



Annual Newsletter (n°3)  
August 2011



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ENERGYPOPLAR (Enhancing Poplar Traits for Energy Applications) is an EC Seventh Framework Programme project aimed at further improving poplar trees as an energy crop. The work is directed to understand and improve traits such as yield and wood properties coupled to Bioethanol production. The project also addresses environmental and economical sustainability questions.

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**Will the project promote environmental sustainability of novel energy plantations in Europe?**

Unlike agricultural 1G biofuel crops, poplars show a low nutrient demand for their growth, so they can be planted on marginal soils. Their survival relies on mutualistic symbiosis with soil microbes, so-called ectomycorrhizal fungi. These fungi are the link between plant and soil, delivering otherwise inaccessible nutrients to their host tree and acting as 'green' fertiliser. Thanks to this symbiosis poplars can produce high biomass without industrial fertilisers, lowering greenhouse emissions. ENERGYPOPLAR is committed to evaluating bioenergy poplars' impact on root-associated fungi and soil microbiome

**How extensively are biofuels currently used as an energy source and what is Europe's position on their development?**

Biofuels (liquid transport fuels used mainly for road transport) currently represent only a few per cent of EU road transport fuel (1 to 5 per cent energy basis depending upon country). But under the Renewable Energy Directive (RED), the EC has mandated renewable energy will comprise 15 per cent of the energy mix by 2020 – and that must include renewable energy being 10 per cent of the transport sector by 2020. This is a big increase from renewable energy levels of about 3 per cent at present. In transport, meeting this target suggests deployment of biofuels (included blending with fossil gasoline and/or diesel) at levels of something between 8 and 12 per cent into road transport fuel by 2020 (subject to deployment of renewable in other transport modes (rail, water, air).

## Project progress: selected results.



### Field trials: Bioethanol yield almost doubled.

Data from a field trial at VIB with GMO poplar shows possibilities for large effects on the different stages of ethanol production. The initial results from this work with down regulated lignin production are promising but it also shows that more research is needed before a commercial product is ready for the market.

For more information see: <http://www.vib.be/en/news/Pages/Initial-field-test-results-GM-poplars-bioethanol-yield-almost-doubled.aspx>

### Identifying new genes for yield and processability

We have developed and used several approaches to unravel the complex molecular nature of optimized yield and processability providing information that are used for the generation of novel genotypes. Selected genes are now being analyzed *in planta* by genetic modification of their activity.



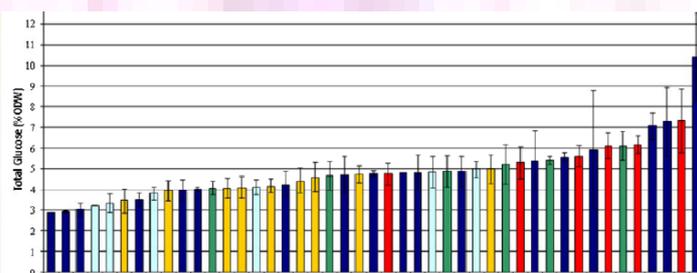
### Mycorrhizia

Poplar is a water demanding species. In symbiotic associations, mycorrhizal fungi ensheath poplar roots with a mantle, improve plant water use efficiency and rescue wood production when water is limiting and the soil is dry. The mechanistic basis of this beneficial influence of soil biota on poplar yield is studied. Also, GM plantations and commercial plantations of poplar are investigated with regard to mycorrhizal diversity and amount.

We are also investigating the developmental process involved in the plant fungi interaction: The first effector protein crucial to symbiosis, MiSSP7 has been identified in the consortium. This helps us understand the biological process underlying this important symbiosis. This can in turn help us produce trees that are more efficient in regarding water and nutrient uptake. <http://www.cell.com/current-biology/abstract/S0960-9822%2811%2900589-6>

### Improved saccharification

One of the major bottlenecks in the saccharification process of wood to fermentable sugars is lignin. A large variation exists in saccharification potential between different genotypes in the mapping and genotype poplar populations used in the consortium. Lignin and cell wall carbohydrates in the F2 POP1 population have been measured by wet chemical analyses. In the end, this information is being used for optimizing saccharification potential by altering cell wall composition. Analysing saccharification potential in existing commercial clones and breeding population reveal large variation in this trait which can be pursued both for clone selection and breeding for future commercial clones.

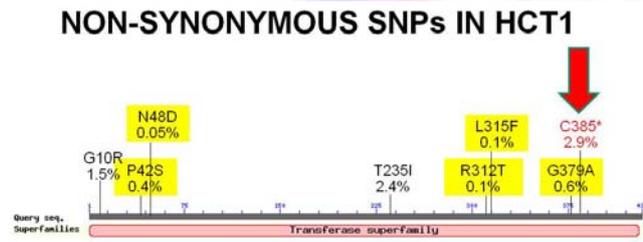
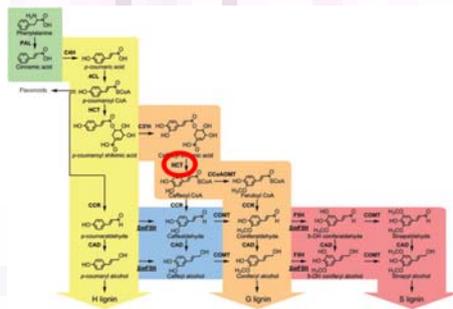


Saccharification of *Populus nigra* genotypes: approx 300% 'range'

## Improvements by using state-of-the-art genome analysis

By screening for natural variation in genes involved in lignin production using state of the art sequencing technologies we have identified new mutants in lignin genes. Experimental crosses within these trees are in progress, and these trees will be analyzed when present.

We have identified missense mutations in several genes and one mutation causing a stop codon in the HCT1 gene related to lignin biosynthesis. (Marroni et al 2011 Plant Journal "Large-scale detection of rare variants via pooled multiplexed next-generation sequencing: towards next-generation Ecotilling"). <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-313X.2011.04627.x/abstract>



**In this study a SNP occurring only once in 1536 chromosomes was identified and confirmed (MAF <0.1%)**

**“This cost effective mutant identification and screening scheme opens up new possibilities to find and use rare alleles in different breeding strategies”**

**“The limiting factor is now starting to be access to large enough populations to start searching for mutants in!”**

This shows the possibility, with basis in the progress of sequencing and genotyping technology, to find and use natural occurring variants of specific genes in breeding strategies. We were able to identify gene variations that only occurred once in a population of 768 trees. Growing evidence suggests that rare functional variants, which are usually missed by genome-wide association scans, play an important role in determining the phenotype. This analysis package can be expanded to any sized tree population, so the size of the population will be determining on which genes useful mutations can be identified. These mutations can then be introduced into breeding projects. We have conducted crosses with some of these mutants and variants in order to create homozygous trees with them. These trees are currently growing and the result will take a few years.

## Environment and economics

A first encompassing LCA (Life cycle analysis) model has been developed that identified key elements of the supply chain that confer environmental benefits or disadvantages.

**“This analysis showed that process optimization for cell wall transformation was identified as a critical element”.**

This underpins the potential importance of advanced breeding techniques (incl. GM technology) to provide poplars with modified cell composition for improved saccharification.



For further information please visit: [www.energypoplar.eu](http://www.energypoplar.eu)