Identification of fad2 mutations and development of Allele-Specific Markers for High Oleic acid content in rapeseed (Brassica napus L.)

Falentin Cyril, Brégeon Michel, Lucas Marie-Odile, Deschamps Max, Leprince Françoise, Fournier Marie-Thérèse, Delourme Régine, Renard Michel

UMR INRA-Agrocampus Rennes, Amélioration des Plantes et Biotechnologies Végétales, BP35327, F-35650 Le Rheu Cedex, France Email: regine.delourm@rennes.inra.fr

Abstract
The quality of rapeseed oil is determined by its constituent fatty acids such as oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3). Most winter rapeseed cultivars normally produce oil with about 60% oleic acid. Development of winter rapeseed cultivars with increased oleic acid is highly desirable for new food and non food markets (better resistance to oxidation...). In this study, we sequenced genomic clones of the B. napus fad2 genes (originating from B. oleracea (fad2C) and B. rapa (fad2A)) amplified from double cycled EMS-induced mutants and wild-type rapeseed cultivars. A comparison of the mutant and wild-type allele sequences of the fad2 genes revealed single nucleotide mutations in each of the genes (fad2C and fad2A).

Detailed sequence analyses suggested mechanisms by which both mutations can cause altered fatty acid contents in the mutants. Based on the sequence differences between the mutant and wild-type alleles, two single nucleotide polymorphism (SNP) markers, corresponding to the fad2C and fad2A gene mutations, were developed. Six doubled haploid populations have been used to characterize the effect of both fad2C and fad2A mutated alleles on oleic acid content. These new molecular markers will be highly useful for direct selection of desirable fad2C and fad2A alleles during marker-assisted trait introgression and breeding of high oleic rapeseed.

Key words: rapeseed, Brassica napus, high oleic acid content, SNP, marker-assisted selection

Introduction
High oleic acid content of vegetable oils is a desirable trait both because of the health benefits and of the stability to oxidation and heat of oleic acid. In particular, it has been shown that oleic acid is effective in lowering plasma cholesterol levels (Bonanome et al., 1988; Liu et al., 2002). Furthermore, its single insaturated bond makes oleic acid (C18:1) a much less vulnerable fatty acid than its multiply insaturated counterparts. For instance, the rate of oxidation of linolenic acid (C18:3) is 100 times that of oleic acid (Debruyne, 2004).

One of the most promising paths towards such plants is to select plants essentially deprived of FAD2 activity. Indeed, FAD2 (delta12 oleate desaturase) catalyses the transformation of oleic acid (C18:1) to linoleic acid (C18:2); plants having lowered FAD2 activity thus have higher oleic acid content thanks to limited catabolism of the latter.

Brassica napus (rapeseed) is an amphidiploid which contains the genomes of two diploid ancestors, B. rapa (the A genome) and B. oleracea (the C genome) (U, 1935). The oleic acid content is 61 % for the traditional oil of rapeseed (Stan Skrypetz, 2005).

The present study notably arises from the obtention of five previously unrecognised mutations in the fad2 genes of Brassica napus plants. These mutations can be used in plant breeding programs by marker-assisted selection.

Material and Methods

Plant Material

Winter rapeseed lines ‘LOR1#S007’ (wild type), ‘LOR1#PR-2601’ (wild type), ‘HOR1#S005’ (mutant type), ‘HOR1#B005’ (mutant type), ‘HOR1#NPZ-12’ (mutant type), ‘HOR2’ (mutant type), ‘HOR3’ (mutant type) and ‘HOR4’ (mutant type) were used in this study for cloning of fad2 (fatty acid desaturase-2) alleles.

‘HOR1#S005’, ‘HOR1#B005’ and ‘HOR1#NPZ-12’ have been obtained by crossing an ethyl methanesulphonate (EMS) mutant line ‘HOR1’ with three classical rapeseed lines (S005, B005 and NPZ-12); these three mutant lines have an oleic acid content at about 80 to 90%.

‘LOR1#S007’ and ‘LOR1#PR-2601’ are rapeseed lines with oleic acid content at about 60%.

‘HOR2’, ‘HOR3’ and ‘HOR4’ are three other independent EMS mutant lines with oleic acid content at about 75 to 85%.

Six doubled haploid (DH) populations were developed by microspore culture (Coventry et al., 1988) from F1 plants of crosses between:

(1) ‘LOR1#S007’ and ‘HOR1#S005’ rapeseeded lines,
(2) ‘LOR1#PR-2601’ and ‘HOR1#NPZ-12’ rapeseeded lines,
(3) ‘LOR1#S007’ and ‘HOR1#B005’ rapeseeded lines,
(4) ‘LOR1#S007’ and ‘HOR1#NPZ-12’ rapeseeded lines,
Results

In this study, several clones were sequenced. The sequence alignment of these clones with *B. rapa* and *B. oleracea* fad2 sequences found in public databases identified:

1. two fad2 genes originating from *B. rapa* (fad2A) and *B. oleracea* (fad2C) for ‘HOR1’ lines,
2. one fad2 gene originating from *B. rapa* (fad2A) for ‘HOR2’ line,
3. one fad2 gene originating from *B. rapa* (fad2A) for ‘HOR3’ line and
4. one fad2 gene originating from *B. rapa* (fad2A) for ‘HOR4’ line.

For ‘HOR1’ lines, the sequence analysis identified a single nucleotide mutation in each fad2 gene (fad2A and fad2C) resulting in amino acid substitutions.

For ‘HOR2’ line, the sequence analysis identified a single nucleotide mutation in the *fad2A* gene resulting in an amino acid substitution.

For ‘HOR3’ and ‘HOR4’ lines, the sequence analysis identified a single nucleotide mutation in the *fad2A* gene creating a stop codon that causes early termination of the polypeptide chain during translation.

Molecular tests were developed for genotyping the 6 doubled haploids populations.
After genotyping all the 399 DH lines, it was found that the alleles distribution was highly correlated to C18:1 content (Fig. 1).

Fig. 1. Histogram showing the relation between the presence of the mutant allele-specific markers and oleic acid content in field (2005) and greenhouse (2003).

Discussion

The analysis of fad2 nucleic sequences obtained after cloning and sequencing showed that there are two copies of the fad2 gene in rapeseed: one from B. rapa (fad2A) and one from B. oleracea (fad2C). The preliminary results of correlation of the oleic acid content with the molecular genotyping highlight the cumulative effect of the transferred alleles fad2A and fad2C on the oleic acid content. Thus, 77% of oleic acid content could be obtained on average when the two mutated alleles fad2A and fad2C were cumulated in the genotypes. The maximum values obtained in greenhouse and in the field were higher than 80%.

Conclusions

This study highlights new SNP mutations in fad2 genes of 4 different mutants. These new molecular markers will be highly useful for direct selection of desirable fad2C and fad2A alleles during marker-assisted trait introgression and breeding of high oleic rapeseed. All information concerning these molecular markers was the subject of a patent filling. For more information, please contact INRA: cyril.falentin@rennes.inra.fr

References


