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Genome-wide association mapping of *Fusarium* head blight resistance in Norwegian spring and winter wheat lines

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Abstract

Fusarium graminearum is a species of fungal pathogens known to cause *Fusarium* head blight impacting grain quality and yield of Norwegian wheat. The genetic background of this disease have been studied over the past two decades. Genome-wide association studies (GWAS), also called association mapping, have proven to be powerful tools in identifying the genetic composition of complex traits. In this thesis, markers from the 90K SNP array with phenotypic data for a core collection of 172 MASBASIS spring and winter wheat lines from 2014 and previous years were analysed with the mixed linear model in Tassel correcting for kinship and population structure. STRUCTURE identified 8 subpopulations in MASBASIS differentiated by geographic origin and spring and winter growth habits. A total of 22 031 markers for spring wheat, and 16091 for winter wheat were used to identify significant markers for earliness, plant height, anther extrusion, *Fusarium* head blight and mycotoxin deoxynivalenol. All traits were discovered to hold significant markers around previously discovered QTL, indicating that genes controlling these traits may be located at positions around the markers of significance. Heritabilities calculated from analyses of variance demonstrated that the percentages of the observed variances resulting from genetics was much lower for FHB than for DON, suggesting a great impact of environmental effects and experimental error, such as weather and scoring, on the rate of disease. The usefulness of using FHB as a trait in genome-wide association mapping, compared to DON, must therefore be individually determined. DON is considered the most important trait due to the yield and quality loss from mycotoxin infected grain, and gave both high heritability and significant markers from the association mapping from this study. From the field trial of 2014, Norwegian breeding lines had relatively low values for DON compared to the susceptible lines, indicating that breeding for Fusarium resistance have been successful. However, compared to the highly resistant source Sumai 3, there is still a long way to go. Further analyses of resistance is recommended to identify the genes underlying traits for resistance and the specific lines carrying these for use in breeding programmes.

Thesis organisation

This thesis focuses on association mapping of quantitative trait loci for *Fusarium* Head Blight and is divided into five chapters. The first chapter contains a detailed review of the literature, aims and the materials and methods used for the study. The second chapter includes heritability and phenotypic results of earliness (DH/HD), plant height (PH) *Fusarium* head blight (FHB) and mycotoxin deoxynivalenol (DON) for the core collection of spring and winter wheat lines (MASBASIS).

A defined population structure and association mapping of earliness and plant height in MASBASIS is presented in chapter three, followed by a forth chapter including the results for the association mapping of anther extrusion, *Fusarium* head blight and deoxynivalenol. Furthermore, the fifth chapter includes general discussion of the thesis and further recommendations.

The thesis includes two manuscripts to be submitted as parts of scientific papers. However, one more year of testing, especially for *Fusarium* head blight and anther extrusion, is required before the paper can be eligible for submission. All references cited are listed after the recommendations, followed by Appendices.

Abbreviations

FHB – *Fusarium* head blight

DON – Deoxynivalenol

DH – Days to heading

HD – Heading date

PH – Plant height

AE – Anther extrusion

FHBreg – Regression for *Fusarium* head blight

DONreg – Regression for deoxynivalenol

QTL – Quantitative trait loci

SNP – Single nucleotide polymorphism

GWAS – Genome-wide association study

MLM – Mixed linear model

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Chapter 1:
General introduction and methodologies

1.1. Literature review

1.1.1. Wheat production

Wheat is consumed worldwide. It is one of the primary grains grown and produced in a wide range of environments and conditions (Dupont & Altenbach 2003). Some of these include hot, cool, dry and moist environments. Both productivity and quality of the grains are subject to variation because of these environmental conditions. According to Dupont and Altenbach (2003) not only wheat growers are dependent on quality yield. Also millers and bakers inquire wheat with functional properties for further flour production and processing.

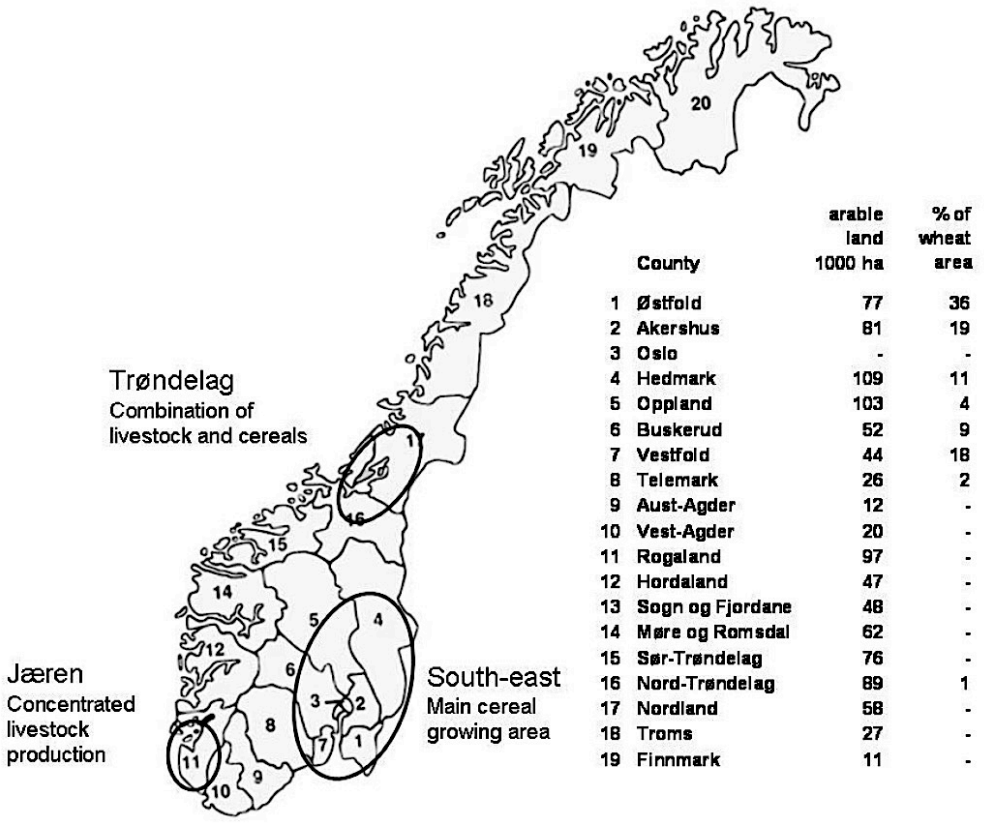
The Food and Agricultural Organization of the United Nations (FAO) has recently stated that "the world's wheat production is anticipated to 722 million tonnes (mt) in 2015"(FAO 2015). However, this is 1% lower than the current estimate of 2014/2015 (728.2 mt). This is an effect of the reduced plantings in the EU, although prospects have been improved (FAO 2015). The consumption of wheat as food is estimated to rise by about 1% (5 mt) every year according to FAO (2015) and IGC (2014) because of the expanding uses of feed and food. Although the food demand per capita is the same as previous years, the increasing population causes this upward trend (IGC 2014). Furthermore, much of the anticipated increase in wheat production directly linked to use for human food. IGC (2014) estimates this to a 1% annual increase, mainly due to increasing popularity of wheat in developing countries in Africa and Asia. The global per capita demand for wheat is still at 66% with the overall increase in consumption in developing regions (IGC 2014).

Norwegian wheat production has been impacted by its geographic position. Short cool summers and long winters are the challenges for the Norwegian cereal industry (Belderok et al. 2000; Yoshida et al. 1998). Early ripening and winter hardiness has been important in Norwegian wheat breeding in the 20th century. The spring wheat is typically sown in the spring, and is ready for harvest around August. Winter wheat is typically sown after September, and harvested around august the next summer depending on the climatic conditions. The winter wheat needs a period of cold for vernalisation in order to transfer to

the generative phase. The wheat plants will lie dormant over the winter until the soil warms up in the spring. Yield will then depend on both conditions around the growing time, and during its dormant stage. Winter wheat in northern regions must possess good resistance to snow mould and cold temperatures to be able to survive the winter (Yoshida et al. 1998).

1.1.2. History of wheat breeding in Norway

Norwegian agriculture dates back to the Early Neolitihic Period around 4000 BC, when pollen analyses indicated changes in the local vegetation around the Oslofjord and Jæren, direct evidence being found around 2500 BC (Lillemo & Dieseth 2011). The main agricultural areas in Norway are the south east, Jæren and Trøndelag (Figure 1).



	County	arable land 1000 ha	% of wheat area
1	Østfold	77	36
2	Akershus	81	19
3	Oslo	-	-
4	Hedmark	109	11
5	Oppland	103	4
6	Buskerud	52	9
7	Vestfold	44	18
8	Telemark	26	2
9	Aust-Agder	12	-
10	Vest-Agder	20	-
11	Rogaland	97	-
12	Hordaland	47	-
13	Sogn og Fjordane	48	-
14	Møre og Romsdal	62	-
15	Sør-Trøndelag	76	-
16	Nord-Trøndelag	89	1
17	Nordland	58	-
18	Troms	27	-
19	Finnmark	11	-

Figure 1. Distribution of arable land and wheat cultivation in Norway divided by county. The main agricultural areas are indicated by circles (Lillemo & Dieseth 2011)

Fossils of grain have been found at many locations in Norway around the coast, and the major trends in cereal growing were established from about 1800 BC onwards (Lillemo & Dieseth 2011). The predominant cereals during the Viking and Medieval times were oat and barley, while wheat was considered a luxury grain utilized at special fields at the big farms (Mikkelsen 1979). A decline in production was reached around 1200 AD and onwards due to worsening of the climate. Norway had to rely more on imported grains, mainly from England and countries around the Baltic Sea (Lunden 2004).

After the World War I it became a political issue to secure a reliable supply of grain because of the growing population, where incentives were placed on the farmers to increase the production (Lillemo & Dieseth 2011). When the State Grain Monopoly was established in 1929, farmers got the right to sell their grain to the State above the current price for the world market. A new decline in domestic cereal production was reached after World War II when in 1950 the combine harvester was introduced with new requirements for acceptable moisture level in the field before harvest (Lillemo & Dieseth 2011). As stated by Lillemo and Dieseth (2011), this was a particular issue because of the frequency of rain during August and September, which in turn was a problem for the long, weak straws, and lack of pre-harvest sprouting resistance. Wheat production in Norway was close to eradication at this time. In the early 1960s, intense breeding efforts were made to develop cultivars with a combination of sprouting resistance and baking quality, causing wheat production to increase again leading Norway towards self-sufficiency (Lillemo & Dieseth 2011). Since 1925, the Norwegian yield has tripled, most of said increase coming from the development of new varieties and improvement in cultivation techniques. In Norway, the political will to protect the domestic grain production by not importing cheap grains led to the integration of the Norwegian economy with the European Economic Area (EEA) and implementation of recent World Trade Organization (WTO) agreements (Lillemo & Dieseth 2011). These agreements have also led to changes in the implementation of the policy, causing the State Grain Monopoly to be removed in 1995, and the obligation by the state to buy all Norwegian grain was abolished in 2001 (Lillemo & Dieseth 2011).

The overall wheat production in Norway has, compared to the global production, increased. Figure 2 displays the Norwegian wheat production from 1989 to 2014 demonstrating that the production has increased over the years. Over the past 25 years the production has increased from 139.600 t in 1989 to 375.000 t in 2014.

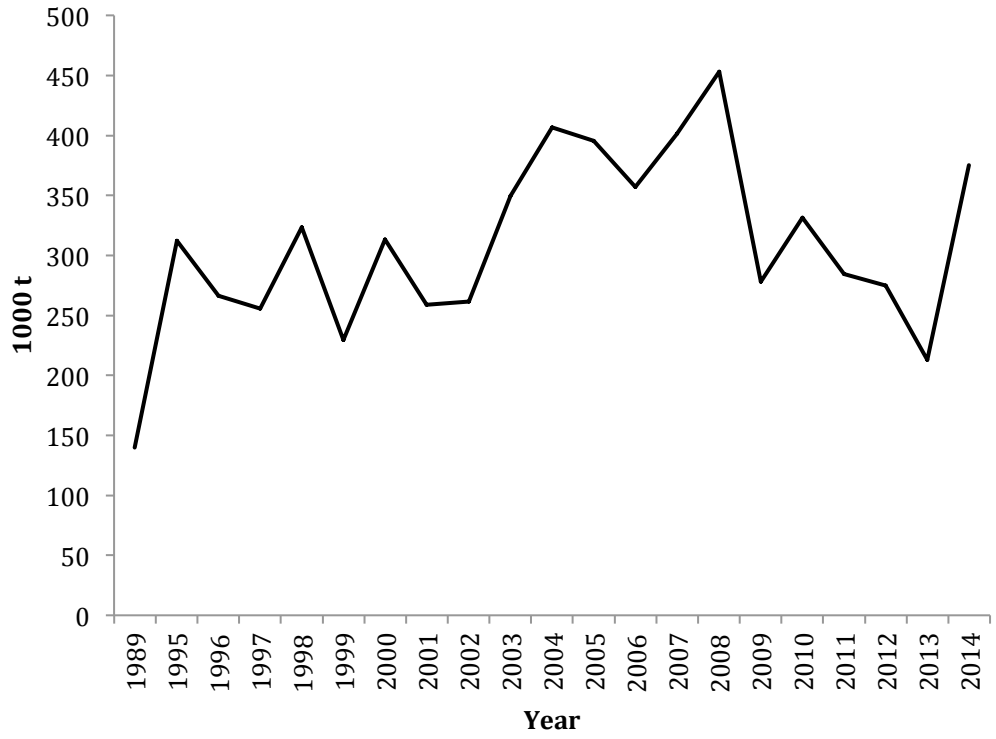


Figure 2. Wheat production in Norway between 1989 and 2014 in 1000 tonnes (t) (SSB 2015)

Evaluation of the Norwegian landraces of wheat was started by Bastian Larsen in 1898 at the Agricultural University of Norway, Ås (Lillemo & Dieseth 2011). Testing and selection of promising lines was carried out at the experimental station while final testing was carried out at a variety of locations around the country. By the beginning of the 20th century, wheat breeding stations were established. The work by Bastian Larsen was continued by Knut Vik, who started to characterize the landraces in more detail. Knut Vik discovered that many of these landraces had complementary traits which could be combined into improved cultivars, which was started in 1913 by target crossing for powdery mildew resistance (Lillemo & Dieseth 2011). Later, traits such as lodging resistance and baking quality were subject to improvement, while winter wheat breeding was especially focused around improving hardiness. Figure 3 and 4 displays the genealogy of the first Norwegian spring and winter wheat varieties (Lillemo & Dieseth 2011).

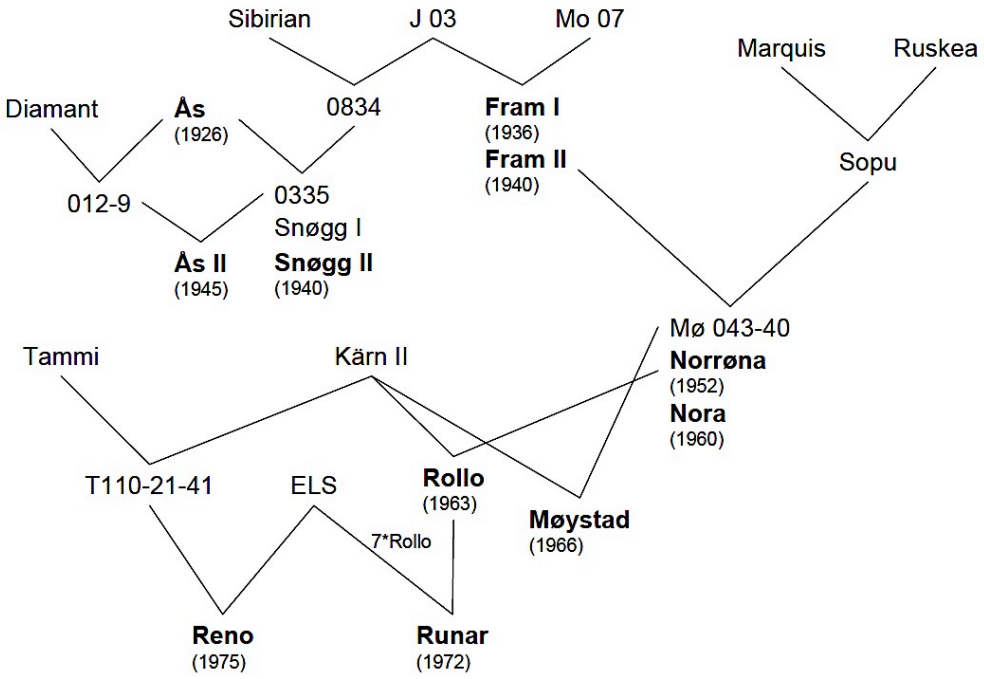


Figure 3. Genealogy of the first Norwegian spring wheat lines with released varieties developed in Norway in bold and the year of release in parenthesis (Lillemo & Dieseth 2011)

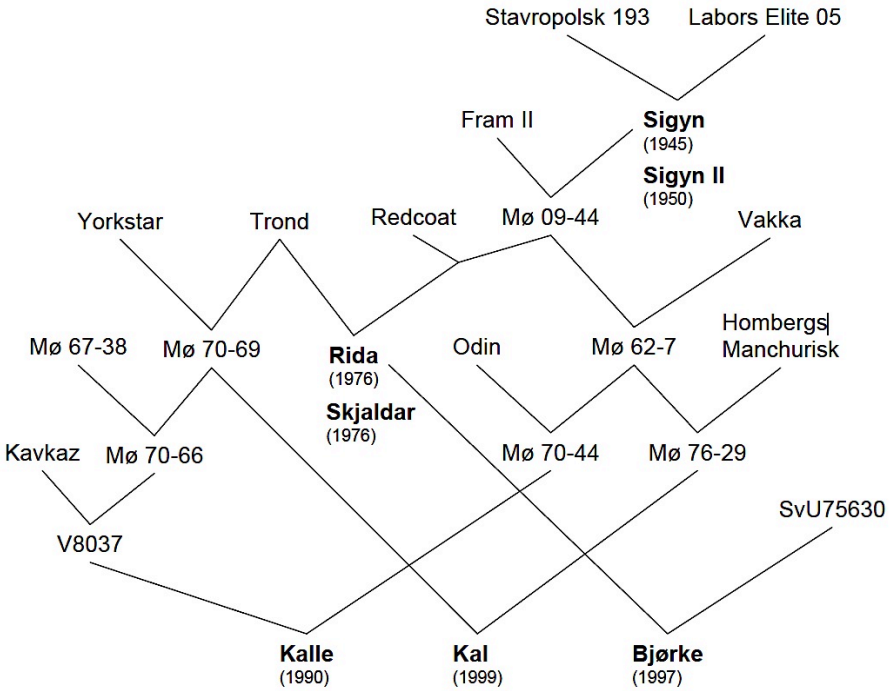


Figure 4. Genealogy of the first Norwegian winter wheat varieties with released varieties in Norway in bold and the year of release in parenthesis (Lillemo & Dieseth 2011)

The general breeding goals around the world are typically; “high yield potential, good agronomic performance, resistance to important diseases and good quality” (Lillemo & Dieseth 2011). Grain quality has been mentioned as one of the primary goals for wheat breeding in Norway. However, breeding for disease resistance is also of high priority due to the cost and potential health and environmental threat of fungicides (Lillemo & Dieseth 2011). “The diseases that most commonly threaten the wheat crops in Norway are powdery mildew (*Blumeria graminis f.sp. tritici*), *Septoria* Leaf Blotch and *Fusarium* Head Blight (FHB)”(Lillemo & Dieseth 2011). For *Fusarium* head blight not much have been done previously with specific screening to improve resistance. There has been an effort to incorporate resistance genes from exotic sources into the Norwegian breeding material (Lillemo & Dieseth 2011). For the past 20 years, the Norwegian University of Life Sciences (NMBU) have worked closely with Graminor for resistance testing and genetic studies of *Fusarium* resistance in wheat and other cereals.

1.1.3. The *Fusarium* genus

The *Fusarium* genus includes a group of fungal pathogens known to cause widespread disease of many plant species (Buerstmayr et al. 2002). The *Fusarium* species are primarily associated with *Fusarium* Head Blight (FHB), also known as scab (Bottalico & Perrone 2002; Nganje et al. 2004). *Fusarium graminearum* causes various diseases in cereal-grains and is the most important *Fusarium* species infecting wheat. However, it is most associated with FHB in wheat and barley in particular, as these cereal-grains constitute around two thirds of the worlds cereal-production (Bottalico & Perrone 2002). In Norway it has also caused great damage in oats. *F. graminearum* took over as the dominating pathogen in Norway in 2004-2005. As the fungus produces ascospores that can be spread by wind, the disease has proven to be difficult to control. It is therefore important for Norwegian cereal grain production to develop resistant lines to reduce the rate of disease.

The *Fusarium* genus contains different species. Some are very pathogenic, while others are less pathogenic causing less damage to the infected plants. The species *F. graminearum*, *F. avenaceum* and *F. culmorum* are the species which are most associated with FHB (Bottalico & Perrone 2002). Deoxynivalenol and zearalenone, produced by *F. graminearum* and *F. culmorum*, are also the most regularly encountered *Fusarium* mycotoxins in Europe (Bottalico & Perrone 2002; Kollers et al. 2013).

1.1.4. *Fusarium* graminearum

F. graminearum typically causes reduced germination of the grains and further develops into the disease known as *Fusarium* head blight (FHB) with the capacity and potential to destroy a high yielding crop (McMullen et al. 1997). FHB was first mentioned in England 1884 when it was described as a major threat to wheat and barley (Goswami & Kistler 2004). The disease has probably also been present in Norway for a long time although it had not been identified as a disease until more recent. Nielsen et al. (2011) found fungal DNA of *F. graminearum* in historical wheat samples in Denmark dating to 1957, suggesting that it has most likely been present also in Norway around this time.

FHB causes multiple threats. Florets become sterile, and kernels become discoloured and withered (Goswami & Kistler 2004; Sun et al. 2002). This, in turn, causes yield and quality loss in addition to mycotoxin production (Nganje et al. 2004). Once there is a loss in yield both marketing, exporting and processing the infected grains becomes difficult (Goswami & Kistler 2004).

Figure 5 displays how the grains look after *Fusarium* infection. The susceptible cultivar Avocet YrA have more withered and grey grains compared to the resistant source Sumai 3. Additionally, for the promising new line Mirakel, there are less damaged grains than for the more susceptible cultivar Vinjett.

Figure 5. FHB on wheat cultivars from field testing in 2013 at Vollebekk, Ås

Figure 6 displays the life cycle of *Fusarium.* As explained by Goswami and Kistler (2004) the disease comes primarily from infected plant debris where the fungus overwinters as saprophytic mycelia, feeding on dead organic material. Ascospores are produced as a result of warm and moist weather conditions around the flowering time of cereal crops (Markell & Francl 2003). These spores are then spread by wind, rain, insects or animals to the host plants (Parry et al. 1995; Sutton 1982).

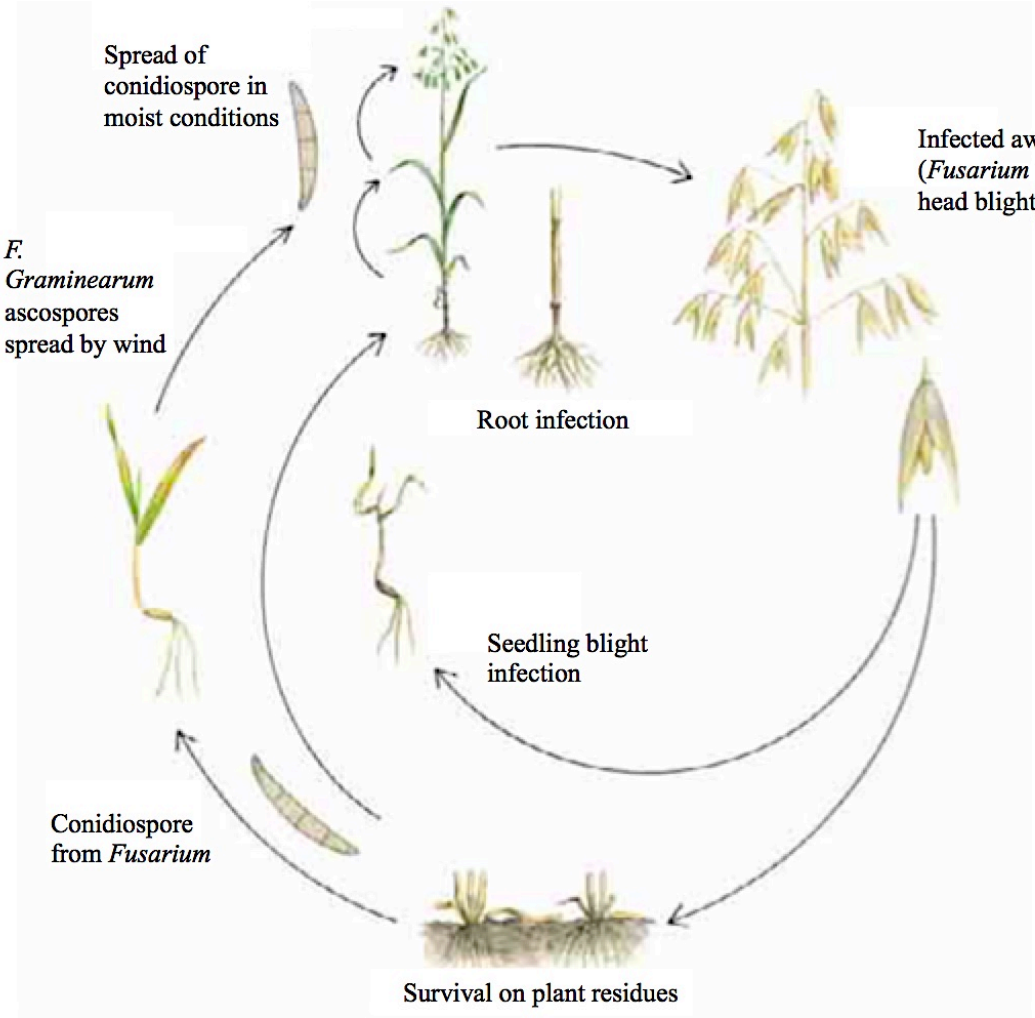


Figure 6. Life cycle of *Fusarium (*Brandsæter et al. 2009)

According to Lillemo et al. (2013) this is a particular problem in Norway and one reason for the difficulty of growing certain cultivars of oats. Ascospores produced by *F. graminearum,* combined with raised temperatures and rainfall during flowering, are particular reasons why FHB is such a problem. It is possible to increase the germination rates of *Fusarium* infected grain with a fungicide seed treatment. However, the method has proven to be less effective if infection is severe (Lillemo et al. 2013), which is probably due to the fact that the infection is located underneath the hulls and can not be attacked by the fungicide unless the shell is removed. In addition, controlling the disease by fungicides is also limited by cost and efficacy (Goswami & Kistler 2004; McMullen et al. 1997). A fungicide with the active ingredient Prothioconazole in the product Proline (Bayer 2015) can reduce DON content of the grains by 50%. This is currently the only active fungicide in Norway, and the most effective against *F. graminearum*. There is to this day no complete protection against FHB and it is therefore

important to be able to breed lines that maintain high germination, and are more resistant to *Fusarium* infection (Lillemo et al. 2013; Liu et al. 2009; Lu et al. 2013). With highly resistant lines and effective fungicides this integrated pest management can effectively reduce the DON content.

There are also several factors associated with the onset of FHB. Climatic conditions, such as rain and temperature at the flowering stage of the plant is of particular importance (Bottalico & Perrone 2002), as it is the time when the fungi is most likely to infect the plant. However, it is also known to infect at a later stage during grain filling. There are also agronomic factors related to the pathogenic onset. These can be "soil cultivation, nitrogen fertilization, fungicides, crop rotation, and host genotype" (Bottalico & Perrone 2002).

Table 1. Mycotoxigenic species isolated from FHB of wheat in Europe (Bottalico & Perrone 2002)

Species	Geographical incidence		Mycotoxin
	North/Centre	South	
F. graminearum	+ + +	+ + +	DON, NIV, ZEN, AcDON, FUS
F. avenaceum	+ + +	+ +	MON, BEA, ENS
F. culmorum	+ + +	+ +	DON, ZEN, ZOH, NIV
F. poae	+ +	+	NIV, BEA, DAS, FUS, ENS
F. equiseti	+ +	+	DAS, ZEN, ZOH
F. tricinctum	+	+	MON
F. cerealis	+	±	NIV, FUS, ZEN, ZOH
F. sporotrichioides	+	±	T2, HT2, T2ol, NEO
F. acuminatum	±	±	T2, NEO
F. subglutinans	±	−	MON
F. solani	±	−	−
F. oxysporum	±	−	−

AcDON = Monoacetyl-deoxynivalenols (3-AcDON, 15-AcDON); BEA = Beauvericin; DAS = Diacetoxyscirpenol; DON = Deoxynivalenol (Vomitoxin); ENS = Enniatins; FUS = Fusarenone-X (4-Acetyl-NIV); HT2 = HT-2 toxin; MON = Moniliformin; NEO = Neosolaniol; NIV = Nivalenol; T2 = T-2 toxin; T2ol = T-2 tetraol; ZEN = Zearalenone; ZOH = zearalenols (α and β isomers).

Table 1 from Bottalico and Perrone (2002) illustrates the mycotoxins associated with the *Fusarium* species in Europe. Here, one can see that both *F. graminearum* and *F. culmorum* are associated with DON. It has been indicated by field surveys that this mycotoxin is "the most frequently encountered mycotoxin in wheat in Europe" (Bottalico & Perrone 2002). The

most important problem with *Fusarium* is the mycotoxins in the grains. Mycotoxins are toxic chemicals, and restrictions are put on the grain to be safely consumed by humans and animals. Although DON is suggested not to have any immediate serious health applications, prolonged exposure to the mycotoxin should be prevented (VKM 2015). For animals, especially pigs, the symptoms of prolonged high DON levels in the grains are refusal to eat, reduced exploitation of the food, impaired immune system, diarrhoea and vomiting (Clasen & Børsum 2012; VKM 2015). The Norwegian Scientific Committee for Food Safety (VKM) is responsible for assessing food safety risks in Norway. As the mycotoxin has been proven to propose health risks in animals, these negative effects could be an indication that prolonged exposure to DON could be harmful for humans. According to VKM (2015), children today ingest too much DON, and considering the possible health risks proposed by VKM (2015) for prolonged exposure, the level of mycotoxins in the grains should be reduced. Therefore, breeding for resistance to FHB is important.

1.1.5. *Fusarium* resistance

Resistance to *Fusarium* has proven to be quantitatively inherited (Buerstmayr et al. 2002; Kollers et al. 2013; Lu et al. 2013; Zhou et al. 2002), which means that the trait is influenced by more than one gene. In addition, Snijders and Vaneeuwijk (1991) and Bai and Shaner (1994) suggested that the influence of the environment on the disease makes reliable phenotyping difficult.

FHB resistance is a complex and quantitative trait where there has been determined five different types of resistance (Table 2) (Mesterházy 1995).

Table 2. Different parameters of *Fusarium* Head Blight (FHB) resistance (Mesterházy, 1995)

Type	Explanation of resistance
I	Resistance to invasion
II	Resistance to fungal spread
III	Resistance to toxin accumulation
IV	Resistance to kernel infection
V	Tolerance

Klahr et al. (2007) and Somers et al. (2003) suggested that taller lines are farther from the soil thus may have a better chance of escaping infection. However, the study by Srinivasachary et al. (2008) indicated that the FHB resistance and plant height relationship may be more complex.

Sun et al. (2002) also stated that "The cultivation of genetically resistant cultivars is the most cost-effective method to control the disease…" (Sun et al. 2002). By investigating the genes for resistance in different wheat cultivars improved lines can be developed, as different resistant sources are likely to possess different resistance genes (Sun et al. 2002). In this particular study by Sun et al. (2002) 35 different spring wheat cultivars were studied for genetic diversity related to *Fusarium* resistance. They concluded that breeding for FHB resistance is difficult for three reasons: Firstly, *Fusarium* resistance is of exotic origin and usually come with very low agronomic traits. Secondly, resistance is determined by a number of genes, making it difficult to pinpoint exactly how it works. In addition, screening for FHB resistance has proven to be expensive, time-consuming and environmentally biased (Sun et al. 2002).

The semi-dwarf allele *Rht-D1b* has been demonstrated to have a negative effect on FHB resistance to type I infection compared to the wild type allele *Rht-D1a* (Srinivasachary et al. 2008). Furthermore, another semi-dwarf allele associated with FHB resistance is *Rht-B1b*. This specific allele has proven to, similarly to *Rht-D1b,* decrease type I resistance. However, whilst *Rht-D1b* was shown to have little effect on type II resistance, the *Rht-B1b* allele increased the FHB resistance (Srinivasachary et al. 2008; Srinivasachary et al. 2009). This suggests that choice of semi-dwarf gene should be specifically considered in breeding programmes for FHB resistance.

AE is also associated with resistance to *Fusarium*. When there is low AE, anthers getting trapped between the glumes provide dead tissue. This dead tissue is then readily colonized by *Fusarium* (Lu et al. 2013; Skinnes et al. 2008; Skinnes et al. 2010). Lines with low AE are therefore more susceptible to *Fusarium* infection. Consequently, the developing of lines with high AE is important for further disease resistance. In Japan, closed flowering has been used as a strategy of alternative breeding (Kubo et al. 2010). Among the recombinant inbred lines used for the study, there was less initial FHB infection for the closed flowering lines than for the open flowering lines. However, Kubo et al. (2010) found no significant differences in

grain deterioration and mycotoxin accumulation between the 2 groups.

Lu et al. (2013) studied a recombinant inbred line (RIL) population developed from bread wheat line Shanghai-3/Catbird (SHA3/CBRD) and Naxos to identify quantitative trait loci (QTL) for FHB resistance in a non-*Fhb1* germplasm. Sumai 3 is known to carry *Fhb1*, which is a major FHB resistance gene (Cuthbert et al. 2006). For this reason, the line is heavily dependent on in wheat breeding worldwide (Lu et al. 2013). SHA3/CBRD was used because of the high level of type I FHB resistance (Srinivasachary et al. 2008; Srinivasachary et al. 2009) and high anther extrusion. Naxos on the other hand has low anther extrusion and the *Rht-B1a* wild-type gene. Lu et al. (2013) found a relationship between FHB and PH. The association between FHB and PH/AE was found to be more linked to severity of infection rather than other FHB traits. For the study, there was an observed relationship of increasing AE and PH with reduced FHB severity. Furthermore, this was confirmed by QTL analysis where both low AE and reduced PH increased FHB severity (Lu et al. 2013).

Resistance sources from Asia are also frequently used in studies of FHB resistance. Wheat growing regions in Asia have suffered from FHB and thus breeding for resistance has been a long tradition (Buerstmayr et al. 2009). The Chinese cultivars Sumai 3, Ning 7840 and Ning 8331 have been successful in breeding and distributed to other parts of the world for use in breeding programmes. Furthermore, they have been used in early projects to determine the genetic basis of *Fusarium* resistance (Buerstmayr et al. 2009).

1.1.6. Genotyping

With an increase in demand for food and feed, and diseases impacting the cereal production, the use of plant breeding is important. The emergence of molecular marker technology has been helpful (Gupta et al. 2001). Molecular markers are heritable DNA sequence differences, also called polymorphisms, associated with a certain location on the genome and usually identified using hybridization methods or a polymerase chain reaction (PCR)(Talbert et al. 1994).

There are a series of different marker systems developed over the last two decades. Gupta et al. (2001) divided these into three generations of markers. The first generation molecular markers are the Restricted Fragment Length Polymorphisms (RFLPs) and Random Amplified

Polymorphic DNA (RAPD). These types of markers are not frequently used, as they require large amounts of sample DNA and because screening genotypic material with these markers is time consuming. However, the second-generation markers, including the microsatellites Short Tandem Repeats (STRs), Amplified Fragment Length Polymorphisms (AFLPs) and Simple Sequence Repeats (SSRs) especially, are used in analyses for kinship (pattern of relationships) and populations (Laidò et al. 2013). These are typically co-dominant microsatellites with a repeat length of 2-5 basepairs (bp).

The third generation are the SNP marker technologies including Single Nucleotide Polymorphisms (SNPs), Kompetitive Allele Specific PCR (KASP) and Genotyping-By-Sequencing (GBS) (Gupta et al. 2001). These markers are readily used today for individual genotyping. SNPs are mostly used when genotyping large amounts of markers for each line, making it a high-throughput type of sequencing technology. Genotyping by sequencing (GBS) is also a high-throughput type of technology which uses genotyping instead of SNP-chips by the use of for instance the Illumina systems (Illumina 2015). This next generation of sequencing has been a revolutionary step in marker technology as whole genomes can be genotyped in a short amount of time. Although, SNP chips are expensive as they are developed to contain large amounts of markers. KASP markers are however more useful if there are only a few markers of interest that should be genotyped on many samples. In plant breeding there is usually a need to genotype only a few markers of interest. Therefore, KASP markers are the most cost effective option. SNP-chips however, are more cost effective in cases when many markers are genotyped on few samples.

Association mapping, also known as and linkage disequilibrium (LD) mapping, are genome-wide association studies (GWAS) used to identify marker alleles associated with phenotypic traits with the use of high-throughput markers like SNPs (Gómez et al. 2011). This type of mapping uses ancestral recombination from the species' gene pool to identify associations between markers and traits (Gómez et al. 2011). This works on the presumption that some markers are in LD with, or actually are, causative SNPs. To be in LD with means that there is a "correlation of alleles on different sites" (Remington et al. 2001). However this method has some limitations, such as reduced genetic diversity and difficulties in terms of time and labour building segregating populations and the presence of only one meiotic generation (Gómez et al. 2011). Where QTL mapping uses analyses of variance (ANOVA) and regression with data from pedigree of one cross (Young 1996), association mapping uses phenotypic and

genotypic data combined with a kinship model and often population structure for mapping QTL related to traits of interest (Gómez et al. 2011).

Association mapping have been usefully applied to wheat studies. Zanke et al. (2014a) discovered the genetic architecture of QTL for heading in European winter wheat, whereas Zanke et al. (2014g) applied whole-genome association mapping to discover the genetics behind plant height, also for winter wheat. Whole genome association mapping have also been used to study FHB resistance (Kollers et al. 2013). Wang et al. (2014) recently developed high-density SNP arrays to genotype different types of wheat, where the method proved essential in identifying genes in economically important crops. However, the allotetraploid durum wheat (*Triticum turgidum* subsp. *durum*) and allohexaploid bread wheat (*Triticum aestivum L.*) was difficult to analyse as the ratio of allelic variations in polyploids deviated from the observed ratio in diploid organisms, which made analyses based on genotype calling softwares difficult (Wang et al. 2014). To solve this issue, a wheat SNP iSelect array including roughly 90 000 gene-associated SNPs was used to genotype the polyploidy genome (Wang et al. 2014). In the study, a total of 46 977 SNP-markers were genetically mapped, making it possible to identify major genes for complex traits like for instance resistance to disease (Wang et al. 2014).

Meta-analysis has been used as a method to estimate confidence intervals of identified QTL in different sources of resistance (Liu et al. 2009). Liu et al. (2009) used 249 FHB resistance QTL from 46 unique wheat lines from 45 studies to cluster the estimated QTL confidence intervals. A total of 209 QTL were found for resistance types I, II, III and IV. These were positioned in 43 clusters on 21 chromosomes. Among these, 119 QTL were significant and 116 QTL explained more than 10% of the phenotypic variation. The 19 confirmed QTL are displayed in Table 3 and includes QTL from chromosomes 3A, 5A, 7A, 1B, 3BS, 5B, 6B and 2D (Liu et al. 2009). Confirmed QTL are reportedly discovered in multiple sources while unique QTL are exclusive to one particular line. The confirmed QTLs were found from cultivars Sumai 3, Frontana, Wangshuibai and Arina. For the unique QTLs additional sources included NK93604, Renan, Cansas, Goldfield, CJ9306, Ritmo, Apache, Pirat, DH181, Chokwang and Romanus.

Table 3. Confirmed and unique QTL for FHB resistance in wheat based on a meta-analysis of QTL in 46 lines from 45 studies reported from 2001-2009 (Liu et al. 2009)

Chromosome locations	Type of resistance	Sources of resistance
Confirmed QTL		
3A	II	Frontana
5A	I, II, III	Sumai 3
5A	I	Wangshuibai
7A	II	Wangshuibai
1B	II	Wangshuibai
3BS	I, II, III, IV	Sumai 3
3BS	II	Wangshuibai
3BSc	II	Wangshuibai
5B	II	Wangshuibai
6BS	I, II, IV	Sumai 3
6B	II	Arina
2DL	II	Sumai 3
Unique QTL		
1A	II	Wangshuibai
2A	III	NK93604
2A	IV	Wangshuibai
3A	I	Wangshuibai
5AL	II	Renan
7A	II	Frontana
1B	I, II	Cansas, Arina
2B	I	Goldfield
5B	I, I, II	Cansas, Wangshuibai, Arina
7B	I, I, II	Cansas, Goldfield, CJ9306
1D	I, II, II, IV	Ritmo, Apache, Pirat, DH181
3D	I, II	Cansas, Arina
5D	II	Chokwang
6D	II, II, II	Arina, Renan, Romanus
7D	IV	Wangshuibai

Furthermore, Liu et al. (2009) found that QTL for FHB resistance is associated with specific types of resistance. For instance gene *Fhb5* on chromosome 5A and QTL on 3A contribute more to type I resistance and less to type II (Lu et al. 2013). More than 100 QTL analyses of FHB resistance have been reported recently and summarized by Liu et al. (2009) and (Buerstmayr et al. 2009).

When it comes to Norwegian wheat, recent studies have been conducted on the most important and promising Norwegian breeding-lines. Lillemo et al. (2013) studied the resistance towards *F. graminearum* by artificially field-testing different Norwegian wheat-, barley-and oat cultivars. These lines were harvested and analysed with 'mixed linear modelling' in SAS for DON.

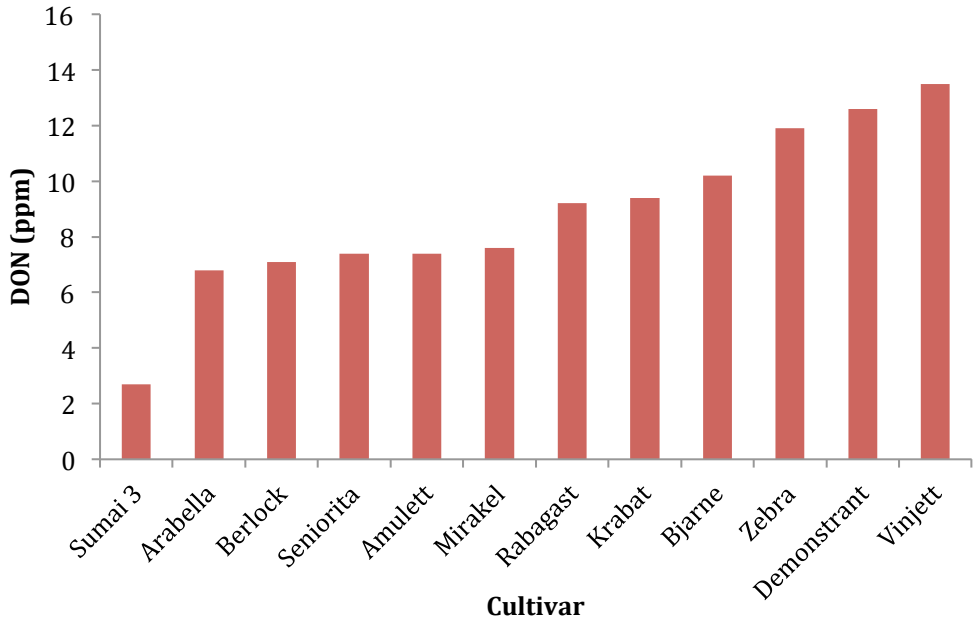


Figure 7. Average DON content for chosen cultivars of spring wheat between 2008-2013 (Lillemo et al. 2013)

Figure 7 shows the differences between the DON-values for the six different spring wheat cultivars in this study (Lillemo et al. 2013). Especially the cultivars Zebra and Demonstrant have high values, while Bjarne and Berserk, have lower values. Furthermore, the new cultivars Arabella, Berlock, Seniorita, Amulett and Mirakel show a considerable improved resistance against *F. graminearum* compared to Zebra and Demonstrant. Therefore, breeding for resistance towards FHB has proven to be successful. However, there is still a large gap between the resistant adapted cultivars and the resistance source Sumai 3. Furthermore, it is

also important to note that there are differences in DON values from one year to the other (Figure 8). This is an indication that the yearly climate has an impact on the degree of FHB infection and mycotoxin levels. From Figure 8 it is clear that Zebra and Demonstrant consistently have the highest levels of DON of the current spring wheat cultivars. Although Lillemo et al. (2013) has shown that it is possible to develop cultivars with improved FHB resistance, more information concerning the major genes associated with FHB in Norwegian wheat is yet to be studied.

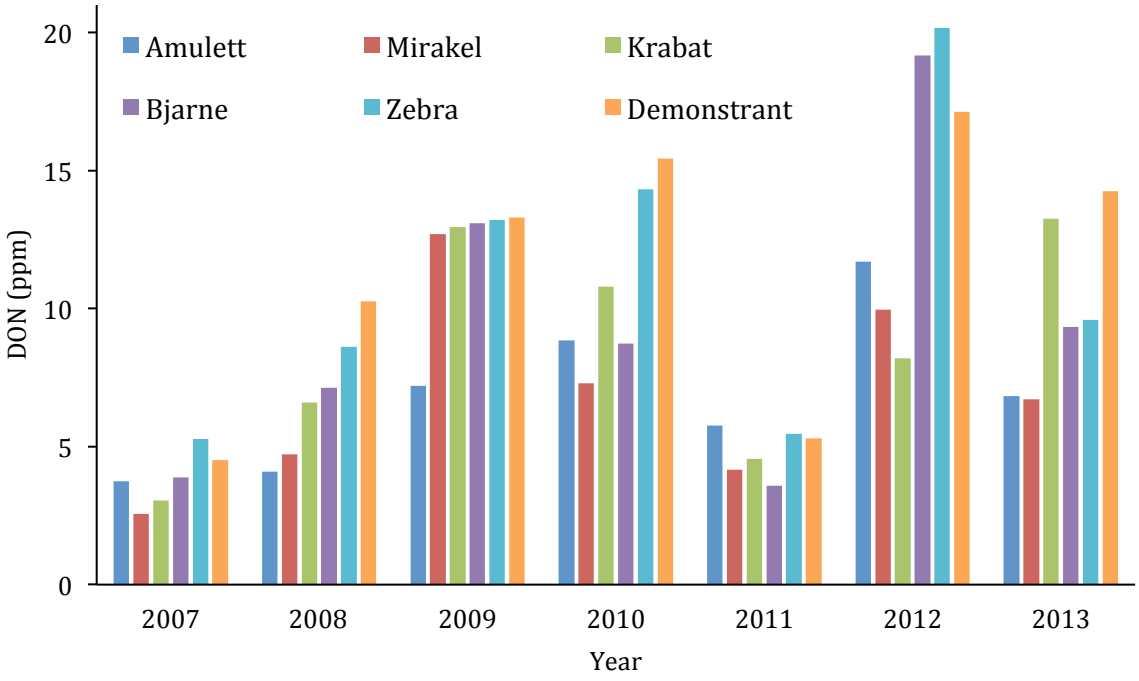


Figure 8. The comparisons of DON for Norwegian spring wheat cultivars from 2007-2013 (Lillemo et al. 2013)

1.1.7. Aims of the study

The objective of this research was to identify the most important QTL for *Fusarium* resistance in Norwegian spring and winter wheat lines using the data from the 90K SNP assay genotyping and phenotyping for DH, PH, AE, FHB. Both NMBU and Graminor have, through systematic testing, discovered resistance differences in different wheat lines. However, little is known about which genes are causing these differences. This thesis is important for the development of genetic markers for FHB resistance in the Norwegian breeding material. To this date, for the most part, exotic lines have been thoroughly examined.

The breeding for FHB resistance is difficult, and time consuming. The identification of QTL and genes responsible for resistance is a good step towards effective breeding of resistant cultivars. The use of markers and association mapping of marker-trait relationships can be helpful in identifying these genetic resistances and thus the future development of resistant wheat cultivars.

For this thesis I wanted to investigate and identify the most important QTL for FHB resistance by means of a population structure analysis and association mapping to identify markers showing significant effect to phenotypic traits for DH, PH, AE, FHB and DON. By ruling out the QTL for DH, PH and AE, the major QTL for FHB resistance and DON can be identified. The genetic foundation for *Fusarium* resistance in Norwegian spring wheat, and the markers developed in this thesis will contribute to further studies towards more effective breeding scheme of future Norwegian wheat lines with better resistance to FHB.

1.2. Materials and methodology

1.2.1. Plant material

For the thesis, 240 spring wheat lines and 80 winter wheat lines, mainly MASBASIS with some additional lines from Graminor, representing the genetic variation in Norwegian and Nordic breeding material were tested for *F. graminearum* infection. The lines were sown and tested under high infectious pressure in field trials at Vollebekk, Ås.

Figure 9. Grain spawn isolates prepared for cultivation

The grain spawn included a mix of four different isolates of *F. graminearum*. Infection of the kernels were performed based on a protocol from Dr. Bernd Rodemann, Julius Kühn Institute, Braunchweig, Germany, as described by Lu et al. (2013). Each of the isolates was produced on petri dishes (Figure 9) and cultivated 7 days in a liquid culture containing 1g oat flour in 100 ml ionized water (Figure 10).

Figure 10. Vials containing oat flour (left) mixed with ionized water and cultivated for 7 days (right)

The isolates were then mixed with sterile oat kernels in bags and stored in room temperature and ambient light for 3 weeks until abundant development of mycelium (Figure 11). The bags were held closed with cotton tops to allow air in and contain the infection. The isolates were then kept on trolleys at room temperature for approximately 3 weeks being irrigated daily with water to stimulate perithecia development (Figure 12). The isolates were then mixed and scattered in the field experiment with a density of 5g/m^2. High moisture level and night dew was provided by mist irrigation.

Figure 11. Infected oat kernels stored for 3 weeks (left) with mycelium produced (right)

MASBASIS is a collection of all historically important current cultivars on the market in Norway. These include important sources of disease resistance and quality traits, crossing parents and advanced breeding lines from Graminor. MASBASIS therefore contains all the genetic diversity the breeding programme is using. MASBASIS has previously been genotyped with SNP markers (Illumina iSelect 90K wheat array) as a part of the wheat genome sequencing project at NMBU, and was used for this study. Previously tested SSR and KASP markers were also added to the SNP markers. Statistical methods were used to find associations between markers and *Fusarium* resistance, correcting for population structure and kinship. The strongly significant SNP markers from this study will then be converted to KASP-markers and tested on Graminor's breeding material to examine their practicality in association to *Fusarium* resistance.

Figure 12. Two of the four isolates on trolleys with daily water irrigation

1.2.2. Data collection

The main material for this study was the collection of the 240 spring wheat lines, which were sown at Vollebekk, Ås, in four reps, where each of the reps was divided into sub blocks (Figure 13). This was done to help correct for variation in each of the two trial blocks, like for instance soil type, moisture, shadow and wind, which would provide a better estimate of the measured parameters. Where block describes the horizontal lines, PLT (plot) describes the vertical columns of the design. The 80 winter wheat lines were sown in a different design, with 3 reps, each rep divided into four blocks of 20 trial plots, counting up to a total of 240 trial plots (Figure 15). Before sowing, the samples had to be measured in packets of 50 grams each and put in the right order for the alpha lattice design like explained in Figure 13 and Figure 15. Figure 14 displays the field for the spring wheat trial.

The scoring of AE and DH in spring wheat and FHB in winter wheat was performed by me, while others scored HD, PH and AE in winter wheat and PH for spring wheat. Additionally, we were two people scoring FHB in spring wheat; one for each rep. The data used for previous years in this thesis were also performed by others.

Block 12	4901	…4920	…4940
		Rep 1	Rep 2
Etc.			
Block 3			
Block 2	3901	3902 3903… …3920	3921 3922 3923… …3940
Block 1	3801	3802 3803… …3820	3821 3822 3823… …3840
	PLT		

Figure 13. Alpha lattice design for field trial of spring wheat lines sown at Vollebekk, Ås 3/5-2014

Figure 14. Field trial for spring wheat at Vollebekk, Ås

Rep 3	6201	…6220
Rep 2		
Rep 1		
	5101	…5120
	PLT	

Figure 15.Design used for the winter wheat lines sown at Vollebekk, Ås 24/9-2013

A visual evaluation was made by percentage of FHB in addition to spike development and growth, yellowing and plant height for the spring and winter wheat lines. AE evaluation was made on a separate field, from a mildew hillplot, which contained most of the 240 spring wheat lines. In addition, the grains were analysed for DON. Figure 16 displays a typical wheat spikelet killed by FHB.

Figure 16. Head of wheat with spikelet killed (right) compared to a dry head (left)

DH, PH and percentage of FHB infection was scored. With these data it would be possible to relate infection of *Fusarium* with how early the heads emerge. The scoring of heading started in June (10/6 - 2014) and was finished in July (5/7 – 2014), plant height was scored 15/7 – 2014, and FHB infection was scored in August. After harvest, samples of the 240 spring wheat lines, and 80 winter wheat lines, were sent to Minnesota, USA, to be tested for DON.

1.2.3. Fieldwork and phenotypic evaluation

In terms of fieldwork, there were three different designs related to the three different trials; Spring wheat, winter wheat and mildew. The 240 spring wheat lines were sorted into duplicate bags of grain each containing 50 grams. These were then sown 3/5-2014. Heading was scored the date in which approximately 50% of the heads had emerged from the leaf sheath. When all the lines were finished growing, plant height was scored as centimeters from the ground up to the head. Percentage of FHB was scored just before yellowing. This was carried out by choosing 10 heads randomly inside the trial block, counting total spikelets, and

then counting the number of infected spikelets out of the 10 random heads (for instance 19 infected out of the total 190 would be 19/190 = 10%). This was then repeated with another 10 random heads in the same trial plot.

It was slightly difficult determining which spikelets were infected with *F. graminearum*, and which were just gone dry. In addition, two people were scoring, one for each rep, which may have caused dissimilar results. The same traits were scored for the winter lines. The spring wheat lines were harvested the 25/8-2014 and 26/8-2014. The winter wheat lines were sown 24/9-2013 and was harvested 6/8-2014. Results for FHB are displayed as percent (%) and DON as parts per million (ppm). The mildew trial was sown as a hill plot, containing many of the lines from both MASBASIS and Graminor used for the spring wheat trial. These plots were used to score DH and AE. AE was scored on a scale from 1-9 where 1 meant that all the anthers were still inside the spikelet, and 9 where they all had been released.

1.2.4. Analysis of variance (ANOVA)

Analysis of variance (ANOVA) was used to calculate heritability for phenotypic traits scored in the field trials and for previous years of testing in Minitab v.16 software (Minitab 2014). Heritability is the proportion of variation in trait values due to genetic differences. Additionally, it gives an indication of how precise the data is and how heritable the phenotypic trait is. Narrow sense heritabilities (h^2) were calculated using the results from the ANOVA table as described by Singh et al. (1995). High levels of heritability were considered at h^2>80%.

$$h^2 = \frac{\sigma^2 G}{\sigma^2 p}$$

$$h^2 = \frac{\sigma^2 G}{\sigma^2 G + \left(\frac{\sigma^2 e}{r}\right)}$$

$MS_e = \sigma^2_e$

$MS_L = r\,\sigma^2_G + \sigma^2_e$

σ^2_G = genetic variance

σ^2_p = phenotypic variance

σ^2_e = environmental variance

r = number of reps (spring and winter wheat 2014) and number of years (spring wheat over years)

1.2.5. Statistical analysis of phenotypic data

The 240 spring wheat lines and 80 winter wheat lines harvested from the field trials were were checked for errors and residuals using Minitab. The SAS statistical package (SAS 2014) mixed linear model PROC MIXED was used to statistically analyse the traits and calculate least square means (lsmeans) for the phenotypes with lines as fixed effects, and replicates, and blocks within replicates, as random factors. The results from SAS were used as phenotypic data for the association mapping with a selection of 172 lines of both spring (123 lines) and winter (49 lines) wheat. These were selected on basis of the 90K Illumina SNP chip which already had genotype data for these lines.

Data from previous years of testing were also used to compare with the phenotypic results from this study. Spring wheat data were from 2009-2014 for DH, 2009, 2013 and 2014 for PH and 2006, 2008, 2013 and 2014 for anther extrusion AE. Data for FHB and DON were from 2013 and 2014. For winter wheat there were only FHB and DON data from 2014. The lsmeans across years were calculated in PROC MIXED of individual years defining lines as fixed, and years as random effects.

1.2.6.1. Genetic diversity

The markers for the study were chosen based on the 90k iSelect SNP chip. A total of 22031 markers for spring wheat and 16091 for winter wheat were chosen as good markers to be used in further analyses. Markers were considered good if more than 90% of the lines had a genotype for the particular marker, and a minor allele frequency >=5%. The total of markers consisted of a combination of SNP markers, SSR markers and some KASP markers. The

KASP markers have previously been genotyped on MASBASIS based on the *Fhb1* gene on 3BS and the genes *Rht-B1* and *Rht-D1* on chromosome 4B and 4D respectively. Furthermore, the low quality markers were filtered out based on markers being "no call" or had many lines with many heterozygotes. In addition, a final filtration was performed where the markers having less than 5 lines with the most rare allele were removed.

1.2.6.2. Consensus map development

To be able to identify QTL and compare the results, a consensus map proved to be a good resource in locating the genes and QTL from the association mapping. The consensus map used for this study has been produced by Wang et al. (2014) including 46 977 mapped SNPs from the 90K iSelect wheat genotype assay.

1.2.6.3. Defining population structure

Definition of population structure was performed with the STRUCTURE v.2.3.4 software (Pritchard et al. 2000) and Unscrambler X (CAMO 2015). The Structure analysis was carried out with K = 10, 5000 burnin length and 50 000 reps over 3 iterations and the results were run in Structure Harvester (Earl & vonHoldt 2012) for estimated K for the 172 MASBASIS lines and the respective 123 spring and 49 winter wheat lines. "Delta K (ΔK) is based on the rate of change in the log probability of data between successive K values" (Evanno et al. 2005). This was used to determine the number of clusters (K) in the population (Evanno et al. 2005). Unscrambler X was used to perform a Principial Component Analyses (PCA) of the genotypic SNP marker data

1.2.6.4. Genotypic analysis

Association mapping analysis can be a helpful method in identifying the molecular markers significantly linked to traits of interest. In this case mixed linear modelling (MLM) was used to perform the association mapping analyses. MLM includes both population structure and kinship, and reduces type I error due to relatedness and population structure. Both genotype data including SNP markers and phenotype data from the field trials were used along with a kinship matrix constructed from the genotypic data and population structure results from STRUCTURE v. 2.3.4 in the statistical software Tassel v.5.2.7 (Bradbury et al. 2007). The

alleles were also coded as A and T in the analysis which was transformed to allele a and allele b for explanation of allele effects in Appendices 2 and 3.

1.2.6.5. QTL analysis

Markers were analysed three times in Tassel v. 5.2.7 to determine the best-fit model:

- PCA + kinship
- Population structure + kinship
- PCA + population structure + kinship

The three models were compared based on the P-values of the significant markers. All three models displayed similar results, however the population structure + kinship model was determined to be best-fit as it had the lowest P-values for the significant markers.

Regression for FHB and DON was performed in Minitab with DH and PH as factors for correction. The resulting residuals were then added to the original phenotype data run as MLM in Tassel. The association mapping of FHB and DON were run in two separate analyses, one each for FHB and DON and one with regression values corrected for DH and PH (FHBreg and DONreg). The most significant QTL were then compared to integrated consensus maps with SSR markers from published studies to see if the significant QTL found in this analysis have been located before. Significant markers for DH, PH and AE were determined from data from 2014, 2013, 2013-2014 and 2008-2014 for spring wheat and 2014 for winter wheat. FHB, FHBreg, DON and DONreg markers were predominantly from year 2013. Manhattan plots presented contain markers for 2014 for DH/HD, PH and AE, whereas FHB/FHBreg and DON/DONreg are from 2013. The reason for this was due to the low infectious pressure of 2014 impacting the significant effects and markers from the association mapping. Significant markers from 2014 were compared to 2013 and mean over years to determine that the results from 2014 were a good illustration of the marker-trait associations.

The most significant markers were chosen based on –log10(P-value) threshold >2.5 for PH and DH for spring wheat. For AE and FHB the –log10(P-value) were set to >2.5, >2.0 for FHBreg and >3.0 for DON and DONreg. Winter wheat PH was set to >2.3, whereas AE, FHB, FHBreg and DON the threshold was set to >2.0. Additionally DONreg had a

significance threshold at >2.3. Lists of significant markers are attached in Appendix 2 (DH and PH) and Appendix 3 (AE, FHB, FHBreg, DON, DONreg). The positions in cM are based on SNPs which makes comparing these to positions of SSR markers inaccurate. However, the positions give indications of which markers are located in close distance with each other.

Chapter 2:
Heritability and phenotype results

2.1. Heritability

Figure 17 gives an indication that there is non-normality in the spring wheat data from 2014. PH is the phenotypic trait closest to a normal distribution. Both FHB and DON are heavily right skewed with a long ”tail” indicating a non-symmetric distribution of data. AE also gives a very non-normal display of the data.

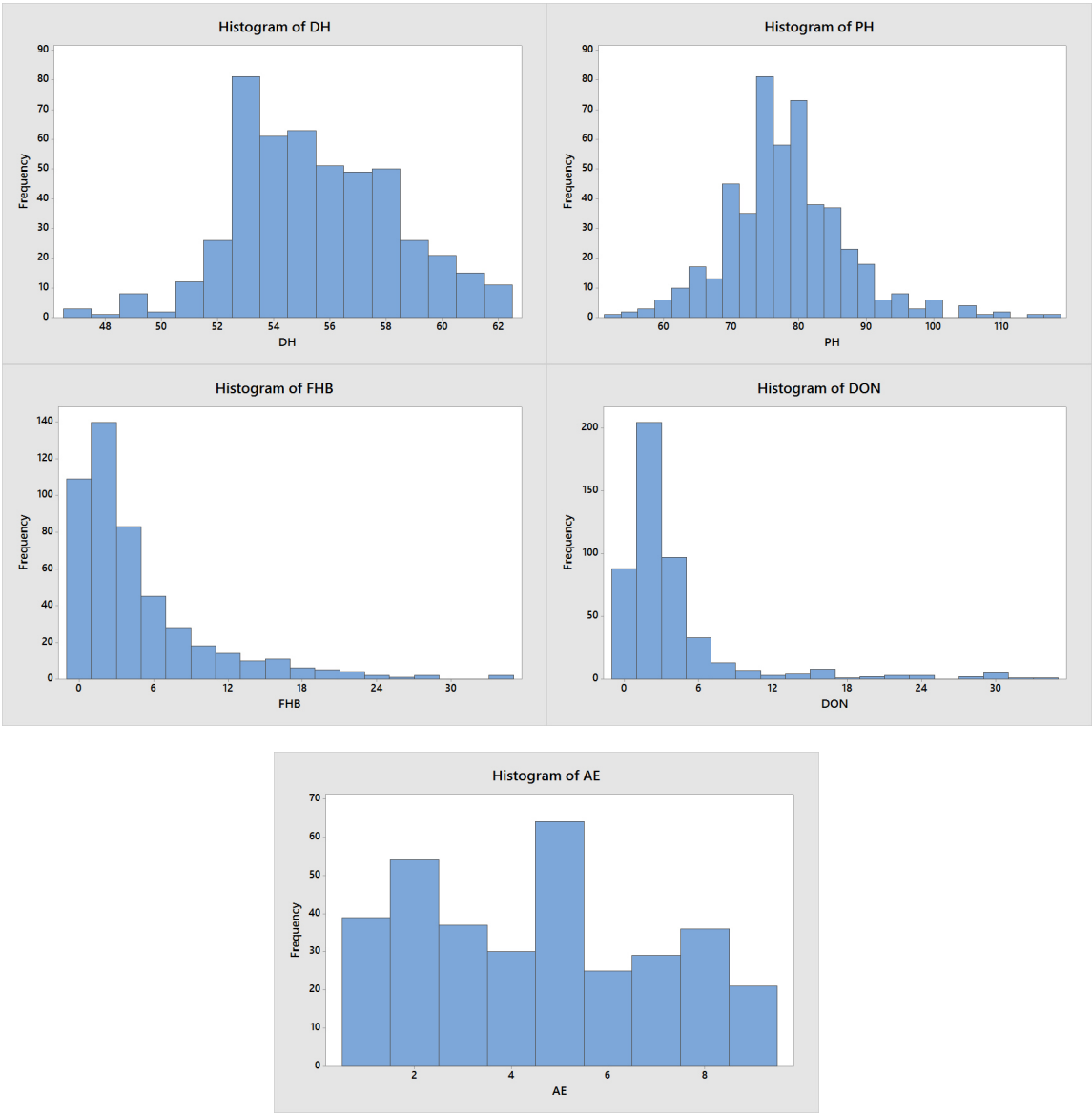


Figure 17. Histograms of phenotypic traits DH, PH, FHB, DON and AE and frequencies for spring wheat from 2014

For DH (Table 4), the ANOVA gave significant values for both line, rep, block within rep and PLT within rep. Rep had the topmost effect of variance (F=22.93) with the most significant P-value (0.000), followed by line with an F of 13.77 and P=0.000. For PH, the sources of variance are also highly significant. PH (Table 5) also had a high effect of line (F=9.01) with a corresponding significant P-value (P=0.000).

Table 4. ANOVA for DH, using adjusted SS for tests for spring wheat 2014

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Line	226	3523.781	3086.995	13.659	13.77	0.000
Rep	1	111.586	106.578	106.578	22.93	0.000
Block(Rep)	22	50.836	46.119	2.096	2.11	0.004
PLT(Rep)	38	71.970	71.970	1.894	1.91	0.003
Error	188	186.525	186.525	0.992		
Total	475	3944.697				

Table 5. ANOVA for PH, using adjusted SS for tests for spring wheat 2014

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Line	226	32121.19	24157.06	106.89	9.01	0.000
Rep	1	1564.97	1386.89	1386.89	8.72	0.007
Block(Rep)	22	1671.56	1649.97	75.00	6.32	0.000
PLT(Rep)	38	1099.16	1099.16	28.93	2.44	0.000
Error	188	2229.39	2229.39	11.86		
Total	475	38686.28				

The results for FHB (Table 6) gave a low effect of line (F=1.52) with a significant P-value of 0.002 indicating very low variances in the data. Rep showed the highest effect (F=54.37) with a corresponding significant P-value of 0.003 suggesting that there were significant differences of phenotypic data between the two reps. However, when looking at DON (Table 7), the effect of line was higher (F=8.23, P=0.000), and rep was lower. The effect of rep on DON (F=14.48) was still found highly significant (P=0.001). Additionally, line and rep within block was also found to be significant, though with a smaller value of F. Furthermore, AE (Table 8) gave significant results for both line and rep, however, the effects were very low (Line F=2.50 and P=0.000).

Table 6. ANOVA for FHB, using adjusted SS for tests for spring wheat 2014

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Line	226	8006.23	7122.20	31.51	1.52	0.002
Rep	1	1208.34	1087.35	1087.35	54.37	0.003
Block(Rep)	22	421.21	401.00	18.23	0.88	0.625
PLT(Rep)	38	872.92	872.92	22.97	1.11	0.323
Error	188	3905.54	3905.54	20.77		
Total	475	14414.24				

Table 7. ANOVA for DON, using adjusted SS for tests for spring wheat 2014

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Line	226	11266.06	9207.77	40.74	8.23	0.000
Rep	1	417.66	411.33	411.33	14.48	0.001
Block(Rep)	22	371.02	368.87	16.77	3.39	0.000
PLT(Rep)	38	222.13	222.13	5.85	1.18	0.233
Error	188	930.45	930.45	4.95		
Total	475	13207.32				

Table 8. ANOVA for AE, using adjusted SS for tests for spring wheat 2014

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Line	163	1428.246	1381.795	8.477	2.50	0.000
Rep	1	9.728	9.547	9.547	2.81	0.095
Block	13	54.055	54.055	4.158	1.23	0.266
Error	157	532.467	532.467	3.392		
Total	334	2024.496				

The calculated heritabilities show how much of the variation in traits can be explained from genes. For spring wheat in 2014 (Table 9) the heritability for DH was the highest at 93%, with PH and DON at 89% and 88% respectively. AE had a heritability of 60%, meaning that the other 40% of the variance can be explained from environmental factors and errors in assessing the trait. Furthermore, FHB has the lowest heritability at 34%. It is also important to pinpoint that yearly variations and conditions makes the heritability only valid under those exact conditions of the specific field trials used to obtain the data.

Table 9. Calculated heritabilities for spring wheat 2014

Phenotypic trait	h^2	Percentage heritability
DH	0.93	93%
PH	0.89	89%
FHB	0.34	34%
DON	0.88	88%
AE	0.60	60%

The histograms for spring wheat over years gave similar results as for 2014 (Figure 18). The DH distribution is slightly right skewed and PH closest to a normal distribution. FHB and DON are also here right skewed with a long “tail” and AE with a slight left skew.

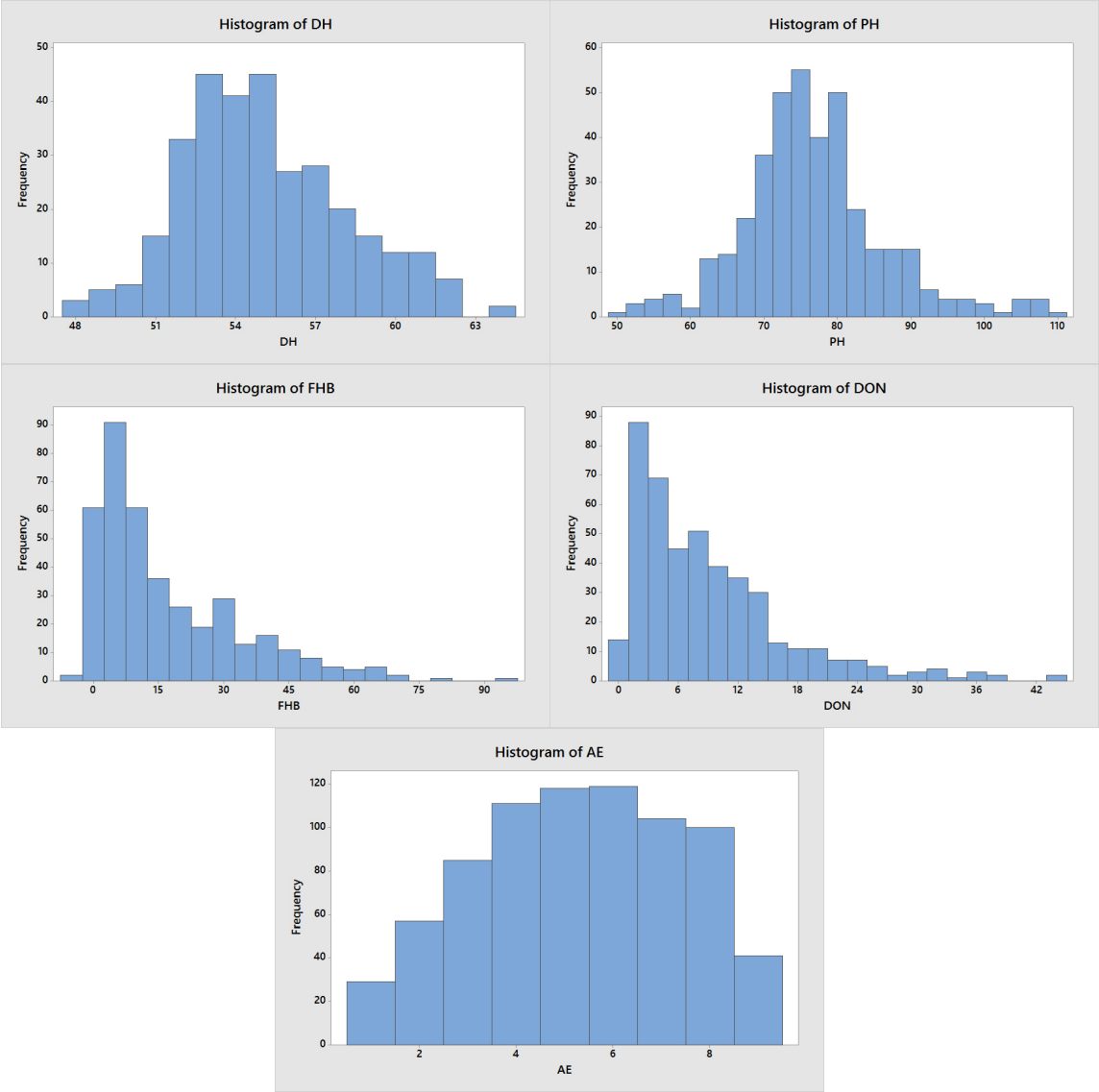


Figure 18. Histograms of phenotypic traits DH, PH, FHB, DON and AE and frequencies for spring wheat over years

The ANOVA for DH (Table 10) gave an effect of year (F=39.28) being highly significant (P=0.000), meaning that years are the main contributor to variation in the data. There is also a high effect of line with F=8.83 and P=0.000. PH however (Table 11), gave no effect or significance of year (F=0.94 and P=0.333), but an indication of an effect of lines (F=7.16 and P=0.000).

Table 10. ANOVA for DH, using adjusted SS for tests for spring wheat for years 2013-2014

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Line	159	2582.115	2587.272	16.272	8.83	0.000
Year	1	72.365	72.365	72.365	39.28	0.000
Error	155	285.532	285.532	1.842		
Total	315	2940.012				

Table 11. ANOVA for PH, using adjusted SS for tests for spring wheat for years 2008, 2009, 2010, 2013 and 2014

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Line	159	22651.62	22654.72	142.48	7.16	0.000
Year	1	18.78	18.78	18.78	0.94	0.333
Error	155	3084.41	3084.41	19.90		
Total	315	25754.80				

For FHB and DON over years there were very high effects of year from the ANOVA (Table 12 and 13) with F=247.79 (P=0.000) for FHB and F=384.05 (P=0.000) for DON. For DON there was also a highly significant effect of lines (F=4.95 and P=0.000), however FHB showed no significant effect of lines (F=1.20 and P=0.123). Furthermore, for AE the ANOVA (Table 14 gave significant effects for both line and year, though the effect of year (F=54.33, P=0.000) was higher than for lines (F=3.32, P=0.000).

Table 12. ANOVA for FHB, using adjusted SS for tests for spring wheat for years 2008, 2009, 2010, 2013 and 2014

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Line	159	33204.3	32827.4	206.5	1.20	0.123
Year	1	42497.8	42497.8	42497.8	247.79	0.000
Error	155	26583.8	26583.8	171.5		
Total	315	102285.9				

Table 13. ANOVA for DON, using adjusted SS for tests for spring wheat for years 2008-2014

Source	DF	Seq SS	Adj SS	Adj MS	F	P
MASBASIS	159	14504.61	14363.22	90.33	4.95	0.000
Year	1	7009.73	7009.73	7009.73	384.05	0.000
Error	155	2829.07	2829.07	18.25		
Total	315	24343.41				

Table 14. ANOVA for AE, using adjusted SS for tests for spring wheat for years 2006, 2008, 2013 and 2014

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Line	163	1513.594	1347.647	8.268	3.32	0.000
Year	3	405.295	405.295	135.098	54.33	0.000
Error	597	1484.579	1484.579	2.487		
Total	763	3403.468				

The heritabilities calculated for spring wheat over years (Table 15) show similar trends as for 2014. The DH, PH and DON all have high percentages of heritability (89%, 87% and 78% respectively). However, FHB over years give a heritability of 17% which is even lower than for the 2014 results. Furthermore, AE show a higher heritability over years compared to 2014 (70%).

Table 15. Calculated heritabilities for spring wheat over years

Phenotypic trait	h^2	Percentage heritability
DH	0.89	89%
PH	0.87	87%
FHB	0.17	17%
DON	0.78	78%
AE	0.70	70%

The distribution of data for winter wheat (Figure 19) shows that there is a non-normal distribution for all traits. Both FHB and DON is similarly to spring wheat right skewed with a long “tail”. PH was additionally less close to a normal distribution for winter wheat than for spring wheat.

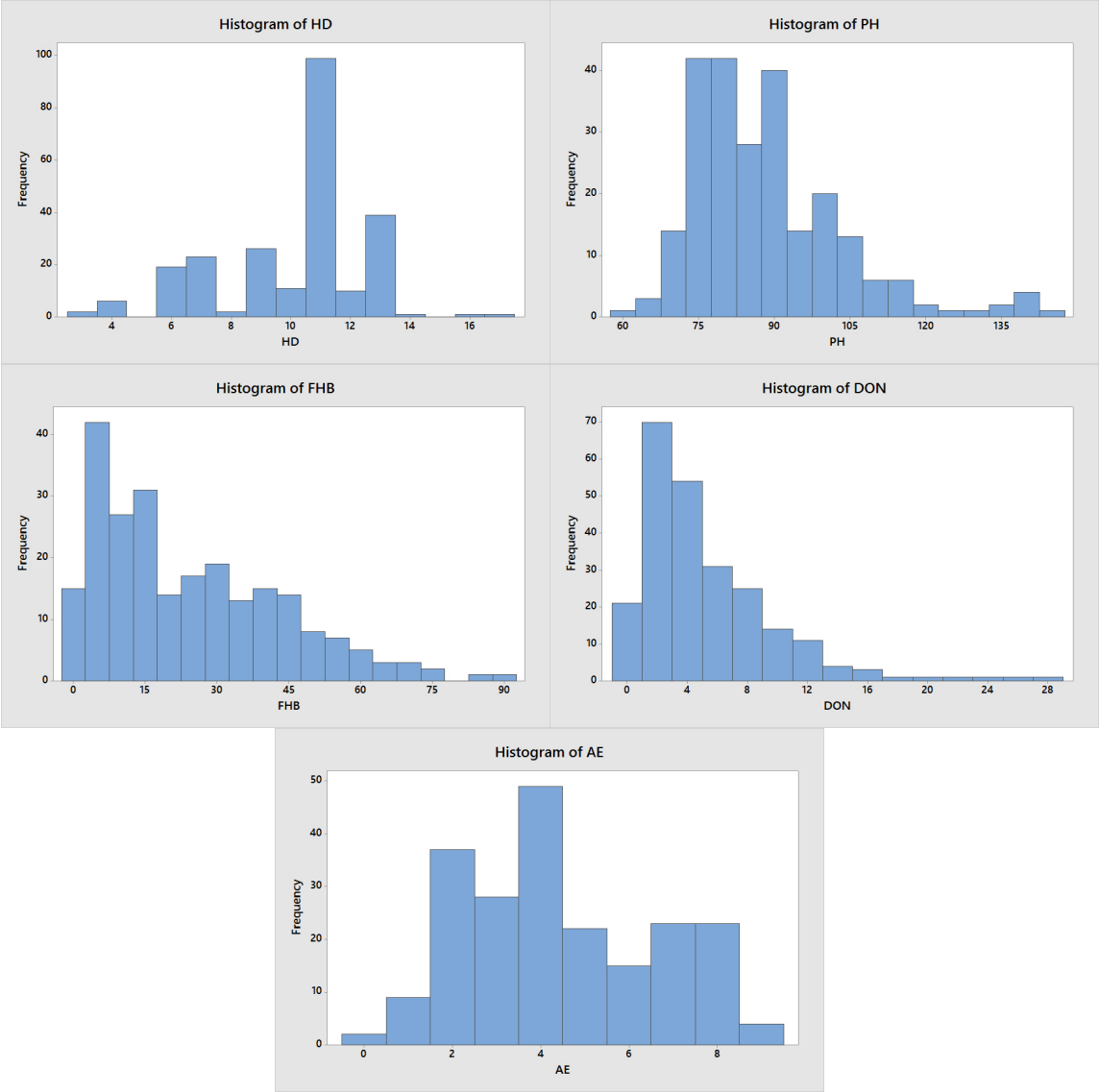


Figure 19. Histograms of phenotypic traits DH, PH, FHB, DON and AE and frequencies for spring wheat over years

The results from the ANOVA of HD in winter wheat (Table 16) gave no significant effect of rep, rep within block or rep within PLT. However, a significant effect was found for lines (F=11.19, P=0.000). Additionally, PH (Table 17) showed no significant effect for rep, rep within block or rep within PLT, but for line (F=26.43, P=0.000).

Table 16. ANOVA for HD, using adjusted SS for tests for winter wheat 2014

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Line	70	1198.555	833.458	11.907	11.19	0.000
Rep	2	7.202	8.863	4.431	1.51	0.268
Block(Rep)	9	15.484	14.184	1.576	1.48	0.166
Plt(Rep)	57	103.870	103.870	1.822	1.71	0.010
Error	97	103.193	103.193	1.064		
Total	235	1428.305				

Table 17. ANOVA for PH, using adjusted SS for tests for winter wheat 2014

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Line	70	50460.20	37603.96	537.20	26.43	0.000
Rep	2	18.55	24.65	12.32	0.65	0.592
Block(Rep)	9	197.79	179.82	19.98	0.98	0.459
Plt(Rep)	57	1120.32	1120.32	19.65	0.97	0.548
Error	97	1971.19	1971.19	20.32		
Total	235	53768.05				

FHB (Table 18) and DON (Table 19) both had significant effects for lines, though the effect was not high (F=5.49, P=0.000 for FHB and F=4.32, P=0.000 for DON). Additionally, for DON (Table 19) there was also an effect of rep within block (F=9.47, P=0.000). The ANOVA for AE (Table 20) in winter wheat gives very low effects for all model responses, although the line effects are highly significant.

Table 18. ANOVA for FHB, using adjusted SS for tests for winter wheat 2014

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Line	70	67735.9	51634.6	737.6	5.49	0.000
Rep	2	815.3	715.7	357.8	11.76	0.607
Block(Rep)	9	1031.6	981.8	109.1	0.81	0.606
Plt(Rep)	57	5054.6	5054.6	88.7	0.66	0.955
Error	97	13022.0	13022.0	134.2		
Total	235	87659.4				

Table 19. ANOVA for DON, using adjusted SS for tests for winter wheat 2014

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Line	70	3220.950	1918.587	27.408	4.32	0.000
Rep	2	79.541	81.126	40.563	0.43	0.663
Block(Rep)	9	551.104	540.718	60.080	9.47	0.000
Plt(Rep)	57	435.047	435.047	7.632	1.20	0.210
Error	97	615.695	615.695	6.347		
Total	235	4902.337				

Table 20. ANOVA for AE, using adjusted SS for tests for winter wheat 2014

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Line	69	481.445	462.317	6.700	1.89	0.001
Rep	2	15.147	15.793	7.896	1.74	0.237
Block(Rep)	15	63.382	63.382	4.225	1.19	0.285
Error	125	442.554	442.554	3.540		
Total	211	1002.528				

The heritabilities for winter wheat (Table 21) gave higher heritabilities for FHB and DON in winter wheat at 81% and 77% respectively, than for spring wheat. Additionally HD and PH had very high heritabilities, at 90% and 96%. AE was the trait with the lowest percentage heritability at 47%.

Table 21. Calculated heritabilities for winter wheat 2014

Phenotypic trait	h^2	Percentage heritability
HD	0.91	90%
PH	0.96	96%
FHB	0.81	81%
DON	0.77	77%
AE	0.47	47%

2.2. Phenotypic results

The phenotypic results display how the different spring and winter wheat lines differ from one another in terms of FHB and DON and how the phenotypic cofactors such as DH, PH and AE affect these. When comparing DON content and FHB symptoms, lines seemed to have a relative correspondence between FHB and DON. Some lines had a very good correspondence, while others did not. However, as it is the mycotoxin level that is of most importance, the DON values are the ones of particular interest.

Figure 20 shows the FHB and DON values for the resistance sources in the breeding material, and Figure 21 shows the 20 lines with the highest DON score, and 20 lines with the lowest DON score. From Figure 21 one can see that the DON values ranges from 0 to 27.3. Avocet-YrA has the highest score for DON at 27.3 ppm. ONPMSYDER-05 also has a high score at 26.5 ppm followed by Pfau/Milan, C80.1/3*QT4522//2*ATTILA and Milan with 24.0 ppm, 22.8 ppm and 22.1 ppm respectively. Furthermore, CBRD/KAUZ and Kukri had a DON value of 20.3 ppm, Chara 19.0 ppm and Bau/Milan-2 18.8 ppm.

For the lower-DON lines, Sumai 3(18.) had low traces of DON, while the other Sumai line (Sumai#3 (12SRSN)) had a DON value of 1.3 ppm, which is also considered a low value. The majority of lines had a DON value less than 5.0 ppm. Therefore, the important sources of resistance have been included in Figure 20. Many of these resistant sources are also found at the lower end of Figure 21 indicating that these do in fact have low DON values.

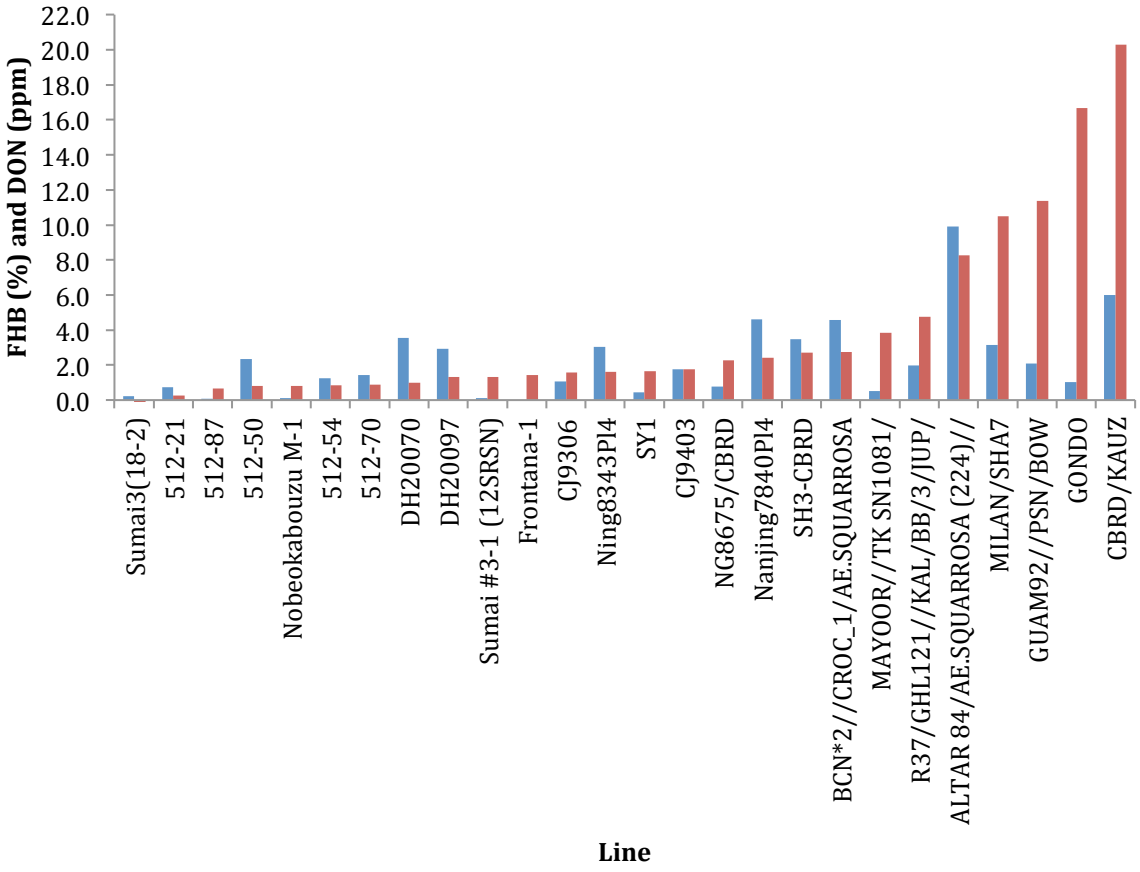


Figure 20. The most important sources of *Fusarium* head blight resistance in the breeding material with FHB (blue) and DON (red) values for 2014

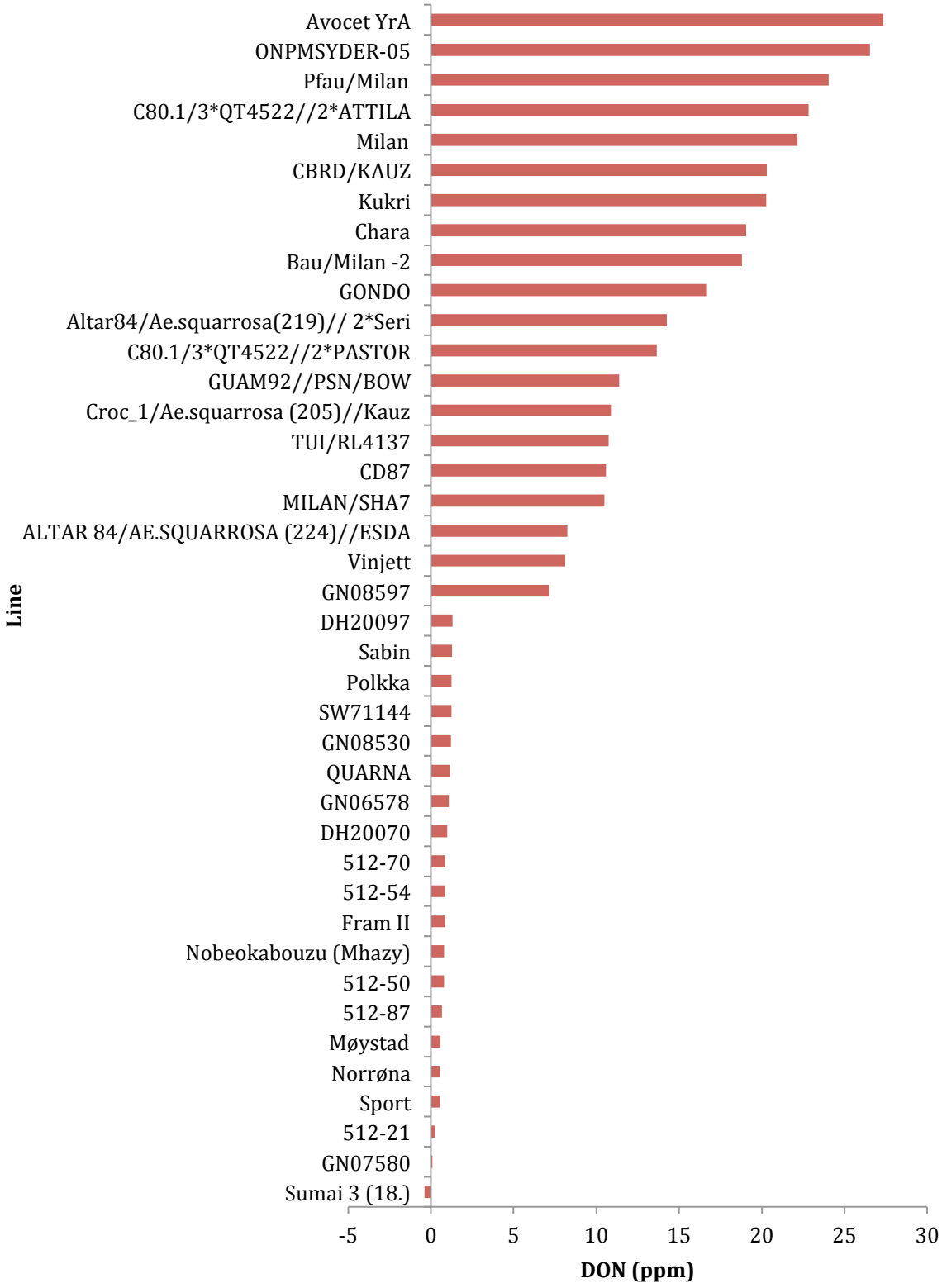


Figure 21. The 20 lines with the highest DON values and 20 lines with the lowest DON values for spring wheat lines for 2014

Figure 22 shows the most important spring wheat cultivars in Norway. The DON values ranges from 1.6 ppm for Krabat, to 3.4 ppm for Bjarne, which compared to the DON values from Figure 21 is at the lower end of the spectrum.

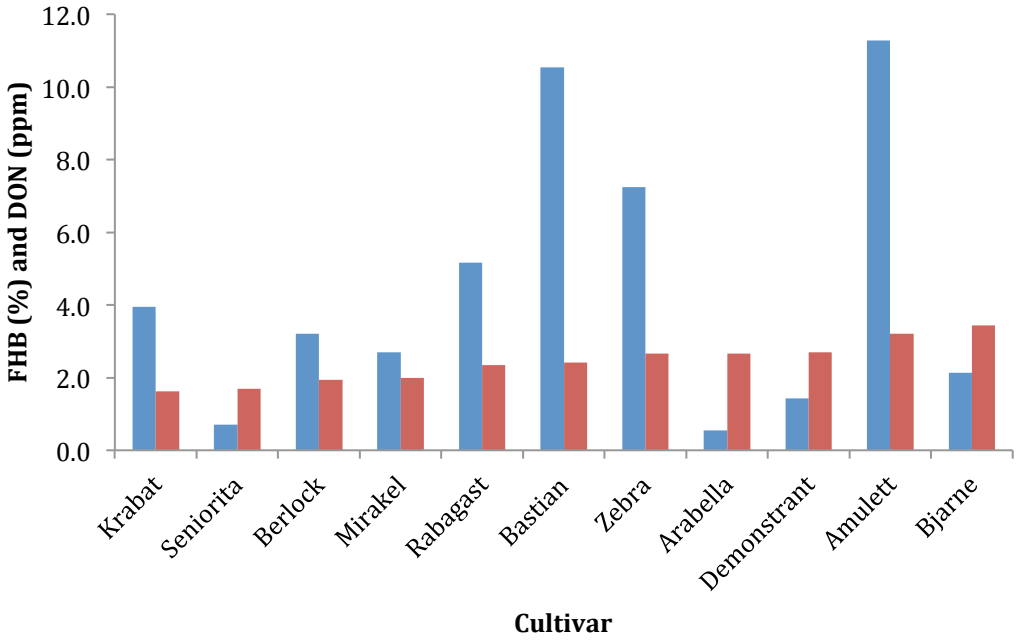


Figure 22. Important breeding cultivars with corresponding values for FHB (blue) and DON (red) for 2014

When looking at the FHB and DON content compared with the phenotypic traits such as DH DON had an R^2 value of 0.11317 (Figure 23). However, there are only a few lines, which has a very high level of DON influencing the positive trend. Without these lines the R^2 value would have been lower. The FHB results gave a very low correlation with DH (R^2 = 0.01874). When looking at Figure 24, the average results over years for earliness gives a very low R^2 value for both FHB (R^2 = 0.02523) and DON (R^2 = 0.02637).

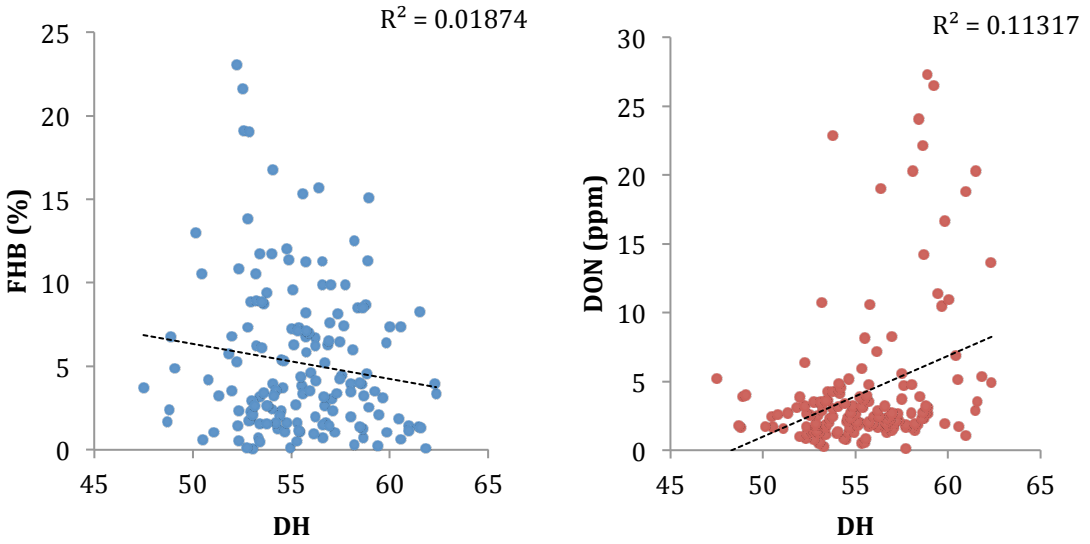


Figure 23. Correlation of *Fusarium* head blight (FHB) and Deoxynivalenol (DON) with phenotypic data for earliness (DH – days to heading) for spring wheat lines for 2014

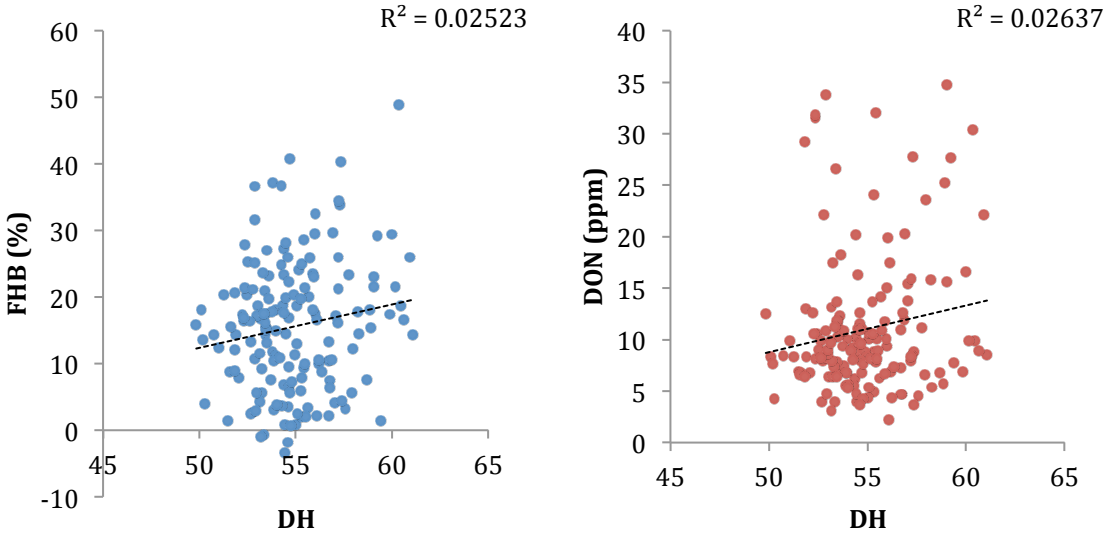


Figure 24. Correlation of *Fusarium* head blight (FHB) and Deoxynivalenol (DON) with phenotypic data for earliness (DH - days to heading) for spring wheat lines over years

The comparisons between PH and FHB and DON are displayed on Figure 25. It would be expected that lines with shorter straws would have more FHB and DON than lines with longer straws. FHB and DON shows in this case $R^2 = 0.05766$ and $R^2 = 0.06997$ respectively, indicating that there is a weak trend towards lines with longer straws having less FHB and DON than lines with short straws (Figure 25). When comparing the phenotypic results from

2014 to the results over years (Figure 26), the results are corresponding relatively well with the DON and FHB R^2 values of 0.08037 and 0.03349 respectively.

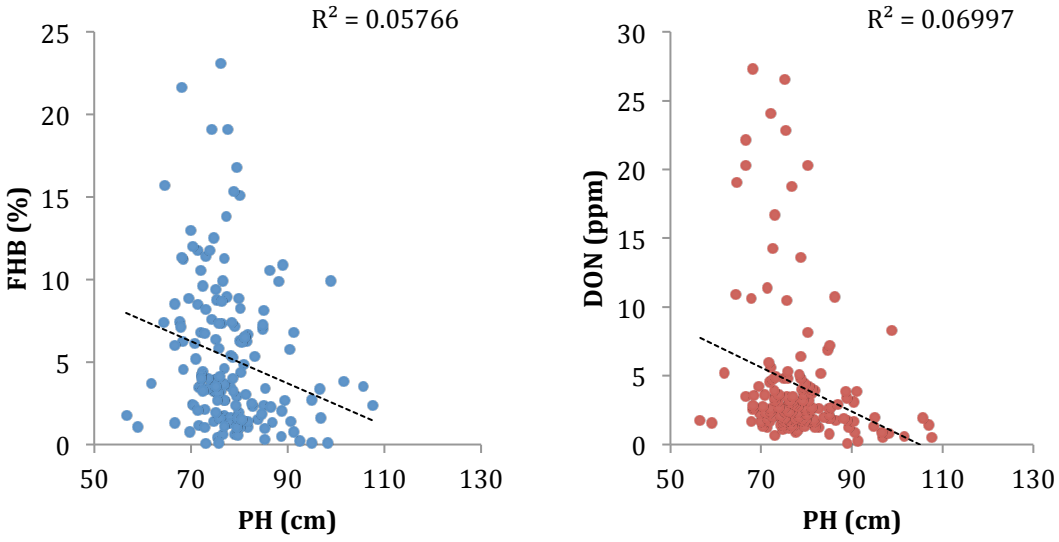


Figure 25. Correlation of *Fusarium* head blight (FHB) and deoxynivalenol (DON) with phenotypic data for plant height (PH) in cm for spring wheat lines for 2014

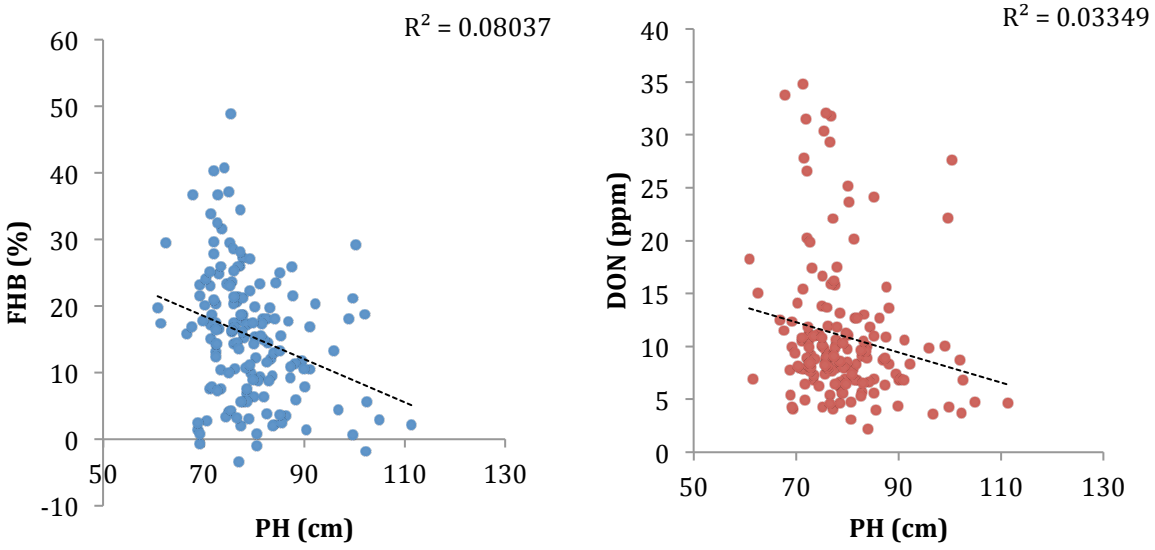


Figure 26. Correlation of *Fusarium* head blight (FHB) and Deoxynivalenol (DON) with phenotypic data for plant height (PH) in cm for spring wheat lines over years

Lines with high anther extrusion never get a lot of *Fusarium*, while lines with poor anther extrusion have a higher spread in infection frequency. From Figure 27, one can see that there is indeed a negative correlation between FHB and AE (R^2 = 0.08144), and DON and AE (R^2

= 0.07019). The explained percentage and ppm was expected to be higher than 8 % (FHB) and 7 % (DON). However, when looking at the phenotypic results for anther extrusion over years (Figure 28) the association gives a better trend at $R^2 = 0.15204$ for FHB and $R^2 = 0.25768$ for DON.

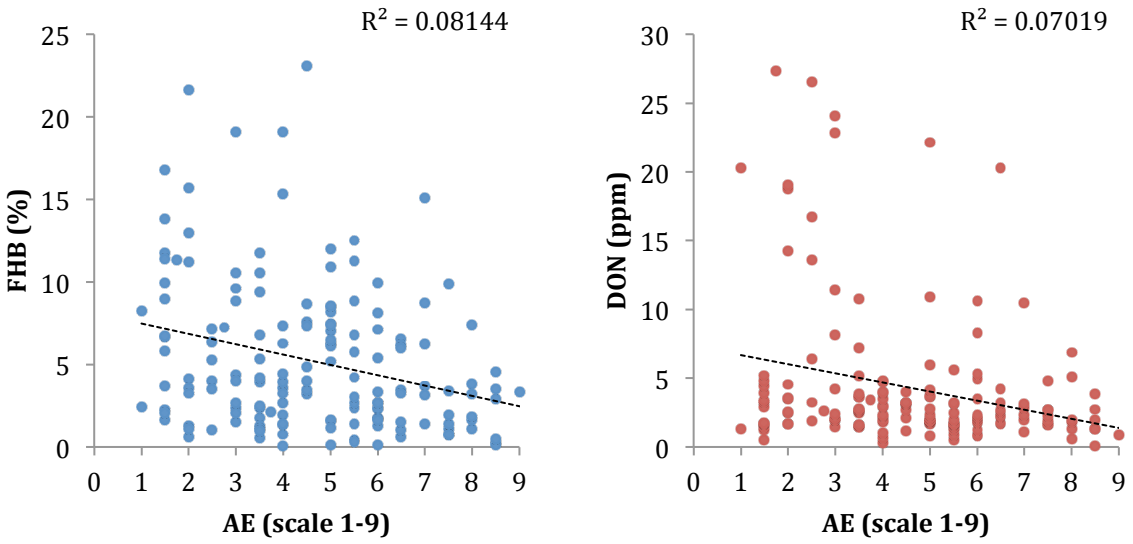


Figure 27. Correlation of *Fusarium* head blight (FHB) and deoxynivalenol (DON) with phenotypic data for anther extrusion (AE) on a scale from 1-9 for spring wheat lines for 2014

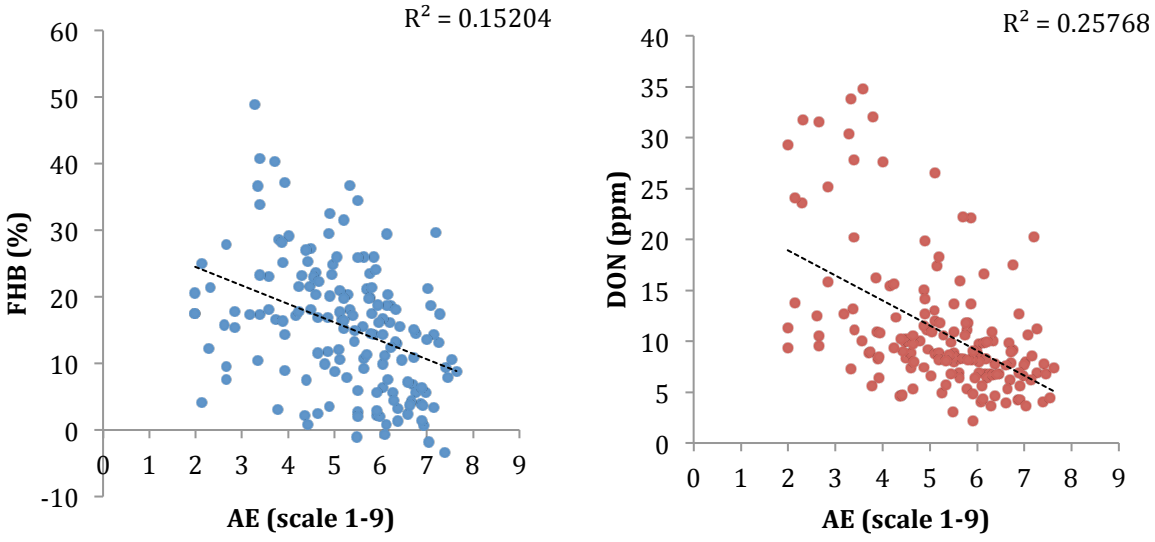


Figure 28. Correlation of *Fusarium* head blight (FHB) and Deoxynivalenol (DON) with phenotypic data for anther extrusion (AE) on a scale from 1-9 for spring wheat lines over years

Figure 29 compares the results for FHB and DON for spring wheat lines in 2014, and Figure 30, the results for the same lines over years. When comparing FHB and DON for spring wheat there was no correlation between the two ($R^2 = 0.00739$). The low R^2 is here caused by the few observations of very high DON. When looking at the average of FHB versus DON over years the trend is much higher, at $R^2 = 0.30127$.

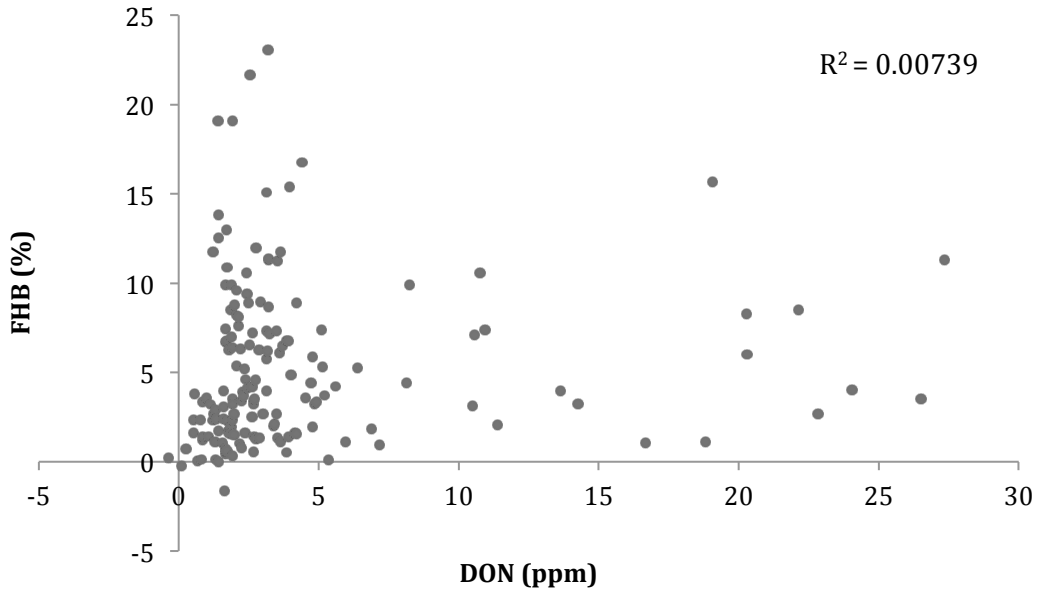


Figure 29. Deoxynivalenol (DON) against *Fusarium* head blight (FHB) for spring wheat lines for 2014

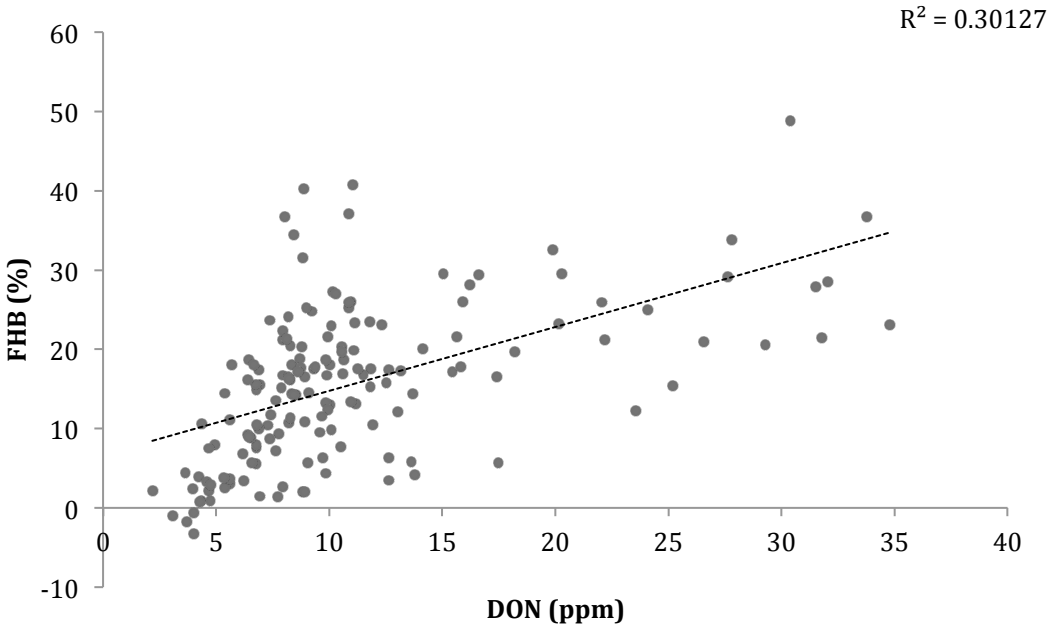


Figure 30. Deoxynivalenol (DON) against *Fusarium* head blight (FHB) for spring wheat lines over years

The winter wheat lines displayed similar trends as the spring wheat. Figure 31 displays the 49 winter wheat lines with their corresponding DON values. The DON values ranges from -0.03 (Mironovskaja808) to 20.1 (Apollo). The top 5 lines, Apollo, Fenman, Ambition and GN05013 and Senat all had DON values over 10 ppm (20.1,15.2, 13.0, 12.1 and 11.0 respectively). At the lower end of the spectrum, Mironovskaja 808 and Regina had -0.03 and 0.01 ppm DON.

Figure 32 shows the important cultivars in Norway. The values for DON are evenly spread when comparing this Figure to Figure 31. Ellvis has the lowest DON value at 2.7 ppm and Skagen the highest at 8.4 ppm. When comparing DON values with FHB, the percentage of FHB did not show any clear correspondence with the DON results.

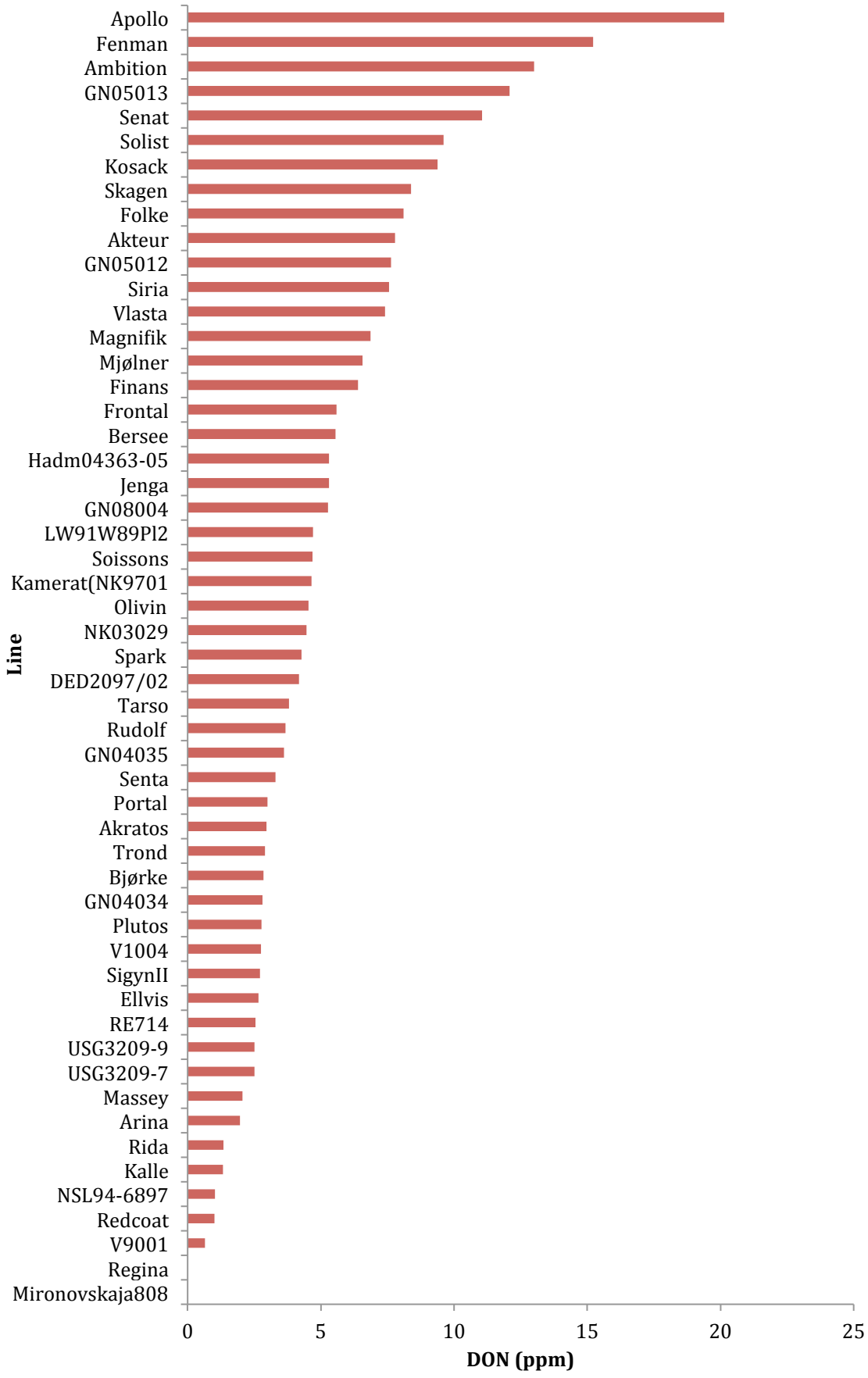


Figure 31. Deoxynivalenol (DON) for winter wheat lines for 2014

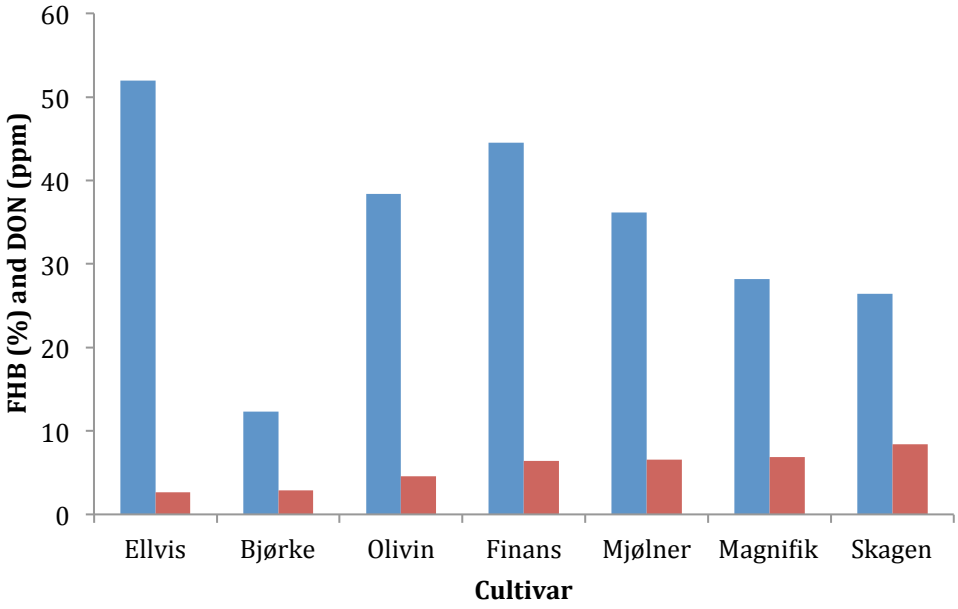


Figure 32. Important breeding lines with corresponding FHB (blue) and DON (red) values for 2014

The comparison of FHB and DON to phenotypic cofactors such as DH, PH and AE did show expected correlations. For DH it is clear to see that there is a positive trend, with early lines having less FHB and DON than later lines (Figure 33). Similar to the spring wheat, the DON R^2 value was higher than the FHB value (DON $R^2 = 0.29257$) (FHB $R^2 = 0.01273$).

PH also show an expected trend. Figure 34 displays a negative trend for plant height for DON with and $R^2 = 0.1808$ compared to FHB with no trend at all ($R^2 = 0.00212$). There were more DON in lines with shorter straws than in lines with longer straws, though this relationship was absent for FHB. However, the correlation between DON and PH is much higher than for FHB.

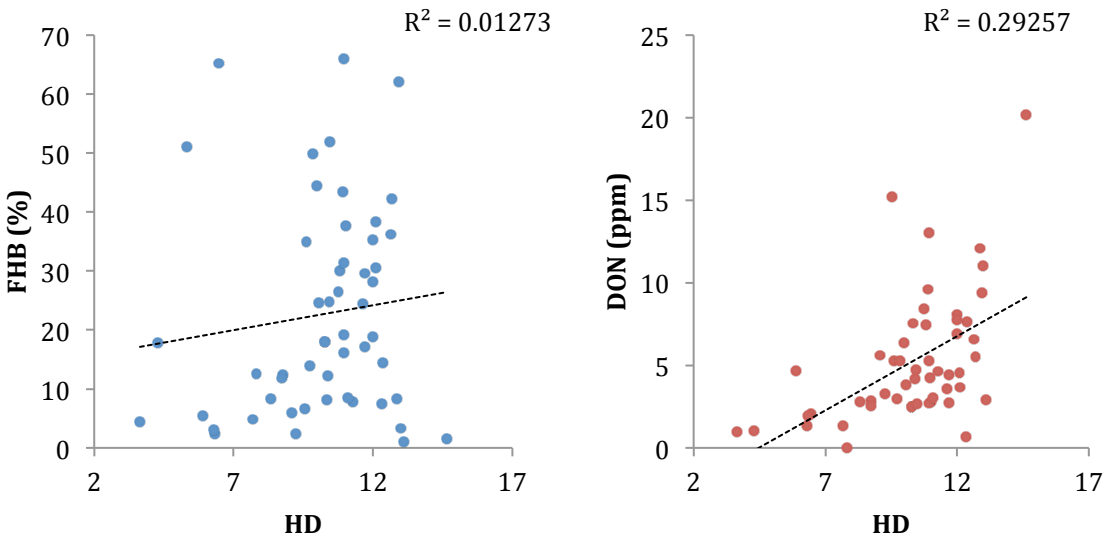


Figure 33. Correlation of *Fusarium* head blight (FHB) and deoxynivalenol (DON) with phenotypic data for heading date (HD) in July for winter wheat lines for 2014

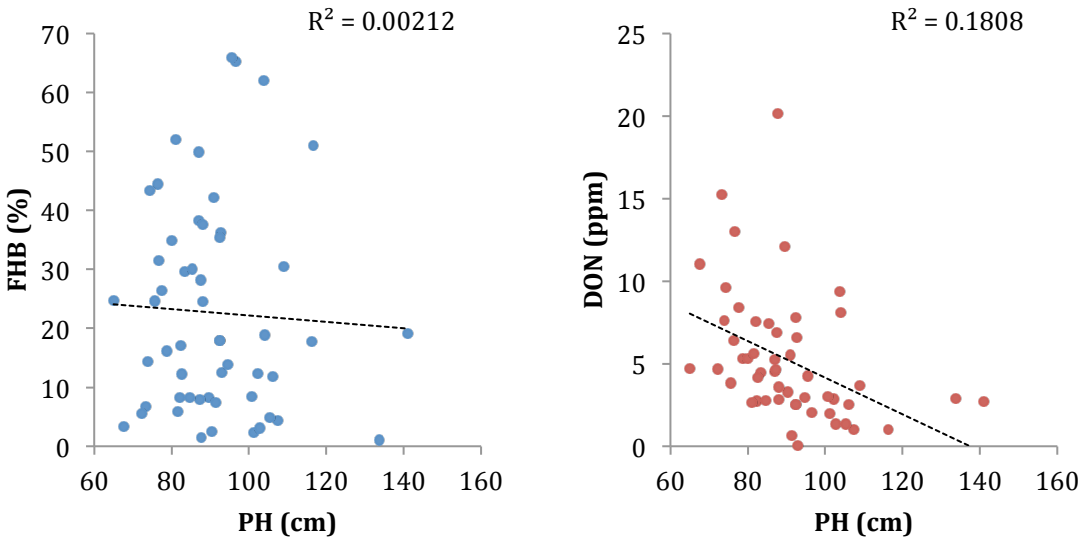


Figure 34. Correlation of *Fusarium* head blight (FHB) and deoxynivalenol (DON) with phenotypic data for plant height (PH) in cm for winter wheat lines for 2014

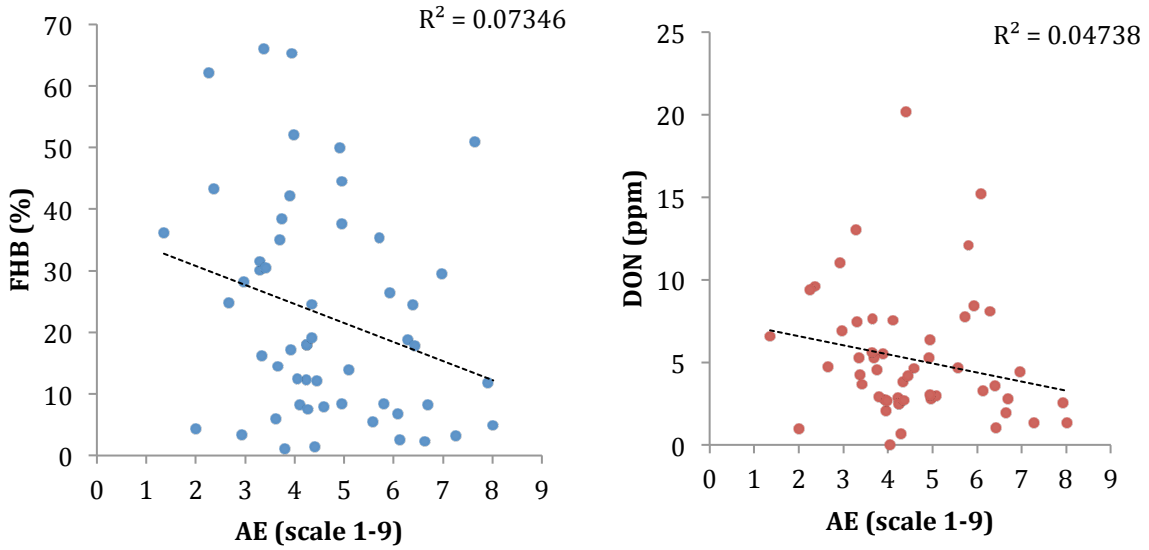


Figure 35. Correlation of *Fusarium* head blight (FHB) and deoxynivalenol (DON) with phenotypic data for anther extrusion (AE) on a scale from 1-9 for winter wheat lines for 2014

Figure 35 shows that there is a negative correlation to FHB (R^2 = 0.07346) and DON (R^2 = 0.04738), showing that lines with good AE had less FHB and DON than lines with poorer AE. Therefore, 7% of FHB is explained by AE, and 5% for DON. Furthermore, as with spring wheat, a figure showing the average FHB and DON compared with AE over years would be a better explanation of the actual trends, but such data was not available. Furthermore, when comparing FHB to DON (Figure 36) there was similarly to spring wheat no correlation between FHB and DON (R^2 = 0.00017)

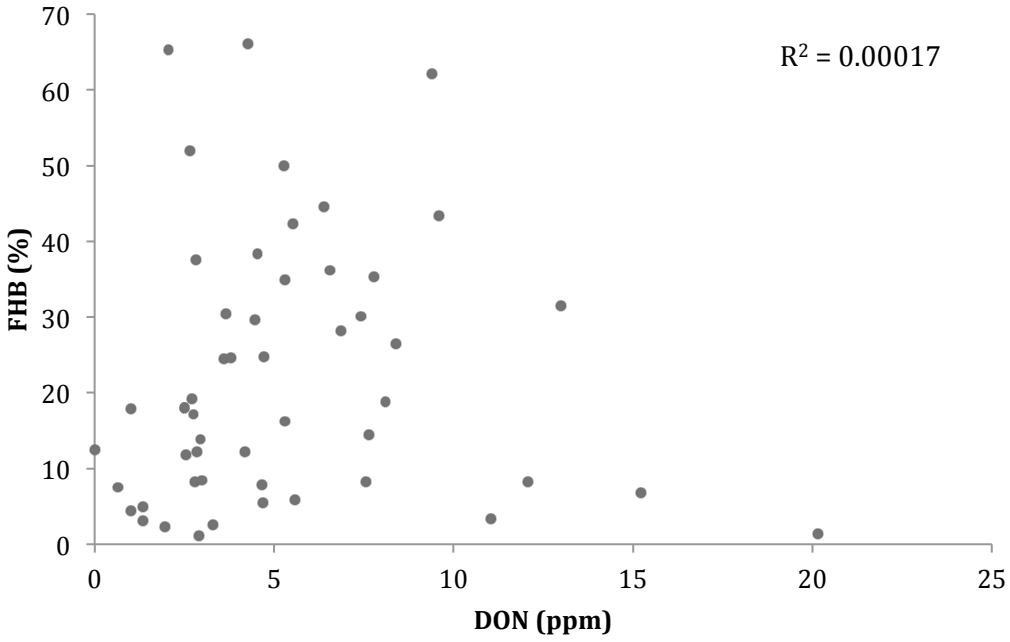


Figure 36. *Fusarium* Head blight (FHB) against Deoxynivalenol (DON) against for winter wheat for 2014

2.3. Discussion

2.3.1. Heritability

For all traits there were a non-normal distribution of data. PH was the trait closest to a normal distribution, possibly due to the trait being quite easy to score compared to the other traits. Additionally, PH had a heritability of 89% for spring wheat and 96% for winter wheat indicating that most of the observed phenotypic effects are due to genetic factors rather than environment. Furthermore, over years for spring wheat the heritability was calculated to 87% again manifesting the genetic impact on the observed variations of the trait. DH gave a percentage of heritability of 93% for spring wheat 2014, 93% over years and 90% for winter wheat 2014, suggesting that the observed phenotypic variation of DH/HD is mainly due to the genetic diversity of MASBASIS.

It would be expected that FHB would display a lower percentage of heritability than for DON due to DON being more accurately scored. This was true for the spring wheat, with 34% FHB and 88% DON for 2014, and 17% FHB and 78% DON over years. One of the reasons for the insignificance of effects of FHB over years is probably due to the different infectious

pressures over the two years. The inoculum used for 2014 was only 5g/m^2, whereas previous years, the inoculum was distributed as 10g/m^2. Additionally, environmental influence eg. differences in weather conditions from one year to another. Furthermore, different evaluations of traits may also have resulted in low heritability over years as people may judge observations differently. However, for winter wheat it is the other way around, with a heritability of 81% for FHB and 77% for DON which is still a similar result for DON as with spring wheat. The high heritability of FHB may most likely have been due to the experienced difficulty of scoring, mistaking FHB infection for dryness. AE heritability were also different. Spring wheat gave heritabilities of 60% for 2014 and 70% over years, while winter wheat for 2014 had 47% suggesting environmental effects impacting the observed phenotypic trait.

2.3.2. Trait relationships

Both spring and winter wheat lines display similar trends in relation to DH (Figure 23 and 33) PH (Figure 25 and 34) and AE (Figure 27 and 35) when it comes to the visual symptoms of FHB and DON. For DH, the high positive trend was caused by a few lines with very high levels of DON. Without these, the correlation between DH and DON would be lower. Additionally the correlation between DH and FHB was slightly negative, potentially being caused by the difficultness scoring visual symptoms of FHB in the field. However, the results over years show that both FHB and DON have low correlations with DH. Furthermore, because some lines flower early and some late the visual symptoms of FHB may not have come to the latest stage for all lines when they are all harvested at the same time.

For PH it would be expected that lines with longer straws had less FHB and DON than lines with shorter straws, first and foremost because of the distance to the soil. The infection in this study was scattered on the soil, thus it would be farther for the pathogen to travel to the higher rather than the lower heads.With Klahr et al. (2007) and Somers et al. (2003) stating that taller lines have a higher chance of escaping infection because of their distance from the soil this may also be the case. For natural infection to occur the ascospores are spread by wind, rain, insects or animals (Parry et al. 1995; Sutton 1982). For the pathogen to infect host plants it would need to be able to attach to their hosts. It would therefore be easiest for the pathogen to attach themselves to wheat with short straws as the longer straws are moving more vigourously in the wind. For this study, lines with higher straws had less FHB and DON than lines with shorter straws. However, the relationship between PH and FHB and DON has been

proven to be more complex. The dwarf gene *Rht-D1b* is suggested by Srinivasachary et al. (2008) to be pleiotropic or linked with a mechanism giving high physiological susceptibility to FHB in the plant. The study by Lu et al. (2013) also found that low PH increased FHB severity.

The conditions around flowering time has been suggested by Bottalico and Perrone (2002) to play an important role in creating differences in FHB severity. It was expected that AE shows a stronger association with FHB than DON, as it has more impact on the infection that cause visual symptoms, than mycotoxin accumulation which is affected by active resistance mechanisms after infection. AE is difficult to score and is also affected by the environment. FHB and DON is also affected by environment. Therefore, a higher association was seen when the average data over years was compared. However, the spring wheat lines were scored in two different field trials; one for scoring FH, PH and FHB, the other AE. This could cause a potentially poorer comparison than if scored in one trial. Furthermore, climate could also impact the results of the different trials, as they were not sown on the same field. Climate is a contributing factor to different results over years, as it is not the same from one year to the next.

The correlation between FHB and DON was very low in the 2014 field trials. Again, this may be due to the differences in infectious pressure ($10g/m^2$ and $5g/m^2$) and also more favourable weather conditions for FHB development. The correlation was therefore much higher when FHB and DON were compared over years. Another reason may have been the weather in 2014. After FHB was scored there was a longer period of rain. In the already infected grain, the FHB could have developed further due to moist conditions which is beneficial for the fungus. Therefore, as the correlations between FHB and DON were much higher over years, one year of testing is not enough to give any significant conclusions.

Chapter 3:

Population structure and association mapping of earliness and plant height

3.1. Population Structure

STRUCTURE v. 2.3.4 was run with a subset containing 338 markers, chosen with an interval of approximately 5 centiMorgans (cM) based on the consensus map. The 172 MASBASIS lines were run in STRUCTURE v. 2.3.4 with K from 1 to 10 giving a ΔK estimation of 2 subpopulations (K = 2)(Figure 37), indicating that STRUCTURE has divided the 172 lines in spring and winter wheat respectively. However, a second peak from Figure 37 suggest that the most optimal further division is in 8 subpopulations. After the STRUCTURE run with K from 1 to 10 for the 129 spring wheat lines and 49 winter wheat lines, the estimated ΔK gave K= 5 for spring wheat (Figure 38) and K= 3 for winter wheat (Figure 39), indicating more or less the same 8 genetic clusters as in the combined analysis of spring and winter wheat. This shows that there is a definite structure in these 172 MASBASIS lines, giving 5 subpopulations of spring wheat, and 3 of winter wheat.

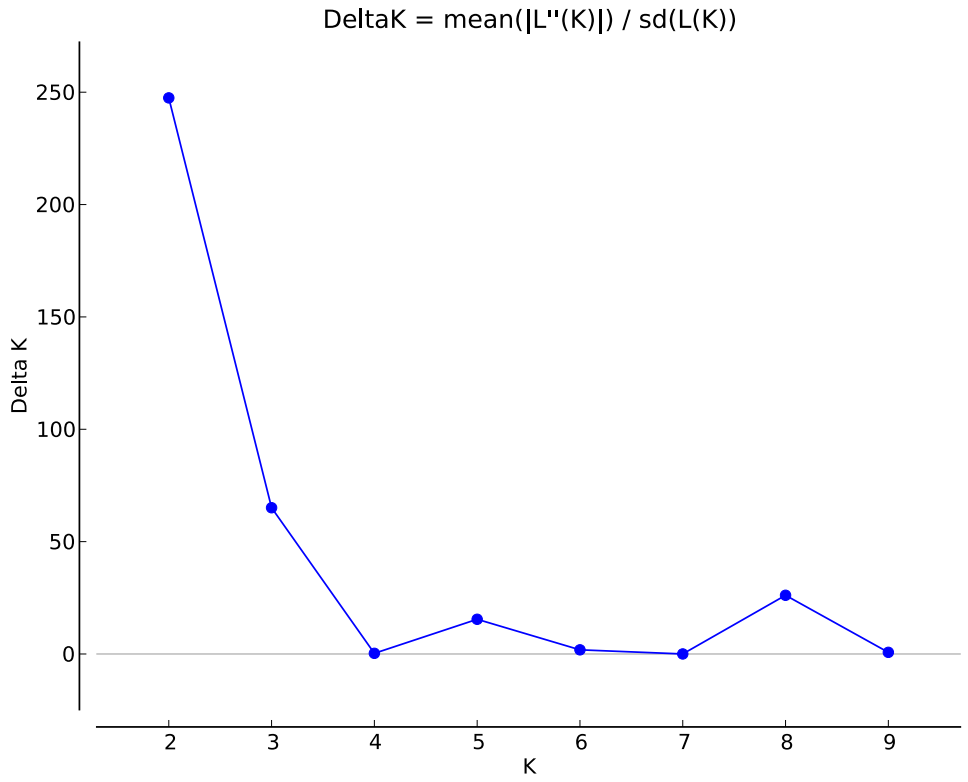


Figure 37. Delta K versus K for 172 MASBASIS lines from Structure Harvester

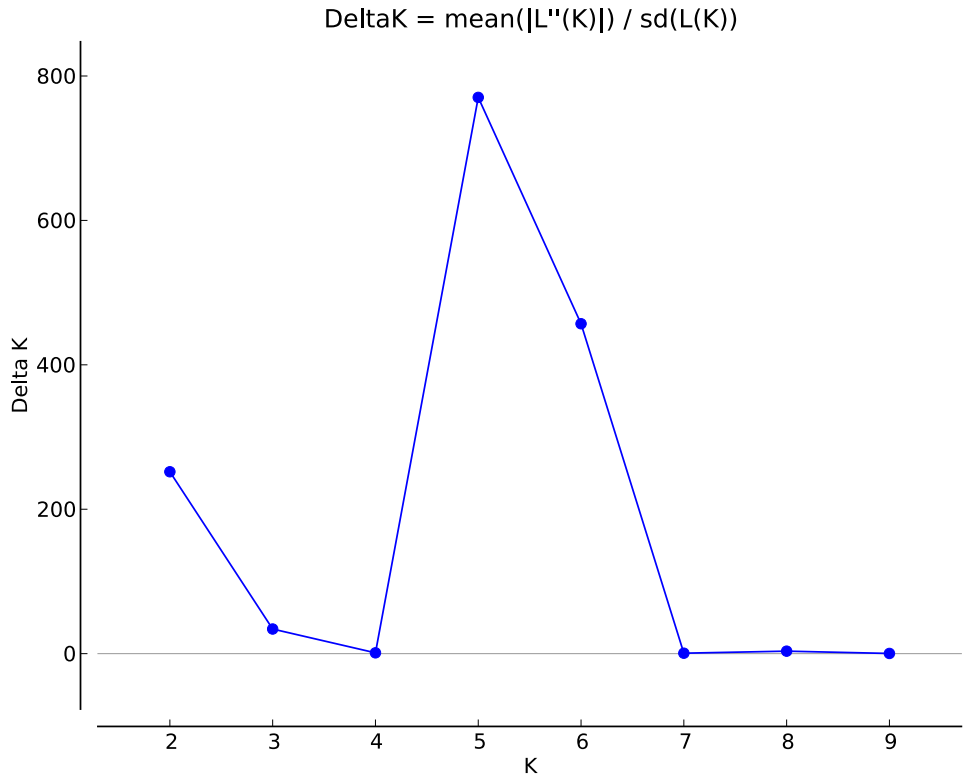


Figure 38. Delta K versus K for spring wheat lines from Structure Harvester

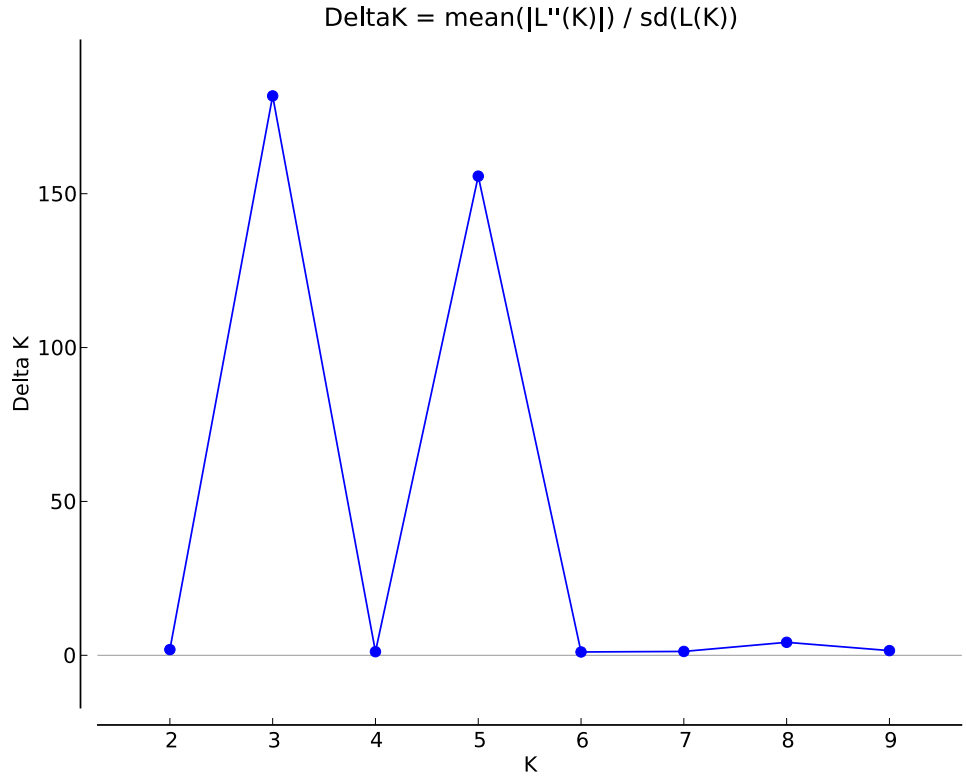


Figure 39. Delta K versus K for winter wheat lines from Structure Harvester

PCA analysis was also performed with the use of the Unscrambler X software (CAMO 2015). A number of 4 Principal components (PC) were used, as more components did not explain any more of the population structure displayed by Tables 22, 25 and 27 for all lines, spring wheat lines and winter wheat lines respectively. PC1-PC4 identified the same 8 subpopulations for MASBASIS in Unscrambler X as in STRUCTURE. In addition, the subpopulations were assigned accessions where all lines with a percentage less than 50 for one subpopulation were considered mixed (Figure 40). Table 23 shows how many lines were in the different groups, and their geographic location.

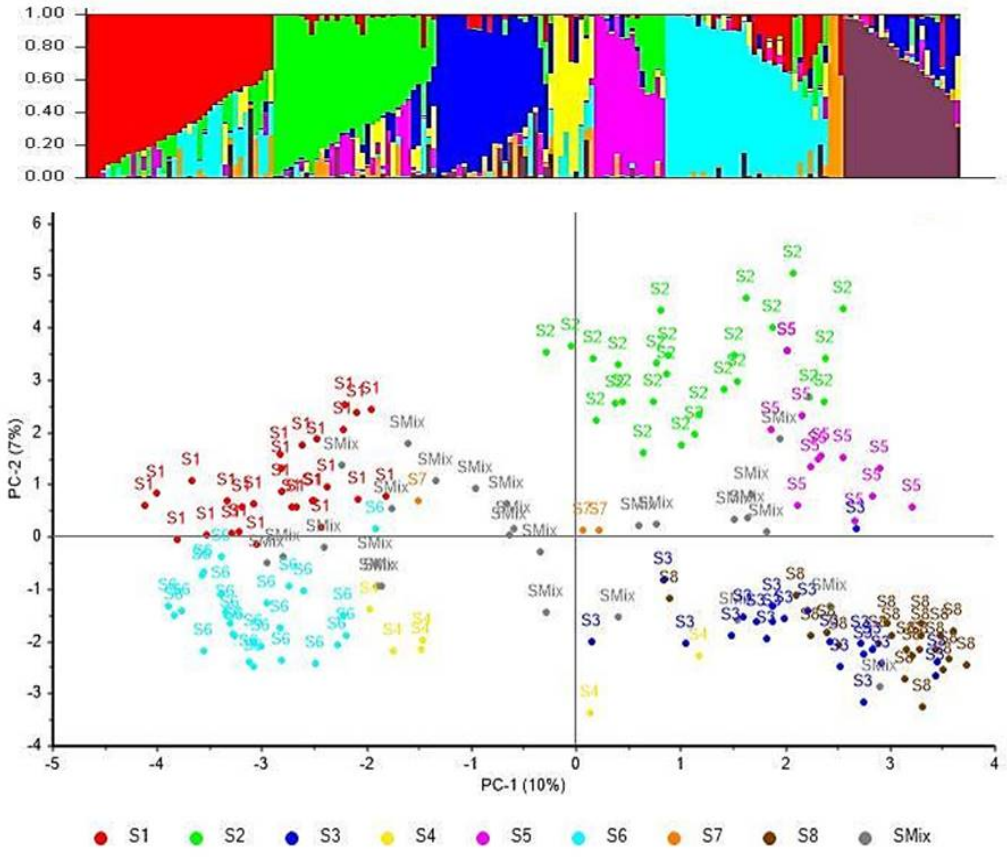


Figure 40. Population structure defined by STRUCTURE and Unscrambler X for 172 MASBASIS lines displaying PC1 against PC2 for 8 subpopulations (S1-S8) and one mixed group containing lines with <50% relation to a single population (SMix)

Table 22. Explained population structure from principal components for 172 MASBASIS lines with explained percentage and variance with sum of squares (SS)

Principal component	Explained percentage (%)	SS	Explained variance (R^2)	
			Calibration	Validation
PC1	10	1027.01	9.74	8.27
PC2	7	717.91	16.57	13.85
PC3	4	429.48	20.64	16.93
PC4	3	343.97	23.92	18.89
PC5	3	290.87	26.69	20.16
PC6	2	257.32	29.13	21.48
PC7	2	225.71	31.29	22.14

Table 23. Distribution of spring and winter wheat lines in groups defined by STRUCTURE and their geographical location

Population	Number of lines	Type of lines	Location
S1	28	Spring	Norway
S2	27	Spring	Mostly CIMMYT
S3	21	Winter	Norway/Sweden
S4	6	Spring/Winter	Europe
S5	13	Spring	Mostly Chinese
S6	26	Spring	Sweden
S7	3	Spring	Norway
S8	21	Winter	Europe
SMix	27	Mix	Mix

Population 1, 6 and 7 consist of spring wheat from Norway (population 1 and 7) and Sweden (population 6). Population 7 (J03, Fram II and Norrøna) clearly group themselves as a separate population from the Norwegian and Swedish spring wheat. Population 2 consists mostly of the CIMMYT (International Maize and Wheat Improvement Center) spring wheat lines, while population 5 is made up of primarily Chinese cultivars and CIMMYT breeding lines with FHB resistance derived from Chinese wheat. Population 3 and 8 are winter wheat lines, where population 3 contains Norwegian and Swedish winter wheat, and population 8, European winter wheat. In addition population 4 group as European spring wheat with one Russian winter wheat line Mironovskaja 808. The complete population structure for the 172 MASBASIS lines and the respective subpopulations is displayed in Appendix 1 (table 9).

Figure 41 displays the grouping of the 123 spring wheat lines with the explained principal components for the analysis in Table 25. Comparing the results from all 172 lines with the 123 spring wheat lines, the grouping of the subpopulations are similar. Subpopulation 1 are mostly CIMMYT lines while subpopulation 5 consist of the Chinese wheats. The Norwegian and Swedish spring wheat group themselves in subpopulation 2 and 4 respectively, while the European spring wheat in subpopulation 3 consist of the very prominent old Norwegian breeding sources J03, Norrøna and Fram II among others.

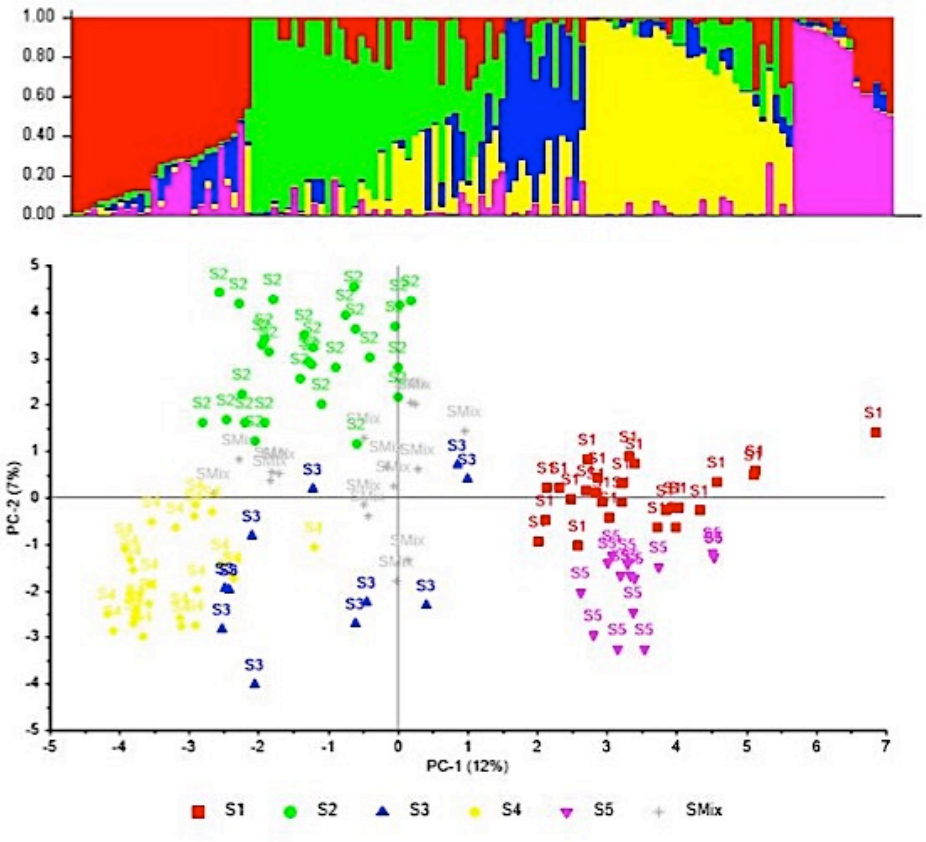


Figure 41. Population structure defined by STRUCTURE and Unscrambler X for 123 spring wheat lines displaying PC1 against PC2 for 5 subpopulation (S1-S5) and one mixed group containing lines with <50% relation to a single population (SMix)

Table 24. Distribution and number of spring wheat in groups and their geographic location

Population	Number of lines	Location
S1	26	Mostly CIMMYT
S2	29	Norway
S3	11	Europe
S4	26	Sweden
S5	15	Mostly Chinese
SMix	16	Mix

Table 25. Explained population structure from principal components for 123 spring wheat lines with explained percentage and variance with sum of squares (SS)

Principal component	Explained percentage (%)	SS	Explained variance (R^2)	
			Calibration	Validation
PC1	12	940.71	11.68	9.79
PC2	7	530.04	18.22	15.01
PC3	5	363.09	22.72	17.60
PC4	4	296.99	26.40	19.76
PC5	3	251.16	29.53	21.46
PC6	3	217.62	32.23	22.44
PC7	2	199.58	34.71	23.54

Following Figure 42, for the winter wheat, subpopulation 1 were European winter wheat lines and subpopulation 3 the Norwegian and Swedish lines. However, subpopulation 2 consisted of lines which were defined as mixed (<50% relatedness to a single population) in the analysis of all the 172 MASBASIS lines. The three lines of subpopulation 2 were Massey, and two line selections of its progenity line USG3209 from Virginia, USA. Table 27 displays the explained principal components for the population structure of the winter wheat lines. The full population structure of MASBASIS spring and winter wheat lines can be found in Appendix 1.

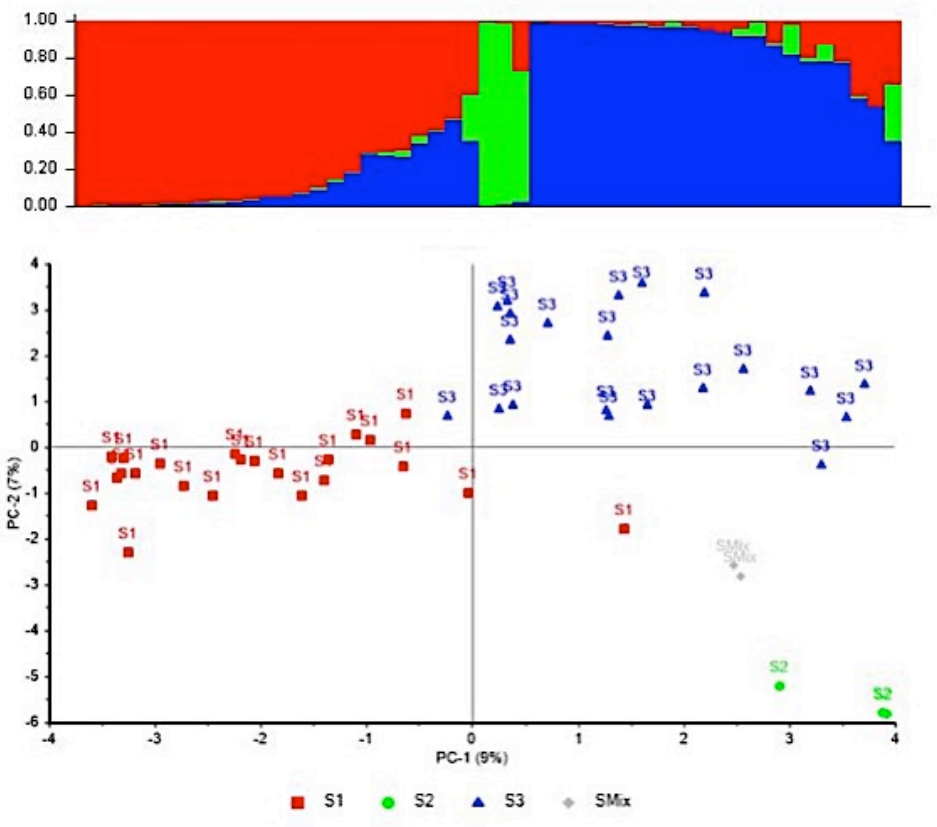


Figure 42. Population structure defined by STRUCTURE and Unscrambler X for 49 winter wheat lines displaying PC1 against PC2 for 3 subpopulations (S1-S3) and one mixed group containing lines with <50% relation to a single population (SMix)

Table 26. Distribution and number of winter wheat in groups and their geographic location

Population	Number of lines	Location
S1	23	European
S2	3	Mostly CIMMYT
S3	21	Norway
SMix	2	Mix

Table 27. Explained population structure from principal components for 49 winter wheat lines with explained percentage and variance with sum of squares (SS)

Principal component	Explained percentage (%)	SS	Explained variance (R^2)	
			Calibration	Validation
PC1	9	257.76	8.91	3.92
PC2	7	220.11	16.41	8.06
PC3	5	159.09	21.87	9.60
PC4	5	142.44	26.71	10.84
PC5	4	126.81	31.07	12.69
PC6	4	118.40	35.18	14.25
PC7	4	110.33	38.99	15.77

3.2. Association mapping of earliness and plant height

The mixed linear model in Tassel gave significant QTL for both DH and PH. The significant markers for DH are displayed in Figure 43. Full lists of significant markers are attached in Appendix 2. The significant markers for 2013 were chosen for FHB/DON for spring wheat because of the low heritability and correlations between FHB and DON for 2014. The significance threshold for DH was set to –log10(P-value) 2.5 (P= 0.003154) due to the high amount of significant markers >2.0. There were a total of 223 markers with a –log10(P-value) >2.0 (P= 0.009991).

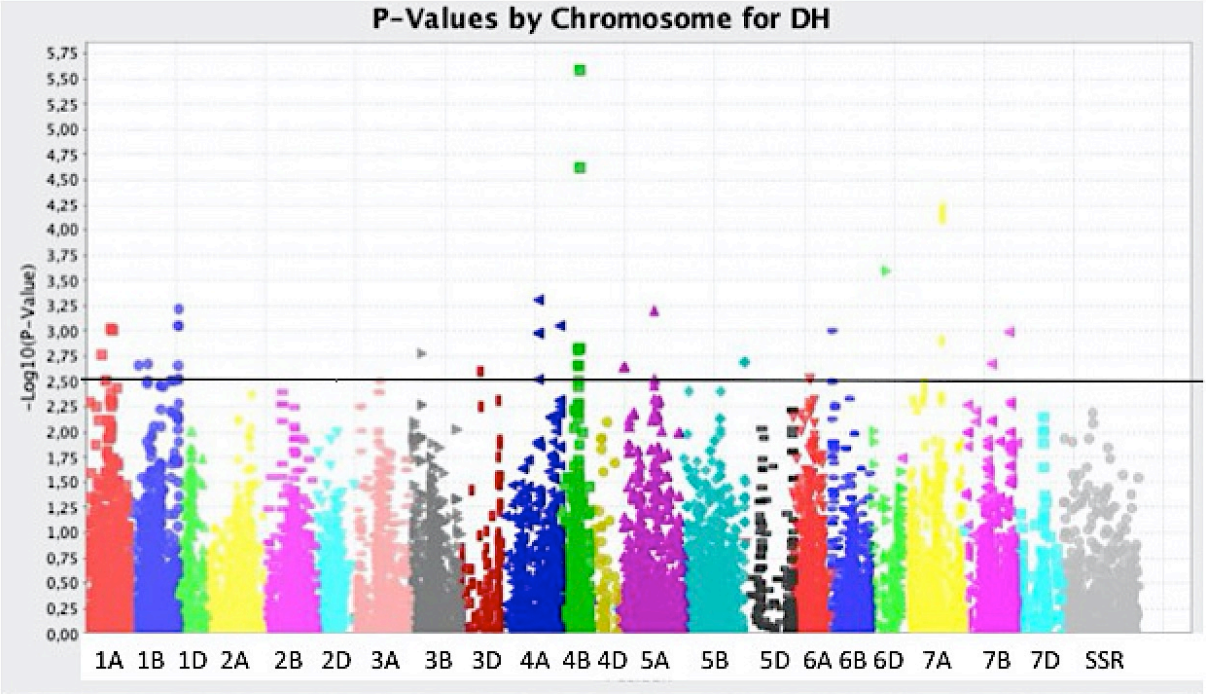


Figure 43. Manhattan plot displaying the markers for earliness (DH) in spring wheat derived from Tassel with marker positions on chromosomes and significance threshold at -log10(P-value) 2.5

Following Figure 43 a total of 58 markers were found to be significant. The most significant markers were found on chromosome 4B with P-values of 0.000024 (wsnp_Ex_c32127_40841791) and 0.000003 (CAP12_c2983_140) at positions 221 and 223 cM. A total of 15 significant markers were found in this area of chromosome 4B. Additionally, there were three significant markers on chromosome 7A, two of these with highly significant P values at position 458 cM (wsnp_Ku_c21665_31431143, P = 0.000061 and Kukri_c57086_133, P=0.000078). Chromosome 6D also had a marker of high significance compared to the rest of the markers on the same chromosome (IACX10982, P=0.000256). On chromosome 1A there were a total of 6 significant markers, three of the most significant of these at position 274 and 289 cM. Chromosome 1B had 13 markers above the significance threshold, most of which had a position around 549 cM. Single significant markers were found on chromosome 3B and 3D. On chromosome 4A there was a significant cluster at position 402 cM. Two highly significant markers were also found on chromosome 6B and 6D with the positions 1 and 183 cM respectively. Four markers were found on chromosome 5A, and three at position 676 cM on chromosome 5B. Additionally, there were also three significant markers on chromosome 7A, two of these at position 458 cM.

Furthermore, two significant markers were found on chromosome 7B at position 292 and 489 cM.

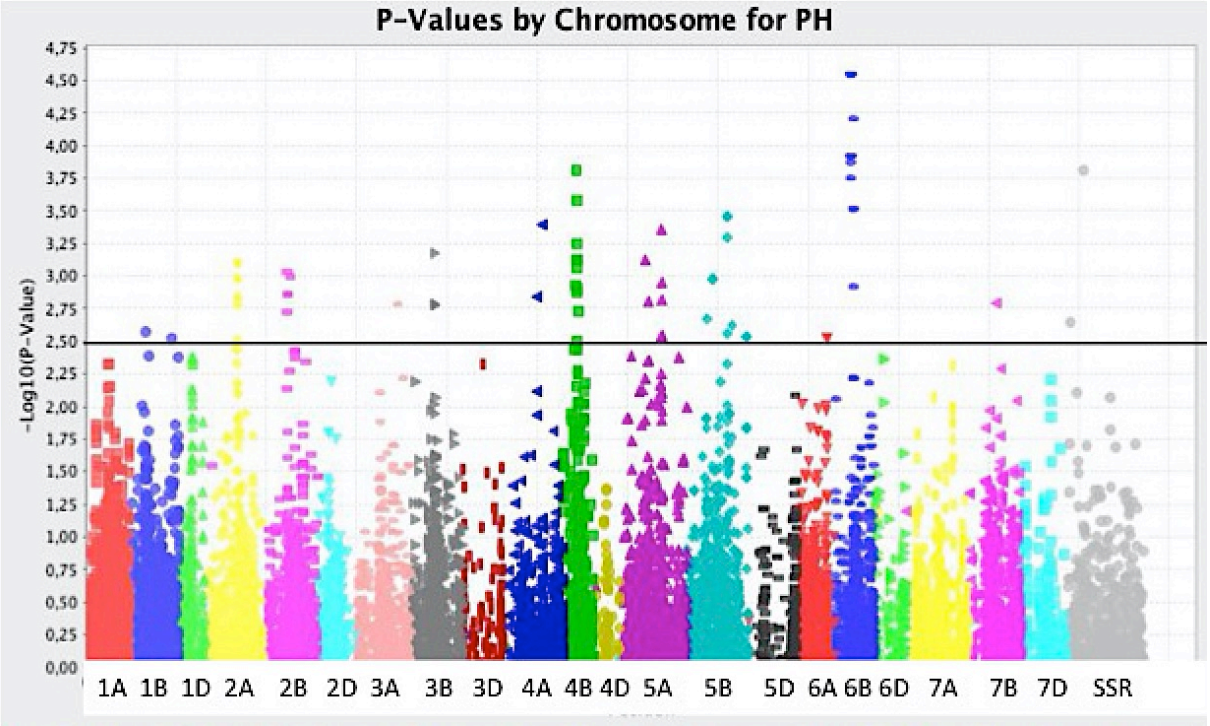


Figure 44. Manhattan plot displaying the markers for plant height (PH) in spring wheat derived from Tassel with marker positions on chromosomes and significance threshold at -log10(P-value) 2.5

The significance threshold –log10(P-value) of 2.5 had a P-value of 0.003145 and contained 79 markers for PH in spring wheat (Figure 44). A total of 190 markers had a –log10(P-value) >2.0 (P= 0.009921). Some of the stand-out values of significance were found on chromosome 4B. Eight highly significant markers were found clustered, ranging from position 159 to 163 cM. The KASP marker Rht-B1 was found at position 159 cM with a P-value of 0.000156. Furthermore, 15 highly significant markers were found clustered on chromosome 6B, 12 of these at position 168 cM and 3 on 198 cM. Additionally, two SSR significant markers, barc130_295 (P=0.002268) and DuPw167_259 (P=0.000155) were found on chromosomes 5DS and 6AL respectively.

Furthermore, two significant markers were found on chromosome 1B at position 182 and 470 cM. 10 markers were found on chromosome 2A at positions 329 and 332 cM. Next, six markers were found on chromosome 2B mostly at positions 254 and 262 cM, and one marker on chromosome 3A (471 cM). A cluster of eight markers were found at position 269 cM on

chromosome 3B, and two on chromosome 4A. Seven markers were found on chromosome 5A, most of these around position 460 cM. Additionally, 10 markers were found on chromosome 5B, the most significant of these at positions 447 and 448 cM. Two markers were also found at position 338 on chromosome 6A.

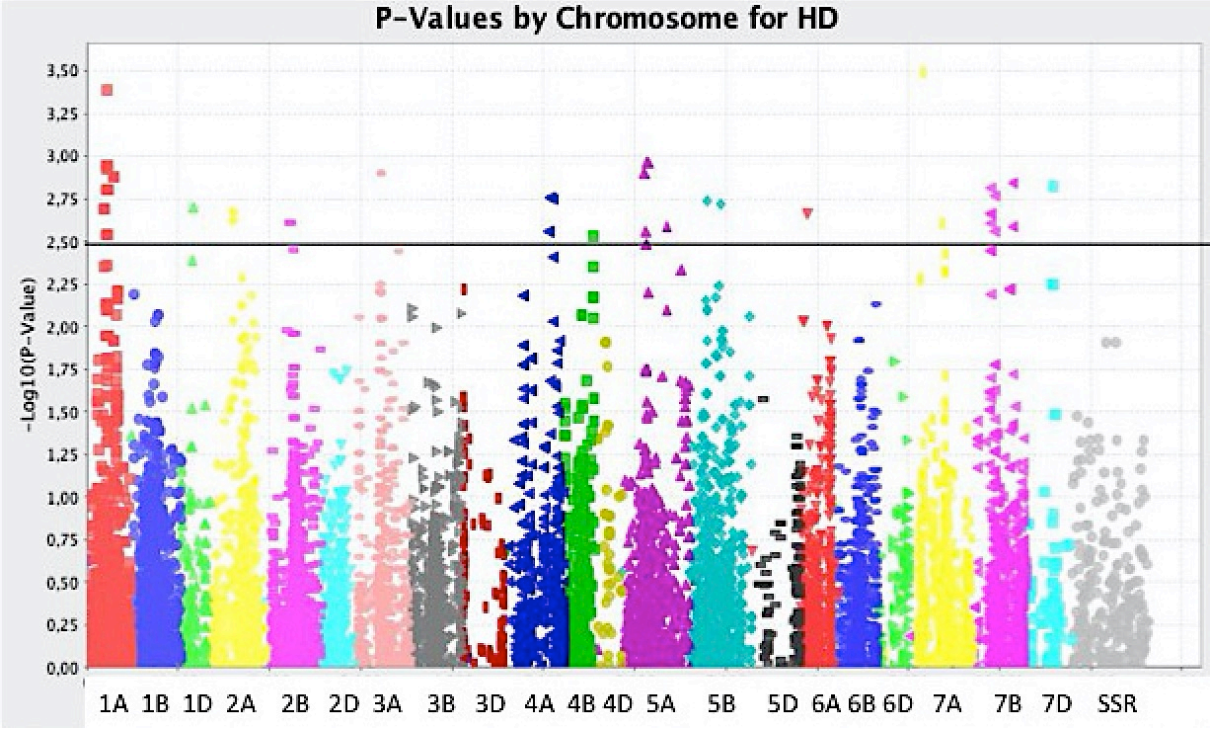


Figure 45. Manhattan plot displaying the markers for earliness (HD) in winter wheat derived from Tassel with marker positions on chromosomes and significance threshold at -log10(P-value) 2.5

The threshold for significance was set to –log10(P-value) 2.5 (P=0.002932) for HD in winter wheat containing 69 significant markers (Figure 45). A total 181 of markers were above the–log10(P-value) 2.0 (P= 0.009927). The chromosome displaying the most significant markers was 1A with 31 significant markers predominantly at positions 184, 216 and 219 cM. The most significant marker from these was wsnp_Ex_c3906_7086162 at 216 cM (P=0.000410). Clusters of significant markers were also found on chromosomes 4A, 5A and 7B. Two significant markers were also found on 7A, one of them (RAC875_c19631_269) highly significant with a P value of 0.000323. There were also significant markers on chromosomes 1D, 2A, 2B, 3A, 4B, 5B, 6A and 7D.

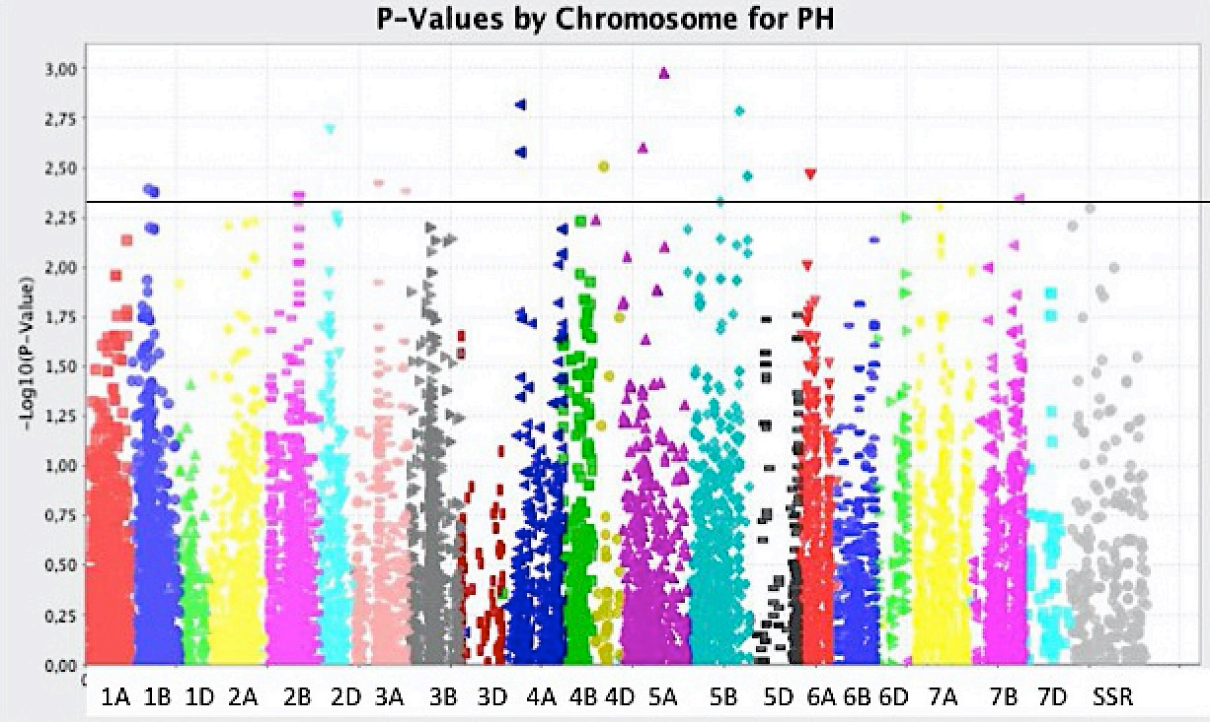


Figure 46. Manhattan plot displaying the markers for plant height (PH) in winter wheat derived from Tassel with marker positions on chromosomes and significance threshold at -log10(P-value) 2.3

For PH in winter wheat (Figure 46) the 38 significant markers had a threshold –log10(P-value) of 2.3 (P= 0.004922). A total of 97 markers had a –log10(P-value) >2.0. The KASP marker Rht-D1 at position 117 cM was the only significant marker on chromosome 4D, with a P-value of 0.003130. Furthermore, three markers were found significant on chromosome 5A, and four on 5B. The most significant markers were CAP11_c1685_149 and Kukri_c6266_260 (P=0.001054) on 5A and RFL_Contig5616_1779 (P=0.001630) on 5B.

There were five significant markers on chromosome 1B, one at position 209 and four on 276 cM. On chromosome 2B six markers came up as significant ranging from positions 363-374 cM. One marker was found on chromosome 2D and three in 3A. However, on chromosome 4A, eight markers were found clustered at position 191 cM. Chromosome 6A had four significant markers from position 131-135, and chromosome 7A had two significant markers at position 364 cM. Additionally, a single marker on chromosome 7B at position 510 cM was found to be significant. Two SSR markers were also found between the –log10(P-value) 2.0 and 2.3, barc128_218 on 5BS and gwm016_174 on unknown chromosome.

3.3 Discussion

3.3.1. Populations

The 172 MASBASIS lines grouped themselves as spring and winter wheat in 8 subpopulations. The Norwegian spring wheat, represented by 28 lines grouped into one subpopulation. Among these were many of the Norwegian cultivars, eg. Møystad, Runar, Bastian, Bjarne and Brakar. Møystad and Runar are two old varieties, released in Norway in 1966 and 1972 respectively (Lillemo & Dieseth 2011). Both Bastian and Bjarne came from these varieties, released in 1989 and 2002. Demonstrant was also found in this group, a variety developed for high yield in 2008 (Lillemo & Dieseth 2011). CIMMYT lines grouped themselves in a separate population. These lines were brought to Ås in the 1960s and has since then been subject to extensive crossing because of their poor adaptation to Norwegian conditions, susceptibility to powdery mildew and Septoria and vulnerability to pre harvest sprouting among other things (Lillemo & Dieseth 2011). However, the CIMMYT lines included in MASBASIS are newer introductions representing good sources of resistance to powdery mildew or FHB.

There is also a population consisting of primarily Chinese lines. Sumai 3 is a highly popular source of resistance to FHB infection (Buerstmayr et al. 2009). Nanjing7840 and Ning8343 are also resistant sources from this area in this population in addition to the Chinese landrace Nobeokabouzu. The Swedish spring wheat also grouped themselves in a separate population. The most important Swedish varieties have also been utilized in Norway; Tjalve (1987), Avle (1996), Vinjett (1999) and Zebra (2001) (Lillemo & Dieseth 2011).

A very strong subpopulation was defined with three lines; J03, Fram II and Norrøna. J03 is a pure line selection from a Norwegian landrace that became an important source of powdery mildew resistance at the start of modern plant breeding in Norway. By crossing with a lodging resistant club wheat line from Montana, USA, it gave rise to Fram II which was released as a Norwegian variety in 1940. Furthermore, in 1952 Norrøna was derived from a cross between Fram II and a Finnish variety Sopu for its superiority in yield and quality.

The European spring wheat was grouped with a single winter wheat line; the Russian variety Mironovskaja 808. This is an old Russian winter wheat variety, suggesting that the European

spring wheat in this subpopulation may have traces of winter wheat in their background. The Norwegian and Swedish winter wheat group together. Sigyn II and Rida are typical Norwegian varieties released in 1950 and 1976 respectively. The Swedish varieties Trond, Folke, Mjølner, Magnifik and Finans have successively been important cultivars in Norway from since the 1960s (Lillemo & Dieseth 2011). The German cultivar Olivin were also grown in Norway from 2006. This cultivar had a strong association to the subpopulation with European winter wheat. This explains how the defined population structure is as expected, and reliable for use in further analyses.

3.3.2. Association mapping of earliness and plant height

The association mapping for earliness for spring wheat discovered several significant markers on chromosomes 1A, 1B, 3B, 4A, 4B, 4D, 6A, 6B, 6D and 7A. Significant markers on these chromosomes may indicate the presence of vernalisation genes, photoperiod response genes or earliness per se genes.

Vernalisation genes regulate the sensitivities and responses to continuous cold temperatures. Usually spring and winter wheat can be differentiated based on the sensitivity or insensitivity to these periods of cold. Spring wheat is therefore expected to be insensitive to cold temperatures, initiating flowering irrespective of cold treatment (Worland & Snape 2001). The major genes for vernalisation have been found on chromosome 5A (*Vrn-A1*), 5B (*Vrn-B1*) and 5D (*Vrn-D1*) (Yan et al. 2003; Zanke et al. 2014a). Both spring and winter wheat had significant markers on chromosome 5A and 5B. Winter wheat would be expected to carry genes for vernalisation sensitivity, as flowering is dependent on a longer period of cold temperatures. According to Worland and Snape (2001), gene *Vrn3* for vernalisation sensitivity are found on chromosome group 1. For winter wheat there was a cluster of significant markers for earliness on chromosome 1A. For spring wheat there was also a cluster on 1A, however more significant markers were found clustered on 1B, especially at position 549 cM.

Another trait influencing earliness is the photoperiod response. The vernalisation genes typically satisfies the vernalisation responses thus flowering time will have little effect. Therefore, to impact flowering time in winter wheat, adjusting for photoperiod response is necessary (Worland & Snape 2001). A photoperiod sensitive variety remain vegetative until

day length increase satisfying photoperiod requirements This in turn enables the plant to promote the onset of initiation of the floral primordia (Worland & Snape 2001). For instance, the *Ppd-D1* has been found to accelerate ear emergence time by 2 to 14 days. Major genes for photoperiod response has been found on chromosomes 2A (*Ppd-A1*), 2B (*Ppd-B1*) and 2D (*Ppd-D1*) (Worland & Snape 2001; Zanke et al. 2014a). Zanke et al. (2014a) found significant markers for both *Ppd-A1* and *Ppd-B1* on chromosomes 2A and 2B respectively. This study also found significant markers on these chromosomes. However, similarly to Zanke et al. (2014a) no significant markers were found on chromosome 2D which is supposedly where *Ppd-D1* is located (2DS). Both Zanke et al. (2014a) and this study used both SNP and SSR markers. A 21cM gap between SSR markers in the genomic region where *Ppd-D1* supposedly is located was suggested to be a reason for the absence of significant markers at this chromosome. Additionally, Kollers et al. (2013) detected a significant marker-trait association for the *Ppd-D1* alleles on FHB severity.

According to Worland and Snape (2001) many of these chromosomes have genes associated with flowering time. This study found two of the most significant markers for earliness on chromosome 4B which was stated by Worland and Snape (2001) to contain *earliness per se* genes affecting flowering time (Hoogendoorn 1985). These genes are known to influence time of flowering without the impact of environmental reactions like day length and temperature. Muira and Worland (1994) also found *earliness per se* genes on chromosomes 3A (Faricelli et al. 2010; Gawronski & Schnurbusch 2012) and 3B. No particularly significant markers were found for chromosome 3A in this study. However, chromosome 4A, 6B and 6D had significant markers which were also discovered to include *earliness per se* genes (Hoogendoorn 1985). Additionally, *earliness per se* effects have been discovered on chromosome 7B (Hoogendoorn 1985). For this study, 7A displayed more significant markers than 7B, although both chromosomes displayed markers of significance.

The association mapping for plant height gave particularly significant clusters of markers on chromosome 4B and 6B. Lu et al. (2013) found QTL for PH on several chromosomes. These included chromosomes 1B, 2D, 4A, 4B, 5A and 6A. Dwarfing genes *Rht-B1b* and *Rht-D1b* have been found to affect plant height. Gosman et al. (2007) found the *Rht-D1b* gene in all the studied UK winter wheat lines at chromosome 4D. This is consistent with this study where the KASP marker *Rht-D1* came up as significant (P=0.003) for plant height in the winter wheat lines. The *Rht-B1* marker was not found significant for winter wheat. However, for the spring

wheat lines, the KASP marker Rht-B1 on chromosome 4B was found highly significant (P=0.000156). Furthermore, Rht-D1 was not found significant for the spring wheat lines.

Additionally the *Rht-B1b* and *Rht-D1b* genes have been discovered to coincide with major QTL for FHB susceptibility (Lu et al. 2013; Srinivasachary et al. 2008; Srinivasachary et al. 2009). The *Rht-D1b* allele is favourable related to improved yield and less lodging in modern agriculture making this a major challenge (Lu et al. 2011). The study by Lu et al. (2011) found that combining two of the strongest QTL for FHB resistance *Fhb1* and QTL on 5A was just enough to balance out the negative effect of *Rht-D1b*. Four significant markers were found on 5A suggesting that these markers could be located around the significant QTL for PH on 5A found by Lu et al. (2013)

A significant SSR marker for PH in spring wheat (DuPw167_259) were found on chromosome 6AL on an unknown position. A possible position for this SSR may be where two significant SNPs were found at 338 cM. The same chromosome was found to contain QTL for PH in the study by Lu et al. (2013) suggesting a QTL for PH at this position. Association mapping for earliness and plant height have proven to give significant QTL corresponding to literature. By correcting for earliness and plant height, underlying QTL directly responsible for *Fusarium* resistance can be identified through association mapping.

Chapter 4:
Major genes for *Fusarium* resistance in spring and winter wheat

4.1. Results

The mixed linear model in Tassel gave significant QTL for AE, FHB and DON in both spring and winter wheat. Significant markers for AE are presented from 2014, while FHB and DON results are from 2013. Lists of significant markers are attached in Appendix 3.

The association mapping gave 35 significant QTL for AE from 2014 in spring wheat (Figure 47) at the significance threshold of –log10(P-value) of 2.5 (P= 0.003135) and 156 above the –log10(P-value) of 2.0 (P=0.009911). A single significant marker was found on position 182 cM on chromosome 1A (RAC875_c14066_452). Chromosome 1B had 10 significant markers on position 215 and 216 cM most of which with a P-value of 0.000686. Three markers were found on chromosome 2A position 355, 386 and 413 cM. On chromosome 2B there were two markers (Excalibur_c7964_1290 and Tdurum_contig57254_254) on position 458 with a P-value of 0.000466.

Chromosome 3A and 3B and 4A had two significant markers each. Three markers were also found on chromosome 5A at position 267 cM. A single highly significant marker on chromosome 5B (P=0.000780) was found at position 88 cM. Additionally, two markers were found on chromosome 6A, one on 6D and three on 7D. Furthermore, 3 SSR markers (barc125_170, barc40_233 and gwm320_275) were found on chromosomes 3D, 5A and 2DL respectively. Especially markers on chromosome 1B, 2B, 4A, 5B and 6A were consistent over years.

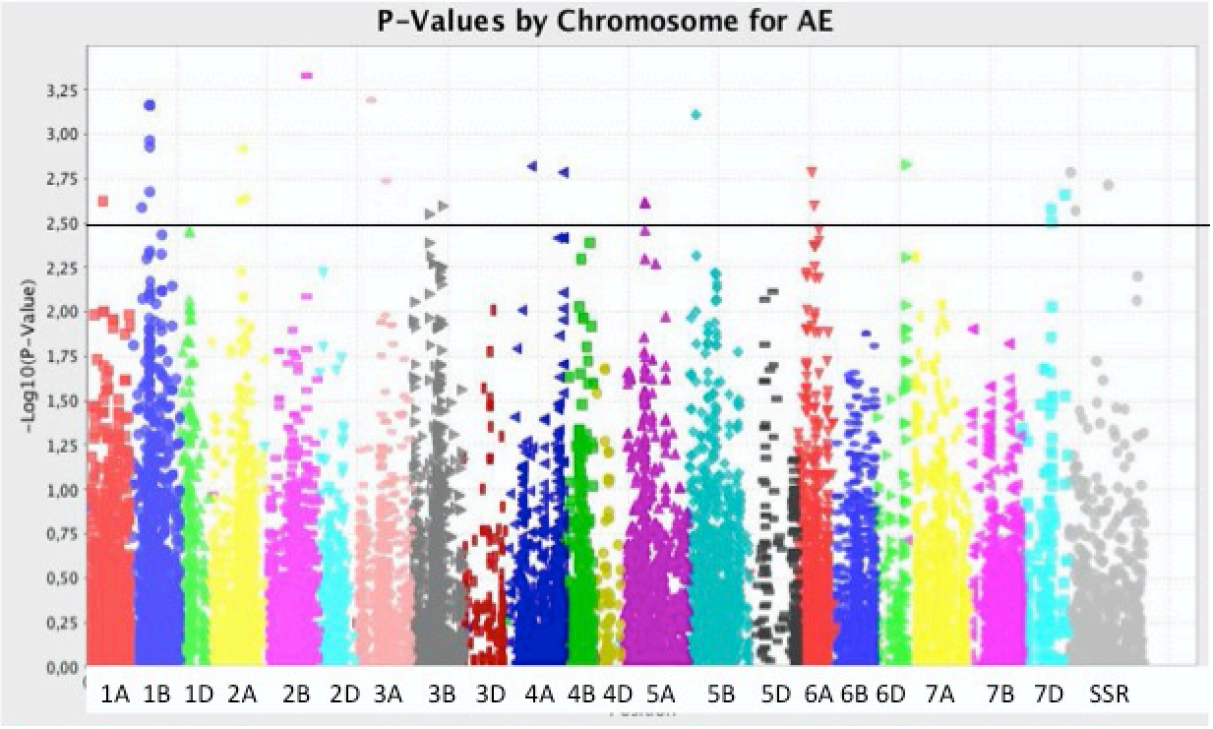


Figure 47. Manhattan plot displaying the markers for anther extrusion (AE) in spring wheat derived from Tassel with marker positions on chromosomes and significance threshold at -log10(P-value) 2.5

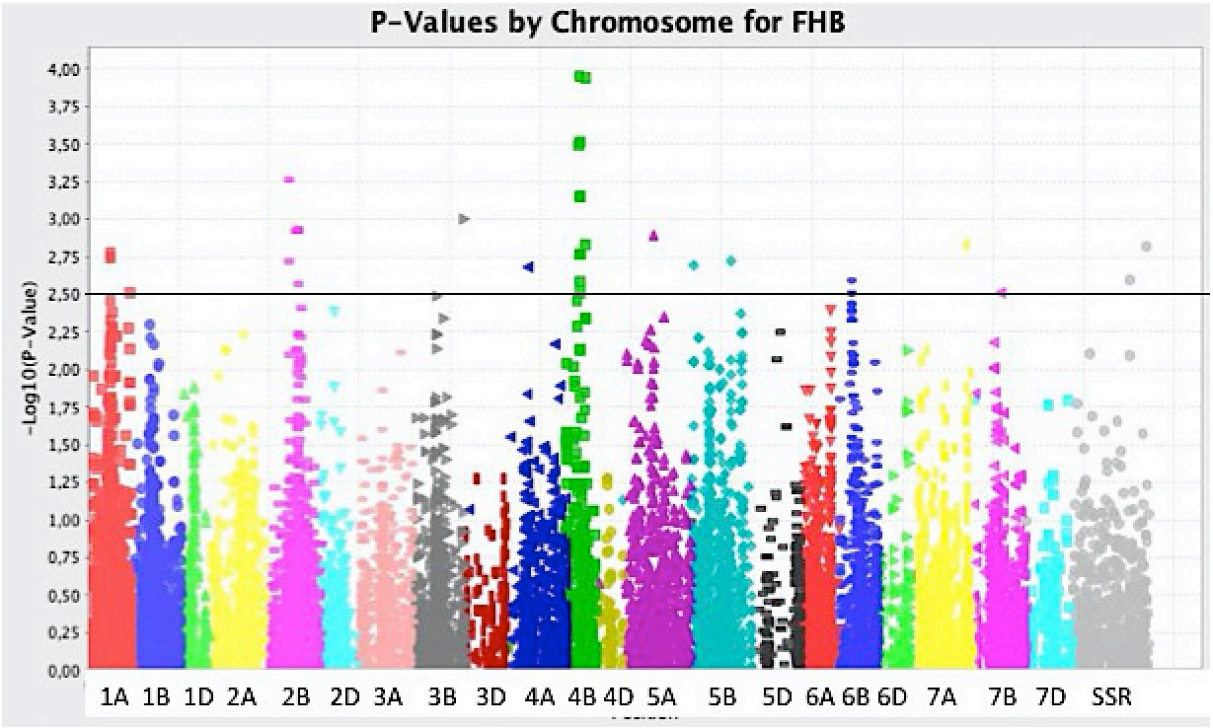


Figure 48. Manhattan plot displaying the markers for *Fusarium* head blight (FHB) in spring wheat derived from Tassel with marker positions on chromosomes and significance threshold at -log10(P-value) 2.5

Figure 48 displays the significant QTL for FHB for 2013 in spring wheat. The significant markers from 2013 were used instead of 2014 because of lack of significant differences between the lines. A total of 191 markers were found to be over the –log10(P-value) of 2.0 (P= 0.009920) and 39 over the significance threshold of 2.5 (P= 0.003122). The stand-out significant markers for FHB were found on chromosome 4B. 12 highly significant markers were found clustered, ranging from position 148 to 223 cM. The KASP marker Rht-B1 was found at position 159 cM with a P-value of 0.000113. There was also another highly significant SNP marker at this position (RAC875_c27536_611). Additionally two SSR markers, mag548a_null and wmc559_274, were located on chromosomes 2BL and 3A respectively.

Four significant markers were located on chromosome 1A, three of these around position 240 cM. 11 markers were found on chromosome 2B, the most significant one at position 245 cM with a P-value of 0.000549 (RAC875_c26469_480). Two single markers were found on chromosome 3B and 4A. Excalibur_c766_705, was located on chromosome 3B at position 558 cM (P=0.000998), and wsnp_Ex_c48449_53350799 at position 228 cM on 4A. One marker was located on chromosome 5A and two on 5B. Furthermore, three markers were found on position 133 cM and 134 cM on chromosome 6B, and a single marker on chromosome 7B. The results were consistent with the significant markers over years, especially for 4B and 6B.

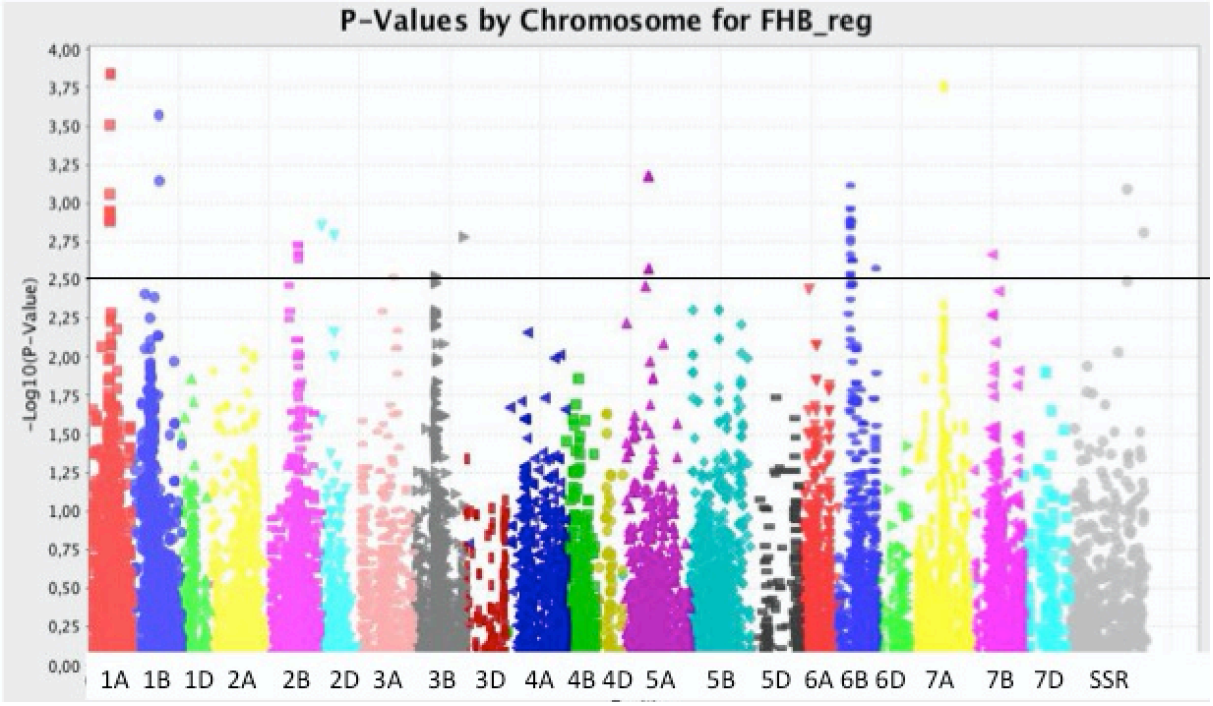


Figure 49. Manhattan plot displaying the markers for *Fusarium* head blight after regression (FHBreg) in spring wheat derived from Tassel with marker positions on chromosomes and significance threshold at -log10(P-value) 2.5

The association mapping of *Fusarium* based on regression gave different results (Figure 49). There were 68 significant markers from year 2013 above the –log10(P-value) threshold of 2.5 (P= 0.003041), and 190 above the –log10(P-value) threshold of 2.0 (P= 0.009991). Again, marker data from 2013 was chosen because of the 2014 result not giving any significant differences The biggest difference between FHB and FHBreg is the effect of *Rht-B1* disappearing, which is also consistent when comparing these results to the results over years. The stand-out significance of markers for this analysis were located on chromosome 6B. A total of 31 markers were found to be significant, 25 of these on position 133-135 cM. Five markers were located at positions 146 and 149 cM and one at 416 cM. The most significant of these were Excalibur_c46399_307 with a P-value of 0.000767. No significant markers were found on chromosome 4B, compared to the association mapping of FHB without the correction for DH and PH. Furthermore, two significant markers were found at position 398 chromosome 7A (P=0.000177). Additionally, two SSR markers were found to be significant (mag548a_null on 2BL and wmc559_274 on 3A), the most significant of these mag548a_null with P-value 0.000815.

Chromosome 1A had 12 significant markers from position 230 to 241 cM. The four most significant were located at 230 cM (BS00063068_51), 235 cM (IAAV213) and 241 cM (wsnp_Ra_c16080_24638622 and RAC875_c38916_66). Two highly significant markers were also found on chromosome 1B (BS00110278_51 and Excalibur_c94658_59) at positions 294 and 301 cM both with a –log (P-value) >3.0. Six significant markers were located on chromosome 2B around position 350 cM, two markers on chromosome 2D, one on 3A and one on 7B. Furthermore, four significant markers were found on chromosome 3B, three around position 250 and one at 558 cM. For this analysis, five markers of significance was located on chromosome 5A from position 279-291 cM.

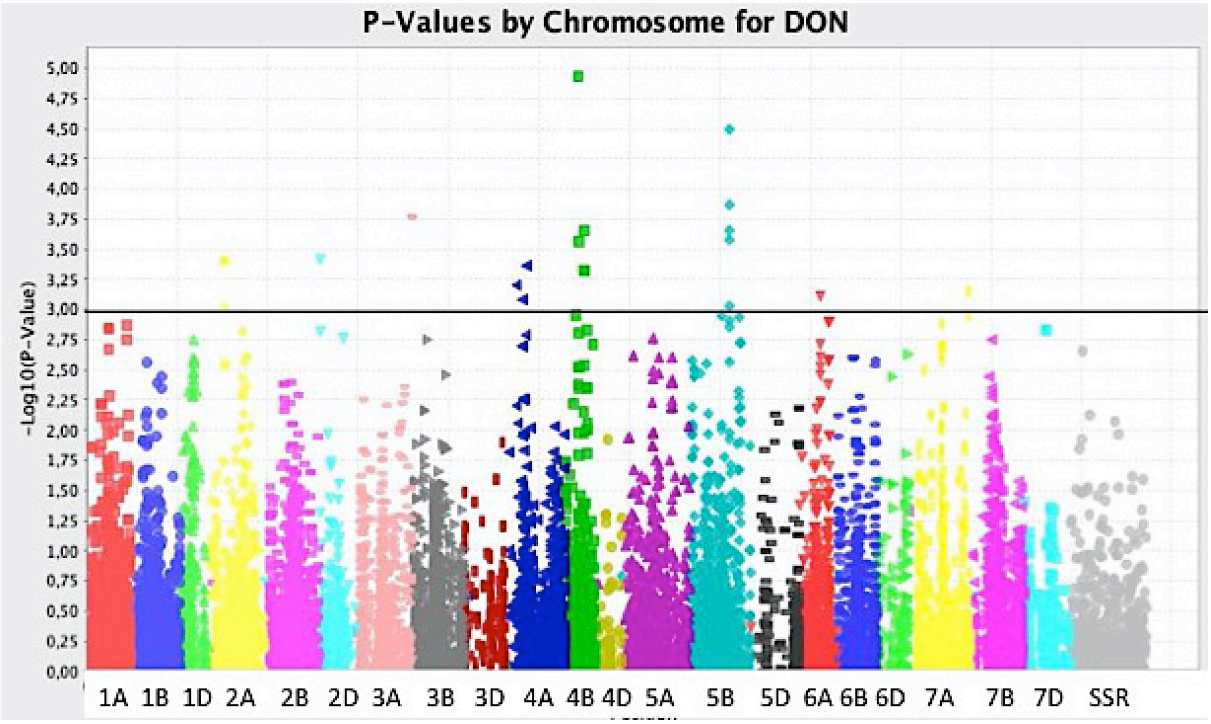


Figure 50. Manhattan plot displaying the markers for deoxynivalenol (DON) in spring wheat derived from Tassel with marker positions on chromosomes and significance threshold at -log10(P-value) 3.0

The significance level for DON was set to –log10(P-value) >3.0 (P= 0.000970) which included 25 markers from 2013 (Figure 50). A total of 305 markers was above the –log10(P-value) of 2.0 (P= 0.009993). Compared to the association mapping of FHB (Figure 48), chromosome 4B is also here a stand-out in the case of significant markers. Five markers were located from 159-230 cM, one of these being the Rht-B1 KASP marker at 159 cM (P= 0.000012). Chromosome 5B also had five highly significant markers, these being clustered

around position 447 and 448 cM, the most significant of these being BS00078784_51 at P=0.000032.

There were seven significant markers on chromosome 2A, all located on position 173 cM. A single significant marker was also found on chromosome 2D (Excalibur_rep_c109101_94). Furthermore, two significant markers were found on chromosome 3A, one at position 604 (RAC875_rep_c109554_198) and one at 617 cM (Excalibur_c17654_166). On chromosome 4A, three significant markers were found, at positions 116, 185 and 228 respectively. Furthermore, significant markers were also found on chromosome 6A and 7A.

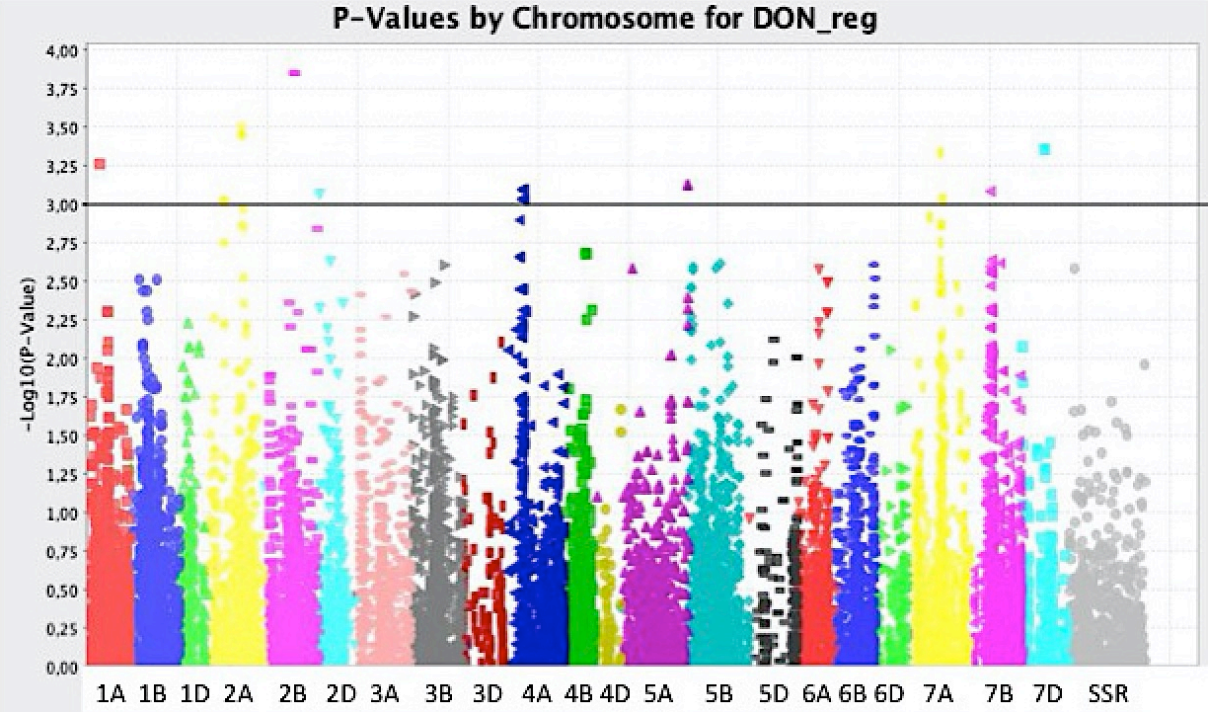


Figure 51. Manhattan plot displaying the markers for deoxynivalenol after regression (DONreg) in spring wheat derived from Tassel with marker positions on chromosomes and significance threshold at -log10(P-value) 3.0

Again, the association mapping of DON based on regression gave different results (Figure 51). The chosen significance threshold at –log10(P-value) of 3.0 (P=0. 0.000939) held 38 markers from 2013. Compared to the association mapping for DON there were more significant markers for DON after correction for DH and PH, with the effect of *Rht-D1* being greatly reduced. A total of 254 markers were above the –log10(P-value) of 2.0 (P= 0.009844). On chromosome 2B, the markers of highest significance for DON were found at position 330 cM (P=0.000140). One marker on chromosome 1A (RAC875_c37934_285 position 149 cM)

was also particularly significant at P=0.000547. Next, 11 markers were found on chromosome 2A clustered at position 173 366 and 368 cM. Position 200 and 203 cM on chromosome 4A contained a cluster of significant markers with P-values 0.000808 and 0.000924. Furthermore, two significant markers were found on chromosome 5A on position 737 cM, three markers at positions 371 and 398 cM at chromosome 7A and one marker (Ex_c68356_553) at position 216 on 7B. Additionally five markers clustered at position 249 on chromosome 7D were found to be significant.

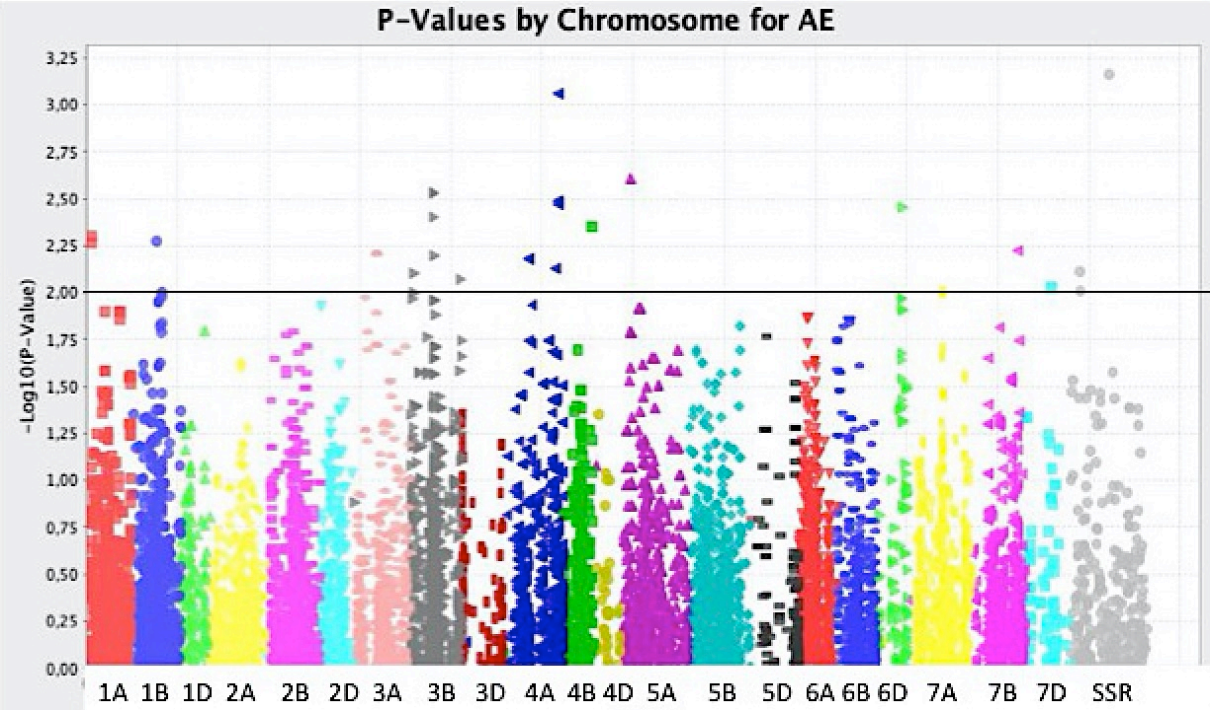


Figure 52. Manhattan plot displaying the markers for anther extrusion (AE) in winter wheat derived from Tassel with marker positions on chromosomes and significance threshold at -log10(P-value) 2.0

A total of 32 markers were found significant above the –log10(P-value) threshold of 2.0 (P=0.009959) for AE in winter wheat from 2014 (Figure 52). Three SSR markers were found to be significant, especially gwm301_239 on 2DL with a P-value of 0.000691. The other two markers (cfd018b_207, P=0.007740 and cfd018b_215, 0.009858) were located on 5D. Significant markers were found on chromosome 1A. Two markers were positioned at 51 cM. There were also two significant markers on chromosome 1B, however at position 287 and 344 respectively. Furthermore, six significant markers were found in a cluster at 246-247 cM on chromosome 3A. Clusters of markers were also present at chromosome 3B, mostly around

270 cM, and chromosome 4A. There were also single markers of significance on chromosome 4B, 5A, 6D and 7B.

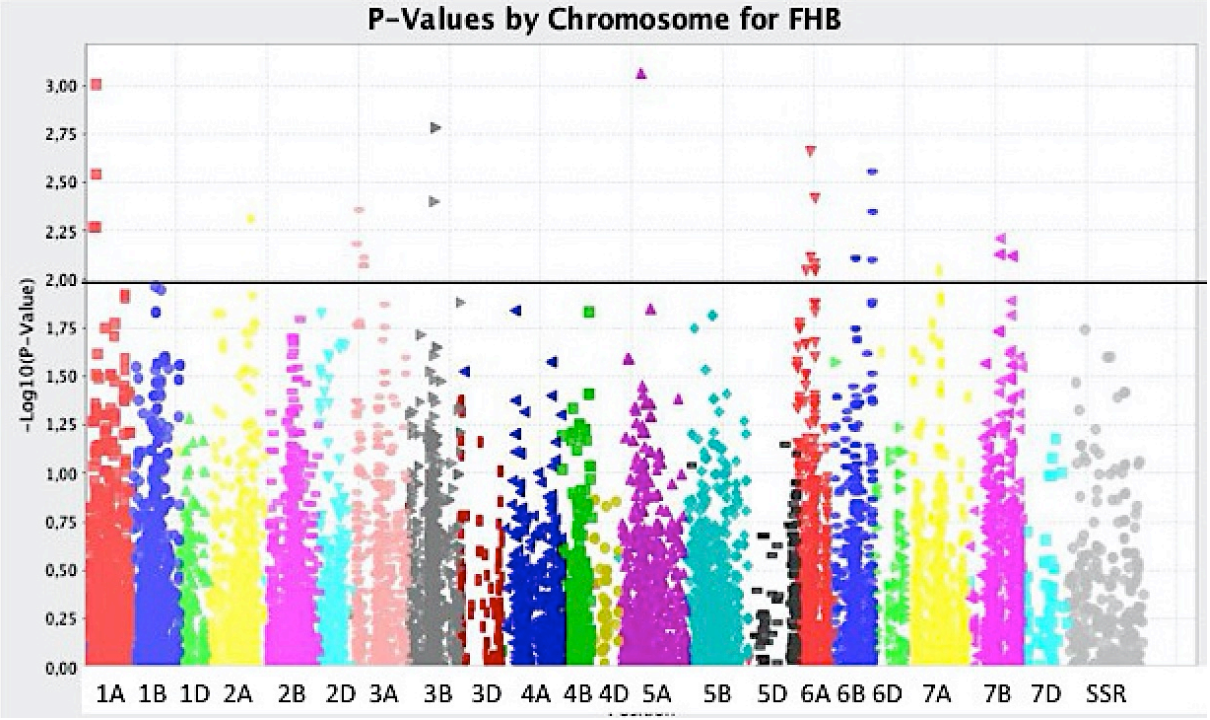


Figure 53. Manhattan plot displaying the markers for *Fusarium* head blight (FHB) in winter wheat derived from Tassel with marker positions on chromosomes and significance threshold at -log10(P-value) 2.0

Markers for FHB in winter wheat from 2014 (Figure 53) were considered significant above the –log10(P-value) of 2.0 (P= 0.009086), counting a total of 38 significant markers. Four markers were found to be significant on chromosome 1A. Two of these had position 117 cM where one had a very high P-value (Kukri_c2121_1334, P=0.000980). A single significant marker was located at chromosome 2A. Nine markers were found to be significant on chromosome 3A where most of these were located at 122 cM. Position 308 on chromosome 3B had two significant markers. A single stand-out marker (Tdurum_contig43874_1129) was found at position 245 cM on chromosome 5A. Next, seven significant markers were found on chromosome 6A, most at position 204 cM, and six markers at chromosome 6B. One marker was found significant on chromosome 7A, whereas six were found significant on chromosome 7B (positions 323, 330, 337 and 464 cM).

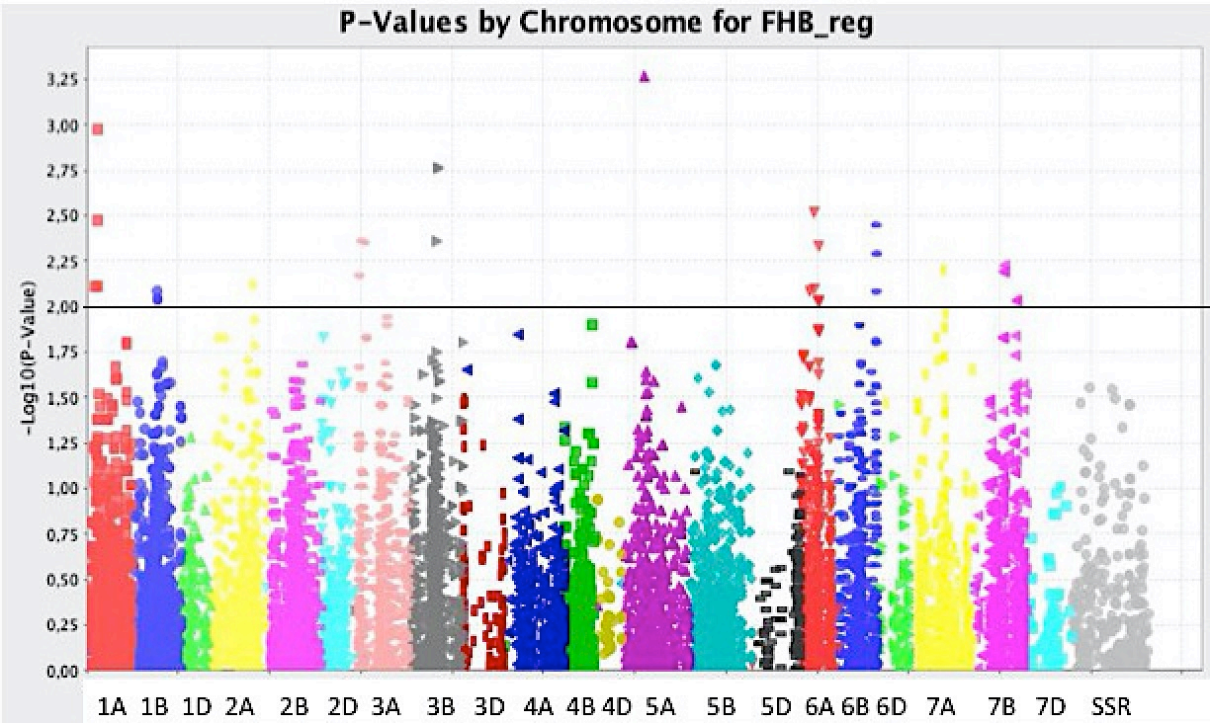


Figure 54. Manhattan plot displaying the markers for *Fusarium* head blight after regression (FHBreg) in winter wheat derived from Tassel with marker positions on chromosomes and significance threshold at -log10(P-value) 2.0

The results for FHBreg (Figure 54) in winter wheat from 2014 were very similar to the association mapping of FHB before regression. The significance threshold was similar to FHB set to –log10(P-value) of 2.0 (P= 0.009522) with a total of 33 markers of significance. Markers on chromosome 1A at position 117 cM was found for both FHB and FHBreg. However, for FHBreg significant markers were present at 1B, all of them around at position 287-289 cM. One marker was found significant on chromosome 2A. Chromosome 3A and 3B both had three markers of significance. However, one new marker had appeared as significance at chromosome 3B (position 286 cM). The same marker was also found at 245 cM on 5A. Furthermore, the same seven markers were also found on chromosome 6B, whereas there were fewer markers for FHBreg on chromosome 6B than for FHB. Additionally, the same markers were found for chromosome 7A and 7B.

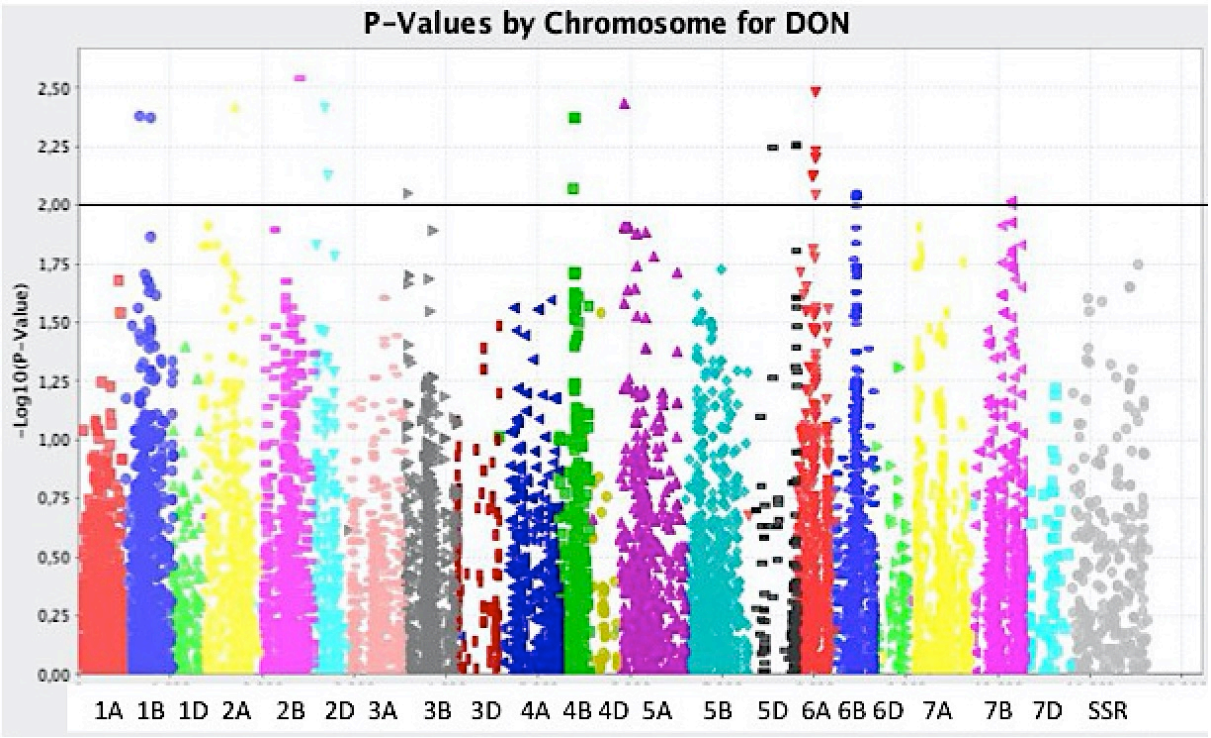


Figure 55. Manhattan plot displaying the markers for deoxynivalenol (DON) in winter wheat derived from Tassel with marker positions on chromosomes and significance threshold at -log10(P-value) 2.0

The level of significance was for DON from 2014 in winter wheat was set to –log10(P-value) 2.0 (P= 0.009920) displayed on Figure 55. A total of 60 markers were found to be significant. Two significant markers on chromosome 1B (BS00066014_51 at 185 cM (P=0.004154) and Tdurum_contig10362_328 at 304 cM (P=0.004235), one on 2A (Ku_c33021_109 at 355 cM (P=0.003892)), one on 2B (CAP12_rep_c3989_239 431 cM (P=0.002869)) and two on 2D (wsnp_BE488779D_Ta_1_2 at 110 cM and BS00011109_51 at 145 cM). The majority of significant markers were found on chromosome 6A. A total of 30 markers were found; 10 on position 172 cM, 10 at 173 cM, and 10 at 204 cM. Additionally there were nine significant markers on chromosome 6B at positions 218 and 226 cM. A single significant marker was also found on chromosome 3B at position 43 cM. Furthermore, six significant markers were found at positions 159, 180 and 183 cM on chromosome 4B. There was also a single significant marker on chromosome 5A at position 79 cM. Finally, three significant markers were located at 427 cM on chromosome 7B.

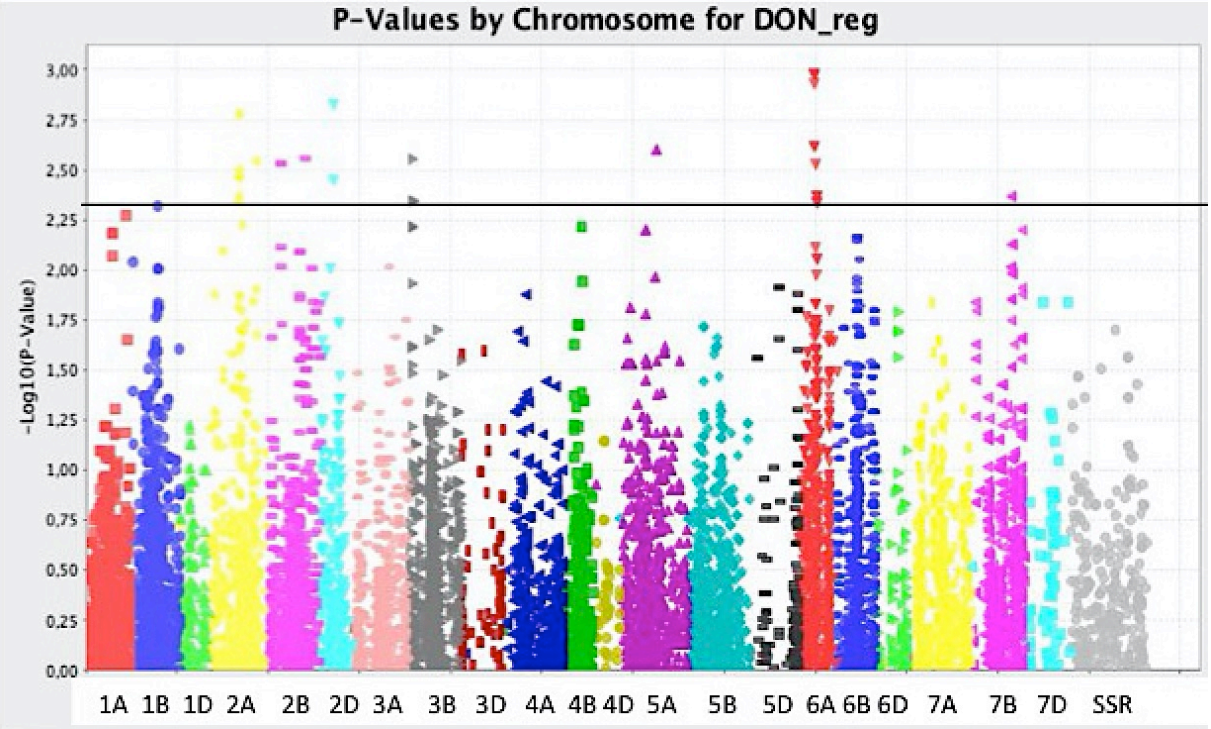


Figure 56. Manhattan plot displaying the markers for deoxynivalenol after regression (DONreg) in winter wheat derived from Tassel with marker positions on chromosomes and significance threshold at -log10(P-value) 2.3

Significance threshold was set to –log10(P-value) of 2.3 (P= 0.004823) for DONreg in winter wheat (Figure 56). The results for DONreg gave 62 significant markers, 98 above the –log10(P-value) of 2.0 (P=0.009920).). The same 30 markers were found at chromosome 6A as for DON, however a new marker was located at position 190 cM (BS00066047_51). One significant marker was found on chromosome 1B Tdurum_contig10362_328 at 304 cM. However, whereas for DON there were only one significant marker on chromosome 2A, the results from DONreg located 19 significant markers (position 329 and 332 cM).

Additionally, three markers were found on chromosome 2B (compared to one for DON). The same markers were found at chromosome 2D for DONreg as for DON, however three new markers with new positions (45 and 56 cM) were found on chromosome 3B. Another significant marker was also found on chromosome 5A, now on position 400 cM (BS00065292_51). Furthermore, two significant markers were found on chromosome 7B (427 cM).

4.2. Discussion

4.2.1. Association mapping of anther extrusion

"Anther extrusion has early on been suggested as one of the resistance mechanisms for FHB as infection occurs via anthers " (Skinnes et al. 2010). However, anther extrusion as a trait has not been as thoroughly investigated. Low temperatures and humidity has been discovered to promote anther extrusion, whereas high temperatures and drought decrease the onset (Devries 1971). Additionally Devries (1971) discovered that there is less than a one hour duration of flowering. A more recent study by Gilsinger et al. (2004) associated open flowering to FHB and found evidence for narrow flowering preventing entry of spores and infection by *F. graminearum.*

Skinnes et al. (2010) found associations of five genomic regions related to anther extrusion on chromosomes 1A, 1B, 4D, 6A and 7A from the cross between the winter wheat Arina and spring wheat NK93604. The QTL on 1A was found to be the most important, and 6A the second, from Skinnes et al. (2010). For this study, a single significant marker was found on 1A and two on 6A. However a cluster of 10 significant markers were found on chromosome 1B indicated that there is a QTL for anther extrusion on this chromosome. Three significant SSR markers were also found on 3D, 5A and 2DL.

Additionally, studies by Sage and Isturiz (1974) and Singh et al. (2007) found positive correlations between lodicule size, anther extrusion and percentage of opening florets, and duration of flower opening. Nair et al. (2010) have successfully isolated the gene controlling cleistogamy (*Cly1*) in barley. Orthologous genes (*TaAP2*) have also recently been discovered on the telomeric ends of chromosome 2AL, 2BL and 2DL in wheat (Ning et al. 2013). From the association mapping of AE in winter wheat, the SSR marker gwm301_239 was significant at the same position as *TaAP2-D* on chromosome 2DL. It is also not unlikely that the significant markers on chromosome 2A and 2B can be caused by allelic variations of *TaAP2-A* and *TaAP2-B*.

According to Devries (1971) anthers may be retained because of the short opening duration and narrow angle between lemma and palea, especially in varieties with compact spike types. Therefore, determining a suitable scale for this trait is important as well as the observed differences in flowering time between both spikes and tillers as proposed by Skinnes et al.

(2010) should be used for scoring. Furthermore, it should also be determined beforehand how often an experiment with genetic variation should be scored. For this study, a scale of 1-9 was used to determine the degree of anther extrusion. However, difficulties arose at flowering time due to weather conditions in addition to the visual difficulties of scoring in the field.

The phenotypic results indicated that high AE is associated with low FHB. These traits were scored in separate fields makes phenotypic correlations poorer than if scored in the same trial. However, correlations between parameters scored in different fields with different environmental conditions have a higher probability of variations being due to genetics. This is especially positive if AE is to be used as an indirect selection method for FHB resistance. Therefore, as it is suggested by Skinnes et al. (2008), although QTL analysis showed that some genetic factors coincided for high AE with low FHB/DON and others did not, there is still possibilities for using AE as an indirect criterion for selection for FHB and DON.

4.2.2. Association mapping of *Fusarium* head blight and mycotoxin deoxynivalenol

The results from the association mapping of both FHBreg and DONreg showed that many SNPs significant for FHB/DON were no longer significant once corrected for earliness and plant height. This was particularly noted for chromosome 4B and 5B, with the KASP marker Rht-B1 present on 4B in the association analysis of FHB and not in FHBreg. This is consistent with the QTL Lu et al. (2013) found for plant height. The chromosome was also found in this study to contain significant SNPs for earliness. Hoogendoorn (1985) discovered *earliness per se* genes suggested to promote earliness (Worland & Snape 2001). Additionally chromosome 5B is known to hold one of the major vernalisation genes *vrn-B1* (Worland & Snape 2001).

DON and DONreg (mycotoxin accumulation) were chosen as the primary results for this analysis because the grain quality is of highest interest in breeding. The primary goal of breeders is to reducing mycotoxin levels in the grains for consumers. Additionally, Lu et al. (2013) stated the importance of including different resistance parameters beyond FHB severity, eg. DON content, as these are under different genetic control. DON is more related to type III resistance than type I and II. Additionally, FHB and AE have been proven difficult to score, thus having lower heritabilities for lines than earliness and plant height. It would be expected that DON would give higher heritabilities due to the fact that it is analytically

scored. However, lower heritabilities are also discovered for DON because it is affected by environment.

The Significant markers in this study for FHB/DON still present in FHBreg/DONreg are most likely markers positioned around QTL for FHB/DON. For FHB and FHBreg in spring wheat the same markers were found on 1A position 241 cM, 2B position 350, 3B position 558, 6B on 133 and 134 cM and two SSR markers on 2BL and 3A, suggesting that there are QTL here for FHB. Lu et al. (2013) located QTL for FHB on 1A and 2BL (Lu et al. 2011), whereas Liu et al. (2009) described QTL for FHB on chromosomes 3BS and 6B. Furthermore, QTL have been found on chromosome 3A (Liu et al. 2009; Lu et al. 2011) suggesting the possibility of a location for a QTL for FHB. Six significant markers on 2A were found for both DON and DONreg at position 173 cM, and one on 2D (6 cM), both chromosomes reported by Lu et al. (2013) to hold QTL for DON.

For winter wheat, most significant markers found for FHB were also found for FHBreg. However, chromosome 1B had significant markers for FHBreg which were absent in the association mapping for FHB. This chromosome was found by Holzapfel et al. (2008) to contain QTL for FHB resistance. The study by Narasimhamoorthy et al. (2006) found QTL for FHB on chromosome 3B, which was also found for this study. Furthermore, significant markers were positioned at some of the chromosomes Miedaner et al. (2011) found to contain QTL for FHB resistance, these being 1B, 3A and 7A. Most of the significant markers for DON were also found for DONreg. Many markers were positioned around 172 cM on 6A suggesting that there is a QTL for DON around this position.

Furthermore, some genes are controlled by both FHB and DON as illustrated by Lu et al. (2013). If significant markers for FHB and DON are found around the same position, there is likely a gene on that position controlling both. Significant markers were found on 2B (position 330-350 cM), 7A (398 cM) and 7B (216 cM) for FHBreg and DONreg indicating overlapping QTL in these regions for spring wheat. Lu et al. (2013) found overlapping QTL for chromosome 2B and 7A suggesting that there might be genes in this area controlling both FHB and DON. For winter wheat there are significant markers for both FHBreg and DONreg at 204 cM on 6A. Additionally there are significant markers on 7B around 464 cM for FHBreg and 427 cM for DONreg suggesting that there might be a QTL for FHB and DON at this chromosome.

Skinnes et al. (2010) and Lu et al. (2013) found major QTL on 1A for these three traits suggesting that there are QTL for these traits around the position of the significant markers. This chromosome was found to hold significant maarkers for AE, FHB and DON. The most significant markers were found for FHBreg at 241 cM (P=0.000146). AE and FHBreg was also found to have significant markers on chromosome 1B which in the study by Skinnes et al. (2010) had overlapping QTL. Similarly 7A was found to hold QTL for FHB and AE which is consistent with the significant markers found in this study. As described, high AE seems to be associated with low infection rate and thus type I resistance. However, also genotypes with high anther extrusion get infected if the infectious pressure is very high (Skinnes et al. 2010) suggesting that breeding for lines with high anther extrusion may be one of many components that need to be combined in order to achieve a high level of FHB resistance. Additionally, FHB and DON can be controlled by different genes. Therefore, QTL for FHB and DON are not necessarily always at the same positions or chromosomes.

The *Fhb1* gene has been reportedly located on chromosome 3BS in studies of Sumai 3 (Cuthbert et al. 2006) and later between inbred lines of Ning 7840/Clark BC7F7 (Bernardo et al. 2011) affecting both type II resistance and DON content where the infection is unable to spread (Gunnaiah et al. 2012). Gunnaiah et al. (2012) has recently discovered that this has something to do with cell wall apposition due to the involvement of hydroxycinnamic acid amides, flavonoids and lignin monomers in the formation of the cell wall. It is expected that the *Fhb1* gene will show an effect on FHB and DON. No effect on this location of the chromosome 3BS was found in this study. A reason for this may be that from the 172 MASBASIS lines, only seven lines are known to hold *Fhb1*; CJ9306, Sumai 3(18.), Nobeokabouzu, Nanjing 7840, Ning 8343, Sumai 3(12SRSN) and Sabin. From these seven, six lines are grouped in subpopulation 5, the seventh line being Sabin from subpopulation 1. MLM removes the effect of *Fhb1* as it corrects for population structure, and thus will not show up as significant for the association mapping.

Some of the significant SSR markers found in this study may be positioned close to significant SNPs. Especially interesting is the SSR marker mag548a_null which shows significance to FHB in spring wheat. It is positioned on chromosome 2BL at 350 cM where there is also a cluster of 5 significant SNPs. A QTL for FHB has been found on this chromosome (Lu et al. 2011) indicating a gene for FHB being positioned on the long arm of chromosome 2B. SSR marker barc40_233 were discovered to be positioned on chromosome

5A for AE in spring wheat with an unknown chromosomal position. Three significant SNPs were found on this chromosome at 267 cM. The SSR marker could be positioned around the SNP markers suggesting the possibility of a gene for AE on this chromosomal position. QTL for AE on this chromosome was found by Skinnes et al. (2010).

Chapter 5:
General discussion and recommendations

5.1. General discussion

There are limitations with association mapping. Marker density on certain chromosomes were very poor in this study. The genes *Rht-B1b* and *Rht-D1b* for instance did not show up on the SNP chip hence the need to include previously screened KASP markers. Markers should also be polymorphic with a best case scenario of a 50/50 distribution of allele frequencies. If an important gene is fixed in a population, it will not give an effect. Würschum et al. (2013) did a similar study as this in winter wheat with the 9K Illumina Infinitum SNP array. After removing low quality markers which were unscorable, monomorphic, showed a high degree of heterozygosity or had a minor allele frequency <5%, only 1 395 (out of 8 630) markers were left for association mapping. Further studies including more SNPs have been recommended by Würschum et al. (2013) and (Langer et al. 2014). Many markers for this study also had to be removed due to monomorphic markers, high heterozygosity and minor allele frequencies, even though the 90K iSelect wheat genotype assay was used. The analysis of linkage disequilibrium has been suggested to be important for association mapping as it detects associations between QTL and the trait indirectly (Würschum et al. 2013). However, because of the inbreeding in wheat, homozygosity is retained making recombinations ineffective thus reducing linkage disequilibrium (Würschum et al. 2013). Furthermore, the correction for population structure and kinship could remove the effect of significant markers, explained by the effect of the *Fhb1* gene disappearing after the correction for population structure in the mixed linear model.

Heritabilities of traits give an indication of how much of the variation in the phenotypes are due to genetic factors, and how much is due to other conditions, eg. differences environmental conditions and evaluators. Only under the exact same conditions can the heritability be compared. However, the calculated heritabilities prove that there are significant variations in the observed phenotypic traits DH, PH and DON. For FHB the effect of rep was highly significant which is explained from different evaluators scoring individual reps. The heritability of FHB was also very low, a reason being the lower infectious pressure of the 2014 field trial. This was also confirmed by there being no correlation between FHB and DON for 2014, but high correlation over years. AE has also been suggested to be difficult

scoring because of the short flowering time (Devries 1971). This was confirmed during the fieldwork of 2014 which may be a reason for the variations of this particular trait.

An expected population structure of MASBASIS with eight subpopulations was used further in association mapping for all traits, giving significant marker-trait associations for previously confirmed QTL. The significant markers for DH were positioned on chromosomes where genes for earliness, vernalisation and photoperiod response had been reported, suggesting that these genes are present in the MASBASIS population. MASBASIS were also found to include semi-dwarf genes with the KASP markers Rht-B1 and Rht-D1 being highly significant from the association mapping of PH. When correcting FHB and DON for DH and PH, the significant markers found in DH and PH disappeared leaving significant markers of possible association to *Fusarium* resistance. The *Fhb1* gene would be expected to have significant markers for FHB and DON on chromosome 3BS. However, no such significance were found due to population structure correcting for this effect in the GWAS. Overlapping QTL have also been described, where there are genes controlling multiple traits. The results gave good correspondence with the study by Lu et al. (2013) describing overlapping QTL on chromosome 2B and 7A for FHB and DON.

Several similar studies have been conducted with intention of improving breeding and increase yield and quality. The results from association studies have already been incorporated into Norwegian breeding programmes through Graminor from the material for FHB and DON from Arina and NK93604 (Semagn et al. 2007) and the association with AE from the same two lines (Skinnes et al. 2010). Results from the association study of AE and PH for SHA7/CBRD x Naxos by Lu et al. (2013) are also incorporated. Additionally, the study described in this thesis is also part of an on-going project to improve Norwegian wheat production.

5.2. Recommendations

Genome-wide association mapping have proved that it is possible to identify significant marker-trait associations which can be linked to confirmed QTL. However, there are limitations to the association mapping. More work should be done in validating markers for the optimal marker-trait associations. Therefore, further analyses are recommended to identify which lines have the genes for FHB resistance so it can be effectively utilized in breeding programmes.

At least one more year of testing should be performed for successful QTL mapping. Two independent experiments (locations or years) are necessary for estimation of repeatability of calculated heritabilities and correlations between experiments as suggested by Buerstmayr et al. (2009). In addition, association mapping results are, similarly to heritabilities unique for the specific year or data. Considering the low infectious pressure of 2014, this is of particular importance. Also, linkage disequilibrium was not analysed for this thesis. Although the linkage disequilibrium is reduced for inbreeding populations, it is still recommended for association mapping.

The value and effectiveness of using visual phenotypic data for FHB, versus the analytical DON data, must be considered as the evaluation of the trait is difficult. Additionally, DON content in the grains are of most concern for yield and quality, thus indicating that limiting the accumulation of DON in the grains should be of high priority.

Significant SNPs markers positioned close to SSR markers found in this study can be analysed further to see if they are associated with the trait of interest. The significant markers found in this study could be effectively utilized in KASP assays for more accurately discrimination of a known SNP. The result from these can then be used and tested on a breeding population to see if the markers show the expected effects. Based on these effects, resistant wheat lines can be effectively used for breeding programmes.

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Appendix 1

Table 28. Population structure of 172 MASBASIS lines with subpopulations (S) 1-8 and mixed

Line	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	S
Bastian	0.909	0.086	0.001	0.001	0.000	0.001	0.002	0.001	1
Bjarne	0.767	0.208	0.001	0.003	0.001	0.017	0.001	0.002	1
Brakar	0.857	0.132	0.002	0.001	0.002	0.003	0.002	0.001	1
Runar	0.996	0.000	0.000	0.000	0.000	0.001	0.001	0.000	1
T2038	0.774	0.041	0.004	0.007	0.113	0.052	0.003	0.005	1
T9040	0.996	0.000	0.000	0.000	0.000	0.001	0.001	0.000	1
T10014	0.509	0.152	0.010	0.250	0.066	0.010	0.003	0.002	1
NK93602(1995)	0.645	0.243	0.003	0.001	0.007	0.045	0.056	0.001	1
D99060	0.875	0.106	0.001	0.000	0.003	0.006	0.007	0.002	1
219-Seri-Avle	0.741	0.124	0.008	0.001	0.012	0.010	0.002	0.102	1
NK93604-1	0.810	0.182	0.001	0.001	0.001	0.001	0.004	0.001	1
512-21	0.994	0.001	0.001	0.001	0.001	0.001	0.002	0.001	1
512-50	0.831	0.001	0.001	0.033	0.091	0.002	0.036	0.004	1
512-54	0.846	0.001	0.001	0.001	0.147	0.002	0.002	0.001	1
512-70	0.952	0.001	0.004	0.008	0.029	0.003	0.002	0.002	1
512-87	0.804	0.001	0.001	0.001	0.188	0.003	0.002	0.001	1
BAJASS-5	0.921	0.001	0.001	0.014	0.001	0.060	0.000	0.002	1
Demonstrant	0.592	0.001	0.001	0.001	0.001	0.404	0.001	0.001	1
Krabat	0.547	0.002	0.001	0.001	0.001	0.445	0.002	0.002	1
GN04528	0.842	0.150	0.001	0.001	0.001	0.001	0.002	0.001	1
GN05580	0.534	0.004	0.003	0.002	0.013	0.443	0.002	0.001	1
GN06557	0.737	0.138	0.003	0.001	0.002	0.112	0.002	0.003	1
GN06573	0.616	0.085	0.013	0.001	0.018	0.251	0.013	0.002	1
NK01565	0.660	0.001	0.005	0.003	0.001	0.321	0.007	0.001	1
GN08504	0.696	0.001	0.001	0.006	0.001	0.291	0.001	0.003	1
GN08531	0.908	0.022	0.001	0.001	0.001	0.064	0.001	0.001	1
GN08588	0.666	0.001	0.002	0.063	0.002	0.257	0.001	0.008	1
Møystad	0.722	0.001	0.001	0.002	0.001	0.088	0.186	0.001	1
Saar-1	0.008	0.866	0.012	0.011	0.062	0.023	0.015	0.002	2
Filin-1	0.003	0.696	0.053	0.008	0.226	0.004	0.005	0.005	2
Milan-1	0.007	0.547	0.001	0.002	0.434	0.002	0.005	0.002	2
Dulus-1	0.041	0.744	0.007	0.010	0.002	0.002	0.008	0.186	2
Gondo-1	0.009	0.719	0.002	0.003	0.243	0.020	0.003	0.001	2
205-Kauz	0.001	0.933	0.018	0.001	0.006	0.001	0.001	0.040	2
219-Seri	0.001	0.991	0.001	0.001	0.001	0.001	0.001	0.003	2
Kariega-2	0.036	0.791	0.058	0.004	0.011	0.013	0.080	0.007	2
Avocet-YrA-1	0.002	0.567	0.012	0.041	0.127	0.046	0.203	0.003	2
SY1	0.003	0.721	0.012	0.000	0.245	0.000	0.007	0.012	2
Frontana	0.004	0.592	0.113	0.002	0.280	0.001	0.005	0.003	2
CBRD/KAUZ	0.002	0.788	0.006	0.001	0.195	0.001	0.003	0.004	2

GUAM92//PSN/BOW	0.001	0.519	0.002	0.001	0.467	0.002	0.001	0.007	2
ALTAR 84/AE.SQUARROSA (224)//ESDA	0.062	0.692	0.002	0.190	0.004	0.009	0.038	0.002	2
BCN*2//CROC_1/AE.SQUARROSA (886)	0.001	0.991	0.002	0.001	0.001	0.001	0.001	0.003	2
MAYOOR//TKSN1081/ AE.SQUARROSA (222)	0.002	0.666	0.253	0.003	0.013	0.001	0.058	0.003	2
AC_Somerset	0.013	0.638	0.005	0.123	0.077	0.016	0.121	0.007	2
CD87-3	0.037	0.845	0.002	0.001	0.001	0.009	0.104	0.002	2
Chara-3	0.003	0.815	0.003	0.016	0.003	0.005	0.150	0.005	2
Kukri-3	0.003	0.946	0.003	0.001	0.036	0.007	0.002	0.001	2
Naxos/2*Saar	0.018	0.603	0.009	0.297	0.018	0.024	0.009	0.021	2
ONPMSYDER-05	0.006	0.901	0.002	0.005	0.001	0.080	0.002	0.002	2
Sabin	0.011	0.591	0.180	0.205	0.003	0.004	0.001	0.004	2
Tom	0.125	0.668	0.052	0.100	0.013	0.015	0.010	0.016	2
RB07	0.016	0.657	0.002	0.199	0.107	0.002	0.010	0.006	2
C80.1/3*QT4522//2*ATTILA	0.035	0.863	0.003	0.058	0.005	0.003	0.001	0.032	2
C80.1/3*QT4522//2*PASTOR	0.127	0.846	0.002	0.002	0.004	0.017	0.001	0.001	2
Folke	0.001	0.001	0.918	0.000	0.070	0.002	0.001	0.008	3
Mjølner	0.002	0.001	0.966	0.001	0.003	0.002	0.002	0.023	3
Magnifik	0.089	0.010	0.870	0.002	0.020	0.002	0.004	0.003	3
Finans	0.002	0.001	0.785	0.002	0.004	0.011	0.010	0.186	3
NK03029	0.002	0.001	0.787	0.004	0.003	0.002	0.198	0.004	3
Soissons	0.002	0.202	0.522	0.003	0.256	0.005	0.004	0.006	3
Apollo	0.011	0.038	0.640	0.060	0.015	0.043	0.059	0.134	3
Regina	0.003	0.005	0.559	0.031	0.002	0.003	0.001	0.396	3
GN04035	0.001	0.001	0.993	0.001	0.001	0.001	0.001	0.002	3
GN04034	0.001	0.001	0.942	0.004	0.001	0.001	0.009	0.041	3
GN05012	0.091	0.001	0.879	0.001	0.001	0.006	0.018	0.003	3
GN05013	0.113	0.001	0.844	0.003	0.002	0.033	0.003	0.001	3
V1004	0.003	0.001	0.720	0.004	0.012	0.002	0.254	0.005	3
V9001	0.003	0.004	0.858	0.001	0.001	0.002	0.130	0.001	3
Tarso	0.002	0.001	0.906	0.009	0.003	0.003	0.010	0.067	3
Kamerat(NK97017)	0.003	0.001	0.740	0.012	0.001	0.001	0.023	0.219	3
Rida	0.083	0.006	0.873	0.004	0.003	0.001	0.030	0.001	3
Trond	0.001	0.001	0.969	0.003	0.002	0.001	0.002	0.021	3
SigynII	0.029	0.093	0.776	0.008	0.002	0.041	0.043	0.007	3
GN08004	0.005	0.001	0.600	0.002	0.002	0.373	0.008	0.008	3
Naxos	0.000	0.000	0.001	0.996	0.000	0.001	0.001	0.001	4
Mironovskaja808	0.003	0.007	0.287	0.663	0.002	0.001	0.001	0.035	4
GN06578	0.017	0.001	0.001	0.697	0.001	0.282	0.001	0.001	4
GN08533	0.287	0.001	0.002	0.693	0.001	0.014	0.001	0.001	4

GN08554	0.031	0.002	0.002	0.643	0.005	0.311	0.004	0.002	4
GN08568	0.003	0.001	0.001	0.860	0.001	0.131	0.002	0.001	4
Bagula-Milan-2	0.002	0.481	0.002	0.001	0.511	0.001	0.002	0.001	5
Catbird-2	0.001	0.344	0.103	0.006	0.526	0.007	0.001	0.010	5
CJ9306	0.024	0.169	0.029	0.001	0.769	0.003	0.002	0.003	5
CJ9403	0.001	0.005	0.101	0.012	0.870	0.006	0.001	0.004	5
SHA3/CBRD	0.002	0.020	0.016	0.003	0.942	0.006	0.001	0.010	5
Sumai3(18-2)	0.007	0.001	0.001	0.002	0.983	0.001	0.004	0.002	5
Nobeokabouzu	0.002	0.330	0.010	0.043	0.508	0.007	0.091	0.008	5
Nanjing7840	0.002	0.001	0.139	0.001	0.842	0.001	0.002	0.012	5
Ning8343	0.003	0.001	0.003	0.004	0.845	0.003	0.138	0.002	5
GONDO	0.002	0.480	0.002	0.001	0.511	0.001	0.003	0.001	5
MILAN/SHA7	0.018	0.131	0.003	0.001	0.837	0.003	0.004	0.003	5
NG8675/CBRD	0.002	0.078	0.026	0.013	0.794	0.028	0.001	0.059	5
Sumai#3-1(12SRSN)	0.001	0.001	0.001	0.001	0.993	0.001	0.002	0.001	5
Tjalve	0.119	0.002	0.093	0.002	0.005	0.739	0.018	0.022	6
Avle	0.002	0.001	0.001	0.001	0.001	0.994	0.000	0.001	6
Zebra	0.145	0.016	0.009	0.030	0.080	0.702	0.017	0.001	6
Vinjett	0.001	0.000	0.000	0.000	0.000	0.997	0.001	0.000	6
GN03531	0.246	0.001	0.001	0.001	0.001	0.749	0.001	0.001	6
SW45126	0.082	0.001	0.003	0.001	0.001	0.909	0.002	0.002	6
SW46375	0.001	0.001	0.001	0.003	0.001	0.992	0.001	0.001	6
GN03597	0.345	0.006	0.002	0.004	0.002	0.639	0.002	0.001	6
SW51069	0.003	0.001	0.002	0.063	0.001	0.919	0.008	0.004	6
SW51114	0.007	0.001	0.014	0.010	0.064	0.900	0.002	0.003	6
Bombona	0.013	0.005	0.002	0.020	0.006	0.800	0.003	0.150	6
GN03529	0.349	0.001	0.001	0.001	0.001	0.645	0.001	0.001	6
SW45204	0.005	0.032	0.002	0.001	0.001	0.920	0.033	0.006	6
GN04526	0.308	0.001	0.001	0.001	0.001	0.688	0.001	0.001	6
GN03503	0.133	0.018	0.011	0.086	0.058	0.678	0.015	0.001	6
GN08564	0.001	0.000	0.001	0.001	0.001	0.996	0.001	0.001	6
GN08595	0.095	0.001	0.001	0.001	0.001	0.899	0.003	0.001	6
GN08596	0.097	0.001	0.001	0.001	0.001	0.898	0.002	0.000	6
GN08597	0.289	0.001	0.001	0.001	0.000	0.705	0.001	0.001	6
GN08647	0.018	0.340	0.001	0.107	0.001	0.529	0.001	0.002	6
SW44333	0.002	0.001	0.065	0.001	0.005	0.924	0.001	0.002	6
SW44431	0.012	0.001	0.001	0.001	0.001	0.984	0.000	0.001	6
SW51127	0.003	0.001	0.042	0.142	0.001	0.793	0.010	0.009	6
SW71127	0.002	0.001	0.002	0.086	0.003	0.902	0.001	0.003	6
SW71144	0.002	0.001	0.007	0.002	0.010	0.903	0.011	0.065	6
SW71237	0.002	0.000	0.004	0.003	0.001	0.954	0.035	0.001	6
J03	0.001	0.000	0.000	0.000	0.000	0.000	0.997	0.000	7
Norrøna	0.368	0.001	0.001	0.001	0.001	0.001	0.626	0.001	7
FramII	0.001	0.000	0.000	0.000	0.000	0.000	0.997	0.000	7
DH20070	0.328	0.003	0.041	0.010	0.002	0.003	0.068	0.546	8
Olivin	0.003	0.001	0.168	0.020	0.002	0.001	0.004	0.800	8

RE714	0.021	0.002	0.047	0.084	0.188	0.003	0.001	0.654	8
Fenman	0.003	0.001	0.002	0.124	0.005	0.003	0.001	0.861	8
Arina	0.002	0.001	0.222	0.007	0.024	0.002	0.024	0.718	8
LW91W89Pl2	0.002	0.001	0.200	0.011	0.004	0.001	0.001	0.780	8
Bersee	0.002	0.001	0.101	0.001	0.027	0.002	0.001	0.865	8
Spark	0.025	0.165	0.002	0.046	0.026	0.025	0.006	0.705	8
Vlasta	0.003	0.007	0.206	0.149	0.009	0.004	0.002	0.620	8
Senat	0.001	0.001	0.004	0.001	0.026	0.001	0.002	0.963	8
Solist	0.002	0.001	0.002	0.001	0.063	0.002	0.001	0.928	8
Ambition	0.007	0.001	0.003	0.001	0.007	0.001	0.006	0.975	8
Jenga	0.003	0.002	0.083	0.003	0.022	0.005	0.021	0.861	8
Siria	0.001	0.001	0.477	0.001	0.001	0.001	0.004	0.514	8
Skagen	0.003	0.014	0.351	0.002	0.006	0.007	0.008	0.609	8
Plutos	0.011	0.002	0.002	0.002	0.000	0.006	0.001	0.975	8
Akratos	0.069	0.001	0.009	0.016	0.004	0.010	0.001	0.891	8
DED2097/02	0.001	0.001	0.288	0.009	0.001	0.001	0.001	0.698	8
Akteur	0.004	0.043	0.349	0.004	0.001	0.038	0.001	0.559	8
Ellvis	0.002	0.001	0.268	0.002	0.001	0.002	0.002	0.723	8
Frontal	0.001	0.001	0.005	0.015	0.001	0.005	0.001	0.972	8
Berserk	0.173	0.258	0.005	0.007	0.016	0.334	0.004	0.203	Mix
T9040(1995)	0.002	0.003	0.009	0.375	0.046	0.257	0.305	0.003	Mix
MS273-150	0.435	0.301	0.003	0.004	0.003	0.015	0.238	0.002	Mix
Paros	0.002	0.051	0.308	0.217	0.007	0.161	0.107	0.149	Mix
D99014	0.461	0.001	0.010	0.214	0.014	0.171	0.122	0.007	Mix
D99159	0.337	0.001	0.003	0.191	0.018	0.188	0.259	0.003	Mix
DH20097	0.438	0.096	0.009	0.007	0.005	0.001	0.018	0.427	Mix
R37/GHL121//KAL/ BB/3/JUP/MUS/4/2 *YMI #6/5/CBRD	0.004	0.369	0.004	0.134	0.482	0.002	0.004	0.002	Mix
Sport-2	0.439	0.001	0.001	0.154	0.002	0.401	0.001	0.001	Mix
GN04537	0.333	0.002	0.122	0.010	0.005	0.363	0.139	0.026	Mix
GN05507	0.402	0.298	0.002	0.001	0.001	0.292	0.002	0.001	Mix
QUARNA	0.005	0.182	0.111	0.204	0.137	0.279	0.070	0.013	Mix
Bjørke	0.014	0.010	0.701	0.006	0.064	0.007	0.002	0.195	Mix
Massey	0.014	0.398	0.286	0.046	0.013	0.022	0.016	0.206	Mix
NSL94-6897	0.032	0.288	0.243	0.253	0.122	0.028	0.003	0.032	Mix
Senta	0.003	0.023	0.231	0.268	0.141	0.005	0.019	0.309	Mix
USG3209-7	0.007	0.420	0.379	0.153	0.008	0.008	0.008	0.018	Mix
USG3209-9	0.017	0.447	0.361	0.109	0.023	0.004	0.010	0.029	Mix
GN05551	0.470	0.002	0.002	0.002	0.001	0.444	0.077	0.002	Mix
GN05589	0.378	0.131	0.001	0.006	0.002	0.471	0.006	0.005	Mix
GN07581	0.345	0.184	0.069	0.002	0.139	0.239	0.018	0.004	Mix
GN08534	0.248	0.311	0.003	0.081	0.003	0.340	0.013	0.002	Mix
GN08541	0.206	0.414	0.003	0.012	0.007	0.332	0.001	0.027	Mix
GN08557	0.480	0.232	0.006	0.001	0.006	0.026	0.046	0.202	Mix
TJALVE/Purpurseed	0.006	0.256	0.036	0.001	0.115	0.486	0.093	0.006	Mix
Granary	0.006	0.116	0.021	0.384	0.015	0.164	0.064	0.231	Mix

Redcoat	0.171	0.251	0.405	0.080	0.005	0.001	0.018	0.068	Mix
Hadm04363-05	0.002	0.001	0.449	0.005	0.001	0.003	0.067	0.473	Mix

Table 29: Population structure for 123 spring wheat lines with subpopulations (S) 1-5 and mixed

Line	Q1	Q2	Q3	Q4	Q5	S
Saar-1	0.902	0.013	0.014	0.023	0.048	1
Filin-1	0.709	0.008	0.015	0.010	0.259	1
Milan-1	0.508	0.011	0.010	0.005	0.465	1
Dulus-1	0.885	0.043	0.038	0.010	0.024	1
Gondo-1	0.719	0.022	0.011	0.027	0.221	1
205-Kauz	0.969	0.005	0.004	0.003	0.019	1
219-Seri	0.984	0.004	0.004	0.003	0.005	1
Kariega-2	0.877	0.032	0.027	0.016	0.047	1
Avocet-YrA-1	0.618	0.008	0.188	0.035	0.150	1
SY1	0.714	0.006	0.001	0.002	0.277	1
Frontana	0.625	0.011	0.012	0.004	0.349	1
CBRD/KAUZ	0.789	0.006	0.005	0.005	0.195	1
ALTAR 84/AE.SQUARROSA (224)//ESDA	0.705	0.048	0.214	0.020	0.013	1
BCN*2//CROC_1/AE.SQUARROSA (886)	0.985	0.004	0.003	0.003	0.005	1
MAYOOR//TK SN1081/ AE.SQUARROSA (222)	0.732	0.018	0.094	0.006	0.150	1
AC_Somerset	0.663	0.026	0.133	0.038	0.140	1
CD87-3	0.924	0.039	0.011	0.020	0.007	1
Chara-3	0.877	0.012	0.090	0.009	0.012	1
Kukri-3	0.948	0.006	0.005	0.009	0.032	1
Naxos/2*Saar	0.581	0.021	0.280	0.080	0.038	1
ONPMSYDER-05	0.927	0.016	0.008	0.044	0.005	1
Sabin	0.655	0.030	0.253	0.029	0.033	1
Tom	0.737	0.071	0.116	0.031	0.044	1
RB07	0.692	0.023	0.223	0.011	0.051	1
C80.1/3*QT4522//2*ATTILA	0.909	0.028	0.037	0.008	0.019	1
C80.1/3*QT4522//2*PASTOR	0.875	0.076	0.007	0.025	0.016	1
Bastian	0.114	0.871	0.003	0.009	0.002	2
Bjarne	0.230	0.727	0.005	0.032	0.007	2
Brakar	0.149	0.829	0.006	0.008	0.008	2
Runar	0.002	0.990	0.002	0.004	0.002	2
T2038	0.092	0.726	0.016	0.084	0.082	2
T9040	0.002	0.989	0.002	0.005	0.002	2
T10014	0.102	0.473	0.253	0.057	0.115	2
NK93602(1995)	0.263	0.656	0.008	0.038	0.034	2
D99060	0.109	0.855	0.004	0.013	0.018	2
219-Seri-Avle	0.226	0.690	0.005	0.048	0.031	2
NK93604-1	0.209	0.778	0.004	0.005	0.003	2
512-21	0.006	0.978	0.003	0.003	0.010	2
512-50	0.014	0.806	0.113	0.007	0.060	2
512-54	0.003	0.840	0.005	0.008	0.144	2
512-70	0.005	0.919	0.018	0.010	0.048	2

512-87	0.002	0.799	0.004	0.012	0.183	2
BAJASS-5	0.009	0.812	0.013	0.164	0.002	2
Demonstrant	0.003	0.594	0.002	0.398	0.003	2
Krabat	0.006	0.565	0.003	0.423	0.003	2
GN04528	0.186	0.802	0.004	0.003	0.005	2
GN05580	0.013	0.516	0.008	0.445	0.018	2
GN06557	0.153	0.712	0.006	0.121	0.008	2
GN06573	0.111	0.602	0.005	0.252	0.030	2
NK01565	0.006	0.663	0.011	0.314	0.006	2
GN08504	0.009	0.613	0.010	0.364	0.003	2
GN08531	0.052	0.796	0.006	0.141	0.005	2
GN08588	0.012	0.604	0.045	0.333	0.006	2
Møystad	0.010	0.805	0.121	0.058	0.007	2
Norrøna	0.010	0.551	0.417	0.006	0.016	2
T9040(1995)	0.006	0.010	0.806	0.160	0.017	3
Naxos	0.005	0.002	0.877	0.112	0.004	3
Paros	0.077	0.014	0.537	0.175	0.197	3
D99159	0.005	0.410	0.510	0.065	0.010	3
J03	0.026	0.287	0.618	0.005	0.064	3
GN06578	0.004	0.021	0.592	0.379	0.004	3
GN08533	0.014	0.182	0.596	0.205	0.004	3
GN08554	0.004	0.026	0.565	0.400	0.006	3
GN08568	0.003	0.006	0.708	0.280	0.003	3
Granary	0.104	0.013	0.676	0.183	0.024	3
FramII	0.026	0.327	0.588	0.005	0.054	3
Tjalve	0.020	0.124	0.035	0.739	0.081	4
Avle	0.004	0.007	0.003	0.979	0.006	4
Zebra	0.033	0.128	0.054	0.690	0.095	4
Vinjett	0.002	0.004	0.002	0.990	0.002	4
GN03531	0.005	0.255	0.003	0.735	0.003	4
SW45126	0.004	0.085	0.003	0.905	0.003	4
SW46375	0.005	0.007	0.009	0.969	0.010	4
GN03597	0.017	0.348	0.009	0.616	0.010	4
SW51069	0.006	0.010	0.083	0.898	0.003	4
SW51114	0.005	0.014	0.022	0.884	0.075	4
Bombona	0.031	0.013	0.155	0.789	0.012	4
GN03529	0.005	0.366	0.004	0.622	0.003	4
SW45204	0.082	0.014	0.008	0.879	0.017	4
GN04526	0.005	0.324	0.003	0.665	0.003	4
GN03503	0.031	0.125	0.106	0.666	0.072	4
GN08564	0.004	0.003	0.006	0.982	0.005	4
GN08595	0.004	0.130	0.005	0.859	0.003	4
GN08596	0.002	0.131	0.003	0.861	0.002	4
GN08597	0.006	0.281	0.004	0.707	0.002	4
GN08647	0.368	0.020	0.060	0.547	0.005	4
SW44333	0.008	0.006	0.009	0.910	0.066	4

SW44431	0.005	0.044	0.004	0.944	0.004	4
SW51127	0.005	0.005	0.192	0.794	0.005	4
SW71127	0.003	0.007	0.089	0.890	0.010	4
SW71144	0.012	0.007	0.041	0.883	0.057	4
SW71237	0.003	0.009	0.036	0.943	0.009	4
Bagula-Milan-2	0.373	0.005	0.005	0.004	0.613	5
Catbird-2	0.302	0.005	0.016	0.025	0.653	5
CJ9306	0.102	0.050	0.007	0.012	0.829	5
CJ9403	0.023	0.004	0.021	0.019	0.932	5
SHA3/CBRD	0.020	0.005	0.015	0.021	0.940	5
Sumai3(18-2)	0.007	0.020	0.012	0.004	0.957	5
Nobeokabouzu	0.324	0.007	0.128	0.011	0.530	5
Nanjing7840	0.008	0.012	0.027	0.005	0.948	5
Ning8343	0.007	0.009	0.081	0.013	0.891	5
GONDO	0.372	0.006	0.004	0.004	0.614	5
MILAN/SHA7	0.059	0.023	0.006	0.010	0.902	5
R37/GHL121//KAL/BB/3/JUP/ MUS/4/2*YMI #6/5/CBRD	0.375	0.011	0.089	0.009	0.516	5
GUAM92//PSN/BOW	0.482	0.004	0.004	0.006	0.505	5
NG8675/CBRD	0.041	0.004	0.053	0.045	0.856	5
Sumai#3-1(12SRSN)	0.004	0.006	0.008	0.003	0.979	5
Berserk	0.354	0.148	0.077	0.360	0.061	Mix
MS273-150	0.339	0.495	0.122	0.024	0.020	Mix
D99014	0.003	0.482	0.429	0.078	0.007	Mix
DH20070	0.076	0.335	0.320	0.042	0.227	Mix
DH20097	0.235	0.387	0.270	0.007	0.101	Mix
Sport-2	0.005	0.384	0.140	0.466	0.005	Mix
GN04537	0.017	0.364	0.250	0.332	0.036	Mix
GN05507	0.306	0.394	0.005	0.290	0.005	Mix
QUARNA	0.122	0.015	0.432	0.258	0.174	Mix
GN05551	0.014	0.487	0.036	0.451	0.012	Mix
GN05589	0.131	0.375	0.017	0.468	0.008	Mix
GN07581	0.210	0.338	0.017	0.252	0.184	Mix
GN08534	0.333	0.216	0.108	0.335	0.009	Mix
GN08541	0.461	0.166	0.016	0.340	0.016	Mix
GN08557	0.366	0.456	0.022	0.095	0.061	Mix
TJALVE/Purpurseed	0.231	0.021	0.016	0.467	0.264	Mix

Table 30: Population structure for 49 winter wheat lines with subpopulations (S) 1-3 and mixed

Line	Q1	Q2	Q3	S
Olivin	0.944	0.005	0.050	1
RE714	0.944	0.006	0.050	1
Fenman	0.986	0.009	0.005	1
Arina	0.809	0.007	0.184	1
LW91W89Pl2	0.963	0.003	0.034	1
Bersee	0.970	0.010	0.020	1
Spark	0.975	0.008	0.017	1
Vlasta	0.854	0.016	0.130	1
Senat	0.987	0.002	0.010	1
Solist	0.990	0.003	0.006	1
Ambition	0.985	0.006	0.009	1
Jenga	0.970	0.004	0.026	1
Senta	0.697	0.035	0.267	1
Mironovskaja808	0.616	0.042	0.342	1
Siria	0.589	0.010	0.401	1
Skagen	0.712	0.009	0.280	1
Plutos	0.993	0.002	0.005	1
Akratos	0.983	0.007	0.010	1
Akteur	0.703	0.020	0.276	1
Hadm04363-05	0.522	0.008	0.470	1
Ellvis	0.926	0.009	0.065	1
Frontal	0.989	0.003	0.008	1
DED2097/02	0.897	0.011	0.093	1
Massey	0.268	0.707	0.025	2
USG3209-7	0.008	0.980	0.011	2
USG3209-9	0.003	0.995	0.003	2
Bjørke	0.195	0.020	0.784	3
Folke	0.028	0.004	0.969	3
Mjølner	0.020	0.003	0.977	3
Magnifik	0.012	0.009	0.978	3
Finans	0.119	0.016	0.865	3
NK03029	0.008	0.004	0.987	3
Soissons	0.407	0.014	0.579	3
Apollo	0.127	0.090	0.783	3
Regina	0.459	0.005	0.536	3
GN04035	0.011	0.003	0.986	3
GN04034	0.058	0.005	0.938	3
GN05012	0.009	0.003	0.988	3
GN05013	0.005	0.003	0.991	3
V1004	0.011	0.015	0.974	3
V9001	0.007	0.077	0.916	3
Tarso	0.024	0.008	0.968	3
Kamerat(NK97017)	0.218	0.009	0.773	3

Rida	0.006	0.024	0.969	3
Trond	0.043	0.006	0.951	3
SigynII	0.017	0.163	0.819	3
GN08004	0.042	0.040	0.918	3
NSL94-6897	0.398	0.247	0.355	Mix
Redcoat	0.336	0.309	0.355	Mix

Appendix 2

Table 31: Significant markers for earliness (DH) at a -log10(P-value) threshold of 2.5 with position on chromosome (cM) and allele effects for spring wheat lines where n number of lines with 'a' having a positive effect and 'b' a negative effect

Marker	Chromosome	Position	P-value	R^2	n = a	n = b	Effect
wsnp_Ku_c34659_43981982	1A	177	0.001722	0.09	**108**	15	2.94
wsnp_Ex_c22963_32183009	1A	219	0.003083	0.08	**13**	110	3.43
Tdurum_contig43203_318	1A	226	0.003083	0.08	**13**	110	3.43
wsnp_Ku_c23012_32893918	1A	274	0.000936	0.10	**94**	29	-2.59
BobWhite_c12977_65	1A	289	0.000989	0.10	**109**	14	3.49
Excalibur_c29605_535	1A	289	0.000989	0.10	**14**	109	-3.49
BobWhite_c17044_155	1B	102	0.002175	0.08	**47**	76	1.85
Kukri_c34519_630	1B	201	0.003124	0.08	**16**	107	-2.33
RAC875_rep_c72356_51	1B	207	0.002124	0.08	**111**	12	2.95
BobWhite_s64871_104	1B	461	0.003154	0.08	**112**	11	2.87
BobWhite_rep_c50057_164	1B	462	0.003154	0.08	**112**	11	2.87
wsnp_Ku_c2797_5284087	1B	502	0.003119	0.08	**104**	19	2.34
Tdurum_contig1631_240	1B	541	0.002224	0.08	**105**	18	2.50
Tdurum_contig48103_1481	1B	542	0.000893	0.10	**104**	19	2.80
wsnp_Ex_c750_1474184	1B	549	0.000605	0.10	**115**	8	3.92
Tdurum_contig60566_269	1B	549	0.000893	0.10	**104**	19	2.80
BS00027006_51	1B	549	0.002990	0.08	**57**	66	-1.73
Excalibur_c25640_110	1B	549	0.002990	0.08	**57**	66	-1.73
wsnp_Ku_c13952_22097895	1B	549	0.002990	0.08	**57**	66	-1.73
wsnp_Ex_c9510_15761235	3A	280	0.003135	0.08	**37**	86	-1.93
Excalibur_c47907_517	3A	288	0.003067	0.08	**38**	85	-1.92
Kukri_c40882_312	3B	136	0.001683	0.09	**22**	101	2.12
Kukri_c42075_156	3D	213	0.002529	0.08	**20**	103	1.96
BobWhite_c7217_317	4A	402	0.000499	0.11	**86**	37	2.81
Excalibur_c4325_1150	4A	402	0.001041	0.09	**89**	34	2.49
Excalibur_c4325_466	4A	402	0.001041	0.09	**89**	34	2.49
CAP11_c18_238	4A	402	0.003041	0.08	**87**	36	2.26
RAC875_c37988_243	4A	641	0.000886	0.10	**111**	12	3.00
IAAV163	4B	203	0.001474	0.09	**76**	47	0.43
RAC875_c104414_76	4B	203	0.001474	0.09	**46**	77	1.93
RAC875_c15807_669	4B	203	0.001474	0.09	**46**	77	1.93
Kukri_c15910_159	4B	203	0.001538	0.09	**46**	77	1.93
Excalibur_c38012_393	4B	203	0.002175	0.08	**47**	76	1.85
Kukri_c32064_629	4B	203	0.002175	0.08	**47**	76	1.85
RAC875_c54178_90	4B	203	0.002175	0.08	**47**	76	1.85
wsnp_Ex_c296_573976	4B	203	0.002175	0.08	**47**	76	1.85
wsnp_Ku_c7453_12833586	4B	206	0.003111	0.08	**40**	83	1.86
wsnp_Ra_rep_c69724_67278233	4B	208	0.001474	0.09	**46**	77	1.93
BS00068851_51	4B	208	0.002175	0.08	**47**	76	1.85
wsnp_Ex_c32127_40841791	4B	221	0.000024	0.16	**11**	112	4.25
CAP12_c2983_140	4B	223	0.000003	0.20	**17**	106	4.03
Tdurum_contig10466_87	4B	226	0.001507	0.09	**113**	10	-3.79

RAC875_c67417_275	4B	230	0.001507	0.09	**10**	113	3.79
Ra_c10762_1137	5A	78	0.002279	0.08	**117**	6	3.88
wsnp_Ku_c9559_15999945	5A	78	0.002279	0.08	**117**	6	3.88
BS00021955_51	5A	410	0.000636	0.10	**110**	13	-3.40
Kukri_rep_c70839_205	5A	414	0.003014	0.08	**30**	93	1.74
Excalibur_c2207_1060	5B	676	0.002013	0.08	**105**	18	2.55
tplb0048o03_953	5B	676	0.002013	0.08	**105**	18	2.55
wsnp_Ku_c16116_24914891	5B	676	0.002013	0.08	**18**	105	-2.55
Excalibur_c56264_188	6A	192	0.003009	0.08	**81**	42	-1.60
TA002381-0322	6B	1	0.000989	0.10	**97**	26	2.05
IACX10982	6D	183	0.000256	0.12	**102**	21	2.68
BS00009926_51	7A	448	0.001239	0.09	**106**	17	2.42
wsnp_Ku_c21665_31431143	7A	458	0.000061	0.14	**17**	106	-3.08
Kukri_c57086_133	7A	458	0.000078	0.14	**18**	105	-3.27
RAC875_c24101_284	7B	292	0.002134	0.08	**10**	113	-3.86
Excalibur_c23777_74	7B	489	0.001038	0.09	**100**	23	2.32

Table 32: Significant markers for plant height (PH) at a -log10(P-value) threshold of 2.5 with position on chromosome (cM) and allele effects for spring wheat lines where n number of lines with 'a' having a positive effect and 'b' a negative effect

Marker	Chromosome	Position	P-value	R^2	n = a	n = b	Effect
Tdurum_contig60037_441	1B	182	0.002667	0.08	**10**	113	8.24
IACX17310	1B	470	0.003006	0.08	**104**	19	-7.95
Kukri_c7914_99	2A	329	0.000785	0.10	**18**	105	8.33
Kukri_rep_c83485_398	2A	329	0.000785	0.10	**18**	105	8.33
BS00036766_51	2A	329	0.001055	0.09	**24**	99	6.38
Excalibur_c37649_125	2A	329	0.001055	0.09	**24**	99	6.38
BS00036767_51	2A	329	0.001664	0.09	**106**	17	-8.12
BS00031466_51	2A	332	0.000785	0.10	**18**	105	8.33
RAC875_c7699_292	2A	332	0.001457	0.09	**19**	104	7.52
Tdurum_contig48302_532	2A	332	0.001490	0.09	**22**	101	6.52
wsnp_BG314532A_Ta_2_1	2A	332	0.001664	0.09	**106**	17	-8.12
BS00039983_51	2A	332	0.003129	0.07	**105**	18	-7.21
Ku_c21663_1390	2B	254	0.000932	0.09	**70**	53	-7.25
wsnp_Ex_c7285_12506938	2B	254	0.001903	0.08	**37**	86	6.43
BS00010055_51	2B	262	0.001391	0.09	**35**	88	6.66
Excalibur_c108170_294	2B	262	0.001391	0.09	**35**	88	6.66
IACX8565	2B	262	0.001391	0.09	**35**	88	6.66
BS00070120_51	2B	291	0.001025	0.09	**27**	96	8.45
BS00065734_51	3A	471	0.001637	0.09	**112**	11	-9.87
Excalibur_c22827_452	3B	269	0.000663	0.10	**12**	111	10.77
Excalibur_rep_c113157_316	3B	269	0.001673	0.09	**10**	113	10.51
RAC875_c30414_343	3B	269	0.001673	0.09	**10**	113	10.51
RAC875_rep_c105184_88	3B	269	0.001673	0.09	**10**	113	10.51
wsnp_BQ168706B_Ta_2_1	3B	269	0.001673	0.09	**10**	113	10.51
wsnp_BQ168706B_Ta_2_2	3B	269	0.001673	0.09	**10**	113	10.51
wsnp_Ex_c21499_30644485	3B	269	0.001673	0.09	**10**	113	10.51
wsnp_Ku_c10291_17065480	3B	269	0.001673	0.09	**10**	113	10.51
RFL_Contig4336_184	4A	356	0.001454	0.09	**96**	27	5.98
Excalibur_c64860_102	4A	416	0.000408	0.11	**115**	8	13.89
Tdurum_contig64772_417	4B	148	0.001194	0.09	**35**	88	-5.86
Rht-B1	4B	159	0.000156	0.13	**78**	45	7.42
Excalibur_c36630_2194	4B	162	0.000559	0.10	**61**	62	6.43
Tdurum_contig42229_113	4B	162	0.000756	0.10	**78**	45	6.66
BS00021984_51	4B	163	0.000265	0.12	**69**	54	6.77
RAC875_rep_c105718_430	4B	163	0.000756	0.10	**78**	45	6.66
Tdurum_contig33737_157	4B	163	0.000756	0.10	**78**	45	6.66
BobWhite_rep_c49034_167	4B	163	0.000875	0.10	**79**	44	6.53
wsnp_Ku_c28756_38667953	4B	163	0.003145	0.07	**44**	79	-5.70
IAAV971	4B	167	0.001335	0.09	**41**	82	-6.53
Excalibur_c56787_95	4B	169	0.001255	0.09	**37**	86	-6.42
Excalibur_c17607_542	4B	183	0.001883	0.08	**88**	35	6.45
BS00023407_51	5A	282	0.000754	0.10	**10**	113	15.78
Kukri_rep_c103857_458	5A	314	0.001564	0.09	**5**	118	15.56
RAC875_rep_c76193_513	5A	460	0.000439	0.11	**36**	87	7.34
BS00088851_51	5A	463	0.001131	0.09	**48**	75	7.34

CAP11_c3209_76	5A	463	0.002852	0.08	**42**	81	6.35
Excalibur_c24051_1028	5A	463	0.002926	0.08	**42**	81	6.23
wsnp_Ex_rep_c101994_87256479	5A	464	0.001505	0.09	**41**	82	6.97
Excalibur_c17055_1451	5B	222	0.002112	0.08	**117**	6	-8.84
BobWhite_c27244_211	5B	281	0.001070	0.09	**103**	20	-5.98
Excalibur_rep_c105399_213	5B	290	0.001070	0.09	**103**	20	-5.98
BS00064853_51	5B	447	0.000349	0.11	**80**	43	8.44
BS00078784_51	5B	448	0.000350	0.11	**81**	42	8.61
BS00028082_51	5B	448	0.000509	0.11	**45**	78	-8.20
RAC875_rep_c111379_93	5B	448	0.002757	0.08	**83**	40	7.65
Excalibur_c92555_283	5B	501	0.002382	0.08	**29**	94	7.24
IACX2594	5B	659	0.002931	0.08	**18**	105	6.85
RAC875_rep_c106589_184	5B	659	0.002931	0.08	**18**	105	6.85
Kukri_c66671_183	6A	338	0.002971	0.08	**114**	9	-11.47
Tdurum_contig70819_393	6A	338	0.002971	0.08	**114**	9	-11.47
BS00067590_51	6B	168	0.000028	0.16	**11**	112	14.44
GENE-0221_350	6B	168	0.000028	0.16	**11**	112	14.44
GENE-0221_721	6B	168	0.000028	0.16	**11**	112	14.44
Kukri_c31032_897	6B	168	0.000028	0.16	**11**	112	14.44
Kukri_c32307_481	6B	168	0.000028	0.16	**11**	112	14.44
TA005332-1378	6B	168	0.000028	0.16	**11**	112	14.44
Excalibur_s111479_146	6B	168	0.000120	0.13	**114**	9	-15.04
RAC875_c10650_90	6B	168	0.000120	0.13	**114**	9	-15.04
RAC875_rep_c116755_285	6B	168	0.000120	0.13	**114**	9	-15.04
RFL_Contig2024_600	6B	168	0.000120	0.13	**114**	9	-15.04
BS00047044_51	6B	168	0.000134	0.13	**13**	110	11.46
RAC875_c6837_468	6B	168	0.000177	0.12	**109**	14	-10.77
RAC875_c17559_3102	6B	198	0.000062	0.14	**13**	110	11.82
BS00084314_51	6B	198	0.000307	0.11	**13**	110	10.77
TA002465-0455-w	6B	198	0.000307	0.11	**13**	110	10.77
Excalibur_c21670_484	6B	198	0.001198	0.09	**112**	11	-10.77
RAC875_c24101_284	7B	292	0.001615	0.09	**10**	113	-11.57
barc130_295	SSR 5DS	-	0.002268	0.08	**114**	9	-14.68
DuPw167_259	SSR 6AL	-	0.000155	0.13	**109**	14	-9.31

Table 33: Significant markers for heading date (HD) at a -log10(P-value) threshold of 2.5 with position on chromosome (cM) and allele effects for winter wheat lines where n number of 'a' lines are indicated by a positive effect and 'b' a negative effect

Marker	Chromosome	Position	P-value	R^2	n = a	n = b	Effect
RAC875_c14066_452	1A	182	0.002022	0.21	42	6	5.40
Excalibur_c7237_1084	1A	184	0.002022	0.21	6	42	-5.40
RAC875_c10090_963	1A	184	0.002022	0.21	6	42	-5.40
RAC875_rep_c111911_116	1A	184	0.002022	0.21	6	42	-5.40
wsnp_Ex_c3906_7086162	1A	216	0.000410	0.28	38	10	2.87
CAP8_rep_c7560_159	1A	216	0.001135	0.23	36	12	3.00
Excalibur_c49588_76	1A	216	0.001135	0.23	36	12	3.00
RAC875_c39125_365	1A	216	0.001135	0.23	36	12	3.00
RAC875_c8515_653	1A	216	0.001190	0.23	37	11	3.09
CAP11_c6014_160	1A	216	0.001580	0.22	31	17	2.98
TA013367-0455	1A	216	0.001580	0.22	31	17	2.98
wsnp_Ex_rep_c101746_87053634	1A	216	0.001580	0.22	31	17	2.98
Ex_c6765_2118	1A	216	0.002877	0.19	13	35	-2.25
RAC875_c38417_246	1A	216	0.002877	0.19	13	35	-2.25
CAP7_c3269_236	1A	219	0.001135	0.23	12	36	-3.00
IAAV749	1A	219	0.001135	0.23	36	12	3.00
wsnp_Ex_c2840_5247386	1A	219	0.001135	0.23	36	12	3.00
wsnp_Ex_c41969_48673442	1A	219	0.001135	0.23	36	12	3.00
CAP8_c4897_397	1A	219	0.001190	0.23	37	11	3.09
BobWhite_c1488_504	1A	219	0.001580	0.22	17	31	-2.98
Kukri_rep_c101316_375	1A	219	0.001580	0.22	17	31	-2.98
wsnp_Ra_c6182_10833256	1A	219	0.001580	0.22	17	31	-2.98
CAP8_c2843_226	1A	219	0.002877	0.19	13	35	-2.25
Excalibur_c14943_695	1A	219	0.002877	0.19	13	35	-2.25
IACX2325	1A	219	0.002877	0.19	13	35	-2.25
Kukri_rep_c103147_745	1A	219	0.002877	0.19	13	35	-2.25
Tdurum_contig48416_335	1A	220	0.002877	0.19	13	35	-2.25
Ra_c11023_679	1A	230	0.001580	0.22	17	31	-2.98
BS00039378_51	1A	291	0.001332	0.23	8	40	-4.55
BS00065430_51	1A	291	0.001332	0.23	8	40	-4.55
BS00039377_51	1A	293	0.001332	0.23	8	40	-4.55
BS00066446_51	1D	135	0.001996	0.21	11	37	-3.01
Tdurum_contig11803_306	2A	247	0.002358	0.20	33	15	1.54
Tdurum_contig11803_475	2A	250	0.002141	0.21	32	16	1.54
Tdurum_contig11803_850	2A	250	0.002141	0.21	32	16	1.54
Tdurum_contig11803_935	2A	250	0.002358	0.20	33	15	1.54
Excalibur_c21752_768	2B	254	0.002462	0.20	4	44	-7.13
Kukri_c12368_82	2B	254	0.002462	0.20	4	44	-7.13
wsnp_Ex_c15269_23491104	3A	284	0.001259	0.23	5	43	-6.17
wsnp_Ex_c15269_23492289	3A	284	0.001259	0.23	5	43	-6.17
Excalibur_c50192_149	4A	479	0.002751	0.19	4	44	-3.84
wsnp_BG313770B_Ta_1_1	4A	479	0.002751	0.19	4	44	3.84
Ra_c60252_743	4A	497	0.001757	0.21	7	41	-3.14
Ra_c60252_1733	4A	497	0.001757	0.21	41	7	3.14
Tdurum_contig43961_607	4A	532	0.001773	0.21	5	43	-3.58

BS00034147_51	4B	333	0.002932	0.19	19	29	-1.67
BobWhite_c2236_111	5A	248	0.001259	0.23	43	5	6.17
BS00109052_51	5A	249	0.001259	0.23	43	5	6.17
Excalibur_c6314_91	5A	265	0.002780	0.19	40	8	2.88
wsnp_Ex_c1279_2451699	5A	278	0.001090	0.24	44	4	3.91
BobWhite_c6759_365	5A	290	0.001090	0.24	44	4	3.91
BobWhite_rep_c64315_180	5A	496	0.002609	0.20	5	43	-5.31
IACX7928	5B	194	0.001835	0.21	40	8	2.65
Excalibur_c5329_1335	5B	342	0.001902	0.21	41	7	2.66
GENE-4826_86	6A	81	0.002197	0.20	9	39	-4.04
RAC875_c19631_269	7A	153	0.000323	0.29	42	6	3.24
Tdurum_contig11557_86	7A	360	0.002462	0.20	4	44	-7.13
GENE-4826_641	7B	182	0.002197	0.20	9	39	-4.04
Tdurum_contig10932_375	7B	182	0.002197	0.20	9	39	-4.04
wsnp_Ku_c665_1371121	7B	186	0.001542	0.22	43	5	3.55
wsnp_Ex_c36325_44308589	7B	187	0.002462	0.20	4	44	-7.13
Excalibur_c41549_276	7B	229	0.002751	0.19	4	44	-3.84
BobWhite_c8027_421	7B	231	0.001703	0.22	6	42	-3.39
RAC875_c57326_85	7B	428	0.001437	0.22	36	12	3.30
IAAV9045	7B	429	0.001437	0.22	36	12	3.30
BobWhite_c28058_232	7B	429	0.002612	0.20	13	35	-3.10
wsnp_Ex_c8400_14157060	7B	429	0.002612	0.20	13	35	-3.10
IAAV4133	7D	299	0.001503	0.22	42	6	4.86

Table 34: Significant markers for plant height (PH) at a -log10(P-value) threshold of 2.3 with position on chromosome (cM) and allele effects for winter wheat lines where n number of 'a' lines are indicated by a positive effect and 'b' a negative effect

Marker	Chromosome	Position	P-value	R^2	n = a	n = b	Effect
tplb0050c03_1003	1B	209	0.004056	0.20	**40**	9	-12.15
BS00055866_51	1B	276	0.004173	0.20	**35**	14	-15.95
GENE-0487_644	1B	276	0.004173	0.20	**35**	14	-15.95
IACX11274	1B	276	0.004173	0.20	**35**	14	-15.95
RAC875_c87950_333	1B	276	0.004173	0.20	**35**	14	-15.95
Excalibur_c7449_587	2B	363	0.004314	0.20	**14**	35	17.12
Tdurum_contig10219_295	2B	363	0.004314	0.20	**14**	35	17.12
Kukri_c52356_96	2B	364	0.004727	0.19	**14**	35	16.20
Excalibur_c46178_303	2B	365	0.004314	0.20	**14**	35	17.12
RFL_Contig3044_346	2B	365	0.004314	0.20	**14**	35	17.12
Ku_c9369_1965	2B	374	0.004314	0.20	**14**	35	17.12
wsnp_BE444144D_Ta_1_1	2D	118	0.002032	0.24	**22**	27	14.55
wsnp_Ex_rep_c101340_86719115	3A	276	0.003777	0.20	**35**	14	-10.70
wsnp_Ex_rep_c101340_86719239	3A	276	0.003777	0.20	**35**	14	-10.70
Tdurum_contig67686_851	3A	578	0.004137	0.20	**42**	7	-18.06
RAC875_c37611_302	4A	191	0.001510	0.25	**39**	10	-22.88
wsnp_Ex_c13091_20706489	4A	191	0.001510	0.25	**39**	10	-22.88
Ex_c5979_1449	4A	191	0.002666	0.22	**44**	5	-27.64
IAAV6309	4A	191	0.002666	0.22	**44**	5	-27.64
Kukri_c61419_550	4A	191	0.002666	0.22	**44**	5	-27.64
RAC875_c42756_168	4A	191	0.002666	0.22	**44**	5	-27.64
Ra_c45147_1600	4A	191	0.002666	0.22	**44**	5	-27.64
tplb0051b16_1324	4A	191	0.002666	0.22	**44**	5	-27.64
Rht-D1	4D	117	0.003130	0.21	**27**	22	13.28
Kukri_c2781_719	5A	252	0.002521	0.22	**41**	8	-28.58
CAP11_c1685_149	5A	484	0.001054	0.27	**14**	35	15.87
Kukri_c6266_260	5A	484	0.001054	0.27	**14**	35	15.87
Kukri_c5685_1066	5B	359	0.004674	0.19	**10**	39	14.73
RFL_Contig5616_1779	5B	571	0.001630	0.25	**34**	15	-10.36
RAC875_rep_c106589_784	5B	659	0.003467	0.21	**6**	43	16.40
Tdurum_contig92922_58	5B	659	0.003467	0.21	**6**	43	16.40
Kukri_c35661_63	6A	131	0.003436	0.21	**31**	18	-13.44
Kukri_rep_c104648_439	6A	131	0.003436	0.21	**31**	18	-13.44
Tdurum_contig62141_496	6A	135	0.003436	0.21	**31**	18	-13.44
Tdurum_contig62141_93	6A	135	0.003436	0.21	**31**	18	-13.44
BS00011072_51	7A	364	0.004922	0.19	**33**	16	-10.71
wsnp_Ex_c5341_9442913	7A	364	0.004922	0.19	**33**	16	-10.71
wsnp_BE445506B_Ta_2_4	7B	510	0.004510	0.20	**32**	17	-11.16

Appendix 3

Table 35: Significant markers for anther extrusion (AE) at a -log10(P-value) threshold of 2.5 with position on chromosome (cM) and allele effects for spring wheat lines where n number of 'a' lines are indicated by a positive effect and 'b' a negative effect

Marker	Chromosome	Position	P-value	R^2	n = a	n = b	Effect
RAC875_c14066_452	1A	182	0.002393	0.08	**111**	12	2.19
BS00025965_51	1B	129	0.002585	0.08	**35**	88	-1.39
Tdurum_contig28899_127	1B	215	0.000686	0.10	**93**	30	-1.85
BS00022581_51	1B	216	0.000686	0.10	**30**	93	1.85
BS00060270_51	1B	216	0.000686	0.10	**30**	93	1.85
BS00071555_51	1B	216	0.000686	0.10	**30**	93	1.85
Excalibur_c57972_116	1B	216	0.000686	0.10	**30**	93	1.85
Tdurum_contig8081_2331	1B	216	0.000686	0.10	**30**	93	1.85
Tdurum_contig57127_56	1B	216	0.001085	0.09	**33**	90	1.70
BobWhite_c11460_291	1B	216	0.001181	0.09	**31**	92	1.76
Kukri_c20927_339	1B	216	0.002129	0.08	**29**	94	1.72
wsnp_CAP8_c2677_1394934	2A	355	0.002389	0.08	**35**	88	1.46
BS00000297_51	2A	386	0.001206	0.09	**37**	86	1.59
wsnp_Ex_c3808_6925015	2A	413	0.002293	0.08	**50**	73	-1.31
Excalibur_c7964_1290	2B	458	0.000466	0.11	**19**	104	1.97
Tdurum_contig57254_254	2B	458	0.000466	0.11	**19**	104	1.97
wsnp_Ex_c18883_27772081	3A	169	0.000643	0.10	**97**	26	-1.72
BS00056089_51	3A	333	0.001827	0.08	**29**	94	-1.87
BS00056258_51	3B	206	0.002840	0.08	**82**	41	-1.33
IAAV5302	3B	347	0.002534	0.08	**92**	31	-1.74
Ku_c10913_2542	4A	293	0.001527	0.09	**97**	26	1.83
Excalibur_c65272_341	4A	641	0.001636	0.09	**112**	11	-2.37
BobWhite_c11405_356	5A	267	0.002411	0.08	**104**	19	2.00
GENE-3493_612	5A	267	0.002411	0.08	**104**	19	2.00
Jagger_c1611_158	5A	267	0.002411	0.08	**104**	19	2.00
Kukri_rep_c103150_398	5B	88	0.000780	0.10	**68**	55	-1.46
BobWhite_c5782_825	6A	158	0.001636	0.09	**118**	5	3.86
BS00041481_51	6A	186	0.002521	0.08	**28**	95	2.03
wsnp_Ex_c4480_8054926	6D	352	0.001491	0.09	**57**	66	-1.33
BS00083421_51	7D	323	0.002644	0.08	**65**	58	1.46
BS00023150_51	7D	332	0.003136	0.08	**103**	20	-1.65
D_contig65328_393	7D	474	0.002207	0.08	**15**	108	1.84
barc125_170	SSR 3D	205	0.001636	0.09	**118**	5	3.86
barc40_233	SSR 5A	-	0.002712	0.08	**111**	12	2.13
gwm320_275	SSR 2DL	-	0.001938	0.08	**110**	13	1.99

Table 36: Significant markers for *Fusarium* head blight (FHB) at a -log10(P-value) threshold of 2.5 with position on chromosome (cM) and allele effects for spring wheat lines where n number of 'a' lines are indicated by a positive effect and 'b' a negative effect

Marker	Chromosome	Position	P-value	R^2	n = a	n = b	Effect
BS00021889_51	1A	240	0.001687	0.08	52	69	-13.38
wsnp_Ra_c16080_24638622	1A	241	0.001803	0.08	73	48	-11.58
RAC875_c38916_66	1A	241	0.001803	0.08	48	73	11.58
BS00035273_51	1A	462	0.003109	0.07	89	32	11.55
RAC875_c26469_480	2B	245	0.000549	0.10	91	30	-16.35
RAC875_c34516_316	2B	245	0.001897	0.08	21	100	16.84
RAC875_c15396_90	2B	327	0.001186	0.09	100	21	-16.45
Kukri_c50842_573	2B	334	0.001186	0.09	21	100	16.45
Tdurum_contig13653_255	2B	334	0.001186	0.09	21	100	16.45
BS00100563_51	2B	342	0.001186	0.09	100	21	-16.45
TA004152-0921	2B	342	0.001186	0.09	21	100	16.45
IAAV4899	2B	350	0.001170	0.09	73	48	-13.42
IACX5919	2B	350	0.001170	0.09	73	48	-13.42
RAC875_rep_c74537_344	2B	350	0.001170	0.09	73	48	-13.42
BS00016650_51	2B	350	0.002692	0.07	72	49	-12.30
Excalibur_c766_705	3B	558	0.000998	0.09	45	76	12.68
wsnp_Ex_c48449_53350799	4A	228	0.002077	0.08	11	110	19.42
Tdurum_contig64772_417	4B	148	0.000319	0.11	35	86	14.08
Rht-B1	4B	159	0.000113	0.12	77	44	-15.28
RAC875_c27536_611	4B	159	0.000702	0.09	81	40	16.76
Tdurum_contig42229_113	4B	162	0.001695	0.08	76	45	-12.32
Excalibur_c36630_2194	4B	162	0.002623	0.07	59	62	-11.68
BS00021984_51	4B	163	0.000303	0.11	67	54	-13.33
BobWhite_rep_c49034_167	4B	163	0.000700	0.09	77	44	-13.38
RAC875_rep_c105718_430	4B	163	0.001695	0.08	76	45	-12.32
Tdurum_contig33737_157	4B	163	0.001695	0.08	76	45	-12.32
RAC875_c19303_228	4B	163	0.003123	0.07	68	53	-11.48
wsnp_Ex_c32127_40841791	4B	221	0.001477	0.08	11	110	18.84
CAP12_c2983_140	4B	223	0.000116	0.12	17	104	19.46
wsnp_Ku_c51039_56457361	5A	335	0.001280	0.08	45	76	15.49
RAC875_c79649_582	5B	36	0.002015	0.08	88	33	15.00
BS00078784_51	5B	448	0.001909	0.08	79	42	-16.01
Kukri_c11992_240	6B	133	0.002555	0.07	31	90	12.00
RFL_Contig5693_807	6B	134	0.003100	0.07	14	107	16.51
Tdurum_contig33428_272	6B	134	0.003100	0.07	14	107	16.51
Kukri_c33620_129	7A	624	0.001473	0.08	69	52	12.43
BobWhite_c23455_184	7B	287	0.003095	0.07	68	53	-11.31
mag548a_null	SSR 2BL	350	0.002525	0.07	46	75	11.51
wmc559_274	SSR 3A	-	0.001526	0.08	82	39	-11.56

Table 37: Significant markers for *Fusarium* head blight after regression (FHBreg) at a -log10(P-value) threshold of 2.5 with position on chromosome (cM) and allele effects for spring wheat lines where n number of 'a' lines are indicated by a positive effect and 'b' a negative effect

Marker	Chromosome	Position	P-value	R^2	n = a	n = b	Effect
BS00063068_51	1A	230	0.000880	0.08	57	64	-10.30
BobWhite_rep_c49207_243	1A	230	0.001128	0.08	67	54	10.15
IAAV213	1A	235	0.000310	0.10	43	78	1.14
wsnp_Ex_c25734_34995416	1A	235	0.001128	0.08	67	54	10.15
wsnp_Ku_c557_1166684	1A	235	0.001128	0.08	67	54	10.15
BS00033469_51	1A	235	0.001321	0.08	63	58	9.75
RAC875_c41275_131	1A	235	0.001321	0.08	63	58	9.75
Ra_c58315_265	1A	235	0.001321	0.08	63	58	9.75
wsnp_Ex_c56097_58351893	1A	235	0.001321	0.08	63	58	9.75
wsnp_Ex_c56097_58352130	1A	235	0.001321	0.08	63	58	9.75
wsnp_Ra_c16080_24638622	1A	241	0.000146	0.11	73	48	-12.51
RAC875_c38916_66	1A	241	0.000146	0.11	48	73	12.51
BS00110278_51	1B	294	0.000267	0.10	21	100	15.04
Excalibur_c94658_59	1B	301	0.000713	0.09	96	25	-12.99
Tdurum_contig10380_87	2B	344	0.001828	0.07	34	87	-11.48
BS00010012_51	2B	350	0.001869	0.07	44	77	10.65
IAAV4899	2B	350	0.002106	0.07	73	48	-10.47
IACX5919	2B	350	0.002106	0.07	73	48	-10.47
RAC875_rep_c74537_344	2B	350	0.002106	0.07	73	48	-10.47
BS00016650_51	2B	350	0.002358	0.07	72	49	-10.27
wsnp_Ex_c13686_21480826	2D	8	0.001411	0.08	9	112	-17.03
Kukri_c26676_225	2D	145	0.001631	0.08	99	22	11.83
Ex_c24554_1583	3A	391	0.003016	0.07	41	80	8.62
Kukri_rep_c102621_659	3B	249	0.003042	0.07	35	86	11.41
Kukri_c20199_83	3B	250	0.003042	0.07	35	86	11.41
Kukri_rep_c101837_143	3B	250	0.003042	0.07	35	86	11.41
Excalibur_c766_705	3B	558	0.001644	0.08	45	76	9.90
wsnp_Ex_c1279_2451699	5A	278	0.000666	0.09	109	12	17.56
Ku_c47168_563	5A	285	0.002691	0.07	107	14	14.28
BobWhite_c6759_365	5A	290	0.000666	0.09	109	12	17.56
wsnp_Ex_c7168_12311649	5A	290	0.000666	0.09	109	12	17.56
wsnp_Ex_c1279_2451582	5A	291	0.002691	0.07	107	14	14.28
RAC875_c2291_123	6B	133	0.001085	0.08	25	96	12.26
Kukri_c11992_240	6B	133	0.002147	0.07	31	90	10.09
Kukri_rep_c104879_103	6B	133	0.002903	0.07	51	70	9.97
Excalibur_c46399_307	6B	134	0.000767	0.09	21	100	13.22
BS00110803_51	6B	134	0.001085	0.08	25	96	12.26
TA004901-0137	6B	134	0.001269	0.08	12	109	15.55
BS00090070_51	6B	134	0.001295	0.08	111	10	-17.43
RAC875_c13920_747	6B	134	0.001295	0.08	111	10	-17.43
RAC875_rep_c72491_171	6B	134	0.001295	0.08	111	10	-17.43
BS00003891_51	6B	134	0.001295	0.08	10	111	17.43
GENE-0418_209	6B	134	0.001295	0.08	10	111	17.43
IACX6021	6B	134	0.001295	0.08	10	111	17.43

IACX6023	6B	134	0.001295	0.08	10	111	17.43
IACX9024	6B	134	0.001379	0.08	108	13	-14.96
Kukri_c61725_362	6B	134	0.001379	0.08	108	13	-14.96
Kukri_c61725_545	6B	134	0.001379	0.08	108	13	-14.96
Tdurum_contig9612_971	6B	134	0.001379	0.08	13	108	14.96
RFL_Contig5693_807	6B	134	0.001393	0.08	14	107	14.51
Tdurum_contig33428_272	6B	134	0.001393	0.08	14	107	14.51
RAC875_c19425_903	6B	134	0.001434	0.08	11	110	16.54
BS00090069_51	6B	134	0.001688	0.08	19	102	12.42
Kukri_c61725_298	6B	134	0.001784	0.07	107	14	-13.98
Tdurum_contig62941_85	6B	134	0.002903	0.07	51	70	9.97
D_F1BEJMU01AK2KX_99	6B	134	0.002985	0.07	12	109	14.19
RAC875_c13920_836	6B	135	0.001295	0.08	10	111	17.43
Kukri_c8343_228	6B	146	0.002367	0.07	76	45	-10.26
tplb0024k14_744	6B	146	0.002367	0.07	76	45	-10.26
wsnp_Ex_c8011_13585237	6B	146	0.002367	0.07	76	45	-10.26
tplb0024k14_2098	6B	149	0.002367	0.07	45	76	10.26
tplb0024k14_829	6B	149	0.002367	0.07	45	76	10.26
wsnp_Ku_c5160_9203385	6B	416	0.002669	0.07	54	67	8.65
wsnp_Ex_c5177_9174930	7A	398	0.000177	0.11	48	73	12.51
wsnp_RFL_Contig2864_2688208	7A	398	0.000177	0.11	57	64	11.48
RAC875_rep_c72877_159	7B	216	0.002168	0.07	76	45	-12.86
mag548a_null	SSR 2BL	350	0.000815	0.09	46	75	10.84
wmc559_274	SSR 3A	-	0.001555	0.08	82	39	-10.05

Table 38: Significant markers for deoxynivalenol (DON) at a -log10(P-value) threshold of 3.0 with position on chromosome (cM) and allele effects for spring wheat lines where n number of 'a' lines are indicated by a positive effect and 'b' a negative effect

Marker	Chromosome	Position	P-value	R^2	n = a	n = b	Effect
Tdurum_contig42153_5854	2A	173	0.000398	0.11	106	15	8.97
RAC875_rep_c111906_144	2A	173	0.000398	0.11	15	106	-8.97
Tdurum_contig42153_5214	2A	173	0.000398	0.11	15	106	-8.97
Tdurum_contig42153_5454	2A	173	0.000398	0.11	15	106	-8.97
Tdurum_contig42153_6232	2A	173	0.000398	0.11	15	106	-8.97
Tdurum_contig42153_7329	2A	173	0.000398	0.11	15	106	-8.97
Tdurum_contig42153_6439	2A	173	0.000970	0.09	107	14	8.40
Excalibur_rep_c109101_94	2D	6	0.000389	0.11	110	11	-10.00
RAC875_rep_c109554_198	3A	604	0.000170	0.12	114	7	-13.97
Excalibur_c17654_166	3A	617	0.000170	0.12	114	7	-13.97
wsnp_BE405275A_Ta_1_1	4A	116	0.000631	0.10	12	109	9.59
Excalibur_c10390_104	4A	185	0.000828	0.09	101	20	8.62
wsnp_Ex_c48449_53350799	4A	228	0.000430	0.11	11	110	11.45
Rht-B1	4B	159	0.000012	0.17	77	44	-8.28
BS00021984_51	4B	163	0.000273	0.11	67	54	-6.30
CAP12_c2983_140	4B	223	0.000223	0.12	17	104	8.59
Tdurum_contig10466_87	4B	226	0.000469	0.10	111	10	-12.12
RAC875_c67417_275	4B	230	0.000469	0.10	10	111	12.12
BS00064853_51	5B	447	0.000136	0.12	78	43	-8.49
Ra_c45135_456	5B	447	0.000219	0.12	76	45	-7.93
Excalibur_c45488_148	5B	447	0.000946	0.09	48	73	6.47
BS00078784_51	5B	448	0.000032	0.15	79	42	-9.58
BS00028082_51	5B	448	0.000264	0.11	44	77	7.96
BS00062781_51	6A	239	0.000768	0.10	34	87	-6.25
wsnp_JD_c1635_2290177	7A	676	0.000702	0.10	104	17	8.54

Table 39: Significant markers for deoxynivalenol after regression (DONreg) at a -log10(P-value) threshold of 3.0 with position on chromosome (cM) and allele effects for spring wheat lines where n number of 'a' lines are indicated by a positive effect and 'b' a negative effect

Marker	Chromosome	Position	P-value	R^2	n = a	n = b	Effect
RAC875_c37934_285	1A	149	0.000547	0.10	5	116	-11.21
Tdurum_contig42153_5854	2A	173	0.000939	0.09	106	15	6.69
RAC875_rep_c111906_144	2A	173	0.000939	0.09	15	106	-6.69
Tdurum_contig42153_5214	2A	173	0.000939	0.09	15	106	-6.69
Tdurum_contig42153_5454	2A	173	0.000939	0.09	15	106	-6.69
Tdurum_contig42153_6232	2A	173	0.000939	0.09	15	106	-6.69
Tdurum_contig42153_7329	2A	173	0.000939	0.09	15	106	-6.69
BS00022896_51	2A	366	0.000314	0.11	104	17	-8.28
BS00012320_51	2A	368	0.000350	0.11	18	103	7.79
RAC875_c38018_278	2A	368	0.000350	0.11	18	103	7.79
RFL_Contig4517_1300	2A	368	0.000350	0.11	103	18	-7.79
Excalibur_c42364_134	2B	330	0.000140	0.12	9	112	-11.05
GENE-1667_528	2B	330	0.000140	0.12	9	112	-11.05
Excalibur_rep_c109101_94	2D	6	0.000857	0.09	110	11	-7.49
BobWhite_c13322_215	4A	200	0.000808	0.09	11	110	8.65
wsnp_Ex_c1563_2986030	4A	200	0.000808	0.09	11	110	8.65
wsnp_Ex_rep_c101638_86971861	4A	200	0.000808	0.09	11	110	8.65
wsnp_Ex_rep_c66706_65037564	4A	200	0.000808	0.09	110	11	-8.65
wsnp_Ex_c2403_4502745	4A	200	0.000924	0.09	9	112	9.34
BobWhite_c1593_539	4A	200	0.000924	0.09	112	9	-9.34
BobWhite_c4931_170	4A	200	0.000924	0.09	112	9	-9.34
Jagger_c2057_97	4A	200	0.000924	0.09	112	9	-9.34
wsnp_BG604678A_Ta_1_2	4A	200	0.000924	0.09	112	9	-9.34
wsnp_Ex_c12933_20488438	4A	200	0.000924	0.09	112	9	-9.34
wsnp_Ex_c64593_63334637	4A	200	0.000924	0.09	112	9	-9.34
Kukri_c29625_198	4A	203	0.000924	0.09	112	9	-9.34
wsnp_Ex_c829_1620518	4A	203	0.000924	0.09	112	9	-9.34
wsnp_Ex_c20899_30011827	5A	737	0.000740	0.10	106	15	-7.51
BobWhite_c47401_491	5A	737	0.000740	0.10	15	106	7.51
RAC875_rep_c76772_850	7A	371	0.000462	0.10	117	4	12.75
wsnp_Ex_c5177_9174930	7A	398	0.000916	0.09	57	64	4.47
wsnp_RFL_Contig2864_2688208	7A	398	0.000916	0.09	57	64	4.47
Ex_c68356_553	7B	216	0.000823	0.09	18	103	-8.59
RAC875_c4453_2678	7D	248	0.000435	0.10	109	12	7.94
wsnp_Ex_c20320_29383710	7D	248	0.000435	0.10	109	12	7.94
RAC875_c1863_3196	7D	248	0.000435	0.10	12	109	-7.94
wsnp_Ex_c20320_29383285	7D	248	0.000435	0.10	12	109	-7.94
wsnp_Ex_c20320_29383733	7D	248	0.000435	0.10	12	109	-7.94

Table 40: Significant markers for anther extrusion (AE) at a -log10(P-value) threshold of 2.0 with position on chromosome (cM) and allele effects for winter wheat lines where n number of 'a' lines are indicated by a positive effect and 'b' a negative effect

Marker	Chromosome	Position	P-value	R^2	n = a	n = b	Effect
BS00064205_51	1A	51	0.005042	0.18	**21**	28	-1.32
BobWhite_c31470_532	1A	51	0.005477	0.18	**20**	29	-1.31
TA005251-0278	1B	287	0.005348	0.18	**32**	17	-1.46
Kukri_c66214_59	1B	344	0.009959	0.15	**42**	7	1.61
BS00065468_51	3A	246	0.006183	0.17	**34**	15	-1.35
BS00007502_51	3A	247	0.006183	0.17	**34**	15	-1.35
BobWhite_c11298_512	3A	247	0.006183	0.17	**34**	15	-1.35
Excalibur_c24123_165	3A	247	0.006183	0.17	**34**	15	-1.35
IAAV2646	3A	247	0.006183	0.17	**34**	15	-1.35
Kukri_c28917_96	3A	247	0.006183	0.17	**34**	15	-1.35
Ra_c17608_960	3B	56	0.007910	0.16	**15**	34	-1.26
BS00084883_51	3B	268	0.002963	0.21	**38**	11	-1.83
BobWhite_c11540_60	3B	268	0.003982	0.19	**37**	12	-1.72
tplb0031e09_1230	3B	269	0.002963	0.21	**38**	11	-1.83
tplb0031e09_1763	3B	269	0.002963	0.21	**38**	11	-1.83
wsnp_Ex_c238_460841	3B	277	0.006397	0.17	**6**	43	1.92
Kukri_c29615_377	3B	561	0.008471	0.16	**19**	30	1.37
wsnp_Ex_c19207_28125072	4A	272	0.006649	0.17	**13**	36	1.44
Tdurum_contig97887_268	4A	276	0.006649	0.17	**13**	36	1.44
RAC875_rep_c117027_577	4A	575	0.007486	0.17	**5**	44	2.41
RAC875_c11702_1015	4A	598	0.003397	0.20	**39**	10	-1.82
Tdurum_contig75584_1118	4A	603	0.000878	0.27	**11**	38	2.02
RAC875_c55173_65	4A	603	0.003233	0.20	**39**	10	-1.82
BS00034148_51	4B	331	0.004488	0.19	**27**	22	1.38
wsnp_Ex_c16551_25061395	5A	113	0.002494	0.22	**11**	38	-1.66
Excalibur_c5612_711	6D	295	0.003500	0.20	**15**	34	1.60
BS00021666_51	7B	497	0.005941	0.18	**44**	5	-2.02
Kukri_c34056_329	7D	297	0.009371	0.16	**12**	37	1.63
wsnp_bm138650D_Ta_2_2	7D	297	0.009371	0.16	**12**	37	1.63
cfd018b_207	SSR 5D	-	0.007740	0.16	**37**	12	-1.38
cfd018b_215	SSR 5D	-	0.009858	0.15	**34**	15	1.25
gwm301_239	SSR 2DL	-	0.000691	0.28	**44**	5	-2.73

Table 41: Significant markers for *Fusarium* head blight (FHB) at a -log10(P-value) threshold of 2.0 with position on chromosome (cM) and allele effects for winter wheat lines where n number of 'a' lines are indicated by a positive effect and 'b' a negative effect

Marker	Chromosome	Position	P-value	R^2	n = a	n = b	Effect
BS00110709_51	1A	89	0.005425	0.19	**8**	41	22.15
BS00022270_51	1A	109	0.005425	0.19	**8**	41	22.15
Kukri_c2121_1334	1A	117	0.000980	0.27	**15**	34	20.65
wsnp_JD_c7522_8606553	1A	117	0.002869	0.22	**14**	35	18.75
BS00011478_51	2A	466	0.004929	0.19	**35**	14	-17.96
BS00062974_51	3A	47	0.006575	0.18	**10**	39	18.25
RAC875_rep_c77067_347	3A	67	0.004374	0.20	**6**	43	31.59
RAC875_c371_251	3A	107	0.008478	0.17	**31**	18	-14.66
IAAV1328	3A	122	0.007713	0.17	**42**	7	22.31
Excalibur_c4548_2505	3A	122	0.008438	0.17	**8**	41	-20.76
Excalibur_c4548_2697	3A	122	0.008438	0.17	**8**	41	-20.76
wsnp_Ex_c4548_8166555	3A	122	0.008438	0.17	**8**	41	-20.76
wsnp_Ra_c9738_16173810	3A	122	0.008438	0.17	**8**	41	-20.76
wsnp_Ra_c9738_16174002	3A	122	0.008438	0.17	**8**	41	-20.76
RAC875_rep_c113906_294	3B	286	0.003978	0.20	**43**	6	-30.60
RAC875_c530_354	3B	308	0.001651	0.25	**5**	44	40.49
wsnp_Ex_c6245_10887043	3B	308	0.001651	0.25	**5**	44	40.49
Tdurum_contig43874_1129	5A	245	0.000869	0.28	**37**	12	-22.93
Excalibur_c23748_1233	6A	103	0.009086	0.16	**40**	9	-21.25
BS00023627_51	6A	150	0.002182	0.23	**16**	33	-18.36
wsnp_JD_c2180_3000498	6A	151	0.007677	0.17	**18**	31	-15.99
wsnp_CAP11_c1137_665073	6A	204	0.003818	0.20	**26**	23	-17.30
wsnp_CAP11_c1137_665340	6A	204	0.008286	0.17	**17**	32	-16.41
CAP11_rep_c6843_59	6A	204	0.009011	0.16	**18**	31	-15.47
wsnp_CAP11_c303_253438	6A	204	0.009011	0.16	**18**	31	-15.47
CAP12_rep_c4571_181	6B	229	0.007782	0.17	**44**	5	28.81
Kukri_c16568_287	6B	229	0.007782	0.17	**44**	5	28.81
TA003659-1136	6B	229	0.007782	0.17	**44**	5	28.81
BS00011795_51	6B	408	0.002772	0.22	**12**	37	22.37
Kukri_c45876_157	6B	411	0.007922	0.17	**13**	36	19.02
IACX7844	6B	416	0.004473	0.20	**11**	38	22.21
Kukri_c46218_66	7A	376	0.008944	0.16	**14**	35	-15.85
wsnp_Ex_c10193_16730348	7B	323	0.007415	0.17	**10**	39	17.65
BS00039502_51	7B	330	0.006194	0.18	**11**	38	17.61
BS00080621_51	7B	337	0.007415	0.17	**10**	39	17.65
BS00110528_51	7B	464	0.007556	0.17	**34**	15	-15.80
CAP12_c1587_70	7B	464	0.007556	0.17	**34**	15	-15.80
CAP12_c1587_142	7B	464	0.007556	0.17	**15**	34	15.80

Table 42: Significant markers for *Fusarium* head blight after regression (FHBreg) at a -log10(P-value) threshold of 2.0 with position on chromosome (cM) and allele effects for winter wheat lines where n number of 'a' lines are indicated by a positive effect and 'b' a negative effect

Marker	Chromosome	Position	P-value	R^2	n = a	n = b	Effect
BS00110709_51	1A	89	0.007770	0.17	**8**	41	21.26
BS00022270_51	1A	109	0.007770	0.17	**8**	41	21.26
Kukri_c2121_1334	1A	117	0.001051	0.27	**15**	34	20.48
wsnp_JD_c7522_8606553	1A	117	0.003331	0.21	**14**	35	18.38
Tdurum_contig83079_133	1B	287	0.008227	0.17	**15**	34	15.28
BobWhite_c39656_106	1B	289	0.009133	0.16	**13**	36	15.69
Ku_c9909_1766	1B	289	0.009133	0.16	**13**	36	15.69
Kukri_rep_c112383_588	1B	289	0.009133	0.16	**13**	36	15.69
BS00011478_51	2A	466	0.007581	0.17	**35**	14	-16.94
BS00062974_51	3A	47	0.006760	0.18	**10**	39	18.30
RAC875_rep_c77067_347	3A	67	0.004352	0.20	**6**	43	31.61
RAC875_c371_251	3A	107	0.004445	0.20	**31**	18	-15.85
RAC875_rep_c113906_294	3B	286	0.004335	0.20	**43**	6	-30.29
RAC875_c530_354	3B	308	0.001725	0.24	**5**	44	40.07
wsnp_Ex_c6245_10887043	3B	308	0.001725	0.24	**5**	44	40.07
Tdurum_contig43874_1129	5A	245	0.000541	0.30	**37**	12	-23.92
Excalibur_c23748_1233	6A	103	0.008252	0.17	**40**	9	-21.44
BS00023627_51	6A	150	0.003031	0.21	**16**	33	-17.66
wsnp_JD_c2180_3000498	6A	151	0.007980	0.17	**18**	31	-15.89
wsnp_CAP11_c1137_665073	6A	204	0.004603	0.19	**26**	23	-16.90
CAP11_rep_c6843_59	6A	204	0.009189	0.16	**18**	31	-15.51
wsnp_CAP11_c303_253438	6A	204	0.009189	0.16	**18**	31	-15.51
wsnp_CAP11_c1137_665340	6A	204	0.009522	0.16	**17**	32	-16.12
BS00011795_51	6B	408	0.003554	0.21	**12**	37	20.92
Kukri_c45876_157	6B	411	0.008215	0.17	**13**	36	18.55
IACX7844	6B	416	0.005133	0.19	**11**	38	21.12
Kukri_c46218_66	7A	376	0.006302	0.18	**14**	35	-16.63
wsnp_Ex_c10193_16730348	7B	323	0.006486	0.18	**10**	39	18.15
BS00039502_51	7B	330	0.005910	0.18	**11**	38	17.86
BS00080621_51	7B	337	0.006486	0.18	**10**	39	18.15
CAP12_c1587_142	7B	464	0.009195	0.16	**15**	34	15.37
BS00110528_51	7B	464	0.009195	0.16	**34**	15	-15.37
CAP12_c1587_70	7B	464	0.009195	0.16	**34**	15	-15.37

Table 43: Significant markers for deoxynivalenol (DON) at a -log10(P-value) threshold of 2.0 with position on chromosome (cM) and allele effects for winter wheat lines where n number of 'a' lines are indicated by a positive effect and 'b' a negative effect

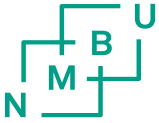
Marker	Chromosome	Position	P-value	R^2	n = a	n = b	Effect
BS00066014_51	1B	185	0.004154	0.20	**14**	35	3.63
Tdurum_contig10362_328	1B	304	0.004235	0.20	**32**	17	3.04
Ku_c33021_109	2A	355	0.003892	0.20	**39**	10	-3.62
CAP12_rep_c3989_239	2B	431	0.002869	0.22	**21**	28	4.06
wsnp_BE488779D_Ta_1_2	2D	110	0.003854	0.20	**25**	24	-2.46
BS00011109_51	2D	145	0.007490	0.17	**6**	43	5.03
BS00058861_51	3B	43	0.008885	0.16	**6**	43	4.80
Tdurum_contig81905_502	4B	159	0.008585	0.17	**9**	40	5.98
BS00062691_51	4B	180	0.004226	0.20	**9**	40	3.48
BS00067428_51	4B	180	0.004226	0.20	**9**	40	3.48
BS00074440_51	4B	180	0.004226	0.20	**9**	40	3.48
TA004522-0516	4B	180	0.004226	0.20	**9**	40	3.48
GENE-2129_76	4B	183	0.004226	0.20	**9**	40	3.48
wsnp_Ex_c9301_15450818	5A	79	0.003679	0.21	**32**	17	3.36
BS00066144_51	5D	264	0.005712	0.18	**34**	15	2.81
wsnp_Ex_c11055_17928283	5D	525	0.005545	0.19	**29**	20	-3.42
BS00079664_51	5D	525	0.005630	0.18	**26**	23	-3.09
wsnp_Ex_c24145_33394644	5D	525	0.005630	0.18	**26**	23	-3.09
BS00109576_51	6A	172	0.007467	0.17	**30**	19	-3.41
Kukri_c21943_466	6A	172	0.007467	0.17	**30**	19	-3.41
Kukri_c48959_337	6A	172	0.007467	0.17	**30**	19	-3.41
RAC875_c39200_260	6A	172	0.007467	0.17	**30**	19	-3.41
RAC875_c64852_655	6A	172	0.007467	0.17	**30**	19	-3.41
TA005243-1174	6A	172	0.007467	0.17	**30**	19	-3.41
wsnp_Ex_c25300_34566908	6A	172	0.007467	0.17	**30**	19	-3.41
wsnp_Ex_c55340_57883276	6A	172	0.007467	0.17	**30**	19	-3.41
wsnp_Ex_rep_c69191_68104835	6A	172	0.007467	0.17	**30**	19	-3.41
wsnp_Ku_c18534_27848426	6A	172	0.007467	0.17	**30**	19	-3.41
BS00067619_51	6A	173	0.007467	0.17	**30**	19	-3.41
BobWhite_c15977_107	6A	173	0.007467	0.17	**30**	19	-3.41
CAP11_c862_116	6A	173	0.007467	0.17	**30**	19	-3.41
Excalibur_c44694_414	6A	173	0.007467	0.17	**30**	19	-3.41
GENE-3798_1143	6A	173	0.007467	0.17	**30**	19	-3.41
Kukri_c12641_1185	6A	173	0.007467	0.17	**30**	19	-3.41
wsnp_Ex_c14975_23127669	6A	173	0.007467	0.17	**30**	19	-3.41
wsnp_Ex_c32765_41369642	6A	173	0.007467	0.17	**30**	19	-3.41
wsnp_Ex_c55340_57883479	6A	173	0.007467	0.17	**30**	19	-3.41
wsnp_Ex_c99215_85409445	6A	173	0.007467	0.17	**30**	19	-3.41
Tdurum_contig13240_523	6A	204	0.003273	0.21	**20**	29	-3.37
wsnp_Ex_c17185_25829084	6A	204	0.003273	0.21	**20**	29	-3.37
wsnp_Ex_c902_1745108	6A	204	0.003273	0.21	**20**	29	-3.37
GENE-4194_514	6A	204	0.005875	0.18	**21**	28	-3.10
Tdurum_contig42125_5972	6A	204	0.005875	0.18	**21**	28	-3.10
Kukri_c8148_2719	6A	204	0.006317	0.18	**25**	24	-2.90
wsnp_Ex_c11348_18326787	6A	204	0.006317	0.18	**25**	24	-2.90

wsnp_Ex_c11348_18327861	6A	204	0.006317	0.18	**25**	24	-2.90
IAAV1652	6A	204	0.006317	0.18	**24**	25	2.90
Tdurum_contig10194_765	6A	204	0.009092	0.16	**30**	19	-3.20
BS00060838_51	6B	218	0.009157	0.16	**19**	30	-2.00
BS00063174_51	6B	218	0.009157	0.16	**19**	30	-2.00
CAP8_c4223_13	6B	218	0.009157	0.16	**19**	30	-2.00
RAC875_c10122_113	6B	218	0.009157	0.16	**19**	30	-2.00
RAC875_c35008_398	6B	218	0.009157	0.16	**19**	30	-2.00
RAC875_c70_598	6B	218	0.009157	0.16	**19**	30	-2.00
BS00076093_51	6B	218	0.009354	0.16	**31**	18	2.00
BS00049942_51	6B	226	0.008901	0.16	**28**	21	-2.34
TA005016-0827	6B	226	0.008901	0.16	**28**	21	-2.34
BS00083578_51	7B	427	0.009597	0.16	**41**	8	-4.74
BS00022522_51	7B	427	0.009597	0.16	**8**	41	4.74
wsnp_Ex_c7934_13467460	7B	427	0.009920	0.16	**26**	23	2.99

Table 44: Significant markers for deoxynivalenol after regression (DONreg) at a -log10(P-value) threshold of 2.3 with position on chromosome (cM) and allele effects for winter wheat lines where n number of 'a' lines are indicated by a positive effect and 'b' a negative effect

Marker	Chromosome	Position	P-value	R^2	n = a	n = b	Effect
Tdurum_contig10362_328	1B	304	0.004823	0.19	**32**	17	2.87
BS00039209_51	2A	329	0.003146	0.21	**24**	25	-3.28
Kukri_c7914_99	2A	329	0.003452	0.21	**25**	24	-2.92
Kukri_rep_c83485_398	2A	329	0.003452	0.21	**25**	24	-2.92
Excalibur_c37649_125	2A	329	0.004249	0.20	**27**	22	-2.81
BS00036766_51	2A	329	0.004574	0.19	**29**	20	-3.00
Excalibur_rep_c111743_194	2A	332	0.001679	0.24	**26**	23	-3.25
Tdurum_contig48302_532	2A	332	0.001679	0.24	**26**	23	-3.25
BS00031466_51	2A	332	0.003452	0.21	**25**	24	-2.92
BS00066978_51	2A	332	0.003452	0.21	**25**	24	-2.92
Excalibur_c15733_252	2A	332	0.003452	0.21	**25**	24	-2.92
Excalibur_c3108_5476	2A	332	0.003452	0.21	**25**	24	-2.92
Kukri_c47534_1509	2A	332	0.003452	0.21	**25**	24	-2.92
RAC875_c13116_116	2A	332	0.003452	0.21	**25**	24	-2.92
RAC875_c20700_853	2A	332	0.003452	0.21	**25**	24	-2.92
RAC875_c7699_292	2A	332	0.003452	0.21	**25**	24	-2.92
Tdurum_contig48302_539	2A	332	0.003452	0.21	**25**	24	-2.92
Tdurum_contig5311_67	2A	332	0.003452	0.21	**25**	24	-2.92
Tdurum_contig82812_213	2A	332	0.003452	0.21	**25**	24	-2.92
Kukri_c35516_93	2A	522	0.002864	0.22	**22**	27	-2.67
Tdurum_contig29819_61	2B	166	0.002923	0.22	**15**	34	2.93
Tdurum_contig54634_846	2B	166	0.002923	0.22	**15**	34	2.93
CAP12_rep_c3989_239	2B	431	0.002781	0.22	**21**	28	3.80
Kukri_c54059_654	2D	145	0.001491	0.25	**41**	8	-4.14
BS00011109_51	2D	145	0.003542	0.21	**6**	43	4.61
Excalibur_rep_c66331_1967	3B	45	0.002776	0.22	**35**	14	-3.45
Kukri_c49752_254	3B	56	0.004547	0.19	**41**	8	-5.48
RAC875_rep_c118229_56	3B	56	0.004547	0.19	**41**	8	-5.48
BS00065292_51	5A	400	0.002494	0.22	**13**	36	3.26
BS00109576_51	6A	172	0.001053	0.27	**30**	19	-3.90
Kukri_c21943_466	6A	172	0.001053	0.27	**30**	19	-3.90
Kukri_c48959_337	6A	172	0.001053	0.27	**30**	19	-3.90
RAC875_c39200_260	6A	172	0.001053	0.27	**30**	19	-3.90
RAC875_c64852_655	6A	172	0.001053	0.27	**30**	19	-3.90
TA005243-1174	6A	172	0.001053	0.27	**30**	19	-3.90
wsnp_Ex_c25300_34566908	6A	172	0.001053	0.27	**30**	19	-3.90
wsnp_Ex_c55340_57883276	6A	172	0.001053	0.27	**30**	19	-3.90
wsnp_Ex_rep_c69191_68104835	6A	172	0.001053	0.27	**30**	19	-3.90
wsnp_Ku_c18534_27848426	6A	172	0.001053	0.27	**30**	19	-3.90
TA004440-0664	6A	172	0.001177	0.26	**31**	18	-3.51
BobWhite_c43699_393	6A	172	0.002414	0.23	**21**	28	3.91
GENE-3703_114	6A	172	0.002414	0.23	**21**	28	3.91
BS00067619_51	6A	173	0.001053	0.27	**30**	19	-3.90
BobWhite_c15977_107	6A	173	0.001053	0.27	**30**	19	-3.90
CAP11_c862_116	6A	173	0.001053	0.27	**30**	19	-3.90

Excalibur_c44694_414	6A	173	0.001053	0.27	**30**	19	-3.90
GENE-3798_1143	6A	173	0.001053	0.27	**30**	19	-3.90
Kukri_c12641_1185	6A	173	0.001053	0.27	**30**	19	-3.90
wsnp_Ex_c14975_23127669	6A	173	0.001053	0.27	**30**	19	-3.90
wsnp_Ex_c32765_41369642	6A	173	0.001053	0.27	**30**	19	-3.90
wsnp_Ex_c55340_57883479	6A	173	0.001053	0.27	**30**	19	-3.90
wsnp_Ex_c99215_85409445	6A	173	0.001053	0.27	**30**	19	-3.90
BS00066047_51	6A	190	0.002969	0.22	**24**	25	3.00
IAAV1652	6A	204	0.004312	0.20	**24**	25	2.93
Kukri_c8148_2719	6A	204	0.004312	0.20	**25**	24	-2.93
wsnp_Ex_c11348_18326787	6A	204	0.004312	0.20	**25**	24	-2.93
wsnp_Ex_c11348_18327861	6A	204	0.004312	0.20	**25**	24	-2.93
Tdurum_contig13240_523	6A	204	0.004585	0.19	**20**	29	-3.19
wsnp_Ex_c17185_25829084	6A	204	0.004585	0.19	**20**	29	-3.19
wsnp_Ex_c902_1745108	6A	204	0.004585	0.19	**20**	29	-3.19
RAC875_c41938_471	7B	427	0.004325	0.20	**22**	27	-2.60
RAC875_rep_c78007_394	7B	427	0.004325	0.20	**22**	27	-2.60



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