QTL Mapping Of Sugarcane Enhanced By High Throughput RADseq Genotyping

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Sugarcane QTL Mapping

1. Identification of markers linked to yellow spot disease resistance - M134/75 X R570 (RxS)

-Up to 25% yield loss in super-humid regions

Control: Avoid cultivation of susceptible varieties in superhumid regions & breed for resistant varieties

Objective:
Apply MAS to improve on selection efficiency and shorten breeding cycle



Identification of markers linked to early ripening CP 67412 x M 245/76 (early x late)

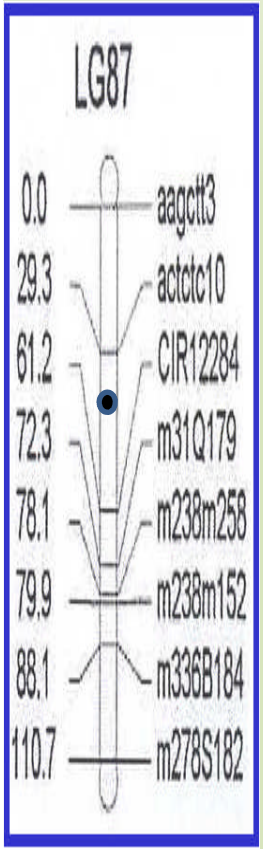
Objective:
Start the cane harvest season 1 month earlier

Discovery of Markers Associated With YSDR

Previous work

Mapping population of 227 individuals derived from M 134/75 (R) and R 570 (S) genotyped using AFLP and gSSR

Major QTL identified, flanked by AFLP marker *actctc10* (14cM) & SSR marker CIR 12284 (17cM)



Further screen mapping population with EST-SSR markers

- **Sugarcane EST-SSR primers**

(Pinto et al., 2004 , Oliveira et al., 2007, ICSB- R. Ming).

- **Sorghum EST-SSR**

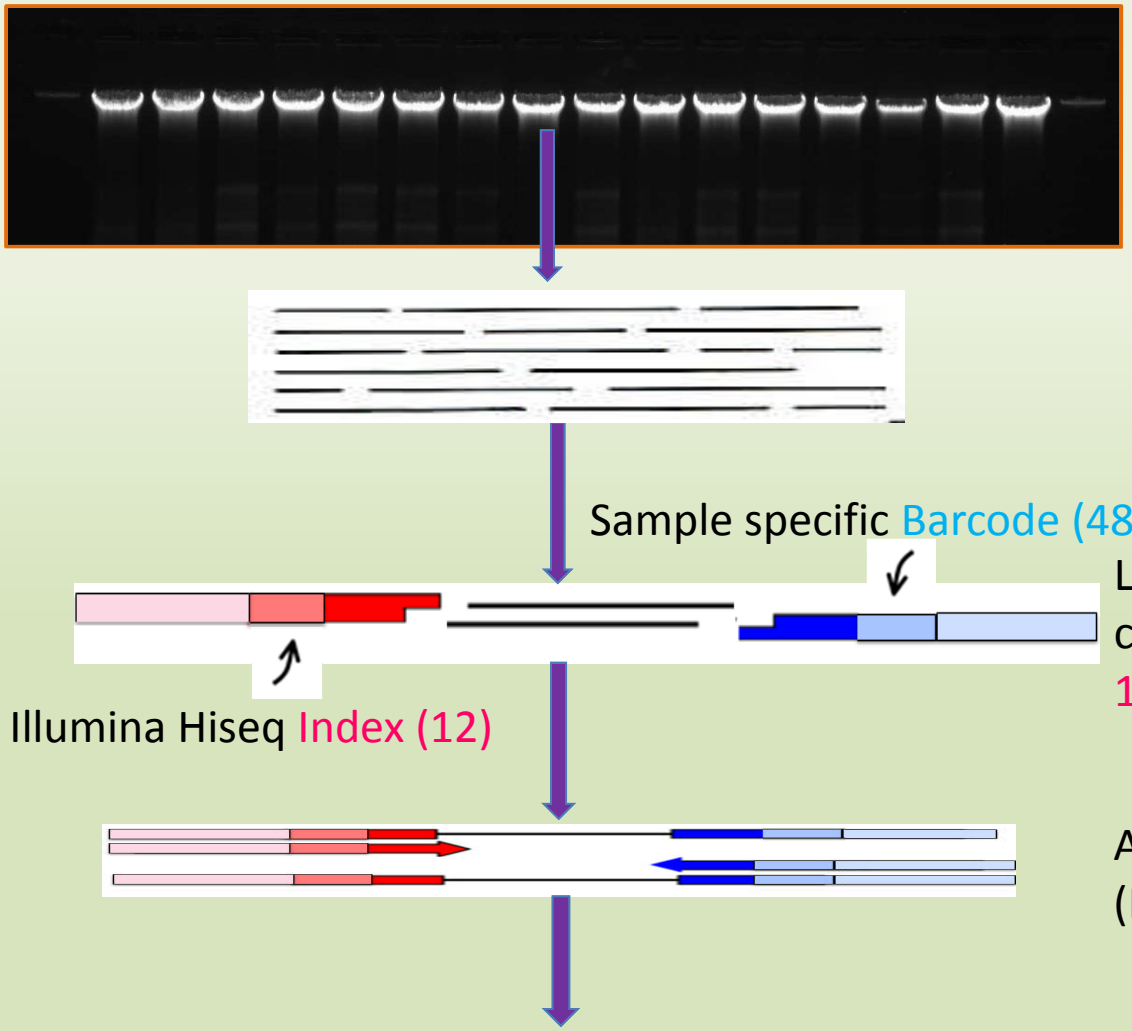
(24- Wang et al., 2005 and 600- ICRISAT)

67 EST-SSR primer pairs were used for population screening and 142 markers scored for M 134/75

Application Of NGS To SNP Genotyping In Sugarcane

Restriction Site Associated DNA Sequencing (ddRADseq)

RADseq Sample Processing



Digest
DNA with a pair of restriction enzymes (*NsiI* and *MseI*)

Sample specific Barcode (48)

Ligate fragments with adaptors containing barcode and index
12 x 48 combinations

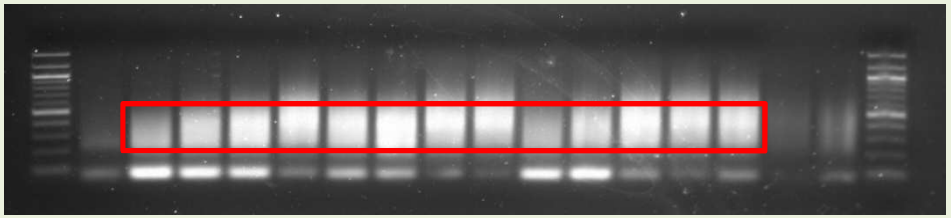
Illumina Hiseq Index (12)

Amplify adaptor-ligated fragments (RADtags) max 18 cycles

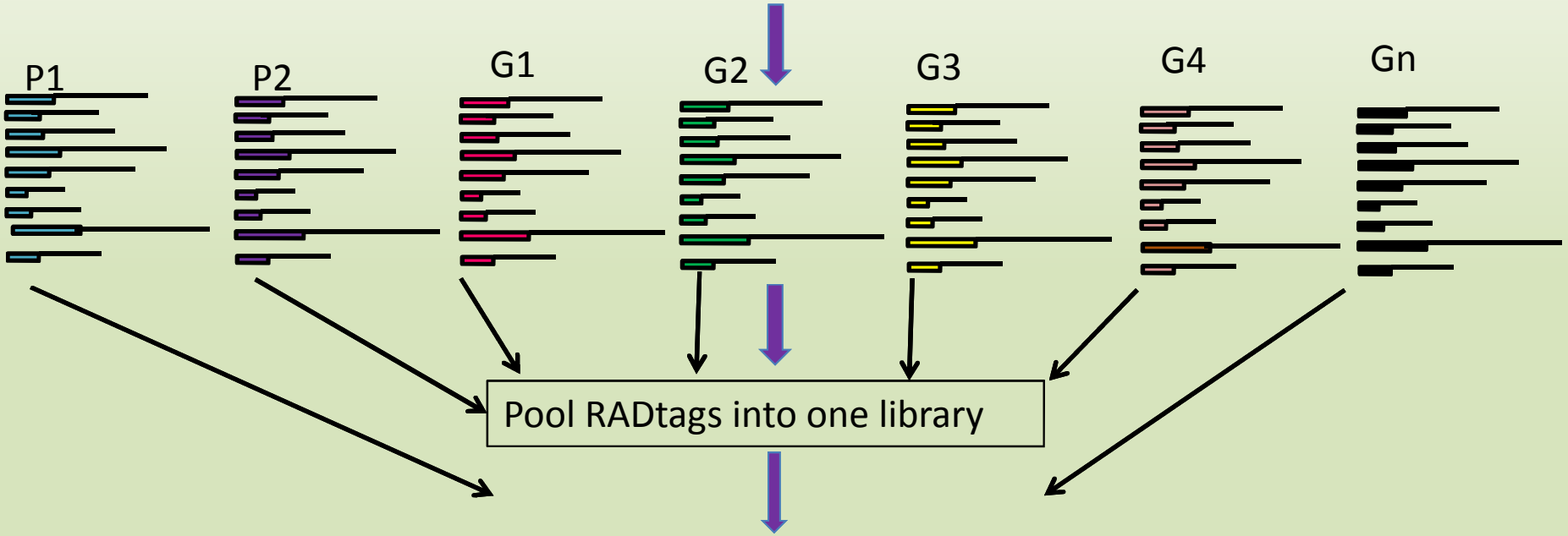
Enrich a reduced representation of the sugarcane genome

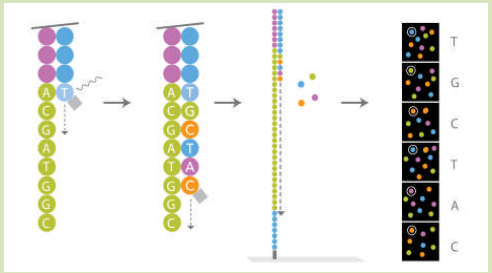
RADseq Sample Processing

Run amplified RADtags on agarose gel



Size-select amplified RADtag fragments 200-500 bp, clean up, quantify

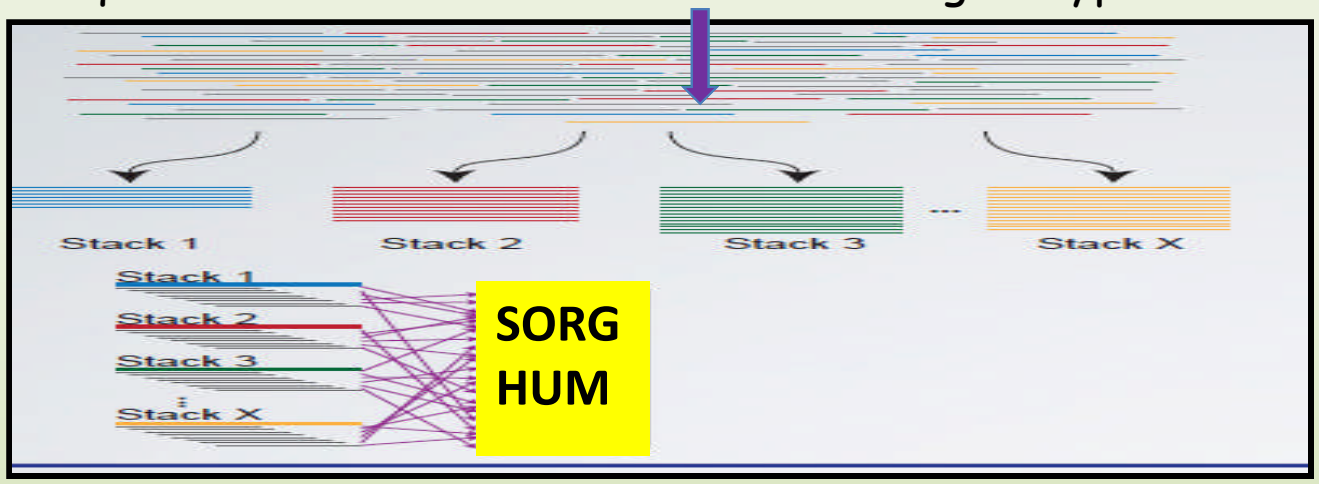




Illumina Sequencing

RADseq Sequence Data Analysis

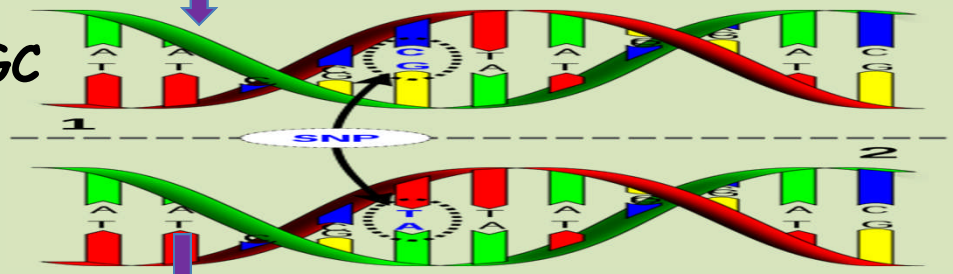
Demultiplex reads according to index and barcodes
Equate barcodes/index combination to genotype



Stack similar reads from each genotype, align to sorghum genome and define locus

M 134/75 Chr _10_12345678GC

R 570 Chr_10_12345678TA



Identify SNPs between mapping parents and determine zygosity

Assign zygosity of genotype from mapping population and tabulate results in Joinmap format (nnxnp, lmxll, hkxhk)

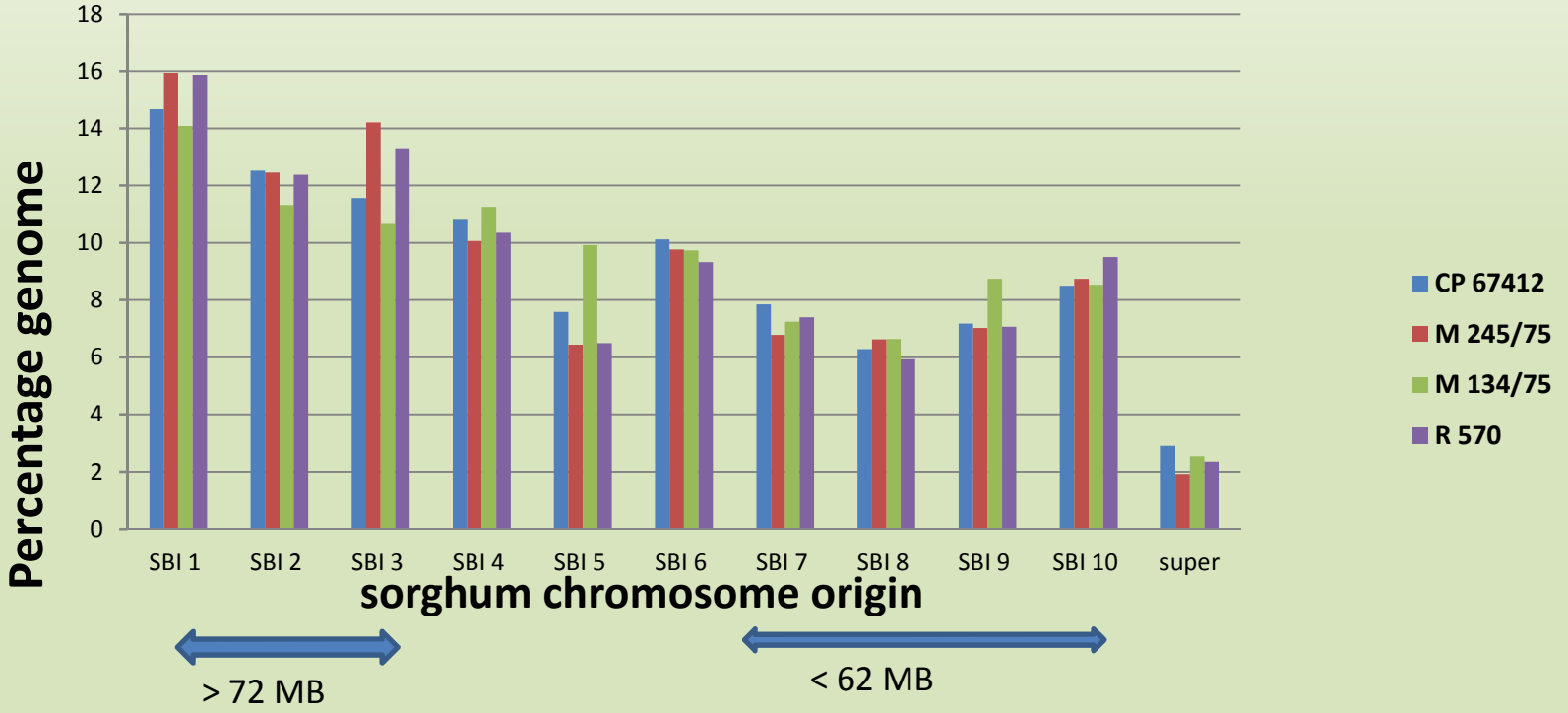
Results – SNP Distribution

Yellow spot

- 8,873 SNPs for the YSR variety M 134/75
- 12,161 SNPs for R 570 (susceptible)

Early ripening

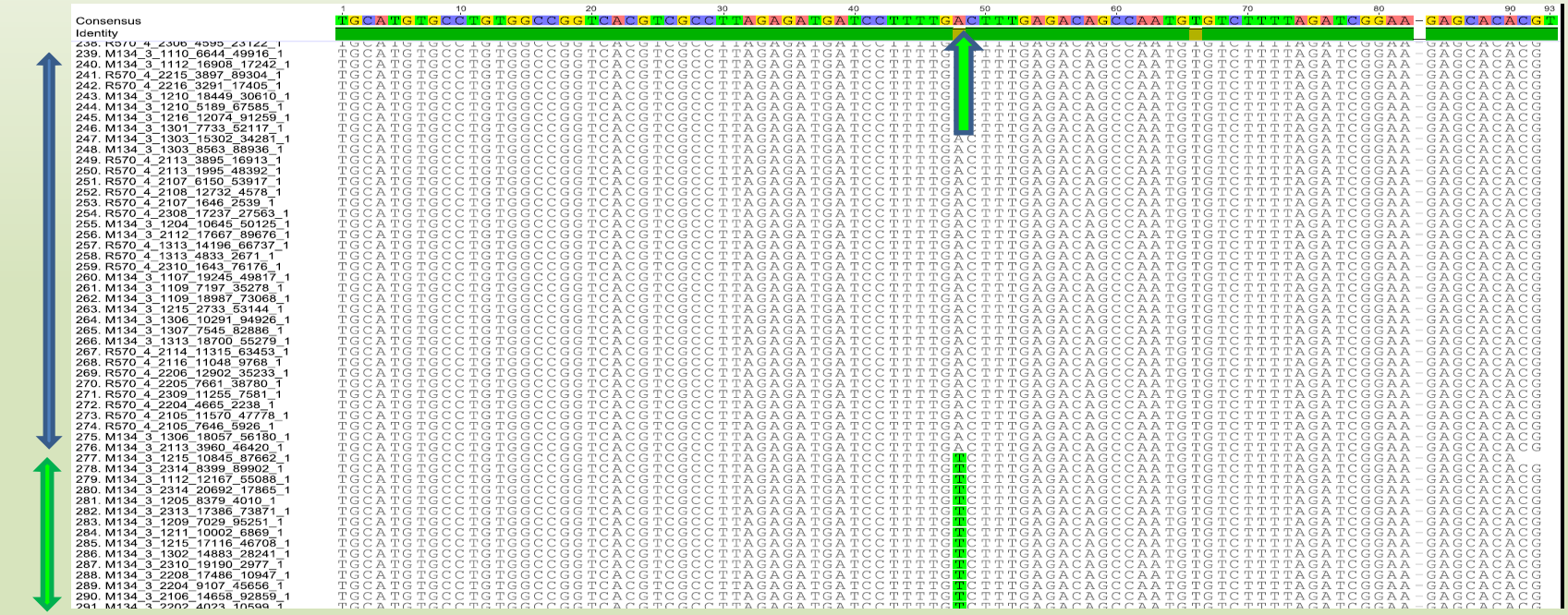
- 9500 SNP for CP 67412 (early) and
- 7560 SNPs for M 245/76 (late)



Even genome coverage of markers across chromosomes using *MseI* and *NsiI* restriction enzymes

Validation Of Radseq SNP Markers

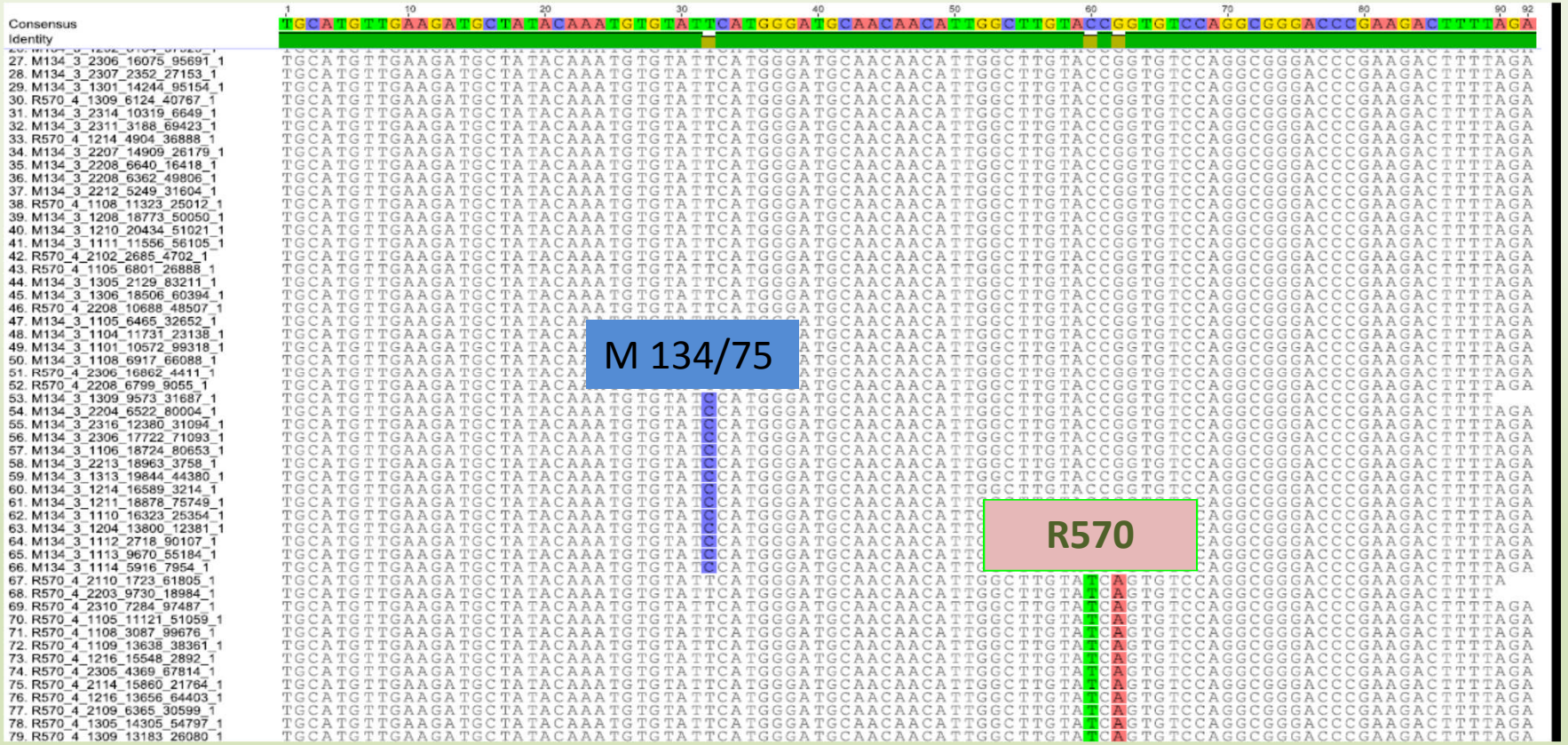
- Extract similar sequences from mapping parents M 134/75 and R 570
- Use Geneious software to align sequences
- Locate position of SNP from RADtag read: present in M 134/75 at position 48



Blast RADtag sequence to *S. bicolor* genome sequence on Phytozome
Identify best hit and calculate SNP position

All SNPs lie within +/- 1 bp to SNP identified from STACKS SNP calling pipeline

SNP Calling Validation



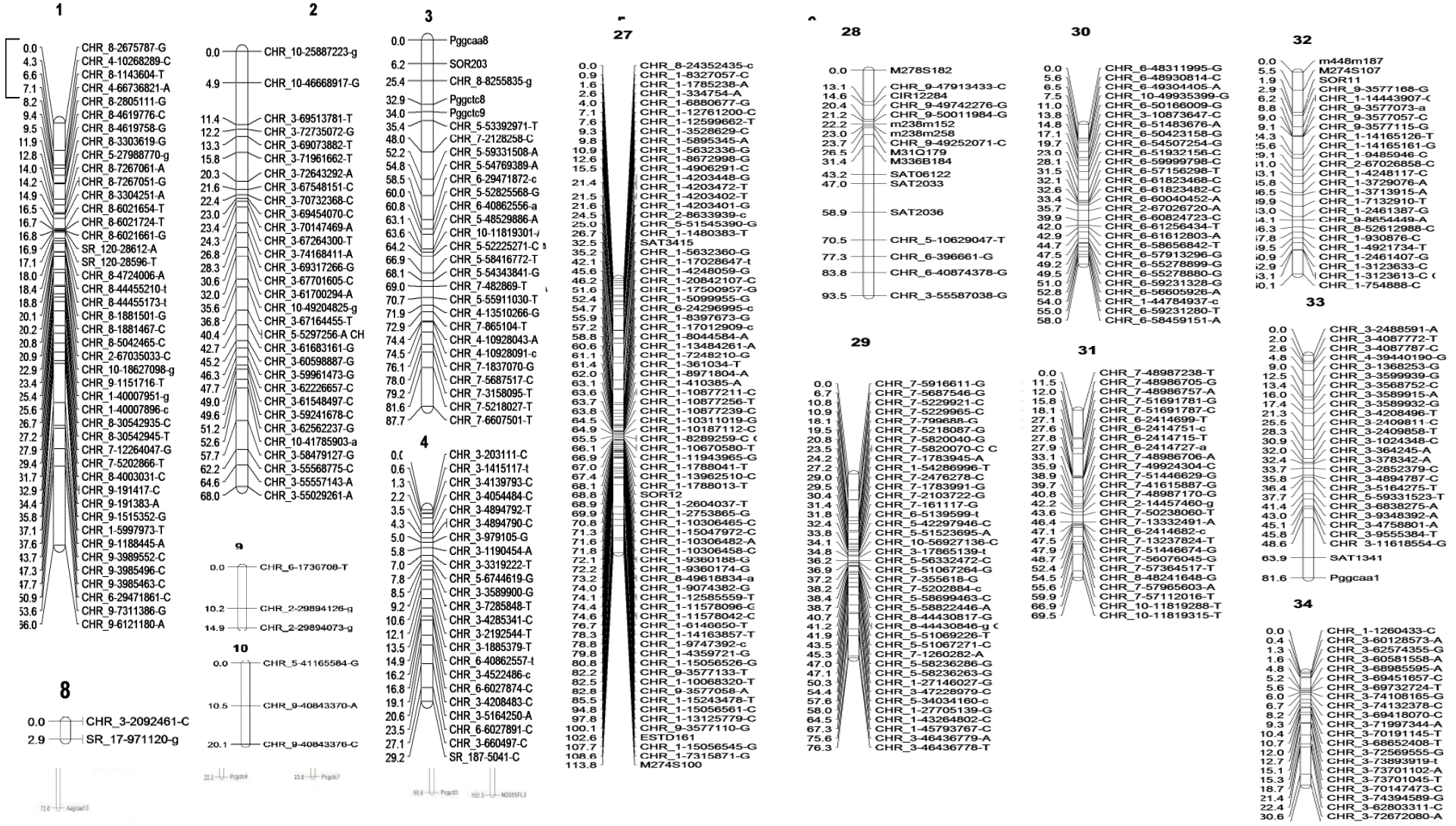
SNP variants identified in both parents from the same RADtag read

- Very useful for comparative mapping and assembling composite maps

Linkage Map Construction

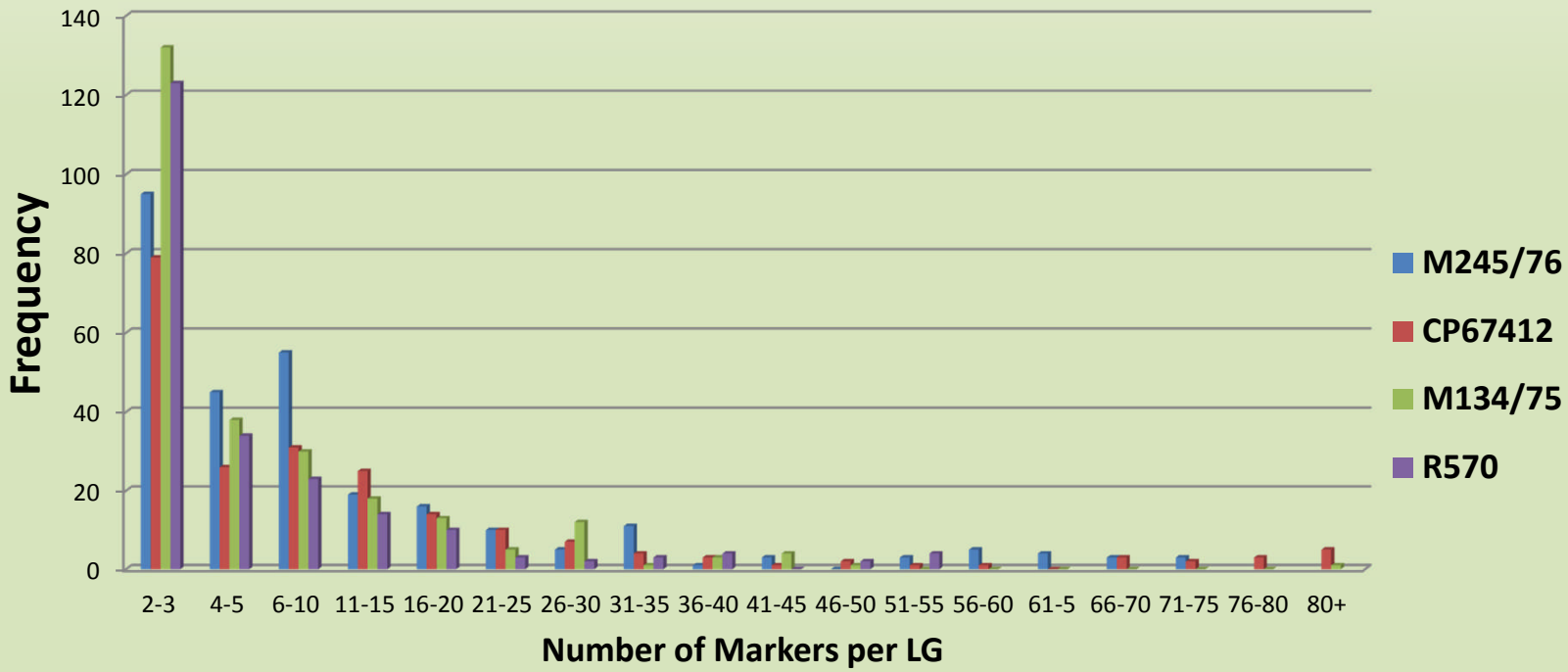
Construct linkage map of R 570, CP 67412 and M 245/76 using RADseq single dose markers using Joinmap

M 134/75; combine 528 AFLP and SSR markers to RADseq markers

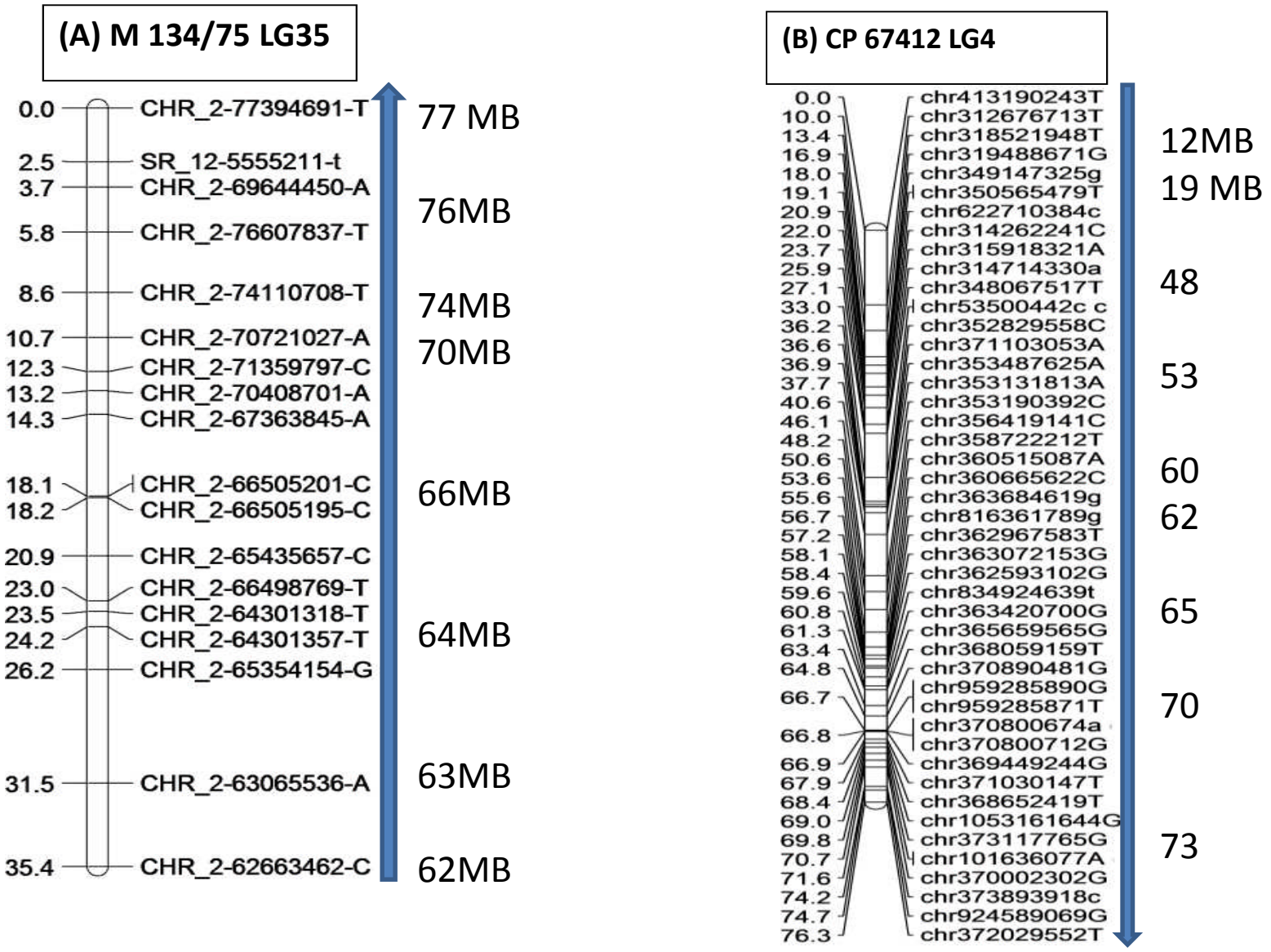


Linkage Map Construction

	Mapping Parents			
	CP 67412	**M 245/76**	**M 134/75**	**R 570**
Total size (cM)	8103	6609	8277	4779
Number of markers	3293	3066	2048	1768
Number of linkage group	233	281	286	222
Average markers distance (cM)	2.4	2.15	4.06	2.7

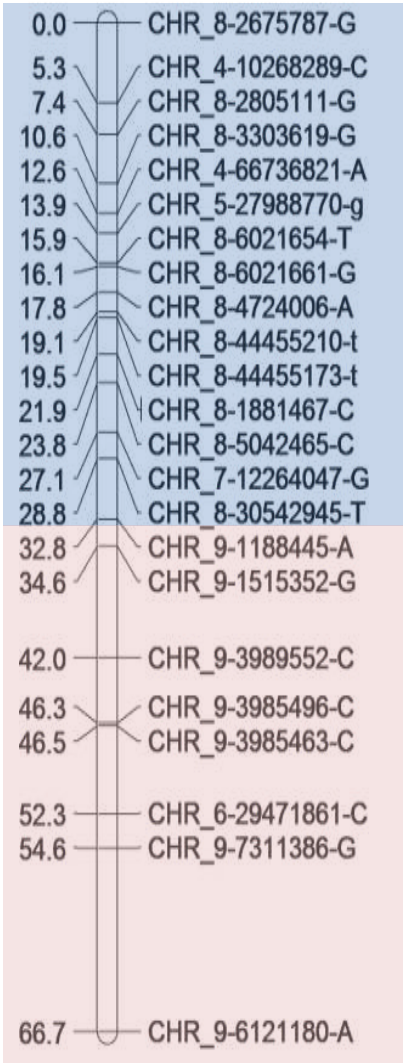


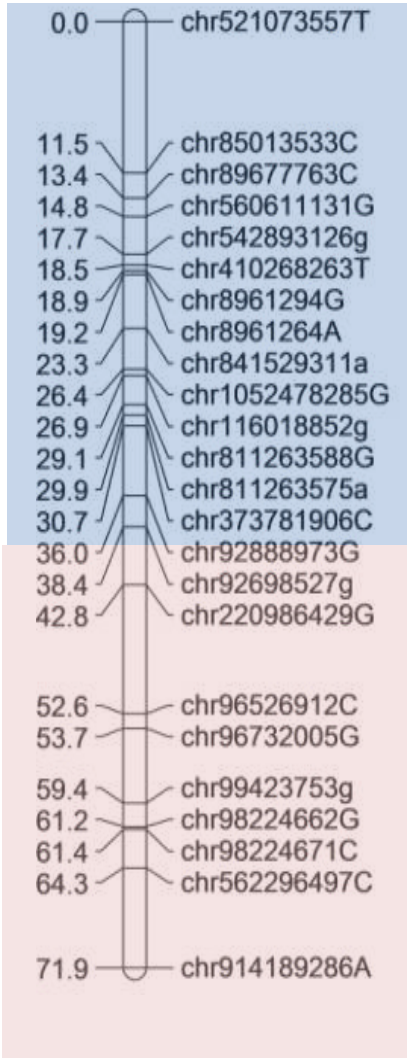
RADseq Marker Collinearity



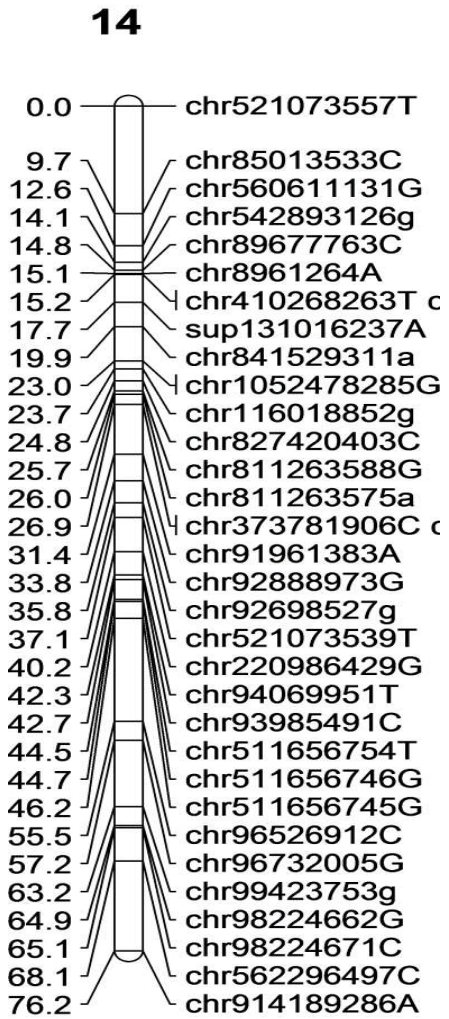
Linkage mapping

Fused chromosomes

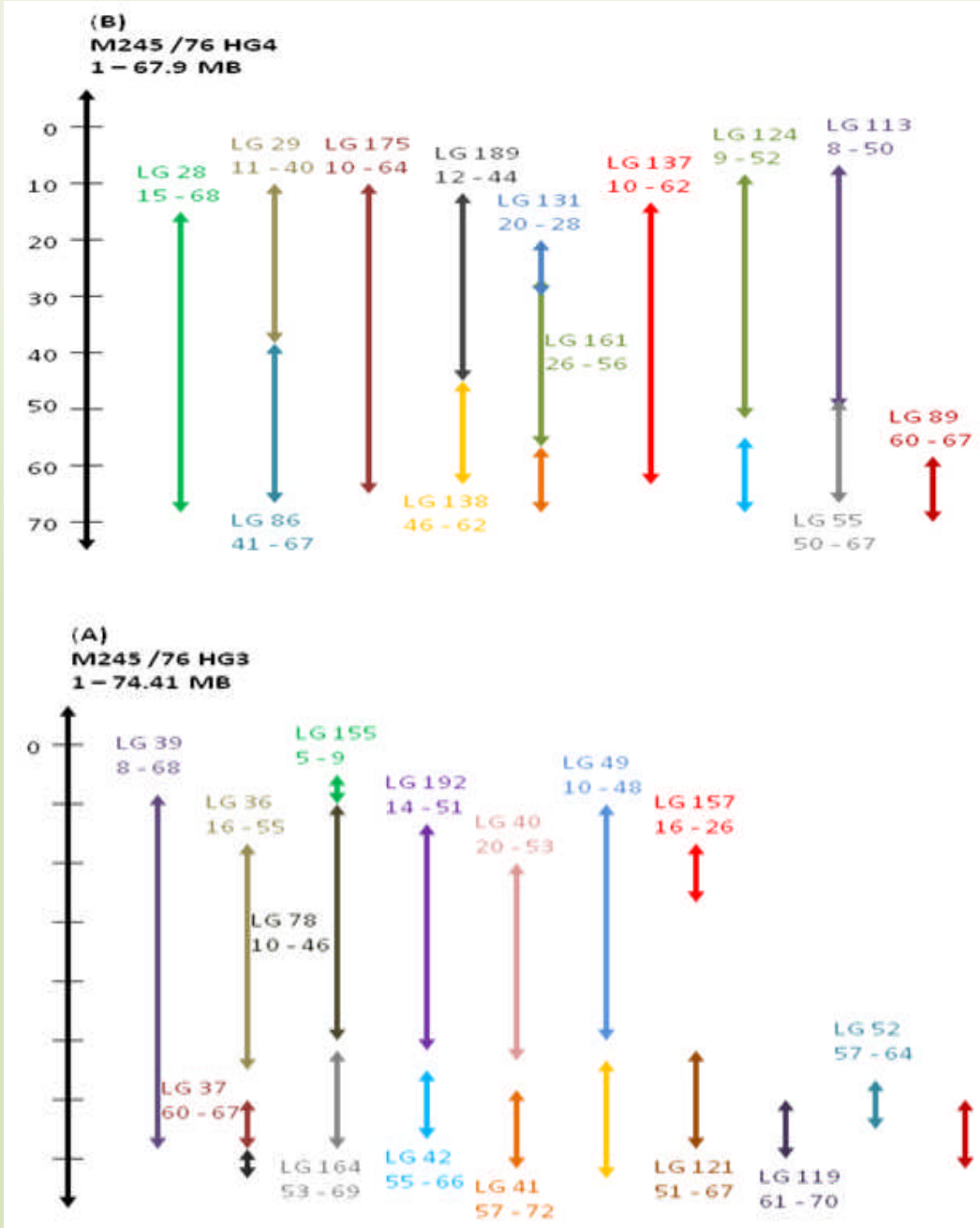


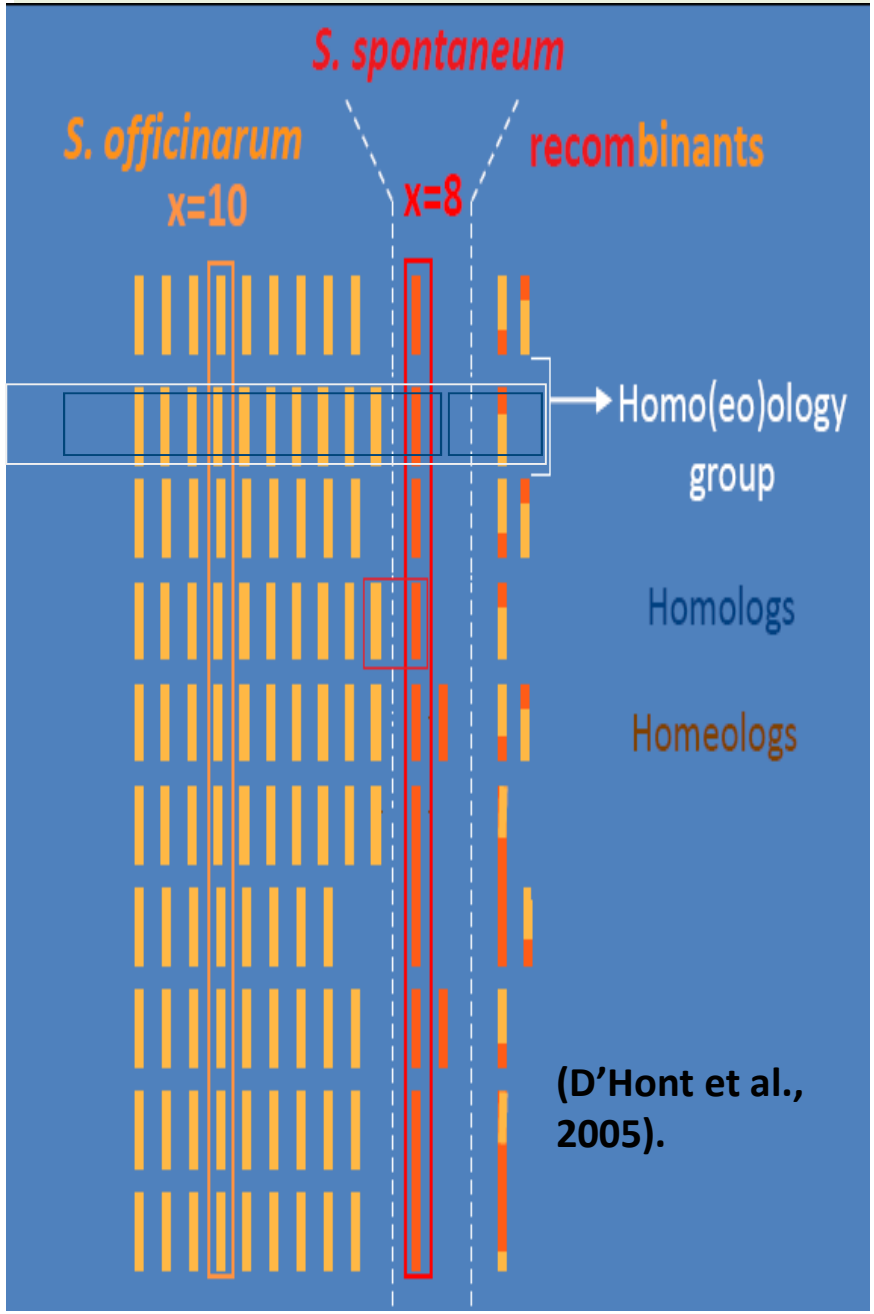


Undetermined origin



Genome Coverage

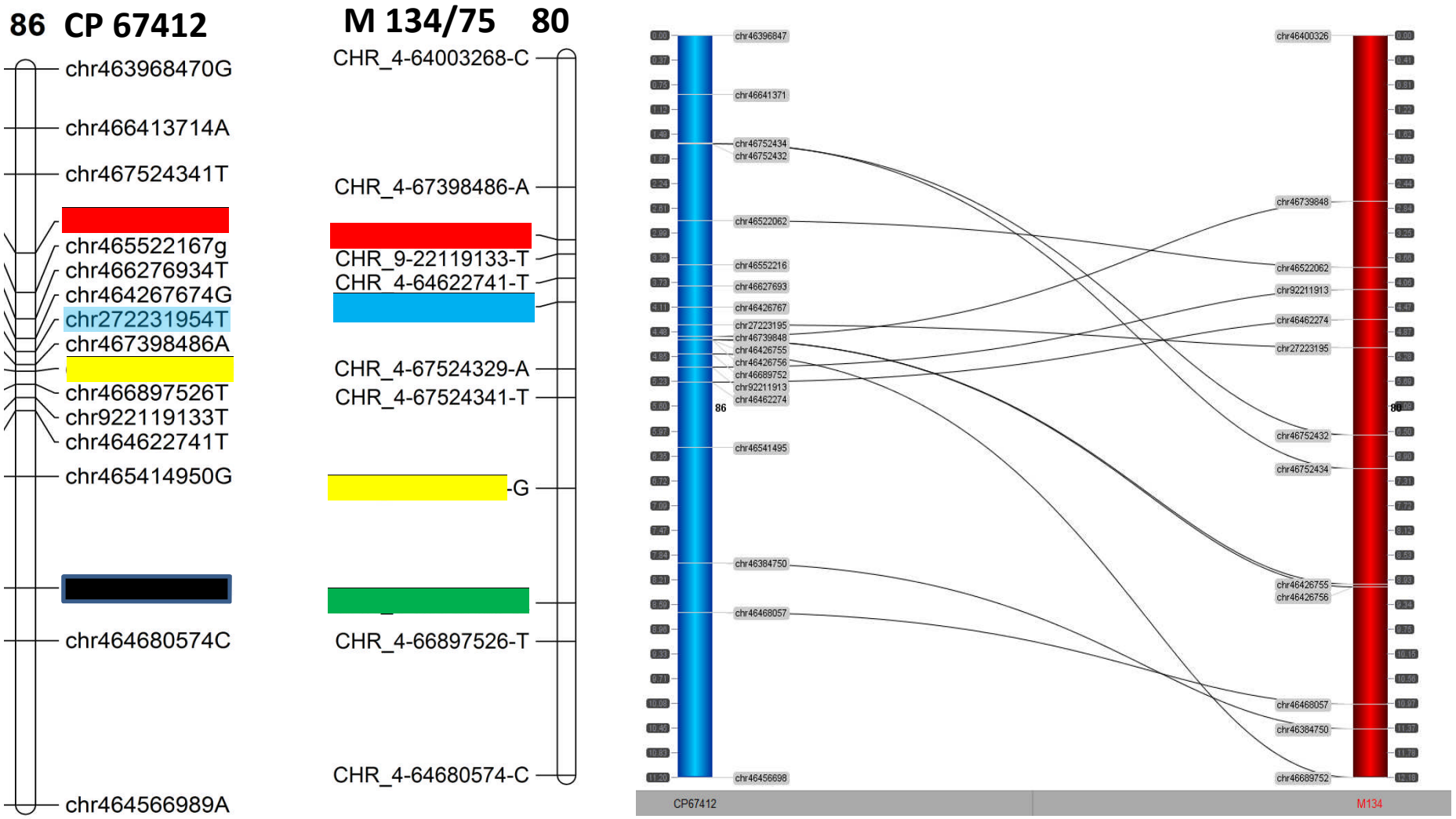




(D'Hont et al., 2005).

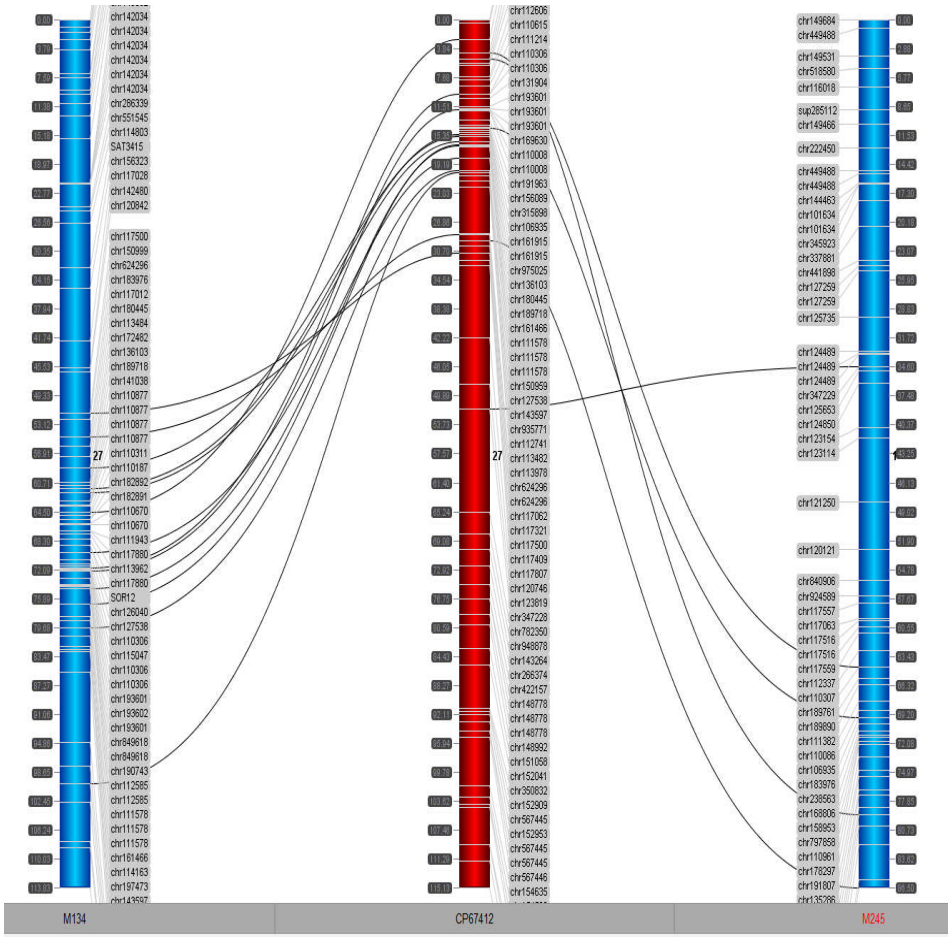
Comparative Mapping

Identify LGs with common markers between mapping parents
Assess level of synteny between LGs

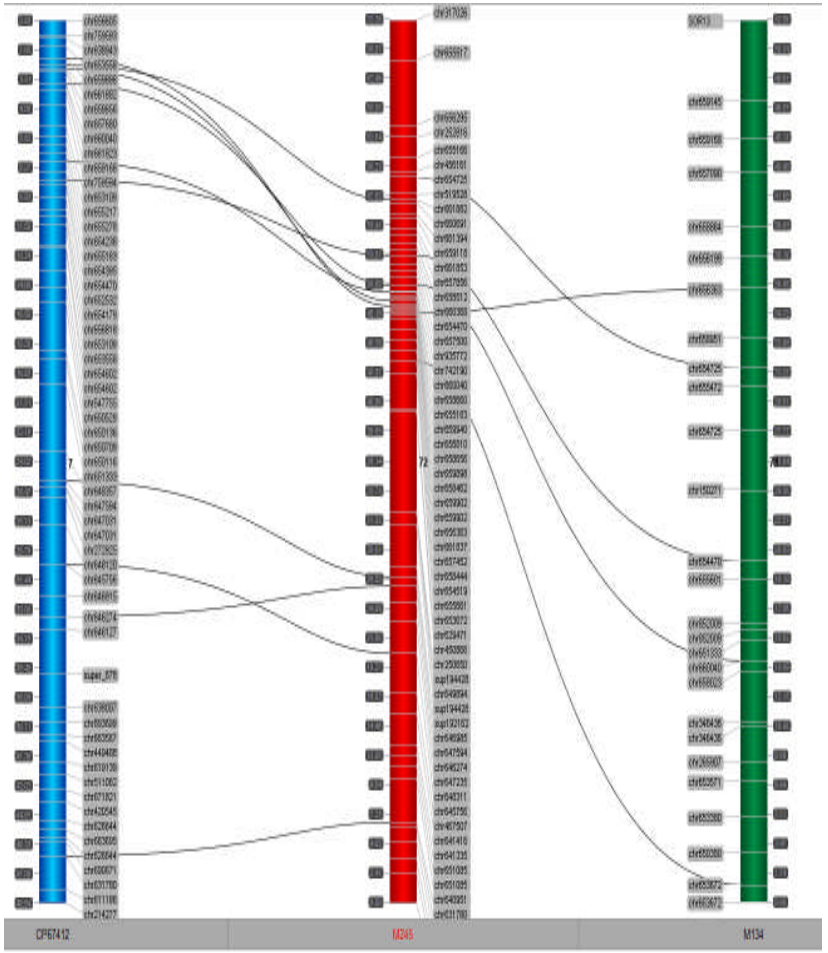


Comparative Mapping

M 134/75 CP 67412 M 245/76



CP 67412 M 245/76 M 134/75



Comparison between similar LGs from 3 varieties

Merits of RADseq

Wet lab:
Carried out within a period of 6 months from DNA extraction to scoring of marker across mapping population.
Lower cost of consumables

Marker Scoring:
- **Automated** SNP calling
- Marker scoring highly **accurate**

Bi-parental cross: Enable simultaneous scoring of both parents

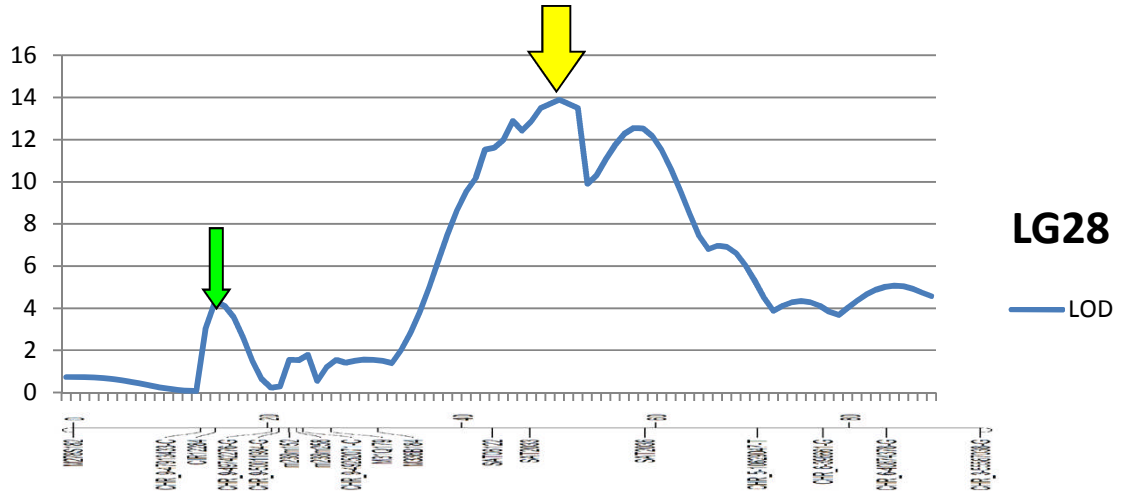
Collinearity:
Direct interpretation of SNP markers to physical location on sorghum map. Comparative mapping made easy

Marker information:

Direct information of sequence flanking SNPs. Useful for sequence manipulation eg PCR marker development

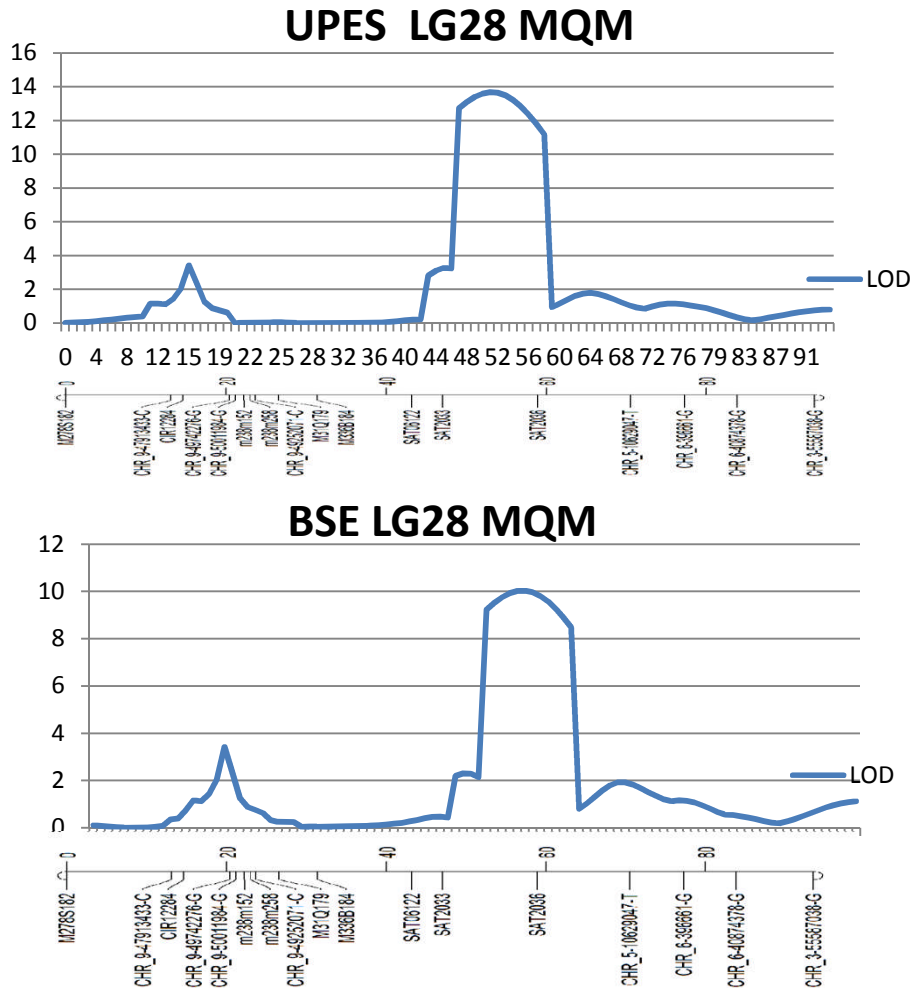
Limitations
- Use of sorghum genome as reference
- Missing data

QTL Mapping (SIM) For YSDR



Group	Position	Locus	LOD	mu_A	mu_H	% Expl.	Additive	GIC
28	0	M278S182	0.73	12.0	8.1	2.3	3.9	1.00
28	13	CHR_9-47913433-C	0.08	10.2	8.7	0.3	1.5	0.99
28	15	CIR12284	4.31	14.7	5.5	12.9	9.2	1.00
28	20	CHR_9-49742276-G	0.23	10.5	8.0	0.7	2.5	0.97
28	21	CHR_9-50011984-G	0.29	10.6	7.7	0.9	2.9	0.92
28	22	m238m152	1.54	12.7	7.1	4.8	5.6	1.00
28	24	CHR_9-49252071-C	0.55	10.9	7.2	1.7	3.7	0.98
28	26	M31Q179	1.42	12.8	7.4	4.4	5.5	0.99
28	31	M336B184	1.4	12.7	7.3	4.4	5.4	0.99
28	43	SAT06122	12	17.5	3.6	28.7	13.8	0.99
28	47	SAT2033	12.9	18.2	3.3	32.8	14.8	0.99
28	59	SAT2036	10.31	18.0	4.1	28.1	13.9	0.99
28	71	CHR_5-10629047-T	6.81	15.4	3.9	19.6	11.6	0.96
28	77	CHR_6-396661-G	3.87	14.3	5.4	11.6	8.9	0.96
28	84	CHR_6-40874378-G	3.68	13.7	4.7	11.1	9.0	0.91
28	94	CHR_3-55587038-G	4.57	4.6	141.3	9.9	0.9	0.93

Stability Of YSDR QTL Between Environments (MQM)



Validate markers SAT2033, SAT2036 and SAT06122 with

- A second population derived from the cross M 134/75 x R570 and
- Germplasm Collection

QTL Mapping –early ripening

- 300 individuals RADseq processed

- Trait scores:
  - Sucrose accumulation
  - Fibre content
  - Tillering phase
  - Flowering habit
  - Cane elongation

Acknowledgements

MSIRI Management

European Union : ACP-SRP scheme

Ray Ming: University of Illinois at Urbana-Champaign

Fine Mapping of YSDR QTL

