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Full Length Research Paper

Interspecific cross between improved cytoplasmic male sterile lines and enrich Chinese cabbage genetic resources

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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 BC_1 which was from F_1×kale, by pollinated several times, while the average compatibility index was only 0.0014. There were sluggish chromosomes in BC_1 PMCs anaphase.

Key words: CMS Chinese cabbage, kale embryo culture, chromosome, F_1, BC_1.

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 interspeific 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).

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, F_1 and BC_1 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 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 $mg \cdot L^{-1}$ 6-benzyladenine, 0.1 $mg \cdot L^{-1}$ naphthyl acetic acid (NAA) and 30 $g \cdot L^{-1}$ 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

F_1 root tips, which were from earlier plantlets *in vitro*, were pretreated with saturated solution of 1,4-dichlorobenzene for 2 - 5 h at 20 then killed with Carnoy's fluid (3 alcholol + 1 acetic acid) for 5 - 16 h and hydrolyzed in equal volume raw HCl with 95% alcholol solution at 20°C for 10 - 15 min and stained in carbol fuchsin lastly. BC_1 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 5^{th} day after manual pollination, multicellular spherical embryos were clear, while endosperm nuclei were surrounded slightly by cell wall (Figure 1). The 10^{th} day after pollination, numbers of cells of spherical embryo increased a lot, while endosperm walls were more obvious (Figure 2). The 14^{th} 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 6^{th} to 8^{th} day after pollination is lower than that of 9^{th} to 11^{th} and the best period of embryos rescue was from the 9^{th} to 11^{th} day after pollination, when 14.29% embryos could become plantlets. From the 15^{th} to 20^{th} day after pollination, we have not got plantlets from young embryos *in vitro*.

F_1 morphology and bolting characteristics

Table 2 shows that the F_1 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, the F_1

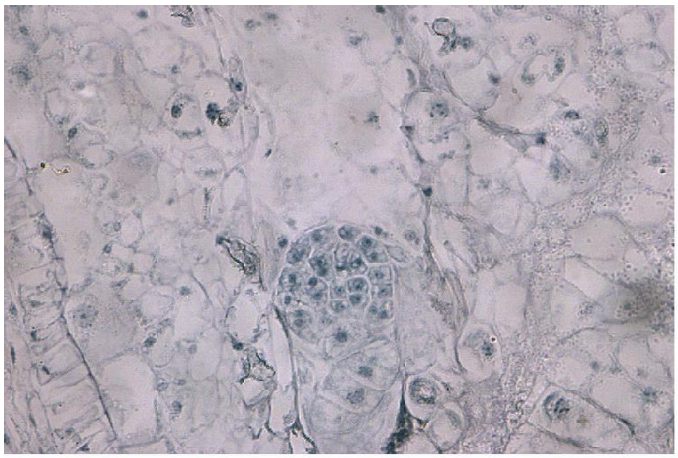

Figure 1. Multicellular spherical embryo, endosperm nuclei were surrounded by walls slightly (400×).

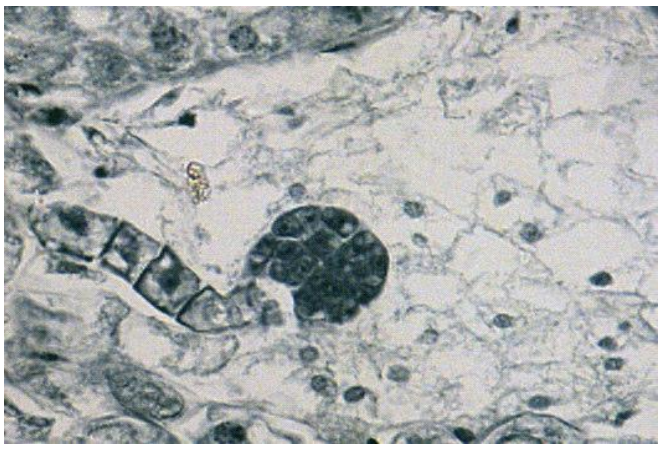

Figure 2. Multicellular spherical embryo, endosperm walls was obvious (400×).

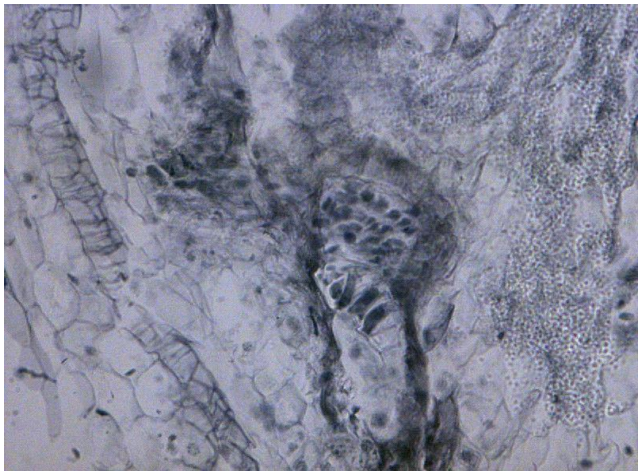

Figure 3. Embryo was crushed extremely and ovule tissue collapsed (400×).

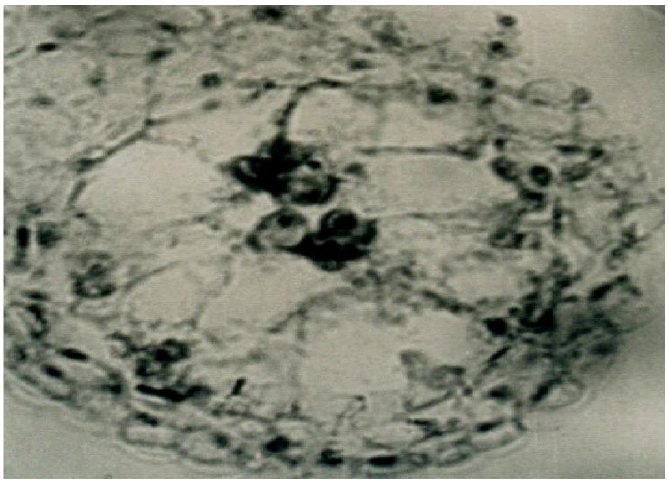

Figure 4. Tapetum cells vacuolation and inflation (400×).

maintained rosette other than bolting when treated in the same conditions with female parent, which was bolting clearly. On the other hand, the F_1 bolting more easily and earlier than male parents, whose bolting was very strict to low temperature and the size of plants.

F_1 pollen fertility and microspore development

Table 2 also shows the F_1 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 F_1 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).

F_1 cytology

According to our observations, one of the typical F_1 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 %), which

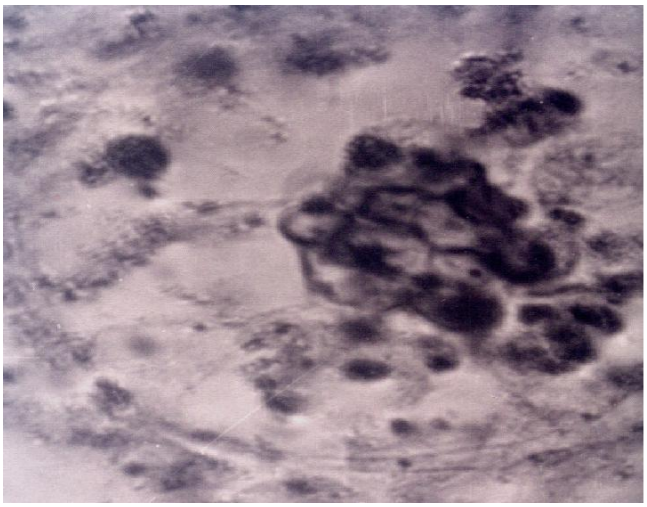

Figure 5. Microspore abortion (400×).

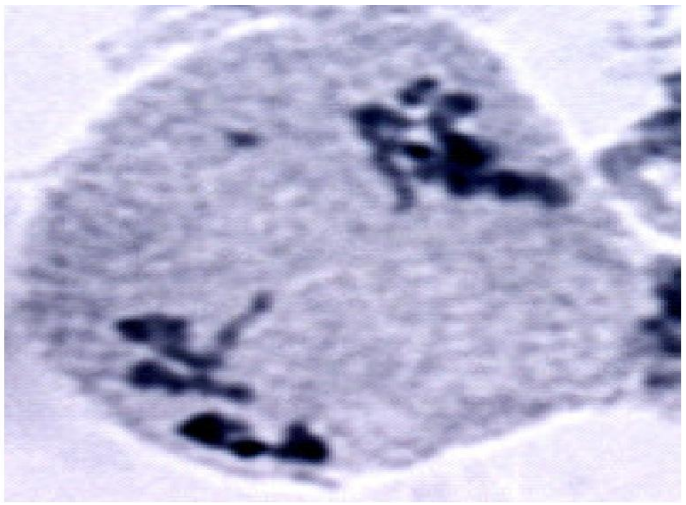

Figure 8. Meiophase anaphase, indicating sluggish chromosome (1000×).

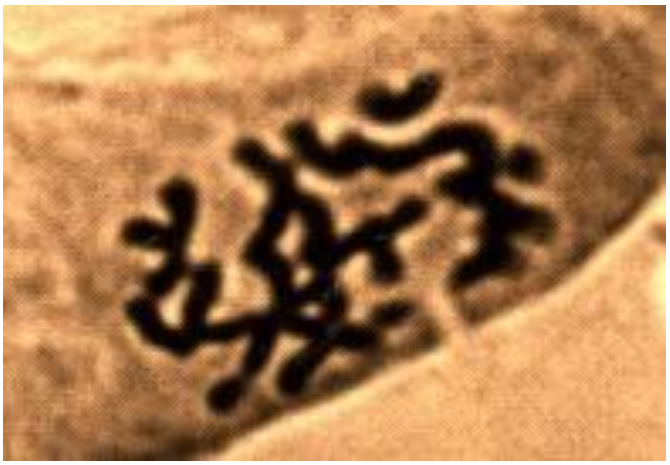

Figure 6. Number of root tip cells chromosome of F_1, 2n = 19.

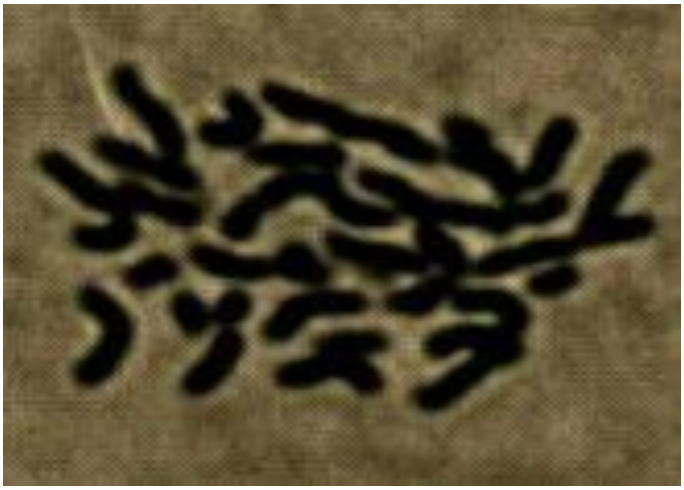

Figure 7. Number of root tip cells chromosome of F_1, 2n = 38.

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

BC1 generation

The average compatibility index of BC_1, which was from F_1×kale by manual pollinating several times by cutting buds before blossoming, was only 0.0014. We observed sluggish chromosome in BC_1 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 F_1 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

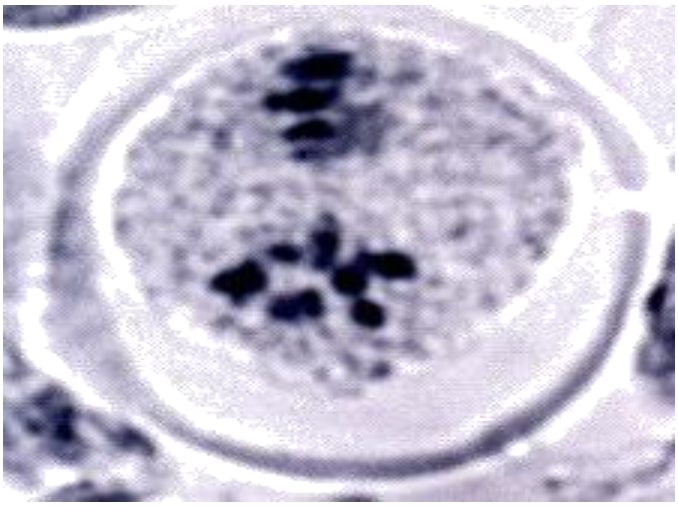

Figure 9. Metaphase, indicating chromosomes lay in equatorial plate (1000×).

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 F_1 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 BC_1, 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).

REFERENCES

Akbar MA (1989). Resynthesis of Brassica napus Aiming for Improved Earliness and Carried out by Different Approaches. Hereditas 111: 239-246.

Bradshaw JE, Williamson CJ (1991). Selection for Resistance to Clubroot (Plasmodiophora brassicae) in Marrowstem Kale (*Brassica oleracea* var. *acephala* L). Annals Appl. Biol. 119(3): 501-511.

Fang ZY (1984). Preliminary Results on Cabbage Cytoplasmic Male Sterility Lines Breeding. China Vegetables 4: 42-43.

Hosoda T, Sarashima M, Namai H (1969). Studies on the Breeding of Artificially Synthesized Napus Crops by means of Interspecific Crosses between n=10 Group and n=9 Group in Genus Brassica. Memo. Fac. Agri. Tokyo Univ. Educ. 15: 193-209.

Huang BQ, Chang L, Ju CM, Chen JG (2001). Production and Cytogenetics of Intergeneric Hybrids Between Ogura CMS *Brassica campestris* var. *purpuraria* and *Raphanus sativus*. Acta Genetica Sinica 28(6): 556-561.

Inomata N (1980). Hybrid Progenies of the Cross, *Brassica campestris* × *Brassica oleracea*. Cytogenetical Studies on F1 Hybrids. Japan. J. Genet. 55(3): 189-202.

Inomata N (1983). Hybrid Progenies of the Cross, *Brassica campestris* ×*B. oleracea*. Crossing Ability of F1 Hybrids and Their Progenies. Jpn. J. Genet. 58: 433-449.

Inomata N (1996). Overcoming the Cross-incompatibility through Embryo Rescue and the Transfer of Characters within the Genus *Brassica* and between Wild Relatives and *Brassica* Crops. Academic Report Okayama University 85: 79-88.

Li MX, Zhang ZP (1996). Crop Chromosomes Technology. Beijing: China Agriculture Press p. 1.

Liang ZL, Jiang RQ, Zhong WN (1992). Studies on Chromosome Behaviour of F1 and Fertility Restoration in Hybrid of *Gossypium hirtutum* × *G. bickii*. J. Integrative Plant Biol. 34: 931-936.

Luo P, Fu HL, Lan ZQ, Zhou SD, Zhou HF, ALuo Q (2003). Phytogenetic Studies on Intergeneric Hybridization between *Brassica napus* and *Matthiola incana*. Acta Botanica Sinica 45(4): 432-436.

Mohapatra D (1986). Hybridization in *Brassica juncea* ×*B.campestris* through Ovule Culture. Euphytica 37(1): 83-88.

Nagaharu U (1935). Genome-analysis in Brassica with Special Reference to the Experimental Formation of *B. napus* and Peculiar Mode of Fertilization. Japan. J. Bot. 7: 389-452.

Nomura K, Minegishi Y, Kimizuka-Takagi C (2005). Evaluation of F2 and F3 plants introgressed with QTLs for Clubroot Resistance in Cabbage Developed by using SCAR Markers. Plant Breeding 124(4): 371-375.

Olsson G (1960). Species Crosses within the Genus *Brassica*. II. Artificial *napus* L. Hereditas 46: 315-386.

Sang WB, Yukio K, Yasuo M (1996). Production of Intergeneric Hybrids between Raphanus and Sinapis and the Cytogenetics of Their Progenies. Breeding Sci. 46: 45-51.

Zhang GQ, Tang GX, Song WJ, Zhou WJ (2004). Resynthesizing Brassica napus from Interspecific Hybridization between *Brassica rapa* and *B. oleracea* through Ovary Culture. Euphytica 140(3): 181-187.

Zhu PF, Wei YT (2004). Preliminary Studies on Interspecific Cross between *Brassica campestris* L.ssp. *pekinensis* and *B. oleracea* var.*acephala*. China Vegetables pp. 39-11.