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Quantitative trait loci influencing β-glucan content in oat (*Avena sativa*, 2n=6*x*=42)

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Abstract The β-glucan content of oat grain is of interest due to its positive human health role as a dietary component influencing serum cholesterol levels and its relation to the energy intake of livestock feed. Two recombinant inbred populations sharing a common parent (Kanota × Ogle and Kanota × Marion), and containing 137 individual lines each, were used to identify genomic regions that influence the β-glucan content in cultivated oat. Single-factor ANOVA, a backward elimination process, simple interval mapping (SIM) and simplified composite interval mapping (sCIM) were used to identify quantitative trait loci (QTLs). Regions on linkage groups 11 and 14 of the hexaploid oat RFLP map influenced β-glucan levels in both populations and over environments. Other genomic regions were identified whose effects varied depending on the genetic background, but were significant over measurements for a given population. Kanota and Ogle exhibit similar β-glucan levels and each parent contributed about the same number of positive β-glucan alleles in the Kanota × Ogle cross. Marion is higher in β-glucan content than Kanota and contributed all of the positive alleles in the Kanota × Marion cross. Three of the β-glucan QTL regions identified have been previously implicated as having a significant influence on the groat oil content in oat. These correlated QTL regions were either in coupling phase, with a region from one parent having the same effect on both traits, or were in repulsion phase. Identification of coupling- and repulsion-phase QTL regions for β-glucan and oil content facilitates the use of markers in manipulating these traits in oat breeding.

Key words Oat · QTL analysis · β-glucan · Oil content · Coupling-phase QTL

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Introduction

Various food products derived from oat, such as oat porridge and oat bran, are known to reduce serum cholesterol levels in humans (Ripsin et al. 1992). This effect is attributed to the high content of water-soluble fiber of mixed linkage (1→3), (1→4) β-D-glucans (β-glucans) in oat groats (de-hulled kernels) compared to that in most other cereals (Chen et al. 1981; Klopfenstein and Hoseney 1987; Jennings et al. 1988). Feed rations high in β-glucan, on the other hand, can be detrimental to the weight gain of animals due to the energy differential in metabolism (Anderson et al. 1978). The development of oat cultivars with β-glucan contents different from those currently available is desirable to make the grain better suited to serve the needs of both the food and the feed markets.

Grain β-glucan contents among oat cultivars range from 28 g kg^{-1} to 68 g kg^{-1} on a dry weight basis (Welch and Lloyd 1989; Peterson 1991; Welch et al. 1991; Wood et al. 1991; Lim et al. 1992; Cho and White 1993; Miller et al. 1993). This trait is controlled by multiple genetic

loci with primarily additive effects (Holthaus et al. 1996). Genotype by environment (G×E) interaction for this trait has been reported but its relative importance is not clear. In certain instances, the G×E effect is believed to be minor with genotypes generally showing a similar ranking for β-glucan content among environments (Holthaus et al. 1996). In other cases this effect has been considered significant even when tested via the mean square for year by variety (Peterson 1991; Lim et al. 1992). This trait does not show a consistent correlation with agronomic characters (Peterson et al. 1995; Holthaus et al. 1996). Broad-sense heritability for β-glucan is intermediate, estimated at around 0.55 (Holthaus et al. 1996). Based on existing genetic variability for β-glucan levels among oat cultivars, long-term gains due to selection are clearly possible (Peterson et al. 1995; Holthaus et al. 1996). However, molecular genetic dissection of this trait and the use of molecular markers in a marker assisted selection (MAS) program could help expedite and possibly reduce the cost of modifying the β-glucan levels in oat, especially since accurate measurements of β-glucan are difficult and expensive (Wood 1986).

The recent explosion in the development of restriction fragment length polymorphism (RFLP) linkage maps and their use for identifying genomic regions affecting quantitatively inherited traits are well documented (Phillips and Vasil 1994; Tanksley and McCouch 1997). A molecular marker study relating to β-glucan content and β-glucanase activity in barley grain, which is relatively high in β-glucan content, has been reported (Han et al. 1995). This report corroborated a previous study identifying 3–5 additive genetic loci or genomic regions influencing the β-glucan content in barley (Powell et al. 1989; Han et al. 1995). The regions identified each explained between 10.5% and 19.2% of the phenotypic variance for β-glucan content and together accounted for 34% of the variance (Han et al. 1995). More interestingly, in barley these regions did not show significant QTL by environment variation for β-glucan content (Han et al. 1995). Considering the hexaploid nature of the oat genome, up to three times as many genetic loci are expected to influence this trait in oat compared to diploid barley. Due to the increased number of loci, they each may have a reduced effect on β-glucan content making them more difficult to identify in oat compared to barley.

Herein we report a study aimed at identifying genomic regions that significantly influence groat β-glucan content in two recombinant inbred oat populations sharing a common parent. This trait was evaluated over diverse environments providing a unique opportunity to identify and evaluate the interaction between β-glucan QTLs and environments in two genetic backgrounds. The objectives of this study were to identify common regions affecting β-glucan content in two different genetic backgrounds, to determine the consistency of QTLs identified across environments, to compare regions influencing β-glucan content with loci previously shown to influence groat oil content in the same recombinant inbred populations, and to evaluate the potential use of markers in a MAS program to manipulate the β-glucan content in oat.

Materials and Methods

Genetic material

Two $F_{2:6}$-derived recombinant inbred populations developed by single-seed descent were used in this study: a population of 137 recombinant inbred lines (RILs) developed from the cross of a facultative winter type cultivar, Kanota, by a spring cultivar, Ogle, and a second population of 137 RILs from the cross of Kanota by another spring cultivar, Marion. The second population was chosen on the basis of having a common parent, Kanota, to provide a degree of "biological replication." These materials were grown in 1991–1995 in a randomized complete block design at the following locations:

Kanota × Ogle (KO): Aberdeen, Idaho, 1991, 1992 and 1993; Ithaca, New York, 1993; and St. Paul and Rosemount, Minnesota, 1994.

Kanota × Marion (KM): Aberdeen, Idaho, 1992; St. Paul and Rosemount, Minnesota, 1994; and St. Paul, Minnesota, 1995.

At Aberdeen, Idaho, the materials were grown in three replicates of four-row plots 2.5-m long, 30 cm between rows with the center two rows harvested. At Ithaca, New York, the materials were grown in three replicates of six-row plots 4-m long and 18 cm between rows. At St. Paul and Rosemount, Minnesota, the materials were grown in four replicates of hill plots with 30 seeds planted per hill on a 30-cm grid spacing. The exact parents used to generate the populations were also included as single or multiple entries in each replicate

β-glucan analysis

To limit the number of samples for costly β-glucan analysis, equal amounts of sample from each replicate at a given location/year were bulked to produce a single balanced sample per line. This resulted in seven measurements for each RIL in the KO population (Aberdeen, Idaho, 1992, samples were measured by two different laboratories) and four for the KM population.

For β-glucan measurements, 20–40 g of dried oat seeds were de-hulled with an impact type de-huller and were dried at 70°C for 18 h. After cooling and cleaning, about 5 g of each sample were ground in a Retsch Ultra Centrifugal Mill Model ZM1 with a 0.5-mm screen. The β-glucan content of samples was determined by the flow injection analysis (FIA) method developed by Jørgensen and Aastrup (1988) and described by Peterson (1991). Each value given as g kg^{-1} of β-glucan on a groat dry weight basis is the mean of nine individual FIA measurements; three portions of each ground sample were evaluated three times each with FIA.

RFLP analysis and map development

Techniques of DNA extraction, gel blotting and hybridization have been described previously (Kianian et al. 1997). Briefly, 20–30 µg of total DNA samples from each RIL and the parental lines were digested with the appropriate restriction endonuclease and electrophoresed overnight in 1× TAE (0.04 M Tris-acetate, 0.002 M EDTA) at a constant 30 V (Sambrook et al. 1989). After transfer to a nylon membrane, DNA blots were hybridized with a radioactively labeled probe (Feinberg and Vogelstein 1983; Kianian et al. 1997). The source and characteristics of the randomly selected RFLP probes BCD, CDO, OG and UMN have been described previously (O'Donoughue et al. 1995; Kianian et al. 1997). Methods of bacterial growth, plasmid isolation, and insert recovery were those reported by Hosaka et al. (1990).

A hexaploid oat RFLP map containing 561 loci has been generated from 71 RILs in the KO population (O'Donoughue et al. 1995). To take advantage of the entire population and to add power to our analysis, the remaining 66 RILs in this population were also mapped. Markers were chosen at random and to-date 153 loci have been mapped with these 66 individuals. An RFLP map based on all 137 individuals and the 153 mapped loci was generated and was in accord with the published map (O'Donoughue et al. 1995), except for linkage group 3. An inter-varietal translocation is believed to involve this linkage group. In the final analysis, we used the published map for this region. Additional information from aneuploid analysis (Kianian et al. 1997) was also taken into account when two linkage groups were assigned to the same chromosome. Thus, linkage groups 5 and 7 assigned to chromosome 5C, linkage groups 6 and 20 assigned to chromosome 16, and linkage groups 4 and 12 assigned to chromosome 21 were analyzed as three distinct units. These 153 loci cover most of the genome except for linkage groups 9, 18, 19, 31, 35, 37 and 38.

The results from our initial QTL analysis on the first 71 RILs of the KO population for β-glucan and groat physical traits were used to select the markers for mapping with the KM population. A total of 60 loci were mapped on the 137 RILs in the KM population. These loci mapped to 19 of the 38 linkage groups that had been identified in the KO population (linkage groups 1, 2, 4, 5, 8, 9, 18, 19, 21, 24, 25, 26, 30, 33, 34, 35, 36, 37 and 38 were not covered). The linkage groups not covered were, in general, small groups consisting of only two to a few markers in the KO map. The data from the KM population generated an additional linkage group of two loci, and 13 markers remained unlinked. These loci cover all the linkage groups showing association with β-glucan content in the first 71 RILs of the KO population except for group 37.

Statistical analysis

Phenotypic data of the traits evaluated were analyzed using SAS (ANOVA and GLM procedures; SAS Institute 1990). In the analysis of trait data, each location/year combination was considered as one environment. To determine significant associations of individual marker loci with β-glucan content, a single factor ANOVA was performed on each individual combination (PROC GLM, SAS Institute 1990). A genomic region was considered to significantly influence this trait if it fit the following criteria: (1) the association was significant when considering the average of all the environments ($P \leq 0.01$); (2) the association was also significant ($P \leq 0.05$) over a majority of environments (5 of 7 in KO and 3 of 4 in KM); and (3) closely linked markers within that region, when available, also showed significant associations. A multi-locus model containing the most significant markers was constructed by a "backward elimination" process as described by Kennard and Havey (1995). Briefly, a model is first constructed which includes all the independently tested significant ($P<0.05$) regions for the entire genome (only the most significant marker in a given region is used, i.e., no linked markers). Then markers are excluded one at a time based on the criterion of least significant ($P<0.05$) type-III sums of squares. The final model includes all markers that are significant ($P<0.05$) when tested against the full model variation.

To corroborate our results from ANOVA, MQTL software (Tinker and Mather 1995a, b) was used to perform Simple Interval Mapping (SIM) and Simplified Composite Interval Mapping (sCIM). The advantages and details of this procedure have been described elsewhere (Tinker and Mather 1995a, b; Tinker et al. 1996). The reasons we used this software package were: (1) the advantage of searching for one QTL at a time while simultaneously accounting for the effects of other segregating QTLs; (2) separate analyses are performed for each environment circumventing the problem of dealing with QTL by environment (QTL×E) interaction and complications due to environmental heterogeneity; and (3) permutation tests are performed to establish thresholds for the control of type-I error rate. In the sCIM procedure, 30 loci were randomly chosen and specified as background markers in the KO population and five loci as background markers in the KM population. Threshold estimates for QTL main effects and QTL×E interaction were determined by randomizing the phenotypic values in relation to the RFLP scores for each individual and calculating the test statistics for these data. This procedure was repeated 1000 times to determine the threshold for a genome-wise type-I error rate of 0.05. Tinker and Mather (1995a) found this procedure provided a good control of type-I error rate for SIM but not for sCIM. The significance of QTL regions identified by MQTL is reported as the test statistics $TS=n\times\ln(RSSr/RSSf)$ where n is the number of observations, RSSf is the residual sums of squares for the full model, and RSSr is the residual sums of squares from the model without the effect being tested. This test statistic is similar to the likelihood ratio, and approximately equal to the F statistic. For a single environment, TS can be converted to the LOD score computed by Mapmaker QTL by multiplying by 0.22 or dividing by $2\ln(10)$.

Significant QTL regions were determined as described by Tinker et al. (1996). These regions were grouped in a multi-locus linear model to estimate the overall variance explained by all significant QTLs simultaneously. However, we used an additional criterion of at least 10-cM spacing between significant markers to be included in this final model; the population size was not large enough to differentiate between closely linked QTLs and a single QTL.

Results

Trait data

Both the KO and the KM oat populations showed a relatively continuous distribution for β-glucan content with a majority of the individuals having values within one standard deviation of the parents (Fig. 1 and Table 1). Among the parents, Marion had the highest β-glucan values followed by Ogle and then Kanota. Consequently, the difference in β-glucan content between parents was greater in the KM population (9.9 g kg^{-1} β-glucan) than in the KO population (3.9 g kg^{-1} β-glucan). Also, the individuals in the KM population had, on average, a 5 g kg^{-1} higher β-glucan content than the KO individuals (Table 1). Large numbers of individuals having values outside the range of the parents were observed in KO and some were found in KM (Table 1). In the KO population, more transgressive segregants were observed for high β-glucan values than for low ones (Fig. 1).

The correlation coefficients (r) between various measurements of β-glucan content were all significant but not very high (Table 2). As expected, this trait was somewhat influenced by the environment. Values for the KO population had an average r of 0.58 with the lowest value of 0.40 between materials collected at Aberdeen, Idaho, 1991 and St. Paul, Minn., 1994 (Table 2). The highest correlation was for the measurements of the same set of samples by two different laboratories, Aberdeen 1992 samples measured at the University of Minnesota (Minn.) and the Quaker Oats Company (QOC), with r=0.79. This indicates the difficulty in accurately measuring this trait and the variation observed in laboratory measurements. The values for the KM population showed a similar high amount of variability with an average r of 0.58 and a lowest value of 0.35 for samples from Aberdeen, Idaho, 1992 and St. Paul, Minn., 1995 (Table 2).

Table 1 β-glucan content[a] and percent transgressive segregants in two recombinant inbred oat populations. SD=standard deviation, Min.=minimum, Max.=maximum, X=number of individuals and QOC=The Quaker Oats Company

Population (P1×P2)	Environment	Laboratory	Parents		Population				% Transgressive segregants	
			P1	P2	Mean	SD	Min.	Max.	X<P1-SD	X>P2+SD
Kanota × Ogle	Aberdeen 91	Minnesota	39.0	41.0	44.8	6.0	32.0	63.0	2	39
	Aberdeen 92	Minnesota	38.3	45.3	44.7	4.2	36.3	60.7	0	18
	Aberdeen 92	QOC			40.0	3.7	31.9	52.0		
	Aberdeen 93	QOC	42.8	44.8	43.4	3.8	34.4	54.4	20	13
	Ithaca 93	QOC	44.6	49.0	47.4	4.2	38.3	56.9	5	11
	St. Paul 94	QOC	43.6	47.4	48.9	6.1	34.5	62.8	3	26
	Rosemount 94	QOC	44.6	49.3	47.7	4.8	37.0	58.6	4	9
	Average		42.2	46.1	45.3	3.7	37.2	57.6	5	18
Kanota × Marion	Aberdeen 92	Minnesota	42.7	54.0	48.7	4.3	39.3	58.3	0	0
	St. Paul 94	QOC	44.2	56.9	50.0	4.3	42.0	60.3	0	0
	Rosemount 94	QOC			50.1	4.3	39.6	62.5		
	St. Paul 95	QOC	49.2	58.3	53.6	4.4	43.2	66.6	2	5
	Average		45.4	55.3	50.6	3.7	42.8	59.6	0	1

[a] All β-glucan values are in g kg^{-1}. Blank cells represent missing values

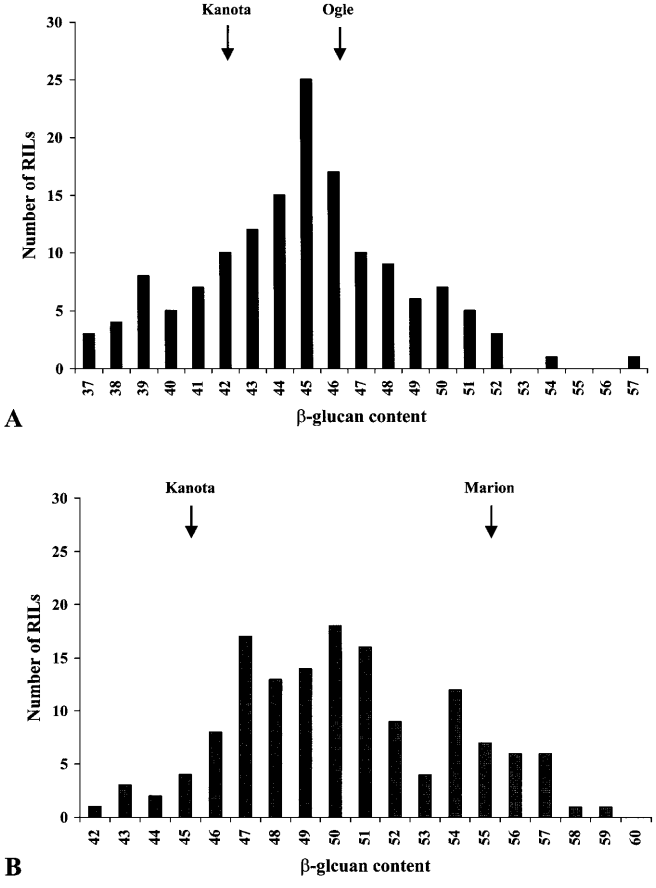

Fig. 1A, B Histogram of β-glucan content averaged over all measurements relative to the number of recombinant inbred lines (RILs). **A** The distribution for the Kanota × Ogle population, and **B** the distribution for the Kanota × Marion population.

QTL analysis

Kanota × Ogle population

The power of QTL detection relates to the size of a population; therefore, the entire KO population was analyzed. This analysis identified regions on linkage groups 3, 6, 11, 13, 14, 17 and 20 as significantly contributing to the β-glucan content (Table 3; $P \leq 0.05$ for most of the individual environments and $P \leq 0.01$ using means across environments). The region on linkage group 3 was the most significant one accounting for a 3.5 g kg^{-1} change in β-glucan content and 12.5% of the phenotypic variance averaged across environments. The next most important region was on linkage group 11 near the *Xcdo665* locus accounting for 5.3% of the phenotypic variance and a 2.3 g kg^{-1} difference in the β-glucan content (Table 3). The region on linkage group 37, identified using the 71 RILs employed in generating the oat hexaploid RFLP map (O'Donoughue et al. 1995), could not be verified due to a lack of RFLP data for the entire population. Kanota contributed three and Ogle contributed four of the alleles for the loci that had a positive influence on this trait. Overall, the alleles from Kanota contributed 7.4 g kg^{-1} to the β-glucan content compared with 6.5 g kg^{-1} from Ogle. As expected, each identified QTL showed a varied effect depending on the environment, as illustrated by the range of phenotypic variance each explained. Regions on linkage groups 3, 6, 11, 13 and 14 are considered the most important and remained in the full model after the backward elimination process (Table 4). These regions account for 14% of the phenotypic variance and a 10.7 g kg^{-1} change in β-glucan content. The source of increased β-glucan content alleles for three of these five loci is Kanota, the parent with the slightly lower content of β-glucan (Tables 1, 4).

The model generated by using the MQTL software contained some of the most significant markers identi-

Table 2 Phenotypic correlations (*r*) for β-glucan content between environments. All significant, $P<0.0001$. Laboratory designation for sample evaluations, MN=Minnesota and QOC=The Quaker Oats Company

Population	Location						
		AB91 (MN)	AB92 (MN)	AB92 (QOC)	AB93 (QOC)	IT93 (QOC)	SP94 (QOC)
Kanota × Ogle	Aberdeen 92 (MN)	0.52					
	Aberdeen 92 (QOC)	0.44	0.79				
	Aberdeen 93 (QOC)	0.56	0.70	0.76			
	Ithaca 93 (QOC)	0.56	0.58	0.66	0.73		
	St. Paul 94 (QOC)	0.40	0.42	0.44	0.47	0.53	
	Rosemount 94 (QOC)	0.43	0.57	0.63	0.70	0.78	0.44
		AB92 (MN)	SP94 (QOC)	RS94 (QOC)			
Kanota × Marion	St. Paul 94 (QO)	0.53					
	Rosemount 94 (QOC)	0.55	0.82				
	St. Paul 95 (QOC)	0.35	0.63	0.60			

Table 3 Genomic regions influencing β-glucan content in oat as identified by single-factor analysis of variance

Linkage group[a]	Locus[b]	β-glucan[c] (g kg^{-1})	%R^{2} [d]
Kanota × Ogle			
3, K	*Xcdo346A*	3.5	12.5**
6, K	*Xcdo82*	1.6	2.3*
11, K	*Xcdo665B*	2.3	5.3*
13, O	*Xcdo549B*	1.7	2.6*
14, O	*Xcdo400*	1.6	2.3*
17, O	*Xcdo1340*	1.5	2.0*
20, O	*Xcdo57B*	1.7	2.4*
Total	Kanota	7.4	
	Ogle	6.5	
Kanota × Marion			
11, M	*Xcdo665B*	2.2	5.5**
14, M	*Xcdo400*	2.6	7.6***
5X, M[e]	*Xcdo1385H*	2.0	4.4*
10X, M[e]	*Xumn409A*	2.0	4.3*
Total	Kanota	0.0	
	Marion	8.8	

* Indicates significance at $P\leq0.01$, ** $P\leq0.001$ and *** $P\leq0.0001$ for the means across all environments. $P\leq0.05$ for majority of the individual environments

[a] The linkage group and parent contributing the positive allele (O=Ogle, K=Kanota and M=Marion)

[b] Locus with the highest %R^2 within the linkage group

[c] Substitution of the source alleles will cause this change (based on overall mean of values from seven measurements in KO and four in KM) in β-glucan content

[d] %R^2 is the average (over all the environments) amount of phenotypic variance explained by this locus

[e] More markers are needed to verify the tentative linkage-group assignments of these markers

Table 4 Multi-locus models for β-glucan content in oat

Population	ANOVA Model[a]	MQTL Model[b]
Kanota × Ogle	*Xcdo346A* (3, K) *Xcdo82* (6, K) *Xcdo665B* (11, K) *Xcdo549B* (13, O) *Xcdo400A* (14, O)	*Xcdo346A* (3, K) *Xcdo82* (6, K) *Xcdo549B* (13, O) *Xcdo1340* (17, O)
Total % R^2	14.0	20.0
Kanota × Marion	*Xcdo665B* (11, M) *Xcdo400A* (14, M) *Xcdo1385H* (5X, M) *Xumn409A* (10X, M)	*Xcdo665B* (11, M) *Xcdo400A* (14, M) *Xcdo1385H* (5X, M) *Xumn409A* (10X, M)
Total % R^2	20.8	20.0

[a] The linkage group and parent contributing the positive allele are depicted in parenthesis [i.e., linkage group 6 with the Kanota allele being positive (6, K)]. The models are based on seven environments for KO and four in KM

[b] The VI/VP [(variance explained by the QTL(s)+variance QTL×E) over the phenotypic variance)] is presented as the %R^2 for MQTL

fied by the ANOVA backward-elimination process (Table 4 and Fig. 2). The loci included in this model were *Xcdo346A* on linkage group 3, *Xcdo82* on linkage group 6, *Xcdo549B* on linkage group 13 and *Xcdo1340* on linkage group 17. Considering the variation due to the environment, these loci account for 20% of the phenotypic variance [Variance Main effect (VM)/Variance Phenotypic (VP)=16%, Variance QTL × environment Interaction and VM (VI)/VP=20% and Variance Genetic from background markers (VG)/VP=3%)]. The only locus considered significant for having a test statistics (TS) value above the threshold of 50.2 was *Xcdo346A* on linkage group 3 (TS = 54.7). This locus accounts for 2.4 g kg^{-1} change in β-glucan content (0.8 g kg^{-1} to 2.6 g kg^{-1} depending on the environment). Since the threshold estimate is found to provide a good control of the type-I error rate for SIM but not for sCIM (Tinker and Mather 1995a), secondary loci can be inferred when either SIM or sCIM give evidence for QTLs (Tinker et al. 1996). The *Xcdo82* and *Xcdo549B* loci were considered significant as secondary QTLs based on this reasoning (Fig. 2). These markers showed a large variation due to the environment, and *Xcdo1340* on linkage group 17 was identified due to a significant QTL×E peak (TS=22.5; Fig. 2). The allele from Ogle contributed on average a 1.9 g kg^{-1} change in β-glucan content for this locus except for the samples from St. Paul, Minn., 1994, where the positive contribution came from Kanota, a shift in the allelic value. Overall, in the MQTL model, two of the signifi-

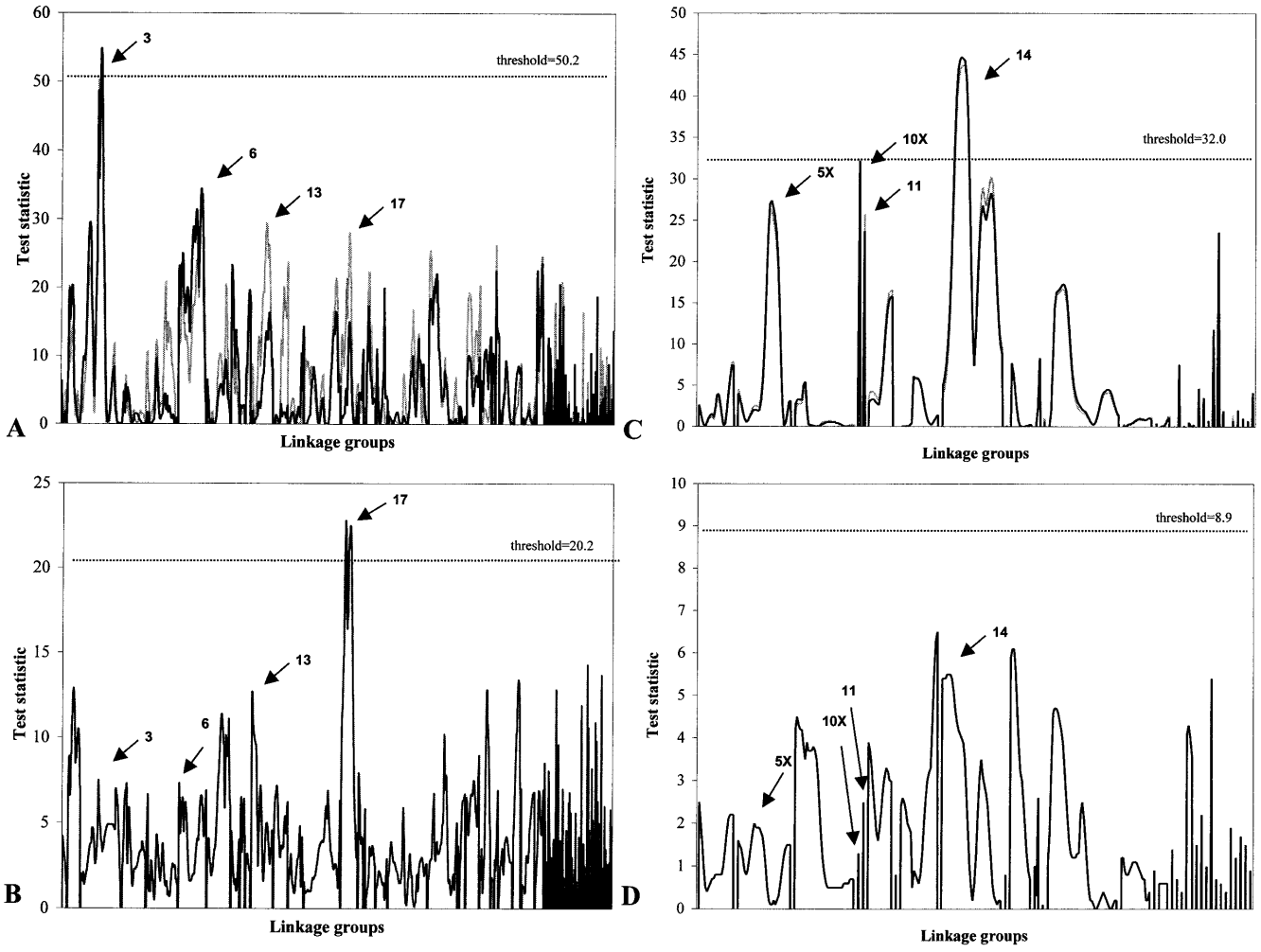

Fig. 2A–D Scan of test statistics generated by MQTL for the two recombinant inbred populations. **A** and **B** are the main effect and QTL×E interaction graphs for the Kanota × Ogle (KO) population, respectively. **C** and **D** are the main effect and QTL×E interaction graphs for the Kanota × Marion (KM) population, respectively. Thresholds for a type-I error rate of 5% after 1000 replications are reported on the graphs. *Solid lines* represent simple interval mapping, *dashed lines* simplified composite interval mapping, and *arrows* indicate the peak for linkage groups in the MQTL model (Table 4).

cant loci were contributed by Kanota and two by the Ogle parent. Together these loci caused a change of 8.5 g kg^{-1} in β-glucan content (5.5 g kg^{-1} to 12.6 g kg^{-1} depending on the environment).

Kanota × Marion population

Single-factor analysis of variance revealed four genomic segments significantly associated with β-glucan content in the KM population (Table 3; $P<0.05$ for individual environment and $P<0.01$ using means across environments). These were regions on linkage groups 11, 14, 5X and 10X (groups 5X and 10X have been tentatively assigned to linkage groups 5 and 10 but more markers are needed to accurately place them on the hexaploid oat RFLP map). As would be expected from the parental values and population distribution (Table 1), Marion contributed the alleles for all of the important regions affecting this trait. The overall parental contribution to β-glucan content was 8.8 g kg^{-1} for Marion (Table 3). Regions on linkage groups 11 and 14 were in common with those identified in the KO population. All loci remained in the full model after the backward elimination process (Table 4). The four loci in the full model collectively accounted for 20.8% of the phenotypic variance and a change of 8.8 g kg^{-1} in β-glucan content.

MQTL analysis gave results similar to the ANOVA process (Table 4). Linkage groups 11, 14, 5X and 10X were all considered significant. The most important locus was *Xcdo400* on linkage group 14 with a TS value of 44.7 accounting for a change of 3.7 g kg^{-1} in β-glucan content (Fig. 2). Including the environmental variability, these four regions accounted for up to 20% of the phenotypic variance (VM/VP=18%, VI/VP=20% and VG/VP=1%). The environmental influence on the QTL peaks was not significant (Fig. 2). The four regions together accounted for a 8.7 g kg^{-1} change in β-glucan content (7.6 g kg^{-1} to 10.6 g kg^{-1} depending on the environment). Marion contributed the positive alleles at all the loci.

Table 5 Relationship between genomic regions significantly influencing both β-glucan and groat oil content in oat

Kanota × Ogle			Kanota × Marion		
Linkage group[b]	β-Glucan source[a]	Groat oil source[a]	Linkage group[b]	β-Glucan source[a]	Groat oil source[a]
6	Kanota	Kanota	11[c]	Marion	Kanota
11[c]	Kanota	Kanota	5X	Marion	Kanota

[a] Parental source of the allele which contributes positively to the trait
[b] Linkage groups significantly associated with both traits (Kianian et al. 1999, for oil QTLs)
[c] Associated with the *XaccaseA* locus identified by the Acetyl-CoA Carboxylase cDNA clone from oat

β-Glucan and groat oil content

Oat groats are unique among cereals for the high level and the embryo-plus-endosperm localization of lipids. A locus having a major effect on oil content in oat groats was located on linkage group 11 by the QTL analysis methods described here (Kianian et al. 1999). A partial oat cDNA clone for plastidic acetyl-CoA carboxylase (ACCase), which catalyzes the first committed step in de novo fatty acid synthesis, identified a polymorphism linked to this major QTL (Kianian et al. 1999). Similar QTL and ACCase locus placements were obtained with both the KO and KM populations. The QTL linked to *XAccaseA* on linkage group 11 accounted for up to 48% of the phenotypic variance for groat oil content. Other QTLs were identified in both populations which accounted for an additional 10–20% of the phenotypic variance (Kianian et al. 1999).

The genomic regions on linkage groups 6 and 11 identified as being significantly associated with the groat β-glucan content in the KO population were those previously reported to be involved in alteration of groat oil content (Kianian et al. 1999; Table 5). Similarly for the KM populations, regions identified for β-glucan on groups 5X and 11 were also identified for groat oil content (Table 5). These results were obtained despite the fact that the correlation between the two traits was minimal and not significant (0.34 in KO, –0.14 in KM). The regions on linkage groups 6, 11 and 5X are considered most important and were included in the full model(s) for both traits. For instance, the region on linkage group 11 that explains up to 48% of the phenotypic variance for groat oil content and is linked to *XaccaseA* also explains about 5.3% of the phenotypic variance for β-glucan content (Table 3; Kianian et al. 1999). This region is important for both traits in both populations (Table 5). In the KO population, the Kanota allele was the source of increased groat oil and β-glucan content. However, in the KM population the Kanota allele contributes to increased groat oil content while the Marion allele increases the β-glucan content.

Discussion

For molecular markers to be an effective tool in a marker-assisted selection breeding program, they have to contribute significantly in terms of saving time, costs, or both. The β-glucan content in oat is controlled by many loci with additive effects and strong environmental influences (Peterson 1991; Lim et al. 1992; Holthaus et al. 1996). Because, the β-glucan content in oat is expensive to measure accurately, is influenced by the environment, and is controlled by several genes, it represents an excellent example of a trait for molecular quantitative trait dissection and manipulation in a marker-assisted selection breeding program.

The number of progenies required to map a QTL by linear regression methods is inversely proportional to the square of the allelic effect of the QTL, except when the allelic effect is large enough for the trait to be considered qualitative (Soller et al. 1976). Thus a large number of individuals are needed to detect small phenotypic effects; however, this number tends to be determined more by economical and genetic limitations of the study than theoretical considerations. The analysis of the KO population highlights the danger in identifying QTLs for a trait such as β-glucan content in oat using small populations. None of the linkage groups identified in the original 71 KO recombinant inbred lines [lines used in the generation of the hexaploid oat RFLP map (O'Donoughue et al. 1995)] were the same as those identified in the remaining 66 individuals (data not shown). If we reduce the threshold of detection, many of the regions are recognized in both portions of the KO population. This leads to the detection of false positives (Type-I error). Conversely, increasing significance levels leads to rejection of the association between a marker and a QTL when it exists (Type-II error). As has been pointed out by Zehr et al. (1992), the appropriate probability level for this type of application is not clear. Increasing the population size could remedy some of the problems associated with the analysis of traits affected by many loci with small effects. The majority of the QTL regions identified in each part of the KO population were also detected when the analysis was performed on the entire population. This finding is in agreement with the conclusions of Beavis (1998), based on modeling experiments using different population sizes, that small populations tended to not identify all QTLs and often over-estimated the magnitudes of the QTLs identified.

Various other genetic and quantitative-trait analysis tools can be used to identify those segments of the genome having a consistent influence on a trait. These in-

clude multiple replications of the same genetic material in different environments and analysis of the phenotype in multiple genetic backgrounds. Regions on linkage groups 11 and 14 identified in this study represent segments of the genome that consistently, over environmental as well as genetic replication, influenced the β-glucan content in two populations of hexaploid oat sharing a common parent. The degree to which these regions affected this trait varied with the environment, but a striking similarity between the two populations was observed for the effect. For example, a region on linkage group 11, near the *Xcdo665B* RFLP locus, caused an average of about a 2.2 g kg^{-1} change in the β-glucan content in both recombinant inbred populations explaining up to 20% of the phenotypic variance depending on the environment. However, whereas Kanota contributed the positive allele in the KO population, Marion contributed the positive allele in the KM population. In other words, the allelic values for this locus are a 0, 2.2, and 4.4 g kg^{-1} change in the β-glucan content for Ogle, Kanota and Marion, respectively. Thus, the effect of this locus can be more pronounced in a population generated from a cross between Ogle and Marion.

Consistency in the identification of important regions in a given population over environments and QTL analysis methods permits verification of regions influenced by the genetic background. Regions on linkage groups 3, 6 and 13 in the KO population and groups 11, 14, 5X and 10X in the KM population are good examples of such verification. The Kanota by Ogle cross involves two parents with similar β-glucan content values. In the KO population it is expected that both parents would contribute equally to this trait, as was observed. Therefore, a locus such as the one detected in the KO population on linkage group 6 may have a minor effect that was undetectable in the KM population which was generated from more diverse parents. On the other hand, since the QTL region on linkage group 3 had such a significant influence in the KO population, its effect most likely would be recognizable in the KM population (which it was not) unless Marion has the same positive allele as Kanota.

The Kanota by Marion population was developed from more diverse parents than the KO population with respect to their β-glucan content. It is expected that the parent with the higher β-glucan content would contribute more QTL regions with a positive influence on the trait, as was observed here. Therefore, genomic segments from Marion such as those on linkage groups 5X and 10X, which are consistently identified over environments and analysis methods, will be important in the β-glucan improvement of adapted cultivars.

Results from other QTL studies in the same population can help identify linked or pleiotropic effects of other traits on β-glucan content. It is intriguing that some of the major loci identified as associated with groat oil content in oat (Kianian et al. 1999) also are associated with variations in β-glucan content. Locus *XaccaseA* on linkage group 11 (tightly linked to *Xcdo665B*) has a dramatic influence on the groat oil content explaining almost 50% of the phenotypic variance in some cases. The same region is also important in affecting β-glucan content. It is possible that a change in one aspect of groat chemical characteristics has an influence on other such traits or else that there exists an intermediate controlling mechanism.

The changes in various groat chemical characteristics may not show a direct correlation, as is the case for β-glucan and groat oil content, where an increase in one trait may not necessarily cause a direct increase or decrease in the other trait. Selection for the Kanota allele at the *XaccaseA* locus on linkage group 11 is expected to cause a dramatic increase in the groat oil content and a significant increase in the β-glucan content of the grain in populations with Kanota as the donor parent for positive alleles (coupling-phase QTL). The opposite scenario would be true for the Ogle allele, in which the coupling association would give reductions in values of both traits. On the other hand, a selection for the Marion allele in a population with Ogle as the other parent would cause an increased β-glucan content and a reduced groat oil content. Curiously, this difference in parental allelic value (repulsion-phase QTL), where groat β-glucan and oil content were influenced by the same genomic region, is true of both cases in the KM population but in neither of the two situations in the KO population.

Results from other quantitative studies of various plant and grain morphological traits (grain shape, size and ratio of primary and secondary to tertiary kernels) in oat indicate that linkage groups 11 and 13 have some influence on test weight. This is further evidence of the role of these genomic segments in oat groat morphology and physiology (Cakir et al. 1996; Siripoonwiwat et al. 1996; Holland et al. 1997). In contrast, linkage group 17 is believed to have a strong influence on heading date, plant height and other associated agronomic characteristics. This region showed a coupling-phase association with respect to these physical-characteristic QTLs (Siripoonwiwat et al. 1996; Holland et al. 1997). That is to say, for linkage group 17 Kanota contributed the allele causing the later heading date and taller plants. In the case of pleiotropy, we might predict the same allele to contribute to both an increased oil and β-glucan content because of an increased nutrient partitioning in groat due to a longer grain-development time. However, on linkage group 17 the allele from Ogle increases the β-glucan content indicating linkage rather than pleiotropy. These studies on quantitative traits in oat are instrumental in identifying genomic regions which have an influence on multiple traits, and for defining a selection scheme(s) based on the relationship between parental alleles affecting each trait.

Homoeologous relationships of various chromosomes in hexaploid oat are not clear. Segmental homoeology instead of whole-chromosome homoeology appears to best describe the genome organization in oat (Kianian et al. 1997). Homoeology between regions on different chromosomes showing significant association with a given trait further substantiates that relationship. Regions

identified on linkage groups 11 and 14 are both flanked by marker sequences recognized by probes CDO665 and CDO1509, and thus represent possible homoeologous segments. Linkage group 5 maps to chromosome 5C of hexaploid oat (Kianian et al. 1997) which shares a homoeologous segment with group 6 located between marker sequences detected by the probes CDO1385 and CDO1357. Thus, two sets of homoeologous segments account for the association with a major portion of the variation in β-glucan content. Relationships between these regions and those of other grass species such as maize and rice have been described (Ahn and Tanksley 1993; Van Deynze et al. 1995; Kianian et al. 1999). The regions identified in maize and rice are also associated with genes known to have a major influence on grain or kernel chemical composition (Ahn and Tanksley 1993; Van Deynze et al. 1995). The regions identified to have a strong influence on β-glucan content in barley also correspond well to the regions retained in the multi-locus reported here. For instance, two of the major QTL loci in barley are on chromosomes 1 and 2 which correspond to the regions identified on linkage groups 3 and 11 in the present study (Han et al. 1995). This comparative evidence further re-inforces the association of these genomic regions with β-glucan and other components of oat grain, such as oil content.

Grain chemical composition and physical characteristics, as well as yield, are among the most important characteristics in crop plants. Changes in the groat chemical composition are desired to develop oat cultivars more adapted to the needs of consumers. Higher β-glucan and lower oil-content groats would suit the food market, while low β-glucan and high oil-content grain better meet the demands of the feed industry. The study and identification of genetic loci affecting these traits, the determination of their relative influence in varying environments and genetic backgrounds, and an analysis of their relationship with other important traits are needed for the modification of existing germplasm. For markers to be useful in a MAS breeding program, their value, predictability over environments and genotypes, and ease of use have to exceed the gains made due to selection through more conventional breeding methods. In this study, we used two recombinant inbred populations sharing a common parent, obtained a replicated measurement of groat β-glucan content over multiple environments and analyzed the data by various statistical procedures to identify QTL regions that consistently influence this trait. The relationship between identified QTL regions, due to linkage or pleiotropy and homoeologous segments help identify the associations. Groat β-glucan content in oat and its relationship with groat oil content present an interesting case study in the identification of markers useful for a MAS breeding program.

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