
Review

Possibilities of direct introgression from *Brassica napus* to *B. juncea* and indirect introgression from *B. napus* to related Brassicaceae through *B. juncea*

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The impact of genetically modified canola (*Brassica napus*) on biodiversity has been examined since its initial stage of commercialization. Various research groups have extensively investigated crossability and introgression among species of Brassicaceae. *B. rapa* and *B. juncea* are ranked first and second as the recipients of cross-pollination and introgression from *B. napus*, respectively. Crossability between *B. napus* and *B. rapa* has been examined, specifically in terms of introgression from *B. napus* to *B. rapa*, which is mainly considered a weed in America and European countries. On the other hand, knowledge on introgression from *B. napus* to *B. juncea* is insufficient, although *B. juncea* is recognized as the main Brassicaceae weed species in Asia. It is therefore essential to gather information regarding the direct introgression of *B. napus* into *B. juncea* and indirect introgression of *B. napus* into other species of Brassicaceae through *B. juncea* to evaluate the influence of genetically modified canola on biodiversity. We review information on crossability and introgression between *B. juncea* and other related Brassicaceae in this report.

Key Words: introgression, genetically modified, *Brassica napus*, *Brassica juncea*, Brassicaceae, crossability.

Introduction

The global cultivation area of genetically modified (GM) canola (*Brassica napus*) has continuously increased each year, encompassing approximately 9.2 million hectares in 2012 (James 2012), with Japan importing most of its canola from Canada (Ministry of Finance 2013). While the production of GM canola should be increased, the impact of biodiversity by introgression from GM canola to related Brassicaceae has aroused public concern and led to a global debate (Wilkinson and Tepfer 2009). Introgression from GM canola derived from spilled seeds has also been considered and monitored in non-cultivating countries of GM canola, such as Japan (Aono *et al.* 2011, Mizuguti *et al.* 2011), the United Kingdom (Claessen *et al.* 2005a, 2005b, Crawley and Brown 1995, 2004) and other countries (Devos *et al.* 2011, Pivard *et al.* 2008, Reuter *et al.* 2008, von der Lippe and Kowarik 2007a, 2007b).

A list of potential recipient species for *B. napus* is currently available, including their crossability and hybrid fitness (OECD 2012, OGTR 2011). *B. rapa* is the most likely recipient of introgression from *B. napus* (Scheffler and

Dale 1994) and is one of the most highly distributed weed in Europe, Atlantic, Africa, Asia, North and South America, and Australia (Warwick *et al.* 2009). Because *B. rapa* is an allogamous plant with self-incompatibility, its crossability, including interspecific and intergeneric hybridization, is generally higher than that of autogamous plants (OECD 2012). In fact, many spontaneous hybrids can be obtained by hybridization between *B. napus* and *B. rapa* (CFIA 1999, OECD 2012, OGTR 2011).

At present, 17 Brassicaceae species are recognized, including *B. juncea*, *B. carinata*, *B. nigra*, *B. oleracea*, *B. fruticulosa*, *B. maurorum*, *B. tournefortii*, *Diplotaxis catholica*, *D. muralis*, *D. erucoides*, *Eruca sativa*, *Erucastrum gallicum*, *Hirschfeldia incana*, *Raphanus sativus*, *R. raphanistrum*, *Sinapis alba*, and *S. arvensis*. These species have also been listed as recipients of *B. napus* (OECD 2012). *B. juncea* is considered to have the second highest crossability with *B. napus* after *B. rapa* (Scheffler and Dale 1994).

An interspecific hybrid is easily produced between *B. juncea* and *B. napus* by artificial pollination (Mason *et al.* 2011a, Mohammad and Sikka 1940, Rao and Shivanna 1997, Tsuda *et al.* 2011). *H. incana* and *R. raphanistrum* are hybrids that were generated by artificial pollination with *B. napus* (Kerlan *et al.* 1992). However, the probability of introgression into these two species is low (OECD 2012)

because of poor seed productivity and fertility of the F₁ progeny of *H. incana* (Chadoeuf *et al.* 1998) and *R. raphanistrum* (Guéritaine *et al.* 2003) as recipients. *R. sativus* (Huang *et al.* 2002) and *S. arvensis* (Moyes *et al.* 2002) have extremely low crossability with *B. napus* and barely produce F₁ hybrids with *B. napus* under natural conditions. Moreover, crossability between *B. napus* and 12 of its relatives, namely *B. carinata*, *B. nigra*, *B. tournefortii*, *D. muralis*, *B. fruticulosa*, *B. maurorum*, *B. oleracea*, *E. sativa*, *D. catholica*, *D. eruroides*, *Erucastrum gallicum*, and *S. alba*, is very low (OECD 2012) and thus, spontaneous hybridization will not occur. Therefore, introgression from *B. napus* to these 12 closely related species relatives is highly unlikely, resulting in the identification of *B. rapa* and *B. juncea* as major candidate recipients of introgression from *B. napus*.

B. juncea was introduced from China before the 9th century (Hoshikawa 1998) and eventually became a naturalized species of Japan (Shimizu *et al.* 2003). *B. rapa* was also introduced to Japan in the 8th century (Hoshikawa 1998). Weedy *B. rapa* is also derived from cultivated varieties, and is distributed as an escaped plant from fields in Japan (Osada 1980). Based on these historical accounts, the Japanese government has exempted *B. rapa* and *B. juncea* from its list of protected native plants, because biodiversity is based on the Cartagena protocol, despite the occurrence of spontaneous hybridization with *B. napus*. However, indirect introgression from *B. napus* to related Brassicaceae may still occur with *B. rapa* and/or *B. juncea* acting as bridge species.

Hybridization between *D. tenuifolia* and *B. napus* has not been reported, although *B. rapa* can hybridize with *D. tenuifolia* (Salisbury 1989), which in turn, suggests that some traits of *B. napus* were transferred into *D. tenuifolia* through *B. rapa*. We consider this case as a typical example of indirect introgression.

Because *B. juncea* is widely distributed around the world, yet centrally focused on Asia, this species has the potential role of acting as a mediator of introgression from *B. napus* to related members of the Brassicaceae, as observed in the case of *B. rapa* in America and Europe. Several research groups have previously investigated crossability, and spontaneous hybridization frequency between *B. napus* and *B. rapa* has been reported and reviewed by many research groups (CFIA 1999, OECD 2012). However, the information about *B. juncea* and *B. napus* is not sufficient (CFIA 2012, Warwick *et al.* 2009). This review will perform two tasks. First, we will review and summarize intro-

gression and crossability between *B. juncea* and *B. napus*. Second, we will show and discuss the potential of indirect introgression through *B. juncea* by crossability between *B. juncea* and other members of the Brassicaceae.

Introgression from *B. napus* to *B. juncea*

Spontaneous hybridization frequency

Spontaneous hybridization frequency is greatly influenced by the field experimental design, such as the size of the pollen donor and the isolated distance from the donor (Scheffler *et al.* 1993, Tsuda *et al.* 2012a). This section summarizes the effects of distance between the donor and the recipient on spontaneous hybridization frequency (Table 1).

In mixed cultivation experiments, spontaneous hybridization frequency varied from 0.13 to 5.91% in *B. juncea* × *B. napus* (Bing *et al.* 1991, 1996, Heenan *et al.* 2007, Huiming *et al.* 2007, Jørgensen *et al.* 1998, Liu *et al.* 2010, Tsuda *et al.* 2012a). On the other hand, the frequency in *B. napus* × *B. juncea* ranged from 1.1 to 1.3% (Bing *et al.* 1991, 1996, Heenan *et al.* 2007, Jørgensen *et al.* 1998). Spontaneous hybridization in Brassicaceae under isolated cultivation, wind-pollination (Becker *et al.* 1992, McCartney and Lacey 1991), and insect-pollination (Free and Spencer-Booth 1963, Free 1993) have been previously reported. However, spontaneous hybridization is generally difficult because *B. juncea* is a self-compatible species (Ohsawa and Namai 1987, Tsuda *et al.* 2012a) reported that hybridization frequency under isolated conditions between the donor and the recipients was 0.05% at a distance of 1 m and 0.03% at a distance of 17.5 m, and no hybrids were observed under more distant isolated conditions, ranging from 20 to 27.5 m. These results also indicate the difficulty of hybridization when *B. juncea* is isolated from *B. napus*. In Japan, it has been previously reported that the flowering period of *B. juncea* and *B. napus* overlap (Matsuo and Itoh 2001). However, when the flowering period of the donor and the recipient completely overlapped, the spontaneous hybridization frequency of *B. juncea* × *B. napus* was not high compared with that of *B. rapa* × *B. napus* (Tsuda *et al.* 2012a). Based on these reports, it has thus been estimated that spontaneous hybridization results in low hybrid production.

Hybrid production by artificial pollination

Several research groups have examined seed productivity and hybrid productivity by artificial pollination (Table 2). It is desirable to evaluate hybrid productivity on the basis of

Table 1. Spontaneous hybridization frequency between *B. juncea* and *B. napus*

Cross-combinations	Cultivation condition	Frequency (%)	References
<i>B. juncea</i> × <i>B. napus</i>	Mixed cultivation	0.13–5.91	Tsuda <i>et al.</i> (2012a), Heenan <i>et al.</i> (2007), Jørgensen <i>et al.</i> (1998), Huiming <i>et al.</i> (2007), Liu <i>et al.</i> (2010), Bing <i>et al.</i> (1991, 1996)
	Isolated cultivation (1.0–27.5 m)	0.00–0.05	Tsuda <i>et al.</i> (2012a)
<i>B. napus</i> × <i>B. juncea</i>	Mixed cultivation	1.10–1.30	Heenan <i>et al.</i> (2007), Jørgensen <i>et al.</i> (1998), Bing <i>et al.</i> (1991, 1996)

Table 2. Hybrid production efficiency between *B. juncea* and *B. napus* by artificial pollination

Cross-combinations	Number of seeds/ pollination		References	Number of hybrids/ pollination		References
	Range	Average		Range	Average	
	<i>B. juncea</i> × <i>B. napus</i>	0.21–16.50		4.96	Sharma and Singh (1992), Frello <i>et al.</i> (1995), Heenan <i>et al.</i> (2007), Tsuda <i>et al.</i> (2011), Mathias (1985), Bing <i>et al.</i> (1991, 1996), Choudhary and Joshi (1999), GhoshDastidar and Varma (1999), Mohammad and Sikka (1940)	
<i>B. napus</i> × <i>B. juncea</i>	0.01–26.20	1.99	Frello <i>et al.</i> (1995), Heenan <i>et al.</i> (2007), Choudhary and Joshi (1999), Yamagishi and Takayanagi (1982), GhoshDastidar and Varma (1999), Mohammad and Sikka (1940)	0.00–0.32	0.07	Choudhary and Joshi (1999), Mohammad and Sikka (1940), Sabharwal and Doelžel (1993), Mason <i>et al.</i> (2011a)

production efficiency of true hybrids per pollination because false hybrids can be produced by artificial pollination in Brassicaceae (Kakizaki 1925, Nishi and Hiraoka 1962, Nishi *et al.* 1964, Terao 1934), especially in *B. juncea* × *B. napus* (Tsuda *et al.* 2011) and *B. napus* × *B. juncea* (Mohammad and Sikka 1940). It has been determined that false hybrids are generated by pseudogamy, which is a type of apomixis (Nishi and Hiraoka 1962). False hybrids do not present any morphological characteristics and genomic regions of its pollen parent (Ammitzbøll and Jørgensen 2006, Nishi and Hiraoka 1962, Tsuda *et al.* 2011). Therefore, we identified features that could assist in distinguishing true hybrids from false hybrids.

The range and average of hybrid production efficiency are shown in Table 2. Mohammad and Sikka (1940) could not obtain hybrids from *B. juncea* × *B. napus*, whereas Rao and Shivanna (1997) could obtain 13.5 seeds per pollination. Despite an average of 4.05 seeds per pollination in *B. juncea* × *B. napus* cross (Table 2), hybrid production efficiency may vary among cultivars and experimental conditions. On the other hand, the progeny seeds produced only 0.32 seeds per pollination at maximum and 0.07 hybrid/pollination on average in *B. napus* × *B. juncea*. Therefore, introgression from *B. napus* to *B. juncea* showed a higher potential than the direction of *B. juncea* to *B. napus*. The cross of *B. juncea* × *B. napus* should be assessed with the assumption that false hybrids may occur. Furthermore, low seed productivity of the F₁ hybrid may be a major limiting factor in the extension of introgression from *B. napus* to *B. juncea*.

Hybrid production by tissue culture techniques

Ovary, ovule, and embryo culture procedures have been used to rescue the hybridized embryos of Brassicaceae (Bajaj 1990, Kaneko *et al.* 2009). Tissue culture has also been utilized in the production of hybrids between *B. napus* and *B. juncea* (Bajaj *et al.* 1986, Sacristán and Gerdemann 1986, Sharma and Singh 1992, Zhang *et al.* 2003). Their range and average hybrid production efficiencies are presented in Table 3. In the cross *B. juncea* × *B. napus*, the average hybrid production efficiency ranged from 24.9 to 43.4% in each culture technique, and hybrids of *B. napus* × *B. juncea* were stably produced, within the range of 10.2–39.8%. A higher efficiency was observed in *B. juncea* × *B. napus* than in *B. napus* × *B. juncea*, but the efficiency of *B. napus* × *B. juncea* was sufficient to produce F₁ hybrids. Therefore, the production of hybrids between *B. juncea* and *B. napus* by tissue culture was considered stable.

Fertility of hybrids and their progenies

Introgression may be affected by the productivity of hybrids and their progenies. To evaluate the productivity of hybrid progenies, seed fertility of F₁ derived from hybridization between *B. juncea* and *B. napus* and their self-progenies, as well as backcross progenies, were examined.

The F₁ hybrid from *B. juncea* (AABB, 2n = 36) × *B. napus* (AACC, 2n = 38) showed a genome composition of AABC (Sabharwal and Doležel 1993, Tsuda *et al.* 2012b) or AAB (Sabharwal and Doležel 1993). Most of the hybrids had chromosomes and an AABC genome composition

Table 3. Proportion of hybrid plants to cultured tissues

Cross-combinations	Culture method	Range (%)	Average (%)	References
<i>B. juncea</i> × <i>B. napus</i>	Ovary	0.4–67.6	43.4	Bajaj <i>et al.</i> (1986), Sharma and Singh (1992)
	Ovule	30.4–49.5	42.4	Bajaj <i>et al.</i> (1986)
	Embryo	4.2–73.0	24.9	Bajaj <i>et al.</i> (1986), Zhang <i>et al.</i> (2003)
<i>B. napus</i> × <i>B. juncea</i>	Ovary	5.4–14.9	10.2	Bajaj <i>et al.</i> (1986)
	Ovule	37.5–42.0	39.8	Bajaj <i>et al.</i> (1986)
	Embryo	0.8–60.9	22.6	Sacristán and Gerdemann (1986), Bajaj <i>et al.</i> (1986), Zhang <i>et al.</i> (2003)

Table 4. Production efficiencies of progenies in F₁ by backcrossing and selfing

Cross-combinations	Seeds/pollination	References
F ₁ produced from <i>B. juncea</i> × <i>B. napus</i>		
Backcrossing		
<i>B. juncea</i> × F ₁	0.65–1.9	Song <i>et al.</i> (2010), Frello <i>et al.</i> (1995)
F ₁ × <i>B. juncea</i>	0.06–1.2	Song <i>et al.</i> (2010), Liu <i>et al.</i> (2010), Heenan <i>et al.</i> (2007)
<i>B. napus</i> × F ₁	NR ^a	Schelfhout <i>et al.</i> (2006), Prakash and Chopra (1990)
F ₁ × <i>B. napus</i>	0–1.6	Mathias (1985), Liu <i>et al.</i> (2010), Heenan <i>et al.</i> (2007)
Selfing (F ₁ × F ₁)	NR	Liu <i>et al.</i> (2010), Schelfhout <i>et al.</i> (2006), Roy (1984), Choudhary and Joshi (2001)
F ₁ produced from <i>B. napus</i> × <i>B. juncea</i>		
Backcrossing		
<i>B. juncea</i> × F ₁	NR	Schelfhout <i>et al.</i> (2006)
F ₁ × <i>B. juncea</i>	NR	Schelfhout <i>et al.</i> (2006), Kirti <i>et al.</i> (1995)
<i>B. napus</i> × F ₁	NR	Schelfhout <i>et al.</i> (2006)
F ₁ × <i>B. napus</i>	NR	Schelfhout <i>et al.</i> (2006)
Selfing (F ₁ × F ₁)	NR	Schelfhout <i>et al.</i> (2006), Roy (1984)

^a NR: not reported, progeny seeds were obtained, but the number of seed sets per pollinated flower was not described.

(Choudhary and Joshi 1999, Tsuda *et al.* 2012b). Pollen fertility of F₁ ranged from 0 to 35.6% (Choudhary and Joshi 1999, Frello *et al.* 1995, Heenan *et al.* 2007, Liu *et al.* 2010, Sandhu and Gupta 2000, Song and Qiang 2003), with the highest fertility (78%) observed in nine samples of the F₁ hybrid. On the other hand, the F₁ hybrid from *B. napus* × *B. juncea* showed a genome composition of AABC or AABBC (Sabharwal and Doležal 1993), and pollen fertility was 95.4% (Heenan *et al.* 2007). Therefore, male fertility of F₁ derived from *B. juncea* × *B. napus* was lower than that of F₁ from *B. napus* × *B. juncea*. However, only one report (Heenan *et al.* 2007) described male fertility of F₁ from *B. napus* × *B. juncea*, prompting further investigations to assess the occurrence of introgression. Investigations on the mechanisms underlying the variations in genome composition in F₁ hybrids from each pollen parent of *B. juncea* and *B. napus* are warranted. Because the A–C chromosomal pairing affinity is higher than that of A–B and B–C in the F₁ hybrid with AABC genome composition from *B. juncea* × *B. napus* (Mason *et al.* 2010), hybrids of the combinations of the A genome derived from *B. juncea* and the C genome derived from *B. napus* might easily occur during hybridization between *B. juncea* and *B. napus*. Female fertility of the F₁ hybrids was assessed by comparative analysis of the productivity of BC₁ seeds using backcrossing (Table 4). Seed productivity of BC₁ was remarkably reduced compared with that of both parents (Choudhary and Joshi 2001, Frello *et al.* 1995, Heenan *et al.* 2007, Kirti *et al.* 1995, Liu *et al.* 2010, Mathias 1985, Roy 1984, Schelfhout *et al.* 2006, Song *et al.* 2010), which was indicative of the degradation of female and male fertility of F₁.

Fertility of BC₁, BC₂, and BC₃ generations from F₁ (*B. juncea* × *B. napus*) × *B. juncea* was reported by Song *et al.* (2010) and Tsuda *et al.* (2012b). Seed productivities of BC₂ and BC₃ were promptly recovered to the same level as those of the parent lines (Table 5). However, seed productivity of BC₁ [(*B. juncea* × *B. napus*) × *B. napus*] was not reported. It is possible that BC₂ produced by reciprocal cross-pollination between BC₁ and *B. napus* was extremely low.

Table 5. Production efficiencies of progenies from BC₁ derived from *B. juncea* × *B. napus*

Cross-combinations	Seeds/pollination	References
BC ₁ from <i>B. juncea</i> × F ₁		
<i>B. juncea</i> × BC ₁	5.6–10.6	Song <i>et al.</i> (2010), Tsuda <i>et al.</i> (2012b)
<i>B. juncea</i> × BC ₂	5.8–16.0	Song <i>et al.</i> (2010), Tsuda <i>et al.</i> (2012b)
<i>B. juncea</i> × BC ₃	15.5–16.0	Song <i>et al.</i> (2010)
BC ₁ from F ₁ × <i>B. juncea</i>		
BC ₁ × <i>B. juncea</i>	3.8	Song <i>et al.</i> (2010)
BC ₂ × <i>B. juncea</i>	3.9–15.0	Song <i>et al.</i> (2010)
BC ₃ × <i>B. juncea</i>	9.7–15.2	Song <i>et al.</i> (2010)
<i>B. juncea</i> × <i>B. juncea</i>	6.2–16.2	Song <i>et al.</i> (2010), Tsuda <i>et al.</i> (2012b)

On the other hand, F₂ to F₇ derived from F₁ (*B. juncea* × *B. napus*) were obtained (Roy 1984), whereas F₃ was not obtained from F₁ (*B. napus* × *B. juncea*) (Liu *et al.* 2010, Roy 1980).

Indirect introgression from *B. napus* to other Brassicaceae through *B. juncea*

Spontaneous hybridization between *B. juncea* and *B. napus* has been extensively examined (Bing *et al.* 1991, 1996, Heenan *et al.* 2007, Huiming *et al.* 2007, Jørgensen *et al.* 1998, Liu *et al.* 2010, Tsuda *et al.* 2012a). However, Bing *et al.* (1991, 1996) reported that spontaneous hybridization of *B. nigra* × *B. juncea* and *S. arvensis* × *B. juncea* was unsuccessful, and no other reports on spontaneous hybridization between *B. juncea* and other members of the Brassicaceae have been published.

On the other hand, numerous reports have described the production of inter- and intra-specific hybrids by artificial pollination. Crossability and hybrid production efficiency between *B. juncea* and seven other species were evaluated previously, and the results are listed in Table 6. Progeny seeds were obtained from all cross-combinations, except *S. pubescens* × *B. juncea*. On the other hand, hybrid plants

Table 6. Interspecific and intergeneric cross compatibility by artificial pollination between *B. juncea* and other members of the Brassicaceae

Species	Parent status	Seeds/pollination		References	Hybrids/pollination		References
		Range	Average		Range	Average	
<i>B. nigra</i>	Pollen	0.00–10.20	2.11	Mohammad and Sikka (1940), Bing <i>et al.</i> (1991, 1996), Prasad <i>et al.</i> (1997), GhoshDastidar and Varma (1999), Morinaga (1934)	0.03–0.10	0.06	Rao and Shivanna (1997), Bing <i>et al.</i> (1991, 1996)
	Seed	0.00–0.01	0.01	Mohammad and Sikka (1940), Bing <i>et al.</i> (1991, 1996), GhoshDastidar and Varma (1999)	0.01	0.01	Bing <i>et al.</i> (1991, 1996)
<i>B. rapa</i>	Pollen	0.00–2.96	0.39	Sharma and Singh (1992), Rhee <i>et al.</i> (1997), GhoshDastidar and Varma (1999), Kakizaki (1925), Song and Qiang (2003), Choudhary and Joshi (1999), Takeshita <i>et al.</i> (1980), Mohammad and Sikka (1940)	0.00–5.66	0.85	Rao and Shivanna (1997), Choudhary and Joshi (1999), Mohammad and Sikka (1940)
	Seed	0.00–10.40	0.07	Kakizaki (1925), GhoshDastidar and Varma (1999), Prasad <i>et al.</i> (1997), Choudhary and Joshi (1999), Yamagishi and Takayanagi (1982), Mohammad and Sikka (1940), Nishi <i>et al.</i> (1964), Rhee <i>et al.</i> (1997), Takeshita <i>et al.</i> (1980)	0.00–0.50	0.00	Choudhary and Joshi (1999), Mohammad and Sikka (1940), Nishi <i>et al.</i> (1964)
<i>B. oleracea</i>	Pollen	0.00–0.08	0.02	Rao and Shivanna (1997), Kakizaki (1925), GhoshDastidar and Varma (1999)	0.00	0.00	Kakizaki (1925)
	Seed	0.00–0.12	0.03	Yamagishi and Takayanagi (1982), Nishi <i>et al.</i> (1964), Kakizaki (1925), GhoshDastidar and Varma (1999)	0.00	0.00	Nishi <i>et al.</i> (1964)
<i>B. carinata</i>	Pollen	0.22–0.75	0.48	Sharma and Singh (1992), Mason <i>et al.</i> (2011b), GhoshDastidar and Varma (1999)	0.02–0.2	0.48	Mason <i>et al.</i> (2011b), Rao and Shivanna (1997)
	Seed	0.04	0.04	GhoshDastidar and Varma (1999), Getinet <i>et al.</i> (1997), Mason <i>et al.</i> (2011a)	–	–	–
<i>R. sativus</i>	Pollen	0.00–0.31	0.09	Kakizaki (1925), Rhee <i>et al.</i> (1997)	–	–	–
	Seed	0.00–0.51	0.14	Nishi <i>et al.</i> (1964)	0.00–0.06	0.03	Nishi <i>et al.</i> (1964)
<i>S. arvensis</i>	Pollen	0.07	0.07	Bing <i>et al.</i> (1991, 1996)	0.03	0.03	Bing <i>et al.</i> (1991, 1996)
	Seed	0.02	0.02	Bing <i>et al.</i> (1991, 1996)	0.00	0.00	Bing <i>et al.</i> (1991, 1996)
<i>S. pubescens</i>	Pollen	0.15	0.15	Inomata (1991)	0.05	0.05	Inomata (1991)
	Seed	0.00	0.00	Inomata (1991)	–	–	–

were not obtained in cross-combinations of *B. oleracea* × *B. juncea*, *B. juncea* × *B. oleracea*, and *S. arvensis* × *B. juncea*. Differences between seed/pollination and hybrid/pollination showed the occurrence of false hybrids in inter- and intra-specific crossings (Table 6).

Although artificial pollination of *S. pubescens* × *B. juncea* did not generate seeds or hybrids, a few seeds or hybrid plants were obtained by artificial pollination of *B. juncea* × *S. pubescens*. However, these hybrids were barely produced under natural conditions (Inomata 1991). Furthermore, hybridization between *S. pubescens* and *B. napus* has not been reported in the literature. It is thus possible that

S. pubescens could be a new candidate of indirect introgression from *B. napus*.

Cross-pollination between *B. juncea* and other relatives, such as *B. oxyrrhina* (Bijral and Sharma 1999b, Katiyar and Chamola 2007, Salisbury 1989), *B. gravinae* (Nanda Kumar *et al.* 1989), *D. eruroides* (Bhat *et al.* 2006, Inomata 1998) as male and female parents, *D. catholica* (Banga *et al.* 2003), *D. berthautii* (Bhat *et al.* 2008), *D. tenuifolia* (Salisbury 1989), *Sinapidendein fruticulosa* (Herberd and McArthur 1980) as female parent, *R. raphanistrum* (Kamala 1983), *E. sativa* (Bijral and Sharma 1999a), *Moricandia arvensis* and *B. cossoniana* (Herberd and McArthur 1980)

as male parent, produced seeds by artificial pollination, although the data on seed/pollinated were not reported. The following nine species, namely *B. tounefortii* (GhoshDastidar and Varma 1999, Goyal *et al.* 1997, Lokanadha and Sarla 1994), *D. virgata* (Herberd and McArthur 1980, Inomata 1994, 2003) as male and female parents, *D. siifolia* (Ahuja *et al.* 2003, Batra *et al.* 1990), *D. siettiana* (Sarmah and Sarla 1995), *Erucastrum abyssinicum* (Rao *et al.* 1996, Sarma and Sarla 1997) as a female parent, *Erucastrum virgatum* (Inomata 2001), *Orychophragmus violaceus* (Li *et al.* 1998), *Crambe abyssinica* (Wang and Luo 1998), and *Enarthrocarpus lyratus* (Gundimeda *et al.* 1992) as a male parent, produced hybrids with *B. juncea* by tissue culture. However, these species have not been reported for hybrid production by artificial pollination. Moreover, hybridization between *B. juncea* and *B. fruticulosa* (used as male and female parents), *D. siifolia* (used as a male parent), *E. sativa*, and *M. arvensis* (used as a female parent) have not been reported by artificial pollination or tissue culture.

In this review, we summarized the possibility of introgression from *B. napus* to a closely related species through *B. juncea*. There has been no report of crossability and productivity of hybrids between *B. juncea* and other Brassicaceae species, except for the six species described above.

Conclusions and Remarks

B. juncea is currently considered as the species that generated hybrids with *B. napus* by spontaneous hybridization, artificial pollination, and tissue culture. Scheffler and Dale (1994) reported that *B. juncea* shows the second highest crossability with *B. napus* after *B. rapa*. Moreover, investigations on *Hortus siccus* revealed that the flowering periods of *B. juncea* and *B. napus* in Japan completely overlap (Matsuo and Itoh 2001) and that *B. juncea* is distributed in all prefectures of Japan (Abe *et al.* 2004, Hokkaido 2010, Kanai *et al.* 2008, Konta *et al.* 2006, MAFF 2012, MLIT 2005, National Museum of Nature and Science 2000, Tsuda and Tabei 2014). Under these conditions, some feral GM canola cultivars have been identified each year since it was initially reported in 2003 (MAFF 2012). Therefore, spontaneous hybridization between *B. juncea* and *B. napus* is possible. However, hybrids between *B. juncea* and GM canola have not been discovered, although hybrids between *B. rapa* and GM canola have been observed around feral GM canola (MAFF 2012). It is possible that *B. juncea* is an autogamous plant, and this trait minimizes cross-pollination under natural conditions. In fact, the maximum spontaneous hybridization between *B. juncea* and *B. napus* was 5.91% in mixed planting (Heenan *et al.* 2007), and spontaneous hybrids were not detected among plants separated at distances of 20 m (Tsuda *et al.* 2012a). Moreover, Devos *et al.* (2011) concluded that feral GM canola has not invaded areas outside its cultivated fields; these plants also occupy ruderal habitats because of its low spontaneous hybridization frequency and fertility. Introgression between crop and feral

and between feral and wild relatives could therefore be very difficult.

On the other hand, Di *et al.* (2009) reported that F₁ hybrids from wild *B. juncea* × GM canola showed higher fertility than that reported previously (Bing *et al.* 1991, 1996, Frello *et al.* 1995, Tsuda *et al.* 2012b). Di *et al.* (2009) also reported that the vigorous vegetative and reproductive growth of wild *B. juncea* resulted in higher fertility rates of the F₁ hybrids. Because wild *B. juncea* consists of multiple genotypes, discussion on introgression potential should also consider genotypic variation, and further investigations should be conducted.

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