Breeding for oil quality in European oil crops:

I. Introduction

II. Some background information

III. Case study

IV. Conclusion

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Breeding for oil quality in European oil crops:

I. Introduction:

- Which crops ? Sunflower, Oilseed rape, Soybean (not really European crop…)

- Oil quality: a challenge for the breeders in terms of breeding targets

• Increasing diversity of end uses, leading sometimes to contradictory expectations.
• Relative uncertainty of nutritional recommendations.

But despite demonstrated effects of climate and agronomic pratices, an overall good heritability for the traits involved in oil quality and quantity.

- Oil quality: What are we talking about ?

• fatty acid composition
• other components (tocopherols / Vitamine E, phytosterols, …)

Breeding for oil quality in European oil crops:

II. Background:

➢ Fatty acid profiles: Basic considerations (*):

Fatty Acid	Palmitic *(C16:0)*	Stearic *(C18:0)*	Oleic *(C18:1) ("Omega 9")*	Linoleic *(C18:2) ("Omega 6")*	Linolenic *(C18:3) ("Omega 3")*	
Quality for Health						(1)
Quality for Food Industry						
Standard CROP *(% of the Oil)*						
Soybean	11	4	23	54	8	(2)
Rapeseed *(0 erucic)*	4	2	61	20	11	
Sunflower	7	3	30	60	0	(3)

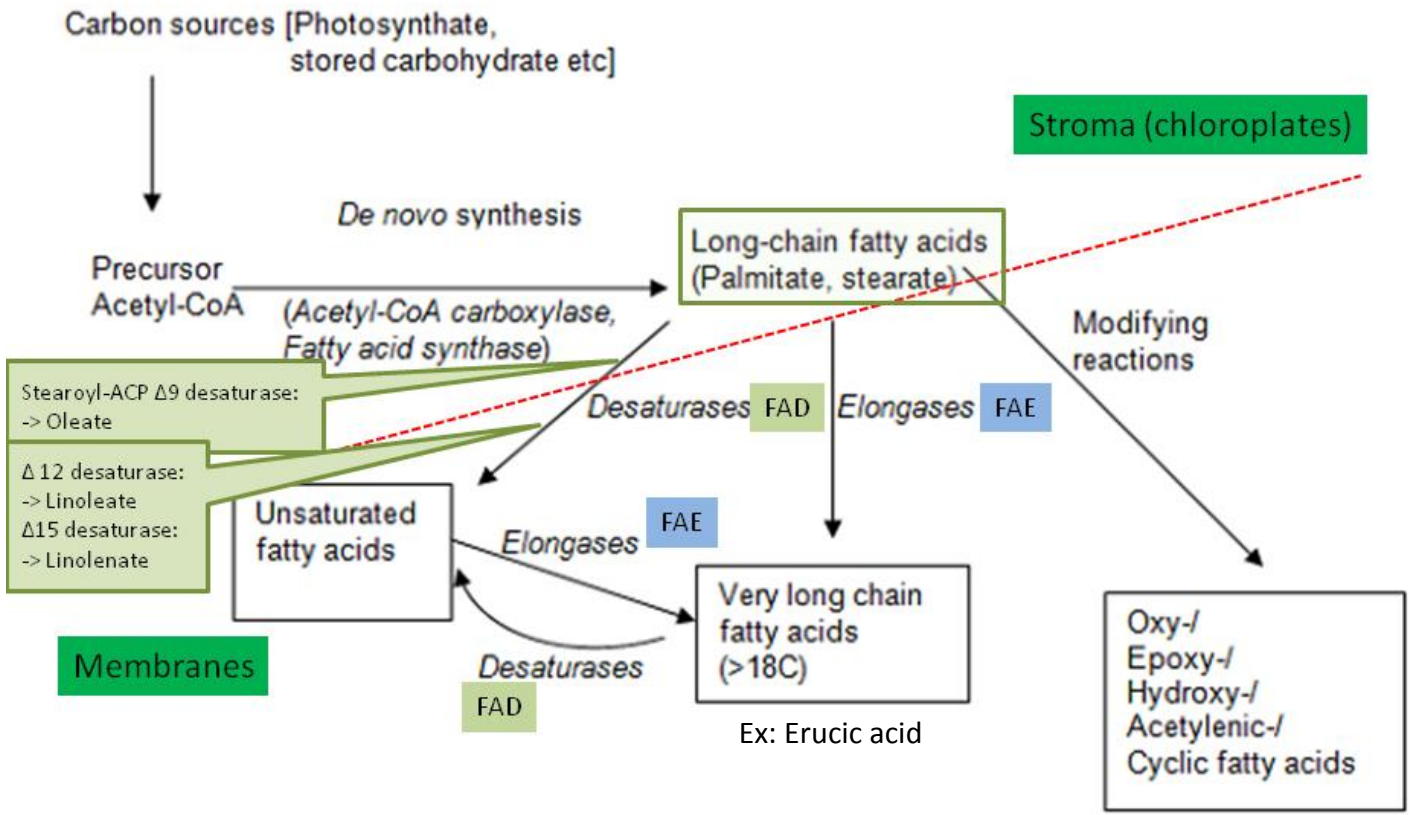
(1): to be discussed !
(2): Soybean is the most cultivated oilcrop over the world, but does not present today the better profile.
(3): No genetic variability within the Helianthus genus to get some Omega 3

(*) Average profiles of standard crops from Baldini et al., 2014, CAB Reviews 9 (021):1-16

Breeding for oil quality in European oil crops:

II. Background:

- Fatty acid biosynthesis: A short (and summarized) reminder



Derived from http://lipidlibrary.aocs.org/plantbio/fa_biosynth/index.htm

Breeding for oil quality in European oil crops:

II. Background:

➢ For the 3 considered oilcrops, mutagenesis provided mutants which, when transferred by classical breeding in agronomically adapted germplasm, offered the possibility to diversify the production without demonstrated negative (*) impact :

Fatty Acid	High Palmitic *(C16:0)*	High Stearic *(C18:0)*	High Oleic *(C18:1)*	Low Linolenic *(C18:3)*
Quality for Health				
Quality for Food Industry				
Existing mutants for the CROP *(% of the Oil)*				
Soybean	20-40	19-28	77-86	1-2
Rapeseed *(0 erucic)*	-	-	71-80	2-3
Sunflower	25-28	26-37	90	-

Source: from Baldini et al., 2014, CAB Reviews 9 (021):1-16 . Each range of values represents the maximum percentage of fatty acid obtained across the different mutation events.

(*) Effect of the sunflower HO/HS genotype on germination ? Belo et al., 2014

Breeding for oil quality in European oil crops:

III. Case study (historical case!): The fatty acid profile of oilseed rape had a tremendous impact on the development of this crop: the « 0-erucic » target :

- From studies on laboratory animals (1970's), some negative effects (heart diseases) of the erucic acid content (C22:1) were expected from human comsumption => « 0-erucic » varieties.
- Involvement of FAE1 (ex: Sun et al., 2013)
- However, high number of industrial uses. With a « niche » market for each ?
- Toward very high content (85-90%)?

Breeding for oil quality in European oil crops:

III. Case study : Reducing the ALA (18:3) content of oilseed rape through mutagenesis in *B.oleracea*

- A typical case where there is an antagonism between requirements for health and for food industry (?)
- HOLLi (High oleic / Low alpha-linolenic) varieties are already available (~ 3% ALA instead of ~ 11%)
- Oilseed rape = *B.napus* (AACC) = 2 genomes (*B.rapa*: AA, *B.oleracea*: CC)
- EMS mutagenesis in *B.oleracea* to obtain a new mutant in one of the FAD3 gene.
- Impact on *B.napus* fatty acid profile through « resynthesis » ?

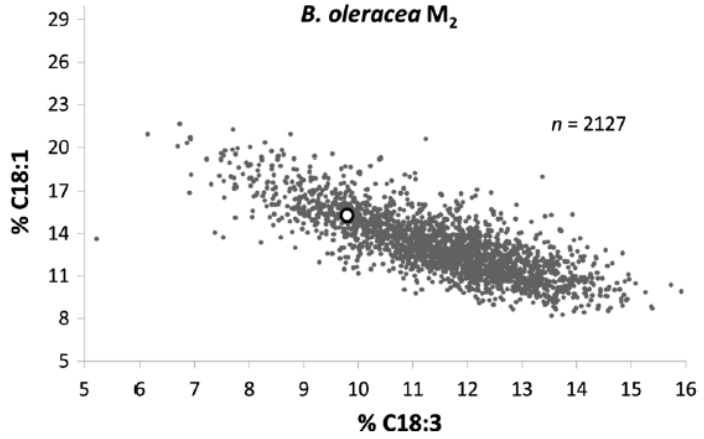
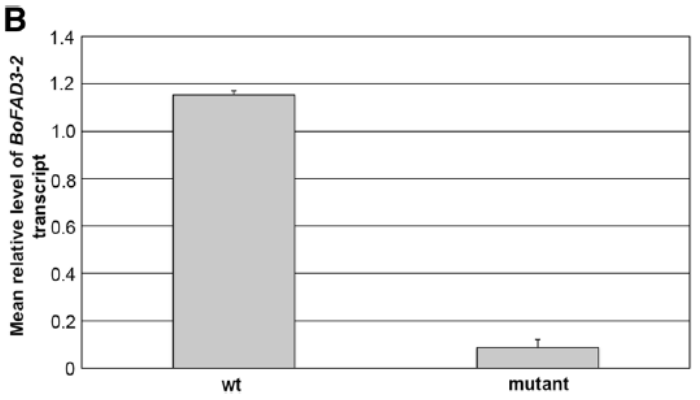


Figure 1 Scatter diagram of percent α-linolenic acid (C18:3) plotted against oleic acid (C18:1) in the seed oil of an M_2 population of *Brassica oleracea* var. *alboglabra* generated from seed mutagenized with 0.5% ethyl methanesulphonate (EMS). Open circle = *wt B. oleracea* control.



Source: Singer et al., 2014
See also Rhaman et al., 2013

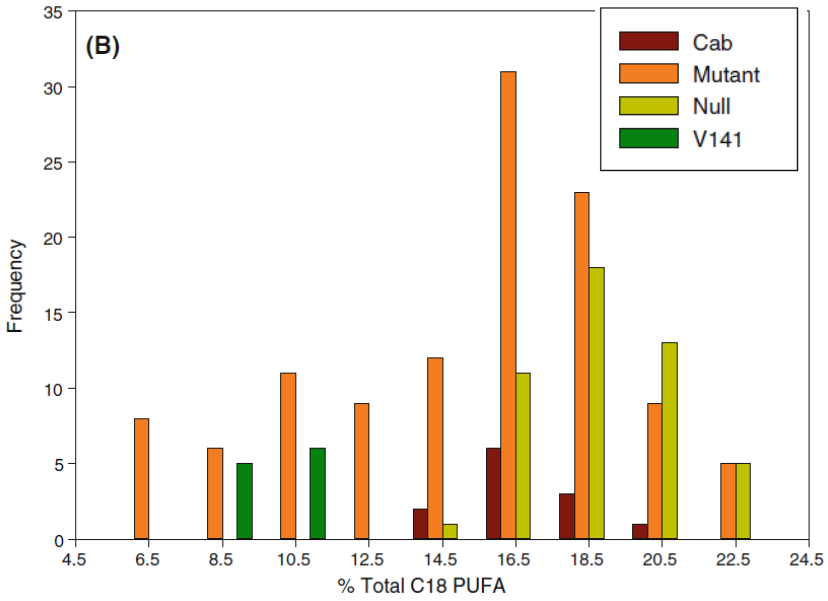
Breeding for oil quality in European oil crops:

III. Case study : In the context of Next-Generation Sequencing technologies, mutagenesis remains a powerful tool to create and identify mutants of interest: an example in oilseed rape:

- EMS mutagenesis on the variety CABRIOLET (75% oleic, 16% polyunsaturated fatty acids /PUFA) , which was hypothesized to have only one (out of four) FAD2 functional gene.
- Mutations in the BnaC.FAD2.a gene were obtained, allowing a further decrease of PUFAs.

MGAGGRMQVSPPSKKSETDTIKRVPCETPPFTVGELKKAIPPH
CFKRSIPRSFSYLIWDIIIASCFYYVATTYFPLLPHPLSYFAW
PLYWACQGCVLTGVWVIAHECGHHAFSDYQWLDDTVGLIFHSF
LLVPYFSWKYSHRRHHSNTGSLERDEVFVPKKKSDIKWYGLYL
NNPLGRTVMLTVQFTLGWPLYLAFNVSGRPYDGGFACHFHPNA
PIYNDRERLQIYISDAGILAVCYGLFRYAAAQGVASMVCFYGV
PLLIVNGFLVLITYLQHTHPSLPHYDSSEWDWLRGALATVDRD
YGILNKVFHNITDTHVAHHLFSTMPHYHAMEATKAIKPILGEY
YQFDGTPVVKAMWREAKECIYVEPDRQGEKKGVFWYNNKL*

Fig. 2 Phenotypic effects of altering amino acids in the desaturase encoded at *BnaC.FAD2.a*. *Red* mutation results in polyunsaturated fatty acid content below 7 % and oleic acid content over 80 %. *Blue* mutation results in fatty acid composition similar to wild type. *Orange* mutation results in intermediate fatty acid composition. The amino acid position mutated to a stop codon in line M0643 is *boxed*



Source: Wells et al., 2014

Breeding for oil quality in European oil crops:

III. Case study :HO sunflowers:

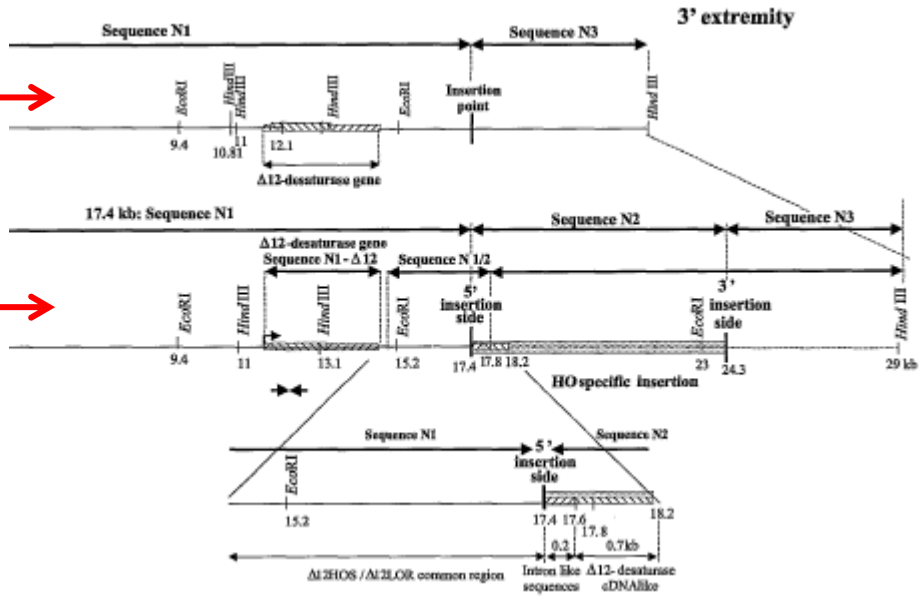
➢ The « Pervenets » HO mutation (Soldatov, 1976, Hongtrakul et al., 1998, Lacombe et al., 2001, 2009):

• Genetic markers have been proposed to help the breeders in transferring the « Pervenets » mutation event into the non-HO, classical germplasm, by classical breeding method (« backcross »).

• Due to the presence of « modifiers », such a transfer did not results systematically in very high and stable HO content, depending on the « receiving » germplasm. The genetic of these « modifiers » is not clearly elucidated today.

LO →

HO →



The « Pervenets » mutation event results from an insertion within a Δ12 desaturase.
Berville et al., 2005, Patent WO2005106022 (A2)

Breeding for oil quality in European oil crops:

III. Case study : HO/HS sunflowers:

➢ A strong research investment (15 years) of CSIC (Spain, Osorio et al., 1995, Perez-Vich et al., 1999) together with UGA (USA) and Advanta (Perez-Vich et al., 2002, 2004) provided information on several fatty acid mutants in sunflowers. This investment resulted in the HS/HO concept.

Table 1. Mutant lines and mutagenic treatments used to obtain lines.

Mutant line	Phenotype†	Original line	Mutagenic treatment
CAS-3	Very high 18:0	RDF-1-532	EMS‡; 70 m*M*
CAS-4	High 18:0	RDF-1-532	Sodium azide; 2 m*M*
CAS-5	Very high 16:0 and high 16:1	BSD-2-691	X-rays; 160 Gy
CAS-8	High 18:0	RDF-1-532	Sodium azide; 2 m*M*

† 16:0 = palmitic acid; 16:1 = palmitoleic acid; 18:0 = stearic acid.
‡ EMS = ethyl methanesulfonate.

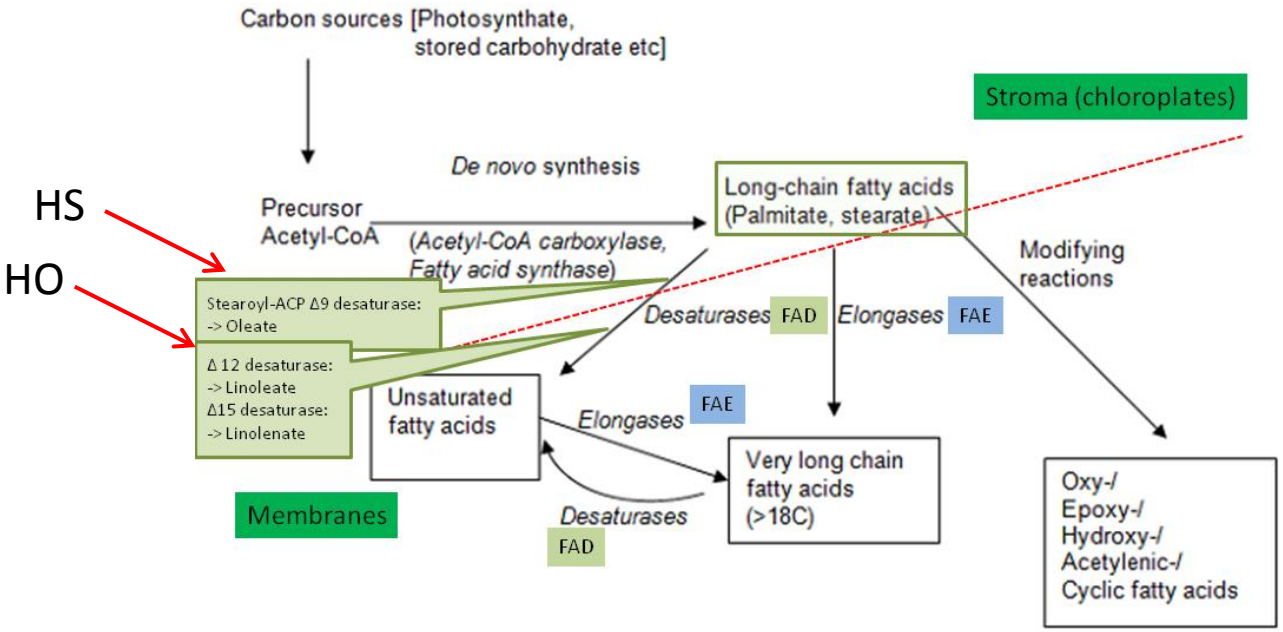
Source: Osorio et al., 1995, Crop Sci . 35 (3):739-742

Breeding for oil quality in European oil crops:

III. Case study : HO/HS sunflowers:

➢ Molecular aspects of the HO/HS concept (Perez-Vich et al., 2002):

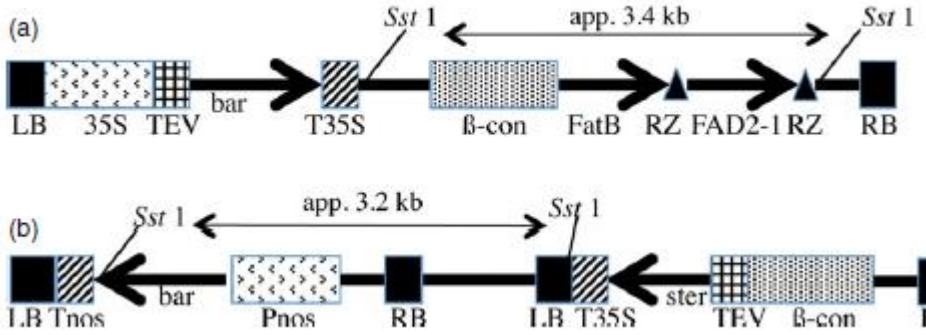
• The HS genotype is provided by the mutant CAS3 and cosegregate with a stearoyl-ACP desaturase locus, mapped on LG1.
• The HO genotype is provided by the « Pervenets » mutant and cosegregate with oleoyl-PC desaturase locus , mapped on LG14 (cf. ? Lacombe et al., 2001, Berville et al., 2005, Lacombe et al., 2009)



See Patents EP1185160 and US2004088758

Breeding for oil quality in European oil crops:

III. Case study : Stacking two GMO events to obtain HS/HO soybeans :



Event 1: « 335-13 »: Silencing of FatB and FAD2-1

Event 2: « 680-2 »: Stearoyl-ACP thioesterase from mangosteen (*Garcinia mangostana*)

Table 4. Fatty acid profile of soybean events under field conditions in 2009

Event/Stack	Palmitic%	Stearic%	Oleic%	Linoleic%	Linolenic%
WT (Thorne)	11.3 ± 0.5	3.0 ± 1.9	15.4 ± 1.7	51.0 ± 1.7	14.3 ± 1.2
680-2	8.4 ± 0.6	10.2 ± 1.4	15.1 ± 1.8	48.0 ± 1.5	15.2 ± 1.6
335-13 × 680-2	4.1 ± 0.9	12.7 ± 2.2	67.3 ± 5.4	4.1 ± 2.6	8.0 ± 1.9

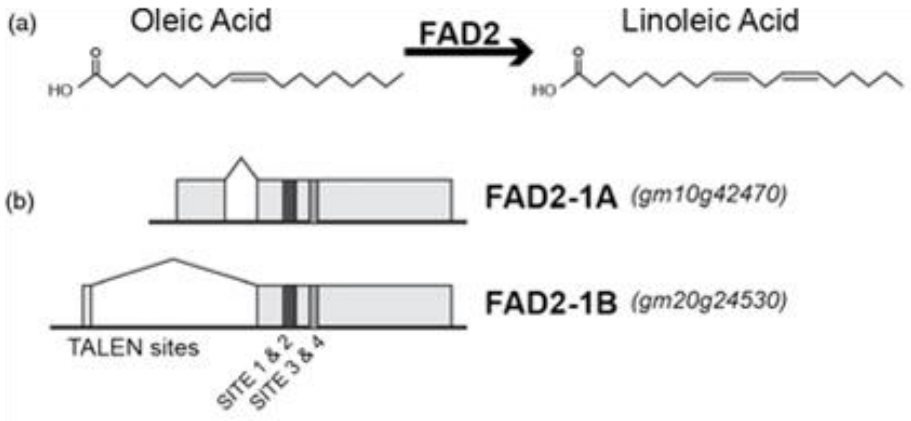
Event/Stack column refers to control seed (genotype Thorne), event 680-2 and stack line with high oleic acid event 335-13. Numbers within the columns are mean percentages of corresponding fatty acid ± standard deviations based on 50–90 samples.

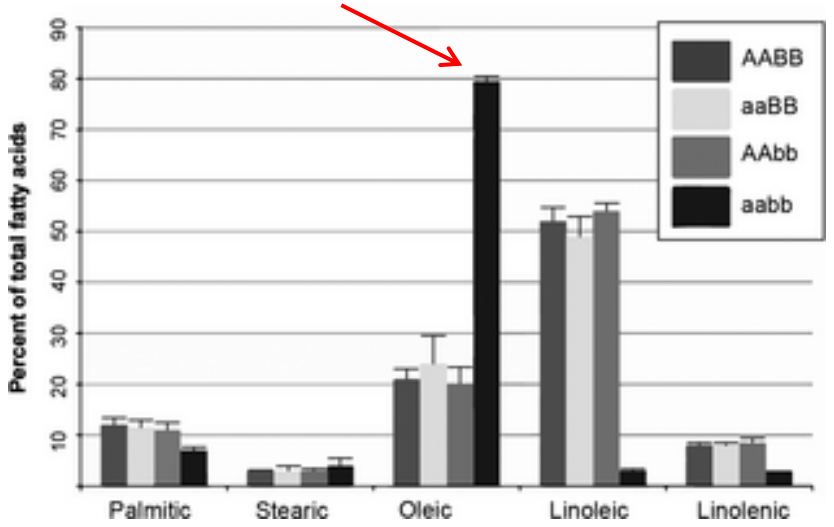
Source: Park et al., 2014

Breeding for oil quality in European oil crops:

III. Case study : HO soybeans: Genome Editing to provide GMO solutions ?

- Transcription activator-like effector nucleases (TALENs) were used to target FAD2 genes in soybean, resulting in strong modifications of oleic acid content (Haun et al., 2014).
- Selected resulting events are TALEN free.





Source: Haun et al., 2014 (CELLECTIS Co.)

Breeding for oil quality in European oil crops:

III. Case study : Resequencing large collection of genetic resources to discover new (*non GMO*) alleles: an example in soybean

- 302 accessions from a world collection, including wild soybeans and landraces.
- Genome Wide Association (GWA) studies for several traits, including fatty acid profiles.

Supplementary Table 14 Fatty acid synthesis genes located in the selection sweeps and oil QTLs

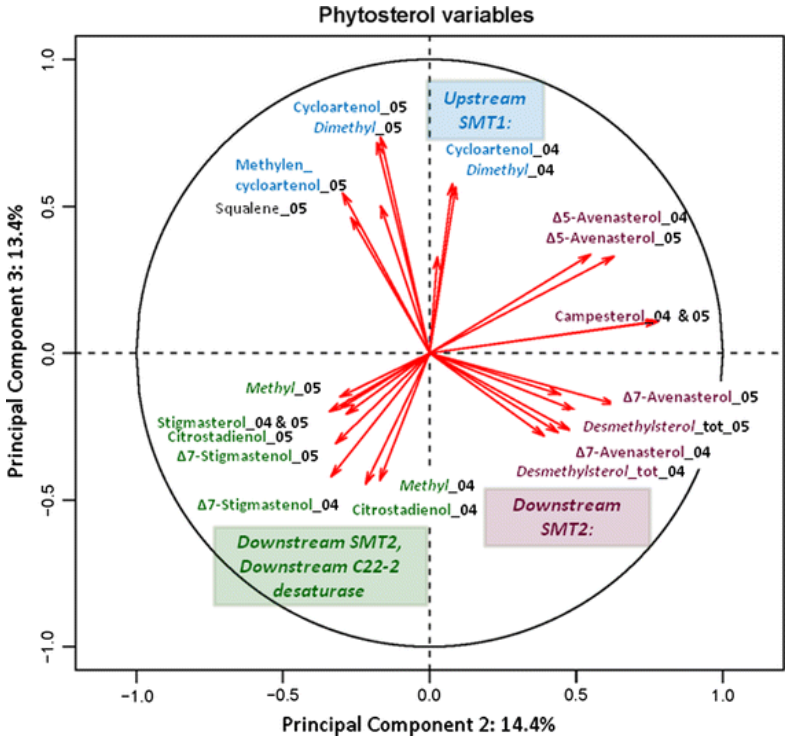
Oil synthesis pathway genes		
Gene	**Position**	**Annotation**
Glyma08g01180	Gm08:687,470-689,239	*ACP4* , acyl carrier protein 4
Glyma08g45990	Gm08:45,223,982-45,229,809	*MOD1* , NAD(P)-binding Rossmann-fold superfamily protein
Glyma10g42470	Gm10:49,416,645-49,418,380	*FAD2* , fatty acid desaturase 2 ←
Glyma13g31440	Gm13:33,848,800-33,850,851	*DES-1-LIKE* ,fatty acid desaturase family protein
Glyma14g27990	Gm14:34,322,879-34,325,575	Plant stearoyl-acyl-carrier-protein desaturase family protein ←
Glyma15g00550	Gm15:276,266-278,043	*KAS III* , 3-ketoacyl-acyl carrier protein synthase III
Glyma15g05800	Gm15:4,116,287-4,120,919	Thioesterase superfamily protein
Glyma15g07900	Gm15:5,561,199-5,563,177	*DES-1-LIKE* , fatty acid desaturase family protein
Glyma15g11230	Gm15:8,229,206-8,233,359	NAD(P)-binding Rossmann-fold superfamily protein
Glyma15g15310	Gm15:11,724,822-11,730,419	*LPD1* , lipoamide dehydrogenase 1
Glyma17g12940	Gm17:9,856,959-9,861,288	*FATB* , fatty acyl-ACP thioesterases B ←

Source: from Zhou et al., 2015

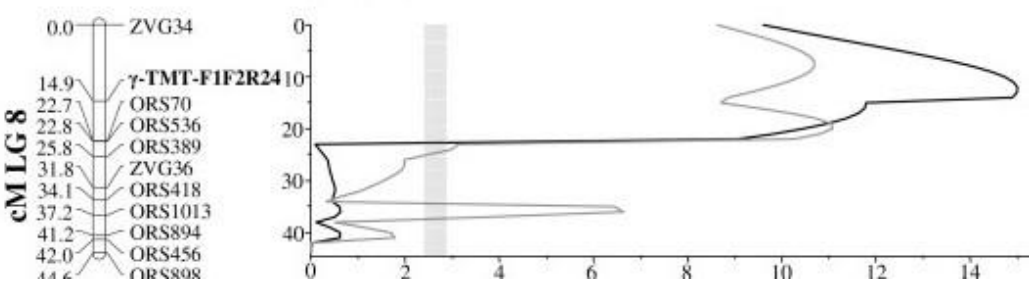
Breeding for oil quality in European oil crops:

III. Case study : Examples of other oil quality traits: Genetic variability for tocopherol (Vitamine E) and phytosterol content in sunflower:

- High throughput phenotyping tools allow to finely describe the metabolomic variation in segregation populations or association panels.
- Depending on the interest of the industry, it could be certainly possible to modify some "minor" traits of the oil quality.



Source: [Merah et al., 2012]



Main QTL of Gamma Tocopherol
Source: [Garcia-Moreno et al., 2012]

Breeding for oil quality in European oil crops:

IV. Conclusion:

- **Increasing knowledge on the genes involved in the fatty acid profile variation and on their polymorphism.**

- **Mutagenesis already provided a range of mutants which are used is cultivated varieties. The perspective of this methodology is still promising.**

- **New technologies for genome editing might allow to produce « cleaner » GMO (?) events.**

- **The play between the seed companies and the food or chemical industry remains complicated.**