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Genetic linkage mapping and the detection of SNB resistance QTLs in Avocet x T9040 wheat field trials in the period 2012-2022

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Abstract

With the large scientific strides made in recent years when it comes to genome sequencing, techniques for plant breeding based on genomic data is on the rise. With the ever-changing environment and the increase of pesticide-resistance in pathogens, new technologies for protecting yield must be found. Mapping of resistance loci for various infectious agents is a relatively novel technique that shows great promise for the advancement of breeding for disease resistant crops.

In this thesis, the focus has been to map resistance QTLs in wheat for the fungal disease Septoria leaf blotch. Phenotypic and genotypic data encompassing six years of field trials in the 2012-2022 period has been analysed and compared to find important resistance QTLs. While several QTLs were found overall, three major resistance QTLs showing great consistency over the years were identified, of which two were previously known and one appears to be novel. Hopefully these QTLs can be used in breeding, and the data used to identify more resistance loci in the future.

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1. Introduction and theory

1. 1. About wheat

Wheat is the common name for any of several species of the genus *Triticum,* belonging to the grass family, Poaceae. These grasses are among the oldest and most important of the cereal crops; any of the many species cultivated for its edible grain. Among the thousands of varieties known, one of the most important species of wheat is the common wheat, *Triticum aestivum*, used for making bread, as well as durum wheat, *T. durum*, used for making pasta (Britannica, 2023). Of *T. aestivum,* there are early growing varieties, called spring wheat, which are sown in early spring and are ready for harvesting during summer or early autumn, and winter wheat, which are varieties that are sown in autumn. Winter wheat varieties sprout before winter, with the leaves and stems surviving the winter. Winter wheats are dependent on a longer, cooler period with temperatures of 2-4 degrees Celsius to induce blooming. In Norway, spring wheat is most commonly grown, although with the climate becoming milder, more winter wheat is being grown in Norway as well (Brød og Korn, 2022).

The nutritional composition of wheat will vary somewhat with the environment and soil composition. Usually it consists of about 70% carbohydrates and 12% protein, with smaller amounts of fats and minerals (Britannica, 2023). Harder wheat types with higher protein content and strong gluten are better for bread making, while softer wheat types with less protein and weak gluten can be used for softer bakes and as household flour (Britannica, 2023).

The common wheat, *T. aestivum,* has one of the most complicated genomes known to science, according to Zimin et al., 2017. The genome is hexaploid, meaning it has six copies of each chromosome. It contains an enormous number of near-identical sequences scattered throughout, largely due to long transposable elements (Li et al, 2004), and a complete haploid size of more than 15 billion bases. (Zimin et al, 2017). The first full sequencing effort of the wheat genome was conducted in 2012 by Brenchley et al. It used an earlier generation of sequencing technology, and only succeeded in sequencing approximately one third of the genome. In 2014, a new try to sequence the wheat genome was conducted by the International Wheat Genome Sequencing Consortium (IWGSC). The IWGSC succeeded in sequencing about two thirds of the wheat genome. Yet another assembly was attempted by

Clavijo et al in 2017, resulting in approximately 78% coverage. Then, the first near-complete assembly of the full genome was published by Zimin et al in 2017. This assembly was compiled using a combination of short Illumina reads and very long Pacific Biosciences reads. Given all this, and the relatively recent nature of publishing of the near-complete genome, a lot of research still needs to be done to uncover the function of different genes on the wheat genome, including identifying resistance genes for diseases.

1. 2. Septoria nodorum blotch (SNB)

Wheat is one of the most economically important grain crops in Norway. In 2020, the Norwegian wheat consumption was at 77kg per person (Brød og Korn, 2022). It is also one of the crops that can easily be grown in the Norwegian climate. However, wheat, like all other commercial crops, is grown as a monoculture. Monoculture effectivizes agriculture because of ease of harvesting and processing, but it also increases the amount of crop lost to disease. This is because of the lack of other plants and animals to function as natural barriers (Balogh, 2021). When planting the individual plants close together, the pathogen also easily spreads from one individual to the next. Each year, about 20% of the wheat crop is lost to disease (Singh et al, 2022). Due to this, current agriculture is heavily dependent on insecticides and pesticides (Balogh, 2021), but environmental concerns make the finding of new ways to combat pathogens a priority.

One well-known wheat disease is Septoria nodorum blotch (SNB). SNB is one of the most economically important diseases of both spring and winter wheat in Norway (Brodal & Elen, 2022). The disease is caused by the fungal agent *Parastagonospora nodorum*. *P. nodorum* has gone through several name changes as its placement in the fungal phylogenetic tree has been revised. While *P. nodorum* is its currently accepted scientific name, the disease name comes from the synonym and previously accepted name, *Septoria nodorum*. The fungal disease affects all above-ground parts of the plant. An infection usually starts as small necrotic spots near the mid-ribs on older leaves, and gradually spreads in size to become large brown lesions. In severe SNB epidemics, several lesions can affect each leaf and coalesce, covering the whole leaf and killing the leaf tissue. The infection can spread from the leaves to the glumes of the plant. The infection on glumes causes shriveled kernels, which decrease crop yield and quality (Mehra et al, 2019). When the pathogen infects the glumes, the disease is called Stagonospora or Septoria glume blotch (SGB). The disease spreads to the glumes

usually by spores splashing from the leaves (Freije & Wise, 2015). While the same fungus causes both SNB and SGB, the genetic mechanisms behind disease resistance are not the same for the two diseases. Further, both diseases are subject to extensive genotype-by-environment interactions (Francki et al, 2021), complicating the mapping of resistance QTLs. In this thesis, the focus will be on Septoria nodorum leaf blotch.

Fig 1: Infection of *Parastagonospora nodorum* on a wheat leaf (Freije & Wise, 2015)

Parastagonospora nodorum can survive up to two years as spores and mycel in discarded infected plant tissue. The pathogen survival rate is highest if the infected tissue is left on the soil surface, but the pathogen can also be transferred with infected seeds for sowing, and it can overwinter in autumn wheat. Because of this, it's extra important to use certified disease-free seeds when planting (Brodal & Elen, 2022).

P. nodorum belongs to the fungal division Ascomycota. The spores of *P. nodorum* are dependent on free water to release and spread. Conidia, the asexual fungal spores of the Ascomycota, are spread from one plant to the next through droplets hitting the leaves, in rainy weather or with overhead irrigation. As such, the weather is crucial for the development of disease. In hot, rainy weather, the fungus can complete its life cycle, from infection to creation of new spores, in as little as ten days. In addition to local infection with conidia, the fungus can also create sexual ascospores, which are carried by the wind. This can account for

new infections in fields in the absence of previously infected plant material and despite the use of certified infection-free seeds (Brodal & Elen, 2022).

1. 3. Testing for SNB infection

Scoring for SNB is done both by inoculation of seedlings in greenhouses, and by scoring of percentage disease-affected leaf areal in field trials. The pathogen produces several nectrotrophic effectors, of which the three factors SnToxA, SnTox1, and SnTox3 have been cloned (Hafez et al, 2020). Phenotyping for sensitivity for these effectors is done in greenhouse trials by inoculation of seedlings plants. However, the effects of the effectors are not consistent between greenhouse trials and field trials (Francki, 2013), and the full relevance of the effectors for resistance mapping is yet to be determined.

The material used in this thesis is a cross between the two wheat cultivars Avocet and T9040. These two cultivars show different reactions to SnToxA and SnTox1. Avocet is sensitive to both, while T9040 is insensitive to both (Ruud et al, 2018). Further, the plants show different reactions to SnTox3 when inoculated in the greenhouse. Avocet is highly sensitive and shows necrotic reaction, also called Tox3 reaction type 3, whereas T9040 is less sensitive and only has a chlorotic reaction, or Tox3 reaction type 2 (Ruud et al, 2018). While this might suggest that SnTox3 resistance is connected to disease resistance, only a minor connection between SnTox3 sensitivity and field disease resistance has so far been found. When the lines were tested in field between 2010 and 2016, 2010 was the only year that the link between SnTox3 sensitivity and development of disease in field was significant (Ruud et al, 2018). However, Ruud et al 2018 found that insensitivity to SnToxA showed high correlation with field SNB resistance, and as such, screening for SnToxA sensitivity in seedling plants might be an inexpensive way to increase SNB field resistance.

This thesis is based on phenotypic data collected through several field trials of the Avocet x T9040 population. According to Liu et al. 2015, association mapping located SnTox3 sensitivity in wheat to the short arm of chromosome 5B. Additionally, several QTLs for SnToxA resistance are found throughout chromosome 5B (Friesen et al, 2006, Lin et al, 2020, Liu et al, 2015, Virdi et al, 2016). A theory is thus that if sensitivity to these nectrothrophic effectors indicates field disease resistance, a resistance QTL for Septoria nodorum blotch will be found at chromosome 5B.

1. 4. QTLs and QTL-mapping

A QTL, or quantitative trait locus, is a locus that corresponds with a phenotypic variation in a quantitative trait in a population (Illumina, 2023). Rather than a phenotypic variation controlled by a single gene, a quantitative trait is polygenic, meaning controlled by two or more different genes. The effect of a single QTL on the phenotype will vary; the phenotype can be controlled by several QTLs which all contribute a little to the complete phenotype of a single trait, or fewer QTLs which all contribute a lot to the phenotype. Often, it will be a mix of both, with all QTLs contributing to different degrees to the phenotypic expression of the trait (Miles et al, 2008). QTLs are most commonly associated with traits that show continuous variance (Illumina, 2023) and are normally distributed across the mean. These traits can also be influenced by other factors than genotype, such as sex, environment, and epistatic effects with other QTLs (Miles et al, 2008). Common examples of such traits are height, time to maturity, and disease resistance.

Identifying important QTLs for any given phenotype is becoming increasingly important in plant breeding. Pesticide and fungicide resistance is ever increasing, and genotyping is becoming more efficient and cost-effective day by day. By knowing which QTLs correspond with which phenotype, it is possible to predict how a plant will grow and respond to a variety of factors. Especially in food production, by identifying QTLs related to drought resistance, disease resistance, cold resistance, and yield, it will be possible to grow food more efficiently and adapt agriculture to a changing climate.

A QTL analysis is a statistical method that links collected phenotypic data of desired complex traits with genotypic data. This way, complex traits can be linked to specific regions of chromosomes, and creates the basis for finding specific genes that code for certain traits. To perform a QTL analysis, certain requirements must be met. First, two or more cultivars or strains that differ in phenotype for the traits that are to be analyzed need to be identified and crossed. An example of this can be a very tall plant cultivar and a very short one, or one that’s receptive to a disease and one that’s resistant. These two lines must be crossed to create a heterozygotic but phenotypically uniform F1 generation, which will then show phenotypic variance in the following F2 generation. Second, a multitude of polymorphic molecular markers must be identified in the two parent cultivars. Polymorphism means that the marker

exist as two or more different alleles. These molecular markers can be SNPs (single nucleotide polymorphisms; a difference in a single nucleotide), microsatellites (a repeat of a simple sequence), RFLPs (restriction fragment length polymorphisms; a difference in length in a sequence cut by a restriction enzyme), or transposable element positions (Casa et al., 2000; Vignal et al., 2002; Gupta & Rustgi, 2004; Henry, 2006). Markers like this are preferred for QTL analyses since they're unlikely to affect the trait of interest (Miles et al, 2008). In cases where the phenotypic trait is controlled by enough different genes (tens to hundreds of genes), the parental lines don't even need to have different phenotypes; they simply need to have different alleles for the molecular markers, so that recombination in the offspring generations produce a variety of phenotypic values. Third, the number of offspring in each crossing need to be large enough to give statistically significant results. (Miles et al 2008). Currently, SNPs are the most commonly used markers for QTL-analyses.

To perform the analysis itself, the phenotype and genotype of each individual in the trial is recorded. Then, the genotypic data and the phenotypic data can be input into any appropriate statistical program for analysis. A marker that is close to a gene of the desired trait is likely to be inherited together with the gene, and as such the approximate position of genes influencing any quantitative trait can be inferred (Miles et al 2008). This is often recorded as a LOD score. LOD, or logarithm of the odds, is a statistical estimate of the probability that two loci are located close together and thus are likely to be inherited together (Brody, L, 2023). In a QTL analysis, these two loci would be the molecular marker, for which the position and genotype is known, and the locus influencing the phenotypic trait, for which the position and phenotype are unknown.

Table 1: Compilation of known SNB resistance QTLs in adult leaves of wheat plants. Excerpt from a longer table compiled by my co-supervisor Min Lin.

Resist. source	Plant tissue	Population	NE-Snn	Chr.	QTL name/chr	Markers	Reference	Start (Mbp)	End (Mbp)
SHA3/CBRD	Adult plant leaf	SHA3/CBRD x Naxos		1A	*1A*	IWA2995	Ruud et al. 2017	472.16893	472.16913
	Adult plant leaf	GWAS 232 lines (global origin)		1A		IWB10679	Francki et al. 2020	485.228882	485.228982
EGA Blanco	Adult plant leaf	EGA Blanco x N		1B	*QSnl07.daw-1B*	IWB64368,wPt-8949	Francki et al. 2018	1.844152	4.320956
EGA Blanco	Adult plant leaf	EGA Blanco x Millewa		1B	*QSnl.daw-1B*	wPt-8949 – wPt-2575	Francki et al. 2011	18.44632	24.121806
EGA Blanco	Adult plant leaf	EGA Blanco x N		1B	*QSnl08.daw-1B*	IWB55607,wPt-8267	Francki et al. 2018	31.47872	45.18355
Wyalkatchem	Adult plant leaf	Calingiri x Wyalkatchem		1B	*Qsnb.fcu-1BS*	gpw7059a - *wPt-2654*	Phan et al. 2016	49.66299	74.68668
	Adult plant leaf	GWAS 232 lines (global origin)		1B		IWB7076	Francki et al. 2020	8.559698	8.559798
Naxos	Adult plant leaf	SHA3/CBRD x Naxos		1B	*1B*	wmc619	Lu and Lillemo 2014	65.28268	65.283285
BR34	Adult plant leaf	BR34 x Grandin		1B	*QSnb.fcu-1BS*	*fcp267 – barc240*	Friesen et al. 2009	389.990067	389.990043
	Adult plant leaf	GWAS 232 lines (global origin)		1B		IWB53316,IWB65947	Francki et al. 2020	586.300643	586.30091
	Adult plant leaf	Liwilla × Begra doubled haploid		1B	*QSnl.ihar-1B*	wPt-7160	Czembor et al. 2019	629.675435	629.675751
	Adult plant leaf	GWAS 232 lines (global origin)		1B		IWB29150	Francki et al. 2020	645.571228	645.571328
	Adult plant leaf	GWAS 232 lines (global origin)		1B		IWB29151	Francki et al. 2020	645.571368	645.571468
Naxos	Adult plant leaf	SHA3/CBRD x Naxos		1B	*1B*	SCM9	Ruud et al. 2017	NA	NA
P92201D5	Adult plant leaf	P92201D5 x P9	2A	2A	*QSnl05.daw-2A*	IWB22268,wPt-8464	Francki et al. 2018	2.336338	19.649717
P92201D5	Adult plant leaf	P92201D5 x P9	2A	2A	*QSnl04.daw-2A*	IWB32474,IWB9206	Francki et al. 2018	15.608826	16.321845
	Adult plant leaf	NIAB MAGIC		2A	*QSnb.niab-2A.2*	Kukri_c24852_466 and BS00008805_51	Lin et al. 2020	78.84436	111.457371
P92201D5	Adult plant leaf	P92201D5 x P91193D1		2A	*QSnl.daw-2A*	gwm614a – *wPt-7056*	Francki et al. 2011	218.395896	218.39588
	Adult plant leaf	NIAB MAGIC		2A	*QSnb.niab-2A.3*	JD_c2056_506 and BS00022241_51	Lin et al. 2020	410.872829	663.329017
	Adult plant leaf	BMWpop MAGIC		2A	*QSnb.nmbu-2A.1*	Kukri_c11327_977 and Tdurum_contig33398_106	Lin et al. 2021	507.691373	718.885511
	Adult plant leaf	NIAB MAGIC		2A	*QSnb.niab-2A.4*	wsnp_Ra_c6586_11477949 and Tdurum_contig8350_350	Lin et al. 2020	755.929525	780.714672
	Adult plant leaf	GWAS 121 spring wheat lines		2B	*2B*	IWB2427	Ruud et al. 2019	68.201551	68.201651
	Adult plant leaf	BMWpop MAGIC		2B	*QSnb.nmbu-2B.1*	Excalibur_rep_c66577_159 and Ra_c71978_532	Lin et al. 2021	572.591268	648.083659
	Adult plant leaf	BMWpop MAGIC		2D	*QSnb.nmbu-2D.1*	D_F1BEJMU01A0OMY_356 and BS00071755_51	Lin et al. 2021	14.636197	15.115231
	Adult plant leaf	NIAB MAGIC		2D	*QSnb.niab-2D.1*	wsnp_JD_rep_c63957_40798083 and BobWhite_c59161_181	Lin et al. 2020	14.897896	27.859806
6HRWSN125	Adult plant leaf	WAWHT2074 x 6HRWSN125		2D	*QSnl.daw-2D*	cfd11 – *gwm30*	Shankar et al. 2008	79.231376	142.336663
	Adult plant leaf	6HRWSN125/W	2D	2D	*QSnl03.daw-2D*	IWB65834,IWB38687	Francki et al. 2018	32.622088	461.301312
	Adult plant leaf	GWAS 121 spring wheat lines		2D	*2D*	gwm301, IWB35134, IWB30879, IWB30880, IWB34359	Ruud et al. 2019	642.266688	650.327186
	Adult plant leaf	NIAB MAGIC		3A	*QSnb.niab-3A*	RAC875_c46403_277 and BS00066230_51	Lin et al. 2020	1.031135	32.325166
Naxos	Adult plant leaf	SHA3/CBRD x Naxos		3A	*3AS*	gwm2	Lu and Lillemo 2014	60.200997	60.201123
Naxos	Adult plant leaf	SHA3/CBRD x Naxos		3A	*3AS.1*	gwm2, IWB35234	Ruud et al. 2017	60.200997	61.348708
SHA3/CBRD	Adult plant leaf	SHA3/CBRD x Naxos		3A	*3AS.2*	IWB39383, IWB27319	Ruud et al. 2017	522.187649	537.509025
SHA3/CBRD	Adult plant leaf	SHA3/CBRD x Naxos		3B	*3B*	wPt-4127	Lu and Lillemo 2014	325.832733	325.833271
Naxos	Adult plant leaf	SHA3/CBRD x Naxos		3B	*3BL*	wPt-4933	Lu and Lillemo 2014	786.347562	786.348263
Naxos	Adult plant leaf	SHA3/CBRD x Naxos		3B	*3BL*	wPt-4933	Ruud et al. 2017	786.347562	786.348263
	Adult plant leaf	NIAB MAGIC		4A	*QSnb.niab-4A*	BS00072025_51 and IAAV6581	Lin et al. 2020	598.590361	605.713556
	Adult plant leaf	GWAS 232 lines (global origin)		4B		IWB32911	Francki et al. 2020	12.528899	12.528999
	Adult plant leaf	GWAS 121 spring wheat lines		4B	*4B*	IWB6422	Ruud et al. 2019	16.056666	16.056766
Oberkulmer	Adult plant leaf	Forno x Oberkulmer		4B	*QSnl.eth-4B*	*glk348 – psr921*	Aguilar et al. 2005	NA	NA
	Adult plant leaf	BMWpop MAGIC		5A	*QSnb.nmbu-5A.1*	Tdurum_contig54785_216 and RAC875_c25339_200	Lin et al. 2021	558.69278	571.683315
	Adult plant leaf	GWAS 232 lines (global origin)		5A		IWA675	Francki et al. 2020	609.872751	609.872951
SHA3/CBRD	Adult plant leaf	SHA3/CBRD x Naxos		5B	*5BS*	IWB11709	Ruud et al. 2017	6.648497	6.648597
SHA3/CBRD	Adult plant leaf	SHA3/CBRD x Naxos		5B	*5B.2*	wPt-5914	Ruud et al. 2017	29.366719	29.367128
	Adult plant leaf	GWAS 232 lines (global origin)		5B		IWA5670	Francki et al. 2020	514.929756	514.929956
	Adult plant leaf	EGA Blanco x N	5B	5B	*QSnl07.daw-5B*	IWB22904,IWA4103	Francki et al. 2018	541.343146	558.625587
SHA3/CBRD	Adult plant leaf	SHA3/CBRD x Naxos		5B	*5BL*	fcp1	Lu and Lillemo 2014	546.147138	546.147495
	Adult plant leaf	GWAS 232 lines (global origin)		5B		IWB14942	Francki et al. 2020	546.704065	546.704161
	Adult plant leaf	EGA Blanco/Mi	5B	5B	*QSnl08.daw-5B*	IWB73666,VrnB1	Francki et al. 2018	571.709975	573.81607
	Adult plant leaf	GWAS 232 lines (global origin)		5B		IWB1546,IWB40363,IWB72592	Francki et al. 2020	617.141962	617.142015
EGA Blanco	Adult plant leaf	EGA Blanco x Millewa		5B	*QSnl.daw-5B*	*wPt-3457 – wPt-0935*	Francki et al. 2011	NA	597.287799
SHA3/CBRD	Adult plant leaf	SHA3/CBRD x Naxos		5B	*5BS*	wPt-5346	Lu and Lillemo 2014	NA	NA
	Adult plant leaf	GWAS 232 lines (global origin)		6A		IWA6999	Francki et al. 2020	11.114893	11.115093
	Adult plant leaf	GWAS 232 lines (global origin)		6A		IWA4961,IWB40335,IWB55352,IWB55355,IWB75134	Francki et al. 2020	16.566143	16.571563
	Adult plant leaf	GWAS 232 lines (global origin)		6A		IWB1211,IWB44959,IWB61273	Francki et al. 2020	17.664493	17.913074
	Adult plant leaf	NIAB MAGIC		6A	*QSnb.niab-6A.1*	IAAV5188 and RFL_Contig3088_949	Lin et al. 2020	74.025753	249.160705
	Adult plant leaf	GWAS 232 lines (global origin)		6A		IWB113	Francki et al. 2020	610.200062	610.200162
Calingiri	Adult plant leaf	Calingiri x Wyalkatchem		6B	*Qsnb.cur-6BS*	*wPt-3168 - barc146a*	Phan et al. 2016	NA	NA
	Adult plant leaf	GWAS 232 lines (global origin)		7A		IWB31999	Francki et al. 2020	6.997578	6.997679
Naxos	Adult plant leaf	SHA3/CBRD x Naxos		7A	*7A*	wmc603	Lu and Lillemo 2014	488.729983	488.730318
	Adult plant leaf	GWAS 232 lines (global origin)		7A		IWB41146	Francki et al. 2020	546.041678	546.041778
Naxos	Adult plant leaf	SHA3/CBRD x Naxos		7A	*7A*	IWB53887	Ruud et al. 2017	553.993765	553.993865
	Adult plant leaf	GWAS 121 spring wheat lines		7B	*7B*	IWB57207, IWB73685, IWB70085	Ruud et al. 2019	6.153848	6.393796
	Adult plant leaf	GWAS 232 lines (global origin)		7B		IWA809	Francki et al. 2020	62.687493	62.687576
	Adult plant leaf	GWAS 232 lines (global origin)		7B		IWB7527	Francki et al. 2020	83.030027	83.030127
Naxos	Adult plant leaf	SHA3/CBRD x Naxos		7B	*7B*	wPt-0963	Lu and Lillemo 2014	260.281322	260.282086
Forno	Adult plant leaf	Forno x Oberkulmer		7B	*QSnl.eth-7B*	*mwg710a – glk576*	Aguilar et al. 2005	NA	NA
	Adult plant leaf	GWAS 232 lines (global origin)		7D		IWB18914	Francki et al. 2020	55.073662	55.073874
	Adult plant leaf	Liwilla × Begra doubled haploid		7D	*QSnl.ihar-7D*	gdm46	Czembor et al. 2019	536.500011	536.500152
	Adult plant leaf	NIAB MAGIC		7D	*QSnb.niab-7D.3*	RAC875_c10022_23 and JD_c2708_1512	Lin et al. 2020	629.325776	724.128709

The analysis starts by creating a genetic linkage map. A genetic linkage map links different markers together based on recombination frequency. Two markers that sit closely together will rarely recombine, while two markers that are located far apart recombine more frequently. By analysing the genomic sequence of the individuals in a trial and finding recombination frequencies for several gene pairs, the linkage of different loci and their relative distance to each other can be calculated. New markers can be found and placed by testing for relative linkage between previously identified markers. All the markers that are known to be linked together form a linkage group (CD Genomics). When the linkage maps are made, they can be compared and analysed together with collected phenotypic data to determine the most likely markers associated with the phenotype.

In this thesis, the two programs used for calculating the QTL positions are Joinmap 4 and MapQTL 6, both by Kyazma. Joinmap uses genotypic data of the population to create high quality genetic linkage maps. MapQTL then uses the collected phenotypic data, the genotypic data, and the linkage maps created in JoinMap to calculate the likely positions of QTLs.

2. Materials and methods

2. 1. Plant material

For crossing, the two inbred wheat cultivars Avocet and T9040 have been used. The cross was made both using plants from each cultivars as both pollen donor and pollen recipient. From the cross, over a hundred individual lines were genotyped. The genotyping was done by scientists at NMBU using the TraitGenetics 25K SNP-chip. This chip contains 24145 markers. Then a filtering has been done to exclude monomorphic (existing as only one allele) markers, and exclude markers or individuals that show a high level of heterozygosity. The final dataset I was provided for this thesis contains 3150 genotyped polymorphic markers and 110 individual wheat lines.

The wheat lines were tested in several different field trials, first at Staur (shortened st) in Stange kommune in 2012, and thereafter at Vollebekk (shortened vb) in Ås kommune in

2017 and 2019-2022. At these six field trials, days to heading (DH) and development of Septoria leaf blotch disease (LB) in percentage of tissue affected was recorded for each line. For the trials Vollebekk 2019-2022, plant height (PH) was also recorded. Two parallells were run, and the development of leaf blotch disease presented as the mean of the two.

2. 2. Data analyzation

JoinMap ® 4 (Van Ooijen, J. W., 2006)

In JoinMap, a new project was created and the genomic data was input as a rlx population. Rlx are retrotransposons, segments of DNA that are replicated throughout the genome. Used as genetic markers population (Ghonaim et al, 2020). JoinMap does not support full marker names, so shorthand marker names using a value and the chromosome on which the marker is found were used. The data was calculated into linkage groups, starting with a high linkage LOD-value of 20, then decreasing as the groups got smaller. The large LOD-value was selected to get closely linked groups without any gaps. For each group, the data was checked for suspect linkages, and markers that didn't fit were removed. In total, this created 71 linkage groups.

MapQTL ® 6 (Van Ooijen, J. W., 2009)

The linkage groups created in JoinMap were put into one single map-document, sorted by chromosome. This map, along with an excel file containing the phenotype data for all the lines as well as a file containing all the genotype data were loaded into MapQTL. First, an interval mapping analysis were run for the three phenotypes scored; days to heading (DH), plant height (PH) and leaf blotch (LB). The LB data was already corrected for DH. After the interval mapping, a multiple QTL mapping was done on LB to confirm the data.

While LB data corrected for DH existed, no such correction was previously done for PH. Thus, another interval mapping was done, using the raw data of LB with DH and PH as cofactors and correcting for both. This mapping only comprises the Vollebekk field trials years of 2019-2022, as plant height was not recorded in the previous trials.

After the mapping in MapQTL was done, the markers in the map-document, still in shorthand from being formatted for JoinMap, were replaced with the proper marker names. This map is included as appendix 1. The group names in the map document (appendix 1) correspond to

the order and name they got when they were being mapped in JoinMap, and have been preserved as such in the document to make it easier to find back to the corresponding .loc and .map files in JoinMap in the future.

Mapchart 2.32 (Wageningen University & Research, 2017)

The relevant groups from the map-document were input in Mapchart, along with the LOD-values and positions of the markers from MapQTL. This created a figure of the genetic linkage group as well as a LOD-curve for the markers. This was done for the three most relevant QTLs for LB. The groups used are group 252, group 42 and group 12 from appendix 1 respectively. For the Mapchart outputs they have been renamed group 2A (group 252), group 6A (group 42) and group 6B (group 12), to correspond with the chromosome they are placed on.

3. Results

3. 1. Linkage maps

The full genetic linkage map is included as appendix 1. It contains 71 linkage groups, with 2768 out of 3150 markers placed. This leaves 382 markers unmapped. The average is 39 markers in each linkage group.

The longest linkage group contains 157 markers, and the shortest contains 2. The longest linkage group is 150.553 cM long, and the shortest is 0.471 cM.

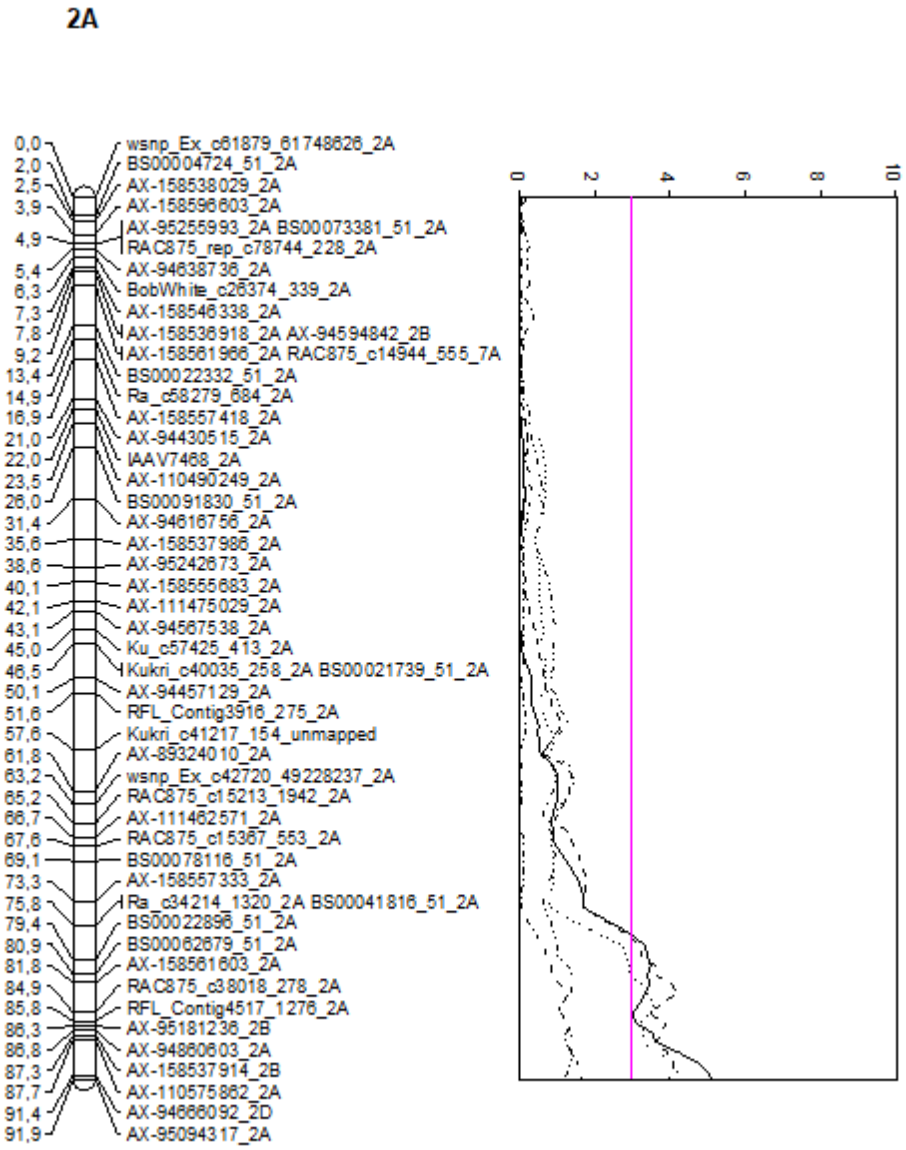

Fig 2: Figure of the genetic linkage group containing the QTL *QSnb.nmbu-2A* (table 5), with accompanying LOD-curve.

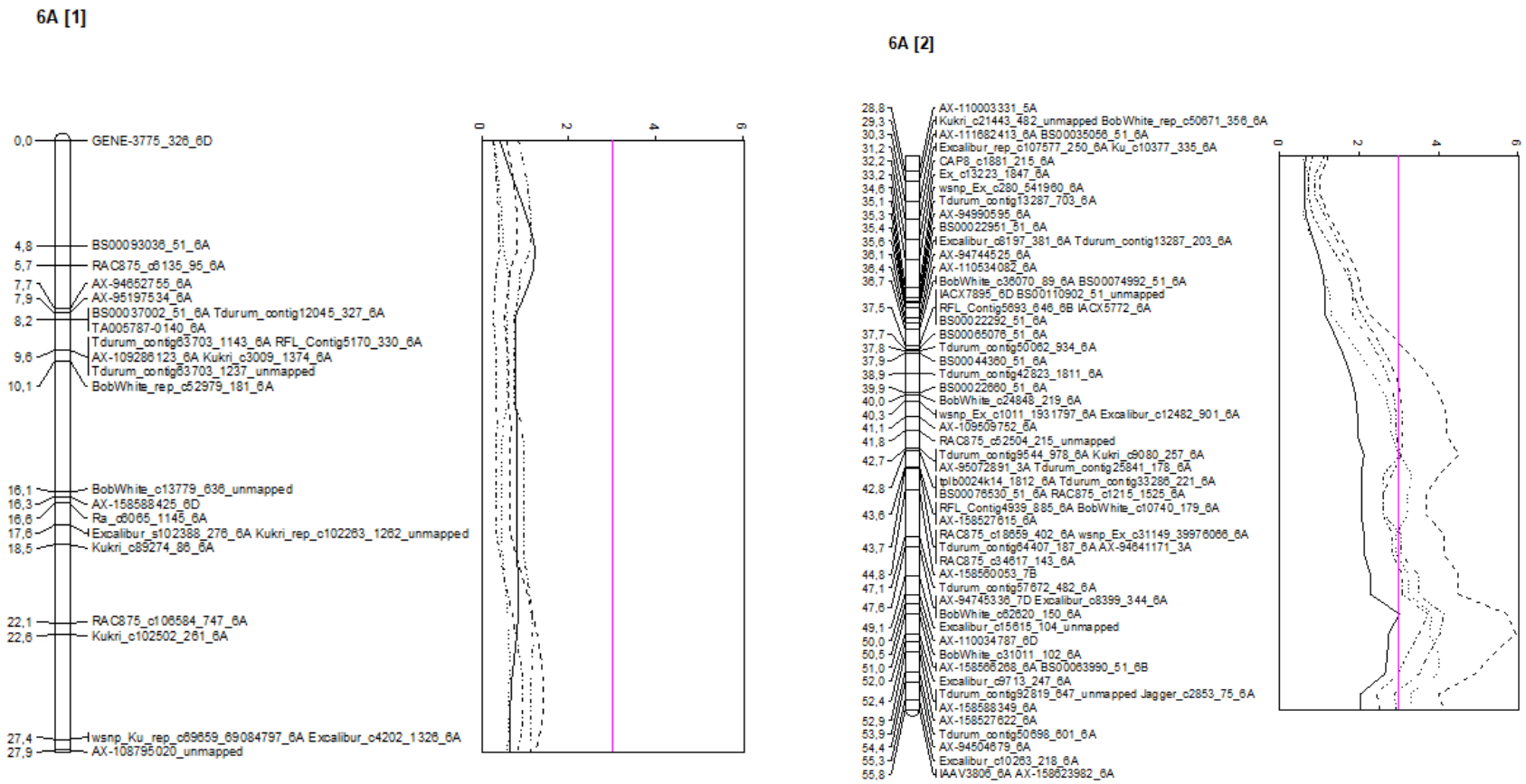

Fig 3: Figure of the genetic linkage group containing the QTL *QSnb.nmbu-6A* (table 5), with accompanying LOD-curve. The group has been divided into two sections in the figure due to the size of the linkage group.

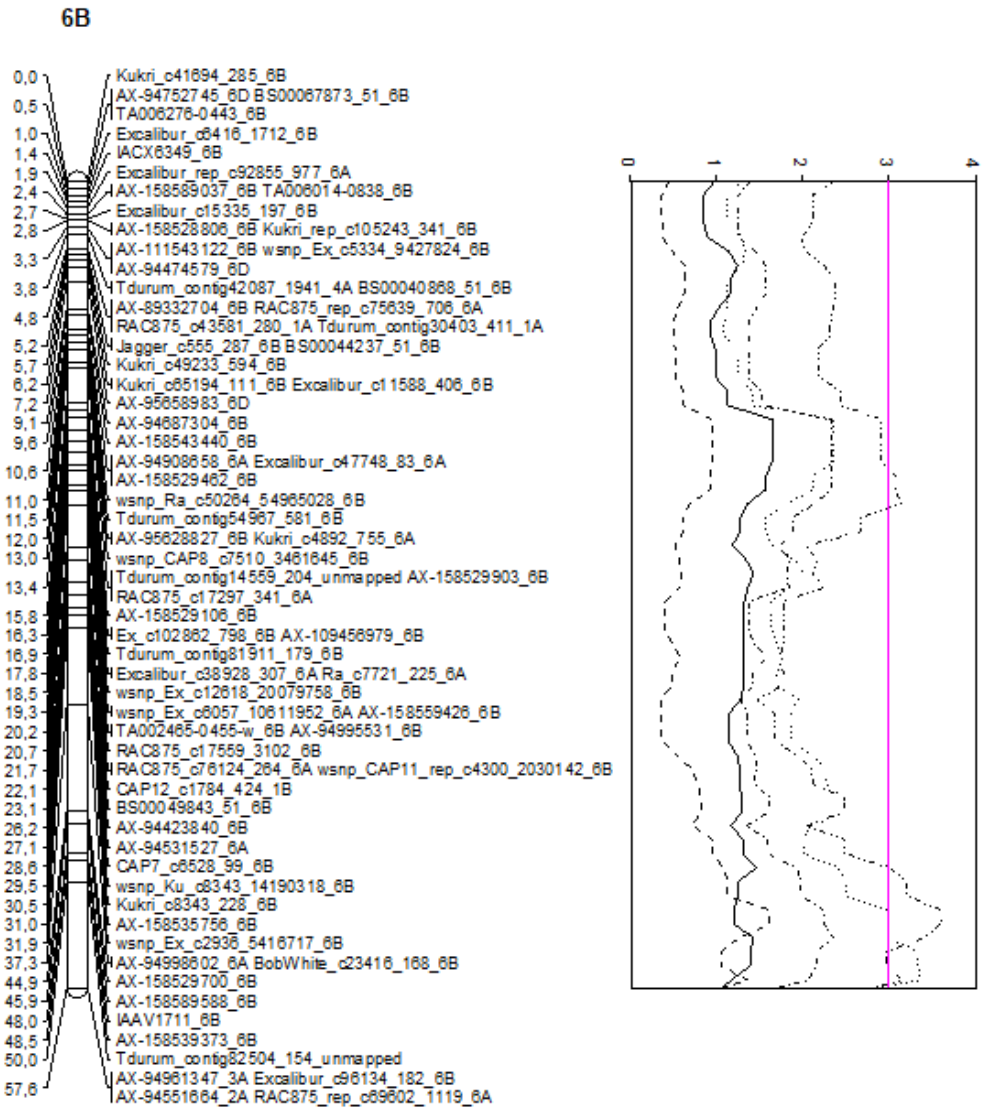

Fig 4: Figure of the genetic linkage group containing the QTL *QSnb.nmbu-6B* (table 5), with accompanying LOD-curve.

For the QTLs, a LOD-value over 3 is considered significant. Figures 2-4 show the maps for the linkage groups of the three most significant resistance QTLs found in these trials. The details of the QTLs are outlined in table 5. A LOD-value of 3 is drawn in as a constant in the accompanying LOD-curves for the figures, showing at which point in the group the LOD-value is over the significant threshold.

3. 2. Phenotypic data

Phenotypic data of the different field trials were collected over the years. Here presented as several figures. The complete phenotypic data is included as appendix 6.

Leaf blotch

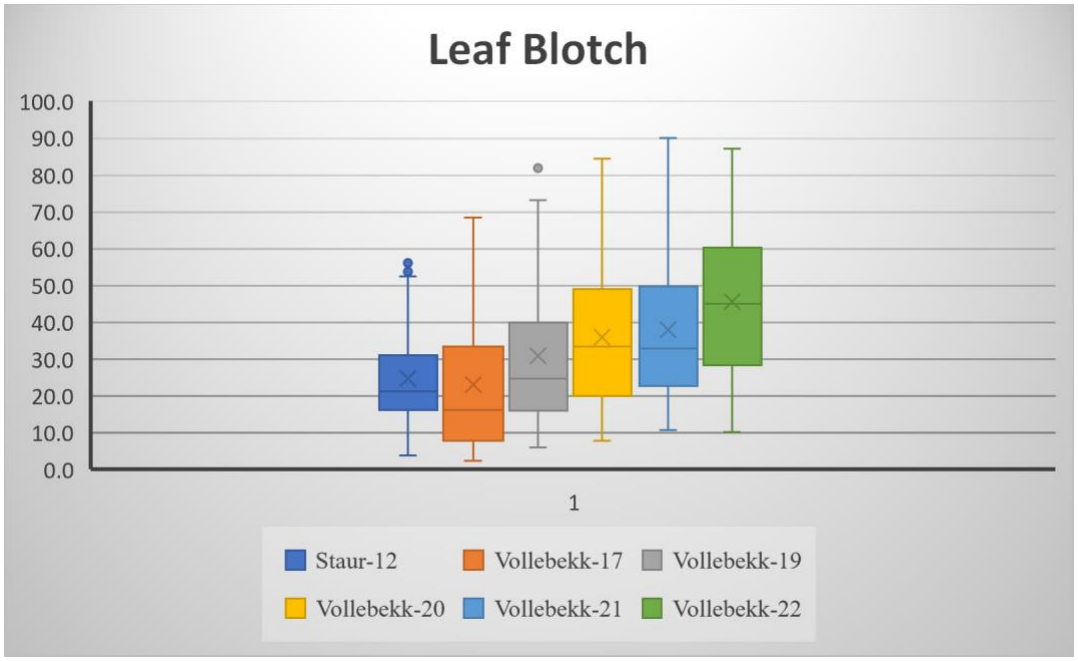

Fig. 5: Leaf blotch, given as the average between two parallels, for each year the trial has been run. Values on y-axis given as percentage of infection.

The comparison data in figure 5 shows that there has been a steady increase in infection, with the earlier field trials having the least amount of infection and the more recent field trials having the most infected leaf tissue.

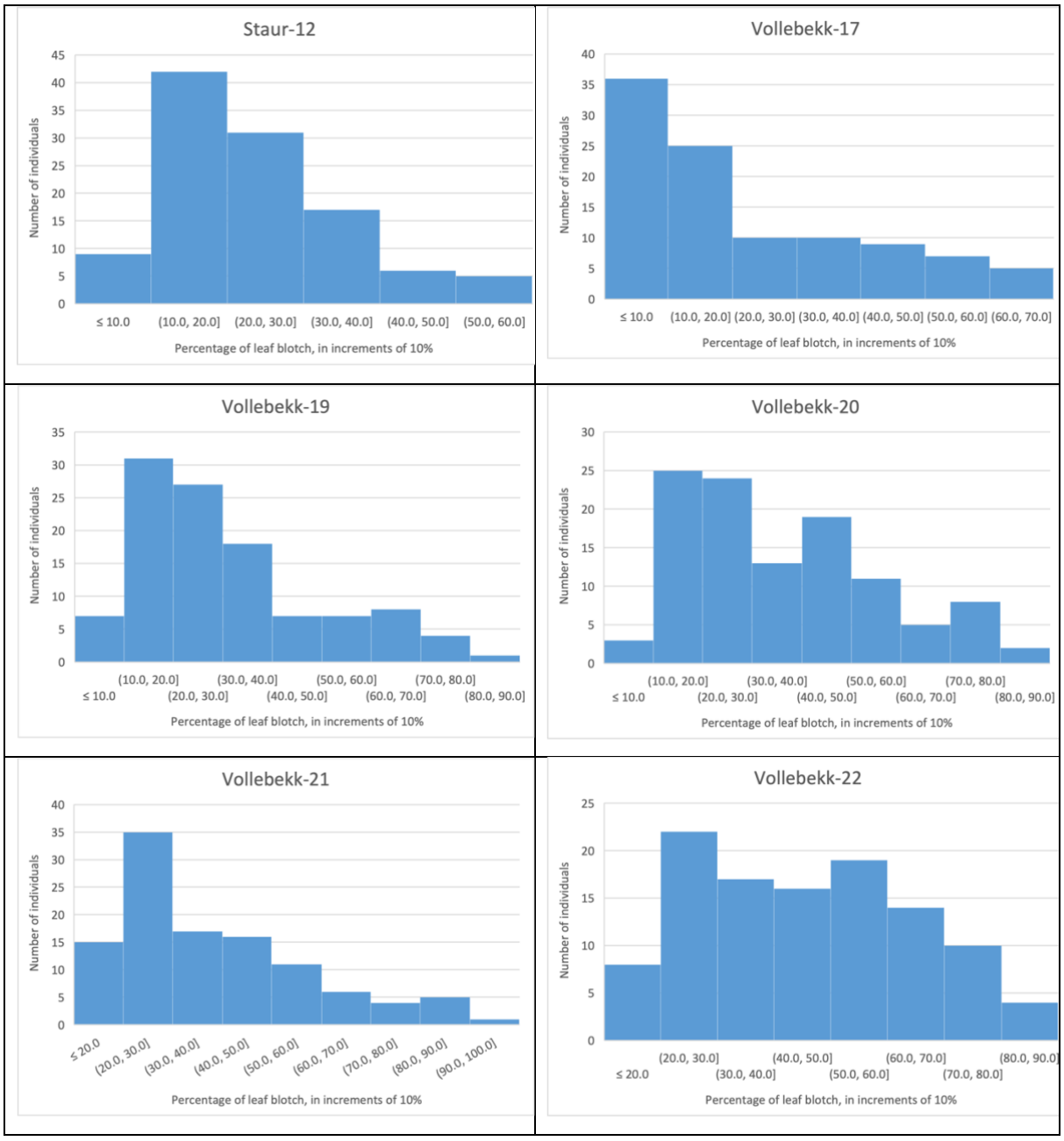

Fig. 6: Histograms showing the percentage of leaf blotch in all the field trials; Staur 2012 and Vollebekk 2017-2022. Each bin is given as a 10 percent interval, and the Y-axis shows number of individuals belonging to each bin.

As can be seen in figure 6, the leaf blotch for each year shows a normal distribution, although the extremes are skewed toward resistant. Vollebekk-17 shows the lowest average spread of disease, with a mean of 23% infected tissue, while Staur-12 shows the lowest extremes,

having a maximum infection of 54% and a minimum infection of 3.9%. The highest average infection is found in Vollebekk-22, with an average of 46% infected tissue.

Days to heading

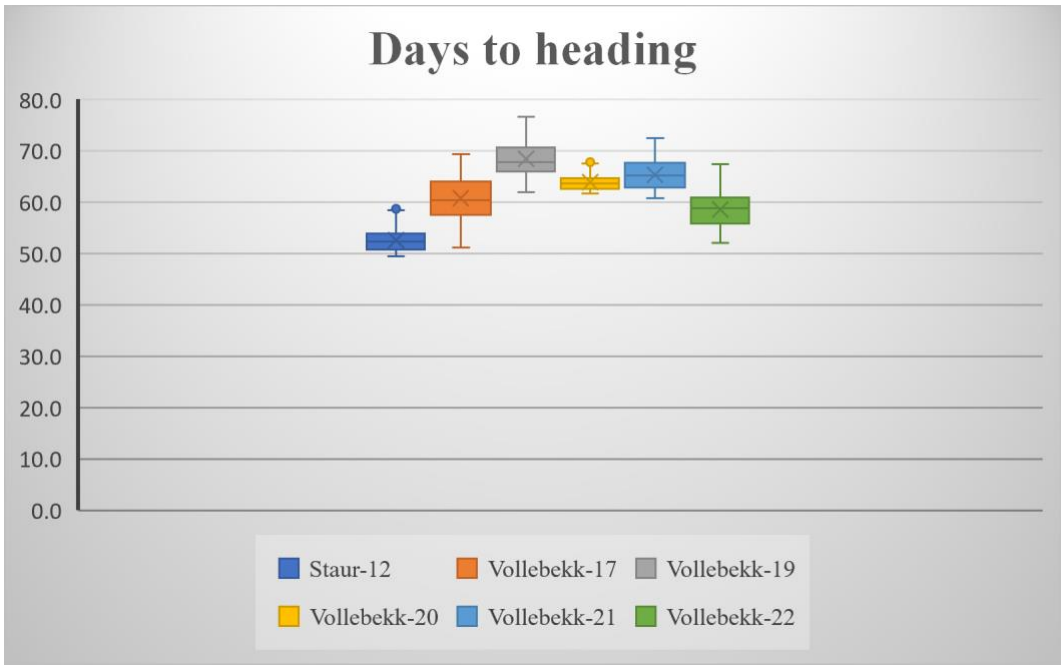

Fig. 7: Days to heading for all the trials. Y-axis given as number of days.

Days to heading is the number of days from sowing of seed to the shooting of the first flower stem. As can be seen in fig. 7, all plants used at least 50 days to heading. Staur-12 had the shortest average, with a mean of 53 days to heading, and a minimum of 50 days. The longest average was found in Vollebekk-19, with a mean of 68 days to heading, and a maximum of 77 days.

Plant height

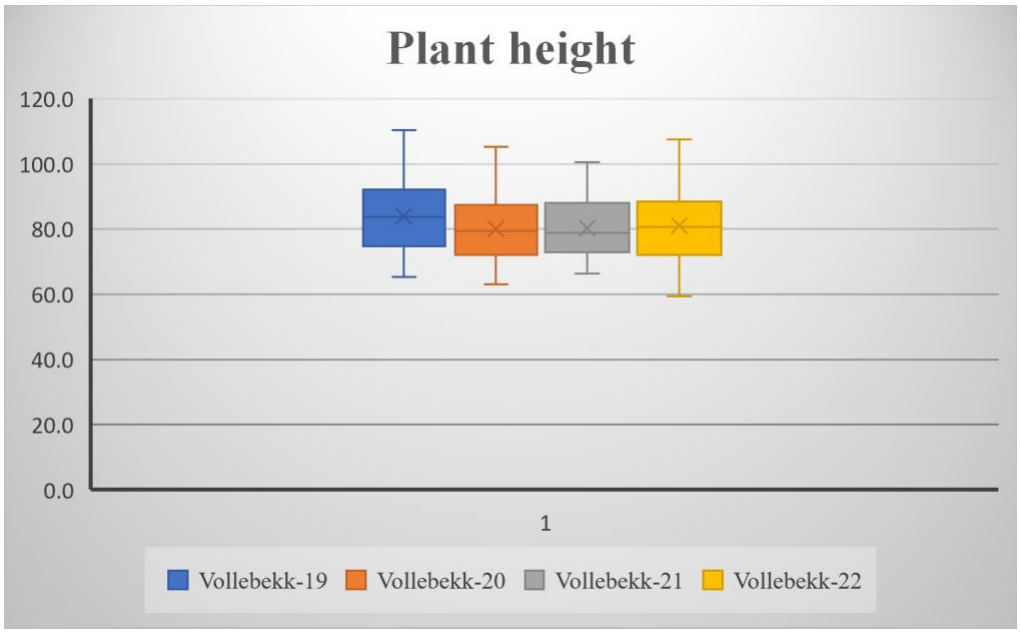

Fig. 8: Plant height for all trials where height was recorded. Y-axis values given in cm.

As shown in fig. 8, the average height of plants is fairly consistent through all trials, with the smallest average of 80,2cm found in Vollebekk-20, and the largest average at 84cm found in Vollebekk-19. The shortest plant came in at 59cm and was found in the Vollebekk-22 trial, and the tallest plant was 110cm, measured in the trial at Vollebekk in 2019.

3. 3. QTL-mapping

Which parent contributes most to the phenotype can be seen by the additive effect in the raw data (appendix 3). A negative additive effect means that T9040 contributes most to the phenotype, while a positive additive effect means that Avocet contributes most to the phenotype. In some cases, the most closely associated marker to the QTL a specific year is a marker known from a different chromosome than the QTL itself. In these cases, the physical position of the marker closest has been chosen for the interval. The physical positions of the markers have been obtained from a file compiled by my co-supervisor Min Lin, and is attached as appendix 4.

Leaf Blotch

Table 2: QTLs found using interval mapping, for leaf blotch corrected for days to heading. Most closely associated marker (the one giving the highest LOD value) for each. Numbers for each year are in percentage of phenotypic variation explained by each QTL.

Most closely associated marker(s)	**Physical position (Mbp)**	**Placement of QTL**	**Parent mostly contributing to QTL**	**vb22**	**vb21**	**vb20**	**vb19**	**vb17**	**st12**
AX-94760290	580.42483	chr 1A	T9040	12.7					
AX-110575862 (vb21), AX-95181236 (vb20), RFL_Contig4517_1276 (vb19), RAC875_c38018_278 (vb17)	639.988448-671.703270	chr 2A	T9040	(9.6)	16.0	26.5	26.5	30.4	
Excalibur_c20277_483	5.585696	chr 3B	Avocet		14.4				
AX-94685504	30.595306	chr 4B	Avocet	26.9					

Kukri_c16087_281	70.2166174	chr 5A	Avocet					13.8	
AX-94734229	463.992592	chr 5B	T9040						12.1
Tdurum_contig50698_601 (vb22), CAP8_c1881_215 (vb21 & vb17), wsnp_Ex_c280_541960 (vb20), Ex_c13223_1847 (vb19)	17.638471-51.952876	chr 6A	T9040	17.6	14.5	15.4	22.0	12.8	
TA002465-0455-w (vb22), wsnp_CAP11_rep_c4300_2030142 (vb20)	115.701042-117.841394	chr 6B	T9040	14.0		13.1			
wsnp_Ex_c46061_51675763	46.34941	chr 7B	T9040						19.2

As can be seen from table 2, several of the QTLs were only present in one or a few of the experiments, but the QTL on chromosome 2A and the QTL on chromosome 6A both explained a significant amount of the phenotypic variation in all the Vollebekk trials. The Staur trial did not share significant QTLs with any of the Vollebekk trials.

After the interval mapping, a multiple QTL mapping was run on the dataset. These results are presented in table 3.

Table 3: QTLs found using multiple QTL mapping, for leaf blotch corrected for days to heading. Most closely associated marker (the one giving the highest LOD value) for each. Numbers for each year are in percentage of phenotypic variation explained by each QTL.

Most closely associated marker(s)	**Physical position (Mbp)**	**Placement of QTL**	**Parent most contributing to QTL**	**vb22**	**vb21**	**vb20**	**vb19**	**vb17**	**st12**
AX-94760290 (vb22), AX-158605698 (vb21 & vb20)	580.188586-580.424830	chr 1A	T9040	9.8	8.7	7.3			
AX-95181236 (vb20), AX-158561603 (vb19 & vb17)	618.268569-663.328967	chr 2A	T9040			16.6	13.4	20.4	
Ku_c19185_1569_2D	461.301238	chr 2D	Avocet					8.4	
Excalibur_c20277_483	5.585696	chr 3B	Avocet		15.8				
AX-94685504 (vb22), TG0010a (vb20 & vb19)	30.595306-30.861559	chr 4B	Avocet	24.0		8.0	7.4		
AX-94734229	463.992592	chr 5B	T9040						12.2
Tdurum_contig50698_601 (vb22), Ex_c13223_1847 (vb19)	17.910881-51.952876	chr 6A	T9040	14.6			11.7		
TA002465-0455-w (vb22), AX-89332704 (vb21), CAP12_c1784_424 (vb20)	106.513871-199.080025	chr 6B	T9040	11.6	8.4	11.0			
wsnp_Ex_c46061_51675763	463.4941	chr 7B	T9040						15.4

The MQM mostly returned the same results as the regular interval mapping. However, an additional QTL at chromosome 2D was found for the field trial at Vollebekk 2017.

All the previous results were corrected for earliness (days to heading) but not for plant height. Another analysis, using interval mapping, was therefore run to correct for plant height. This analysis only comprises the years 2019-2022 at Vollebekk, since plant height was not recorded the previous years. These results are presented in table 4.

Table 4: QTLs found using interval mapping, for leaf blotch corrected for days to heading and plant height. Most closely associated marker (the one giving the highest LOD value) for each. Numbers for each year are in percentage of phenotypic variation explained by each QTL.

Most closely associated marker(s)	**Physical position (Mbp)**	**Placement of QTL**	**Parent most contributing to QTL**	**vb22**	**vb21**	**vb20**	**vb19**
RAC875_c38018_278 (vb22 & vb19), RFL_Contig4517_1276 (vb21), AX-95181236 (vb20)	639.988448-663.328967	chr 2A	T9040	8.0	13.1	24.4	19.4
Excalibur_c20277_483	5.585696	chr 3B	Avocet		7.5		
Kukri_c16087_281	702.166174	chr 5A	Avocet			9.2	
Excalibur_c6057_281	63.775359	chr 6A	T9040	5.7			
CAP8_c1881_215 (vb22, vb21 & vb19), wsnp_Ex_c280_541960 (vb20)	17.638471-17.911219	chr 6A	T9040	9.7	9.7	11.6	14.2

Jagger_c555_287 (vb22 & vb21), wsnp_CAP11_rep_c4300_2030142 (vb20)	117.841394-191.991853	chr 6B	T9040	6.5	7.9	9.9	

The results from this mapping eliminated some QTLs found in the previous two mappings. However, the prominent QTLs at chromosome 2A and chromosome 6A were still equally prominent. Based on this mapping, a summary of the QTLs which were prominent in three or more trials are presented in table 5. While the trial at Vollebekk in 2017 did not record plant height, it showed significance for two of the three most prominent QTLs in the interval mapping. Therefore, the values from the interval mapping (table 2) for Vollebekk-17 have been added to the summary.

As these results present the most conclusive resistance QTLs, the QTLS have here been named after the international standard for naming QTLs, according to Boden et al (2023).

Table 5: Summary of the most significant SNB resistance QTLs, using the results from table 3, and the vb17 data from table x. QTLs named after international standard.

Most closely associated marker(s)	**Physical position (Mbp)**	**QTL name**	**Parent contributing to QTL**	**vb22**	**vb21**	**vb20**	**vb19**	**vb17***
RAC875_c38018_278 (vb22,vb19 & vb17), RFL_Contig4517_1276 (vb21), AX-95181236 (vb20),*	639.988448-663.328967	*QSnb.nmbu-2A*	T9040	8.0	13.1	24.4	19.4	30.4
CAP8_c1881_215 (vb22, vb21, vb19 & vb17), wsnp_Ex_c280_541960 (vb20)*	17.638471-17.911219	*QSnb.nmbu-6A*	T9040	9.7	9.7	11.6	14.2	12.8
Jagger_c555_287 (vb22 & vb21), wsnp_CAP11_rep_c4300_2030142 (vb20)	117.841394-191.991853	*QSnb.nmbu-6B*	T9040	6.5	7.9	9.9		

Days to heading

Table 6: QTLs found using interval mapping, for days to heading (DH). Most closely associated marker (the one giving the highest LOD value) for each. Numbers for each year are in percentage of phenotypic variation explained by each QTL.

Most closely associated marker(s)	**Physical position (Mbp)**	**Placement of QTL**	**Parent contributing to QTL**	**vb22**	**vb21**	**vb20**	**vb19**	**vb17**	**st12**
AX-94611746 (st12, vb22)	749.958893	2A	Avocet	15.6					13.7
RAC875_c59673_188 (st12, vb19, vb20, vb21, vb22), wsnp_JD_c27162_22206547 (vb17)	664.154322 - 681.669144	4A	T9040	22.0	24.2	22.3	19.3	16.1	16.7
AX-89380014(st12), TG0010b (vb20), AX-111081978 (vb22)	30.861559-36.447963	4B	Avocet	12.3		11.9			16.6
Excalibur_c26671_57 (st12, vb17, vb19, vb20, vb21, vb22)	591.31917	5A	Avocet	28.2	22.9	21.8	25.7	17,1	27
RAC875_rep_c102342_470 (st12, vb20, vb22), AX-158537212 (vb17, vb19)	573.495707 - 589.372425	5B	Avocet	18.7		14.2	17.6	15.7	14.7
RAC875_c36779_148 (vb20)	548.429667	5B	Avocet			11.8			
Kukri_rep_c69164_94 (vb19, vb22)	100.761613	7A	Avocet	15			12.1		
IACX11443 (vb19)	701.404681	7A	Avocet				12.2		

Table 6 contains the QTLs that show significance for days to heading. The QTLs for DH were mapped to assure the quality of the leaf blotch data. The DH QTLs and LB QTLs show no overlap.

Plant height

Table 7: QTLs found using interval mapping, for plant height (PH). Most closely associated marker (the one giving the highest LOD value) for each. Numbers for each year are in percentage of phenotypic variation explained by each QTL.

Most closely associated marker(s)	**Physical position (Mbp)**	**Placement of QTL**	**Parent contributing to QTL**	**vb22**	**vb21**	**vb20**	**vb19**
RAC875_c19303_228 (vb22), AX-158618580 (vb21), AX-95215762 (vb20), AX-111081978 (vb19)	31.881864-85.873885	4B	Avocet	33.9	33.2	49.0	33.4

As shown in table 7. Only one significant QTL was found for plant height, on chromosome 4B.

4. Discussion

4. 1. QTLs

The phenotypic data show the traits to be normally distributed. Both parent cultivars seem to be slightly more resistant than receptive, as the distribution of the phenotypes in the offspring are skewed towards resistant (fig. 6). Staur-12 and Vollebekk-17 show the least amount of infection, with average leaf disease of 25 and 23 percent, respectively. The following years, the average amount of disease increased, from 30 percent in Vollebekk-19 to 46 percent in Vollebekk-22. This could be due to environmental factors, changes in the infectious agent, or fewer resistance QTLs. As all the three most significant QTLs are present in the more recent field trials, environment or difference in the pathogen seem to be the most likely cause. Wetter summers is a change in environment that might cause increased fungal infection. Comparing historical data from Yr.no for Vollebekk, Ås, the summers of 2017, 2019 and 2020 were all slightly wetter than the summers of 2021 and 2022. Therefore there seems to be no correlation with rainfall and infection rate these years, as the wettest years show the most LB resistance and the driest years show the most LB sensitivity. Therefore, a change in the infectious agent; either a mutation or a different strain, seems the most likely cause.

The resistance QTLs found for the trial at Staur in 2012 are entirely different from the resistance QTLs found at Vollebekk 2017-2022. This can be due to a variety of factors. It likely connects to the difference in environment, as QTLs are subject to genotype-by-environment interactions. It can also be due to different fungal strains in the two areas, or random mutations.

Three significant resistance QTLs were identified in the data from these field trials. The QTL *QSnb.nmbu-2A* (physical position 639.988448-663.328967 Mbp) found in this population of Avocet x T9040, corresponds to the published QTL *QSnb.niab-2A.3* (physical position 612.422267-677.529836 Mbp) identified in field trials at Vollebekk and greenhouse inoculation of seedling leaves in the population NIAB Magic by Lin et al (2020). The overlap in position shows that this is likely to be the exact same QTL, and the somewhat narrower position interval found as a result in this thesis narrows the physical position of the resistance locus on the chromosome further down. The fact that this QTL is conserved across both

breeding populations cements its place as an important SNB resistance locus. Since this is not a newly discovered QTL, it'll be referred to as *QSNb.niab-2A.3* from here.

Similarly, the *QTL QSnb.nmbu-6A* (physical position 17.638471-17.911219 Mbp) in this population of Avocet x T9040 overlaps with an unnamed QTL (physical position 17.664493-17.913074 Mbp) published by Francki et al in 2020. It was found to be a major QTL in a study comprising 71 wheat lines from Australian origin, 72 inbred and commercial lines from Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) and 78 inbred lines from International Center for Agricultural Research in the Dry Areas (ICARDA) (Francki et al, 2020). Not only does this QTL seem to be conserved across several breeding lines, as it was found of significance both in Australia and Norway it also is conserved in lines from opposite sides of the globe. Further, cultivars adapted for the Australian climate are significantly more drought resistant than cultivars adapted for the wetter Norwegian climate. As such, the QTL also shows significance as a resistance QTL in very different environments. This versatility may be of interest for future breeding.

The QTL *QSnb.nmbu-6B* (physical position 117.841394-191.991853 Mbp) seem to be novel. Its position does not match any previously published QTLs. Further studies may confirm or deny its significance.

While the focus of this thesis is on leaf blotch (LB), the population was also mapped for QTLs relating to days to heading (DH) and plant height (PH) for quality assurance. If a LB QTL were to show up as significant for DH or PH, that would make it less likely to be a resistance QTL. The data shows no overlap of QTLs for LB and DH, but the first interval mapping returned a resistance QTL at 4B which also shows up as significant for PH. In fact, it was the only significant QTL for PH. When the LB data was corrected for PH, the QTL on 4B disappeared from the LB QTL output. This shows that the QTL has significance for PH rather than LB. This makes sense as chromosome 4B in wheat is known to contain dwarf genes (Chai et al, 2021), which have a large impact on plant height.

It is also notable that the QTLs connected to plant height and days to heading are fairly conserved across all the populations, including Staur, while the QTLs for leaf blotch resistance are much more varied. This implies that LB resistance is much more subject to the environment than growth (PH and DH) is.

The LB resistance QTLs are mainly inherited from T9040 (table 5), confirming it is the more resistant parent. However, the DH and PH QTLs are mostly inherited from Avocet. This shows that Avocet contain more genes that influence early blooming and height of plants.

In some of the QTLs, the marker having the highest LOD score was originally isolated from a different chromosome than the one the QTL is found on. This is related to the nature of SNPs and the nature of the wheat genome. With SNPs being very short sequences and the wheat genome containing large amounts of retrotransposons and other repeating sequences, the same short sequence may be found several places along the wheat genomic code. This is a more likely explanation than recombination in the case of SNPs.
A major point of interest for this study was to see if any QTLs on 5B could be identified for field resistance. As written earlier, the necrotrophic effectors SnToxA and SnTox3 which have large effects on seedling plants in greenhouse inoculation trials correspond to loci on chromosome 5B. If resistance QTLs on 5B were found in this study, it would make the link between SnToxA and SnTox3 and field resistance more probable. However, as seen from the QTL tables in the results section, no such QTL was found. The only resistance QTL found on chromosome 5B was in the trials at Staur 2012, and it's not enough to establish a probable link. The relationship between SnToxA and SnTox3 and field SNB resistance remains unresolved in this study.

4. 2. Linkage groups

When working on this thesis, by far the most time was spent creating the linkage groups. I started out with a maximum LOD-value of 10, but this created linkage groups with large gaps and many suspect linkages. Therefore, the LOD-value was increased to a maximum of 20. This gave linkage groups with fewer gaps and fewer suspect linkages but led to a single chromosome containing several linkage groups.

In the end, I decided to keep it this way. I found it to be more useful to keep the linkage groups of good quality to get good quality QTL data, rather than try and merge several together and compromise on quality. Another issue is the unmapped markers. Several markers would not fit into the map, and yet other markers would only fit into a linkage group of their own. However, the unmapped markers also show up in the QTL mapping in

MapQTL. When analysing the data, none of the unmapped markers showed LOD-values of especially high interest, so I decided not to try and place them.

5. Conclusion

Three high-quality resistance QTLs were found in this study. Two previously published, and one novel. They have the names *QSNb.niab-2A.3* (as named per the previous publisher of this QTL), *QSnb.nmbu-6A* (while previously published, this one was not previously named), and *QSnb.nmbu-6B* (novel QTL for this study). All three show consistency across the different field trials. No resistance QTL on chromosome 5B were found, on which QTLs for SnToxA and SnTox3 sensitivity are placed. While SnToxA and SnTox3 are linked to disease sensitivity in the greenhouse, no correlation could be found in these field trials .

6. Supplementary material

Appendix 1: Genetic linkage map of the Avocet x T9040 population
Appendix 2: Raw data map with short marker names from JoinMap
Appendix 3: Raw data from MapQTL
Appendix 4: Physical position of the markers on the genome, compiled by Min Lin
Appendix 5: Genotypic data provided to me
Appendix 6: Phenotypic data provided to me

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