

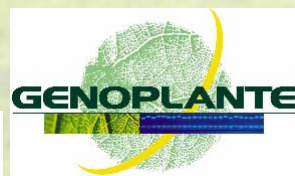


## Maize and grasses for the future

### Breeding maize and grasses

- for improved intake and digestibility in cattle
- for improved biofuel production

based on the understanding of cell wall biogenesis



ProMaïs  
ProSorgho





**Observed genetic variation  
for intake and digestibility  
traits in maize**





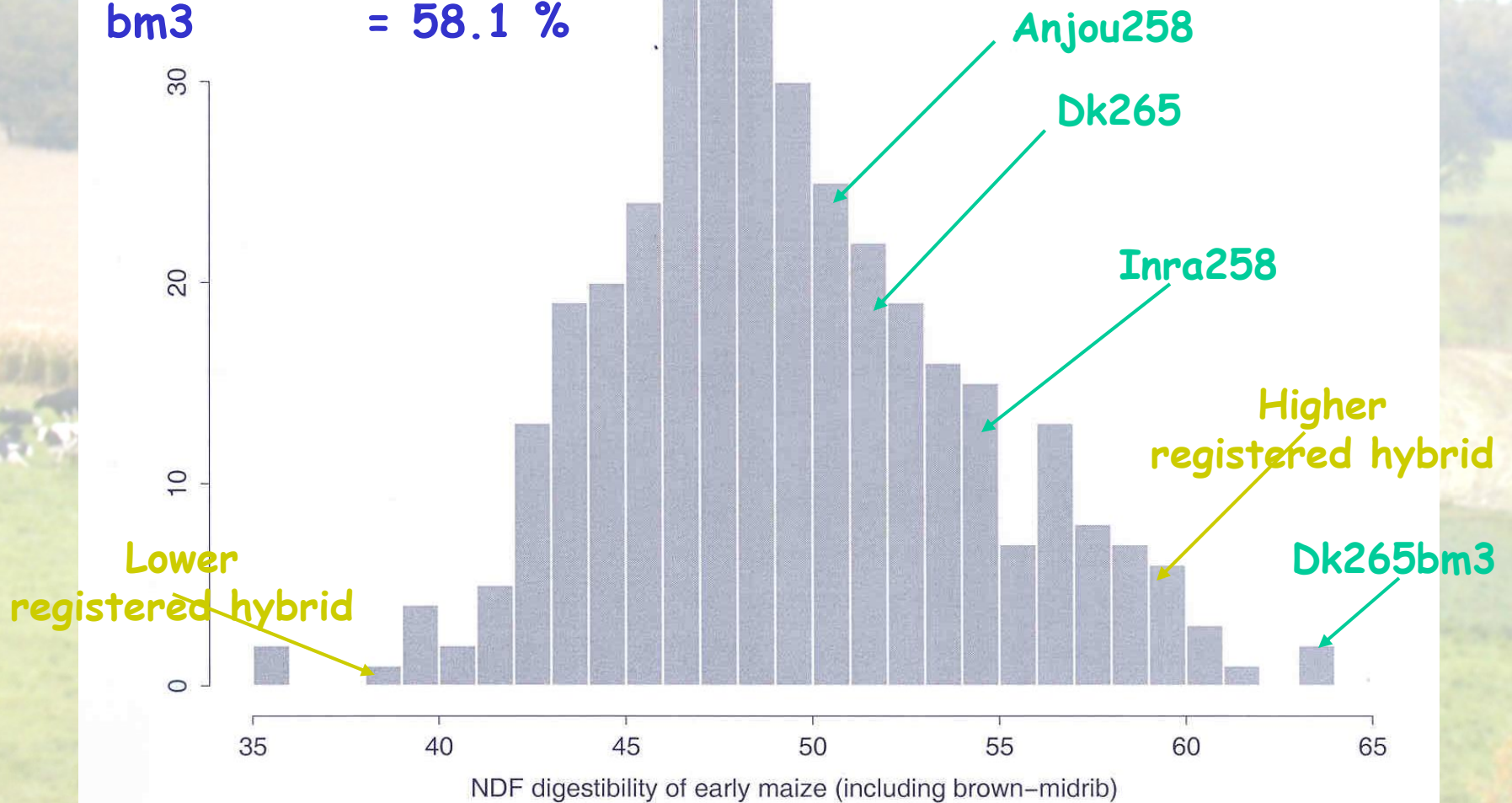
# Genetic variation for in vivo cell wall digestibility in a set of 376 early maize hybrids

## NDFD means

Normal = 48.4 %

Registered = 47.9 %

bm3 = 58.1 %





## Decrease of average cell wall digestibility since 1958

Registered early hybrids	1958	1981	1989	1994	1999	2002
	1980	1988	1993	1999	2002	2010
NDFD < LG11	32 %	63 %	75 %	84 %	93 %	xx

with correlative decrease of energy value

(NDFD = in vivo cell wall digestibility)

Genetic drift during breeding for grain related traits

Adverse effect during breeding for stalk mechanical resistance

Insufficient use of relevant genetic resources



## Genetic variation for intake



	Intake (kg/cow/day)	NDFD %
DK265bm3	18.1	62.1
DK265	17.0	51.4
Brutus	16.0	51.7
Anjou285	14.5	43.3

Higher Digestibility and Intake  $\Leftrightarrow$  Economical Benefit  
from 6,000 to 10,000 Euros / 75 cows . year

### Maize

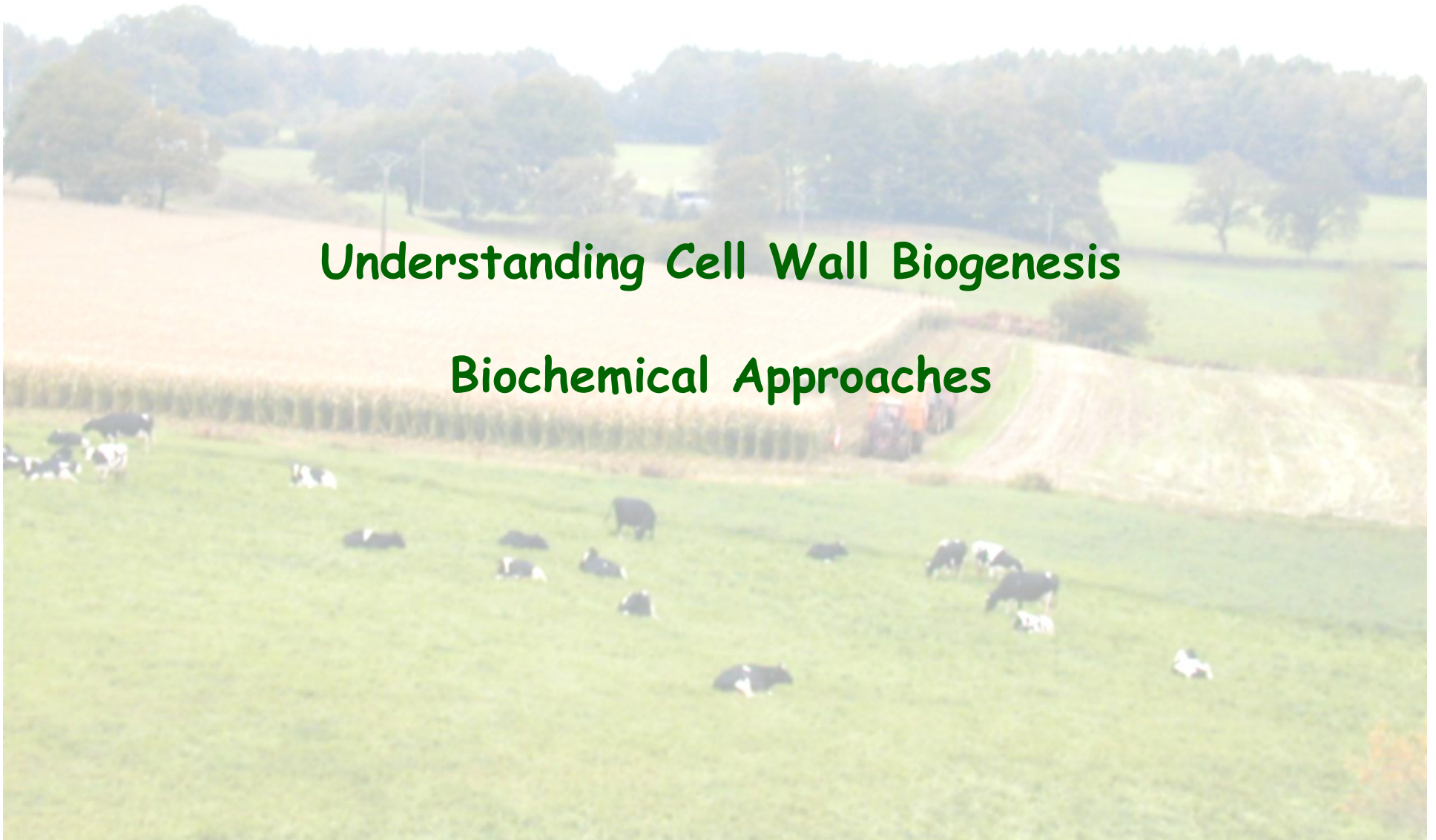
Energy value  $\Leftrightarrow$  66 % cell wall digestibility + 33 % starch

Intake  $\Leftrightarrow$  50 % cell wall digestibility  
+ 50 % tissue friability (chewing speed)



# Understanding Cell Wall Biogenesis

## Biochemical Approaches

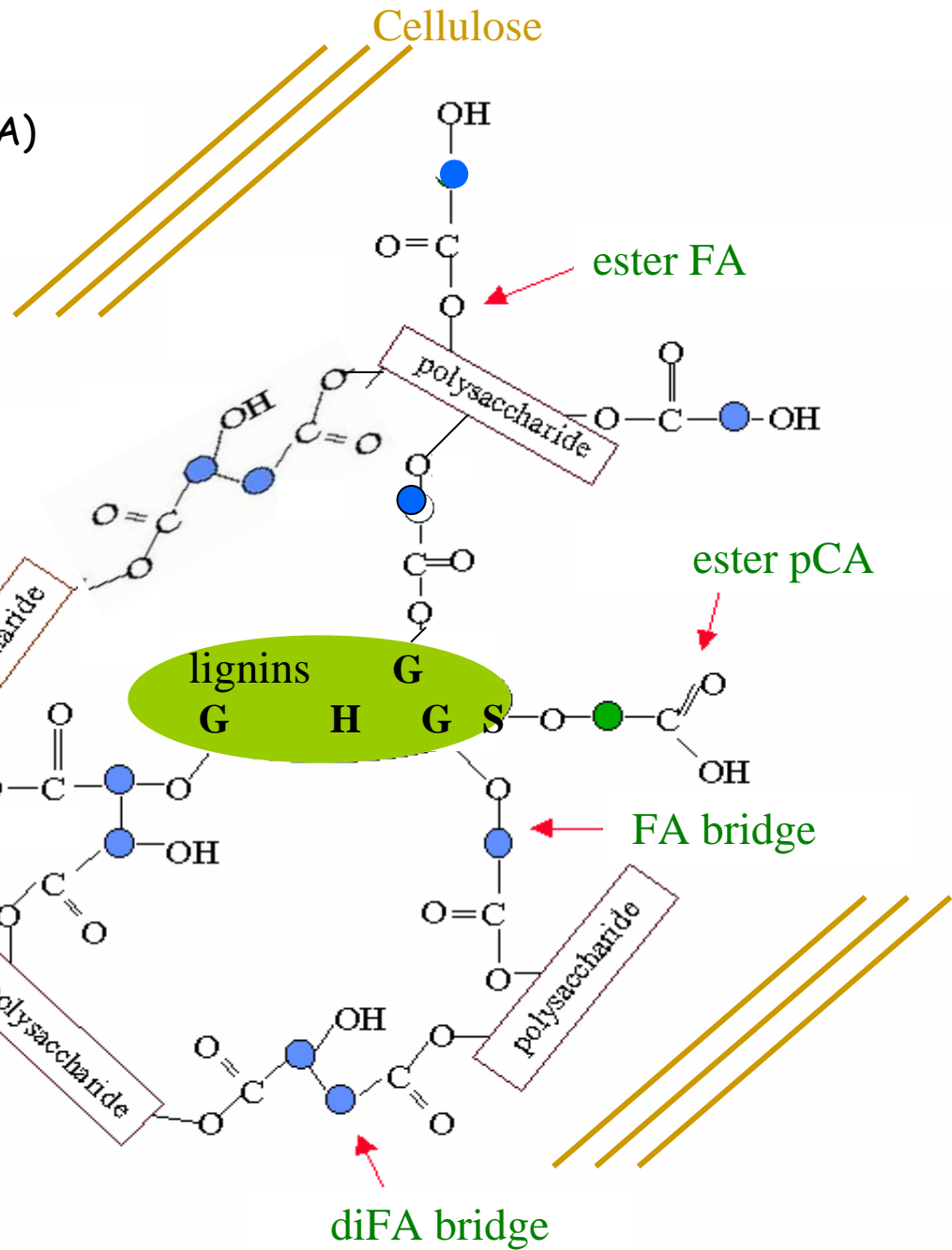
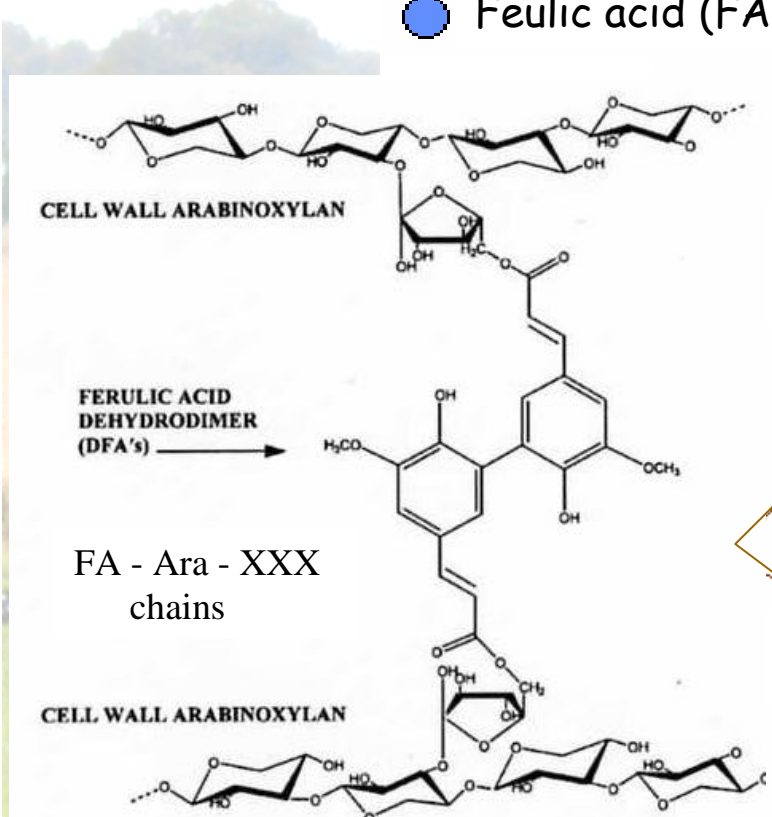




# The grass cell wall

● pCoumaric acid (pCA)

● Feulic acid (FA)

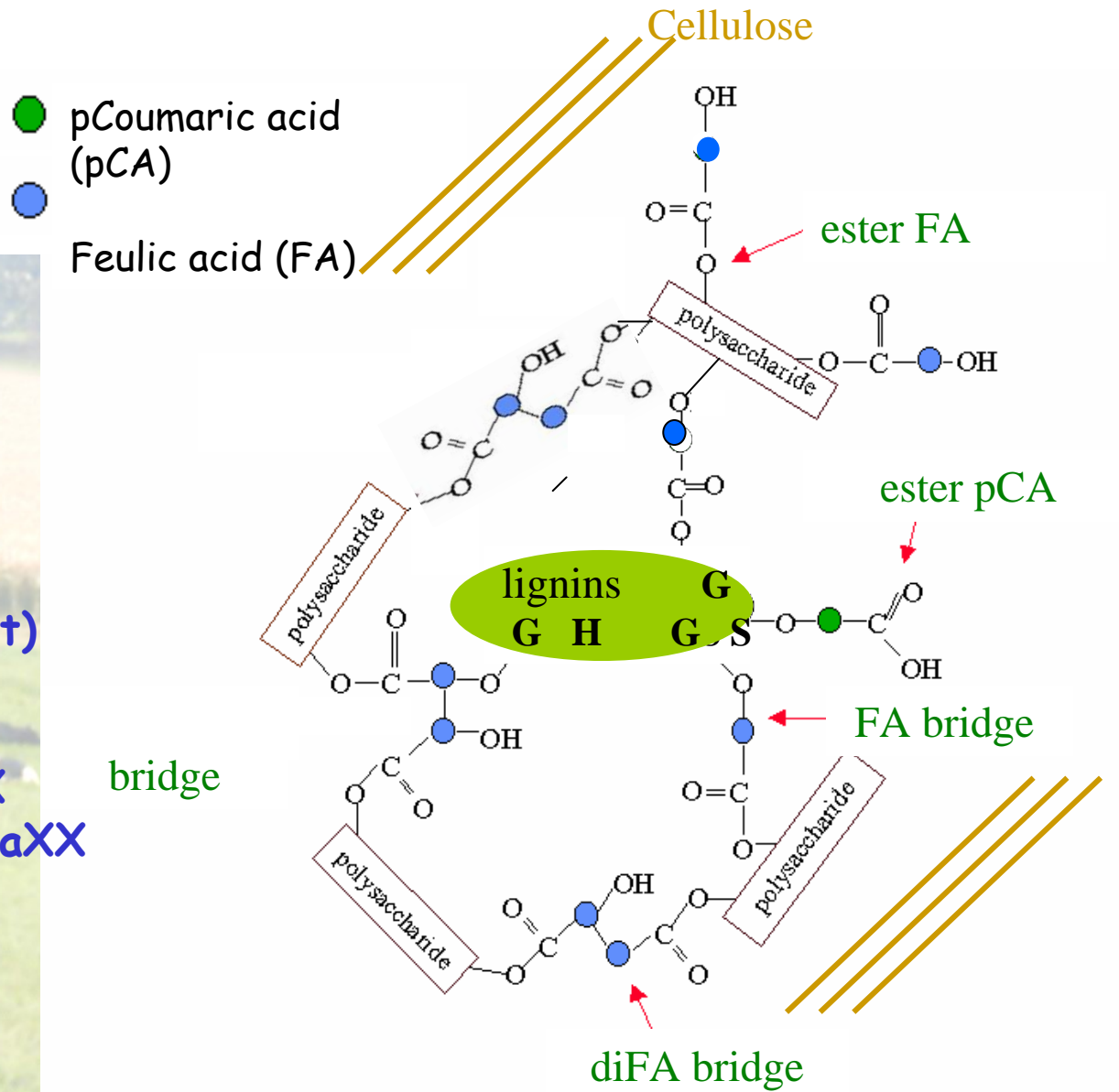
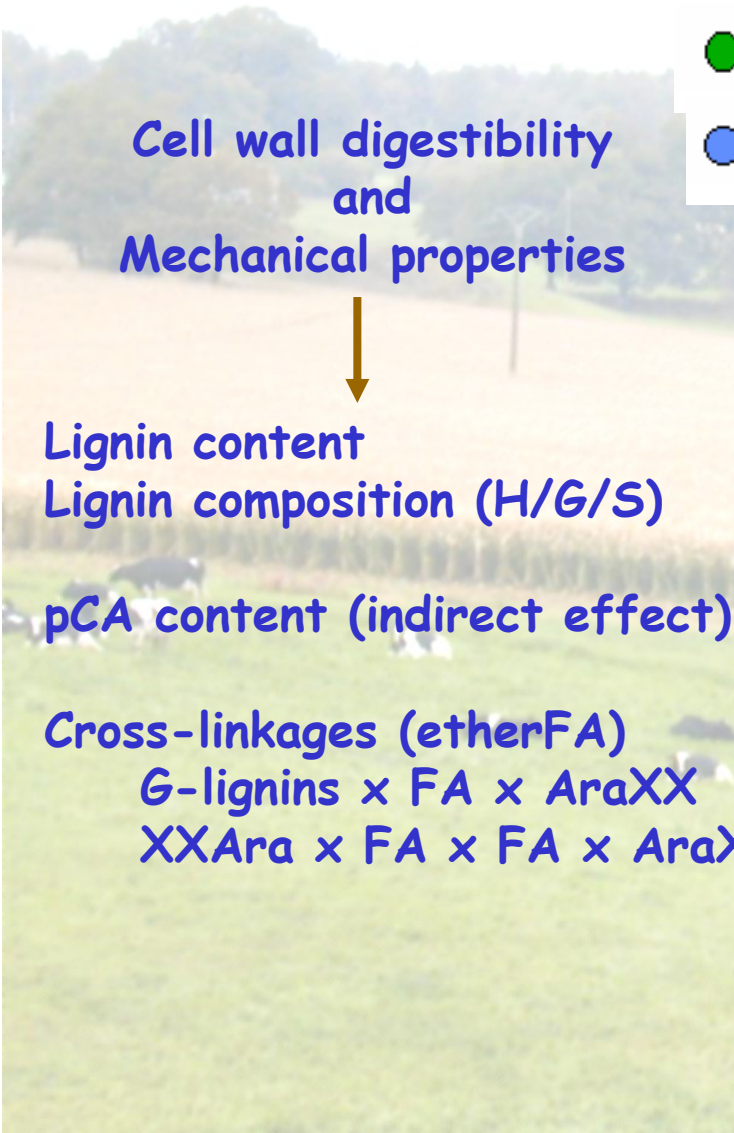


Lignins <==>

Polymer of hydroxyphenyl (H)  
coniferyl (G)  
syringyl (S) alcohols

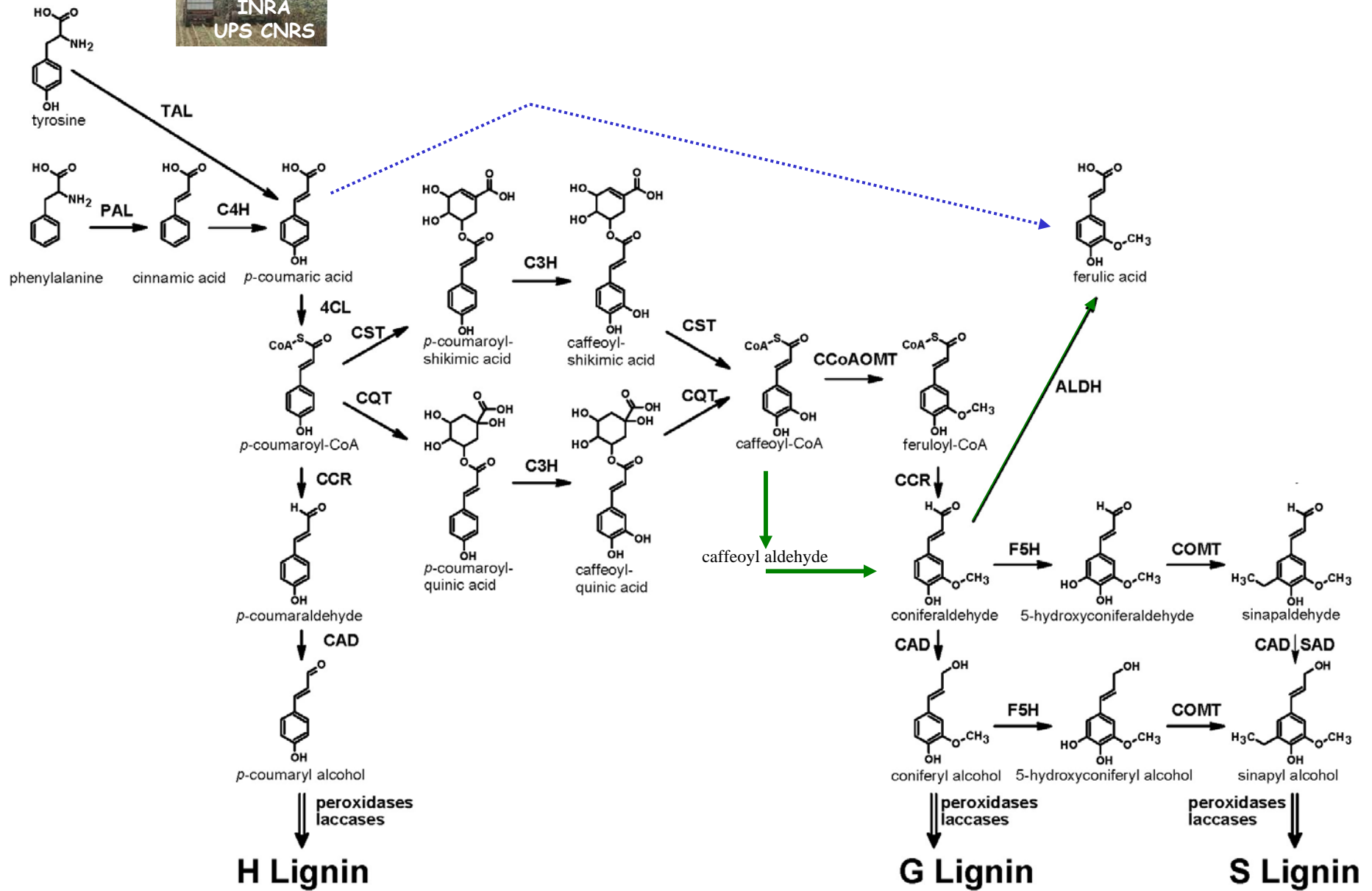


# The grass cell wall





# Current knowledge on the lignin pathway in grasses





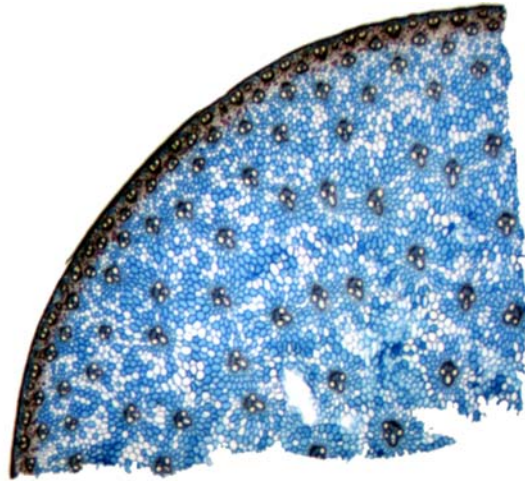
## Genetic variation for p-hydroxycinnamates and lignin monomeric units in flint maize lines

	dNDF	ADL/ NDF	pCA	FAester	FAether	VN (G)	SN (S)
<b>F4</b>	<b>38.16</b>	<b>3.88</b>	<b>7.01</b>	4.98	1.24	<b>4.47</b>	<b>5.42</b>
<b>F2</b>	<b>30.48</b>	4.92	<b>11.64</b>	<b>5.70</b>	<b>1.30</b>	<b>6.65</b>	<b>7.96</b>
<b>F286</b>	<b>31.71</b>	<b>5.27</b>	<b>11.43</b>	<b>5.85</b>	<b>1.16</b>	<b>6.51</b>	<b>7.47</b>
<b>F268</b>	<b>22.93</b>	<b>6.43</b>	<b>10.82</b>	<b>4.70</b>	<b>1.57</b>	<b>6.37</b>	<b>8.75</b>
<b>L1</b>	<b>22.55</b>	<b>5.63</b>	<b>13.15</b>	<b>5.85</b>	<b>1.39</b>	<b>7.35</b>	<b>9.45</b>
<b>L3</b>	<b>23.09</b>	<b>7.14</b>	<b>12.96</b>	<b>4.05</b>	<b>2.36</b>	<b>7.59</b>	<b>11.25</b>



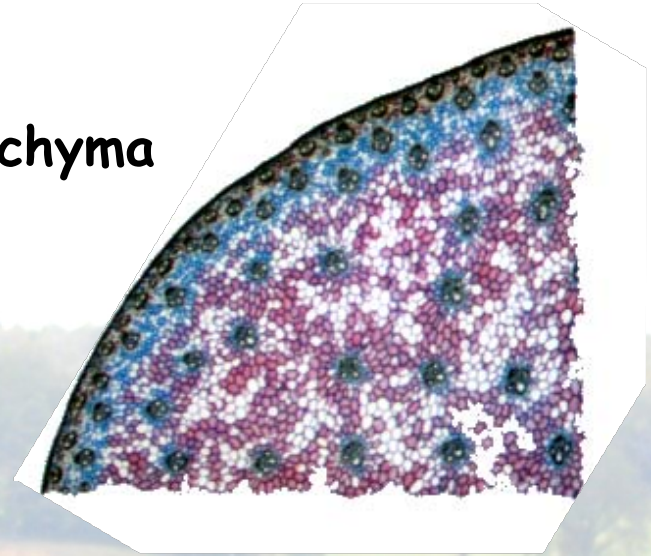
# Genetic variation for histological traits

Fasga staining  
Blue = non-lignified  
Red = lignified

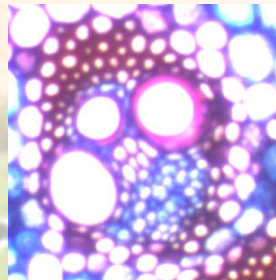


Cm484

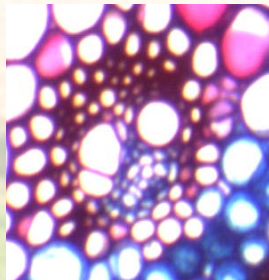
Parenchyma



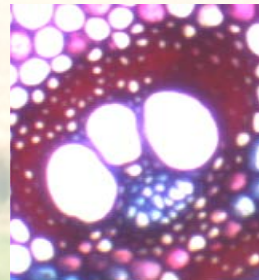
F2



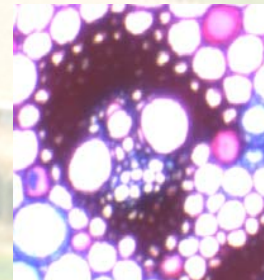
F7025



F4

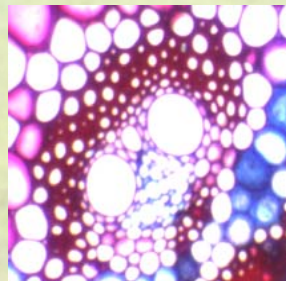


Cm484

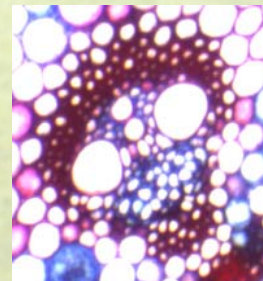


F251

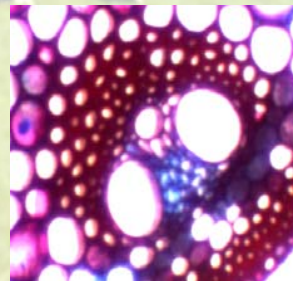
Vascular fibers



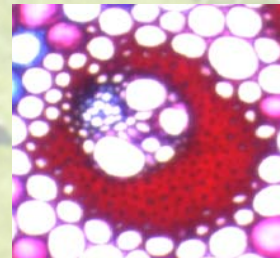
W117



F2



F271

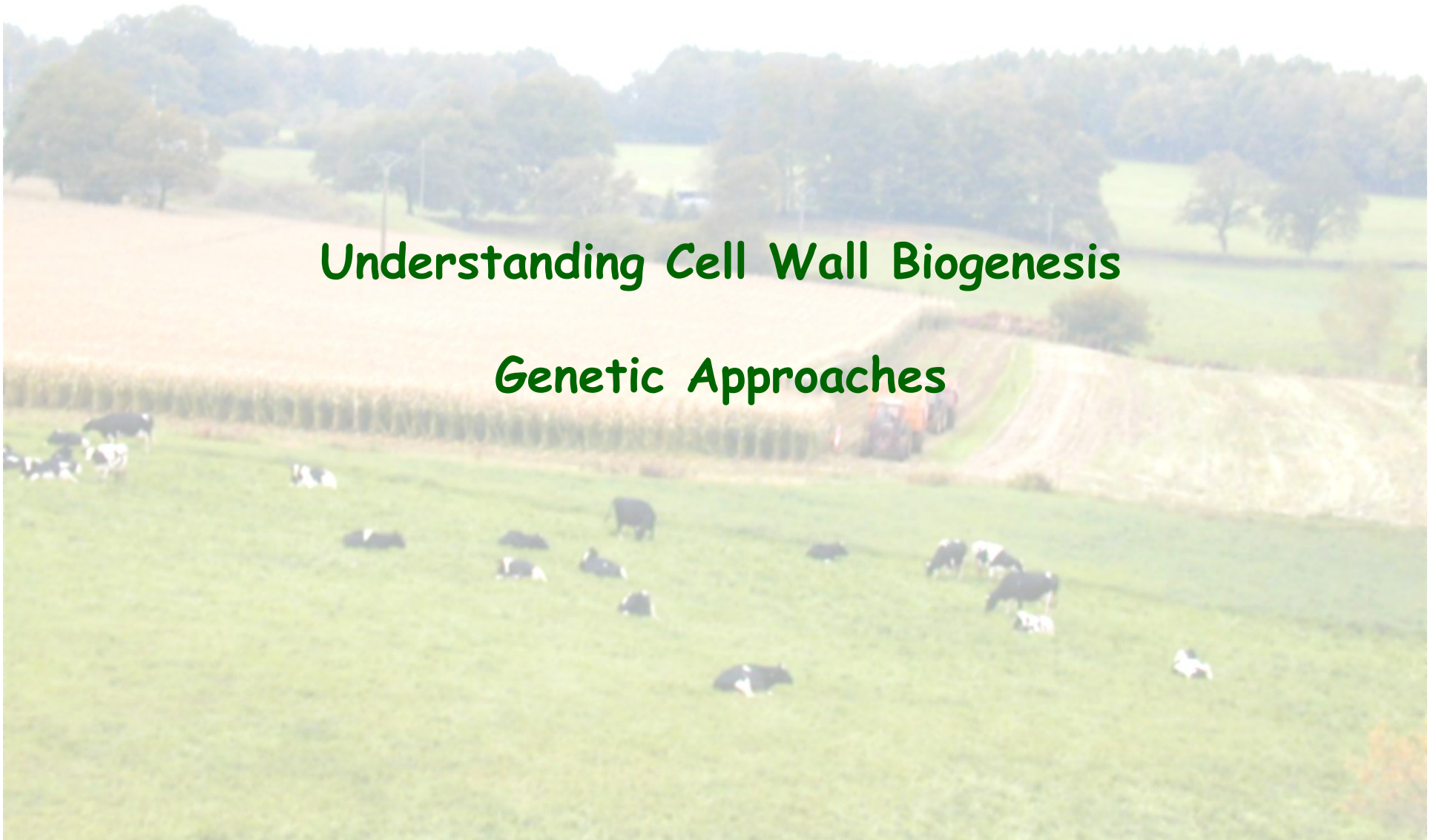


LH224



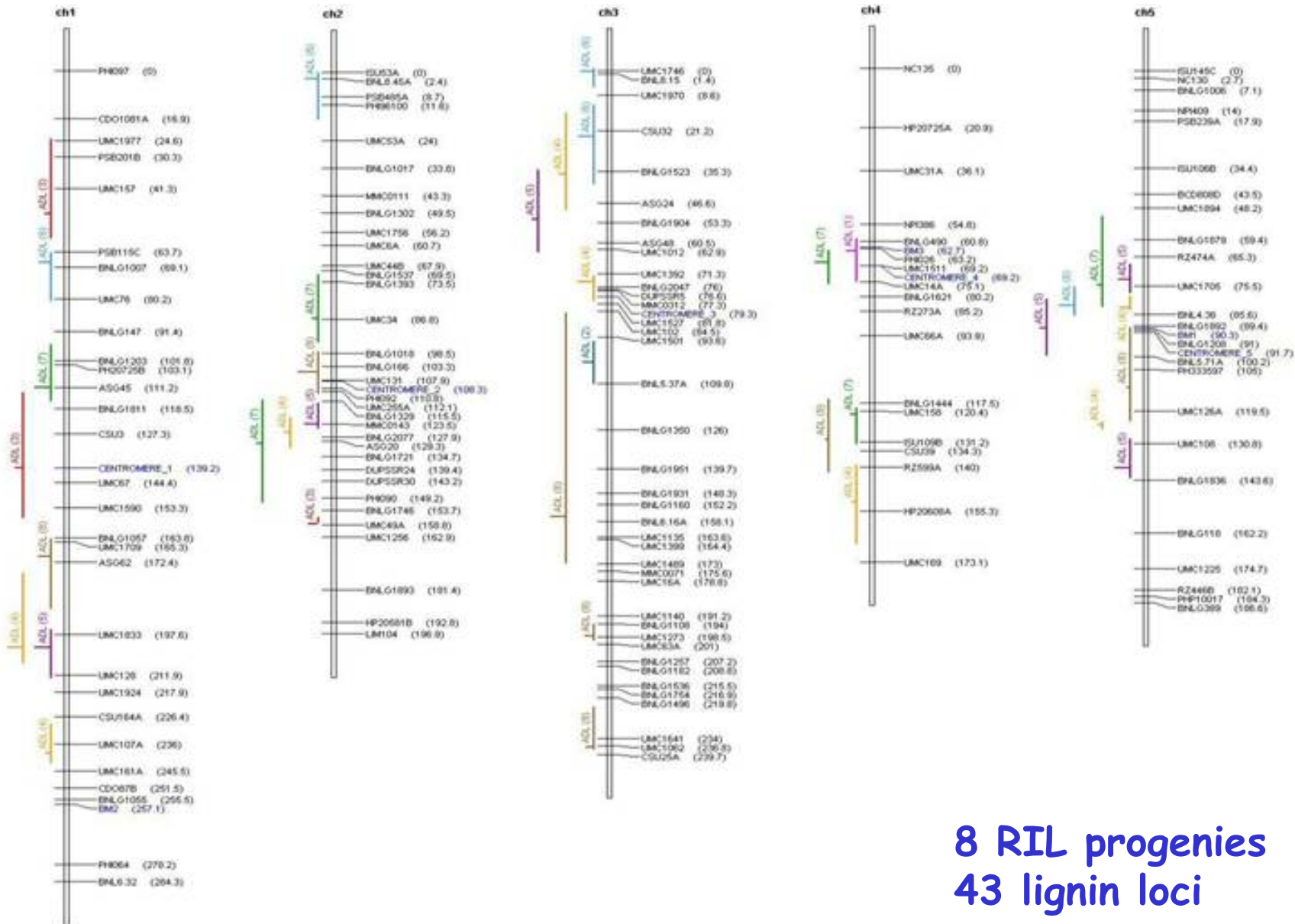
# Understanding Cell Wall Biogenesis

## Genetic Approaches



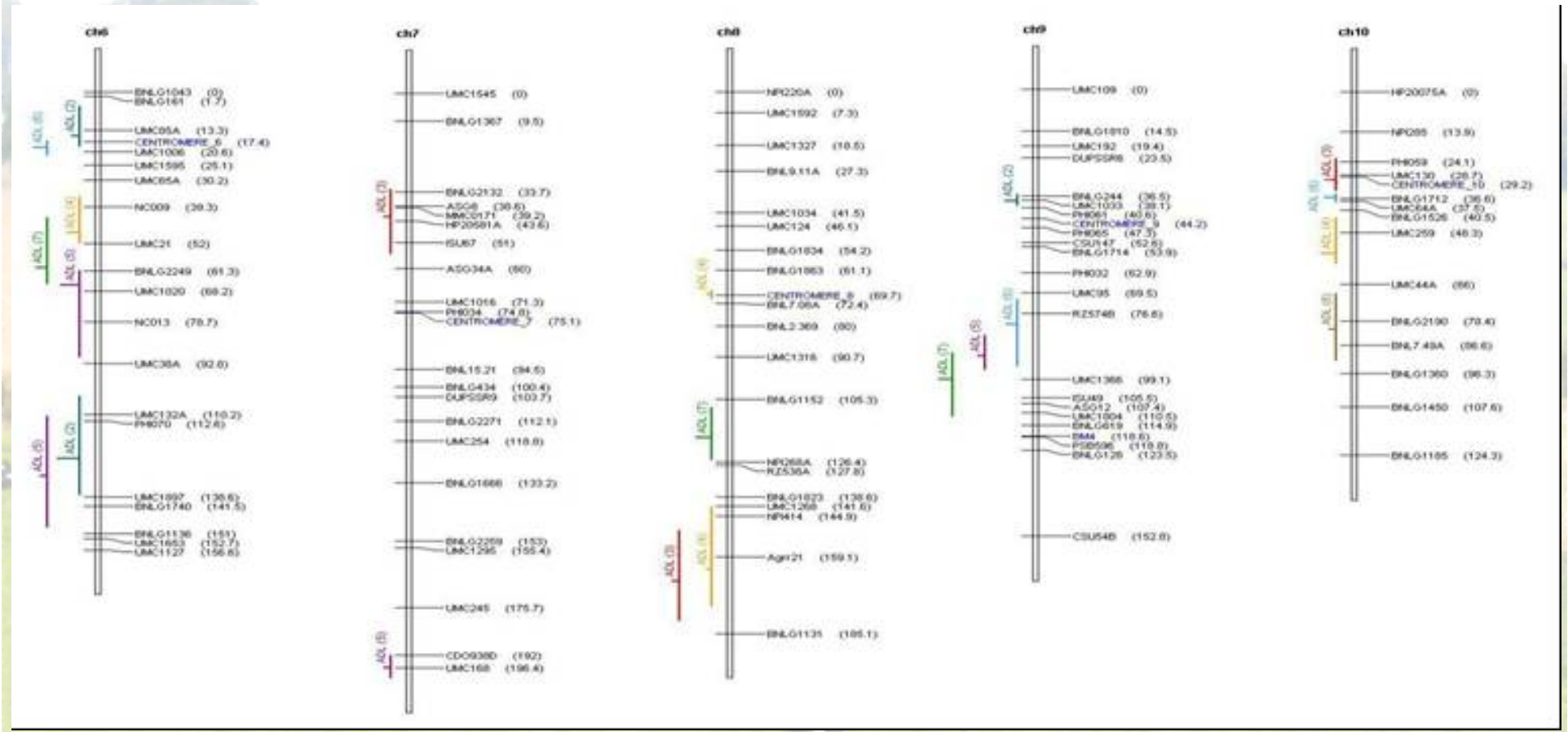


# QTL meta-analysis for lignin (ADL) content





# QTL meta-analysis for lignin (ADL) content



8 RIL progenies  
43 lignin loci



# QTL analysis in the F838 x F286 RIL progeny

**Colocalization bin 1.07**  
**Favorable allele F838**

trait	closest marker	dist clo-m	R <sup>2</sup>	chr pos
FA ether	bnlg1556	- 1	7.6	1-224
pCA ester	bnlg1556	1	9.1	1-226
Syringaldehy	bnlg1556	1	8.0	1-226
IVNDFD	bnlg1556	3	10.8	1-228
ADL/NDF	bnlg1556	3	6.4	1-228

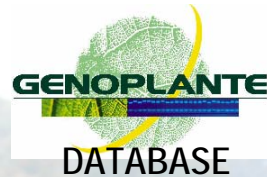




# QTL analysis in the F838 x F286 RIL progeny

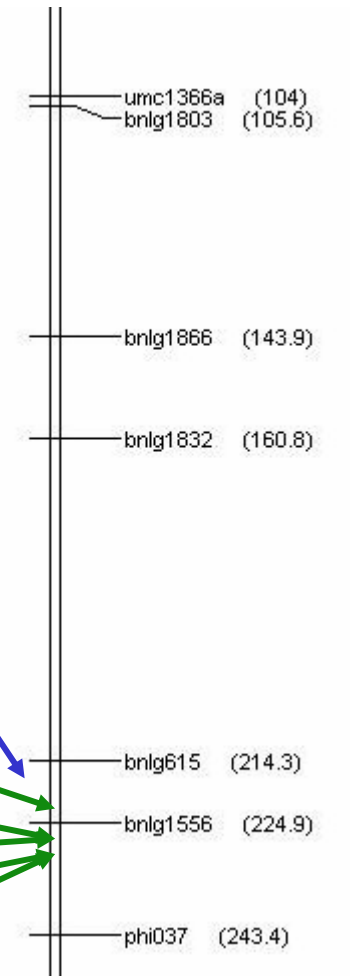
## Candidate genes

ProMaïs



Transcription factors  
 Peroxydase  
 CCR, F5H, CAD

trait	closest marker	dist clo-m	R <sup>2</sup>	chr pos
FA ether	bnlg1556	- 1	7.6	1-224
pCA ester	bnlg1556	1	9.1	1-226
Syringaldehy	bnlg1556	1	8.0	1-226
IVNDFD	bnlg1556	3	10.8	1-228
ADL/NDF	bnlg1556	3	6.4	1-228



Colocalization bin 1.07  
 Favorable allele F838

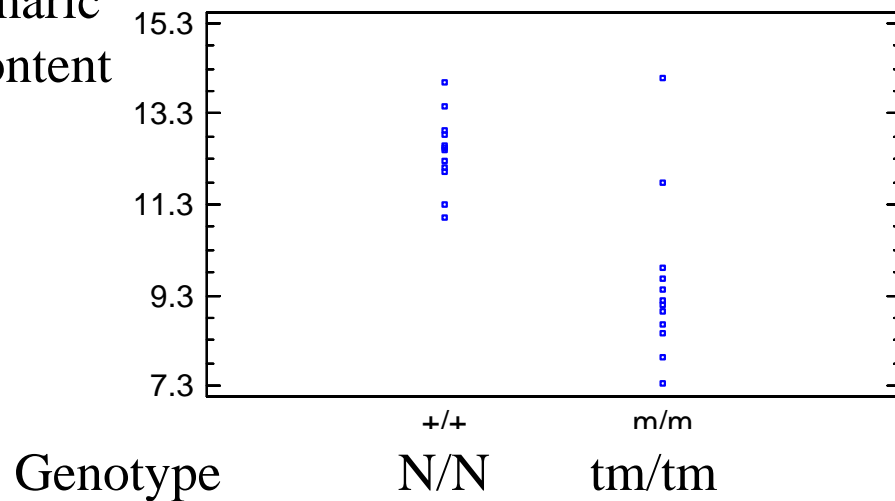


## Validation of candidate genes

Gene deregulation by transgenesis (RNAi, ...)

Gene down-regulation or silencing with MUTATOR

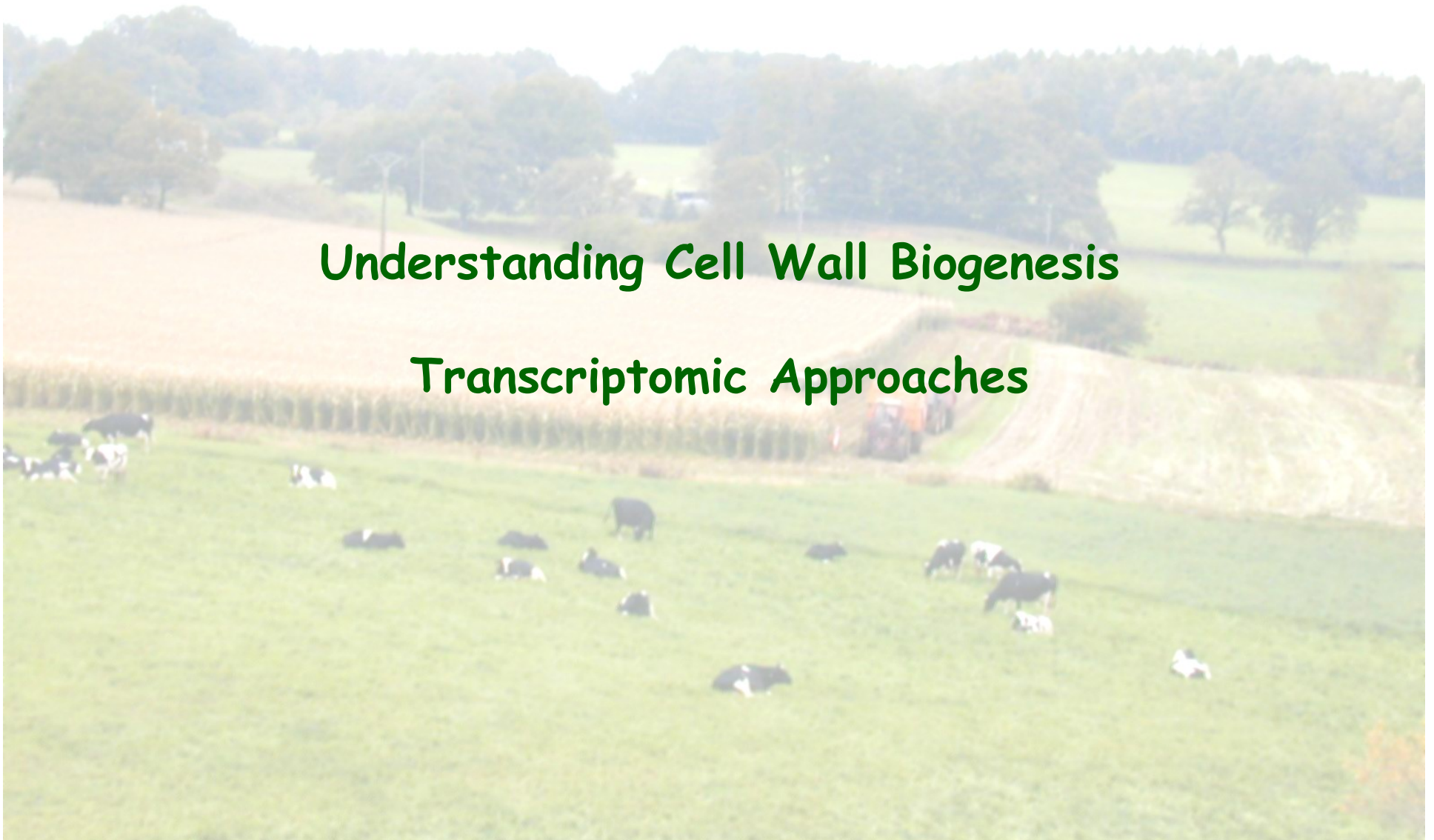
pCoumaric  
acid content





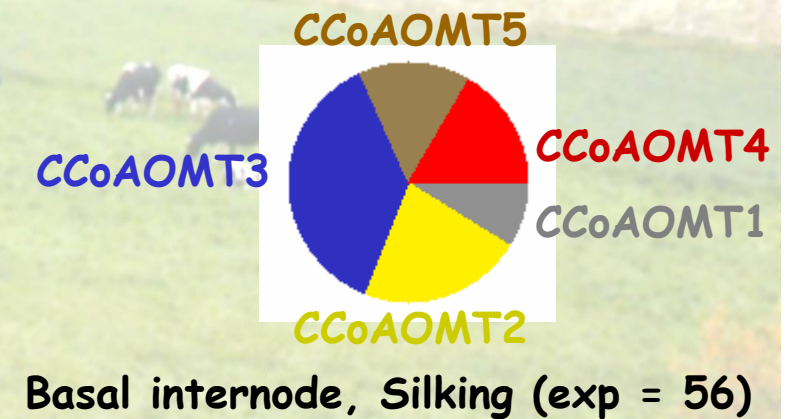
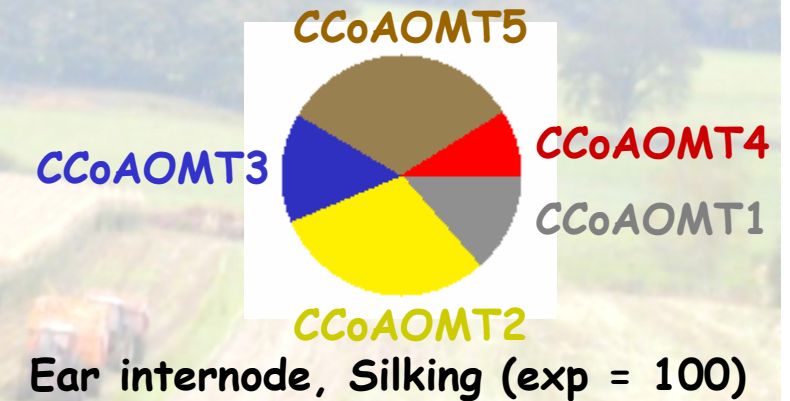
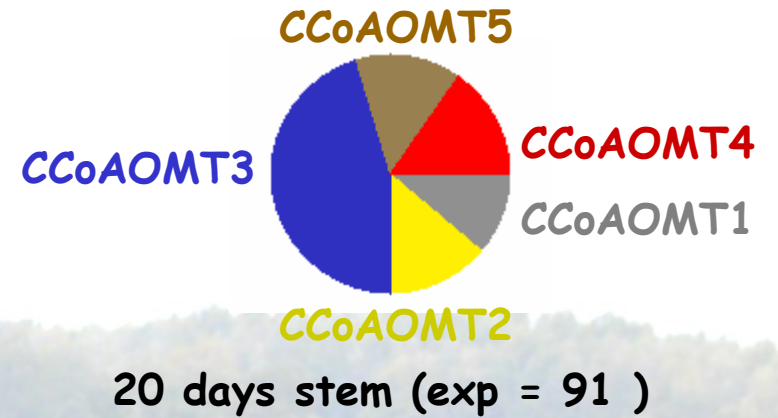
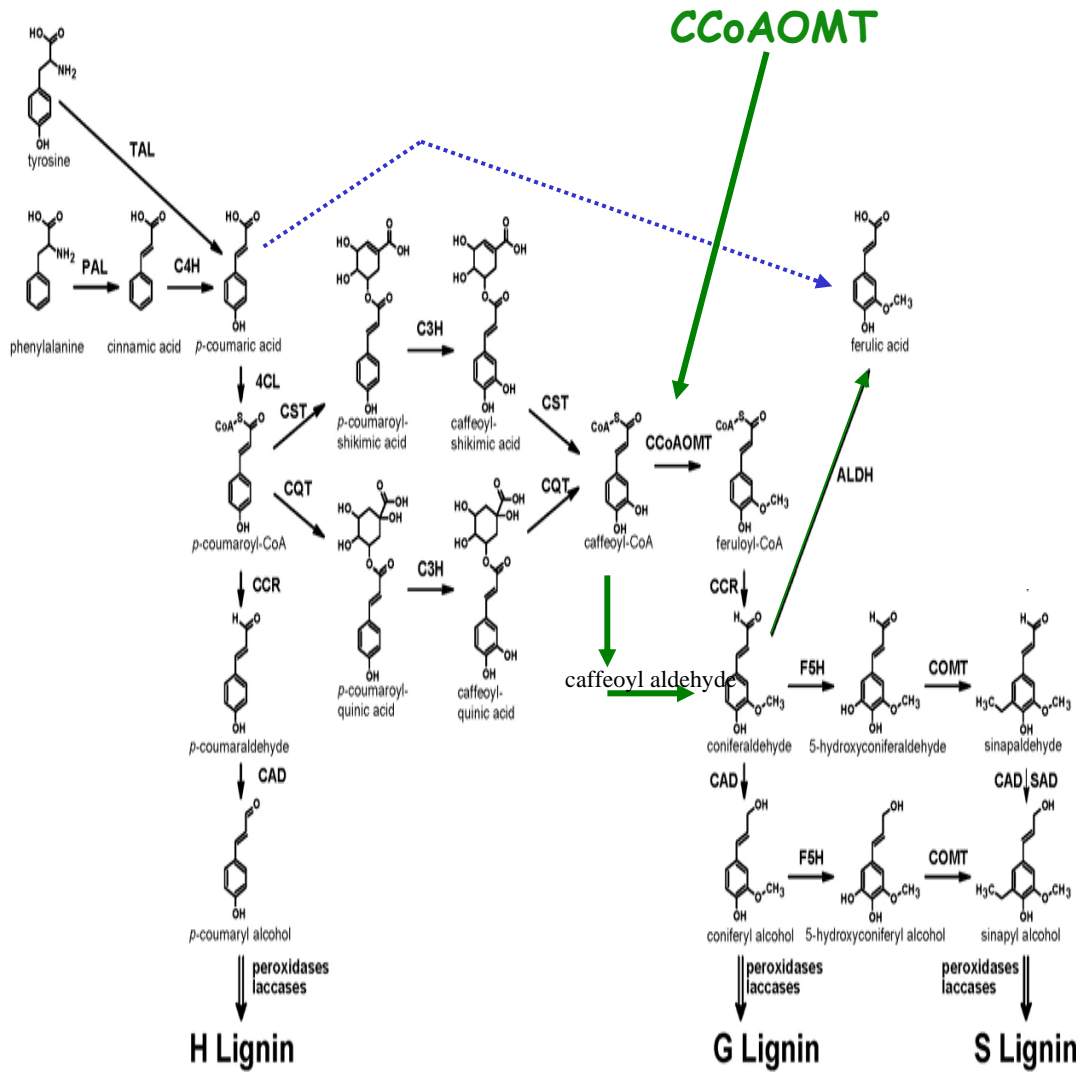
# Understanding Cell Wall Biogenesis

## Transcriptomic Approaches





# Transcriptomic approach INRA F2 inbred line





## Transcriptomic approach Comparison of F268 and F286 lines

Expression in ear internode of silking plants	F268	F286 / F268
Caffeoyl CoA O-methyltransferase (CCoAOMT1)	9656	0.77
Caffeoyl CoA O-methyltransferase (CCoAOMT2)	13612	0.74
Caffeoyl CoA O-methyltransferase (CCoAOMT3)	18453	0.82
Caffeoyl CoA O-methyltransferase (CCoAOMT4)	7923	0.79
Caffeoyl CoA O-methyltransferase (CCoAOMT5)	71160	0.39

	dNDF	ADL/ NDF	pCA	FAester	FAether	VN (G)	SN (S)
F286	31.71	5.27	11.43	5.85	1.16	6.51	7.47
F268	22.93	6.43	10.82	4.70	1.57	6.37	8.75



## Transcriptomic approach ZmCAD2 / bm1 mutant



Bin 5.04



CAD/bm1  
ZmCAD2

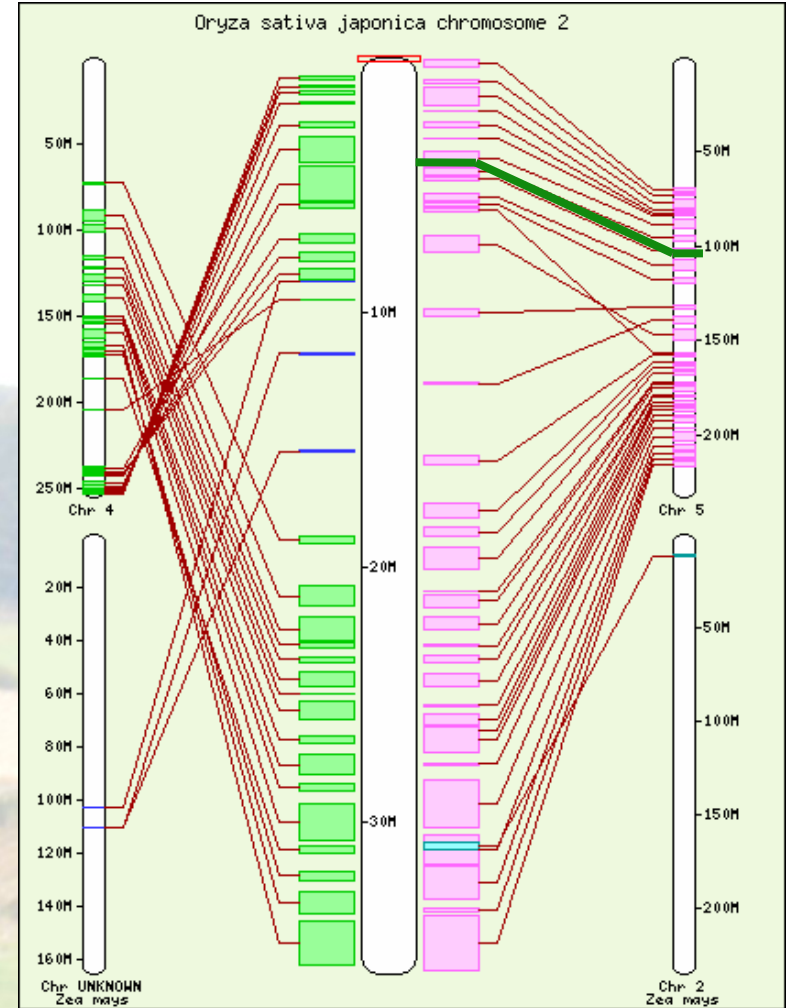
ZmCAD2 activity is reduced by 70 % in bm1 plants (Halpin et al 1998)

	F2bm1/F2
<b>Cinnamyl alcohol dehydrogenase (ZmCAD2, bm1)</b>	<b>0.38</b>
<b>Putative cinnamyl alcohol dehydrogenase</b>	<b>0.29</b>
<b>Putative cinnamyl alcohol dehydrogenase</b>	<b>0.22</b>
<b>Putative cinnamyl alcohol dehydrogenase</b>	<b>0.44</b>
<b>Sinapyl alcohol dehydrogenase</b>	<b>0.36</b>
<b>Cinnamyl alcohol dehydrogenase (ZmCAD1)</b>	<b>0.45</b>

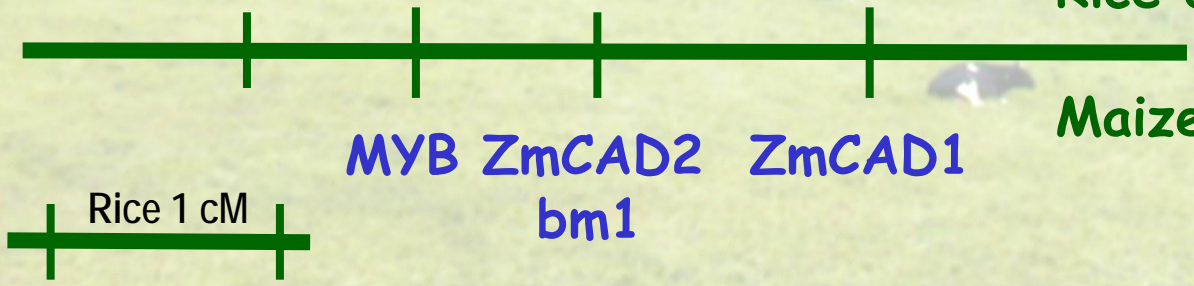


# Transcriptomic approach ZmCAD2 / bm1 mutant

	F2bm1/F2
ZmCAD2, bm1	0.38
Putative CAD	0.29
Putative CAD	0.22
Putative CAD	0.44
SAD	0.36
ZmCAD1	0.45
Argonaute	0.43



Os02g08480  
Os02g07310 Os02g09480  
AGO MYB OsCAD2

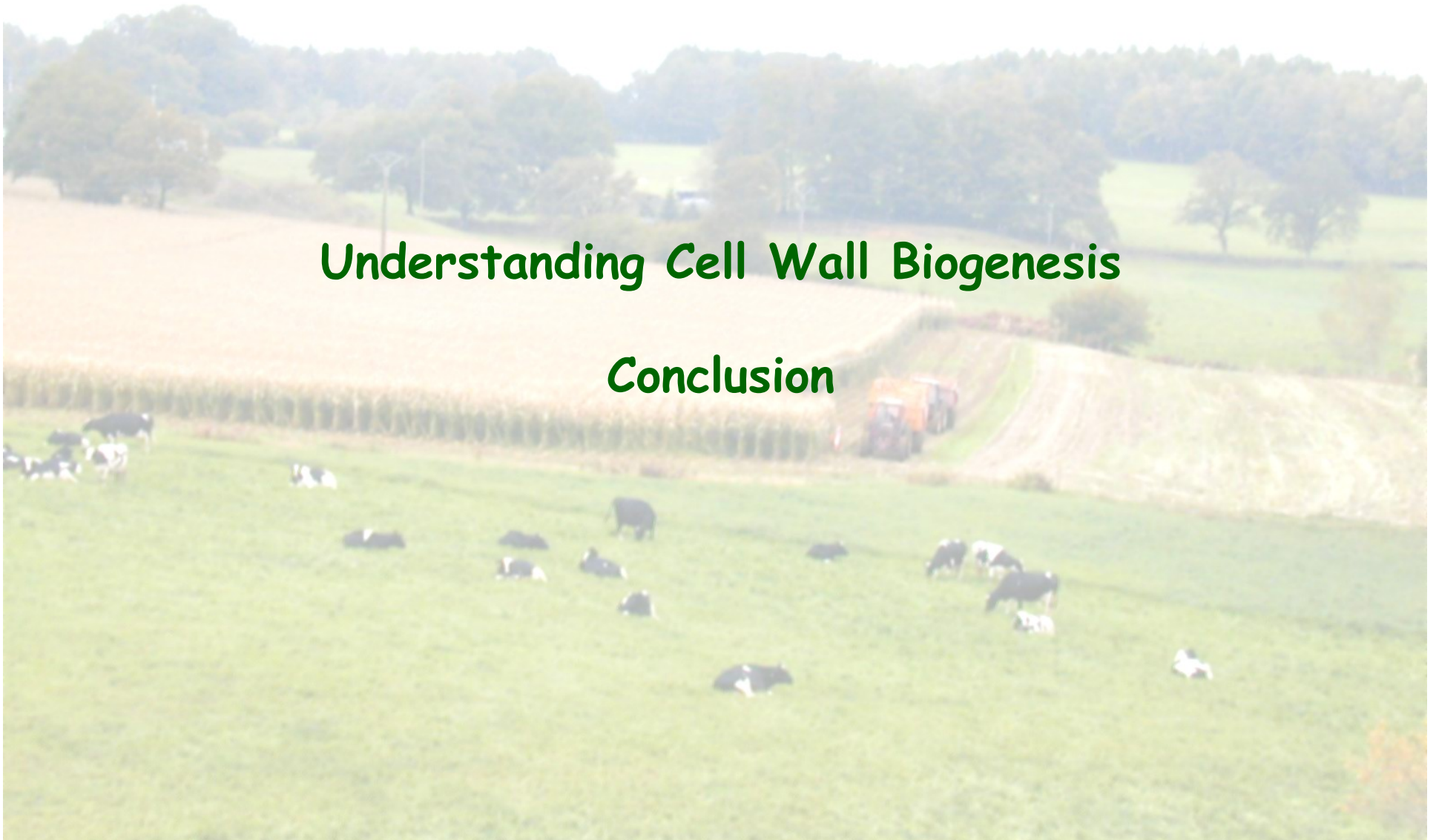


Rice chr 2  
Maize bin 5.04



# Understanding Cell Wall Biogenesis

## Conclusion





## Conclusion

Understanding cell wall biosynthesis  
in maize and grasses  
for silage and biofuel production

Dissection of cell wall traits  
into their underlying  
biochemical, genetic, genomic components



- Biochemical components, cross-linkages
- QTL analysis, basal biochemical traits
- Expression studies, candidate genes

Association studies  
Marker assisted selection  
Improvement of cell wall quality traits





Thanks for your invitation to the EPSO meeting

Thanks for your attention

Thanks to all colleagues  
involved in the project

