# Novel mutants optimized for lignin, growth and biofuel production via re-mutagenesis

**Investigators**
In Division of Plant Sciences, College of Life Sciences, University of Dundee:
Claire Halpin, Professor and Deputy Head of Division
Gordon Simpson, Lecturer
Yuguo Xiao, Postdoctoral researcher
Christopher McClellan, Postdoctoral researcher
Abdellah Barakate, Postdoctoral researcher
Michael Skelly, Technician
Kirsty Graham, Technician

External Collaborators:
Wout Boerjan, Professor, University of Ghent, Belgium
Simon McQueen Mason, Professor, University of York, UK

**Abstract**

This project aims to identify and manipulate novel genes that might be useful for plant biomass improvement for bioenergy applications. We are doing this by mutagenising existing Arabidopsis mutants in lignin biosynthesis genes and isolating secondary novel mutations that enhance growth or sugar release from plant cell walls. So far, along with our collaborators, we have confirmed that some mutants in lignin biosynthesis have improved saccharification properties as evaluated in a high-throughput semi-automated saccharification screen. One of these mutants has been remutagenised to identify 14 putative novel secondary mutations that restore normal growth to the 'founder' mutant. Further analysis will determine how useful these mutant might be in a bioenergy context and will attempt to identify and clone the secondary mutation. More rounds of remutagenesis are planned for other 'founder' lignin mutants in the coming months.

**Introduction**

This project aims to take a simple but new approach to identifying and manipulating novel genes that connect to lignin biosynthesis using a functional screen that will specifically isolate lignin-modified plants with improved saccharification yield and normal or enhanced growth. These novel genes would represent good targets for manipulation to improve saccharification and biofuel yield from dedicated biomass crops.

**Background**

Interest continues to grow on the possibilities of manipulating cell walls and particularly lignin biosynthesis in order to improve plant raw materials for biofuel applications. We are part of several large research networks aimed at optimizing cell walls for bioenergy applications (including an EU-funded project RENEWALL, and the BBSRC Sustainable Bioenergy Centre - BSBEC), and many other international projects have started. Our GCEP project, to our knowledge, still remains distinctive, however, in focusing exclusively on remutagenesis to identify novel genes, and discovering

unappreciated gene combinations, that might be manipulated to further improve biofuel production from biomass. Knowledge transfer between our different projects is enhancing the GCEP work; for example, data on the saccharification yield of modified-lignin plants (tobacco, poplar, barley) from other projects is identifying the best candidate genes to focus on within GCEP i.e. those that can effect consistent improvements in saccharification when manipulated in different species. Although out GCEP work focuses on Arabidopsis, through these other projects we are also maintaining contact with groups involved in perennial biomass crop (e.g. Miscanthus, willow, poplar) improvement who might ultimately use the results of our GCEP work in a commercial bioenergy context.

**Results**

Very good progress has been made on several fronts in this first year of the project. Saccharification data from our own and collaborator's laboratories was used to identify Arabidopsis mutants that, compared to wild type, released more sugars from stem cell walls after a simple pretreatment followed by an enzyme incubation. A short-list of these mutants has been drawn up for future remutagenesis. One mutant was selected for the first proof-of-concept screen. This mutant is in a Columbia (Col) background and has reduced lignin and a very significant improvement in sugar release in the saccharification assay but grows much less vigorously than wild type plants. This 'founder' mutant has been used in a suppressor screen to identify further mutations that maintain the cell wall and saccharification changes but restore a normal growth phenotype. Seed for the selected mutant was bulked up and 10,000 seed was remutagenised with 0.3% (v/v) ethylmethane sulphonate (EMS) to induce random point mutations. The M1 seed was bulked into 250 families comprised of 25 seeds each and screening continued into the M2 generation where 320 seeds from each family were screened to identify recessive mutations. Due to space limitations and the large number of plants to be grown, only 40% of the families have been evaluated in our initial screen. The result has been incredibly exciting and, already, 14 putative mutants ('putants') with various degrees of restored growth have been identified. These have all been genotyped to confirm that they truly contain the original 'founder' mutation. All 'putants' are currently being grown for further comparative analysis to include growth characteristics, lignin and cell wall analysis, and saccharification properties. Genetic analysis will include allelism tests to identify which 'putants' are, in fact, mutants in the same gene. Luckily, the 'founder' mutations was also available in a *erecta* (Ler) background, avoiding the necessity of introgressing the 'founder' mutation into Ler in order to facilitate mapping of the suppressor gene. Col x Ler crosses represent the most commonly used Arabidopsis mapping populations and have the greatest number of markers and mapping resources available. Although caution still needs to be exercised in evaluating the results of this first experiment at such an early stage, the data is extremely encouraging. Armed with this apparent success, we are now growing up the remaining 60% of the families to try to identify further 'putants'.

A second mutant has recently been selected from our short-list, seed has been bulked, and a second EMS mutagenesis experiment is about to be implemented. In this case, the 'founder' mutant grows normally but has cell wall changes that lead to significant saccharification improvement. Enhancer mutations will be identified using a high-

throughput semi-automated saccharification assay that has been developed and tested by our collaborator Prof. Simon McQueen Mason at the University of York to try to identify 'putants' that further improve saccharification yield over that of the 'founder' mutant.

**Progress**

Significant progress has been made towards our goal of identifying novel mutations which could be useful for improving plant biomass as a feedstock for carbon-neutral or carbon-negative biofuel production. We have identified several putative mutants which, if confirmed, could combine usefully with mutations in lignin biosynthesis genes to yield normal phenotype plants with modified cell walls from which it is easier to release sugars as a biofuel fermentation substrate.

**Future Plans**

Our future plans are to implement further suppressor and enhancer screens using different lignin mutant 'founder' genotypes to add further to our library of useful secondary mutations. The secondary mutants we have already identified will be characterized genetically and biochemically (e.g. for cell wall to confirm that the changes to lignin persist) and the mutants genes will be mapped and identified.

**Publications**

No publications yet, although the existence and aims of the GCEP project have been mentioned in several presentations including:

"Lignin biosynthesis and manipulation" presented by Claire Halpin at the Gordon Conference on "Cellulosomes, Cellulases & Other Carbohydrate Modifying Enzymes" July 26-31, 2009, Proctor Academy, Andover, NH.

"Barley Straw – A fuel for the Future?" presented by Claire Halpin at the BCPC09 (British Crop Production Council Congress 2009), November 2009, Glasgow, UK.

**Contacts**

Claire Halpin: c.halpin@dundee.ac.uk
Gordon Simpson: g.g.simpson@dundee.ac.uk
Abdellah Barakate: abdellah.barakate@scri.ac.uk
Christopher McClellan: Christopher.mcclellan@scri.ac.uk
Yuguo Xaio: yuguo.xaio@scri.ac.uk
Michael Skelly: Michael.skelly@scri.ac.uk
Kirsty Graham: Kirsty.graham@scri.ac.uk