Institution:
Cardiff University

Unit of Assessment:
UoA5_Casestudy4

Title of case study:
New data analysis methods drive transgenic research to raise yield in oil crops

1. Summary of the impact (indicative maximum 100 words)

By 2020 global demand for plant-derived oils is set to increase by 23%. Researchers at Cardiff University have developed an approach using a method called flux control analysis to identify key biomolecular nodes which, when manipulated, enhance oil production. This research has informed the successful improvement of oil crops and has already increased oil yields in new strains of oilseed rape in the field by over 8%. The methodology is currently being applied to other major oil crops (oil palm, soybean), and has changed industry practice in crop development.

2. Underpinning research (indicative maximum 500 words)

Edible oil crops are a vital agricultural resource, grown for human and animal consumption, specialised products, renewable chemicals and biofuels. Since 1993 global demand for plant-derived oils/lipids has more than doubled, a rise which has only been met through a proportional increase in agricultural land used for oil-crop production.

The Organisation for Economic Co-operation and Development (OECD) projects that the global growth in oilseed demand will require a further 23% increase in production by 2020 (source OECD-FAO Agricultural Outlook 2011-2020). The pressure on the supply/demand equation for oilseed crops is intense, driving agricultural producers to explore innovative ways to intensify production.

Research in Cardiff has focused on all three of the major oilseed crops (oil palm, soybean and oilseed rape) as well as the high-value olive. Cardiff researchers, under the leadership of Professor John Harwood (Professorial appointment 1984 to present), have, over the last 15 years, participated in national and international collaborations to identify the specific parts of the crops' metabolic pathways which are most important for regulation and therefore exert the most control over oil yields.

Flux control analysis

The Cardiff team was the first to apply a technique called flux control analysis to investigate lipid biosynthetic pathways in any organism.

Plants synthesise triacylglycerols (the main oil constituents) by forming fatty acids and then assembling these into complex lipids via biosynthetic pathways. Flux control analysis is a mathematic method that uses data from metabolic experiments to see where in a biosynthetic pathway there are constraints. It is analogous to evaluating traffic flow down a motorway and identifying places where flow is slowed - e.g. at a tunnel, bridge, or because of an accident. Working with several international partners and with funding from BBSRC and industrial companies, the Cardiff lab showed how flux control analysis could be used to identify potential genes for manipulation[3.1-3.3].

DGAT in oil biosynthesis

The flux control analysis identified several limiting steps in crop oil biosynthetic pathways which could be targeted for genetic manipulation to improve the flow of carbon down the pathway and, ultimately increase oil yields. In particular, the analyses by the Cardiff team showed that diacylglycerol acyltransferase (DGAT) was a key node for flux control, thus confirming the biochemical predictions of Perry HJ et al[3.4] (Cardiff University fellow & Post Doctoral Researcher, 1990-1999)

The validity of using flux control analysis to predict important regulatory parts of the biosynthetic pathway was then confirmed by collaborative studies between Cardiff and Canadian researchers, which compared the predictions of flux control analysis with the actual flux control characteristics of the canola lines genetically modified to up-regulate DGAT[3.5].

In further research with our Canadian collaborators the Cardiff team successfully manipulated

DGAT to increase activity in developing canola seeds; the resulting transgenic oilseed rape lines had enhanced oil yields and were also resistant to drought stress[3.6]. The market size for these new higher yield DGAT manipulated lines of oilseed rape is worth up to $2 billion for the major producers (EU, China, India, Canada and Japan) (source: AOCS Lipid Library http://lipidlibrary.aocs.org).

In collaboration with DuPont (Wilmington), the Cardiff team has also applied flux control analysis to identify constraints in the overall pathway for lipid biosynthesis in soybean. This has led to the development of high value products and the Cardiff research has also been adopted as a key tool by companies and institutes worldwide to aid efficient crop improvement.

3. References to the research (indicative maximum of six references)

3.1 Taylor D.C., **Harwood J.L.** *et al* (2009) Molecular modification of triacylglycerol accumulation by over-expression of DGAT1 to produce canola with increased seed oil content under field conditions, Botany 87, 533-543. http://dx.doi.org/10.1139/B08-101

3.2 **Ramli US, Baker, D.S., Quant, P.A.**, **Harwood, J.L**. (2002) Control analysis of lipid biosynthesis in tissue cultures of oil crops shows that flux control is shared between fatty acid synthesis and lipid assembly. *Biochem J.* 364, 393-401. http://dx.doi.org/10.1042/BJ20010203

3.3 **Ramli US**, **Salas JJ,** Quant PA, **Harwood JL**, (2005). Metabolic control analysis reveals an important role for diacylglycerol acyltransferase in olive oil but not in oil palm lipid accumulation. *FEBS J.* 272, 5764-5770. http://dx.doi.org/10.1111/j.1742-4658.2005.04964.x

3.4 **Perry HJ,** Bligny R, Gout E, **Harwood JL**, (1999). Changes in Kennedy pathway intermediates associated with increased triacylglycerol synthesis in oil-seed rape. *Phytochemistry* 52, 799-804. http://dx.doi.org/10.1016/S0031-9422(99)00294-0

3.5 Weselake RJ, **Tang M, Harwood JL** *et al*., (2008). Metabolic control analysis is helpful for informed genetic manipulation of oilseed rape *(Brassica napus)* to increase seed oil content. *J. Exptl. Botany* 59, 3543-3549. http://dx.doi.org/10.1093/jxb/ern206

3.6 **Mingguo Tang**, **Irina A. Guschina, John L Harwood** *et al*. (2012) Metabolic control analysis of developing oilseed rape (Brassica napus cv Westar) embryos shows that lipid assembly exerts significant control over oil. New Phytologist 196,414-426. http://onlinelibrary.wiley.com/doi/10.1111/j.1469-8137.2012.04262.x/pdf

4. Details of the impact (indicative maximum 750 words)

The Cardiff research team have carried out biochemical experiments and flux control analyses on the three most important oil crops (oilseed rape, soybean and palm oil) which together account for 75% of global edible oil production with a combined annual market value of $135 billion (based on 2011/12 production[5.1a] and the mean combined market price for the three crops[5.1b]). The research has also extended to the high-value olive which has an annual production value of $10 billion (based on 2011/12 production[5.1a] at 2011/12 prices http://www.indexmundi.com/commodities/?commodity=olive-oil&months=60), with best quality extra-virgin oils being worth over ten times the value of bulk commodity edible oils. As a result, higher yield rape and soybean crops have been developed, while one of the world's largest producers of palm oil has adopted Cardiff's flux control methodology in the development of new lines.

Increasing yields of oilseed rape and soybean

The early work by Perry[3.4] and colleagues in the Harwood laboratory and the later application of flux control analysis to oilseed rape biosynthetic pathways identified the enzyme DGAT as a primary target for genetic manipulation in oilseed rape.

Transgenic canola varieties with modified (up-regulated) DGAT have been developed by the Alberta Innovates Phytola Centre; as a result of this work a patent was filed on DGAT in 2009[5.2]. This patent deals with the use of flax diacylglycerol acyltransferase (DGAT) enzymes to increase the production of seed oil in camelina. The Alberta Centre's scientific director Randall Weselake states that the patent (source 5.2) is founded on the early demonstration by Harwood's group that DGAT

was limiting oil production in oilseed rape[5.3]. Subsequent work by Harwood and Weselake showed how flux control analysis could be used for informed genetic manipulation. Weselake adds: "These key observations have laid the foundation for industrial exploitation of the basic science"[5.3] The first new transgenically manipulated canola lines developed by the Phytola centre have entered field trials. In successive years (2010/11-2011/12) an 8% increase in oil yield has been observed, which is estimated to be worth an additional $190 million in Canada alone at current market prices. The increased drought resistance of these lines could also save more than 30% in lost yield during dry years.

This approach has also been exploited by DuPont (a Fortune 100 Company) over an extended period in close collaboration with Professor Harwood. The objective of this work has been to improve commercial soybean lines; DuPont's main crop of interest. DuPont have shown that over-expression of various genes encoding DGAT enzymes in soybean seeds leads to lines with a more than 20% increase in seed oil content. These genes are now pivotal to DuPont's strategy to create a new generation of commodity soybean[5.4]. DuPont has committed to this strategy of commercialising the new soybean strains over the next ten years, based on the company's calculation it will add hundreds of millions of dollars to the soybean market.

One successful product is already on the market; 'Plenish' by DuPont/Pioneer. Currently demand is outstripping supply whilst seed production is increased during the first 2-3 years of release onto the market.

Changing industrial practice in the oil palm industry

Oil palm is produced mainly in Malaysia and Indonesia; it is the major (and most productive) oil crop worldwide. As a measure of its importance, palm oil contributed 11.3% of the total Malaysian exports in 2010[5.5].

Projections by the OECD suggest that even with land restrictions and environmental regulations taking effect, combined palm oil output sales have the capability to expand by almost 45%, raising this product's share of global edible oil output to 36% by 2020[3.7]. Malaysia is the world's second largest producer of palm oil and the country's palm oil industry employs over 500,000 people[5.6].

The role of flux control analysis within the palm oil industry has now been recognised as a significant new approach in the development of high yield crop lines. The Malaysian Palm Oil Board (MPOB) approached the Harwood laboratory to train individuals in flux control analytical techniques and methodologies through PhDs. These individuals have embedded the use of flux control within their sections (Metabolics, led by U Ramli; Gene Expression, led by A. Manaf). Since their appointment in 2010 a further dozen high quality posts have been created to exploit flux control analysis techniques in developing transgenic oil palm strains that produce higher yields[5.6].

The Director of the MPOB's Advanced Biotechnology and Breeding centre states that "The benefits of these practices include more reliable and efficient techniques and methodologies to identify potential genes for manipulation, which in turn allow us to produce oil palm strains with higher oil yields"[5.6]. Harwood is also supporting the application of flux control analysis techniques to a major Palm oil producer in Malaysia (Sime Darby) through his appointment as an international member of the Programme Advisory Committee (PAC) for the MPOB[5.7], of which Sime Darby are also a member.

The data from flux control analyses are not only being used by MPOB, but also by the U.S. Department of Energy's "Center for advanced Biofuel Systems" who are developing strains of enhanced camelina suitable for the production of biodiesel and jet fuel.[5.8]

5. Sources to corroborate the impact (indicative maximum of 10 references)

5.1 The lipid Library, Global production http://lipidlibrary.aocs.org/market/ofo6-07.htm (5.1a) & prices http://lipidlibrary.aocs.org/market/prices.htm (5.1b). Online source of information verifying the scale and value of the global edible oils market, including details of major crops Harwood has researched.

5.2 Weselake R. et al., (2011) Diacylglycerol acyltransferases from flax-camelina; U.S. Patent Application no. 12/381,183; notice of allowance issued Dec. 5, 2011. (Source 5.3. confirms that the patented technology is underpinned by the work of Professor Harwood)

(Document available on request)

5.3 Statement from Canada Research Chair in Agricultural Lipid Biotechnology (Uni of Alberta) & Scientific Director, Alberta Innovates Phytola Centre. Confirming the impact of Professor Harwoods research as the underpinning technology to a patent (5.2) now being exploited commercially. (Statement available on request)

5.4 Statement from Research Director, DuPont Agricultural Biotechnology, Wilmington, USA. Confirming DuPonts adoption of flux control analysis as a core technology for the development of new Soybean strains (eg Plenish). (Statement available on request)

5.5 Malaysian Oil Palm review for 2010, corroborating that Oilpalm accounts for 11.3% of Annual Exports. http://www.statistics.gov.my/portal/images/stories/files/LatestReleases/trade/bi/Dec10/External_Trade_DisBI.pdf

5.6 Statement from Director, Advanced Biotechnology and Breeding Centre, Malaysian Palm Oil Board. Verifying the adoption of Flux Control techniques and description of the resulting strategic expansion of MPOB's scientific capability. (Statement available on request)

5.7 Statement from Chief Scientist, Sime Darby Technology Centre, Malaysia. Confirming the adoption of Cardiff's research as a key tool in the development of new lines of oil palm. (Statement available on request)

5.8 Statement from Director, Centre for Plant Science Innovation, University of Nebraska-Lincoln, U.S.A. Confirming the adoption of Flux control analysis in developing optimised biofuels for the U.S. Department of Energy via the "Center for Advanced Biofuel Systems" (Statement available on request)