DEVELOPMENT OF DNA-BASED MARKERS FOR LOW LINOLENIC ACID CONTENT IN RAPESEED

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ABSTRACT

To lower linolenic acid (LA) content is an important breeding objective for the improved quality of rapeseed oil. Progress in lowering LA in rapeseed oil has been slow in the past two decades due to its low response to phenotype selection. DNA-based marker-assisted selection would increase efficiency for the selection of this traits. We have successfully identified two DNA-based markers associated with LA content in a segregating population derived from crossing the cultivar, 'Duplo' and a low LA line, 3637-1. The first marker, K01-1100, an 1100 bp DNA fragment amplified with RAPD primer K01, was identified with the trait-based approach, Two subpopulations consisting of either high or low LA content individuals sampled from the two extremes of the F_2 distribution displayed significantly different frequencies for these marker. The cloned K01-1100 fragment segregated in a codominant fashion when used as an RFLP probe on DNA from individuals of this population. The second marker, L01+L09-600, was identified with bulked segregant analysis in conjunction with a modified RAPD technique using two primers. It is estimated that K01-1100 and L01+L09-600 account for 29% and 31% of the genetic variation of LA content in this population, respectively. The segregation of these two markers fit 1:2:1 ratio and are linked to each other at a distance of 3.2 cM. In separate linkage experiments, a distance of 13 cM was found between K01-1100 and a desaturase gene in *B. oleracea*. Additional experiments are needed to determine the potential use of the markers as selection tools for low linolenic acid content in the breeding populations derived from mutant 3637-1.

INTRODUCTION

Linolenic acid LA content is of considerable importance for oil quality and is the primary cause of flavor instability (Scarth *et al.*, 1991). In rapeseed oil, one important breeding objective has been to lower the LA content for quality improvement. It is expected to develop improved cultivars with 3% or less LA with the mutants induced by chemical mutagen(Robbelen and Nitsch 1975; Roy and Tarr 1986). Genetic analysis indicated that LA content is determined mainly by the genotype of the embryo, involving maternal effects and the interaction of two or three loci with influence of environmental factors (Brunklaus-Jung and Robbelen 1987; Diepenbrock and Wilson 1987; Pleines and Friedt 1989 and Chen and Bevesdorf 1990). Because of this LA content showed low response to selection based on phenotype. Therefore, progress in lowering LA in rapeseed oil has been slow in the past two decades.

The introduction of DNA-based markers in genetic mapping has facilitated the development of detailed linkage groups in many plants, including all three diploid Brassica species: *B. campestris* (Song *et al* 1991; McGrath and Quiros, 1991; Chy *et al.*, 1992), *B. oleracea* (Slocum *et al.*, 1990; Landry *et al.*, 1992; Kianian and Quiros, 1992; Kennard *et al.*, 1994; Kowalski *et al.*, 1994), and *B. nigra* (Truco and Quiros, 1994), and an amphidiploid species *B. napus* (Landry *et al.*, 1991). These work have led to the comparison of the genome organization and evolutionary relationships between genomes. We reported in this paper the development of DNA-based markers associated to LA content, which may be useful for efficient selection of Low LA content rapeseed lines.

MATERIALS AND METHODS

The segregating population used in the study was derived from a cross between two doubled haploid parental lines, 'Duplo' , a German rapeseed cultivar, and a low LA line, 3637-1, selected from the progeny of two low LA lines, 'Mutant Oro' (Robbelen and Nitsch, 1975) and 'IXLIN' (Roy and Tarr, 1986). LA content was determined on each of the 132 plants from the F_2 population grown in Germany, following the method of Thies (1971). Seeds from each individual F_2 plant of this population were grown at Davis, California, for the gene mapping experiments.

DNA was extracted from 3 to 4 weeks old F_3 seedlings following the procedure of Kianian and Quiros (1992). Approximately similar amounts of tissue from 8 to 10 plants of each F_3 family were used for DNA extraction in order to determine the genotype of their maternal F_2 plant.

The procedure for RAPDs reported by Hu and Quiros (1991) was followed. RAPD primers (Operon Technologies) were used to amplify the DNA samples from the parents, F_1 and the F_2 for polymorphism detection. A modified RAPD technique using two primers in the reaction was also used.

For RFLP analysis, 3 to 5 µg DNA samples prepared from each family were digested with the restriction endonuclease *Eco RI* and electrophoresed in 0.8% agarose gel in 1x TAE buffer, and transferred onto Zeta-Probe GT (Bio-Rad) nylon membranes. About 50 ng of probe DNA was labeled with the Multiprimer DNA labeling kit (Amersham). Hybridization was performed in a hybridization incubator (Robbins Scientific) at 65 °C overnight. Post hybridization washes and autoradiography were carried out by following the manufacturer's protocols.

A segregating *B. oleracea* population consisting 69 plants derived from a cross between braccoli and collard was used in analysis of linkage between K01-1100 and three desaturase genes.

RESULTS AND DISCUSSION

The mean LA content for the two parents and F_1 hybrid were 10.4%, 2.2% and 4.7%, respectively. The F_2 LA content exhibited a slightly skewed continuous distribution, thus making discrete classification of the individuals impossible. Maternal effect, partial dominant action of the genes involved, and environmental influence may be responsible for the deviation between F_2 and the mid-parent value (6.3%) and the F_2 skewness. We concluded that LA content was mainly determined by nuclear genes since the F_2 mean (6.2%) was practically identical to the mid-parent value (6.3%), which is in agreement with previous reports (Brunklaus-Jung and Robbelen 1987; Diepenbrock and Wilson 1987; Pleines and Friedt 1989; Chen and Bevesdorf 1990).

In spite of the LA segregation, this progeny showed very low level of polymorphism at DNA level in our preliminary screening. For RFLP, , only about 10% (4 of the 38 tested) of the mapped probes (Kianian and Quiros 1992) detected polymorphism between the parental DNA samples digested independently with three restriction enzymes, *Eco* RI, *Bam* HI and *Hind* III. For RAPD, although a single reaction amplifies 7 to 10 bands, only 30% of the primers (19 of 60 tested) produced polymorphic bands. Because of the low level of polymorphism between the two parents of the segregating population, we adapted the trait-based approach of Lebowitz *et al* (1987). Twelve F_3 families at each end of the distribution (12 lowest, 2.1-4.4%, and 12 highest, 8.1-10.5%, LA content), two parental lines and the F_1 were used in the screening for markers associated with LA content. The RAPD marker, K01-1100, displayed significantly different frequencies between two subpopulations consisting of either high or low linolenic acid content individuals sampled from the two extremes of the F_2 distribution. Marker K01-1100 converted to an RFLP segregated in a codominant fashion on DNA from individuals of the population. The linolenic acid content means for the three resulting RFLP genotypes were 4.8% (homozygous 3637-1 allele), 6.4% (heterozygous), and 7.5% (homozygous 'Duplo' allele), respectively. It was estimated that this marker accounts for 29% of the genetic variation of linolenic acid content in this population (Hu et al, 1994).

Bulked segregant analysis (Michelmore et al 1991) was used in an attempt to identify other markers tightly linked to the locus governing LA content and marker K01-1100. We bulked equal amount of DNA samples from five families with respect to LA content as well as K01-1100 genotypes. Only homozygotes confirmed by RFLP analysis were used. Two bulked tubes for each genotype and the two parental tubes were amplified with 500 RAPD primers, but no polymorphic bands were found between the two bulks. In the search for new markers we developed the two-primer RAPD technique which uses two primers in the reaction. With this modified RAPD technique, we successfully identified a second marker, L01+L09-600, linked to K01-1100 at a distance of 3.2 cM. It seems that this marker is closer to the gene determining LA than K01-1100 since approximately 31% of the variation could be explained by it.

The Biochemical processes controlled by the alleles that result in different content of LA are not known. LA in the rapeseeds is believed to be the end product in a sequential desaturation of oleic acid. In separate experiments in *B. oleracea*, we detected linkage between marker K01-1100 and three *B. napus* DNA sequences coding for desaturases in *B. oleracea*. Because of low level of polymorphism, only one of the three sequences was mapped in *B. napus* population: Desaturase clone, CD3-3, coding for the omega-3-fatty acid desaturase (Arondel et al, 1992), produced four fragments. Two loci, one codominant and one dominant could be scored from the three segregating bands. A fourth band perhaps corresponding to a third locus did not segregated. None of them showed association to linolenic acid content in the population. The other two probes, BND9 and BND19, coding for Stearoyl ACP delta-9 desaturases, were monomorphic on the southern blots containing parental DNA samples of *B. napus* digested with eight different restriction enzymes. However, all three desaturase probes along with the cloned K01-1100 fragment were mapped in the segregating *B. oleracea* population. It was interesting to found the K01-1100 is linked with these sequences. Six loci disclosed by three clones (CD3-3a and CD3-3c; BND9a and BND9b and K01200a and K01900) were mapped on chromosome 5 and three loci (BND19a, K01-200d and CD3-3b) on chromosome 7. Although most of these genes clustered on two chromosomes, they were not closely linked to suggest associations on the basis of function. They were located at least 10 cM apart with the exception of BND9b and CD3-3a on chromosome 5 which were at distance 3.4 cM. K01-200a and K01-900a were linked at a distance of 12.7 cM from CD3-3c and 22.2 cM from BND9b. This

observation implies that the association of marker K01-1100 to LA content might have resulted from the linkage relationship between K01-1100 and desaturase genes. However, additional experiment is needed to validate the use of these markers as a tool in the selection for low LA in rapeseeds.

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