# **Biodiesel from Afghanistan Poppies**

Proposal from CSIRO Plant Industry, Canberra. Dr Philip Larkin March, 2007

## Background

Current world diesel consumption is about 1087 billion litres p.a. Biodiesel currently accounts for 0.4% of the total, but this is increasing due to mandated targets in Europe. Europe currently accesses much of its biodiesel from palm oil. However palm oil has poor "cold flow" properties (high viscosity when cold). Preferred so-called "soft" oils have good cold flow properties. Poppy seed oil is a soft oil composed of 12% palmitic (16:0), 19% oleic (18:1) and 56% linoleic (18:2) fatty acids. Unmodified poppyseed oil biodiesel would blend well with diesel in Europe.

Afghanistan is very dependent on imported fuel, not least to power many thousands of local electrical generators. Shortages are frequent and power stoppages very disruptive to the economy. Village-scale biodiesel processing is available technology. Poppyseed biodiesel would be a carbon-neutral energy source, produced and processed in very many localities in Afghanistan and becoming the foundation of a major export industry, especially into the rapidly expanding European market. The business model might involve villages jointly owning the production, crushing and biodiesel processing equipment.

Poppy seed yields are already impressive, being about 1.8 tonnes/ha seed (cf. 2.4 for canola). The oil content is exceptional at 45-50% oil content per unit seed weight (cf. 40% in canola). Poppy yields about 0.8 tonnes oil per ha. The area sown to illegal poppy in Afghanistan in 2004 was 131,000 ha. This is less than 2% of the arable land and therefore could be greatly expanded when the crop becomes legalized. Even the 2004 crop would have produced 100,000 tonnes of oil, equivalent to 2.5% of current world biodiesel consumption.

We are proposing three phases of conversion of the Afghan poppy crops into a legal and internationally valued agribusiness.

- Phase 1. Biodiesel from poppy seed oil using opium-less, opiate-less poppy varieties.
- Phase 2. Higher value high oleic poppy biodiesel, using metabolically engineered poppies.
- Phase 3 (optional and contingent). Industrial products using poppies as a production platform.

Poppy is an adaptable crop, able to tolerate the extremes of environment in Afghanistan and able to grow where most crops fail. Furthermore Afghan farmers have a long history of poppy cultivation and are already expert in poppy agriculture. Consequently the shift to a legal poppy crop would require little initial cultural shift, and would be motivated by potential increased profitability, the attraction of participation in a legal and supported agribusiness, and the potential to alleviate pressing local and national energy and fuel needs.

For **Phase 1**, we propose making available to growers a mutant poppy that produces no opium (latex resin) and does not accumulate opiates. Such a mutant has already been produced at CSIR Lucknow India, called Sujata. As part of the process of creating a legal agribusiness the new varieties would come with the support of agronomists and breeders to improve productivity and sustainability of the industry. Investment would be required in the pressing and processing equipment and expertise. The deployment of Sujata derived varieties would displace the opium poppies and begin to have an effect on the supply of heroin worldwide. The deployed opium-less mutants would be readily distinguished from the opium poppies. We suspect that a thorough market and pricing analysis will show that growers would return more per hectare from the poppyseed than for the opium. There are

opportunities for use of most of the waste stream e.g. poppy straw for ethanol fuel (and one of the catalysts for the biodiesel transesterification process); potassium sulphate fertilizer; protein "meal" by-product from the seed as food and feed supplement. After the protein meal, the biggest waste stream is glycerol which quickly exceeds uses such as for soap manufacture. Some biodiesel plants are using glycerol mixed with some biodiesel to burn in electrical generators to power the plant.

**Phase 2** would involve adding value to the oil by changing it to a high oleic acid (monounsaturated) oil. Oleic acid oil is ideal for biodiesel because it is "soft" but also resists oxidation to polymer particles in hot engines. High oleic poppyseed oil is readily achievable through genetic modification. CSIRO is expert at oilseed modification (1-6) and has produced high oleic oils in soybean, canola, flax and cottonseed. CSIRO is expert at poppy transformation and biotechnology (7-13). The high oleic gene technology should be readily transferable to poppy. These oils are also the most healthy if some of the oil is diverted for food.

**Phase 3** is an optional future phase whereby value is added to the crop by using it as a platform for the production of higher value products such as specialist industrial oils or polymers. Although oilseeds have advantages for production and purification of pharmaceutical proteins, such as antibodies and enzymes, it is unlikely in the foreseeable future that Afghanistan would achieve the level of identity preservation and stewardship required for this.

# **CSIRO** Technical Feasibility Project

### Minimal Funding.

A twelve month technical feasibility study would require a Technical Officer (100%), Dr Larkin (10%) and estimated funding of **A\$ 265,850**. The tasks to be undertaken would establish the feasibility of both Phases 1 and 2 and would include:

- The genetics and chemistry of Sujata opium-less poppy. Gain access for research purposes. Do crosses and determine the genetic control of the trait. Produce seed and determine oil composition relative to normal poppy.
- Test genetic transformation efficiency of the Sujata poppy Transform with test genes.
- Pilot production of biodiesel from Poppyseed

Obtain 500 kg poppy seed.
Crush and extract oil (outsourced)
Process to biodiesel by transesterification (outsourced to CSIRO Molecular Health Technology in 250 or 500 L batches).
Test the qualities of the biodiesel (outsourced e.g. T&S Lab Services, Sydney).

# **Desired Funding**

If funding was at the level of **A\$ 424,400**. We would employ a Technical Officer (100%), Technical Assistant (100%) and Dr Larkin (10%). In addition to the tasks above, we would:

- Confirm that the available seed-specific gene promoters work in Sujata poppy.
- Isolate and sequence target poppy genes for the high oleic oil modification, namely  $\Delta 12$ -desaturase and FATB thioesterase

#### Scoping Activity by Others

It is envisaged that in parallel others (Tenix, Sandia or SDSU??) would undertake the scoping of possibilities in Afghanistan including:

- 1. Political support in Afghanistan for poppy biodiesel.
- 2. Biodiesel processing options best suited to the Afghan environment and conditions.
- 3. The scale of production and processing plants required for different localities in Afghanistan.
- 4. Exploration of market scope, both local and export.

#### **Citations:**

- 1. Liu, Q., Singh, S. and Green, A. (2002) High-Oleic and High-Stearic Cottonseed Oils: Nutritionally Improved Cooking Oils Developed Using Gene Silencing. *Journal of the American College of Nutrition* **21**: 2058-2118.
- Robert, S.S., Singh, S.P., Zhou, X.R., Petrie, J.R., Blackburn, S.I., Mansour, P.M., Nichols, P.D., Liu, Q. and Green, A.G. (2005) Metabolic Engineering of Arabidopsis to Produce Nutritionally Important Dha in Seed Oil. *Functional Plant Biology* 32: 473-479.
- 3. Singh, S., Thomaeus, S., Lee, M., Green, A. and Stymne, S. (2000) Inhibition of Polyunsaturated Fatty Acid Accumulation in Plants Expressing a Fatty Acid Epoxygenase. *Biochemical Society Transactions* 28: 940-942.
- 4. Singh, S., Thomaeus, S., Lee, M., Stymne, S. and Green, A. (2001) Transgenic Expression of a Delta 12-Epoxygenase Gene in Arabidopsis Seeds Inhibits Accumulation of Linoleic Acid. *Planta* **212**: 872-879.
- Stoutjesdijk, P.A., Hurlestone, C., Singh, S.P. and Green, A.G. (2000) High-Oleic Acid Australian Brassica Napus and B-Juncea Varieties Produced by Co-Suppression of Endogenous Delta 12-Desaturases. *Biochemical Society Transactions* 28: 938-940.
- Wesley, S.V., Helliwell, C.A., Smith, N.A., Wang, M.B., Rouse, D.T., Liu, Q., Gooding, P.S., Singh, S.P., Abbott, D., Stoutjesdijk, P.A., Robinson, S.P., Gleave, A.P., Green, A.G. and Waterhouse, P.M. (2001) Construct Design for Efficient, Effective and High-Throughput Gene Silencing in Plants. *Plant Journal* 27: 581-590.
- Chitty JA, Allen RS, Fist AJ, Larkin PJ (2003) Genetic transformation in commercial Tasmanian cultivars of opium poppy, *Papaver somniferum L.*, and movement of transgenic pollen in the field. Functional Plant Biology 30: 1045-1058
- 8. Allen R, Millgate A, Chitty J, Thistleton J, Miller J, Fist A, Gerlach W, Larkin PJ. (2004). Replacing morphine with the non-narcotic reticuline in opium poppy by RNAi gene silencing. Nature Biotechnology **22**: 1559-1566.
- 9. Millgate AG, Pogson BJ, Wilson IW, H. Zenk MH, Gerlach WL, Fist, AJ and Larkin PJ (2004) Analgesia morphine-pathway block in top1 poppies. Nature **431**:413-414.
- Frick S, Chitty JA, Kramell R, Schmidt J, Allen RS, Larkin PJ, Kutchan TM. (2004) Transformation of opium poppy (*Papaver somniferum* L.) with antisense berberine bridge enzyme gene (anti-bbe) via somatic embryogenesis results in an altered ratio of alkaloids in latex but not in roots. Transgenic Research 13: 607–613.
- 11. Miller JAC, Henning L, Heazlewood VL, Larkin PJ, Chitty J, Allen R, Brown PH, Gerlach WL, Fist AJ. (2005) Pollination biology of oilseed poppy, *Papaver somniferum* L. Aust J Agric Res **56**: 483-490.
- Chitty JA, Allen RS, Larkin PJ (2006). Opium Poppy (*Papaver somniferum*). Methods in Molecular Biology, vol. 344: Agrobacterium Protocols, 2/e, volume 2 Edited by: Kan Wang © Humana Press Inc., Totowa, NJ. chapter 35, pages 383-391.
- Larkin PJ, Miller JAC, Allen RS, Chitty JA, Gerlach WL, Frick S, Kutchan TM, Fist AJ (2007) Increasing morphinan alkaloid production by overexpressing codeinone reductase in transgenic *Papaver somniferum*. Plant Biotechnology Journal 5: 26-37.