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ORIGINAL PAPER

Quantitative trait analysis of seed yield and other complex traits in hybrid spring rapeseed (*Brassica napus* L.): 2. Identification of alleles from unadapted germplasm

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Abstract Unadapted germplasm may contain alleles that could improve hybrid cultivars of spring oilseed *Brassica napus*. Quantitative trait loci (QTL) mapping was used to identify potentially useful alleles from two unadapted germplasm sources, a Chinese winter cultivar and a re-synthesized *B. napus*, that increase seed yield when introgressed into a *B. napus* spring hybrid combination. Two populations of 160 doubled haploid (DH) lines were created from crosses between the unadapted germplasm source and a genetically engineered male-fertility restorer line (P1804). A genetically engineered male-sterile tester line was used to create hybrids with each DH line (testcrosses). The two DH line populations were evaluated in two environments and the two testcross populations were evaluated in three or four environments for seed yield and other agronomic traits. Several genomic regions were found in the two testcross populations which contained QTL for seed yield. The map positions of QTL for days to flowering and resistance to a bacterial leaf blight disease coincided with QTL for seed yield and other agronomic traits, suggesting the occurrence of pleiotropic or linked effects. For two hybrid seed yield QTL, the favorable alleles increasing seed yield originated from the unadapted parents, and one of these QTL was detected in multiple environments and in both populations. In this QTL region, a chromosome rearrangement was identified in P1804, which may have affected seed yield.

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Introduction

Although germplasm that is unadapted to a particular environment is inferior to adapted germplasm, it may contain superior alleles at some loci that could improve specific traits when introgressed into the adapted germplasm. In oilseed *Brassica napus*, unadapted germplasm has been used to improve seed yield of spring hybrid combinations. Introgression of alleles from a French winter cultivar into a parent of a spring hybrid substantially increased seed yields over open-pollinated cultivars, commercial hybrids, and experimental spring × spring hybrids (Butruille et al. 1999a). QTL analysis was used to investigate the effects of introgressing winter alleles from the German winter cultivar Ceres into the spring backgrounds of the cultivar Marnoo (Australian) and the cultivar Westar (Canadian; Butruille et al.

1999b). This introgression revealed two QTL (*HSY3* on N3 and *HSY14* on N14) for which winter alleles increased seed yield of hybrids. Recently, Quijada et al. (2004a) confirmed the favorable effects of the winter allele at *HSY3* in spring Australian and European genetic backgrounds. Additional QTL studies of French winter germplasms have identified genomic regions containing alleles from unadapted germplasm that improve seed yields of Canadian × European spring canola testcrosses (Quijada et al. 2004b; 2006).

In addition to European winter germplasm, other unadapted *B. napus* germplasm, such as Asian winter types, also may contain favorable alleles, which improve seed yield of spring hybrids. Asian winter germplasm is intermediate to spring and European winter germplasm in growth habit and responsiveness to vernalization, and it is also distinct based on molecular markers (Diers et al. 1994; Becker et al. 1995). Lefort-Buson et al. (1987) observed a correlation between genetic distance and heterosis in European and Asian winter lines. Asian winter germplasm has not been evaluated directly for heterotic effects with spring germplasm because the hybrids have greatly delayed maturity. However, alleles from Asian germplasm that are introgressed into spring hybrids could improve seed yields, as suggested by our previous phenotypic analysis (Udall et al. 2004).

The extant diploid progenitors of the allotetraploid *B. napus*, *B. rapa* (A genome) and *B. oleracea* (C genome), also represent germplasm sources which may contain novel alleles for increased seed yield in spring hybrid *B. napus*. Both diploid *Brassica* species contain more genetic diversity than *B. napus*, probably because the natural allopolyploidization event(s) caused a genetic bottleneck (Song et al. 1993). One method of allele introgression from the diploids to *B. napus* employs inter-specific hybridization of *B. rapa* and *B. oleracea* and subsequent chromosome doubling of the F_1 amphihaploid to create resynthsized *B. napus* lines. Because *B. rapa* is also bred and cultivated as an oilseed crop, some of its alleles may directly improve quantitative traits of oilseed *B. napus*. Resynthesized *B.napus* has been previously introgressed into cultivated *B. napus* germplasm with the primary objective of introducing specific traits, such as seed color (Chen et al. 1988), photoperiod insensitivity (Akbar 1989), clubroot resistance (Bradshaw et al. 1997; Manzanares-Dauleux et al. 2000), and silique shattering resistance (Prakash and Chopra 1990; Morgan et al. 1998). Kräling (1987) investigated the use of resynthesized *B. napus* to improve seed yield of winter hybrids and reported some seed yield improvement of cultivar × resynthesized and cultivar × (cultivar × resynthesized) winter hybrids. We also reported positive effects on seed yield from introgression of alleles from resynthesized *B. napus* into a spring hybrid combination (Udall et al. 2004).

In this study, we use QTL analysis to identify alleles from two unadapted sources of germplasm that affect seed yield and other traits when introgressed into a spring hybrid combination. We used two segregating populations of doubled haploid (DH) lines independently developed by crossing a Canadian spring line to an Asian winter line and to a resynthsized *B. napus* line. These populations were previously evaluated as DH lines and testcrosses in several environments, and in this study genetic linkage maps are used to identify QTL for seed yield and other important agronomic traits.

Materials and methods

Plant materials

The hybrid combination, P124 × P1804, was selected as a Canadian × European heterotic combination in which to evaluate the effects of introgressing alleles from a Chinese winter cultivar and a resynthesized *B. napus* line. The female, P124, segregates for male-sterility due to the barnase transgene (Mariani et al. 1990) and was a parent of commercial hybrids owned by Bayer CropScience. P1804 contains the barstar transgene (Mariani et al. 1992), which restores male-fertility (*Rf*), and sister lines of P1804 have been used as male parents in commercial hybrid cultivars released by Bayer CropScience.

The Chinese cultivar Hua-dbl2 and a resynthesized *B. napus* line, TO1141, were selected as unadapted germplasm sources. Hua-dbl2, a open-pollinated winter cultivar with a facultative vernalization requirement for flowering, was kindly provided by Dr. Jinling Meng, Huazhong Agricultural University, China. RV289 was a single plant selected from Hua-dbl2. TO1141 was a single plant created by crossing oilseed *B. rapa* cv. Reward as a female with rapid cycling *B. oleracea* TO1000 as a male and chromosome doubling with colchicine.

Two populations of DH lines (HUA DH and SYN DH) were developed by microspore culture from the F_1 crosses RV289 × P1804 and TO1141 × P1804, respectively (for more details, see Udall et al. 2004). One hundred and seventy DH lines of each population were selected to contain the *Rf* gene by selecting for the presence of the linked herbicide resistance gene. Testcross seed was produced using the HUA and SYN DH lines as males and P124 as the female tester line

(HUA and SYN testcross populations) by Bayer CropScience in field cages in Canada during the summer of 1998 and in Australia during the summer of 1999–2000 (see Udall et al. 2004 for more details on the development of these materials).

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, 2000, and 2001. Because of seed production problems in 1998, 148 DH lines of the HUA population were evaluated in 1999 and 2000 and 160 DH lines of the SYN population were evaluated in 2000 and 2001. 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 and 2001, the plots were 6.1 m long and were trimmed to 4.9 m long 2 weeks before swathing. Seeds were planted during the last 3 days of April each year.

The HUA and SYN testcrosses were evaluated during the summers of 1999, 2000, and 2001 at the same WI locations as the inbreds and at Saskatoon, Saskatchewan (SK), Canada during 1999 and 2000. We evaluated 155 and 157 HUA testcrosses in 1999 and 2000, respectively; and 154 and 133 SYN testcrosses were evaluated in 2000 and 2001, 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 (Hua-dbl2 or TO1141 and P1804), tester (P124) and some commercial cultivars used as checks (for more details, see Udall et al. 2004).

Several traits were measured for each plot, including days to flowering (dtf), plant height (ph), lodging (ld), seed yield (sy), test weight (tw), and seed weight (sw). A bacterial leaf blight (blb) disease caused by *Pseudomonas syringae* was evaluated only for the DH lines, since the testcrosses were resistant presumably due to a dominant resistance allele(s) in the tester. The data collection methods were reported previously (Udall et al. 2004). Additional data on seed and silique characteristics were measured on the SYN DH populations for this study and were not previously reported. Seed oil content (oil) and total glucosinolates (gls) were measured for DH lines of the WI1999 HUA population by David Syme, Bayer CropScience, Canada, as described by Toroser et al. (1995). Silique characteristics, including ease of shattering (sh), pod length (ln), and beak length (bk) were measured for the WI2001 SYN population. Between 14 and 21 days after swathing the WI2001 field, three branches containing ten or more siliques were collected from each replication of each DH line. These siliques were stored and their ease of shattering was assessed in an environmentally controlled chamber at 25°C and 25% humidity. Six to ten siliques were cut (20 total) from each of the three branches and placed in a round plastic container (radius = 9 cm) with five steel ball-bearings. The container was attached to a shaking platform (5 Hz) and the number of broken siliques was counted every 20 or 30 s until there were no unbroken siliques remaining. Silique length and beak length was measured from the remaining septa. The better fit of two regression models (linear or log) for the shattering time course was chosen for each sample and the number of seconds for one-half of the siliques to rupture was estimated from the regression line.

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. Parents of the DH lines or parents of the testcrosses were excluded from the analysis. In each experiment, environment was considered as a fixed effect and genotypes (DH lines or testcrosses), 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 (CIs) 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 twelve 3-week-old plants of the parents and the 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 DNA clones used as probes were the same ones used in previous studies (Teutonico and Osborn 1994; Ferreira et al. 1994; Thormann et al. 1996; Kole et al. 1997; Butruille et al. 1999b); however, probe nomenclature of Parkin et al. (1995) and Sharpe et al. (1995) was used due to its greater simplicity and ease of cross-referencing studies (Udall et al. 2005). Additional probe information is available at http://www.osbornlab.agronomy.wisc.edu/research.html. In addition, four homologues of *FLOWERING LOCUS C* (*FLC*), corresponding to *BrFLC1*, *BrFLC2*, *BrFLC3*, and *BrFLC5* from *B. rapa* (Schranz et al. 2002), and *FLOWERING LOCUS T* (*FT*) (Kardailsky et al. 1999) were also used as probes.

The HUA DH map had 243 marker loci at an average marker density of 7.3 cM along a 1,460 cM genetic map. The SYN DH map had 312 marker loci at an average density of 6.0 cM along a 1,668 cM genetic map. Each map had 19 linkage groups which probably corresponded to the individual chromosomes of *B. napus* ($n = 19$). These linkage maps are described in more detail elsewhere (Udall et al. 2005). The linkage groups were named N1–N10 (homologous to the A genome of *B. rapa*) and N11–N19 (homologous to the C genome of *B. oleracea*), according to the convention established by 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 HUA and SYN mapping populations (Udall et al. 2005).

QTL analysis

The QTL cartographer suite of computer programs was used to identify significant QTL by composite interval mapping (CIM) using a maximum likelihood approach (Basten et al. 2002). CIM was used to scan the genetic map and estimate the likelihood of a QTL and its corresponding effects at every 2 cM while using significant cofactors (loci) to adjust the phenotypic effects associated with other positions in the genetic map. Two to fifteen cofactors for each trait were initially selected by SRmapqtl using a forward-backward regression algorithm (cofactor included for $P < 0.01$; cofactor dropped for $P > 0.05$). Cofactors within 10 cM on either side of the QTL test site were not included in the Zmapqtl QTL model (model 6) to avoid the inclusion of putative tail effects in the model of the tested chromosome position. By convention, the likelihood of the presence of a QTL at each 2 cM interval reported here is expressed as a LOD score (Liu 1997). On each side of the QTL peak, 1 and 2 LOD CIs were extracted from the Zmapqtl output. Jzmapqtl was used to estimate QTL × environment interaction. CIM experiment-wise LOD threshold levels were determined for each trait by 1,000 permutations which shuffled the phenotypes (adjusted means) with the genotypes (markers; Doerge and Churchill 1996). Each permutation included putative cofactor selection for each model by SRmapqtl using a Perl script to iterate and parse the output of the DOS-based suite of programs. 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 $P < 0.05$.

Results

Quantitative genetic variation

A detailed analysis of seed yield and other traits were reported previously for each population, including histograms, least significant differences, and performance of the populations relative to check cultivars (Udall et al. 2004). Significant differences were found among lines in each population for nearly every trait in each environment, including seed yield. Heritability and genetic correlations are presented in Tables 1 and 2 for the DH and testcross populations, respectively. Generally, both SYN DH and SYN testcross populations had higher trait heritability estimates than HUA DH and HUA testcross populations, respectively. The difference may have been due to greater allelic differences in the SYN DH population than the HUA DH population. The DH populations generally had higher heritability estimates than their respective testcross populations, probably because the genetic contribution of the tester buffered genetic variation in the testcross populations. Days to flowering and plant height had the highest estimates of heritability, while those of seed yield were generally moderate or low. The heritability estimates showed similar trends to those reported by Butruille et al. (1999b) for four populations of spring canola IBLs with winter germplasm introgression and by Quijada et al. (2006) for two similar types of populations of DH and testcross lines; however, the estimates for IBL populations were generally lower, especially those for plant height and seed yield.

Table 1 Heritabilities and genotypic correlations of seed yield and other traits in two populations of DH lines (HUA and SYN)

Trait	SYN[a] (h^2)	CI[b]	Seed yield	Days to flowering	Plant height	Lodging[c]	Test weight	Seed weight	Leaf blight	HUA[a] (h^2)	CI
Seed yield	0.76	(0.68, 0.83)		−0.48***	0.15	−0.14	0.56***	−0.05	−0.77***	0.59	(0.43, 0.71)
Days to flowering	0.88	(0.84, 0.92)	−0.12		0.64***	−0.55***	−0.42***	−0.36***	0.22*	0.79	(0.71, 0.85)
Plant height	0.83	(0.77, 0.88)	0.44***	0.47***		−0.51***	−0.12	−0.26*	−0.34***	0.78	(0.70, 0.85)
Lodging	0.65	(0.51, 0.74)	−0.07	−0.47***	−0.36***		−0.05	0.06	0.06	0.51	(0.32, 0.65)
Test weight	0.40	(0.17, 0.56)	0.44***	0.15	0.50***	−0.07		0.25	−0.28	0.40	(0.16, 0.57)
Seed weight	0.76	(0.67, 0.83)	0.02	−0.14	0.20*	−0.29**	0.05		−0.05	0.62	(0.47, 0.73)
Leaf blight	0.59	(0.44, 0.70)	−0.76***	0.08	−0.50***	0.32***	−0.11	−0.09		0.62	(0.46, 0.72)

On the left (SYN) and right sides (HUA) of the table are listed trait heritabilities (h^2) and their respective 95% confidence intervals (CIs). Genotypic correlations are reported in the center of the table with values for the HUA population above the diagonal and values for the SYN population below the diagonal

*, **, ***Three levels of significance ($P \leq 0.05, 0.01, 0.001$, respectively). Sequential Bonferoni correction was used to declare significance of the multiple correlation tests (Rice 1989)

[a] SYN and HUA are populations of *Brasscia napus* L. DH lines derived from RV289 x P1804 and TO1141 x P1804, respectively

[b] Exact 95% CIs were calculated for each heritability (Knapp et al. 1985)

[c] Lodging estimates were only based on WI data

Table 2 Heritabilities and genotypic correlation of seed yield and other traits in two testcross populations (TxHUA and TxSYN)

Trait	TxSYN[a] (h^2)	CI[b]	Seed yield	Days to flowering	Plant height	Lodging[c]	Test weight	Seed weight	TxHUA[a] (h^2)	CI
Seed yield	0.43	(0.23, 0.57)		−0.17	0.05	−0.78***	NE	0.25*	0.36	(0.16, 0.52)
Days to flower	0.86	(0.81, 0.90)	−0.17		0.99***	−0.55***	NE	−0.67***	0.83	(0.78, 0.87)
Plant height	0.67	(0.55, 0.75)	−0.10	0.88***		−1.16[d]	NE	−1.14	0.32	(0.11, 0.49)
Lodging	0.57	(0.40, 0.70)	0.30**	−0.54***	−0.56***		NE	0.36***	0.57	(0.40, 0.70)
Test weight	0.41	(0.20, 0.56)	0.53***	0.33**	0.31**	−0.27*		NE	−0.20	(−0.57, 0.10)
Seed weight	0.66	(0.52, 0.75)	0.06	−0.58***	−0.43***	0.13	0.08		0.43	(0.26, 0.57)

On the left (TxSYN) and right sides (TxHUA) of the table are listed trait heritabilities (h^2) and their respective 95% CIs. Genotypic correlations are reported in the center of the table with values for the HUA testcross population above the diagonal and values for the SYN testcross population below the diagonal

NE No estimates available because the genotypic variance component of test weight was less than zero

*, **, ***Three levels of significance ($P \leq 0.05, 0.01, 0.001$, respectively). Sequential Bonferoni correction was used to declare significance of the multiple correlation tests (Rice 1989)

[a] TxSYN and TxHUA are testcross populations of *Brasscia napus* L. lines derived from testcrosses between two respective populations of doubled haploid lines and the tester, P124

[b] Exact 95% CIs were calculated for each estimate of heritability (Knapp et al. 1985)

[c] Lodging estimates were only based on WI data

[d] Genotypic correlation values greater than 1 were perhaps due to sampling variance

The genetic correlations between days to flowering and several other traits were highly significant in each population (Tables 1, 2). For example, days to flowering and plant height were positively correlated while both of these traits were negatively correlated with lodging. Days to flowering and plant height were not consistently correlated with seed yield. For example, days to flowering was negatively correlated with seed yield in the HUA DH population, yet plant height was positively correlated with seed yield in the SYN DH population. Other traits were also correlated with seed yield. In the DH populations, for example, seed yield was negatively correlated with bacterial leaf blight levels in both populations of DH lines. In the testcross populations, seed yield had significant positive correlations with lodging (TxSYN), seed weight (TxHUA), and test weight (TxSYN), and significant negative correlations with lodging (TxHUA).

QTL analysis

Significant QTL × environment interactions were observed for seed yield and other traits (data not shown), and some QTL were detected in only a portion of the environments. Thus, QTL analyses were conducted separately for each environment and are reported

here. QTL that mapped to the same genomic position with overlapping CIs in different environments in the DH and testcross populations were assumed to be the same. QTL were detected for each trait in each population [Figs. 1, 2; Tables S1, S2 of the Electronic Supplementary Material (ESM)], and for traits with high heritability, such as days to flowering and plant height, QTL in the same position were often found in both DH and testcross populations and in multiple environments. Different traits with highly significant QTL and overlapping LOD CIs often had significant genetic correlations, such as days to flowering and plant height. QTL for traits with moderate or low heritability, such as lodging, seed yield, test weight and seed weight were less often detected in the same position in both DH and testcross populations or in multiple environments.

Several seed yield QTL were detected in both populations. For most of the seed yield QTL, the unadapted parental alleles decreased seed yield; however, an increase of seed yield was attributed to the unadapted parental alleles at one QTL in the SYN DH population (*sy14.3*, $R^2 = 9.2\%$), at two QTL in the TxSYN population (*hsy3.3*, $R^2 = 9.6$; *hsy10.3*, $R^2 = 9.3\%$), and at one QTL in the TxHUA population detected in two environments (*hsy10.2*, $R^2 = 10.6\%$; *hsy10.4*, $R^2 = 14.4\%$). Thus, both testcross populations contained segregating alleles from the unadapted parent on N10, which increased seed yield of testcross lines. These segregating alleles were detected in approximately the same position on their respective genetic maps (Figs. 1, 2). In the HUA DH population, a seed yield QTL on N10 was detected near this region (*sy10.1* $R^2 = 14.1\%$ and *sy10.3* $R^2 = 7.9\%$). This QTL was considered different than the QTL detected in the testcross populations because the unadapted parental allele decreased seed yield and co-localized with a QTL allele for susceptibility to bacterial leaf blight in the WI environments (see below).

Several of the days to flowering and plant height QTLs (Figs. 1, 2) were detected in multiple environments and in overlapping positions on both genetic maps. Some of these individual QTL explained large portions of the phenotypic variance (e.g. *hdtf2* in the HUA testcross population: $R^2 = 28.0\%$ for *hdtf2.1*, $R^2 = 24.8\%$ for *hdtf2.2*, $R^2 = 32.1\%$ for *hdtf2.3*, $R^2 = 44.1\%$ for *hdtf2.4*); and, with one exception on N6 in the SYN DH and SYN testcross populations, they were nearly always detected in a region that included a mapped *BnFLC* locus. Large amounts of the total phenotypic variation were explained by the QTL for days to flowering and plant height within an environment in both DH and testcross populations (Tables S1, S2). For example, the QTL for days to flowering explained up to 62.5% and 68.8% of the phenotypic variation in the HUA DH and HUA testcross populations within an environment, respectively. The frequent overlapping of days to flowering and plant height QTL was not surprising because of their highly significant genotypic correlation ($P < 0.001$) in every population.

The QTL for other traits with significant genetic correlations were also detected in overlapping genomic positions, such as QTL for bacterial leaf blight, plant height and seed yield; and QTL for silique length and silique shattering. A negative correlation was found between the QTL for bacterial leaf blight and both seed yield and plant height in the HUA DH population. As previously mentioned, a QTL for seed yield was detected on N10 (*sy10.1*, $R^2 = 14.1\%$; *sy10.3*, $R^2 = 7.9\%$), and one also was detected on N16 (*sy16.3*, $R^2 = 25.4$). Bacterial leaf blight QTL (*blb10.1*, $R^2 = 8.8$; *blb16.1*, $R^2 = 34.3\%$; and *blb16.3*, $R^2 = 23.5\%$) and a plant height QTL (*ph16.3*, $R^2 = 7.3\%$) were also detected in these genomic regions. In both regions, the RV289 allele was associated with an increase of bacterial leaf blight and a decrease of seed yield and plant height. In the SYN DH population, silique length and silique shattering time were positively correlated (0.48, $P < 0.001$). Both traits had overlapping QTL on N9 (*ln9.5*, $R^2 = 25.3\%$; *sh9.1*, $R^2 = 11.0\%$) and the unadapted allele was associated with a decrease in silique length and a decrease in shattering time. In addition to these examples, many other overlapping QTL of correlated traits were also detected.

Discussion

Seed yield

Compared to alleles in adapted germplasm, most of the alleles from unadapted germplasm are expected to have negative or neutral effects on agronomically important traits. However, previous studies have shown that unadapted germplasm can have favorable alleles when introgressed into adapted germplasm, and in this study, alleles from two unadapted germplasm sources were identified that increased seed yield of spring *B. napus* DH lines (one region on N14) and hybrids (two regions on N3 and N10). The QTLs on N3 and N10 were in different positions than seed yield QTLs detected in a previous study, for which the alleles from the winter cultivar Ceres increased seed yield in a spring hybrid (Butruille et al. 1999b; Quijada et al. 2004a). QTL on N10 were detected in the same region in both segregating testcross populations of our study,

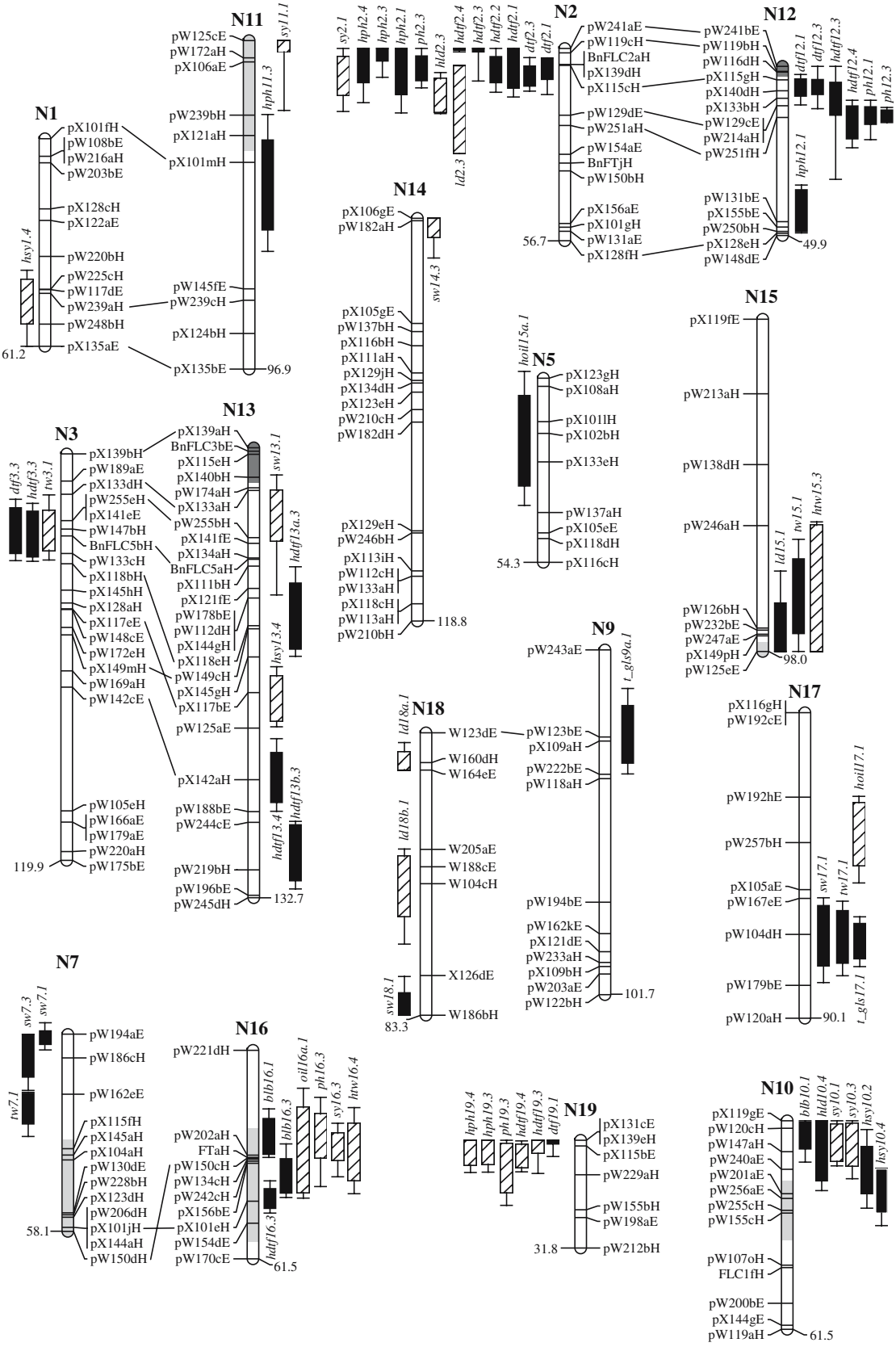
N1
pX101fH
pW108bE
pW216aH
pW203bE
pX128cH
pX122aE
hsy1.4
pW220bH
pW225cH
pW117dE
pW239aH
pW248bH
pX135aE
61.2
pX101mH
N11
pW125cE
pW172aH
pX106aE
pW239bH
pX121aH
hph11.3
sy1.1
pW145fE
pW239cH
pX124bH
pX135bE
96.9
N2
sy2.1
hph2.4
hph2.3
hph2.1
ph2.3
hld2.3
hdf2.4
hdf2.3
hdf2.2
hdf2.1
dtf2.3
dtf2.1
ld2.3
pW241aE
pW119cH
BnFLC2aH
pX139dH
pX115cH
pW129dE
pW251aH
pW154aE
BnFTjH
pW150bH
pX156aE
pX101gH
pW131aE
pX128fH
56.7
N12
pW241bE
pW119bH
pW116dH
pX115gH
pX140dH
pX133bH
pW129cE
pW214aH
pW251fH
hdtf12.1
dtf12.3
hdtf12.3
hdtf12.4
ph12.1
ph12.3
hph12.1
pW131bE
pX155bE
pW250bH
pX128eH
pW148dE
49.9
N14
pX106gE
pW182aH
sw14.3
pX105gE
pW137bH
pX116bH
pX111aH
pX129jH
pX134dH
pX123eH
pW210cH
pW182dH
pX129eH
pW246bH
pX113iH
pW112cH
pW133aH
pX118cH
pW113aH
pW210bH
118.8
N5
hoil15a.1
pX123gH
pX108aH
pX101lH
pX102bH
pX133eH
pW137aH
pX105eE
pX118dH
pX116cH
54.3
N15
pX119fE
pW213aH
pW138dH
pW246aH
pW126bH
pW232bE
pW247aE
pW149pH
pW125eE
98.0
ld15.1
sw15.1
htw15.3
N3
dtf3.3
hdtf3.3
tw3.1
pX139bH
pW189aE
pX133dH
pW255eH
pX141eE
pW147bH
BnFLC5bH
pW133cH
pX118bH
pX145hH
pX128aH
pX117eE
pW148cE
pW172eH
pX149mH
pW169aH
pW142cE
pW105eH
pW166aE
pW179aE
pW220aH
pW175bE
119.9
N13
pX139aH
BnFLC3bE
pX115eH
pX140bH
pW174aH
pX133aH
pW255bH
pX141fE
pX134aH
BnFLC5aH
pX111bH
pX121fE
pW178bE
pW112dH
pX144gH
pX118eH
pW149cH
pX145gH
pX117bE
pW125aE
pX142aH
pW188bE
pW244cE
pW219bH
pW196bE
pW245dH
132.7
sw13.1
hdtf13a.3
hsy13.4
hdtf13.4
hdtf13b.3
N9
pW243aE
t_gls9a.1
pW123bE
pX109aH
pW222bE
pW118aH
pW194bE
pW162kE
pX121dE
pW233aH
pX109bH
pW203aE
pW122bH
101.7
N18
ld18a.1
W123dE
W160dH
W164eE
ld18b.1
W205aE
W188cE
W104cH
X126dE
W186bH
sw18.1
83.3
N17
pX116gH
pW192cE
pW192hE
pW257bH
pX105aE
pW167eE
pW104dH
pW179bE
pW120aH
90.1
hoil17.1
sw17.1
tw17.1
t_gls17.1
N7
sw7.3
sw7.1
tw7.1
pW194aE
pW186cH
pW162eE
pX115fH
pX145aH
pX104aH
pW130dE
pW228bH
pX123dH
pW206dH
pX101jH
pX144aH
pW150dH
58.1
N16
pW221dH
pW202aH
FTaH
pW150cH
pW134cH
pW242cH
pX156bE
pX101eH
pW154dE
pW170cE
61.5
blb16.1
blb16.3
hdtf16.3
oil16a.1
ph16.3
sy16.3
htw16.4
N19
hph19.4
hph19.3
ph19.3
hdtf19.4
hdtf19.3
dtf19.1
pX131cE
pX139eH
pX115bE
pW229aH
pW155bH
pW198aE
pW212bH
31.8
N10
pX119gE
pW120cH
pW147aH
pW240aE
pW201aE
pW256aE
pW255cH
pW155cH
pW107oH
FLC1fH
pW200bE
pX144gE
pW119aH
61.5
blb10.1
hld10.4
sy10.1
sy10.3
hsy10.2
hsy10.4

Fig. 1 Quantitative trait loci (*QTL*) for seed yield and other traits detected in the HUA doubled haploid (*DH*) population and its testcross population. QTL were designated using the trait name initials 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* (*solid* RV289 allele and *hatched* P1804 allele) and whiskers represent 1 LOD and 2 LOD confidence intervals, respectively for significant QTL based on 1000 permutation tests for each trait in each environment. The allele that increased trait value is indicated by *hatched* (P1804) or *solid* (HUA) *boxes*. Linkage group designations follow the convention of Parkin et al. (1995). *Light and dark shaded regions* within LGs represent approximate locations of segregating chromosomal rearrangements detected in this population from the P1804 and Hua-dbl2 parents, respectively

and QTL in this region were also detected on two additional segregating testcross populations which used the same Canadian line as a parent (P1804) and the same tester (P124) (Quijada et al. 2006). P1804 appeared to have a chromosomal rearrangement on N10 which may have caused reduced seed yield and was corrected by replacement with regions of normal N10 chromosomes from the unadapted parents (discussed further below). Segregating chromosomal rearrangements may also have accounted for some of the QTL that affected seed yield in the DH populations. For other QTL detected in the DH populations, seed yield may have been affected by loci that had primary effects on other traits, such as flowering time and disease resistance (see below).

Days to flowering

The unadapted parents also contributed alleles that affected flowering time, many of which were in the same genomic regions in the different populations. However, the allelic effects from the two unadapted parents were in opposite directions: alleles from RV289, which is late-flowering, generally delayed flowering, whereas alleles from TO1141, which is early flowering, generally reduced days to flowering.

Most of the QTL for days to flowering were in regions having homology to the top of *Arabidopsis thaliana* chromosome 5 where the flowering time gene *FLC* is located (Osborn et al. 1997). Two of these QTL were on N2 and N3 in regions that were previously found to have QTL for flowering time and named *VFN4* and *VFN3*, respectively (Osborn et al. 1997; Butruille et al. 1999b). Indeed, flowering time variation in three *B. rapa* populations was associated with *B. rapa FLC* homologs on linkage groups R2 (*BrFLC2*), R3 (*BrFLC3* and *BrFLC5*), and R10 (*BrFLC1*) (Kole et al. 2001; Schranz et al. 2002), which are homologous to N2, N3, and N10 of *B. napus*. Kole et al. (2001) and Schranz et al. (2002) also observed that the number of late alleles had a clear cumulative effect in delaying flowering time. In this study, we found that six of the eight polymorphic *BrFLC* loci mapped within the CI of QTL for days to flowering in one or both populations and that together these QTL in *BnFLC* genomic regions could explain up to 68% of the flowering time variation within a single environment (HUA testcross population in WI2000).

Only one non-*FLC* genomic region (middle of N6) was associated with flowering time in these and two additional populations (Quijada et al. 2006). DNA sequences of the N6 RFLP probes indicated homology between the middle of N6 and the bottom of *A. thaliana* chromosome five (At5), as had been reported previously (Parkin et al. 2002). Based on this alignment, a candidate locus for the N6 flowering QTL is the *B. napus* homolog of *A. thaliana MAF2* (Ratcliffe et al. 2003). *MAF2* is a MADS box gene that is highly related to *FLC* by DNA sequence and function, and this gene also may regulate the autonomous flowering pathway in *B. napus*.

The five regions including *BnFLC* loci and the region on N6 containing significant QTL for days to flowering also had QTL with overlapping CIs for other traits. Such overlapping QTL can be explained either by closely linked loci with independent phenotypic effects or by pleiotropy. Since the time to flowering plays a central role in developmental processes of annual crop species such as *B. napus*, it is likely that at least some of these overlapping QTL were the result of pleiotropy. For example, significant QTL for lodging (N2 HUA; N19 SYN), seed weight (N13 HUA; N12 SYN), test weight (N3 HUA; N19 SYN), and seed yield (N2 HUA; N6 SYN) all had overlapping CIs with QTL for days to flowering. Many of these co-segregating QTL were detected in the Wisconsin environments where the populations were exposed to heat stress in late summer. Heat stress (temperatures above 29.5°C) can cause male-sterility (Morrison and Stewart 2000) and/or seed abortion, possibly affecting seed weight, test weight and seed yield. This temperature threshold was exceeded during the last eleven days of July 1999 and the first ten days of August 2001 in Arlington, WI (Udall et al. 2004) and late flowering lines that flowered

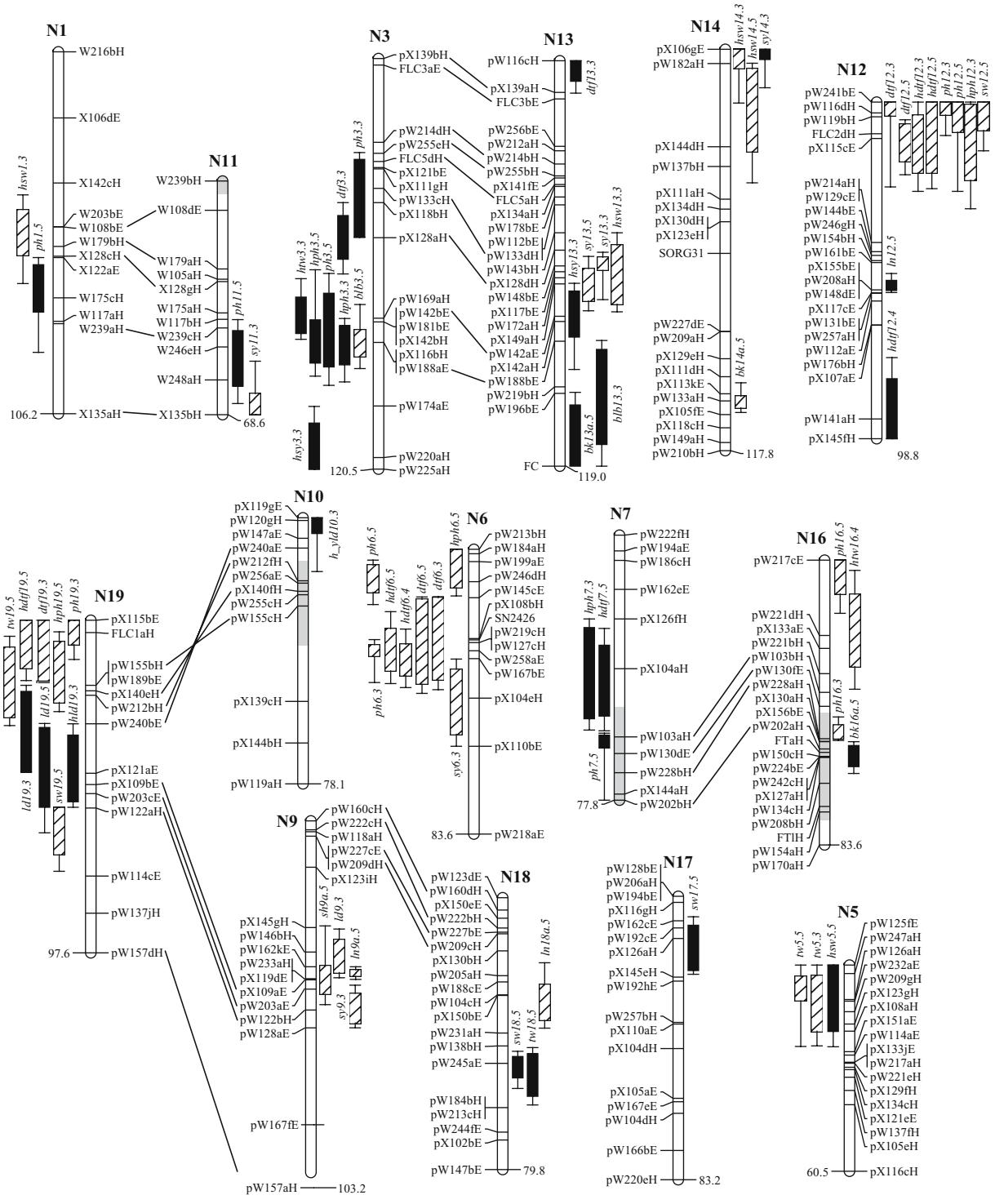

Fig. 2 Quantitative trait loci for seed yield and other traits detected in the SYN DH population and its testcross population. QTL were designated using the trait name initials followed by a number identifying the LG. After the LG, a number was used to indicate the environment where the QTL was detected: *3* WI2000, *4* SK2000, and *5* WI2001. 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* (*solid* TO1141 allele and *hatched* P1804 allele) and *whiskers* represent 1 LOD and 2 LOD CI, 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* (HUA) *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 from the P1804 parent

during or close to these times may have had reduced seed yield due to the effects of heat on fertility.

Silique characters

Although the resynthesized and P1804 parents had similar levels of shattering resistance, this trait appeared to segregate in the SYN population in WI2000 and it was measured in WI2001. A QTL for silique shattering (*sh9.5*) was detected on N9 (Table S1), and the allele(s) from TO1141 decreased the number of seconds required for the siliques to rupture (i.e., increased ease of shattering). This region from TO1141 also reduced silique length (*ln9.5* in Table S1); and thus, the increased ease of shattering may have been a pleiotropic effect of an allele that reduced silique length. TO1141 alleles in this region also had a negative effect on seed yield in one environment (*sy9.3* in Table S1).

Bacterial leaf blight

Bacterial leaf blight occurred only in the Wisconsin environments and only affected the DH populations. QTL for this disease were found in both DH populations. In the SYN population, two QTL for bacterial leaf blight disease were detected in homoeologous regions of N3 and N13. Homologs of *A. thaliana* bacterial disease resistance gene (*RPM1*) were previously reported in these regions in other *B. napus* lines, but they were determined to be non-functional (Grant et al. 1998; Sillito et al. 2000). In this study, two homoeologous loci on N3 and N13 appeared to have played a role in disease response, although the effects of unadapted alleles were in opposite directions (less disease with the TO1141 allele than the P1804 allele on N3; more disease with the TO1141 allele than the P1804 allele on N13). As was the case with days to flowering, QTL for other traits had overlapping CIs with those for bacterial leaf blight. In the SYN populations, for example, plant height and test weight QTL had overlapping CIs with bacterial leaf blight QTL, and lines with the TO1141 allele at *blb3.5* generally had less symptoms of the bacterial disease, greater plant height, and greater test weights than lines with the alternative allele (P1804).

Different QTL for bacterial leaf blight were detected in the HUA DH population (*blb10* and *blb16*). The *blb10* QTL had a relatively small effect and overlapped with a seed yield QTL (*sy10*). DH lines with the RV289 allele in this region generally had more disease and less seed yield. Thus, the *sy10* QTL for DH seed yield may have been due to the effects of a RV289 allele conferring disease susceptibility, and was probably different than the N10 QTL for seed yield detected in the testcross population (see below). QTL for bacterial leaf blight and seed yield were also detected in the same genomic region on N10 in two other DH mapping populations grown in the same Wisconsin environments with similar interpretations (Quijada et al. 2006). The QTL for bacterial leaf blight with the largest effects were detected on N16 (*blb16.1* $R^2 = 34.3\%$, and *blb16.3* $R^2 = 23.5\%$) in the same position as QTL for plant height (*ph16.3*), seed yield (*sy16.3*), and test weight. A search for candidate genes of these QTL in the region of the *A. thaliana* genome with homology to N16 did not reveal any likely candidates. As suggested above, clustering of these QTL may have been due to the pleiotropic effects of a segregating allele for susceptibility from RV289. Alternatively, the clustering of QTL on N16 (in both populations) could have been due to the segregation of a chromosomal rearrangement found in the parent P1804 (see below; Udall et al. 2005). Clusters of QTL, for bacterial leaf blight, days to flowering, and seed yield also were found within chromosomal rearrangements in other mapping populations (Quijada et al. 2006).

Effects of chromosomal rearrangements

The presence of chromosomal rearrangements were previously reported for the two populations used in this study (Figs. 1, 2; Udall et al. 2005), and for additional mapping populations developed from genetically diverse parents (Udall et al. 2005; Sharpe et al. 1995; Parkin et al. 1995). Nearly all of the observed rearrangements can be explained by infrequent recombination between pairs of homoeologous chromosomes resulting in homoeologous reciprocal (HRT) and homoeologous non-reciprocal transpositions (HNRT) between the two sub-genomes of *B. napus*. Udall et al. (2005) provided a more detailed explanation of HRT and HNRT than described here, including method of detection. Homoeologous recombination events that occurred during F_1 meiosis (i.e., de novo) resulted in transpositions only in the derived line. However, different transpositions existed in the natural *B. napus* lines used as mapping parents (i.e., P1804 and RV289), which segregated in the mapping populations, and in some cases phenotypic effects co-segregated with the rearrangements.

Two parents of these experimental populations, P1804 and RV289, contained chromosomal transpositions which segregated among the DH lines, including HNRTs on N12.N2(T) and N13.N3(T) in RV289 and on N11.N1(T), N12.N2(T), N15.N5(T) and N10.N19(T)

in P1804, and an HRT on N7.N16(T) and N16.N7(T) in P1804 (Figs. 1, 2; Udall et al. 2005). Clusters of QTL for various traits were found in or near the N10.N19(T), N12.N2(T), N15.N5(T), and N16.N7(T) HNRTs in the HUA map and the N16.N7(T) HRT in the SYN map. While pleiotropic effects of single genes is one possible explanation for QTL clustering (see above), large allelic differences of several linked loci between the normal chromosome (e.g. N2, *B. rapa* homolog) and the chromosomal transposition [e.g. N2.N12(T), *B oleracea* homolog] could also account for these quantitative differences among segregating DH or their testcross progenies.

Several seed yield QTL were associated with chromosomal rearrangements. The QTL detected on N16.N7(T) in the HUA DH population mapped in approximately the same position as QTL in two other DH and testcross populations (Quijada et al. 2006). A seed yield effect was also associated with segregation of a N7–N16 HRT in four other mapping populations (Osborn et al. 2003). These authors observed reduced seed yield in lines with the non-parental configurations of the HRT (equivalent to lines with an HNRT), and they suggested this was due to an increase in intergenomic homozygosity. In our study, the HUA population was fixed for the N7.N16 (T) chromosome from P1804 due to selection for herbicide resistance gene near or in the rearrangement. Lines with the unrearranged N16 from RV289 had lower seed yield, perhaps due to increased intergenomic homozygosity from the presence of N7.N16 (T) and N16.

For the seed yield QTL on N10, alleles from the unadapted parents increased seed yield in the HUA and SYN testcross populations, and in the testcross populations reported by Quijada et al. (2006). All four of these populations shared a common parent, P1804, which had a HNRT on N10 [a segment of N19 (*B. oleracea* homolog) on the *B. rapa* homolog N10] and the testcross lines were all produced using a common tester P124, whose chromosomal configuration is undetermined. The N10 chromosomes from RV289 and TO1141 were normal; thus, the higher testcross seed yields associated with N10 allele(s) from these unadapted parents may have been due to the effects of normal chromosomal regions on increasing intergenomic heterozygosity. The fact that alleles in this region from the unadapted parents decreased seed yield in the DH populations could be due to the impact of linkage drag from alleles for disease susceptibility located in this same genomic region. The effect of this linkage drag appears to be covered up in the testcross populations due to a resistance allele in the tester, allowing us to see the positive effect of this region from the unadapted germplasms.

Additional studies are needed to validate the effects of these QTL alleles in this genetic background, and to determine their effects in other genetic backgrounds. These studies might also resolve questions of linkage *vs.* pleiotropy, and they could provide additional information on the relationship of the QTL to chromosomal rearrangements. Similar types of studies with other germplasm might provide evidence that chromosomal rearrangements have broad effects on allelic diversity in oilseed *B. napus*. The results could be used to improve hybrids by selecting for recombination events that correct deleterious rearrangements.

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