

Accelerating development and use of genomic and genetic resources in chickpea to improve crop productivity in Asia and sub-Saharan Africa



3000 -

RLD (cm cm ⁻³)	Moderate drought		Severe drought	
	YLD	HI	YLD	HI
0-15cm	0.344	0.354	0.442	0.404
15-30cm	0.699 *	0.672 *	0.718 **	0.709 **
30-45cm	0.406	0.544	0.779 **	0.616 *
45-60cm	0.405	0.496	0.576 *	0.355
Total	0.613 *	0.681 *	0.659 *	0.565

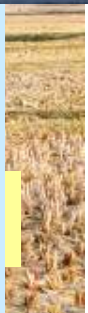


Total pr

Low tem

Ascochy

Pod boreal



Precise and high throughput phenotyping



Crane for lifting root cylinders for moisture under water stress determinations

Experiment of chickpea root and growth in ROS in 2008-2009

Insect resistance phenotyping

Natural field conditions

Detached leaf assay



- Data recorded on
- damage rating
 - larvae counted on plants
 - overall resistance score
 - pod damage
 - grain yield

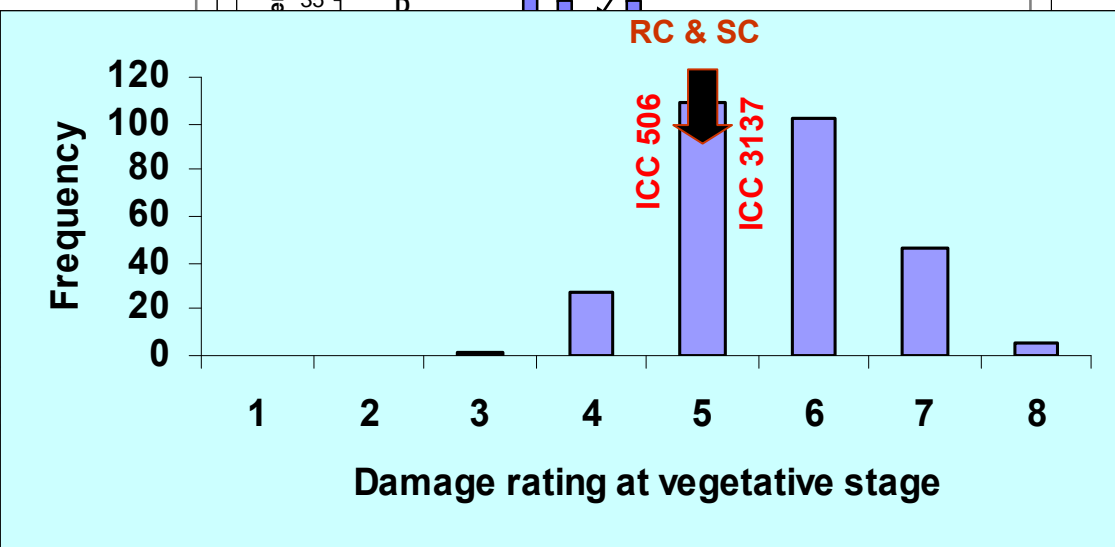
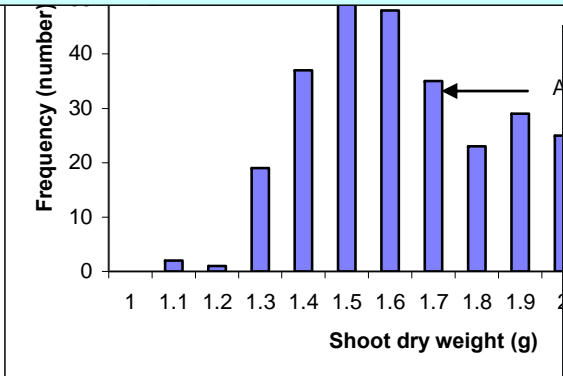
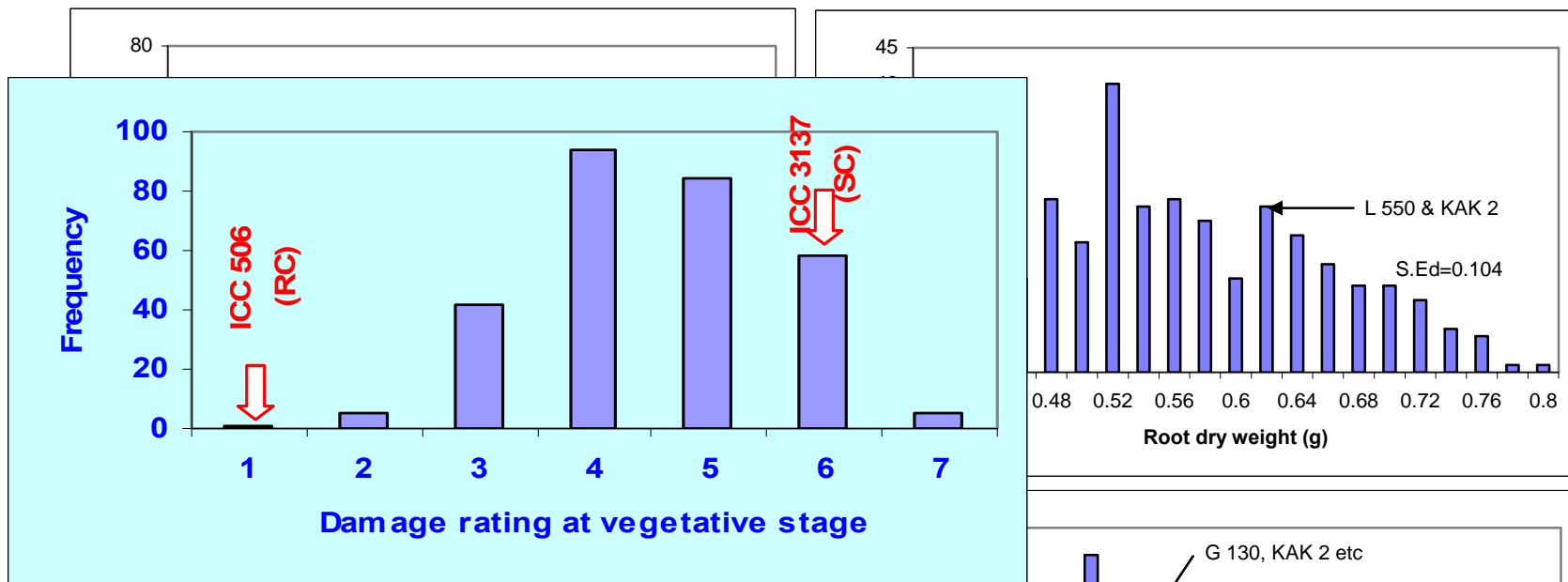
- cut branches placed on agar plate
- counted number of 10 larvae released on cut branches
- observations recorded on leaf damage, larval survival and weight gained by insects

Genetic resources and phenotyping

- Reference collection (300 lines)
 - drought related traits e.g. root traits, HI, yield
 - insect resistance in both field and lab conditions
 - $\delta^{13}C$, SLA and SCMR (see Poster # 3.16)
- Interspecific population (131 RILs)
 - phenotyped for insect resistance
- Two intraspecific populations
 - ICC 4958 x ICC 1882 -264 RILs
 - phenotyped for root traits, HI and 13-C
 - ICC 283 x ICC 8261- 281 RILs
 - phenotyped for root traits and HI

Phenotyping completed for 2 seasons: 2007/08 and 2008/09

Reference collection phenotyping @ Patancheru



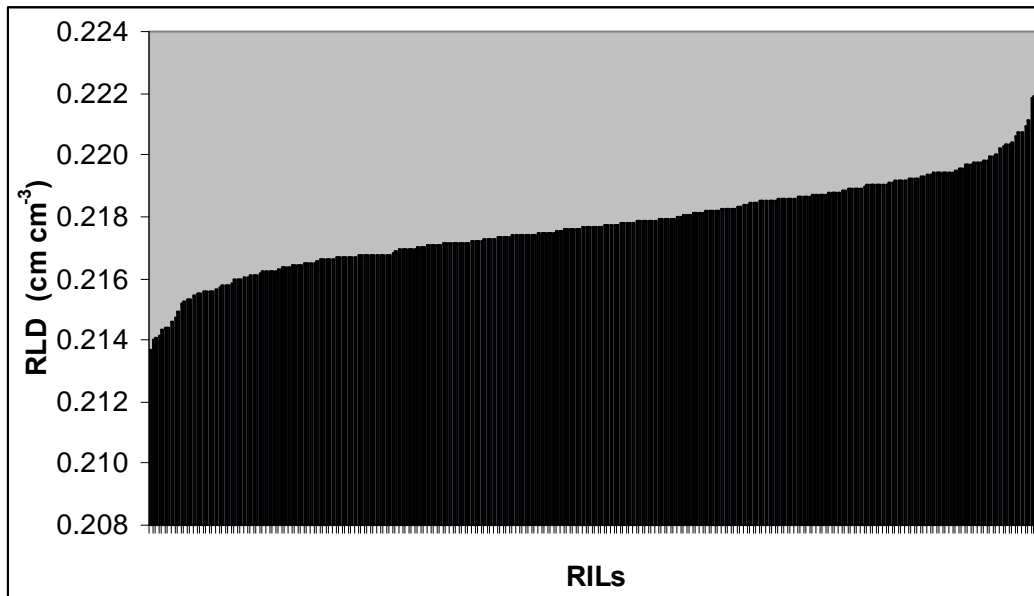
Range for (A) maximum root weight (g) and (D) root length post-rainy season 2008-09

A snapshot of phenotyping by Egerton Univ (Paul Kimurto)

Genotype	yield	HI
ICCC1052	4409	0.55
ICC4958	3440	0.54
ICCRIL03-0215	2125	0.51
ICCRIL04-0239	3313	0.49
ICCRIL03-0216	3247	0.48
Annigeri	4135	0.47
ICC15333	4276	0.47
ICCRIL03-0177	2913	0.45
ICCRIL03-0135	3617	0.45
ICCRIL03-0168	1939	0.45

See Poster 3.12

Intraspecific population (ICC 4958 x ICC 1882)



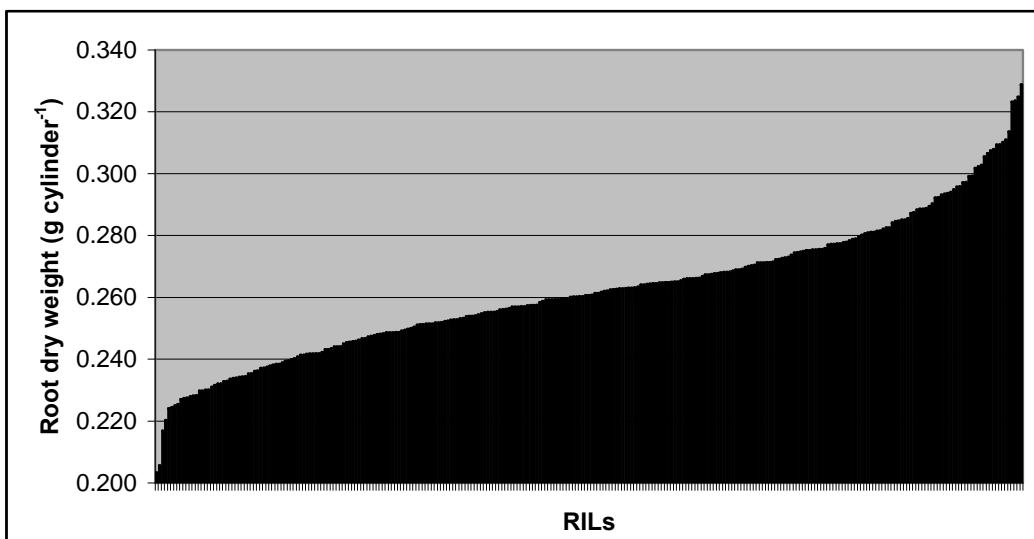
<i>Accession</i>	<i>Root depth (cm)</i>	<i>Root dry wt (g)</i>
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ICC 8261	123.3	1.25
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ICCV 283	91.6	0.73
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ICC 4958	116.6	1.06
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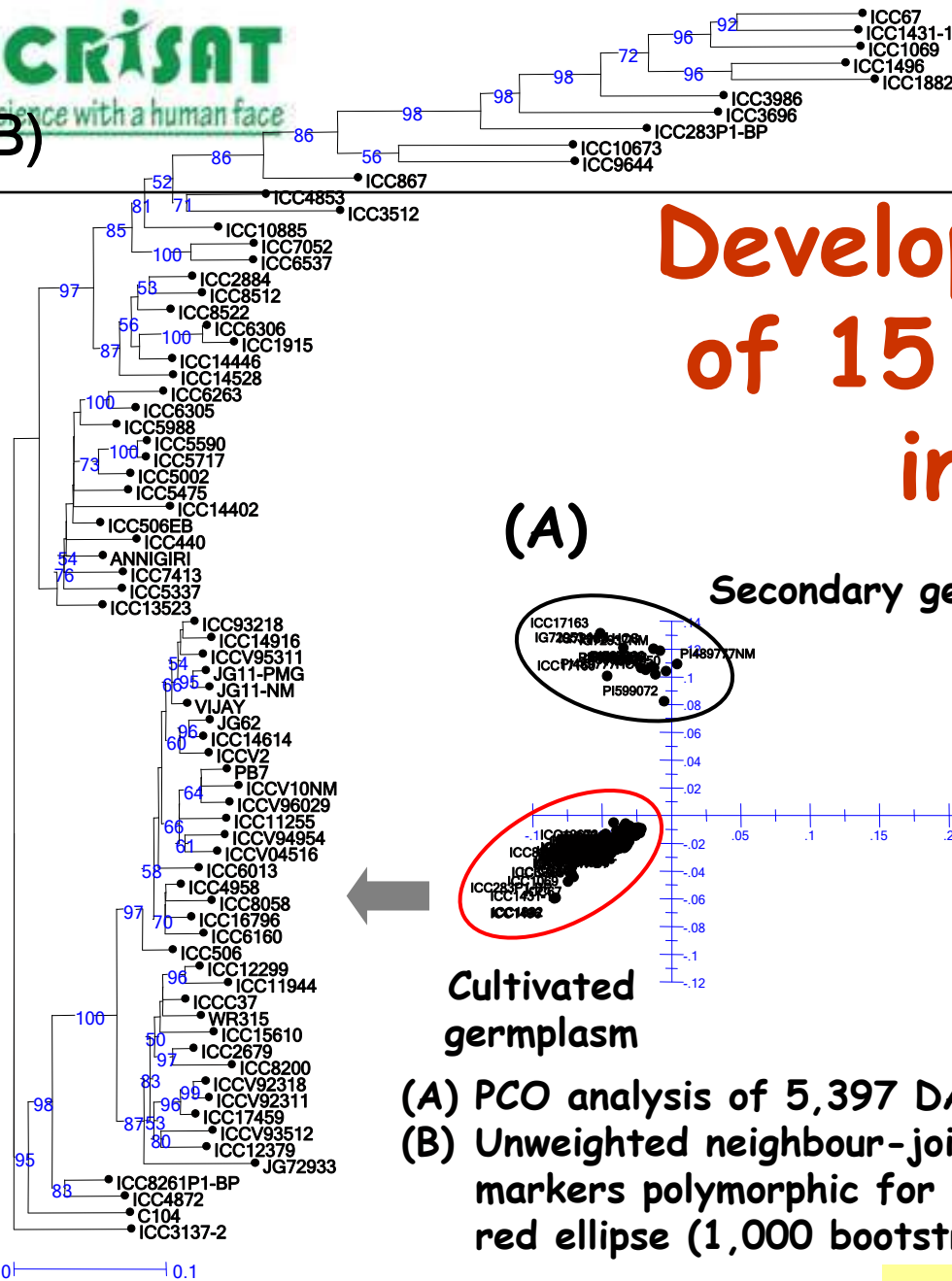
ICC 1882	83.9	0.71
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**Already phenotyped
for two environments**

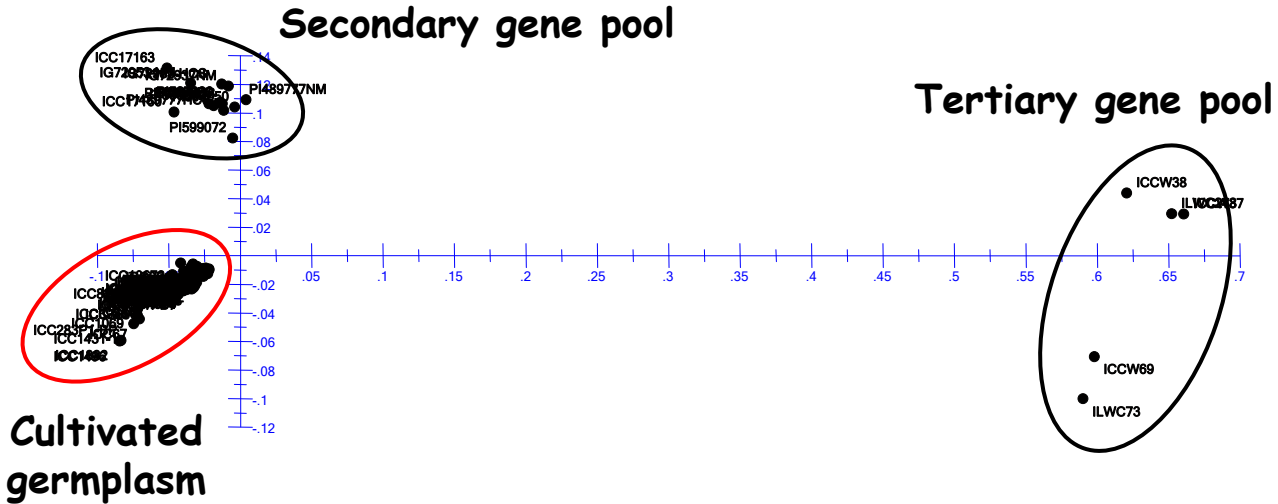
library	SSR enriched sequences	BAC-end
Number of clones	288	25,000
Sequences surveyed	457	46,270
Amount seq Data (bp)	286,718	33,217,120
SSRs identified	643	6,845
SSR frequency	1 / 4.5 kb	1/ 4.9 kb
Primers designed	311	4,964
Primers synthesized	311	1,344
Primers tested	311	1,344 total 1,655

(B)



Development and use of 15 K DArT array in chickpea

(A)



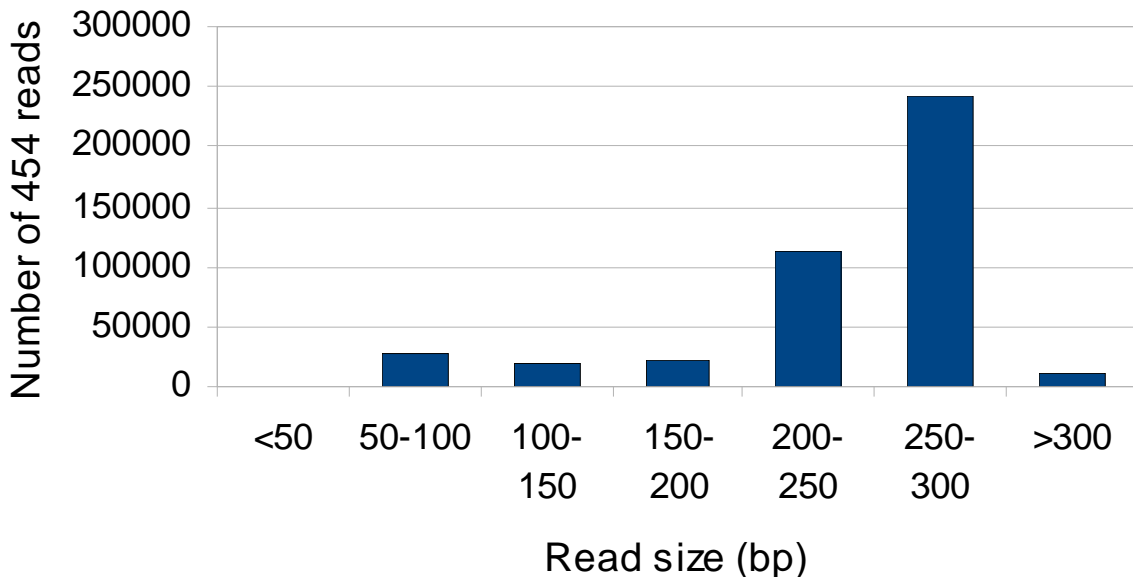
- (A) PCO analysis of 5,397 DArT markers
- (B) Unweighted neighbour-joining dendrogram based on 901 DArT markers polymorphic for the set of 75 cultivated lines in the red ellipse (1,000 bootstrap replicates)

in collaboration with A. Killian

Trait/Genotype	Total ESTs	High quality ESTs	Unigenes
Drought :			
ICC 4958	5982	5461	2656
ICC 1882	5919	5535	2392
Total Drought responsive (ICC 4958 + ICC 1882)	11901	10996	4558
Salinity:			
JG 11	3798	3132	1707
ICCV 2	4460	4307	1251
Total Salinity responsive (JG 11 + ICCV 2)	8258	7439	2595
ESTs from public domain	7097		4059
Total Chickpea ESTs	27257	25532	9569

Cataloguing chickpea genes !

C1



C9



C



issues	
eryo	
ys old seedlings	
ys old seedlings	
ys old plant	
ves	
old plants	
essed	

Total numbers of FLX ESTs : 443,969

Average read length : 221 bp

Number of ESTs with >200 bp : 347,583

Transcript Analysis (Sanger + 454 FLX ESTs)

Number of contigs : 42,852

Pooled F
develop
generat
subject
at JCVI



Drought sensitive ICC 1882



Drought tolerant ICC 4958

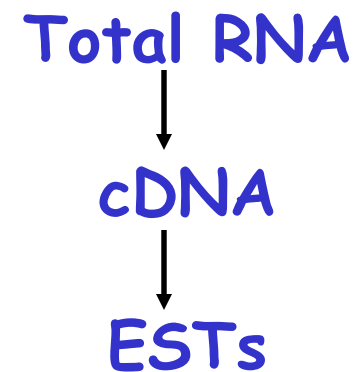


Comparison of ICC 1882 and ICC 4958 differing in root traits

15-days old plants in hydroponic solution

Green house conditions

Field grown materials



Analysis of Solexa sequencing data

http://chickpea-test.ncgr.org - pileup - Mozilla Firefox

gnl|lis|1655300|gb|AJ884609.1 | case: ICC1882

variant filter

Restrict where at least reads call variant SNP nsSNP in/del

Restrict where at least uniquely aligned reads call variant variant in coding region

Restrict where at least reads cover position variant causes premature stop

Restrict where between % and % of reads call variant variant not near homopolymer

Read quality average Max.

UPDATE CLEAR

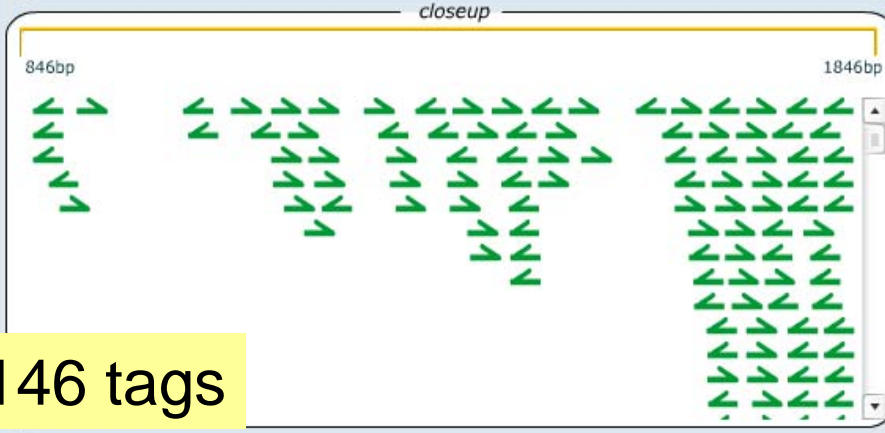
overview

SNP nsSNP in/del CDS/exon ambiguous alignment

0 1846

closeup

846bp 1846bp



146 tags

Transferring data from chickpea-test.ncgr.org...

http://chickpea-test.ncgr.org - pileup - Mozilla Firefox

gnl|lis|1655300|gb|AJ884609.1 | case: ICC4958

variant filter

Restrict where at least reads call variant SNP nsSNP in/del

Restrict where at least uniquely aligned reads call variant variant in coding region

Restrict where at least reads cover position variant causes premature stop

Restrict where between % and % of reads call variant variant not near homopolymer

Read quality average Max.

UPDATE CLEAR

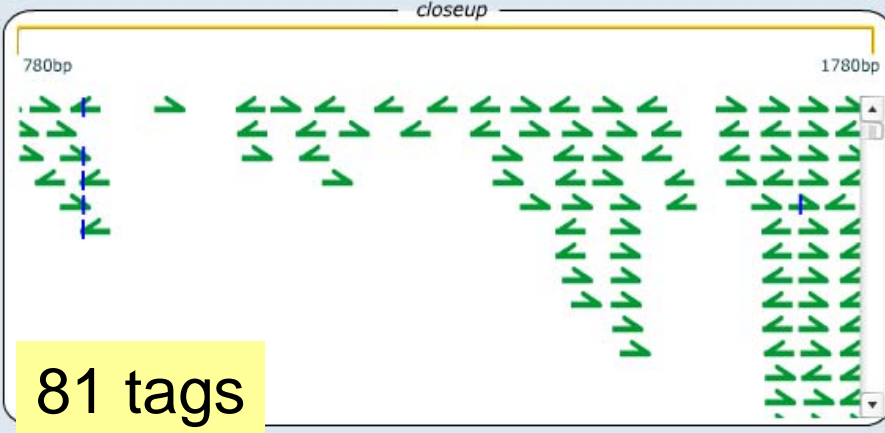
overview

SNP nsSNP in/del CDS/exon ambiguous alignment

0 1846

closeup

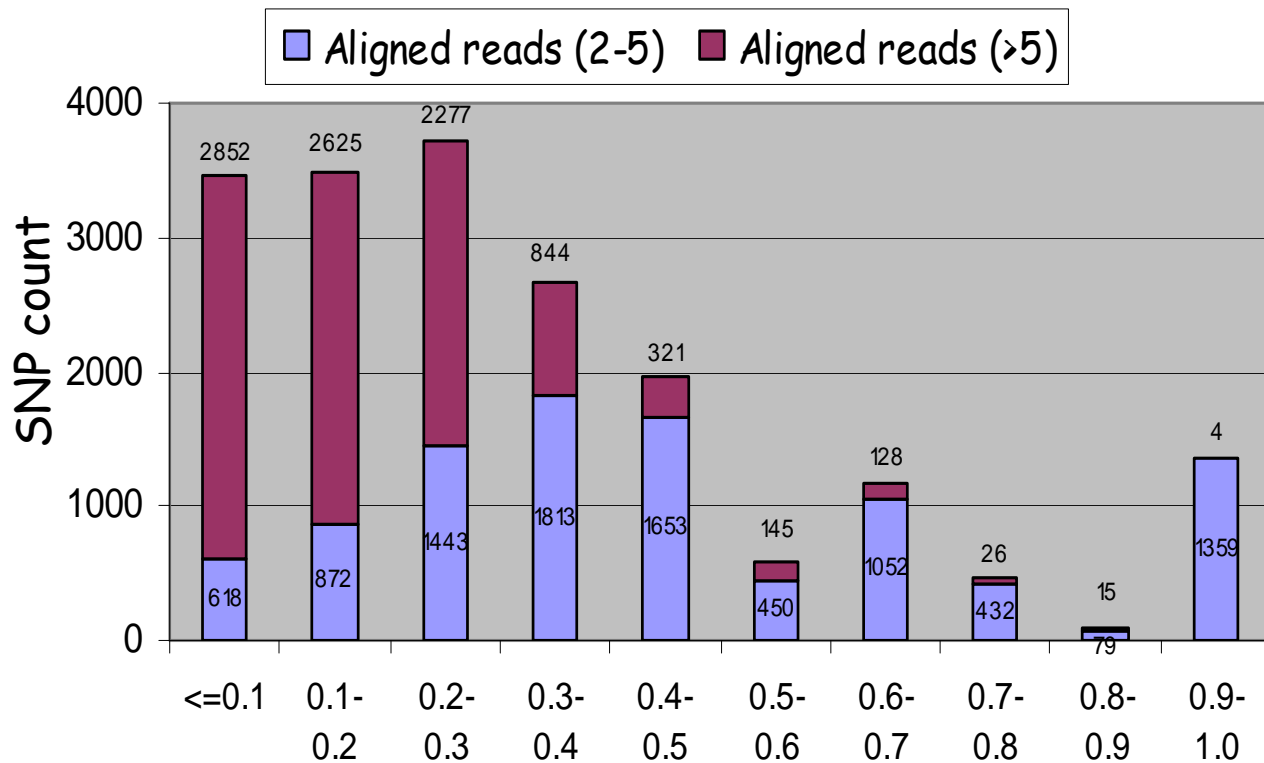
780bp 1780bp



81 tags

Transferring data from chickpea-test.ncgr.org...

Distribution of SNPs in ICC 4958/ICC 1882



SNPs identified: 26,082
 No. of contigs: 6,908
 High quality SNPs: 9,237

Frequency difference of reference allele between ICC 4958 and ICC 1882

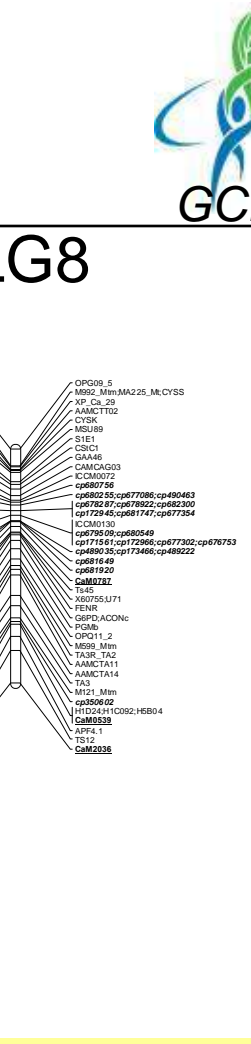
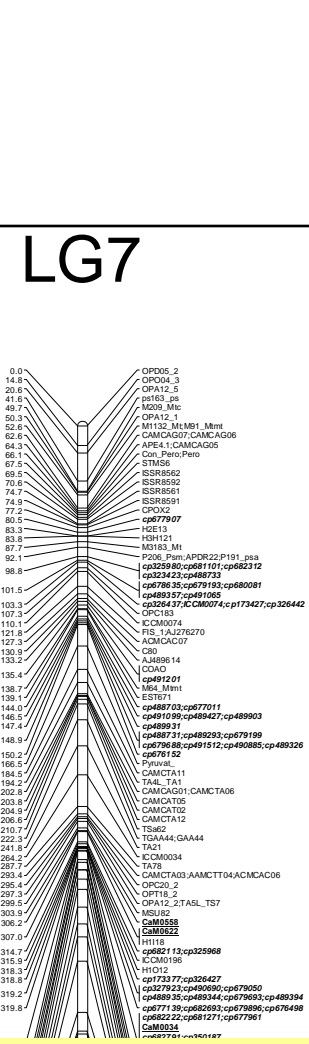
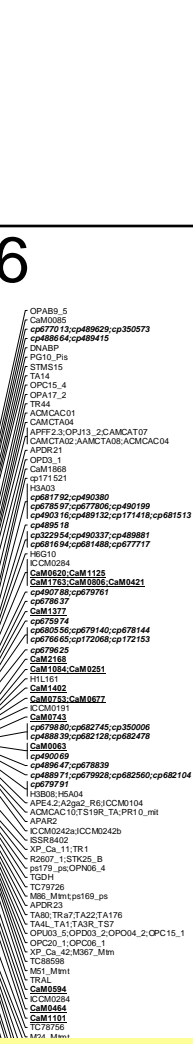
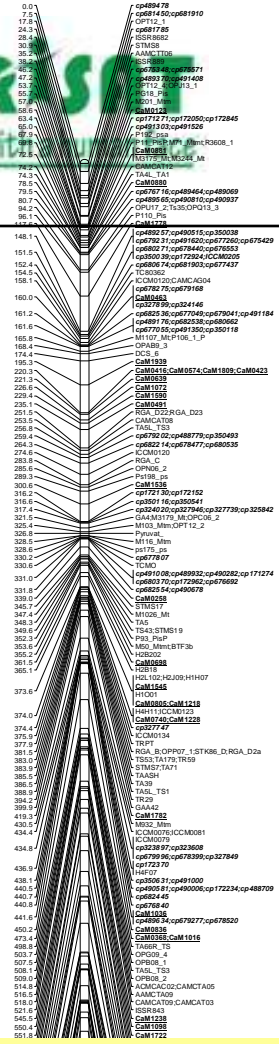
Illumina GoldenGate assay for 768 SNPs

LG5

LG6

LG7

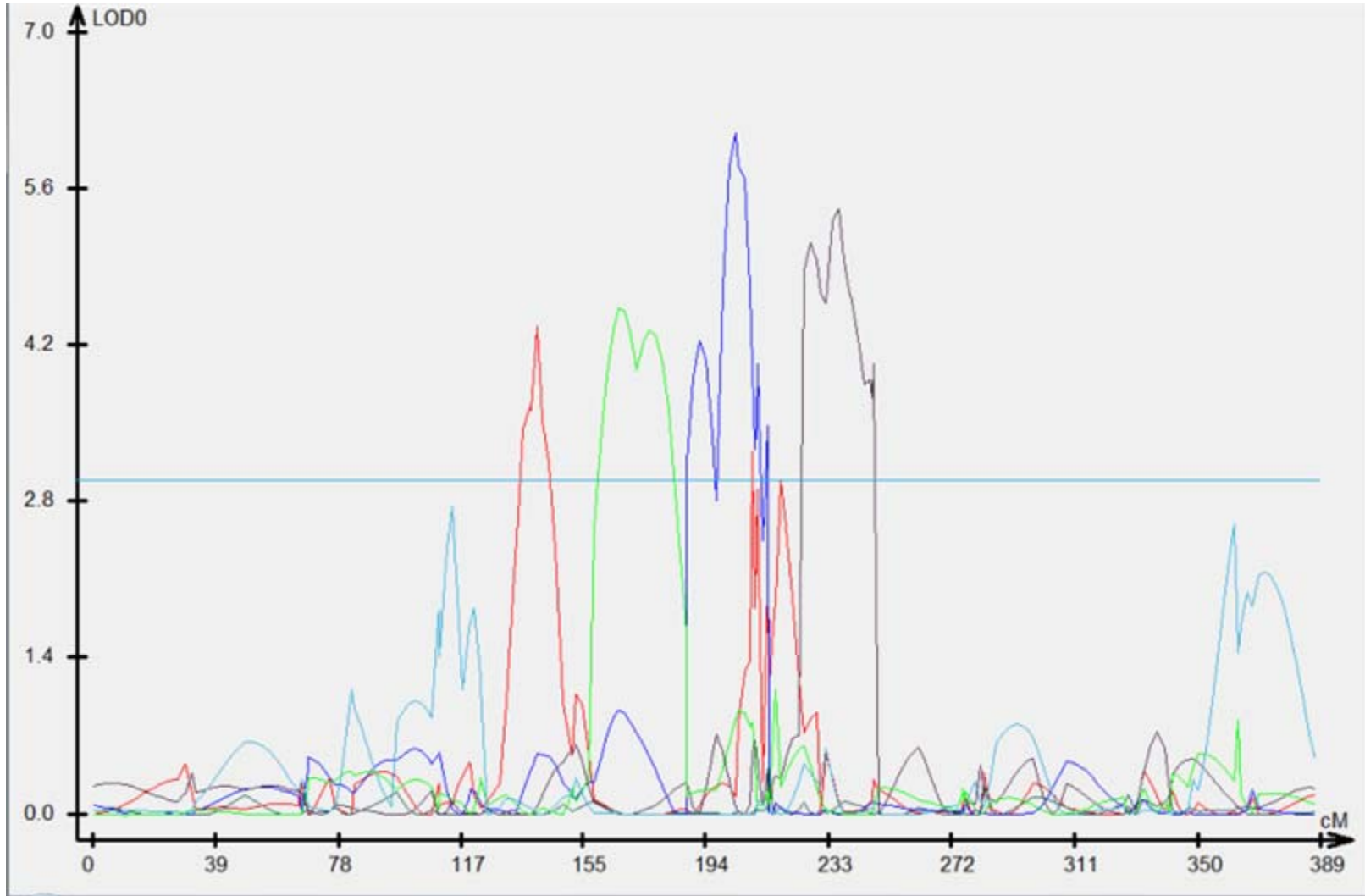
LG8



- ❖ Average number of marker loci per linkage group: 174
- ❖ Total map distance: 4243.8 cM
- ❖ Average inter-loci distance: 3.05 cM

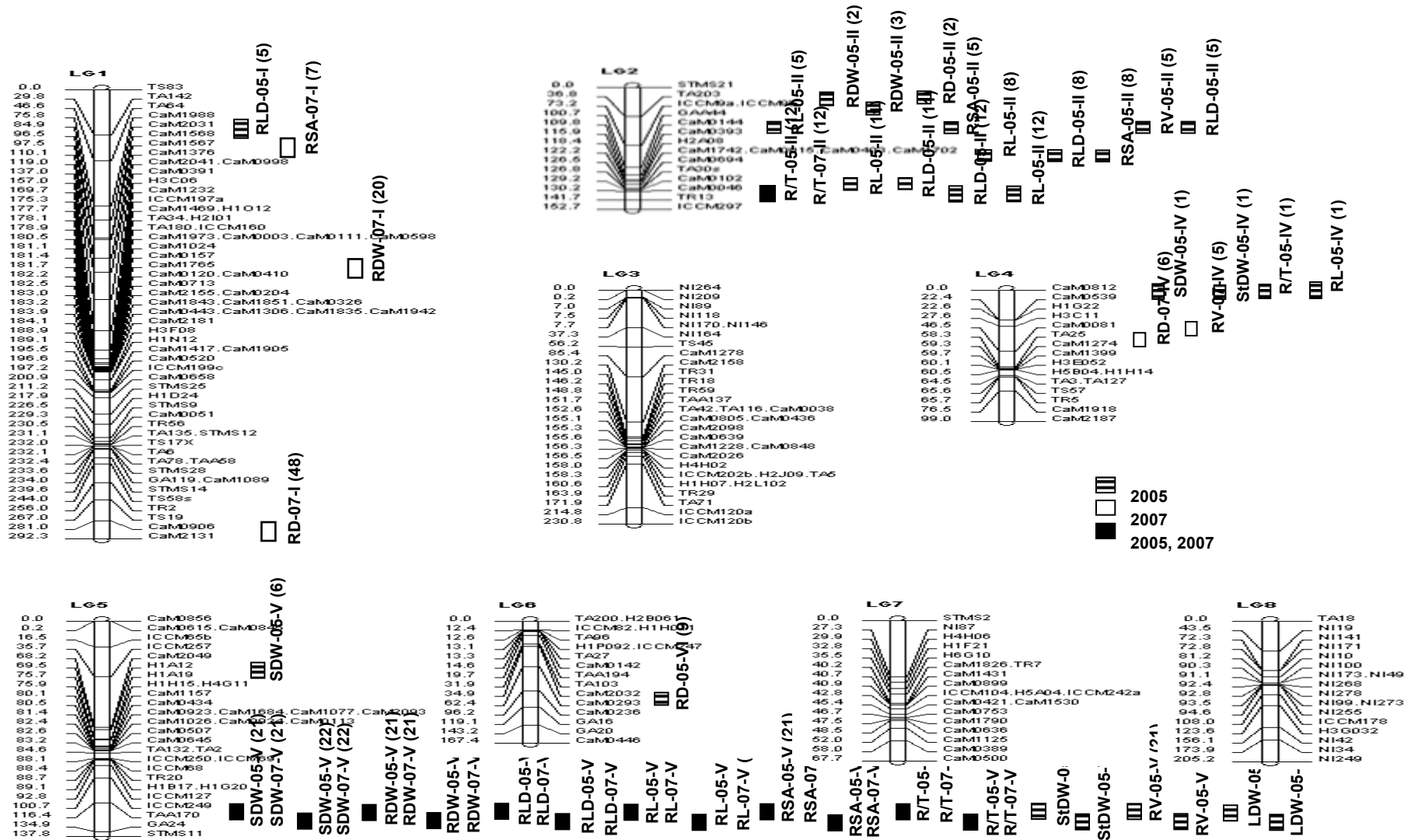


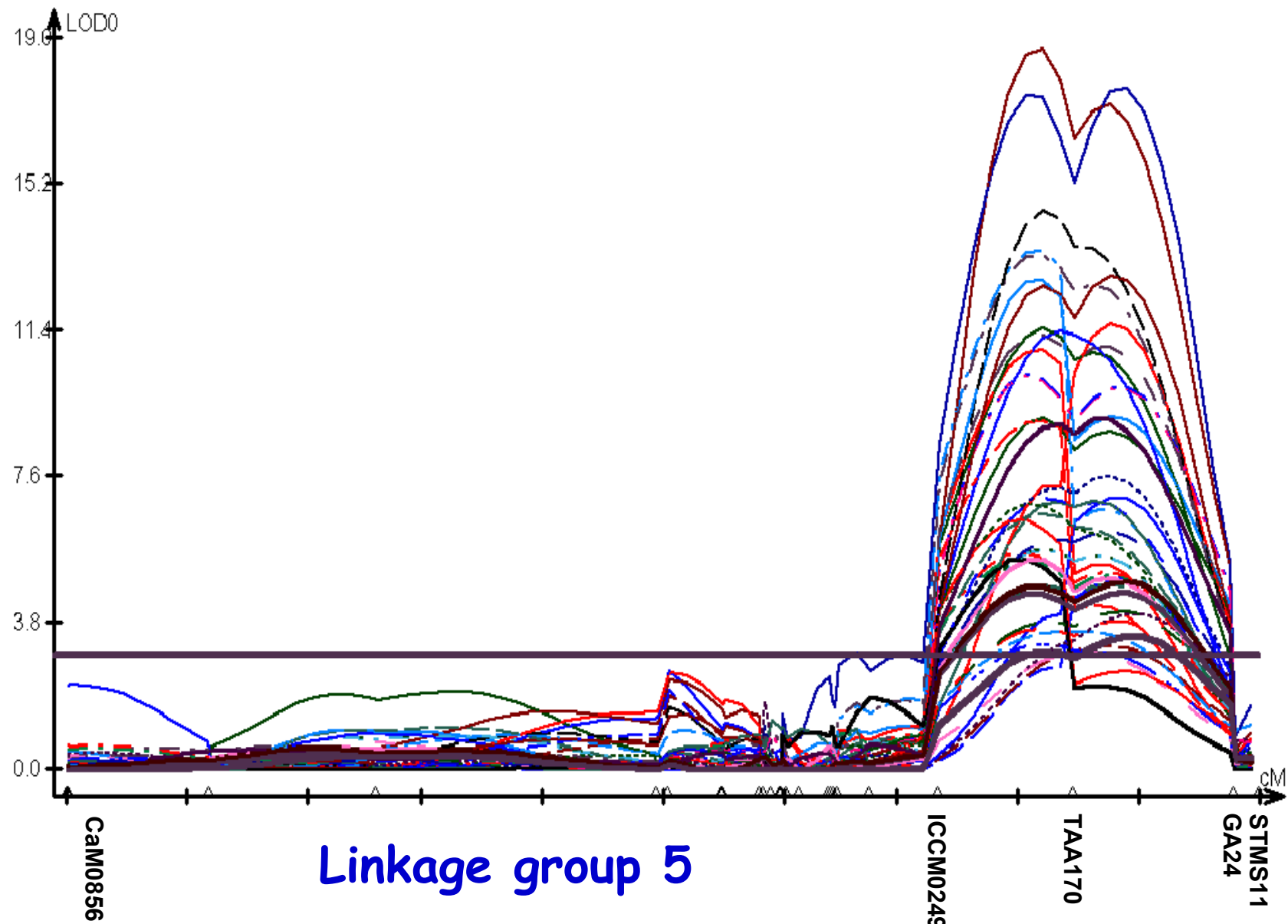
QTLs for insect resistance (ICC 4958 X PI 489777)



Chromosome 4

Root trait QTL map (ICC 4958 x ICC 1882)





Linkage group 5

**Grain Yield
(under drought) = T x TE x HI**

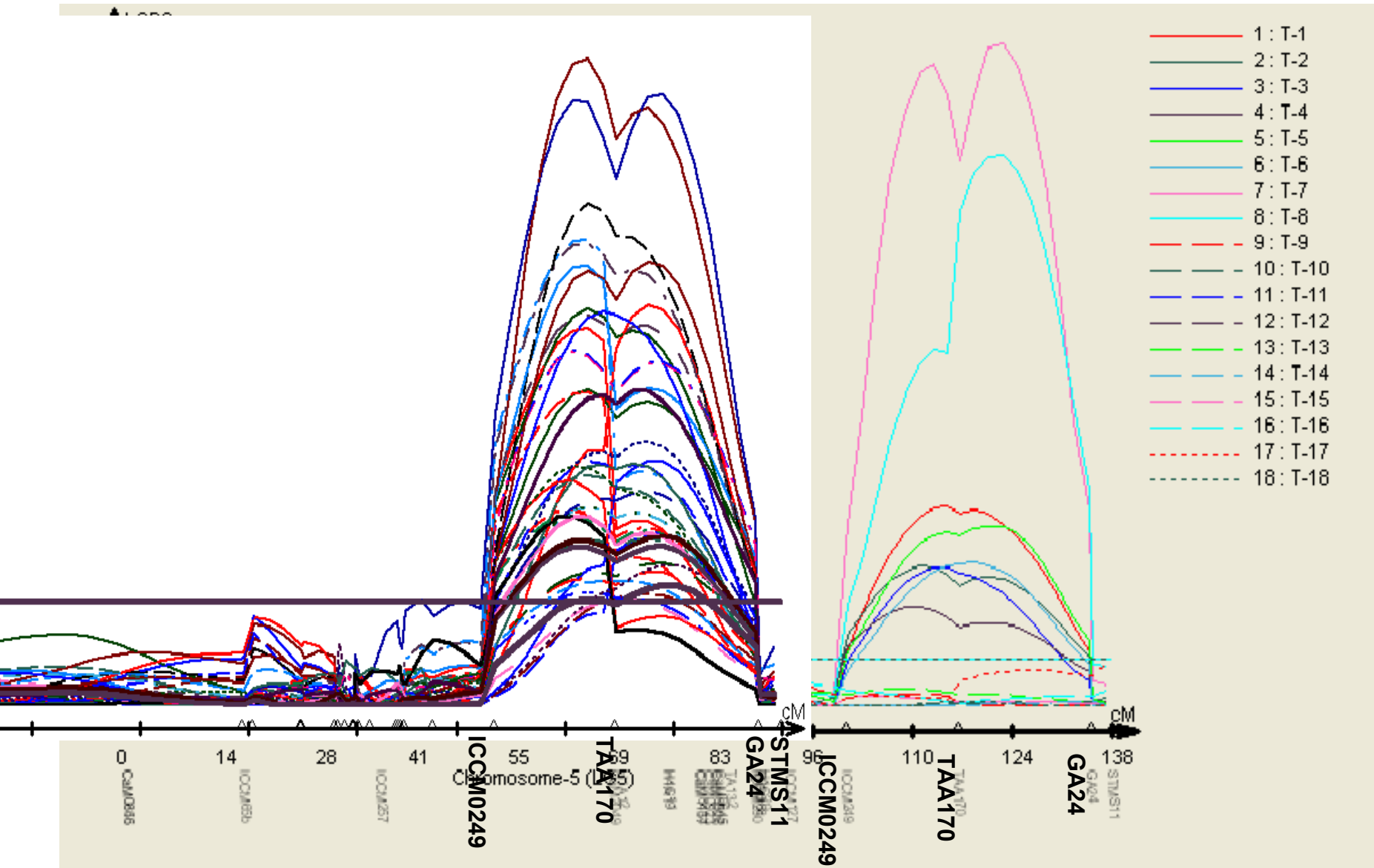
Passioura (1977)

ICC 4958 x ICC 1882 RILs have been evaluated for HI, grain yield and $\delta^{13}\text{C}$ at 4 locations during 2008/09

Correlation of grain yield with biomass, HI and $\delta^{13}\text{C}$

	Nandyal	Patancheru	Sehore	Durgapura
Biomass yield	0.98**	0.84**	0.96**	0.76**
Harvest Index	0.82**	0.41**	0.22**	0.97**
$\delta^{13}\text{C}$	-	0.23**	0.10	0.17**

QTLs in the same region...



MABC for improving drought tolerance

Crosses:

3 Cultivars x 2 Donors for root traits

Donors

BC1:

Cultivar x F1



BC1F1

BC 2:

Cultivar x BC1F1



BC2F1

Subjected to foreground and background selection

BC3:

Cultivar x BC2F1

As in BC 2



BC3F1

Selected heterozygous plants for QTL-linked markers and over 90% genome of the recurrent parent



BC3F2

Select homozygous plants for QTL-linked markers



BC3F3

Seed multiplication



Multilocation evaluation BC3F4 lines



Cultivars



MARS for improving drought tolerance

Five good-by-good crosses involving cultivars from Africa and Asia

Set 1 (2 crosses)

2008/09

Genotyping of F3 plants



F4 (Seed multiplication)



Multilocation evaluation of F5 progenies and QTL analysis



Selection and inter-crossing of F3s

Set-2 (3 crosses)

F2 grown



Genotyping of F3 plants



F4 (Seed multiplication)



Multilocation evaluation of F5 progenies and QTL analysis



Towards a bright future...

- ❖ Resource development through community efforts (Lack of polymorphism and density of genetic maps)
- ❖ Beginning of utilization of novel genetic variation through genomics tools and strategies
- ❖ Precision phenotyping critical, but possible
- ❖ Moving QTLs in breeding programme initiated

- **ICRISAT, Patancheru, India:** Rajeev Varshney, Pooran Gaur, L Krishnamurthy, Junichi Kashiwagi, Hari Sharma, Vincent Vadez, Hari Upadhyaya, Spurthi Nayak, Siva Kumar, Shailesh Tripathi, Nicy Vargehese, Thudi Mahender, Gudipati Srivani.
- **UC-Davis, USA:** Doug Cook, R Varma Penmetsa
- **NCGR, Santa Fe, USA:** Greg May, Andrew Farmer
- **ICRISAT, Nairobi, Kenya:** NVPR Ganga Rao, Said Silim
- **Egerton University, Kenya:** Paul Kimurto, Richard Mulwa
- **EIAR, Addis Abba, Ethiopia:** Million Eshete
- **LZARDI, Debre Zeit, Tanzania:** Robert Kileo
- **UAS-Dharwad, India:** S Sheshashayee
- **IIPR-Kanpur, India:** M Ali, SK Chaturvedi, Aditya Garg
- **Uni Frankfurt, Germany:** Peter Winter, Guenter Kahl
- **DArT P/L, Yaramula, Australia:** Andrzej Killian





Thanks for your attention