Cytogenetic studies of polyhaploids of *Brassica napus* L.

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Abstract More than 40 polyhaploid plants (2n=2X=19, genome formula AC) of *Brassica napus* (2n=4X=38, AACC) were cytogenetically studied. Some of these polyhaploids were found among field populations and the rest were derived through anther culture of regular *B. napus*. Meiotic behaviour at diakinesis and metaphase I revealed nine bivalents and one univalent in more than forty percent of the greater than 400 pollen mother cells analyzed. However, when the chromosome number of the polyhaploids was doubled using colchine, 19 bivalents were observed. These plants (2n=4X=38, AACC) also had meiotic behaviour identical to a regular *B. napus*. Quadrivalents association were observed when the chromosome numbers were doubled to the octaploid level (2n=8X=72, AAAACCCC). It is suggested that 'A' and 'C' are homoeologous genomes. If homologous partners are present, chromosomes would pair within the same genome to form bivalents as toccurred in the tetraploids and to form quadrivalents as occurred in the octaploids. However, when a homologous partner is not available, the homoeologous chromosomes would then pair to form bivalents in those polyhaploids.

Introduction *Brassica napus* L. has been recognized as an allotetraploid which has derived its two genomes of chromosomes from *B. campestris* (2n=2X=20, AA) and *B. oleracea* (2n=2X=18, CC) (U 1935). The chromosomes of *B. napus* form 19 bivalents during first meiosis. Occasionally, one to two quadrivalents have been observed (Catcheside 1934, Broda et al 1979). However, no satisfactory theory has been provided to explain the origin of these quadrivalents. Attia and Robblen (1986) reported a higher degree of meiotic pairing (chiasmata/cell) in the AC amphiploid than in AB and BC amphiploids and suggested that the genomes A and C are more closely related to each other than to the B genome.

U (1935) observed mostly bivalents with occasional univalents in his synthetic *Brassica napus*. Sarashima (1964) noticed abnormalities such as quadrivalents and trivalents which persisted up to the fifth generation after synthesis. Analysis of meiosis of synthetic *Brassica napus* produced by somatic fusion also showed a wide variation in chromosome pairing with many univalents and multivalents (Schenck and Robblen 1982).

Various authors have suggested partial homology between chromosomes belonging to the A and C genomes (Mizushima 1952, Robblen 1966, Inomata 1980, Prakash and Hinata 1980). The present paper intends to ascertain the phylogenetic relationship between the A and C genomes and the origin of quadrivalent association in Brassica napus by studying dihaploids (2n=2X=19, AC) of spontaneous origin and plants derived through anther or microspore culture.

Materials and Methods Three regular plants of B. napus variety 'Regent' were used to supply anthers and microspores for tissue culture. Twenty five dihaploid plants were used in this study. Seven dihaploids were discovered in the rapeseed breeding field. Eighteen dihaploid plants were obtained through tissue culture. Stem cuttings were treated with a colchicine solution to double their chromosome number. Eleven plants were obtained with doubled chromosome numbers and one plant was a mixoploid with both 4X and 8X cells.

The techniques of Keller and Armstrong (1978, 1979) were used to culture anthers and microspores. Approximately 65% of the plants had their chromosome numbers doubled after the colchicine treatment. For cytogenetic investigation, flower buds were fixed in a Farmer's solution. The cetocarmine smear technique was used to prepare microslides for cytogenetic observation. For each meiotic interpretation, a minimum of 25 pollen mother cells (PMC's) were examined.

Pollen viability was tested by the staining reaction in a solution of I2-KI. Those that stained darkly and were round and full were considered as viable. Lightly stained and shrunken pollen grains were classified as inviable.

Results Meiotic behaviour in the three regular B. napus plants was identical to those reported in the literature. Among 71 PMC's observed, 69 cells or 97.18% of the total had a typical 19 bivalents at diakinesis and metaphase I. One cell had one quadrivalent and another cell had two quadrivalents. At anaphase I, among a total of 56 PMC's examined, over 95% of them had their dyad chromosomes separated equally and they migrated toward each of the opposite two poles. Only one cell each had lagging chromosomes and a chromatin bridge, respectively. Chromosomes also behaved normally at other meiotic stages and no micronucleus was detected at the quartet stage.

Spontaneously occurring dihaploid plants of B. napus (2n=2X=19) have been reported by Olsson and Hagberg (1955), Stringam and Downey (1973), and others. Morphologically, these plants are similar to a regular B. napus plant but their growth is usually stunted with narrower leaves. They are always highly sterile. Dihaploid B. napus plants have also been produced through anther or microspore culture in various laboratories including those of Stringam (1977) and Keller and Armstrong (1978, 1979).

Meiotic chromosome behaviour of spontaneously occurring and tissue culture derived dihaploids are very similar. Among 96 PMC's examined at diakinesis, over 50% of them had a chromosome association of $(19^{II} + 1^{I})$ and about 44% of the 104 cells observed at metaphase I had the same pairing pattern. Overall, of the 200 PMC's examined at diakinesis and metaphase I, 188 cells or 96% of the total had a minimum of five bivalents. The average chromosome association in the dihaploid plants was $7.73^{II} + 2.54^{I}$.

A total of 170 PMC's were counted at anaphase I. Almost 40% of the them had a (10-9) segregation. Another 37% of the cells had either lagging chromosomes or one to two chromatin bridges. Thirty nine cells (23%) had chromosomes clumped together and were not countable. But they had no laggards, bridges, or any other types of apparent meiotic irregularities.

Chromosome counts were made in 18 colchicine treated plants. Eleven plants had their chromosome numbers doubled to the tetraploid level (2n=4X=38) and one plant was a mixoploid with both tetraploid (2n=4X=38) and octaploid (2n=8X=76) cells. The overall doubling rate of this colchicine treatment was about 67%.

Chromosome behaviour of the artificially induced tetraploids is very similar to their regular B. napus counterpart. The chromosomes form 19 bivalents at diakinesis and metaphaseI with occasional quadrivalents. At anaphase I, regular (19-19) segregation was observed in most of the PMC's examined. No laggards or bridges were observed.

The tetraploid cells of the mixoploid plant behaved similarl to those in a regular tetraploid plant. The ratio between tetraploid and octaploid cells is about 4:1.

Only 25 cells, 21 at diakinesis and 4 at metaphase I, respectively, had their chromosomes separated well enough that reliable cytological interpretation could be made. Three cells had 38 bivalents which were all at late metaphase I when separation had already started in some of the chromosomes. The one cell examined at earlier metaphase I and all 21 cells at diakinesis had a minimum of four quadrivalents. The highest number of quadrivalents reached 16 and the average chromosome association was $6.88^{IV} + 24.3^{II}$. No trivalent or univalent was observed.

Pollen viability, as expressed by staining reaction in an I2-KI solution, of all plants is listed in Table 7. Pollen stainability of the three regular B. napus approached 100%. The dihaploids had only 6.8% stainable pollen. The colchicine doubled tetraploids had 72% stainable pollen. The mixoploid plant had 17% stainable pollen. Among those stainable pollen grains, two size classes were quite obvious and the ratio was similar to the ratio between tetraploid and octaploid cells. Therefore, the large and small pollen grains were considered as products of tetraploid and octaploid PMC's respectively.

The pollen stainability in all plants reflected their meiotic chromosome behaviour. The colchicine doubled tetraploid plants had lower pollen stainability than a regular B. napus plant probably because of genetic imbalance in a newly doubled amphiploid. Similar situation is true in colchicine treated materials in many other plant species. The mixoploid plant was expected to have lower stainability because irregular chromosome segregation could occur due to multivalent chromosome association at metaphase I.

Discussion U (1935) observed as many as eight bivalents in a hybrid between B. campestris and B. oleracea and suggested that the A genome of B. campestris and the C genome of B. oleracea are closely related. Mizushima (1950) suggested that the A genome chromosomes could form two "autosyndedic" pairs and the C genome chromosomes could form one "autosyndedic" pair. He suggested that at least five of the eight bivalents observed by U were "allosyndedic" and also concluded that A and C are closely related genomes. Attia and Robbelen (1986) reached the same conclusion by using information on chromosome pairing and chiasma frequencies in amphihaploid hybrids.

Rieger et al (1976), based on an original paper by G. L. Stebbins (1947), defined 'autosyndesis' as "the pairing of complete or partial homologous contained in the same gametes at fertilization". Therefore, what appeared in rapeseed literature in the past as "autosyndesis", pairing of chromosomes within A or C genomes, should be called homologous pairing or intra-genomic pairing. And "allosyndesis", pairing of chromosomes between A and C genomes, should be termed homoeologous pairing or inter-genomic pairing.

Olsson and Hagberg (1955) counted five to eight bivalents, may be $8^{II} + 3^{I}$, in their spontaneously occurring dihaploid B. napus. In the photomicrographs of stem cultured plants of B. napus haploids published by Stringam (1977), several loosely paired bivalents could be recognized. Mostly bivalents could be identified in the doubled material.

Results of the present study are clearly in agreement with the data published in the literature that a high number of bivalents are present in plants with one A and one C genomes of chromosomes. One possible explanation for the lack of quadrivalent association in tetraploid plants, regular B. napus plants or tetraploid plants obtained through doubling of dihaploids, is that the homoeologous (inter-genomic) pairing is genetically suppressed. Suppression of homoeologous pairing in other species of the genus Brassica has been reported by Prakash (1974), Harberd (1976) and Harberd and McArthur (1980). Similar suppression is well know in many other plant species (Devorak 1983).

Apparently, genic suppression is removed in the newly synthesized hybrid between B. campestris and B. oleracea and in the spontaneously occurring and anther culture derived dihaploids

in which each genome is only present once (2n=2X=19, AC) and mostly bivalents are observed. When the the chromosome number of dihaploids is doubled, a high number of quadrivalents should be present in the tetraploids (2n=4X=AACC). On the other hand, if the presence of quadrivalents in a regular B. napus plant is caused by tetrasomy as suggested by Mizushima (1950), there should be a similar number of octavalents present in the octaploid cells. Neither has been found to be true in the present study

A similar situation is reported in a tetraploid barley Hordeum jubatum by Starks and Tai (1974). Hordeum jubatum is a tetraploid with two homoeologous genomes. The homoeologous chromosomes would pair only when there is no competition from a more closely related homologous partner

In a tetraploid B. napus, spontaneous or induced, each chromosome of either A or C genome has a homologous partner and they pair to form bivalents. When the chromosome number is reduced to the dihaploid level, inter-genomic pairing prevails between homoeologous chromosomes of the A and C genomes. The presence of quadrivalents in a regular B. napus plant can be attributed to occasional secondary pairing between homoeologous bivalents. Secondary association in the genus Brassica has been reported by Prakash and Hinata (1980) and in this laboratory by Tang (1985).

Literature Cited

Attia, T., and G. Robbelen. 1986. Cytogenetic relationship within cultivated Brassica analyzed in amphihaploids from the three diploid ancestors. Can. J. Genet. Cytol. 28: 323-329.

Broda, I. A., P. Rouselle, and M. Renard. 1979. Investigation of two kinds of cytoplasmic male sterility in rapeseed (Brassica napus L.). Genet. Polonica. 2: 487-497.

Catcheside, D. G. 1934. The chromosomal relationships in the swede and turnip groups of Brassica. Ann. Bot. 48: 601-633.

Dvorák, J. 1983. Evidence for genetic suppression of heterogenetic chromosome pairing in polyploid species of Solanum, sect. Petota. Can. J. Genet. Cytol. 25: 530-539.

Harberd, D. J. 1976. Cytotaxonomic studies of Brassica and related genera. In: The Biology and Chemistry of the Cruciferae. (Ed. J. G. Vaughan, A. J. MacLeod, and B. M. Jones). AP. London, N.Y., San Francisco.

Harberd, D. J., and E. D. McArthur. 1980. Meiotic analysis of some species and genus hybrids in the Brassiceae. In: Brassica Crops and Wild Allies. Biology and Breeding. (Ed. Tsunoda, S., Hinata, K., and Gomez-Campo, C.). Japan Sci. So. Press, Tokyo.

Inomata, N. 1980. Hybrid progenies of the cross Brassica campestris x B. oleracea. I. Cytogenetical studies on F1-hybrids. Japan J. Genet. 55: 189-202.

Keller, W. A., and K. C. Armstrong. 1978. High frequency production of microspore-derived plants from Brassica napus anther culture. Z. Pflanzenzuchtg 80: 100-108.

Keller, W. A. and K. C. Armstrong. 1979. Stimulation of embryogenesis and haploid production in Brassica campestris anther culture by elevated temperature treatment. Theor. Appl. Genet. 55: 65-67

Mizushima, U. 1950. Karyogenetic studies of species and genus hybrids in the tribe Brassiceae of Cruciferae. Tohoku J. Agric. Res. 1: 1-14.

Muzushima, U. 1952. Karyo-genetical studies on Brassiceae. Gihodo, Tokyo.

Olsson, G. 1960. Species crosses within the genus Brassica II. Artificial Brassica napus L. Hereditas 46: 351-396.

Olsson, G. and Hagberg, A. 1955. Investigations on haploid rape. Hereditas 41: 223-237.

Prakash, S. 1974. Probable basis of diploidization of Brassica juncea Coss. Can. J. Genet. Cytol. 16. 232-234.

Prakash, S., and K. Hinata. 1980. Taxonomy, cytogenetics and origin of crop Brassicas, a review Opera Botanica 55. Lund Bot. Soc., Borgstroms, Motala.

Rieger, R., A. Michaelis, and M. M. Green. 1976. Glossary of Genetics and Cytogenetics. Springer-Verlag, Berlin, Heidelberg, N.Y.

Röbbelen, G. 1966. Beobachtungen bei interspezifischen Brassica-Kreuzungen insbesondere über die Entstehung matromorpher. Pflanzen. Angewandte Botanik 39: 205-221.

Sarashima, M., 1964, Studies on the Breeding of artificially synthesized rape (Brassica napus) I F1 hybrids between Brassica campetris group and Brassica oleracea group and the derived F2 plants. Japan J. Breed. 14: 226-236.

Schenck, H. R. and G. Röbblen. 1982. Callus and root regeneration from mesophyll protoplasts of basic Brassica species: Brassica campestris, Brassica oleracea, Brassica nigra. Z. Pflanzenzuchtg. 89: 278-288.

Stringam, G. R. 1977. Regeneration in stem explants of haploid rapeseed (Brassica napus, L.). Plant Sci. Lett. 9: 115-119.

Stringam, G. R., and R. K. Downey. 1973. Haploid frequencies in Brassica napus. Can. J. Plant Sci. 53: 229-231.

Tang, Hoang van. 1985. Tissue culture studies of Brassica napus L. and Brassica campestris L. and a cytogenetic investigation of tissue culture regenerated plants of Brassica napus L. M.S. Thesis. University of Manitoba, Winnipeg, Manitoba, Canada

U, N. 1935. Genomic analysis in Brassica with special reference to the experimental formation of Brassica napus and peculiar mode of fertilization. Japan J. Bot. 7: 389-452.