Occurrence of metaxenia and false hybrids in Brassica juncea L. cv.
Kikarashina × B. napus

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Imported genetically modified (GM) canola (Brassica napus) is approved by Japanese law. Some GM canola varieties have been found around importation sites, and there is public concern that these may have any harmful effects on related species such as reduction of wild relatives. Because B. juncea is distributed throughout Japan and is known to be high crossability with B. napus, it is assumed to be a recipient of B. napus. However, there are few reports for introgression of cross-combination in B. juncea × B. napus. To assess crossability, we artificially pollinated B. juncea with B. napus. After harvesting a large number of progeny seeds, we observed false hybrids and metaxenia of seed coats. Seed coat color was classified into four categories and false hybrids were confirmed by morphological characteristics and random amplified polymorphic DNA (RAPD) markers. Furthermore, the occurrence of false hybrids was affected by varietal differences in B. napus, whereas that of metaxenia was related to hybridity. Therefore, we suggest that metaxenia can be used as a marker for hybrid identification in B. juncea L. cv. Kikarashina × B. napus. Our results suggest that hybrid productivity in B. juncea × B. napus should not be evaluated by only seed productivity, crossability ought to be assessed the detection of true hybrids.

Key Words: Brassica juncea, B. napus, GM canola, gene flow, metaxenia, false hybrid.
Japan (Shimizu 2003). From these reports, it appeared that *Raphanus*, *B. nigra* and *B. oleracea* could not be recipient candidates for *B. napus*.

On the other hand, both *B. rapa* and *B. juncea* have high crossability with *B. napus* and can be hybridized by both artificial and open pollination (Bing *et al.* 1991, Jørgensen *et al.* 1998, Roy 1980, Scheffler and Dale 1994). *B. juncea* is more widely distributed in Japan than *B. rapa*, and therefore, has a higher possibility of being a recipient of *B. napus* pollens.

Therefore, we have started investigation to clarify introgression from *B. napus* to *B. juncea*. We evaluated the crossability and efficiency of hybrid production in *B. juncea × B. napus*, and we also observed metaxenia in seed coat and that the occurrence of metaxenia and false hybrids was affected by varietal differences in *B. napus*.

Some studies have reported metaxenia in date palm (Swingle 1928), apple (Nebel and Trump 1932), corn (Pinter *et al.* 1987), cotton (Harrison 1931), common bean (Freytag *et al.* 1987), and poppy (Bernáth *et al.* 1987), and *Brassica* species has not yet been reported.

The occurrence of false hybrids (sometimes referred to as exhibiting “matromorphy” or called a matromorphic plant) in *Brassica* species was initially confirmed by observation of same morphological characteristics with wild plants such as the width and thickness of the leaves and the flower and capsule size (Kakizaki 1925). Subsequently, Terao (1934) and Nishi *et al.* (1964) also identified false hybrids in many cross combinations by observing morphological characteristics (number, length, width, and shape of leaves; length of petiole; and root color). Nishi and Hiraoka (1962) estimated that a kind of apomixsis caused false hybrid. Although Mohammad and Sikka (1940) obtained 44 hybrids from 57 pollinated flowers of *B. juncea × B. napus*, identical to our cross combination, they did not detect any false hybrids. Ammitzbøll and Jørgensen (2006) evaluated the hybridity of progeny from *B. napus × R. raphanistrum* using inter-simple sequence repeat (ISSR) analysis and any genetic region derived from *R. raphanistrum* were not detected from some progenies, then these progenies were concluded *B. napus*-like plant, and these were false hybrid.

Here we report the crossability of *B. juncea × B. napus* to reveal the possibility of introgression from *B. napus* to *B. juncea*. This is the first report to reveal metaxenia and false hybrids in progeny of *B. juncea × B. napus*. We also confirmed that the differences in paternal varieties affected the rate of hybrid occurrence.

**Materials and Methods**

*Plant materials*

*B. juncea* L. cv. Kikarashina (Takii & Co., Ltd., Kyoto, Japan) with a yellow seed coat was used as the maternal parent. *B. napus* L. cv. Isuzu-natane (provided by Dr. Yasunobu Ohkawa of the National Institute of Agrobiological Sciences [NIAS]), Norin 16 (Kaneko Seeds Co., Ltd., Gunma, Japan) and Westar (Genebank of NIAS, JP No. 40734) were used as the paternal parents. Isuzu-natane and Norin 16 are Japanese winter-type varieties and Westar is a Canadian spring-type variety, used as a transgenic host. The seed coat color of *B. napus* varieties was dark brown. Seeds were germinated in Petri dishes on filter paper moistened with sterile distilled water (25°C, 48 h). Germinated seeds were incubated at 4°C for 2 weeks for vernalization. They were then transplanted into 15-cm plastic pots and grown in a glass greenhouse programmed at day/night temperatures of 25°C/22°C.

**Interspecific hybridization**

Artificial bud pollination in *B. juncea* L. cv. Kikarashina × *B. napus* was performed to evaluate interspecific crossability. And each siring of parent species were also performed by artificial bud pollination as same as interspecific hybridization. Kikarashina plants were grown in the same greenhouse but in another room to avoid cross pollination. Blooming Kikarashina flowers were emasculated and covered with a parchment bag. Parental plants were also covered with a parchment bag before blooming to avoid contamination with pollen from other varieties. After 1 day, parchment bags were removed and pollen of the paternal parent was applied to the stigma of the maternal parent. After pollination, plants were again covered with parchment bags. About 10 days after pollination, parchment bags were removed, then, the mature pods were harvested ca. 30 days after pollination. Forty buds per plant were crossed and five plants were used to examine crossability. Seed productivity by artificial pollination was reported as the number of seeds per pollinated bud.

**Classification of seeds according to seed coat color**

The progeny seeds from *B. juncea* L. cv. Kikarashina × *B. napus* were classified into four groups (Fig. 1) on the basis of seed coat color: BJ, level 1 (Lv 1), level 2 (Lv 2) and level 3 (Lv 3). Seeds in the BJ group had yellow (Kikarashina) seed coats, those in the Lv 1 group had less than one-third brown area, those in the Lv 2 group had more than one-third brown area with an area that was nearly yellow ochre, and those in the Lv 3 group had completely brown but not as dark as that of *B. napus* (designated BN). The funiculus of the seed was not included as part of the brown area in the seed groups.

**Observation of brown area on seed coat**

To confirm the occurrence of a brown area in the tissue of seed coat and mature embryo, cross sections of the seed, seed coat and embryo were observed. Before preparing the cross sections, seeds were soaked in water in a Petri dish for 6 to 8 h and cut in half before germination. Seeds were then soaked again in water with for 1 to 2 days, after which the embryo was separated from the seed coat. All samples were observed using a stereoscopic microscope (Leica MZ16FA, Leica Microsystems K. K., Tokyo, Japan).
Confirmation of hybridity

Hybridity of progeny from *B. juncea* cv. Kikarashina × *B. napus* was evaluated by observing their morphological characteristics and then confirmed by random amplified polymorphic DNA (RAPD) analysis.

Morphological characteristics observed included flower organ size, the shape of the leaf margin, the leaf rugose, the leaf fairness, waxy leaf and the difference in flowering time.

RAPD analysis was performed according to the following method. Total DNA was extracted from young leaves using ISOPLANT II (NIPPON GENE Co., Ltd., Toyama, Japan) and used as the template for polymerase chain reaction (PCR). Nineteen primers from RAPD 10-mer kits A and B (Operon Technologies, Inc., Alameda, CA, USA) were used (Table 1). PCR fragments were amplified using Gene Taq (NIPPPON GENE CO., Ltd.). The PCR reaction mixture consisted of 2.5 µl of 10× Gene Taq Universal Buffer, 4 µl of dNTP mixture (2.5 mM each), 1.25 µl of primer (20 µM), 0.25 µl of Gene Taq (5 U/µl), 0.5 µl of template DNA and 16.5 µl of water. PCR was performed at 94°C for 5 min; 35 cycles at 94°C for 1 min, 36°C for 1 min and 72°C for 2 min; 72°C for 2 min. PCR was performed using the GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA, USA). The amplified PCR products were mixed with loading dye and loaded on 2.0% agarose gel prepared in 1× TAE buffer. After electrophoresis, the gel was stained with ethidium bromide and bands were observed using an ultraviolet illuminator. RAPD analysis was performed in duplicate.

Results

Seed productivity in interspecific hybridization of *B. juncea × B. napus*

The number of seeds per pollinated bud (seeds/pollination) was calculated as an indicator of seed productivity in interspecific hybridization of *B. juncea × B. napus* (Table 2). The seeds/pollination obtained due to self-pollination of Kikarashina, Isuzu-natane, Norin 16, and Westar was 10.4, 14.2, 15.6 and 18.9, respectively, whereas seeds/pollination in the interspecific cross combination of Kikarashina × Isuzu-natane, Kikarashina × Norin 16 and Kikarashina × Westar was reduced to 3.9, 4.0 and 5.8, respectively. Although the seed productivity in all interspecific cross combinations decreased compared to that in self-pollination of parent varieties, progeny seeds were still obtained.

| primer name | 5’ to 3’ | *B. juncea* specific marker | *B. napus* specific marker |
|-------------|---------|-----------------------------|---------------------------|
|             | number  | band size                   | number                    | band size                   |
| OPA 01      | CAGGCCTTC | 2 300, 600                  | 2 250, 400                |
| OPA 05      | AGGGGTCTTG | 1 550                       | 2 350, 750                |
| OPA 08      | GTGACGTTAG | 1 600                       | 1 450                     |
| OPA 10      | GTGATCGCAG | 3 500, 700, 800             | 1 600                     |
| OPA 12      | TCGGGGATAG | 1 400                       | — a                       |
| OPA 13      | CAGCACCCAC | — —                        | 2 750, 900                |
| OPA 14      | TCTGTCGGTG | 2 800, 1500                | 1 250                     |
| OPA 15      | TTCCGAAACC | 2 400, 1000                | 1 900                     |
| OPA 17      | GACCGCTTGT | — —                        | 1 800                     |
| OPA 19      | CAAACGTGCG | 3 400, 600, 1000           | 1 200                     |
| OPA 20      | GTTGGCATCC | — —                        | 2 500, 800                |
| OPB 03      | CATCCCCCTG | 2 200, 600                 | 2 450, 700                |
| OPB 05      | TGGCCCTTTC | 4 200, 300, 450, 700       | 4 500, 900, 1100, 1200   |
| OPB 08      | TTCCACCTTC | 1 750                       | 1 450                     |
| OPB 10      | CTCTGCTCAC | 1 550                       | — —                       |
| OPB 11      | GTAGACCCGT | 1 400                       | 2 950, 1300               |
| OPB 12      | CTTGGACAGC | 2 250, 650                 | 3 750, 1000, 1200         |
| OPB 17      | AGGGAACGAG | — —                        | 1 500                     |
| OPB 19      | ACACCCGAAG | 1 1000                     | 4 650, 800, 1300, 1600    |

Table 1. A list of primer sequence, the number and band size of each marker used in RAPD analysis

| primer name | 5’ to 3’ | *B. juncea* specific marker | *B. napus* specific marker |
|-------------|---------|-----------------------------|---------------------------|
|             | number  | band size                   | number                    | band size                   |
| OPA 01      | CAGGCCTTC | 2 300, 600                  | 2 250, 400                |
| OPA 05      | AGGGGTCTTG | 1 550                       | 2 350, 750                |
| OPA 08      | GTGACGTTAG | 1 600                       | 1 450                     |
| OPA 10      | GTGATCGCAG | 3 500, 700, 800             | 1 600                     |
| OPA 12      | TCGGGGATAG | 1 400                       | — a                       |
| OPA 13      | CAGCACCCAC | — —                        | 2 750, 900                |
| OPA 14      | TCTGTCGGTG | 2 800, 1500                | 1 250                     |
| OPA 15      | TTCCGAAACC | 2 400, 1000                | 1 900                     |
| OPA 17      | GACCGCTTGT | — —                        | 1 800                     |
| OPA 19      | CAAACGTGCG | 3 400, 600, 1000           | 1 200                     |
| OPA 20      | GTTGGCATCC | — —                        | 2 500, 800                |
| OPB 03      | CATCCCCCTG | 2 200, 600                 | 2 450, 700                |
| OPB 05      | TGGCCCTTTC | 4 200, 300, 450, 700       | 4 500, 900, 1100, 1200   |
| OPB 08      | TTCCACCTTC | 1 750                       | 1 450                     |
| OPB 10      | CTCTGCTCAC | 1 550                       | — —                       |
| OPB 11      | GTAGACCCGT | 1 400                       | 2 950, 1300               |
| OPB 12      | CTTGGACAGC | 2 250, 650                 | 3 750, 1000, 1200         |
| OPB 17      | AGGGAACGAG | — —                        | 1 500                     |
| OPB 19      | ACACCCGAAG | 1 1000                     | 4 650, 800, 1300, 1600    |

a No specific bands were detected.
Occurrence of metaxenia in seed coats of progeny seeds

Seed coat colors of *B. juncea* and *B. napus* were yellow and dark brown, respectively (Fig. 1). Because the seed coat is derived from mother tissue, the seed coat color of progeny seeds obtained from *B. juncea* × *B. napus* was predicted to be yellow; however, the seed coat color of progeny seeds varied from yellow to brown (Fig. 1), with mottled brown areas on yellow seed coats (Fig. 2E). The brown areas were not observed in the mature embryo (Fig. 2B, 2H) and were limited to the seed coats (Fig. 2B, 2E). Winburne (1962) and Soule (1985) defined this phenomenon as metaxenia.

All seeds were classified into four groups according to the extent of metaxenia (Fig. 1 and Table 3). The occurrence rates of seed color type were as follows: BJ: 37%, Lv 1: 28%, Lv 2: 24%, Lv 3: 11% in Kikarashina × Isuzu-natane; BJ: 32%, Lv 1: 24%, Lv 2: 30%, Lv 3: 13% in Kikarashina × Norin 16; and BJ: 55%, Lv 1: 33%, Lv 2: 11%, Lv 3: 1% in Kikarashina × Westar. Metaxenia occurred in the seed coats of all tested interspecific cross combinations, and most seeds were categorized in BJ seeds, while Lv 3 seed was lowest appearance frequency.

Occurrence of false hybrid plants

Some progeny obtained from Kikarashina × Isuzu-natane and Kikarashina × Norin 16 were confirmed hybrids; however, the plant type of numerous progeny closely resembled *B. juncea*. This plant was named “*B. juncea*-like plant.” Because the occurrence of hybrids and false hybrids was closely related to that of metaxenia, we investigated the relationship between metaxenia and hybridity in detail.

First, hybridity of the progeny was evaluated by the representative morphological characteristics and differences in flowering times (Fig. 3). Most characteristics indicated intermediate between *B. juncea* and *B. napus*, however, flowering time was controlled by the dominant trait derived from *B. napus*. Flower organ size of *B. napus* was larger than that of *B. juncea*, and hybrid plants showed an intermediate size (Fig. 3A). For leaf characteristics, *B. napus* had undulate margins, *B. juncea* was highly rugose with incised margins, and hybrid plants were less rugose with toothed margins (Fig. 3B). *B. napus* had waxy leaves, while *B. juncea* had

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**Table 2.** Cross combinations and their seed productivity in *B. juncea* × *B. napus* by artificial pollination

| Cross combination (♀ × ♂) | Pollinated flowers | Seeds per pollination* |
|--------------------------|-------------------|------------------------|
| *B. juncea* × *B. napus* |                   |                        |
| Kikarashina × Isuzu-natane | 193               | 3.9 ± 2.3              |
| Kikarashina × Norin 16    | 181               | 4.0 ± 3.0              |
| Kikarashina × Westar      | 194               | 5.8 ± 3.4              |
| Mean of seeds per pollination | —                | 4.5 ± 1.1              |
| *B. juncea* selfing       |                   |                        |
| Kikarashina               | 204               | 10.4 ± 3.6             |
| *B. napus* selfing         |                   |                        |
| Isuzu-natane              | 100               | 14.2 ± 2.8             |
| Norin 16                  | 100               | 15.6 ± 3.5             |
| Westar                    | 100               | 18.9 ± 3.2             |
| Mean of seeds per pollination | —                | 16.2 ± 2.4             |

*a* Seeds per pollination shows the number of obtained seeds per pollinated flowers and the standard deviation for seeds per pollination in each pollinated plant.

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**Fig. 2.** Cross section of seed, seed coat and embryo of *B. juncea* L. cv. Kikarashina, metaxenia-containing seeds (Lv 2) and Westar. (A, D and G) Kikarashina; (B, E and H) metaxenia-containing progeny seeds from Kikarashina × Westar (Lv 2); (C, F and I) Westar. A, B and C are cross sections of seeds. D, E and F are seed coats that detached from the embryo after stimulation of germination. G, H and I are embryos.

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**Table 3.** Seed number and occurrence rate of each metaxenia level in interspecific hybridization and self pollination of parent species

| Cross combination (seed coat color) | Total number of each metaxenia level | Seed number and occurrence rate of each metaxenia level |
|-------------------------------------|--------------------------------------|--------------------------------------------------------|
| *B. juncea* × *B. napus* (Yellow × Dark brown) |                                  |                                                        |
| Kikarashina × Isuzu-natane          | 748                                  |                                                        |
| BJ                                  | 295                                  | 37 ± 23                                                |
| Lv 1                                | 230                                  | 28 ± 8                                                 |
| Lv 2                                | 164                                  | 24 ± 11                                                |
| Lv 3                                | 59                                   | 11 ± 11                                                |
| Kikarashina × Norin 16              | 846                                  |                                                        |
| BJ                                  | 274                                  | 32 ± 26                                                |
| Lv 1                                | 204                                  | 24 ± 22                                                |
| Lv 2                                | 255                                  | 30 ± 14                                                |
| Lv 3                                | 113                                  | 13 ± 9                                                 |
| Kikarashina × Westar                | 1116                                 |                                                        |
| BJ                                  | 613                                  | 55 ± 21                                                |
| Lv 1                                | 364                                  | 33 ± 14                                                |
| Lv 2                                | 126                                  | 11 ± 3                                                 |
| Lv 3                                | 13                                   | 1 ± 1                                                  |

**B. juncea** selfing (Yellow × Yellow)

| Cross combination | Total number of each metaxenia level | Seed number and occurrence rate of each metaxenia level |
|-------------------|--------------------------------------|--------------------------------------------------------|
| Kikarashina × Kikarashina | 2117 |                                                        |
| BJ                 | 2117                                  | 100                                                    |

**B. napus** selfing (Dark brown × Dark brown)

| Cross combination | Total number of each metaxenia level | Seed number and occurrence rate of each metaxenia level |
|-------------------|--------------------------------------|--------------------------------------------------------|
| Isuzu-natane      | 694                                   | 694 100                                                |
| Norin 16          | 582                                   | 582 100                                                |
| Westar            | 824                                   | 824 100                                                |

*a* Occurrence rate shows the average and the standard deviation for number of seed in each metaxenia level.

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*B. napus*. Flower organ size of *B. napus* was larger than that of *B. juncea*, and hybrid plants showed an intermediate size (Fig. 3A). For leaf characteristics, *B. napus* had undulate margins, *B. juncea* was highly rugose with incised margins, and hybrid plants were less rugose with toothed margins (Fig. 3B). *B. napus* had waxy leaves, while *B. juncea* had
abundant leaf fairness; hybrid plants expressed slight wax and had modest fairness. In addition, delay in flowering time was a typical trait for identifying hybridity. On the other hand, the *B. juncea*-like plants did not have any characteristics of either hybrid plants or *B. napus* (Fig. 3). The *B. juncea*-like plants were evaluated to be putative false hybrids, and hybridity was confirmed by identifying molecular characteristics using RAPD markers.

We selected 19 random primers for detection of hybrid plants by RAPD analysis (Table 1). *B. juncea*- and/or *B. napus*-specific PCR fragments could be detected with these selected primers, and therefore, 27 *B. juncea* species-specific markers and 31 *B. napus* species-specific markers were used (Table 1). All species-specific markers were detected from hybrid plants (Fig. 4d, 4g, 4j and Table 4); whereas, all *B. juncea*-like plants had only *B. juncea*-specific markers; no *B. napus*-specific bands were detected (Fig. 4c, 4f, 4i). The hybridity identified using morphological characteristics completely matched the results of RAPD analysis. These results showed that hybridity can be determined using morphological characteristics, and we concluded that the *B. juncea*-like plants were false hybrids.

**Table 4. Identification of hybridity (false hybrid or hybrid) in progeny plant by morphological characteristic and RAPD analysis**

| Cross combination          | Metaxenia level | Number of observation | Plant type |
|----------------------------|-----------------|-----------------------|------------|
|                            | BJ              | Hybrid                | B. juncea  |
| *B. juncea* × *B. napus*   |                 |                       | 0          |
| Kikarashina × Isuzu-natane |                  |                       | 0          |
| Bj                         | 70              | 0                     | 0          |
| Lv 1                       | 60              | 4                     | 0          |
| Lv 2                       | 93              | 93                    | 0          |
| Lv 3                       | 22              | 22                    | 0          |
| Total                      | 245             | 126                   | 119        |
| Kikarashina × Norin 16     | Bj              |                       |            |
| Lv 1                       | 46              | 40                    | 0          |
| Lv 2                       | 80              | 80                    | 0          |
| Lv 3                       | 39              | 39                    | 0          |
| Total                      | 213             | 88                    | 125        |
| Kikarashina × Westar       | Bj              |                       |            |
| Lv 1                       | 72              | 2                     | 70         |
| Lv 2                       | 76              | 1                     | 75         |
| Lv 3                       | 85              | 85                    | 0          |
| Total                      | 240             | 3                     | 237        |
| *B. juncea* selfing        |                 |                       |            |
| Kikarashina                | Bj              |                       |            |
| Isuzu-natane               | BN              |                       |            |
| Norin 16                   | BN              |                       |            |
| Westar                     | BN              |                       |            |
|                            |                 |                       | 30         |
|                            |                 |                       | 0          |
|                            |                 |                       | 0          |
|                            |                 |                       | 30         |

*B. napus* selfing

| Cross combination | Species Variety | Metaxenia level | Number of observation | Plant type |
|-------------------|-----------------|-----------------|-----------------------|------------|
| Kikarashina       | Bj              |                 | 30                    | 30*        |
| Isuzu-natane      | BN              |                 | 30                    | 0          |
| Norin 16          | BN              |                 | 30                    | 0          |
| Westar            | BN              |                 | 30                    | 0          |

* These plants were not false hybrids, and they were obtained from selfing of Kikarashina.
Relationship between metaxenia and hybridity

Progeny seeds were categorized into four groups according to the extent of metaxenia (Fig. 1 and Table 3). The false hybrids or hybrids of progeny were identified. The relationship between metaxenia and hybridity is summarized in Table 4. All seeds categorized in Lv 2 and Lv 3 groups were hybrids. Furthermore, most progeny from Kikarashina × Westar in of BJ and Lv 1 groups were hybrids; however, most progeny from Kikarashina × Isuzu-natane and Kikarashina × Norin 16 in of BJ and Lv 1 groups were false hybrids as identified by morphological characteristics and RAPD analysis. When Westar was used as the paternal parent, 2 out of 72 plants in the BJ group and 1 out of 76 plants in the Lv 1 group were false hybrids. The occurrence rate of false hybrids was extremely low, and we found no relationship between the occurrence of metaxenia and hybridity.

On the other hand, in both Kikarashina × Isuzu-natane and Kikarashina × Norin 16, all progeny of BJ were false hybrids. Although the progeny of Lv 1 were nearly classified as false hybrids, 4 out of 60 and 6 out of 46 from Kikarashina × Isuzu-natane and Kikarashina × Norin 16 were hybrids. Consequently, hybrid formation efficiencies were 49% and 59% in Kikarashina × Isuzu-natane and Kikarashina × Norin 16, respectively.

The occurrence of metaxenia and hybridity was related and the occurrence of false hybrids was affected by varietal differences.

Discussion

Seed coat develops from integument palisade and pigment cells of maternal parents. Although the maternal parent B. juncea L. cv. Kikarashina has a yellow seed coat, seed coats of the progeny seeds from B. juncea L. cv. Kikarashina × B. napus varied from yellow to brown (Fig. 1, 2). The expression of brown area was affected by paternal varieties; this phenomenon is called metaxenia. Winburne (1962) and Soule (1985) provided a similar definition of metaxenia, a direct effect of the paternal parent on the seed and fruit outside the embryo, endosperm and embryo sac. Metaxenia has been reported in several plants such as seed weight and date of fruit ripening in the date palm (Swingle 1928), pH value and acidity in apples (Nebel and Trump 1932), grain weight of corn (Pinter et al. 1987), pod size of the common bean (Frehtag 1979), and morphine content of the capsule of the poppy (Bernath et al. 2003). For metaxenia, length of the lint and quantity of fuzz were reported in seed coat of cotton (Harrison 1931) and Radics (1977) reported the color and surface of seed coats in interspecific hybridization among six Rorippa species belonging to the Brassicaceae family.

Although numerous interspecific hybrids have been produced during Brassica breeding programs, metaxenia has not been reported in this genus. We speculated that because the seed coats of most B. juncea are brown or dark brown, it is very difficult to detect metaxenia by seed coat color change in this species. Since the maternal parent B. juncea L. cv. Kikarashina has a yellow seed coat, it is easy to detect seed coat color change from yellow to brown. We also speculate that maternal varieties also affect the occurrence of metaxenia, however, we could not use other maternal varieties with yellow seed coat in this experiment. Subsequently, maternal varietal difference should be evaluated.

According to some previous reports on seed coat color, brown is dominant over yellow (Liu et al. 2005, Mingli et al. 2009, Vera et al. 1979). Liu et al. (2005) reported that the occurrence of brown seed coats in B. juncea is controlled by two dominant genes. We observed that seed coats of all F2 seeds (total 14 seeds) obtained from three cross combinations were brown, and this brown color is thought to be dominance over yellow. However, it is not clear that relation between brown color in F2 seeds and these dominant genes.

We obtained many progeny seeds from artificial interspecific hybridization (Table 2). Our results of seed productivity were similar to those of previous reports (Bing et al. 1991, Frello et al. 1995); however, 126 out of 245 seeds (51%) and 88 out of 213 seeds (41%) were false hybrids when Isuzu-natane and Norin 16 were used as the paternal parent, respectively (Table 4). When Westar was used as the paternal parent, only three out of 240 seeds (1.3%) were false hybrids (Table 4). This result showed that the occurrence of false hybrids was significantly affected by varietal differences. Nishi and Hiraoka (1962) observed false hybrids generated from unfertilized egg cells and they thought that false hybrids were a form of apomixis. Moreover, the occurrence of false hybrids is defined as apomixis (Solntseva 2003). However, the mechanism of false hybrids remains to be clarified.

False hybrids have been reported in various hybridizations in Brassicaceae (Ammitzbøll and Jørgensen 2006, Banga 1986, Ito et al. 1948, Kakizaki 1925, Mohammad and Sikka 1940, Nishi and Hiraoka 1962, Nishi et al. 1964, Noguchi 1935, Terao 1934). Most false hybrids mentioned in previous reports had been confirmed by observation of morphological characteristics. As the B. napus-like plants from B. napus × R. raphanistrum did not have any ISSR marker of Raphanus genome (Ammitzbøll and Jørgensen 2006), the B. napus-like plant is assumed to be a false hybrid. Nishi and Hiraoka (1962) observed that the egg cell began embryonic development after stimulation by pollination without fertilization. They estimated that the false hybrid was generated by pseudogamy, a type of apomixis; however, the mechanism for the occurrence of false hybrids has never been completely analyzed. We considered that the B. juncea-like plants in this study are false hybrids, generated by pseudogamy because these plants were confirmed to be related to B. juncea using morphological characteristics and RAPD analysis (Table 4 and Figs. 3, 4).

Because B. juncea has a greater ability to form hybrid progeny with B. napus, B. juncea is ranked second to B. rapa among the recipient species when crossed with B. napus (Scheffler and Dale 1994). The gene flow in B. juncea × B. napus has been investigated by several groups (Bing et al. 1991, 1996, Choudhary and Joshi 2001, Frello et al. 1995, 1997).
Huiming et al. 2007, Liu et al. 2010, Mohammad and Sikka 1940, Song et al. 2010), but false hybrids in B. juncea × B. napus have not been reported. We obtained numerous false hybrids when Isuzu-natane and Norin 16 were used as parental parents. Thus, our results suggest the possibility that introgression cannot be evaluated by only seed productivity.

In cross combination of Kikarashina × Isuzu-natane and Kikarashina × Norin 16, a relationship between metaxenia and hybridity was observed. Most progeny in the BJ and Lv 1 groups were false hybrids and all progeny in Lv 2 and Lv 3 groups were hybrids. In these combinations, the occurrence of metaxenia appeared to be a useful visual marker for estimating hybridity in progeny.

If we reveal the possibility of introgression and the effect of the biosafety from B. napus, we will have to understand the productivity and fitness of the true hybrid. The data of only seed number by interspecific hybridization will lead to overestimate the introgression possibility. The accurate possibility for introgression must be evaluated by recognition of false hybrid. We assure that introgression from B. napus to B. juncea should be assessed by not only true hybrid productivity in progeny but also fitness of hybrid progeny for further study. The next step would be to investigate the fitness of hybrids from B. juncea × B. napus.

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