Ridesa
Universidade Federal de Viçosa
Molecular Plant Physiology Laboratory
Plant Physiology Graduate Program
Plant Biology Department

Sugarcane biological diversity, breeding and molecular characterization of elite genotypes for ethanol biomass production

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Actual importance of lignocellulosic alcohol in the brazilian context

➔Increase in internal demand (flexi-fuel vehicles)
➔ increase in brazilian exportation of alcohol (increased international demand)
➔ high petroleum prices
➔ brazilian production of biodiesel (ethanol used in the transesterifications of FA;5%)

Key points in lignocellulose alcohol technology (Workshop Energy Cane 2007)

✓Characterization of variability in sugarcane culm cell wall e its effect in alcohol production
✓Establish standard technologies to evaluation of lignin
✓ Identify germoplasm and breeding for energy cane
✓Development of pre-treatment and hydrolysis technology of sugarcane bagasse
✓ Improvement of fermentation technology

What sugarcane breeding can help us to improve lignocellulosic alcohol production?

RIDESA

Rede Interuniversitária para o Desenvolvimento do Setor Sucroalcooleiro
(Academic Network for the Development of Sugar-Alcohol Sector)

Network responsable for 57% of sugarcane cultivated area

Successful interaction with private companies: no need of public money

Universidade Federal de Viçosa
Centro de Ciências Agrárias
Departamento de Fitotecnia

http://www.pmgca.com.br/site/

Português
Inglês

Domingo, 7 Setembro, 2008

Programa de Melhoramento Genético da Cana-de-Açúcar

Home | PMGCA | Histórico | Equipe | Cultivares e clones RB | Infra-estrutura | Parcerias | Publicações | Contato | Acesso restrito

Seja bem vindo ao Programa de Melhoramento Genético da Cana-de-Açúcar, PMGCA, do Departamento de Fitotecnia da Universidade Federal de Viçosa.

O PMGCA - UFV tem por objetivo principal desenvolver cultivares de cana-de-açúcar por meio da cooperação técnica firmada com usinas e destilarias produtoras de açúcar, álcool e energia em Minas Gerais.

A UFV possui o único programa público de melhoramento genético da cana-de-açúcar em Minas Gerais e desenvolve cultivares e clones RB em parceria com outras universidades federais que constituem a **RIDESA**.

:: Equipe PMGCA / UFV

:: Parcerias

Rede de Experimentos

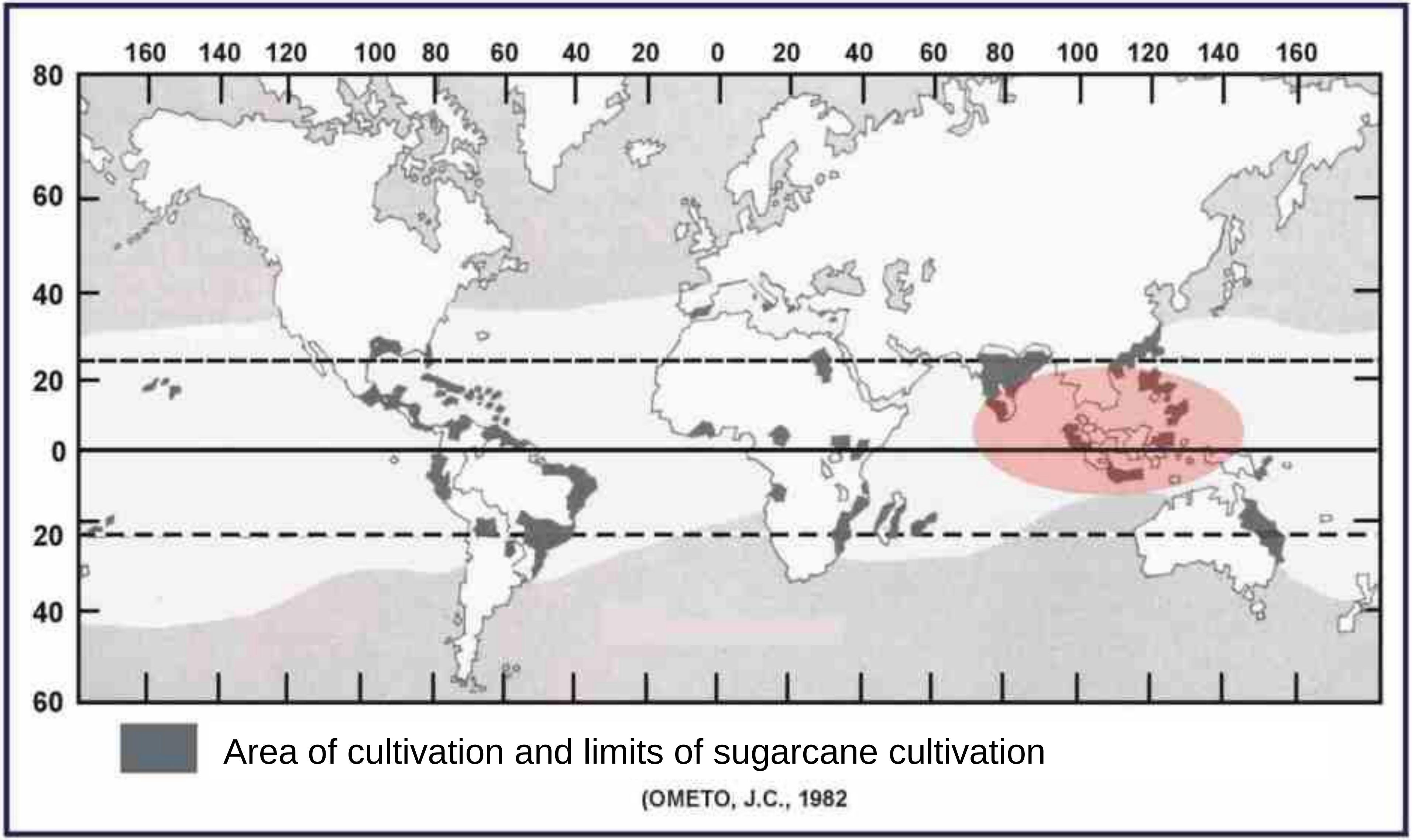
Usinas e Destilarias

Genotype evaluation in 34 different field trials experiments annualy

MD7 Layouts

Yearly, only at Ridesa/UFV, 200000 clones are evaluated

Sugarcane origin and cultivation



Stalk growth is inhibited by temperatures lower than 21ºC

Sugarcane Germplasm

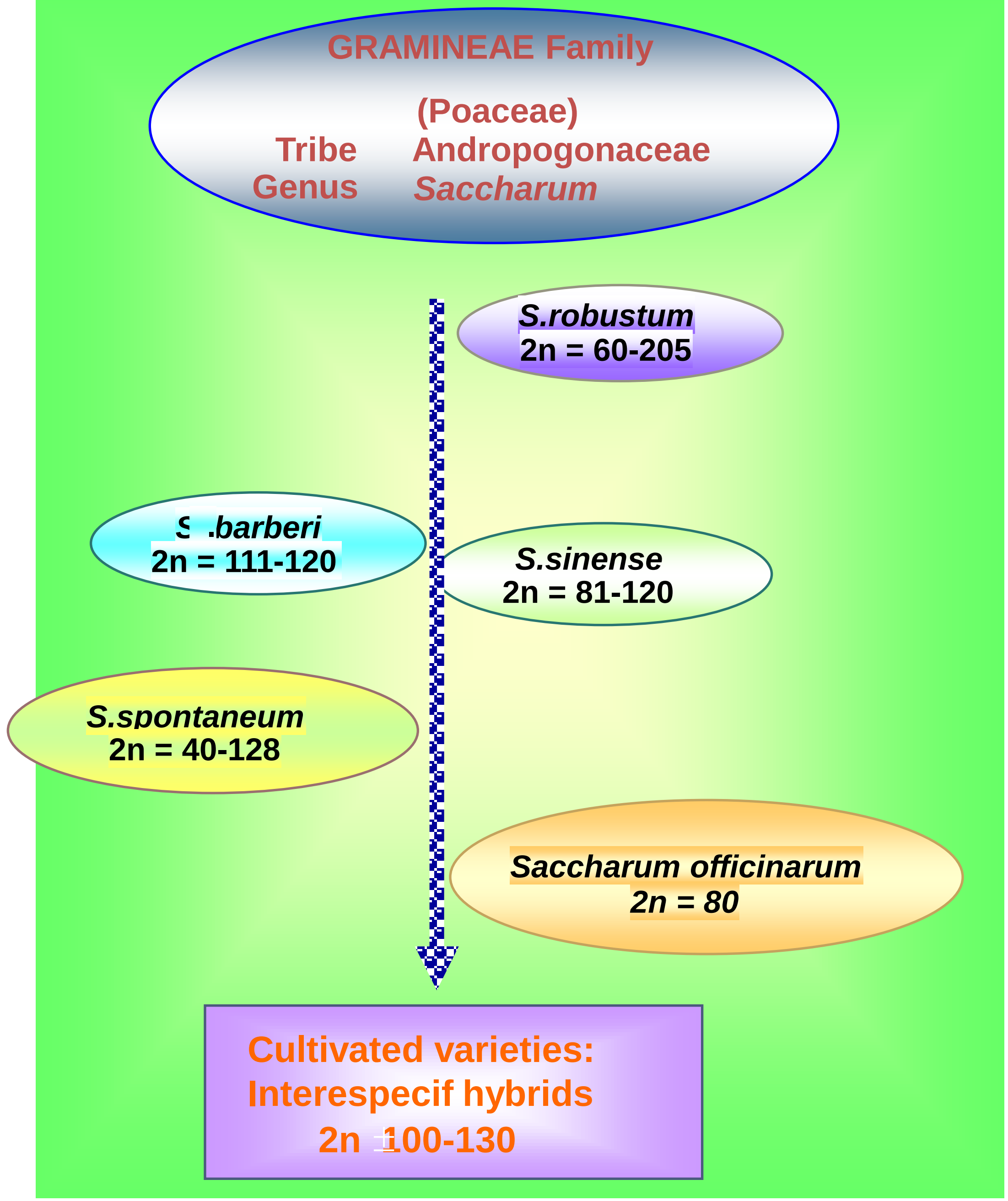
- **Wild cane (*S. spontaneum*)**
- **Wild cane (*S. robustum*)**
- **Wild cane (*Erianthius arundinaceus*)**
- **Wild cane (*Miscanthus species*)**
- **Noble cane (*S.officinarum*)**
- **Sugarcane (advanced interspecific hybrids)**

***S. officinarum*: high sugar, low fiber**, low yield, poor ratooning (“soca”) and low pest resistance

S. *spontaneum*: **low sugar, high fiber**, low yield, **excellent ratooning and disease resistance**, hybrid vigour when crossed

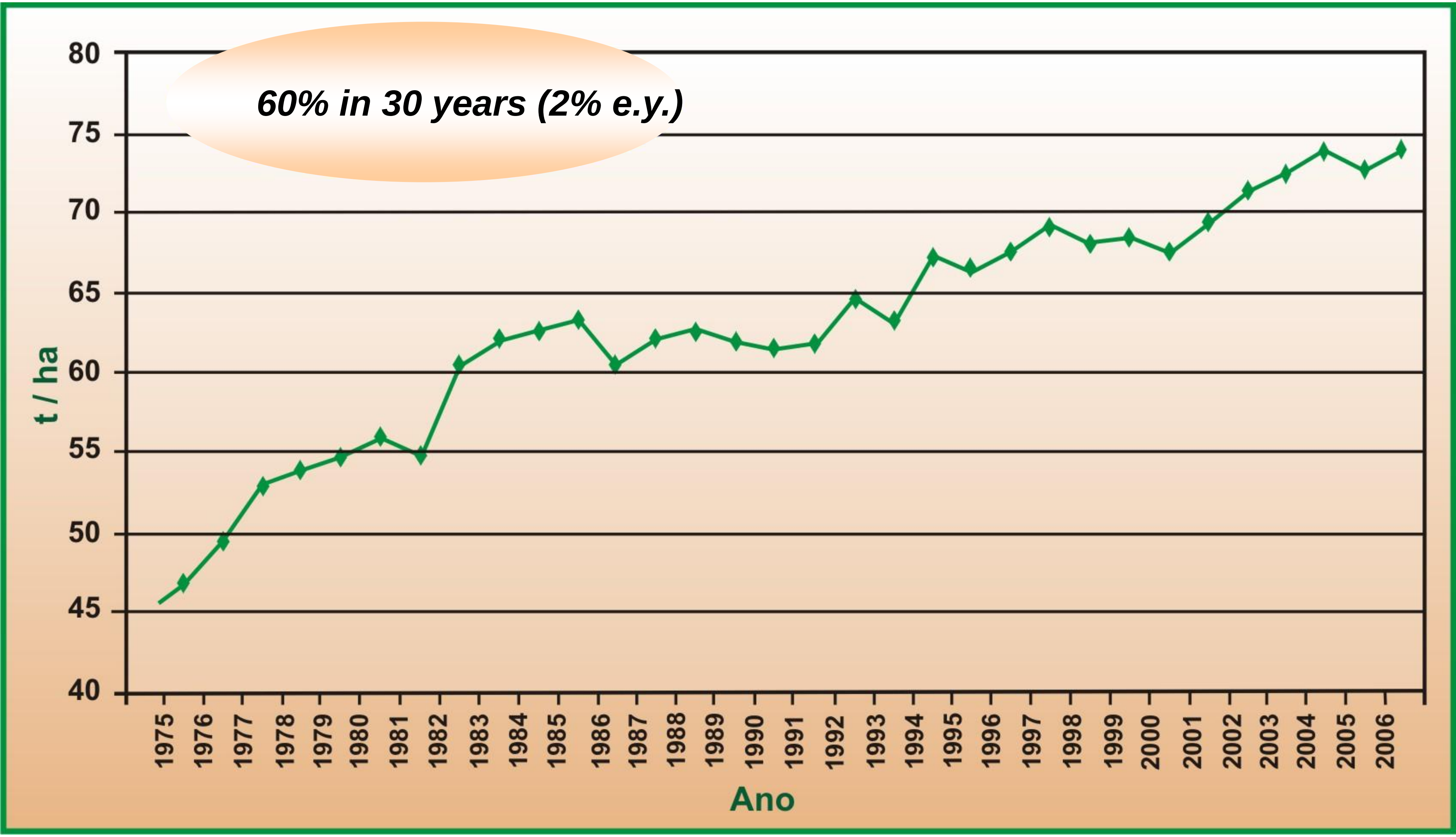
- **S. *robustum*: Medium sugar, high fibre**, low yield, medium ratooning
- **E*rianthus*:** low sugar, high fibre, medium yield, excellent ratooning
- ***Miscanthus***: low sugar, high yield, adopted to cooler climate

Polyploids
Aneuploids



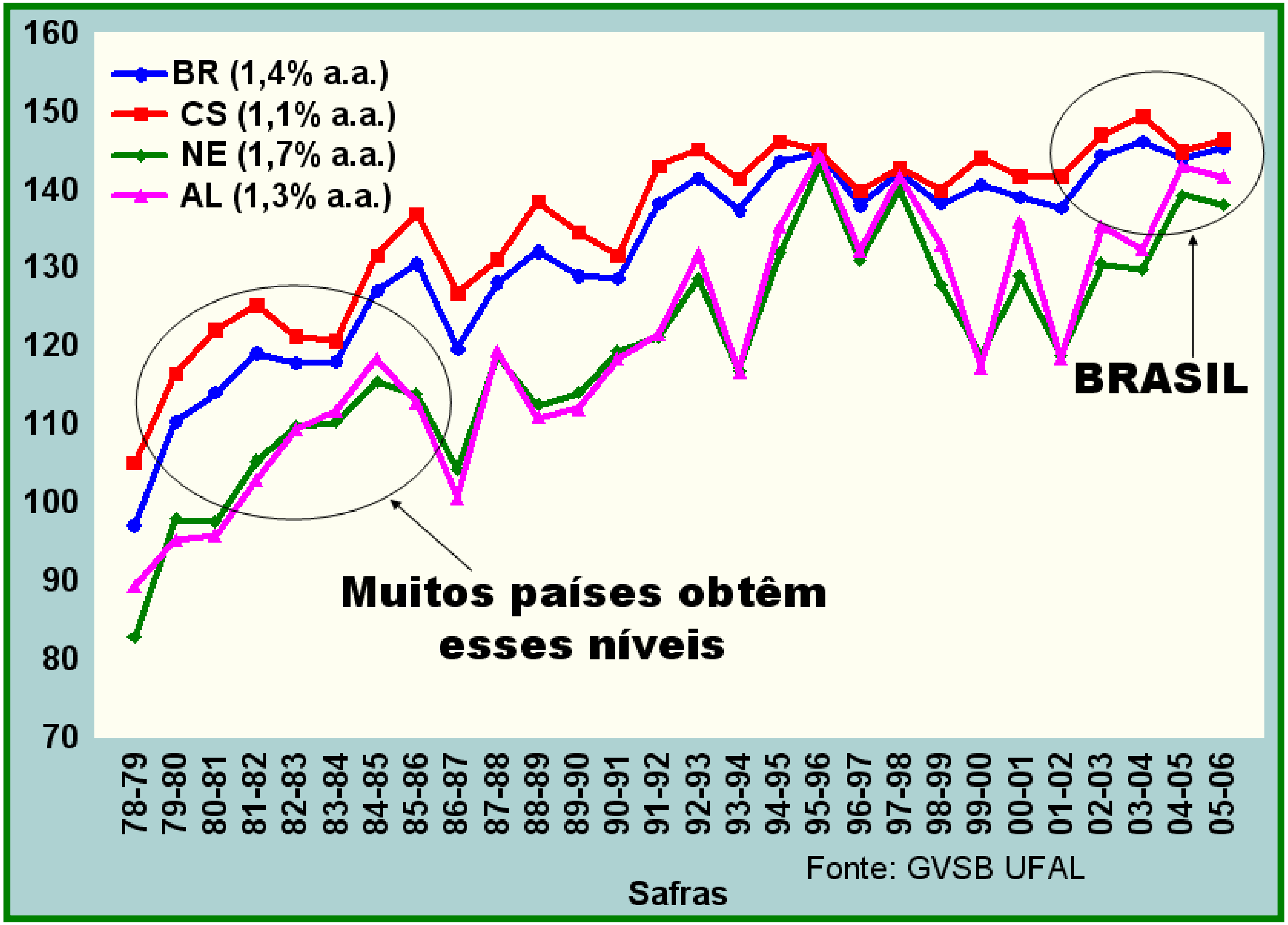
Complexity of the sugarcane genome ➔ higher than any important polyploid crop

Evolution of sugarcane yeld in Brazil (t/ha)



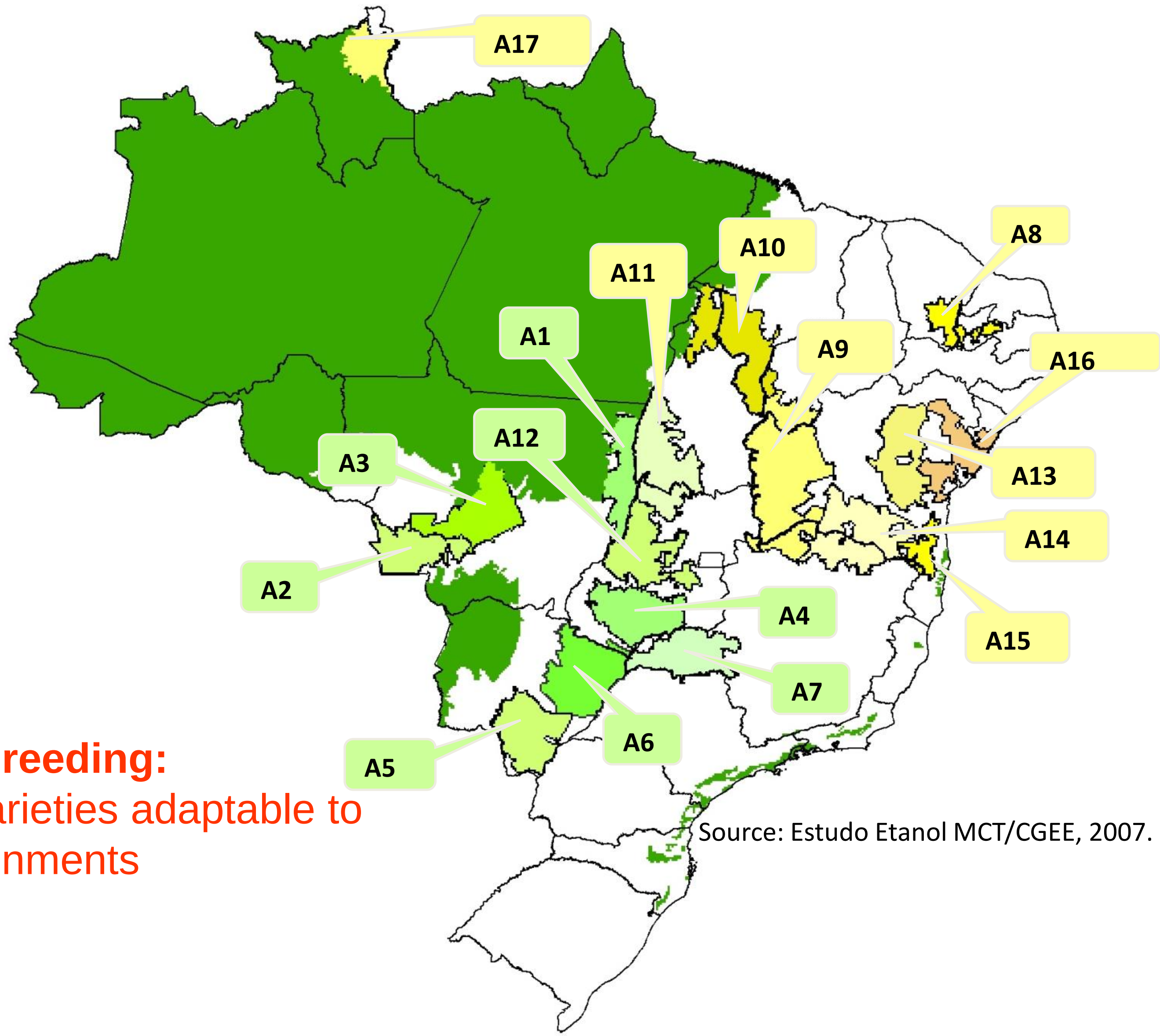
Theoretical potential: 350 t/ha

Yield evolution of sugar production of sugarcane cultivation (Kg/ton culms)



Fron

2004	Area (10^6 ha)
Soybean	22
Maize	12
Sugarcane	6
Agriculture Total	60
Pastures	200
Agriculture Potential	320

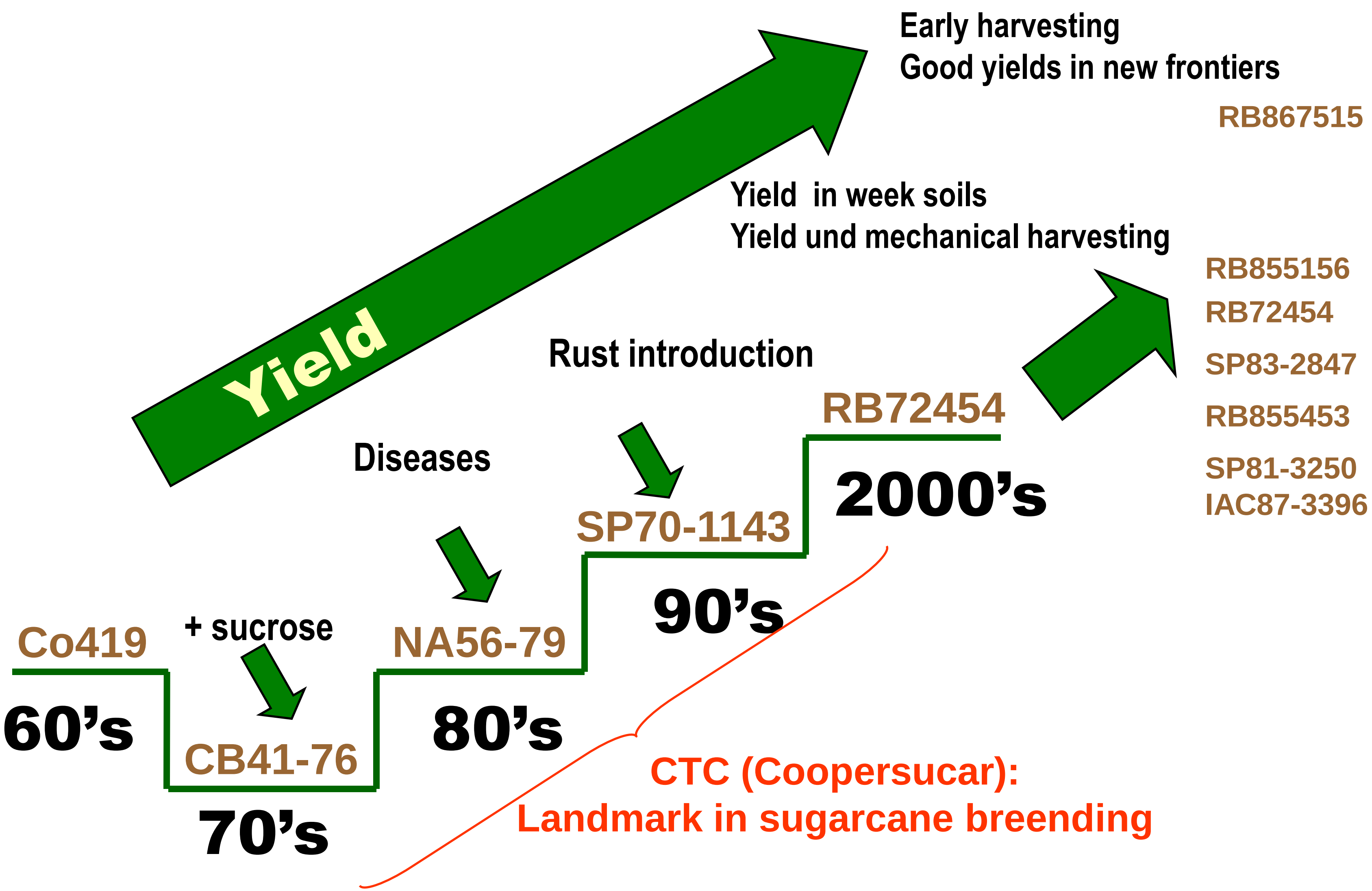


Source: Estudo Etanol MCT/CGEE, 2007.

Challenge for breeding:

Need for different sets of varieties adaptable to different environments

Main Brazilian sugarcane cultivars in the last 50 years



Method of choice for sugarcane breeding

➔***Method of recurrent selection***

Characteristics of recurrent selection

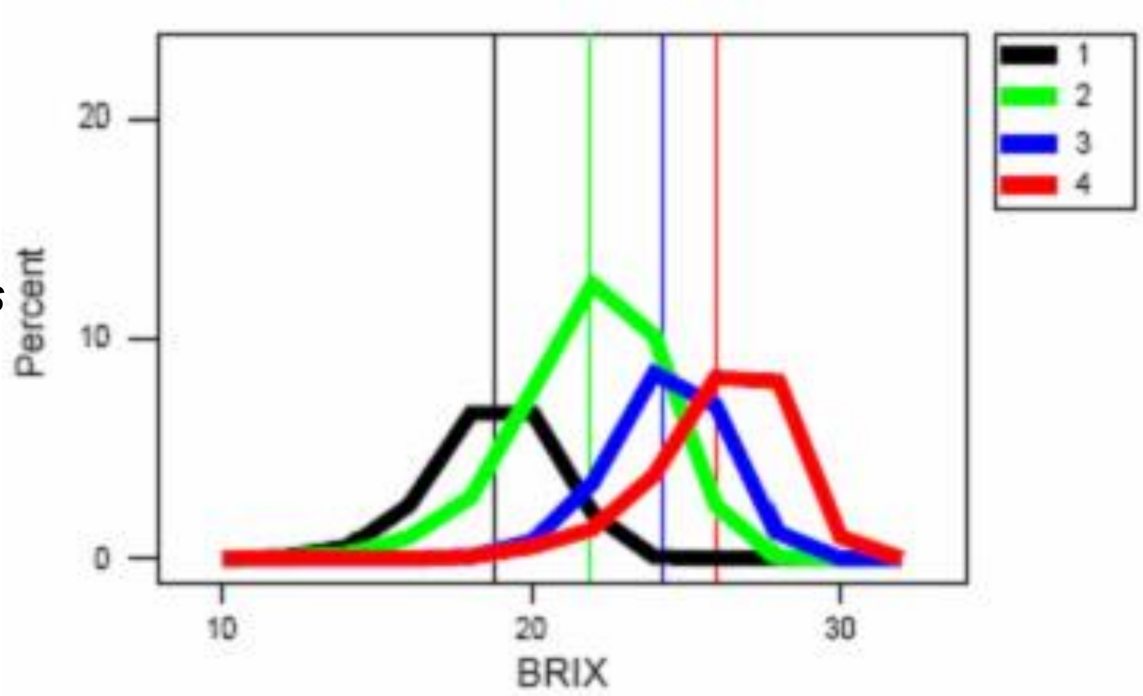
✓Increasing the frequency of favourable alleles ➔ repeated cycles of selection and crossing

✓Favor additive genetic effects

✓Maintaining genetic variation in breeding populations

✓Ex; Increase sugar levels

Distribution of Brix in four cycles of recurrent selection



Some examples of crosses used in recurrent selection

Multiparental cross

Biparental crosses

Actual RIDESA breeding parameters

- ✓ Productivity
- ✓ Stalk sucrose content
- ✓ Stalk sprouting after mechanical harvesting
- ✓Longevity
- ✓Disease resistance
- ✓Low susceptibility to flowering

New RIDESA breeding at UFV beggining in 2008

Energy cane:

- ✓ Higher biomass production keeping or increasing sugar production/area
- ✓Low lignin and/or changed lignin composition

Actual sugarcane interespecific hybrids culm composition

Water	73-76
Total Solids	24-27
Total Soluble Sugars	10-16
Fiber (DW)	11-16%

50% of its dry weight as sucrose

Component	Energy (MJ/tc)
150 kg sugars	2500
135 kg of stalk fiber	2400
140 kg of leaf fiber	2500
Total	7400 (0.176 Toe)

Conversion Efficiency

1st Generation

1) 86 L ethanol + 10.8 kg bagasse (DM) = 2200 MJ ➔ efficiency = 29.8%

2) 86 L ethanol + 60 kWh = 2230 MJ ➔ ***efficiency = 30.1%***

Alcohol production using only sucrose : wasting of energy

Alcohol production using sucrose and burning bagasse for thermical energy : still waste of energy***: 70% of energy present in sugarcane is lost***

Energy cane: the future of alcohol production

Sugarcane & Multipurpose cane

Varieties>	B77602	WI81456
Cane t/ha	77.6	125.4
Brix t/ha	15.1	15.3
Fibre t/ha	11.5	30.0
Dry matter (Brix+fibre) t/ha	26.6	45.3
Tops %	17.8	17.3
Biomass (C+T) t/ha	91.4	147.1

Source: Rhao S. (2006)

Production of lignocellulosic alcohol:

Possible to double the ethanol production: increase from 6.000 L/ha to 12.000 L/ha

Potential : 1 ton cana→ 160 L cellulosic ethanol: 19000L/ha

Sugarcane have different cell walls than Dicots

Approximate composition[a] (% dry weight) of typical dicot and grass primary and secondary cell walls

	Primary wall		Secondary wall	
	Grass	Dicot	Grass	Dicot
Cellulose	20–30[b,c]	15–30[c,d,e]	35–45[c,f]	45–50[c]
Hemicelluloses				
Xylans	20–40[d]	5[c]	40–50[c,g]	20–30[c,g]
MLG	10–30[d]	Absent	Minor	Absent
XyG	1–5[c,d,g]	20–25[g]	Minor	Minor
Mannans and glucomannans	Minor	5–10[d]	Minor	3–5[g]
Pectins	5[c]	20–35[d]	0.1[c]	0.1[c]
Structural proteins	1[d]	10[d,e]	Minor	Minor
Phenolics				
Ferulic acid and p-coumaric acid	1–5[c,d]	Minor (except order Caryophyllales)	0.5–1.5[c]	Minor (except order Caryophyllales)
Lignin	Minor	Minor	20[c]	7–10[c]
Silica			5–15[c]	Variable

Source: Vogel (2008)

Our sugarcane cultivars:

50% em α-cellulose,

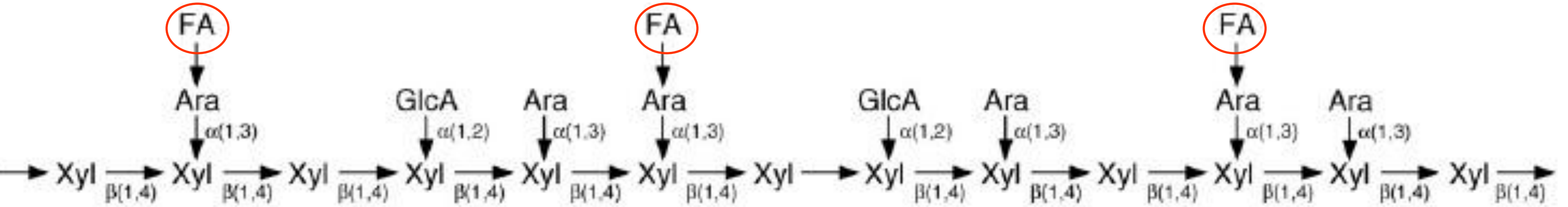
25-30% hemicelluloses

10-25% de lignina

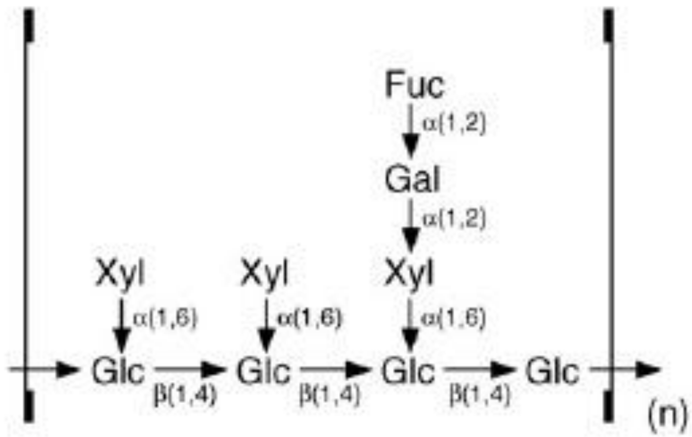
Importance of hemicellulose composition for ethanol production

- Ferulic acid crosslinkage of hemicellulose inhibits saccharification
- We do not know nothing about the dinamic of this process
- We do not know about the structure of this crosslinkages

Hemicellulose structures and functions in grasses



Glucuronoarabionoxylans (GAXs) ➔ major role in crosslinking cellulose microfibrils



β- glucans (MLG) ➔ tightly coat the cellulose microfibrils

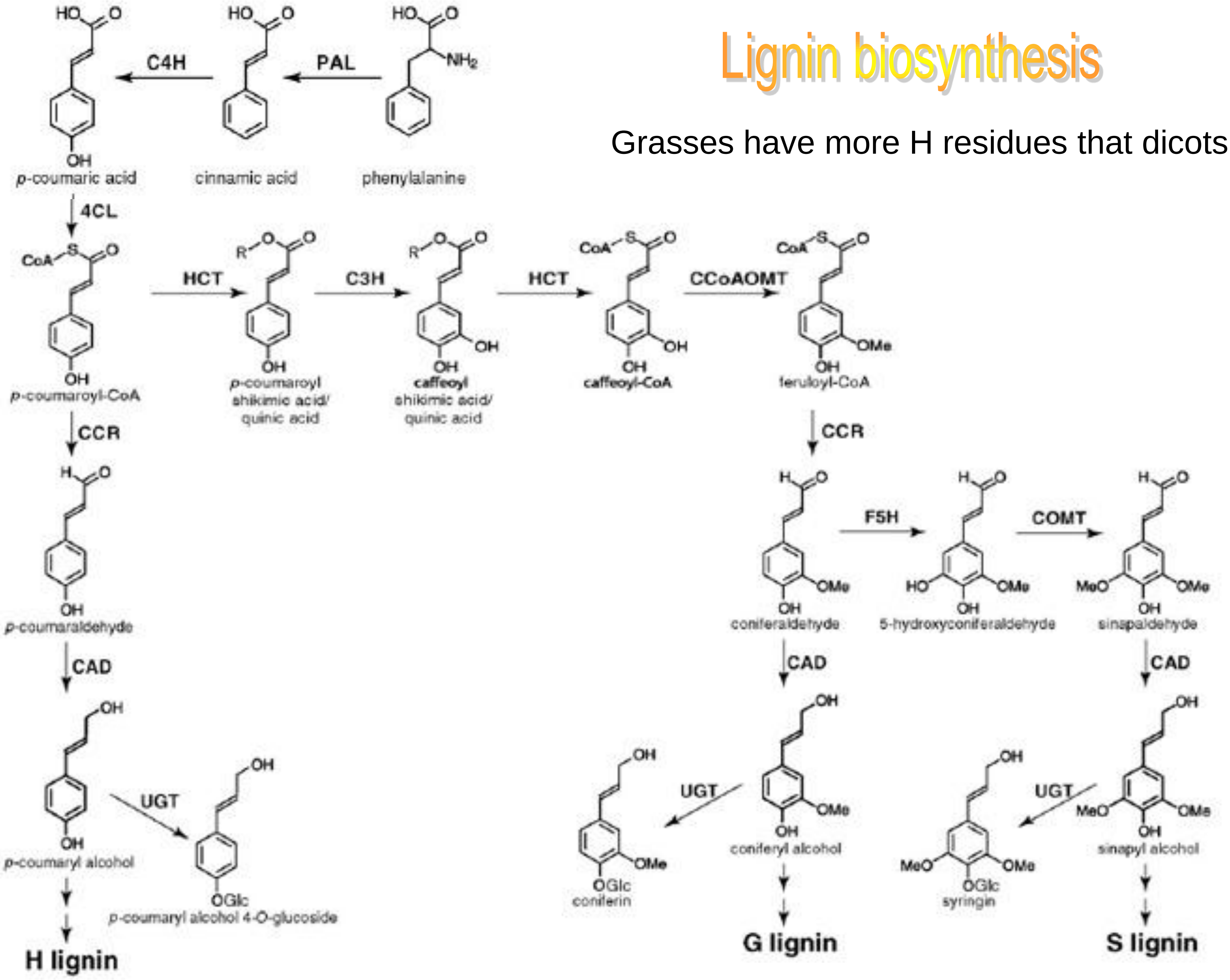
Importance of lignin for ethanol production

- ✓Lignin produces inhibition of saccharification enzymes
- ✓Reduction in cellulose hidrolytic activities due to adsorbtion of enzymes to lignin
- ✓Inhibition of fermentation

- ➢Lignin: key control point in determining the efficiency of biofuels production
- ➢Challenge for sugarcane lignocellulosic ethanol biotechnology:
  - ➢to mitigate the negative effects of lignin in cellulosic ethanol production

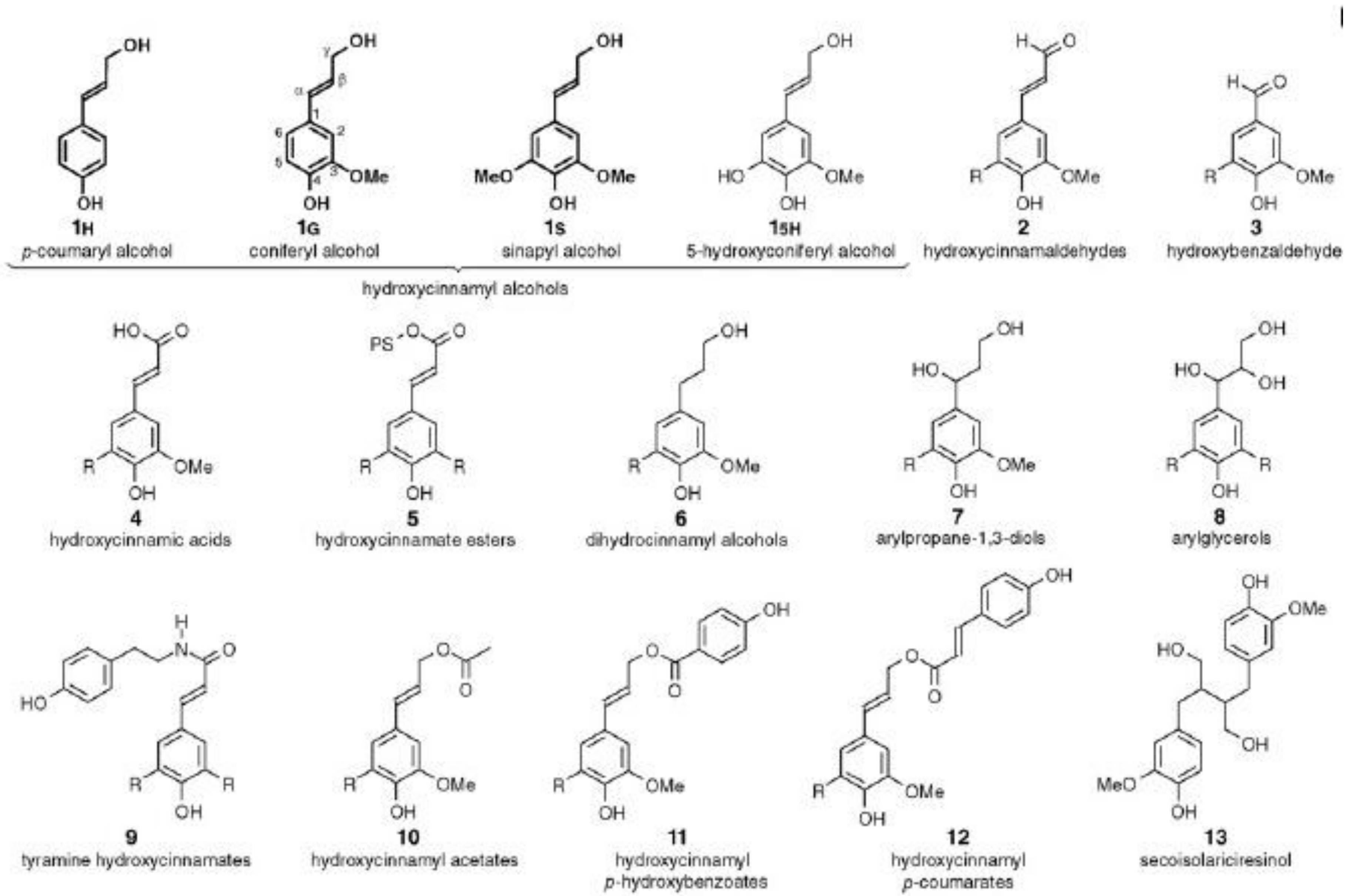
Lignin biosynthesis

Grasses have more H residues that dicots



Lignins are more complex that says the textbook

Not only H, G, S, but …



Lignin can be engineered with large changes in H/G/S rations

Effects on lignin content and H/G/S composition in various mutant and transgenic plants with altered monolignol biosynthesis

wild type

Gene(s)	Total lignin	H	G	S	S/G	References
PAL↓	↓	↓	↓	↓	↓/↑	[3,6,49]
PAL↑	↑	n.a.	↑/No changes	↓/No changes	↓/No changes	[3]
C4H↓	↓	↓	↓	↓	↓	[3,6]
C4H↑	No changes	n.a.	No changes	No changes	No changes	[3]
4CL↓	↓	↑	↓	↓	No changes	[3]
HCT↓	↓	↑	↓	↓	↑	[6,13,15••]
C3H↓	↓	↑	↓	↓	n.a.	[3,14•]
CCoAOMT↓	↓	↑	↓	↓/No changes	↓/No changes/↑	[3,6,10]
CCR↓	↓	↓	↓	↓	↓/↑	[3,7,17•]
F5H↓	↓/No changes	n.a.	↑	↓	↓	[3,6]
F5H↑	↓/No changes	n.a.	↓	↑	↑	[3]
COMT↓	↓/No changes/↑	n.a.	↓/↑	↓	↓	[3,6,10]
COMT↑	No changes	n.a.	No changes	No changes	No changes	[3]
CAD↓	↓/No changes	n.a.	↑/No changes	↓/No changes	↓/No changes	[3,12]
4CL↓ *F5H*↓	↓	n.a.	n.a.	n.a.	↑	[3]
CCoAOMT↓ *COMT*↓	↓/No changes	n.a.	↓/No changes	↓	↓	[3,10]
CCR↓ *COMT*↓	↓	n.a.	n.a.	n.a.	↑	[3]
CCR↓ *CAD*↓	↓	n.a.	↓	↓	↑	[3]
COMT↓ *CCR*↓ *CAD*↓	↓	n.a.	n.a.	n.a.	n.a.	[3]

Vanholme et al (2008)

Frequently other undesirable phenotypes: dwarfing, collapse of vessel elements and increased susceptibility to fungal pathogens, etc

Dixon group (2007): alfalfa: transgenic idenpendently downregulated in 6 lignin genes: Doubling in sachararification efficiency

Possible targets that could mitigate the negative effects of lignin in ethanol production

✓Reducing the amount of lignin

✓Changing the lignin composition (reducing coniferyl and guaiacyl content)

✓Changing the patterns of lignin polymerization:

✓ through manipulation of the activity of monolignol-specific oxidases (peroxidases and laccases)

✓Introducing molignols more eaily degradable (phenolic esters: p-coumarate and p-hydroxybenzoate, hidroxycinnamic acid amides

✓Reduce the acetylation of lignin and transform plants (inducible promoters)

✓To discover new enzymes to degrade lignin (complete genomic sequence of *Phanerochaete chrysosporium* ("white rot fungi") and termites (metagenome sequencing of gut flora of *Nasutitermes*)

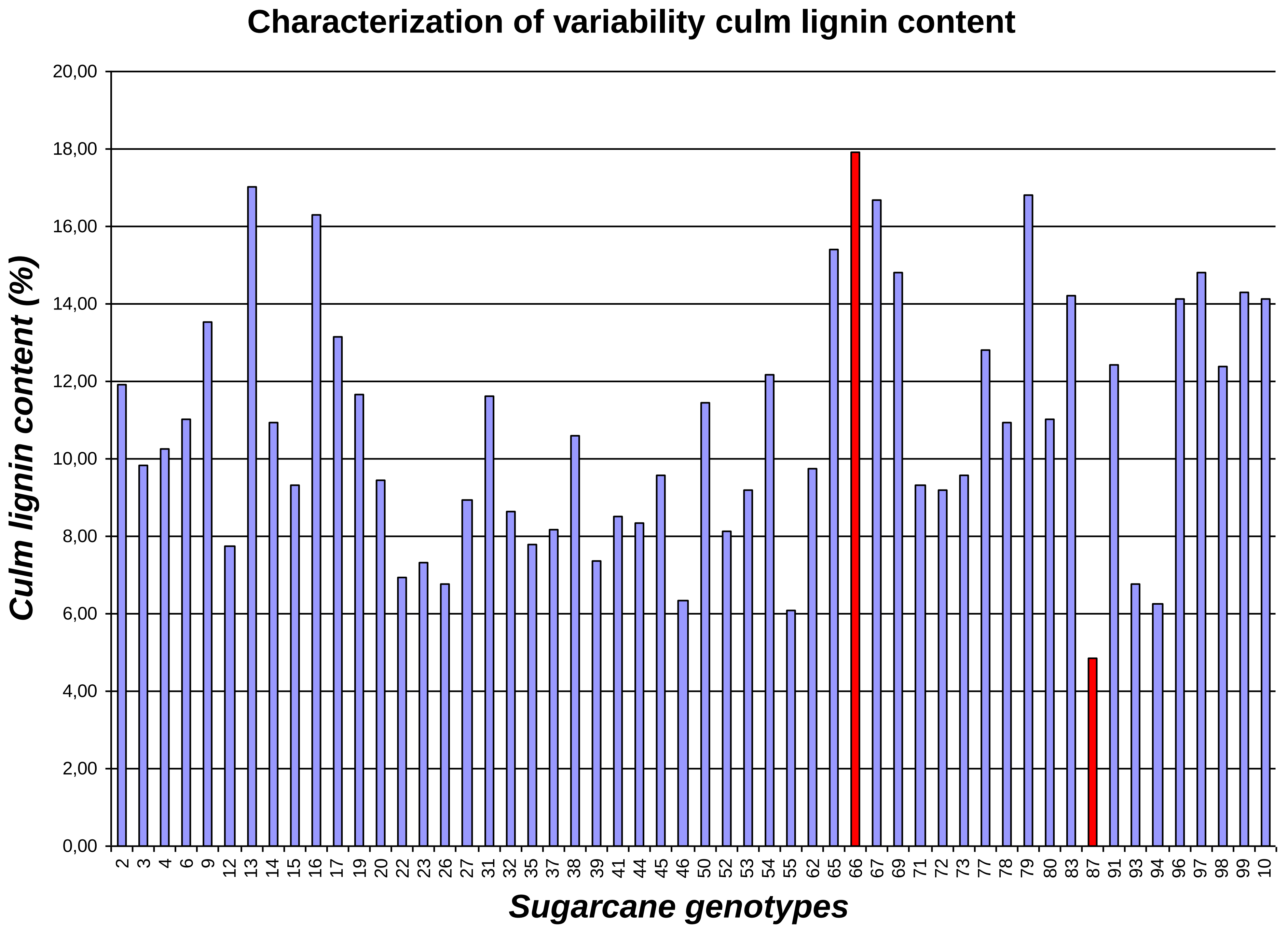
RIDESA: Breeding for energy cane

Considerable genetic potential for biomass is present in the sugarcane germplasm
• Extensive and long term breeding program is needed to produce high biomass varieties with desired characteristics (8-10 years)
• Important to get adequate number of families and seedlings are needed to maintain high probability of selecting right variety

Past selection may have altered genome composition and shifted gene frequencies
Need to ensure right genes for appropriate fiber occur in the breeding population

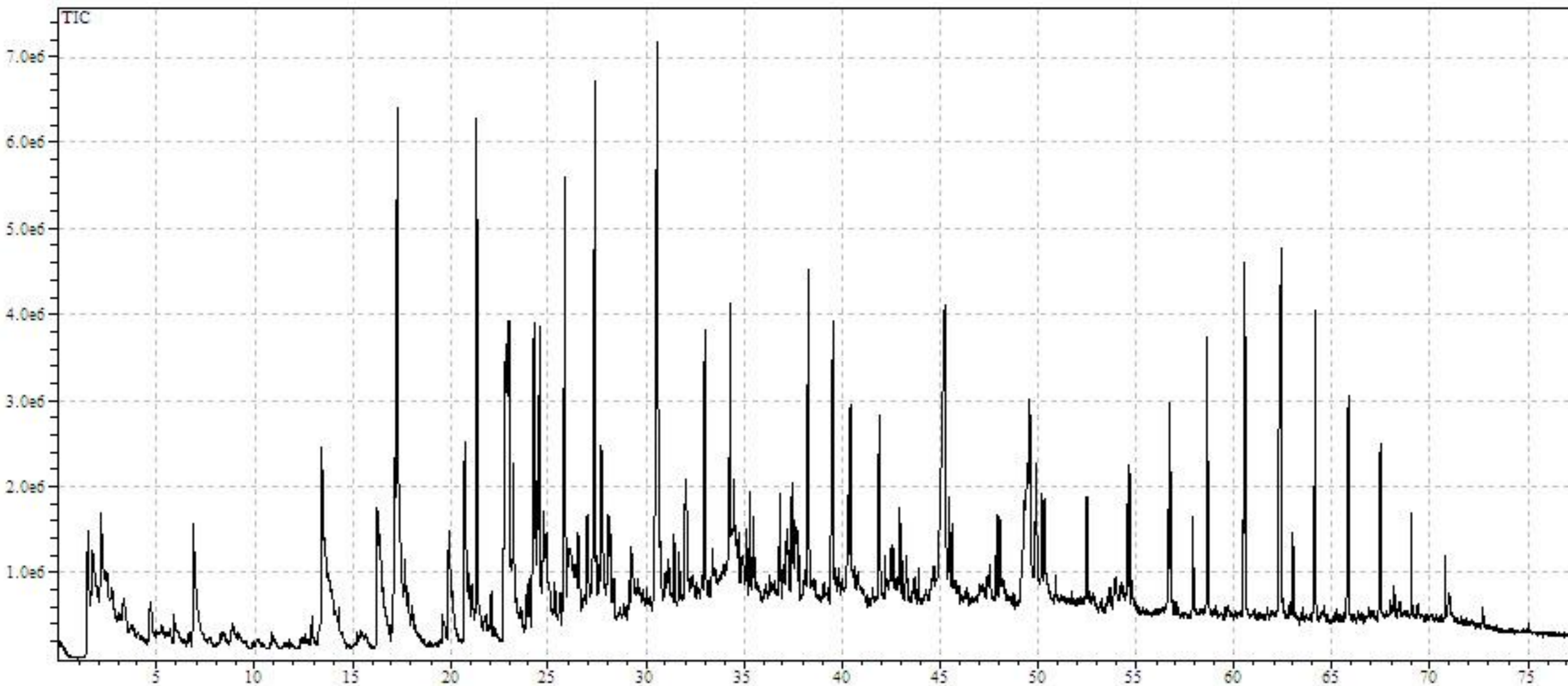
What to do to beginning?

1) Characterize genetic diversity of culm cell wall compositon
2) Develop analytical methods for cell wall characterization for large scale phenotyping
3) Test the the effects of diversity in cell wall composition
4) Select the progenitors for recurrent selection

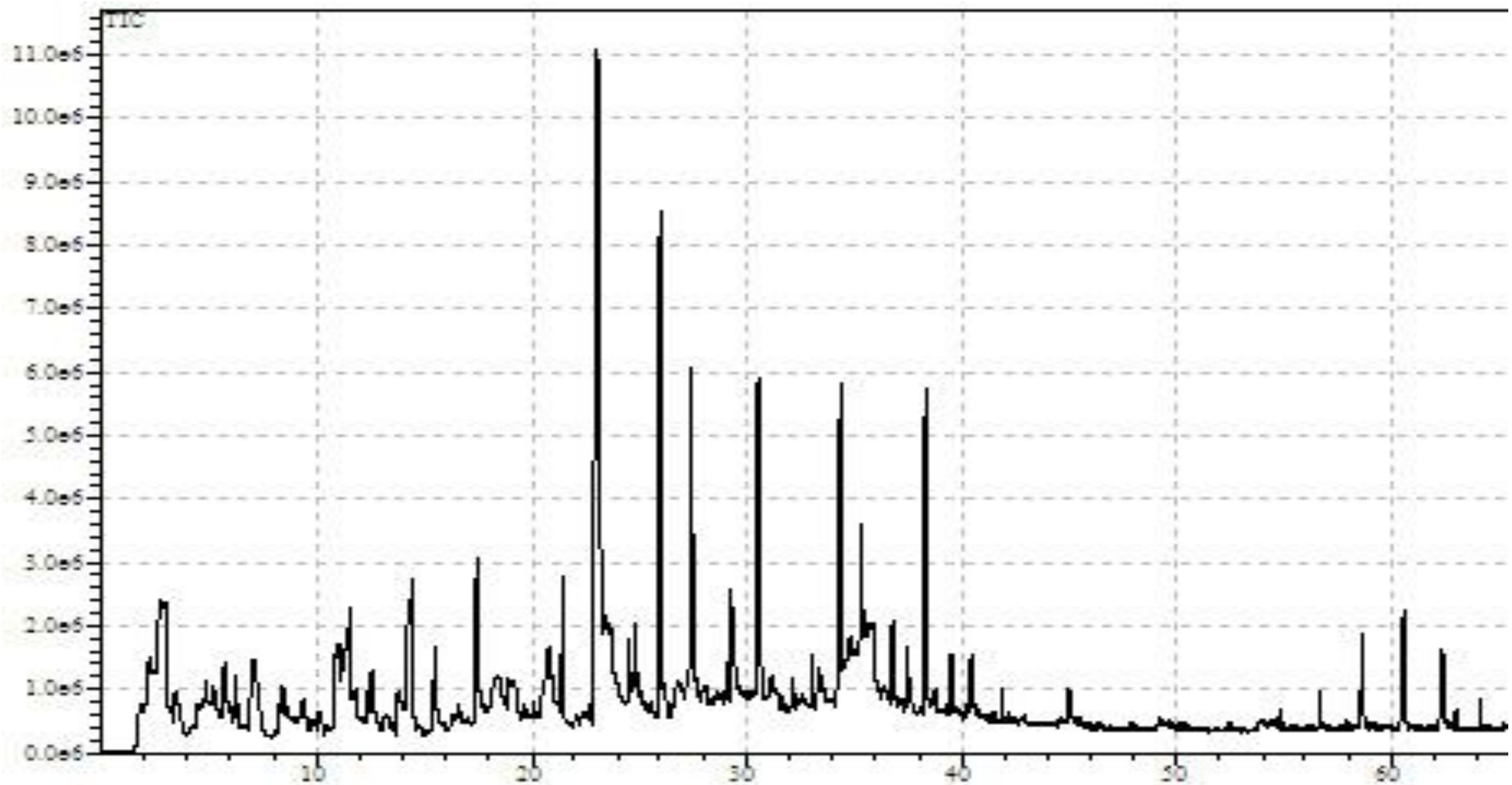

Characterization of variability culm lignin content
Culm lignin content (%)
20,00
18,00
16,00
14,00
12,00
10,00
8,00
6,00
4,00
2,00
0,00
2 3 4 6 9 12 13 14 15 16 17 19 20 22 23 26 27 31 32 35 37 38 39 41 44 45 46 50 52 53 54 55 62 65 66 67 69 71 72 73 77 78 79 80 83 87 91 93 94 96 97 98 99 10
Sugarcane genotypes

Methods for large scale cell wall phenotype characterization

Pyrolysis+GC/MS: purified lignin from sugarcane stalks (TIC)



Pyrolysis+GC/MS: extracted cell walls from sugarcane stalks (TIC)



	A	B	C	D	E	F	G	H	I
3	RT	area	Rel. Area(RA)						
4	6,871	10.335.933	1,234	furfural	C				
5	13,377	19.105.567	2,282	fenol	LH		**Guaiacila**	Area	Abs. Area
6	16,273	12.873.203	1,537	2-metilfenol	LH		Guaiacol	5,727	47.953.161
7	17,151	10.962.318	1,309	p-metilfenol	LH		4-Metilguaiacol	4,659	39.006.239
8	17,302	47.953.161	5,727	guaiacol	LG		4-Vinilguaiacol	0,47	3.938.201
9	19,926	8.984.299	1,073	2.6-dimetilfenol	LH		Vanilina	0,943	7.897.125
10	20,737	15.963.174	1,906	3.5-dimetilfenol	LH			11,799	98.794.726
11	21,369	39.006.239	4,659	4-metilguaiacol	LG				
12	22,796	28.487.109	3,402	1.2-benzenodiol	LH				
13	22,948	30.059.521	3,590	2.3-diidrobenzofurano	C		**Siringila**		
14	23,127	3.878.301	0,463	2.3-diidrobenzofurano	C		4-Etilsiringol	1,451	12.152.408
15	23,227	11.575.426	1,382	m-isopropilfenol	LH		4-Vinilsiringol	2,155	18.044.788
16	24,279	25.433.819	3,038	3-metoxicatecol	LM		Homosiringaldeído	1,019	8.531.164
17	24,527	14.675.723	1,753	4-etilguaiacol	LG		Acetosiringona	2,247	18.817.394
18	24,645	2.872.992	0,343	2-metilbenzeno-1.4-diol			Siringilacetona	1,547	12.954.562
19	24,86	3.938.201	0,470	4-vinilguaiacol	LG			8,419	70.500.316
20	26,973	7.929.216	0,947	Phenol, 3,4-dimethoxy-					
21	27,384	47.268.378	5,645	siringol	LS				
22	27,703	15.631.309	1,867	3.4-dimetoxifenol	LS		**S/G**	0,713535	0,713604044
23	28,372	2.170.437	0,259	tetradecano					
24	29,069	2.197.936	0,263	eugenol	LG				
25	29,252	7.897.125	0,943	vanilina	LG				
26	30,569	49.519.322	5,914	metilsiringol	LS				
27	31,406	3.716.676	0,444	G-CH=C=CH$_2$	LG				
28	31,631	2.784.585	0,333	G-CH=C=CH$_2$	LG				
29	32,009	11.039.243	1,318	acetoguaiacona	LG				
30	32,983	12.152.408	1,451	4-etilsiringol	LS				
31	33,344	1.452.582	0,173	guaiacilcetona	LG				
32	34,252	18.044.788	2,155	4-vinilsiringol	LS				
33	34,508	11.167.448	1,334	hexadeceno					
34	34,709	2.798.232	0,334	hexadecano					
35	35,327	4.031.210	0,481	4-alilsiringol	LS				
36	35,492	3.322.868	0,397	4-propilsiringol	LS				
37	36,787	4.453.314	0,532	cis-4-propenilsiringol	LS				
38	37,442	8.531.164	1,019	siringaldeido	LS				
39	38,278	18.233.009	2,178	trans-4-propenilsiringol	LS				
40	39,488	18.817.394	2,247	acetosiringona	LS				
41	40,459	12.954.562	1,547	siringilacetona	LS				
42	41,879	10.699.088	1,278	propiosirigona	LS				
43	42,578	2.493.674	0,298	ftalato					
44	43,07	2.932.034	0,350	hidrocarboneto					
45	45,232	41.474.762	4,953	ácido hexadecanóico					
46	45,457	3.957.666	0,473	hidrocarboneto					
47	45,593	2.365.471	0,283	hidrocarboneto					
48	47,885	3.580.374	0,428	nonadeceno					

Plan1 | **Lignina** | com extrativo | sem extrativo

	RT	area	Rel. Area		
2	2,808	39.640.462	5,704	ácido acético	C
3	5,744	8.704.882	1,253	butanodial	C
4	6,052	1.516.268	0,218	3-furaldeido	C
5	11,033	26.710.080	3,844	2(5H)-furanona	C
6	11,545	39.706.155	5,714	5-metil-2(3H)-furanona	C
7	17,408	26.341.133	3,791	guaiacol	LG
8	21,422	12.898.507	1,856	4-metilguaiacol	LG
9	23,075	182.190.293	26,218	2.3-diidrobenzofurano	C
10	24,59	12.255.880	1,764	4-etilguaiacol	LG
11	24,816	23.244.766	3,345	3-metoxicatecol	LM
12	26,031	62.116.320	8,939	4-vinilguaiacol	LG
13	27,511	53.085.180	7,639	siringol	LS
14	29,119	3.135.916	0,451	eugenol	LG
15	29,35	15.027.989	2,163	vanilina	LG
16	30,553	33.300.939	4,792	4-metilsiringol	LS
17	31,213	5.976.019	0,860	homovanilina	LG
18	32,129	2.856.412	0,411	4-hidroxivinilguaiacol	LG
19	33,022	2.900.640	0,417	4-etilsiringol	LS
20	33,44	4.726.653	0,680	guaiacilcetona	LG
21	34,347	29.211.258	4,204	4-vinilsiringol	LG
22	35,373	16.906.111	2,433	4-alilsiringol	LS
23	36,826	5.580.072	0,803	cis-4-propenilsiringol	LS
24	37,484	8.563.836	1,232	siringaldeido	LS
25	38,338	30.547.044	4,396	trans-4-propenilsiringol	LS
26	39,508	6.595.086	0,949	acetosiringona	LS
27	40,494	6.724.280	0,968	siringilacetona	LS
28	40,874	782.076	0,113	propiosirigona	LS
29	45,014	3.249.892	0,468	ácido hexadecanóico	
30	56,7	2.962.999	0,426	hidrocarboneto	
31	58,671	6.591.559	0,949	hidrocarboneto	
32	60,568	8.632.186	1,242	hidrocarboneto	
33	62,335	9.288.074	1,337	hidrocarboneto	
34	63,047	1.094.386	0,157	hidrocarboneto	
35	64,145	1.839.321	0,265	hidrocarboneto	
36		694.902.674	100,000		

Guaiacila	AR
Guaiacol	3,791
4-Metilguaiacol	1,856
4-Vinilguaiacol	8,939
Vanilina	2,163
	16,749
Siringila	
4-Etilsiringol	4,792
4-Vinilsiringol	4,204
Homosiringaldeido	1,232
Acetosiringona	0,968
Siringilacetona	0,968
	12,164
S/G	0,73

Similar values relative to the results from purified lignin

Plan1 | Lignina | **com extrativo** | sem extrativo

Analytical time used:

1) Method with lignin purification

Purify lignin: 4 days

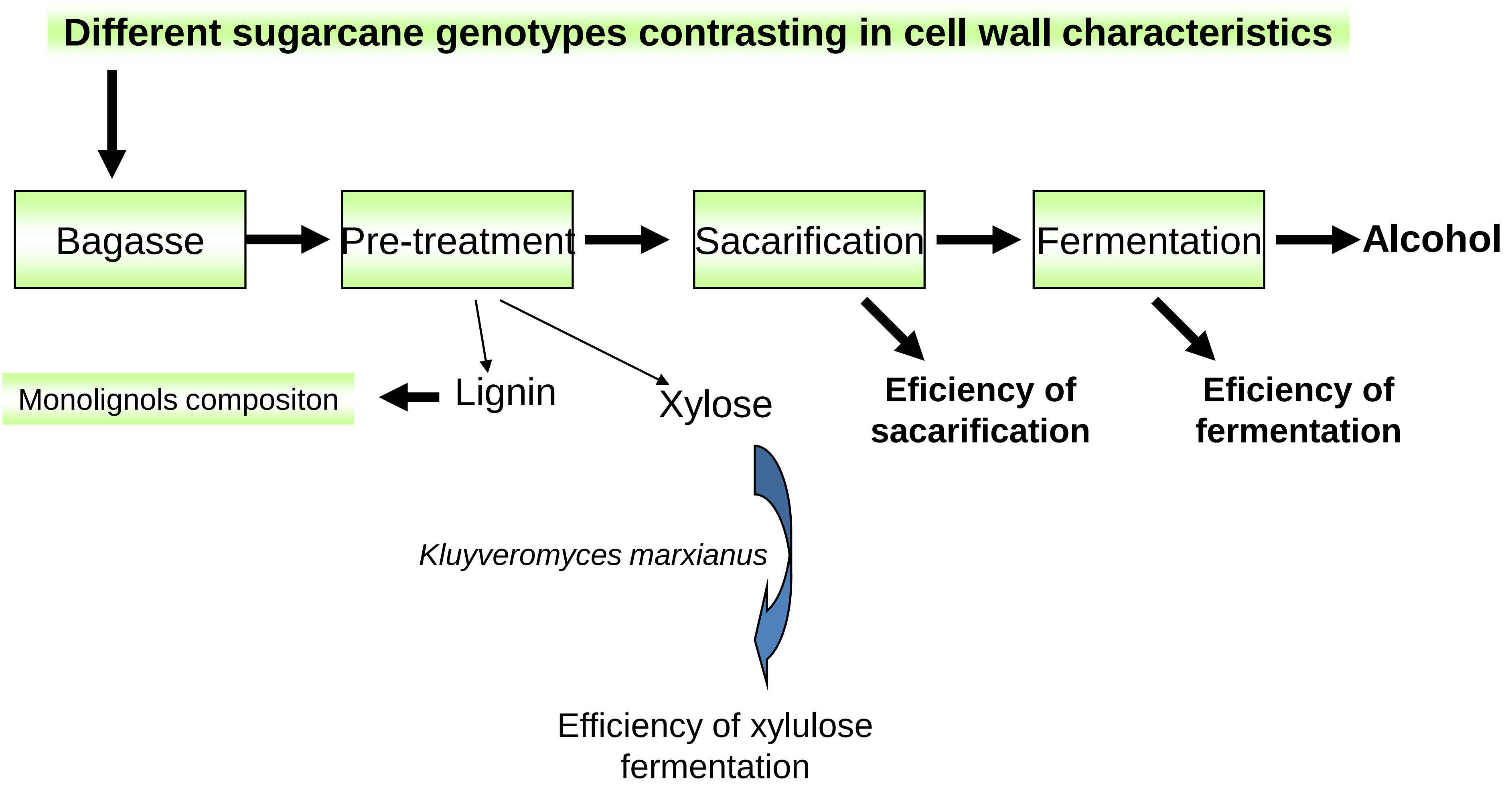
Pyrolysis + GC/MS = 60 min

2) Method without lignin purification:

Extraction: 1 hour

Pyrolysis + GC/MS = 60 min

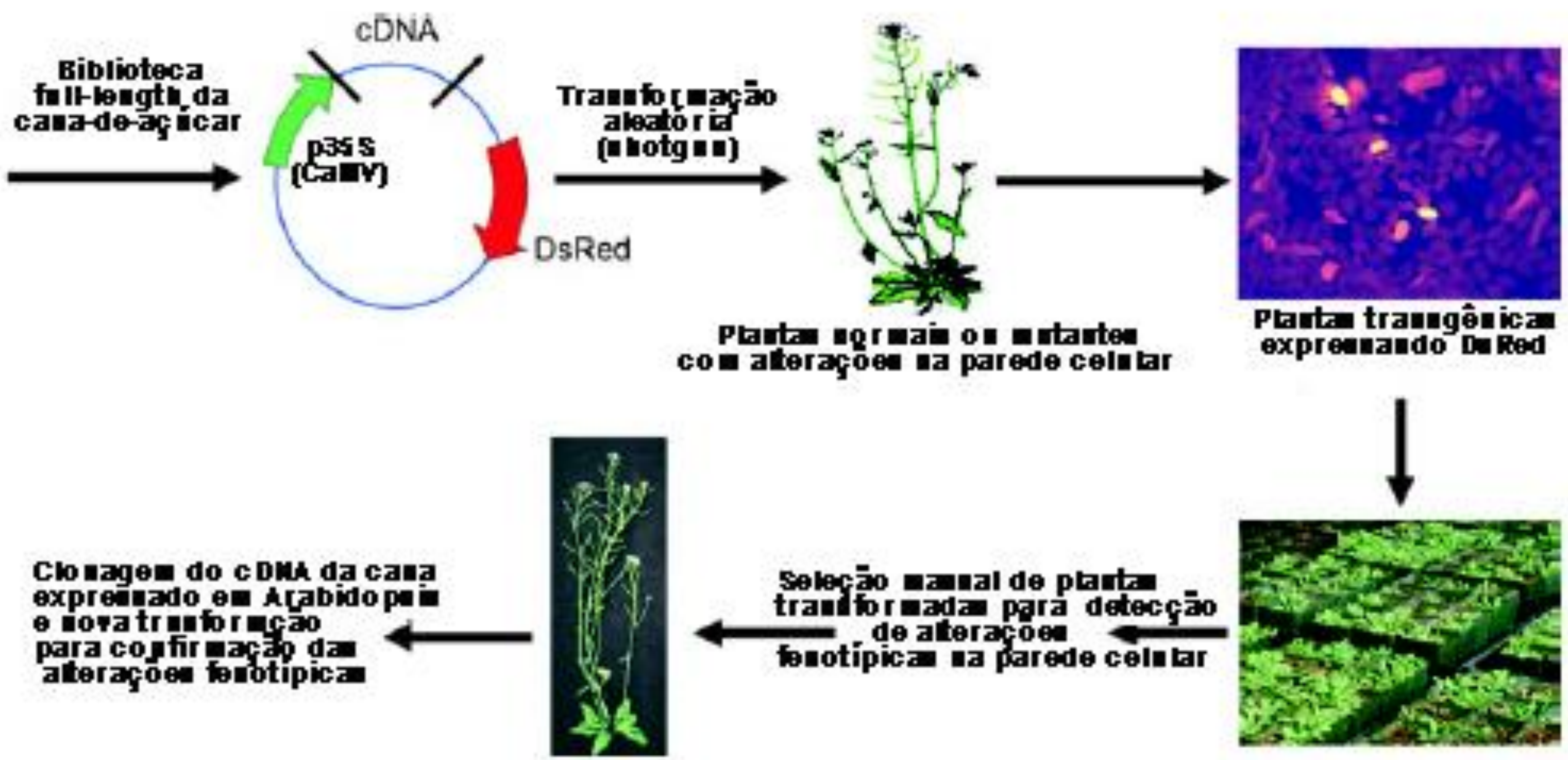
“Mini” UFV Bioen



Gene discovery and functional genomics: urgently needed in sugarcane

10% of plant genome ➔ genes to construction and rearrangement of their cell walls
Arabidopsis ➔ ~ 2500 genes
Sugarcane ➔ 4300 genes?
Buckeridge and coworkers: 469 cell wall genes identified in sugarcane (~10%)
Gene discovery in sugarcane is needed to further identify other cell wall genes

Simultaneous gene discovery and functional characterization strategy



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