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A linkage map of spring turnip rape based on RFLP and RAPD markers

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A linkage map of spring turnip rape *(Brassica rapa* ssp. *oleifera)* was constructed from an F_2 population of a cross Jo4002 x Sv3402. The map contained 22 RFLP loci, 144 RAPDs, one microsatellite, and one morphological marker (seed colour). All ten *B.rapa* linkage groups could be identified and the total map distance was 519 cM. A proportion of the markers (13%), most of which were located in two linkage groups, showed segregation distortion.

Key words: DNA polymorphism, microsatellite, segregation distortion

Introduction

The development of highly polymorphic DNA markers has facilitated the construction of genetic linkage maps. During the last few years linkage maps have been developed for many plant species, e.g. in the genus *Brassica* for *B.oleracea* (Slocum et al. 1990, Kianian and Quiros 1992, Landry et al. 1992), *B.napus* (Landry et al. 1991, Ferreira et al. 1994, Uzunova et al. 1995), and *B.rapa* (Song et al. 1991, Chyi et al. 1992, Teutonico and Osborn 1994).

The most commonly used type of DNA marker in linkage studies has been restriction fragment length polymorphism (RFLP). Recently developed marker types based on use of the polymerase chain reaction (PCR) such as random amplified polymorphic DNA (RAPD), have several advantages over RFLPs. RAPD analysis is easy to perform and rapid, and does not require the use of radioactivity. In addition, because only minute amounts of crude template DNA are needed, it is possible to use rapid small-scale DNA extraction methods. A disadvantage is that the dominant nature of RAPD markers can cause problems if an F_2 intercross population is used. In such cases, estimation of recombination frequency is very inefficient between repulsion phase markers (Ott 1985) and, there-

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fore, two maps including only coupling phase markers have to be constructed.

Existing *B.rapa* linkage maps are mostly composed of RFLP markers. Our aim here was to construct a linkage map of spring turnip rape (*B.rapa* ssp. *oleifera*) consisting mainly of RAPD markers. RFLP markers were used to integrate our map with the existing *B.rapa* map (Teutonico and Osborn 1994).

Material and methods

Plant material

The F_2 mapping population was derived by self-pollinating five F_1 individuals from a cross between two individuals of repeatedly selfed spring turnip rape lines Jo4002 and Sv3402. The linkage data are mostly based on 77 F_2 individuals; 28 additional plants were scored to confirm linkages between some markers.

DNA of the plants was extracted by a method slightly modified from that of Dellaporta et al. (1983), as described by Tanhuanpää et al. (1993).

Markers

RFLP analysis was performed using standard methods (Maniatis et al. 1982) with restriction enzymes *Eco*RI or *Hin*dIII as described by Tanhuanpää et al. (1994). The F_2 progeny was screened with 24 DNA clones from *B.rapa* or *B.napus* (Teutonico and Osborn 1994) and two PCR-amplified genomic sequences of *Brassicaceae*: the *Brassica* self-incompatibility gene SLG-8_c (Dwyer et al. 1991), and the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene from *B.napus* (Gasser and Klee 1990).

RAPD primers were either synthesised on an Applied Biosystems 392 DNA/RNA Synthesizer (Table 1) or purchased from Operon Technologies (Alameda, California, USA). RAPD analysis was performed as described in Tanhuanpää et al. (1995a) with minor modifications. Putative allelism of two RAPD markers was investigated by hybridisation using one of the RAPD bands as a probe.

Table 1. RAPD primers used to analyse the F_2 progeny of the *B.rapa* ssp. *oleifera* cross, Jo4002 x Sv3402. In addition, primers from Operon Technologies were used.

Primer	Sequence 5' to 3'			
10	GCT	GCT	CGA	GT
11	CGT	CCT	TAA	GC
14	GCA	CTG	TCG	AC
19	CGC	TCT	AGA	CC
20	TGC	CAG	TTA	CG
25	GCG	TGT	AGG	CT
26	GGA	ATC	TCG	GT
33	CCG	CTT	AGT	TC
45	AGA	CGA	TGT	AC
63	GAC	CGT	GAG	AC
65	ACG	TGC	ATG	G
72	TGG	ACT	CGA	G
74	GCT	GAC	TCG	AG
75	CGA	ACC	TGA	TC
76	ATC	GTC	GAT	GC
77	GCT	AGC	TAC	TG
78	AGT	CGA	CTT	C
90	ACG	CTA	GAC	CT
93	GGT	ACT	CGA	CT
101	ATG	CGT	CAG	TC
102	TGA	TCG	ACT	CG
103	CGT	TCG	AGT	CT
105	TGC	ATC	GTA	C
107	GAC	TCG	AGA	C
110	ACG	CCG	TAC	G
111	TCG	GAA	GGA	C
112	GGA	CAC	TAC	T
117	GCG	CAA	GTG	AA
118	CGT	CGC	TGT	T
123	ACT	GAG	CGT	G
127	CAG	CTC	AGG	CT
129	GTC	CAC	GTA	GC
130	ACT	CTG	GCA	G
134	GAC	TGT	GCA	T
137	CTA	CAT	GCA	CG
138	GTC	CAC	AGA	T
140	ACG	CTA	TGA	C
141	CTG	ATC	TGC	A
146	GCT	TCA	TCG	TG
147	CGT	TCA	CCT	C
148	CCG	ACT	TCC	A
149	TGC	CAG	TCT	CC
164	AGA	AAT	GGG	G

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Table 2. *B.napus* microsatellites used to search for polymorphism between the parents of the *B.rapa* ssp. *oleifera* cross, Jo4002 and Sv3402. '–' indicates no identifiable amplification.

locus	product size range (bp)	repeat sequence	flanking sequences 5' to 3'
MB4[a]	71	$(TG)_{10}$	TGT TTT GAT GTT TCC TAC TG GAA CCT GTG GCT TTT ATT AC
MB5[a]	–	$(AT)_3GT(AT)_4(GT)_8$	AAC ATC TTT TTG CGT GAT AT AAT AGC ATT GAA GCC TTA C
X64257[b]	–	$(ATA)_n$	GTC TGC TCT CCA GAA CTA CTG TAC CTT TGG TTT CGG
X61097[b]	–	$(CT)_n$	AAC GAC CCT TTT CCG TCA GGC CGC TCA CAT TTG TAT
12A[c]	314	$(GA)_{11}(AAG)_4$	GCC GTT CTA GGG TTT GTG GGA GAG GAA GTG AGA GCG GGA AAT CA
35D[c]	222–234	$(GA)_{13}$	GCA GAA GGA GGA GAA GAG TTG G TTG AGC CGT AAA GTT GTC ACC T
38A[c]	155	$(TG)_{11}$	TGG TAA CTG GTA ACC GAC GAA AAT C ACG CTG TCT TCA GGT CCC ACT C
59A1[c]	–	$(CA)_{11}$	TGG CTC GAA TCA ACG GAC TTG CAC CAA CAA GTC ACT AAA GTT
72A[c]	277	$(TAA)_5(GA)_9$	GCC CAC CCA CCT TCT TGT CCT CCC TTC ATC CAA ACT CCT CCT CGT
83B1[c]	196	$(GA)_{11}$	GCC TTT CTT CAC ACC TGA TAG CTA A TCA GGT GCC TCG TTG AGT TC
92A1[c]	–	$(A)_{28}$	ACC GCC CGT GAC CAA A CCC ACC CCG TTA ACA TAT AAG TC
9B[c]	204	$(GA)_{28}$	GAC CGT GGA AGC AAG TGA GAA TG CCA AGC TTA TCG AGC CAT CCC
25C2[c]	132	$(GA)_{10}$	AAA CCT CCT CAA AAA CCC CTA AAC G TCC CCT CTT TCC TCT CTC TCT AGG C
19A[c]	–	$(GA)_8$	CAC AGC TCA CAC CAA ACA AAC CTA C CCC CGG GTT CGA AAT C

[a] Lagercrantz et al. (1993)

[b] Microsatellites from the EMBL and GenBank databases

[c] Kresovich et al. (1995), Dr A. Szewc-McFadden, pers. comm.

PCR programs used are those in the respective articles, microsatellites from databases amplified with the program described by Lagercrantz et al.

Microsatellites are simple DNA sequences consisting of repeated nucleotide motifs, and show extensive polymorphism due to the occurrence of different numbers of repeat units. The microsatellites (Table 2) were amplified in PCR using a pair of flanking primers, one primer of each pair labelled with fluorescein. The amplified products were visualised with ALF DNA Sequencer (Pharmacia).

One morphological marker, seed colour, which exhibits dominant inheritance ('brown' dominant over 'yellow'), was scored visually in the F_2 population.

Nomenclature

RFLP probes and the respective loci (Fig.1) were named according to Teutonico and Osborn

(1994): with the prefix WG (genomic DNA clones from *B.napus* cv 'Westar'), TG (genomic DNA clones from *B.rapa* cv 'Tobin') or EC (cDNA clones from *B.napus* cv 'Westar').

RAPD loci (Fig.1) were named by the primer: self-synthesised primers with plain numbers, and Operon primers with a letter and a number. Different polymorphic markers produced by the same primer were assigned with a small letter following the number of the primer (Table 3).

The microsatellite marker on the map has the prefix MS.

The nomenclature of ten *B.rapa* linkage groups (LG1-LG10) follows that on the previous map (Teutonico and Osborn 1994), the groups being identified by the common RFLP loci. Unassigned groups were named with capital letters (A–C, Fig. 1).

Statistical analysis

Because the inbred lines Jo4002 and Sv3402 contained residual heterozygosity, the F_1 seed was not uniform. Some marker loci were homozygous in some of the five F_1 individuals, leading to genetically uniform (with respect to these loci) F_2 progeny which had to be omitted in the linkage analysis. Therefore, the number of segregating individuals within the pooled F_2 population varied from locus to locus.

Goodness-of-fit to the expected F_2 segregation at marker loci was tested by chi-square analysis. Linkage relationships were evaluated by the MAPMAKER 3.0 computer program (Lander et al. 1987). Markers were grouped with a LOD score of 4.0 and a maximum recombination fraction of 0.4 as linkage criteria. On a few occasions, the LOD score threshold for linkage was decreased to 2.0 to include additional RFLP loci (indicated with a dashed line in Fig. 1) on the map. Map distances in centiMorgans were computed by Haldane's mapping function. Separate linkage analyses were performed for data set A (dominant markers originating from Jo4002) and data set B (dominant markers from Sv3402). Codominant markers were present in both data sets.

The map was built in two phases. First, a framework map was constructed from data set A, using only those markers that could be ordered with a LOD score difference > 3.0 (in some cases 2.0) in favour of the best map. To build up the final linkage map, all the other markers linked to each group with a LOD score > 4.0 were placed to the side of the closest framework locus (markers from data set A and codominant markers to the left and markers from data set B to the right).

Results

A high level of DNA polymorphism was observed in the mapping population: 67% of the

Fig.1. Linkage map of *B.rapa* ssp. *oleifera* constructed from the F_2 population of a cross, Jo4002 x Sv3402. For grouping markers, a LOD score threshold of 4.0 was used, except for TG1H12 and WG1G6, which were attached to the framework using a LOD score of 2.0 (indicated with a dashed line). For ordering, a LOD score difference >3.0 (wider line) or >2.0 (LGs 4, 6, 7, slim line) in favour of the best map was used. Dominant RAPD markers on the framework and on its left side are derived from Jo4002 (data set A), on the right side from Sv3402 (data set B). Marker distances are shown in centimorgans; for markers not included in the framework, two point map distances between the marker and the nearest framework locus are shown (LG9 includes four markers, 65a, 93a, 147b, 140d, which did not show linkage to any framework markers but only to markers from data set B). Linkage groups are named after the previous *B.rapa* RFLP map (Teutonico and Osborn 1994); the orientation of groups LG1, 6, 7 and 8, where only one locus is common with the previous map, is arbitrary. Codominant markers are underlined, loci common with the previous map printed in italics. The nomenclature of markers is described in Material and methods. Loci exhibiting aberrant segregation are indicated with *(P<0.05), **(P<0.01) or ***(P<0.001). LG10 is split into two parts, which probably represent distal segments of the same chromosome, because in data set B the codominant markers in these segments map to opposite ends of the same linkage group. Groups B and C contain markers from data set B only.

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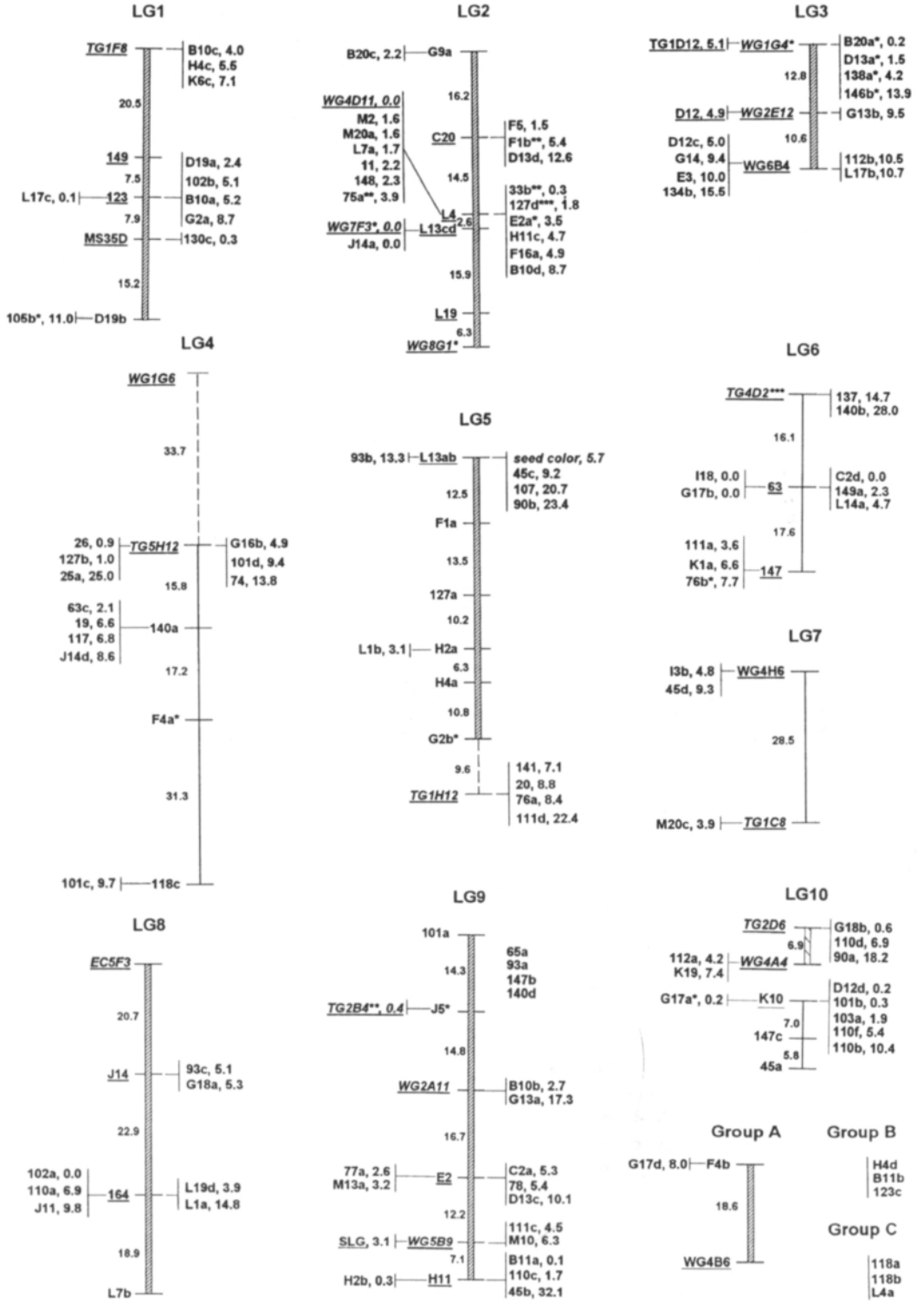

Primer	Marker size (kb)
11	0.9
19	0.7
20	1.6
25	2.2 (a)
26	0.4
33	0.9 (b)
45	1.0 (a), 1.1 (b), 1.6 (c), 0.6 (d)
63	1.4 (b), 0.5 (c), 1.3 (d)
65	2.1 (a)
74	1.4
75	0.5 (a)
76	0.9 (a), 0.6 (b)
77	1.6 (a)
78	1.2
90	1.6 (a), 1.2 (b)
93	1.2 (a), 0.6 (b), 0.6 (c)
101	1.4 (a), 1.6 (b), 1.2 (d)
102	1.7 (a), 1.2 (b)
103	0.2 (a)
105	1.0 (b)
107	0.7
110	2.5 (a), 2.2 (b), 2.1 (c), 1.4 (d), 0.8 (f)
111	1.9 (a), 1.0 (c), 1.3 (d)
112	2.5 (a), 0.9 (b)
117	1.8
118	2.0 (a), 1.8 (b), 1.5 (c)
123	1.5 (a), 1.3 (b), 1.0 (c)
127	2.4 (a), 2.2 (b), 1.2 (d)
130	1.1 (c)
134	0.5 (b)
137	0.5
138	1.8 (a)
140	3.0 (a), 2.3 (b), 0.9 (d)
141	0.9
146	0.7 (b)
147	2.3 (b), 2.2 (c), 1.0 (e), 0.9 (f)
148	0.4
149	2.0 (a), 1.3 (b), 1.3 (c)
164	0.8 (a), 0.7 (b)
B10	1.4 (a), 1.0 (b), 0.7 (c), 0.3 (d)
B11	1.0 (a), 0.8 (b)
B20	2.1 (a), 0.8 (c)
C02	1.1 (a), 0.5 (d)
C20	1.9 (a), 1.9 (b)
D12	2.0 (a), 1.9 (b), 1.7 (c), 1.6 (d)
D13	0.8 (a), 0.6 (c), 0.5 (d)
D19	1.3 (a), 0.8 (b)
E02	1.2 (a), 0.5 (b), 0.5 (c)
E03	1.0
F01	1.3 (a), 1.0 (b)
F04	1.5 (a), 1.0 (b)
F05	0.9
F16	2.5 (a)
G02	2.0 (a), 1.1 (b)
G09	1.9 (a)
G13	1.4 (a), 0.9 (b)
G14	1.0
G16	1.4 (b)
G17	1.4 (a), 1.3 (b), 0.5 (d)
G18	1.3 (a), 1.2 (b)
H02	1.0 (a), 0.9 (b)
H04	0.8 (a), 0.6 (c), 0.4 (d)
H11	1.9 (a), 1.8 (b), 1.3 (c)
I03	1.8 (b)
I18	1.0
J05	0.7
J11	1.2
J14	1.3 (a), 1.2 (b), 1.2 (c), 0.7 (d)
K01	2.8 (a)
K06	1.1 (c)
K10	0.7 (a), 0.6 (b)
K19	0.6
L01	1.8 (a), 1.4 (b)
L04	0.6 (a), 0.5 (b), 0.4 (c)
L07	1.4 (a), 0.9 (b)
L13	2.4 (a), 2.3 (b), 1.3 (c), 1.3 (d)
L14	0.7 (a)
L17	1.1 (b), 0.8 (c)
L19	1.5 (b), 1.4 (c), 1.1 (d)
M02	0.9
M10	1.2
M13	1.0 (a)
M20	0.8 (a), 0.7 (c)

Table 3. The approximate size of the RAPD markers included in the *B.rapa* map. The markers have the same name as the respective primer; in cases where a primer produces more than one polymorphic marker, lower case letters differentiate between the markers. Codominant markers are underlined; lower case letters are not used in their name on the map (Fig. 1).

81 RFLP probes and 79% of the 340 RAPD primers tested detected polymorphism between the parents of the cross, Jo4002 and Sv3402. Only one (35D) of the 14 microsatellites tested could be used as a marker; the others either detected no polymorphism, could not be inter-

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preted, or the primers failed to amplify detectable products (Table 2). The F_2 population was scored with a total of 26 RFLP probes, 90 RAPD primers, one microsatellite and one morphological marker. The 90 RAPD primers amplified 176 reproducible polymorphic loci, of which 15 exhibited codominant inheritance.

The 114 loci in data set A were arranged into twelve linkage groups, 3–16 markers each. In data set B (132 loci) 11 linkage groups with 3–20 markers each were found. Twenty markers in data set A and 27 markers in data set B remained unlinked.

Data set A was used for building the framework map, because all ten major linkage groups identified on the previous map (Teutonico and Osborn 1994) could be found. The framework map consisted of 48 markers, 32 showing codominant inheritance. The length of the linkage groups ranged from 6.9 cM to 98 cM, the total map distance being 519 cM.

The final linkage map, with markers from both data sets, was composed of 58 dominant markers from Jo4002, 71 dominant markers from Sv3402, 38 codominant markers and one morphological marker (Fig. 1). A total of 18 markers (printed in italics) were common with those of the previous map of Teutonico and Osborn (1994). Three triplets of linked markers were unassigned (groups A–C) and 32 individual loci remained unlinked.

Twenty markers (5 RFLPs and 15 RAPDs) on the final linkage map and seven unlinked markers exhibited distorted segregation (13% in total). Most of the mapped markers with skewed segregation clustered to linkage groups LG2 and LG3 and were distorted towards the Jo4002 allele. All except one of the distorted RAPD markers in LG2 and LG3 were derived from data set B (dominant allele from Sv3402).

Discussion

In this study, a linkage map of *B.rapa* ssp. *oleifera* was built from an F_2 population of the cross Jo4002 x Sv3402. Mainly RAPD markers were used, and all ten linkage groups of *B.rapa* could be identified.

Although repulsion phase markers were not used, it was impossible to order all markers accurately; the best order was usually only slightly more probable than the alternatives. There are a couple of explanations for this. First, estimation of recombination frequencies (and thus ordering of loci) between dominant markers is more inefficient than between codominant ones (Ott 1985). This holds true especially when the recombination fraction is small, which was the case in some chromosomal segments where markers appeared to cluster.

Second, the residual heterozygosity in the parents resulted in a reduced size of the F_2 progeny for some loci. This sometimes led to situations where the number of common informative loci between individuals was too low for a reliable estimation of recombination frequency. Finally, errors in genotyping may have caused ambiguity in the placement of loci. The inability to order all the loci reliably resulted in a total map length of only 519 cM; the total length of the map of Teutonico and Osborn (1994) was 1785 cM.

The clustering of loci to some map positions may reflect suppressed recombination in heterochromatic regions (Roberts 1965). It may, however, also be due to limited resolution of the map. Clustering of loci has been reported in maps of various different species (e.g. sugar beet, Barzen et al. 1995; *Arabidopsis,* Reiter et al. 1992; and *Lactuca sativa*, Kesseli et al. 1994).

Interestingly, loci with distorted segregation ratios mapped primarily to LG2 and LG3, and were skewed towards Jo4002 alleles. The clustering of skewed loci may indicate the existence of gametic or zygotic lethal alleles or gametophytic selection, i.e. gametes containing these regions of the Jo4002 genome were more competitive. Similar findings of skewed clusters have been reported in various plant species, e.g. *B.rapa* (Chyi et al. 1992, Teutonico and Osborn 1994), *B.napus* (Landry et al. 1991), *Hordeum vulgare* (Giese et al. 1994), *Lactuca sativa* (Kes-

seli et al. 1994), *Beta vulgaris* (Barzen et al. 1995) and *Medicago sativa* (Echt et al. 1993). Our results agreed with those of Teutonico and Osborn (1994) in having a cluster of skewed loci in LG2.

This is the first reported linkage map mostly consisting of RAPD loci in *B.rapa.* Not all the loci could be ordered unambiguously, which, however, does not diminish the value of the map. The loci can later be mapped more precisely in regions of particular interest by analysing more F_2 individuals. The map has already been used to find a QTL for palmitic acid (in LG9, Tanhuanpää et al. 1995b) and for oleic acid (in LG6, Tanhuanpää et al. 1996), and will be used in future studies.

Our previous work (Tanhuanpää et al. 1996) demonstrates the possibility of transferring RAPD marker information from one cross to another, and thus, the map can provide information for other researchers, too. In that work (Tanhuanpää et al. 1996), we studied the occurrence of a total of 20 markers in two different F_2 populations: one was derived from a cross between one individual from the line Jo4002 and another individual from the line Jo4072; the other population was the same as that used here, i.e. derived from the cross Jo4002 x Sv3402. Ten of the markers studied were derived from the parent, which differed in the two populations, and in those cases the probability of finding the same marker in the two populations was 40%.

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SELOSTUS

RAPD- ja RFLP-markkereista koostuva rypsin kytkentäkartta

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Rypsin kytkentäkartan laatimista varten kasvatettiin F_2 populaatio, jonka vanhempina olivat yksilöt kevätrypsilinjoista Jo4002 ja Sv3402. DNA-polymorfia oli runsasta tässä karttapopulaatiossa: testatuista 81 RFLP-probista 67 % ja testatuista 340 RAPD-primerista 79 % oli polymorfisia risteytysvanhemmissa. Lopullinen kartta koostui 168 markkerista, joista 144 oli RAPD-markkereita, 22 RFLP-markkereita, yksi morfologinen markkeri (siemenen väri) ja yksi mikrosatelliitti. Kaikki rypsin 10 kytkentäryhmää pystyttiin tunnistamaan, ja kartan kokonaispituus oli 519 cM. Markkereista 13 % ei segregoitunut normaalisti, ja suurin osa näistä markkereista kartoittui vain kahteen kytkentäryhmään. Kartta on ensimmäinen julkaistu rypsin kartta, jossa suurin osa markkereista on RAPDeja. Karttaa on jo aiemmin käytetty hyväksi paikallistettaessa palmitiini- ja öljyhappopitoisuuksiin vaikuttavat geenit, ja tulevaisuudessa sitä käytetään myös muiden tärkeisiin ominaisuuksiin vaikuttavien geenien kartoittamiseen.