Observation on chromosome behavior during meiosis of resynthesized *Brassica napus*

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Abstract

Four characters of the chromosome behavior during meiosis were observed in the new resynthesized *Brassica napus* F_0 plants: 1) One group of chromatin separated into two during interphase; 2) Separation and asynchronous division of two groups of chromosomes, suggesting that the two groups of chromosomes are respectively from A and C genomes, which maintained independent in the PMCs; 3) Chromosome pairing within genomes occurred more frequently than that between A- and C-genome; 4) Meiosis at later stages, such as anaphase I, telophase I and meiosis IIwere generally normal. The study on genome separation and related chromosome behaviorsin new resynthesized *B.napus* is of importance for phylogenic study, breeding and transgenic risk control of this crop. The factors causing chromosome change in the initial generations were discussed in this paper as well.

Key words: resynthesized *B. napus*, chromosome behavior, meiosis

Introduction

It is very important to clarify the chromosome behavior during the evolution of *Brassica* genomes. A model for the genomic evolution of *Brassiceae* was described in the so-called U triangle, which showed that the three secondary species, *B. napus* (AACC, 2n=38), *B. juncea* (AABB, 2n=36) and *B. carinata* (BBCC, 2n=34), were originated from three primary species, *B. rapa*(AA, 2n=20), *B.oleracea*(CC, 2n=18)and *B. nigra* L.(BB, 2n=16). Based on this relationship and the hypothesis of Mikkelsen et al. (1996 a) of possible "safe" integration sites, Li et al.(2006) had obtained C-genome transgenic *B. napus* with low environmental risk by means of artificial resynthesis, and they concluded a low exchange frequency of (trans)gene between A- and C-chromosome. However, studies on genetic relationship revealed that the relationship between A and C genome was so close that chromosome pairing between A and C-genome could occur frequently. Röbbelen had proposed a hypothesis that the primary species may derived from an even older polyploid ancestor (AABBCCDDEEFF, 2n=2x=12), which had a potential pairing of six pairs between A and C- chromosomes. Results of comparative genomics of *Brassica* spp. also showed a lot of chromosome variation during the evolution of *Brassica* genomes, such as duplication, deletion and rearrangement. Undoubtedly, the *Brassica* genomes have undergone restructuring during evolution. However, whether it is resulted from the reciprocity of A and C-chromosome, pairing behavior between A- and C-chromosomes in newly resynthesized *B. napus* accordingly plays an important role in explaining these different standpoints.

Relationship study between A and C-genome on chromosome level has gained prodigious development. Most studies paid more attention to the chromosome behavior in the natural *B. napus* cultivars (Li and Liu, 1994) or offspring of resynthesized *B. napus* after a few generations (Zhang and Niu, 2003). Nevertheless, Li et al.(2006) had showed that the chromosome in hexaploid hybrids could become stable after three generations under artificial selected. They suggested that many changes on the chromosomes occur during the few initial generations. Therefore, using newly resynthesized *B. napus* to observe chromosome behavior could be an optional way to elucidate chromosome change during the evolution of *B. napus*.

In the present research, we particularly observed the chromosome behavior from the genome of *B. rapa* and *B. oleracea* during meiosis of the newly resynthesized *B. napus* lines. By comparing to those results of *B. napus* cultivars and offspring after several generations, more information on the procedure of *B. napus* evolution, the chromosome exchange frequency between A- and C-chromosome, and characters to differentiate chromosome behavior between cultivar and resynthesized *B. napus* during meiosis was obtained.

Material and methods

Newly resynthesized *B. napus* lines were obtained from the ovary culture and chromosome doubling of intergeneric hybrids between *B. oleracea* var. *alboglabra* and *B. rapa* (Li et al., 2006).

For meiotic analysis of PMCs, buds from the terminal inflorescence were fixed in fresh Carnoy's solution at 4°C. PMCs were squeezed out of anthers in a drop of 10% modified carbol fuchsin (Li and Liu, 1996). For mitotic analysis, pistils of flower buds were peeled out, treated with 0.002mol/L 8-hydroxyquinoline for 4h, then fixed in Carnoy's solution for 24h, and stored in 70% ethanol at 4°C until use. The pistils were hydrolyzed in 1*N* HCl at 60°C for about 10 min, squashed in a drop of modified carbol fuchsin and observed under oil.

Results

The meiosis of resynthized *B.napus* started from a relative large chromatin occupying the central position of the PMCs

(Fig.1-a). It separated into two towards the end of the leptotene (Fig.1-b, c). Zygotene, by definition, is the stage of chromosome synapsis, and early zygotene is therefore characterized by the first indications of synapsis. However, this stage in resynthesized *B. napus* characteristically failed to produce well spread preparations (Fig. 1-d, e). This appeared to be caused by polarization, which was accompanied by aggregation of the pericentromeric heterochromatin into a variable number of clumps (Fig. 1-f). At early pachytene stage, the chromosomes are much shorter compared to the earlier prophase stages. All chromosomes are seen to be fully synapsised in double structures, some with obvious chromomere differentiation and others remaining aggregated (Fig. 1-g, h). By late pachytene, the 19 bivalents became more obvious, but seemed to be divided into 9 + 10 bivalents (Fig. 1-i), which might respectively from A- and C-genome (Li et al. 1996). common to all the plants was the abundance of multivalents, comprising mainly tri- and quadrivalents. This is exemplified in Fig.1-i in which auto- or allosyndetic pairing or secondary association is readily identifiable. But penta-, hexa-, and heptavalents were also found in the plants with 38 chromosomes (Fig. 1-i, j). During meiosis II, few abnormal chromosome pairing occurred (Fig. 1-l). And heteromorphic bivalents and multivalents could also be detected at metaphase mitosis (Fig. 1-n). Chromosome number was confirmed to be 38 by observation of somatic cells (Fig. 1-o, p).

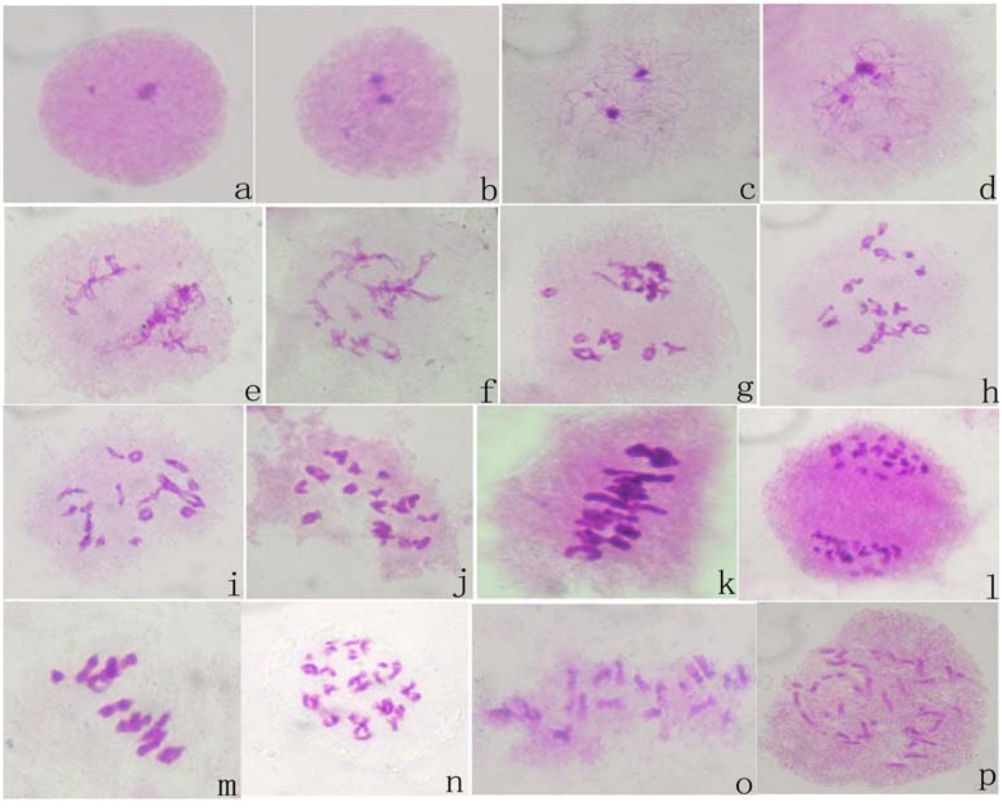

Fig. 1 Chromosome behavior during meiosis of Resynthized *B.napus*

a. One group of chromatin in pollen mother cells (PMCs); b～e. Two groups of chromatin in PMCs; f. PMCs with various shapes of chromosomes; g. PMCs with 9 bivalents and one group of chromosomes; h. PMCs with more than 10 bivalents and one group of chromosomes; i～j. PMCs with 19 bivalents appearing as 9 + 10 biavalents; k. Metaphase I of PMC; l. PMC at telophase I; m. Metaphase Ⅱ of PMC; n. Somatic synapsis of resynthized *B. napus*; o～p. Chromosome counts of resynthized *B.napus*

Based on cytological observation, the distinct characters of meiosis in newly resynthized *B. napus* were : 1)One group of chromatin separated into two during interphase, which showed asynchronous division and separation during the following stage of meiosis, because there were always 9 or 10 bivalents appeared earlier than others. 2) During late pachytene and metaphase, the 19 bivalents obviously divided into 9 + 10 bivalents with an underling boundary. It is suggested that the above two groups of chromosomes are related to A and C genomes, which maintained independent in the pollen mother cells. 3) an allosyndetic association or pairing between A and C- genome is more frequently than within genomes; 4) Later stages of meiosis, such as anaphase I, telophase I and meiosis IIwere generally normal.

Discussion

According to the chromosome formulation of allotetraploid *B. napus* of AAABBCCCDDDDEEFFFF, complicated situation of chromosome pairing should occur frequently. According to Roebblesen (1960), there would be a maximum of homologous and homoeologous pairing of 3 and 6 respectively. However, the meiosis observations of cultivar *B. napus*, *B. rapa* and *B. oleracea* showed a normal chromosome pairing behavior (Heneen et al. 1995, Zhang and Niu 2003; Li and Liu, 1994). While in newly resynthized *B. napus*, there were many abnormal phenomena such as micronuclei at resting stage and broken parts at metaphase I. At late diakinesis in meiosis, common to all the plants was the abundance of multivalents, comprising mainly tri- and quadrivalents, but penta-, hexa-, and heptavalents could also be found (Fig. 1-n). Zhang and Niu

(2003) also detected that chromosome pairing during meiosis in succeeds of systematic selective breeding of resynthized *B. napus* after several generals have variation of chromosome numbers and structures with abnormal behavior of meiosis. However, at anaphase I and meiosis II, abnormal behavior of chromosome had not yet been found.

Li and Liu (1994) suggested that previous studies on chromosome pairing mechanism could not explained the steady chromosome pairing in the amphidiploid which contained two high homologous genomes. They believed that different speed of meiotic division between the chromosomes of A and C genome and independent distribution in pollen cells were the main reasons that resulted in the avoiding of homologous chromosome pairing. While in our observations, the difference of meiotic division speed also existed in new resynthized *B. napus*. Furthermore, we found that multivalences appeared after some of the chromosomes formed. Heneen et al (1995) also observed hexavalents at meiosis in resynthized *B. napus*. Therefore, different speed of meiotic division could not construct the main reason of steady meiosis in *B. napus*. Some studies on resynthized amphidiploid wheat (Okamoto, 1957), oat (Rajhathy and Thomas, 1972) and cotton (Mursal and Endrizzl, 1976) showed that the steady chromosome pairing were obtained by genetic control and structure changing of chromosome, which was in accordance with our observations. That is, in new resynthized *B.napus*, the coexistence of A^r and C^o in initial stages broke up the steady chromosome pairing in the cell of two parents and resulted in abnormal behavior of chromosome pairing. But in the succeeding generations, following the gene recombination and chromosome structure changing, the mechanism of steady chromosome pairing was formed.

The observations of new resynthized *B.napus* during meiosis showed, the frequency of multivalance formed within A or C genome is higher than between genomes. Similar to natural *B. napus* cultivar, asynchronous meiotic division of A and C genome and independent distribution in pollen cells also decreased the frequency of chromosome pairing between them. Qian (2003) found that the mutual contribution rate between A^r and A^n genome chromosome is obviously higher than between A^n and C^n genome chromosome. Quiros et al.(1994) also detected that the genetic linkage groups of 4C, 5C and 6C tended to recombined. Therefore, the genomic difference between synthetic species and basic species evidenced by some previous studies (Song et al. 1989; Teutonicoa and sborn 1994), might largely attributed to the chromosome exchange and recombination within the genome under reciprocity between A and C genome.

During our observations, the two groups of chromatin changed from equal into unequal during meiosis. While DNA content test showed that the three basic species had a similar amount of 470-660 Mbp/lc(Li et al., 2005). It is assumed that the two genomes in the resynthized *B. napus* or *B. napus* cultivars had a different degree of concretion at different stages.

Most studies showed that there was close relationship between pollen fertility and abnormal chromosome behavior (Heath and Earle 1996, 1997). Usually, pollen fertility of new resynthized *B. napus* is very low and it needs to be inbred for 7 or 8 generations to restore (Qian, 2003). This is also confirmed by our observations of pollen fertility and chromosome behavior of new resynthized *B. napus* (Li et al., 2006).

References

Heneen W.K., Chen B.Y., Cheng B.F. (1995). Characterization of the A and C genomes of *Brassica campestris* and *B. alboglabra*, Hereditas. 123, 251-267.

Li M.T., Wang F., Zhang C.Y., M J.L.(2006). The Cytological and Fertility Studies on Intersubgenomic Hybrids($A^rA^nC^oC^n$) in *Brassica napus*. *Acta Agronomica Sinica*, 32, 351-357.

Li Z.Y., Liu H.L. (1994). Observation on chromosome behavior during meiosis of *Brassica napus*. Journal of Huazhong Agricultural University, 13, 418-421.

Li J., Fang X.P., Wang Z., Li J., Luo L.X., Hu Q.(2006). Transgene directionally integrated into C-genome of *Brassica napus.* Chinese Science Bulletin, 51, 1578-1585.

Qian W.(2003). Studies on Intersubgenomic Heterosis between *Brassica napus* and *B.rapa*. Doctor thesis, Wuhan, Huazhong Agricultural University.

^{r}, ^{n}, ^{o} represents the genome from *B.rapa*, *B. napus* and *B. oleracea,* respectively