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Identification of molecular markers associated with linoleic acid desaturation in *Brassica napus*

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Abstract Linolenic acid is a component of canola oil that is readily oxidized, which results in a reduced frying stability and shelf life of the oil. The reduction of linolenic acid in canola seed has therefore been an important breeding objective for many years. The inheritance of linolenic acid concentrations in seed oil is polygenic and is also strongly influenced by the environment. For these reasons, molecular markers are sought to assist in early and reliable selection of desired low linolenic acid genotypes in breeding programmes. Molecular markers associated with low linolenic acid loci were identified in a doubled-haploid population derived from a cross between the *Brassica napus* lines, 'Apollo' (low linolenic) × YN90-1016 (high linolenic) using RAPDs and bulked segregant analysis. A total of 16 markers were distributed over three linkage groups, which individually accounted for 32%, 14% and 5% of the phenotypic variation in linolenic acid content. The rapeseed *fad*3 gene was mapped near the locus controlling 14% of the variation. The mode of inheritance appeared to be additive, and a QTL analysis showed that collectively the three loci explained 51% of the phenotypic variation within this population. PCR fragments for low linolenic acid 'Apollo' alleles (3% linolenic acid) were identified at all three loci. Simultaneous selection for low linolenic acid 'Apollo' alleles at each locus resulted in a group of DH lines with 4.0% linolenic acid. The use of these makers in the breeding programme will enhance the breeding of low linolenic acid *B. napus* cultivars for production in Canada.

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Introduction

There is a concerted effort among canola breeders to reduce the content of linolenic acid in canola seed oil. Linolenic acid is readily oxidized, which imparts a distasteful flavour and reduces the shelf life of the oil. Seed oil of standard *Brassica napus* canola cultivars contain 8–10% linolenic acid, and it is desirable to reduce this level to below 3%. Linolenic acid content in canola seed is influenced by temperature and photoperiod, making selection for low linolenic acid lines difficult. In addition, the inheritance of linolenic acid levels is controlled by three additive genes, which adds to the complexity of making reliable selections (Chen and Beversdorf 1990). So far, no genetic or morphological markers have been identified that are linked to all three loci conditioning low linolenic acid in canola that could be used for selection by breeders.

The source of low linolenic acid in *B. napus* was derived over 25 years ago. Seed of the *B. napus* cultivar 'Oro' was treated with ethanemethylene sulphonate (EMS), and seed of resulting low linolenic acid mutant lines was again treated with EMS to further reduce linolenic acid (Rakow 1973; Robbelen and Nitsch 1975). Several low linolenic acid mutants were identified and used in crosses to develop *B. napus* low linolenic acid cultivars such as 'Stellar' (Scarth et al. 1988) and 'Apollo' (Scarth et al. 1994). Studies have shown that the oil from these low linolenic acid cultivars have improved storage and frying stability and shortened hydrogenation times compared to standard canola oil (Eskin et al. 1989; Przybylski et al. 1993), indicating that linolenic acid reduction in canola oil will in fact result in a more versatile vegetable oil.

The introduction of marker-assisted selection (MAS) techniques into canola breeding programmes might speed up the breeding of low linolenic acid cultivars. With MAS, selections in segregating generations of crosses are based on the presence or absence of molecular markers that are closely linked to the low linolenic acid trait and which are not influenced by the environment. Also, molecular selection can be automated and made inexpensive when polymerase chain reaction (PCR)-based techniques are employed, such as random amplification of polymorphic DNA (RAPD) analysis or sequence characterized amplified regions (SCARs).

Several studies have reported the identification of markers for linolenic acid content in *B. napus*. A single locus marked with a restriction fragment length polymorphism (RFLP) probe explained 27% of the phenotypic variation (Hu et al. 1995), and similarly a single locus was identified with a RAPD marker that explained 23% of the variation (Tanhuanpaa et al. 1995). The RAPD marker was dominant for high linolenic acid. Thormann et al.(1996) identified two quantitative trait loci (QTLs) from an RFLP map based on low and high linolenic acid parents. Together, the QTLs explained 60% of the phenotypic variation. The membrane-associated, rapeseed, fatty acid desaturase *fad*3 gene (Arondel et al. 1992) mapped within one of the QTLs (Thormann et al.1996). Finally, Jourden et al.(1996a) have confirmed these results by identifying two QTLs with RAPD markers and again, the *fad*3 gene mapped within a major QTL. In this case, the two QTLs explained 71% of the phenotypic variation (Jourden et al. 1996b).

All of the previous linolenic acid molecular marker studies used low linolenic acid lines derived from mutants of the cultivar 'Oro' (Rakow 1973; Robbelen and Nitsch 1975). In addition, the mapping populations in previous studies were derived from a low erucic acid parent with a linolenic acid content of 8.7–10.4% and, in one case, high erucic acid rapeseed. The use of high erucic acid rapeseed can be problematic in characterizing linolenic acid levels in that the high erucic acid may mask the expression of genes involved in linolenic acid synthesis where oleic acid has been diverted through chain elongation to erucic acid rather than desaturation to linoleic and linolenic acid. The unique feature in this study was the use of parents with zero erucic acid and equal linoleic acid contents in the doubled haploid (DH) mapping. The marker development was related specifically to loci controlling the desaturation of linoleic to linolenic acid. It was not clear from previous studies what the linoleic acid levels were in the parents and whether the linoleic acid level fluctuated in the segregating populations.

In order for MAS to be most effective, it is important that most or all genes controlling a trait be identified with molecular markers and that the PCR-based markers used be dominant for the desired allele(s) to be selected. In the study presented here three unlinked loci were identified with a collection of RAPD markers. The effectiveness of the markers from the three loci in selecting lines with reduced linoleic acid desaturation activities resulting in low linolenic acid genotypes from an experimental DH population is discussed.

Materials and methods

Plant material

A cross between a canola-type, low linolenic acid source of *Brassica napus* cv 'Apollo' (Scarth et al. 1994) and a canola-type, high linolenic acid line YN90-1016 was used to derive 115 DH lines from F_1 microspores. The DH lines were grown at the AAFC Saskatoon Experimental Farm in 1994 and 1995. The field evaluations in each of the 2 years used reserve seed from the original selfed DH plant and consisted of two replicated rows of 60–80 plants each year. Seed from each row was subjected to a complete fatty acid analysis, including oleic acid, linoleic acid and linolenic acid, by gas chromatography according to the method of Thies (1971) with some procedural modifications (P. Raney, personal communication). The regression analysis of linolenic acid data contents between the 2 years showed an $r^2 = 0.80$ ($P < 0.001$). The mean linolenic acid content for each DH line was calculated from the 2 years of replicated field data.

The DNA analysis of this population used reserve DH seed from the original selfed greenhouse-grown DH plant. Four DH plants of each of the 115 lines and 4 inbred plants of each parent were grown in a controlled environment chamber for 4–6 weeks. Equal amounts of leaf tissue from the 4 plants was bulked, lyophilized, ground to a powder and then stored at $-20°C$.

DNA extraction and PCR

Brassica napus DNA was extracted from 30–50 mg of dry tissue in a 1.5-ml microtube by adding 1 ml of 95°C extraction buffer [0.1 *M* TRIS-HCl (pH 8.0); 10 m*M* EDTA, 1 *M* KCl] and incubating at 95°C for 10 min with occasional vortexing. The homogenate was centrifuged to remove cell debris. The supernatant was treated with RNAse, and DNA was precipitated with isopropanol. The DNA was washed with 70% ethanol, resuspended in water, re-precipitated with ethanol, resuspended in 75 ul of water, then quantified by fluorimetry using Hoechst 33258 stain. Typical yields were 20–30 ug of DNA, and all of the DNA samples were diluted to 5 ng/ul. The bulked segregant analysis (BSA) included DNA of the parents ('Apollo' and YN90-1016) and DNA from low and high linolenic acid bulked lines. The bulked segregants were prepared by combining equal amounts of DNA from each of ten low and nine high linolenic acid lines. A total of 380 random, 10-base pair (bp) oligonucleotide primers from the University of British Columbia (UBC, Vancouver, Canada) were screened using bulked segregant analysis.

Each PCR reaction contained 10 ng DNA as template. Amplification reactions contained 1 U of *Taq* DNA polymerase (BRL, Mississauga, Ont.), 50 m*M* KCl, 2.5 m*M* $MgCl_2$, 200 μ*M* each dNTP, 0.2 μ*M* primer. The DNA amplification protocol was 1.5 min at 95°C, followed by 35 cycles of 20 s at 95°C, 1 min at 36°C, (ramp 1°C/s), 1.5 min at 72°C and a final 7-min cycle at 72°C. In addition, the *fad*3 gene was amplified using primers and a protocol described by Jourden et al. (1996a). Since the parental lines were not polymorphic for the *fad*3 amplicon, several restriction enzymes were tested in order to reveal a sequence polymorphism by adding 5 U of restriction enzyme and the appropriate buffer directly to the completed PCR reaction.

All PCR products were resolved in 2% (w/v) agarose gels in 1 × TAE by electrophoresis at 100 V for 3 h. Gels were stained in ethidium bromide and photographed on a digital gel documentation system (Stratagene).

Data analysis

The RAPD banding pattern and particularly the marker band were shown to be reproducible by repeating the PCR reactions at least three times. Genetic segregation data and QTL analysis for RAPD markers associated with the linolenic acid trait by BSA among the 115 DH lines were analysed using MAPMAKER/EXP V3.0 (Lander et al. 1987, Lincoln et al. 1992a) and MAPMAKER/QTL V1.1 (Paterson et al. 1988; Lincoln et al. 1992b). The grouping and genetic linkage map were generated using a minimum LOD of 3.0 and a maximum recombination fraction of 0.3. Map distances were converted to centiMorgans using the Kosambi function (Kosambi 1944).

The ANOVA was performed using SAS V6.0 (SAS Institute, Cary, N.C.) for each of the 16 markers and for a combination of unlinked markers. The mean ± SE linolenic acid content of each genotypic class was also calculated for each marker and for a combination of unlinked markers. Three linkage groups were identified and were named QTL1, QTL2 and QTL3 in decreasing order of r^2 values from the ANOVA.

The backcross model with two flanking markers described by Doerge et al. (1997) was used to estimate the probabilities for genotype classes having 'Apollo' alleles at QTL1 flanked by the two closest markers. A mixed population distribution of both parental genotypes was used to estimate the mean linolenic acid content for each DH line marker class.

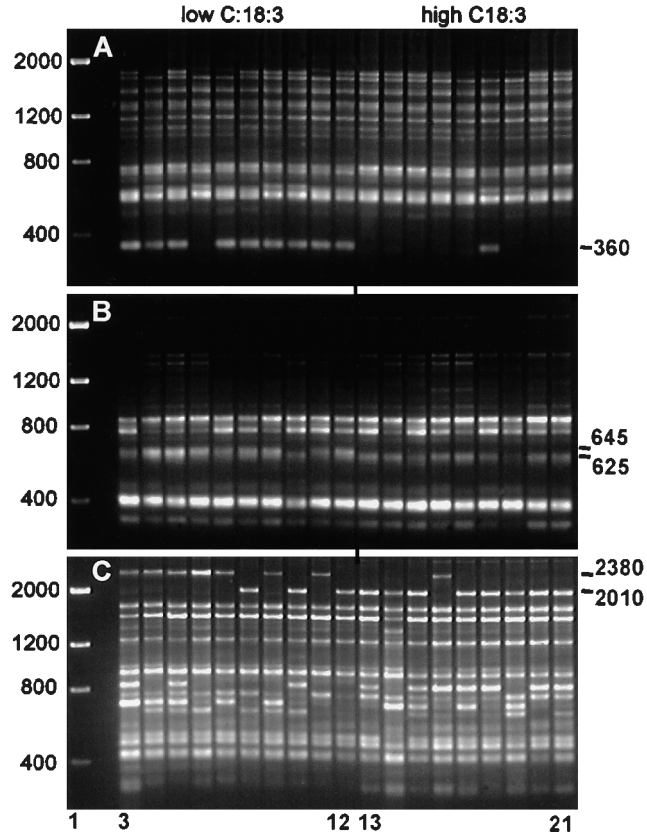

Fig. 1A–C PCR profiles produced by RAPD analysis in *Brassica napus*. PCR reactions were carried out with ten low and nine high linolenic acid (C18 : 3) DH lines. *Numbers* on the *right* in each panel indicate the position of a RAPD band that was closely associated with linolenic acid inheritance in *B. napus*. The linolenic acid markers amplified in each panel (**A, B, C**) are gentically unlinked and represent three loci. *Lane 1* molecular-weight markers, *lanes 3–12* low linolenic acid DH lines (mean 3.1%), *lanes 13–21*, high linolenic acid DH lines (mean 14.9%)

Results

This research was designed to identify molecular markers for loci controlling linoleic acid desaturation (low linolenic acid content) in *B. napus* seed oil. The source of low linolenic acid was the cultivar 'Apollo', which is used extensively as a parent in the Agriculture and Agri-Food Canada, Saskatoon oilseed breeding programme to develop low linolenic acid germplasm.

A total of 380 primers were screened by BSA in search of polymorphisms. In total, 228 primers (60%) amplified an intense and reproducible PCR banding pattern. There were 53 primers (14%) that showed qualitative polymorphisms between the parents, and 41 primers (11%) that showed qualitative or quantitative polymorphisms between the low and high linolenic acid bulked DNA samples. These putative linolenic acid RAPD markers were next screened against the 19 individuals used in the bulked DNA samples, and the best markers were finally tested against the entire 115 individuals in the DH population. An additional marker was amplified using primers homologous to the rapeseed *fad*3 gene sequence (Arondel et al. 1992; Jourden et al. 1996a).

The RAPD analysis using the BSA strategy identified a total of 14 amplified fragments that were all associated with loci controlling linoleic acid desaturation. Figure 1 shows the PCR profiles of 5 markers amplified from the 19 individuals of the bulked DNA samples. Primer LA.12 (bands 2380 and 2010 bp) and LA.11 (bands 645 and 625 bp) amplified pairs of DNA fragments that were associated with low and high linolenic acid content, respectively. Amplification of these bands in the entire 115 DH lines of the population showed that the pairs of bands from each primer were allelic. In all 14 RAPD marker profiles, recombinant lines were identified within the bulk individuals (Fig. 1). We only studied the markers that showed 4 or fewer recombinants at this stage of the BSA in detail. Only 1 marker was eliminated by ANOVA in that it explained an insignificant ($P > 0.05$), marginal amount of the phenotypic variation.

The primers for *fad*3 amplified a single 1900-bp fragment from both parents. Digestion of this fragment with restriction enzymes revealed a single *Eco*RI site within the 'Apollo' *fad*3 allele which resulted in an 1150- and 750-bp fragment (data not shown). This

Table 1 Linolenic acid markers identified in a *Brassica napus* DH population derived from the cross 'Apollo' × YN90-1016

Primer	Band length (bp)	Allele(s)
LA.1	1140	YN90-1016
LA.2	600	YN90-1016
LA.3	700	Apollo
LA.4	340	YN90-1016
LA.5	725	YN90-1016
LA.6	1210	Apollo
LA.7	1020	Apollo
LA.8	360	Apollo
LA.9	535	Apollo
LA.10	940	YN90-1016
LA.11	645/625	Apollo/YN90-1016
LA.12	2380/2010	Apollo/YN90-1016
FAD3	1150-750/1900[a]	Apollo/YN90-1016

[a] Polymorphism was produced by *Eco*RI digestion of amplified fragments

Table 2 Analysis of variance for linolenic acid (C18 : 3) marker(s) on 3 linkage groups

Primer	r^2	MS	*P*	Means[a] (%) ± SE	
				Low C18 : 3	High C18 : 3
LA.1	0.25	283	0.0001	6.3 ± 0.4	10.1 ± 0.4
LA.2	0.25	283	0.0001	6.3 ± 0.5	10.2 ± 0.4
LA.3	0.25	283	0.0001	6.2 ± 0.4	10.1 ± 0.4
LA.4	0.26	304	0.0001	7.0 ± 0.4	10.4 ± 0.5
LA.5	0.16	184	0.0002	6.9 ± 0.5	9.9 ± 0.4
LA.6	0.28	323	0.0001	6.2 ± 0.4	9.8 ± 0.4
LA.7	0.27	314	0.0001	6.5 ± 0.5	10.1 ± 0.4
LA.8	0.29	329	0.0001	6.1 ± 0.4	10.1 ± 0.3
LA.9	0.19	218	0.0001	7.0 ± 0.5	9.6 ± 0.5
LA.10	0.06	68	0.0271	7.4 ± 0.4	9.5 ± 0.5
LA.11	0.13	145	0.0011	7.0 ± 0.5	9.6 ± 0.5
LA.12	0.05	55	0.0481	7.4 ± 0.5	9.3 ± 0.5
FAD3	0.12	140	0.0012	7.0 ± 0.5	9.5 ± 0.5
Interaction:					
LA.8 × 11 × 12	0.47	543	0.0001	4.0 ± 0.3	11.8 ± 0.9

[a] All means are significantly different ($P < 0.001$)

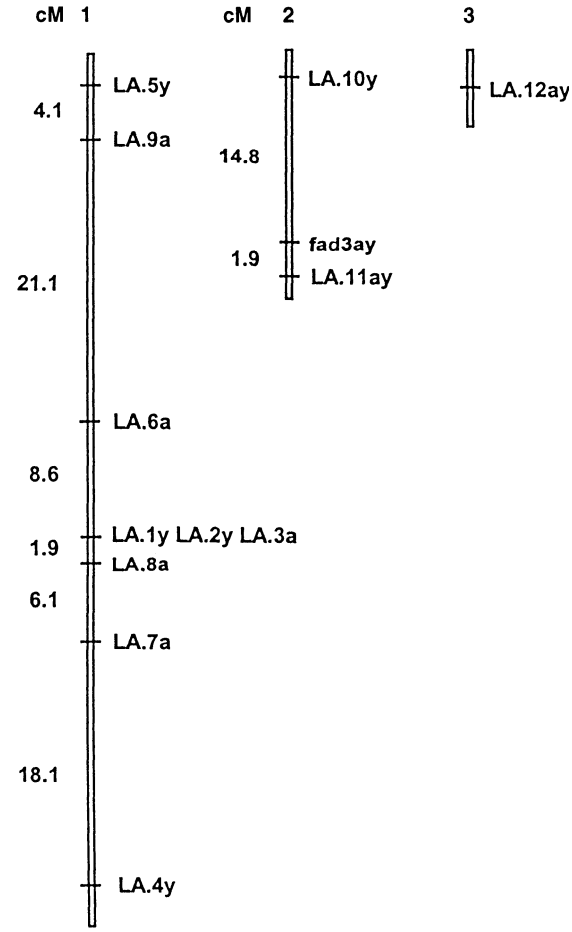

Fig. 2 A linkage map of RAPD markers and the *fad*3 gene which are associated with linolenic acid inheritance *in B. napus. Lowcase a* ('Apollo') and *y* (YN90-1016) denote the allele(s) detected at each locus. Distances are reported in Kosambi centiMorgans units

post-PCR restriction fragment length polymorphism could be used for linkage analysis in the DH population.

Table 1 lists the 14 RAPD markers and the *fad*3 alleles which showed a strong association with low or high linolenic acid. There were 8 markers dominant for low and 8 markers dominant for high linolenic acid. All of the markers segregated in a 1 : 1 ratio among the 115 DH lines as demonstrated by Chi-square analysis. Regression analysis was used to describe the amount of phenotypic variation explained by each marker (Table 2). Marker LA.8 ($r^2 = 0.29$) explained the highest and marker LA.12 ($r^2 = 0.05$) explained the lowest amount of variation in linolenic acid content; marker LA.11 ($r^2 = 0.13$) was intermediate. In all cases, the mean linolenic acid content between the genotypic class for each marker was significantly different ($P < 0.001$) (Table 2).

The set of RAPD markers was assigned to three arbitrary linkage groups by analysis of segregation data with MAPMAKER V3.0. There were 6 markers that clustered into a 16.6-cM region on group 1. One other linkage group was identified with 5 markers, which included the *fad*3 gene and a single unlinked marker was identified (Fig. 2). A QTL analysis revealed a peak in the interval between LA.8 and LA.7 which explained 32% of the phenotypic variation in linolenic acid content (Fig. 3). The QTL analysis also showed the terminal marker (LA.11) on the second linkage group explained 14% of the variation in linolenic acid content in this population. The combination of the two QTLs and the third unlinked locus ($r^2 = 0.05$) explained 51% of the variation of linolenic acid content.

The interaction between markers at the three unlinked loci (LA.8, LA.11, LA.12) explained 47% of the phenotypic variation based on ANOVA. Addition

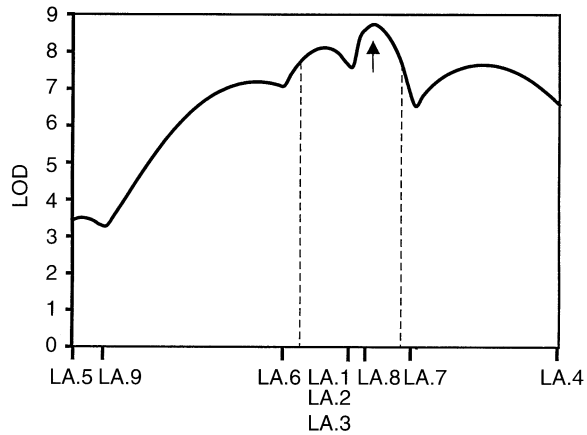

Fig. 3 A QTL scan of linkage group 1 (QTL1) showing the position of the peak (*arrow*) and confidence interval relative to the RAPD markers mapped to this linkage group

of the r^2 values for each of these markers was 0.47 (47%) as well. The linolenic acid content of 'Apollo' was 3% and YN90-1016 was 13%. The mean linolenic acid content of the DH population was $8.4 \pm 0.4\%$, while that of the low bulk was $3.1 \pm 0.4\%$ and the high bulk was $14.9 \pm 0.4\%$. The mean linolenic acid content of the low and high allele classes are indicated for the group of 3 interacting loci (Table 2). Among the 115 DH lines, 14 lines were shown to carry the three 'Apollo' alleles for markers LA.8, LA.11, LA.12 which agreed with the expected number of lines for this genotypic class (14.4) in a DH population of this size. Selection of 'Apollo' alleles at each of the best markers on each linkage group (LA.8, LA.11, LA.12) resulted in a mean linolenic acid content of $4.0 \pm 0.3\%$ ($n = 14$) and a maximum linolenic acid content of 6.9%, which was 2% lower than the linolenic acid content from lines picked with marker LA.8 alone. Further, 13 of 14 individuals in this selected group averaged only $3.7 \pm 0.3\%$ linolenic acid content.

Discussion

This study focused on identifying molecular markers associated with linoleic acid desaturation loci in *B. napus*. The goal was to establish a set of RAPD markers and subsequently SCARs that can be incorporated into the breeding programme where the reduction of linolenic acid in the seed oil is a priority for breeding of *B. napus*, *B. rapa* and *B. juncea*.

The random primer screening was moderately efficient, resulting in 60% of the primers tested amplifying informative RAPD profiles. Through BSA, 14% of the primers were found to show polymorphisms between the bulked DNA samples. This ultimately resulted in testing of 13 primers in the 115 DH line population which amplified a band associated with linolenic acid contents. Fourteen RAPD marker bands and the *fad*3 gene proved to be significantly associated with linoleic acid desaturation loci in this population based on ANOVA (Table 2). These markers clearly segregated the population into two significantly different phenotypic classes based on the mean linolenic acid content of each genotypic class (Table 2).

An important finding in this study was the identification of three unlinked loci with RAPD markers, all significantly associated with linolenic acid content. Previous studies have identified two loci, all using germplasm similar in linolenic acid content to 'Apollo' in that it was derived from 'Oro' (Robbelen and Nitsch 1975). This studied differed from other studies in that the linolenic acid content in the high linolenic acid parent YN90-1016 was 13%, which established a wider range in linolenic acid levels in the DH population. This was an absolute increase in linolenic acid content of 5–6% over corresponding ranges in populations from previous reports. This wider range of phenotype likely facilitated the identification of the three genes in this study.

Mapping the markers placed 9 markers on one linkage group (Fig. 2). A QTL analysis of these data placed the peak of the QTL within 2 cM of marker LA.8 (Fig. 3). The QTL included a cluster of 6 markers separated by 16.6 cM, and the confidence interval surrounding the QTL peak was 14.3 cM in length. The cluster of 6 markers included 4 that showed dominant DNA fragments for the 'Apollo' allele (Table 2, Fig. 3). These attributes will give this set of markers an excellent potential in MAS of low linolenic acid at this locus, given their proximity to the QTL peak and availability of positive tests for 'Apollo' alleles.

The markers distributed over the three loci included 8 markers where 'Apollo' alleles were observed as dominant DNA fragments in gels. In addition, all three loci included at least 1 marker that was dominant for the 'Apollo' allele. This is highly significant in that these markers for 'Apollo' DNA fragments can be used for selection of low linolenic acid alleles at all three loci. Previous reports have included PCR-based markers that are dominant for the high linolenic acid allele (Hu et al. 1995; Tanhuanpaa et al. 1995). Such markers are less useful in a breeding programme as they do not allow for the positive identification of desirable, low linolenic acid alleles introgressed into a variety of different genetic backgrounds.

The oleic (C18 : 1), linoleic (C18 : 2) and linolenic acid (C18 : 3) contents of 'Apollo' and YN90-1016 were 64%, 24%, 3% and 54%, 24%, 13%, respectively. It is most important to note that the linoleic acid concentration was equal in both parents and was not variable among the DH lines. Thus, the markers identified in this population associated specifically with the desaturation step from C18 : 2 to C18 : 3. The linoleic acid desaturation ratio (LDR) (%C18 : 2/%C18 : 2 + %C18 : 3) (Cherif et al. 1975) was highly negatively correlated with the

Table 3 Expected frequency of marker genotypes, probability of 'Apollo' alleles at the marked QTLs and expected mean linolenic acid (C18 : 3) values for each marker genotype

Genotype[a]	Genotype frequency[b]		Probability of Apollo alleles at QTL1[c]	Mean C18 : 3 content (%) ± SE	
	Observed	Expected		Observed	Expected[c]
(including QTL1 only)					
MMNN	42	47.9	1.0	6.2 ± 0.46	4.8 ± 0.12
MMnn	4	3.1	0.69	9.5 ± 0.50	10.4 ± 0.32
mmNN	2	3.1	0.31	5.4 ± 0.71	7.4 ± 0.32
mmnn	54	47.9	00.0	10.2 ± 0.41	13.0 ± 0.30
(including all three QTLs)					
MMNNKKPP	17	12.0	1.0	4.1 ± 0.35	3.0 ± 0.12
mmnnkkpp	16	12.0	00.0	11.8 ± 0.93	13.0 ± 0.30

[a] Uppercase letters, 'Apollo' markers, lowercase letters, YN90-1016 markers; M/m, LA.7; N/n, LA.8; K/k, LA.11; P/p, LA.12

[b] χ_2 was significant for QTL1 genotypes ($P < 0.01$) and for three-QTL genotypes ($P < 0.05$)

[c] Determined on the basis of mixed distributions where QTL1 was flanked by markers M and N with recombination frequencies r(M,N) = 0.061, r(N,QTL1) = 0.020 and r(M,QTL1) = 0.041. QTL2 and QTL3 are assumed to have peak LOD frequencies of markers LA.11 and LA.12, respectively

linolenic acid content, which showed further that the variation in C18 : 3 content in the DH population was directly related to the desaturation of C18 : 2 to C18 : 3 and was not influenced by C18 : 2 levels. The possible role of desaturation factors was also confirmed by the presence of the *fad*3 gene mapping to one of the QTLs (Fig. 2).

The mean linolenic acid content of selected lines from this population demonstrated the effectiveness of the markers at three loci. The best marker alone, based on ANOVA, was LA.8, which explained 29% of the phenotypic variation. This 'Apollo'-derived marker alone selected lines that were 6.1% in mean linolenic acid content. When all three loci were used for selection of 'Apollo' alleles, the mean linolenic acid content was decreased to 4.0% and the three loci explained 47% of the phenotypic variation (Table 2). Similarly, the QTL analysis indicated that the three loci could explain 51% of the variation. Selection for markers at three loci assorting independently among 115 DH lines should result in 14 lines on average. There were only 14 lines carrying 'Apollo' alleles at all three loci in this data set, and 13 of these 14 lines averaged 3.7% linolenic acid. This low mean linolenic acid content in selected lines is noteworthy since 'Apollo' has a linolenic acid content of 3%. These data emphasize the effectiveness of the suit of markers developed in this study for selection of low linolenic acid lines from breeding programmes.

This analysis can be taken further by taking into consideration the genotypic make-up of the DH population and the marker classes showing linkage to QTLs. Each marker class will be composed of a mixture of both parental populations as described in models by Doerge (1995). If the QTL1 position between the flanking markers LA.7 and LA.8 is fixed and QTL2 and QTL3 are fixed at a location that coincides with the peak LOD score on these linkage groups (LA.11 and LA.12, respectively), the frequency of 'Apollo' alleles in each genotypic class in the mapping population can be estimated (Table 3). There were 102 individuals for which marker data was available for all 4 of the above RAPD markers. The data show the extent to which a breeder can expect to improve the population for low linolenic acid if selection is made only on the 2 flanking markers of the major genetic factor, QTL1 (4.8% linolenic acid) (Table 3). When all three QTLs are considered, the observed linolenic acid content of selected lines was 4.1%. The marginal deviation from 'Apollo' (3%) can be explained by (1) undetected QTLs, (2) marker density and recombination fraction around the identified QTLs and (3) the size of the DH population.

The usefulness of the set of markers identified in this study will be the subject of a concurrent project. The markers will be tested for amplification of similar-sized DNA fragments in a variety of germplasm including (1) interspecific crosses between 'Apollo' and *B. rapa* or *B. juncea*, (2) alternative (non-'Oro' derived) *B. napus* low linolenic acid mutant lines and (3) standard high linolenic acid canola cultivars. Since multiple RAPD markers were identified in the study, the chances of introgressing the 'Apollo' genes into different genetic backgrounds is increased. If 1 marker fails to amplify a certain segment of DNA, then a flanking marker may substitute as a positive test for the presence or absence of the Apollo DNA fragment. It is proposed to convert the suit of markers into SCARs for more efficient and automated selection of 'Apollo' alleles at all three loci controlling low linolenic acid.

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