

Il miglioramento degli alberi forestali nell'era della genomica: applicazioni al pioppo ed alle piantagioni intensive

Michele Morgante, Università di
Udine
Istituto di Genomica Applicata



Table 1 Prices of selected energy sources

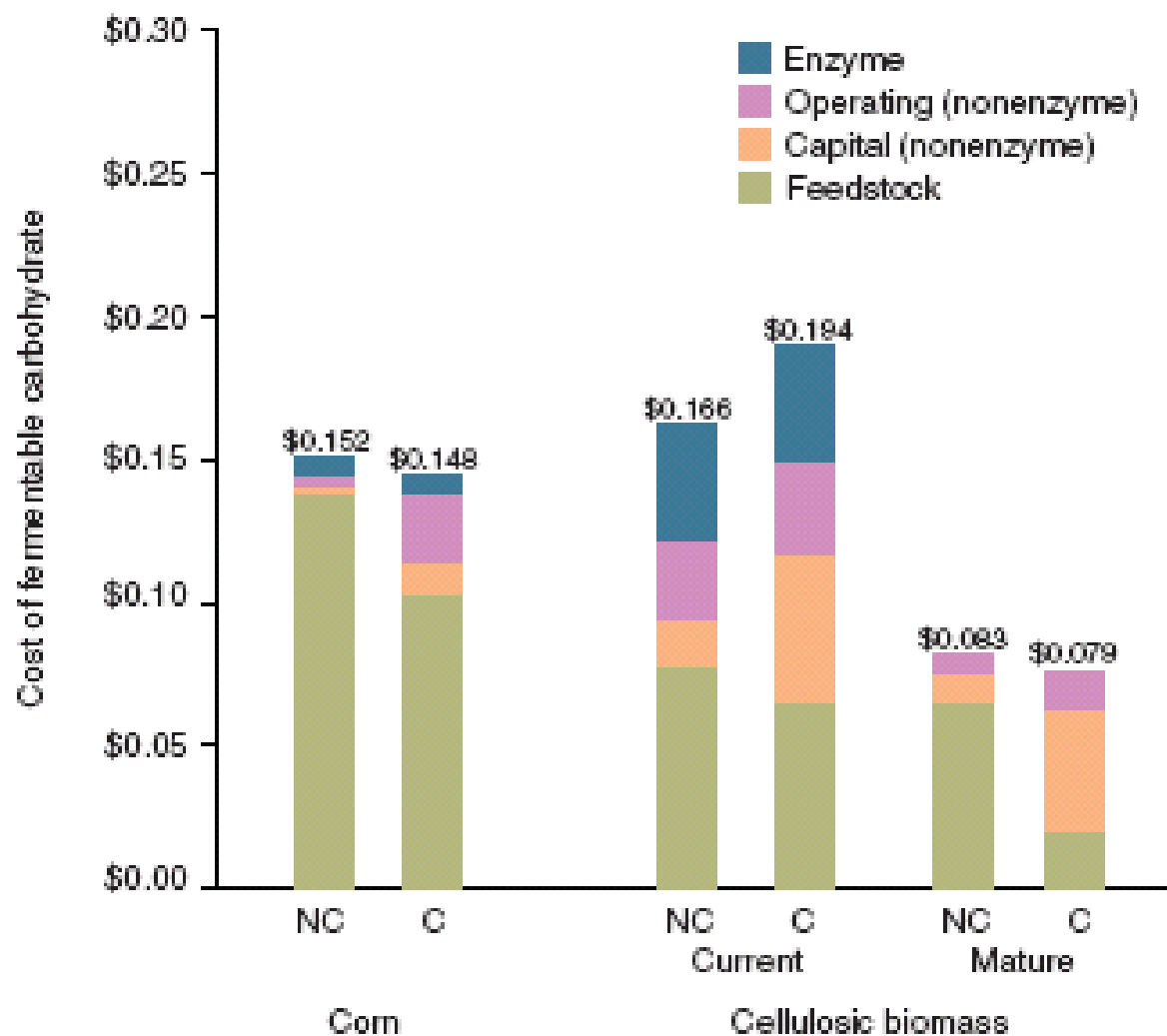
Energy source	Price	
	Common (\$/amount)	\$/GJ ^a
Petroleum	50/bbl	8.7
Gasoline ^b	1.67/gallon	13.7
Natural gas ^c	7.50/scf	7.9
Coal ^d	20/ton	0.9
Coal with carbon capture ^{e,f}	106/ton	4.8
Electricity	0.04/kWh	11.1
Soy oil ^g	0.23/lb	13.8
Corn kernels ^h	2.30/bu	6.6
Cellulosic crops ⁱ	50/ton	3.0

^aAssumed lower heating values: petroleum, 5.8 GJ/bbl; gasoline, 5.1 GJ/bbl; natural gas, 37.3 MJ/m³; coal, 23.3 MJ/kg; soy oil, 36.8 MJ/kg; corn kernels, 16.3 MJ/kg; cellulosic crops, 17.4 MJ/kg. ^bWholesale price, average 2004–2006 (ref. 21). ^c2005 annual average US wellhead price²¹. ^d2004 annual average US open market price²¹. ^eCost of carbon capture assumed to be \$150/ton carbon²². ^fCoal carbon content assumed to be 57% (dry weight basis)²³. ^gAverage price 2004–2005 (ref. 19). ^hAverage price 2002–2005 (ref. 24). ⁱPrice representative of typical values assumed for energy crops in the literature (for example, McLaughlin *et al.*²⁵). bbl, barrel; scf, standard cubic foot.

Lynd *et al.* Nat. Biotechnology, 2008



Costs of fermentable carbohydrate for processing corn and cellulosic biomass



Lynd et al. Nat. Biotechnology, 2008





Main species for biomass production for 2nd generation bioethanol

Switchgrass

Panicum virgatum

Elephant grass

Miscanthus giganteus

Poplar/aspen

***Populus* spp.**

Willows

***Salix* spp.**





What feedstock traits need improving?

Biomass Quantity

- More biomass per hectare
- Limited N and water inputs

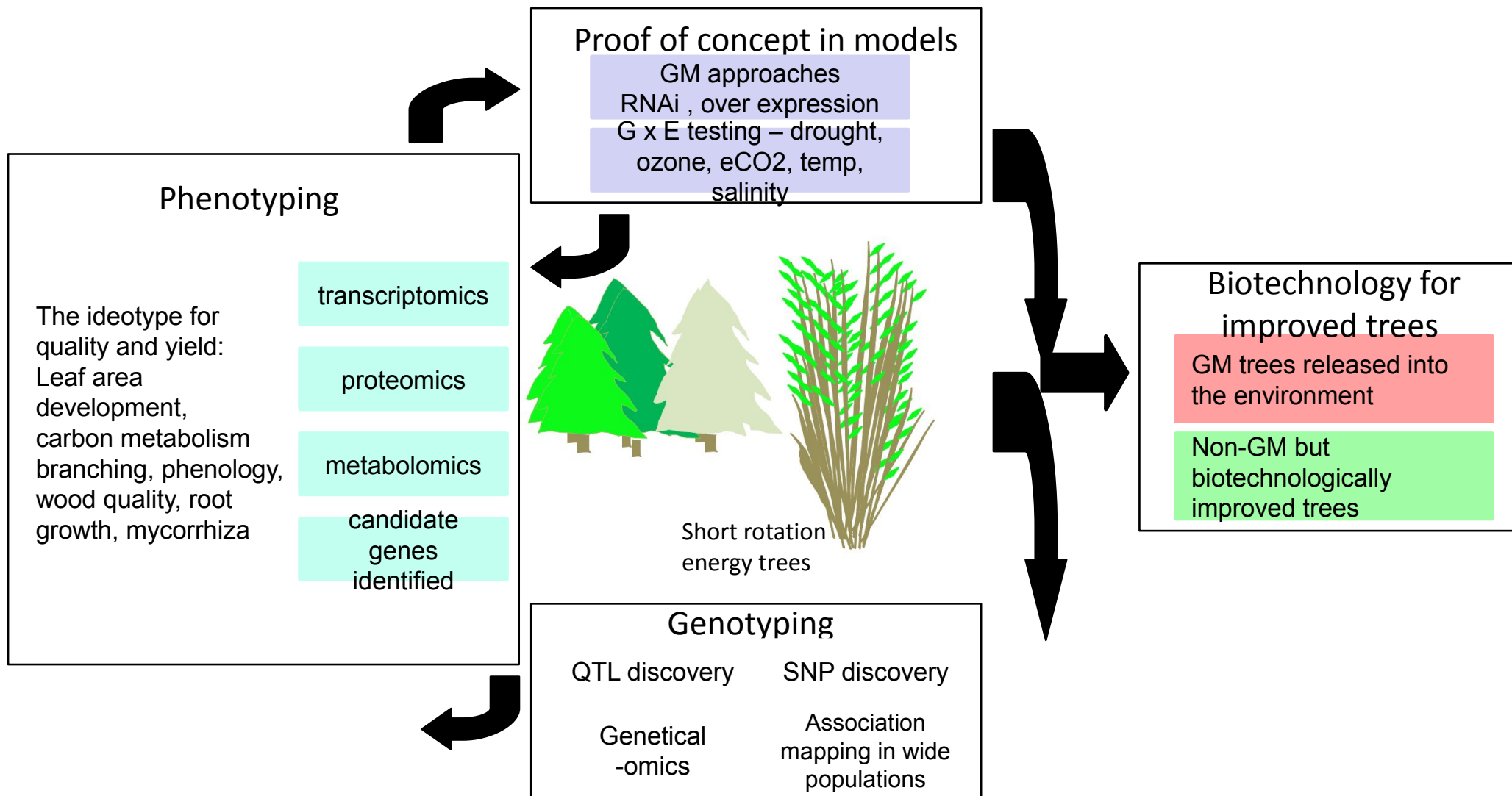
Biomass Quality

Improved quality

- lignin
- cellulose
- hemicellulose
- saccharification
- fermentation

Sustainable – GHG balance, biodiversity, water use,

Genomics for optimised yield and lignocellulosic quality in energy trees



Modified from Karnosky, and Taylor, 2009



Why is poplar the ideal bioenergy tree?



- Full genome sequence (15 Sept 2006)
- Small genome 475 Mbp (similar to rice)
- Diversity of species
- Fast growing and perennial
- Clonal
- Transformation system
- Genetic collections and molecular maps
- Activation tagged populations
- EST and BAC libraries
- Genomic and post-genomic tools



Energy Poplar

Enhancing poplar traits for energy applications

More than 30 species, wide range of climates and soils

Large genetic diversity, large natural provenances (91%)

Interspecific hybridisation

Seventh framework
programme

Food, Agriculture
and Fisheries,
Biotechnology



P. SIGAUD



FAO; Ball, Carle and Del Lungo

J. CARLE





Work Packages

WP1 Optimised yield for bioenergy poplar

WP2 Genetic improvement of poplar wood quality for saccharification

WP3 Generation of novel genotypes

WP4 Environmental and economic sustainability assessment

WP5 Dissemination and Technology Transfer

WP6 Management





WP1 Optimised yield for bioenergy poplar

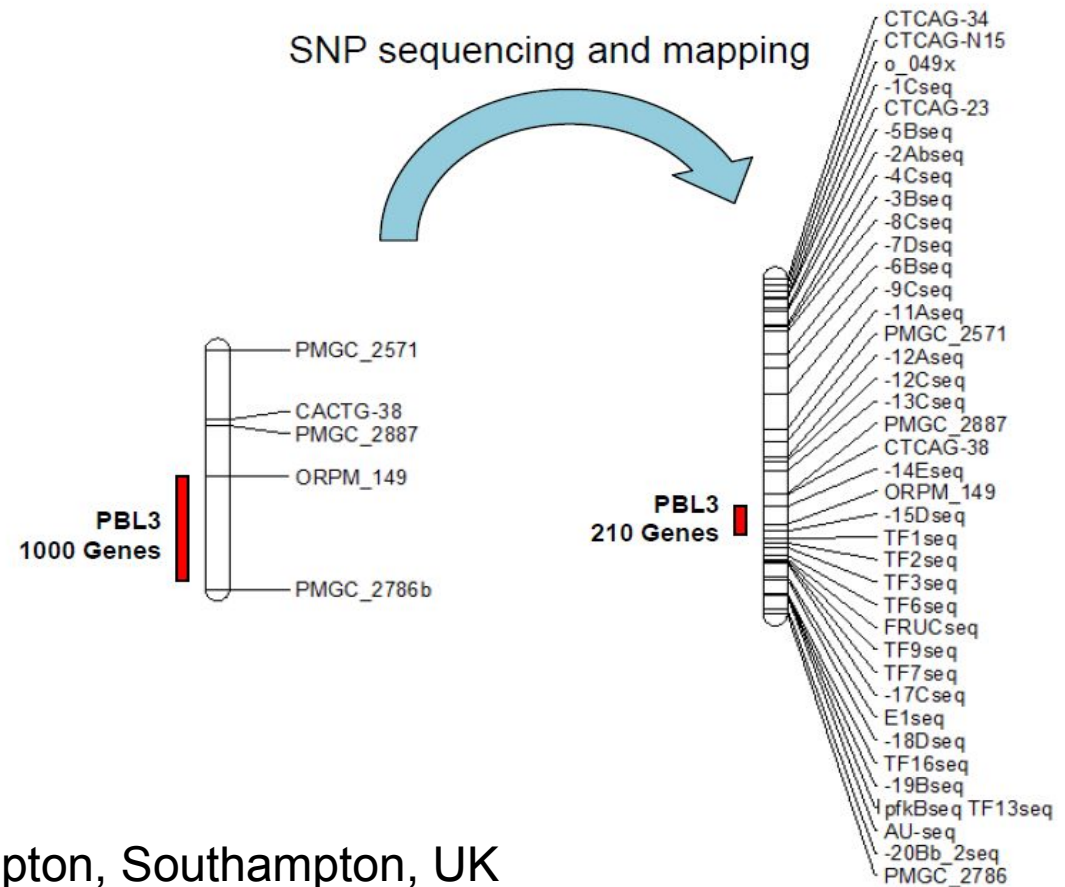
- 1) Identify an area of the Populus genome determining yield and underlying genes → Map genes affecting biomass production. Select/Produce trees carrying a genotype positively affecting yield.
- 2) Optimise root function for nutrient capture and carbon sequestration → Increase in Populus rooting ability is expected to lead to increase in biomass production.



Identify an area of the Populus genome determining yield and underlying genes

Use of classical positional cloning methods to map Quantitative Trait Loci (QTLs) affecting yield in poplar.

Identified a locus harboring several genes/transcription factors affecting biomass, Poplar Biomass Locus (PBL3).



Patrick Stephenson, University of Southampton, Southampton, UK





WP2 Genetic improvement of poplar wood quality for saccharification

1) Establishment of field trials with different species, GM-clones and triploids under Short Rotation Coppice (SRC) → Test saccharification potential (proxy for bioethanol production) of several selected clones under conditions realistic for an industrial application



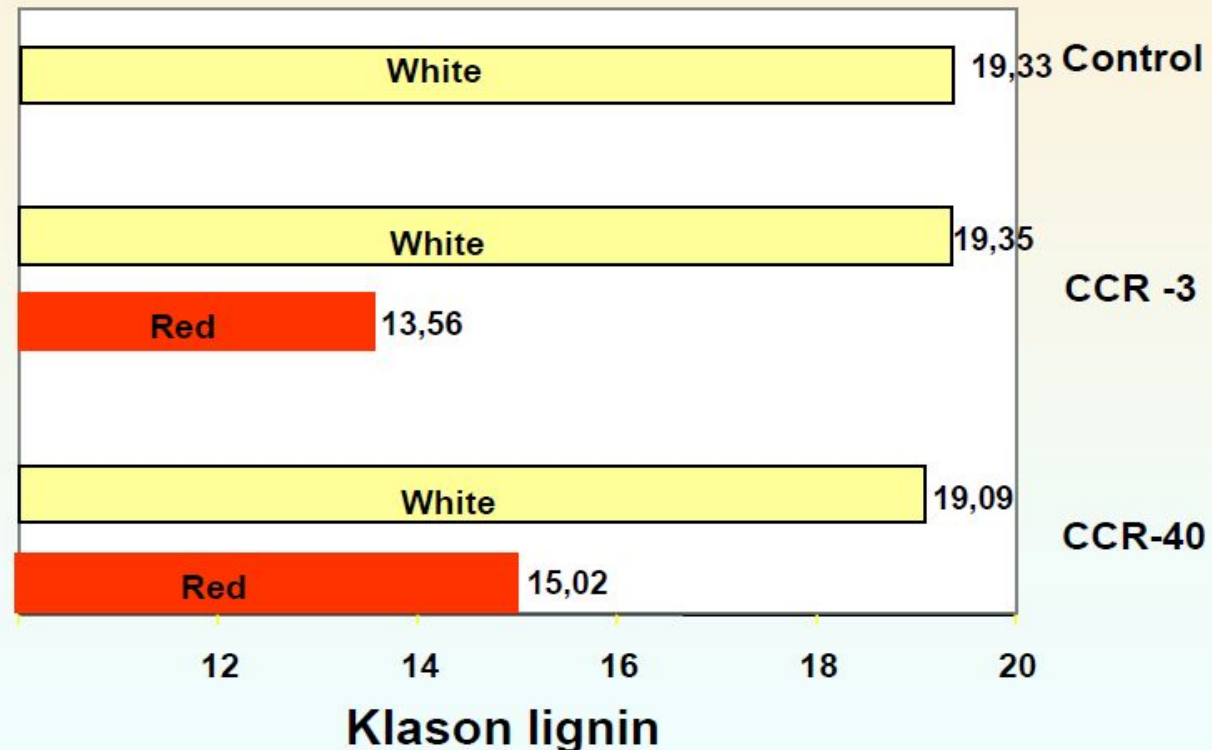
Establishment of field trials with different species, GM-clones and triploids under SRC

Downregulation of *CCR*, involved in lignin biosynthesis
Transgenic trees show spots of red xylem, in which lignin content is substantially reduced.

Leplé *et al*, 2007, The Plant Cell.

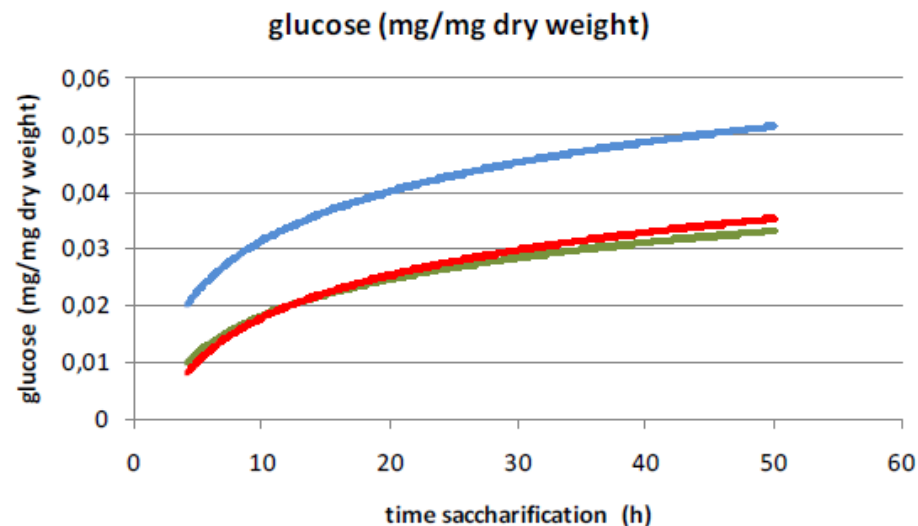


Debarked stem



Establishment of field trials with different species, GM-clones and triploids under SRC

CCR-40 (Blue line) has higher saccharification potential than CCR-3 (Red line) and WT (Green line). All the trees were grown under SRC.



Rebecca van Acker, VIB, Ghent, Belgium





WP3 Generation of novel genotypes

- 1) **Development of locus-specific SNP markers for molecular diagnostics** → Identify SNPs affecting phenotype. Select trees carrying the desired SNPs and use them in conventional breeding program.
- 2) **Development of whole genome markers for genetic distance estimation** → Special interest in heterosis (hybrid vigor).
- 3) **Development of transgenic poplars with multiple transgenes (gene-stacking)** → Combine genes beneficial for biomass and wood composition



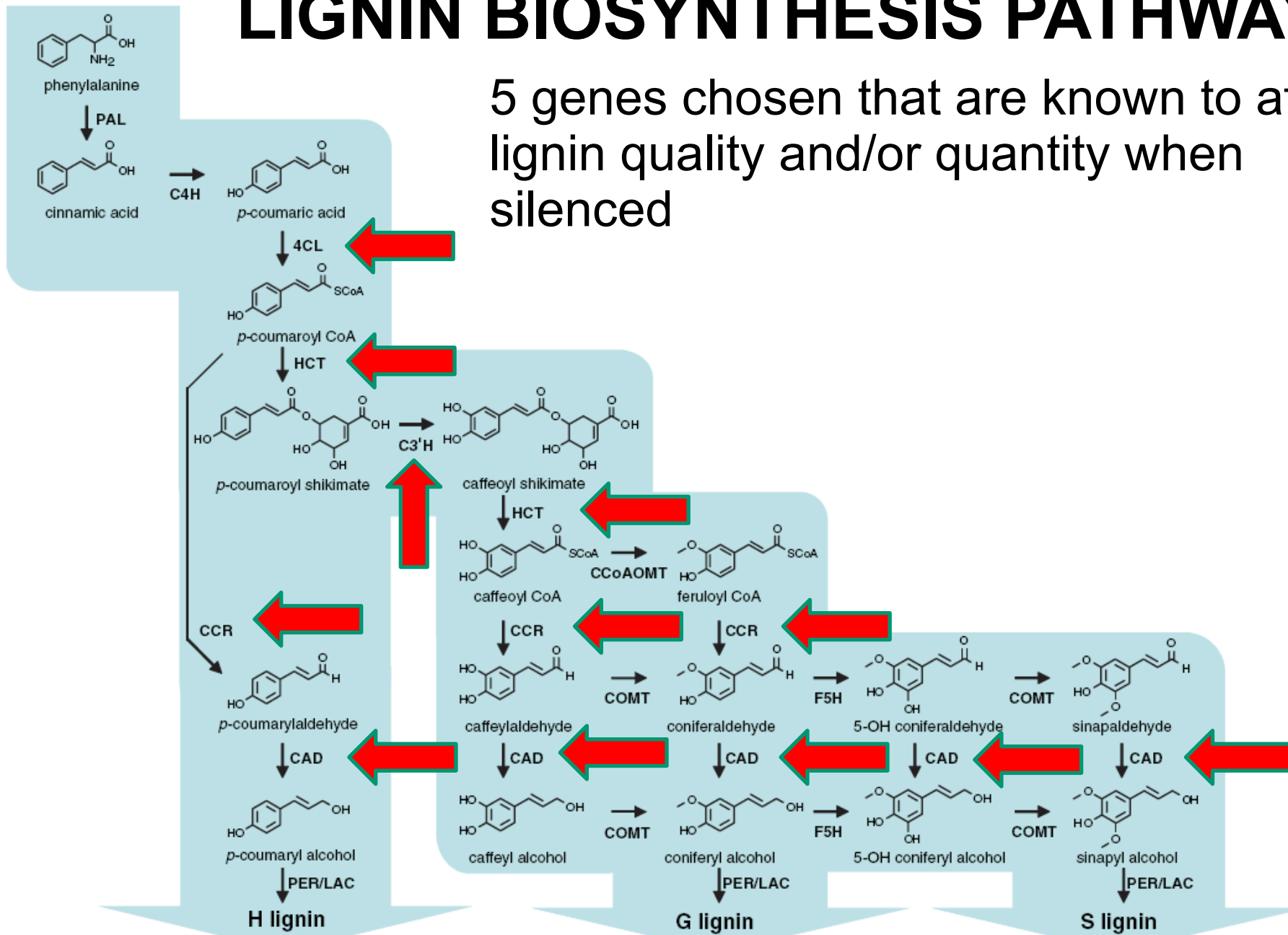


Development of locus-specific SNP markers for molecular diagnostics

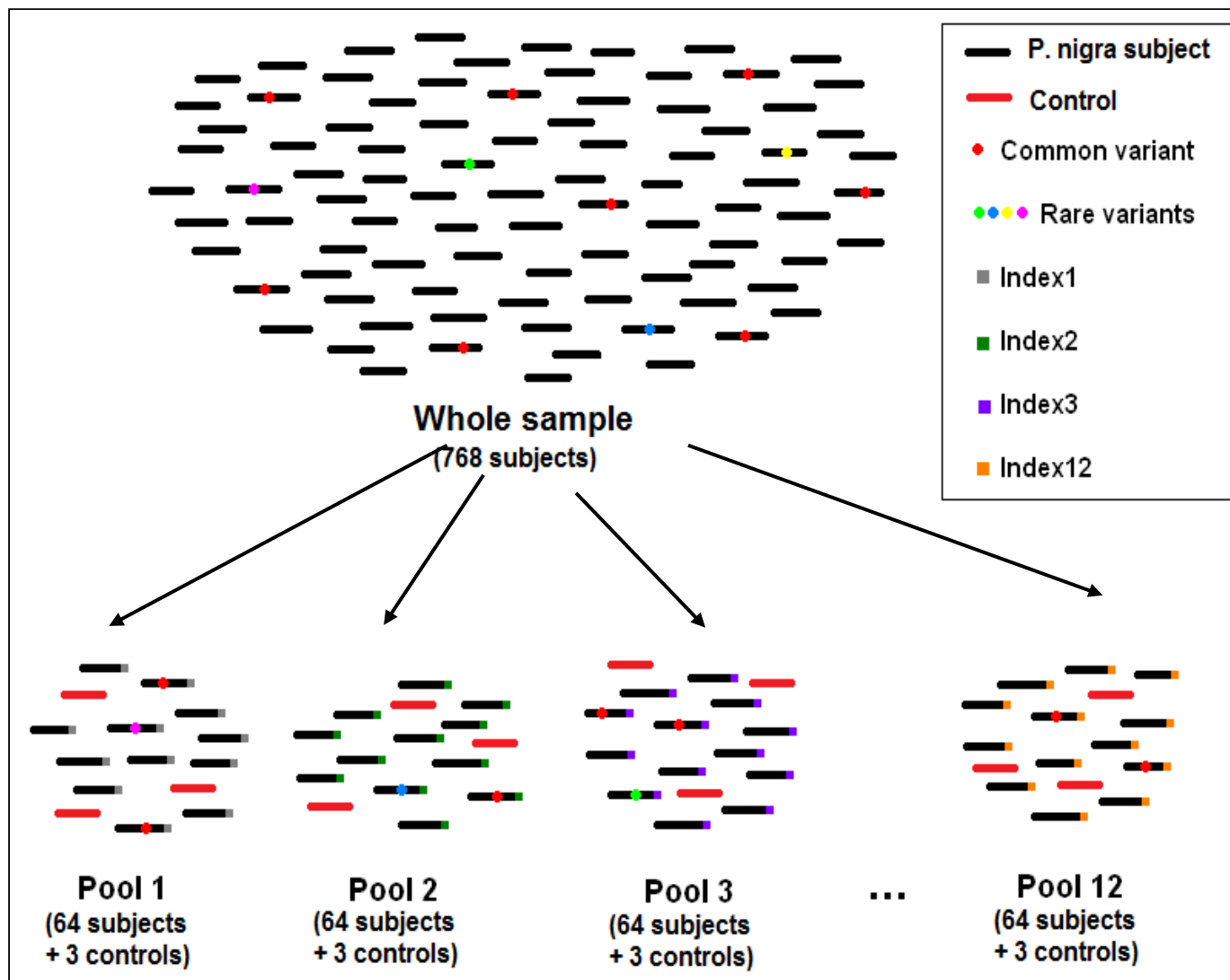
- To improve wood quality in poplar for saccharification and ethanol production by modifying lignin composition
- To identify naturally occurring mutations affecting genes involved in lignin metabolism. Functional variants are likely to be rare (Need large sample size).
- Best case scenario: to identify null alleles (variants causing premature stop codons).



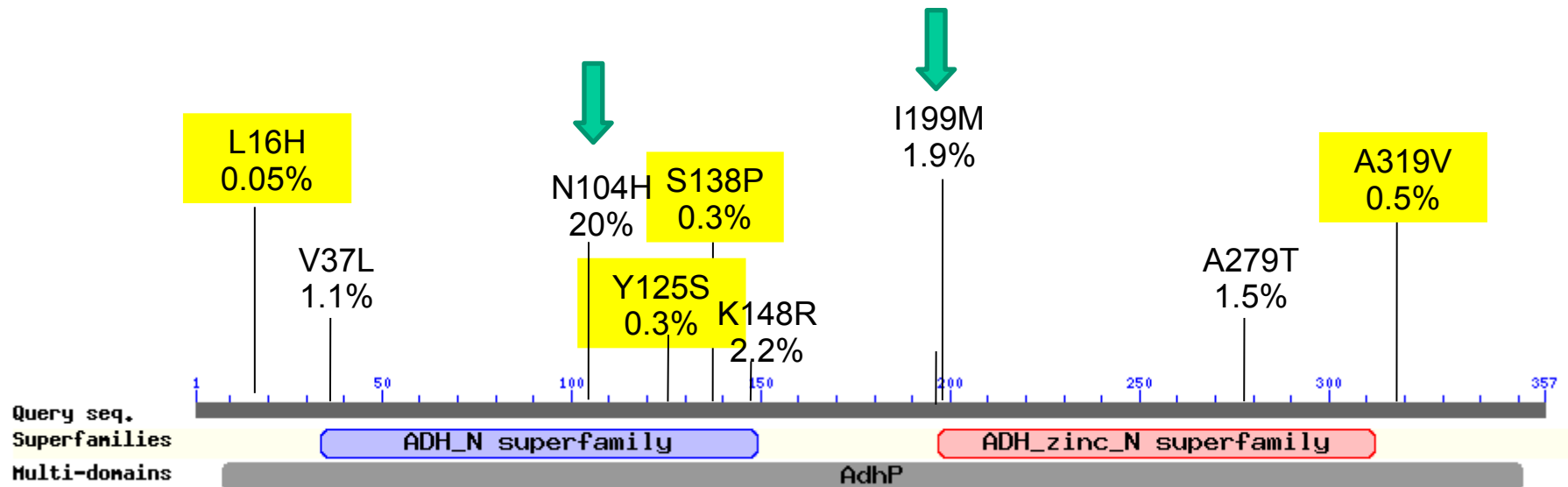
5 genes chosen that are known to affect lignin quality and/or quantity when silenced



MULTIPLEXED POOLED DEEP SEQUENCING OF LIGNIN BIOSYNTHETIC GENES



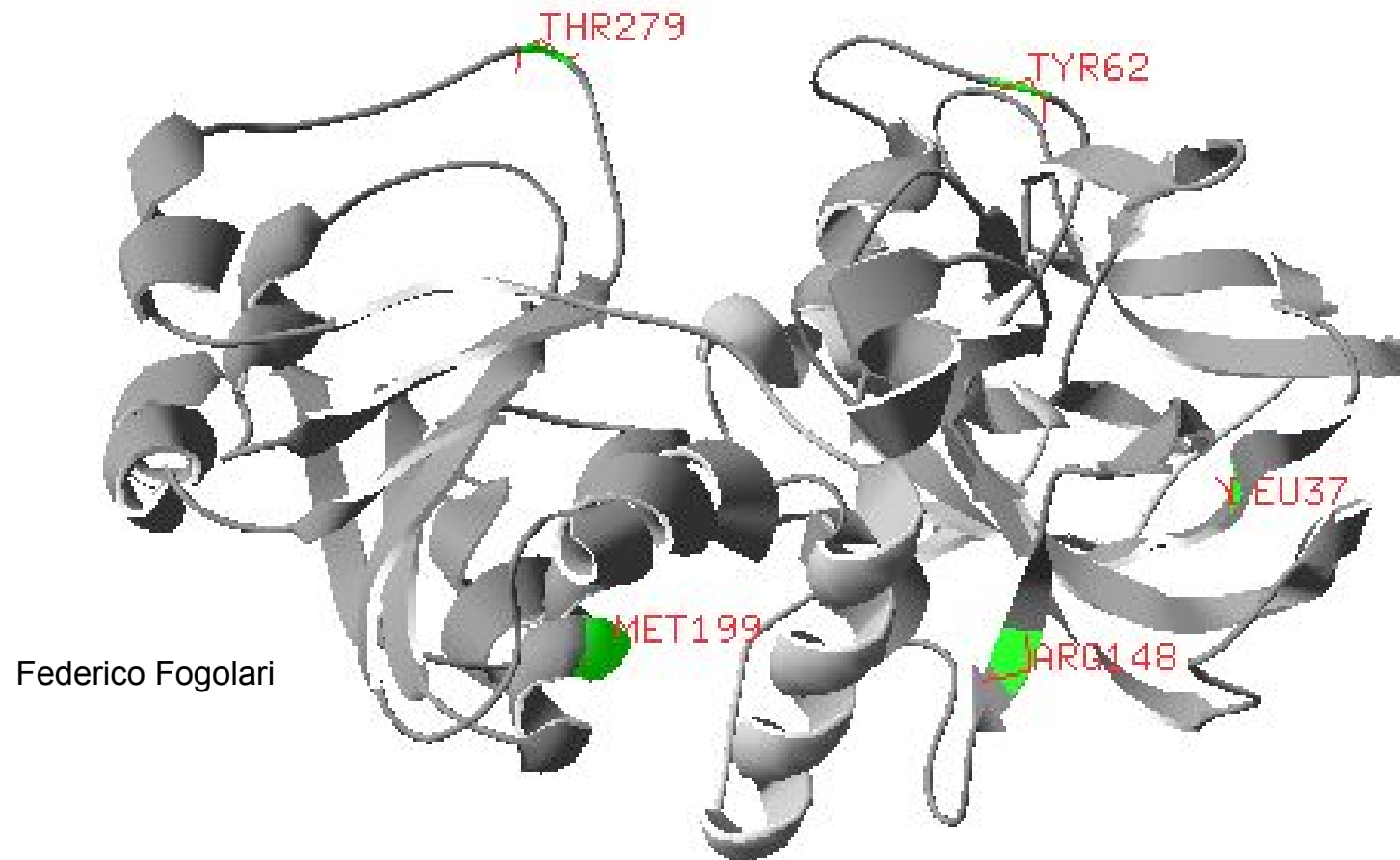
NON-SYNONYMOUS SNPs IN CAD



- ADH_zinc_N, Zinc-binding dehydrogenase
- ADH_N, Alcohol dehydrogenase GroES-like domain
- AdhP, Zn-dependent alcohol dehydrogenases

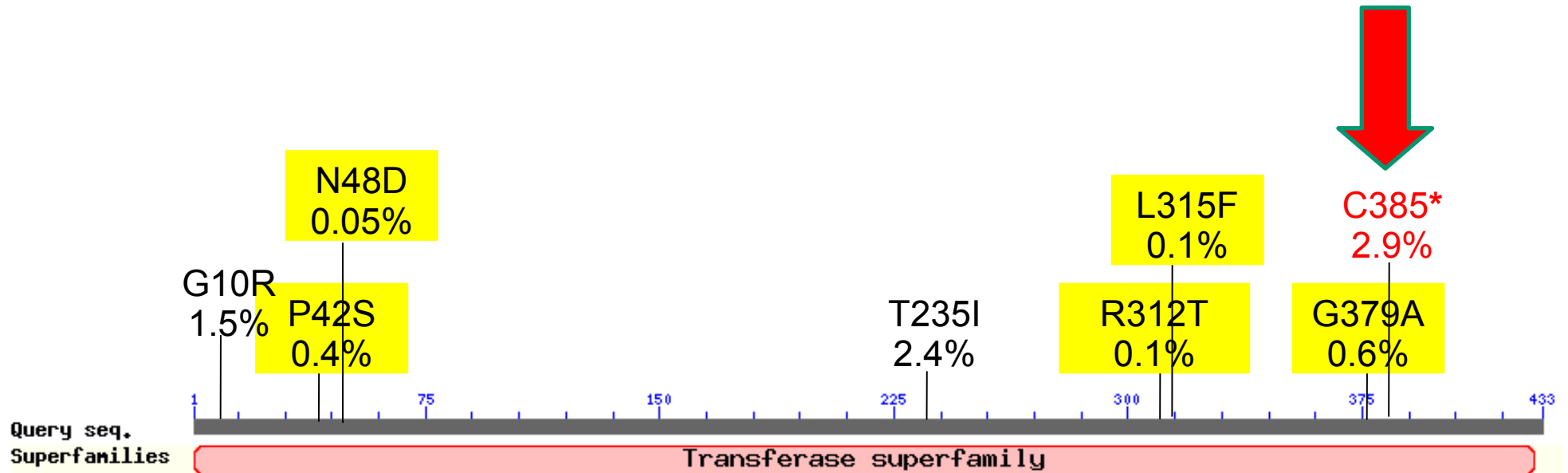


CAD PROTEIN STRUCTURE AND AA. SUBSTITUTIONS



Homology-based modelling of *P. nigra* CAD4 structure
Analysis of effects of aa. substitutions on protein structure and function

NON-SYNONYMOUS SNPs IN HCT1



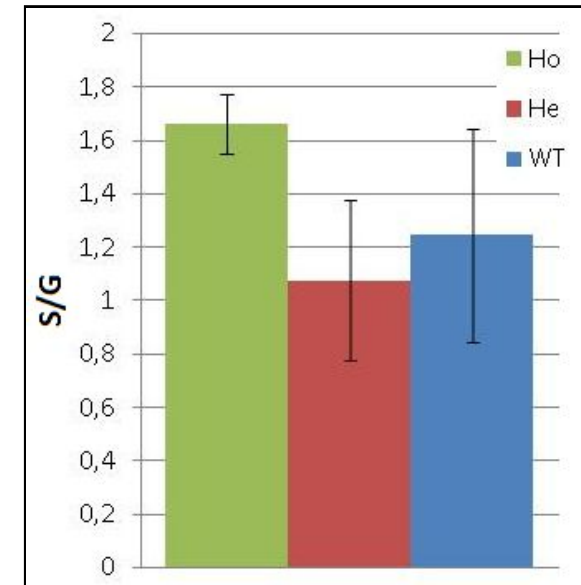
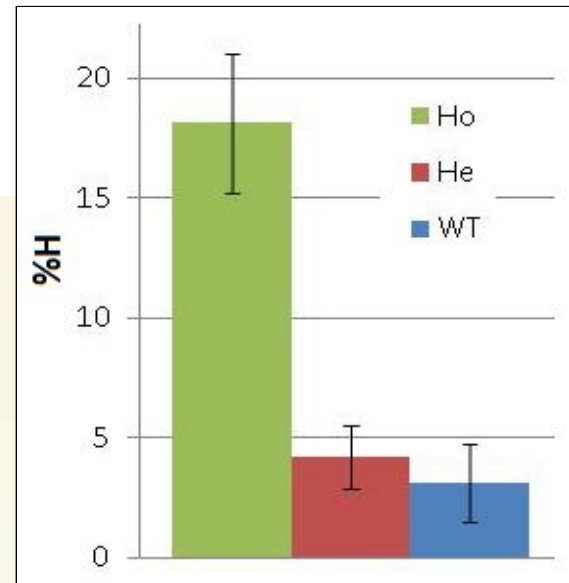
We performed crosses among individuals carrying interesting mutations to create homozygotes and look at phenotypes as well as analysed biochemically wild types, heterozygotes and homozygous nulls



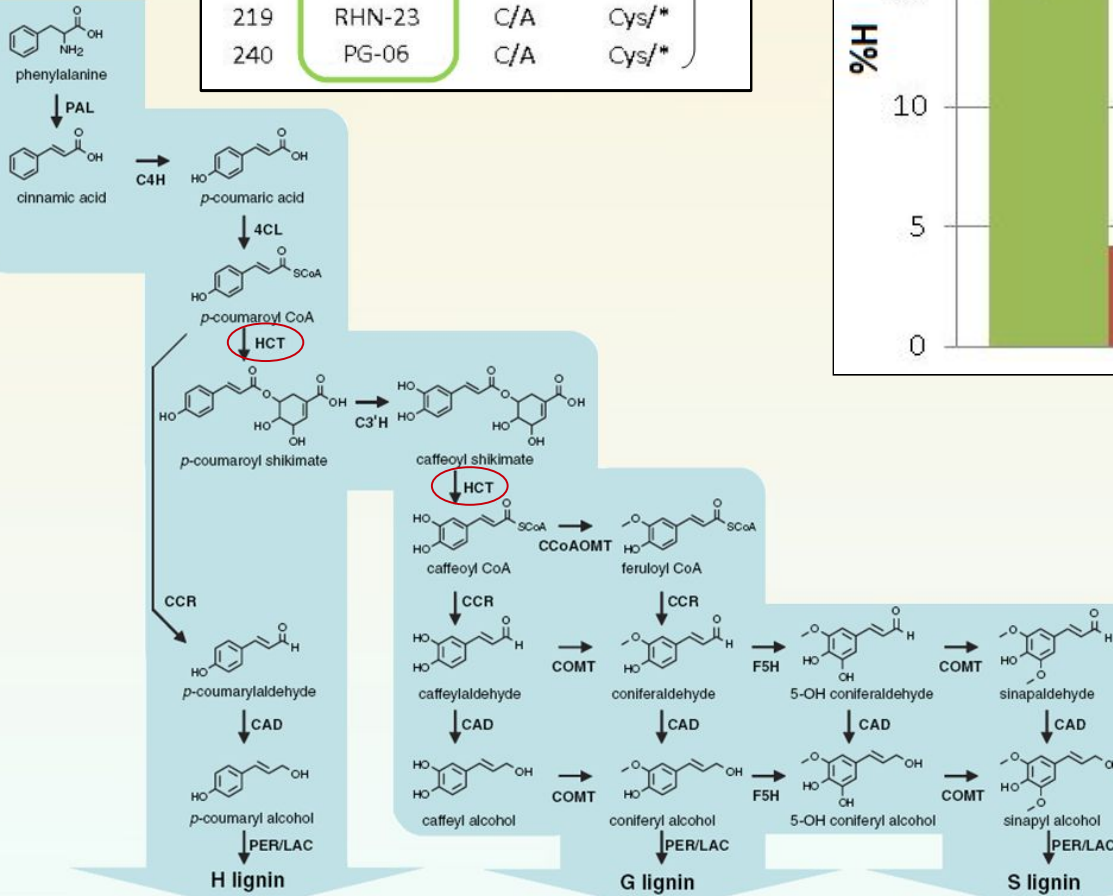
HCT1 MUTANTS: LIGNIN COMPOSITION



Code	Name	Genotype	aa in 385	
F9	71030-501	A/A	*/*	Ho
255	BSL-39	C/A	Cys/*	He
258	SPM-45	C/A	Cys/*	
155	BSL-01	C/A	Cys/*	
156	VDL-47	C/A	Cys/*	
219	RHN-23	C/A	Cys/*	
240	PG-06	C/A	Cys/*	



- ↑ H-units
- ↑ S/G-ratio
- No significant difference in lignin content and saccharification





Development of whole genome markers for genetic distance estimation

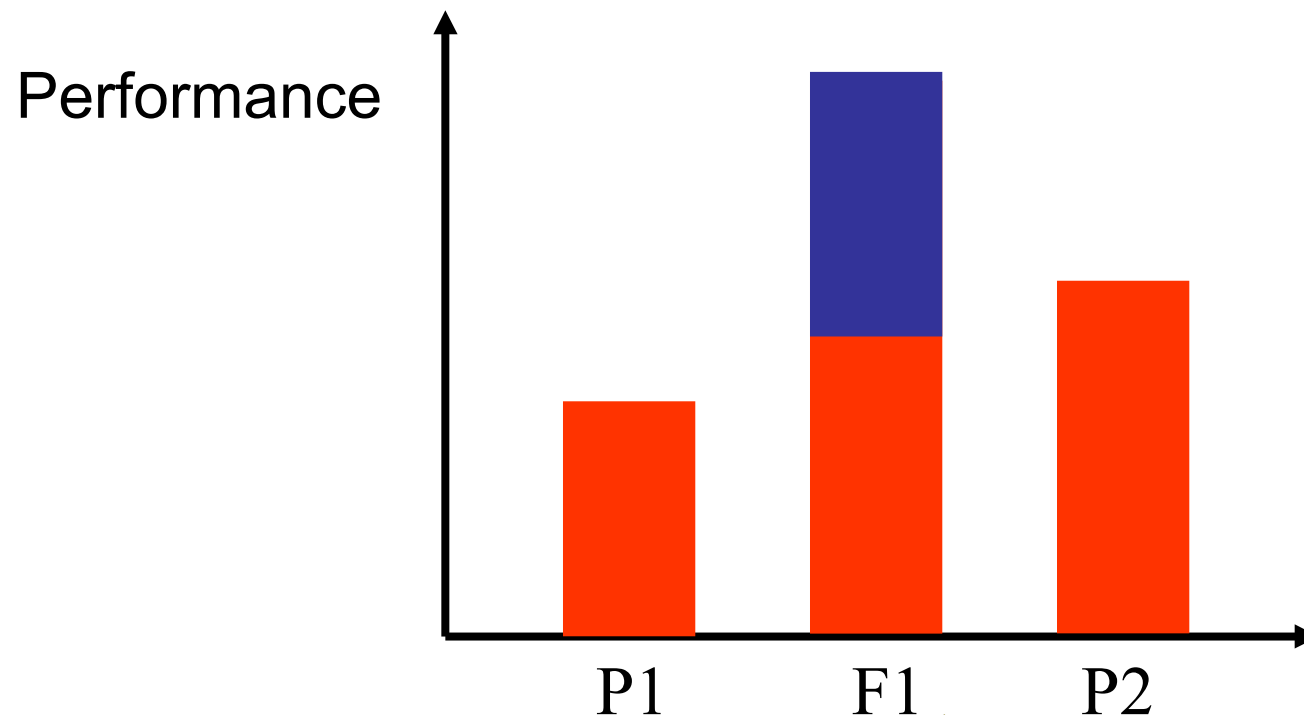
High-throughput resequencing of three *P. nigra* individuals. Joint re-sequencing effort EnergyPoplar, EvoTree, NovelTree.

Aim: To obtain whole genome map of SNPs and structural variants.



Understanding and exploiting heterosis

- Heterosis is the yield advantage of hybrid individuals in comparison to the parents
- In poplar commercial clones are interspecific hybrids

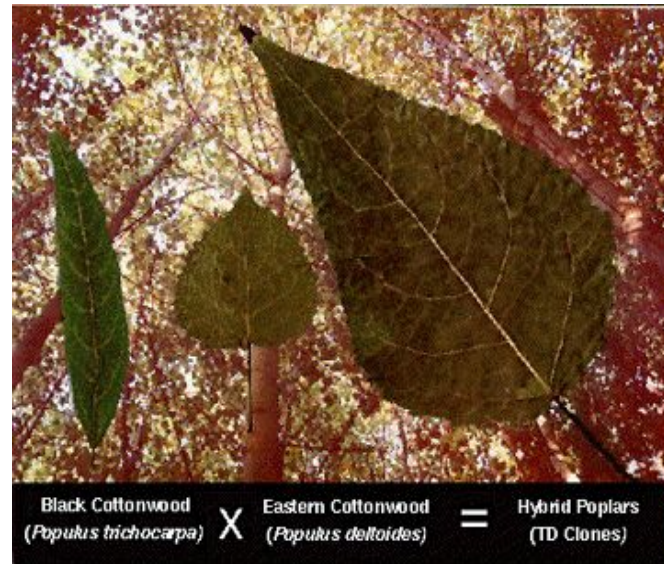


POPLAR SPECIES COMPARED

Populus nigra, *Populus deltoides*, *Populus trichocarpa*

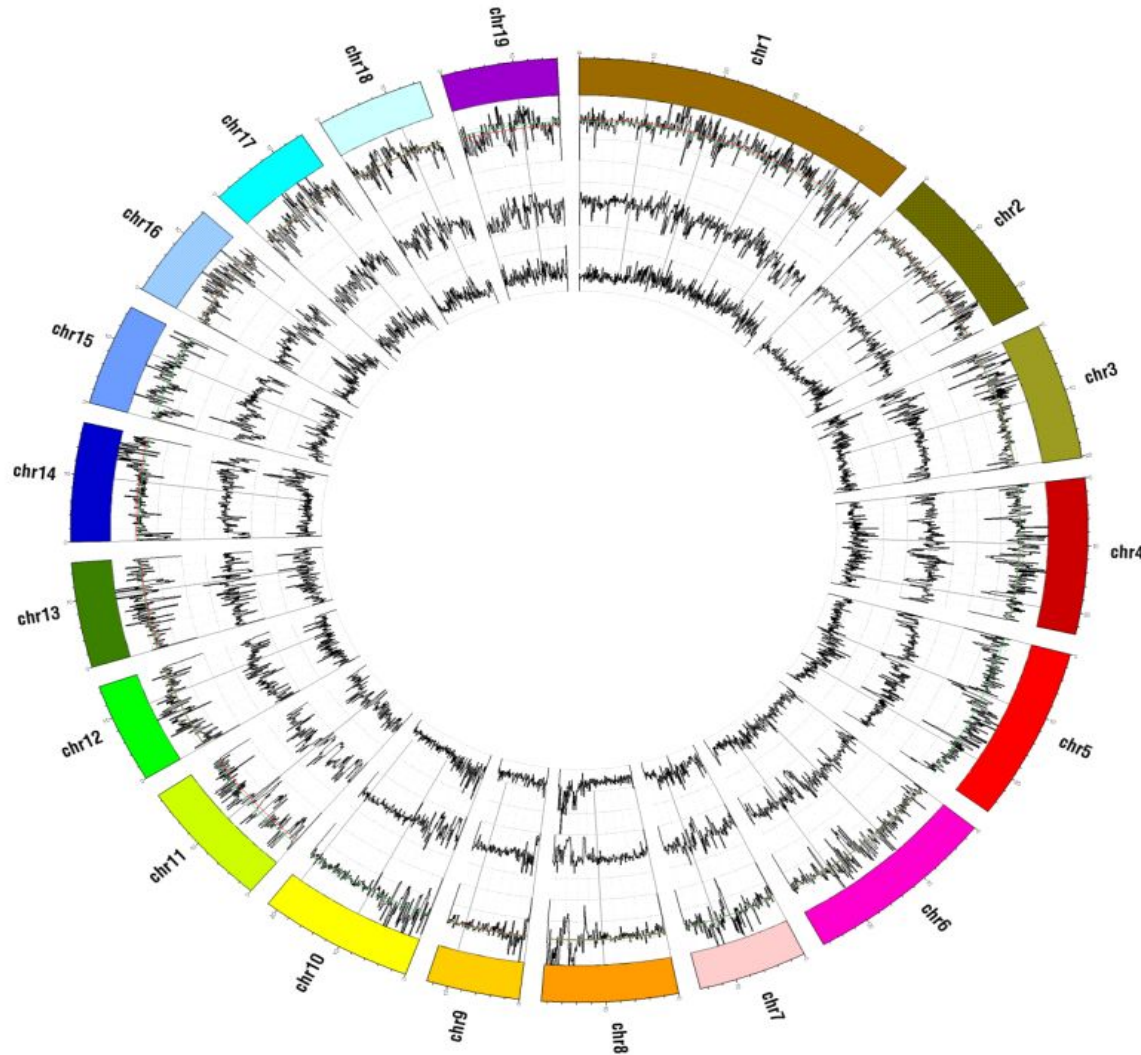


Interfertile →



→ Improved performances in the F1 hybrids: hybrid vigour

Development of whole genome markers for genetic distance estimation: SNP distribution

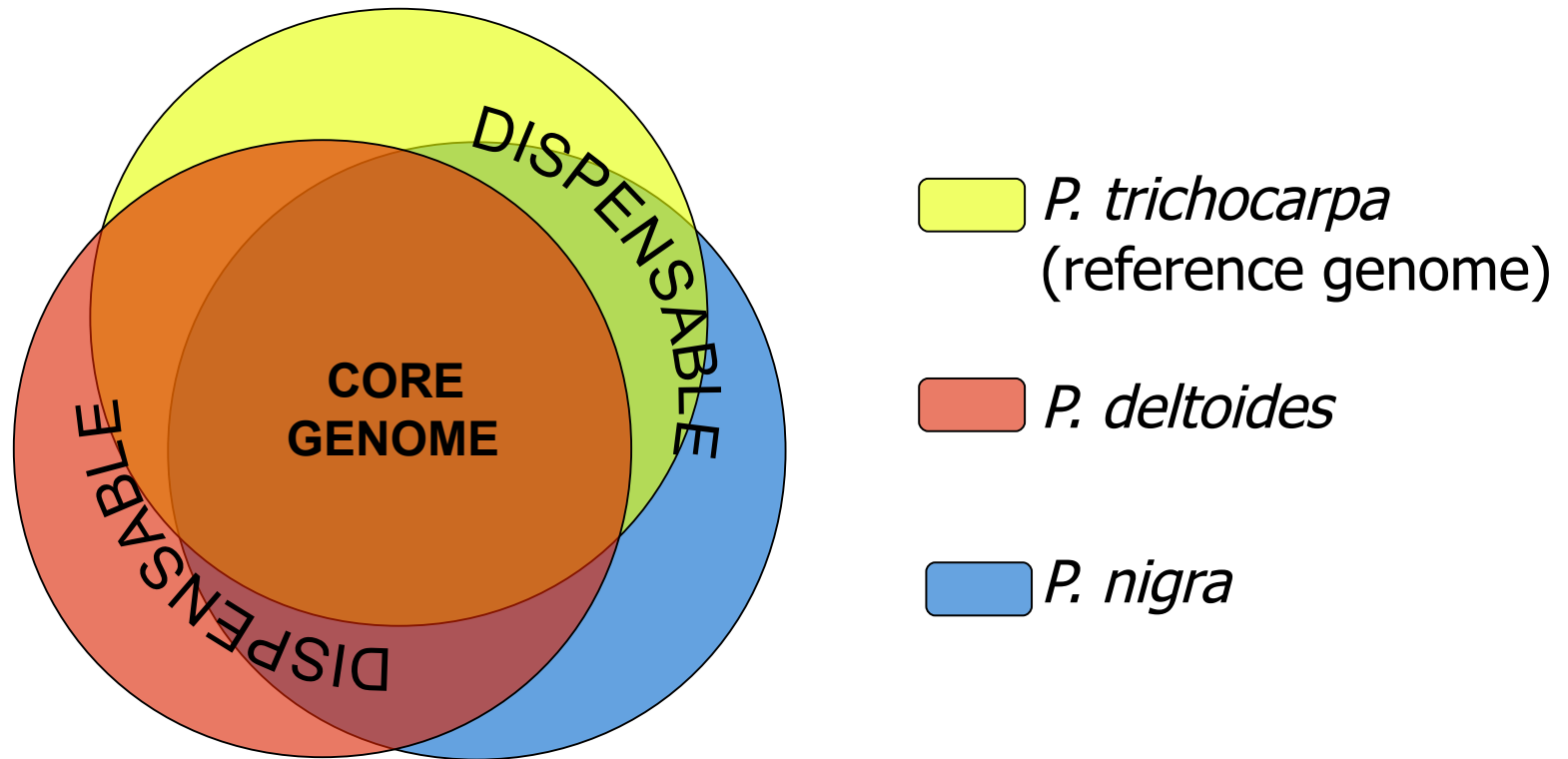


Outer layer: total SNPs
Inner layer: heterozygous SNPs (within *P. nigra* SNPs)
Middle layer: homozygous SNPs (*P. nigra*-*P. trichocarpa* SNPs?)

Stefania Giacomello

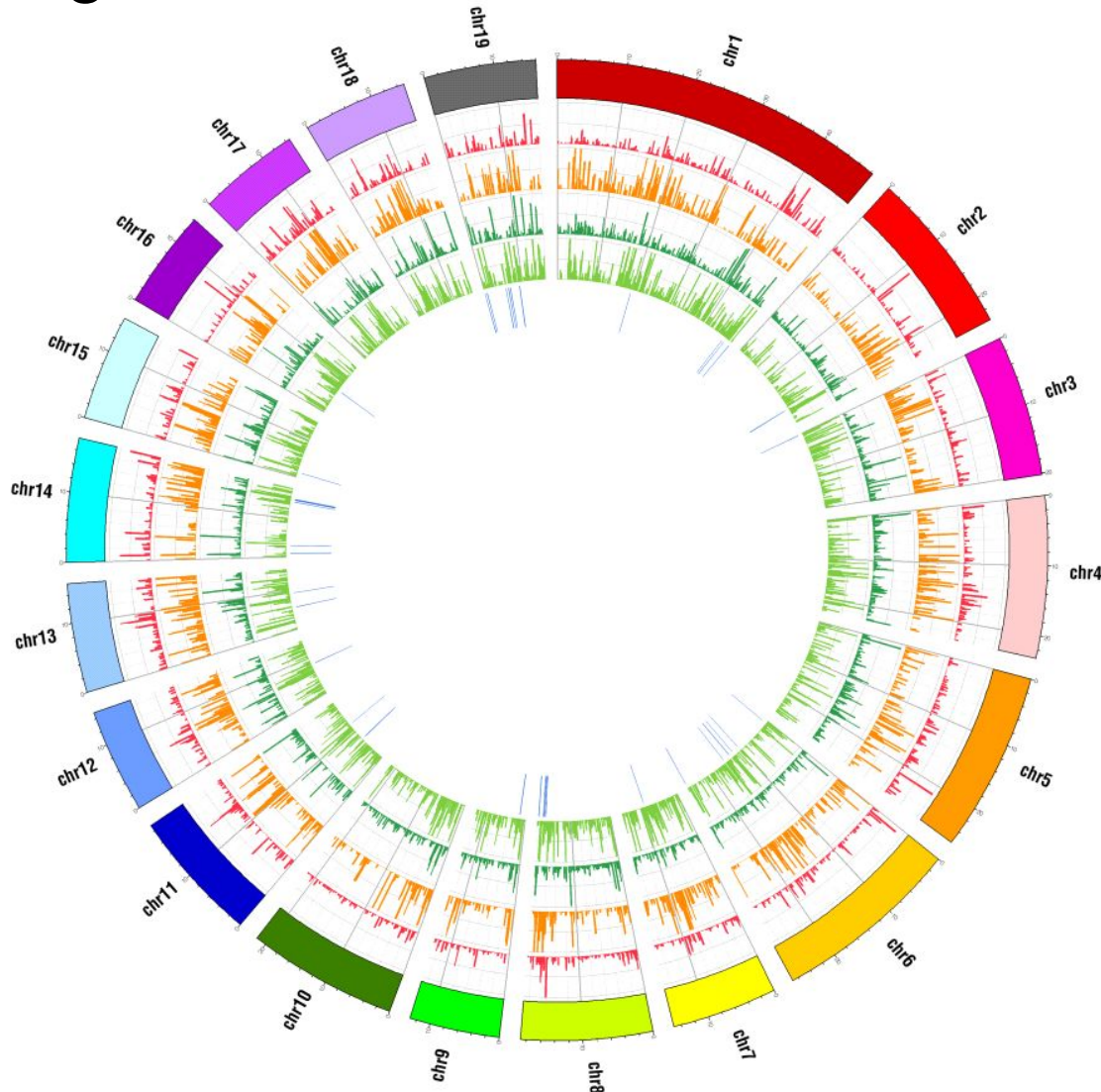


TOWARDS THE POPLAR PAN-GENOME



- Dispensable genome: involved in heterosis?
- Structural variation analysis → short and large deletions and insertions

Development of whole genome markers for genetic distance estimation: SV distribution



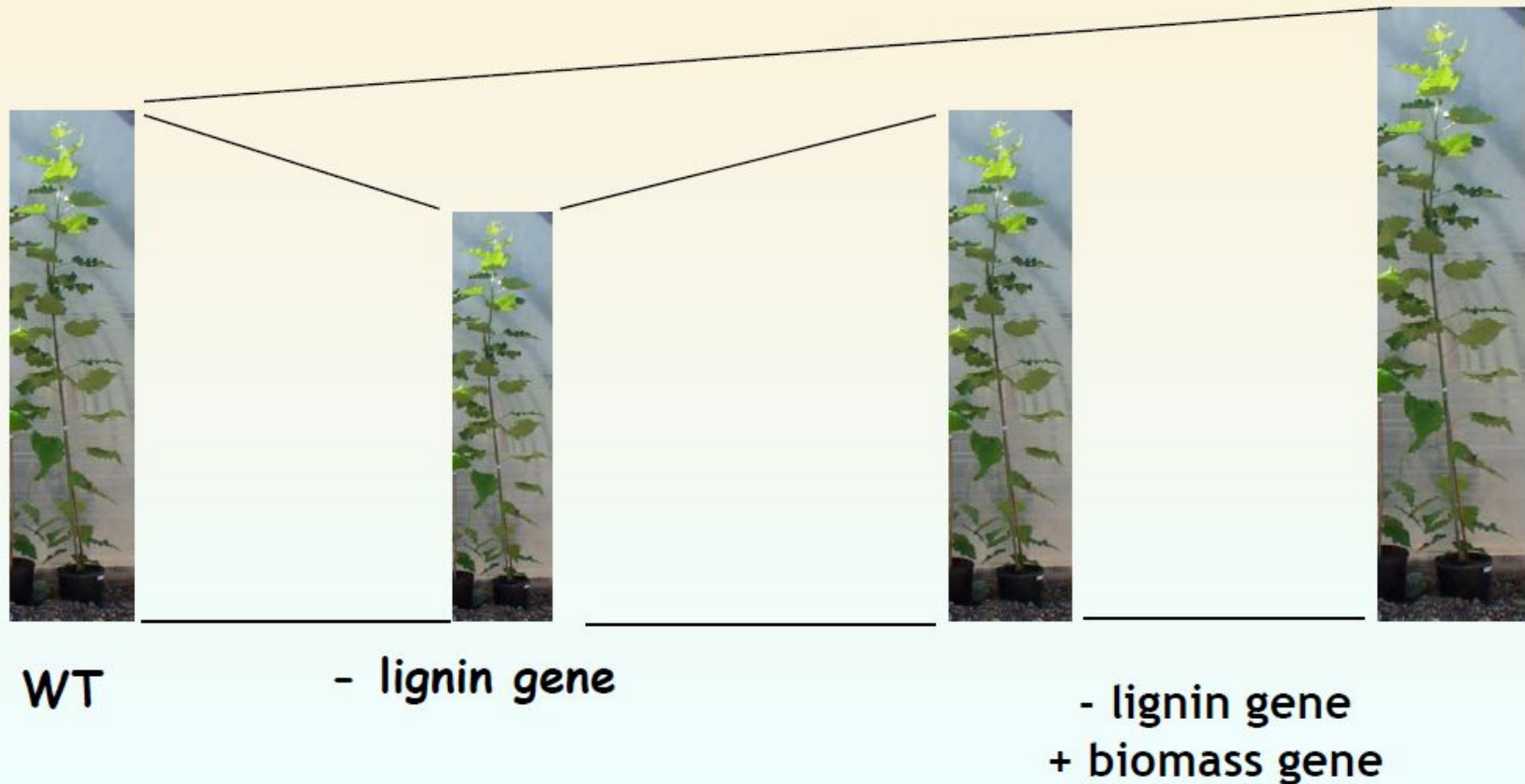
Red bars: deletions in *P. deltoides*
Orange bars: insertions in *P. deltoides*
Green bars: deletions in *P. nigra*
Light green bars: insertions in *P. nigra*

Blue segments represent regions of copy number variation between the two species.



Development of transgenic poplars with multiple transgenes (gene-stacking)

Aim: To combine genes beneficial (e.g.) for biomass and wood composition



Annabelle Déjardin, INRA Orléans, Orléans, France



Involved units

N°	Beneficiary name	Short name	Country	Date enter project	Date exit project
1.	Institut National de la Recherche Agronomique	INRA	France	M1	M48
2.	Sveriges Lantbruksuniversitet	SLU	Sweden	M1	M48
3.	Albert-Ludwigs-Universität Freiburg	ALU	Germany	M1	M48
4.	Flanders Institute for Biotechnology	VIB	Belgium	M1	M48
5.	University of Southampton	SOTON	UK	M1	M48
6.	Istituto di Genomica Applicata	IGA	Italy	M1	M48
7.	Georg-August Universität Göttingen	UGOE	Germany	M1	M48
8.	SwETree Technokogies AB	STT	Sweden	M1	M48
9.	Imperial College of Science, Technology and Medicine	Imperial	UK	M1	M48
10.	INRA Transfert	IT	France	M1	M48





Università degli
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DiSA

Dipartimento di Scienze Agrarie e Ambientali
Department of Agriculture and Environmental Sciences

Stefania Giacomello
Giusi Zaina
Poster 12

Sara Pinosio
Fabio Marroni
Poster 27, 24

Patricia Faivre-Rampant
Veronique Jorge
Catherine Bastien
INRA

Federica Cattonaro
Eleonora Dicenta
Irena Jurman
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Alberto Policriti
Cristian Del Fabbro
Francesco Vezzi
Simone Scalabrin

Wout Boerjan
VIB

Stefan Jansson
UPSC



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