Received: 17 July 2023 | Revised: 22 November 2023 | Accepted: 15 December 2023

DOI: 10.1111/pbr.13156

ORIGINAL ARTICLE

Plant Breeding WILEY

Identification of haplotypes associated with resistance to *Fusarium graminearum* in spring oat (*Avena sativa* L.)

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Funding information
Research Council of Norway; Graminor, Grant/Award Number: 286606

Abstract

Fusarium head blight (FHB) is the predominant disease in oat in Norway caused by the fungus *Fusarium graminearum*. It causes yield loss, reduced seed quality, reduced germination ability and accumulation of deoxynivalenol (DON). The FHB resistance is quantitative, and most genes have small effect. Markers with verified effect in the breeding program could further enhance the resistance breeding. This study aims to use a large and diverse population of 541 lines to identify quantitative trait loci (QTL) associated to FHB resistance in a genome-wide association study (GWAS) and verify their effect in independent breeding material. The material has been tested in six environments over three years and two locations in spawn inoculated and mist irrigated disease trials. The traits tested were germination ability and DON accumulation. A total of 15 significant QTL-regions were detected across 12 different linkage groups. Haplotypes for each region was constructed and the effect of the alleles in each environment was calculated, which identified the most likely resistant and susceptible alleles. Five QTL-regions were validated showing consistent effect in the GWAS population and the breeding material. Stacking of the resistant alleles of these regions from zero to five showed significant decrease in DON values and increased germination ability. The haplotype information of a set of historical and modern Nordic varieties were analysed, and the results could be used to select parents for future crossings. The validated haplotypes from this study can be used either to do marker assisted selection (MAS) or improve genomic prediction models in breeding programs.

KEYWORDS
DON accumulation, *Fusarium graminearum*, genome-wide association study, germination ability, QTL-regions

1 | INTRODUCTION

Oats is one of the most important cereal crops in Norway and is considered a good break crop in rotation with barley and wheat, as they have few diseases in common (Abrahamsen et al., 2016). But one disease that infects all crops and pose major economic challenges, especially in oat, is Fusarium head blight (FHB) (Bernhoft et al., 2013). FHB is caused by a wide range of *Fusarium* species. In Norway, *Fusarium graminearum* stands as the main species responsible for FHB in oats (Hofgaard et al., 2016). This disease reduces yield, seed quality,

germination capacity (Tekle et al., 2013) and produces the mycotoxin deoxynivalenol (DON). DON is known to induce inflammation in the intestines in both humans and animals (Kang et al., 2019). To mitigate health risks, Norway and the EU have set a threshold of 1.75 ppm DON for unprocessed oat (Commission regulation, 2006).

The need for resistant oat varieties in Norway is underscored by three key factors. Firstly, the temperate and humid conditions during Norwegian summers create favourable conditions for fungal growth and spore production of *F. graminearum* (Xu et al., 2008). Secondly, the available fungicides have limited effect, providing only 30–50% reduction in DON content when applied at the optimal timing (Felleskjøpet Agri SA, 2023). Thirdly, *F. graminearum* is shown to have the ability to sexually reproduce in Norway (Aamot et al., 2015), which heightens the risk that the pathogen adapts and gains resistance to fungicides, which could improve aggressiveness, virulence and mycotoxin production (Becher et al., 2010; de Chaves et al., 2022).

Fusarium resistance can be divided into five primary classes (Hautsalo et al., 2018); (i) resistance to initial infection (Schroeder & Christensen, 1963), (ii) resistance to disease spread, (iii) resistance to kernel infection, including germination ability (Mesterházy, 1995), (iv) tolerance (Mesterházy, 1995) and (v) resistance to mycotoxin accumulation (Mesterházy et al., 1999; Miller et al., 1985). Additionally, there are several passive avoidance mechanisms that exhibit strong correlation with FHB resistance. Two key mechanisms are plant height (PH), where taller plants increase the distance from the initial conidia spores from the soil to the heads (Hautsalo et al., 2020), and days to heading (DTH), which affects the risk of plants flowering at the time of high disease pressure (Tekle et al., 2018).

Previous investigations that aimed to detect resistance QTL in oat found several QTL focusing on the traits FHB severity, DON accumulation and Germination ability (Bjørnstad et al., 2017; Haikka et al., 2020 & He et al., 2013). The present study aims to identify and validate QTL along with linked markers for implementation in marker-assisted selection. This will be achieved through a comprehensive genome-wide association study (GWAS) focusing on DON accumulation and germination percentage. The study employs a large and diverse germplasm, subjected to disease trials conducted across multiple environments. Furthermore, the investigation seeks to determine the presence of QTL in independent breeding populations.

2 | MATERIALS AND METHODS

2.1 | Materials

2.1.1 | GWAS panel

The GWAS panel used in this study consisted of 541 oat lines and cultivars selected from a larger pool of 1124 by using a combination of selection strategies evaluated by Sørensen et al. (2023). These strategies were based on marker information and was used to ensure that the GWAS panel had high relationship to the breeding material (Akdemir et al., 2015), and high genetic diversity (Franco et al., 2005). Additionally, lines that exhibit resistance and susceptibility based on historical data from Graminor's databases were included. Lines with very long and short straw as well as naked varieties were excluded to avoid association between the traits and FHB resistance. In total, 440 lines were from Norway, 40 from Sweden, 26 from Germany, nine from Netherlands, nine from Finland, eight from Canada, five from USA, two from Austria, one from Denmark and one from Australia (Supplementary Table S1).

2.1.2 | Validation populations

Three breeding populations (V1, V2 & V3) were used to validate significant markers from the GWAS analysis. V1 and V2 consisted of 242 F_{10} breeding lines from Graminor from the years 2020 and 2021, respectively. F_{10} lines were used as they have not yet been selected for fusarium resistance. V3 consisted of 230 lines where 112 were F_{10} lines from 2022, 88 were F_{11} from V2, 22 were F_{12} from V1 and eight were F_{13} from a breeding population from 2019.

2.2 | Field trials

Field experiments with the GWAS panel were conducted for three years from 2020 to 2022 in two locations, Vollebekk (59.66°N, 1.75°E) and Staur (6.73°N, 11.10°E). The GWAS panel trials were abbreviated as 20S, 20V, 21S, 21V, 22S & 22V for the individual years and locations, and Ov for the overall trial analysis. V1, V2 and V3 were tested in one year each, 2020, 2021 and 2022, respectively in both locations. The experimental design of the GWAS panel, V1 and V2 was alpha lattice with two replicates and sub-block size of 5. The experimental design of V3 was randomized complete block with two replicates (RCBD). All materials were sown in spawn-inoculated and mist-irrigated disease trials (Tekle et al., 2018).

2.3 | Phenotyping

All plots were scored for DTH as the number of days from sowing to at least 50% of the heads had emerged, and PH as the number of centimetres from the ground to the top of the plants. DON was measured on milled seed samples with husks as parts per million (ppm) at Graminor with an Agraquant Deoxynivalenol Plus (0.25/5), 96 Wells ELISA kit developed by Romer Labs Ltd. Germination ability was measured at Graminor as percentage of germinated seeds (GP) using the 'between paper' method described in point 5.6.2.1.1 of the ISTA protocol (International Seed Testing Association, 2021). Two replicates of 50 seeds were used per plot. Plastic bags were used to retain moisture. Samples were stored in 5–10 °C for 7 days, and approximately 20 °C for 6–8 days before analysis. The papers used were of size 220 × 400 mm and 200 × 400 mm with a capillary capacity of 80 mm/10 min. DON and GP were collected for all trials except GP for V3 Staur in 2022.

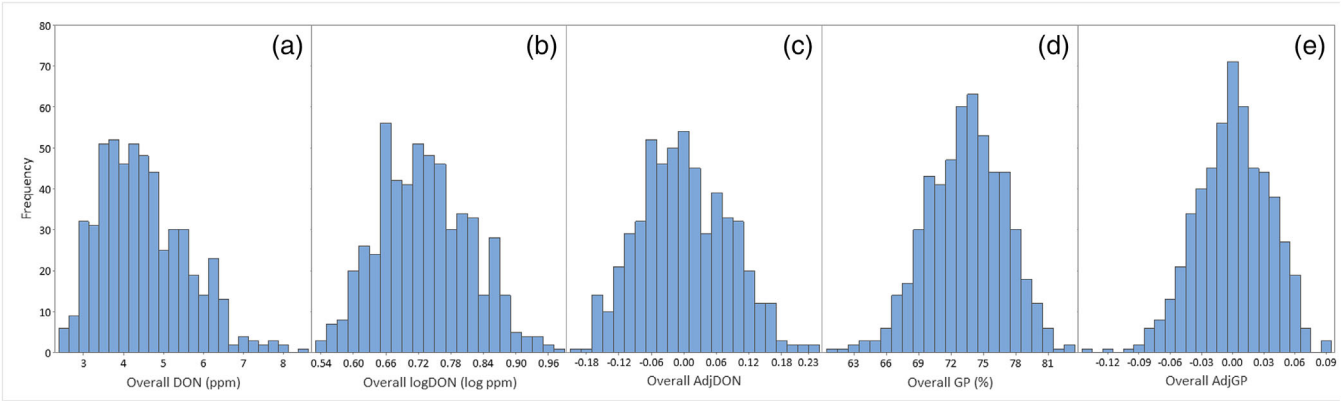

FIGURE 1 Distribution of overall phenotypic values of the GWAS panel for the traits DON values in ppm (a), logtransformed DON values (logDON) (b), logtransformed DON values adjusted for effect of days to heading and plant height (AdjDON) (c), germination percentage (GP) (d) and germination percentage adjusted for effect of days to heading and plant height (AdjGP) (e) with number of genotypes in the Y-axis and phenotypic values in the X-axis.

2.4 | Phenotypic data analysis

The Best linear unbiased estimators (BLUE) were calculated for each genotype in each trial and across trials (Overall), with the META-R software (Alvarado et al., 2016) using the models listed in Supplementary Table S4. The GWAS analysis was performed on data from single trials and overall, while the analysis for V1–3, only the overall values were used. Outliers were not excluded from the dataset. The BLUE DON values (Figure 1a) were log transformed to obtain close to normally distributed values (Figure 1b) (West, 2022) for the GWAS analysis. Both BLUE values of GP (Figure 1d) and logDON values were adjusted for the effect of DTH and PH to avoid false association to the correlated traits, by performing a regression analysis with GP and DON as response variables separately, and DTH and PH as explanatory variables (Nannuru et al., 2022). The resulting adjusted DON (AdjDON) (Figure 1c) and GP (AdjGP) (Figure 1e) have zero correlation with PH and DTH and R^2 of 0.95 and 0.94 with the unadjusted logDON and GP overall values. AdjDON and AdjGP are used as phenotypes in the subsequent GWAS analysis.

2.5 | Genotyping and data preparation

All lines of the GWAS panel were genotyped with a customized, unpublished 20K SNP-chip containing 18,598 markers including all markers from the publicly available 6K SNP-chip (Tinker et al., 2014). The genetic data were filtered with a threshold of 10% for missing values/heterozygotes and 5% MAF based on the GWAS panel, resulting in 3071 polymorphic markers. V1–3 were genotyped using a different customized 7K-SNP chip (Polley et al., 2023) containing 6642 markers where 6587 were the most polymorphic markers from the Nordic 20K SNP-chip. An unpublished consensus map was used to assign markers to linkage groups (LG) representing the 21 oat chromosomes. The map is an updated version of the genetic map developed by Chaffin et al. (2016), updated with six biparental populations from the Nordic breeding programs. Linkage disequilibrium (LD) between each pair of markers was calculated using the TASSEL statistical software (Bradbury et al., 2007).

2.6 | GWAS analysis

The GWAS was performed for the traits AdjGP and AdjDON for individual environments and overall values with the 'farmCPU' method (Liu et al., 2016) in the GAPIT3 package (Wang & Zhang, 2021) with the R statistical software (R Core Team, 2022). FarmCPU were chosen over MLMM based on the QQ-plot results (Supplementary Figure S2). FarmCPU has proven more efficient compared with other models for *F. graminearum* resistance in wheat (Nannuru et al., 2022). This method is considered statistically powerful, it avoids overfitting and reduce the number of false positive and negatives compared with other models (Kaler et al., 2020). The GWAS was not corrected for population structure as a 'model selection' approach in GAPIT revealed that zero principal components were optimal, and a visual inspection of a PCA plot of the marker data supported this (Supplementary Figure S1). Markers with FDR adjusted *p*-value less than .05 were considered significant and were calculated as $\frac{p*n}{r}$ where *p* is the *p*-value, n is the number of markers tested, and r is the rank of the marker from lowest to highest *p*-value (Benjamini & Hochberg, 1995).

2.7 | QTL-regions and haplotype analysis

QTL-regions were determined as the significant markers from the GWAS that were on the same LG and in significant LD with each other. Markers that were not in LD on the same LG were considered as a separate region. Regions with only one marker from single experiments were not analysed further, while single marker detected using the overall data were retained. Haplotypes from each region were

then formed by adding the significant SNP-marker information in the region together from lowest to highest centimorgan position on the consensus map.

The haplotype alleles for each QTL region were analysed in each environment of the GWAS panel and the overall values of V1–3 with a Games–Howell simultaneous pairwise comparison test (Games & Howell, 1976) using Minitab statistical software (Minitab, 2021) on the unadjusted DON and GP. Higher number allelic variations in the regions equal higher number of comparisons. This was done to determine which allele had significantly ($p < .05$) lower DON and higher GP than others in each environment. The ones that most frequently showed to significantly resistant than other in the same region were designated the resistant allele, and similar for the susceptible allele. Resistant alleles that showed consistently lower DON and higher GP than the susceptible allele across environments and populations were considered validated. The effects were summarized as percentage difference in DON to get similar scales across environments. This was calculated as the difference in mean divided by the average between the alleles multiplied by 100.

2.8 | Allele stacking and analysis of historical varieties

QTL-regions validated from the haplotype analysis were further analysed for their additive effect on the phenotypes DON and GP. Games–Howell tests were performed to see if there were significant decrease of DON and increase in GP with increased number of resistant alleles. This was done only in the overall GWAS panel. DTH and PH were also analysed to see if the resistant alleles had any effect on these traits.

To evaluate the trend of resistant alleles through time, and possibly identify resistance sources for crossings, a set of 74 varieties were selected to represent the most important Norwegian material from the last century (Supplementary Table S3). Each variety were given a year as an approximation of the year they became inbred lines determined as six years before release or six years after crossing depending on available information about the variety. The dataset was assembled in groups of different time periods, and the number of genotypes carrying different number of resistant and susceptible alleles were summarized and averaged for each time period.

2.9 | BLAST search

Markers in the validated QTL-regions were BLASTED against the reference genome of OT3098 (PepsiCo, 2021) in the GrainGenes database (Yao et al., 2022). This reference genome was chosen because it contains more annotations with information on gene function than other reference genomes. When the markers got more than one chromosome hit the lowest average E-value among the markers determined which chromosome they were assigned to. The region between the markers and 10 Mbp in each side were investigated for annotated genes described with an effect on disease resistance.

3 | RESULTS

3.1 | Phenotypic correlations

PH showed significant positive Pearson correlation to GP in Staur and Vollebekk in 2020, while the correlation was significantly negative in 2022 (Table 1). The overall values were non-significant and close to zero, as was most other trials. DTH showed significant positive correlation to DON in four experiments, significant negative correlation with GP in three, with r values of .2 and −.25 respectively for the overall values of DON and GP. DON showed significant negative correlation to GP in five of six experiments and the overall values, with r values between −.13 in Vollebekk in 2020 and −.69 in Staur in 2022.

Trial statistics are shown in the Supplementary Table S5. The heritability was relatively high for both DON and GP in all environments except for the GP of 2020 Vollebekk, while the overall heritability were .79 for DON and .62 for GP, which were higher than any individual experiment. The genotype effect was significant below .05 for both traits in all experiments. The overall values of AdjDON and AdjGP were close to normally distributed (Figure 1c and e).

3.2 | GWAS analysis and QTL-regions

A total of 48 significant markers for FHB resistance were detected, 24 for adjDON, 22 for adjGP and two for both. Six LGs (3C, 4C, 8A, 10D, 11A and 14D) had only one significant marker for one trait from a single experiment and were not analysed further. Four unmapped markers were detected, three of them were in LD with the significant markers on LG 1C. The unmapped markers were analysed further. Two markers on 19A and 21D were excluded from further analysis as they were not in significant LD with the other markers in the LGs. The remaining 36 markers were assembled into 15 QTL-regions (Table 2). The markers on 5C, 7C-17A and 18D were split into separate QTL-regions as there was no significant LD between them.

3.3 | Haplotype analysis and ANOVA validation

The haplotype analysis revealed that of the 15 QTL-regions detected in the GWAS, five (1C, 7C-17A-2, 9D, 12D and 18D-1) showed a consistent effect and significant difference in unadjusted DON and GP in comparison between the resistant and susceptible alleles for the overall phenotypes and at least two individual experiments. They also showed the same effect in at least two of three validation populations (Table 3). Of these five, 9D stands out positively, because the difference between the resistant and susceptible alleles were highly significant for DON in all experiments in the GWAS panel and V3 with an effect of 12–22% reduction in DON content.

TABLE 1 Pearson correlation between the BLUE values of traits plant height (PH), days to heading (DTH), DON accumulation in ppm (DON) and germination percentage (GP) from each experiment (20S, 20V, 21S, 21V, 22S, 22V) and the overall values (Ov) of the GWAS panel and their level of significance level with *α > .05, **>0.01 and ***>0.001.

	20S	20V	21S	21V	22S	22V	Ov
PH v DON	−.23***	0.10*	.03	−.04	.05	−.02	−.05
PH v GP	0.10*	.15***	−.04	−.05	−.03	−.20***	−.06
DTH v DON	.00	.29***	.15***	0.33***	.05	.14**	0.21***
DTH v GP	−.03	.01	−.16***	−.34***	.08*	−.34***	−.25***
DON v GP	−.34***	−0.13**	−.02	−.28***	−.69***	−.49***	−.52***
DTH v PH	.30***	.29***	0.38***	0.10*	.29***	.25***	.30***

TABLE 2 QTL-regions determined from significant markers from either single environment or overall GWAS for the traits log-transformed DON and GP adjusted for effect of days to heading and plant height (AdjDON& AdjGP). The table includes the size of the region in cM (span), number of SNPs detected (n-SNP), the number of experiments they were detected in (n-exp), the traits associated to the region (trait) and the range of -10log-transformed *p*-value of the markers (-LOG(p)). The R-HT is the most frequently resistant allele in the number of significance tests (R-HT tests), and the most frequent susceptible-allele (S-HT) from the same number of tests (S-HT).

QTL/LG	Span (cM)	n-SNP	n-Exp	Trait	-LOG(p)	R-HT	R-HT tests	S-HT	S-HT tests
1C	89–92.8	4	3	AdjGP & AdjDON	3.84–14.22	ACAG	19/24	GTCA	10/24
2C	74.2	2	2	AdjGP	3.86–6.04	TC	3/4	CA	3/4
5C-1	48.7–52.4	3	2	AdjGP & AdjDON	4.07–4.73	CAC	5/10	TGA	6/10
5C-2	86.6	1	1	AdjGP	4.42	C	6/6	T	6/6
6C	48.7–65.3	2	2	AdjGP	5.3–6.43	CA	2/3	CG	2/3
7C-17A-1	28.7–72	3	3	AdjGP & AdjDON	3.69–4.94	CGA	18/25	TGG	11/25
7C-17A-2	74.9	1	1	AdjDON	4.32	G	1/1	A	1/1
9D	21.3–35.4	3	4	AdjGP & AdjDON	5.2–5.42	CTC	11/17	TCT	17/17
12D	51–61.1	3	2	AdjGP & AdjDON	4.15–4.93	GGC	19/23	TGT	12/23
15A	87.9	1	1	AdjGP & AdjDON	5.12	A	4/4	G	4/4
18D-1	25–45.9	3	3	AdjDON	4.8–6.54	AGT	10/10	GTC	10/10
18D-2	97.7–99.5	2	2	AdjGP & AdjDON	4.33–4.87	TG	4/5	CG	3/5
19A	30–54.1	2	2	AdjDON	5.11–5.31	AA	3/5	CG	3/5
20D	39.6–7.9	2	2	AdjGP	4.09	TG	4/9	CT	5/9
21D	41.9–87.8	4	3	AdjGP & AdjDON	4.02	AACC	10/13	AACT	5/13

TABLE 3 List of the five most significant QTL-regions and the difference between the resistant and susceptible haplotype alleles listed in Table 2 in all environments of the GWAS panel (20S, 20V, 21S, 21V, 22S, 22V and Ov) and the validation populations (V1–3). DON is shown as percentage difference between alleles ([difference in mean/average between groups] × 100)) while GP is given as the difference in mean percentage points. *α < .05, **<.01 and ***<.001.

QTL	20S	20 V	21S	21 V	22S	22 V	Ov	V1	V2	V3
DON										
1C	−8	−5	−8	−6	−24***	−11*	−12*	−43	31	−14
7C-17A-1	-17	−31*	−18	−26	−29*	−19*	−23**	−32	−6	−33***
9D	−22***	−19***	−14**	−14**	−19***	−12***	−16***	−16	−17	−15*
12D	−15	−26**	−19*	−8	−22**	−15**	−17***	NA	−15	−14
18D-1	−9	−9*	−11**	−15**	−14**	−13***	−12***	−2	−16*	−14**
GP										
1C	1.8	0.1	1.1	1.1	6.9***	7.9***	3.1***	1.5	−1.8	5.6
7C-17A-1	2.2	2.9	0.1	3.8*	4.9	9.4***	4.2***	4.0	.6	12.2
9D	2.9***	.9	0.5	−0.2	3.1*	2.7**	1.6***	0.5	2.0	4.6*
12D	5.3***	.6	−0.1	−0.7	3.7*	4.2**	2.1***	NA	2.7	5.2
18D-1	2.4**	0.7	0.2	0.1	2.9**	3.2***	1.6***	−0.2	2.2*	4.9**

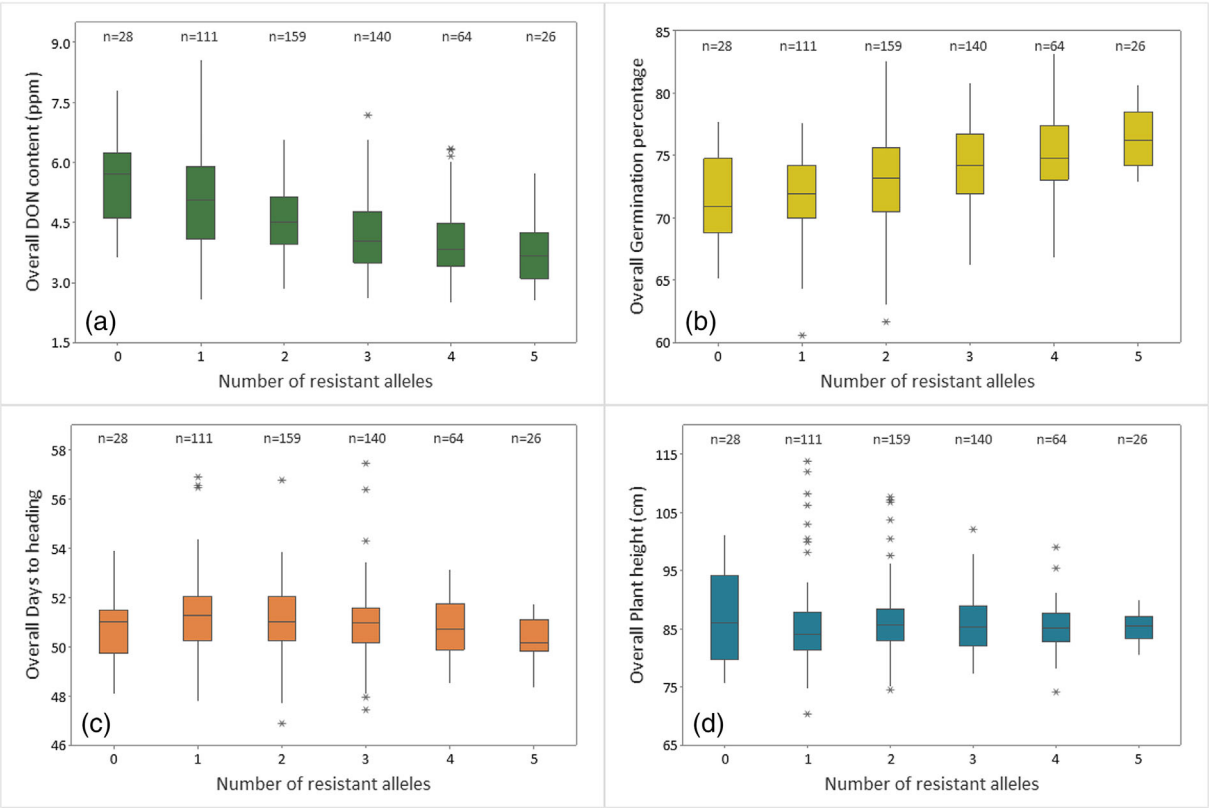

FIGURE 2 Boxplots of overall values from the GWAS panel for the traits DON accumulation in ppm (DON) (a), germination percentage (GP) (b), days to heading (DTH) (c) and plant height (PH) (d) with phenotypes in the Y-axis and the number of resistant alleles from Table 3 in the X-axis. N equals the number of lines that carry different number of alleles.

Table 2 shows which alleles were the most frequently resistant (R-HT) and susceptible (S-HT) in the significance tests. Some QTL-regions had low number of significant comparisons (2C, 6C, 7C-17A-2, 15A, 18D-2 and 19A). Others had low frequency of resistant or susceptible alleles (1C, 5C-1, 7C-17A-1, 12D, 20D and 21D). Some combinations of markers were missing in the populations; hence, all allelic variations were not tested in this study.

3.4 | Allele stacking

The number of validated resistant alleles showed a reduction from a mean DON of 5.47 ppm with zero resistant alleles to 3.71 ppm with five (Figure 2a). GP increased from 71.5% for the group with zero resistant alleles to 76.4% for the one with five (Figure 2b). Both DON and GP had a linear increase in resistance from zero to five alleles, and R^2 between phenotype and number of alleles were .17 and .13 for overall DON and GP respectively which were both highly significant with $p < .001$ (data not shown). There was no significant reduction in DON from zero to one resistant allele, but there was a significant reduction from one to two, and two to three. But no significant reduction from three to four or five. For GP, there was a significant increase from zero to one, one to two, and two to three alleles, and from three to five, but not from three to four or four to five. The number of resistant alleles did not affect the PH (Figure 2d), but for DTH, there were significant reductions from one, two and three to five alleles with approximately one day difference between one and five (Figure 2c).

3.5 | Analysis of historically important varieties

The five validated haplotypes from Table 3 were analysed in a subset of material that includes older important Nordic varieties from 1895 to 1999 and modern Norwegian varieties from 2001 to 2017. The full list of varieties and their haplotypes are listed in Supplementary Table S3. The analysis showed that the average number of resistant alleles (Figure 3a) increased from 1.3 in the period of 1895–1920 to 3.1 in 2001–2009 with a small dip down to 2.7 in 2011–2017. In the oldest varieties, 70% had one or less resistant alleles, while for most of the modern varieties, none had less than two. The number of susceptible alleles (Figure 3b) has been reduced from an average of 1.7 in the period 1895–1920 to 0.3 in 2001–2009 with a small increase to 0.4 in 2011–2017. In the oldest varieties, 60% had two or more susceptible alleles while 65% of the varieties from 2001–2017 had zero. There was almost no difference in resistant allele frequencies between the periods 1991–1999 and 2001–2009 with an increase in average of 0.2, while the

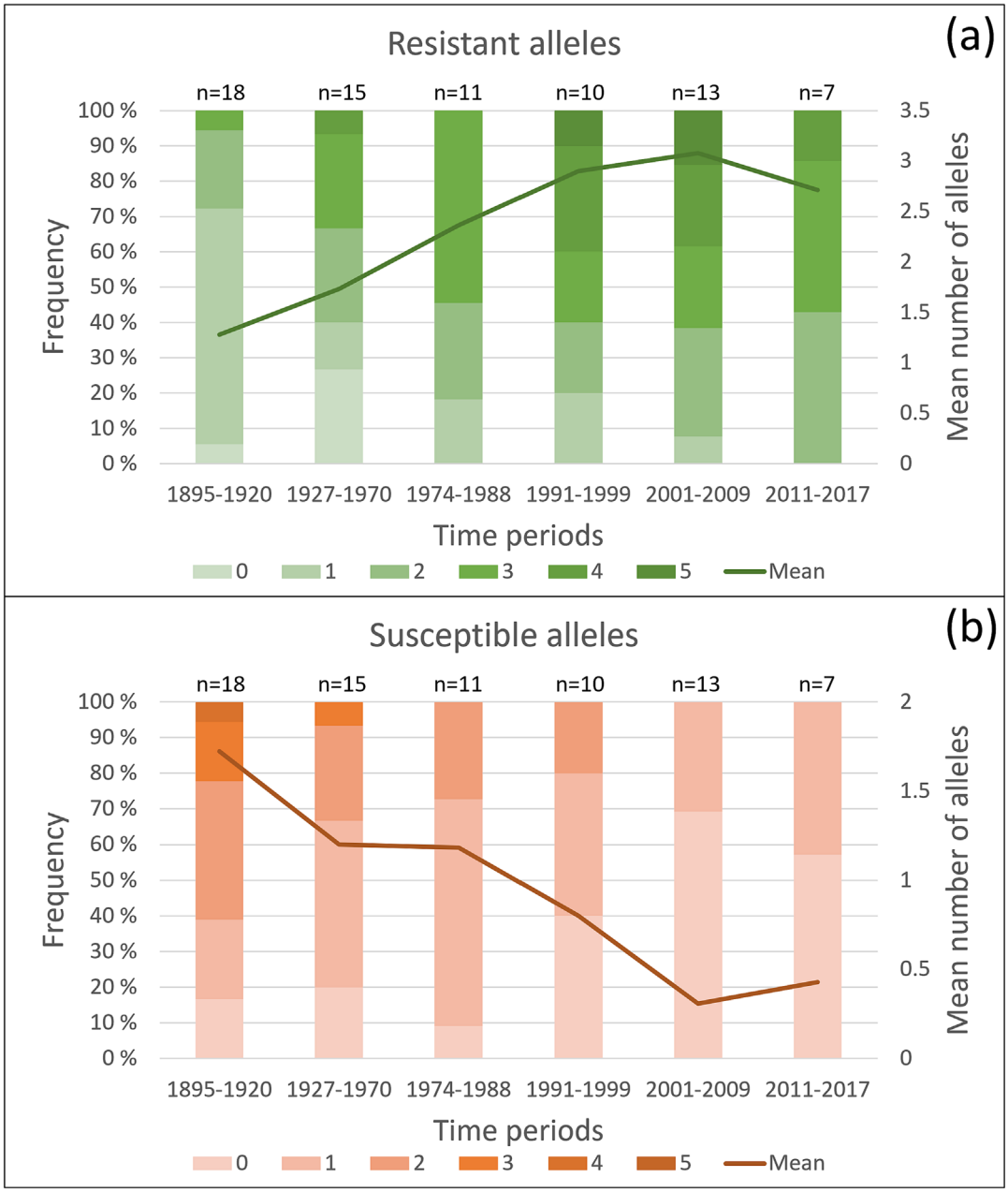

FIGURE 3 Number of resistant (a) and susceptible (b) alleles listed in Table 3 present in a selection of historical Nordic lines from 1891 to 2017. Each column represents a time period of approximately when the cultivars became inbred lines. The left Y-axis is the frequency in percentage and the right is the average numbers of alleles. The different colours are the different number of alleles, and the solid line is the mean number of alleles in each period.

number of susceptible alleles was reduced with an average of 0.5 in the same period.

3.6 | Candidate genes

Based on BLAST searches in the OT3098 reference genome (PepsiCo, 2021), several disease resistance related genes were identified within the five QTL-regions. The QTL-regions 1C, 7C-17A-1, 9D, 12D and 18D-1 spanned 21.2, 47.3, 4.1, 23.2 and 4.2 Mbp, respectively. The genes found in these regions are named *RGA1–5*, *RPM1*, *RPS2*, *Pik-1*, *2* & *6*, *RPP13*, *At3g14460*, *At1g50180*, *EDR2* and *EDR4*. QTL region 1C contained 20 candidate genes, which is the highest among the regions with 16 of them close to the first marker. Regions 7C-17A-1, 12D, 9D and 18D-1 contained 16, 11, five and three candidate genes, respectively. The third SNP in the 7C-17A-1 haplotype did not match the same chromosome as the other two but a homologue, so it appears to not be part of the same QTL region. The full list of candidate genes found in each LG is listed in Supplementary Table S2.

4 | DISCUSSION

4.1 | Quality of data

The phenotypic data showed a highly significant negative Pearson correlation between DON and GP (Table 1), which is expected from previous studies (Hautsalo et al., 2020; Tekle et al., 2012; Tekle et al., 2018). It was expected that the agronomic traits DTH and PH would be highly correlated to DON and GP (Moreno-Amores et al., 2020), which was the case for DTH but not for PH. This could be because the tallest varieties are also oldest and more susceptible either due to few resistant alleles or late heading. The disease pressure varied between the experiments, but this did not affect the difference in ranking as the Pearson correlation between the trials remained high for DON. The CV values for DON ranged between 20 and 43% for the individual experiments, which shows that there is a large variation in DON contents within the trials. But given that the CV values are smaller than similar studies in oat (32.6–63.2) (Haikka et al., 2020; Yan et al., 2010), and that the heritability measures were relatively high means that the experiments were successful, which ensured a good expression of genetic variation. GP had generally lower heritability than DON.

4.2 | Comparison with previous QTL studies

The results from this study can be compared with three mapping studies on resistance to *F. graminearum* in oat. The first (He et al., 2013) used two biparental populations based on crosses between accessions derived from *Avena sterillis*, North American varieties and a Norwegian variety. It detected QTL in three LG that might correspond to our results on 5C, 7C-17A and 9D. The second study (Bjørnstad et al., 2017) used mostly North American breeding lines with a few varieties from Northern Europe. They detected QTL on 6C, 7C-17A and 9D that might correspond to our results. The third (Haikka et al., 2020) used most Finnish breeding lines and varieties. They did not detect any significant markers, but they did detect low *p*-values in regions on 1C and 9D potentially corresponding to our results. To summarize, there is ample evidence to support a major QTL for FHB resistance on 9D and 7C-17A based on our results and previous research. The remaining QTL-regions appear to be novel, except for regions on 1C, 5C and 6C, which might have been detected in previous studies. More precise mapping of physical position of markers is needed to properly evaluate the overlapping regions in these studies.

A recent study from Norway suggests that the ranking of cultivars is partly similar in accumulation of DON and HT2 + T2, which is the mycotoxin produced by *Fusarium langsethiae* (Hofgaard et al., 2022). There was, however, evidence to suggest that some resistance is specifically associated to DON or HT2 + T2. A recent GWAS study on *F. langsethiae* detected a significant QTL on LG 14D (Isidro-Sánchez et al., 2020). This QTL were not significant in our study and could therefore be considered specific to *F. langsethiae*.

4.3 | Candidate genes

There were several different disease resistance genes within the QTL-regions, and some of them were grouped together in smaller clusters. Networks of QTL as a defence response to *F. graminearum* have been previously reported in bread wheat (Kugler et al., 2013) and durum wheat (Sari et al., 2019), which also find the same genes as this study, specifically RGA1, RGA2, RGA4, RPP13 and At3g14460. A study of the *F. graminearum* fungus indicated the presence of *AVR-Pik* effector genes that helps in the infection of plant tissues (Hao et al., 2020). A different study claimed that the *Pik-1* and *Pik-2* genes work as defence genes against these effectors in rice (Maidment et al., 2023). Both of which were present in the QTL-regions of this study.

A recent study on possible DON detoxification genes in oat found two candidates named AsUGT1 and AsUGT2 (Khairullina et al., 2022). These are UDP-Glucotransferases and were annotated in the Sang reference genome (Kamal et al., 2022) found in the GrainGenes database (Yao et al., 2022). The location of these genes does match one of the possible physical positions of the QTL-region on 1C from the BLAST search, but not the same as the one where 20 resistance genes were annotated.

4.4 | Implications for resistance breeding

The stacking of the five resistant alleles indicates that a plateau of resistance is reached in the material with the stacking of three of validated resistance alleles, as further significant increase of resistance requires more than one allele. The analysis of the Nordic material shows that the number of resistant alleles were low in the oldest varieties (1885–1920) and increased to almost three (1990–1999). But it did not increase further when breeding for FHB resistance with inoculated trials started in Norway (2001–2009). But the number of susceptible alleles was reduced to almost zero. It is possible that the screening of material for fusarium resistance resulted in reduction in most susceptible material, and that breeding priorities changed to other traits like yield and quality. An increase in resistance before 2001–2009 probably did not come from targeted breeding against *F. graminearum*, although it is likely that highly susceptible genotypes would have been discarded because of visible symptoms in epidemic years. It is also likely that selection for yield and test weight in years with high disease pressure would have improved the FHB resistance. Future breeding strategies could be to select crosses based on the haplotype information provided in this study, use MAS to select for the QTL with largest effect in early generations and use genomic prediction to select for both small and large effect QTL using genome wide SNP arrays.

4.5 | Conclusion

This study identified 15 significant QTL-regions involved in Fusarium resistance in oats, validated five that showed consistent effect across

environments and populations and identified resistant and susceptible haplotype alleles. The additive effects of the five QTL-regions reduced the DON content by 38%. Several genes associated with resistance against *F. graminearum* in other crops were found to be located within these regions. Our study has confirmed that Fusarium resistance is made up of multiple QTL across the genome with varied effects. An approach to resistance breeding could be to use MAS to select for the QTL with larger effects in early generations and to develop genomic prediction models and make selections that include QTL with both large and small effects. The haplotype information provided in this study could also be used to select crosses for improved fusarium resistance.

AUTHOR CONTRIBUTIONS

ESS analysed data from field trials, organized the germination analysis and performed the GWAS analysis, MA provided funding, CJ provided management of staff for phenotyping DON and field trials at Staur, SW, ML and AKS provided support in GWAS analysis, ESS wrote the manuscript, and MA, SW, ML, AKS and CJ participated in revising the scientific work and writing.

ACKNOWLEDGEMENTS

This research was funded by the Research Council of Norway (The research funds for agriculture and food industry -FFL/JA) and Graminor ((NFR # 286606). The authors wish to thank Graminor for providing the material and facilities for this research, and the technical staff at Graminor and NMBU for helping in field experiments and data collection.

CONFLICT OF INTEREST STATEMENT

All authors have no conflict of interest to declare that are relevant to the content of this article.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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SUPPORTING INFORMATION

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How to cite this article: Sørensen, E. S., Windju, S., Jansen, C., Sonesson, A. K., Lillemo, M., & Alsheikh, M. (2024). Identification of haplotypes associated with resistance to *Fusarium graminearum* in spring oat (*Avena sativa* L.). *Plant Breeding*, 1–11. https://doi.org/10.1111/pbr.13156