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Note

# Resynthesis of *Brassica juncea* for resistance to *Plasmodiophora brassicae* pathotype 3

**Muhammad Jakir Hasan and Habibur Rahman***

*Department of Agricultural, Food and Nutritional Science, University of Alberta, 410 Agriculture/Forestry Centre*, Edmonton, AB, T6G 2P5, Canada

The oilseed crop *Brassica juncea* carries many desirable traits; however, resistance to clubroot disease, caused by *Plasmodiophora brassicae*, is not available in this species. We are the first to report the clubroot resistant resynthesized *B. juncea* lines, developed through interspecific crosses between a clubroot resistant *B. rapa* ssp. *rapifera* and two susceptible *B. nigra* lines, and the stability of the resistance in self-pollinated generations. The interspecific nature of the resynthesized *B. juncea* plants was confirmed by using A- and B-genome specific SSR markers, and flow cytometric analysis of nuclear DNA content. Self-pollinated progeny ($S_1$ and $S_2$) of the resynthesized *B. juncea* plants were evaluated for resistance to *P. brassicae* pathotype 3. The $S_1$ and $S_2$ progenies of one of the resynthesized *B. juncea* lines were resistant to this pathotype. However, resistance was lost in 6 to 13% plants of the $S_2$ progenies derived from the second resynthesized *B. juncea* line; this apparently resulted from the loss of the genomic region carrying resistance due to meiotic anomalies.

**Key Words:** *Brassica juncea*, clubroot, *Plasmodiophora brassicae*, resynthesis.

## Introduction

The oilseed crop species *Brassica juncea* (AABB; $2n = 36$) carry many desired traits, such as tolerance to heat (Gunasekera *et al.* 2006), drought (Wright *et al.* 1995) and silique shatter (Wang *et al.* 2007), and resistance to blackleg (Roy 1978) and leaf blight disease (Wechter *et al.* 2007). This crop species yields greater than *B. napus* in heat- and drought-prone areas as well as in short growing season areas (Burton *et al.* 1999, Potts *et al.* 1999). Although, *B. juncea* possess all these desired properties, resistance to *Plasmodiophora brassicae* Woronin, causing clubroot disease, is not available in this species (Hasan *et al.* 2012). Clubroot disease can result in up to 90% yield loss and about 4–6% decrease in seed oil content (Pageau *et al.* 2006). Resistance to this disease can be found in the two parental species of *B. juncea*, *viz.*, *B rapa* (AA; $2n = 20$) and *B. nigra* (BB; $2n = 16$) (Buczacki *et al.* 1975, Hasan *et al.* 2012). At least eight clubroot resistance genes have been mapped to date in *B. rapa* (reviewed by Piao *et al.* 2009), and this species has been used widely in the breeding of clubroot resistant *B. napus* lines and cultivars (Diederichsen and Sacristan 1996). The objective of the present study was to develop a clubroot resistant *B. juncea* line using a resistant *B. rapa* line, and to investigate the stability of this resistance in the resynthesized *B. juncea* line.

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*Corresponding author (e-mail: habibur.rahman@ualberta.ca)

## Materials and Methods

### *Plant materials*

A *B. rapa* line, homozygous for resistance to *P. brassicae* pathotype 3, was used as female in the interspecific crosses with two susceptible *B. nigra* (BB, $2n = 16$) accessions CR 2136 and CR 2137 as male. The *B. rapa* line was developed through self-pollination of the *B. rapa* ssp. *rapifera* cv. Gelria (AA; $2n = 20$). The cv. Gelria carries the clubroot resistance gene *CRa* (Matsumoto *et al.* 1998, Ueno *et al.* 2012) and *CRb* (Piao *et al.* 2004, 2009); however, recent studies have showed that the *CRa* and *CRb* to be the same locus and located on the chromosome A3 (Hatakeyama *et al.* 2017, Kato *et al.* 2013). Seeds of Gelria were obtained from the Green Gene International, Hill Castles, United Kingdom, and the *B. nigra* accessions were obtained from the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany. The interspecific cross-derived hybrid ovules were cultured *in vitro* following the technique described by Bennett *et al.* (2008). After about 14 days in culture, the visible embryos were transferred to solid B5 medium containing 0.1 mg/L $GA_3$, 20 g/L sucrose and 8 g/L

agar (Coventry *et al.* 1988) for 3–4 weeks until roots and shoots are developed. The plantlets were then planted in six-inch pots containing soil-free growth medium.

Interspecific nature of the plantlets was confirmed by the use of A- and B-genome specific simple sequence repeat (SSR or microsatellite) markers. For this, a total of 29 SSR markers specific to the 10 A-genome linkage groups (A1 to A10) and 36 markers from the eight B-genome linkage groups (B1 to B8) were used. Details of DNA extraction and PCR amplification of the SSR markers is described elsewhere (Hasan and Rahman 2016).

### *Chromosome doubling and generation of resynthesized B. juncea lines*

The $S_0$ plantlets identified as *B. rapa* × *B. nigra* interspecific hybrid were treated with 0.34% (w/v) aqueous solution of colchicine for chromosome doubling. The chromosome-doubled fertile $S_0$ plants were self-pollinated using 5% NaCl solution (Tingdong *et al.* 1992) for $S_1$ seeds. The $S_1$ families were grown in a glasshouse and were self-pollinated by bag isolation for $S_2$ seeds.

### *Assessment of the ploidy level*

The ploidy level of the $S_2$ generation resynthesized *B. juncea* lines and their diploid parents were determined through flow cytometric analysis of nuclear DNA content using a Partec CyFlow® Ploidy Analyzer (www.partec.com). The ultra-violet (UV) light of the instrument was set at 365 nm wavelength and the samples were run at a rate of 20 to 50 nuclei/sec. Data was acquired for 1500 to 2500 nuclei per sample. One canola quality *B. juncea* breeding line from the Canola Breeding Program of the University of Alberta was used as the reference. The ploidy level of the samples was calculated by using the following equation (Dolezel *et al.* 2007);

$$\text{Sample ploidy (integer)} = \frac{\text{(Mean position of the G1 sample peak)}}{\text{(Mean position of the G1 reference peak)}} \times \text{Reference ploidy}$$

### *Evaluation for clubroot resistance*

The $S_1$ and $S_2$ generation resynthesized *B. juncea* lines and their diploid parents were evaluated for resistance to the single-spore derived *P. brassicae* isolate, classified as pathotype 3 based on Williams (1966) differentials. Resting spore suspension (inoculums) was prepared from preserved galls following the protocol described by Strelkov *et al.* (2007). The details of inoculation and screening of the inoculated plants for resistance is described in Hasan and Rahman (2016).

## Results

### *Production of resynthesized Brassica juncea*

A total of 43 interspecific crosses were made which gave 14 silique carrying fertilized ovules (developed to normal size) (**Table 1**). Five siliques of the *B. rapa* cv. Gelria × *B. nigra* CR 2136 cross yielded 34 fertilized ovules, and this translated to 6.8 fertilized ovules/silique. Fifteen (44.1%) of the 34 cultured ovules yielded zygotic embryos of which 13 (38.2%) grew into plantlets. On the other hand, nine siliques of the *B. rapa* cv. Gelria × *B. nigra* CR 2137 cross yielded 56 ovules translating to 6.2 fertilized ovules/silique; only 21 (37.5%) of the 56 ovules yielded zygotic embryos of which 17 (30.4%) developed into plant. All 30 plantlets obtained from the two crosses were treated with colchicine, however, only two (6.67%) plants of *B. rapa* cv. Gelria × *B. nigra* CR 2137 became amphidiploid (AABB). These plants produced fertile pollen and viable seed under self-pollination (**Table 1**). Single silique from each of the two $S_0$ plants, 1578.001 and 1578.002, produced 13 and seven $S_1$ seeds, respectively. A total of eight and seven $S_1$ plants, respectively, of 1578.001 and 1578.002 were grown in a glasshouse of which three of 1578.001 and four of 1578.002 were self-pollinated by bag isolation for $S_2$ seeds.

### *Molecular characterization of the resynthesized B. juncea lines*

Interspecific nature of the $S_0$ plants was confirmed using SSR markers. For this, 190 SSR markers from the 10 A-genome chromosomes (A1 to A10) were screened of which

**Table 1.** Resynthesis of *Brassica juncea* (AABB, 2*n* = 36) though *in vitro* culture of ovules of *Brassica rapa* (AA, 2*n* = 20) × *Brassica nigra* (BB, 2*n* = 16) interspecific cross

| Female | Male | No. pollination | No. silique formed | No. ovule cultured | No. zygotic embryo developed | No. plants transferred | No. resynthesized plants obtained | Plant ID |
|---|---|---|---|---|---|---|---|---|
| *B. rapa* ssp. *rapifera* cv. Gelria, p1 | *B. nigra* (CR 2136), p1 | 9 | 3 | 20 | 9 | 9 | 0 | |
| *B. rapa* ssp. *rapifera* cv. Gelria, p3 | *B. nigra* (CR 2136), p3 | 8 | 2 | 14 | 6 | 4 | 0 | |
| *B. rapa* ssp. *rapifera* cv. Gelria, p1 | *B. nigra* (CR 2137), p1 | 11 | 5 | 26 | 13 | 11 | 1 | 1578.001 |
| *B. rapa* ssp. *rapifera* cv. Gelria, p1 | *B. nigra* (CR 2137), p2 | 15 | 4 | 30 | 8 | 6 | 1 | 1578.002 |
| Total | | 43 | 14 | 90 | 36 | 30 | 2 | |

29 showed clear polymorphism between the A and B genome parental species (**Table 2**). Of the 29 polymorphic markers, 15 amplified the expected alleles in *B. rapa* but showed no amplification product in *B. nigra*; these 15 markers also amplified similar size alleles in the resynthesized *B. juncea* plants. The other 14 markers amplified alleles both in *B. rapa* and *B. nigra*, and similar size alleles were also detected in the resynthesized *B. juncea* plants. Based on this marker analysis, it can be anticipated that all 10 A-genome chromosomes of *B. rapa* were present in the resynthesized *B. juncea* plants.

A total of 48 B-genome specific (chromosome B1 to B8) SSR markers were tested on the two parents; 36 of them amplified alleles only in *B. nigra* (**Table 3**) and these alleles were also detected in the resynthesized *B. juncea* lines. This marker analysis confirmed the presence of all eight B genome chromosomes of *B. nigra* in the resynthesized *B. juncea* lines.

### *Ploidy assessment of the resynthesized B. juncea plants*

A total of 36 plants belonging to seven $S_2$ families were analyzed for nuclear DNA content to determine their ploidy level. Of the seven $S_2$ families, three derived from the $S_1$ line 1578.001 showed a mean ploidy level of 4.10 ± 0.218, which is similar to the natural *B. juncea* (**Table 4**). On the other hand, mean ploidy level of the four $S_2$ families, derived from the $S_1$ line 1578.002, was 4.44 ± 0.119 indicating the occurrence of plants with greater chromosome number in this population.

### *Resistance to Plasmodiophora brassicae*

A total of 15 $S_1$ plants derived from the two resynthesized *B. juncea* lines (1578.001 and 1578.002) were evaluated for resistance to *P. brassicae* pathotype 3. All 15 plants were completely resistant to this pathotype (disease score 0). Seven of the 15 $S_1$ plants were self-pollinated by bag isolation for $S_2$ seeds. The $S_1$ plants showed wide variation for seed set—ranging from as low as 30 seeds per plant to as high as 515 seeds per plant.

A total of 103 plants belonging to seven $S_2$ families were evaluated for resistance to pathotype 3. All $S_2$ plants belonging to three $S_2$ families, 1578.003, 1578.005 and 1578.008 which derived from the $S_1$ family 1578.001, were resistant. On the other hand, 87 to 94% $S_2$ plants belonging to four $S_2$ families, 1578.004, 1578.006, 1578.007 and 1578.009 which derived from the $S_1$ family 1578.002, were resistant to this pathotype; thus, resistance was lost in about 6 to 13% of the $S_2$ plants of these four families during their development

**Table 2.** Evaluation of the resynthesized *Brassica juncea* lines by SSR (microsatellite) markers from the ten A genome linkage groups including those are specific to the A genome of *Brassica rapa*

| Linkage group (LG) | Total no. marker tested | No. marker polymorphic between diploid parents | Primer name | Amplified allele size (bp) in | | |
|---|---|---|---|---|---|---|
| | | | | *B. rapa* ssp. *rapifera* cv. Gelria (AA genome) | *B. nigra* (CR2137) (BB genome) | Resynthesized *B. juncea* (AABB genome) |
| A1 | 22 | 4 | sNRA51nm | 198 | – | 198 |
| | | | sS2136b | 123 | 138 | 123, 138 |
| | | | sN11665 | 276 | 272 | 272, 276 |
| | | | sN11824 (aNP) | 384 | – | 384 |
| A2 | 24 | 3 | sR12095 | 349 | – | 351 |
| | | | sORE27 (aNP) | 213, 239 | 239 | 213, 239 |
| | | | BrSTS-78 | 158 | 162 | 158, 162 |
| A3 | 15 | 3 | sNRA85 | 133 | 162 | 133, 162 |
| | | | sN1087(cNP) | 471 | – | 471 |
| | | | BoGMS1587 | 282 | – | 288 |
| A4 | 31 | 2 | sN2025 | 155 | 138 | 138, 155 |
| | | | Na12-A01C | 135 | – | 135 |
| A5 | 15 | 3 | Na10E02 | 155 | – | 155 |
| | | | CB10080 | 133, 140 | 146 | 140, 146 |
| | | | CB10545 | 96 | – | 96 |
| A6 | 26 | 4 | sN12508II | 324 | 334 | 324, 334 |
| | | | sR12156 | 198 | – | 198 |
| | | | sN1958 (bNM) | 365 | 361 | 365 |
| | | | sN0904 (a) | 234, 247, 255 | 255 | 234, 247, 255 |
| A7 | 14 | 3 | BRAS023 | 207, 217 | – | 207, 217 |
| | | | BnGMS608 | 158 | – | 156 |
| | | | BRMS129 | 276, 295 | 276, 284 | 276, 295 |
| A8 | 12 | 2 | Na12B05a | 191 | – | 191 |
| | | | BRMS185 | 254 | – | 254 |
| A9 | 14 | 3 | CB10373A | 245 | 257 | 245, 257 |
| | | | Ni4-D09 | 209 | 203 | 203, 209 |
| | | | BnGMS81 | 397 | – | 397 |
| A10 | 17 | 2 | CB10524 | 239 | – | 239 |
| | | | BRMS244 | 268 | 252 | 252, 268 |
| Total | 190 | 29 | | | | |

**Table 3.** Evaluation of the resynthesized *Brassica juncea* plants by SSR (microsatellite) markers from the eight B-genome linkage groups

| Linkage group (LG) | No. marker tested | No. markers polymorphic between parents | Primer name | Allele size (bp) in | | |
|---|---|---|---|---|---|---|
| | | | | *B. rapa* ssp. *rapifera* cv. Gelria (AA genome) | *B. nigra* (CR2137) (BB genome) | Resynthesized *B. juncea* (AABB genome) |
| B1 | 6 | 6 | sJ3838F | – | 289 | 289 |
| | | | sJ4933 | – | 360 | 360 |
| | | | sJ84165 | – | 307 | 307 |
| | | | sJ0644 | – | 457 | 457 |
| | | | sJ3891 | – | 123 | 123 |
| | | | sB0563I | – | 459 | 459 |
| B2 | 6 | 3 | sJ3302RI | – | 433 | 420 |
| | | | sJ03104 | – | 405 | 405 |
| | | | sB4817R | – | 270 | 270 |
| B3 | 6 | 6 | sJ3627R | – | 308 | 308 |
| | | | sB1822 | – | 282 | 282 |
| | | | sB1672 | – | 208 | 208 |
| | | | sJ7046 | – | 304 | 304 |
| | | | sB1990F | – | 511 | 511 |
| | | | sB1752 | – | 450 | 450 |
| B4 | 6 | 5 | sA0306 | – | 382 | 351, 382 |
| | | | sB0372 | – | 255 | 255 |
| | | | sB2141AI | – | 401 | 401 |
| | | | sB1935A | – | 275 | 275 |
| | | | sJ8033 | – | 167 | 167 |
| B5 | 6 | 5 | sB3140 | – | 231 | 231 |
| | | | sJ3874I | – | 184 | 184 |
| | | | sJ6842 | – | 355 | 355 |
| | | | sB2556 | – | 268 | 268 |
| | | | sB3872 | – | 197 | 197 |
| B6 | 6 | 3 | sJ7104 | – | 346 | 346 |
| | | | sJ0338 | – | 359 | 359 |
| | | | sJ0502 | – | 268 | 268 |
| B7 | 6 | 5 | sJ39119I | – | 366 | 366 |
| | | | sJ13133 | – | 317 | 317 |
| | | | sJ1536 | – | 231 | 231 |
| | | | sB1937 | – | 280 | 280 |
| | | | sJ4633 | – | 328 | 328 |
| B8 | 6 | 3 | sJ34121 | – | 359 | 359 |
| | | | sJ1668I | – | 325 | 325 |
| | | | sB3739 | – | 397 | 397 |
| Total | 48 | 36 | | | | |

**Table 4.** Ploidy level of the 36 $S_2$ generation resynthesized *Brassica juncea* plants measured through estimation of nuclear DNA content using a flow cytometer

| Family ID | Generation | No. plants tested | Ploidy (Mean ± SE) |
|---|---|---|---|
| *Brassica juncea*[a] | Inbred | 5 | 4.00 ± 0.083 |
| $S_2$ derived from 1578.001 ($S_1$) | | | |
| 1578.003 | $S_2$ | 5 | 4.86 ± 0.254 |
| 1578.005 | $S_2$ | 4 | 3.41 ± 0.185 |
| 1578.008 | $S_2$ | 4 | 3.85 ± 0.222 |
| Sub total | | 13 | 4.10 ± 0.218 |
| $S_2$ derived from 1578.002 ($S_1$) | | | |
| 1578.004 | $S_2$ | 6 | 4.46 ± 0.069 |
| 1578.006 | $S_2$ | 6 | 3.93 ± 0.372 |
| 1578.007 | $S_2$ | 4 | 4.77 ± 0.059 |
| 1578.009 | $S_2$ | 7 | 4.69 ± 0.097 |
| Sub total | | 23 | 4.44 ± 0.119 |

[a] Canola quality *Brassica juncea* breeding line from University of Alberta Canola breeding program.

through self-pollination (**Table 5**). No significant correlation between seed set on the $S_1$ plants and clubroot resistance in the $S_2$ families could be found ($r = -0.523$, $R^2 = 0.274$; $df = 5$, $p < 0.05$).

## Discussion

The present study demonstrated that a clubroot resistant *B. juncea* line in the $S_2$ generation could be achieved through resynthesis of this species by exploiting the resistance available in one of the parental species, *B. rapa*. The allopolyploid resynthesized *B. juncea* lines, theoretically, were assumed to be homozygous and the resistance was expected to be inherited in a stable manner through the self-pollinated generation; however, loss of resistance occurred in some of the $S_2$ plants that obtained from these experiments (**Table 5**). Several researchers have reported that chromosomes in the resynthesized *Brassica* allopolyploids can undergo meiotic anomalies and homoeologous pairing

**Table 5.** Resistance in $S_1$ and $S_2$ generation plants of resynthesized *Brassica juncea* to *Plasmodiophora brassicae* pathotype 3

| Family ID | Generation | No. selfed seed produced | No. plants tested | No. R plant (Score 0) | Number of S plant | | | | Percent resistant plant |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Score 1 | Score 2 | Score 3 | Total S plant | |
| 1578.001 | $S_1$ | 13 | 8 | 8 | 0 | 0 | 0 | 0 | 100.0 |
| 1578.002 | $S_1$ | 7 | 7 | 7 | 0 | 0 | 0 | 0 | 100.0 |
| Sub total | | | 15 | 15 | | | | 0 | 100.0 |
| $S_2$ derived from 1578.001 | | | | | | | | | |
| 1578.003 | $S_2$ | 42 | 9 | 9 | 0 | 0 | 0 | 0 | 100.0 |
| 1578.005 | $S_2$ | 208 | 8 | 8 | 0 | 0 | 0 | 0 | 100.0 |
| 1578.008 | $S_2$ | 54 | 29 | 29 | 0 | 0 | 0 | 0 | 100.0 |
| Sub total | | | 46 | 46 | | | | 0 | 100.0 |
| $S_2$ derived from 1578.002 | | | | | | | | | |
| 1578.004 | $S_2$ | 118 | 18 | 17 | 0 | 0 | 1 | 1 | 94.4 |
| 1578.006 | $S_2$ | 215 | 15 | 13 | 0 | 0 | 2 | 2 | 86.7 |
| 1578.007 | $S_2$ | 30 | 9 | 8 | 0 | 0 | 1 | 1 | 88.9 |
| 1578.009 | $S_2$ | 515 | 15 | 13 | 0 | 0 | 2 | 2 | 86.7 |
| Sub total | | | 57 | 51 | | | | 6 | 89.5 |
| Grand Total | $S_2$ | | 103 | 97 | | | | 6 | 94.2 |

Note: R = Resistant; S = Susceptible.

in their early generations, and this can result in some structural rearrangements including loss or gain of chromosomes (Gaeta *et al.* 2007, Gaeta and Pires 2010, Szadkowski *et al.* 2010, Udall *et al.* 2005, Xiong *et al.* 2011). The mechanisms driving the change in chromosome number and structure in the newly formed polyploid is not well understood; this may result from downsizing of nuclear DNA content, inter- and intra-genomic rearrangements, chromosome breakage and fusion, rDNA change, and loss of repeat sequences (Han *et al.* 2005, Leitch and Bennett 2004, Liu *et al.* 1998, Renny-Byfield *et al.* 2013, Xiong *et al.* 2011, for review, see Renny-Byfield and Wendel 2014). According to Xiong *et al.* (2011), chromosome number in self-pollinated progeny of a resynthesized *B. napus* ($2n = 38$) plant can vary from $2n = 36$ to 42; in this regard, the occurrence of greater nuclear DNA content in $S_2$ progeny of the resynthesized *B. juncea* plant 1578.002 agree with the result reported by Xiong *et al.* (2011). In addition to chromosomal change, allopolyploids can also exhibit a change in gene expression (reviewed by Adams and Wendel 2005, Chen and Ni 2006) which can cause a change in the phenotype. Salmon *et al.* (2005) found DNA methylation in about 30% of the parental fragments in the allopolyploids of *Spartina* spp. Structural rearrangement of chromosomes in resynthesized *B. napus* can also contribute to the variation of a quantitative trait, such as flowering time (Pires *et al.* 2004). In case of qualitative traits, such as self-incompatibility (Rahman 2005) and clubroot resistance (Diederichsen and Sacristan 1996), stability of the trait has often been seen in self-pollinated progeny of a resynthesized *B. napus* plant. The clubroot resistance in the resynthesized *B. juncea* lines developed in this research is derived from the *B. rapa* cv. Gelria. This cultivar reported to carry the major clubroot resistance gene *CRa/CRb*; however, the reason of the loss of resistance in some of the $S_2$ plants was beyond the scope of the present study. The loss of resistance might have resulted from the loss of the genomic region carrying the resistance; further investigation would be needed to resolve this.

The resynthesized *B. juncea* lines obtained in this study showed wide variation for seed set under self-pollination. Poor seed set in a resynthesized allopolyploid is a common phenomenon, especially in their early generations, as reported by Srivastava *et al.* (2004) in *B. juncea*. Meiotic anomalies in the resynthesized *Brassica* allopolyploids, as discussed above, can result in reduced pollen viability and thus poor seed set (Ramsey and Schemske 2002). Xiong *et al.* (2011) found an inverse correlation of seed yield and pollen viability with the increased aneuploidy; they observed the highest fertility in the resynthesized *B. napus* lines carrying the parental chromosomes with least change. Self-incompatibility of the parental species may also have contributed to this reduced seed set under self-pollination in the resynthesized *B. juncea* lines developed in this study. Rahman (2005) also reported the effect of the self-incompatibility genes on reduced seed set in resynthesized *B. napus*.

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## Literature Cited

Adams, K.L. and J.F. Wendel (2005) Polyploidy and genome evolution in plants. Curr. Opin. Plant Biol. 8: 135–141.

Bennett, R., M. Thiagarajah, J. King and H. Rahman (2008) Interspecific cross of *Brassica oleracea* var. *alboglabra* and *B. napus*: Effects of growth condition and silique age on the efficiency of hybrid production, and inheritance of erucic acid in the self-pollinated backcross generation. Euphytica 164: 593–601.

Buczacki, S., H. Toxopeus, P. Mattusch, T.D. Dixon and L.A. Hobolth (1975) Study of physiologic specialization in *Plasmodiophora brassicae*: Proposals for attempted rationalization through an international approach. Trans. Br. Mycol. Soc. 65: 295–303.

Burton, W., S. Pymer, P. Salisbury, J.T.O. Krik and R.N. Oram (1999) Performance of Australian canola quality *Brassica juncea* lines. *In*: Proceedings of 10th International Rapeseed Congress, pp. 2–7.

Chen, Z.J. and Z. Ni (2006) Mechanisms of genomic rearrangements and gene expression changes in plant polyploids. Bioessays 28: 240–252.

Coventry, J., L. Kott and W.D. Beversdorf (1988) Manual for microspore culture technique for *Brassica napus*. Department of Crop Science Technical Bulletin, University of Guelph, OAC Publication Vol. 489.

Diederichsen, E. and M. Sacristan (1996) Disease response of resynthesized *Brassica napus* L. lines carrying different combinations of resistance to *Plasmodiophora brassicae* Wor. Plant Breed. 115: 5–10.

Dolezel, J., J. Greilhuber and J. Suda (2007) Estimation of nuclear DNA content in plants using flow cytometry. Nat. Protoc. 2: 2233–2244.

Gaeta, R.T., J.C. Pires, F. Iniguez-Luy, E. Leon and T.C. Osborn (2007) Genomic changes in resynthesized *Brassica napus* and their effect on gene expression and phenotype. Plant Cell 19: 3403–3417.

Gaeta, R.T. and J.C. Pires (2010) Homoeologous recombination in allopolyploids: the polyploid ratchet. New Phytol. 186: 18–28.

Gunasekera, C.P., L.D. Martin, K.H.M. Siddique and G.H. Walton (2006) Genotype by environment interactions of Indian mustard (*Brassica juncea* L.) and canola (*Brassica napus* L.) in Mediterranean-type environments: II. Oil and protein concentrations in seed. Eur. J. Agron. 25: 13–21.

Han, F., G. Fedak, W. Guo and B. Liu (2005) Rapid and repeatable elimination of a parental genome-specific DNA repeat (pGc1R-1a) in newly synthesized wheat allopolyploids. Genetics 170: 1239–1245.

Hasan, M., S.E. Strelkov, R.J. Howard and H. Rahman (2012) Screening of *Brassica* germplasm for resistance to *Plasmodiophora brassicae* pathotypes prevalent in Canada for broadening diversity in clubroot resistance. Can. J. Plant Sci. 92: 501–515.

Hasan, M.J. and H. Rahman (2016) Genetics and molecular mapping of resistance to *Plasmodiophora brassicae* pathotypes 2, 3, 5, 6, and 8 in rutabaga (*Brassica napus* var. *napobrassica*). Genome 59: 805–815.

Hatakeyama, K., T. Niwa, T. Kato, T. Ohara, T. Kakizaki and S. Matsumoto (2017) The tandem repeated organization of NB-LRR genes in the clubroot-resistant *CRb* locus in *Brassica rapa* L. Mol. Genet. Genomics 292: 397–405.

Kato, T., K. Hatakeyama, N. Fukino and S. Matsumoto (2013) Fine mapping of the clubroot resistance gene *CRb* and development of a useful selectable marker in *Brassica rapa*. Breed. Sci. 63: 116–126.

Leitch, I. and M. Bennett (2004) Genome downsizing in polyploid plants. Biol. J. Linn. Soc. 82: 651–663.

Liu, B., J.M. Vega, G. Segal and L. Bao (1998) Rapid genomic changes in newly synthesized amphiploids of *Triticum* and *Aegilops*. I. Changes in low-copy noncoding DNA sequences. Genome 41: 272–277.

Matsumoto, E., C. Yasui, M. Ohi and M. Tsukada (1998) Linkage analysis of RFLP markers for clubroot resistance and pigmentation in Chinese cabbage. Euphytica 104: 79–86.

Pageau, D., J. Lajeunesse and J. Lafond (2006) Impact de l'hernie des crucifères [*Plasmodiophora brassicae*] sur la productivité et la qualité du canola. Can. J. Plant Pathol. 28: 137–143.

Piao, Z., N. Ramchiary and Y.P. Lim (2009) Genetics of clubroot resistance in *Brassica* species. J. Plant Growth Regul. 28: 252–264.

Piao, Z.Y., Y.Q. Deng, S.R. Choi, Y.J. Park and Y.P. Lim (2004) SCAR and CAPS mapping of *CRb*, a gene conferring resistance to *Plasmodiophora brassicae* in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). Theor. Appl. Genet. 108: 1458–1465.

Pires, J.C., J. Zhao, M.E. Schranz, E.J. Leon, P.A. Quijada, L.N. Lukens and T.C. Osborn (2004) Flowering time divergence and genomic rearrangements in resynthesized *Brassica* polyploids (Brassicaceae). Biol. J. Linn. Soc. 82: 675–688.

Potts, D.A., G.W. Rakow and D.R. Males (1999) Canola quality *Brassica juncea*, a new oilseed crop for the Canadian prairies. *In*: Proceedings of 10th International Rapeseed Congress, Canberra, Australia.

Rahman, M.H. (2005) Resynthesis of *Brassica napus* L. for self-incompatibility: Self-incompatibility reaction, inheritance and breeding potential. Plant Breed. 124: 13–19.

Ramsey, J. and D.W. Schemske (2002) Neopolyploidy in flowering plants. Annu. Rev. Ecol. Syst. 33: 589–639.

Renny-Byfield, S., A. Kovarik, L.J. Kelly, J. Macas, P. Novak, M.W. Chase, R.A. Nicols, M.R. Pancholi, M.-A. Grandbastien and A.R. Leitch (2013) Diploidization and genome size change in allopolyploids is associated with differential dynamics of low- and high-copy sequences. Plant J. 74: 829–839.

Renny-Byfield, S. and J.F. Wendel (2014) Doubling down on genomes: Polyploidy and crop plants. Am. J. Bot. 101: 1711–1725.

Roy, N.N. (1978) A study on disease variation in the populations of an interspecific cross of *Brassica juncea* L. × *B. napus* L. Euphytica 27: 145–149.

Salmon, A., M.L. Ainouche and J.F. Wendel (2005) Genetic and epigenetic consequences of recent hybridization and polyploidy in *Spartina* (Poaceae). Mol. Ecol. 14: 1163–1175.

Srivastava, A., A. Mukhopadhyay, N. Arumugam, V. Gupta, J.K. Verma, D. Pental and A.K. Pradhan (2004) Resynthesis of *Brassica juncea* through interspecific crosses between *B. rapa* and *B. nigra*. Plant Breed. 123: 204–206.

Strelkov, S.E., V.P. Manolii, T. Cao, S. Xue and S.F. Hwang (2007) Pathotype classification of *Plasmodiophora brassicae* and its occurrence in *Brassica napus* in Alberta, Canada. J. Phytopathol. 155: 706–712.

Szadkowski, E., F. Eber, V. Huteau, M. Lode, C. Huneau, H. Belcram, O. Coriton, M.J. Manzanares-Dauleux, R. Delourme, G.J. King *et al.* (2010) The first meiosis of resynthesized *Brassica napus*, a genome blender. New Phytol. 186: 102–112.

Tingdong, F., S. Ping, Y. Xiaoniu and Y. Guangsheng (1992) Overcoming self-incompatibility of *Brassica napus* by salt (NaC1) spray. Plant Breed. 109: 255–258.

Udall, J.A., P.A. Quijada and T.C. Osborn (2005) Detection of chromosomal rearrangements derived from homeologous recombination in four mapping populations of *Brassica napus* L. Genetics 169: 967–979.

Ueno, H., E. Matsumoto, D. Aruga, S. Kitagawa, H. Matsumura and N. Hayashida (2012) Molecular characterization of the *CRa* gene conferring clubroot resistance in *Brassica rapa*. Plant Mol. Biol. 80: 621–629.

Wang, R., V.L. Ripley and G. Rakow (2007) Pod shatter resistance evaluation in cultivars and breeding lines of *Brassica napus*, *B. juncea* and *Sinapis alba*. Plant Breed. 126: 588–595.

Wechter, W.P., M.W. Farnham, J.P. Smith and A.P. Keinath (2007) Identification of resistance to peppery leaf spot among *Brassica juncea* and *Brassica rapa* plant introductions. HortScience 42: 1140–1143.

Williams, P. (1966) A system for the determination of races of *Plasmodiophora brassicae* that infect cabbage and rutabaga. Phytopathology 56: 624–626.

Wright, P.R., J.M. Morgan, R.S. Jessop and A. Cass (1995) Comparative adaptation of canola (*Brassica napus*) and Indian mustard (*B. juncea*) to soil water deficits: Yield and yield components. Field Crops Res. 42: 1–13.

Xiong, Z., R.T. Gaeta and J.C. Pires (2011) Homoeologous shuffling and chromosome compensation maintain genome balance in resynthesized allopolyploid *Brassica napus*. Proc. Natl. Acad. Sci. USA 108: 7908–7913.