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Genetic determination of wood traits in Scots pine - analysis of candidate genes influencing wood quality

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Introduction

- With a few exceptions, little is known about the cellular, molecular, and developmental processes that underlie wood formation.
- Wood formation is controlled by exogenous (photoperiod and temperature) and endogenous (phytohormones) factors and by interaction between them.
- Wood (secondary xylem) formation four major steps : cell division, cell expansion (elongation and radial enlargement), cell wall thickening (involving cellulose, hemicellulose, cell wall proteins, and lignin biosynthesis and deposition) and programmed cell death.
- Wood density is one of main characteristics of wood quality.

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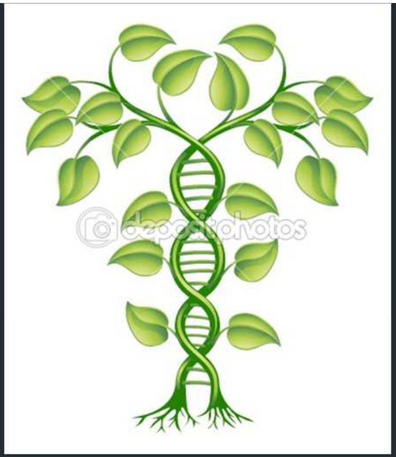
Genetic aspects



- **Wood formation is driven by the coordinated expression of numerous structural (some of known function) and regulatory genes (mostly unknown).**
- **The very large genomes of all *Pinus* species, long life cycle, large multigenic families and high metabolistic elasticity are some of the reasons why pines were less studied at molecular level.**
- **Now there are linkage maps, EST databases and gene expression data available for *P.taeda* and *P.radiata* so comparative genomics approach can be used for information transfer to other important pine species.**
- **Gene copy number variation (CNV) is type of genetic variation. In human genomes CNV affects gene expression. In *Arabidopsis thaliana* and *Sorghum bicolor* gene duplications altered ion transport.**

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Aim

- To find out if it is possible to find link between wood density and genetic parameters in Scots pine
- For future dream- marker assisted selection for Scots pine.
- Taking in consideration one genetic variation types –CNV.

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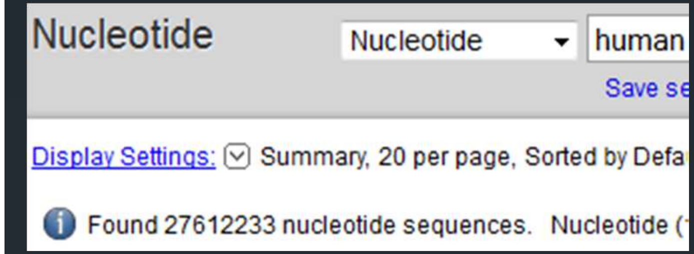
Nucleotide

Nucleotide ▾ human

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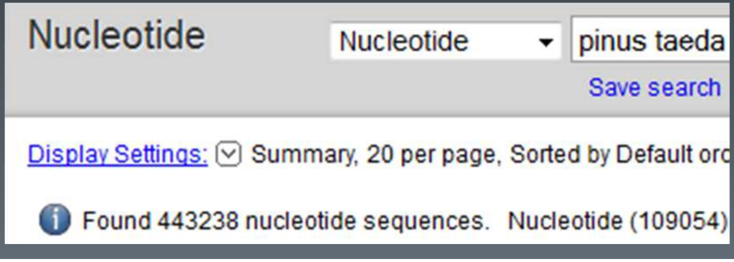
Found 27612233 nucleotide sequences. Nucleotide (

Methods

- Wood density measured with Pilodyn instrument.
- Selected 25 trees with higher wood density and 25 with lower, belonging to different tree families.
- Literature analysis and searching in databases for candidate genes.
- Sample gathering (Spring and Autumn), DNA, RNA extracting, purification, PCR, Real time PCR optimization.
- Quantitative RT PCR for detecting gene copy number variation.
- Relative standart curve RT PCR for gene expression analysis.
- Data analysis


Nucleotide

Nucleotide ▾ pinus taeda

Save search

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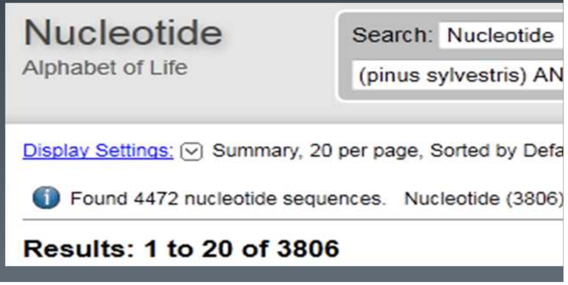
Found 443238 nucleotide sequences. Nucleotide (109054)


Nucleotide

Alphabet of Life

Search: Nucleotide

(pinus sylvestris) AN

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Found 4472 nucleotide sequences. Nucleotide (3806)

Results: 1 to 20 of 3806

Candidate genes

- At first nine genes were taken in consideration (*CAD1, CESA1, CESA7, CESA4, AGP1, aquaporin like, aquaporin gene, Susy1*).
- Candidate genes were selected of which three are involved in lignin biosynthesis – phenylalanine ammonia-lyase gene (*PAL1*), cinnamyl alcohol dehydrogenase gene (*CAD1*), Cinnamoyl-CoA reductase gene (*CCR1*);

sucrose synthase gene (*Susy1*) in sucrose metabolism ; Aquaporin like gene (*Aqual1*) involved in water transport, in attempts to reveal whether link can be established between candidate gene expression, gene copy number and wood density .

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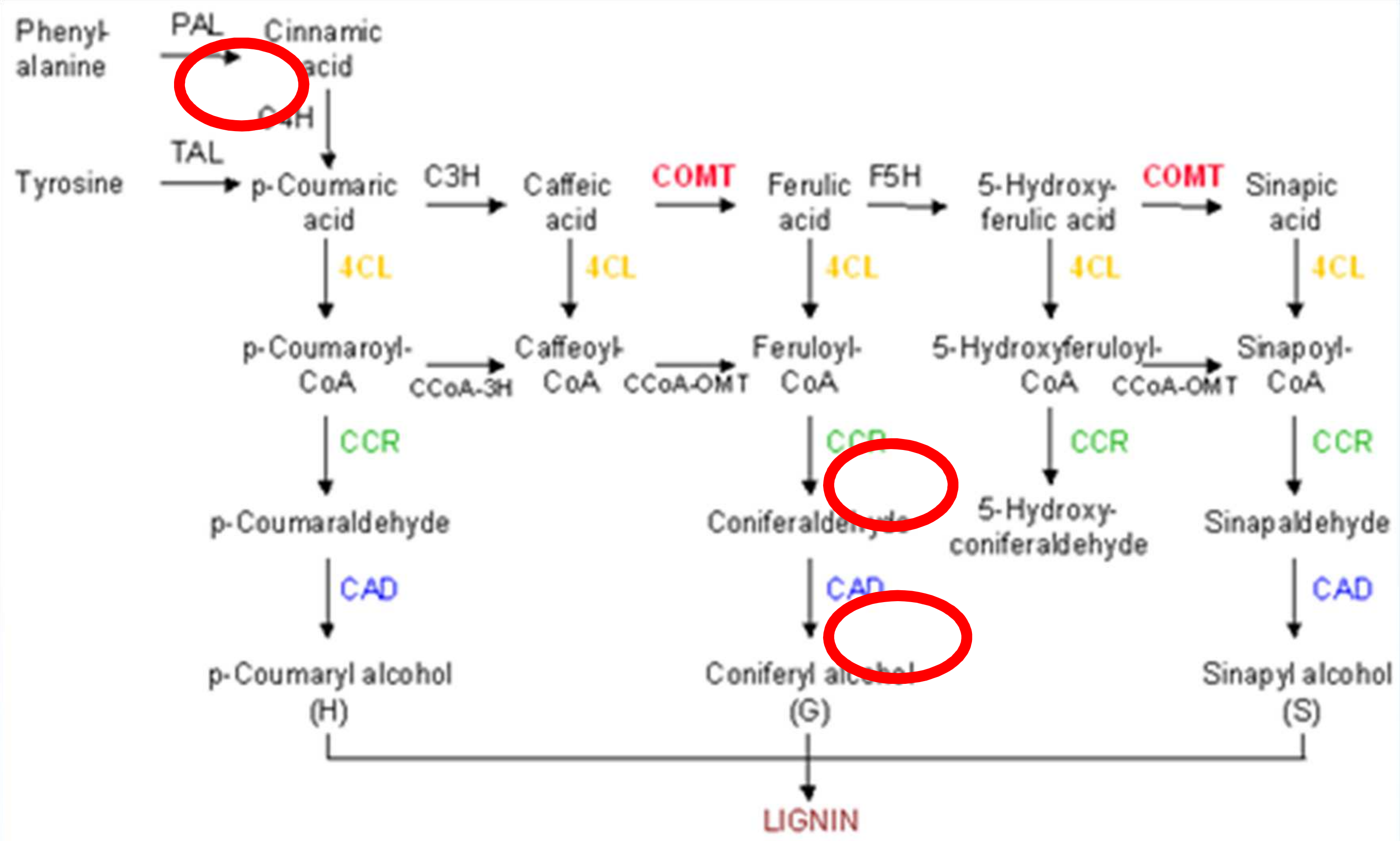
Lignin biosynthesis



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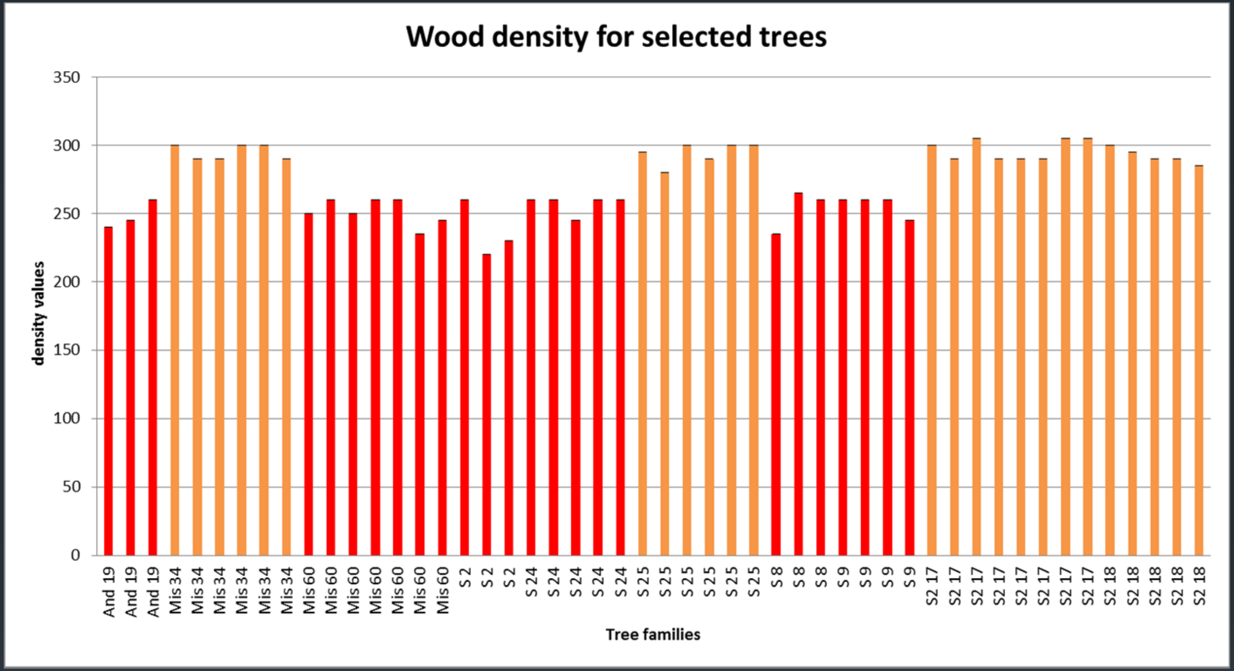
Wood density



Orange - trees with higher wood density

Red - with lower wood density

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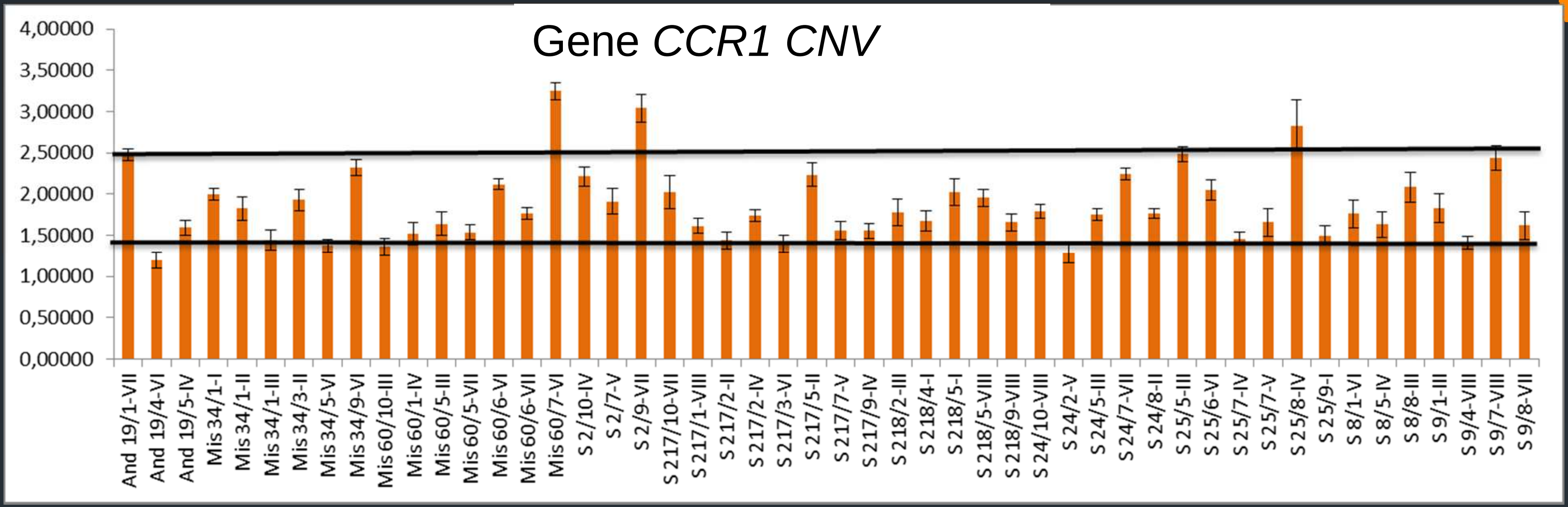
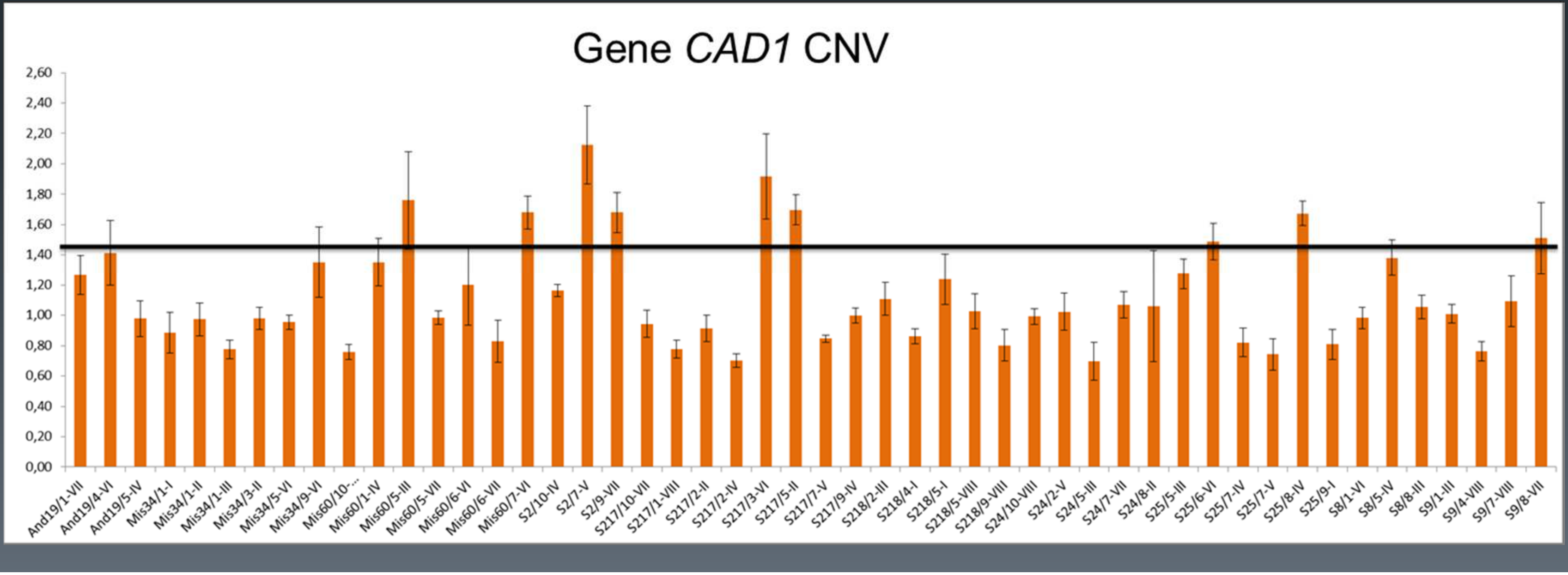
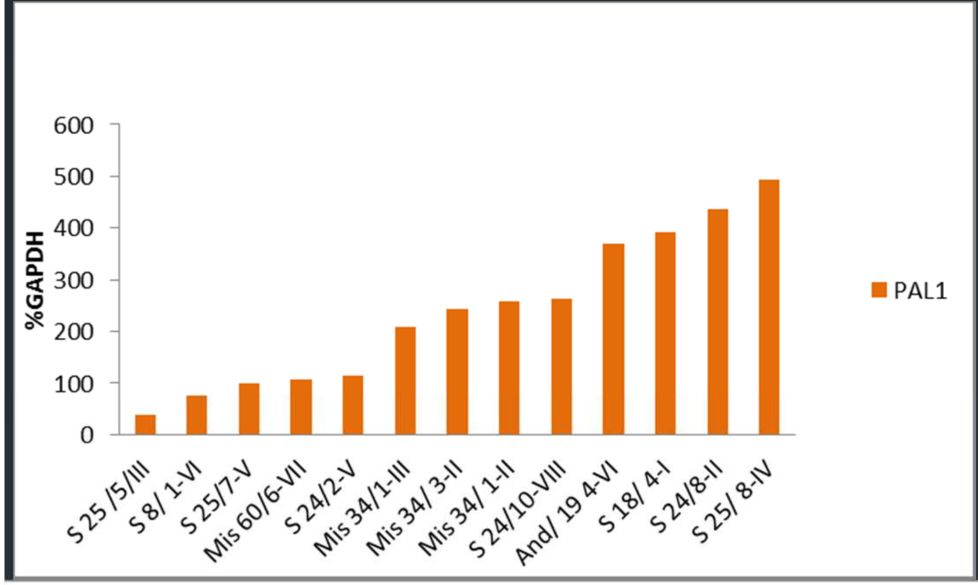
Copy number variation of gene *CCR1* and *CAD1*

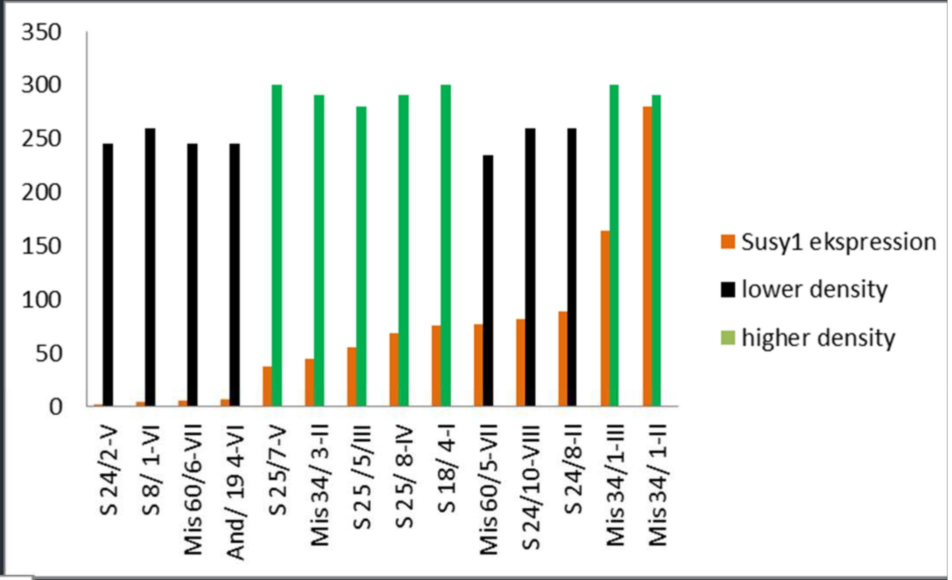
|Z| < 2

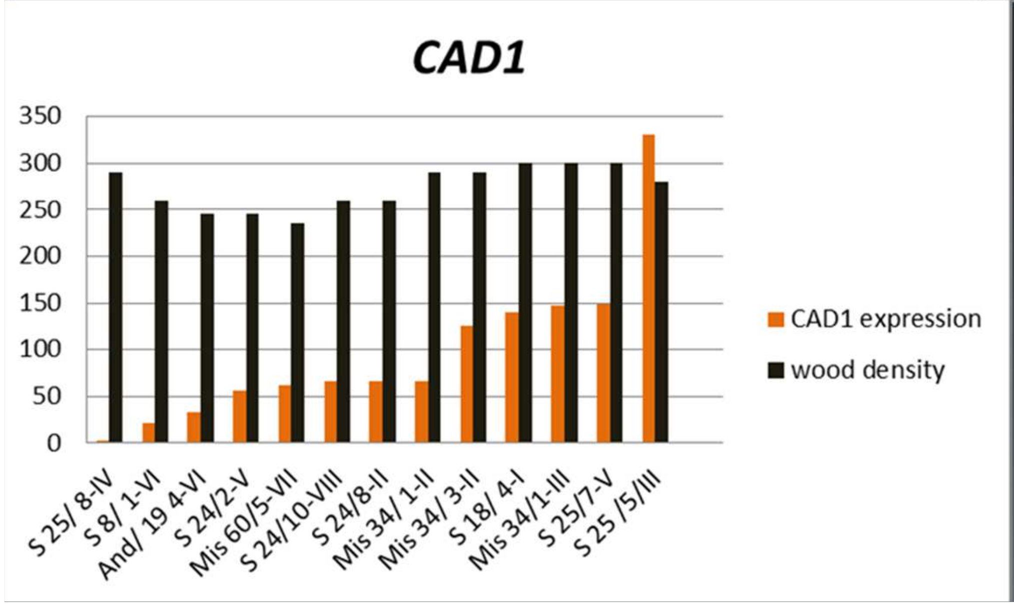




Gene *PAL1, CAD1, Susy1* expression(spring)







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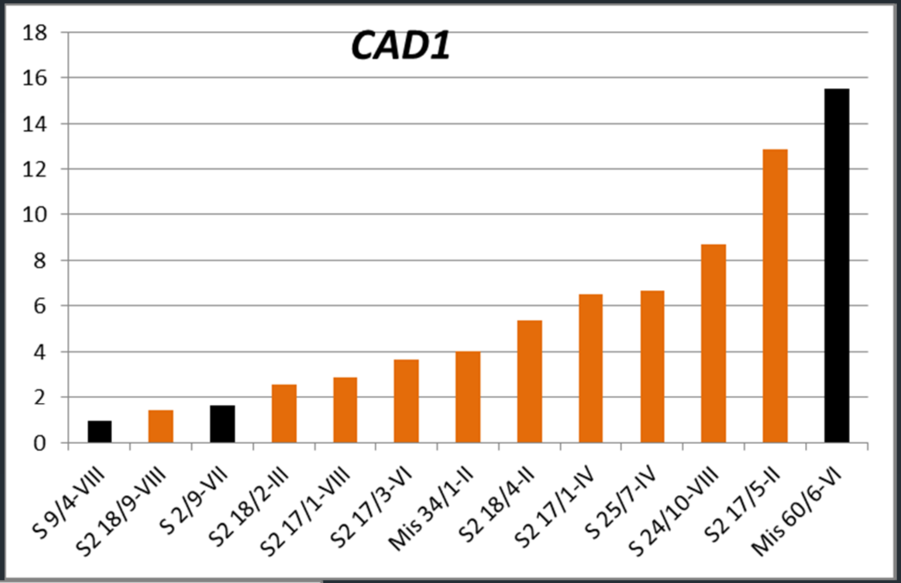
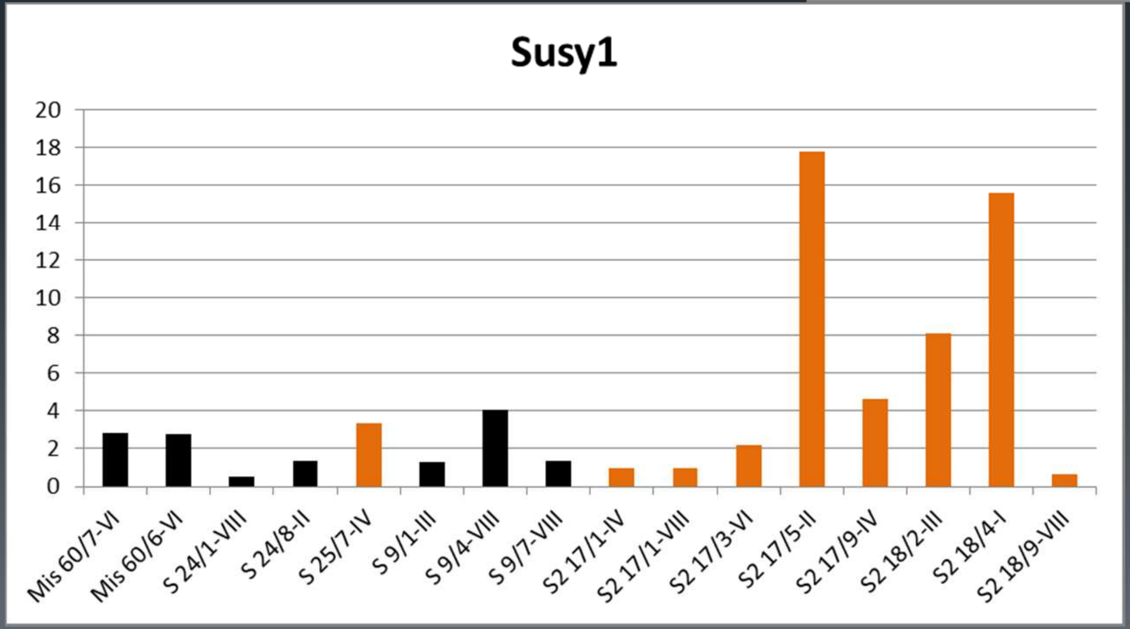
Autumn





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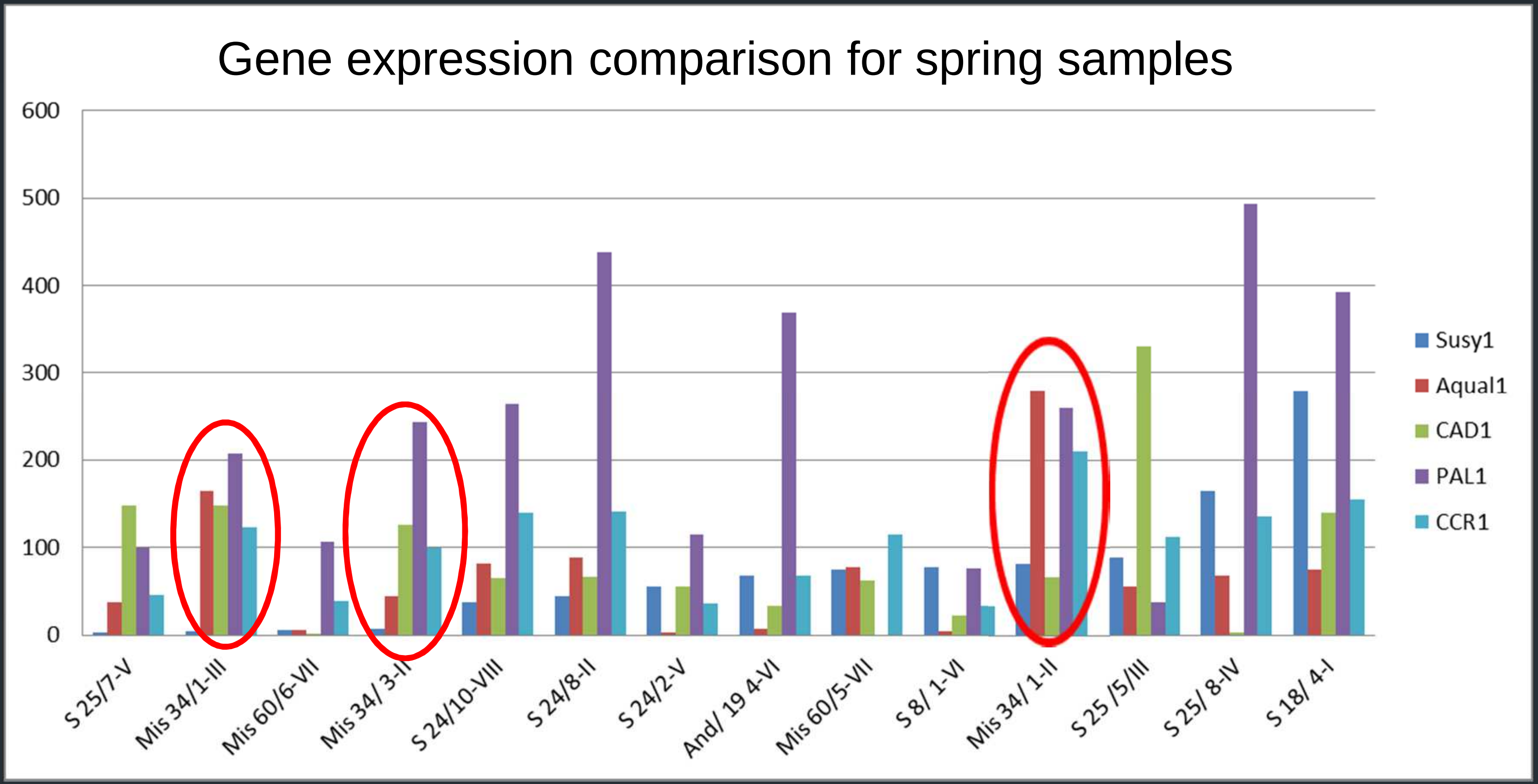
Gene expression comparison for spring samples

PAL1 ekspression higher 2x than for others
Mis 34 perspective

	Susy1	**Aqual1**	**CAD1**	**PAL1**	**CCR1**
Susy1	1				
Aqual1	0,063774	1			
CAD1	0,043073	0,11905	1		
PAL1	0,4313	0,205608	-0,3006	1	
CCR1	0,400035	0,838829	0,166369	0,492318	1

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Some conclusions

- Quantitative RT PCR can be used to determine gene copy number variation for *P.sylvestris.*
- CNV data confirms sugestions about genes PAL1 and CAD1- that there are single functional gene copy in pines.
- There are positive regression between gene *SuSy1* and *CAD1* expression in spring and wood density, so ve can assume thatearly wood density is affected significantly by carbohydrate availabilty and last step of monolignol biosynthesis .
- Between genes *CCR1* and *Aqual1* exists strong correlation for spring samples, which my indicate about gene interraction im wood formation process.

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Future research plans

- Continue gene expression determination for genes *CAD1, PAL1, Aqual1,Susy1,CCR1* for all samples and possibly more.

- Analyse more candidate genes, for example transcription factors *MYB1*, *MYB2.* Search for genes envolved in monolignol transportation (coniferyl alcohol glucosyltransferase).

- Microscopic evaluation of celulose/lignin content, cell wall thickness. Or at least earlywood/latewood proportion measurment.

- Sequencing in hope to find gene regions suitable for marker development

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Conferences and result presentations

- 69. Annual Scientific Conference of University of Latvia, Plant Biology section, 22 february, 2011 (Oral Presentation).

- Scientific conference «Advances in plant biotechnology in Baltic Sea Region» , 30-31 March, 2011, Lithuania, Abstract book p.53-54, (Poster) .

- 2011. Master degree in Biological Sciences in University of Latvia.

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Research funding

European Social Fund’s Project “Importance of Genetic Factors in Formation of Forest Stands with High Adaptability and Qualitative Wood Properties” (No 2009/0200/1DP/1.1.1.2.0/09/APIA/VIAA/146)

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Thank you for attention!