

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

Identification of a RAPD marker for palmitic-acid concentration in the seed oil of spring turnip rape (*Brassica rapa* ssp. *oleifera*)

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Abstract F₂ progeny (105 individuals) from the cross Jo4002 × Sv3402 were used to identify DNA markers associated with palmitic-acid content in spring turnip rape (*Brassica rapa* ssp. *oleifera*). QTL mapping and ANOVA analysis of 140 markers exposed one linkage group with a locus controlling palmitic-acid content (LOD score 27), and one RAPD (random amplified polymorphic DNA) marker, OPB-11a, closely linked (1.4 cM) to this locus. Palmitic-acid content in the 62 F₂ plants with the visible allele of marker OPB-11a was $8.45 \pm 3.15\%$, while that in the 24 plants without it was $4.59 \pm 0.97\%$. As oleic-acid concentration is affected by a locus on the same linkage group as the palmitic-acid locus, this locus probably controls the chain elongation from palmitic acid to oleic acid (through stearic acid). Marker OPB-11a may be used in future breeding programs of spring turnip rape to simplify and hasten the selection for palmitic-acid content.

Key words *Brassica rapa* · RFLP · RAPD · QTL · Palmitic acid

Introduction

Fatty acid composition determines the quality of *Brassica* oil. Breeding strategies aim at fatty acid profiles that are more suitable for the production of table margarine and cooking oil. The present goals include the increase of thermostable oleic acid (18:1) for improved cooking

oil, the increase of dietary essential linoleic acid (18:2), and the reduction of linolenic acid (18:3) to prevent taste impairment resulting from double-bond oxidization. For the production of table margarine, *Brassica* oil should include more (10–12%) 16-carbon fatty acids, such as palmitic (16:0) and palmitoleic (16:1) acid, because they reduce the tendency of margarines made from low erucic oils to form large internal crystals on storage. This propensity is presently inhibited either by blending another oil (e.g. palm oil) to margarine or by chemical hardening.

Fatty acid biosynthesis follows a pathway progressing through enzymatically controlled carbon-chain elongation and desaturation steps (Thompson 1983). Fatty acid concentrations are usually determined by gas chromatography from half-seed or bulked-seed samples.

An alternative method to select for the desired fatty acid composition, would be to use DNA markers closely linked to loci affecting the trait (marker-assisted selection). The aim of the present study was to identify DNA markers (RFLPs = restriction fragment length polymorphisms and RAPDs = random amplified polymorphic DNAs) tightly linked to the locus controlling palmitic-acid content in spring turnip rape (*Brassica rapa* ssp. *oleifera*).

Material and methods

Plant material

The mapping population originated from a cross between two individuals of repeatedly selfed spring turnip rape lines Jo4002 and Sv3402 which differed for their seed palmitic-acid content, being < 4% and > 11%, respectively. Five F₁ individuals from this cross were selfed to produce the F₂ population. Most of the linkage data is based on 77 F₂ individuals; an additional 28 plants were scored for some markers to confirm their linkages. Quantitative-trait data comprises only 67 (or 94) individuals because some of the F₂ plants did not produce enough seed for fatty acid determination. F₃ progeny of some F₂s were analysed to reveal the genotype of the F₂ individuals (hetero- or homo-zygous for the visible RAPD marker).

DNA of the parents and F₂ individuals was extracted by a slightly modified Dellaporta method (Dellaporta et al. 1983), while DNA of

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P. 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

F₂ individuals was prepared by the Edwards method (Edwards et al. 1991) as described by Tanhuanpää et al. (1993).

Markers

The markers used in this study included one morphological marker (seed colour), RFLPs and RAPDs.

RFLP analysis was performed by standard methods (Maniatis et al. 1982), as described by Tanhuanpää et al. (1994), using mainly genomic DNA clones from *B. rapa* or *B. napus* from Dr. T. Osborn (University of Wisconsin-Madison). The probes used in screening the F₂ progeny comprised 22 genomic DNA clones, a PCR-amplified genomic sequence of a *Brassica* self-incompatibility gene SLG-8_c (Dwyer et al. 1991), and a PCR-amplified sequence of the 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS) gene from *B. napus* (Gasser and Klee 1990).

The 220 RAPD primers were either synthesised (denoted by 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. (1995) with minor modifications. Different polymorphic markers produced by the same primer are assigned with small letters after the number of the primer. The genotype of some individuals was ascertained by hybridisation analysis using the RAPD marker excised from the gel as a probe.

Fatty acids

Fatty acid composition was analysed using gas chromatography of the fatty acid methyl esters (modified method of Thies 1968) from two different samples of ten seeds obtained by selfing. The amount of palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acid in the seed (calculated as a percentage of total fatty acids) of the parents were 4.6, 0.3, 1.8, 45.4, 30.9 and 15.3, respectively, for Jo4002 and 15.8, 5.7, 1.2, 38.6, 21.2 and 15.9, respectively for Sv3402. The mean percentage of palmitic acid in the F₂ progeny was 7.38 ± 3.18 , and the average deviation between the two different samples of ten seeds from a F₂ individual was 0.81 ± 0.89 .

Statistical analysis

The inbred lines Jo4002 and Sv3402 contained residual heterozygosity. This resulted in genetic variation between five F₁ parents of the pooled F₂ population. Marker loci were sometimes heterozygous in certain F₁ plants and segregating among the resulting F₂ progeny, but homozygous in other F₁ plants, leading to genetically uniform F₂ progeny. For this reason, the number of segregated 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 a chi-Square analysis. Linkage relationships were evaluated by the MAPMAKER 3.0 computer program (Lander et al. 1987) with a LOD score > 3.0 and a recombination fraction of 0.40 as linkage criteria. Distances in centiMorgans were computed by Haldane's mapping function. Quantitative trait data were analysed by MAPMAKER/QTL 1.1 using a LOD score threshold of 3.0 to declare the presence of a putative quantitative trait locus (QTL), and standard analysis of variance (ANOVA).

Results

Sixty-seven percent of the 74 RFLP probes, and 75% of the 220 RAPD primers tested, identified polymorphisms between the parents of the cross, Jo4002 and Sv3402. The F₂ population was analysed using 24 RFLP probes and 116 reproducible RAPD markers generated by 53 primers. Three of the RAPDs were

co-dominant. Loci defined by six RFLPs and 14 RAPDs exhibited distorted segregation ratios ($P < 0.01$).

Because estimation of recombination frequencies is very inefficient in repulsion matings of dominant markers (Ott 1985), two linkage maps were generated using only RAPDs in coupling phase. The visible (= fragment present) RAPD alleles were derived from Jo4002 in map A, and from Sv3402 in map B. There were 72 markers in data set A and 96 markers in data set B (RFLPs and co-dominant RAPDs are present in both sets).

Nine linkage groups with 3–14 markers each were discovered in both maps ($n = 10$ in *B. rapa*). Co-dominant markers enabled the identification of six corresponding linkage groups between the two maps.

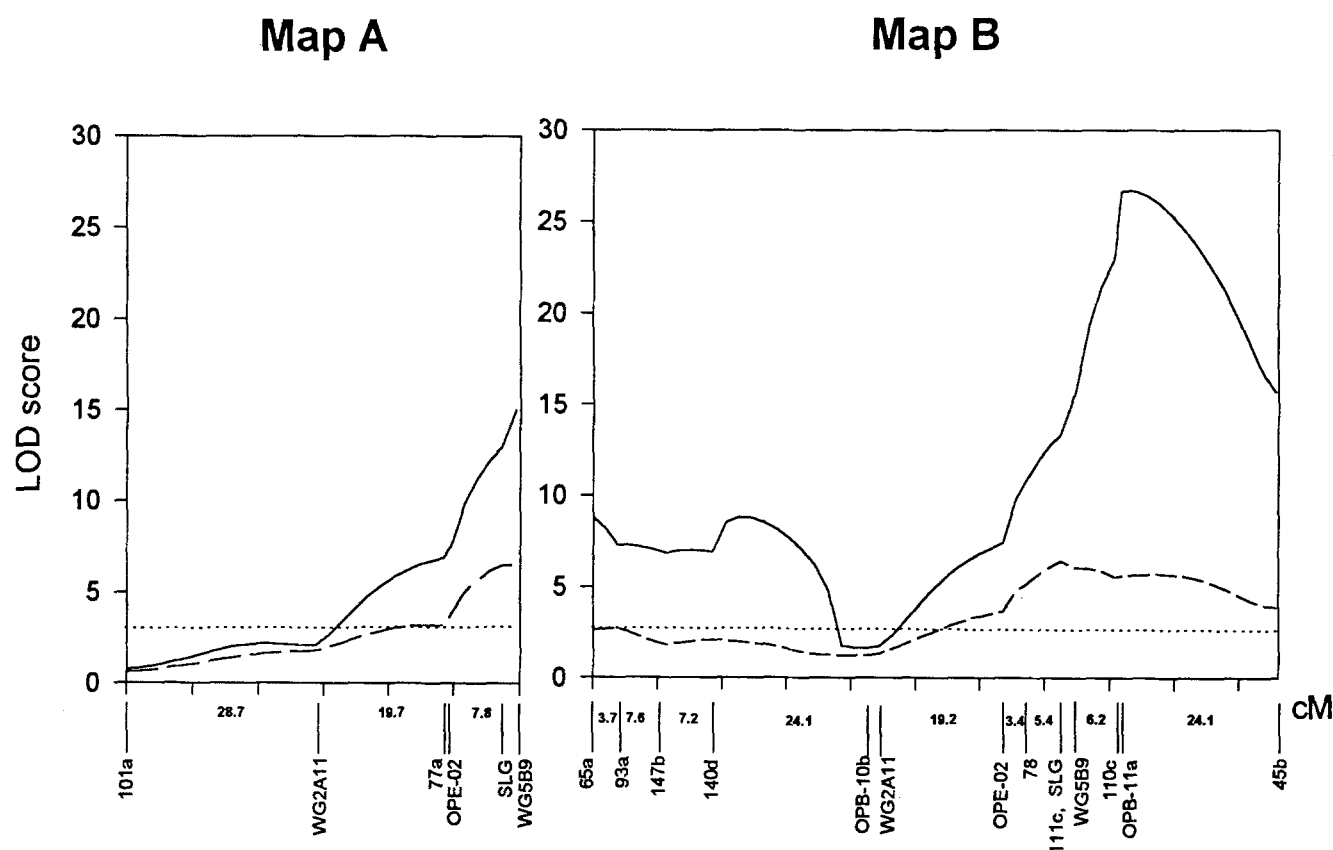
Palmitic-acid content was very strongly associated with one (corresponding) linkage group in both maps. This linkage group corresponds to LG9 in the *B. rapa* RFLP map of T. Osborn (personal communication). The group was composed of six markers extending over 60 cM in map A, and 14 markers extending over 106 cM in map B (Fig. 1).

The exact position of the QTL affecting palmitic-acid content could not be revealed in map A, because the LOD score exhibited a maximum (15.1) at the end of the linkage group (Fig. 1). The variance explained at this point (the percentage decrease in the square root of estimated variance when the QTL is allowed to control a portion of the trait) was 55%.

In map B, the peak LOD score (26.7) for a locus affecting palmitic acid mapped very close (1.4 cM) to RAPD marker OPB-11a. The variance explained at this point was 85%. The linkage group also exhibited another, lower, LOD score peak (8.8) for palmitic acid (Fig. 1). However, the significance of the LOD score peaks may be somewhat misleading, because different subsets of F₂ individuals were analysed at different loci. Testing for the presence of a second QTL (by comparing the maximum LOD scores assuming the presence of only the first QTL, to the maximum LOD score assuming the presence of two QTLs) does not support the existence of two QTLs. Possible reasons for a spurious peak are errors in the linkage data (suggested by the shape of the smaller peak) or doubtful orientation of the two marker groups in map B (which are linked to each other only by one marker pair).

Variance analysis revealed eight markers having significant association ($P < 0.01$) with palmitic acid (Table 1). All these markers are located in the linkage group associated with palmitic acid.

Both MAPMAKER and ANOVA exposed marker OPB-11a mapping very close to a putative QTL affecting palmitic-acid content. The fidelity of the marker was ascertained by repeating the RAPD analysis with varying DNA concentrations for some samples (data not shown). The OPB-11a band (1 kb) was very clearly amplified with template concentrations ranging from 1 to 75 ng. In addition, the OPB-11a band identity was confirmed by hybridisation analysis (data not shown).



The mean seed palmitic-acid content in the 62 F_2 plants with the visible allele of marker OPB-11a (homo- and hetero-zygous individuals) was $8.45 \pm 3.15\%$, while that of the 24 plants without it was $4.59 \pm 0.97\%$ (Fig. 2). This difference is statistically significant at $P < 0.001$ (t test). The genotypes of 12 F_2 s having the OPB-11a band were deduced from the F_3 progeny. When the palmitic-acid concentration was $> 12.0\%$, the F_2 individual was homozygous for the OPB-11a band (12 F_3 individuals tested); when the palmitic-acid content was $< 8.0\%$, the F_2 was usually (six F_2 s out of seven) heterozygous (eight F_3 individuals tested).

Table 1 DNA markers that detect phenotypic variation ($P < 0.01$) with palmitic-acid concentration by variance analysis (F statistic) in the F_2 population of *B. rapa* cross Jo4002 \times Sv3402

Marker ^a	n	R^2 ^b	F	P <
WG5B9	77	0.54	28.21	0.0001
SLG	54	0.43	12.36	0.0008
77a	93	0.27	29.94	0.0001
110c	80	0.26	26.98	0.0001
111c	85	0.17	17.37	0.0004
78	67	0.10	7.62	0.0075
OPB-11a	86	0.31	18.87	0.0001
OPE-02	77	0.29	15.13	0.0001

^a WG5B9 and SLG are RFLPs, OPE-02 is a co-dominant RAPD, the other markers are dominant RAPDs; small letters indicate multiple polymorphic loci detected by one primer

^b R^2 The proportion of total variance explained by the marker-genotype classes

Fig. 1 QTL likelihood maps of the linkage group harboring a locus affecting palmitic-acid (—) and oleic-acid (---) content in the F_2 population of the *B. rapa* cross Jo4002 \times Sv3402. The visible RAPD alleles are inherited from Jo4002 in map A, and from Sv3402 in map B. Markers with a prefix WG or TG, and SLG indicate RFLP markers; OPE-02 is a co-dominant RAPD marker, the other markers are dominant RAPDs. The order and distances (cM) of markers were computed with the MAPMAKER 3.0 computer program. The dashed line indicates a LOD score threshold of 3.0

With regard to the relationship between palmitic acid and other fatty acids, it seemed that oleic acid is the most significantly correlated (negatively) with palmitic acid (Table 2). The palmitic-acid linkage group also exhibited a LOD score peak of 6.5 (variance-explained 30%) for oleic acid (Fig. 1). The exact resolution of the QTL was not possible because the peak was quite flat in both maps. In ANOVA analysis, seven out of the eight markers (all except marker 78, Table 1) associated with palmitic acid were also associated with oleic acid at $P < 0.01$ (data not shown).

Discussion

This study identified a QTL for palmitic-acid content on one linkage group of spring turnip rape. In addition, the same linkage group was associated with oleic-acid content at about the same location. This is not unexpected, because, due to the stepwise biosynthetic formation of fatty acids, the increase of one fatty acid reduces the

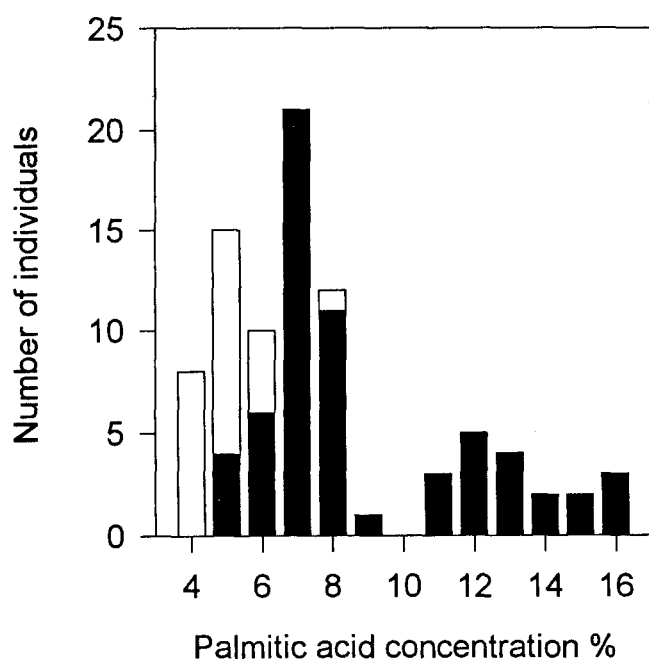


Fig. 2 Frequency distribution of the Jo4002 × Sv3402 F₂ population for palmitic-acid content based on the presence (■) or absence (□) of the OPB-11a band

Table 2 Linear regression analysis between palmitic acid and four other fatty acids in the F₂ progeny of the *B. rapa* cross Jo4002 × Sv3402; *n* = 94 in each case

Fatty acid	Slope	Intercept (%)	<i>r</i>
Stearic acid	−0.043	2.07	0.34***
Oleic acid	−1.15	58.54	0.74***
Linolenic acid	−0.33	25.46	0.31**
Linolenic acid	0.18	13.44	0.30**

** and *** correlation significant at *P* < 0.01 and *P* < 0.001, respectively

amount of some other fatty acid. Therefore, we believe that the QTL found in our study affects the carbon-chain elongation step from palmitic acid to oleic acid (through stearic acid). The QTL appears to be a major gene, because it accounted for 85% of the genetic variation of palmitic acid in this cross.

One RAPD marker, OPB-11a, was found to be tightly linked to the QTL for palmitic acid. This marker proved to be reliable. The tight linkage of the visible allele of OPB-11a in coupling phase with a higher palmitic-acid content enables identification and selection of plants with this trait. Marker-assisted selection would accelerate breeding because RAPD markers are easy to score and interpret. In addition, markers closely linked to the trait may eventually enable the

isolation and analysis of the gene affecting palmitic-acid content.

Molecular markers linked with genes of economic significance have been identified in many crops (e.g. Penner et al. 1993; Dax et al. 1994; Mohan et al. 1994; Tanhuanpää et al. 1995). The linkage reported here is, to our knowledge, the first reported linkage between an economically important trait and a RAPD marker in *B. rapa*.

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