The First Year of ICON

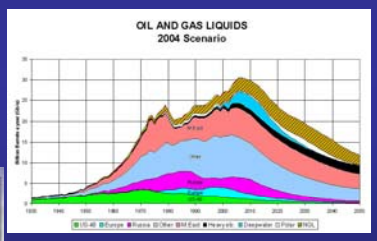
The World's Largest Biotechnology Consortium in Industrial Plant Oils

Setting the scene:

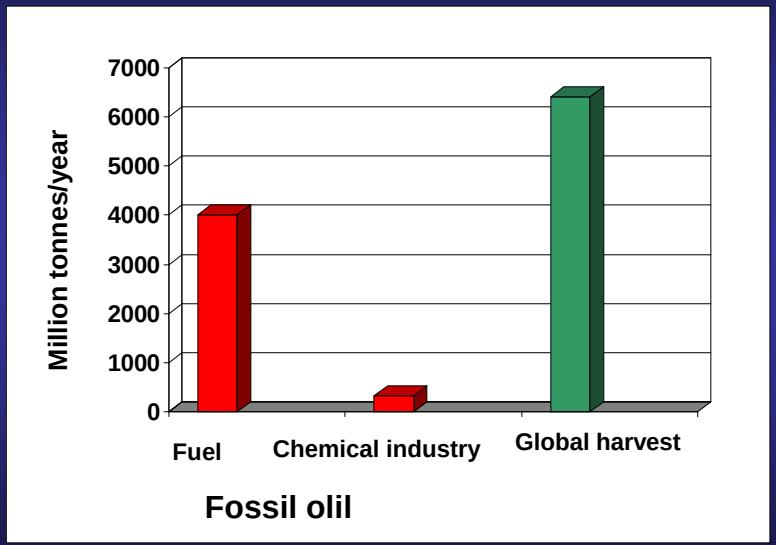
Why Plant Oils for Industrial Uses?

The fossil oil is soon to be depleted and causes huge geopolitical problems and carbon dioxide emissions that cause a global warming.

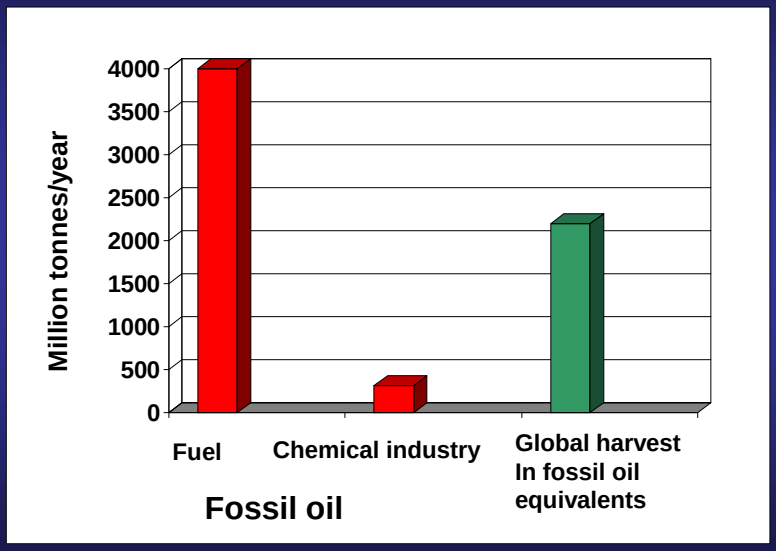
We have to find sustainable alternatives very soon.



How can agriculture contribute to replace fossil oil?



How can agriculture contribute to replace fossil oil?



Everything you can do with fossil oil, you can also do with plant oil

It is just a matter of costs!!

$17 USD $88 USD $70 $100

1997 2009

Fossil oil is old oil - Plant oils are fresh oils!

135 million tonnes of vegetable oil are produced per year.

About 20% (27 million tonnes) are used in industrial applications today.

Soap, detergents
Paints, inks
Plastic
Lubricants
Cosmetics
etc.

Plants are chemical factories

using sunlight, water and carbon dioxide

Oil seeds: The perfect chemical factory!

Petrochemical industry cracks carbon chains and use advanced organic chemistry to build desired products with these building blocks. ***Usually, this require much more energy than the final product contains***

Plants design the complex product direct in the seeds without extra cost and energy, thereby minimizing downstream processing costs..

In many industrial applications, plant oils have successfully competeted with fossil oil despite that their price have been over five times higher.

Plant oils have a huge competitive advantages compared to fossil oil if their chemical structures are optimized for the end uses.

Considerable energy savings in the downstream processing can be done by using plant oils.

NOTE:

For the chemical industry, the biological material is ***the only alternative*** to the reduced carbon from fossil sources

For energy, there are many other alternatives

Vision and Mission:

To replace 60% of the fossil oil in the chemical industry with plant oils in 20 years time.

To replace 60% of the fossil oil in the chemical industry with plant oils

What needs to be done to obtain this?

To replace 60% of the fossil oil in the chemical industry with plant oils

What needs to be done to obtain this?

1. **Optimize the chemical structure of the oil for the chemical industry**

To replace 60% of the fossil oil in the chemical industry with plant oils

What needs to be done to obtain this?

1. **Optimize the chemical structure of the oil for the chemical industry**
2. **Increase the global production of plant oils 3 times**

To replace 60% of the fossil oil in the chemical industry with plant oils

What needs to be done to obtain this?

1. **Optimize the chemical structure of the oil for the chemical industry**
2. **Increase the global production of plant oils 3 times**

Is this really possible?

To replace 60% of the fossil oil in the chemical industry with plant oils

What needs to be done to obtain this?

1. **Optimize the chemical structure of the oil for the chemical industry**
2. **Increase the global production of plant oils 3 times**

Is this really possible?

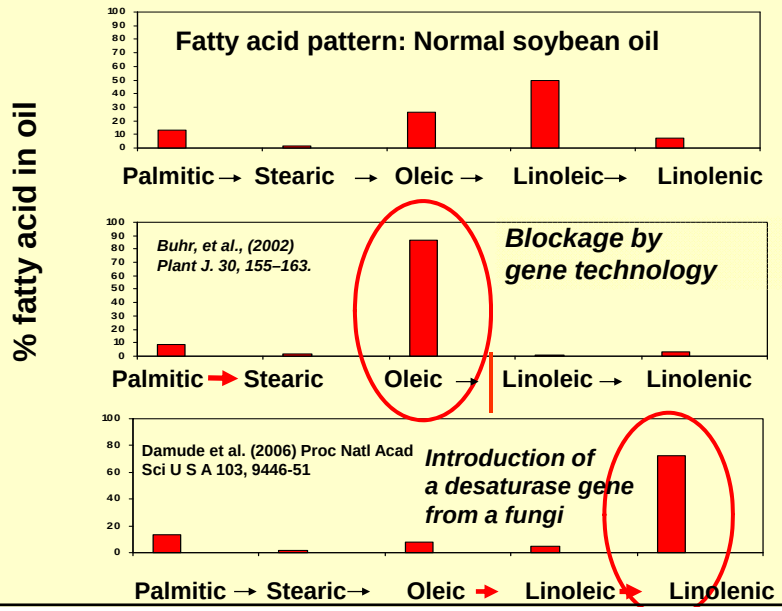
Yes, if we use plant biotechnology!

The chemical structure of the fatty acids in the oil determines the chemical and physical Property and thereby the end use of the oil

Genetic engineering can optimize the chemical structure to make it more economical for chemical industry

We move the chemical factory into the seed

Gene technology can change the relative proportions between the five fatty acids in oil seeds

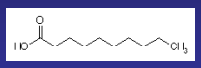


A number of wild plants have very high amount of industrial valuable fatty acids in their seed oils

73% Caprylic acid (8:0)

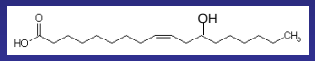
Cuphea painteri,

95% Capric acid (10:0)



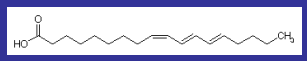
Cuphea koehneana

90% Ricinoleic acid



Castor *(Ricinus communis)*

88% α–Eleostearic acid



Aleurites fordii

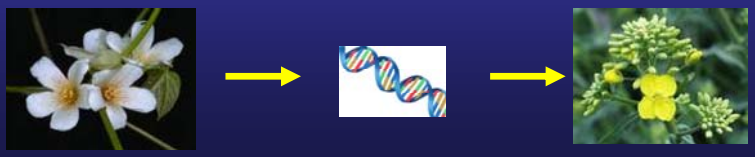
78% Sterculic acid

Sterculia foetida

+ several hundreds of other fatty acid structures….

Cuphea species Castor ***Aleurites fordii*** ***Sterculia foetida***

Transferring the biosynthetic machinery for the production of unsusal fatty acids to high yielding oil crops by genetic engineering could yield production at a low price and with stable supply.



Transgenic rape with medium chain thioesterases

Voelker et al. (1992) Science, 257, 72–74.
Knutzon et al. (1999) Plant Physiol. 120, 739–746.

	% 12:0 in oil	
	In host seed oil	GM-rape
12:0 -TE from California bay tree	60%	67%

Despite the great potentials, the industrial investments in the development of industrial oil by plant biotechnology have decreased.

This have mainly been due to following factors:

Despite the great potentials, the industrial investments in the development of industrial oil by plant biotechnology have decreased.

This have mainly been due to following factors:

•GM plants for non-food uses are problematic

Despite the great potentials, the industrial investments in the development of industrial oil by plant biotechnology have decreased.

This have mainly been due to following factors:
•GM plants for non-food uses are problematic
•IPR spread on many actors

Despite the great potentials, the industrial investments in the development of industrial oil by plant biotechnology have decreased.

This have mainly been due to following factors:
•GM plants for non-food uses are problematic
•IPR spread on many actors
•IPR positions unclear

Despite the great potentials, the industrial investments in the development of industrial oil by plant biotechnology have decreased.

This have mainly been due to following factors:
- GM plants for non-food uses are problematic
- IPR spread on many actors
- IPR positions unclear
- Public distrust in GM technology

Despite the great potentials, the industrial investments in the development of industrial oil by plant biotechnology have decreased.

This have mainly been due to following factors:
- GM plants for non-food uses are problematic
- IPR spread on many actors
- IPR positions unclear
- Public distrust in GM technology
- Volumes and prices of product not high enough to warrant investments (calculations made on substitution on existing markets and not on novel potential applications)

Despite the great potentials, the industrial investments in the development of industrial oil by plant biotechnology have decreased.

This have mainly been due to following factors:

- GM plants for non-food uses are problematic
- IPR spread on many actors
- IPR positions unclear
- Public distrust in GM technology
- Volumes and prices of product not high enough to warrant investments (calculations made on substitution on existing markets and not on novel potential applications)
- Too long time frame before return of investments

Despite the great potentials, the industrial investments in the development of industrial oil by plant biotechnology have decreased.

This have mainly been due to following factors:

- GM plants for non-food uses are problematic
- IPR spread on many actors
- IPR positions unclear
- Public distrust in GM technology
- Volumes and prices of product not high enough to warrant investments (calculations made on substitution on existing markets and not on novel potential applications)
- Too long time frame before return of investments

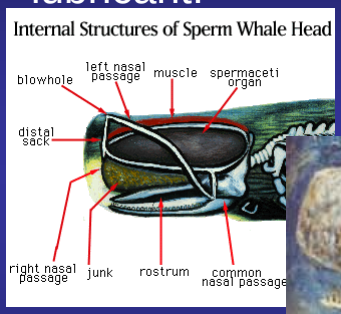
- Scientific bottlenecks that cannot be solved without the input of large parts of the Scientific Community

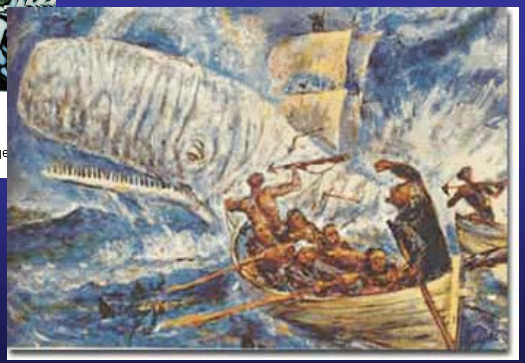
To follow in its wake:
- **Public acceptance of GM plants for industrial use**
- **GM crops with other industrial qualities**
- **GM crops for food**

We need an ice-breaker for industrial oil:

- **A robust and safe platform crop for industrial products**
- **Non-toxic product with a substantial market and added value**
- **Substantial environmental benefits**
- **Development not driven by multinational companies and profit**
- **High probability of technical success in relatively short time frame**

The spermaceti whale was hunted for its spermaceti oil that was widely used as lubricant.





In the infancy of automobile all cars had spermaceti oil or derivatives of that as transmission oils in the gear boxes..

The product was so efficient that the oil lasted the lifetime of the car..

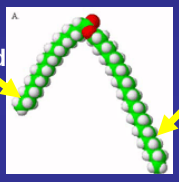
15 million kilos of spermaceti oil was used yearly
until the whale was nearly extinct

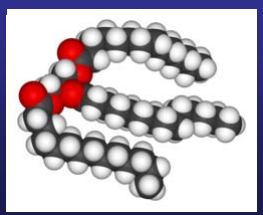
1972 -a total ban on hunting spermaceti whale was introduced

After that no good biological alternative to lubricants
Based on fossil oil was available

50 million tonnes of lubricants are used yearly!

The jojoba plant has seed oil (vax esters) that is similar to the spermaceti oil.





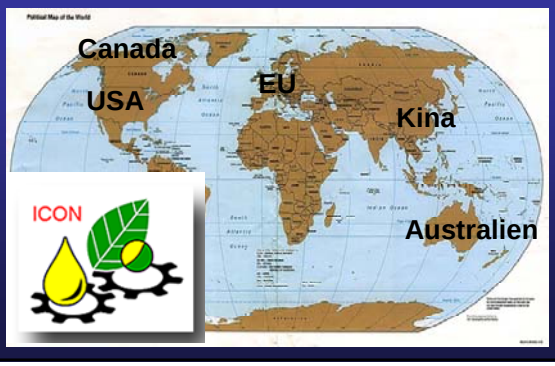
EU 7th frame project approved

ICON

Industrial Crops producing added value Oils for Novel chemicals

Large collaborative 4 yrs project, 11 million €

involving 25 partners



Crambe abyssinica might fullfil the criteria for a robust and safe platform oil crop for industrial products

- Not a food crop (60% erucic acid in its oil)
- Not outcrossing with other oil crops & few wild relatives
- Reasonable oil yield per hectare (same as spring rape)
- Some experience as an agricultural crop
- Excellent fatty acid composition of oil for the conversion into wax esters (due to the 60% erucic acid)

Crambe abyssinica might fullfil the criteria for a robust and safe platform oil crop for industrial products

But:

No efficient transformation protocol was available

Brassica carinata as an alternative industrial oil crop preferred by Canadian partners

- Efficient transformation protocol available (at PBI)
- Drought resistant

Brassica carinata as an alternative industrial oil crop

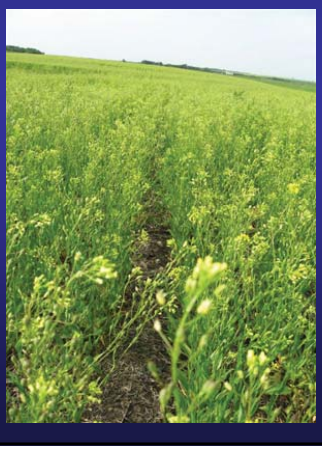
But:

- Some outcrossing with other *Brassica* species
- Limited seed yield

A third industrial crop platform was introduced by US partners in ICON during the first year

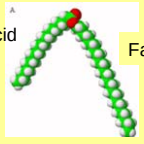
Camelina sativa

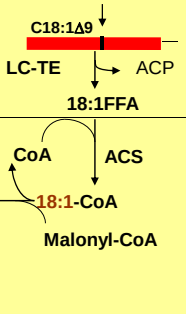
Can be transformed via flower dipping!



Transgenic Arabidopsis with Jojoba FAR and WS genes had replaced up a major portion of their triacylglycerols with wax esters

(Lardizabal *et al.* (2000) Plant Physiology, 122, 645–655)





Target quality should have high oxidation stability and low melting point

Target quality should have high oxidation stability and low melting point

Spermaceti oil:
Cetyl-palmitate (16:0-16:0) *melting point 54°C*

Target quality should have high oxidation stability and low melting point

Spermaceti oil:
Cetyl-palmitate (16:0-16:0) *melting point 54°C*

Known plant waxes:
Jojoba wax:
20:1 and 22:1 FA and alcohols *melting point 9°C*

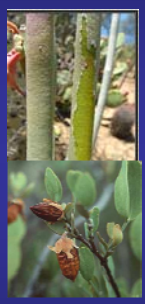
Carnuba and other cuticular wax esters:
C36-C70 saturated wax esters *melting point 60-80°C*

Target quality should have high oxidation stability and low melting point

Spermaceti oil:
Cetyl-palmitate (16:0-16:0) *melting point 54°C*

Known plant waxes:
Jojoba wax:
20:1 and 22:1 FA and alcohols *melting point 9°C*

Carnuba and other cuticular waxes:
C36-C70 saturated wax esters *melting point 60-80°C*



ICON qualities: e.g.

18:1-18:1 *melting point -4°C*

Ricinoyl-ricinoleate *melting point -20 to -30°C??*

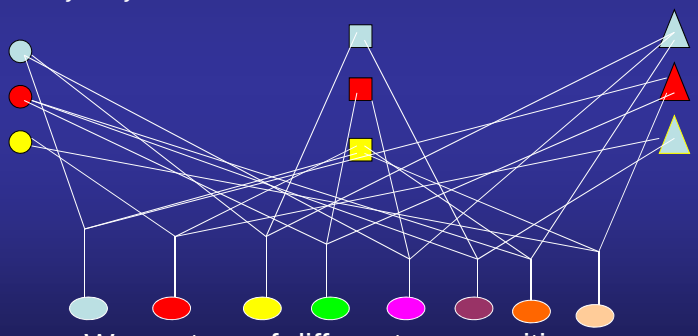
2,4,6,8,12-methyl-20:0-alcohol---2,4,6,8,10,12-methyl-20:0-FA melting point ????

By combining various fatty acid modifying, FAR and WS genes, a huge number of wax esters with different compositions can be achieved

Fatty acid modifying genes
e.g. medium chain thioesterses
RNAi FAD2, hydroxylases

Wax synthase genes with different specificities

FAR genes with different specificities



Wax esters of different compositions

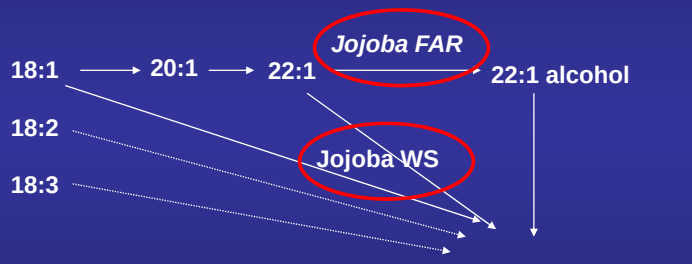
Field tests with Crambe and *Brassica carinata* *with at least 20% wax esters in their oil*

ICON

Lubrication tests by partner

ICON

Target oil quality
Wax esters with endogenous Crambe fatty acid profile using jojoba FAR and jojoba WS.

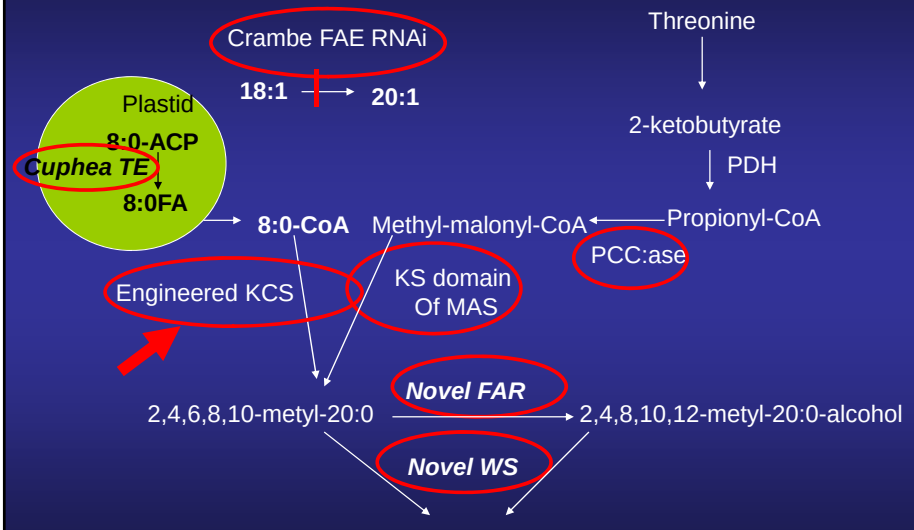


22:1-alcohol –- unsaturated-FA

Wax esters

ICON

Target Oil quality 7b.
Wax esters with methyl-branched chain FA

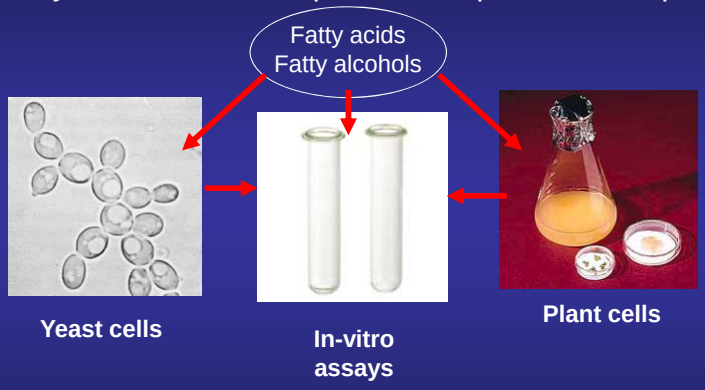


2,4,6,8,12-methyl-20:0-alcohol---2,4,6,8,10,12-methyl-20:0-FA

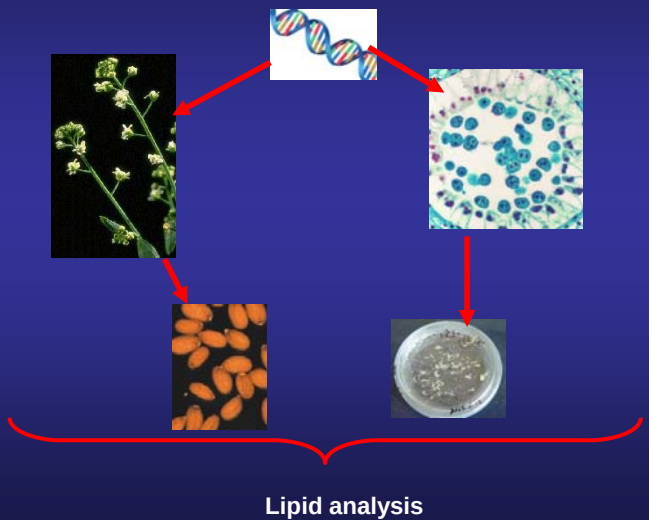
Branched chain wax esters

Enzymes specificity and construction of pathways will first be established in model systems

(E. coli, yeast, transient expression in plant cell suspensions)

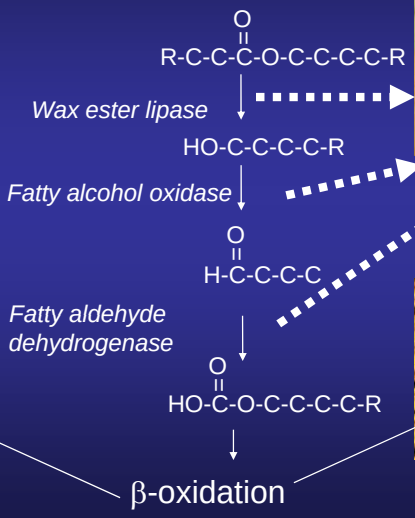


Proof of concepts in seeds will be demonstrated first in Arabidopsis and microspore derived embryos of rape



Severe germination problems in transgenic seeds are expected at high wax ester levels

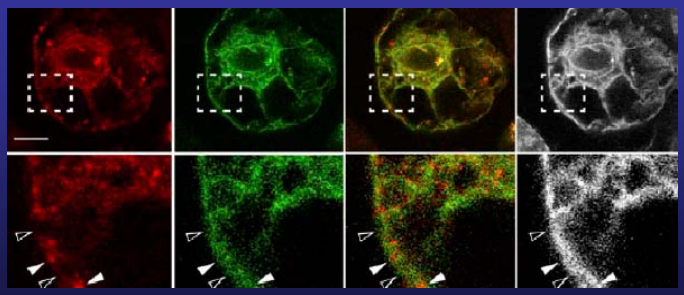
Germination



Cell biology

E.g.

- Are the introduced enzymes placed at the right site in the cell in order to act on the substrates, deposit the wax esters in oil bodies and mobilize them during germination?
- Are the oil body proteins correctly targeted to wax esters
- Is there metabolic channelling?



From Shockey et al. Plant Cell 18, 2294-2314

One ICON partner involved in adding value to seed by-products

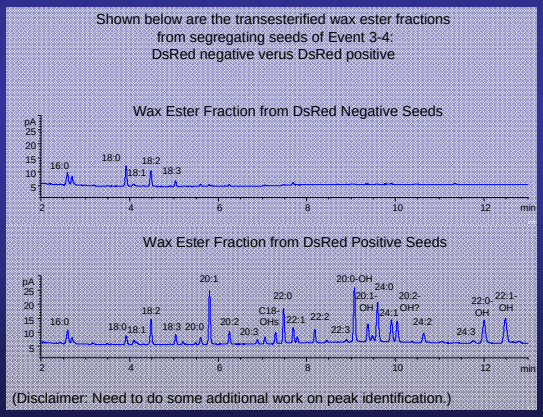
Plastics from seed cake of B. carinata and Crambe

Plastics made out of Crambe seed meal (left) and B. carinata seed meal (right)

In particular B. carinata meal has very interesting properties!

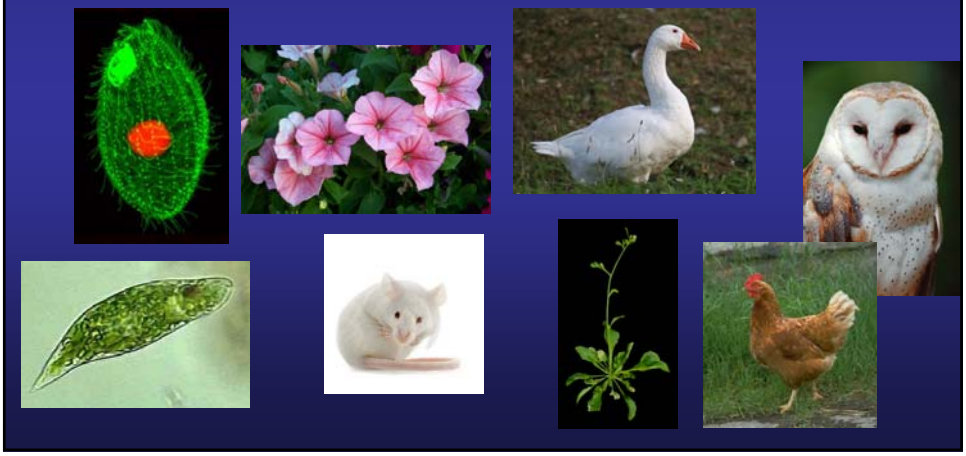
Major achievements the first year of ICON

Wax ester production in Camelina with jojoba genes (about 10% of oil)



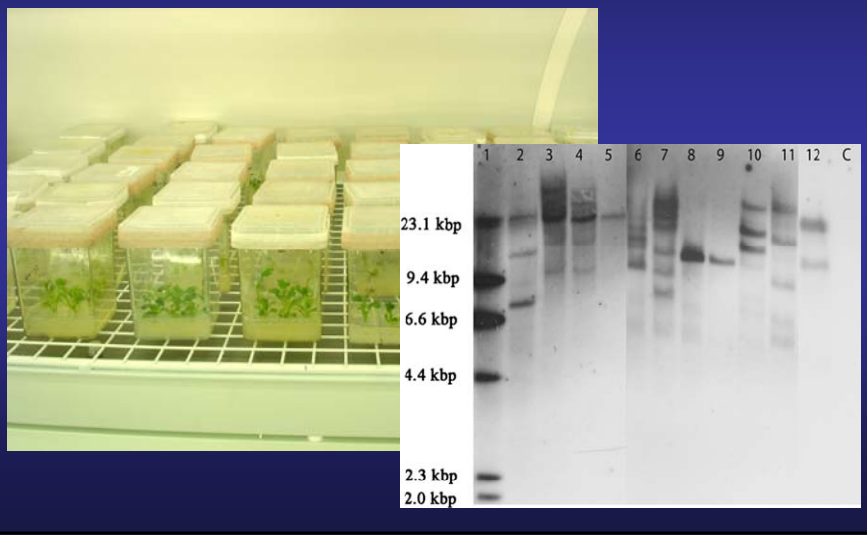
Major achievements the first year of ICON

More than 30 FAR and WS genes isolated and are under characterization



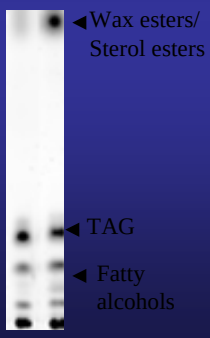
Major achievements the first year of ICON

Robust transformation protocol for Crambe developed



Major achievements the first year of ICON

Successfull subcellular re-targeting of FAR to re-localize with WS giving increased wax ester production in yeast



To replace 60% of the fossil oil in the chemical industry with plant oils

What needs to be done to obtain this?

1. **Optimize the chemical structure of the oil for the chemical industry**
2. **Increase the global production of plant oils 3 times**

We have some ideas

But that is another story…..

The Chemical Factory

2009

2029

Thank you!