

## QTLs and Epistasis Associated with Vernalization Responses in Oat

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### ABSTRACT

Oat (*Avena sativa* L.) genotypes differ in their patterns of growth and development in response to vernalization (cold temperatures applied to germinating seeds). Genomic regions controlling vernalization response in heading date, plant height, and tiller number were mapped in a recombinant inbred (RI) population derived from the cross of oat cultivars 'Kanota' (vernalization-responsive) and 'Ogle' (vernalization-insensitive). Seventy-one F<sub>6</sub>-derived RI lines were subjected to vernalization and no-vernalization treatments, and then grown in growth chambers. A genetic linkage map of 561 (primarily RFLP) loci was used to identify quantitative trait loci (QTLs) affecting the traits in vernalized and non-vernalized plants. Nine to 16 linkage groups and unlinked loci were associated with each trait assessed herein. Individual loci explained up to 37% of the phenotypic variation. Three to five significant loci were included in multiple locus linear models which explained up to 66% of phenotypic variation for each trait. One to 14 interactions between loci were found for each trait. The interactions explained up to 30% of the phenotypic variation not accounted for by the main effects of loci involved in the interactions. Inclusion of epistatic interactions tended to improve the fit of multiple locus models. As much as 83% of phenotypic variation was explained by multiple locus models including epistasis. Numerous epistatic interactions involving at least one locus with no significant main effect were detected.

OAT genotypes vary in their responses to vernalization, a cold temperature treatment applied during germination. Fall-sown "winter oat" cultivars tend to require vernalization in order to flower at an optimum date in their environment, while spring oat cultivars generally neither require nor respond to vernalization (King and Bacon, 1992). Vernalization of winter types tends to decrease their time to heading, plant height, and number of tillers (Sorrells and Simmons, 1992). Changes in such traits would have significant influence on the productivity of genotypes. Little is known about the genetic control or genomic location of factors influencing vernalization response in oat (Brouwer and Flood, 1995). Such information might provide some insight into genotype  $\times$  environment interaction and facilitate gene transfer between the gene pools of winter- and spring-sown oat and those of other crops as well. Rose (1966) reported that three dominant genes with epistatic effects inhibit early initiation of flowering in some oat cultivars with quantitative vernalization and photoperiod requirements. However, Rose's (1966) study was based on classifying segregating progenies simply as either early or late and did not involve a vernalization treatment. Individual genes with qualita-

tive effects on vernalization have been identified in wheat (*Triticum aestivum* L., Flood and Halloran, 1986), barley (*Hordeum vulgare* L., Takahashi and Yasuda, 1971; Laurie et al., 1995), and rye (*Secale cereale* L., Brule-Babel and Fowler, 1989). QTLs affecting vernalization response of flowering have been localized in barley (Hackett et al., 1992; Pan et al., 1994) and several *Brassica* species (Kennard et al., 1994; Ferreira et al., 1995; Teutonico and Osborn, 1995).

O'Donoghue et al. (1995) developed a molecular genetic linkage map based on an RI population of oat produced from the cross of a spring oat cultivar, Ogle, and a 'facultative' winter oat cultivar, Kanota. Siripoon-wiwat et al. (1996) mapped QTLs affecting agronomic characteristics in this population. We used a QTL mapping approach to determine which genomic regions affect vernalization response for plant height and heading date in this population. In addition, we investigated the possibility that epistatic interactions between pairs of loci affect vernalization responses.

### MATERIALS AND METHODS

#### Development of Mapping Population and Maps

Parents of the RI population differ in region of adaptation and response to vernalization. Ogle (C.I. 9401) was developed in the spring oat breeding program at the University of Illinois (Brown and Jedlinski, 1983). Kanota (C.I. 839) was developed from mass selection performed in Kansas in a cultivar, 'Fulghum', developed in Georgia (Stanton et al., 1927; Coffman et al., 1961). Kanota and Fulghum are adapted to both winter sowing and spring sowing (Coffman et al., 1961). The development of the Kanota  $\times$  Ogle RI population, genotypic data collection, and development of the molecular linkage map were described in detail by O'Donoghue et al. (1995). The linkage map including all 561 loci described by O'Donoghue et al. (1995) was used in the QTL mapping study described here. The loci are organized into 38 linkage groups (LGs), and 29 unlinked loci. We evaluated the same 71 F<sub>6</sub>-derived lines and used seed from the original parents used to develop the linkage map in our growth chamber experiments (O'Donoghue et al., 1995).

#### Phenotypic Evaluations

Six seeds of each line were germinated by placing them on filter paper soaked with distilled water at 21°C for 12 h in dark; then they were transferred to 6°C for 21 d in dark to be vernalized. Six non-vernalized seeds of each line were germinated at 21°C for 1 d in dark. Vernalized and non-vernalized seeds were planted at the same time and were at approximately the same stage of germination when planted. Experimental units were 15-cm-diam. pots each containing three vernalized and three non-vernalized seeds of a RI line or a parent. Parent lines were subjected to the same treatments and two replicate pots of each were included in each growth chamber. The potting medium consisted of 200 g kg<sup>-1</sup> soil, 400 g kg<sup>-1</sup> cana-

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**Abbreviations:** cM, centimorgan; LG, linkage group; QTL, quantitative trait locus; RI, recombinant inbred; RFLP, restriction fragment length polymorphism; SS, sums of squares.

dian peat moss, and 400 g kg<sup>-1</sup> perlite. A randomized complete block design using two growth chambers as blocks was used. The first replicate growth chamber was set at 16-h photoperiod and 22°C day, 18°C night temperatures. The second replicate growth chamber was set at 16-h photoperiod and 20°C day, 18°C night temperatures. Growth chamber lights were a combination of fluorescent and incandescent bulbs producing 960 μmol<sup>-2</sup> s<sup>-1</sup> photon flux density. These conditions represent optimum growing conditions for most oat varieties (Sorrells and Simmons, 1992). Days from emergence to heading (complete panicle extension) and plant height at heading were recorded on each plant. Vernalization response for each trait was calculated for each line in each replication as the mean of the non-vernalized plants minus the mean of the vernalized plants in the same pot. Thus, a positive response to vernalization signifies that plants headed earlier and achieved shorter stature following the vernalization treatment as compared to the no vernalization treatment.

### Analyses of Phenotypic Variation and QTLs

Analyses of variance were performed for each trait to determine if significant variation existed among RI lines. Means of each RI line were calculated for use in QTL detection analyses. Parental means were also computed to investigate the possibility of transgressive segregation by comparing them to RI line means.

Single-factor analyses of variance were computed for each locus-trait combination, and additive effects of loci were calculated following Edwards et al. (1987). Two-factor analyses of variance were also computed for each possible pair of loci to determine main effects of the two loci plus their interaction (Damerval et al., 1994; Edwards et al., 1987). Preselection techniques were used to reduce the number of factors to be considered for building linear additive models for each trait. Main effects of loci were considered for model building if they were significant at  $P \leq 0.05$ . To reduce problems of collinearity among loci due to linkage, each significant locus on a linkage group (LG) was paired with each other significant locus on that LG to create two-factor models without interactions. Only if both loci were significant at  $P \leq 0.05$  in the two-factor model would they both be considered in the model-building step. Otherwise, the locus with the greatest individual  $r^2$  value was chosen to represent the effect of the putative QTL on the LG. If two loci remained significant in a two-factor model, both loci would be considered in the model building step, on the supposition that two QTLs could be present in the LG. If the locus with the largest  $r^2$  value was not among those, it would also be included in the model building step. In only one case (heading date in vernalized plants, LG 17), two different significant pairs of loci on the same LG were considered for the model building step.

Preselection was also performed on the epistatic interactions before considering them for model building. Only those interactions with full model probability levels of less than  $10^{-5}$  were considered for model building. This number was chosen because 703 independent combinations exist among the 38 LGs analyzed. A comparison-wise error rate of  $7 \times 10^{-5}$  for 703 independent tests would correspond approximately to an experiment-wise error rate of 0.05. This is a liberal estimate of the genome-wise error rate for epistatic interactions because more than 703 tests were made, although there was a great deal of dependency among tests. A full model (main effects plus interaction) significant at  $P \leq 0.05$  did not necessarily imply that the interaction alone was significant. Therefore, the  $F$ -test of the interaction based on Type III sums of squares for each remaining model was examined, and pairs with non-

significant interactions were eliminated. In addition, the effects of linkage on both loci involved in an interaction were considered by choosing the interaction model with the highest  $R^2$  among those interaction models involving the same two LGs. Another interaction between those two LGs would be allowed only if at least one of the loci involved in it were more than 50 centimorgans (cM) from loci involved in the first interaction.

“Partial  $R^2$ ” parameters were estimated for each interaction effect and refer to the amount of variation explained by the interaction effect after accounting for the main effects of loci. This was calculated by dividing the Type III sums of squares for the interaction effect by the total sums of squares for the model which included both main effects plus the interaction effect.

The genome-wise  $\alpha = 0.05$  threshold levels for additive effects of each trait were determined from permutation tests (Churchill and Doerge, 1994). We used QTL Cartographer software to perform 1000 permutations of whole genome linear regression analyses for each trait. This provided estimates of the genome-wise threshold values for likelihood ratio statistics. We estimated the threshold levels for additive effects from markers with likelihood ratio statistics approximately equal to the threshold level.

### Linear Additive Model Selection

Linear additive model building techniques were used to identify best models including and excluding epistatic terms. “Best” models were those with greatest  $R^2$  values under the restriction that all factors in the model remained significant at  $P \leq 0.05$  when Type III sums of squares were considered. The only exception was that main effects of loci included in interaction terms were not restricted to be significant, although the interactions themselves were. The best model excluding epistatic terms was identified by considering all of the single locus factors remaining after preselection and using SAS Proc Reg (SAS Institute, 1987) to select the ten best models based on  $R^2$  values for each subset of models with a given number of loci. All of these models were then treated as linear additive models, with the loci considered class variables and re-analyzed by SAS Proc GLM (SAS Institute, 1987). All models from this step in which all loci were significant at  $P \leq 0.05$  were used in further model building steps. The single best model without epistasis was chosen from among these based on  $R^2$  value, without regard to number of loci.

Models including epistasis were developed by first constructing all possible models containing two preselected interaction terms and their main effects. The ten best models (those with greatest  $R^2$  values and with both interaction terms remaining significant) were advanced to the next step, which was to attempt to add a third interaction term to each of the ten models. All preselected interaction terms were added to the 10 models and the 10 best models from among all of these with three significant interaction terms were advanced to the next step. This process was continued in an iterative fashion, adding all preselected interaction terms to the ten best models from the previous step and selecting the 10 best models that resulted, until no further models could be developed in which all interaction terms remained significant.

Next, all preselected interaction terms (main effects plus interaction) were combined with each of the preselected single locus factors one at a time. The 10 best models with both a significant single locus and a significant interaction factor were chosen for the next step. All possible interaction terms were added to the selected models and evaluated in the same manner, keeping the ten best models with the single locus and

both interaction terms remaining significant. Again, this process was continued until no further epistatic terms could be added to develop models which met the restrictions.

All possible epistatic terms were then added to the models composed of two significant locus main effects selected from the multiple regression step. The iterative process of adding epistatic terms to these models was executed in the manner described for models with one single factor term. This process was also applied to all of the models chosen from the multiple regression step possessing more than two single factor terms, as long as all single factors were significant. Ultimately, for each trait, a large number of models with a range of numbers of both single locus factors and epistatic interaction factors were developed. The model with the greatest  $R^2$  value and with all single locus terms and all interaction terms significant was chosen from among all of these models to be the best model that included epistasis.

## RESULTS AND DISCUSSION

### Trait Statistics

Analysis of variance results indicated that variation among lines for plant height and heading date traits was highly significant, and these were analyzed for QTLs. Parental means, overall population means, population extremes, and least significant differences for mean comparisons for each trait are presented in Table 1. Continuous variation in the population was observed for each trait. Transgressive segregation was observed for each trait except for vernalization response in heading date (Table 1). Although the two parents had nearly identical heading dates after vernalization treatment, the range of the population was nearly as great for this trait as for heading date without vernalization, for which the parents differed greatly. This result suggests that the parents differ for genes controlling time to heading, and that some of the genes are sensitive to vernalization.

While the difference between Ogle and Kanota heading dates was essentially eliminated by vernalization, they did not differ significantly for plant height with or without vernalization (Table 1). However, the parents differed significantly for vernalization response because Ogle's height was not affected by vernalization, while Kanota's height was reduced following vernalization. Also, vernalization response for height varied in sign to a greater extent than did response for heading (Table 1). Thus, while most lines were shorter following vernalization, some lines were significantly taller following vernalization. One possible interpretation is that Kanota

possesses a gene or genes that promote reduced plant height to a greater extent than the alleles from Ogle when activated by vernalization, but when not activated they confer increased plant height compared to alleles from Ogle. In addition, the situation is further complicated by the apparent presence of a gene or genes that promote increased plant height following vernalization, which caused some lines to have negative vernalization responses (Table 1).

### Heading Date QTLs

Marker loci considered for building multiple locus models for heading date based on the selection criteria described are presented in Table 2. The genomic regions represented by these marker loci can be classified as vernalization sensitive or vernalization insensitive based on their association with vernalization response for heading date. A region on LG 24 (e.g., Xbcd1968B) had the strongest association with the vernalization response for heading date. It was also associated with heading date in both vernalized and non-vernalized plants, but the association was reduced by the vernalization treatment. In non-vernalized plants the Kanota allele at this locus was associated with an increase in 4.4 d to heading, and was the most important locus for the trait, explaining 37% of the phenotypic variance. In vernalized plants the Kanota allele was also significantly associated with later heading, but the effect was reduced to 2.2 d and it was no longer the most important locus for the trait, explaining only 14% of the phenotypic variance. As a result, the Kanota allele was associated with a vernalization response of 2.2 d, the difference between its effect in non-vernalized and vernalized plants, and Xbcd1968B explained 29% of the phenotypic variance for vernalization response.

Another locus associated with vernalization response for heading date was unlinked locus Xumn624, which was also associated with heading date in non-vernalized plants, but not in vernalized plants. At this locus the Ogle allele was associated with later heading and vernalization response.

Other loci associated with significant vernalization responses had significant effects on heading time in neither vernalized nor non-vernalized plants. Some of these loci, including Xbcd1819 on LG 2, Xbcd1876 on LG 13, Xbcd1380A on LG 15, Xcdo1428A on LG 20, Xbcd880B on LG 33, and Xbcd1840B on LG 35, appeared to act

**Table 1. Parental and population means, population maxima and minima, and least significant differences (LSD)<sup>†</sup> for mean comparisons for heading date and plant height traits.**

	Heading date			Plant height		
	Non-vernalized	Vernalized	Response	Non-vernalized	Vernalized	Response
	d			m		
Kanota	52.6	37.6	15.0	0.806	0.689	0.116
Ogle	37.9***	37.5	0.4***	0.744	0.746	-0.002***
LSD <sub>1</sub> (0.05)	3.7	3.3	4.0	0.097	0.095	0.081
Pop. Mean	44.9	40.6	4.7	0.752	0.691	0.063
Pop. Min.	32.2*	32.5*	-3.6	0.589***	0.435***	-0.107*
Pop. Max.	64.2**	60.2***	16.3	0.918	0.925**	0.282***
LSD <sub>2</sub> (0.05)	4.6	4.0	5.0	0.118	0.116	0.099

\*, \*\*, \*\*\* Designate parental differences or differences between population extreme and most similar parent significant at  $P \leq 0.05$ , 0.01, and 0.001, respectively.

<sup>†</sup> LSD<sub>1</sub> (0.05) is the LSD for parental comparisons at  $P \leq 0.05$ . LSD<sub>2</sub> (0.05) is the LSD for comparing a single RI line (such as a population extreme) to a parent at  $P \leq 0.05$ .

as vernalization-sensitive genes that had small effects just below the threshold level in one treatment, and nil effect in the other. Two loci with significant vernalization responses, unlinked locus Xcdo447 and Xbcd1643C on LG 22, had small effects on heading time that were positive in one treatment and negative in the other. The effects were not significant in either treatment, but the relatively great difference between the effects in the different treatments caused a significant vernalization response.

Other loci appeared to affect heading date in a vernalization-insensitive manner. These regions affected heading in a consistent manner in both vernalized and non-vernalized plants, and therefore, did not affect vernalization response. Loci that typified this behavior included Xcdo962B on LG 7, Xisu1364A on LG 17, and Xisu0582B on LG 23. A region on LG 17 characterized by Xisu1364A had the greatest effect on heading in vernalized plants, explaining 19% of the phenotypic variance. The Kanota allele at this locus was associated with an increase of about 2.5 heading days in both vernalized and non-vernalized plants, and was not associated with vernalization response. Xisu0582B differed slightly in that its Kanota allele was associated with earlier heading, which helps to explain the transgressive

segregation for heading observed in both vernalized and non-vernalized plants. These vernalization-insensitive loci contributed to the high positive correlation ( $r = 0.81$ ,  $P < 0.001$ ) observed between heading date in non-vernalized and vernalized plants.

Other loci listed in Table 2 were significantly associated with heading date in only treatment, but did not have significant vernalization responses. These loci are more difficult to classify according to vernalization response. It is possible that some of these regions are vernalization insensitive and affect heading date with or without vernalization, but their effects in one treatment were not detected. On the other hand, it is possible that some of these regions were associated with a vernalization response, but the response was not detected. Finally, it is possible that no QTLs exist in some of these regions and that they were detected as false positives because of the liberal genome-wide Type I error rate.

Response to vernalization for heading date was highly correlated with heading date in non-vernalized plants ( $r = 0.60$ ,  $P < 0.001$ ) but not in vernalized plants ( $r = 0.16$ ,  $P > 0.05$ ). Among the 12 LGs with significant vernalization responses for heading date, seven had positive responses (Kanota contributed the more vernalization-sensitive allele) and five had negative responses

**Table 2. Loci associated with heading date considered for multiple regression models.**

Linkage group†	Position	Marker	Non-vernalized		Vernalized		Vernalization response	
			Additive effect‡§	<i>r</i> <sup>2</sup>	Additive effect	<i>r</i> <sup>2</sup>	Additive effect	<i>r</i> <sup>2</sup>
-	-	Xumc44	-2.1*	<i>0.086</i>	-1.3	NS	-1.0	NS
-	-	Xumn624	-2.8***	<i>0.158</i>	-1.2	NS	-1.9***	<i>0.212</i>
-	-	Xumn159	1.4	NS	1.5*	<i>0.069</i>	0.1	NS
-	-	Xumn5032	-1.5	NS	-1.7*	<i>0.080</i>	0.1	NS
-	-	Xcdo447	0.5	NS	-0.3	NS	1.0*	<i>0.064</i>
2	6.1	Xbcd1095A	1.7*	<i>0.063</i>	0.6	NS	0.9	NS
2	20.1	Xbcd1819B	1.2	NS	0.0	NS	1.1*	<i>0.069</i>
3	4.1	Xbcd1405	1.4	NS	2.2**	<i>0.141</i>	-0.5	NS
5	89.8	Xcdo1433	-1.6	NS	-2.0**	<i>0.124</i>	0.2	NS
6	49.0	Xcdo420C	-1.5	NS	-1.5*	<i>0.067</i>	-0.2	NS
6	79.5	Xisu1755A	-1.0	NS	-1.7*	<i>0.084</i>	0.4	NS
7	14.4	Xcdo962B	-1.8*	<i>0.069</i>	-2.0**	<i>0.118</i>	0.1	NS
8	39.2	Xbcd1950B	-1.4	NS	-1.6*	<i>0.080</i>	0.3	NS
13	38.6	Xcdo460A	1.8*	<i>0.062</i>	0.9	NS	0.6	NS
13	60.0	Xbcd1876	1.2	NS	0.2	NS	1.2*	<i>0.088</i>
14	28.1	Xumn339A	-0.2	NS	1.0	NS	-1.0*	<i>0.071</i>
15	51.4	Xbcd1380A	-0.2	NS	-1.2	NS	1.0*	<i>0.062</i>
16	42.2	Xbcd1117	-0.9	NS	-0.1	NS	-1.0*	<i>0.069</i>
17	13.9	Xcdo1467A	1.9*	<i>0.079</i>	2.4***	<i>0.180</i>	-0.3	NS
17	18.0	Xumn370	2.5**	<i>0.138</i>	2.4***	<i>0.176</i>	0.3	NS
17	26.9	Xisu1755B	2.1*	<i>0.093</i>	2.4***	<i>0.174</i>	0.0	NS
17	37.6	Xisu1364	2.4**	<i>0.126</i>	2.5***	<i>0.193</i>	0.1	NS
17	55.1	Xisu1247B	2.3**	<i>0.105</i>	1.8*	<i>0.092</i>	0.1	NS
17	61.0	Xisu1900A	2.4**	<i>0.118</i>	1.9**	<i>0.110</i>	0.0	NS
20	17.5	Xcdo1428A	1.3	NS	-0.2	NS	1.3**	<i>0.100</i>
22	0.0	Xumn287	-1.3	NS	-1.6*	<i>0.076</i>	0.0	NS
22	56.5	Xbcd1643C	-0.9	NS	0.7	NS	-1.2*	<i>0.100</i>
23	0.0	Xisu0582B	-2.4**	<i>0.122</i>	-2.0**	<i>0.122</i>	-0.6	NS
24	52.2	Xbcd1797D	3.3***	<i>0.220</i>	1.4*	<i>0.058</i>	2.1***	<i>0.268</i>
24	57.5	Xbcd1968B	4.4***	<i>0.369</i>	2.2**	<i>0.139</i>	2.2***	<i>0.291</i>
24	62.9	Xcdo484B	3.6***	<i>0.282</i>	1.6*	<i>0.090</i>	2.1***	<i>0.260</i>
24	67.2	Xcdo1523A	3.4***	<i>0.233</i>	1.7*	<i>0.087</i>	1.7***	<i>0.188</i>
28	35.5	Xbcd1212	-1.5	NS	-1.4*	<i>0.058</i>	0.1	NS
33	-1.0	Xbcd880B	1.2	NS	0.0	NS	1.0*	<i>0.061</i>
35	0.0	Xbcd1840B	1.3	NS	0.1	NS	1.4**	<i>0.101</i>
37	6.7	Xcdo1414B	-2.0*	<i>0.079</i>	-1.2	NS	-0.8	NS
Genome-wise $\alpha = 0.05$ threshold			3.9		2.5		1.9	

\*\*\* Designate significant additive effects at  $P \leq 0.05$ ,  $P \leq 0.01$ , and  $P \leq 0.001$ , respectively, based on comparison-wise  $\alpha$  levels.

† "-" denotes unlinked loci.

‡ Additive effects were calculated as  $0.5 \times$  (mean of plants homozygous for the Kanota allele at the locus - mean of plants homozygous for Ogle allele at the locus) in units of days.

§ Loci with additive effects and  $r^2$  values in italic print for a given trait were considered for linear additive model building steps for that trait.

(Ogle contributed the more vernalization-sensitive allele). All seven vernalization-sensitive regions contributed by Kanota were associated with later flowering in non-vernalized plants, but only two were in vernalized plants. Of the five vernalization-sensitive regions from Ogle, four contributed to later flowering in non-vernalized plants, while three did in vernalized plants. Thus, 11 of 12 vernalization-sensitive regions were associated with later flowering in non-vernalized plants, but only five were in vernalized plants. This result suggests that vernalization-sensitive alleles tended to promote later flowering compared to insensitive alleles, but the difference between alleles was reduced following vernalization.

A possible molecular mechanism for this type of response is that the products of these genes are required to initiate flowering, and vernalization-sensitive alleles tend to produce much less gene product unless they have been vernalized. Burn et al. (1993) suggested that vernalization acts to reduce DNA methylation and this promotes expression of genes required for early flowering.

### Plant Height QTLs

Loci associated with plant height and chosen with the selection techniques described are presented in Table 3. These loci can be classified according to vernalization response as were those affecting heading time. Kanota-

derived alleles at vernalization-sensitive regions detected on LGs 6 (e.g., Xumn361), 13 (Xbcd1495), 24 (Xbcd1643A), and 33 (Xbcd269) were associated with shorter plants, with larger effects detected in vernalized compared with non-vernalized plants. On the other hand, the Kanota allele of Xisu2013 on LG 17 was associated with taller plants; but again, the effect was greater in vernalized than in non-vernalized plants. As was the case for heading time, some loci showed relatively small, insignificant effects in both treatments, but the effects had opposite sign in vernalized compared with non-vernalized plants, contributing to significant vernalization responses. This was observed for unlinked locus Xcdo393C and loci on LGs 15 (Xbcd1823B), 28 (Pgm), and 30 (Xptb17).

Vernalization-insensitive regions included loci associated with plant height in both vernalized and non-vernalized plants, such as unlinked loci Xcdo677 and Xumn51B, Avn-B on LG 5, Xcdo1090A on LG 6, Xcdo1090E on LG 14, Xbcd1797A on LG 22, and Xbcd829B on LG 37. Loci on LGs 16 (Xumn13), 20 (Xcdo1428A), 23 (Xumn339B), and 25 (Xbcd1235) also had no detectable vernalization response and had consistent effects in the two treatments. However, their effects on plant height were significant only in non-vernalized plants because of greater error variance in vernalized plants. These regions contributed to the positive phenotypic correlation between plant heights in the two treatments ( $r = 0.71$ ,  $P < 0.001$ ).

**Table 3. Loci associated with plant height considered for multiple regression models.**

Linkage group†	Position	Locus	Non-vernalized		Vernalized		Vernalization response	
			Additive effect‡§	$r^2$	Additive effect	$r^2$	Additive effect	$r^2$
		<b>m</b>			<b>m</b>		<b>m</b>	
–	–	Xcdo677	<i>0.027**</i>	<i>0.131</i>	<i>0.029**</i>	<i>0.090</i>	0.000	NS
–	–	Xumn51B	<i>–0.021*</i>	<i>0.087</i>	<i>–0.033**</i>	<i>0.125</i>	0.010	NS
–	–	Xcdo393C	0.006	NS	–0.013	NS	<i>0.017*</i>	<i>0.072</i>
5	37.7	Xcdo1165A	<i>–0.020*</i>	<i>0.076</i>	<i>–0.014</i>	NS	–0.008	NS
5	90.8	Avn-B	<i>–0.031***</i>	<i>0.176</i>	<i>–0.039***</i>	<i>0.164</i>	0.006	NS
6	52.3	Xcdo56	–0.006	NS	<i>–0.029*</i>	<i>0.095</i>	<i>0.021**</i>	<i>0.099</i>
6	58.0	Xumn361	–0.005	NS	<i>–0.030*</i>	<i>0.091</i>	<i>0.023**</i>	<i>0.116</i>
6	84.5	Xcdo595	<i>–0.024**</i>	<i>0.096</i>	<i>–0.038**</i>	<i>0.139</i>	0.011	NS
6	86.7	Xcdo1090A	<i>–0.026**</i>	<i>0.121</i>	<i>–0.040***</i>	<i>0.175</i>	0.012	NS
11	17.3	Xbcd1270	–0.017	NS	<i>–0.026*</i>	<i>0.079</i>	0.008	NS
13	29.8	Xbcd1443B	<i>–0.022*</i>	<i>0.068</i>	<i>–0.029*</i>	<i>0.072</i>	0.004	NS
13	69.0	Xbcd1495	–0.005	NS	<i>–0.028*</i>	<i>0.079</i>	<i>0.024**</i>	<i>0.128</i>
13	70.2	Xog49	–0.009	NS	<i>–0.029*</i>	<i>0.087</i>	<i>0.018*</i>	<i>0.072</i>
14	34.4	Xcdo1090E	<i>0.023*</i>	<i>0.096</i>	<i>0.030**</i>	<i>0.103</i>	–0.006	NS
14	36.0	Xcdo57D	<i>0.021*</i>	<i>0.088</i>	<i>0.030**</i>	<i>0.109</i>	–0.007	NS
15	7.2	Xbcd1823B	0.002	NS	–0.015	NS	<i>0.017*</i>	<i>0.067</i>
16	31.3	Xumn13	<i>–0.018*</i>	<i>0.061</i>	<i>–0.021</i>	NS	0.001	NS
17	55.1	Xisu1247B	<i>0.019*</i>	<i>0.063</i>	<i>0.038**</i>	<i>0.149</i>	<i>–0.017*</i>	<i>0.063</i>
17	83.7	Xisu2013	0.011	NS	<i>0.034**</i>	<i>0.129</i>	<i>–0.021**</i>	<i>0.103</i>
20	17.5	Xcdo1428A	<i>0.024**</i>	<i>0.104</i>	0.022	NS	0.000	NS
21	2.5	Xbcd782	–0.006	NS	–0.020	NS	<i>0.016*</i>	<i>0.059</i>
21	3.5	Xbcd1250	–0.013	NS	<i>–0.026*</i>	<i>0.072</i>	0.015	NS
22	62.5	Xbcd1797A	<i>–0.024**</i>	<i>0.125</i>	<i>–0.030**</i>	<i>0.120</i>	0.004	NS
23	22.4	Xumn339B	<i>–0.021*</i>	<i>0.084</i>	–0.017	NS	–0.002	NS
24	46.9	Xbcd1643A	<i>0.021*</i>	<i>0.077</i>	–0.005	NS	<i>0.026**</i>	<i>0.144</i>
24	59.8	Xumn220	<i>0.021**</i>	<i>0.079</i>	0.010	NS	0.013	NS
25	0.0	Xbcd1235	<i>–0.021*</i>	<i>0.085</i>	–0.023	NS	0.000	NS
28	12.1	Pgm	0.013	NS	–0.014	NS	<i>0.025*</i>	<i>0.171</i>
30	0.0	Xptb17	–0.006	NS	0.012	NS	<i>–0.017*</i>	<i>0.068</i>
33	14.2	Xbcd269	<i>–0.020*</i>	<i>0.070</i>	<i>–0.036**</i>	<i>0.155</i>	<i>0.016*</i>	<i>0.067</i>
36	12.5	Xbcd1407	<i>0.026**</i>	<i>0.127</i>	0.017	NS	0.008	NS
37	0.0	Xbcd1829B	<i>–0.024**</i>	<i>0.107</i>	<i>–0.039***</i>	<i>0.171</i>	0.014	NS

\*\*\*, \*\*\*, \* Designate significant additive effects at  $P \leq 0.05$ ,  $P \leq 0.01$ , and  $P \leq 0.001$ , respectively, based on comparison-wise  $\alpha$  levels.

† “–” denotes unlinked loci.

‡ Additive effects were calculated as  $0.5 \times (\text{mean of plants homozygous for the Kanota allele at the locus} - \text{mean of plants homozygous for Ogle allele at the locus})$  in units of days.

§ Loci with additive effects and  $r^2$  values in italic print for a given trait were considered for linear additive model building steps for that trait.

Response to vernalization for plant height was highly negatively correlated with plant height in vernalized plants ( $r = -0.60$ ,  $P < 0.001$ ) but not in non-vernalized plants ( $r = 0.12$ ,  $P > 0.05$ ). This pattern was opposite to that observed for heading date response. All eight of the vernalization-sensitive regions from Kanota were associated with shorter plant height in vernalized plants, but only four were in non-vernalized plants. The two vernalization-sensitive regions from Ogle were associated with shorter plant height in vernalized plants, while only one was in non-vernalized plants. In sum, 10 vernalization-sensitive regions were associated with shorter plant height in vernalized plants, while only five were in non-vernalized plants. Thus, the effects of vernalization-sensitive regions associated with plant height tended to increase when vernalized. This is in contrast to the general effect of vernalization on vernalization-sensitive regions associated with heading date, which was to reduce differences between allelic effects. One possible interpretation is that vernalization-sensitive alleles of genes affecting plant height tended to promote shorter growth even when they are not vernalized. Their ability to promote shortness could have been enhanced by vernalization, possibly via increased gene expression as postulated previously.

Avn-B on LG 5 had the largest effect on plant height in non-vernalized plants, explaining 18% of the phenotypic variation. It also had a major effect in vernalized plants, explaining 16% of the variation for plant height in that treatment. However, two other regions had slightly larger effects on plant height in vernalized plants. Xcdo1090A on LG 6 explained 18% of the variation, while Xbcd1829B on LG 37 explained 17% of the variation. Interestingly, the region with the largest effect on vernalization response for plant height was on LG 30, where the isozyme locus, Pgm, explained 17% of the variation. This locus had no significant association with plant height in vernalized or non-vernalized plants, but the estimated non-significant effects were of opposite sign in vernalized compared to non-vernalized plants.

### Epistatic Interactions between QTLs

Five pairs were found with significant interaction effects on heading date in vernalized plants, 11 pairs were significant for heading date in non-vernalized plants, and 14 pairs had significant interaction effects on vernalization response in heading date (Table 4). Individual epistatic interactions explained up to 30% of the phenotypic variation for heading date in vernalized plants, 21% in non-vernalized plants, and 25% in vernalization response, after accounting for main effects of the markers. Various forms of epistatic interactions are illustrated in the two-locus homozygous genotypic means in Table 4. For example, both non-parental homozygous genotypes involving loci Xumc44 and Xbcd1716A, had earlier mean heading dates in vernalized plants than did the parental two-locus combinations. Thus, the difference between the Kanota and Ogle homozygous classes at the Xbcd1716A locus was positive when the Xumc44 locus was homozygous for the Kanota allele and negative when Xumc44 was homozygous for the Ogle allele.

By itself, the interaction effect of these two loci accounted for 30% of the phenotypic variance remaining after accounting for main effects of the two loci. The interaction effect of loci Xbcd1950B and Xisu1755A explained 17% of the phenotypic variation after accounting for the main effects of the loci. One of the non-parental two-locus homozygous genotypes had a significantly later heading date in vernalized plants than did the other three genotypes, which all had similar means, suggesting complementary gene action. On the other hand, Xcdo99 and Xog41 interacted significantly because one of the parental homozygous combinations had a significantly later heading date than did the other three homozygous combinations, which had similar means. These three types of epistatic interactions were observed among the other heading date traits as well. Individual epistatic interactions often had large effects on flowering time variables, but the largest epistatic effects were not as great as the most important individual locus additive effects.

Fewer epistatic effects were found for plant height variables. Only one interaction effect was found for each of the three variables, with the most important effect being the interaction between Xcdo665A and Xcdo1090E on plant height in non-vernalized plants, with a partial  $R^2$  value of 27%. Interestingly, this interaction had a greater effect than any of the single-locus additive effects and both loci involved in this epistatic effect are on LG 14, located 34 cM apart. While Xcdo1090E is located in a region considered to have an effect on plant height, Xcdo665A is not. It is possible there are two QTL on LG 14 that interact epistatically.

It was of interest to consider what proportion of these interaction effects would have been detected by testing interactions only between loci with significant main effects, rather than between all loci, regardless of their individual effects. Among all traits, only four of the 35 significant interactions involved loci which both had significant main effects. However, 28 of the other interactions involved one locus which had a significant effect on the trait. Three interactions involved loci which were both individually non-significant. It is clear, therefore, that many significant epistatic interactions would have remained undetected if interactions only between significant individual loci had been examined. The finding that important epistatic interactions were detected between loci with significant main effects and loci without significant main effects has implications for marker-assisted selection of QTLs. The effects of QTLs that exhibit interactions with unlinked genes may be altered dramatically when they are incorporated into a genetic background different from the one in which they were mapped.

### Multiple Locus Models with and without Epistasis

Best linear additive models including and excluding epistatic terms are presented in Table 5. Inclusion of epistasis in models had the greatest effect for those traits in which numerous epistatic interactions were detected. The best models including epistasis for heading date traits had  $R^2$  values from 10 to 28 percentage points

Table 4. Locus pairs exhibiting epistatic interactions.

Locus 1			Locus 2			Two-locus genotypic means‡				
Linkage group	Position	Name	Linkage group	Position	Name	Partial $R^2$ †	Kanota-Kanota	Kanota-Ogle	Ogle-Kanota	Ogle-Ogle
<b>Heading date (non-vernalized plants)</b>										
–	–	Xbcd1414A	24	60.0	Xumn220	0.07	47.56	43.88	49.67	38.58
–	–	Xisu0707A	24	54.0	Xbcd1104	0.23	49.09	41.07	47.50	38.21
3	120.0	Xumn706A	24	54.0	Xbcd1104	0.14	48.39	39.29	47.33	44.12
7	19.2	Xbcd1049	24	60.0	Xumn220	0.09	49.57	37.55	47.07	43.18
12	15.2	Xcdo187	24	60.0	Xumn220	0.09	51.15	40.53	47.28	39.31
14	0.0	Xcdo665A	24	54.0	Xbcd1103	0.19	49.14	41.92	46.36	34.89
16	6.0	Xbcd709	24	57.5	Xbcd1968B	0.06	47.24	41.83	49.79	37.41
18	19.5	Xisu1372B	24	60.0	Xumn220	0.10	46.56	43.19	50.12	37.67
19	3.5	Xisu1247A	24	57.5	Xbcd1968B	0.08	47.55	42.26	49.09	35.30
22	0.0	Xumn856A	24	54.0	Xbcd1103	0.21	47.64	42.26	49.49	38.57
22	74.0	Xbcd1842B	24	57.5	Xbcd1968B	0.10	46.58	39.17	50.92	37.67
<b>Heading date (vernalized plants)</b>										
–	–	Xumc44	36	16.8	Xbcd1716A	0.30	42.16	38.00	39.01	47.63
3	4.1	Xbcd1405	17	10.7	Bcdo1378A	0.06	48.13	39.59	39.87	36.99
8	39.2	Xbcd1950B	17	26.9	Xisu1755a	0.17	38.68	38.83	45.72	38.00
17	19.0	Xcdo99	38	5.6	Xog41	0.12	45.14	38.84	37.69	38.83
17	35.0	Xisu1364A	22	5.0	Xbcd1588	0.11	40.29	46.18	38.57	36.94
<b>Heading date (vernalization response)</b>										
–	–	Xumn624	4	14.0	Xumn341B	0.12	1.78	2.00	6.99	5.26
–	–	Xumn624	10	12.2	Xcdo1388	0.20	3.02	1.60	4.56	7.75
–	–	Xumn624	22	41.4	Xumn114	0.16	1.92	2.06	5.19	7.01
–	–	Coleo	24	57.5	Xbcd1968B	0.04	5.91	3.73	6.77	0.90
–	–	Xumn110	24	57.5	Xbcd1968B	0.14	5.61	1.08	6.85	1.23
–	–	Xbcd880A	24	60.0	Xumn220	0.05	6.16	4.39	7.07	1.45
–	–	Xcdo1385B	24	60.0	Xumn220	0.05	6.80	0.56	6.10	3.35
2	20.0	Xisu2182A	24	57.5	Xbcd1968B	0.07	8.96	–0.15	5.44	1.48
3	35.3	Xbcd115A	24	54.0	Xbcd1103	0.25	6.40	1.42	6.92	2.36
6	41.6	Xumn815B	24	57.5	Xbcd1968B	0.08	6.02	–0.14	5.80	2.28
7	19.2	Xcdo1385C	24	60.0	Xumn220	0.04	7.94	1.69	5.49	2.48
10	15.2	Xcdo1328	24	54.0	Xbcd1797D	0.12	4.51	3.17	7.71	0.69
24	57.5	Xbcd1968B	29	10.3	Xcdo58A	0.11	6.77	5.59	0.90	1.52
24	57.5	Xbcd1968B	36	23.6	Xcdo1321A	0.09	5.92	6.46	1.58	0.72
<b>Plant height (non-vernalized plants)</b>										
14	0.0	Xcdo665A	14	34.4	Xcdo1090E	0.27	0.752	0.779	0.834	0.698
<b>Plant height (vernalized plants)</b>										
6	84.5	Xcdo595	8	39.2	Xbcd1950B	0.11	0.690	0.658	0.686	0.789
<b>Plant height (vernalization response)</b>										
14	30.8	Xcdo1192B	28	12.0	Pgm	0.14	–0.052	–0.055	–0.155	–0.026

† Partial  $R^2$  estimated the amount of phenotypic variance explained by the interaction after accounting for the main effects of loci involved in the interaction. It was calculated as (Interaction SS/Total SS) where the Type III SS from the full model including both main effects and interaction are used.

‡ Two-locus genotypic means were calculated only for doubly homozygous classes. The first parent named refers to genotypes homozygous for the parental allele at the first locus of the pair, while the second parent named refers to genotypes homozygous for the allele from that parent at the second locus of the pair. Units are days (d) or meters (m).

greater than the corresponding best models excluding epistasis. In contrast,  $R^2$  values of the best models for plant height including or excluding epistasis differed by at most 11 percentage points. For plant height in vernalized plants, the best model excluding epistasis had a slightly higher  $R^2$  than did the best model including epistasis. Thus, in general, inclusion of appropriate epistatic terms produced models with higher  $R^2$  values than those without epistasis, although the increase in  $R^2$  values was quite variable, and in one case no improvement was obtained by inclusion of epistatic terms. The model with the greatest  $R^2$  value (0.83) among all of the traits was the model including epistasis for heading date in non-vernalized plants. This model had no significant main effects of loci and only contained significant inter-

action effects. All of the models excluding epistasis had four loci, except heading date in non-vernalized plants, which had five, and plant height vernalization response, which had three. For most traits, the best model with epistasis included some, but not all, of the loci included in the best model without epistasis.

We cannot be sure that the methods used in this study to develop multiple-factor models will always produce the best possible models. We used a method that is similar to forward stepwise regression techniques for model selection. Jansen (1993) suggested that backward elimination would have a better probability of finding the best possible model. In the present study, beginning model selection by backward elimination from the full model of 561 loci would have been impossible because

**Table 5. Best linear models including and excluding epistasis for each trait.**

Models excluding epistasis			Models including epistasis		
Linkage group	Factor	Type III SS	Linkage group	Factor	Type III SS
<b>Heading date (non-vernalized plants)</b>					
2	Xbcd1095A	142.2	—	(Xisu0707A)	58.2
17	Xcdo1467A	239.3	24	(Xbcd1103)	14.0
17	Xisu1247B	159.9	-,24	Xisu0707A × Xbcd1103	308.1
23	Xisu0582B	227.6	7	(Xbcd1049)	0.9
24	Xcdo1523A	468.9	24	(Xumn220)	26.2
	Full model $R^2 = 0.55$		7,24	Xbcd1049 × Xumn220	78.0
			22	(Xbcd1842B)	16.0
			24	(Xbcd1968B)	19.4
			22,24	Xbcd1842B × Xbcd1968B	170.5
				Full model $R^2 = 0.83$	
<b>Heading date (vernalized plants)</b>					
3	Xbcd1405	204.2	3	Xbcd1405	94.7
5	Xcdo1433	182.9	17	Xcdo1467A	80.2
17	Xcdo1467A	454.7	24	Xbcd1968B	108.8
24	Xbcd1968B	168.8	—	(Xumc44)	22.9
	Full model $R^2 = 0.57$		36	Xbcd1716A	62.3
			-,36	Xumnc44 × Xbcd1716A	103.8
			8	(Xbcd1950B)	22.0
			17	(Xisu1755B)	15.3
			8,17	Xbcd1950B × Xisu1755B	97.2
				Full model $R^2 = 0.75$	
<b>Heading date (vernalization response)</b>					
—	Xcdo447	45.3	22	Xbcd1643C	49.6
2	Xbcd1819B	37.2	24	Xbcd1968B	77.2
22	Xbcd1643C	38.2	10	(Xcdo1328)	26.4
24	Xbcd1968B	306.4	24	(Xbcd1797D)	22.7
	Full model $R^2 = 0.66$		10,24	Xcdo1328 × Xbcd1797D	30.5
			2	(Xisu2182A)	12.1
			2,24	Xisu2182A × Xbcd1968B	39.7
				Full model $R^2 = 0.76$	
<b>Plant height (non-vernalized plants)</b>					
5	Avn-B	518.8	5	Avn-B	269.7
13	Xbcd1443B	571.4	13	Xbcd1443B	267.2
23	Xumn339B	418.8	23	Xumn339B	112.2
24	Xumn220	273.1	24	Xumn220	131.9
	Full model $R^2 = 0.49$		14	Xcdo1090E	118.4
			14	(Xcdo665A)	14.4
			14,14	Xcdo1090E × Xcdo665A	244.8
				Full model $R^2 = 0.60$	
<b>Plant height (vernalized plants)</b>					
5	Avn-B	748.8	13	Xog49	699.6
21	Xbcd1250	382.6	6	Xcdo595	537.0
33	Xbcd269	182.6	8	Xbcd1950B	658.1
37	Xbcd1829B	500.6	6,8	Xcdo595 × Xbcd1950B	823.2
	Full model $R^2 = 0.53$			Full model $R^2 = 0.51$	
<b>Plant height (vernalization response)</b>					
—	Xcdo339C	353.4	15	Xbcd1823B	322.2
6	Xumn361	193.0	14	Xcdo1192B	203.4
13	Xbcd1495	407.4	28	Pgm	289.3
	Full model $R^2 = 0.46$		14,28	Pgm × Xcdo1192B	378.6
				Full model $R^2 = 0.54$	

we had data on only 71 lines. On the other hand, it is possible that by reducing the set of loci to be considered for model-building to only those which were individually significant, we may have excluded terms that could have been significant in linear additive models with other terms. Examples of where this did occur in our study can be seen in comparisons of models including and excluding epistasis. For example, Xbcd1716A had no significant effect on heading date in vernalized plants and was not considered for inclusion in the models excluding epistasis. However, it was involved in a significant interaction with another locus for that trait. When included in the best model including epistasis, the main effect of Xbcd1716A, as well as its interaction with Xumc44, was significant. Similar situations were ob-

served for Xbcd1950B for plant height in vernalized plants, and for Xcdo1192B for plant height vernalization response. Thus, while we cannot be confident that we have identified the best possible models using the techniques described here, we were also not able to use backward elimination techniques because of sample size restrictions. The techniques used here were at least able to identify models with reasonably high  $R^2$  values for the situation of small sample size and limited replication.

### Comparison-Wise and Genome-Wise Type I Error Rates

The great number of statistical tests performed in this study raises the issue of genome-wise Type I error rates.

We estimated threshold additive effects that maintained genome-wide error rates of  $\alpha = 0.05$  for the single-factor analyses using permutation tests (Table 2). However, these permutation tests did not account for the simultaneous testing of all possible epistatic interactions. A genome-wide threshold level incorporating both single-factor and two-factor interaction tests could not be determined easily with current software. Based on these threshold levels, only a single region was significantly associated with each heading date variable. A region on LG 24 was associated with heading date in non-vernalized plants and with heading date vernalization response, while a region on LG 17 was associated with heading date in vernalized plants (Table 2). No markers were associated with any plant height variables using the genome-wide threshold levels (thus, the threshold additive effect is not presented in Table 3). Despite this, we wanted to compare our results with those of Siripoonwiwat et al. (1996) who mapped genomic regions associated with plant height and heading date in field experiments in this same population, and considered any region associated with a trait at a comparison-wise  $\alpha = 0.05$  probability level to be a candidate QTL. The use of a less dense map for detecting QTLs in this study would probably have been a wiser choice, as it would have required less stringent genome-wide error rates.

It was not possible to estimate threshold levels for epistatic interactions via permutation tests, but a reasonably conservative threshold level of  $\alpha = 10^{-5}$  was used for the epistatic interactions. This level was chosen based on the idea that at least 703 independent interactions among 38 independent linkage groups were tested, and  $\alpha = 0.5/703 = 7 \times 10^{-5}$ . While 157 080 interactions were actually tested for each trait, each test was statistically dependent on other tests because of linkage (often very tight linkage) between markers. Thus, it is not clear what the comparison-wise error rate should have been to maintain a genome-wide error rate of  $\alpha = 0.05$ , but the rate suggested here, while probably too liberal, is not unreasonable. A total of  $157\,080 + 561 = 157\,641$  single-marker and epistatic interaction tests were performed for each trait. One can compute the probability of making one or more Type I errors from this number of tests based on a comparison-wise error rate of  $\alpha = 10^{-5}$  and assuming that each test is independent, which would clearly provide an overly conservative estimate. Using the assumption of independence among tests, we expect to make 1.6 Type I errors. The probability of no more than one Type I error = 0.533 and the probability of no more than three Type I errors is 0.923. We emphasize that these probabilities apply only to independent tests, which was not the case here. Furthermore, after performing the tests we generally eliminated many significant loci and interactions because they were linked to other significant loci and interactions. Thus, while we most likely have made at least one Type I error here, the numerous locus main effects and epistatic interactions detected cannot all be considered false positives.

From the practical point of view, it would be best to consider the markers and marker interactions presented

as potentially linked to candidate QTLs. We want to compare our results with those of Siripoonwiwat et al. (1996) and to make our results available for comparisons with future studies. For this reason, Type II errors (i.e., not detecting an existing QTL) within this experiment are associated with greater costs than are Type I errors. Furthermore, Beavis (1994) concluded from simulation studies that Type II errors were a greater problem than Type I errors when QTL detection studies were performed in small populations, as in the present study.

Markers and interactions retained in multiple factor models are probably the best candidates for markers linked to QTLs, but the problems associated with developing multiple marker models in small populations must be considered. It is likely that markers associated with QTLs could have been excluded from best models because of small sample size and collinearity with other markers. It is also possible that markers not associated with QTLs could have remained in best models because of collinearity (because of the small population rather than linkage) with markers truly linked to QTLs. Additional information from studies in other oat populations or in larger samples of this same population will be necessary to sort out the regions that have highest likelihood of containing QTLs for these traits.

### Pleiotropic Effects

Candidate regions exhibiting pleiotropic effects might be detected by comparing the genomic distribution of regions affecting heading date with those affecting plant height. We caution, however, that resolution of the QTL maps in this study is not sufficient to distinguish QTLs with pleiotropic effects from tightly linked QTLs affecting the different traits. LG 17 loci had strong effects on heading date and plant height. Interestingly, loci such as Xisu1247B and Xisu1900A, which had effects on both traits, did not show any indication of vernalization sensitivity for heading date, but did have significant vernalization responses for plant height. Loci on LG 24 also had significant effects on both traits. These effects were greater in non-vernalized than in vernalized plants and the vernalization responses for both heading time and plant height were significant. These pleiotropic regions contributed to the significant phenotypic correlations observed between heading date and plant height in both vernalized ( $r = 0.36$ ,  $P < 0.001$ ) and non-vernalized treatments ( $r = 0.41$ ,  $P < 0.001$ ). This association has a developmental basis in that plants that flower later have a longer vegetative growth phase in which to grow taller.

Loci in different regions of LG 6 had different responses to vernalization. Loci in the region of position 49 to 58 cM, such as Xcdo56 or Xumn361, had major effects on plant height in vernalized plants, but not in non-vernalized plants, and had a major effect on vernalization response. Loci in the region of position 84 to 9 cM also had major effects on plant height in vernalized plants, but they also had smaller, yet significant effects on plant height in non-vernalized plants. Although a small vernalization response was associated with this

region, it was not significant. It is possible that two QTLs for plant height exist on LG 6, and they are distinguished by different responses to vernalization. These regions also affected heading date, but in a manner consistent across the two regions. Loci from both regions of LG 6 could be simultaneously fit as significant factors into linear additive models for both heading date and plant height in vernalized plants, lending further support to the hypothesis that two QTL exist in the LG.

### Distribution of QTLs among Homoeologous Regions

Several groups of QTLs mapped to homoeologous regions of the genome. For example, clone Xisu1755 detected QTLs for heading date in vernalized plants on LGs 6 (Xisu1755A) and 17 (Xisu1755B, Table 2). Xbcd1840B on LG 35 was associated with a vernalization response for heading date and an orthologous locus, Xbcd1840A, is linked to Xbcd880B on LG 33, which was also associated with a similar effect. LGs 22, 24, and 28 are included in homoeologous group E (O'Donoghue et al., 1995), and each LG was associated with major effects on traits measured here. Xbcd1797A on LG 22 was associated with vernalization-insensitive effects on plant height; Xbcd1797D on LG 24 was associated with vernalization response for plant height (data not shown, but the effect of this region was represented by linked marker Xbcd1643A in Table 3), and vernalization-sensitive effects on heading date; and Xbcd1797B and Xbcd1797C, both on LG 28, were associated with vernalization response for plant height. (In order to illustrate homoeology of certain regions, we include here results from some significant markers which were not included in Tables 2 and 3 because they are linked to markers with greater effects.) Xcdo962A and Xbcd1280B on LG 17 and orthologous loci Xcdo962B and Xbcd1280A on LG 7 were associated with heading date. Xcdo1378A and Xcdo1467A are closely linked (5.8 and 2.6 cM, respectively) to Xcdo962A and Xbcd1280B on LG 17 and were associated with the same effects. Their homoeologous loci, Xcdo1378B and Xcdo1467B on LG 6, were associated with heading date and plant height traits. Another locus on LG 6, Xcdo1090A, was associated with plant height, and two orthologous loci, Xcdo1090E on LG 14 and Xcdo1090C on LG 20, were associated with, or closely linked to loci associated with plant height and vernalization response for heading date.

It has been suggested that disomy in self-fertilizing polyploids provides opportunities to maintain allelic interactions in the form of additive by additive epistatic interactions among orthologous loci (Mac Key, 1970). However, the interactions between loci reported here did not consistently occur between loci on homoeologous regions.

### Comparisons with QTLs Detected in Other Studies and Other Species

The results of this study can be compared with those of Siripoonwiwat et al. (1996) in which QTLs for agro-

nomous traits including heading date, plant height, and grain yield were mapped in this same population based on phenotypic data collected from 2 yr at two locations (Ithaca, NY, and Aberdeen, ID). The mean heading date of Kanota across all environments was 2 d later than that of Ogle, indicating that in those environments, some vernalization did occur, but not to the same extent as in the present study's vernalization treatment. Siripoonwiwat et al. (1996) detected loci with greatest effects on heading date on LGs 3,7,8,11,12,17, and 24. Except for those loci on LGs 11 and 12, we detected similar regions in the present study in vernalized plants, with consistent, although not necessarily significant, effects in the non-vernalized plants. The parent allele which contributed to lateness was consistent across the two studies, as was the identification of LGs 17 and 24 as the regions with the greatest effects on flowering time.

Siripoonwiwat et al. (1996) detected loci with greatest effects on plant height on LGs 3,7,9,11,16,17,20,22,23,24,33, and 37. Except for those loci on LGs 3,7, and 9, we detected regions with similar effects in non-vernalized plants. Some of these regions were also detected in vernalized plants, and some of the regions were classified as vernalization sensitive, while others were classified as vernalization insensitive. The parent allele contributing to tallness was consistent across the two studies. Some of the QTLs that Siripoonwiwat et al. (1996) detected with effects on plant height and heading date had pleiotropic effects on other agronomic traits, such as grain yield and test weight. The regions on LGs 17 and 24, which we also suggested had pleiotropic effects on both heading time and plant height, also affected straw yield, grain yield, and test weight. The effect on heading date is probably the causal factor in these relationships because plants that flower sooner have shorter vegetative growth phases, so are often shorter, and will have lower straw yields. Also, the partitioning of photosynthate between vegetative and reproductive organs will be affected by heading date, which will influence grain yield. Finally, the timing of heading affects the initiation and duration of grain filling, both of which affect test weight.

The relationships to the QTLs controlling heading date and plant height in this oat population have already been compared with those in related cereal crop species. Siripoonwiwat et al. (1996) and Van Deynze et al. (1995) noted that the position of the heading date QTL on LG 17 corresponded to a vernalization gene found in wheat, a photoperiod response gene in rice, and a QTL controlling days to anthesis in maize. While the QTL we detected for heading date on LG 17 appeared to be vernalization insensitive, the plant height QTL detected in the same region was vernalization sensitive. Siripoonwiwat et al. (1996) and Van Deynze et al. (1995) reported that the QTL affecting heading date vernalization response on LG 24 of oat (also detected in our study) corresponded to vernalization genes in wheat and barley and a QTL controlling days to tasselling and silking in maize. They also suggested that the heading date QTL detected by Siripoonwiwat et al. (1996) (and ourselves) on LG 5 corresponded to a photoperiod-sensitivity gene in rice

and a heading date QTL in barley. Siripoonwiwat et al. (1996) also reported that QTLs associated with plant height on oat LGs 7 and 17 appeared to correspond to QTLs with similar effects in barley, wheat, and maize. However, the QTL on LG 7 was not detected in our study.

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