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GENETIC TRANSFORMATION AND HYBRIDIZATION

Production and genetic analysis of partial hybrids in intertribal crosses between *Brassica* species (*B. rapa*, *B. napus*) and *Capsella bursa-pastoris*

Hai-Feng Chen · Hua Wang · Zai-Yun Li

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Abstract *Capsella bursa-pastoris* (L.) Medic ($2n = 4x = 32$) is a natural double-low (erucic acid < 1%, glucosinolates < 30 μmol/g) germplasm and shows high degree of resistance to *Sclerotinia sclerotiorum*. Hybridizations were carried out between two *Brassica* species viz. *B. rapa* ($2n = 20$) and *B. napus* ($2n = 38$) as female and *C. bursa-pastoris* as male parent to introduce these desirable traits into cultivated *Brassica* species. Majority of F_1 plants resembled female parents in morphology and only a few expressed some characters of male parent, including the white petals. Based on cytological observation of somatic cells, the F_1 plants were classified into five types: two types from the cross with *B. rapa*, type I had $2n = 27–29$; type II had $2n = 20$; three types from the crosses with *B. napus*, type III was haploids with $2n = 19$; type IV had $2n = 29$; type V had $2n = 38$. One to two chromosomes of *C. bursa-pastoris* were detected in pollen mother cells (PMCs) of type I plant by genomic in situ hybridization (GISH), together with chromosomal segments in ovary cells and PMCs of some F_1 plants. Amplified fragment length polymorphism (AFLP) bands specific for the male parent, novel for two parents and absent bands in *Brassica* parents were generated in majority of F_1 plants, even in *Brassica*-types and haploids, indicating the introgressions at various levels from *C. bursa-pastoris* and genomic alterations following hybridization. Some *Brassica*-type progeny plants had reduced contents of erucic acid and glucosinolates associated with improved resistance to *S. sclerotiorum*. The cytological and molecular mechanisms behind these results are discussed.

Keywords *Brassica* species · *Capsella bursa-pastoris* · GISH · AFLP · Introgression

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H.-F. Chen · H. Wang · Z.-Y. Li (✉)
National Key Lab of Crop Genetic Improvement,
National Center of Crop Molecular Breeding Technology,
National Center of Oil Crop Improvement (Wuhan),
Huazhong Agricultural University, Wuhan 430070, China
e-mail: zaiyunli@yahoo.com.cn

Introduction

Wide hybridization plays an important role in crop improvement and has been used successfully to transfer desired traits from wild germplasm to large number of crop species, including rape (Peterka et al. 2004; Ma et al. 2006; Snowdon 2007), rice (Amante-Bordeos et al. 1992), wheat (Martin-Sanchez et al. 2003), coffee (Prakash et al. 2004), sunflower (Ronicke et al. 2004). However, investigations on intertribal sexual hybrids are not frequent and reported only for *Avena sativa* × *Zea mays* (Riera-Lizarazu et al. 1996) and *Brassica napus* × *Matthiola incana* (Luo et al. 2003).

The double-low *Brassica* varieties have the seed oil with the desired fatty acid composition for human nutrition (i.e., <1% erucic acid and ~60% oleic acid) and the seed meal suitable for animal feed (<30 μmol glucosinolates/g oil free seed meal). The *Brassicaceae* family comprises a large number of wild species which are potential sources of nuclear genes for many economically important traits, such as resistance to biotic and abiotic stresses, and novel fatty acid compositions. The crucifer *Capsella bursa-pastoris* (L.) Medic of tribe *Lepidieae* is an annual to biennial predominantly autogamous species with worldwide distribution. It has been used traditionally as vegetable and medicinal plant in China and some other countries for many centuries (Zhou 1987). *Capsella*, *Brassica* and *Arabidopsis* show close genetic relationships. The divergence

times for the species pairs *Arabidopsis-Brassica*, *Capsella-Brassica* and *Capsella-Arabidopsis* are 12.2–24 (Yang et al. 1999; Koch et al. 2000; Acarkan et al. 2002), 12.4–19.5 (Acarkan et al. 2002) and 6.2–14 (Acarkan et al. 2002; Koch and Kiefer 2005) million years ago, respectively. The seed oil of *C. bursa-pastoris* has considerably lower erucic acid content compared to other *cruciferous* plants (Park 1967). A population of this species collected in the campus of Huazhong Agricultural University is found to be a natural double-low germplasm (0.68% erucic acid and 15.68 μmol glucosinolates/g oil free seed meal). *C. bursa-pastoris* has been reported to be highly resistant to *Alternaria brassicae* (Conn et al. 1988; Sigareva and Earle 1999). In the present study we have observed that it possesses high resistance to *Sclerotinia sclerotiorum*, one of the most devastating diseases of rapeseed in China. It has the ability to tolerate cold, salt and drought (Liu et al. 2004a). These facts indicate the utility of *C. bursa-pastoris* as a potential source of agronomic important traits for introgression into *Brassica* crops. Intertribal somatic hybrids between *C. bursa-pastoris* and *B. oleracea* have been produced, but no progeny plants could be obtained because of sterility, thus their use in further breeding program is limited (Sigareva and Earle 1999). The present investigation reports production of sexual hybrids between *Brassica* species (*B. rapa*, *B. napus*) and *C. bursa-pastoris* and their morphological and chromosomal/genomic characterizations for the first time.

Materials and methods

Plant materials and crosses

Cultivars used in the present study are *B. rapa var. chinensis* cv. Aijuehuang and *B. napus* cvs. Oro (the first *B. napus* cultivar with low content of erucic acid in the world), Huashuang no. 3 (double-low cultivar), Zhongyou 821 (high content of erucic acid and glucosinolates, but high yield and resistance to *S. sclerotiorum*).The double-low *C. bursa-pastoris* was collected in Huazhong Agricultural University. *B. rapa* and *B. napus* had yellow petals and black seeds, while *C. bursa-pastoris* had small deep-green leaves, basal clustering branches, short plant stature (30–50 cm), white petals and very small brown seeds in the heart-shaped pods. The crosses between *Brassica* species and *C. bursa-pastoris* with the latter as pollen parent were performed in the fields by hand emasculation and pollination at Wuhan in 2002 and 2003, at Xining, Qinghai Province in 2004. About 2–3 weeks after pollination, some immature embryos were cultured on MS agar medium (Murashige and Skoog 1962) and others left on plants to obtain mature seeds.

Cytology

To determine the chromosome numbers of hybrids, young ovaries were treated with 2 mM 8-hydroxyquinoline for 3–4 h at 22°C, and fixed in a mixture of ethanol: acetic acid (3:1, v:v) for 24 h, stored at –20°C. For meiotic analysis, flower buds were fixed in a mixture of ethanol: acetic acid (3:1, v:v) for 24 h, transferred to fresh mixture and stored at –20°C. Mitotic and meiotic observations were made according to the methods of Li et al. (1995). Pollen fertility was determined as the percentage of pollen grains stained with 1% acetocarmine.

Probe labeling and GISH analyses

DNA was extracted and purified from young leaves according to the method of Dellaporta et al. (1983). The DNA of *Brassica* species was sheared to 300–500 bp fragments by boiling for 15 min and used as block. The DNA of *C. bursa-pastoris* was labeled with bio-11-dUTP (SABC in China) by nick translation method and used as probe. The length of the probe DNA fragments averaged approximately 500 bp.

The young ovaries and anthers with pollen mother cells (PMCs) at suitable stages were digested in an enzyme mixture containing 0.6% cellulase Onozuka RS (YAKULT HONSHA Co., LTD, Japan), 0.4% pectinase (MERCK, Germany) and 0.5% snailase (Beijing Baitai Biochem Co., China) at 37°C for about 63 and 68 min, respectively. The chromosome preparations for GISH mainly followed the procedures of Zhong et al. (1996). In situ hybridization was carried out according to the protocol by Leitch et al. (1994). Hybridization signals of the *C. bursa-pastoris* probe were detected using Cy3-labeled streptavidin (Sigma, USA), and chromosomes were counterstained with 0.2% 4'-6-Diamidino-2-phenylindole (DAPI) solution (Roche, Basel, Switzerland), mounted in antifade solution (Citifluor) and examined under a Leica DMLB fluorescent microscope (Wetzlar, Germany) equipped with CCD (LEICA DC 300F).

AFLP analysis

AFLP fingerprints were generated based on the protocol of Vos et al. (1995), and DNA bands were visualized by silver staining (Bassam et al. 1991). The bands with 80–800 bp were scored.

Fatty acids and glucosinolates analysis

Fatty acids of the seed oil were analyzed on gas chromatography machine (HP 6890, Germany). A bulk seed sample (0.2 g) per plant was crushed and transferred into

glass tube with 1 ml mixture of diethyl ether: petroleum ether (1:1, v:v) added, for extraction of seed oil at room temperature over 8 h. After 1 ml methanol (with 5% KOH) was added to the tube for esterification for 40 min and 2 ml H_2O added, 0.5 µl of the upper phase containing fatty acid methyl esters as sample was injected into the gas chromatography machine equipped with a fused-silica capillary column (30 m × 0.25 mm). The injector and flame ionization detector were held at 250 and 180°C, respectively. The carrier gas flow was 30 ml/min (H_2), 300 ml/min (air) and 25 ml/min (N_2). To directly determine the content of glucosinolates, about 3 g seeds were scanned by near-infrared reflectance spectroscopy (NIRS) (Vector 22/N, Bruker, Germany, OPUS/QUANT4.0 software).

Culture of *Sclerotinia sclerotiorum* and infection

Sclerotinia sclerotiorum isolate was collected from infected *B. napus* plants in the fields of Huazhong Agricultural University. Fungal mycelia were cultured on solid Potato/Dextrose/Agar (PDA, 20% potato, 2% dextrose and 1.5% agar) medium. Mycelial agar disks of 5-mm diameter punched from the growing periphery of the 2-day old culture of *S. sclerotiorum* on PDA were used as inoculums to infect the plants.

Leaves excised from plants at the 9–12 leaf stage were inoculated with inoculums, covered with plastic bags to provide adequate humidity for infection at 20°C. The lesion diameter was measured at 48 h after inoculation to evaluate the level of resistance. Plants in the field were inoculated 3 weeks before harvest. The inoculums were affixed to the stems surface with parafilm and remained in contact with the stems surface until lesion developed (Li et al. 2004). The lesion length along the stems was measured 8 days after inoculation.

Results

Crossability and morphology of F_1 plants

B. rapa × *C. bursa-pastoris*

From 7,513 pollinations, 185 F_1 plants were obtained (0.025 seeds/silique). Fourteen plants were distinguished by their phenotypes and cytology and grouped according to their chromosome numbers into two types, viz. type I (no. 1) and type II (nos. 2–14) (Table 1). The plant no. 1 was morphologically intermediate between the two parents (Fig. 1a1, a5), but conspicuous in expressing some traits of male parent, such as small deep-green leaves (Fig. 1a2), nanism, basal clustering branches and white petals (Fig. 1c2). The original seed-plant was multiplied in vitro by culturing its buds on MS medium and the cloned plants showed the same phenotype and chromosome number (see below). Some plants exhibited purple petiole (Fig. 1a3) and cleft leaves (Fig. 1a4) of *C. bursa-pastoris*. Two plants (nos. 1, 4) had yellow seeds. Except plant no. 1, all F_1 plants with various pollen fertility (47.6–98.4%) had good seed-set after selfing. The seed-, or cloned- plant no. 1 had very poor pollen (16.7%) and seed fertility. No seed was obtained after selfing, and only few seeds could be obtained following pollination with the female parent.

B. napus × *C. bursa-pastoris*

From 9,248 pollinations, 169 F_1 plants were obtained (0.018 seeds/silique). Of these, 22 were selected and grouped according to their chromosome numbers into three types, type III (nos. 15, 16), type IV (nos. 17–19) and type V (nos. 20–36) (Table 1). These plants exhibited some traits of *C. bursa-pastoris* (Fig. 1a5, c1, d3) having small sized dark-green, deeply divided leaves (Fig. 1b), nanism, basal clustering branches and white petals (Fig. 1d2), and the flowers of one plant had curly petals (Fig. 1c3). Plants of types III and IV had poor pollen fertility and produced no seeds after selfing and only one to three seeds in a silique after pollination by the female parent. Except plant no. 30, all F_1 plants of type V with varying pollen fertility (66.8–98.5%) had good seed-set after selfing. The plant no. 30 which resembled female parent (Fig. 1d1) in morphology was male sterile with rudimentary stamens (Fig. 1c4), however, the pistil was normal and had good seed-set after pollination by the female parent. Most hybrids had black seeds as female (Fig. 1e1), however, four plants had red brown or yellow brown seeds (Fig. 1e2, e3, Table 1), were similar to male parent (Fig. 1e5), and some progenies of plant no. 30 produced yellow seeds (Fig. 1e4). All hybrid plants from the crosses with *B. napus* and *B. rapa* produced the pods of *Brassica*-type, not the heart-shaped pods of *C. bursa-pastoris*.

Cytogenetic and GISH analyses of F_1 plants

B. rapa × *C. bursa-pastoris*

Capsella bursa-pastoris had $2n = 32$ (Fig. 2a1) and the PMCs at diakinesis had 16 bivalents (Fig. 2a2). The single plant of type I (no. 1) had $2n = 27–29$ with a preponderance of 29 in ovary cells (85.3%). Majority of its PMCs at diakinesis showed 13 II + 3 or 4 I, 14 II + 3 or 4 I. However, the sum of chromosomes in two polar groups of PMCs at anaphase I (AI) were $2n = 28–36$, though $2n = 29$ was still the most frequent (34.4%), $2n > 29$ appeared in 63.6% cells. One to five laggards were observed in 71.2% AI PMCs (Fig. 2b) and rarely in second divisions. Plants of type II (nos. 2–14) had $2n = 20$, same as *B. rapa* (Table 1).

Table 1 Phenotypes, chromosome numbers of ovary cells and AFLP bands in individual F_1 plants from the crosses *Brassica* species × *C. bursa-pastoris*

Types[a]	Plant no.	2*n*	Morphology[b]	Seed color[c]	Pollen fertility (%)	Polymorphic AFLP bands[d] (%)			
						S	N	A	Total
I	1	27–29	BB,L1,L2,N,WP	Y	16.7	5.9	35.4	14.6	55.9
II	2–14	20	BB(3),L1(5),L2(2),N(1)	B(12),Y(1)	47.6–98.4	0–3.6	11.0–27.0	7.4–20.2	26.2–42.0
III	15–16	19		B	31.8–40.5	0.8–1.3	8.3–10.0	6.6–8.0	15.7–19.3
IV	17–19	29	BB(1),L1(2),L2(3),N(1)	B	37.7–50.0	0.6–2.1	10.0–1.8	6.6–14.3	19.1–26.4
V	20–36	38	BB(5),L1(2),L2(4), MS(1),N(3),S(2),WP(2)	B(12),RB(3), YB(1),Y[e] (1)	0, 66.8–98.5	0–2.3	5.8–13.6	5.0–11.3	15.6–24.8

[a] Types I, II: *B. rapa* × *C. bursa-pastoris*; types III, IV, V: *B. napus* × *C. bursa-pastoris*

[b] *BB* basal clustering branches, *L1* small leaves, *L2* deep-green leaves, *MS* male sterility, *N* nanism, *S* small seeds, *WP* white petals. The numbers in brackets are the numbers of plants with the traits

[c] *B* black, *RB* red brown, *Y* yellow, *YB* yellow brown. The numbers in brackets are the numbers of plants with the seed color

[d] *S* bands specific for *C. bursa-pastoris*, *N* bands novel for two parents, *A* bands absent in female parents

[e] Seed color of BC_1F_1

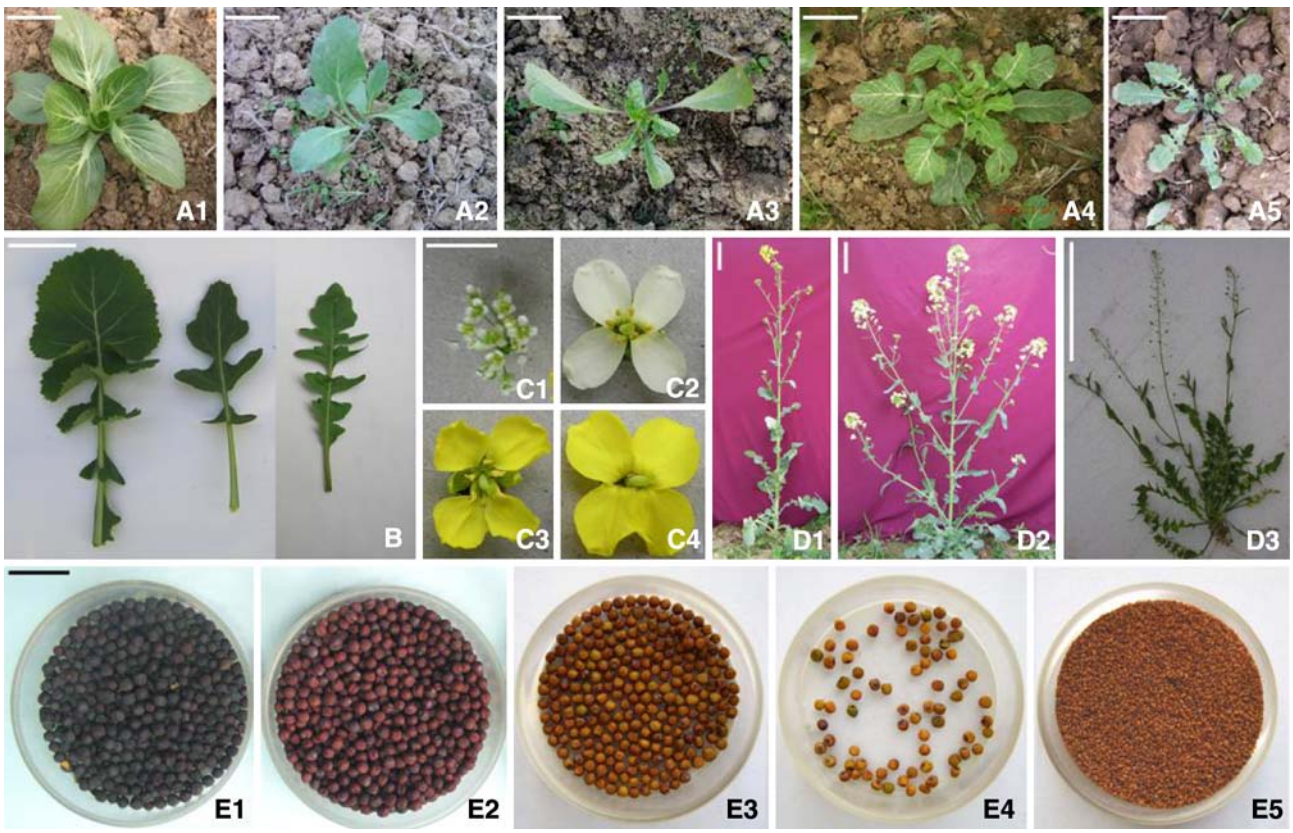

Fig. 1 Phenotypes of F_1 plants from the crosses between *Brassica* species and *C. bursa-pastoris*. **a1**–**a5** Young plants of *B. rapa* cv. Aijuehuang, hybrids nos. 1, 4 (purple petioles), 14 and *C. bursa-pastoris*. *Bar*: 5 cm. **b** Leaves of *B. napus* cv. Oro, hybrid no. 17 and *C. bursa-pastoris* (from *left* to *right*). *Bar*: 5 cm. **c1**–**c4** Flowers of *C. bursa-pastoris* (one inflorescence), hybrids no. 1 with white petals, no. 29 with curly petals, no. 30 (male sterility). *Bar*: 1 cm. **d1**–**d3** Flowering plants of *B. napus* cv. Oro, hybrid no. 22 with basal clustering branches and white petals and *C. bursa-pastoris*. *Bar*: 20 cm. **e1**–**e5** Seeds of *B. napus* cv. Oro, hybrids nos. 21 (*red brown*), 28 (*yellow brown*), 30 (seeds of BC_1F_1, *yellow*) and *C. bursa-pastoris* (*brown*). *Bar*: 1 cm

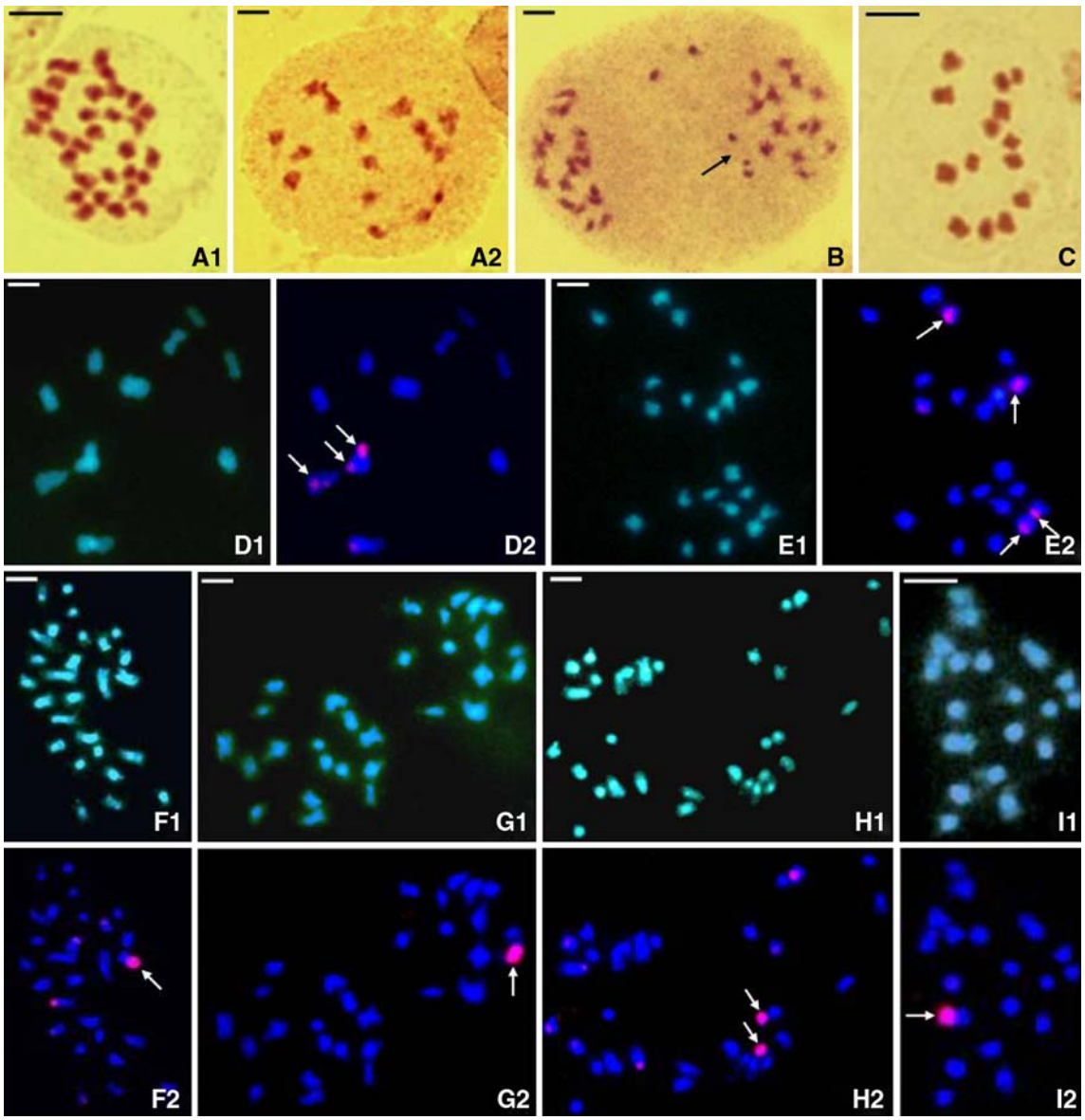

Fig. 2 Cytology of hybrids between *Brassica* species and *C. bursa-pastoris*. **a1**, **a2** Mitotic cell ($2n = 32$) and PMC at diakinesis with 16 bivalents of *C. bursa-pastoris*. **b** One AI PMC of hybrid no. 1 with 16 (*left*): 13 (*right*) segregation, 2 laggards and 3 segments (*arrow*). **c** One ovary cell ($2n = 14$) of plant no. 15. **d1**–**i1** DAPI (*blue*) and **d2**–**i2** merged images (*red signals* from the *C. bursa-pastoris* probe) of ovary cells and PMCs. **d1**–**d2**, **e1**–**e2** The distribution of GISH signals (*arrows*) of *C. bursa-pastoris* probe on chromosomes of one diakinesis (**d2**) and one AI (**e2**) PMC of *B. rapa* cv. Aijuehuang. **f1**–**f2** One ovary cell ($2n = 27$) of hybrid no. 1 with one chromosomal arm (*arrow*) labeled. **g1**–**g2** One AI PMC of hybrid no. 1 with 13 (*right above*): 16 (*left below*) segregation and one chromosome (*arrow*) labeled *red*. **h1**–**h2** One AI PMC of hybrid no. 1 with 31 chromosomes and two (*arrows*) labeled red. **i1**–**i2** One ovary cell ($2n = 19$) of hybrid no. 15 with one chromosomal arm (*arrow*) labeled *red*. *Bar*: 5 μm

As *C. bursa-pastoris* probe was applied to the preparations of *B. rapa* cv. Aijuehuang (the DNA of itself as block), signals of large size and strong intensity were mainly located at two terminals of one v-shaped bivalent and centromeric part of another one at diakinesis (Fig. 2d1, d2), at terminal or centromeric parts of two chromosomes in each polar group of AI PMCs (Fig. 2e1, e2). The same hybridization pattern on 'Aijuehuang' was also observed using *Orychophragmus violaceus* probe (Liu and Li 2007). Judged from its morphology, the bivalent with its two terminals being strongly labeled was most likely the satellited chromosome pair of *B. rapa* (Cheng et al. 1994; Liu and Li 2007). This made it more reliable to detect *C. bursa-pastoris* chromosomes/chromosomal segments in these hybrids. Extensive GISH investigations showed that one chromosomal arm in ovary

cells of plant no. 1 was fully covered by signals of the *C. bursa-pastoris* probe (Fig. 2f1, f2). One chromosome was fully labeled in 36.8% AI PMCs with various chromosome numbers (Fig. 2g1, g2), and two chromosomes in 8.7% PMCs (Fig. 2h1, h2). In plants of type II, signals of small size or weak intensity were located mainly at terminal and centromeric parts of the chromosomes in mitotic and meiotic cells, which showed that no intact chromosomes or large segments of *C. bursa-pastoris* origin were contained.

B. napus × *C. bursa-pastoris*

Plants of type III (nos. 15, 16) were haploids with $2n = 19$, however, plant no. 15 had $2n = 13–18$ (Fig. 2c) in 9.4% somatic cells. Plants of type IV (nos. 17–19) had $2n = 29$; type V (nos. 20–36) had $2n = 38$, same as *B. napus* (Table 1). In plants of type IV, 60.5% PMCs at diakinesis had 1 III + 9 II + 8 I, the remaining had 10 II + 9 I and showed segregations 14:15, 13:16, 12:17, 11:18 and 10:19. However, in plant no. 17 of type IV, 4.8% PMCs had $2n = 30$ and 31, and 26.7% AI PMCs had 1–2 lagged chromosomes. The chromosome pairing (19 II) and segregation (19:19) were normal in PMCs of type V. Only one chromosomal arm was fully covered by the signal from the probe of *C. bursa-pastoris* in ovary cells of plant no. 15 (Fig. 2i1, i2), while weak or minor signals appeared at terminal or centromeric parts of some chromosomes in ovary cells and PMCs of these plants.

AFLP analyses of F_1 plants

Polymorphic AFLP bands were amplified in the hybrids from the randomly selected fifteen pairs of primers. Three kinds of bands, i.e., specific for *C. bursa-pastoris*, novel for two parents and absent in *Brassica* parents were detected in F_1 plants except for three plants (nos. 13, 24, 32) which had no specific bands, and the respective numbers in individual plants were 0–28, 39–168 and 32–80 for the cross with *B. rapa*, and 0–11, 25–65 and 23–60 for the cross with *B. napus*. The numbers of the specific (28) and novel (168) bands of plant no. 1 were the highest among F_1 plants with 55.9% polymorphic bands. The percentages of polymorphic bands in plants from the cross with *B. rapa* were all over 30% expect for plant no. 2, being higher than in plants from cross with *B. napus* (about 20%) (Table 1). In haploid plants of type III, three kinds of bands were also detected with comparable percentages to other plants. Some polymorphic loci were the same in F_1 plants (Fig. 3, arrowed), indicating that the introgressions were not entirely random.

Erucic acid and glucosinolate contents of F_1 plants and progenies

B. rapa × *C. bursa-pastoris*

Reduced erucic acid content of varying degrees from 51.7% of *B. rapa* to 8.58–37.78% was observed in F_1 plants of type II (Table 2). Similarly, the glucosinolates content in seeds also decreased from 116.57 μmol/g oil free meal of *B. rapa* to 41.53–85.39 μmol/g, but none reached the level of *C. bursa-pastoris* (15.68 μmol/g). Most profiles of the selfed seeds of F_2 plants derived from one F_1 plant were similar to each other and their F_1 plants (data not shown).

B. napus × *C. bursa-pastoris*

The changes in erucic acid content were observed in some F_1 plants of type V (Table 2). For the cross with *B. napus* cv. Oro, the content of glucosinolates was reduced in most F_1 plants and progenies, some being <30 μmol/g; however, the content of erucic acid of two plants (nos. 23, 25) increased. For the cross with Huashuang no. 3, F_1 plants still had the double-low quality as the female, and the content of glucosinolates was reduced in some plants. Most F_1 plants from the cross with Zhongyou 821 possessed reduced contents of erucic acid and glucosinolates, and one plant (no. 35) reached double-low standard. The content of glucosinolates of F_2 plants was deviated from those of some F_1 plants, but the content of erucic acid remained same (data not shown).

Resistance to *Sclerotinia sclerotiorum* in progenies

Some lines (F_2 or F_3) derived from some F_1 plants showed significantly higher resistance to *S. sclerotiorum* compared to female parents (Table 3). Plants nos. 1 and 13, derivatives of the cross with *B. rapa* cv. Aijuehuang, showed the smallest leaf and stem lesions, 1.8 and 4.3 cm, respectively. Four lines derived from plant nos. 1, 2, 4, 13 had significantly higher resistance expect for leaf infection of plant no. 13, one line derived from plant no. 1 showed significantly ($P < 0.01$) lower damage on leaves and stems than female parent. For cross with *B. napus* cv. Oro, the smallest leaf and stem lesions were 1.1 and 3.3 cm on plant nos. 29 and 17, respectively. Six lines derived from plant nos. 17, 20, 21, 22, 29, 30 showed significant resistance except for stem infection of plant no. 29, two lines derived from plant nos. 17 and 30 showed significantly ($P < 0.01$) lower damage on leaves and stems than female parent. For crosses with Huashuang no. 3 and Zhongyou 821, only few lines showed

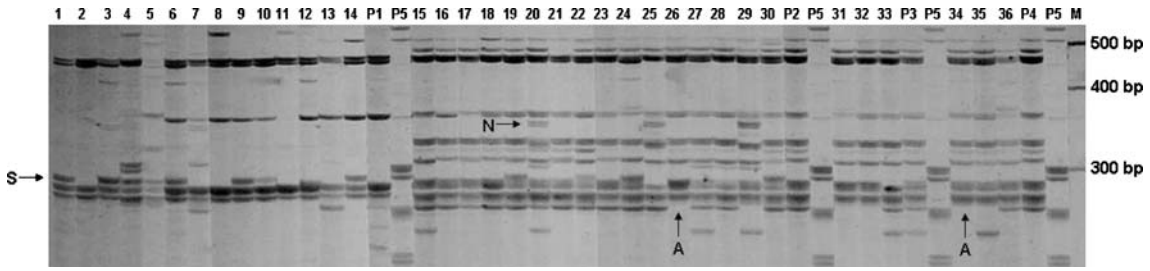

Fig. 3 AFLP profiles generated from the primer combinations 5′-GACTGCGTACCAATTCACT-3′ and 5′-GAT-GAGTCCTGAGTAACGA-3′ of *B. rapa* cv. Aijuehuang (*P1*), *B. napus* cvs. Oro (*P2*), Huashuang no. 3 (*P3*), Zhongyou 821 (*P4*), *C. bursa-pastoris* (*P5*), hybrids of P1 × P5 (nos. 1–14), P2 × P5 (nos. 15–30), P3 × P5 (nos. 31–33), and P4 × P5 (nos. 34–36). The *arrows* indicate polymorphic fragments: specific for *C. bursa-pastoris* (*S*), novel for two parents (*N*) and absent in female parent (*A*). *M*: Marker

Table 2 The contents of erucic acid (%) and glucosinolates (μmol/g oil free meal) of seeds from partial F_1 plants

Origin[a]	Plant no.	Erucic acid	Glucosinolates
P1 × P5	2	37.78	52.37
	5	11.06	41.53
	7	23.28	52.56
	10	32.37	85.39
	12	8.58	49.46
P2 × P5	20	0.14	43.81
	23	8.08	49.70
	25	11.78	52.44
	26	0.13	27.19
	27	0.45	41.23
	29	0.28	15.08
P3 × P5	32	1.04	17.81
	33	0.13	6.33
P4 × P5	35	0.61	29.26
	36	0.63	37.18
P1		51.70	116.57
P2		0.96	73.78
P3		0.77	24.38
P4		30.59	97.26
P5		0.68	15.68

[a] P1: *B. rapa* cv. Aijuehuang; P2: *B. napus* cv. Oro; P3: *B. napus* cv. Huashuang no. 3; P4: *B. napus* cv. Zhongyou 821; P5: *C. bursa-pastoris*

significantly lower damage than female parents, probably due to the high resistance of female parents.

Discussion

Progenies from the intertribal sexual hybridizations between *Brassica* species and *C. bursa-pastoris* were investigated on morphology, cytology and molecular characteristics, which enabled us to determine the hybridity status of each plant and to quantify the level of hybridization occurrence in these crosses. However, the progenies produced were not the hybrids with the expected chromosome complements. Only few F_1 plants were morphologically intermediate between the parents (Table 1, Fig. 1) and the rest resembled the female parents. GISH (Fig. 2) and AFLP (Table 1, Fig. 3) analyses revealed that the hybridization events occurred at various levels. Some *B. napus*-like progenies were also obtained after crossings with *C. bursa-pastoris* and new lines with higher yield and resistance to *S. sclerotiorum* were selected (Zhao et al. 1995), however, no genetic study was carried out.

Plant no. 1 of type I from cross with *B. rapa* expressing some morphological characters of the male *C. bursa-pastoris* (Table 1, Fig. 1) was a mixoploid with $2n = 27–29$ in somatic cells, while 63.6% PMCs had more chromosomes ($2n = 30–36$). GISH analysis demonstrated that only one or two *C. bursa-pastoris* chromosomes were included and chromosomal fragments translocated in PMCs and somatic cells (Fig. 2). These results suggested that the original hybrid cells ($2n = 26$) underwent chromosome duplication once or twice during mitotic divisions of the zygotes or plants, eliminating most male chromosomes, and extra duplication of partial chromosomes during meiotic DNA synthesis phase. The similar results were observed in *B. rapa* × *O. violaceus* intergeneric cross, where the chromosome doubling in hybrid cells and successive elimination of *O. violaceus* chromosomes accompanied by the introgression and recombination were responsible for producing *B. rapa*-type plants with modified genetic constitutions and phenotypes (Liu and Li 2007). This mechanism would be valid for explaining the present results. Partial hybrids with a haploid complement from female parent (oat) and some chromosomes (1–4) of male parent (maize) were reported in oat × maize cross (Riera-Lizarazu et al. 1996).

AFLP analysis performed on 474 loci for plant no. 1 indicated that it contained 5.9% DNA fragments putatively derived from *C. bursa-pastoris*, however, 55.9% genomic loci were changed (Table 1), suggesting that other reasons

Table 3 *Sclerotinia sclerotiorum* resistance of progenies

Origin[a]	Plant no.	Leaf lesion (cm)	Stem lesion (cm)
P1 × P5	1	1.8 ± 0.12**	5.9 ± 0.87**
	2 [b]	1.8 ± 0.39*	6.9 ± 2.40*
	4	2.1 ± 0.35*	6.8 ± 1.99*
	13	2.5 ± 0.53	4.3 ± 0.38**
P2 × P5	17	1.2 ± 0.08**	3.3 ± 0.97**
	20[b]	1.9 ± 0.17*	3.6 ± 1.52**
	21	1.9 ± 0.23*	3.8 ± 1.36**
	22	1.8 ± 0.21*	3.7 ± 1.80**
	29[b]	1.1 ± 0.41**	7.6 ± 0.32
	30	1.7 ± 0.31**	4.1 ± 1.91**
P3 × P5	31	1.6 ± 0.05*	2.4 ± 0.79**
	33[b]	1.2 ± 0.12**	1.3 ± 0.4**
P4 × P5	36	1.6 ± 0.42	4.4 ± 0.91*
P1		2.6 ± 0.17	19.5 ± 8.8
P2		2.2 ± 0.21	9.0 ± 2.27
P3		2.1 ± 0.25	5.9 ± 1.22
P4		2.0 ± 0.21	5.3 ± 0.20
P5		1.0 ± 0.25	2.5 ± 0.67

[a] The same as Table 2

[b] Progenies of F_2, others were F_3

*, **Significant difference for female parent at $P < 0.05$ and $P < 0.01$ in a Student's t test, respectively

were also involved in these genomic variations. Extensive alteration in DNA methylation patterns (Natali et al. 1998; Liu et al. 2004b; Wang et al. 2005), some mobile genetic elements (transposons and retrotransposons) (Liu and Wendel 2000; Shan et al. 2005; Wang et al. 2005), rapid sequence elimination in synthetic hybrids and allopolyploids (Song et al. 1995; Shaked et al. 2001), and genomic rearrangements in the hybrids were the causes for the genomic variations. The high frequency of the novel bands (35.4%) for two parents and deleted bands (14.6%) in *B. rapa* might be due to some of these reasons.

Matroclinous plants of types II (AA, $2n = 20$) and V (AACC, $2n = 38$) could arise due to the complete elimination of the *C. bursa-pastoris* chromosomes accompanied by alien introgression, and doubling the haploid genome during embryo development. The morphological traits of male parent and specific bands for *C. bursa-pastoris* indicated the occurrence of alien introgression. This kind of partial hybrids with the same chromosome numbers of female parents but altered genomic compositions had been reported in coffee (Lashermes et al. 2000), rapeseed (Cheng et al. 2002; Hua et al. 2006), and sunflower (Faure et al. 2002). Though the loci of *C. bursa-pastoris* were only 0–3.6% in these plants, 11.8–39.6% genomic loci were changed. Similarly, extensive genomic variations detected by AFLP analysis were up to 30% loci in rice recombinant inbred lines with <0.1% alien introgressed DNA (Wang et al. 2005). Plants of type III were *B. napus* haploids and were outcome of complete elimination of the *C. bursa-pastoris* chromosomes, together with fragment translocations (one chromosomal arm was labeled in ovary cells of plant no. 15) (Fig. 2) and introgressions (0.8–1.3% specific bands for *C. bursa-pastoris*). In plant no. 15, 9.4% somatic cells had $2n < 19$ (Fig. 2), suggesting that several chromosomes of *B. napus* in some cells were also eliminated.

Plants of type IV had $2n = 29$ and their PMCs at diakinesis had 10 II + 9 I and 1 III + 9 II + 8 I, suggesting that the genome of these plants consisted of ten bivalents and nine univalents. Cheng et al. (2002) obtained this kind of plants from *B. napus* × *O. violaceus* with similar results of cytology and molecular markers. Because these plants were obtained following crosses in 3 years and they expressed some traits of *C. bursa-pastoris*, such as basal clustering branches, small deep-green leaves and nanism and on the other hand had 0.6–2.1% specific bands for *C. bursa-pastoris*, suggested that they originated from true hybridizations with *C. bursa-pastoris*, not from the pollen contamination of *B. rapa*. According to their chromosome pairing configurations, their genomic constitution was proposed as 20A + 9C where one C genome was lost from the complement of *B. napus*. The plants ($2n = 29$) with all chromosomes of *B. napus* origin were also obtained in the crosses of *B. napus* with *O. violaceus* ($2n = 24$) (Hua and Li 2006) and *Lesquerella fendleri* ($2n = 12$) (Du et al., unpublished). One possible reason for this could be attributed to the dominance of rRNA genes from the two ancestors of *B. napus*, for the hierarchy of rRNA gene transcriptional dominance is *B. nigra* >*B. rapa* >*B. oleracea* and *B. rapa* rRNA transcripts are readily detected in natural *B. napus*, but *B. oleracea* transcripts are not detectable (Chen and Pikaard 1997). Similarly, more chromosomes from *B. oleracea* than from *B. nigra* were lost in cells with partial *B. carinata* complements ($2n < 34$) in hybrids between *B. carinata* and *O. violaceus* (Hua et al. 2006). The expression of *B. rapa* rRNA genes might help to stabilize the chromosomes of A genome in *B. napus* (Li and Ge 2007). Same as the plants of other types, genomic alterations of type IV were obvious and 19.1–26.4% genomic loci were changed.

The first *B. napus* cultivar 'Oro' with low erucic acid was selected from one local variety 'Liho' in Germany, while the only donor conferring the low glucosinolates in almost all the *B. napus* varieties was 'Bronowski' from Poland. Thus, the search for new gene source for double-low quality of rapeseed through suitable approaches including wide hybridization is pivotal for further genetic improvement. New *B. napus* inbred lines with increased levels of oleic and linoleic acids derived from one *B. napus* cv. Oro × *O. violaceus* hybrid, and reduced content

of glucosinolates (<30 μmol/g oil free meal) was obtained (Ma et al. 2006). The genomic compositions of these new lines were substantially altered from that of *B. napus* cv. Oro, as revealed by AFLP analysis. These changes should be extensive and affected many genes including plant phenotypes, the synthesis of fatty acids and glucosinolates. The reduction of erucic acid and glucosinolates content in our hybrids and their progenies (Table 2) might be caused by the introgression of related genes from *C. bursa-pastoris* or the genomic alteration consecutive to hybridization. Progenies with yellow seeds could be used in rapeseed breeding for higher oil content (Daun and DeClercq 1988).

The combination of cytological and molecular techniques was successful to determine the chromosomal/genomic constitutions of partial/introgressive *Brassica* hybrids (Cheng et al. 2002; Hua et al. 2006; Ma et al. 2006; Liu and Li 2007). In the present study, application of GISH and AFLP techniques better characterized the intertribal hybrids with very limited amount of alien genetic elements. In conclusion, the introgressive hybrids (types II and V) provided an opportunity to rapidly and successfully introduce useful traits of *C. bursa-pastoris* into *Brassica* species and to produce lines with improved oil quality and higher resistance to *S. sclerotiorum*.

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