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Molecular-mapping analysis in *Brassica napus* using isozyme, RAPD and RFLP markers on a doubled-haploid progeny

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Abstract We have undertaken the construction of a *Brassica napus* genetic map with isozyme (4%), RFLP (26.5%) and RAPD (68%) markers on a 152 lines of a doubled-haploid population. The map covers 1765 cM and comprises 254 markers including three PCR-specific markers and a morphological marker. They are assembled into 19 linkage groups, covering approximatively 71% of the rapeseed genome. Thirty five percent of the studied markers did not segregate according to the expected Mendelian ratio and tended to cluster in eight specific linkage groups. In this paper, the structure of the genetic map is described and the existence of non-Mendelian segregations in linkage analysis as well as the origins of the observed distortions, are discussed. The mapped RFLP loci corresponded to the cDNAs already used to construct *B. napus* maps. The first results of intraspecific comparative mapping are presented.

Key words *Brassica napus* · L. Genetic mapping · RFLP and RAPD markers · Segregation distortions · Comparative mapping

Introduction

The expansion of molecular tools has led to the construction of genetic maps for a large number of plant species over the last 10 years. Many species of the Cruciferae family, a world-wide plant group of agronomical and economical importance, have benefited from this approach, and especially the species constituents of the U (1935) triangle. Thus, genetic maps have been published for the diploid species *Brassica oleracea* (Slocum et al. 1990; Kianian and Quiros 1992; Landry et al. 1992; Bohuon et al. 1994; Chyi et al. 1994; Le Corre et al. 1994), *B. rapa* (McGrath and Quiros 1991; Song et al. 1991; Chyi et al. 1992; Teutonico and Osborn 1994), and *B. nigra* (Truco and Quiros 1994; Lagercrantz and Lydiate 1995). With the exception of *B. carinata*, the amphidiploid species, *B. napus* (Landry et al. 1991; Lydiate et al. 1993; Chyi et al. 1994; Ferreira et al. 1994; Parkin et al. 1995; Sharpe et al. 1995; Uzunova et al. 1995) and *B. juncea* (Mohapatra et al. 1995) have also been mapped. Most of the genetic maps were constructed from F_2 populations although some recent *Brassica* maps were derived from doubled-haploid populations (Bohuon et al. 1994; Ferreira et al. 1994; Parkin et al. 1995; Sharpe et al. 1995; Uzunova et al. 1995) since most of them, and especially *B. napus*, are species amenable to in vitro androgenesis. DH progenies represent a reference population on which genetic mapping can be easily coupled to quantitative trait studies. A DH population, compared to an equal sized F_2 population, is also recommended for genetic mapping when dominant markers are mainly used since a better estimate of the recombination frequency is obtained.

For all the published *Brassica* maps, markers were mainly RFLP-derived from cDNA libraries, therefore mapping coding genomic regions. However, the availability of new types of markers has recently led to a diversification of the markers employed (Truco and Quiros 1994). Each type of marker has its merits and drawbacks (price, ease of use, scoring of co-dominants, availability in large numbers, relation to loci of known function) and their use is dependent on laboratory facilities as well as the aim of the research program.

The existing genetic maps of *Brassica* have already been used for an interspecific comparison of genome organisation (McGrath and Quiros 1991; Lydiate et al. 1993, Chyi et al. 1994; Parkin et al. 1995). Comparisons

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between species were enlarged within the Cruciferae through the analysis of the well-known *Arabidopsis thaliana* (Kowalski et al. 1994), a useful model system for basic genetic studies, for which many genetic maps have been published (Koornneef et al. 1983; Chang et al. 1988; Nam et al. 1989; Reiter et al. 1992; Hauge et al. 1993; Lister and Dean 1993; McGrath et al. 1993). By contrast, to our knowledge, no intraspecific comparisons have been published although this may be an interesting way to determine genomic regions associated with chromosomal rearrangements.

The present paper reports on the development of a *Brassica napus* genetic map on a doubled-haploid population. The parental lines were chosen on the basis of two criteria: (1) high polymorphism level between them, and (2) the occurrence of many segregating agronomic traits. A cross between 'Darmor-*bzh*' and 'Yudal' was selected since it was segregating for oligogenic, as well as polygenic, traits concerning plant development (dwarfism, earliness of flowering), seed quality (erucic acid and glucosinolates contents) and disease resistance (to *Phoma lingam, Cylindrosporium concentricum*).

Segregation studies were mainly done with RAPD markers due to the ease of the technique and in order to map randomly coding, as well as non-coding, sequences (Williams et al. 1990). Isozymes, commonly used in our laboratory were mapped. RFLP markers corresponding to selected cDNA probes already used for the construction of *B. napus* maps (Landry et al. 1991; Dion and Landry, unpublished data) were employed. The RFLP markers represented evenly spaced landmarks of the *B. napus* genome and were used to speed up the development of our map.

Here we report on the management of markers showing distorted segregation ratios in linkage analysis. The interest of using different types of markers is discussed, and first results of intraspecific comparisons using RFLP markers are described.

Materials and methods

Parental lines and mapping progeny

A doubled-haploid (DH) population was derived from isolated microspore cultures (as described by Polsoni et al. 1988) of a single F_1 hybrid plant obtained from the cross 'Darmor-*bzh*' × 'Yudal'. 'Darmor-*bzh*' is a dwarf isogenic line (BC_3F_3) resulting from the introduction of the dwarf *Bzh* gene (Foisset et al. 1995) in a French Winter cultivar, 'Darmor'. 'Yudal' is a Spring Korean line (F_9) that behaves as an early-flowering winter type in temperate climates. More than 400 DH lines were produced either by spontaneous chromosome doubling or colchicine treatment (Foisset et al. 1996).

A total of 152 DH lines were randomly selected, from lines with good seed set, for segregation analyses and genetic mapping. A DH population of this size provides estimates of the recombination frequency (γ) so that the standard deviation S γ is $<1/2\,\gamma$ for every $\gamma > 0.03$ (which is equivalent to an F_2 population of 90 individuals scored with co-dominant markers). Isozyme assays on fresh leaves and DNA extraction for RAPD and RFLP markers were performed on H_1 plants (first generation of selfing of the derived DH lines).

Isozyme analysis

Nine isoenzymatic systems were tested: acid phosphatase (APS), aconitase (ACO), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6 PGD), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), shikimic dehydrogenase (SDH) and triosephosphate isomerase (TPI). The isozyme nomenclature was that of Chèvre et al. (1995). All systems except APS were tested after electrophoresis on starch gels. Preparation of starch gels and total protein extraction from young leaves have been described in Chèvre et al. (1995). The gel/electrode buffers proposed by Shields et al. (1983) were used: morpholine citrate pH 6.1 (buffer G) for LAP, MDH, and 6 PGD; histidine tris citrate pH 7.0 (buffer E) for ACO, PGI and PGM; tris citrate borate pH 7.5 (buffer N) for SDH; and tris citrate lithium borate pH 8.3 (buffer C) for TPI. Staining procedures were as described by Arus and Orton (1983) for LAP, Wendel and Stuber (1984) for ACO, and Vallejos (1983) for the others. APS was run on acrylamide gels. Acrylamide gel electrophoresis conditions were as described in Chèvre et al. (1995).

DNA extraction

Young leaves were harvested from each DH line (several plants were collected) and kept at –80°C until DNA extraction. Leaves were ground in a mortar with liquid nitrogen. DNA was extracted as described in Doyle and Doyle (1990).

RAPD and PCR-specific analysis

RAPD reactions were performed in 1.9 mM $MgCl_2$, 150 µM dNTP (Boerhinger), 0.2 µM primer, 0.5 unit *Taq* polymerase (Eurobio), 1.25 µl 10×*Taq* polymerase buffer (Eurobio) and 12.5 ng genomic DNA. The final volume was adjusted to 12.5 µl with sterile double distilled H_2O. DNA was amplified in a 480 or 9600 Perkin Elmer Cetus DNA thermocycler, using a cycling profile of 30 s at 94°C followed by 45 cycles of 30 s at 94°C, 1 min at 35°C and 3 min at 72°C. At the end of the 45 cycles, the reactions were held at 72°C for 5 min. Amplified products were separated on 1.8% agarose gels prepared in 1×TAE buffer. Gels were run 5 h at 3 V/cm and then stained in a 0.5 µg/ml of ethidium bromide solution.

Random primers were from Operon Technologies Inc. They are designated by an 'OP' prefix, followed by the kit letter and primer number. RAPD loci are then specified by adding the size (in base pair) of the corresponding amplified DNA. When RAPD markers are co-dominant, the sufix 'cd' followed by a number (indicative of the number of co-dominant markers obtained with the same primer) is added to the name instead of the molecular weight of the two bands. Three specific-PCR markers were also mapped: *Slg*, a self-incompatibility gene isolated from *B. oleracea* (Brace et al. 1994), the *Fad3.1900* locus, a gene coding for a Δ15 desaturase (Arondel et al. 1992), and the nga 162 locus, a microsatellite of *A. thaliana* (Bell and Ecker 1994).

RFLP analysis

A total of 56 RFLP probes representing loci evenly spaced on the *B. napus* maps established by Landry et al. (1991) and Dion and Landry (unpublished data), were selected, and the corresponding cDNA probes used. Most of them correspond to anonymous clones from a cDNA library from a *B. napus* embryo (Harada et al. 1988), and were designated as 1ND1 to 6NF3. Four seedling-specific cDNA clones named pCa15, pCa25, pGs43 and pCot44 (Harada et al. 1988) and a cruciferin cDNA clone, pC1 (Simon et al. 1985), corresponding to a major storage protein in *Brassica*, were also used as probes. Probe preparation and Southern blot analyses were as described in Cloutier et al. (1995). Only one restriction enzyme, *HindIII*, was used. Duplicated loci detected by a single clone were designated by the same name followed by a different lower case letter as described in Landry et al. (1991).

Genetic linkage analysis

Goodness of fit to expected Mendelian ratios for each segregating locus was tested by chi-square analysis (α=1%). Markers with too great distortion values (chi-square>50) were excluded. Linkage analyses were performed on Mapmaker/exp version 3.0 (Lincoln et al. 1992). Linkage groups were first established with a minimum LOD score of 4.0 and a maximum recombination frequency of 0.4. Markers within each linkage group were then ordered using only highly informative and evenly spaced markers ("Compare" command, LOD>3.0). The remaining markers were sequentially placed ("Try" command, LOD>2.0) using first the most informative markers. The framework was verified with the "Order" command (LOD>3.0). The best one was confirmed by permuting all adjacent triplets of markers ("Ripple" command, LOD>2.5). Arrangements of the linkage groups with distorted segregation ratios were confirmed by a chi-square (Mather 1957) to test the independence of two segregations, conditional on their marginal frequencies; tests (α=1%) were applied between pairs of markers within each group and between groups. Distances were re-estimated with the product formula of Bailey (1949). Centimorgan distances were expressed with the Kosambi function (Kosambi 1944).

Results

Polymorphism observed between 'Darmor-*bzh*' and 'Yudal' and marker characteristics

Only markers that were reproducible, and consequently could be scored unambiguously on the corresponding DH progeny, were analysed. The polymorphism level was expressed as a function of the isozyme system, the RAPD primer or the RFLP probe that detected at least one polymorphic locus.

Isoenzymatic markers

From the nine systems tested, all but one (PGI) were polymorphic between the two parental lines, which represented an isozyme polymorphism level of 89%. Two loci for the MDH and TPI systems were polymorphic. A total of ten isoenzymatic loci were then mapped. Isozyme markers corresponded to co-dominant allozymes but six were scored as dominant due to unresolved alleles that migrated to the same location as a band of a monomorphic locus. Duplicated loci have been demonstrated in rapeseed for all systems studied (Delourme and Foisset 1991).

RAPD markers

A total of 266 RAPD primers, randomly chosen, were tested. Most of them (89.5%) were polymorphic between 'Darmor-*bzh*' and 'Yudal'. Each primer gave on average 1.9 markers (1 to 5 per primer). Then, 183 RAPD markers revealed with 95 primers were analysed on the DH progeny, of which ten were scored as co-dominant.

RFLP markers

From the 56 clones directly tested on both the parental and the progeny DNA cut with only one enzyme (*Hin*dIII), nine were monomorphic; that is to say, 84% of them gave at least one segregating band in the DH progeny. Each probe revealed on average 1.6 markers (1 to 3 per probe). Sixty nine RFLP loci were used for mapping. Fifty one loci gave a co-dominant scoring. The others were analysed as dominant due to the overlapping of bands with a monomorphic locus or the absence of the alternate band. Nineteen probes detected two to three polymorphic loci. Thus, the majority of the probes detected only one polymorphic locus while hybridizing to additional monomorphic bands. Only one probe, named 1NH6, displayed a hybridization profile that would correspond to a single-copy DNA sequence.

Segregation analysis in the doubled-haploid progeny

In a DH population, a 1:1 Mendelian segregation ratio is expected when no selection occurs. Out of the 266 markers assayed, 34.6% did not segregate according to this expected Mendelian ratio (α=1%), with an equal number of loci favouring the alleles of each parental line. The three types of genetic markers revealed some loci with biased segregation [isozyme (30%), RFLP (30%) and RAPD (36.6%)] and the proportions of loci with a distorted segregation ratio were not significantly different between the three types of marker (chi-square test value for contingency=2.1, P=35.1%).

Linkage analysis with markers showing non-Mendelian segregation

Linkage groups and frameworks were first established with the Mapmaker program. Loci with biased segregation were included in the analysis. The use of a LOD≥4.0 generated 17 linkage groups containing at least four markers, from a raw data file of 266 markers. One of these groups was in fact a spurious linkage group that was split into three distinct groups – DY6, DY7 and DY11. These three groups are quite exclusively made up with markers that show segregation distortion in favour of 'Yudal' alleles. This phenomenon arose early during the construction of the map when less markers were available. During these preliminary analyses, two groups were established: one corresponding to DY7 and another one to the association of DY6 and DY11. Through the addition of new markers, DY6 and DY11 could be split, but three markers (3NF11a, 1NG2a and 1NH6a) of the final DY6 group remained at the end of DY11 (Fig. 1A). Chi-square tests of independence were in fact not significant (P>1%) between those three markers and the other markers of the DY11 group, implying independence. Bailey's estimate confirmed the inaccuracy of the distances given by Mapmaker (Fig. 1A). Then, linkage was detected by chi-square test between those three mark-

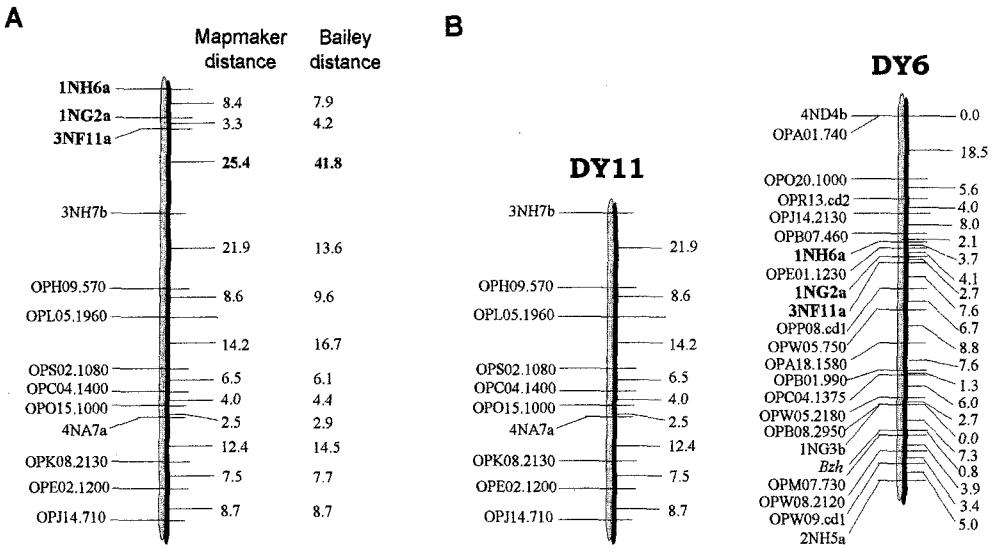


Fig. 1 Linkage group construction with distorted markers and correction. **A** Linkage group DY11 constructed with Mapmaker without considering the existence of distortions. Comparison to distances derived by the estimate of Bailey. Inconsistancies between both estimates, as well as doubtful markers, are indicated in *bold face* type. **B** Structure of linkage groups DY11 and DY6 constructed by integrating distortion. Distances are expressed in Kosambi cM

ers and linkage group DY6 ($P<1\%$). For both DY6 and DY11, frameworks were then re-estimated with Mapmaker taking into account a chi-square test of independence correction (Fig. 1B). With the raw data file of 266 markers, a LOD≥6.0 was needed to separate correctly the DY6, DY7 and DY11 groups.

For all the other groups, chi-square tests of independence between all pairs of markers within each group, and between groups comprising markers with biased segregation, confirmed the grouping established with Mapmaker. Distances between pairs of markers along each framework were re-evaluated using Bailey's estimate. Observed values were close to the output results delivered by Mapmaker. Therefore, Mapmaker distances were kept in this study. The structure of the linkage groups was confirmed by running Mapmaker only with markers showing Mendelian segregation. The assignment and order of the Mendelian markers were both identical to those obtained when loci with biased segregation were included in the analysis.

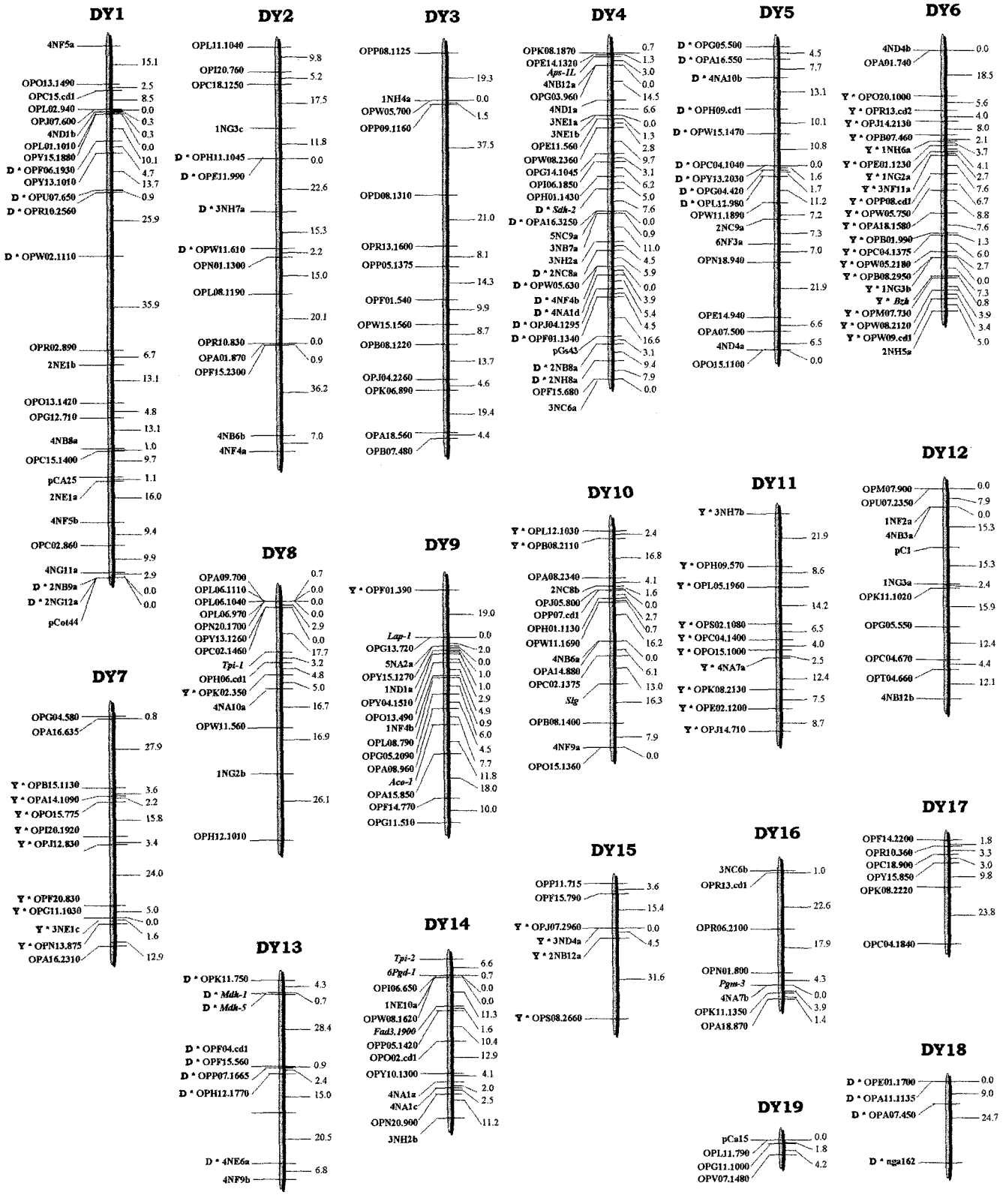
Genetic linkage map

The genetic linkage map derived from the cross 'Darmor-*bzh*' × 'Yudal' comprised 254 markers assembled into 19 linkage groups (Fig. 2). From the 266 segregating loci tested, 12 remained unlinked. The linked loci covered 1765 cM; linkage groups varied in size from 6.5 to 206 cM, with an average spacing of 7 cM between loci. Spacing between markers ranged from 0 to 37.5 cM. Markers with distorted segregation ratios tended to cluster in eight specific linkage groups, each of the clusters comprising loci exclusively favouring the alleles of the same parental line (Fig. 2). Four of these groups contained loci with an excess of 'Yudal' alleles (DY6, DY7, DY11 and DY15) whereas the four others were skewed towards 'Darmor-*bzh*' alleles (DY4, DY5, DY13 and DY18).

The genetic map was constructed mostly with RAPD markers (68% of the segregating markers) but also included isozyme (4%) and RFLP (26.5%) loci. A microsatellite derived from *Arabidopsis thaliana* (Bell and Ecker 1994) was also mapped (nga 162, linkage group DY18). The distribution of each kind of tested marker was generally even on the linkage groups, although DY3, DY7, DY17 and DY18 were essentially composed of RAPD markers. If the markers were randomly distributed throughout the genome, the number of markers within each linkage group should be directly correlated with the length of the group; the longer a linkage group is, the more markers it should have. The correlation coefficient r (calculated without the loci positioned on the map by targeted mapping), between the size of the linkage groups and their number of loci ($r=0.79$; $P=10^{-4}$) confirmed this hypothesis. However, DY2, DY3 and DY4 deviate slightly from the linear regression model; the number of markers per 10 cM varied from 0.7 for DY2 and DY3 to 2.1 for DY4.

Most markers were of unknown function except for the isozyme loci that are involved in the metabolic pathway of plant cells and five cDNA clones corresponded to the specific genes described in Materials and methods. A morphological marker (*Bzh*, group DY6) which corresponds to a dwarf *B. napus* gene (Foisset et al. 1995) was also localized on the linkage map, as well as two specific PCR markers including *Slg* (linkage group DY10), a *B. oleracea* self-incompatibility gene (Brace et al. 1994) and *Fad3.1900* (linkage group DY14; Jourdren et al. 1996a), a Δ15 desat-

Fig. 2 Linkage map of *B. napus*. Linkage groups are named DY1 to DY19 in decreasing order of size. Map distances, in Kosambi cM, are indicated on the right side of the linkage groups and locus name (according to the nomenclature described in Materials and methods) is given on the left. Names of loci with biased segregation are preceded by an *asterisk* and the letter *D* (for 'Darmor-*bzh*') or *Y* (for 'Yudal') according to the favoured parental line



Unlinked markers:

1NF7a, 2NB8d, Y * OPD08.875, OPG15.560, OPA16.1820, Y * OPY04.2130, OPW03.550, Y * OPD20.760, OPD20.820, Y * OPV19.510, OPV19.870, OPV07.1000

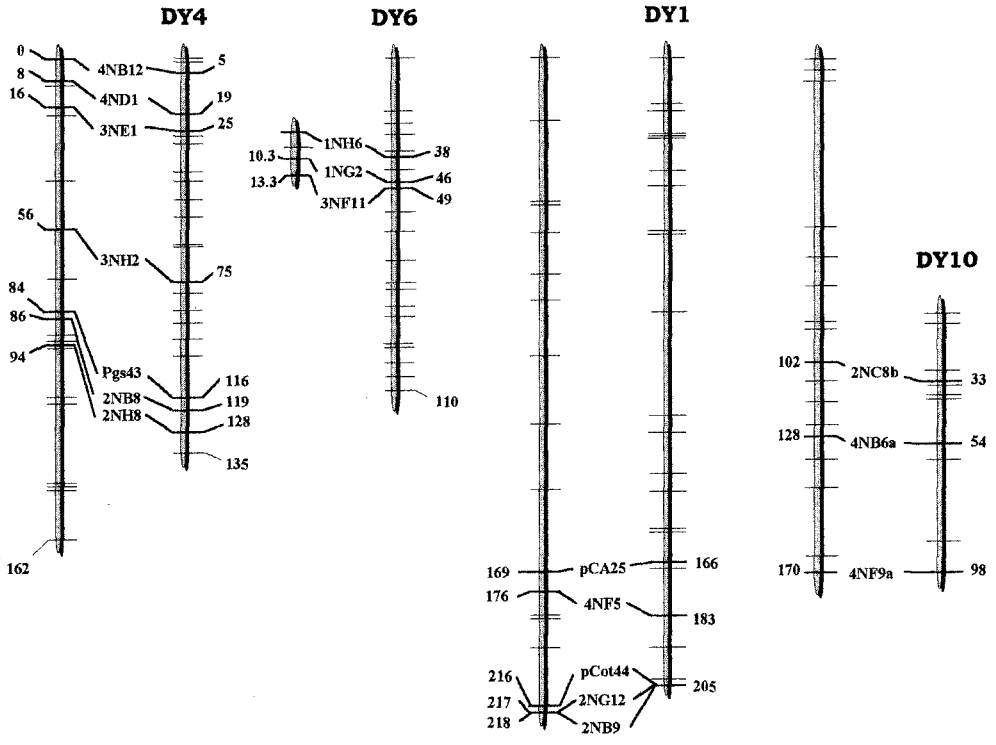


Fig. 3 Intraspecific *B. napus* comparative mapping between linkage groups from the cross 'Darmor-*bzh*' × 'Yudal', on the right side, and 'Westar' × 'Crésor' on the left (Dion and Landry unpublished data). Distances are indicated in Kosambi cM. Mapped loci are indicated by a *perpendicular dash* on the linkage groups. Only the names of common loci between both maps are mentioned

urase encoding-gene (Arondel et al. 1992) involved in fatty acid biosynthesis.

Intraspecific comparative mapping approach

The cDNA probes employed were previously used to construct *B. napus* genetic maps (Landry et al. 1991; Dion and Landry, unpublished data). Direct testing of the co-linearity of linkage groups revealed conserved RFLP loci organisation between four of our linkage groups (DY1, DY4, DY6 and DY10) and Dion and Landry's *B. napus* map (unpublished data) based on the cross 'Westar' × 'Crésor' (Fig. 3).

Discussion

Genetic mapping and segregation distortions

Segregation ratios that do not follow the expected Mendelian ratios are common. The construction of detailed genetic maps in several plant species has highlighted this phenomenon and has offered the opportunity to study genetic segregation more extensively by considering a greater part of the genome. However, the consequences of segregation distortion in linkage analysis must not be under-estimated since statistical tests may themselves be biased (Lorieux et al. 1995a, b). One of the assumptions underlying the commonly used software, Mapmaker, is the absence of disturbed segregation (Lander et al. 1987). Its approach to multipoint linkage analysis is based on the maximum-likelihood estimate, with linkage and marker order being supported through the LOD score test (Morton 1955). In this case, segregation distortion may generate false-positive linkages, leading to the aggregation of two or several linkage groups (Lorieux et al. 1995a,b). The determination of the order may also be inaccurate. Complementary methods to Mapmaker software have been proposed (Lorieux et al. 1995a, b). Chi-square tests of independence and especially Bailey's (1949) estimate, have been recommended (Lorieux et al. 1995a) for linkage analyses and order determination of loci displaying segregation distortion. In maize, a chi-square test of independence has been shown to be more suitable than the LOD score for reducing the number of spurious linkages when mapping loci with biased segregation, although 97% of the DH map length established with the LOD score test was consistent with the corresponding F_2 map obtained using loci with normal Mendelian segregation (Bentolila et al. 1992). In the present study, 34.6% of the segregating markers demonstrated a non-Mendelian segregation. Linkage data derived from Mapmaker were consistent with the chi-square test of independence and with Bailey's estimate except for the genomic regions corresponding to DY6 and DY11, where grouping error was detected and corrected. Markers with too strong deviations were excluded from genetic mapping since several frameworks could be proposed, with the impossibility to detect the best order. A similar conclusion

was drawn by Cloutier (1994). The use of a LOD≥6.0 was necessary to properly separate markers included in the present linkage groups DY6 and DY11. However, this threshold could not be applied to the whole map because it would have led to the break up into small groups of some of other linkage groups which did not contain markers with biased segregation.

Caution is, therefore, necessary when large proportions of loci with biased segregation are observed, and chi-square test of independence and Bailey's estimate are a useful complement to Mapmaker. However, only the comparative mapping with *B. napus* maps derived from different crosses where most markers show Mendelian segregation will validate linkages. Encouraging results have been obtained for the 'Darmor-*bzh*' × 'Yudal' map through the comparison with a *B. napus* map comprising fewer loci with biased segregation (21.5%, $P \leq 1\%$; Dion, personal communication). Co-linearity was observed between Dion and Landry's map (unpublished data) and the distorted linkage groups DY4 and DY6.Distorted segregation ratios have often been reported on androgenic plant material in various species (for review see Foisset and Delourme 1996) and especially in rapeseed (Ferreira et al. 1994; Uzunova et al. 1995). Two main hypotheses may be proposed for their existence: gametic selection or selection specific to in vitro microspore culture. Genomic regions involved in androgenic DH production have been reported in maize (Armstrong et al. 1992; Wan et al. 1992) and *B. napus* (Cloutier et al. 1995) and in most cases a clustering of loci with biased segregation, coupled to a deviation towards the parental line that responded well to in vitro microspore culture was observed (Graner et al. 1991; Heun et al. 1991; Bentolila et al. 1992; Murigneux et al. 1993). Our data indicated a clustering of biased loci on 8 linkage groups out of the 19 mapped. The number of loci with non-Mendelian segregation favoured the alleles of each parental line equally, although recent investigations have identified an obvious difference in the in vitro microspore culture capacity of both the parental lines used (Foisset et al. 1996; Lucas, personal communication). A relationship between segregation distortion and the genes controlling in vitro microspore culture responsiveness is assumed, but it would be necessary to analyse the segregation of the loci with biased segregation on the corresponding F_2 or BC populations in order to distinguish between genomic regions concerned with gametic selection from those involving in vitro androgenic controlling genes. Cloutier et al. (1995) reported a potential genomic region involved in microspore culture responsiveness (linkage group 1) that would correspond to linkage group DY4. The fact that this group is biased towards the alleles of the responsive parental line ('Darmor-*bzh*') reinforces such an assumption. However, we may suppose that one locus surrounding the marker 1NG2a on linkage group DY6 would control the observed distortion and would have a gametic origin. RFLP markers in this region were observed to be biased on a F_2 population as well as on a DH population from the cross 'Westar' × 'Topas' (Landry et al. 1991; Cloutier et al. 1995).

Genetic map structure

The present genetic map is 1765 cM in length but remains unsaturated since the size of the linkage groups increased as more markers were added and because 13 markers were unlinked. The formula proposed by Chakravarti et al. (1991) allows us to estimate the size of genome coverage from an incomplete map. On this basis, the genetic map we have established would cover 71% of the rapeseed genome. According to the formula proposed by Lange and Boehnke (1982), and considering a genome coverage equal to 0.95, 500 markers would be needed to cover the entire genome with an average spacing of 15 cM.

A linear relation between the length of the groups and the number of markers has been observed. However three linkage groups do not seem to fit this model. We may then suppose a higher or lower rate of crossing-over on these groups. Large differences in the density of markers per linkage group have already been reported in *B. napus* (Uzunova et al. 1995). The rate of crossing-over is known to differ from one species to another, as well as between genotypes and between genomic regions (Tanksley et al. 1992; Lukaszewski and Curtis 1993; Jones 1994). Therefore, it would be interesting to compare the linkage groups DY2, DY3 and DY4 in different genetic backgrounds to confirm their characteristics. Nevertheless, marker densities along each group had distinct profiles. In tomato, marker density appeared to be higher near centromeric and telomeric regions and some genes were positioned in genomic regions where recombination was highly reduced (Tanksley et al. 1992). Positioning of preferential sites of crossing-over with regard to genes of agronomic importance could help to resolve problems of trait transfer in breeding schemes or problems encountered in attempts to reduce the size of introgressed segments by classical crosses.

The novelty of this *B. napus* genetic map is its construction through the use of different types of marker: isozyme, RAPD and RFLP. The simultaneous use of different types of marker could be of value to an even-coverage of the entire genome. In pepper Lefèbvre (1993) reported the clustering of RAPD markers mainly in two of the linkage groups observed. Kesseli et al. (1992) have mentioned the clustering of RAPD in alternation with RFLP markers. In our case, RFLP and RAPD markers do not seem to have a special distribution, although linkage group DY3, DY7, DY17 and DY18 were deficient in RFLP markers. However, the fact that few RFLP markers were used and that they were selected from unsaturated *B. napus* maps must be taken into consideration.

Intraspecific comparative mapping approach

The availability of various types of marker made an intraspecific comparative mapping approach possible in different ways. First results using RFLP markers revealed a good conservation of the order of the markers and of the relative distances between markers observed in two distinct *B. napus* genetic backgrounds. The genomic regions cor-

responding to linkage groups DY1, DY4 DY6 and DY10 could be related to stable *B. napus* genomic regions within these two populations. More extensive comparisons will be possible when more common probes become available for the entire genome. RAPD markers were used to localize genes that were tagged in other segregating populations. They were very efficient in rapidly identifying markers tightly linked to genes of interest because they were easily coupled with bulked segregant analysis (Michelmore et al. 1991). Jourdren et al. (1996b) mapped the two genes involved in linolenic acid content in a DH population derived from the cross 'Stellar' × 'Drakkar'. Then, using common segregating RAPD markers, the two linkage groups surrounding the linolenic acid genes were related to the DY14 and DY3 linkage groups. In a similar manner, Baranger et al. (submitted) have used RAPD markers to rapidly map the insertion site of a herbicide resistance transgene in a DH population derived from the cross 'Westar T5' × 'Miyuki' and to compare the linkage group obtained with that of DY8. These results indicate that RAPD markers could be easily transferred from one cross to another even if the parental lines employed have very different genetic origins.

RAPD markers coupled with the BSA method were also used to localize the genomic regions involved in oligogenic traits segregating in the mapping population. It proved very efficient to enrich these regions with markers. This approach was used to map the genes responsible for dwarfism (Foisset et al. 1995a) and erucic-acid content (Jourdren et al. 1996c).

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