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

Allan Green | Research Director - Bioproducts
INNOVATION in crop species
INNOVATION in technology

• F1 hybrid varieties

• GM herbicide tolerance

• GM insect resistance (cottonseed)
INNOVATION in oil quality

• High-oleic sunflower

• Linola (low linolenic linseed)

• Canola quality *Brassica juncea*

• High-oleic, low linolenic, reduced sats canola
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
Expanding market demands

- Current production
- Increased food oil demand
- Petrochemical replacement

Production trebled in 25 years
Can it be trebled again in 40 years?

Current production

Can it be trebled again in 40 years?
Biotech oils in the pipeline

Metabolic engineering oil synthesis
Biotech oils in the pipeline
Omega-3 canola
Engineering canola to produce EPA & DHA

Engineered oilseed crops with fish oil DHA levels

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

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.

- Developing an oilseed source of omega-3 long-chain (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, 18:3n3) and stearidonic acid (SDA, 18:4n3) (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 short-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).
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
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

<table>
<thead>
<tr>
<th>Retail value ($ per 100 gm LC-PUFA)</th>
<th>Affordable</th>
<th>Safe</th>
<th>Sustainable</th>
</tr>
</thead>
<tbody>
<tr>
<td>$75</td>
<td>$25-$45</td>
<td>$4</td>
<td></td>
</tr>
</tbody>
</table>

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.
First publication of DHA synthesis in seeds (June 2005)

2% EPA & 1% DHA

Proof of concept with various gene sources
Assembling a more efficient DHA pathway

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Product</th>
<th>Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ12-des</td>
<td>18:2ω6, LA</td>
<td>31% → 46%</td>
</tr>
<tr>
<td>ω3-des</td>
<td>18:3ω3, ALA</td>
<td>83%</td>
</tr>
<tr>
<td>Δ6-des</td>
<td>18:4ω3, SDA</td>
<td>66% → 78%</td>
</tr>
<tr>
<td>Δ6-elo</td>
<td>20:4ω3, ETA</td>
<td>74% → 96%</td>
</tr>
<tr>
<td>Δ5-des</td>
<td>20:5ω3, EPA</td>
<td>34% → 90%</td>
</tr>
<tr>
<td>Δ5-elo</td>
<td>22:5ω3, DPA</td>
<td>91%</td>
</tr>
<tr>
<td>Δ4-des</td>
<td>22:6ω3, DHA</td>
<td>91%</td>
</tr>
</tbody>
</table>

Sources:
- *Lachancea kuyveri*
  - Unpublished
- *Pichia pastoris*
  - Yeast 25:21-27 (not CSIRO)
- *Micromonas pusilla*
  - Metab Eng. 12:233-240
- *Pyramimonas cordata*
  - Marine Biotechnol. 4:430-438
- *Pavlova salina*
  - Phytochem. 6:785-796
Fish oil-like levels of DHA in leaf and seed

Benth leaf TAG
- DHA 22:6ω3 15.9%
- DPA 22:5ω3

Arabidopsis seed
- DHA 22:6ω3 15.1%
Metabolic Engineering Plant Seeds with Fish Oil-Like Levels of DHA

James R. Petrie, Pushkar Shrestha, Xue-Rong Zhou, Maged P. Mansour, Qing Liu, Srinivas Belide, Peter D. Nichols, Surinder P. Singh

15% DHA in Arabidopsis seed oil
Equivalent to bulk fish oil
Progress in DHA biosynthesis in seed oils

1 HA canola = 10,000 fish

A joint venture between CSIRO, GRDC and NuSeed
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

Production trebled in 25 years

Can it be trebled again in 40 years?

- Petrochemical replacement
- Increased food oil demand
- Current production

Current production

Increased food oil demand

Petrochemical replacement

Can it be trebled again in 40 years?

Production trebled in 25 years

Can it be trebled again in 40 years?
Oil plants can be genetically engineered to 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.
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
Oleic acid
[C18:1\(\Delta^9\)]

Chemical oxidation

Separation & purification

Pelargonic acid
C9

Azelaic acid
C9

HMDA
C6

PA6,9 nylon

Industrial Oils
Oleic Acid feedstock for BioNylon
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

<table>
<thead>
<tr>
<th></th>
<th>Saturates</th>
<th>Oleic</th>
<th>Linoleic</th>
<th>Linolenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Linoleic</td>
<td>9</td>
<td>17</td>
<td>73</td>
<td>OL/OL</td>
</tr>
<tr>
<td>High Oleic</td>
<td>7</td>
<td>77</td>
<td>16</td>
<td>ol/ol</td>
</tr>
</tbody>
</table>

- 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)

<table>
<thead>
<tr>
<th></th>
<th>Palmitic (16:0)</th>
<th>Stearic (18:0)</th>
<th>Oleic (18:1)</th>
<th>Linoleic (18:2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High linoleic safflower</td>
<td>6</td>
<td>3</td>
<td>11</td>
<td>80</td>
</tr>
<tr>
<td>High oleic safflower</td>
<td>5</td>
<td>2</td>
<td>78</td>
<td>15</td>
</tr>
</tbody>
</table>
Super high-oleic safflower oil (SHO)

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<th>Linoleic</th>
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<tr>
<td>16:0</td>
<td>18:0</td>
<td>18:1</td>
<td>18:2</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
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</tr>
<tr>
<td>High-oleic safflower</td>
<td>5</td>
<td>2</td>
<td>78</td>
<td>15</td>
</tr>
<tr>
<td>SHO safflower</td>
<td>2</td>
<td>1</td>
<td>95</td>
<td>2</td>
</tr>
</tbody>
</table>
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

18:0 \rightarrow 18:1 \rightarrow 18:2 \rightarrow VA

OH
\begin{array}{c}
\text{hydroxylated}
\end{array}

RA

ESA

\begin{array}{c}
\text{conjugated}
\end{array}

DHSA

CA

\begin{array}{c}
\text{cyclic}
\end{array}

\begin{array}{c}
\text{epoxygenated}
\end{array}

\begin{array}{c}
\text{acetylenic}
\end{array}
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

Diagram showing the processes involved in oil synthesis, including:

- Cytosol
- Chloroplast
- Endoplasmic Reticulum
- Peroxisome

Key processes:
- PUSH:
  - Sucrose
  - FFA
- PULL:
  - Kennedy
  - PC editing
  - Lipase
  - TAG
- PROTECT:
  - β-oxidation

TAGs are transferred between these processes.
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

TAG content

WT

Best T₀ plant

TAG (% DW)

0 2 4 6 8 10 12 14 16 18 20

TAG

Origin

WT #1

WT

Best T₀ plant

WT

Best T₀ plant

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

Thomas Vanhercke¹, Anna El Tahchy¹, Qing Liu¹, Xue-Rong Zhou², Pushkar Shrestha¹, Uday K. Divi¹, Jean-Philippe Ral¹, Maged P. Mansour³, Peter D. Nichols³, Christopher N. James⁴, Patrick J. Horn⁴, Kent D. Chapman⁴, Frederic Beaudoin⁵, Noemi Ruiz-López⁵, Philip J. Larkin², Robert C. de Feyter², Surinder P. Singh¹, James R. Petrie¹,*

Article first published online: 24 OCT 2013
**Push-Pull-Protect leads the way**

- N. tabacum (WT)
- James et al. (2010)
- Sanjaya et al. (2011)
- Petrie et al. (2012)
- Sanjaya et al. (2013)
- Bouvier-Nave et al. (2000)
- Slocombe et al. (2009)
- Winichayakul et al. (2013)
- Andrianov et al. (2010)
- Kelly et al. (2013)
- Fan et al. (2013) (At)
- Soybean seed
- CSIRO, N. tabacum leaf
- Canola seed

Tag (% of dry tissue weight)

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**Figure:** Diagram showing the TAG (triglyceride) content in different materials:

- **PUSH** section with WRI1, Sucre, FFA, PC editing, and ENDOPPLASMIC RETICULUM.
- **PULL** section with DGAT, TAG, and Oleosin.
- **PROTECT** section with CGI58, LEC, Oleosin, and SDP1.

The diagram highlights the TAG content across different materials, with a specific focus on Canola seed reaching 30%+.
Leaf oil could match oil palm productivity

- Soybean
- Canola
- Tobacco leaf (current)
- Dual purpose tobacco (seed & leaf)
- High biomass crop (Miscanthus) with 27% oil
- Oil palm

Oil yield (tons/ha)
Tobacco leaf
Dedicated leaf crop
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 or cut for biomass 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

<table>
<thead>
<tr>
<th>Crop</th>
<th>Production Potential</th>
<th>Utilization Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macadamia</td>
<td>70%</td>
<td>60%</td>
</tr>
<tr>
<td>Sesame</td>
<td>60%</td>
<td>48%</td>
</tr>
<tr>
<td>Sunflower</td>
<td>48%</td>
<td>12%</td>
</tr>
<tr>
<td>Rapeseed</td>
<td>48%</td>
<td>12%</td>
</tr>
<tr>
<td>Peanut</td>
<td>48%</td>
<td>12%</td>
</tr>
<tr>
<td>Linseed</td>
<td>42%</td>
<td>18%</td>
</tr>
<tr>
<td>Safflower</td>
<td>38%</td>
<td>22%</td>
</tr>
<tr>
<td>Camelina</td>
<td>35%</td>
<td>25%</td>
</tr>
<tr>
<td>Soybean</td>
<td>19%</td>
<td>41%</td>
</tr>
<tr>
<td>Cottonseed</td>
<td>18%</td>
<td>42%</td>
</tr>
<tr>
<td>Lupin</td>
<td>8%</td>
<td>52%</td>
</tr>
</tbody>
</table>

60%
Converting lupin to an oilseed?

### Values at 8% and 45% Lupin Moisture

<table>
<thead>
<tr>
<th></th>
<th>@ 8%</th>
<th>@ 45%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>-</td>
<td>$450</td>
</tr>
<tr>
<td>Meal*</td>
<td>-</td>
<td>$120</td>
</tr>
<tr>
<td>Total</td>
<td>-</td>
<td>$570</td>
</tr>
<tr>
<td>Crush</td>
<td>-</td>
<td>$80</td>
</tr>
<tr>
<td>Seed</td>
<td>$250</td>
<td>$490</td>
</tr>
</tbody>
</table>

**Total:** $570

*Assuming meal value of $220/T

**Lupin Values:**

- **Oil**
  - 2001: $0
  - 2002: $200
  - 2003: $400
  - 2004: $600
  - 2005: $800
  - 2006: $1,000
  - 2007: $1,200
  - 2008: $1,400
  - 2009: $1,600
  - 2010: $1,800

- **Protein**
  - 2001: $0
  - 2002: $200
  - 2003: $400
  - 2004: $600
  - 2005: $800
  - 2006: $1,000
  - 2007: $1,200
  - 2008: $1,400
  - 2009: $1,600
  - 2010: $1,800

**Note:**

- * Assuming meal value of $220/T

**Additional Information:**

- **Crush** + 96%
Thank You

CSIRO Food, Nutrition & Bioproducts Flagship
Allan Green

t  +61 2 6246 5154
e  Allan.Green@csiro.au

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www.csiro.au