ANALYSIS OF MEIOTIC PAIRING AND OFFSPRING OF INTERSPECIFIC BRASSICA HYBRIDS CONTAINING THREE GENOMES

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SUMMARY

By crossing digenomic and monogenomic Brassica species 5 different trigenomic hybrids were synthesized all containing 27 chromosomes of the A, B and C genomes. Depending on the mother type the trigenomic amphihaploids contained different cytoplasms. Backcrosses with B.napus resulted in alloplasmatic and aneuploid B.napus progenies. Whereever resitution nuclei with the haploid genome occurred seeds did develop. Offspring with the B.napus genome and additional B.nigra chromosomes was examined. Meiotic pairing was analyzed in metaphase I. Results were evaluated in respect to the evolutionary relations between the Brassica genomes as well as towards an identification of single chromosome addition lines.

INTRODUCTION

The close relationship between the cultivated Brassica species allows wide interspecific hybridizations. In order to obtain trigenomic amphihaploids combining three genomes A, B and C, Ton BOSCHLOO in 1983 in our Institute crossed the digenomic Brassica species with that monogenomic ones not existent in the digenomic parent (Fig. 1). All trigenomic hybrids contained 27 chromosomes. But the same genome formula ABC was derived from three different sources; e.g. the A genome originated from B.napus, B.juncea and B.campestris, respectively.

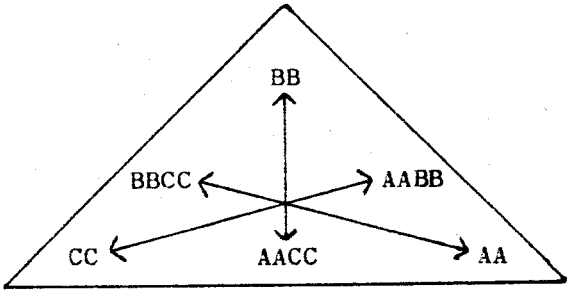


Fig. 1: Crossing scheme to produce trigenomic amphihaploids in Brassica

Reciprocal crosses were also successful. Thus the trigenomic amphihaploids consisted of 6 different cytoplasms depending on mother type:

BB	x	AACC	B.AC
AACC	x	BB	AC.B
AA	x	BBCC	A.BC
BBCC	x	AA	BC.A
CC	x	AABB	C.AB
AABB	x	CC	AB.C

Meiotic pairing in these hybrids was analyzed by ATTIA and RÖBBELEN (1986) and BUSSO et al. (1987). Comparisons with digenomic haploids led to the conclusion that a high rate of homeologous pairing occurred between the A and the C genomes, while the B genome was only involved in intergenomic pairs to a very low degree. There were no cytoplasmic effects on the regulation of pairing.

Five combinations, except AB.C, produced seeds by open pollination with B.napus. The offspring of these backcrosses was examined for chromosome number and metaphase pairing.

MATERIALS and METHODS

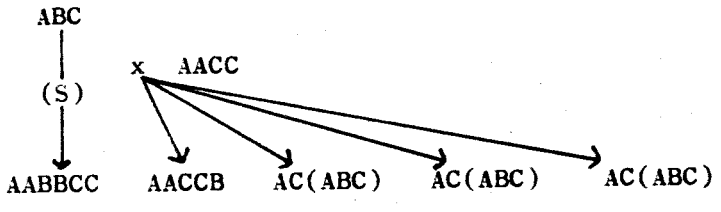
The parents of the interspecific hybrids were taken from the Brassica collection of the Institute in Göttingen. By in vitro embryo culture a sufficiently large number of hybrids was raised. Later, plants were transferred to soil, but since 1983 some are still left in sterile culture at 10°C and 8 h illumination. For the following generations B.napus varieties served as pollinators. The first backcross resulted from open pollination, the second from crossings with the Canadian summer rape 'Andor'.

For the determination of chromosome numbers the main problem was to get actively growing root tips with enough cell divisions. Root tips were taken 3 to 5 days after transplanting from the initial 7x7 cm to 9x9 cm pots in the greenhouse. Young root tips were harvested and placed in cold water (3-5°C) for 24 h. Afterwards they were fixed in acetic acid, hydrolyzed with 1 N HCl for 9 min , stained with leucobasic fuchsin (Feulgen) and squashed in a drop of acetocarmine after short heating.

For meiotic analyses, plants with 4-6 leaves were transferred into a growth room with 5°C, 70% humidity and 16 h illumination. Before the fixation of appropriate buds in Carnoy solution (4 parts dichloromethane, 3 alcohol, 1 acetic acid) the plants were brought for 4-5 h into sunlight at 18°C. For crossings the plants were carried back into the greenhouse (16 h illumination, 15°C - 25°C). Anthers were squashed in acetocarmine and pairing configurations were recorded in pollen mother cells (PMCs) with the help of a phase-contrast microscope; fotos were taken with a connected camera.

RESULTS and DISCUSSION

The examined progenies of the trigenomic amphihaploids had between 40 and 54 chromosomes (Fig. 2).



Chromosomes	54	46	44	42	40
No of plants	4	22	5	1	1

Fig. 2: Examined offspring of trigenomic amphihaploids from selfing (S) and backcrossing with B.napus

In all trigenomic haploids meiotic pairing (BUSSO et al. 1987) had shown an average of 6-9 bivalents irrespective of the origin of the genomes. Accordingly gametes of around 14 chromosomes might have been expected from the 6 to 9 bivalents and additional univalents and multivalents, giving around 33 chromosomes after fusion with a B.napus gamete. But none of the investigated plants had less then 40 chromosomes. This indicates that obviously seeds only developed if unreduced (or nearly unreduced) gametes occurred (Fig. 3). Plants with 54 chromosomes indicated that restitution might be possible from both pollen and egg cells, although hexaploid trigenomics (AABBCC) could also result from later mitotic defects. These plants had irregular meiosis and no seeds developed. HEYN (1977) also reported unreduced gametes. Hence, these are obviously of rather common occurrence in interspecific Brassica crosses.

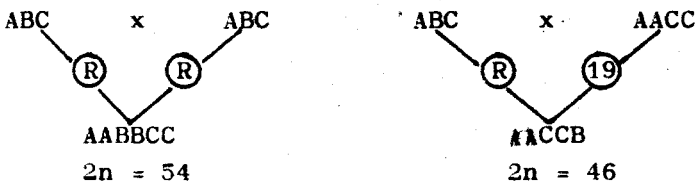


Fig. 3: Restitution nuclei (R) forming eu-triploid gametes with 27 chromosomes in trigenomic Brassica haploids as revealed by chromosome numbers after selfing (2n=54; left) and pollination with B.napus (2n=46; right)

Meiotic analysis in metaphase I of PMCs from plants with 46 chromosomes exhibited an average of 19 bivalents and 8 univalents (Tab. 1), fitting in the assumption of full genome AACCB pentaploids, the genome B yielding the 8 univalents. BUSSO et al. (1987) discussed the possibility of a pairing suppressor effect conditioned by the B genome. But they discovered no influence of the B genome on the pairing of A and C even in the trigenomic amphihaploids. In the AACCB pentaploids the A and C chromosomes find their true homologues for regular pairing. There the B genome contributes to pairing only occassionally with 1 or rarely 2 bivalents. Quadrivalents always occur together with 8 or more univalents, indicating that the B genome is not involved.

Table 1: Metaphase I of AACCB plants with 46 chromosomes

Trigenomic ♀	No cells	Min. I	Max. I	mostly	$\bar{x}$		
		I - II	I - II	I - II	I	II	IV
B.AC	24	4 - 21	10 - 18	8 - 19	7.6	19	0.1
AC.B	10	6 - 20	10 - 18	8 - 19	7.7	19.15	-
A.BC	29	6 - 20	10 - 18	8 - 19	7.6	19.2	-
BC.A	75	4 - 21	12 - 17	8 - 19	8,5	18.67	0.04
C.AB	59	6 - 20	10 - 18	8 - 19	7.66	19.05	0.07

I = univalent II = bivalent IV = quadrivalent

The relatively regular chromosome pairing in the pentaploids made backcrossings with B.napus possible to receive rape addition lines with B.nigra chromosomes (Fig. 4). In the first backcross progeny plants have 37 to 44 chromosomes (mainly 39 - 44). First selections resulted in 22 plants with 39 chromosomes which have to be established to be monosomic addition lines (AACC + 1B). After selfing, the identification of 8 different disomic addition lines (AACC + 2B) will be tried.

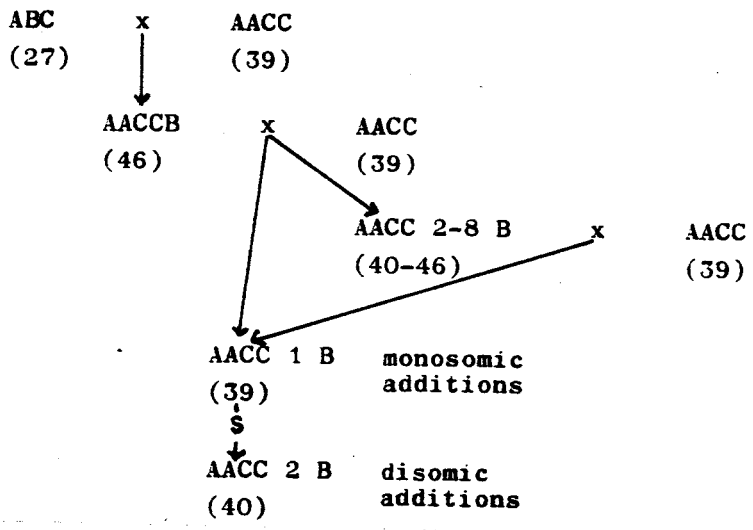


Fig. 4: Crossing scheme for the establishment of addition lines of B.napus with B.nigra chromosomes starting from trigenomic haploids. In brackets = chromosome numbers

B.nigra addition lines of B.napus are expected to contribute to the genetic analysis of many useful traits, especially disease or drought resistance of the plant and quality characteristics of the seeds. They will also facilitate their transfer into rapeseed cultivars.

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