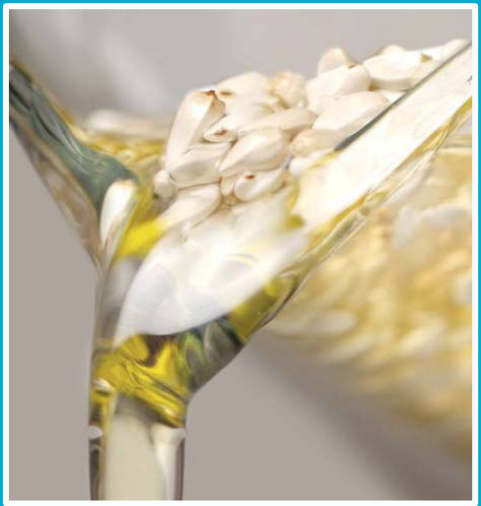





Innovation in oilseeds: new oils for the food and industrial markets

Allan Green | Research Director - Bioproducts

AUSTRALIAN GRAINS INDUSTRY CONFERENCE – MELBOURNE, JULY 29, 2014
www.csiro.au

CSIRO

INNOVATION in crop species






INNOVATION in technology

- F1 hybrid varieties
- GM herbicide tolerance
- GM insect resistance (cottonseed)

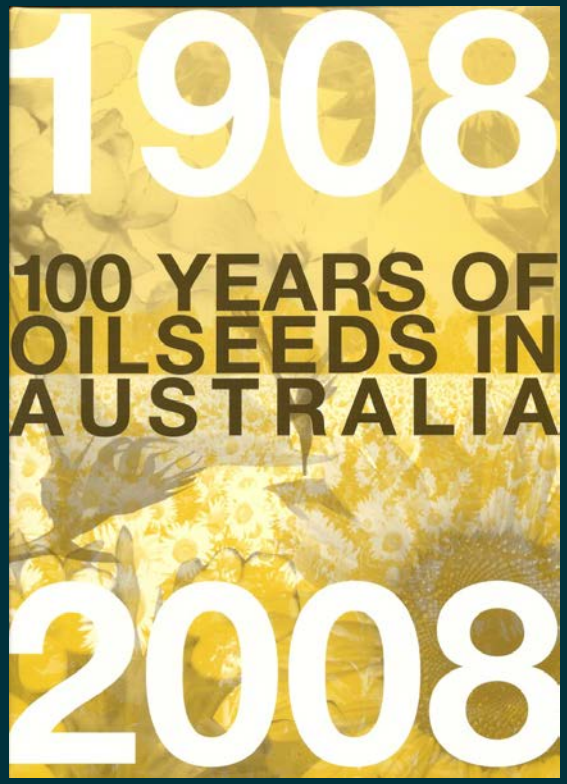
CSIRO

INNOVATION in oil quality

- High-oleic sunflower
- Linola (low linolenic linseed)
- Canola quality *Brassica juncea*
- High-oleic, low linolenic, reduced sats canola

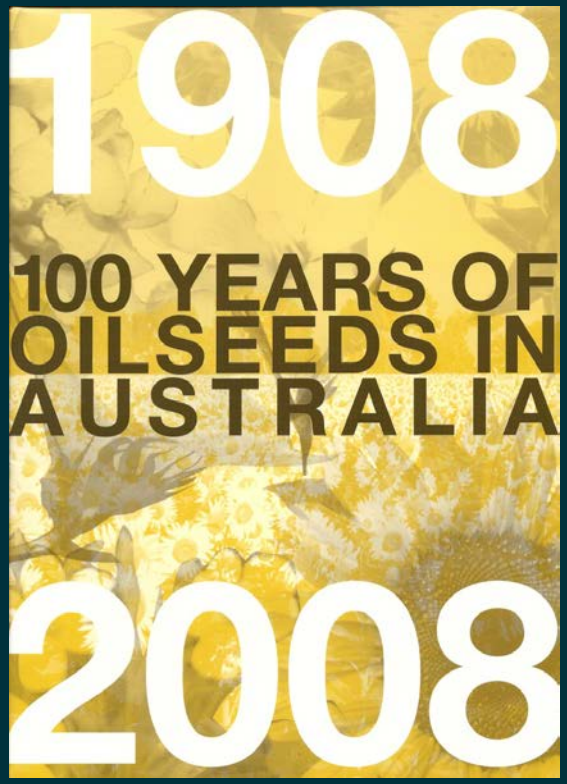
CSIRO

Oilseeds industry built on INNOVATION



Now a **$2 billion** industry producing up to 4 MT of oilseed annually, mainly for export …

Oilseeds industry built on INNOVATION



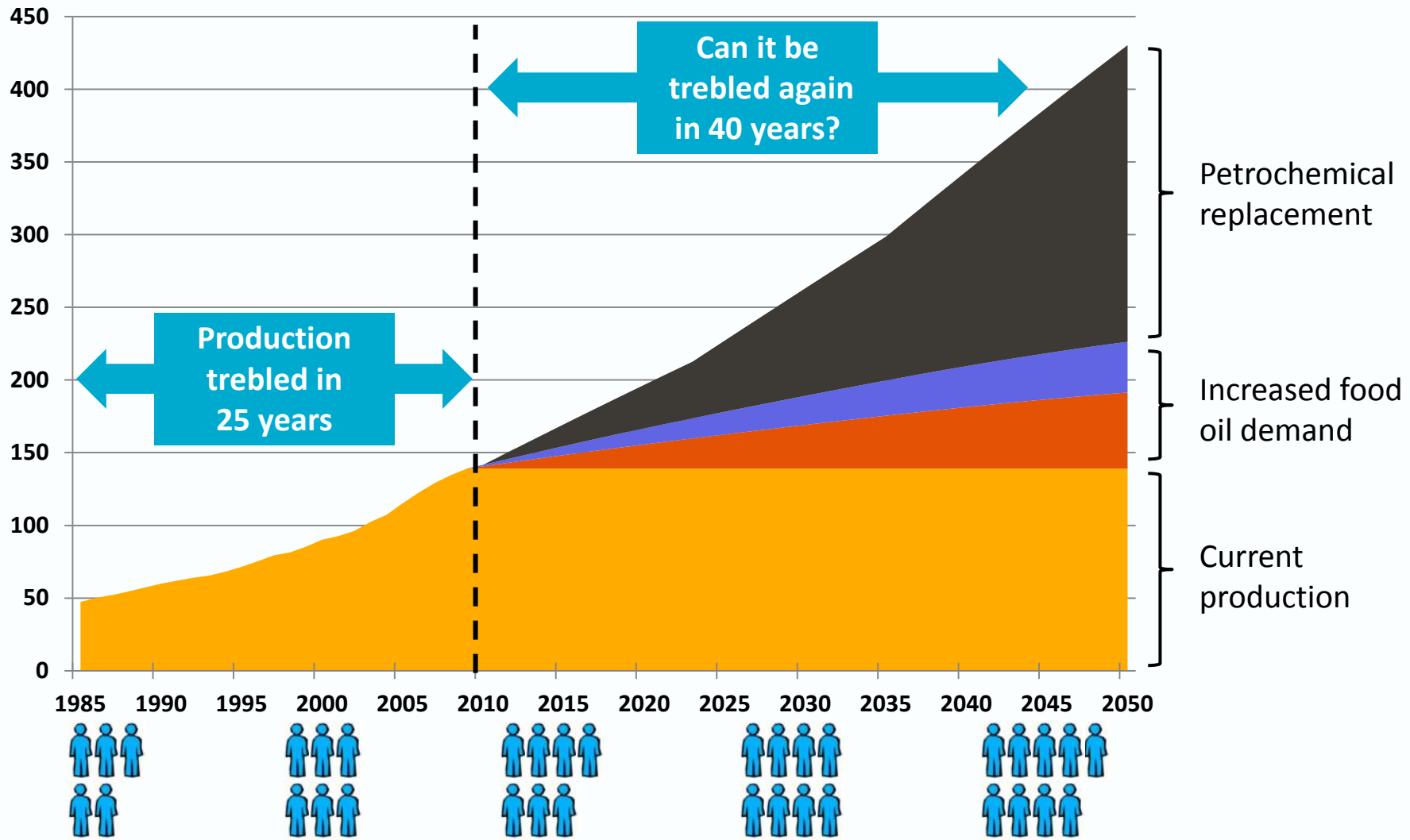
Now a $2 billion industry producing up to 4 MT of oilseed annually, mainly for export …

"… well equipped and positioned to go forward with confidence to meet the demand for traditional food and feed products and the expectation of exciting new markets for fuel and a new generation of specialty products to suit changing consumer needs."

Robert Green, President AOF

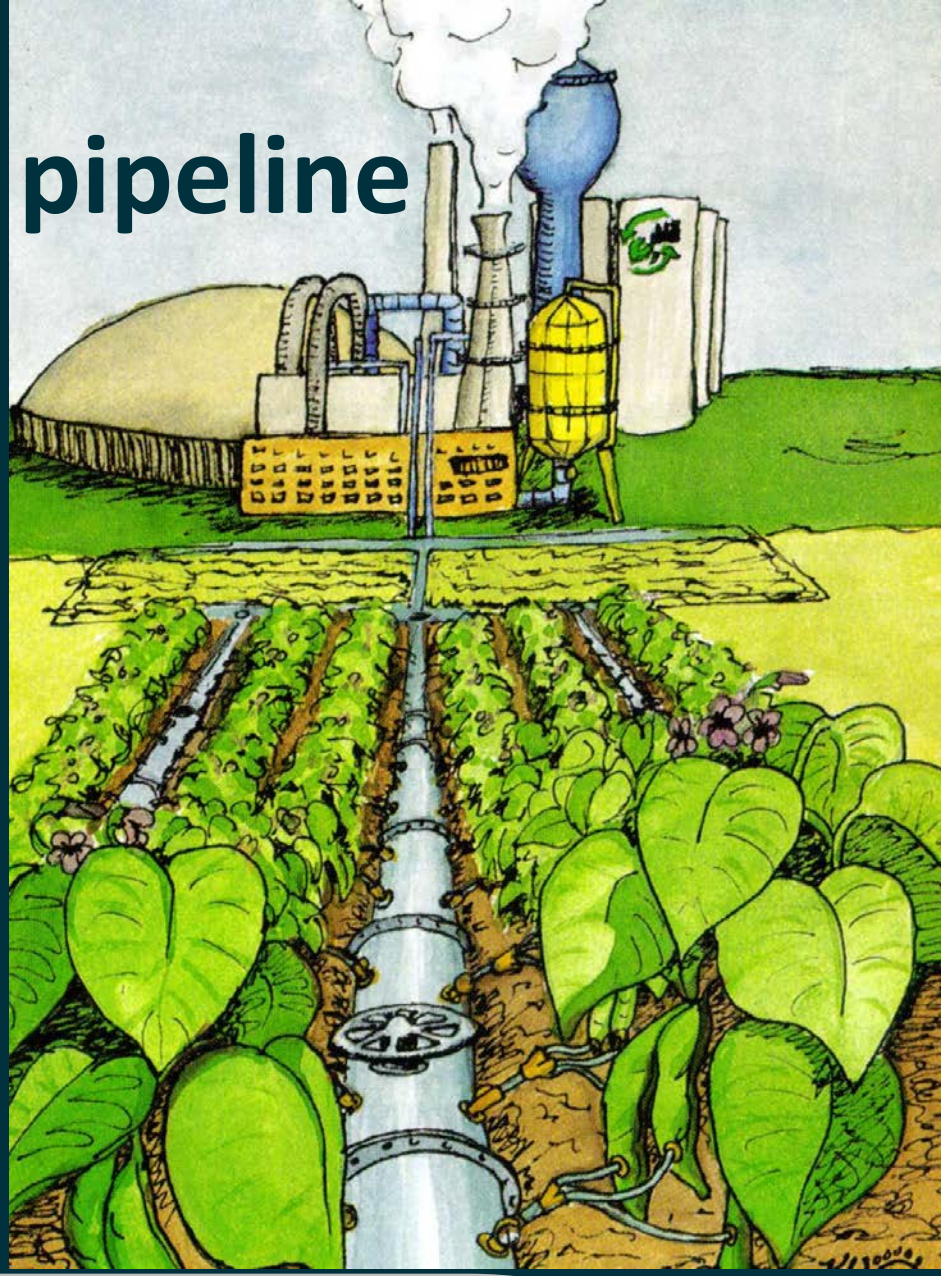
CSIRO

Expanding market demands

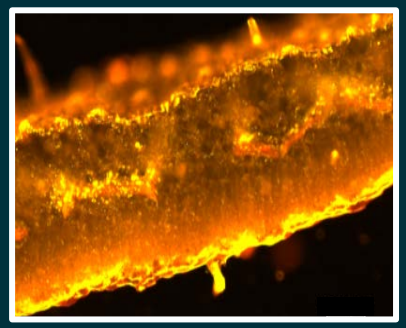


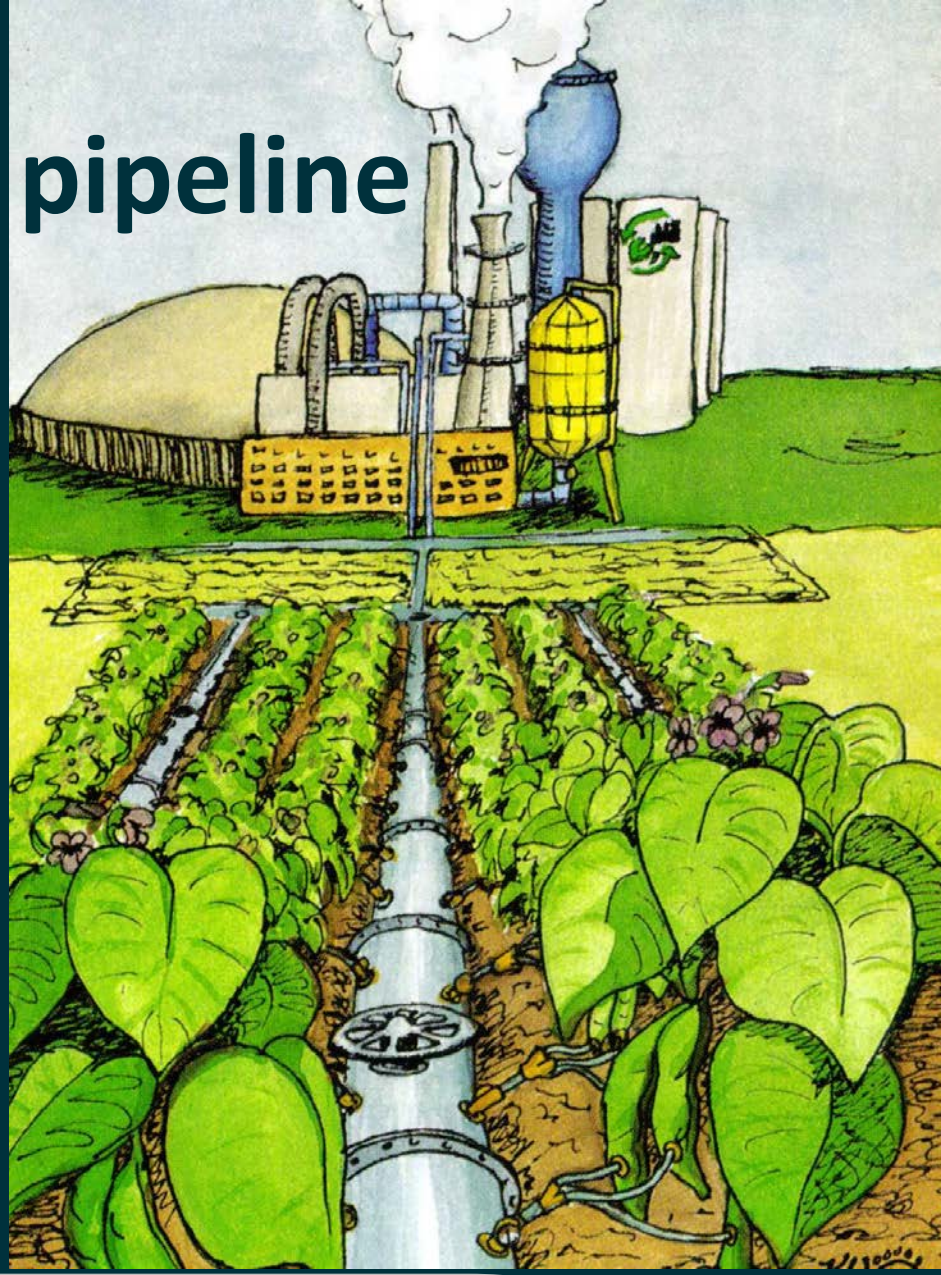
Biotech oils in the pipeline

Metabolic engineering oil synthesis

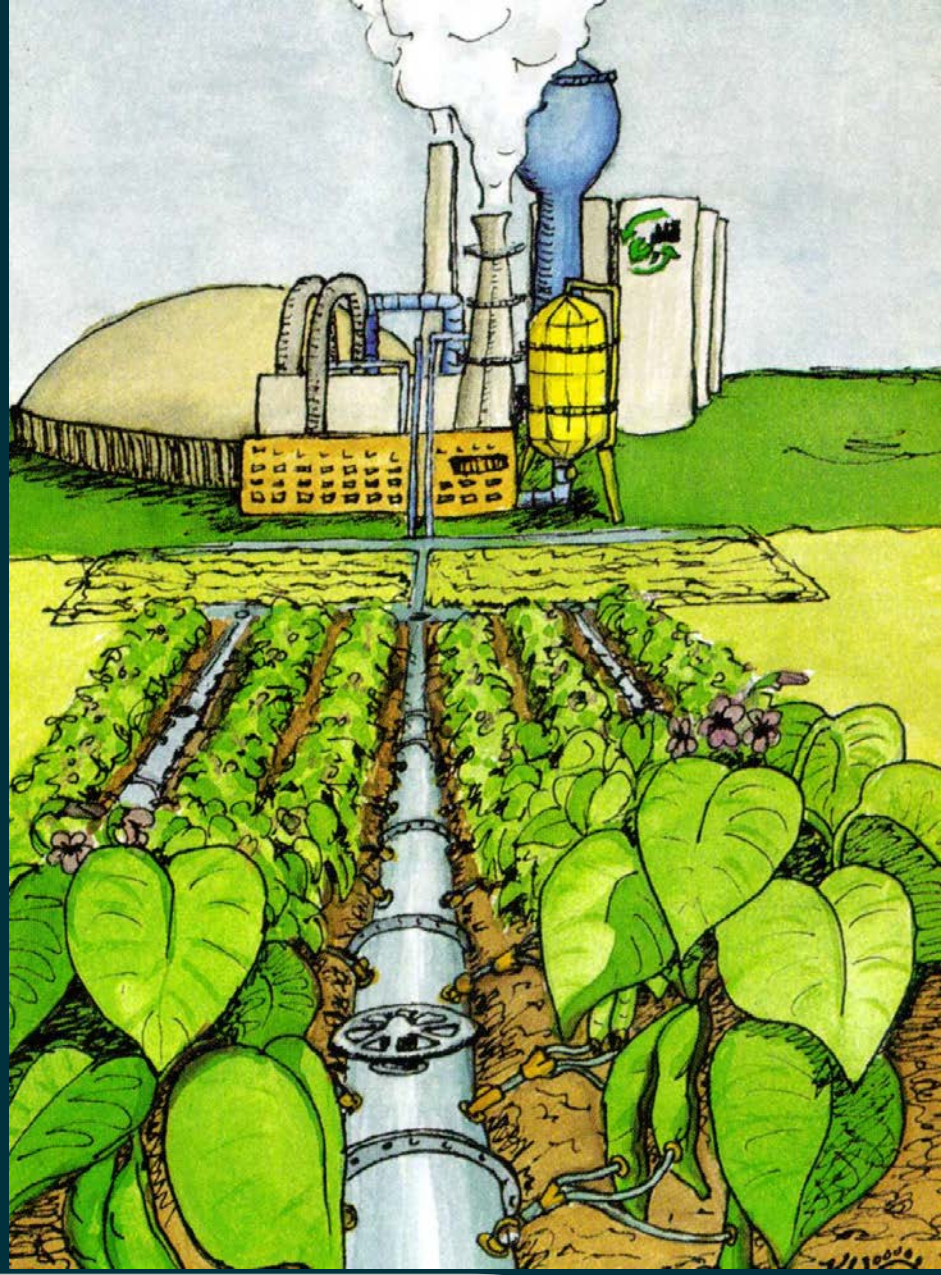


Biotech oils in the pipeline

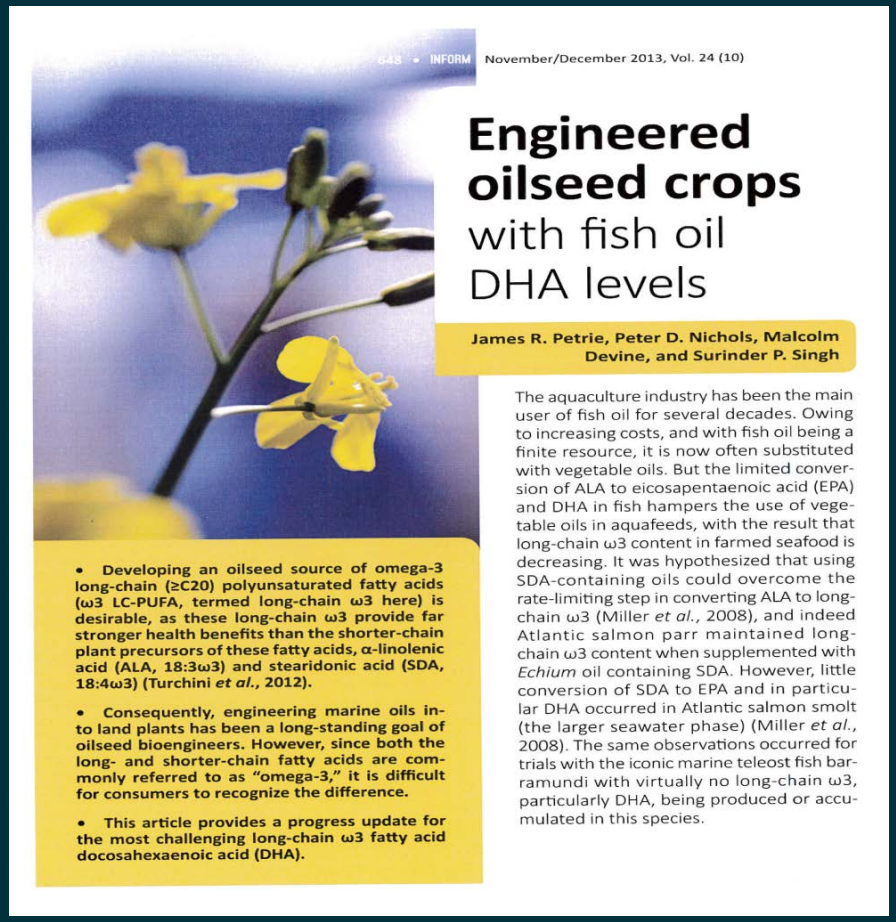




Omega-3 canola



Engineering canola to produce EPA & DHA



648 • INFORM November/December 2013, Vol. 24 (10)

Engineered oilseed crops with fish oil DHA levels

James R. Petrie, Peter D. Nichols, Malcolm Devine, and Surinder P. Singh

- **Developing an oilseed source of omega-3 long-chain (≥C20) polyunsaturated fatty acids (ω3 LC-PUFA, termed long-chain ω3 here) is desirable, as these long-chain ω3 provide far stronger health benefits than the shorter-chain plant precursors of these fatty acids, α-linolenic acid (ALA, 18:3ω3) and stearidonic acid (SDA, 18:4ω3) (Turchini *et al.*, 2012).**
- **Consequently, engineering marine oils into land plants has been a long-standing goal of oilseed bioengineers. However, since both the long- and shorter-chain fatty acids are commonly referred to as "omega-3," it is difficult for consumers to recognize the difference.**
- **This article provides a progress update for the most challenging long-chain ω3 fatty acid docosahexaenoic acid (DHA).**

The aquaculture industry has been the main user of fish oil for several decades. Owing to increasing costs, and with fish oil being a finite resource, it is now often substituted with vegetable oils. But the limited conversion of ALA to eicosapentaenoic acid (EPA) and DHA in fish hampers the use of vegetable oils in aquafeeds, with the result that long-chain ω3 content in farmed seafood is decreasing. It was hypothesized that using SDA-containing oils could overcome the rate-limiting step in converting ALA to long-chain ω3 (Miller *et al.*, 2008), and indeed Atlantic salmon parr maintained long-chain ω3 content when supplemented with *Echium* oil containing SDA. However, little conversion of SDA to EPA and in particular DHA occurred in Atlantic salmon smolt (the larger seawater phase) (Miller *et al.*, 2008). The same observations occurred for trials with the iconic marine teleost fish barramundi with virtually no long-chain ω3, particularly DHA, being produced or accumulated in this species.

AOCS Inform

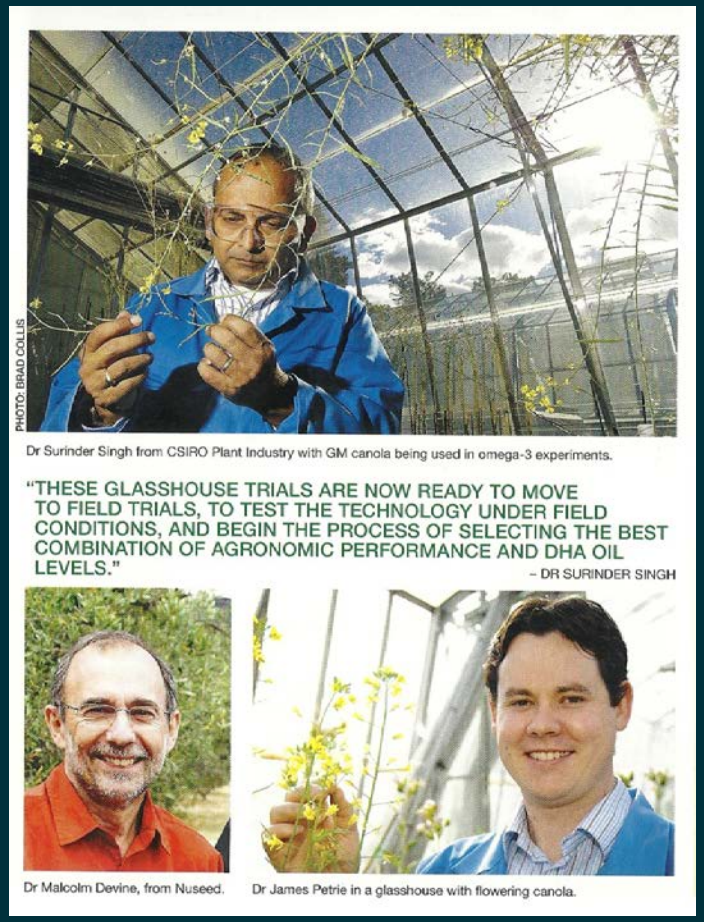


PHOTO: BRAD COLLIS

Dr Surinder Singh from CSIRO Plant Industry with GM canola being used in omega-3 experiments.

"THESE GLASSHOUSE TRIALS ARE NOW READY TO MOVE TO FIELD TRIALS, TO TEST THE TECHNOLOGY UNDER FIELD CONDITIONS, AND BEGIN THE PROCESS OF SELECTING THE BEST COMBINATION OF AGRONOMIC PERFORMANCE AND DHA OIL LEVELS."

– DR SURINDER SINGH

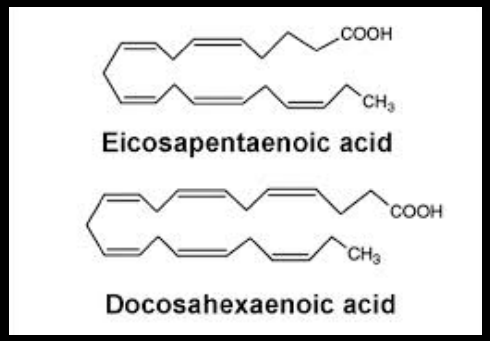
Dr Malcolm Devine, from Nuseed.

Dr James Petrie in a glasshouse with flowering canola.

GRDC Ground Cover

Engineering canola to produce EPA & DHA

Long-chain ω3 PUFA (EPA & DHA)



- EPA (20:5) and DHA (22:6) are essential ω3 LC-PUFA found in every cell membrane in the body
- Widely recognised for ability to improve many aspects of human health

Foetal growth & development

Brain growth. Visual development.

Brain growth

Cognition & mood

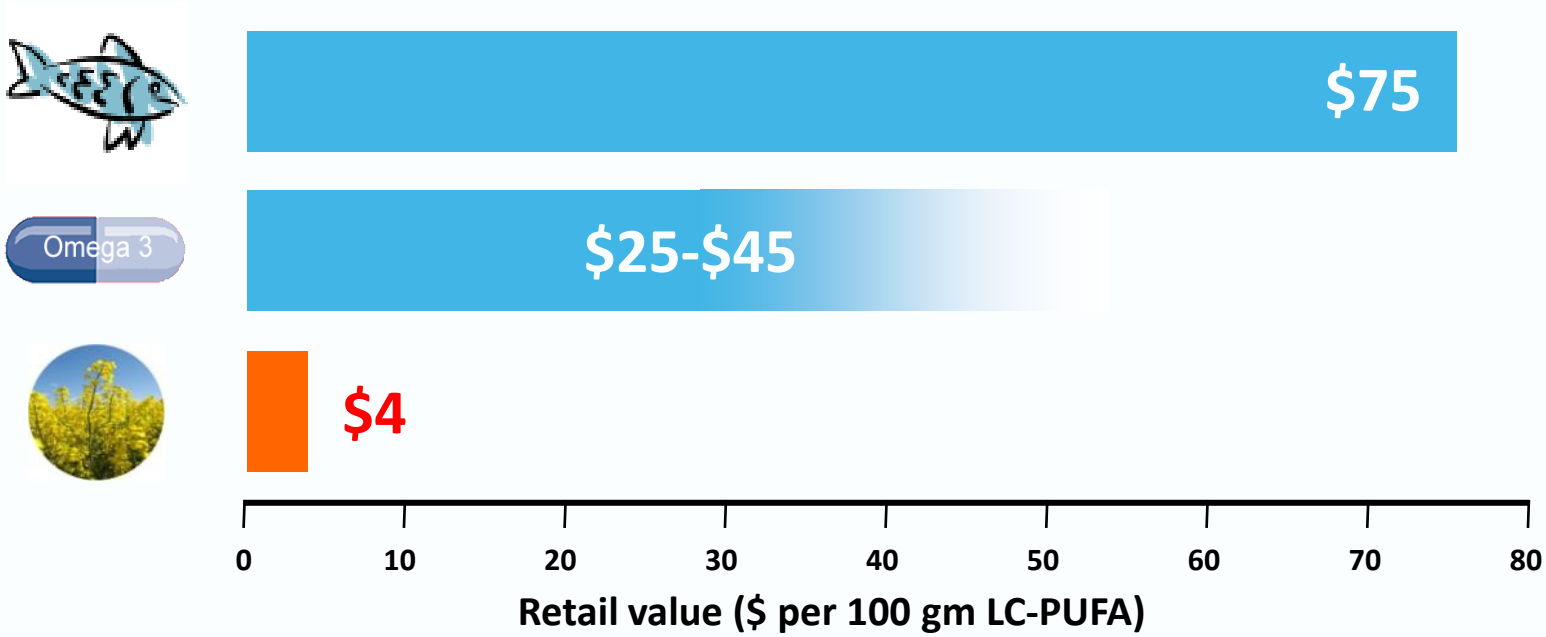
Alzheimers. Cellular health.

Engineering canola to produce EPA & DHA

Why develop plant sources?

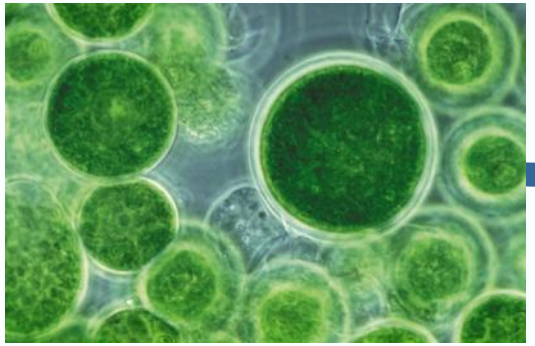
- Most people should consume more
- Plant ω3 (ALA) is poorly converted to DHA
- Wild fisheries are under threat or have collapsed
- Aquaculture relies unsustainably on fish-feed
- Safe, affordable and sustainable sources are needed

Plants can be low-cost sources of EPA & DHA



Affordable **Safe** **Sustainable**

Salmon @ $25/kg, 10% oil; Capsules @ $15 for 90x 1gm; EVOL @ $12 per L; All oils 33% EPA+DHA



Genes for synthesis of ω3 LC-PUFA can be transferred to plants

Palmitic 16:0 → **Stearic** 18:0 → **Oleic** 18:1 → **Linoleic** 18:2 → **Linolenic** 18:3

↓

Stearidonic
18:4 $^{\Delta 6,9,12,15}$
SDA

↓

Eicosopentaenoic
20:5 $^{\Delta 5,8,11,14,17}$
EPA

↓

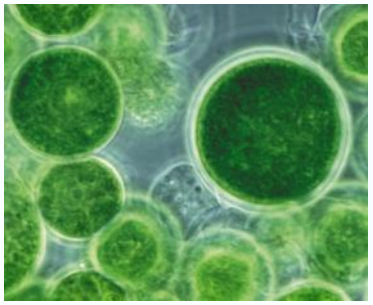
Docosohexaenoic
22:6 $^{\Delta 4,7,10,13,16,19}$
DHA

National Research FLAGSHIPS Food Futures — CSIRO

First publication of DHA synthesis in seeds (June 2005)

2% EPA & 1% DHA

Proof of concept with various gene sources



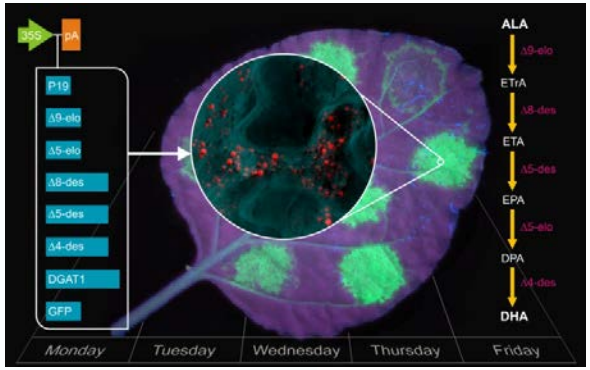
CSIRO

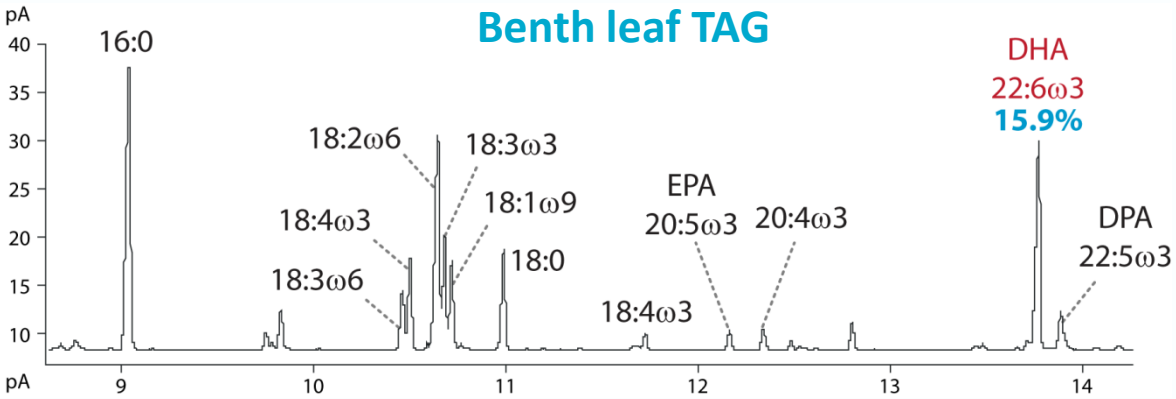
Assembling a more efficient DHA pathway

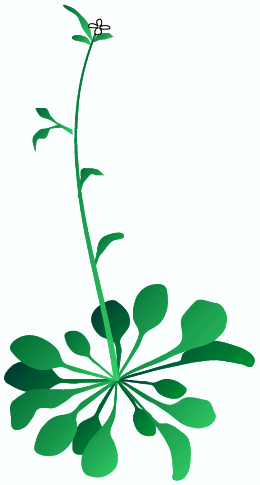
Australian National Algae Culture Collection

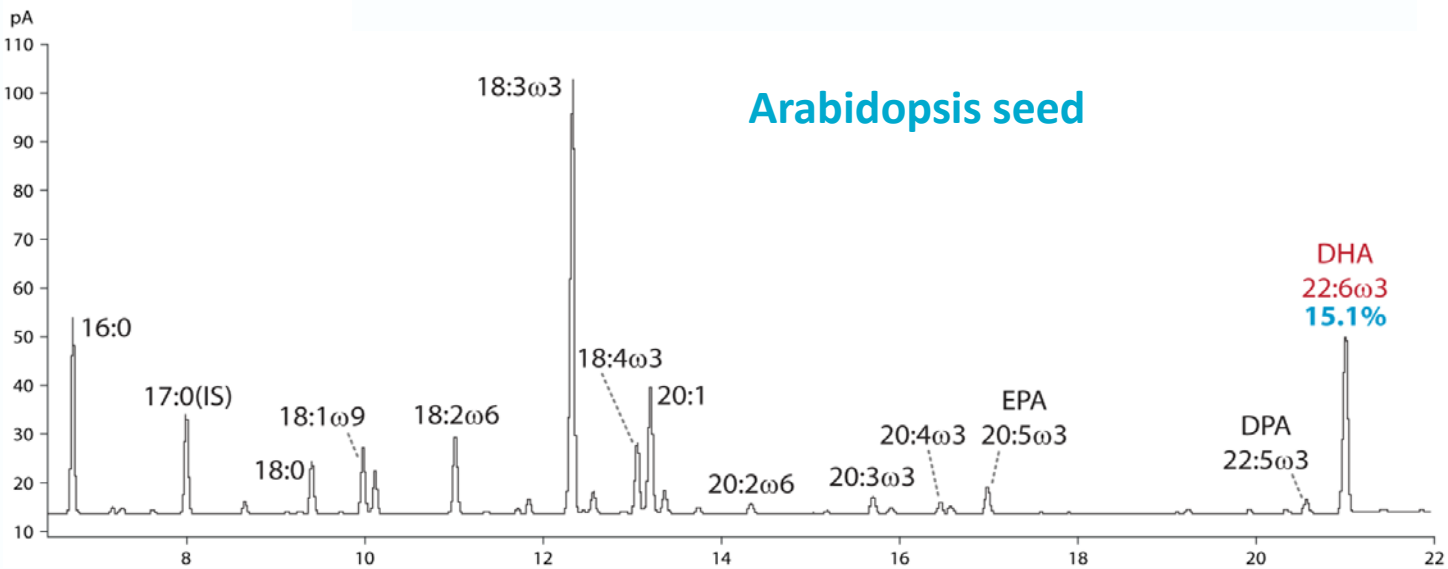
	18:1ω9, OA				
Δ12-des	↓			83%	*Lachancea kluyveri* **Unpublished**
	18:2ω6, LA				
ω3-des	↓			91%	*Pichia pastoris* **Yeast 25:21-27 (not CSIRO)**
	18:3ω3, ALA				
Δ6-des	↓	31%	→	46%	*Micromonas pusilla* **Metab Eng. 12:233-240**
	18:4ω3, SDA				
Δ6-elo	↓	66%	→	78%	*Pyramimonas cordata* **Marine Biotechnol. 4:430-438**
	20:4ω3, ETA				
Δ5-des	↓	74%	→	96%	*Pavlova salina* **Phytochem. 6:785-796**
	20:5ω3, EPA				
Δ5-elo	↓	34%	→	90%	*Pyramimonas cordata* **Marine Biotechnol. 4:430-438**
	22:5ω3, DPA				
Δ4-des	↓	91%		91%	*Pavlova salina* **Phytochem. 6:785-796**
	22:6ω3, DHA				

Fish oil-like levels of DHA in leaf and seed









Palmitic		Stearic		Oleic		Linoleic		Linolenic
16:0	→	18:0	→	18:1	→	18:2	→	18:3

↓

Stearidonic

18:4 $^{\Delta 6,9,12,15}$

SDA

↓

Eicosopentaenoic

20:5 $^{\Delta 5,8,11,14,17}$

EPA

↓

Docosohexaenoic

22:6 $^{\Delta 4,7,10,13,16,19}$

DHA

PLOS | ONE A peer-reviewed, open access journal

RESEARCH ARTICLE OPEN ACCESS

Metabolic Engineering Plant Seeds with Fish Oil-Like Levels of DHA

James R. Petrie[1], Pushkar Shrestha[1], Xue-Rong Zhou[2], Maged P. Mansour[1,3], Qing Liu[1], Srinivas Belide[1], Peter D. Nichols[1,3], Surinder P. Singh[1*]

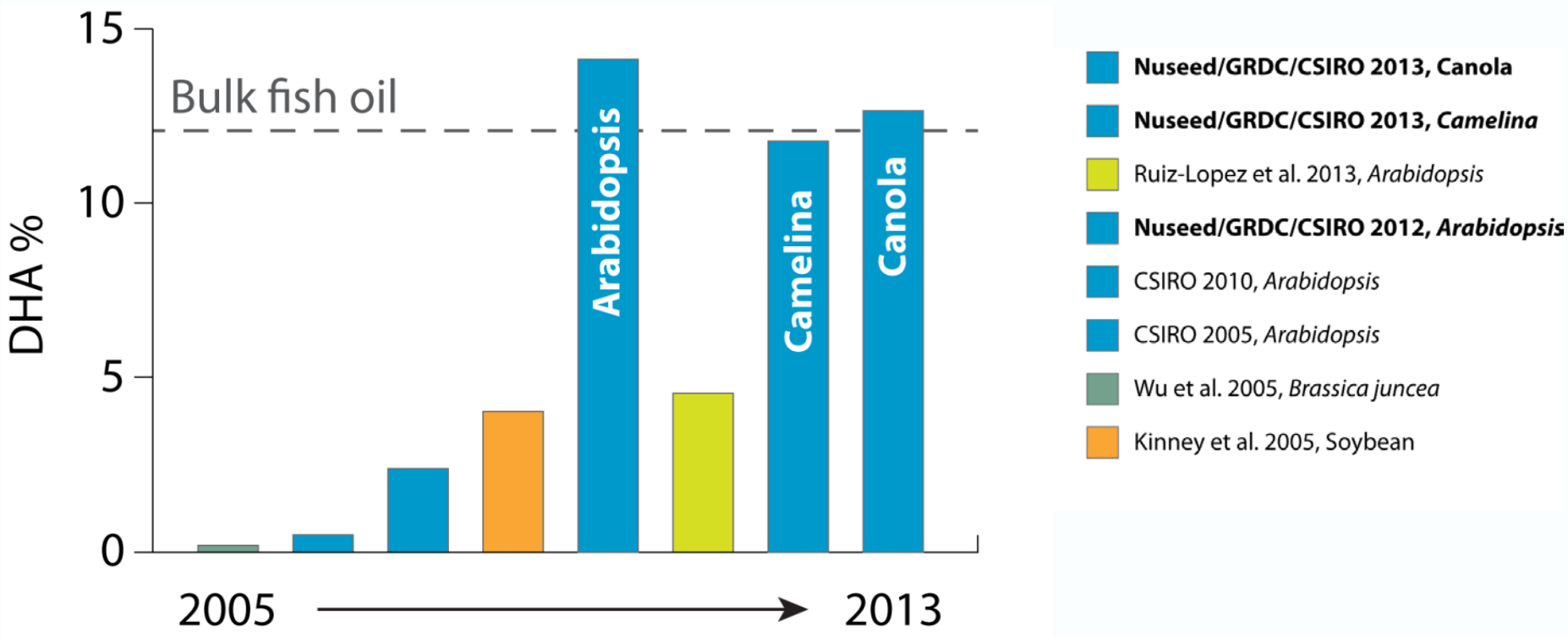
1 CSIRO Food Futures National Research Flagship, Canberra, Australian Capital Territory, Australia, 2 CSIRO Plant Industry, Canberra, Australian Capital Territory, Australia, 3 CSIRO Marine and Atmospheric Research, Hobart, Tasmania, Australia

PLoS ONE 7(11): e49165. doi:10.1371 (2012)

15% DHA in Arabidopsis seed oil

Equivalent to bulk fish oil

Progress in DHA biosynthesis in seed oils



1 HA canola = 10,000 fish

CSIRO

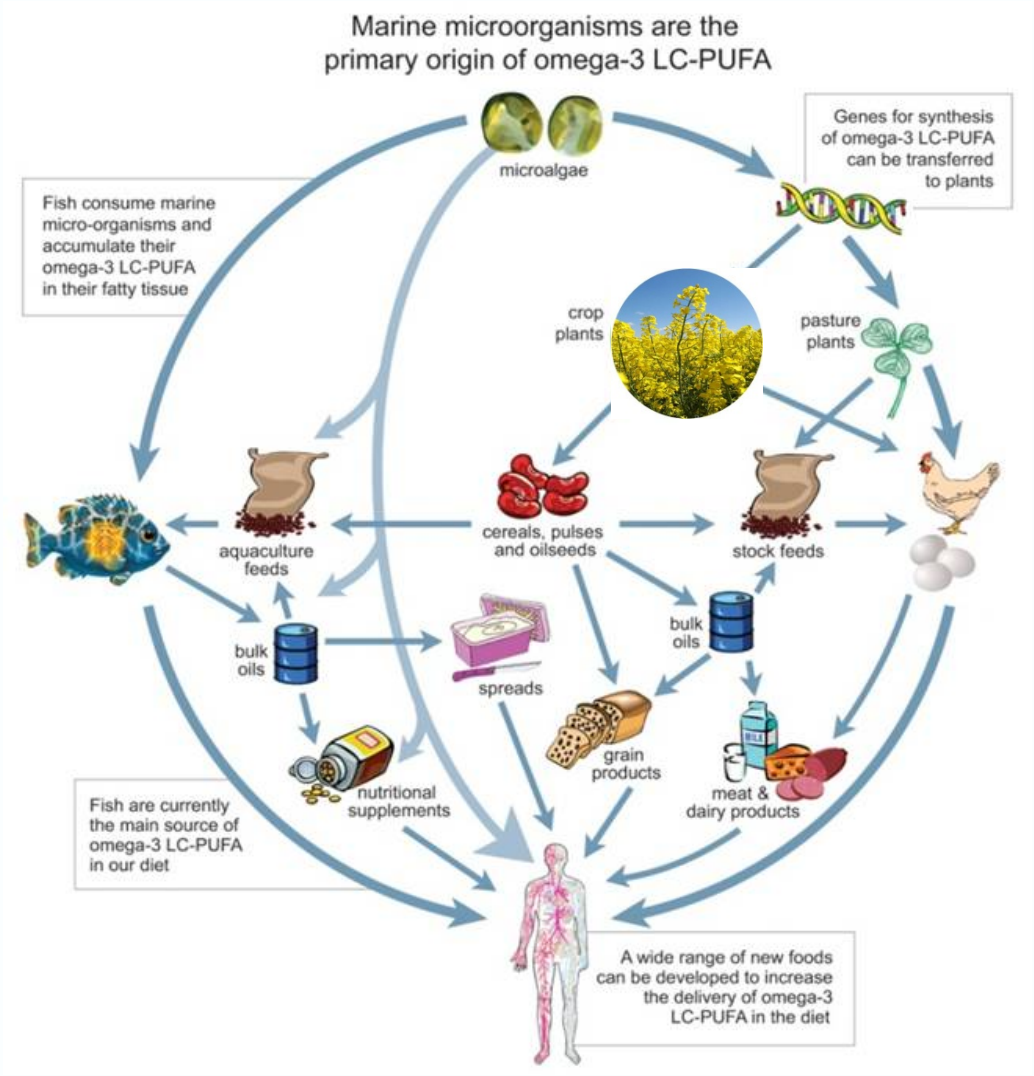
GRDC Grains Research & Development Corporation

nuSEED

DHA canola – moving to field trials in 2014

Selecting the best combination of DHA levels and agronomic performance. Target is commercial release in 2018.

Expanded LC-PUFA delivery routes



- **Whole-grains**

  multigrain breads, muesli products

- **Extracted oils**

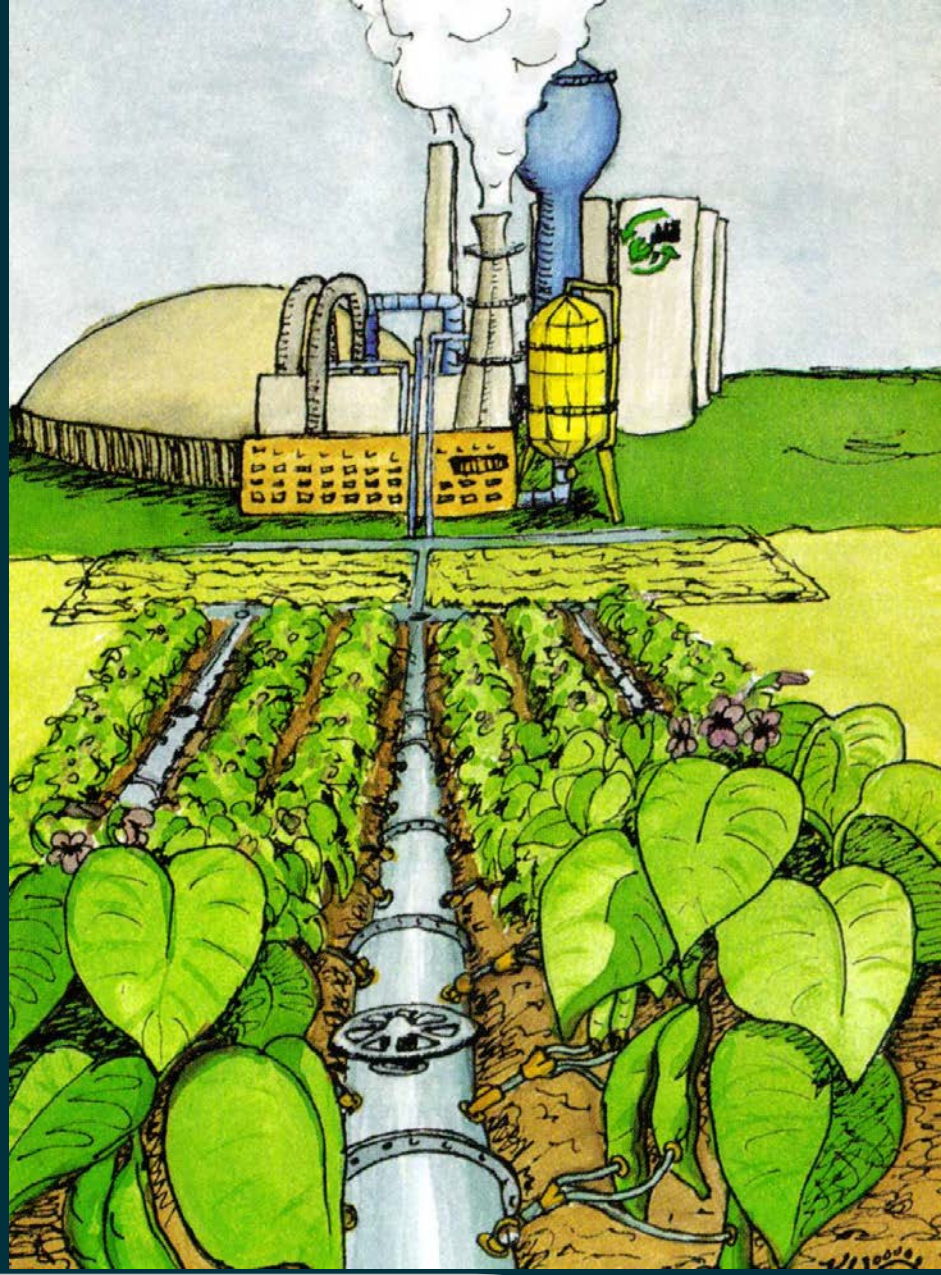
  spreads, salad oils, microencapsulated food ingredients

- **Livestock feeds**

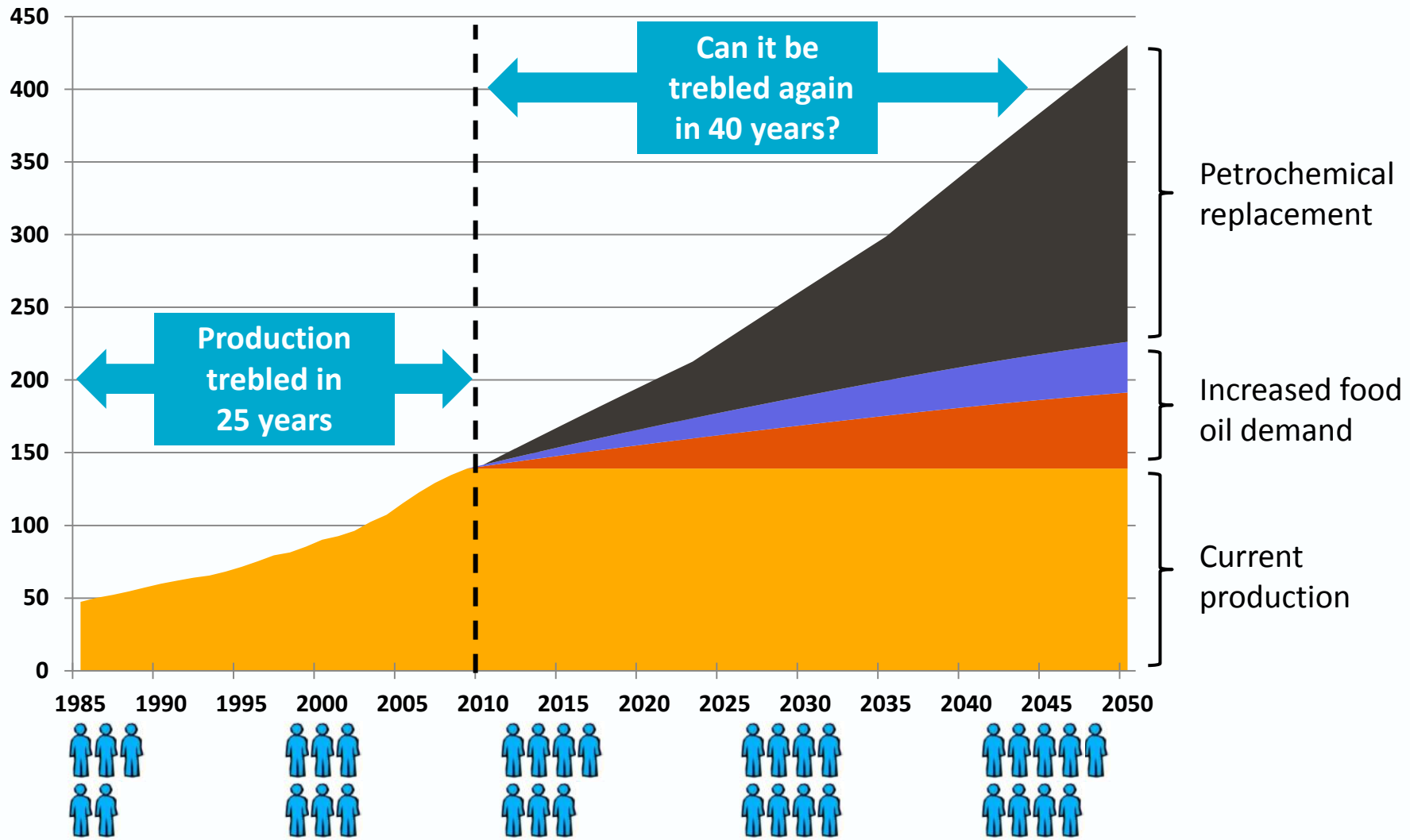
  enriched meat, milk and egg products

- **Aquaculture feeds**

SHO safflower



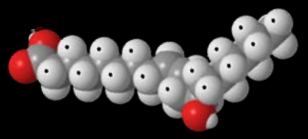
Expanding market demands

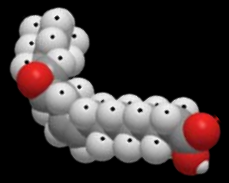


Oil plants can be genetically engineered

Introduce industrial functionality

- **Altered chain length**
  - Shorter chain (*FatB* & *KASII*)
  - Longer chain (acyl-CoA elongases)
- **Novel unsaturation**
  - Position and number of C=C
- **Novel functionalities**
  - Hydroxy, epoxy
  - Acetylenated, conjugated
  - Wax esters
- **High purity of specific fatty acids**
  - Enriched triglycerides





Provided oils can still be used for energy during germination

Crop Biofactories Initiative

Enabling a bio-based industrial economy

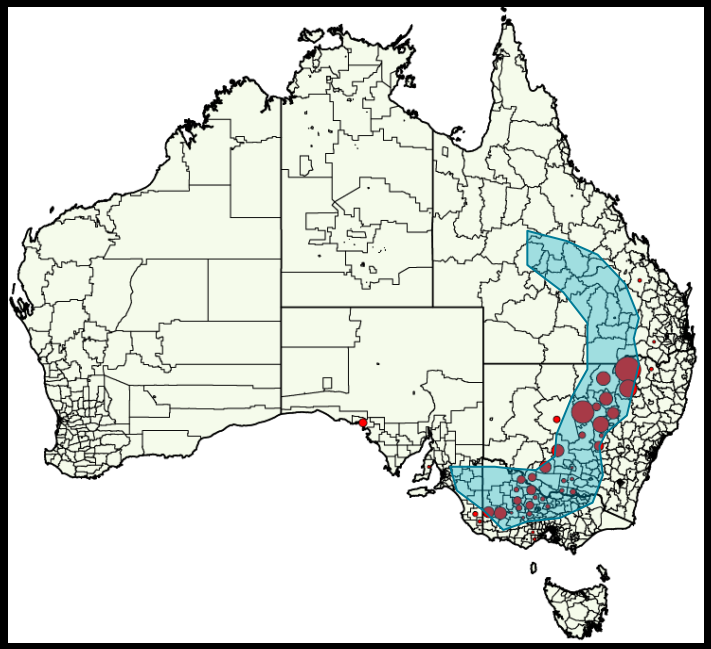
A strategic alliance between CSIRO and GRDC to develop high-value industrial crops for Australian growers.

CSIRO

Australian Government
Grains Research and Development Corporation

Why Safflower?

A known quantity

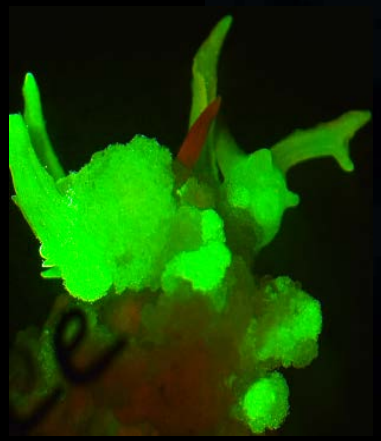


- ✓ Chosen by the grains industry
- ✓ Well-understood agronomy
- ✓ Potential to expand production
- ✓ Need for rotation crops in warmer regions
- ✓ Low use as food crop in Australia
- ✓ Industrial/GM segregation achievable

Why Safflower?

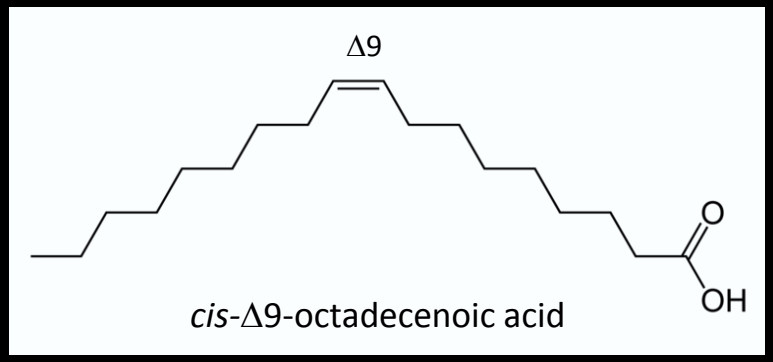
Technically ready

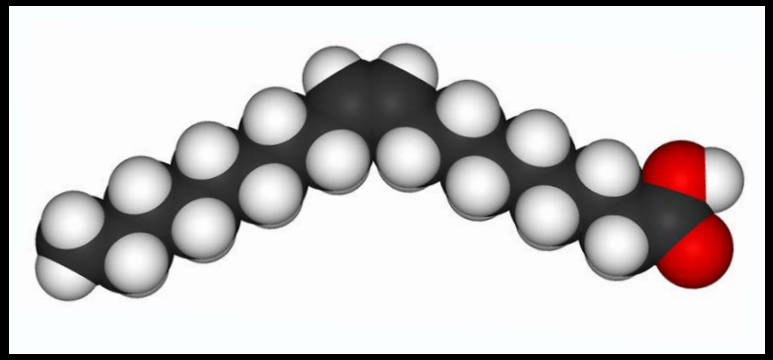
- ✓ Transformable (*Agrobacterium*)
- ✓ Fatty acid biosynthetic genes cloned
- ✓ Seed promoters available
- ✓ Favourable starting oil profiles
  - High-linoleic
  - High-oleic
  - No linolenic
- ✓ Genomic tools available



Industrial Oils

Oleic Acid





1. High oxidative stability
2. Biodegradability
3. High human safety
4. Direct use in bio-lubricants, hydraulic and dielectric fluids
5. Oleochemical precursor for biopolymer production
6. Platform for higher value derivatives

Industrial Oils

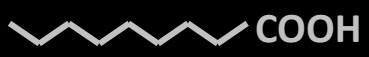
Oleic Acid feedstock for BioNylon

Oleic acid

[C18:1$^{\Delta 9}$]

Chemical oxidation

Separation & purification



Pelargonic acid

C9

Azelaic acid

C9

+

HMDA

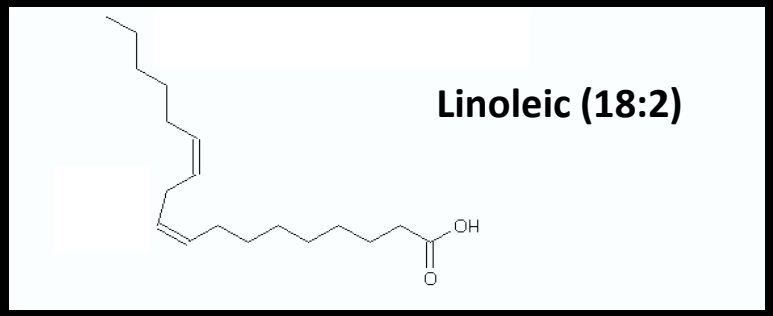
C6

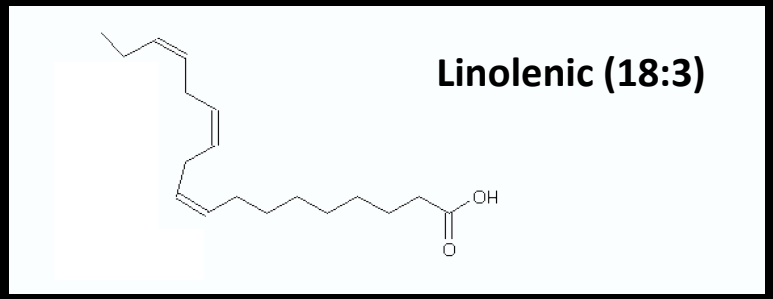
PA6,9 nylon

Industrial Oils

Polyunsaturates are problems

Several plant oils are good sources of oleic acid but they also have significant levels of undesirable polyunsaturates.





1. Promote auto-oxidation
   - 18:1 < 18:2 < 18:3 (1:4:10)
2. Produce short-chain (C3, C6) monomer contaminants during oxidative cleavage of oleic acid
   - Difficult/costly to separate

Maximising oleic acid levels

	Saturates	Oleic	Linoleic	Linolenic	
High Linoleic	9	17	73		***OL/OL***
High Oleic	7	77		16	***ol/ol***

- High-oleic safflower lines have around 75-80% oleic.
- Rest is mainly polyunsaturate (18:2) and saturates (16:0 & 18:0)
- Can oleic acid be raised to very high levels (> 90%) by further reducing the biosynthesis of polyunsaturates and saturates?

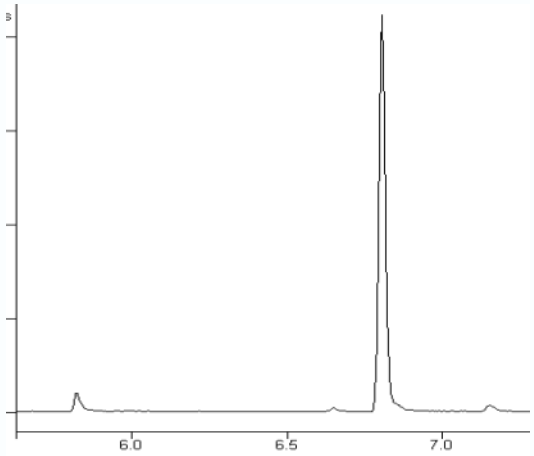
Super high-oleic safflower oil (SHO)

Palmitic		Stearic		Oleic		Linoleic
16:0	⇨	**18:0**	⇨	**18:1**	⇨	**18:2**

	16:0	18:0	18:1	18:2
High linoleic safflower	6	3	11	80
High oleic safflower	5	2	78	15

Super high-oleic safflower oil (SHO)

	16:0	18:0	18:1	18:2
High linoleic safflower	6	3	11	80
High-oleic safflower	5	2	78	15
SHO safflower	2	1	**95**	2

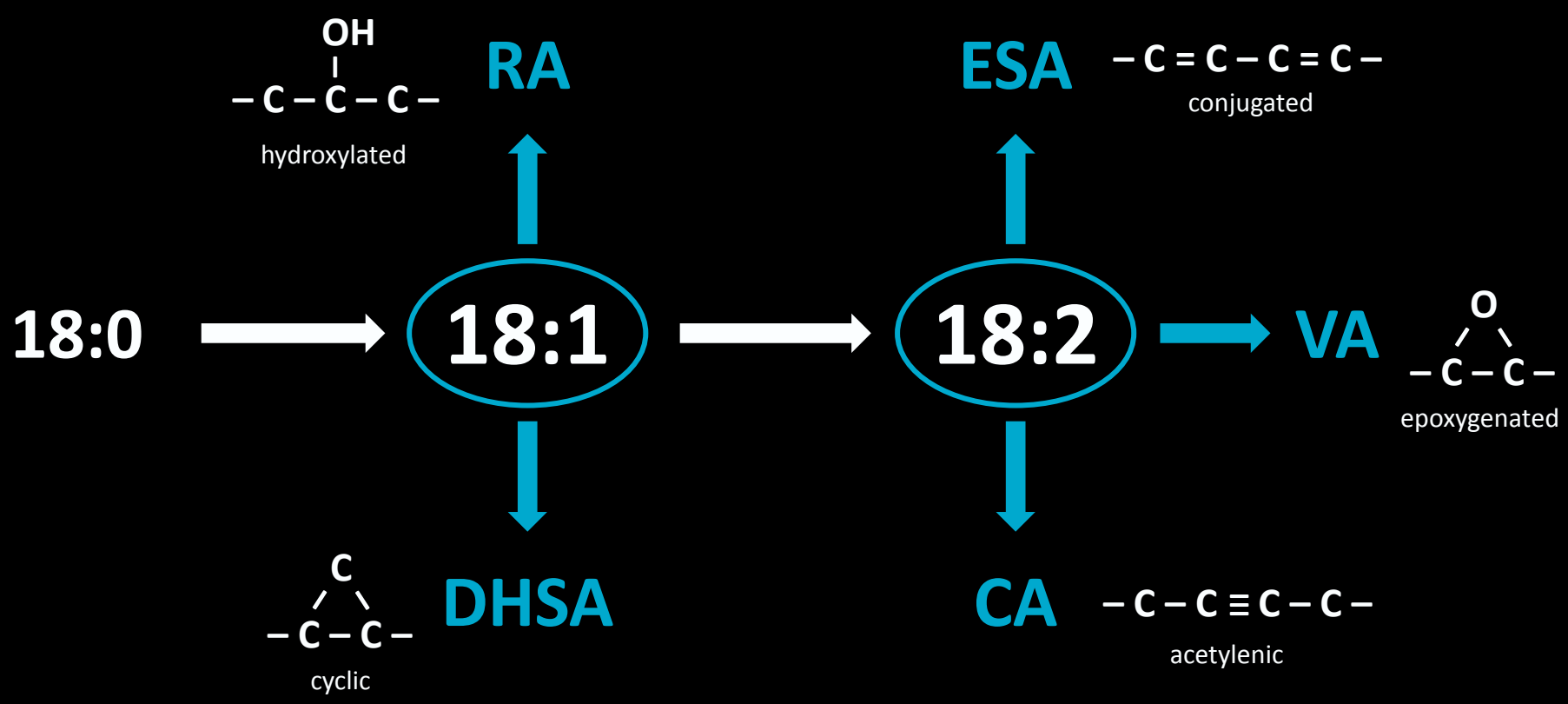


Development and commercialisation

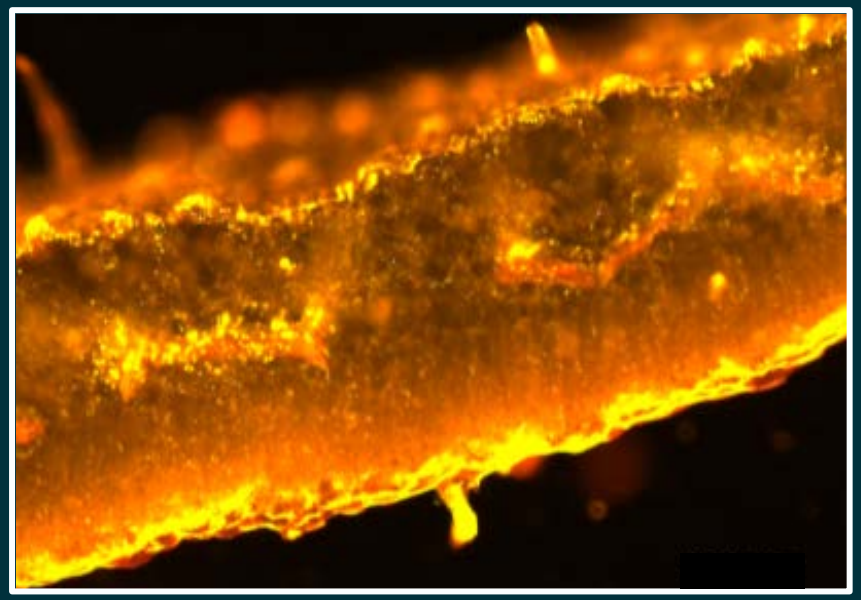
- Field trials Ginninderra (2013), Kununurra (2014)
- Expression of interest (under negotiation)
- 2018 target for commercialisation of SHO-Saff

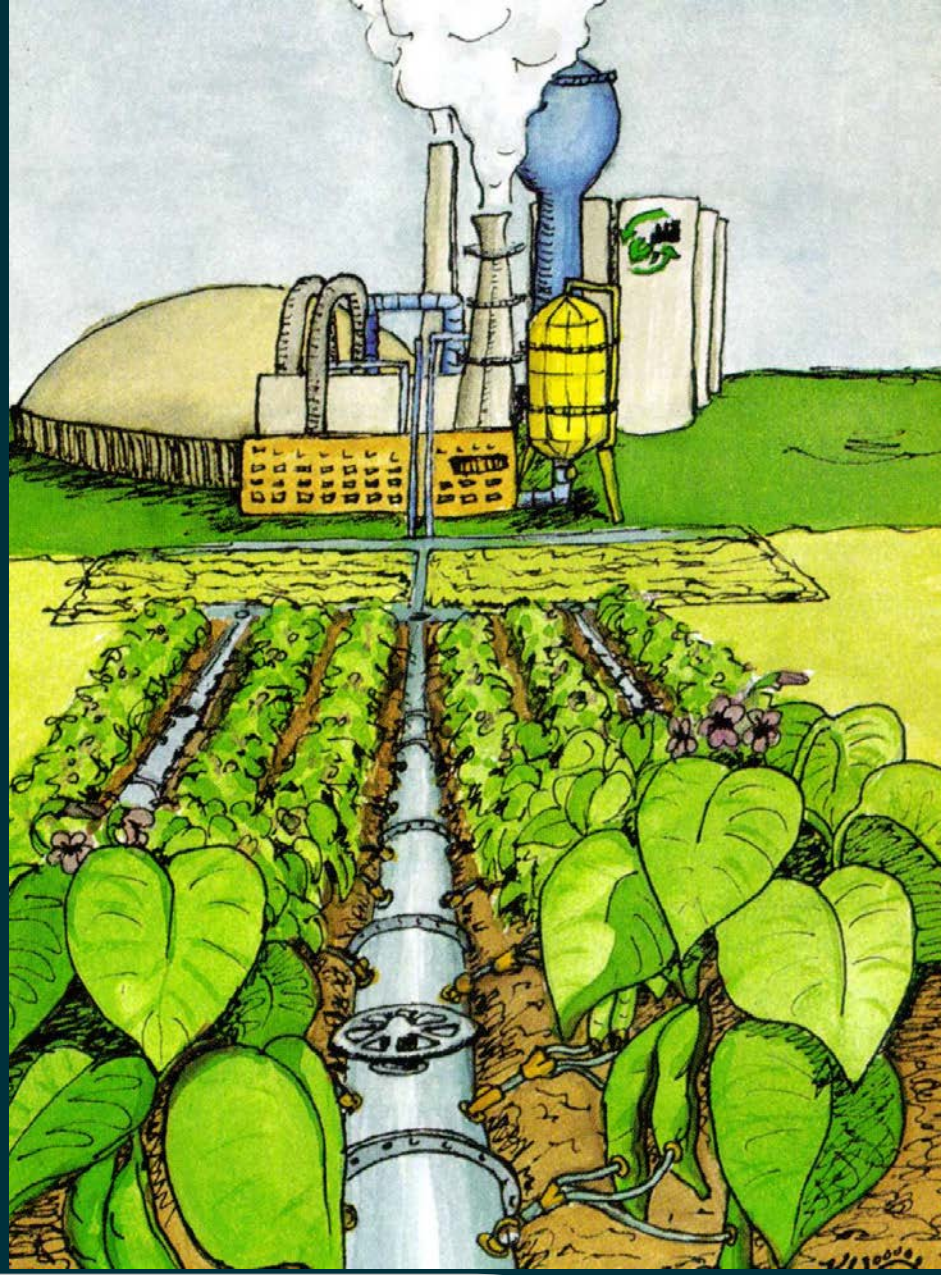
Industrial Fatty Acids

High-value derivatives of Oleic Acid



Leaf oils?





Global Plant Oil Supply Challenge

Can we treble global plant oil production by 2050 to provide enough surplus oil to replace 40% of petroleum products with renewables?

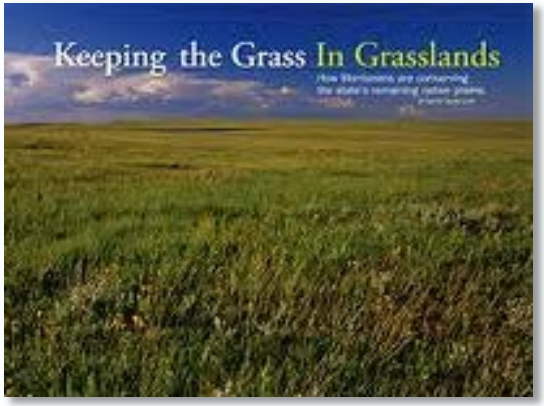
We can’t rely on acreage expansion

Arable land area is under increasing pressure...

Population

Degradation

Conservation



We can’t rely on acreage expansion

Arable land area is under increasing threat.

Rate of expansion will slow down significantly

- where can we expect to get the land needed?
- especially while increasing other food grains by 1 BT

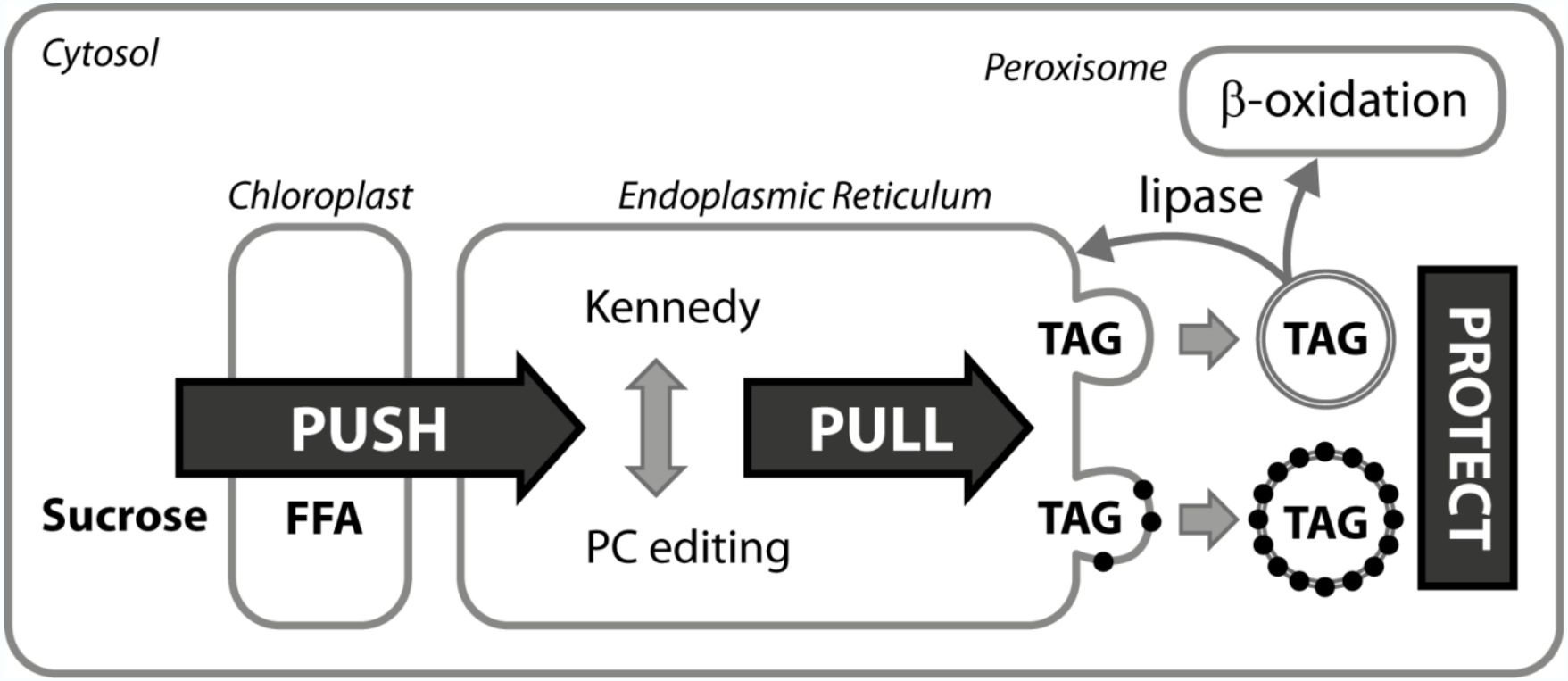
200 Mha + 60 Mha + 25 Mha

We can’t rely on acreage expansion

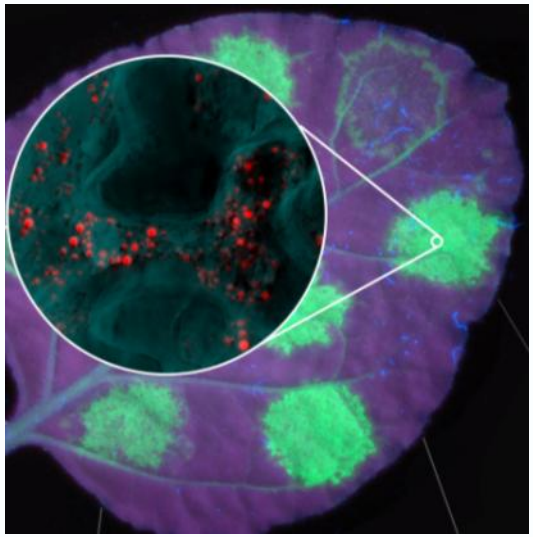
How can we engineer the **intensification** of plant oil production systems to produce more oil products from same area?

Exploring production of oils in non-seed tissues (leaves, tubers, etc)

Up-regulating oil synthesis in leaves

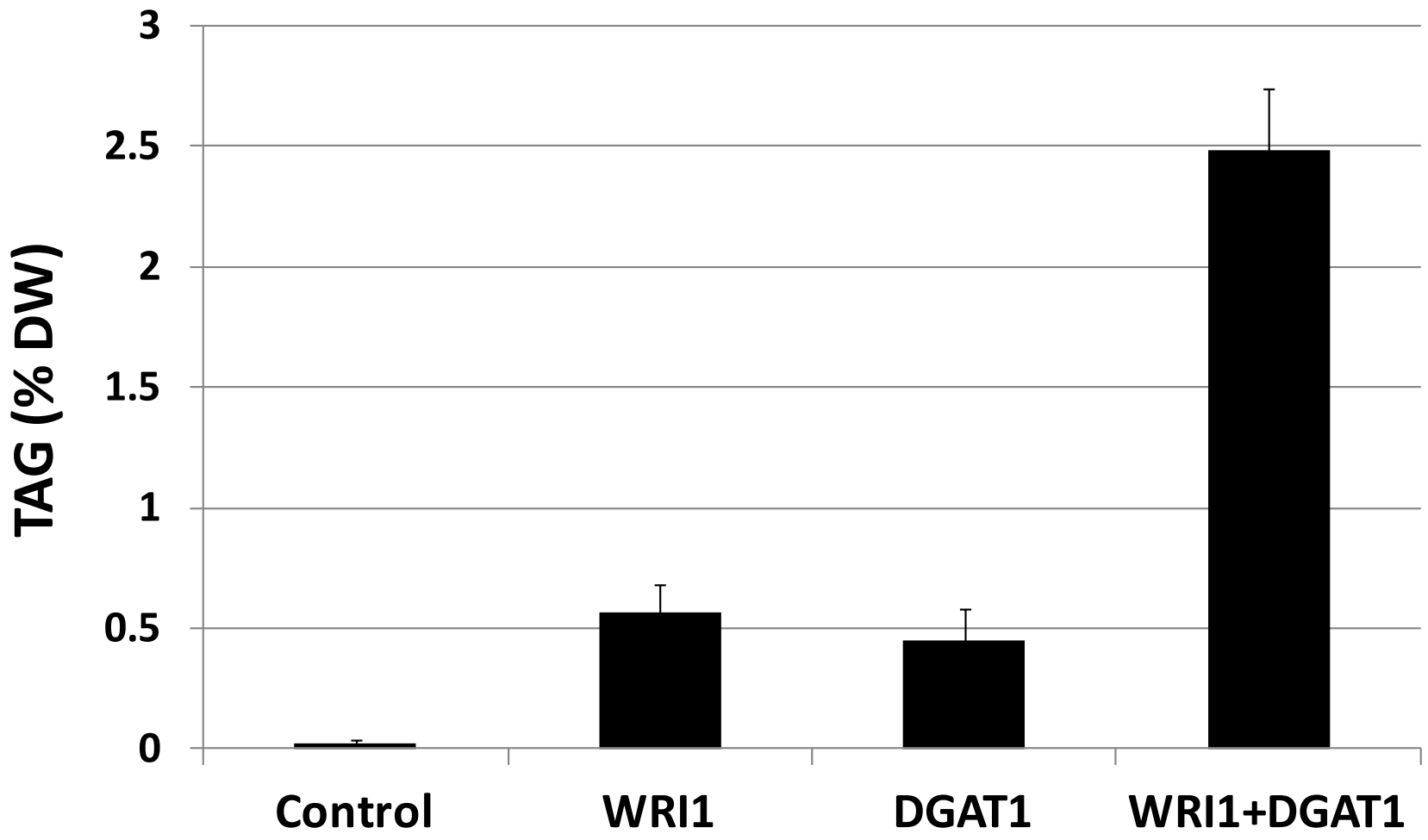


Transient expression in *N. benthamiana*



Rapid system (5 days) for *in planta* gene expression.
Enables combinatorial metabolic engineering.

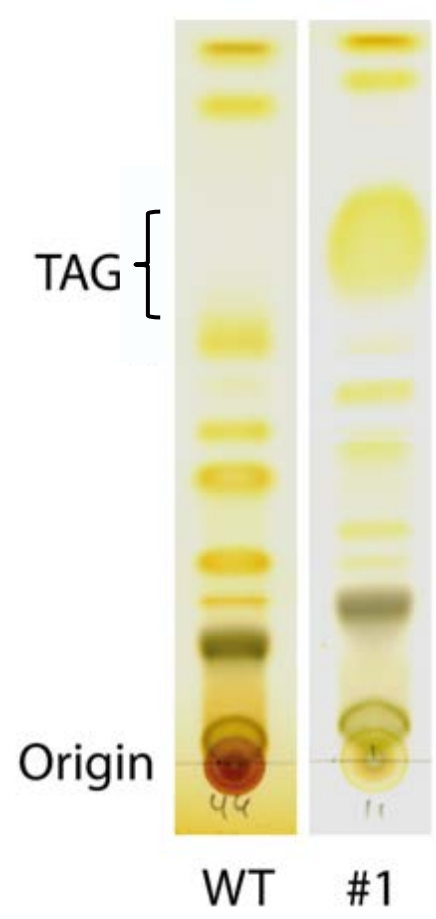
Push + Pull synergy demonstrated

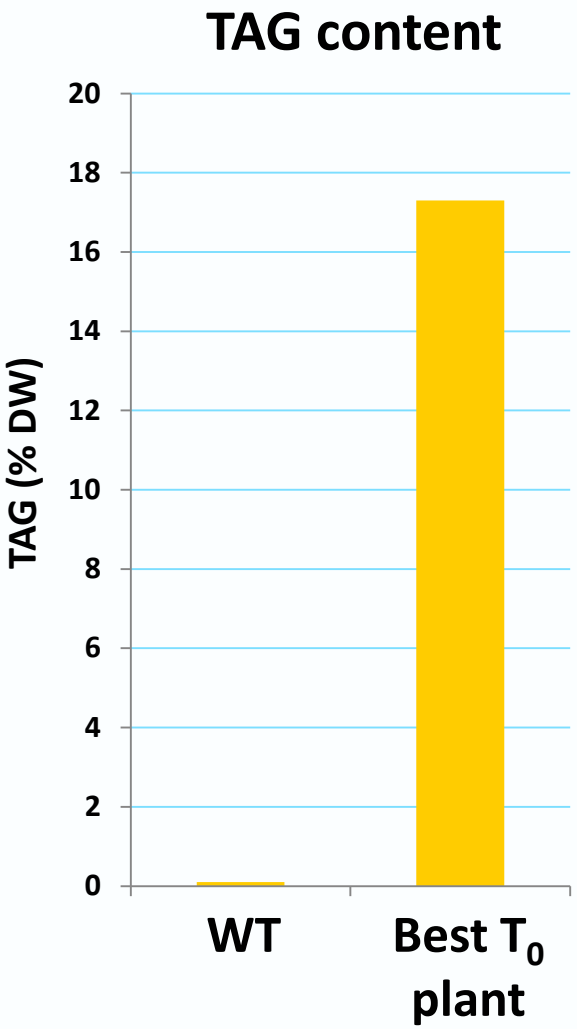


Testing in tobacco

High biomass species with stable transformation system

Testing in tobacco





Plant Biotechnology Journal

Research Article

Metabolic engineering of biomass for high energy density: oilseed-like triacylglycerol yields from plant leaves

Thomas Vanhercke[1], Anna El Tahchy[1], Qing Liu[1], Xue-Rong Zhou[2], Pushkar Shrestha[1], Uday K. Divi[1], Jean-Philippe Ral[1], Maged P. Mansour[3], Peter D. Nichols[3], Christopher N. James[4], Patrick J. Horn[4], Kent D. Chapman[4], Frederic Beaudoin[5], Noemi Ruiz-López[5], Philip J. Larkin[2], Robert C. de Feyter[2], Surinder P. Singh[1], James R. Petrie[1,*]

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Issue

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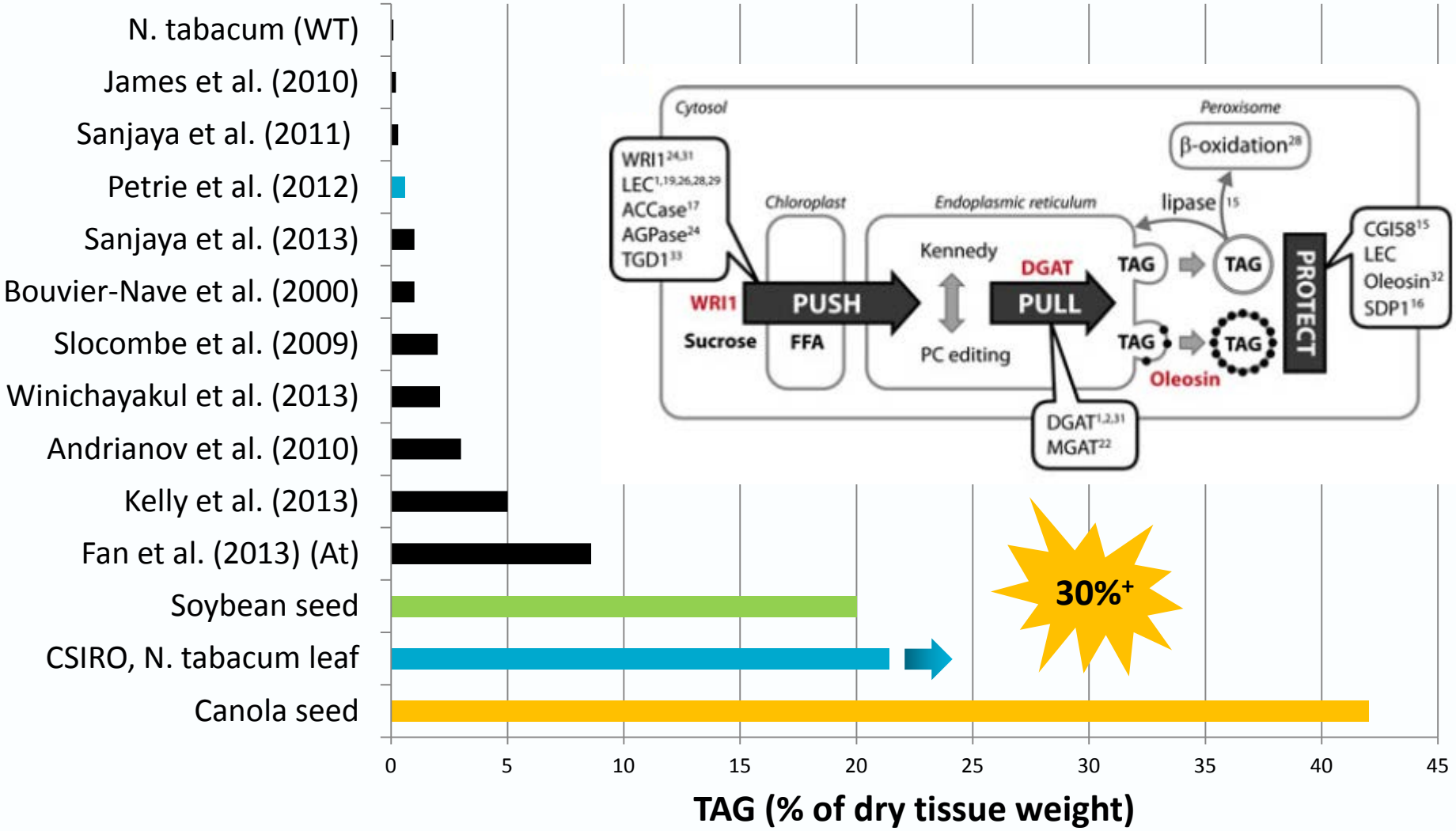
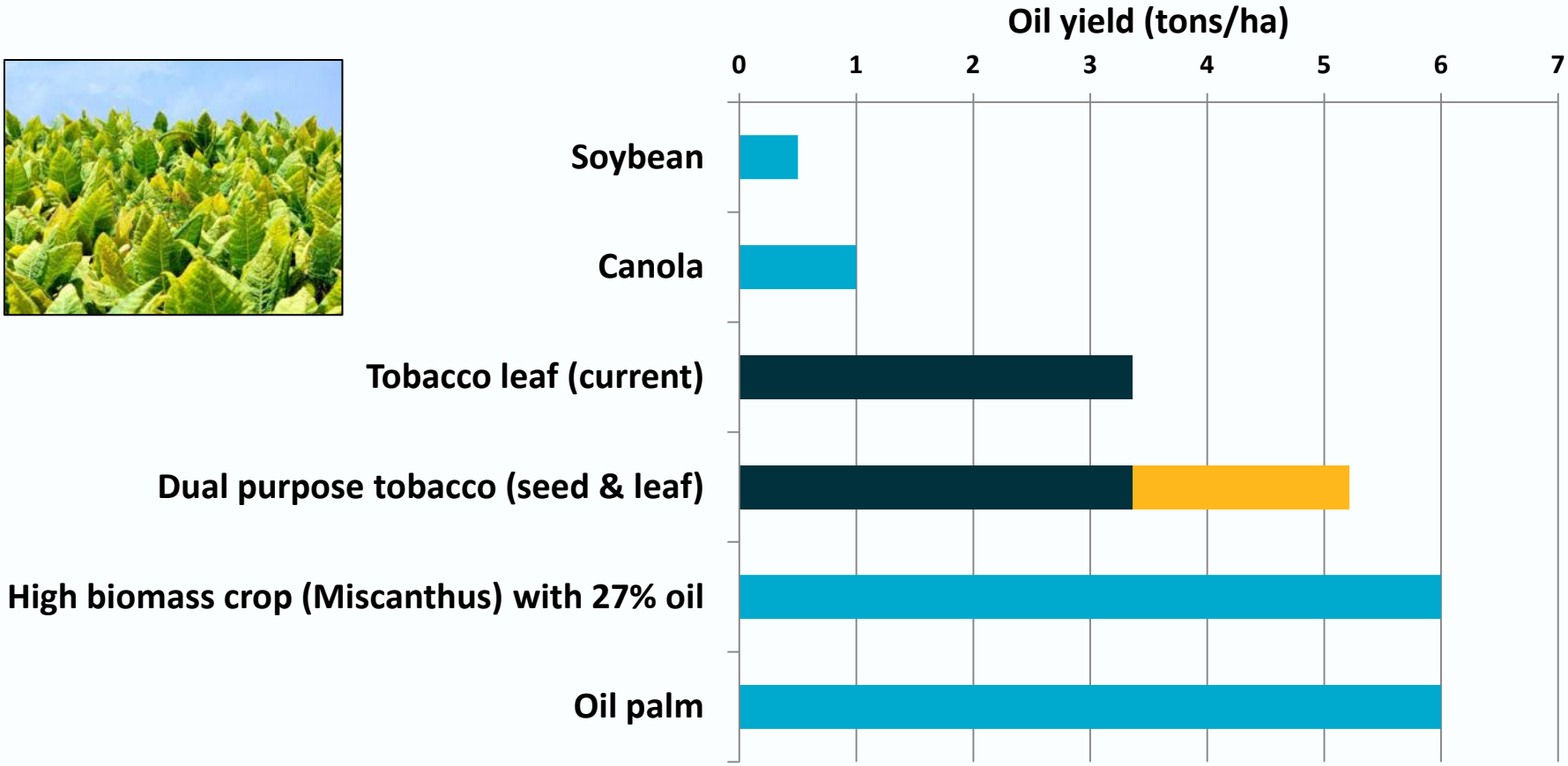
Push-Pull-Protect leads the way



Leaf oil could match oil palm productivity



Tobacco leaf

Dedicated leaf crop

Sorghum

Feed grain & fodder

Corn stover

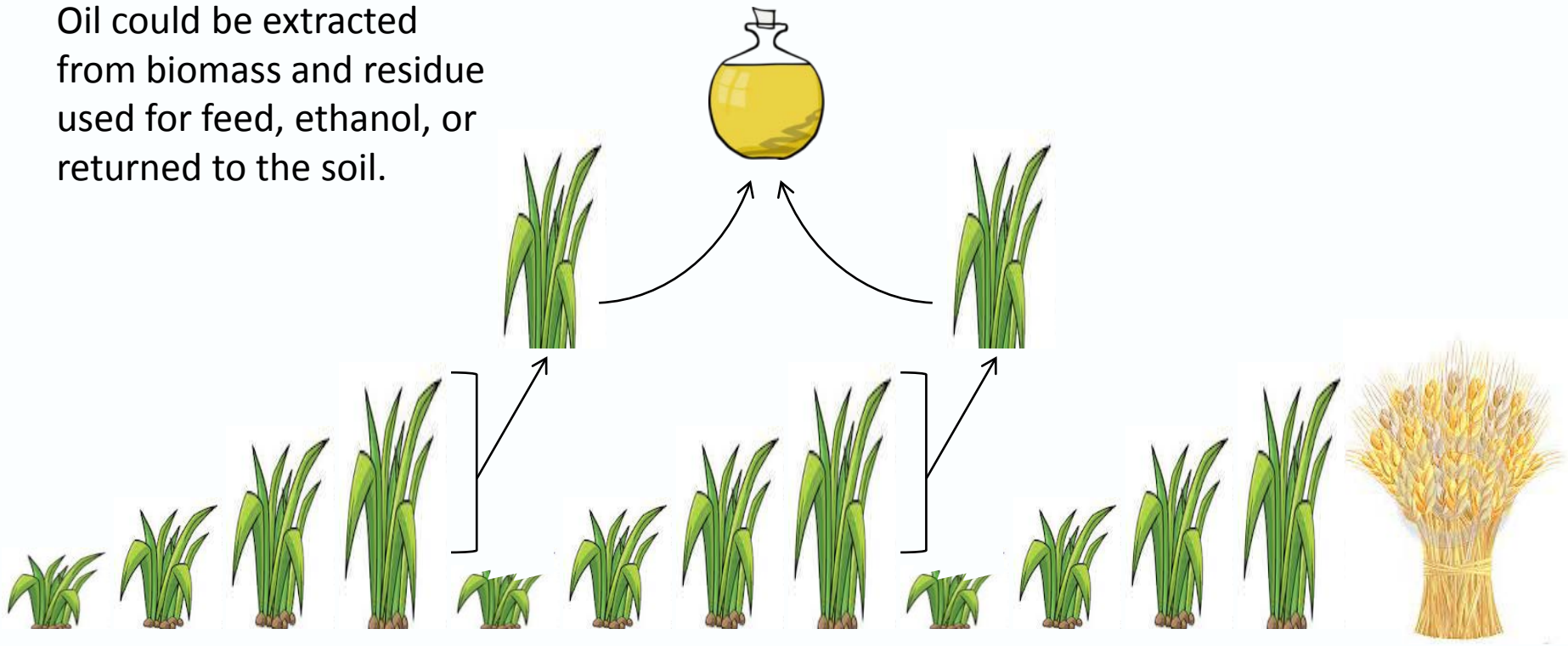
Crop residue

Cereal straw

Crop residue

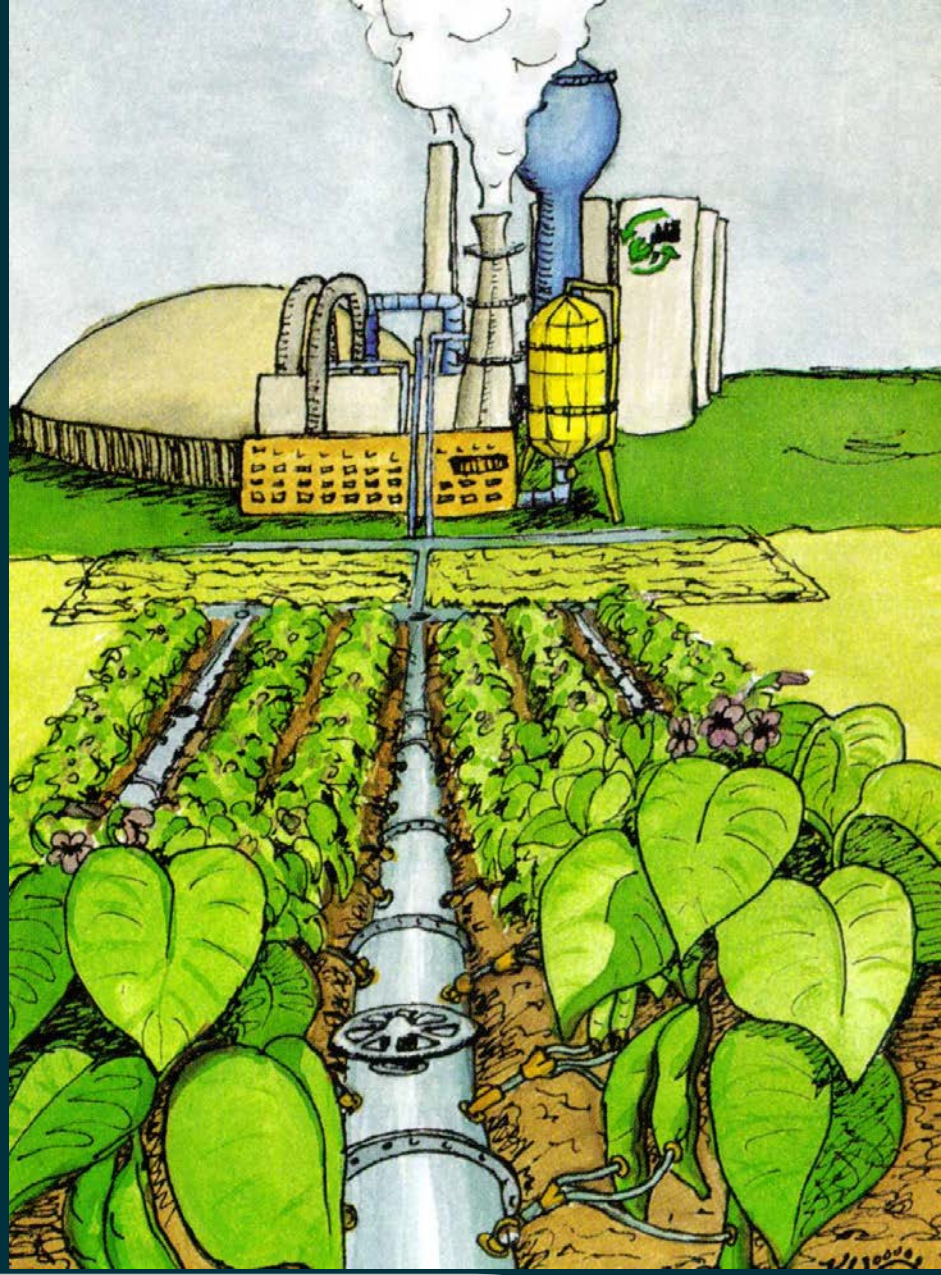
Dual-purpose biomass oil & grain crops

Oil could be extracted from biomass and residue used for feed, ethanol, or returned to the soil.



Long-season (winter) wheats can be repeatedly grazed <u>or cut for biomass</u> during growing season and still produce high grain yield at maturity

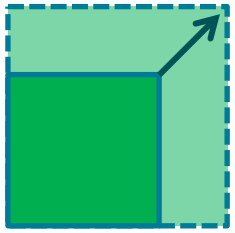
Oilseed lupin?



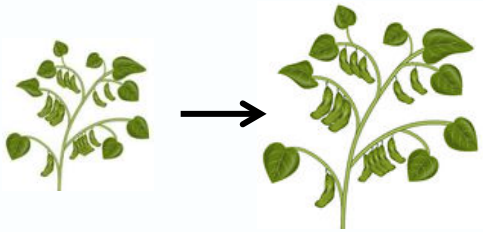
Past production increase factors

Trebling plant oil production over past 20 yrs was achieved by:-

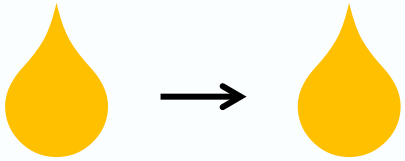
- **Doubling the area** sown to oil crops, especially oil palm, soybean and rapeseed/canola



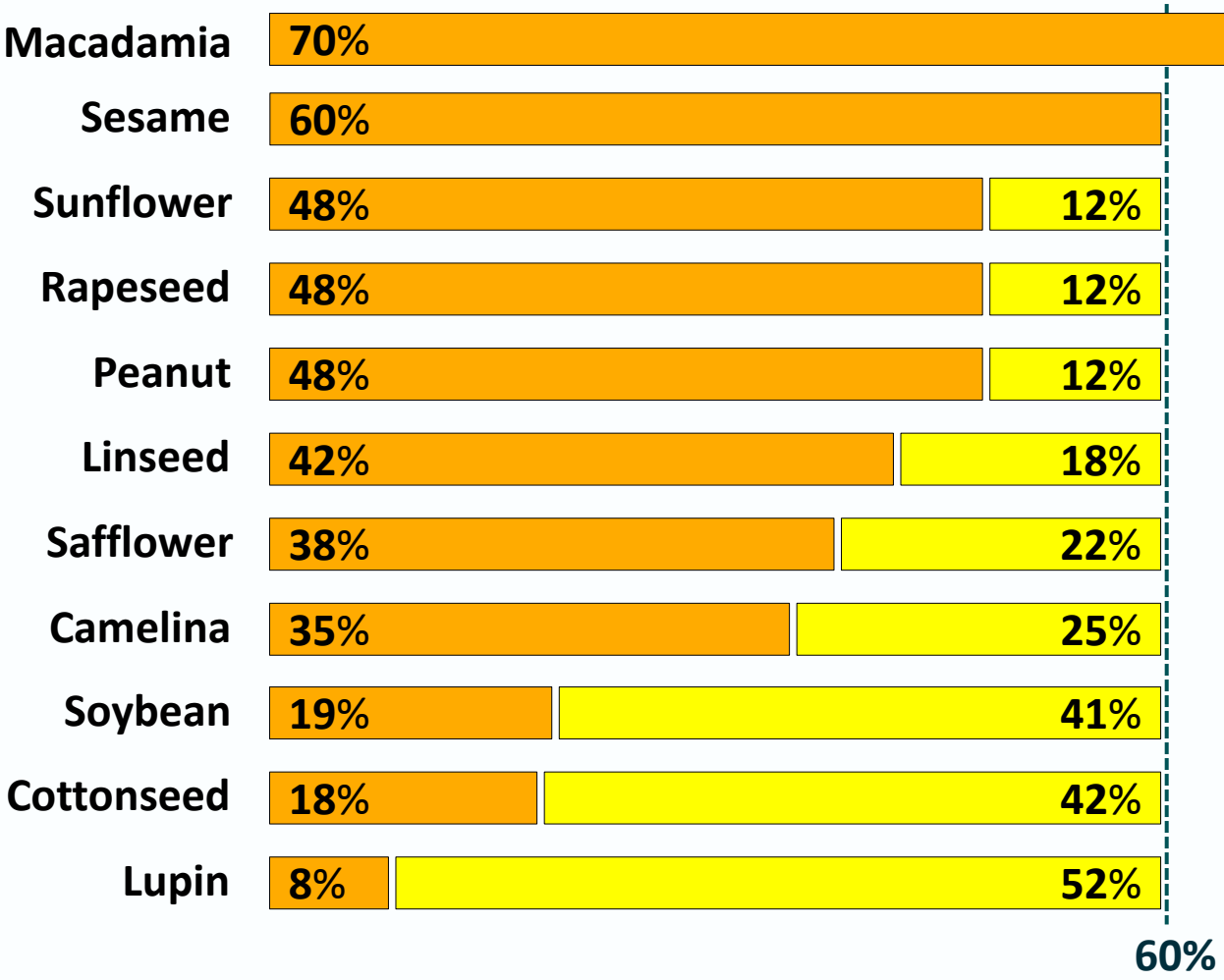
- Strong gains in oil yield per hectare through 40-50% **improvement in crop productivity**



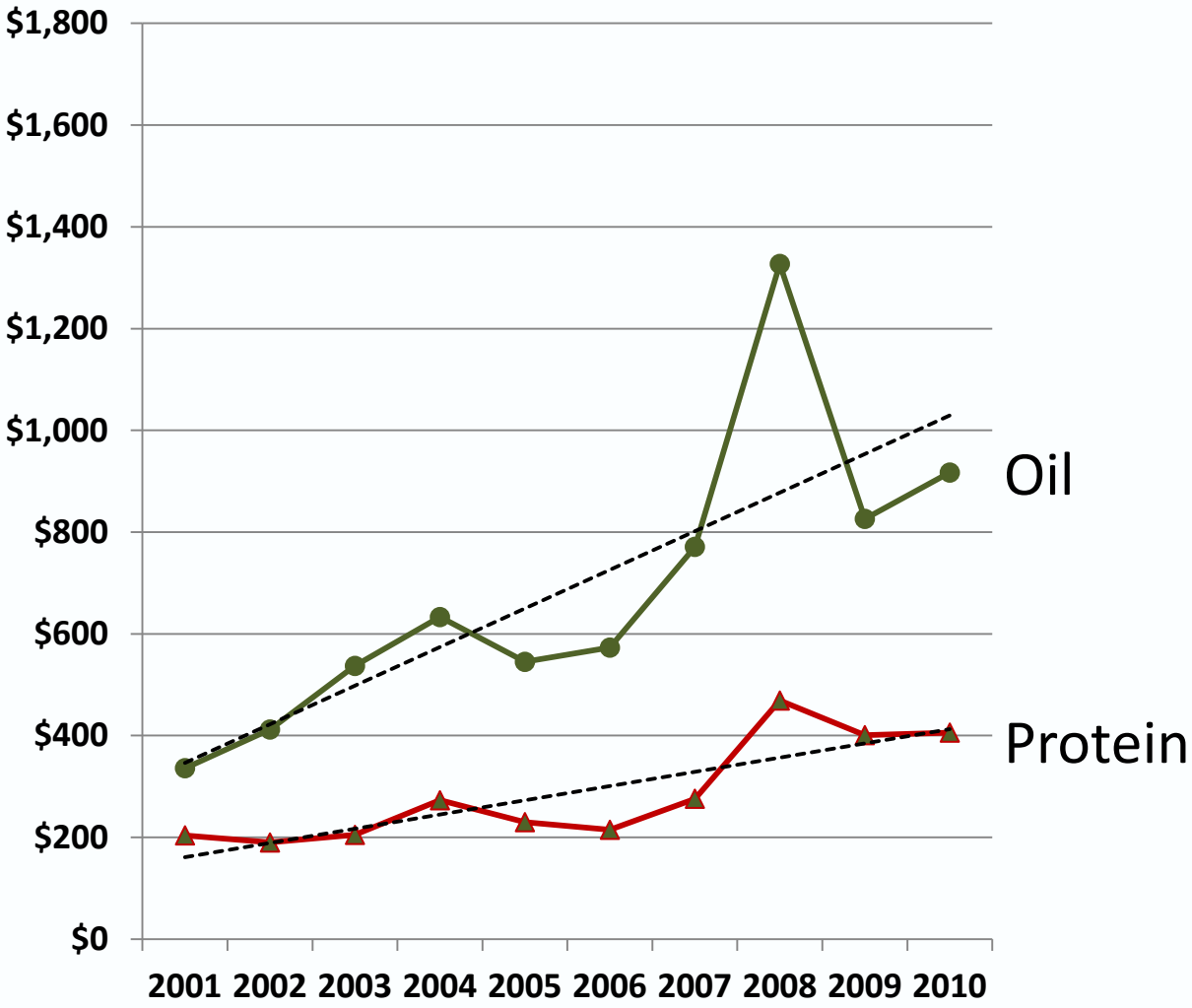
- Improvements in **oil content** were relatively minor



“Untapped” oil production potential



Converting lupin to an oilseed?



	@ 8%	@ 45%
Oil	-	$ 450
Meal*	-	$ 120
Total	-	**$ 570**
Crush	-	$ 80
Seed	**$ 250**	**$ 490**
		+ 96%

** Assuming meal value of $220/T*

Thank You

CSIRO Food, Nutrition & Bioproducts Flagship

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