PROGRESS IN THE GENETIC MODIFICATION OF SUNFLOWER OIL QUALITY

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Abstract

Sunflower oil has been traditionally appreciated as a high quality commodity in the world oil market. However, new emerging markets together with an increasing concern about health risks of foods are demanding changes in the oil quality. Sunflower breeders have been tremendously successful in developing novel oil types over the past 30 years. Together with the standard sunflower oil, with a fatty acid profile made up of 11% saturated fatty acids, 20% oleic acid, and 69% linoleic acid, there is currently available a vast diversity of other sunflower oil types, for example low saturated (<7%), high palmitic (>25%), high stearic (>25%), high oleic (>85%), high linoleic (>75%) as well as a number of oils with intermediate levels and combinations among them. Similarly, the standard sunflower oil with 95% of the tocopherols in the alpha-tocopherol form has been modified to produce oils with high levels of beta-tocopherol (>75%), gamma-tocopherol (>95%), and delta-tocopherol (>65%). The novel fatty acid and tocopherol traits are controlled by the genotype of the developing embryo and they are in all cases governed by a reduced number of genes, which considerably facilitates their management in plant breeding programs. All this variation for fatty acid and tocopherol profile, developed in all cases by conventional selection after germplasm screening or mutagenesis, opens up the possibility of tailoring specialty oils for specific food and nonfood applications, which guarantees a very promising future to sunflower oils in the global world market.

Introduction

Sunflower (*Helianthus annuus* L.) is the fourth largest source of vegetable oil at a world level, after soybean, palm, and canola. Sunflower oil has many potential applications, both in the food and nonfood industry. For many of such applications, standard sunflower oil needs additional processing and chemical additives, which in most cases results in a final product with detrimental implications for human health. Thus, common uses of sunflower oil such as deep frying or margarine production require a previous hydrogenation or hardening of the oil. This process produces t*rans* and positional isomers related to heart disease (Willett and Ascherio, 1994). Similarly, standard sunflower oil is not optimally suited to some potential uses, for example those requiring a high oxidative stability. Fortunately, plant breeders have been successful in overcoming the limitations of standard sunflower oil by developing a wide range of novel and healthier sunflower oil types with optimal properties for many specific applications, both in the food and nonfood industry. The most relevant achievements in this field over the past thirty years are briefly summarized in this review.

Discussion

Oil Quality and Breeding Objectives. Oil quality refers to both the nutritional and functional properties of the oil. There is not an optimal oil quality, as it will depend on the final use of the oil. In all cases, the main parameters defining the quality of the oil are fatty acid composition, the distribution pattern of fatty acids within the triacylglycerol molecule, and the total content and profile of several families of polyisoprenoid lipids present in the oil, mainly tocopherols and sterols.

Fatty acids are commonly classified into saturated, monounsaturated, and polyunsaturated, according to the number of unsaturations (double bonds) present in the fatty acid chain. From a nutritional point of view, saturated fatty acids are regarded as undesirable for human consumption, as they have a detrimental atherogenic effect by raising both serum total cholesterol content and low-density lipoprotein (LDL) levels as compared with mono- and polyunsaturated fatty acids (Mensink and Katan, 1989; Mensink et al., 1994).

From a technological point of view, key aspects for most applications are plasticity and resistance to oxidation, particularly at high temperatures. Plastic fats are widely required in the food industry for the production of shortenings, margarines, and many specialty products. Because standard sunflower oil is mainly made up of unsaturated fatty acids, it is liquid at room temperature. Accordingly, its utilization by the food industry usually requires a previous chemical hardening to change to a solid or semisolid state, usually conducted by hydrogenation. However, hydrogenation also induces *cis-trans* isomerisation of fatty acids (Tatum and Chow, 1992), resulting in the production of *trans*-fatty acids related to heart disease (Willett and Ascherio, 1994).

Hydrogenation is not only used for transforming liquid sunflower oil into a plastic fat, but also to extend the shelf life of sunflower oil and foods that are fried in it. Standard sunflower oil is rich in polyunsaturated linoleic acid, with two double bonds in its molecule. Double bonds are the active sites of lipid oxidation, as they react rapidly with oxygen in the air in a process involving the production of free radicals, which are implicated in a number of diseases, tissue injuries and in the process of aging (Shahidi, 1996). Furthermore, the breakdown products of fatty acid oxidation are the major source of off-flavors in oils, which reduce their shelf life (Tatum and Chow, 1992).

Sunflower oil contains small quantities of polyisoprenoid lipids of great nutritional and technological value, the most important of which, according to our current knowledge, are the tocopherols. The tocopherols exhibit antioxidant activity both in vivo and in vitro. In vivo they exert vitamin E activity, protecting cellular membrane lipids against oxidative damage (Muggli, 1994). In vitro they inhibit lipid oxidation in oils and fats, as well as in foods and feeds containing them (Kamal-Eldin and Appelqvist, 1996). Alpha-tocopherol is the biologically most active tocopherol, but it shows a low in vitro antioxidant activity (Traber and Sies, 1996). The other tocopherols (beta-, gamma-, and delta-tocopherol) have a lower vitamin E activity than alpha-tocopherol, but they are better in vitro antioxidants (Pongracz et al., 1995).

According to the previous discussion, there are multiple breeding objectives depending on the intended use of sunflower oil. Standard sunflower oil has an average fatty acid composition of 11% saturated fatty acids, palmitic (16:0) and stearic acid (18:0), 20% oleic acid (18:1), and 69% linoleic acid (18:2), although the ratio linoleic/oleic acid is environmentally dependent, with a range of variation from 0.8 to 5.0 depending on the temperature during seed development. Total tocopherol content in standard refined sunflower oil is around 700 mg/kg, with about 95% of the total tocopherols in the alpha-tocopherol form. Applications demanding a high nutritional

value in the oil (e.g., salad oil) will require a reduction of saturated fatty acids and enhancement of the vitamin E (=alpha-tocopherol) content of the oil. The use of sunflower oil in the food industry requiring plastic fats (e.g., margarines and shortenings) will demand an increased concentration of saturated fatty acids in order to avoid hydrogenation. High temperature processes (e.g., frying oil industry, biolubricants) need a sunflower oil highly resistant to thermoxidation, with a low concentration of linoleic acid and a high concentration of in vitro antioxidants (mainly gamma and delta-tocopherol). Any other application of sunflower oil will probably require specific modifications of the nutritional and/or functional properties of the oil.

Development of Modified Sunflower Oils. The search for natural variability in cultivated and wild sunflower germplasm, and to a more of an extent, the use of mutagenesis has allowed for the development of a great deal of variation in the content of all the fatty acids and tocopherols.

Screening Procedures. An indispensable prerequisite for improving seed oil quality traits is the availability of adequate screening procedures to measure them. In sunflower, both the fatty acid and the tocopherol profile of the seeds are governed by the genotype of the developing embryo. Accordingly, selection for these oil quality traits can be conducted at the single-seed level, provided that single-seed fatty acid composition can be accurately measured in a nondestructive way. Downey and Harvey (1963) developed the half-seed technique for nondestructive analysis of the fatty acid composition of single seeds of rapeseed (*Brassica napus* L.). The technique has been adapted to sunflower (Conte et al., 1989), and it is additionally used for the nondestructive analysis of tocopherol composition (Demurin et al., 1996) or even for both tocopherol content and composition (Velasco et al., 2004b).

An additional step in facilitating selection for seed quality at a single-seed level has been the utilization of near-infrared spectroscopy (NIRS) for analyzing the fatty acid profile of intact or husked achenes. In a first step, Sato et al. (1995) demonstrated the feasibility of NIRS for measuring the concentration of linoleic acid in the oil of single husked achenes of sunflower. Velasco et al. (1999) reported that NIRS permitted the discrimination of single unhusked achenes of sunflower for oleic and linoleic acid concentration in the seed oil. More recently, Velasco et al. (2004a) used NIRS for large-scale screening for high stearic acid concentration in husked sunflower achenes. The NIRS technique has major advantages over conventional chromatographic procedures, as NIRS analyses are faster and more cost-effective. Accordingly, NIRS can be used to speed up selection programs.

Sunflower Oils with Modified Saturated Fatty Acids. Many industrial uses require oils that are enriched in saturated fatty acids, preferably stearic acid. Conversely, the reduction of total saturated fatty acid content is also an important objective for specific dietary uses. Mutagenesis of dry seeds by X-ray irradiation allowed the isolation of single mutants with high palmitic acid content above 25% (275HP, Ivanov et al., 1988; CAS-5, Osorio et al., 1995) (Table 1). A double mutant CAS-12, showing high palmitic acid levels (of about 30%) and an increased oleic acid content (about 56%), was also developed by mutagenizing a high oleic acid line isogenic to the original low oleic acid line used to obtain CAS-5 (Fernández-Martínez et al., 1997). Demurin (2003) also identified high palmitic acid levels (23.9%) after evaluation of a world germplasm collection. All the high palmitic acid mutants exhibit palmitoleic acid (16:1) in their seed oil, which is absent in standard sunflower oil. More recently, the mutant CAS 37, with high palmitic acid levels and presence of palmitolinoleic acid (16:2) and asclepic acid (18:1Δ11) has been reported (Salas et al., 2004; Table 1).

Table 1. Fatty acid composition (%) of the seed oil of the principal induced and natural mutants of sunflower, in comparison with the standard type. The concentrations of the most altered fatty acids are printed in bold.

Mutant	Oil type	Fatty acid composition (%) [1]					Reference
		16:0	16:1	18:0	18:1	18:2	
Standard[2]	High 18:2	7.0	----	4,0	17.0	72.0	Dorell and Vick, 1997
		6.5	----	3.0	40.9	49.6	Fernández-Martínez et al., 1993
Pervenets	High 18:1	-----	----	-----	**79.3**	14,8	Soldatov, 1976
HO lines[3]	High 18:1	4.9	----	2.9	**90.3**	1.8	Fernández-Martínez et al., 1993
M-4229	High 18:1	3.4	----	4.1	**86.1**	3.9	Vick and Miller, 1996
M- 3067	Mid 18:1	3.9	----	5.2	**54.6**	33.9	Vick and Miller, 1996
2698-1	High 18:2	----	----	----	----	**78.0**	Miller and Vick, 2001
F6 sel.	High 18:2	-----	----	-----	----	**77.3**	Simpson et al., 1989
275HP	High 16:0	**25.1**	6.9	1.7	10.5	55.8	Ivanov et al., 1988
CAS-5	High 16:0	**25.2**	3.7	3.5	11.4	55.1	Osorio et al., 1995
HP line	High 16:0	**23.9**	3.4	2.0	20.4	50.7	Demurin, 2003
CAS-12	High 16:0 Mid 18:1	**30.7**	7.6	2.1	**56.0**	3.1	Fernández-Martínez et al., 1997
CAS-37	High 16:0 High 16:1	**29.5**	**12.3**[4]	1.4	5.4	38.7	Salas et al., 2004
CAS-14	High 18:0	8.4	**----**	**37.3**	12.4	38.0	Fernández-Moya et al., 2002
CAS-3	High 18:0	5.1	----	**26.0**	13.8	55.1	Osorio et al., 1995
CAS-4	Mid 18:0	5.4	----	**11.3**	34.6	48.0	Osorio et al., 1995
CAS-19	Mid 18:0	6.8	----	**15.3**	21.5	56.4	Pérez-Vich et al., 2004a
CAS-8	Mid 18:0	5,8	-----	**9.9**	20.4	63.8	Osorio et al., 1995
CAS-20	Mid 18:0	5.7	----	**7.7**	35.9	50.5	Pérez-Vich et al., 2004a
LS-1	Low 18:0	5.6	----	**4.1**	20.2	67.4	Miller and Vick, 1999
LS-2	Low 18:0	8.6	----	**2.0**	10.8	75.0	Miller and Vick, 1999
LP-1	Low 16:0	**4.7**	----	5.4	23.8	63.7	Miller and Vick, 1999
RS1	Low sat	**3.9**	----	**2.6**	40.1	51.8	Vick et al., 2003
RS2	Low sat	**4.4**	----	**3.2**	42.9	47.7	Vick et al., 2003
NMS2229	Low sat	**3.9**	----	**1.9**	-----	-----	Seiler, 2004

Chemical mutagenesis with EMS and sodium azide also allowed the isolation of three mutants, CAS-8, CAS-4 and CAS-3, with >10%, >13%, and >25% of stearic acid, respectively (Osorio et al., 1995). More recently, a very high stearic acid mutant CAS-14, with stearic acid levels higher than 35% was isolated using mutagenesis with sodium azide (Fernández-Moya et al., 2002). Two more lines, CAS-19 and CAS-20, with mid-stearic acid levels (Table 1) were further developed from crosses between CAS-3 and a line with standard fatty acid profile (Pérez-Vich et al., 2004a).

Low saturated fatty acids have been developed by using both mutagenesis and germplasm evaluation. A mutant with 4.7% of palmitic acid, compared to 6.3% in the

check, and two low stearic mutants with 4.1 and 2.0 %, compared to 4.8 % in the check, were obtained using chemical mutagenesis (Miller and Vick, 1999). Similarly, two germplasm lines, RS1 with 7.7 % and RS2 with 7.6 % of total saturated fatty acids, were selected from a cultivated sunflower line (Vick et al., 2002). In both lines, a reduction in stearic content was largely responsible for the decrease in total saturated fatty acids. Low saturated fatty acid levels (3.9% of palmitic acid and 1.9% of stearic acid, totaling 5.8% for both) were also identified in a population of wild *H. annuus* (Seiler, 2004).

Sunflower Oils with Modified Oleic and Linoleic Acid Content. As mentioned above, oleic acid is both nutritionally appropriate and very stable to oxidation. The first approach to develop materials with elevated oleic acid levels was the evaluation of cultivated and wild germplasm. High oleic acid levels up to 85% were identified in wild *H. annuus*, but they were not stable under different temperature conditions during seed development (Fernández- Martínez and Knowles, 1987). The most successful source of high oleic acid in sunflower was obtained by Soldatov (1976). He developed the variety "Pervenets," with oleic acid content above 75%, after seed treatment with dimethyl sulfate and further selection for high oleic acid during several generations. High oleic acid lines derived from Pervenets have been shown to be very stable, with oleic acid contents higher than 83% under different temperature regimes (Fernández-Martínez et al., 1986; Miller et al., 1987). Vick and Miller (1996) also developed, using EMS treatments, high and mid oleic acid mutants (Table 1).

An alternative breeding objective has been the development of higher-than-normal, stable levels of linoleic acid. Several sources have been obtained by evaluation and selection in cultivated (Simpson et al., 1989; Miller and Vick, 2001) and wild (De Haro and Fernández-Martínez, 1991; Seiler, 1992) germplasm.

Sunflower Oils with Modified Tocopherols. The modification of the tocopherol profile through a partial substitution of alpha-tocopherol, with weak in vitro antioxidant action, by other tocopherol derivatives is an important goal for developing sunflower oil with improved oxidative stability. Breeding efforts in this direction have led to the development and characterization of several sources of modified tocopherol profile in sunflower. After extensive evaluation of germplasm of wild and cultivated sunflower, Demurin (1993) and Demurin et al. (1996) isolated two lines with modified tocopherol profiles: the line LG-15, with increased concentration of beta-tocopherol (50% of the total tocopherols), and the line LG-17, with increased concentration of gamma-tocopherol (95% of the total tocopherols). Crosses between LG-15 and LG-17 produced the line LG-24, with 84% gamma and 8% delta-tocopherol (Demurin, 1993).

Velasco et al. (2004b) developed the line T589 with increased levels of beta-tocopherol (>30% of the total tocopherols) from an accession of the open-pollinated cultivar Peredovik, as well as from the line T2100 with high gamma-tocopherol content (>85% of the total tocopherols) from another accession of Peredovik. The application of chemical mutagenesis to seeds of Peredovik led to the isolation of two additional mutants with increased levels of gamma tocopherol (>95%), IAST-540 and IAST-1 (Velasco et al., 2004c). Crosses between IAST-1 and T589 produced an unexpected transgressive segregation for high concentrations of beta- or delta-tocopherol. Selection from such a transgressive segregation led to the isolation of IAST-4, with a high concentration of delta-tocopherol (>65%), and IAST-5, with a high concentration of beta-tocopherol (>75%) (Velasco et al., 2004c).

Genetic Control and Environmental Effects: Inheritance Studies. In general, genetic modifications altering either the fatty acid or the tocopherol profile have been found to be qualitative rather than quantitative, i.e., they are controlled by a reduced number of genes and they are less affected by environmental factors than by quantitative traits such as oil content. Another feature of the genetic control of these traits is that in

most cases they are determined by the genotype of the developing embryo with little or no maternal influence. The latter is crucial in breeding programs, as a low weight of maternal inheritance allows selection to be carried out at a single-seed level.

Saturated Fatty Acids. Several researchers have carried out genetic studies on the inheritance of high and low content of saturated fatty acids in sunflower. The results are summarized in Table 2. The inheritance of the high palmitic acid mutant 275HP was studied by Ivanov et al. (1988) and was found to be partially recessive and gametophytic. The character was treated as a quantitative trait and the number of genes involved was not identified. The palmitic acid content of the mutant CAS-5 was found to be controlled by alleles at each of three independent loci (*P1, P2, P3*) with partial dominance for low concentration and no maternal effects (Pérez-Vich et al., 1999a). The genotypes with a high palmitic acid concentration are homozygous recessive at the *P1* locus (*p1p1*) and either at the *P2* (*p2p2*) or *P3* (*p3p3*) locus (Table 2). The genetic study on CAS-5 revealed that the *p2* and *p3* alleles were already present in the line BSD-2-691 used for mutagenesis, while only the *p1* allele was the result of the mutagenic treatment. The genetic control of the high palmitic acid trait in CAS-12 was found to be similar to that in CAS-5 (Pérez-Vich et al., 2002a).

Genetic characterization of the high stearic acid content of the sunflower mutant CAS-3 concluded that the trait is controlled by two alleles (*es1, es2*) at two different loci, *Es1* and *Es2*, with partial dominance for low concentration (Pérez-Vich et al., 1999b). The effect of the *Es1* locus on stearic acid concentration was found to be greater than that of the *Es2* locus, but both loci had an additive effect on stearic acid content. Studies on the inheritance of the medium stearic acid content of mutant CAS-4 concluded that the trait was controlled by alleles at the *Es1* and *Es2* loci identified in CAS-3 or at tightly linked loci (Pérez Vich et al., 2002b). The CAS-4 alleles at the *Es2* locus were those present in CAS-3 (*es2es2*), whereas the alleles at the *Es1* locus were different from those of CAS-3 (*es1es1*), and they were designated *es1bes1b*. The results indicated that the *es2* allele was present in the original line RDF-1-532, and that the mutation only induced the change of *Es1* to *es1* in CAS-3 and of *Es1* to *es1b* in CAS-4.

The low palmitic and low stearic acid content of the mutants RHA-274-LP-1 and HA-821-LS-1 were found to be controlled by single genes with additive gene action, designated *fap1* and *fas1*, respectively (Miller and Vick, 1999). The low stearic acid content of the mutant RHA-274-LS-2 showed two modified genes, designated *fas2* and *fasx* (Miller and Vick, 1999). Genetic characterization of the lines RS1 and RS2 indicated that reduced saturated fatty acid content was partially dominant and that the reduced stearic acid content was controlled by more than one gene (Vick et al., 2002).

Oleic and Linoleic Acids. A number of studies have been conducted on the inheritance of the high oleic acid line Pervenets, although no general agreement has been reached so far (Table 3). The first studies determined that the high oleic acid content was controlled by a single dominant (Urie, 1984) or partially dominant (Fick, 1984) gene designated *Ol*. However, although the segregation 3 high: 1 low + intermediate, indicating a single dominant gene, was observed by other authors (Schmidt et al., 1989;

Table 2. Theoretical genotypic and phenotypic classes for palmitic (16: 0) and stearic acid (18: 0) levels in crosses segregating for these fatty acids.

Trait	Line	Genetic model	Expected F_2 genotypes[1]	Freq.	16:0 or 18:0 phenotypes	References
High 16:0	275HP	Quantitative trait				Ivanov et al., 1988
High 16:0	CAS-5 CAS 12	Three partially recessive genes	*P1P1 _ _ _ _ , _ _ P2P2 P3P3*	19/64	Low	Pérez-Vich et al.,1999a Pérez-Vich et al., 2002a
			P1p1 p2_ _ _ , P1p1 _ _ p3_ , *p1p1 P2p2 P3P3; p1p1 P2P2 P3p3* *p1p1 P2p2 P3p3*	38/64	Intermediate	
			p1p1 p2p2 _ _ , p1p1 _ _ p3p3	7/64	High	
High 18:0	CAS-3	Two partially recessive genes	*es1es1 es2es2*	1/16	High	Pérez-Vich et al., 1999b
			Es1_ Es2_ , Es1_ es2es2, *es1es1 Es2_*	15/16	Low + Intermediate	
Mid 18:0	CAS-4	Two partially recessive genes	$es1^{b}es1^{b}es2es2$	1/16	Medium	Pérez-Vich et al., 2002b
			Es1_ Es2_ , Es1_ es2es2, $es1^{b}es1^{b}$ *Es2_*	15/16	Low + Intermediate	
Mid 18:0	CAS-19	One partially recessive gene	*es1es1 Es2Es2*	1/4	Medium	Pérez-Vich et al.,2004a
			Es1_ Es2Es2	3/4	Low + Intermediate	
Mid-low 18:0	CAS-20	One partially recessive gene	*Es1Es1 es2es2*	1/4	Medium-low	Pérez-Vich et al., 2004a
			*Es1Es1 Es2_*	3/4	Low + Intermediate	
Low 16:0	LP-1	One gene with additive gene action	*fap1fap1*	1/4	Reduced	Miller and Vick, 1999
			*Fap1_*	3/4	Low + Intermediate	
Low 18:0	LS-1	One additive gene	*fas1fas1*	1/4	Reduced	Miller and Vick, 1999
			*Fas1_*	3/4	Low + Intermediate	
Low 18:0	LS-2	Two additive genes	*fas1fas1 fasxfasx*	1/16	Reduced	Miller and Vick, 1999
			Fas1_ Fasx_, Fas1_fasxfasx *fas1fas1 Fasx_*	15/16	Low+ Intermediate	
Low sat	RS1 RS2	Continuous distribution, with reduced saturated content partially dominant				Vick et al., 2002

[1] "_ _" indicates that any allelic configuration may occur at those loci.

Fernández-Martínez et al., 1989; Pérez-Vich et al., 2002a) (Table 3), further studies demonstrated that the genetic control of the high oleic acid trait was more complex. Urie (1985) reported the existence of modifying genes as well as reversal in the dominance of the gene *Ol*. Several hypotheses have been advanced to explain the inheritance of modifiers; Miller et al. (1987) described the *Ml* locus acting as a modifier of the *Ol* locus. According to their model, the high oleic acid concentration was only expressed in genotypes having one dominant allele of the *Ol* gene combined with the recessive allele *ml* in a homozygous condition (genotypes: *Ol_mlml*). Fernández et al. (1999) also postulated a two-gene model, and suggested that *Ml* was a gene complex. Fernández-Martínez et al. (1989) and Pérez-Vich et al. (2002a) observed three different F2 segregations (1:3; 7:9, and 37:27) for low-intermediate and high oleic acid classes, and proposed a model based on the existence of three complementary genes *Ol1*, *Ol2* and *Ol3* controlling the high oleic acid trait (Table 3). Velasco et al. (2000), in a controlled environment, observed a number of different segregations both in the F1 and F2 generations, which were explained with a genetic model including five genes. The reversal of dominance mentioned by Urie (1985) has been further confirmed in several works (Fernández-Martínez et al., 1989; Demurin and Škorić, 1996; Velasco et al., 2000; Pérez-Vich et al., 2002a). In short, although there is a general agreement on the presence of a principal gene, *Ol1*, the high oleic acid content is a very complex trait which involves a number of modifying genes whose number and function are still to be determined.

In the only study on the inheritance of high linoleic acid content, Simpson et al. (1989) postulated that this trait was governed by a partially recessive gene with maternal influence.

Tocopherol Content and Composition. Tocopherols are accumulated in the seeds as the result of a biosynthetic pathway independent from that determining the accumulation of triacylglycerols. Accordingly, the final concentration of tocopherols in the raw oil will depend on both the concentration of oil in the seeds and the concentration of tocopherols in the seeds. Several studies have focused on the relative weight of genotypic and environmental effects on total tocopherol content of sunflower seeds. Marquard (1990) concluded that total tocopherol content was significantly influenced by both location and genotype, the effect of location being more important than the effect of genotypes. Alpaslan and Gündüz (2000) evaluated ten sunflower varieties during two years, concluding that both the genotype and the environment influenced significantly the tocopherol content. In that study, the effect of varieties was larger than that of years. Velasco et al. (2002), in a study conducted with 36 varieties over 13 locations, found that the effect of the genotype was larger than those of the environment and the genotype x environment interaction.

Genetic characterization of the line LG-15 concluded that the increased levels of beta-tocopherol were produced by recessive alleles at the *Tph1* locus (Demurin et al., 1996). Velasco et al. (2003) reached a similar conclusion for the line T589, with increased beta-tocopherol content. A genetic comparison between both lines has not been conducted yet.

Increased levels of gamma-tocopherol in the line LG-17 were the result of recessive alleles at the *Tph2* locus (Demurin et al., 1996). Velasco et al. (2003) also found that the high gamma-tocopherol content of the line T2100 was produced by recessive alleles at a single locus, although it still has to be determined whether the alleles for high gamma-tocopherol content in T2100 are located at the *Tph2* or at a different locus.

Table 3. Theoretical genotypic and phenotypic classes for oleic acid (18:1) levels in crosses segregating for this fatty acid

Line	Genetic model	Expected F_2 genotypes[1]	Freq.	18:1 Phenotype	References
Pervenets selection	One partially dominant gene	*Olol* *Ol_*	1/4 3/4	Low High+ intermediate	Fick, 1984
Pervenets selection	One dominant gene	*Olol* *Ol_*	1/4 3/4	Low-intermediate High	Urie, 1985 Schmidt et al., 1989
Pervenets selection	Single, dominant and recessive modifier	*Olol_ _* *Ol_Ml_* *Ol_mlml*	4/16 9/16 3/16	Low Intermediate High	Miller et al., 1987
R 978	Single, recessive and recessive modifier	*Ol_Ml_*, *Ol_mlml*, *olol_Ml_* *olol mlml*	15/16 1/16	Low + intermediate High	Fernández et al., 1999
HAOL-9	One dominant gene	$ol_1ol_1\ OL_2OL_2\ OL_3OL_3$ $OL_1\ _\ OL_2OL_2\ OL_3OL_3$	1/4 3/4	Low-intermediate High	Fernández-Martínez et al., 1989 Pérez-Vich et al., 2002a
HAOL-9	Two dominant genes	$ol_1ol_1\ _\ _\ OL_3OL_3$ or $_\ _\ ol_2ol_2\ OL_3OL_3$ $OL_1\ _\ OL_2\ _\ OL_3OL_3$	7/16 9/16	Low-intermediate High	Fernández-Martínez et al., 1989 Pérez-Vich et al., 2002a
HAOL-9	Three dominant genes	$ol_1ol_1\ _\ _\ _\ _$ or $_\ _\ ol_2ol_2\ _\ _$ or $_\ _\ _\ _\ ol_3ol_3$ $OL_1\ _\ OL_2\ _\ OL_3\ _$	37/64 27/64	Low-intermediate High	Fernández-Martínez et al., 1989 Pérez-Vich et al.,2002a
HAOL-9	One recessive gene	$OL_1\ _\ OL_2OL_2\ OL_3OL_3$ $ol_1ol_1\ OL_2OL_2\ OL_3OL_3$	3/4 1/4	Low-intermediate High	Fernández-Martínez et al., 1989 Pérez-Vich et al., 2002a
LG-27	One recessive gene	$OL_$ ol_1ol_1	3/4 1/4	Low Mid	Demurin et al., 2000
HAOL-9	Five genes				Velasco et al. 2000
High linoleic selection	Partially recessive gene				Simpson et al., 1989

[1] "_ _" indicates that any allelic configuration may occur at those loci.

Relationship Between Quality Traits. The recombination of several seed oil quality traits in a single phenotype constitutes a further step in the improvement of oil quality. This process is facilitated when the traits to be recombined are independently inherited. Accordingly, the study of the relationships between different quality traits has been the subject of a number of investigations.

The relationship between the high palmitic and the high stearic acid traits was studied in crosses between the mutant lines CAS-5 and CAS-3. The results indicated that the complete recombination of the high palmitic and the high stearic acid contents was not possible because of an epistatic effect of the loci controlling high palmitic acid content on the loci responsible for high stearic acid (Pérez-Vich et al., 2000a). Similarly, the genetic relationships between the high stearic and the high oleic acid contents were investigated in crosses involving the high stearic acid line CAS-3 and the high oleic acid line HAOL-9. In that study, it was concluded that the complete recombination of the high oleic and high stearic acid levels of the parents was hindered by a genetic linkage between the *Es2* and *Ol* loci (Pérez-Vich et al., 2000b). Conversely, the high palmitic acid and high oleic acid traits were found to be independently inherited (Pérez-Vich et al., 2002a). Similarly, genes controlling fatty acid levels seem to be independently inherited from those controlling the tocopherol profile, which allowed the combination of different fatty acid and tocopherol profiles (Demurin et al., 1996).

Molecular Studies. The molecular basis of a modified fatty acid content in the seed oil of sunflower has been studied through a QTL and a candidate gene approach. A number of sunflower genes, coding for enzymes involved in the fatty acid biosynthetic pathway in seeds, have been cloned and their polymorphism studied in cultivated sunflower. The pathway of fatty acid synthesis (reviewed in Harwood, 1996; Somerville et al., 2000) in oilseeds starts with malonyl-CoA, the product of acetyl-CoA carboxylase, from which short medium chain (C8-C14) fatty acids as well as palmitic acid are synthesized after repeated condensations of malonyl-acyl carrier protein (ACP), a process mediated by the fatty acid synthases III and I (FAS III and FAS I). Palmitoyl-ACP can be exported out of the plastid or elongated to stearoyl-ACP by the enzymatic complex fatty acid synthase II (FAS II). Stearoyl-ACP can be exported out of the plastid or desaturated to oleoyl-ACP by the stearoyl-acyl carrier protein (ACP) desaturase (SAD). In sunflower, oleoyl-ACP is the major final product of de novo fatty acid biosynthesis in the plastid. Export of acyl-ACPs out of the plastid requires the hydrolysis mediated by acyl-ACP thioesterases, which separate the fatty acid from the ACP. Free fatty acids move through the plastid membrane and are converted to CoA thioesters by acyl-CoA synthase. The 18:1-CoA may be incorporated into membrane phospholipids, where it is desaturated to linoleate by the action of the oleoyl phosphatidyl-choline desaturase (OLD).

Hongtrakul et al. (1998a) reported the isolation of two stearoyl-acyl carrier protein (ACP) desaturase genes (SAD17 and SAD6) in sunflower that were highly expressed in seeds. Sunflower SAD genes have also been isolated by Serrano-Vega et al. (2003) and Kabbaj et al. (1996a, b). The genes were homologous either to SAD17 (Serrano-Vega et al., 2003) or SAD6 (Lacombe and Bervillé, 2001). Candidate gene and QTL analysis revealed the co-location of a major QTL affecting stearic acid content in the high stearic acid mutant CAS-3 (genotype *es1es1es2es2*) with a SAD gene. The SAD17A locus was found to co-segregate with *Es1* (Pérez-Vich et al., 2002c). Using RFLP-AFLP linkage maps constructed from two different mapping populations derived from CAS-3, the SAD17A locus was mapped to linkage group (LG) 1 of the public sunflower genetic map (Tang et al., 2002), and it was found to underlie the major QTL affecting the concentration of stearic acid. This QTL explained about 80% of the phenotypic variance of this fatty acid (Pérez-Vich et al., 2002c) and it was named *st1-SAD17A*. Other minor QTLs affecting stearic acid content, which mapped to LG3 (*st2.1*), LG7, and LG13

(*st2.3*), were detected in that study, although none of them was consistent enough to be considered as a strong candidate for the *Es2* locus (Pérez-Vich et al., 2002c).

Since the highly significant effect of the macromutation *Es1* effectively reduced the power of the QTL analyses to identify QTLs with smaller effects on stearic acid levels, another mapping population in which stearic acid segregated independently of *Es1* was developed from the CAS-20 line (genotype *Es1Es1es2es2*) (Pérez-Vich et al., 2004b). An RFLP-SSR genetic linkage map from this population allowed the identification of three QTLs affecting stearic acid, located on LG3 (*st2.1*), LG11 (*st2.2*), and LG13 (*st2.3*). In total, the QTLs explained 43.6% of the phenotypic variation (Pérez-Vich et al., 2004b). Additionally, four candidate genes (two stearate desaturase genes, SAD6 and SAD17, as well as a FatA and a FatB thioesterase gene) were placed on the QTL map. On the basis of positional information, the QTL on LG11 was suggested to be a SAD6 locus.

Marker studies related to high oleic acid content in sunflower began with the identification of two RAPD markers linked to the *Ol1* gene (Dehmer and Friedt, 1998). Later studies demonstrated that the *Ol1* gene cosegregates with an OLD gene (*FAD2-1*) that is strongly expressed in normal-type (low oleic) and weakly expressed in mutant (high oleic) lines (Hongtrakul et al., 1998b; Lacombe and Bervillé, 2001; Martínez-Rivas et al., 2001). The *Ol1-FAD2-1* locus mapped to LG14 (Pérez-Vich et al., 2002c) of the public sunflower genetic map, and was found to underlie a major oleic acid QTL explaining 56% of the phenotypic variance (Pérez-Vich et al., 2002c).

Several studies have also been conducted to map modifying genes affecting oleic acid content. Pérez-Vich et al. (2002c) described the existence of a minor QTL on LG8 which showed an epistatic interaction with the major QTL for oleic acid at the *FAD2-1* locus on LG14. Lacombe et al. (2001, 2002) identified a locus which suppressed the effect of the *FAD2-1* locus, probably through a mechanism of "gene silencing."

Environmental Effects. In general, the phenotypic expression of seed oil quality traits in sunflower, governed by a reduced number of genes, is less affected by the environment than in the case of quantitative traits such as oil content. Temperature during seed formation is the main factor affecting oil quality traits in oil crops (Canvin, 1965). In sunflower, lower temperatures cause an increase in the unsaturation level, affecting significantly the oleic to linoleic acid ratio (Harris et al., 1978). However, not all the genotypes are identically affected by the temperature, showing a strong genotype x environment interaction. For example, the oleic to linoleic acid ratio is much more stable across environments in mutants with very high oleic acid content than in standard types (Fernández-Martínez et al., 1986; Garcés et al., 1989), high linoleic acid lines (Fernández-Martínez et al., 1986; Simpson et al., 1989), or some wild species (De Haro and Fernández-Martínez, 1991; Seiler, 1992). A genotype x environment interaction was also reported for saturated fatty acid contents after evaluating high palmitic and high stearic acid mutants under contrasting temperature conditions (Martínez-Force et al., 1998). Both mid stearic and high palmitic acid mutants were more affected by temperature conditions during seed development than the high stearic acid mutant CAS-3, which was revealed to be relatively stable. Conversely, the stearic acid levels of the very high stearic mutant CAS-14 were found to be highly dependent on temperature during seed development (Fernández-Moya et al., 2002).

Conclusions

Important research efforts have been carried out during the last thirty years to improve oil quality in sunflower. Most of such efforts concentrated on the modification of the fatty acid composition of the seed oil, but more recently other aspects such as tocopherol content and composition as well as synergistic effects among different traits are being considered. A tremendous range of variation for the relative concentration of all

the individual fatty acids and tocopherols present in sunflower seeds has been obtained through mutagenesis or selection from naturally occurring variation. This progress was made possible by advances in analytical techniques which allow very rapid, cheap, reliable, and nondestructive analyses of fatty acid and tocopherols at a single-seed level. Nowadays, a wide range of sunflower lines with contrasting fatty acid profiles have been developed, for example with high and low levels of saturated fatty acids, mid and high levels of oleic acid, and high concentration of linoleic acid, as well as different ranges of intermediate levels and combinations among them. Similarly, lines with high levels of beta-, gamma-, and delta-tocopherol have been developed. With few exceptions, the modified traits in these variants are relatively stable, governed by a reduced number of major genes, and controlled by the genotype of the developing embryo. Accordingly, the modified fatty acid and/or tocopherol profiles can be easily managed in plant breeding programs. Moreover, progress has been made in the last few years in the development of molecular markers for some of the modified oil quality traits, which will contribute to improving breeding efficiency, especially for those traits controlled by recessive genes or those more affected by the environment. These profound changes in sunflower oil quality, with the possibility of combining several quality traits in a single phenotype to tailor specialty oils, provide essentially “new crops” for specific market niches both in the food and nonfood industry.

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