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M. Uzunova · W. Ecke · K. Weissleder · G. Röbbelen

Mapping the genome of rapeseed (*Brassica napus* L.). I. Construction of an RFLP linkage map and localization of QTLs for seed glucosinolate content

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Abstract A linkage map of the rapeseed genome comprising 204 RFLP markers, 2 RAPD markers, and 1 phenotypic marker was constructed using a F_1 derived doubled haploid population obtained from a cross between the winter rapeseed varieties 'Mansholt's Hamburger Raps' and 'Samourai'. The mapped markers were distributed on 19 linkage groups covering 1441 cM. About 43% of these markers proved to be of dominant nature; 36% of the mapped marker loci were duplicated, and conserved linkage arrangements indicated duplicated regions in the rapeseed genome. Deviation from Mendelian segregation ratios was observed for 27.8% of the markers. Most of these markers were clustered in 7 large blocks on 7 linkage groups, indicating an equal number of effective factors responsible for the skewed segregations. Using cDNA probes for the genes of acyl-carrier-protein (ACP) and β-ketoacyl-ACP-synthase I (KASI) we were able to map three and two loci, respectively, for these genes. The linkage map was used to localize QTLs for seed glucosinolate content by interval mapping. Four QTLs could be mapped on four linkage groups, giving a minimum number of factors involved in the genetic control of this trait. The estimated effects of the mapped QTLs explain about 74% of the difference between both parental lines and about 61.7% of the phenotypic variance observed in the doubled haploid mapping population.

Key words *Brassica napus* L
Restriction fragment length polymorphism (RFLP)
Linkage map · Seed glucosinolate content
Quantitative trait loci (QTL)

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M. Uzunova · W. Ecke (✉) · K. Weissleder · G. Röbbelen
Institute of Agronomy and Plant Breeding, University of Göttingen, Von-Siebold-Str. 8, D-37075 Göttingen, Germany

Introduction

Botstein et al. (1980) were the first to propose the use of restriction fragment length polymorphisms (RFLPs) as genetic markers. The advantages of RFLP markers generally include abundance, phenotypic neutrality and, usually, codominance. In many plant species, the availability of large numbers of RFLPs has led to the development of dense linkage maps comprising most of the genome (Pillen et al. 1992; Tanksley et al. 1992; Kishimoto et al. 1993; Kleinhofs et al. 1993). These RFLP linkage maps have been proven to be very useful tools for studying genome structure and evolution, for identifying introgressions, and for comparing genome organization and gene order of different species (Bonierbale et al. 1988; Tanksley et al. 1988; Hosaka et al. 1990). RFLP markers have made it possible to localize genes of interest (Barone et al. 1990; Jung et al. 1990; Klein-Lankhorst et al. 1991), and tightly linked markers have already served as starting points for the isolation of genes characterized only by their phenotypic effect by map-based cloning approaches (Martin et al. 1991, 1993; Arondel et al. 1992). In the analysis of traits showing quantitative variation new approaches to identify and localize the genetic factors contributing to these traits and estimating their effects have become available through the use of RFLP linkage maps (Lander and Botstein 1989; Haley and Knott 1992). In an interspecific cross in tomato, quantitative trait loci (QTLs) affecting fruit size, soluble solids concentration, and pH have been mapped by interval mapping (Paterson et al. 1991). Using the same approach Heun (1992) was able to map QTLs for powdery mildew resistance in barley.

In rapeseed and related *Brassica* species cloned genomic sequences have been shown to reveal high levels of polymorphism (Figdore et al. 1988). This feature has been utilized to study *Brassica* taxonomy (Song et al. 1988a, b, 1990) and to construct linkage maps of the diploid progenitors of *B. napus*, *B. oleracea*, and *B. rapa* (Slocum et al. 1990; Song et al. 1991; Chyi et al. 1992; Kianian and Quiros 1992; Landry 1992). A first linkage map of *Brassica*

napus comprising 120 markers was developed in a cross between two closely related spring rapeseed varieties (Landry et al. 1991).

One of the main objectives in rapeseed breeding during the last 25 years has been the reduction of the amount of glucosinolates in the seed. Glucosinolates are plant secondary metabolites with decomposition products that are toxic to animals and fungi. With respect to this, a function of glucosinolates in protecting the plant against insect pests and fungal diseases is discussed (Chew 1988; Mithen et al. 1986). On the other hand, as the products of glucosinolate decomposition are also toxic to domesticated animals, the seed glucosinolate content directly determines the usefulness of the rapeseed meal in livestock feeding. The present rapeseed varieties with low glucosinolate content were developed from the spring variety 'Bronowski', which was found to have a very low seed glucosinolate content of only 10 µmol/g seed. Attempts to elucidate the genetic control of this trait proved to be difficult as segregating populations showed a continuous distribution of seed glucosinolate content. Estimates of the number of genes involved ranged from two genes with overdominance to four or five genes with only small dominance effects (Lein 1972; Gland 1985; Mou and Liu 1988; Rücker 1993).

We have used genomic probes to develop an RFLP linkage map of the rapeseed genome in a cross between two winter rapeseed varieties. Gene specific cDNA clones were used to map genes involved in fatty acid biosynthesis. Using the linkage map, we located QTLs controlling seed glucosinolate content by interval mapping.

Materials and methods

Plant material

The segregating population used for mapping was developed from a cross between doubled-haploid (DH) lines from the winter rapeseed varieties 'Mansholt's Hamburger Raps' and 'Samourai'. 'Mansholt' is an old landrace with a high content of both erucic acid and glucosinolates. 'Samourai' is a new French variety of canola quality. Two DH lines from 'Mansholt', DH5.1 and DH5.2, were crossed with 1 DH line from 'Samourai', DH11.4, and segregating DH populations of 70 and 81 lines were derived from microspores of the respective F_1 plants. Since no differences in marker alleles and allele frequencies have been observed between the two populations they have been regarded as one population of 151 DH lines.

In 1992/1993 the parental DH lines, the F_1, and the lines of the DH population were grown at Göttingen-Reinshof in separate but neighboring blocks in the field. Plots consisted of two rows, 2.5 m in length. Distance between rows was 0.33 m and between plants within rows, 0.12 m. Flower color was scored visually. Seed samples of 4–5 g harvested separately from 1–12 plants of each double row were used to measure the glucosinolate content by near infrared reflection spectroscopy (NIRS, Reinhardt 1992).

Probes and markers

For RFLP mapping a total of 153 genomic probes, 5 cDNAs and 1 simple repeat specific oligonucleotide were used. Thirty probes designated WG and 10 probes designated TG were derived from cloned *Pst*I and *Eco*RI fragments from total DNA of *B. napus* cv. 'Westar' and *B. rapa* cv. 'Tobin', respectively. These probes were kindly provided by Thomas C. Osborn (University of Madison, Wisconsin, USA). One hundred and thirteen probes with the designation pRP have been derived from a DNA library of cloned *Pst*I fragments from total DNA of *B. napus* cv 'Duplo'. The *Pst*I digested DNA had been size fractionated, and only fragments in the size range from 0.7 kb to 2.0 kb have been used for cloning. Three cDNAs for the genes of acyl-carrier protein (ACP), β-ketoacyl-ACP-synthase I (KASI), and acetyl-CoA-carboxylase (ACC) and 2 anonymous cDNAs were kindly provided by Reinhard Töpfer (MPI, Köln Vogelsang, Germany). The digoxigenized simple repeat specific oligonucleotide $(GATA)_4$ was purchased from Fresenius (Oberursel/Taunus, Germany). The designations of the markers are derived from the probe enzyme combination used for mapping the respective marker. For example, marker RP1415.H2 is the second marker that could be mapped with probe pRP1415 using the enzyme *Hin*dIII.

DNA isolation

Plant total DNA was extracted from 5–10 g of young leaves from greenhouse grown plants according to a modification of the CTAB procedure of Rogers and Bendich (1988). Leaf material was frozen in liquid nitrogen and ground with a mortar and pestle to a fine powder. Ten milliliters of hot (65°C) 2 × CTAB buffer (100 m*M* TRIS-HCl pH 8.0, 20 m*M* EDTA, 1.4 *M* NaCl, 1% PVP 40 000, 2% CTAB) was added to the powder and, after the addition of 100 µg/ml proteinase K, incubated for 30 min at 65°C with slight agitation. After extraction with one volume of chloroform/isoamyl alcohol (24:1), 1/10 volume of a 10% CTAB solution (10% CTAB, 0.7 *M* NaCl) was added to the aqueous phase, and the extraction was repeated. The aqueous phase was removed and mixed with an equal volume of hot (65°C) CTAB precipitation buffer (50 m*M* TRIS HCl pH 8.0, 10 m*M* EDTA, 1% CTAB). The resulting CTAB/DNA complex was immediately pelleted by centrifugation for 10 min at 10 000*g* at 20 °C. The resulting pellet was resuspended in 2 ml high-salt buffer (10 m*M* TRIS-HCl pH 8.0, 1 m*M* EDTA, 1 *M* NaCl), and the DNA was precipitated by addition of two volumes of cold 100% ethanol. After pelleting the nucleic acids, cold 70% ethanol was added to remove salts. The final pellet was resuspended in 1 ml TE buffer (10 m*M* TRIS-HCl pH 8.0, 1 m*M* EDTA).

RFLP and RAPD analysis

For RFLP analysis the DNA was digested with *Eco*RI and *Hin*dIII, respectively. After digestion, the DNA concentrations were estimated by fluorometry in a Mini-Fluorometer TKO 100 from Hoefer (Hoefer Scientific Instruments, San Francisco, Calif.), and approximately 5 µg of DNA per genotype was separated on 0.8% agarose gels using TAE buffer (Sambrook et al. 1989). The DNA was transferred to Hybond N^+ by the Southern capillary blotting procedure followed by an alkaline fixation as recommended by the supplier (Amersham Buchler, Braunschweig, Germany). Probes were labelled by random primed labelling according to the method of Feinberg and Vogelstein (1983) with α-[^{32}P]dCTP using the Prime-It II Random Primer Labelling Kit from Stratagene (La Jolla, Calif.). Blots were hybridized by 65°C in a hybridization oven from Appligene (Illkirch, France) according to the method of Bernatzky and Tanksley (1986). Final washes were with 0.5×SSC, 0.025% SDS at 65°C. Hybridization filters were reprobed up to 15 times after the removal of hybridized probes by treatment with 0.2 *M* NaOH followed by 0.2 *M* TRIS-HCl pH 7.5, 0.1% SDS, 0.1×SSC at 45°C for 30 min each.

Ten-mer primers for random amplified polymorphic DNA (RAPD) analysis were purchased from Operon (Primer Kits A-D, Q-T, AG-AJ, Operon Technologies, Alameda, Calif.). Amplifications were performed according to Quiros et al. (1991) with 1 U *Taq* DNA polymerase from AGS (Angewandte Gentechnologie Systeme GmbH, Heidelberg, Germany) in a Perkin Elmer Cetus Thermal Cycler TC480. Polymerase chain reaction (PCR) products were analyzed by electrophoresis in 1.8% agarose gels using TAE buffer.

Bulked segregant analysis was performed according to Giovannoni et al. (1991) with DNA from 12 selected DH lines per bulk.

Segregation analysis and map construction

Deviations of the marker allele frequencies from the segregation ratio of 1:1 expected in a DH population and the heterogeneity between the two DH populations were tested by χ^2 analysis (Mather 1957). Linkage analysis and map construction were performed using the computer program MAPMAKER v1.9 (Lander et al. 1987). To group the RFLP loci into linkage groups two-point analysis with a minimum LOD score of 4.0 and a maximum recombination frequency of 0.4 was used. These linkage criteria excluded the occurrence of spurious linkages that were observed when reducing the minimum LOD score to less than 3.2. A combination of three-point and multipoint analysis was applied to find the most probable locus order within each linkage group. Double-crossover events were examined, and the original scores were rechecked to find potential scoring errors. The internal consistency of the linkage groups was verified by estimating the multipoint LOD score for linkage of each pair of adjacent markers using MAPMAKER's "link" command. Recombination frequencies were transformed into centiMorgans (cM) using the Kosambi mapping function (Kosambi 1944).

QTL mapping

Mean phenotypic values of marker genotypes were compared by the *t*-test to indicate candidate regions of the linkage groups for QTLs. As more than 200 markers were tested, a significance threshold of $P \leq 0.001$ was applied. QTLs were mapped by interval mapping using the computer program MAPMAKER/QTL (Lander and Botstein 1989). A LOD score threshold of 2.8 was used, giving approximately a 5% chance of falsely declaring a QTL to exist anywhere on the map (Lander and Botstein 1989). The QTLs were first localized by scanning the linkage groups in 2-cM steps. When we used models with fixed QTLs, the LOD score attributable to the putative QTL at the scanned position was calculated as the difference between the total LOD score and the LOD score derived from the fixed QTLs only. MAPMAKER/QTL's "map" command was used in conjunction with multiple QTL models to precisely determine the most likely positions of QTLs and to estimate the additive effects of the mapped QTLs.

Results

Probes, markers, and linkage groups

To identify probes useful for mapping the rapeseed genome, about 665 probes were tested against the parents and the F_1 of the mapping population using the restriction enzymes *Eco*RI and *Hin*dIII. About 42.4% of the probes proved to reveal polymorphisms. The observed hybridization patterns were generally complex with an average of 3–6 fragments per probe (Fig. 1). Probes hybridizing to only 1 or 2 fragments proved to be the exception.

One hundred and fifty-eight probes giving strong hybridization signals and showing clearly distinguishable polymorphisms between 'Mansholt' and 'Samourai' were selected for mapping. With these probes it was possible to follow the segregation of 204 RFLP markers in the mapping population. About 43% of these markers proved to be of a dominant nature. In several cases restriction fragments segregating in a 3:1 fashion were observed in addition to at least 2 fragments showing the expected 1:1 segregation. Segregation analysis showed these hybridization signals to represent 2 identically sized fragments from two different loci, that were allelic to the 1:1 segregating fragments. The rest of the markers displayed the codominant nature expected for RFLP markers. In addition to the RFLP markers 1 RAPD marker, OPB15.1200, and a phenotypic marker, pale yellow (*pale*), due to a monogenically determined difference in flower color between 'Mansholt' and 'Samourai', were used for mapping.

From the 206 markers, linkage analysis initially demonstrated that 202 constitute 18 linkage groups. Of the 4 remaining markers, 2, RP1266.E1 and RP981.H1, appeared to be linked if the LOD score threshold for linkage was reduced from 4.0 to 2.9. To confirm this linkage, bulked segregant analysis was used to find additional markers in this region. Two hundred and sixty decamer primers were tested with two DNA pools, each pool derived from 12 genotypes with only 'Samourai' or 'Mansholt' alleles, respectively, for both markers. Two RAPD markers showed a clear difference between the pools, but only 1 marker, OPT9.862 proved to be linked with markers RP1266.E1 and RP981.H1. These 3 markers formed a further linkage group, giving a total of 19 linkage groups with 205 mapped markers (Fig. 2). With the exception of the 2 peripheral markers on linkage group 15, all adjacent markers showed multipoint linkage LOD scores well above 6.0, with a mean of 33.70 and a standard deviation of 12.54, indicating a high internal consistency

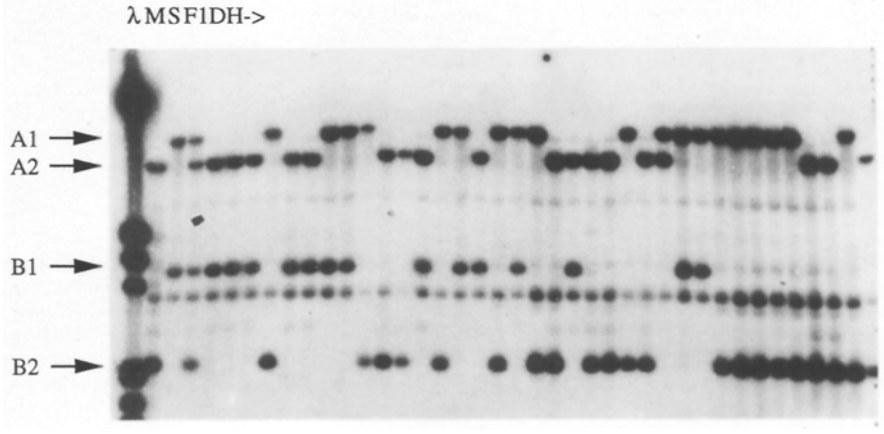

Fig. 1 Segregation of RFLP loci detected by probe RP984. *A1/A2, B1/B2*: Alleles of two loci detected by probe pRP984 in combination with the restriction enzyme *Hin*dIII; *M*: 'Mansholt's Hamburger Raps'; *S*: 'Samourai'; *DH*: doubled haploid lines from the mapping population. The symbol λ indicates lambda DNA double digested with *Eco*RI and *Hin*dIII as molecular weight marker

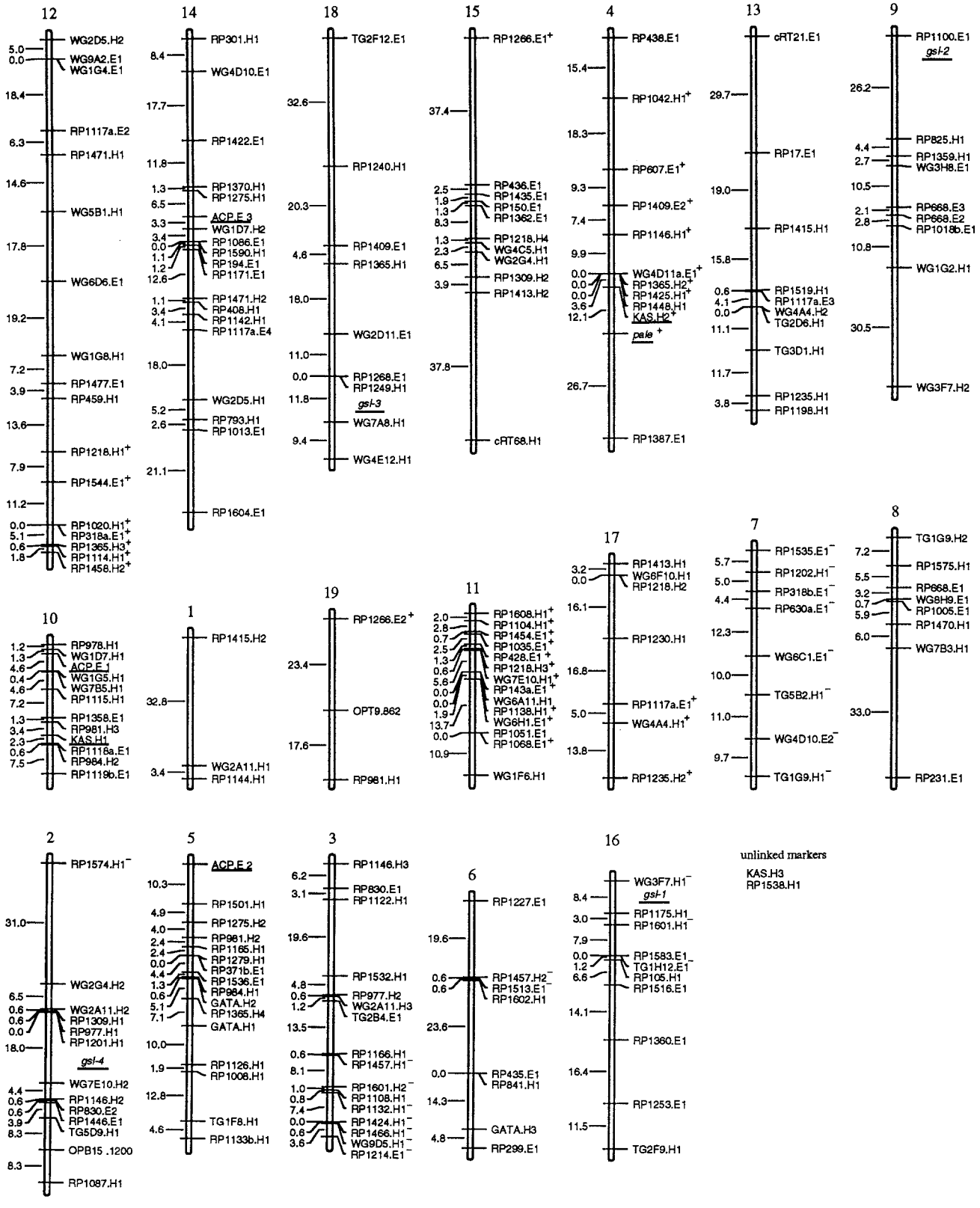

Fig. 2 RFLP linkage map of winter rapeseed. Distances between markers are given in cM, calculated from recombination frequencies according to the Kosambi mapping function. '+' and '−' indicate markers showing significant deviations ($P \leq 0.05$) from the expected 1:1 segregation ratio in favor of 'Mansholt' or 'Samourai' alleles, respectively. Mapped genes and QTLs are *underlined*. *pale*: Pale yellow flower color, *ACP*: acyl-carrier-protein, *KAS*: β-ketoacyl-ACP-synthase I, *gsl*: QTLs for seed glucosinolate content

of the observed linkage groups. The total length of the linkage map was 1441.3 cM (Table 1).

The number and density of the markers differed considerably between different linkage groups, ranging from as few as 3 markers on linkage groups 1 and 19 to 19 markers on linkage group 14 and a marker density of 0.7 markers per 10 cM on linkage group 19, to 3.5 markers per 10 cM on linkage group 10 (Table 1). In addition, on several linkage groups a clustering of markers in certain regions is evident, for example at the end of linkage group 12 or in the central parts of linkage groups 14 and 15.

From the mapped markers 57 (27.8%) showed a significant deviation ($P< 0.05$) from the expected 1:1 segregation ratio. For about two thirds of these markers an excess of 'Mansholt' alleles was observed. The markers showing skewed segregations are not randomly distributed throughout the genome. With the exception of 5 markers on linkage groups 2, 6, 15, and 19, these markers constitute seven large blocks on linkage groups 3, 4, 7, 11, 12, 16, and 17 (Fig. 2).

Table 1 Linkage group size, number of markers, and marker density per linkage group.

Linkage group	Size [cM]	Number of markers	Density (markers/cM)
1	36.2	3	0.083
2	82.7	13	0.157
3	71.0	16	0.225
4	102.8	12	0.117
5	71.8	16	0.223
6	63.6	8	0.126
7	58.1	8	0.138
8	61.4	8	0.130
9	90.0	9	0.100
10	34.3	12	0.350
11	42.1	14	0.333
12	132.8	17	0.128
13	95.9	10	0.104
14	122.8	19	0.155
15	103.2	11	0.107
16	69.0	10	0.145
17	54.9	7	0.128
18	107.7	9	0.084
19	41.0	3	0.073
$\Sigma/\bar{x}$	1441.3	205	0.153

Duplicated regions in the rapeseed genome

From the 158 probes used for mapping, 31 hybridized to more than 1 segregating marker locus. Of these probes, 22 detected 2 markers, 6 detected 3 markers, and 3 probes detected even 4 markers, giving a total of 74 duplicated markers. In only one case duplicated markers were found in 1 linkage group. Probe pRP668 identified 2 markers in linkage group 9, RP668.E2 and RP668.E3, 2.1 cM apart from each other. In all other cases, the duplicated markers were found in different linkage groups. On 11 linkage groups ten different clusters of 2–4 duplicated loci were found, indicating larger duplicated regions (Table 2). These regions comprise 380.4 cM or 26.4% of the total length of the RFLP map. An example of such duplications is presented in Fig. 3. Linkage group 17 appears to be completely duplicated, consisting of three separate blocks that were also detected on linkage groups 12, 13, and 15.

Mapping of genes by gene-specific probes

To map loci for known genes within the linkage map cDNAs for the genes of acyl-carrier protein (ACP), β-ketoacyl-ACP-synthase I (KASI), and acetyl-CoA-carboxylase (ACC), all involved in fatty acid biosynthesis, were used as probes. The cDNA for ACC hybridized to 3 *Eco*RI as well as to 3 *Hin*dIII fragments, indicating three loci for this gene (data not shown). As none of the hybridizing fragments was polymorphic in the mapping population, these loci could not be mapped.

The cDNA of ACP showed 2 strongly hybridizing polymorphic fragments in both 'Mansholt' and 'Samourai', and in both genotypes several additional fragments giving weak hybridization signals. The 2 main fragments defined two major loci, ACP.E1 and ACP.E2, that could be mapped to linkage groups 10 and 5, respectively. Of the weakly hybridizing fragments, only 1 proved to be polymorphic and was mapped as dominant marker on linkage group 14 (Fig. 2).

The probe for KASI hybridized to a larger number of fragments (>10). Three *Hin*dIII-fragments proved to be polymorphic, defining 3 dominant RFLP markers. Two loci, KAS.H1 and KAS.H2, could be mapped on linkage groups 10 and 4, respectively. The 3rd marker, KAS.H3, is one of the 2 remaining markers showing no linkage to any one of the other markers (Fig. 2).

Mapping of QTLs for glucosinolate content in the seed

In Table 3 the average seed glucosinolate content of the parental lines, the F_1, and the DH population of the cross 'Mansholt' × 'Samourai' is shown. The difference between the parental lines amounts to more than 50 μmol glucosinolates per gram of seed. The mean value of the F_1 is significantly different from the midparent value, indicating dominance effects in favour of a high glucosinolate content. The seed glucosinolate content was estimated for 115 lines of the DH population. In accordance with the quantitative nature of the trait no phenotypic classes could be distinguished. The values proved to be normally distributed (skewness: 0.02, kurtosis: −0.01).

In a first approach to identify QTLs controlling seed glucosinolate content, the mean values of the marker classes were compared for all markers using a *t*-test. At a significance threshold of $P \leq 0.001$, only markers on linkage groups 16 and 18 and the marker RP1100.E1, terminal on linkage group 9, showed a significant association to seed glucosinolate content (Table 4), indicating the presence of at least 3 QTLs on the respective linkage groups.

Table 2 Location and size of duplicated regions in the RFLP linkage map of winter rapeseed

Duplication 1			Duplication 2		
Marker	Linkage group	Size (cM)	Marker	Linkage group	Size (cM)
RP1146.H2 RP830.E2 RP977.H2 WG2A11.H2	2	24.2	RP1146.H3 RP830.E1 RP977.H1 WG2A11.H3	3	34.3
RP1309.H1 WG2G4.H2	2	7.1	RP1309.H2 WG2G4.H1	15	6.5
RP1409.E1 RP1365.H1	4	17.3	RP1409.E2 RP1365.H2	18	4.6
RP984.H1 RP981.H2 ACP.E2	5	29.7	RP984.H2 RP981.H3 ACP.E1	10	24.5
ACP.E2 RP1275.H2	5	15.2	ACP.E3 RP1275.H1	14	6.5
WG1D7.H1 ACP.E1	10	1.3	WG1D7.H2 ACP.E3	14	3.3
WG2D5.H2 RP1117a.E2 RP1471.H1	12	29.7	WG2D5.H1 RP1117a.E4 RP1471.H2	14	26.6
RP1117a.E2 RP1218.H1	12	82.6	RP1117a.E1 RP1218.H2	17	32.9
RP1117a.E3 WG4A4.H2 RP1235.H1	13	26.9	RP1117a.E1 WG4A4.H1 RP1235.H2	17	18.8
RP1413.H2 RP1218.H4	15	14.0	RP1413.H1 RP1218.H2	17	3.2

Fig. 3 Duplicated regions on linkage groups 12, 13, 15, and 17. Duplicated loci are *underlined*. Identically marked regions indicate duplications between the respective linkage groups

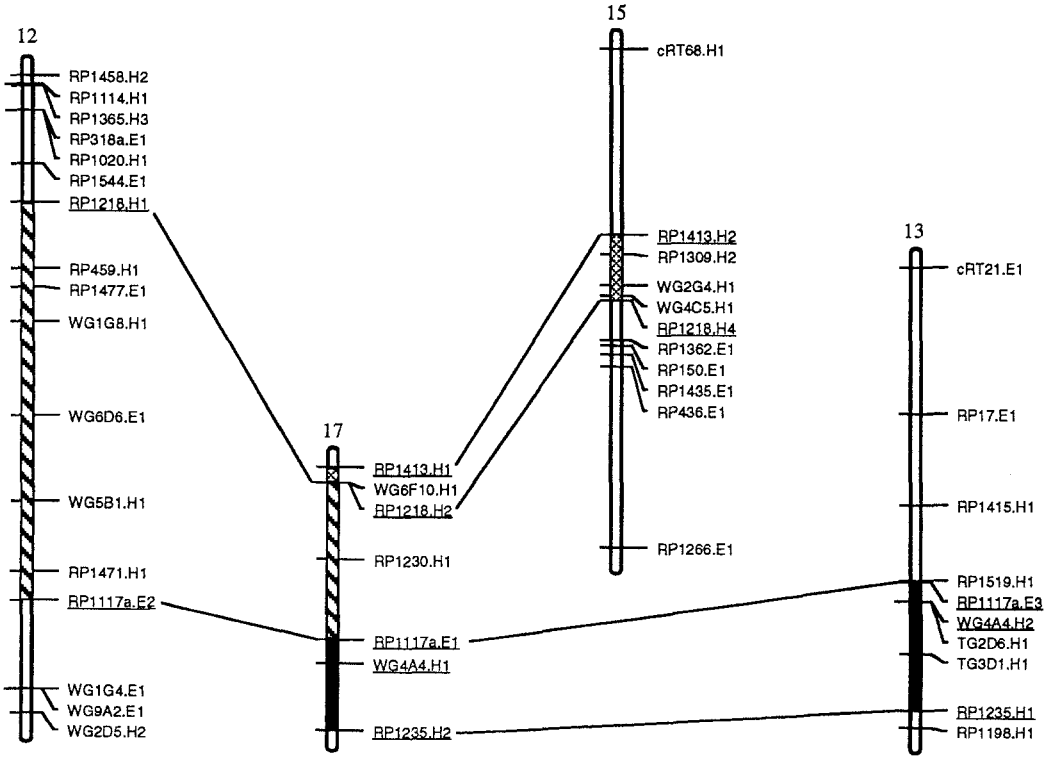

Table 3 Average seed glucosinolate content in different generations of the cross 'Mansholt's Hamburger Raps' × 'Samourai'

Generation	n^a	$\bar{x}$ [μmol/g]	s	t-test[b]
Mansholt	120	62.3	11.05	
Samourai	87	11.2	2.62	
F_1	141	51.5	8.42	b
DH population[c]	115	35.1	12.27	a
Midparent value		36.7		a

[a] Parental lines; F_1: number of plants analyzed, DH population; number of DH lines analyzed
[b] Comparison of generation means with midparent value: different letters indicate significant differences at $P \leq 0.05$
[c] Values for individual DH lines were obtained as a mean from up to 12 plants

Table 4 Markers with significant association ($P \leq 0.001$) to seed glucosinolate content

Marker	Linkage group	t-value	df
RP1100.E1	9	3.43	109
WG3F7.H1	16	6.01	108
RP1175.H1	16	6.70	111
RP1601.H1	16	5.70	110
RP1583.E1	16	4.35	107
TG1H12.E1	16	4.32	110
RP105.H1	16	4.66	111
RP1516.E1	16	4.53	108
RP1268.E1	18	4.00	108
RP1249.H1	18	3.79	106
WG4E12.H1	18	3.39	106

In a second approach, interval mapping was used to localize the putative QTLs. Scanning the 19 linkage groups, we found the highest LOD scores to be on linkage group 16 in the interval between markers WG3F7.H1 and RP1175.H1, in accordance with the t-test, which resulted in the highest t-value for marker RP1175.H1. On linkage group 18 significant LOD scores were found in the intervals around markers RP1268.E1 and RP1249.H1. With a maximum value of 2.32 at marker RP1100.E1 the LOD scores on linkage group 9 did not reach significance (Fig. 4). A rescan of the RFLP map with the QTL on linkage group 16 fixed at its most likely position increased the LOD score on linkage group 9 to a maximum of 4.44, clearly exceeding the significance threshold. In addition, on linkage group 2 a fourth QTL could be localized in the interval between markers RP1201.H1 and WG7E10.H2 with a maximum LOD score of 3.57 (Fig. 4).

To test for the presence of further QTLs, the remaining linkage groups were rescanned with the four QTLs on linkage groups 2, 9, 16, and 18 fixed. An additional LOD score peak was observed on linkage group 3. With a maximum value of 1.6 this peak did not reach significance, but nevertheless may be indicative of an additional QTL with a small effect. On all other linkage groups the LOD-scores remained well below 1.

The additive effects and most likely positions of the four mapped QTLs controlling seed glucosinolate content in rapeseed are presented in Table 5. The additive effects of these QTLs sum up to 38 μmol/g seed for homozygous genotypes and explain about 74% of the difference between 'Mansholt' and 'Samourai' and about 61.7% of the variance observed in the DH population (Table 3).

Discussion

The linkage map

In this study genomic DNA sequences were used as probes for the development of an RFLP linkage map of winter rapeseed. In accordance with the results of Figdore et al. (1988) a high degree of polymorphism was found with this type of probe. The markers assembled into 19 linkage groups that may represent the 19 chromosomes of rapeseed. Large differences were observed in the number and density of markers per linkage group, leading to a considerable variance in the length of the linkage groups. These differences and the clustering of markers in certain regions of some linkage groups may be in part due to differences in chromosome length and in recombination frequency in different parts of the genome. In cytogenetic studies on pachytene chromosomes of *B. oleracea* and *B. rapa,* different chromosomes were found to vary in length up to 2.5 fold (Röbbelen 1960). In wheat and rye the recombination frequency has been shown to increase exponentially with the physical distance from the centromere, the actual distribution of recombination depending on the length of the chromosome arm (Lukaszewski 1992; Lukaszewski and Curtis 1993), in a high density map of tomato highly increased marker densities were observed near centromeres and in some instances also near telomeres, indicating a suppression of recombination in these areas (Tanksley et al. 1992). In addition to variation in chromosome length and recombination frequency, differences of chromosomes or chromosomal regions in the amount of repetitive sequences may also account for part of the observed variation in the number of markers per linkage group. Due to the selection against repetitive DNA during the screening for informative probes, such differences would have led to differences in the representation of these regions in the probe set used for mapping.

Two markers segregating in the mapping population could not be linked to any other marker, indicating that part of the rapeseed genome has not yet been mapped. To include the areas represented by the unlinked markers in the linkage map, it will be necessary to place new markers specifically in these regions. As has been shown with markers RP1266.E1 and RP981.H1, which represent the 19th linkage group, this can be done by bulked segregant analysis using RAPD markers. To conclusively map the whole extent of the rapeseed genome, the ends of the chromosomes have to be mapped. Hybridizations of a telomere specific probe from *Arabidopsis thaliana* (Richards and

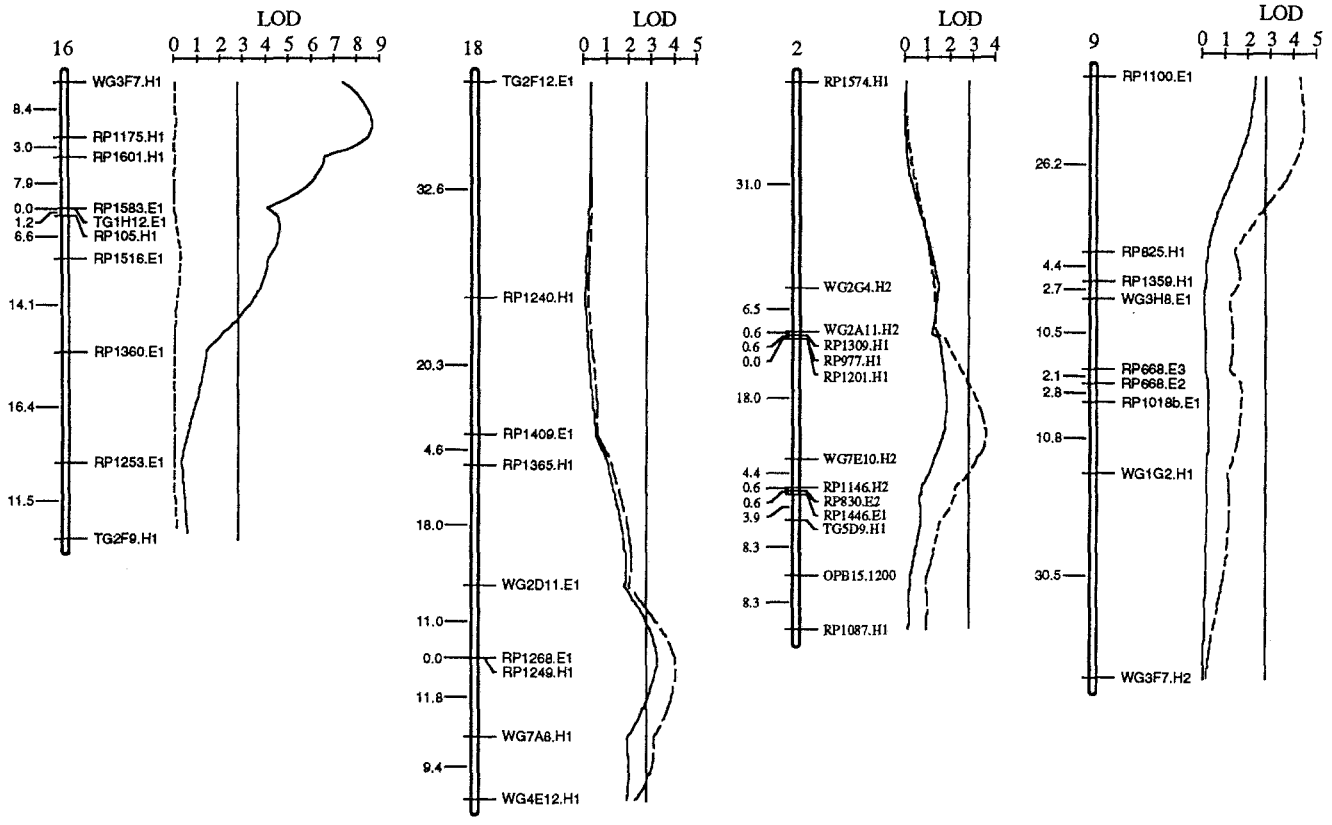

Fig. 4 Interval mapping of QTLs for seed glucosinolate content on linkage groups 16, 18, 2, and 9. The LOD curves represent the likelihood for the presence of a QTL at the corresponding positions of the linkage groups (1) without fixed QTLs (*continuous line*) and (2) with the QTL on linkage group 16 fixed at its most likely position, at a distance of 6.4 cM from marker WG3F7.H1 (*dotted line*). The *vertical line* indicates the significance threshold of LOD=2.8. Distances between markers are given in cM (Kosambi)

Table 5 Additive effects and most likely positions of the mapped QTLs for seed glucosinolate content

Linkage group	Marker interval	QTL position[a] (cM)	Additive effect[b] (μmol/g seed)
2	RP1201.H1-WG7E10.H2	12.5	3.4
9	RP1100.E1	1.1	4.3
16	WG3F7.H1-RP1175.H1	3.3	7.8
18	RP1249.H1-WG7A8.H1	5.4	3.5

[a] Distance to the first marker of the indicated interval
[b] Estimated for the substitution of an 'Samourai' allele by an allele of 'Mansholt's Hamburger Raps'

Ausubel 1988) against *Eco*RI- or *Hin*dIII digested DNA from lines of the mapping population have not resulted in scorable banding patterns (data not shown). Here it will be necessary to use different enzymes or to directly clone subtelomeric sequences as has been demonstrated in rice (Ashikawa et al. 1993).

Duplicated regions and dominant markers

Brassica napus is an amphidiploid, comprising the A and C genome from *B. oleracea* and *B. rapa* or from close relatives of these species (Song and Osborn 1992). Cytogenetic studies have indicated a high degree of homology between the two genomes, since nine bivalents can usually be observed in amphihaploids of rapeseed (Prakash and Hinata 1980; Attia and Röbbelen 1986). This was confirmed by RFLP analysis, which showed most genomic probes to be hybridizing to both genomes, thereby indicating a high sequence homology between the A and the C genome (Figdore et al. 1988). In addition, linkage analysis in *B. oleracea* and *B. rapa* has demonstrated a high amount of duplications in the diploid genomes (Slocum et al. 1990; Song et al. 1991; Chyi et al. 1992). This situation is reflected in the complex hybridization patterns of the probes used for mapping. As the majority of these probes hybridized to only 1 segregating locus while hybridizing to additional monomorphic fragments, the duplicated regions that could be mapped in this study have to be regarded as a lower limit of the extent of duplications in the rapeseed genome.

The high prevalence of duplications in rapeseed may also explain the large number of dominant markers. Directly after the speciation event leading to the diploid ancestors, or following the duplication event within the diploid genomes, the duplicated loci will have had identical alleles. After the occurrence of a new allele by mutation at 1 of the loci, a dominant marker would appear since the original allele would be masked by the monomorphic

fragment from the second locus. Only with two new alleles at 1 locus would a codominant marker be observed. One new and 1 original allele at both loci would lead the fragment representing the latter to segregate in a 3:1 fashion, as has been observed in some cases.

Disturbed segregations

A high percentage of the mapped markers showed a significant deviation from Mendelian segregation ratios. As has been shown in several studies, disturbed segregations are a common feature of microspore derived DH populations in a number of species (Orton and Browers 1985; Guiderdoni et al. 1991; Thompson et al. 1991), including rapeseed (Foisset et al. 1993). This is generally explained by selection interfering with in vitro androgenesis or the subsequent plant regeneration, since in several cases no such distortions could be observed in the corresponding F_2 generations. This hypothesis could be corroborated with the phenotypic marker *pale*. While this marker and neighboring markers on linkage group 4 show a strong deviation from Mendelian segregation ratios in the doubled haploid mapping population, no such deviations could be observed for *pale* in the F_2 and BC_1 populations of the cross 'Mansholt' × 'Samourai' (data not shown).

Most of the markers showing skewed segregation observed in this study were located in seven large blocks on 7 separate linkage groups. This distribution implies a minimum number of seven factors segregating in the mapping population that might be effective in in vitro androgenesis and/or plant regeneration. The favorable alleles of four of these factors were inherited from 'Mansholt'. For the three remaining factors 'Samourai' was the donor. The rest of the skewed markers, distributed on 4 additional linkage groups, may indicate extra factors with minor effects.

Genetic control of seed glucosinolate content

It has been possible to map four QTLs for seed glucosinolate content, setting a lower limit for the number of genes involved in the genetic control of this trait. The additive effects of these QTLs ranged from 7.8 to 3.4 μmol/g seed, in total explaining about 74% of the difference between 'Mansholt' and 'Samourai'. As the mapped QTLs cannot explain the total difference between the parents, the presence of further QTLs, most likely with smaller effects, has to be assumed. One indication of such a QTL may be the LOD score peak on linkage group 3. The dominance effects of the mapped QTLs could not be estimated due to the doubled-haploid nature of the mapping population, but the F_1 data show that significant dominance is involved in the expression of this trait. The DH lines of the mapping population have been backcrossed to 'Mansholt' and 'Samourai'. It should be possible to estimate the dominance effects in these crosses without further marker analysis.

RFLP mapping in DH populations

A DH population of 151 lines was used for the construction of the linkage map. The development of large DH populations requires a greater effort and is more time consuming than the development of a similar sized F_2 or BC_1. This effort is compensated for by the many advantages of this type of population. In a DH population all types of markers are of equal use. While codominant markers give as much as twice the linkage information in a F_2 population compared to DH and BC_1 populations, dominant markers give principally less information. In the case of 2 tightly linked dominant markers in repulsion nearly no linkage information at all is available in a F_2. In a BC_1 on the other hand, all dominant markers with the visible allele from the recurrent parent are useless for mapping. In addition, the scoring of phenotypic markers, like flower color, may be much easier in DH populations, as no intermediate phenotypes or partial dominance effects may obscure the identification of genotypes. The greatest advantage in using DH populations is found in QTL mapping. Contrary to F_2 and BC_1 populations, the lines of a DH population can be propagated indefinitely. The population size required to detect a QTL with a given effect is approximately proportional to the phenotypic variance observed for the analyzed trait (Lander and Botstein 1989). Through the calculation of means over several individuals from 1 DH line, the environmental variance can be significantly reduced in DH populations, thereby reducing the observed phenotypic variance. Consequently, with a given population size QTLs with smaller effects can be mapped in a DH population than would be possible in a F_2 or BC_1. Furthermore, when using doubled haploids as parents it will be possible to increase the size of the mapping population at any time by the production of additional F_1-derived DH lines. In addition, the doubled haploid mapping population can be tested in different locations and years, thereby providing an opportunity to analyze the interaction between individual QTLs and the environment without further marker analysis. These advantages of DH populations will be most pronounced when analyzing quantitative traits with low heritability.

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