	7.3333	38.5	19.45
8501	7390001	Z	51.5927	69	5.5	28.2434	21.0737
8502	7390001	Z	51.5927	69	6.5	37.2434	23.0737
8503	7390001	Z	51.0927	71	5.5	39.2434	22.5737
8505	7390009	Z	52.5927	70	5	20.2434	21.4737
8506	7390009	Z	52.0927	63	5.5	31.2434	28.2737
8613	7399021	B	54.6667	78	6.3333	25	6.85
8614	7399021	B	54.6667	77	7.6667	24.5	10.35
8616	7399021	B	54.3333	75	7.6667	30	7.35
8510	7398002	Z	55.0927	80	7	24.2434	5.8737
8511	7398002	Z	55	77	7.6667	33.5	11.75
8512	7398002	Z	54.6667	77.5	7.3333	27.5	7.8
8513	7398005	Z	54.6667	73	7.6667	20.5	9.5
8514	7398005	Z	55	73.5	7.6667	22	8.45
8515	7398005	Z	54.6667	78	8.3333	31.5	27.75
8516	7398007	Z	53.0927	61	8.5	20.2434	17.0737
8517	7398007	Z	54	65	8.6667	35.5	21.5
8608	7385021	B	55	72	8	30	28.35
8609	7385021	B	52.6667	67.5	8.3333	26.5	12.9
8611	7385021	B	51	63.5	9	21.5	14.1
8612	7385021	B	53.6667	72.5	9	20.5	15.85
8617	7401002	B	54	61	8.3333	27	26.65
8618	7401002	B	55	58	6	32	47.05
8619	7401002	B	55.0927	55	8.5	35.2434	36.9737
8709	7410014	B	55.6667	69	8.3333	26	9.95
8710	7410014	B	54.0927	63	7.5	37.2434	6.8737
8711	7410014	B	54.6667	66	8.3333	33	12.1
8713	7410019	B	55.6667	65	7.3333	26.5	10.4
8714	7410019	B	55.6667	64.5	8.3333	33.5	14.15
8715	7410019	B	55.0927	67	8	21.2434	12.9737
8716	7410019	B	55.0927	65	8.5	31.2434	7.6737