# Towards a rational design of sustainable biomass feedstocks using poplar tree genomics

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# Acknowledgments


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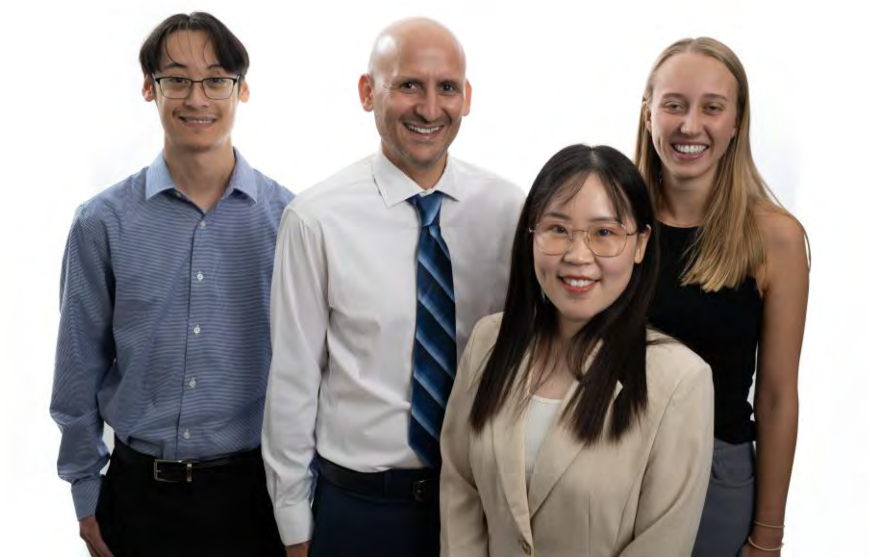
- But most importantly
  - ❑ Weiwei Zhu
  - ❑ Rachel Weber
  - ❑ Max Bentelspacher
  - ❑ Vadivel Irulappan

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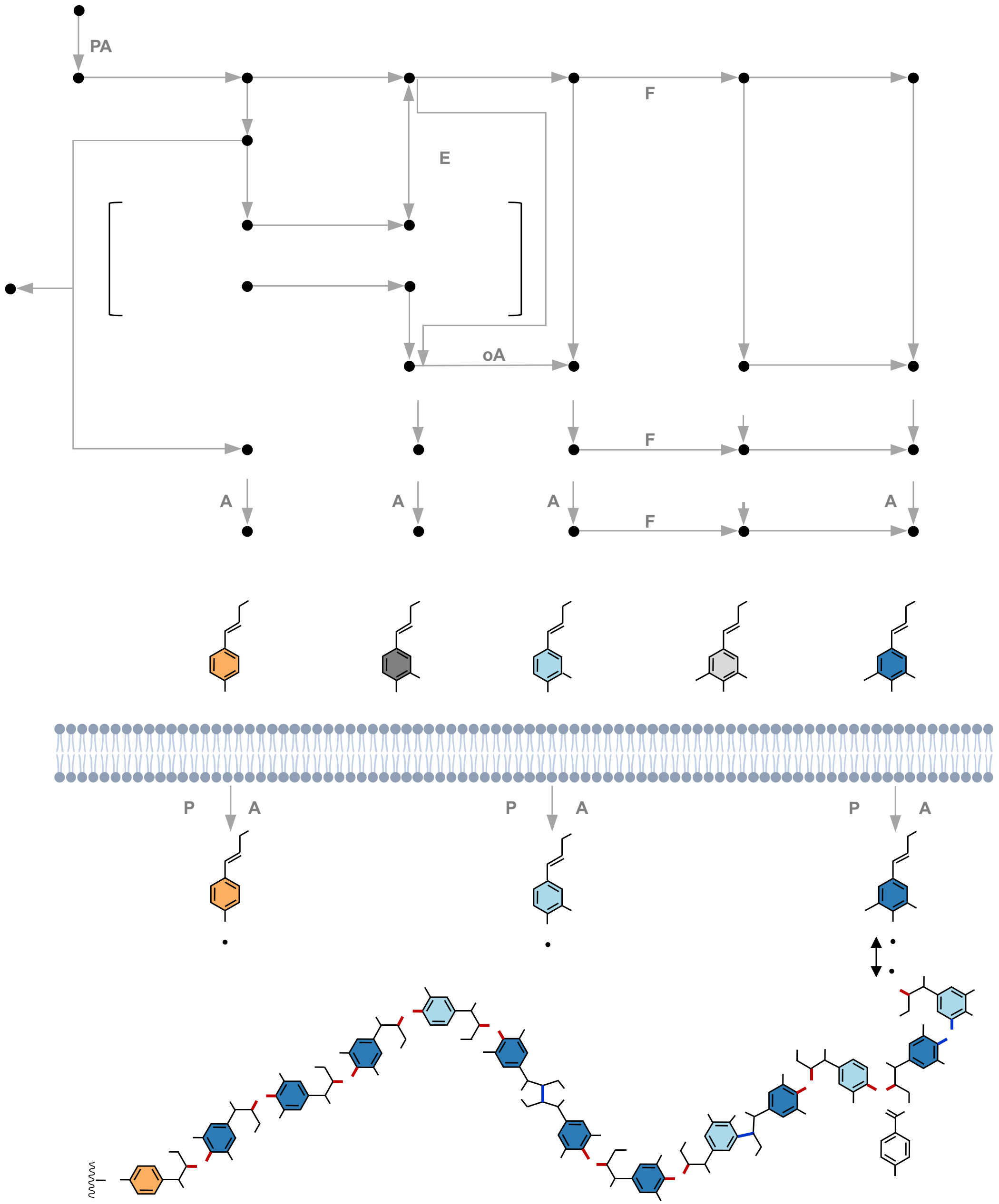
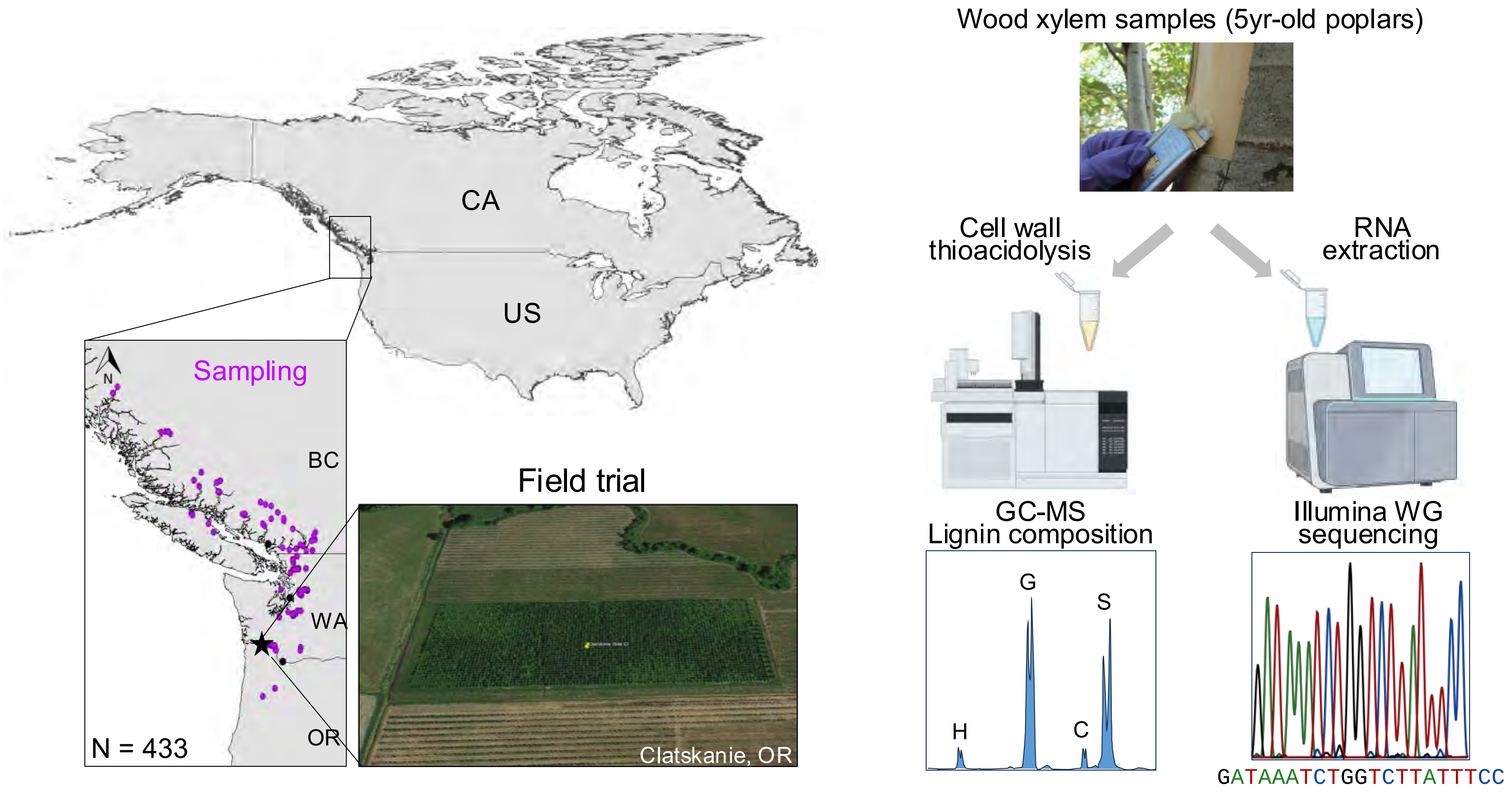
# Lignin biosynthesis in poplar

Biosynthesis

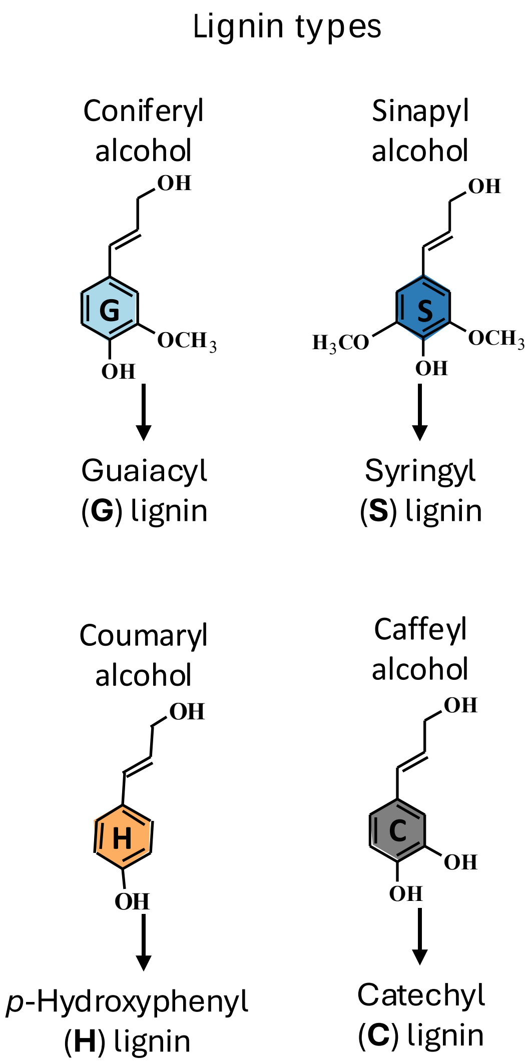
Polymerization

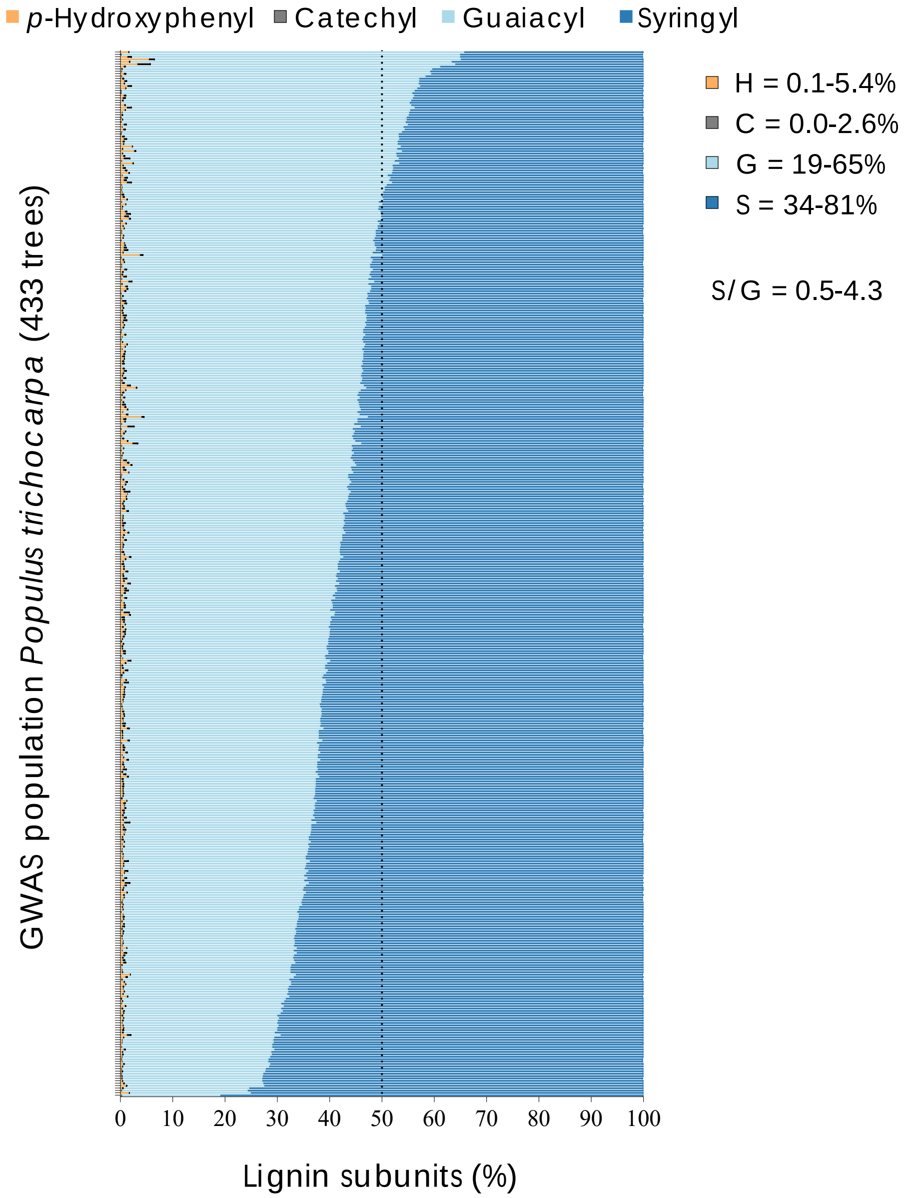



# Experimental design

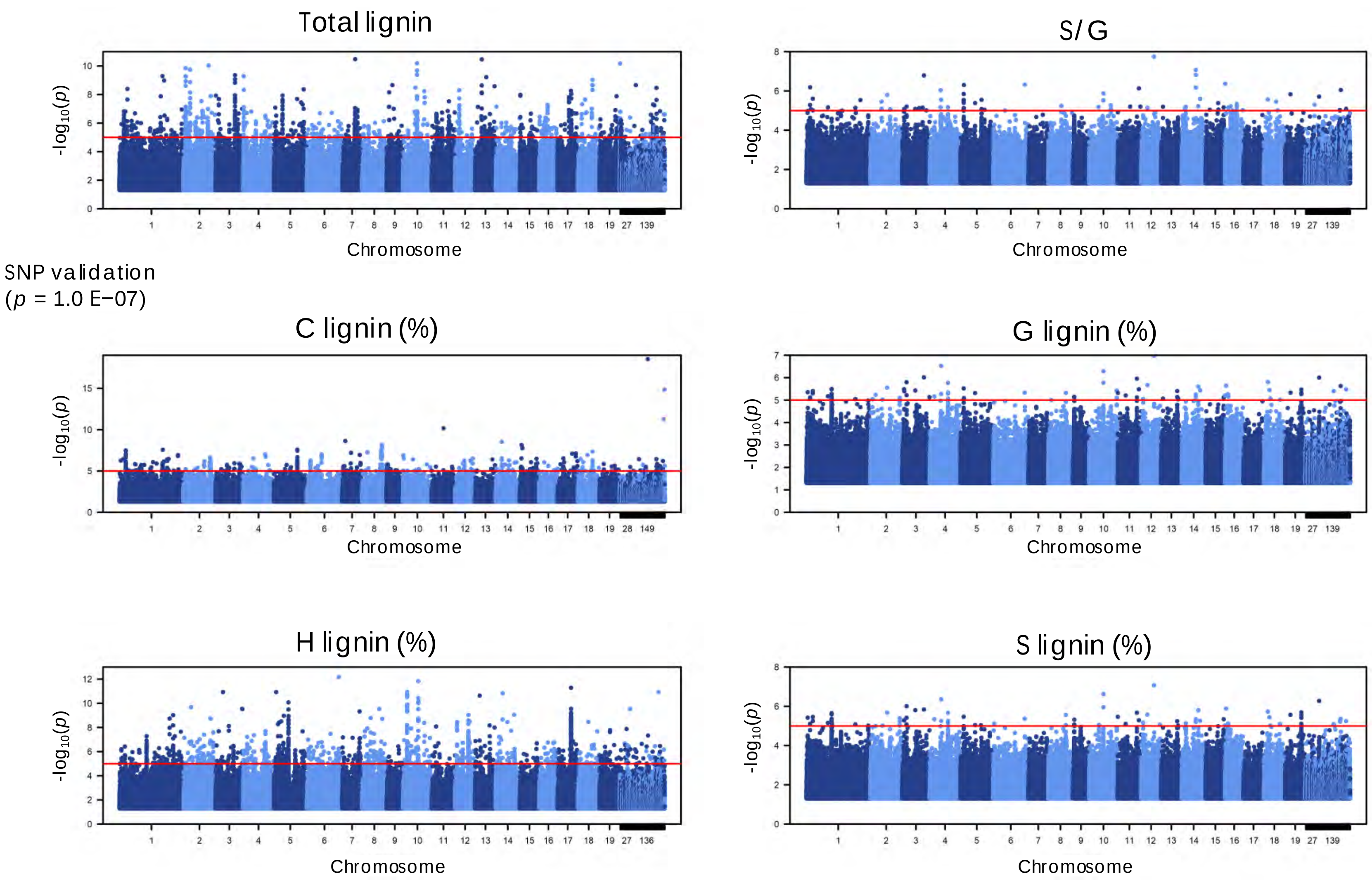


# Natural variation of lignin composition



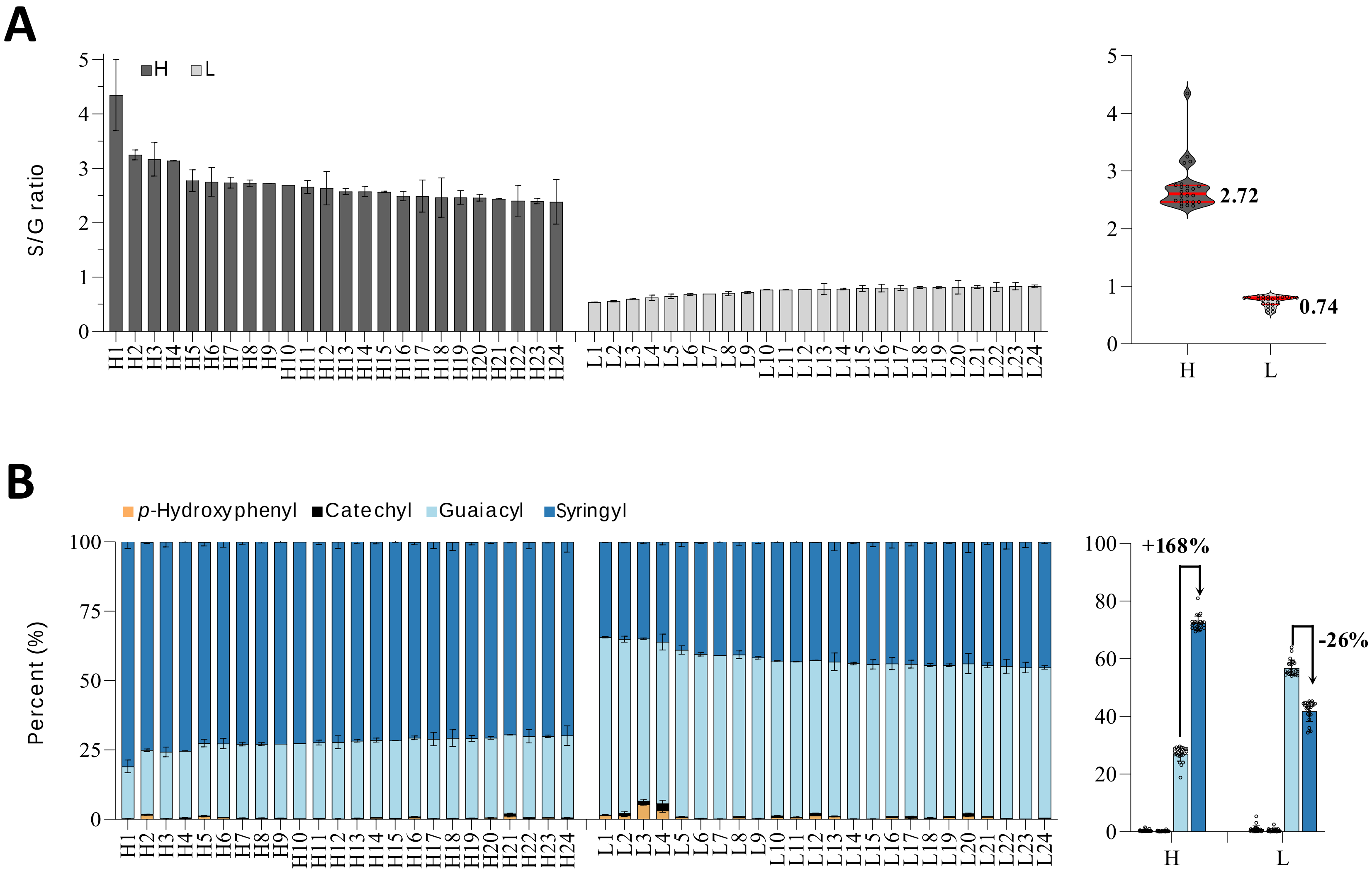



# GWAS identified hundreds of potential lignin genes

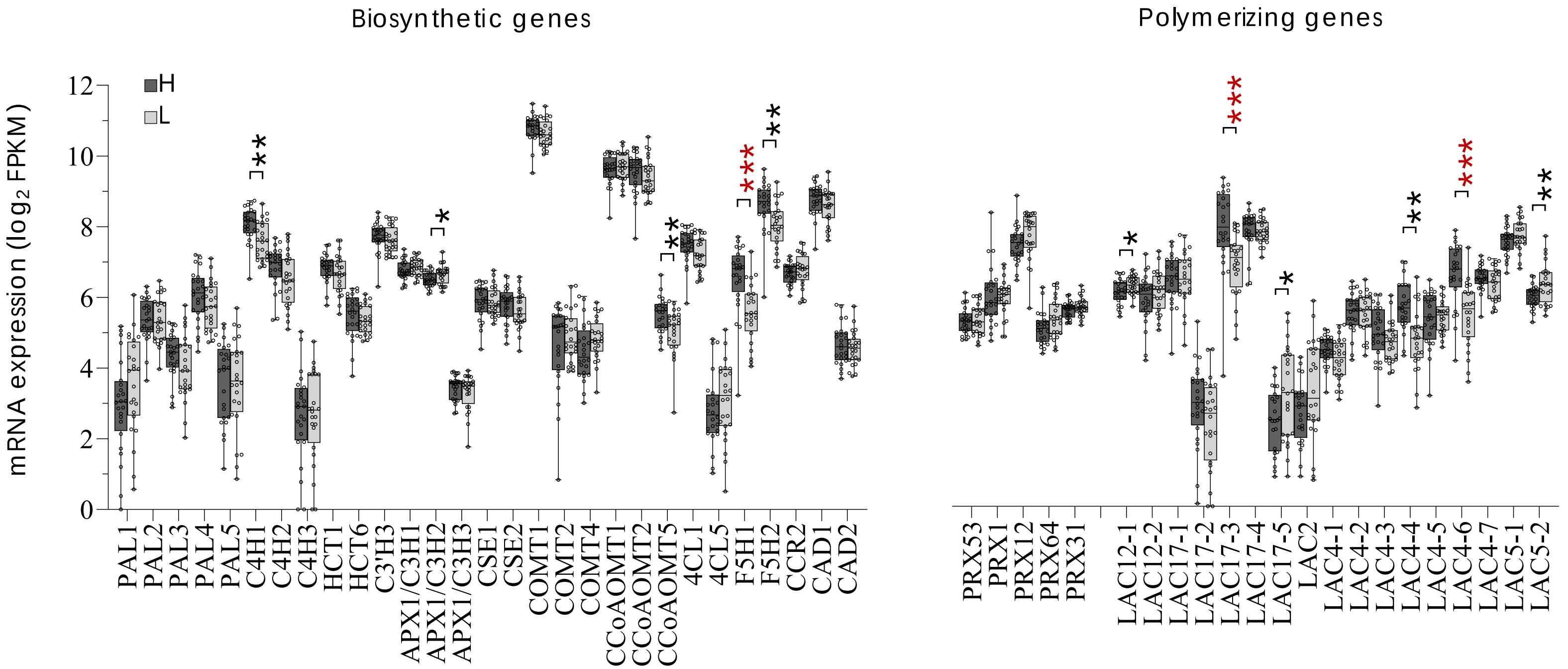



These results suggests that lignin composition is a complex polygenic trait (significant associations might have very small effects)

# Trees with extreme highest (H) and lowest (L) S/G ratios



# Laccases expression is correlated with differences in lignin composition




UP with S-units

| | H | L | H − L | *P* value |
|---|---|---|---|---|
| *C4H1* | 282 | 206 | 76 | 0.0019 |
| *CCoAOMT5* | 49 | 36 | 13 | 0.0049 |
| *F5H1* | 110 | 55 | 55 | 0.0001 |
| *F5H2* | 427 | 289 | 138 | 0.0018 |
| *LAC17-3* | 295 | 136 | 159 | 0.0001 |
| *LAC4-4* | 57 | 31 | 26 | 0.0007 |
| *LAC4-6* | 110 | 52 | 58 | 0.0001 |

*n* = 24

UP with G-units

| | H | L | H − L | *P* value |
|---|---|---|---|---|
| *APX1/C3H2* | 92 | 102 | -11 | 0.034 |
| *LAC12-1* | 69 | 78 | -9 | 0.042 |
| *LAC5-2* | 63 | 86 | -23 | 0.007 |

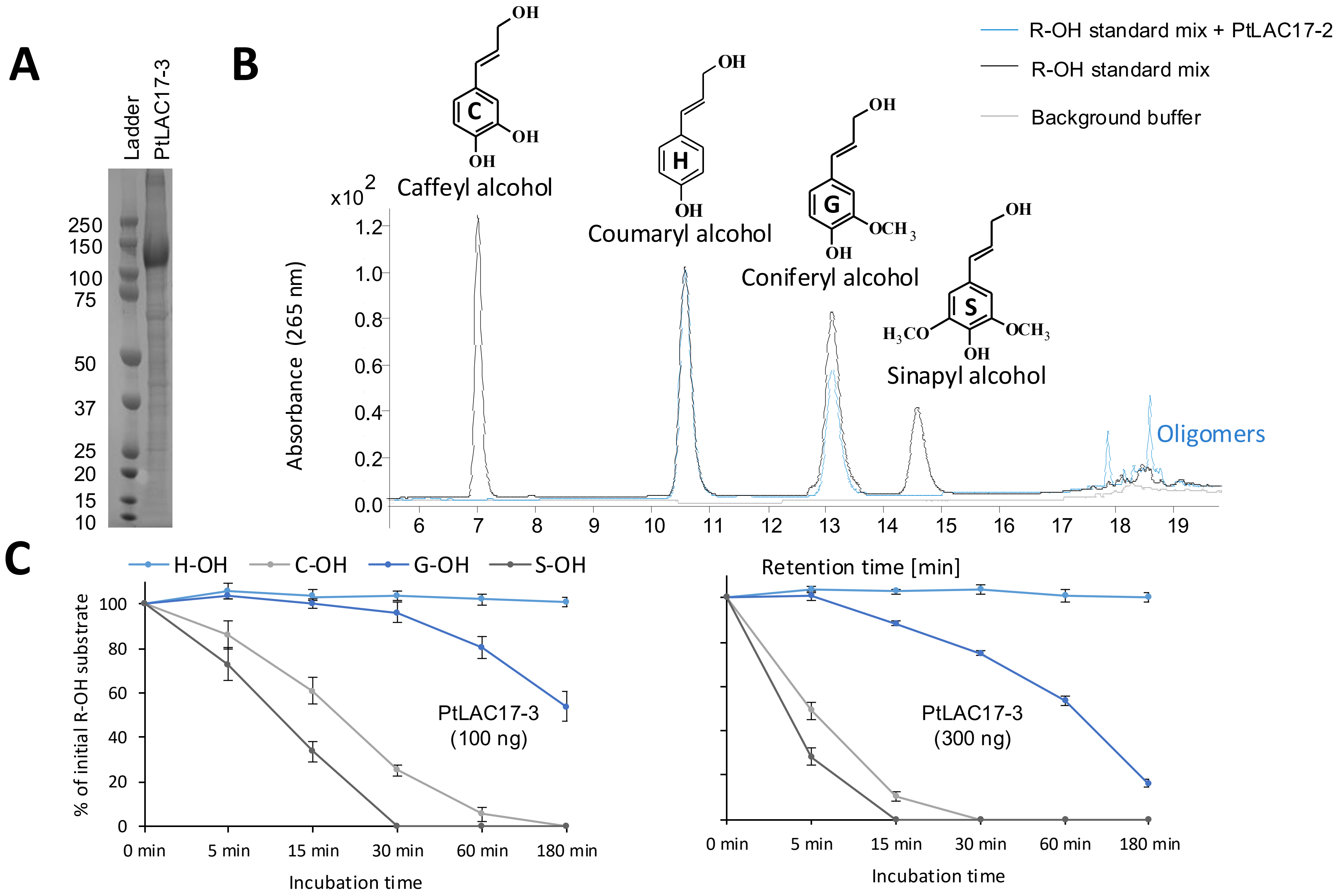
*n* = 24

# Selection of laccases for recombinant protein expression

| name | gene ID | UniProt ID | mRNA expression *p*<0.05 | *p*<0.01 | *p*<0.001 | preference | Transcript %COMT1 |
|---|---|---|---|---|---|---|---|
| PtLAC12-1 | Potri.008G073800 | B9HHV8 | * | ns | ns | G-OH | 0.6% |
| PtLAC12-2 | Potri.010G183500 | B9HSI5 | ns | ns | ns | | |
| PtLAC17-1 | Potri.001G401300 | B9N1I1 | ns | ns | ns | | |
| PtLAC17-2 | Potri.011G120300 | B9HZV6 | ns | ns | ns | | |
| PtLAC17-3 | Potri.006G087100 | A0A2K1ZZ60 | * | ** | *** | S-OH | 13% |
| PtLAC17-4 | Potri.006G087500 | B9HBT3 | ns | ns | ns | | |
| PtLAC17-5 | Potri.001G184300 | A0A2K2BZT5 | * | ** | ns | G-OH | 0.6% |
| PtLAC2 | Potri.009G156800 | A0A2K1Z8I1 | * | ns | ns | G-OH | 0.6% |
| PtLAC4-1 | Potri.006G096900 (IRX12) | A0A2K1ZZP5 | ns | ns | ns | | ~4.5% |
| PtLAC4-2 | Potri.006G097000 | A0A2K1ZZN3 | ns | ns | ns | | |
| PtLAC4-3 | Potri.006G097100 | U7DW34 | ns | ns | ns | | |
| PtLAC4-4 | Potri.008G064000 | B9HHK7 | * | ** | *** | S-OH | 2.6% |
| PtLAC4-5 | Potri.009G042500 | B9HP74 | ns | ns | ns | | |
| PtLAC4-6 | Potri.010G193100 | B9HT10 | * | ** | *** | S-OH | 2.6% |
| PtLAC4-7 | Potri.016G112000 | A0A2K1XE42 | ns | ns | ns | | |
| PtLAC5-1 | Potri.008G073700 | A0A2K1ZDD2 | ns | ns | ns | | |
| PtLAC5-2 | Potri.010G183600 | B9HSI6 | * | ** | ns | G-OH | 4.3% |

PtLac17-3 was well expressed but PtLac5-2 expression didn't work.

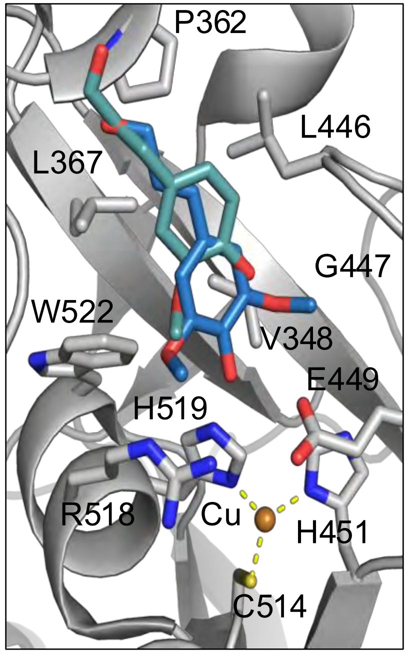
# Heterologous expression of PtLac17-3 in HEK293 cells



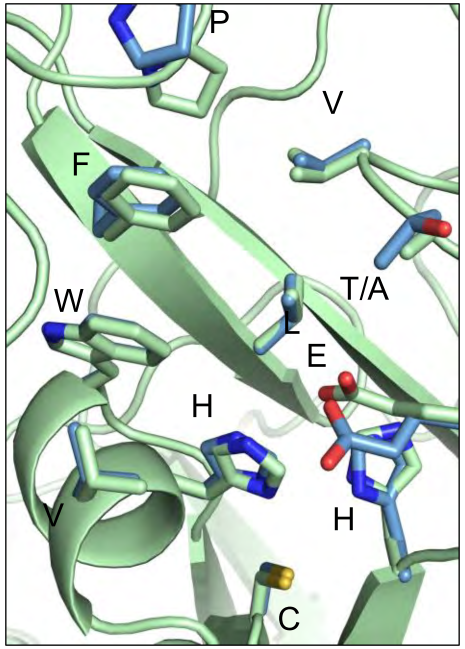

PtLAC17 has preference for S-OH and polymerizes C-OH with similar efficiency.
PtLAC17 does not oxidize H-OH, even at long incubation times.

# Comparison of active site residues

ZmLac3




ZmLac3/PtLac17-3/PtLac5-2




| ZmLac3 | PtLac17-3 | PtLac4-4 | PtLac4-6 | PtLac5-2 | PtLac17-5 | PtLac2 | PtLac12-1 | PtLac14 |
|---|---|---|---|---|---|---|---|---|
| V348 | L345 | V337 | L337 | L339 | L344 | L344 | L342 | L338 |
| P362 | P359 | - | - | P356 | P358 | P358 | P360 | G349 |
| L367 | F365 | A351 | A351 | F361 | F363 | F365 | F365 | V354 |
| L446 | V443 | I429 | I429 | V440 | F441 | F441 | P444 | I431 |
| G447 | A444 | A430 | A430 | T441 | G442 | G442 | T445 | S432 |
| E449 | E446 | E432 | E432 | E443 | E444 | E444 | E447 | E434 |
| H451 | H448 | H434 | H434 | H445 | H446 | H446 | H449 | H436 |
| R518 | V515 | V501 | V501 | V512 | V513 | V513 | V516 | V503 |
| H519 | H516 | H502 | H502 | H513 | H514 | H514 | H517 | H504 |
| W522 | W519 | W505 | W505 | W516 | W517 | W517 | W520 | W507 |

**S-OH** (ZmLac3, PtLac17-3, PtLac4-4, PtLac4-6) **G-OH** (PtLac5-2, PtLac17-5, PtLac2, PtLac12-1, PtLac14)

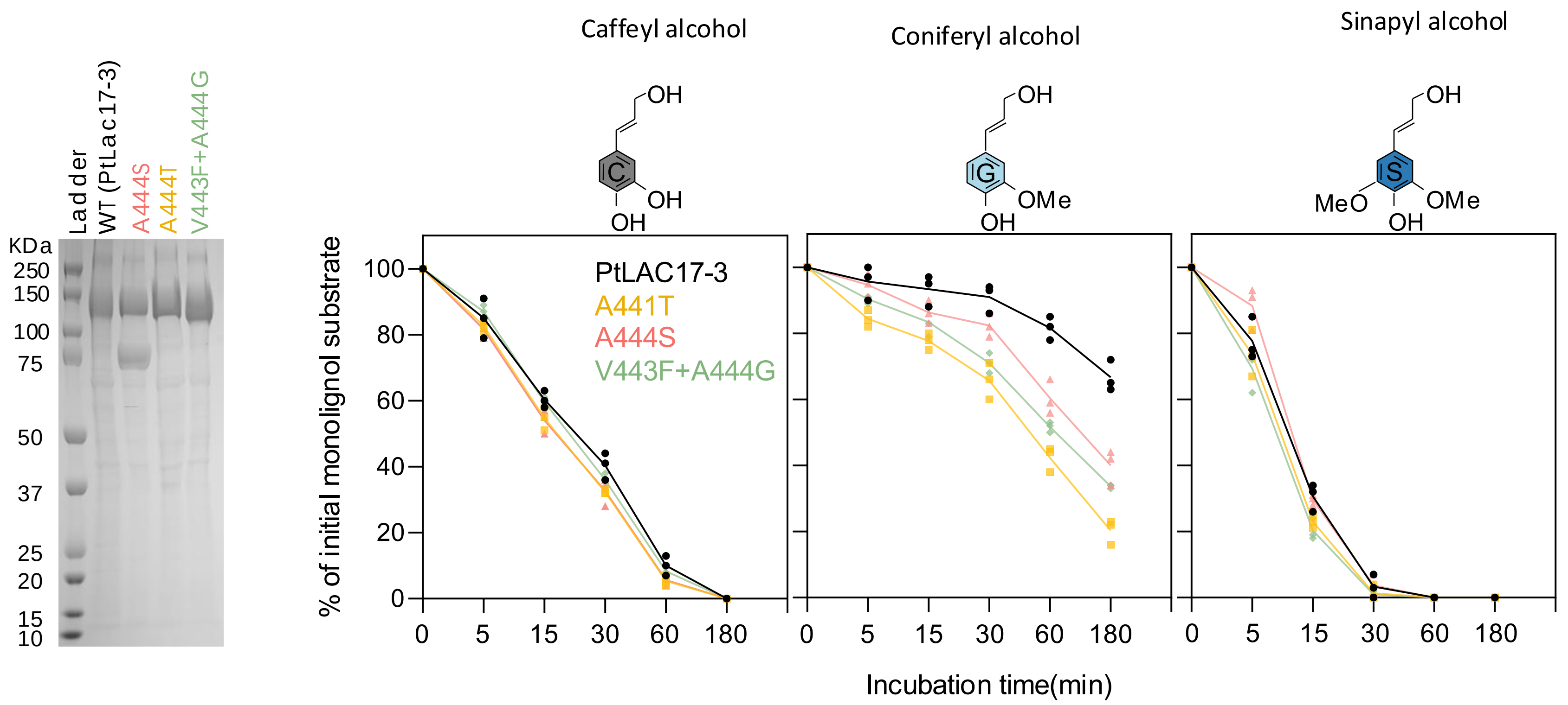
*Hypothesis: Small nonpolar side chains (G447 in ZmLac3 and A444 in PtLac17-3) may create a pocket capable of accommodating the second methoxy group in S-OH. However, the larger, polar Thr residue in PtLac5-2 fills this space and may facilitate the binding of G-OH.*

Parks Lab (ORNL)

Jerry Parks

Generated PtLAC17-3 mutants:
Ala444Thr, Ala444Ser, and double Val443Phe + Ala444Gly

# A single PtLAC17-3 mutation boosts G-monomer oxidation



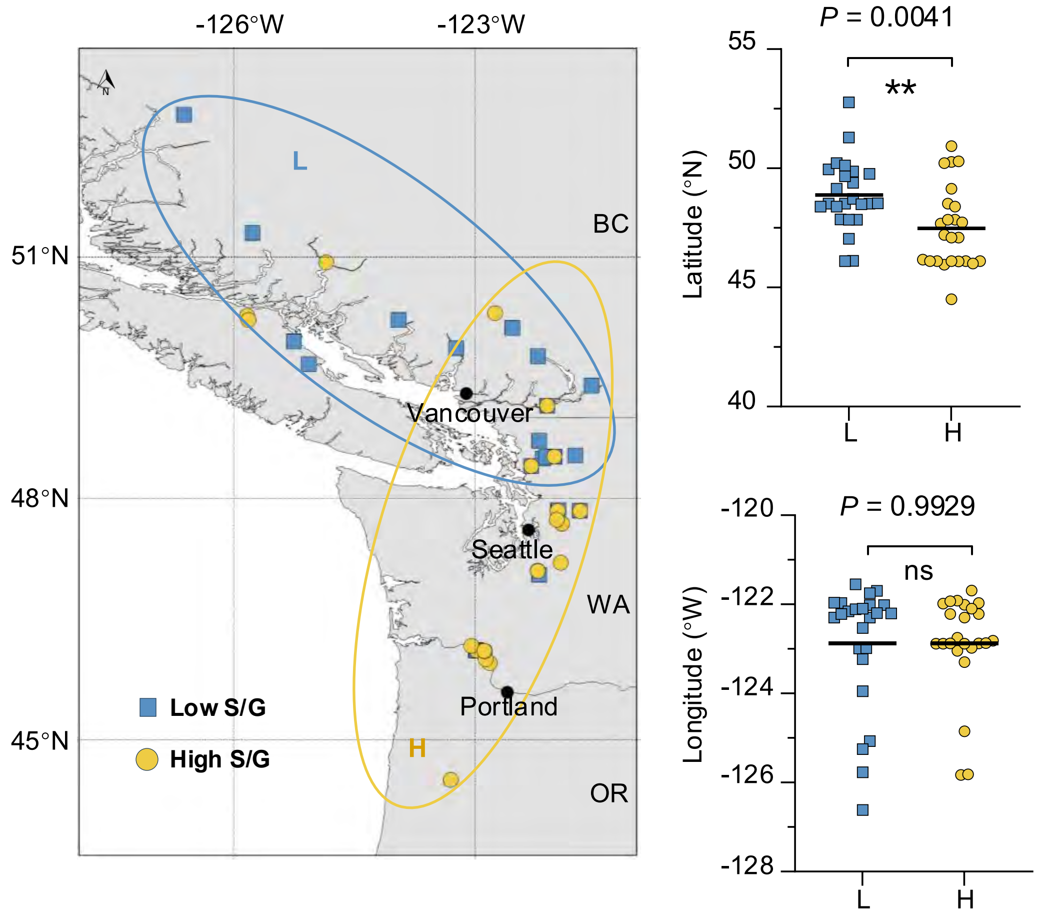

The larger polar Thr residue in PtLac5-2 and PtLac12-1 may better accommodate the hydroxyl group in G-OH facilitating a more effective monomer binding and oxidation.

# Goals

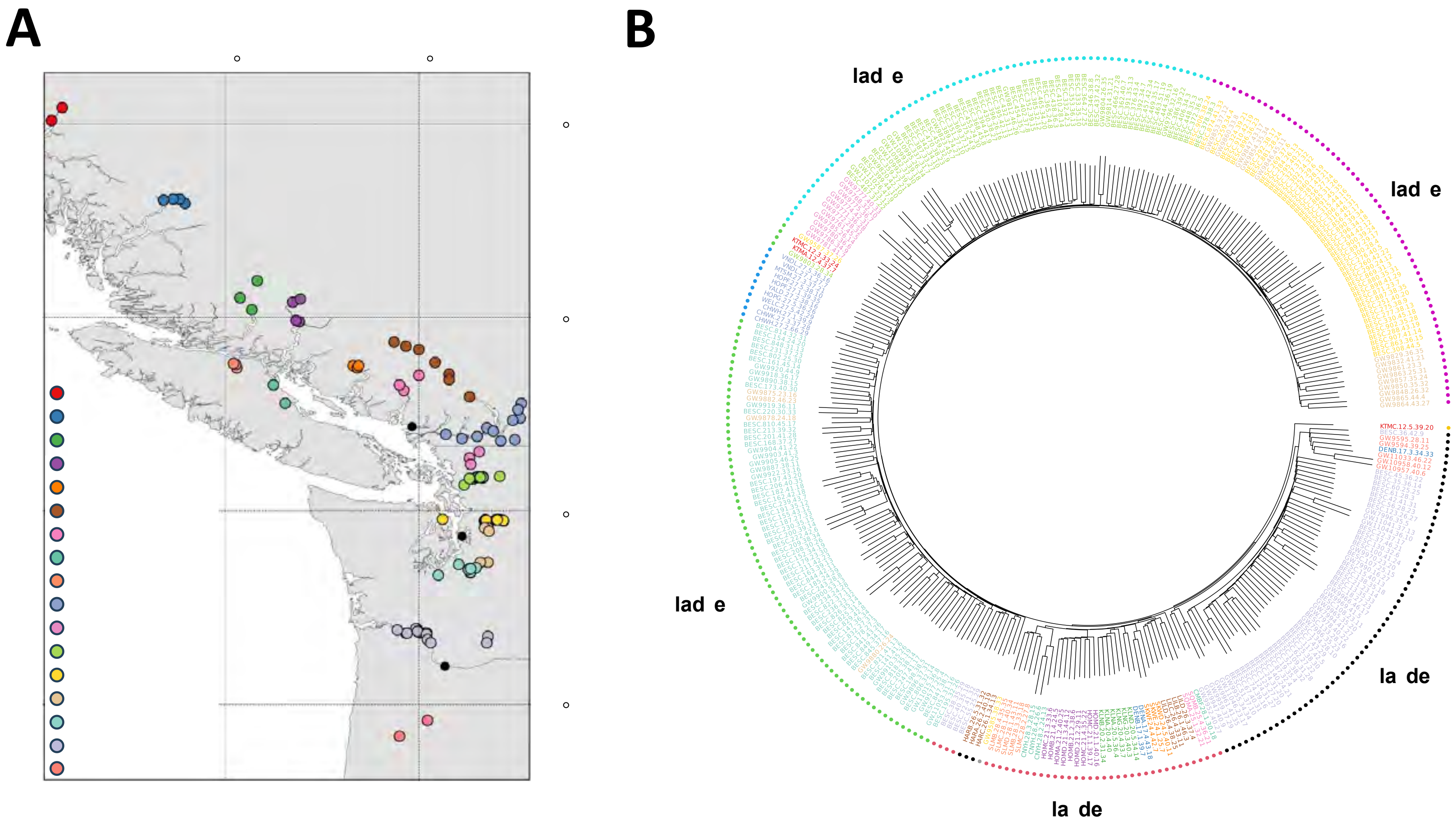
1. Identify genes associated with the natural variation in lignin composition ✓

2. Investigate the ecological significance of the observed broad natural variation in lignin composition

# Poplars with lower S/G shift to higher latitudes

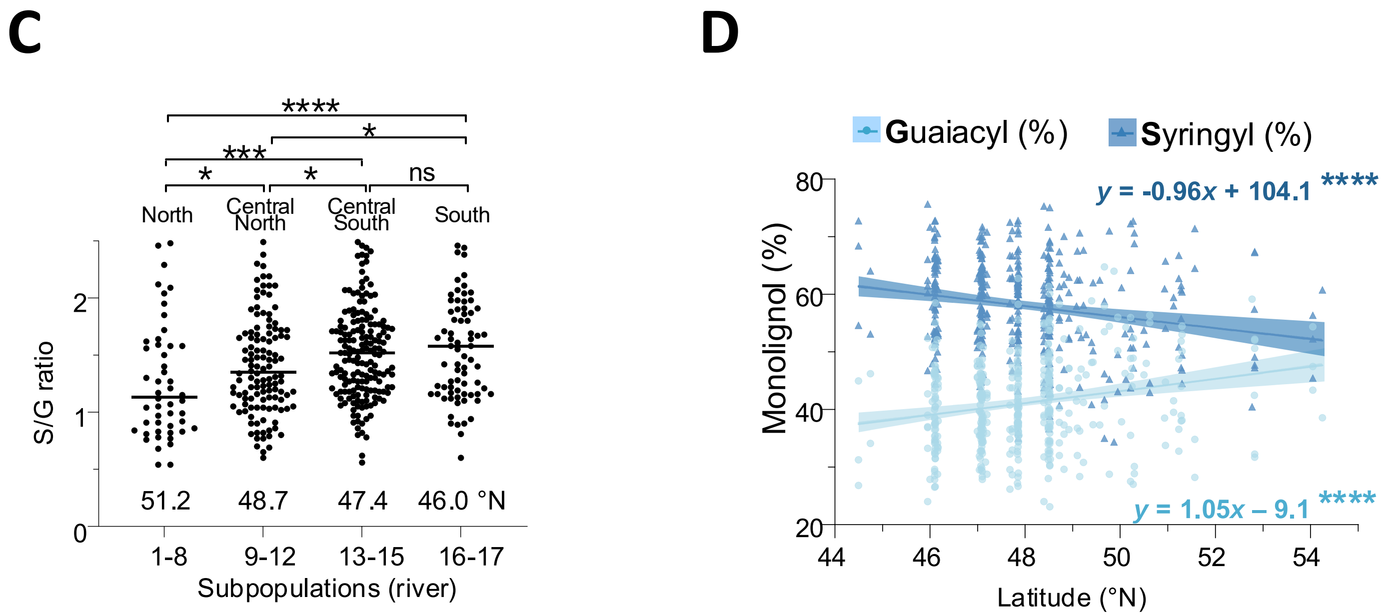


# Population has genome-wide genetic structure at spatial scale



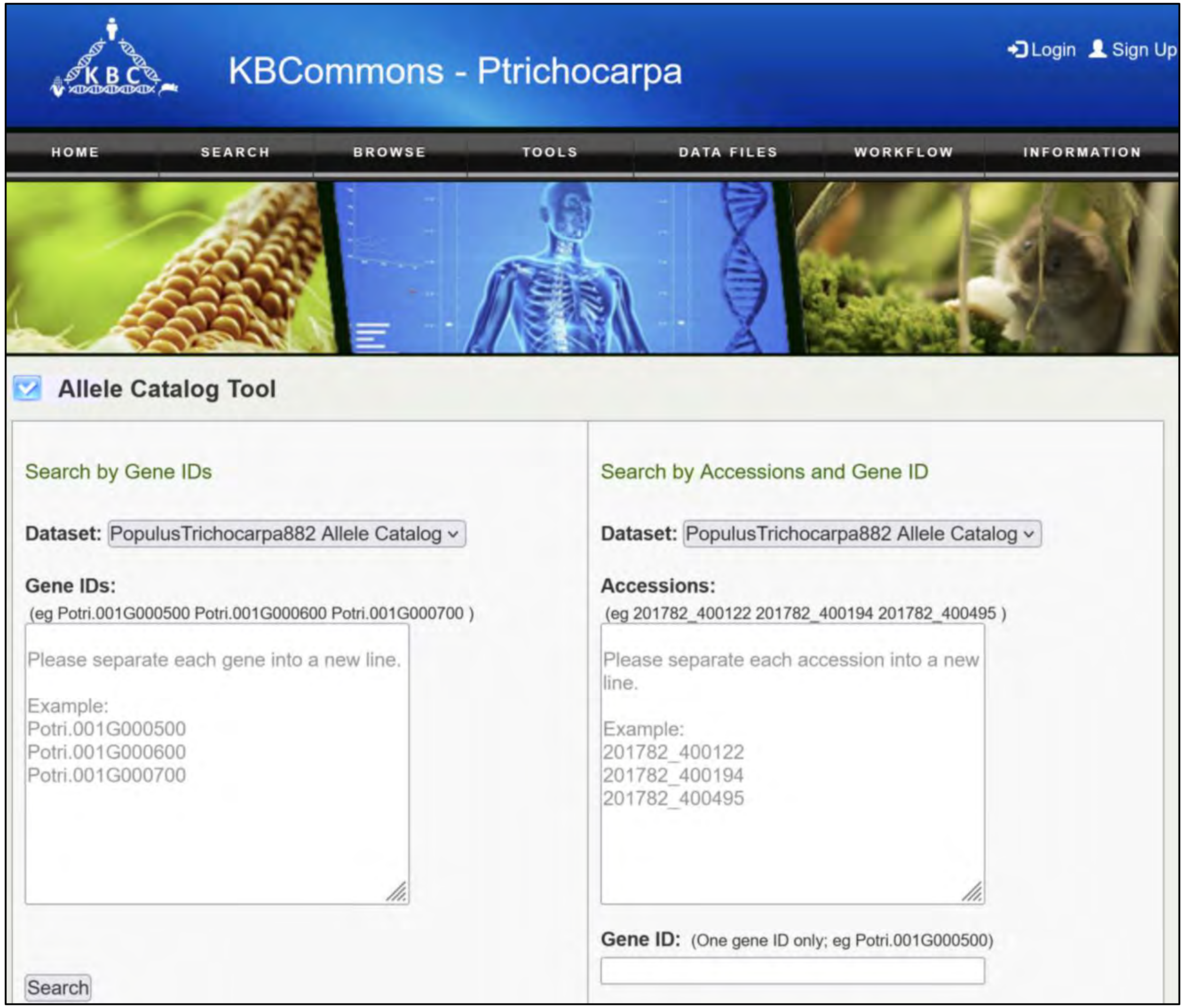

Five major genetic groups, clades, or subpopulations clustering poplar variants with the same geographical origin.

# Clinal increase in lignin S/G from northern to southern populations driven by natural selection




These results suggest that lignin heterogeneity is an evolutionary strategy enabling poplar adaption to different latitudes.

# Allele Catalog Tool for *Populus Trichocarpa*




Joshi Lab (MU)


Trupti Joshi Yen On Chen

https://kbcommons.org/system/tools/AlleleCatalogTool/Ptrichocarpa

# Allele Catalog Tool for *Populus Trichocarpa*

| | Total | Gene | Chromosome | 55582 | 56086 | 56348 | 59246 |
|---|---|---|---|---|---|---|---|
| ☐ | 537 | Potri.001G000700 | Chr01 | A\|K292I | G\|Ref | T\|L161M | T\|Ref |
| ☐ | 285 | Potri.001G000700 | Chr01 | A\|K292I | G\|Ref | A\|Ref | A\|I79F |
| ☐ | 30 | Potri.001G000700 | Chr01 | A\|K292I | G\|Ref | T\|L161M | A\|I79F |
| ☐ | 26 | Potri.001G000700 | Chr01 | A\|K292I | G\|Ref | A\|Ref | T\|Ref |
| ☑ | 2 | Potri.001G000700 | Chr01 | T\|Ref | G\|Ref | A\|Ref | A\|I79F |
| ☐ | 2 | Potri.001G000700 | Chr01 | A\|K292I | T\|D207E | T\|L161M | A\|I79F |

Nucleotide/Protein alterations

Genotypes:

| Accession | CBI_Coding_ID | Gene | Chromosome | 55582 | 56086 | 56348 | 59246 | Imputation |
|---|---|---|---|---|---|---|---|---|
| 300661_403487 | BESC-890 | Potri.001G000700 | Chr01 | T\|Ref | G\|Ref | A\|Ref | A\|I79F | - |
| 852_1010036 | GW-9953 | Potri.001G000700 | Chr01 | T\|Ref\|+ | G\|Ref | A\|Ref | A\|I79F | + |

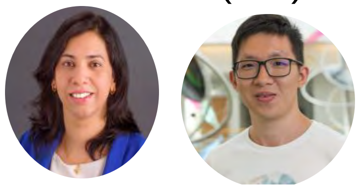
Lignin Phenotype:

| Chromosome | Position | Accession | CBI_Coding_ID | Genotype | Functional_Effect | Imputation | S_Lignin_Percentage |
|---|---|---|---|---|---|---|---|
| Chr01 | 55582 | 300661_403487 | BESC-890 | T | Ref | | 52.52256785 |
| Chr01 | 55582 | 852_1010036 | GW-9953 | T | Ref | + | 34.76897682 |

Link genotype-with lignin phenotype

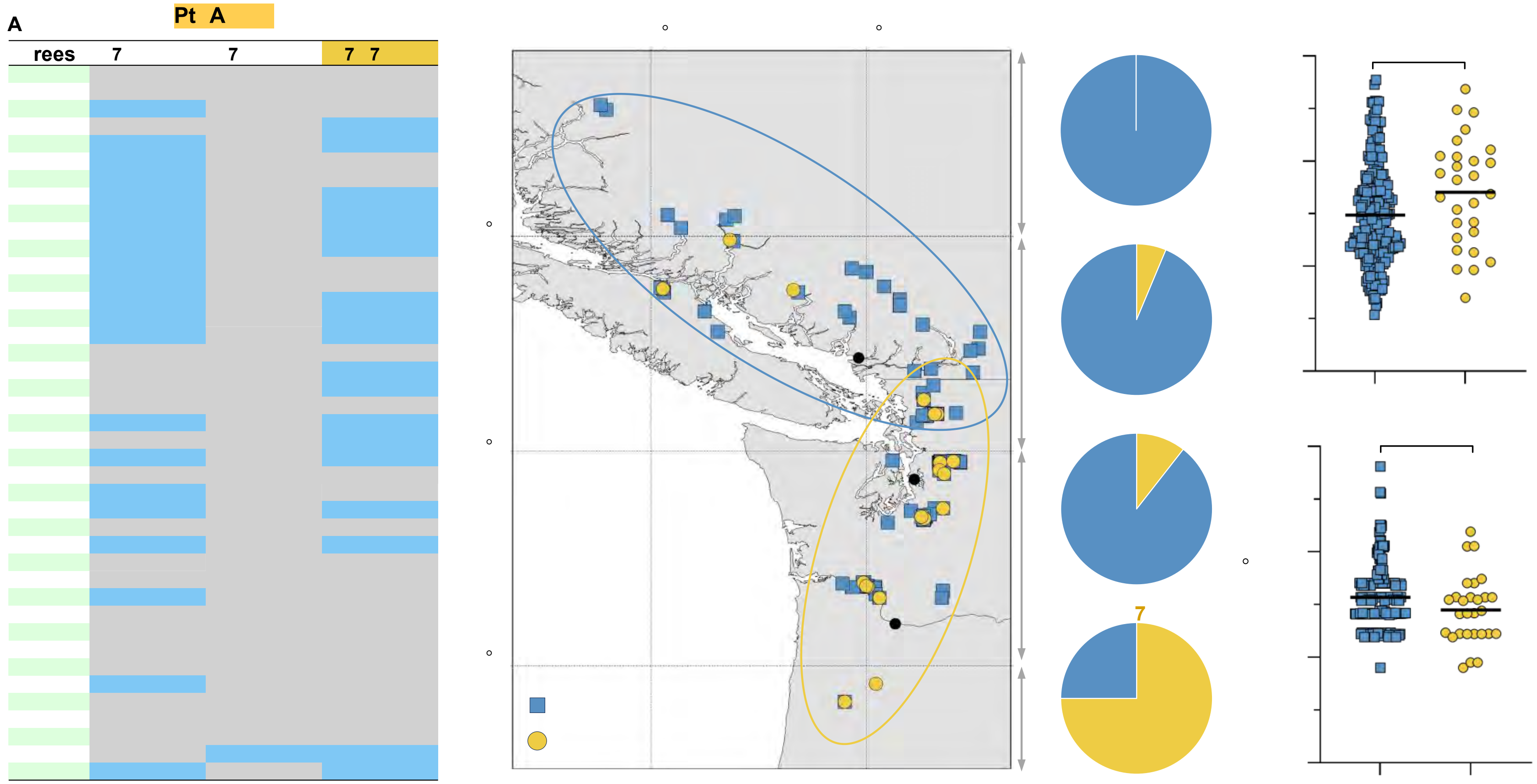

Phenotype measurement

Joshi Lab (MU)



Trupti Joshi Yen On Chen

# His64Asp in PtLAC4-2 is linked to higher S/G and contributes to adaptation of poplars to lower latitudes




Increased proportion of PtLac4-2 mutant alleles from higher to lower latitudes.

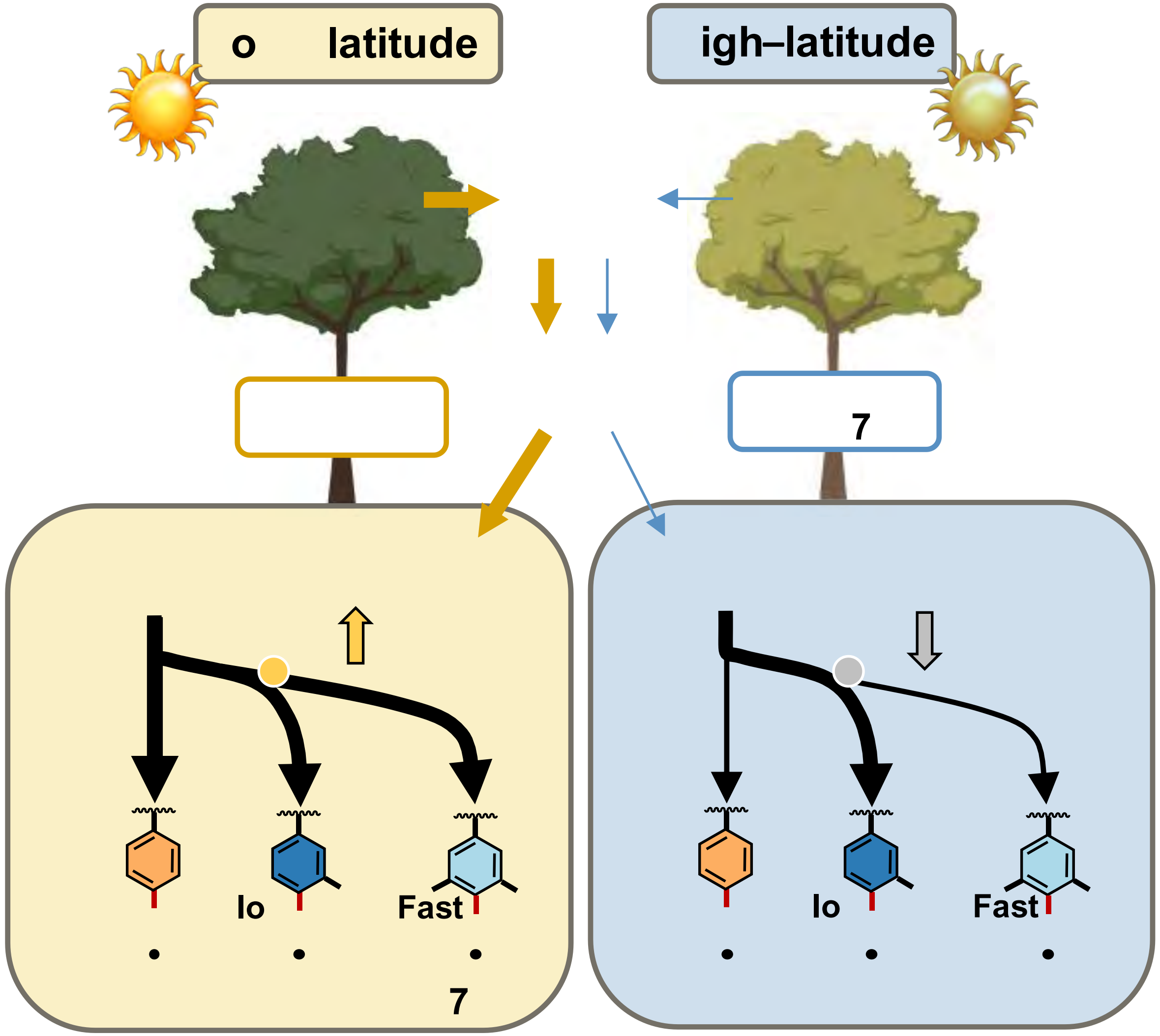
## Lignin laccases in *Populus trichocarpa* trees

PtLAC17-3
(A0A2K1ZZ60)

PtLAC4-2
(A0A2K1ZZN3)

Rachel Weber
(MU)

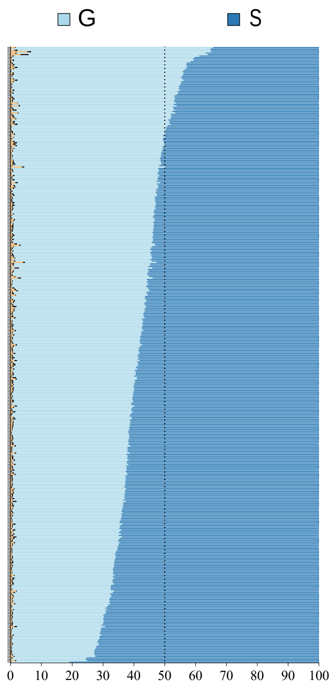
# Natural lignin response to environmental stimuli



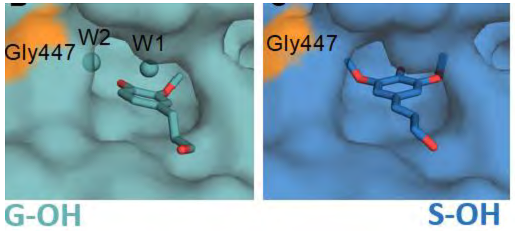

e and substrate
e cell wall.

# Conclusions

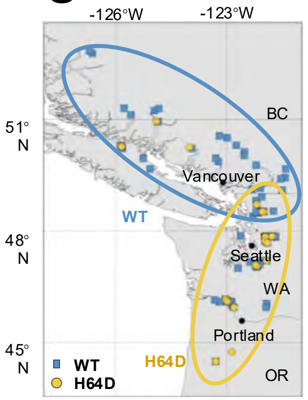
i. Broad natural phenotypic variation for lignin




ii. Structural basis of laccase polymerization




iii. Ecological role of lignin heterogeneity




v. Community resource: Poplar allele catalog tool