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Genomics assisted breeding in alfalfa

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► **To cite this version:**

Bernadette Julier, Marie Pégard, Julien Leuenberger, Philippe Barre. Genomics assisted breeding in alfalfa. EUCLEG Workshop on Genomics assisted breeding in forage and grain legumes, Sep 2021, online, United Kingdom. hal-03365357

HAL Id: hal-03365357

<https://hal.inrae.fr/hal-03365357>

Submitted on 5 Oct 2021

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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

INRAE

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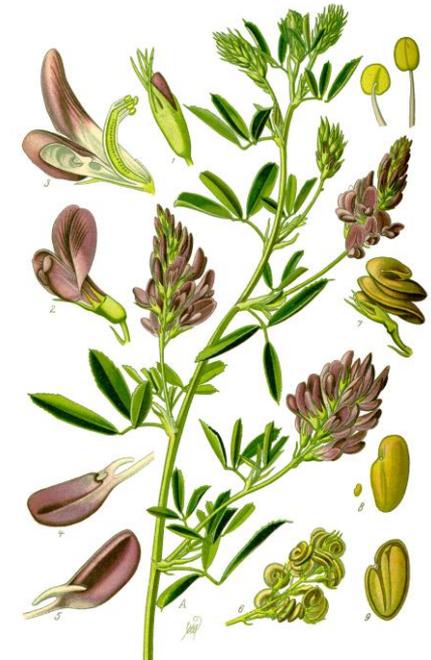
A major legume species

- 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

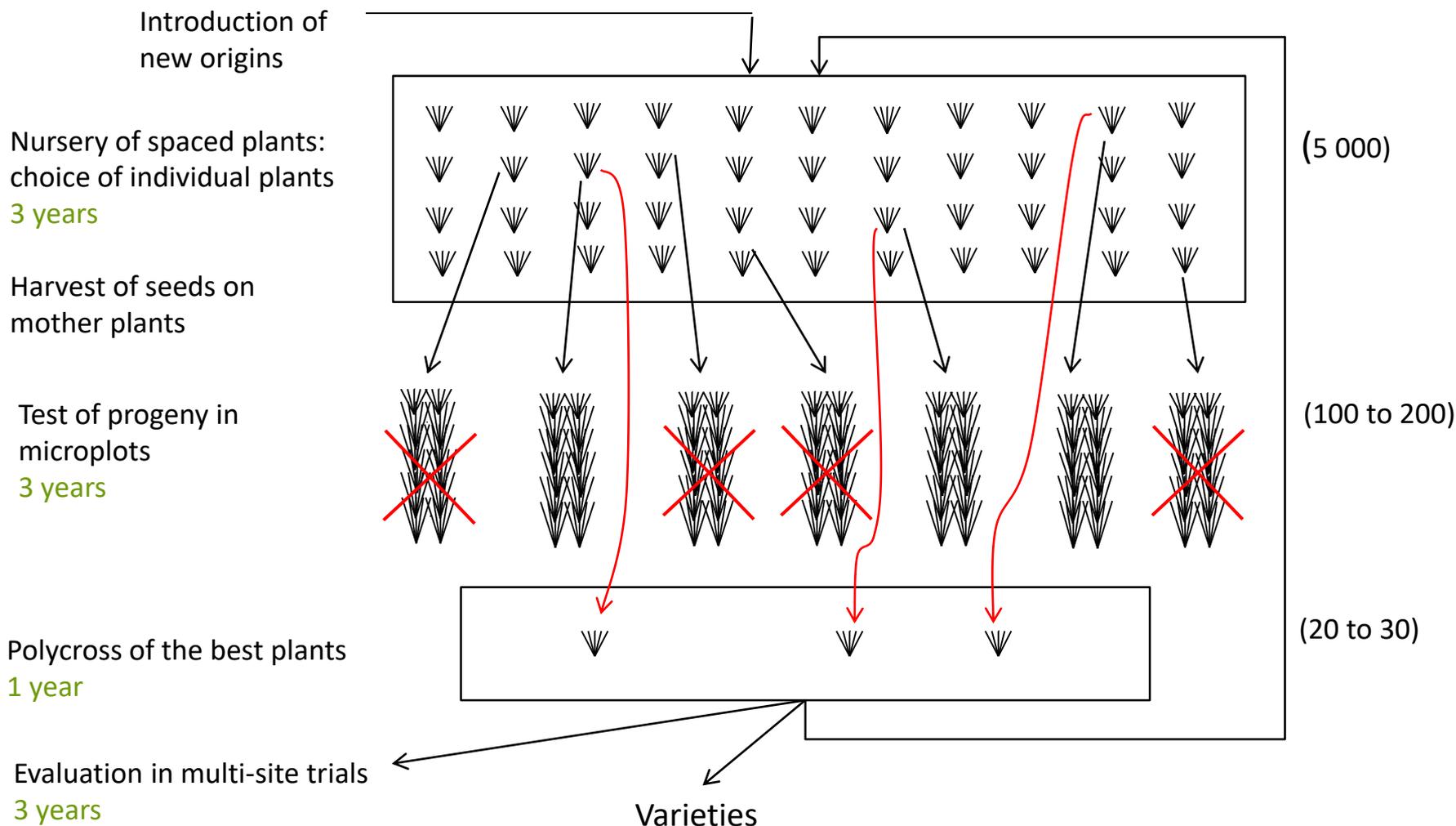


- Based on phenotypic traits
- First step in nursery of spaced plants
- Second step in progeny testing



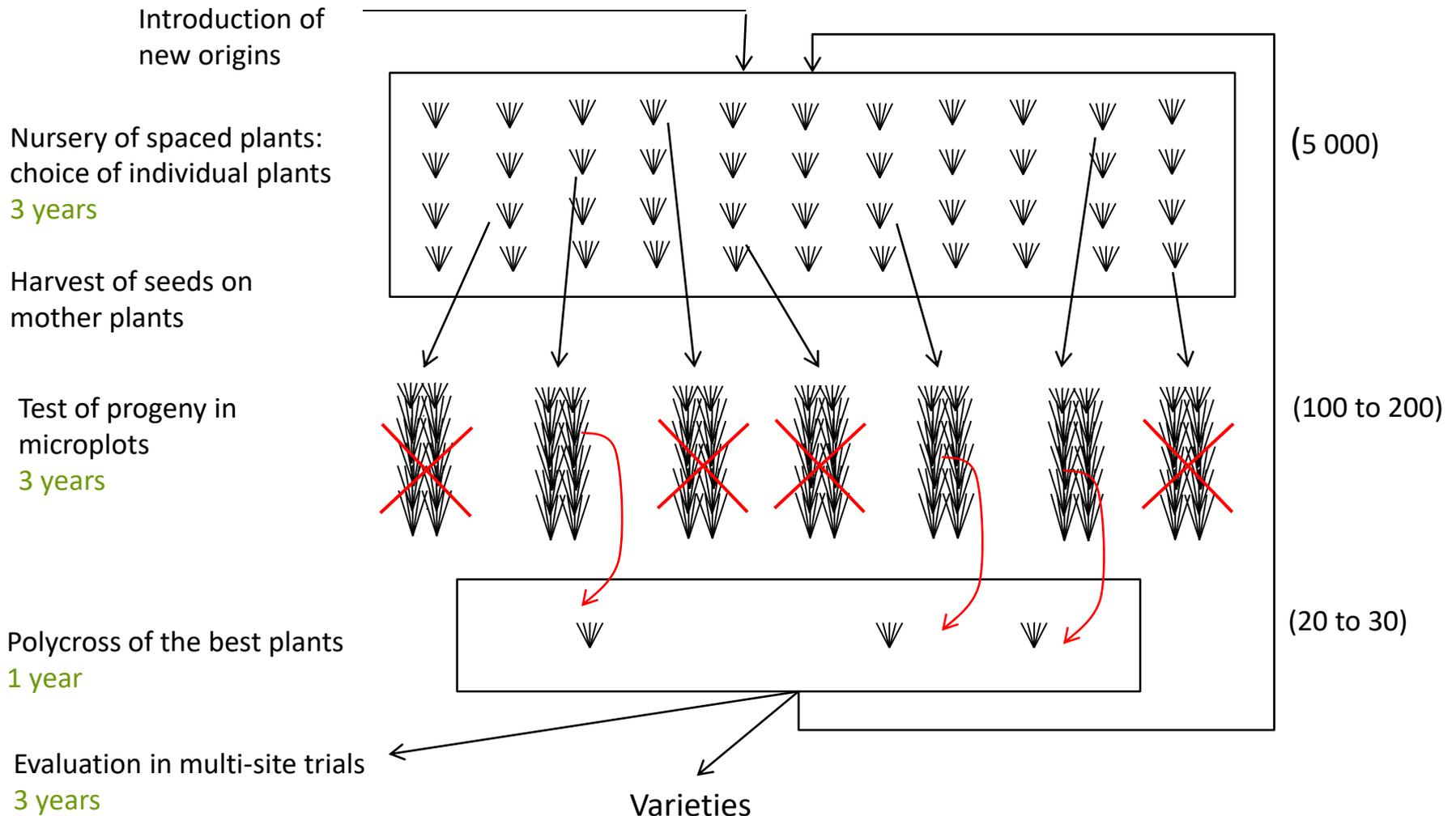
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Traditional breeding methodology



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Traditional breeding methodology



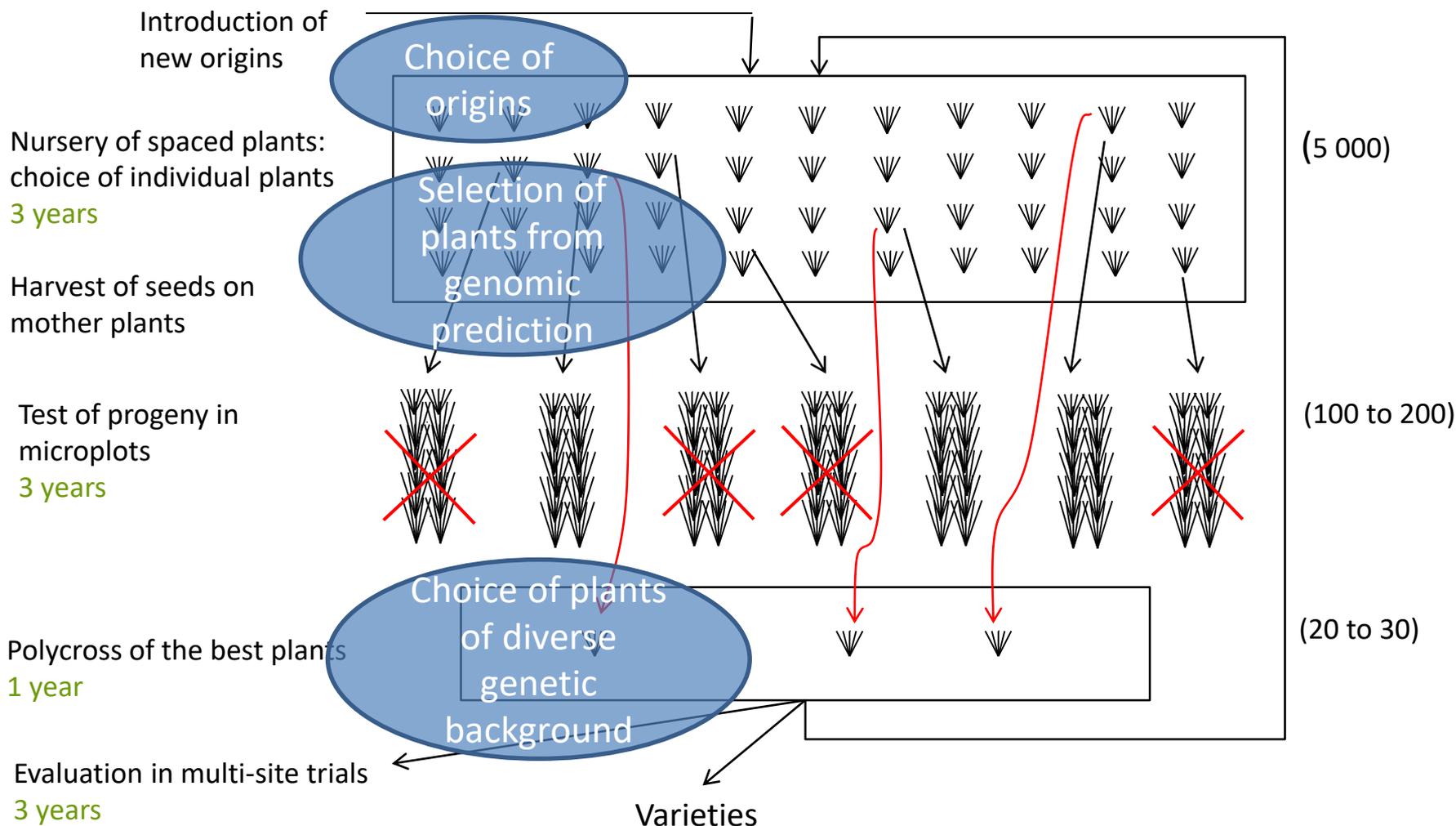
Strength

- 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



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Genomics assisted breeding



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



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.*

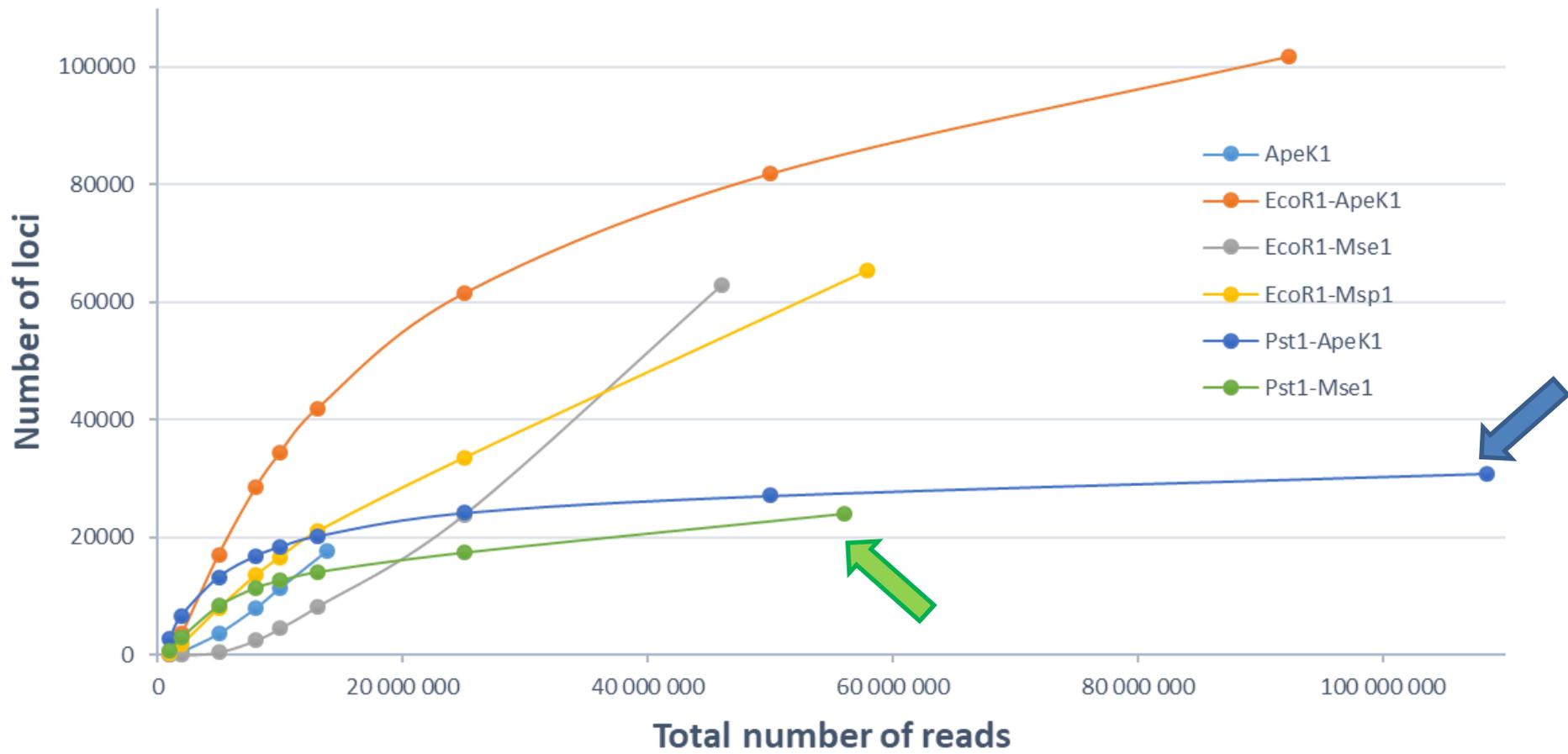
Before EUCLEG:

- 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



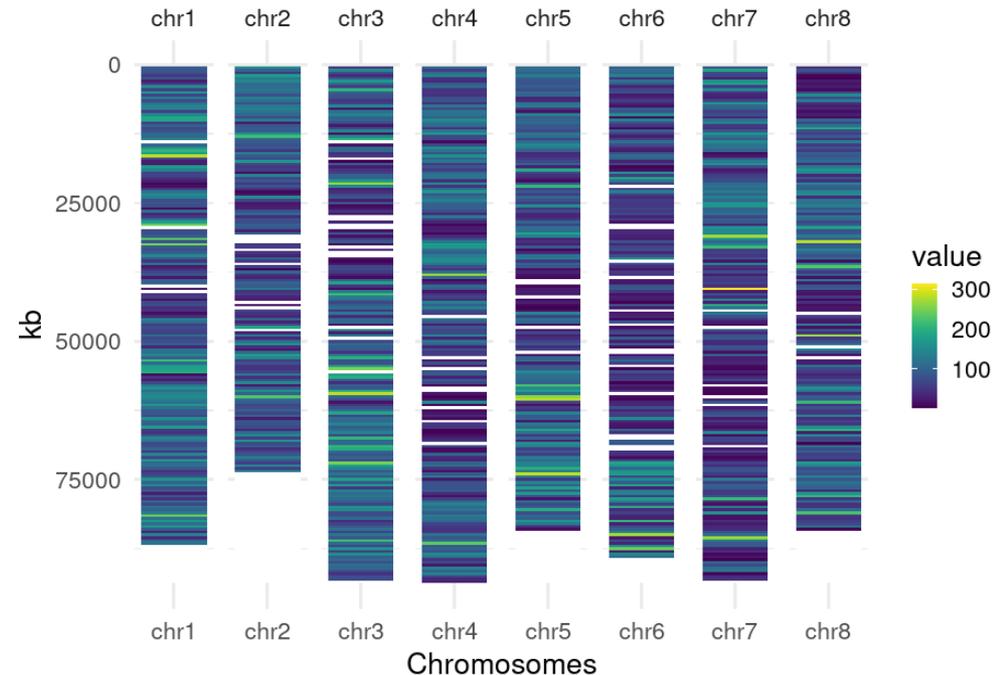
EUCLEG: an improved GBS protocol

- 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

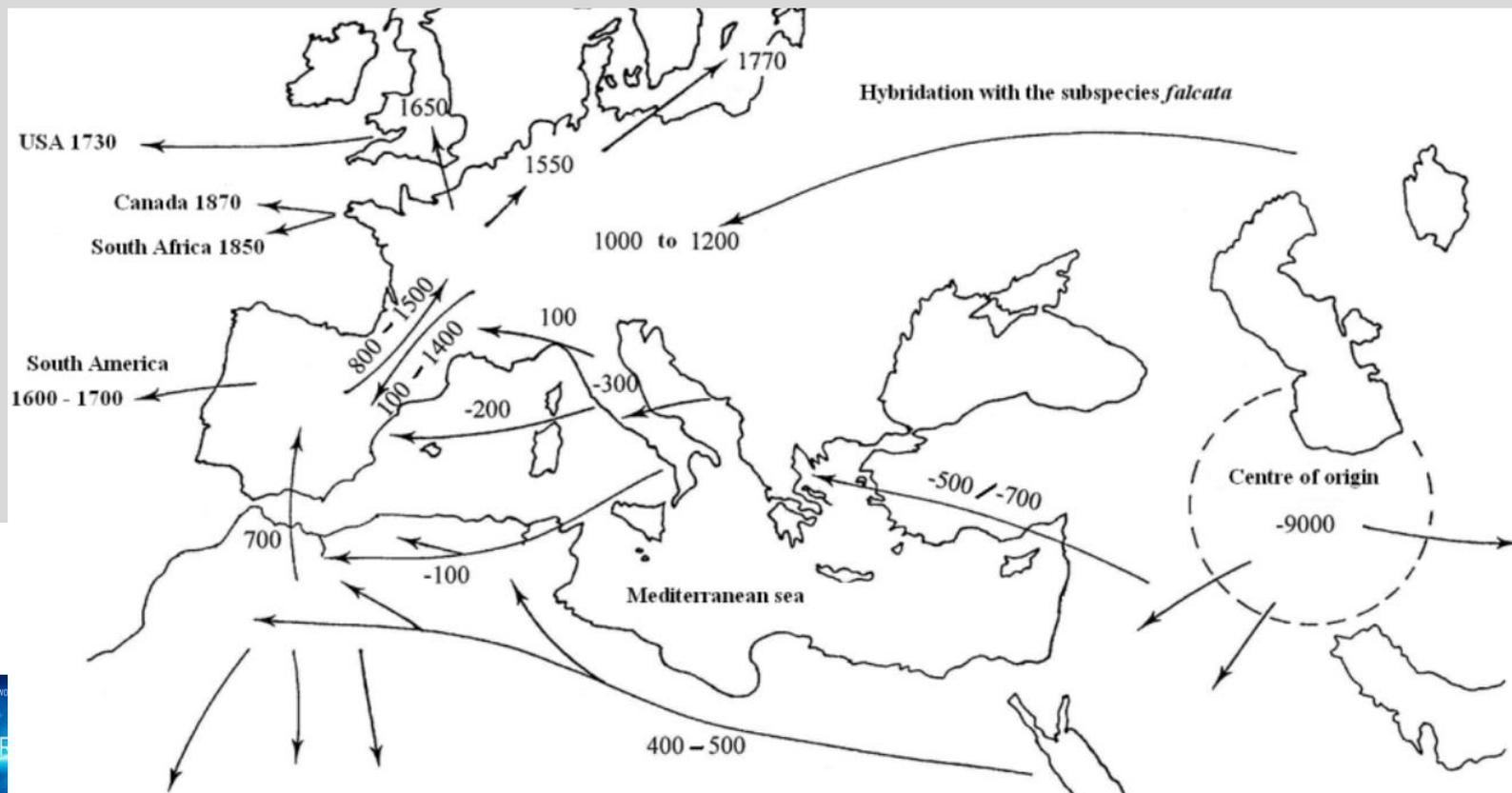
On 1 061 accessions:

- 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			

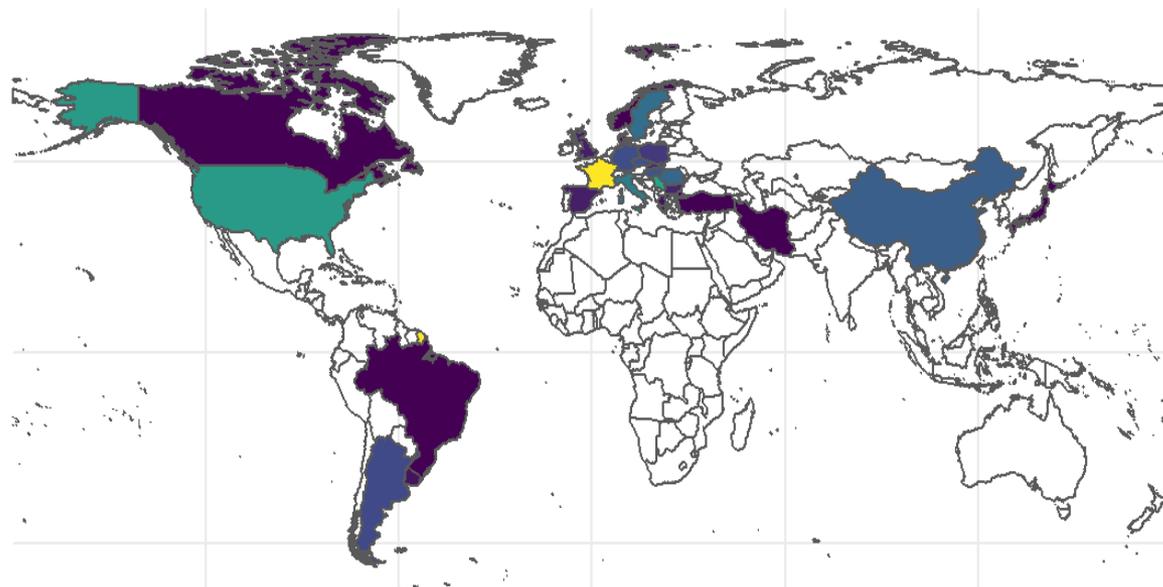
Herrmann et al., 2010

Julier et al. 2000

EUCLEG: a revision of diversity overview

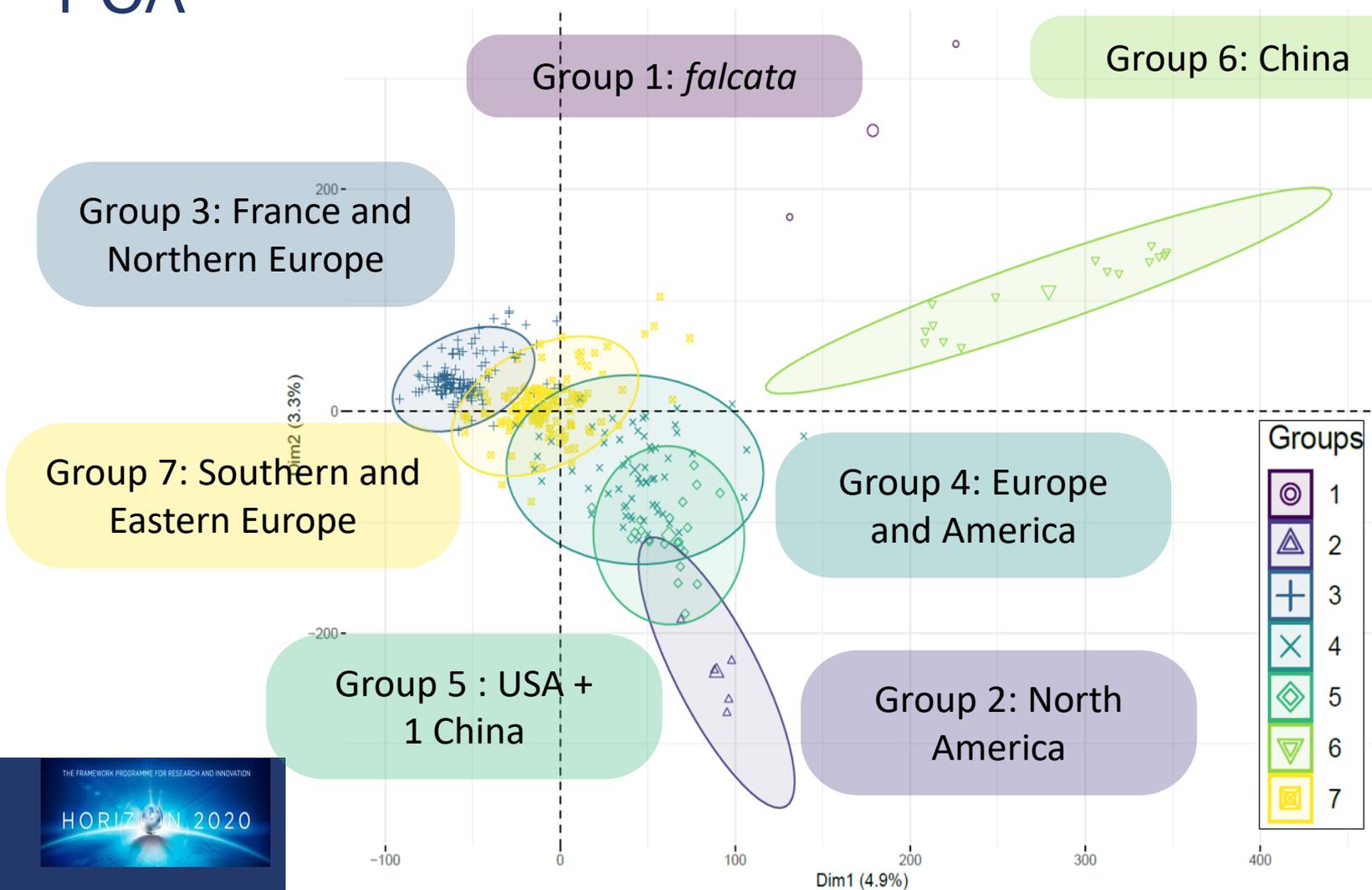
400 accessions: landraces and cultivars, dormancy 3 – 7:

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



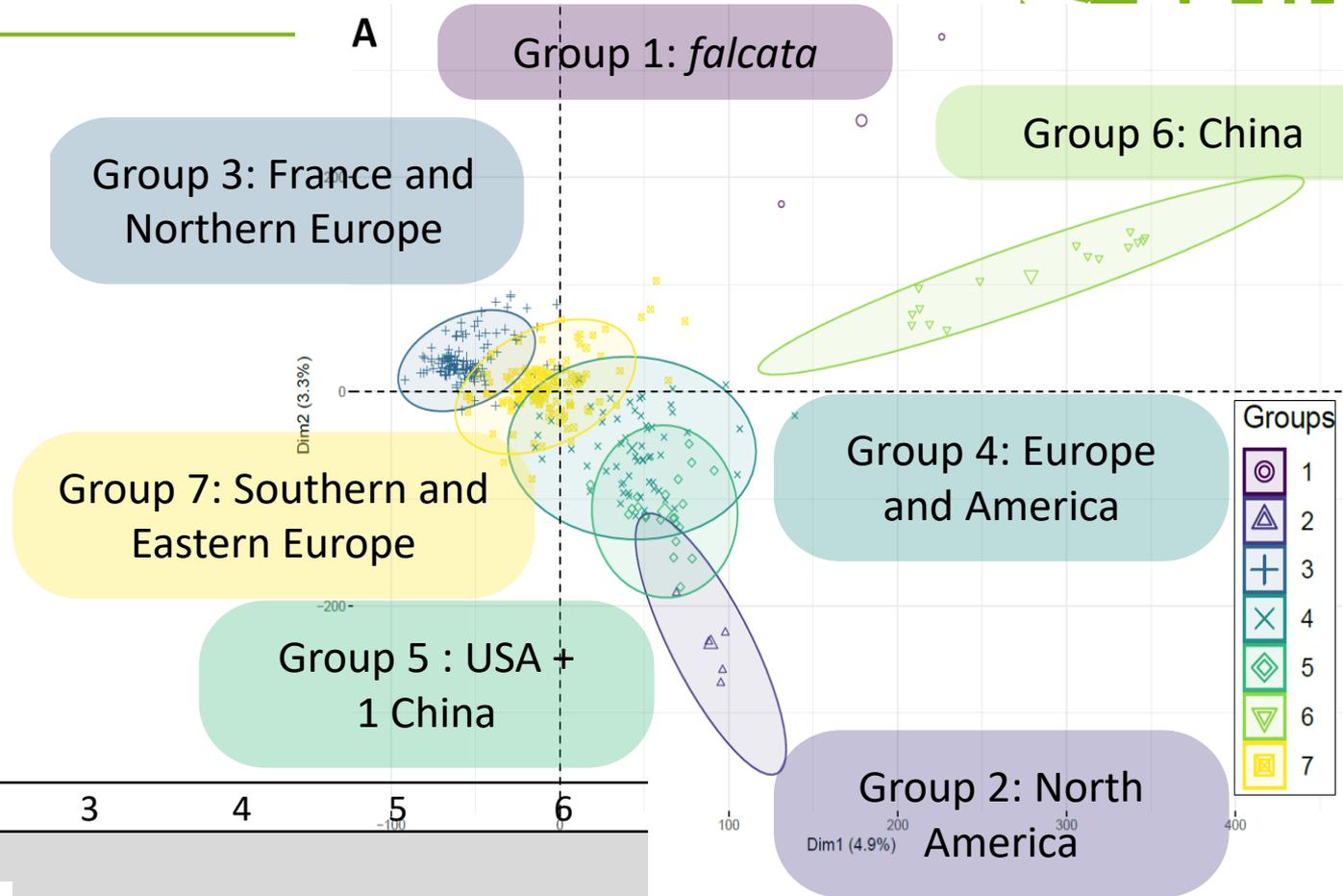
Management of genetic diversity

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

Management of genetic diversity

PCA : accessions colored with autumn dormancy score

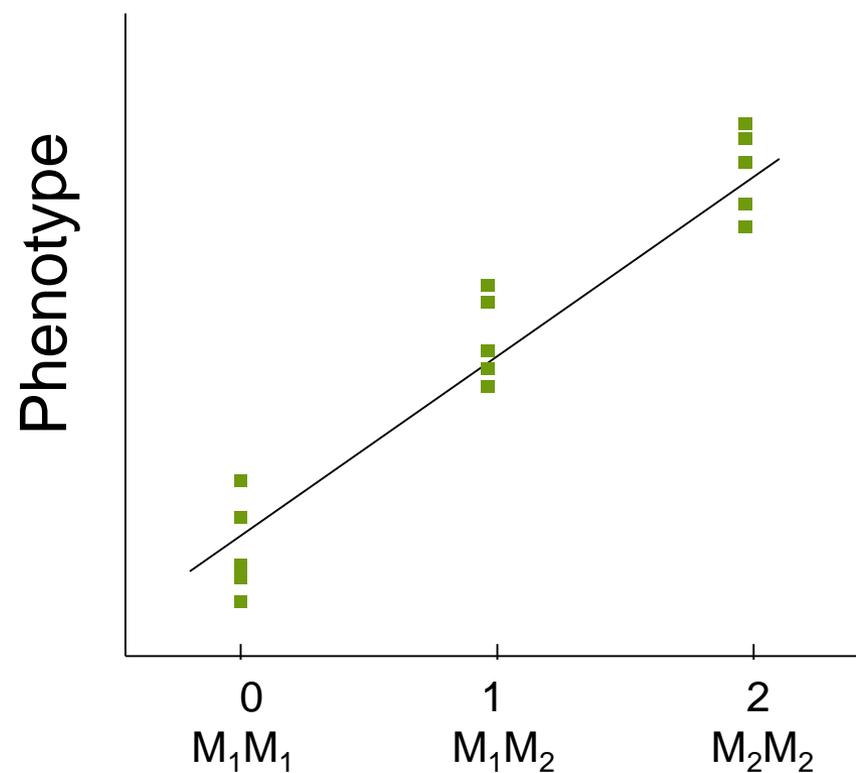
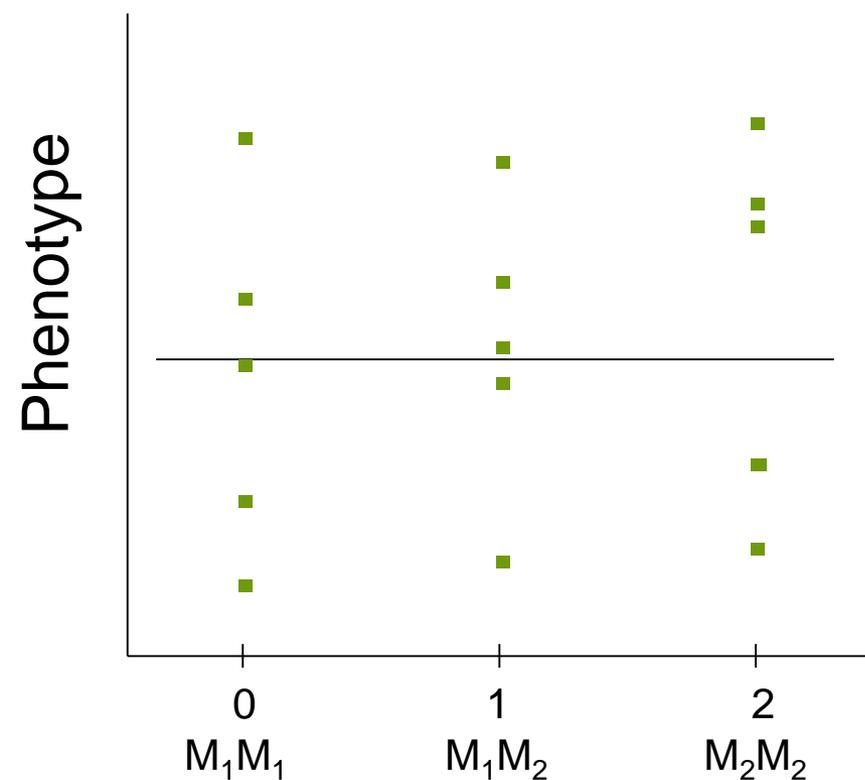


EUCLEG: a revision of diversity overview

- Diversity: China \leftrightarrow Europe + America
- Diversity: Europe \leftrightarrow America
- Structure is not associated to autumn dormancy

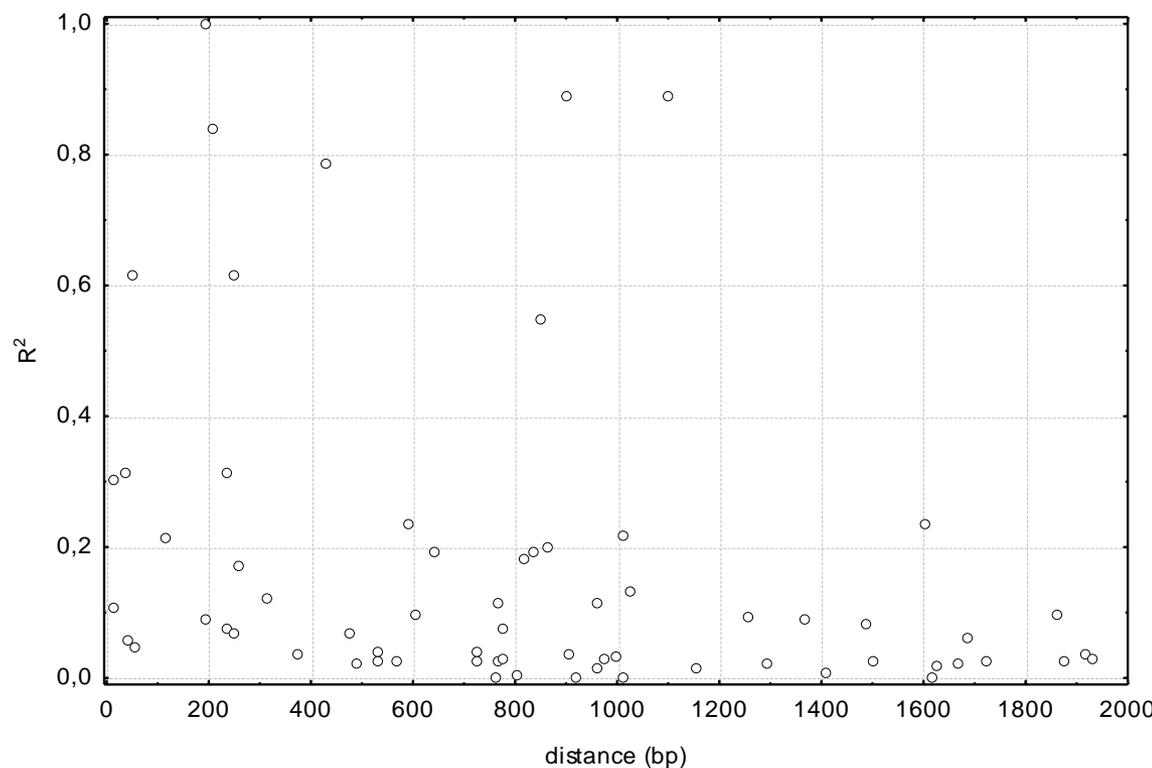
Genome wide association study

For each marker: is it associated to trait variation?



Before EUCLEG:

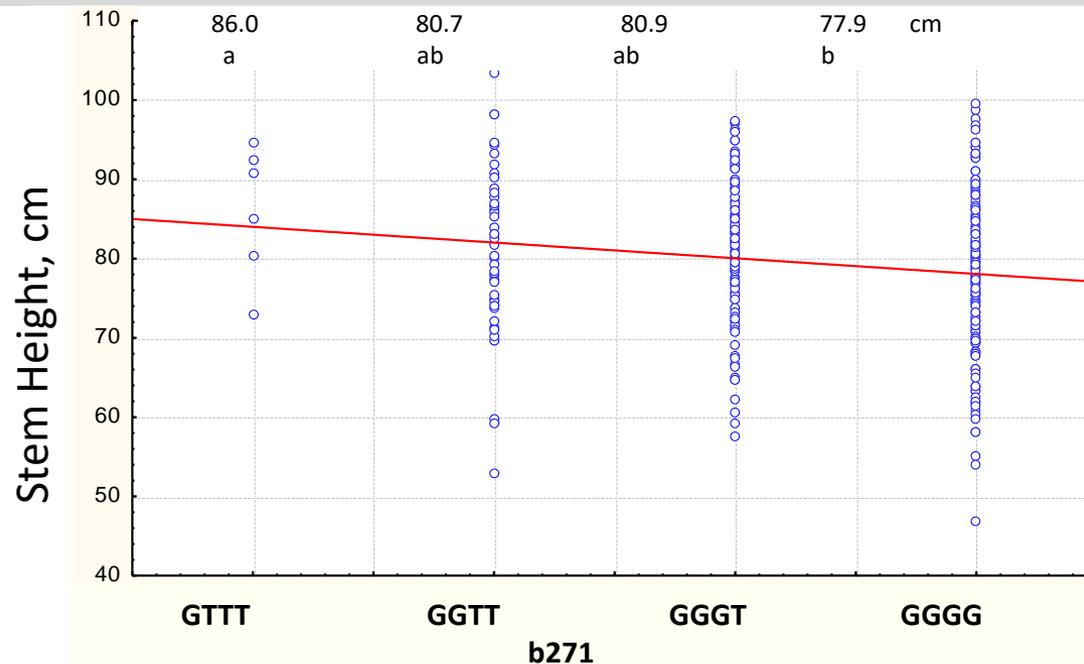
- Low marker density
- Short linkage disequilibrium



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

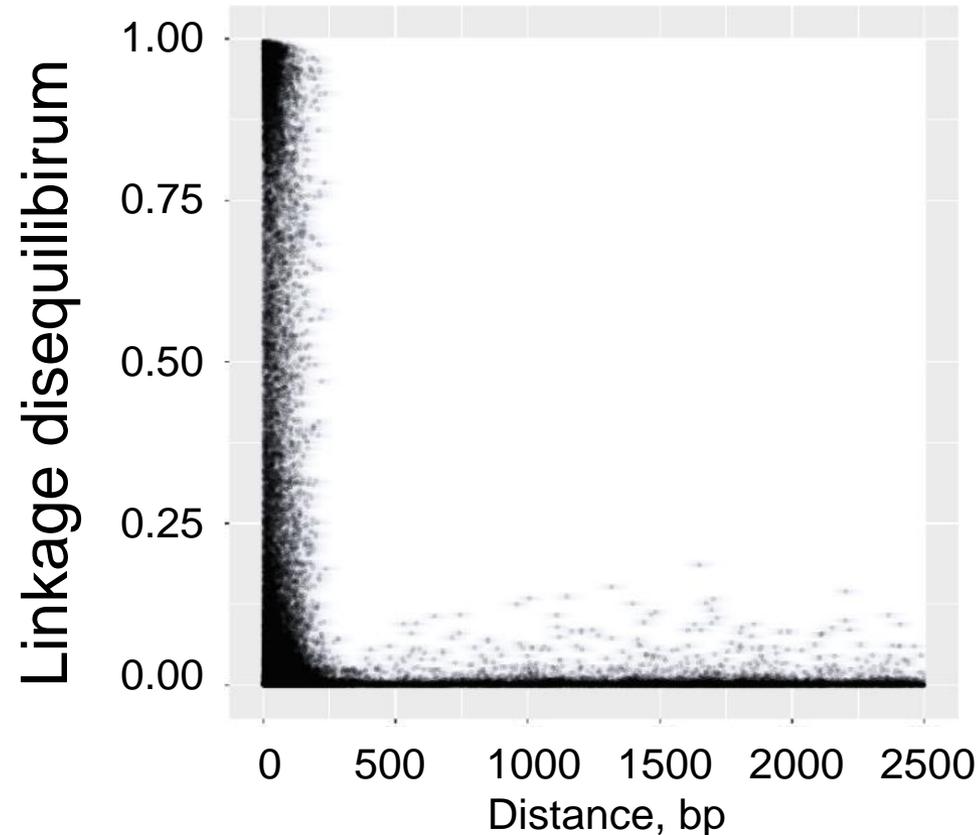
Before EUCLEG:

- 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

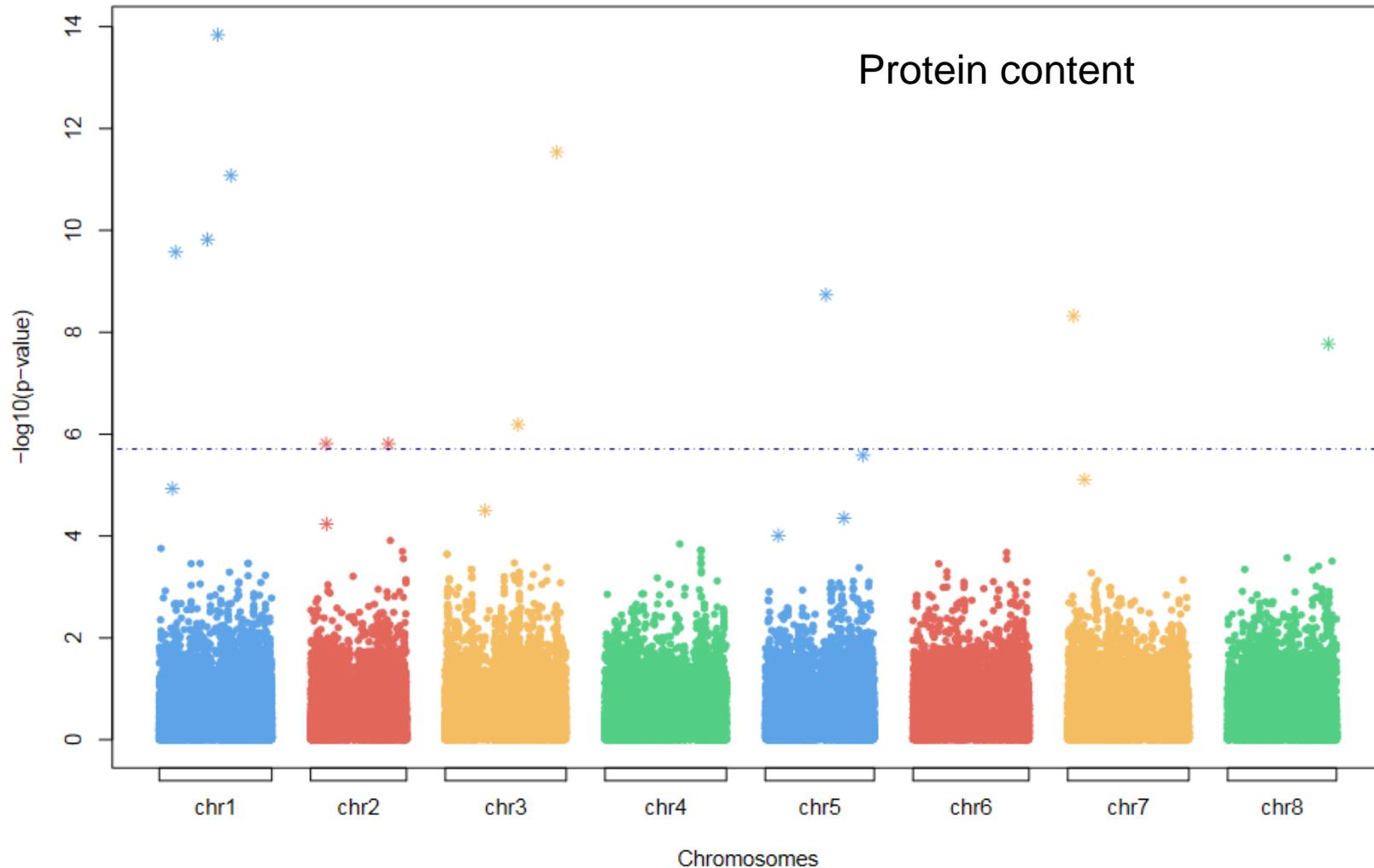
Method: Multi Locus Mixed Model (MLMM)



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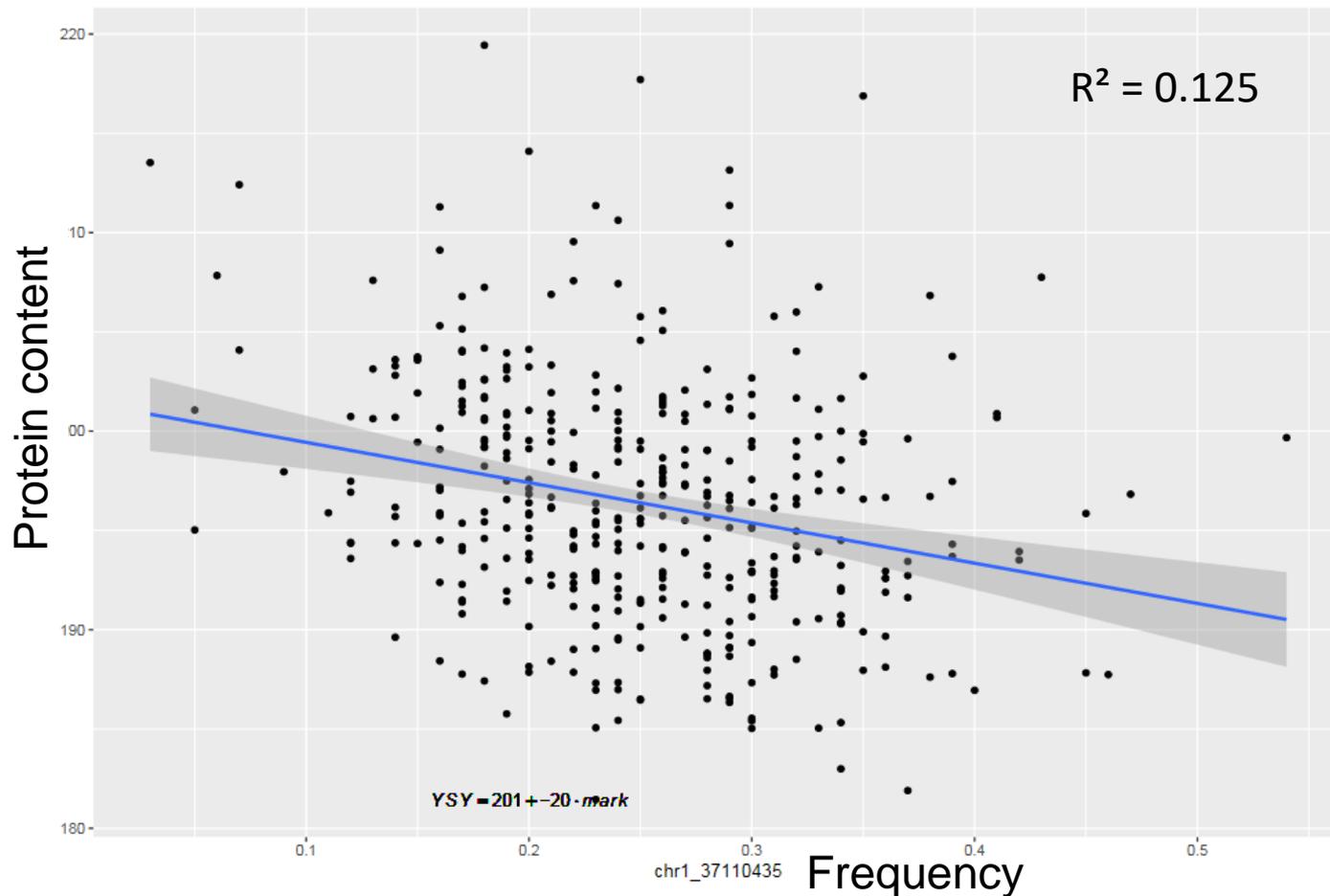
Genome wide association study

EUCLEG: Detection of major QTL

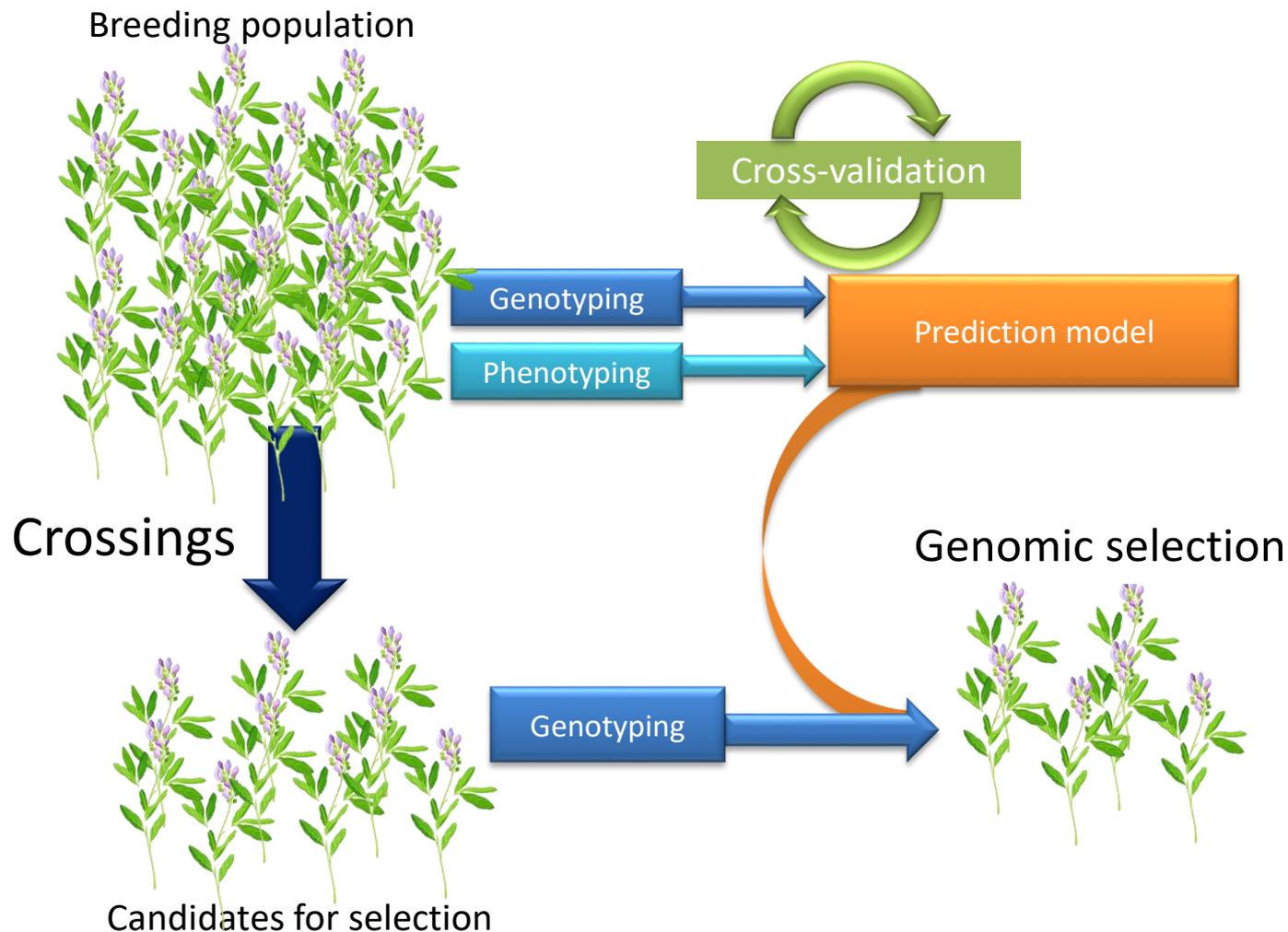


EUCLEG: Detection of major QTL

- Up to 10 – 20% of variation



Genomic selection



Before EUCLEG

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

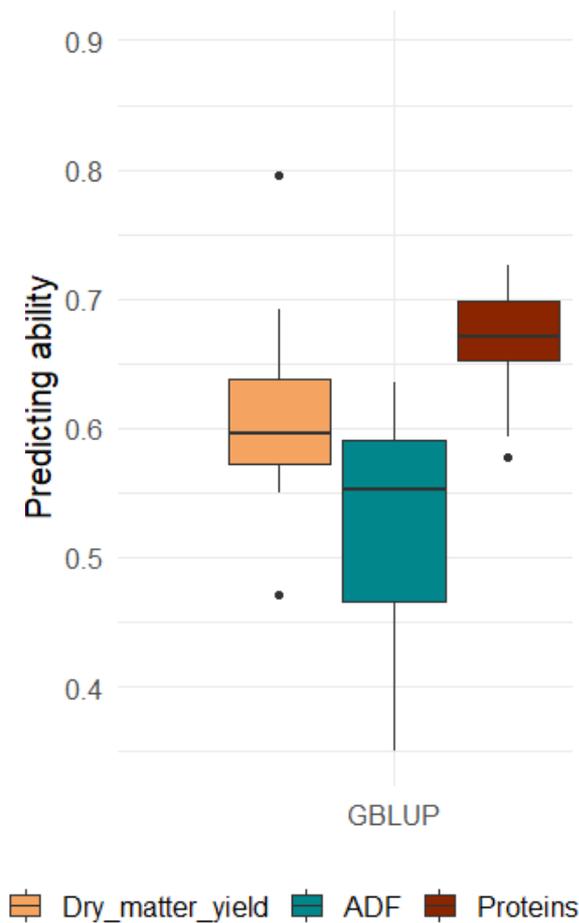
Genomic selection

EUCLEG

- GBLUP

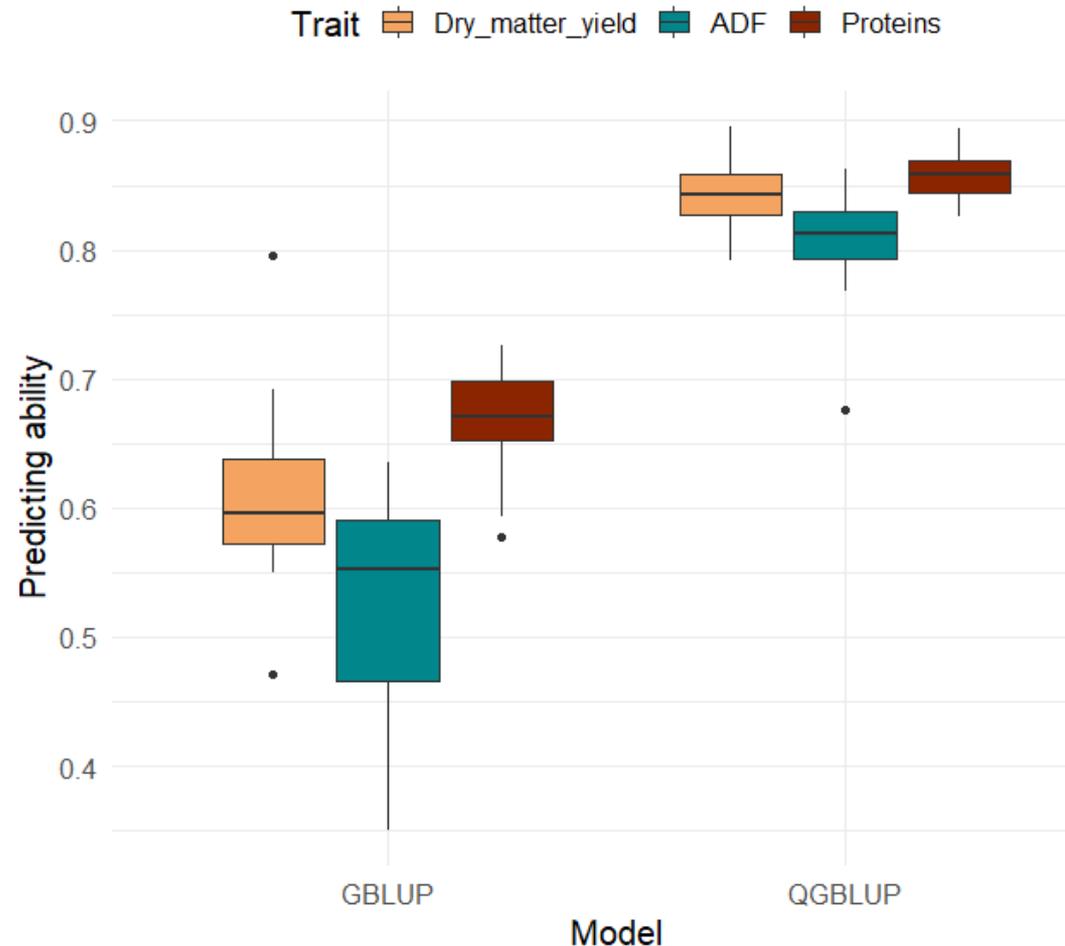
- A good predicting ability:

$0.52 < P < 0.66$



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

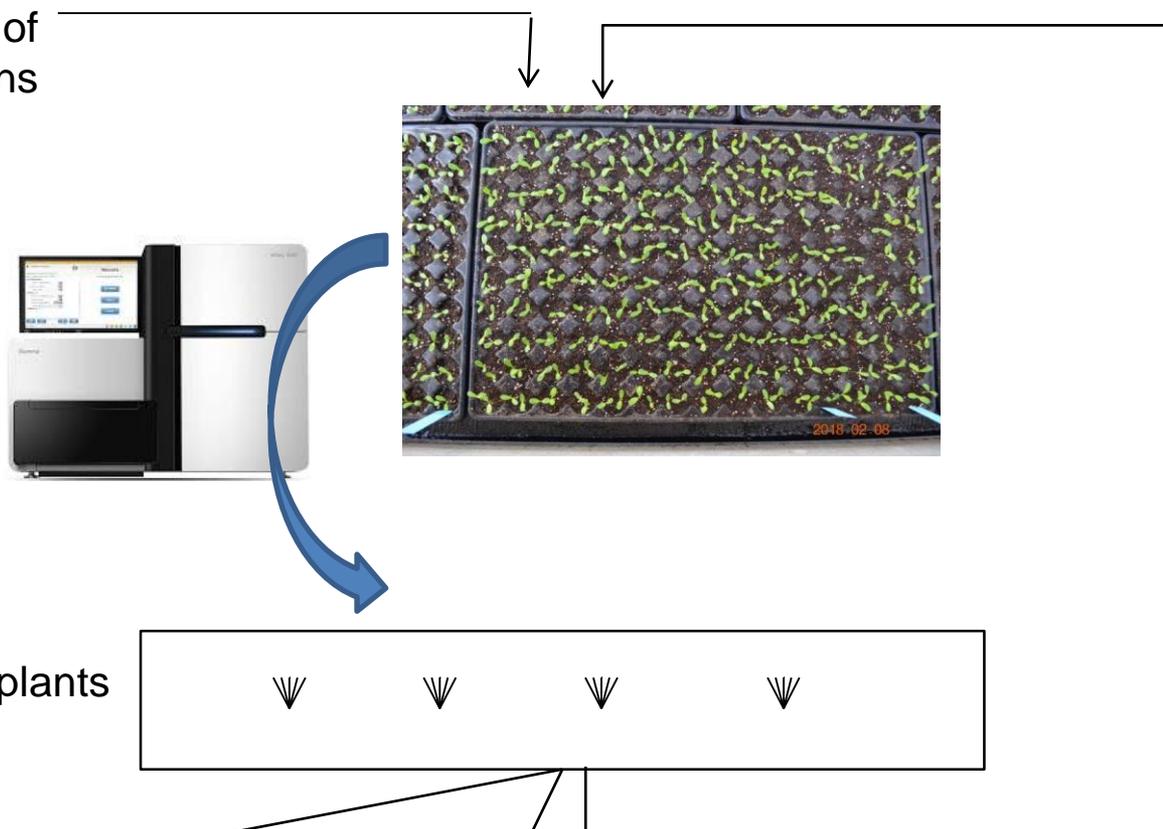
→ To be used to select promising individuals in breeding pools

- 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)

(20 to 30)

Polycross of the best plants
1 year

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?

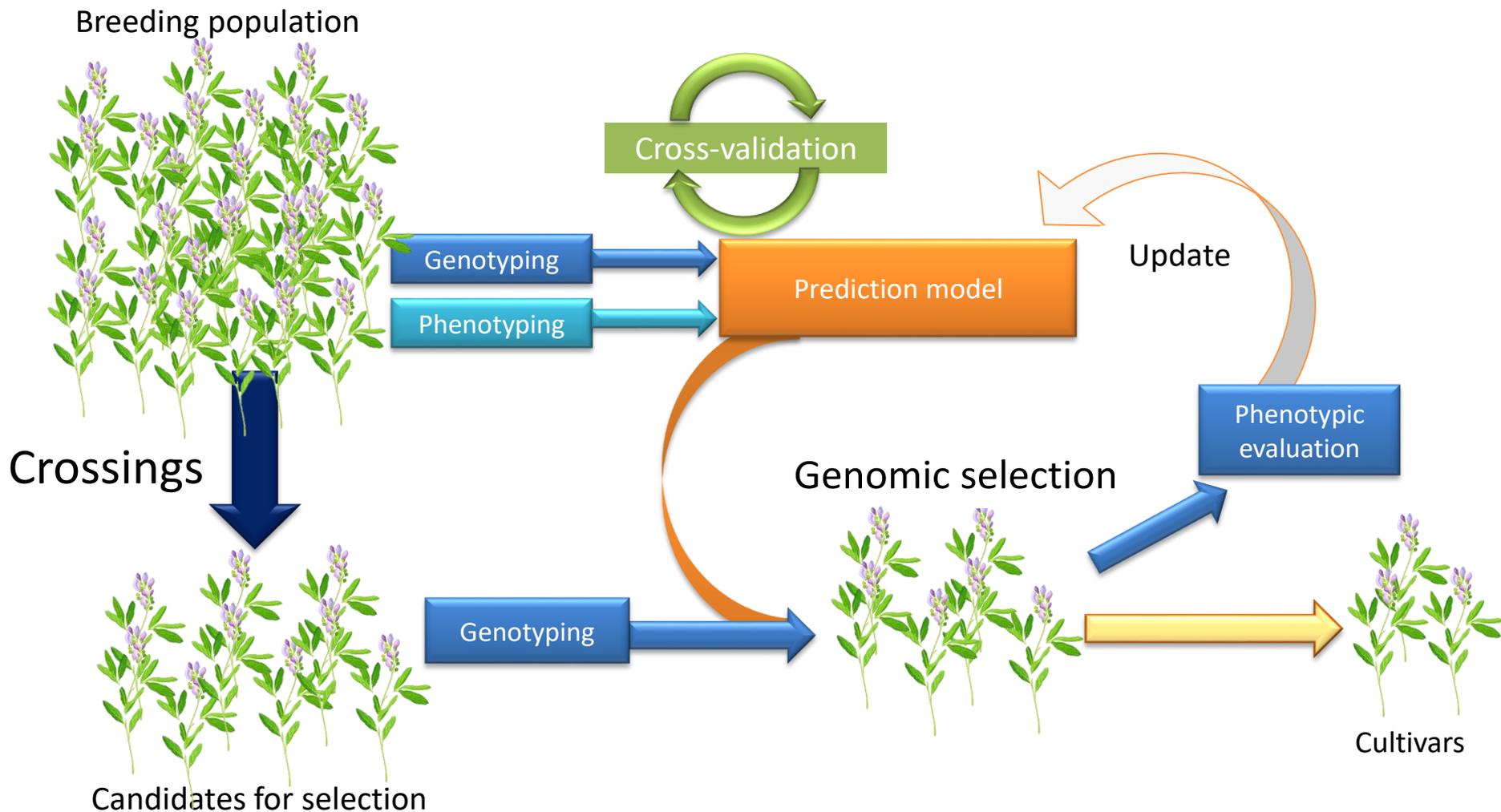
Weakness

- 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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EUCLEG.eu