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Studying the landscape of nucleotide diversity in candidate genes for wood quality in *Pinus pinaster*.

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Improvement of wood quality related traits is currently hampered by costly chemical and technological assays and the necessity to wait until the trees are nearly mature to evaluate wood properties. The availability of a vast quantity of genomic data opens now a new avenue to identify early selection criteria based on molecular information and therefore increase selection efficiency. Association mapping is becoming a method of choice to identify QTN (quantitative trait nucleotide) that contribute to complex trait variation. The implementation of this approach requires on the one hand knowledge of the molecular mechanisms underlying trait variation and polymorphism within candidate genes, and on the other hand the availability of phenotypically well characterized genetic material. We are developing this strategy in the frame of the maritime pine breeding program, an economically important forest tree species in the South Western Europe. This poster reviews the results obtained in respect to the pattern of nucleotide diversity for a set of wood quality candidate genes (structural genes of the lignin and cellulose biosynthesis, transcription factors). In addition to the estimation of diversity levels and extent of linkage disequilibrium, this study also provides some clues about compatibility between nucleotide diversity patterns and neutral models of molecular evolution.

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General approach

Availability of phenotypically well characterized genetic material (breeding program)

Knowledge of nucleotide polymorphism and linkage disequilibrium patterns within candidate genes for wood properties

Association mapping

Identification of QTNs that contribute to complex trait variation

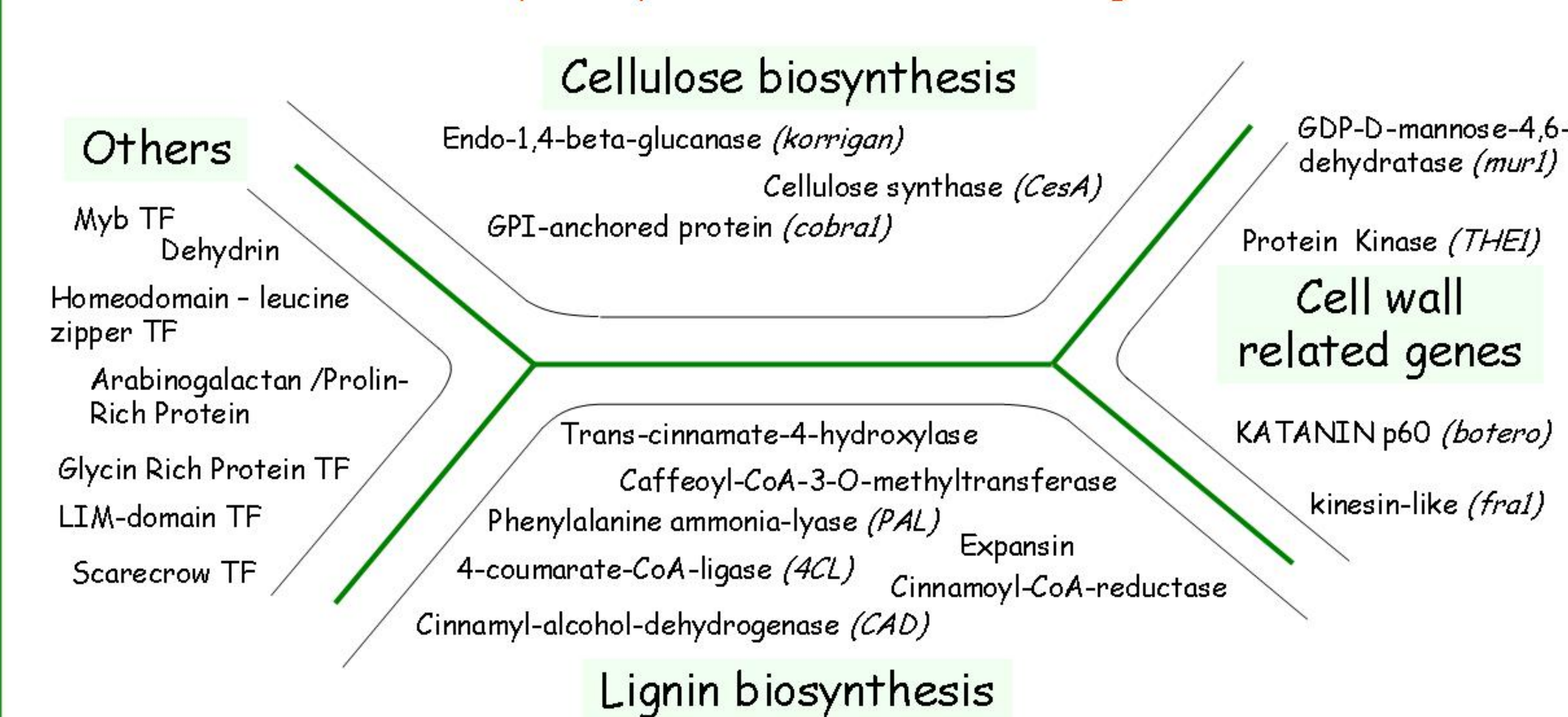
Improvement of wood quality related traits by marker assisted selection

Questions

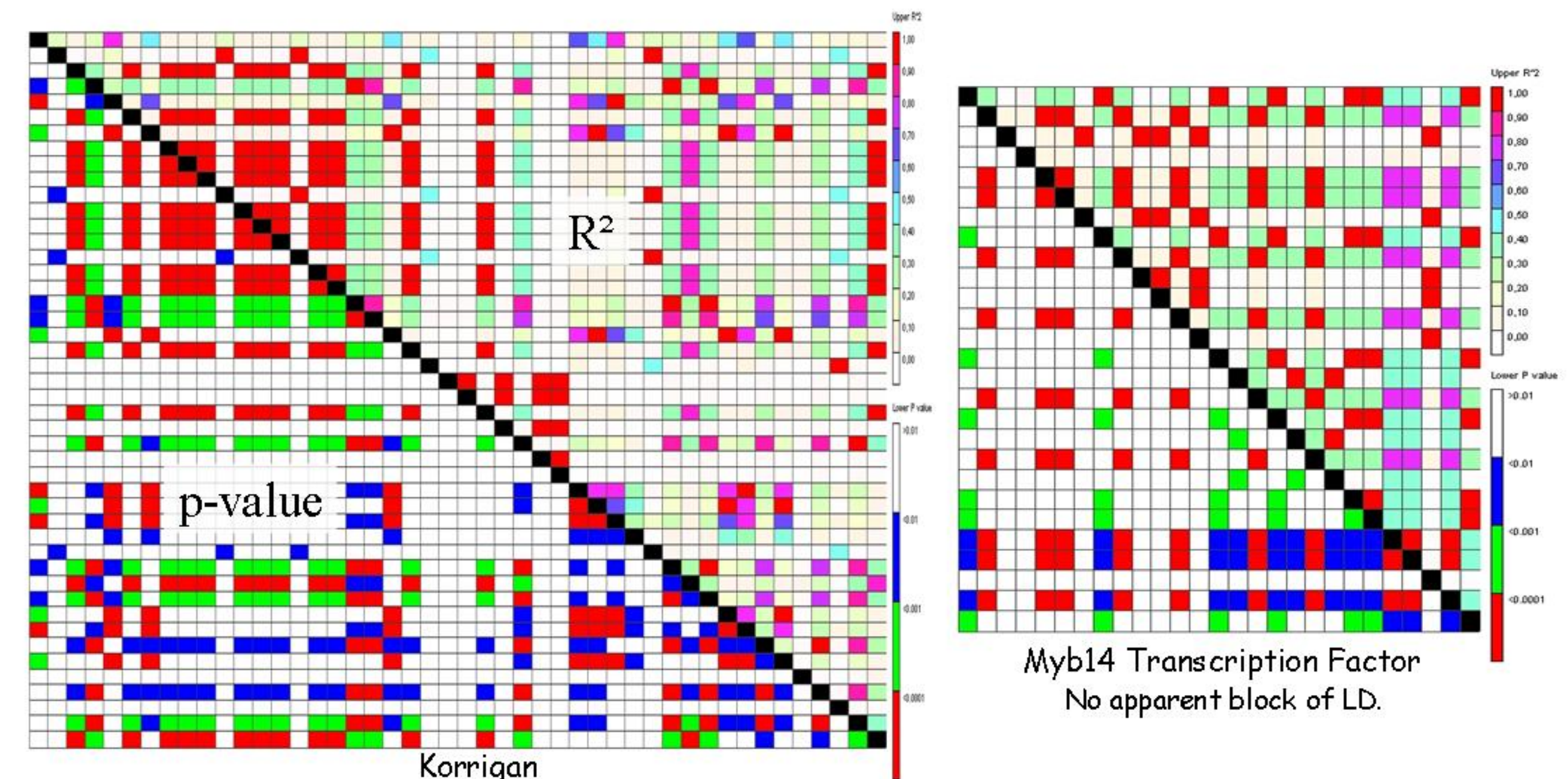
- Levels of candidate genes diversity ?
- Extent of linkage disequilibrium within and between genes ?
- Molecular signatures of natural selection ?



Wood quality related candidate genes



Linkage disequilibrium between polymorphic sites



Short extent of LD, as expected from previous experimental results in conifer species (e.g. Gonzales Martinez *et al.* 2006)

→ potential high resolution for association mapping

Nucleotide and haplotype diversity

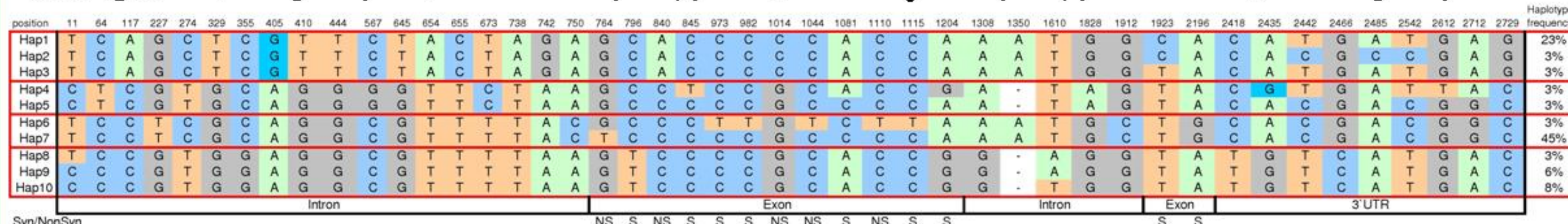
Genes	Sample Size	Length (bp) (coding + non coding)	Number of polymorphic sites			Average SNP Frequency (bp)	Number of haplotypes	Hd	$\pi \times 10^3$		
			Total	Syn. Coding	Syn. Non Coding					Non Syn	
Transcription factors	<i>Myb related</i>	8	1383 (689+694)	0	-	0	1	-	-		
	<i>Myb8</i>	10	1345 (1212+133)	1	-	0	2	-	-		
	<i>Myb1</i>	40	1301 (939+362)	8	1	4	3	162	0.51	3.19	
	<i>Myb14</i>	28	846 (480+366)	27	4	16	7	31	5	0.69	7.77
Cellulose biosynthesis	<i>Scarecrow Like</i>	22	901 (901+0)	9	5	0	4	100	4	0.52	2.34
	<i>Korrigan</i>	35	2765 (1170+1595)	46	7	32	7	60	10	0.75	5.21
	<i>Cobra 1-1</i>	35	2184 (924+1260)	41	3	34	2	53	15	0.90	5.05
Cell wall related genes	<i>Cobra 1-2</i>	34	751 (215+536)	9	0	8	1	84	10	0.87	3.31
	<i>Botero</i>	38	385 (0+385)	3	0	3	0	128	4	-	-
	<i>THE1</i>	38	551 (44+507)	11	0	11	0	50	6	0.65	7.70
	<i>Mur1</i>	8	592 (264+328)	1	0	1	0	-	2	-	-

→ Several genes identified as expressional candidates for wood quality are not polymorphic

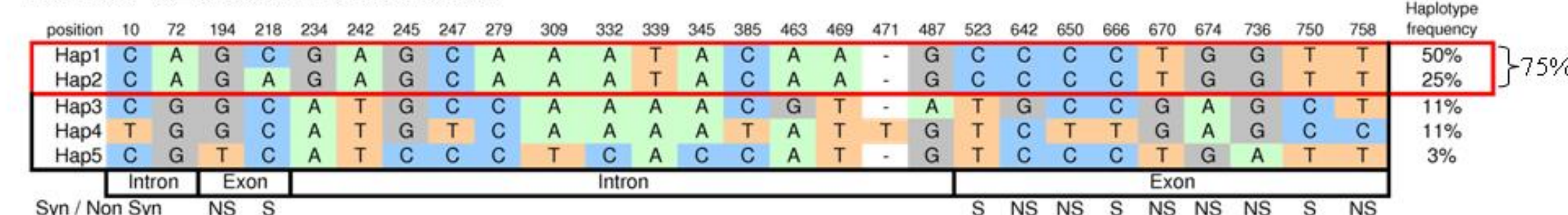
→ As expected, large variation for Hd and π across the other candidate genes

Example: two genes with strong haplotype structure

Korrigan : four groups of related haplotypes & two major haplotypes in 2 different groups



Myb14 : two major related haplotypes plus additional ones (distant & in lower frequency), numerous NS mutations



Hypothesis 1 : Divergent haplotypes maintained by balancing selection ?

Hypothesis 2 : Amplification of two different genes from the Myb multigenic family ? Can be confirmed by genetic mapping of SNPs from the two putative paralogs.

Neutrality tests

Gene	Length (bp)	4Ne.r	Tajima's D ¹	Fu's Fs ²	Fay & Wu H ³
<i>Myb14</i>	846	0.5	<0 ^{ns}	>0 ^{**}	<0 ^{ns}
<i>Scarecrow Like</i>	901	0	<0 ^{ns}	>0 ^{ns}	<0 ^{ns}
<i>Korrigan</i>	2765	2	>0 ^{ns}	>0 ^{**}	>0 ^{ns}
<i>Cobra 1-1</i>	2184	0	>0 ^{ns}	>0 ^{ns}	<0 ^{ns}
<i>Cobra 1-2</i>	756	6	>0 ^{ns}	<0 ^{ns}	>0 ^{ns}

ns : not significant * : significant test at Pr<0.05 ** : significant test at Pr<0.01

→ Absence of population structure inferred from SSR data (not shown) and demographic equilibrium assumed (from known population history)

→ Observed departures from neutrality can be interpreted as natural selection events (alternative hypotheses of departures from demographic equilibrium will also be tested in the future).

Korrigan & Myb14 : Significant and positive Fs values

→ Deficit of haplotypes compared to neutral expectation (due to maintenance of distant haplotypes in intermediate frequency)

→ Signatures of balancing selection ?

Ongoing activities

→ 4300 trees phenotyped for wood quality traits (lignin and cellulose contents, micro-density...), quantitative genetic parameters estimated for 545 mother trees (2002-2007)

→ Implementation of the SNPlex technology to genotype a subset of polymorphisms representative of haplotype diversity

Test and validation of candidate genes polymorphisms' effects on phenotypic variation

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