Theor Appl Genet (1999) 98: 884–894

S. F. Kianian · M. A. Egli · R. L. Phillips
H. W. Rines · D. A. Somers · B. G. Gengenbach
F. H. Webster · S. M. Livingston · S. Groh
L. S. O'Donoughue · M. E. Sorrells · D. M. Wesenberg
D. D. Stuthman · R. G. Fulcher

Association of a major groat oil content QTL and an acetyl-CoA carboxylase gene in oat

Received: 2 July 1998 / Accepted: 3 November 1998

Joint contribution of the Minnesota Agricultural Experiment Station and USDA-ARS. Scientific Journal Series paper no 98-1-13-0077 of the Minnesota Agricultural Experiment Station, St. Paul.

Communicated by B. S. Gill

S. F. Kianian[1] (✉) · M. A. Egli · S. M. Livingston · S. Groh · D. D. Stuthman
Department of Agronomy and Plant Genetics,
University of Minnesota, St. Paul, MN 55108, USA
Fax: + 1 701 231-8474
e-mail: kianian@badlands.nodak.edu

R. L. Phillips · D. A. Somers · B. G. Gengenbach
Department of Agronomy and Plant Genetics, Plant Molecular Genetics Institute, University of Minnesota, St. Paul, MN 55108, USA

H. W. Rines
Plant Science Research Unit, USDA-ARS, and Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108, USA

F. H. Webster
The Quaker Oats Co. John Stuart Research Laboratories, Barrington, IL 60010, USA

L. S. O'Donoughue
DNA LandMarks Inc., P.O. Box 6, St. Jean Sur Richelieu, Quebec, J3B 6Z1, Canada

M. E. Sorrells
Department of Plant Breeding, 252 Emerson Hall, Cornell University, Ithaca, NY 14853, USA

D. M. Wesenberg
USDA-ARS, National Small Grains Germplasm Research Facility, P.O. Box 307, Aberdeen, ID 83210, USA

R. G. Fulcher
Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN 55108, USA

Present address:
[1] Department of Plant Sciences, North Dakota State University, Fargo, ND 58105, USA

Abstract Oat groats are unique among cereals for the high level and the embryo-plus-endosperm localization of lipids. Genetic manipulation of groat quality traits such as oil is desired for optimizing the value of oat in human and livestock diets. A locus having a major effect on oil content in oat groats was located on linkage group 11 by single-factor analysis of variance, simple interval mapping and simplified composite interval mapping. 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. Similar QTL and ACCase locus placements were obtained with two recombinant inbred populations, 'Kanota' × 'Ogle' (KO) and 'Kanota' × 'Marion' (KM), containing 137 and 139 individual lines, respectively. By having a common parent these populations provide biological replication of the results in that significant genomic regions should be evident in analyses of multiple cross combinations. The KO population was mapped with 150 RFLP loci distributed over the genome and was grown in five diverse environments (locations and years) for measurement of groat oil content. The KM population was mapped with 60 RFLP loci and grown in three environments. The QTL linked to *AccaseA* on linkage group 11 accounted for up to 48% of the phenotypic variance for groat oil content. These results provide strong support for the hypothesis that ACCase has a major role in determining groat oil content. Other QTLs were identified in both populations which accounted for an additional 10–20% of the phenotypic variance.

Key words Oat · Oil content · Acetyl-CoA carboxylase · Molecular markers · Candidate gene

Introduction

Cultivated oat, *Avena sativa* L. ($2n = 6x = 42$), is grown mostly in temperate regions. In many parts of the world, oat grain is produced for multiple purposes such as for animal feed, human food and cosmetic and pharmaceutical additives (Webster 1986). Among the cereals, oat groats (hulless caryopses) have the highest lipid concentration (Morrison 1978), but due to their high fiber content whole oat grain is lower in energy value than many other cereals when used for livestock feed. Oat is unique among the cereals because more than 50% of total seed lipid is deposited in the starchy endosperm rather than the embryo of developing caryopses (Peterson and Wood 1997; Youngs 1986). An increase in oil content is desirable for making oat a higher-energy feed grain. On the other hand, a decrease in oil content would make oat a more attractive commodity for use in many modern human diets where oat is desired for the type and concentration of its fiber (Ripsin et al. 1992).

A polygenic pattern of inheritance with primarily additive gene action (Thro and Frey 1985) has been indicated for groat oil content with heritability estimates ranging from 63% to 93% (Schipper and Frey 1991a). Genotype-by-environment interaction for this trait is small (Gullord 1986; Branson and Frey 1989). After five cycles of a recurrent selection program, Schipper and Frey (1991b) produced oat lines with a groat oil content greater than 162 $g\,kg^{-1}$. This response to selection in oat is similar to that found in the classical Illinois long-term selection experiments for oil content in maize (*Zea mays* L.) which resulted in dramatic progress toward altering the oil concentration of the original variety (Dudley et al. 1974). These studies demonstrate that, except when physiological limits are encountered, long-term selection can prove successful for a quantitatively inherited, highly heritable and environmentally stable trait. Analysis of 5987 oat samples representing a wide range of *Avena* germplasm showed a range of 20 to 120 $g\,kg^{-1}$ for free non-polar lipids, with polar lipids contributing an additional 20 to 30 $g\,kg^{-1}$ (Youngs et al. 1982). Combinations of alleles for changing the groat oil content beyond this range may exist because this is mostly an unselected trait that exhibits large phenotypic diversity. Genetic dissection of this trait could help identify candidate genes and expedite a program aimed at modifying the oil level of oat.

The recent explosion in the development of restriction fragment length polymorphism (RFLP) linkage maps and their use in identifying genomic regions that affect quantitatively inherited traits is well-documented (Phillips and Vasil 1994). Molecular genetic studies of seed oil content have been reported for major oil-producing crop plants such as rape seed (*Brassica rapa* ssp. *oleifera*, Tanhuanpaa et al. 1995a,b), soybean (*Glycine max* L., Diers et al. 1992; Mansur et al. 1993; Brummer et al. 1997) and maize (Goldman et al. 1994; Alrefai et al. 1995). These studies indicate that genomic regions can be identified which significantly influence seed oil content. In the maize Illinois long-term selection material, two populations were analyzed to identify genomic regions influencing kernel oil content, the high-oil by low-oil population (Alrefai et al. 1995) and the high-protein by low protein population (Goldman et al. 1994). In both populations a region on chromosome 6 was identified near the *linoleic acid-1* locus. This region explained 63% of the phenotypic variation in the ratio of oleic to linoleic (18 : 1 to 18 : 2) fatty acids and also influenced total kernel oil content in the high- by low-oil population (Alrefai et al. 1995). As would be expected, the amount of phenotypic variance for total oil content explained by this region was less in the high-protein by low-protein cross; however, the influence of this chromosomal segment on oil content was still evident (Goldman et al. 1994). To date, oat plants with an altered fatty acid composition as a result of a single genetic mutation have not been identified, possibly because of the hexaploid nature of the genome. However, as demonstrated by the studies in maize, genomic regions which greatly influence fatty acid content/composition of the kernel can be identified which may represent alterations in a known genetic locus.

Acetyl-CoA carboxylase (ACCase, EC 6.4.1.2) catalyzes the ATP-dependent formation of malonyl-CoA from acetyl-CoA and bicarbonate, the first committed step in de novo fatty acid synthesis (Harwood 1988). In plants, fatty acids containing up to 18 carbons are synthesized in plastids. Biochemical, inhibitor and genetic transformation experiments suggest that ACCase plays a major regulatory role in fatty acid synthesis (reviewed by Ohlrogge and Jaworski 1997).

The ACCase of monocot plastids is a multifunctional polypeptide that functions as a homodimer (Ohlrogge and Jaworski 1997). Monocot ACCase also appears to regulate fatty acid biosynthesis. Light-stimulated lipid synthesis in maize leaves is modulated by changes in chloroplast pH, Mg and ATP, which are known to alter ACCase activity (Nikolau and Hawke 1984). High levels of ACCase transcripts are found near the basal meristem of greening wheat leaves (Gornicki et al. 1994) in which chloroplast membrane synthesis is maximal. Graminicides cause plant cell death by inhibiting plastidic ACCase and subsequent fatty acid biosynthesis (Burton et al. 1989). Based on the apparent flux control coefficient for ACCase in barley and maize leaves exposed to low levels of graminicides, ACCase could be shown to be the major flux-controlling enzyme for light–stimulated lipid synthesis (Page et al. 1994). Certain maize cell lines that grow in the presence of normally lethal concentrations of graminicides exhibit up to a 2.6-fold increase in ACCase activity relative to the wild type and can synthesize fatty acids in the presence of herbicide (Parker et al. 1990). Other results point to the role of ACCase in regulating seed

oil deposition. Maize embryos from herbicide-insensitive *Acc1* mutant plants exhibit increased herbicide-insensitive ACCase activity in parallel with the reported time course of oil deposition in this tissue (Somers et al. 1993).

We have identified a genomic region in oat with a strong influence on groat oil content and its possible candidate gene, ACCase. In this study, two recombinant inbred populations sharing a common parent were examined for their RFLP genotype and groat oil content to assess the biological reproducibility of the quantitative trait locus (QTL) results and attach a degree of confidence to regions identified. Oil content was evaluated over diverse environments, providing an opportunity to identify and analyze the interaction between oil QTLs, ACCase allelic variation and environments in two genetic backgrounds. Our main objectives were to identify genomic region(s) and candidate gene(s) that can be manipulated with a desirable and predictable outcome on groat oil content.

Materials and methods

Genetic material

Two oat $F_{2:6}$-derived recombinant inbred populations developed by single-seed descent were used in this study: a population of 137 recombinant inbred lines (RILs) from the cross of a facultative winter-type cultivar, 'Kanota', by a spring cultivar, 'Ogle', and a second population of 139 RILs from the cross of 'Kanota' by another spring cultivar, 'Marion'. A portion (71 RILs) of the first population was used in previously reported oat mapping and QTL studies (O'Donoughue et al. 1995; Siripoonwiwat et al. 1996; Holland et al. 1997). 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 1992–1995 in a randomized complete block design at the following locations:

'Kanota' × 'Ogle' (KO): Aberdeen, Idaho, 1992 and 1993; Ithaca, New York, 1993; and St. Paul and Rosemount, Minnesota, 1994.
'Kanota' × 'Marion' (KM): 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 of 2.5 m in length, 30 cm between rows, with the two center rows harvested. At Ithaca, New York, the materials were grown in three replicates of six-row plots of 4 m in length 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 30-cm grid spacing. The parents used to generate the populations (involved in the original cross) were included as single or multiple entries in each replicate.

Oil content analysis

To reduce the number of samples for analysis, we bulked equal amounts of sample from each replicate at a given location/year so as to represent a single balanced sampling per line. This resulted in five measurements for each RIL in the KO population and three for the KM population.

Groat oil content was measured by a Bran + Luebbe InfraAlyzer 500 on dehulled ground samples at The Quaker Oats Co., John Stuart research laboratories, Barrington, Illinois. The Near Infrared (NIR) calibration was based on total fat as determined by acid hydrolysis. The calibration set contained 348 oat samples (representing commercial and elite breeding lines from North American oat breeding programs) with fat contents ranging from 50 to 140 $g\,kg^{-1}$ dry weight. The calibration equation had a correlation coefficient of 0.985 with a standard error of 0.299, and a F-value of 11 500. Since acid hydrolysis extracts the total lipid content of the groat, the reported values represent the sum of neutral lipids (triglycerides) plus polar lipid fractions (Youngs et al. 1982). These values are higher than those obtained from ether extracts, which only contain the neutral lipid fraction.

Oat ACCase clone

An amplified 'Prairie' oat developing groat cDNA library (R. Skadsen, USDA-ARS, Madison, Wis.) cloned into the *Eco*RI site of λZAPII (Stratagene Corp) was screened with a [^{32}P]-dCTP-labelled probe (Ausubel et al. 1997) derived from an *Eco*RI digest of maize ACCase cDNA clone 18–5 (nucleotides 2020-5927; Egli et al. 1995). Plaques from 11 partially purified positives were eluted in suspension medium (Ausubel et al. 1997) and excised in vivo to obtain pBlueScript plasmids carrying oat cDNA inserts, according to the manufacturer's instructions (Stratagene Corp). Five colonies per transformation reaction were screened by colony lift (Ausubel et al. 1997) with an AflII-BbrPI fragment of maize ACCase cDNA (4281-5797; Egli et al. 1995). One clone, oat-91, hybridized to the maize ACCase probe and was sequenced at the Advanced Genetic Analysis Center, University of Minnesota, St. Paul, Minnesota. The oat-91 sequence was compared to other plant ACCases with the BESTFIT program (BESTFIT, Wisconsin Package V. 9.1, Genetics Computer Group, Madison, Wis.). The oat-91 clone has been entered into GenBank as accession no AF072737.

RFLP and statistical analysis

The methods of RFLP analysis, probe preparation and their description are as given in Kianian et al. (1997). Two statistical procedures were used to determine the location, effect and QTL-by-environment interaction (QTL × E) for significant QTLs. The first procedure was single-factor ANOVA (ANOVA and GLM procedures; SAS Institute 1990) with the added criteria for consistency in association of genomic regions with a trait over environments and linkage distances. In other words, the association was significant when the average of all the environments was considered; the association was also significant over the majority of the environments (3 of 5 in KO and 2 of 3 in KM), and closely linked markers within that region, when available, also showed significant associations. A "backward elimination" process (Kennard and Havey 1995) was used to construct a multi-locus model containing the most important markers identified by the ANOVA procedure. Marker loci were excluded one at a time from this model based on the criterion of least significant ($P < 0.05$) type-III sum of squares (Kennard et al. 1994). In addition, MQTL software (Tinker and Mather 1995a, b) was used to strengthen and corroborate the ANOVA results. This software performs Simple Interval mapping (SIM) and Simplified Composite Interval mapping (sCIM). It also has the advantages of searching for one QTL while simultaneously accounting for the effect of others, providing separate analyses for each environment, and calculating a threshold to control the type-I error rate. We performed 1000 permutations to estimate the threshold with a type-I error rate below 5%. Significant QTL regions were determined as described by Tinker et al. (1996). Tinker and Mather (1995a) found this procedure provided a good control of type-I error rate for SIM but not for

Table 1 Groat oil content[a] and percentage of transgressive segregants in two recombinant inbred oat populations

Population (P1 × P2)	Location	Parents		Population				Percentage transgressive segregants			
		P1	P2	Mean	SD	Minimum	Maximum	X < P2	X > P1	X < P2-SD	X > P1 + SD
Kanota × Ogle	Aberdeen 92	115	83	97	13	75	140	13	8	0	2
	Aberdeen 93	105	84	101	16	72	154	18	35	0	8
	Ithaca 93	109	71	90	14	65	136	8	8	0	2
	St. Paul 94	109	77	92	14	66	134	13	10	0	2
	Rosemount 94	111	84	90	13	67	126	30	5	5	1
	Average	110	80	94	14	69	138	16	13	1	3
Kanota × Marion	St. Paul 94	129	102	102	10	80	133	47	1	12	0
	Rosemount 94	–[b]	92	104	12	75	142	10	–	1	–
	St. Paul 95	100	84	88	7	71	108	26	2	4	1
	Average	115	93	98	10	75	128	28	2	6	1

[a] All groat oil values are in grams per kilogram
[b] Blank cells represent missing values

Table 2 Phenotypic correlations (r)[a] for groat oil content between environments

Population	Location				
Kanota × Ogle		Aberdeen 92	Aberdeen 93	Ithaca 93	St. Paul 94
	Aberdeen 93	0.94			
	Ithaca 93	0.94	0.97		
	St. Paul 94	0.90	0.91	0.92	
	Rosemount 94	0.91	0.95	0.96	0.94
Kanota × Marion		St. Paul 94	Rosemount 94		
	Rosemount 94	0.87			
	St. Paul 95	0.80	0.71		

[a] Values are all significant at $P < 0.0001$

sCIM. Significance of QTL regions identified by MQTL is reported as test statistics (TS = n 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 equals to the F statistic. For a single environment, TS can be converted to LOD computed by MAPMAKER QTL by multiplying 0.22 or by dividing 2 ln(10). These regions were grouped in a multi-locus linear model to estimate the overall phenotypic variance explained by the model. However, we used additional criteria of 10 cM spacing between QTLs to include markers in the final MQTL model.

Results

Trait data

Groat oil content for both the KO and KM populations showed a relatively continuous distribution with many of the individuals having values within one standard deviation of the parental values (Table 1). Among the three parents 'Kanota' had the highest groat oil content followed by 'Marion' and then 'Ogle'. The groat oil content value for the 'Kanota' line derived from the parent used in generating the KM population was higher than that of the 'Kanota' line used for the KO population (Table 1). This is not unexpected since 'Kanota' is a heterogeneous population showing variation for other traits. Thus, individual 'Kanota' parent lines used in population development are maintained as controls for each population. Of the individuals having values outside the parental range, more tended toward lower groat oil content rather than the higher (Table 1). The KO population contained individuals with the highest and lowest groat oil contents, possibly due to a larger difference in parental values (30 $g\,kg^{-1}$ oil between 'Kanota' and 'Ogle', 20 $g\,kg^{-1}$ between 'Kanota' and 'Marion'; Table 1).

The correlation among the different environments for oil content was high (Table 2). Measurements for the KO population had an average r of 0.93 with the lowest value of 0.90 between material grown at Aberdeen, Idaho (1992) and St. Paul, Minnesota (1994) ($P < 0.0001$). Values for the KM population were less correlated with an average r of 0.79 with the lowest correlation value of 0.71 between the samples grown in Rosemount, Minnesota (1994) and St. Paul, Minnesota 1995 ($P < 0.0001$).

Table 3 Genomic regions significantly influencing groat oil content in oat identified by single-factor analysis of variance

Kanota × Ogle					Kanota × Marion				
Linkage group	Locus[a]	Source	R^2	Oil[b] ($g\,kg^{-1}$)	Linkage group	Locus[a]	Source	R^2	Oil[b] ($g\,kg^{-1}$)
6	*Xcdo1357C*	Kanota	0.11	8	3	*Xbcd1562A*	Kanota	0.03	4
11	*Xcdo665B*	Kanota	0.43	20	11	*Xcdo665B*	Kanota	0.20	7
37[c]	*Xcdo1414B*	Kanota	0.07	13	22	*Xcdo419*	Kanota	0.06	3
Unlinked[c]	*Coleoptal*	Kanota	0.13	11	5X[d]	*Xcdo1199C*	Kanota	0.13	7
Total				28 (52[c])					21

[a] Locus with the highest R^2 within the linkage group
[b] Substitution of the source alleles will cause on average (based on averages of values from each of five environments in KO and three in KM) this change in groat oil content. $P < 0.0001$ for the means across all environments, except for *Xbcd1562A*, *Xcdo1357c*, *Xcdo1414B* and *Coleoptal* with $P < 0.01$ and *Xcdo419* with $P < 0.05$. $P < 0.05$ for each individual environment
[c] Based on only the first 71 individuals of this population
[d] More markers are needed to accurately place this linkage group on the hexaploid oat RFLP map

'Kanota' × 'Ogle' population

RFLP data

A hexaploid oat RFLP map containing 561 loci has been generated from 71 RILs in the KO population (O'Donoughue et al. 1995). Initial quantitative analysis for oil content was performed using this subset of the KO population, the available trait data, and 360 of the 561 loci that are mostly co-dominantly segregating and evenly distributed across the oat genome. To take advantage of the entire population and to add power to our analysis, we also mapped the remaining 66 RILs in this population. 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 2 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.

Essentially the same QTLs were detected with either the subset (360 markers and 71 individuals) or the entire data set (153 markers and 137 individuals) of the KO population, except in two situations. In both cases, QTLs were detected in the subset, but not in the entire population due to the absence of genetic data for the remaining 66 RILs. To have complete coverage of the genome for SIM and sCIM, we used 344 mapped loci (191 loci scored on only 71 RILs) in the MQTL analysis. Results for the entire population will be presented here except for the aforementioned differences.

Quantitative analysis

Analysis of variance, using the criteria mentioned, revealed 2 linkage groups significantly associated with groat oil content ($P < 0.05$ for an individual environment. These regions are on linkage groups 6 and 11 (Table 3). Two additional genomic segments, one on linkage group 37 and an unlinked locus, *Coleoptal*, were significant in tests of the first 71 individuals of the mapping population but could not be verified on the whole population due to the lack of data for the entire set. The positive alleles (those that cause an increase in groat oil content) for all of the genomic regions influencing this trait were from the 'Kanota' parent (Table 3). Overall, the alleles from 'Kanota' contributed 28 $g\,kg^{-1}$ (52 $g\,kg^{-1}$ if linkage group 37 and *Coleoptal* loci were included) to the groat oil content. Regions on linkage groups 6 and 11 were considered highly significant and remained in the full model after the backward elimination process (Table 4). However, the 2 additional loci from linkage group 37 and *Coleoptal* were also included in the model when only the first 71 individuals in this population were analyzed. Locus *Xcdo665B* on linkage group 11 accounted for 36–48% (depending on the environment) of the phenotypic variance for this trait ($P < 0.0001$). For this locus a substitution of the 'Kanota' allele for its 'Ogle' counterpart accounted for an average increase of 20 $g\,kg^{-1}$ in groat oil content. Substitution of the 'Kanota' allele for the 'Ogle' allele at the locus *Xcdo1414B* (or *Xumn51B*) on linkage group 37 accounted for an average increase of 13 $g\,kg^{-1}$ in groat oil content and was the second most significant locus when only the first 71 individuals in this population were considered.

Quantitative analysis using the MQTL software was more conservative than ANOVA in identifying QTL regions. Similar to ANOVA results, a highly significant locus, *Xcdo665B*, located on linkage group 11, was identified at a test statistic (TS) value of 290.2. This locus overshadowed any other region and accounted

Table 4 Multi-locus models for groat oil content in oat

Population	ANOVA model[a]	MQTL model[b]
Kanota × Ogle	*Xcdo 1357C* (6, K) *Xcdo665B* (11, K)[c] *Xcdo1414B* (37, K)[d] *Coleoptal* (Unlinked, K)[d]	*Xcdo665B* (11, K)[c] *Xcdo 1414B* (37, K) *Coleoptal* (Unlinked, K)
Total R^2	0.45 (0.67)[e]	0.48
Kanota × Marion	*Xcdo665B* (11, K)[c] *Xcdo419* (22, K) *Xcdo1199C* (5X, K)	*Xcdo665B* (11, K)[c] *Xcdo419* (22, K) *Xcdo1199C* (5X, K)
Total R^2	0.28	0.22

[a] The linkage group and parent contributing the positive allele are depicted in parentheses (i.e. linkage group 11 with 'Kanota' allele being positive (11, K)). The models are based on five environments in KO and three 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

[c] *XaccaseA* is closely linked to *Xcdo665B* and gives the same relative effects as *Xcdo665B*

[d] These additional loci fit the model when the analysis included only the first 71 individuals in this population

[e] Total R^2 calculation includes *Xcdo1414B* and *Coleoptal* loci

for more than 38% of the phenotypic variance [variance QTL main effect (VM)/variance phenotypic (VP) = 38%, variance QTL × environment interaction and VM (VI)/VP = 38% and variance genetic from background markers (VG)/VP = 7%)] and a change of 20 g kg^{-1} in groat oil content (Fig. 1 A). However, secondary loci below the threshold value (threshold TS = 58.2 after 1000 permutations) were inferred when either SIM or sCIM (provided) evidence of QTLs (Tinker et al. 1996). *Xumn51B* (or *Xcdo1414B*) on linkage group 37 and the *Coleoptal* locus, which is unlinked, had test statistic values of 49.9 and 44.8, respectively. These loci plus *Xcdo665B* accounted for 48% of the phenotypic variance and a change of 33 g kg^{-1} in groat oil content (VM/VP = 47%, VI/VP = 48% and VG/VP = 7%). The 'Kanota' parent contributed the positive allele for all 3 loci. Locus effects were remarkably consistent over the five environments; thus, no significant peaks were inferred from the QTL × E interaction scan (Fig. 1B).

'Kanota' × 'Marion' population

RFLP data

The results from an initial quantitative analysis of the KO population (first 71 RILs) for groat physical and chemical traits were used to determine markers for mapping the KM population. The markers mapped were not chosen for their association with groat oil content in the KO subset population but for their association with other groat chemical and physical characteristics. Most probes detected sequences mapping to more than one location and were widely distributed throughout the genome. Sixty loci were mapped with the 139 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, 24–26, 30, 33–38 were not covered). The linkage groups not covered were, in general, small groups consisting of only 2 to a few markers in the KO map. The data from the KM population generated an additional linkage group of 2 loci, and 13 markers remained unlinked. Except for linkage group 37 and the *Coleoptal* locus, the KM map covered all the regions that were significantly associated with groat oil content in the KO population.

Quantitative analysis

Single factor ANOVA revealed 4 linkage groups that were significantly associated with groat oil content in the KM population (Table 3). These were groups 3, 11, 22 and 5X ($P < 0.05$ for individual environment). As in KO, all of the alleles favoring higher oil content were from the 'Kanota' parent. Overall, the 'Kanota' genomic regions contributed 21 g kg^{-1} to the groat oil content. Regions on linkage groups 11, 22 and 5X (group 5X has been tentatively assigned to linkage group 5 but more markers are needed to accurately place it on the hexaploid oat RFLP map) are considered highly significant and remained in the full model after the backward elimination process (Table 4). As in the KO population, locus *Xcdo665B* on linkage group 11 had a highly significant effect on groat oil content ($P < 0.0001$) and accounted for 16–20% (depending on the environment) of the phenotypic variance. The effect of substituting a 'Kanota' allele for the 'Marion' counterpart at this locus accounted for an average increase of only 7 g kg^{-1} in groat oil content in this population. Overall, the 3 significant loci explained 28% of the phenotypic variance and together

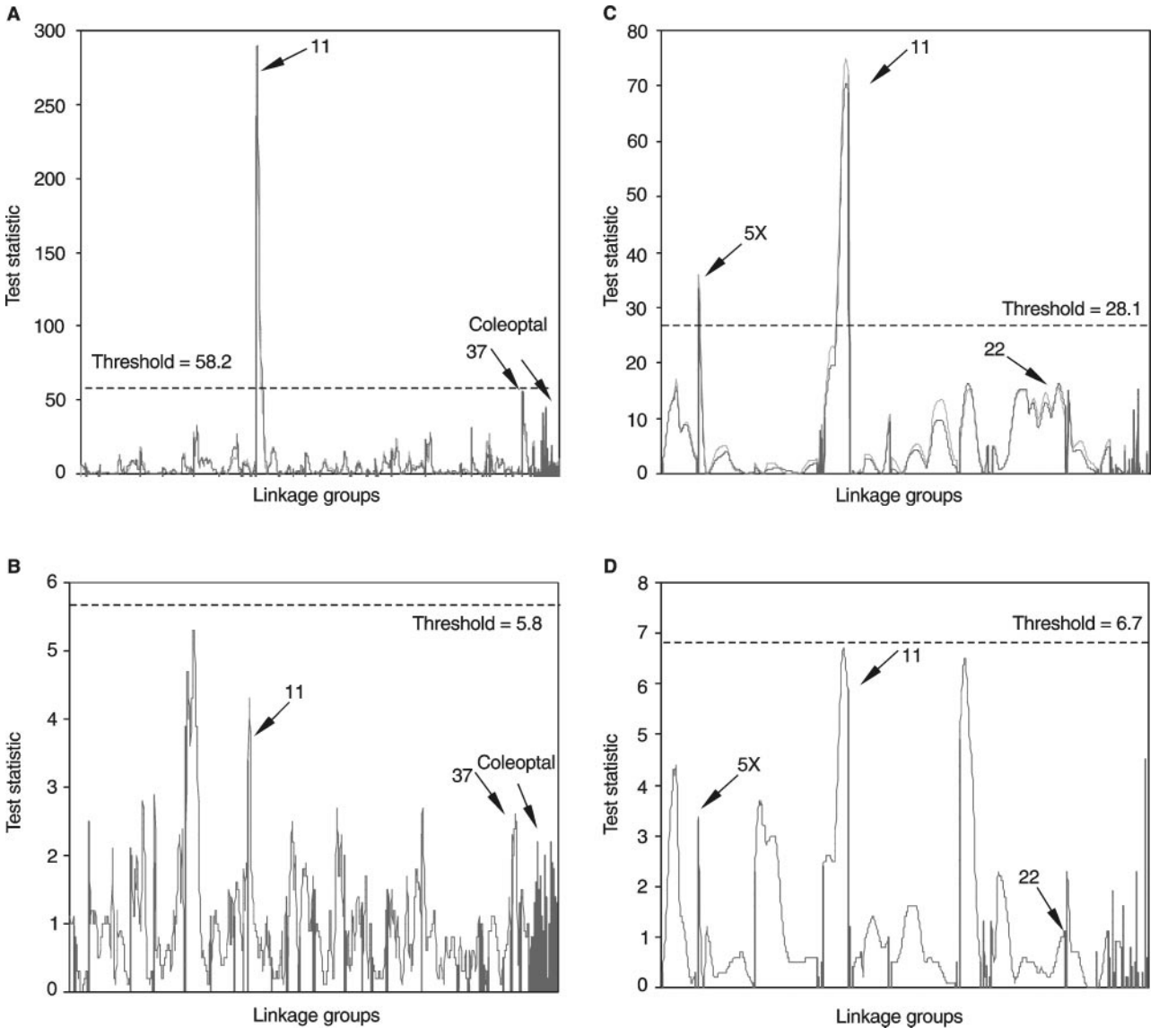

Fig. 1A–D Scan of test statistic generated by MQTL for the two recombinant inbred populations. **A** and **B** are the main effect and QTLxE interaction graphs for the 'Kanota' × 'Ogle' (KO) population, respectively. **C** and **D** are the main effect and QTL × E interaction graphs for the 'Kanota' × 'Marion' (KM) population, respectively. Thresholds for a type-I error rate of 5% after 1000 replications are reported on the graphs. —— Simple interval mapping, - - - - - simplified composite interval mapping, ↙ indicates the peak for linkage groups in the MQTL model (**Table 4**)

accounted for a total difference of 17 g kg^{-1} in groat oil content.

Analysis by SIM or sCIM gave analogous results to ANOVA (Table 4). Linkage groups 11, 22 and 5X were significant in the KM population. Locus *Xcdo665B* on linkage group 11 was significant at a test statistic value of 70.5. This locus was not as influential in the KM population as it was in the KO population (Fig. 1), however, it explained 18% of the phenotypic variance (VM/VP = 18%, VI/VP = 19% and VG/VP = 3%) and accounted for a 7 g kg^{-1} change in groat oil content. The other significant locus above the threshold value was *Xcdo1199C* on linkage group 5X (TS = 33.1), accounting for a change of 7 g kg^{-1} in groat oil content. A secondary locus inferred was *Xcdo419* on linkage group 22, which accounted for a 3 g kg^{-1} change in oil content. These 3 loci together accounted for 22% of the phenotypic variance and 15 g kg^{-1} in groat oil content (VM/VP = 20%, VI/VP = 22% and VG/VP = 3%). As expected from the trait data, these loci showed a varied effect over the three environments and only one interaction, on linkage group 11, is notable (Fig. 1D). The QTLxE interaction peak on linkage group 11 was at TS = 6.6, slightly below the significant threshold (TS = 6.7), and was due to a range of 5 g kg^{-1} in groat oil content as previously explained [the values for the groat oil content were 6 g kg^{-1} (St. Paul, Minn., 1995) and 11 g kg^{-1} (Rosemount, Minn., 1994)].

ACCase and its relationship to groat oil content QTLs

An oat groat cDNA clone was isolated based on its hybridization to a maize cDNA fragment which included portions of the less conserved mid-peptide sequence and the transcarboxylase domain of ACCase (Shorrosh et al. 1994). Sequence identity indicated that the oat cDNA (3031 bp) corresponded to maize ACCase nucleotides 3124–6169 (Egli et al. 1995). The 1010 amino acid-predicted oat polypeptide was nearly identical to portions of wheat (89%) and maize (84%) plastidic multifunctional ACCases (Egli et al. 1995; Gornicki et al. 1997) but had a lower identity to cytosolic ACCase, including wheat (67.5%) and alfalfa (64.6%) (Gornicki et al. 1994; Shorrosh et al. 1994). Therefore, the oat ACCase cDNA is likely to encode a plastid-targeted enzyme.

The 3031-bp oat ACCase cDNA clone hybridized strongly to three fragments (in *Dra*I and *Hin*dIII digests, 2 with *Eco*RV and 1 with *Eco*RI) and weakly to two fragments (in *Dra*I, *Hin*dIII and *Eco*RI digests and 4 with *Eco*RV) on DNA blots. Sequence information indicated that these fragments represent three copies of this gene (1 for each genome in hexaploid oat) and possibly two pseudogenes or genes for cytosolic isoforms (the weak signals). A single polymorphic locus in the KO population (*AccaseA*) and 2 polymorphic loci in the KM population (*AccaseA* and *AccaseB*) were mapped using the enzyme *Dra*I and scoring those fragments with a strong signal. The *AccaseA* locus maps 1.3 cM from *Xcdo665B* and 4.7 cM from *Xisu2287* on linkage group 11. The *AccaseB* locus maps 11 cM from *Xcdo665D* and 7.3 cM from *Xbcd1729B* on an unassigned linkage group in KM. In the KO population, *AccaseA* explains on average 37%, and in KM 18%, of the phenotypic variance for groat oil content (for both cases $P < 0.0001$ for means across all environments). The slight difference in the KO population between the amount of phenotypic variance explained by *AccaseA* and *Xcdo665B* is due to missing genotype data for the latter locus. Individuals in both populations can be divided into two groups based on their genotype for this locus and their mean groat oil content across all environments (Fig. 2). These two classes differ in their oil content by an average of 16.7 g kg^{-1} in KO and 7.8 g kg^{-1} in KM. The result of QTL analysis for the *AccaseA* locus is the same as that for *Xcdo665B* (Table 3). The *AccaseB* locus had no detectable effect on groat oil content in the KM population.

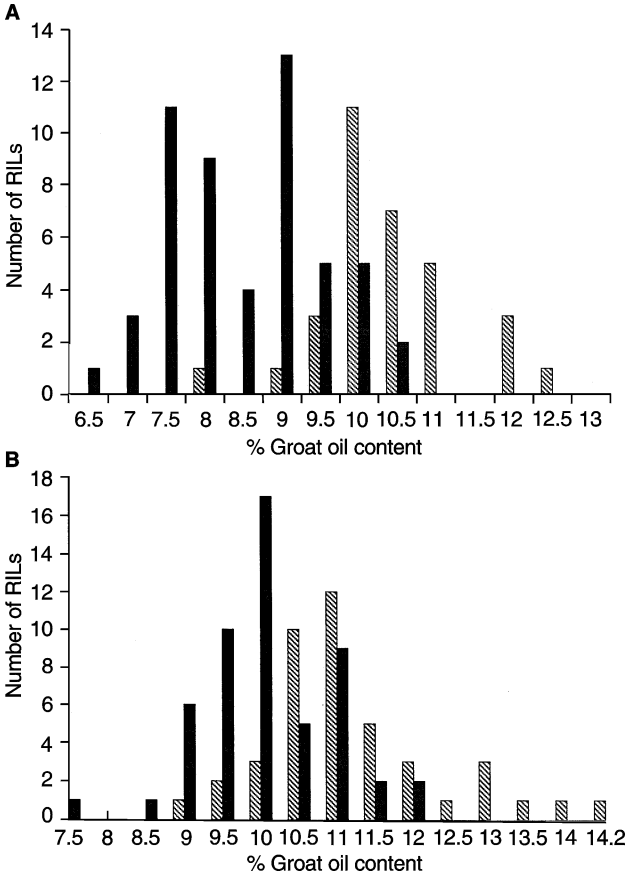

Fig. 2A, B Histogram of % groat oil content for the mean of all environments relative to the number of recombinant inbred lines (*RILs*) with each parental genotype for the *XaccaseA* locus. **A** The distribution for the 'Kanota' × 'Ogle' population, **B** distribution for the 'Kanota' × 'Marion' population. ■ RILs with the 'Kanota' genotype for the *XaccaseA* locus, ■ RILs with the 'Ogle' (**A**) 'Marion' (**B**) genotype for the *XaccaseA* locus

Discussion

The ACCase cDNA clone from developing oat seeds is linked to a QTL on linkage group 11 having a major influence on the total lipid content of oat groats. The influence of this locus was detected in various environments and in two genetic backgrounds, confirming the value of biological as well as environmental replication. These results support the hypothesis that ACCase has a major role in determining oil content in crop plants. Either allelic variation in ACCase activity controls oil content in oat groats, or a gene(s) closely linked to ACCase is responsible. In other studies of oat directed at transferring a major crown rust resistance locus on linkage group 11 (Bush et al. 1994; Wilson and McMullen 1997; L. S. O'Donoughue, personal communications), lines with the introduced rust resistance gene often had altered groat oil content (M. S. McMullen, personal communication). These results further support the likely presence on linkage group 11 of a genetic locus with a critical role in the fatty acid biosynthetic pathway in oat. Mutations that cause a gradual but demonstrable major effect on a biochemical pathway are believed to be the most common type in polyploids (Sears 1972).

Identification of a major QTL influencing the biochemical composition of grain is not unusual. Studies of fatty acid, starch, and protein concentrations and composition provide good examples (Goldman et al. 1993, 1994; Alrefai et al. 1995; Hu et al. 1995). In many of these cases the significant region is in close proximity to a known genetic locus with a demonstrated effect on the trait of interest. For instance, in maize, a major genomic region for fatty acid concentration and composition has been identified near the *linoleic acid-1* locus, and one influencing protein and starch concentration was identified near the *Shrunken-2* locus (Goldman et al. 1993, 1994; Alrefai et al. 1995). These and numerous other reports bolster the hypothesis that allelic variants at known genetic loci may be responsible for quantitative effects (Robertson 1985).

Reliable identification of genomic regions with an influence on a particular quantitative trait has always been difficult. In a number of studies conducted on a given quantitatively inherited trait in various populations and environments, the most significant regions tended to be in common (Goldman et al. 1994; Alrefai et al. 1995). However, the accuracy of identifying a quantitative trait locus will depend, in part, on the degree to which it influences the trait in a given population. This is demonstrated in our study by the locus on linkage group 11, which has similar but varied effects in different populations. 'Marion' has a higher groat oil content than 'Ogle' and may have more loci that positively influence this trait. The locus on linkage group 11 increased the groat oil content to a lesser degree in the KM population than in the KO population. Correspondingly, the relative proportion of variance due to the environmental influence on this locus was much higher in the KM population than in the KO population. Our results for KO versus KM illustrate the power of biological replication (i.e. having populations with a common parent) in identifying QTLs.

Identification of the most significant locus does not preclude the description of other less important loci. The consistency in the identification of significant regions by various quantitative analysis methods in either the KO or KM population is a good example. In the KM population, regions on linkage groups 3, 11, 22 and 5X are considered to make important contributions to groat oil. The *Xcdo1199C* locus in the KM population has nearly the same effect as the *Xcdo665B-AccaseA* region on linkage group 11. This QTL was not detected in the KO population, presumably because either the 'Ogle' and 'Kanota' alleles had a similar influence on groat oil content or the hexaploid oat RFLP linkage map of KO does not cover this region. The other two significant loci in the KM population have relatively minor effects on groat oil and would be more difficult to detect in a population segregating for regions with a larger influence, such as in the KO population. However, the results presented here clearly demonstrate the difficulty in identifying QTLs with minor effects consistently across populations.

Results from other QTL studies can help identify possible pleiotropic effects of traits such as heading date or vernalization response on groat oil content. A region on linkage group 3 is described as having a major influence on heading date and, possibly, yield; thus it is unlikely to have a direct effect on groat oil content (Siripoonwiwat et al. 1996; Holland et al. 1997). 'Ogle' was found to have alleles on linkage groups 22 and 37 that increase plant height in the KO population (Siripoonwiwat et al. 1996). However, the relationship between groat oil content and plant height is not obvious, especially since the positive allele for increasing the oil came from 'Kanota'. Loci on linkage group 11 are believed to have an influence on test weight and other groat physical characteristics, thus providing additional evidence of the role this genomic segment plays in oat groat morphology and physiology (Siripoonwiwat et al. 1996; Cakir et al. 1996). These and other studies of quantitative traits in oat are helping identify genomic regions that influence the growth, development and physiology of grain, the primary product. Thus, genetic and molecular manipulation of oat to improve grain quality and quantity can be directed at critical segments of the genome.

Cultivated oat is a hexaploid composed of three ancestral genomes designated A, C and D (Rajhathy and Thomas 1974). Recent studies have indicated that assigning the organization of hexaploid oat genomes into discrete homoeologous groupings, such as that of hexaploid wheat, is difficult (Rooney et al. 1994; O'Donoughue et al. 1995; Kianian et al. 1997). Oat C-genome chromosomes, due to their distinctiveness, have been used as a basis to identify homoeologous segments among the other chromosomes (Kianian et al. 1997). A type of segmental homoeology instead of whole chromosome homoeology appears to best describe the genome organization in hexaploid oat (Kianian et al. 1997). The association of homoeologous genomic segments with a quantitative trait further substantiates that relationship. The region marked by the probe CDO1414 can identify both the significant region on linkage group 11 and the one on linkage group 37, indicating possible homoeology of these regions.

In hexaploid wheat, plastidic ACCase maps to the short arm of homoeologous group 2 near the telomere (Gornicki et al. 1997). Plastidic ACCase genes in wheat are transcriptionally active in seedling leaves (Gornicki et al. 1997), but their effects on seed lipid content is unknown. Plastidic ACCase in maize has been mapped to the short arm of chromosome 2 near the centromere and to the long arm of chromosome 10 (Maize DB 1998; Van Dee 1994). A QTL with a moderate effect on oil content in progeny of crosses between Illinois high- and low-oil maize lines was located near the ACCase locus on maize chromosome 2 (Berke and Rocheford

1995). There also are numerous *defective kernel* (*dek*), *reduced endosperm* (*ren*) and *Endosperm factor* (*Ef*) loci, which influence grain morphology and physiology, located on chromosomes 2 and 10 of maize near these syntenic segments (MaizeDB 1998). In maize, oil is stored in embryos, and embryo size is associated with the oil content of whole seeds (Dudley 1974). Unlike maize, oat grains contain a significant amount of lipid in the endosperm (Peterson and Wood 1997), thus QTLs affecting pathway flux could be more important than those altering seed anatomy. The regions in wheat and maize agree well in terms of conserved synteny and correspond to chromosome 4 of rice, linkage group C of diploid oat and linkage group 11 of the hexaploid oat (Ahn and Tanksley 1993; Van Deynze et al. 1995). This comparative evidence from other grass species further support the association of these genomic regions with groat oil content and composition in oat.

The goal of any study on the genetics and inheritance of a quantitatively inherited trait is to identify the loci important in controlling the trait. Studies directed at grain morphology and physiology are vital in agronomically important crop plants since the objective is to improve the quality and quantity of the final product with concomitantly little effect on the overall plant morphology. If we are to develop oat cultivars more adapted to the food and feed markets, a change in groat oil content is desired. Co-segregation of the oat ACCase locus with the major groat oil QTL not only provides a useful tool for manipulating this trait but also the opportunity to analyze the underlying biochemical processes.

Acknowledgments We thank Drs. P. Rayapati, B.-C. Wu, G. Penner, D. Hoffman and R. Hautea for their assistance in development of the initial oat RFLP map. We thank Drs. O. Riera-Lizarazu, W. Kennard, J. Orf, D. De Koeyer and R. de la Pena for valuable discussion and suggestions. We also are grateful for the technical support of M. Neiss, P. Avoles, D. Aldecorn, R. Halstead, L. Gulbranson, R. Caspers and J. Suresh and computer support provided by D. Flanders. This study was supported in part by The Quaker Oats Company and by a USDA competitive research grant No. 9600765 to B.G.G. and M.A.E.

References

Ahn S, Tanksley SD (1993) Comparative linkage maps of the rice and maize genomes. Proc Natl Acad Sci USA 90: 7980–7984

Alrefai R, Berke TG, Rocheford TR (1995) Quantitative trait locus analysis of fatty acid concentrations in maize. Genome 38: 894–901

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (1997) Current protocols in molecular biology. John Wiley and Sons, New York

Berke TG, Rocheford TR (1995) Quantitative trait loci for flowering, plant and ear height, and kernel traits in maize. Crop Sci 35: 1542–1549

Branson V, Frey KJ (1989) Recurrent selection for groat oil content in oat. Crop Sci 29: 1382–1387

Brummer EC, Graef GL, Orf J, Wilcox JR, Shoemaker RC (1997) Mapping QTL for seed protein and oil content in eight soybean populations. Crop Sci 37: 370–378

Burton JD, Gronwald JW, Somers DA, Gengenbach BG, Wyse DL (1989) Inhibition of corn acetyl-CoA carboxylase by cyclohexanedione and aryloxyphenoxypropionate herbicides. Pest Biochem Physiol 34: 76–85

Bush AL, Wise RP, Rayapati PJ, Lee M (1994) Restriction fragment length polymorphisms linked to genes for resistance to crown rust (*Puccinia coronata*) in near-isogenic lines of hexaploid oat (*Avena sativa*). Genome 37: 823–831

Cakir M, Reeves DL, Kahler AL (1996) Linkage analysis of RFLP markers and quantitative traits in hexaploid oats. In: Agronomy abstracts. American Society of Agronomy, Madison Wis., p 163

Diers BW, Keim P, Fehr WR, Shoemaker RC (1992) RFLP analysis of soybean seed protein and oil content. Theor Appl Genet 83: 608–612

Dudley JW, Lambert RJ, Alexander DE (1974) Seventy generations of selection for oil and protein concentration in the maize kernel. In: Dudley JW (ed) Seventy generations of selection for oil and protein in maize. Crop Sci Soc Am, Madison, Wis., pp 181–212

Egli MA, Lutz SM, Somers DA, Gengenbach BG (1995) A maize acetyl-coenzyme A carboxylase cDNA sequence. Plant Physiol 108: 1299–1300

Goldman IL, Rocheford TR, Dudley JW (1993) Quantitative trait loci influencing protein and starch concentration in the Illinois Long Term Selection maize strains. Theor Appl Genet 87: 217–224

Goldman IL, Rocheford TR, Dudley JW (1994) Molecular markers associated with maize kernel oil concentration in an Illinois high protein X Illinois low protein cross. Crop Sci 34: 908–915

Gornicki P, Podkowinski J, Scappino LA, DiMaio J, Ward E, Haselkorn R (1994) Wheat acetyl-coenzyme A carboxylase: cDNA and protein structure. Proc Natl Acad Sci USA 91: 6860–6864

Gornicki P, Faris J, King I, Podkowinski J, Gill B, Haselkorn R (1997) Plastid-localized acetyl-CoA carboxylase of bread wheat is encoded by a single gene on each of the three ancestral chromosome sets. Proc Natl Acad Sci USA 94: 14179–14184

Gullord M (1986) Oil and protein content in oats (*Avena sativa* L.). In: Lawes DA, Thomas H (eds) Proc 2nd Int Oats Conf. Martinus Nijhoff Publ, Dordrecht, The Netherlands, pp 210–213

Harwood JL (1988) Fatty acid metabolism. Annu Rev Plant Physiol Plant Mol Biol 39: 101–138

Holland JB, Moser HS, O'Donoughue LS, Lee M (1997) QTLs and epistasis associated with vernalization responses in oat. Crop Sci 37: 1306–1316

Hu J, Quiros CF, Arus P, Struss D, Robbelen G (1995) Mapping of a gene determining linolenic acid concentration in rapeseed with DNA-based markers. Theor Appl Genet 90: 258–262

Kennard WC, Havey MJ (1995) Quantitative trait analysis of fruit quality in cucumber: QTL detection, confirmation, and comparison with mating-design variation. Theor Appl Genet 91: 53–61

Kennard WC, Slocum MK, Figdore SS, Osborn TC (1994) Genetic analysis of morphological variation in *Brassica oleracea* using molecular markers. Theor Appl Genet 87: 721–732

Kianian SF, Wu B-C, Fox SL, Rines HW, Phillips RL (1997) Aneuploid marker assignment in hexaploid oat with the C genome as a reference for determining remnant homoeology. Genome 40: 386–396

Maize DB http://probe.nalusda.gov:8300/cgi-bin/browse/maizedb

Mansur LM, Lark KG, Kross H, Oliveira A (1993) Interval mapping of quantitative trait loci for reproductive, morphological, and seed traits of soybean (*Glycine max* L.). Theor Appl Genet 86: 907–913

Morrison WR (1978) Cereal lipids. In: Pomeranz Y (ed) Advances in cereal science and technology, Vol. 2. Am Assoc Cereal Chem, St. Paul, Minn., pp 221–348

Nikolau BJ, Hawke JC (1984) Purification and characterization of maize leaf acetyl-CoA carboxylase. Arch Biochem Biophys 228: 86–96

O'Donoughue LS, Kianian SF, Rayapati PJ, Penner GA, Sorrells ME, Tanksley SD, Phillips RL, Rines HW, Lee M, Fedak G, Molnar SJ, Hoffman D, Salas CA, Wu B, Autrique E, Van Deynze A (1995) A molecular linkage map of cultivated oat. Genome 38 : 368–380

Ohlrogge HB, Jaworski JB (1997) Regulation of fatty acid synthesis. Annu Rev Plant Physiol Plant Mol Biol 48 : 109–136

Page RA, Okada S, Harwood JL (1994) Acetyl-CoA carboxylase exerts strong flux control over lipid synthesis in plants. Biochem Biophys Acta 1210 : 369–372

Parker WB, Somers DA, Wyse DL, Keith RA, Burton JD, Gronwald JW, Gengenbach BG (1990) Selection and characterization of sethxoydim-tolerant maize tissue cultures. Plant Physiol 92 : 1220–1225

Peterson DM, Wood DF (1997) Composition and structure of high oil oat. J Cereal Sci 26 : 121–128

Phillips RL, Vasil IK (1994) DNA-based markers in plants. Kluwer Academic Publ, Dordrecht, The Netherlands

Rajhathy T, Thomas H (1974) Cytogenetics of oats (*Avena* L.). In: Wilkes A (ed) Genetics Society of Canada miscellaneous publication no. 2. Genetics Society of Canada, Ottawa, On., pp 1–90

Ripsin CM, Keenan JM, Jacobs DR, Elmer PJ, Welch RR, Van Horn L, Liu K, Turnbull WH, Thye FW, Kestin M, Hegsted M, Davidson DM, Davidson MH, Dugan LD, Demark-Wahnefried W, Beling S (1992) Oat products and lipid lowering. J Am Med Assoc 267 : 3317–3325

Robertson DS (1985) A possible technique for isolating genic DNA for quantitative traits in plants. J Theor Biol 117 : 1–10

Rooney WL, Jellen EN, Phillips RL, Rines HW, Kianian SF (1994) Identification of homoeologous chromosomes in hexaploid oat (*A. byzantina* cv 'Kanota') using monosomics and RFLP analysis. Theor Appl Genet 89 : 329–335

SAS Institute (1990) SAS procedures guide, version 6, 3rd edn. SAS Institute Inc, Cary, N.C.

Schipper H, Frey KJ (1991a) Selection for groat-oil content in oats grown in field and greenhouse. Crop Sci 31 : 661–665

Schipper H, Frey KJ (1991b) Observed gains for three recurrent selection regimes for increased groat-oil content. Crop Sci 31 : 1505–1510

Sears ER (1972) The nature of mutation in hexaploid wheat. Symp Biol Hung 12 : 73–82

Shorrosh BS, Dixon RA, Ohlorogge JB (1994) Molecular cloning, characterization, and elicitation of acetyl-CoA carboxylase from alfalfa. Proc Natl Acad Sci USA 91 : 4323–4327

Siripoonwiwat W, O'Donoughue LS, Wesenberg D, Hoffman DL, Barbosa-Neto JF, Sorrells ME (1996) Chromosomal regions associated with quantitative traits in oat. JQTL (http://probe.nalusda.gov:8000/otherdocs/jqtl/jqtl1996–03)

Somers DA, Keith RA, Egli MA, Marshall LC, Gengenbach BG, Gronwald JW, Wyse DL (1993) Expression of the *Acc*1 gene-encoded acetyl-coenzyme-A carboxylase in developing maize (*Zea mays* L.) kernels. Plant Physiol 101 : 1097–1101

Tanhuanpaa PK, Vikki JP, Vikki HJ (1995a) Identification of a RAPD marker for palmitic-acid concentration in the seed of spring turnip rape (*Brassica rapa* ssp. *oleifera*). Theor Appl Genet 91 : 477–480

Tanhuanpaa PK, Vikki JP, Vikki HJ (1995b) Association of a RAPD marker with linolenic acid concentration in the seed oil of rapeseed (*Brassica napus* L.). Genome 38 : 414–416

Thro AM, Frey KJ (1985) Inheritance of groat-oil content and high-oil selection in oats (*Avena sativa* L.). Euphytica 34 : 251–263

Tinker NA, Mather DE (1995a) Methods for QTL analysis with progeny replicated in multiple environments. JQTL (http://probe.nalusda.gov:8000/otherdocs/jqtl/jqtl1995–01)

Tinker NA, Mather DE (1995b) MQTL: software for simplified composite interval mapping of QTL in multiple environments. JQTL (http://probe.nalusda.gov:8000/otherdocs/jqtl/jqtl1995–02)

Tinker NA, Mather DE, Rossnagel BG, Kasha KJ, Kleinhofs A, Hayes PM, Falk DE, Ferguson T, Shugar LP, Legge WG, Irvine RB, Choo TM, Briggs KG, Ullrich SE, Franckowiak JD, Blake TK, Graf RJ, Dofing SM, Saghai Maroof MA, Scoles GJ, Hoffman D, Dahleen LS, Kilian A, Chen F, Biyashev RM, Kudrna DA, Steffenson BJ (1996) Regions of the genome that affect agronomic performance in two-row barley. Crop Sci 36 : 1053–1062

Van Dee KL (1994) Evidence for two independently segregating loci encoding acetyl-CoA carboxylase in *Zea mays*. MSc thesis, University of Minnesota, St. Paul, Minn.

Van Deynze AE, Nelson JC, O'Donoughue LS, Ahn SN, Siripoonwiwat W, Harrington SE, Yglesias ES, Braga DP, McCouch SR, Sorrells ME (1995) Comparative mapping in grasses. Oat relationships. Mol Gen Genet 249 : 349–356

Webster FH (1986) Oat utilization: past, present and future. In: Webster FH (ed) Oat chemistry and technology. Assoc Cereal Chem, St. Paul, Minn., pp 413–427

Wilson WF, McMullen MS (1997) Recombination between a crown rust resistance locus and an interchange breakpoint in hexaploid oat. Crop Sci 36 : 1694–1698

Youngs VL (1986) Oat lipids and lipid-related enzymes. In: Webster FH (ed) Oat chemistry and technology. Assoc Cereal Chem, St. Paul, Minn., pp 205–226

Youngs VL, Peterson DM, Brown CM (1982) Oats. In: Pomeranz Y (ed) Advances in cereal science and technology, vol 5. Am Assoc Cereal Chem, St. Paul, Minn., pp 49–105