Theor Appl Genet (1998) 97:976–981 

K. Srinivasan · V. G. Malathi · P. B. Kirti
S. Prakash · V. L. Chopra

# Generation and characterisation of monosomic chromosome addition lines of *Brassica campestris - B. oxyrrhina*

Received: 20 October 1997 / Accepted: 31 March 1998

**Abstract** Monosomic chromosome addition lines of *Brassica oxyrrhina* in the background of alloplasmic *B. campestris* carrying *B. oxyrrhina* cytoplasm were generated and characterised through morphology, cytology and molecular (RAPD) analysis. Four successive backcrosses of the synthetic alloploid *B. oxycamp* with *B. campestris* yielded 24 monosomic addition plants that were grouped into seven different synteny groups based on morphological similarity and RAPD patterns. Each synteny group exhibited morphological features diagnostic for the presence of individual *B. oxyrrhina* chromosomes including some novel phenotypes. Meiotic studies of the addition lines revealed the homoeology of four *B. oxyrrhina* chromosomes (synteny groups 1, 3, 5 and 6 ) with *B. campestris* chromosomes as indicated by trivalent associations, with the highest homoeology (44.23%) in synteny group 1 and the lowest (6.1%) in synteny group 3. Seed fertility of the addition lines ranged from 94.85% (synteny group 1) to 56.98% (synteny group 5). All of the addition lines were male-sterile except synteny group 6 which had 12–16% stainable pollen. Ovule transmission of the *B. oxyrrhina* chromosomes added to the progenies of addition lines ranged from 23.52% (synteny group 6) to 14% (synteny group 7). RAPD analysis confirmed the validity of synteny grouping based on morphological observations. Approximately 45% of the primers studied were informative, giving *B. oxyrrhina*-specific RAPD bands unique for each synteny group, except group 6.

Communicated by G. E. Hart

K. Srinivasan · P. B. Kirti · S. Prakash (✉) · V. L. Chopra
National Research Centre on Plant Biotechnology,
Indian Agricultural Research Institute, New Delhi-110012, India

V. G. Malathi
Division of Plant Pathology, Indian Agricultural Research Institute,
New Delhi-110012, India

**Key words** Chromosome addition line · Synteny group · *Brassica campestris–Brassica oxyrrhina* · Monosomic · Alloplasmic · RAPD

## Introduction

Alien chromosome addition lines have considerable importance in crop improvement because they allow the localisation of genes of agronomic value to individual chromosomes. Different series of such lines were generated following the dissection of the basic diploid genomes of *B. nigra* and *B. oleracea* in the genus *Brassica* and then characterized using molecular markers (Quiros et al. 1987, 1991; Jahier et al. 1989; Chevre et al. 1991, 1994, 1996; Struss et al. 1991, 1992, 1996). A naturally occurring wild species *B. oxyrrhina* ($2n = 18$, OO) is a repository of desirable genes for water stress, *Alternaria* leaf spot resistance and white rust resistance. It has been shown that the cytoplasm of this species induces male sterility in *B. campestris*/*B. juncea* (Prakash and Chopra, 1990). However, fertility-restoring genes could not be located in existing cultivars and natural accessions. To initiate attempts for exploiting the desirable genes of *B. oxyrrhina* and to address the problem of fertility restoration for *oxy*-based cytoplasmic male-sterile (CMS) systems in *Brassica*, we initiated a programme to generate chromosome addition lines of *B. oxyrrhina* on *B. campestris* carrying *B. oxyrrhina* cytoplasm. These addition lines were characterised by their morphology and cytology and by molecular random amplified polymorphic DNA (RAPD) analysis.

## Materials and methods

### Generation of addition lines

The synthetic amphidiploid *Brassica oxycamp*, $2n = 38$, OOAA (*B. oxyrrhina*, $2n = 18$, OO × *B. campestris* spp. *oleifera* var brown

sarson, $2n = 20$, AA; Prakash and Chopra 1988), was successively backcrossed with *B. campestris* for generating the addition lines. On the basis of various diagnostic morphological features of *B. oxyrrhina*, we isolated 7 monosomic additions from a population of 201 plants that were analysed cytologically in the $BC_2$ generation. The following year 9 monosomic additions were identified in a population of 102 $BC_3$ plants. Some of the $BC_3$ families which had two to three univalents of *B. oxyrrhina* were further examined in the $BC_4$ generation and monosomic additions were identified. A total of 24 monosomic additions isolated from various backcross generation families were studied.

### Meiotic studies

Anthers were fixed in aceto-alcohol and squashed in 2% acetocarmine. Pollen mother cells (PMCs) were analysed for determinimg chromosome number and meiotic metaphase-I pairing behaviour.

### Maternal transmission

Maternal transmission of *B. oxyrrhina* univalents in the addition lines of various synteny groups was determined by counting the number of $2n + 1$ progeny from each $(2n + 1) \times 2n$ cross for a minimum of 50 progenies.

### Female fertility

Female fertility was determined for each synteny group of the addition lines by counting the number of well–formed seeds among the total number of seeds in one pod from each of 15 plants.

### Plant material and DNA isolation

Total genomic DNA from leaf samples was isolated using the method of Mohapatra et al. (1992) from *B. campestris* spp. *oleifera*, CMS (*oxy*)*B. campestris*, *B. oxyrrhina*, *B. oxycamp* and *B. campestris* – *oxyrrhina* chromosome addition lines.

### Polymerase chain reaction (PCR) conditions

Purified, RNAase-treated genomic DNA was used as the template for amplification using random decameric primers (Operon Technologies, USA). The protocol of Williams et al. (1990) for detecting PCR products was followed with the following modifications. Each reaction volume of 25 µl contained: total genomic DNA (20 ng), 1 × PCR buffer, 2.5 mM $MgCl_2$, 100 µM of each dATP, dTTP, dCTP and dGTP (Perkin Elmer), 0.2 µM (15 ng) of random primer (Operon Technologies USA) and 0.5 U (Genei, India) or 1.0 U (Promega Corp, USA) of *Taq* DNA polymerase. A control reaction containing all of the components except for genomic DNA was also performed with each primer. Reactions were performed in a Perkin Elmer Cetus Gene Amp 9600 DNA thermocycler. The temperature profiles followed were as per the specifications given by Jain et al. (1994). Amplified products were analysed by electrophoresis on 1.2% agarose gels with 1 × TBE buffer along with a standard molecular-weight marker (1-kb ladder, Stratagene, USA) at a constant voltage of 45 V for 4–5 h. The gel was photographed under UV light after ethidium bromide staining.

### Data analysis

Results of RAPD analysis represented a consensus of three repetitions. Readily detectable and reproducible amplification products were scored on the basis of their presence or absence. The nomenclature for PCR products and detection of chromosome-specific RAPD bands are similar to that of Quiros et al. (1991). The addition lines were grouped into different synteny groups after the RAPD data were integrated with the available morphological and cytological data.

## Results

### Characterisation of addition lines

All except 4 of the 24 monosomic additions showed morphological markers diagnostic for the presence of a *B. oxyrrhina* chromosome, and these were classified accordingly (Table 1). The markers were of two types. Spines on stem, leaves and flower buds (Fig. 1c), stunted habit, rossette nature of leaves, angular style (Fig. 1d) in the flower and purplish anthocyanin pigmentation on plant parts were specific to *B. oxyrrhina* (synteny groups 1, 2, 3, 5 and 7), while cupped leaves (Fig. 1a), oval leaf shape and crinkled petals (Fig. 1b) were novel phenotypes that were not present in either *B. campestris* or *B. oxyrrhina* (synteny groups 2, 4 and 5). Spines on the stems and leaves were expressed in all of the addition lines except groups 2 and 6, indicating that this trait is polygenic. The novel phenotype crinkled petal was strongly correlated (100%) with the presence of a *B. oxyrrhina* chromosome. Other characters showed a correlation that ranged between 50–80% (data not shown). Synteny group 6 exhibited delayed flowering, and group 2 had a slightly angular style as in *B. oxyrrhina*. Four monosomic additions did not exhibit specific morphological characters. All of the addition lines exhibited severe chlorosis on young leaves, a feature characteristic of alloplasmic (*oxyrrhina*) *B. campestris*, and there were no deleterious floral modifications. Nectaries were well-developed, and anthers were normal with sterile pollen except for synteny group 6 which exhibited 12–16% pollen fertility. Differences in height were not significant among the addition lines except for synteny group 2 which showed a considerable reduction in height and a bushy habit that resembled *B. oxyrrhina*.

All of the addition lines exhibited complete male sterility except group 6, which showed 12–16% fertile pollen. Attempts to self-fertilise group 6 plants and pollinations of CMS (*oxy*) *B. campestris* did not yield seed in the first two seasons. However, in the third season, ten seeds from the cross CMS (*oxy*) *B. campestris* × group 6 produced progenies with low fertility (5–12% stainable pollen).

In all of the additions isolated in the present study, a univalent of *B. oxyrrhina* was observed at diakinesis of prophase I that was never associated with the nucleolus. At metaphase I, $10^{II} + 1^{I}$ configurations were observed in a majority of the PMCs. However $9^{II} + 1^{III}$ configurations, indicating trivalent formation

**Table 1** Characteristics of *B. campestris–oxyrrhina addition* lines

| Synteny group (s) | Morphological marker (s) | Chromosome behaviour (% trivalents) | Maternal transmission of univalents (% $2n + 1$ progenies) | Seed fertility (%) | Pod length (cm) | RAPD marker(s) |
|---|---|---|---|---|---|---|
| S1 | Spines on leaves and stem | 44.23 | 9.72 | 94.85 | 7.56 | OPM 03–750; OPM 10–650; OPN 10–400; OPN 11–850; OPN 13–950; OPN 16–1100 |
| S2 | Stunted habit, oval-shaped lower leaves | 0.0 | 11.11 | 71.34 | 7.66 | OPM 16–750;OPN03–500; OPN 16–800 |
| S3 | Spines on buds, incomplete leaf clasping, reduced style | 6.1 | 10.42 | 90.24 | 9.3 | OPN 12–950 |
| S4 | Typical crinkling of flower petals | 0.0 | 8.18 | 74.33 | 7.4 | OPA 16–1200;OPN 07–1300; OPN 08–650;OPB 18–1200 |
| S5 | Leaves cupped, purplish tinge on leaf margins flower buds and anthers | 8.93 | 8.57 | 56.88 | 9.1 | OPM 18–700 |
| S6 | Anthers show stainable, fertile pollen 5–16% | 11.49 | 11.76 | 84.38 | 8.96 | OPN 05–625;OPN 17–1000 |
| S7 | Lower leaves form a rossette arrangement., occasionally a seed in pod beak | 0.0 | 7.14 | 89.6 | 11.0 | OPM 07–1450;OPM 16–700 |
| NG-1[a] | – | 0.0 | 13.64 | 56.55 | 7.36 | OPN 04–1100 |
| NG-2 | – | 12.5 | 15.79 | 92.51 | 7.4 | OPA 16–1600;OPN 17–550 |
| NG-3 | – | 13.56 | 9.52 | 50.48 | 8.9 | – |

[a]NG, Unmarked additions


**Fig. 1a–d** Morphology of *B. campestris – oxyrrhina* addition lines; **a** cupped leaf – synteny group 5, **b** crinkled petals – synteny group 4, **c** spine on flower bud (*arrow*) – synteny group 3, **d** crooked style – synteny group 2

between a *B. campestris* bivalent and the *B. oxyrrhina* univalent, were also observed. The individual chromosomes involved in the trivalent formation could not be identified. *B. oxyrrhina* univalents either persisted until telophase II and were included in one of the daughter nuclei or were eliminated as early as telophase I.

## Random amplified polymorphic DNA (RAPD) analysis

### *Nature and pattern of amplified PCR products*

Out of 54 random primers tested, 42 detected polymorphic differences between *B. campestris* and *B. oxyrrhina* genomes. Based on clearly distinguishable polymorphic bands, a total of 34 primers were used for characterising the different *B. oxyrrhina* chromosomes present in the addition lines. Amplification products appearing in the overall profile were classified into three categories:

(1) RAPD(s) that distinguish *B. campestris* and *B. oxyrrhina*, genomes. (2) RAPDs that distinguish alloplasmic (*oxy*)*B.campestris* from the euplasmic *B. campestris* genome and (3) RAPDs specific only for some members of a synteny group which were either derived from the same mother plant or from different backcross generation families (Fig. 2). A total of 94 RAPDs from 34 primers specific for the *B. oxyrrhina* genome were scored in different addition lines. The number of amplification products for each primer ranged from 2 to 12. Amplification products which revealed polymorphism ranged in size from 250 bp to 1450 bp.

*Synteny classification*

Table 2 presents a summary of the RAPD analysis of the addition lines. Both group 6 and an unmarked addition line (NG-3) could not be identified with any informative primer.Three products from 3 primers were specific for the other 2 unmarked addition lines. Twenty-four RAPDs representing more than one synteny group were amplified from *B. oxyrrhina* chromosomes, 9 products from as many different primers identified two different *B. oxyrrhina* chromosomes, and 5 products from 5 primers were amplified from three synteny groups. Some RAPDs were amplified from both marked and unmarked addition lines. Marker OPN 02–800 was specific for all the unmarked addition lines, and 36 RAPDs were amplified only from certain plants in a synteny group. The latter were omitted from synteny group classification (data not shown). Fourteen PCR products that were specific for *B. oxyrrhina* were not identified in any of the chromosome addition lines.

## Discussion

In the genus *Brassica*, addition lines have been generated by separating out the three basic genomes. While some could not be identified by morphological markers(Quiros et al. 1987; This et al. 1990), others were distinguished by phenotypic markers such as puckered leaf type (McGrath and. Quiros 1990), seed and flower colours (Chevre et al. 1994; Cheng et al. 1994) and resistance to *Phoma lingam*, sinigrin

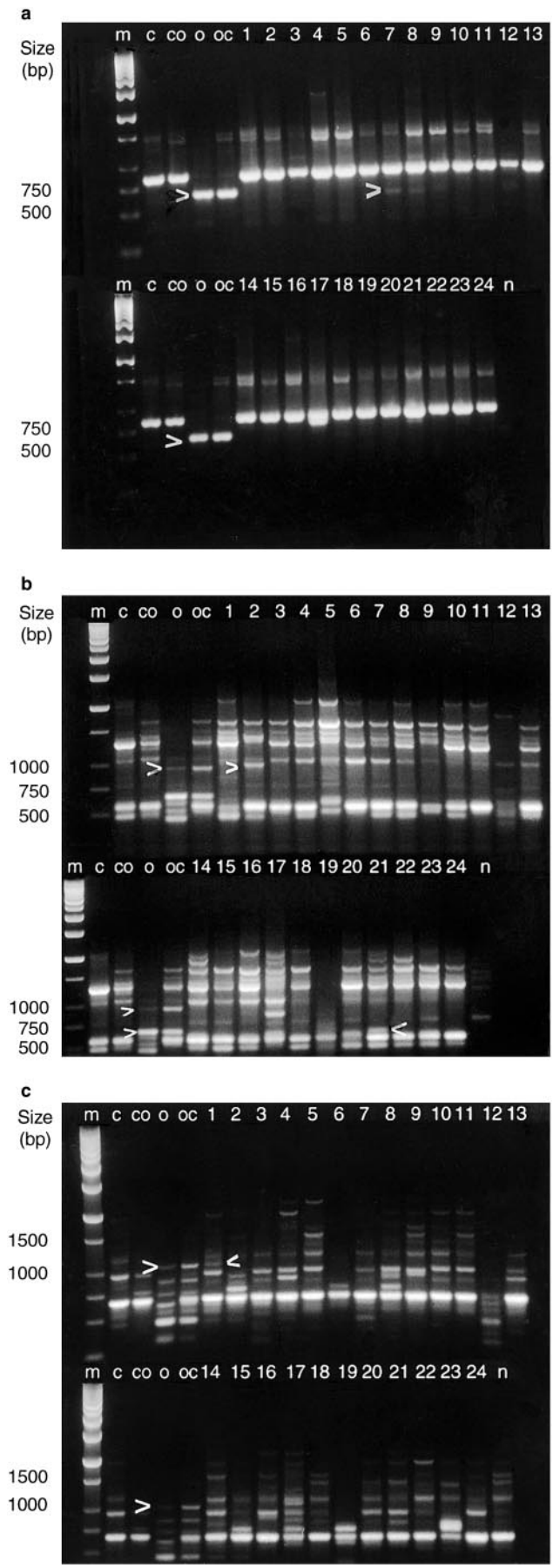


**Fig. 2a–c** RAPD analysis of *B. campestris–oxyrrhina* additions. **a** Agarose gel showing OPM08 marker (650 bp) specific for snyteny group 4 (*lanes 7* and *8*). **b** Agarose gel showing OPM16 markers (950 bp, 700 bp) specific for synteny group 2 (*lane 2*) and synteny group 7 (*lanes 20* and *21*), respectively. **c** Agarose gel showing OPN16 marker (1100 bp) specific for synteny group 1 (*lane 1*) *m* 1-kb ladder, *c B. campestris*, *co* CMS(*oxy*) *B. campestris*, *o B. oxyrrhina*; *oc*: *B. oxycamp*; *lanes 1–24* chromosome addition lines of *B. campestris-oxyrrhina*, *n* control

**Table 2** RAPD markers useful for the identification of *B. campestris – oxyrrhina* chromosome addition lines

| Synteny group(s) | Number of primers identifying the synteny group | Number of PCR products specific for *B. oxyrrhina* | Number of PCR products specific for the synteny group | Percentage of informative markers |
|---|---|---|---|---|
| 1 | 14 | 14 | 6 | 42.9 |
| 2 | 11 | 11 | 3 | 27.3 |
| 3 | 9 | 10 | 1 | 10.0 |
| 4 | 8 | 9 | 4 | 44.4 |
| 5 | 14 | 14 | 1 | 7.1 |
| 6 | 2 | 2 | 0 | 0.0 |
| 7 | 7 | 8 | 2 | 25.0 |
| NG-1 | 4 | 4 | 1 | 25.0 |
| NG-2 | 6 | 7 | 2 | 28.6 |
| NG-3 | 5 | 6 | 0 | 0.0 |

(glucosinolate) and erucuc acid contents (Struss et al. 1991, 1996). In the present investigation, all of the seven synteny groups were identified by reliable morphological markers which were specific for either the genome of *B. oxyrrhina* or for novel phenotypes which were presumably caused by interactions between the *B. oxyrrhina* and *B. campestris* genomes. The reliability of diagnostic morphological markers was complete for synteny 4 ( crinkled petals), and it ranged from 50% to 80% for the other synteny groups, indicating either less than 100% penetrance or, less likely, the occurrence of spontaneous introgression of the *B. oxyrrhina* chromosome segments into the *B. campestris* genome. Novel phenotypes were also observed in *D. erucoides–nigra* and *B. campestris–oleracea* additions (Quiros et al. 1988; McGrath and Quiros 1990) for which epigenetic mechanisms were suggested to be the cause. The presence of combined phenotypic markers in the unmarked *B. campestris–oxyrrhina* additions indicates the possibility that intra-genomic recombinations or translocations occurred between different *B. oxyrrhina* chromosomes due to autosyndesis during backcross generations, as suggested by multivalent associations in hyperploid plants.

Interaction between the *B. oxyrrhina* cytoplasm and the *B. campestris* nucleus did not cause any major deleterious effect on floral morphology. Anther morphology, except for size reduction, was normal in all the addition lines. With one exception, monosomic additions of *B. oxyrrhina* did not exhibit any adverse effect on seed fertility except group 5. On the contrary, *B. campestris – oleracea* addition lines (Lee and Namai 1992) suffered from an unexpected low seed set. *B. campestris – oxyrrhina* additions were also male sterile except group 6, which had 12–16% pollen fertility indicating that the additional chromosome possesses a gene (s) responsible for fertility restoration. Disomic additions of *B. oxyrrhina* were not obtained by selfing these plants, presumably due to the self-incompatible nature of *B. campestris* var 'brown sarson'. In alloplasmic *Nicotiana* and wheat the nucleolar organizer region (NOR) has been implicated in fertility restoration (Gerstel et al. 1978; Suzuki et al. 1994 ). However, in our investigation, the added chromosome was never observed to carry a NOR.

The genome of *B. oxyrrhina* has partial homoeology with the *B. campestris* genome, as indicated by the occurrence of up to four bivalents in the hybrids (Prakash and Chopra 1990). However, trivalent formation in the addition lines (synteny groups 1, 3, 5 and 6) suggested allosyndesis between the chromosomes of these genomes. Frequent occurrence of diploid sibs with markers unique for addition lines indicated introgression due to homoeologous pairing. The absence of trivalent associations in synteny group 4 was consistent with the presence of the morphological marker crinkled petal, which was 100% correlated with the presence of a *B. oxyrrhina* chromosome. Using chromosome-specific morphological markers, we were able to trace the transmission of individual *B. oxyrrhina* chromosomes from sesquiploid hybrids through various backcross generations. The observed maternal transmission rate for individual *B. oxyrrhina* chromosomes suggested a 35–80% reduction in transmission frequency relative to the theoretical expectation. There were no significant differences in chromosome transmission frequencies among different backcross generations. However, variable chromosome transmission frequencies were observed among the different backcross generations in *B. campestris–oleracea* additions (McGrath and Quiros 1990). The use of individual chromosome transmission frequencies for the identification of added chromosomes, as suggested for *B. napus-nigra* additions (Chevre et al. 1991), was not possible in the present study.

Characterisation of *B. oxyrrhina* chromosomes added to *B. campestris* using RAPDs supported the synteny group classification based on morphology and cytology. Approximately 45% of the informative primers identified *B. oxyrrhina* chromosomes of the different synteny groups and, with one exception (synteny group 6), RAPDs specific for each synteny group were detected.

In *B. napus* – *D. erucoides* additions (Chevre et al. 1994), the occurrence of intergenomic recombination due to multivalent associations between *B. napus* and *D. erucoides* chromosomes made it difficult to produce a complete set of additions. In the present study, the hyperploid progenies of *B. campestris* – *oxyrrhina* showed multivalent associations at very low frequencies, thereby enabling the identification of the addition lines. As observed in the case of *B. napus* – *nigra* additions (Quiros et al. 1991; This et al. 1991; Struss et al. 1992), absence of *B. oxyrrhina* specific RAPDs in the addition lines suggested the incomplete set of additions.

Amplification by individual primers displaying the same size of PCR products from more than one synteny group indicates the possibility that intragenomic recombinations have occurred among different *B. oxyrrhina* chromosomes. Speculation regarding the duplicated genomic regions scattered over different chromosomes warrants restriction fragment length polymorphism (RFLP) analyses using *B. oxyrrhina*-specific genomic fragments. Observations on duplicated loci derived from RFLP and RAPDs in different *Brassica* addition line series have been reported (McGrath et al. 1990; This et al. 1990, 1991; Chevre et al. 1991; Struss et al. 1996).

The results of the present investigation open up the possibility of transferring genes of agronomic importance from *B. oxyrrhina* to *B. campestris*. A comprehensive investigation is in progress to construct fertility restorers by exploiting allosyndetic recombination between the *B. oxyrrhina* chromosome of synteny group 6 and *B. campestris* chromosomes.

**Acknowledgements** We acknowledge Dr. M. Lakshmikumaran, Tata Energy Research Institute, New Delhi, India for her help and suggestions during this investigation.

## References

Cheng BF, Chen BY, Heneen WK (1994) Addition of *Brassica alboglabra*, Bailey chromosomes to *B. campestris* L. with special emphasis on seed colour. Heredity 73 : 185–189

Chevre AM, This P, Eber F, Deschamps M, Renard M, Delseny M, Quiros CF (1991) Characterisation of disomic addition lines *Brassica napus – Brassica nigra* by isozyme, Fatty acid and RFLP markers. Ther Appl Genet 81 : 43–49

Chevre AM, Eber F, Quiros CF, Delourme R (1994) Creation and characterisation of *Brassica napus – Diptotaxis erucoides* addition lines. Cruciferae Newsl 16 : 28–29

Chevre AM, Eber F, This P, Barret P, Tanguy X, Brun H, Delseny M, Renard M (1996) Characterisation of *Brassica nigra* chromosomes and of blackleg resistance in *B. napus – B. nigra* addition lines. Plant Breed 115 : 113–118

Gerstel DU, Burns JA, Burk LG (1978) Cytoplasmic male sterility in *Nicotiana*. restoration of fertility and the nucleolus. Genetics 89 : 157–169

Jahier J, Chevre AM, Tanguy AM, Eber F (1989) Extraction of disomic addition lines of *Brassica napus – B. nigra*. Genome 32 : 408–413

Jain A, Bhatia S, Banga SS, Prakash S, Lakshmi Kumaran M (1994) Potential use of random amplified polymorphic DNA (RAPD) technique to study the genetic diversity in Indian mustard (*Brassica juncea*) and its relationship to heterosis. Theor Appl Genet 88 : 116–122

Lee KH, Namai H (1992) Stabilization of new types of diploids ($2n = 22, 24$) through selfing of aneuploids ($2n = 21, 22$) derived from crossing of sesquiploids ($2n = 29$, AAC) and *Brassica campestris*. Euphytica 60 : 1–13

McGrath JM, Quiros CF (1990) Generation of alien chromosome addition lines from synthetic *Brassica napus*: morphology, cytology fertility and chromosome transmission. Genome 33 : 374–383

McGrath JM, Quiros CF, Harada JJ, Landry BS (1990) Identification of *Brassica oleracea* monosomic alien chromosome addition lines with molecular markers reveals extensive gene duplication. Mol Gen Genet 233 : 198–204

Mohapatra T, Sharma RP, Chopra VL (1992) Cloning and use of low copy sequence genomic DNA for RFLP analysis of somaclones in mustard (*Brassica juncea* L. Czern and Coss). Curr Sci 62 : 482–484

Prakash S, Chopra VL (1988) Synthesis of alloplasmic *Brassica campestris* as a new source of cytoplasmic male sterility. Plant Breed 101 : 253–255

Prakash S, Chopra VL (1990) Male sterility caused by cytoplasm of *Brassica oxyrrhina* in *B. campestris* and *B. juncea*. Theor Appl Genet 79 : 285–287

Quiros CF, Ochoa O, Kianian SF, Douches D (1987) Analysis of the *Brassica oleracea* genome by the generation of *B. campestris-oleracea* chromosome addition lines: characterization by isozyme and rDNA genes. Theor Appl Genet 74 : 758–766

Quiros CF, Ochoa O, Douches D (1988) Exploring the role of $x = 7$ species in *Brassica* evolution : Hybridization with *B. nigra* and *B. oleracea*. J. Hered 79 : 351–358

Quiros CF, Hu J, This P, Chevre M, Delseny M (1991) Development and chromosomal localization of genome specific markers by polymerase chain reaction in *Brassica*. Theor Appl Genet 82 : 627–732

Struss D, Quiros CF, Robbelen G (1991) Construction of different B genome addition lines of *Brassica napus*. In: McGregor DI (ed) Proc 8th Int Rapeseed Congr, Vol 2. GIRC, Saskatoon, Canada, pp 358–363

Struss D, Quiros F, Robbelen G (1992) Mapping of molecular markers on *Brassica* B genome chromosomes added to *Brassica napus*. Plant Breed 108 : 320–323

Struss D, Quiros CF, Pleiske J, Robbelen G (1996) Construction of *Brassica* B genome synteny groups based on chromosomes extracted from three different sources by phenotypic, isozyme and molecular markers. Theor Appl Genet 93 : 1026–1032

Suzuki T, Nakamura C, Mori N, Iwasa Y, Kaneda C (1994) Homoeologus group l chromosomes of *Agropyron* restore nucleus cytoplasmic compatibility in alloplasmic common wheat with *Agropyron* cytoplasms. Jpn J Genet 69 : 41–51

This P, Ochoa O, Quiros CF (1990) Dissection of the *Brassica nigra* genome by monosomic addition lines. Plant Breed 105 : 211–220

This P, Chevre AM, Quiros CF, Delseny M (1991) RFLP characterisation of *Brassica napus – Brassica nigra* addition lines. In: McGregor DI (ed) Proc 8th Int Rapeseed Congr Vol 2. GCIRC, Saskatoon, Canada, pp, 1050–1054

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18 : 6531–6535