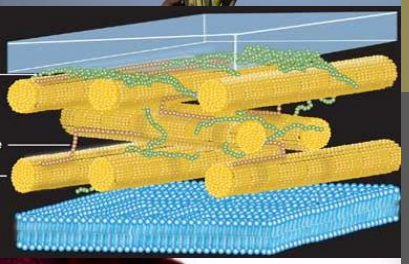


XXII EUCARPIA Maize and Sorghum Conference
June 19-22, 2011, Opatija, Croatia

Association mapping of biomass and cell wall related traits in sorghum

David Pot
UMR AGAP
Genetic Resources team
david.pot@cirad.fr

Renewable energy and Animal Nutrition
Ideotype definition

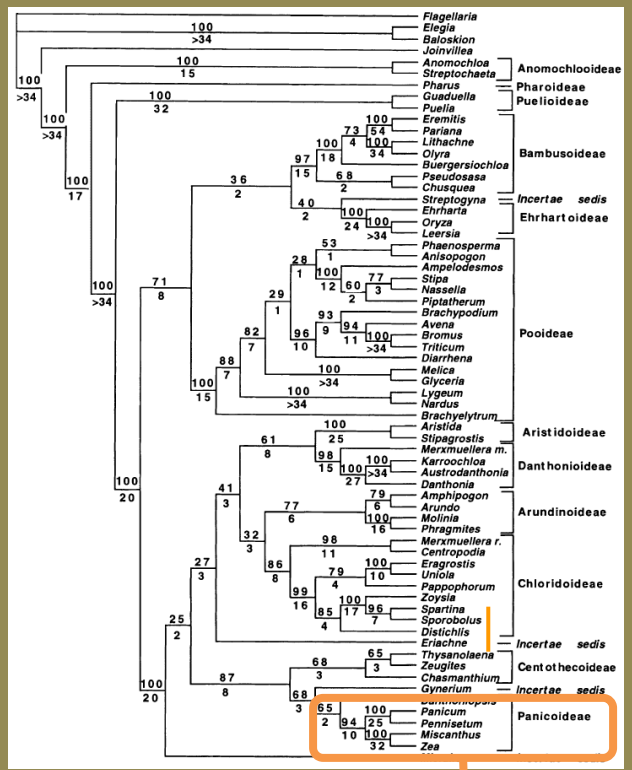
- Common targets
  - ✓ Increase biomass yield under low inputs
  - ✓ Optimizing biomass composition and properties

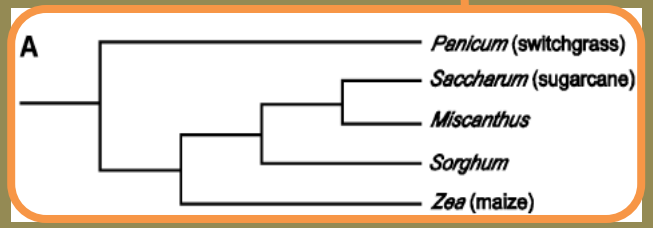
Accession	Race	Flowering	Height	Basal Diameter	Tillers	DM% harvest	DM yield (T/Ha)	ADL/NDF	INNDFD
IS 2156	B	No	325	1.99	**4.30**	20.0	18.74	8.17	20.6
IS 25596	C	No	355	2.45	0.30	20.7	19.39	8.57	24.7
IS 16186	D	No	255	**2.73**	0.23	17.4!	14.41	**6.97**	**29.6**
IS 12531	B	No	290	2.47	0.20	NH	NH	8.79	23.5
IS 15148	C	18/08/2011	313	2.28	0.20	NH	NH	8.31	16.4
IS 16396	G	No	335	2.46	1.30	18.8!	18.16	9.75	16.0
IS 23100	G	No	285	**2.64**	2.00	18.1	10.51	8.21	**25.1**
IS 23777	G	No	350	2.17	1.60	22.4	**23.07**	8.68	17.1
IS 32569	D	03/09/2010	295	2.16	0.80	**29.9**	**25.72**	8.62	19.3
SSM 12	D	No	325	2.37	0.21	NH	NH	7.73	23.8
BIOMASS 140 (T)	B	26/08/2010	**380**	2.25	0.40	**30.5**	**31.44**	8.94	12.7

- Flowering required to optimize DM% at harvest and Yield
- High Tillering is not relevant
- Large variability for lignin content and cell wall degradability
  - •High genetic gains are expected

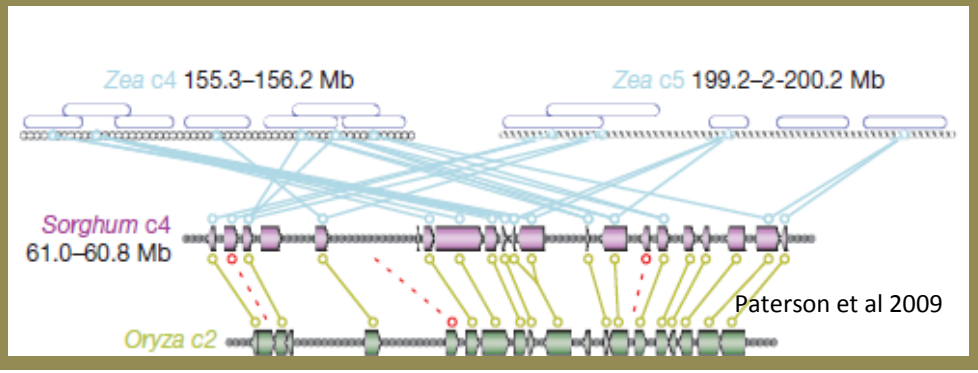
Maize and Sorghum : models for bioenergy and silage quality C4 grasses

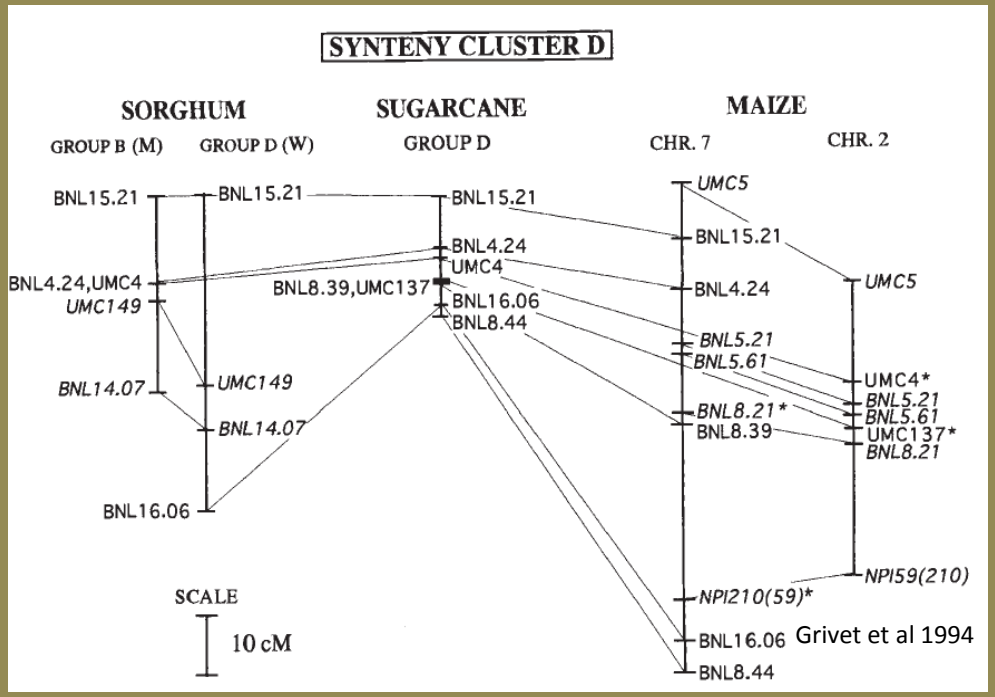
Poaceae Phylogeny





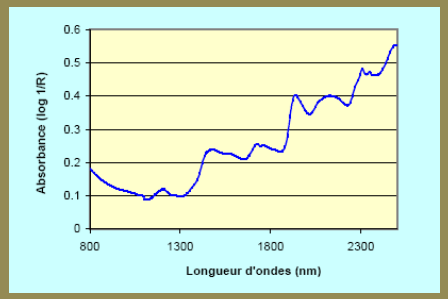
Syntheny and colinearity between C4 grasses





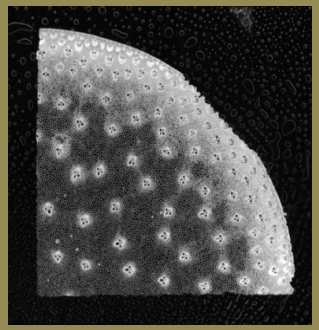
Characterizing the biomass / Cell Walls

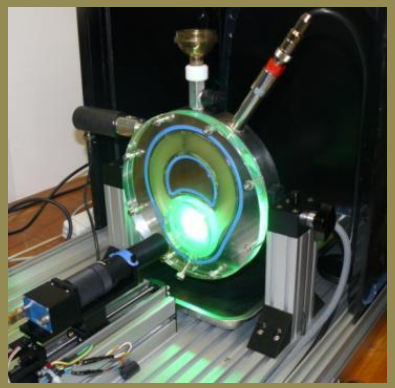
- NIRS calibration curve establishment for Biomass composition and CW related traits



=> Fibers, Lignin, Cellulose, hemicellulose, Nitrogen content, Non structural sugars (underway), Cell wall degradability

- Tools development for phenotyping at the Histological, biochemical and degradation kinetics levels





See : Poster : « Towards a sorghum ideotype with optimized cell wall degradability: insights from histological, degradation kinetics, biochemical, and pre-treatment analyses”

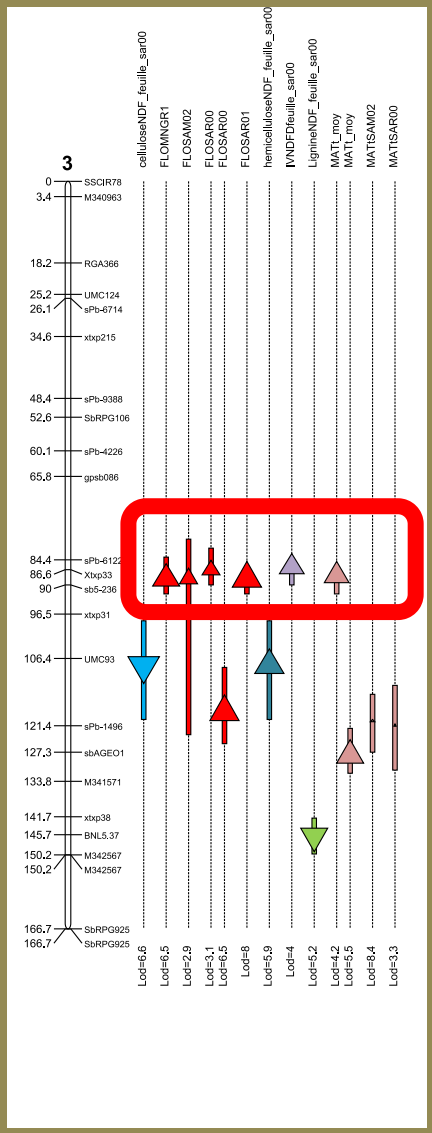
Deciphering the genetic architecture of biomass yield and composition
1- Biparental QTL mapping

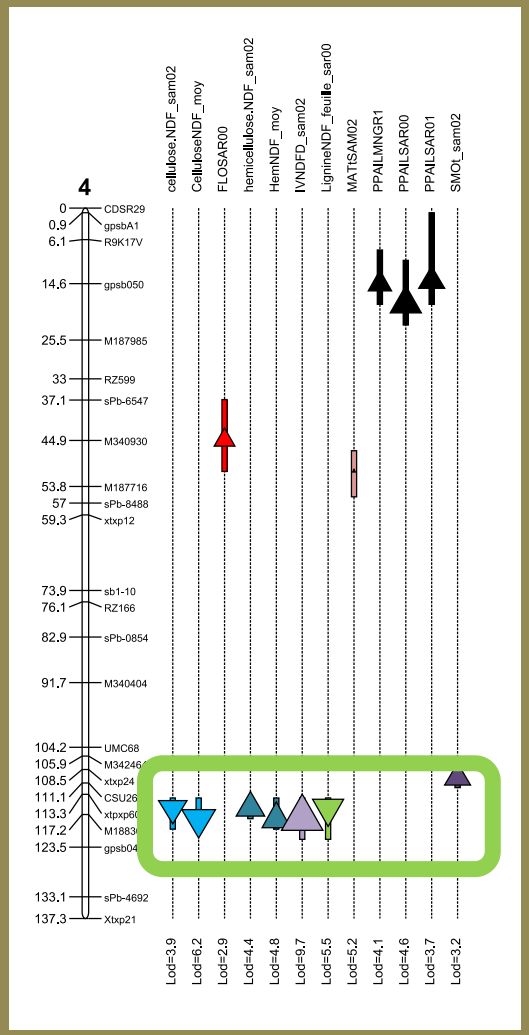
- **Population of 183 RILs** : SARIASO10 (caudatum breeding line) x SSM249 (guinea landrace). Highly contrasting in terms of biomass composition (but also cycle length…)

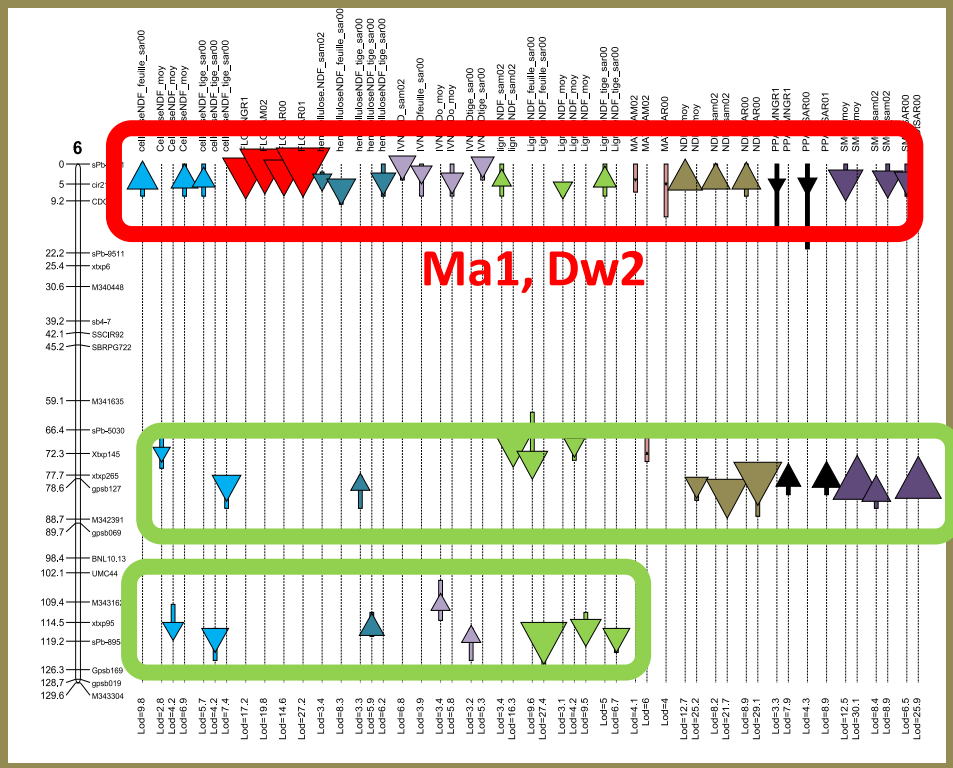
- **Two locations** (2 years)
  - ✓Burkina Faso
  - ✓Mali
  - ✓3 reps

- **Quantitative traits**
  - ✓NIRS leaves and shoots (fibers, cellulose, lignins…)
  - ✓Yield (grain)
  - ✓Biomass
  - ✓Agromorphologic measures

« Validation » of QTL over years / sites
Pleiotropic effects of Ma Dw genes

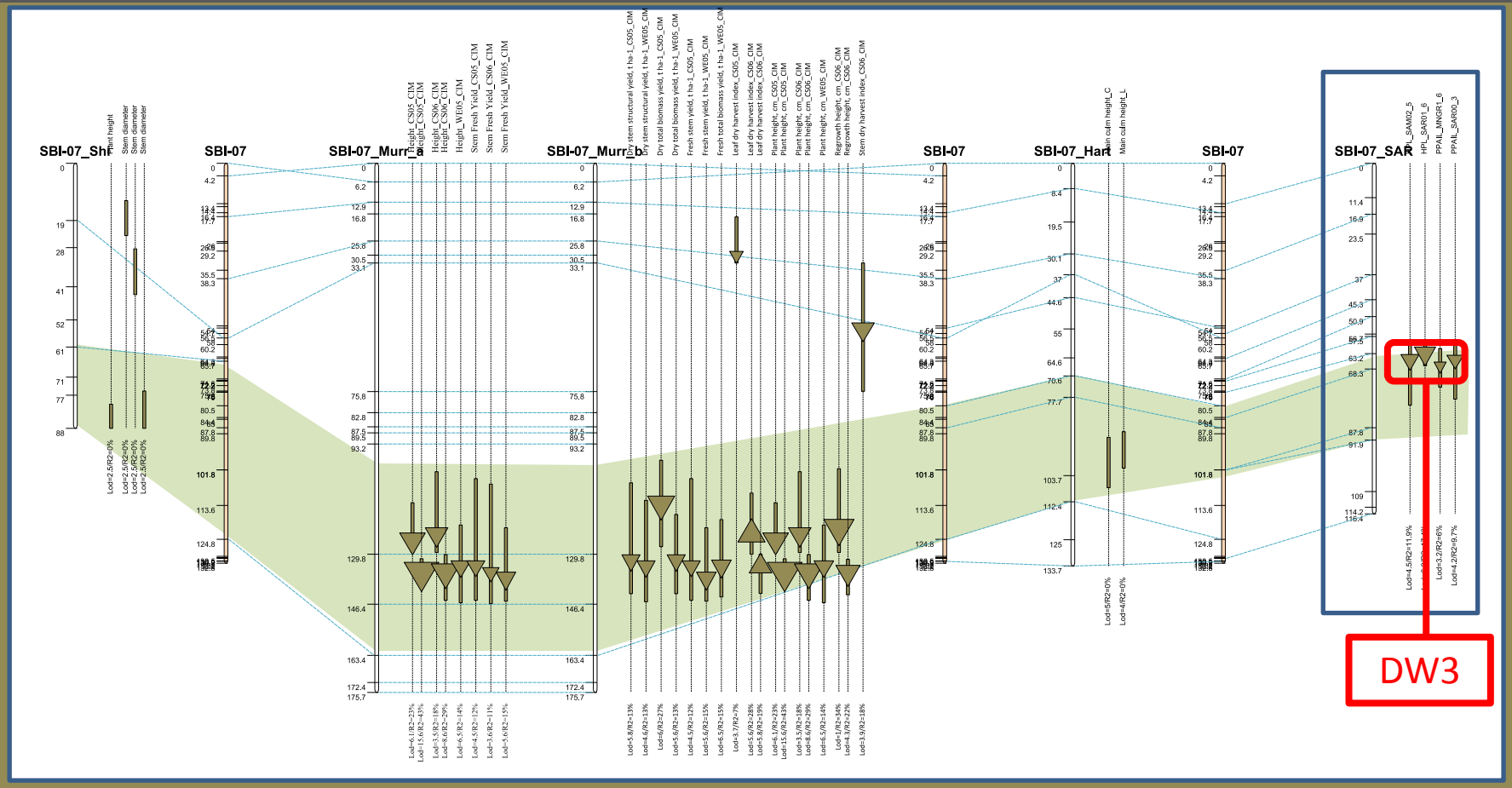






Biomass
Flowering
Brix
MAT
NDF
Cellulose
Hemicellulose
Lignin
Biomass Degradability
Cell Wall Degradability

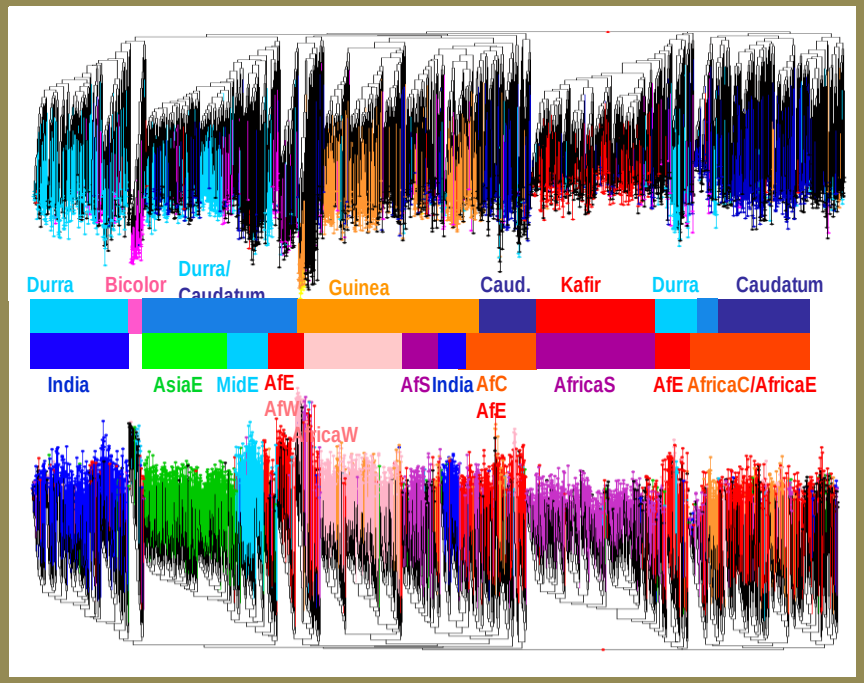
Comparative QTL mapping of biomass production



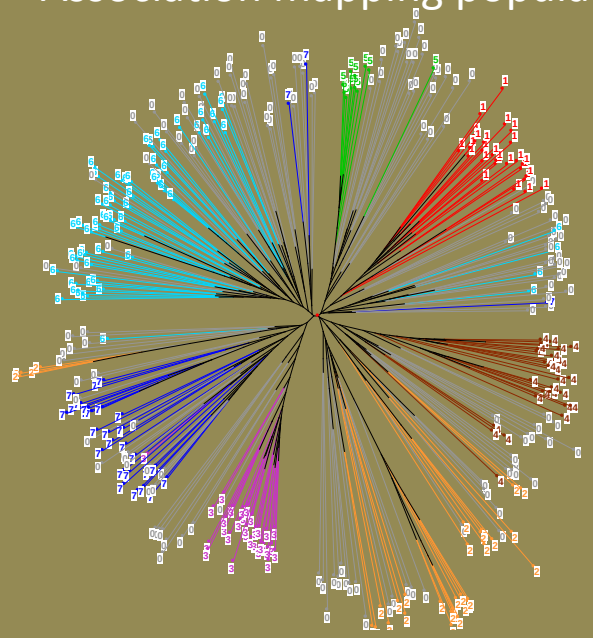
- No obvious QTL colocations for Biomass composition (not exactly the same traits considered…different genetic backgrounds… : Murray et al 2008a,b)
- Confidence intervals extremely large
- Not really useful for Breeding : « real life multiparental background »

Deciphering the genetic architecture of biomass yield and composition
2- Association Mapping : A strategy anchored on the analysis of the natural diversity

- Generation Challenge Program : 3367 accessions x 41 SSR



- A panel 413 accessions as a broad based Association mapping population



- 7 genetic groups
- Results are expected to be relevant for the whole sorghum community
- Some limitations (Flowering, Height)

Phenotyping of natural variation of Biomass and Cell Wall related traits

413 accessions analyzed

Cirad Core collection (210)

GCP Ref Set (384)

PHENOTYPING

2008

Mali (Samanko) Dec 2007-June2008 : 210

France (Montpellier) May -Oct 2008 : 100

Temperate subset

Mali (Sotuba) July -Nov 2008 : 362

2009

France (Montpellier) May -Oct 2009 : 100

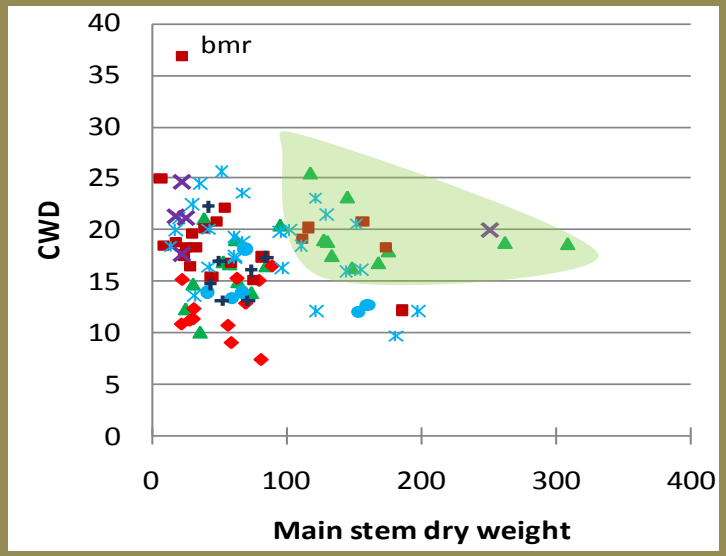
Temperate subset

Mali (Sotuba) June -Nov 2009 : 362

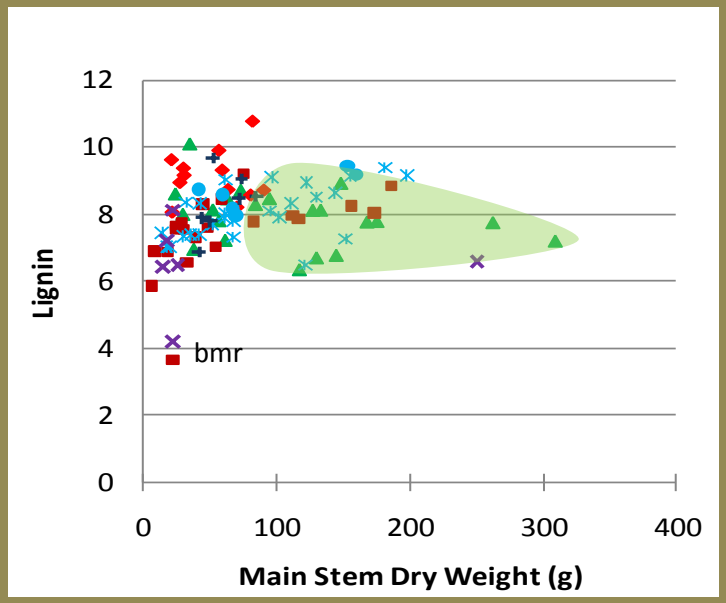
From Sylvain.Gutjahr (CIRAD)

Heritability and variability of Biomass and Cell Wall related traits

- Heritabilities between 0.5 and 0.98
- Coefficient of variation between 7 and 10 % for cell wall composition and higher for Cell Wall degradability
- Significant genetic gains are expected

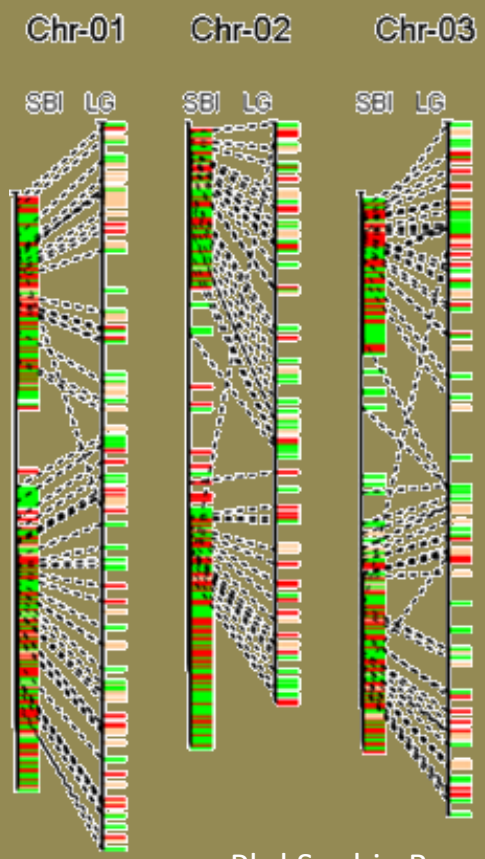


- Strong effects of the genetic group on the Biomass related traits
- Durra (East Africa and India), Kafir and Caudatum genotypes combining biomass yield, CWD and low lignin

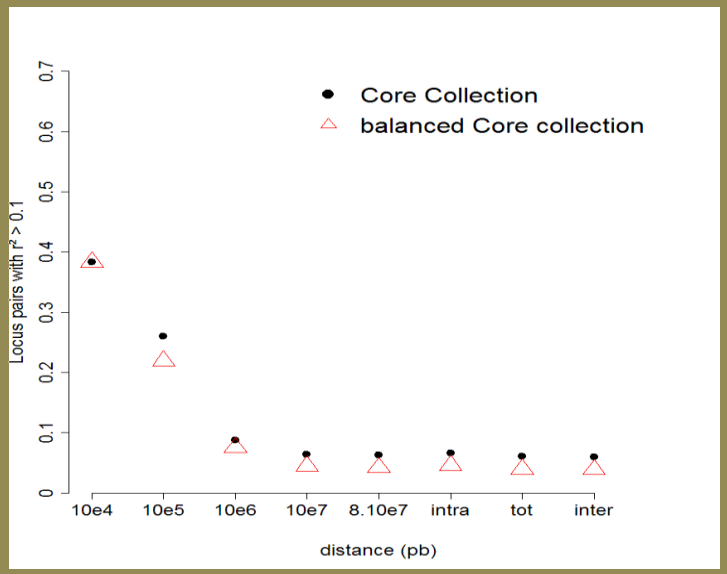


« A low density Whole Genome Scan » based on physically anchored DArT Markers

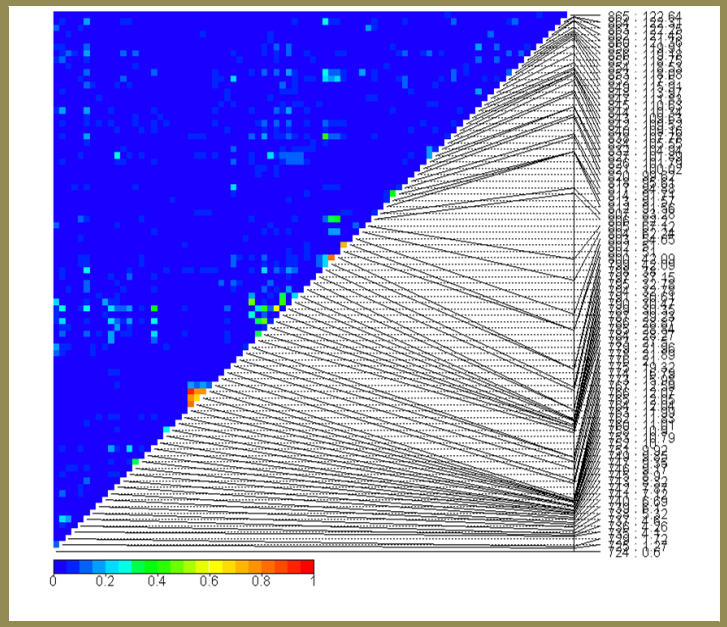
- 1122 DArT Markers physically anchored



Phd Sophie Bouchet



- r^2 = 0.2 at 10 kb



- More than 73 000 markers necessary to reach a satisfying coverage (QTL effect = 10 %,panel of 400 accessions)

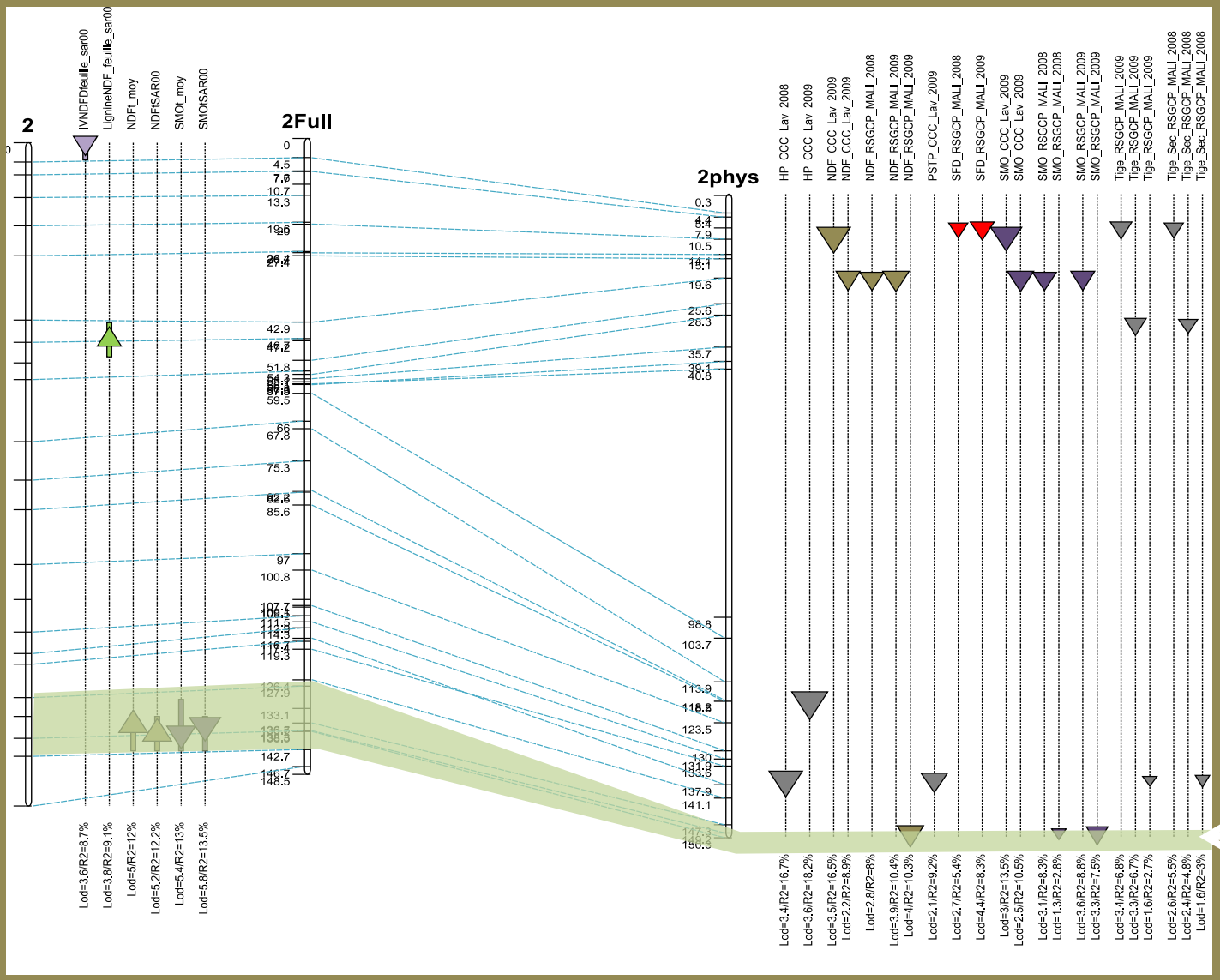
Association mapping in structured population

- **3 models explored**
  - Naive (no structure and no Kinship effects) : Yij= μ + Mi + eij
  - (Structure Yijk = μ + Qi + Mj+ eijk)
  - Structure + Kinship Mixed model

Trait class	# traits	MLM, pvalue <1.10E-3	% Signif tests	% test Signif MLM / Naive	Detected in Multiple Env with pvalue <=1.10E-3	Detected in Multiple Env with pvalue <=0.05
Biomass	5	16	0.29	6.02	1	12
Flowering	7	24	0.31	6.32	4	18
Nitrogen	5	9	0.16	_	0	2
Fibers	5	5	0.09	_	0	5
Brix	3	1	0.03	0.65	0	0
Cellulose	8	2	0.02	0.36	0	0
Hemicellulose	5	4	0.07	1.25	0	0
Lignin	5	1	0.02	0.47	0	1
CWD	8	9	0.10	2.37	0	6
Biomass degradability	5	7	0.12	_	1	6

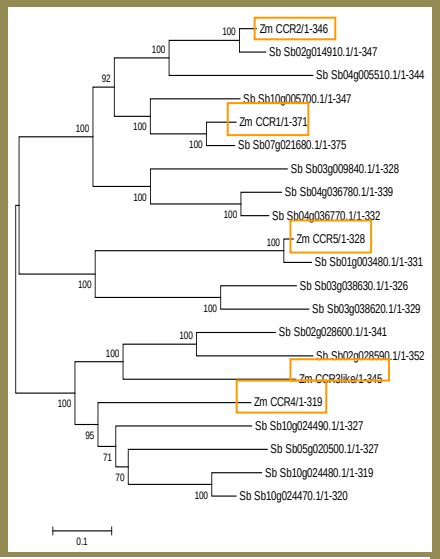
- Structure and Kinship need to be considered
- Some associations validated in different trials

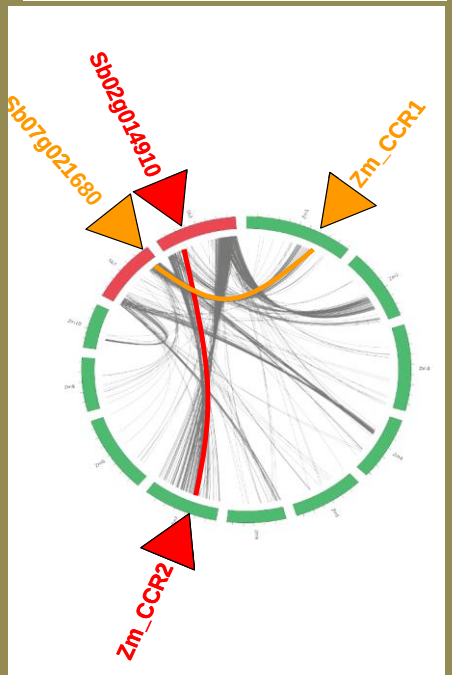
A peroxidase gene identified in a refined QTL interval for Fiber content and Biomass degradability



- Sb02g040690
- 8Kb from the DArT marker
- 54 % similarity with Peroxidase, Evalue = 8e-60 , identified by Shi et al 2006 as down regulated in bm mutant **(F2/F2bm1)**

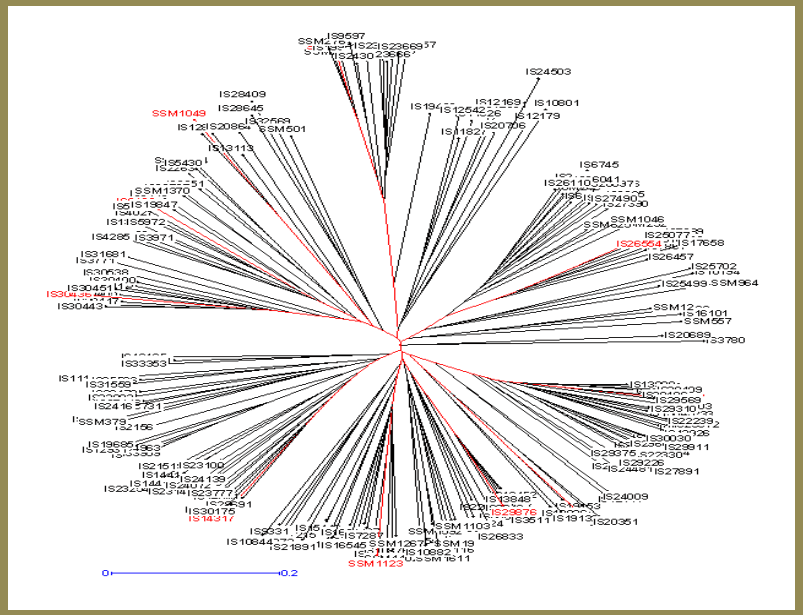
A candidate gene approach focused on the lignin biosythesis pathway





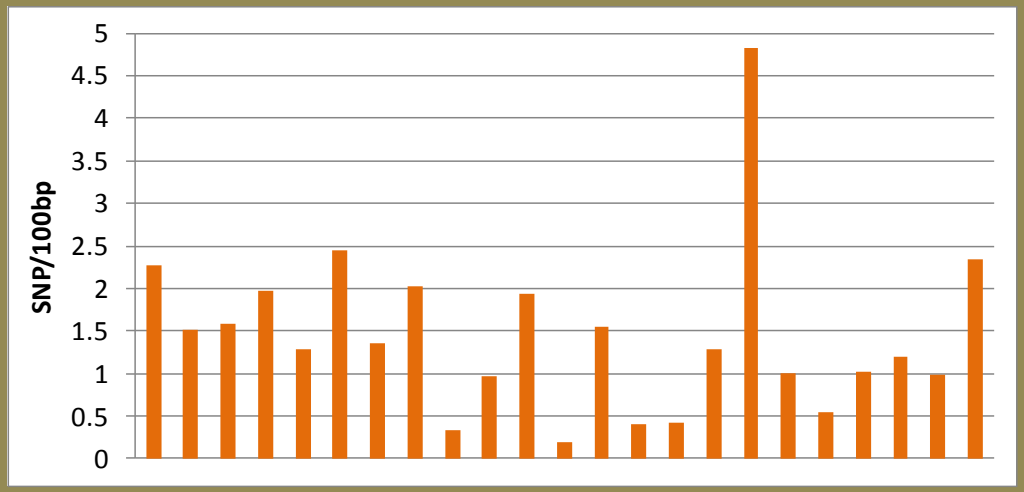
- 23 genes of the lignin biosynthesis pathway
  - ✓functional, expressional, and genetic informations from Zm, Os and Sb
  - ✓A combined phylogeny and syntheny based approach

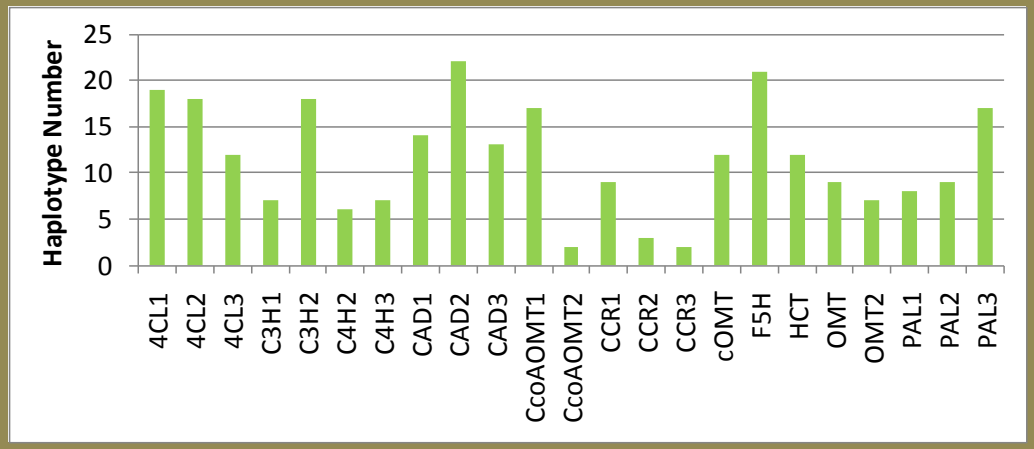
- Sequencing on 24-32 genotypes representative of the worldwide diversity



Lignin biosthesis genes : different patterns of diversity

- 37 Kb sequenced
- 585 polymorphisms identified
- 468 SNPs
- Large variability of nucleotide diversity patterns
- Different evolutionnary histories





Targeted Association Mapping

- 111 SNP from 22 genes analyzed in 413 genotypes (between 1 and 14 SNP / gene)

- MLM analysis

Trait	Trial	Marker	pvalue	errordf	r^2
CWD	1	4CL2	8.93E-05	357	5.00
Cellulose	1	4CL2	0.00139097	357	3.37
CWD	2	4CL2	0.03858877	357	1.77
Lignin	3	4CL2	0.03929415	98	4.20
Cellulose	2	C4H3	7.52E-04	351	4.04
Hemicellulose	2	C4H3	0.012016	351	2.42
Cellulose	4	OMT2	8.66E-04	98	12.42
Hemicellulose	4	OMT2	0.04392029	98	4.03

- More SNP have been genotyped in order to capture the different haplotypes

- Analyses on-going…

CIRAD (Montpellier) : Genetics and Breeding
Gilles Trouche
Jean-François Rami
Ronan Rivallan
Julien Maleyrat
Jacques Chantereau

CIRAD Montpellier : Animal Nutrition
Denis Bastianelli
Laurent Bonnal

CIRAD (Mali) : Genetics and Breeding
Michel Vaksmann
Benoit Clerget
Fabrice Sagnard

INRA / CNRS
Colleagues from the Grassbiofuel project

ANR GENOPLANTE

Funded by ANR Genoplante Grassbiofuel Project coordinated by Yves Barrière

Ideotype definition for high biomass genotypes in France

10 high biomass genotype analyzed over 2 years

Accession	Race	Flowering	Height	Basal Diameter	Tillers	DM% harvest	DM yield (T/Ha)	ADL/NDF	INNDFD
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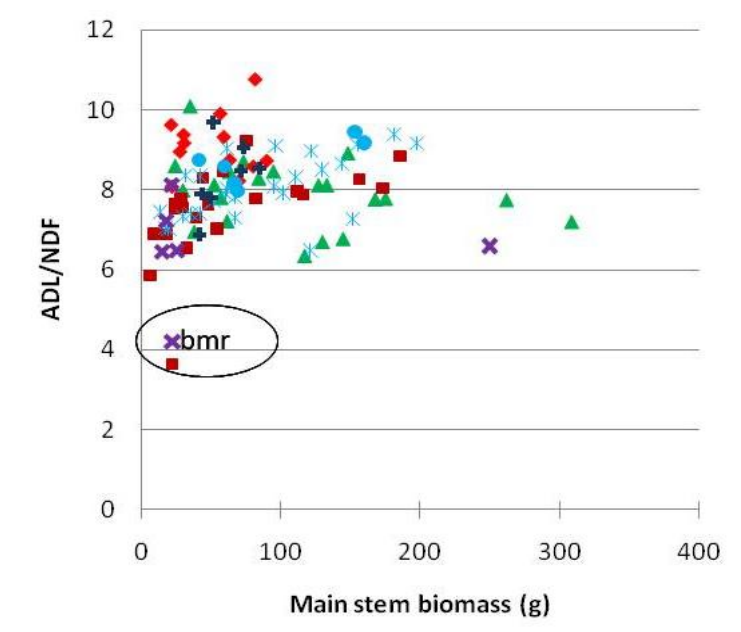
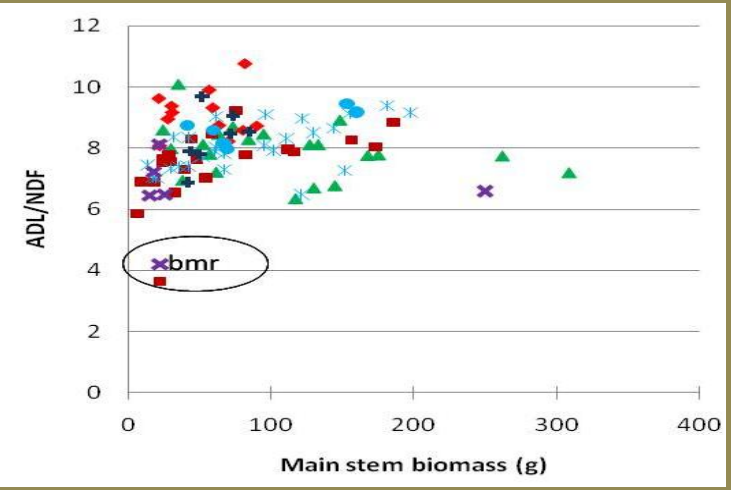
- Flowering required to optimize DM% at harvest and Yield
- High Tillering is not relevant
- Large variability for lignin content and cell wall degradability
  - High genetic gains are expected

Heritability and variability

trait	Year	h²sl	cv*
Biomass	2008	0.75	68.14
Biomass	2009	0.91	78.85
Flowering	2008	0.89	22.45
Flowering	2009	0.98	29.84
NDF	2008	0.81	11.72
NDF	2009	0.78	7.39
Lignin	2008	0.57	8.35
Lignin	2009	0.61	8.71
Cellulose	2008	0.48	2.46
Cellulose	2009	0.74	3.41
Hemicellulose	2008	0.51	4.65
Hemicellulose	2009	0.73	7.29
Biomass Degradability	2008	0.80	18.65
Biomass Degradability	2009	0.76	30.15
CWD	2008	0.55	34.80
CWD	2008	0.68	28.03
BRIX	2009	0.82	40.25

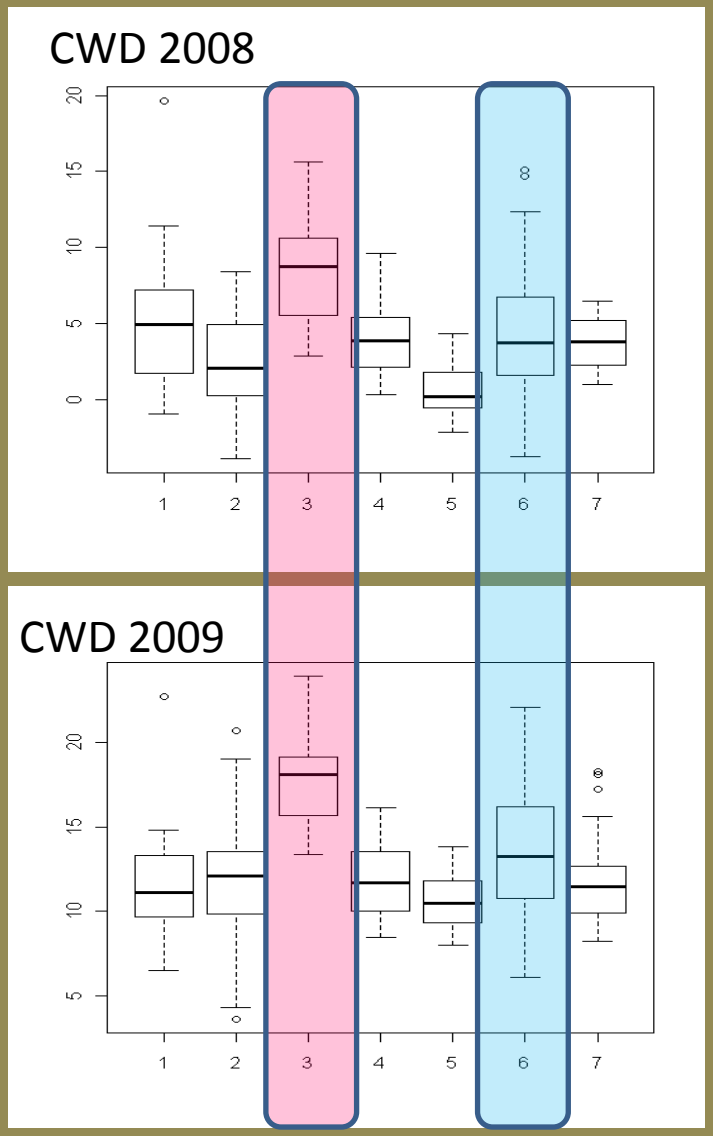


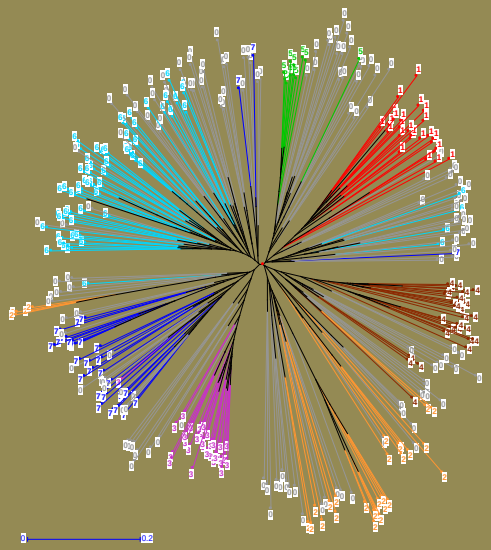


- Within Durra East Africa and India (mainly), kafor and Caudatum genotypes combining biomass yeild, CWD and low lignin ▲

Strong effect of the genetic groups on the target traits

Trait	Year	pvalue
Biomass	2008	1.62E-11
Biomass	2009	5.60E-20
Flowering	2008	2.09E-12
Flowering	2009	1.15E-22
Nitrogen	2008	1.78E-10
Nitrogen	2009	7.67E-10
Fibers	2008	1.07E-15
Fibers	2009	1.25E-12
lignin	2008	6.72E-12
lignin	2009	4.42E-10
Cellulose W	2008	2.94E-17
Cellulose W	2009	2.31E-15
Cellulose VS	2008	4.35E-09
Cellulose VS	2009	1.39E-17
Hemicellulose	2008	8.18E-14
Hemicellulose	2009	1.57E-18
Biomass Degradability	2008	2.05E-16
Biomass Degradability	2009	1.85E-11
CWD	2008	1.32E-14
CWD	2009	1.95E-09
Brix	2009	7.42E-22

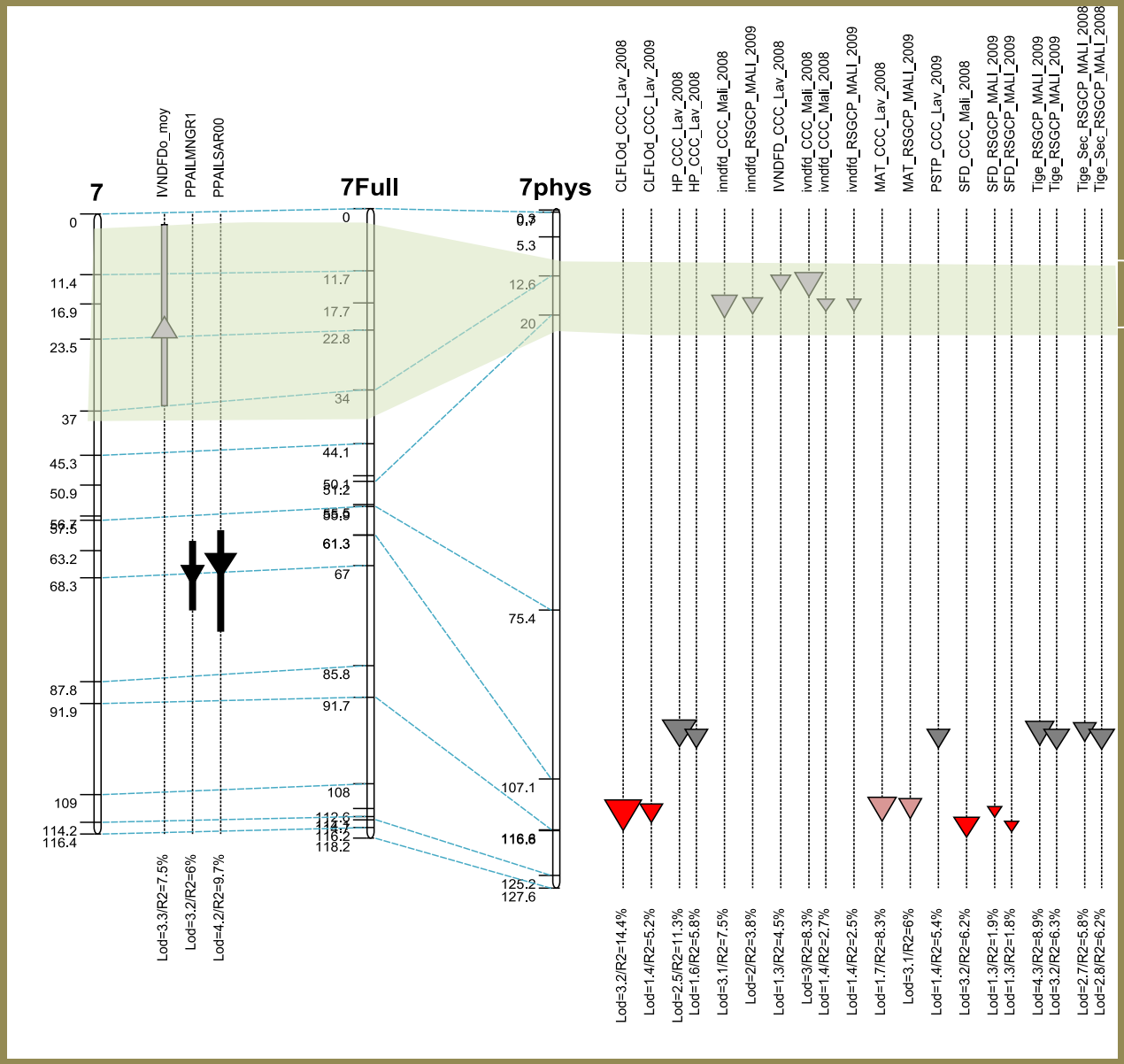




Kafir (South Africa)

Caudatum (East and some West African)

Reduction of the confidence Interval for a CWD QTL



- 2.2 Mb
- 2 different QTLs ?
- Several genes in the region to analyze