**LOUISIANA STATE UNIVERITY**
**College of Agriculture**
**School of Plant, Environmental, and Soil Sciences**
**AGRO 7075 Prediction-based Breeding**

# Genome-Wide Association Studies

**Prof. Roberto Fritsche-Neto**

rfneto@agcenter.lsu.edu

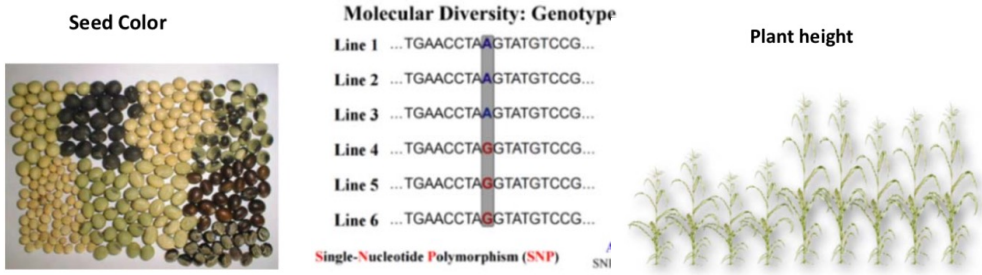
**Baton Rouge, April 3rd, 2023**

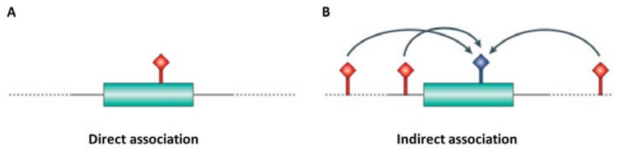
# Introduction

- Qualitative traits - few genes, with large effect, low environmental bias, and high $h^2$
- Quantitative traits - many genes, small effect, with high environmental bias, and low $h^2$

- How to select these traits?
- "Traditional" Breeding
- Molecular markers associated with quantitative trait loci (*QTL*)
- *Marker Assisted Selection (major QTL or QTL with large effect)*
- *Genomic Selection (many QTL with small effect)*

- GWAS is more interested in finding the causal relationship between genetic polymorphism within a specie than the phenotypic differences observed between individuals
- Also, how it is passed to the next generation




**A**) Functional SNP (red) was genotyped and an a direct association was identified.

**B**) Functional SNP (blue) was not genotyped, but number of other SNPs (red), in high LD with the functional SNP, were genotyped. So an indirect association was identified for these SNPs.

# Trait-marker association

- **How to identify trait-marker association?**
- QTL mapping based on biparental population
- It is still a powerful method to identify regions of the genome that co-segregate with a given trait
- $F_{2:3}$ populations or Recombinant Inbred Line (RIL) families

- **Limitations of QTL mapping:**
- **Low allelic diversity:**
- It is limited to two parents (two alleles) of a particular cross
- **Lower resolution**:
- Few recombination events happen during the creation of the RIL
- When the resolution is low, the QTL interval is large
- 10 Mb interval in maize might have more than 200 genes
- **A biparental population needs to be created**
- It takes a long time

# GWAS *vs.* QTL mapping

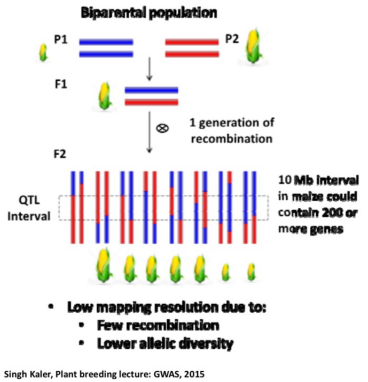
- A GWAS is an approach that uses whole genome markers to find genetic variations associated with a trait

**Biparental population vs Natural populations:** Mapping resolution

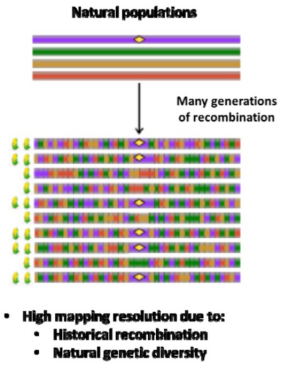
**Genotyping**

| | | | | | | |
|---|---|---|---|---|---|---|
| Individua 1 | A | C | G | A | G | 1.3 m |
| Individua 2 | A | C | G | A | T | 1.4 m |
| Individua 3 | A | T | A | A | G | 1.5 m |
| Individua 4 | C | T | A | G | T | 1.8 m |
| Individua 5 | A | C | G | G | T | 2.0 m |
| Individua 6 | A | T | G | G | G | 2.1 m |
| | A/C | T/C | G/A | A/G | G/T | |

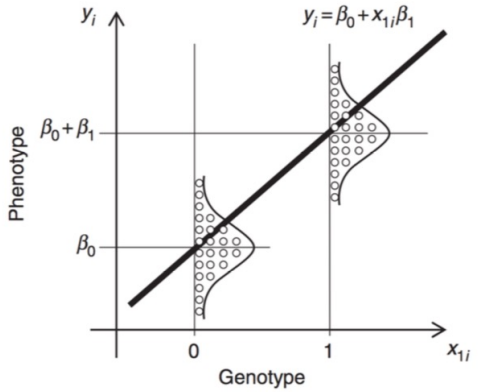
**Phenotyping**




Singh Kaler, Plant breeding lecture: GWAS, 2015




- High-resolution power due to a high amount of historical recombinatio
- Low LD
- High genetic diversity (diverse populations)
- Biological adaptation and geographical distribution
- No need to create a mapping population (saving time)
- Study various regions of the genome simultaneously
- Greater capacity for detecting more alleles



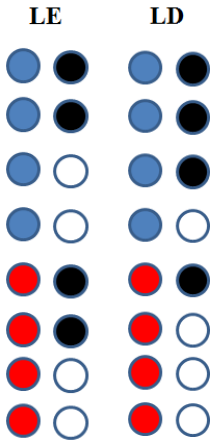
# GWAS limitations and advantages

- **Limitations**
- **Reproducibility:** sometimes, results are not replicated across populations
- **Results need to validate** by replication in independent samples in different populations (validation test)
- **Size of population:** population should be enough large to detect a QTL (statistical power)
- **Marker dataset size:** a large number of markers is required to cover the whole genome
- **Detects association, not causation**
- **Noncoding variants with unknown effect:** most of the identified variants in GWAS are far from discovered protein-coding gene
- **Detection of rare variants:** detects only variants with frequency >5% in a population
- **SNPs only explain a small fraction of the phenotypic variation of a trait**

- **Advantages**
- **Discover novel candidate genes or QTL for a measured trait(s)**
- **Determine aspects of the genetic architecture of complex traits:**
- Number of loci that contributed to the phenotype
- The respective contribution of loci to the phenotype

# Linkage disequilibrium (*LD*)

- Non-random association between two or more loci
- Not necessarily on the same chromosome
- Some combinations are more frequent than expected




$$p(A) = 0,7 \quad p(AB) = 0,35$$
$$p(a) = 0,3 \quad p(ab) = 0,05$$
$$p(B) = 0,6 \quad p(Ab) = 0,25$$
$$p(b) = 0,4 \quad p(aB) = 0,35$$

$$D = p(AB)p(ab) - p(Ab)p(aB)$$

$$D = 0,35.0,05 - 0,25.0,35 = -0,07$$

$$r^2 = \frac{D^2}{p(A)p(a)p(B)p(b)}$$

$$r^2 = \frac{0,0049}{0,7.0,3.0,6.0,4} = 0,10$$

- LD after t gerations
- *Recombination rate (c)*

$$D_t = D_o(1-c)^t \qquad \frac{D_t}{D_o} = (1-c)^t$$

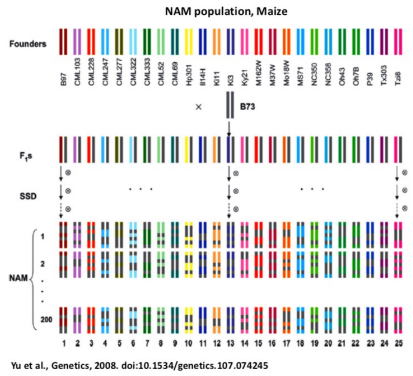
$$t = ln\left(\frac{D_t}{D_o}\right)/ln(1-c)$$

- How many generations to reduce 20% of LD?
- $c = 0.05$

$$t = ln(0.8)/ln(0.95) = 4.35$$

# GWAS assumptions and populations

- Assumptions
- Genetic variants contribute to the development of a trait
- A marker associated with a certain trait is in or near a gene that contributes to that trait
- Common variants explain a significant proportion of the genetic variation in the population
- Population homogeneity
- Populations normally used
- A pool of genotypes from a breeding program
- Multiple cross populations: *NAM, MAGIC*
- Lines derived from diallel crosses
- Germplasm collection: *landraces, accessions*



Yu et al., Genetics, 2008. doi:10.1534/genetics.107.074245

**Populations used in GWAS**

**Table 8.3** The relevant features of various mapping populations available for association analysis in plant breeding programs

| Feature | Germplasm bank | Elite breeding material | Synthetic population |
|---|---|---|---|
| Sample | Core collection accessions | Lines and cultivars developed in breeding programs | Individuals or lines drawn from the population |
| The composition of sample | Does not change | Changes with time as new materials are developed | Changes with time as the generation advances |
| Traits analyzed | Highly heritable and domestication traits | Low heritability traits like yield | Depends on the evaluation scheme |
| Level of LD | Low | High | Intermediate |
| Population structure | Medium | High | Low |
| Allelic diversity in the sample | High | Low | Intermediate |
| Resolution of AM | High | Low | Intermediate; increases with generation |
| Power of association analysis | Low | High | Intermediate; decreases with generation |
| The use of markers associated with the target traits | Marker-aided selection (MAS) | MAS | Incorporated in selection index |

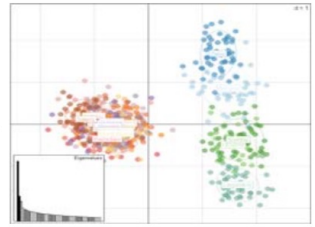
Based on Breseghello and Sorrels (2006)

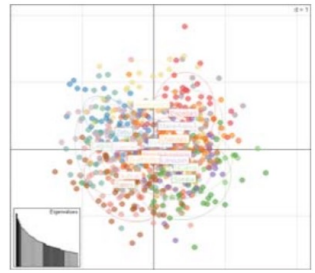
# Power of GWAS

- **The proportion of phenotypic variation explained by the SNP –** increases the heritability
- **The effect size of the two allelic variants:** how they differ in their phenotypic effect (no way to change)
- **Sample size** (evaluate more individuals)
- **Frequency of allelic variants in the sample** (change the mating design, increasing the rare alleles)
- **Population structure:** introduce heterogeneity resulting in an association that is not true
- *Geographical distribution*
- *Growth habit: winter and spring wheat*
- *Unequal familial relationship*
- *Different LD pattern*

# Population structure

- A systematic difference in allele frequencies between subpopulations
- This may be due to different ancestry: *geographical and climate distance, familial relationship*, …
- Violates assumptions: *population homogeneity*
- It ends up in spurious association ➔ *False positives (Type I error)*
- Over-estimation of associations' significance



- **Solution**: regression on covariates - **quantitative (PCs ) or binary (sex, origin)**
- For instance, including 1-3 PCs in the mixed linear model

- **Example: SNP1**
- Assumed the SNP is associated with plant height or disease resistance
- North American lines are:
  - Taller and susceptible
  - Allele **T** could be associated with either trait
- South American lines are
  - Shorter and tolerant
  - Allele **G** could be associated with either trait

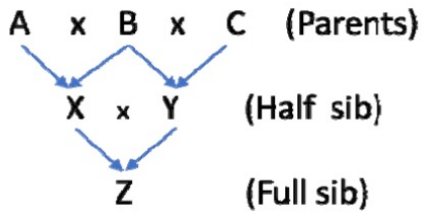
| | North America | | | | | | | | | | South America | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Plant Ht. | 10 | 10 | 12 | 11 | 13 | 9 | 11 | 10 | 13 | 12 | 4 | 6 | 5 | 7 | 6 | 6 | 4 | 5 | 9 | 5 |
| Dis. Res. | S | S | S | T | S | S | S | S | T | S | T | S | T | T | T | T | T | S | T | T |
| SNP1 | T | T | T | G | T | T | T | T | G | T | G | G | G | G | G | G | T | G | T | G |

# Unequal familial relationship

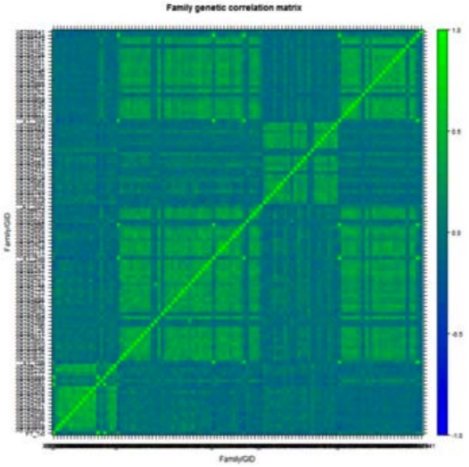
- Coefficient of coancestry: the probability that **an allele selected randomly from individual X** and **an allele selected randomly from the same autosomal locus of individual Y** is in identity by descent (IBD)

- **K (kinship)** = twice of the coancestry

- **Genomic relationship matrix (G or K )**
- Molecular markers are used to estimate relationships
- Two individuals sharing lots of genotypes at SNPs are likely belong to the same family



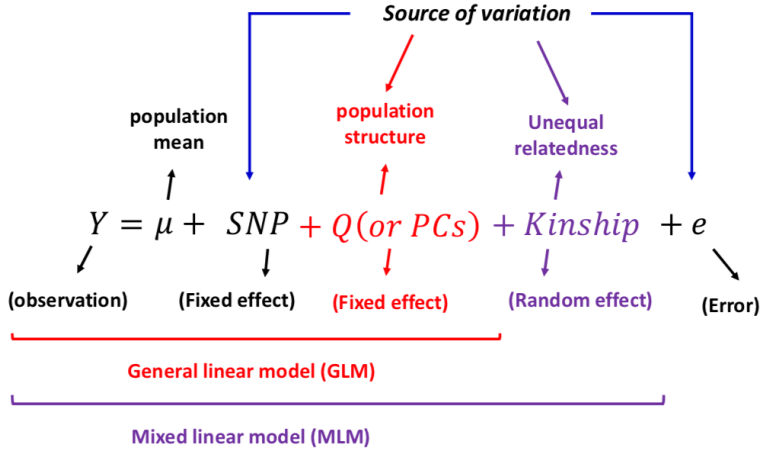




# GWAS models

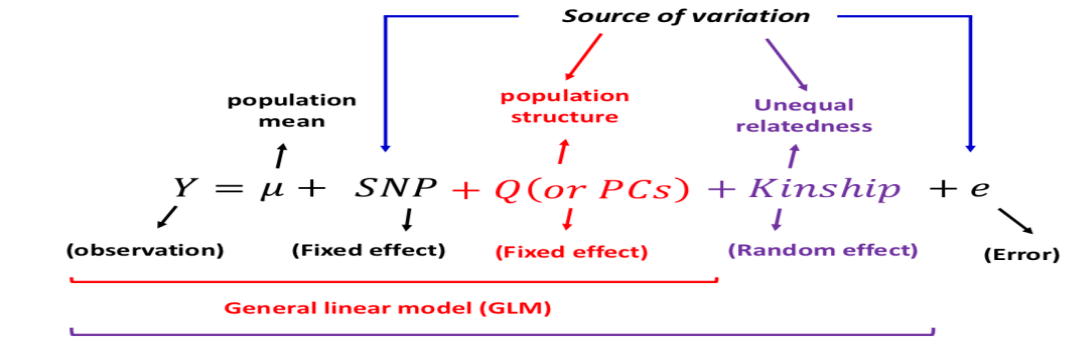
- **GLM:** all the factors included in a GLM are fixed effects
- This model is built and solves for each trait and marker information
- **Includes:**
- **Phenotypic dataset** *(observation for each trait)*
- Each individual could have several observations *(e.g., replicates, locations, years)*
- The adjusted mean value for each genotype is used in GWAS
- M**arker data** (e.g., SNP)
- **Covariates**
- Any covariates that can be used to control field variations, and individuals *(e.g., winter and spring wheat, geographical distribution, fertility variation of field,...)*
- 1-3 PCs (or Qs) to control population structure

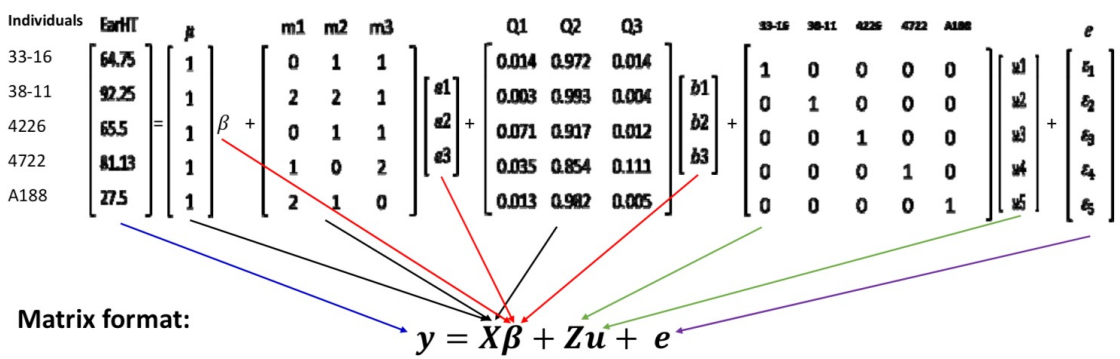
- **MLM:** Factors in MLM include both fixed and random effects
- Individuals in MLM are random
- The kinship matrix controls the unequal familial relationship



# GWAS model example

**Crop:** Maize
**Trait:** Ear height




**Matrix format:**

$$y = X\beta + Zu + e$$

**y**: a vector of phenotypic observation for trait of interest.
$\boldsymbol{\beta}$: an unknown vector containing fixed effects, including genetic marker effect and population structure (**Q or PC**).
$\boldsymbol{e}$: a vector for random residual $e \sim N(0, \sigma_e^2 I)$
$\boldsymbol{u}$: an unknown vector of random additive genetic effects from multiple background QTL for individuals $u \sim N(0, \sigma_a^2 K)$
$\boldsymbol{X}$ & $\boldsymbol{Z}$ : known design matrices

# How is marker-trait tested?

- Testing the full model over the reduced one to see if SNP has a significant effect on the trait

$$Y = \mu + SNP + Q + e \quad \text{(Full model)}$$

$$Y = \mu + Q + e \quad \text{(Reduced model)}$$

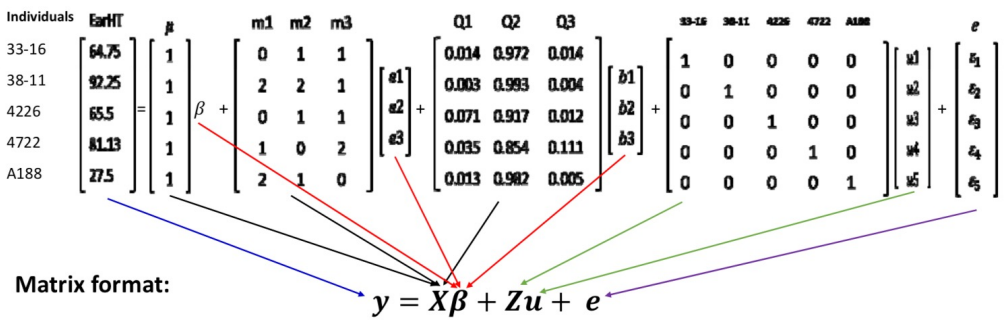
$$\frac{Full\ model}{Reduced\ model}, \qquad P\ value$$

- LRT = Chi- square test $\chi 2\ (df = 1)$
- anova(Full.m, Red.m) in R
- Compare the p-value with the threshold p-value (0.05)

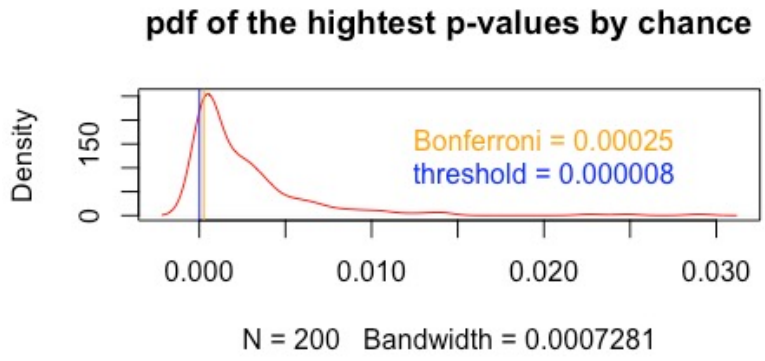
- **Multiple hypothesis testing**
- In GWAS, we perform many marker-trait hypothesis-tests (#tests = #markers)
- It creates a challenge with Type I errors called **Multiple testing problem**
- For N independent testes ==> N*0.05. So, by increasing N, we make lots of errors
- Thus, the p-value by Bonferroni is equal to 0.05/N

# Building your own threshold

- Resampling method
- First, the phenotypic values are shuffled, breaking their association with the markers
- Then, the random association between all markers to the phenotype is estimated
- The corresponding best marker score (minimum *p*-value among all markers) is recorded
- This procedure is repeated hundreds of times for each trait – a distribution of random p-values
- Based on that, define the 95 % quantile
- It is defined as the newest threshold (based on your data) to declare a significant association

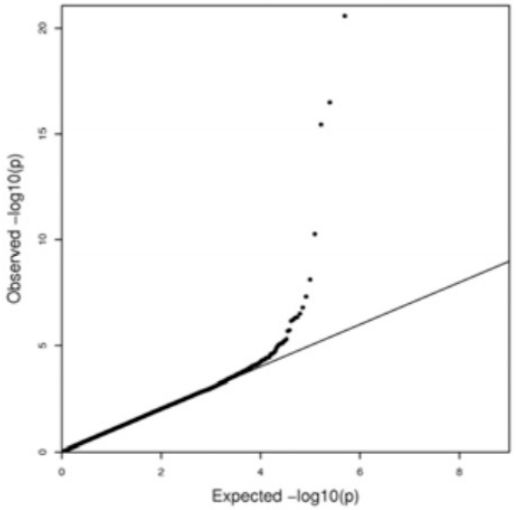



**y**: a vector of phenotypic observation for trait of interest.
$\boldsymbol{\beta}$: an unknown vector containing fixed effects, including genetic marker effect and population structure (**Q or PC**).
$\boldsymbol{e}$: a vector for random residual $e \sim N(0, \sigma_e^2 I)$
$\boldsymbol{u}$: an unknown vector of random additive genetic effects from multiple background QTL for individuals $u \sim N(0, \sigma_a^2 K)$
$\boldsymbol{X}$ & $\boldsymbol{Z}$ : known design matrices



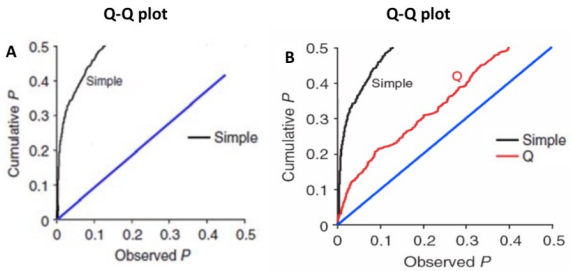
# Quantile–Quantile (QQ) plot

- It is a plot of the quantile distribution of observed p-values (on the y-axis) on the quantile distribution of expected p-values (on the x-axis)
- The expected p-values have a random, uniform distribution
- If a QQ plot is a line with a tail, there are some casual polymorphisms
- A few of the p-values are in LD with a causal polymorphism and had significant p-values.
- It is a statistical tool used to visualize GWAS output and power
- Most of the observed p-values have a uniform distribution (*not in LD with a causal polymorphism*)




**QQ plot: Correction for population structure (model selection)**

**Trait:** Flowering time
**Population structure:** High




$Y = \mu + aSNP + e$

**Simple model**

$Y = \mu + aSNP + bQ + e$

**GLM model (Q model)**

**K model:** $Y = \mu + aSNP + cK + e$

**Q + K model:** $Y = \mu + aSNP + bQ + cK + e$

**MLM model**

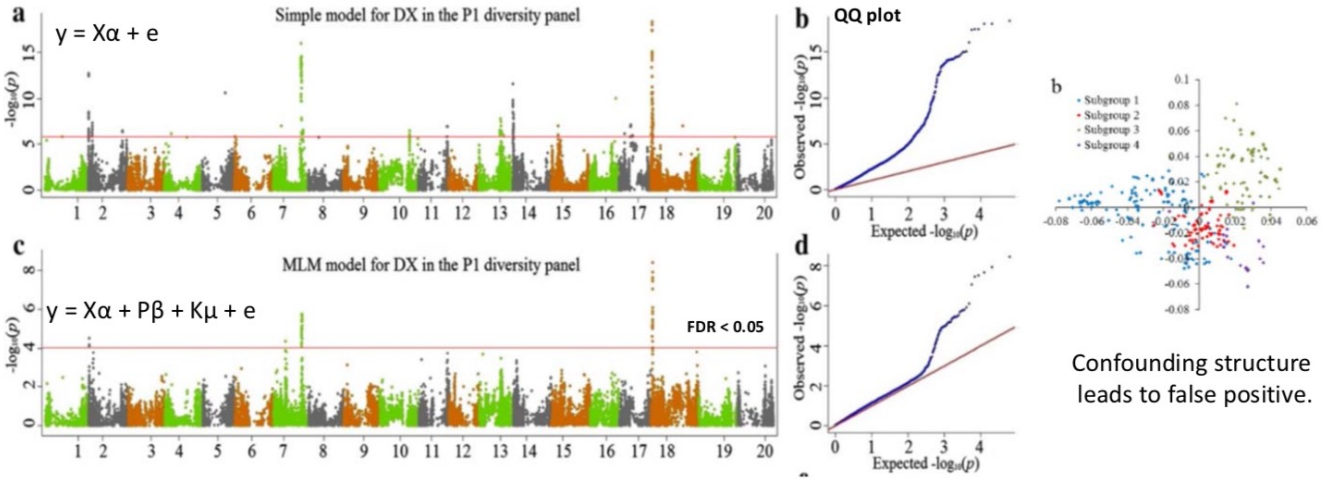
Yu et al. 2006 NATURE GENETICS Volume 38, No 2.

Results: More complex populations need more complex model!

# Manhattan plot

- It is a graphical tool to show significant hits associated with the trait under test
- Each data point represents a genotyped SNP, ordered across the chromosomes (x-axis)
- y-axis = $-\log_{10}$(p-value)

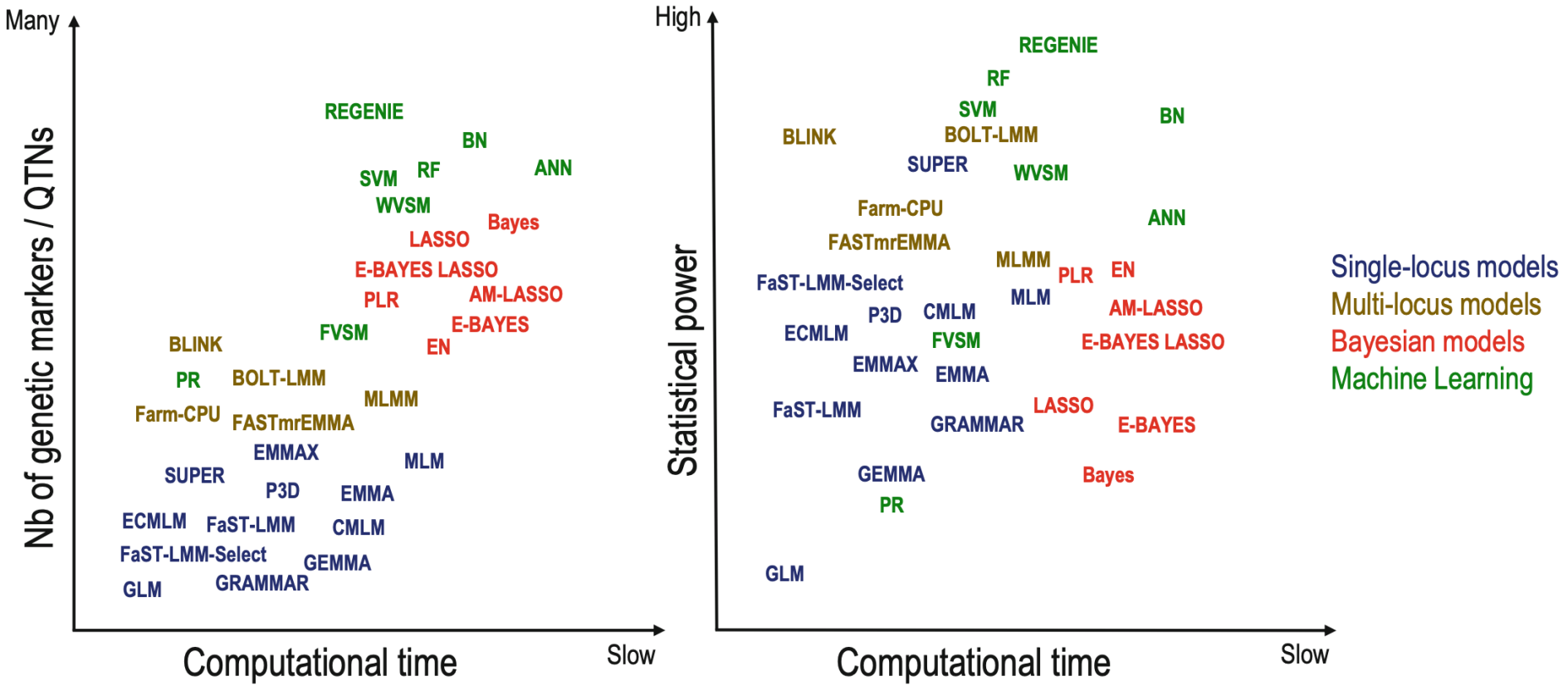
- Soybean cultivars (392 individuals)
- Sudden death syndrome (SDS) disease index (DX)
- The simple model (using only SNPs) leads to heavily inflated p-values




Zixiang Wen et al. 2014 (*BMC Genomics*)

Confounding structure leads to false positive.

# GWAS algorithms



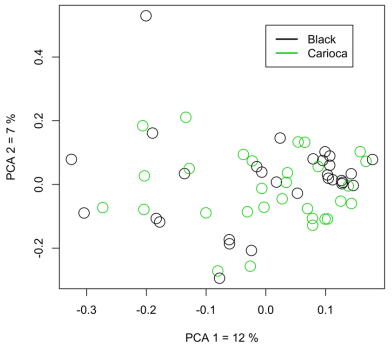
DOI: http://dx.doi.org/10.1590/1678-992X-2017-0306

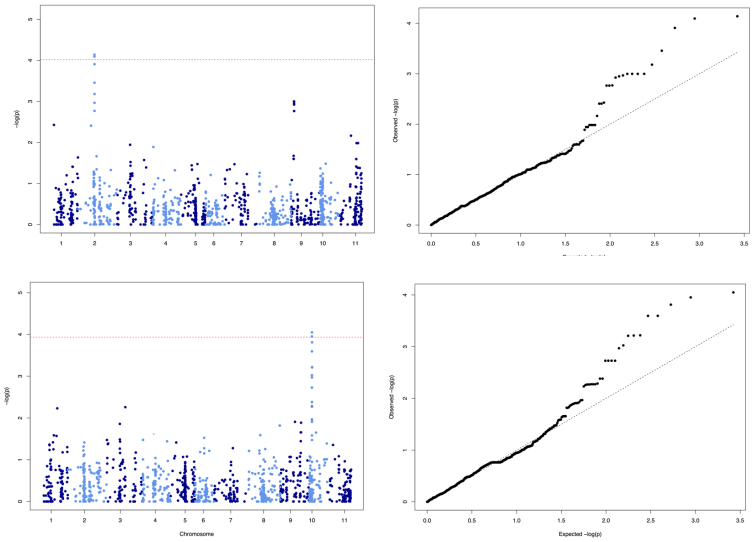
ISSN 1678-992X

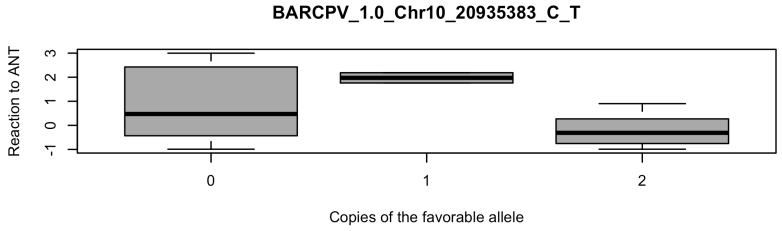
SCIENTIA AGRICOLA

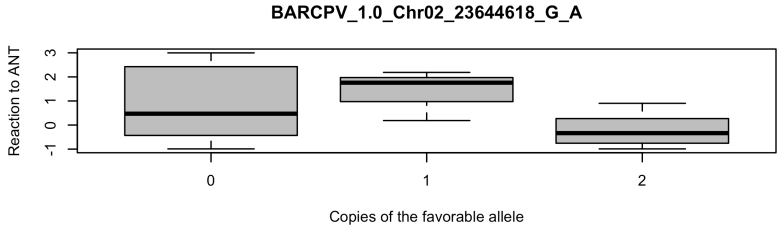
# Association mapping in common bean revealed regions associated with Anthracnose and Angular Leaf Spot resistance

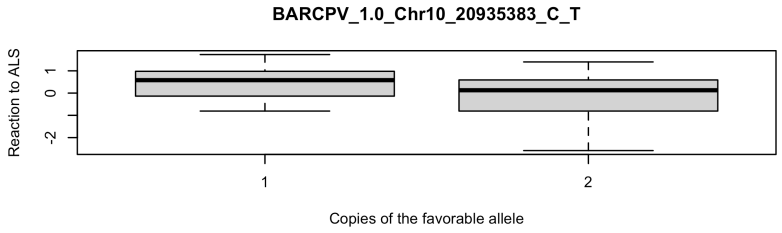
Roberto Fritsche-Neto[1]*, Thiago Lívio Pessoa Oliveira de Souza[2], Helton Santos Pereira[2], Luís Cláudio de Faria[2], Leonardo Cunha Melo[2], Evandro Novaes[3], Itaraju Junior Baracuhy Brum[4], Jean-Luc Jannink[5]




| Trait | SNP ID | Chr | SNP Position | R without SNP | R with SNP | MAF | Annotation |
|---|---|---|---|---|---|---|---|
| AN | BARCPV_1.0_Chr02_23542475_A_G | Pv02 | 23542475 | 0.02 | 0.25 | 0.29 | Intron of Phvul.002G115900 (Interleukin-1 Receptor-Associated Kinase 4) |
| | BARCPV_1.0_Chr02_23644618_G_A | | 23644618 | 0.02 | 0.25 | 0.18 | Intergenic region upstream of gene Phvul.002G116400 (Rab escort protein) |
| ALS | BARCPV_1.0_Chr10_20935383_C_T | Pv10 | 20935383 | 0.02 | 0.19 | 0.14 | Intergenic region downstream of gene Phvul.010G072700 (Scarecrow-like protein) |