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Comparative mapping of loci controlling winter survival and related traits in oilseed *Brassica rapa* and *B. napus*

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Abstract

Winter survival is an important characteristic of oilseed *Brassica* that is seeded in the fall in northern climates, and it may be affected by genetic variation for other cold-regulated traits, such as freezing tolerance and vernalization responsive flowering time. We analyzed immortalized populations of oilseed *Brassica rapa* (recombinant inbred lines) and *B. napus* (double haploid lines) derived from crosses of annual and biennial types in order to compare the map positions and effects of quantitative trait loci controlling winter survival, nonacclimated and acclimated freezing tolerances, and flowering time. The *B. napus* population was evaluated in multiple winters, and six of the 16 total significant QTL for winter survival were detected in more than one winter. Correspondence in the map positions of QTL controlling different traits within species provided evidence that some alleles causing greater acclimated freezing tolerance and later flowering time also contributed to increased winter survival. Correspondence in the map positions of QTL between species provided evidence for allelic variation at homologous loci in *B. rapa* and *B. napus*. The potential role of some candidate genes in regulating these traits is discussed.

Introduction

Winter survival is an important characteristic for overwintering herbaceous crops, such as oilseed *Brassica*, and depends on the expression of many interacting traits. One of these traits is freezing tolerance, and this can be increased in some genotypes by acclimating plants to cold temperatures (Kacperska-Palacz 1978; Palta 1992). Acclimated and non-acclimated freezing tolerances were found to have separate genetic control in an interspecific cross of diploid potato species (Stone et al. 1993), and different putative quantitative trait loci (QTL) were identified for these two traits in oilseed *B. rapa* (Teutonico et al. 1995). Flowering habit also can be related to winter survival, especially in crop species having variation in flowering response to vernalization. In barley (Hayes et al. 1993a, 1993b; Pan et al. 1994) and wheat (Galiba et al. 1995; Storlie et al. 1998), a homologous chromosome region in each species was found to affect both vernalization responsive flowering time and cold hardiness.

Oilseed *B. rapa* and *B. napus* include both annual and biennial types, the latter of which require vernalization to flower and are grown as an over-wintering crop in northern climates. Biennial forms generally have a higher frequency of winter survival; and winter survival was correlated with acclimated freezing tolerance in a study including annual and biennial cultivars (Teutonico et al. 1993). We previously ana-

lyzed segregating populations of these species derived from crosses of annual and biennial types for molecular markers (Ferreira et al. 1994; Teutonico and Osborn 1994), flowering time (Ferreira et al. 1995; Teutonico and Osborn 1995; Osborn et al. 1997) and freezing tolerance (Teutonico et al. 1995) in order to map and compare QTL controlling these cold related traits. Most of the variation in vernalization responsive flowering time was controlled by a few genes in regions that are homologous between the species (Osborn et al. 1997). In *B. rapa*, we mapped loci controlling non-acclimated and acclimated freezing tolerance to different regions of the genome that were also different from those of flowering time genes. Most freezing tolerance effects were due to over-dominance for freezing sensitivity (the heterozygous class had less tolerance than either homozygous class). In a *B. napus*, none of the genome regions covered by markers were significantly associated with freezing tolerance (Teutonico et al. 1995).

In the following paper, we report on QTL analyses for winter survival in *B. rapa* and *B. napus*. For *B. napus*, we used the same population assayed previously for freezing tolerance (Teutonico et al. 1995), and we also reanalyzed the freezing tolerance data using a more complete molecular marker linkage map. For *B. rapa*, we use a recombinant inbred population derived from the same parents studied before (Teutonico et al. 1995). This population, like the *B. napus* population of DH lines, will segregate only for additive genetic variation; and thus, results from this population are more directly comparable to those from *B. napus*. The *B. rapa* RI lines also were analyzed for freezing tolerance QTL. The map position of QTL for winter survival and freezing tolerance, and for flowering time reported previously for these populations (Osborn et al. 1997), are compared both within and between species.

Materials and methods

Linkage maps

A set of 87 recombinant inbred (RI) lines in *B. rapa* was used to construct a genetic linkage map of 143 RFLP loci and 3 trait loci (seed color, leaf pubescence and resistance to *Albugo candida*) (Kole et al. 1997). This map spans 890 cM over 10 major linkage groups designated BR1-10 and two small groups designated BRA and BRB. These RI lines were developed by single-plant-descent from individual F_2 plants derived from a cross between Per, a biennial, winterhardy, freezing tolerant turnip rape cultivar that requires vernalization to flower and has seeds with low erucic acid content, and R500, an annual, cold sensitive sarson cultivar that flowers early and has seed with high erucic acid content.

For *B. napus,* a genetic linkage map of 132 RFLP loci was previously constructed using a segregating population of 105 doubled haploid (DH) lines (Ferreira et al. 1994). These lines were derived from a cross between Major, a biennial, winterhardy, freezing tolerant rapeseed cultivar that requires vernalization to flower and contains high erucic acid, and Stellar, an annual, cold sensitive canola cultivar that contains low erucic acid and flowers early. A subset of 90 DH lines were analyzed for additional 348 marker loci including 71 RFLP loci, 5 isozyme loci, 4 trait loci (two each for disease resistance and erucic acid), and 268 AFLP loci. An enriched map was constructed for these 90 DH lines that included 480 loci organized into 19 linkage groups covering 2007 cM (Osborn et al. (1997), unpublished data).

Trait measurement

Winter survival: For *B. rapa*, winter survival was determined from field trials near Madison, Wisconsin in 1994–95 and 1995–96. Sixty RI lines along with the parents were grown by planting 25 seeds in late August or early September using 1m rows spaced 0.6m apart in a randomized complete block design (RCBD) with three replications. For *B. napus*, 103 DH lines along with their parents were grown as described for *B. rapa* near Madison, Wisconsin in 1992–93, 1993–94, 1994–95, 1995–96, 1996–97, 1997–98 and 1999–00. Winter survival was measured as percentage of plants surviving the winter by counting seedlings in each plot after germination in the fall and counting surviving plants in the spring after plants begin to regrow. Data from each winter which caused variable survival were subjected to analysis of variance, and data sets showing significant line effects were used for QTL analysis. The percentage survival was averaged over replications used for QTL analysis.

In vitro freezing tolerance: A set of 77 *B. rapa* RI lines and the parents of these lines were evaluated for nonacclimated (FTN) and acclimated freezing tolerance (FTA) following a previously described procedure (Teutonico et al. 1995). By this procedure, we determined the freezing temperature at which 50%

ion leakage occurred. Data on FTN and FTA for the subset of 90 DH lines of *B. napus*, which were collected previously (Teutonico et al. 1995), were reanalyzed using the enriched marker map. Acclimation ability was not included in the analysis because in a previous study the gene effects were very similar to those found for FTA (Teutonico et al. 1995). Data from the freeze assay were subjected to analysis of variance and used for QTL analyses as described previously (Teutonico et al. 1995).

The flowering time data for these populations were the same as reported previously (Osborn et al. 1997); however, we reanalyzed these data for QTL using the same analysis methods as for the new traits presented in this study.

QTL analysis

Trait and marker genotype data were analyzed using QtlCart with the Composite Interval Mapping option, abbreviated CIM (Zeng 1994). A set of markers was chosen using SRmapqtl (a component of QTLCart package) which, when considered in a linear model, provided the best fit with the trait. The markers acted as surrogates for other possible QTL in the other marker intervals. Up to fifteen markers were allowed as background markers in our analysis to ensure that a surrogate existed for every putative QTL in our final analysis. If this was not done, the other QTLs would inflate the residual sum-of-squares, and reduce the power to detect of a putative QTL in the region of interest. A 10 cM "window" size was chosen in the analysis. This prevented any background markers within 10 cM of a putative QTL from being included in the analysis, since this could result in masking the effect of the QTL. As in Interval Mapping, CIM performed a scan of the genome, looking for likely locations for QTL. LOD scores, magnitudes of effects, and R^2 values were estimated for each location. Putative QTL were chosen based on one the following criteria: a LOD score of 3.0 or above, a LOD score of 2.5 and above with an R^2 value of 10% or more, or a LOD score of 2.5 or more and location in a genome region that contained significant QTL for other traits.

Results and discussion

Quantitative trait loci for winter survival

The *B. rapa* RI population was tested for survival in two winters, but no plants survived in 1995–96. In 1994–95, 82% of the plants from the winterhardy parent, Per, survived, no R500 plants survived, and at least some plants survived the winter for about one-fourth (28%) of the RI lines (Figure 1a). Three QTL exceeded the threshold and together these loci explained about one-third of the variation in winter survival (Table 1). Two QTL were linked on BR7 and these had allelic effects with opposite signs; the alleles from the winterhardy parent, Per, increased survival for the third QTL on BR3. We recognized two additional QTL with LOD scores and R^2 values just below the 3.0 LOD threshold on BR2 and BR1 because these regions were significantly associated with other traits related to winter survival.

The *B. napus* DH population was tested for survival in seven winters. No plants survived in 1995–96 and 1996–97. In 1993–94 and 1997–98, nearly all plants survived, including the winter sensitive parent Stellar (Figure 1c and 1e), but DH line effects from analysis of variance were significant ($p < 0.001$, 1993–94; $p < 0.01$, 1996–97). The largest ranges in percentage winter survival were in 1992–93 and 1994–95, when the winterhardy parent, Major, survived and no Stellar plants survived (Figure 1b and 1d) and 1999–00 when both parents survived at high levels (Figure 1f). DH line effects from analysis of variance were highly significant for these winters ($p < 0.0001$). The F_1 (1992–93 only) and many DH lines surpassed the hardy parent Major in survival (Figure 1b and 1d), suggesting that some DH lines may contain favorable alleles from both parents. This was supported by results from QTL mapping, in which alleles increasing winter survival were detected from both parents (Table 2).

One to five QTL for winter survival were above the threshold in each of the five *B. napus* test winters having some survival data, and these loci explained a total of 18–50% of the variation in each year (Table 2). The alleles from the winterhardy parent, Major, increased survival for only one of the five QTL detected in 1992–93, but alleles from Major increase survival for a majority of the loci detected in other years. We recognized an additional QTL on N2 that was just below the 3.0 LOD threshold in 1994–95 (data not shown) because this genome region was also

Table 1. Quantitative trait loci (QTL) for winter survival and related traits detected in a *Brassica rapa* population of recombinant inbred lines derived from Per (winter turnip rape) × R500 (spring sarson).

Trait	QTL	LG[a]	MP (cM)[a]	Confidence interval[a]	LOD[b]	R^2 [b]	Add[b]
Winter survival 1994–95 (%)							
	WS94R1	BR3 (R3)	119.8	wg9a2b+12.1-pCHS3.8c+5.2	4.52	12.9	4.79
	WS94R2	BR7 (R1)	65.7	tg5e11b+1.4-pC1b+0.1	3.13	9.5	–5.57
	WS94R3	BR7 (R1)	44.2	wg6f3b+1.8-pPG11+3.6	3.00	14.8	6.44
	WS94R4	BR2 (R2)	38.6	wg5a6+0.3-wg7f3+1.5	2.90	9.9	3.85
	WS94R5	BR1 (R8)	37.1	wg4d5+1.5-wg1f6b+8.6	2.88	7.6	3.42
Freezing tolerance - nonacclimated (°C)							
	FTNR1	BR9 (R7)	11.3	wg6e9a+6.9-wg2c4b+2.3	2.66	11.2	–0.12
Freezing tolerance – acclimated (°C)							
	FTAR1	BR9 (R7)	19.5	wg2c4b+2.2-COR47a+12.6	2.83	19.5	–0.42
	FTAR2	BR1 (R8)	33.5	wg1g3d+26.7-wg1g3a+1.3	2.65	17.0	–0.41
Flowering time nonvernalized (days)							
	VFR2	BR8 (R10)	34.8	pCHS3.8a+7.1-ec5f3+1.2	16.26	39.3	20.54
	VFR1	BR2 (R2)	59.6	wg7f3+10.9-wg6b10a+2.1	7.32	12.5	11.02
	FR2	BR3 (R3)	101.8	wg4a4b+5.1-wg9a2b+2.1	4.27	7.0	8.15
	VFR3	BRA	5.6	wg3c5b+0.9-tg5h12b+0.0	4.22	7.5	8.35
	–	BR9 (R7)	20.5	wg2c4b+3.9-COR47a+13.1	3.89	8.5	8.87
	–	BR10 (R4)	16.3	ec2f1b+0.6-ec4c5+10.1	3.02	5.3	7.03

[a] Linkage group designations (LG), map positions of peak LOD scores (MP in centiMorgans), and markers delineating the 1 LOD confidence interval for significant QTL. LG designations in parentheses correspond to those of Lagercrantz and Lydiate (1996) (unpublished data)
[b] Log likelihood of the odds ratio (LOD) that a QTL is present vs. absent, percentage variation explained by the QTL (R^2), and additive effect (Add) of the allele from Per in trait values, as determined by using QTL Cartographer (see Materials and Methods)

significantly associated with winter survival in 1993–94 (WS93N3, Table 2). A second QTL detected in 1993–94 was in a region of N5 that contained the only QTL detected in 1997–98. A third QTL detected in multiple years (1994–95 and 1999–00) was in a different position on N2. For each of the three regions detected in multiple years, alleles from Major increased winter survival in both years. The detection of different sets of significant QTL in each winter suggests that different allelic effects were expressed in each winter, perhaps due to different types of weather conditions that affect winter survival.

Quantitative trait loci for freezing tolerance

The *B. rapa* RI lines were normally distributed for nonacclimated (FTN) and acclimated freezing tolerance (FTA) (Figure 2). In the analysis of variance, both traits had significant effects due to RI line, temperature and line × temperature interactions ($p < 0.001$). For FTN, the parents had similar values and most transgressive segregants were more freezing sensitive. One QTL was above the threshold (Table 1). It accounted for 11% of the variation and alleles from Per decreased the temperature of 50% ion leakage (more freezing tolerant). Acclimation increased the freezing tolerance of all the lines and parents; Per was the most tolerant among all genotypes, R500 was moderately tolerant, and many RI lines were less tolerant than either of the parents (Figure 2b). Two QTL for FTA were above the threshold and together they explained about one-third of the variation (Table 1). For both QTL, alleles from Per increased freezing tolerance. The FTA QTL on BR9 was in the same region as the FTN QTL detected on BR9, suggesting these effects were due to the same locus. This is in contrast to a previous study using F_3 families from the same *B. rapa* cross (Teutonico et al. 1995) in which no single genome region was found to contain QTL for both traits. In this previous study, QTL for FTA and FTN were identified in different genomic regions than reported in the current study. Most of the gene effects among the F_3 families were due to over-dominance for winter sensitivity, and only small additive effects were detected. In RI populations, only additive effects can be detected, and this may explain why different QTL were identified compared to the previous study.

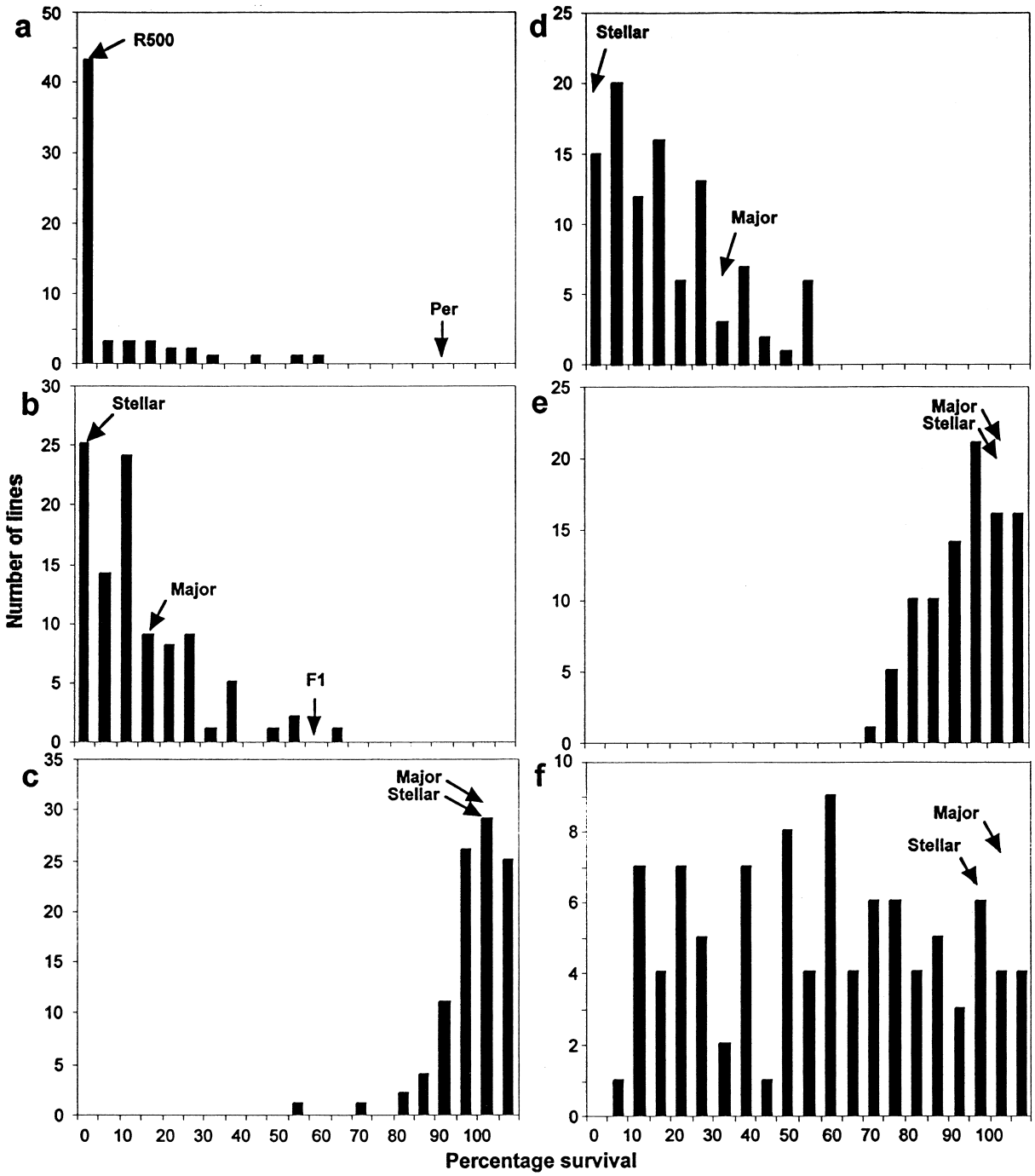

Figure 1. Phenotypic distributions of *Brassica rapa* recombinant inbred lines in 1994–95 (a) and *B. napus* double haploid lines in 1992–93 (b), 1993–94 (c), 1994–95 (d), 1997–98 (e), and 1999–00 (f) for winter survival. Positions of the parents of the populations are shown in each distribution.

The *B. napus* DH lines were also normally distributed for FTN and FTA (Figure 3). For both traits, the F_1 surpassed the parents, and for FTA many DH lines were more freezing tolerant than the parents and F_1. In the analysis of variance, both traits had significant effects due to DH line, temperature and line × temperature interactions ($p < 0.001$). No QTL were detected for FTN, and only one QTL was above the

Table 2. Quantitative trait loci (QTL) for winter survival and related traits detected in a *Brassica napus* population of double haploid lines derived from Major (winter rapeseed) × Stellar (spring canola).

Trait	QTL	LG[a]	MP (cM)[a]	Confidence interval[a]	LOD[b]	R^{2} [b]	Add[b]
Winter survival 1992–93 (%)							
	WS92N1	N8	28.4	wg6g9+1.5-E33M62.99+1.8	8.04	15.2	–5.17
	WS92N2	N10	27.2	E33M47.182a+5.2-wg9d6b+3.5	5.00	9.1	–3.96
	WS92N3	N17	56.2	E32M50.325+5.1-E35M47.170+7.4	4.83	8.6	–3.96
	WS92N4	N1	70.4	ec5d5+5.2-wg1g10a+4.0	4.79	8.5	3.92
	WS92N5	N3	36.4	E33M49.165+0.5-ec4g7b+2.0	4.30	8.2	–5.86
Winter survival 1993–94 (%)							
	WS93N1	N4	15.7	ec3b4+4.8-E32M59.302+7.8	3.21	7.9	–2.43
	WS93N2	N5	67.7	ec6b2+2.9-ec4h9+1.1	3.09	7.7	4.59
	WS93N3	N2	157	ec2d1a+7.9-tg2f12+0.0	3.06	7.3	2.46
Winter survival 1994–95 (%)							
	WS94N1	N2	82.6	wg6b10+3.3-wg8g1b+7.0	6.10	13.9	7.07
	WS94N2	N6	31.3	E32M62.75+0.3-E32M59.330+3.2	5.36	13.2	5.76
	WS94N3	N2	110.8	tg6a12+9.5-E38M50.133+11.2	3.05	6.7	–4.75
Winter survival 1997–98 (%)							
	WS97N1	N5	56.7	E32M59.161+0.2-ec6b2+7.2	4.25	17.6	7.77
Winter survival 1999–00 (%)							
	WS99N1	N2	89.6	wg8g1b+2.7-tg6a12+0.0	5.01	11.2	10.54
	WS99N2	N17	5.9	E35M47.199+1.3-E33M60.71+8.3	4.83	11.2	–9.71
	WS99N3	N9	30.6	wg4d11+1.1-E38M62.461+4.8	3.47	6.8	–9.25
	WS99N4	N19	51.8	ec3f1+18.0-E35M60.107+9.3	3.04	6.4	7.84
Freezing tolerance – acclimated (°C)							
	FTAN1	N8	40.7	E38M50.157+3.4-wg6d9+0.0	4.22	17.3	–0.91
Flowering time nonvernalized (days)							
	VFN1	N2	66.4	E33M59.59+0.8-E33M59.59+6.2	25.87	35.9	21.3
	VFN3	N3	106.8	E32M47.252+0.2-wg6b2+2.0	13.33	14.7	12.95
	VFN2	N10	43.3	wg7b3+6.8-E33M59.64+7.7	13.14	13.8	12.77
	VFN4	N2	154	ec2d1a+8.0-tg2f12+0.0	10.69	9.8	11.13
	–	N13	126.7	wg5b1b+2.3-ec3d2+2.5	3.24	3.0	–5.78

[a] Linkage group (LG) designations, map positions of peak LOD scores (MP, in centiMorgans), and markers delineating the 1 LOD confidence interval for significant QTL. LG designations correspond to those reported by Parkin et al. (1995); Butruille et al. (1999)

[b] Log likelihood of the odds ratio (LOD) that a QTL is present vs. absent, percentage variation explained by the QTL (R^{2}), and additive effect (Add) of the allele from Major in trait values, as determined by using QTL Cartographer (see Materials and Methods)

threshold for FTA (Table 2). Alleles from Major increased FTA at this locus. The same freezing tolerance data were analyzed previously using a less complete linkage map, and no significant QTL were detected. The use of a more complete linkage map and/or different analysis methods may have allowed us to detect a QTL effect in the current study.

Quantitative trait loci for flowering time

The reanalysis of the flowering time data for these populations grown without vernalization gave very similar results (Tables 1 and 2) as previous analyses using MapMaker QTL (Osborn et al. 1997). All QTL reported previously for this trait (VFR1, VFR2, and VFR3 in *B. rapa*, and VFN1, VFN2, and VFN3 in *B. napus*) were detected using QTL Cartographer. FR2 in *B.rapa* was detected in this reanalysis, although it was significant only in the vernalized population previously. A few additional QTL with LOD scores just above the threshold were detected in the reanalysis. Only VFN4 was named so it could be identified in Figure 4.

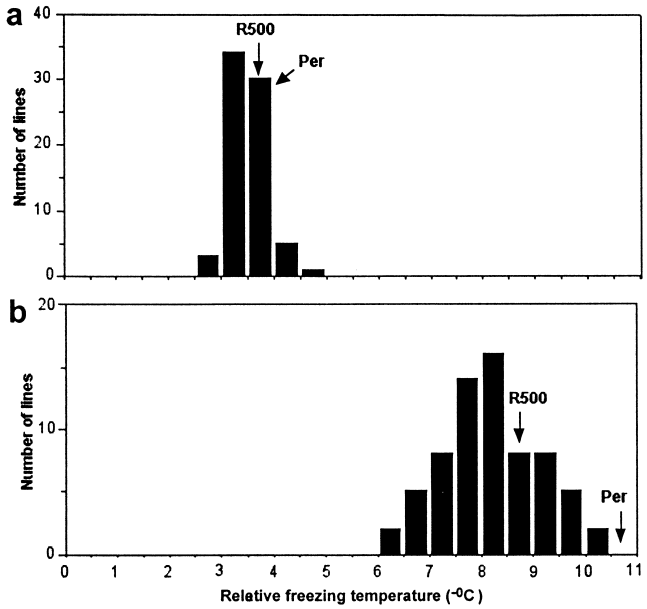

Figure 2. Phenotypic distributions of *Brassica rapa* recombinant inbred lines for nonacclimated freezing tolerance (a) and acclimated freezing tolerance (b). Positions of the parents of the populations are shown in each distribution.

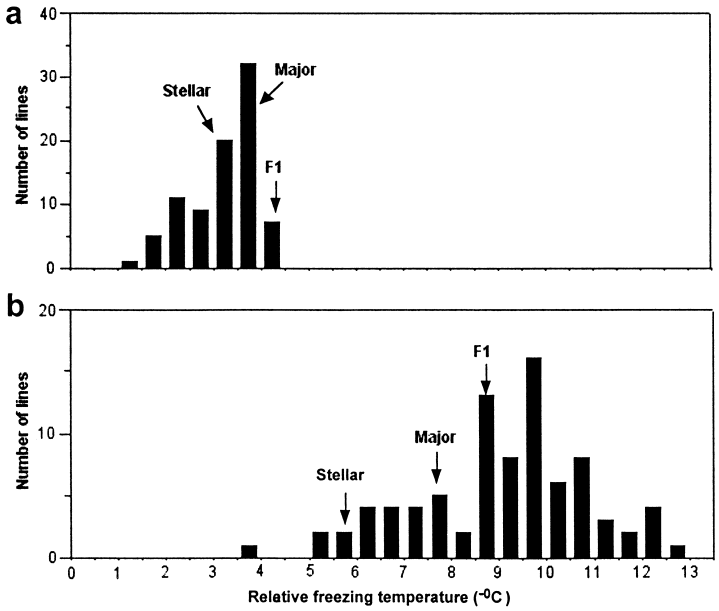

Figure 3. Phenotypic distributions of *Brassica napus* double haploid lines for nonacclimated freezing tolerance (a) and acclimated freezing tolerance (b). Positions of the parents of the populations are shown in each distribution.

Comparisons of QTL for winter survival, freezing tolerance and flowering time

Winter survival may be related to the other cold-response traits analyzed in this study, and comparison of QTL map position could provide evidence for genes that control winter survival through regulation of these traits. The strongest evidence for correspondence between winter survival and freezing tolerance loci was found on BR1 in *B. rapa* where the confidence intervals for FTAR2 and WS94R5 overlapped (Figure 4a). At these loci, Per contributed allele for greater winter survival and more freezing tolerance (Table 1). Winter survival and freezing tolerance loci

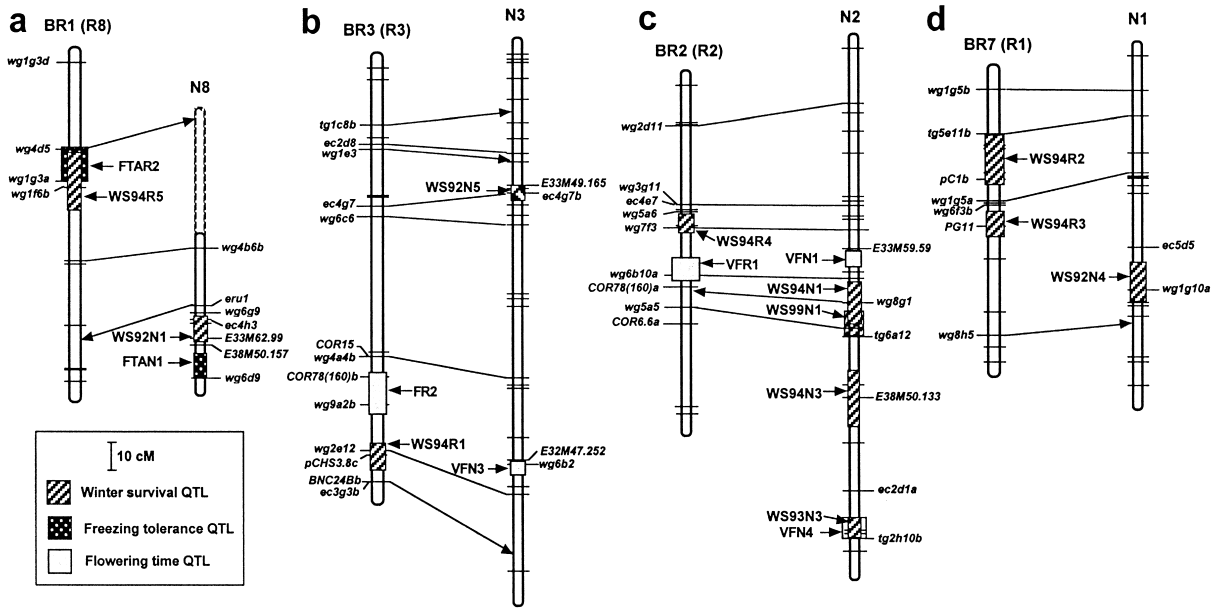

Figure 4. Map positions of QTL controlling winter survival (WS-----), acclimated freezing tolerance (FTA--), and flowering time (VF-- or F--) in linkage groups of *Brassica rapa* (BR- or R-) and *B. napus* (N-). Marker loci linked to QTL or those mapped in both species are shown on the right or left of the linkage groups. All marker loci were detected as RFLPs, except those beginning with "E" which were detected as AFLPs. Marker loci connected by lines were mapped in homologous linkage groups in both *B. rapa* and *B. napus*. Connecting lines with arrows indicate approximate position of markers mapped in other populations of *B. rapa* (Teutonico and Osborn 1994) or *B. napus* (Butruille et al. 1999). The dashed lines on N8 represent an extension of this linkage group as determined by Butruille et al. (1999). Shaded areas indicate the 1 LOD confidence interval for significant QTL. Magnitudes of allelic effects are not indicated.

also mapped very close to each other on N8 of *B. napus*; however, they did not have over-lapping confidence intervals (Figure 4a) and the alleles from Major had opposite effects (less winter survival and more freezing tolerance, Table 2) from those expected if a single locus affected both traits.

There also was some evidence for correspondence between winter survival and flowering time loci. On BR3 and BR2 of *B. rapa*, a locus for each of these traits mapped in close proximity to each other (Figure 4b and 4c) and alleles from Per delayed flowering and increase winter survival on both linkage groups (Table 1). Several winter survival loci were detected on N2 of *B. napus*; two were very close to VFN1, which had a major effect on flowering time, and one overlapped with a minor flowering time locus, VFN4 (Figure 4c). For all of these loci, alleles from Major delayed flowering and increased winter survival.

Alleles that delay flowering time usually affect growth habit by keeping plants in a rosette form longer into the fall and winter. Morphological characteristics, such as rosette formation and lack of stem elongation are known to be associated with cold tolerance of rapeseed cultivars (Kacperska 1984; Kacperska-Palacz 1978). In our previous study using F3 families derived from Per × R500, internode length was measured and a QTL was mapped to BR7 (Teutonico et al. 1995). This QTL occurred in the same region of BR7 as the winter survival locus WS94R2 detected in this study (Figure 4d), and alleles from Per increased winter survival (Table 1) and decreased internode length (Teutonico et al. 1995). Thus, alleles that delay flowering and shorten internodes may also improve the winter survival of plants containing those alleles. Evidence for this relationship also has been reported in barley (Hayes et al. 1993a, 1993b; Pan et al. 1994) and wheat (Galiba et al. 1995; Storlie et al. 1998).

Comparison of QTL between species

Brassica napus is an amphidiploid species derived by hybridization of *B. rapa* and *B. oleracea* (or close relatives of these), and the organization of the *B. napus* genome is highly conserved with the combined genomes of *B. rapa* and *B. oleracea* (Parkin et al. 1995). Thus, the map positions of QTL detected in our study can be compared between *B. rapa* and *B. napus* to provide evidence for or against allelic vari-

ation at homologous loci. This is possible because many of the same RFLP probes were used to construct the two linkage maps, and comparisons of the common marker loci have allowed us to identify the most probable pairs of homologous linkage groups between *B. rapa* and *B. napus* (Osborn et al. 1997). We use the *B. napus* linkage group nomenclature of Parkin et al. (1995) which identifies the *B. rapa* homologs of *B. napus* as N1-N10 and the *B. oleracea* homologs as N11-19. The *B. rapa* linkage groups are labeled according to Kole et al. (1997); Teutonico et al. (1993); Teutonico and Osborn (1994, 1995) to allow comparison to those studies, but we also include the R1–R10 designations of Lagercrantz and Lydiate (1996) which correspond numerically to their homologous counterparts in *B. napus* (N1–N10)

We previously reported evidence for homology of flowering time loci VFR1 in *B. rapa* and VFN1 in *B. napus* which mapped to corresponding positions on BR2 (R2) and N2, respectively. In this study, we found winter survival QTL near these loci (Figure 4c) which may be due to effects of the homologous flowering time loci on plant morphology, as discussed above, or due to homologous loci that are linked to these flowering time loci. The map positions of other flowering time loci (FR2 on BR3 or R3 and VFN3 on N3, Figure 4b) also provide evidence for homology; however, only one of these (FR2) is closely linked with a winter survival locus (WS94R1). Additional testing may reveal a winter survival QTL on N3 that is closely linked to VFN3 and due to the effects of this flowering time locus. Finally, there was some evidence for homologous loci controlling winter survival on BR7 (R1) and N1 (Figure 4d). It is not possible to determine if the confidence intervals of these loci (WS94R3 and WS94N4) overlap in homologous regions due to the lack of homologous marker loci in the regions; however, the winter parents contributed alleles for increased winter survival at both QTL (Tables 1 and 2) and they may represent homologous loci controlling winter survival.

Candidate genes

Specific genes that control winter survival have not been identified. Our results suggest that alleles affecting flowering time in oilseed *Brassica* species may also affect winter survival. Based on results from fine mapping, the flowering time gene *VFR2* has been identified as a homolog of the flowering time gene *FLC* from *Arabidopsis thaliana* (Kole et al. 2001). Although winter survival QTL linked to VFR2 were not identified, one was found linked to FR2 which is also in an *FLC* homologous region and may represent a duplicate homolog of *FLC* (our unpublished data). Other QTL we identified for flowering time may be homologous to other flowering time genes in *A. thaliana* (see, for example, Osborn et al. (1997)), many of which have been cloned. These will provide sources of candidate genes for testing effects of homologous loci in *Brassica* species.

Genes related to cold acclimation and cold tolerance also have been cloned in *A. thaliana* and in *B. napus*. The COR (Hajela et al. 1990) and BNC (Saez-Vasquez et al. 1994) genes, which are transcriptionally activated by low temperatures, were included as probes for mapping RFLPs in *B. rapa*. BNC24B mapped near WS94R1 on BR3 and two COR sequences mapped near flowering time QTL on BR 2 and BR3 (Figure 4b and 4c). These are potential candidate genes for the cold related traits in *B. rapa*; however, their expression has not been shown to be required for expression of cold-related phenotypes. The COR gene transcriptional activator, CBF1, was cloned recently from *A. thaliana* and shown to protect nonacclimated plants from freezing injury when constitutively overexpressed (Jaglo-Ottosen et al. 1998). BLAST analysis revealed that the CBF1 gene occurs on chromosome 4 of *A. thaliana*. Two RFLP probes used in our study, EC4E7 and EC4H3, have sequences that match *A. thaliana* sequences at positions 4 Mb on either side of CBF1 (data not shown). These clones map near winter survival (WS94N1) and acclimated freezing tolerance (FTAN1) QTL on N8 and a winter survival QTL (WS94R4) on BR2 (Figure 4a and 4c). If synteny between *Brassica* and *A. thaliana* genomes is maintained over a several mega-base distance in this region, homologs of CBF1 may also occur in these regions and could be responsible for the variation in winter survival. A more direct test of this hypothesis would be to analyze the phenotypic effects of allelic variants of CBF1 homologs from Brassica in backcross progeny or in transgenic plants.

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