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ORIGINAL PAPER

Quantitative trait analysis of seed yield and other complex traits in hybrid spring rapeseed (*Brassica napus* L.): 1. Identification of genomic regions from winter germplasm

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Abstract The introgression of winter germplasm into spring canola (*Brassica napus* L.) represents a novel approach to improve seed yield of hybrid spring canola. In this study, quantitative trait loci (QTL) for seed yield and other traits were genetically mapped to determine the effects of genomic regions introgressed from winter germplasm into spring canola. Plant materials used comprised of two populations of doubled haploid (DH) lines having winter germplasm introgression from two related French winter cultivars and their testcrosses with a spring line used in commercial hybrids. These populations were evaluated for 2 years at two locations (Wisconsin, USA and Saskatchewan, Canada). Genetic linkage maps based on RFLP loci were constructed for each DH population. Six QTL were detected in the testcross populations for which the winter alleles increased seed yield. One of these QTL explained 11 and 19% of the phenotypic variation in the two Canadian environments. The winter allele for another QTL that increased seed yield was linked in coupling to a QTL allele for high glucosinolate content, suggesting that the transition of rapeseed into canola could have resulted in the loss of favorable seed yield alleles. Most QTL for which the introgressed allele decreased seed yield of hybrids mapped to genomic regions having homoeologous non-reciprocal transpositions. This suggests that allelic configurations created by these rearrangements might make an important contribution to genetic variation for complex traits in oilseed *B. napus* and could account for a portion of the heterotic effects in hybrids.

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Introduction

The introgression of alleles from unadapted to adapted germplasm, in combination with molecular marker analysis, is a powerful approach for identifying and manipulating novel alleles that can improve complex trait of interest. In oilseed *Brassica napus* (canola or rapeseed), two major forms occur that are adapted to very different environments: annual or spring forms (planted and harvested in the same growing season) and biennial or winter forms (planted in the fall and harvested the following growing season, requiring vernalization to induce flowering). Spring cultivars are grown in Canada, northern Europe, Australia and parts of Asia. Winter cultivars are grown in regions of Europe and Asia with mild winters. Within each form, the highest yielding

hybrids are created by crossing genetically diverse cultivars from different geographical regions and pedigrees (Lefort-Buson et al. 1987; Brandle and McVetty 1990; Diers et al. 1996). Genetic diversity is greater between spring and winter forms than within each form (Diers and Osborn 1994), probably because breeders have kept the spring and winter forms as distinct gene pools crossing them primarily to transfer genes for canola quality. Although the winter germplasm is completely unadapted to spring environments, it represents an untapped source of genetic diversity to introgress into the spring canola germplasm in order to broaden its genetic base and potentially boost seed yields of spring hybrids. Some form of introgression is needed because hybrids produced directly between spring and winter forms are difficult to produce and not well adapted to either spring or winter environments. The introgression process is simplified by the fact that the annual versus biennial growth habit difference is controlled by only a few QTL (Ferreira et al. 1995a; Osborn et al. 1997).

To test the effects of introgressing winter germplasm into spring hybrids, Butruille et al. (1999a) evaluated seed yield of doubled haploid (DH) lines in hybrid combinations with the two spring canola testers. The DH lines were derived from a F_1 of the winter cultivar Major and the spring cultivar Stellar. They found that the average yield of the experimental hybrids was higher in each test environment than the yield of spring open-pollinated and hybrid cultivars and spring-by-spring experimental hybrids included in the trials. Butruille et al. (1999b) also developed and analyzed four populations of inbred backcross lines and their testcrosses to a spring canola cultivar and used these to map genomic regions introgressed from the German cultivar Ceres into spring hybrid combinations. They found two putative QTL for seed yield in the testcross populations that together explained about 10% of the observed genetic variation, in which the donor alleles positively contributed to yield. Recently, Quijada et al. (2004a) confirmed the effects of the QTL that explained a higher proportion (6.6%) of the genetic variation in two different genetic backgrounds.

Additional sources of unadapted germplasm might also contain alleles that when introgressed increase seed yield of spring hybrids. Results from mapping the genome location of these alleles will indicate if different germplasm sources have novel alleles at different loci, and they will identify linked markers that can be used for marker-assisted selection. Information on genome location may also reveal linkage relationships with loci for other important traits and this information could provide historical insights and affect future strategies for utilizing favorable alleles. For example, the conversion from rapeseed to canola quality involved the use of elite alleles at two loci that reduce erucic acid in the oil (Kondra and Stefansson 1965; Jonsson 1977; Thormann et al. 1996) and at least three loci that reduce glucosinolates in the meal (Kondra and Stefansson 1970; Toroser et al. 1995; Uzunova et al. 1995). In this conversion, alleles for increased seed yield may have been lost at loci linked to the erucic acid and glucosinolates loci. Crosses between lines containing high and low erucic acid and glucosinolate alleles in mapping studies could reveal such linkages.

We previously developed two populations of spring type DH lines containing germplasm introgressed from two related French winter cultivars. The DH lines and their hybrids with a spring canola tester (testcrosses) were evaluated in field trials during 2 years at two very different locations (Quijada et al. 2004b). In this study, we use genetic maps of the two populations to determine the effects of genomic regions having winter introgression on seed yield and other complex traits in spring *B. napus*. The use of these two related winter germplasms (an old rapeseed cultivar and a modern canola quality cultivar) allowed us to determine if these sources had different genes that enhance seed yield of spring hybrids and if some of these genes are linked to genes controlling canola quality traits.

Materials and methods

Plant materials

Two populations of 150 DH lines (MF and RV) were developed by culturing microspores from the F_1 hybrids of MF216 × P1804 and RV128 × P1804, respectively (for more details, see Quijada et al. 2004b). MF216 is a non-canola quality (46% erucic acid and 47 μmol g^{-1} glucosinolates, listed as DH 416 in Butruille et al. 1999a) DH line derived from a F_1 hybrid between the spring canola cultivar Stellar and the winter French cultivar Major, an old rapeseed cultivar with high levels of erucic acid and glucosinolates (Ferreira et al. 1994). RV128 is a canola quality BC_2S_2 line developed by backcrossing alleles for spring growth habit from 'Westar' into 'Samourai', a winter canola cultivar derived by backcrossing canola quality genes into the winter rapeseed cultivar 'Bienvenu', which was presumably derived from Major (Sernyk, personal communication). Both MF216 and RV128 have spring growth habit, although they are later flowering than commercially grown spring cultivars. P1804 is a DH line having the restorer gene of the genetically

engineered male-sterility system, Seedlink® (Mariani et al. 1990, 1992; Goldberg et al. 1993).

Testcross seed was produced, using the MF and RV DH lines as males and P124 as the female tester line (MF and RV testcross populations), in field crossing cages by Bayer CropScience in Outlook, Saskatchewan, Canada during the summer of 1998 and in Australia during the summer 1998–1999. P124 has the male-sterile gene of the transgenic system cited above. Selfed seed of each DH line was also produced for use in field trials (Quijada et al. 2004b).

Field trials and trait measurements

The DH lines were evaluated at the Arlington Agricultural Research Station in Wisconsin (WI), USA during the summers of 1999 and 2000. Because of seed production problems in 1998, only 114 of MF DH lines and 96 of the RV DH lines were evaluated in 1999. The experiments conducted in 2000 included 144 DH lines in each population. The experimental design was a randomized complete block design (RCBD) with two replications. In 1999, the plots were seven rows wide, with 0.15 m between rows and 4.9 m long, and were not trimmed before swathing. In 2000, the plots were 6.1 m long and trimmed to 4.9 m long 2 weeks before swathing. Seeds were planted during the last 3 days of April each year.

The MF and RV testcrosses were evaluated during the summers of 1999 and 2000 at the same WI locations as the inbreds and at Saskatoon, Saskatchewan (SK), Canada. We evaluated 128 and 150 MF testcrosses in 1999 and 2000, respectively; and 114 and 150 RV testcrosses in 1999 and 2000, respectively. The experimental design at both locations was an RCBD with two replications. In WI, planting dates and plot sizes were the same as for the inbred experiments conducted during the same years. In the field trials conducted in SK, the plots were five rows wide, with 0.19 m between rows and 6 m long, and plots were not trimmed before swathing. Seeds were planted in SK on May 28 in 1999 and on May 20 in 2000. Both DH line and testcross evaluations included the parental lines (MF216 or RV128 and P1804), tester (P124) and some commercial cultivars used as checks (for more details, see Quijada et al. 2004b).

The traits evaluated in each experiment included days to flowering (dtf), plant height (ph), lodging (l), seed yield (sy), test weight (tw) and seed weight (sw) in both DH and testcross populations, and bacterial leaf blight (blb) in the DH populations (Quijada et al. 2004b). Total glucosinolates contents (gls) were determined by David Syme, Bayer CropScience Canada, from a sample of each replicate of the MF DH lines evaluated in WI1999 as described by Toroser et al. (1995).

Data analysis

Statistical analyses were conducted using the MIXED procedure of SAS (Littell et al. 1996), where the source 'Entry' included the DH lines or their testcrosses; cultivar checks, parents of the DH lines or parents of the testcrosses were excluded from the analysis. Components of variance for the DH lines and the testcrosses were calculated with the MIXED procedure. In each experiment, environment was considered as a fixed effect and genotypes (DH lines or testcrosses) and, genotype by environment (GE) interaction and replicates within environment were treated as random effects. The variance components were used to estimate the narrow-sense heritability (h^2) on a mean basis for each trait as described by Hallauer and Miranda (1988). Exact 95% confidence intervals of h^2 were calculated according to Knapp et al. (1985). Genetic correlations (r_g) among traits in each experiment were estimated as described by Mode and Robinson (1959). The covariance estimates were calculated using the SAS GLM procedure with the 'Manova' statement (SAS Institute 2000). The significance of each genetic correlation was determined using a t test of the correlation coefficient (Edwards 1976) with a significance level corrected according to the Bonferroni–Holm sequential method (Rice 1989).

Molecular markers and linkage map

Genomic DNA was isolated from lyophilized leaf tissues collected from 12, 3-week old plants of the parents and 150 DH lines of each population using the CTAB procedure described by Kidwell and Osborn (1992). The procedures used for restriction enzyme digestion, gel electrophoresis, Southern blotting, probe radiolabeling and membrane hybridization are described elsewhere by Ferreira et al. (1994).

The majority of the RFLP probes included in this study had been used in previous studies (Teutonico and Osborn 1994; Ferreira et al. 1994; Thormann et al. 1996; Kole et al. 1997; Butruille et al. 1999b), and came from three libraries: a *Pst*I genomic library, cDNA library from *B. napus* cultivar Westar, and *Eco*RI genomic library from *B. rapa* cultivar Tobin that was constructed and screened as described for the Westar genomic library (Ferreira et al. 1994). These and other probes along with their nomenclature are described in more detail by Udall et al. (2005).

Genotype data for 218 RFLP loci in the MF population and 205 RFLP loci in the RV population were used to construct linkage maps, which are described in more detail elsewhere (Udall et al. 2005). The RFLP linkage maps spanned 1,398 and 1,453 cM for the MF and RV populations, respectively, and each consisted of 19 linkage groups (LGs), which probably correspond to the 19 chromosomes of *B. napus*. The average spacing between markers was 7.5 cM in each population. These results are similar to those reported for other *B. napus* maps (Landry et al. 1991; Ferreira et al. 1994; Cloutier et al. 1995; Uzunova et al. 1995; Sharpe et al. 1995; Foisset et al. 1996; Cheung et al. 1997; Kelly et al. 1997). Linkage groups were designated following the convention of Parkin et al. (1995), which was possible due to the use of RFLP probes that had been used in previous studies and alignment to a map based on a resynthesized *B. napus* parent (Udall et al. 2005). *Brassica napus* is an allo-tetraploid comprised of A and C genomes (U 1935). Linkage groups N1–N10 represent the A genome (*B. rapa*) and LGs N11–N19 represent the C genome (*B. oleracea*) of *B. napus*, respectively (Parkin et al. 1995). A homoeologous reciprocal transposition (HRT) including N7 and N16 and several homoeologous non-reciprocal transpositions (HNRTs) were segregating in each of the MF and RV mapping populations (Udall et al. 2005).

QTL analysis

Quantitative trait loci affecting seed yield and other traits in each population were analyzed using the composite interval mapping (CIM) procedure described by Zeng (1994) with the software QTLCartographer (QTLCart) v1.16 (Basten et al. 2002). The experiment-wise significance thresholds for QTL detection were determined for each trait by permutation analysis (Doerge and Churchill 1996). This procedure randomly permuted trait data and recalculated likelihood-ratios (LRs) across all genetic intervals. The extreme LR value from each permutation was saved and used to generate a distribution of extreme LRs to which empirical data were then compared. Empirical LR values equal to or exceeding the 5% highest LR values in the distribution of extreme permutation values were considered significant at $P < 0.05$. One thousand permutations were performed for each trait.

Results

Quantitative genetic variation

The traits evaluated in the DH lines and testcross populations showed highly significant differences among genotypes ($P < 0.001$), with the exception of plant height in the RV DH population (Quijada et al. 2004b). For most of the traits, the DH lines showed higher heritabilities than the testcross populations (Table 1). The higher heritability estimates were associated with larger genetic variances among lines within the DH populations. The distributions of least squares mean of DH lines and testcrosses (T × DH lines) for seed yield illustrate these differences in variation between DH and testcross populations (Quijada et al. 2004b). Narrow-sense heritability estimates ranged from 0.60 (lodging in the RV population) to 0.91 (days to flowering in the MF population) in the DH populations. In the testcross populations, the heritability estimates ranged from 0.14 (lodging in the MF testcross population) to 0.92 (days to flowering in the MF testcross population). The highest narrow-sense heritability estimates in the DH lines and testcross populations were those for days to flowering and these estimates were approximately equal in the DH lines and in their testcrosses. The heritability estimates showed a similar trend to those reported by Butruille et al. (1999b) for four populations of spring canola IBLs with winter

Table 1 Narrow-sense heritability estimates for seed yield and other traits in two populations (MF and RV) of doubled haploid lines (DH) and their testcross progenies

Characteristics	MF DH lines	MF testcrosses	RV DH lines	RV testcrosses
Seed yield	0.79 (0.70,0.86)	0.41 (0.24,0.54)	0.77 (0.67,0.84)	0.58 (0.46,0.67)
Days to flowering	0.91 (0.87,0.94)	0.92 (0.90,0.94)	0.90 (0.85,0.93)	0.89 (0.86,0.92)
Plant height	0.83 (0.75,0.88)	0.54 (0.41,0.64)	0.68 (0.64,0.83)	0.59 (0.47,0.68)
Test weight	0.69 (0.55,0.78)	0.53 (0.39,0.64)	0.73 (0.60,0.81)	0.53 (0.40,0.64)
Lodging	0.64 (0.47,0.75)	0.14 (−0.1,0.34)	0.60 (0.41,0.72)	0.29 (0.09,0.45)
Seed weight	0.61 (0.43,0.73)	0.29 (0.02,0.49)	0.74 (0.61,0.82)	0.44 (0.75,0.87)
Bacterial leaf blight	0.85 (0.78,0.89)	n/a	0.75 (0.64,0.83)	n/a

Values in parenthesis correspond to the 95% confidence intervals calculated according to Knapp et al. (1985)

n/a not evaluated

germplasm introgression; however, our estimates were generally higher, especially those for plant height and seed yield.

Significant genetic correlations were found between most of the traits evaluated in the DH line experiments (Table 2). Seed yield showed a significant ($P < 0.001$) negative association with the leaf blight disease caused by *P. syringae* in both DH populations and with days to flowering in the RV population, and a positive significant association with plant height and test weight. Days to flowering and the bacterial disease were significantly correlated with each other and with almost every other trait in both DH populations. Thus, it is possible that variation for many of the traits was affected by these two traits, as discussed further below.

The testcross populations showed a higher percentage than the DH populations of genetic correlation estimates that were significantly different from zero (73 vs. 55%, Table 3). Seed yield and lodging in both populations had significant positive correlations. The genetic associations between seed yield and other traits followed different patterns in the MF and RV testcross populations. In the MF testcross population, seed yield was negatively associated ($P < 0.05$) with days to flowering and positively correlated ($P < 0.001$) with test and seed weight. In the RV population, seed yield had no association with days to flowering and test weight, and its association with seed weight was negative. Plant height showed a strong positive association with seed yield in the RV population, while there was no association between these two traits in the MF testcross population. Days to flowering showed a very strong correlation ($r_g = 0.90$; $P < 0.0001$) with plant height in both populations. The high association of lodging with plant height in both populations and with days to flowering in the RV testcross population could be partially due to the relationship between days to flowering and plant height.

QTL analysis

Analyses across environments revealed highly significant QTL × environment interactions for seed yield and other traits (data not shown). Therefore, we analyzed the data for each population and test environment separately. QTL detected in each environment

Table 2 Genetic correlations among traits in MF doubled haploid lines (above diagonal) and RV doubled haploid lines (below diagonal) grown in Wisconsin during 1999 and 2000

	Seed yield	Days to flowering	Plant height	Test weight	Lodging	Seed weight	Bacterial leaf blight
Seed yield		−0.24 ns	0.54**	0.76**	−0.10ns	0.07 ns	−0.87**
Days to flowering	−0.46**		0.52**	−0.34**	−0.05ns	−0.48**	0.25*
Plant height	0.27*	0.54**		0.18 ns	−0.14 ns	−0.32**	−0.63**
Test weight	0.66**	−0.54**	0.08 ns		−0.06 ns	0.38**	−0.53**
Lodging	0.05 ns	−0.22 ns	−0.25*	0.21 ns		−0.01 ns	0.03 ns
Seed weight	−0.12 ns	−0.27*	0.13 ns	0.27*	0.19 ns		−0.12 ns
Bacterial leaf blight	−0.84**	0.28*	−0.55**	−0.36**	0.02 ns	−0.24 ns	

ns not significant at the 0.05 probability level

*, **Significant at the 0.05, and 0.001 probability levels, respectively. Significance levels were corrected according to the Bonferroni–Holm sequential method

Table 3 Genetic correlations among traits in the MF testcross population (above diagonal) and RV testcross population (below diagonal) grown in four environments

	Seed yield	Day to flowering	Plant height	Test weight	Lodging	Seed weight[†]
Seed yield		−0.24*	0.02	0.43***	−0.39***	0.51***
Day to flowering	0.03 ns		0.92***	−0.16 ns	−0.06 ns	−0.79***
Plant height	0.35***	0.87***		−0.03 ns	−0.63***	−0.92***
Test weight	0.06 ns	0.15 ns	−0.36 ***		0.27**	0.24*
Lodging	−0.55***	−0.54***	−0.64***	−0.16 ns		−0.63***
Seed weight	−0.24*	−0.65***	−0.99***	0.39***	0.25**	

Seed weight was evaluated in only two environments (WI1999 and WI2000)

ns not significant at the 0.05 probability level

*, **, ***Significant at the 0.05, 0.01, and 0.001 probability levels, respectively. Significance levels were corrected according to the Bonferroni–Holm sequential method

were labeled separately, although those with similar effects and map positions (overlapping 1 LOD confidence intervals) were assumed to represent the same QTL detected in different environments. Detailed information on the map position, confidence interval, LOD score, proportion of the phenotypic variance explained by a QTL (R^2) and additive effect of each detected QTL is presented in Table S1 (Electronic Supplementary Material). Although the donor parents contained alleles that originated from a spring cultivar in some genomic regions, they contained alleles that originated from their winter parents for all of the QTL we detected, except for a few as indicated.

Seed yield

The QTL analyses of the DH lines revealed a region on N15 (*sy15.3*) at which the donor allele significantly increased seed yield in both populations in WI2000 (Figs. 1, 2). QTL on N10 (*sy10*) and N16 (*sy16*), for which the donor alleles decreased seed yield, were detected in both environments and in both populations. In the MF DH population, two other genomic regions (*sy3.1*, *sy11.1*) were identified at which donor alleles also decreased seed yield in WI1999. The donor alleles present in these genomic regions originated from Stellar, the spring parent of MF216. Moreover, these genomic regions appeared to be segregating for chromosomal rearrangements including the N7–N16 HRT and several HNRTs (Figs. 1, 2).

Six QTL significantly affected seed yield in each testcross population. A QTL on N10 for which the donor allele increased seed yield was detected in both populations in the two Canadian environments. This QTL explained 10.5 (1999) to 12.5% (2000) of the phenotypic variance with an additive effect of 260 (1999) to 329 kg ha^{-1} (2000) in the MF testcross population (Fig. 1), and it explained 16.1 (1999) to 21.3% (2000) of the phenotypic variance with an additive effect of 327 (1999) to 453 kg ha^{-1} (2000) in the RV testcross population (Fig. 2). In the RV population, there were three additional QTL (*hsy6.4*, *hsy7.2,4* and *hsy15.2*) for which donor alleles increased seed yield of the hybrids in at least one environment. One of these (*hsy7*) was detected in both Canadian environments, and together with *hsy10* these two QTL explained 34 and 30% of the phenotypic variance and had total additive effects of 862 and 774 kg ha^{-1} in SK1999 and SK2000, respectively. One additional QTL (*hsy2.1*), for which the donor allele increased seed yield (220 kg ha^{-1}), was detected only in the MF testcross population, and this QTL explained 10.8% of the observed phenotypic variation in WI1999. At the remaining four QTL, detected in the MF testcross population, the donor allele decreased seed yield. Three of these QTL (*hsy11.1–3, hsy13.1,3* and *hsy16.3*) were mapped to genomic regions, which were segregating for the N7–N16 HRT or HNRTs (Fig. 1).

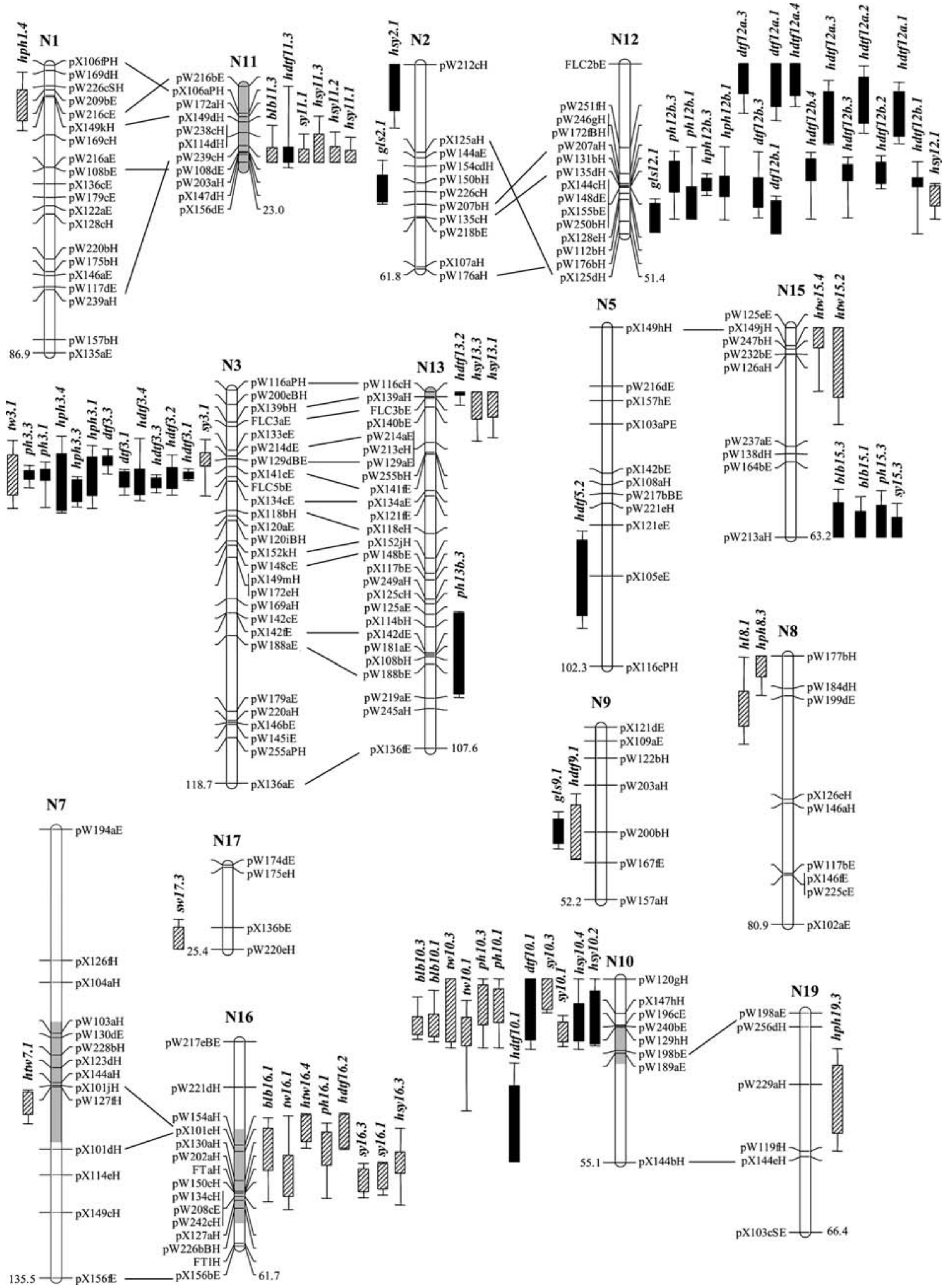
Days to flowering

Three QTL (*dtf3*, *dtf10* and *dtf12a*) for which the donor allele delayed flowering were identified in both DH populations in both environments (Figs. 1, 2). The region containing *dtf3* had the largest effect in the MF DH population (average $R^2 = 28\%$ and additive effect of donor allele = 2.3 days delay in flowering), while *dtf3* ($R^2 = 13.4\%$, additive effect of donor allele = 2 days delay in flowering) and *dtf12a* ($R^2 = 22.7\%$, additive effect of donor allele = 2 days delay in flowering) had the largest effects in the RV population. A fourth QTL (*dtf12b)*, for which the donor allele delayed flowering, was detected in the MF population. The combined effects of these four QTL detected in the MF population accounted for 55 and 38% of the phenotypic variance with the donor alleles delaying flowering 5.2 and 6.6 days in WI1999 and WI2000, respectively. The combined effects of the three QTL detected in the RV population accounted for 35 and 56% of the variance with the donor allele delaying flowering 3.2 and 5.6 days in WI1999 and 2000, respectively. In the RV population, two QTL (*dtf6.1* and *dtf11.1*) were identified for which donor alleles caused earlier flowering and together they explained an additional 16.3% of the variance and decreased flowering time by 2.3 days in WI1999.

Three QTL affecting days to flowering (*hdtf3, hdtf12a* and *hdtf12b*) were detected in the MF testcross population in each environment (Fig. 1). The combined effect of these QTL accounted for 35–59% of the phenotypic variance and donor alleles at these QTL delayed flowering 3.0–6.8 days in the four environments. Two of these QTL (*hdtf3* and *hdtf12a*) were also detected in the RV testcross population (Fig. 2) and together they accounted for 43–64% of the phenotypic variance and donor alleles delayed flowering 2.4–4.4 days in the four environments. The position of these QTL suggested that they were the same as those detected in the MF and RV DH populations.

Plant height

Most of the main QTL detected for plant height in both DH populations (Figs. 1, 2) mapped in the same genomic regions as those detected for days to flowering. With the exception of *ph10*, the donor allele contributed to increase plant height in these genomic


N1
hph1.4
pX106fPH
pW169dH
pW226cSH
pW209bE
pW216cE
pX149kH
pW169cH
pW216aE
pW108bE
pX136cE
pW179cE
pX122aE
pX128cH
pW220bH
pW175bH
pX146aE
pW117dE
pW239aH
pW157bH
86.9
pX135aE
N11
pW216bE
pX106aPH
pW172aH
pX149dH
pW238cH
pX114dH
pW239cH
pW108dE
pW203aH
pX147dH
pX156dE
23.0
blb11.3
hdtf11.3
sy11.1
hsy11.3
hsy11.2
hsy11.1
N2
hsy2.1
gls2.1
pW212cH
pX125aH
pW144aE
pW154cdH
pW150bH
pW226cH
pW207bH
pW135cH
pW218bE
pX107aH
61.8
pW176aH
N12
FLC2bE
pW251fH
pW246gH
pW172fBH
pW207aH
pW131bH
pW135dH
pX144cH
pW148dE
pX155bE
pW250bH
pX128eH
pW112bH
pW176bH
pX125dH
51.4
gls12.1
ph12b.3
ph12b.1
hph12b.3
hph12b.1
dtf12a.3
dtf12b.3
dtf12b.1
dtf12a.1
hdtf12a.4
hdtf12b.4
hdtf12a.3
hdtf12b.3
hdtf12a.2
hdtf12b.2
hdtf12a.1
hdtf12b.1
hsy12.1
N3
tw3.1
ph3.3
ph3.1
hph3.4
hph3.3
hph3.1
dtf3.3
dtf3.1
hdtf3.4
hdtf3.3
hdtf3.2
hdtf3.1
sy3.1
pW116aPH
pW200eBH
pX139bH
FLC3aE
pX133eE
pW214dE
pW129dBE
pX141cE
FLC5bE
pX134cE
pX118bH
pX120aE
pW120iBH
pX152kH
pW148cE
pX149mH
pW172eH
pW169aH
pW142cE
pX142fE
pW188aE
pW179aE
pW220aH
pX146bE
pW145iE
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hsy13.3
hsy13.1
ph13b.3
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pX139aH
FLC3bE
pX140bE
pW214aE
pW213eH
pW129aE
pW255bH
pX141fE
pX134aE
pX121fE
pX118eH
pX152jH
pW148bE
pX117bE
pW249aH
pX125cH
pW125aE
pX114bH
pX142dE
pW181aE
pX108bH
pW188bE
pW219aE
pW245aH
pX136fE
107.6
N5
pX149hH
pW216dE
pX157hE
pX103aPE
pX142bE
pX108aH
pW217bBE
pW221eH
pX121eE
hdtf5.2
pX105eE
102.3
pX116cPH
N15
htw15.4
htw15.2
pW125eE
pX149jH
pW247bH
pW232bE
pW126aH
pW237aE
pW138dH
pW164bE
blb15.3
blb15.1
ph15.3
sy15.3
pW213aH
63.2
N8
hl8.1
hph8.3
pW177bH
pW184dH
pW199dE
pX126eH
pW146aH
pW117bE
pX146fE
pW225cE
80.9
pX102aE
N9
gls9.1
hdtf9.1
pX121dE
pX109aE
pW122bH
pW203aH
pW200bH
pW167fE
52.2
pW157aH
N7
pW194aE
pX126fH
pX104aH
pW103aH
pW130dE
pW228bH
pX123dH
pX144aH
pX101jH
pW127fH
htw7.1
pX101dH
pX114eH
pX149cH
135.5
pX156fE
N17
sw17.3
pW174dE
pW175eH
pX136bE
25.4
pW220eH
N16
pW217eBE
pW221dH
pW154aH
pX101eH
pX130aH
pW202aH
FTaH
pW150cH
pW134cH
pW208cE
pW242cH
pX127aH
pW226bBH
FT1H
pX156bE
61.7
blb16.1
tw16.1
htw16.4
ph16.1
hdtf16.2
sy16.3
sy16.1
hsy16.3
N10
blb10.3
blb10.1
tw10.3
tw10.1
ph10.3
ph10.1
hdtf10.1
dtf10.1
sy10.3
sy10.1
hsy10.4
hsy10.2
pW120gH
pX147hH
pW196cE
pW240bE
pW129hH
pW198bE
pW189aE
55.1
pX144bH
N19
hph19.3
pW198aE
pW256dH
pW229aH
pW119fH
pX144eH
pX103cSE
66.4

Fig. 1 Quantitative trait loci (QTL) for seed yield and other traits detected in the MF doubled haploid (DH) population and its testcross population. QTL were designated using the trait name initials (see Materials and methods: Field trials and trait measurements) followed by a number identifying the linkage group (LG). After the LG, a number was used to indicate the environment where the QTL was detected: *1* WI1999, *2* SK1999, *3* WI2000 and *4* SK2000. For the QTL detected in the testcross populations, an "h" was added before the trait name initials to distinguish these designations from those used in the DH populations. *Lines* between LGs represent homoeologous polymorphic marker loci. *Boxes and whiskers* represent 1 LOD and 2 LOD confidence intervals, respectively for significant QTL based on 1,000 permutation tests for each trait in each environment. The allele that increased trait value is indicated by *hatched* (P1804) or *solid* (MF26) *boxes*. Linkage group designations follow the convention of Parkin et al. (1995). *Shaded regions* within LGs represent approximate locations of segregating chromosomal rearrangements detected in this population

regions. Five additional plant heights QTL that were not associated with flowering time were found in the DH and testcross populations (Figs. 1, 2): three (*hph1.4, hph8.3* and *hph19.3*) in the MF testcross population, one (*ph17.1*) in the RV DH population and one (*hph6*.3) in the RV testcross population. For these five QTL, donor alleles decreased plant height.

Lodging and seed related traits

QTL for lodging were identified only in the testcross populations. One QTL was mapped in the MF testcross population (*hl18.1*) for which the donor allele decreased lodging (Fig. 1). Three QTL (*hl6.3, hl14*.1 and *hl16*.3) were mapped in the RV testcross population for which the donor allele increased lodging (Fig. 2).

For most of the QTL affecting test and seed weight, the donor allele decreased trait values. Only in the RV populations (Fig. 2) were QTL detected for which donor alleles increased test weight (*tw17.1* and *htw6.1*) and seed weight (*hsw19.3*).

Glucosinolate content was analyzed only in the MF DH population in WI1999 (Fig. 1). Three detected QTL were mapped to N2 (*gls2.1*), N9 (*gls9.1*) and N12 (*gls12.1*). The combined effects of these QTL account for 65% of the phenotypic variance and on average, donor alleles increased glucosinolate content by 29.4 μmol g^{-1}. *gls9.1* and *gls12.1* corresponded to the main QTL mapped in the previous studies (Toroser et al. 1995; Uzunova et al. 1995; Howell et al. 2003). Uzunova et al. (1995) found another minor QTL that could be equivalent to *gls2.1* and might correspond to the N2 locus hypothesized by Howell et al. (2003).

Bacterial leaf blight

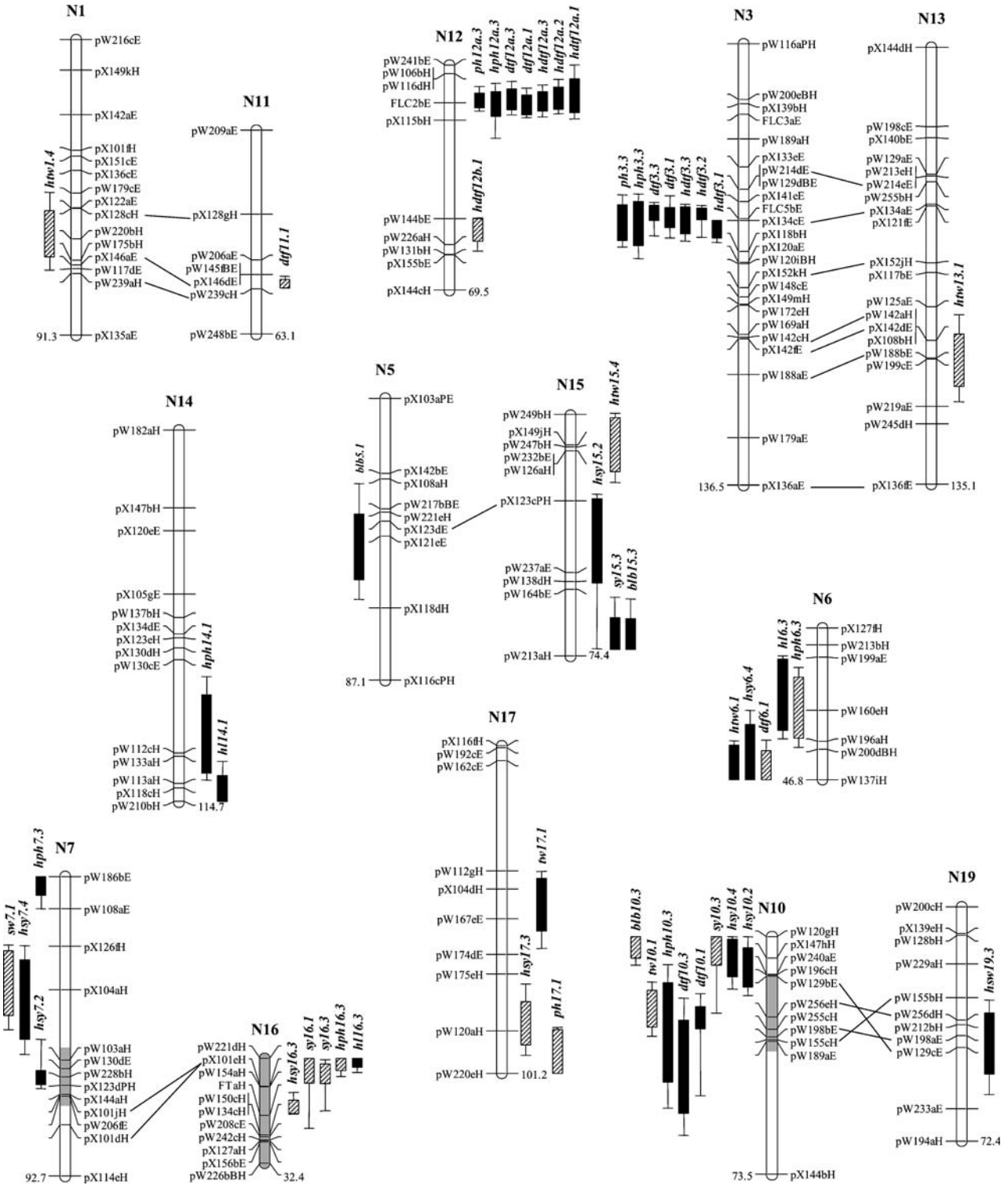
In both DH populations, a QTL for the bacterial leaf blight disease was detected at the top of N10, for which the donor allele increased susceptibility (Figs. 1, 2). This QTL explained a much higher percentage of the variance in the MF population (24.5%) than that in the RV population (14.8%). Another QTL for which the donor allele from Stellar increased susceptibility mapped on N11 (*blb11.3*) in the MF population. Two QTL for which the donor alleles increased resistance to the leaf blight disease were located on N15 (*blb15*) in both populations and on N5 (*blb5*) in the RV population.

Clustering of QTL and correlated traits

The QTL for several traits mainly clustered in the regions of LGs that contains QTL for bacterial disease resistance (N10 and N15 in the DH populations; Figs. 1, 2) and QTL for days to flowering (N3, N10 and N12). Other clusters of QTL were detected in LGs for which we had evidence of segregating HNRTs (N11 and N13 in the MF DH and testcross populations; Fig. 1) and HRT (N16 in the MF and RV DH and testcross populations) (Udall et al. 2005). Clustering of QTL was consistent with the strong genetic correlations observed among most of the traits (Tables 2, 3).

Discussion

Using an approach that combined introgression of unadapted germplasm, development of DH lines and molecular marker analysis, we were able to identify five QTL in testcross populations for which donor alleles, all of which originated from winter cultivars, increased seed yield of spring hybrids. One of these QTL (*hsy10*) was detected in both populations and explained up to 19% of the phenotypic variance in the Canadian environments. The QTL identified in this study were different from those identified by Butruille et al. (1999b), who found two QTL for seed yield in four IBL populations evaluated as testcrosses. The detection of more QTL in our study may be due, in part, to the use of balanced populations (populations having equal representation of alleles from each parent, e.g., F_2, BC_1, recombinant inbred lines and doubled haploid lines), which have greater power than unbalanced populations (e.g., inbred backcross line and advanced backcross line populations) to detect QTL (Tanksley and Nelson 1996; Kaeppler 1997). Our results provide further support for the hypothesis that



winter germplasm, which is completely unadapted as spring-seeded crop, contains desirable alleles that can improve seed yield in spring genetic backgrounds, and they indicate that different genetic sources contain favorable alleles at different loci.

One of the reasons for using parents with introgression from two related winter French germplasms was to determine if the conversion to canola quality might have eliminated genomic regions that contribute to increase seed yield in oilseed *B. napus*. Our analyses

Fig. 2 Quantitative trait loci (QTL) for seed yield and other traits detected in the RV doubled haploid (DH) population and its testcross population. QTL were designated using the trait name initials (see Materials and methods: Field trials and trait measurements) followed by a number identifying the linkage group (LG). After the LG, a number was used to indicate the environment where the QTL was detected: *1* WI1999, *2* SK1999, *3* WI2000 and *4* SK2000. For the QTL detected in the testcross populations, an "h" was added before the trait name initials to distinguish these designations from those used in the DH populations. *Lines* between LGs represent homoeologous polymorphic marker loci. *Boxes and whiskers* represent 1 LOD and 2 LOD confidence intervals, respectively, for significant QTL based on 1,000 permutation tests for each trait in each environment. The allele that increased trait value is indicated by *hatched* (P1804) or *solid* (RV128) *boxes*. Linkage group designations follow the convention of Parkin et al. (1995). *Shaded regions* within LGs represent approximate locations of segregating chromosomal rearrangements detected in this population

provide some evidence that alleles for high seed yield could have been lost in the transformation of rapeseed to canola. We analyzed the MF DH population, which was segregating for some canola quality genes, for QTL affecting glucosinolate content (Fig. 1). A QTL for which the winter allele increased glucosinolate content mapped to N2 (*gls2.1*). In the same LG, about 20 cM from *gls2.1*, a QTL (*hsy2.1*) was detected for which the winter allele increased seed yield in the MF testcross population. The low glucosinolate trait was introduced into modern *B. napus* cultivars from 'Bronowski', an agronomically inferior canola cultivar. Sharpe and Lydiate (2003) identified 15 residual segments of the donor genotype (Bronoswki) in the modern canola cultivar Tapidor. These segments occupy approximately 29% of the *B. napus* genome. The large number of donor segments probably contributed to the reduced agronomic performance of Tapidor compared with 'Bienvenu', the elite cultivar into which the low glucosinolate trait was introgressed from Bronowski. These results indicate that alleles for high seed yield are linked in repulsion phase to some canola quality alleles, a feature that could lead to their removal during breeding for canola quality. Molecular makers offer a valuable tool to break these linkages and combine favorable allele at both loci.

Many of the flowering time QTL identified in this study were detected in the same genomic regions where flowering time QTL had been previously detected in *B. napus* (Ferreira et al 1995a; Osborn et al. 1997; Butruille et al. 1999b) and *B. rapa* (Teutonico and Osborn 1994; Osborn et al. 1997; Kole et al. 1997, 2001; Schranz et al. 2002). Genomic regions on N3, N10 and N12 that contained flowering time QTL have high homology with a region at the top of chromosome 5 of *A. thaliana*, where several flowering time genes [*FLC* and *CONSTANS* (*CO*), among others] are located (Osborn et al. 1997; Lagercrantz 1998; Parkin et al. 2002; Schranz et al. 2002). Recently, four *B. rapa* homologs of *FLC* have been cloned (Schranz et al. 2002), and three (*BrFLC1*, *BrFLC2* and *BrFLC5*) co-segregated with loci controlling flowering time in populations derived by backcrossing alleles from a winter *B. rapa* into a spring form of this species (Kole et al. 2001; Schranz et al. 2002). The four *BrFLC* clones were used to map RFLP loci in our populations, and two of these clones (*BrFLC2* and *BrFLC5*) detected RFLP loci at the peak of two flowering time QTL detected in the DH line and testcross populations. These findings support *FLC* as a likely candidate gene for the QTL located on N3 (*dtf3*) and N12 (*dtf12a*). *BrFLC1* was not polymorphic on N10, and the marker locus (*pW189aE*) nearest to the peak position of the flowering time QTL on N10 (*dtf10b*) had a DNA sequence that aligned to the 5.34 Mb position at the top of *A. thaliana* chromosome 5 (data not shown), about 250 Kb from *CO*. *FLC* is located at the 3.13 Mb position, 2.21 Mb away from the QTL peak and outside of the confidence interval. Therefore, *CO* is a better candidate gene for this QTL on N10.

The DNA sequences of probes that detected RFLP loci in genomic regions containing the bacterial leaf blight QTL were compared to the entire *A. thaliana* genome sequence available at TAIR (The Arabidopsis Information Resource) database (http://www.arabidopsis.org, data not shown) by BLASTn analysis (Altschul et al. 1997; Lukens et al 2003). The markers surrounding *blb11.3* and *blb10* aligned to *A. thaliana* chromosome 3, near the location of *RPS3* (*RPM1*), a *P. syringae* pathovar *maculicola* resistance gene. The markers near *blb15* and *blb5* aligned to the 4.0 Mb region at the top of *A. thaliana* chromosome 1. This region includes *RPS5*, another member of the *RPS* gene family, and *PBS2*, a signal transduction gene linked to *RPS5*. Possible candidate genes for the *P. syringae* resistance QTL located on N11, N5 and N15 have been reported in comparative studies between *A. thaliana* and *B. napus* (Grant et al. 1998; Sillito et al. 2000). Grant et al. (1998) positioned the *RPM1* (*RPS3*) gene on N11 in an interval between marker loci *pW108c* and *pO12c* using a *B. napus* DH population developed by Sharpe et al. (1995); and in this study, pW108 detected an RFLP locus that mapped 2.8 cM from the peak of a bacterial disease resistance QTL on N11. Sillito et al. (2000) mapped two homologs of *RPS5*, a *P. syringae* resistance gene located on chromosome 1 of *A. thaliana*, to N6 and N15

in a subsample of the DH population developed by Parkin et al. (1995). These two previous studies support our finding of *RPM1* and *RPS5* as candidate genes for *blb11* and *blb15*, respectively. A good candidate gene for *blb5* is *PBS2*, a putative signal transduction gene linked to *RPS5* on *A. thaliana* chromosome 1, that acts downstream of *RPS2, RPS5* and *RPM1* (Warren et al. 1999). The QTL detected at the top of N10 (*blb10*) has not been reported previously in comparative studies of *A. thaliana* and *Brassica*, and based on genomic alignments, as described above, *RPM1* is a candidate gene for this QTL. Candidate genes were not identified for the QTL found on N16.

The findings of QTL for days to flowering and plant height mapping essentially in the same genomic regions (overlapping confidence intervals between QTL) are probably due to a pleiotropic effect of the flowering time genes on plant height, rather than linkage between QTL affecting these two traits. Later, flowering lines grew taller by producing more leaves before transition to reproductive stages, similar to results reported by Butruille et al. (1999b) and Udall et al. (2006) in *B. napus* and by Ungerer et al. (2002) in *A. thaliana*. This observation is consistent with the strong genetic correlations observed between these traits (Tables 2, 3). In the case of overlapping QTL for the bacterial disease, seed yield and other traits, either pleiotropy or linkage, may be the possible cause of this clustering. As Conner (2002) pointed out, by using QTL mapping, we cannot distinguish linkage from pleiotropy because these analyses rely on crosses between distinct inbred lines (linkage disequilibrium) and few recombination events.

A striking finding of this study was the mapping of seed yield QTL to genomic regions for which there was evidence of chromosomal rearrangements (Udall et al. 2005). One of the QTL for which the winter allele decreased seed yield was detected in a genomic region having the N7–N16 HRT (Figs. 1, 2). Osborn et al. (2003) reported that segregation of this HRT in other populations affected seed yield. The common parent (P1804) of the two populations used in this study was homozygous for this HRT. Therefore, this HRT segregated in both MF and RV populations (Udall et al. 2005). The N7 LG of P1804 contained the herbicide resistance gene and thus our populations were highly skewed for the transposed form of N7. In the HRT region on N16, very significant seed yield reductions were associated with winter alleles in both the MF and RV DH population and their testcross progenies. This agrees with a previous report of reduced seed yields for genotypes having a combination of one normal and one rearranged form of N7 and N16, resulting in greater intergenomic homozygosity for the rearranged region (Osborn et al. 2003). We also detected a QTL for seed yield on N7 (*hsy7.2* and *hsy7.4*) in the RV testcross population in both Canadian environments for which the winter allele increased seed yield; however, segregation distortion on N7 prevented accurate estimation of QTL effects. This QTL effect could have been due to segregation for the HRT or segregation of a gene for resistance to *Leptosphaeria maculans* on N7 (Ferreira et al. 1995b; Mayerhofer et al. 2005). Blackleg disease caused by *L. maculans* was present in the Canadian experiments, although reaction to this disease was not measured in our populations.

Two of the QTL for which donor alleles decreased seed yield in the MF DH population were associated with segregating HNRTs on N11 and N13. MF216 had a N11.N1(T) HNRT (Udall et al. 2005) and MF216 alleles in this segment were associated with lower seed yield in the DH line and testcross populations. In the MF DH lines, the presence of the HNRT increases intergenomic homozygosity and this may have affected seed yield, as was observed for segregation of the HRT, through a mechanism analogous to inbreeding depression. Since a similar effect was detected in the testcross population, the tester may also have had a HNRT in this region. This could also explain the effect detected in the MF testcross population on N13 for which MF216 had the N13.N3(T) HNRT.

Some of the genomic regions from winter germplasm that increased seed yield in the hybrid spring background may also be due to the effects of segregating chromosomal rearrangements. The QTL having the highest R^2 and additive effects in the MF and RV testcross populations occurred on a region of N10 in which the spring parent, P1804, contains a HNRT [N10.N19(T); Udall et al. 2005, 2006; Kramer, personal communication]. The winter parents did not contain a rearranged N10 and their alleles had positive effects on seed yield in testcross populations. However, the winter alleles decreased seed yield for the same QTL detected in the DH populations. This discrepancy might be attributed to the impact of linkage drag associated to disease susceptibility loci located within the same genomic region of the seed yield QTL. This linkage drag effect appears to have been covered up in the hybrids due to a resistance allele in the tester, allowing expression of the positive effect of this non-rearranged chromosome.

Our hypothesis that chromosomal rearrangements may account for allelic variation at some seed yield QTL can be extended to provide a general explanation for a portion of the seed yield heterosis in oilseed *B. napus*. The higher seed yield of hybrids compared to

their inbred may be due, in part, to increased intergenomic heterozygosity in regions containing HNRTs, as it is the case of the N10.N19(T) HNRT reported in this study. Thus, chromosomal rearrangements could have a major impact on genetic variation for seed yield and other complex traits in hybrid cultivars of oilseed *B. napus*. Molecular markers could be very useful in identifying chromosomal rearrangements and their association with traits, and in selecting desirable configurations in hybrid combinations.

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