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ORIGINAL PAPER

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Genetic analysis of agronomic and quality traits in mustard (*Brassica juncea*)

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Abstract To develop an efficient mustard (*Brassica juncea*) breeding programme, a better knowledge of the genetic control and relationships of the main selected characters is needed. Thus, doubled haploid (DH) lines were evaluated over 2 years in the field. Days to flowering, plant height, thousand-seed weight, fatty acid composition, seed oil content, sinigrin, gluconapin and total glucosinolate contents were determined in the DH population. The influence of seed coat colour was estimated. Results showed significant differences between yellow and brown seeds only for oil and fatty acid contents. Molecular analysis revealed that seed coat colour is associated with two Mendelian trait loci: *Bjc1* [on linkage group (LG) 3] and *Bjc2* (on LG6). The quantitative trait loci associated with characters—detected by composite interval mapping—were not co-localised and revealed a genetic independence. The results obtained in this study show that the most important agronomic and quality traits of brown mustard could be bred independently. Correlation between the studied traits is also discussed.

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Introduction

There are two main groups of *Brassica juncea* that differ in many morphological and seed quality traits. The first group originated from India/Pakistan, has brown testa seed, a high erucic acid level, contains both butenyl and propenyl glucosinolates (GSLs) and is earlier flowering and shorter in stature. The second group from China/East Europe has yellow testa seeds, a low erucic acid level, contains only propenyl GSLs and it is tall and late flowering (Vaughan et al. 1963). These great variations among *B. juncea* genotypes offer a suitable genetic resource for breeding programmes. In addition, several authors have shown interactions and associations between traits. Morgan et al. (1998) studied in 67 genotypes of *B. juncea* the variation of seed traits, including seed size, colour, lipid and protein content. They observed, as did Woods (1980), George and Rao (1983), Abraham and Bhatia (1986) and Singh et al. (1996), that total lipid and protein were correlated with seed weight, and that yellow seeds had higher oil content than brown seeds. Woods (1980) observed also that a larger seed size was associated with a yellow seed coat. These studies implied that breeding objectives could be complicated or restricted due to the pleiotropic association among characters that have opposite effects on other important agronomic traits.

Advances in molecular genetics permit the dissection of agronomic traits and, with the development of molecular markers linked to the variation of quantitative traits, this analysis is a new tool to describe the genetic control of some important agronomic and quality traits. Several quantitative trait loci (QTLs) controlling characters were already mapped in *Brassica* species and in *B. juncea*. Axelsson et al. (2001) identified multiple QTLs for flowering time in three diploid *Brassica* species. Mukherjee et al. (2001) identified two RAPD markers linked to the *Albugo candida* resistance locus which could be used in marker-assisted selection. Cheung et al. (1998), Lionneton et al. (2002) and Mahmood et al. (2003a) mapped QTLs associated with the fatty acid profile in *B. juncea*. QTLs controlling aliphatic GSLs in mustard were

mapped by Cheung et al. (1998) and more recently by Mahmood et al. (2003b). However, few studies correlated quality traits with seed coat colour in mustard. Vera et al. (1979) and Vera and Woods (1982) confirmed that seed coat colour in *B. juncea* is under the control of two genes at two loci. Thiagarajah and Stringam (1993) showed that seeds are yellow when both alleles are recessive and brown when a single dominant allele is present. Upadhyay et al. (1996) studied, using analysis of variance (ANOVA), the association of 25 RFLP markers arranged on nine linkage groups with quantitative traits and one locus controlling seed coat colour in *B. juncea*. Negi et al. (2000) identified AFLP fragments linked to this trait without homology with known genes. Nevertheless, these studies were based on single-marker analysis. Moreover, few works analysed the phenotypic and genetic relationships among these traits in *B. juncea*. In a preliminary study (Lionneton et al. 2002), we described a genetic map of a double haploid (DH) population. A QTL analysis of fatty acid content was performed, allowing the identification of several genomic regions associated with these traits. However, this analysis was based on 1-year data. In order to widen our investigations on *B. juncea*, we have selected other traits, such as GSL level, which are of interest for mustard seeds use in Burgundy. In these areas, brown mustard is used for the production of the condiment 'Moutarde de Dijon' because of the high sinigrin level in the seeds responsible for the savour of the condiment. A breeding programme was developed to breed many characters, including GSL level, oil content, seed weight, seed yield and late flowering. Until now, mustard selection has been based on phenotypic selection methods to improve quantitative traits, but the environment plays an important role on the variation of these traits, and they are difficult to manipulate in breeding. Molecular markers are complementary tools to traditional selection. They can help in obtaining knowledge of selected characters and their genetic association, which may modify the breeding objectives. After the development of an AFLP genetic linkage map of condiment brown mustard (Lionneton et al. 2002), we describe in this paper the mapping of QTLs associated with quantitative traits based on 2 years of experiments and confirmed by preliminary results obtained in 2001. The objectives of the study were to investigate the genetic control of important selected traits in condiment mustard by the identification of genes and QTLs associated with trait variation and to describe the relationships among them.

Materials and methods

Plant material

A population of DH lines was produced from an F_1 plant of a cross between BJ-99, a tall and late-flowering oriental type with yellow seeds, and BJ-70, a short and early-flowering Indian type with brown seeds. The DH population was obtained via microspore culture, according to the procedure described by Lionneton et al. (2001). An AFLP genetic linkage map has been previously constructed using 131 DH lines (Lionneton et al. 2002). This map included 273 markers over 18 linkage groups and covered 1,641 cM.

Traits were evaluated on 118 lines of the mapping DH population in 2001 and 111 in 2002 in spring field plots sown at the INRA experimental field at Dijon. Two 2-m rows of each DH plant and of the two parents of the cross were replicated as three randomised blocks.

Trait measurement and analysis

For seed coat colour, brown and yellow seeded genotypes were clearly distinguished, so seed coat colour was visually noticed. Days to flowering (DF) were determined as the number of days from seed sowing to the appearance of the first open flower. Plant height (PH, in centimetres) was determined on the standing crop by measuring the height of ten random plants on two plots at maturity and averaging the measurements. Thousand-seed weight (TSW, in grams) was estimated from the weight of 500 grains. Fatty acid composition, seed oil content, sinigrin (SIN), gluconapin (GNA) and total GSL contents of the parents and DH plants were directly determined by near infrared spectrometry (NIRS Foss sytem, WinISI, version 1.04 software), on samples of 5–10 g seeds, following Unilever's protocol. The spectrum was calibrated for lipid and GSL content with a series of genotypes covering variability in the mustard collection and production in Burgundy. Each year the database was enriched with several samples from the same area. The amount of oil content in seeds was expressed as a percentage of the seed dry matter: the percentage of palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), eicosenoic acid (20:1) and erucic acid (22:1) in seed was expressed as a percentage of the total oil content. Contents of GSL were expressed in micromoles per gram of seed.

Statistical analyses and QTL analysis

Trait means, Spearman rank-order correlations, ANOVAs and *t*-tests were all performed with the software SYSTAT 10 (SPSS, USA).

QTL detection was performed by composite interval mapping (CIM, Zeng 1994), using QTL Cartographer software (Basten 1999). A forward–backward stepwise regression was performed to choose co-factors before QTL detection by CIM. A window size of 10 cM around the test interval, where the co-factors were not considered, was chosen with P_{in}=0.05 and P_{out}= 0.05 (model six of QTL Cartographer). Empirical statistical significance thresholds (experiment-wise P<0.05) for declaring the presence of a QTL were determined by performing 500 permutations tests of the data set (Churchill and Doerge 1994).

Results

ANOVA detected significant differences between DH lines for each trait in the 2 years. The interaction genotype by year contributed for 88–98% of the variation for DF, PH, TSW, SIN, GNA and GSL between 2001 and 2002. Therefore, each year was analysed separately.

Weather conditions

Rainfall, temperature and evapotranspiration, obtained from the meteorological station at INRA experimental field in Dijon, are presented in Table 1. Cumulative rainfalls, as well as averaged temperature during the two

plant cycles (March–August), were closely similar in both growing seasons. However, the evaporative demand in 2001 was nearly double that in 2002 (Table 1).

Trait variation and correlation in the DH population

The two parental lines were different for most traits in 2001 and 2002 (Table 2). The extreme phenotype values of some DH lines were higher than the highest parent value or were lower than the lowest parent value, indicating transgressive segregation.

Correlation between traits in the DH population for 2001 and 2002 are presented in Table 3. In both years, a highly negative correlation between SIN and GNA levels and a positive correlation between SIN and total GSL contents were observed (Table 3). DF was significantly correlated with PH in 2002. For the other traits, no evident correlation was observed, excepted between seed oil content and total GSL content and between DF and seed oil content.

The influence of the seed coat colour on the other traits was estimated by a *t*-test between the means calculated for the two types of seeds (Table 4). Data from 2002 showed that yellow seeds contain 6.23% more oil than brown ones. Concerning the level of each fatty acid, in 2001 and 2002, yellow seeds contain more C16:0, C18:0, C18:1 and C18:2 than brown ones, in contrast with the level of C20:1 and C22:1 that are significantly higher in brown seeds than in yellow ones. In 2002, a significant difference was observed between the two types of seeds for PH—yellow-seeded plants being shorter than brown types. For DF, TSW, SIN, GNA and GSL, no significant differences were observed.

QTL detection

QTLs detected with QTL Cartographer, their additive effect of alleles of each parent and the 1-LOD likelihood confidence interval are shown in Fig. 1.

Agronomical traits

In 2001, one QTL was detected for DF on linkage group (LG) 16, which explained 14.6% of the variation of this trait (Table 5). Alleles of BJ-70 at this QTL increase the number of DF. In 2002, two other QTLs were detected and localised on LG2 and LG6 explaining, respectively, 11.8% and 8.9% of the variation of this trait, and alleles of BJ-99 acted positively at these QTLs. For both trials, two distinct QTLs were detected for PH on linkage group LG5, with favourable effects from BJ-99 (Table 5). These QTLs explained 10.4–13% in 2001 and around 11% in 2002. In contrast, for TSW, three QTLs with small effects were detected only for the 2001 trial (Table 5). They were located on LG1, LG6 and LG13 and explained 7–8% of the variation. The alleles from the parent with higher seed size (BJ-70) influenced these QTLs positively. However, the most detected QTLs for agronomical traits present small effects.

Table 1 Weather conditions recorded from March to August in 2001 and 2002

Weather conditions	2001	2002
Rainfall (R, mm)	70.9	71.2
Evapotranspiration (ET, mm)	445.5	246.0
R/ET	0.16	0.29
Temperature (°C)	14.2	14.2

Seed coat colour

Of the 131 DH plants observed, 100 had brown seed coats and 31 had a yellow seed coat colour. This 3:1 segregation ratio (Yates corrected χ^2=0.0012, P=0.97) suggests a Mendelian segregation of two loci determining seed coat colour in our population. These Mendelian trait loci (MTL) were assigned on two distinct linkage groups (Fig. 1): Bjc_1 co-segregates with the marker E3 M3_7 on LG3, and Bjc_2 co-segregates with the marker E8 M7_4 on LG6.

Seed oil and fatty acid content

In 2002, two QTLs were detected for oleic acid (C18:1), on LG2 at marker E1 M7_11 and at E7 M8_1, which explains 45.7% and 48.4% of the phenotypic variation of this trait. For linoleic acid (C18:2), two QTLs were detected on LG2 at marker E1 M7_10, explaining 38% of the phenotypic variation (Table 5) and at marker E7 M8_1, explaining 41.5% of the variation of C18:2. QTLs involved in the control of C18:1 and C18:2 indicated that alleles from BJ-99 conferred a higher content of these fatty acids in seeds. With data from 2002, two QTLs were mapped for linolenic acid (C18:3)—one on LG2 at marker E6 M8_6 and one on LG6 at marker E1 M7_14—they explained 13.7% and 15.5% of the phenotypic variation of this trait, respectively. The QTL located on LG2 indicated that the allele from BJ-99 conferred a higher content of linolenic acid, while the allele of BJ-70 acted positively for the QTL on LG6. A major QTL was detected for erucic acid (C22:1) on LG2 at marker E1 M7_11, with another one at marker E7 M8_1, and they explained 55–64.8% of the phenotypic variation of erucic acid. For these two QTLs, the alleles from the parent with a lower erucic acid content (BJ-99) decrease erucic acid concentration in seed.

GSL content

Two major QTLs associated with SIN and GNA content of the seeds were detected for both years on linkage groups LG12 and LG14 (Table 5). These QTLs explained 8–18%

Table 2 Phenotypic value of the parents and the double haploid (DH) population from each trial in 2001 and 2002

Character	Mapping									
	2001					2002				
	BJ-70	BJ-99	Mean	Minimum	Maximum	BJ-70	BJ-99	Mean	Minimum	Maximum
Days to flowering (DF)	58[b]	66	61	58	65	64[b]	80	69	62	76
Plant height (PH, cm)	95[b]	124	110	70	144	145[b]	192	170	111	205
Thousand-seed weight (TSW, g)	7.9[b]	4.1	5.1	3.4	8.0	5.8[a]	3.7	4.1	2.4	6.2
Total seed oil content (%)	35.8[b]	31.7	31.2	26.4	37.9	35.7	34.4	34.1	27.1	39.4
C16:0 (% of oil)	2.4[a]	3.5	2.9	2.2	4.1	3.4	4.0	3.5	2.9	4.3
C18:0 (% of oil)	0.7[b]	1.3	1.0	0.6	1.8	1.3	1.5	1.4	0.9	1.8
C18:1 (% of oil)	4.4[b]	8.9	7.7	1.8	15.4	14.0[a]	19.3	15.2	6.8	23.3
C18:2 (% of oil)	10.0[b]	14.1	13.1	9.0	20.4	17.2[a]	22.9	19.2	14.6	24.7
C18:3 (% of oil)	11.4	11.3	10.5	8.6	12.9	14[b]	15.5	14.8	11.2	18.5
C20:1 (% of oil)	12.7[a]	10.1	10.1	3.9	14.2	11.2	11.2	11.2	10.1	12.2
C22:1 (% of oil)	48.8[b]	29.9	39.0	20.6	51.8	48.4[b]	25.2	39.4	26.8	49.5
Sinigrin (SIN, μmol/g)	42.0[b]	148.9	89.6	15.6	179.1	23.6[b]	120.8	68.7	18.6	140.6
Gluconapin (GNA, μmol/g)	108.0[b]	16.8	58.7	1.0	128.6	86.7[b]	18.5	46.8	0.0	97.1
Glucosinolate (GSL, μmol/g)	145.3[a]	164.7	148.4	112.3	185.7	123.6[b]	153.2	128.2	97.5	164.5

[a,b]Significantly different from BJ-99 at $P<0.05$ and $P<0.01$, respectively

Table 3 Linear correlation (Pearson's) between traits in the population of DH lines

	DF	PH	TGW	Oil	SIN	GNA
Traits 2001						
PH	0.04					
TSW	−0.01	0.22				
Oil	−0.36[a]	−0.07	0.10			
SIN	0.16	0.14	−0.02	0.07		
GNA	−0.15	−0.11	0.11	−0.22	−0.91[b]	
GSL	0.13	0.12	0.12	−0.31	0.51[b]	−0.16
Traits 2002						
PH	0.60[b]					
TSW	0.04	0.26				
Oil	−0.22	−0.14	−0.18			
SIN	0.27	0.31	0.08	−0.03		
GNA	−0.31	−0.25	0.09	−0.17	−0.90[b]	
GSL	−0.02	0.21	0.32	−0.38[a]	0.53[b]	−0.13

[a,b]Significant correlation at the $P<0.01$ and $P<0.001$ probability level

of the variation of SIN in 2001 and 2002, and between 10% and 15% of the variation of GNA in both years. The highest levels of SIN were associated with the allele from BJ-99, and the highest levels of GNA were associated with the alleles from BJ-70 at the two QTLs. Two minor QTLs were also detected in 2002 for the control of SIN on LG6 and on LG1 for GNA. Concerning the total seed content of GSLs, two QTLs on LG1 and LG7 were detected in 2001 and 2002, which explained 4.7–14.6% of the trait variation, depending on the year, and had opposite allelic effect. In 2002, one more QTL was localised on the linkage group LG6 close by a QTL for SIN content (Fig. 1), which explained 5.8% of the GSL variation and was affected by the allele from BJ-70.

Discussion

The differences noticed in mean values of the measured traits between the two sowing seasons were probably caused by differences in weather conditions recorded during the plant cycles. Teulat et al. (2001) used the ratio of total rainfall to evapotranspiration to characterise the climatic conditions during the plant cycle. In our study, this ratio was nearly twofold higher in 2002 than in 2001 (Table 1). This ratio could probably explain an important part of the differences observed between 2001 and 2002 for DF and PH.

A positive correlation was observed between DF and PH in 2002 (Table 3). A similar relationship has been already observed in *B. napus* (Foisset 1995), with co-localisation of QTLs controlling these two traits and in other species such as pea (Dirlewanger et al. 1994) and barley (Bezant et al. 1996; Teulat et al. 2001). In our trials, no QTL for the control of PH and DF were co-localised, which could explain this relation. Moreover, some of the studies above reported inconsistent QTLs for agronomical traits across years or environments and with small effects, in agreement with our results.

We observed a 3:1 Mendelian segregation ratio for seed coat colour in our DH population, which is consistent with the hypothesis of two loci controlling this trait as already reported by several authors with different populations of *B. juncea*, in an F_2 (Vera and Woods 1982; Thiagarajah and Stringam 1993; Negi et al. 2000) and in DH lines (Thiagarajah and Stringam 1993). These two loci, Bjc_1 and Bjc_2, were mapped on two linkage groups, LG3 and LG6, respectively, of the *B. juncea* linkage map. These interesting results could be exploited to develop STS markers in order to incorporate them in marker-assisted selection programs.

Table 4 Differences between yellow and brown seeds in the DH population. *SD* Standard deviation

Character	2001					2002				
	Mean of brown seed (*n*=92)	SD	Mean of yellow seed (*n*=26)	SD	*t*-value	Mean of brown seed (*n*=89)	SD	Mean of yellow seed (*n*=22)	SD	*t*-value
	61	2	61	2	0.58 NS[c]	69	3	68	3	0.82 NS
PH (cm)	111	13	107	13	1.36 NS	172	17	161	14	3.1[b]
TSW (g)	5.0	0.7	5.4	0.9	1.77 NS	4.1	0.7	4.3	0.8	1.09 NS
Total seed oil content (%)	31.0	1.8	32.0	2.9	1.77 NS	33.7	2.3	35.8	2.1	3.96[b]
C16:0 (% of oil)	2.9	0.3	3.3	0.4	4.51[b]	3.5	0.3	3.6	0.3	1.07 NS
C18:0 (% of oil)	1.0	0.2	1.2	0.3	2.90[b]	1.3	0.2	1.4	0.2	2.19[a]
C18:1 (% of oil)	7.3	2.8	9.2	3.0	2.88[b]	14.8	3.2	16.9	3.1	2.73[a]
C18:2 (% of oil)	12.8	2.09	14.1	2.0	2.88[b]	18.9	2.4	20.5	2.8	2.36[a]
C18:3 (% of oil)	10.6	0.9	10.5	0.8	0.68 NS	15.0	1.5	14.0	1.3	2.99[b]
C20:1 (% of oil)	10.2	2.4	9.7	2.1	0.99 NS	11.2	0.4	11.2	0.3	1.23 NS
C22:1 (% of oil)	40.4	7.0	34.0	8.2	3.6[b]	40.2	7.2	36.0	7.4	2.3[a]
SIN (μmol/g)	89.5	35.5	90.1	33.4	0.07 NS	71.0	31.8	58.9	24.2	1.89 NS
GNA (μmol/g)	58.2	29.4	60.8	27.3	0.42 NS	44.8	27.3	55.9	25.7	1.72 NS
GSL (μmol/g)	148.0	14.4	149.6	16.5	0.45 NS	128.4	14.5	126.9	10.7	0.52 NS

[a,b]Significantly different at $P<0.05$ and $P<0.01$, respectively
[c]*NS* Not significantly different

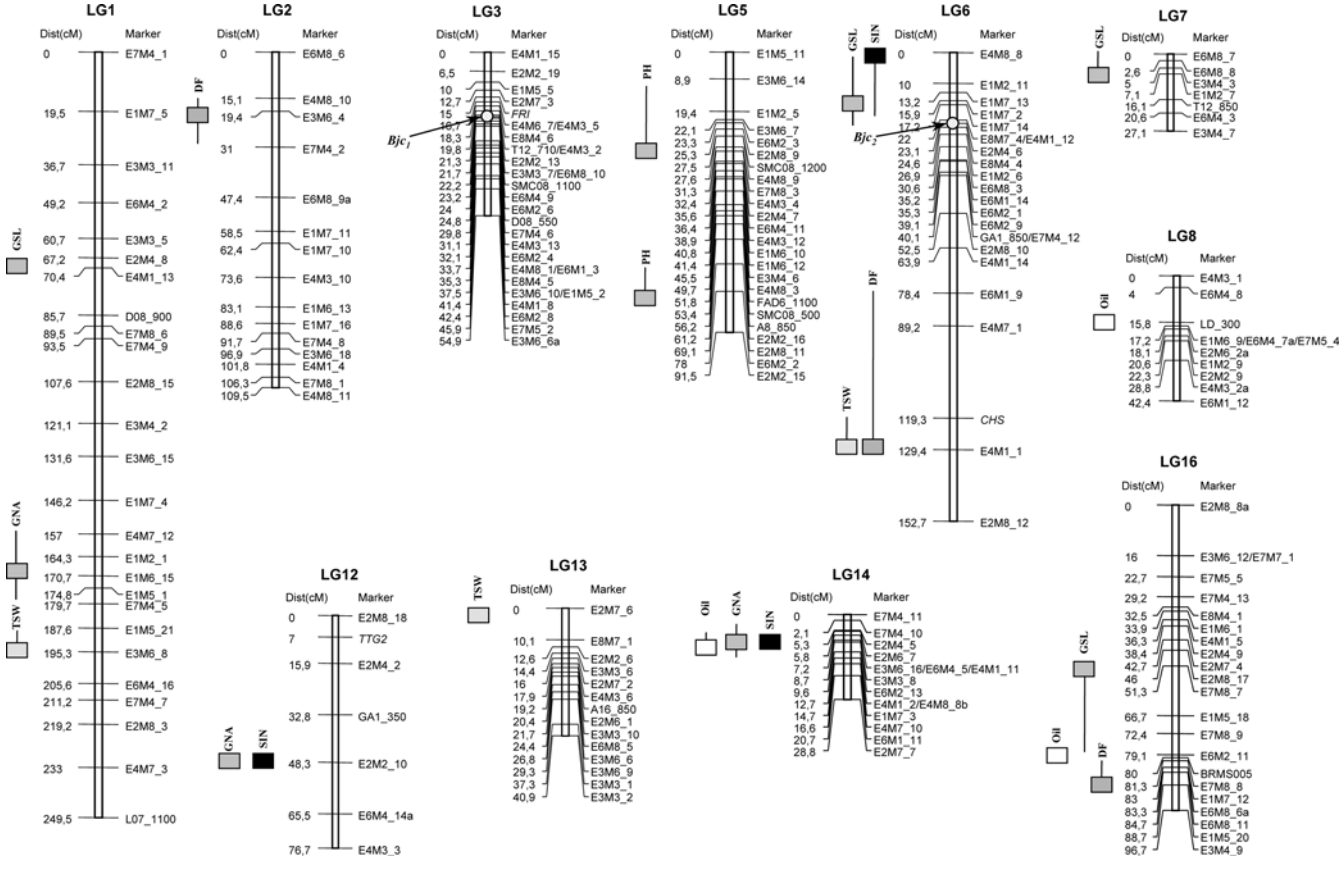

Fig. 1 Mapping of QTLs for days to flowering (*DF*), plant height (*PH*), thousand-seed weight (*TSW*), seed oil content (*Oil*), sinigrin (*SIN*), gluconapin (*GNA*) and total glucosinolate (*GSL*) contents on the AFLP linkage map of *Brassica juncea*. Bjc_1 and Bjc_2 indicate the two loci controlling seed coat colour. Distances between markers are in Kosambi centiMorgans. The position of each quantitative trait locus (QTL) is indicated by a *box* to the *left* of the linkage group. The 1-LOD (tenfold) likelihood confidence interval of each QTL is indicated by a *bar*

Table 5 QTLs detected by composite interval mapping with QTL Cartographer for agronomic and quality traits, measured in 2001 and 2002. *a* Additive effect

Trait	Year	Linkage group	Marker[a]	Confidence interval[b]	LOD	Seuil[c]	R^{2d}	a^{e}
DF	2001	LG 16	E1 M5_20	BRMS005- E1 M5_20	5.28	4.56	14.6	1.7
	2002	LG 2	E3 M6_4	E4 M8_10- E7 M4_2	3.00	4.13	11.8	−1.0
		LG 6	E4 M1_1	E6 M1_9-E4 M1_1	2.20		8.9	−0.9
PH	2001	LG 5	E4 M8_9	E4 M8_9- E1 M6_10	2.84	3.9	10.4	−4.3
		LG 5	E6 M2_2	E6 M2_2	3.6		13.2	−4.8
	2002	LG 5	E3 M6_14	E1 M5_11-E7 M8_3	3.06	5.86	11.9	−6.1
		LG 5	E6 M2_2	A8_850- E6 M2_2	2.84		11.2	−5.8
TSW	2002	LG 1	E3 M6_8	E1 M5_21-E3 M6_8	2.89	5.43	8.2	0.2
		LG 6	E4 M1_1	*CHS* - E4 M1_1	2.61		7.5	0.2
		LG 13	E2 M7_6	E2 M7_6	2.94		8.5	0.2
OIL	2001	LG 14	E4 M1_2	E2 M6_7- E4 M8_8b	2.83	4.12	8.0	−0.6
	2002	LG 8	LD_300	LD_300	2.19	3.09	8.7	0.7
		LG 16	BRMS005	BRMS005	2.54		10.1	−0.8
C18:1	2002	LG 2	E1 M7_11	E1 M7_11	14.6	3.91	45.7	−2.2
		LG 2	E7 M8_1	E7 M8_1	15.9		48.4	−2.3
C18:2	2002	LG 2	E1 M7_10	E1 M7_10	11.4	5.43	38.0	−1.6
		LG 2	E7 M8_1	E7 M8_1	12.8		41.5	−1.7
C18:3	2002	LG 2	E6 M8_6	E6 M8_6- E7 M4_2	3.5	5.21	13.7	−0.6
		LG 6	E1 M7_14	E1 M7_2- E1 M7_14	4.0		15.5	0.6
C22:1	2002	LG 2	E1 M7_11	E1 M7_11	19.1	4.77	55.2	5.5
		LG 2	E7 M8_1	E4 M1_4-E7 M8_1	25.1		64.8	6.0
SIN	2001	LG 12	E2 M2_10	E2 M2_10	3.29	4.34	8.6	−10.9
		LG 14	E4 M1_2	E6 M2_13-E4 M7_10	3.65		10.0	−11.6
	2002	LG 6	E4 M8_8	E4 M8_8-E1 M7_14	2.44	4.77	7.7	8.8
		LG 12	E2 M2_10	E2 M2_10	6.13		18.4	−13.4
		LG 14	E6 M2_13	E6 M2_13- E4 M1_2	3.44		10.4	−10.2
GNA	2001	LG 12	E2 M2_10	E2 M2_10	4.34	4.99	15.4	11.4
		LG 14	E4 M1_2	E6 M2_13- E4 M1_2	4.00		14.3	11.2
	2002	LG 1	E1 M6_15	E4 M7_12-E7 M4_5	2.69	4.77	7.3	7.7
		LG 12	E2 M2_10	E2 M2_10- E6 M4_14a	4.82		13.5	10.2
		LG 14	E6 M2_13	E6 M2_13- E4 M1_2	3.81		10.3	9.0
GSL	2001	LG 1	E4 M1_13	E4 M1_13-D08_900	4.15	4.34	10.1	5.1
		LG 7	E6 M8_8	E6 M8_7-E1 M2_7	2.53		5.9	−3.8
	2002	LG 1	E4 M1_13	E4 M1_13-E7 M8_6	2.00	6.29	4.7	3.3
		LG 6	E1 M7_2	E4 M8_8-E8 M7_4	2.48		5.8	3.4
		LG 7	E3 M4_3	E6 M8_7-E3 M4_3	5.77		14.6	−5.5

[a]Left flanking marker of test position
[b]These boundaries indicate the width of the peaks before the likelihood drops tenfold (an LOD drop of 1.0)
[c]Significance of thresholds of detected QTLs obtained by performing permutation test at a 0.05 probability level
[d]Percentage of variance explained by the QTL
[e]The allele providing the higher value for the trait is coded by a positive sign for BJ-70 and a negative one for BJ-99

Mapping of MTLs controlling seed coat colour and QTLs associated with agronomic and seed quality traits did not show any co-localisation among them. This result tends to prove that loci for seed coat colour segregate independently from QTLs involved in the control of time to flowering, PH, seed size, lipid and GSL contents. Morgan et al. (1998) evoked pleiotropic association between characters such as seed coat colour and seed size, which have opposite effects, increasing the difficulty for breeding objectives. Our results tend to demonstrate the opposite, as most characters studied were genetically independent. The relation between seed coat colour and lipid content observed in the DH population and in other studies (Vanghesdale and Fournier 1980; Woods 1980; George and Rao 1983; Kirk and Hurlstone 1983; Abraham and Bhatia 1986; Morgan et al. 1998) was probably due to physiological relationships between traits. Morgan et al. (1998) showed that the difference in lipid content between yellow and brown seeds disappeared when it was expressed per embryo weight because yellow seed coats have thinner testas than brown. The ratio testa: embryo weight in yellow and brown seeds should explain the link observed with oil content.

We showed that the composition of lipids varied between brown and yellow seeds from a DH population of *B. juncea* (Lionneton et al. 2002). Total oil seed content, palmitic, stearic, oleic and linoleic acid levels were higher in yellow seeded lines, but the level of erucic acid was lower in yellow than in brown seeds. These results confirmed those obtained in *B. juncea* by Morgan et al. (1998), Abraham and Bhatia (1986) and Woods (1980), which showed that yellow seeds were associated with higher oil content. Kirk and Hurlstone (1983) and Vanghesdale and Fournier (1980) also showed that erucic acid content was higher in brown seeds and could be linked to their geographic origin. QTLs associated with

fatty acids, detected with the field data of 2002, confirmed those mapped by Lionneton et al. (2002). Two major QTLs associated with fatty acid content in mustard seed were located on LG2, which may correspond to fatty acid elongase genes and a minor QTL detected on LG6, as already discussed by Lionneton et al. (2001).

As already observed by Morgan et al. (1998) in 67 brown and yellow genotypes of *B. juncea*, and in spite of these differences in seed composition, no correlation between the seed coat colour and the seed size (TSW) was observed in our DH population. However, this result contrasted to those obtained by Woods (1980) who, in a backcross population of *B juncea*, reported an association between seed size and yellow seed coat colour. Excepting total GSL level that was slightly linked to seed oil content (Table 3), lipid content in seeds was not correlated with the other traits. This correlation could be explained by a co-localisation of a QTL associated with seed oil and GSL content on linkage group LG16. For GSLs, it was generally admitted that the seed levels depend also on the geographic origin (Josefsson 1972; Gland et al. 1981; Love et al. 1990). In our work, the SIN and GNA level and the total GSL content were not linked to the seed coat colour. The analysis of correlations showed that SIN level exhibited a highly negative correlation to GNA level in the DH population due to their common metabolic pathway (Halkier and Du 1997). GSL was positively associated with SIN (Table 3); this correlation was expected because SIN constitutes 54–60% of GSL in the DH progeny. The distribution and the analysis of the SIN and GNA levels in the DH population did not permit us to make a conclusion concerning the genetic control of these traits. Nevertheless, the QTL detection revealed two important regions on LG12 and LG14 which control the presence or absence of GNA in the seed. This result tends to confirm the presence of at least two loci controlling these two GSLs. Love et al. (1990), Cheung et al. (1998) and Mahmood et al. (2003b) had already showed the existence of these two loci and mapped two common QTLs for propenyl and butenyl GSLs in *B. juncea*. The allelic effects showed that high levels of SIN were associated with the alleles from BJ-99 at the QTL on LG12 and LG14 when they were associated with the alleles from BJ-70 at the QTL on LG6. The alleles of BJ-70 influenced the amount of GNA at the two major QTLs on LG12 and LG14 and at the minor one on LG1. These results would suggest that two duplicated regions controlled the side-chain elongation of GSLs in the DH population and were analogous to *Gsl-elong* genes described by Magrath et al. (1994). Loci on LG12 and LG14 may control the side-chain elongation step from propyl to butenyl, and loci on LG1 and LG6 may control the side-chain elongation step upstream propyl GSL. The results obtained were similar to those observed by Cheung et al. (1998) and Mahmood et al. (2003b) in *B. juncea*, and by Magrath et al. (1994) in *B. napus*, where two duplicated genes originated from the two amphidiploid genomes.

The results obtained in these studies provide a better knowledge of the interaction among the most important economical characters in condiment mustard. Only yellow-seed coat colour was linked to seed oil content (for 1 year only), due to physiological relationships between others traits and not to a genetic interaction. The other characters were independent, and no genetic interactions or QTL co-localisations were observed. This may imply that each trait could be bred independently. Moreover, the localisation of QTL and loci involved in the control of traits and the identification of markers closely linked to them, such as the seed coat colour or the absence of GNA in seed, could be used as PCR-based markers to facilitate and help breeders.

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