Cytology of multisomic addition line "NJ04-8089" in *Brassica napus* L.

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Abstract

A B genome chromosome multi-somic addition line "NJ04-8089" in *Brassica napus* (AACC 2n=38)was identified from offsprings derived from $BC1F_1$of *Brassica napus*/*Brassica carinata* (BBCC 2n=34)//*B. napus.* Results from cytological observation showed that somatic chromosome number was 2n=45 observed from either root-tip or baby ovary cells of "NJ048089". 7 univalent scattered around the equator at Metaphase I(MI) of pollen mother cells (PMCs) meiosis. A chromosome configuration of 19II + 7I formed at MI. Some lagged chromosomes formed at Anaphase I (AI). The 19 divalent observed could be the result of the homologous pairing of AACC chromosomes and the 7 additional chromosomes were the chromosomes of B genome coming from *B. carinata* according to known relationships between chromosome of A-A, C-C, A-C, B-A and B-C genome. It indicates that the 7 chromosomes likely that they originated from B genome of *Brassica carinata*.

An unequal chromosome number with a wide range of distribution from 2n=38 to 2n=51 found in root-tip cells of seeds harvested from "NJ04-8089" plant after bagging which resulted in an unequal chromosome number in each of the gametes produced from it. Out of the total root-tip observed there was 17.28%, 13.58% and 20.99% having a chromosome number of 2n=43, 2n=44 and 2n=45 respectively. It indicated that the 7 univalent was randomly assorted to each of the polar at AI but some of them acted as a group staying in one side of the cell in stead of random assorting.

Key words: *Brassica napus*, *Brassica carinata*, Multisomic, Additional line, Cytology

The genus *Brassica* crops includes three cultivated diploid species: *Brassica rapa* L (2n=20, genome AA), *B. oleracea* L (2n=18, genome CC) and *B. nigra* Koch (2n=16, genome BB), and three amphidiploids species: *B. napus* L. (2n=38, AACC), *B. juncea* Czern and Coss (2n=36, AABB), *B. carinata* Brou (2n=34, BBCC)[1]. Results from cytological studies demonstrated that the amphidiploids crops were originated from a spontaneous hybridization between each pair of the diploid crops accomplished with chromosome doubling.[1, 2] Based on the information developed by comparative studies on chromosome structure and relations among them, It assumes that genome of the genus *Brassica* crops is derived from an ancestral genome of x=5 or 6 chromosomes undergoing duplication by amphi-ploidization.[2,3,4,5] It is believed that the B genome is more distantly related to both A and B genomes than the latter two are each other.[3,4,5]

In genus *Brassica* several chromosome addition lines have been developed and been used as a tool in dissecting of chromosomes in a given genome and comparison of the chromosomes as well as location of a gene(s) controlling an important agronomic traits.[6,7,8,1o,11,12] These chromosome addition lines obtained includes monosomic addition line of *B. napus-Raphanus sativa* carrying a white flower trait, and *B. napus-Diplotex* carrying a male sterile trait and *B. rapa-oleracea* carrying high erucic content. Scientists are making effort to construct a set of alien chromosome addition lines from a given genome into a common genome. Results of identification of a B chromosome addition line derived from the descents of a cross between *B. napus* and *B. carinata* is reported in present paper.

1 Materials and methods

1.1 Materials

All the plant materials used in this research are kindly supplied by Institute of Industrial Crops (IIC), Jiangsu Academy of Agricultural Sciences (JAAS). It includes "Ningyou 10" (*B. napus* L.), a yellow-seeded cultivar with low erucic content, and "NJ04-8089" a descent from a cross of "Ningyou 10" × "Dodolla" (*B. carinata*), and the hybrid seed of "NJ04-8089" × "Ningyou 10". Seed was sow in breeding nursery of IIC in autumn of 2004. Each line planted 2 rows in 3 m long with a row apace of 40cm and inter- plant space of 20cm respectively.

1.2 Cytological observation

1.2.1 Somatic cells chromosome number counting

The root tips or young ovaries were used to identify chromosome number in somatic cells of the material observed in this research.

Young ovary (size:2mm) ware treated with0.002*M* 8-hydroxyquinoline for 3 h at 22° C and then fixed in Carnoy's solution for 24 h, and transferred to a fresh Carnoy's solution keeping in refrigerator at 4 °C.

Seeds were in a patric dish spreading with 2 layer of tissue paper wetted with adequate water for germination. Root tips ware treated with0.002*M* 8-hydroxyquinoline for 4 h then took it out and after watered fixed in Carnoy's solution.

The root tips and young ovaries took out from fixation solution were hydrolyzed in 1 M HCl at 60°C for 6-9 min, squashed in one drop of 10% modified carbol fuchsin and examined under microscope of "Olympus".

1.2.2 Meiosis observation of pollen mother cells (PMCs)

Young buds were collected 9-11oclock in morning and fixed in Carnoy's solution for 24 h, then transferred to 70% ethanol and keeping at 4°C. Anthers were dissected out of the buds and the PMCs squeezed out in a drop of 10% modified carbol fuchsin [13,14]examined under microscope " Olympus".

2 Results and Analysis

Chromosome number in somatic cells of NJ04-8089 plant

Chromosome number in somatic cells of NJ04-8089 was 2n=45 resulting from ovary cells observation (fig 1).

Chromosome behavior in pollen mother cells meiosis of 04-8089

Cytological observation revealed that 19 divalent and 7 univalent formed at MI in PMCs of 04-8089 and no trivalent detected (fig 2A). However, the 7 univalent were asserted independently to each pole of a given PMC. Several lagged chromosome fund at AI (fig 2B, 2C, showed in arrow). As a consequence dyads formed randomly at AI (fig 2B) in which chromosome number varied from 18:27 to 22:23. It demonstrates that 04-8089 is a multi-somic addition line of *B. napus* in which 7 alien chromosomes from B genome of *B.carinata* added to the AACC genomes of *B. napus.*

Chromosome number in root-tip of the selfing offspring (seeds) of NJ04-8089

Chromosome number in root-tip cell of the seeds descending from NJ04-8089 plant by bagging were varied from 2n=40 - 2n=51(fig3 and table 1). Out of the total root-tip (81) observed 67% had a chromosome number of 2n=42, 2n=43, 2n=44 and 2n=45. Among them 20% had a chromosome number of 2n=45 (table 1). These results suggested that chromosome number in each of gametes both male and female produced by NJ04-8089 were not equal and varied from n=19 to n=26. Among them gametes having a chromosome number of n=22 and n=23 were much more than the others because more offspring derived from NJ04-8089 showed a somatic chromosome number of 2n=45.

The morphology of NJ04-8089 was abnormal and characterized with short (98cm) in plant height. Its pollen grains were stick together and shaded irregularly with a poor viability of pollen resulting in a low rate of seed-set by bagging. It could be related to an uneven assortment of chromosome during the process of PMCs meiosis as well as the disorder of physiological and genetic disturbing caused by more extra- or alien chromosomes.

3 Discussions

NJ04-8089 was segregated from a cross of *B. napus/B. carinata//B. napus*. Obviously its fundamental chromosomes is the one of AACC genome and the added one is the B genome chromosomes since no trivalent found at MI of PMCs meiosis of NJ04-8089 in this study. Cytological studies evidences that B chromosomes are more distantly related to A or C one than the latter two are each other. Thus chromosomes from B genome have much difficulties and nearly no possibility to pair with that either from A or C theoretically. A conclusion can be made from the cytological observation in this paper is that the 7 added alien chromosomes are the chromosomes of B genome originated from Dodolla, a cultivated form of *B. carinata* (genome BBCC, 2n=34).

In the process of PMCs meiosis all the added chromosomes might be 7 single chromosomes. Not only can they not pair to each other, but also they can not pair to any of one of the chromosomes of A or C genome because of the distant relations. Consequently the 7 alien chromosomes acted as univalent dispersed outside of equator plate at MI and lagged chromosome(s) formed at AI and AII resulting in an uneven assortment of the added chromosome and the gametes both male and female having unequal chromosome number produced. Chromosome number in the gametes can be divergent from n=19, the *B. napus* type, to n=26, the extreme one which the 7 chromosomes assorted to one cell at AI. Seeds which carry a chromosome number of 2n=51 in its somatic cells were detected from descends of NJ04-8089. Consequentially, individual plants which has various chromosome number ranged from 2n=38 to 2n=52 could be segregated easily from descends of NJ04-8089 by ovary sample observation and might become a useful materials in dissection of B genome as well as location of important gene(s).

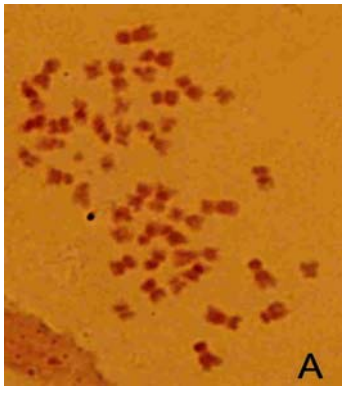

Fig1Showing chromosome number in somatic (ovary) cells of NJ04-8089 (2n=45)

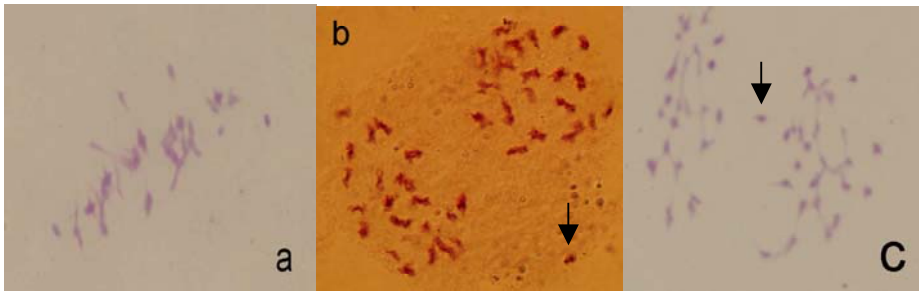

Fig 2 Chromosome behavior in PMCs meiosis of NJ04-8089

a) Showing 19II + 7I at MI; b～c) Showing lagged chromosome(arrow) at AI in the dyad with a chromosome number of 22:23(b) and 19:26(c)

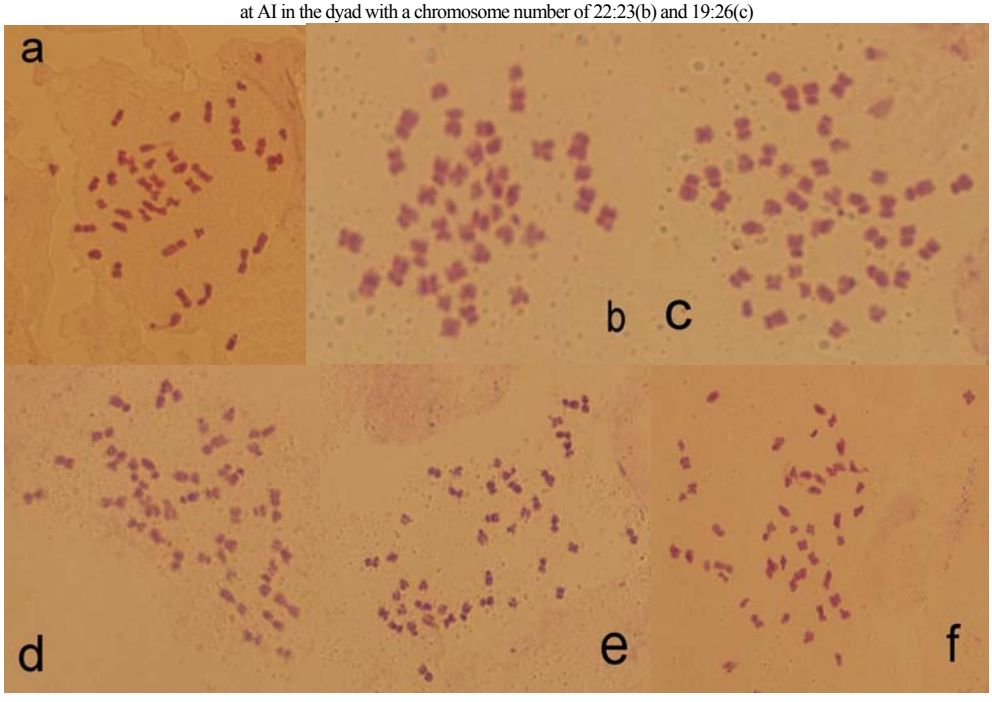

Fig 2.3 Chromosome number in root tip cell of isolated seeds from NJ04-8089

a) 2n=43，b) 2n=40，c) 2n=44， d)2n=41，e) 2n=42，f) 2n=48

Table 1 Chromosome number in root-tip cell of inbred seeds of NJ04-8089

Chromosome number (2n)	Root tips observed	%of total observed	Chromosome number (2n)	Root tips observed	%of total observed
40	5	6.17	46	5	6.17
41	3	3.70	47	7	8.64
42	12	14.81	48	3	3.70
43	14	17.28	49	1	1.23
44	11	13.58	50	2	2.46
45	17	20.99	51	1	1.23

Fig 2.4 Plant morphology of NJ04-8089

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