Theor Appl Genet (1996) 93: 512–518
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Specific molecular marker of the genes controlling linolenic acid content in rapeseed

Received: 9 August 1995 / Accepted: 24 November 1995

Abstract In rapeseed, which is an agronomically important oilseed, variation in the linolenic acid content of the oil has been obtained through chemical mutagenesis treatment. Conventional breeding of this quantitative trait, however requires specific molecular markers. By means of biochemical experiments, we have established that the induced variation in linolenic acid content is associated with the *fad3* gene encoding the microsomal Δ^{15} desaturase. Using a pair of primers specific to this gene and a doubled haploid progeny derived from a low linolenic×high linolenic acid F_1hybrid, we have identified a polymorphism of the *fad3* alleles between the low- and the high-linolenic acid genotypes. The structure exon/intron of the *fad3* DNA sequence seems to be very similar to that of the *Arabidopsis fad3* gene. The choice of the primer pair allows specific amplification of one of the two rapeseed *fad3* genes. The value and contribution of specific markers to conventional plant breeding is discussed.

Key words *fad3* gene · Δ^{15} Microsomal desaturase · Linolenic acid · Mutant · Rapeseed · Specific gene mapping

Introduction

Vegetable alimentary oil consumption in the world has increased to 32% in the last 10 years (Rieux 1994), with the main alimentary oils being palm, soya, rapeseed and sunflower oils. The quality of the oil can be changed dependent on its intended use (for example margarine, cooking oil), which has been accomplished by obtaining changes in the fatty acid (FA) composition through conventional breeding, chemical mutagenesis and molecular techniques (Miquel and Browse 1995). The first notable change in FA composition was the decrease in erucic acid content in rapeseed oil from around 40% to 0% of the total FA composition (Morice 1979). However, this new rapeseed oil was not optimal for large use in human nutrition. As a matter of fact, this low-erucic acid oil contained a relatively high level of α-linolenic acid (C18:3), a polyunsaturated FA associated with flavour instability (Röbbelen and Nitsch 1975). In addition, the nutritional quality of alimentary oil is improved by reducing the C18:3 level in favour of linoleic acid (C18:2) (Diepenbrock and Wilson 1987).

At present, nutritionists consider that the ideal alimentary oil should contain about 3% C18:3. In fact C18:3, as well as the C18:2, are 'essential FAs' and not synthesised by mammalian species in general and by humans in particular. In higher plants, C18:3 and C18:2 represent 70% of leaf cell FAs, C18:3 up to 90% of the major chloroplast thylakoid membrane glycerolipids (Pham Thi 1984). These two FAs can be also accumulated in the storage lipids in oilseeds of rapeseed and soya oils. The desaturation of C18:2 leads to the formation of C18:3 via Δ^{15} desaturases through a complex process inserting a third double bond between the 15th and the 16th carbon atom (Mazliak 1994). The desaturations occur either on the phosphatidylcholine via the Δ^{15} desaturase of the endoplasmic reticulum or on monogalactosyldiacylglycerol (or phosphatidic acid) via the chloroplastic Δ^{15} desaturase (Yadav et al. 1993). The C18:3 of the green parts of the plant mainly derives from this last enzyme, while that of the seed cotyledons mainly derives from the first one (Somerville and Browse 1991; Yadav et al. 1993). Rapeseed oil contains a relatively high level of C18:3 (10%) even in the high-erucic acid rapeseed oil, which is reserved for industrial uses. No substantial variation in C18:3 content has been found in rapeseed (Auld et al. 1992). Chemical mutagenesis programmes led to the creation of the first low-C18:3 rapeseed variety, 'Stellar' (Scarth et al. 1988) and more recently, Somerville's group has characterised a collection of *Arabidopsis*

Communicated by G. Wenzel

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mutants varying in FA compositions (Somerville and Browse 1991). Several genes encoding the membrane bond desaturases of the endoplasmic reticulum have now been cloned. Wada et al. (1990), working on *Synechocystis*, were the first to isolate a desaturase gene implicated in the C18:1 to C18:2 desaturation. The *fad3* gene of the endoplasmic Δ^{15} desaturase was located by chromosome walking and cloned in *Arabidopsis* (Arondel et al. 1992). Subsequently, other genes have been isolated such as *fad2* (Okuley et al. 1994) and *fad8* (McConn et al. 1994). Complementation experiments using these mutants showed that the mutation occurred in structural genes coding for enzymatic proteins such as the Δ^{15} endoplasmic desaturase (Reddy et al. 1993; Arondel et al. 1992).

With respect to oligogenic traits derived from mutant gene expression, it should be possible to map the mutant gene and to use the mutation occurring in this gene to obtain a specific marker. At the present time genetic disease detection in humans is carried out with such markers. Oligogenic traits derived from mutant gene expression are especially common for seed fatty acid composition. 'Stellar', for example, exhibits just such an oligogenic mutant trait: its low C18:3 level in seeds. In this paper, we describe the tagging of a mutant gene implicated in the low-linolenic acid trait. Our objective was to find a specific marker of the target genes having an allelic polymorphism related to the C18:3 level in the seed. Using various 'Oro'-mutants, Brunklaus-Jung and Röbbelen (1987) determined two independent genes with additive effects that control the level of C18:3 in the seed. The issue was to test biochemically certain possible steps in the biosynthesis of C18:3 in order to obtain information on the possible mutation. The first part of this study addressed the identification of putative candidate genes by means of biochemical studies. In fact, several genes could be associated with this trait because the seed C18:3 level is the result of two biosynthetic chains, that of the polyunsaturated FA and that of the triacylglycerols. Consequently, these genes could include phosphatidylcholineacyltransferase and diacylglycerolacyltransferase together with endoplasmic desaturases. Alternatively, the enzymatic system of FA desaturation located in the endoplasmic reticulum membrane comprises several proteins, such as the flavoprotein (NADH oxidase), cytochrome b5 reductase and ferredoxin oxidase, in addition to the desaturase (Mazliak 1994). It was ultimately considered possible that, contrary to the situation in *Arabidopsis* (Okuley et al. 1994; McConn et al. 1994) or *Synechocystis* (Wada et al. 1990) mutants, the alterations occurred in regulatory genes. As a result of the biochemical analyses, one gene was targeted: using the polymerase chain reaction (PCR) technique, we amplified a genomic sequence corresponding to the *fad3* gene encoding the endoplasmic Δ^{15} desaturase and used this to search for polymorphism in the parentals and a doubled haploid (DH) progeny derived from one 'Stellar' × 'Drakkar' F_1 hybrid plant.

Materials and methods

Biological material

The spring Canadian variety 'Stellar' with a low seed C18:3 content (3%) was crossed with another spring variety 'Drakkar', which has a 'normal' seed C18:3 level (9–10%). The F_1seeds obtained were harvested at maturity and then were grown in a greenhouse. Microspores from an individual F_1hybrid plant were cultured according to Polsoni et al. (1988) to provide 300 doubled haploid plants (H0), on which H1 seeds were produced by self-pollination. H1 seeds from 150 genotypes were developed in a greenhouse up to the five-leaf stage. Young leaves were removed, washed under water, dried on filter paper and stored in plastic bag at –80°C until extraction of DNA. These H1 plants were then planted out in field, in a randomised incomplete block design. This design consisted with 18 blocks, 27 genotypes and 2 controls (one 'Stellar' and one 'Drakkar' line) per block. Five plants per line were self-pollinated to produce H2 seeds, which were harvested at maturity. Mature seeds of 'Stellar' and 'Drakkar' were harvested at maturity in the field and used for the experiments on the FA composition of the lipid classes of the seed.

For the experiments on microsomal membranes, 15 plants of 'Stellar' and 'Drakkar' were grown in a greenhouse. Sufficient 24- to 26-day-old seeds were harvested to yield about 5 *g* from the two varieties, and these seeds were stored at –80°C until the microsomal membrane preparation.

Preparation of microsomal membrane and enzymatic activity measure

Microsomal membranes were prepared according to Galle et al. (1993). The protein concentration in the obtained suspensions was determined according to Lowry et al. (1951), with bovine serum albumin as the standard protein.

The activity of the NADH-ferricyanide reductase was measured according to Lee et al. (1967), and its activity was expressed as µmoles of ferricyanide reduced per minute per milligram of protein. The activity of the NADH-cytochrome C reductase was measured according to the protocol described by Hackett et al. (1960) and expressed as µmoles of cytochrome reduced per minute per milligram of protein

Analytical procedures

Lipids from mature seeds of 'Stellar' and 'Drakkar' were extracted according to Bligh and Dyer (1959). Separation of the classes of lipids was performed by thin layer chromatography, and spot transmethylation was realised as described by Trémolières and Garnier (1990). Analysis of the FA composition of the methyl esters was carried out by gas-liquid chromatography on a 12m×0.32 mm i.d. glass column packed with FFAP Carbowax 20M.

FA methyl esters from all the H1, H2 seeds and control seeds from the experimental design were obtained through Thies's protocol (1971) and were analysed by gas-liquid chromatography on the same column.

DNA extraction

Frozen young leaves (1–2 *g*) were ground to powder in a mortar with liquid nitrogen. The powder was poured into tubes containing 15 ml of buffer CTAB and 150 µl of b-mercaptoethanol (Doyle and Doyle 1990). The tubes were incubated at 60°C for 30 min. Phenol-chloroform (10 ml) was added, and the tubes were centrifuged for 40 min at 12 000 *g*. The supernatants were recovered and one volume of chloroform/isoamyl alcohol was added. The blended suspensions were centrifuged for 10 min at 12 000 *g* and supernatants were again recovered. A two-thirds volume of isopropanol was added to precipitate the DNA. After 5 min of centrifugation, the supernatants were

discarded, and the DNA resuspended in 5 ml of 70% ethanol. After 30 min the tubes were centrifuged for 15 min and the pellets obtained were put in sterile Eppendorf tubes containing 1 ml of TE buffer. Finally, 10 μl RNAse (40 mg/ml) was added, and the suspension was incubated at 37°C for 1 h. The DNAs from 94 genotypes were thus extracted and stored at 4°C until use.

PCR reactions

The two specific primers were chosen so as to amplify only the microsomal Δ15 desaturase gene (*fad3*) and not the chloroplastic one (*fad7*). Primers were selected on the BNALINDES sequence (rapeseed *fad3*) and ATHCPFADD sequence (*Arabidopsis fad7*) with the aid of the OLIGO programme Mac Version 4, which indicates the compatible primer pool. The analysis of the corresponding amino acid sequence, displayed on the OLIGO screen, was used to locate the 3′ end of the primers in order to obtain α positive amplifications on different species of Brassica. The selected primers 5′→3′ are:

BNLINDES385: GTG GAC ATG GGA GTT TYT CNG A (which we have named the UP primer);

BNALINDES1020: TGG CAT CGA CCA ART GRT ART G (which we have named the LP primer).

Y = {C, T}, N = {A, G, C, T} et R = {A, G}.

DNAs from 'Stellar' and 'Drakkar' were first amplified to detect a polymorphism between the mutant and the wild gene. The observed polymorphism was then searched for on the low and high C18:3 individuals from the DH progeny, and finally within the progeny. The PCR reaction mixture (25 μl) contained 75 ng of genomic DNA, 1.5 mM $MgCl_2$, 150 μM each of dATP, dGTP, dCTP, dTTP (Boehringer, BP 29. F-38242 Meylan Cedex), 0.25 pmoles of the two primers (UP and LP), 0.5 units of *Taq* polymerase (Eurobio, F-91953 Les Ulis Cedex), 1.25 μl of 10×*Taq* polymerase buffer (Eurobio, F-91953 Les Ulis Cedex), sterile water (q.s.p. 25 μl). The amplification parameters on the Perkin Elmer 480 machine were: 1 cycle of 2 min 30 s at 94°C, 10 min at 60°C, 2 min at 72°C; 40 cycles of 30 s at 94°C, 1 min 30 s at 60°C and 2 min at 72°C; and 5 min at 72°C.

Amplification products were separated according to their size on 2% agarose gels and visualised under ultraviolet light after ethidium bromide staining. The molecular weight marker used was Lambda DNA digested by *Hin*dIII and *Eco*RI. Ten microliters of the amplified DNA was digested by the *Hae*III restriction enzyme (Boehringer). The digestion products were separated according to their size on 4% agarose gels and visualised as described above.

Genetic analysis

Statistical analyses of C18:3 content were performed using the GLM procedure of SAS software (SAS Institute 1989). The analysis of variance of residuals has revealed a lack of normality, so a LOG transformation was applied on the percentage of C18:3 content data to normalise the residuals. Estimates of the seed C18:3 levels were calculated from the results of the GLM procedure.

Results

FA composition of the seed lipid classes

In each of the lipid classes except for LYSO(PE+PC) (see Table 1) C18:3 content was lower in 'Stellar' than in 'Drakkar' while the reverse was true for C18:2 content (Table 1). In contrast, the C18:1 content was generally lower in 'Stellar' than in 'Drakkar' (except in the PA class in which it was higher). The C18:1 to C18:2 desaturation ratio [(%C18:2+%C18:3)×100/(%C18:1+%C18:2 +%C18:3)] ranged from 20% to 50%. This ratio was slightly higher in 'Stellar' for each lipid class. The C18:2 to C18:3 desaturation ratio [(%C18:3)×100/(%C18:2 +%C18:3)] ranged from 10% to 35% in 'Drakkar', depending on the lipid class, and from 5% to 10% in 'Stellar'. In all of the lipid classes, this ratio was substantially lower in 'Stellar' than in 'Drakkar'. Thus, the main difference between 'Stellar' and 'Drakkar' is the C18:2 desaturation ratio as a consequence of a lack of C18:3 desaturation.

Table 1 FA composition of the mature seed lipid classes of vars 'Stellar' and 'Drakkar'. The #0 symbol indicates that the desaturation ratio was considered to be nul because only traces of C18:3 could be detected

	Lipid class	C18:1(%)	C18:2(%)	C18:3(%)	C18:2 desaturation ratio	C18:3 desaturation ratio
PC[a]	'Stellar'	59.9	29.3	0.5	33.0	2.0
PC	'Drakkar'	61.6	23.0	2.7	29.0	10.0
LYSO	'Stellar'	25.5	29.2	Traces	53.0	#0
LYSO	'Drakkar'	34.9	28.1	4.6	48.0	14.0
DAG	'Stellar'	57.6	20.8	Traces	26.0	#0
DAG	'Drakkar'	70.3	15.7	4.4	22.0	22.0
PA	'Stellar'	39.1	26.5	Traces	40.0	#0
PA	'Drakkar'	36.1	19.5	2.5	38.0	11.0
PE	'Stellar'	33.6	38.2	Traces	53.0	#0
PE	'Drakkar'	40.0	25.6	2.6	41.0	9.0
FFA	'Stellar'	56.7	22.4	1.3	29.0	5.0
FFA	'Drakkar'	66.8	14.8	5.7	23.0	28.0
TAG	'Stellar'	62.4	30.7	0.7	33.0	2.0
TAG	'Drakkar'	70.8	14.6	7.7	24.0	34.0

[a] PC, Phosphatidylcholine; LYSO, Lyso-phosphatidylcholine + Lyso-phosphatidylethanolamine; DAG, Diacylglycerol; PA Phosphatidic acid; PE Phosphatidylethanolamine; FFA, free fatty acids; TAG, Triacylglycerol

Table 2 Enzymatic activities in the microsomal membrane fraction extracted from immature seeds of vars 'Stellar' and 'Drakkar'

	Reduced cytochrome C (nmoles/min/µg protein)	Reduced ferricyanide (nmoles/min/µg protein)	Seed lipid level (%)	Protein content (µg/µl)
'Stellar'	26	23	23.8	11
'Drakkar'	29	27	18.2	10

Microsomal enzyme activities

The enzymatic activities of the immature seed microsomal fraction (Table 2) of either the NADH-cytochrome C reductase or NADH-ferricyanide reductase were not different between the varieties 'Stellar' and 'Drakkar'. These activities were similar to those expected for each of the two varieties (A. M. Galle, personal communication). The lipid content in 'Stellar' seeds was higher than that in 'Drakkar' ones, possibly suggesting that the seeds of the latter variety were not as mature as those of 'Stellar'.

Δ^{15} desaturase gene polymorphism and linkage with the C18:3 trait

The specific primer pair amplified one DNA segment of 1900 bp from the genomic DNAs of 'Stellar' and 'Drakkar'. No polymorphism was evident between the two varieties on 2% agarose Southern blotting. Therefore, the amplified DNA segment was digested by the restriction enzyme *Hae*III, generating five smaller bands of 200–600 bp in size. Three of these bands were polymorphic. Under the same amplification and enzymatic digestion conditions, the DNAs from low C18:3 DH individuals exhibited the same molecular fingerprint as the 'Stellar' parent and the DNAs from high C18:3 DH individuals the same as the 'Drakkar' parent (Fig. 1). The three polymorphic bands segregated together and cosegregated with the C18:3 content trait. These bands derived from the single amplicon of 1900 bp, which therefore constituted a specific marker of *fad3* that we have named $FAD3^{1900}$. We have used random amplified polymorphic DNA (RAPD) as markers of C18:3 content (Jourdren et al. 1996) and assigned $FAD3^{1900}$ to one of the two linkage groups determined for this trait (Fig. 2). The putative position of this marker in this group was located in the confidence interval around the quantitative trait locus (QTL) summit estimated on MAPMAKER-QTL software (Lander et al. 1987) (Fig. 3). The low-C18:3 content trait is controlled by two independent major genes with additive effects. The marker $FAD3^{1900}$ cosegregated with one of these two genes situated in one of the two QTLs. The segregation ratio was 52:32, while the one expected was 42:42. Thus, there was a significant distortion in the segregation ratio, with only 38% of the DH individuals exhibiting the same fingerprint as 'Drakkar' and 62% the same as 'Stellar'.

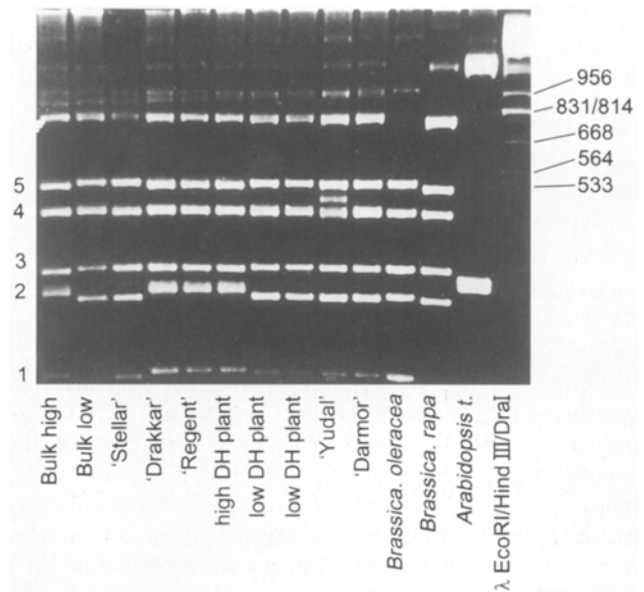

Fig. 1 Molecular fingerprint of the amplified DNAs after digestion by *Hae*III. DNAs from bulks and DH plants of these bulks, rapeseed varieties 'Stellar', 'Drakkar', 'Regent', 'Yudal' and 'Darmor' and three crucifers (*Brassica oleracea*, *B. rapa* and *Arabidopsis thaliana*) were digested. Bands 2, 3 and 5 were polymorphic

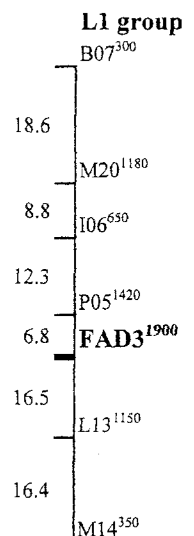

Fig. 2 L1 linkage group. Map distances in cM are indicated on the *left side* of the linkage groups and locus names are on the *right*. L1 locus was located using a MAPMAKER-QTL analysis (Jourdren et al. 1996)

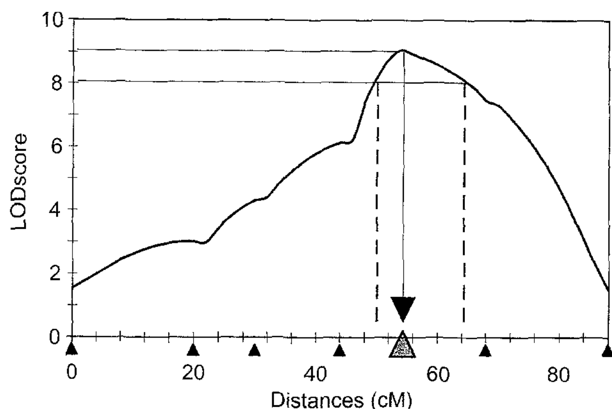

Fig. 3 Position of the FAD3[1900] marker (*grey arrow*) with respect to the L1 loci. The confidence interval is indicated by *dotted lines*. Positions of the other markers are indicated by *small black arrows*

Discussion

Our first objective was to determine putative mutant genes implied in the control of C18:3 level in the seed. The purpose of the biochemical experiments was to invalidate or confirm some of the hypotheses with respect to the mutations that occur in the triacylglycerol (TAG) biosynthesis chain. In fact, while none of these experiments confirmed any one hypothesis definitely, they did allow us to discard some of them. The results obtained were consistant with the hypothesis of an alteration in endoplasmic Δ^{15} desaturase activity. In a previous positional analysis of TAG (Jourdren and Renard 1995), it was shown that the C18:2 to C18:3 desaturation ratio, which is an indication of Δ^{15} desaturase activity, was lower at all three positions of TAG from 'Stellar' than from 'Drakkar'. In addition, all of the lipids from the eukaryotic pathway, phospatidylcholine (PC), phosphatidic acid (PA), diacylglycerol (DAG), phosphatidylethanol amine (PE) and TAG exhibited a lower C18:3 level and a higher C18:2 level in the mature seeds of the mutant variety 'Stellar' than in the 'Drakkar' variety, thereby resulting in a lower C18:2 desaturation ratio. This data suggests that a mutation of a Sn-2 specific acyltransferase cannot explain the mutant phenotype. If, as discussed by Norman and St John (1986), the Δ^{15} desaturase was Sn-2 PC-specific, a decrease in the C18:3 level at both the Sn-1 and the Sn-3 positions of TAG should have occurred in a mutant deficient in lysoPC-acylCoA acyltransferase activity, while the degree of unsaturation of PC should be higher. An alteration in Δ^{15} desaturase activity is consistant with the results of the analysis of the FA composition of the lipid classes (Table 1), since in typical developing oilseeds, acyl exchanges occurring between DAG, PC, PA and acylCoA, determine the degree of unsaturation of TAG (Somerville and Browse 1991). In fact, even if the C18:2 desaturation occurs in PC, the exchange between the Sn-2 position of PC and the acylCoA pool enriches the acylCoA pool with C18:3, which is then available for PA biosynthesis. Thus, both the Sn-1 and Sn-2 positions of PA and of DAG would have a low C18:3 content. Such mutations have already been proposed for some of the well-known *Arabidopsis* mutants derived from Ethylmethanesulfonate (EMS)-treated plants (Browse et al. 1986; James and Dooner 1990). Lemieux et al (1990) have characterised a low C18:3 *Arabidopsis* mutant, which was described as being deficient in the C18:2 to C18:3 desaturation taking place on cytoplasmic PC. Alterations in plastid C18:1 or C18:2 desaturases have also been identified (McConn et al. 1994; Falcone et al. 1993; Watahiki and Yamamoto 1994). EMS-induced mutants generally carry punctual mutations that can occur relatively frequently (Fowler and Stefansson 1975). It seems that this chemical mutagen provokes alkylation of the N-7 and the O-6 positions of guanine that initiates, at least for the O-6 methylation, to the change of the GC pair of bases into an AT pair (McCourt and Somerville 1987).The cloning of the *fad* genes implicated in the mutant phenotype described above has led to an understanding that mutations reside in structural genes. From the biochemical angle, the enzymatic activity of the protein encoded by the chosen candidate-gene could not be tested. In fact, the activity of the Δ^{15} microsomal desaturase requiring molecular oxygen and NADH (Mazliak 1994), had never been detected *in vitro*, whereas the activity of the Δ^{12} desaturase requiring cytochome b5 and NADH:cytochome b5 reductase (Smith et al. 1990) had been (Galle et al. 1993).

We suspected that the mutations occurring in 'Stellar' affected the microsomal Δ^{15} desaturase or microsomal ω-3 desaturase. The pair of specific primers (UP/LP) chosen on the basis of the ω-3 desaturase cDNA (or DNA) sequences available in the databanks allowed us to amplify a 1900-bp DNA segment. A partial direct sequencing (using the UP and LP primers) confirmed that this DNA segment corresponds to the *fad3* gene. According to the rapeseed microsomal ω-3 desaturase cDNA described by Arondel et al. (1992), this pair of primers should amplify a DNA segment containing 653 bp corresponding to the exon sequences and an unknown number of base pairs corresponding to the intron sequences. The homologous DNA of the *Arabidopsis fad3* gene that encodes a microsomal ω-3 desaturase has been sequenced and found to consist of eight exons and seven introns (Nishiuchi et al. 1994). A very strong homology (93%) was found at the amino acid level between this *Arabidopsis fad3* sequence and that of rapeseed (Yadav et al. 1993). Thus, the DNA segment amplified by the (UP/LP) primer pair probably consisted of six exons and five introns (Fig. 4), which should be confirmed by establishing a restriction map of FAD3[1900] DNA. Nishiuchi et al. (1994) reported that the genomic nucleotide sequence of the *Arabidopsis* microsomal ω-3 desaturase was very similar to that of the *fad7* gene (Iba et al. 1993). Differences occurred only in the sequences of the introns and the size of the three first introns; the exon/intron structure was conserved. On this basis, the size of the five introns of our DNA segment could be the same as those of the *Arabidopsis fad3* gene since the 1900-bp segment contained 650 bp of exon sequences and 1250 bp of intron se-

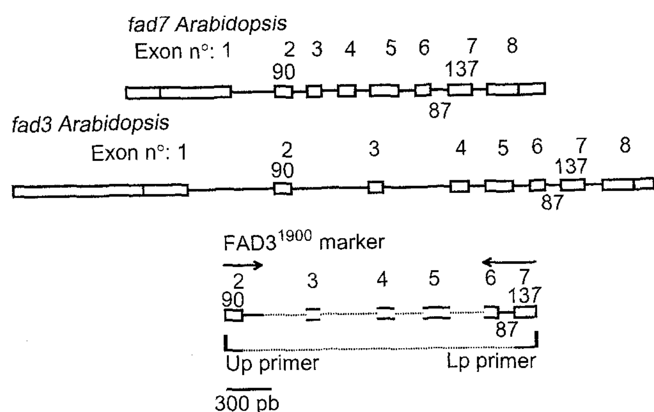

Fig. 4 Comparison of the genomic DNA structure of the *Aradopsis* Δ15 desaturase genes, *fad3* (Nishiuchi et al. 1994) and *fad7* (Iba et al. 1993) and the FAD3[1900] segment. *Arrows above* the FAD[1900] segment indicate the sequenced parts

quences that closely correspond to the sizes of the sequences of the second, third, fourth, fifth and sixth intron of the *Arabidopsis fad3* gene (547, 479, 87, 92 and 87 bp, respectively).

The digestion of this 1900-bp amplicon by the restriction enzyme *Hae*III produced five products, thereby showing that a polymorphism exists between the *fad3* sequence of 'Stellar' and that of 'Drakkar'. This polymorphism is closely associated with the C18:3 level of the seeds from the individuals of the DH progeny. In addition, 'Regent', which was the recurrent variety used to create 'Stellar', exhibited the same molecular pattern as 'Drakkar' (data not shown). The three polymorphic bands are considered to be one *fad3* marker, which was located in one of the two linkage groups previously described (Jourdren et al. 1996). Two major QTLs were then identified for the C18:3 level trait, and these explained about 71% of the total phenotypic variation. The proximity of *fad3* to one of these two QTLs indicates that one of the Δ^{15} desaturase genes is located in this QTL and may even correspond to one of the two mutant genes. However, at this stage it is not yet possible to state absolutely that the mutation occurred in this gene and that it is responsible for the detected polymorphism. In fact, the confidence interval around the QTL summit in which the mutant gene is located covers 22 cM, that is to say about 4400 kb (according to an estimate of Landry and Hubert (1991) on rapeseed genome size that gives 1 cM for 200 kb). Hence, several genes could be clustered and correspond to this QTL. Consequently, a mutation occurring on any of these genes would be closely associated to the trait. Such gene clusters have already been demonstrated by Reddy et al. (1993) in *Synechocystis*. Clear-cut proof that the mutation does occur on the Δ^{15} desaturase gene would be provided by complementation of the mutation in 'Stellar'.

Related to this homology between the *Arabidopsis* and the rapeseed microsomal *fad3* gene, was the very high homology expected between the *fad3* genes of the two rapeseed parental genomes, i.e. *B. oleracea* and *B. rapa* genomes, and the even higher one between the *fad3* gene of the mutant and that of the non mutant rapeseed varieties. Thus, it is possible that only one DNA sequence was amplified; however, it would then consist of a mixture of DNA sequences of a similar size but different intron sequences. It is highly unlikely, however, that all the intron sequences of the *fad3* gene from the *B. oleracea* genome and those from the *B. rapa* genome would have been conserved in exactly the same size. In addition, the three digested polymorphic products cosegregated as a single locus. This indicates that only one *fad3* gene was amplified by the UP/LP pair of specific primers. The nucleotidic sequence of the partially determined FAD3[1900] marker revealed that the first 310 bases of the 5′ end correspond to the second exon together with a fragment of the second intron, while the last 250 bases of the 3′ end correspond to the sixth exon together with the sixth intron and the seventh exon (data not shown). Work is in progress to complete the sequencing data on the 'Stellar' and 'Drakkar' *fad3* gene sequences and to determine new primers allowing the amplification of the two *fad3* genes.

The most important aspect of this study is that one specific marker was identified for C18:3 content in rapeseed that corresponds to the Δ^{15} desaturase gene. This specific gene mapping would be particularly useful for pedigree breeding. Random markers (RAPD; restriction fragment length polymorphism, RFLP) might not be polymorphic in all crosses. Thus, the RAPD primer K11 identified by Hu et al. (1994) in rapeseed and linked to one gene implicated in this same trait was not polymorphic in our cross. In contrast, specific markers would be valid for various genetic backgrounds. What would be more useful would be to have an original marker for each allelic form of one gene. In the case of the oligogenic traits derived from mutant allele expression, mapping the mutation would provide the most accurate and useful marker for plant breeding.

Acknowledgements The authors thank Dr. A. M. Galle (Institut National de la Recherche Agronomique) and Dr. A. Trémolières (Centre National de la Recherche Scientifique) for their advice and help in the biochemical experiments, and P. Dupuy for his technical assistance at INRA from Rennes. We would also like to thank Dr. T. Roscoe for critical reading of the manuscript.

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