

Genetic linkage and QTL analysis in two bi-parental sweetpotato mapping populations

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17th Annual Sweetpotato Speedbreeders Meeting, Nairobi, Kenya, June 5-8, 2018

New Kawogo x Beauregard (NKB) mapping population

Trait	New Kawogo	Beauregard
Dry matter (%)	30-34	18-20
B-carotene content	White (low)	Orange (very high)
SPVD resistance	Resistant	Susceptible
Weevil resistance	Resistant	Susceptible



Benard Yada



NKB mapping population – 287 individuals



Genomic DNA extraction, NaCRRI, Uganda



Illumina HiSeq 2500 - Genomic Science Laboratory, NCSU



287 + 2 parents (6 lanes)
64-plex, 4 plates, 6 lanes each

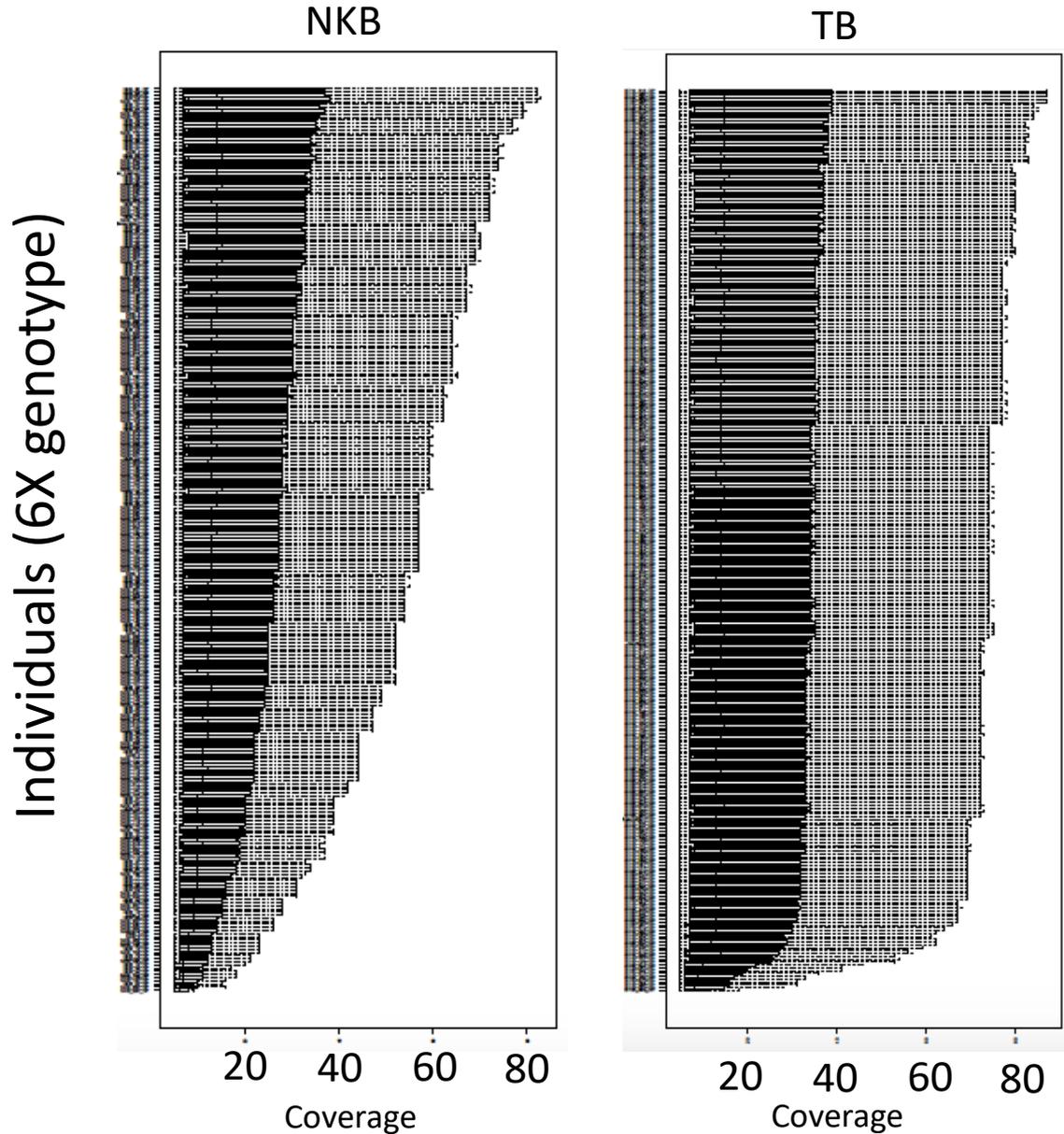


96 samples re-sequenced
6 lanes

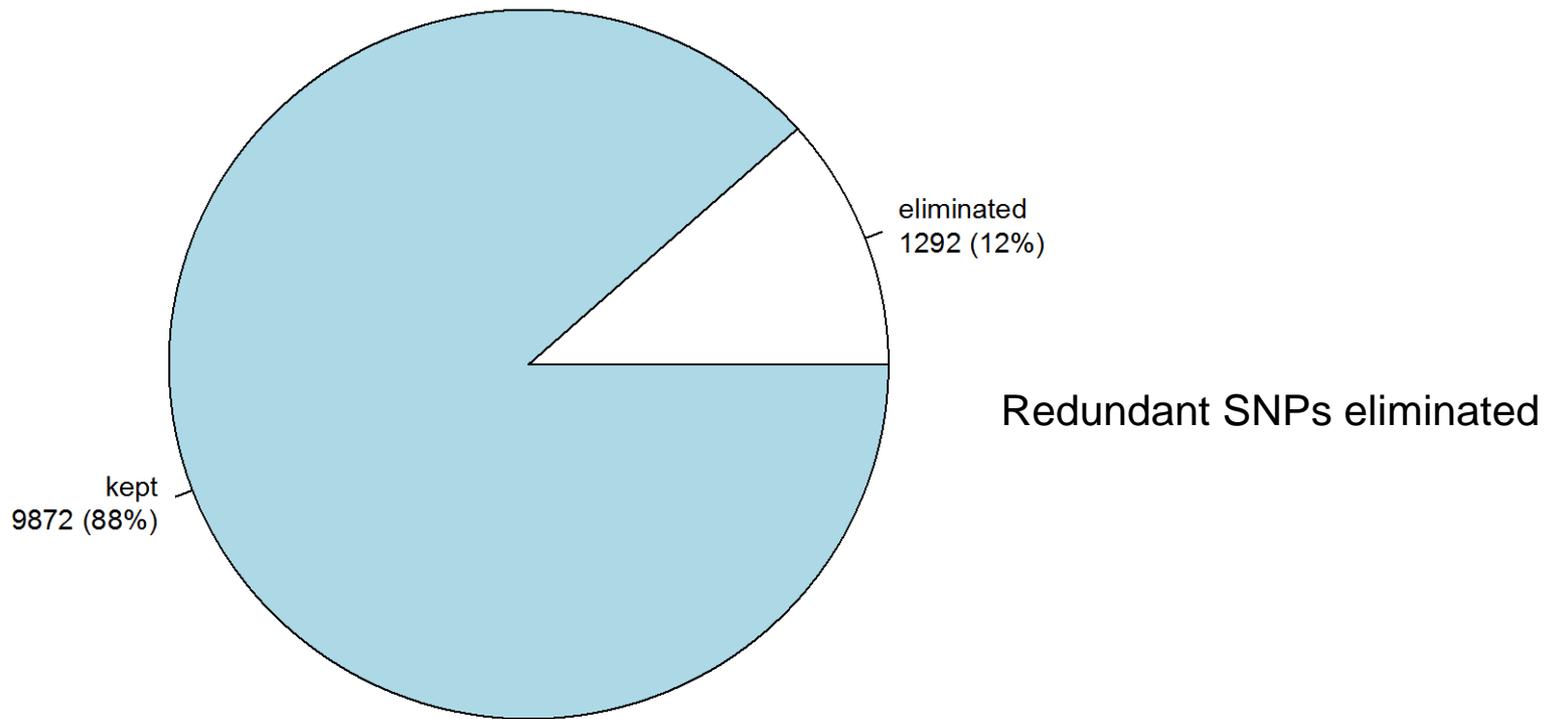
Total = 36 lanes

Normalize sequence coverage per individual

Boxplots showing sequence coverage in NKB and TB



Reference	Alignment	Total #SNPS	Ploidy filter (SuperMASSA)	Segregation distortion & missing data
<i>I. trifida</i>	74.2%	138,509	57,956	11,164



SNP genotypes

SNPs allow for the direct observation of allelic dosage at a given locus

One locus example:

AAAAAA, AAAAAa, AAAAaa, AAAaaa, AAaaaa, Aaaaaa and aaaaaa

Using the A allele as a reference

The #of copies of A is the dosage of that allele

Using SSR, AFLP or other marker system;

1 = allele present (for all AAAAAA, AAAAAa, AAAAaa, AAAaaa, AAaaaa, Aaaaaa)

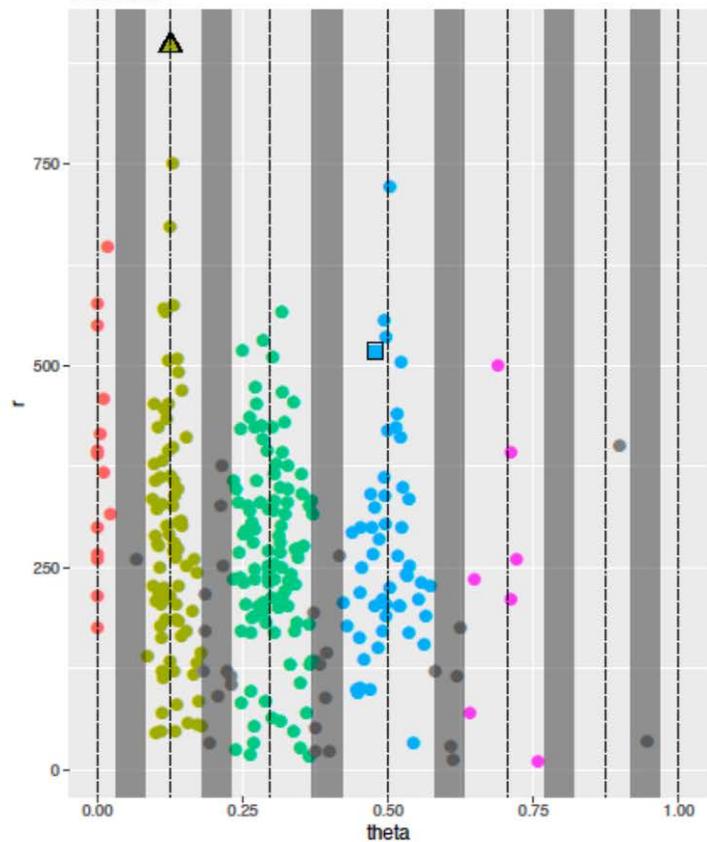
0 = allele absent (for aaaaaa)

Using SNPs;

The scores will include **allele dosage**

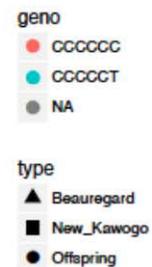
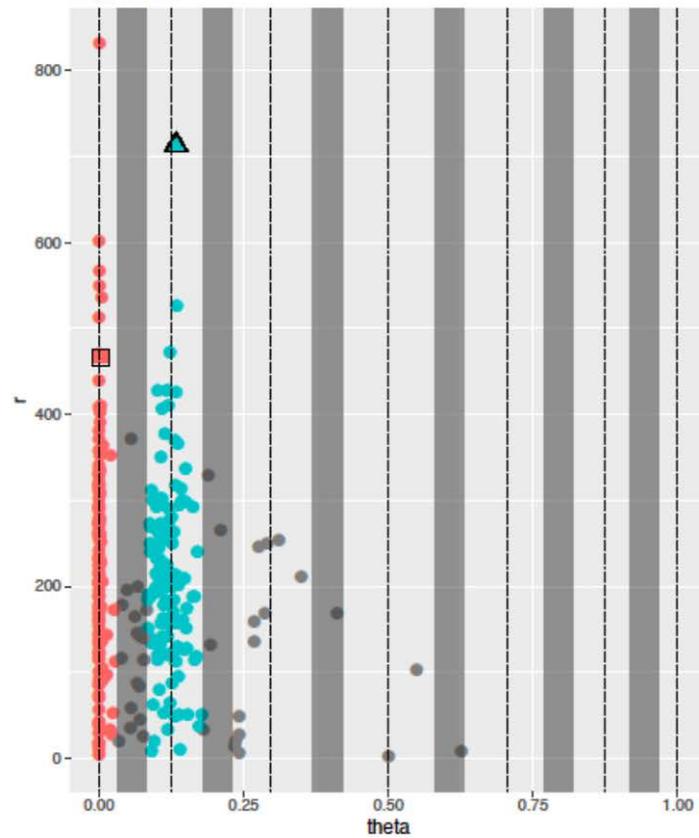
SNP 1

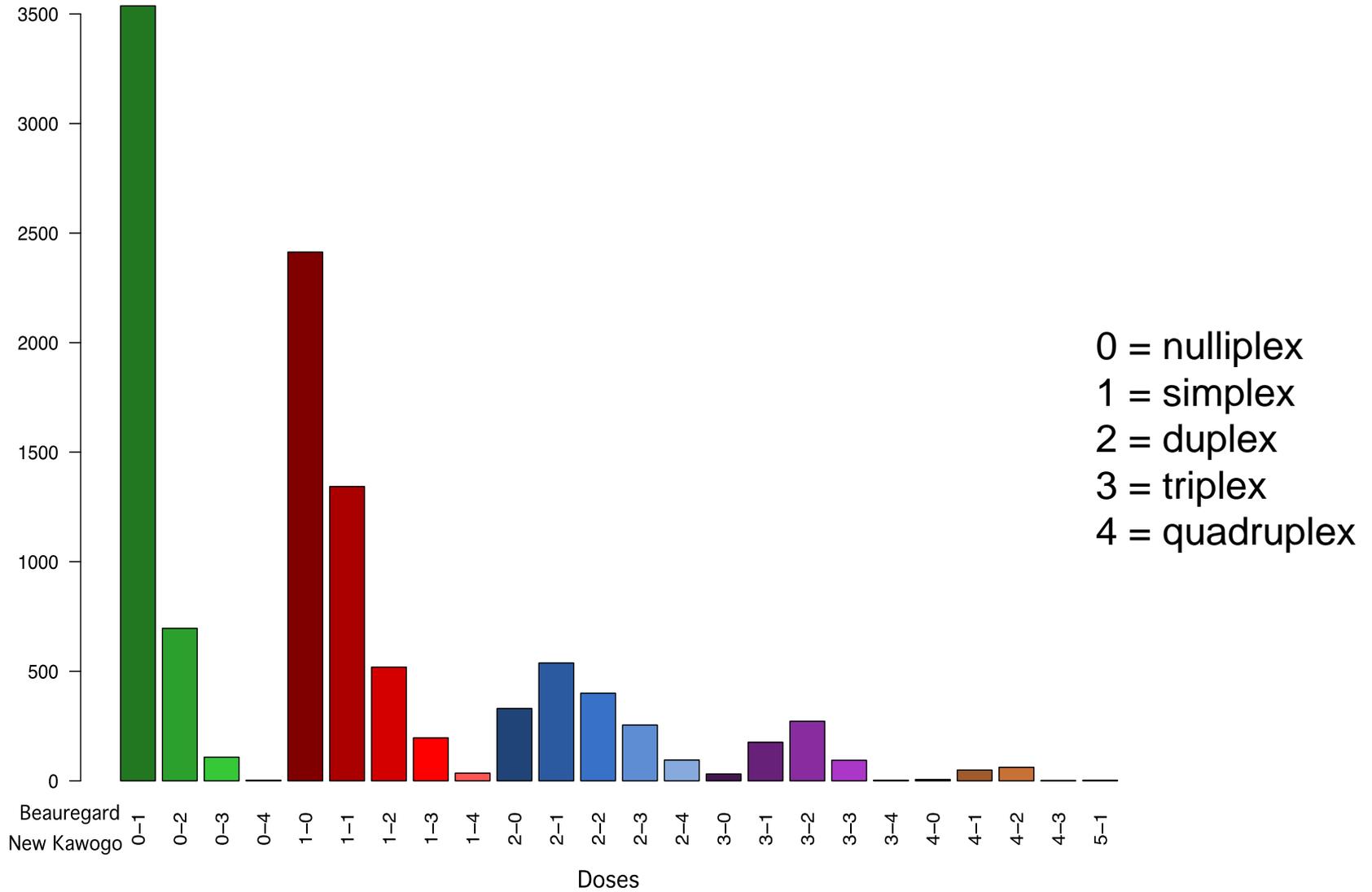
S1_29275

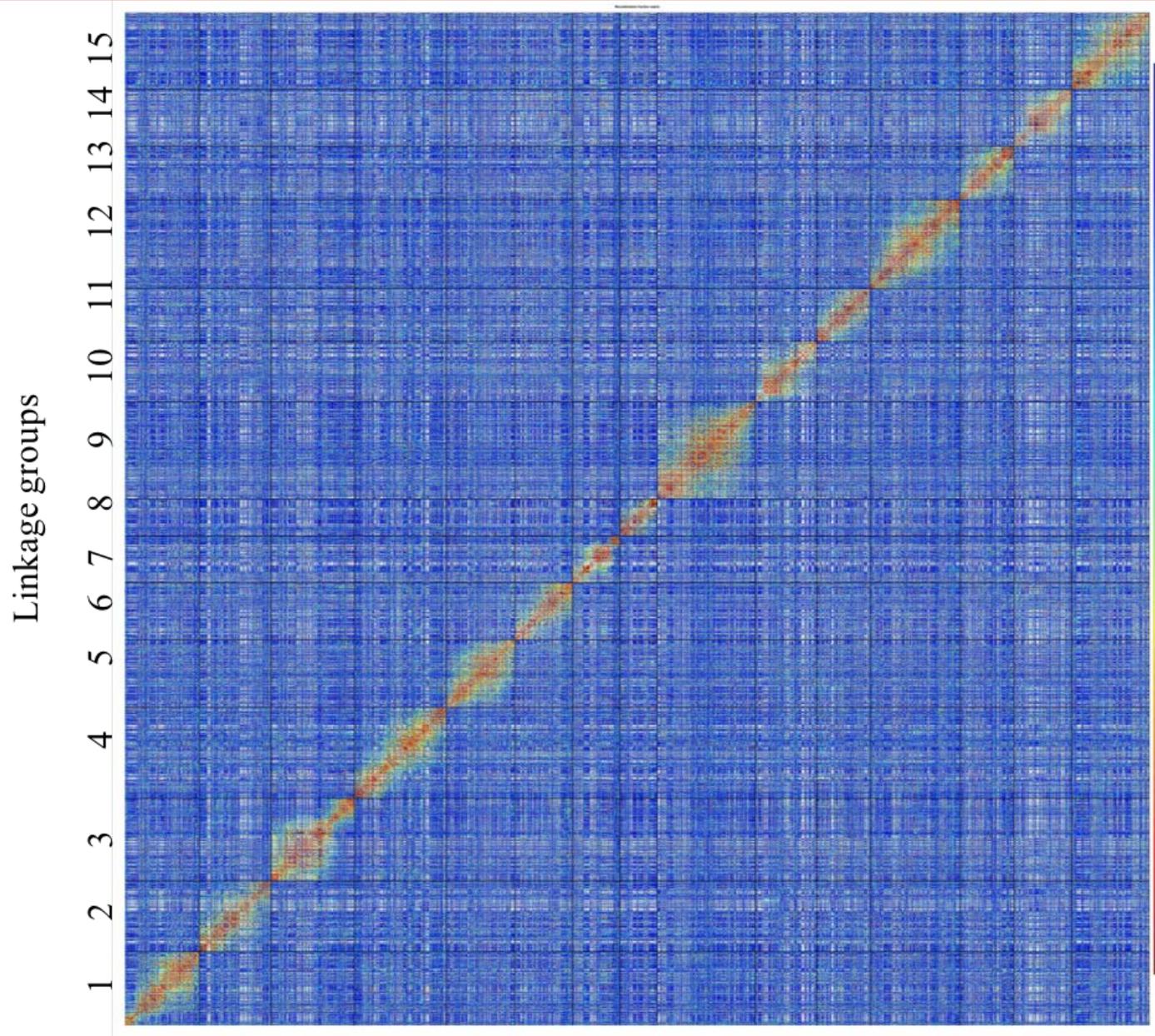


SNP 2

S1_57849





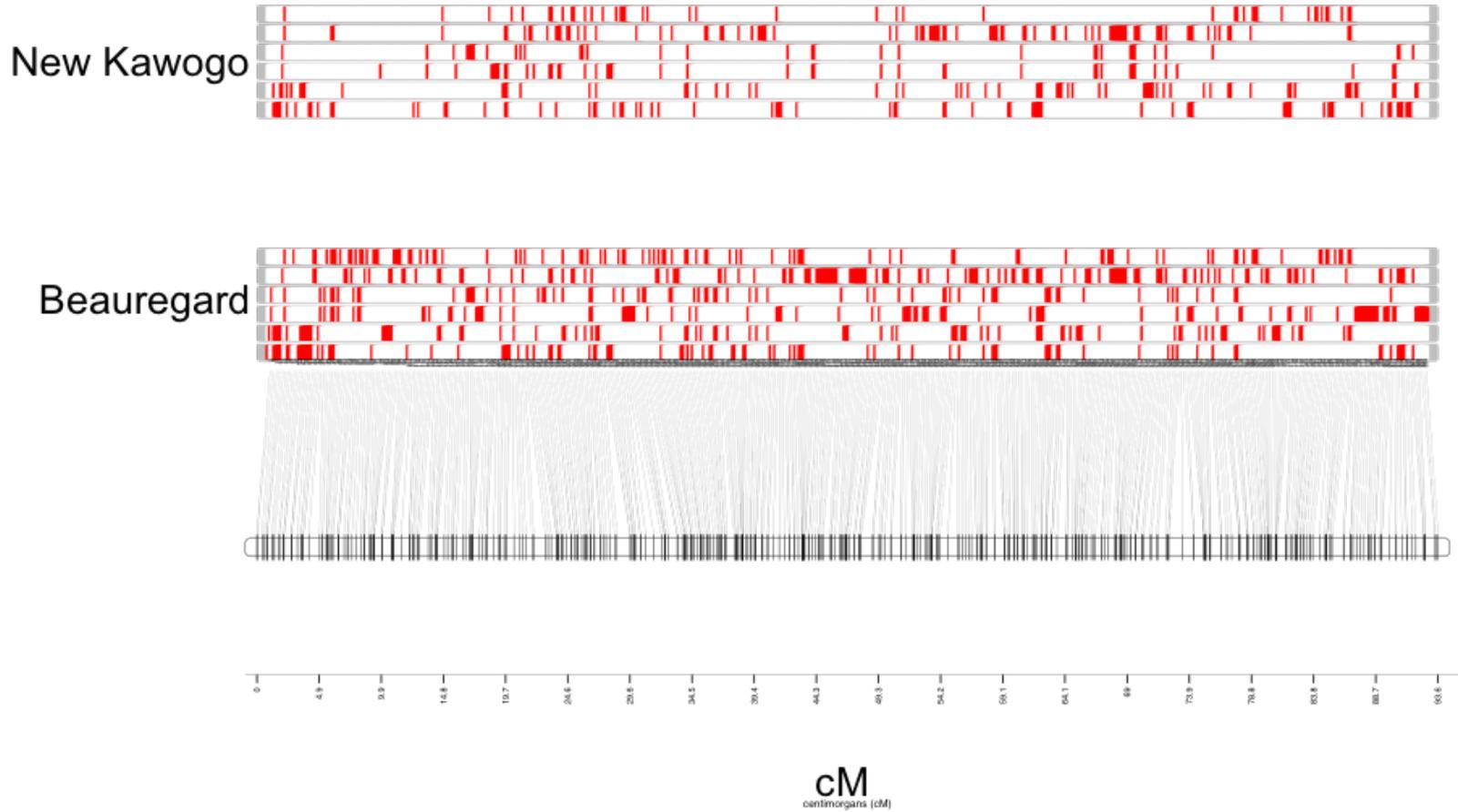


Genome scaffolds of *I. trifida*

Summary of NKB linkage map

Chromosome	cM	No. of SNPS	SNPs/cM
Ch1	93.63	520	5.55
Ch2	94.87	502	5.29
Ch3	103.03	586	5.69
Ch4	99.79	646	6.47
Ch5	89.92	480	5.34
Ch6	92.25	405	4.39
Ch7	87.52	333	3.80
Ch8	85.37	263	3.08
Ch9	86.77	688	7.93
Ch10	87.29	430	4.93
Ch11	89.66	373	4.16
Ch12	91.07	631	6.93
Ch13	83.76	379	4.52
Ch14	100.60	404	4.02
Ch15	86.32	544	6.30
Total	1371.86	7184	5.24

NKB map – Chromosome 1 (520 markers), 93.6 cM



QTL analysis for SPVD in NKB population

1) $y_{ijmn} = \mu + l_m + s_n + b_{j(mn)} + g_{imn} + e_{ijmn}$ - joint adjusted means

2) NKB linkage map

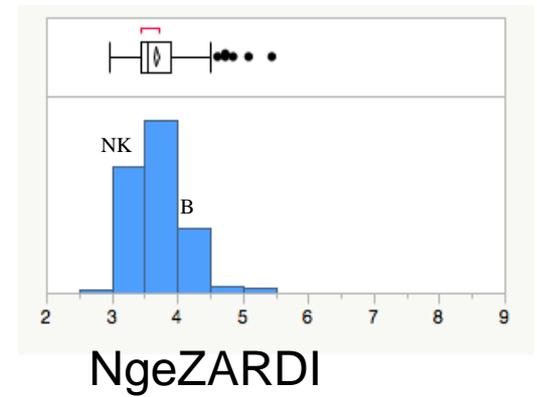
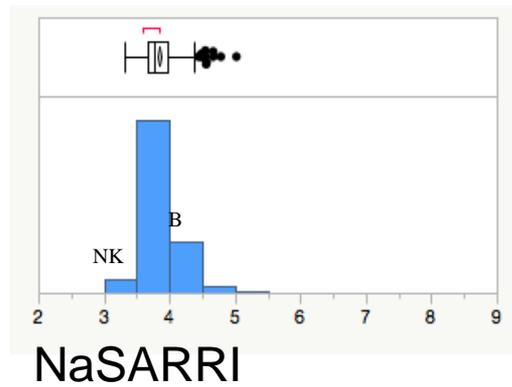
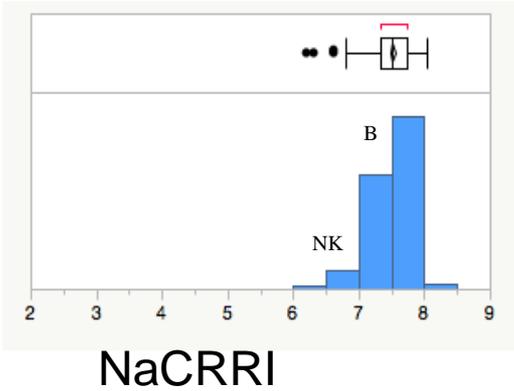
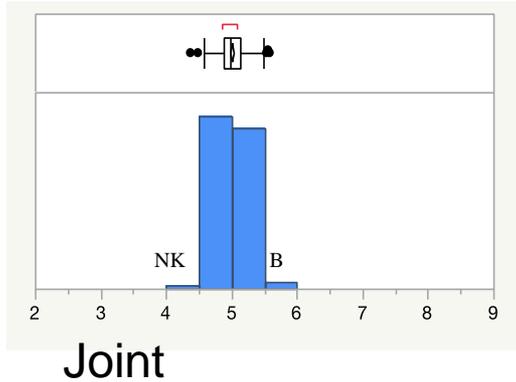
3) Conditional genotype probabilities

Polyqtl

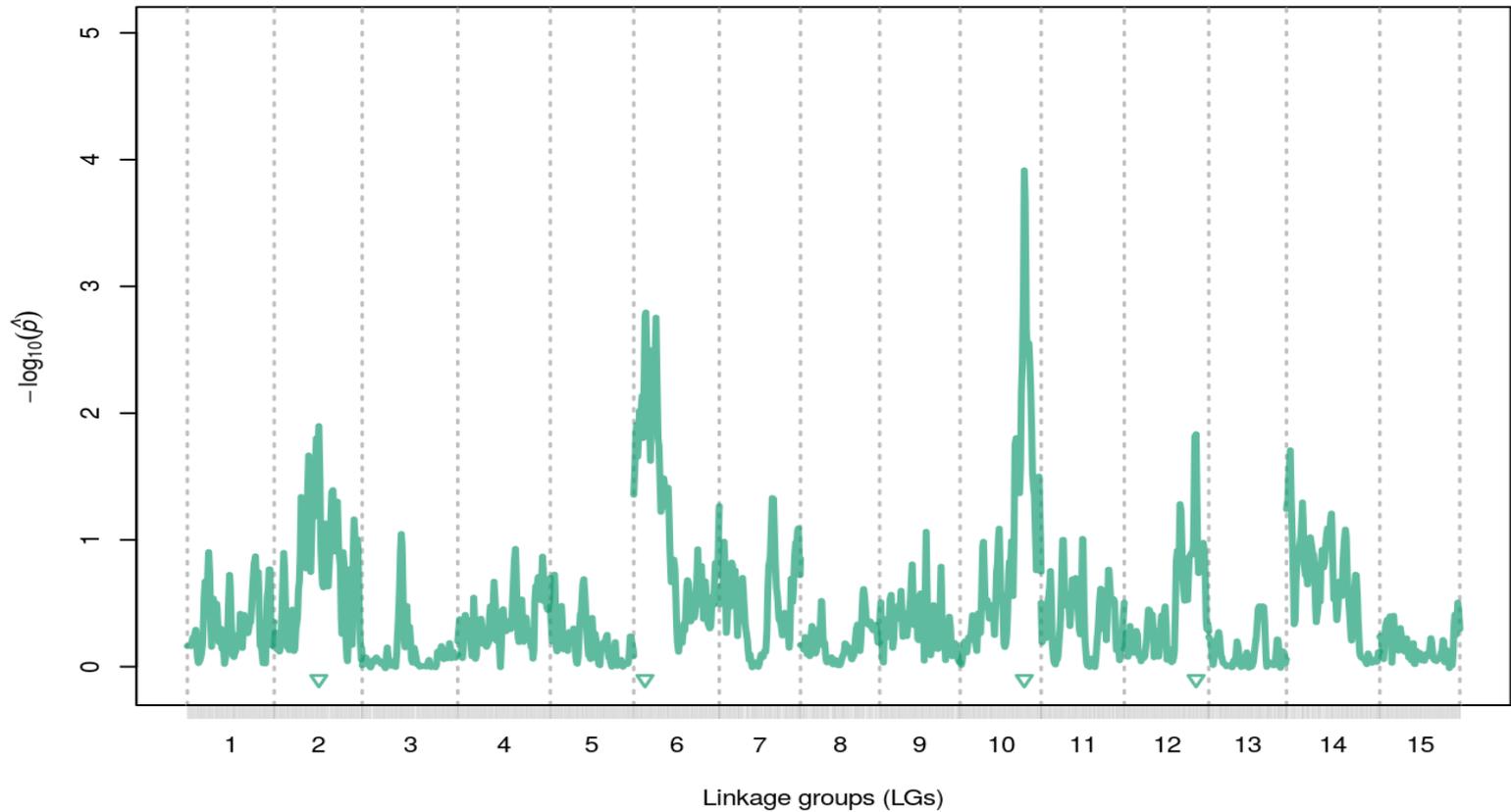
$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ge}^2}{MN} + \frac{\sigma_e^2}{JMN}}$$

$$H^2 = 0.54$$

Plots of joint and marginal means for SPVD

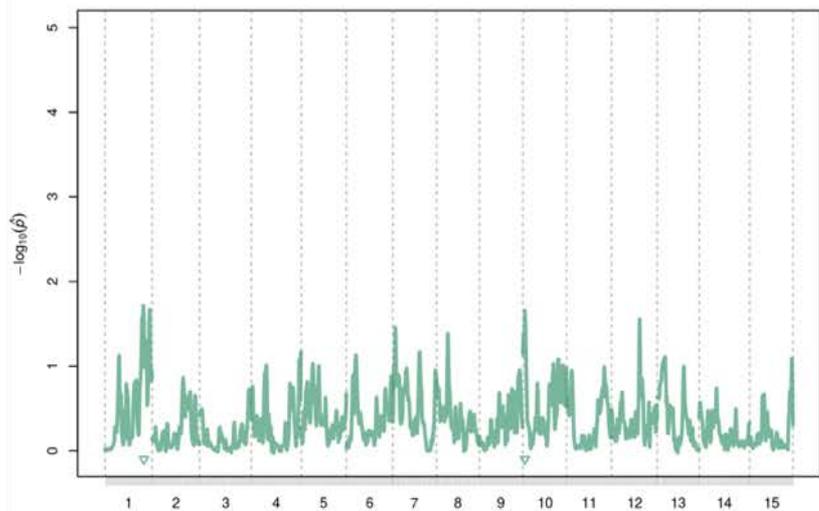


Joint_SiteSeason

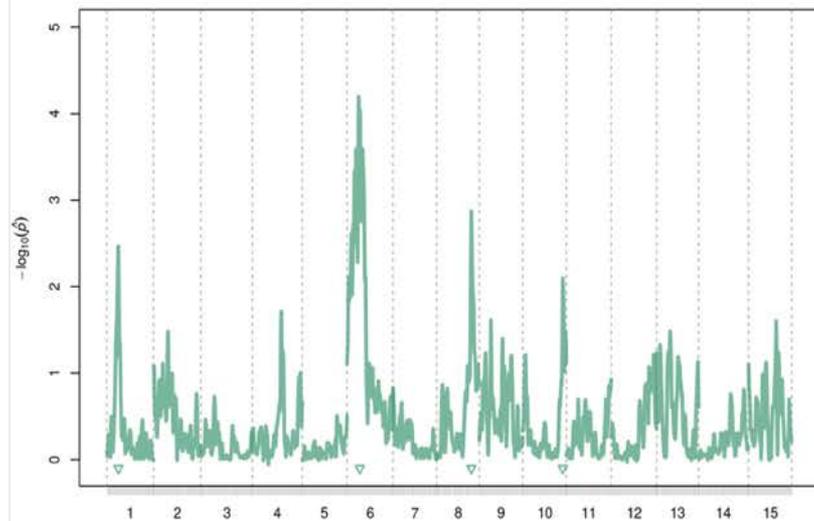


QTL	Chr.	Position	Marker	p-value	σ_{QTL}	H^2_{QTL}
1	2	48.1	S2_12201472	1.24e-02	0.0031	0.061
2	6	12.1	S6_13090342	8.45e-04	0.0038	0.074
3	10	69.1	S10_22000763	1.24e-04	0.0081	0.159
4	12	77.4	S12_22681845	1.22e-02	0.0047	0.091
Total						0.385

NaCRRI

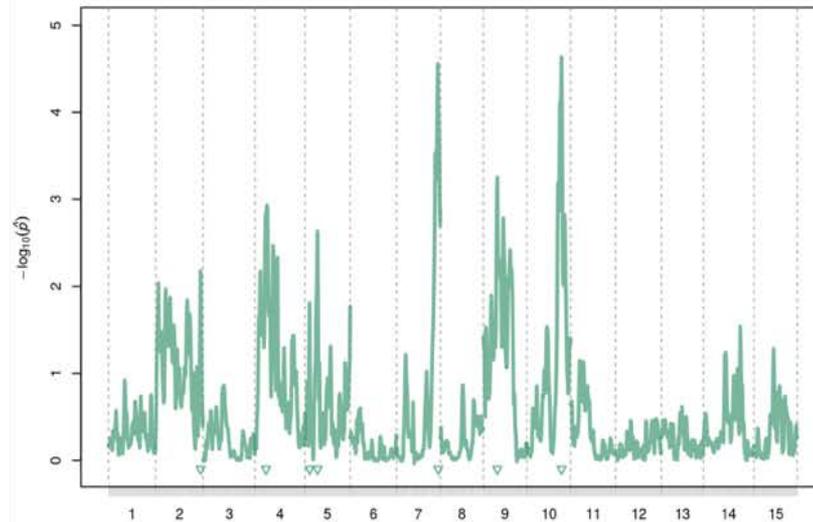


NaSARRI

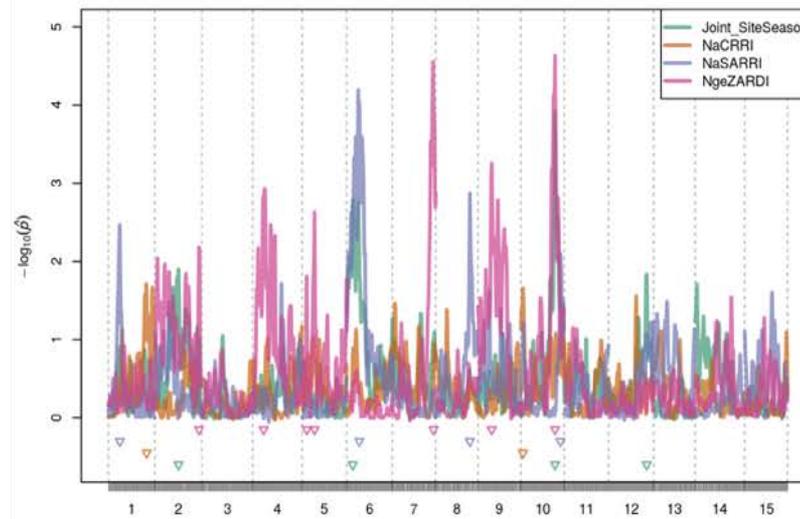


Linkage groups (LGs)

NgeZARDI



SPVD (Site)



Linkage groups (LGs)

Sweetpotato weevil



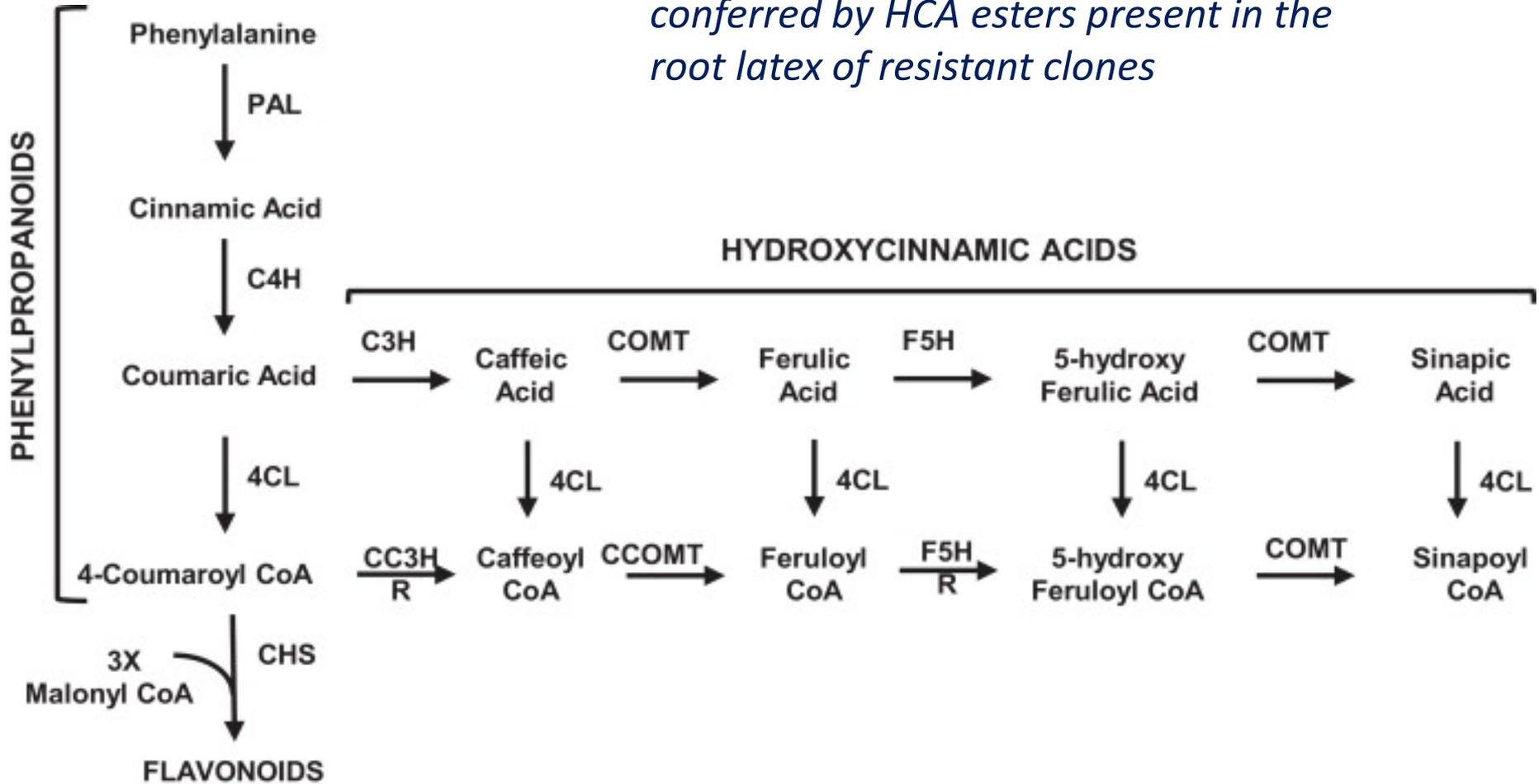
C. puncticollis



C. brunneus

- Mechanistic basis of resistance is poorly understood

Resistance is an active process conferred by HCA esters present in the root latex of resistant clones



Sweetpotato damage parameters	Total hydroxycinnamic acid esters concentration	Field root infestation	Field stem infestation
Field root infestation ($n = 852$)	0.603 ($P \leq 0.0105$) $r^2 = 0.36$		
Field stem infestation ($n = 1704$)	-0.618 ($P \leq 0.05$)	0.497 ($P \leq 0.001$)	
Mean number of feeding holes in lab study ($n = 1734$)	-0.64 ($P \leq 0.05$) $r^2 = 0.4$	0.501 ($P \leq 0.05$) $r^2 = 0.25$	0.405 ($P \leq 0.05$)

Anyanga *et al.* 2017

QTL analysis for SPW in NKB population

1) $y_{ijmn} = \mu + l_m + s_n + b_{j(mn)} + g_{imn} + e_{ijmn}$ - joint adjusted means

2) NKB linkage map

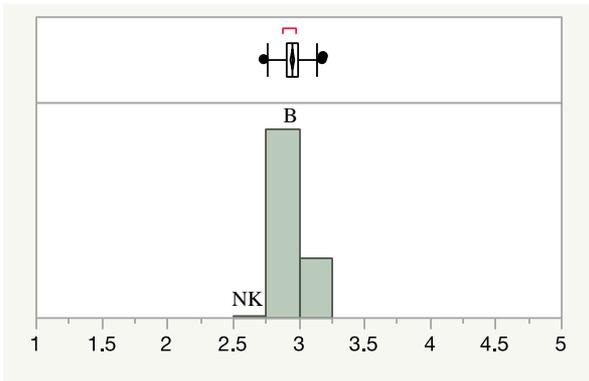
3) Conditional genotype probabilities

Polyqtl

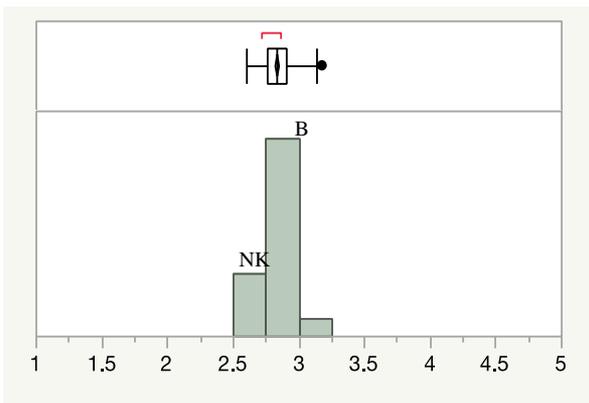
$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ge}^2}{MN} + \frac{\sigma_e^2}{JMN}}$$

$$H^2 = 0.49$$

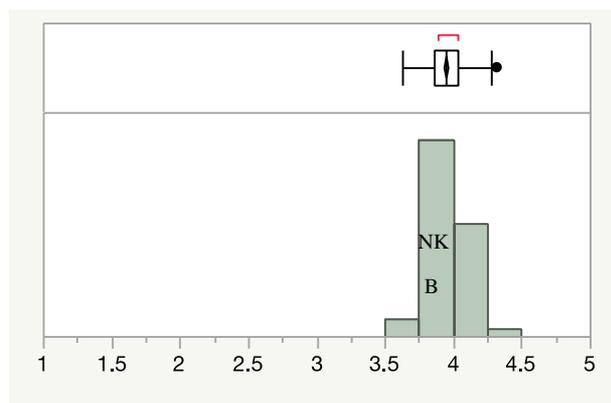
Plots of joint and marginal means for SPW



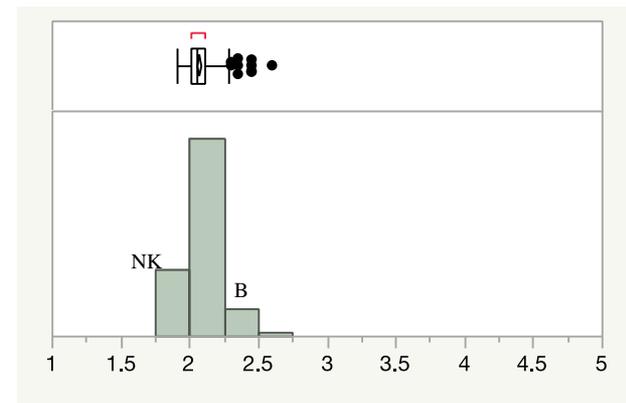
Joint



NaCRRRI



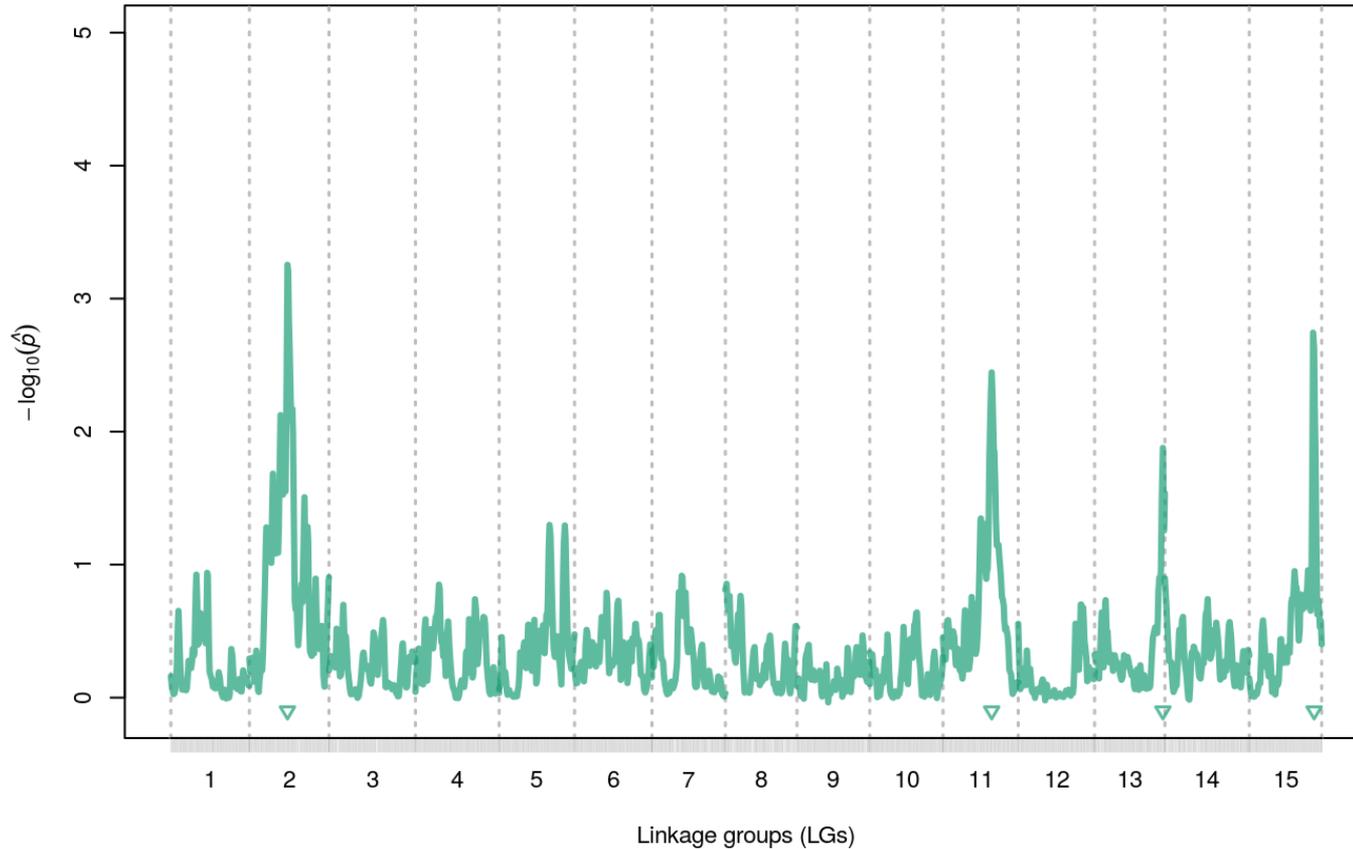
NaSARRI



NgeZARDI

QTL for SPW

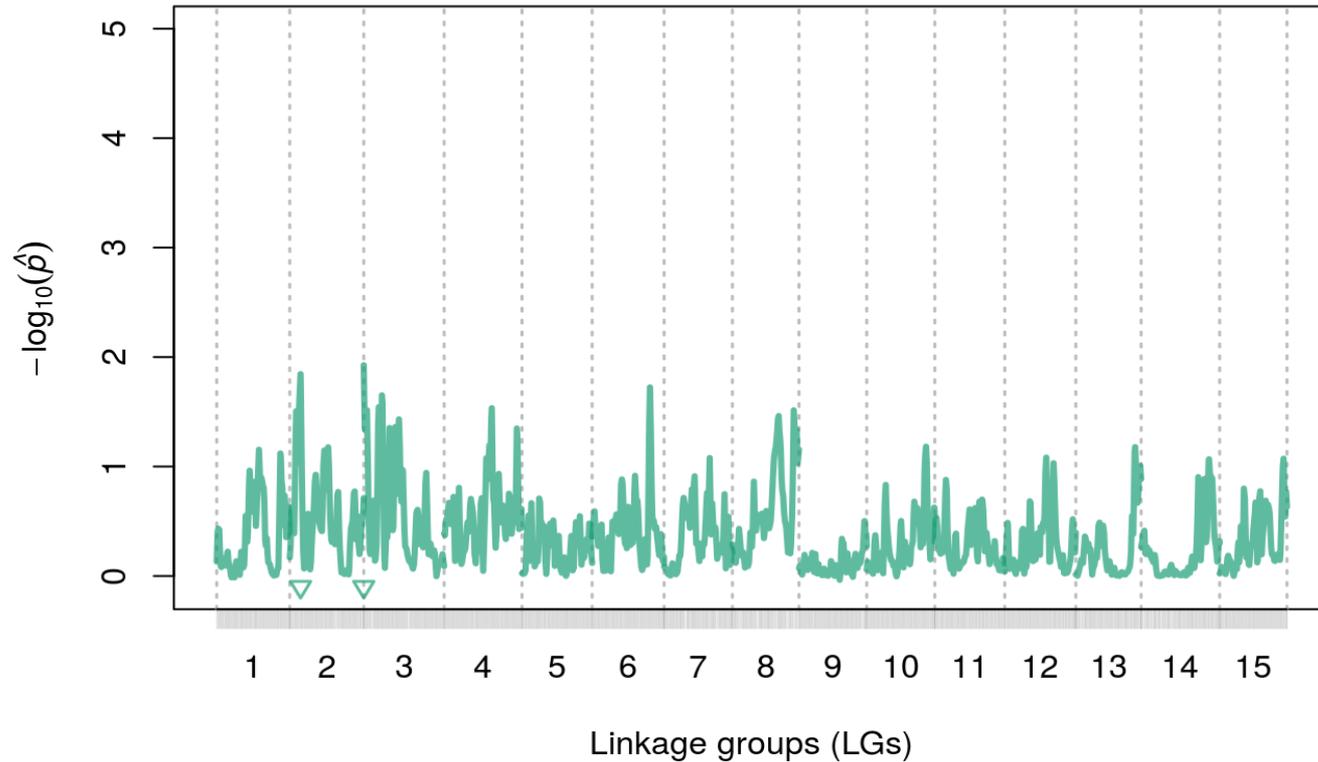
Joint_SiteSeason



QTL	Chr.	Position	Marker	p-value	σ_{QTL}	H^2_{QTL}
1	2	45.2	S2_11881018	4.85e-04	0.0008	0.114
2	11	58.0	S11_7044584	4.34e-03	0.0004	0.055
3	13	81.4	S13_22624948	1.06e-02	0.0006	0.078
4	15	77.3	S15_19475897	1.33e-03	0.0009	0.129
Total						0.377

QTL for HCA

MeanCZ

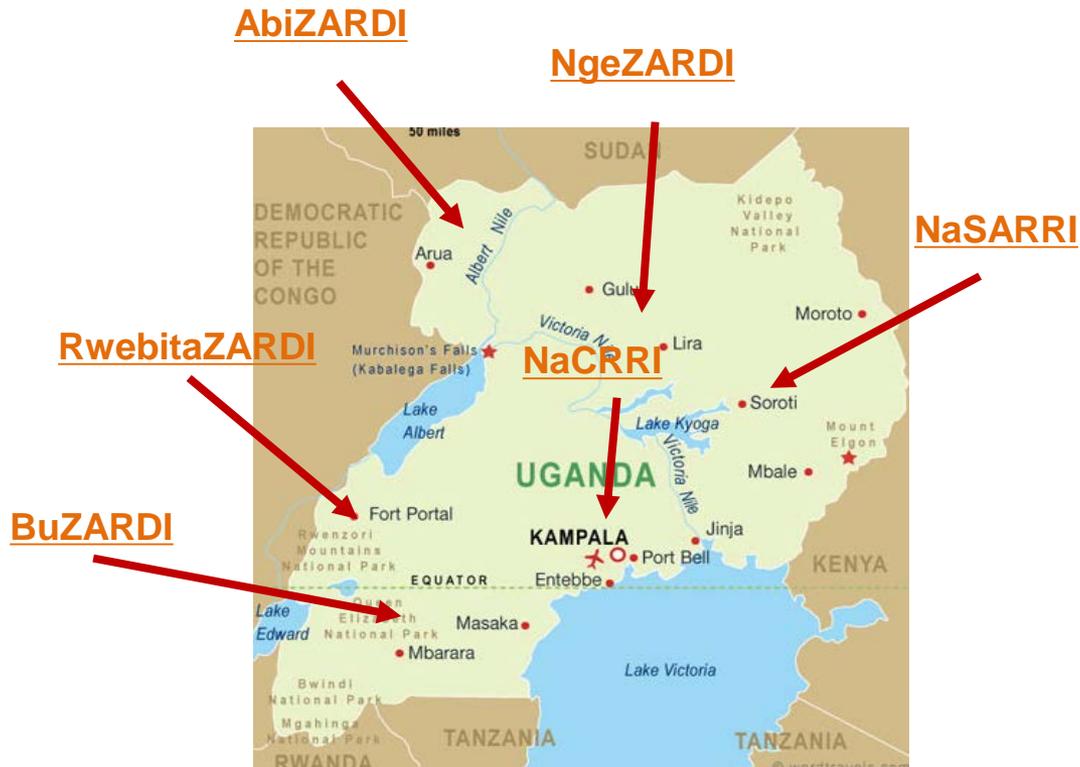


QTL	Chr.	Position	Marker	p-value	σ_{QTL}	H^2_{QTL}
1	2	13.9	S2_7940387	1.16e-02	230.01	0.075
2	3	0.0	S3_132184	1.16e-02	258.12	0.084
Total						0.159

SPW bioassay at NaCRRI

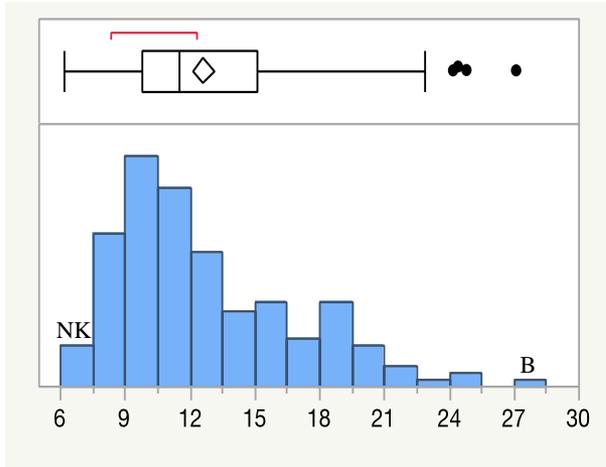


SPW bioassay at NaCRRI

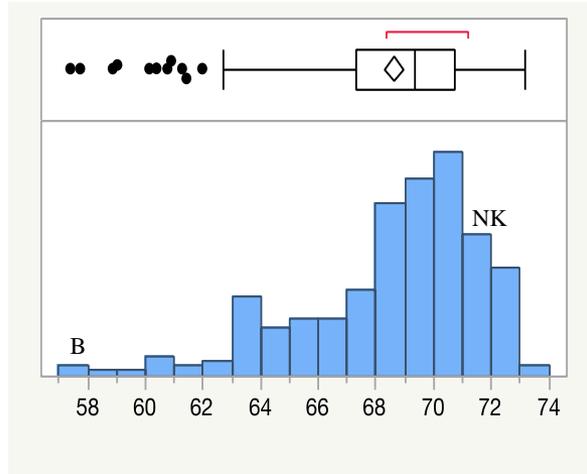


- One root per site
- Very high env. variation
- Error cannot be estimated
 - NO reps, NO controls

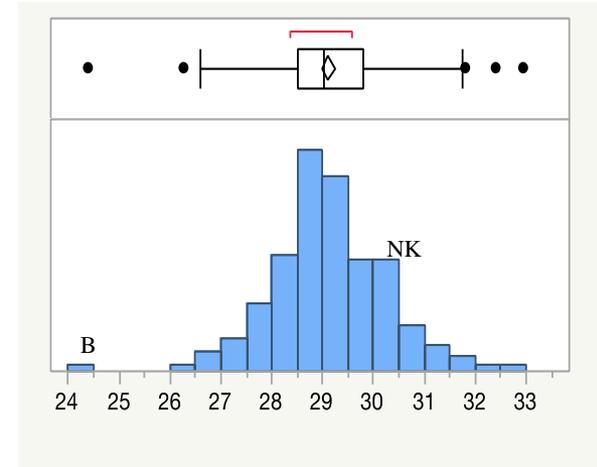
QTL for storage root β -carotene, starch and dry matter



β -carotene content



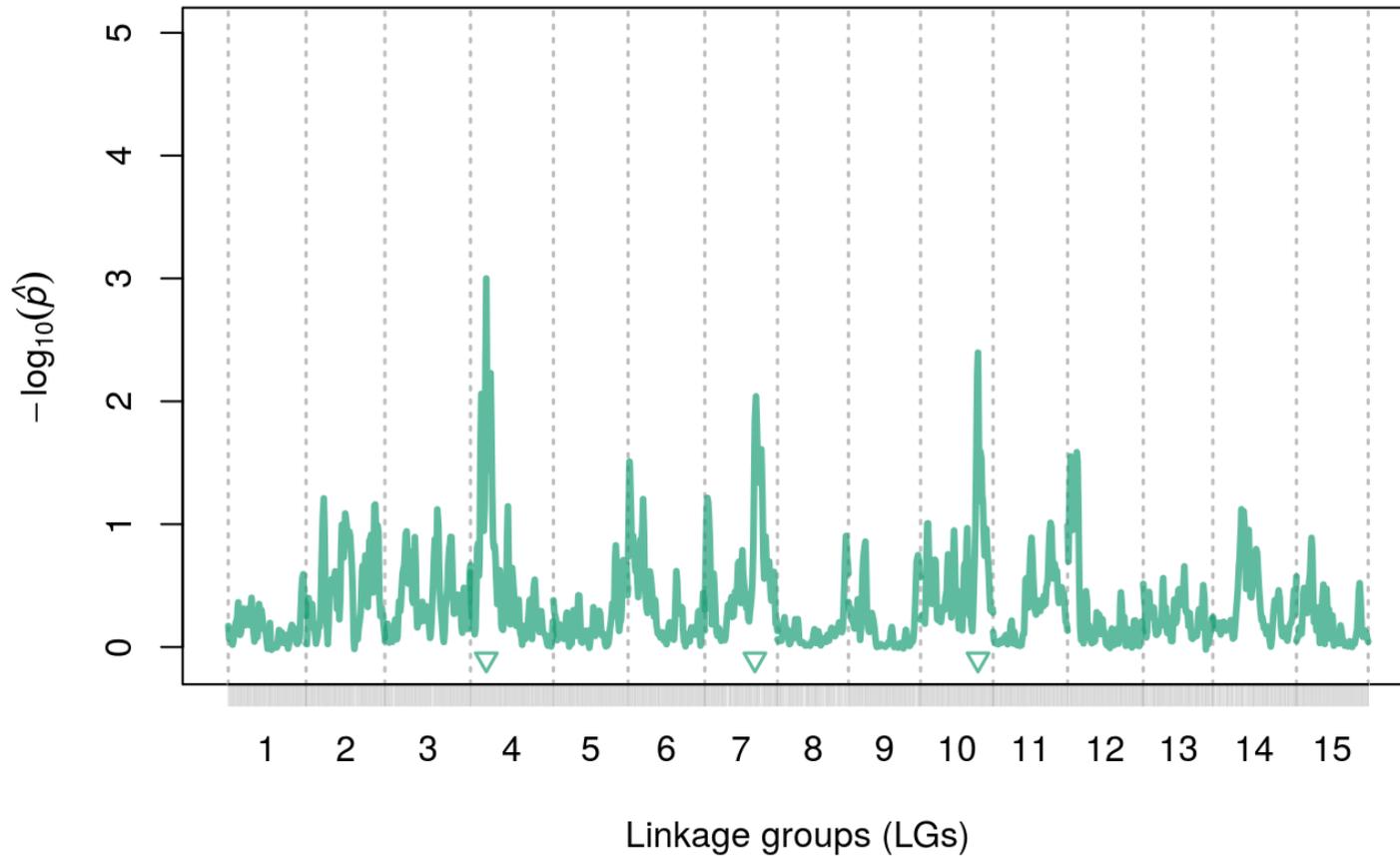
Starch content



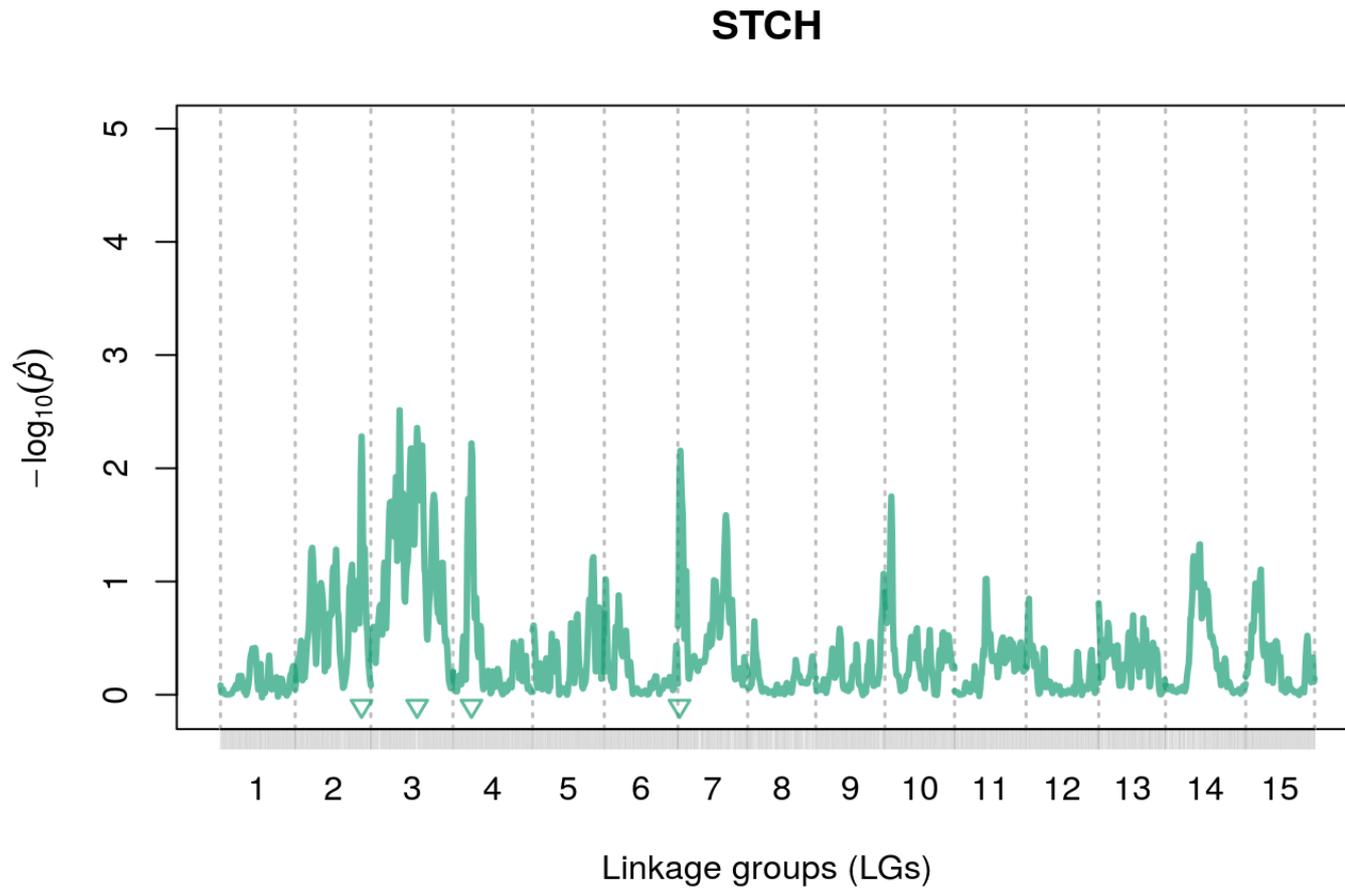
Dry matter content

Storage root β -carotene content

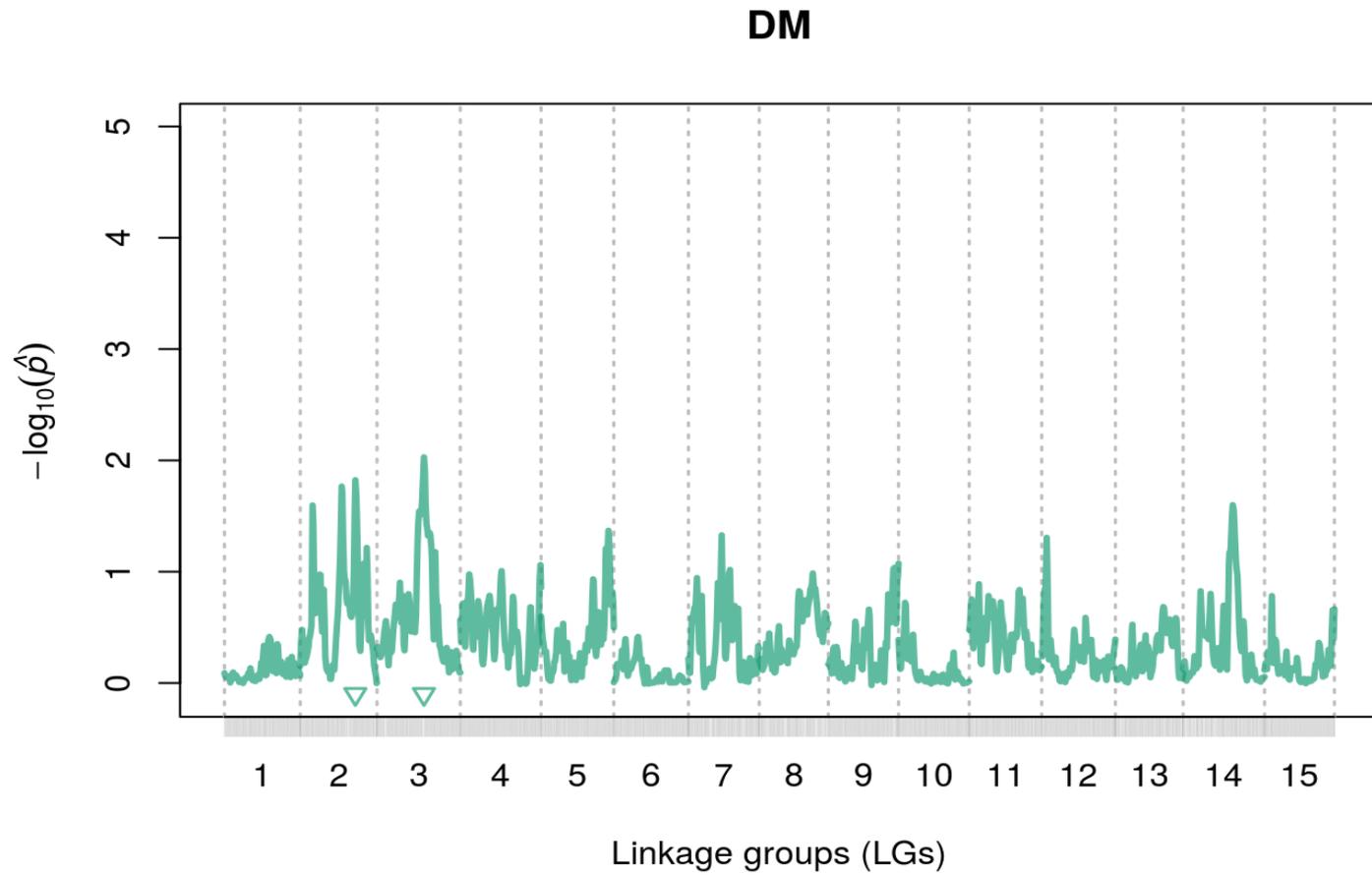
BC



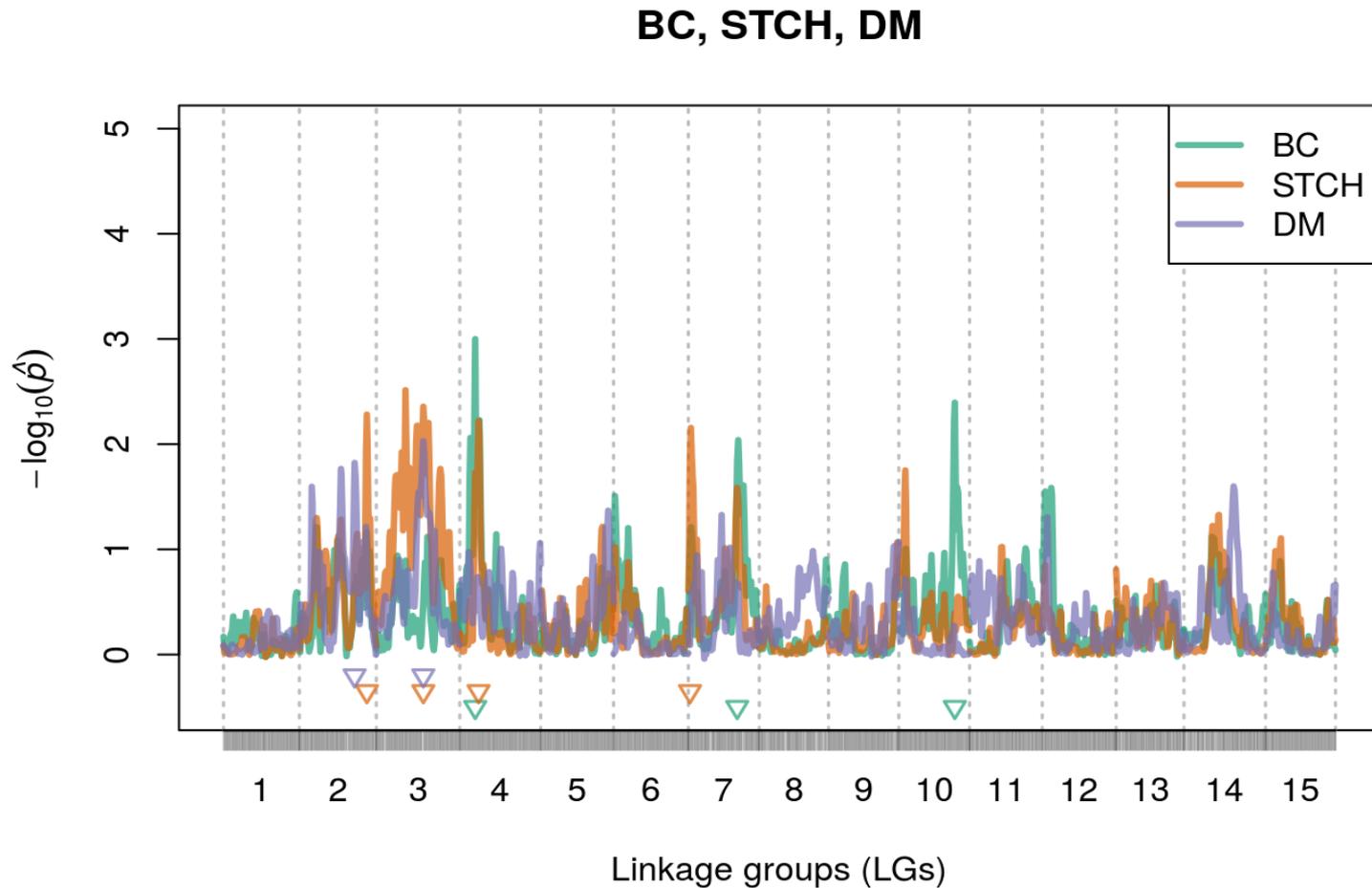
Storage root starch content



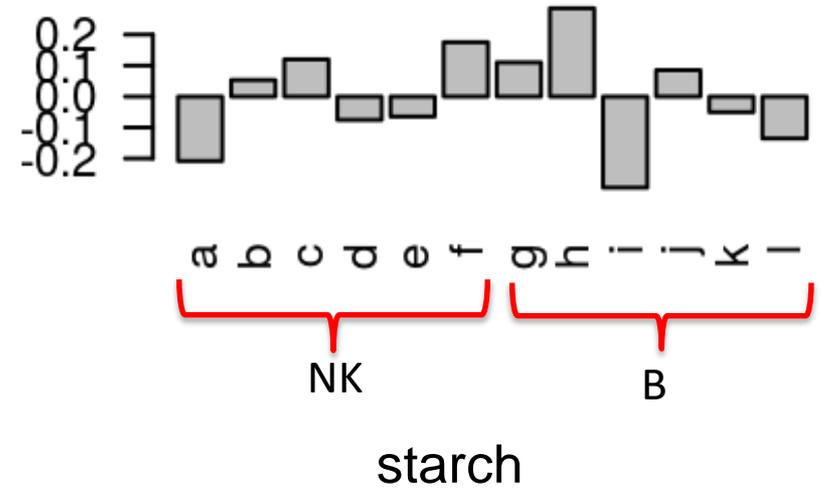
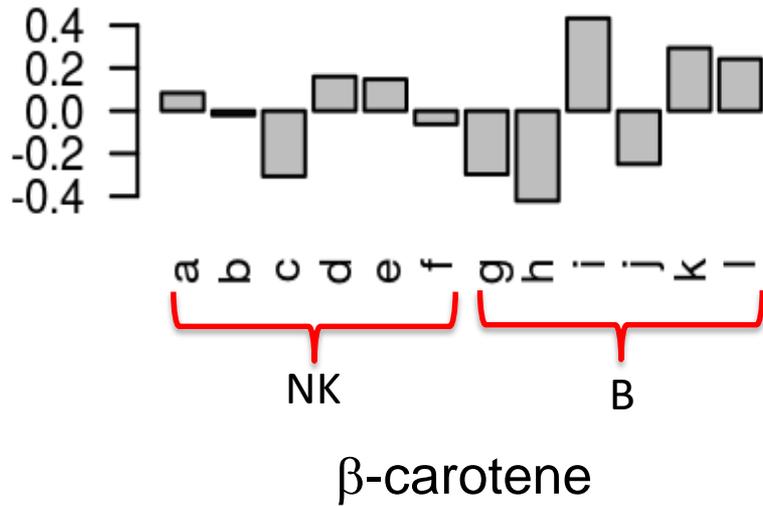
Storage root dry matter content



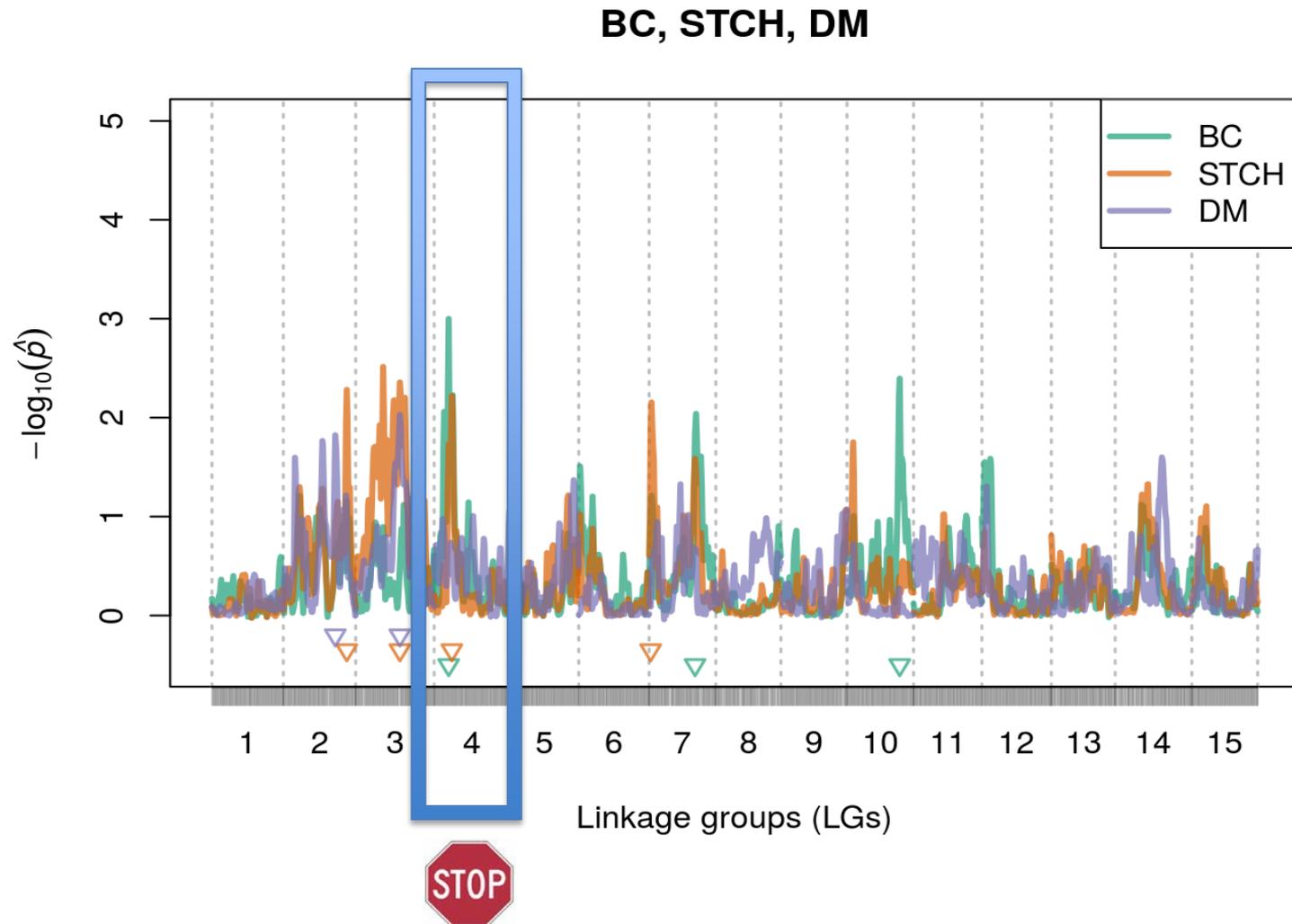
β -carotene, starch and dry matter content



Additive effect for BC QTL 1, and starch QTL 3



β -carotene, starch and dry matter content



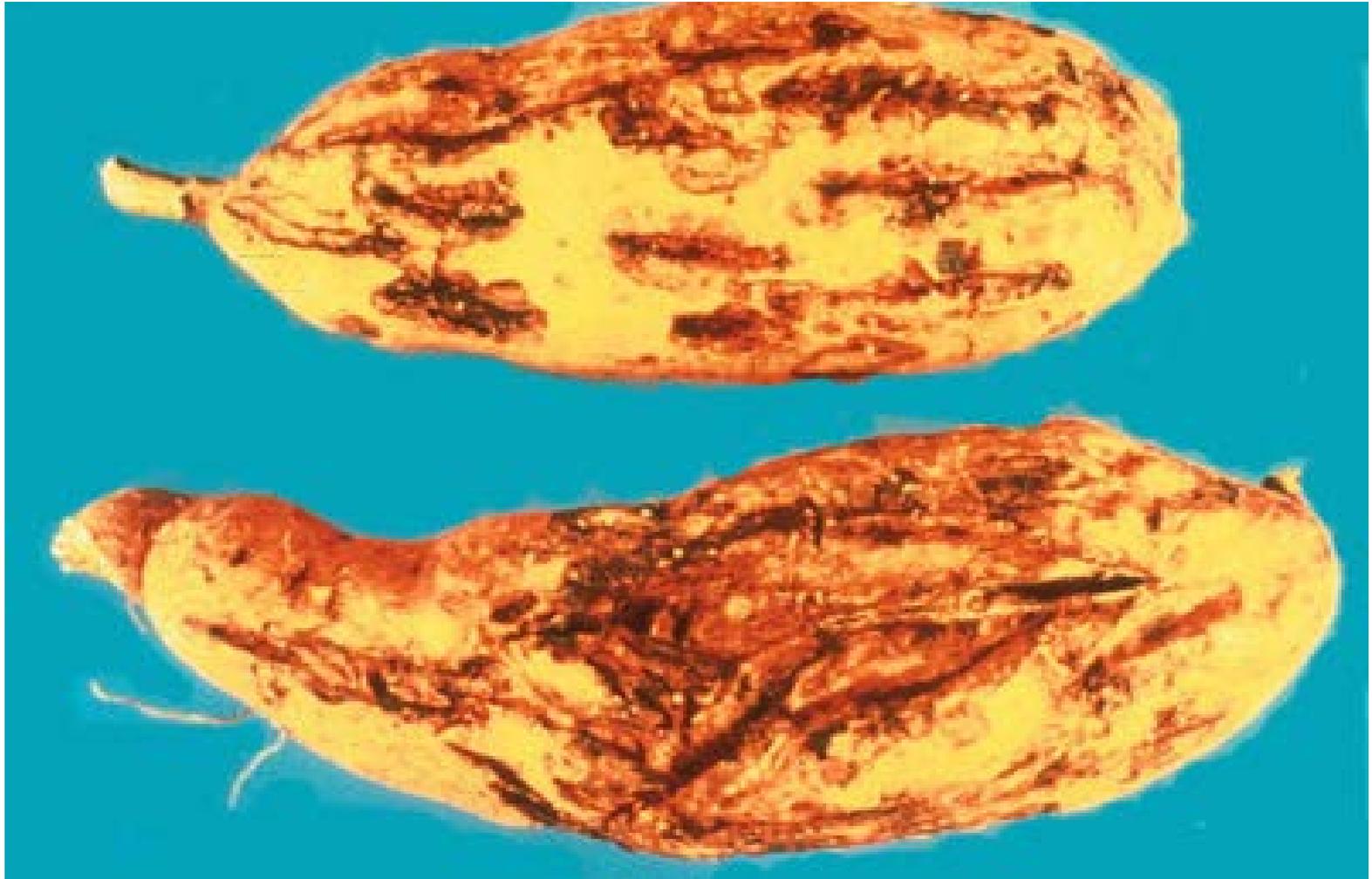
Conclusion

1. 9,872 SNP markers in NKB population
 2. SNP-based integrated map for NKB is now available
 3. QTL identified for SPVD, SPW, HCA, BC, STCH, DM
 - 4 for SPVD
 - 4 for SPW
 - 2 for HCA
 - 3 for BC
 - 4 for Starch
 - 2 for dry matter
- *Sets the stage for MAS in future studies – MDP*

Root knot nematode in sweetpotato



Root knot nematode in sweetpotato



(© Clark and Moyer 1988)

Tanzania x Beauregard (TB) mapping population

Trait

Tanzania

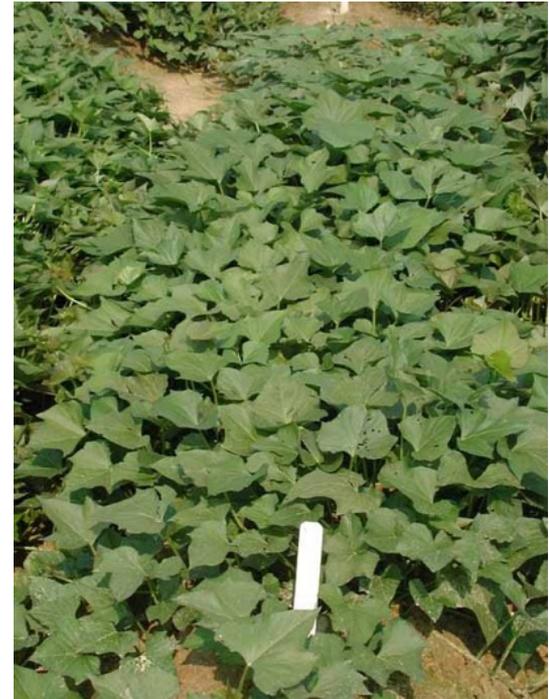
Beauregard

RKN Resistance

Resistant

Susceptible

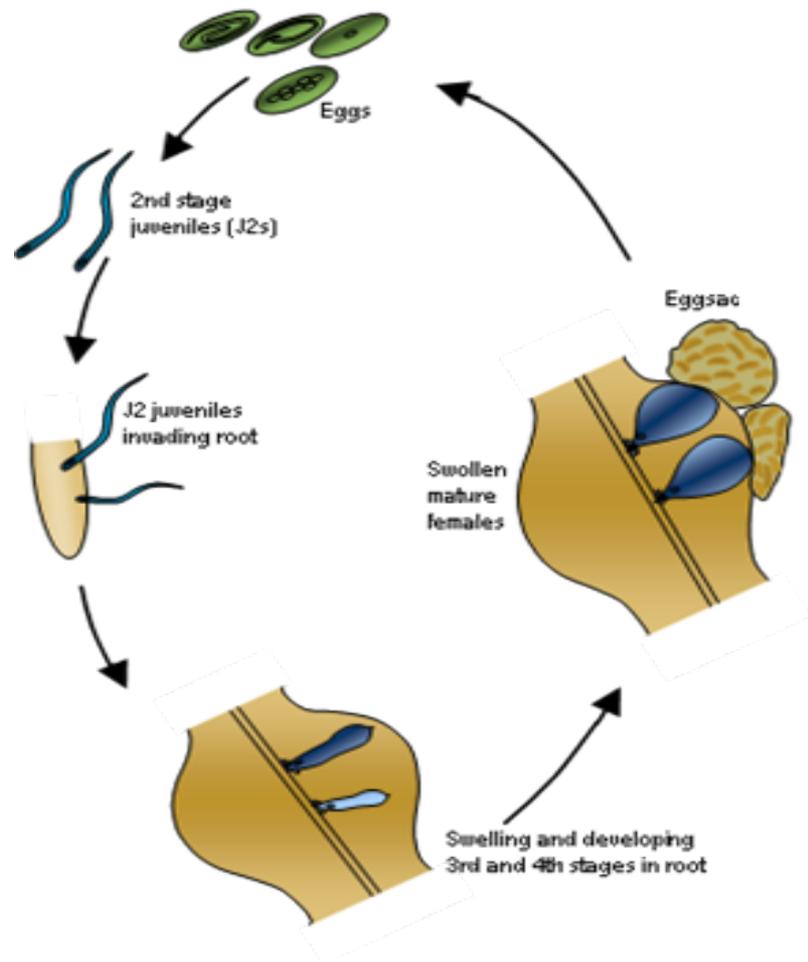
- 250 individuals



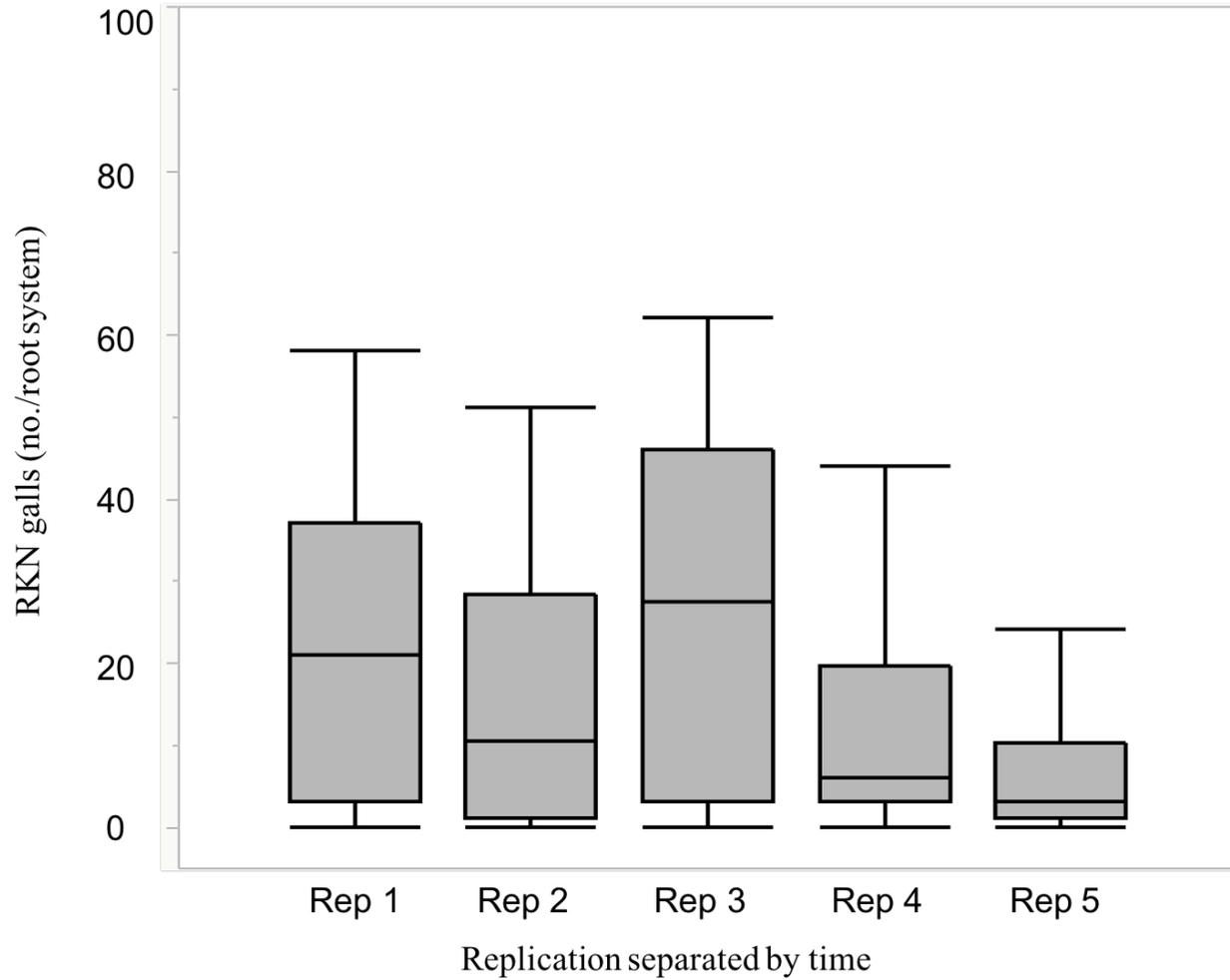
TB mapping population – RKN assay

- TB phenotyping:
 - ✓ 5 reps
 - ✓ CRD`
 - ✓ 20,000 RKN eggs



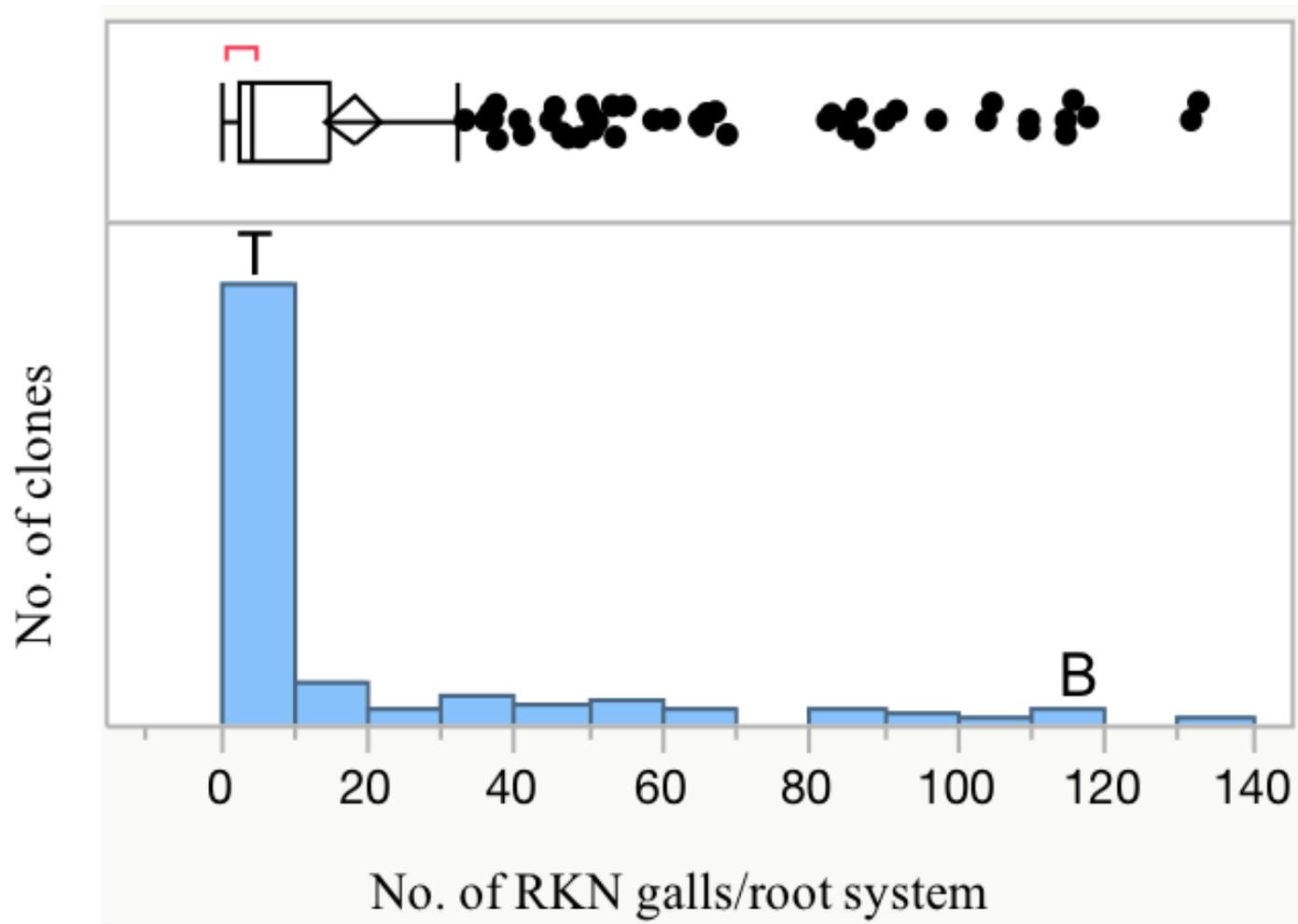






$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{R}}$$

$$H^2 = 0.95$$



QTL for RKN in TB population detected using AFLP

J. AMER. SOC. HORT. SCI. 133(6):844-851. 2008.

Detection of Quantitative Trait Loci and Inheritance of Root-knot Nematode Resistance in Sweetpotato

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Robert O.M. Mwangi
 Namulonge Agricultural and Animal Production Research Institute, P.O. Box 7084, Kampala, Uganda

ADDITIONAL INDEX WORDS. *Ipomoea batatas*, *Meloidogyne incognita*, RKN, quantitative trait loci

ABSTRACT. Resistance to root-knot nematodes [*Meloidogyne incognita* (Kofoid & White) Chitwood] in sweetpotato [*Ipomoea batatas* (L.) Lam.] was studied in a mapping population consisting of 240 progeny derived from a cross between 'Beauregard', the predominant cultivar in the United States, and 'Tanzania', an African landrace. Quantitative trait loci (QTL) analyses to locate markers associated with resistance to root-knot nematodes (RKN) were performed using genetic maps based on parental segregation in 'Beauregard' and 'Tanzania' consisting of 726

- 9 QTL were identified
- 7 in Tanzania and 2 in Beauregard
- In Tanz. 4 QTL had +ve effects ~ 21%

Note

- ✓ Single dose AFLP markers
- ✓ 2 separate maps were used
- ✓ IM & CIM

Summary of TB linkage map

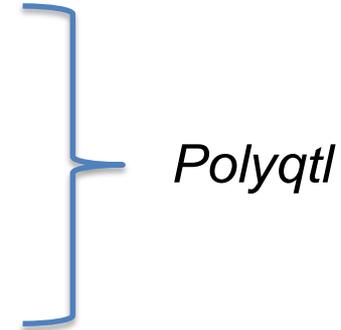
Chromosome	No. of markers	Length	SNPs/cM
CHR 1	359	306.6	1.2
CHR 2	274	213.7	1.3
CHR 3	358	275.6	1.3
CHR 4	424	367.5	1.2
CHR 5	285	240.6	1.2
CHR 6	266	189.1	1.4
CHR 7	258	193.5	1.3
CHR 8	180	142.1	1.3
CHR 9	442	306.9	1.4
CHR 10	364	263.1	1.4
CHR 11	231	179.5	1.3
CHR 12	454	284.6	1.6
CHR 13	232	175.1	1.3
CHR 14	222	222.8	1.2
CHR 15	367	264.4	1.4
Total	4,716	3,627.36	

QTL analysis

1) $y_{ij} = \mu + r_j + g_i + e_{ij}$ - joint adjusted means

2) TB linkage map

3) Conditional genotype probabilities

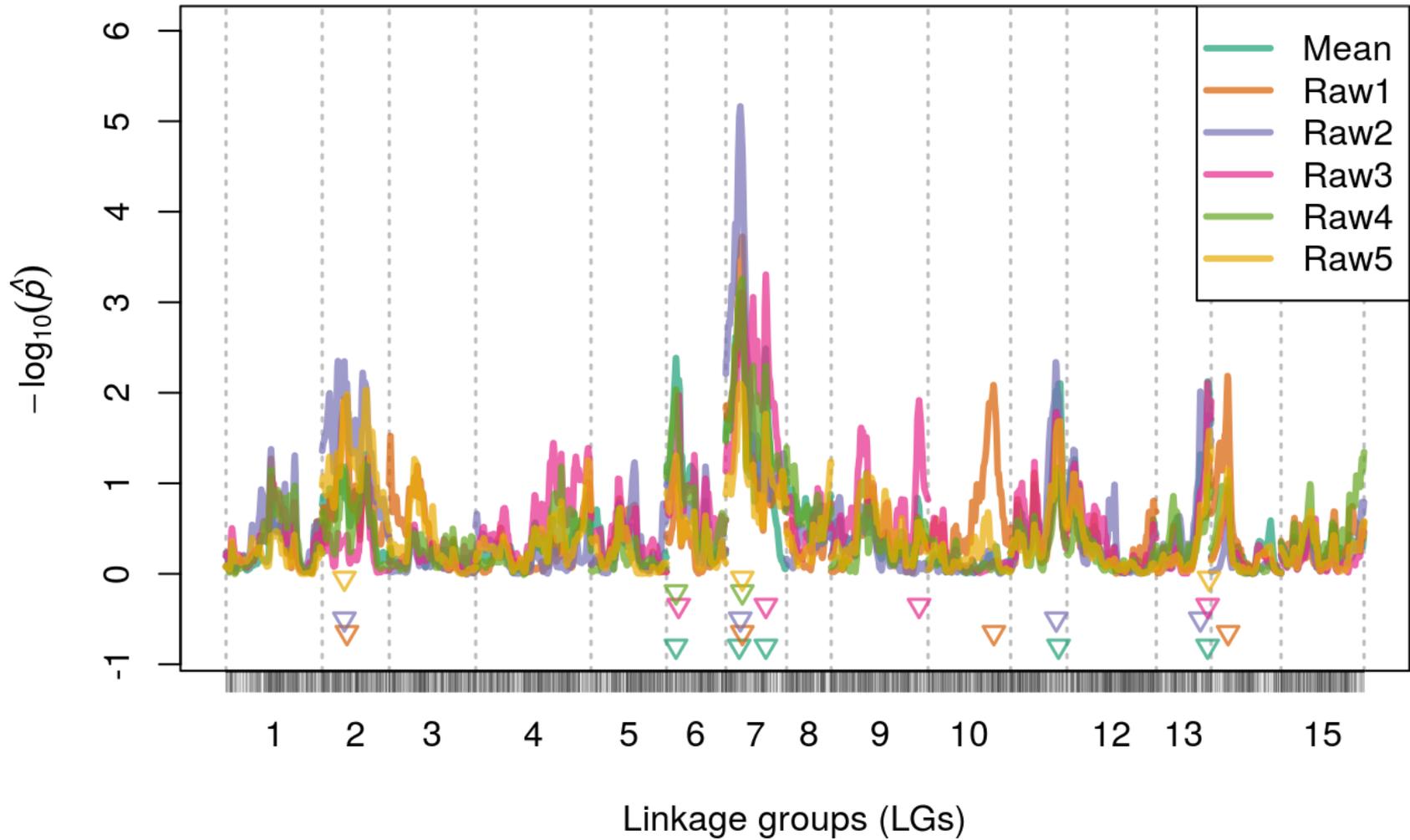


Polyqtl

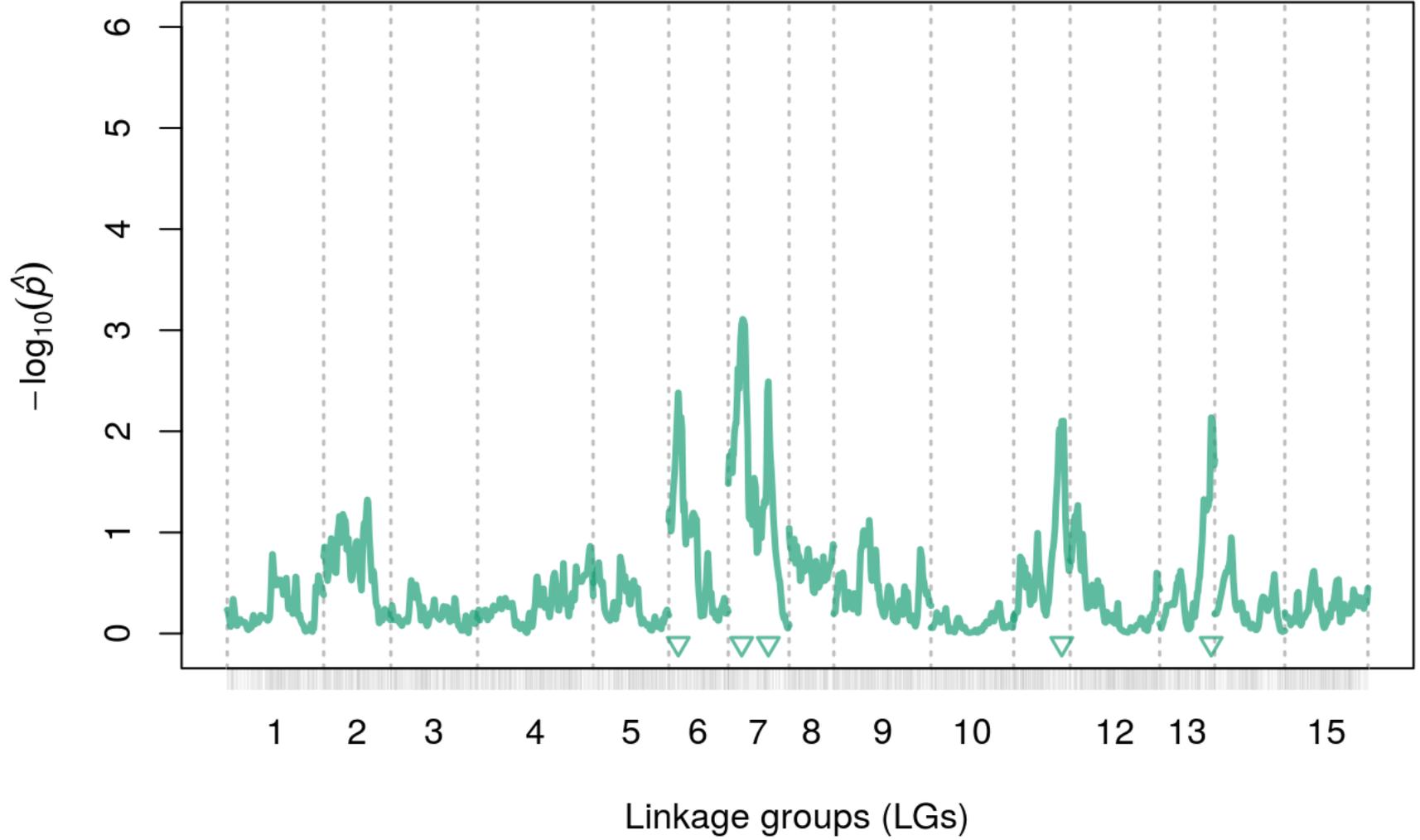
```
This is an object of class 'polyqtl.data'
```

```
Ploidy level:          6
No. individuals:      245
No. linkage groups:   15
Map size:              3627.36 cM ( 1981 markers )
```

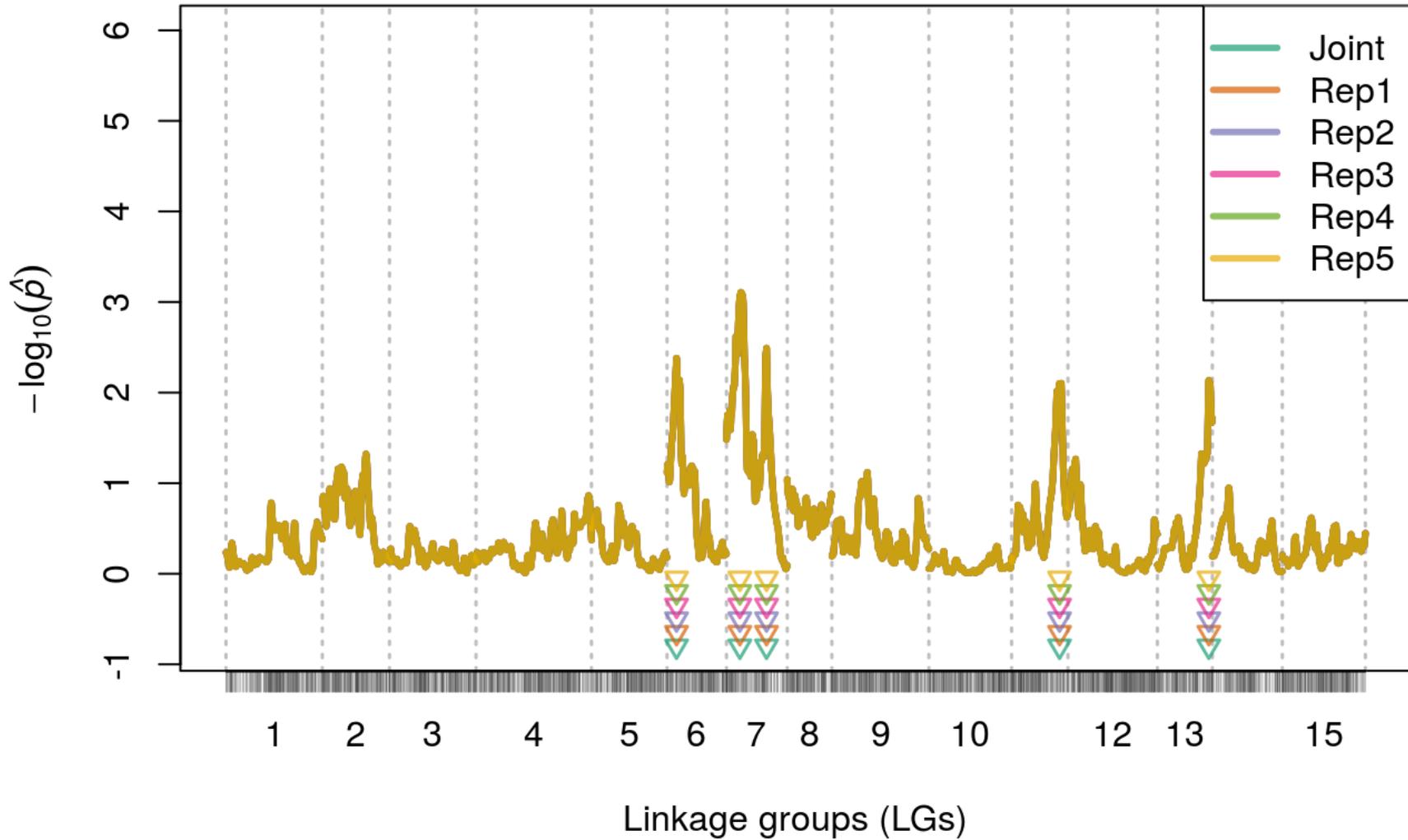
Raw data



Joint



Adjusted means



QTL	Chr.	Position	Marker	p-value	σ_{QTL}	H^2_{QTL}
1	6	30.19	S6_15936007	4.19e-03	93.6	0.079
2	7	42.63	S7_3375600	9.05e-04	143.2	0.122
3	7	127.56	S7_21032190	3.22e-03	144.7	0.098
4	11	152.71	S11_9037208	7.76e-03	135.6	0.155
5	13	163.74	S13_21933180	6.29e-03	104.7	0.089
Total						0.503

Genome Track View Help

0 5,000,000 10,000,000 15,000,000

mRNA itf07g04740.t2

Primary Data

Type	exon
Position	Chr07:3095273..3095451 (+ strand)
Length	179 bp

Attributes

Seq_id	Chr07
Source	gt4sp_hc_repr

Region sequence

 FASTA

```
>Chr07 Chr07:3095273..3095451 (+ strand) class=exon
length=179
GAGTCAGGAGCAAATTTCTTACTACCATCTCCTATAGCTTCAACAAATGAGTTGGTTACA
GTGTATGACCCAGAGGTTGGAAAGATAAAATCCCTCAGAGCATAAGCAGCTAAAGCTTGCA
AGGAGCTTAAATCGTGGAATCATTGACAGAGATCTTAAACCAAGCTCTACTGAAAGAAA
```

CHR	Marker	Region sequence	Length (bp)	Predicted description	E value
7	S7_21032190	Chr07:21030876..21033777 (+strand)	2,902	Protein kinase superfamily protein	0.0
	S7_3095392 [†]	Chr07:3093037..3100120 (+strand)	7,084	Vacuolar protein sorting	1e-64
				phosphatidylinositol 3-kinase ^{††}	2e-58
13	S13_21933180	Chr13:21932800..21935570 (-strand)	2,771	Tetratricopeptide repeat (TPR)-like superfamily protein	1e-62

- **Vacuolar protein sorting**

- ✓ Autophagy
- ✓ Ion secretion
- ✓ Stress responses

- **Phosphatidylinositol 3-kinase**

- ✓ Endocytosis
- ✓ ROS production
- ✓ Vesicle trafficking
- ✓ Involved in activating signaling molecules

- **TPR-like superfamily protein**

- ✓ Suppresses effector-triggered immunity

NC State Sweetpotato and Potato Breeding Team





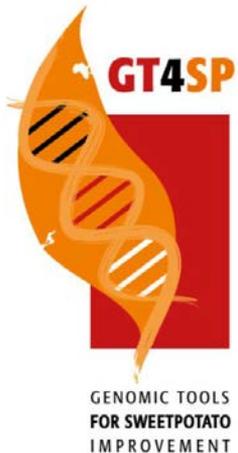


Afwoyo!
Awadifo!
Webale!

Neyanziza!

Asante!

Keitobon! Thank you!



BILL & MELINDA
GATES *foundation*

