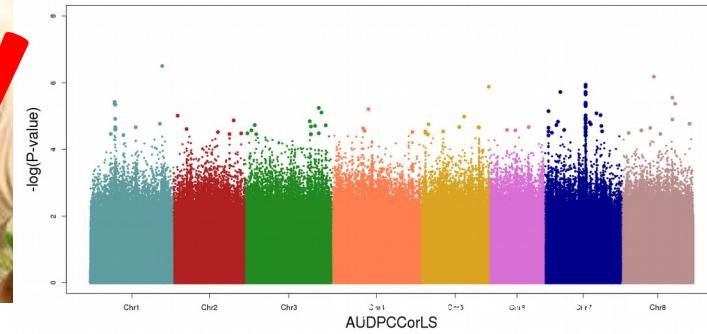
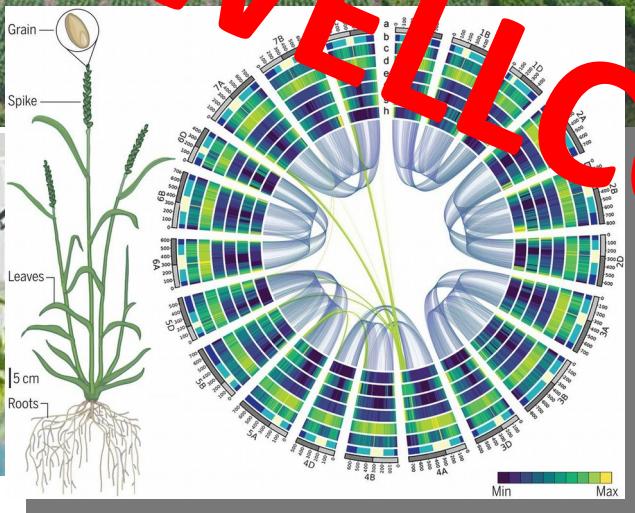
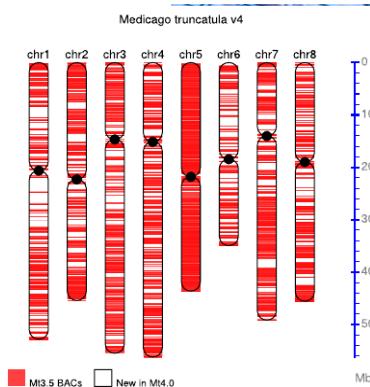
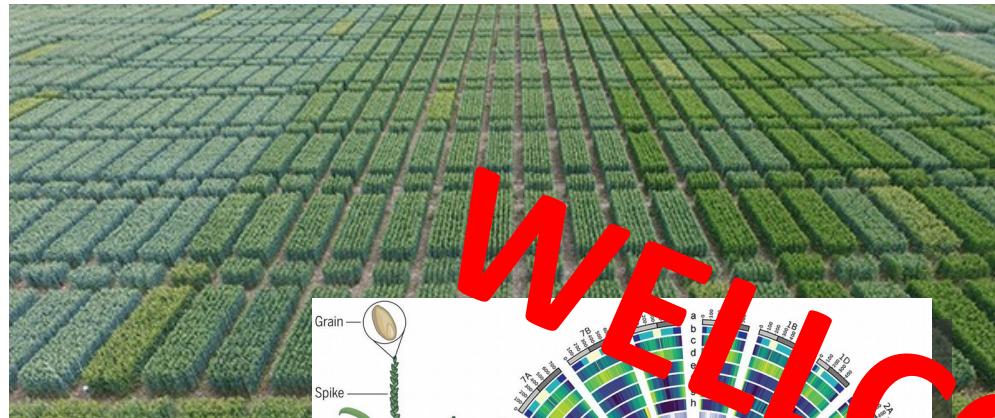


Scientific Training for Plant Biotechnology

Course in Modern Plant Breeding - Regular Level

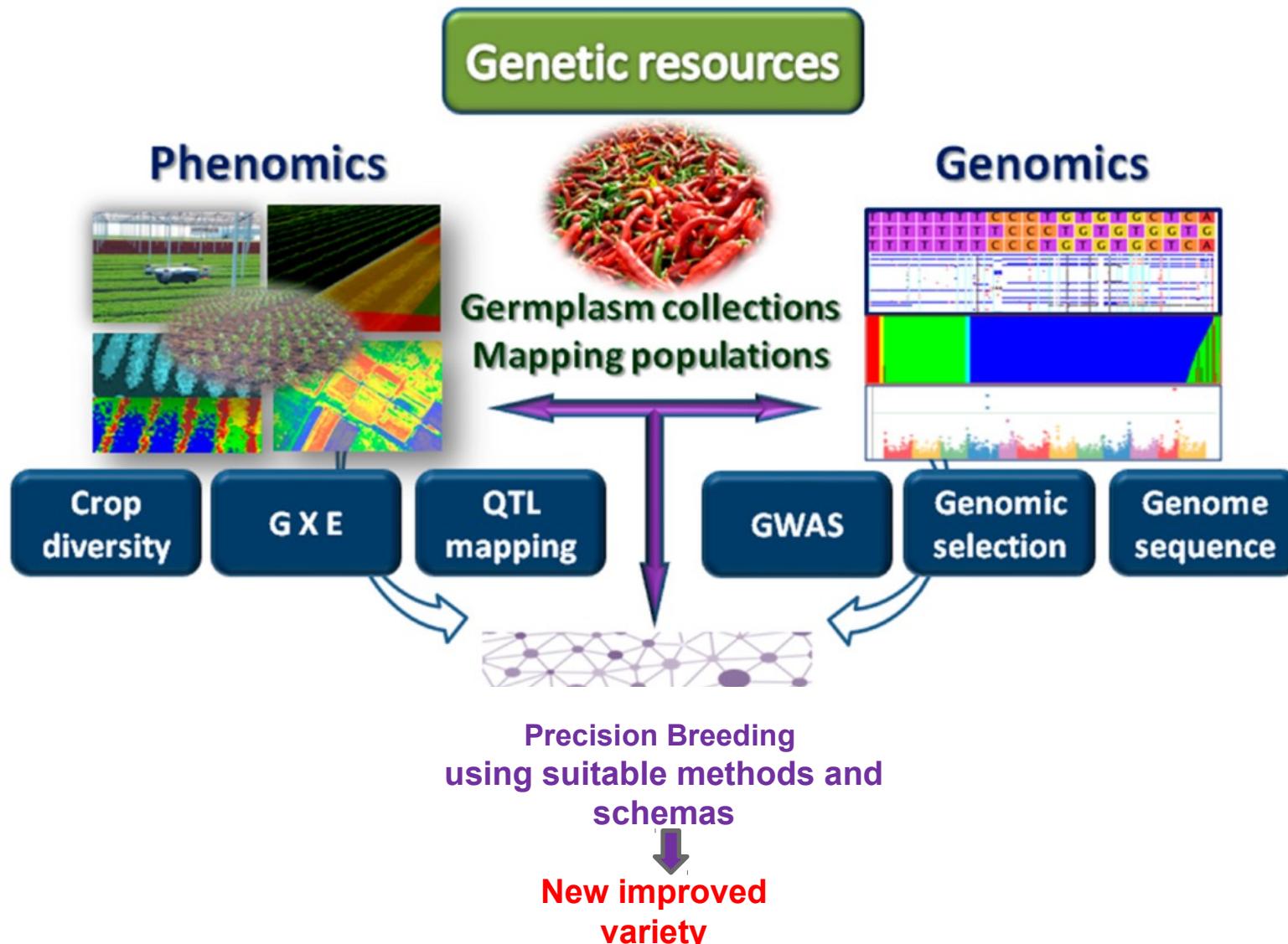
10-21 October 2022

WELCOME!



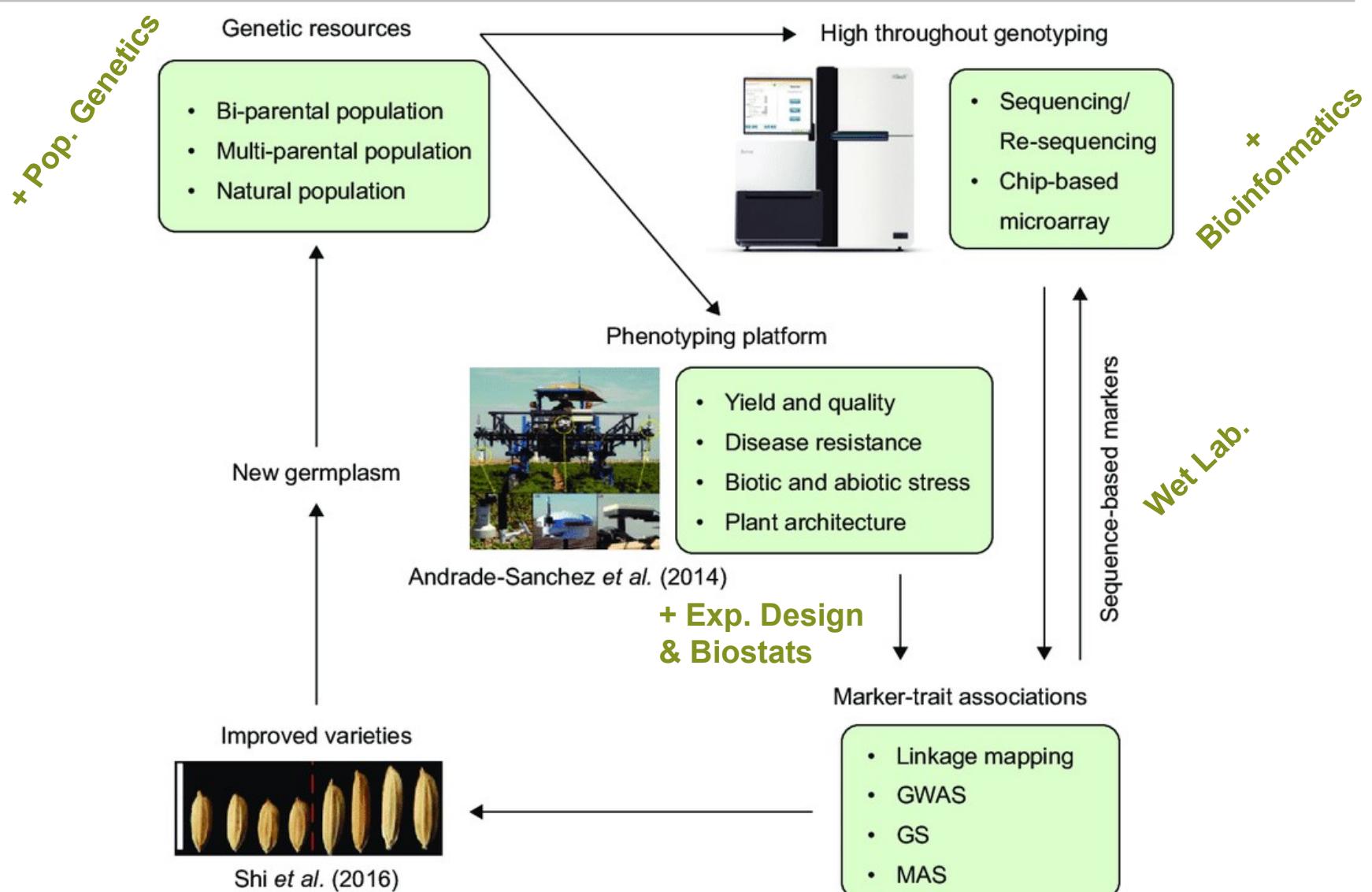
What will we talk about? What we learn in M.P.B. Regular course?

Part 1. Genome-assisted breeding. Integration of genomics and phenomics for the exploitation of genetic resources & development of molecular markers



What will we talk about? What will we learn?

Flowchart for genome-assisted breeding.



From: Leng et al. (2017). Genomics-assisted breeding – A revolutionary strategy for crop improvement. *Journal of Integrative Agriculture*. 16. 2674-2685. 10.1016/S2095-3119(17)61813-6.

GWAS, genome-wide association study; GS, genomic selection; MAS, marker-assisted selection.

What will we talk about? What we learn in M.P.B. Regular course?

How does genome-assisted breeding make a breeding program more efficient and profitable?

To use the form 2 of the breeder's equation:

$$\text{Genetic gain over time} \longrightarrow R_t = \frac{i \cdot r_A \cdot \sigma_A}{y}$$

Selection intensity Selection accuracy Genetic variance
Years per cycle

```
graph LR; A[Genetic gain over time] --> B[Rt = i * rA * sigmaA / y]; C[Selection intensity] --> D[i]; E[Selection accuracy] --> F[rA]; G[Genetic variance] --> H[sigmaA]; I[Years per cycle] --> J[y]
```

Selection intensity

- Increase (to a limit)
- Need bigger populations

Selection accuracy

- Increase
- Reduce errors
- More precise measurements
- **Correct for environment**

Genetic variance (diversity)

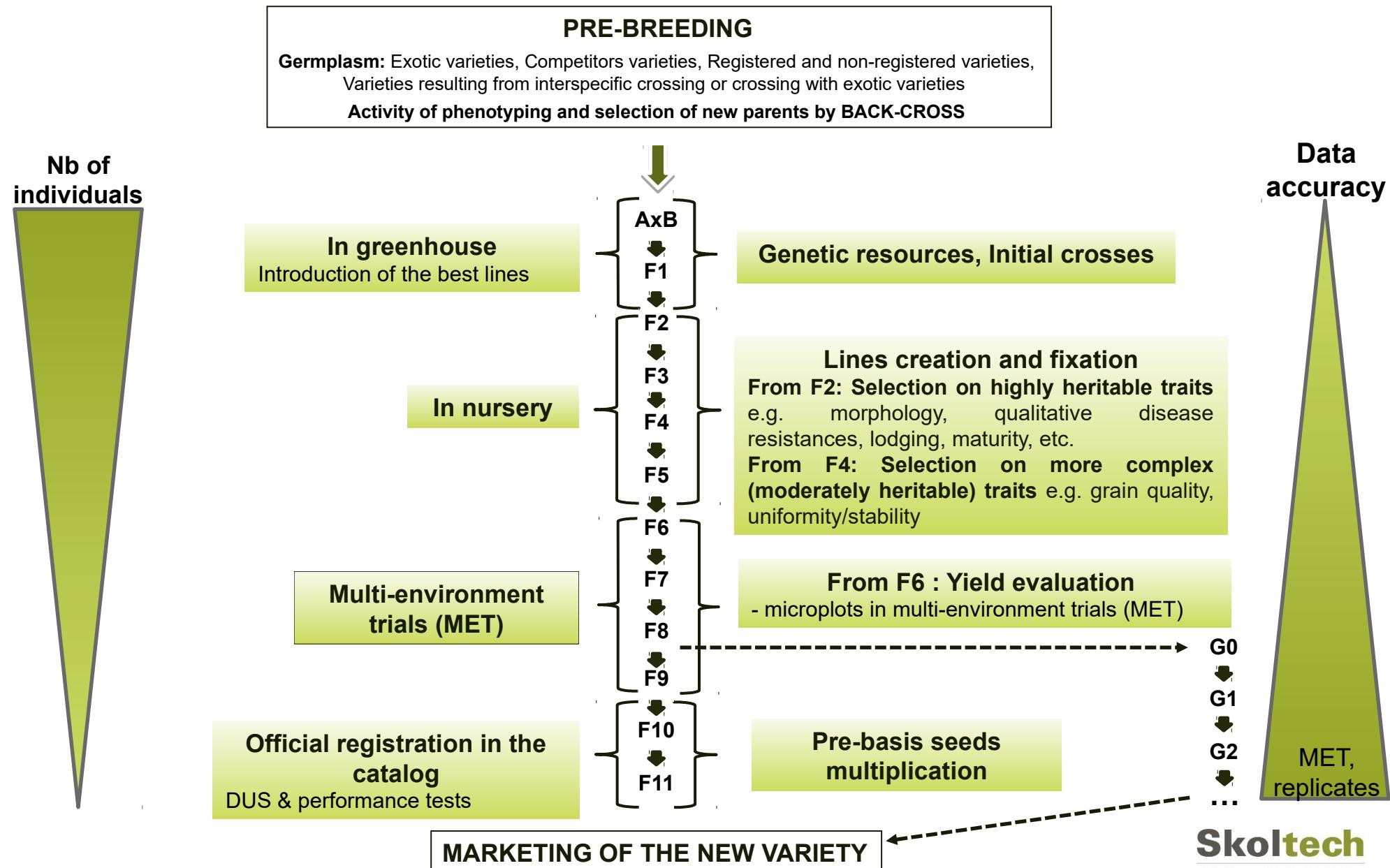
- Increase
- A must have

Year per cycle

- Decrease !!
- Constant 'rate' of return

What will we talk about? What we learn in M.P.B. Regular course?

How does genome-assisted breeding make a breeding program more efficient and profitable?



What will we talk about? What we learn in M.P.B. Regular course?

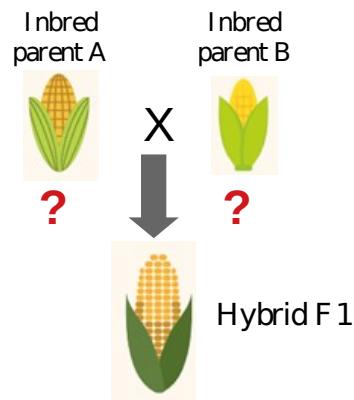
Part 2. How to optimize hybridization in plant breeding? A practical approach

1. How to choose the best parental lines to initiate the breeding program ?

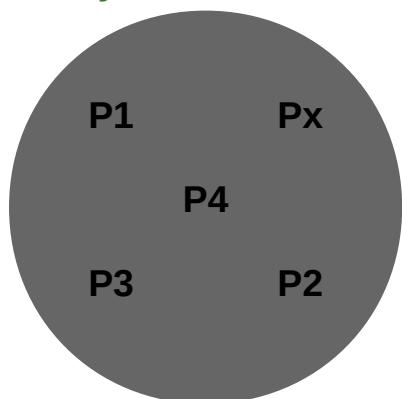
Evaluation of genetic values using well-suited mating designs.

Diallel

	P1	P2	...	Px
P1	P1x P1	P1x P2	P1x ...	P1x Px
P2	P2x P1	P2x P2	P2x ...	P2x Px
...
Px	Px x P1	Px x P2	Px x ...	Px x Px



PolyCross



Line X Tester

	T1	T2
P1	P1x T1	P1x T2
P2	P2x T1	P2x T2
...
Px	Px x T1	Px x T2

What will we talk about? What we learn in M.P.B. Regular course?

Part 2. How to optimize hybridization in plant breeding? A practical approach

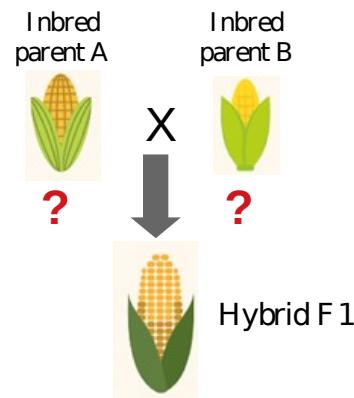
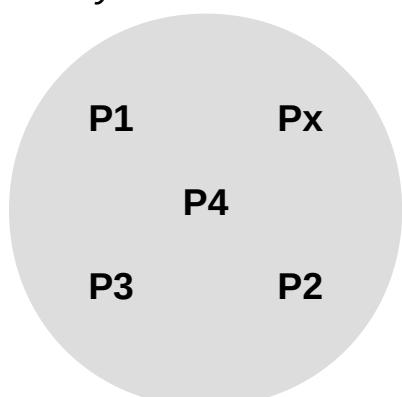
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...
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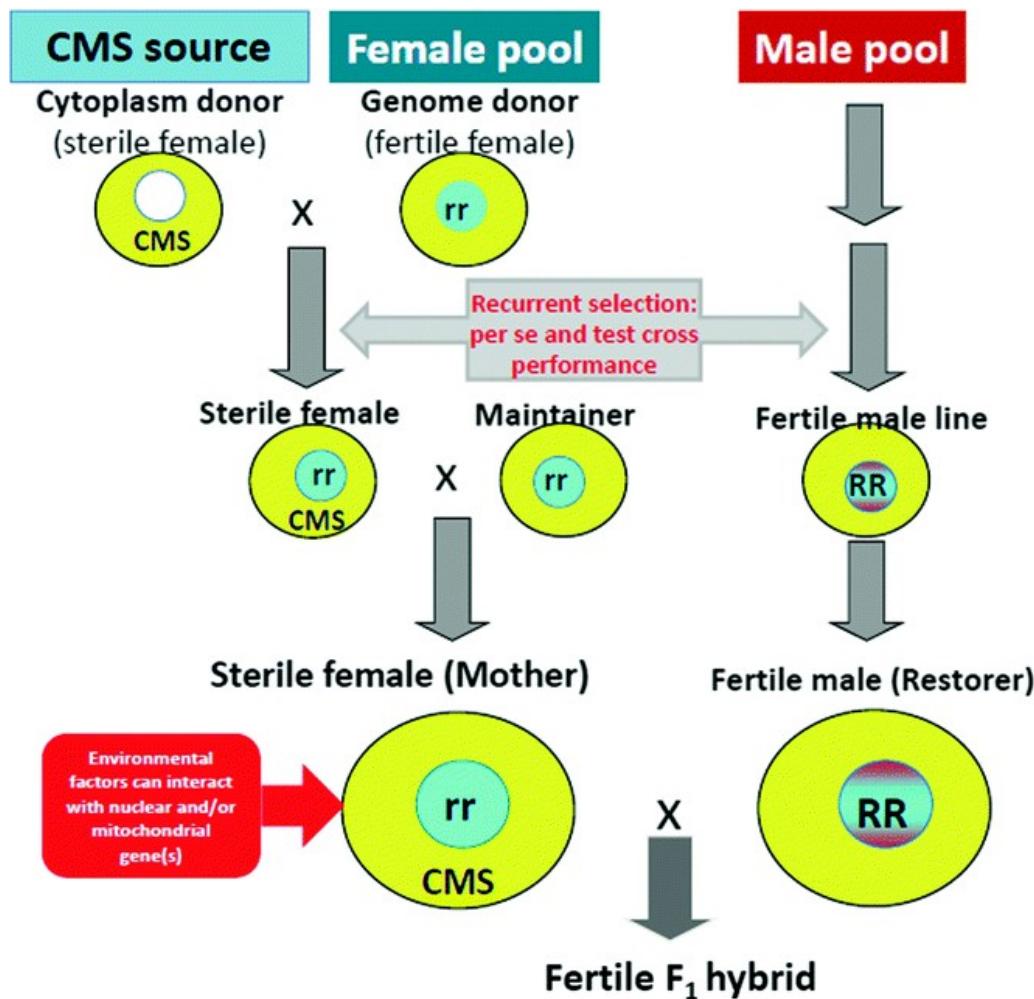
PolyCross



Line X Tester

	T1	T2
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P2	P2x T1	P2x T2
...
Px	Px x T1	Px x T2

2. Efficient use of (nucleo-cytoplasmic) male sterility (CMS)



Part 1. Genome-assisted breeding.

A brief introduction and guideline for the course

Genetics of Quantitative Resistance against *Verticillium* wilt in *Medicago truncatula*

As an example

Laboratory of Functional Ecology and Environment
ECOLAB, CNRS/INP-ENSAT/UPS



Legumes (*Fabaceae*)



- Protein-rich nutrition for humans and animals
- Valuable forage crops
- Green manuring crops
- Symbiosis with nitrogen-fixing rhizobia

Soybean in Russia (based on Rosstat data 2018)

Sown areas in 2018 : 2.919 thousand ha.

- Over the year they increased by 10.7%
- Over 5 years - by 90%,
- Over 10 years - by 290%.



<http://agrotime.info/?p=18970>



Legumes (*Fabaceae*)



- Protein-rich nutrition for humans and animals
- Valuable forage crops
- Green manuring crops
- Symbiosis with nitrogen-fixing rhizobia

- **Plant diseases contribute 10–16% losses in global harvests each year, costing an estimated US\$ 220 billion.** *Balasundram et al. 2020*
- **Root diseases are among the most devastating diseases worldwide** due to:
 - ✓ Broad range of host plants
 - ✓ Difficulty to control (persistence of survival structures, inefficiency of chemical controls)

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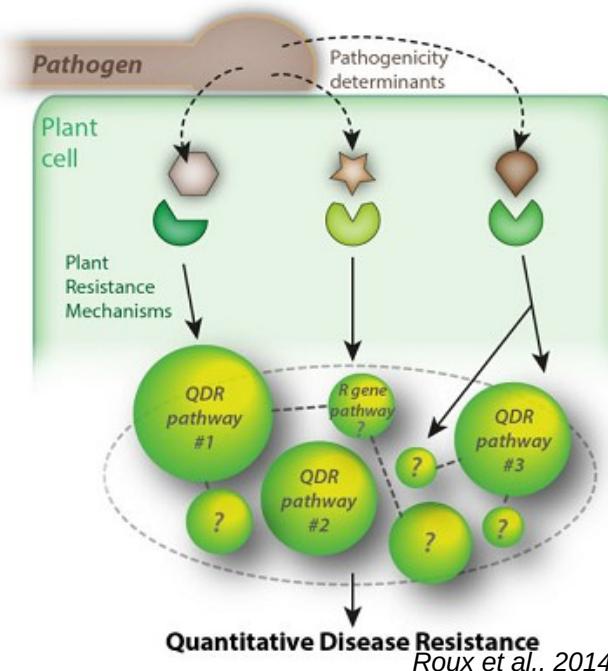
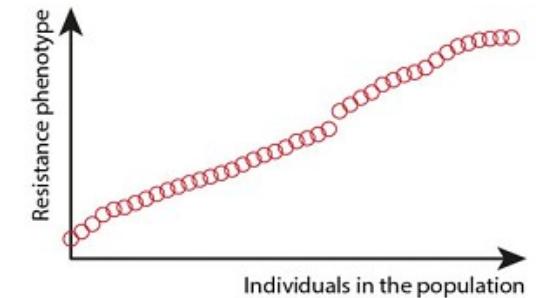
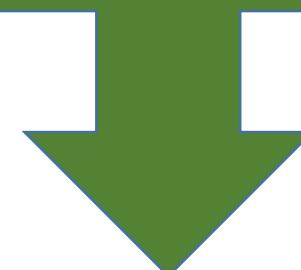
<http://agritime.info/?p=18970>



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Quantitative Disease Resistances 'QDR'

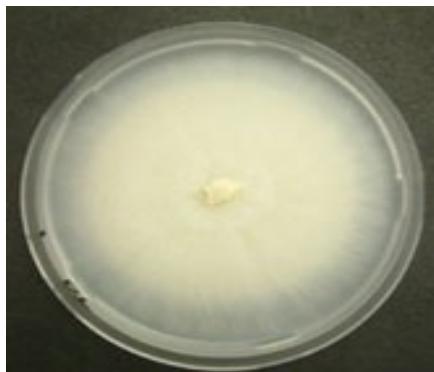
Partial resistance
Polygenic control
Environmental effect
Broad spectrum
Sustainability



Breeding for root QDR in legumes is of great agricultural importance and major aim to secure food/feed production

Response to Verticillium wilt in *Medicago truncatula* is a QDR

- Soil-borne pathogens: Threats difficult to control
- Wilt diseases in more than 300 plant species



Verticillium alfalfae



Verticillium wilt in Medicago truncatula



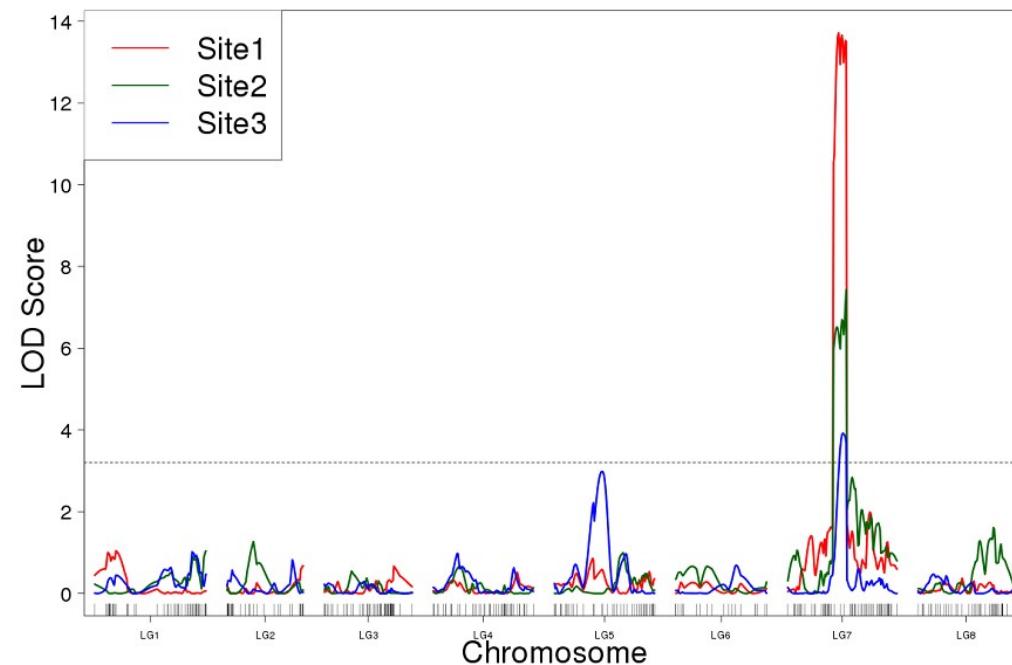
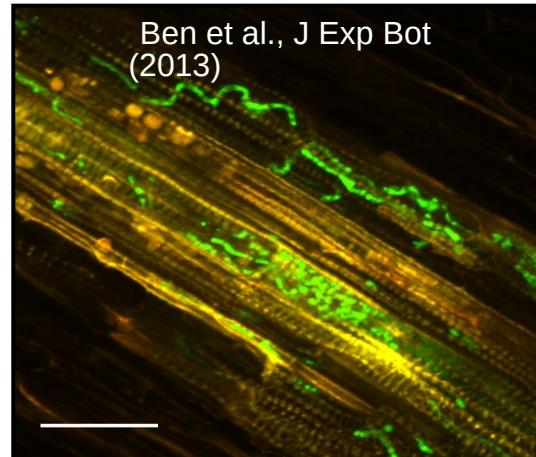
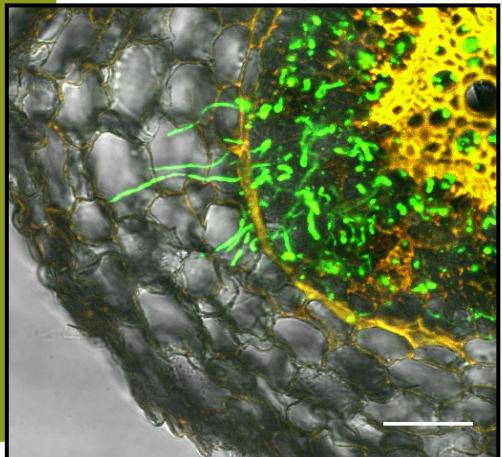
Verticillium wilt in alfalfa



Olea europaea



Solanum lycopersicum

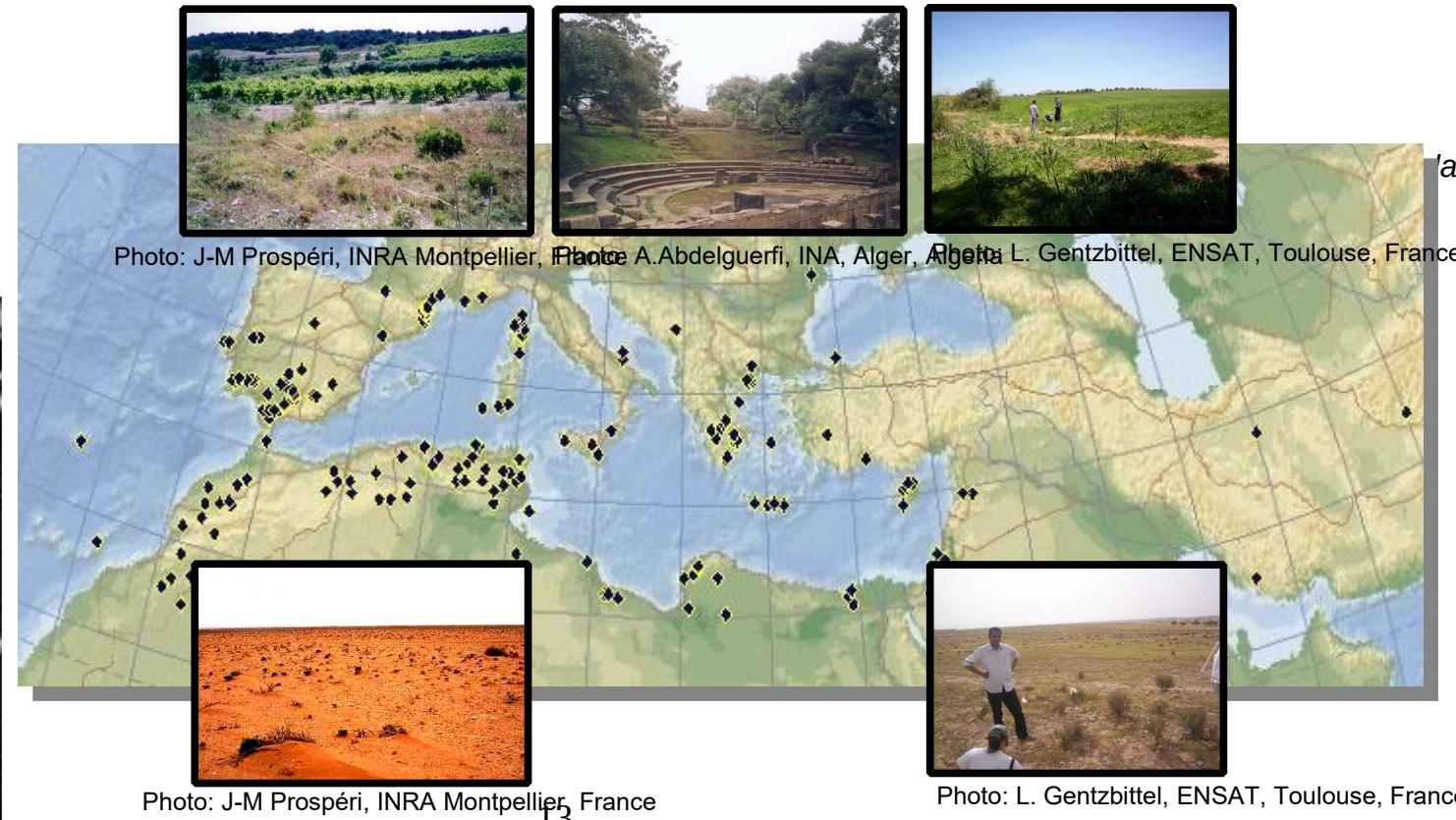
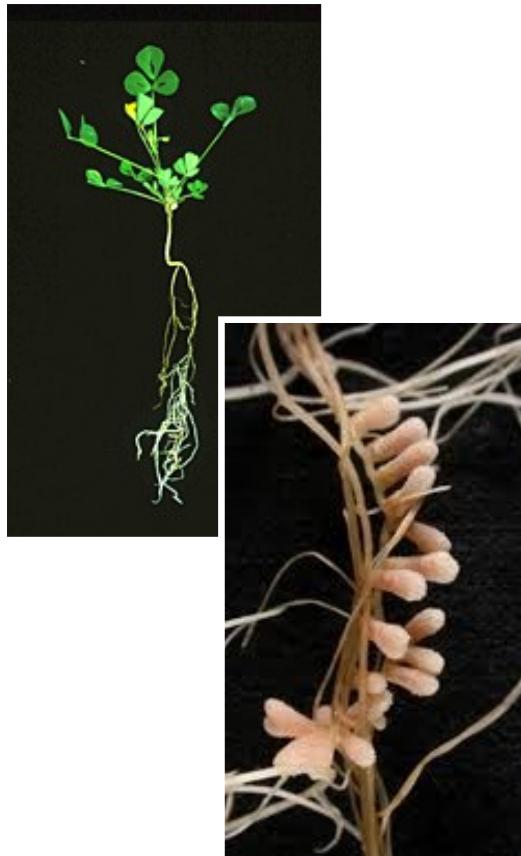




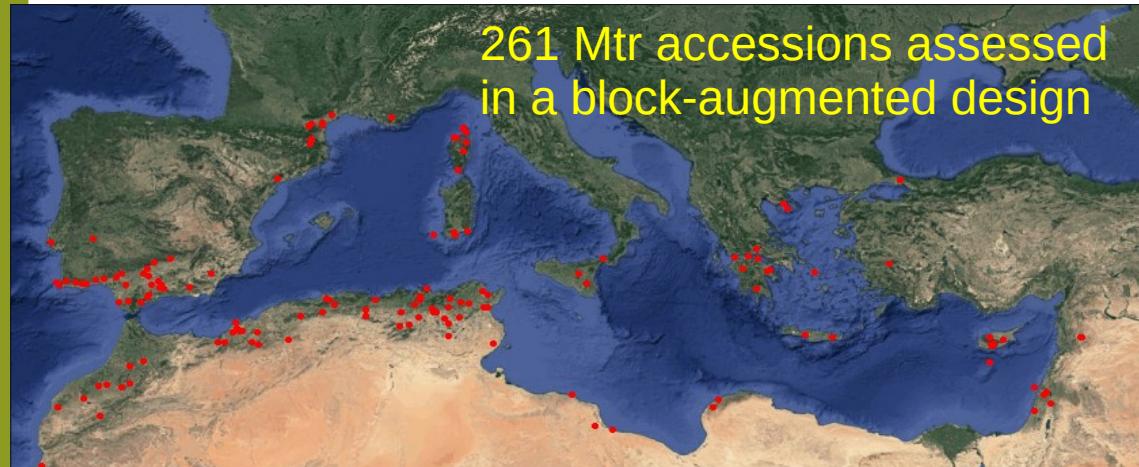
M. truncatula : a model legume plant



- Short life cycle, self fertile
 - Small diploid ($2n=16$) genome, accession A17 sequenced (450Mb, V4.0, Tang *et al.*, 2014)
 - Large biodiversity, SNPs on 288 accessions of *Medicago* genus (>16.5 Millions SNPs, *Medicago* HapMap, <http://www.medicagohapmap.org>)
 - Genetic & Genomic resources (mutants, RIL populations, genetic map)
 - Biotechnology tools (genetic transformation)

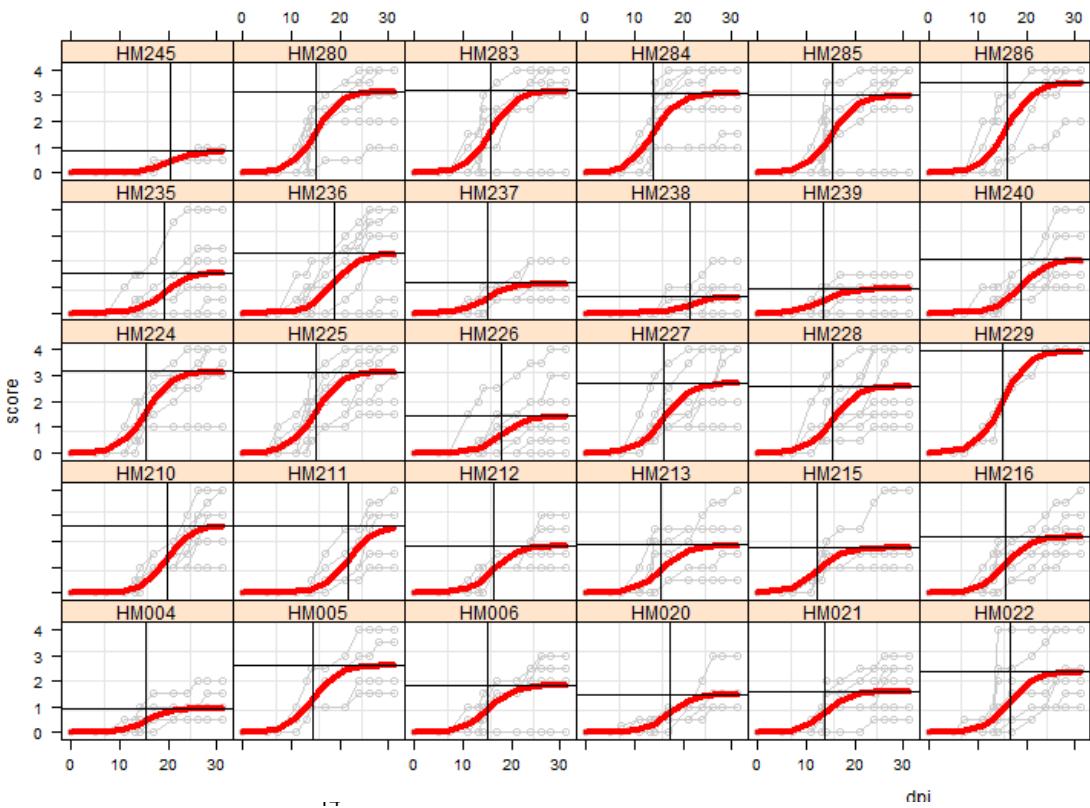


Disease functional parameters varied largely among *M. truncatula* ecotypes

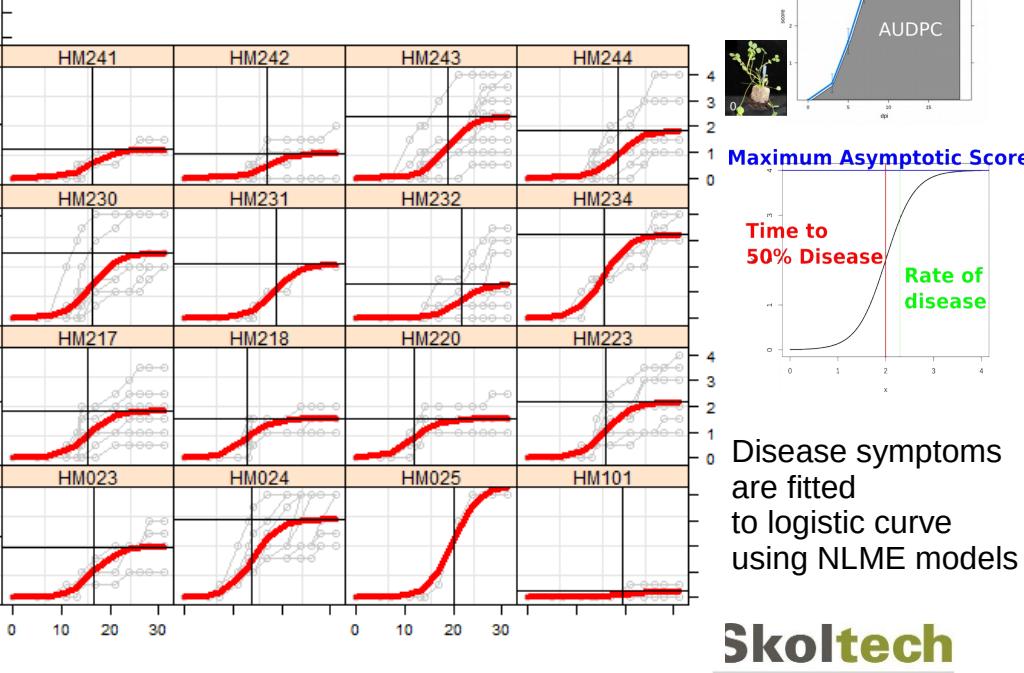


V. alfalfae symptoms on susceptible *M. truncatula*

Ben et al. 2013



Disease progress curves in a subset of 46 accessions



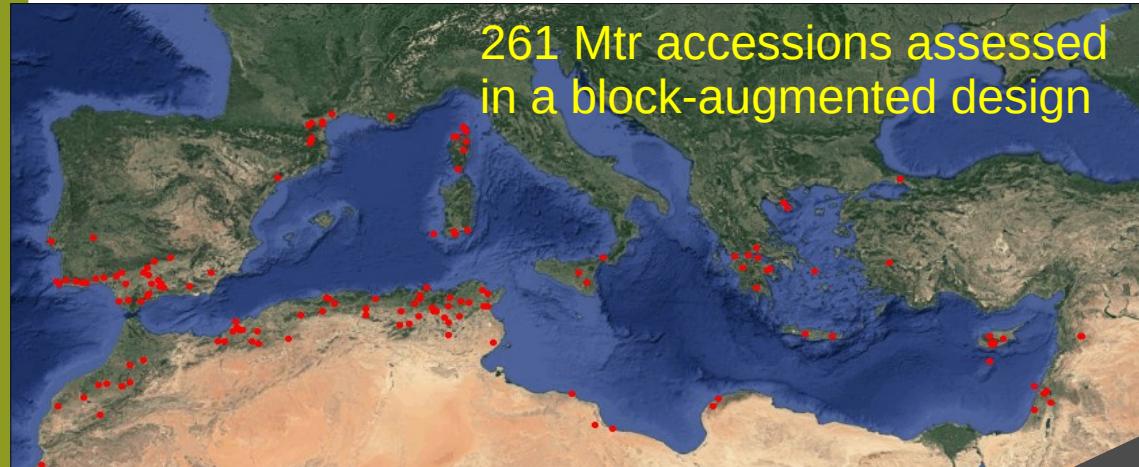
Inoculation with *Vaa* V31-2, three biological replicates

Skoltech

Skolkovo Institute of Science and Technology

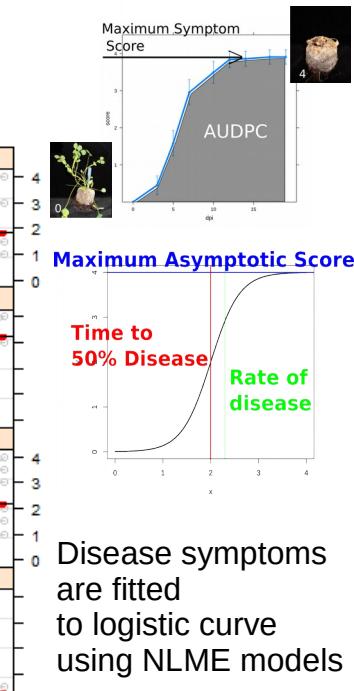
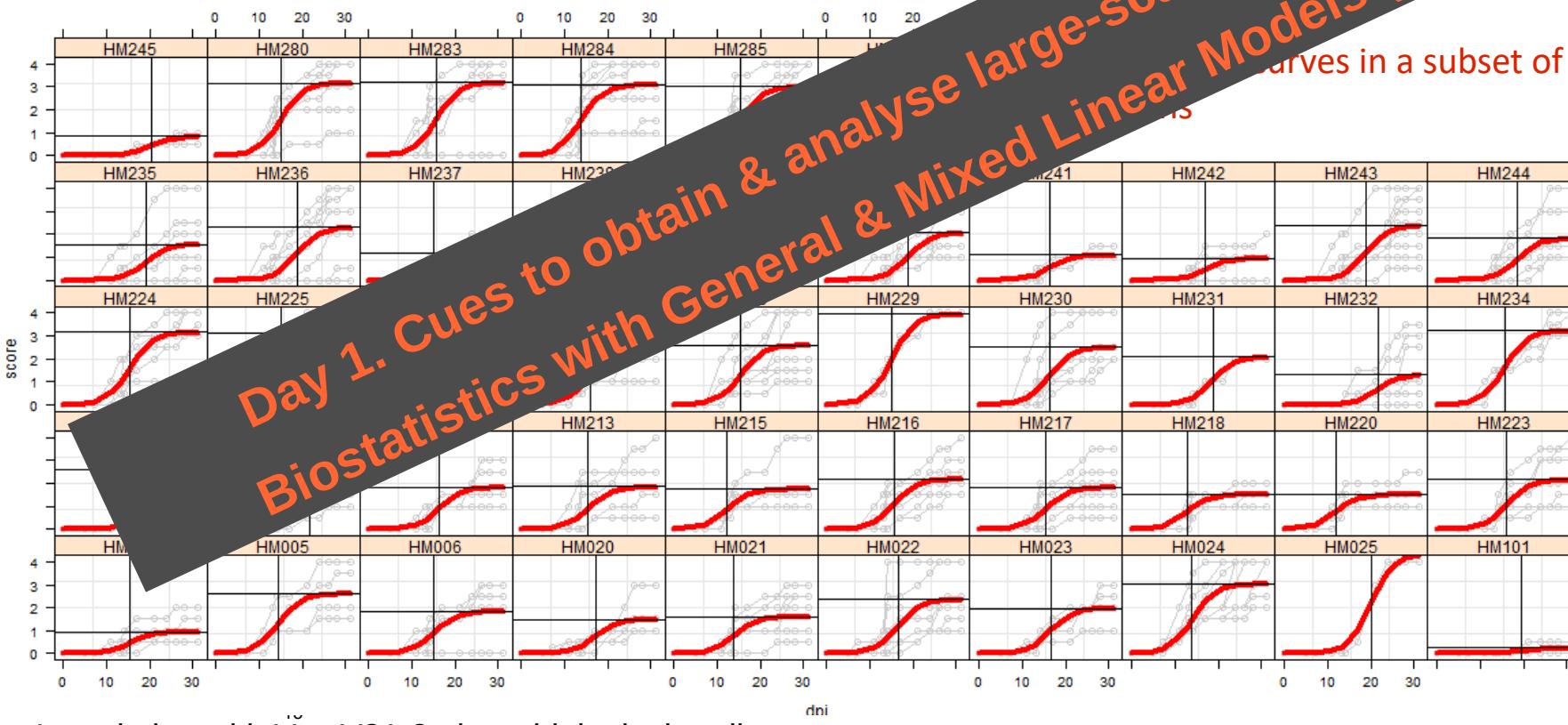
Mazurier et al., in preparation

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V. faba
M. truncatula

Ben et al. 2013



Skoltech

Skolkovo Institute of Science and Technology

Mazurier et al., in preparation

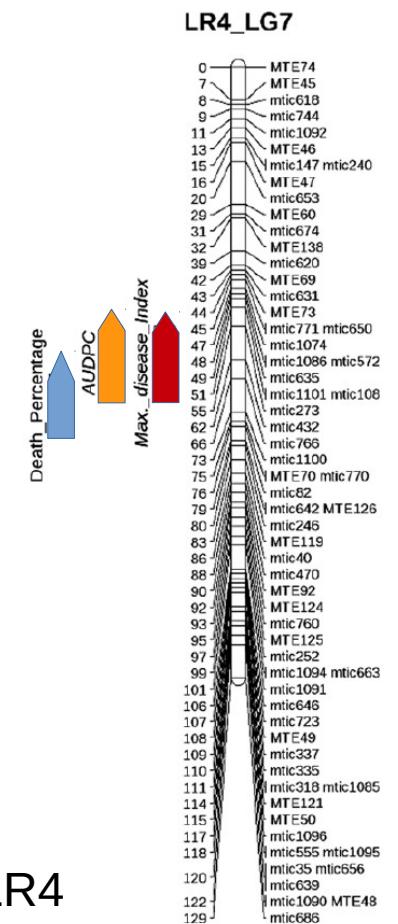
Genetic control of *Verticillium* resistance in *M. truncatula* : QTL analysis on RIL populations

<i>Verticillium</i> species	Population	Genomic loci for resistance
<i>Verticillium alfalfa</i> V31.2	LR3 F83005.5 [S]xDZA45.5 [R]	Chromosomes 2 et 6
<i>Verticillium alfalfa</i> V31.2	LR4 A17[R] x DZA315.16 [S]	Chromosome 7
<i>Verticillium alfalfa</i> V31.2	LR5 A17[R] x F83005.5 [S]	Chromosome 7
<i>Verticillium nonalfalfa</i> LPP0323	LR5 A17[S] x F83005.5 [R]	Chromosomes 1,2, 6 et 8

Genomic loci for resistance are dependent on *Verticillium* species and on plant parental accessions, suggesting different mechanisms.

- * Are there other sources of resistance in *M. truncatula* natural biodiversity?
- * Candidate genes for resistance towards *Verticillium*?

A17 (R) × DZA315.16 (S)

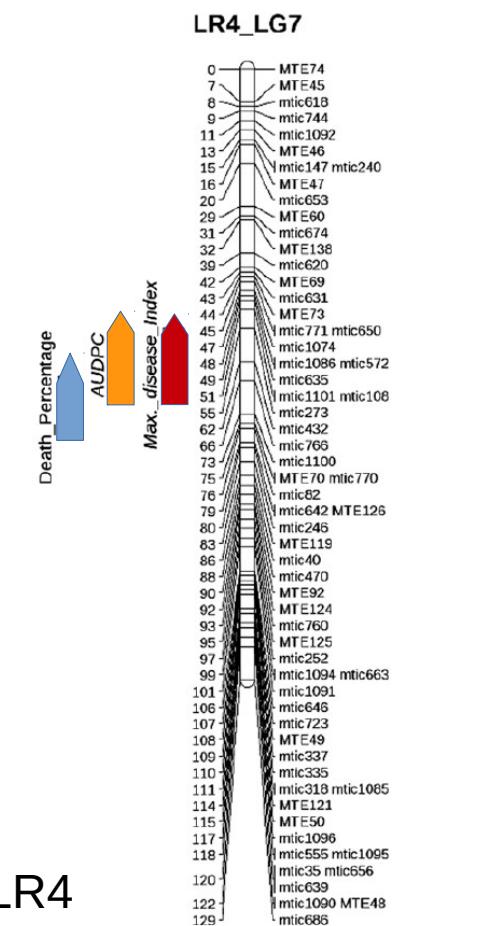


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QTLs for resistance are dependent on *Verticillium* species and on plant parental accessions, suggesting different mechanisms.

Day 2 & 3. Linkage mapping & QTL detection in biparental populations

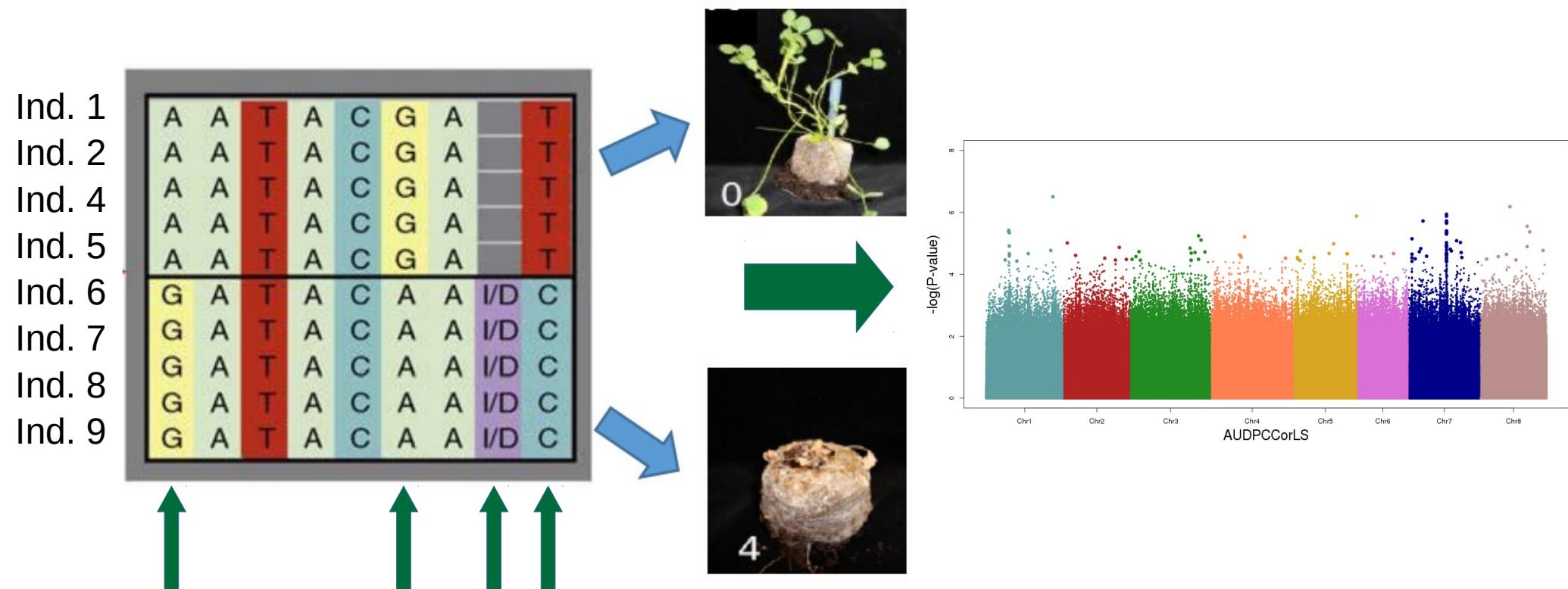


- * Are there other sources of resistance in *M. truncatula* natural biodiversity?
- * Candidate genes for resistance towards *Verticillium*?



Genome-Wide Association Study -GWAS - to get a broad overview of QTLs for resistance to Verticillium in *M. truncatula* species

GWAS :
Scanning the genomic natural biodiversity of a species
to find SNPs associated with a phenotype



Example of GWAS, adapted from Rafalsky et al., 2010

Identification of SNPs within a large germplasm collection

Medicago truncatula

HAPMAP PROJECT

Home Hapmap Tools Downloads Resources Contact

Current state :
288 resequenced accessions
Total : 16.516.721 SNPs

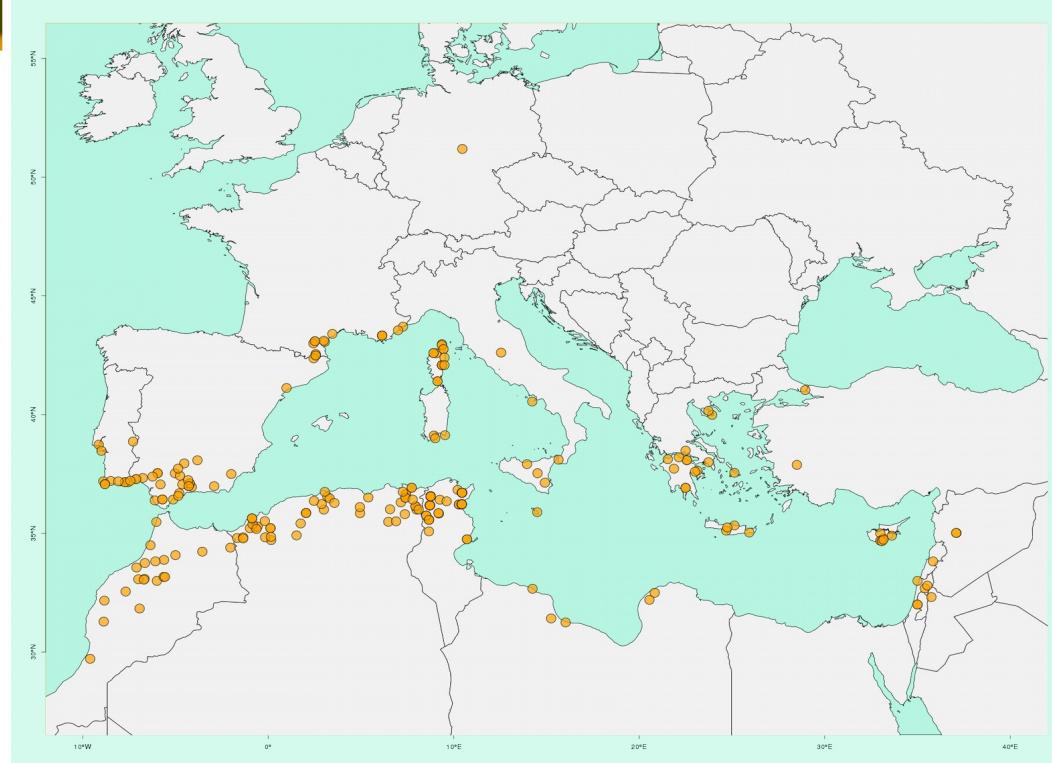


Medicago Hapmap

We are building a hapmap based on short-read sequencing of approximately 330 inbred *Medicago truncatula* accessions. This provides a foundation for discovering single nucleotide polymorphisms (SNPs), insertions/ deletions (INDELs) and copy number variants (CNV's) at very high resolution among the *Medicago* lines. The resulting database of sequence variants establishes a basis for describing population structure and identifying genome segments with shared ancestry (haplotypes) - and thereby creates a long-term, community resource for genome-wide association studies.

About this Project

We are developing a *Medicago* Hapmap as part of an international consortium consisting of the University of Minnesota, the National Center for Genome Resources (NCGR), Boyce Thompson Institute (BTI), J. Craig Venter Institute (JCVI) Hamline University, the University of Southern California, INRA-Montpellier, ENSAT-Toulouse, and the Noble Foundation.



Identification of SNPs within a large germplasm collection

Medicago truncatula
HAPMAP PROJECT

Current state :
288 resequenced accessions
Total : 16.516.721 SNPs

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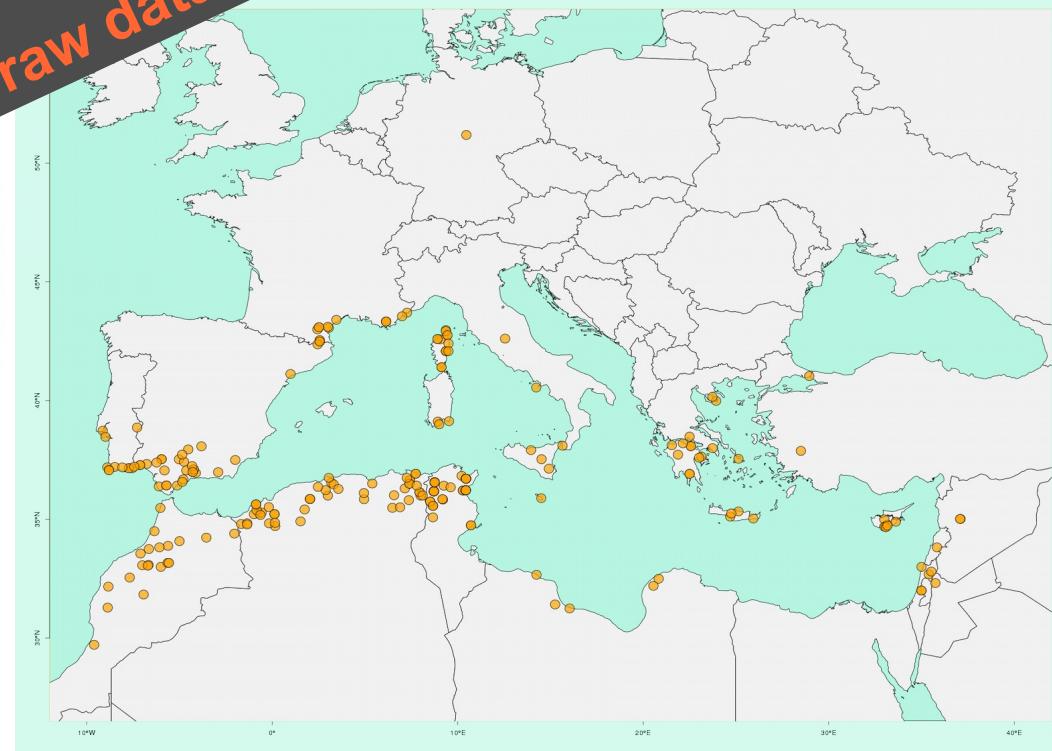


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We are building a hapmap for *Medicago truncatula*, approximately 330 individuals. This provides a foundation for studying nucleotide polymorphisms (SNPs) and copy number variations (CNVs) among the *Medicago* accessions. Sequence variants will be used to study population structure and shared ancestry (haplotypes) - a long-term, community resource for studies.

About this Project

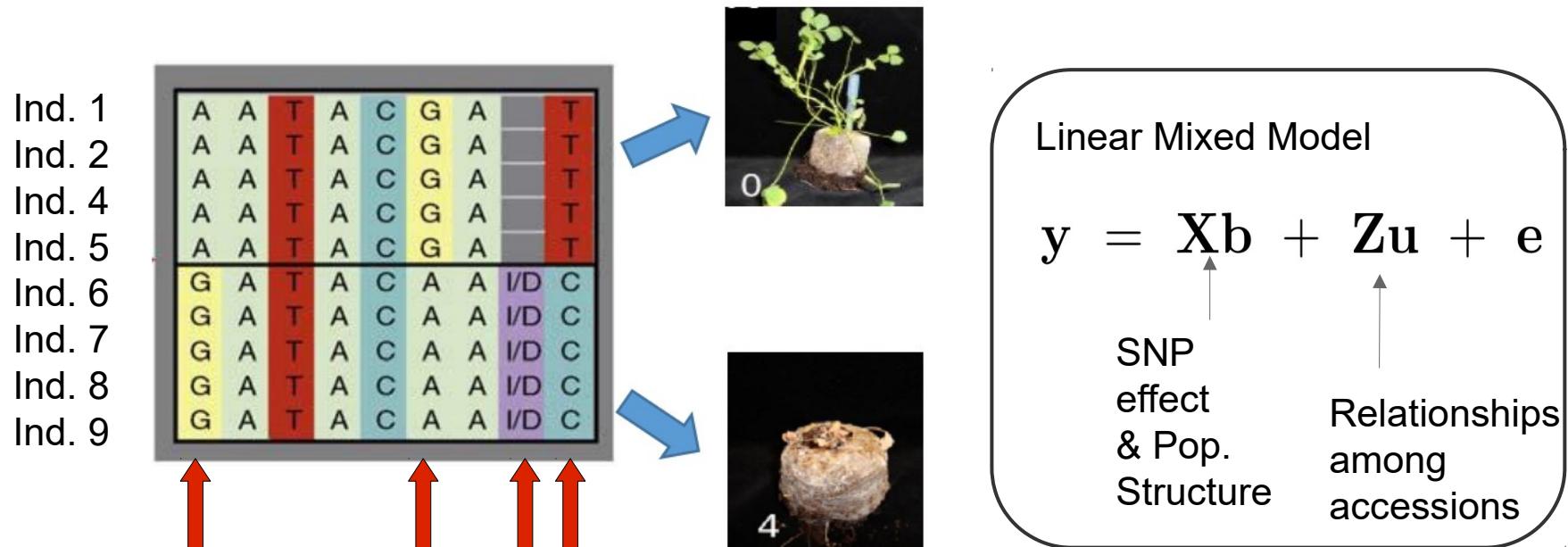
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Day 4. Focus on some useful bioinformatics pipelines to deal with large-scale SNP raw datasets

Statistical models for GWAS can take into account population structure and relatedness among accessions

GWAS :
Scanning the genomic natural biodiversity of a species
to find SNPs associated with a phenotype



adapted from Rafalsky et
al., 2010

Previous studies on population structure of *M. truncatula* species

C Ronfort et al, 2006

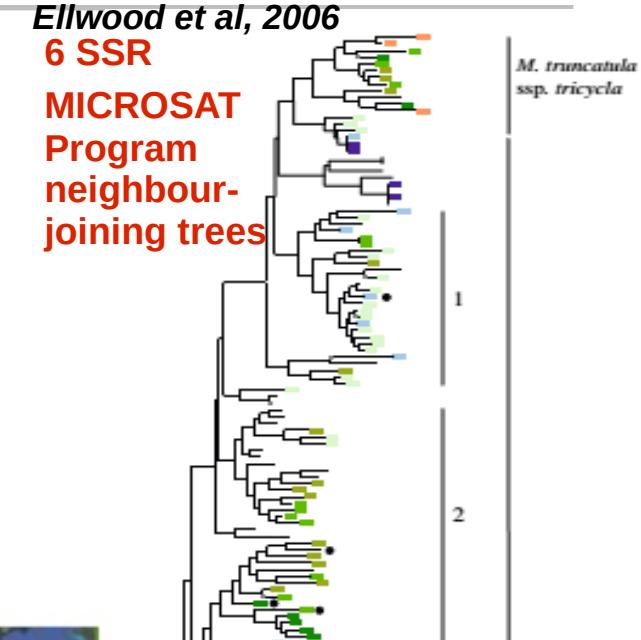
13 SSR,
STRUCTURE Software



Ellwood et al, 2006

6 SSR

MICROSAT
Program
neighbour-
joining trees



Bonhomme et al, 2014

>35,000 SNP (HAPMAP)

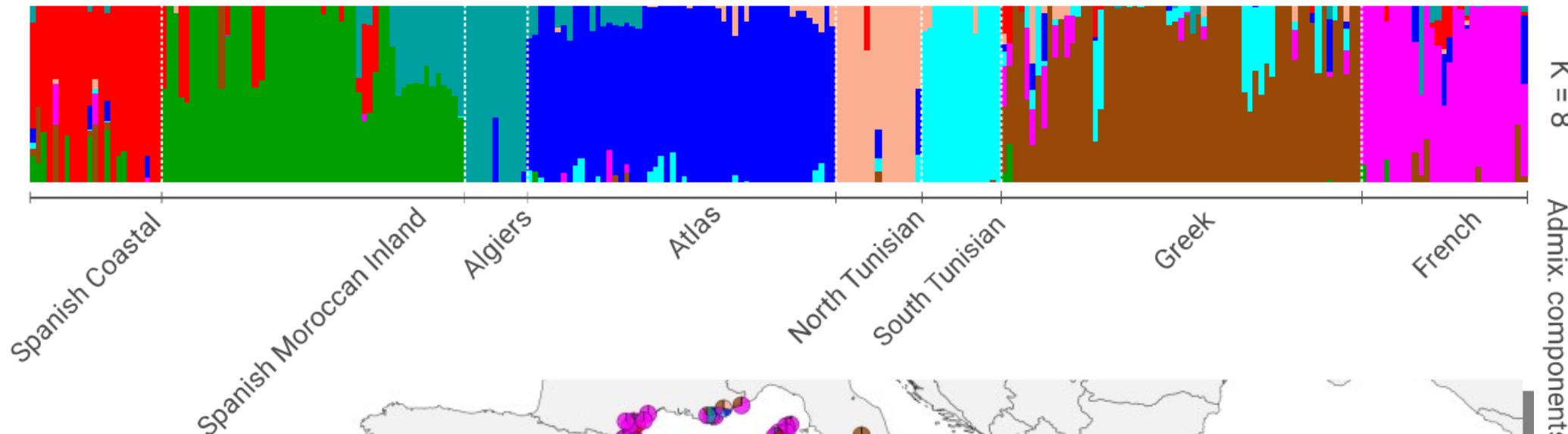
STRUCTURE Software &
DAPC



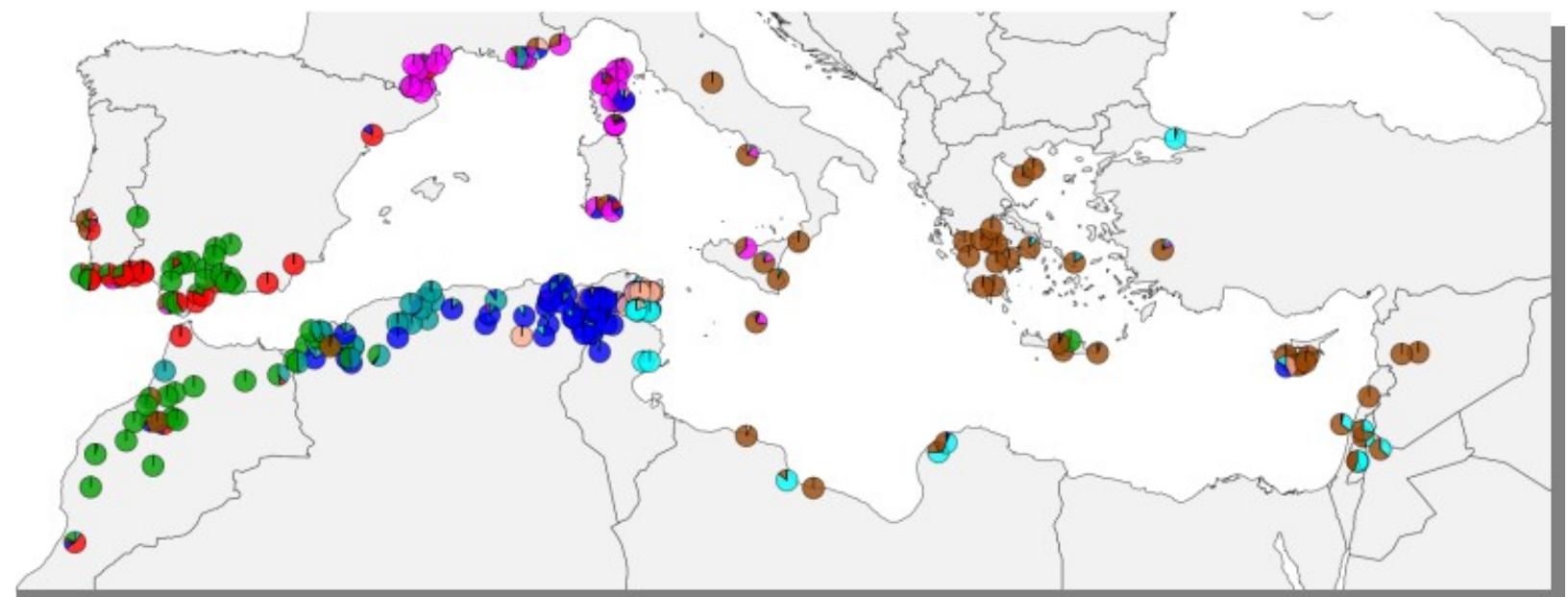
M. truncatula
ssp. *truncatula*

- Libya
- Tunisia
- Algeria
- Morocco
- Europe (except Balkans)
- Greece
- Iberia
- Cyprus
- Near East

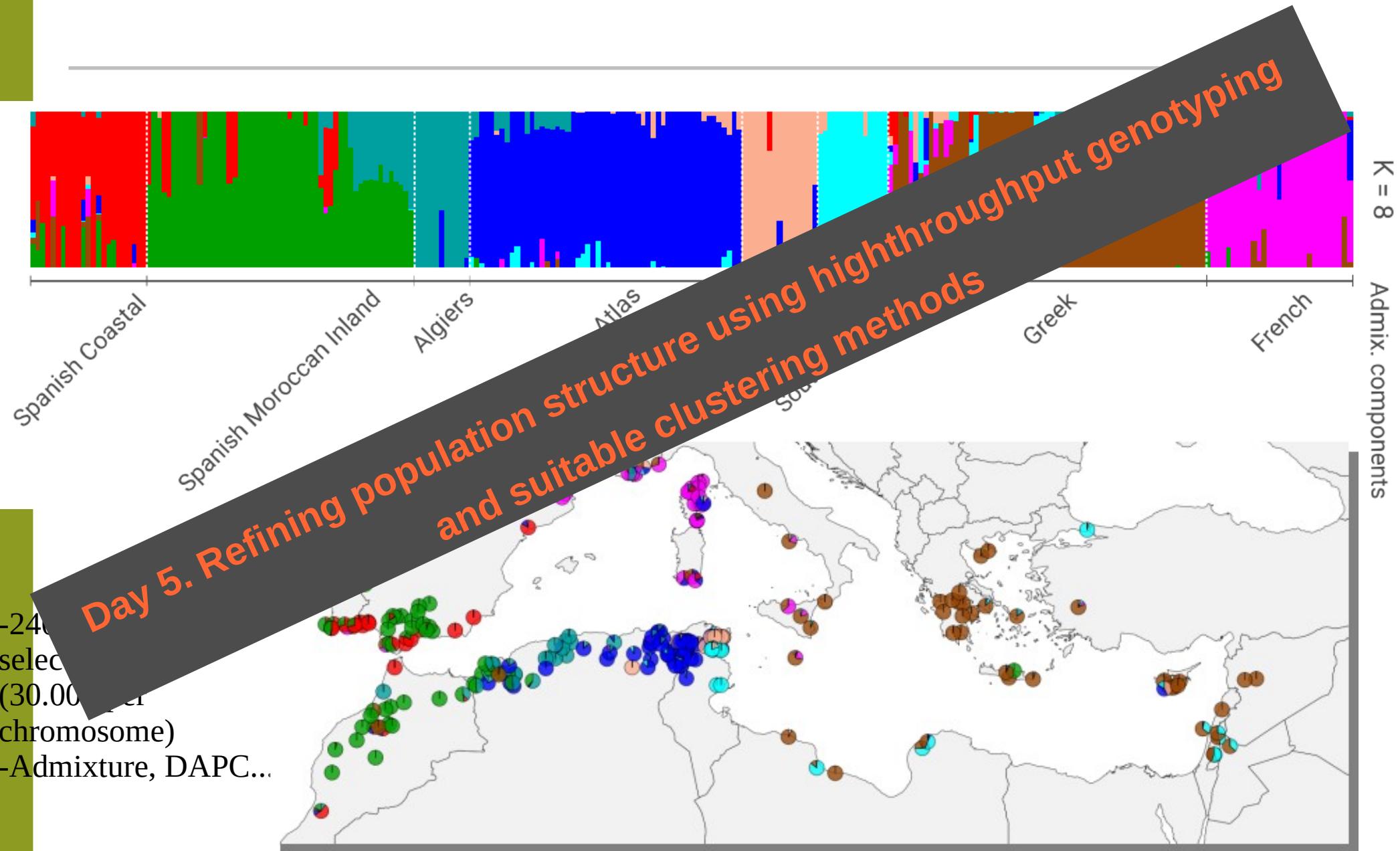
A refined population structure for *M. truncatula* species



-240.000 randomly selected SNPs
(30.000 per chromosome)
-Admixture, DAPC.

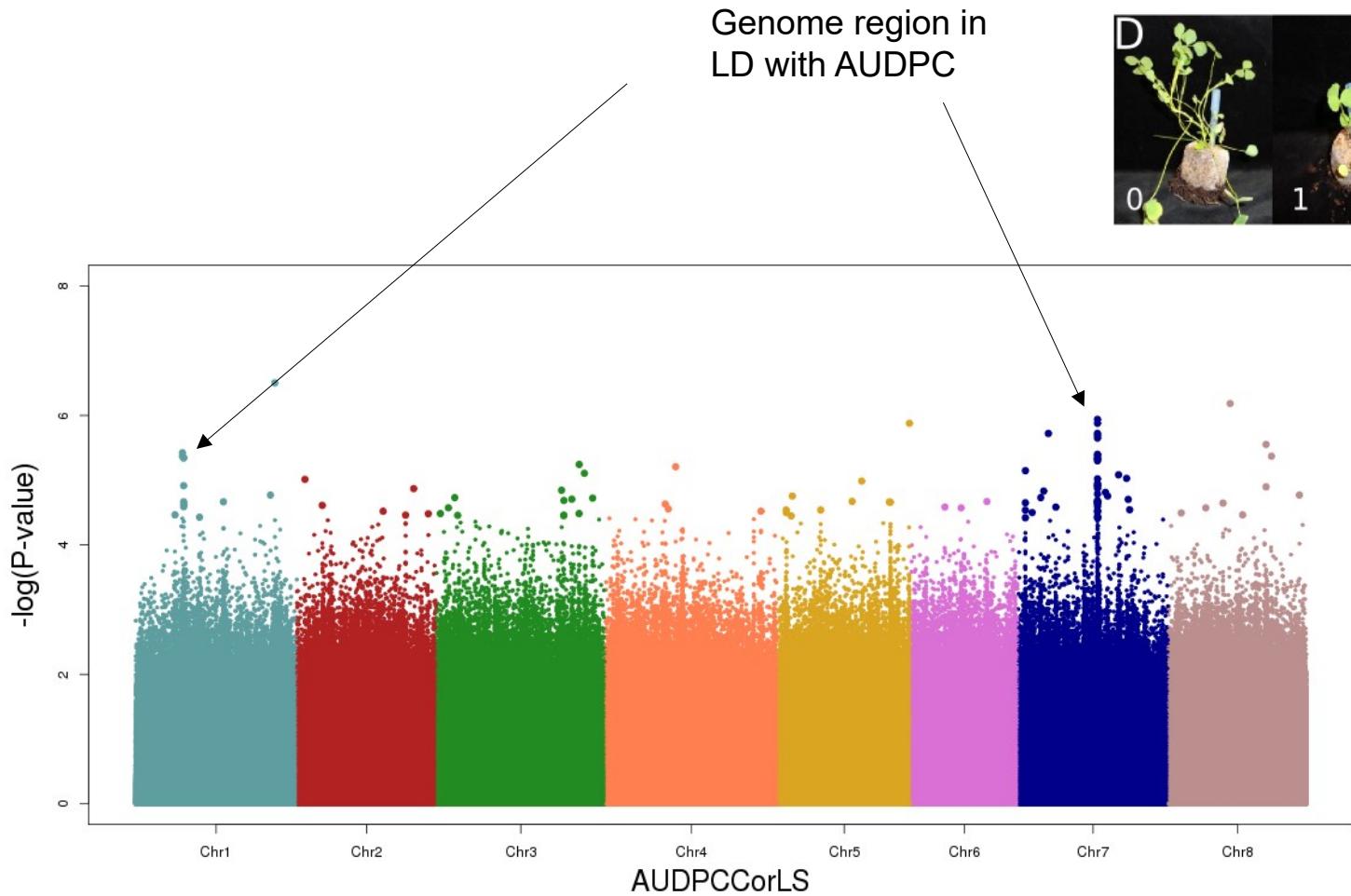


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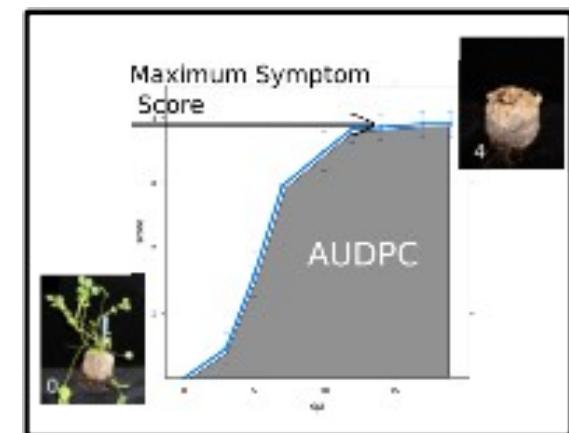




GWAS for Quantitative Resistance to Verticillium wilt – Ex. AUDPC disease parameter

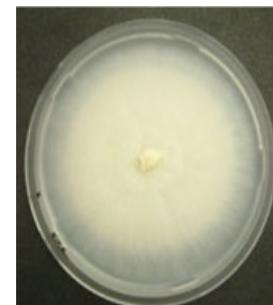


V. alfalfae symptoms on susceptible *M. truncatula*



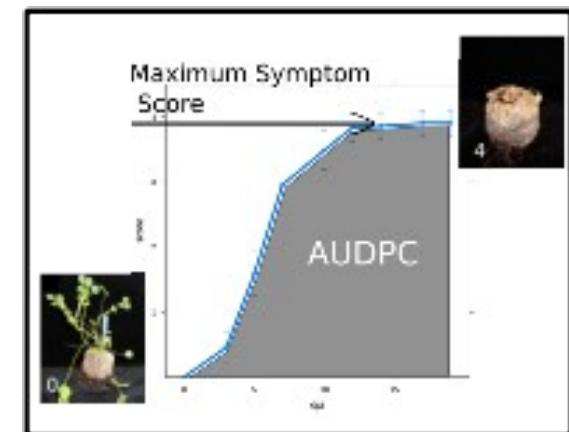
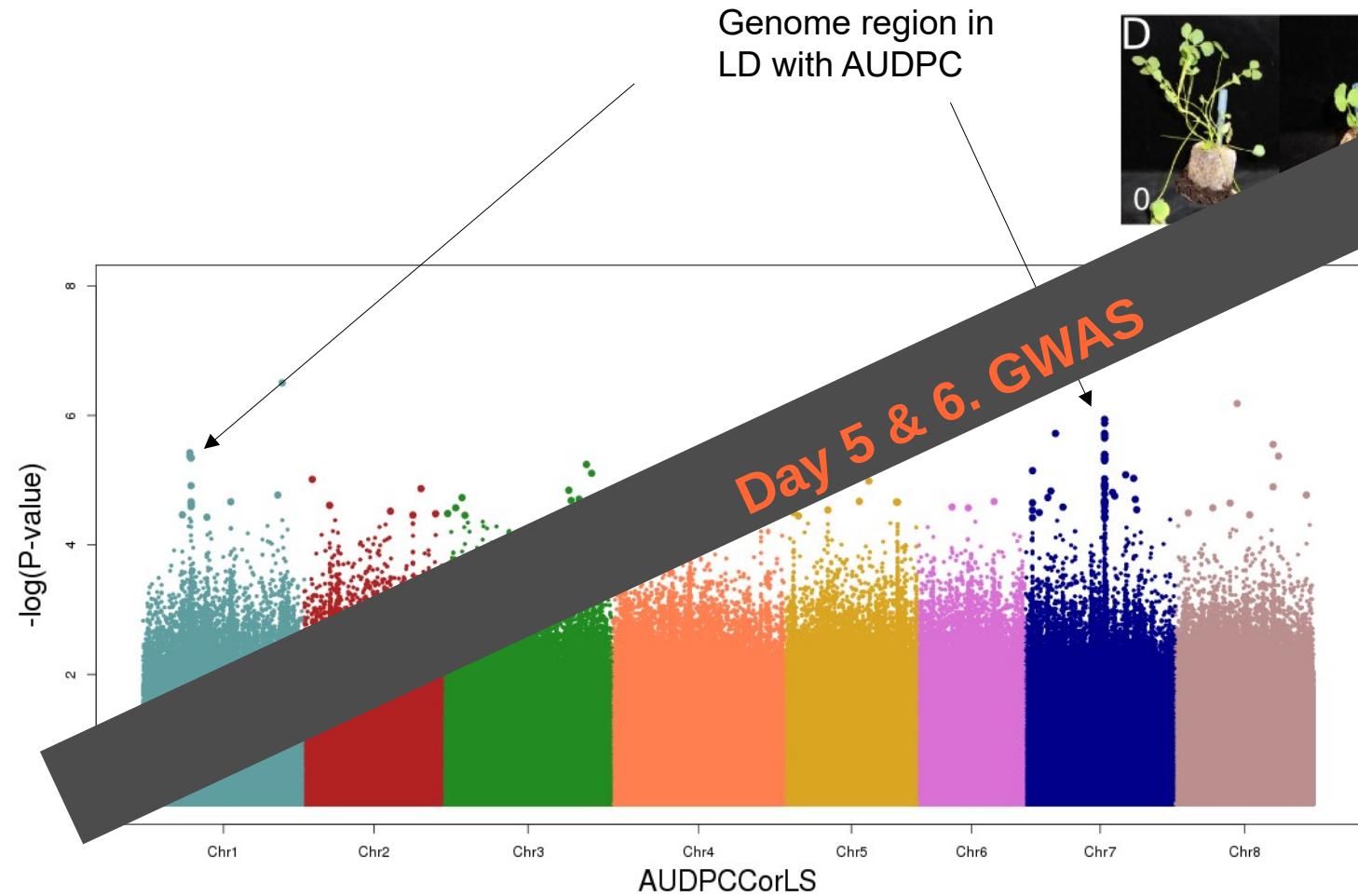
AUDPC : Area Under the Disease progression Curve

- 261 Mtr accessions ; c.a. 5.6 millions SNPs
- 3 independent replications, 10 plants/assay, time-course individual scoring
- 5 different phenotypes : AUDPC, three functional disease parameters, Re-isolation
- EMMAX with K matrix & Q matrix with height admixture components (TASSEL)



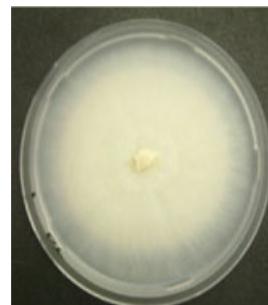
Verticillium alfalfae

GWAS for Quantitative Resistance to Verticillium wilt – Ex. AUDPC disease parameter



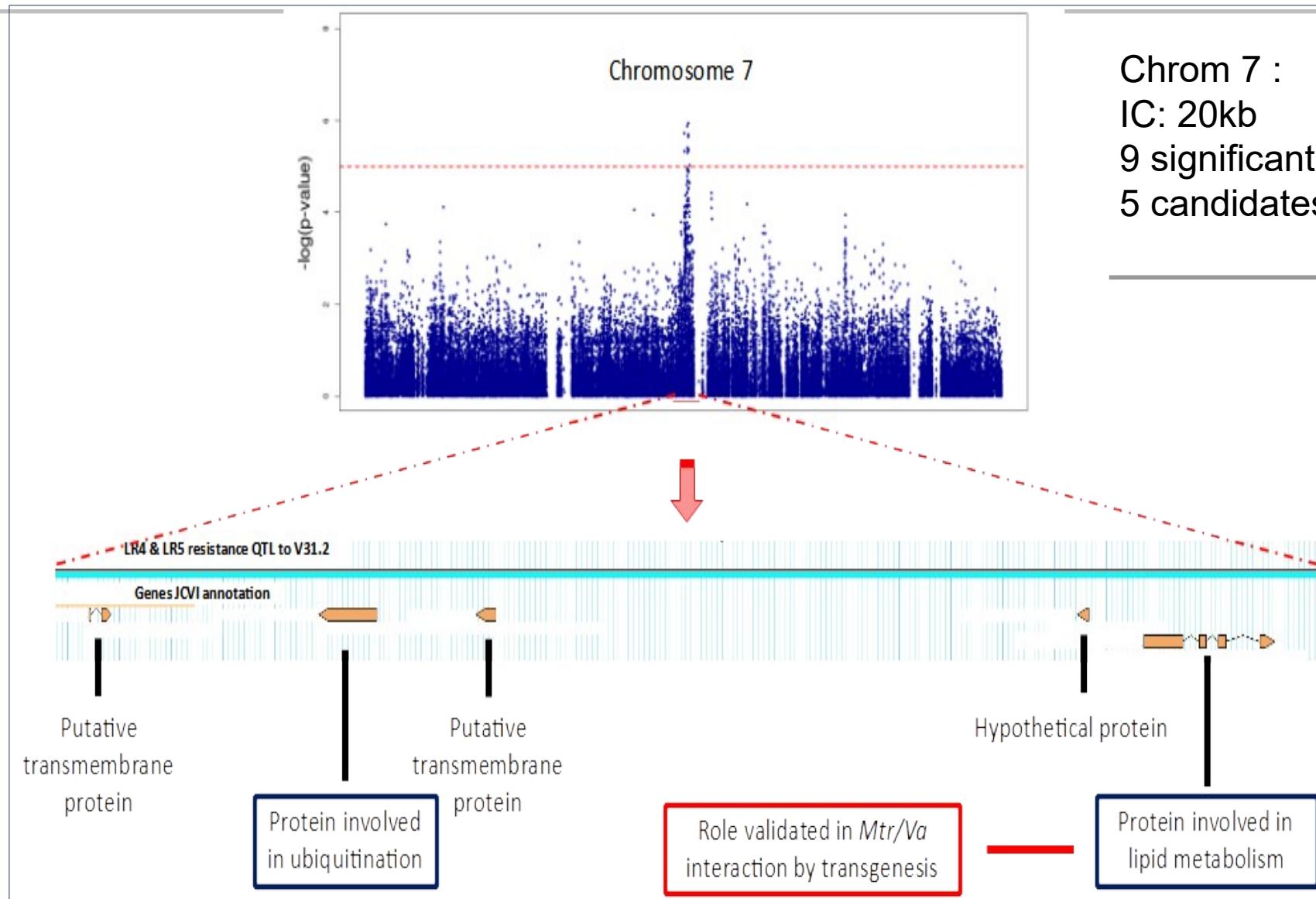
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Verticillium alfalfae

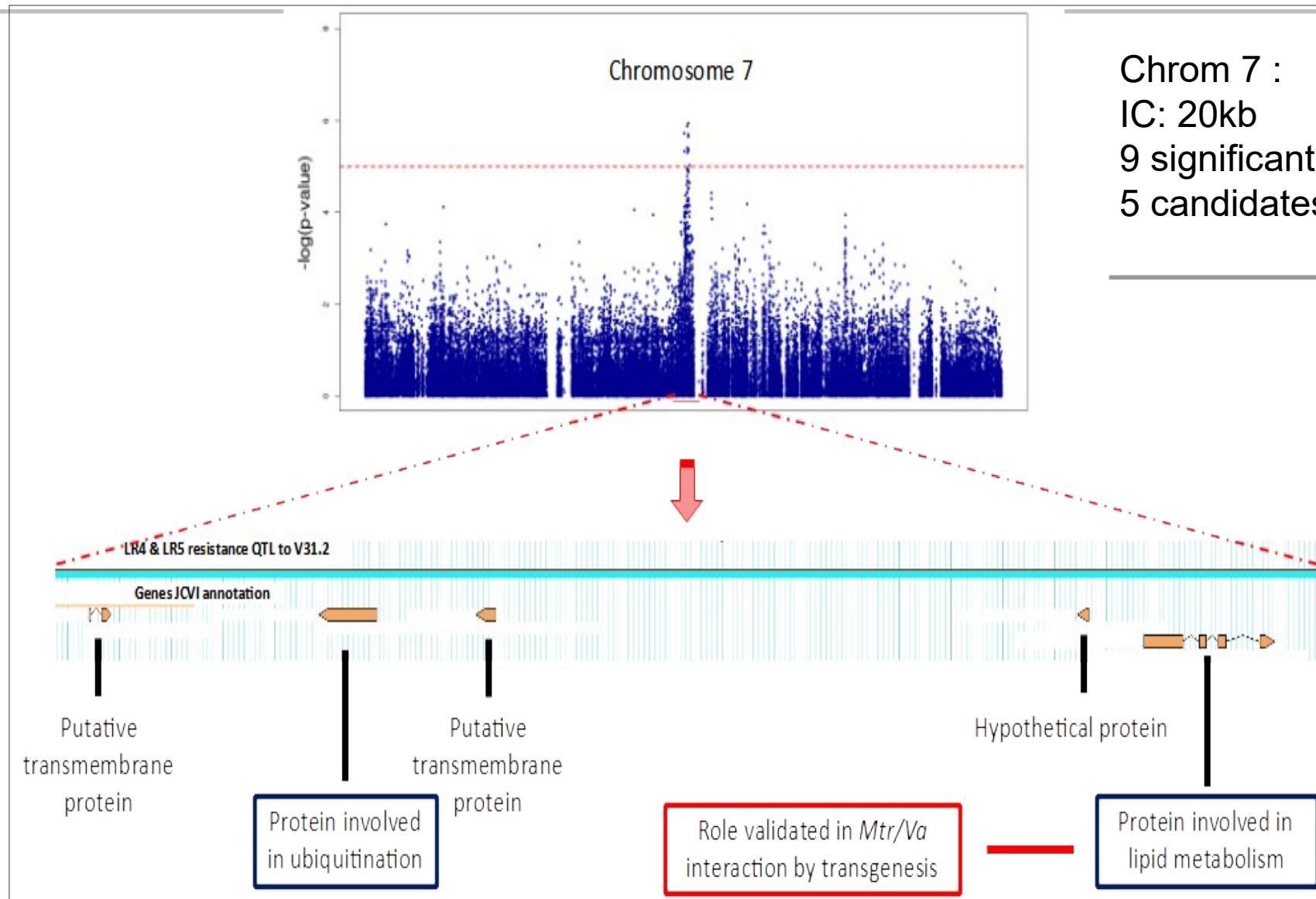
Combining GWAS, QTLs and transcriptome data to pinpoint potential candidates



Mazurier et al., in preparation

Among five candidate genes, only two genes are expressed in *M. truncatula* roots

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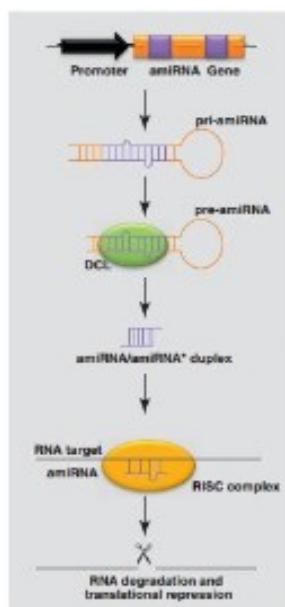
Among five candidate genes, only two genes are expressed in *M. truncatula* roots

Functional validation of the role of SEC14 gene in *M. truncatula* response to *Verticillium* wilt

* Genetic transformation:

amiR silencing => Necessary for resistance/susceptibility ?

35S overexpression => Sufficient for resistance/susceptibility ?



Transformation of young seedlings by
Agrobacterium rhizogenes



2 weeks on selective medium



Transgenic hairy roots
Inoculation with *Verticillium* spores

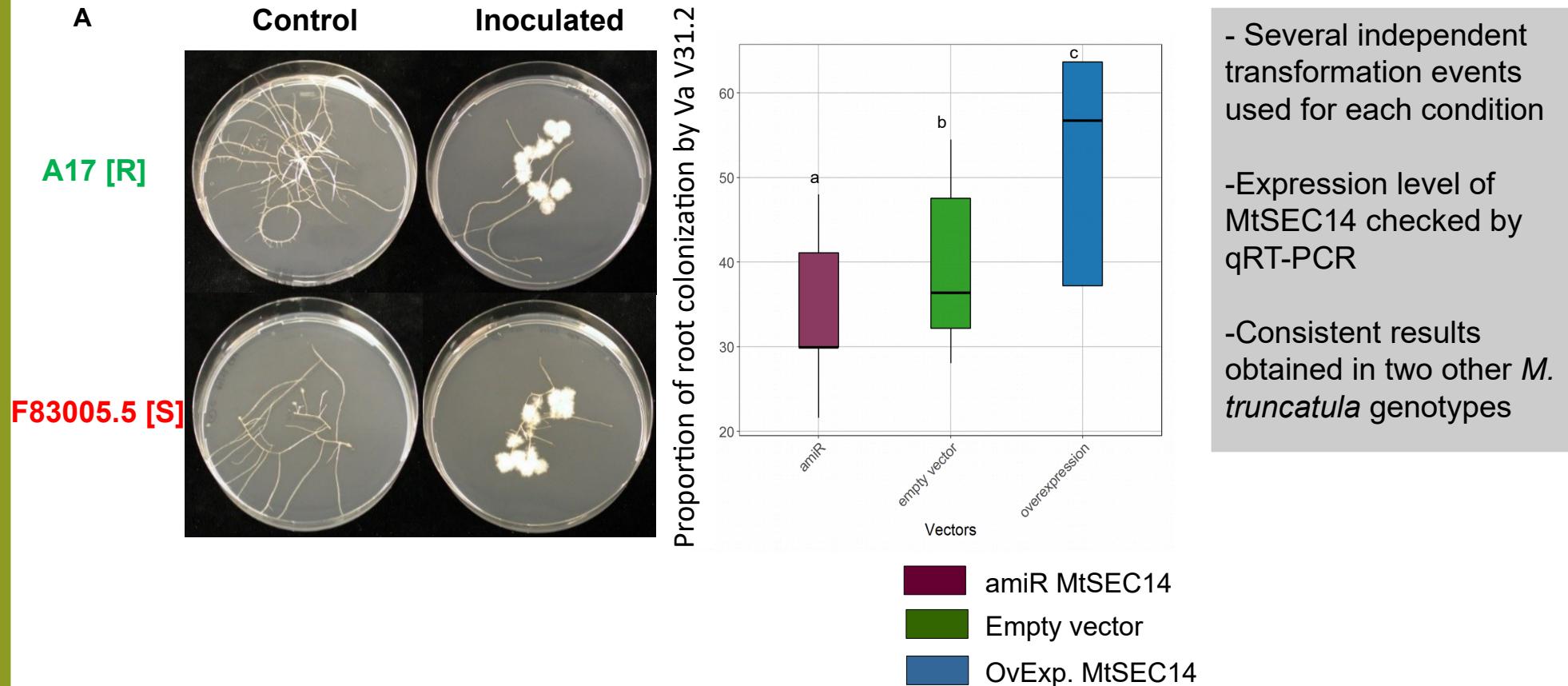


Image analysis with Image J (SmartRoot)

V. alfalfae resistance in *M. truncatula* takes place in the root.
Toueni et al., 2016

Proportion of colonized root
(Length of colonized root at 7dpi / Total root length at 7dpi)

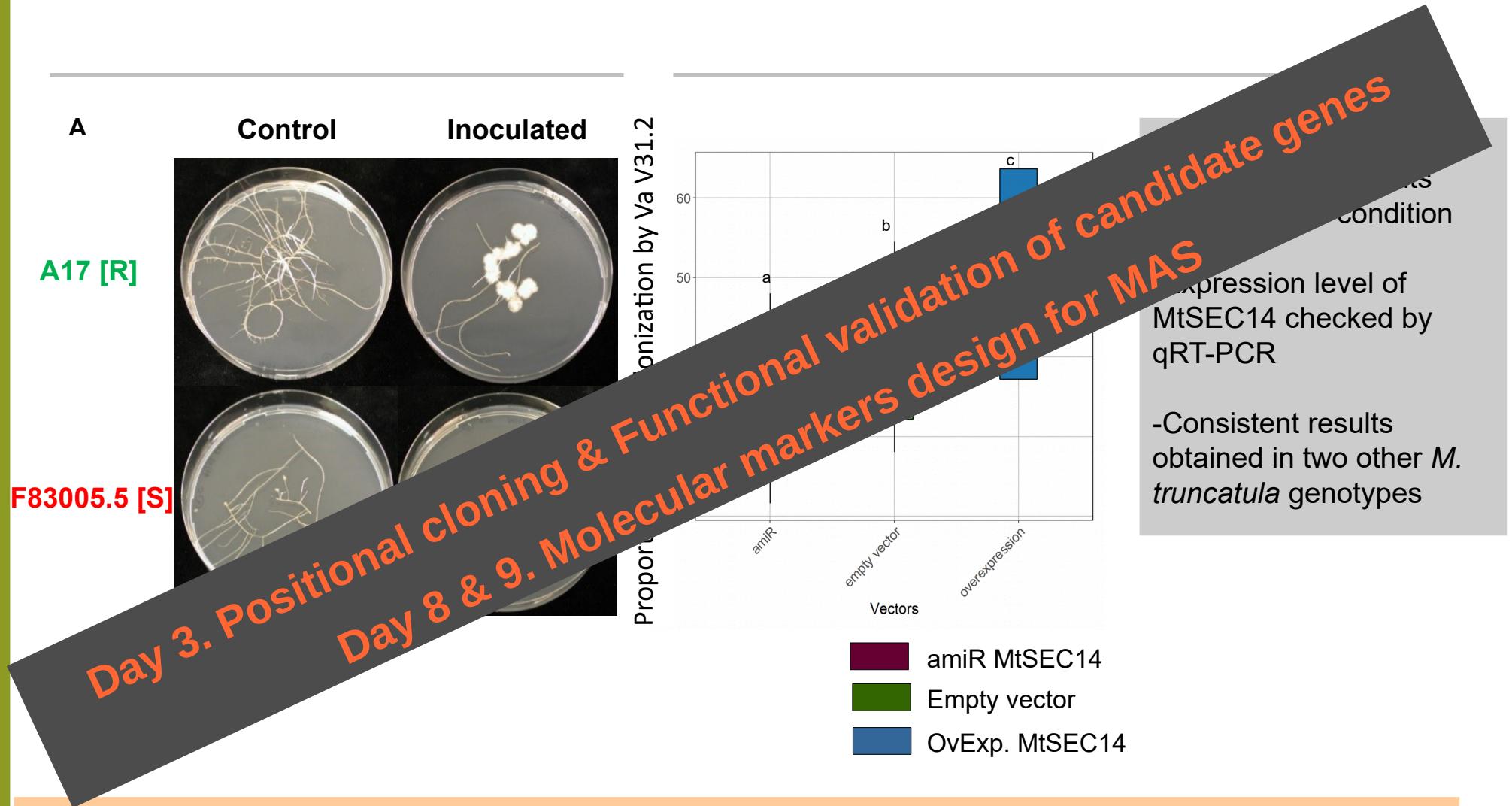
Hairy roots colonization depends on MtSEC14 expression



Medtr7g070480 gene encoding MtSEC14 appears to be a susceptibility gene

- Silencing of the gene decreases root colonization in Resistant accession
- Overexpression of the gene in hairy roots of resistant accession increases root colonization

Hairy roots colonization depends on MtSEC14 expression

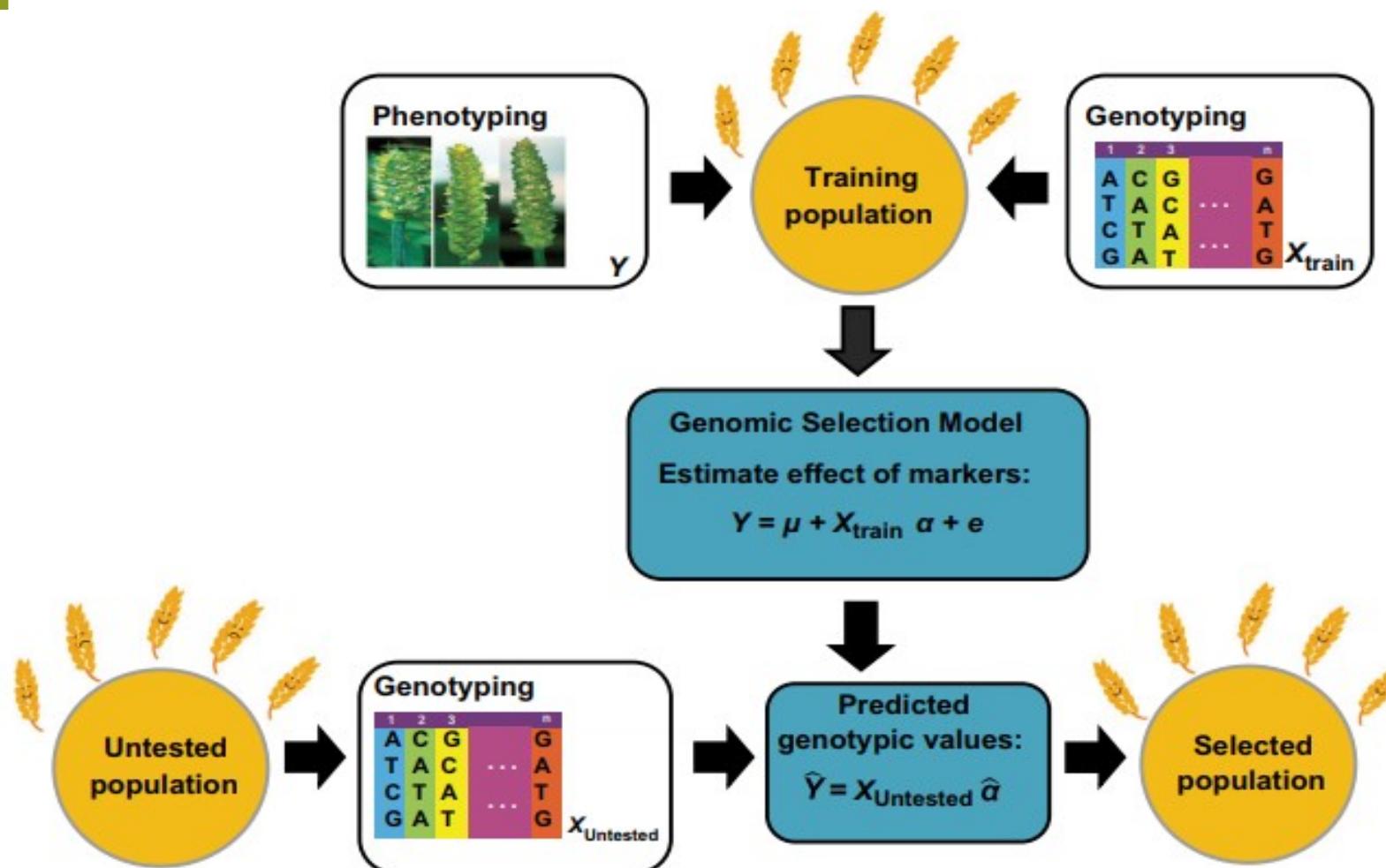


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Genomic selection a « new » powerful approach to predict genetic values of plant genotype

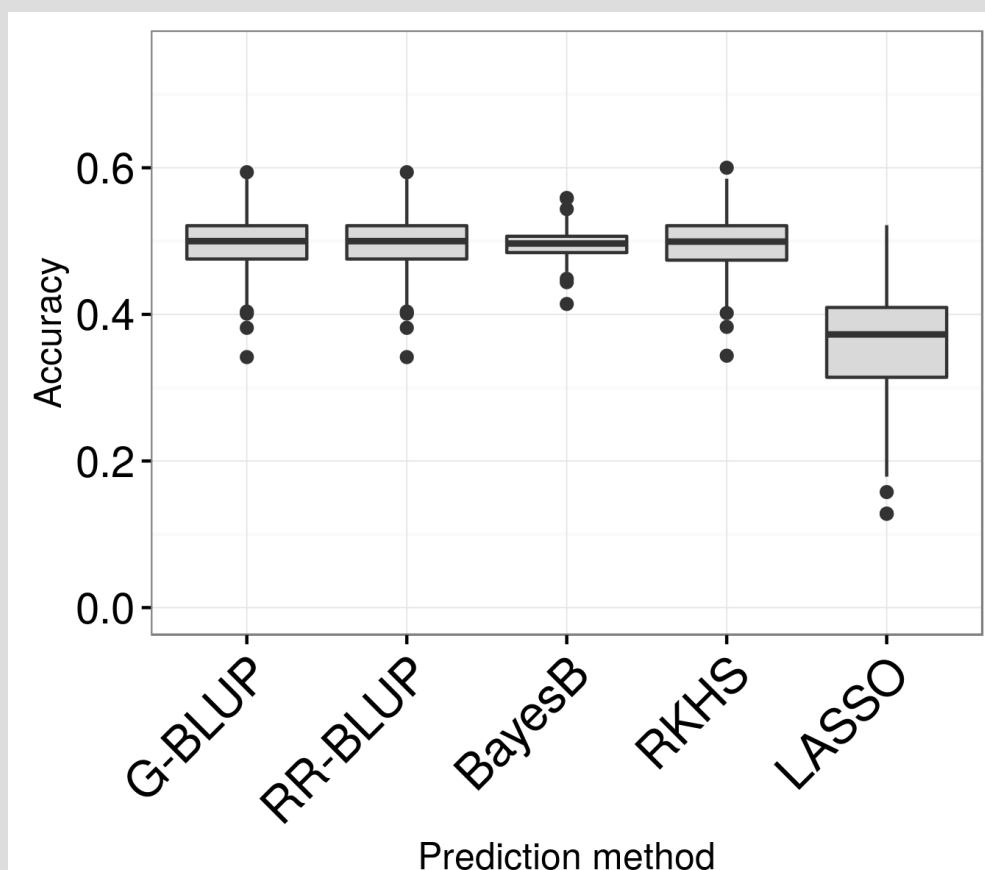
- GS estimates (=predicts) the genetic additive value of accessions based on whole genome data.
- Contrary to GWAS, GS goal is not to identify candidate loci.



Source : Zhao Y., Mette MF., Reif JC., 2015, Genomic selection in hybrid breeding. *Plant Breed* 134:1–10. doi: 10.1111/pbr.12231

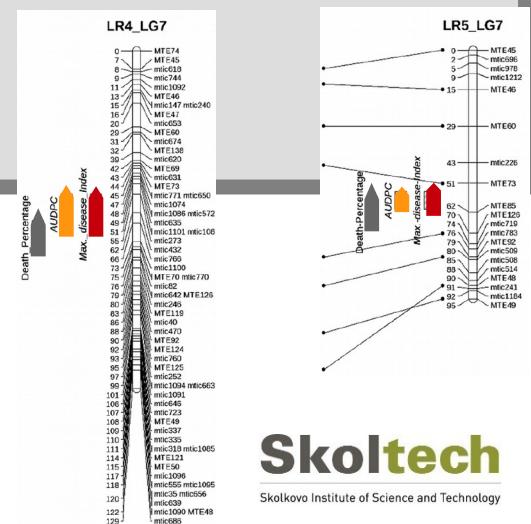
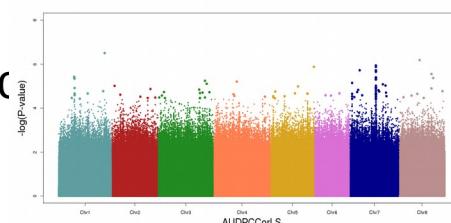
Genomic Selection analysis suggests that additional loci act in establishing this QDR

- 840,000 SNPs
 - Proportional sampling of training set among eight populations
 - 50 rounds of 5-fold cross-validation

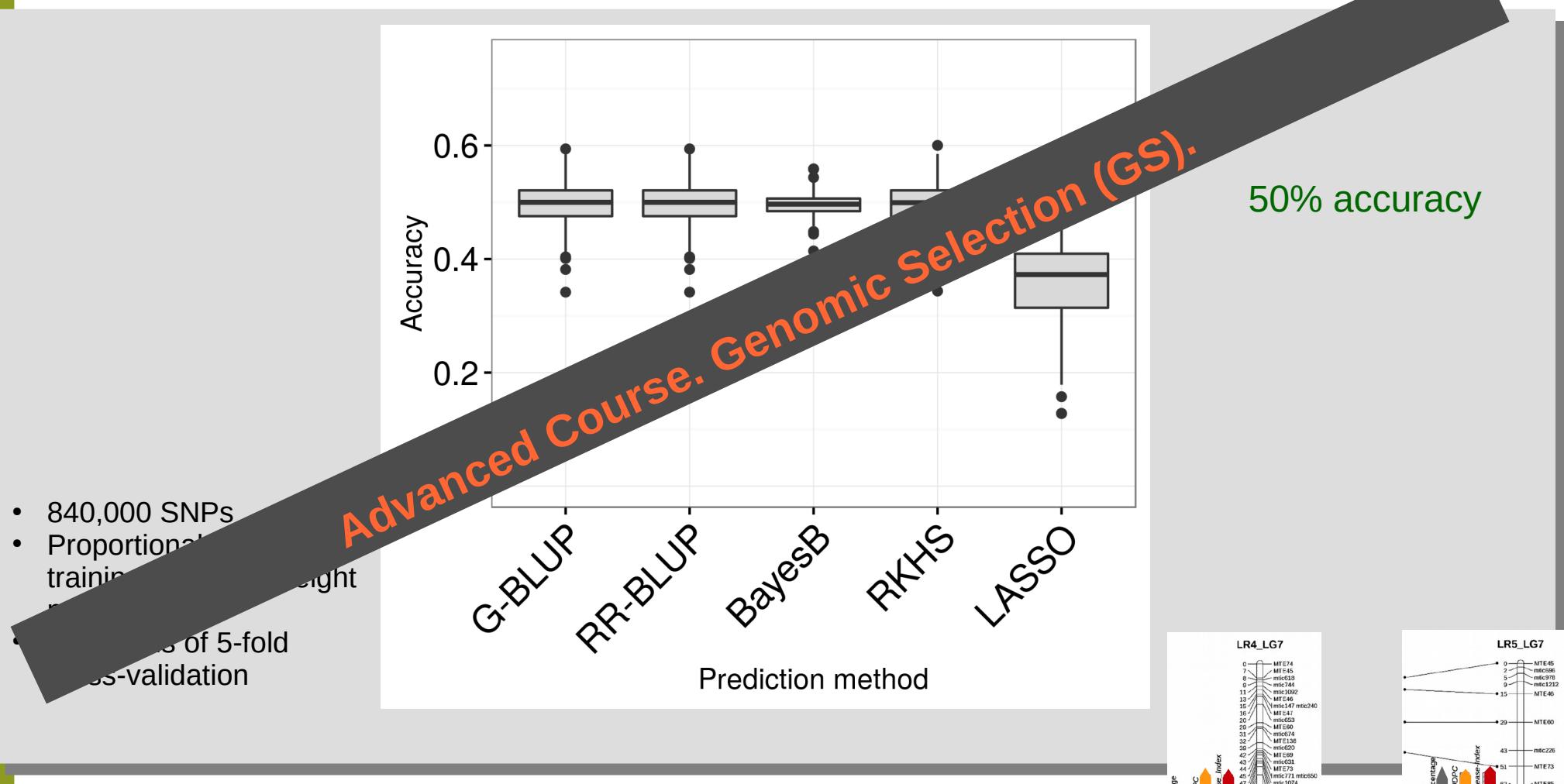


50% accuracy

- QTL analysis : 30% of phenotype for QTL on chrom.7
 - GWAS : 36 % of phenotype for SNPs on chr. 1



Genomic Selection analysis suggests that additional loci act in establishing this QDR

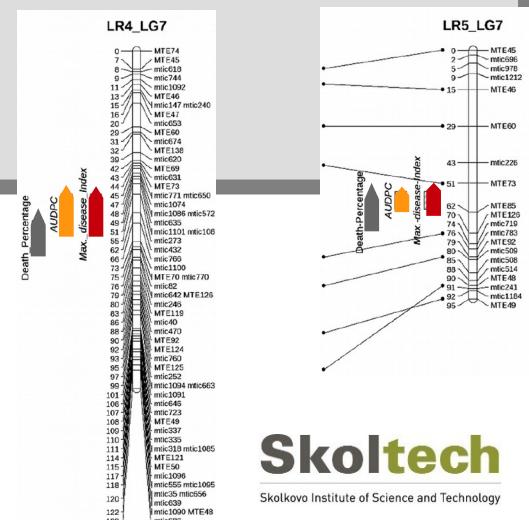
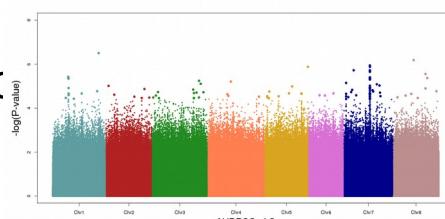


- 840,000 SNPs
 - Proportional training

A

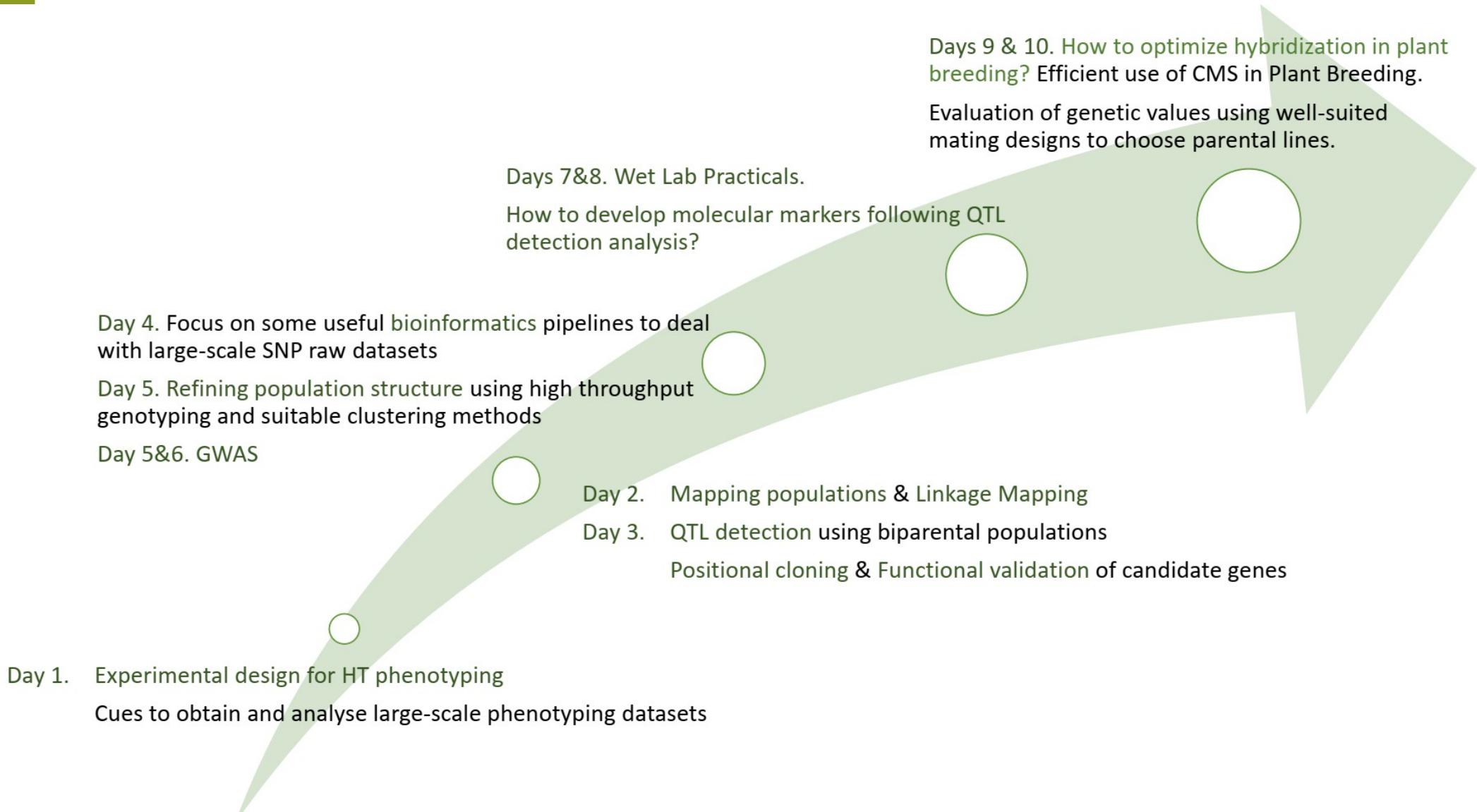
of 5-fold cross-validation

- QTL analysis : 30% of phenotype for QTL on chrom.7
 - GWAS : 36 % of phenotype for SNPs on chr. 1



Modern Plant Breeding – Regular level

Program of the course with learning-by-doing



A multidisciplinary teaching team with complementary expertise (*alphabetical order*)



Dr Alina Baik
Plant Molecular Biology



Pr Cécile Ben
Plant Genetics
& Breeding



Pr Laurent Gentzbittel
Biostatistics
& Quantitative Genetics



Yawar Habib
Data analysis



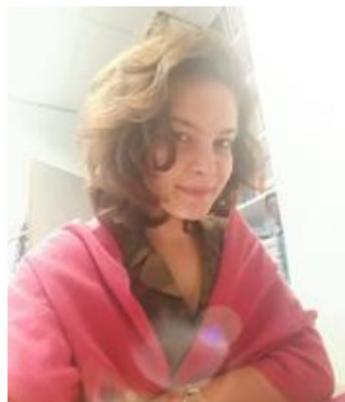
Daria Kostyrko
Plant Molecular Biology



Dr Elena Martynova
Plant Molecular Biology
& Virology



Pr Elena Potokina
Plant Genetic Diversity &
Genomics



Anastasiia Stepanova
Plant Biotech.
& Phytopathology



Alexey Zamalutdinov
Bioinformatics &
Computational Biology

ADMINISTRATIVE TEAM



Natalia Klimova
Deputy Head for Administration



Vladislav Sergeev
Team Assistant



Ekaterina Tolmacheva
Senior Specialist for Admin.

Thank you for your
attention !

