Indian J. Genet., **56** (1): 27–33 (1996)

FORMATION OF AMPHIDIPLOIDS BETWEEN *BRASSICA NAPUS* L. AND *RAPHANUS SATIVUS* VAR. *OLEIFERA* MAKINO AND ITS STABILITY

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(Received: January 9, 1995; accepted: June 11, 1995)

ABSTRACT

The F_1 hybrids obtained by crossing *Brassica napus* cv. *Oro* and *Raphanus sativus* var. *oleifera*, were cultured in the medium (Ms + 0.2 mg/litre NAA + 3 mg/litre BA + 1 g/litre colchicine + 30 g/litre sugar + 8 g/litre agar) for 5 days. Many buds obtained carried 56 chromosomes. With the above technique, it is possible to obtain stable amphidiploids by the routine hybridization between *Brassica* and related species. 28 bivalents were formed in the PMC of the amphidiploids. Sometimes 26 bivalents and 1 quadrivalent were found. There are homologous or homoeologous chromosome in one genome or between two genomes of the amphidiploids. Another type of meiosis was discovered in the amphidiploids in which 56 chromosomes did not form bivalents and the chromatids could be detected in MI, several chromosome masses formed at anaphase and the pollen was sterile. This is the first cytological evidence of sterility in the amphidiploids of rapeseed. Unbalanced division was observed in the *R. sativus* var. *oleifera* at anaphase I of meiosis which formed two types of pollen, one with 8 chromosomes, and another with 10 chromosomes. This could be the starting point where two basic chromosome numbers contributed to the genome evolution.

Key words: Amphidiploids, *Brassica*, *Raphanus*, colchicine, meiosis, genomic number.

Brassica napus and other related species have been resynthesized since 1935. Several methods were used by Akbar [1]. The amphidiploid can be got from the unreduced gametes occasionally. Sometimes the amphidiploids are obtained by colchicine treatment when the distant hybrid F_1 was soaked in the liquid containing colchicine. The colchicine could poison the plants, and it is difficult to obtain amphidiploids.

The F_1 hybrids of *Brassica napus* x *Raphanus sativus* var. *oleifera* contain 28 chromosomes. The higher the chromosome number, the more difficult it is to obtain an amphidiploid. It is difficult to obtain amphidiploids with 56 chromosomes.

In order to transfer the chromosomes of *R. sativus* var. *oleifera* to *B. napus*, the amphidiploids were obtained through the culture medium containing colchicine.

MATERIALS AND METHODS

B. napus cv. Canadia Twin, *R. sativus* var. *oleifera*, the distant hybrid F_1 *B. napus* cv. Oro x *R. sativus* var. *oleifera* were used in this study.

Tissue Culture. The F_1 explant embryo was cultured in the MS medium containing 0.2 mg/litre NAA + 3 mg/litre BA + 30 g/litre sugar + 8 g/litre agar at 20°C.

The amphidiploids resynthesized. F_1 embryos cultured for five days on the MS + 0.2 mg/litre NAA + 3 mg/litre BA + 1 g/litre colchicine + 30 g/litre sugar + 8 g/litre agar medium were transferred to the MS + 0.2 mg/litre NAA + 3 mg/litre BA + 30 g/litre sugar + 8 g/litre agar medium and incubated for 20 days at 20°C. Several buds were obtained which had 56 chromosomes.

Growing root. The amphidiploids were then transferred to MS + 0.5 mg/litre NAA + 30 g/litre sugar + 8 g/litre agar medium at 20°C for 30 days, where they developed roots.

Cytological study. The anthers of amphidiploids and related species were fixed in Carnoy's fluid, chromosome numbers were counted at metaphase I of PMC.

RESULTS

The distant hybrid F_1 explant of *B. napus* x *R. sativus* var. *oleifera* were cultured in the medium containing 1 g colchicine/litre for five days. The tissue upon transfer to the colchicineless medium developed into callus. After one month, many buds were obtained from callus which had 56 chromosomes.

The amphidiploids upon transfer to MS + 0.5 mg/litre NAA + 30 g/litre sugar + 8 g/litre agar medium for one month developed many roots. The amphidiploids were hardened on soil which contained sterilized peat. At 14°C, the seedling survival was 90%.

THE NORMAL MEIOSIS OF AMPHIDIPLOIDS

There were two types of meiosis in the amphidiploids, one normal, another abnormal. In the normal meiosis, 28 bivalents formed at metaphase I (Fig. 1: 3, 4, 5). Occasionally, 26 bivalents and 1 quadrivalent formed in the amphidiploids (Fig. 1: 2). There were two pairs of chromosomes which were homologous or homoeologous in the amphidiploids. At anaphase I, the chromosomes of 28 bivalents separated to the two poles (Fig. 1: 6, 7). The pollen grains were large, ellipsoid and fertile (Fig. 1: 9). Seeds were obtained on selfing.

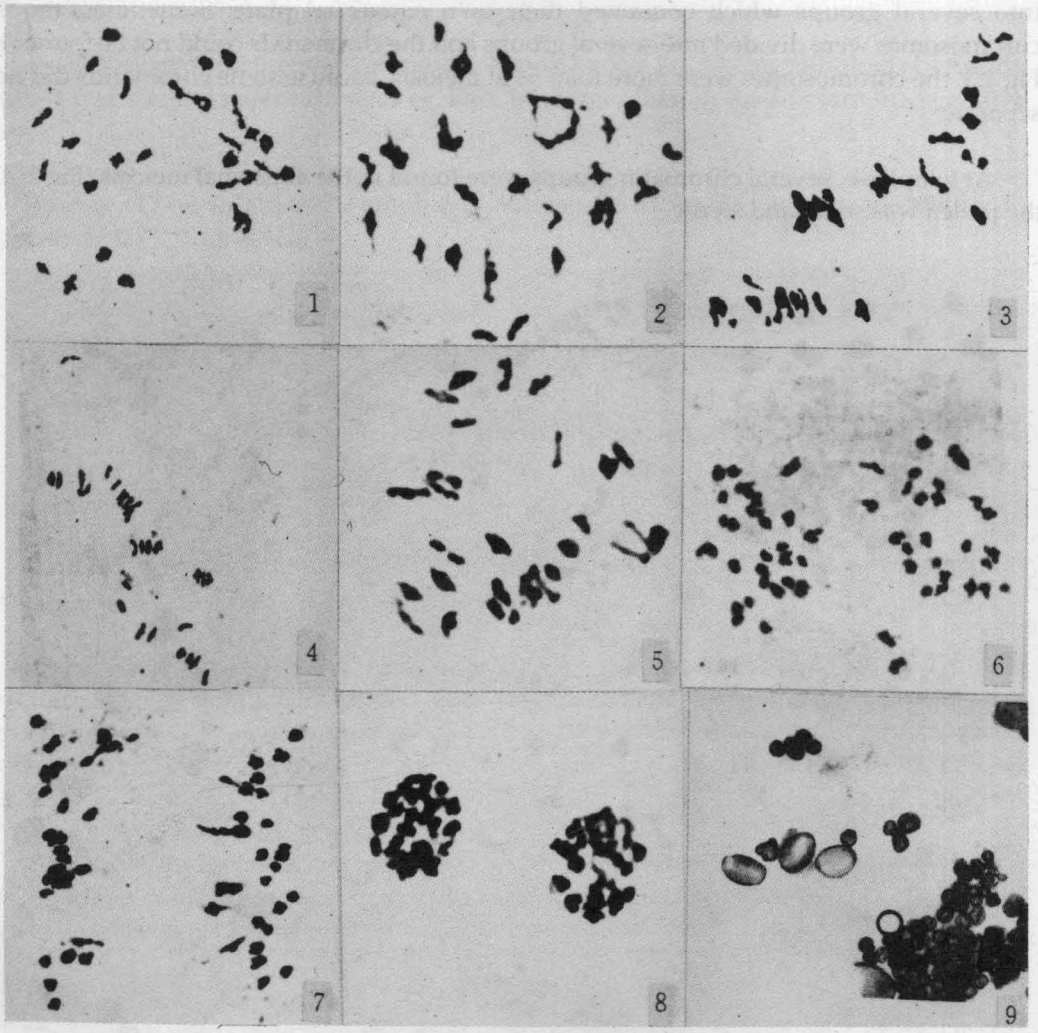

Fig. 1. Normal meiosis of the amphidiploids beltwen *Brassica napus* and *Raphanus sativus* var. *oleifera* Makino. 1, 2, 3, 4, 5) Meiotic metaphase I. 6, 7) Meiotic anaphase. 8) Meiotic telophase I. 9) pollen.

ABNORMAL MEIOSIS OF AMPHIDIPLOID

The abnormal meiosis in the amphidiploids led to sterile pollen which were small (Fig. 2: 1–9).

At metaphase I, the 56 chromosomes remained unpaired, it was like the metaphase of mitosis (Fig 2: 1). The chromatids could be found occasionally (Fig 2: 2), they were divided

into several groups which contained their own equatorial plate. Sometimes, the 56 chromosomes were divided into several groups and the chromatids could not be found. In Fig 1:3, the chromosomes were more than 56 at meiosis, because some chromatids did not separate.

At telophase, several chromatin groups were found in the abnormal meiosis (Fig 2: 4), the pollen was small and sterile.

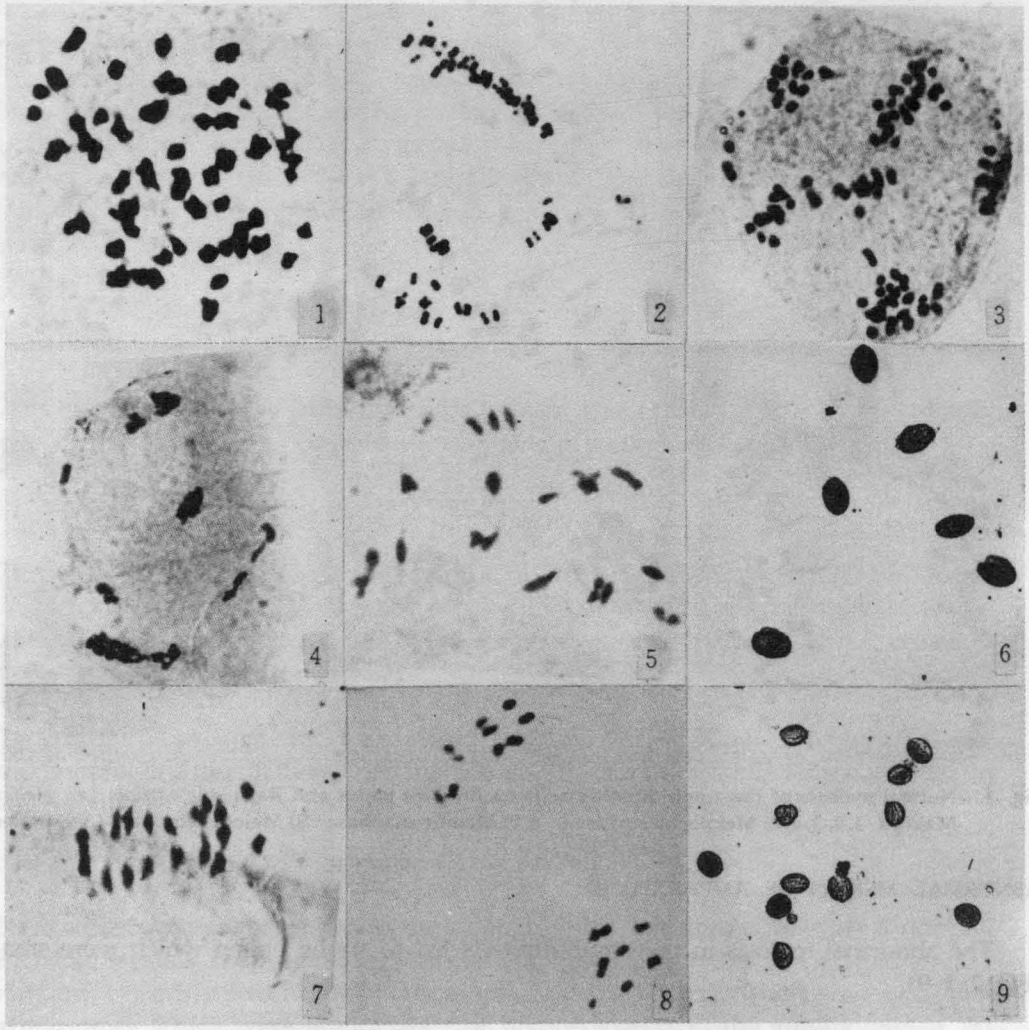

Fig. 2. Abnormal meiosis of the amphidiploids between *B. napus* and *R. sativus* var. *oleifera*. 1, 2, 3, 4) Different stages of chromosomal separation. 5, 6) Metaphase I and pollen of *B. napus*. 7, 8, 9) Metaphase I, anaphase I, and pollen of *R. sativus* var. *oleifera*, respectively.

MORPHOLOGY AND FERTILITY OF AMPHIDIPLOIDS

It was difficult to distinguish the morphology of hybrid F_1 and amphidiploid at seedling stage. It was like the parent *B. napus* cv. Oro, but larger than in the F_1 plant in vegetative stage.

The size of flower bud of the amphidiploids was 0.8 x 0.4 x 0.4 cm^3, and the buds of F_1 plants were 0.7 x 0.3 x 0.3 cm^3.

The flowers of amphidiploid were white. Many seeds were formed in amphidiploids.

THE STABILITY OF *B. NAPUS* AND *R. SATIVUS* VAR. *OLEIFERA*

B. napus and *R. sativus* var. *oleifera* are stable species. Meiosis in them was mostly normal. Some chromosomes were very long, others were very short. So there was some instability in the two species.

There were 19 bivalents at meiosis of metaphase I in *B. napus* (Fig 2: 5), which includes spindle-like and dumbbell-like bivalents (Fig. 2: 6).

There were 9 bivalents at metaphase I of *R. sativus* var. *oleifera* which included spindle-like and dumbbell-like bivalents (Fig. 2: 7). The spindle-like bivalents may separate too late or may not separate, so at anaphase of meiosis cells with 8 chromosomes at one pole and 10 chromosomes at another were found (Fig. 2: 8). The pollen were elllipsoid (Fig. 2: 9).

In *B. napus* and related species, some chromosomes were long and others short. The chromosomes of bivalents separated early or late, and some long chromosomes of bivalents do not separate at anaphase I. The meiosis was thus unbalanced.

DISCUSSION

THE RESYNTHESIS AND STABILITY OF THE AMPHIDIPLOID

The F_1 were cultured on the medium which contained colchicine. Many amphidiploid buds were obtained from the culture, which contained 56 chromosomes. The amphidiploids carried 28 bivalents at metaphase I of meiosis, their pollen was fertile and many seeds were obtained. The amphidiploids could become stable species.

26 bivalents and 1 quadrivalent were formed at metaphase I occasionally. There were homologous or homoeologous chromosomes from one or two genomes in the amphidiploids.

In another type of meiosis the chromosomes did not pair and free chromatids could be seen. The pollen from this unpaired meiosis was sterile. This was a special kind of sterility caused by failure of meiosis. In distant hybridization sterile plants have been reported in the progenies of backcross [2, 3].

There were 2 to 4 univalents in the cells of resynthesized *B. napus* [4], but the cultivated *B. napus* contains 19 bivalents at meiosis. The resynthesized *B. napus* expressed the genes of both parents. Some genes had been described in cultivated species [5].

Stable amphidiploid can be obtained from the amphidiploids resynthesized from *B. napus* and *R. sativus* var. *oleifera.*

THE EVENTS LEADING TO DIFFERENT GENOMIC NUMBER

The 8:10 type (8 chromosomes at one pole, 10 at the other) anaphase I was found at meiosis of *R. sativus* var. *oleifera,* in which two types of gametes could be formed. In the progenies two new species may be obtained, one with 8 and another with 10 chromosomes.

Different types of chromosomes (long, short, telocentric, metacentric, subtelocentric) were found at meiosis. They separated early or late, and a few unbalanced meiosis occurred in such cases.

There were quadrivalent also at meiosis of the resynthesized amphidiploids. If the quadrivalent was in one genome, it was a tetrasome. If the chromosomes of the quadrivalent were in two genomes, then these were homologous or homeologous chromosomes in two different genomes.

The basic chromosome number of *B. napus* and related species is proposed to be 5, 6 or 7 [5–8]. Our study supports this hypothesis.

Thus, A = 10, C = 9, B = 8 genomes originated from rearrangements in aneuploids and polyploids.

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