Theor Appl Genet (1987) 74:758–766

Analysis of the *Brassica oleracea* genome by the generation of *B. campestris-oleracea* chromosome addition lines: characterization by isozymes and rDNA genes

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Received April 27, 1987; Accepted June 16, 1987
Communicated by C. S. Khush

Summary. This study aimed at generating chromosome addition lines and disclosing genome specific markers in *Brassica.* These stocks will be used to study genome evolution in *Brassica oleracea* L., *B. campestris* L. and the derived amphidiploid species *B. napus* L. *B. campestris-oleracea* monosomic and disomic chromosome addition plants were generated by crossing and backcrossing the natural amphidiploid *B. napus* to the diploid parental species *B. campestris.* The pollen viability of the derived sesquidiploid and hyperploid ranged from 63% to 88%, while the monosomic and disomic addition plants had an average pollen fertility of 94% and 91%, respectively. The addition lines were genetically characterized by genome specific markers. The isozymes for 6PGD, LAP, PGI and PGM, and rDNA *Eco* RI restriction fragments were found to possess the desired genome specificity. Duplicated loci for several of these markers were observed in *B. campestris* and *B. oleracea,* supporting the hypothesis that these diploid species are actually secondary polyploids. A total of eight monosomic and eight disomic addition plants were identified and characterized on the basis of these markers. Another 51 plants remained uncharacterized due to the lack of additional markers. rDNA genes were found to be distributed in more than one chromosome, differing in its restriction sites. Intergenomic recombination for some of the markers was detected at frequencies between 6% and 20%, revealing the feasibility of intergenomic gene transfer.

Key words: *Brassica* – Cole crops – Cytogenetics – Genome – Markers

Introduction

The genus *Brassica* has been the subject of numerous cytogenetic studies (Prakash and Hinata 1980). The large number of diploid species with genomic numbers ranging from x = 7 to x = 12, and derived amphidiploids in the genus, makes it an attractive research subject (Mizushima 1980). Most of the cytogenic research in *Brassica* has centered around the elucidation of the origin of the cultivated amphidiploids, *B. napus* L., *B. carinata* A. Br. and *B. juncea* L. by Karpechenko (1922), Morinaga (1934), and U (1935). This early work was based on the synthesis of amphidiploids by hybridization of the three basic diploid cultivated species *B. nigra* L. (x = 8, genome b), *B. oleracea* L. (x = 9, genome c) and *B. campestris* L. (x = 10, genome a). A result of this research was the postulated triangle of U, which is a diagrammatic representation of diploid and amphidiploid species relationship (U 1935). More recently research by Robbins and Vaughan (1983) on Rubisco and by Palmer et al. (1983) and Erickson et al. (1983) on chloroplast DNA not only confirmed the validity of the triangle of U, but also resolved the direction of the crosses leading to two of the amphidiploids.

Very little is known about the evolution of the diploid species and the origin of the different genomic numbers. On the basis of chromosome pairing in haploids (Keller and Armstrong 1983), di-genomic and tri-genomic hybrids (Mizushima 1980; Prakash and Hinata 1980; Catcheside 1934, 1937; Sikka 1940), and pachytene karyotypes (Robbelen 1960), it is assumed that the basic genome of *Brassica* is x = 6. Since no species with this genomic number has ever been reported, they are presumed to be extinct. Robbelen (1960) found six basic types of chromosomes, designating them with the letters A to F, on the basis of heterochromatic knobs and centromere position. He proposed that the composition of each genome in the cultivated diploids was genome

a = AABCDDEFFF, b = ABCDDEEF and c = ABBCCDEEF.

Two chromosomes in each genome, namely A and C in *B. nigra* and *B. oleracea,* and A in *B. campestris,* were found to be involved in the organization of the nucleolus. Thus, the diploid species are considered to be secondary polyploids, since presumably they have some of the basic chromosome types forming part of their genome in duplicate or even in triplicate. The fact that chromosomes of the same type within or between species are not identical, indicates that rearrangements have taken place during the evolution of the diploid species.

In order to test these hypotheses based mostly on cytological observations, cytogenetic stocks useful to dissect each genome and genome specific chromosome markers are required. This approach has been very useful in *Triticum* and related genera (Hart and Tuleen 1983), and *Allium* (Peffley et al. 1985). Genome specific isozyme loci in the cultivated *Brassica* species have already been described (Coulthart and Denford 1982; Arus and Orton 1983; Arus 1984; Quiros et al. 1985).

In this study, we report the use of isozyme markers and rDNA genes in the characterization of *B. campestris-oleracea* monosomic and disomic addition lines obtained by crossing and backcrossing *B. napus* to *B. campestris.* These lines will permit studies of genome organization and evolution in *Brassica.*

Materials and methods

Plant material

The following accessions were used: *B. napus,* rapid cycling CrGC-05 and rapid cycling cytoplasmic male sterile CrGC-14; *B. campestris,* rapid cycling CrGC-01 and rapid cycling cytoplasmic male sterile CrGC-13; rapeseed cv 'Torch'; Chinese cabbage cv 'Kwan-Hoo Choi'. The rapid cycling lines were obtained from Dr. Paul Williams at the University of Wisconsin, USA (Williams and Hill 1986). Also, the turnip cv 'White Lady' was used to study the inheritance of the triose phosphate isomerase isozymes.

Development of addition lines

The amphidiploid species *B. napus* was crossed either as pistillate or pollen parent to the diploid *B. campestris.* Embryos were rescued from developing ovules about 15 days after pollination and cultured in sterile medium (Nitsch and Nitsch 1969). The resulting sesquidiploid hybrids were backcrossed to *B. campestris.* Resulting progeny with more than 2n = 21 chromosomes were backcrossed one or two more times to *B. campestris.* Monosomic addition lines, 2n = 21, were selfed to obtain disomic addition lines (2n = 22). Among all the crosses a total of about 350 plants were generated. The majority of these were studied cytologically and electrophoretically.

Chromosome counts

Flower buds were fixed in propionic acid : absolute ethanol (1 : 3) with ferric chloride added as a mordant (Swaminathan et al. 1954). After 24 h the buds were rinsed and stored in 70% ethanol. Anthers were dissected and squashed in a drop of 1% acetocarmine. For chromosome counts, 10 to 30 cells were examined. Pollen viability was determined on the basis of pollen stainability in 1% acetocarmine. A minimum of 100 pollen grains was used for this determination.

Isozyme markers

Horizontal starch gel electrophoresis was used to separate the enzymes obtained from a crude extract of young leaves and pollen leachates (Weeden and Gottlieb 1980). Details of this technique have been previously described (Quiros and McHale 1985). The following enzymes were assayed: 6phosphogluconase dehydrogenase (6PGD), phosphoglucoisomerase (PGI), leucine aminopeptidase (LAP), and phosphoglucomutase (PGM). The inheritance for the enzyme systems LAP, PGI and PGM have been reported in *B. oleracea* by Arus and Orton (1983). Although we did not carry out a formal genetic analysis in *B. campestris,* the similarity between the zymograms of both species indicate that they have equivalent loci coding for these enzymes.

rDNA genes

Total genomic DNA was isolated from leaves of individual plants according to the protocol of Saghai-Maroof et al. (1984) with the following modifications: the leaf tissue was homogenized with dry ice in a coffee mill (Moulinex/Regal) (Landry and Michelmore 1985). After extraction, the DNA was digested for 8 h with the endonuclease *Eco* RI according to the manufacturer (BRL). The DNA fragments were separated by horizontal agarose electrophoresis and transfered by Southern blotting to Zeta-Probe membranes (Maniatis et al. 1982). Cloned DNA from wheat rDNA, probes pTA71 (Saghai- Maroof et al. 1984) and pTA250-2 (Appels and Dvorak 1982) was nick translated using standard techniques (Maniatis et al. 1982) and hybridized to the membranes. DNA fragments were sized using lambda DNA as a reference. Radish (*Raphanus sativus* L.) DNA was used as a control because the rDNA genes of this close relative of *Brassica* are well characterized (Delseny et al. 1983).

Results

Sesquidiploid hybrids

The cross *B. napus* × *B. campestris* and its reciprocal resulted in seeds devoid of endosperm. A total of seven hybrids were obtained after rescuing and culturing embryos contained in these seeds. Four of the hybrids were male-sterile due to the use of the cytoplasmic male-sterile stocks as pistillate parents. The absence of fertility restorers in the pollen parents made impractical the use of these hybrids in the development of the addition lines. Therefore, our efforts were concentrated in the partially fertile hybrid, 85B138, with 65% pollen viability (Table 1). A second partially fertile hybrid, 85B137, derived

Table 1. Chromosome numbers and pollen viability of *Brassica napus* × *B. campestris* hybrids

Plant no.	2n	% Pollen viability
85B69-1	30	15
85B66-2	29	cms[b]
85B76-1	62	cms
85B146-1	29	cms
85B137-1	29	67
85B138-1	29	65
85B65-1[a]	29	cms

[a] Reciprocal cross
[b] Cytoplasmic male sterile

Table 2. Frequency of chromosome numbers and average pollen viability in plants derived from crossing various hyperploid plants to *B. campestris*

2n	29×20	26×20	24×20	23×20	22×20	21×20	% Pollen viability
20	0	2	1	3	3	8	96.3
21	1	6	3	7	10	6	94.2
22	3	3	6	2	3	5	91.2
23	2	0	5	2	0	0	87.6
24	2	2	7	0	0	0	85.0
25	1	1	0	0	0	0	83.1
26	3	0	1	0	0	0	83.3
Other[a]	2 (30)	1 (40)	1 (34)	1 (19)	0	1 (30)	

[a] Chromosome numbers in parentheses

from the same cross, had the same level of pollen viability but it died shortly after flowering.

Five of the seven hybrids had the expected chromosome number of 2n = 29, whereas hybrids 85B69-1 and 85B76-1 had 2n = 30 and 2n = 62 chromosomes, respectively. The last two perhaps derived by aneuploid gametes, followed by chromosome doubling for 85B76-1. In general, the resulting hybrids morphologically resembled the *B. campestris* parent, characterized by slender leaves and spindly stems.

Hyperploid derivatives

After back crossing the sesquidiploid hybrid 85B138 to *B. campestris* (2n = 29 × 2n = 20) 12 plants were obtained. Two different strains of the latter were used, the rapid cycling accession CRGC-01 and the rapeseed cv 'Torch'. The hybrid was used as the pistillate parent since the reciprocal cross resulted in poor seed set. The seeds developed normally, with a full endosperm, making unnecessary the use of embryo culture. The plants derived from the rapid cycling *B. campestris* strain flowered quite early and ceased development shortly after flowering. In view of this problem, we started using 'Torch' as an alternative pollinator in some of the crosses.

The pollen viability of the 14 plants derived from the 2n = 29 × 2n = 20 crosses was higher than in the sesquidiploid; it ranged from 64% to 96%, with an average of 81.1%. The average chromosome number of the hyperploid plants was 23.7, ranging from 2n = 26 to 2n = 21. As the number of chromosomes increased, the pollen viability in these plants decreased. Thus, from the first backcross to the diploid parent it was possible to obtain at least one monosomic addition line (Table 2). In general, these plants very much resembled their *B. campestris* parent.

The plants resulting from the 29×20 progeny were crossed to *B. campestris*, Chinese cabbage cv 'Kwan Hoo Choi' in order to maximize leaf tissue and flower bud production for the biochemical and cytological determinations. From these crosses, a number of plants with 2n = 21 were obtained. The average pollen viability of these was 94.2%. The frequency of chromosome numbers in the pooled progenies of these plants is shown in Table 3. Upon selfing, they yielded 2n = 21 (25%) and 2n = 22 plants (19%), presumably monosomic and disomic addition lines, respectively (Fig. 1a, b). The rest were diploids. The *B. oleracea* extra chromosomes could not be distinguished from the *B. campestris* chromosomes by the acetocarmine technique. All the 2n = 22 plants derived either from 2n = 21 plants or from higher hyperploids were tentatively classified as either double trisomics or as disomic addition lines on the basis of chromosome pairing during diakinesis and metaphase I. The putative disomic lines displayed 11II in 60% to 80% of the cells, while the double trisomics displayed 10II + 2I for most of the cells. Upon crossing the 2n = 21 plants to *B. campestris* (2n = 20), about 30% of the plants in the resulting progenies had extra chromosomes. Selfing of double trisomic plants (2n = 22) or crossing them to diploid *B. campestris* plants yielded around 80% of plants with extra chromosomes. Selfing of the latter, however, resulted in twice as many plants with 2n = 22 chromosomes than selfing 2n = 21 plants (Table 3). Only the progeny of a single disomic addition line for 6PGD-2 was investigated. Upon selfing or crossing to a diploid *B. campestris* plant, it yielded about

Table 3. Chromosome numbers in pooled progenies of 2n = 21 and 2n = 22 *B. campestris-oleracea* addition lines

Progeny	*n*[a]	Frequency of chromosome nos. (%)			
		20	21	22	Other
2*n* = 21 × 2*n* = 20	4	19 (66)	6 (21)	3 (10)	1 (3)
2*n* = 21⊗	4	8 (50)	4 (25)	3 (19)	1 (6)
2*n* = 22 × 2*n* = 20	4	6 (20)	13 (65)	4 (15)	0 (0)
2*n* = 22⊗	3	9 (26)	3 (37)	4 (37)	0 (0)

[a] No. of families

50% and 30% of plants with extra chromosomes, respectively. About 50% of these had 2n = 22 chromosomes.

Genome specific markers

The four enzyme systems listed in the "Materials and methods" section were satisfactory for identifying the extra *B. oleracea* chromosomes present in the hyperploid plants. Other systems were tried, but the overlapping or the complexity of the zymograms precluded their use as reliable markers. The best diagnostic enzyme system was 6PGD, since it was found to be monomorphic for a number of accessions of *B. oleracea, B. campestris* and *B. napus* (Fig. 2a). This monomorphism has meant that formal genetic tests have not been possible for the determination of the number of loci involved in the synthesis of these isozymes. The zymograms of the diploids showed clearly two activity zones, the more anodal one, named 6PGD-1, is composed of three equidistant bands, of which the most anodal band is the only one shared by both species (Fig. 2a, b). For the more cathodal zone, named 6PGD-2, most *B. oleracea* accessions display a three banded phenotype, while *B. campestris* has only one band overlapping with the *B. oleracea* band of slowest migration. Only the 6PGD-2 isozymes persisted in pollen leachates in both diploids, indicating cytosolic location. Furthermore the three banded pattern observed in the leaves of *B. oleracea* was also retained in pollen leachates revealing duplicated loci for 6PGD-2 (Weeden and Gottlieb 1980). Conversely, the 6PGD-1 isozymes did not persist in pollen leachates indicating plastid location. The isozymes from both diploid species were accounted for in *B. napus,* confirming the hybrid nature of this amphidiploid species. The multiple banded phenotypes for 6PGD-1 and 6PGD-2 bred true in selfed or sib progenies for each of the three species confirming the existence of duplicated loci.

Duplicated loci were also observed for the genes coding for the enzyme triose phosphate isomerase (*Tpi-2* and *Tpi-2'*). In the two diploid cultivated species, *B. oleracea* and *B. campestris,* non segregating multiple banded phenotypes were observed in leaves and in pollen leachates, thus indicating cytosolic location. On the other hand, the isozymes of the more anodal zone, TPI-1 were located in plastids, following the criteria of Weeden and Gottlieb (1980). *B. campestris* plants of the turnip cv 'White Lady' heterozygous for one of the duplicated loci permitted the observation of intergenic heterodimers forming between the monomorphic locus and the two alleles of its polymorphic duplicate locus (Fig. 2c). A sib cross between two TPI-$2'^1$/TPI-$2'^2$ heterozygotes segregated in the expected 1:2:1 ratio, 10($2'^1$):20($2'^1/2'^2$):9($2'^2$), $X^2 = 0.03$, $P = 0.99$. For PGM and LAP, isozymes migrating closer to the cathode and common in *B. oleracea* were diagnostic for extra *B. oleracea* chromosomes (Fig. 3c).

rDNA genes were found to be extremely useful for the characterization of the hyperploid lines. Each diploid species has a typical restriction pattern with *Eco* RI, although a few fragments in common were also observed. The restriction pattern of *B. napus* had all the fragments found in both diploid species (Fig. 3a). Thus, the 6PGD and the rDNA systems were very reliable for confirming the hybrid origin of *B. napus.* The restriction pattern of the radish used as control was a combination of those of *B. oleracea* and *B. campestris* suggesting a close relationship between the two genera. In *B. oleracea,* the following fragments were observed: 6.0, 3.4, 1.8, 1.6, 1.4 and 0.8 Kb, while *B. campestris* displayed 5.3, 4.1, 2.6, 1.6 and 1.4 Kb fragments. Radish had a pattern similar to *B. oleracea,* except for a 6.0 Kb fragment which is replaced by the two 5.3 and 4.1 Kb fragments of *B. campestris.* All these fragments lighted

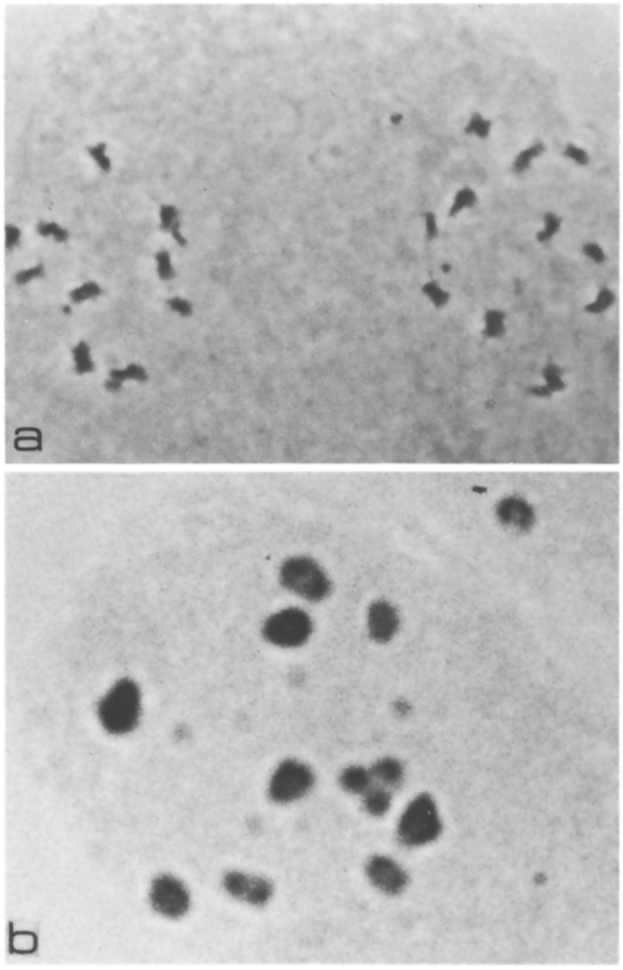

Fig. 1a, b. Pollen mother cells. **a** Metaphase II for monosomic addition line (2n = 21); 10 chromosomes at the left and 11 at the right pole. **b** Metaphase I for a monosomic addition line (2n = 21), showing a trivalent association

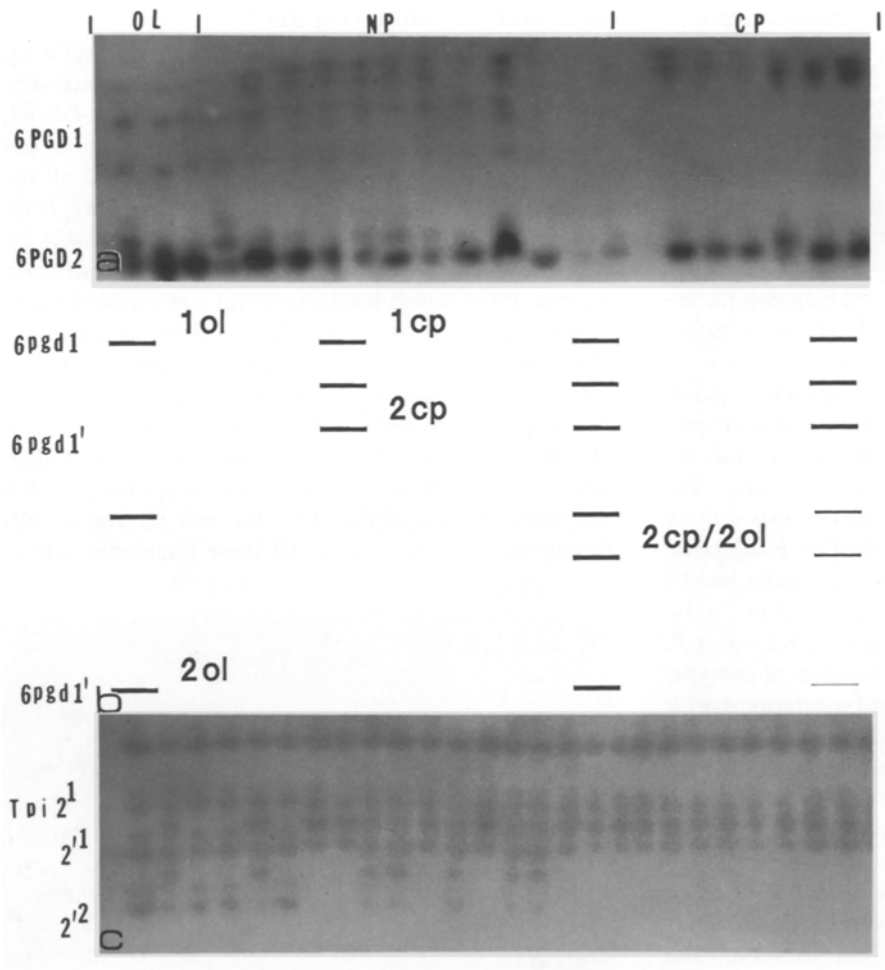

Fig. 2. a 6PGD zymograms for *Brassica oleracea, B. campestris* and *B. napus. B. oleracea* and *B. campestris* specific isozymes for 6PGD-1 and 6PGD-2 are present in the hybrid species *B. napus.* **b** Interpretative diagram for 6PGD-1. *6pgd-1* and *6pgd-1'* are duplicated loci. Isozymes *1o1* (*B. oleracea*) and *1cp* (*B. campestris*) at *6pgd-1* have the same migrations. Isozymes *2o1* and *2cp* at *6pgd-1'* form heterodimers with the *6pgd-1* isozymes. The amphidiploid *B. napus* (*second line* from *right*) and *B. campestris-oleracea* addition lines (*far right*) display an additional heterodimer formed by the *2cp* and *2o1* polypeptides. The intensity of *2o1* depends on the number of extra 6PGD. *B. oleracea* chromosomes. **c** Duplicated loci for the enzyme TPI, *Tpi-2* monomorphic for the isozyme 1 and *Tpi-2'* polymorphic for the allozymes 1 and 2. *First 16 lines from left* show segregating progeny (1:2:1) for *Tpi-2'* allozymes 1 and 2. Heterozygous individuals for *Tpi-2'* (for example, *line 2*) form two heterodimers, one between the *Tpi-2* isozyme and the *Tpi-2'* allozyme 1 and another between the allozymes 1 and 2 of *Tpi-2'*. Homozygous individuals for *Tpi-2'* for a single heterodimer with isozyme 1 of *Tpi-2.* (i.e. *line 1, TPI-2'*^{2}, *line 7, TPI-2'*^{1}). Lines 17 to the end show the progeny of a double homozygous plant of phenotype *TPI-2*^{1} and *TPI-2'*^{1} breeding true. The band at the middle is the interlocus heterodimer

Table 4. Distribution of *B. oleracea*-specific alleles in *B. campestris-oleracea* hyperploid plants and transmission to their progenies

Plant no.	2n	PGI-1	6PGD-1	6PGD-2	LAP-1	PGM-2	RA	RB
85B224-1	24	*			*			
86B25-3	21				*			
86B25-8	–	*			*			
86B29-2	22				*			
86B29-6	–	*						
85B224-3	22	*						
86B44-1	21	*						
85B268-1	24	*	*	*	*	*	*	
86B55-3	22				*			
86B56-2	22		*		*			
85B268-2	22	*		*				
86B50-1	21			*				
86B170-3	21			*				
86B170-6	22			*				
86B50-2	22			*			*	
86B50-3	21			*			*	
86B50-7	22			*			*	
86B183-6	22			*				
85B268-4	26		*				*	*
86B155-1	21		*					
85B268-7	26	*		*	*	*		
86B109-2	22					*		

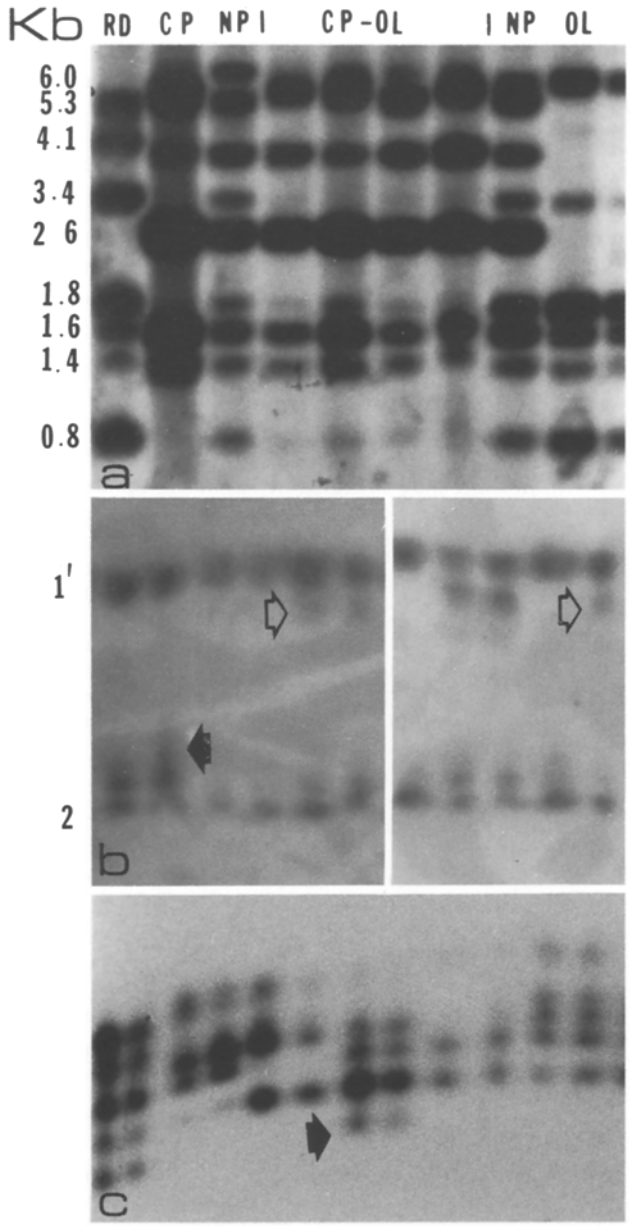

Fig. 3a–c. Characterization of the *B. campestris-oleracea* addition lines by chromosome markers. **a** rDNA *Eco*RI fragments for radish (rd), *B. campestris* (*cp*), *B. napus* (*np*), four *B. campestris-oleracea* hyperploid plants (2n=23, 2n=24, 2n=26, 2n=25) and *B. oleracea*. Fragments of *B. oleracea* and *B. campestris* add up in the amphidiploid *B. napus*. Hyperploid plants lack the *B. oleracea* 3.4 Kb fragment. **b** *B. campestris B. oleracea* monosomic addition lines for 6PGD-1′ nd 6PGD-2. *Arrows point to B. oleracea*-specific isozymes. **c** *B. campestris-oleracea* monosomic addition line for *Pgm-2* (lines 7 and 8, arrow points to *B. oleracea*-specific isozyme). *First two lines* correspond to *B. napus;* the rest show the *B. campestris* PGM phenotype

Table 5. Number of monosomic and disomic addition plants generated from *B. napus* × *B. campestris* crosses

Marker	Monosomic	Disomic
PGI-1	1	1
LAP-1	1	2
6PGD-1	1	1
6PGD-2/RA	5	4
PGM-2	0	1
Unmarked	32	19

in the autoradiograms after hybridization with the probe pTA250.2 which carried only transcribed sequences.

Genetic characterization of hyperploid plants

Plants from all the crosses and backcrosses described above were systematically surveyed for *B. oleracea* isozymes. Progenies from hyperploid plants carrying *B. oleracea* specific markers were screened for chromosome number and for the presence of these markers. Table 4 shows a sample of the phenotypes of some of the hyperploid plants and the transmission of the *B. oleracea* specific isozymes to 2n=21 and 2n=22 individuals found in their progenies. We were able to characterize eight 2n=21 plants as monosomic addition lines, and nine 2n=22 plants as disomic addition lines, by the presence of *B. oleracea* specific alleles for the loci sampled. It was found that 32 2n=21 and 19 2n=22 plants remained uncharacterized due to the lack of additional markers (Table 5). For the rDNA determination, we screened four hyperploid plants with 2n=23, 24, 25 and 26 chromosomes. Two of them showed the simultaneous presence of the *B. oleracea* 1.8 and 0.8 Kb fragments in addition to the *B. campestris* fragments indicating the presence of a *B. oleracea* chromosome carrying these fragments. The rest had the *B. campestris* or *B. napus* rDNA phenotypes. The presence of these two fragments was designated as RA phenotype (Fig. 3a). The presence of the *B. oleracea* 3.4 Kb fragment was designated as RB phenotype. Thus, plants carrying all three fragments had RA, RB phenotype. Three plants of 2n=21 and one of 2n=22 chromosomes derived from the hyperploid parents mentioned above displayed the same phenotype as the parental plants. The presence of the *B. oleracea* specific fragments 1.8 and 0.8 Kb was accompanied by the *B. oleracea* 6PGD-2 isozymes, indicating the genes coding for these isozymes and the rDNA genes are located on the same chromosome. On the other hand, the presence of the *B. oleracea* 6PGD-1 isozymes were independent from that of the 6PGD-2 isozymes revealing that these loci are on different chromosomes (Fig. 3b). Similarly the presence

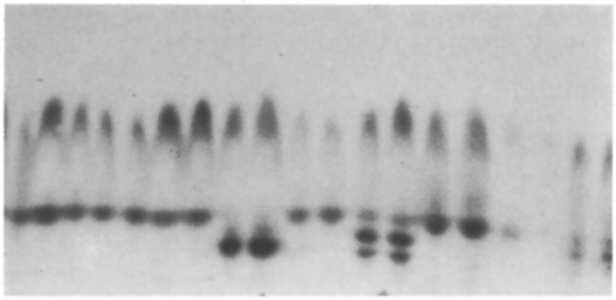

Fig. 4. Progeny from hyperploid *B. campestris-oleracea* plant carrying a *PGI-2 B. oleracea*-specific chromosome. Addition lines are heterozygous (3 banded phenotype) and diploid *B. campestris* are homozygous for the isozymes of faster migration. The plant homozygous for the slower isozyme may have originated by intergenomic recombination

Table 6. Frequency of intergenomic recombinants observed in the progenies of hyperploids

Progeny	n[a]	Recombination frequency (%)	Loci
85B138-1	5	20.0	*Pgi-1*
86B150-1	10	10.0	*Pgi-1*
86B150-7	12	8.3	*6pgd-2*
85B268-4	16	6.2	*6pgd-1*

[a] Progeny size

of LAP-1, PGI-1 and PGM-2 *B. oleracea* specific isozymes were found to be independent of each other.

Intergenomic recombinants were observed in some of the progenies from hyperploid plants. A few diploid plants showing both *B. oleracea* and *B. campestris* isozymes (Table 6) were detected. In addition, two plants were homozygous for a *B. oleracea* PGI isozyme, lacking the *B. campestris* isozymes (Fig. 4). The sporadic presence of multivalents in some of the addition lines explains the origin of these recombinants (Fig. 1 b).

In progenies from hyperploid plants we detected eight self-compatible plants of either 2n = 21 or 22 chromosomes derived from crosses not involving the Chinese cabbage 'Kwan-Hoo Choi', a cultivar with a very relaxed self-incompatibility. In addition, two diploid plants derived from the same hyperploid parents were also found to be self-compatible.

Discussion

The high pollen viability in the sesquidiploid hybrids and in the subsequent hyperploid derivatives permits generation of a series of alien chromosome addition lines in *Brassica*. A good example of the high tolerance for extra chromosomes in *B. campestris* is shown by 2n = 26 plants displaying a pollen viability of 83%. The tolerance for extra chromosomes might have evolved in this species as a step to alloploidization in the generation of the hybrid polyploid *B. napus*. A similar situation occurs in wheat where a whole series of aneuploid stocks has been constructed (Sears 1969). The monosomic and disomic addition lines generated in our study had a pollen viability of at least 90%. Furthermore, the transmission of the extra chromosome through the ovules of 2n = 21 plants was on the average 21%. The presence of disomic addition lines at a frequency of 10% indicates that the extra chromosome undergoes non-disjunction in the female gametes. Selfing of monosomic addition plants increased the transmission of the extra chromosome resulting in 25% trisomics and 19% tetrasomic plants in the progeny. This indicates that the extra chromosome may be transmitted through pollen.

The 6PGD loci and the rDNA phenotypes confirm the origin of *B. napus* as a hybrid of *B. campestris* and *B. oleracea*. Although the number of loci duplicated for 6PGD could not be pinpointed by genetic analysis due to the lack of polymorphism, they can be extrapolated by inspection of the zymograms. Figure 2 b shows the interpretation for 6PGD1 based on 2 loci designated *6pgd-1* and *6pgd-1'* for each of the diploid species. Locus *6pgd-1* seems to be monomorphic for both species, carrying the allozymes *1ol* and *1cp* of identical migration. The duplicated locus *6pgd-1'* has genome specific allozymes *2ol* and *2cp* for *B. oleracea* and *B. campestris*, respectively. The middle bands for each diploid are interlocus heterodimers. In *B. napus* the allozymes *2ol* and *2cp* form an additional interlocus, interspecific heterodimer right below the *B. oleracea* interlocus heterodimer. The same pattern is observed in the monosomic addition lines for 6PGD-1, except that the allozyme *2ol* is weak due to the presence of only one copy of the *B. oleracea* chromosome.

The distinct pattern of rDNA fragments in *B. oleracea* and *B. campestris* is useful for the detection and characterization of the addition lines. The main difference is the replacement in *B. campestris* of the *B. oleracea* fragments 1.8 and 0.8 Kb by a 2.6 Kb fragment, resulting from the loss of a restriction site. The selective loss of the rDNA *B. oleracea* 3.4 Kb fragment (RB phenotype) and the simultaneous presence of the 1.8 and 0.8 Kb fragments in the addition lines indicates that more than one chromosome carry these genes in *Brassica* and that they have different restriction sites. One chromosome carries the 1.8 and the 0.8 Kb fragments while other carries the 3.4 Kb fragment. This difference in restriction sites indicates initial duplication and subsequent divergence of these sequences. The lighter intensity of the 1.8 and 0.8 Kb fragments in the addition lines is explained by the presence of a single copy of the RA *B. oleracea* chromosome versus two copies in *B. napus*. In our limited survey for rDNA phenotypes we did not detect any addition lines of only RB phenotype. Further testing of the unmarked addition lines is expected to disclose individuals with this phenotype. The organization of the other fragments in these two chromosomes is not known at this point.

An important observation was the presence of possible recombinants in some of the progenies. Intergenomic recombination has been reported between the *B. oleracea* and *B. campestris* genomes by Chiang and Crete (1983) after transferring a disease resistance gene from *B. napus* to *B. oleracea.* In the amphidiploid no evidence of recombination between the two genomes has been reported. This lack of recombination is likely due to the high diploidization of *B. napus* resulting in 19II in meiosis. After backcrossing it to the diploid *B. campestris* parent, the loss of pairing partners for some of the chromosomes might result in an increased change of multivalent formation and intergenomic recombination. A possibility that needs further exploration is the presence of a pairing control mechanism similar to that reported in wheat (Riley et al. 1959; Attia and Robbelen 1986). In any event, recombination opens the possibility of exchanging genes among genomes, an important alternative for the *Brassica* breeder. Recombination between the *B. oleracea* and *B. campestris* genomes supports the view that these species have originated from a common ancestral genome by aneuploidy and chromosome repatterning. These changes might have been expedited by translocations which not only rearrange the chromosomes in novel combinations but also yield tertiary trisomics by disturbances in chromosome disjunction (Stebbins 1971; Khus 1973).

The breakdown of self-incompatibility in some of the derivatives is also an interesting finding. Self-compatibility could not be associated with any specific *B. oleracea* marker, or even with the extra chromosomes, since two diploids were found to be compatible. The possibility exists, however, that having the *S* locus in a trisomic condition might weaken its expression, resulting in self-compatible plants, as occurs in the natural amphidiploid species which are self-compatible. In such a case, it might be possible to locate the *S* locus by using these addition lines.

Our work opens the possibility of generating useful cytogenetic stocks for the genetic and evolutionary characterization of *Brassica* species. The presence of duplicated loci in the *B. oleracea* and *B. campestris* genomes agrees with the hypothesis of Robbelen (1960), which suggests that the basic diploids are indeed secondary polyploids. Other evidence is the remarkable tolerance of aneuploidy and the high fertility of the aneuploids found in this study. Generation of addition lines for each of the diploid genomes, including x = 7 genomes from several wild species is underway. This will allow a comparative study of the *Brassica* genomes, as additional markers are developed. A further step will be the generation of synthetic amphidiploids between diploids carrying agronomically useful genes such as those determining disease resistance, plant architecture and presence of important chemical compounds. Generation of addition lines from these will provide information on the location of genes determining the various horticultural traits observed in the diploids, i.e. curd in cauliflower, heading in cabbage, root enlargement in turnips and axillary bud enlargement in Brussels sprouts.

Acknowledgements. We are indepted to Charles Rick, Steve Tanksley, Judy Greenlee and Margi Oard for reviewing the manuscript; to Vince D'Antonio, Janet Stites and Mitch McGrath for technical assistance and to Jane Johnson for typing the manuscript. Supported by a USDA competitive grant 86CRCR-1-1926.

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