Introgression of B-genome chromosomes in a doubled haploid population of *Brassica napus* × *B. carinata*

Z.K. Navabi, I.A.P. Parkin, J.C. Pires, Z. Xiong, M.R. Thiagarajah, A.G. Good, and M.H. Rahman

Abstract: The *Brassica* B-genome species possess many valuable agronomic and disease resistance traits. To transfer traits from the B genome of *B. carinata* into *B. napus*, an interspecific cross between *B. napus* and *B. carinata* was performed and a doubled haploid (DH) population was generated from the BC_2S_3 generation. Successful production of interspecific DH lines as identified using B-genome microsatellite markers is reported. Five percent of DH lines carry either intact B-genome chromosomes or chromosomes that have deletions. All of the DH lines have linkage group J13/B7 in common. This was further confirmed using *B. nigra* genomic DNA in a fluorescent in situ hybridization assay where the B-genome chromosomes were visualized and distinguished from the A- and C-genome chromosomes. The 60 DH lines were also evaluated for morphological traits in the field for two seasons and were tested for resistance to blackleg, caused by *Leptosphaeria maculans*, under greenhouse conditions. Variation in the DH population followed a normal distribution for several agronomic traits and response to blackleg. The lines with B-genome chromosomes were significantly different ($p < 0.01$) from the lines without B-genome chromosomes for both morphological and seed quality traits such as days to flowering, days to maturity, and erucic acid content.

Key words: Brassica napus, *Brassica carinata*, B genome, introgression, SSR markers, cytogenetics, GISH, agronomic traits.

Résumé : Les espèces à génome B au sein du genre *Brassica* possèdent plusieurs caractéristiques agronomiques et résistances à des maladies utiles. Pour transférer ces caractéristiques du génome B du *B. carinata* à celui du *B. napus*, un croisement interspécifique entre le *B. napus* et le *B. carinata* a été réalisé et une population d'haploïdes doublés (HD) a été générée à partir de la génération BC_2S_3. Le succès dans la production de lignées HD interspécifiques a été vérifié en employant des microsatellites spécifiques du génome B. Cinq pourcent des lignées HD portaient soit des chromosomes intacts du génome B ou des chromosomes ayant des délétions. Toutes les lignées HD avaient en commun J13/B7. Ceci a été confirmé en employant l'ADN génomique du *B. nigra* lors d'une hybridation in situ en fluorescence où les chromosomes du génome B ont été visualisés et distingués de ceux des génomes A et C. Ces 60 lignées HD ont également été évaluées pour leurs caractéristiques morphologiques au champ pendant deux saisons de même qu'en serre pour leur résistance à la jambe noire, causée par *Leptosphaeria maculans*. La variation au sein de la population de lignées HD présentait une distribution normale pour plusieurs des caractéristiques agronomiques et la résistance à la jambe noire. Les lignées avec des chromosomes du génome B étaient significativement différentes ($p < 0{,}01$) des lignées sans chromosome du génome B tant pour les caractères morphologiques (floraison, maturité) que les propriétés des graines (contenu en acide érucique).

Mots-clés : Brassica napus, *Brassica carinata*, génome B, introgression, marqueurs SSR, cytogénétique, GISH, caractères agronomiques.

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Introduction

One of the goals of *Brassica* oilseed research programs is the stable introgression of novel traits from wild or closely related species into cultivated canola plants (*Brassica napus*) through inter- and intraspecific crosses (Ky et al. 2000). The *Brassica* species containing the B genome (*B. nigra*, *B. carinata*, and *B. juncea*) possess many valuable agronomic traits including blackleg resistance (Schelfhout et al. 2006), aluminium tolerance (Huang et al. 2002), salinity tolerance (Malik 1990), and heat and drought tolerance (Kumar et al. 1984). Transfer of blackleg resistance and silique shatter resistance from the B genome of *B. juncea* to *B. napus* has been attempted, but these traits have not been successfully introduced into commercial germplasm (Chèvre et al. 1997; Dixelius and Wahlberg 1999; Gerdemann-Knörck et al. 1995; Prakash and Chopra 1988; Roussel et al. 1999; Roy 1984).

Blackleg is a fungal disease (caused by *Leptosphaeria maculans*) that attacks the leaves and stems of many *Brassica* species (Ferreira et al. 1995). Major genes and several minor genes are involved in *B. napus* seedling and adult plant resistance to blackleg (Ballinger and Salisbury 1996; Bansal et al. 1994; McNabb et al. 1993; Pang and Halloran 1996; Stringam et al. 1995). These include *Rlm1*, *Rlm3*, *Rlm4*, *Rlm7*, and *Rlm9*, all mapped to linkage group A7 of the A genome (Ansan-Melayah et al. 1998; Balesdent et al. 2001, 2002; Delourme et al. 2004), and *Rlm2* on A10 (Ansan-Melayah et al. 1998; Delourme et al. 2004, 2006). These resistance genes were mapped in *B. napus*, while an additional resistance gene, *Rlm8*, was described from *B. rapa* but has yet to be mapped (Balesdent et al. 2002). The majority of B-genome-containing *Brassica* species also carry blackleg resistance genes, such as *Rlm5* and *Rlm6*, originally identified in *B. juncea*, one of which maps to linkage group J13/B7 (Balesdent et al. 2002; Christianson et al. 2006; Rimmer and Vandenberg 1992), and *Rlm10* from the B genome of *B. nigra* (Chevre et al. 1996). Efforts to transfer resistance genes derived from the *B. juncea* B genome to *B. napus* by incorporating large introgressions (Barret et al. 1998; Chèvre et al. 1997; Saal et al. 2004) have failed, presumably because of the low levels of homoeology between B-genome and A- or C-genome chromosomes (Leflon et al. 2007).

In this study we successfully introgressed specific B-genome chromosomes (linkage groups) from *B. carinata* into *B. napus* germplasm using an advanced backcross approach (Tanksley and Nelson 1996). More specifically, an interspecific cross between *B. napus* and *B. carinata* was used to generate a doubled haploid (DH) mapping population from BC_2S_3 lines, i.e., lines developed through two backcross generations with *B. napus* and three rounds of self-pollination. This resulted in the production of interspecific DH lines that carry stable B-genome chromosomal segments. We also analyzed these B-genome linkage groups using previously mapped microsatellite markers and integrated the data with molecular cytogenetics to study the nature of the chromosomal segments in this population. It was observed that the B-genome segments are primarily inherited as intact chromosomes. Further, the genetic association of these chromosomes with several agronomic traits is reported.

Materials and methods

Plant material

An accession of *Brassica carinata* (acc. 17-009) from the canola breeding program at the University of Alberta which carries resistance to multiple fungal diseases (V.K. Bansal et al., unpublished data) was used to make a series of crosses with *Brassica napus* 'Westar'. The pedigree of the interspecific cross is shown in Fig. 1. In each generation, selection was applied for blackleg resistance and *B. napus* morphology and optimal seed set. DH lines were developed through bulk microspore cultures performed on three or four BC_2S_3 plants, all of which had been selected as being tolerant to blackleg. The evaluation of blackleg resistance was performed using a cotyledon-based test, as described by Bansal et al. (1999). Over 600 DH lines were produced from this bulk and 60 DH lines were selected randomly for use in this study.

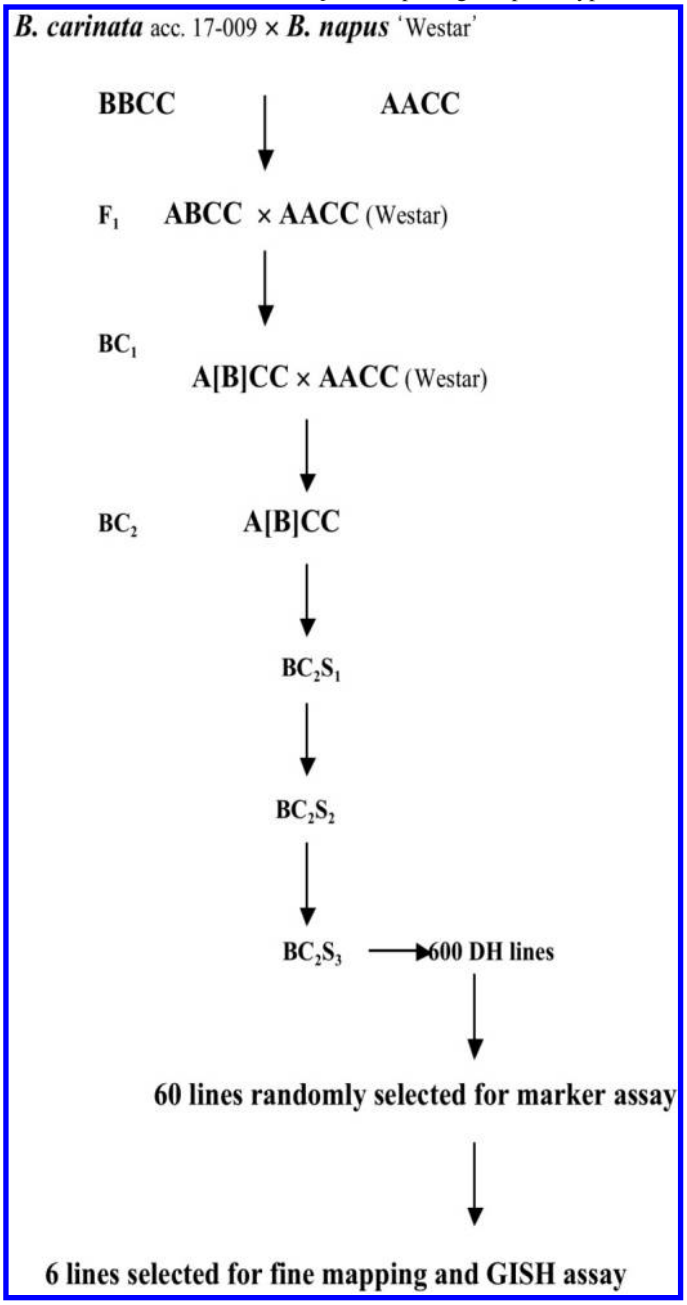
Phenotyping

Sixty DH lines and the parental lines were grown in Metro Mix 290 (Grace Horticultural Products, Ajax, Ontario, Canada) in the greenhouse at 21 °C (day) and 18 °C (night) with a 16 h photoperiod. Plants were fertilized every 2 weeks with 200 ppm Peres 20–20–20 (N–P–K) complete fertilizer with micronutrients (Plant Products, Brampton, Ontario). Fresh leaf tissue was collected from seedlings at the 4–5-leaf stage for DNA extraction using a GenElute Plant Genomic DNA Miniprep Kit (Sigma).

The DH lines, parental lines, and four controls (*B. carinata* lines 070 and 065 and *B. napus* genotypes PSA12 and 'Topas') were evaluated in field plot trials at the University of Alberta Edmonton Research Station in 2006 and 2007. An 8 × 8 unbalanced square lattice design with two replications was used. Each plot consisted of four rows 2 m long with 20 cm row spacing. The morphological characteristics leaf shape, number of lobes, days to flowering, days to maturity, pod attitude, pedicel length, silique length, beak length, and number of seeds per pod were recorded following the *Brassica* descriptors according to the International Board for Plant Genetic Resources (IBPGR 1990). Oil content was determined on the seed harvested from each plot in each year, following the protocol of Daun et al. (1989). The seeds of one of the DH lines, designated DH39, did not germinate in any of the replications, so data for many traits are absent from this line.

Blackleg cultures of isolate V77 (pathogenicity group 2; kindly provided by Dr. Steven Strelkov, University of Alberta) were grown on V8 agar plates at room temperature under a 12 h photoperiod. After 2 weeks a suspension of pycnidiospores was prepared and adjusted to 1×10^6 spores/mL and stored at –80 °C until use. Inoculation was performed on all 60 lines, in two replications with four plants in each replicate test, on needle-wounded stems before flowering according to Bansal et al. (1994). Three weeks after inoculation (after pruning the first new leaves to keep the cotyledons alive), the adult plants were scored for the length of internal and external lesions, which were measured with respect to total plant height.

Fig. 1. Pedigree and crosses used in the development of the DH lines used in this study. Selection was performed in each generation for disease resistance and *B. napus* morphological phenotypes.



Statistical analysis

Data from each season were subjected to analysis of variance following the linear model $Z_{ijkl} = \mu + b_i + I_j(b_i) + T_k + e_{ijkl}$, where for each trait Z_{ijkl} is the phenotypic value of each DH line in each replication, μ is the grand mean, I_j is the effect of incomplete block nested in block (b_i), T_k is the effect of treatment, and e_{ijkl} stands for the experimental error.

A mixed model analysis (Littell et al. 1996) was performed using PROC MIXED in SAS (SAS Institute Inc. 1989) in which genotype was considered a fixed effect and block and incomplete block (block) were considered random effects. Based on marker data, the DH lines were divided into two groups: the first group consisted of three lines carrying B-genome chromosomes, hereafter referred to as "B+", and the second group consisted of three lines with no B-genome content, hereafter referred to as "B–". Single degree of freedom contrasts were performed using the ESTIMATE statement in PROC MIXED to make comparisons between these two groups. A two-tailed *t* test was performed to compare individual lines having different chromosomal contents. A combined analysis of variance was also performed using PROC MIXED, in which genotype was considered fixed while the year, block, incomplete block (block), and all corresponding interactions were considered random. The combined analysis was also followed by single degree of freedom contrasts and the *t* tests were done as described for single-year analyses.

Genotyping

From a total of 1242 B-genome microsatellite (SSR) markers developed at Agriculture and Agri-Food Canada (AAFC), Saskatoon Research Station, 220 were selected based on their location on the B-genome linkage groups (as determined using an AAFC mapping population derived from *B. juncea*) and their ability to amplify strong bands in the AAFC *B. juncea* reference mapping population. The benefit of these markers is that they have been used for mapping in other populations and therefore provided the opportunity to cross-reference our data with other map data. Of these markers, 48 are publicly available, and the locations and *B. carinata* allele sizes are reported in Table S1.[3] Initially, all 60 DH lines and the parental lines were genotyped with these 48 markers in a nested PCR (Schuelke 2000). The PCR mix contained 2 pmol of forward primer, 8 pmol each of reverse and labeled M13 primer (5′-TGTAAAACGACGGCCAGT-3′ with FAM, HEX, or TET), 1 U of *Taq* polymerase, 0.2 mmol/L dNTPs, 10 ng of template DNA, 1.6 mmol/L $MgCl_2$, and 1.5 µL of 10× buffer in a total volume of 15 µL. The PCR consisted of 5 min of initial denaturation at 94 °C; 30 cycles of 94 °C (30 s), 57 °C (45 s), and 72 °C (45 s); 8 cycles of 94 °C (30 s), 55 °C (45 s), and 72 °C (45 s); and a final extension step of 10 min at 72 °C. Subsequently PCR products were diluted 10 times in formamide solution, resolved on an ABI 3730 DNA Analyzer along with GeneScan 500 TAMRA size standard (Applied Biosystems), and sized using GeneMapper software (Applied Biosystems).

The remaining 172 SSR primers (kindly provided by AAFC) were used for fine mapping of the six selected DH lines; unlike the publicly available primers, they were already fluorescently labeled. PCR assay preparation for these primers involved adding 0.5 µL each of forward and reverse primer (5 µmol/L) to 5.0 µL of AmpliTaq Master Mix (Applied Biosystems). Forty nanograms of genomic DNA was used in a total volume of 10 µL for a 384-well plate. The PCR conditions were as follows: 95 °C (10 min), followed by 8 cycles of 94 °C (15 s), 50 °C (15 s), and 72 °C (30 s); 27 cycles of 89 °C (15 s), 50 °C (15 s), and 72 °C (30 s); and a final extension at 72 °C for 10 min. Pooled PCR products, labeled with three different fluorescent dyes, were di-

[3] Supplementary data for this article (Tables S1–S3) are available on the journal Web site (http://genome.nrc.ca).

Table 1. B-genome chromosome (linkage group, LG) content and cytological evaluation of the parental and doubled haploid (DH) lines.

Linkage group	Westar	17-009	O70	DH21	DH31, DH107	DH39	DH45	DH51	No. of markers evaluated per LG
J11/B5	–	+	+	–	–	–	–	–	13
J12/B8	–	+	+	–	–	74%	–	–	26
J13/B7	–	+	+	–	–	+	71%	71%	27
J14/B6	–	+	+	–	–	+	17%	–	16
J15/B2	–	+	+	–	–	–	–	–	25
J16/B4	–	+	+	–	–	–	–	–	20
J17/B1	–	+	+	–	–	+	–	–	14
J18/B3	–	+	+	–	–	+	65%	–	15
No. of chromosomes	38	36	36	37	38	na	38	38	

Note: "+" indicates that all of the markers along the linkage group amplified in the line, indicating the presence of the complete linkage group; "–" indicates the absence of the linkage group in the line. For linkage groups that are not entirely present in the DH lines (either the tip or the middle of the chromosome is lost), the percentage of the LG present (in cM) is shown.

luted 10 times in 0.1% Tween 20, resolved on a MegaBACE 1000 DNA Analysis System (GE Healthcare) with ET ROX 550 size standard (Applied Biosystems), and analyzed using Fragment Profiler (MegaBACE) software.

To identify B-genome alleles, a panel of four *B. napus* lines (Westar, Delta, Topas, PSA12) and three *B. carinata* lines (17-009, 070, and 065) was used. A second panel of four parental and 60 DH lines was used to detect those DH lines carrying B-genome chromosome segments (data not shown). Based on the preliminary marker data, 6 of the 60 DH lines were selected for fine mapping, 3 that carried whole B-genome chromosomes or segments of the chromosomes and 3 that lacked any B-genome content.

GISH analysis

Three positive lines having at least one B-genome chromosome (DH45, DH51, and DH39) and three negative lines (DH21, DH31, and DH107) were selected for genomic in situ hybridization (GISH). Immature flower buds were collected from plants for mitotic and meiotic chromosome spreads. Flower buds were treated with nitrous oxide for 1 h followed by ice-cold 90% acetic acid for 10 min to fix chromosomes and stored in 70% ethanol at –20 °C until use. Slides were prepared following the enzyme maceration method of Kato et al. (2004). *Brassica nigra* and *B. oleracea* genomic DNA and repeated sequences were labeled with fluorescein-12-dUTP, Cy3-dCTP, Cy5-dUTP, or both fluorescein-12-dUTP and Cy3-dCTP (Perkin Elmer Life Sciences, Boston, Massachusetts) using a nick translation procedure (Kato et al. 2004). We also used 45S rDNA as a probe to detect nucleolar organizing regions. Fluorescent in situ hybridization was performed following the method of Kato et al. (2004) with slight modifications as described by Lamb and Birchler (2006). After hybridization and washes, a drop of Vectashield mounting medium containing 100% DAPI (4′,6-diamidino-2-phenylindole; Vector Laboratories Inc., Burlingame, California) was applied and the cells were covered with a 24 mm × 50 mm cover glass. Visualization was performed using an Olympus BX61 fluorescence microscope with a 60× plan apo oil immersion lens, and digital images were captured using the Olympus Microsuite 5 software package. Images were cropped and sized, and contrast was optimized using only functions affecting the whole image, with Adobe Photoshop 9.0.2 (Adobe Systems Inc.).

Results

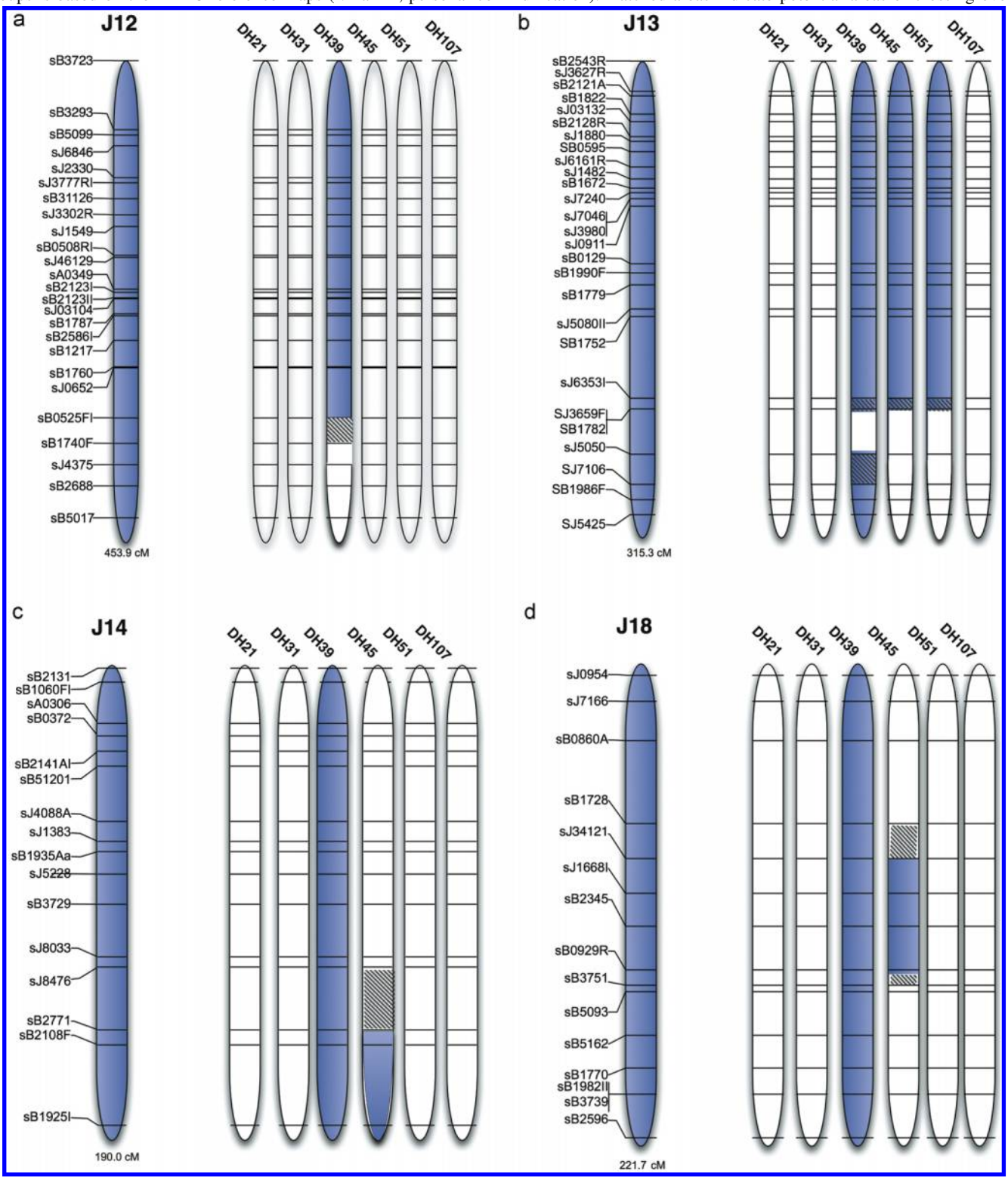
An interspecific cross of *Brassica carinata* and *Brassica napus* was performed with the initial intention of introgressing B-genome-derived blackleg resistance into a *B. napus* background (Fig. 1). Sixty DH lines and 48 publicly available SSR markers were used for the initial phenotyping and genotyping. Six of these DH lines and an additional 172 proprietory SSR markers were selected for detailed genotyping.

Genotypic analysis of the selected DH lines

Out of 220 microsatellite markers, 32 failed to amplify and 4 were not polymorphic between the parents. Of the 184 informative markers, 85 also amplified an allele in the *B. napus* controls, in some cases producing a stronger signal than in the B-genome-containing lines. We were able to assign these alleles to the A or C genome by comparison with the *B. rapa* and *B. oleracea* progenitors but could not map them, owing to the nature of the backcross.

We found three DH lines carrying one or more B-genome linkage groups and in most cases these appeared to occur as independent B-chromosome segments (Table 1, Table S2, Fig. 2).[3] After a number of backcrosses, there was preferential maintenance of five specific B chromosomes: J12/B8, J13/B7, J14/B6, J17/B1, and J18/B3. The "J" and "B" designations refer to the linkage maps of Ramichiary et al. (2007) and Panjabi et al. (2008), respectively. DH39 carried J12/B8, J13/B7, J14/B6, and J18/B3 (but with deleted terminal and internal segments) and also carried segments of J17/B1 (not shown in Fig. 2). DH45 carried the majority of J13/B7 as well as segments of J14/B6 and J18/B3. DH51 carried J13/B7 but was missing the terminal end of the chromosome (Table 1, Table S2, Fig. 2). J13/B7 was the only chromosome maintained in all three lines, J18/B3 and J14/B6 were maintained in two lines, and segments of J12/B8 and J17/B1 were maintained in one line. Figure 2 illustrates the linkage groups and demonstrates that in these lines, the B-genome linkage groups tend to lose the terminal segments, presum-

Fig. 2. Representation of the B-genome linkage groups maintained in the DH lines. Location of the markers on the four B-genome linkage groups is based on the AAFC reference maps (I. Parkin, personal communication). Hatched areas indicate potential areas of crossing-over.

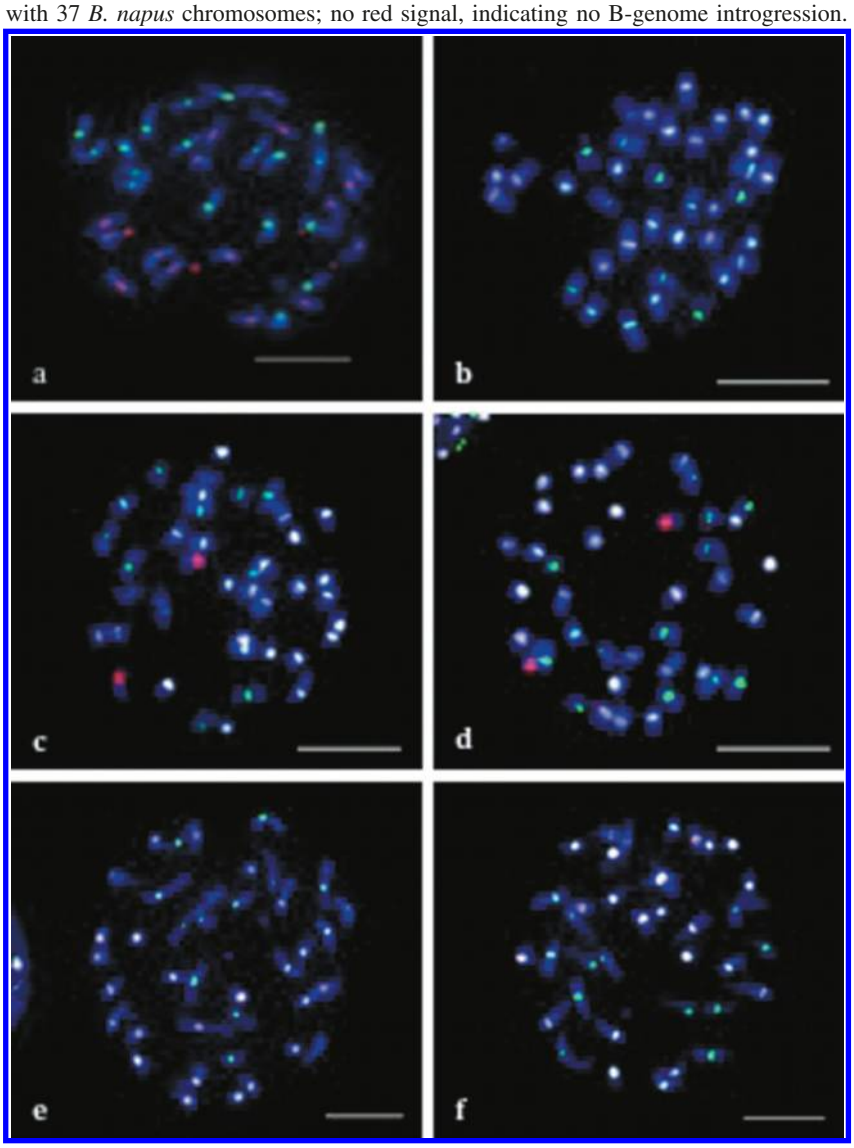


ably through deletions. Interestingly, the breakpoint on J13/B7 in both DH45 and DH51 appears to be conserved, while two of the markers on J13 do not amplify in DH39, suggesting that there may be an internal deletion in J13/B7.

GISH analysis

Figure 3 shows the images from the GISH assay. *Brassica carinata* displayed 8 pairs of chromosomes that were painted Cy3 fluorescent (red) (Fig. 3*a*), while *B. napus*

Fig. 3. Fluorescent in situ hybridization of chromosomes of (*a*) parent *B. carinata*, (*b*) parent *B. napus*, and (*c–f*) doubled haploid progeny lines. In all images, chromosomes are stained with DAPI (blue) and probed with *B. nigra* genomic DNA (B genome) fluorescently labeled with Cy3 (red) and *B. oleracea* genomic DNA (C genome) fluorescently labeled with Cy5 (green). (*a*) *Brassica carinata*, parental line with 34 chromosomes: 8 pairs of red BB centromere signals and 9 pairs of green CC signals; the 4 bright red signals are rDNA loci. In *b–f*, centromere repeat 1 is white and centromere repeat 2 is Cy5 fluorescent (green). (*b*) *Brassica napus*, parental line with 38 chromosomes; no prominent red BB signals aside from background hybridization to repetitive regions. (*c*) DH51, 38 chromosomes: 2 red *B. nigra* chromosomes and 36 *B. napus* chromosomes (consistent with marker data for presence of J13). (*d*) DH45, 38 chromosomes: 2 red *B. nigra* chromosomes and 36 *B. napus* chromosomes (consistent with marker data for presence of J13, but segments of J14 and J18 detected by markers cannot be confirmed). (*e*) DH17, 38 *B. napus* chromosomes; no red signal, indicating no B-genome introgression (consistent with marker data). (*f*) DH21, aneuploid with 37 *B. napus* chromosomes; no red signal, indicating no B-genome introgression. Bars = 10 μm.



($2n$ = 38, Fig. 3*b*) showed 19 pairs of Cy5 fluorescent (green) signals. This indicated that genomic DNA from *B. nigra* is capable of specifically detecting B-genome chromosomes in the allopolyploid species, while genomic DNA from *B. oleracea* can paint both A- and C-genome chromosomes without distinction (Figs. 3*a*, 3*b*). We counted 17 pairs of chromosomes in the *B. carinata* ($2n$ = 34) parent, 8 of which emitted a Cy3 fluorescent (red) signal characteristic of the B-genome chromosomes (Fig. 3*a*). These signals were very strong around the centromere but difficult to visualize on the arms, which prevented us from detecting translocations (Fig. 3). For DH51 and DH45, we counted 38 chromosomes; 2 of them had strong Cy3 fluorescent (red) signals, indicating they were B-genome chromosomes. Therefore, as expected from the marker data indicating the presence of J13/B7 in these lines, we propose that these lines contain a pair of J13/B7 chromosomes ($2n$ = 36+II (J13/B7)) substituting for the original A or C chromosomes (Figs. 3*c*, 3*d*). Based on our original marker data, DH39 appeared to carry four B-genome chromosomes (J12/B8, J13/B7, J14/B6, J18/B3). However, selfed seed from the DH39 plants used for marker analysis did not germinate, nor did any of the seed from DH39's sibling plants; therefore, we could not include this line in our GISH assay. Among the

Table 2. Comparison and statistical analysis of the parental and DH lines in two field trial seasons for key agronomic traits.

	Trait						
	Leaf margin	Days to flowering	Days to maturity	Pod attitude	Silique length (mm)	Beak length (mm)	No. of seeds/ silique
Least square means (standard error) for parental and DH lines							
Population mean	2.8 (0.02)	40 (0.5)	97 (1.1)	3.0 (0.05)	51.5 (0.36)	5.3 (0.10)	22 (0.29)
Mean of DH lines	2.8 (0.02)	39 (0.5)	96 (1.1)	3.0 (0.05)	52.0 (0.34)	5.3 (0.10)	22 (0.28)
17-009	2.2 (0.18)	52 (5.9)	114 (15.4)	1.0 (0.48)	42.1 (2.35)	3.8 (0.85)	13 (1.7)
Westar	2.4 (0.18)	43 (5.9)	98 (15.4)	4.5 (0.48)	53.2 (2.35)	7.0 (0.85)	19 (1.7)
DH45	1.7 (0.18)	48 (5.9)	99 (15.4)	2.5 (0.48)	47.9 (2.35)	8.7 (0.85)	23 (1.7)
DH51	1.9 (0.18)	40 (5.9)	99 (15.4)	3.0 (0.48)	43.8 (2.35)	7.8 (0.85)	17 (1.7)
B–	2.9 (0.08)	38 (1.6)	96 (4.9)	3.3 (0.22)	52.1 (1.46)	5.1 (0.31)	23 (0.98)
Single degree of freedom contrasts between lines with and without B-genome content							
B+ vs. B–	**	**	ns	ns	ns	**	**
B– vs. DH39	ns	**	ns	ns	ns	ns	**
B– vs. DH45	**	**	ns	ns	ns	**	ns
B– vs. DH51	**	ns	ns	ns	**	**	**
DH45 vs. DH51	ns	**	ns	ns	ns	ns	*
Two-tailed *t* test comparison for individual lines							
17-009 vs. Westar	ns	**	**	**	**	**	**
17-009 vs. DH39	*	ns	ns	*	**	ns	ns
17-009 vs. DH45	ns	ns	*	*	ns	**	**
17-009 vs. DH51	ns	**	*	**	ns	**	ns
Westar vs. DH39	ns	*	ns	ns	ns	ns	**
Westar vs. DH45	**	ns	ns	**	ns	*	ns
Westar vs. DH51	ns	ns	ns	*	**	ns	ns
Year	ns	ns	ns	ns	ns	ns	.
Line	**	**	**	*	**	**	**
Line × year	*	*	**	**	**	ns	.

Note: "B+" is a group of three DH lines that carry at least one B-genome linkage group. "B–" is a group of three DH lines that fine mapping proved to be without any B-genome content. *, significant at $p < 0.01$; **, significant at $p < 0.05$; ns, not significant.

DH lines with no B-genome chromosomes, DH17 carries 19 pairs of chromosomes ($2n = 38$) with no Cy3 fluorescent (red) signals (Fig. 3*e*), while DH21, with 37 chromosomes ($2n = 38$–I), has one missing chromosome (Fig. 3*f*).

Phenotypic evaluation of DH lines

Variation within the 60 DH lines followed a normal distribution for blackleg lesion length and for the following agronomic traits: silique length, pedicle length, days to flowering, days to maturity, number of seeds per silique, and beak length (Table 2). However, while the distribution of phenotypic traits followed a normal distribution, there was also significant transgressive segregation for all these traits (data not shown).

There are significant differences ($p < 0.01$) in the DH population for blackleg lesion length, both internal and external (Table 3). The *B. napus* cultivar Westar is susceptible to blackleg and showed a significant difference from the *B. carinata* parent 17-009 ($p < 0.01$). However, most of the DH lines used in this study were not significantly different from Westar, except for DH39, which was similar to its *B. carinata* parent. Both DH45 and DH21 were not significantly different from Westar for any of the blackleg resistance traits that we measured. The only DH line that displayed any resistance was DH51, which displayed moderate resistance for internal lesion length with a value of 5.5 ± 1.71 cm (Table 3).

The DH population was evaluated for agronomic and seed quality traits in two seasons of field trials and data are presented in Table 2 and Table S3.[3] Significant differences were observed between the parental lines and the DH lines for all the investigated traits (Tables 2 and S3). The two parents, Westar and *B. carinata* (17-009), were different for all traits ($p < 0.01$) except pedicel length, leaf margin, and myristic acid (C14:0) content. In the two seasons of measurements, year did not have a significant effect on any of the morphological or agronomic traits (Tables 2 and S3).

The effect of B-genome linkage groups on traits

The two groups of DH lines, those with ("B+") and without ("B–") B-genome chromosomes, were statistically different for the following traits: leaf margin, days to flowering, beak length, number of seeds per silique, C14:0 (myristic acid), C18:0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid), C20:0 (arachidic acid), C20:1 (gadoleic acid), C20:2 (eicosadienic acid), C22:0 (behenic acid), C22:1 (erucic acid), C24:0 (lignoceric acid), C24:1 (nervonic acid), and saturated fatty acids. These *F* values changed slightly when the "B–" group was compared with lines of the "B+" group individually (Tables 2 and S3). More specifically, it was found that DH45 differed from the "B–" group for days to flowering ($p < 0.01$; Table 3); this line was not significantly different from its *B. carinata* parent but was different from its *B. napus* parent ($p < 0.05$) for

Table 3. Analysis of blackleg resistance in the DH population and a subset of DH lines.

	Internal blackleg lesion length (cm)	Relative internal lesion length (cm)	External lesion length (cm)	Relative external lesion length (cm)
Max.	21	0.27	16.16	0.29
Min.	1.5	0.02	0.5	0.008
Mean	7.14	0.11	5.2	0.08
F value	**	**	*	**
B. napus 'Westar'	9.5a	0.15a	7a	0.11a
B. carinata 17-009	3.75b	0.05b	2.6b	0.033b
DH39	3.7b (1.56)	0.04b (0.03)	2.6b (2.11)	0.03b (0.02)
DH45	9.5a (1.56)	0.16a (0.03)	7.0a (2.11)	0.11a (0.03)
DH51	5.5c (1.71)	0.20a (0.03)	6.7a (2.59)	0.20a (0.03)
DH21	10.1a (1.56)	0.17a (0.03)	7.8a (2.11)	0.13a (0.03)

Note: *, significant at $p < 0.01$; **, significant at $p < 0.05$. Within a column, values followed by different letters are significantly different based on a Tukey test.

this trait. DH51 was significantly different from the "B–" group for number of seeds per silique and not different from its *B. carinata* parent but had lower seed set (17 ± 1.7 seeds) than the other DH lines. For all measured traits except days to flowering and number of seeds per silique, DH45 and DH51 were not significantly different from each other but were different from the "B–" group ($p < 0.01$).

Discussion

This is the first study that integrates molecular mapping with molecular cytogenetics to analyze the inheritance of B-genome chromosomes and traits in a *B. napus* background. We were able to generate a molecular karyotype of the B-genome linkage groups in each of six DH lines, using a set of 184 informative markers (approximately 23 markers per linkage group). To the best of our knowledge, this is the first successful development of doubled haploid lines from an interspecific *Brassica* cross including the B genome and the first detailed identification and characterization of B-genome-containing substitution lines. *Brassica carinata* has not been used to date in an interspecific cross for the purpose of transferring blackleg resistance, despite the fact that this species has been shown to have very high levels of field resistance in Australia (Marcroft et al. 2002). All the blackleg resistance genes that have been mapped to date are located on linkage group N7 of the A genome. Therefore, by using *B. carinata* rather than *B. juncea*, we believed that we could eliminate the concern about two potential resistance sources, one from the B genome and one from N7.

During the generations of backcrossing and selfing used in developing this material (Fig. 1), one of the selection criteria was a resistant response to the blackleg fungus *Leptosphaeria maculans.* Chèvre et al. (2007) argued that selection pressure can significantly affect the presence of additional chromosomes in hybrid plants. In our study we observed the presence of the J13/B7 chromosome in all the DH lines carrying B-genome linkage groups. Christianson et al. (2006) reported a blackleg resistance gene to be located on J13/B7 of *B. juncea.* Additonally, the "E block" of *Arabidopsis* chromosome 1, which aligns with J13/B7 (Panjabi et al. 2008), also aligns with the segment of N7 of *B. napus* where other blackleg resistance genes have been mapped (Ferreira et al. 1995; Mayerhofer et al. 2005). Therefore, we were optimistic that these lines would contain some degree of resistance to blackleg. However, in the present study, no significant correlation between the presence of J13/B7 or any of the other B chromosomes and resistance to blackleg was found. This could be due to the sample size of the lines that were found to carry B-genome segments, which might have been limited by the number of markers used at the first genotypic screening (48 markers on eight linkage groups), causing smaller chromosomal segments to be ignored. Another possibility is that it may be due to the presence of multiple sources of resistance for this disease (Delourme et al. 2006; Ferreira et al. 1995; Pilet et al. 1998, 2001). While most resistance genes were found to be located on the A genome, new C-genome sources of resistance also have been identified by Ananga et al. (2006) and Sebastian et al. (2000).

The microsatellite markers for this study were specifically chosen to amplify B-genome alleles; however, 46% of these primers also generated PCR products in the A genome and to a lesser degree in the C genome, based on a comparison of the alleles derived from the A and C genomes in reference genotypes. This is not surprising, since Panjabi et al. (2008) showed that three of the B chromosomes (B4, B5, B6) have regions of homology with three of the A-genome linkage groups (A4, A5, A6, respectively) and that there are additional blocks in the A and B genomes of *B. napus* and *B. juncea* that align with identical regions of the *Arabidopsis* genome.

While it is now clear that the B genome shares some regions of homology with the A and C genomes (Lagercrantz and Lydiate 1996; Panjabi et al. 2008), the *Brassica* B-genome chromosomes do not pair with chromosomes of the A and C genomes in interspecific crosses (Meng et al. 1998). The B genome has significantly diverged from the A and C genomes (Axelsson et al. 2000; Warwick et al. 1992), as evident from cytological observations of preferential pairing between homologous chromosomes in digenomic triploids (BBC and CCB) generated from interspecific hybridization between *B. carinata* and *B. nigra* and *B. carinata* and *B. oleracea* (Attia et al. 1987). It has been estimated that the *B. nigra* (B) and *B. rapa*/*oleracea* (A/C) (Warwick et al. 1992) lineages separated from each other about 7.9 million years ago, followed by the splitting of the *B. rapa* and *B. oleracea* lineages (A and C) approximately

1 million years ago (Lagercrantz 1998). Comparative mapping studies indicate that the A and C genomes have undergone chromosomal rearrangements such as inversions and translocations, presumably due to homoeologous recombination (Parkin and Lydiate 1997; Parkin et al. 1995, 2002, 2005; Sharpe et al. 1995).

There are considerable difficulties in mapping introgressions in the *Brassica* genomes because of the occurrence of sequence repetition, chromosomal rearrangements, and insertion–deletions, which can result in altered chromosomal structure and gene complement as well as abnormal recombination frequencies (Mayerhofer et al. 2005; Parkin et al. 2005). In our study we were able to overcome these problems to some extent by using the AAFC reference maps and knowing the size and location of each allele on the B-genome linkage maps. We used a GISH assay with *B. nigra* genomic DNA to allow us to visualize and distinguish the B-genome chromosomes from the A- and C-genome chromosomes (Ge and Li 2007; Hasterok et al. 2005; Maluszynska and Hasterok 2005; Snowdon et al. 1997). This allowed us to track the B-genome chromosomes and to determine the number of chromosomes in the DH lines. We were able to show that the DH lines carrying the B-genome chromosomes contained them as a pair, which is not surprising, given the doubled haploid nature of the lines. This indicates that these lines were aneuploid before they went through the process of becoming doubled haploid lines. However, the GISH technique did not allow us to visualize the chromosome arms because of preferential painting of the centromeres. Therefore, cytological confirmation of segments that are translocated would require chromosome-specific DNA probes, which are currently not available. The presence of multiple B-genome linkage groups (five of eight chromosomes) in DH39 is one possible reason why we never succeeded in germinating seeds from this line after doubling of the haploid. The cytology study also provided us with information on the chromosome number in each DH line. In most cases the number of chromosomes in the negative controls (DH lines without B-genome chromosomes) was the same as in their *B. napus* parent ($2n$ = 38); the exception was DH21, which was randomly selected to be a negative control and had lost a single A or C chromosome. In multiple spreads and chromosome counts, this line was always observed to have 37 chromosomes ($2n$ = 38–I). In this study, we were able to identify three DH lines that contained B-genome chromosomes from a population of 60 lines (5%). Five of the B chromosomes (J12/B8, J13/B7, J14/B6, J17/B1, J18/B3) were maintained in the BC_2S_3 plants. The low frequency of lines carrying B-genome linkage groups is not surprising given that in each generation there was selection for *B. napus*–type plants and possibly natural selection against gametes carrying a B-genome linkage group.

Our field data indicated consistent differences in a number of agronomic traits between the lines containing B-genome linkage groups and those lacking them. For example, lines DH45 and DH51 were significantly different from the "B–" group in days to flowering and number of seeds per silique. The DH population shows a significant transgressive segregation for the following traits: days to flowering, number of seeds per silique, oleic acid (C18:1) content, and gadoleic acid (C20:1) content (Tables 2 and S3). Since the DH population can potentially carry C-genome chromosome segments from the *B. carinata* parent, the variation cannot be attributed exclusively to the presence of the B genome. Resolving this would require a more detailed investigation of the chromosome architecture of the lines.

In summary, we have demonstrated that B-genome chromosomes can be transferred into *B. napus* using a backcrossing program and that "stable" addition lines carrying two copies of the specific B-genome chromosome can be produced and maintained. However, there are still significant challenges to developing genotypes with introgressed B-genome material. This is because the B-genome chromosomes appear to be inherited as either whole chromosomes or chromosomes with terminal deletions.

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References

Ananga, A. O., Cebert, E., Soliman, K., Kantety, R., Pacumbaba, R.P., and Konan, K. 2006. RAPD markers associated with resistance to blackleg disease in *Brassica* species. Afr. J. Biotechnol. **5**: 2041–2048.

Ansan-Melayah, D., Balesdent, M.H., Delourme, R., Pilet, M.L., Tanguy, X., Renard, M., and Rouxel, T. 1998. Genes for race-specific resistance against blackleg disease in *Brassica napus* L. Plant Breed. **117**(4): 373–378. doi:10.1111/j.1439-0523.1998.tb01956.x.

Attia, T., Busso, C., and Röbbelen, G. 1987. Digenomic triploids for an assessment of chromosome relationships in the cultivated diploid *Brassica* species. Genome, **29**: 326–330. doi:10.1139/g87-053.

Axelsson, T., Bowman, C.M., Sharpe, A.G., Lydiate, D.J., and Lagercrantz, U. 2000. Amphidiploid *Brassica juncea* contains conserved progenitor genomes. Genome, **43**(4): 679–688. doi:10.1139/gen-43-4-679. PMID:10984181.

Balesdent, M.H., Attard, A., Ansan-Melayah, D., Delourme, R., Renard, M., and Rouxel, T. 2001. Genetic control and host range of avirulence toward *Brassica napus* cultivars Quinta and Jet Neuf in *Leptosphaeria maculans.* Phytopathology, **91**(1): 70–76. doi:10.1094/PHYTO.2001.91.1.70. PMID:18944280.

Balesdent, M.H., Attard, A., Kühn, M.L., and Rouxel, T. 2002. New avirulence genes in the phytopathogenic fungus *Leptosphaeria maculans.* Phytopathology, **92**(10): 1122–1133. doi:10.1094/PHYTO.2002.92.10.1122. PMID:18944223.

Ballinger, D.J., and Salisbury, P.A. 1996. Seedling and adult plant evaluation of race variability in *Leptosphaeria maculans* on *Brassica* species in Australia. Aust. J. Exp. Agric. **36**(4): 485–488. doi:10.1071/EA9960485.

Bansal, V.K., Kharbanda, P.D., Stringam, G.R., Thiagarajah, M.R., and Tewari, J.P. 1994. A comparison of greenhouse and field screening methods for blackleg resistance in doubled haploid lines of *Brassica napus.* Plant Dis. **78**: 276–281.

Bansal, V.K., Thiagarajah, M.R., Stringam, G.R., and Tewari, J.P. 1999. Inheritance of partial resistance to race 2 of *Albugo candida* in canola-quality mustard (*Brassica juncea*) and its role in

resistance breeding. Plant Pathol. **48**(6): 817–822. doi:10.1046/j.1365-3059.1999.00408.x.

Barret, P., Guérif, J., Reynoird, J.P., Delourme, R., Eber, F., Renard, M., and Chèvre, A.M. 1998. Selection of stable *Brassica napus-Brassica juncea* recombinant lines resistant to blackleg (*Leptosphaeria maculans*). 2. A 'to and fro' strategy to localise and characterise interspecific introgressions on the *B. napus* genome. Theor. Appl. Genet. **96**(8): 1097–1103. doi:10.1007/s001220050844.

Chevre, A.M., Eber, F., This, P., Barret, P., Tanguy, X., Brun, H., et al. 1996. Characterization of *Brassica nigra* chromosomes and of blackleg resistance in *B. napus–B nigra* addition lines. Plant Breed. **115**(2): 113–118. doi:10.1111/j.1439-0523.1996.tb00884.x.

Chèvre, A.M., Barret, P., Eber, F., Dupuy, P., Brun, H., Tanguy, X., and Renard, M. 1997. Selection of stable *Brassica napus–B. juncea* recombinant lines resistant to blackleg (*Leptosphaeria maculans*). 1. Identification of molecular markers, chromosomal and genomic origin of the introgression. Theor. Appl. Genet. **95**: 1104–1111. doi:10.1007/s001220050669.

Chèvre, A.M., Adamczyk, K., Eber, F., Huteau, V., Coriton, O., Letanneur, J.C., et al. 2007. Modelling gene flow between oilseed rape and wild radish. I. Evolution of chromosome structure. Theor. Appl. Genet. **114**(2): 209–221. doi:10.1007/s00122-006-0424-x. PMID:17091264.

Christianson, J.A., Rimmer, S.R., Good, A.G., and Lydiate, D.J. 2006. Mapping genes for resistance to *Leptosphaeria maculans* in *Brassica juncea.* Genome, **49**(1): 30–41. doi:10.1139/g05-085. PMID:16462899.

Daun, J.K., DeClercq, D.R., and McGregor, D.I. 1989. Analysis of fatty acid in canola and rapeseed. Method of the Canadian Grain Commission, Grain Research Laboratory. Agriculture and Agri-Food Canada, Saskatoon, Sask.

Delourme, R., Pilet-Nayel, M.L., Archipiano, M., Horvais, R., Tanguy, X., Rouxel, T., et al. 2004. A cluster of major specific resistance genes to *Leptosphaeria maculans* in *Brassica napus.* Phytopathology, **94**(6): 578–583. doi:10.1094/PHYTO.2004.94.6.578. PMID:18943482.

Delourme, R., Chevre, A.M., Brun, H., Rouxel, T., Balesdent, M.H., Dias, J.S., et al. 2006. Major gene and polygenic resistance to *Leptosphaeria maculans* in oilseed rape (*Brassica napus*). Eur. J. Plant Pathol. **114**(1): 41–52. doi:10.1007/s10658-005-2108-9.

Dixelius, C., and Wahlberg, S. 1999. Resistance to *Leptosphaeria maculans* is conserved in a specific region of the *Brassica* B genome. Theor. Appl. Genet. **99**(1–2): 368–372. doi:10.1007/s001220051246.

Ferreira, M.E., Rimmer, S.R., Williams, P.H., and Osborn, T.C. 1995. Mapping loci controlling *Brassica napus* resistance to *Leptosphaeria maculans* under different screening conditions. Phytopathology, **85**(2): 213–217. doi:10.1094/Phyto-85-213.

Ge, X.H., and Li, Z.Y. 2007. Intra- and intergenomic homology of B-genome chromosomes in trigenomic combinations of the cultivated *Brassica* species revealed by GISH analysis. Chromosome Res. **15**(7): 849–861. doi:10.1007/s10577-007-1168-4. PMID:17899408.

Gerdemann-Knörck, M., Nielen, S., Tzscheetzsch, C., Iglisch, J., and Schieder, O. 1995. Transfer of disease resistance within the genus *Brassica* through asymmetric somatic hybridization. Euphytica, **85**(1–3): 247–253. doi:10.1007/BF00023953.

Hasterok, R., Ksiazczyk, T., Wolny, E., and Maluszynska, J. 2005. FISH and GISH analysis of *Brassica* genomes. Acta Biol. Cracov. Ser. Bot. **47**: 185–192.

Huang, B., Liu, Y., Xue, X., and Chang, L. 2002. Comparison of aluminium tolerance in the brassicas and related species. Plant Breed. **121**(4): 360–362. doi:10.1046/j.1439-0523.2002.00723.x.

IBPGR. 1990. Descriptors for *Brassica* and *Raphanus*. International Board for Plant Genetic Resources, Rome.

Kato, A., Lamb, J.C., and Birchler, J.A. 2004. Chromosome painting using repetitive DNA sequences as probes for somatic chromosome identification in maize. Proc. Natl. Acad. Sci. U.S.A. **101**(37): 13554–13559. doi:10.1073/pnas.0403659101. PMID:15342909.

Kumar, A., Singh, P., Singh, D.P., Singh, H., and Sharma, H.C. 1984. Differences in osmoregulation in *Brassica* species. Ann. Bot. (Lond.), **54**: 537–541.

Ky, C.L., Barre, P., Lorieux, M., Trouslot, P., Akaffou, S., Louarn, J., et al. 2000. Interspecific genetic linkage map, segregation distortion and genetic conversion in coffee (*Coffea* sp.). Theor. Appl. Genet. **101**(4): 669–676. doi:10.1007/s001220051529.

Lagercrantz, U. 1998. Comparative mapping between *Arabidopsis thaliana* and *Brassica nigra* indicates that *Brassica* genomes have evolved through extensive genome replication accompanied by chromosome fusions and frequent rearrangements. Genetics, **150**(3): 1217–1228. PMID:9799273.

Lagercrantz, U., and Lydiate, D.J. 1996. Comparative genome mapping in *Brassica.* Genetics, **144**(4): 1903–1910. PMID:8978073.

Lamb, J.C., and Birchler, J.A. 2006. Retroelement genome painting: cytological visualization of retroelement expansions in the genera *Zea* and *Tripsacum.* Genetics, **173**(2): 1007–1021. doi:10.1534/genetics.105.053165. PMID:16582446.

Leflon, M., Brun, H., Eber, F., Delourme, R., Lucas, M.O., Vallée, P., et al. 2007. Detection, introgression and localization of genes conferring specific resistance to *Leptosphaeria maculans* from *Brassica rapa* into *B. napus.* Theor. Appl. Genet. **115**(7): 897–906. doi:10.1007/s00122-007-0616-z. PMID:17668174.

Littell, R.C., Milliken, G.A., Stroup, W.W., and Wolfinger, R.D. 1996. SAS® system for mixed models. SAS Institute Inc., Cary, N.C.

Malik, R.S. 1990. Prospects for *Brassica carinata* as an oilseed crop in India. Exp. Agric. **26**(01): 125–129. doi:10.1017/S0014479700015465.

Maluszynska, J., and Hasterok, R. 2005. Identification of individual chromosomes and parental genomes in *Brassica juncea* using GISH and FISH. Cytogenet. Genome Res. **109**(1–3): 310–314. doi:10.1159/000082414. PMID:15753591.

Marcroft, S.J., Purwantara, A., Salisbury, P.A., Potter, T.D., Wratten, N., Khangura, R., et al. 2002. Reaction of a range of *Brassica* species under Australian conditions to the fungus, *Leptosphaeria maculans*, the causal agent of blackleg. Aust. J. Exp. Agric. **42**(5): 587–594. doi:10.1071/EA01112.

Mayerhofer, R., Wilde, K., Mayerhofer, M., Lydiate, D., Bansal, V.K., Good, A.G., and Parkin, I.A.P. 2005. Complexities of chromosome landing in a highly duplicated genome: toward map-based cloning of a gene controlling blackleg resistance in *Brassica napus.* Genetics, **171**(4): 1977–1988. doi:10.1534/genetics.105.049098. PMID:16143600.

McNabb, W.M., Vandenberg, C.G.J., and Rimmer, S.R. 1993. Comparison of inoculation methods for selection of plants resistant to *Leptosphaeria maculans* in *Brassica napus.* Can. J. Plant Sci. **73**: 1199–1207.

Meng, J.L., Shi, S.W., Gan, L., Li, Z.Y., and Qu, X.S. 1998. The production of yellow-seeded *Brassica napus* (AACC) through crossing interspecific hybrids of *B. campestris* (AA) and *B. carinata* (BBCC) with *B. napus.* Euphytica, **103**(3): 329–333. doi:10.1023/A:1018646223643.

Pang, E.C.K., and Halloran, G.M. 1996. The genetics of blackleg

[*Leptosphaeria maculans* (Desm.) Ces. et De Not.] resistance in rapeseed (*Brassica napus* L.). II. Seedling and adult-plant resistance as quantitative traits. Theor. Appl. Genet. **93**(93): 941–949. doi:10.1007/BF00224097.

Panjabi, P., Jagannath, A., Bisht, N.C., Padmaja, K.L., Sharma, S., Gupta, V., et al. 2008. Comparative mapping of *Brassica juncea* and *Arabidopsis thaliana* using Intron Polymorphism (IP) markers: homoeologous relationships, diversification and evolution of the A, B and C Brassica genomes. BMC Genomics, **9**(1): 113. doi:10.1186/1471-2164-9-113. PMID:18315867.

Parkin, I.A., and Lydiate, D.J. 1997. Conserved patterns of chromosome pairing and recombination in *Brassica napus* crosses. Genome, **40**(4): 496–504. doi:10.1139/g97-066. PMID:18464842.

Parkin, I.A., Sharpe, A.G., Keith, D.J., and Lydiate, D.J. 1995. Identification of the A and C genomes of amphidiploid *Brassica napus* (oilseed rape). Genome, **38**(6): 1122–1131. doi:10.1139/g95-149. PMID:18470236.

Parkin, I.A., Lydiate, D.J., and Trick, M. 2002. Assessing the level of collinearity between *Arabidopsis thaliana* and *Brassica napus* for *A. thaliana* chromosome 5. Genome, **45**(2): 356–366. doi:10.1139/g01-160. PMID:11962633.

Parkin, I.A., Gulden, S.M., Sharpe, A.G., Lukens, L., Trick, M., Osborn, T.C., and Lydiate, D.J. 2005. Segmental structure of the *Brassica napus* genome based on comparative analysis with *Arabidopsis thaliana.* Genetics, **171**(2): 765–781. doi:10.1534/genetics.105.042093. PMID:16020789.

Pilet, M.L., Delourme, R., Foisset, N., and Renard, M. 1998. Identification of QTL involved in field resistance to light leaf spot (*Pyrenopeziza brassicae*) and blackleg resistance (*Leptosphaeria maculans*) in winter rapeseed (*Brassica napus* L.). Theor. Appl. Genet. **97**(3): 398–406. doi:10.1007/s001220050909.

Pilet, M.L., Duplan, G., Archipiano, H., Barret, P., Baron, C., Horvais, R., et al. 2001. Stability of QTL for field resistance to blackleg across two genetic backgrounds in oilseed rape. Crop Sci. **41**: 197–205.

Prakash, S., and Chopra, V.L. 1988. Introgression of resistance to shattering in *Brassica napus* from *Brassica juncea* through nonhomologous recombination. Plant Breed. **101**(2): 167–168. doi:10.1111/j.1439-0523.1988.tb00283.x.

Ramchiary, N., Bisht, N.C., Gupta, V., Mukhopadhyay, A., Arumugam, N., Sodhi, Y.S., et al. 2007. QTL analysis reveals context-dependent loci for seed glucosinolate trait in the oilseed *Brassica juncea*: importance of recurrent selection backcross scheme for the identification of 'true' QTL. Theor. Appl. Genet. **116**(1): 77–85. doi:10.1007/s00122-007-0648-4. PMID:17898985.

Rimmer, S.R., and Vandenberg, C.G.J. 1992. Resistance of oilseed *Brassica* spp to blackleg caused by *Leptosphaeria maculans.* Can. J. Plant Pathol. **14**: 56–66.

Roussel, S., Nicole, M., Lopez, F., Ricci, P., Geiger, J.P., Renard, M., and Brun, H. 1999. *Leptosphaeria maculans* and cryptogein induce similar vascular responses in tissues undergoing the hypersensitive reaction in *Brassica napus.* Plant Sci. **144**(1): 17–28. doi:10.1016/S0168-9452(99)00043-6.

Roy, N.N. 1984. Interspecific transfer of *Brassica juncea* type high blackleg resistance to *Brassica napus.* Euphytica, **33**(2): 295–303. doi:10.1007/BF00021125.

Saal, B., Brun, H., Glais, I., and Struss, D. 2004. Identification of a *Brassica juncea*-derived recessive gene conferring resistance to *Leptosphaeria maculans* in oilseed rape. Plant Breed. **123**(6): 505–511. doi:10.1111/j.1439-0523.2004.01052.x.

SAS Institute Inc. 1989. SAS/STAT. SAS Institute Inc., Cary, N.C.

Schelfhout, C.J., Snowdon, R., Cowling, W.A., and Wroth, J.M. 2006. Tracing B-genome chromatin in *Brassica napus* × *B. juncea* interspecific progeny. Genome, **49**(11): 1490–1497. doi:10.1139/G06-103. PMID:17426764.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nat. Biotechnol. **18**(2): 233–234. doi:10.1038/72708. PMID:10657137.

Sebastian, R.L., Howell, E.C., King, G.J., Marshall, D.F., and Kearsey, M.J. 2000. An integrated AFLP and RFLP *Brassica oleracea* linkage map from two morphologically distinct doubled-haploid mapping populations. Theor. Appl. Genet. **100**(1): 75–81. doi:10.1007/s001220050011.

Sharpe, A.G., Parkin, I.A.P., Keith, D.J., and Lydiate, D.J. 1995. Frequent nonreciprocal translocations in the amphidiploid genome of oilseed rape (*Brassica napus*). Genome, **38**(6): 1112–1121. doi:10.1139/g95-148. PMID:18470235.

Snowdon, R.J., Köhler, W., and Köhler, A. 1997. Chromosomal localization and characterization of rDNA loci in the *Brassica* A and C genomes. Genome, **40**(4): 582–587. doi:10.1139/g97-076. PMID:18464849.

Stringam, G.R., Bansal, V.K., Thiagarajah, M.R., Degenhardt, D.F., and Tewari, J.P. 1995. Development of an agronomically superior blackleg resistant canola cultivar in *Brassica napus* L. using doubled haploidy. Can. J. Plant Sci. **75**: 437–439.

Tanksley, S.D., and Nelson, J.C. 1996. Advanced backcross QTL analysis: a method for simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor. Appl. Genet. **92**(2): 191–203. doi:10.1007/BF00223376.

Warwick, S.I., Black, L.D., and Aguinagalde, I. 1992. Molecular systematics of *Brassica* and allied genera (subtribe Brassicinae, Brassiceae) — chloroplast DNA variation in the genus *Diplotaxis*. Theor. Appl. Genet. **83**(83): 839–850. doi:10.1007/BF00226706.

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1. Dandan Hu, Wenshan Zhang, Yikai Zhang, Shihao Chang, Lunlin Chen, Yingying Chen, Yongdi Shi, Jinxiong Shen, Jinling Meng, Jun Zou. 2019. Reconstituting the genome of a young allopolyploid crop, Brassica napus, with its related species. *Plant Biotechnology Journal* **17**:6, 1106-1118. [Crossref]
2. Wenliang Wang, Rui Guan, Xing Liu, Haorui Zhang, Bo Song, Qiwu Xu, Guangyi Fan, Wenbin Chen, Xiaoming Wu, Xin Liu, Jianbo Wang. 2019. Chromosome level comparative analysis of Brassica genomes. *Plant Molecular Biology* **99**:3, 237-249. [Crossref]
3. M. Harunur Rashid, Georg Hausner, W. G. Dilantha Fernando. 2018. Molecular and phenotypic identification of B-genome introgression linked to Leptosphaeria maculans resistant gene Rlm6 in Brassica napus×B. juncea interspecific hybrids. *Euphytica* **214**:11. . [Crossref]
4. M. Harunur Rashid, Zhongwei Zou, W. G. Dilantha Fernando. 2018. Development of molecular markers linked to the Leptosphaeria maculans resistance gene Rlm6 and inheritance of SCAR and CAPS markers in Brassica napus × Brassica juncea interspecific hybrids. *Plant Breeding* **137**:3, 402-411. [Crossref]
5. Changcai Teng, Yan Niu, Dezhi Du, Qinglan Yu, Zhigang Zhao. 2018. Production and genetic analyses of novel Brassica rapa L. introgressions from interspecific crosses with Brassica juncea L. landraces native to the Qinghai-Tibet Plateau. *Euphytica* **214**:2. . [Crossref]
6. Chen Tan, Cheng Cui, Yi Xiang, Xianhong Ge, Zaiyun Li. 2017. Development of Brassica oleracea-nigra monosomic alien addition lines: genotypic, cytological and morphological analyses. *Theoretical and Applied Genetics* **130**:12, 2491-2504. [Crossref]
7. Arif Hasan Khan Robin, Nicholas J. Larkan, Rawnak Laila, Jong-In Park, Nasar Uddin Ahmed, Hossein Borhan, Isobel A. P. Parkin, Ill-Sup Nou. 2017. Korean Brassica oleracea germplasm offers a novel source of qualitative resistance to blackleg disease. *European Journal of Plant Pathology* **149**:3, 611-623. [Crossref]
8. Inderpreet Dhaliwal, Annaliese S. Mason, Shashi Banga, Sakshi Bharti, Beerpal Kaur, Allison Mary Gurung, Phillip Anthony Salisbury, Jacqueline Batley, Surinder Singh Banga. 2017. Cytogenetic and Molecular Characterization of B-Genome Introgression Lines of Brassica napus L. *G3: Genes|Genomes|Genetics* **7**:1, 77-86. [Crossref]
9. Jun Zou, Dandan Hu, Peifa Liu, Harsh Raman, Zhongsong Liu, Xianjun Liu, Isobel A. P. Parkin, Boulos Chalhoub, Jinling Meng. 2016. Co-linearity and divergence of the A subgenome of Brassica juncea compared with other Brassica species carrying different A subgenomes. *BMC Genomics* **17**:1. . [Crossref]
10. Debamalya Chatterjee, Shashi Banga, Mehak Gupta, Sakshi Bharti, Phillip Anthony Salisbury, Surinder Singh Banga. 2016. Resynthesis of Brassica napus through hybridization between B. juncea and B. carinata. *Theoretical and Applied Genetics* . [Crossref]
11. Annaliese S. Mason, Junko Takahira, Chhaya Atri, Birgit Samans, Alice Hayward, Wallace A. Cowling, Jacqueline Batley, Matthew N. Nelson. 2015. Microspore culture reveals complex meiotic behaviour in a trigenomic Brassica hybrid. *BMC Plant Biology* **15**:1. . [Crossref]
12. Annaliese S. Mason, Jing Zhang, Reece Tollenaere, Paula Vasquez Teuber, Jessica Dalton-Morgan, Liyong Hu, Guijun Yan, David Edwards, Robert Redden, Jacqueline Batley. 2015. High-throughput genotyping for species identification and diversity assessment in germplasm collections. *Molecular Ecology Resources* **15**:5, 1091-1101. [Crossref]
13. Pu Chu, Huijuan Liu, Qing Yang, Yankun Wang, Guixia Yan, Rongzhan Guan. 2014. An RNA-seq transcriptome analysis of floral buds of an interspecific Brassica hybrid between B. carinata and B. napus. *Plant Reproduction* **27**:4, 225-237. [Crossref]
14. Jun Zou, Harsh Raman, Shaomin Guo, Dandan Hu, Zili Wei, Ziliang Luo, Yan Long, Wenxia Shi, Zhong Fu, Dezhi Du, Jinling Meng. 2014. Constructing a dense genetic linkage map and mapping QTL for the traits of flower development in Brassica carinata. *Theoretical and Applied Genetics* **127**:7, 1593-1605. [Crossref]
15. Rudolph Fredua-Agyeman, Olivier Coriton, Virginie Huteau, Isobel A. P. Parkin, Anne-Marie Chèvre, Habibur Rahman. 2014. Molecular cytogenetic identification of B genome chromosomes linked to blackleg disease resistance in Brassica napus × B. carinata interspecific hybrids. *Theoretical and Applied Genetics* **127**:6, 1305-1318. [Crossref]
16. Mingli Yan, Xianjun Liu, Chunyun Guan, Lili Liu, Jianhua Xiang, Ying Lu, Zhongsong Liu. 2014. Cloning of TTG1 gene and PCR identification of genomes A, B and C in Brassica species. *Genetica* **142**:2, 169-176. [Crossref]
17. H. Rahman, G. Peng, F. Yu, K.C. Falk, M. Kulkarni, G. Selvaraj. 2014. Genetics and breeding for clubroot resistance in Canadian spring canola (Brassica napus L.). *Canadian Journal of Plant Pathology* **36**:sup1, 122-134. [Crossref]
18. Margaret B. Uloth, Ming Pei You, Patrick M. Finnegan, Surinder S. Banga, Shashi K. Banga, Prabhjot S. Sandhu, Huang Yi, Phillip A. Salisbury, Martin J. Barbetti. 2013. New sources of resistance to Sclerotinia sclerotiorum for crucifer crops. *Field Crops Research* **154**, 40-52. [Crossref]

19. Rahman Habibur. 2013. Review: Breeding spring canola (Brassica napus L.) by the use of exotic germplasm. *Canadian Journal of Plant Science* **93**:3, 363-373. [Abstract] [Full Text] [PDF] [PDF Plus]

20. Zahra-Katy Navabi, Terry Huebert, Andrew G Sharpe, Carmel M O'Neill, Ian Bancroft, Isobel AP Parkin. 2013. Conserved microstructure of the Brassica B Genome of Brassica nigra in relation to homologous regions of Arabidopsis thaliana, B. rapa and B. oleracea. *BMC Genomics* **14**:1, 250. [Crossref]

21. Dan Liu, Ling Xu, Xinxin Geng, Yuanfei Zhou, Zhenchao Zhang, Bing Wang, Weijun Zhou. Distant Hybridization Involving Different In Vitro Techniques 23-44. [Crossref]

22. Sheng Chen, Matthew N. Nelson, Anne-Marie Chèvre, Eric Jenczewski, Zaiyun Li, Annaliese S. Mason, Jinling Meng, Julie A. Plummer, Aneeta Pradhan, Kadambot H. M. Siddique, Rod J. Snowdon, Guijun Yan, Weijun Zhou, Wallace A. Cowling. 2011. Trigenomic Bridges for Brassica Improvement. *Critical Reviews in Plant Sciences* **30**:6, 524-547. [Crossref]

23. Zahra K. Navabi, Kiersten E. Stead, J. Chris Pires, Zhiyong Xiong, Andrew G. Sharpe, Isobel A. P. Parkin, M. Habibur Rahman, Allen G. Good. 2011. Analysis of B-Genome Chromosome Introgression in Interspecific Hybrids of Brassica napus × B. carinata. *Genetics* **187**:3, 659-673. [Crossref]

24. Zhiyong Xiong, J. Chris Pires. 2011. Karyotype and Identification of All Homoeologous Chromosomes of Allopolyploid Brassica napus and Its Diploid Progenitors. *Genetics* **187**:1, 37-49. [Crossref]