



**Seventh framework programme
Food, Agriculture and Fisheries, and Biotechnology**

Specific International Co-operation Actions
Small or medium scale focused research project



Sweet Sorghum an **alternative energy Crop**

Grant Agreement n° 227422

Deliverable 4.5:

*QTLs developed for traits for high
sugar accumulation*

Composition of the consortium

CIRAD
ICRISAT
EMBRAPA
KWS
IFEU
UniBO
UCSC
ARC-GCI
UANL
WIP



Two fertility restorer lines for cytoplasm A1, BR501R (Brandes) and BR505R (Wray), both released sweet sorghum cultivars were crossed with the objective to develop a Recombinant Inbred Line (RIL) population by single seed descent (SSD) for the purpose of identifying QTLs associated with plant tillering, total sugars in the juice and sucrose in the juice. Brandes is a high tillering sweet sorghum cultivar with low purity (low sucrose) and Wray is a non-tillering cultivar with high purity. The F1 was self pollinated to produce a large F2 population. Two hundred seventy-five F_{2:2} RILs were advanced to the S₈(F_{2:9}) generation. This RIL population has been phenotyped for both agronomic and industrial characteristics (2010/2011, 2011/2012, and 2012/2013) at Embrapa, DNA was extracted for 273 RILs and the two parents and genotyping by sequencing (GBS) was completed in 2012/2013. Phenotypic evaluations obtained for two years (2011 and 2012) for a sub-population of 223 sweet sorghum RILs and the two parental sweet sorghum lines were analyzed with the GBS data base. Data were obtained from seven to nine industrial and agronomic traits characters (Table 1). Ratings for disease resistance were discontinued and therefore not entered in the analysis of this report. Consistently, henceforth, the abbreviations in Table 1 will be used in the text, figures and tables. For use only in calculations that other measures derived (CONSECANA-SP, 2006), EC, PBU, PBS and MS were also excluded from further analyzes.

Table 1—Agronomic and Industrial Parameters of 273 sweet sorghum RILs.

<i>Agronomic Parameters</i>	<i>Abbreviation</i>	<i>Unit</i>
Flowering	TFL	days
Plant Height	ALT	m
Fresh Weight Production	PMV	t ha ⁻¹
Juice Extraction (500 g sample)	EC	%
Press cake (fresh)	PBU	g
Press cake (dry)	PBS	g
Press cake	MS	%
<i>Industrial Parameters</i>	<i>Abbreviation</i>	<i>Unit</i>
Brix (extracted juice)	Brix	Brix
Pol(Apparent sucrose)	POLCa	%
Apparent sucrose purity	PUR	%
Reduced sugars (glucose + fructose)	ARCo	%
Fiber	FIB	%
Total recoverable sugar	ATR	%
Total sugars (juice)	ARCa	%
Alcohol yield	VAH	L t ⁻¹
Alcohol production	ALC	L ha ⁻¹

Genotypic analysis

SNP data were obtained for 272 individuals of the RIL population and both parents, through the technique called genotyping by sequencing (GBS), at the IGD-Cornell University, United States, in accordance with the procedure developed by Elshire et al. (2011). The missing data were imputed using the software Npute (ROBERTS et al., 2007), testing a window for charging ranging from 5 to 150 bases for each chromosome. The set of filtered data allocated to lower allele frequencies (Minor Allele Frequency - MAF) of more than 0.40.

The performance of GBS in the population of RILs sweet sorghum resulted in over a million putative SNPs, 58,845 being kept for further analysis after imputation and filtering steps (Table 2). On average, each chromosome of the population contained 5,884 markers. In a typical scenario of an experimental population of self-pollinated plants, this number is considered excellent. SNPs are physically mapped against the genome of sorghum. For charging in Npute (ROBERTS et al., 2007), windows software for imputation was established for each chromosome separately and ranged 47-89 bases.

Table 2 - Number of SNPs obtained via GBS before and after filtering for the population of RILs sweet sorghum.

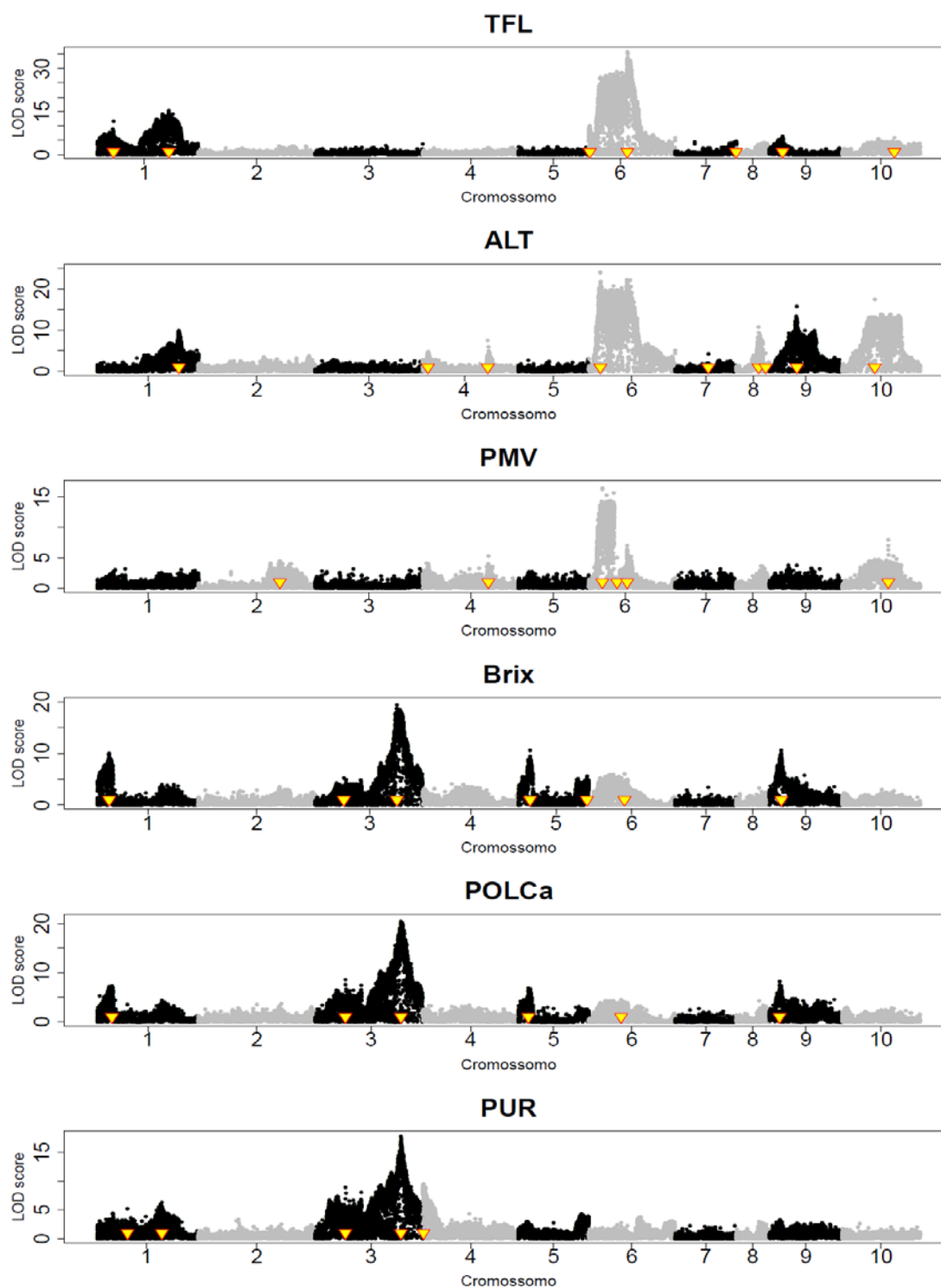
Chromosome	Unfiltered	Filtered
1	158.402	7.231
2	136.341	8.342
3	138.874	7.710
4	116.120	6.839
5	88.939	5.032
6	95.037	6.183
7	81.092	4.369
8	73.555	2.374
9	86.369	5.214
10	91.681	5.551
Total	1.066.410	58.845

Table3- Values of the Akaike criteria (AIC), corrected AIC(AIC_c) and Bayesian (BIC) for hree levels of significance in mapping multiple intervals (MIM) formulti variate agro-industrial characters of the population of 223 sweet sorghum RILs.

Character	Sig-score	AIC	AICc	BIC	N° de QTLs
TPF	0.10	1989.29	1991.95	2043.66	6
	0.15	1989.29	1991.95	2043.66	6
	0.20	1968.23	1972.01	2032.79	7
ALT	0.10	546.28	542.89	485.11	7
	0.15	578.06	572.95	503.30	9
	0.20	578.06	572.95	503.30	9
PMV	0.10	2494.26	2494.93	2521.44	2
	0.15	2494.26	2494.93	2521.44	2
	0.20	2418.89	2420.93	2466.47	6
Brix	0.10	1087.65	1091.03	1148.81	7
	0.15	1087.65	1091.03	1148.81	7
	0.20	1087.65	1091.03	1148.81	7
POLCa	0.10	1148.12	1150.46	1199.10	5
	0.15	1129.09	1131.76	1183.46	6
	0.20	1129.09	1131.76	1183.46	6
PUR	0.10	2404.08	2405.84	2448.26	4
	0.15	2385.07	2387.41	2436.04	5
	0.20	2385.07	2387.41	2436.04	5
ARCo	0.10	2341.68	2338.66	2283.91	6
	0.15	2341.68	2338.66	2283.91	6
	0.20	2341.68	2338.66	2283.91	6
FIB	0.10	98.03	99.53	138.81	5
	0.15	41.71	44.72	99.47	8
	0.20	41.71	44.72	99.47	8
ATR	0.10	2846.01	2850.21	2913.97	8
	0.15	2846.01	2850.21	2913.97	8
	0.20	2846.01	2850.21	2913.97	8
ARCa	0.10	1065.56	1068.95	1126.73	7
	0.15	1047.70	1051.48	1112.26	8
	0.20	1047.70	1051.48	1112.26	8
VAH	0.10	2573.21	2576.59	2634.37	7
	0.15	2555.33	2559.11	2619.89	8
	0.20	2555.33	2559.11	2619.89	8
ALC	0.10	6309.33	6310.83	6350.11	5
	0.15	6309.33	6310.83	6350.11	5
	0.20	6309.33	6310.83	6350.11	5

Eighty three (83) QTLs were detected for a total of 12 traits, with the number of QTLs ranging 5-9 per character. Additionally, significant QTL×environment interactions were observed.

Figure1- Profile of LOD scores resulting from multiple interval mapping (MIM) for multi variate agro-industrial characters in the RILs population of sweet sorghum.



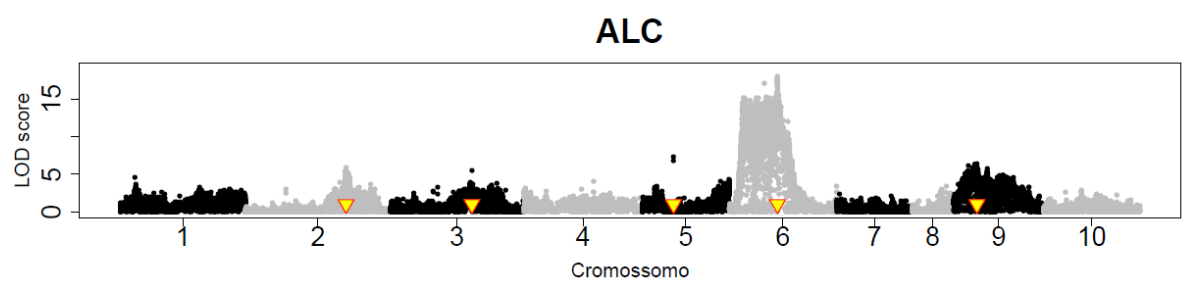
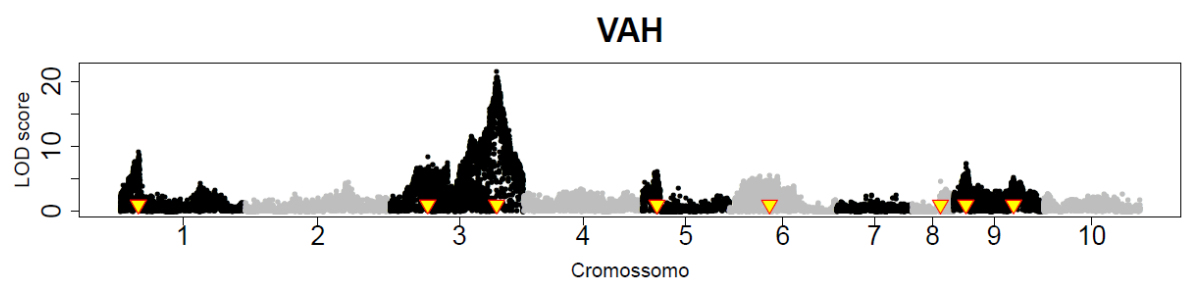
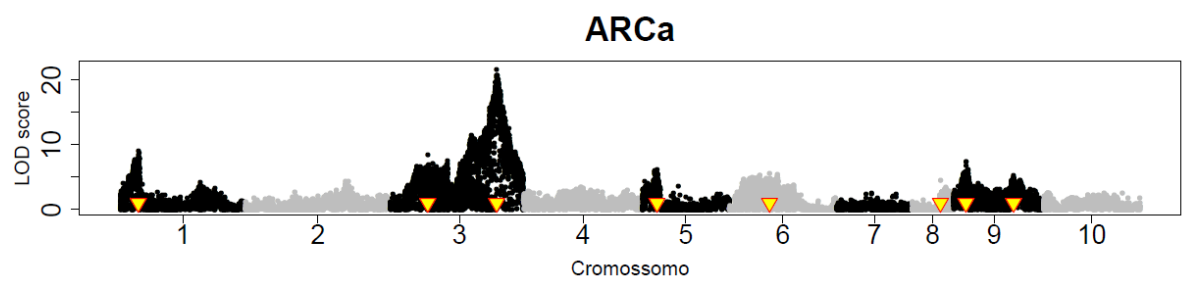
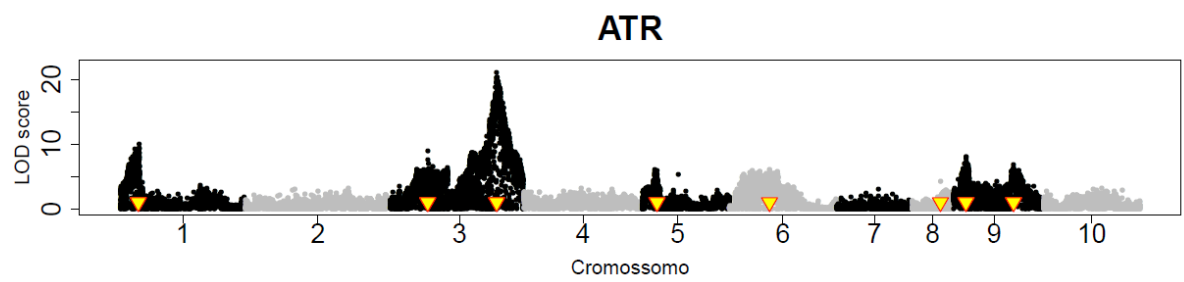
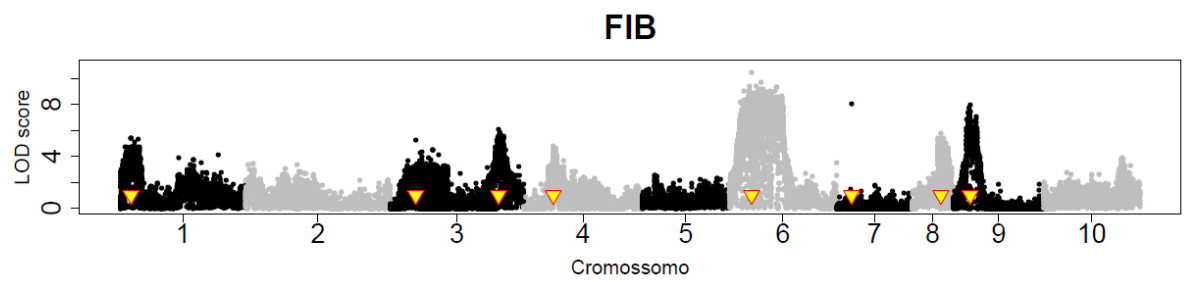
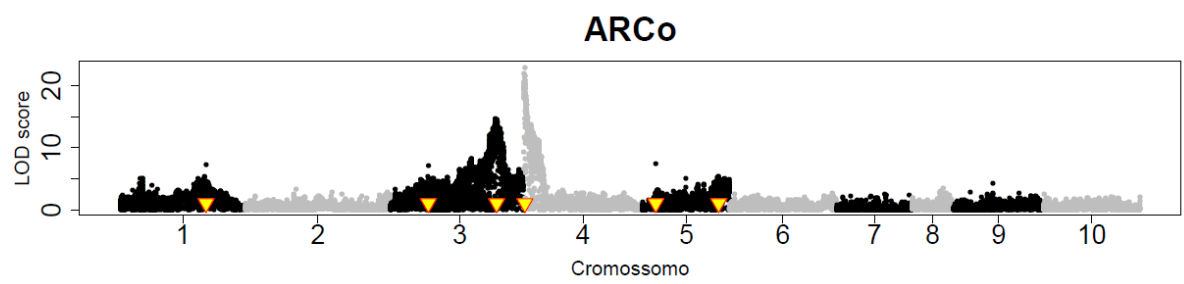


Figure2- Twelve agro-industrial characters of the sweet sorghum RIL population. Histograms of the mean for each character set (diagonal) and dispersion graphs (below diagonal) and values of the genetic correlations (above diagonal) between pairs of characters are shown.



Note the high correlation between Brix and the following characteristics; Pol or sucrose (0,94), total recoverable sugars (ATR) (0,95, total sugar in the juice (ARCa) (0,95) and ethanol yield (VAH) (0,95). These analyses will be repeated including the third year (2013/2014) of phenotyping data at Embrapa. Following this, molecular markers will be developed for selected QTLs for use in selection of superior genotypes in sweet sorghum molecular breeding programs.