

# Dissecting Alfalfa Dormancy Using Selection Mapping

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# Outline

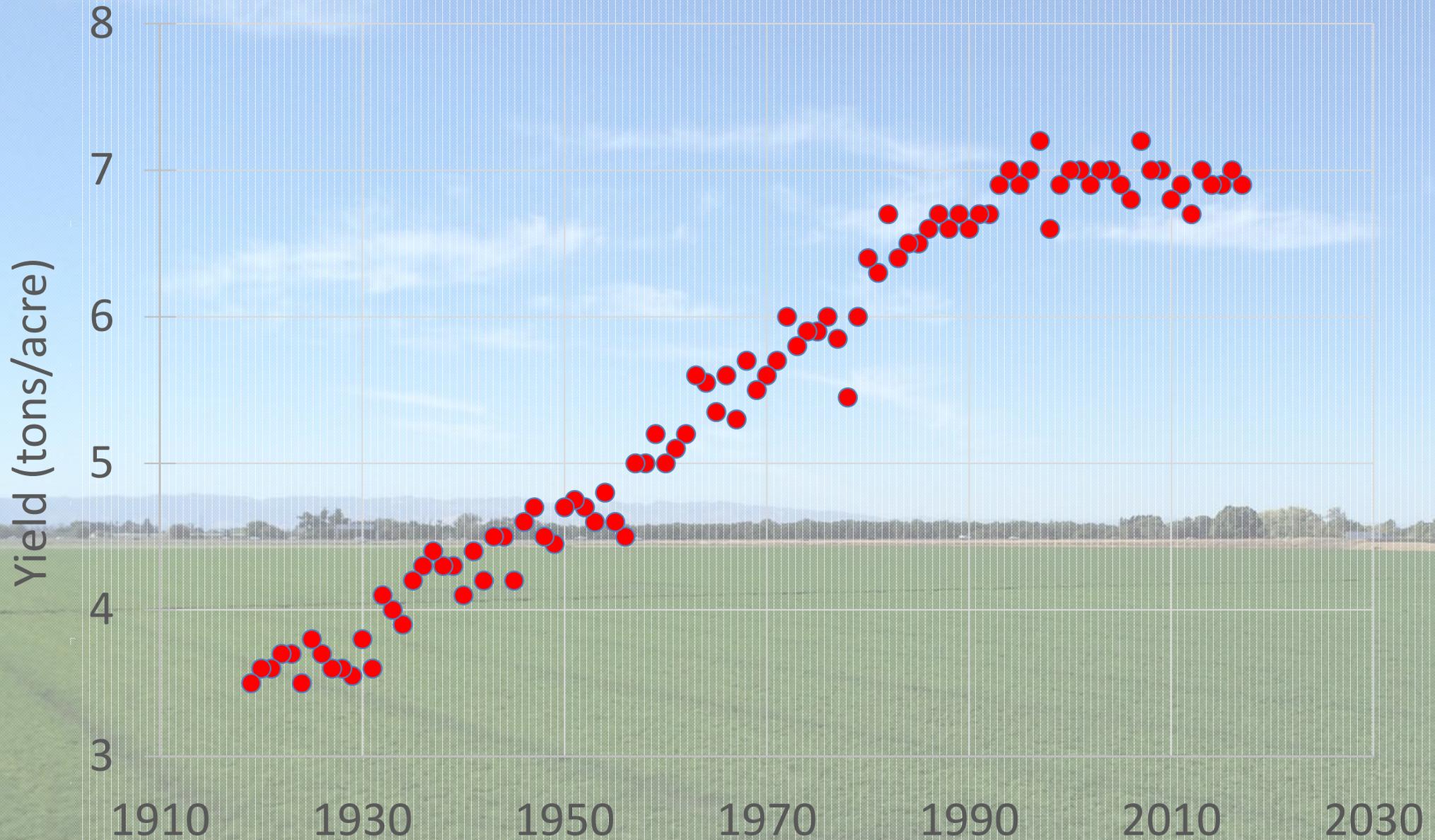
Yield and Fall Dormancy

Selection Mapping

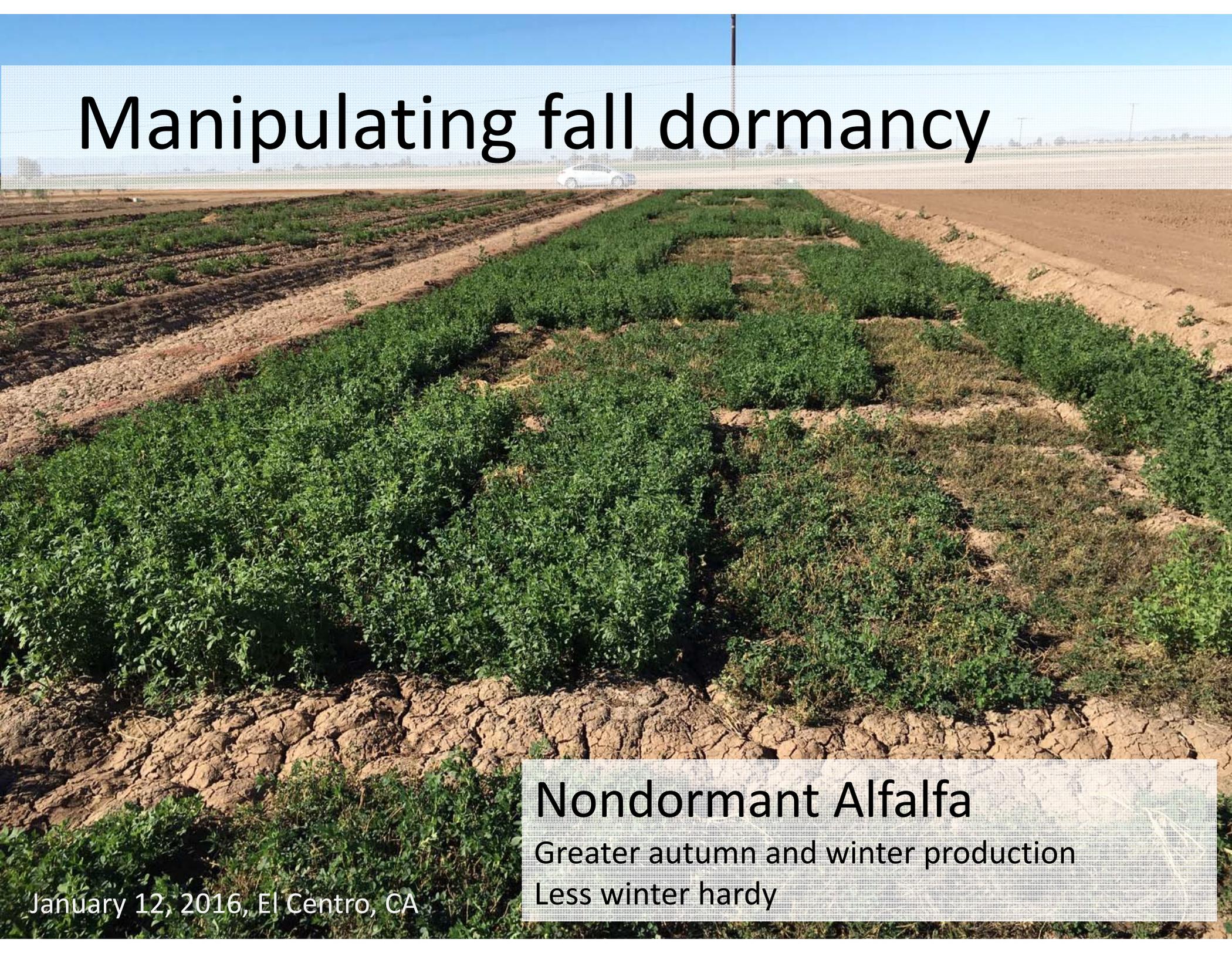
GWAS in a breeding population



# California Alfalfa Yield – USDA Ag Statistics



# Manipulating fall dormancy



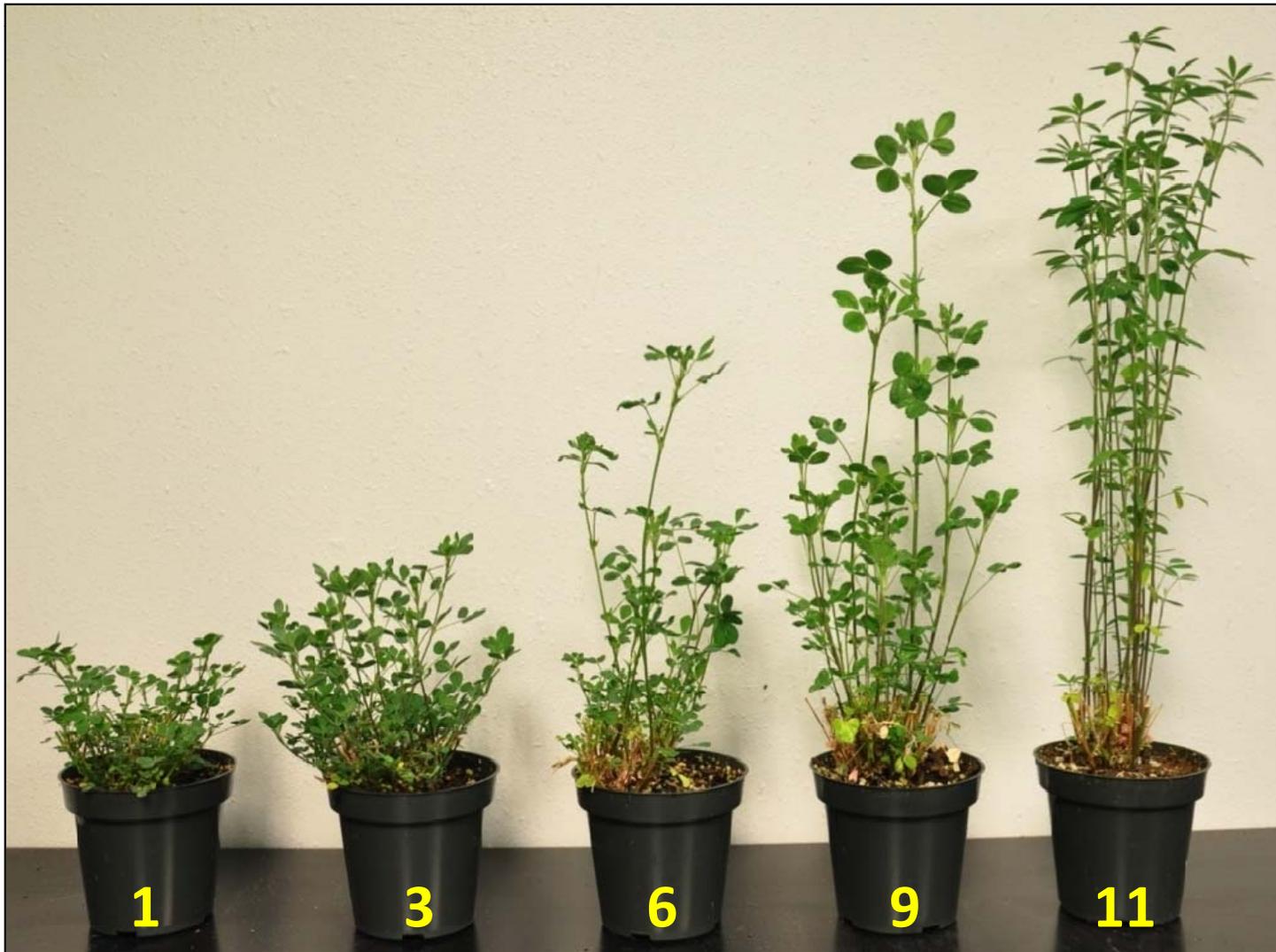
## Nondormant Alfalfa

Greater autumn and winter production

Less winter hardy

January 12, 2016, El Centro, CA

Dormancy is measured by height of regrowth in autumn



Dormancy phenotype observed in five of 11 standard check cultivars when in growth chamber under decreasing temp and photoperiod

# Divergent selection for fall dormancy

Larry Teuber's dormancy selections

Norseman

FD = 1

Saranac

FD = 4

Lahontan

FD = 6

Mesilla

FD = 8

**CUF101**

**FD = 9**

Wadi

FD = 11

For each cultivar

C+3 **H**

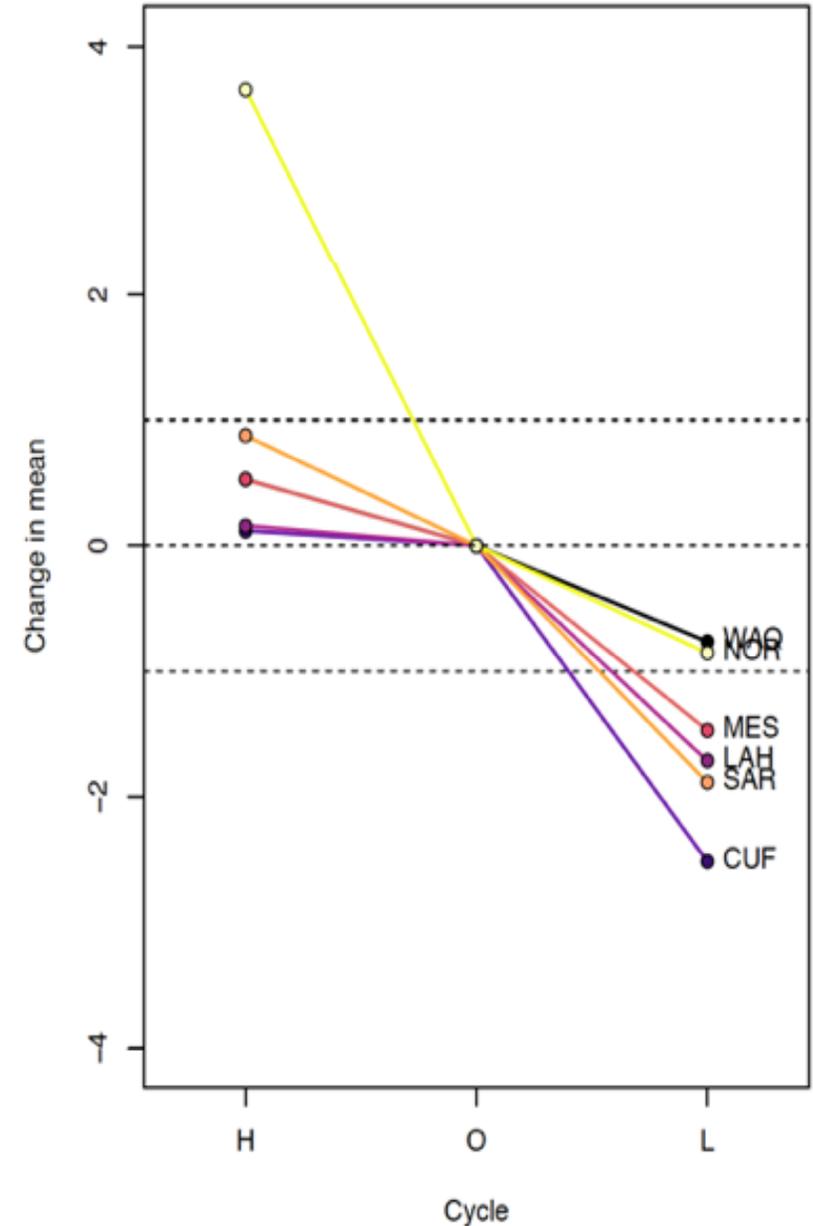
*Taller in autumn*

Less Dormant

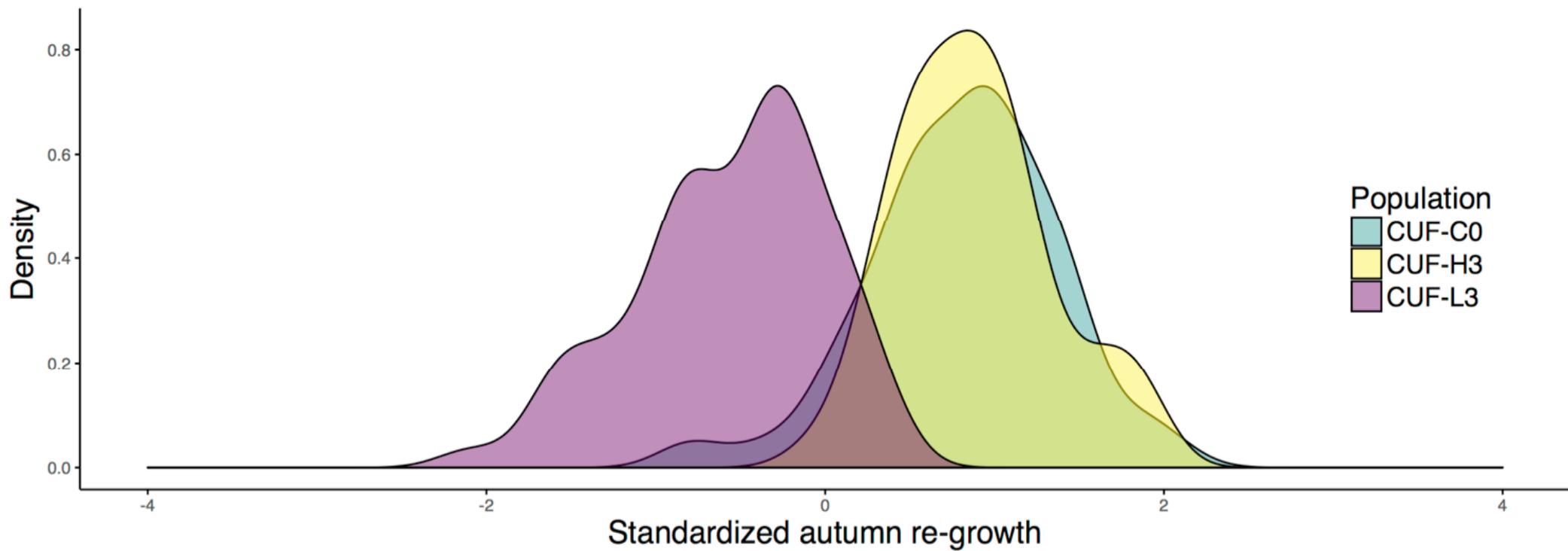
C-3 **L**

*Shorter in Autumn*

More Dormant



# CUF101: 'L' selection increased dormancy



'H' selection did not decrease dormancy

# GBS marker discovery and filtering

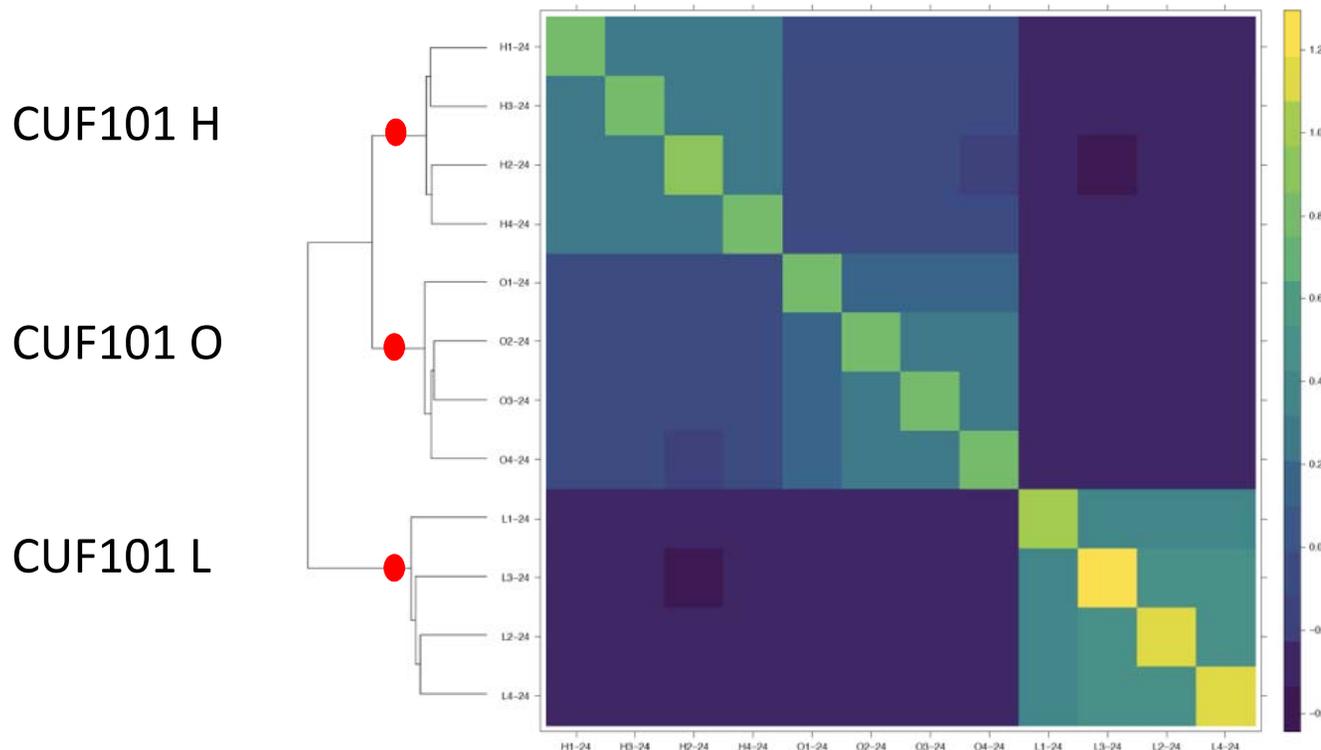
Modified GBS-SNP-CROP (BMC Bioinformatics. 2016. 17:29)

Retained marker tags that aligned to *Medicago* and/or alfalfa

~ 85,000 SNP at **≥100 reads/population**; ~17,000 alfalfa specific SNP

96 genotypes/population - 4 pools of 24 plants each per pop

Computed frequencies for each allele in each pool

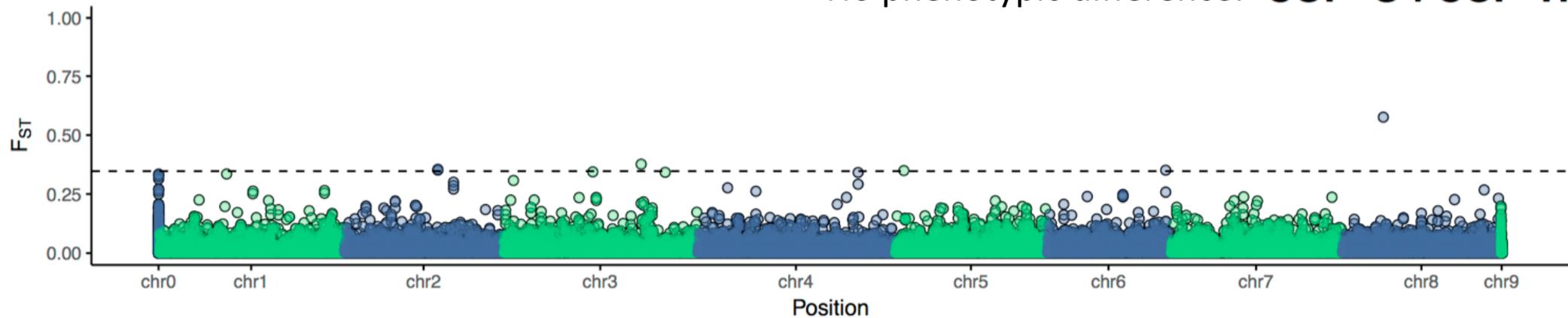


Replicate pools  
cluster together

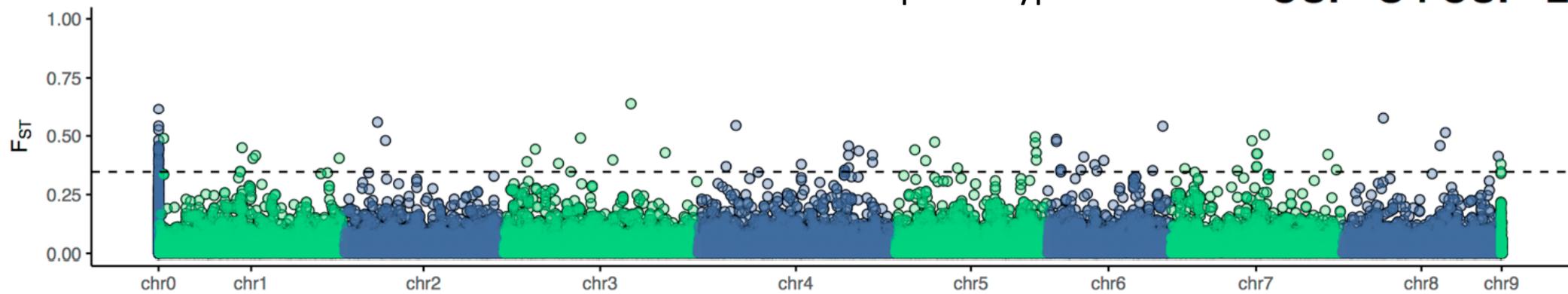
The 'L' population  
which was  
phenotypically  
distinct was also  
genetically distinct

# Loci possibly under selection – CUF101

No phenotypic difference: **CUF-O | CUF-H**



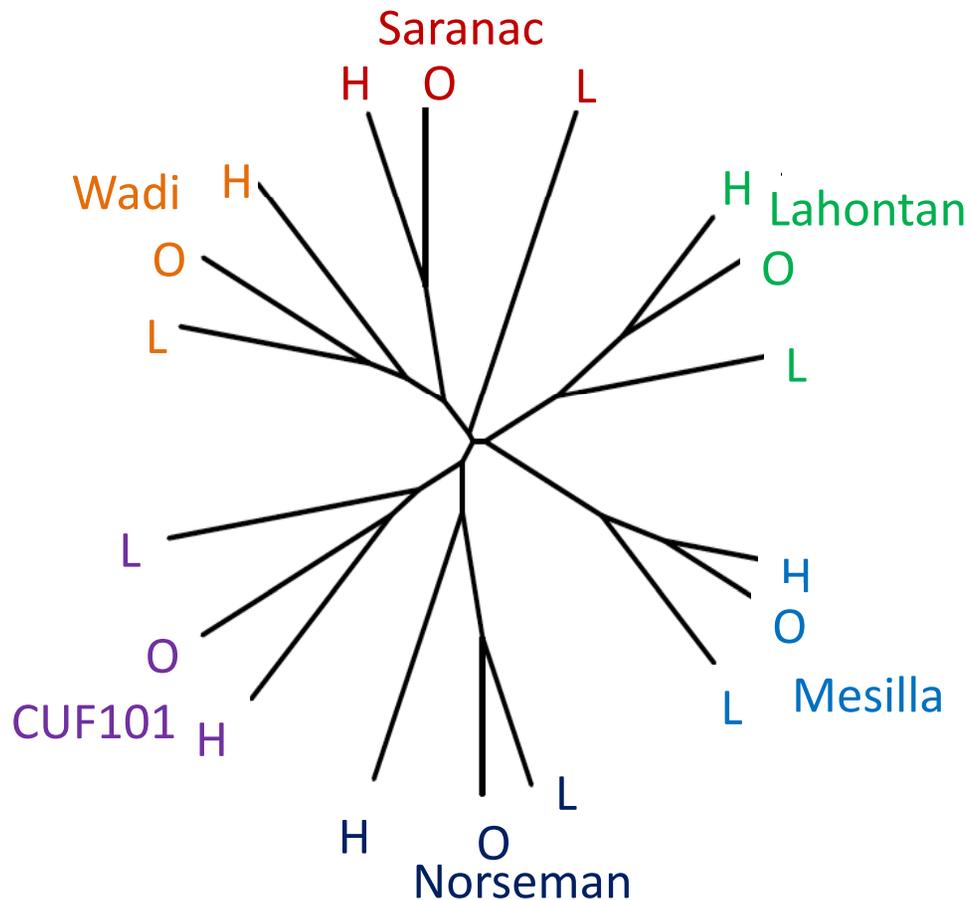
Clear phenotypic difference: **CUF-O | CUF-L**



Position on *Medicago truncatula*

SNP loci that align to alfalfa scaffolds but not to *Medicago*

# Evaluating selection across all populations



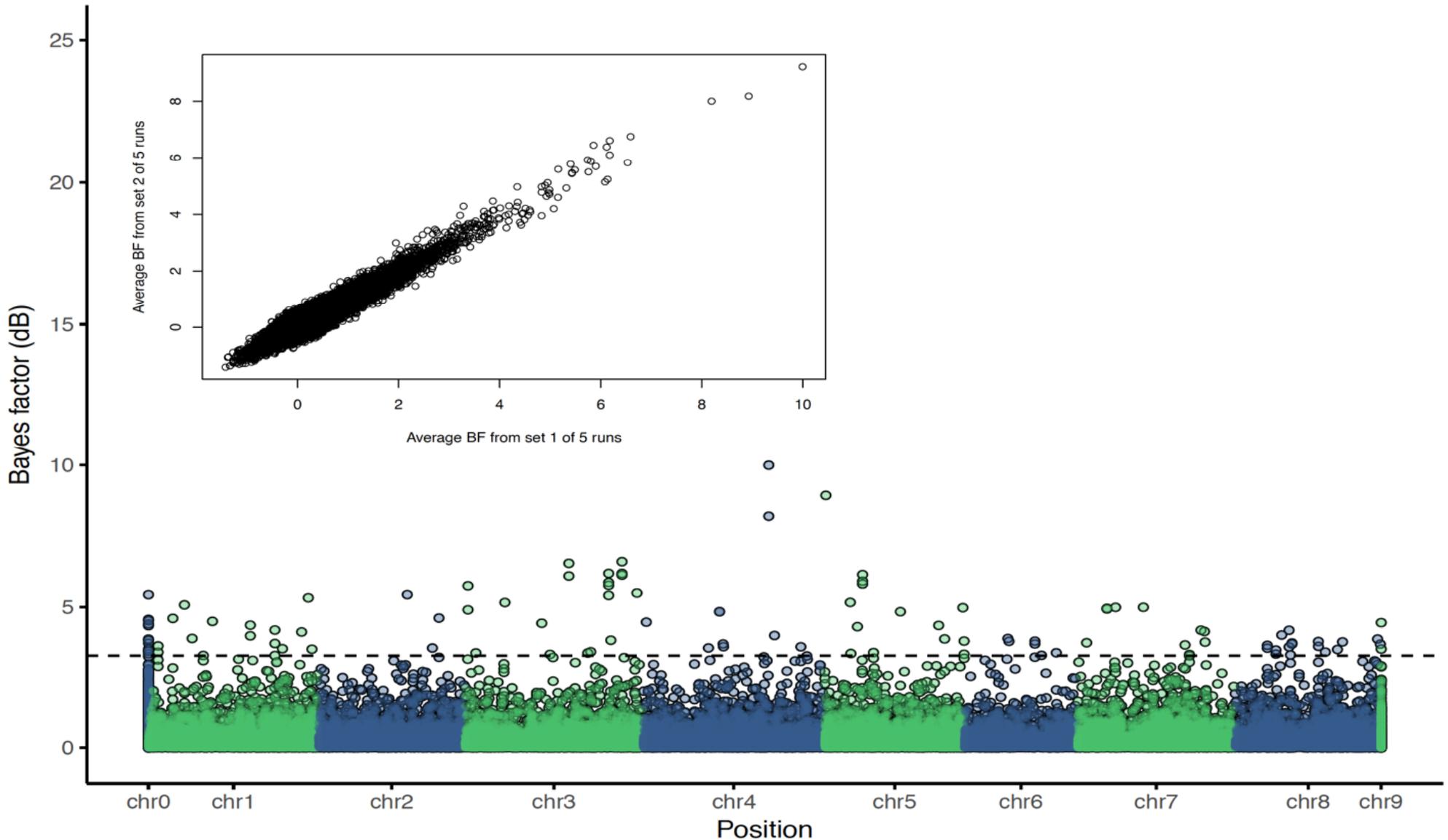
Few markers with >100 reads/population

Model allele frequencies using Bayesian analysis

Account for population structure

Identify selection signature across H – O – L pops

# Markers associated with dormancy across all cultivars



# GWAS in a breeding population

Forage Genetics, Intl. breeding population

268 families, 2 reps

Measured fall height on all plants

Genotyped one plant per plot with GBS

Associate height with markers for chosen plant

# Determining genotypes with low-read GBS data

Assigning discrete *genotype calls* to individuals for marker loci is problematic in autopolyploids

Need substantial depth to unequivocally call the heterozygote classes (AAAa, AAaa, Aaaa)

Two alternative approaches:

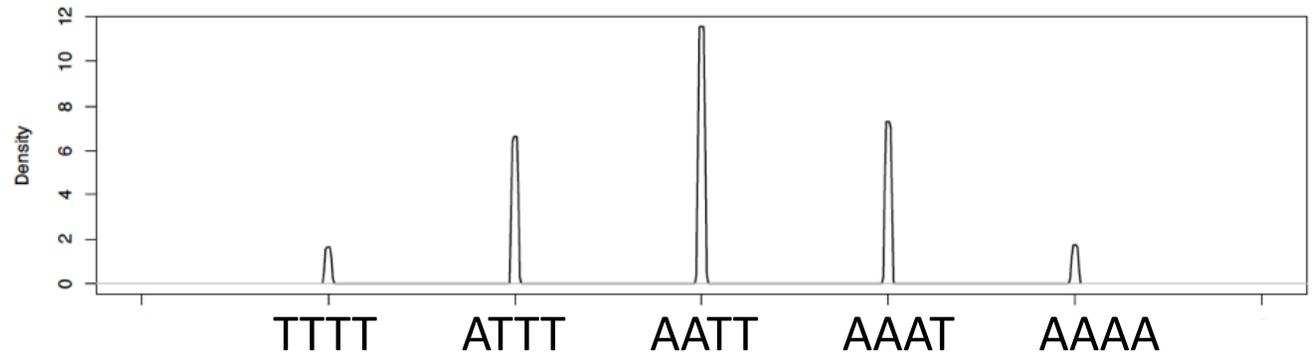
“Diploidize data – only call three genotypic classes (AAAA, heterozygotes, aaaa)

Model the uncertainty

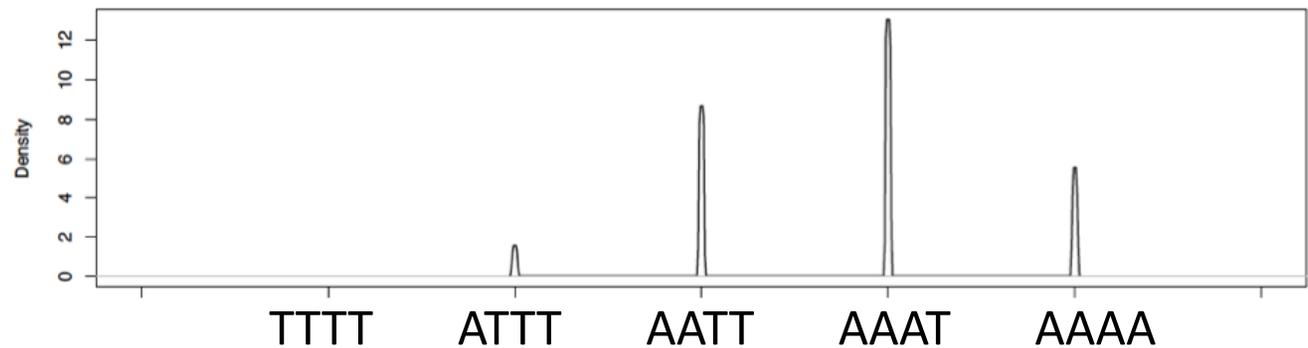
# Modelling auto-tetraploid genotypes

Assume 2 alleles (A/T) with pop freq = 0.5; sampled an "AAAA" genotype

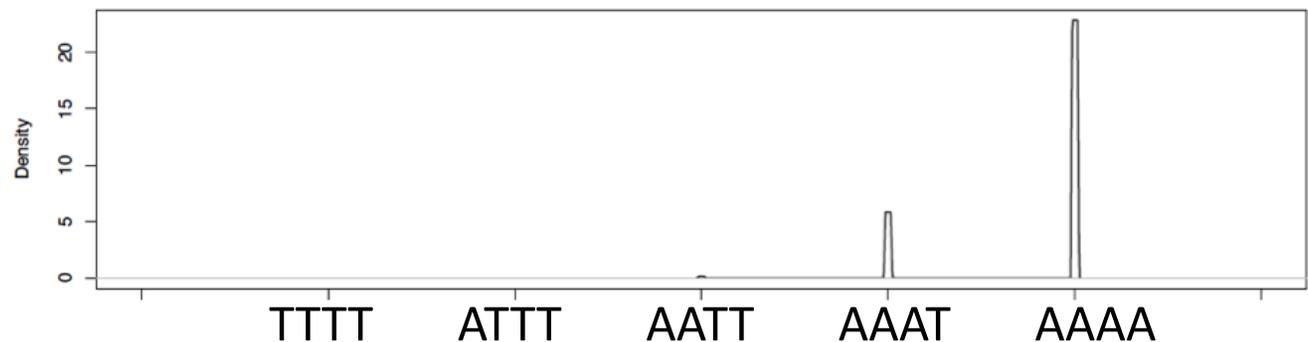
*No reads*



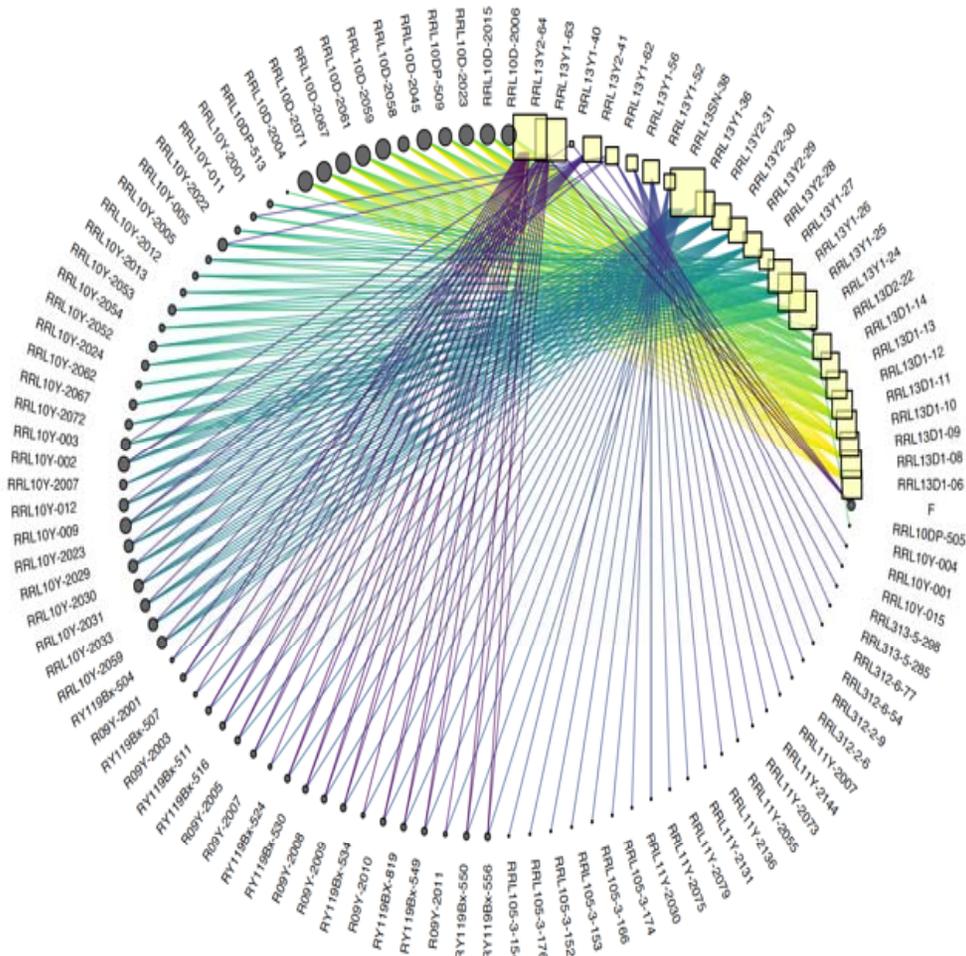
*2 Reads, Both "A"*



*10 Reads, All "A"*



# GWAS with modeled genotypic data



Pedigree of FGI breeding population

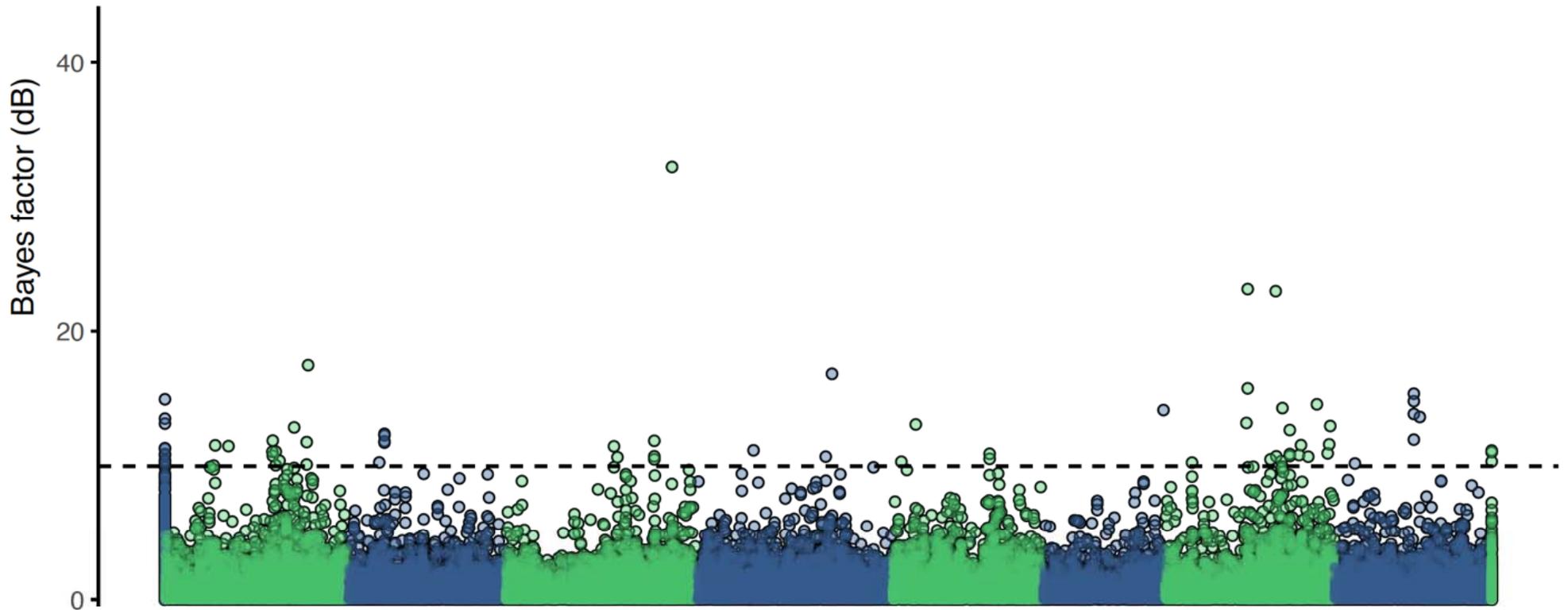
Structure related to pedigree so used a design matrix in the model

Modeled genotypes based on population allele frequency and read depth for each individual

Developed a model to find associations between markers and plant height

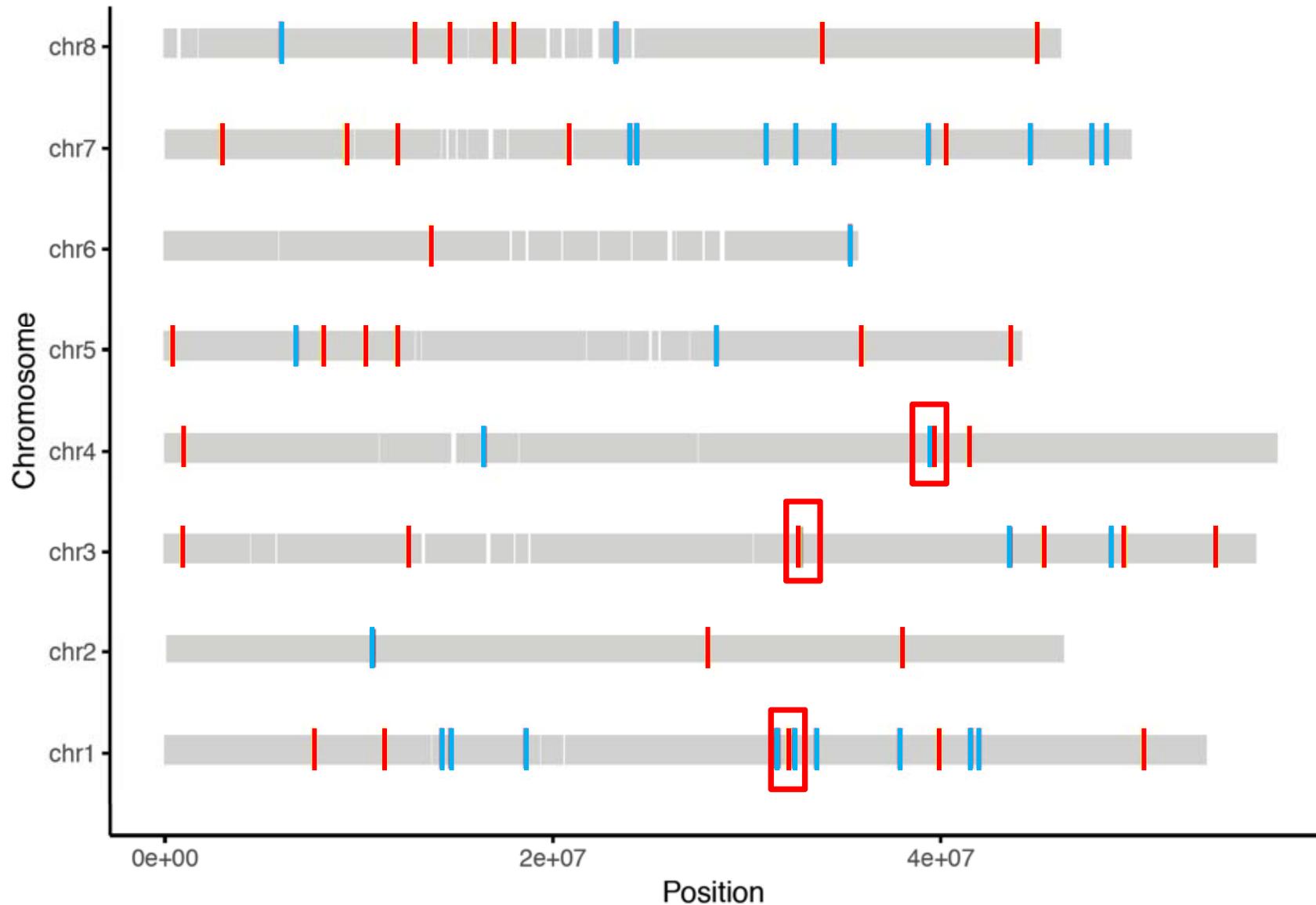
Model fitting performed with *JAGS* using *rjags* (Bayesian Graphical Models using MCMC)

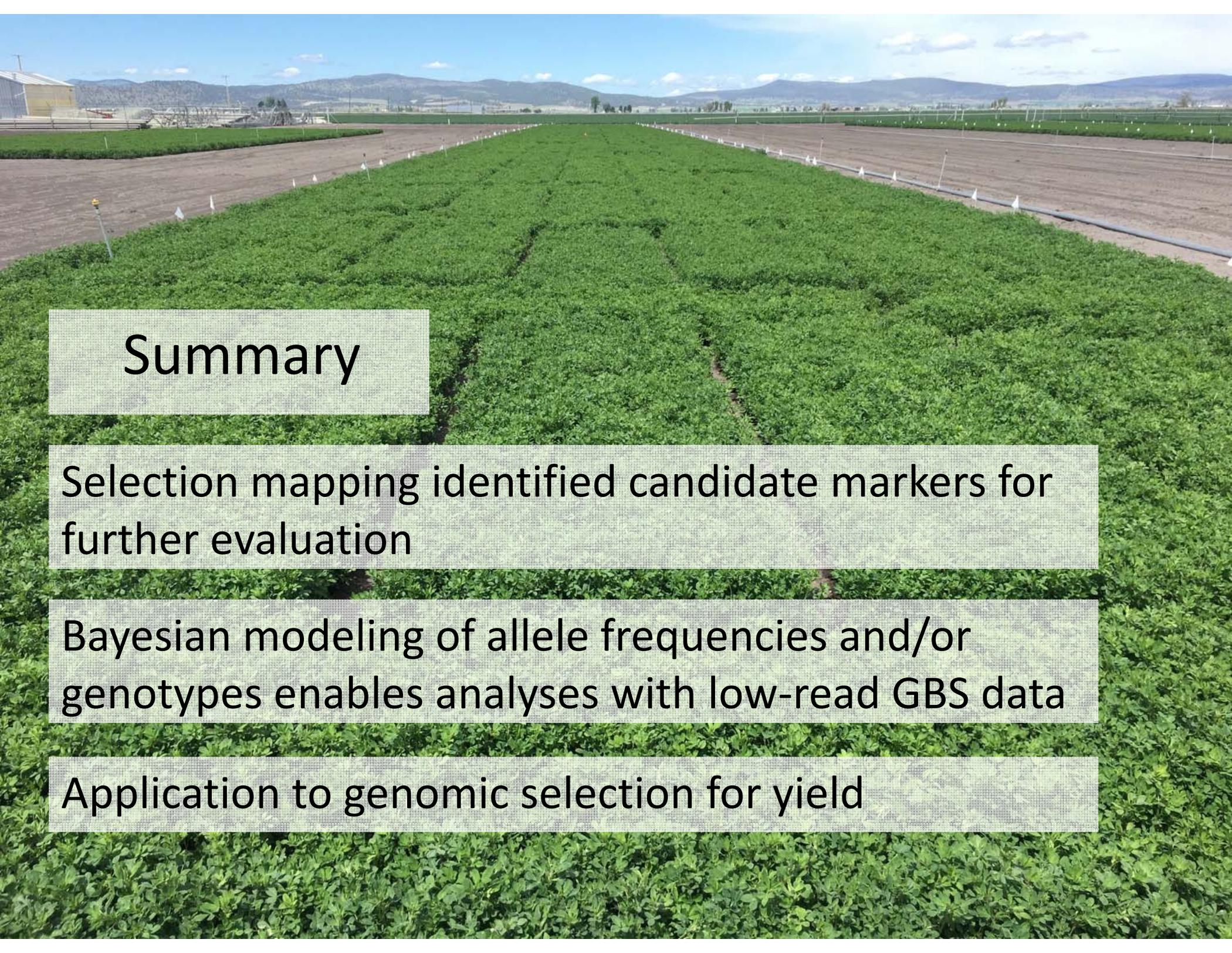
# Markers associated with height



Manhattan plot of Bayes factors in dB units ( $10 \cdot \log_{10}(\text{BF})$ ). Dashed line represents an empirical cut-off implying 10:1 odds in favor of association. Mapping positions are based on alignment to the *M. truncatula* reference genome. The synthetic chromosomes “chr0” and “chr9” represent markers that do not align to *M. truncatula* chromosomes but have valid alignments to both CADL and *M. truncatula* scaffolds (“chr9”) or CADL scaffolds only (“chr0”).

# Localization of top associated markers from GWAS (blue) and selection mapping (red)





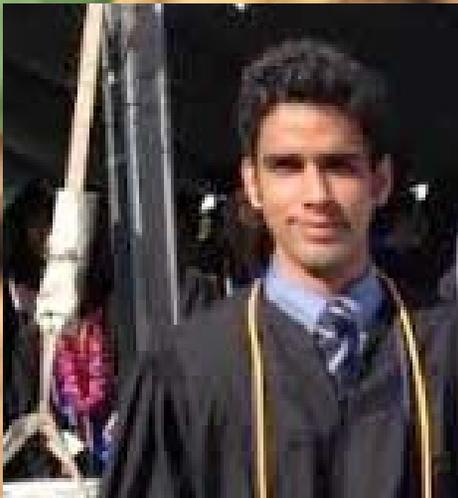
## Summary

Selection mapping identified candidate markers for further evaluation

Bayesian modeling of allele frequencies and/or genotypes enables analyses with low-read GBS data

Application to genomic selection for yield

Thank you!



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