Breeding for biomass sorghum in Europe: a global review on target traits and plant ideotypes depending on cropping conditions and transformation process.

G. Trouche, D. Pot, D. Luquet, S. Braconnier
UMR AGAP/CIRAD

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Economic and Political Context for crop biomass production in Europe

✓ International demand in energy will increase of 50 % before 2030

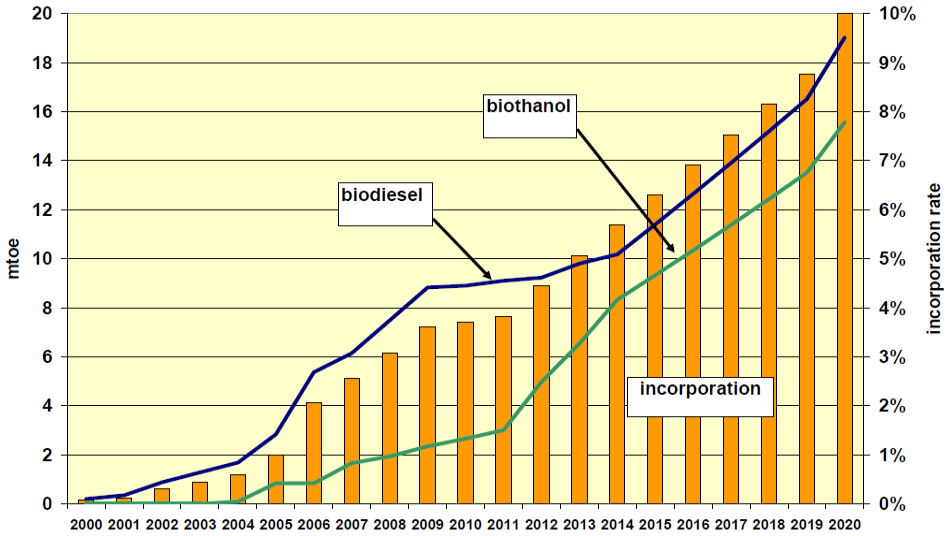
✓ Fossil resources are limited (40-60 years for oil, 70 years of natural gaz, 200 years of charcoal

✓ Global warming

Targets 2020 for Europe :

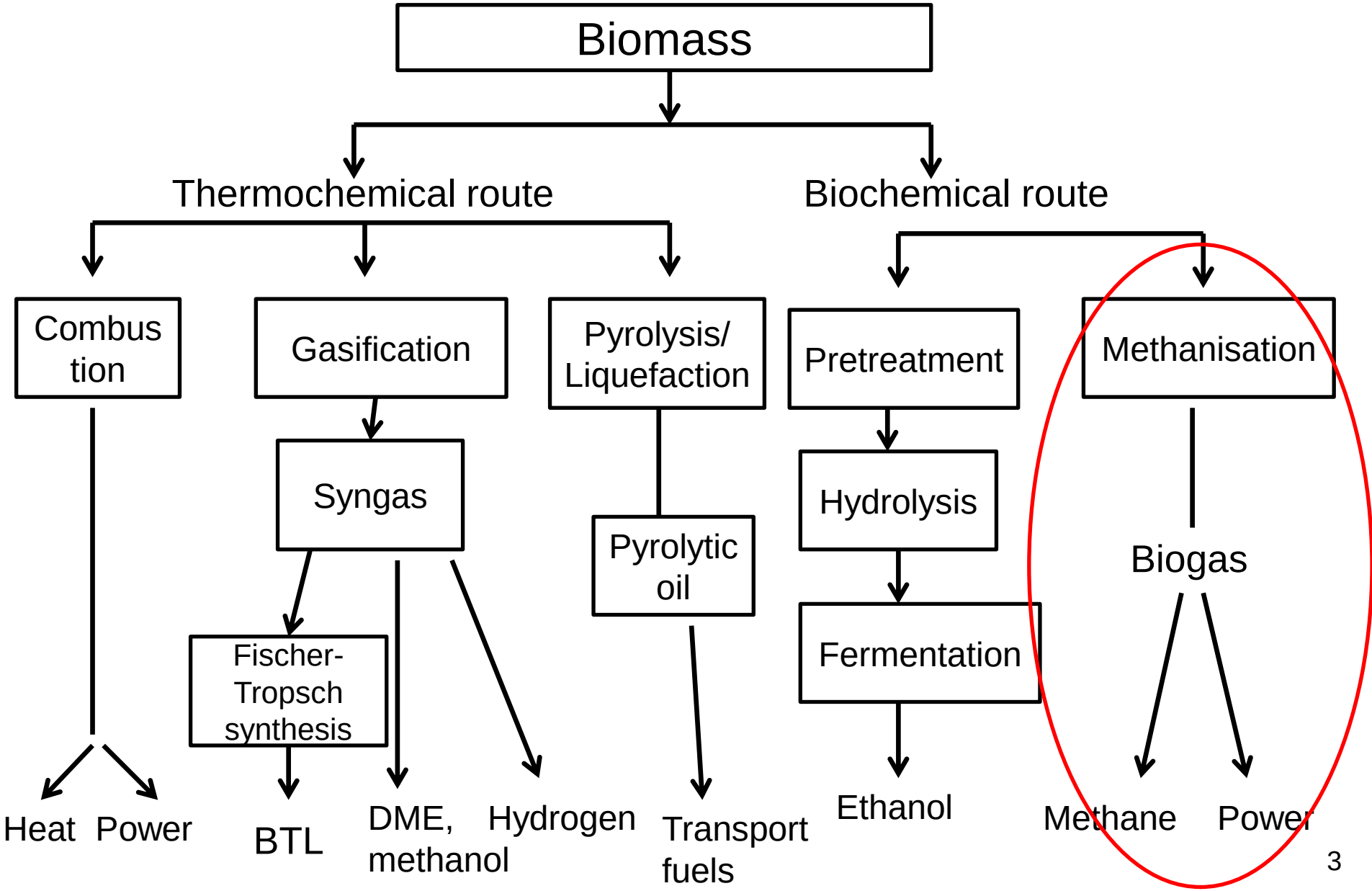
✓20 % of energy and 10% of transport fuels from renewable origin

⇒ Future large areas for crops dedicated to energy production (e.g. France: to 1.2 millions ha)



Sustainable fuel production has to be based on lignocellulose products and by-products

Routes for transformation of lignocellulosic biomass to biofuels and/or energy



Advantages/disavantages of high lignin content depending of transformation process

Route	Transformation process	Value	Sources
Biochemical	Enzymatic hydrolysis and fermentation for ethanol production	- -	Several
	Methanization	- -	Several
Thermochemical	Fast pyrolysis	+	Brown, 2010
	Gazification	+	Folkedahl, 2010
	Liquefaction	?	
	Combustion/cogeneration	++	Several

Methanization: a local route for biomass transformation

- A **local route** through a silage stage **giving flexibility** to choose for dairy cows feeding or heat/electricity production at farm level

- About 0.6 millions ha of biomass crops (maize, sunflower, sorghum…) planted in Germany for this purpose, just starting in France and some other EU countries…

- Need for high biomass productivity/ha with >28-30% of dm and high fibre digestibility (according to private seed sector)

Biomass valorization as biomaterials

✓ Biomaterials for building: blocks, *light weight concrete, …*

➢ Biopolymers : bioplastic composites for car industry and other novel applications…

➢Based on previous studies on Miscanthus need for high cellulose content and long fibres

➢To be better assessed in a new project Biomass crops for the future

Cropping systems for biomass sorghum

Debates still exist about:

- Dedicated summer crop (early spring planting) versus double-cropping systems (late planting of sorghum in june after a winter crop)?
- With or without irrigation?
- Marginal or better soils (eg South of France)?

Biomass sorghum (as summer dedicated crop)

= a sorghum producing high ligno-cellulosic biomass with a biochemical composition required by the chain value

- 20-40 t/ha dry matter result of a cycle duration of 4 to 5 months and plant height >3.5 m
- Early growth vigor and tolerance to cool temperatures for exending the cycle duration
- Lodging resistance
- Dry matter content at harvest time > 28-30%
- High WUE and NUE
- No objective for grain production

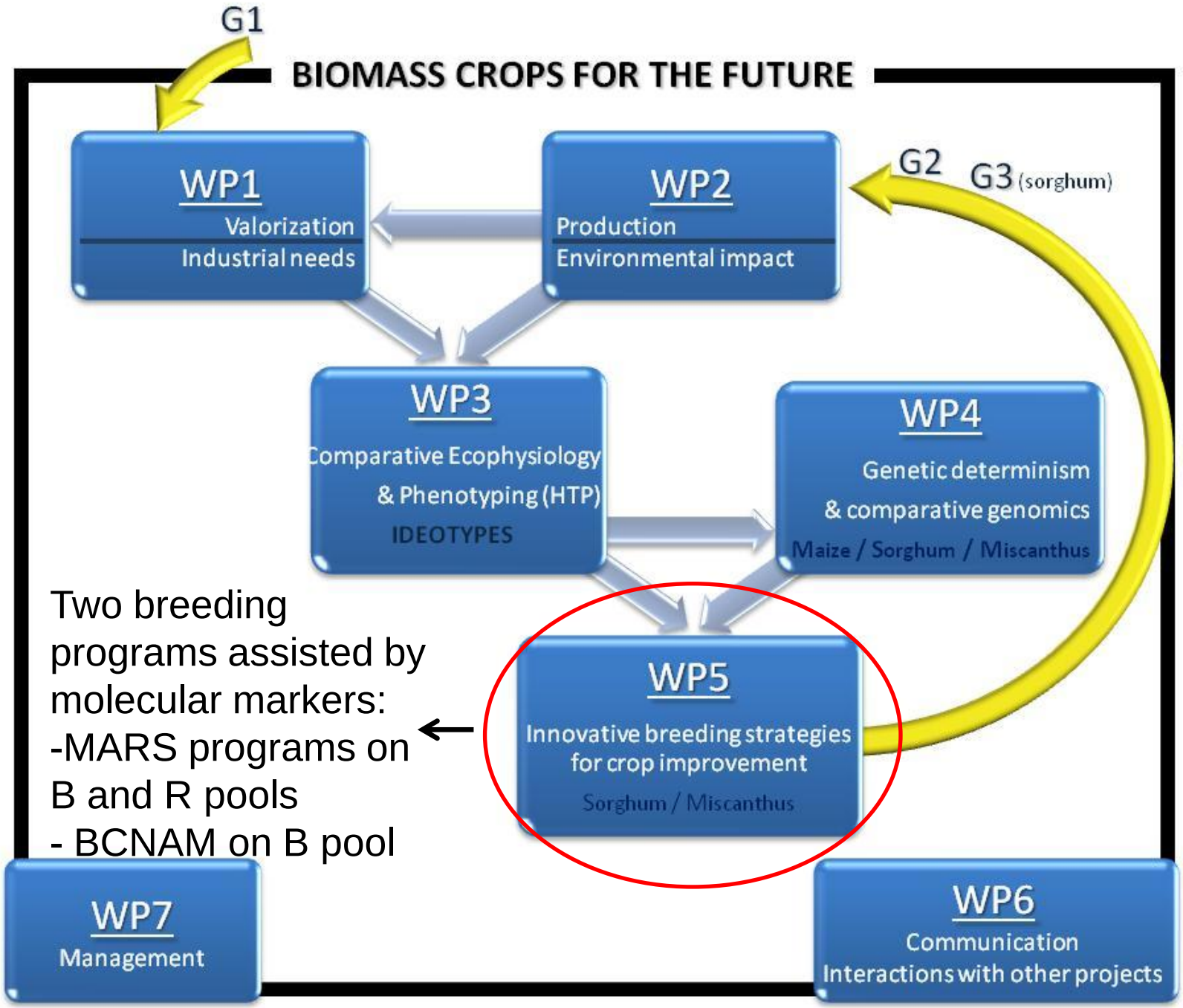
Lignin and cellulose content /fibre digestibility in phase with the requirements of transformation process

Research projects on biomass sorghum in Cirad

- ANR GrassBiofuel 2008-2011 : characterization of the sorghum variability for cell wall composition (France + Mali)

- FP7 UE Sweetfuel WP1 2009-2013: breeding for biomass sorghum for temperate areas (Europe)

- Biomass crops For the Future (BFF) 2013-2020: integrated breeding project with industrial users for multi-purpose biomass sorghums in France



Two breeding programs assisted by molecular markers:
-MARS programs on B and R pools
- BCNAM on B pool

Sorghum breeding for biomass: Components of biomass accumulation

Dry biomass yield is a combination of :

- stems per m²
- internodes per stem
- internode length
- internode diameter
- water content at harvest
- specific weight

plants/m² ← Planting density

tillering

duration of vegetative phase & phyllochron

Dwarf genes

?

Genetic control & varietal aptitudes

Breeding for biomass sorghum: optimizing the biomass production by

- Increasing the number of stem internodes
- Increasing length and diameter of stem internodes
- Increasing tillering ?

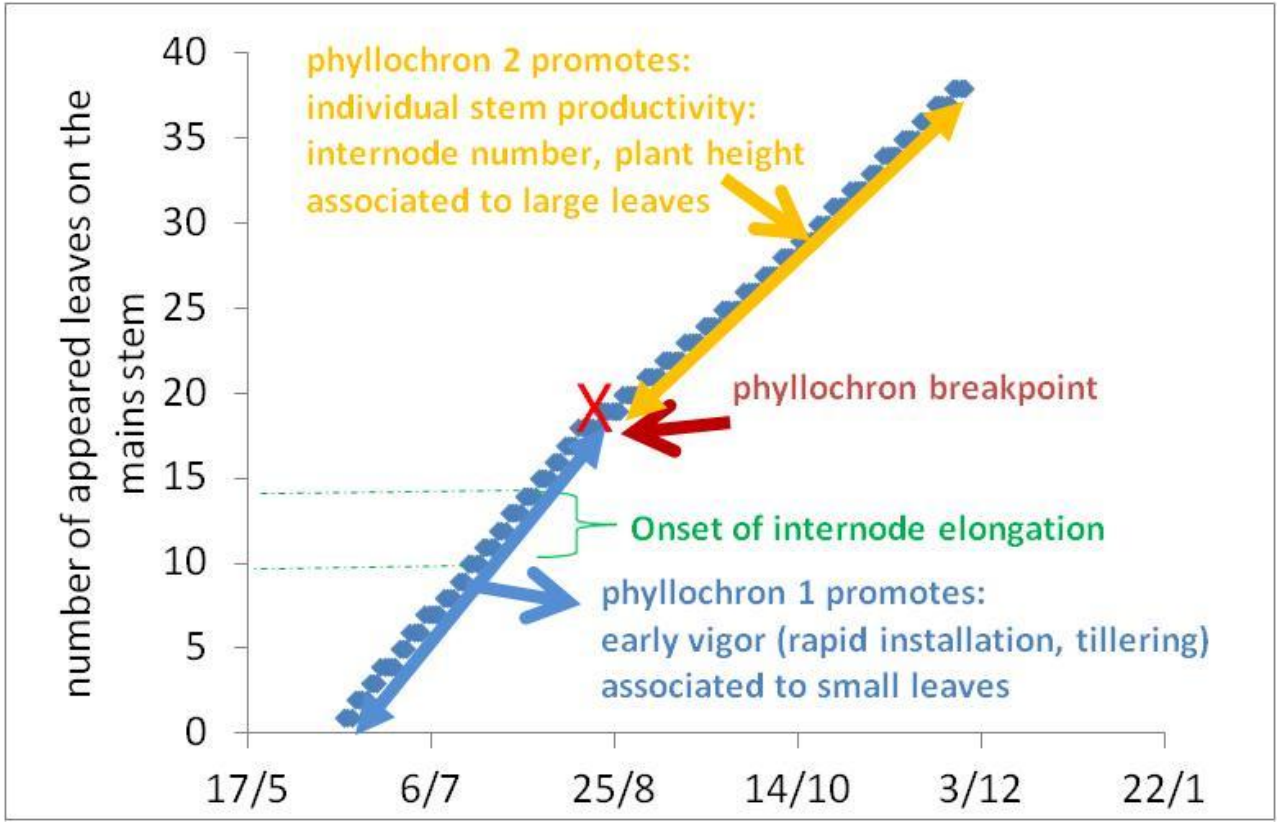
$\Rightarrow$ Hybrids cultivars: need to optimize heterosis by the identification of heterotic pools

Increasing the number of internodes by increasing the duration of vegetative phase

- Increase BVP duration for photoperiod-insensitive sorghums (low variability)
- Use photoperiod-sensitive sorghums: broadening the working genetic base for sorghum improvementin temperate areas
- Improve tolerance to cold temperatures at seedling stage for allowing early plantings (variability exists!)

Exploring the variability of phyllochron and its effects on biomass yield

Preliminary study on 11 long-cycle photoperiodic sorghums in South of France 2010



- Phyllo 1 promotes **whole plant productivity** and phyllo 2 **main stem productivity** but they are not negatively linked (r^2 0.13)

  => **"Trade off"** to find between both phyllochrons?

- To be more explored: impact of **phase 1** & **phase 2 duration with broader diversity**

  => timing of breakpoint & internode elongation onset?

Increase the size of internodes: length and diameter

- Internode length controled by 4 major dwarf genes increasing length when dominant: DW1, DW2, DW3, DW4

- Stem diameter: huge phenotypic variability to better explore and use
  ex sorghum of durra race: thick stems with anchoring roots

Increasing tillering ?

- A limited potential in cultivated sorghums with some genetic differences
- Start at 4-5 leaves on the main stem, stop when LAI becomes constant (about 50 dae), many tillers die during the growth
- A trait with some disavantages in intensive agriculture thus eliminated in the grain sorghum breeding programs

Towards the definition of plant idiotype for biomass production in South of France– Evaluation of various plant architecture and phenology

Accession	date to flag leaf	number of tillers at mid-cycle	number of tillers at harvest	plant height (cm)	DM (%)	Average dry weight of one plant (g))	ADL/NDF	INNDFD
IS 15148	16-août	0.07	0.07	247	**34.3**	196	7.8	20.0
IS 26833	30-août	0.64	0.33	340	**32.4**	289	9.0	15.8
IS 4285	03-sept	0.27	0.27	290	25.0	236	7.4	22.0
IS 32569	10-sept	1.47	**1.40**	308	**29.6**	**368**	7.7	20.6
IS 23777	15-sept	3.21	**2.80**	238	20.3	**310**	6.7	23.6
IS 31559	not reached	1.13	0.33	207	16.6	241	**5.8**	**28.9**
IS 25596	not reached	0.00	0.00	261	15.7	155	6.9	26.7
IS 2156	not reached	3.13	**2.36**	221	19.8	226	7.0	21.5
IS 16396	not reached	2.57	1.57	213	17.7	287	7.1	22.7
IS 11026	not reached	2.14	0.76	247	19.7	**384**	8.0	19.4
BIOMASS 140 (CH)	24-août	1.27	0.40	317	30.3	**365**	8.2	18.8
total mean		1.45	0.94	263	23.8	278	7.4	21.8
Genotype effect		***	***	***	***	**	***	***
flowering		1.13	0.97	285	**28.3**	**280**	7.7	20.4
not flowering		1.79	1.01	230	17.9	258	**7.0**	**23.8**

- Higher biomass yield for **late but flowering** sorghums (final august/first days of september)
- Some **moderate tillering** seems to be profitable for yield
- Higher fiber digestiblity for no flowering sorghum

Biomass quality concerns

- Cultivars with high lignin content are useful for thermochemical routes but not for biochemical (enzymatic) routes

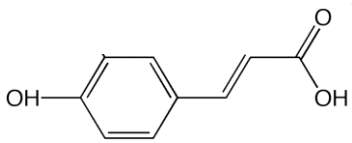
- For methanization and animal feeding, need to develop biomass cultivar with high degradability of cell wall:
  - low lignin content: use of bmr genes
  - understand better the effect of the different lignin (S and G) and p-coumaric and ferulic acids, and the influence of the localization of lignins in the tissues on cell wall degradability

Lodging concern: breeding for very low lignin content seems to increase lodging problems in certain conditions

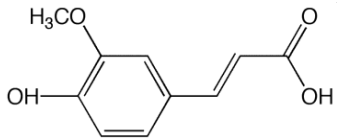
The Grass Cell Wall: Complex Hetero-Polymers

Phenolics

p-Hydroxycinnamic acids

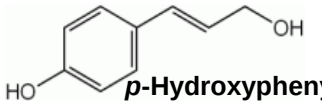


p-Coumaric acid

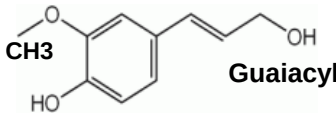


Ferulic acid

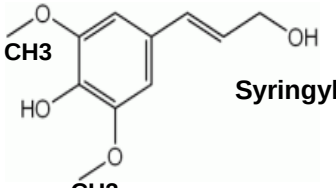
Monolignols



p-Hydroxyphenyl (H) 4 %



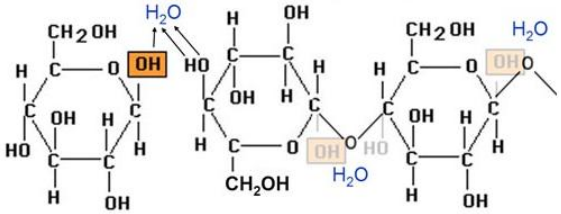
Guaiacyl (G) 45 %

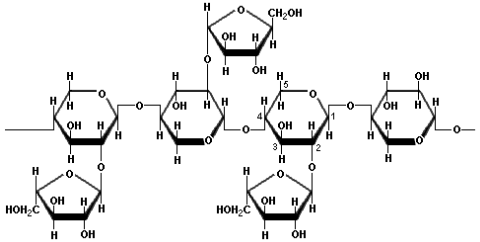


Syringyl (S) 61 %

Carbohydrates

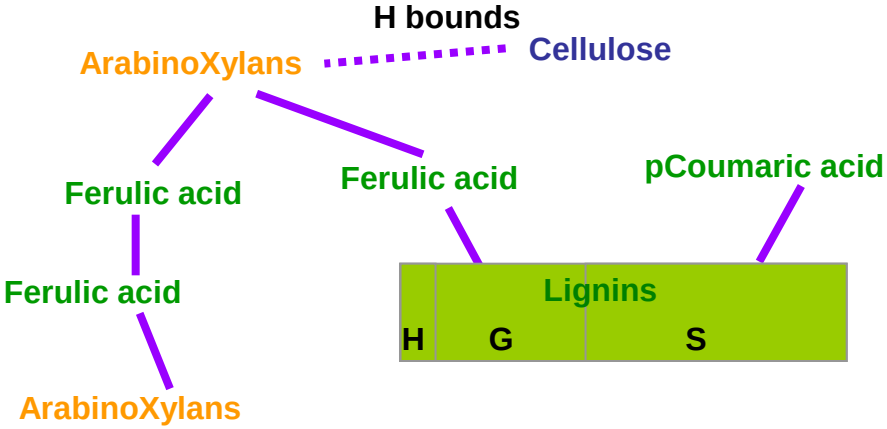
Cellulose

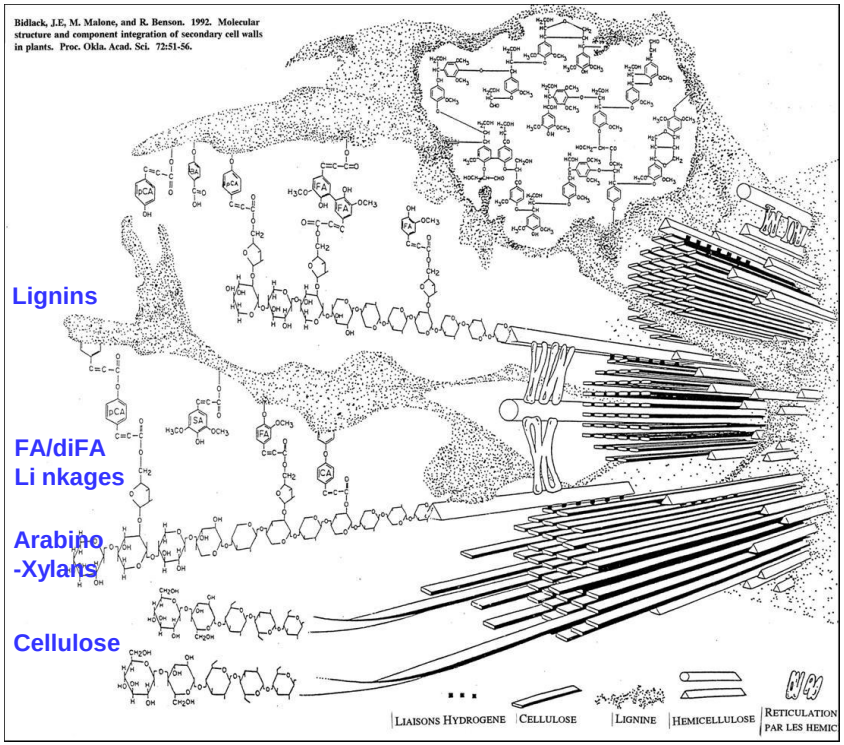




ArabinoXylans

With Complex Cross-linkages

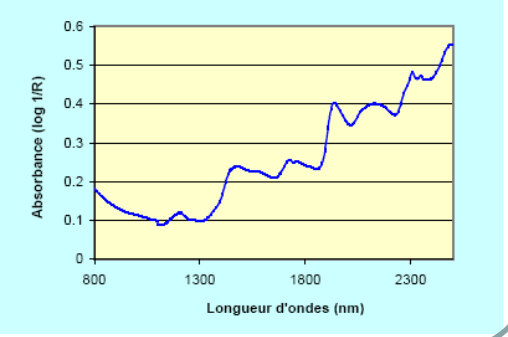




A variability in stem composition to be better characterized

Evaluation of 100 accessions from the Cirad Core Collection flowering in South of France

NIRS calibration for degradability related traits



Stem composition parameters	Range
NDF (% dm)	35-82
ADF (% dm)	19-51
ADL (% dm)	1.2-8.7
ADL/NDF ratio	3-11

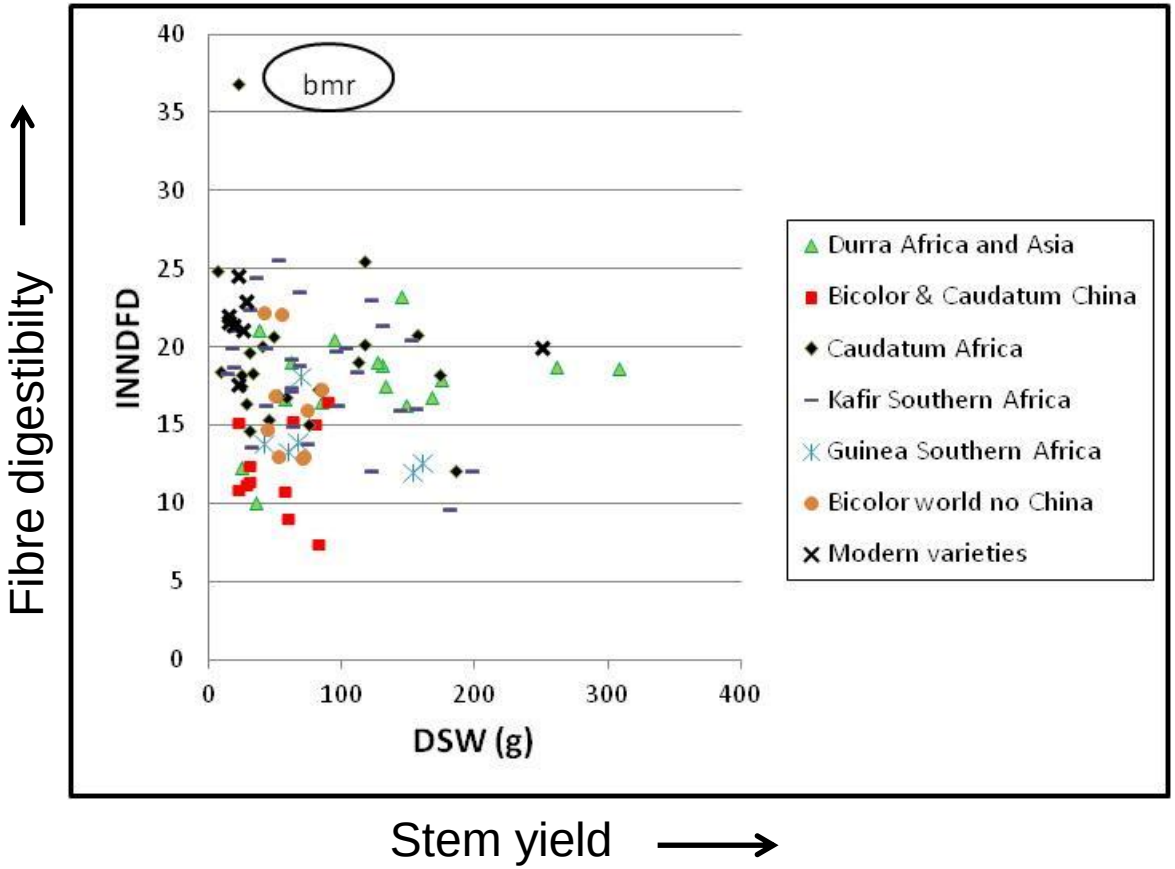
A racial structure for variability in stem composition?

Genetic Cluster*	Number	DM	NDF	ADL	ADL/NDF	CELNDF	INNDFD
Durra Africa & Asia	16	33.35	58.32	4.64	7.91	51.24	17.73
Caud et Bicolor China	11	34.06	**73.86!**	**6.81!**	**9.19**	51.02	12.32
Caud Africa no photop	22	26.79	63.09	4.75	7.53	51.03	**19.28**
Kafir Southern Africa	29	28.20	61.44	4.95	8.04	51.50	**18.36**
Guinea Southern Africa	6	39.65	61.69	5.37	8.69	51.19	14.01
Bicolor world (except China)	9	29.50	63.62	5.20	8.17	51.36	16.51
Cluster effect		***	**	***	***	ns	***

* Genetic cluster according to Deu et al. 2006
! significant different with other cluster at $p < 0.05$ (lsd test)

- Significant effect of genetic cluster for all parameters exept cellulose content
- Chinese sorghums sources of high total fiber (NDF) and high lignin
- No photoperiodic Caudatum from Africa and Kafir source of high stem digestibility

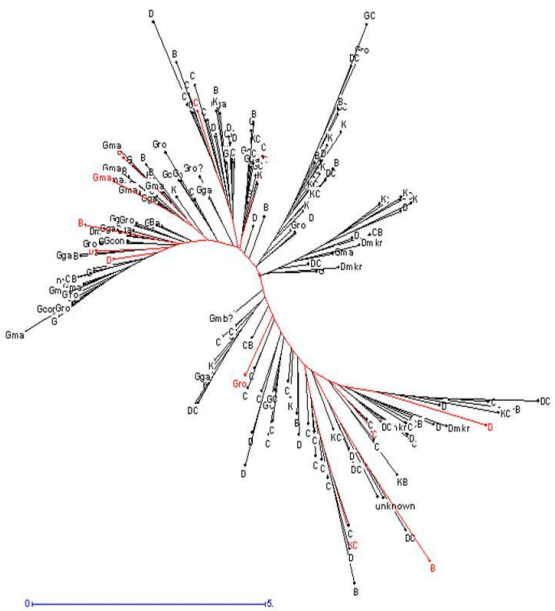
No genetic limitations to improve both yield and digestibility



- It seems possible to develop high yielding biomass sorghums with high fibre digestibility

Fine cell wall characterization

In-depth biochemical analysis of the cell wall composition of 13 genotypes representative of the sorghum biochemical stem variability (NIRS profile)



Selection of the 13 accessions among 384 to represent the sorghum stem biochemical variability

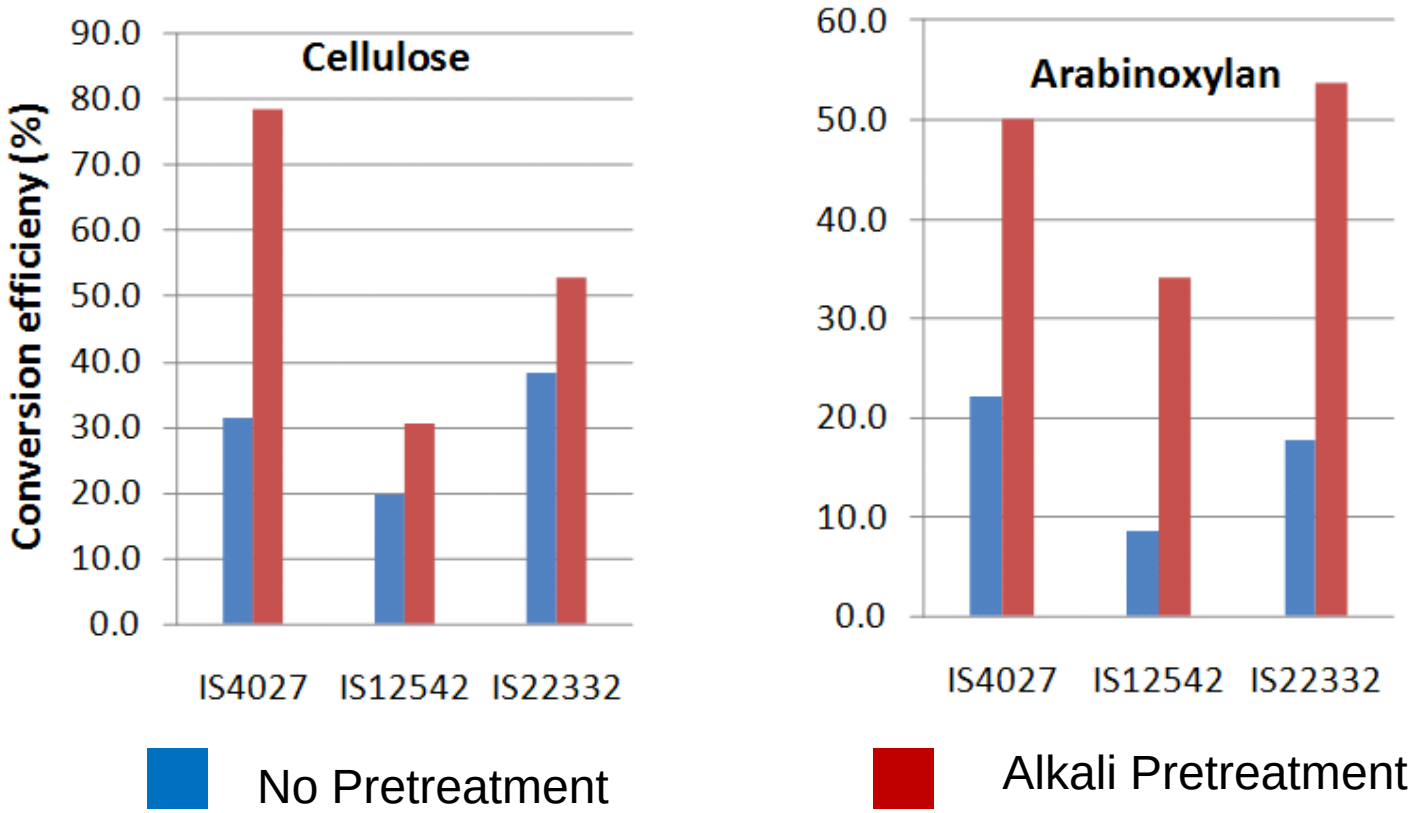
Variability of cell wall components for a sub-panel representative of the worldwide sorghum diversity

Trait	mean	Cv %
Cell wall residue (% DM)	70.32	14.83
Lignin Klason content (% CWR)	16.32	9.05
G µmol/g LK	407.00	10.57
S µmol/g LK	361.01	19.29
Lignin S/G	0.89	14.71
Esterified p-coumaric acid (mg/g CWR)	16.13	12.18
Esterified ferulic acid (mg/g CWR)	4.75	8.89
Etherified ferulic acid (mg/g CWR)	4.06	9.60
Total Sugars (% CWR)	75.56	4.35
Arabinose (% CWR)	4.58	21.21
Galactose (% CWR)	1.09	40.43
Glucose (% CWR)	41.18	14.81
Xylose (% CWR)	28.72	10.74

- A significant variability for all the cell wall components was detected

Source: D. Pot et al., 2011. ANR Grassbiofuel project

Effects of Alkaline Pretreatment



- **Alkaline pretreatments improved significantly the conversion yields** of cellulose and arabinoxylan
- but **they did not eliminate the genetic variability**

Source: D. Pot et al., 2011. ANR Grassbiofuel project

Conclusion

- ❑ Breeding for biomass sorghum is still recent but may take advantage of the great variability existing in sorghum and novel genetic tools
- ❑ One quiet well defined target: biogas-silage
- ❑ For 2G-ethanol processes, several locks to be broken:
  - ✓ Biomass composition (cell wall) need to be optimized
  - ✓ Technological challenges: set up viable industrial processes (low-cost pretreatments, enzymatic cocktails, …)
  - ✓ Economic rentability of the chain need to be proved
- ❑ Several other opportunities for utilization of biomass sorghums as biomaterials and bioplastics…

THANK YOU FOR YOUR ATTENTION!