Introduction

The Brassicaceae comprise about 330 genera and 3700 species, of which two genera (*Brassica* and *Raphanus*) are widely grown for edible oils, vegetables, spices, ornamental flowers, and forage crops around the world. The genus *Brassica* contains three monogenomic diploid species, namely *B. rapa* L. (2n = 20, AA genome), *B. nigra* (L.) Koch (2n = 16, BB), and *B. oleracea* L. (2n = 18, CC), and their naturally produced allotetraploid species, *B. napus* L. (2n = 38, AACC), *B. juncea* (L.) Czern. & Coss. (2n = 36, AABB), and *B. carinata* A. Braun (2n = 34, BBCC). In contrast, the genus *Raphanus* contains only one species of agricultural importance, *R. sativus* (2n = 18, RR) (Mizushima 1950). Other crop species in this family include *Eruca sativa* L. (rocket salad), *Nasturtium officinale* R. Br. (watercress), *Wasabia japonica* Matsumura (wasabi), *Matthiola incana* (L.) R. Br. (stock), and *Erysimum cheiri* (L.) Crantz (English wallflower). A number of wild relatives, on the other hand, have been evaluated as genetic resources in the development of new cultivars with biotic and abiotic stress resistance for use in agriculture in response to the diversification of ecotypes of diseases and pests, changing food preferences, advances in production technology, the use of new approaches such as in vitro breeding programs, and the need for economical production of F₁ seed. To produce potential new cultivars, interspecific and intergeneric hybridizations have been performed between cultivated species and between cultivated species and their wild relatives. Furthermore, interspecific and intergeneric hybrids have been successfully produced using embryo rescue techniques. In this paper, we review the interspecific and intergeneric incompatibilities between Brassicaceae crops and their wild relatives, and the production, characterization, and improvement of synthetic amphidiploid lines, alien gene introgression lines, alloplasmic lines, monosomic alien chromosome addition lines, and monosomic alien chromosome substitution lines. The goal is to provide useful materials to support practical breeding strategies and to study the genetic effects of individual chromosomes on plant traits, the number of genes that control a trait, their linkage relationships, and genetic improvement in Brassicaceae crops.

Key Words: interspecific and intergeneric hybridization, chromosomal engineering, Brassicaceae, amphidiploid, monosomic alien chromosome addition, alloplasmic, alien gene introgression.
Hybridization of Brassicaceae crops

1. Hybridization barriers in interspecific and intergeneric hybridizations

The barriers to interspecific and intergeneric hybridization during sexual reproduction can be divided between those that operate before and after fertilization. Stebbins (1958) suggested that the pre-fertilization barriers might be due to failure of pollen germination, pollen tube growth, or pollen tube penetration of the ovule, whereas post-fertilization barriers would arise from degeneration of the hybrid embryo, male and female sterility in the hybrid plants, and lethality in the hybrid progeny. Khush and Brar (1992) surveyed the hybridization barriers in distant hybridization, and offered effective techniques to overcome both pre- and post-fertilization barriers.

Several researchers have investigated the nature of the pre-fertilization barriers for interspecific and intergeneric hybridizations in the Brassicaceae. For example, Matsuzawa (1983) evaluated the magnitude of the pre-fertilization barriers using a pollen germination index:

\[
P.G.I. = \frac{(1b + 2c + 3d + 4e)}{(a + b + c + d + e)}
\]

where \(0 \leq P.G.I. \leq 4\), and \(a, b, c, d,\) and \(e\) represent the numbers of pistils in which no pollen grain is recognized on the stigma (with a score of 0), pollen grains do not germinate on the stigma (1), pollen grains germinate on the stigma but do not enter (2), pollen tubes reach the style tissue (3), and pollen tubes penetrate the style tissue to reach near or to the ovule (4), respectively. Kerlan et al. (1992) proposed a different equation for the index of pollination compatibility \(I\):

\[
I = x + 2y + 3z
\]

where \(x, y,\) and \(z\) are the corresponding scores for the numbers of pollen grains that germinate divided by the number that arrived on the stigma (with a score of 0, 1, 2 or 3 assigned to the ratio values of 0, 0–0.5, 0.5–0.7 and 0.7–1, respectively), of pollen tubes that grew into the style tissue (with a score of 0, 1 and 2 corresponding to 0, 1 to 5 and more than 6 pollen tubes, respectively), and of pollen tubes that penetrated the ovule (with a score of 0, 1 and 2 assigned to the same scores used for the \(y\) parameter), respectively.

In general, the pre-fertilization barriers between cultivated species and their wild relatives in interspecific and intergeneric hybridizations can be overcome by means of bud pollination and using wild species as the pistillate parent, although the proportion of successful outcomes may depend on the direction and combination in each crossing. In particular, when a self-compatible line was used as the pistillate parent, the pollen tubes of cultivated species grew well and penetrated into the ovule. Therefore, the pre-fertilization barriers in interspecific and intergeneric hybridizations seem to be similar to the self-incompatibility observed in Brassicaceae species, although genetic analysis using the \(F_2\) population between an interspecific-incompatible line and a self-compatible cultivar could not confirm the responsibility of the line and the cultivar in the differences.

---

Fig. 1. Schematic diagram of the distant hybridization breeding system between AA and BB genome species concerned (quoted from Matsuzawa et al. 1996, revised). (I): Synthetic amphidiploid line (SADL), (II): Alien gene(s) introgression line (AGIL), (III): Alloplasmic line (ALPL), (IV): Monosomic alien chromosome addition line (MAAL), (V): Monosomic alien chromosome substitution line (MASL) 1) AB, AABB and AAB (ABB) show genomes for amphihaploid, amphidiploid and sesquidiploid, respectively. 2) A’ (B’) means some genetic modification via recombination between each comple-

---

studies of the growth of hybrid embryos, Nishi et al. (1970) and Inomata (1977) produced interspecific and intergeneric hybrids through embryo and ovary culture techniques, respectively.

Matsuzawa et al. (1996) suggested a system for the use of interspecific and intergeneric hybridizations to develop five types of hybrid lines: synthetic amphidiploid lines, alien gene introgression lines, alloplasmic lines, monosomic alien chromosome addition lines, and monosomic alien chromosome substitution lines (Fig. 1). These hybrid lines would be valuable genetic resources both for breeding more productive cultivars with novel agronomic traits and for research to better understand each chromosome and gene in these hybrids. In each instance, it is first necessary to develop true \(F_1\) hybrids and as many of their progeny as possible.

In this communication, we review current knowledge of the interspecific and intergeneric cross-incompatibility between Brassica crops and their wild relatives, and the production, characterization, and improvement of the five types of hybrid lines.
of the interspecific incompatibility phenotypes (Udagawa et al. 2010).

In contrast to pre-fertilization barriers, Wilmar and Hellendoorn (1968) evaluated post-fertilization barriers by means of an anatomical survey of the development and growth of embryos of Brussels sprouts (B. oleracea). The abortion of hybrid embryos in interspecific and intergeneric hybridizations resulted from abnormal development of the endosperm, and the underlying mechanisms have been explained by various hypotheses: the endosperm balance number (Johnston et al. 1980), activation of the polar nuclei (Nishiyama and Yabuno 1978), and genomic imprinting in the endosperm (Kinoshita 2007). Recently, Tonosaki et al. (2013) performed quantitative trait locus analysis during the formation of hybrid seed in intergeneric hybridization between B. rapa and R. sativus. Researchers have developed effective in vitro culture techniques using pollinated flowers, ovaries, ovules, and embryos to rescue hybrid embryos that would otherwise degenerate during the early stages of their development in interspecific and intergeneric hybridizations. Bang et al. (1996 to 2009) and Jeong et al. (2003 to 2009) inspected the post-fertilization barriers between cultivated species and wild relatives in intergeneric hybridizations, and produced many potential hybrid progeny through in vitro procedures for each of the five breeding strategies shown in Fig. 1.

2. Production of novel F1 hybrid plants by means of embryo rescue

Until the 1980s, F1 hybrid plants had been produced from interspecific and intergeneric hybridizations mainly among B. rapa, B. nigra, B. oleracea, B. napus, B. juncea, B. carinata and R. sativus. Subsequently, a number of F1 hybrid plants were produced from interspecific and intergeneric hybridizations between Brassica crops and wild relatives in the genera Brassica, Sinapis, Diplotaxis, Moricandia, Eruca, and Orychophragmus. At least 45 species of wild relatives were reported in reviews of the literature on the production of F1 hybrids (Harberd and McArthur 1980, Kaneko et al. 2009, Prakash et al. 1999). Numerous novel F1 hybrids have been produced through the development of various embryo rescue techniques: embryo culture (Nishi et al. 1962, 1970), ovary culture (Inomata 1977, 1978a, 1978b, Matsuzawa 1983), ovule culture (Momotaz et al. 1998) and placenta culture (Bang et al. 2007), as well as through combinations of these techniques, namely ovary culture followed by embryo or ovule culture, placenta culture followed by embryo culture, and successive ovary, ovule, and embryo culture. Ovary culture has been widely performed to overcome abortion of hybrid embryos during early stages of development, but this technique failed when a species such as B. oleracea with high regeneration ability was used as the pistillate parent. When R. sativus and B. oleracea are used as the pistillate parent, embryo culture is effective, although the hybrid embryo must develop to at least the heart-shaped stage before culture can begin. Placenta culture, in which placenta-attached ovules are removed and cultured starting about 15 days after pollination, may be more effective than ovule and embryo culture when B. oleracea is used as the pistillate parent.

3. Synthetic amphihaploid lines

Synthetic amphihaploid lines have been developed by doubling the chromosome number of F1 hybrid plants as well as by means of somatic cell fusion. Researchers have sporadically obtained amphihaploid plants in the F2 generation of amphihaploid F1 hybrids through fusion of the unreduced F1 female and male gametes. These amphihaploid lines show low pollen and seed fertility due to unequal chromosome segregation in pollen mother cells in initial generations. With advancing generations, however, the hybrids generally develop high pollen and seed fertility as a result of increasing meiotic stabilization (Howard 1938, Kato and Tokumasu 1976, Sarashima 1973). Six artificial amphihaploid Brassica lines, four Raphanobrassica lines and three Brassicoraphanus lines have been developed and maintained for use as breeding materials (Table 1 and Fig. 2).

Synthetic fertile amphihaploid lines derived from hybrids between cultivated crops and their wild relatives have been used as genetic stocks in breeding programs, and as bridging materials for the transfer of desirable traits from wild species into cultivated ones. The fertile amphihaploid lines obtained through doubling of the chromosome number of sterile hybrids or through somatic hybridization between a cultivated crop and wild relatives have led to novel crops, including ‘Hakuran’ (Brassica campestris × B. oleracea), ‘Radicole’ (Raphanus sativus × B. oleracea), and ‘Raparadish’ (B. campestris × R. sativus) (Namai 1987). As a result, several novel cultivars, ‘Gifu-Green’ (Takada 1985), ‘Senpou no. 1 and no. 2’ (Nagano 1988), ‘Hanakkori’

| Cross combination | Genome | Generations | No. of individuals observed | Seed fertility |
|--------------------|--------|-------------|-----------------------------|---------------|
| B. rapa × B. oleracea | AACC | F4 | 6 | 0.72 ± 0.63 |
| B. oleracea × B. rapa | CCAA | F4 | 10 | 1.19 ± 2.11 |
| B. rapa × B. nigra | ABBB | F7 | 1 | 7.51 |
| B. nigra × B. rapa | BBAA | F6 | 3 | 2.21 ± 1.19 |
| B. nigra × B. oleracea | BBBB | F6 | 4 | 9.16 ± 2.48 |
| B. oleracea × B. nigra | CCBB | F7 | 5 | 1.55 ± 0.99 |
| R. sativus × B. oleracea | RRCC | F10 | 3 | 2.39 ± 0.88 |
| B. oleracea × R. sativus | CCRR | F4 | 5 | 0.76 ± 0.46 |
| R. sativus × B. rapa | RRRA | F7 | – | 1.3 |
| B. rapa × R. sativus | AARR | F7 | – | 0.01 |

B. napus ‘N350’ is 21.15 seeds, B. juncea ‘Aso-Takana’ 11.54, and B. carinata.

Ca115 8.72, respectively.

a Data from Akaba et al. 2009b.

b Data from Matsuzawa et al. 2000.
Hybridization of Brassicaceae crops

The natural hybridization of Brassicaceae crops has been extensively studied, particularly in the context of developing new varieties with improved traits. For example, the artificial hybridization of *Raphanobrassica* (2n = 36, RRCC) derived from hybrid between *R. sativus* (2n = 18, RR) and *B. oleracea* (2n = 18, CC) has been a focal point in breeding programs for leafy vegetables. This hybrid has traits from both parents, including disease resistance and improved yields, making it a valuable tool in genetic diversity.

![Fig. 2. Artificial synthetic amphidiploid plants. (A) Raphanobrassica (2n = 36, RRCC) derived from hybrid between *R. sativus* (2n = 18, RR) and *B. oleracea* (2n = 18, CC), (B) Brassicoraphanus (2n = 34, FFFRR) derived from hybrid between *B. rapa* (2n = 16, FF) and *R. sativus* (2n = 18, RR), (C) Brassicoraphanus (2n = 50, FFFFR) derived from hybrid between *B. fruticulosa* (2n = 32, FFFF) and *R. sativus* (2n = 18, RR), (D) Brassicoraphanus (2n = 36, OORR) derived from hybrid between *B. oxyrrhina* (2n = 18, OO) and *R. sativus* (2n = 18, RR).](image)

This artificial hybridization, along with natural hybridizations, has led to the development of new varieties with enhanced leafy salad vegetable characteristics. For instance, the amphidiploid line *B. rapa* × *D. tenuifolia* (Jeong et al. 1997) and 'F1-Hosoda-Wase' (Takada 2006) were developed from amphidiploids between *B. rapa* and *D. tenuifolia*, and have been released by Japanese seed companies. Recently, we produced a number of amphidiploid lines between crop cultivars and wild relatives: *Brassica mauroorum* × *R. sativus* (Bang et al. 1997, 2006), *Brassica fruticulosa* × *R. sativus* (Bang et al. 2007), *Brassicoraphanus* × *D. tenuifolia* (Bang et al. 1996, 2012), and *B. rapa* × *D. tenuifolia* (Jeong et al. 1997, Matsuzawa et al. 1997, 2000, Fig. 2C), *Brassicoraphanus* × *D. tenuifolia* (Bang, unpublished data), *B. oxyrrhina* × *B. oleracea* (Bang, unpublished), *B. rapa × Diplotaxis tenuifolia* (Jeong et al. 2009), and *B. oleracea × D. tenuifolia* (Bang, unpublished).

Diplotaxis tenuifolia has considerable potential as a healthy leafy salad vegetable because of its content of bioactive photochemicals, such as those that are involved in intermediate C3–C4 photosynthetic activity, and is commonly eaten as "rocket", a name it shares with some *Eruca* species (Martinez-Sanchez et al. 2007). The amphidiploid line of *B. rapa × D. tenuifolia* has desirable morphological characteristics, vigorous growth, and the attractive fragrance of *D. tenuifolia*, but its seed fertility was insufficient for commercial production as a crop. However, the line produced abundant seeds that grew into sesquiploid plants, which inherited desirable traits when they were backcrossed with commercial cultivars of *B. rapa* (Jeong et al. 2009). Therefore, the amphidiploid lines of *B. rapa × D. tenuifolia* and *B. oleracea × D. tenuifolia* could both be useful genetic resources for the breeding of new leafy salad vegetables.

### 4. Monosomic alien chromosome addition lines

Monosomic alien chromosome addition lines (MAALs), have been examined for genetic analysis in breeding programs for agronomic traits, and in studies of genes that are assumed to be located on the added chromosome. The advantages of using MAALs involve the possibility of assigning species-specific genes or characteristics to particular chromosomes, and the potential to transfer desirable agronomic traits between species (Matsuzawa et al. 1996, McGrath and Quiros 1990, Namai 1987, Prakash and Chopra 1990). Since Kaneko et al. (1987) produced MAALs that combined *R. sativus* with *B. oleracea* and Quiros et al. (1987) produced MAALs that combined *B. rapa* with *B. oleracea*, several MAALs have been bred through interspecific and intergeneric hybridizations between crop species and wild relatives. These include combinations of *B. campestris* with *Brassica alboglabra* (Chen et al. 1992), *R. sativus* with *E. sativa* (Bang 1996), *R. sativus* with *Sinapis arvensis* (Bang 1996), *B. campestris* with *B. oxyrrhina* (Srinivasan et al. 1998), *R. sativus* with *Moricandia arvensis* (Bang et al. 2002), *B. napus* with *Sinapis alba* (Wang et al. 2005), *B. napus* with *R. sativus* (Akaba et al. 2009b), and *B. napus* with *B. juncea* (Takashima et al. 2012).

To produce and classify MAALs, the cross-incompatibility that exists in successive backcrosses with the background species (as the recurrent parent) must be overcome, and it is essential to use specific markers for the chromosome from the donor species to assist in selection. The cross-incompatibility that often appears in successive backcrosses with the background species and the chromosome donor. These barriers in the first backcross may be reflected in male and female sterility of the amphidiploid as well as in pre- and post-fertilization barriers (Table 2). In the intergeneric hybridization between *M. arvensis* and *R. sativus* (Bang et al. 1996a), the amphidiploid plants showed more stable chromosome association at metaphase I in pollen mother cells.

### Table 2. Seed fertility by backcrossing between amphidiploid plants and their parents

| Pistillate parent | Genome | Male parent | Genome | Seed fertility |
|-------------------|--------|-------------|--------|---------------|
| Artificial *B. napus* | AACC   | *B. rapa*   | AA     | 2.50 ± 1.55   |
| Natural *B. napus*  | AACC   | ditto       | AA     | 9.47 ± 3.42   |
| Artificial *B. napus* | CCAA   | ditto       | AA     | 3.06 ± 3.13   |
| Artificial *B. juncea* | AABB  | *B. rapa*   | AA     | 0.26          |
| Natural *B. juncea* | AABB   | ditto       | AA     | 9.01 ± 3.40   |
| Artificial *B. juncea* | BBAA | ditto       | AA     | 2.25 ± 0.62   |
| Artificial *B. carinata* | BBCC  | *B. oleracea* | CC    | 1.72 ± 0.58   |
| Natural *B. carinata* | BBCC   | ditto       | CC     | 0.37 ± 0.04   |
| Artificial *B. carinata* | CCBB  | ditto       | CC     | 0.01 ± 0.01   |
| *Raphanobrassica* | RRCC   | *R. sativus* | RR    | 0.12 ± 0.06   |
| *Raphanobrassica* | RRAA   | *R. sativus* | RR    | 0.03 ± 0.03   |
| *Brassicoraphanus* | AARR   | *B. rapa*   | AA     | 0.95 ± 1.59   |

* Data from Kaneko et al. (1987).
and higher pollen fertility than the amphihaploid plants. In
the backcross of the amphidiploid with the parent R. sativus,
there was no pre-fertilization barrier, but a post-fertilization
barrier appeared in the form of abortion of the hybrid em-
byos, although this was overcome using an embryo rescue
 technique. Occasionally, male and female sterility of the
BC1 hybrid plants (sesquidiploids) and subsequent pre- and
post-fertilization barriers also appear in successive back-
crossing (usually by BC2). On the other hand, these barriers
in the first and second backcrosses were not observed in
other cross-combinations to R. sativus × S. arvensis (Bang
1996), R. sativus × E. sativa (Bang 1996), and B. campestris
× B. oxyrrhina (Srinivasan et al. 1998). However, whole
MAALs have not been developed from any cross-
combinations in the Brassicaceae thus far. The genetic sys-
tem that controls hybridization barriers in interspecific and
intergeneric hybridizations seems to function between the
nuclear and cytoplasmic genomes in the recurrent parent’s
genetic background and a few chromosomes from the donor
genome.

These MAALs have been effectively exploited to ana-
yze various agronomic and genetic traits to homoelogous
relationships in the genus Brassica (Kaneko et al. 2002);
disease resistance to blackleg (Leptosphaeria maculans;
Chevre et al. 1996), turnip mosaic virus (TuMV; Kaneko
et al. 1996), beet cyst nematode (Heterodera schachtii;
Peterka et al. 2004), and clubroot (Plasmodytophora brassicae;
Akaba et al. 2009a); and photorespiratory characteristics
(Bang et al. 2009); and in restoring pollen fertility (Akaba
et al. 2009b, Budahn et al. 2008) and avoiding lethality in
an alloplasmic line (Tsutsui et al. 2011).

5. Alloplasmic lines

The production of F1 hybrid seed of Brassica crops is cur-
cently based on either self-incompatibility or cytoplasmic
male sterility (CMS). The self-incompatibility controlled by
S alleles is not sufficiently stable because it is affected by
various environmental factors (Horisaki and Niikura 2004).
CMS is a maternally inherited trait, and has been ascer-
tained to be more successful because of the stable expres-
sion of pollen sterility without any obvious changes in vege-
tative growth or female fertility. Alloplasmic lines have
been developed by exchanging the cytoplasm through inter-
specific and intergeneric hybridizations followed by suc-
cessive backcrossing to the nuclear genome’s donor species.
These hybrids acquire the advantages of CMS and useful
agronomic traits, such as herbicide resistance, modification
of flavor and nutrient content, and morphogenetic potential
(Namai 1987).

Sarashima et al. (1990a, 1990b, 1990c) produced many
kinds of alloplasmic lines of monogenic Brassicaceae
crops (B. rapa, B. oleracea, and R. sativus), but most of
them did not exhibit any agronomically useful traits, except
for CMS of B. oleracea, which carried R. sativus cytoplasm
but no R. sativus nuclear genetic material. The CMS in the
latter alloplasmic line may have been induced by a lack of
genetic affinity between the mitochondrial genome and the
nuclear genome. The cytoplasm from several wild relatives
has been introduced as a source of CMS into several
Brassica crops (B. rapa [B. campestris], B. juncea, and
B. napus) through interspecific and intergeneric hybridiza-
tions followed by successive backcrossings. These wild rela-
tives include Arabidopsis thaliana, Diplotaxis berthautii,
Diplotaxis muralis, Diplotaxis catholica, Diplotaxis siiolia,
Diplotaxis erucoides, B. oxyrrhina, Brassica tournefortii,
Enarthrocarpus lyratus, E. sativa, Eruc a vesicaria,
M. arvensis, and Trachystoma ballii (reviewed by Kaneko
et al. 2009, Prakash et al. 1999). Recently, some new CMS
sources have been introduced into several Brassica crops in
addition to R. sativus, including B. maurorum (Bang et al.
2011), B. oxyrrhina (Shim et al. 2010, 2011a, 2012),
D. tenuifolia (Shim et al. 2011b), and B. fruticulosa (Tsutsui
2013).

When alloplasmic lines are produced through interspecif-
ic and intergeneric hybridizations followed by successive
backcrossings, there are various affinity natures that can be
observed in cross-combinations and in different genera-
tions. For example, two alloplasmic B. juncea lines were
produced using amphidiploid lines of D. erucoides ×
B. campestris and D. berthautii × B. campestris as cytoplas-
mic donor plants (Malik et al. 1999). Alloplasmic B. napus
carrying A. thaliana cytoplasm was obtained from six back-
crosses of the somatic hybrid to B. napus (Leino et al.
2003). Alloplasmic B. campestris with E. sativa cytoplasm
was produced via a sesquidiploid plant obtained from an
amphihaploid F1 hybrid by open pollination (Matsuzawa
et al. 1996), and alloplasmic R. sativus with E. vesicaria cyto-
plasm was produced via the same method (Bang 1996).
Alloplasmic R. sativus with M. arvensis cytoplasm was also
produced via a sesquidiploid plant generated from back-
crossing of an amphihaploid F1 hybrid to R. sativus, fol-
lowed by embryo rescue (Bang et al. 1996a, 2002). As
Namai (1976) indicated, sesquidiploid plants can be useful
as a bridge plant to generate an alloplasmic line with no fer-
tilization barriers in various interspecific and intergeneric
hybridizations.

Occasionally, male or female sterility of sesquidiploid
plants and subsequent pre- and post-fertilization barriers
also appear in successive backcrossings. When this hap-
pens, an alloplasmic line cannot be generated. The pre- and
post-fertilization barriers can often be overcome by using
bud-pollination and embryo rescue, respectively. The fe-
male sterility of sesquidiploid plants, which is a primary
cause of hybrid breakdown, can also be overcome by using
F1 cultivars as the pollen parents and subsequently as the
recurrent parent because male and female gametes with di-
verse genotypes can be confirmed in each generation. In
sesquidiploid hybrids between Brassica crop species and
their wild relatives, Tsutsui et al. (2011) found that many
progeny involving alloplasmic lines could be generated
through repeated pollination without embryo rescue, but
that no progeny could be obtained by bud pollination and subsequent embryo rescue techniques owing to severe chlorosis. It is likely that the female sterility in the sesquidiploid plants was caused by delayed maturation of the egg cells. As mentioned above, using synthetic alloplyploids between different combinations as bridge plants to transfer cytoplasm from wild relative species and ascertaining the cause of sexual incompatibility barriers in the hybrid plants as accurately as possible may provide practical ways to improve the efficiency of producing alloplasmic lines.

6. Alien gene introgression lines

Until the 1980s, alien gene introgression lines had been produced by hybridization mainly between the Brassicaceae crops, resulting in the development of novel cultivars with desirable agronomic traits. Examples include Chinese cabbage (B. rapa) cultivars resistant to bacterial soft rot (Shimizu et al. 1962), early-maturing cultivars of B. napus (Namai 1976, 1987), and virus-resistant cultivars of B. oleracea (Namai 1976). Warwick (1993) described wild genera of the tribe Brassiceae as the sources of various agronomically interesting traits, such as hairiness, resistance to pod shattering, photosynthesis, soil adaptation and disease resistance. A large collection of wild relative species of Brassica crops have been screened as sources of resistance to blackleg (also called stem canker), alternaria leaf spot (Alternaria spp.), and clubroot. Interspecific and intergeneric hybrids between wild relatives and Brassica crop species, and their progeny, would certainly provide valuable breeding materials for the introgression of agronomically useful traits. Siemens (2002) reviewed the possibility of using interspecific and intergeneric hybridizations between B. napus and some wild relatives in the tribe Brassiceae to introduce resistance to important fungal pathogens. As Namai (1987) indicated, the introgression of desirable genes from one species to another in the Brassicaceae has been accomplished through meiotic allozygenesis in Brassica crops. Bang (1996) described the homoeologous pairing between an R. sativus chromosome and an S. arvensis chromosome in an S. arvensis monosomic addition line of R. sativus (MAAL-d type). A pollen fertility restoration gene from the cytoplasmic donor species has been transferred to alloplasmic Brassica oilseed crops through homoeologous pairing between the AB and M chromosomes in an M. arvensis MAAL of B. juncea (Prakash et al. 1999), and between the A and E chromosomes in an E. lyra MAAL of B. rapa (Banga et al. 2003). Recently, resistance to the beet cyst nematode and clubroot has been transferred from R. sativus to B. napus using R. sativus MAALs of B. napus (Akaba et al. 2009a, Budahn et al. 2008). Analysis of genes and genomes and the efficient induction and identification of recombination for introgression might be prerequisites for the development of an alien gene introgression line. The genetic linkage maps that have been recently constructed for some Brassica crops will offer useful tools to support genetic introgression.

7. Monosomic alien chromosome substitution lines

Many disomic alien chromosome substitution lines (DASLs) have been bred in wheat cultivars. In these lines, one member of a chromosome pair is replaced by a homoeologous chromosome from Secale, Hordeum, and Aegilops (Khush 1973). Such DASLs can be used to study the genetic effects of individual chromosomes, and to estimate the number of genes that control a given trait and their linkage relationships. These lines can be generally developed in the progeny after self-fertilization of a monosomic alien chromosome substitution line (MASL). In this breeding system, the MASLs can be generated only when the two corresponding chromosomes are homoeologous and complement each other, especially within diploid species. In the Brassicaceae, DASLs and MASLs generated using the technique described above have not been found, as far as we know. DASLs are known to occur spontaneously during successive backcrossings after interspecific hybridization. By analyzing the meiotic configuration, Banga (1988) reported the development of C genome chromosome substitution lines in B. juncea that arose spontaneously in an interspecific hybridization between B. juncea and B. napus. Although phylogenetic relationships have been suggested in Brassica (Armstrong and Keller 1981, 1982, Röbbelen 1960) as well as between Brassica and allied genera (Quiros et al. 1988), we cannot currently confirm the generation of MASLs among segregated amphidiploid lines, as techniques are not currently available for the critical analysis and identification of each chromosome. Chen et al. (1997) produced B. alboglabra chromosome substitution B. rapa lines. In this context, in situ hybridization procedures, the production of somatic chromosome maps by various imaging methods, and the development of species-specific markers would strongly support the development and analysis of MASLs.

Conclusions

Five categories of hybrid progeny can be produced through interspecific and intergeneric hybridization: synthetic amphidiploid lines, alien gene introgression lines, alloplasmic lines, monosomic alien chromosome addition lines, and monosomic alien chromosome substitution lines. These approaches can provide materials that are useful both for developing practical breeding strategies and for studying the genetic effects of individual chromosomes on plant traits. This includes estimating the number of genes that control a trait, describing their linkage relationships, and using this knowledge for genetic improvement of Brassicaceae crops. For these research programs to be successful, it will be necessary to produce and maintain as many true lines as possible of these five hybrid types.

Literature Cited

Akaba, M., Y. Kaneko, K. Hatakeyama, M. Ishida, S.W. Bang and
Y. Matsuzawa (2009a) Identification and evaluation of clubroot resistance of radish chromosome using a Brassica napus-Raphanus sativus monosomic addition line. Breed. Sci. 59: 203–206.

Akaba, M., Y. Kaneko, Y. Ito, Y. Nakata, S.W. Bang and Y. Matsuzawa (2009b) Production and characterization of Brassica napus-Raphanus sativus monosomic addition lines mediated by the synthetic amphidiploids “Raphanobrassica”. Breed. Sci. 59: 109–118.

Armstrong, K.C. and W.A. Keller (1981) Chromosome pairing in haploids of Brassica campestris. Theor. Appl. Genet. 59: 49–52.

Armstrong, K.C. and W.A. Keller (1982) Chromosome pairing in haploids of Brassica oleracea. Can. J. Genet. Cytol. 24: 735–739.

Bang, S.W. (1996) Studies on the intergeneric crossability and productivity of intergeneric hybrids between the C3- and C4-species. PhD Thesis. Univ. of Tokyo Agric. and Technol., Tokyo, pp. 1–276.

Bang, S.W., Y. Kaneko and Y. Matsuzawa (1996a) Production of intergeneric hybrids between Raphanus sativus and Moricandia. Plant Breed. 115: 385–390.

Bang, S.W., Y. Kaneko and Y. Matsuzawa (1996b) Production of intergeneric hybrids between Raphanus and Sinapis and the cytogenetic properties of their progenies. Breed. Sci. 46: 45–51.

Bang, S.W., D.Iida, Y. Kaneko and Y. Matsuzawa (1997) Production of new intergeneric hybrids between Raphanus sativus and Brassica wild species. Breed. Sci. 47: 223–228.

Bang, S.W., Y. Kaneko and Y. Matsuzawa (1998) Cytogenetical stability and fertility of an intergeneric amphidiploid line synthesized from Brassica mauroorum Durieu and Raphanus sativus L. Bull. Coll. Agric., Utsunomiya Univ. 17: 23–29.

Bang, S.W., Y. Kaneko and Y. Matsuzawa (2000) Cytogenetical stability and fertility in an intergeneric amphidiploid line synthesized from Brassica fruticulosa Cyr. sp. mauritanica (Coss.) Maire and Raphanus sativus L. Bull. Coll. Agric., Utsunomiya Univ. 17: 67–73.

Bang, S.W., Y. Kaneko, Y. Matsuzawa and K.S. Bang (2002) Breeding of Moricandia arvensis monosomic chromosome addition lines (2n = 19) of alloplasmic (M. arvensis) Raphanus sativus. Breed. Sci. 52: 193–199.

Bang, S.W., Y. Mizuno, Y. Kaneko, Y. Matsuzawa and K.S. Bang (2003) Production of intergeneric hybrids between the C1-C3 intermediate species Diplotaxis tenuifolia (L.) DC. and Raphanus sativus L. Breed. Sci. 53: 231–236.

Bang, S.W., K. Sugihara, B.H. Jeong, R. Kaneko, E. Satake, Y. Kaneko and Y. Matsuzawa (2007) Production and characterization of intergeneric hybrids between Brassica oleracea and a wild relative Moricandia arvensis. Plant Breed. 126: 101–103.

Bang, S.W., O. Ueno, Y. Wada, S.K. Hong, Y. Kaneko and Y. Matsuzawa (2009) Production and photoreproductive characteristics of Raphanus sativus (C3)-Moricandia arvensis (C3-C2 intermediate) monosomic and disomic addition lines in each parental cytoplasmic background and their photoreproductive characteristics. Plant Prod. Sci. 12: 70–79.

Bang, S.W., K. Tsutsui, S.H. Shim and Y. Kaneko (2011) Production and characterization of the novel CMS line of radish (Raphanus sativus) carrying Brassica mauroorum cytoplasm. Plant Breed. 130: 410–412.

Bang, S.S. (1988) C-genome chromosome substitution lines in Brassica juncea (L.) Coss. Genetica 77: 81–84.

Bang, S.S., P.B. Bhaskar and I. Ahuja (2003) Synthesis of intergeneric hybrids and establishment of genomic affinity between Diplotaxis catholica and crop Brassica species. Theor. Appl. Genet. 106: 1244–1247.

Budahn, H., O. Schrader and H. Peterka (2008) Development of a complete set of disomic rape-radish chromosome-adding lines. Euphytica 162: 117–128.

Chen, B.Y., V. Simonsen, C. Lanner-Herrr and W.K. Heneen (1992) A Brassica campestris-alboglabra addition line and its use for gene mapping, intergeneric gene transfer and generation of trisomics. Theor. Appl. Genet. 84: 592–599.

Chevre, A.M., F. Ever, P. This, P. Barret, X. Tanguy, H. Brun, M. Delseny and M. Renard (1996) Characterization of Brassica nigra chromosomes and of blackleg resistance in B. napus-B. nigra addition lines. Plant Breed. 115: 113–118.

Harberd, D.J. and E.D. McArthur (1980) Meiotic analysis of some species and genus hybrids in the Brassicaceae. In: Tsunoda, S., K. Hinata and C. Gomez-Campo (eds.) Brassica Crops and Wild Allies, Biology and Breeding, Jpn. Soc. Press, Tokyo, pp. 65–87.

Horisaki, A. and S. Niikura (2004) Effectiveness of insect-pollination to evaluate the level of self-incompatibility and genetic variation in Brassica rapa L. Breed. Sci. 54: 291–295.

Howard, H.W. (1938) The fertility of amphidiploids from the cross Raphanus sativus × Brassica oleracea. J. Genet. 36: 239–273.

Inomata, N. (1977) Production of interspecific hybrids between Brassica campestris and Brassica oleracea by culture in vitro of excised ovaries. I. Effects of yeast extract and casein hydrolysate on the development of excised ovaries. Jpn. J. Breed. 27: 295–304.

Inomata, N. (1978a) Production of interspecific hybrids between Brassica campestris and B. oleracea by culture in vitro of excised ovaries. II. Development of excised ovaries in various culture media with coconut milk and casein hydrolysate. Jpn. J. Genet. 53: 1–11.

Inomata, N. (1978b) Production of interspecific hybrids between Brassica campestris and B. oleracea by culture in vitro of excised ovaries. III. Development of excised ovaries in the crosses of various cultivars. Jpn. J. Genet. 53: 161–173.

Jeong, B.H., S.W. Bang, S. Niikura, Y. Kaneko and Y. Matsuzawa (2003) Wide cross-compatibility and production of hybrid progenies between Brassica crops and wild relatives. Breed. Res. 5 (Suppl 2): 282.

Jeong, B.H., S.W. Bang, S. Niikura, Y. Kaneko and Y. Matsuzawa (2004) Intergeneric cross-compatibility and production of hybrid progenies between Moricandia arvensis and the inbred lines of Brassica crops. Breed. Res. 6 (Suppl 2): 194.

Jeong, B.H., S.W. Bang, S. Niikura, Y. Kaneko and Y. Matsuzawa (2006a) Progenies of intergeneric hybrid between Moricandia arvensis and Brassica crops. Breed. Res. 8 (Suppl 1): 208.

Jeong, B.H., S.W. Bang, S. Niikura, Y. Kaneko and Y. Matsuzawa (2006b) Intergeneric cross-compatibility and production of hybrid progenies between Diplotaxis spp. and the inbred lines of Brassica crops. Breed. Res. 8 (Suppl 2): 136.

Johnston, S.A., T.P.M. Nijs, S.J. Peloquin and R.E. Hanneman (1980) The significance of genic balance to endosperm development in interspecific crosses. Theor. Appl. Genet. 57: 5–9.

Kaneko, Y., Y. Matsuzawa and M. Sarashima (1987) Breeding of the chromosome addition lines of radish with single kale chromosome.
Hybridization of Brassicaceae crops

Jpn. J. Breed. 37: 438–452.
Kaneko, Y., T. Natsuki, S.W. Bang and Y. Matsuzawa (1996) Identification and evaluation of turnip mosaic virus (TuMV) resistance gene in kale monosomic addition lines of radish. Breed. Sci. 46: 117–124.
Kaneko, Y., N. Nagasawa, S.W. Bang and Y. Matsuzawa (2002) Homologous relationships between the f chromosome of Brassica rapa and the e chromosome of Brassica oleracea. Plant Breed. 121: 171–173.
Kaneko, Y., S.W. Bang and Y. Matsuzawa (2009) 11 Distant Hybridization. In: Gupta, S.K. (ed.) Biology and Breeding of Crucifers, Taylor & Francis Group, New York, pp. 207–247.
Karpchenko, G.D. (1928) Polyploid hybrids of Raphanus sativus L. × Brassica oleracea L. Z. Ind. Abstr. Vererbgl. 48: 1–85.
Kato, M. and S. Tokumasu (1976) The mechanism of increased seed fertility accompanied with the chance of flower color in Brassicoraphanates. Euphytica 25: 761–767.
Kerlan, M.C., A.M. Chevre, F. Eber, A. Baranger and M. Renard (1992) Risk assessment of outcrossing of transgenic rapeseed to related species: I. Interspecific hybrid production under optimal conditions with emphasis on pollination and fertilization. Euphytica 62: 145–153.
Krush, S.G. (1973) Cytogenetics of alien addition and substitution. In: Cytogenetics of Aneuploids, Academic Press, New York, pp. 238–248.
Krush, S.G. and D.S. Brar (1992) Overcoming the barriers in hybridization. In: Kalloo, G. and J.B. Chowdhury (eds.) Distant hybridization of crop plants, Springer-Verlag, Berlin, pp. 47–61.
Kinosita, T. (2007) Reproductive barrier and genomic imprinting in the endosperm of flowering plants. Genes Genet. Syst. 82: 177–186.
Leino, M., R. Teixeira, M. Landgren and K. Glimelius (2003) Brassica napus lines with rearranged Arabidopsis mitochondria display CMS and a range of developmental aberrations. Theor. Appl. Genet. 106: 1156–1163.
Malik, M., P. Vyas, N.S. Rangaswamy and K.R. Shivanna (1999) Development of two new cytoplasmic male-stereile lines in Brassica juncea through wide hybridization. Plant Breed. 118: 75–78.
Martinez-Sanchez, A., L. Rafael, I.G. Maria and F. Federico (2007) Identification of new flavonoid glycosides and flavonoid profiles to characterize rocket leafy salads (Eruca vesicaria and Diplotaxis tenuifolia). J. Agric. Food Chem. 55: 1356–1363.
Matsumoto, O., Y. Okafuzi, K. Kaneko and S. Katakawa (1997) Breeding of new vegetable ‘Hanakkori’ by ovule culture. Bull. Yamaguchi Agric. Expt. Stn. 48: 21–24.
Matsuzawa, Y. (1983) Studies on the interspecific and intergeneric crossability in Brassica and Raphanus. Special Bull. Coll. Agric., Utsunomiya Univ. 39: 1–86.
Matsuzawa, Y., Y. Kaneko and S.W. Bang (1996) Prospects of the wide cross for genetics and plant breeding in Brassicaceae. Bull. Coll. Agric., Utsunomiya Univ. 16: 5–10.
Matsuzawa, Y., T. Minami, S.W. Bang and Y. Kaneko (1997) A new Brassicoraphanates (2n = 36); the true-breeding amphidiploid line of Brassica oleracea Coss. (2n = 18) × Raphanus sativus L. (2n = 18). Bull. Coll. Agric., Utsunomiya Univ. 16: 1–7.
Matsuzawa, Y., T. Funayama, M. Kamibayashi, M. Konnai, S.W. Bang and Y. Kaneko (2000) Synthetic Brassica rapa-Raphanus sativus amphidiploids lines developed by reciprocal hybridization. Plant Breed. 119: 357–359.
McGrath, J.M. and C.F. Quiros (1990) Generation of alien chromosome addition lines from synthesis Brassica napus: morphology, cytology, fertility, and chromosome transmission. Genome 33: 374–383.
Mizushima, U. (1950) Karyogenetic studies of species and genus hybrids in the tribe Brassiceae of Cruciferae. Tohoku J. Agri. Res. 1: 1–14.
Momotaz, A., M. Kato and F. Kakihara (1998) Production of intergeneric hybrids between Brassica and Sinapis species by means of embryo rescue techniques. Euphytica 103: 123–130.
Nagano, K. (1988) Senpou Kajii. 1 and No. 2. In: Fujii, K. (ed.) The monograph 'New varieties of vegetable crops': 10. Seibundo-Shinkosha, Tokyo, p. 129.
Namai, H. (1976) Cytogenetic and breeding studies on transfer of economic characters by means of interspecific and intergeneric crossing in the tribe Brassiceae of Cruciferae. Memoirs Fac. Agric., Tokyo Univ. Education 22: 101–171.
Namai, H. (1987) Inducing cytotgenetical alterations by means of interspecific and intergeneric hybridization in brassica crops. Gamma Field Symp. 26: 41–89.
Nishi, S., J. Kawada and M. Toda (1962) Studies on the embryo culture in vegetable crops. Part 2. Breeding of interspecific hybrids between cabbage varieties and Chinese cabbage varieties through the application of embryo culture techniques. Bull. Hort. Res. Sta., Japan, Ser. A1: 111–156.
Nishi, S., M. Toda and T. Toyoda (1970) Studies on the embryos culture in vegetable crops. III. On the conditions affecting to embryo culture of interspecific hybrids between cabbage and Chinese cabbage. Bull. Hort. Res. Sta., Japan, Ser. A9: 73–88.
Nishiyama, I. and T. Yabuno (1978) Causal relationships between the polar nuclei in double fertilization and interspecific cross-incompatibility in Avena. Cytologia 43: 453–466.
Peterka, H., H. Budahn, O. Schrader, R. Ahne and W. Schutze (2004) Transfer of resistance against the beet cyst nematode from radish (Raphanus sativus) to rape (Brassica napus) by monosomic chromosome addition. Theor. Appl. Genet. 109: 30–41.
Prakash, S. and V.L. Chopra (1990) Male sterility caused by cytoplasm of Brassica oleracea in B. campestris and B. juncea. Theor. Appl. Genet. 79: 285–287.
Prakash, S., Y. Takahata, P.B. Kirti and V.L. Chopra (1999) Cytogenetics. In: Gomez-Campo, C. (ed.) Biology of Brassica coenospecies. Elsevier, Amsterdam, pp. 59–106.
Quiros, C.F., O. Ochoa, S.F. Kianian and D. Douches (1987) Analysis of the Brassica oleracea genome by the generation of B. campestris-oleracea chromosome addition lines: characterization by isozymes and rDNA genes. Theor. Appl. Genet. 74: 758–766.
Quiros, C.F., O. Ochoa and D.S. Douches (1988) Exploring the role of x = 7 species in Brassica evolution: hybridization with B. nigra and B. oleracea. J. Hered. 79: 351–358.
Röbelen, G. (1960) Beiträge zur analyse des Brassica-genoms. Chromosoma (Berl.) 11: 205–228.
Sarashima, M. (1973) Studies on the breeding of artificially synthesized forage rape (Brassica napus ssp. oleifera) by means of interspecific crosses between B. campestris and B. oleracea. Special Bull. Coll. Agric., Utsunomiya Univ. 29: 1–117.
Sarashima, M., M. Matsuzawa and S. Suto (1990a) Synthesis of alloplasmic Brassica campestris. Bull. Coll. Agric., Utsunomiya Univ. 14: 1–10.
Sarashima, M., M. Matsuzawa and S. Suto (1990b) Synthesis of alloplasmic Brassica oleracea L. Bull. Coll. Agric., Utsunomiya Univ. 14: 11–26.
Sarashima, M., M. Matsuzawa and S. Suto (1990c) Synthesis of
alloplasmic *Raphanus sativus* L. Bull. Coll. Agric., Utsunomiya Univ. 14: 27–34.

Shim, S., W. Yamada, S.W. Bang and Y. Kaneko (2010) Production of interspecific hybrids between *Brassica* wild species and *B. oleracea*. Breed. Res. 12 (Suppl. 2): 239.

Shim, S., S.W. Bang and Y. Kaneko (2011a) Production and characterizations of *Brassica oleracea* carrying *Diploptaxis* cytoplasm. Breed. Res. 13 (Suppl. 1): 208.

Shim, S., S.W. Bang and Y. Kaneko (2011b) Production and versatility of the CMS *Brassica rapa* line carrying *B. oxyrrhina* cytoplasm. Breed. Res. 13 (Suppl. 2): 202.

Shimizu, S., K. Kanazawa and T. Kobayashi (1962) Studies on the breeding of Chinese cabbage for resistance to soft rot. Part III. The breeding of the resistant variety “Hiratsuka No. 1” by interspecific crossing. Bull. Hort. Res. Sta., Japan, Ser. A1: 157–174.

Siemens, J. (2002) Interspecific hybridization between wild relatives and *Brassica napus* to introduce new resistance traits into the oilseed rape gene pool. Czech J. Genet. Plant Breed. 38: 155–157.

Srinivasan, K., V.G. Malathi, P.B. Kirti, S. Prakash and V.L. Chopra (1998) Generation and characterization of monosomal chromosome addition lines of *Brassica campestris*-*B. oxyrrhina*. Theor. Appl. Genet. 97: 976–981.

Stebbins, G.L. (1958) The inviability, weakness and sterility of interspecific hybrids. Adv. Genet. 9: 147–215.

Takada, M. (1985) Gihu-Green. *In*: Hujii, K. (ed.) The monograph ‘New varieties of vegetable crops’: 9, Seibundo-Shinkosha, Tokyo, p. 144.

Takada, M. (2006) F1-Hosoda Wase (Hakuran). *In*: Ito, T. (ed.) The monograph ‘New varieties of vegetable crops’: 16, Seibundo-Shinkosha, Tokyo, p. 88.

Takashima, M., S.W. Bang and Y. Kaneko (2012) Production and characterization of monosomal addition lines of autoplasmic and alloplasmic *Brassica napus* with each B-genome chromosome of *Brassica juncea*. Breed. Res. 14: 95–105.

Tonosaki, K., K. Michiba, S.W. Bang, H. Kitashiba, Y. Kaneko and T. Nishio (2013) Genetic analysis of hybrid seed formation ability of *Brassica rapa* in intergeneric crossings with *Raphanus sativus*. Theor. Appl. Genet. 126: 837–846.

Tsutsui, K. (2013) Breeding utilization of cytoplasmic male sterile lines in cruciferous carrying cytoplasm of their related species. PhD Thesis. Univ. of Tokyo Agric. and Techni., Tokyo, pp. 1–205.

Tsutsui, K., B.H. Jeong, Y. Ito, S.W. Bang and Y. Kaneko (2011) Production and characterization of an alloplasmic and monosomal addition line of *Brassica rapa* carrying the cytoplasm and one chromosome of *Moricandia arvensis*. Breed. Sci. 61: 373–379.

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

Udagawa, H., Y. Ishimaru, F. Li, Y. Sato, H. Kitashiba and T. Nishio (2010) Genetic analysis of interspecific incompatibility in *Brassica rapa*. Theor. Appl. Genet. 121: 689–696.

Wang, Y.P., X.X. Zhao, K. Sonntag, P. Wehling and R.J. Snowdon (2005) Behaviour of *Sinapis alba* chromosome in a *Brassica napus* background revealed by genomic in-situ hybridization. Chromosome Res. 13: 819–826.

Warwick, S.I. (1993) Guide to the wild germplasm of *Brassica* and allied crops. Part IV. Wild species in the tribe *Brassicaceae* (*Cruciferae*) as sources of agronomic traits. Centre for Land and Biological Resources Research, Research Branch, Agriculture Canada, Ottawa, Ont. Technical Bulletin 1993-17E. 1–19.

Wilmar, J.C. and M. Hellendoorn (1968) Embryo culture of Brussels sprouts for breeding. Euphytica 17: 28–37.