DEVELOPMENTS IN THE BREEDING OF EDIBLE OIL IN *BRASSICA NAPUS* AND *B. RAPA*

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ABSTRACT

Oil quality in the two oilseed *Brassica* species *B. napus* and *B. rapa* (syn. *campestris*) has been modified using a number of techniques and selection methods. Three approaches to the creation of the desired variation: selection, mutagenesis and transformation are covered in the review with examples of the modified oil qualities which have been produced. Once the variation is created, the challenge is to successfully select for the desired oil quality while improving other characteristics such as productivity. Methods to increase selection efficiency for fatty acid composition are also reviewed.

INTRODUCTION

Twenty years ago, the simplicity of one oilseed rape crop-one oil quality changed when the fatty acid composition of oilseed rape was modified by selection, reducing the erucic acid (C22:1) content from 40% to almost 0%. The change was motivated by a desire to enhance the quality of the oil for the edible oil market and to address nutritional concerns. The result was an oil low in C22:1 and high in the mono- and poly-unsaturated C:18 fatty acids. Subsequently, the fatty acid composition of the oilseed rape species *Brassica napus* L. and oilseed turnip rape *B. rapa* have been further modified with the objective of improving oil quality and increasing the applications of the oil. I will present this review using the different approaches of selection, mutagenesis and transformation which have produced the modifications of oilseed rape quality, followed by the selection techniques used to develop the new germplasm.

METHODOLOGIES FOR OIL QUALITY MODIFICATION

Selection

The first approach, selection, requires the identification of a source of the desired oil quality and its incorporation into an adapted background.

A survey was made of the fatty acid composition of a large number of *B. napus* germplasm accessions at the Tohoku National Agricultural Experiment Station in Japan (Ishida *et al.* 1995). There was a wide range in the content of the C18 fatty acids including oleic (C18:1), linoleic (C18:2), and α-linolenic (C18:3) as well as C22:1. The range in C18:3 was from 3.3 to 13.1%, suggesting that the germplasm may be available to develop low C18:3 strains.

Selection was used in the modification of C18:2 and C18:3 levels in spring turnip rape (*B. rapa*) (Laakso *et al.* 1995). Single plants were selected from a population on the basis of a high C18:2/C18:3 ratio, followed by pair-wise crosses. This approach has been successful in identifying progeny with C18:2/C18:3 ratios of >3 as a result of a reduction in C18:3. There was a high variation in the C18:2/C18:3 ratio and a wide range in the polyunsaturated fatty acid content (C18:2+C18:3) in the breeding material.

Identification of a single plant with increased levels of C18:1 (69%) in spring turnip rape was the basis for the high oleic breeding program described by Vilkki and Tanhuanpää (1995). The half-seed technique was used to screen the single plant progeny, followed by selfing and recurrent selection for high oleic content. The sixth selfed generation of high C18:1 plants shows a distinct fatty acid profile from the original parental material (line B.r.-HO in Table 1).

Table 1. A sample of the variation available in the C18 fatty acid composition of low linolenic (C18:3), and high oleic (C18:1) canola lines in comparison to the canola cultivar Westar.

	C16:0 + C18:0 (%)	C18:1 (%)	C18:2 (%)	C18:3 (%)
Westar	5.6	63	22	9.3
Apollo[a]	5.7	67	24	1.7
IMC-02[b]	6.0	67.9	20.7	2.1
DP-LL[c]	5.4	69	21.3	1.4
B.r.-HO[d]	n.a.	85-90	1-3	3-6
PH-HO-1[e]	7-8	78-80	7	6
PH-HO-2[e]	6	86	3	4
IMC-HO[b]	5.2	87.1	2.6	2.8
DP-HO[c]	6.9	83.0	6.3	4.0

[a]Scarth *et al.* 1995, [b]Loh, W.H.-T. 1994. Pers. comm., [c]Fader *et al.* 1995, [d]Vilkki and Tanhuanpää. 1995, [e]Charne. 1993.

Mutagenesis

Mutagenesis has been used successfully in the modification of fatty acid composition in oilseed rape *B. napus*. The mutagenesis is general and random. Therefore the desired mutation must be identified and selected for stability of expression.

The objective of reducing the C18:3 content of canola oil is a result of studies linking the high C18:3 content with reduced stability and the development of undesirable room odor during frying (Vaisey-Genser *et al.* 1994). The low C18:3 trait was not available in oilseed rape germplasm. With the objective of altering the desaturation step between C18:2 and C18:3, mutation work was carried out by Dr. Röbbelen and co-workers (Röbbelen and Nitsch. 1975). Several mutation lines were isolated with altered C18:2/C18:3 ratios. One of these mutations, M11, was used in the development of the low C18:3 cultivar Stellar at the University of Manitoba. Further selection for reduced C18:3 levels has resulted in the fatty acid profile of the low C18:3 cultivar Apollo (Table 1).

A recent study by Rücker and Röbbelen (1995) used mutagenesis in the development of high C18:1 oilseed rape. The mutagenic treatment created a wide range of C18:1 values in the M1 generation. The M3 generation of five M1 plants selected for high C18:1 had a mean C18:1 content of 77%, with single plant levels ranging from 56.4% to 80.4%. The higher C18:1 content was highly correlated with a reduction in C18:2

High C18:1 mutations have also been produced using chemical mutagenesis applied to isolated microspores by researchers with Pioneer Hi-Bred (Charne, 1993). The desired mutations were identified in doubled haploid (DH) lines which were then crossed to low C18:3 germplasm to produce high C18:1, low C18:3 genotypes (PH-HO-1, PH-HO-2, Table 1). High C18:1 commercial cultivars are currently being developed.

Transformation

The final methodology is transformation using *Agrobacterium* vectors to transform isolated oilseed rape cells with recombinant DNA constructs, followed by selection for a successfully integrated gene construct with stable expression. In the modification of oil quality using transformation, gene expression can either be enhanced or suppressed (Fader *et al.* 1995). The genes for the enzymes that desaturate C18:1 to C18:2 (Δ12 desaturase) and C18:2 to C18:3 (Δ15 desaturase) have been isolated, cloned and then joined with a seed specific promoter. Both high C18:1 (DP-HO, Table 1) and low C18:3 (DP-LL, Table 1) germplasm has been developed. The advantage of transformation over mutagenesis is the simpler inheritance of the genes producing the modified oil quality.

Researchers at CalGene have used a seed specific antisense construct of a stearoyl-ACP transferase to suppress gene activity in the first step of the C18 desaturation pathway. The first generation of transformants T1 had increased levels of C18:0 up to 40% and subsequent generations have shown stable inheritance (Thompson, 1993).

Both mutagenesis (IMC-02, Table 1) and transformation (IMC-HO, Table 1) have been used in the modification of canola oil quality by researchers with Cargill Ltd.

SELECTION TECHNIQUES

Techniques which increase selection efficiency are especially advantageous in the modification of oil quality as the embryo phenotype may be influenced by such factors as maternal genotype and environment.

DNA-based markers

DNA-based markers are currently being developed as a selection tool for a number of economically important traits in oilseed rape and related species. Hu *et al.* (1995) have targeted the identification of DNA-based markers associated with the expression of C18:3, with the objective of increasing the efficiency of selection for low C18:3 lines. An F2 population was created between a conventional oilseed rape cultivar Duplo (10.4% C18:3) and a low C18:3 line (2.2% C18:3) produced from a cross between two low C18:3 sources (Röbbelen and Thies, 1975; Roy and Tarr, 1986). The F2 mean C18:3 content was 6.2% which was very close to the mid-parent value of 6.3% and confirmed the major influence of nuclear genes. Only 10% of the mapped probes detected polymorphisms between the parental DNA, a very low level of polymorphism. However, a marker was identified which accounted for 29% of the genetic variation. RFLP analysis using the marker K01-1100 identified homozygotes for RAPD analysis. Using two RAPD primers simultaneously in a modified RAPD technique, a second marker L01-L09-600 was identified which accounted for 39% of the variation for C18:3 content in the population. Hu *et al.* (1995) are pursuing a possible linkage relationship between K01-1100 and desaturase genes which were mapped in segregating *B. oleracea* populations.

Pollen selection

The correlation between the fatty acid composition of pollen and seeds is examined as a possible method to increase selection efficiency (Jourdren *et al.* 1995). Doubled haploid (DH) lines were developed using microspore culture of the F1 of a cross between the low C18:3 cultivar Stellar and a conventional oilseed rape cultivar Drakkar. The DH lines were analysed for the fatty acid composition of both pollen and seed using the same methods of extraction, esterification and fatty acid methyl esters analysis. The results were expressed as %C18:3 of the total fatty acid content and as the Linolenic Desaturation Ratio (LDR = %C18:3 x100/ %C18:3 + %C18:2) (Pleines, 1989). The correlation between pollen LDR and seed LDR provided the best selection with a better efficiency for selection of low C18:3 versus high C18:3. The segregation of the DH lines on the basis of pollen C18:3 content corresponded well to the results obtained by seed analysis. Both analyses support the segregation of two genes controlling C18:3 content in the cross.

CONCLUSIONS

The number of modifications in edible oil quality of oilseed rape are increasing rapidly, as the technology for producing the necessary variation and the ability to select efficiently for the desired genotypes are being refined. The remaining components are the identification of the desirable oil quality for particular applications and the integration of the modified oil quality into productive oilseed rape cultivars.

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