Chromosome elimination, addition and introgression in intertribal partial hybrids between *Brassica rapa* and *Isatis indigotica*

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- **Background and Aims** Partial hybrids with female-parent-type phenotypes and chromosome numbers but altered genomic compositions have been reported in wide crosses of several plants. In order to introgress desirable genes from a wild relative, *Isatis indigotica* (a dye and medicinal plant; 2n = 14), into *Brassica* crops, intertribal sexual hybridizations were carried out with *B. rapa* (2n = 20), and the resulting hybrids and their progenies were characterized.

- **Methods** Using genomic *in situ* hybridization (GISH) and amplified fragment length polymorphism (AFLP), chromosomal/genomic components of the hybrids and their progenies were analysed.

- **Key Results** Many hybrid plants were obtained from the mature seeds harvested from the *B. rapa × I. indigotica* cross, and these exhibited different morphological traits. However, the majority of them did not survive and only three plants grew to maturity. These three hybrids showed poor growth and much smaller stature than the two parents, but had some morphological traits and chemical composition of *I. indigotica*. One plant had 2n = 10, the haploid chromosome number of *B. rapa*, and was absolutely sterile. The other two plants had 20 and 22 somatic chromosomes and were male sterile but produced seeds following pollinations with *B. rapa*. All back-cross progenies over several generations maintained a *B. rapa*-type phenotype and also displayed some variations in morphological characters and fatty acid compositions. They were all 2n = 20 and showed good seed-set. The hybrid with 2n = 22 produced some progeny plants with 2n = 21 and 2n = 22. GISH detected two chromosomes of *I. indigotica* in the hybrid with 2n = 22 but none in the one with 2n = 20. AFLP bands specific for *I. indigotica*, novel for two parents or absent in *B. rapa*, were detected in the two hybrids and their progenies. These progeny plants were novel *B. rapa* types with an altered genomic constitution or alien additions.

- **Conclusions** Complete or partial chromosome elimination and diploidization with genomic rearrangements were considered to lead to the formation of partial hybrids in this cross.

**Key words:** *Brassica rapa*, *Isatis indigotica*, intertribal hybridization, partial hybrids, chromosome elimination, alien addition, introgression, genomic *in situ* hybridization (GISH), amplified fragments length polymorphism (AFLP).

**INTRODUCTION**

Currently there is much interest in hybridization in evolutionary biology. Since it was first emphasized as an evolutionary force by Lotsy (1916), it has been recognized that hybridization and introgression are of wide occurrence among natural plant populations (Anderson, 1949; Anderson and Stebbins, 1954; Grant, 1981; Arnold, 1992, 2004; Dowling and Secor, 1997). Hybridization has been reported to contribute to speciation and introgression in three ways: allopolyploidy from the duplication of chromosome complement in a hybrid (Otto and Whitton, 2000); homoploid hybrid and recombinational speciation (Rieseberg and Wills, 2007); and introgression from backcrossing of the hybrids to parents (Rieseberg and Carney, 1998). The importance of hybridization for speciation has been emphasized in recent years (Rieseberg et al., 2003; Cronn and Wendel, 2004; Baack and Rieseberg, 2007; Hegarty and Hiscock, 2008). Empirical studies of hybrid speciation using molecular genetic tools over the past decade (Rieseberg et al., 1995; Arnold, 1997, 2004; Wendel, 2000; Cronn and Wendel, 2003; Rieseberg et al., 2003; Doyle et al., 2004; Seehausen, 2004) suggest that interspecific hybridization may be a more powerful and creative evolutionary process than envisioned by the early proponents of hybridization (Cronn and Wendel, 2003), such as Anderson (1949), Stebbins (1950) and Grant (1981). Although introgressive hybridization does not necessarily lead to immediate hybrid speciation, it is an important mechanism for the transfer and/or *de novo* origin of traits related to ecological adaptations and therefore plays an important role in facilitating speciation in changing niches (Arnold, 2004). There is also much debate about the significance of wide hybridization (Chapman and Burke, 2007; Buggs et al., 2008).

Gene transfer via wide hybridization still remains a potent approach to broaden gene pools and increase genetic variability in breeding programmes and genetic research aimed at creating novel plant forms and improving the quality of crops. Related or distant species of cultivated crops constitute a large reservoir of genes conferring desirable traits, such as resistance to biotic or abiotic stresses. The genus *Brassica* contains several species that are major sources of edible oil and...
vegetables. These have been hybridized with wild and weedy species of primary, secondary and even tertiary gene pools to synthesize entirely new hybrids, and there are many examples of successfully transferring alien genes to them (Prakash et al., 2009).

Partial hybrids having a phenotype and chromosome number similar to their female parents and carrying some DNA sequences specific for the male parent, or novel for the two parents but absent in the female parent, have been reported in coffee (Lashermes et al., 2000), rapeseed (Cheng et al., 2002; Hua et al., 2006; Chen et al., 2007; Liu and Li, 2007; Du et al., 2008), rice (Liu et al., 1999) and sunflower (Faure et al., 2002a, b). Several examples of intertribal sexual hybrids involving Brassica species have been reported that are partial hybrids with or without alien chromosomes or chromosomal segments but with altered genomic compositions: between B. napus, B. carinata, B. rapa and Orychophragmus violaceus (Cheng et al., 2002; Hua et al., 2006; Liu and Li, 2007); between B. napus and Matthiola incana (Luo et al., 2003); between B. napus, B. rapa and Capsella bursa-pastoris (Chen et al., 2007); and between B. napus and Lesquerella fendleri (Du et al., 2008). Of these wild species, Orychophragmus violaceus is phylogenetically placed outside the Brassicaceae tribe by comparative chromosome painting (Lysak et al., 2007). The possible mechanisms in these examples involve the early elimination of alien fragments or chromosomes of the male parent, genome rearrangements consequent to ‘genomic shock’ (McClintock, 1984; Madlung and Comai, 2004) and diploidization (Faure et al., 2002a; Liu and Li, 2007).

*Isatis indigotica* (Chinese woad or tein-cheing, 2n = 14, II), belonging to the *Isatideae* tribe (Al-Shehbaz et al., 2006) of the Brassicaceae family, is a dye crop containing the same indigo precursors indican (indoxyl b-D glucoside) and isatan B (indoxyl ketoglucolurate) in its leaves as those of *L. tinctoria* L. (woad) but with a greater indigo-production capacity (Angelini et al., 2007). As a traditional Chinese medicine, *I. indigotica* contains compounds that may cure many diseases caused by bacteria and viruses and increase the protective immune response (Chen et al., 2005). These compounds may be very beneficial for improving the quality of *Brassica* vegetable crops. Additionally, *I. indigotica* also shows resistance to tobacco mosaic virus (TMV; Wang and Wan, 1988) and stem rot (*Sclerotinia sclerotiorum*), the most serious disease of rapeseed in China (Zhao et al., 1994). Intertribal somatic hybrids between *B. rapa* spp. *chinensis* and *I. indigotica* have been synthesized by symmetric protoplast fusion and characterized (Tu et al., 2008). The purpose of the present study was to transfer genes conferring desirable traits from *I. indigotica* to *B. rapa* through sexual hybridization. Sexual hybrids with *I. indigotica* as the male parent were produced by hand emasculation and pollination and their chromosomal/genomic compositions analysed using the methods of genomic in situ hybridization (GISH) and amplified fragment length polymorphism (AFLP). The results indicate chromosome elimination, addition and introgression leading to the production of partial hybrids and their back-crossing progenies, with some characters from *I. indigotica* and an altered genomic constitution of *B. rapa.*

### MATERIALS AND METHODS

#### Plant material and crosses

The two parents used for crosses were *Brassica rapa* L. subsp. *chinensis* (L.) P. H. Han et *Alijuehuang* (2n = 20, AA) and *Isatis indigotica* Fortune ex Lindl. (2n = 14, II). In order to control soil water and to shelter plants from the high air humidity during the winter and early spring in Wuhan, where the experiment was conducted, *I. indigotica* was planted in a glasshouse as its growth is retarded under humid conditions. Crosses were made by hand emasculation and pollination with *I. indigotica* as the pollen parent on sunny days and mature seeds were harvested for study. The seeds were placed on wet filter paper in a Petri dish for germination and seedlings were planted in small plastic cups filled with soil, then the young plants were transplanted into the field. The hybrid plants identified were maintained on MS medium (Murashige and Skoog, 1962) by culturing the auxiliary buds for further study in the next generation.

#### Cytology and pollen viability

The ovaries from young flower buds were used to determine the chromosome numbers of the hybrids. After pre-treatment with 8-hydroxyquinoline for 3–4 h at 22 °C, the ovaries were fixed in an ethanol/acetic acid solution (3:1), and stored at −20 °C. The flower buds were fixed directly in 3:1 ethanol/acetic acid and then stored at 4 °C for meiotic analysis. Determination of chromosome numbers and meiotic observations were made according to the method of Li et al. (1995). Fertility based on pollen viability was determined as the percentage of pollen grains stained with aceto-carmine (1%).

#### Probe labelling and GISH analysis

Total genomic DNA from young leaves was extracted and purified according to the method of Dellaporta et al. (1983). DNA of *I. indigotica* was labelled with bio-11-dUTP (Sino-American Biotechnology Company, China) by the nick-translation method and used as a probe; the length of the probe DNA fragments averaged approx. 500 bp. DNA of *B. rapa* subsp. *chinensis* ‘Alijuehuang’ was sheared to 300–500-bp fragments by boiling for 15 min and used as the block. The content of probe and blocking DNA in the hybridization mixture was 3 μg mL⁻¹ and 20 μg mL⁻¹, respectively. Slide preparations of chromosomes for GISH mainly followed the procedures of Zhong et al. (1996), and GISH was carried out according to Tu et al. (2008).

#### AFLP analysis

Amplified fragment length polymorphism analysis was performed following the protocol of Vos et al. (1995). Genomic DNA from young leaves of the hybrids, parents and progeny was entirely restricted by *EcoRI* and *MseI*, then *EcoRI* and *MseI* adapters were ligated to the restriction fragment ends. After two steps of PCR (preselective PCR and selective PCR, in turn), the PCR products were loaded on the gel and resolved. Finally, the AFLP profile was obtained by silver staining (Bassam et al., 1991) and the bands of 80–800 bp
were scored. Twenty-one pairs of primers were randomly selected and used for AFLP fingerprint analyses of the hybrids, and ten or 12 pairs for back-cross progeny.

**Fatty acid analysis, and diethyl ether and ethanol extraction of leaves**

Fatty acids of the seed oil were analysed using a gas chromatography instrument (HP 6890, Germany) following the procedures of Chen et al. (2007).

The basal leaves (1.0 g) from two parents and hybrids were immersed in 4 mL diethyl ether for 4 h, then removed and dried in air; the extraction solutions were retained for observation. The dry leaves were then immersed in 4 mL of 95% ethanol for 4 h and the extracts were used for study (Sun et al., 2007).

**RESULTS**

**Production and morphology of hybrids and progenies**

With *I. indigotica* as the male parent, the majority of seeds harvested from the female parent *B. rapa* germinated and produced seedlings and young plants. At the seedling stage, 14 of the *F*₁ plants were morphologically different from the female parent, *B. rapa* (Fig. 1A11), and expressed some characters of *I. indigotica*, such as short stature and spatulate leaves (Fig. 1A-2, A-3). Some seedlings had three cotyledons (Fig. 1A-4). The rest were very similar to *B. rapa*. After transplantation into the field, only three of the 14 plants (designated H1–H3) grew to maturity; the others did not survive, including those with three cotyledons. The rest were very similar to *B. rapa*. After transplantation into the field, only three of the 14 plants (designated H1–H3) grew to maturity; the rest did not survive, including those with three cotyledons. The plants similar to *B. rapa* developed well and showed high pollen stainability (>90%) and normal seed-set, and consequently no further studies were made on them.

All three hybrids (H1–H3) were poor in growth with smaller stature than the two parents. However, they expressed some traits of *I. indigotica*, such as spatulate and dark-green leaves, basal clustering branches and small petals. The H1 plant, which showed poor growth during the whole growth period, had spatulate leaves (Fig. 1B-2, C) and was green, as in *B. rapa* (Fig. 1B-1). The H2 plant (Fig. 1B-3) grew more slowly than H1, and also had spatulate, but dark-green, leaves (Fig. 1B-3, E), as in *I. indigotica* (Fig. 1B-4); its leaves were also smaller than those of H1. The colour of the diethyl ether extract of the leaves of the two plants was nearly the same as that of the two parents; however, the colour of the ethanol extraction of *I. indigotica* was of
Twenty-one BC-F₁ plants were raised, which expressed morphological characters mainly of the female parent, *B. rapa*. They had high pollen viability (>90 %, except for two plants with approx. 80 %) and good seed-set after selfing.

Cytogenetic and GISH analyses of hybrids and progenies

When the *I. indigotica* probe was applied to the preparations of *B. rapa* ‘Ai juehuang’ (the DNA of itself as the block), signals of large size and strong intensity were mainly located at the two terminals of one V-shaped bivalent or the centromeric part of another bivalent at diakinesis, or at the terminal or centromeric parts of two chromosomes in each polar group of pollen mother cells (PMCs) at anaphase I. This made it easier to distinguish parental chromosomes/chromosomal segments in these hybrids.

H₁ had 2n = 20 in ovary cells and PMCs, the same as the female parent, *B. rapa*. PMCs showed normal chromosome pairing with ten bivalents (II) at diakinesis and segregation of 10:10 at anaphase I (A-I), but one lagged chromosome appeared in 2.3 % of A-I PMCs (Table 1). GISH with the *I. indigotica* probe detected no obvious signals on chromosomes of PMCs, except for strong signals on two chromosomal terminals of one bivalent (Fig. 3A), and on the terminal or central part of one chromosome in each polar group (Fig. 3B), which was nearly the same as in *B. rapa* and probably resulted from the cross-hybridization of repetitive DNA, such as rDNA. All 54 BC-F₁ plants had 2n = 20 in their ovary cells and produced PMCs with ten bivalents at diakinesis and segregation of 10:10 at A-I, but also occasionally with one lagged chromosome (Table 1).

H₂ possessed 2n = 22 in ovary cells and produced PMCs mainly with 11 bivalents at diakinesis. Chromosomes segregated mainly as 11:11 (43.2 %; Fig. 3C) and 10:12 (45.4 %; Fig. 3E) in A-I PMCs, but 9:13 and other ratios with one or two laggards at lower frequency (11.4 %; Table 1). The segregation of 22:22 also appeared occasionally, which was probably the result of precocious division of the chromosomes. Meiotic irregularities occurred with frequent anaphase bridges and lagging chromosomes. Two entirely or partly labelled chromosomes were observed in A-I PMCs with the segregations of 11:11 or other ratios, which were equally or unequally distributed in each polar group (Fig. 3D–F). The unlabeled laggards also appeared in some A-I PMCs (Fig. 3F).

Table 1. Cytology of hybrids and back-crossing progenies from a *B. rapa* × *I. indigotica* cross

<table>
<thead>
<tr>
<th>Origin</th>
<th>2n in ovary cells</th>
<th>No. of plants</th>
<th>Pairings</th>
<th>10:10</th>
<th>10:11</th>
<th>10:12</th>
<th>11:11</th>
<th>Others*</th>
<th>Total no. of cells</th>
</tr>
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<tbody>
<tr>
<td>H₁</td>
<td>20</td>
<td>1</td>
<td>10II</td>
<td>173</td>
<td>97.7</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2.3</td>
</tr>
<tr>
<td>H₂</td>
<td>22</td>
<td>1</td>
<td>11III</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>11.4</td>
</tr>
<tr>
<td>BC-F₁ of H₁</td>
<td>20</td>
<td>54</td>
<td>10II</td>
<td>374</td>
<td>98.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4.9</td>
</tr>
<tr>
<td>BC-F₁ of H₂</td>
<td>20</td>
<td>8</td>
<td>10II</td>
<td>216</td>
<td>95.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>11.4</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>9</td>
<td>10II/II</td>
<td>–</td>
<td>–</td>
<td>235</td>
<td>81.3</td>
<td>–</td>
<td>54.9</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>4</td>
<td>11III</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>81</td>
<td>46.6</td>
<td>17.9</td>
</tr>
</tbody>
</table>

*a Other segregations: a 10:9:1; b 9:13, 10:10:2, 10:11:1; c 10:10:1.
Fig. 3. Cytology of hybrids between *B. rapa* and *I. indigotica*, and progenies. DAPI (blue) and merged images of each pollen mother cell (PMC) are shown (red signals from the *I. indigotica* probe). The labelled chromosome fragments and chromosomes are indicated by arrowheads and arrows, respectively. (A-1, A-2) One diakinesis PMC with 10II from hybrid plant H1 (A-1), and two chromosomal terminals of one V-shaped bivalent are strongly labelled (arrowheads, A-2). (B-1, B-2) One A-I PMC with 10 : 10 segregation from H1 (B-1), and the terminal part of one chromosome in the left group (arrowhead) and central part of one chromosome in the right group (arrowhead) are labelled (B-2). (C-1, C-2) One PMC at early A-I from plant H2 with 2n = 22 (C-1), having two chromosomes (arrows) and two chromosomal terminals (arrowheads) labelled (C-2). (D-1, D-2) One A-I PMC with segregation 11 : 11 from H2 (D-1); each polar group contains one wholly (arrows) and one partly (arrowheads) labelled chromosome (D-2). (E-1, E-2) One A-I PMC with 10 : 12 (top left/bottom right) segregation from H2 (E-1); each polar group contains one wholly (arrows) and one partly (arrowheads) labelled chromosome (E-2). (F-1, F-2) One A-I PMC with 10 : 11 (top left/bottom right) + 1 laggard from H2 (F-1); each polar group has one wholly labelled chromosome but one group (top left) has two partly labelled chromosomes, and the laggard is unlabelled (F-2). (G–J) From BC-F1 with 2n = 21: (G) one mitotic cell with 2n = 21, (H) one diakinesis PMC with 10II + 1II (white arrow), (I) one A-I PMC with segregation 10 : 11, and (J) M-I PMCs with one laggard (white arrows). (K–N) From BC-F1 with 2n = 22: (K) one mitotic cell with 2n = 22, (L) one diakinesis PMC with 11II (arrow indicates one bivalent of smaller size), (M) one A-I PMC with segregation 11 : 11, and (N) one with 10 : 12 (left/right). Scale bars = 10 µm.
Among the 21 BC-F1 plants from H2, eight had \(2n = 20\), nine had \(2n = 21\) and four had \(2n = 22\) chromosomes. These plants did not show major differences in morphological characters and were shorter in height than *B. rapa*. Plants with \(2n = 20\) produced PMCs with ten bivalents at diakinesis and A-I segregation of 10 : 10 (95.2 %), and also occasionally with one laggard chromosome (4.9 %; Table 1). Their pollen viability ranged from 76.2% to 98.4 %, and averaged 92.3 %. Plants with \(2n = 21\) (Fig. 3G) had PMCs with ten bivalents and one univalent at diakinesis (Fig. 3H) and A-I segregation of 10 : 11 (Fig. 3I). One laggard chromosome was usually observed at metaphase I (Fig. 3J). The percentages of segregation of 10 : 11 and 10 : 10 : 1 (laggard chromosome) at A-I were 81.3 and 18.7 %, respectively (Table 1). Their pollen viability was high (92.8–96.8 %), and averaged 94.6 %. Plants with \(2n = 22\) (Fig. 3K) formed 11 bivalents at diakinesis (Fig. 3L) and had A-I segregations of 11 : 11 (43.7 %; Fig. 3M), 10 : 12 (46.6 %; Fig. 3N), and others: 9 : 13, 10 : 10 : 2 and 10 : 11 : 1, (9.8 %; Table 1). Their pollen viability was 79-4 to 93-3 %, with an average of 88.7 %. It was notable that the laggard in Fig. 3J, the univalent in H, and the one bivalent arrowed in Fig. 3L were small sized. Chromosomes of *I. indigotica* were observed to be smaller as compared to *B. rapa* in their somatic hybrids (Tu et al., 2008).

H3 had \(2n = 10\) in ovary cells, the haploid number of *B. rapa*. Ten univalents were seen in most of the diakinesis PMCs. A-I PMCs showed segregations of 5 : 5 and 6 : 4 predominantly, and 7 : 3, 8 : 2 and 9 : 1 at low frequency.

**AFLP analyses of hybrids and progenies**

Among the polymorphic bands amplified (Fig. 4, Table 2), those specific for *I. indigotica* were present at a lower frequency in H1 (12.3 %) than in H2 (17.4 %), but those absent in *B. rapa* had higher frequency in H1 (22.5 %) than in H2 (16.7 %), while those novel for the two parents were comparable (9.9 % for H1, 10.6 % for H2). Therefore, there was a positive association between morphology (Figs 1, 2), cytology (Fig. 3) and genomic composition (Fig. 4, Table 2) in these two hybrids. All BC-F1 plants from H1 and H2 also possessed the three kinds of AFLP bands (Fig. 4), and those from H2 had higher means of specific bands and also novel or absent bands (Table 3). On average, all BC-F1 plants had fewer specific bands than the two hybrids, but more novel and absent bands, except the novel bands in those plants from H1 (Table 3).

**Fatty acid composition of progenies**

In the seed oil of 30 randomly selected BC-F5 plants from H1, the contents of seven fatty acids had obvious changes as compared to the parent *B. rapa* (data not shown). Most plants had increased linolenic acid and reduced erucic acid contents. The seed oil of 18 BC-F1 plants from H2 contained variable contents of seven fatty acids. The levels of linolenic and eicosenoic acids were generally higher, but that of erucic acid lower than in *B. rapa* (data not shown).

**DISCUSSION**

Due to very distant relationships, which cause strong reproductive barriers and/or pre- and post-zygotic sexual incompatibilities, sexual intertribal hybridizations between *Brassica* crops and the species of other tribes have not frequently been reported (Luo et al., 2003; Chen et al., 2007; Du et al., 2008; Prakash et al., 2009). In the present study, progenies from sexual crosses of *B. rapa × I. indigotica* were derived from mature seeds after repeated hand emasculations and pollinations.
alleles (Rieseberg, 2007). The high frequency of transgressive or novel characters in hybrids or later generations is explained by epistatic or dominance effects (Baack and et al., 2003). The poor performance of the three hybrids is probably related to epigenetic factors, which might result in a hybrid displaying a phenotype either substantially better or worse than both of its progenitors (Madlung et al., 2002; Osborn et al., 2003; Wang et al., 2006; Chen, 2007; Hegarty and Hiscock, 2008), or to the numerous impacts of alien introgressions on gene regulation, largely due to epistatic or dominance effects (Baaack and Rieseberg, 2007). The high frequency of transgressive or novel characters in hybrids or later generations is explained by the complementary action of new combinations of normal alleles (Rieseberg et al., 2003), the placement of unexpressed (or expressed) alleles in a new background (epistasis), the fixation of recessive alleles present in the heterozygous form in the parent (dominance), reduced developmental stability, and simple heterosis (over-dominance; Rieseberg and Carney, 1998).

The GISH signals from the I. indigotica probe were mainly located at the terminal or centromeric parts of individual bivalents or chromosomes of B. rapa ‘Aijuehuang’. The similar hybridization pattern on ‘Aijuehuang’ was also observed using Orychophagus violaceus (Liu and Li, 2007) and Capsella bursa-pastoris probes (Chen et al., 2007). Judged from its morphology, the bivalent with its two terminals strongly labelled was most likely the satellite chromosome pair of B. rapa (Cheng et al., 1994; Hasterok et al., 2005; Liu and Li, 2007). GISH signals covered the entire length of I. indigotica chromosomes in the somatic hybrids with B. napus (Du, 2008), but mainly the centromeric parts of the Brassica chromosomes. This made detection of I. indigotica chromosomes/chromosomal segments in these hybrids more reliable.

Characterization of morphology, cytology and genomic composition confirmed hybridity in these crosses and the progeny plants. However, the derived progeny plants were not complete Mendelian hybrids carrying the summation of parental genomes, but were partial hybrids mainly with chromosomal genomic constituents from the female parent. It is notable that the majority of young F1 plants that differed in phenotype from B. rapa failed to survive to maturity. The three plants that flowered showed retarded growth and much smaller stature than the two parents (Fig. 1). Despite this, they maintained the intact genome of B. rapa, with (plant H2) or without (H1) individual I. indigotica chromosomes. This suggests that the normal growth in these progeny plants is disturbed by the introgression of alien DNA segments or genetic rearrangements (Fig. 3; Table 2), and also that the diploid B. rapa buffers the genetic changes less than the allotetraploid B. napus (Chen et al., 2007; Du et al., 2008) and more easily expresses the novel or alien traits (Figs 1, 2). In particular, chemical components from I. indigotica are also possibly produced in the hybrids (Fig. 2), as ethanol can extract cholesterol, indigotin, indirubin, p-hydroxybenzadehyde, salicylic acid, lariciresinol-4, glucopyranoside and other compounds in the plant (Sun et al., 2007).

The poor performance of the three hybrids is probably related to epigenetic factors, which might result in a hybrid displaying a phenotype either substantially better or worse than both of its progenitors (Madlung et al., 2002; Osborn et al., 2003; Wang et al., 2006; Chen, 2007; Hegarty and Hiscock, 2008), or to the numerous impacts of alien introgressions on gene regulation, largely due to epistatic or dominance effects (Baaack and Rieseberg, 2007). The high frequency of transgressive or novel characters in hybrids or later generations is explained by the complementary action of new combinations of normal alleles (Rieseberg et al., 2003), the placement of unexpressed (or expressed) alleles in a new background (epistasis), the fixation of recessive alleles present in the heterozygous form in the parent (dominance), reduced developmental stability, and simple heterosis (over-dominance; Rieseberg and Carney, 1998).

The possible processes of cytological and genome rearrangements proposed for the formation of partial hybrids in other cases (Faure et al., 2002a; Liu and Li, 2007) would be valid for explaining the present results. When haploids (plant H3) and aneuploids (H2) are obtained, chromosome elimination possibly occurs prior to diploidization after fertilization. Complete elimination of the I. indigotica chromosomes with segmental introgression and subsequent chromosome doubling led to the formation of plant H1, and partial elimination of the I. indigotica chromosomes with segmental introgression and chromosome doubling led to the formation of H2. The production of plant H3 resulted from the complete elimination of the I. indigotica chromosomes and segmental introgression, for it also showed some characters from I. indigotica. Partial elimination without diploidization leading to production of stable additions was reported in an oat × maize cross (Riera-Lizarazu et al., 1996).

Genomic rearrangements in partial hybrids prior to meiosis have been reported in Helianthus (Natali et al., 1998; Faure et al., 2002a, b) and explained as responses to genomic shock following the interspecific cross (McCleintock, 1984). Genomic shock may be one reason for the genomic changes in our hybrids. Recombination from the introgressions of I. indigotica fragments into Brassica chromosomes is another reason; however, most of the introgressed fragments were out of the range of GISH resolution (Fig. 3). The fact that the frequencies of specific AFLP bands for I. indigotica are usually lower than those of bands novel for the two parents and absent for B. rapa, especially in back-cross progenies (Tables 2, 3) suggests that other mechanisms are also involved in these genomic variations; for example: rapid sequence elimination in synthetic hybrids and allopolyploids

### Table 2. Numbers and percentages of AFLP bands in hybrids from a B. rapa × I. indigotica cross

<table>
<thead>
<tr>
<th>Origin</th>
<th>No. of plants</th>
<th>Specific for I. indigotica</th>
<th>Novel for two parents</th>
<th>Absent in B. rapa</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. indigotica</td>
<td>517</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>B. rapa</td>
<td>618</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>H1</td>
<td>625</td>
<td>77.2</td>
<td>62.9</td>
<td>139.22</td>
</tr>
<tr>
<td>H2</td>
<td>726</td>
<td>126.17</td>
<td>77.10</td>
<td>103.16</td>
</tr>
</tbody>
</table>

21 pairs of randomly selected primers were used.

### Table 3. Percentages of AFLP bands in BC-F1 plants from a B. rapa × I. indigotica cross

<table>
<thead>
<tr>
<th>Origin</th>
<th>No. of plants</th>
<th>Specific for I. indigotica</th>
<th>Novel for two parents</th>
<th>Absent in B. rapa</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>40</td>
<td>6.1 ± 2.2 (1.1–10.4)</td>
<td>13.4 ± 1.9 (9.7–18.7)</td>
<td>18.4 ± 3.4 (12.1–24.7)</td>
</tr>
<tr>
<td>H2</td>
<td>14</td>
<td>9.5 ± 1.9 (6.1–12.7)</td>
<td>19.2 ± 3.4 (12.7–27.0)</td>
<td>25.9 ± 6.4 (14.5–35.9)</td>
</tr>
</tbody>
</table>

Means ± s.e. are given for each category, ranges are given in brackets.

a Ten, or b 12 pairs of primers were used.
(Song et al., 1995; Shaked et al., 2001); genomic rearrangements in the hybrids; extensive alteration in DNA methylation patterns (Natali et al., 1998; Liu et al., 2004; Wang et al., 2005) or activation of mobile genetic elements (transposons and retrotransposons; Liu and Wendel, 2000; Shan et al., 2005; Wang et al., 2005). Similarly, in rice recombinant inbred lines with <0.1% introgressed alien DNA, extensive and genome-wide de novo variations occur in up to 30% of the loci, with the loss of parental bands being more frequent than the gain of novel bands (Wang et al., 2005).

Hybrid formation also probably depends on the level of genetic and structural relatedness between the genomes of crops and wild plants (Leflon et al., 2006) and the similarity of the centromere structure in the two parents, or expression of the relevant function factors of the alien chromosones in a foreign genetic background (Riera-Lizarazu et al., 1996). Tribe Isatidieae, to which Isatis belongs, was first recognized by Hayek (1911) as a subtribe of the Arabidieae and by Schulz (1936) as a subtribe of the Lepidieae (Al-Shehbaz et al., 2006). In the new tribal classification scheme for the family Brassicaceae, the two tribes Brassiceae and Isatidieae are placed in one of three larger monophyletic lineages (Bailey et al., 2006; Al-Shehbaz et al., 2006), which suggests a closer phylogenetic relationship between the species in the two tribes. The somatic hybrids of I. indigotica with B. rapa (Tu et al., 2008) and B. napus (Du, 2008) are morphologically intermediate and show vigorous growth. The somatic hybrids with the same cultivar of B. rapa as used in this study have 2n = 48 (genomes AAIIII) and are female sterile, but partially male fertile (Tu et al., 2008). Back-cross progeny plants have also been obtained following pollinations with B. rapa. Most of the hybrids with B. napus have 2n = 52 (genomes AACCII), the sum of the two parents, and are partially female fertile and produce back-cross progenies (2n = 45, AACCII) after pollination by B. rapus. Furthermore, the intragenomic chromosome pairing among the seven chromosomes of the I genome and intergenomic pairing between some chromosomes of the I and A/C genomes in progenies with 2n = 45 are revealed by GISH analysis (Du, 2008), indicating the homoeologous relationship between the genomes of these two species from the two tribes. The present successful synthesis of partial hybrids with genetic elements and phenotypes from I. indigotica also suggests that the species of the two tribes are related to some extent. Interestingly, the chromosome elimination occurs only in sexual but not in somatic hybrids, which may be caused by impaired centromere function (Laurie and Bennett, 1989) due to failure to recruit centromere-associated structures as kinetochore components (Mochida et al., 2004). The suppression and silencing of centromeres from one parent in hybrids most likely results from the silencing of the genes for centromere proteins and then the incongruence between their centromeric repeats and the proteins encoded by the genes of another parent. It is probable that the expression of genes from the male parent is delayed in the first zygotic divisions in the cytoplasm of the female parent, while genes from the female parent are preferentially expressed. However, in the somatic hybrid cells from parental protoplasts, where the genes from two parents are expressed, the competition and interaction of parental genes may result in dominance or co-expression. Whether the genes for centromeric proteins from I. indigotica are dominant over, or co-expressed with, those of B. napus needs to be investigated.

The occurrence of intertribal partial hybrids can provide an opportunity to rapidly alter the genomic constitution of B. rapa and introgress useful traits of I. indigotica into Brassica crops. A wide range of phenotypic variations in back-crossing progenies (2n = 20) with normal meiotic behavior can result from the introgression of alien genes, deletion of segments from incipient genomes and the production of novel segments, silencing of genes by the insertion of alien segments, or epigenetic interactions. Besides the morphological characters, variations in fatty acid content and chemical composition (Fig. 2) were also observed, but the question of whether or not medicinal or indigo components of I. indigotica are produced still needs further research. The weak plants among the progenies may be used to identify genes involved in plant development. The disomic additions with 2n = 22 are useful for introducing valuable traits and mapping genes and markers on alien chromosomes.

In conclusion, the successful synthesis of intertribal hybrids of B. rapa with I. indigotica can be viewed as a significant step towards the development of novel B. rapa types by introgressing genes of interest from I. indigotica, which will be beneficial to further broaden the Brassica gene pool. The occurrence of partial hybrids could have evolutionary consequences (Faure et al., 2002a), as mature seeds of intertribal partial hybrids that have undergone a natural process of diploidization are produced and a number of back-cross progeny plants exhibit normal growth and good seed-set. If this process takes place under natural conditions, it could lead to gene introgression between very distantly related species.

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