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P. K. Tanhuanpää · J. P. Vilkki · H. J. Vilkki

Mapping of a QTL for oleic acid concentration in spring turnip rape (*Brassica rapa* ssp. *oleifera*)

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Abstract Bulk segregant analysis was used to search for RAPD (random amplified polymorphic DNA) markers linked to gene(s) affecting oleic acid concentration in an F_2 population from the *Brassica rapa* ssp. *oleifera* cross Jo4002 x a high oleic acid individual from line Jo4072. Eight primers (= 8 markers) out of 104 discriminated the 'high' and 'low' bulks consisting of extreme individuals from the oleic acid distribution. These markers were analysed throughout the entire F_2 population, and their association with oleic acid was studied using both interval mapping and ANOVA analysis. Six of the markers mapped to one linkage group. A quantitative trait locus (QTL) affecting oleic acid concentration was found to reside within this linkage group with a LOD score > 15. The most suitable marker for oleic acid content is OPH-17, a codominant marker close (< 4 cM) to the QTL. The mean seed oleic acid content in the F_2 individuals carrying the larger allele of this marker was 80.14 ± 9.76%; in individuals with the smaller allele, 54.53 ± 6.83%; in the heterozygotes, 65.47 ± 8.15%. To increase reproducibility, the RAPD marker was converted into a SCAR (sequence characterized amplied region) marker with specific primers. Marker OPH-17 can be used to select spring turnip rape individuals with the desired oleic acid content.

Key words *Brassica rapa* · RAPD · SCAR · Bulked segregant analysis · QTL · Oleic acid

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P. K. Tanhuanpää (✉)
Plant Breeding Section, Institute of Crop and Soil Science, Agricultural Research Centre of Finland, FIN-31600 Jokioinen, Finland

J. P. Vilkki
Boreal Plant Breeding, FIN-31600 Jokioinen, Finland

H. J. Vilkki
Animal Breeding, Institute of Animal Production, Agricultural Research Centre of Finland, FIN-31600 Jokioinen, Finland

Introduction

The fatty acid composition of *Brassica* oil determines its physical and chemical properties. The aim of many breeding strategies of spring turnip rape is the production of more thermostable oils by increasing the amount of oleic acid (18:1). At present, the normal amount of oleic acid in the seed oil of spring turnip rape is around 60%, whereas oleic acid concentrations higher than 70% have already been reached in rapeseed.

In breeding programmes, the fatty acid concentrations of individual plants are usually determined on seeds or a half-seed sample by gas chromatography. There is a need for a genotype-based analysis for fatty acid composition because environmental factors (temperature, day length) may influence the manifestation of the phenotype (Appelqvist 1968, 1971; Jönsson 1975; van Hal and Baarsel 1974). Selection based on the genotype is possible with genetic tags. A tag is an easily scored marker closely linked to the gene that controls the desired character. Such a tag can be found with the aid of a marker map, but it is also possible using near-isogenic lines or bulked segregant analysis (Michelmore et al. 1991).

We report the identification of RAPD (random amplified polymorphic DNA) markers linked to a QTL (quantitative trait locus) affecting oleic acid concentration in spring turnip rape (*Brassica rapa* ssp. *oleifera*). The RAPDs were identified using bulked segregant analysis (BSA) and could be positioned to a specific linkage group with the aid of our previously constructed marker map of spring turnip rape. The most suitable RAPD marker for oleic acid content was converted into a SCAR (sequence characterized amplified region) marker (Paran and Michelmore 1993) with specific primers.

Material and methods

Plant material

A population of 100 F_2 plants segregating for oleic acid originated from a cross between one individual from the repeatedly selfed Finnish spring turnip rape line Jo4002 and another from the high oleic acid material (found by J. P. Vilkki) derived from line Jo4072. The quantitative trait data is based on fatty acid content in seed samples. Because some of the F_2 plants did not produce enough seeds, the data contain only 94 individuals.

The F_2 mapping population originated from a cross between two individuals of repeatedly selfed spring turnip rape lines Jo4002 and Sv3402 (Tanhuanpää et al. 1995b).

DNA was extracted by a slightly modified Dellaporta method (Dellaporta et al. 1983), except that DNA from the single F_2 plants of the oleic acid population was prepared by the Edwards method (Edwards et al. 1991) as described by Tanhuanpää et al. (1993).

Fatty acid determination

The fatty acid content of each individual was measured by gas chromatography of the fatty acid methylesters (modified method of Thies 1968) present in two different seed samples of 10 seeds each obtained by selfing. In addition, fatty acids were measured in a half-seed sample, i.e. from the outer cotyledon of the freshly germinated seed; the rest of the embryo was still able to produce a complete plant.

In the oleic acid population, the amount of palmitic, stearic, oleic, linoleic and linolenic acid in the seeds (calculated as a percentage of total fatty acids) was 4.9%, 2.1%, 42.0%, 30.6% and 17.9%, respectively, for the parent Jo4002 and 3.3%, 1.5%, 69.2%, 13.4% and 10.2%, respectively, for the high oleic acid parent. In the F_2 individuals, the oleic acid concentrations as determined from the analysis of seed samples varied from 39.7% to 88.2%, the mean being $65.1 \pm 11.7\%$. The corresponding concentrations from half-seed analysis were higher, but the two results were positively correlated ($r = 0.80$).

Bulking of F_2 individuals

In order to save time, F_2 individuals were bulked on the basis of fatty acid analysis of half-seeds. The 'high' and 'low' oleic acid bulks each contained 9 individuals from the extreme ends of oleic acid distribution (their oleic acid concentration varied from 86% to 90% and 60% to 64%, respectively). When fatty acid concentrations were measured from the samples, it turned out that 1 individual in the 'high' bulk was pooled incorrectly (oleic acid concentration: 50%). This might have complicated the finding of markers discriminating the bulks.

RAPD analysis

RAPD primers were either synthesized (assigned with numbers) on an Applied Biosystems 392 DNA/RNA Synthesizer or purchased (prefix OP) from Operon Technologies (Alameda, Calif.). RAPD analysis was carried out as described in Tanhuanpää et al. (1995a) with the following modifications for the oleic acid populations: a reaction volume of 25 μl, *Taq* polymerase purchased from Promega and a DNA concentration (GeneQuant, Pharmacia) of F_2 individuals of 60–120 ng. The programme consisted of 35 cycles of 30 s at 95 °C, 30 s at 40 °C and 2 min at 72 °C in a MJ Research PTC-100 thermal cycler. Different polymorphic markers produced by the same primer were designed by small letters following the number of the primer. The homology of the RAPD bands with similar molecular weights was ascertained by hybridization using the RAPD fragment excised from the gel as a probe.

SCAR analysis

The RAPD bands of interest (codominant markers OPH-17b and -c) were excised from the agarose gel and purified by the SpinBind DNA Recovery System (FMC BioProducts). The bands were reamplified, excised from the gel, purified and ligated with a Sureclone ligation kit (Pharmacia). Recombinant plasmids were chemically transformed, and the correct clones were picked up after hybridization with the purified RAPD band. DNA sequencing of the cloned RAPD fragments was carried out in both directions with ALF DNA Sequencer (Pharmacia). SCAR primers were designed from the sequence information: one primer (CAC TCT CCT CCA CCA TAC ACT CCC) contained the original 10 bases of the RAPD primer plus the next 14 internal bases; the other (GGT AGG CAG CAA CGC AAG AAA CGC) was designed at the first possible site on the other end of the sequence. The annealing temperature for the primers was 66 °C.

Statistical analysis

Goodness-of-fit between observed and expected F_2 segregation ratios at marker loci was tested by a chi-square analysis. Linkage relationships were evaluated by the MAPMARKER 3.0 computer programme (Lander et al. 1987) with a LOD score > 3.0 and a recombination fraction of 0.40 as linkage criteria. Haldane's mapping function was used to compute the distances in centiMorgans (cM) from the recombination fractions. Separate linkage maps for each parent were generated using RAPDs only in coupling phase. QTLs were identified using MAPMAKER/QTL 1.1 with a LOD score threshold of 3.0 to declare the presence of a putative QTL and standard analysis of variance (ANOVA).

Results

Out of the 153 primers tested, 120 (78%) exhibited polymorphism between the parents of the oleic acid population. Of these, 104 polymorphic primers were tested for differences between the two bulks. Eight primers (= 9 markers) met this criterium: 5 markers were associated with high and 4 with low oleic acid content (Table 1). Hybridization analysis indicated (result not shown) that the markers OPH-17b and -c were codominant alleles, and these were subsequently treated

Table 1 RAPD markers identifying polymorphism between the bulks constructed on the basis of oleic acid concentration analysed from half-seeds. The individuals in the bulks are from the F_2 population of *Brassica rapa* ssp. *oleifera* cross Jo4002 × a high oleic-acid individual from the line Jo4072

Marker[a]	Primer sequence	High bulk[b]	Low bulk[b]
25	GCG TGT AGG CT	−[c]	+
65	ACG TGC ATG G	−	+
OPH-17b	CAC TCT CCT C	+	−
OPH-17c		−	+
OPI-18	TGC CCA GCC T	−	+
OPJ-17	ACG CCA GTT C	+	−
OPK-19	CAC AGG CGG A	+	−
OPL-11	ACG ATG AGC C	+	−
OPL-14	GTG ACA GGC T	+	−

[a] Small letters indicate multiple polymorphic loci detected by one primer

[b] Oleic acid concentration > 80% and < 70%, respectively

[c] +/−: presence/absence of the marker

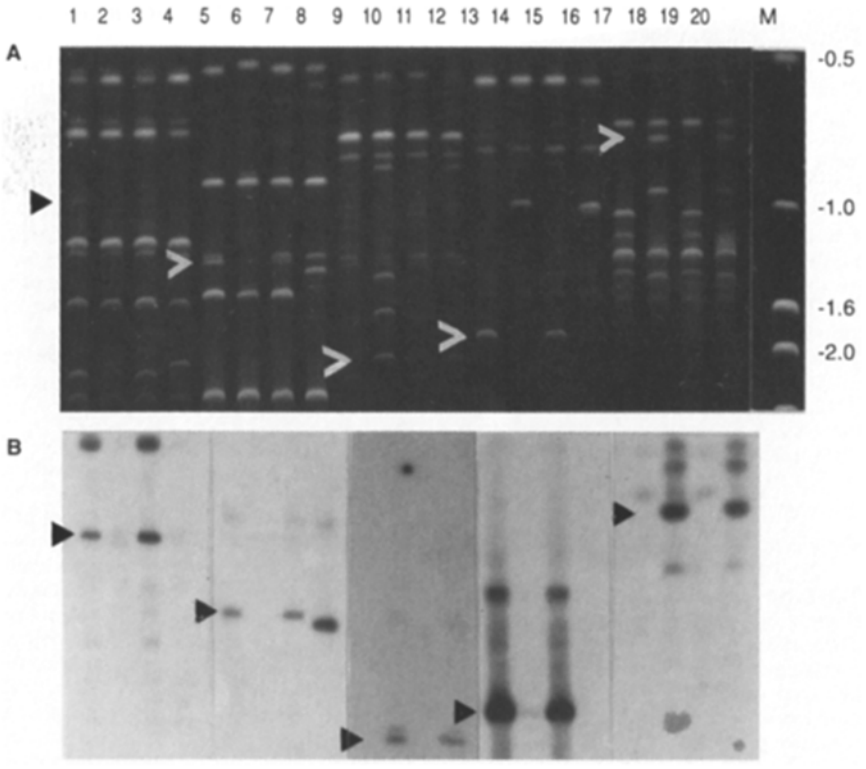

Fig. 1 A, B Verification of the homology of RAPD fragments between the parents of two different *Brassica rapa* ssp. *oleifera* populations. The parents of the population segregating for oleic acid are Jo4002 (*lanes 1, 5, 9, 13, 17*) and the high oleic-acid individual from line Jo4072 (*lanes 2, 6, 10, 14, 18*); and those of the mapping population, Jo4002 (*lanes 3, 7, 11, 15, 19*) and Sv3402 (*lanes 4, 8, 12, 16, 20*). **A** RAPD amplification products using primers OPI-18 (*lanes 1–4*), 63 (*lanes 5–8*), 140 (*lanes 9–12*), 111 (*lanes 13–16*), and OPL-14 (*lanes 17–20*). The markers of interest are indicated by an *arrow*. The molecular weight marker (M)–is a 1-kb DNA ladder (BRL). **B** Hybridization of the RAPD fragments from the oleic acid parent to a Southern blot of the individuals shown in **A**. The fragments were homologous in the two popu-lations. In addition, one codominant allele for primer 63 was found in the mapping population (*lane 8*)

as a single marker locus (OPH-17). The entire F_2 oleic acid population was screened with the 8 markers.

Six of these 8 markers mapped to one linkage group. In order to identify this group among the nine linkage groups found in our earlier study, the parents of the mapping population (Tanhuanpää et al. 1995b) were screened with these markers. Two (OPL-14 and OPI-18) were found to be polymorphic and subsequently analysed in the mapping population. The homology of these 2 markers in the two different populations was ascertained by hybridization (Fig. 1). The markers mapped to a linkage group harbouring the RFLP marker TG4D2 (Fig. 2), which is known to be located on LG6 in the published *B. rapa* RFLP map (Teutonico and Osborn 1995).

Some of the RAPD markers on the linkage group corresponding to LG6 (Tanhuanpää, unpublished) had not been tested for polymorphism between the parents of the oleic acid population. Markers 140b, 63d and 111a revealed polymorphism and were scored throughout the entire population. The identity of markers across the two populations was confirmed (Fig. 1). Primer 63 produced a codominant marker (alleles b and d) in the mapping population, but in the oleic acid population 63d exhibited dominant inheritance. In the oleic acid population, primer 140 generated another marker (140f), in addition to 140b, that seemed to be associated with oleic acid when tested in the bulks. Markers 140b and 140f do not cross-hybridize and are thus probably not (overlapping) alleles.

The oleic acid population had now been analysed with a total of 12 RAPD markers, which all segregated

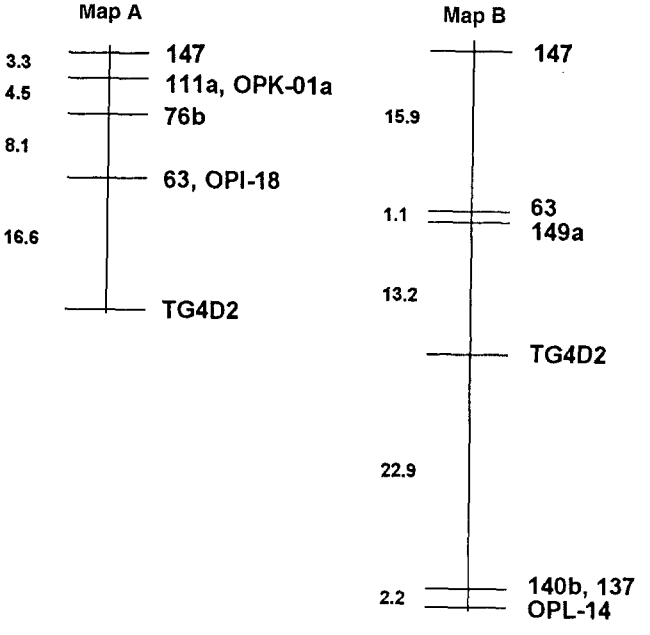

Fig. 2 Genetic maps of linkage group LG6 constructed from the *Brassica rapa* ssp. *oleifera* cross Jo4002 × Sv3402. Distance on the *left* are in centiMorgans (cM). TG4D2 is an RFLP marker; the other markers are RAPDs. RAPD markers 63 and 147 exhibit codominant inheritance. The visible RAPD alleles are inherited from Jo4002 in map A and from Sv3402 on map B. The order and distances of markers were computed with MAPMAKER 3.0 computer programme

with the expected ratios. Two datasets were generated: the visible RAPD alleles were derived from Jo4002 in dataset A (markers 25, 63d, 65, 111a, 140f, OPI-18) and from the high oleic acid parent in dataset B (markers

140b, OPJ-17, OPK-19, OPL-11, OPL-14). Codominant marker OPH-17 was present in both datasets.

In dataset A, all markers except 111a mapped to the same linkage group, which exhibited a peak LOD score of 15.3 (Fig. 3) for a QTL affecting oleic acid content. This QTL accounted for 53.2% of the variation in oleic acid content.

In dataset B, markers OPL-14, 140b, OPK-19 and OPH-17 mapped to one linkage group (Fig. 3), which exhibited a QTL for oleic acid with a peak LOD score of 16.4 and variance-explained 71.2%. OPL-11 and OPJ-17 were linked only to each other.

The precise location of the QTL remained unsettled in both maps. The maximum LOD score for QTL was situated at the end of the linkage group in map A, and the likelihood surface for the QTL was too flat in map B. However, in both maps the QTL was situated near marker OPH-17 (< 4 cM), which is the most suitable marker for oleic acid content because of its codominant nature. The larger allele (b) of this marker was associated with high oleic acid content: the mean seed oleic acid content in the 17 F_2 individuals carrying the allele was $80.14 \pm 9.76\%$, while that in the 26 F_2 individuals carrying the smaller allele (c) was $54.53 \pm 6.83\%$. The mean seed oleic acid content in the 51 heterozygous F_2 individuals was $65.47 \pm 8.15\%$ (Fig. 4). The differences are statistically significant at $P < 0.001$ (t-test).

The codominant bands OPH-17b and -c (approximately 1040 and 1020 bp, respectively) were sequenced and the sequences found to be identical as far as they were readable (250–300 bp from both ends). Therefore, the size difference between the two bands was not due to differences at the primer binding site but to insertion or deletion in the middle of the fragment. Marker OPH-17 was converted into a SCAR marker; the RAPD and SCAR primers amplified bands of similar size.

Variance analysis confirmed the association of the markers on the QTL maps with oleic acid (results not shown), variance–explained being highest (53%) for OPH-17. In addition, it revealed the association of markers OPJ-17 and OPL-11 with oleic acid (variance–explained 12%).

Oleic acid content was related with palmitic (16:0), linoleic (18:2) and linolenic (18:3) acid contents. Firstly, it showed a negative correlation with these three fatty acids in regression analysis (Table 2). Secondly, ANOVA analysis revealed that all of the markers on LG6 were also associated with palmitic, linoleic and linolenic acid (results not shown). Finally, QTLs affecting palmitic, linoleic and linolenic acid contents mapped approximately to the same region as that for oleic acid. It appears that the same locus affects the seed content of palmitic, oleic, linoleic and linolenic acids.

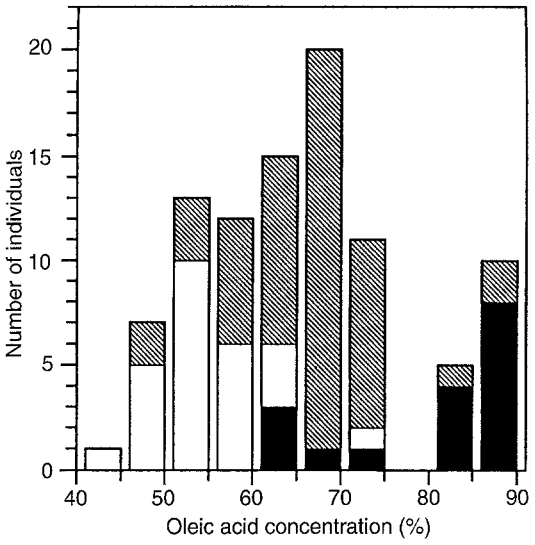

Fig. 4 Distribution of the alleles (c = □,b = ■ and both = ▨) of the codominant marker OPH-17 among classes with different percentages of oleic acid in the F_2 population from the *Brassica rapa* ssp. *oleifera* cross Jo4002 × a high oleic acid individual from line Jo4072

Fig. 3 QTL likelihood maps for oleic acid concentration in the F_2 population from the *Brassica rapa* ssp. *oleifera* cross Jo4002 × a high oleic acid individual from line Jo4072. The visible RAPD alleles are inherited from Jo4002 in map A and from the high oleic acid parent in map B. Orientation of the two maps is arbitrary. The LOD scores were computed using the MAPMAKER/QTL 1.1 computer programme

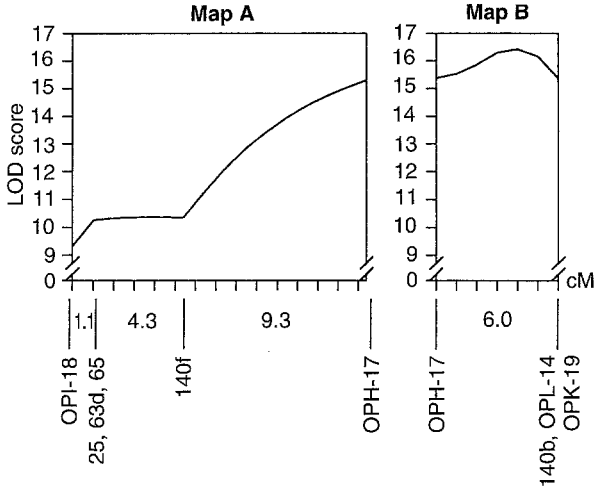

Table 2 Correlation between oleic acid and four other fatty acids based on linear regression analysis in the F_2 progeny of the *Brassica rapa* ssp. *oleifera* cross Jo4002 × a high oleic-acid individual from line Jo4072. $n = 94$ in each case

Fatty acid	Slope	Intercept (%)	r
Palmitic acid	−0.03	5.26	0.82***
Stearic acid	−0.0004	1.44	0.017
Linoleic acid	−0.682	61.07	0.99***
Linolenic acid	−0.30	30.47	0.95***

*** Correlation significant at $P < 0.001$

Discussion

The codominant RAPD marker OPH-17 linked to a QTL controlling oleic acid content in spring turnip rape was found by means of bulk segregant analysis. The existence of a previously constructed marker map enabled positioning of the QTL to linkage group LG6. Accordingly, for the first time it was possible to transfer RAPD marker information from one cross to another.

The QTL for oleic acid also influenced the seed content of palmitic, linoleic and linolenic acids. This was expected because fatty acids are formed by means of enzymatically controlled carbon chain elongations and desaturations (Thompson 1983), and therefore a change in the amount of one fatty acid generally affects the others as well. This QTL is either controlling the chain elongation (18:1 → 22:1) or desaturation (18:1 → 18:2) step. In a population free of eicosenoic (20:1) and erucic (22:1) acids, the latter controls and leads to a negative correlation between oleic and linoleic (and linolenic) acid content (Chen and Beversdorf 1990). This correlation was evident in the present study, and thus the QTL appears to be a major locus affecting the fatty acid desaturation step from oleic to linoleic acid.

The QTL for oleic acid is the third QTL found by the authors to control the fatty acid synthesis chain in *Brassica*. Although all of these QTLs alter the amounts present in the seed of several fatty acids, they exercise their main influence on only one fatty acid: linolenic acid (Tanhuanpää et al. 1995a), palmitic acid (Tanhuanpää et al. 1995b) or oleic acid.

In addition to the markers in linkage group LG6, 2 other linked markers were found to be associated with oleic acid. Because the markers were not polymorphic in the mapping population, it was not possible to localize them to a certain linkage group. They are possibly linked to another QTL with a smaller effect on oleic acid.

Because RAPDs are generally known to be sensitive to reaction conditions, marker OPH-17 was converted into a SCAR marker with specific primers. Marker-assisted selection with marker OPH-17 will accelerate breeding for oleic acid content in spring turnip rape. In addition, the marker will provide a starting point for map-based cloning of the gene affecting oleic acid.

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