La cameline, culture oubliée mais avec un bel avenir?

Mark Tepfer

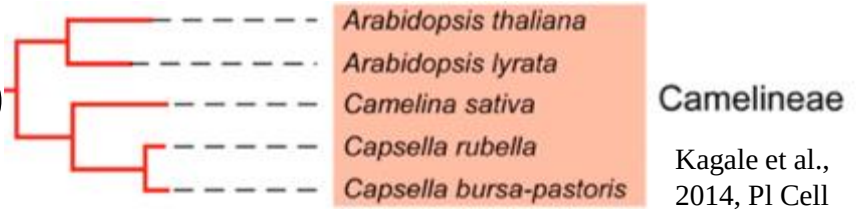
Institut Jean-Pierre Bourgin
INRA-Versailles

What is Camelina?

- Camelina (*Camelina sativa*) is an oilseed crop in the family Brassicaceae
- Domesticated in the Neolithic, grown in France since the Bronze Age, progressive decline since ~1900
- Has undergone little genetic improvement
- Both Spring and Winter varieties are available
- Camelina is an allohexaploid, n = 20 chromosomes (7 + 7 + 6)
- Is beginning to attract renewed attention, both as a crop and as a model for translational research.

Camelina is a model crop for translational research

- High sequence identity with arabidopsis genome
- Easy to transform (floral dip and screen)
- Short life cycle (90-100 days)
- Large plants: in greenhouse 1-5 g seeds per plant



Kagale et al., 2014, Pl Cell

- A model for fundamental research (when you need more plant material)
- A model for translational research: it's a real crop species
  - Proof of principle, including field trials
  - Improvement of camelina as a crop

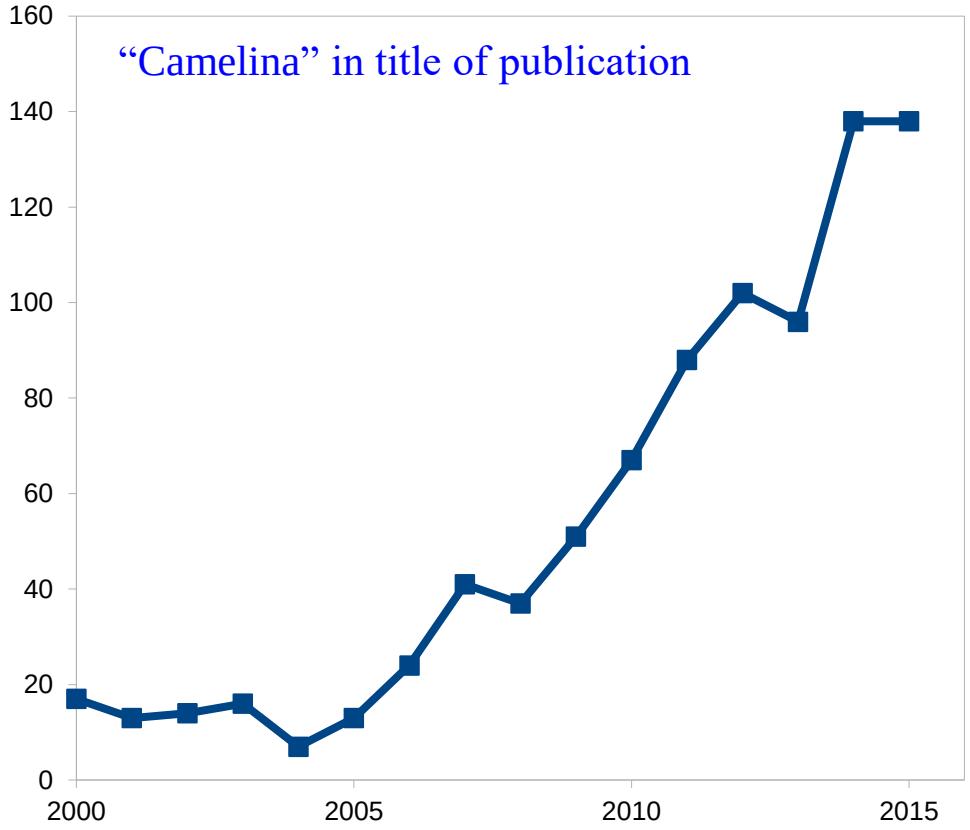
Camelina from an agro-ecological perspective

- Grows well on marginal soils under highly diverse conditions (from Spain to Ukraine to Ireland)
- Requires few or no treatments (herbicides, pesticides, fertilizer)
- Resistant to many pests and pathogens of other crucifers (e.g. OSR)
- Allelopathic (inhibits weeds)
- Plant it, walk away, harvest
- Good yield: 10-25 q/ha

A species with potential for enhancing crop diversification

Already being tested in mixtures and for double-cropping (minimize bare soil)

Camelina is a model crop for translational research



Faure JD & Tepfer M. 2016. Camelina, a Swiss knife for plant lipid biotechnology. OCL.

Special issue Plant Translational biology Science 16 Sept 2016

Potentially important uses of camelina

Food

Oil rich in omega-3 fatty acids, tocopherol

Biofuel

Spain

Iberia completes first commercial biofuel flight

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 the biofuel.

Cosmetics

Animal feed

Camelina cake

Innovations will facilitate integration of camelina in eco-friendly agrosystems

Considering the uncertainty regarding the future role of camelina in Europe, much depends on increasing yield and/or increasing the value of camelina oil.

Our point of view: concerted use of traditional and advanced breeding in conjunction with modern biotechnology can greatly enhance integration of camelina in sustainable, eco-friendly agricultural systems.

Camelina is a model crop

Improving camelina oil yield

Dalal *et al.* 2015; Zhang *et al.*, 2012; Roy Choudhury *et al.*, 2014; An & Suh, 2015; Li *et al.*, 2015

Improving camelina oil composition

Camelina producing an equivalent to fish oil

Ruiz-Lopez *et al.*, 2014; Petrie *et al.*, 2014; Usher *et al.*, 2015 (field trial); Betancor *et al.*, 2015 (aquaculture feeding trial); Tejera *et al.*, 2016 (mouse feeding trial)

Camelina producing Nervonic acid (component of breast milk, important brain lipid)

Huai *et al.*, 2015

Camelina producing oil with improved composition for industry

Kim *et al.*, 2015; Nguyen *et al.*, 2015; Kang *et al.*, 2011; Nguyen *et al.*, 2013; Liu *et al.*, 2015

Camelina producing oil enriched in oleic acid (CRISPR knockout of FAD2)

Morineau *et al.*, 2016

For further details, see:

Faure & Tepfer, 2017, “Camelina, a Swiss knife for plant lipid biotechnology”, OCL

Camelina at IJPB: who is currently doing what

Using transgenes and CRISPR/Cas9 to modify seed oil composition

Jean-Denis Faure et al.

Using CRISPR/Cas9 to create short-cycle cultivars

Mark Tepfer, Fabien Nogué & Jean-Denis Faure et al.

Modulating seed mucilage production to increase oil yield and extractability

Helen North, Bertrand Dubreucq et al.

Modification of seed lipid and protein quality and quantity

Bertrand Dubreucq, Loïc Lepiniec, Martine Miquel et al.

Gene flow to camelina's close relative, *Camelina microcarpa*

Mark Tepfer & Eric Jenczewski et al.

CamelinOil project (3BCAR)

JD Faure et al. (and Zephirin Mouloungui et al. UCAI Toulouse)

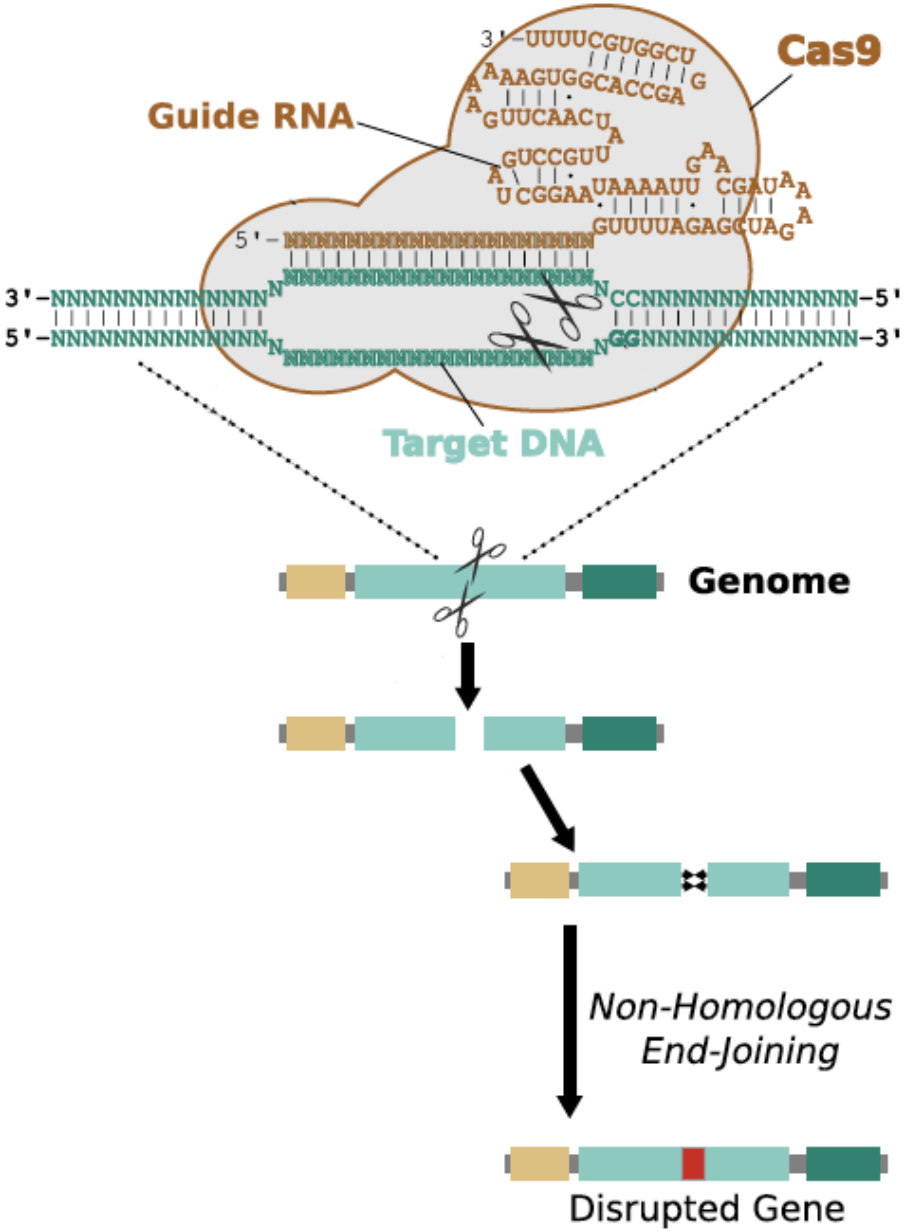
Objective

Create camelina lines that accumulate high levels of oleic acid

How

CRISPR/Cas9 knockout of FAD2 desaturase

Gene knockout with CRISPR/Cas9



Esvelt et al 2014

Creation of camelina enriched in oleic acid by CRISPR/Cas9 mutation of the *FAD2* gene

stearic acid C18:0 → oleic acid C18:1 (n-9) 16%

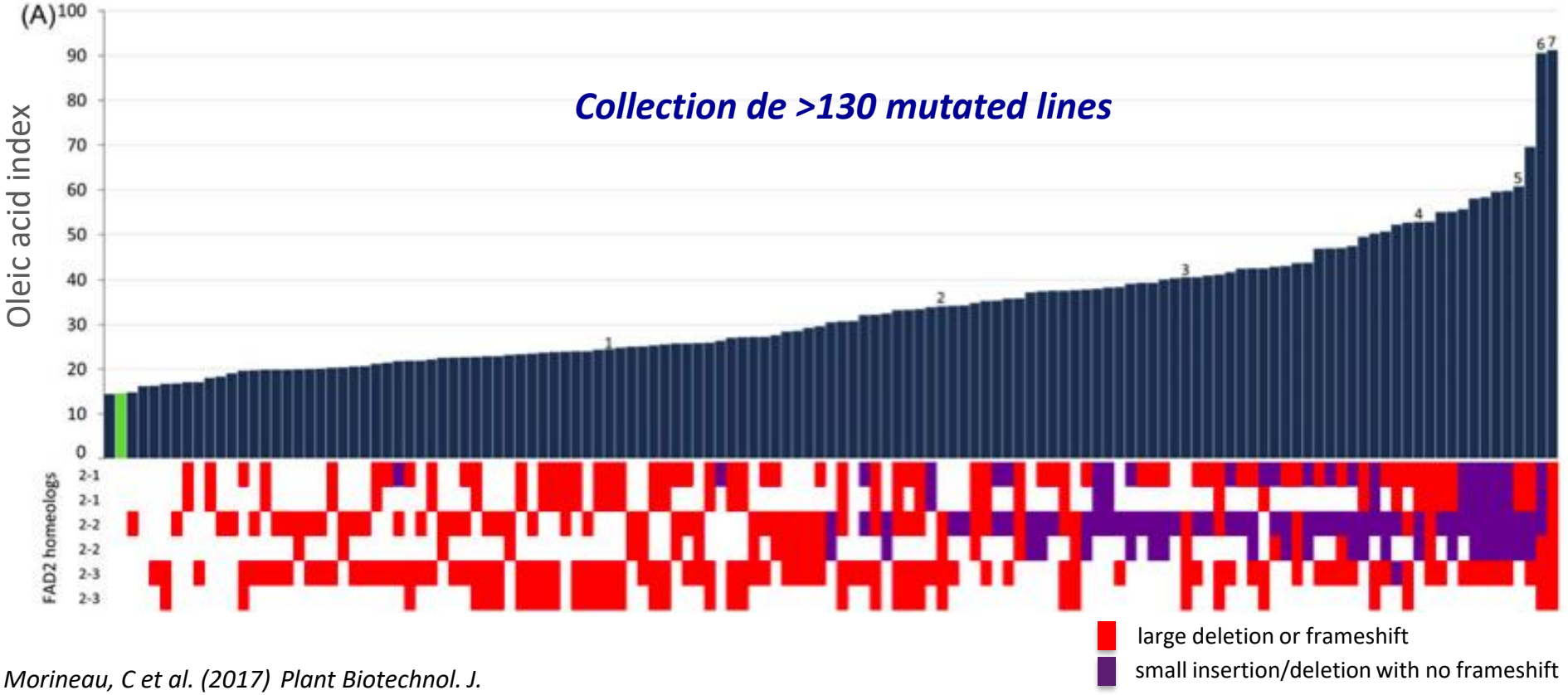
saturated/mono-unsaturated

Δ12 desaturase

CsFAD2-1
CsFAD2-2
CsFAD2-3

linoleic acid C18:2 (n-6,9) 23% → linolenic acid C18:3 (n-3,6,9) 31%

polyunsaturated



Morineau, C et al. (2017) Plant Biotechnol. J.

Can camelina be used in novel cropping systems?

Camelina harvest in Versailles in 2013

15 - 20 quintaux/ha

ShortCam project (dBAP): Using CRISPR/Cas to create short-cycle camelina

M Tepfer, F Nogué, JD Faure et al.

Why?

Enhance the possibility of using camelina in double-cropping systems

- Increased soil cover
- Increased crop diversity

fall	winter	spring	summer	fall	winter	

Winter camelina → Spring soybean (Gesch et al., 2014)

Winter cereal → Spring camelina (Groeneveld et al., 2014)

ShortCam project (dBAP):
Using CRISPR/Cas9 to create short-cycle camelina

fall	winter	spring	summer	fall	winter	

Winter barley → Spring camelina

Versailles field trials: 2017 and 2018

ShortCam project (dBAP):
Using CRISPR/Cas9 to create short-cycle camelina

fall	winter	spring	summer	fall	winter	

Winter barley → Spring camelina

Versailles field trials: 2017 and 2018

Would short-cycle camelina solve the problem?

How?

Use CRISPR/Cas9 to inactivate negative regulation of the floral transition

Using CRISPR/Cas to create short-cycle camelina

We have implemented the Targeted Induced Genetic Variation (TIGV) strategy, which consists in the simultaneous mutagenesis of several genes (Nogué et al., 2016).

- Identify several genes whose inactivation will accelerate the floral transition.
- Develop CRISPR/Cas9 transgenes that will target a total of 10 sites in 5 genes.
- Transform *Camelina sativa* cv Céline.
- Screen progeny for early flowering over several generations if necessary.
- Sequence target sites to determine which have been mutated.
- Validate in field trials (:-D)

Screen of 3800 T3 progeny for early flowering

What next?

- Further improvements: both classical genetics and modern biotechnology
- Development of improved tools: e.g. viral vectors to facilitate CRISPR/Cas9
- Field trials: essential for evaluation of pleiotropic effects
- Integration of camelina in the European agro-economy
  - For what end-products?
  - Monoculture? Double-cropping? Mixed?

The way forward: molecular biology + classic genetics + agronomy

Other potentially emergent Brassicaceae oilseed crops

- **Field pennycress (*Thlaspe arvense*)**

  Under domestication

  Not for food uses

- **Crambe (*Crambe abyssinica*)**

  Cultivated for high levels of erucic acid

- **Camelina (*Camelina sativa*)**

  Food, feed, industrial uses

  Domesticated for millennia

  Advanced research and development


Thank you for your attention