Altered gene dosage in hexaploid *Camelina sativa*

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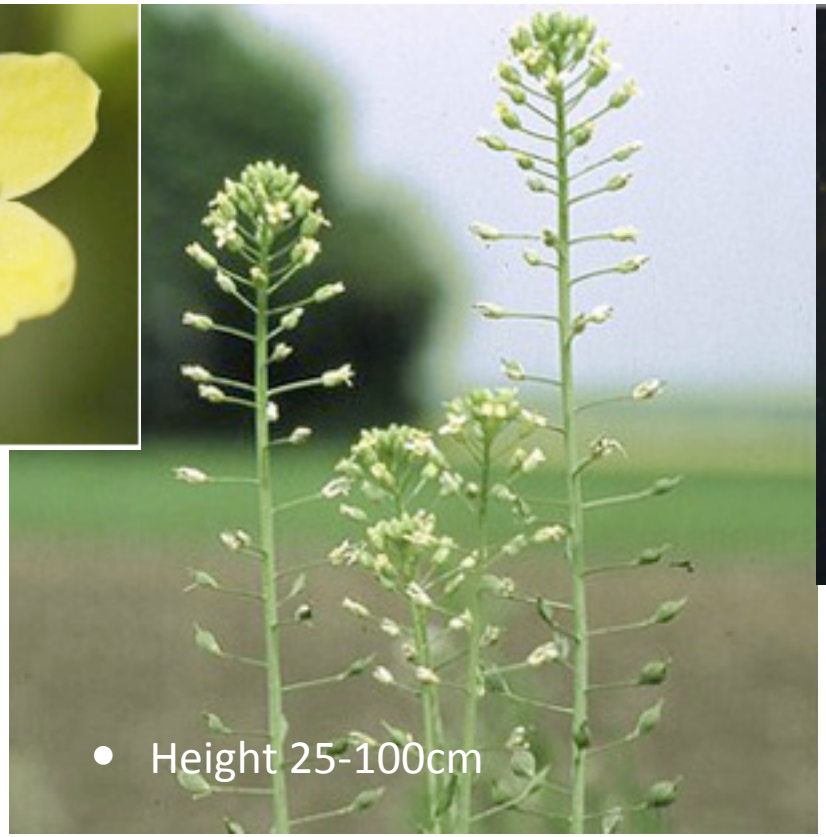
Camelina sativa

- Low input crop
- Winter and spring cultivars
- Cycle of 90-100 days
- Yield 1.25 – 2.5t/ha
- authorized for food (1999)



Russia => 268 000 ha (2014)
25% of spring rapeseed ha
(USDA 2015)

Oilseed profiles

Type	Formula	Name	Camelina (%)	Rapeseed (%)	Flax (%)	Sunflower (%)	Soybean (%)
saturated	C16:0	Palmitic	7,8	6,2	5,3	6	11
	C18:0	Stearic	3	0	3,1	4	4,5
	C20:0	Arachidic	0	0	0	0	
		Total:	10,8%	6,2%	8,4%	10%	15,5%
Mono-unsatured	C18:1	Oleic	16,8	61,3	16,2	16,5	22,75
	C20:1	Gadoleic	12	1,2	0	0	
	C22:1	Erucic	2,8	0	0,9	0	
		Total:	31,6%	61,3%	17,1%	16,5%	38,25%
Poly-unsatured	C18:2	Linoleic	23	21,6	14,7	72,4	54,5
	C18:3	α-linolenic	31,2	6,6	59,6	0	7
		Total:	54,2%	28,2%	74,3%	72,4%	61,5%
others	C20:1	Eicosenic	16,4	1,4	-	0	
	C20:2	Eicosadienoïc	2	0,1	0,1?	0.06	
	C20:3	Eicosatrienoic	1	-	-	-	
		others	2,1	-	-	-	
		Total:	21,5%				

ω3

Source: *Camelina*, D.T. Ehrensing & S.O.Guy, 2008

Camelina oil is already on market for high polyunsaturated fatty acids

Yves Saint Laurent Golden glosses are non–sticky, moisturizing, gleaming gold-infused glosses. The gloss base is filled with countless gold specks (24 carate gold -0.2%) that remain on the lips after the gloss wears off. The glosses are not scent -free; they smell fruity like mango but that can be unpleasant for some people. I found it quite strong the first time I wore it, but I got more accustomed to it after a few applications. Yves Saint Laurent Golden glosses are pretty moisturizing and it might be because of a few exotic plants' oils like CAMELINA SATIVA SEED OIL and NIGELLA SATIVA SEED OIL.

Future uses?

- Aquaculture feed with EPA/DHA enriched oil
- Bioplastics, new synthons for chemistry
- Biofuels
- …

biofuels

Spain

Iberia completes first commercial biofuel flight

Jueves, 06 de octubre de 2011

REM

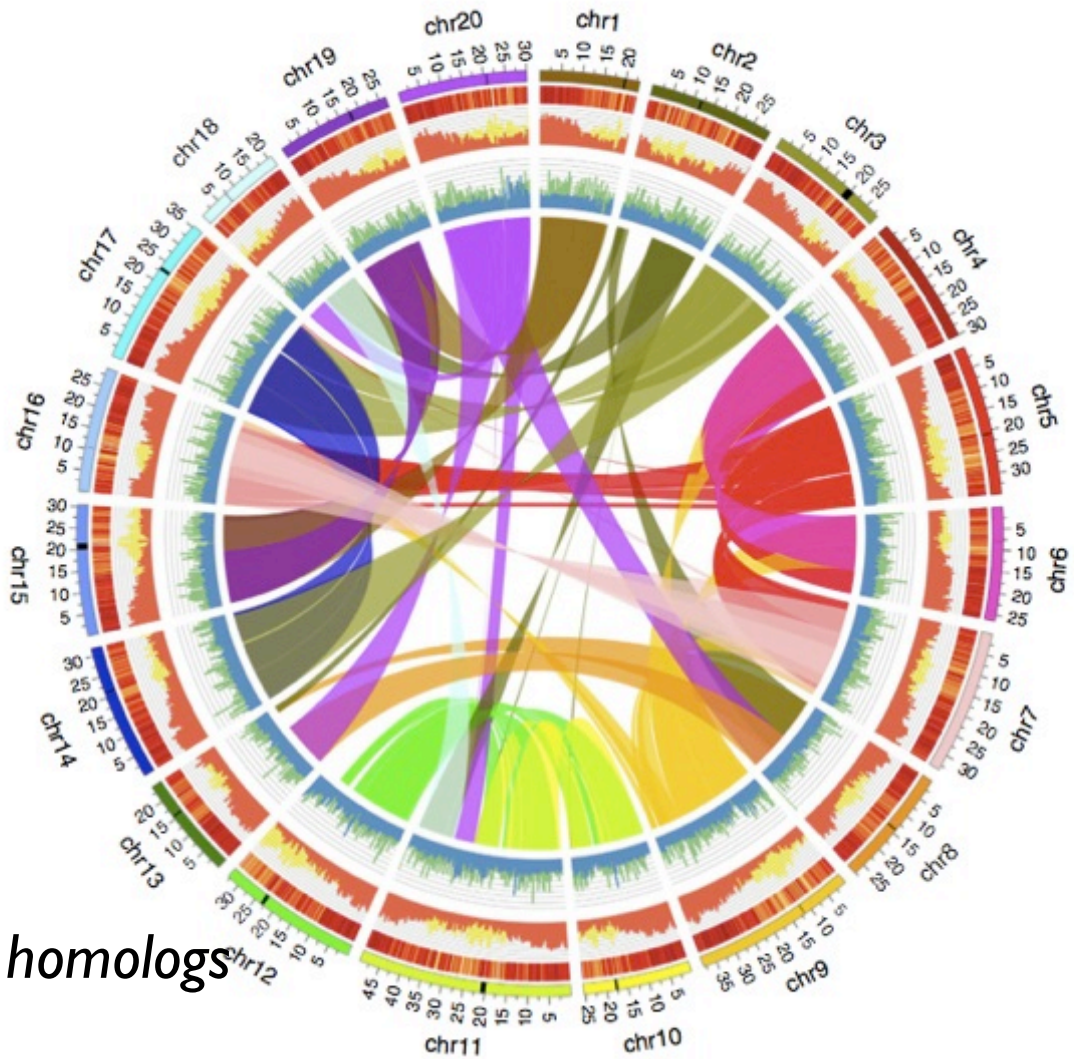
On Monday, Iberia and Repsol staged Spain's first commercial flight powered by biofuel. A conventional Iberia Airbus A320 flying from Madrid to Barcelona burned some 2,800 kg. of a mixture of 75% conventional A-1 jet fuel and 25% biofuel derived from the camelina sativa plant. According to Iberia, carbon dioxide emissions were cut by nearly 1,500 kg as a result of using this biofuel.

The second-generation biofuels is a mixture made by the Mexican government agency Aeropuertos y Servicios Auxiliares (ASA) of synthesized paraffin bio-kerosene produced by Honeywell-UOP and evaluated and certified by Repsol at its Technological Centre, which allows an almost 20% reduction in emissions of greenhouse gases.

Camelina sativa is an inedible oil plant that grows widely and can be cultivated in Spain. Unlike other plants used for biofuels, camelina enriches the soil in which it is grown.

Iberia provided the aircraft, crew, handling services, etc. for the test, and used the Peugeot electric ground vehicles it is now testing for airport use. Iberia also supplied the technical and maintenance services at Madrid Barajas airport. Together with the Airbus company and the engine manufacturers it closely monitored the technical and operational certification of this new biofuel. Repsol produced and delivered the fuel, which was evaluated under high-performance conditions and approved at its research facility. The company has long experience in the development of biofuels at its Technological Center, one of the most advanced of its kind in Europe.

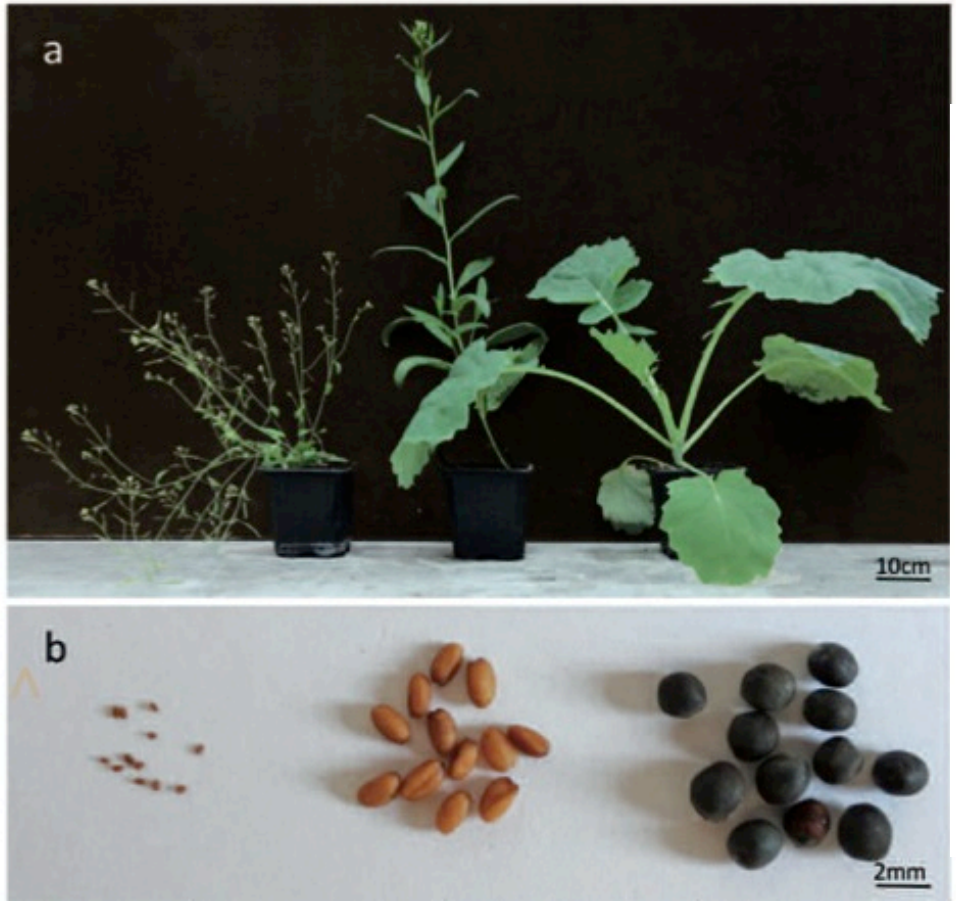
Camelina genome



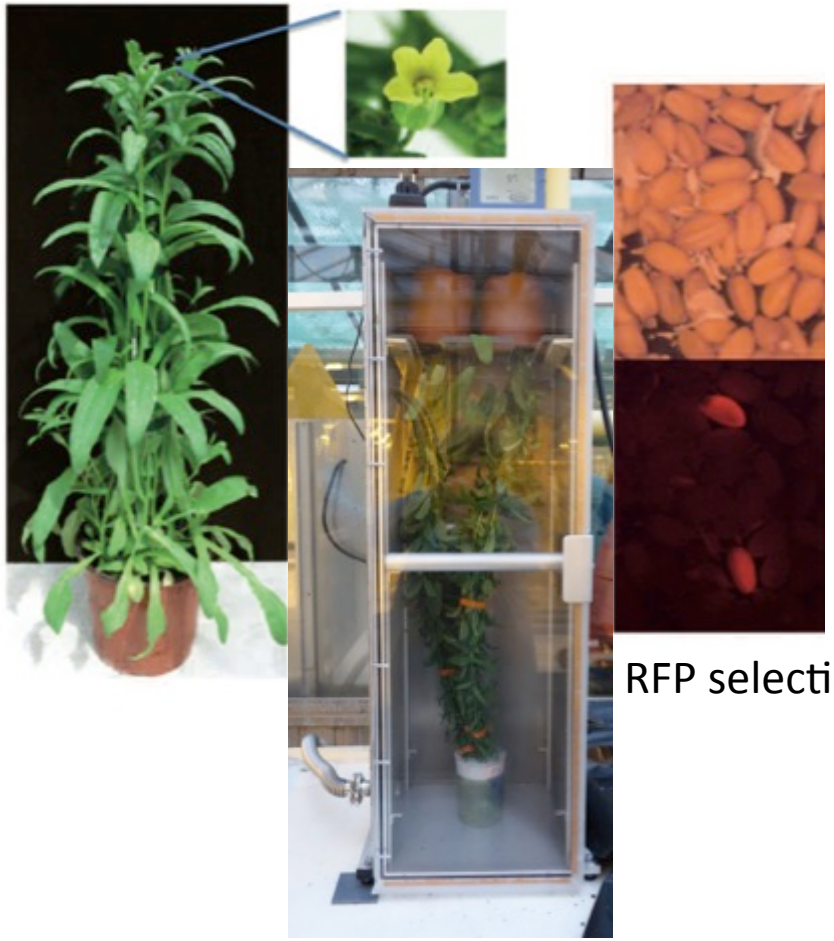
- 785 Mb
- *20 chromosomes*
- *Hexaploid*
- *88 418 genes*
- *3 very close subgenomes*
- *>97% genes have Arabidopsis homologs*
- *>90% identity Cs/At gene sequences*
- *>90% genes are expressed*

Kagale et al. Nat. Com 2014

Camelina sativa, an ideal chassis for lipid metabolic engineering



Flower dip transformation



RFP selection

Tepfer, Dubreucq and Faure, Biofutur 2016

Camelina ploidy a limitation for genetic engineering?

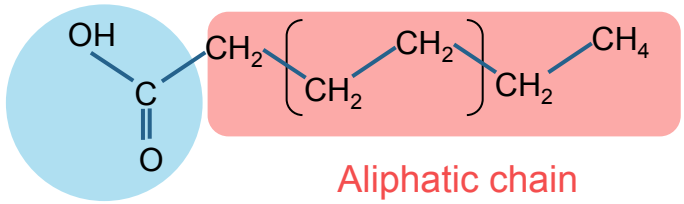
Selective mutagenesis in polyploid camelina?

→ How efficient is CRISPR/Cas9 editing in polyploid camelina? How the closely related camelina genomes will affect CRISPR editing?

→ Is selective gene dosage achievable? Does it impact the phenotype?

⇒ Target FAD2 desaturase to obtain oleic enriched camelina lines

Fatty acid desaturation

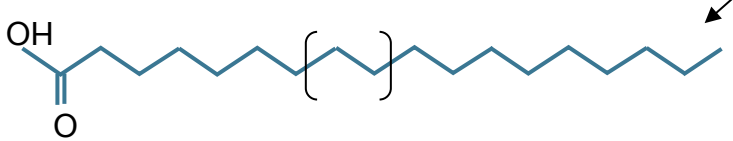


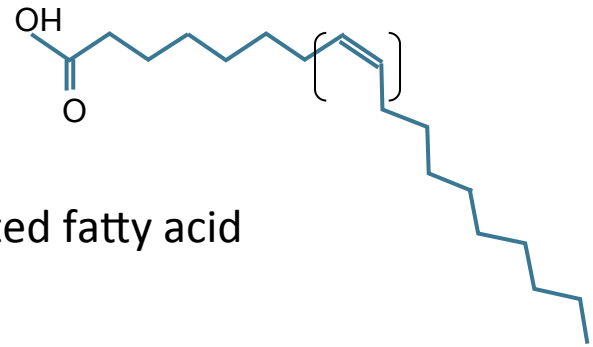
Aliphatic chain

Carboxylic acid

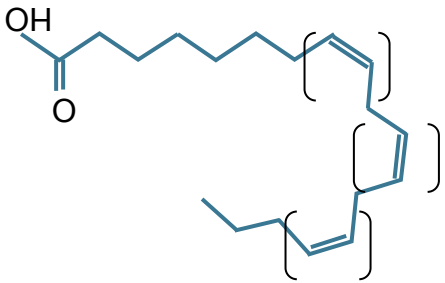
Acyl chain desaturation

saturated fatty acid



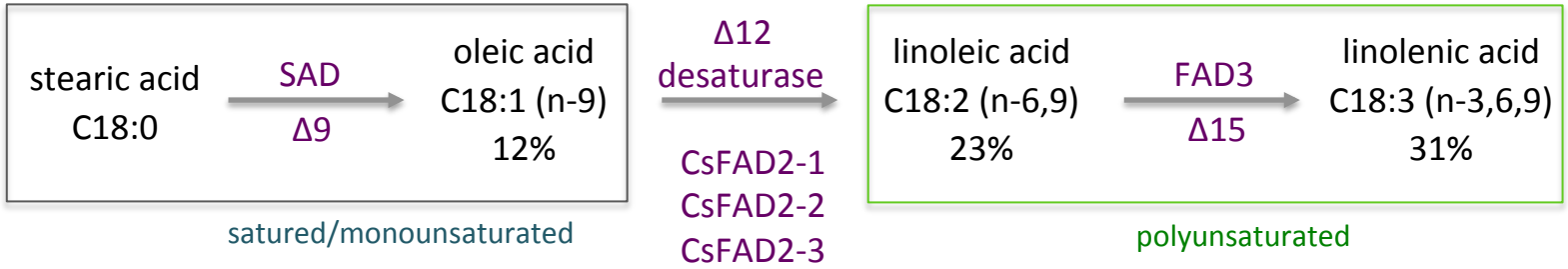


monounsaturated fatty acid



polyunsaturated fatty acid

Choosing the sgRNA



Design sgRNA with CRISPOR : http://tefor.net/crispor/crispor.cgi

Arabidopsis and camelina genomes available

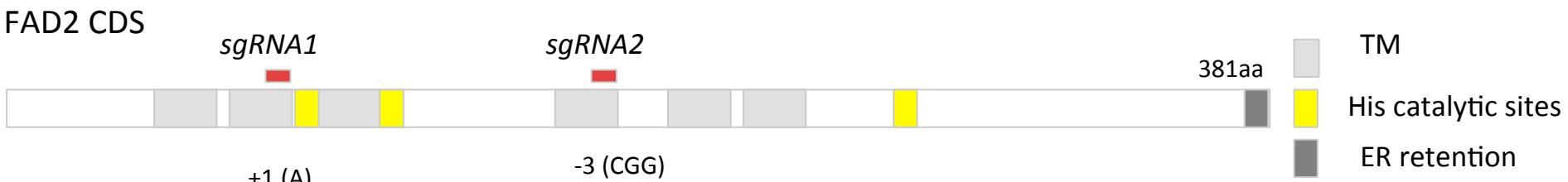
Position/ Strand	Guide Sequence + *PAM* Restriction Enzymes	Specificity Score	Efficacy Score	Prox. GC	Out-of-Frame Score	Off-targets for 0-1-2-3-4 mismatches + next to PAM	Genome Browser links to matches sorted by off-target score exons only Chr3 only
288 / rev	CTATGACCCAGATACCAGTT AGG PCR primers	100	24	-	59 %	0-0-0-0-1 0-0-0-0-0 1 off-targets	4:Chr4 3.30 Mbp
294 / fw	TGTCAAGGCTGTGTCCTAAC TGG PCR primers	100	15	-	70 %	0-0-0-0-0 0-0-0-0-0 0 off-targets	
301 / fw	GCTGTGTCCTAACTGGTATC TGG PCR primers	100	6	-	59 %	0-0-0-0-2 0-0-0-0-0 2 off-targets	4:Chr5 21.20 Mbp 4:Chr5 20.73 Mbp
505 / fw	AATCAGCAATCAAGTGGTAC GGG PCR primers	100	12	-	74 %	0-0-0-0-4 0-0-0-0-0	4:Chr3 23.10 Mbp 4:Chr5 17.32 Mbp

Generation-dependent mutagenesis rate

- sgRNA clones under Camelina U3 and U6 promoters in modified pDE-Cas9 vector (Nguyen et al., 2013)
- Selection of > 120 T1lines
- Genotyping T1 lines for the 3 homeologous genes
- Genotyping T2, T3, ... for each homeologous genes

Guide RNA	T1	T1 Mutation (%)	T2	T2 Mutation (%)	T3	T3 Mutation (%)
gRNA1	99	0	68	55,9 (38/68)	30	100
gRNA2	19	26 (5/19)	49	87,8 (43/49)	146	100

CRISPR/Cas9 editing induced a large array of mutations

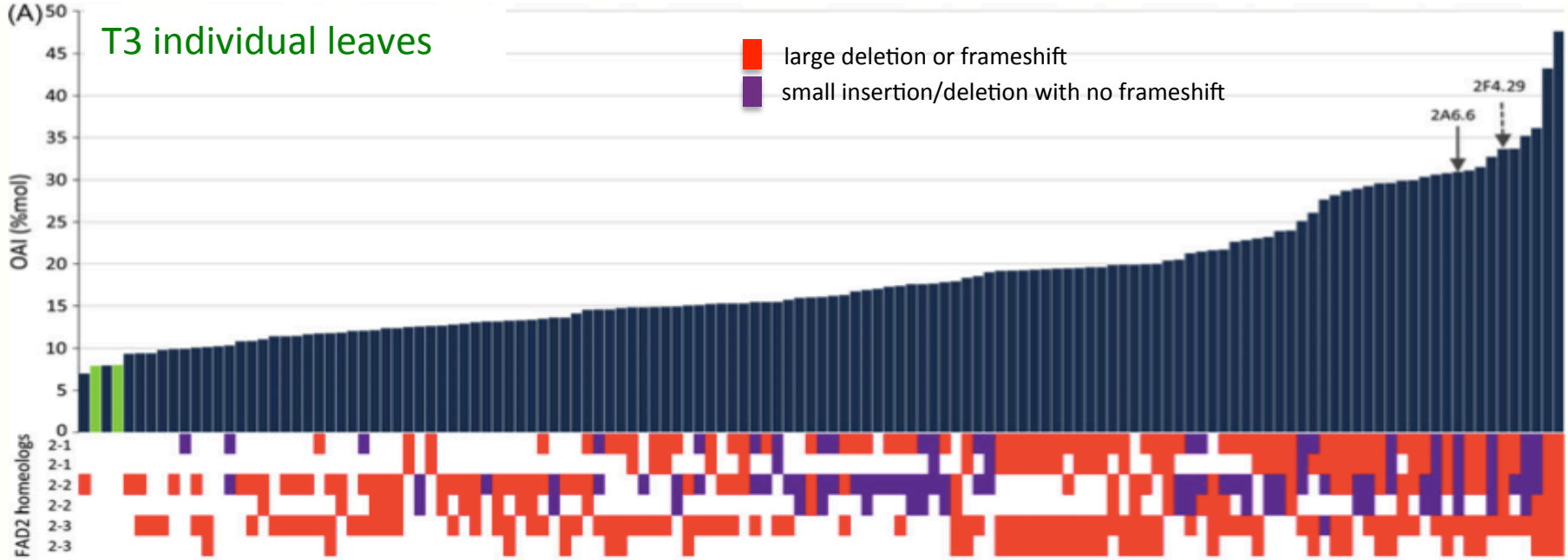


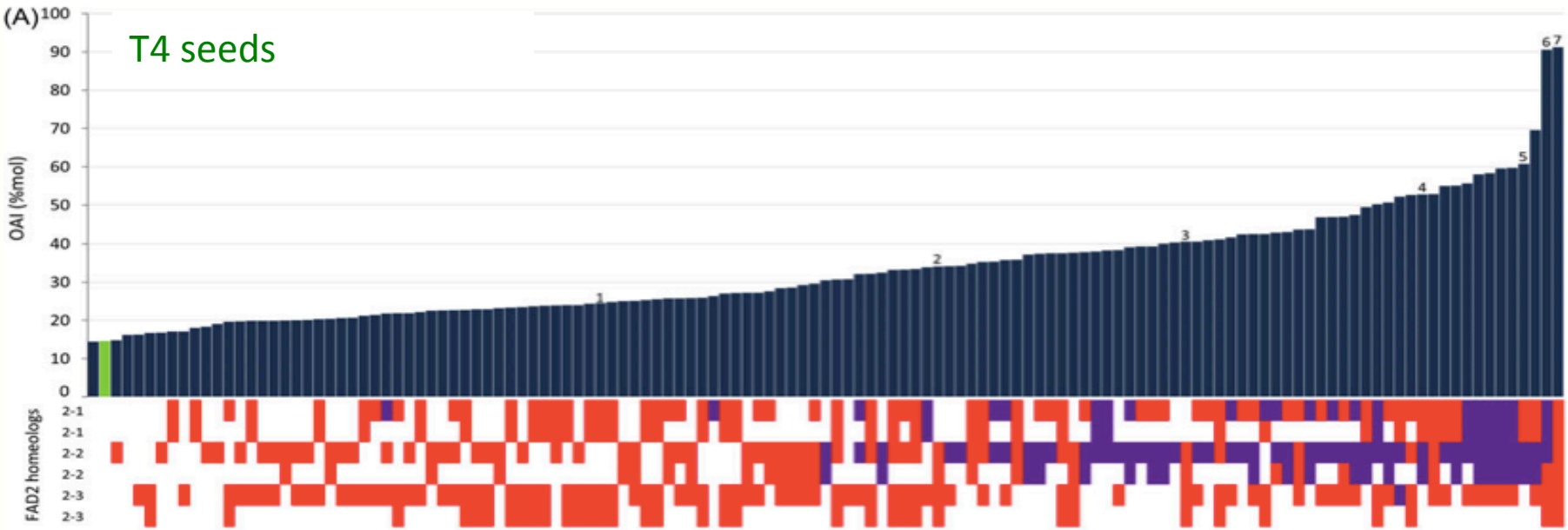
+1 (A)
+1 (A)
+1 (T)
-2 (CC)

-3 (CGG)
-3 (TGG)
-6 (GTGGCC)
-9 (GGGTGGCCC)
-18 (GTC…TTG)
-33 (CGG…CTC)
-3 (TGG),+77
-1 (G)
-1 (T)
-10 (TCCTCGGGTG)
-10 (TTGTCCTCGG)
-40 (ACG…TGG)
+1 (A)
+1 (G)
+1 (T)
-2 (GG)
-2 (GT)
-2 (TG)
-5 (CTCGG)
-5 (GTGGC)
-8 (CCTCGGGT)
-11(CGGGTGGCCCT)
-17 (TGT…CCT)
-4 (GTGG),+78

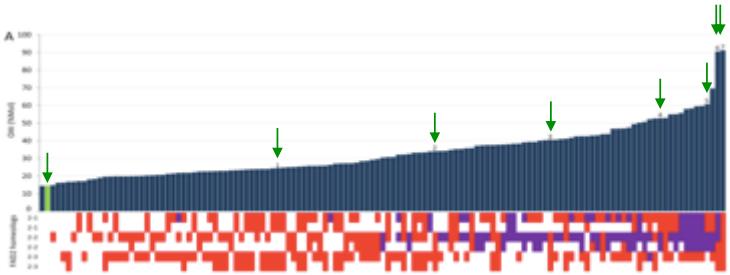
Combinatorial mutations at FAD2 loci

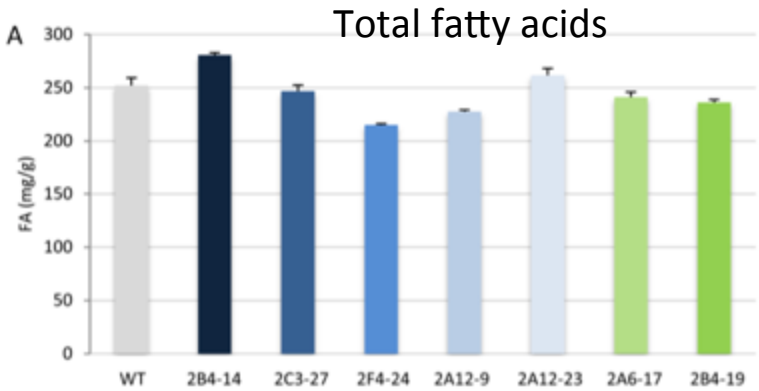
A collection of 133 T3 lines (sgRNA2)

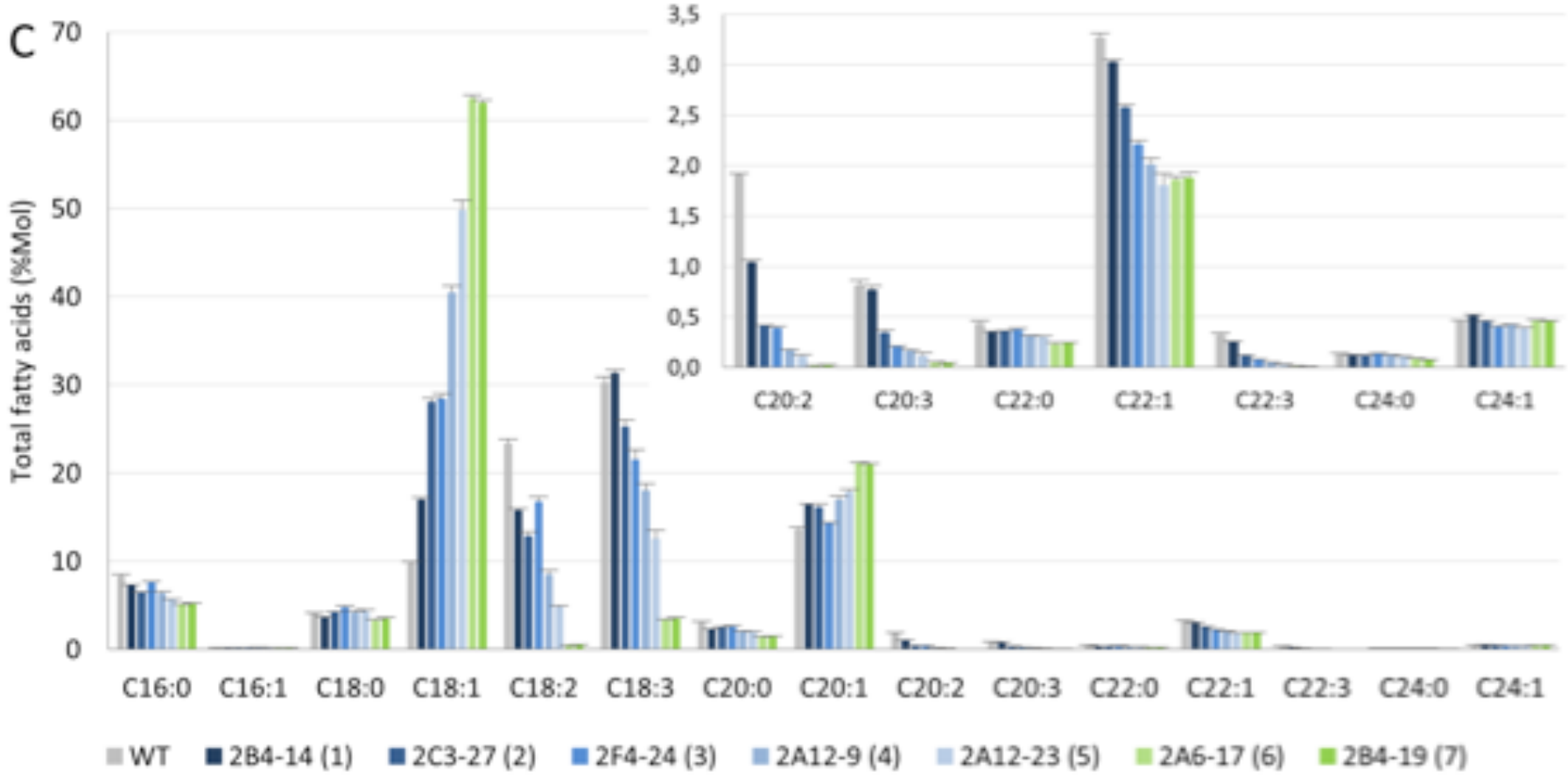




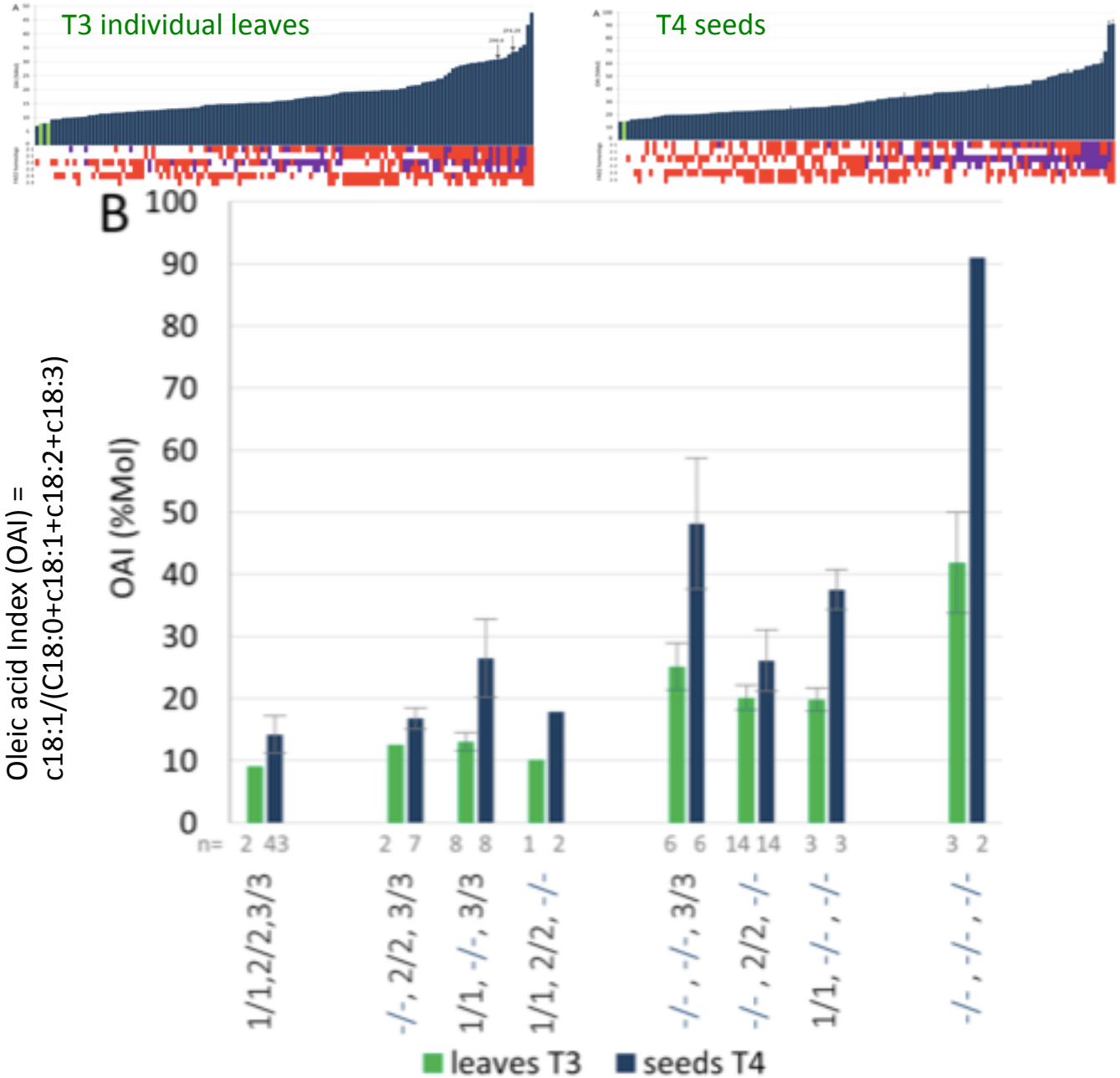
Combinatorial mutations define oleic acid variability



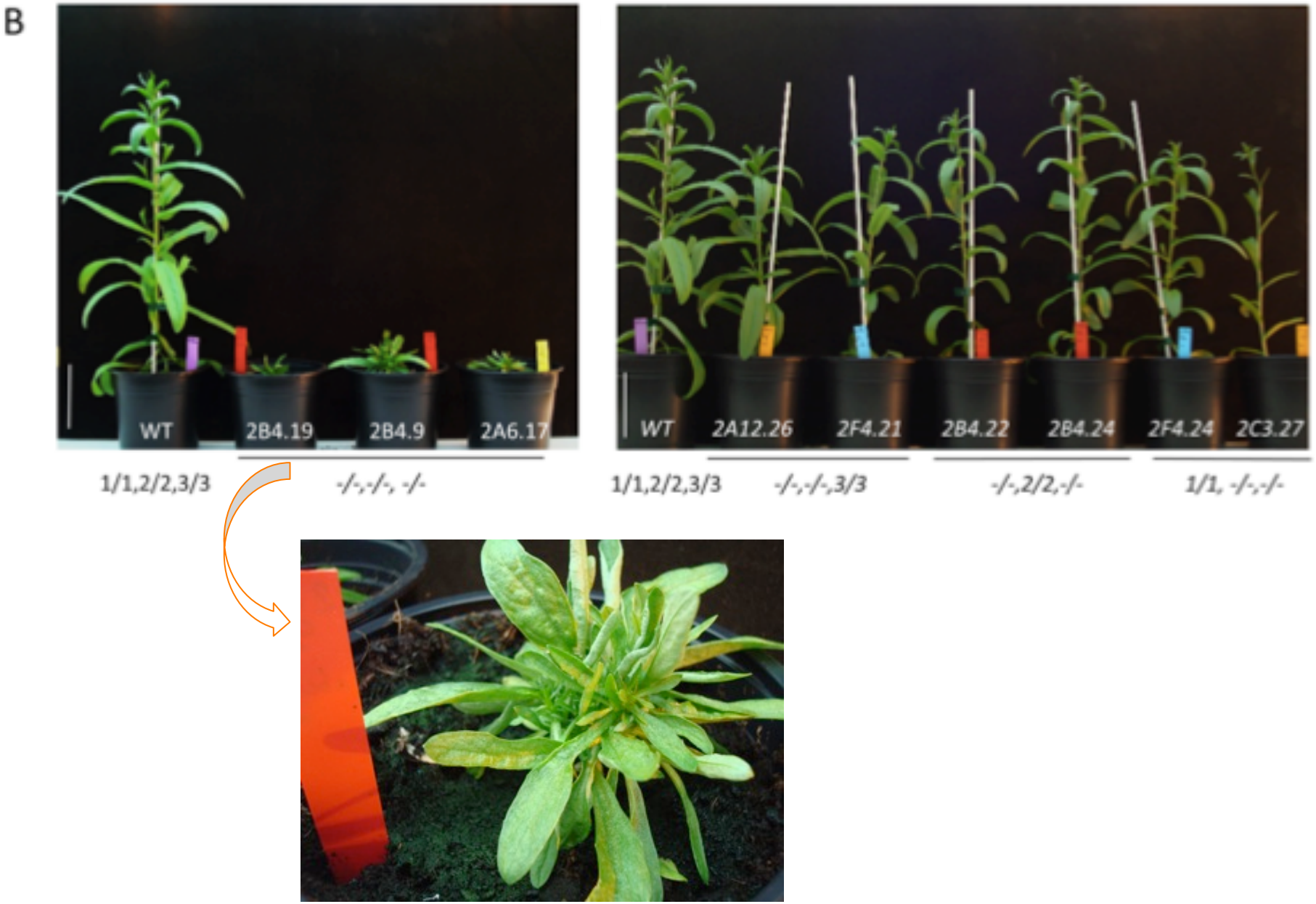




FAD2 homeologous genes and oleic acid accumulation

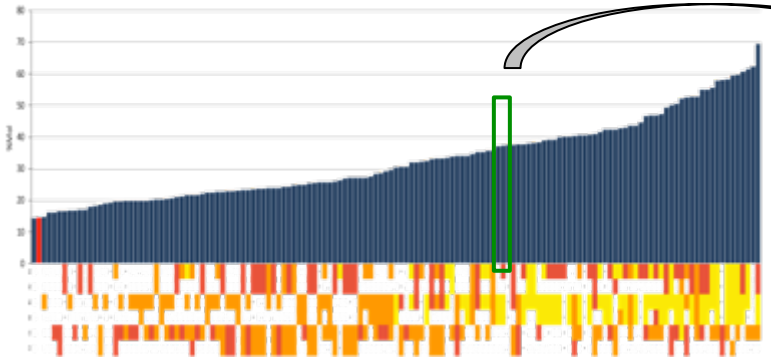


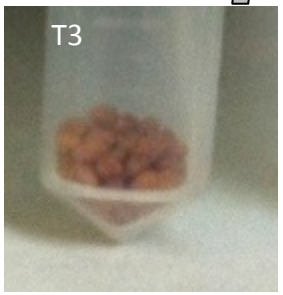
FAD2 triple ko reveals a developmental phenotype in camelina



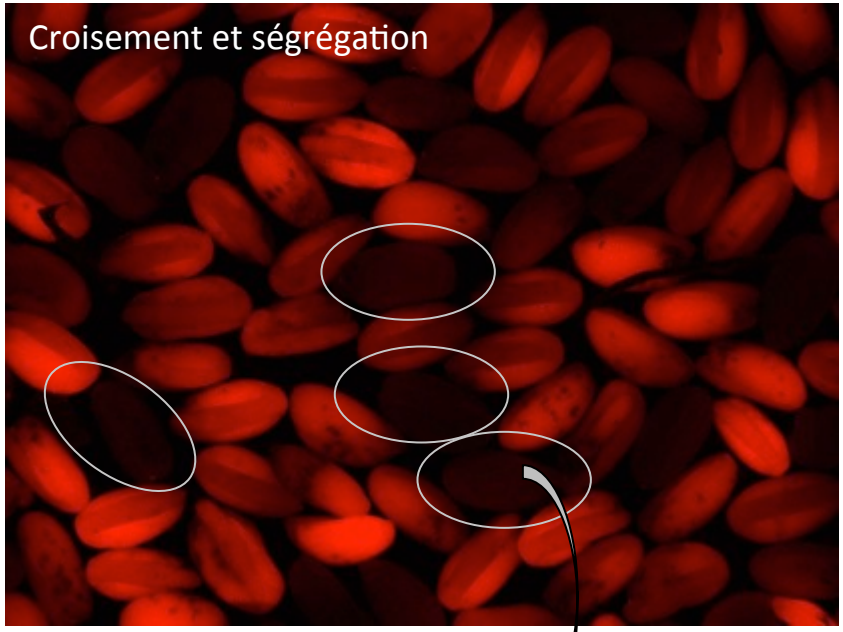
Next : selection of CAS9 defective plants

Selection of appropriate lines





Removal of CAS9 transgene by segregation or crossing



Seed amplification

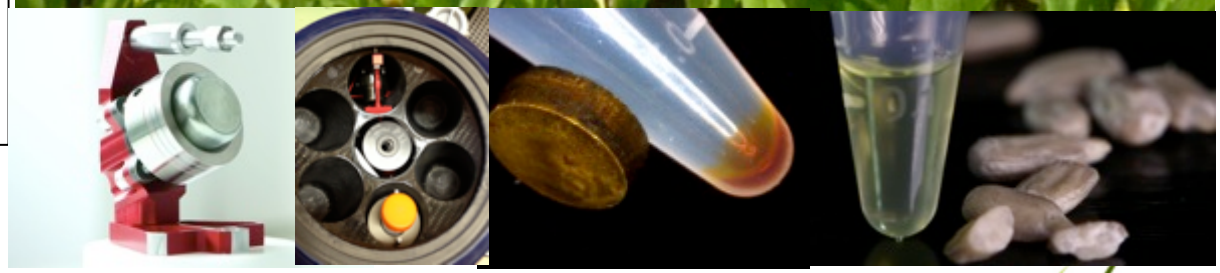
A complete cycle could be carried out in 18 months for segregating lines and 30 months for crossed lines

- The ultimate step : field trials of the oleic camelina lines ...

A camelina "pipeline" for biotech

Camelina lines
- vectors with
  - neutral markers
  - seed promoters
- In planta infiltration
- CRISPR/Cas9

Seed & oil phenotyping
- micropress
- lipidomic



Production
- Glasshouse
- field

Oil & cake fractions
- minipress
- Modification

What did we learn?

- ✓ Gene editing is sgRNA- and most probably gene- dependent
- ✓ Genotyping or phenotyping screens (possibility of forward screen on multiplexed editing)
- ✓ The large targeted genetic diversity is useful for
  understanding gene function in polyploid species
  as a ressource for breeding

=> easy to select an ideotype (oil profile) minimizing negative effects by selecting the nature of the alleles and their most efficient genetic combination

Morineau, C., Bellec, Y., Tellier, F., Gissot, L., Kelemen, Z., Nogue, F. and Faure, J.-D. (2016)
Selective gene dosage by CRISPR-Cas9 genome editing in hexaploid Camelina sativa.
***Plant Biotechnol. J.**, doi: 10.1111/pbi.12671*

M Azzopardi

JL Cacas

Y Bellec,

M Froissard

L Gissot

K Hématy

Z Kelemen

E Laruelle

C Morineau*

JC Palauqui

F Tellier

MTepfer

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F Nogué
C Collonnier

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