Theor Appl Genet (1996) 93: 282–286

C. E. Thormann · J. Romero · J. Mantet · T. C. Osborn

Mapping loci controlling the concentrations of erucic and linolenic acids in seed oil of *Brassica napus* L.

Received: 27 September 1995 / Accepted: 17 November 1995

Abstract The quality of plant oil is determined by its component fatty acids. Relatively high levels of linolenic acid reduce the oxidative stability of the oil, and high levels of erucic acid in the diet have been associated with health problems. Thus, oilseed *Brassica napus* cultivars with low linolenic and low erucic acid contents are highly desirable for edible oil production. In order to identify genes controlling the levels of erucic and linolenic acids, we analyzed the oil composition of 99 F_1-derived doubled haploid lines from a cross between cv 'Major' (high levels of erucic and linolenic acids) and cv 'Stellar' (low levels of both fatty acids). A molecular marker linkage map of 199 loci for this population was used to identify quantitative trait loci (QTL) controlling oil composition. We identified two regions that accounted for nearly all of the phenotypic variation in erucic acid concentration and one region that accounted for 47% of the variation in linolenic acid concentration. The QTL associated with linolenic acid concentration mapped near a RFLP locus detected by a cDNA clone encoding an omega-3 desaturase, suggesting that the low linolenic acid content of 'Stellar' may be due to a mutation in this gene.

Key words *Brassica napus* · RFLP linkage map · QTL · Erucic acid · Linolenic acid

Communicated by G. E. Hart

C. E. Thormann · T. C. Osborn (✉)
Department of Agronomy, University of Wisconsin, Madison, WI 53706, USA

J. Romero
Mycogen Plant Sciences, 5649 E. Buckeye Rd., Madison, WI 53716, USA

J. Mantet
Semences Cargill, Boissay – BP. 17, 28310 Toury, France

Introduction

Seeds of the two major oilseed rape species, *Brassica napus* and *B. rapa* (syn. *campestris*), commonly contain 40–44% oil on a dry weight basis (Downey and Rakow 1987). The quality of the oil is determined by the proportion of its main constituent fatty acids: erucic (C22:1), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids. Oils with high concentrations of erucic acid are desirable for the production of high-temperature lubricants, nylon, plasticizers, water repellents, waxes and surface-active agents (Princen and Rothfus 1984). Yet, most of the rapeseed oil currently produced is used for salad and cooking oils, margarine and shortenings. High levels of erucic acid in the diet have been associated with health problems (Beare et al. 1963), and although there are no indications of erucic acid toxicity in man, it is known to cause cardiac lipidosis and necrosis in rats (Laryea et al. 1992). Thus, cultivars with low erucic acid content are highly desirable for edible oil.

The erucic acid content of *B. napus* seed oil is under embryo genotype control (Harvey and Downey 1964), and there is evidence that this trait is controlled by two genes with multiple alleles acting in an additive manner (Kondra and Stefansson 1965; Jonsson 1977). The manipulation of these genes can fix the levels of erucic acid at values ranging from less than 1% to above 60% of the total oil content (Krzymanski and Downey 1969). A single locus controlling erucic acid levels has been mapped in *Brassica rapa* (Teutonico and Osborn 1994), but the map positions of loci in *B. napus* have not yet been reported.

Existing canola quality cultivars (low erucic acid, low glucosinolates) produce oils with about 55–65% oleic acid, 14–18% linoleic acid, and 8–12% linolenic acid. High levels of polyunsaturated fatty acids reduce the oxidative stability of canola oil, thereby affecting the flavor and quality of the oil, and chemical hydrogenation is required for oils to be used for cooking. However, genetic variation for reduced levels of polyunsaturated fatty acids is limited in *Brassica* germplasm (Auld et al. 1992). In order to over-

come this obstacle, researchers have used chemical mutagens (such as ethyl methanesulfonate) to induce point mutations in plant genomes, followed by selection for plants with alterations in their seed oil linolenic:linoleic acid ratios (Robbelen and Nitsch 1975). Scarth et al. (1988) crossed a mutant line having 3% linolenic and 28% linoleic acids with a canola quality cultivar to develop the cultivar 'Stellar', the first canola quality summer rape cultivar with reduced linolenic and increased linoleic acid contents.

Linolenic acid synthesis occurs by desaturation of 18:2 predominantly in the endoplasmic reticulum, with smaller amounts synthesized in the plastids (Somerville and Browse 1991). A locus controlling the levels of linolenic acid in the oil of rapeseed has been recently identified (Hu et al. 1995), but the genetic regulation of linolenic acid content in rapeseed is not clearly understood. Using mutants of *Arabidopsis*, Arondel et al. (1992) identified and cloned the coding sequence of an omega-3 desaturase (*fad3*) that converts C18:2 to C18:3. One possible explanation for low levels of linolenic acid in the cultivar 'Stellar' could be the inactivation of this fatty acid desaturase.

The purpose of the study described here was to map genes controlling fatty acid composition in *B. napus*. This information allowed confirmation of two major genes controlling erucic acid content and association of a *fad3* homologue with the expression of reduced levels of linolenic acid in the cultivar 'Stellar'.

Materials and methods

Plant material and linkage mapping

A single plant of the *B. napus* cv 'Major' (high concentrations of erucic and linolenic acids) was crossed to a doubled haploid (DH) line derived from cv 'Stellar' (low concentrations of both fatty acids), and 105 segregating lines were obtained by microspore culture of a single F_1 hybrid plant. The 105 recombinant DH lines were used previously to construct a linkage map of 138 restriction fragment length polymorphism (RFLP) loci (Ferreira et al. 1994). A revised map including additional RFLP and isozyme loci was used for this study. The map contained a total of 199 loci: 130 loci detected by 92 nuclear genomic DNA clones from either a *B. rapa* cv 'Tobin' library (*tg* prefix) or a *B. napus* cv 'Westar' library (*wg* prefix); 58 loci detected by 40 cDNA clones from a *B. napus* cv 'Westar' library (*ec* prefix); 4 loci detected by four cloned genes; 5 isozyme loci (diaphorase, leucine aminopeptidase, phosphoglucose isomerase, aconitase and isocitrate dehydrogenase); and 1 locus each controlling resistance to *Albugo candida* (Ferreira et al. 1995a) and *Leptosphaeria maculans* (Ferreira et al. 1995b). RFLPs were detected as described by Thormann et al. (1994), and the isozyme variants were detected following the procedures of Chen et al. (1989).

Linkage analysis and map construction were performed using the MAPMAKER V 2.0 computer program (Lander et al. 1987; Lincoln et al. 1992). A maximum recombination frequency of 0.30 and a minimum LOD ($\log_{10}$ of the odds ratio) score of 4.0 were used to initially distribute the marker loci into potential linkage groups. Multipoint analyses were performed to find the best order of marker loci within each linkage group. Groups containing no more than 6 loci were ordered using the 'compare' command, and the most likely order of loci was selected. The alleles scored in adjacent loci were examined in order to detect possible double-crossovers, and LOD tables (giving the LOD score and recombination frequency for all pair of loci) were carefully examined to check the consistency of the orders. Groups containing more than 6 marker loci were ordered by establishing a framework of 6 loci with interval support of LOD 3.0, followed by multipoint analyses using the 'try' command (Ott 1992). After adding a new locus to a linkage group, the 'ripple' test was used to verify the orders of groups of 3 adjacent loci. The recombination frequencies were corrected based on Kosambi's map distance function (Kosambi 1944).). The final map included 196 loci assembled into 20 linkage groups and five pairs covering 1506 cM, plus 3 unlinked loci.

Trait measurements

Seeds from the two parents and the recombinant DH lines were obtained by controlled self-pollination of plants grown in field plots in Madison, Wisconsin during the summer of 1992 as described by Ferreira et al. (1995c). Erucic (*cis*-13-docoseinoic, C22:1) and a-linolenic (*cis,cis,cis*-9,12,15-octadecatrienoic, C18:3) acid concentrations were measured for both parents and for 99 of the 105 DH lines used for mapping. The lipids were extracted from seeds using heptane and transmethylated with 0.5 *N* sodium methoxide in methanol. Concentrations of specific fatty acids were determined using a Hewlett-Packard 3890 gas chromatograph with an FID detector (modified from Christie 1982) and expressed as a percentage of total seed oil.

Quantitative trait locus analysis

Quantitative trait loci (QTL) associated with the concentrations of erucic and linolenic acids were located by interval mapping using the MAPMAKER/QTL V 1.1 computer program (Lander and Botstein 1989; Lincoln et al. 1992). A LOD threshold of 3.0 was used to identify regions potentially affecting the concentrations of either fatty acid. Additional regions associated with concentrations of erucic or linolenic acids were searched by fixing the QTL with the highest LOD value and re-scanning the entire genome. New loci were considered associated with the trait when their LOD score exceeded the LOD score of the fixed locus by 3.0. Multi-locus models were developed by including the effects of putative QTL that increased the LOD score by at least 3.0. Markers that were unlinked in the map were tested for association with erucic and linolenic acid concentration using one-way analysis of variance.

Results and discussion

Erucic acid

The erucic acid concentration was less than 0.1% in the seed oil of 'Stellar', 50.5% in 'Major' and 28.4% in the F_1. The 99 recombinant DH lines were distributed in three groups; the lower group included 31 lines with less than 1% erucic acid, the intermediate group included 33 lines ranging from 22% to 35% and the higher group included 35 lines ranging from 38% to 50% (Fig. 1a). The detection of three phenotypic classes among the DH lines suggests that two major genes were governing this trait, with the low erucic acid lines having alleles from 'Stellar' at both loci, intermediate lines having alleles from 'Stellar' at 1 locus and alleles from 'Major' at the other locus and the high lines having alleles from 'Major' at both loci. However, the phenotypic ratio (31:33:35) did not fit the 1:2:1 ratio expected for this model (χ^2_{2df}=11.32, P<0.01).

Two putative QTL controlling erucic acid concentration were identified by scanning the genome covered by our

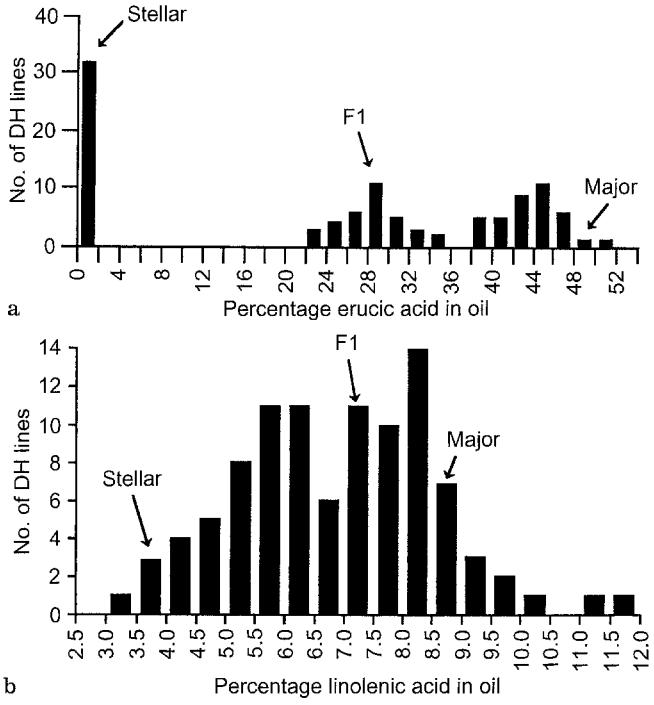

Fig. 1 Phenotypic distributions of 99 F1-derived double haploid (DH) lines for erucic acid (**a**) and linolenic acid (**b**) concentrations as percentages of total seed oil. The relative positions of the parents, 'Major' and 'Stellar', and the F_1 are indicated in the distributions

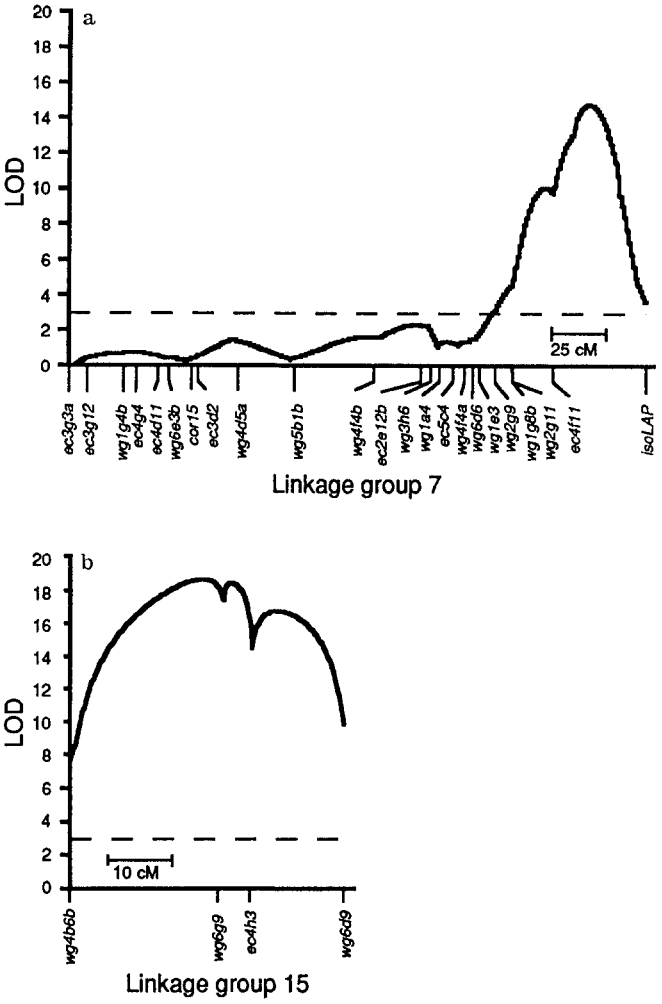

Fig. 2 LOD profiles for loci controlling erucic acid content on linkage groups 7 (**a**) and 15 (**b**) of *Brassica napus*. The Y axis shows the LOD score and the *X* axis shows the marker loci

linkage map. One QTL (peak LOD 14.7) was detected between marker loci *ec4f11* and *isoLAP* (leucine aminopeptidase) in linkage group (LG) 7 (Fig. 2a). A second region associated with this trait (peak LOD 18.6) was detected between the marker loci *wg4b6b* and *wg6g9* in LG 15 (Fig. 2b). Since this linkage group is fairly small, the scanning procedure resulted in significant associations between erucic acid and all 4 marker loci of this group. When the QTL in LG 7 was fixed, only the QTL in LG 15 was detected (peak LOD 29.3), and when the QTL in LG 15 was fixed, only the QTL in LG 7 was detected (peak LOD 33.4). A multi-locus model including the effects of both QTL explained 95% of the phenotypic variation, and no other regions were significantly associated with the trait.

Since only two QTL were identified and they accounted for nearly all of the variation in erucic acid concentration, these loci probably represent the two major genes previously hypothesized to control this trait (Kondra and Stefansson 1965). Genotypes of marker loci flanking the peak LOD positions on LGs 7 and 15 were inspected to determine if they were consistent with segregation at 2 loci resulting in low, intermediate and high erucic acid lines. Low lines had 'Stellar' alleles at 1 or both flanking markers for both loci, and intermediate lines had flanking markers consistent with 'Stellar' alleles at 1 locus and 'Major' alleles at the other. Most of the high lines had 'Major' alleles at one or both flanking markers for both loci; however, 4 high lines had 'Stellar' alleles at both marker loci flanking one of the two QTL. This result suggests that high erucic phenotypes could have resulted from having 'Major' alleles at only 1 locus and may explain why the numbers of lines observed for the low, intermediate and high groups did not fit the expected 1:2:1 ratio. In addition, 9 of the high erucic acid lines had 'Stellar' alleles at 1 of the marker loci flanking one or the other QTL and, thus, some of these lines also may have had 'Stellar' alleles at one of the erucic acid loci.

The phenotypic effects of the high erucic acid alleles at the 2 loci were compared by calculating the mean erucic acid percentage of lines having 'Major' alleles at either one or the other erucic acid loci. Lines with 'Major' alleles at the locus on LG 15 had a slightly, but non-significantly, higher erucic acid concentration (31.1%) compared to lines with 'Major' alleles at the locus on LG 7 (29.9%).

Two of the probes that detected RFLP loci (*wg4b6b* and *wg6g9*) in LG 15 of *B. napus* also detected loci in LG 1 of *B. rapa* (Teutonico and Osborn 1994), which contains a locus controlling the levels of erucic acid in that species. This result suggests that the erucic acid locus detected in LG 15 of *B. napus* may be homologous to the locus detected in LG 1 of *B. rapa*.

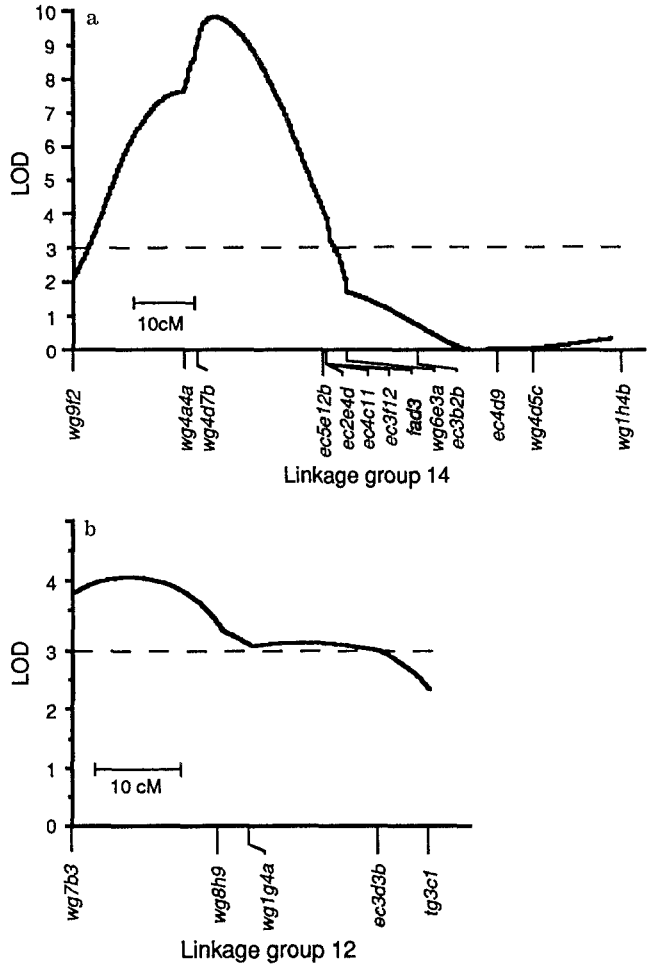

Fig. 3 LOD profiles for linkage group (LG)14 (**a**) of *Brassica napus* containing the major locus controlling linolenic acid content and for LG 12 (**b**) containing a minor locus controlling this trait that was detected when the effect on LG 14 was fixed. The Y axis shows the LOD score and the X axis shows the position of the marker loci

Linolenic acid

The DH population appeared to be normally distributed for linolenic acid concentration and had values ranging from 3.4% to 11.6% of the total seed oil content (Fig. 1b). 'Stellar' was in the lower part of the distribution with 3.6%; the F_1 was close to the middle with 7.1%; and 'Major' was in the higher end of the distribution with 8.8% linolenic acid in the oil (Fig. 1b). One putative QTL controlling this trait was identified by scanning the entire genome. The peak LOD score (9.8) was located between marker loci *wg4d7b* and *ec5e12b* in LG 14 and was only 15 cM from an RFLP locus detected by the omega-3 desaturase clone *fad3* (Fig. 3a). This QTL explained 47% of the variance for linolenic acid content. When this QTL was fixed, a second putative QTL (LOD 4.2) was identified in LG 12 (Fig. 3b). A multi-locus model including the effects of both QTL explained 60% of the variance for this trait. For both QTL, alleles from 'Stellar' conferred lower linolenic acid concentration.

The low linolenic acid phenotype of 'Stellar' originated from mutagenesis of the cultivar 'Oro' (Scarth et al. 1988). The close proximity of the LOD peak for the major QTL on LG 14 to an RFLP locus detected by the *fad3* clone suggests that the low linolenic content of 'Stellar' may be due to a mutation in this gene. This could be tested further by introgressing the RFLP allele detected by the *fad3* clone from 'Stellar' into the 'Major' background while retaining the *wg4d7b* RFLP allele from 'Major' (or visa versa). Analysis of seed oil composition in the recombinant lines could provide evidence that an allele at *fad3* has a major effect on the expression of linolenic acid content in the cultivar 'Stellar'. Additional evidence could be obtained by comparing the DNA sequence and gene product of the *fad3* allele from 'Stellar' to those of the progenitor *fad3* allele from 'Oro'.

Acknowledgements We thank Brian Yandell and Jaya Satagopan for helpful suggestions on data analysis, and Chris Somerville and John Shanklin for providing the *fad3* clone. Support was provided by a fellowship from CNPq, Brazil to CET, by 15 companies to TCO for molecular marker research on oilseed *Brassica*, and by the College of Agriculture and Life Sciences, University of Wisconsin, Madison.

References

Arondel V, Lemieux B, Hwang I, Gibson S, Goodman HM, Somerville CR (1992) Map-based cloning of a gene controlling omega-3 fatty acid desaturation in *Arabidopsis*. Science 258: 1353–1355

Auld DL, Heikkinen MK, Erickson DA, Sernyk JL, Romero JE (1992) Rapeseed mutants with reduced levels of polyunsaturated fatty acids and increased levels of oleic acid. Crop Sci 32:657–662

Beare JI, Campbell JA, Youngs CG, Craig BM (1963) Effects of saturated fat in rats fed rapeseed oil. Can J Biochem Physiol 41:605–612

Chen BY, Haneem WK, Simonsen V (1989) Comparative and genetic studies of isozymes in resynthesized and cultivated *Brassica napus* L., *B. campestris* L., and *B. alboglabra* Bailey. Theor Appl Genet 77:673–679

Christie WW (1982) Lipid analysis, 2nd edn. Pergamon Press, Sydney

Downey RK, Rakow GFW (1987) Rapeseed and mustard. In: Fehr WR (ed) Principles of cultivar development, vol 2. Macmillan, New York, pp 437–486

Ferreira ME, Williams PH, Osborn TC (1994) RFLP mapping of *Brassica napus* using doubled haploid lines. Theor Appl Genet 89:615–621

Ferreira ME, Williams PH, Osborn TC (1995a) Mapping of a locus controlling resistance to *Albugo candida* in *Brassica napus* using molecular markers. Phytopathology 85:218–220

Ferreira ME, Williams PH, Osborn TC (1995b) Mapping loci controlling *Brassica napus* resistance to *Leptosphaeria maculans* under different screening conditions. Phytopathology 85:213–217

Ferreira ME, Satagopan J, Yandell B, Williams PH, Osborn TC (1995c) Mapping loci controlling vernalization requirement and flowering time in *Brassica napus*. Theor Appl Genet 90:727–732

Harvey BL, Downey RK (1964) The inheritance of erucic acid content in rapeseed (*Brassica napus*). Can J Plant Sci 44:104–111

Hu J, Quiros C, Arus P, Struss D, Robbelen G (1995) Mapping of a gene determining linolenic acid concentration in rapeseed with DNA-based markers. Theor Appl Genet 90:258–262

Jonsson R (1977) Erucic-acid heredity in rapeseed (*Brassica napus* L. and *Brassica campestris* L.). Hereditas 86:159–170

Kondra ZP, Stefansson BR (1965) Inheritance of erucic and eicosenoic acid content of rapeseed oil (*Brassica napus*). Can J Genet Cytol 7:505–510

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugenet 12:172–175

Krzymanski J, Downey RK (1969) Inheritance of oleic, linoleic and linolenic acids in seed oil of rapeseed, *Brassica napus*. Can J Plant Sci 55:205–210

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Lander E, Green P, Abrahanson J, Barlow A, Daley M, Lincoln S, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Laryea MD, Jiang YF, Xu GL (1992) Fatty acid composition of blood lipids in Chinese children consuming high erucic acid rapeseed oil. Ann Nutr Met 36:273–278

Lincoln S, Daly M, Lander E (1992) Mapping genes controlling quantitative traits using MAPMAKER/QTL version 1.1: a tutorial and reference manual, 2nd edn. WhiteHead Institute for Biomedical Research Technical Report, Cambridge, Mass

Ott J (1992) Analysis of human genetic linkage. The Johns Hopkins University Press, Baltimore

Princen LH, Rothfus JA (1984) Development of new crops for industrial raw materials. J Am Oil Chem Soc 61:281–289

Robbelen G, Nitsch A (1975) Genetical and physiological investigations on mutants for polyenoic fatty acids in rapeseed, *Brassica napus* L. Z Pflanzenzucht 75:93–105

Scarth R, McVetty PBE, Rimmer SR, Stefansson BR (1988) 'Stellar' low linolenic-high linoleic acid summer rape. Can J Plant Sci 68:509–511

Somerville C, Browse J (1991) Plant lipids: metabolism, mutants, and membranes. Science 252:80–87

Teutonico RA, Osborn TC (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*. Theor Appl Genet 89:885–894

Thormann CE, Ferreira ME, Camargo LEA, Tivang JG, Osborn TC (1994) Comparison of RFLP and RAPD markers for estimating genetic relationships within and among cruciferous species. Theor Appl Genet 88:973–980