Breeding Science 62: 328–333 (2012)
doi:10.1270/jsbbs.62.328

Persistent C genome chromosome regions identified by SSR analysis in backcross progenies between *Brassica juncea* and *B. napus*

Mai Tsuda[1)], Ayako Okuzaki[1)], Yukio Kaneko[2)] and Yutaka Tabei*[1)]

[1)] *Genetically Modified Organism Research Center, National Institute of Agrobiological Sciences (NIAS)*, 2-1-2 Kan-nondai, Tsukuba, Ibaraki 305-8602, Japan

[2)] *Laboratory of Plant Breeding, Faculty of Agriculture, Utsunomiya University*, 350 Minemachi, Utsunomiya, Tochigi 321-8505, Japan

Given that feral transgenic canola (*Brassica napus*) from spilled seeds has been found outside of farmer's fields and that *B. juncea* is distributed worldwide, it is possible that introgression to *B. juncea* from *B. napus* has occurred. To investigate such introgression, we characterized the persistence of *B. napus* C genome chromosome (C-chromosome) regions in backcross progenies by *B. napus* C-chromosome specific simple sequence repeat (SSR) markers. We produced backcross progenies from *B. juncea* and F_1 hybrid of *B. juncea* × *B. napus* to evaluate persistence of C-chromosome region, and screened 83 markers from a set of reported C-chromosome specific SSR markers. Eighty-five percent of the SSR markers were deleted in the BC_1 obtained from *B. juncea* × F_1 hybrid, and this BC_1 exhibited a plant type like that of *B. juncea*. Most markers were deleted in BC_2 and BC_3 plants, with only two markers persisting in the BC_3. These results indicate a small possibility of persistence of C-chromosome regions in our backcross progenies. Knowledge about the persistence of *B. napus* C-chromosome regions in backcross progenies may contribute to shed light on gene introgression.

Key Words: *Brassica napus*, *Brassica juncea*, introgression, backcross progenies, SSR marker, transgenic canola, C genome chromosome.

Introduction

Transgenic canola (*B. napus*, AACC, 2n = 38) is cultivated in Canada, Australia, Chile and the USA and the cultivation area has expanded year by year (James 2011). Because transgenic canola plants derived from spilled seeds have been observed growing along roadsides and in vacant and other spaces in Canada (Yoshimura *et al.* 2006), Japan (Aono *et al.* 2011, Mizuguti *et al.* 2011) and other countries (Claessen *et al.* 2005a, 2005b), the potential of introgression from transgenic canola into wild relatives has aroused public concern and led to worldwide debate (Aono *et al.* 2011, Wei *et al.* 2005, Wilkinson and Tepfer 2009).

B. juncea (AABB, 2n = 36) is cultivated and is also found as a weed and feral plant in Japan (Shimizu *et al.* 2003), Asian countries including China (Di *et al.* 2009), Europe (Hultén and Fries 1986), Australia (OGTR 2011), Canada and the USA (Bryson and DeFelice 2010). Since *B. juncea* is considered the second most likely species after *B. rapa* to be a recipient of *B. napus* genes by virtue of their crossability and weediness (Di *et al.* 2009, OGTR 2011, Scheffler and Dale 1994), the risk assessment regarding introgression from *B. napus* to *B. juncea* should be carried out carefully. Therefore, persistence of chromosome derived from *B. napus* should be investigated in hybrid progenies.

Although *B. juncea* and *B. napus* are crossable and hybrids can be easily produced by artificial pollination (Bing *et al.* 1996, Jørgensen *et al.* 1998, Tsuda *et al.* 2011), the highest spontaneous hybridization frequency was only 3% under a mixed planting condition (Bing *et al.* 1996, Jørgensen *et al.* 1998, Tsuda *et al.* 2012), with the frequency decreasing sharply with distance from *B. napus* as the pollen source (Tsuda *et al.* 2012). Furthermore, the fertility of the F_1 hybrid between *B. juncea* and *B. napus* tends to be poor and less seeds productivity (Bing *et al.* 1996, Frello *et al.* 1995). However, fertility was restored in backcross progenies between *B. juncea* and *B. napus* than in F_1 hybrids (Frello *et al.* 1995, Song *et al.* 2010). If backcross progenies carry any genome regions derived from C-chromosome of *B. napus*, these regions could be introgressions and be inherited to their progeny.

Frello *et al.* (1995) evaluated the persistence of *B. napus*-specific RAPD markers in the BC_1 generation obtained from *B. juncea* × F_1 hybrid, but did not identify the locations of the markers. Distinguishing between A genome chromosomes of *B. juncea* and *B. napus* is currently difficult, but C-chromosomes can be identified using specific SSR markers constructed by Piquemal *et al.* (2005). Then, in order to

Communicated by R. Ohsawa
Received August 21, 2012. Accepted October 30, 2012.
*Corresponding author (e-mail: tabei@affrc.go.jp)

Table 1. Cross combinations and seed productivity of F_1 hybrid and backcross progenies

Plant type produced	Cross combination	No. of used plants		No. of pollinated flowers	No. of seeds	Seeds per pollination[a]	No. of plants for SSR analysis
		♀	♂				
B. juncea	*B. juncea* × *B. juncea*	9	9	276	1,712	6.2 ± 2.7	5
F_1	*B. juncea* × *B. napus*	11	10	231	999	4.3 ± 1.3	7
BC_1	*B. juncea* × F_1	42	50	624	1	0.0016 ± 0.011	1
BC_2	*B. juncea* × BC_1	1	1	25	139	5.6[b]	21
BC_3	*B. juncea* × BC_2	21	21	337	1,955	5.8 ± 3.5	63
BC_1	F_1 × *B. juncea*	48	40	698	0	0	–

[a] Seeds per pollination represents the number of obtained seeds per pollinated flower and the standard deviation for seeds per pollination in each pollinated plant.

[b] Means of standard deviation in seeds per pollination among individual plants could not be calculated, because only one plant was used as seed parents.

investigate the introgression of the *B. napus* genome into *B. juncea*, we evaluated the persistence of C-chromosome regions in F_1 hybrids and their backcross progenies, BC_1, BC_2 and BC_3 generations.

Materials and Methods

Plant materials

B. juncea L. cv. Kikarashina (Takii & Co., Ltd., Kyoto, Japan) and *B. napus* L. cv. Westar (Genebank of NIAS, JP No. 40734) were used as the maternal and paternal parents, respectively. F_1 hybrid plants were obtained by artificial bud pollination in *B. juncea* × *B. napus*. Backcrosses to obtain BC_1 plants were performed by reciprocal crossings between *B. juncea* and the F_1 hybrid by artificial bud pollination. One seed of BC_1 was obtained from backcrossing of *B. juncea* × F_1 and the BC_2 and BC_3 were produced by backcrossing of Kikarashina × BC_1 and Kikarashina × BC_2. Twenty-one seeds were randomly selected from 139 of BC_2 seeds and we distinguished and treated these 21 BC_2 plants as an independent line. A total of 63 BC_3 plants from 21 BC_2 lines were used for SSR analysis. Numbers of plants used as seed or pollen parents are shown in Table 1. Artificial bud pollination, germination and growth conditions were as described by Tsuda *et al.* (2011). Seeds per pollinated flowers was calculated from the numbers of pollinated flowers and obtained seeds (Table 1).

Chromosome preparations

Meiotic chromosome numbers were counted in pollen mother cells (PMCs) using the 1% acetic orcein smear method and were based on at least 20 cells per plant.

Morphological characteristics

Hybridity of F_1 plants was evaluated according to morphological characteristics such as flower organ size, shape of the leaf margin, leaf rugose, leaf fairness, waxy leaf and flowering time as described in Tsuda *et al.* (2011). Morphological characteristics in backcross progenies were evaluated by the same characteristics.

SSR analysis

Genomic DNA was extracted from young leaves by ISOPLANT II (NIPPON GENE CO., LTD., Toyama, Japan) according to the manufacturer's instructions. PCR reactions for SSR analysis were carried out under the following conditions. The composition of the reaction mixture by final concentrations was as follows: 0.5 U/μl Taq DNA polymerase (Gene taq: NIPPON GENE CO., LTD.), 1× PCR Buffer for Gene taq, 0.2 mM dNTP, 0.25 μM forward primer, 0.25 μM reverse primer, 2 ng/reaction DNA. PCR was conducted with a GeneAmp PCR System 9700 (Applied Biosystems) and PCR conditions followed Piquemal *et al.* (2005). The PCR products were electrophoresed on 5% acrylamide gel and visualized by staining with ethidium bromide, and bands were visualized with an ultraviolet illuminator. SSR analyses were performed in duplicate.

We screened applicable 83 C-chromosome specific SSR markers in our research from reported 141 SSR markers located on linkage groups N11–N19 by Piquemal *et al.* (2005). The stability of the screened markers was checked using total DNA of five independent plants each of *B. juncea* and *B. napus*.

Seven F_1 hybrids were randomly selected and used for SSR analysis. Twenty-one plants were randomly selected from 139 BC_2 seeds for analysis. Three BC_3 plants from each of the 21 BC_2 plants were used for SSR analysis, for a total of 63 BC_3 plants. The 83 selected markers were used for analysis of the F_1 and BC_1. Twelve of the 83 markers detected in the BC_1 were used for analysis of the BC_2 and BC_3 generations. In addition, five markers (MR025, CB10036A, CB10109B, CB10234 and CB10504B) undetected in BC_1 were selected from five linkage groups (N11, N13, N14, N16 and N18) of C-chromosomes and used to confirm the absence of these markers in BC_2 and BC_3.

Results

Fertility and morphology of F_1 plants and backcross progenies

Artificial pollination of *B. juncea* × *B. napus* produced

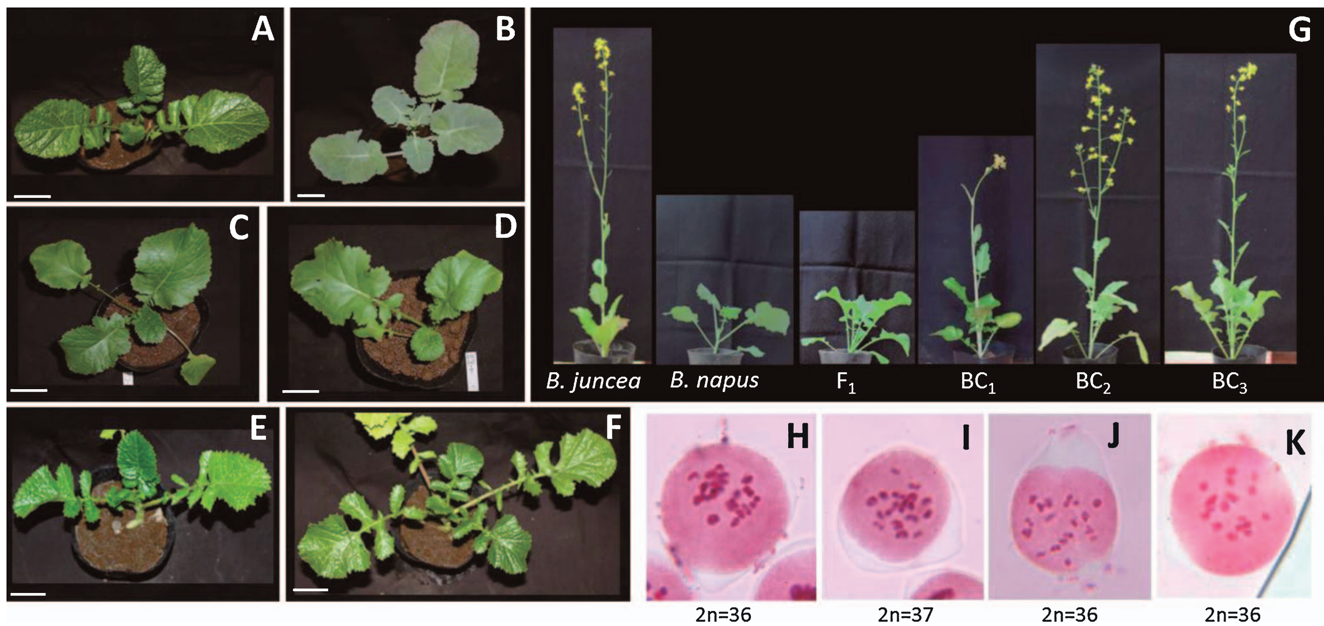

Fig. 1. Morphological characteristics and chromosomal analysis in *B. juncea*, *B. napus*, F_1 hybrid and backcross progenies. A: *B. juncea* plant, B: *B. napus* plant, C: F_1 hybrid plant, D: BC_1 plant, E: Plant BC_2-5 , F: Plant BC_3-5-2, G: Plant types of *B. juncea*, *B. napus*, F_1 hybrid, BC_1, BC_2 and BC_3 plants, H: Chromosomes in PMC of *B. juncea* (2n = 36), I: Chromosomes in PMC of F_1 hybrid (2n = 37), J: Chromosomes in PMC of BC_2 (2n = 36), K: Chromosomes in PMC of BC_3 (2n = 36). Bars = 3 cm.

999 seeds and the production efficiency was 4.3 seeds/pollination (Table 1). Fifty putative F_1 seeds were randomly selected and hybridity of F_1 plants was evaluated by observation of morphological characteristics. These F_1 plants showed intermediate characteristics between *B. juncea* and *B. napus* in flower organ size, shape of leaf margin, leaf rugose, leaf fairness and waxy leaf (Fig. 1C) and the flowering time of these F_1 plants was the same as that of *B. napus* (Fig. 1G). No seed was obtained by 698 bud pollination in F_1 × *B. juncea* and 1 seed was obtained by 624 bud pollination in *B. juncea* × F_1 and the production efficiency was 0.0016 seeds/pollination (Table 1). One hundred and thirty-nine BC_2 seeds were obtained from 25 bud pollinations of *B. juncea* × BC_1 plant (obtained from *B. juncea* × F_1) and the production efficiency was 5.6 seeds/pollination. In total 1955 BC_3 seeds were obtained by 337 bud pollinations and the production efficiency was 5.8 seeds/pollination. Although the fertility of F_1 plants was extremely low, the production efficiencies in the BC_2 and BC_3 were very close to that for *B. juncea* self-pollination (6.2 seeds/pollination) (Table 1). This result was assumed that very low fertility in F_1 hybrids can be recovered rapidly during backcrossing.

BC_1 leaves had more rugose and fairness and less waxy than F_1 plants (Fig. 1D) and the flowering time of the BC_1 was intermediate between F_1 and *B. juncea* (Fig. 1G). BC_2 and BC_3 had morphological characteristics and flowering time similar to those *B. juncea* (Fig. 1G).

Chromosome numbers of F_1 hybrids and backcross progenies

The F_1 hybrids had 37 chromosomes (Fig. 1I). In contrast, 36 chromosomes were observed in BC_1 plant (data not shown). BC_2 and BC_3 plants had also confirmed 36 chromosomes (Fig. 1J, 1K) by observation of 21 plants and 45 plants, respectively. These chromosome numbers in backcross progenies are the same number as *B. juncea*. Chromosome pairing in BC_2 and BC_3 exhibited same manner as that of *B. juncea* and mainly consisted of 18 bivalent (Fig. 1J, 1K).

Screening of SSR markers

To evaluate persistent regions of C-chromosome in this experiment, 83 SSR markers were screened from reported 141 of *B. napus* C-chromosome specific SSR markers by Piquemal *et al.* (2005) (Table 2). These 83 markers were clearly detected in all control plants of *B. napus* and they were not detected in *B. juncea* plants.

Evaluation for persistent C-chromosome regions by SSR analysis

The segregations of detected markers in each backcross generation are shown in Fig. 2. All 83 SSR markers were detected in F_1 hybrids, whereas in BC_1 plant 71 of 83 markers (85%) were deleted and 12 markers were detected. These 12 markers were used for analysis in BC_2 and BC_3 plants. The five markers (MR025, CB10036A, CB10109B, CB10234 and CB10504B) selected for confirmation from the 71 undetected in the BC_1 were also not detected in BC_2 and BC_3 (data not shown).

The 12 markers detected in BC_1 segregated in the BC_2 generation. Nine of 12 markers vanished from more than half of the analyzed BC_2 plants. Other three markers,

Table 2. The list of SSR markers[a] used

Chromosome number	Marker name
N11	CB10587, CB10208, CB10369, CB10443, MR025, Ol12-F11, CB10277, CB10281, CB10258, Na12-C06, Na10-H06, Ol10-A11, Na10-H03, BRAS074
N12	Na12-A01, CB10316, Na14-H11, CB10350, Ol13-G05, CB10026, Ni2-C12, Ol10-H02
N13	Ol13-D03, CB10036A, CB10569, Ol11-B05, Na12-E02, Ol10-B04, CB10132, CB10057, BRAS051, BRAS087, BRAS005, Na10-D03, CB10415B, Na12-F12, Ol13-A10, MR061A, MR061B, MR049A, MR049B, BRAS068, Ol13-H09, Na10-C01A
N14	Ol13-C03, CB10103, Ra2-F11, Ni4-A07, CB10109B, Na12-G04, CB10122, CB10288
N15	Na10-G08, A48350, MR129, Ol12-F02, Na10-A08, Na10-D11, MR097, CB10487
N16	CB10502, CB10234, CB10343, Na12-A02, CB10544, Ra2-A05
N17	CB10297, CB10528, BRAS019, CB10217, Na10-C01B, Na12-F03, BRAS107, CB10299, CB10268, CB10431
N18	CB10139, CB10504B, CB10373, Ni2-F11, Ol12-G04
N19	CB10344, BRAS002

[a] We screened these available SSR markers for our experiment from reported *B. napus* C-chromosome specific markers (Piquemal *et al.* 2005).

Fig. 2. Detection of *B. napus* C-chromosome specific SSR markers in BC_2 and BC_3 plants. Black cells indicate marker detection.

Na14-H11A, CB10415B and CB10288, remained in 13, 21 and 20 BC_2 plants, respectively (Fig. 2).

Of the 12 SSR markers detected in the BC_1, only CB10415B and/or CB10288 were detected and segregated in the BC_3. Of 63 plants, CB10415B was detected in 22 plants and CB10288 in 29 plants. BC_3 plants were classified into four types for persistence pattern of CB10415B and CB10288: both markers were detected in 10 plants of 7 lines, only CB10415B was detected in 12 plants of 7 lines, only CB10288 was detected in 19 plants of 11 lines and neither marker was detected in 22 plants of 12 lines. Both SSR markers were not carried in about one-third of the BC_3 plants after backcrossing. But all three tested plants in lines BC_3-12, -13 and -16 showed the persistence of CB10415B and all tested plants in lines BC_3-1, -6, -7, -9, -12 and -20 carried the CB10288 marker.

Discussion

In *B. juncea* × *B. napus*, it is suggested that the possibility of spontaneous hybridization is generally low (Bing *et al.* 1996, Jørgensen *et al.* 1998, Tsuda *et al.* 2012) and very low fertility of F_1 hybrids in *B. juncea* × *B. napus* (Bing *et al.* 1996, Jørgensen *et al.* 1998). Our results agreed with those of previous researchers in showing seed sterility of F_1 hybrids by reciprocal pollination between F_1 hybrids and *B. juncea* (Table 1). In interspecific and intergeneric hybridization of *Brassica* genus, low seed fertility has been reported often in such as cross combinations of *B. rapa* × *B. oleracea* and *Raphanus sativus* × *B. oleracea* (Namai *et al.* 1980), *B. napus* × *R. raphanistrum* and *B. napus* × *R. sativus* (Ammitzbøll and Jørgensen 2006). And also, F_1 hybrids sterility is common in many plant species (Grant 1981). This observation suggests that introgression from *B. napus* to *B. juncea* is rare in natural environments. However, introgression from transgenic plants to wild relatives through backcrossing is took into account and then many research groups intend to study in *Brassica* (OGTR 2011) and other crops (Andersson and de Vicente 2010, Stewart *et al.* 2003) around the world. Seed productivities of BC_2 and BC_3 generations recovered to the same level as *B. juncea* despite the low seed fertility of the F_1 hybrid (Table 1). Song *et al.* (2010) also reported restoration of seed fertility in backcross progenies between *B. juncea* and *B. napus*. Moreover,

fertility recovery in backcross progenies has been reported in other *Brassica* species (Hauser *et al.* 2003, Snow *et al.* 1999, Song *et al.* 2010), coffee (Coulibaly *et al.* 2003), wheat (Seefeldt *et al.* 1998, Wang *et al.* 2001) and cotton (Jiang *et al.* 2000). Once *B. napus* genome regions are integrated in chromosomes of *B. juncea*, the regions must have persisted and transmitted to subsequent progenies. Therefore, we should reveal how C-chromosome regions would be persisted in backcross progenies.

In BC_1 plant, 71 markers were deleted and 12 markers were persisted. The chromosome number of the BC_1 was 36 and its morphological characteristics were similar to those of *B. juncea*. The 12 markers located on six C-chromosomes (two on N12, six on N13, one each on N14, N15, N16 and N17) showed that the entire C-chromosome was not added to the hybrid progeny.

In BC_3 generation between *B. napus* and *B. carinata*, Navabi *et al.* (2011) speculated that a part of C-chromosome of *B. carinata* was integrated into C-chromosomes of *B. napus* by homologous recombination. *Brassica* species have generally high homoeology among A, B and C genomes (McGrath and Quiros 1991, Prakash and Chopra 1990, Quiros *et al.* 1994, Truco *et al.* 1996, U 1935) and Mason *et al.* (2010) reported that the homologous pairing frequency of allosyndesis in A–C genome chromosome was higher than that of B–C. Bing *et al.* (1996) also proposed the possibility of intergenomic chromosomal recombination resulting in the introgression of C-chromosome region of *B. napus* to *B. juncea*. From these previous reports and our results, we speculated that the persisting C-chromosome regions were integrated into A or B genome chromosomes of *B. juncea* by homologous recombination.

In contrast, the entire chromosome and a large part of B-chromosome were detected in hybrid progeny, F_5 plants (Schelfout *et al.* 2006) derived from *B. napus* × *B. juncea* and BC_3 plants (Navabi *et al.* 2011) derived from *B. carinata* × *B. napus* . It was considered that homologous recombination may hardly occur due to lower homology between B genome and C genome than between A genome and C genome (Mason *et al.* 2010). Therefore, it is speculated that persistent manner of chromosome was affected by homology among A, B and C genomes.

The two SSR markers, CB10415B and CB10288, detected in the BC_3 generation (Fig. 2) are mapped on the C-chromosomes N13 and N14, respectively (Piquemal *et al.* 2005). Akaba *et al.* (2009) reported that chromosomes N11, N15 and N18 of *B. napus* did not undergo pairing with A-chromosomes of *B. rapa*. Our results showed that only one marker, MR129 on N15, was detected in the BC_2, whereas SSR markers on N11 and N18 were not detected. This observation supports the ready elimination of markers on N11, N15 and N18 owing to the lower affinity of these chromosomes to A-chromosomes of *B. juncea*. Thus, studies on chromosomal homology among A, B and C genomes are further progressing (Akaba *et al.* 2009, Ge and Li 2007, Truco *et al.* 1996), at least, N13 and N14 of *B. napus* C-chromosome did not have lower affinity to A and B genomes of *B. juncea* (Akaba *et al.* 2009). We demonstrated the possibility for persistence of some C-chromosome regions in hybrid progeny. The persisting regions were thought to be fixed and inherited to progenies, although most C-chromosome regions had disappeared. In other words, most chromosomal regions from C genome did not remain in hybrid progenies, and this result may have application for controlling introgression of transgenes. Namely, transgenes should disappear in hybrid progeny if the transgenes are integrated into the C-chromosome region with the lowest affinity by novel plant breeding technology e.g., gene targeting technology.

Di *et al.* (2009) reported that the F_1 hybrid from wild *B. juncea* × transgenic canola showed higher fertility than found in our study (Table 1) and reported previously (Bing *et al.* 1996, Frello *et al.* 1995). Di *et al.* (2009) also discussed that vigorous vegetative and reproductive growth of wild *B. juncea* allowed the maintenance of higher fertility in F_1 hybrid. Given that wild *B. juncea* in natural environments is thought to comprise multiple genotypes, a discussion of introgression potential should also take into account this genotypic variation.

Acknowledgments

We are grateful to Mr. Ryouji Yazawa, Mr. Shinya Hirashima, Mr. Takao Komatsuzaki and Ms. Junko Sioda for their technical assistance. This study was supported by Assurance of Safe Use of Genetically Modified Organisms (The Ministry of Agriculture, Forestry and Fisheries of Japan).

Literature Cited

Akaba, M., Y. Kaneko, B.H. Jeong, M. Tsuda, S. Bang, Y. Tabei and Y. Matsuzawa (2009) Estimation of the genetic introgression in hybrid progenies of *Brassica rapa* × *B. napus*. Breed. Res. 11: 267.

Ammitzbøll, H. and R.B. Jørgensen (2006) Hybridization between oilseed rape (*Brassica napus*) and different populations and species of *Raphanus*. Environ. Biosafety Res. 5: 3–13.

Andersson, M.S. and M.C. de Vicente (2010) Gene Flow between Crops and Their Wild Relatives, The Johns Hopkins University Press, Baltimore, Maryland.

Aono, M., S. Wakiyama, M. Nagatsu, Y. Kaneko, T. Nishizawa, N. Nakajima, M. Tamaoki, A. Kubo and H. Saji (2011) Seeds of a possible natural hybrid between herbicide-resistant *Brassica napus* and *Brassica rapa* detected on a riverbank in Japan. GM Crops 2: 201–210.

Bing, D.J., R.K. Downey and G.F.W. Rakow (1996) Hybridizations among *Brassica napus*, *B. rapa* and *B. juncea* and their two weedy relatives *B. nigra* and *Sinapis arvensis* under open pollination conditions in the field. Plant Breed. 115: 470–473.

Bryson, C.T. and M.S. DeFelice (2010) Weeds of the Midwestern United States & Central Canada, The University of Georgia Press, Georgia, p. 143.

Claessen, D., C.A. Gilligan, P.J.W. Lutman and F.V.D. Bosch (2005a) Which traits promote persistence of feral GM crops? Part 1:

implications of environmental stochasticity. OIKOS 110: 20–29.

Claessen, D., C.A. Gilligan, P.J.W. Lutman and F.V. D. Bosch (2005b) Which traits promote persistence of feral GM crops? Part 2: implications of metapopulation structure. OIKOS 110: 30–42.

Coulibaly, I., J. Luarn, M. Lorieux, A. Charrier, S. Hamon and M. Noirot (2003) Pollen viability restoration in a *Coffea canephora* P. and *C. heterocalyx* Stoffelen backcross. QTL identification for marker-assisted selection. Theor. Appl. Genet. 106: 311–316.

Di, K., C.N. Stewart, Jr, W. Wei, B. Shen, Z.X. Tang and K.P. Ma (2009) Fitness and maternal effects in hybrids formed between transgenic oilseed rape (*Brassica napus* L.) and wild brown mustard [*B. juncea* (L.) Czern et Coss.] in the field. Pest Manag. Sci. 65: 753–760.

Frello, S., K.R. Hansen, J. Jensen and R.B. Jørgensen (1995) Inheritance of rapeseed (*Brassica napus*)-specific RAPD markers and a transgene in the cross *B. juncea* × (*B. juncea* × *B. napus*). Theor. Appl. Genet. 91: 236–241.

Ge, X.H. and Z.Y. Li (2007) Intra- and intergenomic homology of B-genome chromosomes in trigenomic combinations of the cultivated *Brassica* species revealed by GISH analysis. Chromosome Res. 15: 849–861.

Grant, V. (1981) Plant Speciation, 2nd edn., Columbia University Press, New York.

Hauser, T.P., C. Damgaard and R.B. Jørgensen (2003) Frequency-dependent fitness of hybrids between oilseed rape (*Brassica napus*) and weedy *B. rapa* (*Brassicaceae*). Am. J. Bot. 90: 571–578.

Hultén, E. and M. Fries (1986) Atlas of North European Vascular Plants. Koeltz Scientific Books Königstein, pp. 495, 1065.

James, C. (2011) Global Status of Commercialized Biotech/GM Crops: 2011. ISAAA Brief No. 43. ISAAA: Ithaca, NY, pp. 217–218.

Jiang, C.X., P.W. Chee, X. Draye, P.L. Morrell, C.W. Smith and A.H. Paterson (2000) Multilocus interactions restrict gene introgression in interspecific populations of polyploidy *Gossypium* (cotton). Evolution 54: 798–814.

Jørgensen, R.B., B. Andersen, T.P. Hauser, L. Landbo, T.R. Mikkelsen and H. Østergård (1998) Introgression of crop genes from oilseed rape (*Brassica napus*) to relative wild species—An avenue for the escape of engineered genes. Acta Hortic. 459: 211–217.

Mason, A.S., V. Huteau, F. Eber, O. Criton, G. Yan, M.N. Nelson, W.A. Cowling and A.M. Chèvre (2010) Genome structure affects the rate of autosyndesis and allosyndesis in AABC, BBAC and CCAB Brassica interspecific hybrids. Chromosome Res. 18: 655–666.

McGrath, J.M. and J.M. Quiros (1991) Inheritance of isozyme and RFLP markers in *Brassica campestris* and comparison with *B. oleracea*. Thor. Appl. Genet. 82: 668–673.

Mizuguti, A., Y. Yoshimura, H. Shibaike and K. Matsuo (2011) Persistence of feral populations of *Brassica napus* originated from spilled seeds around the Kashima seaport in Japan. JARQ 45: 181–185.

Namai, H., M. Sarashima and T. Hosoda (1980) Interspecific and intergeneric hybridization breeding in Japan. *In*: Tsunoda, S., K. Hinata and C. Gomez-Campo (eds.) *Brassica* Crops and Wild Allies, Japan Scientific Societies Press, Tokyo, pp. 191–201.

Navabi, Z.K., K.E. Stead, J.C. Pires, Z. Xiong, A.G. Sharpe, I.A.P. Parkin, M.H. Rahman and A.G. Good (2011) Analysis of B-genome chromosomes introgression in interspecific hybrids of *Brassica napus* × *B. carinata*. Genetics 187: 659–673.

OGTR (Office of the gene technology regulator) (2011) The Biology of *Brassica napus* L. (canola), Version 2.1., http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/canola-3/$FILE/BiologyCanola2011.pdf#search='OGTR 2011 Brassica napus biology'

Piquemal, J., E. Cinquin, F. Couton, C. Rondeau, E. Seignoret, I. Doucet, D. Perret, M.J. Villeger, P. Vincourt and P. Blanchard (2005) Construction of an oilseed rape (*Brassica napus* L.) genetic map with SSR markers. Theor. Appl. Genet. 111: 1514–1523.

Prakash, S. and V. L. Chopra (1990) Reconstruction of allopolyploid *Brassicas* through non-homologous recombination: introgression of resistance to pod shatter in *Brassica napus*. Genet. Res. 56: 1–2.

Quiros, C.F., J. Hu and M.J. Truco (1994) DNA-based marker maps of *Brassica*. *In*: Phillips, R.L. and I.K. Vasil (eds.) DNA-based Markers in Plants, Kluwer Academic Publ., The Netherlands, pp. 199–222.

Scheffler, J.A. and P.J. Dale (1994) Opportunities for gene transfer from transgenic oilseed rape (*Brassica napus*) to related species. Transgen. Res. 3: 263–278.

Schelfout, C.J., R. Snowdon, W.A. Cowling and J.M. Wroth (2006) Tracing B-genome chromatin in *Brassica napus* × *B. juncea* interspecific progeny. Genome 49: 1490–1497.

Seefeldt, S.S., R. Zemetra, F.L. Young and S.S. Jones (1998) Production of herbicide-resistant joined goatgrass (*Aegilops cylindrical*) × wheat (*Triticum aestivum*) hybrids in the field by natural hybridization. Weed Sci. 46: 632–634.

Shimizu, N., H. Morita and S. Hirota (2003) Picture book of Japanese naturalized plants–Plant invader 600 species. Zenkoku Nouson Kyouiku Kyoukai, Japan, pp. 90–91.

Snow, A.A., B. Andersen and R.B. Jørgensen (1999)Cost of transgenic herbicide resistance introgressed from *Brassica napus* into weedy *B. rapa*. Mol. Ecol. 8: 605–605.

Song, X., Z. Wang, J. Zuo, C. Huangfu and S. Qiang (2010) Potential gene flow of two herbicide-tolerant transgenes from oilseed rape to wild *B. juncea* var. gracilis. Theor. Appl. Genet. 120: 1501–1510.

Stewart, Jr. C.N., M.D. Halfhill and S.I. Warwick (2003) Transgene introgression from genetically modified crops to their wild relatives. Nat. Rev. Genet. 4: 806–817.

Truco, M.J., J. Hu, J. Sadowski and C.F. Quiros (1996) Inter- and intragenomic homology of the *Brassica* genomes: implications for their origin and evolution. Thor. Appl. Genet. 93: 1225–1233.

Tsuda, M., K. Konagaya, A. Okuzaki, Y. Kaneko and Y. Tabei (2011) Occurrence of metaxenia and false hybrids in *Brassica juncea* L. cv. Kikarashina × *B. napus*. Breed. Sci. 61: 358–365.

Tsuda, M., A. Okuzaki, Y. Kaneko and Y. Tabei (2012) Relationship between hybridization frequency of *Brassica juncea* × *B. napus* and distance from pollen source (*B. napus*) to recipient (*B. juncea*) under field conditions in Japan. Breed. Sci.

U, N. (1935) Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jpn. J. Bot. 7: 389–452.

Wang, Z., R.S. Zemetra, J. Hansen and C.A. Mallory-Smith (2001) The fertility of wheat × jointed goatgrass hybrid and its backcross progenies. Weed Sci. 49: 340–345.

Wei, W., T.H. Schuler, S.J. Clark, C.N. Stewart, Jr. and G.M. Poppy (2005) Age-related increase in levels of insecticidal protein in the progenies of transgenic oilseed rape and its efficacy against a susceptible strain of diamondback moth. Ann. Appl. Biol. 147: 227–234.

Wilkinson, M. and M. Tepfer (2009) Fitness and beyond: Preparing for the arrival of GM crops with ecologically important novel characters. Environ. Biosafety Res. 8: 1–14.

Yoshimura, Y., H.J. Beckie and K. Matsuo (2006) Transgenic oilseed rape along transportation routes and port of Vancouver in western Canada. Environ. Biosafety Res. 5: 67–75.