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ORIGINAL PAPER

Genetic control of oil content in oilseed rape (*Brassica napus* L.)

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Abstract In oilseed rape (*Brassica napus* L.) like in most oleaginous crops, seed oil content is the main qualitative determinant that confers its economic value to the harvest. Increasing seed oil content is then still an important objective in oilseed rape breeding. In the objective to get better knowledge on the genetic determinism of seed oil content, a genetic study was undertaken in two genetic backgrounds. Two populations of 445 and a 242 doubled haploids (DH) derived from the crosses ''Darmor-*bzh*'' × ''Yudal'' (DY) and ''Rapid'' × ''NSL96/25'' (RNSL), respectively, were genotyped and evaluated for oil content in different trials. QTL mapping in the two populations indicate that additive effects are the main factors contributing to variation in oil content. A total of 14 and 10 genomic regions were involved in seed oil content in DY and RNSL populations, respectively, of which five and two were consistently revealed across the three trials performed for each population. Most of the QTL detected were not co-localised to QTL involved in flowering time. Few epistatic QTL involved regions that carry additive QTL in one or the other population. Only one QTL located on linkage group N3 was potentially common to the two populations. The comparisons of the QTL location in this study and in the literature showed that: (i) some of the QTL were more consistently revealed across different genetic backgrounds. The QTL on N3 was revealed in all the studies and the QTL on N1, N8 and N13 were revealed in three studies out of five, (ii) some of the QTL were specific to one genetic background with potentially some original alleles, (iii) some QTL were located in homeologous regions, and (iv) some of the regions carrying QTL for oil content in oilseed rape and in Arabidopsis could be collinear. These results show the possibility to combine favourable alleles at different QTL to increase seed oil content and to use Arabidopsis genomic data to derive markers for oilseed rape QTL and identify candidate genes, as well as the interest to combine information from different segregating populations in order to build a consolidated map of QTL involved in a specific trait.

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Introduction

Oilseed rape, *Brassica napus* L., is one of the primary sources of vegetable oil for human nutrition and industrial products. For the crushing industry, the main value of oilseed rape is linked to the oil content of the harvested seeds, in spite of some value of the protein part for animal feeding. Increasing oil content remains an important objective of oilseed rape breeding. This is all the more so essential for biodiesel market development for which the production costs must be as low as possible. A better knowledge of genetic determinism of oil content could help the breeder to control the genetic advance for the crop. As some QTLs and genes involved in the change of fatty acid composition have been identified (Ecke et al. 1995; Jourdren et al. 1996a, b; Thormann et al. 1996; Fourmann et al. 1998), little is known on metabolism pathways that interact with total seed oil content. Using genetic mapping, Ecke et al. (1995) and Cheung and Landry (1998) identified a few quantitative trait loci (QTL) for oil content in *B. napus* and *B. juncea* and some of these QTL were linked to the loci controlling erucic acid content. More recently, seven QTL were identified using an intervarietal subset of substitution lines (Burns et al. 2003). Eight QTL with additive effects and nine pairs of loci with additive × additive epistasis with high genotype × environment interactions were detected in a cross involving a Chinese and a European parental line (Zhao et al. 2005, 2006). A complex genetic determinism underlies seed oil content in oilseed rape and a great diversity of favourable alleles might exists when considering different gene pools as in other crop species like sunflower (Leon et al. 2003; Al-Chaarani et al. 2004), soybean (Hyten et al. 2004) or maize (Song et al. 2004).

The aim of our study was to enrich knowledge on QTL involved in oil content in oilseed rape through the genetic analysis of two genetic backgrounds. One cross involves a Korean spring line, ''Yudal'', high in erucic acid and in glucosinolate and a French double low winter line ''Darmor'' in which the dwarf *bzh* gene was introduced (Foisset et al. 1996). The other cross involves two double low French winter oilseed rape lines. The QTL detected in the two crosses are compared and examined in relation to the ones already published in the literature. The effect of genetic background and environment on the QTL detected is discussed.

Fig. 1 Genetic maps and QTL position in the two populations. The name of the linkage groups of each DH population (DY for ''Darmor-*bzh*'' × ''Yudal'' and RNSL for ''Rapid'' × ''NSL96/25'') are aligned to the N1–N19 nomenclature of Parkin et al. (1995). Common segregating loci between DY and RNSL populations are underlined. Confidence intervals of QTL for oil content (*black rectangles*) in the six trials (OILRE01, OILRE02, OILSE02 for DY population and OILVERN, OILCVIL, OILROSEN for RNSL population) and for days-to-flowering (hatched rectangles) in the five trials (DTFRE01, DTFRE02, DTFSE02 for DY population and DTFVERN, DTFCVIL for RNSL population) are indicated on the right side of the linkage groups ▶

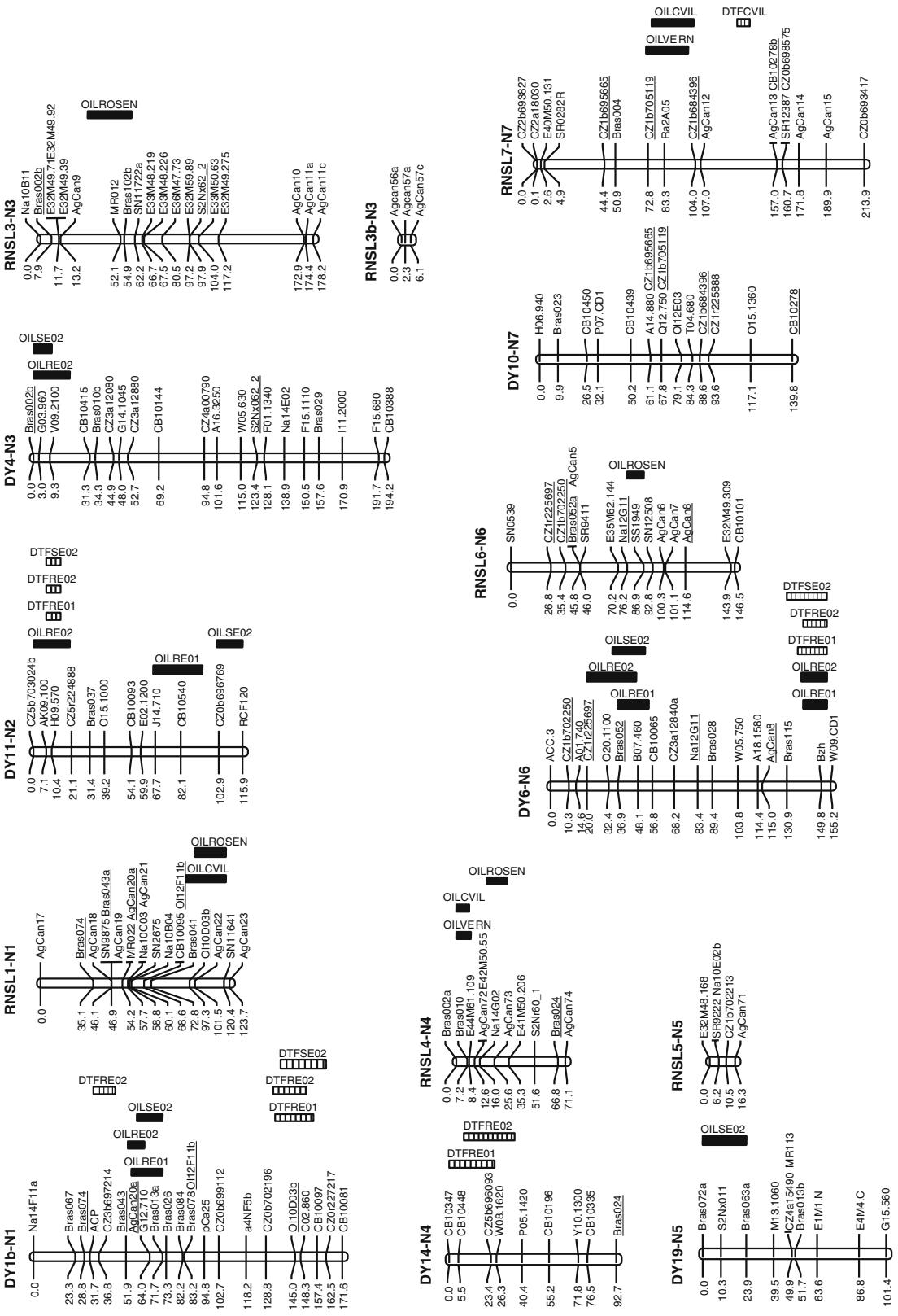
Materials and methods

Plant materials

The segregating DH population derived from the cross ''Darmor-*bzh*'' × ''Yudal'' was obtained as described in Foisset et al. (1996). This cross was chosen as the reference cross for mapping and genetic studies in the INRA group. It consisted of 445 DH lines and was named DY population. ''Darmor-*bzh*'' is a dwarf isogenic line resulting from the introduction of the dwarf *bzh* gene in the French winter cultivar ''Darmor''. ''Yudal'' is a spring Korean line that behaves as an early-flowering winter type in temperate climates. A segregating DH population was derived from the cross ''Rapid'' × ''NSL96/25'' through isolated microspore cultures as described by Coventry et al. (1988) of F1 plants. The two parents were chosen in the elite winter oilseed rape germplasm as they differ in oil content. The population consisted of 242 DH lines and was named RNSL population.

Genetic markers and maps

For DY population, the map published in Lombard and Delourme (2001) was used as a starting point and new PCR markers were then added to progressively replace RFLP, RAPD and AFLP markers. SSR primer pairs prefixed ''BRAS'' and ''CB'' were developed by Celera AgGen consortium. SSR primer pairs prefixed ''MR'' or ''MD'' were developed by the Institute of Agronomy and Plant Breeding of the University of Göttingen (Uzunova and Ecke 1999; Rudolph 2001). All these primer pair sequences are available from the electronic supplementary material of Piquemal et al. (2005). Some public SSRs obtained from http://www.ukcrop.net/perl/ace/search/BrassicaDB were used when necessary to complete the map or to align it with


DY1b-N1
Na14F11a
Bras067
Bras074
ACP
CZ3b697214
Bras043
AgCan20a
Bras013a
G12.710
Bras026
Bras084
Bras078
Ol12F11b
pCa25
CZ0b699112
a4NF5b
CZ0b702196
Ol10D03b
C02.860
CB10097
CZ0r227217
CB10081
OILRE01
OILRE02
OILSE02
DTFRE02
DTFRE01
DTFSE02
RNSL1-N1
AgCan17
Bras074
AgCan18
SN9875
Bras043a
AgCan19
MR022
AgCan20a
Na10C03
AgCan21
SN2675
Na10B04
CB10095
Ol12F11b
Bras041
Ol10D03b
AgCan22
SN11641
AgCan23
OILCVIL
OILROSEN
DY11-N2
CZ5b703024b
AK09.100
H09.570
CZ5r224888
Bras037
O15.1000
CB10093
E02.1200
J14.710
CB10540
CZ0b696769
RCF120
OILRE01
OILRE02
OILSE02
DTFRE01
DTFRE02
DTFSE02
DY4-N3
Bras002b
G03.960
V09.2100
CB10415
Bras010b
CZ3a12080
G14.1045
CZ3a12880
CB10144
CZ4a00790
A16.3250
W05.630
S2Nx062_2
F01.1340
Na14E02
F15.1110
Bras029
I11.2000
F15.680
CB10388
OILRE02
OILSE02
RNSL3-N3
Na10B11
Bras002b
E32M49.771E32M49.92
E32M49.39
AgCan9
MR012
Bras102b
SN11722a
E33M48.219
E33M48.226
E36M47.73
E32M59.89
S2Nx62_2
E33M50.63
E32M49.275
AgCan10
AgCan11a
AgCan11c
OILROSEN
RNSL3b-N3
Agcan56a
Agcan57a
AgCan57c
DY10-N7
H06.940
Bras023
CB10450
P07.CD1
CB10439
A14.880
CZ1b695665
Q12.750
CZ1b705119
Ol12E03
T04.680
CZ1b684396
CZ1r225888
O15.1360
CB10278
RNSL7-N7
CZ2b693827
CZ2a18030
E40M50.131
SR0282R
CZ1b695665
Bras004
CZ1b705119
Ra2A05
CZ1b684396
AgCan12
AgCan13
CB10278b
SR12387
CZ0b698575
AgCan14
AgCan15
CZ0b693417
OILVERN
OILCVIL
DTFCVIL
DY6-N6
ACC.3
CZ1b702250
A01.740
CZ1r225697
O20.1100
Bras052
B07.460
CB10065
CZ3a12840a
Na12G11
Bras028
W05.750
A18.1580
AgCan8
Bras115
Bzh
W09.CD1
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SN0539
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CZ1b702250
Bras052a
AgCan5
SR9411
E35M62.144
Na12G11
SS1949
SN12508
AgCan6
AgCan7
AgCan8
E32M49.309
CB10101
OILRE01
OILRE02
OILSE02
DTFRE01
DTFRE02
DTFSE02
RNSL4-N4
Bras002a
Bras010
E44M61.109
AgCan72
E42M50.55
Na14G02
AgCan73
E41M50.206
S2N60_1
Bras024
AgCan74
OILVERN
OILCVIL
OILROSEN
DY14-N4
CB10347
CB10448
CZ5b696093
W08.1620
P05.1420
CB10196
Y10.1300
CB10335
Bras024
DTFRE01
DTFRE02
OILRE01
OILRE02
OILSE02
RNSL5-N5
E32M48.168
SR9222
Na10E02b
CZ1b702213
AgCan71
DY19-N5
Bras072a
S2Nx011
Bras063a
M13.1060
CZ4a15490
MR113
Bras013b
E1M1.N
E4M4.C
G15.560
OILRE01
OILSE02
DTFRE02

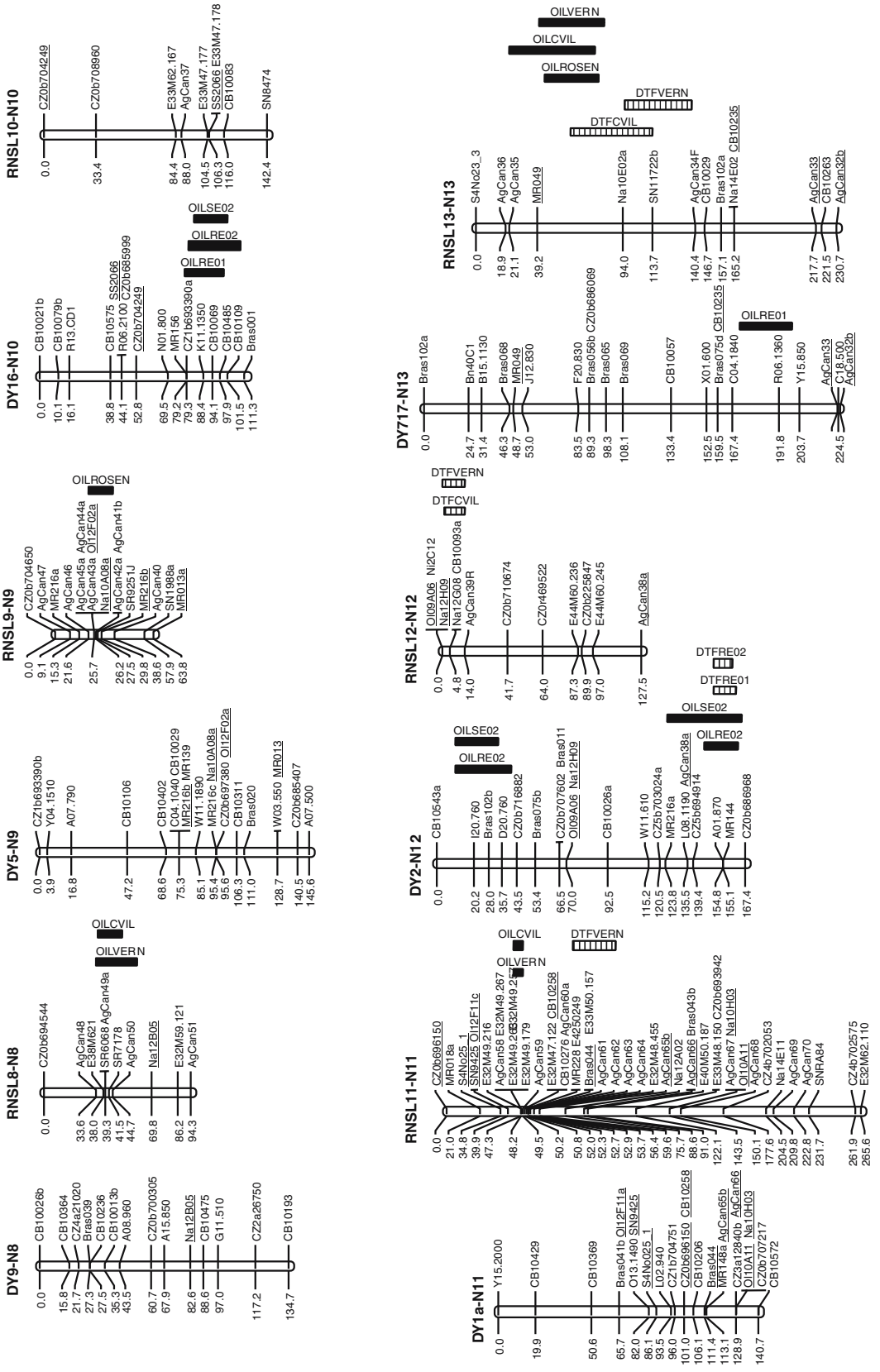

Fig. 1 continued

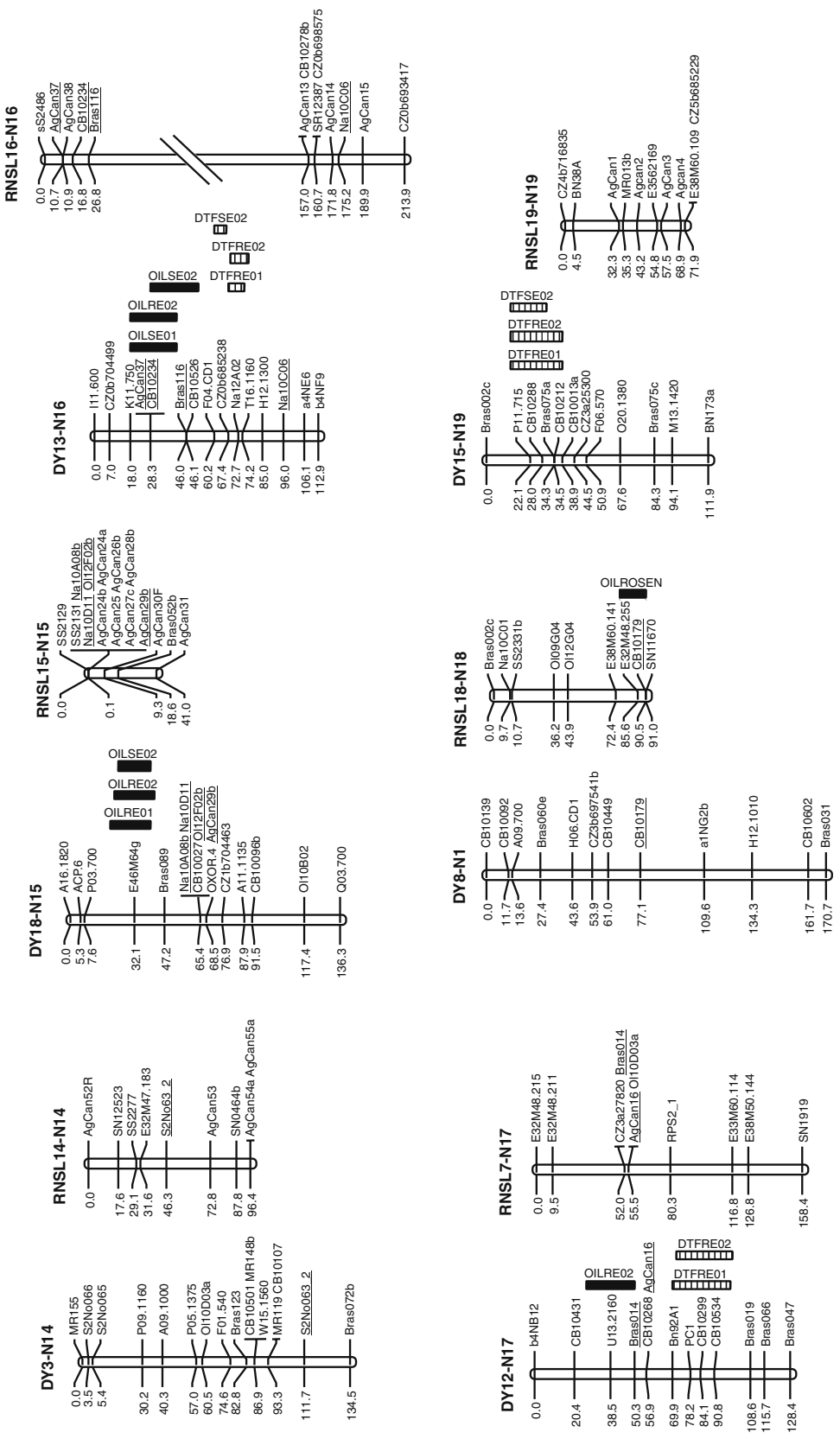

Fig. 1 continued

other published maps. In addition, some SNP (named SnN(o/r/x)nnn) and physical functional markers (PFM, prefixed ''CZ'') markers obtained through Génoplante projects in collaboration with D. Brunel (INRA, Versailles, France) and B. Chalhoub (INRA, URGV, Evry, France) were used.

For RNSL population, the same sets of markers were used. In addition, SSR primer pairs prefixed ''SN'', ''SR'' and ''SS'' were developed through a collaborative research project led by Agriculture and Agri-Food Canada Research Branch and supervised by Andrew Sharpe at the Saskatoon Research Center (SharpeA@AGR.GC.CA). These SSRs are referred to as AgCan SSRs. AFLP markers were used in order to complete the map. AFLP analyses were performed according to Keygene, Wageningen, http://www.keygene.com using the procedure described by Vos et al. (1995). A total of 23 primer combinations involving 10 *Eco*RI fluorescence labelled primers (E32, E33, E35, E36, E38, E39, E40, E41, E42 and E44) and 9 *Mse*I primers(M47, M48, M49, M50, M58, M59, M60, M61, and M62) were used for the selective amplification reaction. The amplification product was loaded on a ABI 3700 capillary electrophoresis system according to the recommendations of the manufacturer.

PCR reactions for SSR markers in DY population were performed in 96-well plates with a volume of 5 µl. The composition of the mix was: Taq DNA polymerase (Eurobio) 0.03 U/µl, Eurobio Taq buffer ten-times diluted, $MgCl_2$ 2 mM, dNTP 75 µM, forward primer coupled to a 19-base M13 tail in 5′ 0.04 µM, reverse primer 0.4 µM and the 19-base M13 tail primer with the 700 nm or the 800 nm chromophore (IR_700 or IR_800) 0.36 µM. DNA (0.5 ng) was added for each reaction. The PCR was performed with the following program: 94°C for 4 min; 12 cycles 94°C for 30 s, annealing temperature for 60 s, 72°C for 30 s, with an annealing temperature starting at 65°C and 1°C decrease at each cycle; then, 25 cycles with 94°C for 30 s, 53°C for 60 s and 72°C for 30 s; then a final elongation step of 10 min. PCR products were loaded on a LI-COR DNA Analyzer, using 25 cm polyacrylamide electrophoresis gels (6.5%) according to the recommendations of the manufacturer.

PCR reactions for SSR markers in RNSL population were performed in 96-well plates with a final volume of 20 µl. The composition of MIX was: Taq DNA polymerase (Invitrogen) 0.02 U/µl; Invitrogen Taq buffer ten-time diluted, $MgCl_2$ 1.5 mM, dNTP 0.2 mM each, forward primer coupled to a 19-base M13 tail in 5′ 0.025 µM, reverse primer 0.25 µM and the 19-base M13 tail primer 0.25 µM. DNA (25 ng) was added for each reaction. The PCR was performed with the following program: 94°C for 5 min; 94°C for 30 s, annealing temperature for 30 s, 72°C for 45 s with 35 cycles. PCR products were loaded on a 3700 capillary electrophoresis according to the recommendations of the manufacturer.

Goodness of fit to expected Mendelian ratios for each segregating locus was tested by Chi-square analysis ($\alpha = 1\%$). MAPMAKER/EXP 3.0 (Lincoln et al. 1992) was used to build the maps. A minimum LOD score of 5.0 and a maximum distance of 37.5 cM were used to group loci into the linkage groups. A multipoint analysis was performed to order the loci on each linkage group with the commands ''order'' and ''ripple'' (LOD = 3.0 and then 2.0). Centimorgan distances were expressed with the Kosambi function (Kosambi 1944).

Field experiment

The field experiment with 442 DH lines of the DY population was conducted in a randomized incomplete block design with 3 replicates and 12 blocks per replicate, the dwarf and tall lines being in separate blocks. The trials were located at le Rheu, France in 2001 (RE01) and 2002 (RE02) (INRA experimental unit) and at Lille, France in 2002 (SE02; Serasem station). Each plot consisted of two rows (2 m long). For RNSL, the field experiment with the 242 DH was conducted as a non-randomized block with two replicates and four blocks per replicate; the trials were located at Verneuil (VERN; LVH station) and Chartainvilliers (CVIL; LVH station), France and at Rosenthal (ROSEN; LVH station), Germany in 2003. Each plot consisted of five rows (2.5 m long). Earliness of flowering was assessed by recording the number of days from the sowing date at which 50% of the plants of a plot was flowered for DY population and 10% for RNSL population. At mature time, a sample of seeds was bulk harvested from the whole plot and oil content was estimated using near-infrared reflectance spectroscopy (Foss NIRS system) (Tillmann 1997).

Statistical analysis and QTL mapping

For each trial, the analysis of variance (ANOVA; proc GLM of Statistical Analysis System, SAS, SAS Institute Inc. 1989) partitioned total variation into effects of lines, replicates, blocks and errors ($P_{ijk} = \mu + L_i + R_j + B_{k/j} + e_{ijk}$ where P_{ijk} is the mean oil content of the ith DH line located in the kth block of the jth replicate, μ the mean of all the data, L_i the DH line i effect, R_j the replicate j effect, $B_{k/j}$ the block k effect in the jth replicate and e_{ijk} the residual) for DY

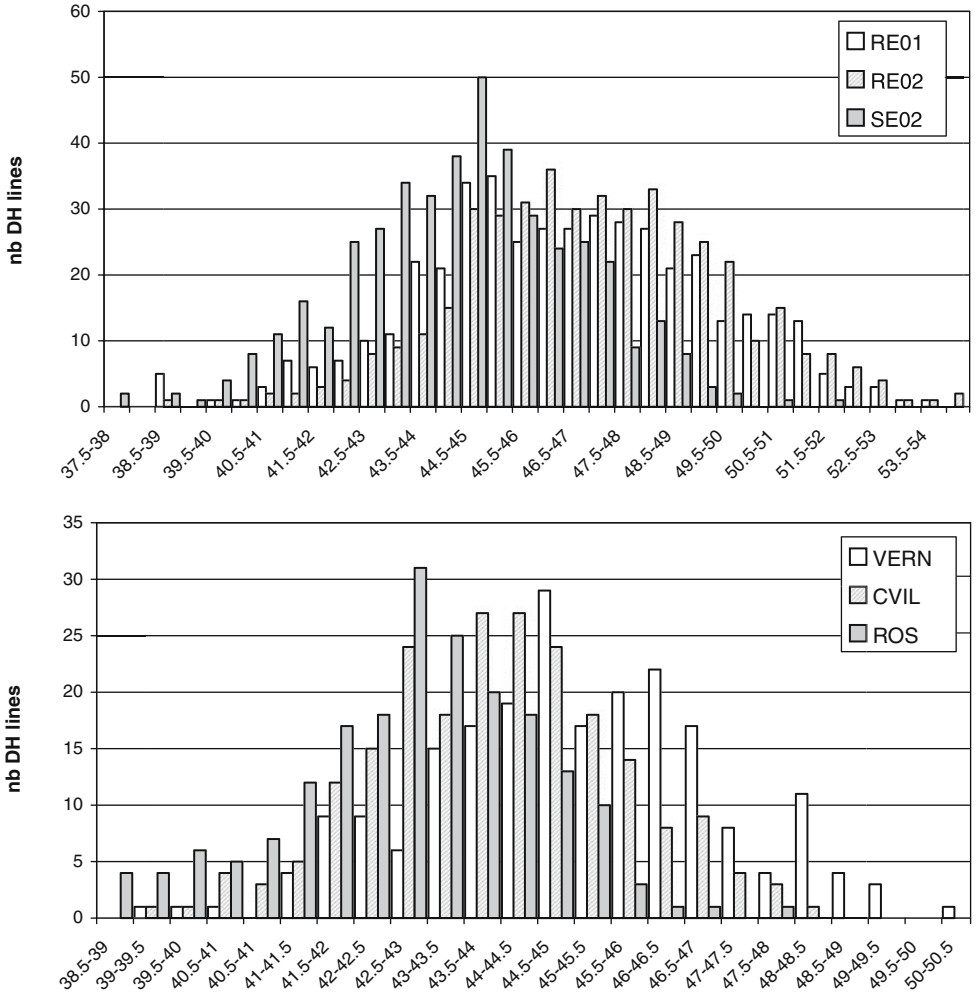

Fig. 2 Frequency distribution of seed oil content (%) for "Darmor-*bzh*" × "Yudal" DH population in the three trials RE01 (Rennes 2001); RE02 (Rennes 2002); SE02 (Serasem 2002) and for "Rapid" × "NSL96/25" DH population in the three trials VERN (Verneuil 2003); CVIL (Chartainvilliers 2003); ROSEN (Rosenthal 2003)

population and into effects of lines, replicates and errors for RNSL population. Oil content and earliness genotypic values of each DH line were assessed from ANOVA after freeing replicate, block and residual components (lsmeans). For each variable, the estimated genotypic value per DH line was the experimental unit for QTL analysis. The Pearson coefficient was calculated with the PROC CORR procedure (SAS) to determine correlations between trials within each population from estimated phenotypic values. Heritability (h^2) was also estimated from ANOVA with the formula: $h^2 = \sigma_g{}^2/[\sigma_g{}^2 + (\sigma_e{}^2/n)]$ with $\sigma_g{}^2$ the genetic variance $[\hat{\sigma}_g^2 = 1/n(\mathrm{MSg} - \mathrm{MSe})]$, $\sigma_e{}^2$ the environmental variance $[\hat{\sigma}_e^2 = \mathrm{MSe}]$ and n the number of replicates per line. QTL detection was performed using composite interval mapping (CIM) with QTL cartographer software (Basten et al. 1997). Five (for earliness in Verneuil and Chartainvilliers trials and oil content in Verneuil trial from RNSL population) or ten markers (all other variables), selected by a forward–backward stepwise regression analysis, were used as cofactors in the CIM procedure, with a 10 cM window size and $P_{\mathrm{in/out}} = 0.05$. The LOD threshold was estimated at 3.0 after 500 permutation tests for each variable. Digenic epistatic interactions were searched for by a two-way ANOVA model with an interaction component (SAS/IML, SAS 1989) using all the mapped markers (significance level threshold: $P = 1.4 \times 10^{-4}$ and 1.6×10^{-4} for DY and RNSL population, respectively; expected five false positives).

Results

Description of the two maps

The DY map comprises 305 loci arranged in 19 linkage groups and covers 2,690 cM. The linkage groups (LG) were named according to Lombard and Delourme (2001). The DY map was aligned to the map of Parkin et al. (1995) using (a) BBSRC microsatellites and comparisons to the map available at http://www.uk-

crop.net/perl/ace/search/BrassicaDB and (b) Celera microsatellites and comparisons to the map of Piquemal et al. (2005). The correspondence between DY nomenclature and N1–N19 nomenclature of Parkin et al. (2005) is indicated in Fig. 1. The RNSL map comprises 259 loci arranged in 19 linkage groups and covers 2,116 cM. It was also aligned to the map of Parkin et al. (1995) using the same comparisons as for DY map but also using AgCan SSRs. The DY and RNSL maps were aligned between each other using 53 common segregating loci and three additional bridges using the comparison to the map published by Piquemal et al. (2005) to which both maps were aligned. From these comparisons, we deduced that the DY and RNSL maps have 18 common linkage groups (Fig. 1). One to ten loci per linkage group are common to the two maps. The LG N2 is missing in the RNSL map.

A high proportion of loci showed segregation distortion in the two populations: 95 (35.7%) and 49 (20%) loci showed distorted segregation ratio in DY and RNSL populations, respectively. Loci with skewed segregation tended to cluster on 10 LGs and 3 LGs for DY and RNSL populations, respectively, each cluster comprising loci exclusively favouring the alleles of the same parental line. In DY population, linkage groups N3, N9, N12, N16 and N15 comprises loci favouring ''Darmor-*bzh*'' allele and linkage groups N2, N5, N6, N13 and N19 comprises loci favouring ''Yudal'' allele. All the skewed loci in RNSL population (on LG N7, N11 and N12) favoured ''Rapid'' allele.

Phenotypic data

Analysis of the experimental designs were performed separately for each trial in both population (DY and RNSL). Frequency distributions of the DH adjusted means for oil content in the different trials are represented in Fig. 2. Adjusted means of parental lines and both populations as well as heritabilities for oil content in the different trials are indicated in Table 1. A great range of variability was observed for oil content within the two DH populations while the difference in oil content between the two parents of each population was not the same (~2% and ~6% between the parents of DY and RNSL populations, respectively). The two NIRS apparatus used for DY and RNSL population evaluation were compared: 215 samples from the DY population ranging from 38.1 to 53.7% in oil content were analysed on the apparatus used for RNSL population. The mean difference for oil content of the 215 samples was very low (0.16%) and the correlation between the two sets of results was very high ($r = 0.97$, $P < 0.0001$). Pearson correlations between each trial within one DH population for the oil content adjusted means were highly significant (Table 2). For RNSL population, the correlation between the two French trials was higher than that between each French trial and the German trial.

Table 2 Correlations between trials within each DH population for oil content

DY population			RNSL population		
	RE02	SE02		CVIL	ROSEN
RE01	0.70	0.75	VERN	0.79	0.59
RE02		0.63	CVIL		0.62

All the correlations are significant at $P = 0.0001$

Identification of additive QTL for oil content

Results from CIM for DY population are summarised in Table 3 and Fig. 1. The QTL are distributed on 11 linkage groups and corresponds to 14 genomic regions. The QTL on LG N1, N6 (upper part), N10, N15 and N16 were detected across the three trials. The QTL on LG N2 (lower part), N3, N6 (lower part) and N12 (upper and lower part) were detected in two trials out of three. The allele increasing oil content is derived either from ''Darmor-*bzh*'' (LG N1, N2, N5, N6-upper part, N10, N12) or from ''Yudal'' (LG N3, N6-lower part, N13, N15, N16, N17). The QTL at the lower part of N6 is localised in the vicinity of *bzh* gene and was only detected in Rennes trials. The estimated phenotypic variation explained by

Table 1 Statistical parameters of DY and RNSL DH populations assessed for oil content (in %) in the different trials

Parameter	DY population			RNSL population		
	RE01	RE02	SE02	VERN	CVIL	ROSEN
Population mean	46.4	47.1	44.5	44.7	43.8	42.7
Range	38–54	43–52	38–48	36.6–50	38–48.2	35.7–47.7
Parental means	D: 48	D: 46.3	D: 45.3	R: 47.5	R: 46.9	R: 45.5
	Y: 46	Y: 48	Y: 44	N: 41.7	N: 41	N: 41
Heritability	0.91	0.79	0.93	0.91	0.87	0.86

Table 3 Oil content QTL detected by CIM in ''Darmor-*bzh*'' × ''Yudal'' DH population in the three trials RE01 (Rennes 2001); RE02 (Rennes 2002); SE02 (Serasem 2002)

Linkage group	Position (cM)	Confidence interval (cM)	LOD	R^{2a}	TR^{2b}	Additive effect[c]
RE01 trial						
N1	68	54–72	14.76	10.9	42.8	0.94
N2	82	67–94	4.29	4.2	32.9	0.58
N6	44	37–54	5.02	4.9	33.4	0.66
N6	145	140–153	10.02	8.9	33.5	–0.85
N10	98	79–100	4.07	3.0	32.2	0.49
N13	184	171–199	4.69	8.9	38.0	–0.84
N15	30	20–40	10.79	12.8	36.9	–1.02
N16	23	18–41	2.85	2.3	32.4	–0.46
RE02 trial						
N1	58	52–62	12.74	8.3	50.4	0.75
N2	13	0–20	3.55	2.5	48.9	0.41
N3	3	*0–20*	*2.23*	*1.2*	*49.5*	–0.28
N6	40	20–47	3.67	2.5	48.8	0.44
N6	150	139–153	15.84	9.5	48.4	–0.80
N10	88	*81–109*	*2.55*	*1.4*	*48.2*	0.31
N12	26	10–40	3.42	2.3	48.8	0.40
N12	153	145–163	11.86	8.5	49.9	0.75
N17	39	26–50	4.37	3.4	49.2	–0.47
N15	30	22–42	15.01	13.4	52.9	–0.96
N16	34	18–41	6.56	4.7	49.6	–0.61
SE02 trial						
N1	68	58–72	7.24	5.9	38.7	0.66
N2	115	102–116	3.94	4.6	39.0	0.49
N3	3	0–10	4.29	2.9	37.1	–0.38
N5	10	0–24	3.09	2.9	38.0	0.40
N6	42	34–52	6.45	5.3	42.4	0.57
N10	98	84–102	6.92	4.9	37.3	0.51
N12	28	10–33	2.88	2.1	37.3	0.33
N12	162	*125–165*	*2.22*	*1.7*	*38.8*	0.30
N15	32	24–40	7.30	7.7	39.8	–0.63
N16	40	*28–52*	*2.52*	*2.1*	*39.1*	–0.36

[a] R^2 is the percentage of variation explained by each QTL

[b] TR^2 is the percentage of variation explained by each QTL and the cofactors

[c] Additive effect is the effect of substitution of one ''Yudal'' allele by one ''Darmor-*bzh*'' allele

individual QTL varied from 2.5 to 13.4%, the overall explained phenotypic variation varying from 35 to 50% according to the trial.

Results from CIM for RNSL population are summarised in Table 4 and Fig. 1. The QTL are distributed on 10 linkage groups and corresponds to 10 genomic regions. The QTL on LG N4 and N13 were detected across the three trials. The QTL on LG N7, N8 and N11 were detected in the two French trials and the QTL on LG N1 was common to ROSEN and CVIL trials. The allele increasing oil content is most often derived from ''Rapid'' (LG N3, N4, N6, N7, N9, N11, N13, N18) but can also come from ''NSL96/25'' (LG N1, N8). The estimated phenotypic variation explained by individual QTL varied from 4.6 to 19%, the overall explained phenotypic variation varying from 38 to 51% according to the trial.

When the genomic regions identified in the two populations are compared, only the QTL on LG N3 appeared to be potentially common. The QTL detected on LG N1 and N13 in the two populations seem to correspond to different regions on each linkage group of the two maps.

QTL identification for earliness of flowering

Results from CIM for both populations are summarised in Table 5, 6 and Fig. 1. In DY population, the QTL are distributed on 8 linkage groups and corresponds to 9 genomic regions. The QTL on LG N1 (lower part), N2, N6, N16 and N19 were detected across the three trials. The QTL on LG N4, N12, N17 were detected in both Rennes trials. The allele increasing earliness is derived from ''Yudal'' for all

Table 4 Oil content QTL detected by CIM in ''Rapid'' × ''NSL96/25'' DH population in the three trials VERN (Verneuil 2003); CVIL (Chartainvilliers 2003); ROSEN (Rosenthal 2003)

Linkage group	Position (cM)	Confidence interval (cM)	LOD	R^{2a}	TR^{2b}	Additive effect[c]
VERN						
N4	0	0–10	3.27	4.8	34.1	0.43
N7	79	72–99	4.43	8.1	35.9	0.57
N8	39	34–60	3.14	6.5	36.4	–0.50
N11	47	43–49	8.66	14.3	35.5	0.77
N13	169	148–190	3.09	13.5	47.6	0.72
CVIL						
N1	110	96–122	5.59	9.3	49.1	–0.51
N4	0	0–8	3.72	5.0	39.9	0.38
N7	93	75–103	4.83	9.5	43.8	0.53
N8	39	35–50	3.04	5.7	41.9	–0.41
N11	47	43–49	8.18	12.4	41.4	0.64
N13	175	154–209	3.16	12.2	48.5	0.59
ROSEN						
N1	114	101–122	4.67	9.2	51.5	–0.50
N3	45	31–60	4.74	8.2	49.8	0.48
N4	26	20–33	3.60	4.7	47.7	0.36
N6	82	76–87	6.23	9.6	50.4	0.52
N9	34	27–42	4.96	7.1	48.4	0.47
N13	169	152–186	4.74	19.0	62.6	0.73
N18	91	75–91	3.54	4.6	47.6	0.37

[a] R^2 is the percentage of variation explained by each QTL

[b] TR^2 is the percentage of variation explained by each QTL and the cofactors

[c] Additive effect is the effect of substitution of one ''RNSL96/25'' allele by one ''Rapid'' allele

QTL but QTL on LG N1 (upper part), N16 and N17. The estimated phenotypic variation explained by individual QTL varied from 1.5 to 27.6%, the overall explained phenotypic variation varying from 33 to 61% according to the trial. In RNSL population, the QTL are distributed on 3 linkage groups and corresponds to 3 genomic regions. The QTL on LG N12 and N13 were detected across the two trials. The allele increasing earliness is derived from ''NSL96/25'' for QTL on N12 and N13 and from ''Rapid'' on the other one. The estimated phenotypic variation explained by individual QTL varied from 5.5 to 9.5%, the overall explained phenotypic variation was 25% in the two trials. No QTL seems to be common to the two populations.

Most of the QTL detected for earliness were not colocalised with QTL for oil content except on LG N6 (at the *bzh* location), N2 (upper part) and N12 (lower part) for DY population and at a lesser extent on LG N13 for RNSL population. In two of these regions, the allele conferring higher oil content is associated with earlier flowering (on LG N6 for DY population and on LG N13 for RNSL population) but, in the other two regions, the allele conferring higher oil content is associated with later flowering (on LG N2 and N12 for DY population).

Search for epistatic QTL for oil content

No interaction was detected using MIM in QTL cartographer. With two-way ANOVA, the numbers of significant interactions were 7, 6 and 8 for OILRE01, OILRE02 and OILSE02 in the DY population, which barely exceeded the expected false positive rate. The numbers of significant interactions were 18, 13 and 6 for OILVERN, OILCVIL and OILROSEN in the RNSL population which exceeded the expected false positive rate for the two first variables. We only considered interactions which involved successive markers in each interacting regions and which relied on sufficient individual numbers in each genotypic class. Then, in DY population, the only interactions observed were between upper part of N2 and upper part of N14 ($P = 3.6 \times 10^{-5}$, $R^2 = 7\%$) and between upper part of N6 and upper part of N12 ($P = 0.00014$, $R^2 = 6\%$). These interactions were detected for OILRE01. For the other two variables, they were under the chosen threshold ($0.002 < P < 0.006$). These interactions involved two regions on LG N2 and N12 that carry additive QTL for one or two of the other variables. In RNSL population, the only notable interaction was observed between LG N15 and lower part of LG N12

Table 5 Days-to-flowering QTL detected by CIM in ''Darmor-*bzh*'' × ''Yudal'' DH population in the three trials RE01 (Rennes 2001); RE02 (Rennes 2002); SE02 (Serasem 2002)

Linkage group	Position (cM)	Confidence interval (cM)	LOD	R^{2a}	TR^{2b}	Additive effect[c]
RE01						
N1	148	134–155	4.63	2.73	52.13	1.79
N2	10.5	7–15	19.04	12.78	54.42	3.89
N4	12	0–25	2.93	2.03	52.38	1.52
N6	141	137–153	11.27	7.69	53.68	2.97
N12	155	150–162	19.36	13.87	55.77	4.03
N16	73	68–76	26.11	20.14	57.93	–6.11
N17	84	70–99	2.66	1.77	52.17	–1.44
N19	22	12–38	6.41	4.23	52.62	2.26
RE02						
N1	36.8	33–45	3.26	1.46	59.27	–1.06
N1	143	133–151	4.33	2.33	59.69	1.35
N2	10.5	7–15	9.69	6.58	61.84	3.19
N4	17	8–36	6.02	3.36	59.99	1.60
N6	145	140–153	19.65	10.74	60.99	2.88
N12	155	150–160	19.01	11.67	62.90	3.00
N16	73	69–78	29.24	19.17	65.21	–4.85
N17	84	72–100	3.53	1.96	59.65	–1.23
N19	30	12–38	4.01	1.98	59.44	1.28
SE02						
N1	152	137–162	3.19	2.81	29.20	0.93
N2	10.5	7–15	9.81	9.48	27.96	1.73
N6	140	131–153	6.11	6.33	27.78	1.39
N16	64	60–67	29.37	27.57	53.97	–3.98
N19	22	12–30	4.87	4.87	27.48	1.25

[a] R^2 is the percentage of variation explained by each QTL

[b] TR^2 is the percentage of variation explained by each QTL and the cofactors

[c] Additive effect is the effect of substitution of one ''Yudal'' allele by one ''Darmor-*bzh*'' allele

($P = 9 \times 10^{-6}$, $R^2 = 9\%$). LG N15 and LG N12 carry an additive QTL in DY population and the region on LG N12 is involved in interaction in both population but not with the same regions. For all these significant interactions, the higher oil content is obtained when the alleles of the same parent are present at both interacting loci.

Discussion

The distribution for oil content and the high number of QTL identified in the two DH populations confirmed the polygenic determinism of this trait. The results from the QTL mapping in the two populations indicate that additive effects are the main factors contributing

Table 6 Days-to-flowering QTL detected by CIM in ''Rapid'' × ''NSL96/25'' DH population in the two trials VERN (Verneuil 2003); CVIL (Chartainvilliers 2003)

Linkage group	Position (cM)	Confidence interval (cM)	LOD	R^{2a}	TR^{2b}	Additive effect[c]
VERN						
N11	89	81–109	3.45	5.5	23.8	0.63
N12	127	113–127	4.81	8.2	23.9	–0.82
N13	106	92–135	4.19	9.5	27.3	–0.82
CVIL						
N12	118	113–126	3.34	6.7	21.9	–1.64
N13	129	117–170	3.69	7.8	24.9	–1.60

[a] R^2 is the percentage of variation explained by each QTL

[b] TR^2 is the percentage of variation explained by each QTL and the cofactors

[c] Additive effect is the effect of substitution of one ''RNSL96/25'' allele by one ''Rapid'' allele

to variation in oil content. Additive effects of individual QTL ranged from 0.2 to 1% in oil content. Very few epistatic effects were identified. This is consistent with previous studies performed in oilseed rape where quantitative genetic studies concluded to the prevalence of additive gene action in the control of seed oil content (Grami and Stefansson 1977; Engqvist and Becker 1991; Shen et al. 2005). The results of QTL mapping in Zhao et al. (2005) indicate that additive effects are main factors contributing to variation in oil content. However, they also showed that a substantial contribution was explained by additive × additive epistatic effects. In our study, the few epistatic QTL we identified involved regions that carry additive QTL in one or the other population.

Fourteen and ten genomic regions involved in oil content variation were identified in DY and RNSL populations, respectively. The alleles increasing oil content were quite equally derived from ''Darmor-*bzh*'' (eight regions) or ''Yudal'' (six regions) in DY population but were mainly derived from ''Rapid'' parent (eight out of ten regions) in RNSL population. This can be related to the difference in oil content between the parental lines of each cross. ''Darmor-*bzh*'' and ''Yudal'' are quite similar in oil content but since they carry different alleles at many QTL, the distribution for oil content in the derived DH population is large. In contrast, the oil content differs more between ''Rapid'' and ''NSL96/25'' and the alleles increasing oil content are mainly derived from the parent with a high oil content, which explained why few transgressive DH lines were obtained in this population.

A strong environmental effect on average oil content was observed. This is shown by the frequency distributions of the DH adjusted means for oil content in the different trials. The oil content in SE02 trial was lower than that obtained in RE01 and RE02 trials. Similarly, the oil content in ROSEN trial was lower than that obtained in CVIL and VERN trials. Interaction with environment is also reflected by the stability of the QTL over the different trials within a population. Five out of the 14 genomic regions were consistent over the three trials and five were consistent in two trials for DY population. Two and four of the ten genomic regions were consistent over three and two trials, respectively in RNSL population. Significant environmental contribution to the oil content variation was also observed by Zhao et al. (2005) who performed the experiments in very contrasted locations (Germany and China). Similar results were obtained in sunflower when QTL studies are performed in different environments (Mestries et al. 1998; Leon et al. 2003).

Seed oil concentration of sunflower is sensitive to environmental conditions such as temperature and radiation during grain filling period (Connor and Hall 1997). Similar environmental effects (temperature, rainfall) have been observed in rapeseed (Pritchard et al. 2000; Si et al. 2003). Since the studied populations were segregating for days-to-flowering, especially the DY one, a relation between oil concentration and earliness could exist. Most of the QTL detected for earliness were not colocalised with QTL for oil content except in three genomic regions for DY population and at a lesser extent in one region for RNSL population. In the regions where a colocalisation was observed, the relation between oil increase and earlier flowering was not consistent. Then, in our analyses, days-to-flowering did not show a major contribution to oil content variation. This is in accordance with the results of Zhao et al. (2006) which shows that most of the variation in oil content occurred independent from the variation in the developmental traits.

Very few common QTL were identified between the two populations DY and RNSL. Only the QTL on LG N3 appeared to be potentially common. It was detected in two trials for DY population and only in ROSEN trial for RNSL population. In addition, from the alignments of our map to the one of Parkin et al. (1995) and from the colinearity observed between some of the linkage groups from the A and C genomes, we were able to deduce that the QTL detected on N1 in DY population is located in a region which is homeologous to the one on N11 carrying a QTL in RNSL population and that the QTL detected on N2 (upper part) and N12 (lower part) in DY population are located in homeologous regions. The same two regions were also revealed for days-to-flowering in DY population. Owing to the amphidiploid nature of oilseed rape genome (AACC, U 1935) and to the intragenomic duplications observed in the Brassica species (Parkin et al. 2003), it is expected that many traits will be controlled by homeologous genes (orthologous or paralogous genes). This has been found for oligogenic traits such as erucic acid content in *B. napus* (Fourmann et al. 1998). Homeologous regions have been shown also to control seed glucosinolates in *B. napus* (Howell et al. 2003) or flowering time in *B. juncea* and the diploid *Brassica* species (Axelsson et al. 2001). In hexaploid wheat, a number of important traits such as flowering time, glutenin synthesis or nematode resistance are controlled by duplicated genes located on homeologous linkage groups (Law et al. 1976; Anderson et al. 1998; Börner et al. 2002; de Majnik et al. 2003).

We compared the results obtained in DY and RNSL populations with the ones obtained in other

populations: ''Darmor'' × ''Samourai'' (DS) DH population (Delourme unpublished); ''Tapidor'' × ''Victor'' (TV) substitution lines (Burns et al. 2003); ''Sollux'' × ''Gaoyou'' (SG) DH population (Zhao et al. 2005) and ''Tapidor'' × ''Ningyou 7'' (TN) DH population (http://www.brassica.bbsrc.ac.uk/IMSORB/; I. Bancroft personal communication). The comparisons with TV and SG populations were more tenuous because of the lack of many common markers. From these comparisons, we found that QTL for oil content were identified over all the linkage groups but some of the QTL appeared to be more consistently revealed across different populations. A QTL was detected on LG N3 in all the populations and was potentially at the same location in DY, RNSL, DS, TN and TV populations. No common marker was available with SG population but a QTL was identified on LG N3 in this population too. On three linkage groups, QTL were identified in at least three populations: On LG N1, a QTL was detected at the same position in DY, DS and TN populations. A QTL was also detected in RNSL population but a slightly different position and in SG population but the identity of the position was not checked due to the absence of common marker. On LG N8, a QTL was detected in RNSL, TN and DS populations. An effect of LG N8 was also found in TV substitution lines (Burns et al. 2003). On LG N13, a QTL was identified in RNSL and TN populations and an effect of LG N13 was also found in TV substitution lines. The QTL on LG N8 and N13 could correspond to the location of the two genes involved in erucic acid content (Jourdren et al. 1996c). QTL for oil content were previously detected at these positions (Ecke et al. 1995; Gül et al. 2003) as well as in TN population. This led to the hypothesis that the increase of molecular mass during the elongation of oleic acid to erucic acid could explain the higher oil content (Ecke et al. 1995). However, in the DY population which is segregating for erucic acid content, no QTL was detected at these positions whereas some QTL were detected in RNSL population not segregating for erucic acid. Then, the genes responsible for higher oil content in these regions might be linked to the genes controlling erucic acid content but not correspond to them. It can be noticed that four QTL are specific to the DY population. For two of them (on LG N5 and N10), the allele increasing oil content is derived from ''Darmor-*bzh*'' and for the two others (on LG N15 and N16) the favourable allele is derived from ''Yudal''. Then, exploring the genetic diversity in various genetic backgrounds will allow to identify the most effective alleles for the regions detected across different populations and to identify original alleles in specific regions.

Recently, genetic control of oil content in seeds of Arabidopsis was studied in a population derived from the cross between ecotypes Landsberg *erecta* (L*er*) and Cape Verde Islands (Cvi) (Hobbs et al. 2004). Two major QTL on chromosome 1 (QTL1t) and chromosome 2 (QTL2) and two minor QTL on chromosome 1 (QTL1b) and chromosome 3 (QTL3) were identified. The alignment between our oilseed rape maps and Arabidopsis map was performed using alignment of the mapped SSR locus sequences with Arabidopsis sequence by BLASTN in TAIR database (http://www.arabidopsis.org/) and using Arabidopsis-derived mapped markers such as the PFM. This was confirmed through the alignment of our maps with the *B. napus* genetic map integrated to Arabidopsis genome (Parkin et al. 2005). We were then able to make the hypothesis that some regions where QTL were detected in oilseed rape could correspond to the regions carrying QTL in Arabidopsis. This seems to be the case for QTL detected on LG N6 (QTL1t from Arabidopsis), N5 (QTL2 from Arabidopsis), N7 (QTL1b from Arabidopsis), N1 and N15 (QTL3 from Arabidopsis). More common markers should be developed in order to ascertain the colinearity between the two species in these QTL regions. In Arabidopsis, there are many candidate genes involved in lipid metabolism underlying the QTL for oil content. This should stimulate the research activities devoted to use the genomic data underlying these Arabidopsis regions to derive markers for oilseed rape QTL and identify candidate genes. Another way to identify candidate genes underlying QTL regions was the use of the *B. napus* microarray obtained from ESTs derived from developing seeds in order to tag genes differentially expressed in bulks of DH contrasted for QTL alleles (Bellamy, Nesi et al., in preparation).

We have shown that a number of QTL control oil content in oilseed rape, some of them being potentially common to different genetic backgrounds but some also specific to some genetic backgrounds. This is not surprising, as every segregating population presents its own potentiality to reveal genetic limiting factors. This offers the possibility to combine favourable alleles to aggregate through marker assisted selection (MAS) from different QTL regions in order to increase oil content. However, due to the negative correlation between oil and protein content, some of the detected QTL might increase one compound while decreasing the other one. Then, genetic analysis and identification of QTL involved in protein content of the meal or conditional QTL mapping of oil content with respect to protein content (Zhao et al. 2006) have to be performed in order to identify regions that influence oil

content independently from protein content if the breeder want to maintain a good value to the meal.

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