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*Full Length Research Paper*

# Morphology and cytology of flower chimeras in hybrids of *Brassica carinata* × *Brassica rapa*

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**Hybridization between white flowered *Brassica carinata* and yellow flowered *B. rapa* were made, and the flower chimeras were observed in a few hybrids. The simple single sequence molecular markers verified the hybridity of those hybrids. Chimeras were justified and totally classified based on the morphological characteristics of the flower petals that appeared in the hybrids of *B. carinata* and *B. rapa*. Two kinds of flower chimeras were observed: one type was different flower petals were with different colour in one branch; another type was that yellow petals were with white variegations, but the variegation size and shape were different in different petals. The meiosis and mitosis analysis showed that the partial or complete separation of parental genomes inferred to occur in pollen mother cells, shoot and early-developed petals in the flower chimeral hybrids, which hinted that the occurrence of complete or partial segregation of parental genomes in the somatic cells might be the reason for the production of flower chimera in the hybrids of *B. carinata* and *B. rapa*.**

**Key words**: *Brassica carinata*, *B. rapa*, SSR, flower chimera.

## INTRODUCTION

As important oilseed crops, *Brassica carinata* (2n = 34, BBCC) and *B. rapa* (2n = 20, AA) have many desirable characteristics, such as self-incompatibility and early maturing of *B. rapa* (Kimber and McGregor, 1995; Ren et al., 2000), drought tolerance and disease resistance in *B. carinata* (Malik, 1990). The hybrids were obtained in order to combine the desirable characteristics of *B. carinata* and *B. rapa* (Li et al., 2005), and the flower chimeras appeared in a few hybrids. Plant chimeras are mosaics in which genetically different cells exist in the shoot apical meristems that give rise to the cells that form the organs of the plant; they can arise by spontaneous or induced mutation, and by artificial synthesis (Burge et al., 2002). The grafting technique was the main method used to produce interspecific chimeras and was widely used between species in the family *Solanaceae* (Goffreda et al., 1990), or between *Brassica* species (Hirata et al., 1990; Hirata et al., 1992; Noguchi et al., 1992; Chen et al., 2006). The morphological characteristics, isozymatic band patterns and PCR analysis were used to confirm those chimeral structures (Hirata et al., 2000; Chen et al., 2006).

Generally, the flower color of rapeseed is yellow; rapeseed with white, ivory-white or orange-yellow flowers has also been reported by some researchers (Yu et al., 2004). Rapeseed with two kinds of flower colour in one plant was also reported in hybrids of Ogura CMS *Brassica rapa* × *Raphanus sativus* × *Brassica napus* (Huang et al., 2002). To date, few studies were about the flower chimeras that spontaneously happened in the hybrids of *B. carinata* and *B. rapa*. In our experiment, flower chimeras were observed in a few hybrids of *B. carinata* and *B. rapa*, which was not reported by other scientists. In this article, we report the characteristics of flower chimera and the special cytological behavior that happened in the hybrids with flower chimera.

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## MATERIALS AND METHODS

### Plant materials

Hybridizations were made by hand between a cultivar of *B. carinata* (BBCC, 2n = 34) with puniceous stalked and white-flower (Field number: 04L07) and a cultivar of *B. rapa* (AA, 2n = 20) with green stalk and yellow-flower (Field number: 04L25) in 2004, the F1 plants were planted in the campus field at Huazhong University of Science and Technology.

### Molecular marker analysis

Total genomic DNA of hybrids and their parents were isolated from young leaves as described by Horn and Rafalski (1992). Ten SSR primer pairs, Ra2-A01, Ra2-B01, Ra2-B02, Ra2-H07, Ra2-H10, Ra2-H11, Ra2-H12, Ra3-C01, Ra3-C04 and Ra3-H09 downloaded from the *Brassica* database (http://www.ukcrop.net), were used for developing SSR markers. The procedure for SSR analysis was performed according to the methods of Saal et al. (2001) and Li et al. (2005a). The PCR reaction profiles were as follows: 94°C at 60 s followed by 35 cycles of 60 s at 94°C, 60 s at 61°C and 1.5 min at 72°C, extension at 72°C for 10 min, and then held at 4°C. The PCR products were checked by 3% agrose gel.

The different color petals of the putative chimeras were analyzed by using Random Amplified Ampifled Polymorphic DNA (RAPD) technique; ten RAPD primers, s1101, s1131, s1036, s1066, s1071, s1073, s1076, s1078, s1090, s1097 (synthesized by SBC Shanghai), were used in the analysis. PCR was carried out in a total volume of 20 µl per reaction, containing 50 ng of genomic DNA, 8 µM of random primers, 0.2 mM of all the four dNTPs, 1 × PCR buffer, 1.5 mM of $MgCl_2$, and 1.5 U of *Taq* DNA polymerase. PCR reactions were as follows: 94°C at 3 min followed by 35 cycles of 60 s at 94°C, 60 s at 40°C and 60 s at 72°C, extension at 72°C for 10 min, and then held at 4°C. The PCR products were checked by 0.8% agrose gel.

### Cytological analysis of flower chimeras

For meiosis of pollen mother cells (PMCs), flower buds were fixed for 24 h in fresh Carnoy's solution and then stored in 70% ethanol; the anthers were dissected out, cut in half and stained with 10% modified Carbol Fuchsin for observation (Li et al., 1999; Li et al., 2001).

## RESULTS

### The identification of hybrids between *B. carinata* and *B. rapa*

In all, 642 seeds were obtained in the hybridization of *B. carinata* and *B. rapa*, and 251 plants survived in natural planting. In the seeding stage, 37 plants with same characteristics as their parents were eliminated. Chromosome analysis showed that the hybrids (genome constitution ABC) with 27 chromosomes, which is the chromosome number of ABC-triploid somatic cells (Li et al., 2005). Ten SSR primer pairs were used for primer screening in the *B. carinata* and *B. rapa*, in which the Ra2-H10 and Ra3-C01 exhibited obvious polymorphism in 3% agrose gel. The interspecific hybrids with 27 chromosomes possessed the specific DNA bands of both parents from Ra2-H10 and Ra3-C01 SSR primer pairs (Figure 1). The hybrids were morphologically intermediate between the two parents; for example, stem colour exhibited continuous variation between red and green, some were closer to *B. carinata*, and others to *B. rapa* (Figure 2a).

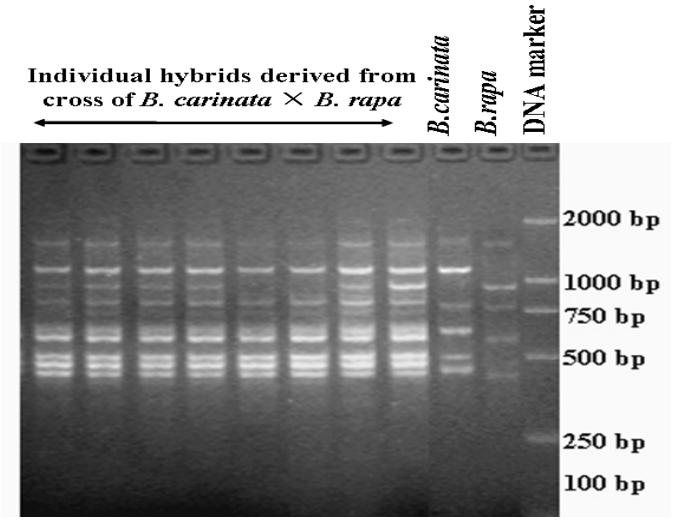


**Figure 1.** The SSR analysis of the hybrids that derived from the hybridization between *B. carinata* and *B. rapa* with the primer of Ra3-C01.

### The characteristic of flower petal chimeras in the hybrids of *B. carinata* and *B. rapa*

Previous research has revealed that the gene which controls white flowers is dominant or incompletely dominant to the gene which controlled the yellow flowers in rapeseed (Zhang et al., 2000; Liu et al., 2004). The parents used in this experiment had white and yellow flowers, respectively. Field observation showed that 95.8% of hybrids did not have white or ivory-white flowers as expected, but yellowish flowers (Figure 2b). We knew that the hybrids derived from *B. carinata* (BBCC) and *B. rapa* (AA) contained three genomes (A, B and C); the gene for white flowers from *B. carinata* was in the B or C genome and the gene for yellow flower of *B. rapa* was in the A genome. So genes, which controlled the petal colour, were different as in other studies.

Two kinds of flower chimera appeared on five hybrid plants with 27 chromosomes: 1) On one early flowering plant, 175 flowers were analyzed, of which 26 flowers had four white petals (Figure 2c, arrowed) and 73 flowers had four yellow petals (Figure 2d, arrowed), other 76 flowers had yellow and white petals in one flower in the same branch (Figure 2c and d). 2); another four plants, 17.4% of the flowers had the white-yellow mosaic petals, and the size and morphology of white stripes varied greatly in different petals (Figure 2e - 4, 5, 6, 7, 8, 9 and

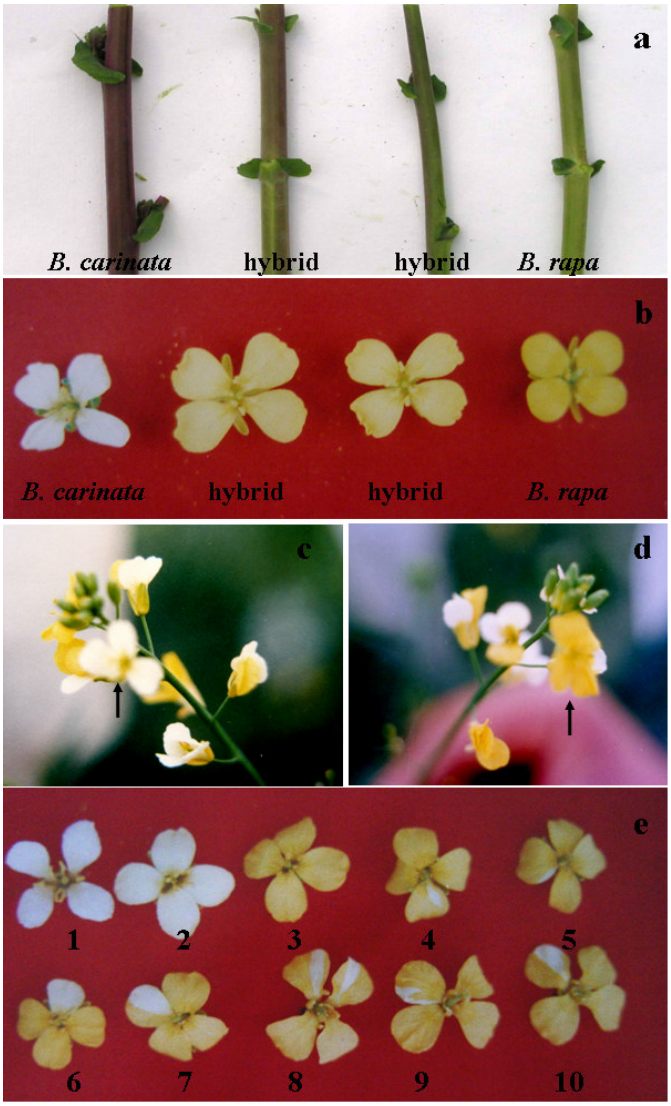


**Figure 2.** The morphological characteristics of interspecific hybrids and the flower chimeral plants in the hybrids of *B. carinata* and *B. rapa*. a and b: The stem color and flower color in the hybrids of *B. carinata* × *B. rapa*. d and e: Two kinds of flower chimeras that spontaneous developed in a few hybrids of *B. carinata* × *B. rapa*.

10), some were larger than 50% of one petal (Figure 2e - 7) and some very small (Figure 2e - 5).

### Cytological analysis of the flower color chimera in the hybrids of *B. carinata* and *B. rapa*

Meiotic behavior of the hybrids was thoroughly observed in our previously studies (Li et al., 2005). Special meiosis behavior also appeared in the PMCs of flower chimeras except for the results reported in our previous work. At diakinesis, two groups of chromosomes were appeared in about 1.5% of PMCs (Figure 3a). At anaphase I, different chromosome numbers were observed in the two polar ends of PMCs, and some of them obviously showing 17: 10 segregation (Figure 3b), which inferred that complete or partial separation of parental genomes during meiosis occurred in flower chimera. The unequally segregated chromosomes were again orientated on the equatorial plate at metaphase II (Figure 3c) and gave rise to AII (anaphase II) with different numbers of chromosomes in different polar positions (Figure 3d); this kind of meiosis behavior may result in the production of parental plants. Three daughter groups of chromosomes at MI and TI also appeared in 0.5% PMCs of flower chimera hybrids (Figure 3e and f), which hinted that the three genomes (A, B and C) also could be separated in a few cases, and the successively divided sister-chromatids at anaphase II resulted in the production of pollen-hexads (Figure 3g).

Abnormal mitotic behavior was also appeared in less than 2% of somatic cells of shoots and early-developed petals. In the cells, the exact 17:10 or the two polar with different chromosome numbers also happened at anaphase (Figure 3h and i), which might indicate the partial or complete separation of parental genomes in the somatic cells of flower chimeras. Considering the occurrence of flower chimeras in the hybrids, that is, the chromosomes with white-petal genes of the BC genome and with yellow-petal genes in the A genome had separated in the mitosis of those somatic cells, the subsequent successive division of those cells with parental genomes led to the production of white-yellow petal chimera, The RAPD method was used on the different petals of the putative chimeras (different flower colours), and the results showed that the yellow and white petals exhibited the DNA patterns of *B. rapa* and *B. carinata*, respectively (Figure 4), which verified the production of somatic cells with parental genomes in the mitosis of somatic cells. Cells with two chromosome groups with obvious different chromosome numbers at prophase and metaphase were also observed in the shoot and early-developed petals (Figure 3j or k), the further research revealed that those cells can enter normal mitosis (Figure 3l).

## DISCUSSION

Many plant chimeras, especially the flower chimeras, are selected by horticulturalists for their distinctive, valuable phenotypes (Burge et al., 2002). Synthetic flower chimeras have been produced in many ways, such as co-culture of pith slices, mixed callus cultures, co-culture of protoplasts and an *in vitro* graft-culture method (Burge et al., 2002). Flower chimeras were also observed in interspecific hybrids, for example, Zhao et al. (1992) and Li et al. (2005) found yellow-salmon pink and red-yellow petal chimeras in the hybrids of *Chrysanthemum* species. Huang et al. (2002) and Yu et al. (2004) found some hybrid plants with yellow-white petals in *Brassica* species. However, little is known about the reasons for the production of flower chimeras in those hybrids.

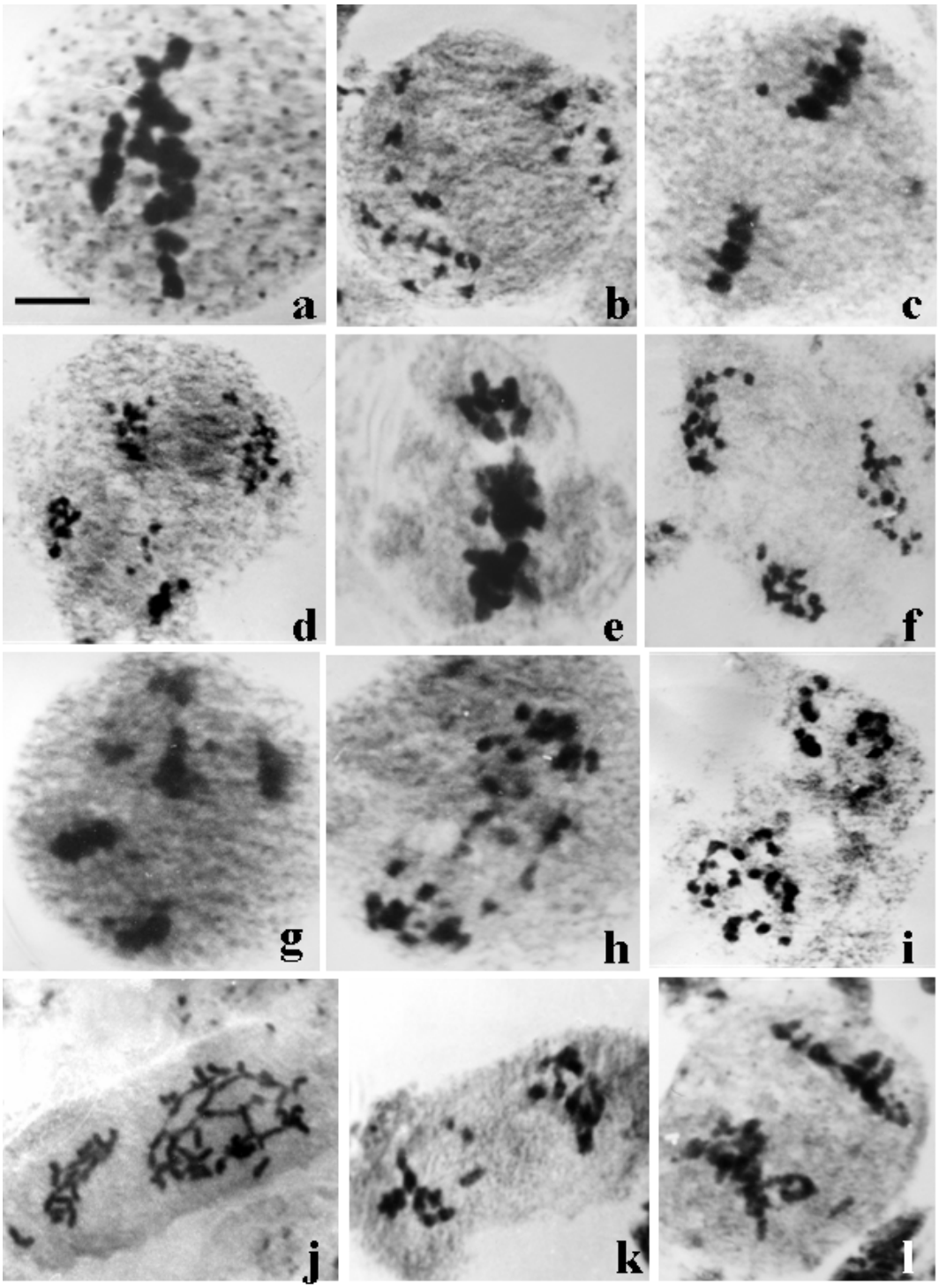


**Figure 3**. The special meiosis and mitosis behavior in the flower chimeral hybrids. a-g. The special meiosis behavior that appeared in the flower chimeral hybrids. a) Two group chromosomes were observed at diakinesis. b) The 27 chromosomes in the PMC were showing 17:10 segregation at anaphase I in some cases. c) and d) The unequal segregated chromosomes were again orientated on the equatorial plate at metaphase II and normally segregated at anaphase II. e) and f) Three chromosome groups were appeared in some PMCs at metaphase I and anaphase I. g) The three complete or partial segregated genomes all can goes into the anaphase II. h – l) The special mitosis behavior that appeared in the shoot and early-developed flower petals. h) and I) The partial or complete genome segregation might happen at anaphase. J), k) and l) Two chromosome groups which with obviously different chromosome numbers at early-prophase, prophase and metaphase were observed in some somatic cells.

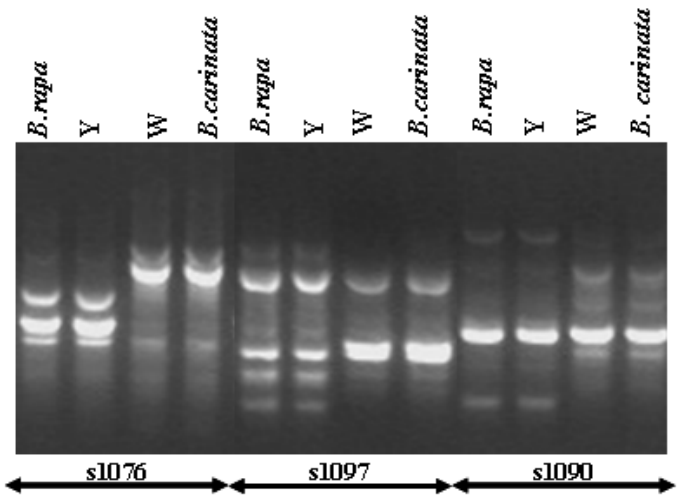


**Figure 4.** The RAPD analysis on the flower petals of the putative chimeras. Y and W represent the yellow and white petals of putative chimeras.

Many reasons have been reported for the production of chimera. Generally, most chimeras have originated spontaneously due to mutation of somatic cells in the apical meristem (Jene, 1992; Burge et al., 2002). Chromosome deletion or the chromosome-bridge formation resulting from interspecific hybridization was the main reason for the production of flower chimeras (Quiros et al., 1988). Recent work using chimeras has shown that transcriptional factors are able to move from cell to cell in plants and remain biologically active (Hake, 2001). Different cells of petals may have different color if the transcriptional factors affected the genes which controlled anthocyanin biosynthesis (van Houwelingen, 1998; Quattrocchio et al., 1999). Both complete and partial separation of the parental genomes during mitosis and meiosis has been proposed to occur in the intergeneric hybrids between *Orychophragmus violaceus* (2n = 24) and the six cultivated *Brassica* species (Li et al., 1995; 1996, 1998, 2002; Li and Heneen, 1999). Chen et al. (2006) considered that the roots of periclinal chimeras that arose in the interspecific chimeras between tuber mustard (*B. juncea*) and cabbage (*B. oleracea*) should originate only from one of the two parents as the cells in a single layer of periclinal chimeras derived from a single parent. In our present study, the complete or partial segregation of parental genomes also appeared possibly in meiosis and mitosis in flower chimeral hybrids of *B. carinata* and *B. rapa*. The complete separation of parental genomes during mitosis in cells of early developed petals led to the production of somatic cells with *B. carinata* and the *B. rapa* complements, and so the successive division of those cells with parental genomes might be the reason for the production of the flower chimeras in the hybrids of *B. carinata* and the *B. rapa*.

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