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 F₂ population of a cross J04002 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 F₂ intercross population is used. In such cases, estimation of recombination frequency is very inefficient between repulsion phase markers (Ott 1985) and, there-
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 F₂ mapping population was derived by self-pollinating five F₁ 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 F₂ 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 _EcoRI_ or _HindIII_ as described by Tanhuanpää et al. (1994). The F₂ 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 SLG8 (Dwyer et al. 1991), and the 5-enolpyruvyl-shikimate-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 F₂ 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     | GGC TGT AGG CT  |
| 26     | GGA ATC TCG GT  |
| 33     | CCG CCT AGT TC  |
| 45     | AGA CGA TGT AC  |
| 63     | GAC CGT GAG AC  |
| 65     | AGC TGC ATG G   |
| 72     | TGG ACT CGA G   |
| 74     | GCT GAC TCG AG  |
| 75     | CGA ACC TGA TC  |
| 76     | ACT 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 GTC A   |
| 107    | GAC TCG AGA C   |
| 110    | ACG CCG TAC G   |
| 111    | TCG GAA GGA C   |
| 112    | GGA CAC TAC T   |
| 117    | GCG CAA GTC T   |
| 118    | CGT CGC TGT T   |
| 123    | ACT GAG CGT G   |
| 127    | CAG CTC AGG CT  |
| 129    | GTC CAC GTA GC  |
| 130    | ACT CTC 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    | CTT TCA CTT C   |
| 148    | CCG ACT TCC A   |
| 149    | TGC CAG TCT CC  |
| 164    | AGA AAT GGG G   |
Table 2. *B.napus* microsatellites used to search for polymorphism between the parents of the *B.rapa* ssp. *oleifera* cross, J04002 and Sv3402. '-' indicates no identifiable amplification.

| locus    | product size range (bp) | repeat sequence | flanking sequences 5' to 3' |
|----------|-------------------------|-----------------|---------------------------|
| MB4<sup>a</sup> | 71                      | (TG)<sub>10</sub> | TGT TTT GAT GTT TCC TAC TG |
|          |                         |                 | GAA CCT GTG GCT TTT ATT AC |
| MB5<sup>a</sup> |                         | (AT)<sub>8</sub>(AT)<sub>8</sub>(GT)<sub>8</sub> | AAG ATG TTT GTG GAT AT |
|          |                         |                 | AAT AGG ATT GAA GCC TTA C |
| X64257<sup>b</sup> |                         | (ATA)<sub>8</sub> | GTC TGC TCT CCA GAA CTA |
|          |                         |                 | CTG TAC CTT TGG TTT CGG |
| X61097<sup>b</sup> |                         | (CT)<sub>8</sub> | AAC GAC CCT TTT CCG TCA |
|          |                         |                 | GGC GCG TCA CAT TTA TAT |
| 12A<sup>c</sup> | 314                     | (GA)<sub>15</sub>(AAG)<sub>4</sub> | GCC GGT CTA GGG TTT GTG GGA |
|          |                         |                 | GAG GAA GTG AGA GCC GGA AAT CA |
| 35D<sup>c</sup> | 222–234                 | (GA)<sub>13</sub> | GCA GAA GGA GGA GAA GAG TTG G |
|          |                         |                 | TGG AGC CGT AAA GTT GTC ACC T |
| 38A<sup>c</sup> | 155                     | (TG)<sub>11</sub> | TGG TAA CTA GTG TCC GAC GAA AAT C |
|          |                         |                 | ACG CTG TCT TCA GGT CCC ACT C |
| 59A1<sup>c</sup> |                         | (CA)<sub>11</sub> | TGG CTC GAA TCA ACG GAC |
|          |                         |                 | TTG CAC CAA CAA GTC ACT AAA GTT |
| 72A<sup>c</sup> | 277                     | (TAA)<sub>9</sub>(GA)<sub>9</sub> | GCC CAC CCA CCT TCT TGT CTT |
|          |                         |                 | CCC TTC ATC CAA ACT CTT CTT CTT GTG |
| 83B1<sup>c</sup> | 196                     | (GA)<sub>11</sub> | GCC TCT CTA ACC TGA TAG CTA A |
|          |                         |                 | TCA GGT GGC TCG TAT AGT TC |
| 92A1<sup>c</sup> |                         | (A)<sub>28</sub> | ACC GCC CGT GAC CAA A |
|          |                         |                 | CCC ACC CGG TTA ACA TAT AAG TC |
| 9B<sup>c</sup> | 204                     | (GA)<sub>28</sub> | GAC CGT GGA AGC AAG TGA GAA TG |
|          |                         |                 | CCA AGC TTA TCA AGC CAT CCC |
| 25C2<sup>c</sup> | 132                     | (GA)<sub>10</sub> | AAA CCT CCT CAA AAA CCC CTA AAC G |
|          |                         |                 | TCC CCT CTT TCC TCT TCT TCT AGG C |
| 19A<sup>c</sup> |                         | (GA)<sub>8</sub> | CAC AGC TCA CAC CAA ACA AAC CTA C |
|          |                         |                 | CCC CGG GGT CGA AAT C |

<sup>a</sup> Lagercrantz et al. (1993)
<sup>b</sup> Microsatellites from the EMBL and GenBank databases
<sup>c</sup> Kresovich et al. (1995), Dr A. Szwec-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<sub>2</sub> population.

**Nomenclature**

RFLP probes and the respective loci (Fig.1) were named according to Teutonico and Osborn.
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(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 F1 seed was not uniform. Some marker loci were homozygous in some of the five F1 individuals, leading to genetically uniform (with respect to these loci) F2 progeny which had to be omitted in the linkage analysis. Therefore, the number of segregating individuals within the pooled F2 population varied from locus to locus.

Goodness-of-fit to the expected F2 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...
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### Table 3

| 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 (g), 0.9 (f) |
| 148    | 0.4             |
| 149    | 2.0 (a), 1.3 (b), 1.3 (g) |
| 164    | 0.8 (g), 0.7 (b) |

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-
interpreted, 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 × 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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References

Barzen, E., Mechelke, W., Ritter, E., Schulte-Kappert, E. & Salamini, F. 1996. An extended map of sugar beet genome containing RFLP and RAPD loci. Theoretical and Applied Genetics 90: 189–193.

Chyi, Y.-S., Hoenecke, M.E. & Sernyk, J.L. 1992. A genetic linkage map of restriction fragment length polymorphism loci in Brassica rapa (syn. campestris). Genome 35: 746–757.

Dellaporta, S.L., Wood, J. & Hicks, J.B. 1983. A plant DNA minipreparation: version II. Plant Molecular Biology Reporter 1: 19–21.

Dwyer, K.G., Balent, M.A., Nasrallah, J.B. & Nasrallah, M.E. 1991. DNA sequences of self-incompatibility genes from Brassica campestris and B. oleracea: polymorphism predating speciation. Plant Molecular Biology 16: 481–486.

Echt, C.S., Kidwell, K.K., Knapp, S.J., Osborn, T.C. & McCoy, T.J. 1993. Linkage mapping in diploid alfalfa (Medicago sativa). Genome 37: 61–71.

Ferreira, M.E., Williams, P.H. & Osborn, T.C. 1994. RFLP mapping of Brassica napus using doubled haploid lines. Theoretical and Applied Genetics 89: 615–621.

Gasser, C.S. & Klee, H.J. 1990. A Brassica napus gene encoding 5-enolpyruvylshikimate-3-phosphate synthase. Nucleic Acids Research 19: 2821.

Giese, H., Holm-Jensen, A.G., Mathiassen, H., Kjær, B., Rasmussen, S.K., Bay, H. & Jensen, J. 1994. Distribution of RAPD markers on a linkage map of barley. Hereditas 120: 267–273.

Kesseli, R.V., Paran, I. & Michelmore, R.W. 1994. Analysis of a detailed genetic linkage map of Lactuca sativa (lettuce) constructed from RFLP and RAPD markers. Genetics 136: 1435–1446.

Kianian, S.F. & Quiros, C.F. 1992. Generation of a Brassica oleracea composite RFLP map: linkage arrangements among various populations and evolutionary implications. Theoretical and Applied Genetics 84: 544–554.

Kresovich, S., Szewc-McFadden, A.K., Blied, S.M. & McFerson, J.R. 1995. Abundance and characterization of simple-sequence repeats (SSRs) isolated from a size-fractionated genomic library of Brassica napus L. (rape-seed). Theoretical and Applied Genetics 91: 206–211.

Lagercrantz, U., Ellefgen, H. & Andersson, L. 1993. The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. Nucleic Acids Research 21: 1111–1115.

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E. & Newburg, L. 1987. MAP-MAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174–181.

Landry, B.S., Hubert, N., Etoh, T., Harada, J.J. & Lincoln, S.E. 1991. A genetic map for Brassica napus based on restriction fragment length polymorphisms detected with expressed DNA sequences. Genome 34: 543–552.

K., Hubert, N., Crete, R., Chang, M., Lincoln, S.E. & Etoh, 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 brassica* (Woronin). Genome 35: 409–419.

Maniatis, T., Fritsch, E.F. & Sambrook, J. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor/NY.

Ott, J. 1985. Analysis of human genetic linkage. John Hopkins University Press, Baltimore, Maryland.

Reiter, R.S., Williams, J.G.K., Feldmann, K.A., Rafalski, J.A., Tingey, S.V. & Scolnik, P.A. 1992. Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. Proceedings of National Academy of Sciences, USA 89: 1477–1481.

Roberts, P.A. 1965. Difference in the behavior of eu- and hetero-chromatin: crossing over. Nature 205: 725–726.

Slocum, M.K., Fiddore, S.S., Kennard, W.C., Suzuki, J.Y. & Osborn, T.C. 1990. Linkage arrangement of restriction fragment length polymorphism loci in *Brassica oleracea*. Theoretical and Applied Genetics 80: 57–64.

Song, K.M., Suzuki, J.Y., Slocum, M.K., Williams, P.H. & Osborn, T.C. 1991. A linkage map of *Brassica rapa* (syn. *campestris*) based on restriction fragment length polymorphism loci. Theoretical and Applied Genetics 82: 296–304.

Tanhuanpää, P.K., VilkkI, H.J., VilkkI, J.P. & Pulli, S.K. 1993. Genetic polymorphism at RAPD loci in spring turnip rape (*Brassica rapa* ssp. *oleifera*). Agricultural Science in Finland 2: 303–310.

–, VilkkI, J.P. & VilkkI, H.J. 1994. Segregation and linkage analysis of DNA markers in microspore derived and F2 populations of oilseed rape (*Brassica napus* L.). Euphytica 74: 59–65.

–, VilkkI, J.P. & VilkkI, H.J. 1995a. Association of a RAPD marker with linolenic acid concentration in the seed oil of rapeseed (*Brassica napus* L.). Genome 38: 414–416.

–, VilkkI, J.P. & VilkkI, H.J. 1995b. Identification of a RAPD marker for palmitic-acid concentration in the seed oil of spring turnip rape (*Brassica rapa* ssp. *oleifera*). Theoretical and Applied Genetics 91: 477–480.

–, VilkkI, J.P. & VilkkI, H.J. 1996. Mapping of a QTL for oleic acid concentration in spring turnip rape (*Brassica rapa* ssp. *oleifera*). Theoretical and Applied Genetics 92: 952–956.

Teutondo, R.A. & Osborn, T.C. 1994. Mapping of RFLP and qualitative trait loci in *Brassica rapa* and comparison to the linkage maps of *B. napus, B.oleracea*, and *Arabidopsis thaliana*. Theoretical and Applied Genetics 89: 885–894.

Uzunova, M., Ecke, W., Weissleder, K. & Röbbelen, G. 1995. Mapping the genome of rapeseed (*Brassica napus* L.). 1. Construction of an RFLP linkage map and localization of QTLs for seed glucosinolate content. Theoretical and Applied Genetics 90: 194–204.

SELOSTUS

**RAPD- ja RFLP-markkereista koostuva rypsin kytentäkartta**

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Rypsin kytentäkartan laatimista varten kasvatettiin F2 populaaatio, jonka vanhempina olivat yksilöt kevätpysilinjoista Jo4002 ja Sv3402. DNA-polymorfia oli runsasta tässä karttapopulaatioissa: testatuista 81 RFLP-probista 67 % ja testatuista 340 RAPD-primeista 79 % oli polymorfisia risteytysvahemmissa. Lopullinen kartta koostui 168 markkereista, joista 144 oli RAPD-markkereita, 22 RFLP-markkereita, yksi RFLP-markkereita, yksi morfologinen markkeri (siemenen väri) ja yksi mikrosatelliitti. Kaikki rypsin 10 kytentäryhmää pystyttiin tunnistamaan, ja kartan kokonaispituus oli 519 cM.

Markkereista 13 % ei segreigoitunut normaalisti, ja suurin osa näistä markkereista kartoittui vain kahteen kytentäryhmään. Kartta on ensimmäinen julkaistu rypsin kartta, jossa suurin osa markkereista on RAPDeja. Kartta on jo aiemmin käytetty hyväksi paikallistettaessa palmiitii- ja öljyhappopitosuksiin vaikuttavat geenit, ja tulevaisuudessa sitä käytetään myös muiden tärkeisiin ominaisuuksiin vaikuttavien geenien kartoittamiseen.