Sunflower mutants with increased levels of palmitic and stearic acids in the oil.

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Abstract

There is a demand of fats and oils with specific fatty acid composition for the purposes of the food industry. Most of these fats are obtained after chemical manipulations, mainly hydrogenation and transesterification, which should be avoided for nutritional reasons. To overcome this problem we developed a mutagenesis programm using chemical and physical mutagens with the aim of inducing genetic variability and select sunflower (*Helianthus annuus* L.) seeds with higher saturated fatty acid content. Five mutants lines have been obtained. Three with high stearic acid content (10, 12 and 25%) and two with high palmitic acid content (25-30%). One high palmitic acid mutant was selected on high oleic background. During selection, seeds of the same plant showed variation in fatty acid composition which indicated that the fatty acid modification was controlled by the genotype of the embryo.

Introduction

The physical and chemical properties of vegetable oils are determined by their fatty acid composition. Normal temperate oil crops produce oils with high proportion of unsaturated fatty acids, by this reason they are liquid at room temperature. For margarine production a minimum degree of plasticity is needed, this is obtained by hydrogenation of the oils. But this procedure not only increases the content of saturated acids but also produce "trans" isomers of the fatty acids that are undesirable. An increase of palmitic or stearic acids content should be useful for margarine consumers and food industry. On the other hand, some vegetable fats with high content of saturate fatty acid, like cocoa, have been found to be not hipercholesteronemic (Bracco, 1994) mainly because the saturated fatty acids are at positions 1 and 3 of the triacylglycerol molecule. The hydrolysis of this type of triacylglycerols by lipase produce free saturated fatty acid that are excreted in a high proportion (Renaud et al., 1995) making it possible to consume a

saturated fat without increasing the cholesterol level. This fatty acid distribution is normally found in most vegetable oils, like olive or sunflower.

Several ways have been used to modify the fatty acid composition of the seed oil. Natural variability has been observed in safflower (*Carthamus tinctorius* L.; Knowles, 1989) and rapeseed (*Brassica napus* L.; Stefanson et al. 1961). Mutagenesis treatments have been utilized to obtain the high oleic acid line in sunflower (*Helianthus annuus* L.; Soldatov, 1976) and the very low linolenic acid line in linseed (*Linum usitatissimum* L.; Green, 1986). Recently, also genetic engineering have been used to modify the fatty acid composition of seed oil. Examples of this are the new rapeseed with either high stearic acid content (Knutzon et al.1992) or high lauric acid content (Voelker et al. 1992).

We have developed a mutagenesis programm to increase the genetic variability in the fatty acid composition of sunflower seed oil (Osorio et al. 1995).

Materials and methods

Seeds of lines BSD-2-691, BSD-2-423 and RDF-1-532 were exposed to chemical or physical mutagenic agents. The line BSD-2-423 has high oleic acid content in the seed oil, the other two lines have normal oil fatty acid composition. Chemical mutagenesis was carried out either with sodium azide or ethyl methanesulfonate (EMS), the physical mutagenesis was carried out using X-rays. In all cases the dose was selected to assure an approximate germination rate of 50%. More than 20.000 seeds were initially mutagenised. M_1 seeds were planted in the field and M_2 seeds harvested, three M_2 seeds from each capitulum were planted to obtain the M_3 seed generation. Two seeds from each M_1 plant and one from each M_2 were used to analyse the fatty acid composition (Garcés et al. 1993).

Results and discussion

For each line and mutagen several treatments were performed. Initially around a hundred putative mutants were selected, but after four selfing and selection only 5 were really stable (Table 1).

Three of them (CAS-3, CAS-4 and CAS-8) have high stearic acid content in the seed oil and were obtained with chemical mutagens on the same original line. The two high palmitic lines, CAS-5 and CAS-12 were obtained after X-rays treatment on two near isogenic lines, except for the oleic acid content. The line BSD-2-423 from which the mutant CAS-12 was obtained was already a high oleic acid mutant line. In all cases the original capitulum had seeds with normal and mutant phenotypes, showing that the mutant character was controlled by the genotype of the embryo.

Table 1. Mutant lines obtained in this work and their origin.

Mutant line	Phenotype	Parental line	Mutagen
CAS-3	Very high 18:0	RDF-1-532	EMS
CAS-4	High 18:0	RDF-1-532	Sodium azide
CAS-5	Very high 16:0	BSD-2-691	X rays
CAS-8	High 18:0	RDF-1-532	Sodium azide
CAS-12	Very high 16:0	BSD-2-423	X rays

The fatty acid composition of the CAS mutants are shown in Table 2. CAS-3 have around 6 times more stearic acid than the normal sunflower seeds, the increase of stearic acid was at the expenses of oleic acid. Mutants lines CAS-4 and CAS-8 have 2-3 times more stearic acid than the control. In CAS-4 a concomitant decrease of linoleic acid is found. CAS-8 have a similar behaviour than CAS-3. This could suggest different modifications in the fatty acid biosynthesis between CAS-4 and the other two.

Table 2. Fatty acid composition of CAS mutant lines and a high oleic (G-8) and a normal (RHA-274) lines as control.

	Fatty acid composition (%)				
Line	16:0	16:1	18:0	18:1	18:2
RHA-274	6.1		4.4	35.0	53.9
G-8	2.4		2.8	88.7	5.5
CAS-3	5.1		26.0	13.8	55.1
CAS-4	5.4		11.3	34.6	48.0
CAS-5	25.2	3.7	3.5	11.4	55.1
CAS-8	5.8		9.9	20.4	63.8
CAS-12	30.6	5.2	3.3	55.5	3.3

The high palmitic acid line have 3-5% of palmitoleic acid. This fatty acid is not present in the normal sunflower, neither is found in most vegetable oils except for olive and cotton seed oils. Compared to the RHA-274 line the CAS-5 line has increased level of palmitic acid and a lower level of oleic acid. Mutant line CAS-12 must be compared with the high oleic control G-8; also in this case oleic acid decreased while no difference was found in linoleic acid content.
An important factor is the parental line. The three high stearic acid mutants were obtained on the same line and the two high palmitic on two near isogenic lines with very different levels of oleic acid.
Future combinations of these characters by sexual crossing could produce tailor made fats for food industry.

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