The oilseed crop *Brassica juncea* carries many desired 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.

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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 S₀ 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 S₀ plants were self-pollinated using 5% NaCl solution (Tingdong et al. 1992) for S₁ seeds. The S₁ families were grown in a glasshouse and were self-pollinated by bag isolation for S₂ seeds.

Assessment of the ploidy level

The ploidy level of the S₂ 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 S₁ and S₂ 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 S₀ plants, 1578.001 and 1578.002, produced 13 and seven S₁ seeds, respectively. A total of eight and seven S₁ 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 S₂ seeds.

Molecular characterization of the resynthesized B. juncea lines

Interspecific nature of the S₀ plants was confirmed using SSR markers. For this, 190 SSR markers from the 10 A-genome chromosomes (A1 to A10) were screened of which

| 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), p₁ | 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, p₂ | B. nigra (CR 2137), p₂ | 15 | 4 | 30 | 8 | 6 | 1 | 1578.002 |

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

### Resistance to Plasmodiophora brassicae

A total of 15 S1 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 S1 plants were self-pollinated by bag isolation for S2 seeds. The S1 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 S2 families were evaluated for resistance to pathotype 3. All S2 plants belonging to three S2 families, 1578.003, 1578.005 and 1578.008 which derived from the S1 family 1578.001, were resistant. On the other hand, 87 to 94% S2 plants belonging to four S2 families, 1578.004, 1578.006, 1578.007 and 1578.009 which derived from the S1 family 1578.002, were resistant to this pathotype; thus, resistance was lost in about 6 to 13% of the S2 plants of these four families during their development.

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

A total of 36 plants belonging to seven S2 families were analyzed for nuclear DNA content to determine their ploidy level. Of the seven S2 families, three derived from the S1 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 S2 families, derived from the S1 line 1578.002, was 4.44 ± 0.119 indicating the occurrence of plants with greater chromosome number in this population.

### 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                                         | –                             | 198                                         |
|                   |                         |                                             | s2136b      | 123                         | 138                                         | 123, 138                      | 123, 138                      |
|                   |                         |                                             | sN11665     | 276                         | 272                                         | 272, 276                      |                                |
|                   |                         |                                             | sN11824 (aNP)| 384                         | –                                           | 384                           |                                |
|                   |                         |                                             | sR12095     | 349                         | 351                                         |                               |                                |
|                   |                         |                                             | sORE27 (aNP)| 213, 239                    | 239                                         | 213, 239                      |                                |
|                   |                         |                                             | BrSTS-78    | 158                         | 162                                         | 158, 162                      |                                |
|                   |                         |                                             | sNRA85      | 133                         | 162                                         | 133, 162                      |                                |
|                   |                         |                                             | sN1087(cNP) | 471                         | –                                           | 471                           |                                |
|                   |                         |                                             | BoGMS1587   | 282                         | –                                           | 288                           |                                |
|                   |                         |                                             | sN2025      | 155                         | 138                                         | 138, 155                      |                                |
|                   |                         |                                             | Na12-A01C   | 135                         | –                                           | 135                           |                                |
|                   |                         |                                             | Na10E02     | 155                         | –                                           | 155                           |                                |
|                   |                         |                                             | CB10080     | 133, 140                    | 146                                         | 140, 146                      |                                |
|                   |                         |                                             | CB10545     | 96                          | –                                           | 96                            |                                |
|                   |                         |                                             | sN12508II   | 324                         | 334                                         | 324, 334                      |                                |
|                   |                         |                                             | sR12156     | 198                         | –                                           | 198                           |                                |
|                   |                         |                                             | sN1958 (bNM)| 365                         | 361                                         | 365                           |                                |
|                   |                         |                                             | sN0904 (a)  | 234, 247, 255               | 255                                         | 234, 247, 255                 |                                |
|                   |                         |                                             | BRAS023     | 207, 217                    | –                                           | 207, 217                      |                                |
|                   |                         |                                             | BnGMS608    | 276, 295                    | 276, 284                                    | 276, 295                      |                                |
|                   |                         |                                             | BRMS129     | 191                         | –                                           | 191                           |                                |
|                   |                         |                                             | BRMS185     | 254                         | –                                           | 254                           |                                |
|                   |                         |                                             | CB10373A    | 245                         | 257                                         | 245, 257                      |                                |
|                   |                         |                                             | Ni4-D09     | 209                         | 203                                         | 203, 209                      |                                |
|                   |                         |                                             | BnGMS81     | 397                         | –                                           | 397                           |                                |
|                   |                         |                                             | CB10524     | 239                         | –                                           | 239                           |                                |
|                   |                         |                                             | BRMS244     | 268                         | 252                                         | 252, 268                      |                                |
| Total             | 190                     | 29                                          |             |                             |                                             |                               |                               |
through self-pollination (Table 5). No significant correlation between seed set on the S1 plants and clubroot resistance in the S2 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 S2 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 S2 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 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               |
|                    |                   |                                        | sB4817R     | 405               | 405               |
|                    |                   |                                        |             | 270               | 270               |
| B2                 | 6                 | 3                                      | sJ3302RI    | 433               | 420               |
|                    |                   |                                        | sJ03104     | 405               | 405               |
|                    |                   |                                        | sB4817R     | 270               | 270               |
| B3                 | 6                 | 6                                      | sJ3627R     | 308               | 308               |
|                    |                   |                                        | sB1822      | 282               | 282               |
|                    |                   |                                        | sJ1672      | 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               |
|                    |                   |                                        | sB3140      | 231               | 231               |
|                    |                   |                                        | sJ3874I     | 184               | 184               |
|                    |                   |                                        | sJ6842      | 355               | 355               |
|                    |                   |                                        | sB2556      | 268               | 268               |
|                    |                   |                                        | sB3872      | 197               | 197               |
| B5                 | 6                 | 5                                      | sJ7104      | 346               | 346               |
|                    |                   |                                        | sJ0338      | 359               | 359               |
|                    |                   |                                        | sJ0502      | 268               | 268               |
|                    |                   |                                        | sJ39119I    | 366               | 366               |
|                    |                   |                                        | sJ13133     | 317               | 317               |
|                    |                   |                                        | sJ1536      | 231               | 231               |
|                    |                   |                                        | sB1937      | 280               | 280               |
|                    |                   |                                        | sJ4633      | 328               | 328               |
| B6                 | 6                 | 3                                      | sJ7104      | 346               | 346               |
|                    |                   |                                        | sJ0338      | 359               | 359               |
|                    |                   |                                        | sJ0502      | 268               | 268               |
|                    |                   |                                        | sJ39119I    | 366               | 366               |
|                    |                   |                                        | sJ13133     | 317               | 317               |
|                    |                   |                                        | sJ1536      | 231               | 231               |
|                    |                   |                                        | sB1937      | 280               | 280               |
|                    |                   |                                        | sJ4633      | 328               | 328               |
| B7                 | 6                 | 5                                      | sJ7104      | 346               | 346               |
|                    |                   |                                        | sJ0338      | 359               | 359               |
|                    |                   |                                        | sJ0502      | 268               | 268               |
|                    |                   |                                        | 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               |

**Table 4.** Ploidy level of the 36 S2 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* | Inbred | 5 | 4.00 ± 0.083 |
| S2 derived from 1578.001 (S1) | 1578.003 | S2 | 5 | 4.86 ± 0.254 |
| | 1578.005 | S2 | 4 | 3.41 ± 0.185 |
| | 1578.008 | S2 | 4 | 3.85 ± 0.222 |
| | Sub total | 13 | 4.10 ± 0.218 |
| S2 derived from 1578.002 (S1) | 1578.004 | S2 | 6 | 4.46 ± 0.069 |
| | 1578.006 | S2 | 6 | 3.93 ± 0.372 |
| | 1578.007 | S2 | 4 | 4.77 ± 0.059 |
| | 1578.009 | S2 | 7 | 4.69 ± 0.097 |
| | Sub total | 23 | 4.44 ± 0.119 |

*Canola quality *Brassica juncea* breeding line from University of Alberta Canola breeding program.
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 \( B. juncea \) \( (2n = 38) \) 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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