# The production of very long chain polyunsaturated fatty acids in transgenic plants

## Towards a sustainable of source of "fish oils"

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Lipgene

**Work-Package 2: Plant Biotechnology** – sustainable sources of omega-3 LC-PUFAs

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## Rothamsted Research

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## Why are fish oils important in human diet?

- Specific fatty acids found in fish oils are prevalent in specialised organs (such as the brain, eyes). These are the n-3/omega-3 long chain polyunsaturates
- Mammals have a very limited ability to synthesise these fatty acids, so we need to obtain them from our diet
- Some human genetic disorders are directly linked to an inability to make these fatty acids. There is also some evidence of a reduced capacity to synthesise them in old age and/or diseased states.
- The fatty acids found in fish oils are NOT the same as those in vegetable oils
- Omega-3 fatty acids play a role in anti-inflammatory responses

## Fish Oils play important roles in the prevention of human diseases

- Moderate daily intake of fish oils can avert progression towards type 2 diabetes and CVD
- Fish oils can help prevent the re-occurrence of cardiac infarction
- A diet rich in fish oils may slow the progression of metabolic syndrome
- Fish oil fatty acids may play roles in moderating arthritic conditions

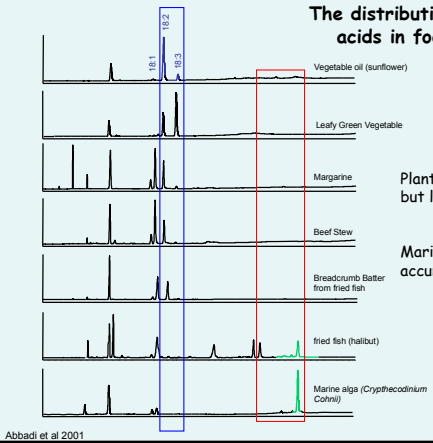

Obesity is now prevalent throughout much of the Western world. This and associated diseases such as CVD and type 2 diabetes represent an imminent public health crisis.

Moderate increases in our consumption of omega-3 long chain polyunsaturated fatty acids found in fish oils can help avert this problem

## Some Fact about Fatty Acids

- Mammals have two Essential Fatty Acids, which they must obtain from their diet: linoleic acid (LA) and $\alpha$-linolenic acid (ALA)
- Fish oils are rich in omega-3 Long Chain polyunsaturated fatty acids (LC-PUFAs)
- Although the EFAs are precursors for LC-PUFAs, animals can only convert them at a low rate (1%)
- Plant oils do NOT contain LC-PUFAs
- Fish do NOT actually synthesise LC-PUFAs; they obtain them from their diet (microalgae at the bottom of the food web)

## The distribution of fatty acids in foodstuffs




Plants are rich in EFAs but lack LC-PUFAs

Marine organisms accumulate LC-PUFAs

Primary Producer

Abbadi et al 2001

## Fish Oil is currently an unsustainable resource

**Considerations:**

Fish oils have a vital role to play in human health & nutrition

Fish oils are known to protect against heart disease & Metabolic Syndrome

Vegetable oils CANNOT provide the same level of protection or health benefits

**Additionally…**

Natural fish stocks are in major decline & suffer from pollution

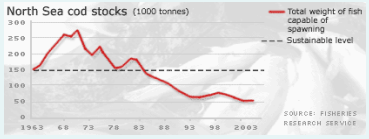
Aquaculture of marine fish requires fish oils (i.e. non-sustainable)

Aquaculture of marine fish CANNOT use vegetable oils as a 100% substitute

Aquaculture is projected to consume 97% of the current production of fish oil

We therefore need an alternative, sustainable source of fish oils for both human nutrition and aquaculture.

There is an urgent need for a sustainable source of fish oils –current marine stocks are in perilous decline




Transgenic plants engineered to produce fish oils can provide a safe & sustainable source of these important compounds for human health and nutrition

## Natural Sources of LC-PUFAs

**Current best natural sources of LC-PUFAs**

| | | |
|---|---|---|
| GLA (18:3, n-6) | Evening primrose, *Mucor* | ~15% |
| SDA (18;4, n-3) | Echium *spp.* | ~20% |
| ARA (20:4, n-6) | *Mortierella alpina* | ~25% |
| EPA (20:5, n-3) | *Phaeodactylum tricornutum* | ~35% |
| DHA (22:6, n-3) | *Crypthecodinium cohnii* | ~45% |
| EPA & DHA | *Isochrysis galabana* | 15%, 8% |

(% of total fatty acids)

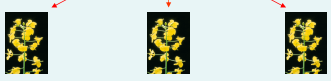
However, not all these sources are easy to cultivate. Some processes are expensive and difficult to optimise or maintain

## Production of $C_{20+}$ PUFAs requires dedicated facilities

## The "Designer" Oilseed Concept

The obvious sources of genes for LC-PUFA biosynthesis are marine algae

Identify gene(s) for trait of interest and transfer into new host (e.g. synthesis of LC-PUFAs)

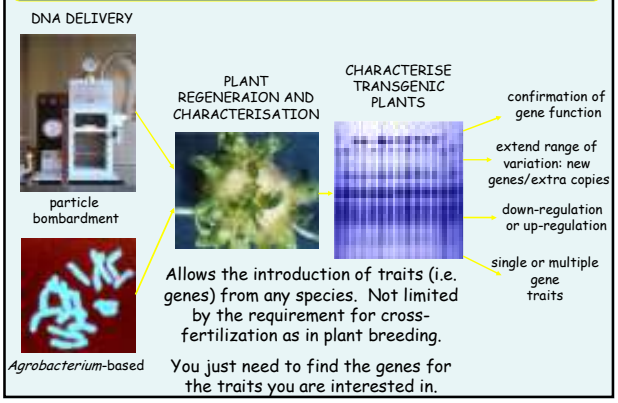


Regenerate transgenic plants with novel fatty acid traits.

## How do you transfer the LC-PUFA biosynthetic pathway into transgenic plants?

- Most oilseeds accumulate linoleic or α-linolenic fatty acids.
- To convert endogenous plant fatty acids into VLC-PUFAs requires the action of multiple enzymes.
- Thus, several "new" genes need to be transferred to the host oilseed to generate the LC-PUFA trait.
- It would be extremely difficult/impossible to generate this trait by a conventional breeding programme.
- This requires transgenic pathway engineering

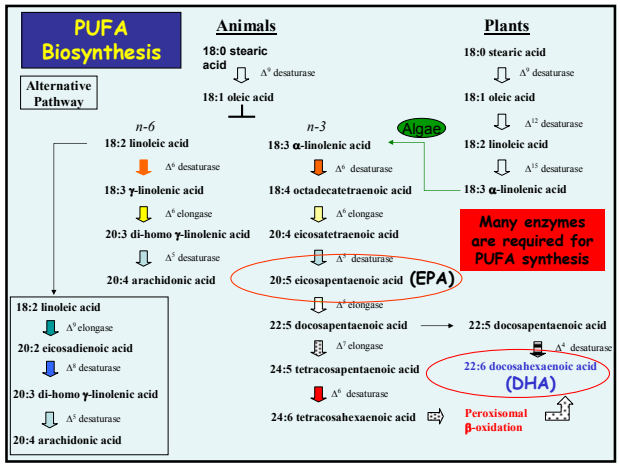
## THE TECHNOLOGIES: TRANSGENESIS



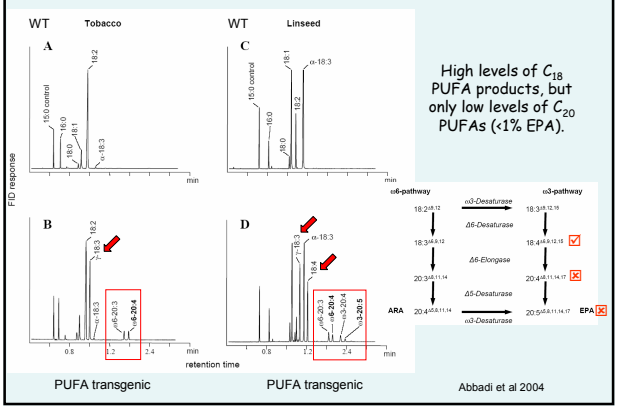

## Background: State-of-the-Art

- **cloned and functionally characterised all the primary enzymes required for $C_{22}$ PUFA biosynthesis (desaturases, elongases)**
- **reconstituted PUFA biosynthesis in yeast**
- **Have suitable seed-specific promoters to drive transgene expression**
- **Have suitable plants to transform with high levels of precursor fatty acids**

**…but can you make fish oils in transgenic plants???**



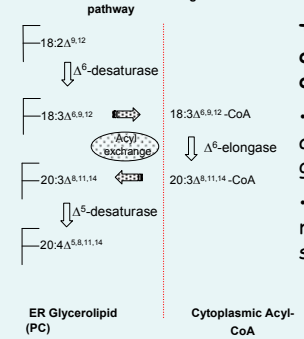

## Expression of LC-PUFA biosynthetic genes in transgenic plants




High levels of $C_{18}$ PUFA products, but only low levels of $C_{20}$ PUFAs (<1% EPA).

Abbadi et al 2004

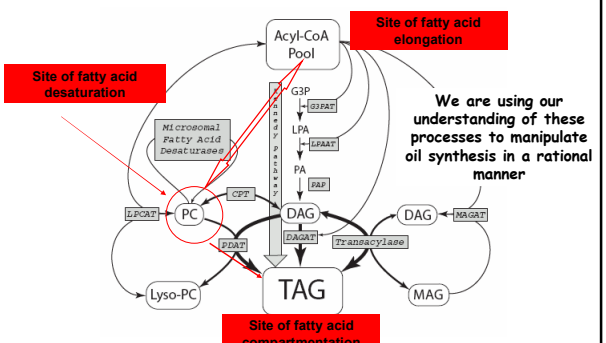
## Heterologous reconstitution of LC-PUFA biosynthetic enzymes is inefficient due to poor acyl-exchange




**This problem can be defined as "substrate dichotomy"**

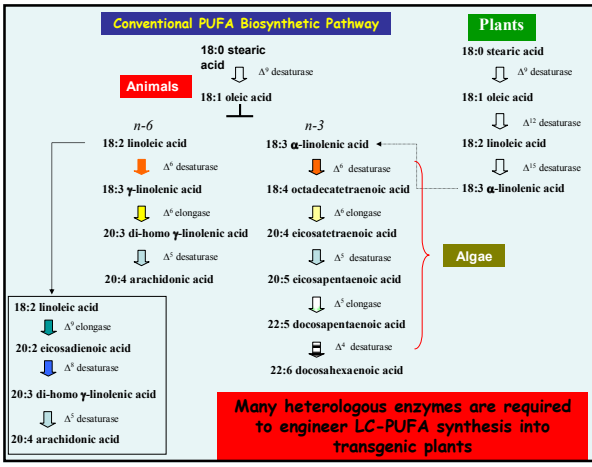
- lower eukaryote fatty acid desaturases require glycerolipid substrates
- fatty acid elongase requires acyl-CoA substrates

## Plant fatty acid metabolism is a complex metabolic network



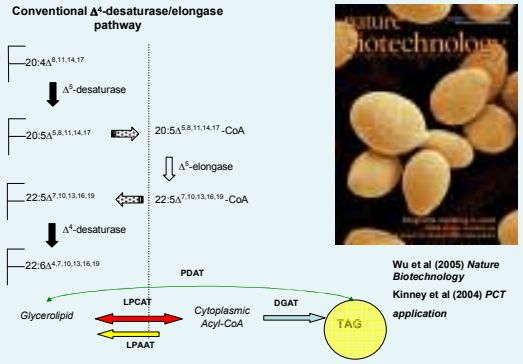

Optimising LC-PUFA synthesis is underway – this is an iterative process ("learning by doing")

## Conventional PUFA Biosynthetic Pathway




Many heterologous enzymes are required to engineer LC-PUFA synthesis into transgenic plants

## Synthesis of DHA in transgenic plants is the next goal



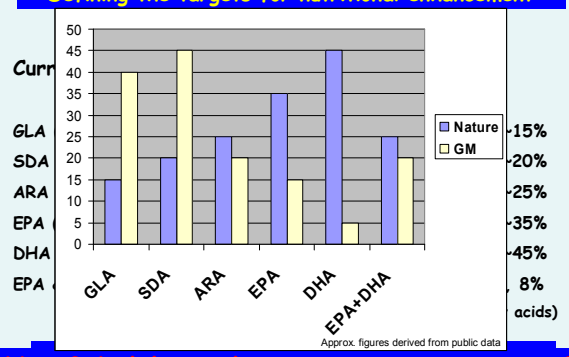



Wu et al (2005) *Nature Biotechnology*

Kinney et al (2004) *PCT application*

Can now make ~15% EPA in transgenic plants, but so far only <5% DHA

## Defining the targets for nutritional enhancement




Has GM delivered improvements on nature?

## Summary of Research on PUFA Biosynthesis

- The production of LC-PUFAs in transgenic plants is feasible and likely to be enhanced by further refinements.
- This should provide a safe, sustainable and environmentally-benign source of these important fatty acids for both human nutrition and aquaculture.
- Using transgenic plants to synthesis health-protective dietary components such as LC-PUFAs may help persuade the public of the benefits of GM food (?).

## Acknowledgements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