



Horizon 2020 of European Union: Call 2016, SFS 44 : “A joint plant breeding programme to decrease the EU's and China's dependency on protein imports”

This project has received funding from the European Union's Horizon 2020 Programme for Research & Innovation under grant agreement n°727312.



Genomics assisted breeding in alfalfa



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Alfalfa - Lucerne

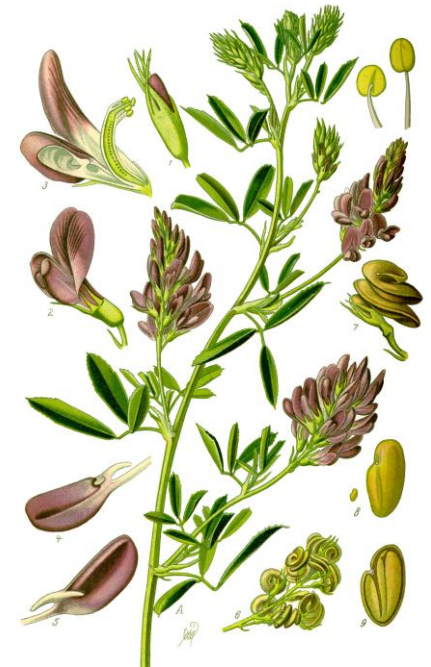
A major legume species

- Highest protein production/ha in temperature climates
- Drought tolerant
- Protein/energy
- Ruminant health
- Positive effects in the rotation

Allogamous reproduction, synthetic varieties

$$2n = 4x = 32$$

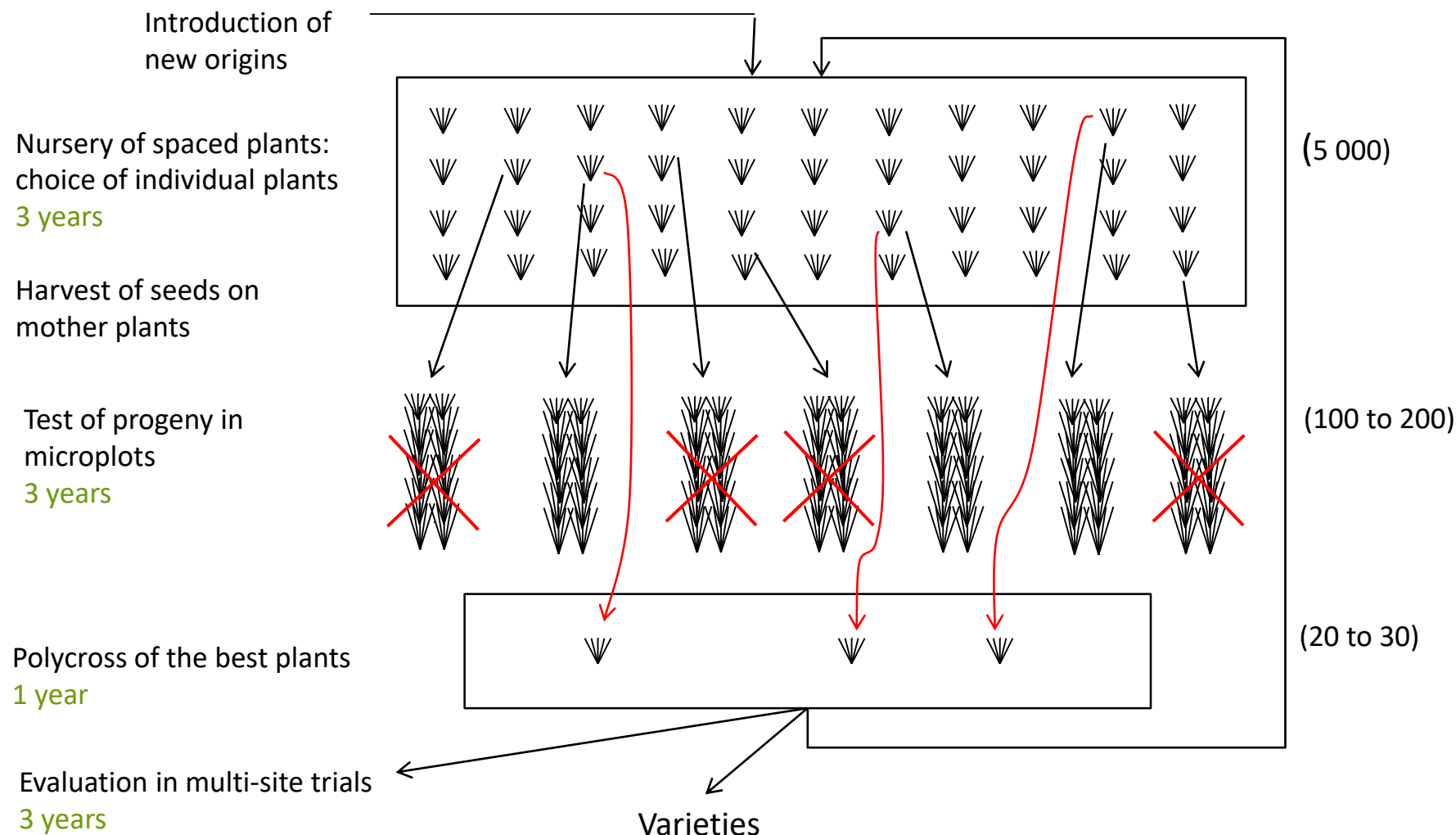
Julier et al. 2017, CABI Publishing



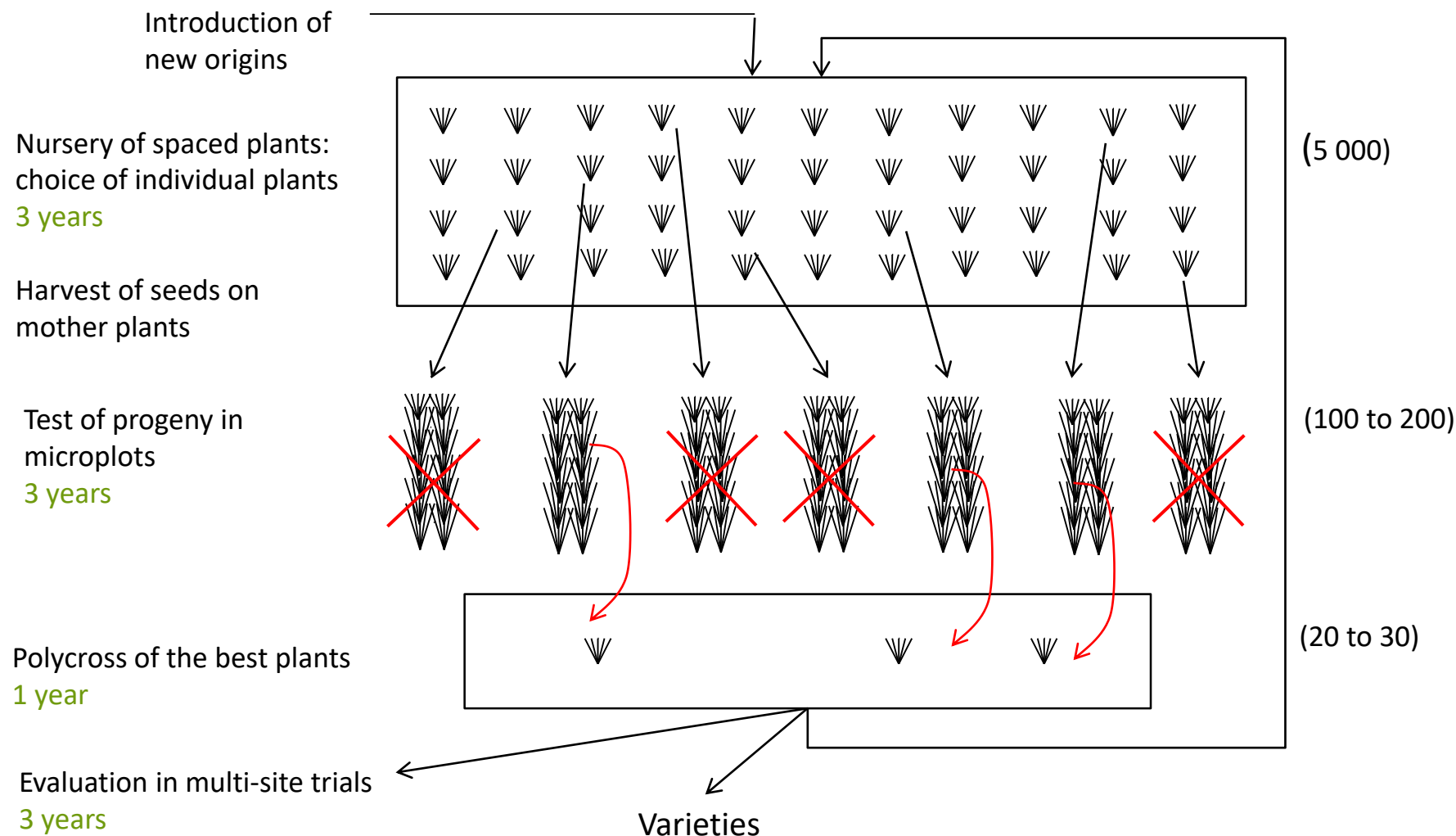
Traditional breeding methodology

- Based on phenotypic traits
- First step in nursery of spaced plants
- Second step in progeny testing

Traditional breeding methodology



Traditional breeding methodology



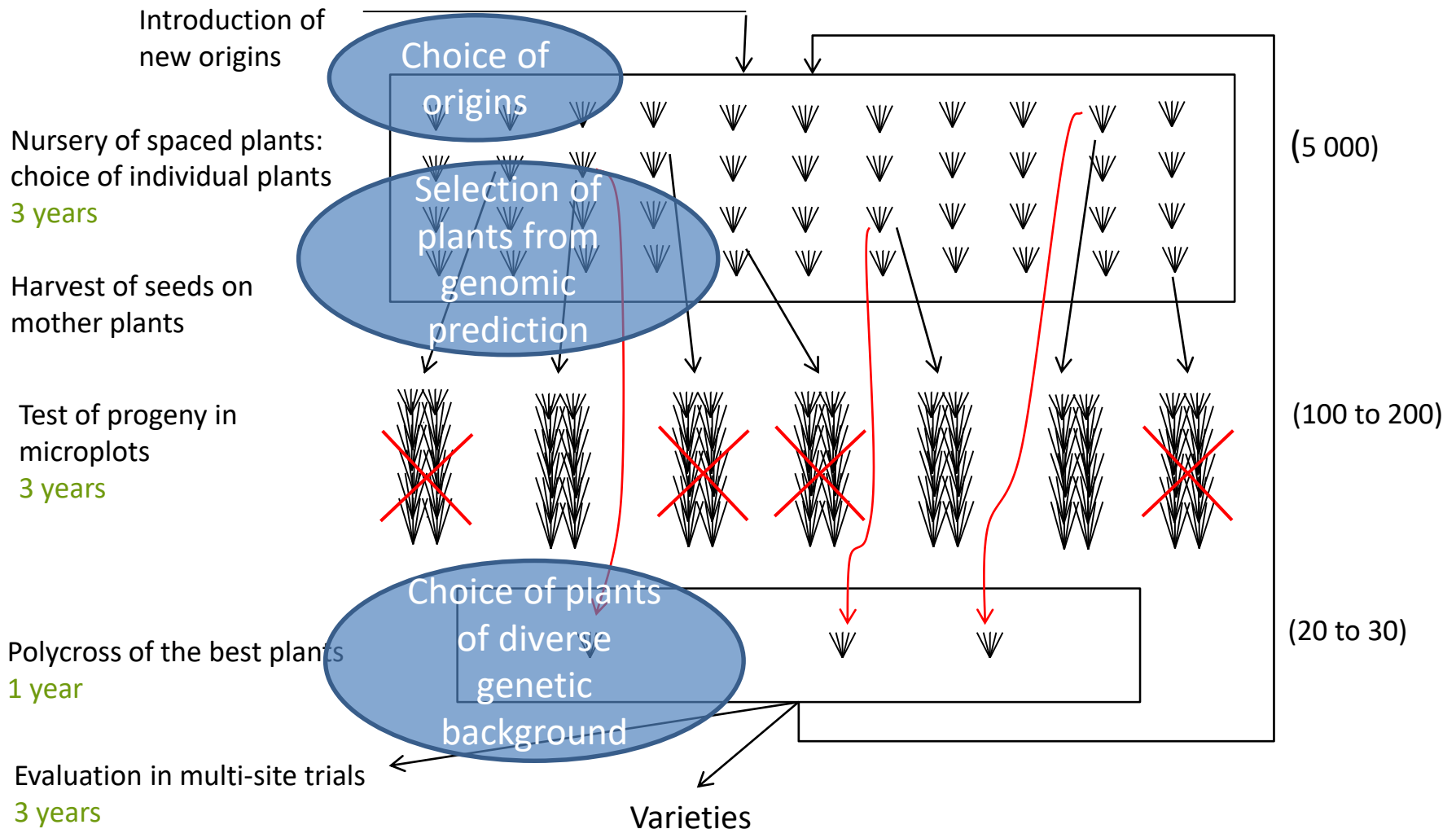
Strength

- Scoring of many traits
- Early selection for heritable traits
- Skilled staffs

Weakness

- Some traits are scored in case of stress occurrence only
- Number of years
- Cost
- Fixation of positive alleles is slow

Genomics assisted breeding



Genomics assisted breeding



- Marker development
- Management of genetic diversity
- Genome wide association study
- Genomic selection



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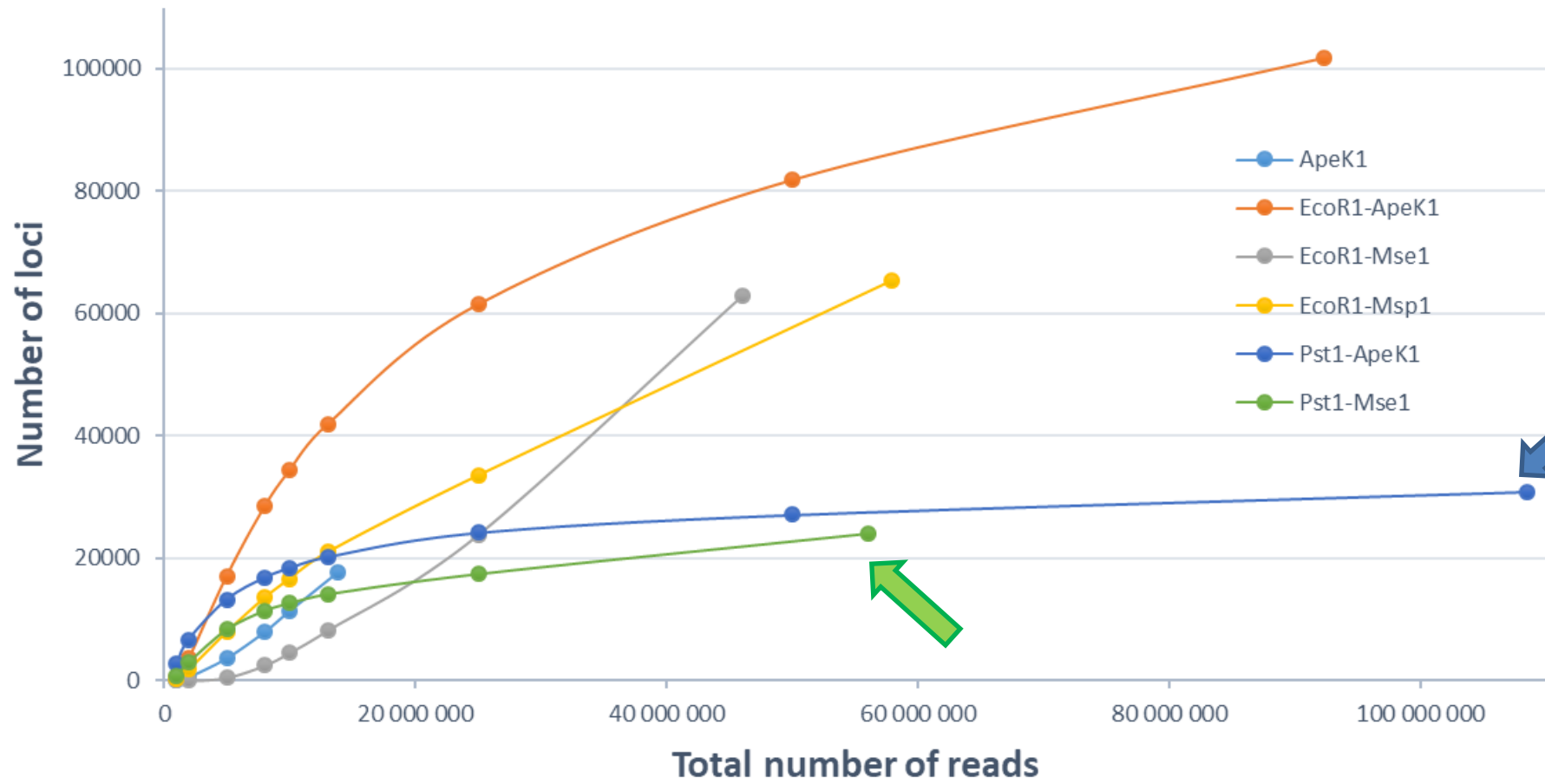
Before EUCLEG:

- Low throughput markers: SSR, AFLP...
- 10k SNP array: too expensive
- GBS: < 40K markers, risk of missing data

EUCLEG: an improved GBS protocol

- Choice of restriction enzymes to reduce missing data

Marker development



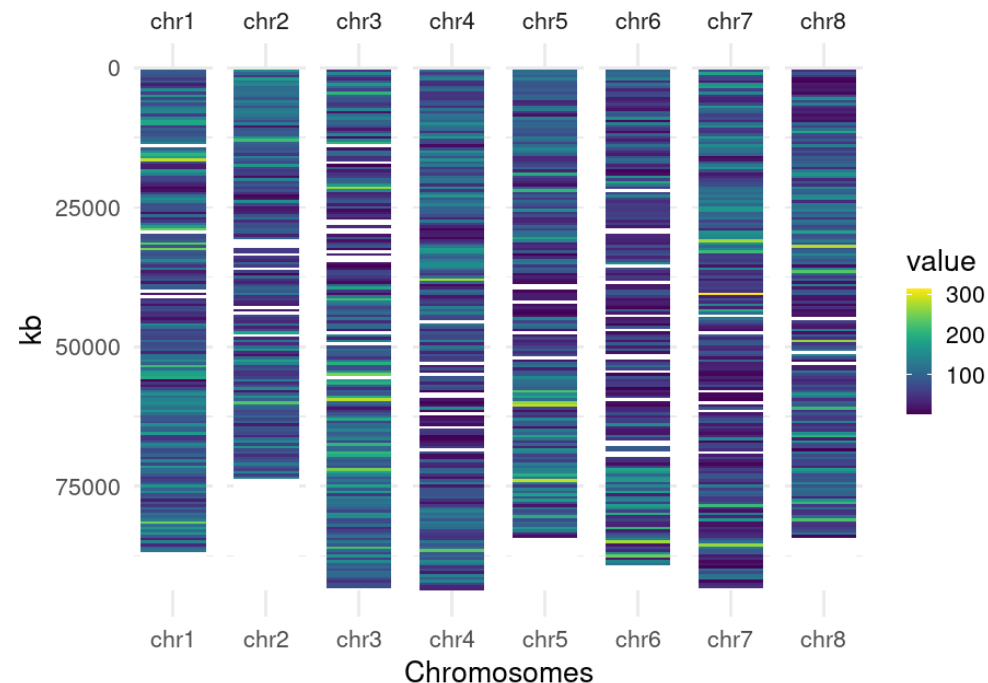
EUCLEG: an improved GBS protocol

- Choice of restriction enzymes to reduce missing data
- Use of a reference genome sequence: Chen et al. 2020
- Allele frequency of each accession

Marker development

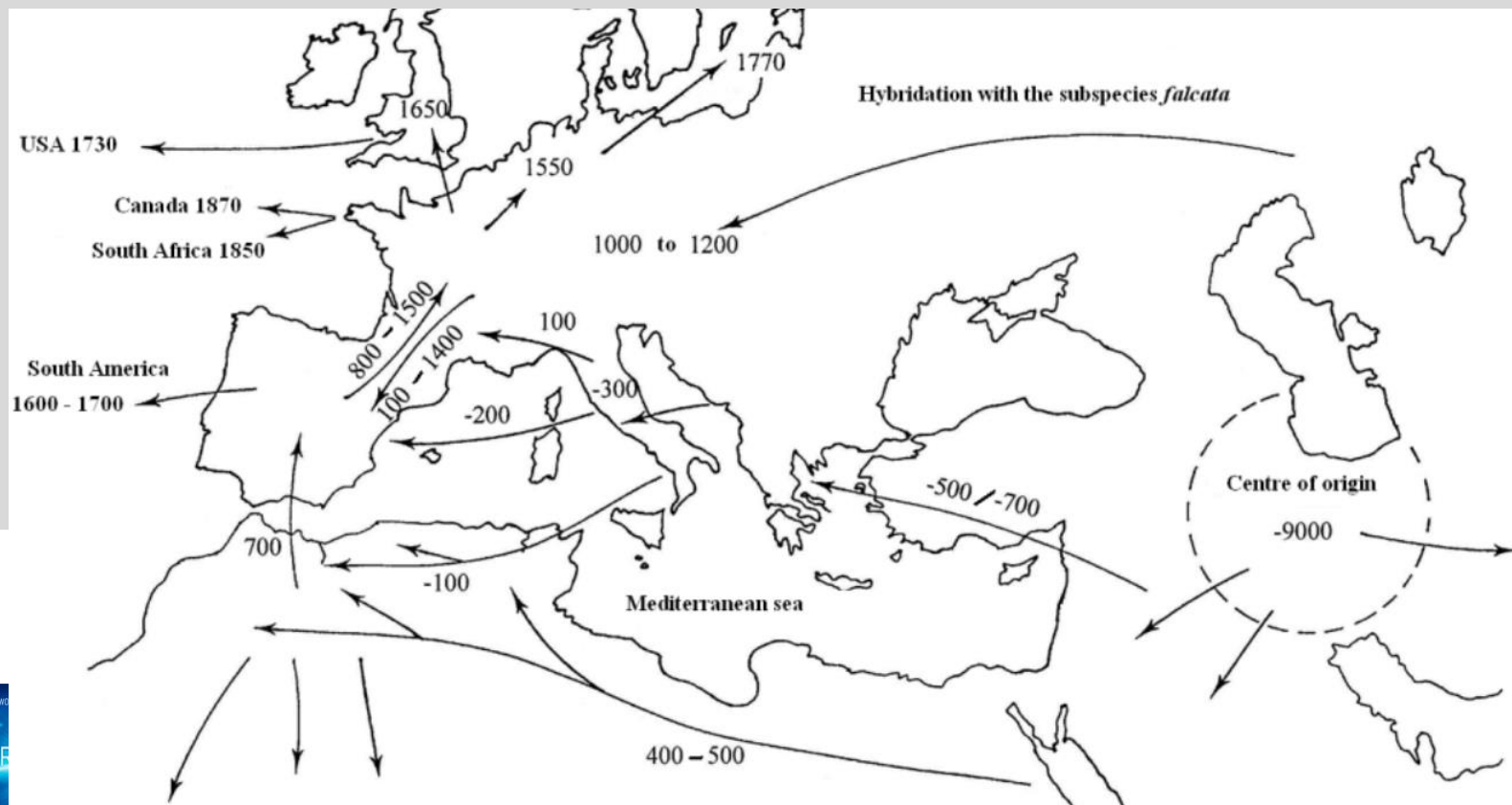
On 1 061 accessions:

- 31 743 loci
- 228 568 SNP with less than 5% missing data per SNP
- 118 421 SNP without missing data



Before EUCLEG:

- Overview of world diversity



Before EUCLEG:

- Overview of world diversity
- Large among-accession diversity
- Huge within-accession diversity

| | <u>10 populations, 40 indiv/pop</u> | | <u>11 populations, 7-20 indiv/pop</u> |
|---------------------------|-------------------------------------|---------------------|---------------------------------------|
| | <u>5 SSR</u> | <u>Plant height</u> | <u>Yield</u> |
| Variance among-varieties | 0.02 | 0.10 | 1.7 |
| Variance within-varieties | 7.56 | 0.30 | 27.7 |
| No structure | | | |

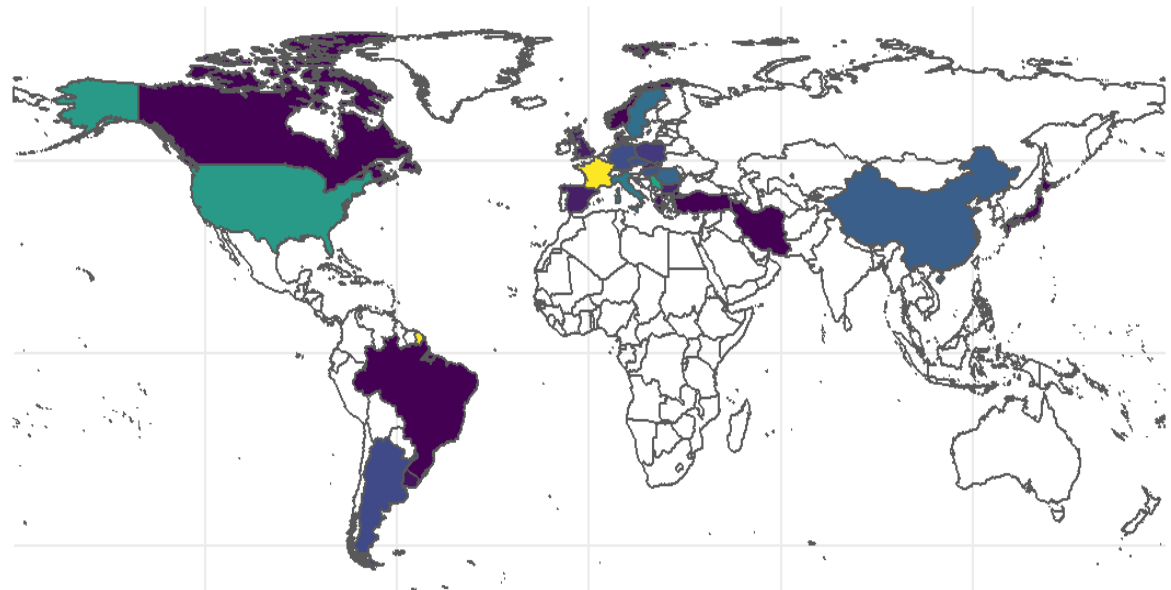
Herrmann et al., 2010

Julier et al. 2000

EUCLEG: a revision of diversity overview

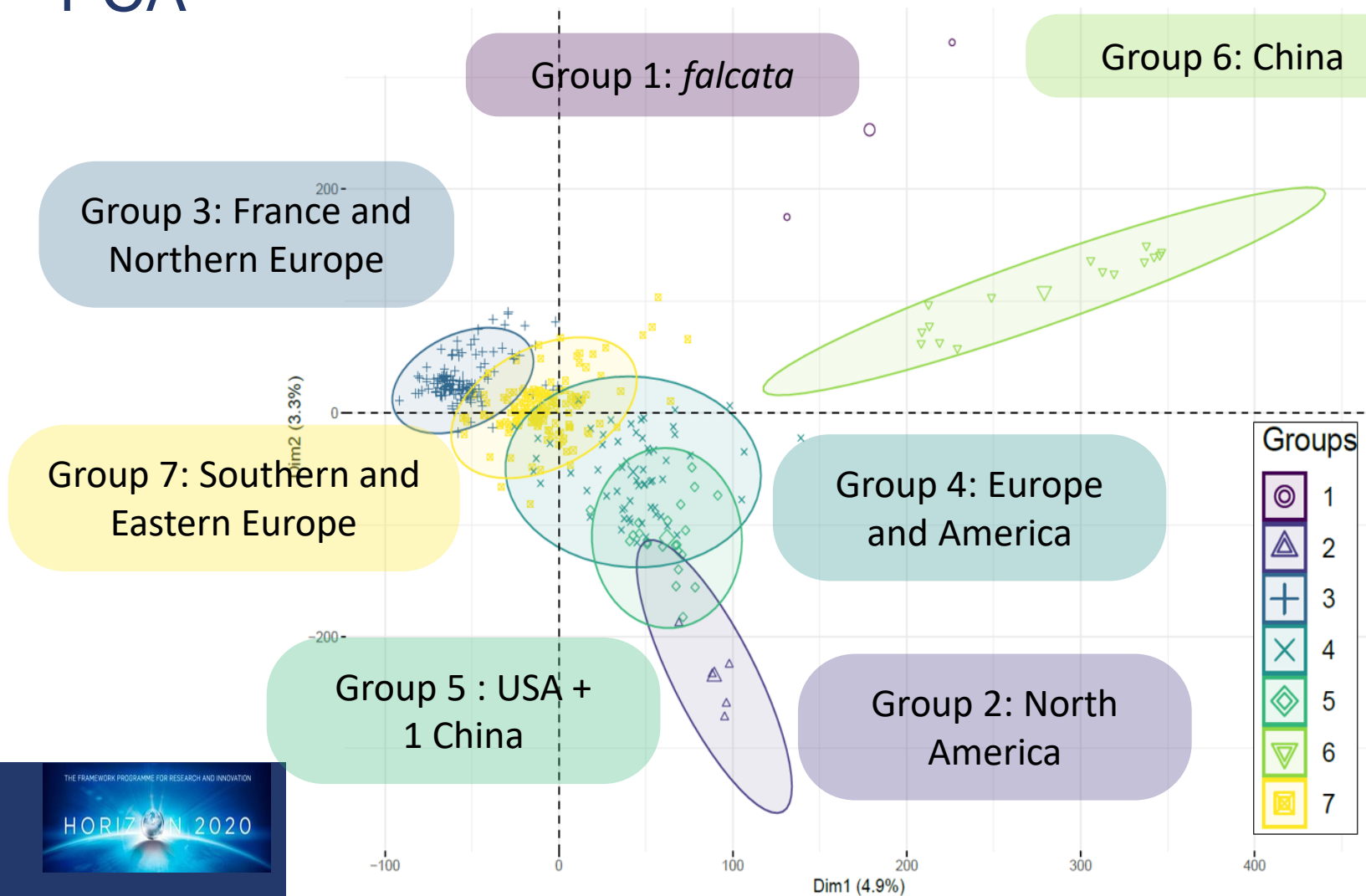
400 accessions: landraces and cultivars, dormancy 3 – 7:

- Europe : 313
- North America : 45
- South America : 16
- China : 17
- Middle East : 3
- Japan : 1



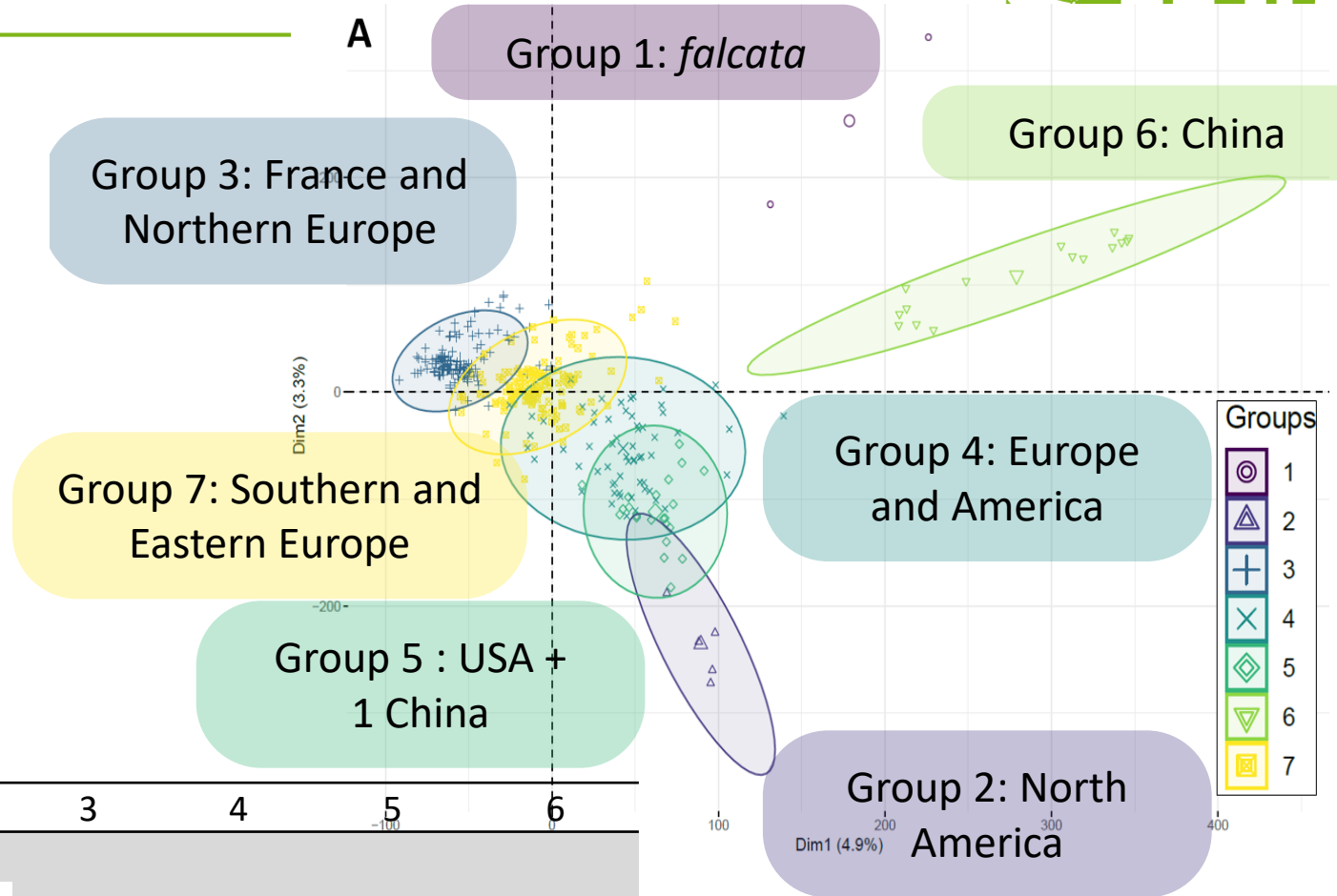
Management of genetic diversity

PCA



Management of genetic diversity

PCA



| Clusters | 1 | 2 | 3 | 4 | 5 | 6 |
|----------|---|-------|-------|-------|-------|-------|
| 1 | | | | | | |
| 2 | | | | | | |
| 3 | | 0.017 | | | | |
| 4 | | 0.013 | 0.003 | | | |
| 5 | | 0.004 | 0.006 | 0.002 | | |
| 6 | | 0.026 | 0.019 | 0.012 | 0.015 | |
| 7 | | 0.016 | 0.001 | 0.001 | 0.005 | 0.015 |

ant breeding programme to decrease the EU's
received funding from the European Union's
reement n°727312.

PCA : accessions colored with autumn dormancy score

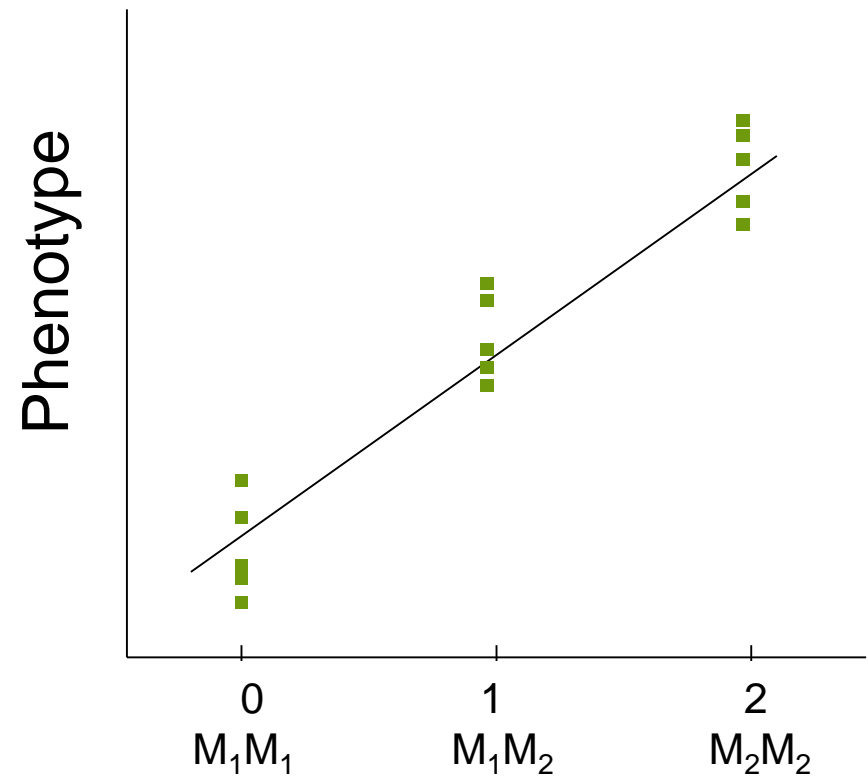
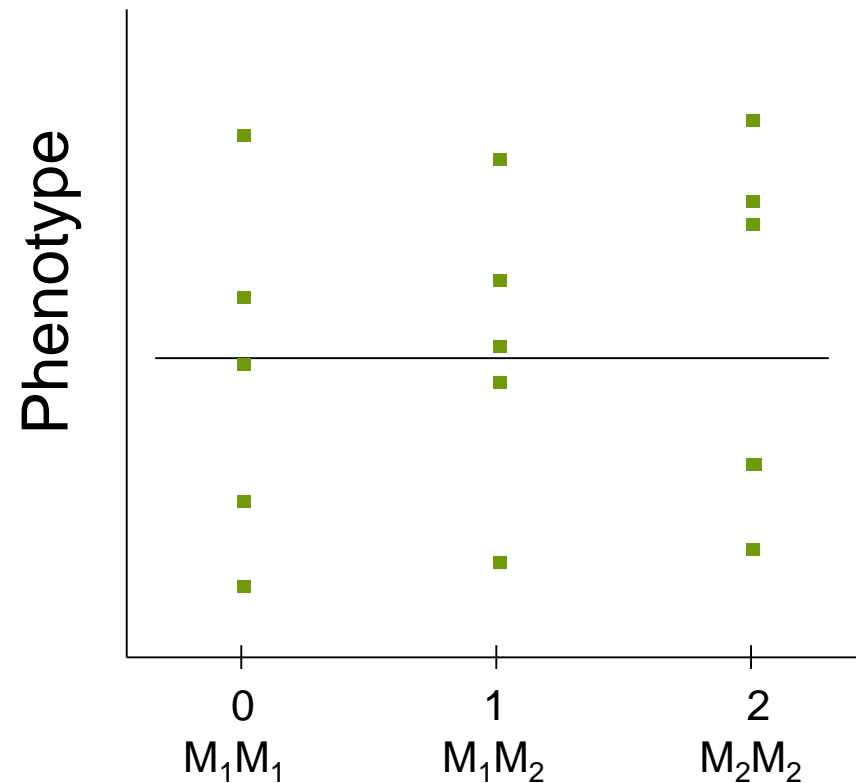


EUCLEG: a revision of diversity overview

- Diversity: China $< >$ Europe + America
- Diversity: Europe $< >$ America
- Structure is not associated to autumn dormancy

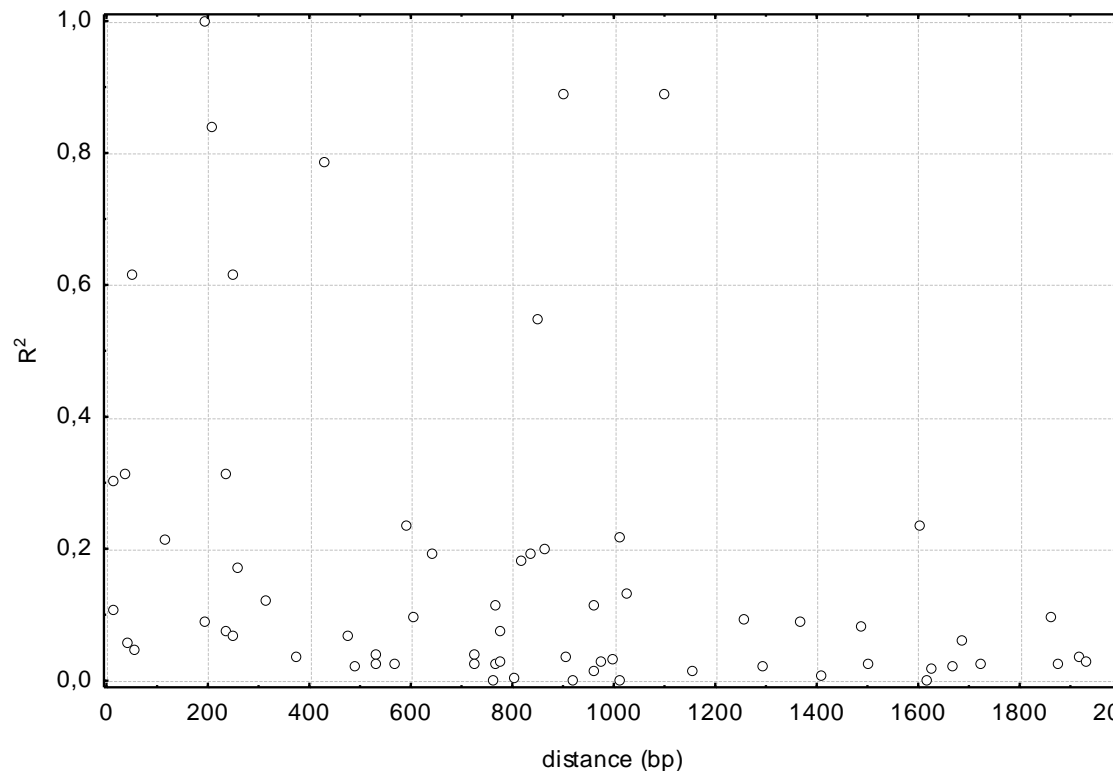
Genome wide association study

For each marker: is it associated to trait variation?



Before EUCLEG:

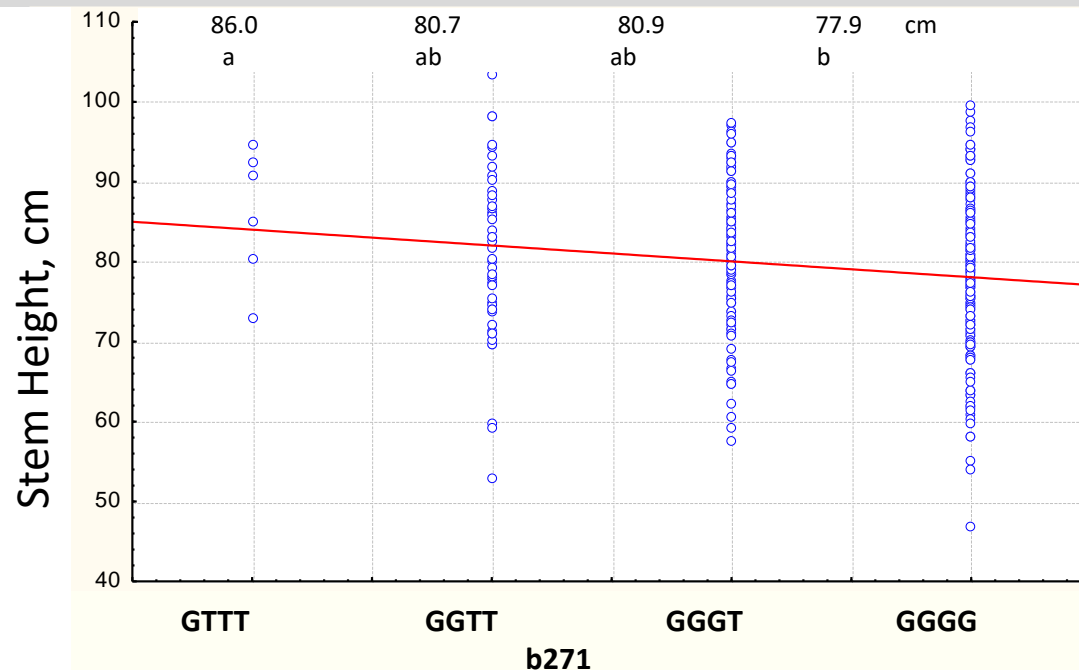
- Low marker density
- Short linkage disequilibrium



12 SNP (66 pairs) in
Constans-like gene
(Herrmann et al.,
2010)

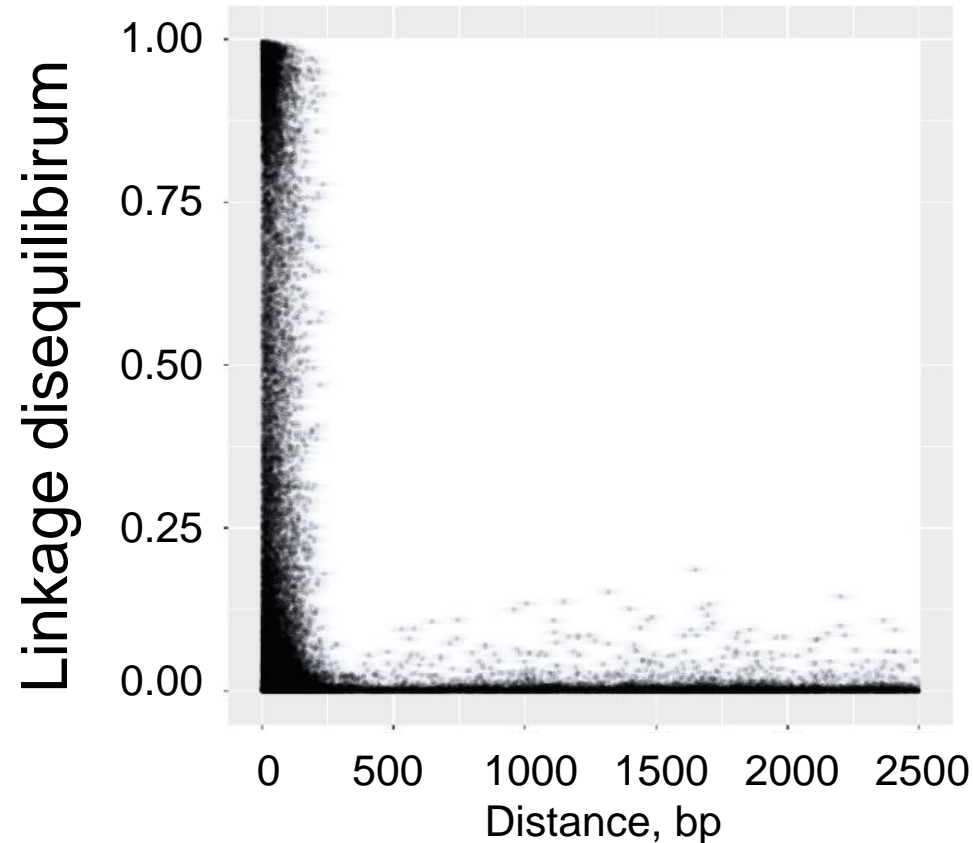
Before EUCLEG:

- Low marker density
 - Short linkage disequilibrium
- Candidate gene approach only



Constans-like gene
(Herrmann et al.,
2010)

EUCLEG: Short linkage disequilibrium over the genome



Yield and quality (proteins, fibres, saponins)

400 accessions x 2 locations x 2 years

+ 100 accessions x 3 locations x 2 years

Germination

Disease resistance: fusarium, anthracnose

Drought and P tolerance

Drought x fusarium

Genome wide association study



EUCLEG: Detection of major QTL

400 accessions x 2 locations x 2 years

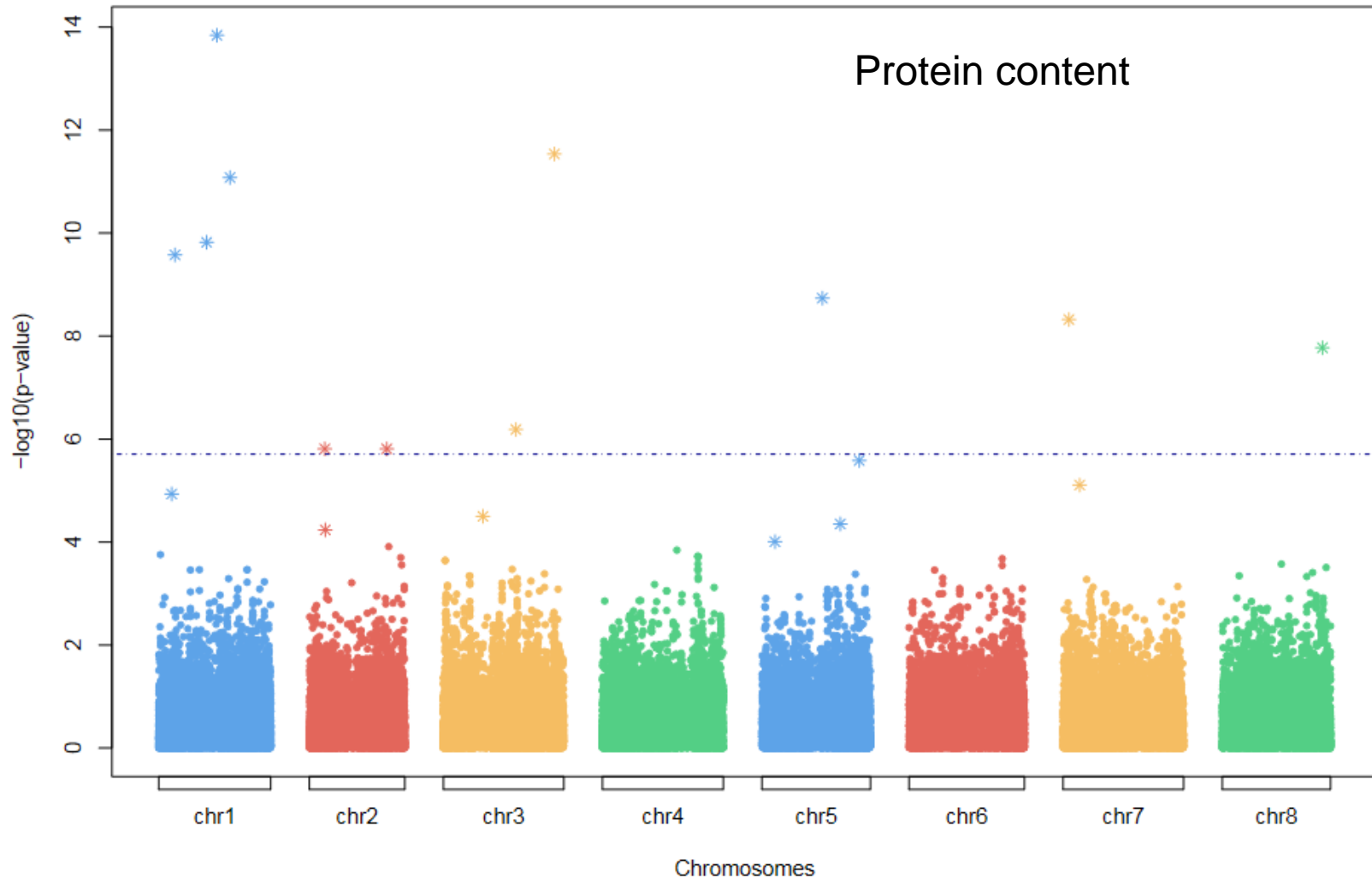
Method: Multi Locus Mixed Model (MLMM)



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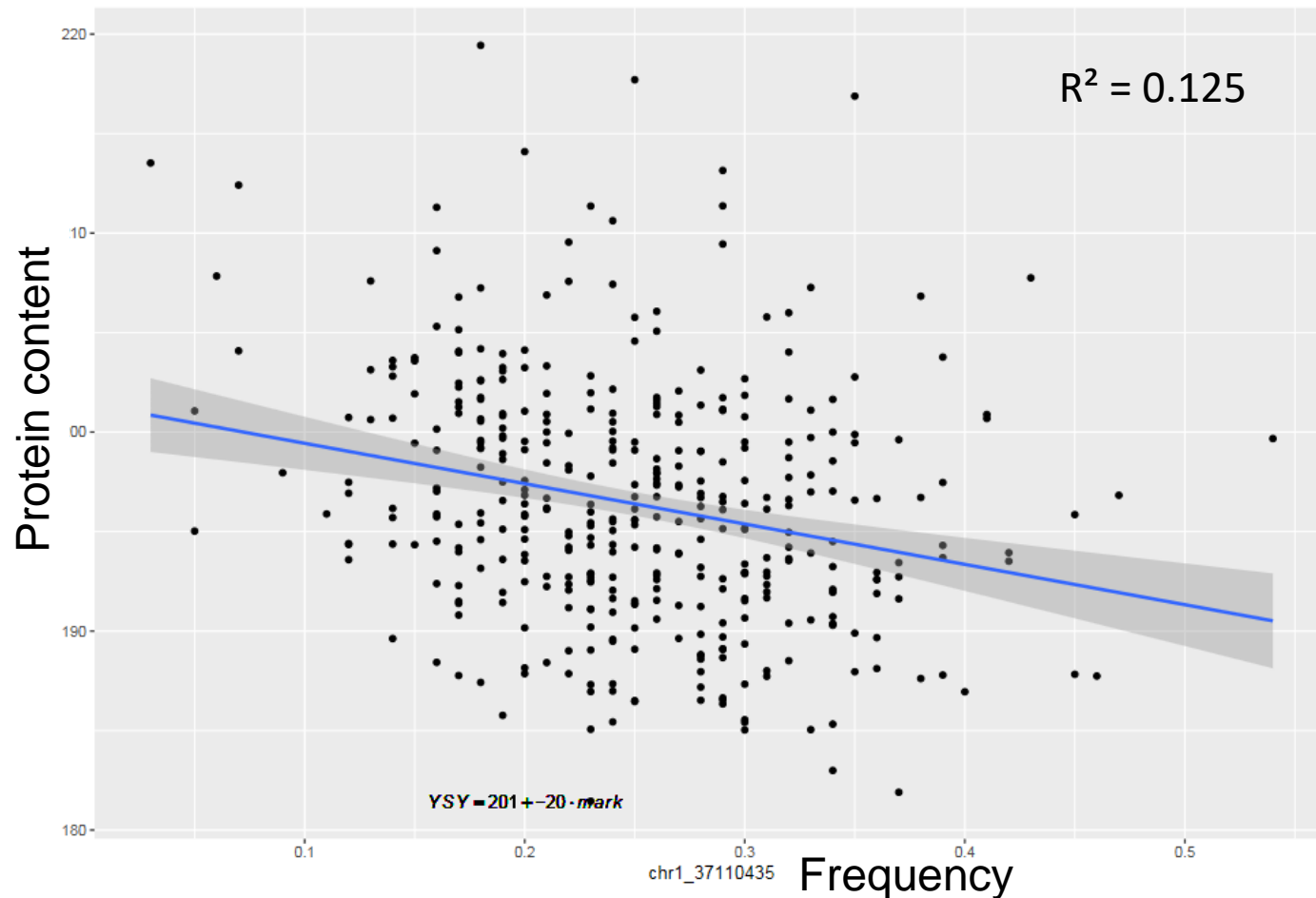
Genome wide association study

EUCLEG: Detection of major QTL

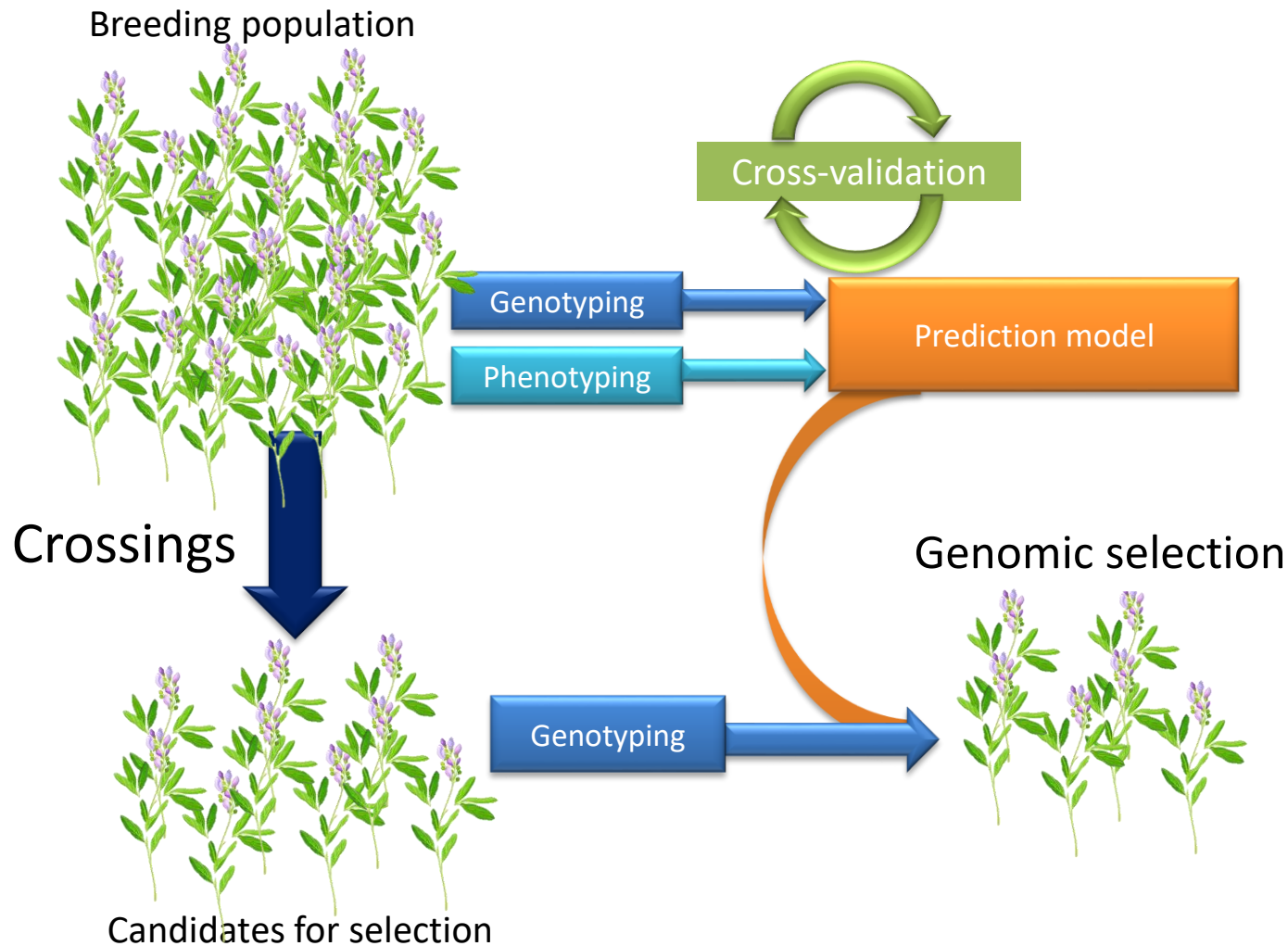


EUCLEG: Detection of major QTL

- Up to 10 – 20% of variation



Genomic selection



Before EUCLEG

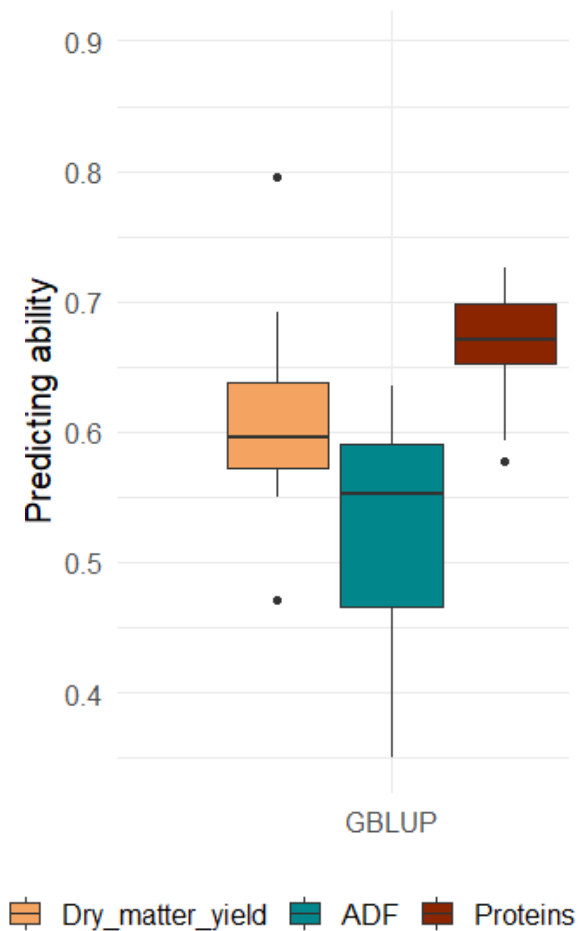
- 8 – 44 K SNP, 75 – 244 individuals
- Promising results, predictive ability ~ 30%

Genomic selection

EUCLEG

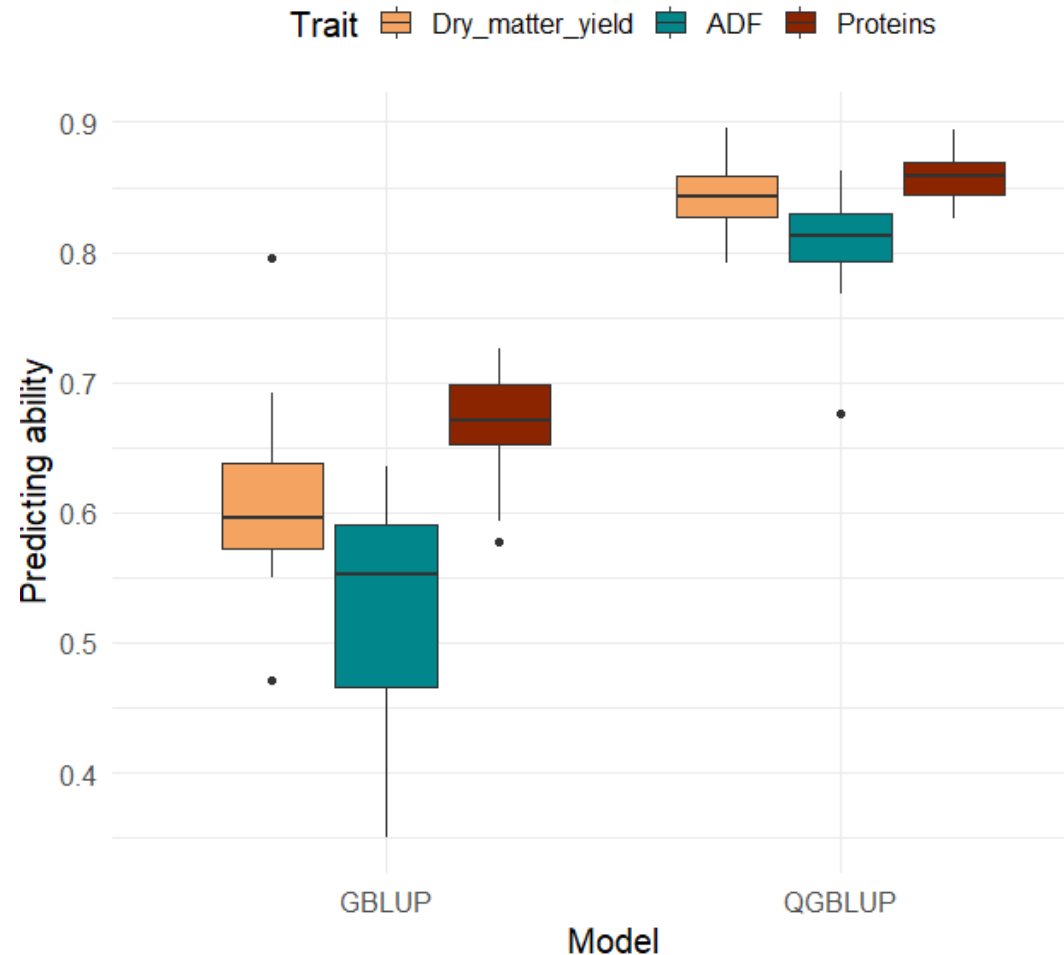
- GBLUP

- A good predicting ability:
 $0.52 < P < 0.66$



Integration of the QTL
information in the
prediction model
(QGBLUP)

→ $P > 0.80$



GBS are efficient markers

- Allele dosage in individuals
- Allele frequency in populations
- Genome coverage
- Low missing data

→ useful to analyse genetic diversity and genetic determinism of breeding traits

Management of genetic diversity

- Some specialisation of the breeding pools in EU, America, China
- GBS markers to decide on the introduction of new genetic diversity in a breeding pool

GS models provide high predictive ability

- Even higher with the inclusion of QTL effect

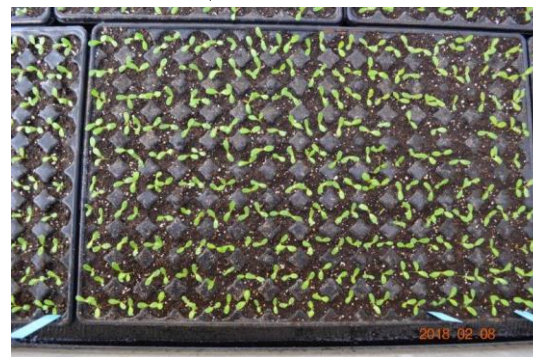
→ To be used to select promising individuals in breeding pools

- Extend the analysis of alfalfa diversity from dormancy 3-7 to the whole species complex
- Improve cost-efficiency of genotyping
- Calculate genetic gain with GS
- Estimate cost-efficiency of GS
- Implement genomic selection in breeding programmes

GS in breeding programmes

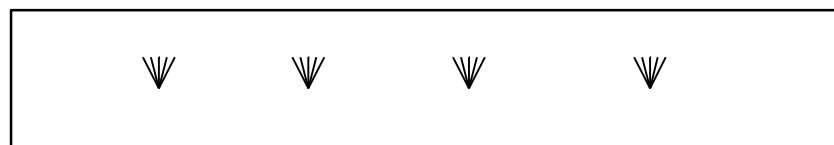
Introduction of
new origins

Genotyping
Analysis of diversity
Genomic prediction
→ Choice of plants
0.5 year



(5 000 to
15 000)

Polycross of the best plants
1 year



(20 to 30)

Evaluation in multi-site trials
3 years

Varieties

Strength

- Reduced field work
- Early selection for all predicted traits
- Reduced number of years
- Fixation of positive alleles is quick

Genetic
gain?

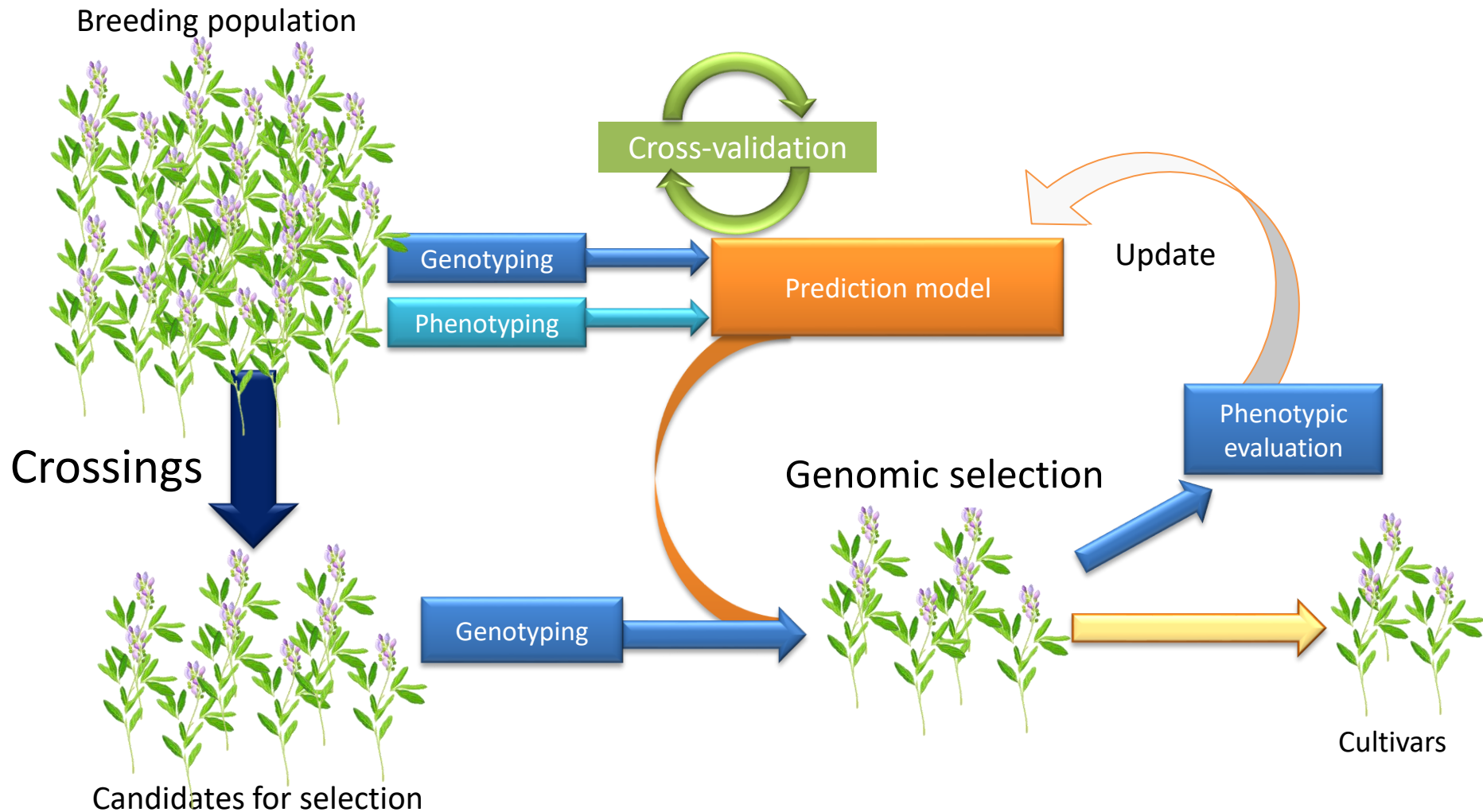
Weakness

- No prediction for some traits
- Staffs have to get new skills

Cost
efficiency ?

- Extend the analysis of alfalfa diversity from dormancy 3-7 to the whole species complex
- Improve cost-efficiency of genotyping
- Calculate genetic gain with GS
- Estimate cost-efficiency of GS
- Implement genomic selection in breeding programmes
- Imagine the updating of GS equations

Genomic selection





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