

## CYTOGENETICAL AND ULTRASTRUCTURAL STUDIES ON INTERGENERIC HYBRIDS BETWEEN *BRASSICA NAPUS* AND *ORYCHOPHRAGMUS VIOLACEUS* AND THEIR DERIVATIVES

### I. CYTOGENETICAL STUDIES

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### INTRODUCTION

Recently, *Orychophragmus violaceus* (or *Moricanda sonchifolia*) belonging to cruciferae was reported to be a valuable oil-seed plant resource (Luo et al. 1991). Its oil contains higher percentage of oleic (20.32%), linoleic (53.17%) and palmitic (14.31%) acids, and lower percentage of linolenic (4.76%) and erucic (0.94%) acids. Intergeneric hybrids between *O. violaceus* and *Brassica* cultivars may be useful for the introduction of good quality of oil traits into brassica crops and for the study of the relationships between these species.

The intergeneric hybrids ( $2n=31$ , ACO) between *B. napus* ( $2n=38$ , AACC) and *O. violaceus* ( $2n=24,00$ ) were produced by means of embryo culture technique with the later as a pollen parent (Li 1992; Li and Liu 1993; Li and Luo 1993). The somatic separation of genomes from two parent species was observed during the mitotic divisions of some hybrid cells (Li et al. 1994). So the hybrid became the mixoploid in nature, consisting of haploid and diploid cells of *B. napus* and 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 were produced by the hybrid. From the selfed progeny of the hybrid, other than mainly two kinds of plants, *B. napus* and the hybrid, two plants ( $2n=25, ACO^B$ ;  $2n=50$ , AACCO) were found. The hybrid plants of the selfed progeny again produced mainly two kinds of plants, *B. napus* and the hybrid.

The present paper reports the cytological characterizations of the pollen mother cells from the hybrid cells in the hybrid and the two plants ( $2n=25,50$ ) in the selfed progeny of the hybrid. Some new facts have been reported in this paper.

### MATERIALS AND METHODS

Plant materials: The intergeneric hybrids between *B. napus* ( $2n=38$ , AACC) cv. Oro and *O. violaceus* ( $2n=24,00$ ) obtained by us, and the two plants ( $2n=25,50$ ) in the selfed progeny of the hybrids were used in this study for cytogenetical studies. The plant ( $2n=25$ ) was backcrossed to parental *B. napus* cv. Oro, and BC1 plants were obtained by embryo rescue.

Cytological methods: For mitotic chromosome counting, root tips were

taken from 3- to 4-week-old plants raised in pots. After treated with 0.002M 8-hydroxyquinoline for 4h, the root tips were fixed in 3:1 alcohol-glacial acetic acid. They were hydrolyzed in 1N HCl 60 °C for 10 min, squashed in a drop of 10% modified carbol fuchsin and observed under oil. For meiotic analysis, buds from the terminal inflorescence were immediately fixed in 3:1 alcohol-glacial acetic acid for 24h. Buds were then stored in 70% ethanol at 4°C. Anthers were dissected out, cut in half and the PMCs squeezed out in a drop of 10% modified carbol fuchsin.

## RESULTS AND DISCUSSIONS

### Meiotic behaviours of genomes in PMCs from the hybrid cells

PMCs produced by the hybrid cells were divided into two types according to their different behaviours of chromosomes during meiosis in the hybrid.

Type I-different moving direction of chromosomes. In this type of PMCs, there was no chromosome pairing and all 31 chromosomes appeared as univalents. Then chromosomes moved poleward with 19 chromosomes at one pole and 12 chromosomes at the other. The chromosomes at the two poles underwent one mitotic division and produced four nuclei, two with 19 chromosomes and two with 12 chromosomes. It is proposed that the chromosomes or genomes from two parent species moved to opposite poles respectively, resulting in the 19 chromosomes at one pole from *B.napus*, and 12 chromosomes at the other pole from *O.violaceus*. Among the four nuclei produced by one mitotic division, two had 19 chromosomes from *B.napus*, and the other two had 12 chromosomes from *O.violaceus*. The gametes with the chromosomes of *B.napus* in the hybrid were the same as those from the parental *B.napus* plants. But the gametes with the chromosomes of *O.violaceus* had the cytoplasm of *B.napus* and the nuclei of *O.violaceus*.

Type II. In this type of PMC, twelve chromosomes appearing as six bivalents always lagged at anaphase I and failed to enter the telophase nuclei. Some of lagging bivalents were included in the telophase nuclei, but were lost in the second meiotic division. It was most likely that the lagged 6 bivalents were from *O.violaceus*. The chromosomes from two parents were also separated during meiosis for their much different moving rate. This indicated that the two species might be very widely related in evolution. This type of PMCs was sterile because the amphihaploid of A and C genomes could not produce fertile gametes after the elimination of O genome in the hybrid cells.

The ratio of two kinds of PMCs above was approximately 15:1 with Type II having much more number of PMCs.

### Morphology and cytogenetics of the special hybrid (2n=25) and its backcross generation (2n=44)

The special hybrid plant was morphologically intermediate between *B.napus* and *O.violaceus* except for the flower colour. The plant was highly male sterile, but probably produced the cytologically non-reduced female gametes with 25 chromosomes, for the BC<sub>1</sub> plants with 44 chromosomes were obtained when the hybrid was backcrossed to the parental *B.napus* cv. Oro.

All eight BC<sub>1</sub> plants with a phenotype similar to the special hybrid were fertile and produced a lot of seeds when selfed. The cytological observations showed that there were 22 bivalents in their pollen mother cells. At anaphase/telophase I, six chromosomes appearing as three bivalents were lagged and probably lost, or partially included in telophase nuclei. The three lagged bivalents were most likely from *O. violaceus*, for the 19 bivalents from *B. napus* were expected to segregate regularly. Finally, there existed one kind of PMCs in which no chromosomes lagged, but 25 chromosomes passed to one pole and 19 ones to the other at anaphase I. So the two kinds of male gametes with 25 and 19 chromosomes should be produced by the PMCs. The percentages of three kinds of PMCs above were 70%, 20%, and 10% respectively. From the selfed progeny of the BC<sub>1</sub> plants, many kinds of plants with 38, 44, 50 and the other number of chromosomes had been identified.

#### Morphology and cytogenetics of intergeneric sesquidiploid (2n=50)

In the F<sub>4</sub> populations of the intergeneric hybrid between *B. napus* and *O. violaceus*, one plant with 50 chromosomes were identified, which was produced most likely by the combination of the unreduced female gamete (n=31) from the hybrid cell and the male gamete (n=19) of *B. napus*. The plant was mainly matroclinous, with a few patroclinous features, such as the oval and hairy leaves and basic clustering stems, but the size of its flowers was larger than that of those from *B. napus* cv. Oro. The plant was fertile and produced a lot of seeds when selfed.

In PMCs of the plant, 25 bivalents were observed at diakinesis and metaphase I. At anaphase I, the equal segregation of 25+25 was observed in most of PMCs, but the unequal segregations of 22+28 and 19+31 were also observed in some PMCs. So the normal gametes with 19, 22, 25, 28, 31 chromosomes were formed by the plant. We are now trying to screen out as many plant types as possible from the selfed progeny of the sesquidiploid plant, which may be valuable for the transfer of good oil quality traits from *O. violaceus* to *B. napus* as expected in the near future.

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#### REFERENCES

- Li, Z.Y. (1992) Study on wide hybridizations between *Brassica napus* and *Orychophragmus violaceus*. *Ph.D. thesis*, Sichuan University, Chengdu, P. R. China.
- Li, Z.Y. and Liu, H.L. (1993). Intergeneric hybrid between *Brassica napus* and *Orychophragmus violaceus*—the mixoploid of two different species. In *The Collection of Papers From the First China Post-doctorial Academic Congress*. Ed. E.B. Feng. pp. 1776-1880, Beijing: National Defence Industry Press.
- Li, Z.Y. and Luo, P. (1993). First intergeneric hybrids of *Brassica napus* × *Orychophragmus violaceus*. *Oil Crops Newsletter*, 10, 27-29.
- Li, Z.Y., Liu, H.L. and Luo, P. (1994). Production and cytogenetics of intergeneric hybrid between *Brassica napus* and *Orychophragmus violaceus*. *Theoretical and Applied Genetics* (in press).
- Luo, P., Lan, Z.Q., Huang, J. and Li, Z.Y. (1991). Study on valuable plant resource *Orychophragmus violaceus* (L.). O. E. S chulz. *Journal of Natural Resources*, 8, 206-210.