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ORIGINAL PAPER

Linkage mapping and identification of QTL affecting deoxynivalenol (DON) content (*Fusarium* resistance) in oats (*Avena sativa* L.)

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Abstract Mycotoxins caused by *Fusarium* spp. is a major concern on food and feed safety in oats, although Fusarium head blight (FHB) is often less apparent than in other small grain cereals. Breeding resistant cultivars is an economic and environment-friendly way to reduce toxin content, either by the identification of resistance QTL or phenotypic evaluation. Both are little explored in oats. A recombinant-inbred line population, Hurdal × Z595-7 (HZ595, with 184 lines), was used for QTL mapping and was phenotyped for 3 years. Spawn inoculation was applied and deoxynivalenol (DON) content, FHB severity, days to heading and maturity (DH and DM), and plant height (PH) were measured. The population was genotyped with DArTs, AFLPs, SSRs and selected SNPs, and a linkage map of 1,132 cM was constructed, covering all 21 oat chromosomes. A QTL for DON on chromosome 17A/7C, tentatively designated as *Qdon.umb-17A/7C*, was detected in all experiments using composite interval mapping, with phenotypic effects of 12.2–26.6 %. In addition, QTL for DON were also found on chromosomes 5C, 9D, 13A, 14D and unknown_3, while a QTL for FHB was found on 11A. Several of the DON/FHB QTL coincided with those for DH, DM and/or PH. A half-sib population of HZ595, Hurdal × Z615-4 (HZ615, with 91 lines), was phenotyped in 2011 for validation of QTL found in HZ595, and *Qdon.umb-17A/7C* was again localized with a phenotypic effect of 12.4 %. Three SNPs closely linked to *Qdon.umb-17A/7C* were identified in both populations, and one each for QTL on 5C, 11A and 13A were identified in HZ595. These SNPs, together with those yet to be identified, could be useful in marker-assisted selection to pyramiding resistance QTL.

This paper is dedicated to the memory of Dr. Kenneth J. Frey (March 23, 1923–July 14, 2013), distinguished oat geneticist and breeder through almost 50 years. Two of the parents in this paper, Z595-7 and Z615-4, come from his vast introgression programme from *Avena sterilis*.

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Introduction

Oats (*Avena sativa* L.) are an important cereal, especially in the northern hemisphere, such as Canada, Russia and the Nordic countries. Oat-based food products are widely acknowledged as beneficial to health, notably in reducing blood cholesterol concentration (Andon and Anderson 2008). However, the prevalence of Fusarium head blight (FHB) poses a great threat to the food and feed safety in oats (Bjørnstad and Skinnes 2008; Campbell et al. 2000). FHB is a devastating fungal disease that occurs in most cereal-growing regions of the world, leading to the accumulation of mycotoxins on grain, of which deoxynivalenol (DON) is the most common (Jones and Mirocha 1999; Müller et al. 1998). Reports on grain samples contaminated with DON have been from Norway (Elen et al. 2003; Langseth and Elen 1996; Langseth et al. 1995; Lindblad et al. 2012), Finland (Hietaniemi et al. 2004; Lindblad et al. 2012), Denmark

(Jorgensen et al. 2011), Sweden (Lindblad et al. 2012), Germany (Müller et al. 1998), Poland (Perkowski and Basinski 2008), Russia (Gagkaeva et al. 2013), Canada (Campbell et al. 2000; Clear et al. 1996; Tekauz 2012; Tekauz et al. 2004), the US (Jones and Mirocha 1999), and to a less extent in the UK, where HT2 and T2 mycotoxins are predominant (Edwards 2009; Scudamore et al. 2007). The European Union has set maximum limits for DON in unprocessed oats (1,750 μg/kg) and oat-based foods (750 μg/kg, European Commission 2006), according to which many samples in the aforementioned reports reached alarming level. It has been reported that most toxins in oats can be removed by simply dehulling (Scudamore et al. 2007; Tekauz et al. 2004; Yan et al. 2010), but this probably reflects late infections (Tekle et al. 2013). Infections around anthesis tend to lead to empty florets with yield losses up to 33 % (Kiecana et al. 2002).

In North America, oats have long been regarded as more FHB resistant than wheat and barley (Campbell et al. 2000; Miller 1994; Tekauz et al. 2004), owing to the long pedicels separating individual florets that prevents the fast spread of fungal mycelia, giving a Type II resistance (Bjørnstad and Skinnes 2008; Langevin et al. 2004; Tekle et al. 2012). In Norway, Elen et al. (2003) also reported a much lower DON content in oats than in spring wheat and barley in comparative trials. Still the same authors found that oats appeared to be more FHB infected and more DON contaminated than wheat and barley (Langseth and Elen 1996; Langseth et al. 1995; Langseth and Stabbetorp 1996), probably reflecting more the way of cultivation of oats and districts prone to epidemics. So the picture is blurred as to epidemic conditions as well as adequate parameters for infection.

To address the latter, the infection process by *Fusarium* in oats was studied by Tekle et al. (2012, 2013). Based on microscopic observation, measurements of DON and germination percentage after inoculation at different stages, it was clear that oats, like other small grain cereals, are most susceptible to infection around anthesis (Parry et al. 1995; Tekle et al. 2012). Fungal hyphae enter through the tip of the glumes, quickly colonize anthers and proceed to the developing caryopsis, which will often be killed. Infection (or secondary proliferation) may also occur at later stages—even up to yellow maturity—under wet field conditions. This mainly leads to depressed germination due to seedling blight, with DON accumulation mainly in the hulls, not affecting seed viability per se, since it may be counteracted by dehulling and seed dressing with fungicides (Tekle et al. 2013), but this is not commercially practiced. The most useful parameter for resistance is then DON concentration, whereas measures of infection reflecting fungal colonization (fungal mass, qPCR or FHB) are less reliable. This also includes the 'freeze-and-blot' parameter advocated by Liu et al. (1997), which means imbibing 100 seeds on a filter paper, freezing them and allowing fungal colonization after subsequent thawing.

This may explain the many conflicting observations in previous studies. Although Tekauz et al. (2004) did not find much visual symptoms in field surveys, they still found high levels of DON contamination and considerable FHB damaged kernels. In Norway, Liu et al. (1997) found limited differences in terms of 'freeze-and-blot test'. This may reflect either the parameter used (rather than DON) or a limited genetic variability for resistance. As shown by He and Bjørnstad (2012) and Tinker et al. (2009), the genetic diversity of cultivated Nordic oats is in general quite restricted. However, we have evidence from our inoculation nurseries during the past 6 years that significant and repeatable differences between cultivars in DON accumulation do occur. Some very susceptible cultivars have even been withdrawn from the market. In recent trials with 480 cultivars from Europe and North America, a much wider range of FHB could also be reliably scored (Tekle, unpublished observations).

Artificial inoculation is usually needed to promote satisfactory FHB epidemics in screening nurseries. In wheat, there are three widely adopted inoculation methods, i.e. spray inoculation, point inoculation, and spawn inoculation (Buerstmayr et al. 2009). In oats, the inoculation methods applied share similarities with that in wheat, while having their own features. Like in wheat, spray inoculation is the most widely adopted method in oats (Kiecana et al. 2002; Liu et al. 1997; Tekle et al. 2012, 2013). However, since the anthesis period among individual spikelets within a panicle or florets within a spikelet is longer (8–9 days, Brown 1980) than that in wheat (5–6 days, De Vries 1971), it is more difficult to target anthesis, the most susceptible time according to Tekle et al. (2012). However, for the same reason there is a window of susceptibility with no decline in DON 5 days after average anthesis of a panicle. Repeated inoculation, e.g. after 3 days, may partly solve this problem (Tekle et al. 2012, 2013). Spawn inoculation is a convenient and labour-saving method, which mimics the situation under natural epidemics, where ascospores reach each flower at the susceptible stage. However, it may not work well if the weather is too cold or rainy, since *F. graminearum* perithecia develop best at temperatures around 20°–25° and ascospores may be trapped by raindrops. Hence, some susceptible plants may escape infection as reported in wheat (Bai and Shaner 1994). Both methods will tend to underestimate DON in susceptible genotypes, since infected florets often die and are not harvested. Both methods are also subject to late (secondary) infections. Which method

is best will be a compound measure depending on seasonal conditions (Tekle et al. 2013). In practice it is more important to achieve a wide enough range. Unlike in wheat, point inoculation is not applicable in oats, due to the high Type II resistance. Langevin et al. (2004) evaluated FHB reaction in six cereal crops using point inoculation, in which oat accessions showed very high resistance and no significant variation was found among oat cultivars. Although no inoculation method is ideal for oats, the spawn method gives reliable ranking of cultivars over years (Lillemo, personal communication).

Breeding FHB/DON resistant cultivars is an economic and environment-friendly way to overcome the abovementioned problems, for which the identification of resistance QTL is a prerequisite. However, the molecular mapping work in oats has lagged far behind the other important cereals, due to its large and allohexaploid genome with frequent genome rearrangements. Needless to say, no QTL analysis has been published for *Fusarium* resistance in oats. Ever since the first publication of a diploid oats map (O'Donoughue et al. 1992), the oats research community has done much efforts in developing oats linkage maps, among which are Kanota × Ogle (O'Donoughue et al. 1995; Tinker et al. 2009; Wight et al. 2003), Ogle × TAM O-301 (Oliver et al. 2010; Portyanko et al. 2001), Ogle × MAM17-5 (Zhu and Kaeppler 2003), Terra × Marion (De Koeyer et al. 2004), Aslak × Matilda (Tanhuanpaa et al. 2008), etc. However, all of those maps are incomplete and fragmented, and a map with 21 linkage groups (LG) well defined by chromosomal assignments was achieved only recently when Oliver et al. (2013) developed a physically anchored consensus map with 21 LGs through SNP mapping in 6 hexaploid oat populations and SNP deletion analysis in a set of monosomic stocks.

Disease resistance QTL in oats have been reported for crown rust, stem rust, barley yellow dwarf and powdery mildew (Rines et al. 2006). The current study—to the best of our knowledge, the first on DON resistance QTL to be reported in oats—was initiated at a time when we relied on fungal infection as the parameter of resistance, rather than DON accumulation. The parent Z595-7 was repeatedly shown to have less fungal infection in freeze-and-blot tests, while the cultivar Hurdal was susceptible. Later the availability of large-scale DON-analyses made us revise this ranking, as it will be shown in this study. The objectives of this study were then to construct linkage maps based on a recombinant-inbred line population (RIL) between these two parents and to identify genomic regions that confer resistance to DON accumulation in oats. The QTL were then validated in a cross of Hurdal with Z615-4, the half-sib oats line of Z595-7.

Materials and methods

Plant materials and field experiments

Two sets of RILs, derived from the crosses of Hurdal × Z595-7 (referred to as HZ595 hereafter) and Hurdal × Z615-4 (HZ615), were used in this study. Hurdal is a locally adapted, early maturing and an average FHB resistant Norwegian oat cultivar, with the pedigree A90017// Gråkall/Tador. Z595-7 and Z615-4 are back cross derivatives developed by Dr. K.J. Frey from the *A. sterilis* accession PI411560 from Eritrea into the US cultivars 'Ogle' and 'Tippecanoe', respectively (Holland et al. 2000). The RILs were made by single seed descent method, and F8 generations were used for mapping, with 184 lines in HZ595 and 91 lines in HZ615.

The field experiments were performed at the Vollebekk Research Farm in the Norwegian University of Life Sciences, Ås, Norway. In 2008, HZ595 was planted in hill plots of 0.40 × 0.45 m with two replications, and in 2009 and 2010 it was sown in plots of 0.75 × 2.0 m by a 14 × 14 incomplete block lattice design with two replications. In 2011, HZ615 was planted in plots with the same style as for HZ595 in 2009 and 2010. The plots were spawn inoculated with *F. graminearum* infected oat kernels following the method of Skinnes et al. (2010), in which an improved protocol was adopted from Dr. Bernd Rodemann, BBA Braunschweig, Germany, where perithecia are developed before spawn grains are spread in the field to ensure ascospore production. The infected oat kernels were scattered in the field at the stage Zadoks 32/33 (Zadoks et al. 1974) with 10 g/m^2. Standard management was adopted before inoculation, and limited irrigation was applied during spore germination. A combination of propiconazole and fenprophimorph was applied 1 week before anthesis at rates of 125–450 g/ha. This treatment does not affect FHB (Henriksen and Elen 2005) but helps to prevent the interference of other foliar diseases.

Phenotyping

For HZ595, DON was analyzed over 3 years from 2008 to 2010. The whole plot was harvested and threshed. Subsampling was done by an automated seed divider from 500 g of seed, and 15 g of seed from the 2008 and 2009 experiments and 70 g from the 2010 experiment were sent to the Department of Plant Pathology, University of Minnesota. Seed samples were ground for 2 min using a Stein Laboratories Mill (Model M-2, Stein Laboratories Inc., Atchison, Kansas), and DON content was determined from a 4-g flour subsample by gas chromatography and mass spectrometry method (Fuentes et al. 2005). FHB severity (FHB)

was only possible to visually estimate in 2009, at 61 and 68 days after sowing. In addition, plant height (PH) and days to heading (DH) in 2009 and 2010 and days to maturity (DM) in 2010 were also scored.

For HZ615, phenotypic traits were measured in 2011, including DON (with the same method as for HZ595), FHB, DH, PH and DM. Field FHB severity was measured at 91 days after sowing, using a linear scale from 0 (no symptom) to 4 (very high infection).

DNA extraction and genotyping

Genomic DNA was extracted from 2-week-old leaves of parents and single plant progenies, using the DNeasy Plant DNA extraction Kit (QIAGEN).

For HZ595, approx. 1,750 markers were firstly applied, including 216 SSRs, 42 *MseI* and *PstI* AFLP primer combinations, and approx. 1,500 DArT markers, using the same protocol as described in He and Bjørnstad (2012). Subsequently, a subset of 51 lines was genotyped with 1,311 SNPs by Oliver et al. (2013) for consensus mapping, and then the whole population was genotyped by selected SNP markers that have shown potential linkage with resistance QTL (Table S1), using the KASPar method following the manufacturer's protocol (KBiosciences, Herts, UK). PCR was done in an ABI 7500 Fast Real Time PCR Machine (Applied Biosystems) and data were collected and analyzed by ABI 7500 software v2.0.5 (Applied Biosystems). In addition, a set of REMAP markers was also scored by the method of Tanhuanpaa et al. (2008). For HZ615, the same sets of SSRs, AFLPs and DArTs were firstly used, and then selected SNPs were applied.

Genetic mapping

For HZ595, a preliminary linkage analysis was performed for only the 51 lines subset included in the Oliver et al. (2013) map with all types of markers including SNPs. Unless specified otherwise, markers with highly distorted segregation ratios ($P < 10^{-4}$) and >10 % missing data points were not used. The software Map Manager QTX (Manly et al. 2001) was used for linkage analysis, with the most stringent P value of 10^{-6}. Some 'non-SNP' markers were thus localized to chromosomes through their linkages with SNPs that have been assigned to chromosomes (Oliver et al. 2013). Another linkage analysis was then conducted for the whole population of 184 lines, using only those 'non-SNP' markers with less than 30 % missing data points and a P value of 10^{-5} in Map Manager QTX for the first round analysis, the P value was then increased to 0.001 for distributing unlinked markers and fragmented linkage groups (LGs). Each LG generated by Map Manager QTX was re-calculated by JoinMap v. 3.0 (Van Ooijen and Voorrips 2001) and the JoinMap version was adopted. The chromosome locations of anchored markers were used to assign LGs to chromosomes. Framework markers were selected based on the JoinMap version of linkage map (sometimes more distorted markers with $P < 10^{-4}$ were also used in chromosome regions where no other markers available), markers closer to a framework marker than 2 cM were also removed. All the markers other than framework markers were placed relatively to the framework map using the M5 program package (Tinker 1999).

For HZ615, a preliminary linkage map was constructed by JoinMap. A LOD score of 5 was set for generating LGs, which were then compared with the HZ595 map to determine their chromosomal locations through shared markers between the two linkage maps. Accordingly, LGs were either combined or split using lower or higher LOD value, respectively, based on the chromosomal information. Framework markers were selected using the same strategy as for HZ595 and other markers were placed by the M5 program.

Statistical analyses and QTL mapping

The phenotypic data were analyzed using the SAS software ver. 9.2 (SAS institute, 2008). The segregation of the RIL lines for phenotypic traits was tested for normality using the PROC UNIVARIATE function. Analysis of variance (ANOVA) was performed with the PROC GLM module, and the information in the ANOVA table was used for calculating the narrow sense heritability for DON content and FHB severity, using the formula $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_E^2/r)$ where σ_g^2 stands for genetic variance, σ_E^2 for error variance, and r for the number of replications (Lu et al. 2013). Pearson correlation coefficients were calculated using the PROC CORR procedure.

QTL analysis was carried out using PLABQTL ver. 1.2 (Utz and Melchinger 2003) based on the JoinMap version of linkage maps of HZ595 and HZ615. Simple interval mapping (SIM) was performed first to detect potential QTL for each trait, followed by composite interval mapping (CIM) for each QTL, using its closest linked markers detected in SIM as cofactors. The LOD threshold for declaring a significant QTL at a type I error rate of $\alpha = 0.05$ in one environment was set to 3.02 for HZ595 and 2.87 for HZ615, based on 1,000 permutations. Minor QTL with LOD values exceeding 2.0 were also scored if they were detected more than once. Linkage maps and LOD curves were drawn by the software MapChart ver. 2.2 (Voorrips 2002).

Results

Phenotypic evaluation

The Hurdal × Z595-7 population

The disease development as reflected in DON content ranged from low infection in 2008 to very high in 2009 (Fig. 1), due to diverse climatic conditions. The distribution of DON content in all the 3 years was continuous, but while the 2009 data showed normal distribution by the Shapiro–Wilk test ($P = 0.059$), the 2008 and 2010 data were skewed towards low DON. Transgressive segregation was apparent in both directions, reflecting that both parents contributed resistance QTL, which was also evidenced by the different ranks of the two parents (Fig. 1). FHB severity in 2009 was much lower than expected from the high DON levels and skewed ($P < 0.0001$, Fig. 1). PH averaged over 2 years showed normal distribution, but DH did not (Fig. 1). ANOVA indicated that the year effects contributed most to the phenotypic variation, but genotypes and genotype-by-environment interactions were also highly significant ($P < 0.0001$, Table 1). Within years, genotypes were the most important source of variation in 2008 and 2010, except in 2009 (although also highly significant at $P < 0.0001$, data not shown).

Table 1 Analysis of variance for DON content in the Hurdal × Z595-7 population

Source of variation	df	Mean squares	*F* value	*P* value
Replication (year)	1	151.48	8.30	0.0041
Year	2	41,440.45	2,270.82	<0.0001
Genotype	183	69.47	3.81	<0.0001
Genotype × year	355	27.04	1.48	<0.0001
Error	522	18.25		
Corrected total	1,063			

The Pearson correlation coefficients of DON 2008 with DON 2009 (0.285) and of DON 2008 with DON 2010 (0.291) were low, while between DON 2009 and DON 2010 it was moderate (0.470), and all were highly significant at $P < 0.001$. Despite the low correlations among years, a few lines showed consistently good resistance (e.g. accessions 7397 and 7398), while some others were always bad (Table S2). In 2009, FHB severity showed a slightly negative correlation with DON (−0.191, $P < 0.01$).There were no significant correlations between DON levels and

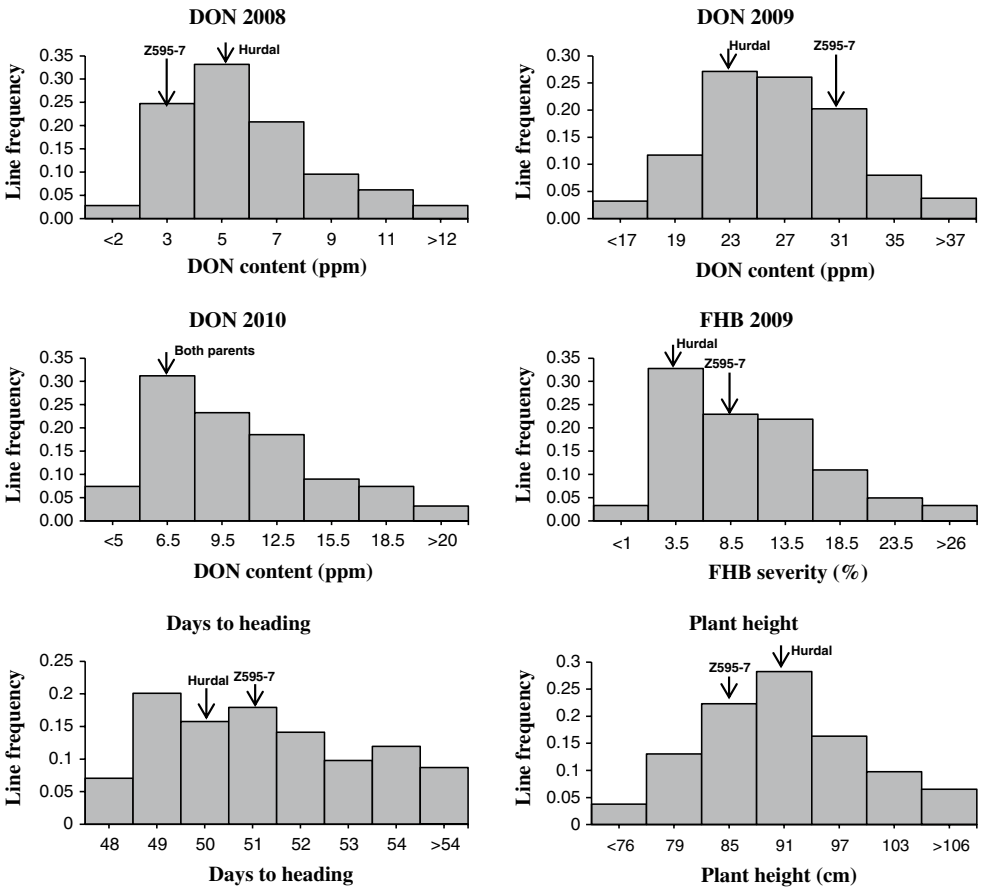


Fig. 1 Frequency distributions of DON content of 2008–2010, FHB severity of 2009, and days to heading and plant height averaged over 2009 and 2010, in the Hurdal × Z595-7 population

Table 2 Pearson correlation coefficients between FHB traits and agronomic traits and narrow sense heritability estimates of the FHB traits for the Hurdal × Z595-7 population and the Hurdal × Z615-4 population

	HZ595				HZ615	
	DON content			FHB severity	DON content	FHB severity
	2008	2009	2010	2009	2011	2011
Days to heading (DH)	−0.080	0.113	−0.380***	−0.722***	−0.373**	−0.419***
Plant height (PH)	−0.150	0.114	−0.212*	−0.660***	0.083	−0.133
Days to maturity (DM)	−0.075	0.103	−0.414***	−0.617***	−0.198	−0.534***
Heritability	0.275	0.516	0.814	0.747	0.575	0.759

For HZ595, averaged values for DH and PH over 2009 and 2010, and FHB severity over two times in 2009, were used for calculating Pearson correlation coefficients, since high correlations were found between the two datasets of each of the traits. *, ** and *** indicate significant at levels of $P < 0.01$, 0.001 and 0.0001, respectively

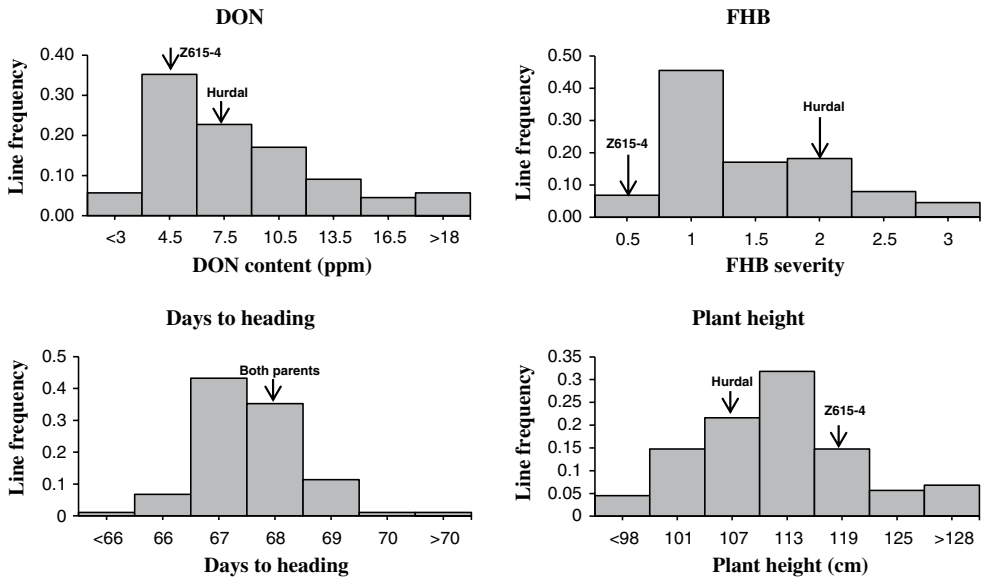


Fig. 2 Frequency distributions of DON content, FHB severity, days to heading and plant height in the Hurdal × Z615-4 population in 2011

agronomic traits in 2008 nor 2009, but DON 2010 and FHB 2009 were significantly correlated with DH, PH and DM (Table 2). The correlations between agronomic traits were all significant and ranged from moderate (0.55, PH vs. DM) to high (0.71, PH vs. DH, and 0.83, DH vs. DM). High heritabilities were detected for DON 2010 and FHB 2009, but were low and moderate for DON 2008 and DON 2009 (Table 2), respectively, due to the low phenotypic variances under low and high disease pressures, respectively.

The Hurdal × Z615-4 population

Similar to HZ595, HZ615 also showed non-normal distributions in DON and FHB, skewed towards low disease (Fig. 2). Z615-4 performed better than Hurdal in both DON and FHB, but transgressions were observed in both directions, especially for DON (Fig. 2). DH was non-normally distributed, with less variation than that of HZ595; PH showed normal distribution (Fig. 2). No significant correlation was detected between DON and FHB (data not shown), but the former was significantly correlated with DH, while the latter with DH and DM (Table 2). Moderate and high narrow sense heritability estimates were detected for DON and FHB in 2011, respectively (Table 2).

Linkage map construction

Initially, 590 markers were scored for HZ595, including 386 DArTs, 147 AFLPs, 32 SSRs and 25 REMAPs. For the subset of 51 lines, 410 SNPs were also added making a total of 1,000 markers (Oliver et al. 2013). After discarding low quality markers, 955 were used for linkage analysis. Based on their linkage with anchored SNPs, 279 non-SNP

markers were assigned to all the 21 oat chromosomes, with numbers ranging from 3 (21D) to 40 (13A).

For the whole population of 184 lines, 522 markers were selected for linkage map construction by MapManager QTX and JoinMap. In total, 30 LGs were constructed by 506 markers, while 16 markers remained unlinked. Based on the anchored markers, 27 LGs were physically assigned, representing all the 21 chromosomes, while 3 LGs were not assigned. Afterwards, 24 SNPs with potential linkages to FHB/DON QTL were scored for HZ595, with 4 located on chromosome 5C, 3 on 11A, 7 on 13A and 10 on 17A/7C.

After marker screening, 148 informative markers were selected as framework markers, and 427 markers were then placed relatively to the framework map and 39 were unplaced, producing a linkage map with genome coverage of 1,132 cM (Fig. 3; Table S3).

For HZ615, 573 markers were scored, including 363 DArTs, 174 AFLPs and 36 SSRs. After marker screening, 401 informative markers were used to construct linkage map using JoinMap. Firstly, thirty-seven LGs were generated by a LOD threshold of 5.0, and then 2 LGs for chromosome 19A were merged at LOD = 3.0, resulting in a linkage map of 36 LGs. Afterwards, 172 markers were selected as framework and 368 more were successfully placed to the framework map by the M5 program, with 33 markers remaining unplaced. Based on alignments of the two linkage maps through shared markers, 29 HZ615 LGs were assigned to 20 chromosomes (lacking chromosome 10D), while the other 7 LGs were not assigned (Fig. S1; Table S4). The HZ615 linkage map spans 946 cM, excluding unlinked markers. The SNPs linked to HZ595 QTL, 4 on chromosome 13A and 6 on 17A/7C, were applied to HZ615, and 3 SNPs on 17A/7C were polymorphic and scored.

Comparison of the two linkage maps

The two linkage maps shared 160 markers, bridging 28 LGs of HZ595 and 31 LGs of HZ615. All LGs aligned well (Fig. S2), except one HZ595 LG that associated with two in HZ615, and one case where two HZ595 LGs associated with one in HZ615. The relative marker orders in alignments were mostly conserved with only minor inversions among adjacent markers.

Skewed segregation of molecular markers

Chi square tests were performed on all the scored markers in both populations. For HZ595, 130 (22 %) markers were significantly skewed at the 0.01 level, of which 53 were skewed towards the female parent Hurdal and 77 were skewed towards the male parent Z595-7. This did not differ between marker types (23 % of DArTs, 19 % of AFLPs, 22 % of SSRs and 24 % of REMAPs). Six LGs showed regions of skewed segregation (9D, 11A, 12D, 13A, 15A and 18D). Similarly, 136 (24 %) markers showed skewed segregation in HZ615, of which 51 were skewed towards Hurdal and 85 towards Z615-4; and the chromosome regions involved were 1C, 5C_2, 8A, 13A_2, 14D, 19A_2 and unknown LGs 1, 3 and 6.

QTL mapping

The Hurdal × Z595-7 population

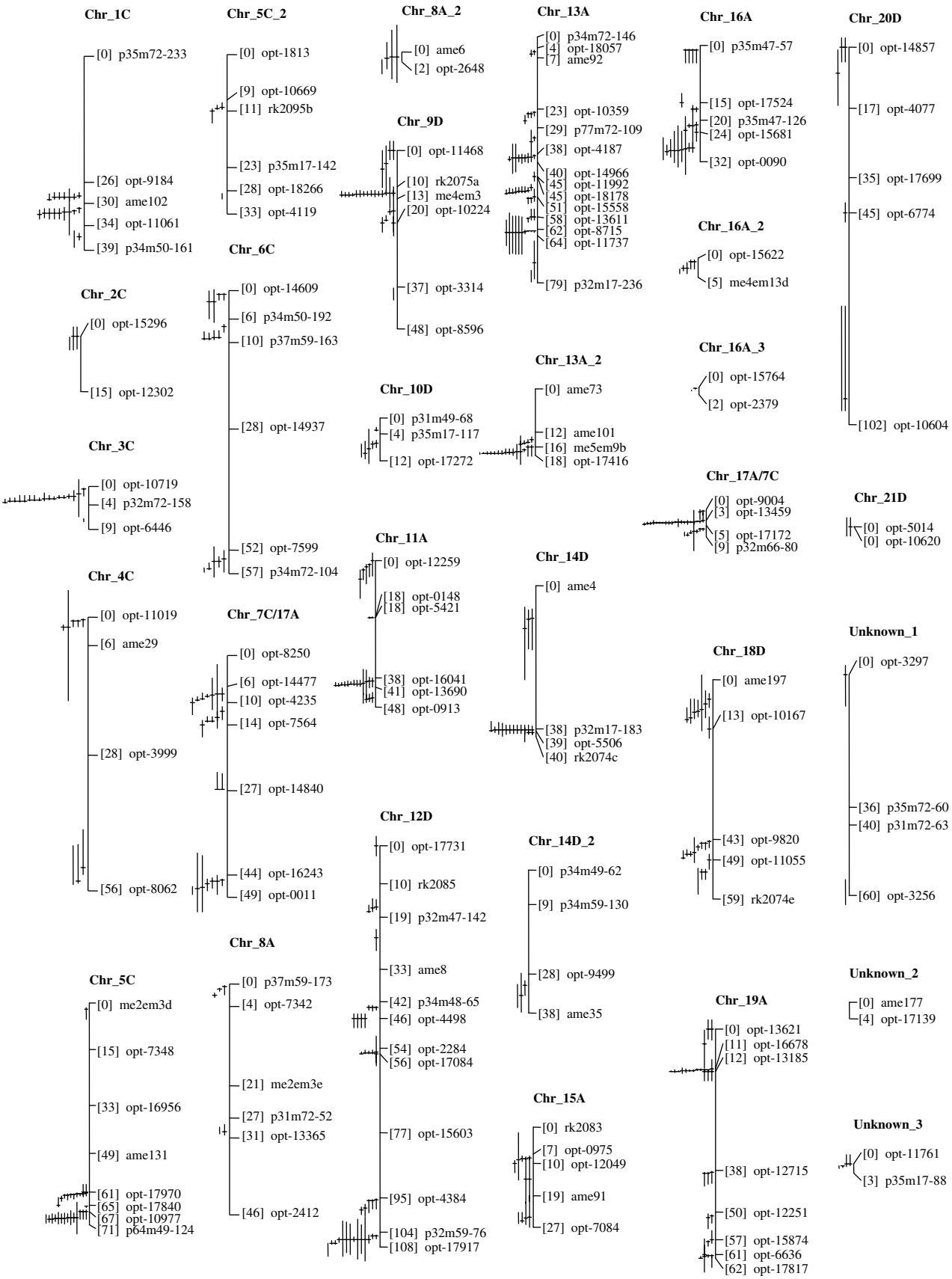
For DON content, a significant QTL was detected in all 3 years between the DArT marker *oPt-9631* and the SNP marker *GMI_ES17_c20752_1084* on chromosome 17A/7C. This was designated tentatively as *Qdon.umb-17A/7C* (Table 3; Fig. 4; 'UMB' being the official acronym of the Norwegian University of Life Sciences). The resistance allele was contributed by Hurdal, explaining phenotypic variations from 13.2 to 27.9 %. In addition, QTL of minor effects for DON content were identified on chromosomes 5C, 9D, 13A, 14D and unknown_3 (Table 3). Most of the minor QTL showed phenotypic effects below 10 % and none were consistently detected across the 3 years. The QTL on chromosomes 9D and 13A coincided with one or all of the agronomic traits (PH, DH and DM).

Only one QTL for FHB severity was detected, on chromosomes 11A, showing minor phenotypic effect and association with agronomic traits and not coinciding with DON QTL (Table 3; Fig. 4).

QTL for the three agronomic traits were found on six chromosomes, with 11A and 13A explaining phenotypic variations higher than 10 %, coinciding with FHB traits and each other (Table 3; Fig. 4), in agreement with the correlations between phenotypic data of the three traits shown previously.

The Hurdal × Z615-4 population

For DON, QTL were detected on chromosomes 7C/17A and 17A/7C, showing slightly higher than 10 % of phenotypic variation explained, with favourable alleles contributed by Z615-4 and Hurdal, respectively (Table 4; Fig. 5). For FHB severity, QTL were identified on chromosomes 13A_3 and unknown_7, with resistance from Z615-4 and Hurdal, respectively. The one on chromosome 13A_3 coincided with DH and DM (Table 4). In addition, QTL for agronomic traits were detected on six chromosomes, all explaining more than 10 % of phenotypic variation, but only the one for DH and DM on chromosome 13A_3 and the one for PH on chromosome 16A_2 had LOD scores exceeding 2.86, a critical value based on 1,000 permutations (Table 4).


Chr_1C
[0] p35m72-233
[26] opt-9184
[30] ame102
[34] opt-11061
[39] p34m50-161
Chr_2C
[0] opt-15296
[15] opt-12302
Chr_3C
[0] opt-10719
[4] p32m72-158
[9] opt-6446
Chr_4C
[0] opt-11019
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[28] opt-3999
[56] opt-8062
Chr_5C
[0] me2em3d
[15] opt-7348
[33] opt-16956
[49] ame131
[61] opt-17970
[65] opt-17840
[67] opt-10977
[71] p64m49-124
Chr_5C_2
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[9] opt-10669
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[23] p35m17-142
[28] opt-18266
[33] opt-4119
Chr_6C
[0] opt-14609
[6] p34m50-192
[10] p37m59-163
[28] opt-14937
[52] opt-7599
[57] p34m72-104
Chr_7C/17A
[0] opt-8250
[6] opt-14477
[10] opt-4235
[14] opt-7564
[27] opt-14840
[44] opt-16243
[49] opt-0011
Chr_8A
[0] p37m59-173
[4] opt-7342
[21] me2em3e
[27] p31m72-52
[31] opt-13365
[46] opt-2412
Chr_8A_2
[0] ame6
[2] opt-2648
Chr_9D
[0] opt-11468
[10] rk2075a
[13] me4em3
[20] opt-10224
[37] opt-3314
[48] opt-8596
Chr_10D
[0] p31m49-68
[4] p35m17-117
[12] opt-17272
Chr_11A
[0] opt-12259
[18] opt-0148
[18] opt-5421
[38] opt-16041
[41] opt-13690
[48] opt-0913
Chr_12D
[0] opt-17731
[10] rk2085
[19] p32m47-142
[33] ame8
[42] p34m48-65
[46] opt-4498
[54] opt-2284
[56] opt-17084
[77] opt-15603
[95] opt-4384
[104] p32m59-76
[108] opt-17917
Chr_13A
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[4] opt-18057
[7] ame92
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[29] p77m72-109
[38] opt-4187
[40] opt-14966
[45] opt-11992
[45] opt-18178
[51] opt-15558
[58] opt-13611
[62] opt-8715
[64] opt-11737
[79] p32m17-236
Chr_13A_2
[0] ame73
[12] ame101
[16] me5em9b
[18] opt-17416
Chr_14D
[0] ame4
[38] p32m17-183
[39] opt-5506
[40] rk2074c
Chr_14D_2
[0] p34m49-62
[9] p34m59-130
[28] opt-9499
[38] ame35
Chr_15A
[0] rk2083
[7] opt-0975
[10] opt-12049
[19] ame91
[27] opt-7084
Chr_16A
[0] p35m47-57
[15] opt-17524
[20] p35m47-126
[24] opt-15681
[32] opt-0090
Chr_16A_2
[0] opt-15622
[5] me4em13d
Chr_16A_3
[0] opt-15764
[2] opt-2379
Chr_17A/7C
[0] opt-9004
[3] opt-13459
[5] opt-17172
[9] p32m66-80
Chr_18D
[0] ame197
[13] opt-10167
[43] opt-9820
[49] opt-11055
[59] rk2074e
Chr_19A
[0] opt-13621
[11] opt-16678
[12] opt-13185
[38] opt-12715
[50] opt-12251
[57] opt-15874
[61] opt-6636
[62] opt-17817
Chr_20D
[0] opt-14857
[17] opt-4077
[35] opt-17699
[45] opt-6774
[102] opt-10604
Chr_21D
[0] opt-5014
[0] opt-10620
Unknown_1
[0] opt-3297
[36] p35m72-60
[40] p31m72-63
[60] opt-3256
Unknown_2
[0] ame177
[4] opt-17139
Unknown_3
[0] opt-11761
[3] p35m17-88

◂ **Fig. 3** Linkage map of the Hurdal × Z595-7 population. *Framework markers* are shown and their positions in centimorgans are given in *parentheses*. Placed markers are represented by *cross-hairs* on the *left* of each linkage group, with the *vertical bars* on cross-hairs indicating their tendency to stretch the interval. The *bars* are 1/4 of the length that the interval would be if the marker was placed at this position. More detailed information on placed markers is presented in Table S3

Based on shared markers between the two linkage maps, putative homologous QTL in the two linkage maps were identified (Fig. 5). *Qdon.umb-17A/7C* in HZ595 was proved to be homologous to the one identified in HZ615 based on 7 shared markers. Furthermore, the QTL on chromosome 13A identified in the two populations for FHB/DON and agronomic traits may be homologous based on three shared markers, and the QTL on unknown_3/unkown_7 adjacent to a DArT marker *oPt-11761* may confer both DON resistance in HZ595 and FHB resistance in HZ615 (Fig. 5).

Discussion

Phenotypic evaluation

Compared to wheat, FHB inoculation is more difficult in oats due to the long flowering period and the long pedicels that hinder the effective application of spray and point inoculation. Spawn inoculation, although having its own drawbacks, showed promising results in our FHB screening nursery, owing to the adoption of an improved protocol that ensured ascospore production and thus eliminated a source of variation. In a recent review of our small grain variety trials from 2007–2012, Lillemo et al. (in preparation) found consistent differences in DON in oats, wheat and barley. The average genotypes tended to interact much more with years, while the best or worst were consistent. This ranking was also consistent in two different testing sites using the same spawn, exhibiting the stability and reliability of this inoculation method.

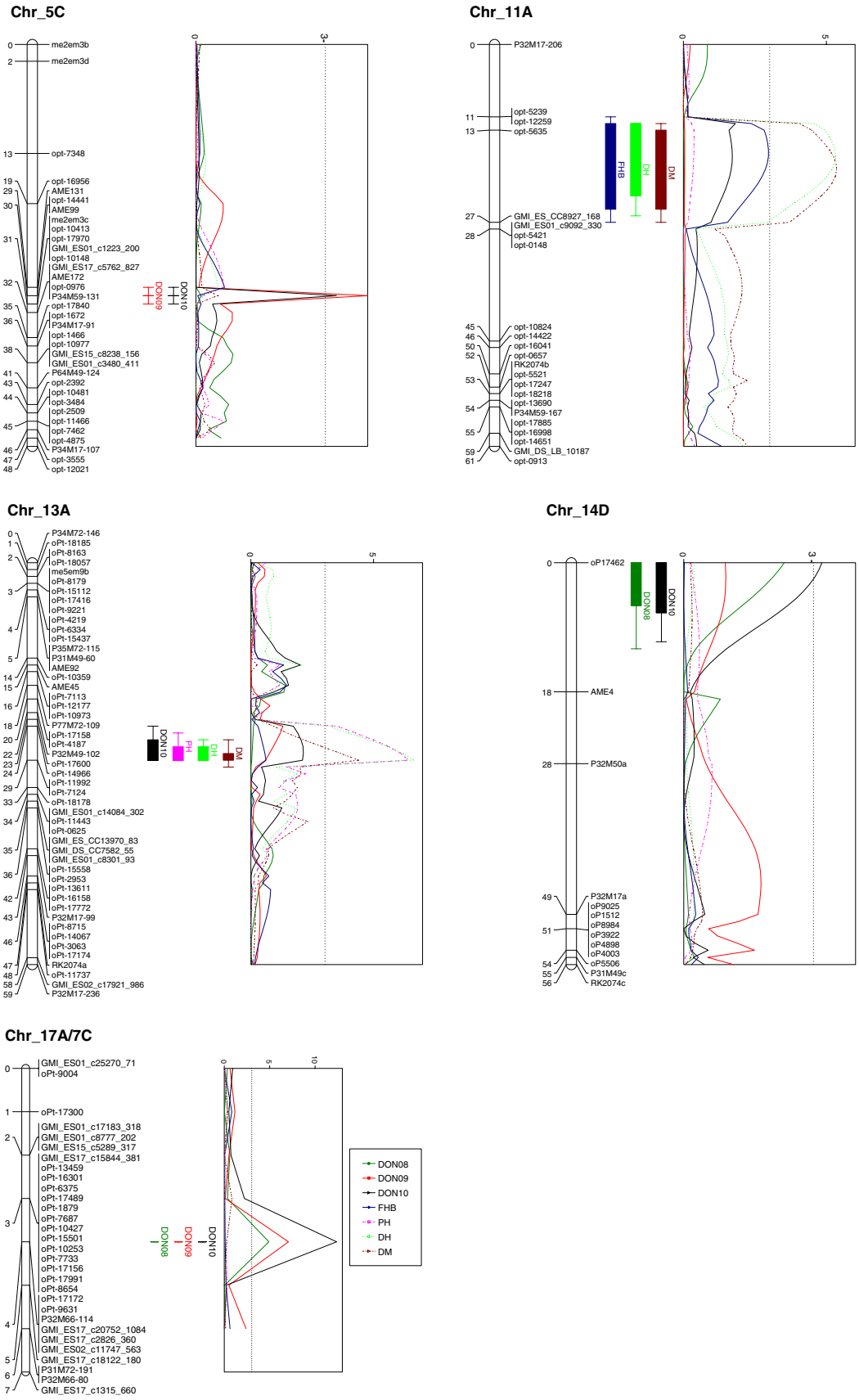
The phenotypic values in a nursery should preferably span the range from ca. 2–20 ppm. In HZ595, the DON levels and range in 2009 was much wider than in 2008 and 2010 (Fig. 1), but with a loss of accuracy. In the adjacent 2009 experiments with advanced breeding lines reported by Tekle et al. (2013), the ranges for spawn and spray inoculation were 8–30 and 3–25 ppm, respectively, with similar accuracy, but spawn had higher DON levels. In HZ595, DON-values were probably confounded by late infections as well as lodging due to inferior agronomy.

According to Langseth et al. (1995), early summer drought and high precipitation in July around flowering are two important factors for high DON content in oats in Norway. Indeed, meteorological data of 2009 indicated a very dry June with only 29.6 mm precipitation and a very wet July with 159.4 mm, while the corresponding values were 78.2/122.6 mm in 2008 and 66.4/104.8 mm in 2010 (http://lmt.bioforsk.no/lmt/index.php?weatherstation=5

Table 3 Results of composite interval mapping (CIM) of DON, FHB and agronomic traits for the Hurdal × Z595-7 population

Chromosome	QTL region (cM)	Marker interval	DON 2008	DON 2009	DON 2010	FHB mean	DH mean	DM 2010	PH mean
5C	29–31	oPt-14441–AME99		9.6/1.8	7.9/1.4				
9D	18–20	oPt-6852–oPt-18114			7.3/−1.3			4.9/−1.2	
11A	11–25	oPt-5635–GMI_ES_CC8927_168				7.2/−2.3	12.5/0.9	12.6/2.1	
13A	23–30	oPt-14966–oPt-11992			5.2/−1.1		15.4/−0.9	10.4/−1.7	14.9/−3.6
14D	0–7	oPt-17462–AME4	6.9/0.8		8.9/1.4				
16A	14–19	oPt-16934–P35M47-126							8.5/2.8
17A/7C	3–5	oPt-9631–GMI_ES17_c20752_1084	12.2/1.0	16.3/2.3	26.6/2.5				
18D	45–48	AME13–P31M72-156					5.2/0.5	5.6/1.2	6.8/2.4
19A	50–63	oPt-12715–oPt-12251							7.5/2.9
unknown_3	0–1	oPt-15884–P44M47-217		10.8/1.8					
Accumulated percentage of the phenotypic variance explained			19.1	36.7	55.9	7.2	33.1	33.5	37.7

Averaged values over 2009 and 2010 for days to heading (DH) and plant height (PH) and the mean of two field FHB severity investigations in 2009 were used for QTL mapping. R^2 values (above the slash) and additive values (below the slash) calculated by PLABQTL are provided for each QTL. It is assumed that the male parent (Z595-7) contributes the high phenotypic value. QTL with LOD scores higher than 2.0 are presented, from which the ones with LOD scores higher than 3.02 (determined by 1,000 permutation tests at $P < 0.05$) are underlined


Chr_5C
me2em3b
me2em3d
opt-7348
opt-16956
AME131
opt-14441
AME99
me2em3c
opt-10413
opt-17970
GMI_ES01_c1223_200
opt-10148
GMI_ES17_c5762_827
AME172
opt-0976
P34M59-131
opt-17840
opt-1672
P34M17-91
opt-1466
opt-10977
GMI_ES15_c8238_156
GMI_ES01_c3480_411
P64M49-124
opt-2392
opt-10481
opt-3484
opt-2509
opt-11466
opt-7462
opt-4875
P34M17-107
opt-3555
opt-12021
DON09
DON10
Chr_11A
P32M17-206
opt-5239
opt-12259
opt-5635
GMI_ES_CC8927_168
GMI_ES01_c9092_330
opt-5421
opt-0148
opt-10824
opt-14422
opt-16041
opt-0657
RK2074b
opt-5521
opt-17247
opt-18218
opt-13690
P34M59-167
opt-17885
opt-16998
opt-14651
GMI_DS_LB_10187
opt-0913
FHB
PH
DH
DM
Chr_13A
P34M72-146
oPt-18185
oPt-8163
oPt-18057
me5em9b
oPt-8179
oPt-15112
oPt-17416
oPt-9221
oPt-4219
oPt-6334
oPt-15437
P35M72-115
P31M49-60
AME92
oPt-10359
AME45
oPt-7113
oPt-12177
oPt-10973
P77M72-109
oPt-17158
oPt-4187
P32M49-102
oPt-17600
oPt-14966
oPt-11992
oPt-7124
oPt-18178
GMI_ES01_c14084_302
oPt-11443
oPt-0625
GMI_ES_CC13970_83
GMI_DS_CC7582_55
GMI_ES01_c8301_93
oPt-15558
oPt-2953
oPt-13611
oPt-16158
oPt-17772
P32M17-99
oPt-8715
oPt-14067
oPt-3063
oPt-17174
RK2074a
oPt-11737
GMI_ES02_c17921_986
P32M17-236
DON10
PH
DH
DM
Chr_14D
oP17462
AME4
P32M50a
P32M17a
oP9025
oP1512
oP8984
oP3922
oP4898
oP4003
oP5506
P31M49c
RK2074c
DON08
DON10
Chr_17A/7C
GMI_ES01_c25270_71
oPt-9004
oPt-17300
GMI_ES01_c17183_318
GMI_ES01_c8777_202
GMI_ES15_c5289_317
GMI_ES17_c15844_381
oPt-13459
oPt-16301
oPt-6375
oPt-17489
oPt-1879
oPt-7687
oPt-10427
oPt-15501
oPt-10253
oPt-7733
oPt-17156
oPt-17991
oPt-8654
oPt-17172
oPt-9631
P32M66-114
GMI_ES17_c20752_1084
GMI_ES17_c2826_360
GMI_ES02_c11747_563
GMI_ES17_c18122_180
P31M72-191
P32M66-80
GMI_ES17_c1315_660
DON08
DON09
DON10
DON08
DON09
DON10
FHB
PH
DH
DM

◂ **Fig. 4** Chromosomes with significant QTL identified in the Hurdal × Z595-7 population, with their LOD curves obtained from CIM presented. Genetic distances in centimorgans are indicated to the *left* of the chromosomes. A threshold of 3.02 calculated through 1,000 permutations is shown by a *dotted vertical line.* DON08, DON09 and DON10 stand for DON content measured in the years 2008, 2009 and 2010, respectively; *FHB* Fusarium head blight severity measured in 2009; *PH* averaged value for plant height measured in 2009 and 2010, *DH* averaged value for days to heading measured in 2009 and 2010, *DM* days to maturity measured in 2010

&loginterval=1&tid=1303897298/). Lodging may have added to the high DON levels in 2009, since Langseth et al. (1995) found that DON increased markedly in oats after lodging. The same effects of lodging have also been reported in wheat, barley, and rice (Nakajima et al. 2008). Although some lodging occurred in 2008 and 2010, it was much more severe in 2009, which may have confounded the DON evaluation and led to the highly significant replication effect. A possible improvement could be to have inoculation nurseries in plastic tunnels, kept open-ended to avoid too high temperatures.

In wheat, FHB severity is usually positively correlated with DON content, but there were also reports on no correlation or negative correlation (Lu et al. 2013). In oats, Rodemann and Niepold (2008) reported a moderate correlation coefficient of 0.375 between the two traits, while Liu et al. (1997) and Tekauz et al. (2004) did not find any correlation, as in the current study for HZ615 and for HZ595 (very low correlations). This may be attributed to a limited variability in FHB resistance in oats, as observed by Tekauz et al. (2004) and in the current study (Figs. 1, 2), which was probably contributed by the efficient Type II resistance in oats due to the long pedicels (Langevin et al. 2004; Tekle et al. 2012). In addition, the accumulation of DON continues throughout the grain filling stage, a much longer process than the development of FHB symptoms (Tekle et al. 2013).

DH is often negatively correlated with FHB severity in wheat, under spray or spawn inoculation (Emrich et al. 2008; Lu et al. 2013). The current study showed significant impact of DH on FHB in both populations (Table 2), implying a similar mechanism in oats. However, since this association was mainly contributed by developmental or epidemiological conditions, selection on late genotypes with low FHB should be avoided (Emrich et al. 2008).

The influence of PH on wheat FHB has been reported in many studies, with mechanisms attributed to pleiotropy, tight linkage, or disease escape (Buerstmayr et al. 2009; Lu et al. 2013). In the seven oat cultivars investigated, Langseth et al. (1995) found that the two tallest showed best FHB resistance, implying a potential association between PH and FHB. However, Gavrilova et al. (2008) did not find a significant correlation between the two traits in a collection of oat accessions with worldwide origin. In the present study, the correlation was significant only for DON 2010 and FHB 2009 in HZ595, making the relationship obscure and further study is needed to validate it.

Linkage mapping

The map sizes of HZ595 (1,132 cM) and HZ615 (946 cM) were much smaller than those of the latest Kanota × Ogle (KO, 2028 cM, Tinker et al. 2009) and Ogle × TAM O-301 (OT, 1862.7 cM, Oliver et al. 2010) maps, covering only 1/3 of the oats genome as estimated by O'Donoughue et al. (1995) of 2,932 cM or by Oliver et al. (2010) of 2,909 cM.

Table 4 Results of composite interval mapping (CIM) for the Hurdal × Z615-4 population, using the 2011 phenotypic data

Chromosome	QTL region (cM)	Marker interval	DON	FHB	DH	DM	PH
3C	11–13	oPt-10719–oPt-13149				11.4/0.6	
7C	3–4	oPt-14477–oPt-2902	10.5/−1.7				
12D	2–4	AEM44–P64M49-76					12.3/3.0
13A_2	1–3	oPt-5044–P34M47-92					11.9/3.0
13A_3	0–14	oPt-9627–P37M59-202		10.4/−0.2	**22.1/0.5**	**15.7/0.8**	
16A_2	8–10	oPt-2130–oPt-7864					19.2/−3.8
16A_2	11–15	P32M47-183–P32M47-175			12.1/0.4		
17A/7C	1–3	GMI_ES02_c11747_563–GMI_ES17_c2826_360	**12.4/1.8**				
unknown_7	11–13	oPt-11761–oPt-4915		**18.5/0.3**			
Accumulated percentage of the phenotypic variance explained			22.9	28.9	34.2	27.1	43.4

R^2 values (above the slash) and additive values (below the slash) calculated by PLABQTL are provided for each QTL. It is assumed that the male parent (Z615-4) contributes the high phenotypic value. QTL with LOD scores higher than 2.0 are presented, from which the ones with LOD scores higher than 2.87 (determined by 1,000 permutation tests at $P < 0.05$) are underlined, and the QTL that may be homologous to those detected in HZ595 are bolded

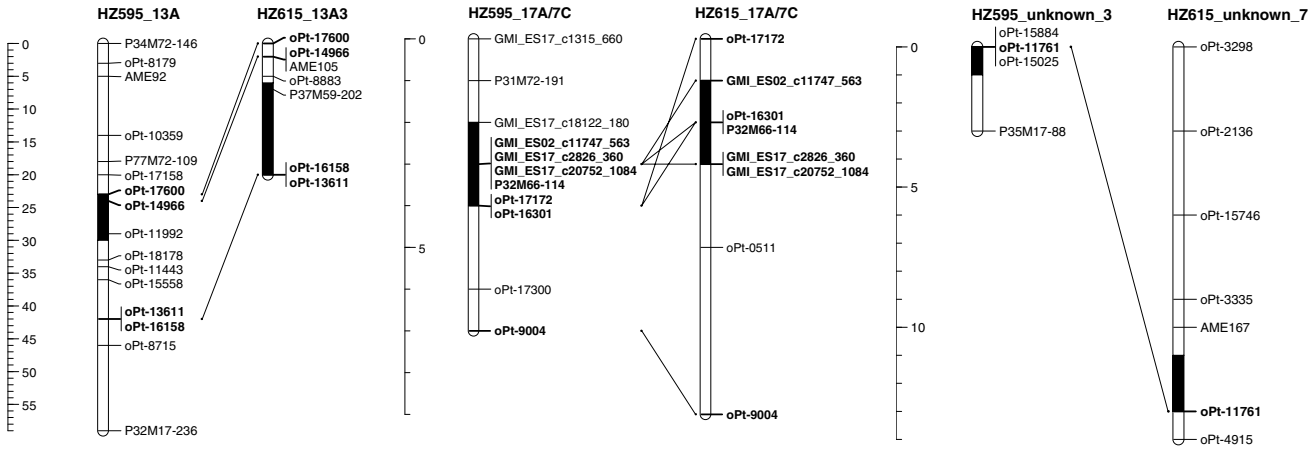


Fig. 5 Alignments of homologous QTL regions of HZ595 and HZ615 on chromosomes 13A, 17A/7C and unknown_3/unknown_7. A ruler is shown to the *left* of each alignment, denoting genetic distance (in centimorgen). The QTL regions are *shaded*, with shared markers *bolded* and connected by *lines*. Only framework makers and shared markers between aligned LGs were shown in this figure

Compared with the consensus map published by Oliver et al. (2013) which covers 1,838.8 cM, the two maps generally showed shorter LGs, particularly 2C, 3C, 10D, 17A/7C, and 21D in HZ595 (Fig. 3), and 7C/17A, 9D, 11A, 17A/7C, 20D, and 21D in HZ615 (Figure S1), which were less than 15 cM. The reasons for the lower genome coverage may be the limited markers applied or the exclusion of low quality markers which would enlarge the maps markedly if included.

Although only a subset of 51 HZ595 lines was used for consensus SNP mapping in Oliver et al. (2013), sufficient non-SNP markers were able to be anchored in this study and then mapped for the whole population, providing robust arguments for chromosomal assignments. This provided further evidence for correct assignments of HZ615 LGs to chromosomes based on the shared markers. Nevertheless, the chromosomal assignments for HZ615 should be taken as tentative, particularly for those supported by only one or two markers (Fig. S2), given the high frequency of chromosome rearrangement that could break the synteny between the two maps. Also, it should be noted that the anchoring of 17A/7C was less robust. In Oliver et al. (2013), only 2 of the 36 SNPs mapped on 17A/7C in HZ595 were localized in other maps, which were then used as anchors to assign non-SNP markers to chromosomes in this study. When compared with the KO map, the LG turned up to be homologous to KO_37 (Table S5), a fragmented and unassigned LG, giving no further evidence for the chromosomal assignment.

Although chromosomal rearrangements may be undetected due to gaps, the collinearity between the two maps was good, with minor inversions among adjacent markers, more likely due to mapping inaccuracies (Wight et al. 2003). The only major chromosomal rearrangement between the two maps was on chromosome 13A, where the projected regions of the two HZ615 LGs on Chr_13A of HZ595 were tightly linked (Fig. 2S), probably due to a translocation event happened between Z595-7 and Z615-4, or when their *A. sterilis* ancestor was introgressed into different genetic backgrounds.

The adoption of DArTs in this study enabled comparison between the current maps and the KO map. While large-scale collinearity was apparent between HZ595 and HZ615, it was far less between HZ595 (HZ615) and KO (Table S5). This was in agreement with previous reports that both macro- and micro-scale of chromosomal rearrangements are present in oats genome (Jellen et al. 1993; Singh and Kolb 1991). The prominent 7C/17A translocation segregating in KO (Tinker et al. 2009; Wight et al. 2003) was homozygous in HZ595 based on karyotyping (both Hurdal and Z595-7 carried the 7C/17A translocation, Jellen, personal communication). The translocation was assumed to be homozygous also in HZ615, given the extensive synteny between HZ595 and HZ615. Additional major chromosomal translocations that differ between HZ595 (HZ615) and KO may happen between HZ_5C_2 (stands for LG 5C_2 of both HZ595 and HZ615) and KO_36, HZ_12D and KO_7_10_28, and HZ_15A and KO_6 (Table S5). Putative translocations indicated by fewer markers could also be derived from inaccurate mapping procedures. From Table S5, KO_37 and KO_48 that were not assigned to chromosomes in Oliver et al. (2013) may reside on chromosomes 17A/7C and 18D, respectively; and HZ595_unknown_1/HZ615_unknown_2 and HZ595_unknown_3/HZ615_unknown_7 in our study may correspond to KO_15 and KO_4_12_13, respectively.

Skewed segregation

Segregation distortion is a common phenomenon in oats and has been found in many studies, owing mainly to genome rearrangements (Rines et al. 2006). In the present study, the relatively high 22–24 % skewed markers may more likely be due to the high genetic distance between parents (He and Bjørnstad 2012), involving chromosomal rearrangements and distorted regions.

Application of highly skewed markers in molecular mapping may lead to false linkages (Portyanko et al. 2001). In the present study, highly skewed markers with $P < 10^{-4}$ were excluded from linkage analyses; but later they were placed relatively to the framework maps, enabling the identification of distorted chromosomal regions. It is noteworthy that none of the skewed regions in HZ595 was found in their homologous regions in HZ615 and vice versa, highlighting a genetic background dependent nature.

QTL mapping

Admittedly, our QTL mapping in HZ595 has its drawbacks. Firstly, DON data from the 3 years showed low correlations, which hindered the consistent detection of minor QTL. Secondly, the incomplete genomic coverage prevented the detection of QTL in chromosome regions not mapped. In addition, the location of *Qdon.umb-17A/7C* on a fragmented LG made its chromosomal designation less robust; although this has no influence on QTL effects and the linked markers. As for HZ615, although its QTL must be considered putative with only 1-year phenotypic data and a rather small population with only 91 lines, those localized on homologous regions of the two maps indicate real QTL.

The most prominent and stable QTL in this study was *Qdon.umb-17A/7C*, detected in both populations over all environments, with its phenotypic effects ranging from 12.2 to 26.6 % in HZ595 (Table 3). The value 26.6 % in 2010 may be the best estimate, considering the too low levels of DON in 2008 and the excessive infection in 2009. Therefore, *Qdon.umb-17A/7C* could be classified as major DON resistance QTL, with phenotypic effect similar to *Fhb1* in wheat, which showed 23 % of average disease reduction and a range from 0–60 % in a set of near-isogenic lines (Pumphrey et al. 2007). The less effect in HZ615 corresponded with this, and studies on other oat materials are needed to further validate the phenotypic effects of *Qdon.umb-17A/7C*.

QTL for FHB or DON often coincide with PH or DH under field conditions (Buerstmayr et al. 2009). However, these QTL may not represent the true resistance genes, but genes showing pleiotropic effects of PH/DH or contributing to disease escape. More attention should thus be paid to QTL showing no association with PH or DH. In addition to *Qdon.umb-17A/7C*, QTL on 5C and 14D in HZ595 were also found repeatedly over 2 years, and the absence of association with PH and DH emphasized their importance as true resistance QTL. Nor were the QTL on unknown_3 in HZ595, 7C/17A and unknown_7 in HZ615 associated with any agronomic QTL. Although they need further validation (since detected only once), the first and the last may reflect the same QTL (Fig. 5), providing an indirect validation.

A QTL responsible for DON or FHB resistance that coincided with agronomic QTL was located on chromosome 13A in both HZ595 and HZ615 (Tables 3, 4). The QTL region was skewed in HZ595 (but not in HZ615), which was in accordance with Holland et al. (2002). They found a DH QTL that coincided with a skewed region on KO_6, which is homoeologous to HZ_13A. The coincidence was also reported later by Portyanko et al. (2005) in the MN841801-1 × Noble-2 population. Several QTL for DH and the vernalization gene *Vrn3* was also mapped on KO_6 (Holland et al. 2002; Nava et al. 2012), and were potential homologs to the QTL in our study. However, the locations of the DH QTL and the *Vrn3* gene in KO differed from that of our QTL based on shared markers. In addition, the QTL regions for PH on KO_6 also differed from ours (Holland et al. 1997), suggesting that the QTL identified in the current study was a new one, or it has undergone a translocation.

Other QTL for DH and/or PH in this study were also compared with those in KO (Holland et al. 2002; Holland et al. 1997; Nava et al. 2012), based on shared markers. Two of them were located in the same regions as their KO counterparts, i.e. the DH QTL on chromosome 11A (homoeologous to KO_13) and the PH QTL on 16A (KO_20) in HZ595. Three QTL were located some distances away from those in KO, i.e. the DH QTL on 16A_2 (KO_24, approx. 10 cM away from DH QTL and *Vrn1*) in HZ615, the PH QTL on 16A_2 (KO_24, 20 cM) in HZ615, and the PH QTL on 19A (KO22, 40 cM) in HZ595. Conclusions for the remaining QTL were not drawn due to insufficient number of shared markers. Further studies are needed to see whether these QTL were the same or not as those in KO.

Marker-assisted selection (MAS)

One of the main goals of QTL mapping is to find linked markers for MAS. Of the marker types applied in this study, AFLPs and DArTs cannot be directly used in MAS unless transformed; SSRs have been widely used in wheat but their availability for oats was very limited (Oliver et al. 2010). SNPs were usually applied in high throughput genotyping, using technologies such as Illumina GoldenGate assay (Oliver et al. 2013); but KASPar technology made them suitable also for low throughput genotyping (Cuppen 2007), offering great flexibility.

Ten SNPs were mapped on chromosome 17A/7C in HZ595, with 6 in the *Qdon.umb-17A/7C* region (Fig. 4); afterwards, 3 of the 6 SNPs, *GMI_ES02_c11747_563*, *GMI_ES17_c2826_360* and *GMI_ES17_c20752_1084* were located in the corresponding region in HZ615 (Fig. 5), these SNPs could be used in MAS for diagnosing *Qdon.umb-17A/7C*. Based on the genotyping data of 5 SNPs linked to *Qdon.umb-17A/7C* (Oliver et al. 2013), 44 of the 108 entries showed resistance haplotype, 32 showed susceptible haplotype, while the remaining 32 were recombinants. Unexpectedly, a very susceptible cultivar, AC Robust, was found to have the resistance haplotype at the *Qdon.umb-17A/7C* locus. This indicates that *Qdon.umb-17A/7C* alone may not provide sufficient protection, and more QTL and their linked markers are thus needed in MAS for pyramiding resistance genes.

In addition, 3 more SNPs were available in HZ595 for identifying their respective QTL, i.e. *GMI_ES01_c1223_200* on chromosome 5C (with a genetic distance of 1 cM) for DON, *GMI_ES_CC8927_168* on 11A (7 cM) for FHB, DH and DM, and *GMI_ES01_c14084_302* on 13A (4 cM) for DON, DH, DM and PH (Fig. 4).

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