RESEARCH ARTICLE

Origin of new *Brassica* types from a single intergeneric hybrid between *B. rapa* and *Orychophragmus violaceus* by rapid chromosome evolution and introgression

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Abstract

Many novel lines were established from an intergeneric mixoploid between *Brassica rapa* ($2n = 20$) and *Orychophragmus violaceus* ($2n = 24$) through successive selections for fertility and viability. Pedigrees of individual F_2 plants were advanced to the 10th generation by selfing. Their breeding habit was self-compatible and different from the self-incompatibility of their female parent *B. rapa*, and these lines were reproductively isolated to different degrees from *B. rapa* and *B. napus*. The lines with high productivity showed not only a wide spectrum of phenotypes but also obvious variations in fatty acid profiles of seed oil and glucosinolate contents in seed meal. These lines had $2n = 36, 37, 38, 39$ and 40, with $2n = 38$ being most frequent (64.56%), and no intact *O. violaceus* chromosomes were detected by genomic *in situ* hybridization (GISH) analysis. Amplified fragment length polymorphism (AFLP) analyses revealed a high extent of variation in genomic compositions across all the lines. *O. violaceus*-specific bands, deleted bands in *B. rapa* and novel bands for two parents were detected in these lines, with novel bands being the most frequent. The morphological and genetic divergence of these novel types derived from a single hybrid is probably due to rapid chromosomal evolution and introgression, and provides new genetic resources for rapeseed breeding.

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Introduction

Interspecific hybridization is common in plants in nature (Peakall *et al*. 1997; Cronn and Wendel 2004), and about 50–70% of angiosperms are thought to have arisen by hybridization (Stace 1987; Martinsen *et al*. 2001). Hybridization has been shown to contribute to speciation in three ways: allopolyploidy from the duplication of a hybrid's chromosome complement, homoploid hybrid and recombinational speciation, and introgression from backcrossing the hybrids to parents. Allopolyploidy is now recognized to be a prominent mechanism of speciation in flowering plants and ferns (Soltis and Soltis 1993; Leitch and Bennett 1997). The recombinational model is the most widely accepted model for homoploid hybrid speciation. In the case of introgression, the products of interspecific crosses, particularly those resulting from backcrossing or introgression, might be favoured by selection and thus contribute to adaptive evolution within populations (Rieseberg and Carney 1998). Experimental microevolutionary studies have provided support for models of hybrid speciation and introgression (Rieseberg and Carney 1998).

Orychophragmus violaceus (L.) O. E. Schulz (syn. *Moricandia sonchifolia* (Bunge) Hook Fil.) of Brassicaceae, with purple flowers, is cultivated as an ornamental plant in China and occurs wild in China and Korea. Although its placement in the tribe Brassiceae has been questioned (Gómez-Campo 1980; Al-Shehbaz 1985), a study on isozyme variation in the tribe supported the inclusion of this genus (Anderson and Warwick 1998). The distant relationship between *O. vio-*

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laceus and the cultivated *Brassica* species in U-triangle (U 1935) was also inferred from the chromosomal behaviours in their hybrids, because the hybrids except the one with *B. oleracea* L. were nonclassical mixoploids, and the separation of parental genomes during mitotic and meiotic divisions was proposed to occur and to be under genetic control (Li *et al*. 1995, 1998; Li and Heneen 1999; Hua and Li 2006; Hua *et al*. 2006; Li and Ge 2007). In this study, the progeny of one mixoploid hybrid between *B. rapa* and *O. violaceus* were successively selected to F_{10} generation for fertility and viability, and many new types, most commonly showing $2n = 38$, but distinctive phenotypes,were obtained. The chromosomal/genomic composition of these lines, analysed by GISH and AFLP, suggests that these lines were established by rapid karyotypic evolution accompanied by recombination and introgression.

Materials and methods

Plant materials

The intergeneric cross between *B. rapa* cv. 'Aijuehuang' ($2n = 20$; genomes AA; self-incompatible) and *O. violaceus* (self-compatible), with the latter as the pollen parent, was made by hand emasculations and pollinations in the campus field at Wuhan in 1995, and only one mixoploid hybrid ($2n = 23 - 42$) with some *O. violaceus* characters was identified (Li and Heneen 1999). This hybrid was partially fertile and produced some seeds of different sizes after selfing and open pollination. F_2 plants, which were grown in the greenhouse at the former Department of Plant Breeding Research, The Swedish University of Agricultural Sciences, Svalöv, Sweden, showed wide variation in phenotypes and chromosome numbers, and nearly half (47 out of 97) produced no seeds after selfing and open pollination, whereas the remaining showed poor and variable seed fertility. F_3 and subsequent generations were developed at Wuhan with one generation per year (from October to May) from 1998 on, with one additional generation (from May to September) in 2001 and 2003 in the experimental field of Qinghai Academy of Agricultural and Forestry Sciences, Xining, Qinghai Province. Progeny from each of the F_2 plants were selfed and selected for viability and seed fertility by using pedigree method, but the pedigrees for most F_2 plants were terminated due to very low fertility of their F_3 and F_4 plants. Thus, the final 59 F_{10} lines used in this study were derived from only 10 F_2 plants (figure 1). Some F_{11} lines were also used for AFLP analysis.

To determine if the F_{10} lines were crossable to *B. rapa* and *B. napus*, 20 flowers on each of three branches of one plant in each line were emasculated and pollinated by *B. napus* cv.'Oro' and *B. rapa* cv. 'Aijuehuang', respectively and seed set was recorded, as well as the pollen fertility of the progeny.

Cytological analysis and pollen stainability

Ovaries from young floral buds and roots from seedlings were used to determine the chromosome number of the progeny. After treatment with 2 mM 8-hydroxyquinoline for 3 h at 22°C, the ovaries and roots were fixed in aceto-alcohol (1:3 v/v) overnight and stored at −20°C until observation. For meiotic analysis of pollen mother cells (PMCs), floral buds from the terminal inflorescence were fixed overnight in aceto-alcohol (1:3 v/v). The preparations were made follow the method of Wu *et al*. (1997). Male fertility was determined by the percentage of pollen grains stained with 1% aceto-carmine solution. At least 300 pollen grains from two flowers of one plant were counted per plant (Li *et al*. 1998).

Genomic in situ hybridization (GISH) analysis

DNA of *B. rapa* cv. 'Aijuehuang' and *O. violaceus* was extracted and purified from young leaves' according to the method of Dellaporta *et al*. (1983). The DNA of *B. rapa* used for block was sheared by boiling for 15 min to produce fragments of about 300–600 bp. The DNA of *O. violaceus* used for probe was labeled with biotin-11-dUTP (SABC, Luoyang, China). The length of probe DNA fragments averaged ~500 bp.

Slide preparations for GISH followed the protocol by Zhong *et al*. (1996). The anthers selected at suitable stages were digested at 37°C for about 65 min in an enzyme mixture containing 0.6% cellulase Onozuka RS (Yakult Honsha Co., Ltd, Tokyo), 0.4% pectinase (MERCK, Darmstadt, Germany) and 0.02% Snailase (Beijing Baitai Biochem Co. Ltd., Beijing, China) .The fixed root tips were digested at 37°C for about 75 min in an enzyme mixture containing 0.2% cellulase Onozuka RS (Yakult Honsha Co., Ltd, Tokyo), 0.2% pectinase (MERCK, Darmstadt, Germany). *In situ* hybridization was carried out according to the protocols of Leitch *et al*. (1994). Hybridization signals of the *O. violaceus* probe were detected using Cy3 (Sigma, St. Louis, USA) and observed under Leica DMLB fluorescence microscope equipped with CCD (Leica, Wetzlar, Germany).

AFLP analysis

Young leaves of one plant per F_{10} line, and two plants per F_{11} line were harvested to extract DNA. AFLP analysis was conducted following the procedure of Vos *et al*. (1995), with some modifications. Genomic DNA was entirely restricted by *Eco*RI and *Mse*I, then *Eco*RI and *Mse*I 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. At last the AFLP profile was obtained with silver staining. Ten pairs of AFLP primes were randomly selected and used for AFLP fingerprint analyses and the bands with 80 to 800 bp were scored. Because most of the progeny lines analysed had $2n = 38$, same as *B. napus* ($2n = 38$; genomes AACC), *B. napus* cv. 'Oro' was included in AFLP analysis.

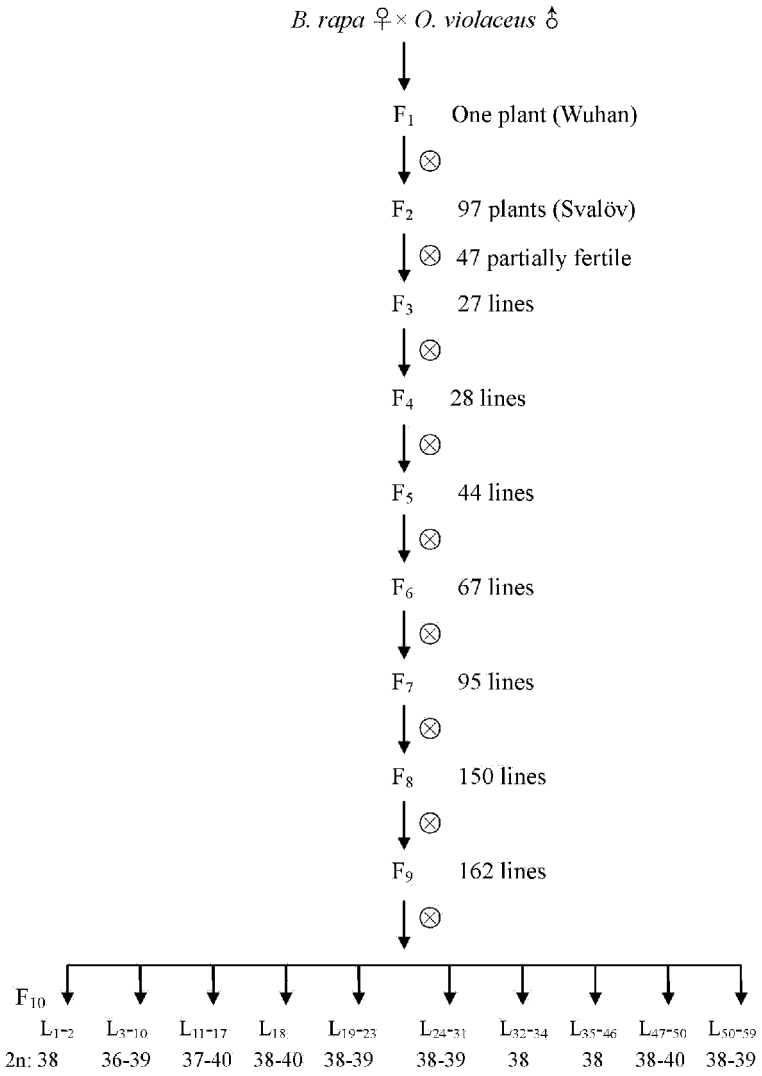

Figure 1. Selection scheme and origin of new plant types from the single hybrid of *B. rapa* and *O. violaceus*. The F_{10} populations originated from ten F_2 plants.

Fatty acid and glucosinolate analysis

Seed oil was extracted and its composition analysed by gas chromatography (HP 6890, Hewlett Packard, Waldbronn, Germany). The glucosinolate content was determined by near-infrared reflectance spectroscopy (NIRS) (Vector 22/N, Bruker, Ettlingen, Germany).

Results

Development and phenotypes of new plant types

The leaf morphology of *B. rapa* cv. 'Aijuehuang' and *O. violaceus* is quite different (figure 2 a,b), and their young hybrid was intermediate, expressing the serrated and basal clustering stems characteristic of *O. violaceus*, with longer and much thinner petioles than those of *B. rapa*. However, the hybrid showed yellow flowers like the female parent *B. rapa*, not purple like the male parent *O. violaceus* (Li and Heneen 1999). Most of the F_3 families grew vigorously but showed poor seed fertility and their pedigrees were stopped. Three to five plants in each family from F_3 and on were selected and bagged to give the seeds for next generations. The fertility segregation in each family continued to F_8 and with the generation progression the seed fertility increased, and by F_9 fertile types became characteristic of whole families. This trend could be attributable to the successive artificial selection for seed fertility. All lines were self-compatible, while their female parent *B. rapa* was self-incompatible.

Plants with normal seed fertility in the same F_{10} lines maintained the same morphology and most of the lines could be distinguished based on their phenotypes. Plants of 17 lines (24.64%) showed the purple colour of *O. violaceus* origin to different degrees on leaves, stems, or pods and some lines had the deep purple colour on all parts of plants,

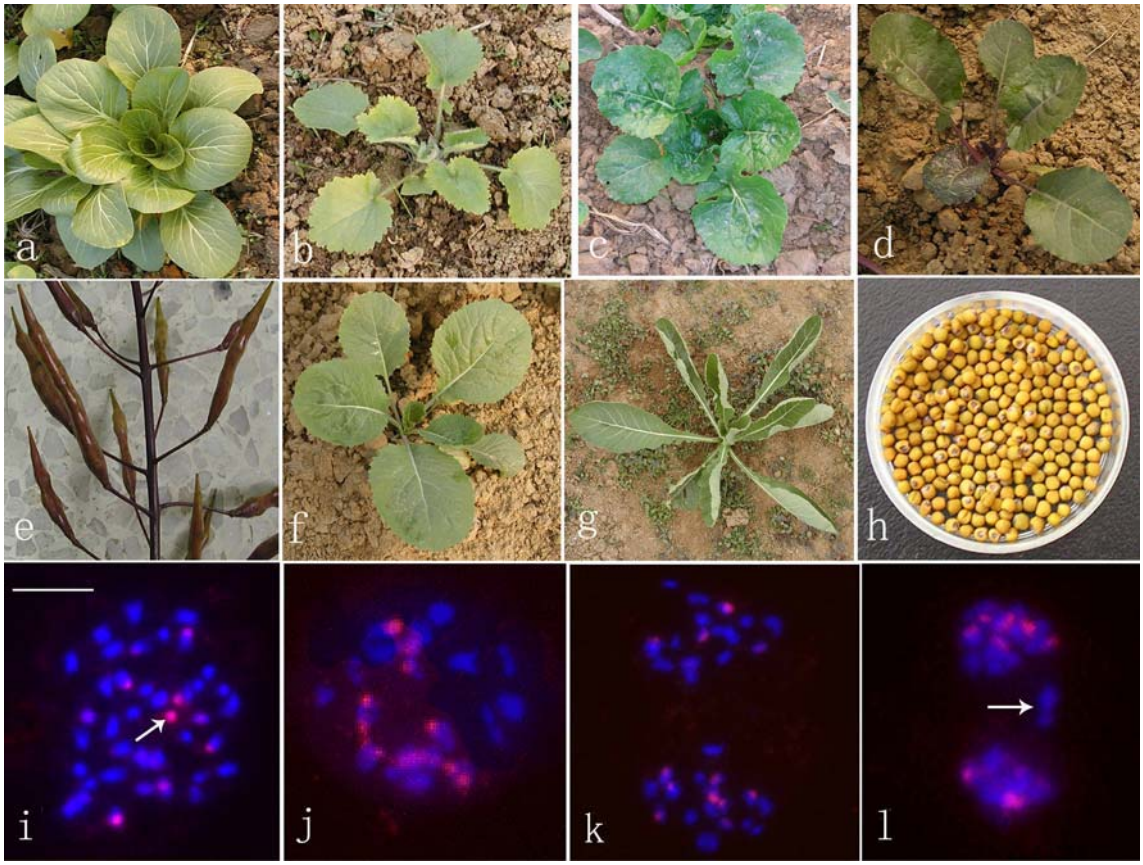

Figure 2. Young plants of (a) *B. rapa* cv. 'Aijuehuang', (b) *O. violaceus*, (c) One waxyless young plant of Line 29, (d, e) Purple young plant and inflorencence of Line 4, (f, g) Plants of Line 5, 41, (h) Yellow seeds of Line 2, (i) One root-tip metaphase (2n = 38) with strong signals of the *O. violaceus* probe at one satellite (arrow), terminal or centromeric parts of several chromosomes, (j) One diakinesis PMC with 19 bivalents and with strong signals of the *O. violaceus* probe at terminal or centromeric parts of several bivalents, (k) One AI PMC with some chromosomes being partially labeled and (l) One telophase I PMC with localized chromatin being labeled but not the laggard (arrow). Bar: 10 μm.

particularly the purple colour of the same line in Xining was deeper than in Wuhan, probably because the sunlight was stronger in Xining. Leaves with serrated edges from *O. violaceus* were expressed by plants of 26 lines (37.68%). The young plants of Line 29 looked more like *O. violaceus* before bolting, but were less waxy (figure 2c). However, the majority of F_{10} populations looked more like *B. rapa* after flowering. The plant heights of these lines, which were much higher than those of *O. violaceus*, were nearly same as *B. rapa* for most lines although some were about 20 cm shorter. The seed yields per single plant of all lines were much higher than *B. rapa* parent, which was attributed to higher number of first branches on main stem, pods per plants, seeds per pods and also much larger seeds (table 1). One line (line 2 in table 1) with highest seed yield produced yellow seeds (figure 2h).

After 21 F_{10} lines from eight F_2 plants were pollinated by *B. rapa* cv. 'Aijuehuang', the seeds per pod were 1.1–9.2 (average of 5.6), being lower in 9 lines and higher in 12 lines than those of the cross between 'Aijuehuang' and *B. napus* 'Oro'. After pollination by *B. napus* cv. 'Oro', the seeds per pod were 1.6–12.2 (average of 6.8), and lower in 13 lines and higher in 8 lines than those of the cross between 'Aijuehuang' and 'Oro'.

Cytology, GISH and AFLP analysis

In 44 F_{10} lines derived from 10 F_2 plants (figure 1), the chromosome numbers ($2n = 36 - 40$) in ovary cells were determined in 79 plants: 1 (1.27%) with $2n = 36$, 8 (10.13%) with $2n = 37$, 51 (64.56%) with $2n = 38$, 15 (18.99%) with $2n = 39$, and 4 (5.06%) with $2n = 40$. Plants with different chromosome numbers appeared in the progeny from seven F_2 plants, and those from the other three F_2 plants had $2n = 38$. So the chromosome number seemed to stabilize at $2n = 38$ for the majority of lines/plants and those with $2n = 36$, 40 were also cytologically stable. In the majority of PMCs of plants with $2n = 36$, 38 and 40, the chromosomes paired normally as 18, 19 and 20 bivalents at diakinesis, and segregated at anaphase I (AI) as 18:18, 19:19 and 20:20, respectively. The plants with $2n = 37$, 39 showed the diakinesis pairing 18 II+1 I and 19 II +1 I and AI segregations as 19:18 and

Table 1. Phenotypic and yield-related traits of typical lines, and seed set in crosses with *B. napus* cv. 'Oro' and *B. rapa* cv. 'Aijuehuang'.

Line	Plant height (cm)	Pods/plant	Seeds/pod	Weight of 1000 seed (g)	Seed yield (g/plant)	Seed colour	Male fertility (%)	Seeds/pod × Oro	Seeds/pod × Ajh
2	168±8.4	654±50.8	16.4±1.4	4.4±0.1	40.4±2.4	yellow	100.00	7.5	6.4
4	180±7.1	557±64.2	18.3±1.7	4.1±0.2	31.6±2.4	black	96.00	5.5	7.5
5	160±8.4	661±41.7	8.6±2.4	5.4±0.2	27.9±3.5	black	98.33	2.7	3.0
10	165±7.6	553±67.4	12.7±2.7	4.6±0.2	29.2±1.8	black	99.00	4.3	5.7
15	169±9.7	696±50.4	11.4±3.4	4.6±0.2	31.3±2.1	black	98.00	1.6	2.6
18	154±8.7	598±41.2	17.5±2.7	3.8±0.3	30.6±1.8	black	95.00	4.8	8.7
20	159±8.1	475±55.6	18.7±2.9	3.9±0.2	30.3±2.8	black	99.00	3.7	1.4
23	165±7.6	719±70.6	5.3±1.4	4.8±0.1	14.2±2.1	black	100.00	4.5	1.2
25	157±7.8	349±48.7	13.4±2.4	3.5±0.2	13.3±1.8	black	95.33	5.3	3.8
29	157±8.7	615±44.5	12.5±4.4	3.4±0.3	21.8±3.2	black	99.00	12.0	2.3
41	149±7.6	310±37.4	15.0±2.1	3.4±0.2	12.1±2.1	black	98.00	4.4	6.8
54	166±5.4	670±41.9	8.22±2.5	4.4±0.1	17.9±2.7	brown	99.33	9.1	8.5
Ajh	171±8.2	483±30.4	11.5±1.4	1.9±0.1	8.2±0.8	black	100.00	4.3	11.4
Oro	175±7.6	482±34.4	14.6±1.2	3.7±0.1	23.2±1.2	black	100.00	14.8	6.2
O.v	53±4.8	93±20.7	24.4±3.2	3.3±0.2	5.3±1.8	brown	99.33		

Ajh, 'Aijuehuang'; Oro, 'Oro'; *O.v.; O. violaceus.* See figure 1 for the origin of lines.

19:20, respectively. One or two laggards and chromosomal bridges were occasionally encountered at metaphase I/II and telophase I/II in PMCs of all plants.

The GISH signals on the *O. violaceus* chromosomes were distributed along their entire lengths (Hua *et al*. 2006; Zhao *et al*. 2007), which was different from strong centromeric signals and only very weak hybridization on chromosome arms seen in *Brassica* (Snowdon *et al*. 1997), making the technique more reliable to detect *O. violaceus* chromosomes in hybrid progeny from the crosses with *Brassica* species. To rule out the hybridization signals being caused by repeats shared by both parental species (for example 45S rDNA), GISH of *O. violaceus* genomic DNA to chromosomes of *B. rapa* cv. 'Aijuehuang' was observed (Liu and Li 2007). Using 'Aijuehuang' genomic DNA as the block, signals of large sizes and strong intensities from the *O. violaceus* probe were mainly located at two terminals of one bivalent and centromeric part of another one in PMCs at diakinesis or metaphase I (MI). Judged from its morphology, the bivalent with its two terminals being strongly labeled most likely was the satellite chromosome pair of *B. rapa* (Cheng *et al*. 1994). The terminal or centromeric parts of one or two chromosomes in each polar groups were covered by signals in AI PMCs and some chromatin was labeled in telophase I (TI) PMCs. Plants expressing obvious morphological characters and many specific AFLP bands (Line 29) of *O. violaceus* origin were subjected to GISH analysis. In their root-tip cells and PMCs at diakinesis, MI, AI/TI, signals of variable sizes and intensities from *O. violaceus* genomic DNA probe were located mainly at terminal and centromeric parts of some mitotic chromosomes and meiotic bivalents at diakinesis or chromosomes at AI, but no fully labeled chromosomes/bivalents were observed (figure 2 i-l), which showed that no intact chromosomes of *O. violaceus* origin were contained in these progeny. For the variation in number, size and intensity of GISH signals among the cells on the chromosome preparations, and also for the hybridization patterns on the chromosomes of original *B. rapa*, it is not possible to correlate the GISH signals with the sequence contributions of the *O. violaceus* parent, or with the number of the *O. violaceus*-specific AFLP bands.

Ten pairs of primers were used to amplify restricted DNA fragments from 41 out of 59 F_{10} lines. AFLP profiles from each pair of primers were highly polymorphic and no identical patterns were shown by any two lines. No lines presented the profiles which were same as or similar to those of *B. napus* cv. 'Oro' and the *B. rapa* parent. Fragments specific for *O. violaceus* were amplified frequently in lines with *O. violaceus*-like phenotype (figure 2 c–f). By using 17 pairs of primers randomly selected, the polymorphic bands were revealed among 28 F_{11} lines derived from 26 F_{10} lines. The average numbers, percentages and ranges of *O. violaceus*-specific bands, deleted bands in *B. rapa* and novel bands for the two parents were 39.4 (33–51), 34.3 (26–44), 85.6 (70–100) and 10.26% (8.5–13.11), 8.8% (6.7–11.3%), 22.3% (18.0–27.7%), respectively. The result that the novel bands with much higher frequency in these lines were more frequent than the other two types might correspond to their chromosome complements having quite different chromosome numbers from that of female *B. rapa*, while the other two types of bands had similar percentages.

Fatty acid composition and glucosinolate content

Fatty acid composition and glucosinolate content were analysed in the selfed seeds of three plants in each line. The seed oil of *O. violaceus* was characterized by high percentage of linoleic (~ 50%) and palmitic (15%) acids and low

Table 2. Fatty acid compositon and glucosinolate content in seeds of one plant in typical lines.

Plants	Fatty acid composition (%)							Glucosinolate (μ mol/g)
	Palmitic	Stearic	Oleic	Linoleic	Linoleic	Eicosenoic	Erucic	
2–1	3.89	0.89	18.97	20.76	9.24	10.35	35.91	119.34
4–1	4.80	2.70	68.35	14.36	6.02	2.15	1.61	93.41
5–7	4.82	1.79	23.59	18.65	10.37	12.78	28.00	94.39
10–3	6.08	3.19	52.79	23.87	13.71	0.07	0.29	58.35
15–3	3.84	0.85	10.20	14.40	10.41	2.99	57.30	84.17
18–2	5.77	3.03	66.29	17.98	5.83	0.95	0.15	42.41
20–4	5.08	2.30	55.53	24.18	11.26	1.40	0.25	29.91
23–3	5.34	3.26	65.76	16.66	8.15	0.78	0.06	61.37
25–1	4.34	3.03	74.48	12.36	3.95	1.66	0.18	86.08
29–1	3.44	0.82	19.94	11.56	5.92	8.50	49.82	29.57
41–4	5.55	2.12	66.57	15.60	8.92	1.00	0.23	53.66
54–2	2.85	1.44	31.58	15.41	7.69	13.85	27.17	68.11
Ajh	2.59	1.40	14.63	14.22	10.60	5.75	50.82	86.50
Oro	4.20	2.14	60.62	18.51	5.71	4.85	3.96	53.40
O.v.	15.31	8.09	15.98	46.67	5.12	4.36	0.86	50.98

Ajh, 'Aijuehuang'; Oro, 'Oro'; *O.V., O. violaceus.* See figure 1 for the origin of lines.

percentage of erucic acid (~1%), that of *B. rapa* cv. 'Aijuehuang' by high percentage of erucic acid (~50%) and low oleic acid (~15%), and that of *B. napus* cv. 'Oro' by high percentage of oleic acid (~60%) and low erucic acid (~3%) (table 2). Fatty acid profiles of the plants in the same lines were similar. However, plants in different lines showed great variation in the percentages of oleic (10.20%–74.48%) and erucic acids (0.06%–57.30%). The percentage of oleic acid was ≥ 65% in nine out of 59 lines, higher than that of 'Oro'.

Wide variation in glucosinolate contents (1.55–139.44 μ mol/g oil free meal, higher or lower than those of the two parents) existed in different lines, but plants of the same line had similar values. Three plants from 3 lines had < 30 μ mol/g glucosinolate (the value for canola standard) and line 29 were double low (< 2% erucic acid also).

Discussion

From the sexual cross *B. rapa* var. *chinensis* cv. 'Aijuehuang' × *O. violaceus* (Li and Heneen 1999), the sole seeded hybrid of mixoploidy nature ($2n = 23 - 42$) was morphologically intermediate, except for its partial fertility and yellow petals similar to that of *B. rapa*. The mixoploidy might have originated from the partial separation of parental genomes and chromosome doubling during mitotic divisions of zygote ($2n = 22$), as proposed for the hybrids of *O. violaceus* with *B. juncea* and *B. carinata* (Li *et al*. 1998), or from the partial loss of *O. violaceus* chromosomes in an embryo with $2n = 44$ as a result of fusion between unreduced gametes or the doubling of zygote chromosomes. The inclusion of *O. violaceus* chromosomes in the hybrid and its progenies was well evidenced by their morphology and cytological behaviour (Li and Heneen 1999), for some large and darkly stained chromosomes were easily distinguishable in its mitotic and meiotic cells and were probably of *O. violaceus* origin, as usually observed in crosses with *B. juncea* (Li *et al*. 1998) and *B. nigra* (Li and Heneen 1999). The larger size of the *O. violaceus* chromosomes than that of *Brassica* ones has been shown by its karyotype (Li *et al*. 2005) and nearly the same DNA content as *B. napus* (Hu *et al*. 2002). F_2 plants showed wide variation in fertility (nearly half sterile), phenotypes and chromosome numbers (still mixoploids, maximum of $2n = 29 - 40$ for each plants observed). However, fertile F_{10} lines with wide variations in phenotypes (figure 2 c-g), fatty acid profile and glucosinolate content (table 2) derived from 10 F_2 plants had only $2n = 36 - 40$, without intact *O. violaceus* chromosomes. This result suggested that these F_{10} lines were partial and rearranged *B. rapa* autotetraploids with diploidized meiotic behaviour and with some introgressed chromosomal fragments from *O. violaceus* (figure 3).

Elegant studies involving hybrids of *Elymus* and *Sitantion* (Stebbins *et al*. 1957), *Gilia malior* and *G. modocensis* (Grant 1966), and *Helianthus annuus* and *H. petiolaris* (Rieseberg and Carney 1998) have shown that the recombinant lineages with a new combination of morphological and cytogenetic features derived from single hybrid plant were typically generated in ten generations or fewer, and isolated strongly from their parents, and that 'a single, partially fertile hybrid individual can suffice as progenitor of a new species' (Ellstrand *et al*. 1996). The establishment of fit hybrid genotypes in a hybrid population may occur through asexual reproduction, selfing, diploid hybrid speciation and the introgression of advantageous alleles (Rieseberg *et al*. 2003). Factors that appear to play a critical role in recombinational speciation include: strong natural selection for the most fertile or viable hybrid segregants, rapid chromosomal evolution and the availability of habitat suitable for the establishment of hybrid neospecies (Rieseberg and Carney 1998).

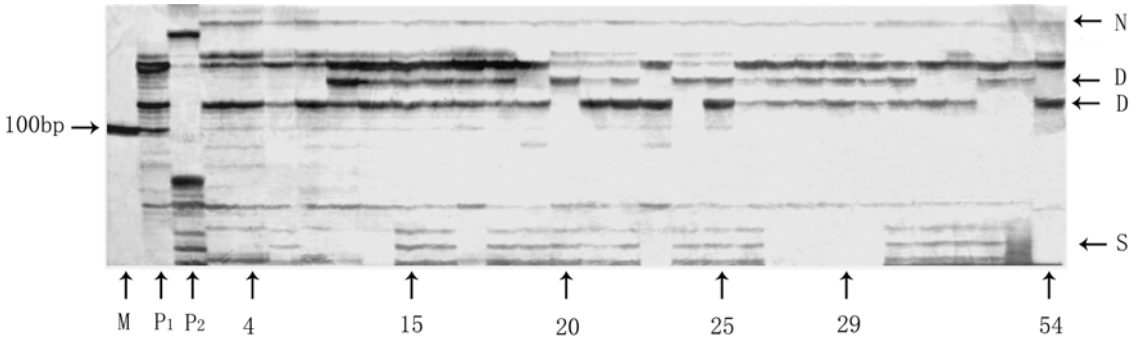

Figure 3. Representative AFLP profiles of 28 F_{11} lines from one pair of primers. The numbers under each lane correspond to those in table 1. S, *O. violaceus*-specific bands; D, deleted bands; N, novel bands; M, Marker; P_1, *B. rapa*; P_2, *O. violaceus*.

Pathway to rapid speciation, other than recombinational speciation, are allopolyploidy, changes in mating system, fixation of chromosomal rearrangements and rapid adaptation to new habitats. Thus, the production of self-compatible lines (their female parent *B. rapa* being self-incompatible) with distinctive phenotypes and genomic compositions from our single hybrid was probably attributable to artificial selfing and selection for seed fertility and viability in successive generations. In an experiment with *Helianthus*, comparison of the genomic composition of the natural (*H. anomalus*) and synthetic hybrid lineages revealed that all the three synthetic hybrid lineages had converged to nearly identical gene combinations (Rieseberg *et al*. 1996), suggesting that deterministic forces such as selection, rather than stochastic forces, largely governed the genomic composition of hybrid species, and that the genomic structure and composition of hybrid species may essentially be fixed after a small number of generations of hybridization and remain relatively static thereafter. The same or similar chromosome numbers in our lines should also be determined in early generations.

At the chromosomal level, these lines were obviously differentiated from their parents *B. rapa* and *O. violaceus*. But the fact that no intact *O. violaceus* chromosomes were included, and the extensive genomic differences with limited DNA fragments from *O. violaceus* among these lines were detected, suggested that chromosomal repatterning with structural changes at a large degree occurred during the selection process. The variation in genomic composition of these lines with the same or similar chromosome numbers might be attributable to structural rearrangements in chromosomes and genomic alterations induced by the hybridization and the subsequent introgressions of the *O. violaceus* chromosomal/DNA fragments (Wang *et al*. 2005). Considering that high crossability existed between *B. rapa* and *B. napus*, and that seed set was usually low in most crosses of these lines with the two *Brassica* species, and that the pollen fertility was also low in the progeny of these crosses (data not shown), reproductive isolation of most of these lines with *B. rapa* and *B. napus* has clearly arisen and they could be considered to be a new *Brassica* type.

As to morphological character expression in hybrids, analysis of many studies revealed several surprising tendencies (Rieseberg and Carney 1998). First, F_1 hybrids were shown to be a mosaic of both parental and intermediate morphological characters, rather than just intermediate ones. A second important finding was the high frequency of transgressive or novel characters in hybrids or in later-generation hybrids. Many phenotypic characters of our lines, including seed yield, were intermediate, novel or transgressive (table 1). Several explanations have been offered to account for the expression of novel or transgressive characters in hybrids (Rieseberg and Carney 1998), such as the complementary action of new combinations of normal alleles (Rieseberg *et al*. 2003), the placement of unexpressed (or expressed) alleles in a new genetic background (epistasis), the fixation of recessive alleles present in the heterozygous form in the parents (dominance), reduced developmental stability, and simple heterosis (overdominance).

It has long been recognized that hybridization and introgression occurs widely among natural plant population (Anderson 1949). Although introgressive hybridization does not necessarily led to immediate hybrid speciation, it is an important means for the transfer and/or *de novo* origination of traits related to ecological adaptations and therefore, plays an important role in facilitating speciation in changing niches (Arnold 2004). A key role of introgressive hybridization is possible to generate extensive stochastic genomic and epigenomic variations that can be translated into phenotypic novelties and upon which natural selection may then act (Wang *et al*. 2005). Even cryptic alien introgression (< 0.1%) can function as a potent mutagen and was seen to induce extensive and genome-wide *de novo* variations affecting up to 30% of the genome in rice recombinant inbred lines with a wide range of phenotypic features (Wang *et al*. 2005). In new *B. napus* inbred lines ($2n = 38$) with variations in phenotypes and fatty acid composition in the F_{12} progenies of one *B. napus* cv. Oro × *O. violaceus* mixoploid hybrid (Li *et al*. 1995), no intact chromosomes of *O. violaceus* origin were detected in their somatic and meiotic cells, while AFLP analysis revealed that substantial genomic changes have occurred (Ma

et al. 2006). However, their phenotypic variations were not so wide as those shown by the present lines, with more *O. violaceus* traits recognizable. Probably the two genomes (A, C) in *B. napus* buffered the expressions of alien *O. violaceus* genes.

The present results are highly relevant for our understanding of hybrid speciation in plants, currently there are not many cases known, where the F_{10} generation hybrids are available for such studies. Moreover, the new lines with high productivity, yellow seed-coat, high oleic acid but low erucic acid and low glucosinolate content should also provide new genetic source for rapeseed breeding.

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