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A molecular linkage map with associated QTLs from a hulless × covered spring oat population

Abstract In spring-type oat (*Avena sativa* L.), quantitative trait loci (QTLs) detected in adapted populations may have the greatest potential for improving germplasm via marker-assisted selection. An F_6 recombinant inbred (RI) population was developed from a cross between two Canadian spring oat varieties: 'Terra', a hulless line, and 'Marion', an elite covered-seeded line. A molecular linkage map was generated using 430 AFLP, RFLP, RAPD, SCAR, and phenotypic markers scored on 101 RI lines. This map was refined by selecting a robust set of 124 framework markers that mapped to 35 linkage groups and contained 35 unlinked loci. One hundred one lines grown in up to 13 field environments in Canada and the United States between 1992 and 1997 were evaluated for 16 agronomic, kernel, and chemical composition traits. QTLs were localized using three detection methods with an experiment-wide error rate of approximately 0.05 for each trait. In total, 34 main-effect QTLs affecting the following traits were identified: heading date, plant height, lodging, visual score, grain yield, kernel weight, milling yield, test weight, thin and plump kernels, groat β-glucan concentration, oil concentration, and protein. Several of these correspond to QTLs in homologous or homoeologous regions reported in other oat QTL studies. Twenty-four QTL-by-environment interactions and three epistatic interactions were also detected. The locus controlling the covered/hulless character (*N1*) affected most of the traits measured in this study. Additive QTL models with *N1* as a covariate were superior to models based on separate covered and hulless sub-populations. This approach is recommended for other populations segregating for major genes. Marker-trait associations identified in this study have considerable potential for use in marker-assisted selection strategies to improve traits within spring oat breeding programs.

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Introduction

Breeding superior oat (*Avena sativa* L.) varieties involves selection for multiple qualitatively and quantitatively inherited disease, agronomic, and quality traits. Knowledge of genomic regions affecting these traits can be used to breed superior varieties through the use of marker-assisted selection. Oat varieties developed in Canada and the northern United States are typically of spring habit (McMullen and Patterson 1992). However, most quantitative trait loci (QTLs) in oat have been identified using recombinant inbred (RI) populations developed from crosses between the facultative winter-type varieties 'Kanota' or 'TAM O-301' and the spring-type varieties 'Ogle' or 'Marion'. QTLs affecting four agronomic and two kernel quality traits (Siripoonwiwat et al. 1996) and vernalization response to heading date and plant height (Holland et al. 1997) were detected in the 'Kanota' ×

'Ogle' (KO) population. Vernalization and photoperiod response QTLs were detected in the 'Ogle' × 'TAM O-301' (OT) population (Holland et al. 2002). QTLs affecting chemical composition and kernel characteristics were found in both the KO and 'Kanota' × 'Marion' (KM) RI populations (Kianian et al. 1999, 2000; Groh et al. 2001). It is uncertain whether QTL linkages identified in populations segregating for vernalization requirements can be transferred to more adapted oat germplasm. For example, only 5 of 40 restriction fragment length polymorphism (RFLP) allele-trait associations were found to be similar between a set of 64 North American spring and winter-type oat accessions and the KO population (Beer et al. 1997). Molecular marker associations have been reported for QTLs affecting barley yellow dwarf virus tolerance (Jin et al. 1998) and hull percentage (Ronald et al. 1997) in spring oat populations. Knowledge of other QTLs affecting important agronomic and quality traits in spring oat germplasm may ultimately be useful for developing genetic tools for marker-assisted selection.

Genetic diversity remains an important factor in selecting the parents of a mapping population but can be problematic for subsequent QTL analysis. Greater diversity favours the identification of increased marker polymorphism and QTLs with larger effects. Selecting parents from divergent germplasm groups can increase genetic diversity. QTL studies have involved populations derived from parents with different end uses, different inflorescence types, and different seed characteristics. Examples of divergent intraspecific barley mapping populations include: "feed" × "malt" quality (Hayes et al. 1993; Tinker et al. 1996), two-rowed × six-rowed (Kjaer and Jensen 1996; Marquez-Cedillo et al. 1999), and covered × hulless (Heun 1992; Pecchioni et al. 1999). Major genes may affect many quantitative traits (Powell et al. 1985a, 1985b, 1990). If these effects are large, they may interfere with the ability to detect other QTLs with more moderate effects. These populations can be divided into sub-populations corresponding to the different alleles at the major locus, such as was done for the *vrs1* locus in barley (Marquez-Cedillo et al. 1999); however, the smaller sub-population sizes will reduce the chances of detecting QTLs. As an alternative to sub-dividing these populations, composite interval mapping (Jansen and Stam 1994; Zeng 1994) and two-locus additive models can be used to incorporate the covariate effects of a major locus as a background marker in the QTL analysis. Epistatic interactions between a major locus and other genomic regions can also be assessed using some QTL analysis software packages, such as MQTL (Tinker and Mather 1995).

In oat, hulless varieties possess a multiflorous spikelet with elongated rachillae and thin lemmas that detach readily from the caryopses during threshing. A review by Marshall and Shaner (1992) indicated that a single, incompletely dominant gene (*N1*) interacting with modifying genes controls the hulless grain characteristic in oat. The expression of the hulless trait is affected by environmental conditions and management practices

(Boland and Lawes 1973; Lawes and Boland 1974; Marshall et al. 1992). Hulless oat varieties have unique end-use advantages compared to covered varieties (Burrows et al. 1993); however, numerous limitations, including low grain yield, have constrained the use of these varieties (Barr et al. 1992; Peltonen-Sainio 1994). Comparatively little breeding effort has been undertaken to improve hulless relative to covered types (Brown and Patterson 1992). For this reason, most hulless by covered oat crosses are made to improve the hulless types; however, these crosses may also transfer desirable alleles to the covered oat germplasm pool.

An RI population derived from a cross between a Western Canadian hulless spring variety and an eastern Canadian, covered spring variety was utilized to develop a molecular linkage map using amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), sequence characterized amplified region (SCAR), and RFLP markers. In this paper, we present this linkage map and summarize the phenotypic and QTL analyses of agronomic and quality traits evaluated on this population in multiple environments in Canada and the United States. The objectives of this study were (1) to develop a new linkage map in oat that can further our understanding of the oat genome, (2) to identify genomic regions affecting agronomic and quality traits in a hulless by covered oat population, (3) to identify the genomic location of the hulless allele (*N1*) and assess its affect on quantitatively inherited traits, and (4) to compare QTL detection methods that account for the variation caused by the *N1* locus.

Materials and methods

Plant material

A 'Terra' × 'Marion' (TM) $F_{5:6}$ RI population was generated by single-seed descent from random F_2 lines of a cross between the hulless oat variety 'Terra' (McKenzie et al. 1981) developed in Manitoba, Canada and the elite covered seeded variety 'Marion' developed in Quebec, Canada. Both parents have a spring growth habit. Marion possesses a relatively high groat β-glucan content compared to other Canadian oat varieties (Miller et al. 1993; Humphreys and Mather 1996). A subset of 101 RI lines, which included 59 covered and 42 hulless lines, was used in the construction of the map and for quantitative trait analyses.

Phenotypic data

The 101 RI lines were grown in 13 environments from 1992 to 1997. The testing sites included one location in the United States (Aberdeen, Idaho) and two locations in Canada (Ottawa, ON and Saskatoon, SK). The local variety testing methods and agricultural practices at each collaborating site were used for field experiments. One to four randomized complete blocks were grown in each environment. Sixteen agronomic, kernel, and chemical composition traits (Table 1) were evaluated in 2 to 13 of the environments (Table 2).

Table 1 Agronomic, kernel, and chemical composition traits measured in the 'Terra' × 'Marion' oat recombinant inbred (RI) population

Trait (abbr.)	Units	Method of measurement
Agronomic traits		
Heading date (HD)	d	Number of days from planting to 50% panicle emergence
Height (HT)	cm	Average plant height from soil surface to tip of panicle
Lodging (LG)	%	Lodging severity, where 0 = no lodging and 100 = complete lodging
Visual score (VS)	1–9	Visual rating for general attractiveness in field, with 1 = worst and 9 = best
Yield (YD)	g m^{-2}	Weight of oat grain harvested per unit area
Kernel traits		
Kernel weight (KW)	g	Average weight per 1,000 kernels
Milling difficulty (MD)	%	Percentage by weight of kernels of a 70 g sample not dehulled during milling yield determination
Milling yield (MY)	%	Groat percentage by weight of a 70 g sample using a laboratory oat dehuller (Codema, Maple Grove, MN)
Plump kernels (PK)	%	Percentage by weight of a 50 g sample remaining above a 5.5/64″ x 3/4″ slotted sieve
Tertiary kernels (TY)	0–5	Visual rating of covered lines for presence of tertiary kernels, with 0 = none
Test weight (TW)	kg hl^{-1}	Bulk density of a sample of oat grain
Thin kernels (TK)	%	Percentage by weight of a 50 g sample passing through a 5.0/64″ × 3/4″ slotted sieve
Chemical composition		
FIA β-glucan (BF)	%	Flow injection analysis (FIA) of Calcofluor (Polysciences, Warrington, PA.) high molecular weight β-glucan complexes (Jorgenson 1988) in ground groat samples on a dry-weight basis.
NIR β-glucan (BN)	%	Near-infrared reflectance (NIR) prediction of mixed-linkage β-glucan [Megazyme (Wicklow, Ireland) assay kit; method 995.16, AOAC 1995] in ground groat samples on a dry-weight basis.
Oil (OL)	%	NIR prediction of acid-hydrolysis oil (method 922.06, AOAC 1995) in ground groat samples on a dry-weight basis
Protein (PN)	%	NIR prediction of Kjeldahl protein (method 976.05, AOAC 1995) in ground groat samples on a dry-weight basis

Table 2 Listing of environments with number of replicates grown and traits measured for the 'Terra' × 'Marion' oat RI population

Environment[a]	Reps	Traits measured[b]
ID92	4	BF[c], OL[c], PN[c]
ID94	3	HD, HT, LG, YD, MD, MY, TY[d], BF, OL, PN
ID95	3	HD, HT, LG, YD, KW, MD, MY, TY, TK, OL, PN
ID96	3	HD, HT, LG, YD, KW, MD, MY, TY, TK, TW, BN, OL, PN
ID97	3	HD, HT, LG, YD, KW, MD, MY, TY, TK, TW, BN, OL, PN
ON93	1	HT, BF, OL, PN
ON94	3	HT, YD, KW, BF, OL, PN
ON95	3	HD, HT, YD, MD, MY, OL, PN
ON96	3	HD, HT, LG, YD, KW, MD, MY, BF[d], BN, OL, PN
ON97	3	HD, YD, KW, MD, MY, TK, BN, OL, PN
SK95	2	HD, HT, VS, YD, KW[c], MD, MY, TW[c], OL, PN
SK96	2	HD, HT, VS, YD, KW, MD, MY, PK, TK, TW, BN[d], OL, PN
SK97	2	HD, HT, VS, YD, KW, MD, PK, TK, TW, BN, OL, PN

[a] Coded by location, where *ID* Aberdeen, Idaho; *ON* Ottawa, ON; *SK* Saskatoon, SK; followed by year
[b] *HD* Heading date, *HT* plant height, *LG* lodging, *VS* visual score, *YD* yield, *KW* kernel weight, *MD* milling difficulty, *MY* milling yield, *PK* plump kernels, *TY* tertiary kernels, *TK* thin kernels, *TW* test weight, *BF* FIA β-glucan, *BN* NIR β-glucan, *OL* oil, *PN* protein
[c] Data from a bulk sample from all reps
[d] Data from one rep only

Marker data and linkage mapping

Tissue preparation, DNA purification, and marker analyses were based on methods described by Wight et al. (2003). Clones for RFLP mapping were chosen from among those mapped previously in the KO population (O'Donoughue et al. 1995; Wight et al. 2003). Marker sources are described in Wight et al. (2003).

AFLP markers were detected using reagents and procedures from the AFLP Analysis System I kit (Invitrogen). Primers were labelled using [γ ^{33}P] ATP. Modifications to the standard protocol included adding 50% more *Taq* DNA polymerase to the selective amplification reaction mixture and increasing the number of cycles after the touch-down phase during selective amplification to 35. Bands were separated by electrophoresis through Long Ranger polyacrylamide gels (BioWhittaker Molecular Applications) and visualized by exposing the dried gels to Kodak BioMax MR-1 film

(Eastman Kodak). Marker names, e.g., acg_cac127, reflect the choice of selective primer pair [3 selective bases (*Eco*RI primer) _3 selective bases (*Mse*I primer)] and location of the band (usually distance run in millimetres) in the first gel analyzed for each primer pair.

The sb978 SCAR marker was derived from the barley cDNA clone bcd978 (Heun et al. 1991). The clone was end-sequenced using an ABI 373 automated DNA sequencer, then a pair of PCR primers (sb978f1 = AAGGTCAGCCAGGTCAAATG and sb978r1 = CGAGATAACCTCCCTCCCTC) was designed and synthesized. SCAR amplification of genomic DNA was performed using a Temptronic thermocycler (Barnstead/Thermolyne) with a standard PCR program of 5 min at 94°C; 33 cycles of 1 min at 94°C, 1 min at 49°C, 1 min 20 s at 72°C; and 5 min at 72°C. PCR reactions were conducted in a volume of 25 μl containing 1–2 mM genomic DNA, 200 nM of each primer, 1.5 mM MgCl$_2$, 200 μM dNTP, 2.5 U *Taq*

DNA polymerase, and 1× PCR buffer. PCR products were separated on 1.8% agarose gels, stained with ethidium bromide, and visualized with UV light.

Linkage mapping was performed using both MAPMAKER, version 3.0 for PC (Lander et al. 1987) and G-MENDEL Win32, version 0.8b (Holloway and Knapp 1993) software. A robust framework map was developed by choosing marker subsets and determining orders that were consistent between both programs. Detailed descriptions of these procedures are provided by Wight et al. (2003). Linkage group numbers are preceded by the designation TM to distinguish them from previously reported oat linkage groups. The linkage group TM_4+16 arose when TM_4 and TM_16 were merged following analysis of additional markers.

Statistical analysis

The phenotypic data for most traits measured in the TM population were first analyzed for the whole RI population, then separately for the covered and hulless sub-populations. Tertiary kernel data were collected only for the covered lines. Analysis of variance for each trait in each environment and over environments was performed using the SAS general linear model procedure (SAS Institute 1985). Due to the varying number of replicates, means for each entry were used to compute the analysis of variance across environments. Both environments and genotypes were considered random. The variance components associated with genetic variance (σ^2_g) and error variance (σ^2_e) were determined using the expected mean squares from the analysis of variance. Heritability (h^2) on an entry mean basis was estimated from the genetic and error variance components using the formula: $h^2=\sigma^2_g/[\sigma^2_g+(\sigma^2_e/r)]$, where r is the number of environments. Factor analysis was conducted on overall entry means using the SAS factor procedure to investigate the relationships among the 15 traits evaluated in the whole population. Extracted factors with eigenvalues greater than 1 were rotated by the varimax method (Kaiser 1958) to allow biologically relevant interpretation of the results. Pairwise comparisons between covered and hulless sub-population means were conducted using t-tests.

Three approaches for QTL detection in the TM population were evaluated using the MQTL software package (Tinker and Mather 1995). These methods of QTL identification included: (1) simple interval mapping (SIM) in the whole population, (2) SIM in the covered and hulless subsets of the population, and (3) testing two-locus additive and epistatic models with N1 as a covariate. Tests for QTL main effects and QTL-by-environment (QTL×E) interactions were conducted using SIM. The two-locus models were used to evaluate QTL main effects only. Permutations ($n=5,000$) were performed to determine threshold test statistics for declaring significant ($P<0.05$) experiment-wide QTL main effects and QTL×E interactions, as described by Tinker et al. (1996). Parameters of QTL main effects and QTL×E interactions were estimated using multi-locus linear models in the full population and within each sub-population. Allelic effects for a given locus were estimated by the average difference between homozygous classes. The proportions of phenotypic variation explained by models with QTL main effects and both QTL main effects and QTL×E interactions were estimated from the coefficients of determination (R^2).

Results and discussion

Parental identity

The hulless parent of this population was originally identified as 'Tibor'. Later, molecular testing (data not shown) confirmed that this parent was actually 'Terra', the only other hulless variety in the crossing block. Supplementary field tests containing 'Terra', 'Tibor',

'Marion', and two RI progeny were conducted (data not shown), and these data supported the phenotypic identity of 'Terra' as a parent. The fact that this information was obtained only after considerable data were collected has several implications for this study. The absence of orthogonal data from 'Terra' relative to the full set of RI lines restricts our ability to draw conclusions regarding transgressive segregation relative to 'Terra'. Preliminary marker surveys including 'Tibor' were not useful for identifying markers that segregated in the RI lines. Surveys including 'Terra' were more accurate, but the seed of the original 'Terra' parent was not available and some inconsistency between alleles in these newly grown 'Terra' plants and those segregating in the RI lines was also observed. This is not unusual, since we have observed considerable marker heterogeneity in different seed sources from identical oat varieties (unpublished). Some differences between alleles in the 'Marion' parent and those in the RI lines were also apparent, so the most reliable surveys were those including a subset of 15 RI lines. This information is important for anyone choosing to conduct further studies using this population.

Molecular marker mapping

A total of 430 polymorphic markers were scored in the RI mapping population. These included 242 AFLP markers, 160 RFLP markers, 26 RAPD markers, and one SCAR marker. The hulless character was also scored and mapped as a phenotypic marker. These markers formed 35 linkage groups at LOD 6. A robust subset of 124 ordered markers was selected from these, favouring RFLP markers where possible. After the construction of a framework map (Fig. 1), the remaining markers were either placed between the two framework markers giving the highest chi-square statistics, or left unplaced when the chi-square statistic was less than 6. The chi-square statistic (testing deviation from independent assortment) was used for computational simplicity, as it is a close approximation of the LOD score. Placement was based on a simple division of the framework distance by the ratio of distances between the placed marker and the two closest framework markers. The total map distance represented by this framework map is 727 cM. However, this does not take into account the linked regions on either end of each linkage group and the regions flanking each of the unlinked markers. Comparison to the KO linkage map (Wight et al. 2003) indicates that this represents approximately 38% of the oat genome, based on the ratio of total map lengths. The mapping of AFLP markers was an attempt to obtain map coverage in regions unrepresented by RFLP markers. A few novel regions were identified by this method (TM_12, TM_32, TM_34, TM_35, and TM_39), but most AFLP markers fell within clusters, such as those on groups TM_1 and TM_15, or remained unlinked. This may be a result of inadequate polymorphism in some regions of the parental genomes.
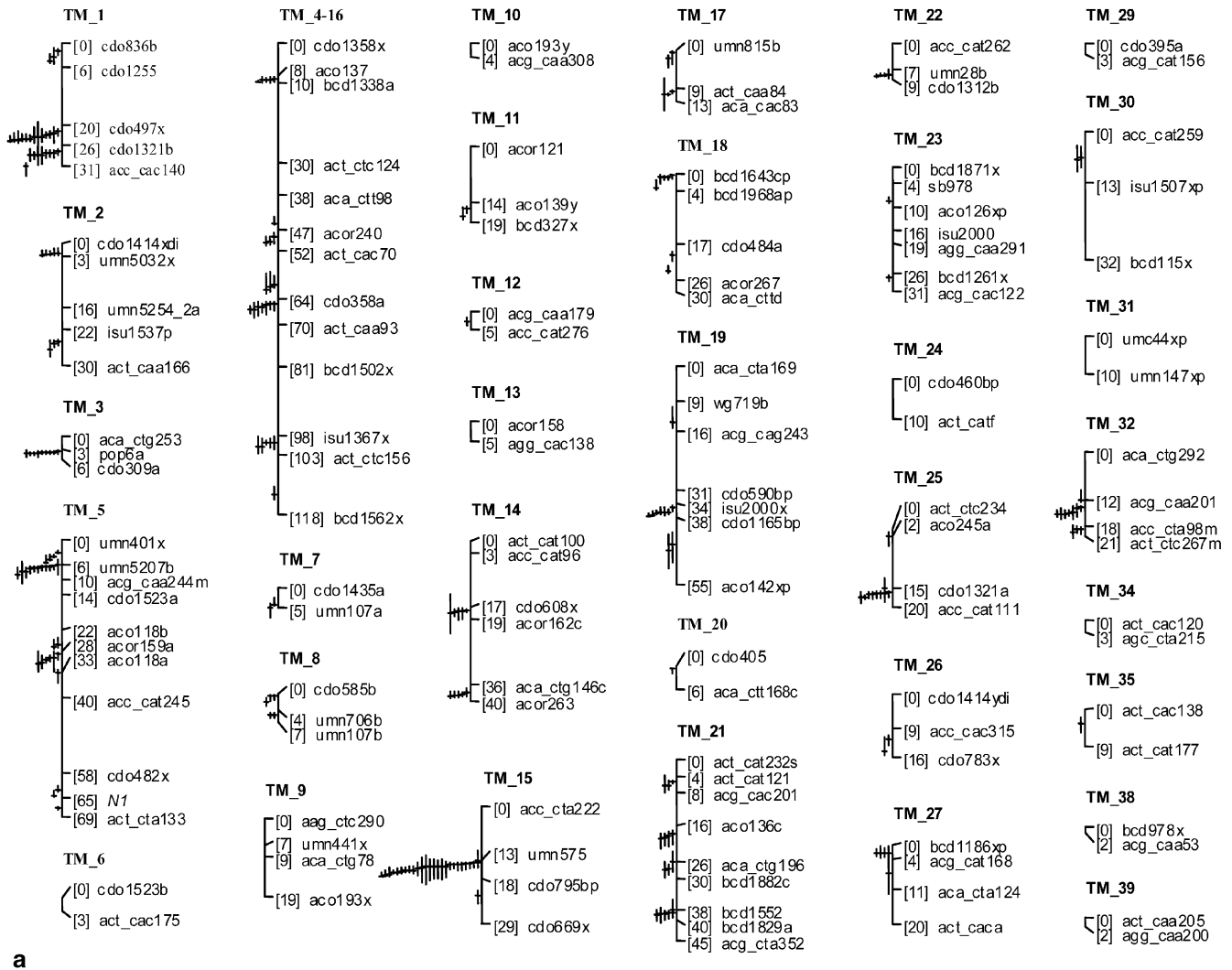
TM_1
[0] cdo836b
[6] cdo1255
[20] cdo497x
[26] cdo1321b
[31] acc_cac140

TM_2
[0] cdo1414xdi
[3] umn5032x
[16] umn5254_2a
[22] isu1537p
[30] act_caa166

TM_3
[0] aca_ctg253
[3] pop6a
[6] cdo309a

TM_5
[0] umn401x
[6] umn5207b
[10] acg_caa244m
[14] cdo1523a
[22] aco118b
[28] acor159a
[33] aco118a
[40] acc_cat245
[58] cdo482x
[65] N1
[69] act_cta133

TM_6
[0] cdo1523b
[3] act_cac175

TM_4-16
[0] cdo1358x
[8] aco137
[10] bcd1338a
[30] act_ctc124
[38] aca_ctt98
[47] acor240
[52] act_cac70
[64] cdo358a
[70] act_caa93
[81] bcd1502x
[98] isu1367x
[103] act_ctc156
[118] bcd1562x

TM_7
[0] cdo1435a
[5] umn107a

TM_8
[0] cdo585b
[4] umn706b
[7] umn107b

TM_9
[0] aag_ctc290
[7] umn441x
[9] aca_ctg78
[19] aco193x

TM_10
[0] aco193y
[4] acg_caa308

TM_11
[0] acor121
[14] aco139y
[19] bcd327x

TM_12
[0] acg_caa179
[5] acc_cat276

TM_13
[0] acor158
[5] agg_cac138

TM_14
[0] act_cat100
[3] acc_cat96
[17] cdo608x
[19] acor162c
[36] aca_ctg146c
[40] acor263

TM_15
[0] acc_cta222
[13] umn575
[18] cdo795bp
[29] cdo669x

TM_17
[0] umn815b
[9] act_caa84
[13] aca_cac83

TM_18
[0] bcd1643cp
[4] bcd1968ap
[17] cdo484a
[26] acor267
[30] aca_cttd

TM_19
[0] aca_cta169
[9] wg719b
[16] acg_cag243
[31] cdo590bp
[34] isu2000x
[38] cdo1165bp
[55] aco142xp

TM_20
[0] cdo405
[6] aca_ctt168c

TM_21
[0] act_cat232s
[4] act_cat121
[8] acg_cac201
[16] aco136c
[26] aca_ctg196
[30] bcd1882c
[38] bcd1552
[40] bcd1829a
[45] acg_cta352

TM_22
[0] acc_cat262
[7] umn28b
[9] cdo1312b

TM_23
[0] bcd1871x
[4] sb978
[10] aco126xp
[16] isu2000
[19] agg_caa291
[26] bcd1261x
[31] acg_cac122

TM_24
[0] cdo460bp
[10] act_catf

TM_25
[0] act_ctc234
[2] aco245a
[15] cdo1321a
[20] acc_cat111

TM_26
[0] cdo1414ydi
[9] acc_cac315
[16] cdo783x

TM_27
[0] bcd1186xp
[4] acg_cat168
[11] aca_cta124
[20] act_caca

TM_29
[0] cdo395a
[3] acg_cat156

TM_30
[0] acc_cat259
[13] isu1507xp
[32] bcd115x

TM_31
[0] umc44xp
[10] umn147xp

TM_32
[0] aca_ctg292
[12] acg_caa201
[18] acc_cta98m
[21] act_ctc267m

TM_34
[0] act_cac120
[3] agc_cta215

TM_35
[0] act_cac138
[9] act_cat177

TM_38
[0] bcd978x
[2] acg_caa53

TM_39
[0] act_caa205
[2] agg_caa200

a

Fig. 1a, b Molecular marker linkage map from the 'Terra' × 'Marion' F_6 recombinant inbred oat population. Markers from the framework map (**a**) are indexed by cM positions in the complete marker list (**b**). Placed markers are represented by the *placement bars* shown to the *left* of each group in the framework map and by *non-indexed marker names* in the complete marker list. The placed marker order in **b** corresponds to a right-to-left reading of the placement bars in **a**. Note that the *N1* locus maps to TM_5. RFLP suffixes reflect the names of the same marker bands in the 'Kanota' × 'Ogle' reference population map or are designated as *x*, *y*, or *z* if the bands were not polymorphic in KO. The suffix *p* indicates the designation is "probable"

Detailed comparisons of this map to other oat maps will be the topic of a future study.

Quantitative traits

Combined environment analysis indicated that there were differences among both lines and environments ($P<0.01$) for all traits. Heritability for the traits ranged from 0.38 for lodging to 1.00 for milling yield (Table 3). Some of the high heritability values can be attributed to the segregation of the hulless allele (*N1*) within this population. Sub-population means were different ($P<0.05$) for 11 of the traits. The covered sub-population had higher lodging, yield, kernel weight, milling difficulty, plump kernels, and FIA β-glucan content and lower visual score, milling yield, test weight, thin kernels, and oil content versus the hulless sub-population. Differences in lodging and oil content would not be expected to be due to differences in kernel type alone. Despite the fact that the hulless parent 'Terra' was not evaluated in this study, useful transgressive segregation was observed relative to 'Marion' for reduced tertiary and thin kernels and increased yield, kernel weight, and plump kernels (Table 3). Within the covered sub-population, there were lines with higher milling yield and test weight than Marion (data not shown).

Factor analysis was conducted to group correlated traits by their association with extracted factors or principal components. Four factors with eigenvalues greater than 1.0 explained 77% of the total variance in the population (Table 4). Eight traits, each showing a

Fig. 1b

TM_1
[0] cdo836b
 acg_cac127
 act_caa218
 bcd1414a
[6] cdo1255
[20] cdo497x
 aco227xp
 act_cac82
 aco134yp
 acg_cat89
 act_cati
 act_ctcf
 acc_cat377
 acor264
 umn128x
 aco171x
 agc_catgc
 agg_cac198
 acor165c
[26] cdo1321b
 cdo780x
 aco187p
 aca_ctt117c
 acc_cta103c
 acg_cag335
 act_ctce
 agg_caa265
 agc_caa142
 aca_ctc333
[31] acc_cac140

TM_2
[0] cdo1414xdi
 bcd1829x
 isu2287x
 cdo783
 cdo1502
 acor160
[3] umn5032x
[16] umn5254_2a
[22] isu1537p
 cdo836ap
 acor109b
 act_caa110
[30] act_caa166

TM_3
[0] aca_ctg253
[3] pop6a
 cdo1385f
 umn162x
 umn341ap
 mog12xp

 umn869x
 agg_caa186
 cdo1423a
 umn420xp
 acc_cta229
[6] cdo309a

TM_4-16
[0] cdo1358x
[8] aco137
 isu1755y
 aca_ctt72
 acor109a
 act_cac174
 agc_catjc
[10] bcd1338a
[30] act_ctc124
[38] aca_ctt98
 acc_ctab
[47] acor240
 cdo212y
 cdo348x
 cdo548x
[52] act_cac70
 agc_cta147
 agc_cta187
 acc_ctaa
[64] cdo358a
 act_ctc172
 cdo203x
 aca_cac162
 agc_cta186
 act_cta93m
 act_cac130
 act_cat133
[70] act_caa93
[81] bcd1502x
[98] isu1367x
 aca_cac98
 aca_cttf
 acor189
 cdo393d
 cdo527x
[103] act_ctc156
 aca_ctt323
[118] bcd1562x

TM_5
[0] umn401x
 aca_cac115
 agc_catb
 agc_catc
 agc_ctt77
[6] umn5207b

 umn5254x
 cdo419xp
 aco183y
 cdo1469c
 cdo1168a
 act_caa143m
 cdo241
 cdo1437a
 aca_cac160
 acc_cac246
 acc_cac248
[10] acg_caa244m
[14] cdo1523a
[22] aco118b
 agg_cac131
 acg_caa161
[28] acor159a
 agc_cta204
 agg_caa93
 bcd1555x
 acc_cat236
 aca_cac384
 aca_ctt287
[33] aco118a
 act_ctcg
[40] acc_cat245
[58] cdo482x
 acor185
 bcd1552x
[65] N1
 acc_cat384
[69] act_cta133

TM_6
[0] cdo1523b
[3] act_cac175

TM_7
[0] cdo1435a
 act_ctc224
 cdo638x
[5] umn107a

TM_8
[0] cdo585b
 aco142bp
 aco238
 agg_caa214
[4] umn706b
 cdo638
 acg_cac232
[7] umn107b

TM_9
[0] aag_ctc290
[7] umn441x
[9] aca_ctg78
[19] aco193x

TM_10
[0] aco193y
[4] acg_caa308

TM_11
[0] acor121
[14] aco139y
 acor179
 aca_ctt360
[19] bcd327x

TM_12
[0] acg_caa179
 acor139
[5] acc_cat276

TM_13
[0] acor158
[5] agg_cac138

TM_14
[0] act_cat100
[3] acc_cat96
[17] cdo608x
 acg_caa295
 aca_ctta
 agg_caa94
 acg_cag142
 act_catdc
[19] acor162c
[36] aca_ctg146c
 agg_caa130
 cdo395b
 acc_cac142
 bcd1414xp
 acc_cac138
[40] acor263

TM_15
[0] acc_cta222
 agg_cac150
[13] umn575
 bcd327
 umn856a
 acor167a
 umn114x
 acor198
 bcd1829z
 umn360a

 act_cat216c
 acg_caa103
 aco183x
 cdo419p
 acor186
 cdo1437b
 acc_cta144
 acg_caa194
 agc_catd
 cdo1196
 aco139x
 umn363x
 agg_caa237
 aca_ctga
 agc_catf
 agc_caa229
 acg_caa196
 agg_cac223c
[18] cdo795bp
 acg_cat231
[29] cdo669x

TM_17
[0] umn815b
 agc_cati
 acc_cac216m
[9] act_caa84
 acor236b
 cdo580x
 acg_cat262
[13] aca_cac83

TM_18
[0] bcd1643cp
 umn401y
 acg_cac105c
 cdo465xp
 act_cta139
 acg_cac170
[4] bcd1968ap
[17] cdo484a
 act_caaa
 acc_cat181
[26] acor267
[30] aca_cttd

TM_19
[0] aca_cta169
[9] wg719b
 agg_cac276
[16] acg_cag243
[31] cdo590bp
[34] isu2000x

b

difference between the covered and hulless sub-population means, had high (>0.5) loadings for the first factor. The four chemical composition traits had a high factor loading for the second factor. Lodging and milling difficulty had high loadings for the third factor, and heading date and height had high loadings for the fourth factor. Only oil had a high loading factor for more than one factor (1 and 2). High milling yield, high test weight, high percentage of thin kernels, high oil content and high visual score were associated with low yield, low kernel weight, and low percentage of plump kernels. High β-glucan (FIA and NIR) and high oil were associated with low protein. There were positive associations between lodging and milling difficulty and between heading date and height. The distribution of factor scores for the 101 TM RI progeny on the first two rotated axes is shown in

Fig. 2. There is a separation of most covered and hulless lines based on the factor 1 scores. These results suggest that the N1 locus is the underlying biological component influencing factor 1. The effect of major genes on quantitative traits has also been demonstrated in barley, where genes controlling height and biomass production were linked to the V locus (Powell et al. 1990). It is plausible that the traits with high loadings in the other factors also have a common genetic basis.

Quantitative trait loci: main effects

For each trait, four QTL main effect tests were conducted: (1) using the whole population, (2) using N1 as a covariate, (3) using only covered lines, and (4) using only

Table 3 Agronomic, kernel, and chemical composition trait data for 101 RI progeny from the 'Terra' × 'Marion' cross and for the hulless and covered sub-populations

Trait	N^a	Covered	Hulless	Whole population				
		μ^b	μ	μ	σ	Min^c	Max	h^{2d}
Heading date (d)	10	60.2	60.6 ns	60.4	1.2	−2.3	3.2	0.94
Height (cm)	12	104.3	103.1 ns	103.8	4.2	−11.2	11.1	0.95
Lodging (%)	5	13.5	6.3**	10.5	6.4	−9.0	18.4	0.38
Visual score (1–9)	3	4.8	5.8**	5.2	0.9	−2.7	1.8	0.61
Yield (g m^{-2})	11	449.6	323.5**	397.6	67.0	−108.0	103.6	0.95
Kernel weight (g)	9	35.7	27.1**	32.1	4.8	−10.3	9.7	0.98
Milling difficulty (%)	10	3.3	2.3*	2.9	2.0	−2.7	8.6	0.76
Milling yield (%)	9	71.6	96.1**	81.8	12.2	−13.7	16.2	1.00
Plump kernels (%)	2	43.1	14.2**	31.1	19.4	−28.6	52.7	0.92
Tertiary kernels (0–5)	4	3.6	–	–	1.0	−2.2	1.4	0.79
Test weight (kg hl^{-1})	5	52.0	58.5**	54.7	3.7	−6.0	9.4	0.95
Thin kernels (%)	6	8.7	37.4**	20.7	16.6	−18.1	51.4	0.97
FIA β-glucan (%)	5	5.3	5.2*	5.3	0.3	−0.6	0.6	0.44
NIR β-glucan (%)	6	5.7	5.6 ns	5.6	0.2	−0.5	0.7	0.85
Oil (%)	13	9.5	10.0**	9.7	0.5	−1.5	1.4	0.98
Protein (%)	13	16.2	15.9 ns	16.1	0.6	−1.7	2.0	0.93

[a] Number of environments
[b] Unadjusted mean across environments
[c] Expressed relative to μ
[d] Proportion of total phenotypic variance attributable to RI lines
Significance of pairwise comparison between covered and hulless subpopulation means: *$P<0.05$, **$P<0.001$, *ns* non-significant

Table 4 Loading of fifteen traits on four factors obtained from factor analysis with a varimax rotation of axes, and the percentage of total variance accounted for by the four factors

Trait	Factor 1	Factor 2	Factor 3	Factor 4
Heading date (d)	0.23	0.16	0.00	0.74
Height (cm)	−0.21	−0.06	0.01	0.86
Lodging (%)	−0.34	−0.03	0.73	0.08
Visual score (1–9)	0.69	−0.06	0.08	0.10
Yield (g m^{-2})	−0.84	0.21	0.24	−0.16
Kernel weight (g)	−0.94	−0.12	0.17	0.16
Milling difficulty (%)	−0.06	0.10	0.82	−0.04
Milling yield (%)	0.88	−0.08	−0.40	0.04
Plump kernels (%)	−0.89	−0.11	−0.01	0.16
Test weight (kg hl^{-1})	0.76	−0.20	−0.47	0.10
Thin kernels (%)	0.92	0.04	−0.20	−0.07
FIA β-glucan (%)	−0.16	0.83	0.06	0.14
NIR β-glucan (%)	−0.15	0.86	−0.03	0.10
Oil (%)	0.62	0.55	0.15	−0.12
Protein (%)	−0.41	−0.64	−0.14	0.34
% Total variance explained	39	16	12	10

Fig. 2 Distribution of 59 covered and 42 hulless lines from the 'Terra' × 'Marion' RI population on the first two rotated factor axes

yield, kernel weight, milling yield, plump kernels, test weight, thin kernels, and oil content mapped at or near the *N1* locus on linkage group TM_5. Other traits mapping to common locations in the genome included oil and protein (unlinked marker bcd1829y), and milling yield and test weight (unlinked marker cdo212x). QTLs affecting heading date, plant height, oil, β-glucan (NIR), and lodging were also detected using the whole population.

N1 as a covariate

Fourteen QTLs were found in the population when *N1* was used as a covariate in the additive genetic model. Multiple-trait QTLs were detected in four regions: linkage groups TM_2 (lodging and yield), TM_5 (test weight and plant height), and TM_35 (lodging, kernel weight, and plump kernels), and with the unlinked marker bcd1829y (oil and protein). QTLs affecting heading date (TM_9), oil (TM_4+16), β-glucan (NIR) (TM_18), and plump

hulless lines. Figure 3a–d shows the result of these analyses for grain yield. Regions of the genome showing significant test statistics for the other traits are summarized in Fig. 4. In total, 34 main effect QTLs were detected using one or more QTL detection methods. These QTLs were located in 19 regions of the genome. Markers linked to some of these QTL are undergoing further study in validation and marker-assisted selection projects.

Whole population

Eighteen QTLs were found in the whole population using SIM. Main effect QTLs affecting lodging, visual score,
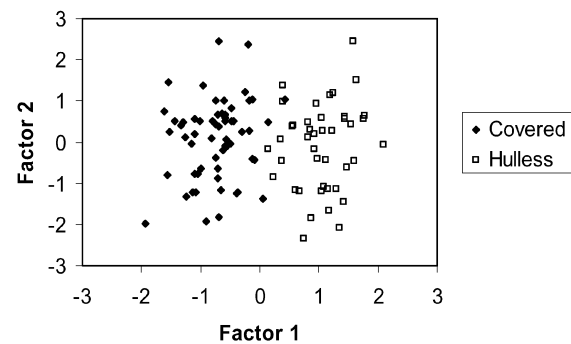
a Yield (g m⁻²) SIM Main Effects - All

b Yield (g m⁻²) Main Effects - 2 Locus Model with N1

c Yield (g m⁻²) SIM Main Effects - Covered
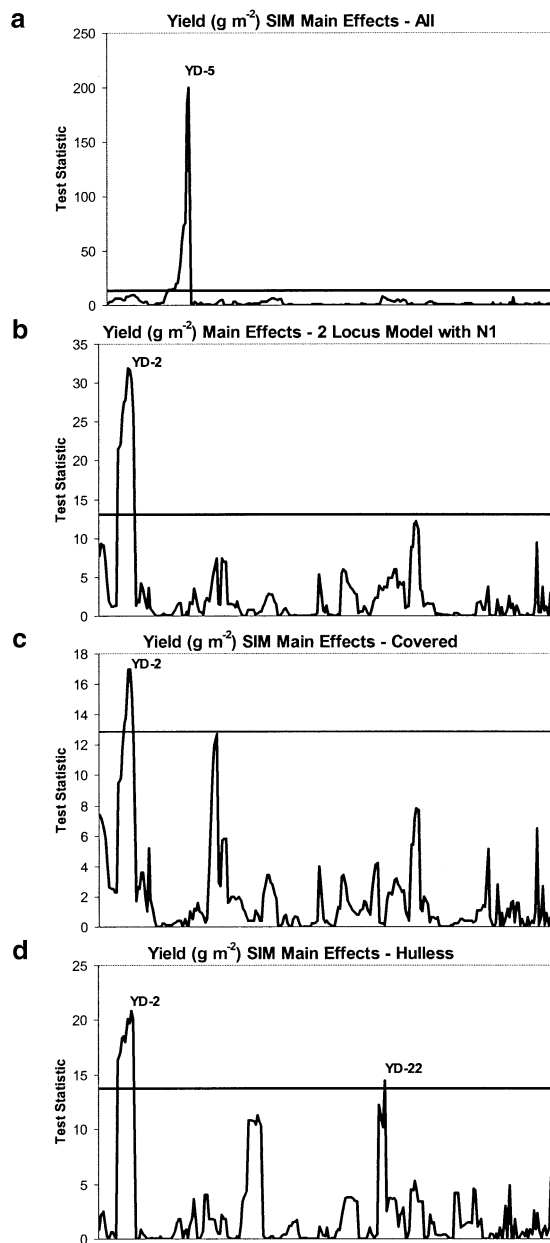
d Yield (g m⁻²) SIM Main Effects - Hulless

Fig. 3 Genome-wide scans of test statistic for simple interval mapping (SIM) of main effects for grain yield using the whole population (**a**), *N1* as a covariate in the model (**b**), covered lines only (**c**), and hulless lines only (**d**). *Horizontal lines* represent threshold value corresponding to the analysis used, based on 5,000 permutations. Peak marker locations are labelled using trait (Table 1) and linkage group designations

kernels (TM_4+16 and unlinked marker bcd1872a) were also detected when *N1* was included as a covariate in the model. Seven of the QTLs detected by using *N1* as a covariate were not found in the previous whole population analysis.

Covered sub-population

Nine QTLs were found in the covered sub-population using SIM. Lodging and yield QTLs mapped to linkage group TM_2 and kernel weight and plump kernel QTLs mapped to linkage group TM_35. Additional QTLs affecting test weight (TM_1), plump kernels (TM_4+16), β-glucan (FIA) (TM_29 and unlinked marker act_cat244), and thin kernels (unlinked marker bcd1872a) were also identified using the covered lines. Four of the QTLs found in the covered sub-population were not identified in the previous analyses.

Hulless sub-population

Eight QTLs were found in the hulless sub-population using SIM. Oil and protein QTLs mapped to the same linkage group (TM_15). QTLs affecting thin kernels (TM_1), test weight (TM_5), oil (TM_4+16), β-glucan (NIR) (TM_21), and yield (TM_22) were identified in the hulless sub-population. Five of the QTLs detected in the hulless sub-population were not identified in the previous analyses.

Localization of the *N1* locus

The hulless condition in oat was previously found to be controlled by a single, incompletely dominant gene (*N1*) interacting with modifying genes (Boland and Lawes 1973). In this study, we have localized the *N1* gene to linkage group TM_5 (homologous to KO_24_26_34). Using an indirect measure of the hulless phenotype, milling yield or groat percentage, we have identified another genomic region (unlinked marker cdo212x) affecting this trait. Further study will be required to confirm that this region contains a gene or genes that interact with the *N1* locus to affect the hulless condition.

QTL-by-environment interactions

For each trait, three QTL-by-environment (QTL×E) interaction tests were conducted using the whole population, the covered lines, and the hulless lines. Figure 5a–c shows the result of these analyses for grain yield. Regions of the genome showing significant test statistics for the other traits are summarized in Fig. 4. In total, 24 QTL×E interactions were detected in ten regions of the genome using one or more detection methods. Thirteen of these QTL×E interactions corresponded to main effect QTLs affecting the same traits. Eleven of the interactions were localized to the region near the *N1* locus.

Fig. 4 Linkage groups containing significant marker-trait associations based on simple interval mapping (*solid bar*), two-locus model containing *N1* locus (*horizontally striped bar*), SIM QTL × environment interactions (Q×E) (*diagonal striped bar*), and epistatic interactions with *N1* (*white bar*). Peak marker locations are labelled using trait (Table 1), sub-population (*A* all, *C* covered, *H* hulless) and 'Terra' × 'Marion' linkage group designations. LOD scores are given on the line below the peak marker designation followed by the additive effects and parental donor of the high allele (*T* 'Terra', *M* 'Marion') at this genomic location
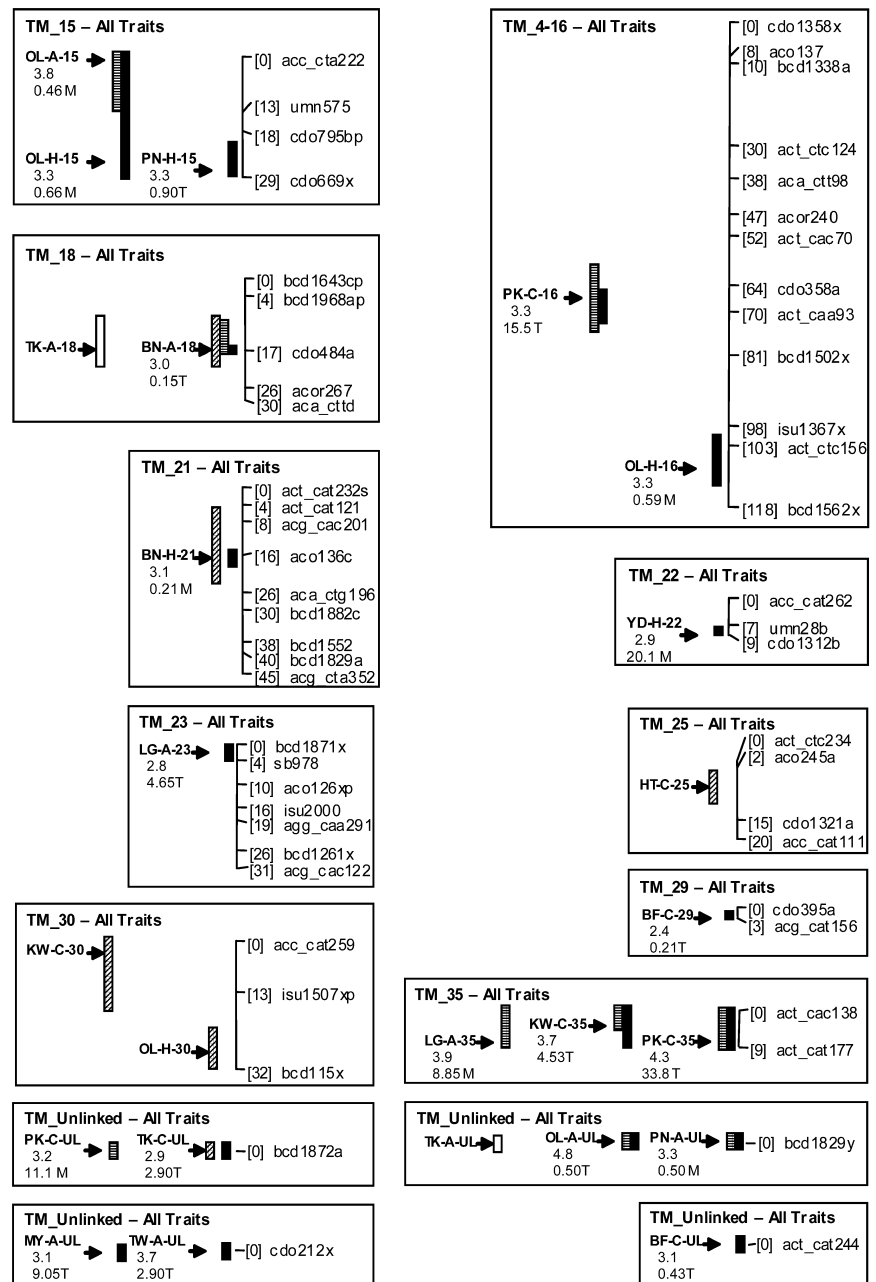
Epistatic interactions with *N1*

Tests for epistasis revealed three regions where QTLs interacted with *N1* to affect thin kernels. These are shown in Fig. 4 and are located on linkage groups TM_1, TM_18, and at the unlinked marker bcd1829y. A main effect QTL affecting thin kernels in the hulless sub-population and test weight in the covered sub-population was located at the same location as the epistatic interaction for thin kernels on linkage group TM_1. QTLs affecting chemical composition traits in the whole population were also localized to the same regions showing significant epistasis with *N1*, including β-glucan (NIR) on linkage group TM_18 and oil and protein at bcd1829y.

These observations suggest that there may be a relationship between thin kernels and other kernel or chemical composition traits. Epistatic interactions between marker loci were also reported in the KO RI population (Holland et al. 1997) and the OT RI population (Holland et al. 2002).

Multiple locus models

Multiple locus models were constructed using markers identified as being linked to QTLs or QTL×E interactions for each of the traits studied (Table 5). The main effect models accounted for 7–95% of the phenotypic variance

1294

Fig. 4 (continued)
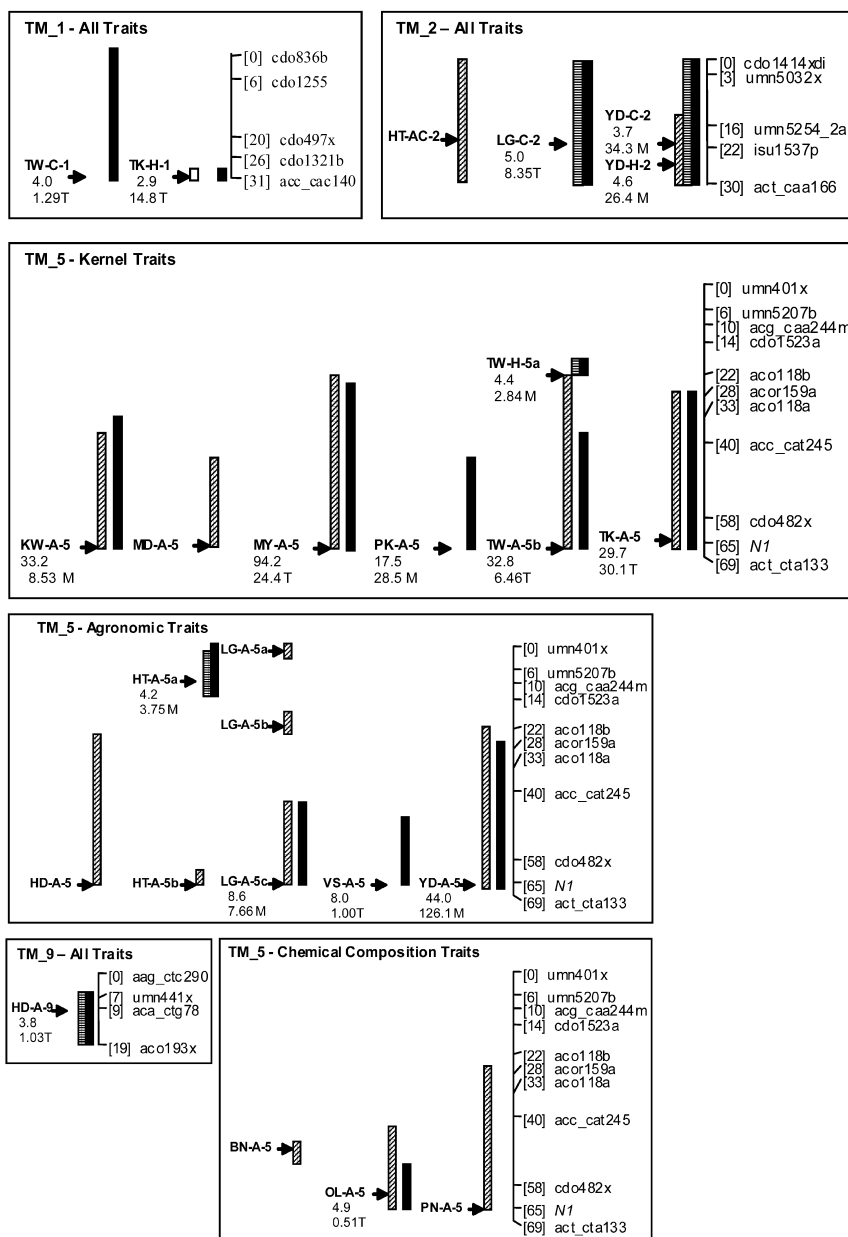
for the various traits in the whole, covered, or hulless sub-populations. The main effect plus QTL×E interaction models accounted for 11–97% of the phenotypic variance. These values are similar to those obtained for heading date, test weight (covered population), β-glucan, and oil content in other oat QTL studies (Siripoonwiwat et al. 1996; Kianian et al. 1999, 2000). For example, MQTL multi-locus models for oil content accounted for 22% and 48% of phenotypic variance in the KM and KO RI populations, respectively (Kianian et al. 1999), compared to 33–39% obtained in our study. For other traits influenced by the *N1* locus such as yield, milling yield (or groat percentage), and test weight in the whole population, our estimates were considerably higher than other studies (Siripoonwiwat et al. 1996; Groh et al. 2001). Estimates for yield (covered population) and plant

height were lower that those obtained by Siripoonwiwat et al. (1996). This could be related to the reduced opportunities for QTL identification resulting from partial genome coverage in the TM map. Inclusion of the epistatic interactions with *N1* for thin kernels increased the proportion of variance explained by the model from 63% (Table 5) to 86%. Multiple locus models for traits associated with vernalization response in KO were also improved by adding epistatic interactions (Holland et al. 1997).

Trait relationships revealed by QTL analysis

Nine genomic regions identified in this study contained QTLs affecting multiple traits. The trait relationships
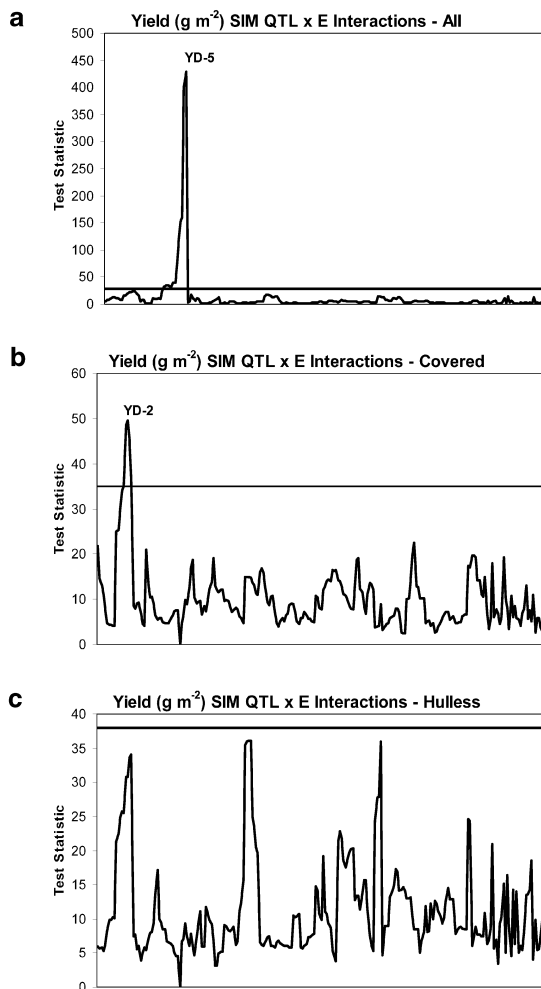
a

b

c

Fig. 5 Genome-wide scans of test statistic for SIM of QTL×E interactions for grain yield using the whole population (**a**), covered lines (**b**), and hulless lines (**c**). *Horizontal lines* represent threshold value corresponding to the analysis used, based on 5,000 permutations. Peak marker locations are labelled using trait (Table 1) and linkage group designations

Table 5 Percentage of phenotypic variance explained by QTLs affecting 15 traits measured on all, covered, or hulless lines from the 'Terra' × 'Marion' RI oat population. *ME* Model including significant main effect QTL only, *ME+QE* model including both main effect QTLs and QTL-by-environment interactions

Trait	Lines	ME	ME + QE
Heading date	All	11	24
Height	All	11	18
	Covered	6	11
Lodging	All	16	44
	Covered	9	20
Visual score	All	17	18
Yield	All	59	75
	Covered	9	15
	Hulless	12	23
Kernel weight	All	72	76
	Covered	19	21
Milling difficulty	All	2	22
Milling yield	All	95	97
Plump kernels	All	65	66
	Covered	34	35
Test weight	All	68	77
	Covered	16	17
	Hulless	27	29
Thin kernels	All	63	71
	Covered	10	17
	Hulless	22	23
FIA β-glucan	Covered	11	16
NIR β-glucan	All	7	14
	Hulless	16	23
Oil	All	36	39
	Hulless	33	39
Protein	All	8	18
	Hulless	18	19

identified by QTL analysis generally agreed with the factor analysis discussed previously in this report. The most important of these regions was on linkage group TM_5 near the *N1* locus. Fourteen traits showed significant QTLs or QTL×E interactions in this region. A study by Ougham et al. (1996) on the biochemical differences between hulless and covered oat lines lead the authors to propose that the *N1* locus encodes a regulatory molecule, as do other homeotic genes previously characterized in plants. Our data support this hypothesis by illustrating the effect this region has on multiple traits, some of which are not kernel characteristics.

The *N1* allele has been shown to affect lignin deposition in developing spikelets (Ougham et al. 1996). This phenomenon could also be responsible for the effect of the hulless gene on lodging if there were differential lignin distribution patterns in the stems of hulless versus covered lines. Lodging resistance is an important goal of

most cereal breeding programs, due to its effect on grain yield and quality (Keller et al. 1999). Lignin plays an important role in plant stem rigidity. Plants with altered lignin composition, such as those containing the brown midrib mutation in maize, have lower stem strength and are more susceptible to lodging (Weller et al. 1985). Understanding the role of the *N1* locus in stem lignin deposition may lead to improved strategies for overcoming this important problem in oat.

The effects of the *N1* allele on agronomic and chemical composition traits may also be due, in part, to agricultural management practices. In our study, the hulless and covered lines were planted, grown, and harvested in the same field experiment with similar treatment given to both types. However, there are slightly different requirements for seeding, threshing, and storage of the two seed types (Burrows et al. 1993), which could have affected trait expression in some environments. Furthermore, identical management practices may have different impacts on the more fragile hulless seeds (Marshall et al. 1992).

Other genomic regions affecting multiple traits were identified from main effect QTLs and QTL×E interaction analyses of the TM population. These include lodging with plant height (TM_2 and 5), grain yield (TM_2), kernel size (TM_35), and test weight (TM_5); test weight with thin kernels (TM_1) and milling yield (unlinked

Table 6 Locations of common QTLs across oat mapping populations: 'Terra' × 'Marion', 'Kanota' × 'Ogle', 'Kanota' × 'Marion', and a recurrent selection population for seven agronomic and quality traits. *TM group* 'Terra' × 'Marion', *KO group* 'Kanota' × 'Ogle', *KM group* 'Kanota' × 'Marion', *RS group* University of Minnesota recurrent selection population

Trait	TM group	KO group	KM group	RS group	Reference
Test weight	1	33	-	-	Siripoonwiwat et al. (1996)
Height	2 (QTL×E)[a]	11	-	11	Siripoonwiwat et al. (1996); De Koeyer et al. (2001)
	5	24	-	-	
Yield	2	-	-	11	De Koeyer et al. (2001)
Plump kernels/ kernel width	4+16	27	-	-	Groh et al. (2001)
Oil	15	-	22	-	Kianian et al. (1999)
	UL[b] (bcd1829)	37(M)[c]	-	-	
Heading date	9	17(M)	-	-	Siripoonwiwat et al. (1996)
β-glucan	18	-	7(M)	-	Kianian et al. (2000)
	18	14(M)	3(M)	-	
	21	3(M)	14(H)[c]	-	
	29	-	-	-	
			-		

[a] *QTL×E* QTL-by-environment interaction
[b] *UL* Unlinked marker
[c] *(M)* Relationship based on marker name only, *(H)* relationship based on homology to KO

marker cdo212x); oil with protein (TM_15 and unlinked marker bcd1829y); and the kernel size parameters kernel weight and plump or thin kernels with each other (TM_35 and unlinked marker bcd1872a). Lodging QTLs have also been shown to map to regions affecting plant height in a wheat × spelt population (Keller et al. 1999) and regions affecting plant height, grain yield, kernel weight, and test weight in barley (Tinker et al. 1996). Several kernel size parameters measured by digital image analysis mapped to the same regions in both KO and KM (Groh et al. 2001). No previous QTL study has reported the oil-protein relationship in oat; however, there have been other reports of a strong negative correlation between these traits (Brown et al. 1966).

Comparison to other QTLs in oat

Accurate comparison of QTLs across populations requires linkage maps with common markers. In hexaploid oat, this process is confounded by the multi-allelic nature of many RFLP markers and the prevalence of inter-varietal chromosome structural rearrangements and duplicated loci (O'Donoughue et al. 1995; Portyanko et al. 2001; Wight et al. 2003). This may be further complicated by the possibility of gene loss during the evolution of allopolyploids, as reported in wheat (Kashkush et al. 2002).

When possible, TM linkage groups were assigned corresponding KO (Wight et al. 2003) or KM (Groh et al. 2001) linkage group numbers to facilitate inter-population map comparison. QTLs for test weight, plant height, grain yield, oil content, and plump kernels (or kernel width) were detected in the same genomic regions in different oat populations (Table 6). When the locations of different RFLP markers associated with identical cDNA clones were analyzed, QTLs were also detected in homoeologous regions in the different oat populations (Table 6). These include heading date, oil content, and β-glucan. Also, the region containing a β-glucan QTL on TM_18 is homoeologous to a region near the oat β-glucanase gene

located on KO_24_26_34. The β-glucanase enzyme catalyzes the hydrolysis of 1,4-β-D-glycosidic linkages in β-D-glucans containing 1,3- and 1,4-bonds (Yun et al. 1993). In addition, a plant height QTL×E interaction on TM_25 maps near umn145 and a second homeologous umn145 locus was previously found to be associated with the dwarfing gene *Dw6* in an 'OT207' × 'Kanota' population (Milach et al. 1997). To our knowledge, this is the first formal report of QTLs affecting lodging, kernel weight, and protein content in oat, so no comparison amongst populations was possible for these traits.

Comparison to QTLs identified in other populations may also provide evidence for determining trait relationships at the genetic level. Three QTLs affecting kernel size (area, length, and width) were detected in KO and KM in regions that correspond to grain yield QTLs in TM. QTLs affecting kernel area and length on KM_11 (homologous to TM_2) and a QTL affecting kernel width on KO_5 (homologous to TM_22) were identified in the KM and KO populations, respectively (Groh et al. 2001). Kernel area and width, measured using digital image analysis, were correlated with kernel weight in a previous study (De Koeyer et al. 1993) and kernel weight is one of three components of grain yield in oat (Grafius 1956). The KO_11 (homologous to KM_11 and TM_2) linkage group is an interesting region of the oat genome, as it has been shown to be the location of QTLs affecting oil (Kianian et al. 1999) and β-glucan (Kianian et al. 2000) in KO and KM and various agronomic characters in KO (Siripoonwiwat et al. 1996), a recurrent selection population (De Koeyer et al. 2001), and TM (current study).

A QTL affecting β-glucan on TM_18 not only corresponds to the genomic region containing the β-glucanase gene on KO_24, but also to the homoeologous location of a heading date QTL on the same linkage group (Siripoonwiwat et al. 1996). Heading date QTLs were also associated with β-glucan QTLs in three genomic locations in previous reports (Kianian et al. 2000; Groh et al. 2001). The common location of heading date and β-glucan QTL could indicate a pleiotropic effect caused when later maturing lines fail to complete the optimum

grain filling period, which results in shrunken kernels with less endosperm and enhanced relative β-glucan content. The unlinked marker bcd1872a, associated with thin and plump kernels in TM, maps to a region on KO_27 where a test-weight QTL in KO is located (Siripoonwiwat et al. 1996). This cross-population trait association is the same as one detected on TM_1 in our study. As more QTL studies in oat are completed, comparison of QTLs across populations will give insight into the genetic basis of traits in the various populations.

QTL detection methods

One-half (9 of 18) of the QTLs identified in the whole population mapped to the region containing the *N1* locus on linkage group TM_5. Additional QTLs were localized using *N1* as a covariate in the additive genetic model and in the two sub-populations. QTLs detected only within the hulless and covered sub-populations had lower LOD scores, which was likely due to the smaller sample sizes. Analysis of the whole population, combined with the use of *N1* as a covariate in the analyses, was the best approach for identifying the most important genomic regions affecting agronomic and quality traits in the TM population. Population size is a critical factor in determining the probability of detecting a QTL within a mapping population (van Ooijen 1992). Therefore, statistical approaches that maintain the full population size are more powerful than approaches that use separate sub-populations. Further studies within larger covered and hulless sub-populations may validate the robustness of the QTLs identified in this study.

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