Meiotic behaviour of extra chromosomes in fertility restored Polima CMS Brassica napus plants[1]

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Abstract Crosses were made between Brassica napus (2n=38) with the 'Polima' cytoplasmic male sterile (CMS) system and variety "Zem" of B. juncea. Fertility was partially restored in backcross progenies with an extra chromosome (2n=39) which was believed to be a member of the B genome of B. juncea. Among the more than 40 self-pollinated offspring plants studied, fertility restoration was transmitted only through those plants with two extra chromosomes (2n=40).

Morphologically, anthers of these plants were either full or shriveled with small swollen segments. Full and round pollen grains were found in full anthers and in the swollen portion of shriveled anthers. These pollen grains were stained darkly with an I_2-KI solution. They were obviously viable because full seed set was obtained from bagged flowers.

Meiotic studies of these plants showed $18^{II} + 1^{IV}$ at diakinesis in most of the more than 600 pollen mother cells analyzed. The chromosomes segregated equally at anaphase I and had normal behaviour at other meiotic stages.

Presence of a single quadrivalent in these backcross progenies was highly consistent and occurred in very high frequencies. It is believed that the extra chromosomes are derived from the B genome and they reside in these quadrivalent rings.

Introduction Fertility restoration gene(s) for the 'Polima' cytoplasmic male sterile system (CMS) of Brassica napus had been found in a B. juncea strain known as 'Zem' (Fan and Tai, 1985). To transfer this fertility restorer gene(s), crosses were made between pol CMS plants of B. napus (2n=4x=38, AACC) and B. juncea 'Zem' (2n=4X=36, AABB). The male fertile hybrid was backcrossed to B. napus cv. Regent for four

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generations. The fertile plants of BC_4 were subsequently selfed to the F_3 family level. Among the 34 BC_4F_3 plants, six plants were found to be monosomics with an extra chromosome (2n=39) which was believed to be a member of the B genome of B. juncea (Fan and Tai, 1985).

The present paper reports on the cytogenetic observation of meiotic behaviour of extra chromosomes in these plants and their selfed progenies in an attempt to identify the origin of the extra chromosomes and to ascertain their relationship to the restoration of fertility in pol CMS B. napus.

Materials and Methods For cytogenetic studies, flower buds were fixed in a Carnoy solution (six parts of absolute ethanol, three parts of chloroform, and one part of glacial acetic acid, v:v:v) or Farmer solution (3 parts of absolute ethanol and one part of glacial acetic acid, v:v). The acetocarmine smear technique was used to prepare microslides for cytological observation. For chromosome association, at least 30 pollen mother cells (PMC's) at diakinesis or metaphase I were observed in detail. For each plant, approximately 20 cells were counted at anaphase II and approximately 100 were observed at the quartet stage.

Pollen viability was tested by the staining reaction in a solution of I_2-KI. Darkly stained, round, and full pollen grains were considered as viable. Lightly stained and shrunken pollen grains were considered as inviable. At least 300 pollen grains in each plant were counted for the stainability reaction in an I_2-KI solution.

Results In general, stamen morphology of rapeseed hybrids can be classified into three groups. The anthers from a sterile plant are usually shriveled with a pointed tip and are pale coloured. Meiosis occurs at an early stage but pollen mother cells degenerate without developing into mature microspores. The microsporangia are almost completely empty. Filament length is greatly reduced. No seed set can be observed.

In a completely normal plant, the anthers and the filaments grow to full size. The anthers are full, rounded, and yellow coloured. Each microsporangium is filled with round pollen grains which can be stained darkly in an I_2-KI solution.

The third type is an intermediate. The anthers are generally shriveled, pointed, and pale coloured as the first type. However, each anther carries one or more 'protrusions' of different sizes. The protrusions are yellow coloured and are filled with pollen grains which can be stained with I2-KI. Usually full seed set can be obtained and these plants are considered to be effectively fertile, even though pollen production is much reduced from normal.

Among 18 Regent-derived BC_4F_3 families, fertility restoration was transmitted in only two lines.

Among the 18 plants observed cytologically, majority of them, 10 or 55.56% of the total, had 2n=38 chromosomes, the chromosome number for a regular B. napus. Chromosomes in these plants also behaved normally as in other B. napus plants with 19 bivalents observed at diakinesis and metaphase I. At anaphase I, 19 dyad chromosomes moved to each of the two opposite poles.

Two plants had 2n=39 chromosomes. They formed 19 bivalents and a univalent at diakinesis and usually segregated in a (19-20) fashion at anaphase I. An additional three plants had 2n=37 chromosomes. Meiotic behaviour of these three was similar to those reported previously in other repeseed monosomics (Fan and Tai 1985, Chang et al 1987).

Only three plants had a chromosome number of 2n=40. Detailed meiotic analysis was carried out in 45 self-pollinated progenies in two of these three families.

Except in three plants of family 7-10, the two plants of family 7-3 and 43 plants of family 7-10 all had very similar chromosome association at diakinesis and metaphase I. About a quarter of the PMC's had 20 bivalents (115 out of a total of 529 PMC's observed) and the remaining cells had 16 bivalents and a quadrivalent (414 PMC's out of 529).

In the other three plants of family 7-10, 50% of a total of 96 pollen mother cells observed showed a $16^{II} + 2^{IV}$ chromosome association. The other 50 % of the PMC's behaved similarly to the other 42 plants.

All plants behaved similarly at anaphase I with over 90% of the cells had an equal segregation of dyad chromosomes, 20 each moved toward opposite poles. Only a small portion of cells had unequal segregation at anaphase I and only two cells had two

lagging chromosomes. No cytological irregularities were observed at anaphase II. The quartet stage also appeared normal with no micronuclei formation.

The three plants with one less chromosome than normal (2n=37) were completely sterile. The 10 plants with normal chromosome complements (2n=38) had normal fertility, good stamen growth and normal pollen grains. Among plants with extra chromosomes (2n>38), fertility restoration was only observed in plants with 2n=40 chromosomes. The rest of them had shriveled, empty, and pale coloured anthers with no stainable pollen grains. There was no seed capsule development and no seeds collected.

Among the 45 plants with 2n=40, 44 of them had round, full, and yellow coloured anthers with darkly stained pollen grains. One plant had the third type of stamen, shriveled anthers with yellow coloured 'protrusions' containing round, full pollen grains. All 45 plants had good seed set.

Discussion Heterosis for seed yield in hybrid rapeseed, B. napus, has been reported by various authors (Sernyk and Stefansson 1983, Buson 1980, Guan 1980, Shuster and Michael 1976, Shiga 1976). Hybrids have shown a 10-40% increase in seed yield over the better parent used in the cross. Other agronomic characteristics also show significant hybrid vigour.

The 'Polima' CMS was derived from spontaneously occurring male sterile plants in a population of B. napus obtained from Poland. Restorer gene(s) had been found in a B. juncea strain known as 'Zem'. The chromosome which carried the restorer gene(s) was recognized as a member of the B genome of B. juncea and this chromosome had been incorporated into B. napus through crosses and backcrosses (Fan and Tai 1985).

Among the offspring plants studied, only those carrying two extra chromosomes (2n=40) had their fertility restoration transmitted. Plants of families 6-2, 7-3, and 7-10 derived from plants which had 39 chromosomes. A (2n+1) plant produced two types of gametes, (n) and (n+1). Plants with 40 chromosomes (2n+2) were results of fusion between two (n+1) gametes. Since both extra chromosomes came from the same origin, they were homologous partners. They should pair normally during meiosis and thus would not cause a reduction in fertility. If they carried the fertility restoration gene(s), they should be able to restore the fertilty in a pol male sterile genotype.

However, none of the plants studied had the expected 20^{II} association. Over 75% of their PMC's had at least one quadrivalent.

One to two quadrivalents appeared also in regular B. napus (Catcheside 1934, Broda et al 1979) and in synthesized B. napus (U 1935, Olsson 1960, Sarashima 1964). Meiosis of synthetic B. napus developed through somatic fusion had many univalents and multivalents (Schenck and Robbelen 1982). Quadrivalents only appeared in less than 3.18% of the cells in those B. napus plants. The authors did not give a satisfactory explanation for the origin of quadrivalents and their effect on the reduction of fertility

A quadrivalent can be formed due to tetrasomy or as a result of reciprocal translocation. In tetrasomy, four homologs are involved in a quadrivalent. Two different pairs of chromosomes are involved in a qudrivalent of reciprocal translocation.

Appearance of quadrivalent association in plants of the present study is much more consistent than those reported in B. napus by other authors. It is likely that these qudrivalents are of a different nature and origin, and the two extra chromosomes are probably involved in the quadrivalent formation. Since no trivalent formation was observed in the original parental plant, these extra chromosomes are probably not members of the A genome of B. juncea which should be homologous with the A genome of B. napus. This practically eliminates the possibility of tetrasomy as the origin of quadrivalent formation.

In the three plants which had two quadrivalents, two plants had two quadrivalents in only two PMC's and the other plant had them in more PMC's. One of the two quadrivalents probably originated from the extra chromosomes and the other quadrivalent had an origin similar to that of other B. napus plants.

The exchange of chromosome segments probably occurred after the B genome chromosome entered the B napus cytoplasm. If the original B genome chromosome differ by one translocation from one of the chromosomes of the A or C genome of B napus, after fusion of two (n+1) gametes, only structure homozygotes would present in the hybrid and they should form bivalents.

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