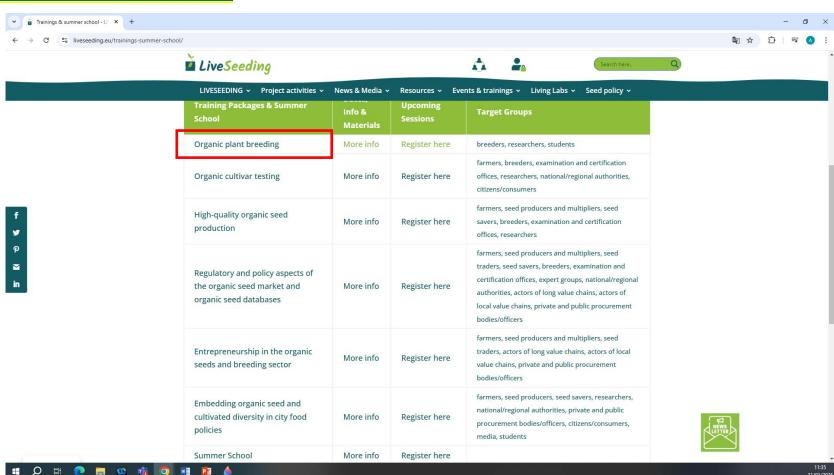# TRAINING IN ORGANIC BREEDING!

LiveSeeding

CONTEXT: Training in LIVESEEDING project

https://liveseeding.eu/trainings-summer-school/

Training in organic breeding organized in 5 Modules

1. **Module 1** - Plant Genetic Resources (PGRs): collection, conservation and exchange to support the increase of agrobiodiversity in farming systems

2. **Module 2** - Phenomics: approaches and tools for genetic resources and breeding material characterization - FEBRUARY 3rd 2025, 9:00 to 17:30 CET

3. **Module 3** - Breeding methods fundamentals - FEBRUARY 13th 2025, 9:00 to 18:00 CET

4. **Module 4** - Development and application of molecular methods in organic breeding - MARCH 4th 2025, 9:00 to 18:00 CET

5. **Module 5** - Organic heterogeneous material (OHM) design and development - MARCH 7th 2025, 9:00 to 18:00 CET

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Training in organic breeding organized in 5 Modules

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Module 4 - Development and application of molecular methods in organic breeding

MARCH 4ᵗʰ 2025   9:00 to   18:00 CET

Unit 4.1: Genome Wide Association and its potential for understanding the genetic basis of complex traits

9:00-11:00   Partner in charge: UPV (Neus Ortega Albero)

11:00-11:30 Break

Unit 4.2: Genomic Selection and its potential in organic breeding

11:30-13:00   Partner in charge: FiBL (Michael Schneider)

13:00-14:30 Lunch Break

Unit 4.3: Application of molecular marker-based selection in practical breeding
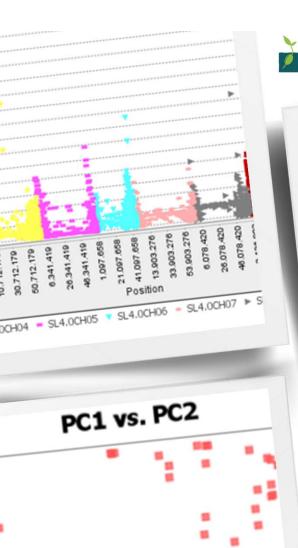
14:30-16:00   Partners in charge: KIS (Barbara Pipan) + FiBL (Lupins; Teresa Lazzaro)

16:00-16:30 Break

Unit 4.4: Overview on Experimental populations (e.g. NILs, RILs, introgression families, MAGIC) and their role in molecular breeding approaches
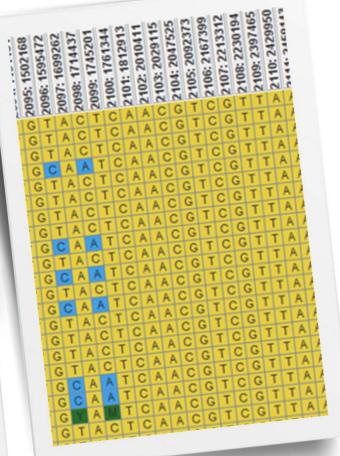
16:30-18:00   Partner in charge: UPV (Neus Ortega-Albero, Adrian Rodríguez-Burruezo) + KIS (Vladimir Meglic)

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Training in organic breeding

Module 4: **Development and application of molecular methods in organic breeding**

Unit 4.1: Genome Wide Association and its potential for understanding the genetic basis of complex traits

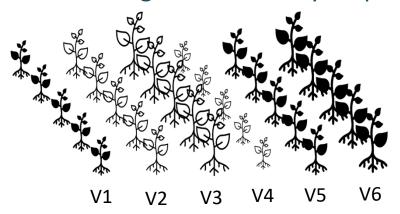Author: **Neus Ortega Albero (UPV)**

UK Research and Innovation

Relationship Gene - Trait

Genotype → Phenotype
Meeting the causing genes.

a) Develop markers to select interesting phenotypes easily (Unit 3)

a) Establishing correlations between traits and finding if the genetic base is the same to select one complex trait through another easy-to-phenotype trait

V1   V2   V3   V4   V5   V6

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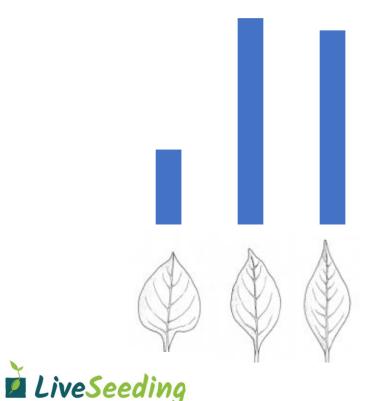
Qualitative vs. Quantitative traits
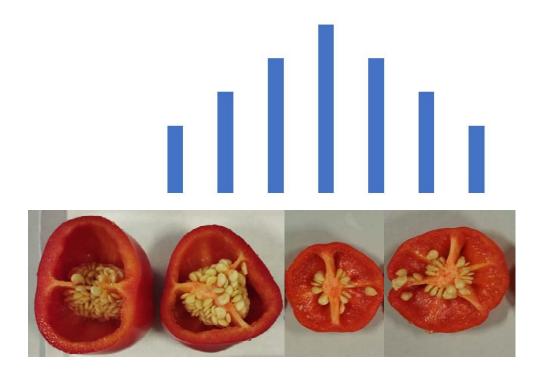
Qualitative traits → categories

Controlled by 1 or few genes

Quantitative traits → continuous expression

Controlled by many genes along the genome
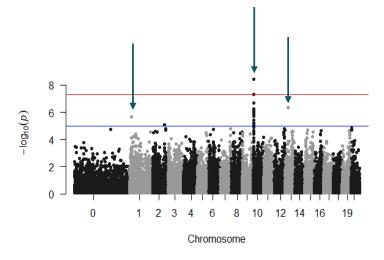
GWAS

Genome-Wide Association Analysis (GWAS)

Quantitative Trait Loci (QTL) → all the points (markers) in the genome associated statistically with the trait. The accumulative effect of these QTLs is the phenotype.

Exploit existing natural variability in a population accumulating recombination during decades.
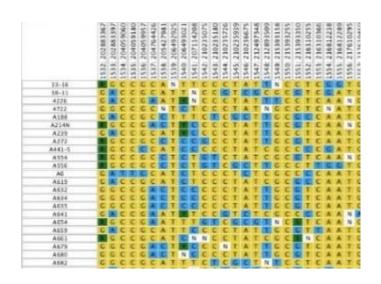
Used in a **collection of varieties** or a **breeding population** embedding variation for the studied trait.

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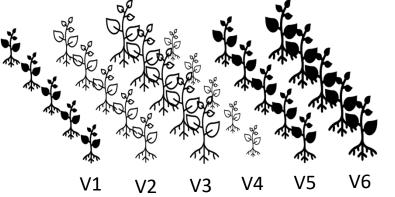
GWAS

Genotype

Phenotype

V1 V2 V3 V4 V5 V6

GWAS

GWAS

ADVANTAGES
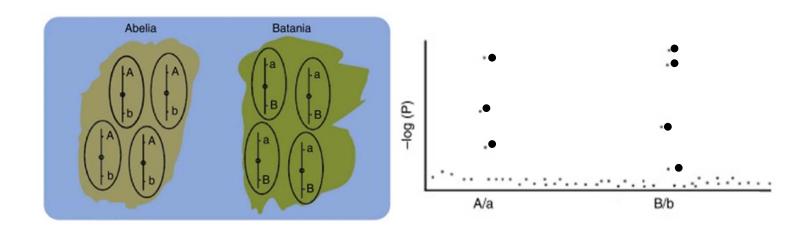
- No need to create a breeding population
- Analyze a wider range of diversity
- Useful for any type of trait
- Work on several traits in very variable populations
- High precisión in gene mapping
- Association for complex traits like adaptation to low input conditions

DISADVANTAGES

- Need variant availability
- The population of study must show variability for the trait
- Avoid population stratification

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Fake Associations
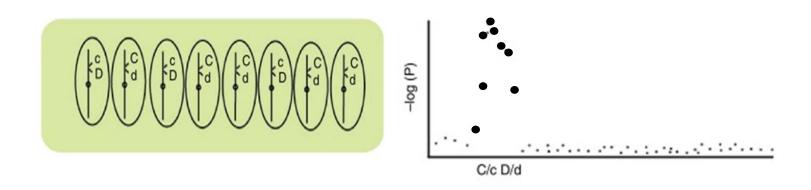
Fixed alleles in the population

When only two combination of haplotypes are fixed in the population, both genes will appear as associated with the trait, even only one is really associated.

![LiveSeeding]

Fake Associations

Ligated Genes or Linkage Disequilibrium
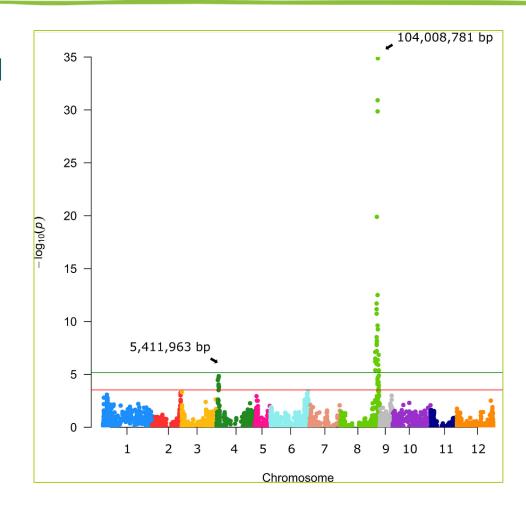
Two proximal genes always inherited together will be both associated to the trait, even only one of them is the cause of the phenotype.

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GWAS examples

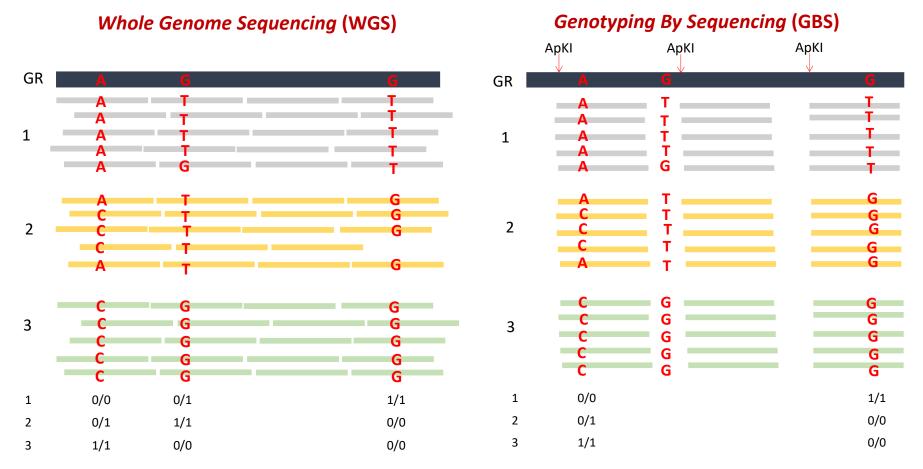Eggplant → pigmentation of the peel
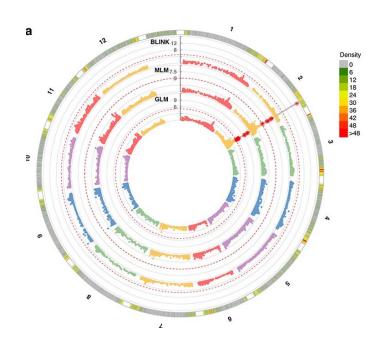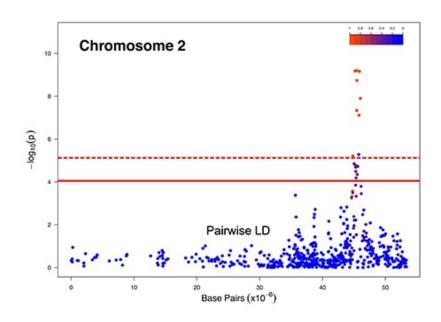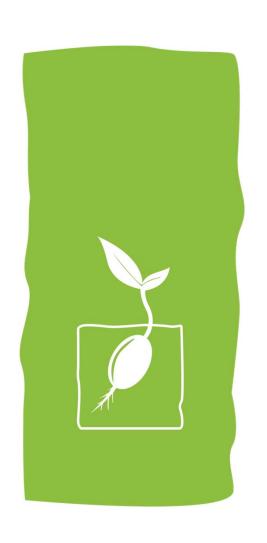
GWAS examples

Tomato → Locule number

PRACTICAL EXAMPLE

WGS vs. GBS

Whole Genome Sequencing (WGS)

Genotyping By Sequencing (GBS)

	ApKI	ApKI	ApKI

GR	A	G	G

WGS:
GR A G G

1
A T T
A T T
A T T
A T T
A G T

2
A T G
C T G
C T G
C T G
A T G

3
C G G
C G G
C G G
C G G
C G G

GBS:
GR A G G

1
A T T
A T T
A T T
A T T
A G T

2
A T G
C T G
C T G
C T G
A T G

3
C G G
C G G
C G G
C G G
C G G

	WGS				GBS		
1	0/0	0/1	1/1	1	0/0		1/1
2	0/1	1/1	0/0	2	0/1		0/0
3	1/1	0/0	0/0	3	1/1		0/0

LiveSeeding

Practical Example
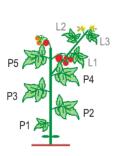
GWAS analysis in a collection of Solanum lycopersicum

Growth habit in tomato
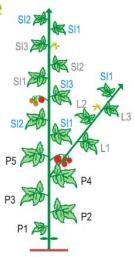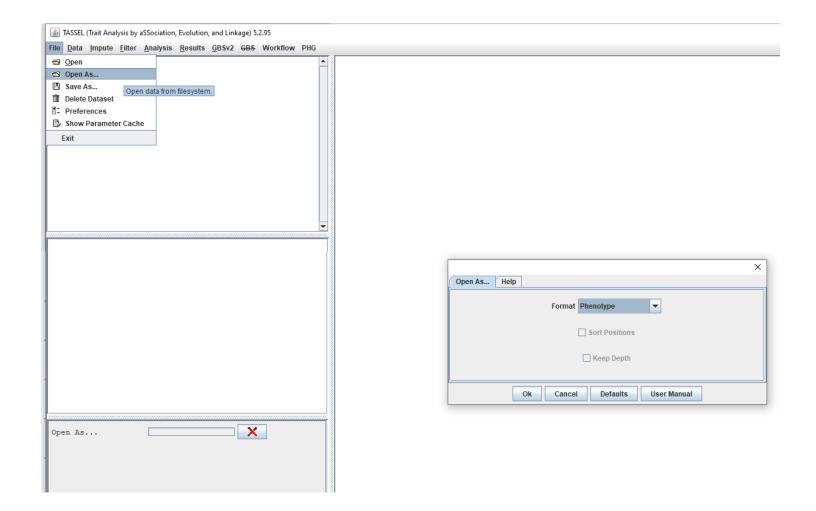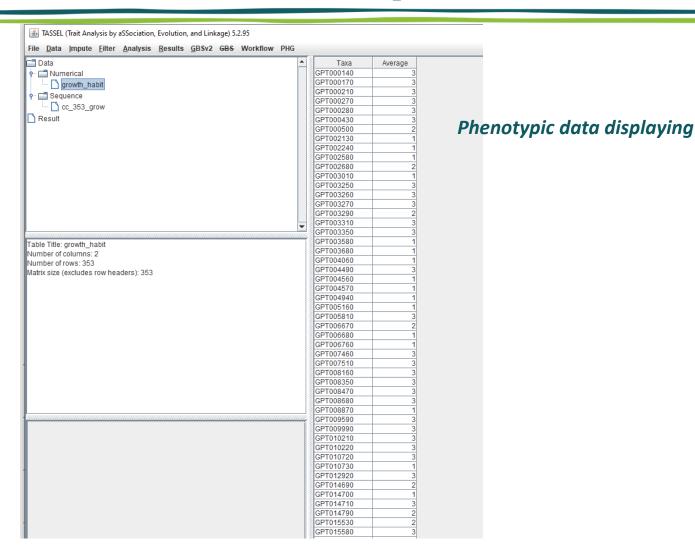
Practical Example

1. Open phenotypic data file

Practical Example

Phenotypic data displaying

Practical Example

2. Open GBS data file

Practical Example

Practical Example

3. Checking population structure with PCA

Practical Example

3. Checking population structure with PCA

Practical Example

Displaying PCA

Practical Example
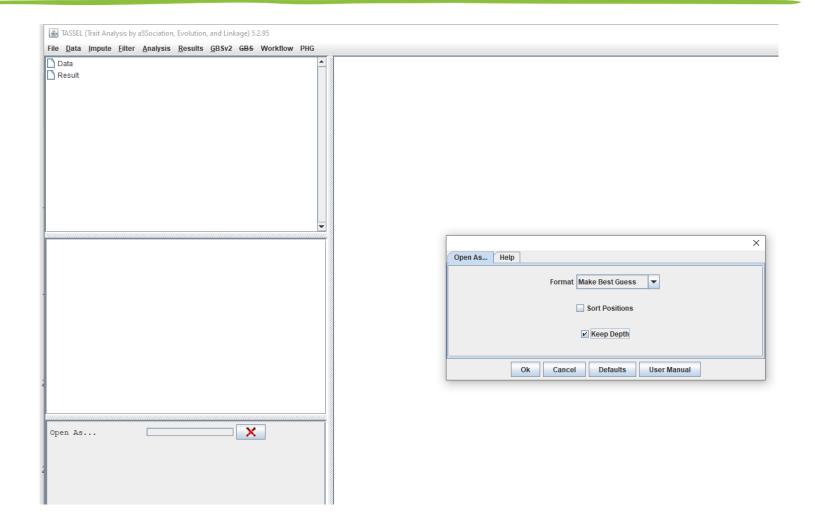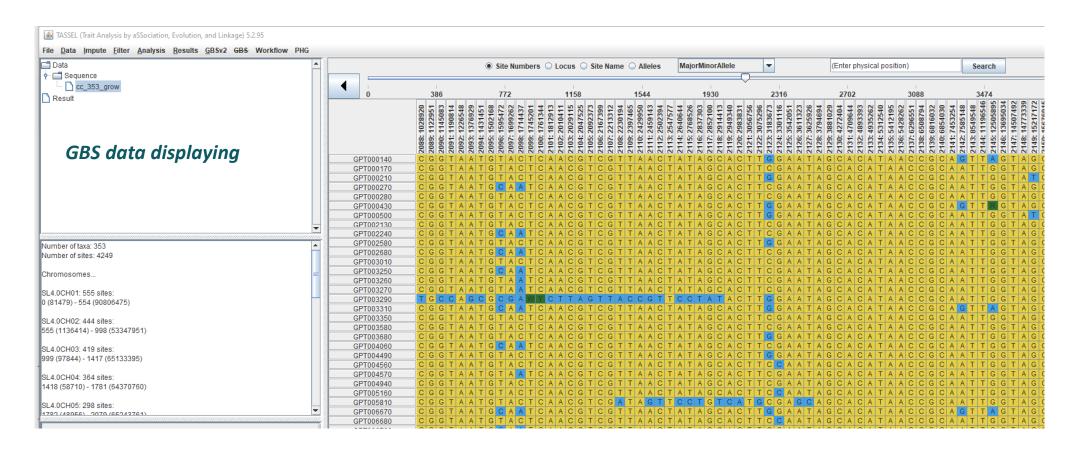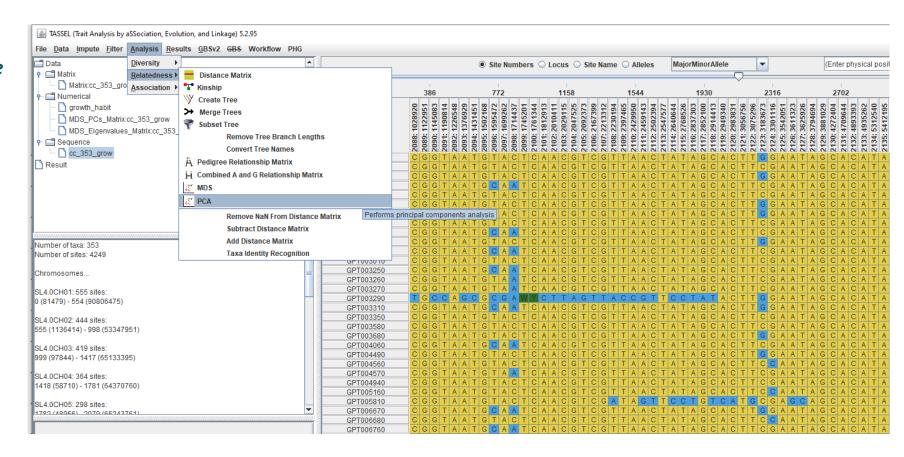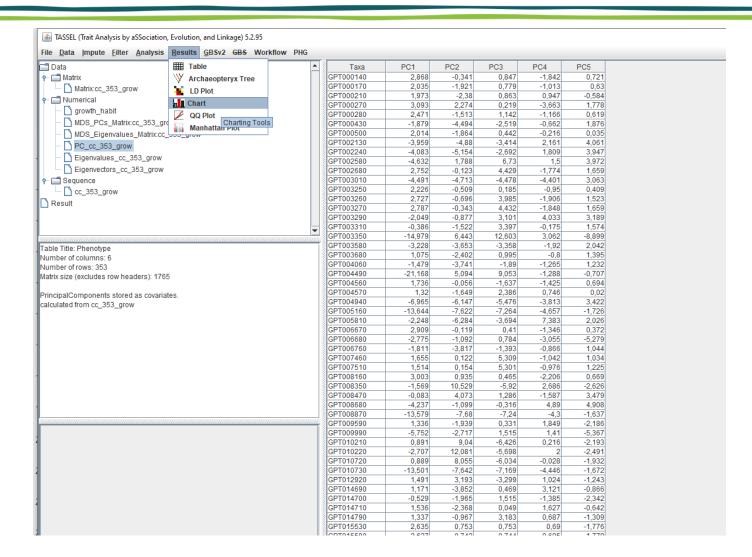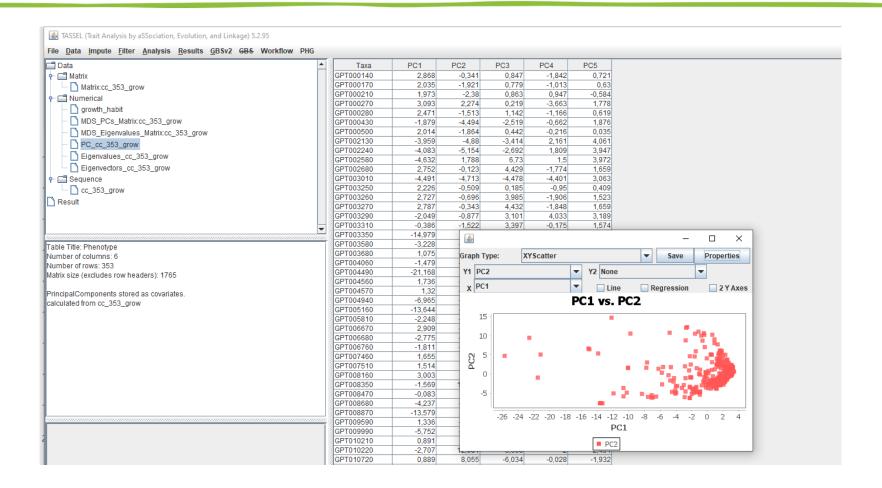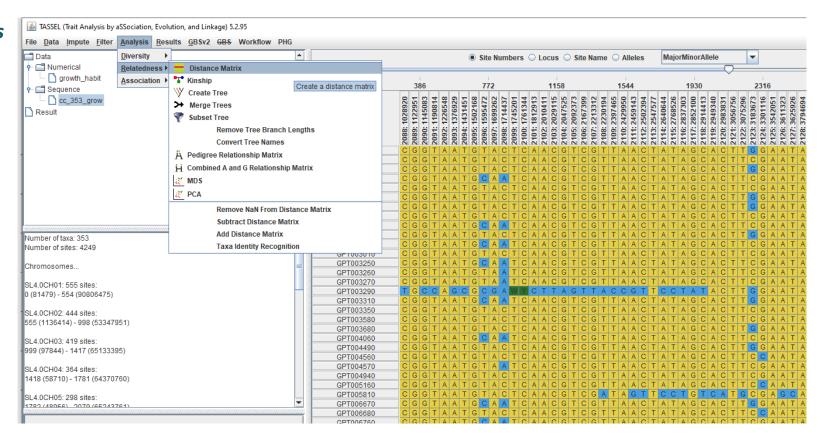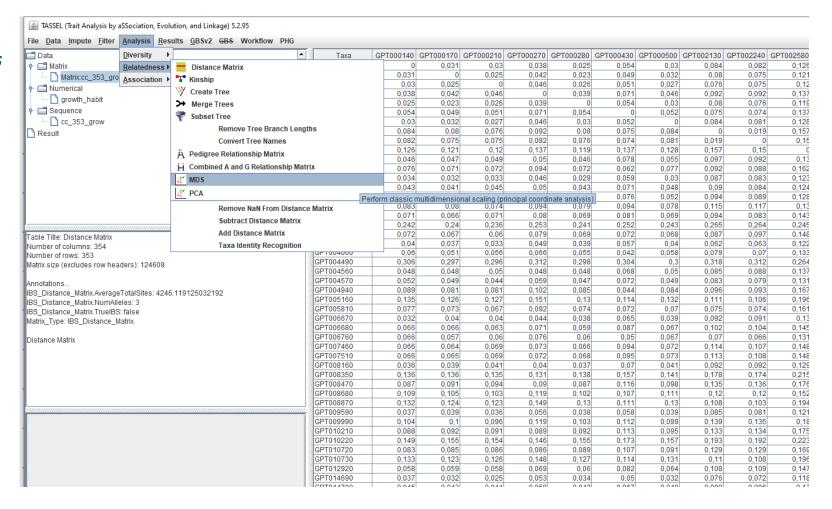
4. Calculating relationships within the population with the Distance Matrix

Practical Example
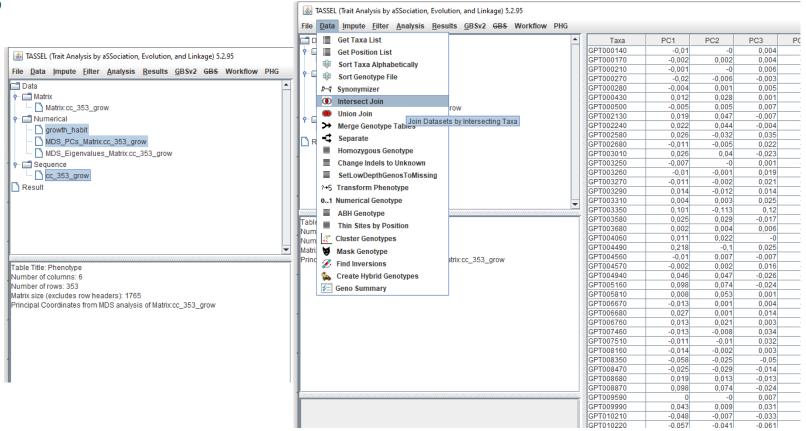
5. Incorporating the relationships to the analysis with the MDS

Practical Example

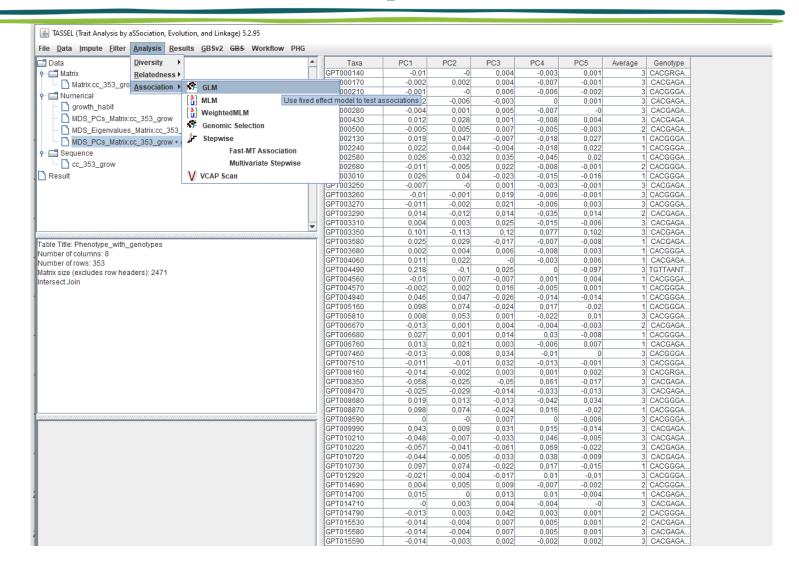**6. Select available data to the analysis (phenotype, genotype and MDS) and Intersect them**

Practical Example
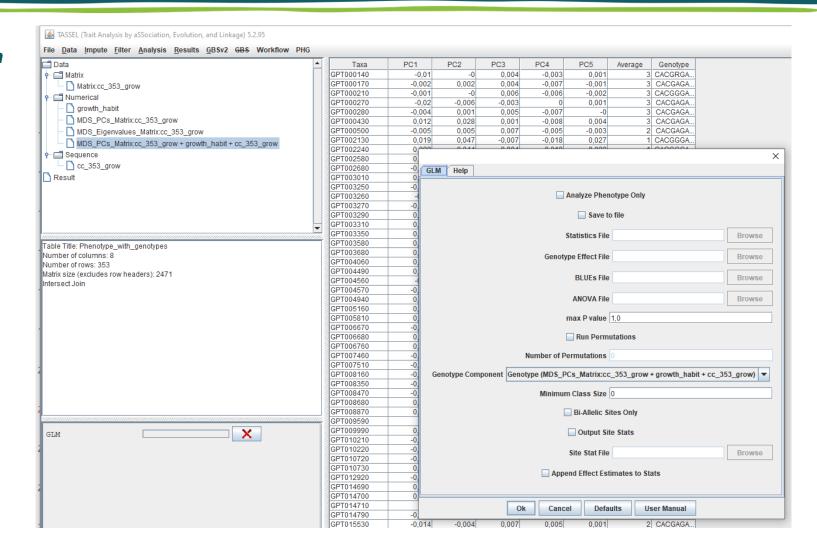
7. Association analysis with GLM

Practical Example

7. Association analysis with GLM
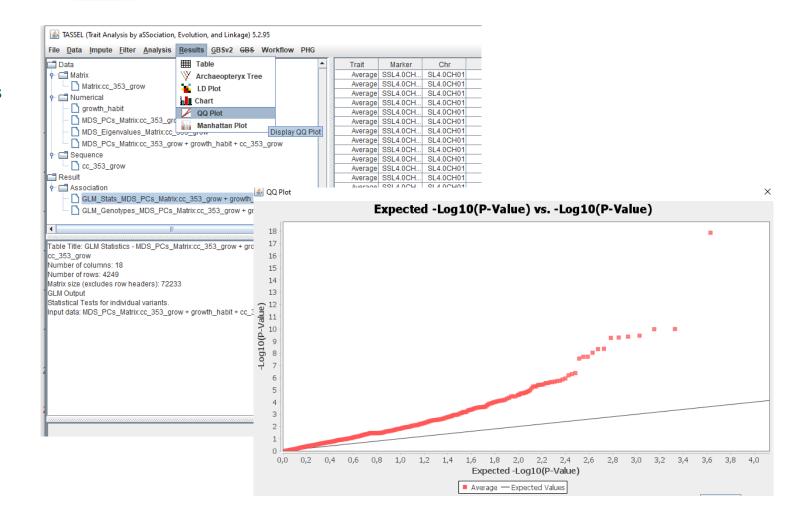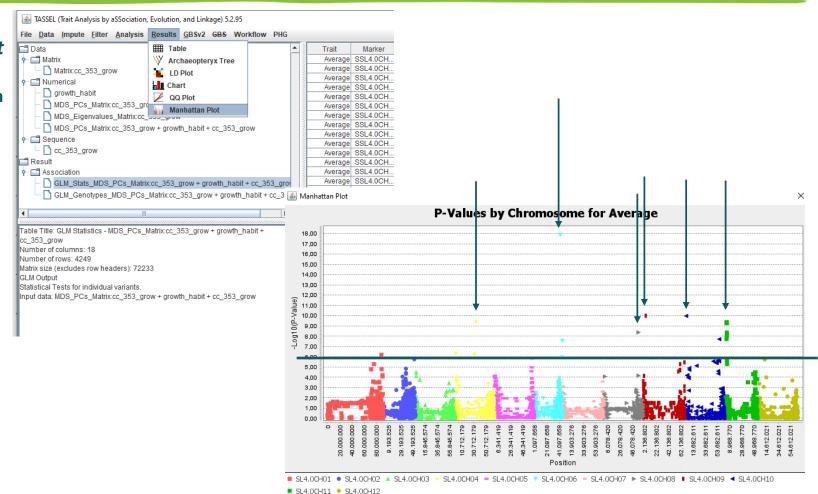
Practical Example

8. Represent QQPlot

The distribution of the markers is according to the expected if any QTL is associated with the trait

Practical Example

9. Represent Manhattan plot

The position of the markers with their association to the trait

Next steps

a) Select the most significant QTLs and establish a region upstream and downstream

b) Use available genomic information of the species to check annotated genes in this region

c) Analyze the function of these genes to know if they could be involved in the expression of the trait

d) Search information in other species and in other databases

Live*Seeding*

Practical Example

GWAS analysis in a collection of Solanum lycopersicum

Fruit shape in tomato

Flattened
1

Slightly flattened
2

Rounded
3

High-rounded
4

Heart-shaped
5

Cylindrical
6

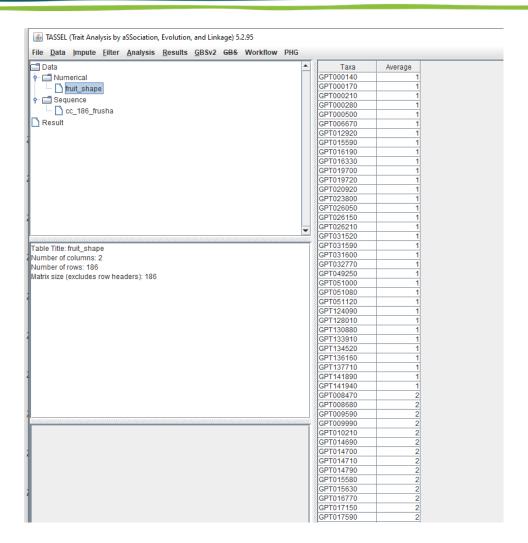Pyriform
7

Ellipsoid
8

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Practical Example

1. Open phenotypic data file

Practical Example
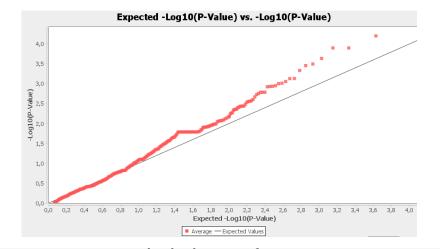
2. Open GBS data file
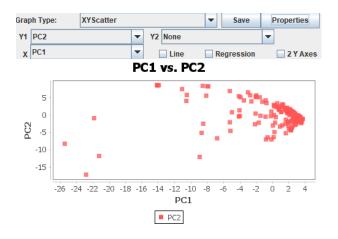
Practical Example

3. PCA
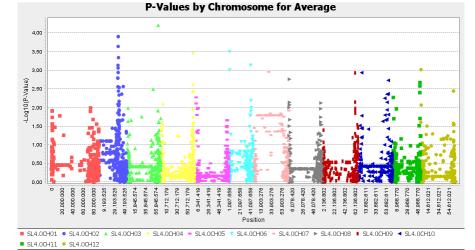
4. Distance Matrix

5. MDS

6. Intersect analysis information

7. Association analysis

8. QQPlot

9. Manhattan plot

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Fast Quiz
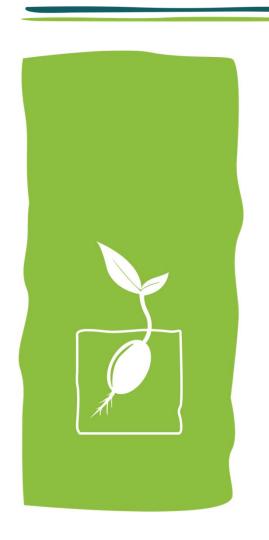
❑ How can the results be affected by the existence of two subpopulations?

❑ Which could be the reason why in this case we do not obtain any associated QTL in the second case? Be aware of the data.

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Take Home Messages

✓ GWAS is used to associate traits with genomic regions

✓ We need a non-structured and variable study population

✓ Specialized programs are required to intersect phenotypic and genotypic data

✓ Significant markers need to be analyzed to find the candidate genes responsible for the trait

Proposed Homework

Try to perform your own analysis with Tassel5 and the data in additional resources (genotyping_data.vcf, phenotyping_data.txt).

Send your results with screenshots of the QQplots and Manhattan plots and explain the obtained results.

Send your results to neuoral@doctor.upv.es, adrodbur@upvnet.upv.es, ac@ips-konzalting.hr

LiveSeeding

Additional Resources

Tassel5
- ❑ https://www.maizegenetics.net/tassel
- ❑ https://www.youtube.com/watch?v=73hD5n-OOJc&list=PLdf-U83sN48OPSQ-DqL3AgqKOvu8nUoMT

Novel High throughput phenotyping platforms:
- ❑ https://www.youtube.com/watch?v=zw02B_oWL9k

Data for homework and presentation:
- ❑ https://github.com/jmartinezpoq/LiveSeeding_Module4/tree/master/Unit1_PracticalExercices

LiveSeeding

Additional Resources

How to Install Tassel5

LiveSeeding