Theor Appl Genet (2001) 102:695–699 © Springer-Verlag 2001

**B. Saal · J. Plieske · J. Hu · C.F. Quiros · D. Struss**

# Microsatellite markers for genome analysis in *Brassica*. II. Assignment of rapeseed microsatellites to the A and C genomes and genetic mapping in *Brassica oleracea* L.

Received: 14 April 2000 / Accepted: 3 July 2000

**Abstract** Microsatellites are highly polymorphic and efficient markers for the analysis of plant genomes. Primer specificity, however, may restrict the applicability of these markers even between closely related species for comparative mapping studies. We have demonstrated that the majority of microsatellites identified in oilseed rape (*Brassica napus* L; AC genome) correspond to loci which can be easily assigned to the A and C progenitor genomes. A study with 63 primer pairs has shown that 54% detect two loci, one from each genome, while 25% and 21%, respectively, are either A or C genome-specific. The distribution of rapeseed microsatellites in the C genome was investigated by genetic mapping in *Brassica oleracea* L. Ninety two dinucleotide microsatellites were screened for polymorphism in an $F_2$ population derived from a cross between collard and cauliflower, for which an RFLP map has been constructed previously. Thirty three primer pairs (35.7%) have yielded either unspecific or no PCR products whereas the remaining primer pairs amplified one or more distinct loci. The level of polymorphism found in the mapping population was 49.2%. A total of 29 primer pairs disclosed 34 loci of which 31 are evenly distributed on 8 of the 9 *B. oleracea* linkage groups. For the remaining three markers linkage could not be established. Our results showed that microsatellite markers from the composite genome of *B. napus* can serve as a useful marker system in genetic studies and for plant-breeding objectives in *B. oleracea*.

**Key words** Microsatellites · *Brassica oleracea* · Genetic mapping

Communicated by H.C. Becker

B. Saal (✉) · J. Plieske · D. Struss
Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Corrensstraße 3, 06466 Gatersleben, Germany

J. Hu
Lipton Tomato Technology Center, 2029 E. Harding Way, Stockton, CA 95205, USA

C.F. Quiros
Department of Vegetable Crops, University of California, Davis, CA 95616, USA

*Present address:*
D. Struss, Department of Pomology, University of California, Davis, CA 95616, USA

## Introduction

In recent years microsatellites, also referred to as simple sequence repeats (SSRs), have gained increasing importance in plant genetics and breeding. High abundance and extensive polymorphism make them an ideal marker system for genetic mapping and the characterization of germplasm, particularly in inbreeding species (Akkaya et al. 1992; Röder et al. 1995; Struss and Plieske 1998). Additionally, analysis is strongly facilitated by the implementation of partial automation and fluorescent detection methods (Mitchell et al. 1997). In contrast to RFLP markers a major drawback of microsatellite markers is their restricted application to very closely related species.

The abundance, characterization and the usefulness of microsatellite markers in *Brassica* species is well documented (Lagercrantz et al. 1993; Kresovich et al. 1995; Szewc-McFadden et al. 1996; Uzunova and Ecke 1999). A large number of microsatellites from rapeseed (*Brassica napus* L.) have been identified and characterized by Plieske and Struss (2000). These authors demonstrated that such microsatellites are not useful as markers for a wide range of species in the family *Brassicaceae*. However, many rapeseed microsatellite flanking primer pairs proved to be functional in the A and C genome species within the genus *Brassica*. These findings support the model of U (1935) who proposed that *Brassica campestris* (syn. *rapa*) (A genome, 2n=20) and *Brassica oleracea* (C genome, 2n=18), or the progenitors thereof, are the ancestors of the amphidiploid *B. napus* (AC genome, 2n=38). Hence, one objective of the present study is the identification of A and C genome-specific microsatellite loci by using resynthesized rapeseed and its respective

parents as well as *Brassica carinata* (BC genome, 2n=34) and *Brassica juncea* (AB genome, 2n=36).

RFLP markers from rapeseed have been successfully used for the construction of molecular-marker linkage maps of *B. oleracea* (Kianian and Quiros 1992; Landry et al. 1992; Hu et al. 1998) and *Brassica nigra* (Truco and Quiros 1994). Apart from that, many RFLP probes detect more than one locus in a single genome, indicating intragenomic duplications and rearrangements. There is a strong effort to establish or complement genetic maps of important crops by means of microsatellite markers (Röder et al. 1998). The high rate of functional primer pairs, derived from *B. napus*, in *B. oleracea* has tempted us to map polymorphic markers in a *B. oleracea* linkage map which has been previously generated by means of RFLP markers (Hu et al. 1998; Hu and Quiros, unpublished). The feasibility of mapping micro-satellites from rapeseed in *B. oleracea*, one of its progenitor genomes, is discussed.

## Materials and methods

### Plant material

Two *B. campestris* parents, Sv03261 and 'Emma', as well as two re-synthesized hybrids were kindly provided by C. Dixelius, SLU Uppsala, Sweden. Since the original C-genome parental plant from the *B. oleracea* variety 'Stor Dansk' was not available another individual of this variety was assayed. The *B. napus* variety 'Vivol' which has been used for the development of the microsatellite markers has been included as a control and for the comparison with re-synthesized *B. napus*.

The generation of the $F_2$ mapping population, derived from a cross between collard and cauliflower, has been described by Kianian and Quiros (1992). Microsatellites were screened for polymorphism between the two parental lines B115 (collard type) and B265 (cauliflower type). Polymorphic microsatellite loci were analysed in 62 individuals of the $F_2$ population.

### Microsatellite PCR

The development of rapeseed microsatellite markers (designated as "BMS") has been described by Plieske and Struss (2000). PCR was carried out in a volume of 25 µl with 50 ng of template DNA, 0.25 µM of each primer, 0.05 mM of each dNTP and 1 U of *Taq* polymerase (Qiagen) in 1× reaction buffer [Tris HCl, KCl, $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, pH 8.7]. The forward primer was labelled with Fluorescein or FAM (6-carboxyfluorescein) at the 5′ end. Thermal cycling was performed in a GeneAmp PCR System 9600 (Perkin Elmer) using the fastest ramp transition. The amplification profile consisted of an initial 3-min denaturation step at 94°C followed by 45 cycles of 1 min at 94°C, 1 min at the annealing temperature, 2-min elongation at 72°C and a final 60-min extension step at 72°C. Depending on the primer pair, annealing temperatures were 60°C (at most), 55°C or 50°C. Samples were stored at 4°C before use. Size standards were produced by PCR from the pBluescript vector (Stratagene) using standard flanking primers of the multiple cloning site.

### Microsatellite analysis

For analysis on an ABI377 sequencer (Applied Biosystems) ROX (6-carboxy-X-rhodamine)-labelled internal size standards of 71, 125, 140, 158, 194 and 227 bp were run together with the samples. Fluorescein-labelled 71- and 227-bp size standards were used for analysis on an A.L.F. sequencer (Pharmacia). Samples, including internal size standards (1 µl on ABI377, 3–4 µl on A.L.F.), were loaded on a 4.5% denaturing polyacrylamide gel (7 M urea, 29:1 acrylamide:bisacrylamide, 12 cm well-to-read). Gel files were documented and genotypes analyzed with the programs Fragment Manager v1.1 for A.L.F. files and GeneScan 3.0 and Genotyper 2.0 (Applied Biosystems) in the case of ABI377 gel files.

### Segregation and mapping analysis

The genotypes of 54 to 62 individuals were assessed for each microsatellite marker. The seg-regation was tested for goodness-of-fit ($\chi^2$) according to the expected Mendelian inheritance. Linkage analysis has been carried out with the MAPMAKER program v2.0 for Macintosh (Lander et al. 1987; Du Pont). The microsatellite genotypic data were combined with the RFLP mapping data for this population and computed together, setting the critical two-point LOD value to 4.0. Marker order was first determined by three-point analysis using a critical LOD value of 3.0. Markers which could not be assigned in this way were sequentially mapped as to their LOD value by multi-point analysis using the 'try' command. Recombination fre-quencies were converted into map distances (cM) by the Kosambi mapping function. The designation of RFLP markers followed Hu et al. (1998).

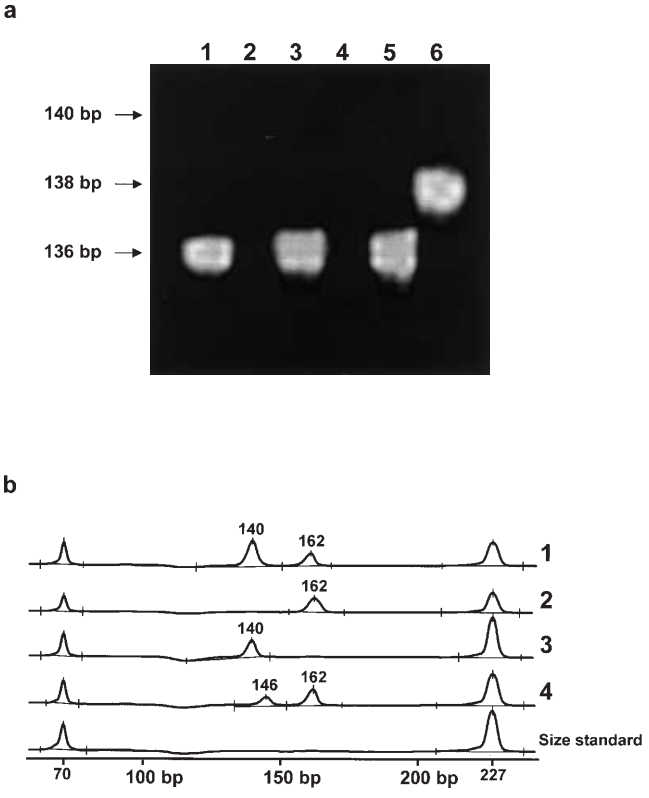


**Fig. 1a, b** Assignment of microsatellite loci from *B. napus* to the *Brassica* A and C genomes. **a** Gel image of the C genome-specific marker BMS 78, analyzed in *1* re-synthesized *B. napus* 2×5, *2 B. campestris* 'Emma', *3* re-synthesized *B. napus* 4×5, *4 B. campestris* Sv03261, *5 B. oleracea* 'Stor Dansk', 6: *B. napus* 'Vivol'. **b** Electropherogram showing two loci detected by BMS92. The larger fragment was assigned to the A, the smaller fragment to the C, genome. *1* Re-synthesized *B. napus* 2×3, *2 B. campestris* Sv03261, *3 B. oleracea* 'Stor Dansk', *4 B. napus* 'Vivol'

## Results

### Assignment of microsatellite loci to the A and C genomes

A total of 120 primer pairs flanking microsatellite loci in *B. napus* derived from a rapeseed genomic library (Plieske and Struss 2001) were used.

Sixty three primer pairs which yielded clearly identifiable bands within the control variety 'Vivol' were considered. A part of the remaining 57 primer pairs either failed to amplify specific loci or else the loci were monomorphic between the parental A and C genomes. Another part produced amplification products in re-synthesized rapeseed but not in the donor species *B. campestris* and *B. oleracea*. Thirty four primer pairs (54%) detected two loci in rapeseed, one derived from the A genome, the other one from the C genome (Fig. 1b). In most cases the loci could be unambiguously assigned to the genomes. Sixteen markers (25%) amplified fragments only in the A, and 13 (21%) only in the C, genome (Fig. 1a). The presence of A and C genome-specific microsatellite loci was also confirmed by analyzing the species *B. juncea* and *B. carinata* (Plieske and Struss 2000) which also carry the A and the C genomes, respectively.

### Screening for polymorphism

A total of 92 microsatellites have been chosen as to their PCR specificity in rapeseed for mapping in a segregating population of *B. oleracea*. These were screened for ampli-fication and polymorphism in both parents of the *B. oleracea* mapping population. Fifty-nine primer pairs (64.1%) generated one or more distinct PCR products of which 49 primer pairs (83.1%) disclosed a single locus. The remaining 33 microsatellite primer pairs were either unspecific, as shown by a smear or superfluous bands (12 primer pairs) or did not amplify at all (21 primer pairs). Twenty nine out of the 59 specific microsatellite loci (49.2%) were polymorphic between the parents.

**Table 1** Segregation data of 31 microsatellite loci in the *B. oleracea* mapping population. An asterisk in the last column indicates significance at the 5% level

| Marker | No. of individuals | Segregation | $\chi^2_{1:2:1}$ | $\chi^2_{3:1}$ | *P* |
|---|---|---|---|---|---|
| BMS6a | 62 | 20:27:15 | 1.84 | | 0.40 |
| BMS6b | 62 | 43:19 | | 1.05 | 0.31 |
| BMS6c | 62 | 46:16 | | 0.02 | 0.89 |
| BMS9 | 62 | 12:33:17 | 1.06 | | 0.59 |
| BMS19 | 60 | 12:31:17 | 0.90 | | 0.64 |
| BMS25 | 55 | 10:26:19 | 3.11 | | 0.21 |
| BMS29 | 58 | 18:24:16 | 1.86 | | 0.39 |
| BMS40 | 61 | 12:27:22 | 4.08 | | 0.13 |
| BMS41a | 60 | 10:32:18 | 2.40 | | 0.30 |
| BMS41b | 61 | 46:15 | | 0.01 | 0.92 |
| BMS41c | 62 | 48:14 | | 0.19 | 0.66 |
| BMS78 | 62 | 16:34:12 | 1.10 | | 0.58 |
| BMS92 | 62 | 48:14 | | 0.19 | 0.66 |
| BMS102 | 54 | 10:24:20 | 4.37 | | 0.11 |
| BMS106 | 55 | 39:16 | | 0.49 | 0.48 |
| BMS107 | 62 | 49:13 | | 0.54 | 0.46 |
| BMS171 | 61 | 12:27:22 | 4.08 | | 0.13 |
| BMS180 | 61 | 14:33:14 | 0.41 | | 0.81 |
| BMS210a | 61 | 8:36:17 | 4.64 | | 0.10 |
| BMS210b | 62 | 50:12 | | 1.05 | 0.31 |
| BMS273 | 60 | 16:26:18 | 1.20 | | 0.55 |
| BMS277 | 62 | 20:27:15 | 1.84 | | 0.40 |
| BMS289 | 57 | 15:29:13 | 0.16 | | 0.92 |
| BMS295 | 58 | 17:29:12 | 0.86 | | 0.65 |
| BMS315 | 62 | 6:37:19 | 7.77 | | 0.02* |
| BMS323 | 62 | 47:15 | | 0.02 | 0.89 |
| BMS325 | 62 | 19:29:14 | 1.06 | | 0.59 |
| BMS341 | 59 | 8:32:19 | 4.53 | | 0.10 |
| BMS354 | 62 | 48:14 | | 0.19 | 0.66 |
| BMS371-1 | 56 | 12:32:12 | 1.14 | | 0.57 |
| BMS397 | 61 | 20:28:13 | 2.02 | | 0.36 |

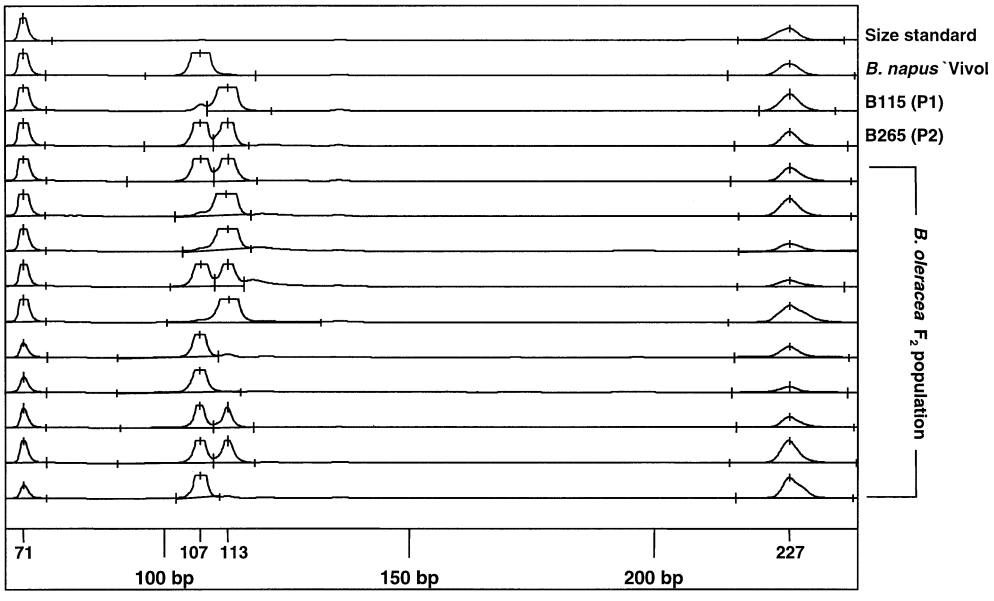


**Fig. 2** Codominant segregation of marker BMS49 in the *B. oleracea* mapping population displayed as an electropherogram. The internal size standards are 71 and 227 bp

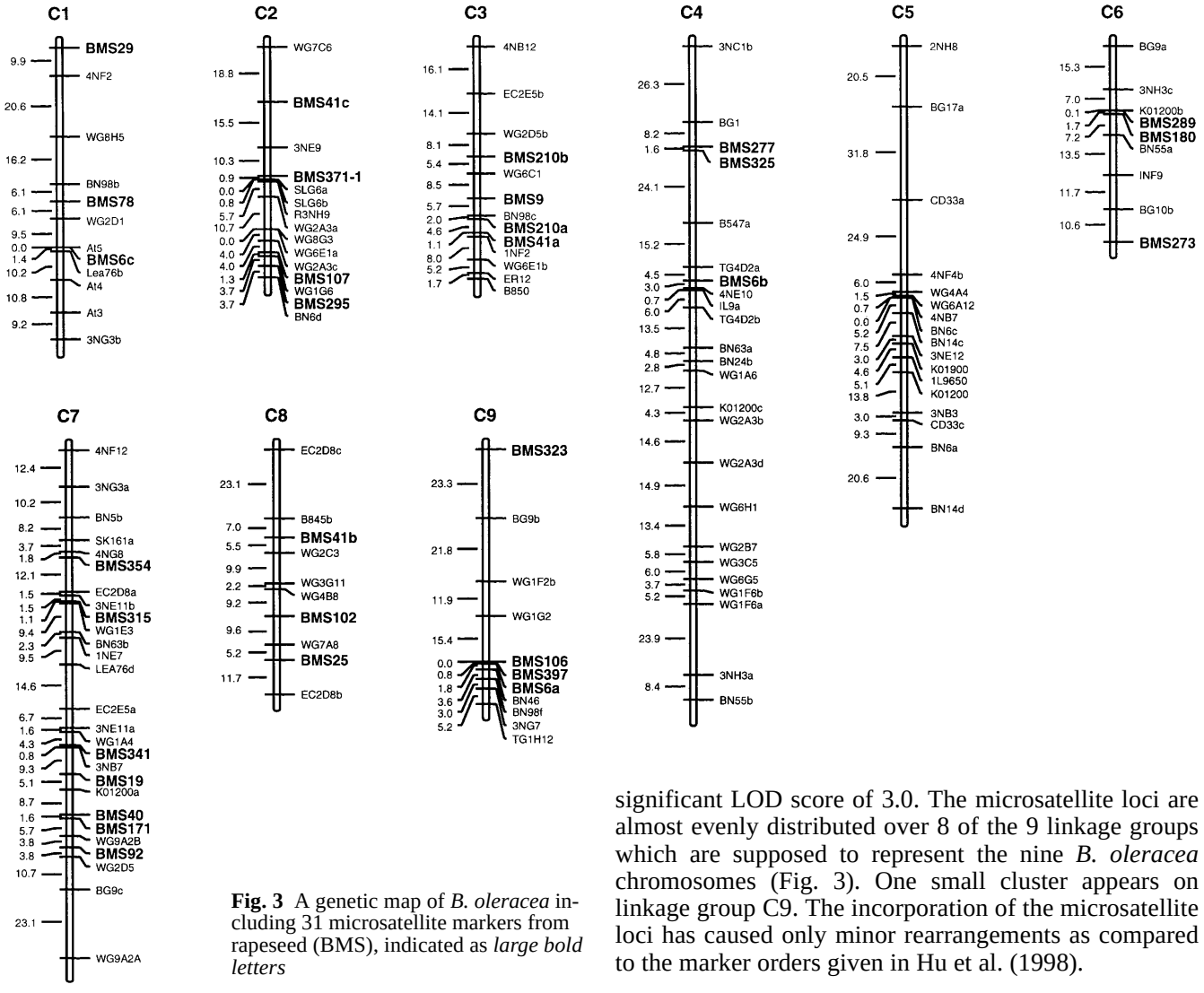


**Fig. 3** A genetic map of *B. oleracea* including 31 microsatellite markers from rapeseed (BMS), indicated as *large bold letters*

Segregation analysis and genetic mapping

As some of these primer pairs disclosed more than one polymorphic locus, a total of 34 marker loci segregated in the mapping population. In detail, markers BMS6 and BMS41 each disclosed three loci and BMS210 two loci. The majority, however, detected single loci. As an example an A.L.F. sequencer electropherogram of the polymorphic marker BMS49 is shown in Fig. 2. In this case one parent is still heterozygous due to the high degree of heterozygosity in the allogamous *B. oleracea*. Twenty-three loci exhibited the expected codominant inheritance while 11 loci segregated as dominant markers. With the exception of marker BMS315, none of the markers showed a significant distortion from the expected segregation ratios of 1:2:1 or 3:1, respectively (Table 1). However, some markers have conspicuously high $\chi^2$ values which are in each case caused by the reduced frequency of the B115 parental allele.

Linkage relationships have been established for 31 loci. The loci BMS49, BMS55 and BMS290, however, could not be mapped to a particular linkage group at a significant LOD score of 3.0. The microsatellite loci are almost evenly distributed over 8 of the 9 linkage groups which are supposed to represent the nine *B. oleracea* chromosomes (Fig. 3). One small cluster appears on linkage group C9. The incorporation of the microsatellite loci has caused only minor rearrangements as compared to the marker orders given in Hu et al. (1998).

## Discussion

Genome specificity of microsatellite markers

Genome specificity is a prominent feature of microsatellite markers. In crops with composite genomes, such as *B. napus*, the applicability of microsatellite markers may be extended to the progenitor species as already known for RFLP markers (Cheung et al. 1997). Microsatellite analysis of *Brassica* A and C-genome species allowed us to assign single micro-satellite loci to the A or C genomes. This study clearly demonstrates that, in most cases where two loci are detected by a single primer pair, this is not the result of duplication events within the A or C genomes but rather due to amplification of single loci from each genome. This also means that homology at the primer sites in the A and C genomes is sufficiently high for PCR-amplification. These segments may be part of larger homoeologous regions where recombination between the A and C genomes can occur.

Earlier investigations on a small set of microsatellites derived from rapeseed (Lagercrantz et al. 1993;

Kresovich et al. 1995; Szewc-McFadden et al. 1996) have already shown their potential as genome-specific markers in *Brassica rapa* and *B. oleracea*, although to a different extent. Mitchell et al. (1997) reported that almost half of the primer pairs failed to amplify in most *B. rapa* accessions whereas the majority were able to generate specific products in *B. oleracea*. These primer sets are presumably A and C genome-specific, respectively. In an extensive study analyzing 81 primer pairs Plieske and Struss (2000) demonstrated that all *Brassica* species carrying the A and/or C genomes exhibited the highest fraction of microsatellite-specific loci relative to rapeseed, while only less than one-third of the markers were present in *B. nigra* (B genome, 2n=16) and other cruciferous species. The identified genome-specific microsatellite loci can therefore be used as markers for the detection of somatic and re-synthezised hybrids, aneuploid, translocation and substitution lines in A- and C-genome species.

### Genetic mapping in *B. oleracea*

For some population genetic studies it is advisable to analyze unlinked loci in order to obtain unbiased estimates. In addition, a good coverage of the genome is a prerequisite to identify markers which are linked to genes controlling important agronomic characters. The high degree of usability of rapeseed microsatellite markers in *B. oleracea* enabled us to integrate these markers into a *B. oleracea* linkage map. The frequency of primer pairs revealing polymorphic loci (49.2%) is slightly higher than for RFLPs and other markers in this mapping population (45%) (Kianian and Quiros 1992), indicating their higher potential to detect polymorphism. It also reflects the wide genetic variation in this allogamous species. Almost every primer pair corresponds to a single marker indicating that only very few microsatellite loci including their flanking regions are present in more than one copy in the C genome. In most cases, however, the variability at the nucleotide level is obviously high enough so that only single sites are tagged.

With the exception of linkage group C5 the markers are almost evenly distributed across the *B. oleracea* genome. First results obtained from 29 polymorphic microsatellite loci in a segregating rapeseed population (data not shown) also confirm that most markers are unlinked and might therefore be well-spread over the rapeseed chromosomes. So far, genetic mapping of microsatellites in *Brassica* species has been scarcely reported (Uzunova and Ecke 1999). But experience from other plant species where the map locations for many microsatellite loci has been determined (Chen et al. 1997; Röder et al. 1998) suggests that these markers represent a valuable source for gene mapping. Saturated mapping of microsatellite markers in *B. oleracea*, as well as in *B. campestris* and *B. napus*, can be successfully used to reveal the homoeologous relationships between *B. napus* and its progenitor genomes. Including re-synthesized *B. napus*, the degree of synteny and the number of rearrangements which have occurred during the evolution of *B. napus* can be assessed.

**Acknowledgements** The authors thank I. Schubert for helpful comments on the manuscript, M. Hantschmann and B. Schmidt for excellent technical assistance.

## References

Akkaya SM, Bhagwat AA, Cregan PB (1992) Length polymorphism of simple sequence repeat DNA in soybean. Genetics 132:1131–1139

Chen X, Temnykh S, Xu Y, Cho YG, McCouch SR (1997) Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). Theor Appl Genet 95:553–567

Cheung WY, Champagne G, Hubert N, Landry BS (1997) Comparison of the genetic maps of *Brassica napus* and *Brassica oleracea*. Theor Appl Genet 94:569–582

Hu J, Sadowski J, Osborn TC, Landry BS, Quiros CF (1998) Linkage group alignment from four independent *Brassica oleracea* RFLP maps. Genome 41:226–235

Kianian SF, Quiros CF (1992) Generation of a *Brassica oleracea* composite RFLP map: linkage arrangements among various populations and evolutionary implications. Theor Appl Genet 84:544–554

Kresovich S, Szewc-McFadden AK, Bliek SM, McFerson JR (1995) Abundance and characterization of simple sequence repeat (SSR) loci isolated from a size-fractionated genomic library of *Brassica napus* L. (rapeseed). Theor Appl Genet 91:206–211

Lagercrantz U, Ellegren H, Andersson L (1993) The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. Nucleic Acids Res 21:1111–1115

Lander ES, Green P, Abrahamson J, Barlow A, Daly M, Lincoln S, Newburg L (1987) Mapmaker: an interactive computer package for constructing primary linkage maps of experimental and natural populations. Genomics 1:174–181

Landry BS, Hubert N, Crete R, Chang MS, Lincoln SE, Etho T (1992) A genetic map of *Brassica oleracea* based on RFLP markers detected with expressed DNA sequences and mapping of resistance genes to race 2 of *Plasmodiophora brassicae* (Woronin). Genome 35:409–420

Mitchell SE, Kresovich S, Jester CA, Hernandez CJ, Szewc-McFadden AK (1997) Application of multiplex PCR and fluorescence-based, semi-automated allele sizing technology for genotyping plant genetic resources. Crop Sci 37:617–624

Plieske J, Struss D (2001) Microsatellite markers for genome analysis in *Brassica*. I. Development and abundance in *Brassica* species. Theor Appl Genet 102:689–694

Röder MS, Plaschke J, König SU, Börner A, Sorrells ME, Ganal MW (1995) Abundance, variability and chromosomal location of microsatellites in wheat. Mol Gen Genet 246:327–333

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Struss D, Plieske J (1998) The use of microsatellite markers for detection of genetic diversity in barley populations. Theor Appl Genet 97:308–315

Szewc-McFadden AK, Kresovich S, Bliek SM, Mitchell SE, McFerson JR (1996) Identification of polymorphic, conserved simple sequence repeats (SSRs) in cultivated *Brassica* species. Theor Appl Genet 93:534–538

Truco MJ, Quiros CF (1994) Structure and organization of the B genome based on a linkage map in *Brassica nigra*. Theor Appl Genet 89:590–598

U N (1935) Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and its peculiar mode of fertilization. Jpn J Genet 7:784–794

Uzunova MI, Ecke W (1999) Abundance, polymorphism and genetic mapping of microsatellites in oilseed rape (*Brassica napus* L.). Plant Breed 118:323–326