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Production and cytogenetics of intergeneric hybrids between *Brassica napus* and *Orychophragmus violaceus*

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Abstract The intergeneric hybrid between *Brassica napus* and *Orychophragmus violaceus* was obtained by means of embryo culture technique with the latter as the pollen parent. The hybrid was morphologically intermediate between its parents, but could produce a lot of seeds when selfed. Somatic separation of the genomes from the two parental species was observed during the mitotic divisions of some of the hybrid cells. Thus, the hybrid became the mixoploid in nature, consisting of haploid and diploid cells of *B. napus*, and a nuclear–cytoplasmic hybrid, with the cytoplasm of *B. napus* and the nuclei of *O. violaceus*, and the hybrid cells. Pollen mother cells with 19, 12 and 6 bivalents, respectively, were produced by the hybrid. From the selfed progeny of the hybrid, mainly two kinds of plants, *B. napus* and the hybrid, were found. The hybrid plants of the selfed progeny again produced two kinds of plants, *B. napus* and the hybrid.

Key words *Brassica napus* · *Orychophragmus violaceus* · Intergeneric hybrid · Somatic separation of genome · Mixoploid

Introduction

Wild species are important as genetic resources for the improvement of cultivated crops. Attempts have been made for many years to expand the pool of accessible genes of oil-yielding *Brassica* crops through sexual crossing. However, wide intergeneric hybridizations of *Brassica* have been mainly limited to such genera, as *Raphanus* (Karpechenko 1928; Takeshita et al. 1980), *Sinapis* (Riley and Arnisson 1990), *Diplotaxis* (Quiros et al. 1988; Fan et al. 1985) and *Moricandia* (Takahata 1990). *Orychophragmus violaceus* belonging to Cruciferae, is a valuable oil-seed plant resource. According to Luo et al. (1991) it is characterized by a superior oil quality. Its oil contains high percentages of oleic (20.32%), linoleic (53.17%) and palmic (14.31%) acids and lower percentages of linolenic (4.76%) and erucic (0.94%) acids. Consequently, intergeneric hybrids between *Brassica* cultivars and *O. violaceus* may be useful for the introduction of good oil quality into rapeseed crops. The present paper reports the first production of hybrids between *B. napus* and *O. violaceus* through embryo culture. In addition, morphological and cytological characterizations of the hybrids were made.

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Materials and methods

Plant materials

Lines and cultivars used in the present study were *B. napus* cvs 'Oro', 'Canadian twinlow', '81008', 'Altex' and 'Senli' ($2n = 4\times = 38$, AACC), *O. violaceus* ($2n = 24$,OO) (supplied by Department of Biology, Sichuan University), and *B. napus* cvs 'Huayou No. 8', 'GR144-149' (supplied by Department of Agronomy, Huazhong Agricultural University). The crosses between *B. napus* and *O. violaceus* were performed in the field by hand emasculation and pollination.

Culture of hybrid seeds and fast multiplication of hybrid buds

To insure the germination of weak seeds from the crosses, embryo culture was used. Seed coats were sterilized using 70% ethanol for 5 min and then 0.15% $HgCl_2$ for 15 min. After the seed coats were removed aseptically on the filter papers and rinsed in sterile water, the embryos were isolated and cultured on Murashige and Skoog (1962) agar medium (MS). The medium was supplemented with sucrose (3% w/v), agar (0.8% w/v), 6-benzyl aminopurine (BAP, 3 mgl^{-1}) and α-naphthalenacetic acid (NAA, 0.2 mgl^{-1}). The pH was adjusted to 5.8 before autoclaving at 1.1 kg/cm^2 for 15 min.

When axillary buds appeared on the seedlings, the terminal and axillary buds were cut off and transferred to fresh medium. The buds developed into plantlets, and clusters of buds appeared on the calli formed at the bottom of the buds. By the successive culture of buds from the shoots and calli, a large number of buds were obtained for application in wide hybridization.

After the buds were cultured on rooting medium (MS supplemented with sucrose (3% w/v), agar (0.8% w/v), 0.5 mgl^{-1} NAA) for 15 days, and the root length reached about 1–2 cm, the plants were transplanted in the field.

Determination of chromosome numbers

The chromosome numbers of the hybrids were determined on fresh leaves, young flower buds and root tips. These were immersed in icewater (0 °C) for 6 h, treated with 2 m*M* 8-hydroxyquinoline for 4 h and then fixed in Carnoy's solution. They were hydrolyzed in 1 *N* HCl at 60 °C for about 10 min, squashed in a drop of 10% modified carbol fuchsin and observed under oil. To observe pollen mother cells (PMC) for meiotic analysis, buds from the terminal inflorescence were fixed immediately after collected in fresh Carnoy's solution for 24 h. Buds were then stored in 70% ethanol at 4 °C. The anthers were dissected out, cut in half and the PMCs squeezed out in a drop of 10% modified carbol fuchsin.

Pollen stainability was determined as the percentage of pollen grains stained with 1% acetocarmine. More than 300 pollen grains from 2 flowers were counted for each plant. Normal pollen grains were fully round and densely stained, and they were easily distinguished from shrunken and lightly stained ones.

Results

Chromosome pairing of *O. violaceus*

The karyotype formula of *O. violaceus* is 2n = 24 = 20 m + 4 sm (4SAT), 20 metacentric and 4 submetacentric chromosomes with satellites (Luo et al. 1991). In the PMCs of *O. violaceus*, more than 30 pairing configurations were observed (Table 1), these mainly 12II (Fig. 1a), 10II + 1IV (Fig. 1b), 9II + 1VI (Fig. 1c), 8II + 2IV (Fig. 1d), 8II + 1VIII (fig. 1e), 7 II + 1 X (Fig. 1f). Thus, multivalents with 4, 6, 8 and 10 chromosomes appeared. The reason for this was the presence of possible homoeology among chromosomes of *O. violaceus*.

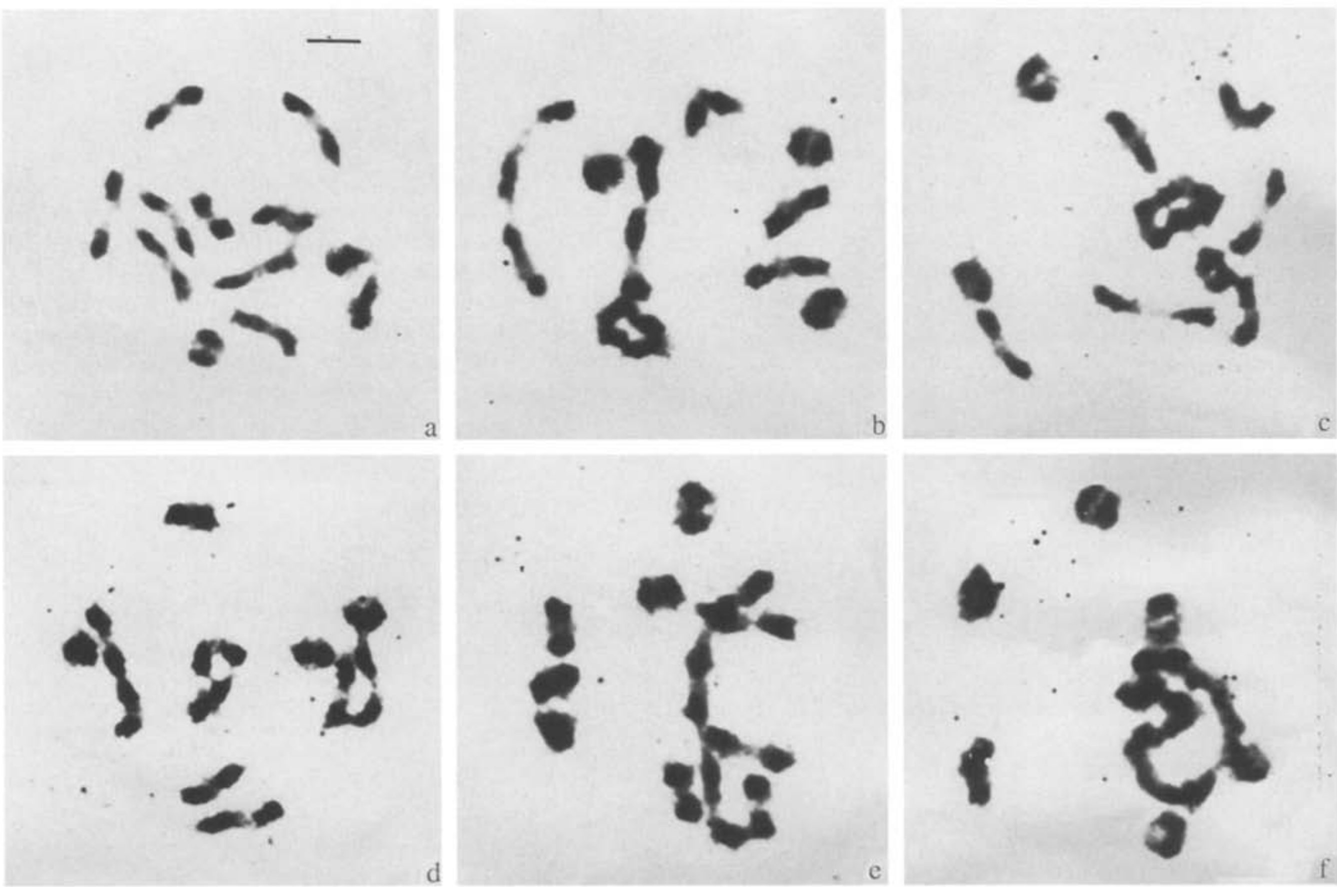

Fig. 1a–f Chromosome pairing of *O. violaceus*. **a** 12 II, **b** 10 II + 1 IV, **c** 9 II + 1 VI, **d** 8 II + 2 IV, **e** 8 II + 1 VIII, **f** 7 II + 1 X. *Bar*: 5 μm

Table 1 Number and percentage of different pairing configurations in PMCs of *O. violaceus*

Configurations	12 II	10 II + 1 IV	9 II + 1 VI	8 II + 2 IV	8 II + 1 VIII	7 II + 1 X	others	Total
Number of PMCs	149	30	20	13	15	11	59	294
Percentage	50.6	10.2	6.8	4.4	5.1	3.7	19.2	100

Production and morphology of the hybrid

When *B. napus* was used as the female parent, hybrid plants were obtained in crosses involving cv 'Oro', 'Huayou No. 8' and 'GR144–149' (Table 2). No hybrid plants were obtained in the crosses using cv 'Canadian twinlow', 'Altex', '81008' and 'Senli' (Table 2). There were great differences among the genotypes of maternal *B. napus* with respect to their crossability with *O. violaceus*. Although a large number of crosses were made with *O. violaceus* as the female parent, no hybrid plants were obtained (Table 2), which indicates that the compatibility between *B. napus* and *O. violaceus* was very low.

Table 2 Results of intergeneric hybridizations for the reciprocal crosses of *B. napus* × *O. violaceus*

Cross	Number of crosses	Number of hybrid plants
Oro × *O. violaceus*	2430	18
Canadian twin-low × *O. violaceus*	632	0
81008 × *O. violaceus*	453	0
Altex × *O. violaceus*	684	0
Senli × *O. violaceus*	735	0
Huayou No. 8 × *O. violaceus*	1550	2
GR144–149 × *O. violaceus*	432	1
O. violaceus × *B. napus*	2350[a]	0

[a] Total number of crosses with all *B. napus* cultivars

The young hybrid plants were characterized by their deep green, oval shape and hairy leaves, characters similar to those of *O. violaceus*. The character of basic clustering stems of *O. violaceus* was also observed in the hybrid plants (Fig. 2a). During the flowering season the hybrid plants displayed the yellow flowers of *B. napus*, not the purple ones of *O. violaceus*. The flowers opened normally and had anthers containing abundant pollen grains. The hybrid plants were fertile, and when selfed, they produced a lot of seeds (Table 3). The siliques of the hybrid were much shorter than those of *B. napus* and *O. violaceus* (Fig. 2b,c). The majority of the seeds from the hybrid plants were shrunken (Fig. 2d) and not germinable (Table 6).

Cytogenetical observations of the hybrid

Somatic separation of genomes during the mitotic divisions of the hybrid cells

The hybrid had the expected chromosome number (2n = 31) in its somatic cells (Fig. 3a). Three genomes were included in the hybrid cells, A and C (from *B. napus*), and 0 (from *O. violaceus*), with 10, 9 and 12 chromosomes, respectively. During the mitotic divisions of the hybrid cells, two groups of chromosomes were formed (Fig. 3b), with the 31 chromosomes arranged in a 19 + 12 formation (Fig. 3c). The two groups of chromosomes then separated gradually (Fig. 3d), ultimately reaching two different cell poles (Fig. 3e). Finally, two kinds of cells with 19 and 12 chromosome, respectively, were produced (Fig. 3f). The two kinds of cells formed after the

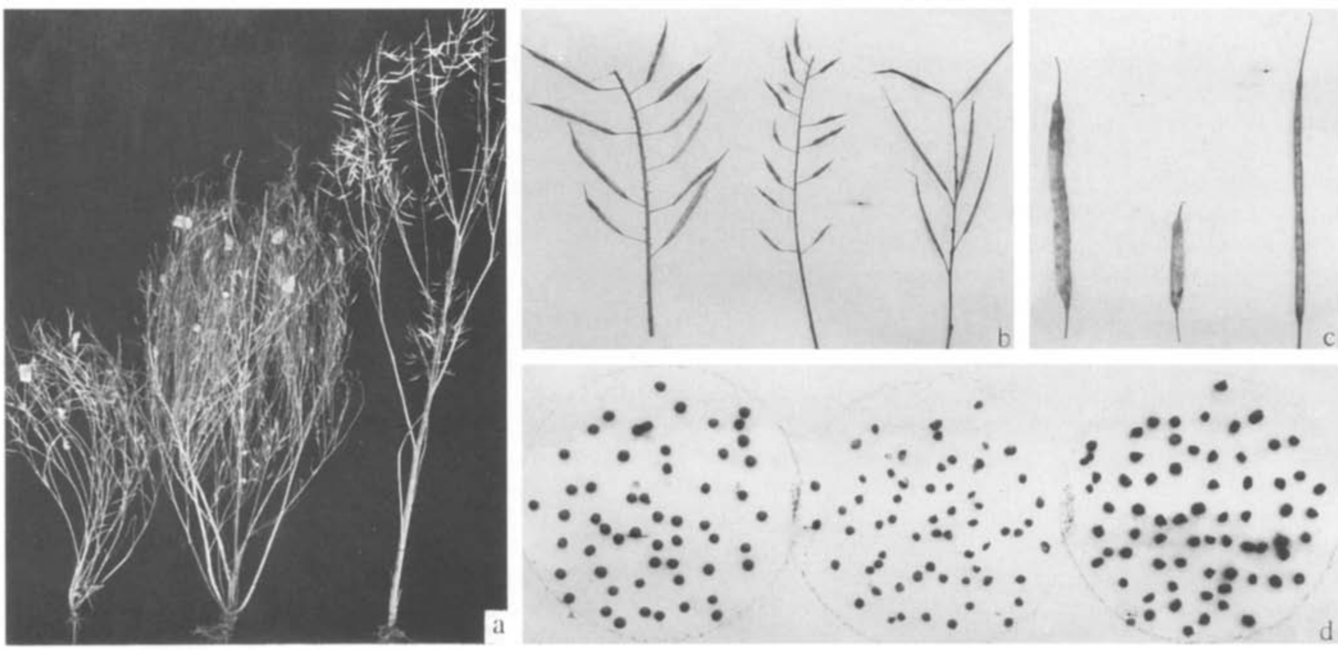

Fig. 2a–d Morphology of the hybrid between *B. napus* cv 'Oro' and *O. violaceus*. **a** Plant comparison of *O. violaceus*, the hybrid and *B. napus* (from *left* to *right*). The hybrid plant displayed the *O. violaceus* character of basic clustering stems. **b–c** The inflorencence and siliques, respectively, of *B. napus*, the hybrid and *O. violaceus* (from *left* to *right*). The siliques of the hybrid were much shorter than those of *B. napus* and *O. violaceus*. **d** The seeds of *B. napus*, the hybrid and *O. violaceus* (from *left* to *right*). Most of the seeds from the hybrid plants were shrunken. The shape and size of the *O. violaceus* seeds were different from those of *B. napus*

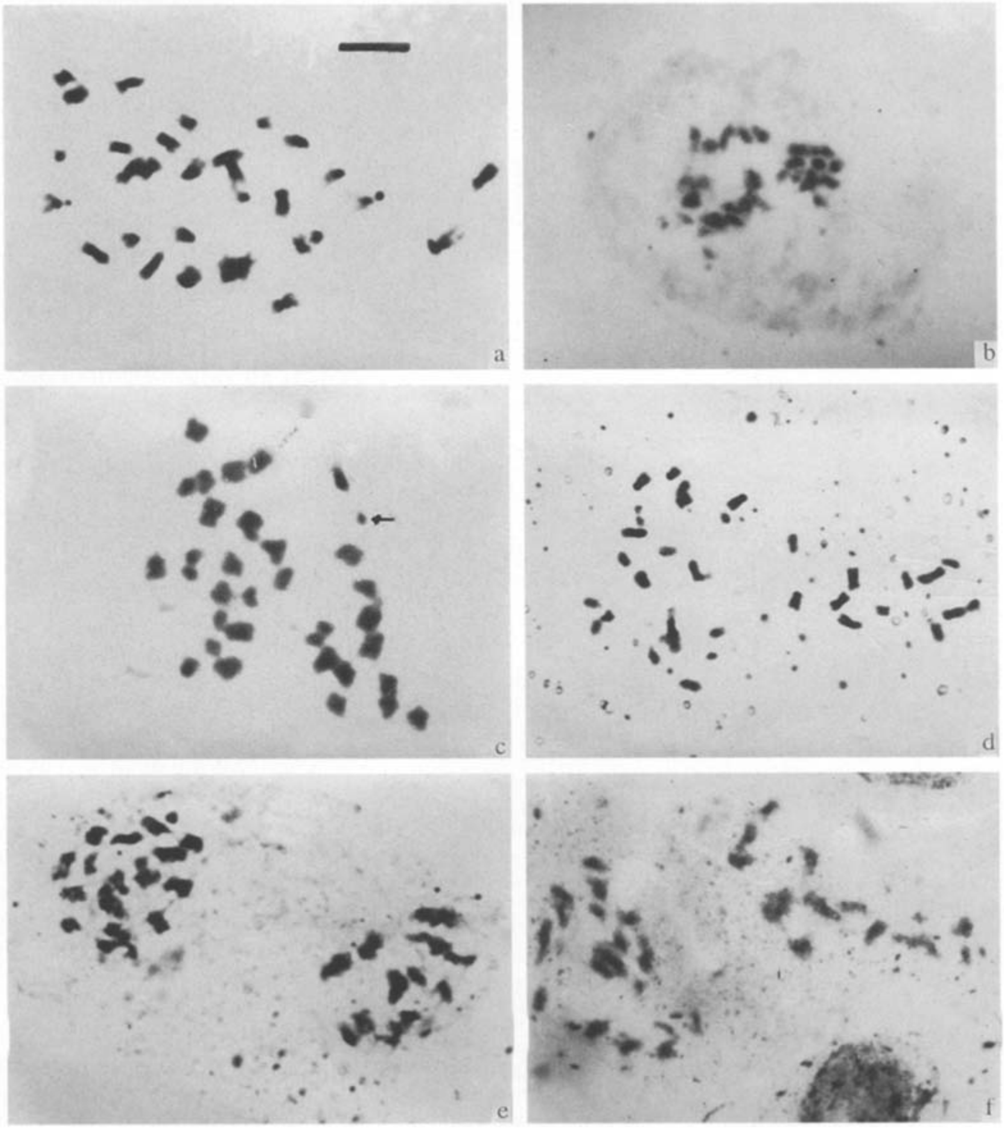

Fig. 3a–f Somatic genome separation from two parents during mitotic division of the hybrid cells. **a** The 31 chromosomes of the hybrid cell. **b** Two groups of chromosomes in the hybrid cell. **c** Arrangement of 31 chromosomes as 19 + 12 in the hybrid cell; *left* 19 chromosomes, *right* 12 chromosomes. Note that the sizes of the 12 chromosomes on the right are larger than those of the 19 chromosomes on the *left*; and that there is little variation in size among the 12 chromosomes and a large variation in size among the 19 chromosomes. On the basis of the number and morphology of the chromosomes, 19 were assumed to come from *B. napus* and 12 from *O. violaceus*. (*Arrow* shows one satellite). **d** Gradual separation of the 19 chromosomes on the *left* from the 12 chromosomes on the *right*. Also, note the difference in chromosome morphology of the two groups. **e** Two groups of chromosomes reached two cell poles, respectively; *left* pole had 19 chromosomes, *right* pole had 12 chromosomes. **f** Formation of two types of cells, one with 19; the other with 12 chromosomes. *Bar*: 5 μm

somatic separation of the genomes, however actually had 38 and 24 chromosomes because of the replication of the chromosomes before the mitosis.

In the fresh leaves of young hybrid plants and the anthers flower buds, various cells were found with 38, 31, 24, 19 and 12 chromosomes, respectively (Table 4). Although most of cells found in the fresh leaves of young hybrid plants were hybrid ones (2n = 31), cells with 38, 24, 19 and 12 chromosomes appeared, indicating that somatic separation of the genomes had occurred in some hybrid cells. The highest number of hybrid cells was still in the anthers of the hybrid. During progressive development of the hybrid plants, the percentage of hybrid cells decreased and that of cells with 38 chromosomes increased. Even more pronounced, the percentage of cells with 2n = 38 was much higher than that of cells with 2n = 24, even though theoretically the two kinds of cells should be present in equal numbers in the hybrid. It may be that the two kinds of cells do not divide at the same rate.

PMCs in the hybrid

Besides the PMCs produced by the hybrid cells (the meiotic pairing of chromosomes in this kind of PMCs will be reported elsewhere), three kinds of PMCs with 19, 12 and 6 bivalents, respectively, were observed in the hybrid (Table 5) (Fig. 4). The pollen stainability of the hybrid was very low, only 46.7% (Table 5).

Plant types in the progeny of the hybrid

There were mainly two kinds of plants in the selfed progeny of the hybrid: *B. napus* (2n = 38) and the hybrid (2n = 31) (Table 6). In the F_3 and F_4 populations, 2 plants with 25 and 50 chromosomes, respectively, were identified. The plants of *B. napus* among the selfed progeny were morphologically similar to the parental ones and produced PMCs with 19 bivalents. Moreover, they showed normal fertility. However, the hybrid

Table 3 Agronomic characters of *B. napus* cv 'Oro', the hybrid and *O. violaceus*[a]

	Plant height (cm)	Branching position (cm)	Number of first branchings	Number of pods on main stem	Number of pods per plant	Length of pods (cm)	Seeds per pod	Weight of 1000 seeds (g)
B. napus	145.6	25.4	8.5	53.4	250.6	6.5	20.5	3.57
Hybrid	116.7	0	9.6	30.6	210.3	4.1	3.8	–
O. violaceus	74.5	0	11.8	15.3	180.6	8.2	35.2	3.92

[a] 20 plants were observed for each character

Table 4 Number and percentage of different kinds of cells in the hybrid between *B. napus* cv 'Oro' × *O. violaceus* at two growth stages

Chromosome number of cells		38	31	24	19	12	Total
Leaves of young plants	Number of cells	20	82	17	9	8	136
	Percentage	15.2	60.5	12.4	6.3	5.6	100
Flower buds	Number of cells	35	43	10	4	5	97
	Percentage	36.1	45.0	10.3	4.1	4.5	100

Table 5 Different kinds of PMCs and pollen stainability in the hybrid of *B. napus* cv 'Oro' × *O. violaceus*

Number of bivalents	19	12	6	HPMC[a]	Total	Stainability (%)
Number of PMCs	560	70	10	760	1400	
Percentage	40.0	5.0	0.7	54.3	100	46.7

[a] This kind of PMCs was produced by the hybrid cells in the hybrid

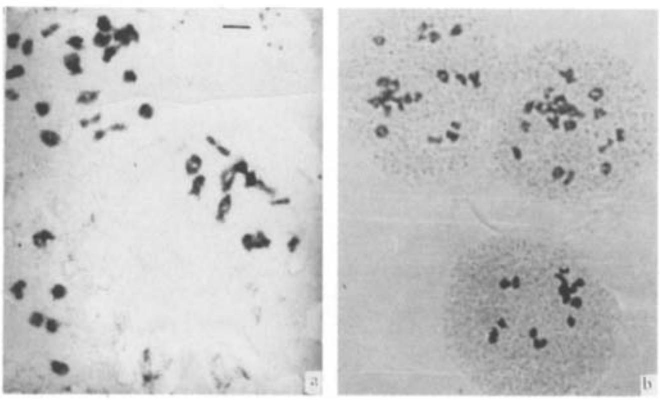

Fig. 4a, b Three kinds of PMCs in the hybrid. **a** Three kinds of PMCs with 19, 12 and 6 bivalents, respectively. **b** Two kinds of PMCs with 19 (two *above*) and 12 (one *below*) bivaleuts, respectively. *Bar*: 5 μm

plants from the selfed progeny of the hybrid again produced PMCs with 19, 12 and 6 bivalents, respectively and plants of *B. napus* and the hybrid by selfing.

Discussion

Complete separation of the genomes from the two parental species during mitosis of hybrid cells

Based on cytological observations, we consider that the separation of genomes from the two parental species primarily occurs during the mitotic divisions of some hybrid cells. The genomes from the two parents were arranged separately in hybrid cells (Fig. 3b, c), they moved to two opposite cell poles (Fig. 3d, e) and they finally were included in two cells (Fig. 3f). The two kinds

Table 6 Types of plants and their percentages among the selfed progeny of the hybrid between *B. napus* cv 'Oro' × *O. violaceus*

Generations[a]	Number of seeds	Number of plants	Percentage of germination	Chromosome number of plants			
				38	31	25	50
F_2	75	38	50.6%	32(84.21%)	6(15.79%)		
F_3	–	1854	–	1762(95.04%)	91(4.91%)	1(0.05%)	
F_4	–	215	–	204(94.88%)	10(4.65%)		1(0.05%)

[a] All F_2 seeds were cultured on medium for germination. All F_3 and F_4 seeds were sowed in the field

of cells produced by the genome separation had 38 and 24 chromosomes, respectively, upon replication of the chromosomes. The somatic separation of the genomes might be followed by the division of the chromosomes at two poles, resulting in formation of two kinds of haploid cells with 19 and 12 chromosomes, respectively. Such behaviors have been observed in PMCs from the hybrid cells (Li et al. in preparation). Five kinds of somatic cells were found in the hybrid; haploid and diploid cells of *B. napus* and the nuclear and cytoplasmic hybrid (NCH) with the cytoplasm of *B. napus* and the nucleus of *O. violaceus* and the hybrid ones. The hybrid became the mixoploid, which consisted of three different kinds of cells; *B. napus*, NCH and the hybrid.

With progessive growth of the hybrid plants, the number of *B. napus* cells (2n = 38) increased, and its percentage was much higher than that of NCH cells in the anthers (Table 4). This shows that NCH cells divided at a slower rate than *B. napus* cells, probably a result of the incongruous relationship between the cytoplasm of *B. napus* and the nuclei of *O. violaceus.*

Genetic analysis of the progeny of the hybrid also indicates the complete separation of genomes from two parents during the mitosis of the hybrid cells. In the selfed progeny of the hybrid, there were always two kinds of plants: *B. napus* and the hybrid. The hybrid plants from the selfed progeny of the hybrid again produced the plants of *B. napus* and the hybrid by selfing (Table 6). Furthermore, the plants of *B. napus* among the progeny were morphologically and genetically similar to the parental ones.

Theoretically, six kinds of plants should be identifiable in the selfed progeny of the hybrid: *B. napus* (2n = 38), the hybrids (2n = 31, 25) and the NCHs (2n = 24, 18 and 12). The absence of NCHs in the selfed population of the hybrid might be caused by the sterility of male gametes with 12 and 6 chromosomes, respectively, of *O. violaceus*, even though the female gametes with 19, 12 and 6 chromosomes, respectively, were fertile and produced the three kinds of plants with 38, 31 and 25 chromosomes when fertilized by the male gamete of *B. napus*. The latter plant (2n = 50) was most likely produced by the combination of the unreduced female gamete (n = 31) from the hybrid cells and the male gamete (n = 19) of *B. napus.*

Genetic basis for the development of NCH cells in the hybrid

Although the NCH cells divided at a slower rate than *B. napus* cells, they still produced fertile gametes and transmitted the chromosomes of *O. violaceus* to the next generation. The strong growth ability of NCH cells in the hybrid is probably related to the genetic constituents of *O. violaceus*.

A great deal of research has been conducted on cell and tissue culture of *O. violaceus*. Plants have been regenerated from flower stalks and buds (Pan and Huang 1986), leaves and petioles (Xu and Xu 1987), mesophyll protoplast (Xu and Xu 1988), and petiole protoplast (Luo and Luo 1991). All of the results have shown that the cells and tissues of *O. violaceus* have a high capacity for plant regeneration in culture and that *O. violaceus* might be an excellent model plant of the Cruciferae in plant tissue and cell culture. The development of NCH cells in the hybrid may be related to its characteristics of high regeneration capacity in vitro.

O. violaceus might be a species with a basic chromosome number of 6

The PMC with 6 bivalents was observed in the mixoploid, and a new hybrid with 25 chromosomes was identified among the selfed progeny of the hybrid. This suggests that *O. violaceus* might be a tetraploid species with the basic chromosome number of 6 (2n = 4x = 24). The chromosome pairing of *O. violaceus* had given us some hints regarding the polyploidy levels of *O. violaceus*. The meiotic pairing of chromosomes in PMCs from the hybrid cells would provide further evidence for this hypothesis.

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