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

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 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 palmitic (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.

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 × = 38, AACC), O. violaceus (2n = 24,00) (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% HgCl₂ 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 mg l⁻¹) and 2-naphthaleneacetic acid (NAA, 0.2 mg l⁻¹). The pH was adjusted to 5.8 before autoclaving at 1.1 kg/cm² 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 mg l\(^{-1}\) 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 mM 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 (PMCs) 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 \(2n = 24 = 20m + 4sm\) (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), 7II + 1X (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 12II, b 10II + 1IV, c 9II + 1VI, d 8II + 2IV, e 8II + 1VIII, f 7II + 1X. Bar: 5 μm](image)

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

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