

Full Length Research Paper

Hybridization Potential and Backcrossing Outcomes Between Improved CMS *Brassica campestris* ssp. *pekinensis* and *B. oleracea* var. *acephala*

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The interspecific cross between improved cytoplasmic male sterility (CMS) *Brassica campestris* ssp. *pekinensis* (Lour.) Olsson and *B. oleracea* var. *acephala* DC. aims to obtain kale male sterile lines and enrich Chinese cabbage genetic resources. The results indicated that there were serious segregations between the 2 parents, so as to descendants could not be obtained by routine pollination methods. Otherwise, interspecific hybrids have been obtained by young embryos culture *in vitro* and the best time of embryo rescue was the 9 - 11 day after pollination. Interspecific embryo collapsed at sphere stage, with endosperm nuclei being surrounded by cell walls. The morphology, fertility, microspore growth, vernalization and numbers of chromosome were conducted to prove the truth of interspecific hybrids. Leaf morphology was intermediate of the 2 parents. Anther shape and plumpness, nectary volume and numbers were matroclinous. The male sterility showed 100% both in degree and ratio. Interspecific hybrids microspore aborted clearly from mononuclear to dinuclear stage. Vernalization was also intermediate of the 2 parents. Chromosome number of hybrids was confirmed to be $2n = 19$, which was the total count of gametophyte chromosome of 2 parents. Also, we obtained BC₁ which was from F₁ × kale, by pollinated several times, while the average compatibility index was only 0.0014. There were sluggish chromosomes in BC₁ PMCs anaphase.

Key words: CMS Chinese cabbage, kale embryo culture, chromosome, F₁, BC₁.

INTRODUCTION

Kale (*Brassica oleracea* var. *acephala* DC.), which is from Europe originally, cultivated as an ornamental plant in China recently. In our previous experiments, it can resist adverse conditions, especially cold temperature. So it has extensive perspectives in landscape North China. Random pollinations cause severe segregation in descendants because kale is cross-pollinated. Application of male sterility is one of the best measures in cabbage (Fang, 1984), as well as in kale. Early studies suggested that diploid *Brassica* species represent "balanced secondary polyploids" exhibiting internal chromosome homeology (Nagaharu, 1935; Olsson, 1960). Quite a few

interspecific and intergeneric hybrids have been synthesized subsequently in *Brassica* and *Cruciferae*, especially oil seeds and vegetables (Nobumichi, 1980; Mohapatra, 1986; Akbar, 1989; Sang et al., 1996; Huang BQ et al., 2001; Luo P et al., 2003). However, we have not found any report on interspecific crosses between improved CMS Chinese cabbage and kale. The present interspecific cross aims to transfer cytoplasmic male sterility (CMS) from Chinese cabbage (*Brassica campestris* ssp. *pekinensis* (Lour.) Olsson) to kale and further improve kale hybrid seed production. Meanwhile, the acquirement of interspecific hybrids can enrich genetic resources of Chinese cabbage, which is a significant vegetable in China for the importing of clubroot resistance (Bradshaw and Williamson, 1991; Nomura et al., 2005).

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Table 1. Results of young embryos growth *in vitro*.

Days of embryos after pollination	Number of excised embryos	Number of plantlets get from excised embryos	Percentage of plantlets (%)
6- 8	40	2	5.0
9-11	56	8	14.29
12- 14	60	4	6.67
15- 17	42	0	0
18- 20	38	0	0

MATERIALS AND METHODS

MATERIALS

Improved CMS Chinese cabbages were supplied by Horticulture Faculty, Shenyang Agricultural University and the original CMS materials were introduced from America in 1996. Seeds of kale were bought on markets in Japan and Dalian, Liaoning Province. Both parents, F₁ and BC₁ were cultivated in the experimental plots in Shenyang Agricultural University.

METHODS

Manual crossing

Improved CMS Chinese cabbages were pistillate parents and kales were pollen parents. Manual pollinations were carried out during bloom.

Embryo growth

Ovaries, which were from female parent manual pollination, were made into wax slices after fixed in FAA (70% ethanol: glacial acetic acid:formaldehyde = 18:1:1) and dyed in Ehrlich's Haematoxylin. The thickness of slices was 8 μ m.

Embryo rescue culture *in vitro*

Embryos of different development days since manual pollination (Table 1) were taken out from ovaries under stereoscope. Media was MS (Murashige and Skoog medium) with 2 mg·L⁻¹ 6-benzyladenine, 0.1 mg·L⁻¹ naphthyl acetic acid (NAA) and 30 g·L⁻¹ cane sugar, were divided into 5 groups of embryos.

Morphology and vernalization

Typical morphological characters were observed during plantlet, lotus and bloom. Development of stages and vernalization characteristics were observed from sowing to bloom.

Fertility and microspore development

Numbers of nectary, shapes of anther, etc. were observed under a stereomicroscope. Vigor of pollen were evaluated by 3,3'-Dimethylbenzidine staining. Microspore was identified under Olympus-BH2 microscope through wax slices.

Cytology

F₁ root tips, which were from earlier plantlets *in vitro*, were pretreat-

ed with saturated solution of 1,4-dichlorobenzene for 2 - 5 h at 20 then killed with Carnoy's fluid (3 alcohol + 1 acetic acid) for 5 - 16 h and hydrolyzed in equal volume raw HCl with 95% alcohol solution at 20°C for 10 - 15 min and stained in carbol fuchsin lastly. BC₁ buds' treatment were the same as root tips'.

RESULTS

Compatibility

We pollinated almost 900 flowers artificially, but none plump seeds were obtained *in vivo* under natural condition. So the reproductive isolation between the 2 parents is quite severe.

Embryos growth and rescue culture

The 5th day after manual pollination, multicellular spherical embryos were clear, while endosperm nuclei were surrounded slightly by cell wall (Figure 1). The 10th day after pollination, numbers of cells of spherical embryo increased a lot, while endosperm walls were more obvious (Figure 2). The 14th day after pollination, embryo was crushed extremely and the whole ovule tissue collapsed (Figure 3). The results shows that embryos collapsed at spherical stage, with endosperm nuclei being surrounded by walls gradually. In the end, ovule tissue inflated extremely and the young embryos crushed and aborted.

Table 1 shows percentage of plantlets got from 6th to 8th day after pollination is lower than that of 9th to 11th and the best period of embryos rescue was from the 9th to 11th day after pollination, when 14.29% embryos could become plantlets. From the 15th to 20th day after pollination, we have not got plantlets from young embryos *in vitro*.

F₁ morphology and bolting characteristics

Table 2 shows that the F₁ progenies were basically intermediate from the 2 parents, such as the color of embryonal axis, the volume of lotus leaf, etc. Some characters such as wax which located on the surface of the leaves were from pollen parents obviously.

Bolting characteristics were also intermediate, that is,

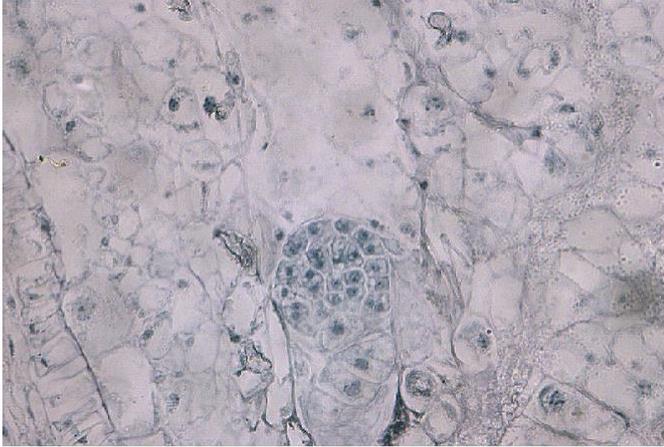


Figure 1. Multicellular spherical embryo, endosperm nuclei were surrounded by walls slightly (400x).

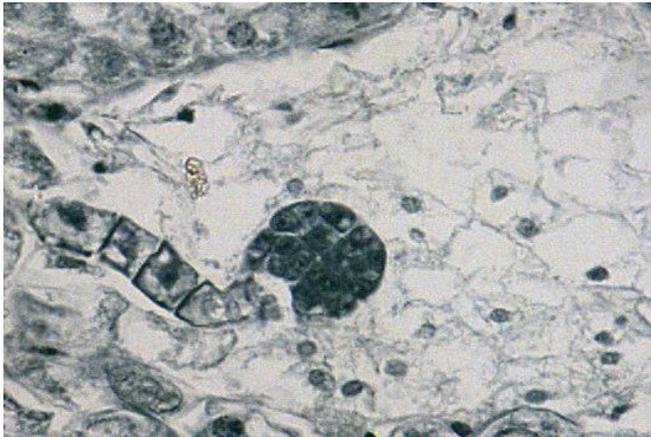


Figure 2. Multicellular spherical embryo, endosperm walls was obvious (400x).

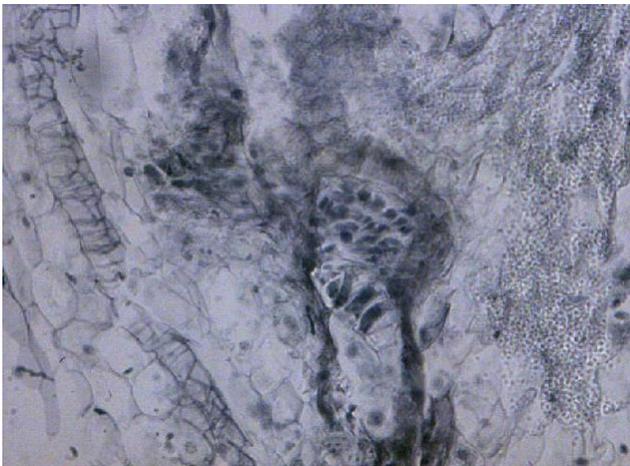


Figure 3. Embryo was crushed extremely and ovule tissue collapsed (400x).

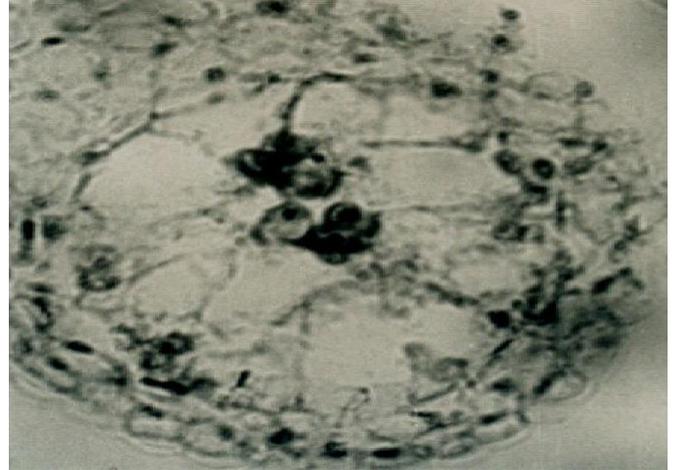


Figure 4. Tapetum cells vacuolation and inflation (400x).

the F₁ maintained rosette other than bolting when treated in the same conditions with female parent, which was bolting clearly. On the other hand, the F₁ bolting more easily and earlier than male parents, whose bolting was very strict to low temperature and the size of plants.

F₁ pollen fertility and microspore development

Table 2 also shows the F₁ hybrids anther shape, size and plumpness, nectary size and numbers, petal length/width were nearly matroclinous. There was no pollen under microscope so we can say that the F₁ progenies were 100% male sterile. In the meanwhile, the percentage of male sterility was also 100%.

Interspecific hybrids microspore aborted clearly from mononuclear to dinuclear stage. Most of tetrad micronucleus grew to uni-nucleate microspore stage and late uni-nucleate microspore stage. Nevertheless, since then, structures of anther wall appeared abnormality without tapetum cells disintegrate and uni-nucleate microspores were expanded and squeezed (Figure 4). In the end, most of uni-nucleate microspores collapsed and shrank and can not grow up to dinuclear microspores (Figure 5).

F₁ cytology

According to our observations, one of the typical F₁ progeny chromosome numbers were $2n = 19$ (Figure 6) and the average cells ratio was 86.3%. Others were $2n = 29$ (cells ratios 9.09%), which were presumably raised by unbalanced pairing of gametes from parents, for example, 20 gametes were from female parent and 9 ones from male parent, that is, double A group and one C group make a triploid of AAC and $2n = 38$ (cells ratios 4.55%),



Figure 5. Microspore abortion (400x).

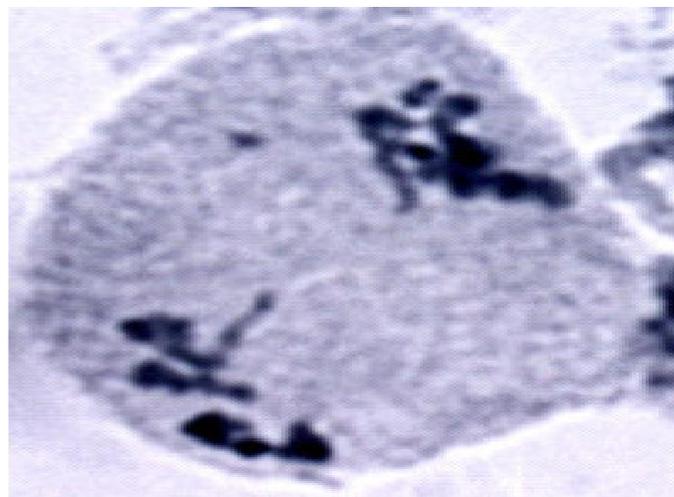


Figure 8. Meiosis anaphase, indicating sluggish chromosome (1000x).

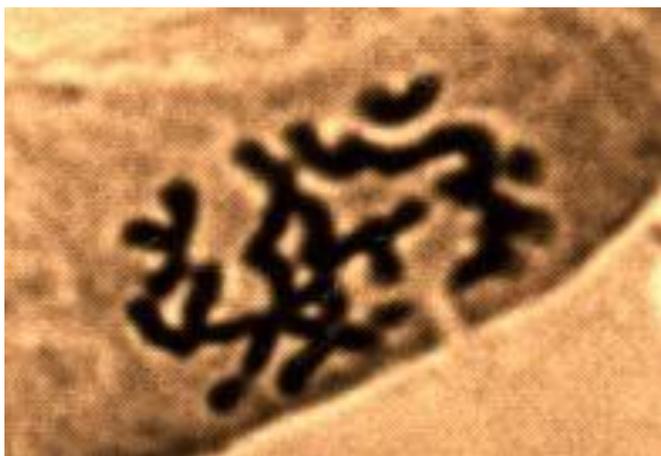


Figure 6. Number of root tip cells chromosome of F₁, 2n = 19.

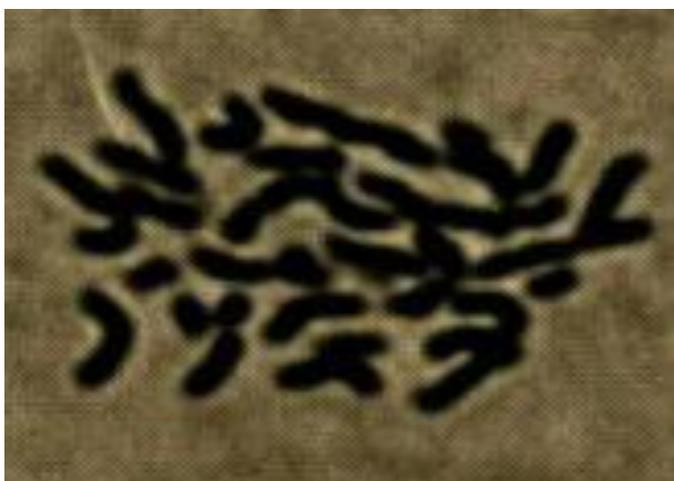


Figure 7. Number of root tip cells chromosome of F₁, 2n = 38.

which is an allotetraploid, were presumably raised by chromosome nondisjunction during meiosis or spontaneous chromosome doubling (Figure 7).

BC₁ generation

The average compatibility index of BC₁, which was from F₁ × kale by manual pollinating several times by cutting buds before blossoming, was only 0.0014. We observed sluggish chromosome in BC₁ PMCs anaphase (Figure 8) and the sluggish chromosome could be the main obstacle of chromosome mating (Liang ZhL et al., 1992). Meanwhile, a few PMCs chromosomes paired in metaphase normally (Figure 9).

DISCUSSION

We got none plump seed *in vivo* under natural condition by pollinating almost 900 flowers artificially. So the reproductive isolation is quite serious between *B. campestris* ssp. *pekinensis* (Lour.) Olsson and *B. oleracea* var. *acephala* DC. Production of interspecific hybrids between *B. campestris* and *B. oleracea* was also very difficult in previous work (Nagaharu, 1935; Hosoda et al., 1963; Hosoda et al., 1969). Crossability between *B. campestris* and *B. oleracea* was increased by excised rescue of ovaries *in vitro* (Inomata, 1983, 1996).

Morphological comparisons are traditional and reliable methods to analysis the truth of interspecific progenies. The F₁ progeny chromosome numbers of most of somatic cells (86.3%) were 2n = 19, that is, the total count of gametophyte chromosome of 2 parents (that is, 9 plus 10). Besides, we found some root tip cells with 29 chromosomes and some 38 with totally sum of 13.64% of

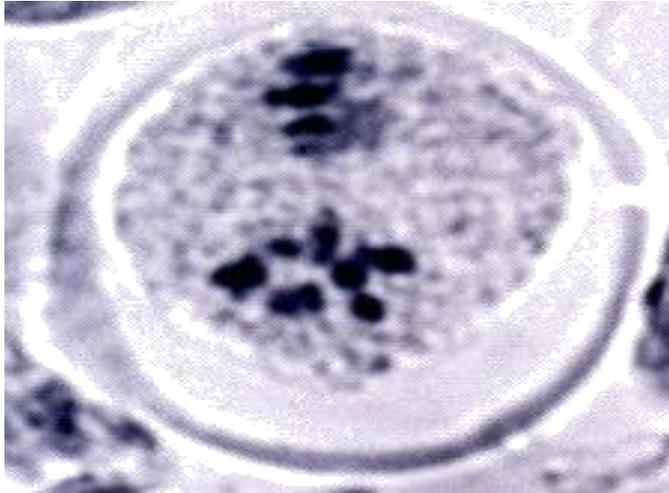


Figure 9. Metaphase, indicating chromosomes lay in equatorial plate (1000x).

the 2 latter types. According to Li and Zhang (1996), we can draw a conclusion from the number of the chromosomes directly that we got true interspecific hybrids.

We can say we have transferred cytoplasmic male sterility gene from Chinese cabbage to kale successfully because we got 100% male sterile F₁ progenies with normal nectaries, which are same to the female parent. Although during the interspecific cross, there is male sterility to some extent. For example, Olsson (1960) obtained hybrid plants from the cross between *B. campestris* and *B. oleracea*, which had 38 chromosomes in root tip cells. Pollen fertility ranged from 69 to 97%, with the mean of 86.8%. Hosoda et al. (1969) artificially synthesized *B. napus*, of which pollen fertility was from 45.0 to 77.5%.

The crushing of spherical embryos dues to the inflating excessively of ovule tissue. The insufficiency and obstacle of nutrient transportation may be the reasons of embryos abortion. Of course, the interspecific reproductive isolation was the key cause of imbalance between the embryos and endosperm. We have also observed embryos growth of *B. campestris* ssp. *pekinensis* × *B. napus*, which is similar to diploid *B. campestris* ssp. *pekinensis* with separable endosperm nuclei without confining of walls. *B. napus* has genome C, which is from *B. campestris* originally and opposite to genome A and B in *Brassica* according to Nagaharu (1935), maybe the reason of coordination between embryo and endosperm development.

Though there were sluggish chromosomes in BC₁, some chromosomes mated normally. These would be keys of backcrossing successfully and obtaining expectant CMS kale for character transfer within the genus *Brassica* was possible by successive backcrossing (Inomata, 1996).

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