

#### Genomics assisted breeding in alfalfa

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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"

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### 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

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# Traditional breeding methodology

HORIZ N 2020





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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



### 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







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## 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





# 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

10 populations, 40 indiv/pop			11 populations, 7-20 indiv/pop
	<u>5 SSR</u>	Plant height	<u>Yield</u>
Variance among-varieties	0.02	0.10	1.7
Variance within-varieties	7.56	0.30	27.7

#### 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





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#### PCA : accessions colored with autumn dormancy score





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EUCLEG: a revision of diversity overview

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





### For each marker: is it associated to trait variation?





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# 

# Before EUCLEG:

- Low marker density
- Short linkage disequilibrium



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

to decrease the EU's *European Union's* 



## Before EUCLEG:

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





### 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





# EUCLEG: Detection of major QTL 400 accessions x 2 locations x 2 years Method: Multi Locus Mixed Model (MLMM)

THE FRAMEWORK PROCOMMENT OR RESEARCH AND INICIDATION



### EUCLEG: Detection of major QTL



Chromosomes



# EUCLEG: Detection of major QTL

• Up to 10 – 20% of variation

HOR



# **Genomic selection**





#### Candidates for selection





### Before EUCLEG

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





# **Genomic selection**

EUCLEG

- GBLUP

HORIZ

A good predicting ability:0.52 < P < 0.66</li>





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0.4

# Genomic selection

Integration of the QTL information in the prediction model (QGBLUP)  $\rightarrow$  P > 0.80



GBLUP

Model





32

QGBLUP



# GBS are efficient markers

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

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GS models provide high predictive ability

- Even higher with the inclusion of QTL effect
- $\rightarrow$  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







# Strength

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

# Weakness

2020

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

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Genetic gain?

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**





#### Candidates for selection



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Horizon 2020 of European Union:

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