

R in Agricultural Research

"Research purposes are the master, whereas statistical methods
are the servant"

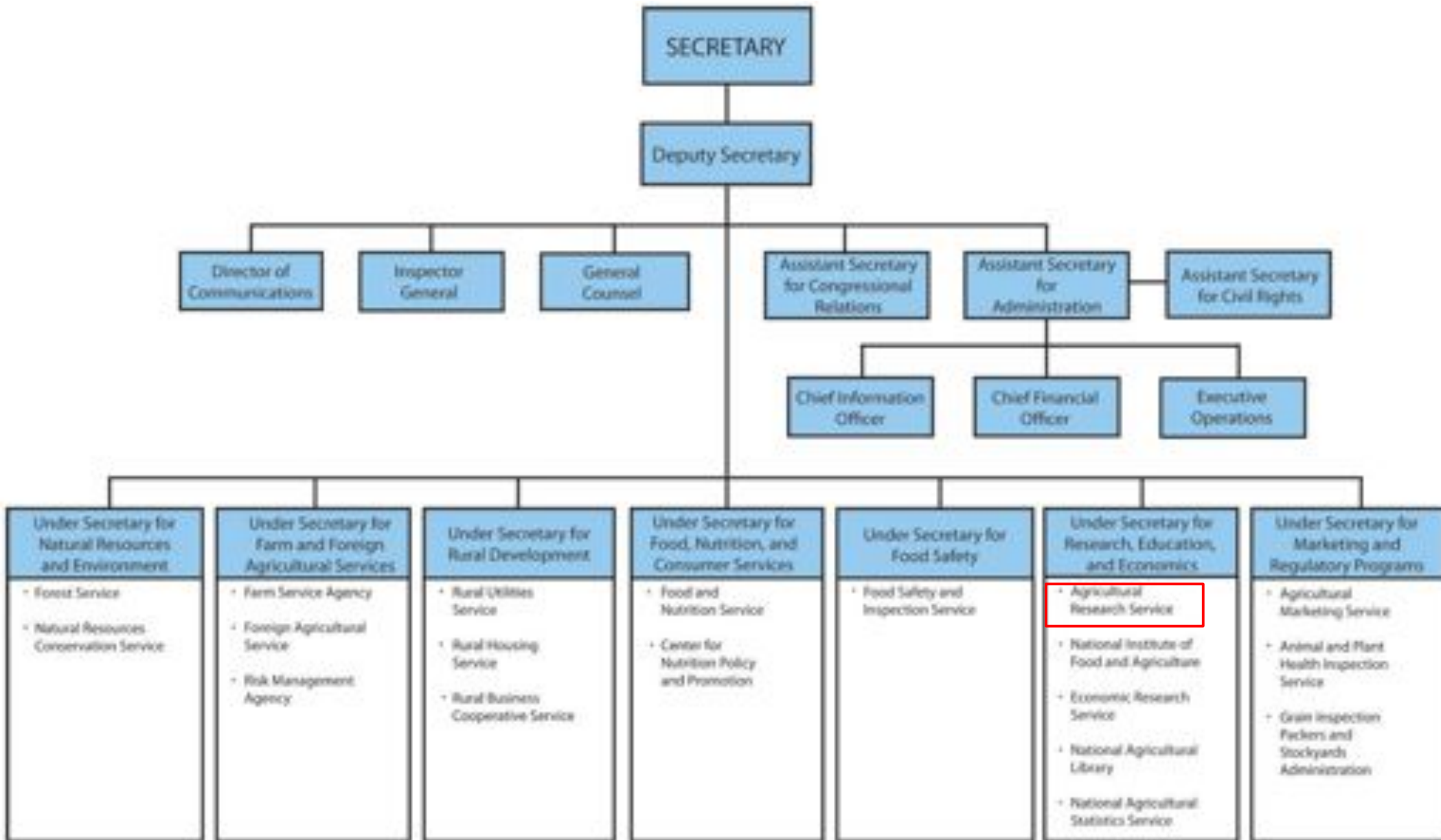
Christian Tobias Ph.D.

Research Molecular Biologist, Project Leader

USDA-ARS Western Regional Research Center



USDA Organization





- **2,100 scientists**
- **1,200 research projects within 21 National Programs**
- **6,000 other employees**
- **100 research locations including a few in other countries**
- **\$1.1 billion fiscal year 2009 budget**



- **President**

- **Secretary of Agriculture**
 - **Deputy Secretary**
 - **Undersecretary for Research Education and Economics**
 - **ARS administrator**
 - **Deputy Administrator**
 - **National Program Leaders/Area Director**
 - **Center Director**
 - **Research Leader**
 - **Lead Scientist**

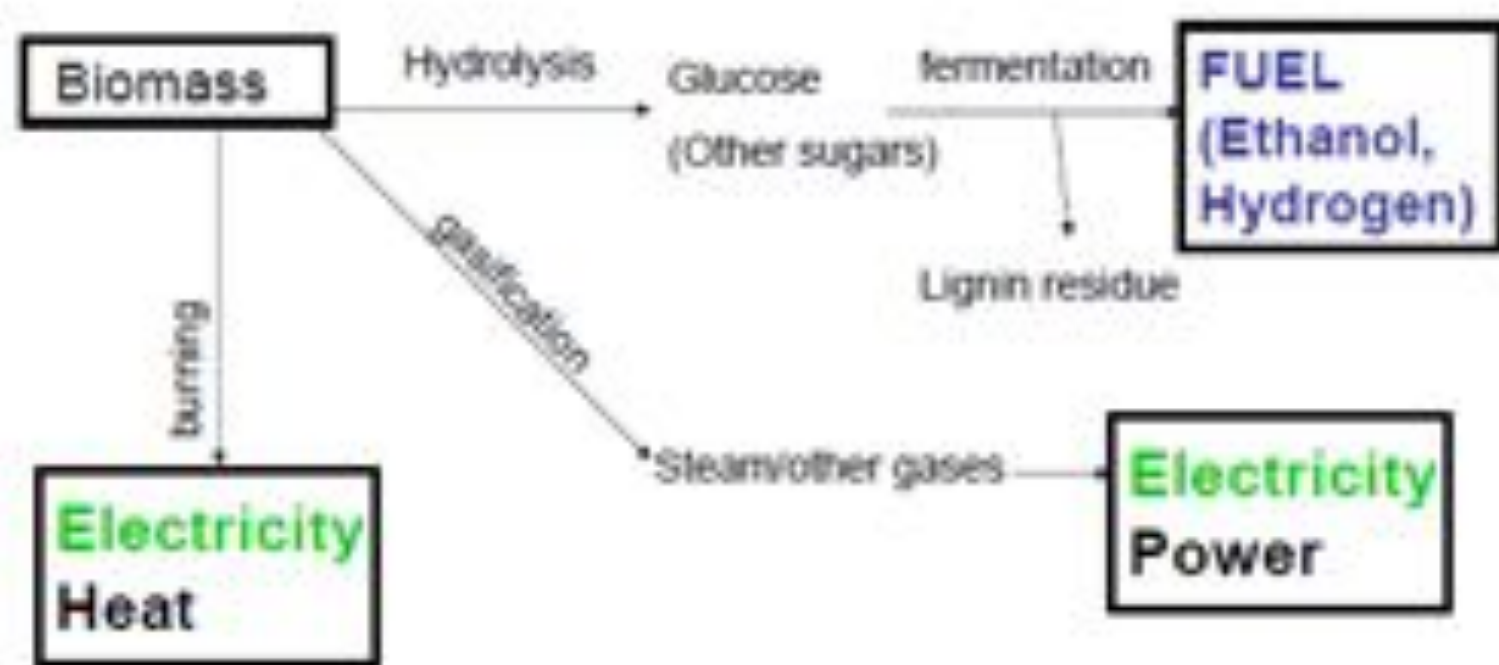


Talk outline:

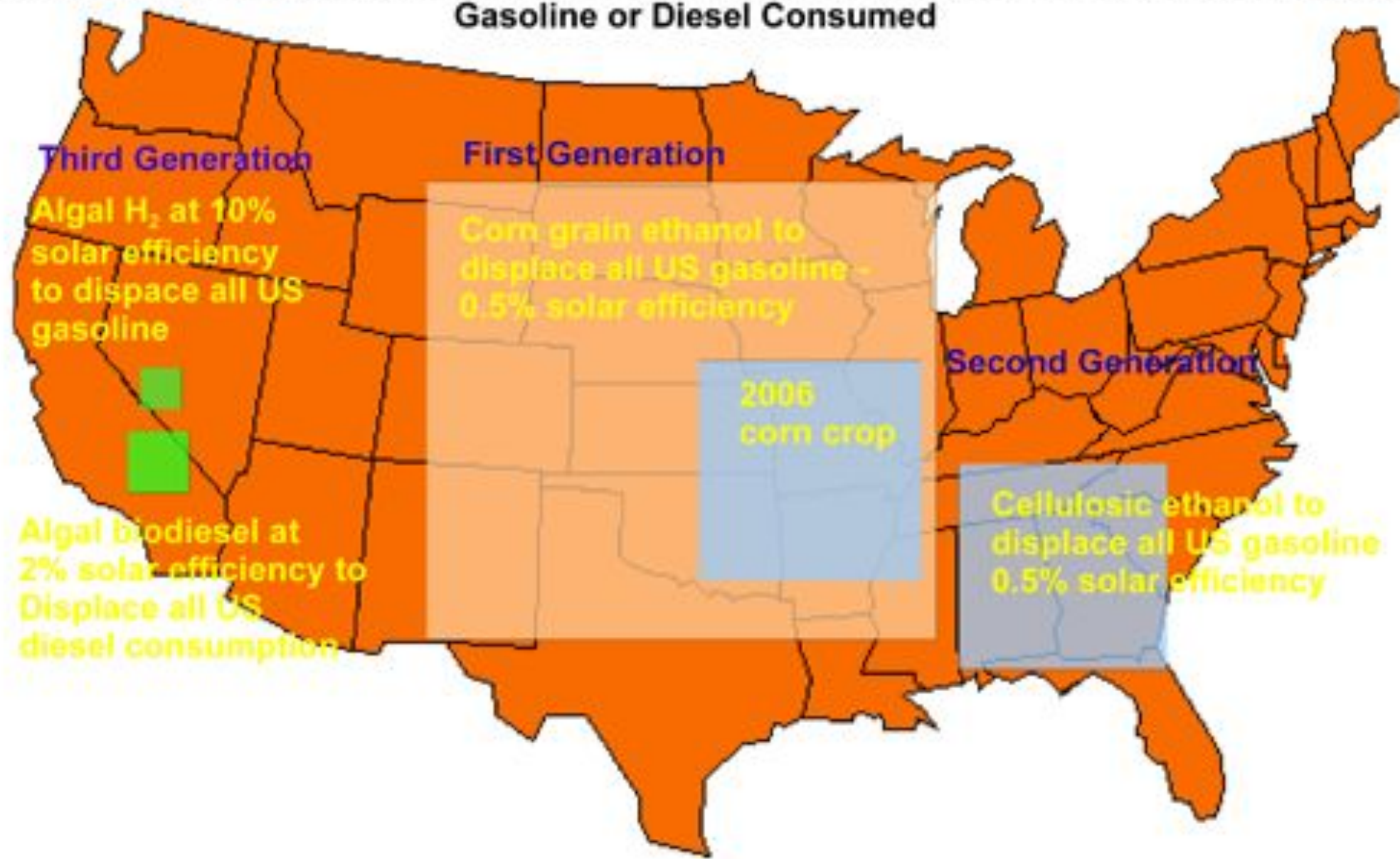
- **Introduction (Bioenergy)**
- **Molecular tools for switchgrass**
- **Specific examples of R usage in:**
 - **marker/trait association**
 - **Graphical display of data**
 - **Interactive presentation tools**
 - **Marker assisted selection/systems biology**

Energy from biomass

Biomass is a Carbon neutral energy resource



Areas of Corn, Switchgrass, and Algal Photobioreactors Required in the US to Displace all Gasoline or Diesel Consumed



Biomass crops



Switchgrass

Harvested annually



Hybrid Poplar

Harvested at age 5 to 10



Willow coppice

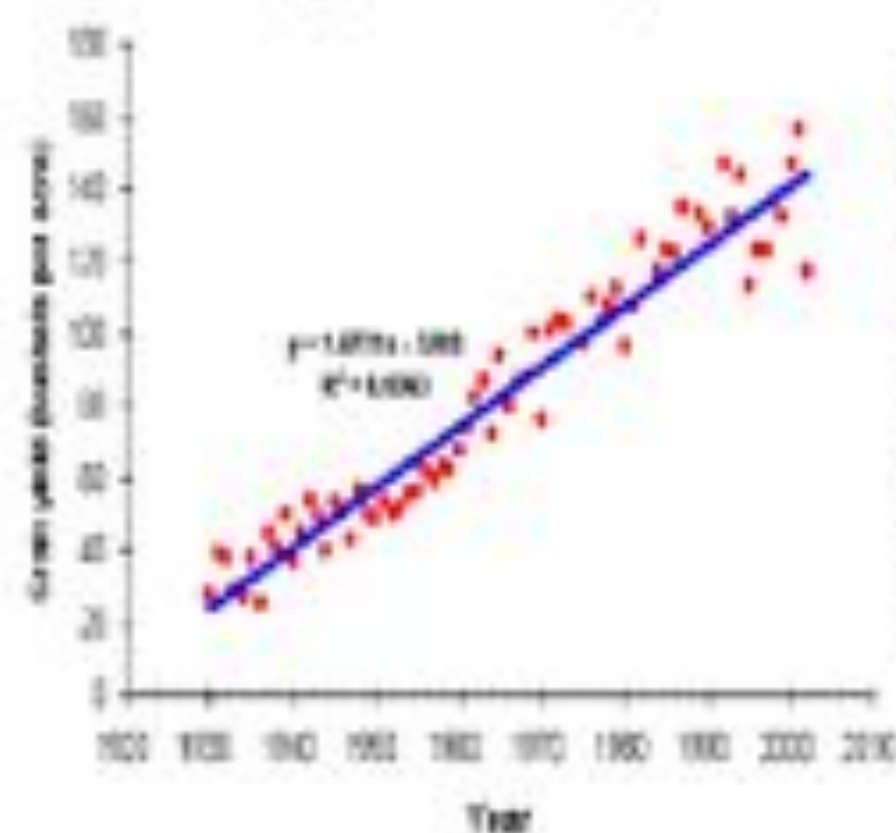
Harvested at age 3 or 4

Geographic distribution of Switchgrass and other Biomass crops



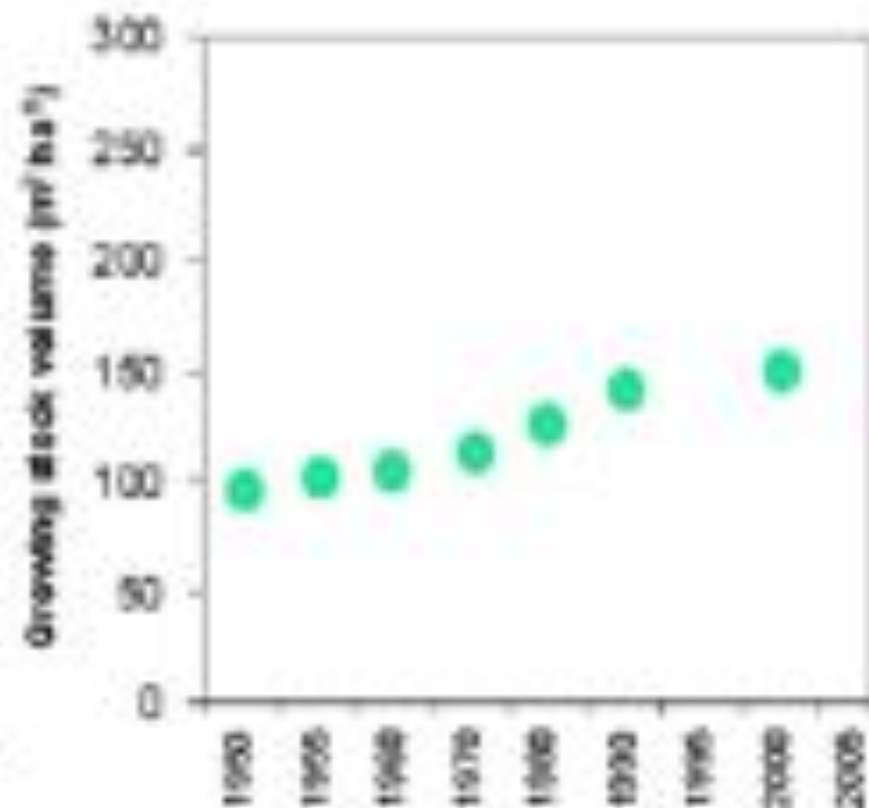
Large increases in biomass species productivity are probably possible through genetic improvement

Average Indiana corn yield



Data Source: Indiana Agricultural Statistics Service

Average European forest yield



Source: European Forest Institute (www.efi.fi)

Comparison of a wild and domesticated tomato



Switchgrass as an energy crop

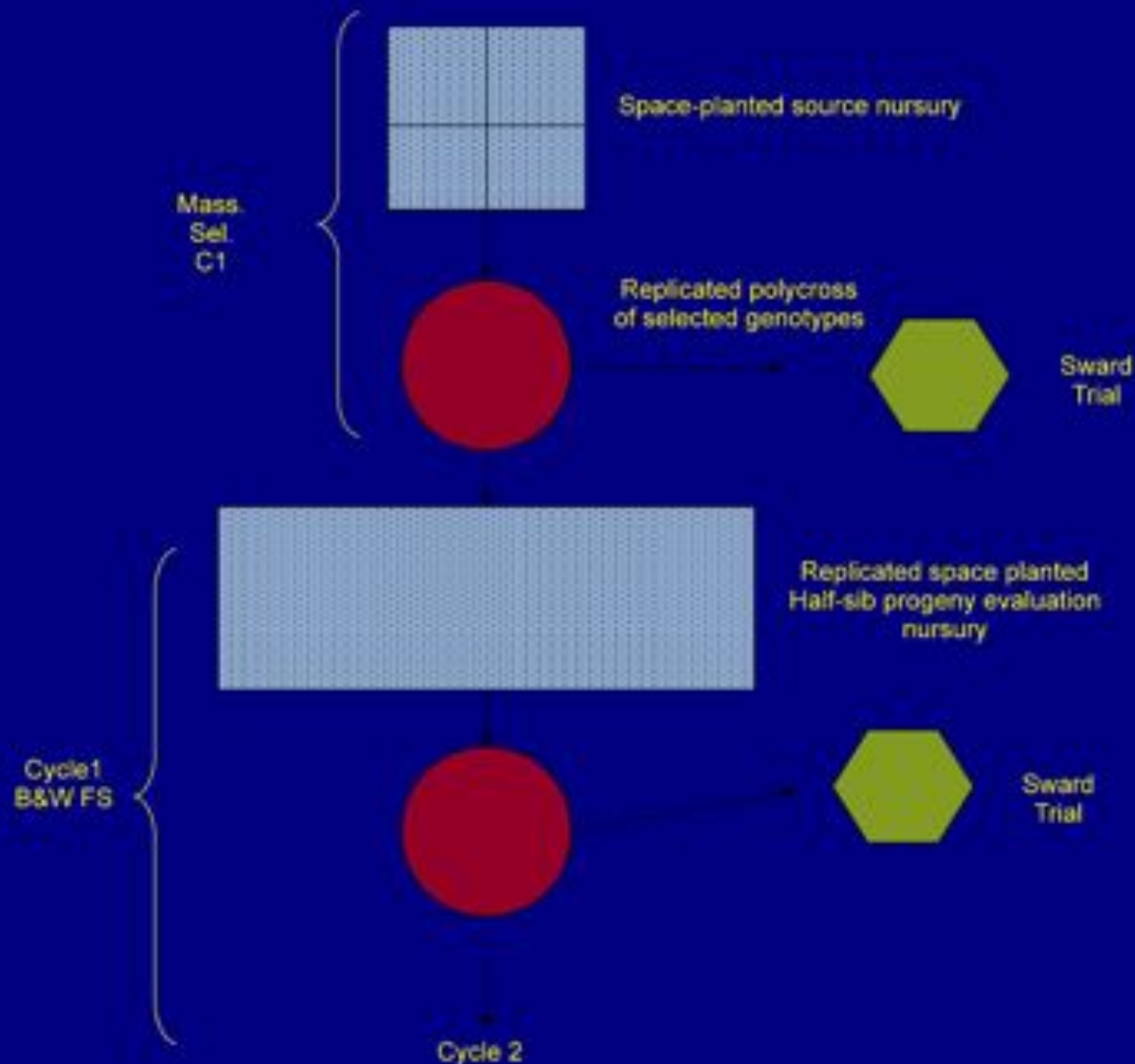
- Compatible with common production systems and farming practices
- Perennial
- C4 metabolism/warm season grass
 - Drought Tolerance
- Efficient nitrogen utilization
- Wide adaptation/large geographical range
- High yielding (13.5 Mg/ha)
- Large fibrous root system/increased soil organic matter
 - Sod Forming
 - Prevents soil erosion
- Tolerance to flooding
 - Wetlands Soil Conservation/Buffer Strips
- Native component of N.A. tallgrass prairie
- Utilized as forage grass
- Good burn qualities for a grass
 - Corn 9-13% Ash
 - Switchgrass 3-6% Ash



Selection of the Best Plants Within a Population



Switchgrass breeding



Switchgrass as a system

- Polyploidy 4x, 8x 10x derivatives
- Difficult to phenotype in greenhouse
- Genetically intractable/cross-pollinated
- Upland and lowland cytotypes
- Transformation and regeneration systems aren't optimal
 - Sterility and somaclonal variation
- Potential weediness/containment
- Targets for breeding and transformation
 - Dry matter yield
 - Abiotic stress
 - Seed dormancy/seed quality/rapid establishment
 - Hybrids/semihybrids
 - Cell Wall Composition
 - Early spring growth

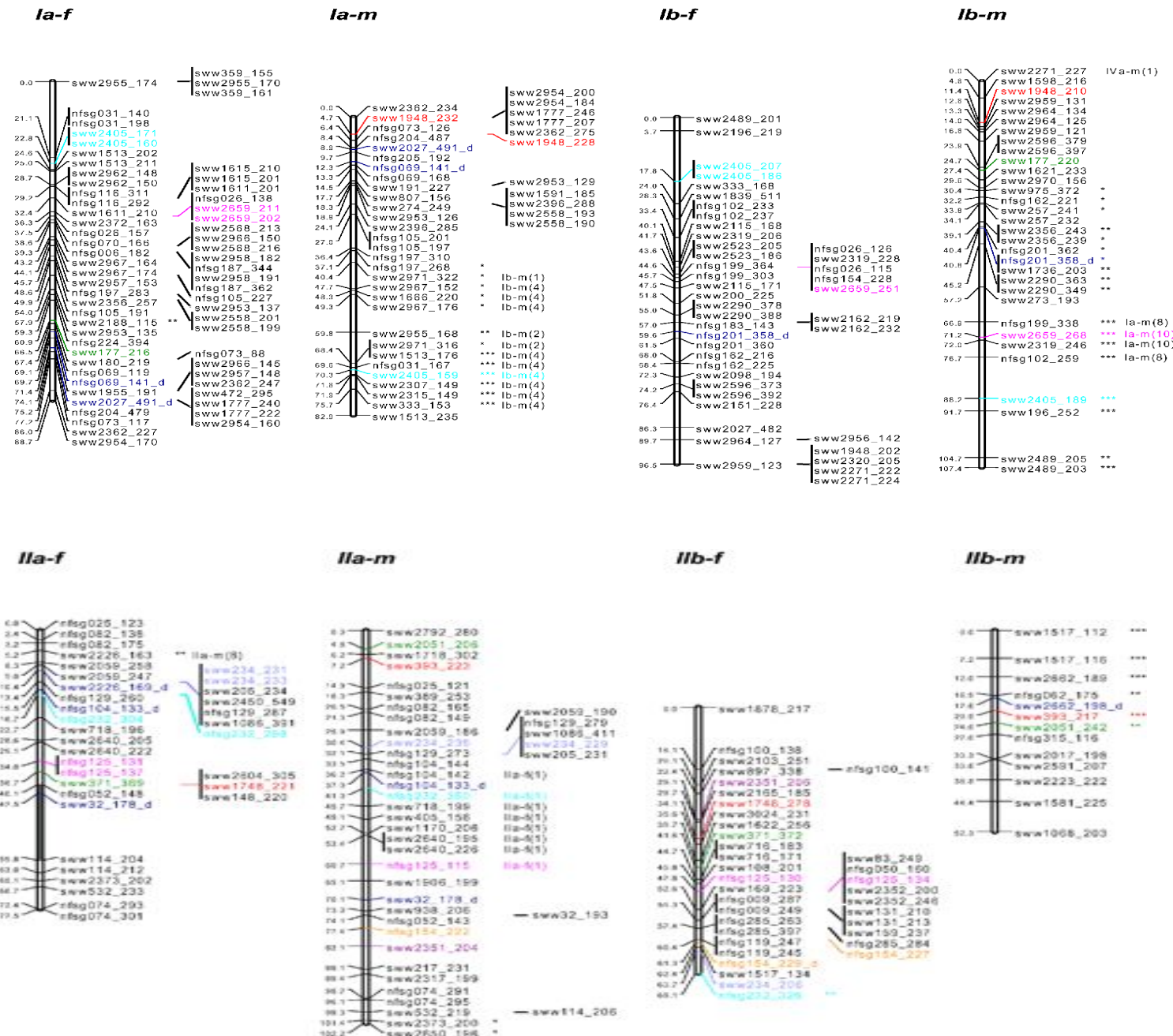


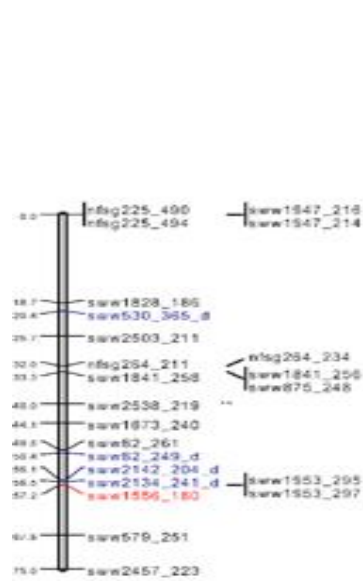
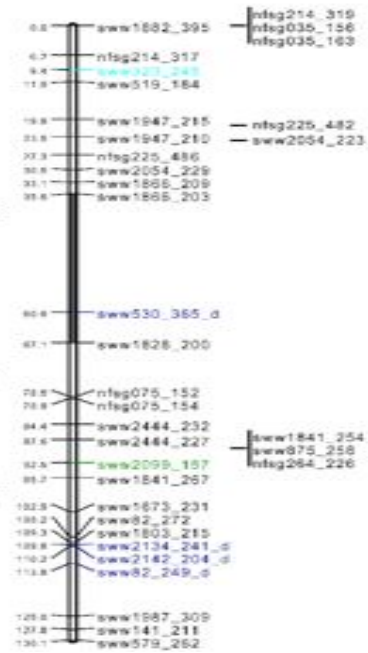
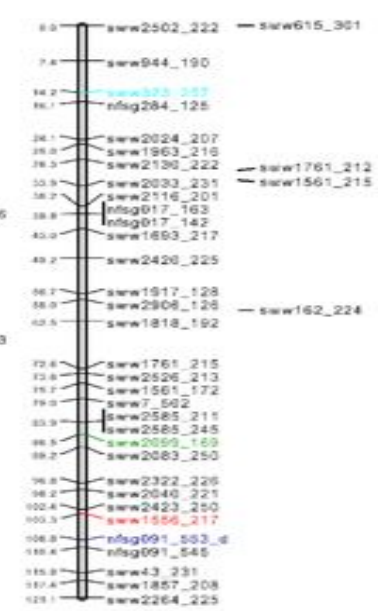
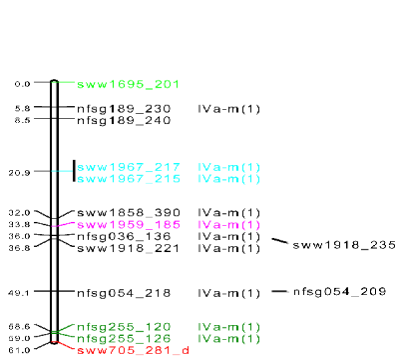
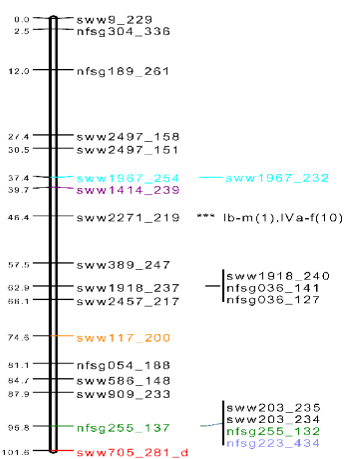
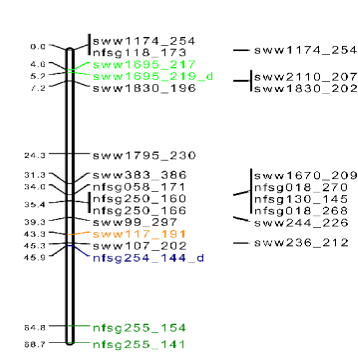
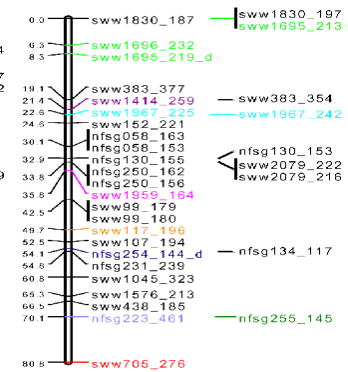
Switchgrass Linkage Map

- pseudo-testbackcross
- Kanlow x Alamo σ^7
- 238 F1 genotyped
- Combination of EST-SSR, EST-STS, and intergenic-SSR markers to allow sequence based comparison
- Scored by capillary gel-electrophoresis, ABI-3730
- greenhouse; Tifton, GA; Austin, TX

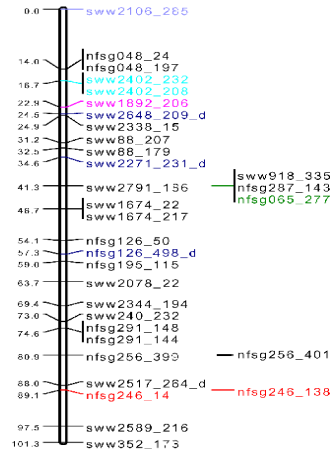


- Linkage Map: Miki Okada
- Raw data available from www.genetics.org
- Cmap data will be included in next version of Gramene (Ken Youens-Clark, personal communication)

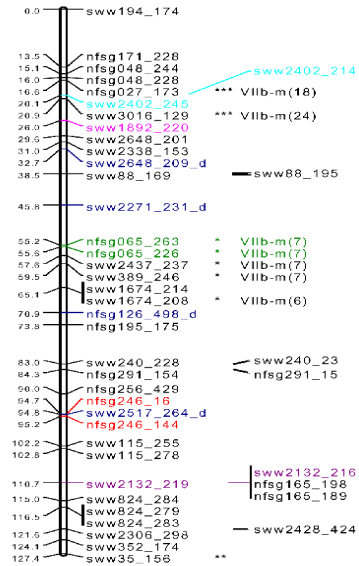


IIIa-f**IIIa-m****IIIb-f****IIIb-m****IVa-f****IVa-m****IVb-f****IVb-m**

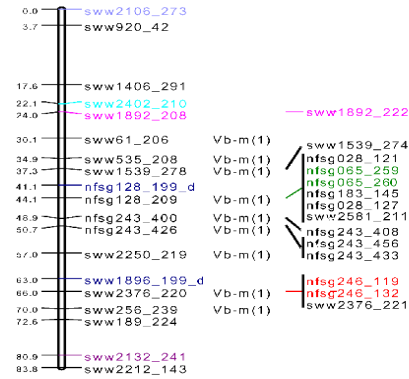
Va-f



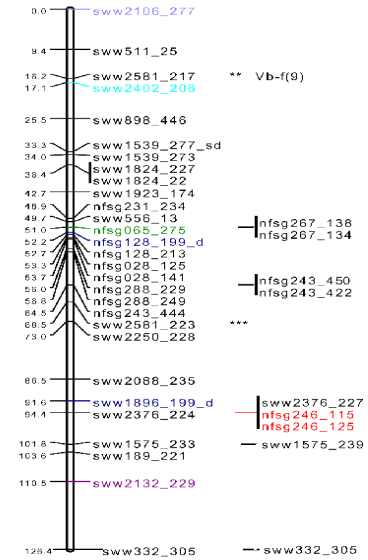
Va-m



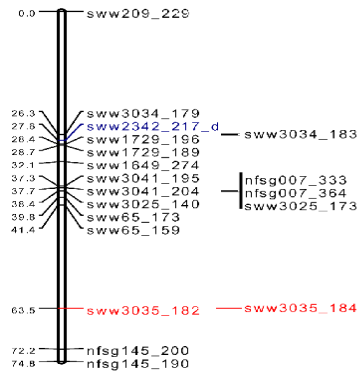
Vb-f



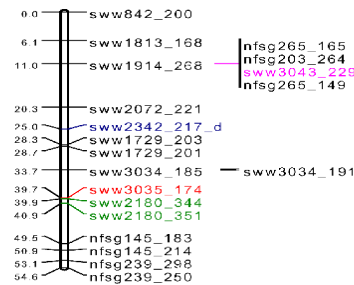
Vb-m



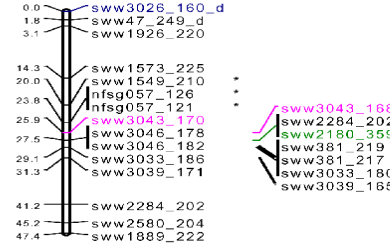
Vla-f



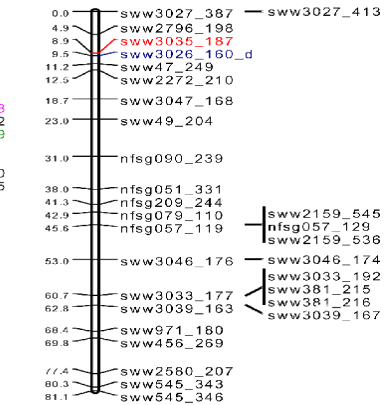
Vla-m

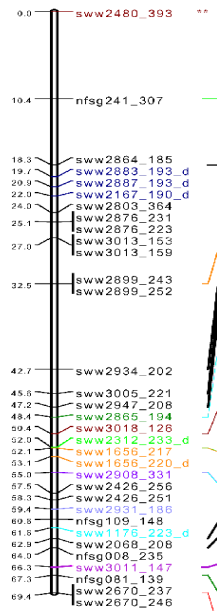
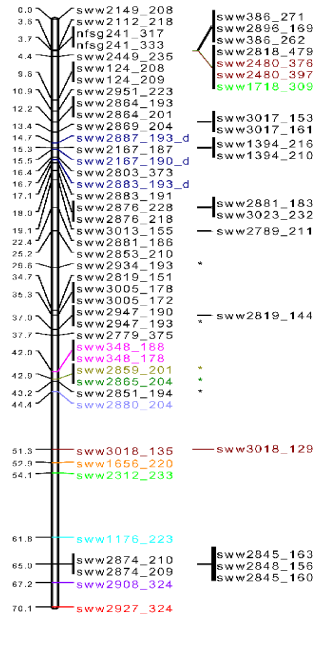
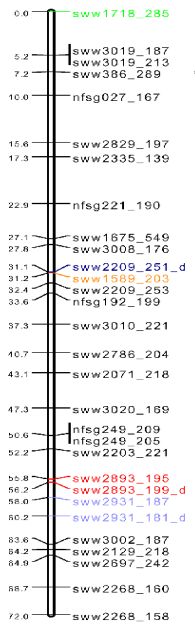
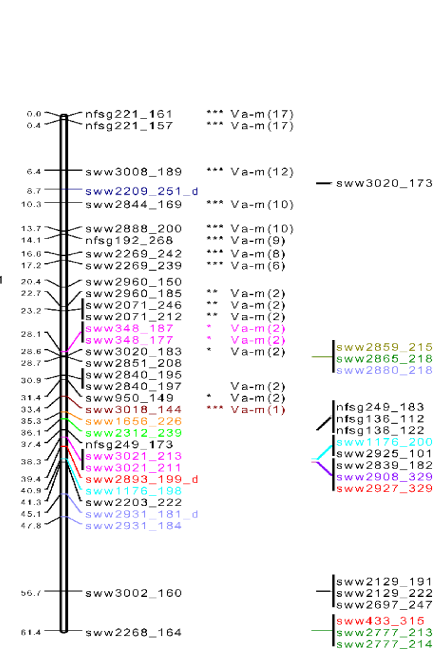
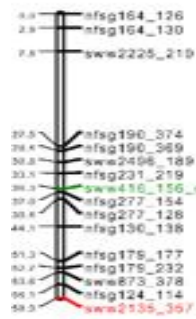
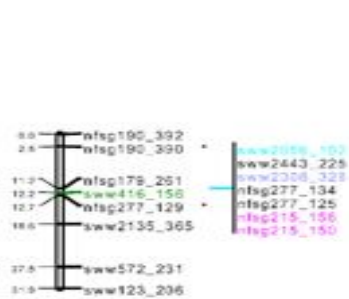
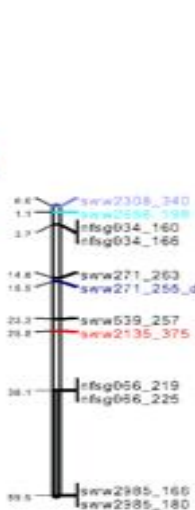
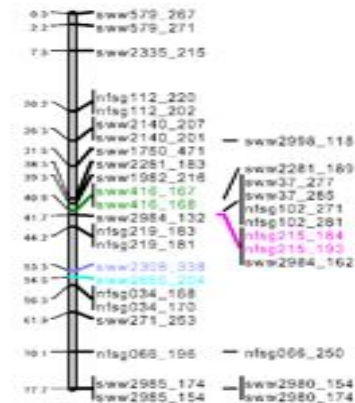


Vlb-f

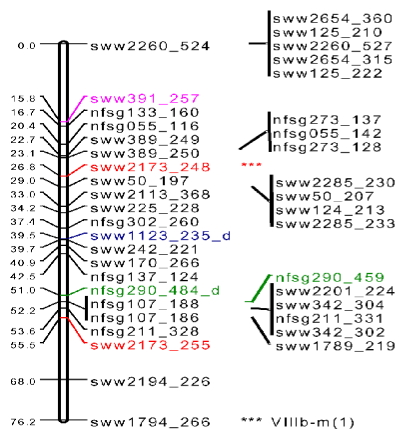


Vlb-m

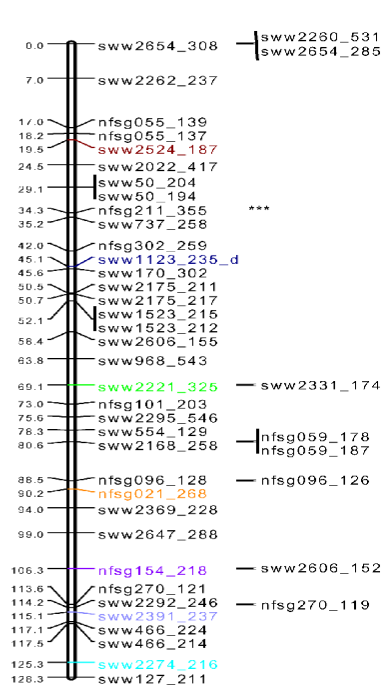


Vila-f**Vila-m****Vilb-f****Vilb-m****Villa-f****Villa-m****Vilb-f****Vilb-m**

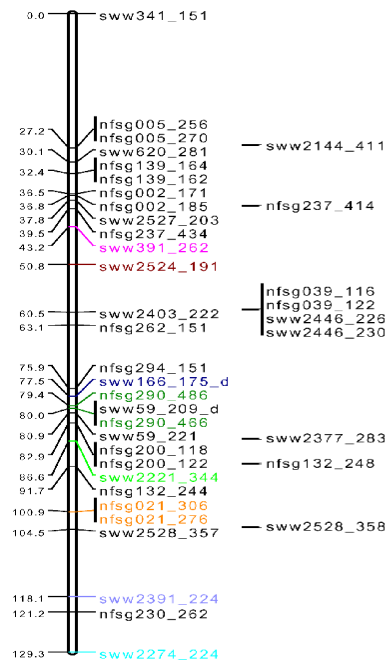
IXa-f



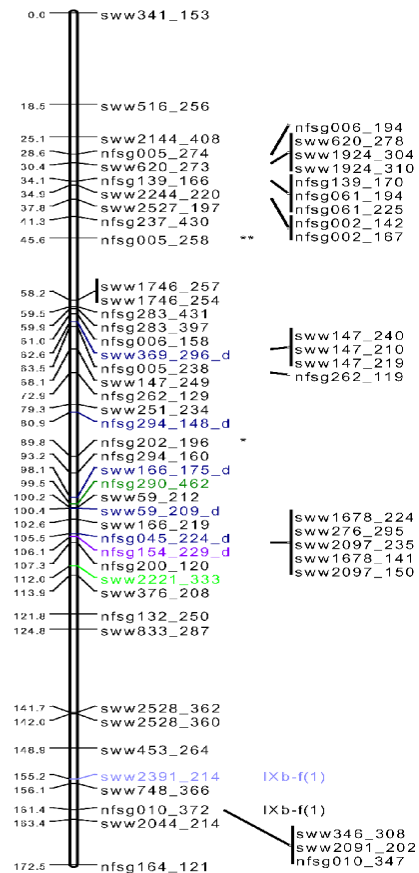
IXa-m



IXb-f



IXb-m



Amplification, polymorphism, and segregation ratios of molecular markers in the switchgrass mapping

population

	Female parent				Male parent				Both parents			
	total	polymorphic	1:1	1:1	3:1	5:1	1:1	1:1	3:1	5:1	3:1	
	probes	amplicons	amplicons	(TRD ^a)				(TRD)				(SDxSD ^b)
EST-SSR	420	1364	945	354	13	2	0	342	62	11	0	91
Genomic-SSR	181	620	509	209	6	2	0	191	24	8	3	29
EST-STS	36	109	55	20	0	0	0	22	4	0	0	6
total	637	2093	1509	583	19	4	0	555	90	19	3	126

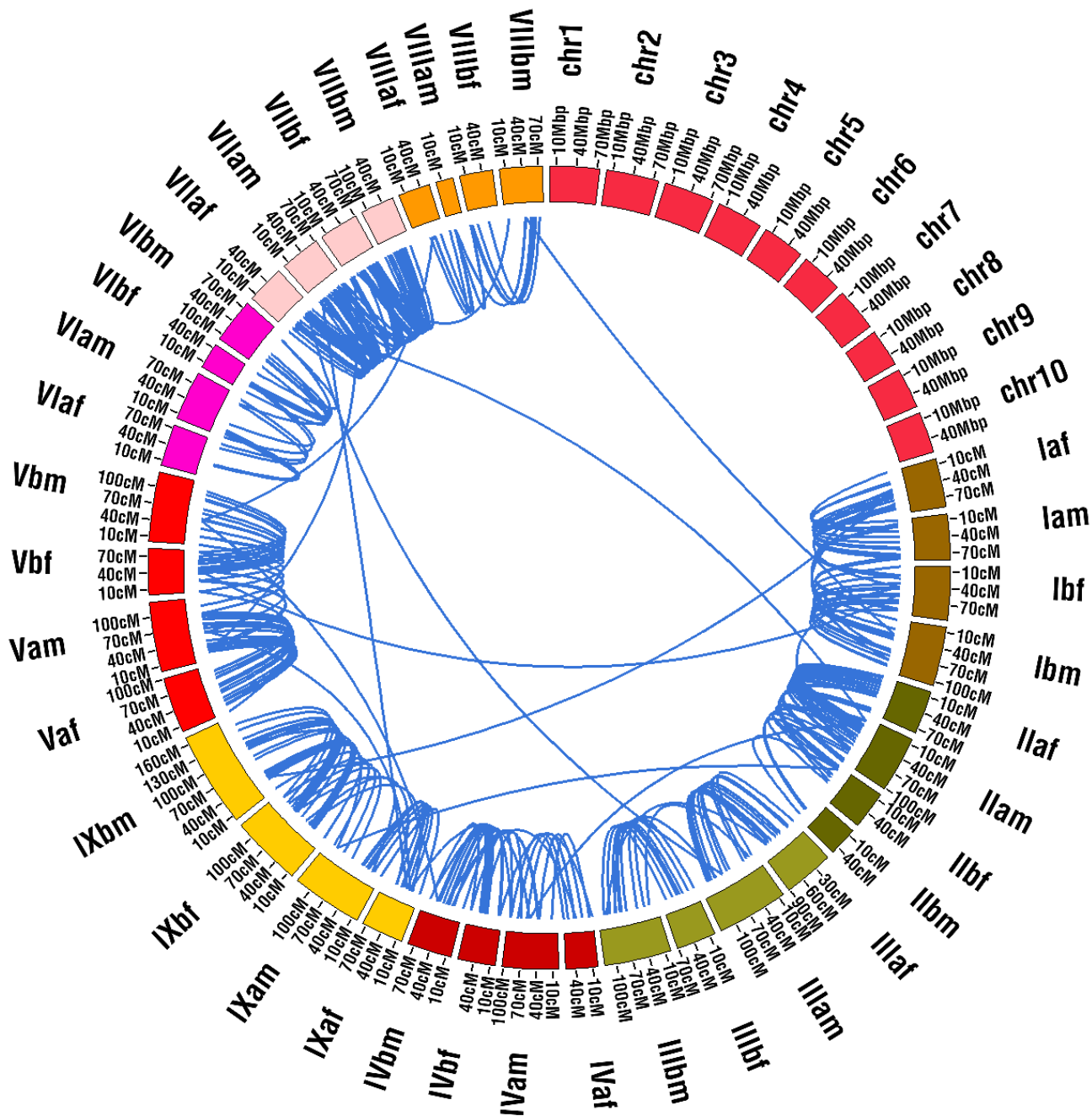
^aTRD, transmission ratio distorted. Markers were considered TRD if segregation ratio was less than 1.73:1 and deviated significantly from 1:1 by the chi-square test ($\alpha = 0.01$)

^bMarkers monomorphic between parents and segregating in 3:1.

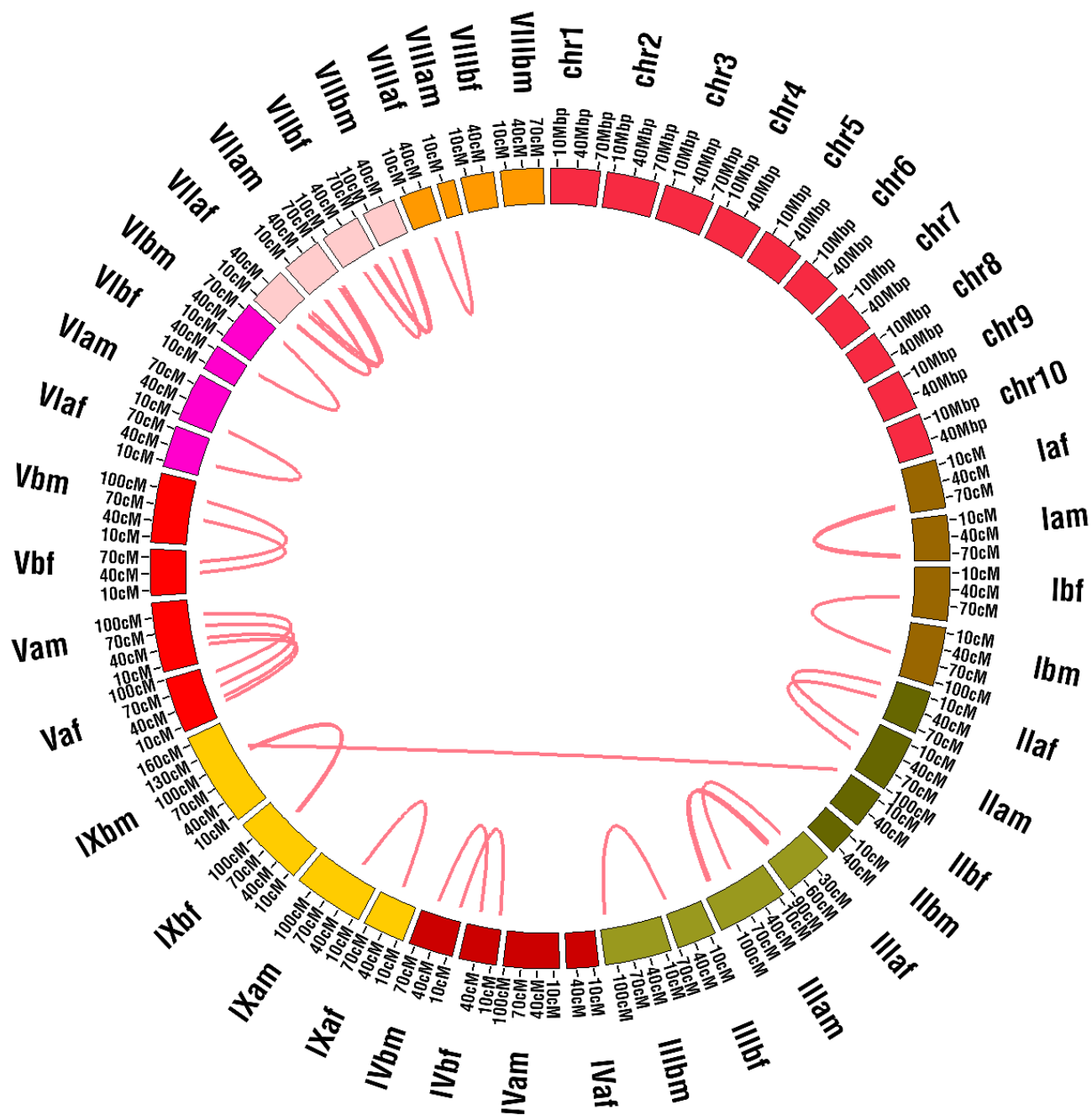
Map Statistics

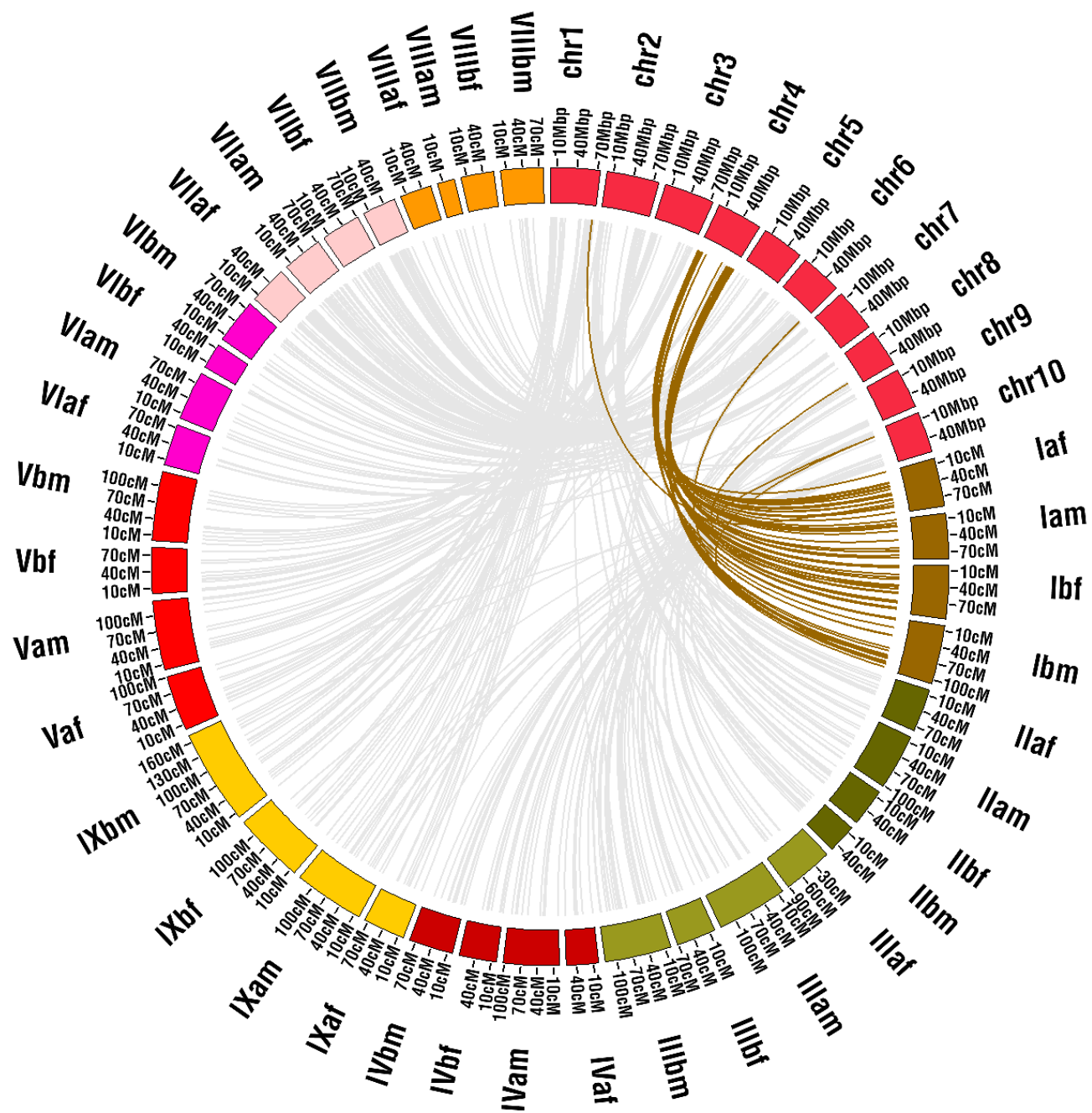
	Framework		with distorted and shared single-dose amplicons included	
	female map	male map	female map	male map
number of mapped polymorphisms				
total	563	542	616	658
single dose (accessory)	338(225)	380(162)	339(225)	381(162)
distorted	n/a	n/a	14	75
single dose x single dose	n/a	n/a	38	40
per linkage group, average (range)	19(9-32)	21(5-35)	22(12-35)	28(8-43)
unmapped single dose	9	4	8	3
total number of linkage groups	18	18	18	18
total map length (cM)	1376	1645	1400	1748
average linkage group length (range) (cM)	76(45-129)	91(27-176)	78(47-129)	97(32-173)

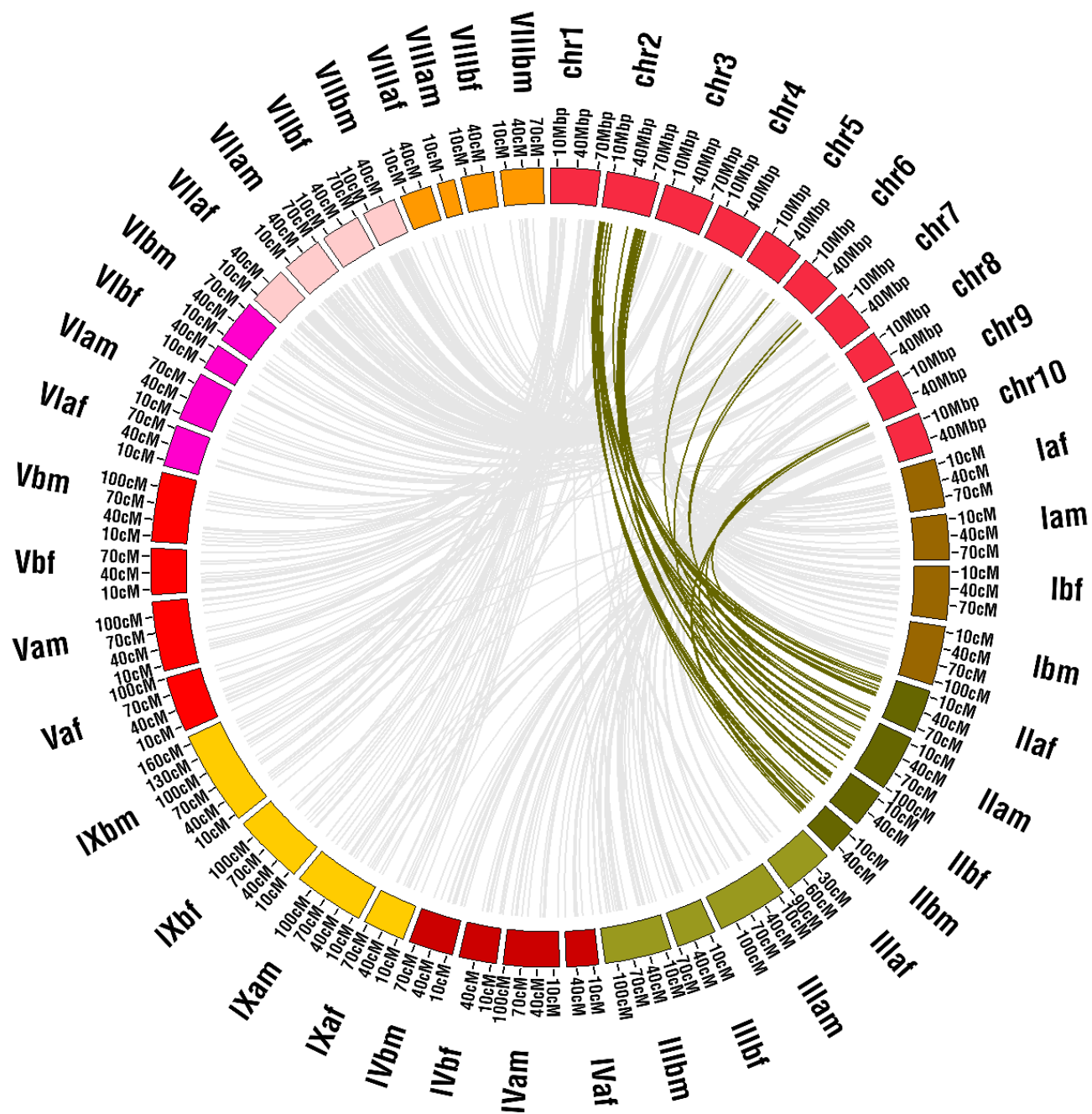
Subgenome Divergence Greater Than Expected by Chance



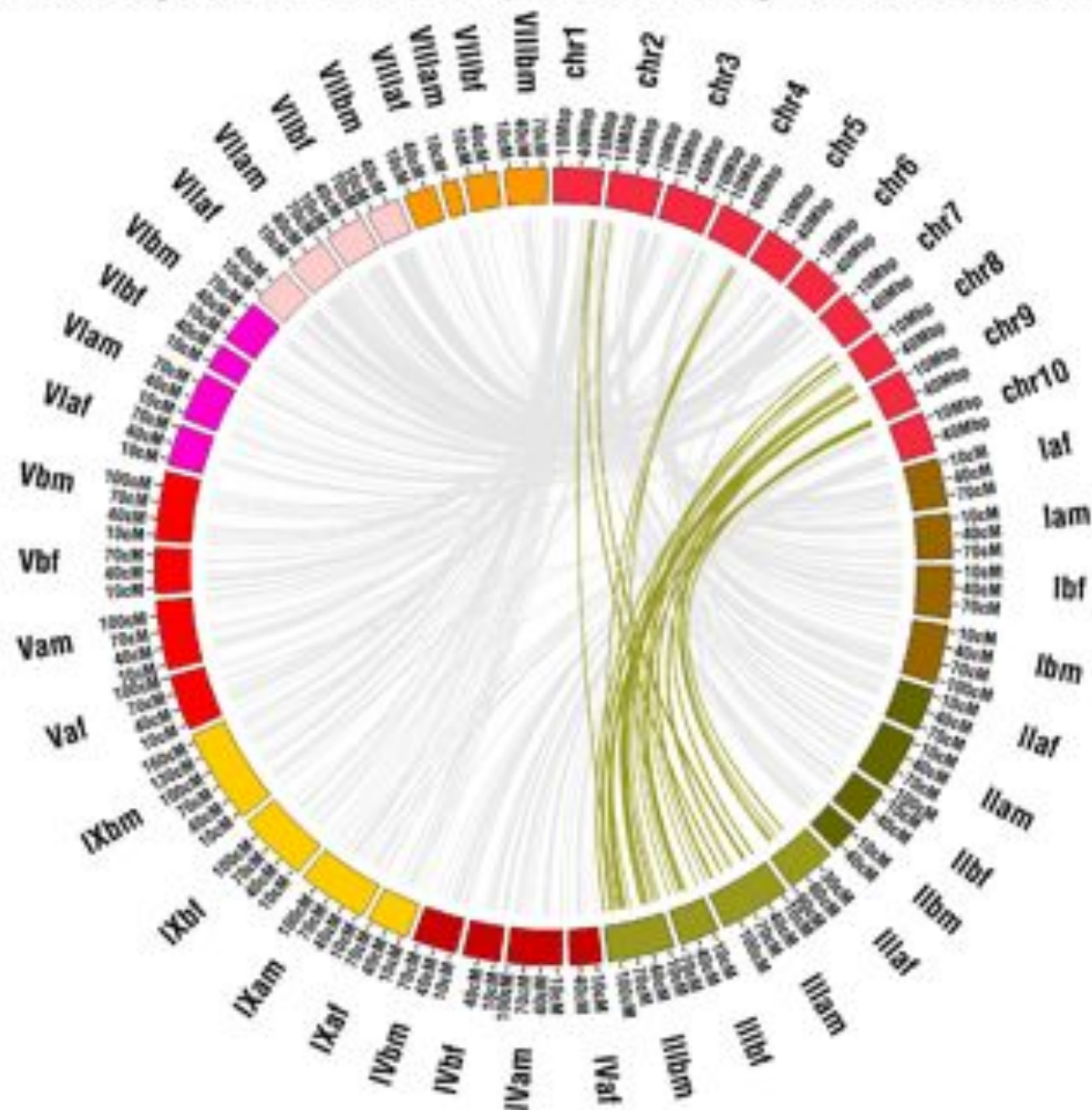
[http://mkweb.bcgsc.ca/circos/
perl](http://mkweb.bcgsc.ca/circos/perl)

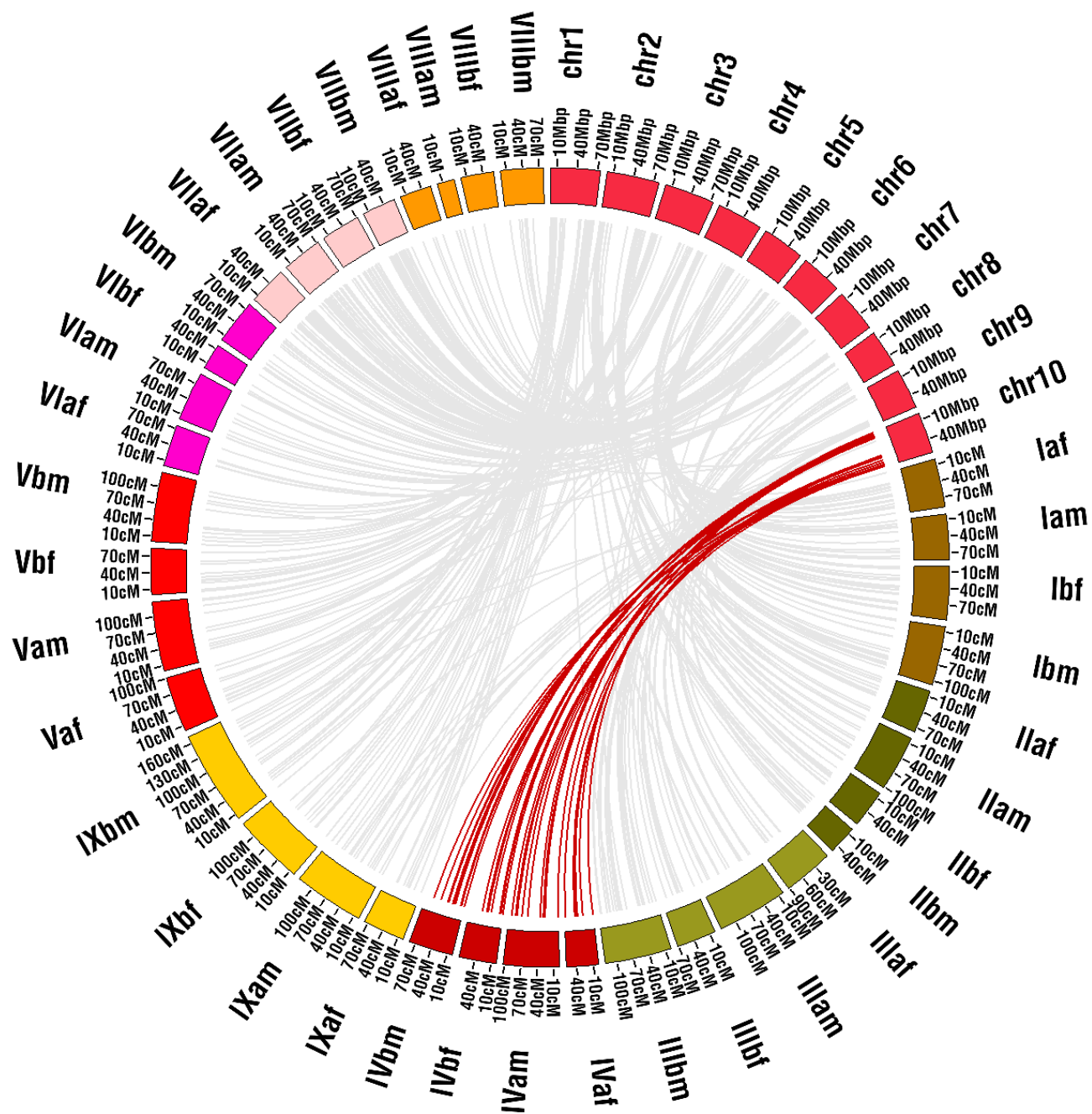


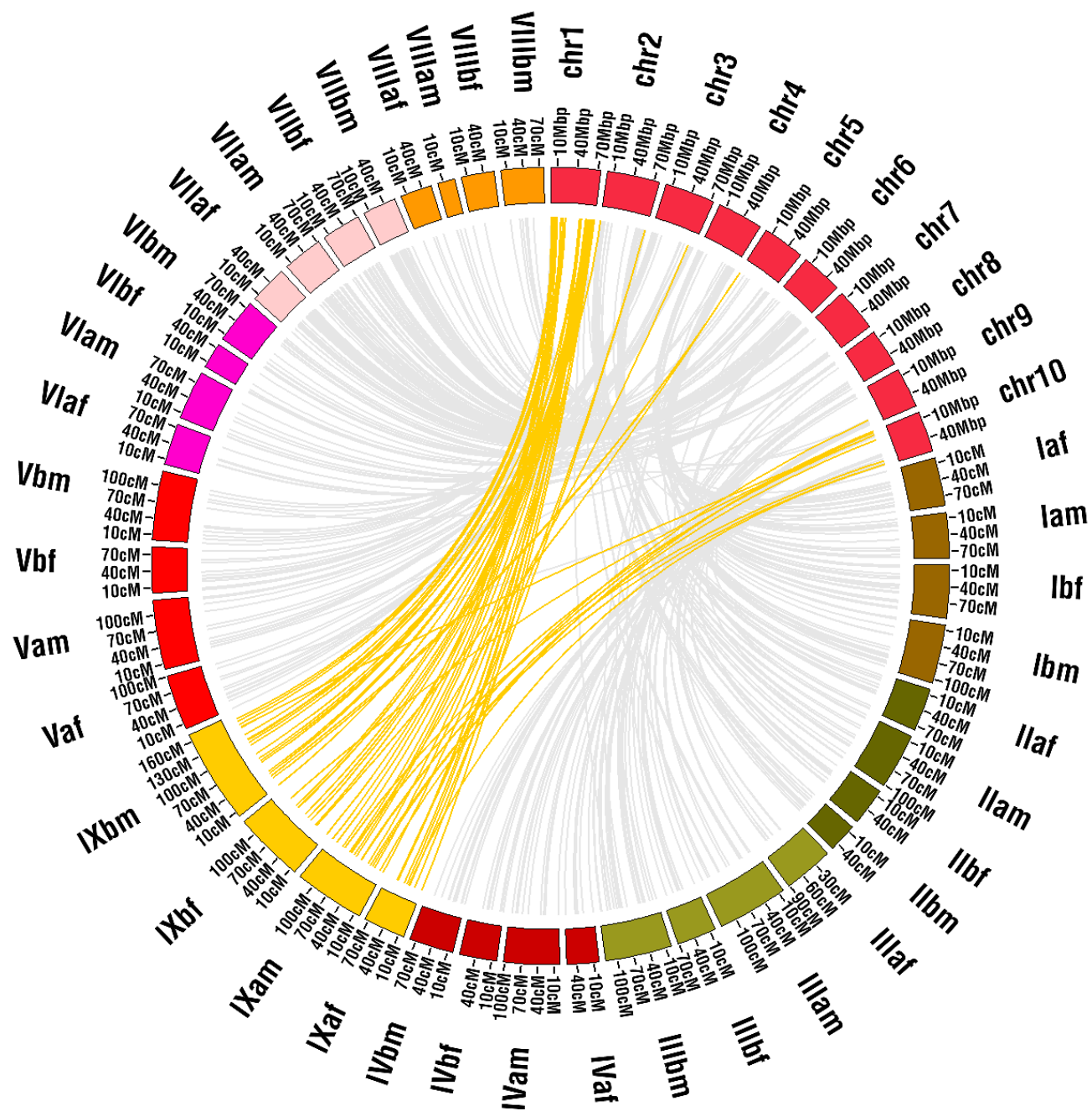


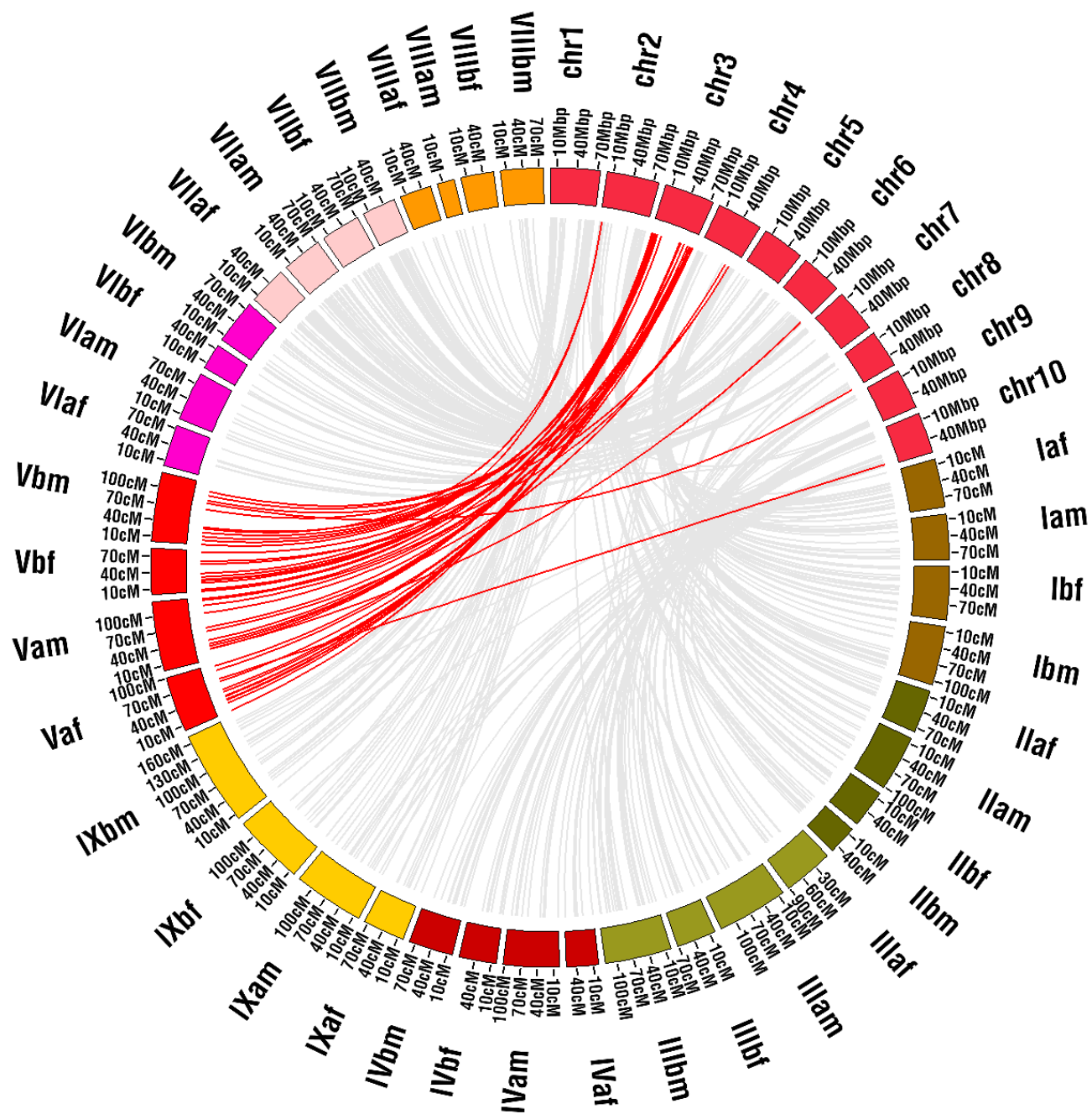


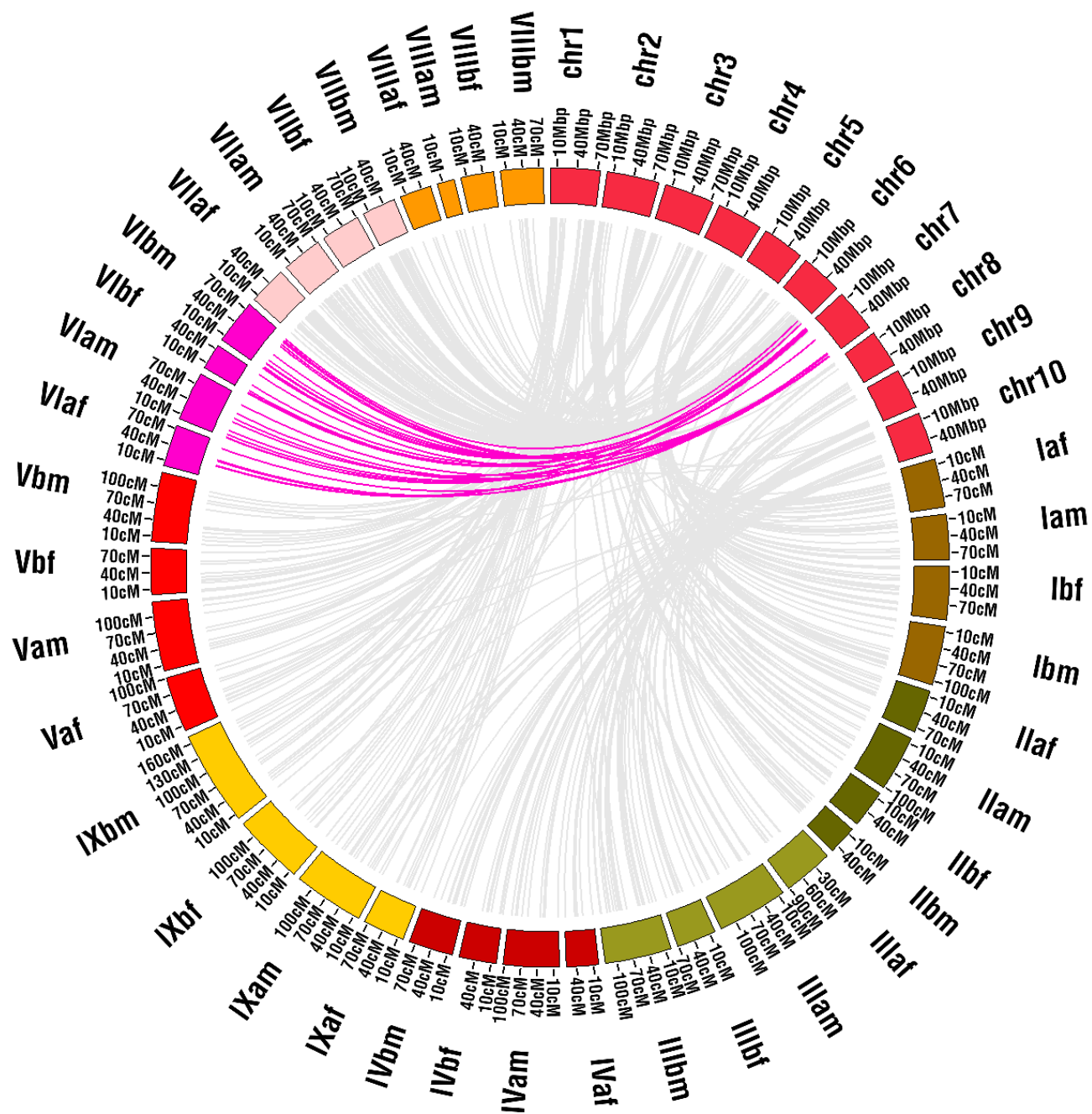
Switchgrass HGIII Corresponds to Sorghum Chr 8 and 9

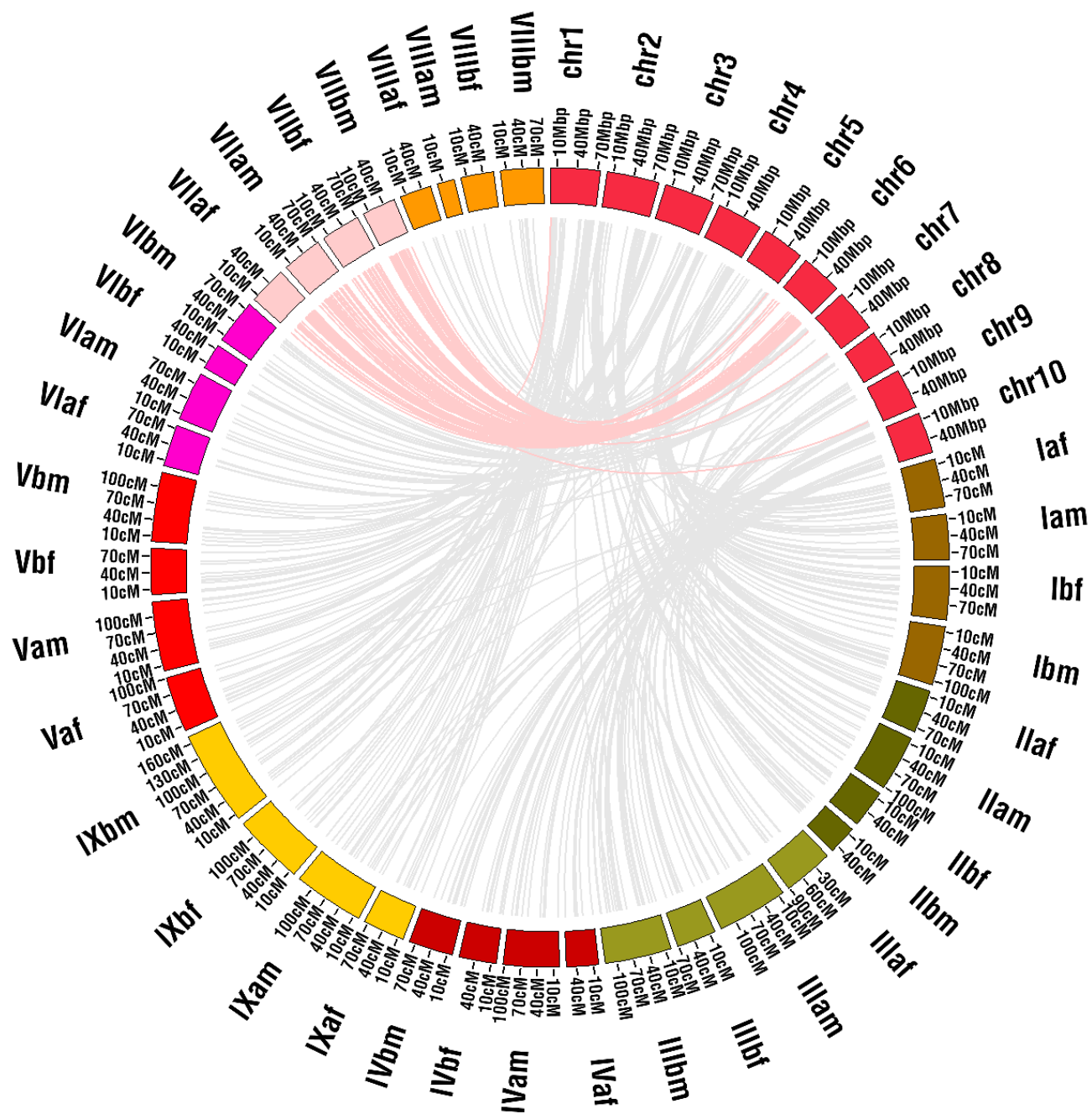


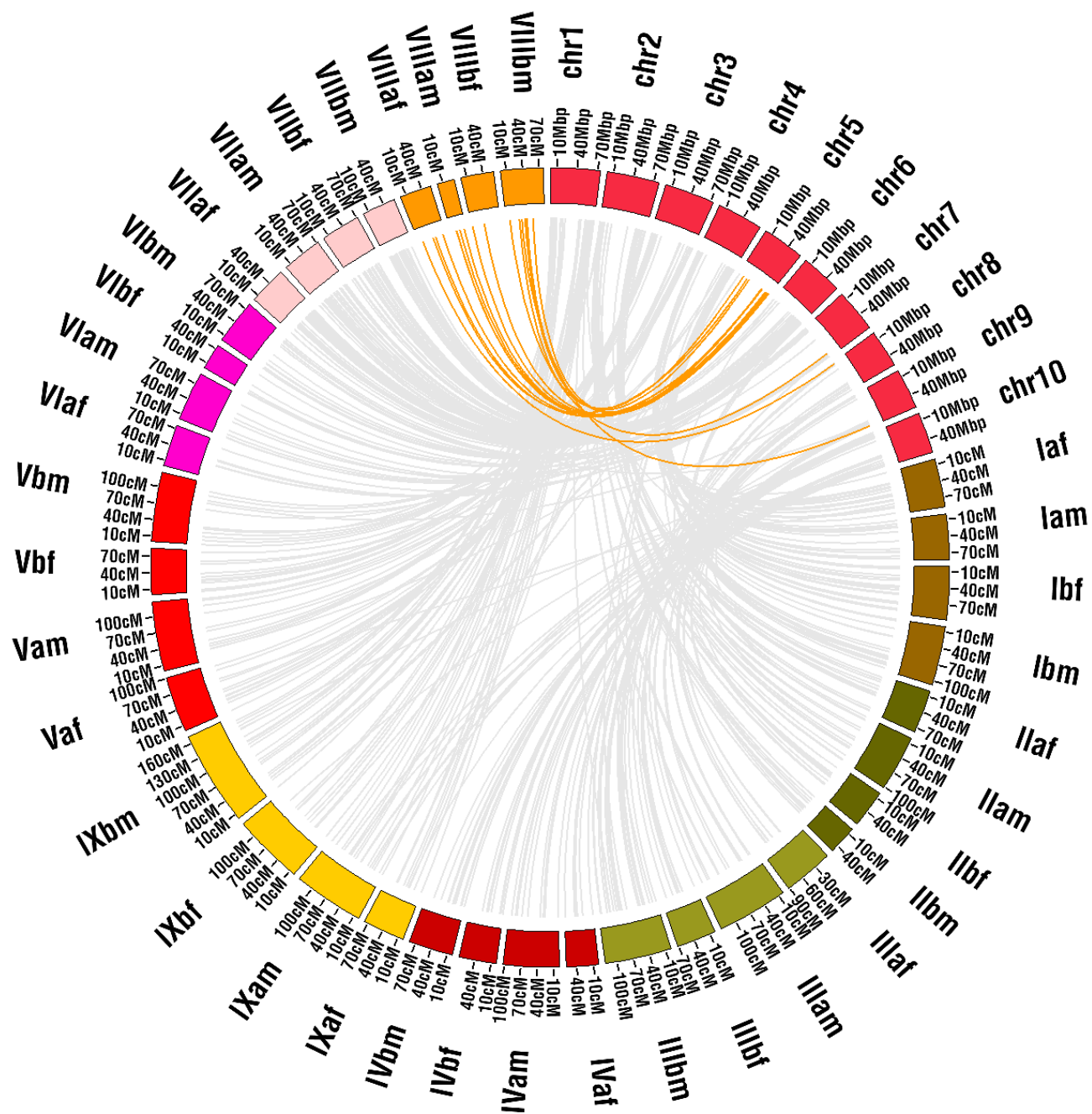




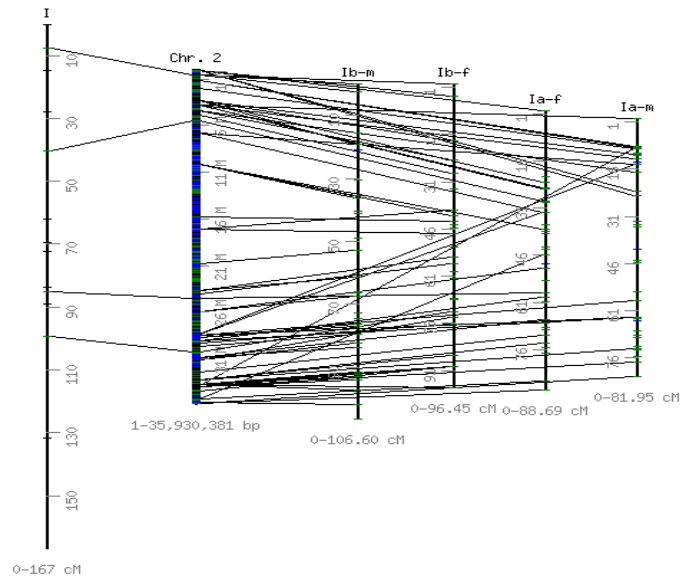




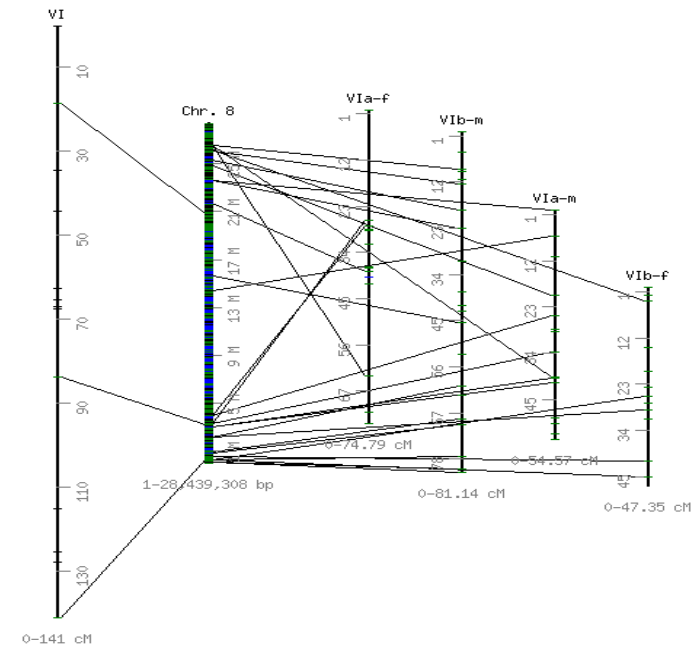
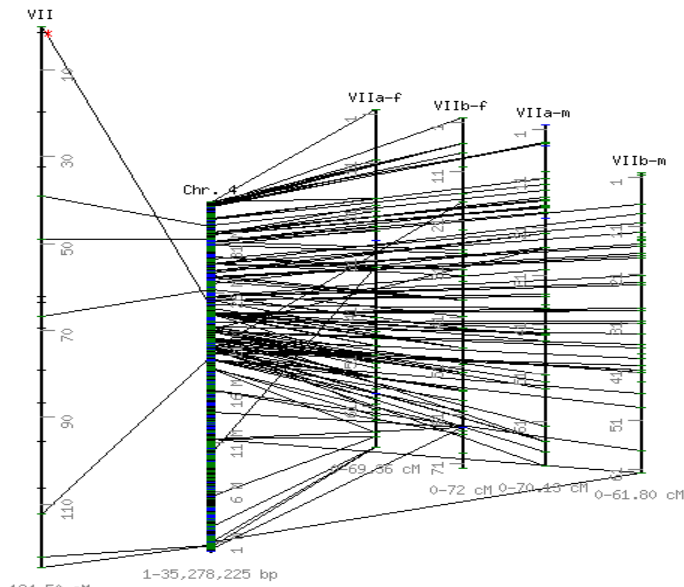
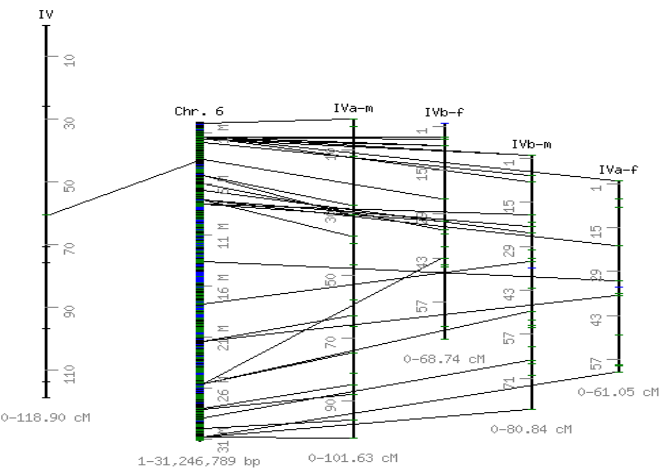
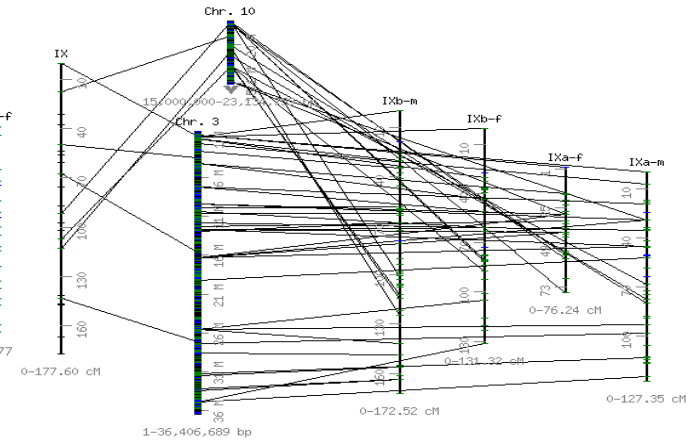
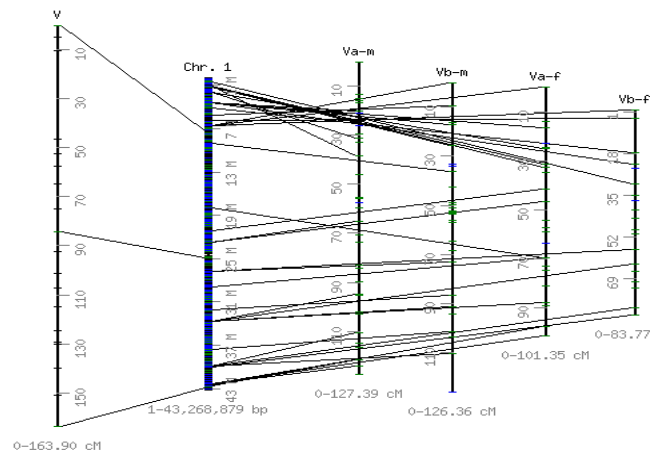




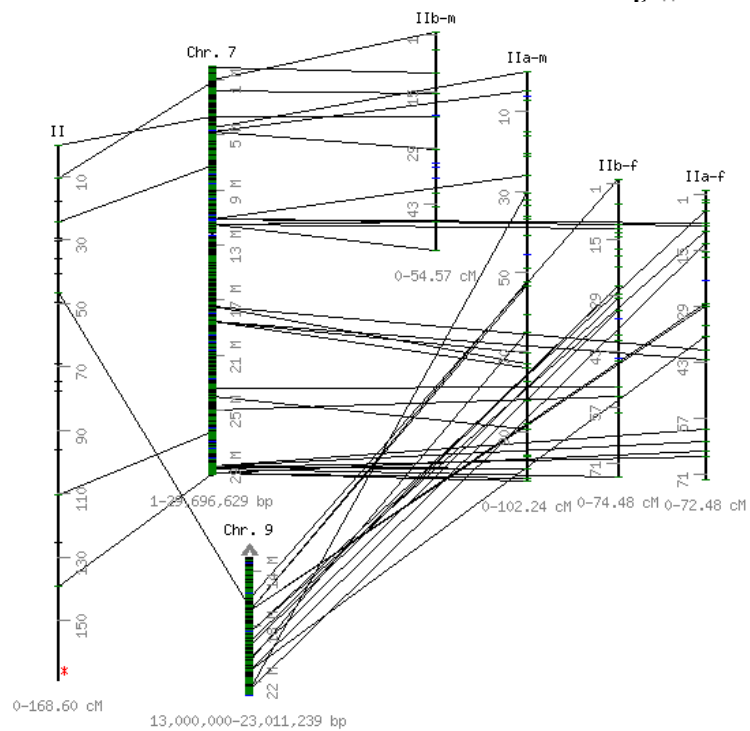
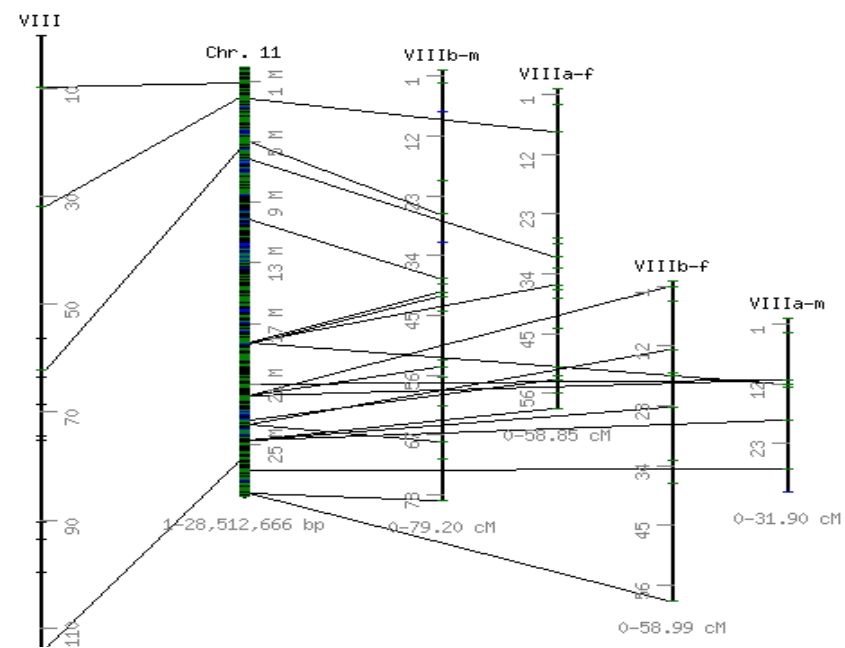
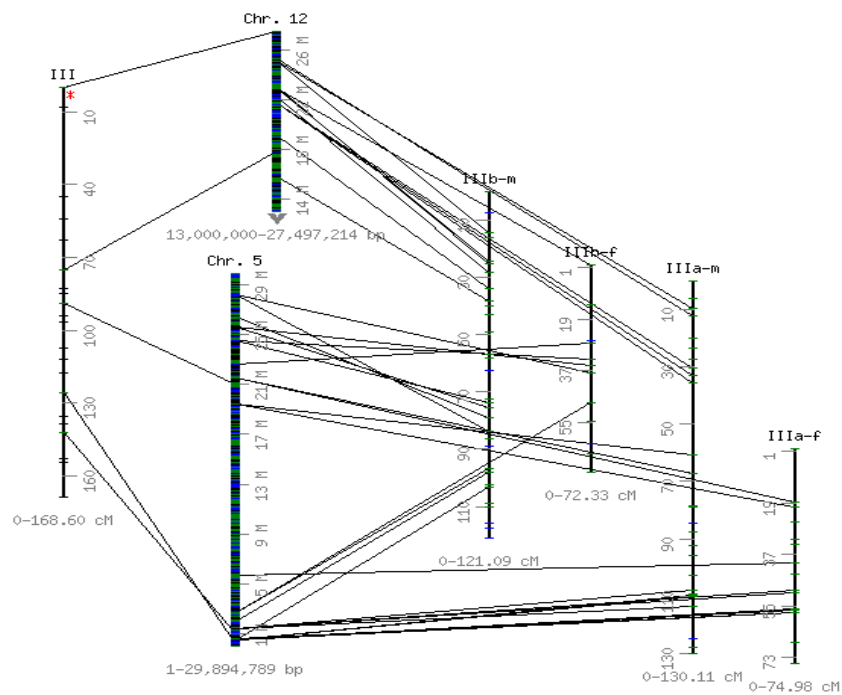
Collinearity of Switchgrass and Foxtail Millet Linkage Maps



CMap of ABC2



web based tool that uses perl



QTL analysis

Bill Anderson, Rongling Wu

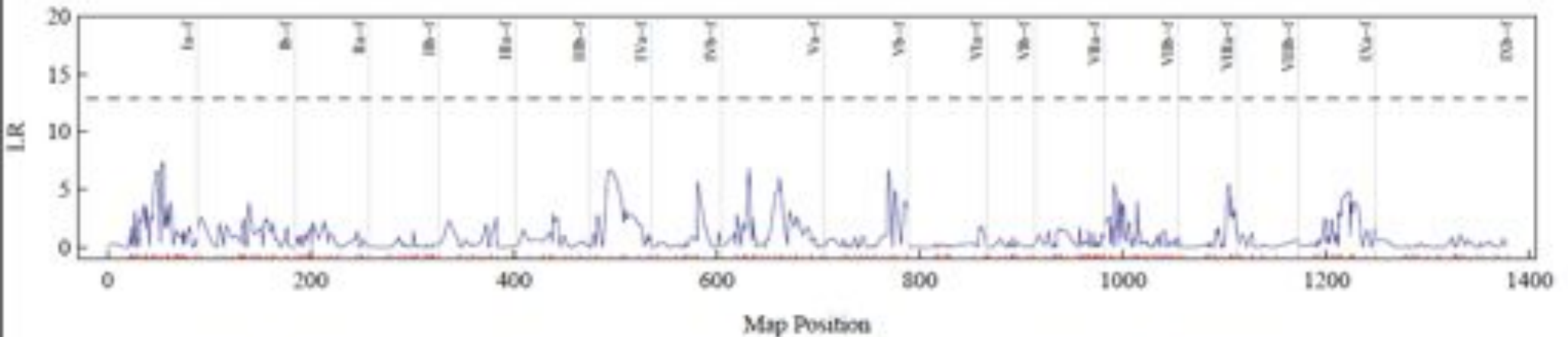


Figure 3a The profile of the log-likelihood ratios for crown width 7/13/07 across all the 18 linkage groups in the maternal map of tetraploid switchgrass. The threshold value based on 1000 permutations for asserting the existence of a QTL at the significant level $p=0.05$ is given as the horizontal dash lines.

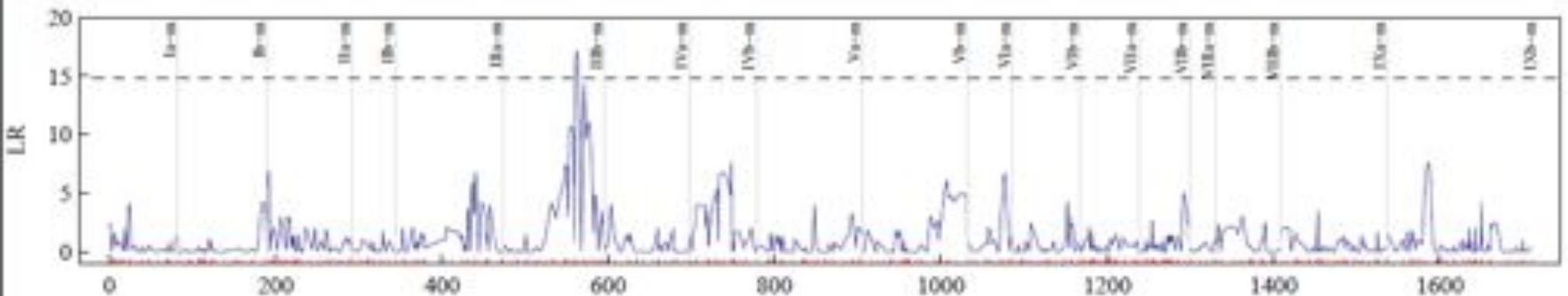


Figure 3b The profile of the log-likelihood ratios for crown width 7/13/07 across all the 18 linkage groups in the paternal map of tetraploid switchgrass. The threshold value based on 1000 permutations for asserting the existence of a QTL at the significant level $p=0.05$ is given as the horizontal dash line.

Most yield traits are controlled by many QTL with small effects

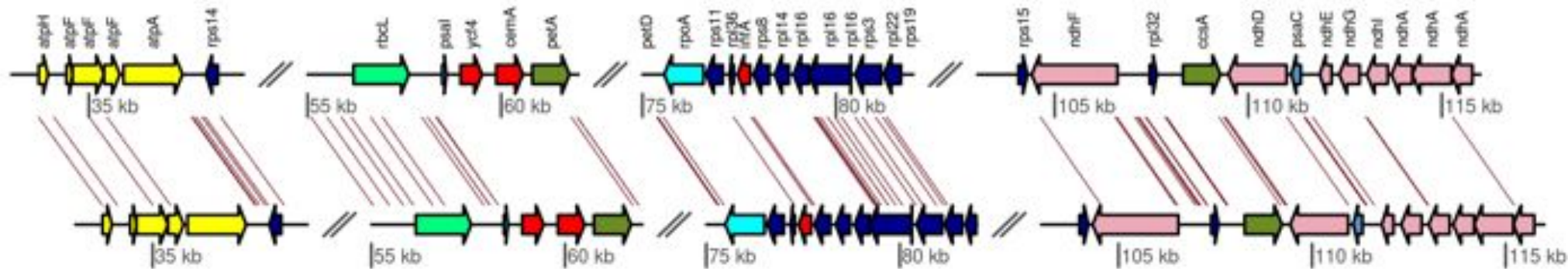
Large effect QTL are infrequent and may have non-additive genetic variance (dominance, epistasis)

Hugh Young

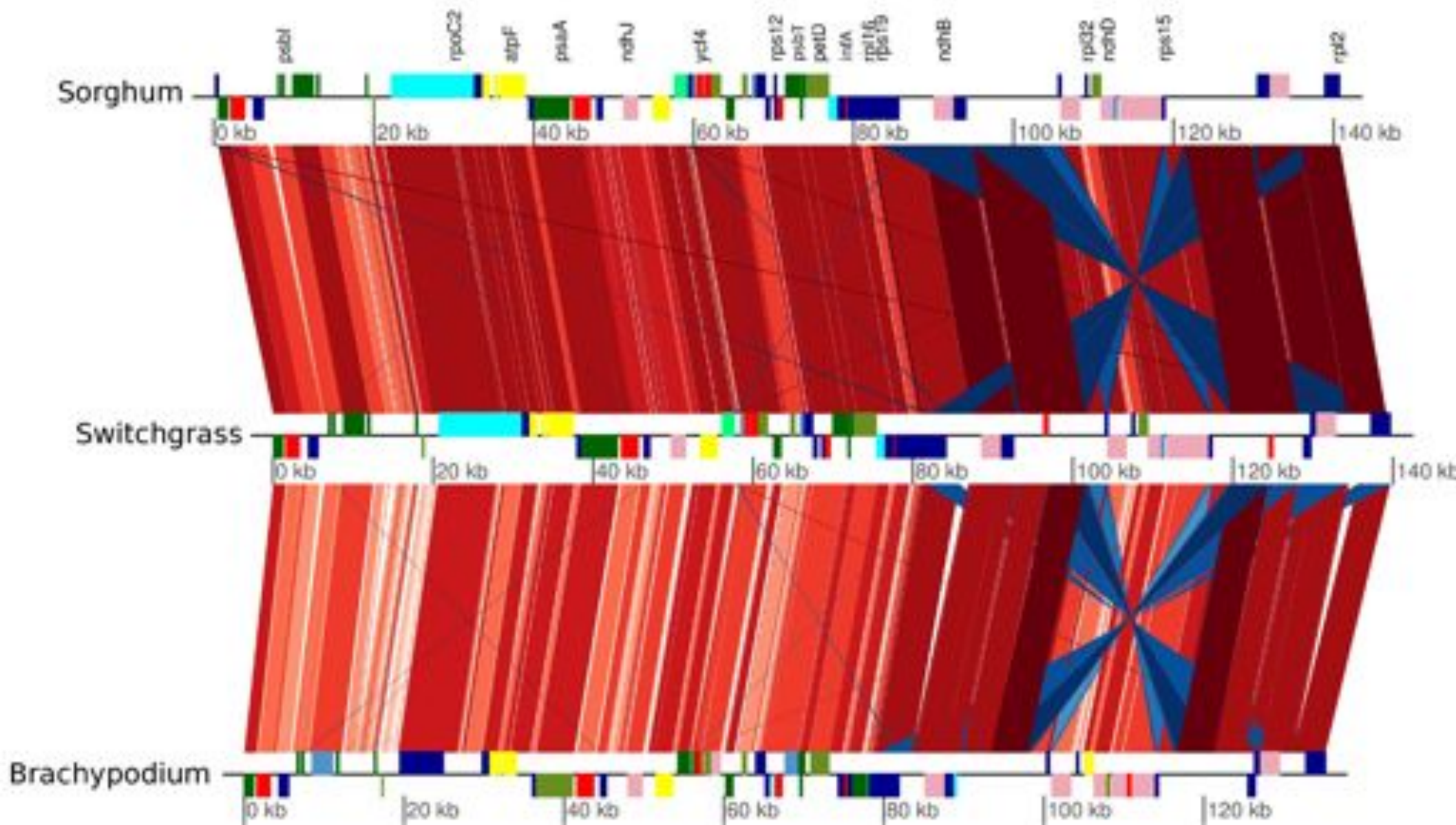
-
- A circular genome map of *P. virgatum_cp_Kanlow*, totaling 139,677 bp. The map displays various genes color-coded by function, with segments labeled with sizes such as 0.00kb, 116.67kb, 93.33kb, 70.00kb, 46.67kb, and 23.33kb. A legend at the bottom left categorizes genes into groups like Rubisco Subunit, Photosystem I proteins, Photosystem II proteins, Cytochrome-related, ATP synthase, NADH dehydrogenase, Ribosomal protein subunit, Ribosomal RNA, Plastid-encoded RNA polymerase, and Other. The DOGMA logo is present in the bottom right corner.

SNPs and Indels in Lowland Switchgrass cv. 'Kanlow' Compared to Upland cv. 'Summer'

Gene	In/Del	Tn	Tv	Nonsyn	Total
<i>atpB</i>		2			2
<i>atpF</i>	1				1
<i>ccsA</i>	1				1
<i>matK</i>	1	1		1	2
<i>ndhA</i>			1		1
<i>ndhD</i>			1	1	1
<i>ndhF</i>	2	1			3
<i>ndhH</i>	2				2
<i>ndhK</i>			1		1
<i>rbcL</i>	1	1		2	2
<i>rpl22</i>			1	1	1
<i>rpl36</i>	1				1
<i>rpoB</i>		1			1
<i>rpoC1</i>		1	1		2
<i>rpoC2</i>	1	2	3	4	6
<i>rps3</i>			2		2
Subtotal coding	2	14	11	8	29
Subtotal noncoding	49	22	44	na	115
Total	51	36	57	8	144



Insertion	position	length	location	sequence
Lowland	6248-6265	17	rps16-psbK	ACTAATAATACAACAAA
Upland	28227-28246	19	rpoC2	AGTATAGGACTCGAGAGGA
Upland	48626-48672	47	rps4-ndhJ	AATTAGGAATGATTATGAAATATAAAATTCTGAATTTTTTTTAGAAT
Lowland	49333-49374	42	rps4-ndhJ	TTTTCTTTCTGGTTCTTTTCTTTTCTTTTCTGGTTCTTTTCT
Lowland	53233-53264	32	ndhC-atpE	ATAATATAATATAATATAAACATACCAATAAT
Lowland	58304-58325	23	rbcL-psaI	AAAAATCCATAAAAAAGTATTCTA
Lowland	63685-63709	25	psbE-petL	AATTCCTTTTTTCTCTTCTTTGTTT
Upland	107092-107108	17	ndhF-rpl32	TAAATTTTTTCTTTT



Reads genbank and several other formats

Takes comparison files from Mauve and blast -m8

Many display options

```
##          PACKAGES TO LOAD
```

```
library("genoPlotR")
```

```
##          Load Genbank Annotations and DNA segments or "ad hoc segments"
```

```
kanlow<-try(read_dna_seg_from_genbank("kanlowcp.gbk"))
```

```
mid_pos_kan <- middle(kanlow)
```

```
annot_kan <- annotation(x1 = mid_pos_kan, text = kanlow$name, rot = 90)
```

```
names <- c("Kanlow", "Sorghum", "Rice", "Brachypodium", "Sugarcane")
```

```
##          Load Mauve or Genbank comparisons
```

```
bbone_file <- "kan.sorg.rice.brach.sacc.Align1.backbone"
```

```
bbone <- read_mauve_backbone(bbone_file, ref = 1, filter_low = 100)
```

```
names(bbone$dna_segs) <- names
```

```
#Calculating the lengths by adding the length on both sides of the comparisons
```

```
for (i in 1:length(bbone$comparisons)) {
```

```
  cmp <- bbone$comparisons[[i]]
```

```
  bbone$comparisons[[i]]$length <- abs(cmp$end1 - cmp$start1) +
```

```
  abs(cmp$end2 - cmp$start2)}
```

```
#reads blast sequence comparisons from m9 formatted output
```

```
kan.sbi<-try(read_comparison_from_blast("kanlowcp.fasta.6.out"))
```

```
#Plot subregions
```

```
xlims <- list(c(1, 80000), c(1, 80000), c(1, 80000), c(1, 80000), c(1, 80000))
```

```
#Plot features incorporates global color scheme for comparisons and many other
```

```
#formatting options
```

```
pdf("output.pdf")
```

```
plot_gene_map(dna_segs = list(kanlow, sorghum, rice, brady, sugarcane),
```

```
  comparisons = bbone$comparisons,
```

```
  annotations = list(annot_kan, annot_sbi, annot_osa, annot_bdi, annot_sof),
```

```
  annotation_height = 0.8, gene_type = "side_blocks",
```

```
  dna_seg_scale = TRUE, scale = FALSE,
```

```
  global_color_scheme = c("length", "increasing", "red_blue"),
```

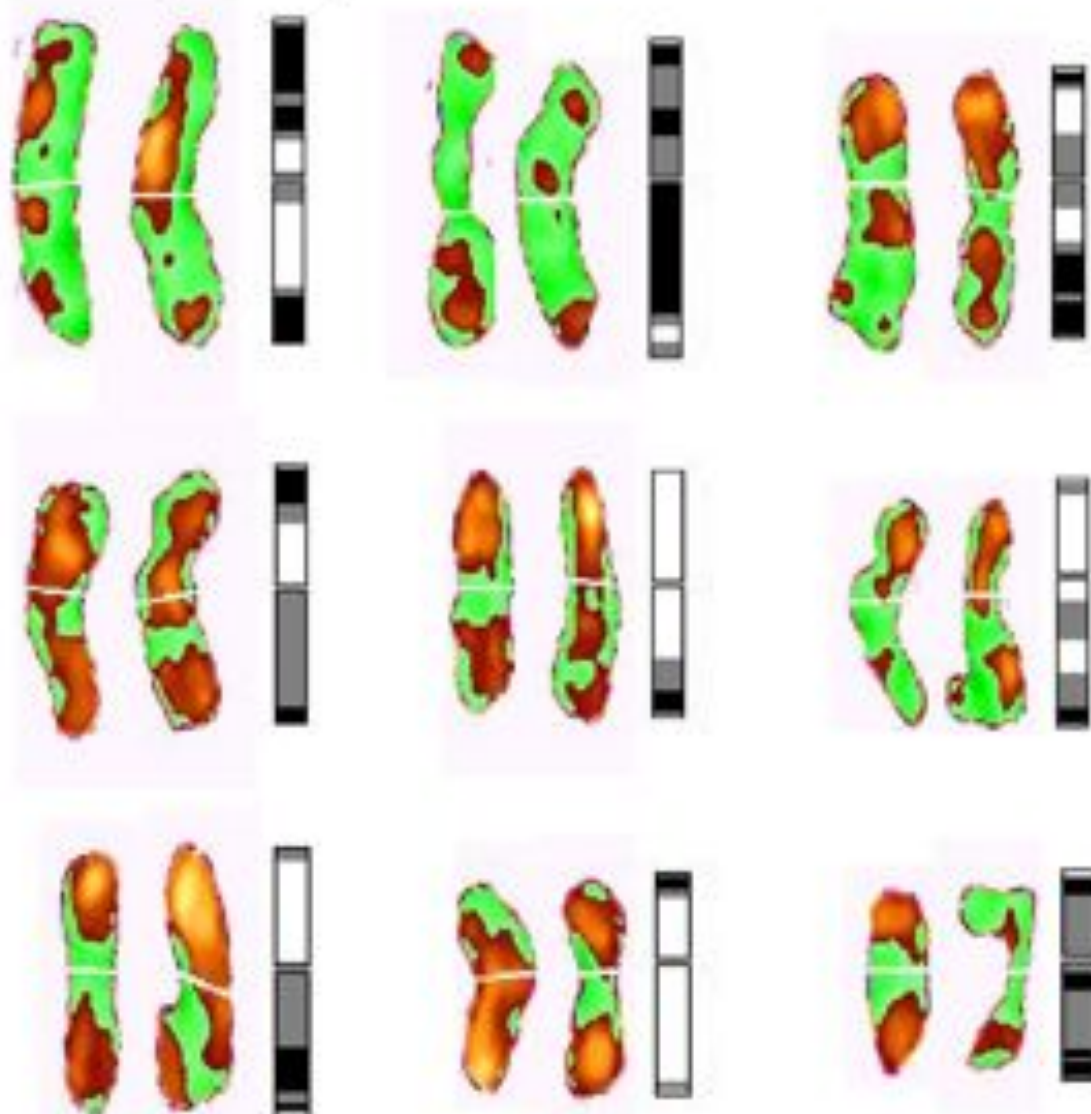
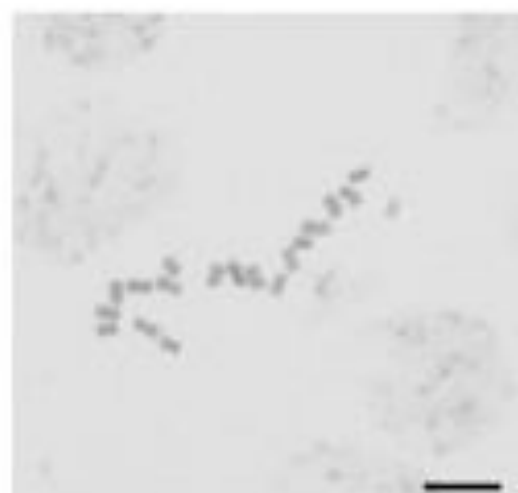
```
  override_color_schemes = TRUE)
```

```
pdf())
```

Cytogenetics

Hugh Young

Diploid Genetic Stocks ALB280, ALB5B have been deposited at Plant Genetic Resources Conservation Unit (PGRCU) Griffin, GA for propagation and public release. We are still working on chromosome doubling, restoring fertility, and FISH



Package agricolae

- **Planning of field experiments:**
lattice, factorial, RCBD, CRD, Latin Square, Graeco, BIB, Alpha design, Cyclic, augmented block, split and strip plot designs.
- **Comparison of multi-location trials:**
AMMI Stability (biplot and triplot),
- **Comparison between treatments:**
LSD, Bonferroni and other p-adjust, HSD, Waller, Student Newman Keuls SNK, Duncan, Scheffe
- **Non parametric tests:**
Kruskal, Friedman, Durbin, Van Der Waerden
- **Analysis of genetic experiments:**
North Carolina designs, LinexTester, Balanced Incomplete Block, Strip plot, Partially Balanced Incomplete Block, analysis Mother and baby trials
- **Resampling and simulation:**
resampling.model, simulation model, montecarlo
- **Ecology:**
Biodiversity Index, Path Analysis
- **Soil Uniformity:**
Smith's Index. Cluster Analysis: Consensus Cluster

HSD test

```
> comparison <- HSD.test(Cal, Sample.Name, df, MSerror, group=TRUE,  
+   main="CAD silencing: Caloric Data")
```

Study: CAD silencing: Caloric Data

HSD Test for Cal

```
.....  
Alpha                0.050000  
Error Degrees of Freedom    12.000000  
Error Mean Square        170.778744  
Critical Value of Studentized Range  4.750231
```

Treatment Means

	Sample.Name	Cal	std.err	replication
1	A22-34 6-1	4460.760	5.101150	3
2	A22-34 6-89	4445.040	3.888907	3
3	A22-91 6-19A	4352.430	6.911920	3
4	A22-91 6-27A	4388.080	12.142019	3
5	A22-91 6-36A	4403.643	5.310823	3
6	A22-91 6-54F	4390.863	8.775195	3

Honestly Significant Difference 35.84025

Means with the same letter are not significantly different.

Groups, Treatments and means

a	A22-34 6-1	4460.76
a	A22-34 6-89	4445.04
b	A22-91 6-36A	4403.643
b	A22-91 6-54F	4390.863
bc	A22-91 6-27A	4388.08
c	A22-91 6-19A	4352.43

Used frequently
in tables

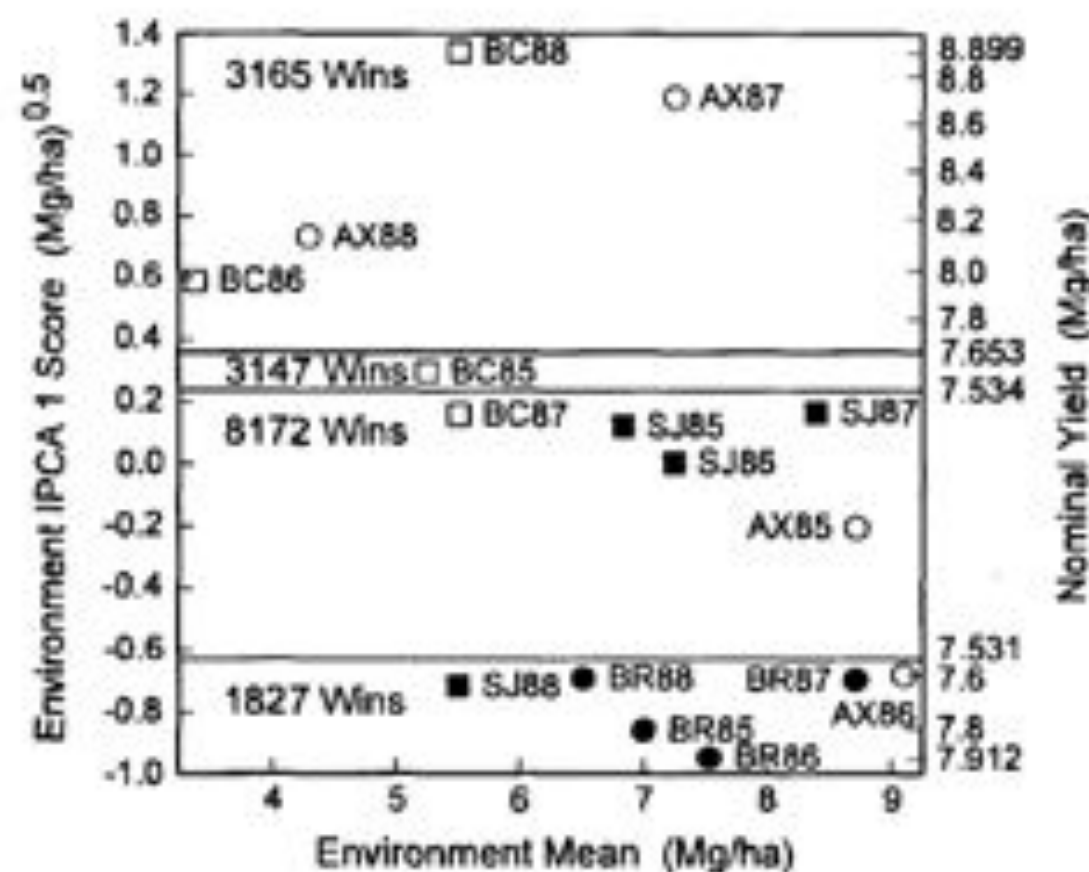
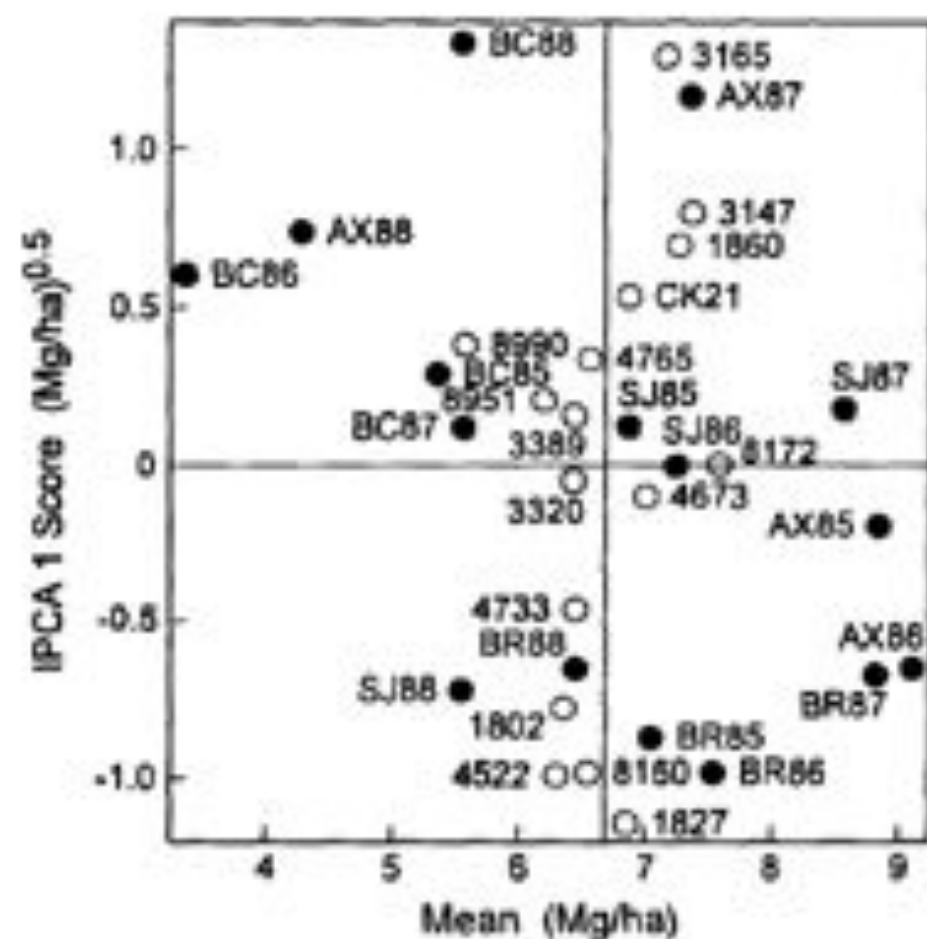
AMMI (Additive Main effects and Multiplicative Interaction (AMMI) model Genotype main effects and Genotype × Environment interaction (GGE) model

Widely used for Multienvironment field trials
determine G×E interactions
visualizing data and gaining accuracy
Combines ANOVA and PCA

$$Y_{ger} - \alpha_g - \beta_e + \mu = \sum_n \lambda_n Y_{gn} \delta_{en} + \rho_{ge} + \varepsilon_{ger}$$

Y_{ger} = yield for genotype g in environment e for replicate r,
 μ = grand mean, summation is done over components $n = 1$ to N
 λ_n = singular value for principal component ,
 Y_{gn} = eigenvector score for genotype g and component n
 δ_{en} = eigenvector score for environment e and component n,
 ρ_{ge} = residual for genotype g and environment e, and
 ε_{ger} = e error for genotype g and environment e and replicate r

For visualizing data n typically is 1 or 2.



Example Potato Yield

A data frame with 504 observations on the following 6 variables.

Genotype

a factor with levels 102.18 104.22 121.31 141.28 157.26 163.9 221.19
233.11 235.6 241.2 255.7 314.12 317.6 319.20 320.16 342.15 346.2
351.26 364.21 402.7 405.2 406.12 427.7 450.3 506.2 Canchan Desiree
Unica

Locality

a factor with levels Ayac Hyo-02 LM-02 LM-03 SR-02 SR-03

Rep

a numeric vector

WeightPlant

a numeric vector

WeightPlot

a numeric vector

Yield

a numeric vector

Example continued

```
library(agricolae)
# Example 1
data(plrv)
#startgraph
# biplot
model<- AMMI(Locality, Genotype, Rep,
             Yield,graph="biplot" number="FALSE")
```

ANOVA Results

Response: Y

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
ENV	5	9607.4	1921.49	284.6352	4.957e-12	***
REP(ENV)	12	81.0	6.75	2.7313	0.00154	**
GEN	27	1367.4	50.64	20.4904	< 2.2e-16	***
ENV:GEN	135	1764.8	13.07	5.2891	< 2.2e-16	***
Residuals	324	800.8	2.47			

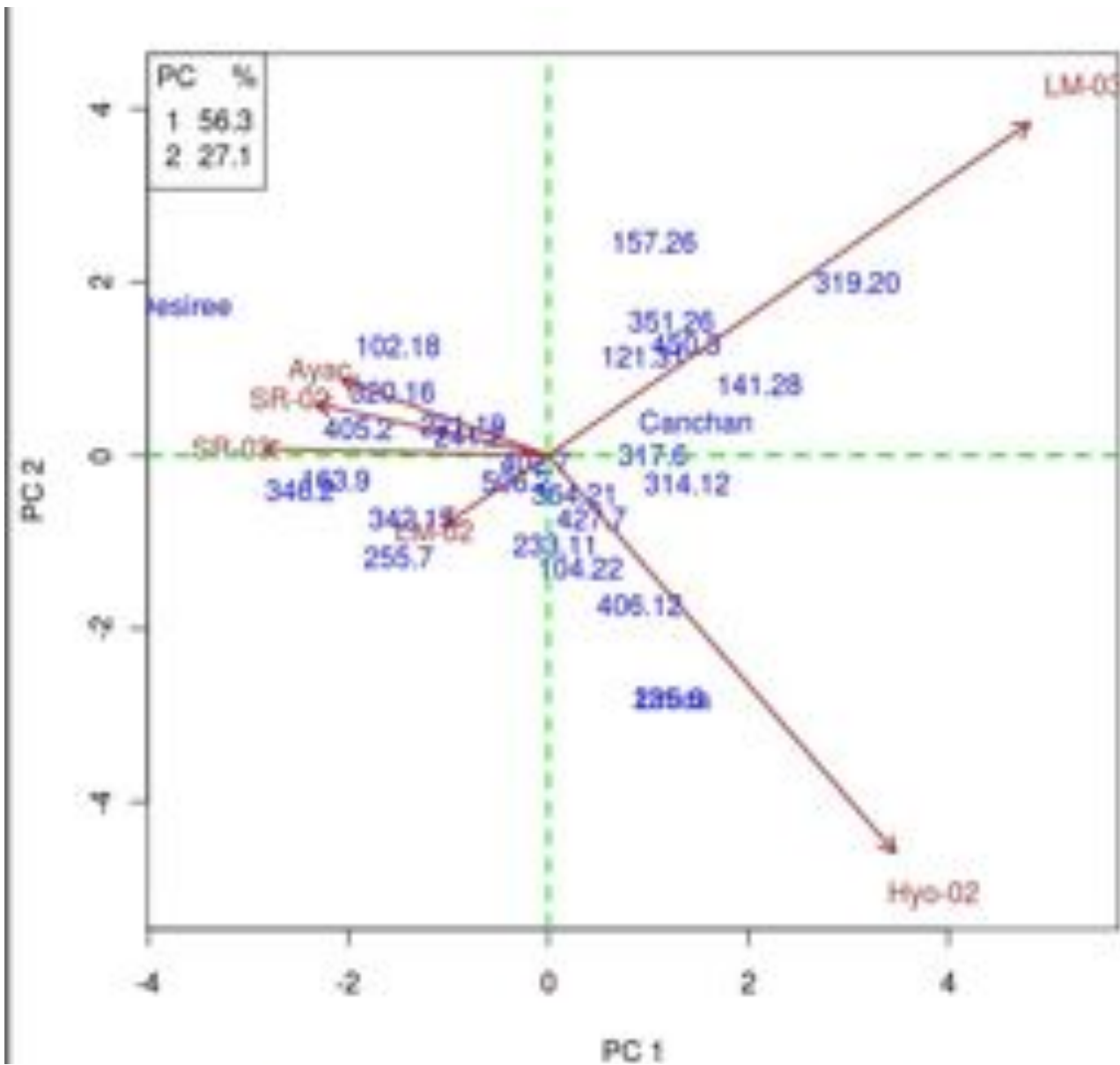
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Coeff var Mean plrv[, 5]
20.07525 7.831188

Analysis

	percent	acum	Df	Sum.Sq	Mean.Sq	F.value	Pr.F
PC1	56.3	56.3	31	13368.5954	431.24501	11.65	0.0000
PC2	27.1	83.4	29	6427.5799	221.64069	5.99	0.0000
PC3	9.4	92.8	27	2241.9398	83.03481	2.24	0.0005
PC4	4.3	97.1	25	1027.5785	41.10314	1.11	0.3286
PC5	2.9	100.0	23	696.1012	30.26527	0.82	0.7059
PC6	0.0	100.0	21	0.0000	0.00000	0.00	1.0000

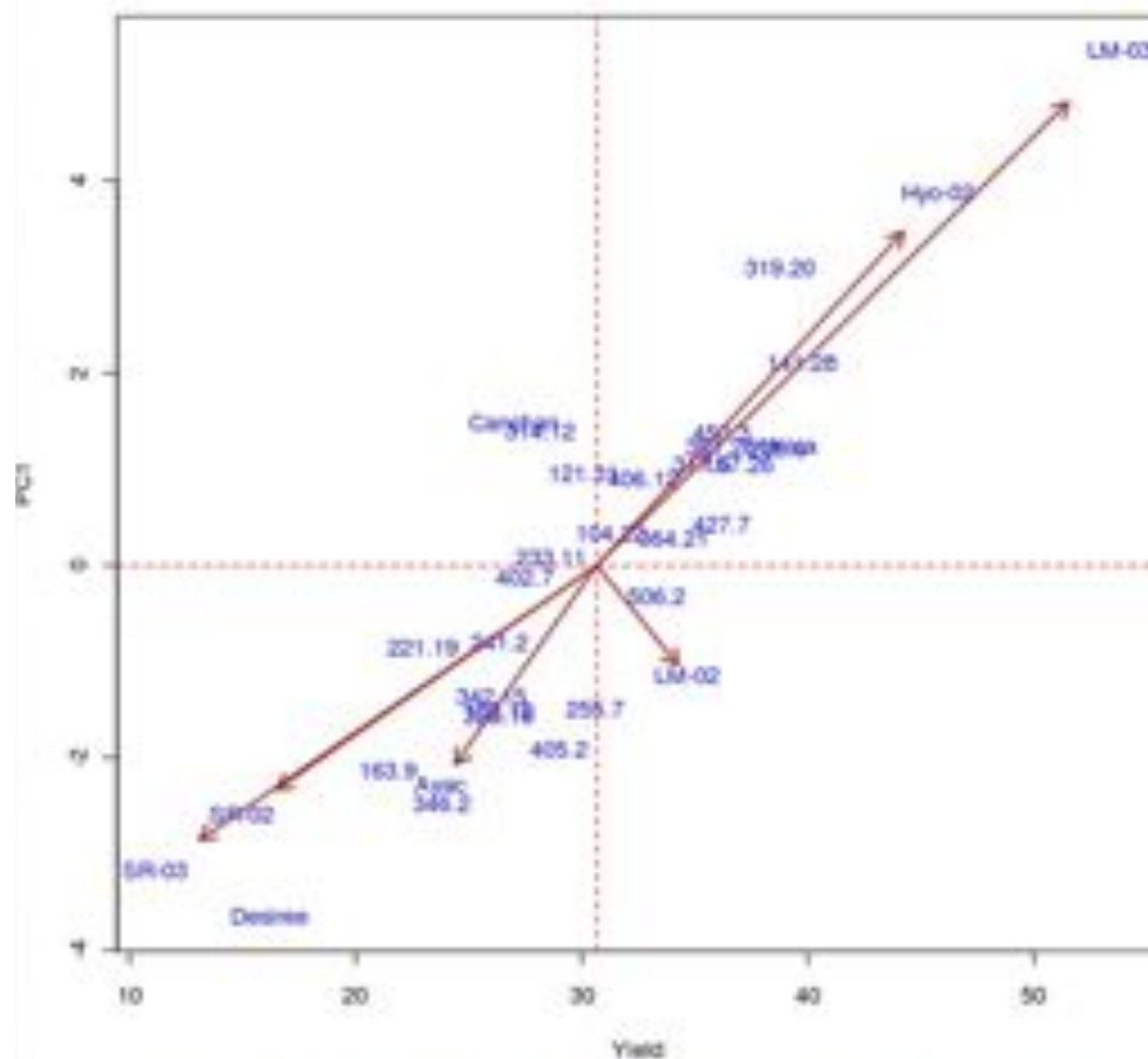
Biplot PC1 & PC2



AMMI1 biplot

```
bplot<-model$bplot[,1:4]
attach(bplot)
#startgraph
par(cex=0.8)
plot(Yield,PC1,cex=0.0,text(Yield,PC1,labels=row.names
(bplot),col="blue"),
     main="AMMI BIPLLOT",frame=TRUE)
MEANS<-mean(Yield)
abline(h=0,v= MEANS,lty=2,col="red")
amb<-subset(bplot,type=="ENV")
detach(bplot)
attach(amb)
s <- seq(length(Yield))
arrows(MEANS, 0, 0.9*(Yield[s]-MEANS)+MEANS,
0.9*PC1[s], col= "brown",
lwd=1.8,length=0.1)
```


AMMI BI PLOT



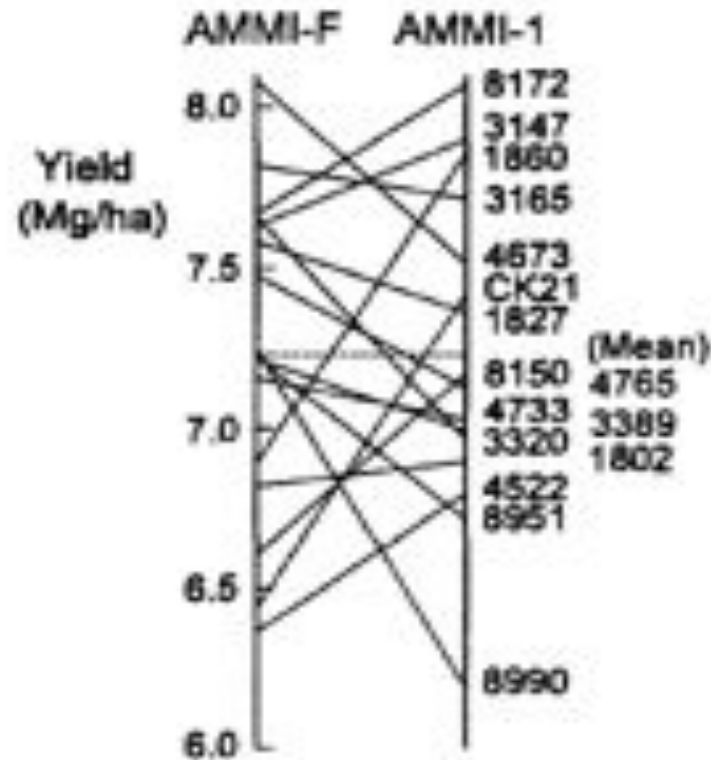


Fig. 6. Additive Main Effects and Multiplicative Interaction (AMMI)-F yield data and AMMI-1 estimates for environment SJ86 of a Louisiana corn trial. Genotype 4673 ranks first in the raw data but ranks fifth in the more predictively accurate AMMI-1 model. AMMI-1 identifies Genotype 8172 as the best genotype in this environment.

Model validation using cross-validation (bootstrapping approach)

BARLEY

CAP

Coordinated Agricultural Project



NIFA

Barley Coordinated Agricultural Project (CAP) USDA-NIFA

Incorporating public breeding
programs data to facilitate
marker assisted selection

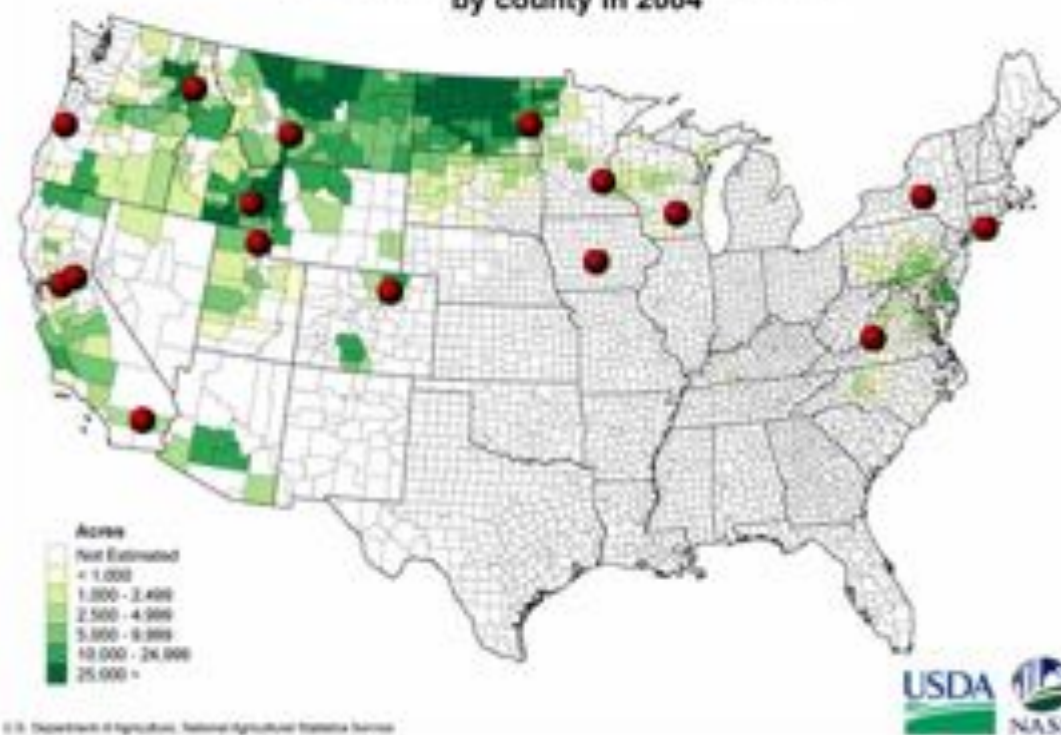
Objectives:

- High-Throughput Marker Development
- Worldwide Web Access
- Genes and Traits
- Superior Germplasm
- Education and Outreach

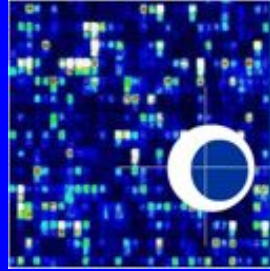
The Hordeum Toolbox (THT)

<http://tht.vrac.iastate.edu/>

Locations of Barley CAP participants
and the number of barley acres planted
by county in 2004







PLEXdb

ATGGCCCTCCTAGGA

00101111000101010110101000001111101011100101010101000001111101
00101111000101010110101000001111101011100101010101000001111101
00101111000101010110101000001111101011100101010101000001111101

Gene expression resources for plants and plant pathogens

PlexDB (<http://www.plexdb.org/>)
USDA-NRI project

MIAME/Plant-compliant database which serves as a public repository for raw and normalized expression data.
PLEXdb also provides annotation for the Affymetrix plant microarrays and for other important microarrays.

(Schnabel lab Iowa state)

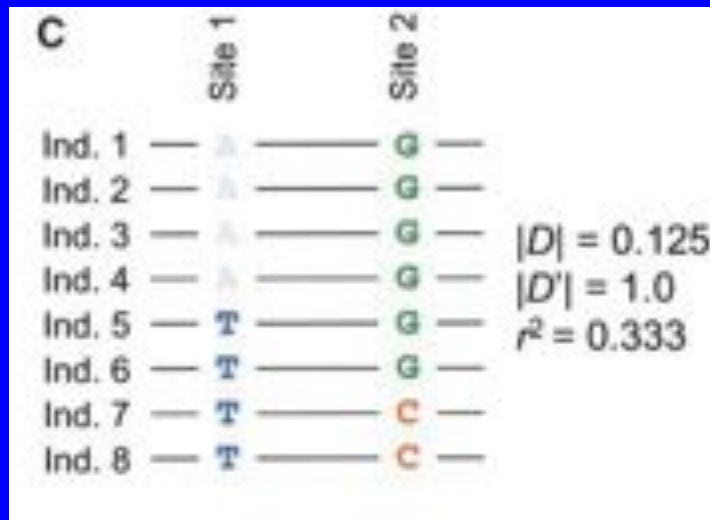
Bioconductor: background adjustment, normalization, and transformation using the robust multiarray average (RMA) approach



Association mapping

Association mapping,

R packages such as gap (genetics analysis package), genetics, GenABEL, mapLD, hwde, SNPmatrix, etc. can perform GWAS

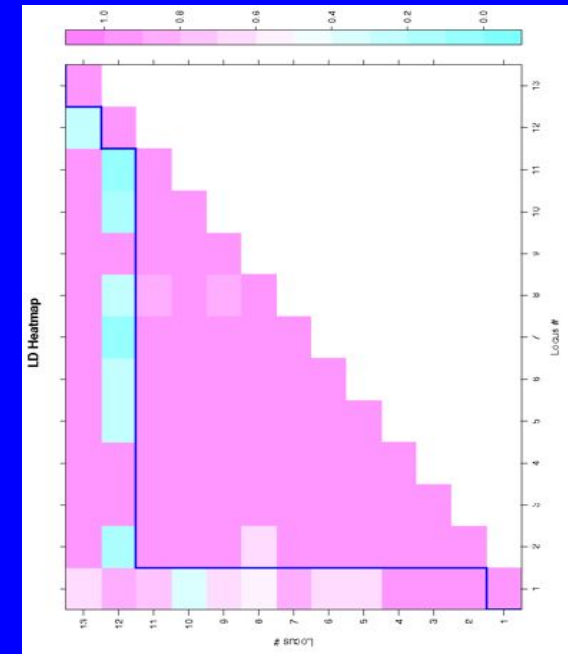


$$D = p_{A1}p_{G1} - (p_{A1})(p_{G1})$$

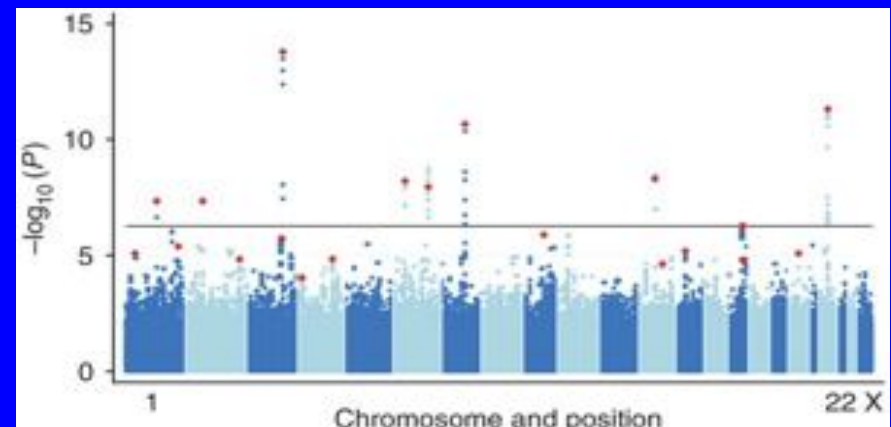
$$r^2 = D^2 / (p_{A1})(p_{G1})$$

$$E[r^2] = 1/1 - 4Nc$$

Also ASREML-R (commercial)
Tassel (ARS) and probably others



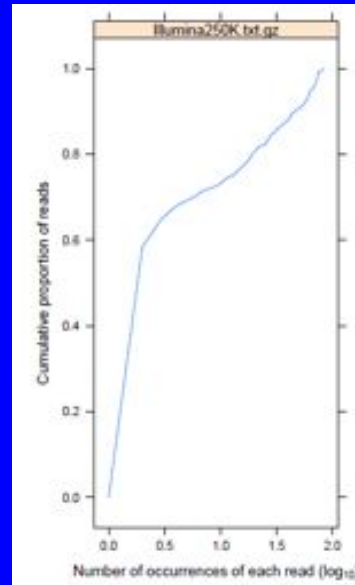
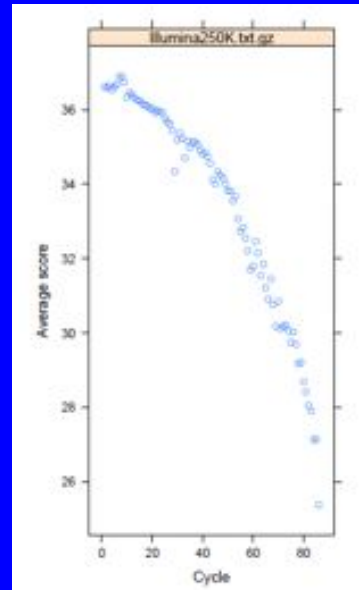
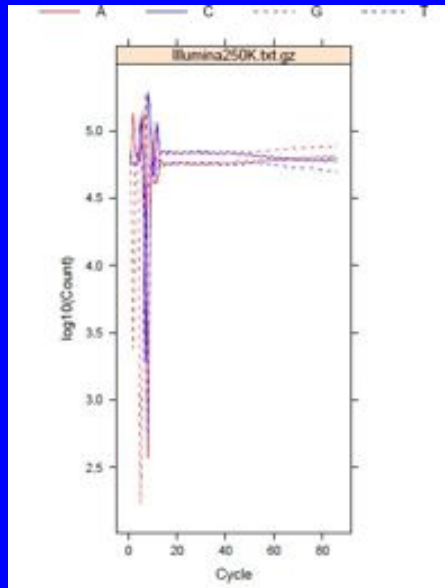
LD heat map showing pairwise LD



'Manhattan Plot' Chromosome location vs. significance threshold for trait assn.

Short Read Sequencing

Sequencing: Packages: Short Read

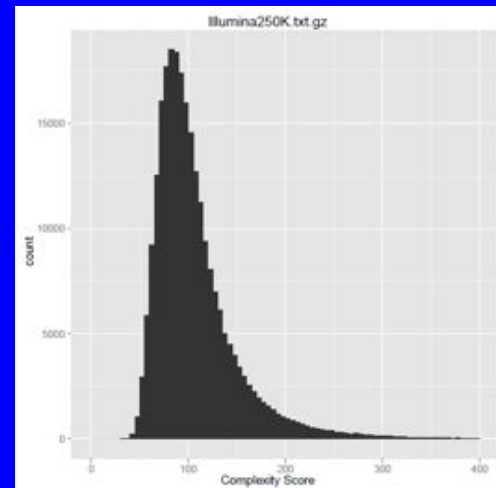
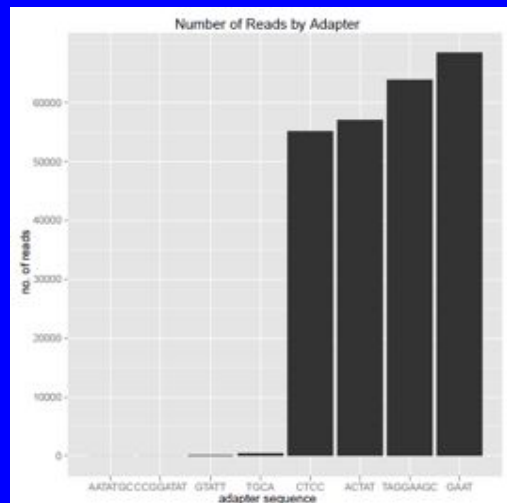


Can be used with multiplexing and reduced representation libraries for highly efficient genotyping.

Map reads to a reference sequence assembly or assemble de novo and call SNPs.

Genotyping can be complicated in plants due to repetitive DNA, large gene families, polyploidy, and nucleotide diversity.

www.maizegenetics.net,
gramene, graingenes and
others for examples of
custom databases, and
GWAS



Genome Wide Selection and GEBVs

Extent and nature of LD

- Biparental populations
- Many founders in mutation-drift-recombination equilibrium

Number and Genetic Architecture of quantitative trait loci (QTL)

- Small number that explain most of variance
- Large number with small effects
- GxG and GxE interactions

Availability of a reference genome

- Missing Data Imputation
- Combining results of different SNP panels

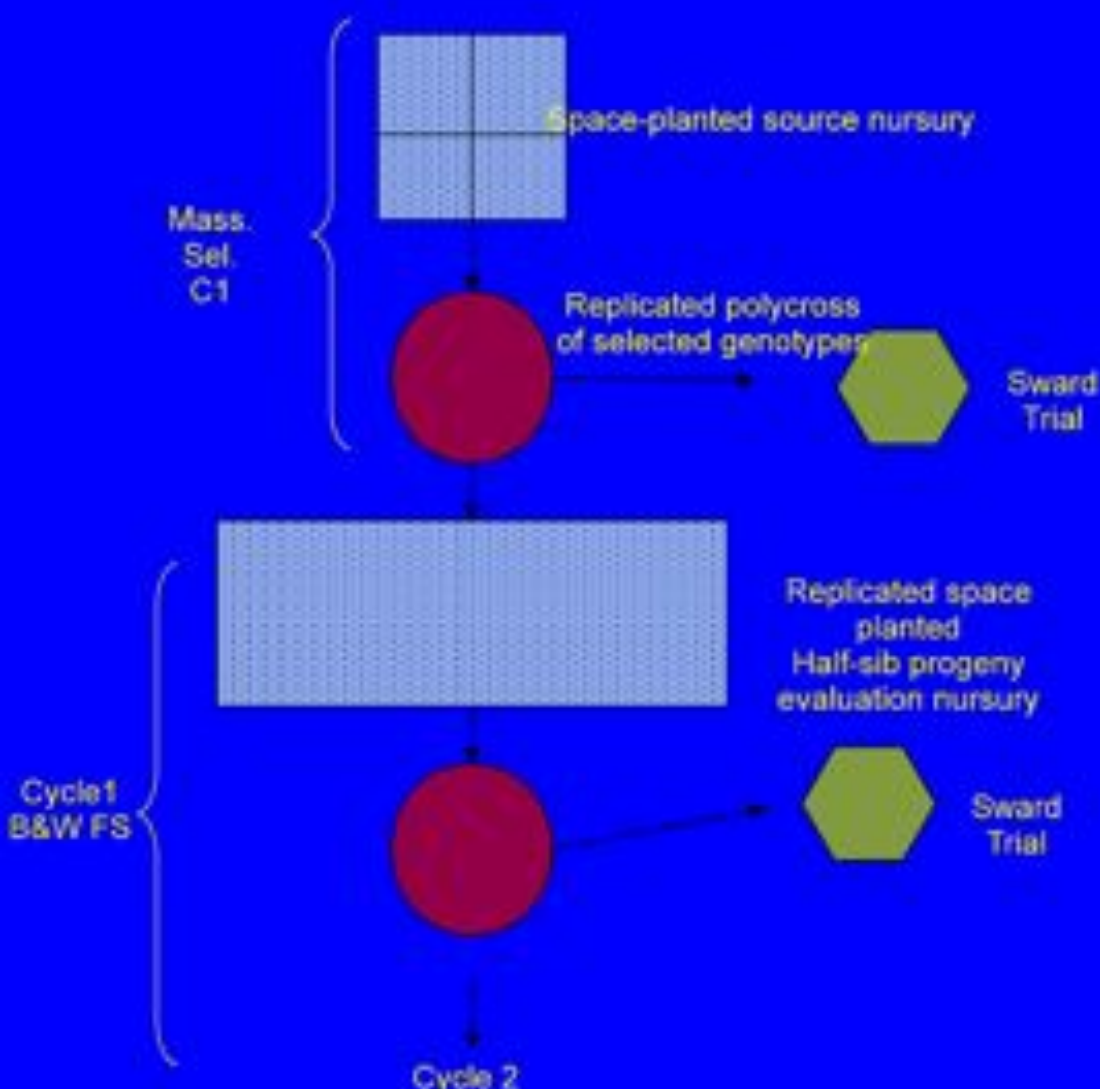
Allocation of resources

- Larger population (increase in power)
- Increased accuracy of phenotypic data
- Number of markers required

Prediction

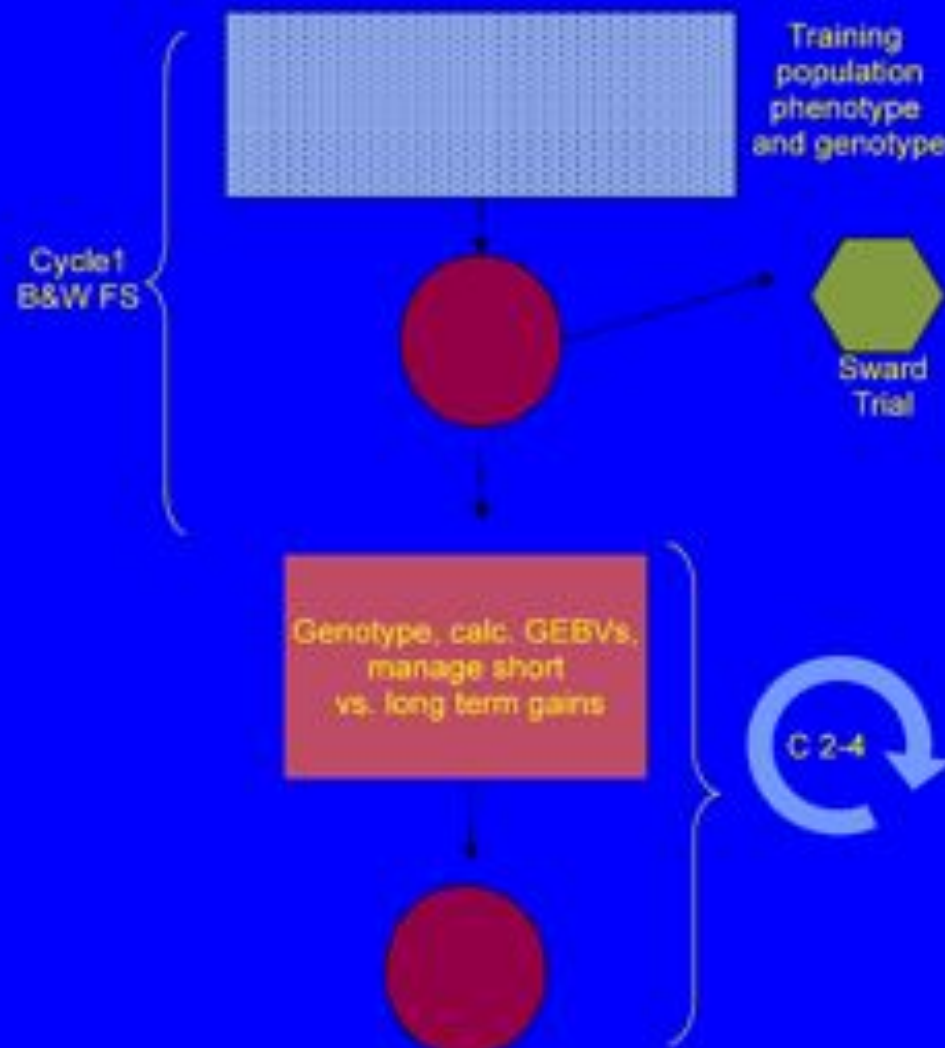
- BLUP, Bayesian, ridge regression, kernel regression, support vector machine, random forest

Phenotypic selection



**2 generations
6 years**

Genome Wide Selection



**4 generations
4.5 – 5 years**

OPPORTUNITIES FOR INTEGRATION OF STATISTICAL MODELING TOOLS IN MICROBIOLOGY, PLANT BIOLOGY AND METAGENOMICS

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NEWS

DOE Systems Biology Knowledgebase Implementation Plan

Now available. (September 2008)

PDF

Contribute to Defining Knowledgebase Requirements and Specifications

A Wiki has been created to provide open access to an interactive resource for viewing, providing comments, and contributing to this community-driven effort to specify the requirements for the DOE Systems Biology Knowledgebase. Go to the [Wiki](#) and select "How to Participate" to join this effort.

Wiki

Systems Biology Knowledgebase for a New Era in Biology

A Report from the May 2008 DOE Workshop

[Download full report or report sections](#)

This foundational report defined the high-level vision for the

DOE Systems Biology Knowledgebase

Experiments by investigators

Individual data

Workflow collaborations

Research centers

Shared tools by data analysis and visualization

Simulation engines

Image analysis

Workflow analysis

Modeling and simulation

Requirements-oriented modeling tools

Model-to-simulation coupling tools

Model-to-dynamics simulation layers

Knowledgebase

Experimental

Modeling

Simulation

Experiments by investigators

Shared tools by data analysis and visualization

Modeling and simulation

Experiments by investigators

Shared tools by data analysis and visualization

Modeling and simulation

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FEATURING

Systems Biology Knowledgebase Implementation Plan

[09/10]

Bioenergy Research Centers Overview

[07/10]

DOE Genomic Science Program Overview PDF

[2/2010]

New Frontiers in Characterizing Biological Systems

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- John Vogel
- Bill Anderson

U. Madison

- Juan Zalapa

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