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Fabien Cormier, Sébastien Faure, Pierre Dubreuil, Emmanuel E. Heumez,
Katia Beauchêne

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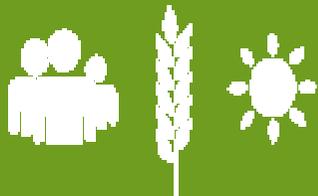
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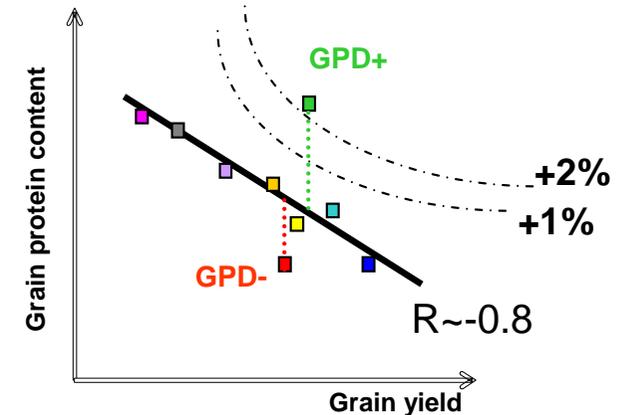
Nitrogen Use Efficiency in Bread Wheat: breeding tools and recent genetic progress

Fabien Cormier, Sébastien Faure, Pierre Dubreuil, Emmanuel Heumez, Katia Beauchêne,
Stéphane Lafarge, Sébastien Praud, Jacques Le Gouis

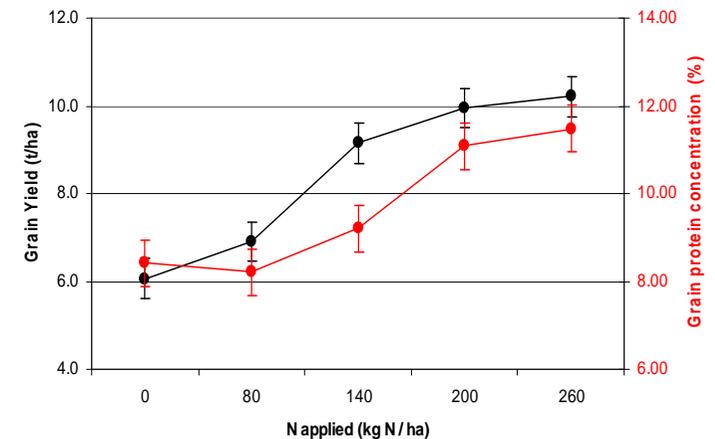


What are the N traits to select for ?

- **GPD** = Grain Protein Deviation
 - Proposed by Monaghan et al (2001)
 - Bonus for GPD+ lines during registration in France (2007)



- **NUE** = Nitrogen Use Efficiency ?
 - First official trials at 3 N levels harvested in 2013
 - Identification of potential criteria after two experimental years
 - Bonus for « efficient » lines by 2016 ?



What is Nitrogen Use Efficiency?

- Two main components

✓ **N Uptake** = total plant N / available N

✓ **N Utilization** = grain yield / total plant N

and sub-components : Straw N content, Grain N content, ...

- Two main possible targets

✓ **Sub-optimal** -> Maintain yield / quality

✓ **Optimal** - > Increase yield / quality

that will depend on Genotype x N level interactions



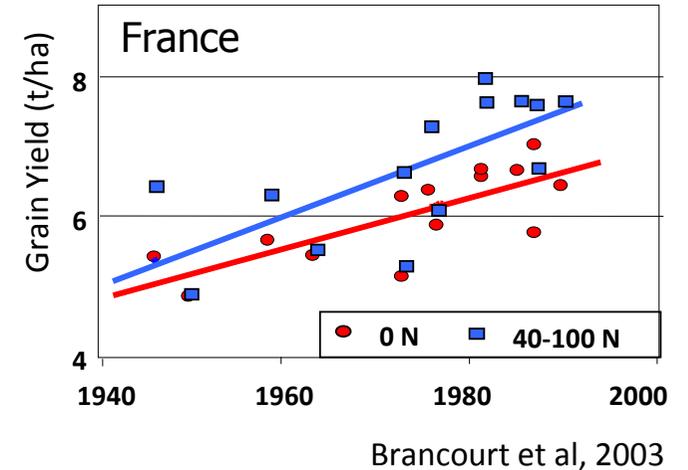
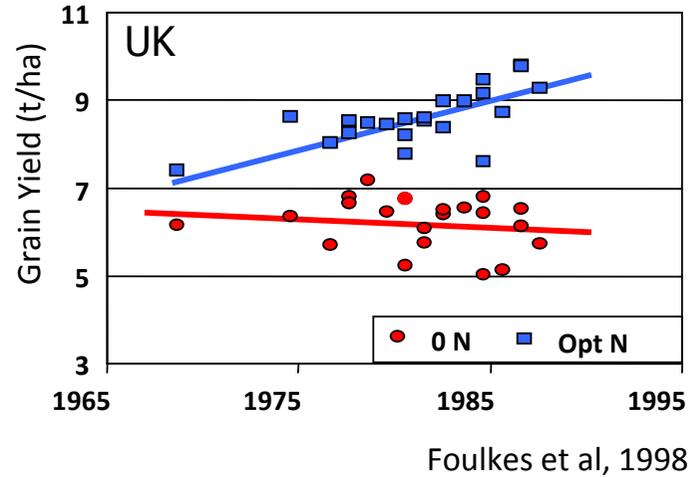
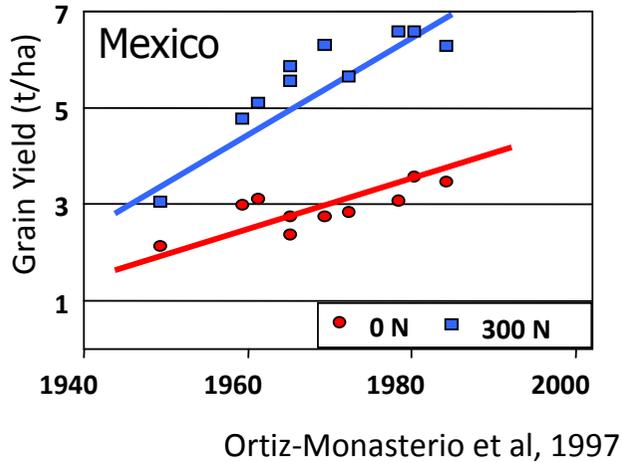
Utilization



Uptake



Can we improve NUE?



NUE = GY if we suppose Available N to be the same for all genotypes

What about recent breeding progress on NUE ?

Has NUE been increased ? How? For which N levels?

What are traits heritabilities?

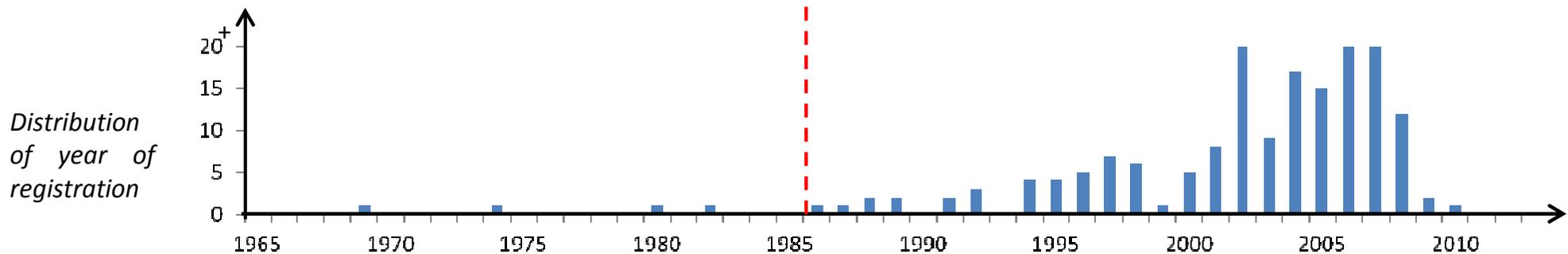
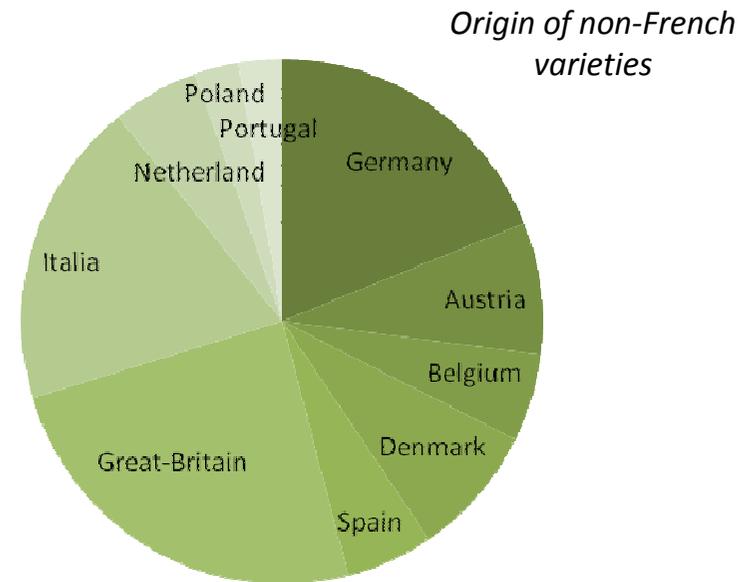
Is there GxN interactions?

In which environment to select?

Is elite diversity sufficient?

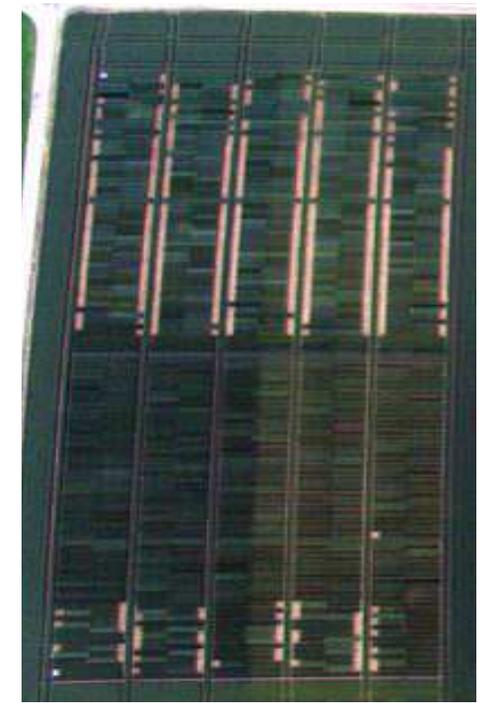
A panel of registered lines

225 European elite varieties
160 "French" + 65 others
Registered from 1985 to 2010



A network of field experiments

Site x Season	Years	Genotypes tested	Residual soil N (kg N ha ⁻¹)	N supply ¹ (kg ha ⁻¹)	
				HN	LN
VB08	07/08	197	106	126	44
VR09	08/09	196	30	220	120
EM08	07/08	207	67	170	70
EM09	08/09	208	30	150	50



Aerial view of EM08

8 trials = 2 N x 4 environments
with environmental characterization (daily temperature, rain,...)

2865 plots - 10 measured traits

182 varieties (out of 225) tested in all trials

Controls: Apache, Orvantis, Caphorn, and Soissons (07/08) or Premio (08/09)

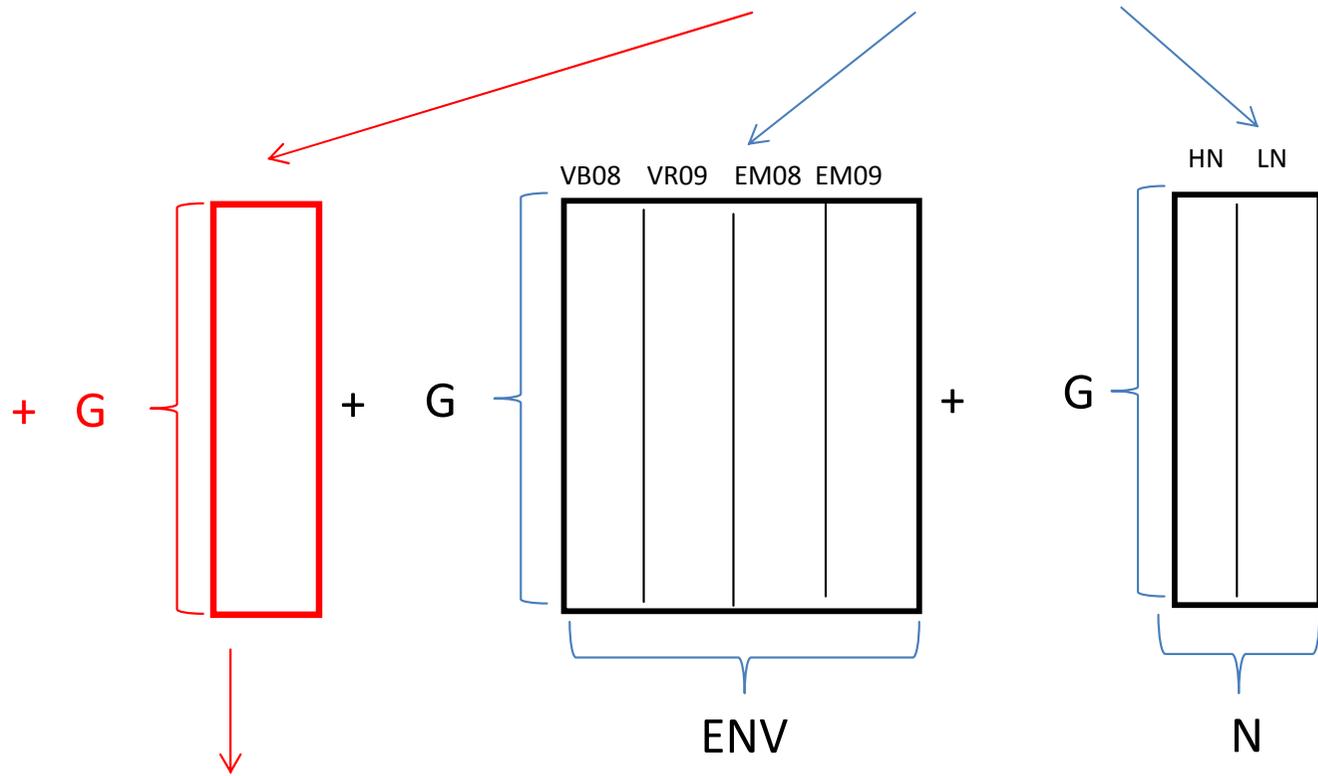


Location

Genetic progress estimation

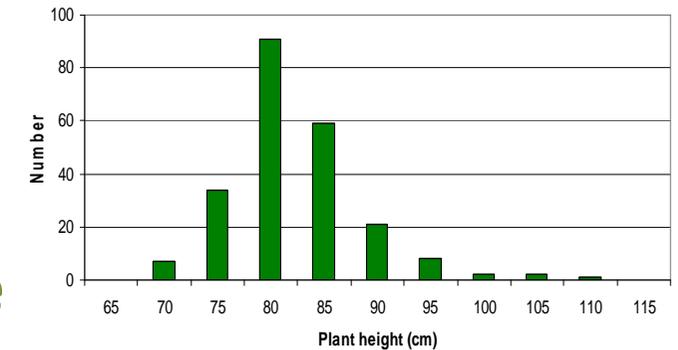
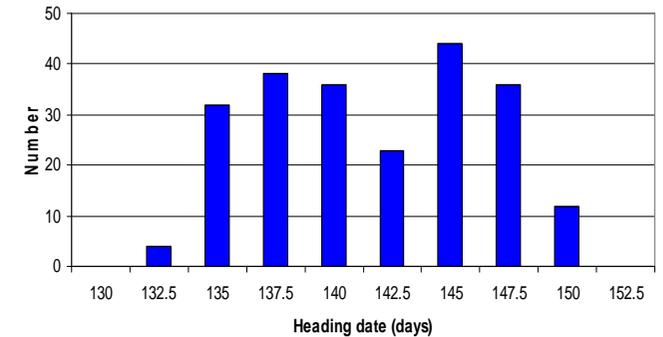
Additive effects

$$P_{ijk} = \mu + N_k + E_j + E_j \times N_k + G_i + G_i \times E_j + G_i \times N_k + \epsilon_{ijk}$$



G = Quality + Precocity + Height + Year of Release

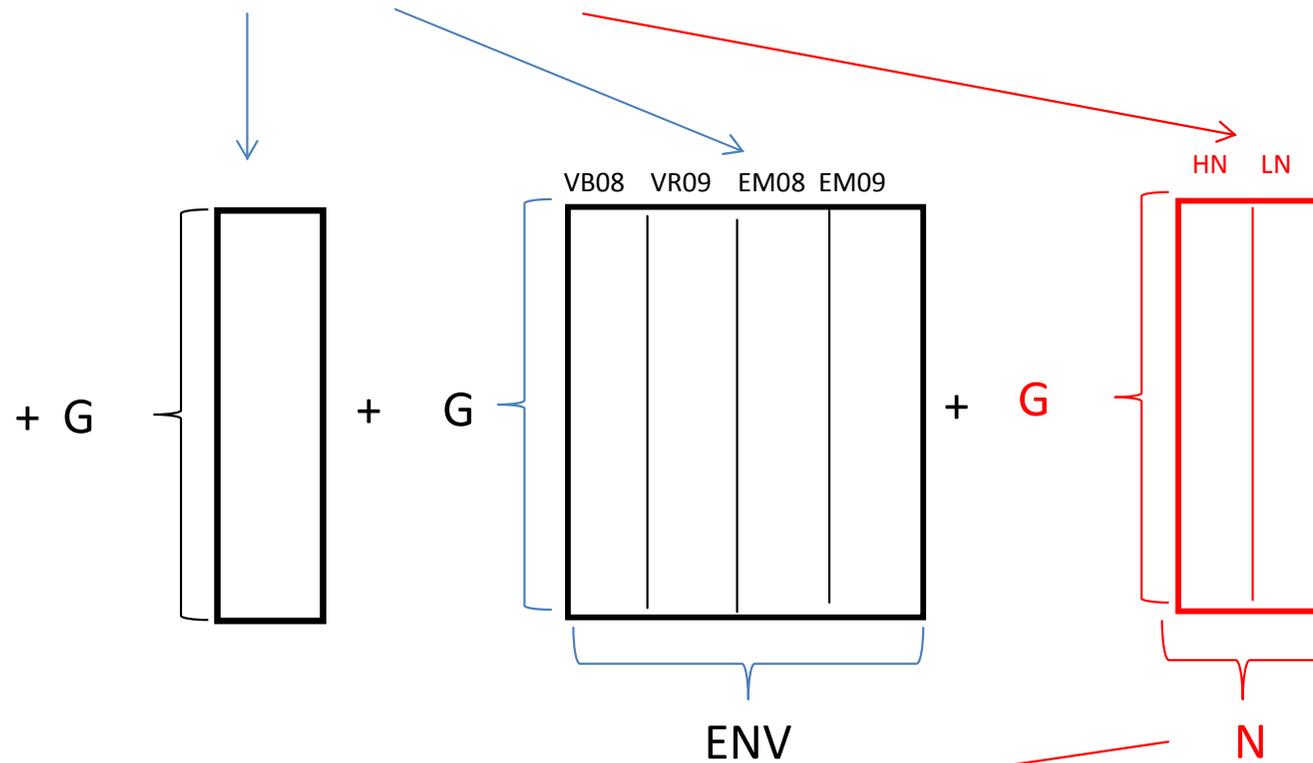
Quality classes	N°	YR means	GPC
High quality	104	2003 a	9.95 b
Good quality	50	2003 a	9.81 b
Biscuit quality	7	2001 ab	9.79 b
Other uses	7	2001 ab	9.20 c
Very high quality	12	1999 b	12.11 a



Genetic progress estimation

Interaction effect

$$P_{ijk} = \mu + N_k + E_j + E_j \times N_k + G_i + G_i \times E_j + G_i \times N_k + \epsilon_{ijk}$$

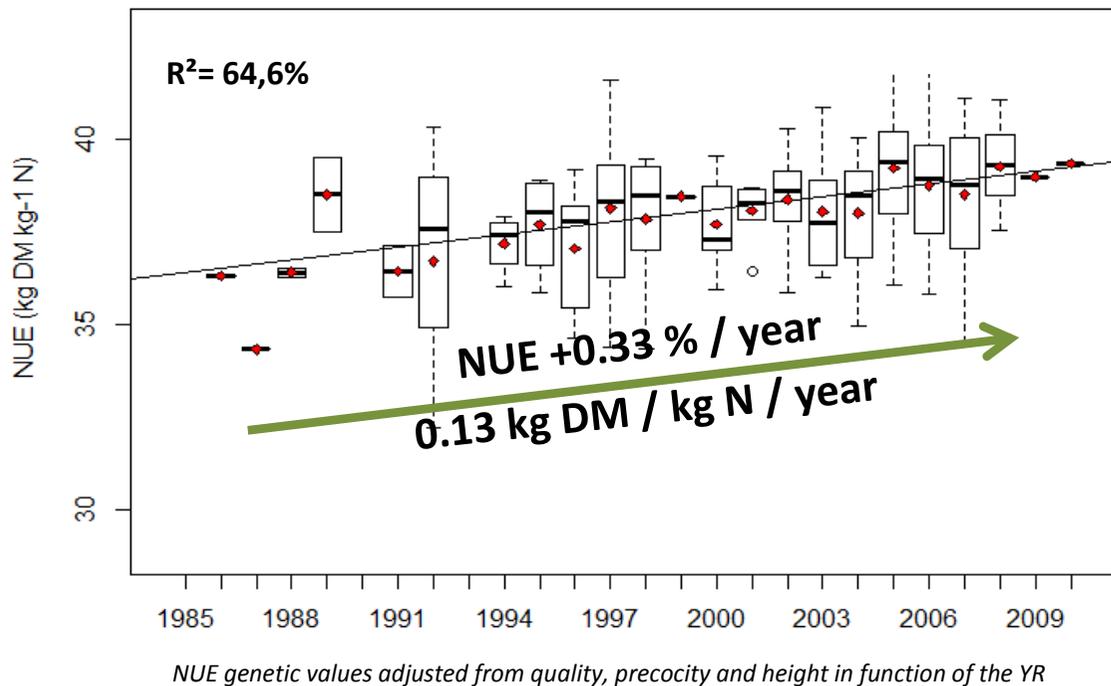


GxN = Quality + Precocity + Height + Year of Release

Genetic progress estimation

Variance components

Trait	h^2	G	G × E	G × N
NupE	0.00	10% ns.	77% ***	13% ns.
NutE	0.79	63% ***	30% ***	7% ***
NUE	0.80	69% ***	26% ***	5% *

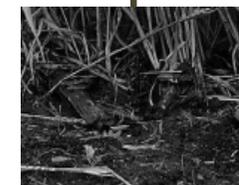


Utilization **



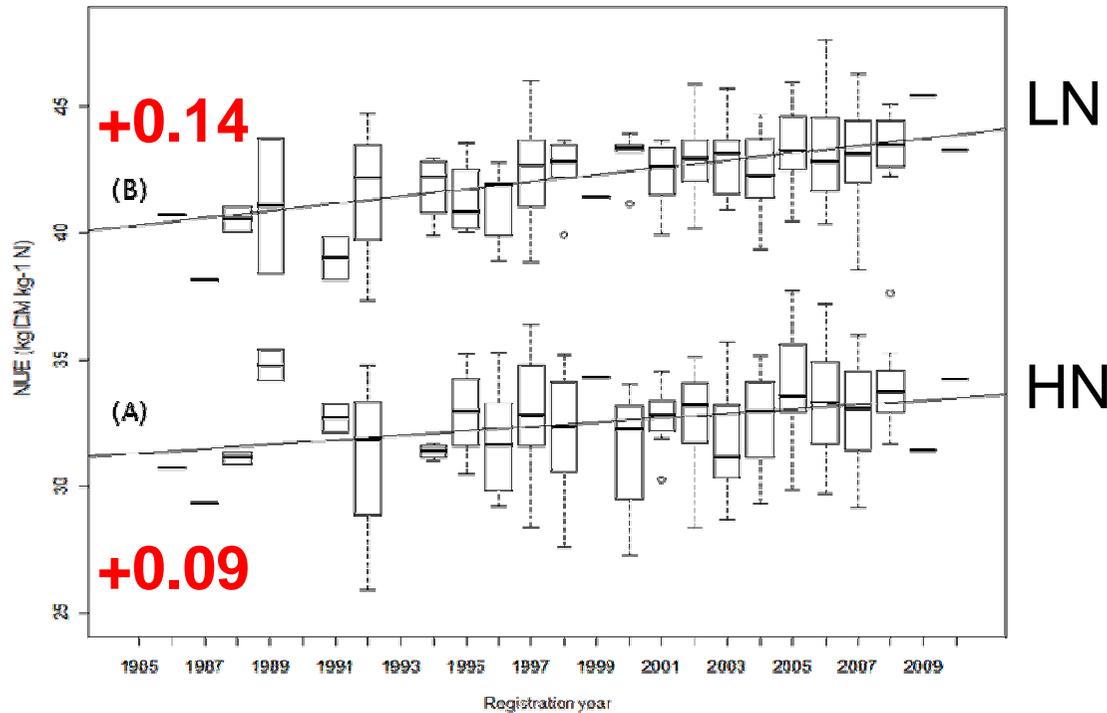
Nitrogen
Use
Efficiency

~~Uptake~~



Genetic progress estimation

Interaction effects



Significant difference between
HN and LN



Gain in yield stability



Based on heritabilities and genetic correlations:
Direct selection at LN is still the more efficient
Indirect Selection Efficiency ISE=0.78

How to maintain / accelerate this improvement?

- ✓ Increase genetic diversity
 - 2 new association panels (4600 INRA collection)
 - 10 advanced backcross populations



✓ Identify linked molecular markers

✓ Develop phenotyping tools

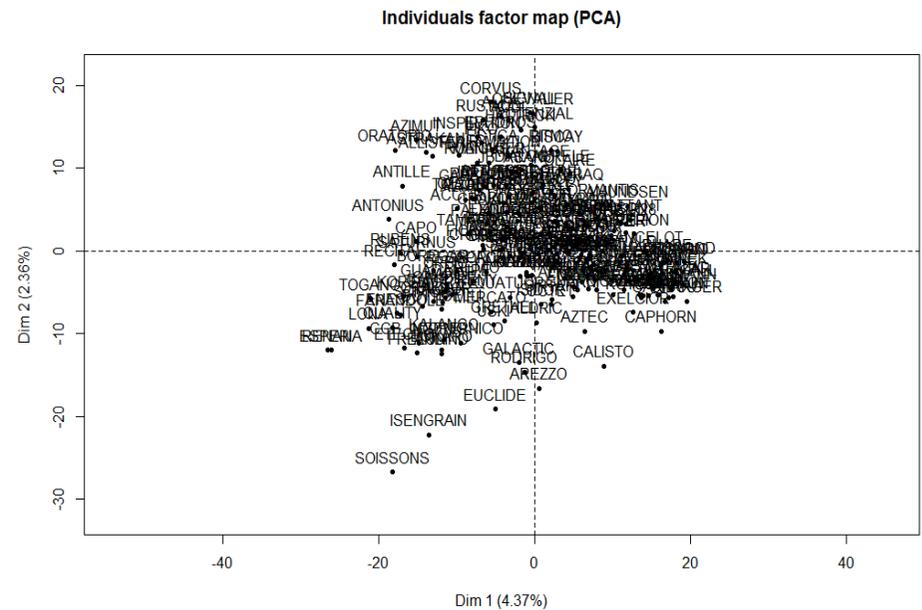
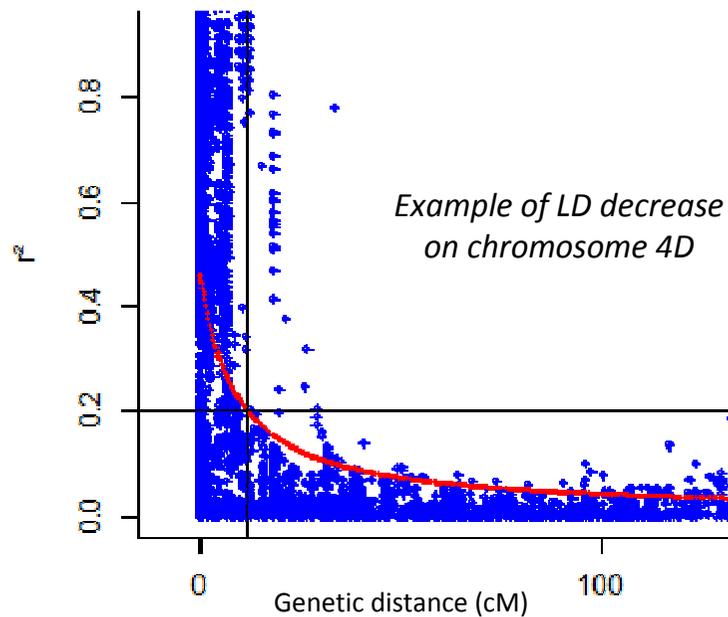
Association genetics parameters

214 genotyped varieties

34 382 polymorphic SNP (Illumina 90K + Biogemma)

Mean Linkage Disequilibrium (LD) decay = 1.1 +/-1.3 cM

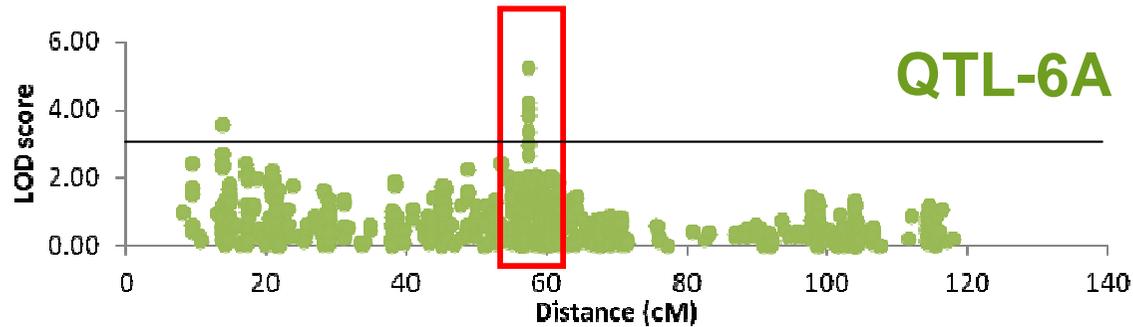
A poorly structured panel



Two first axes of the PCA of varieties by 3 461 non-redondant SNP

Example of an associated region

$$\text{NUE} = \text{ENV} + \text{Quality} + \text{Precocity} + \text{SNP} + \text{SNP} \times \text{N} + \text{K}$$



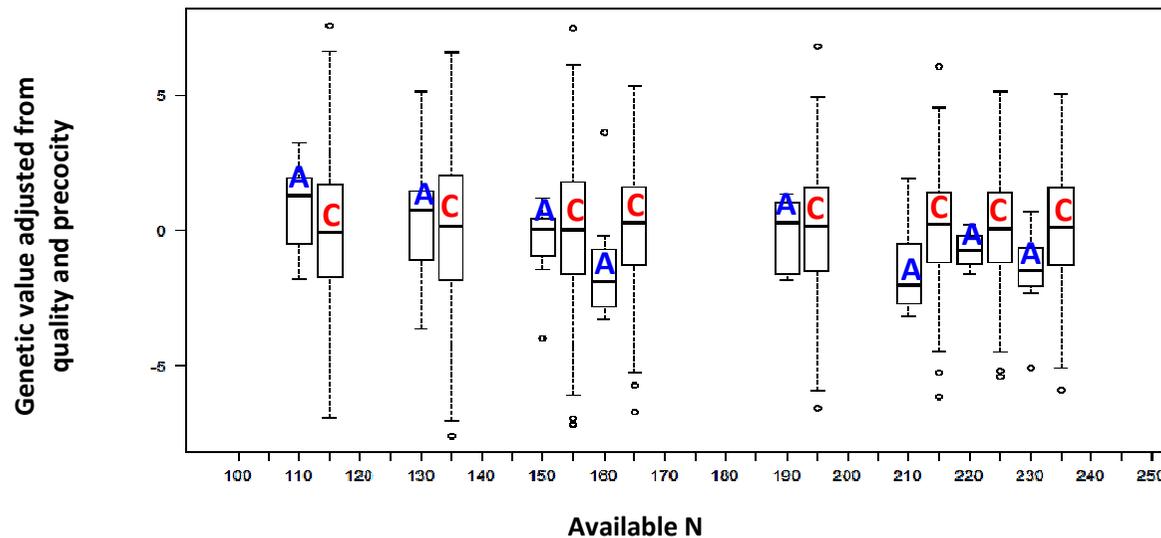
The most significant LOD score of interaction with available N



Effect on remobilization (NHI and %N in straw)



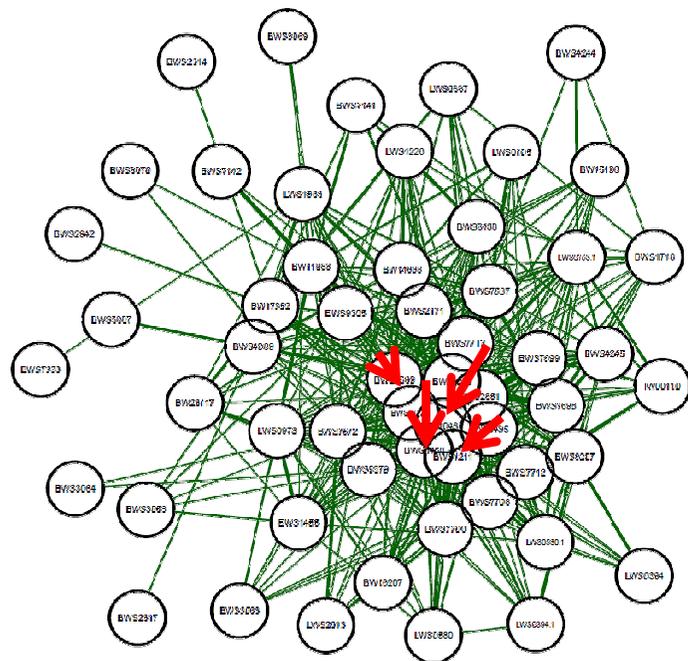
Region already identified in 2 independent yield GWAS (\neq GW2)



Epistasis and QTL6A

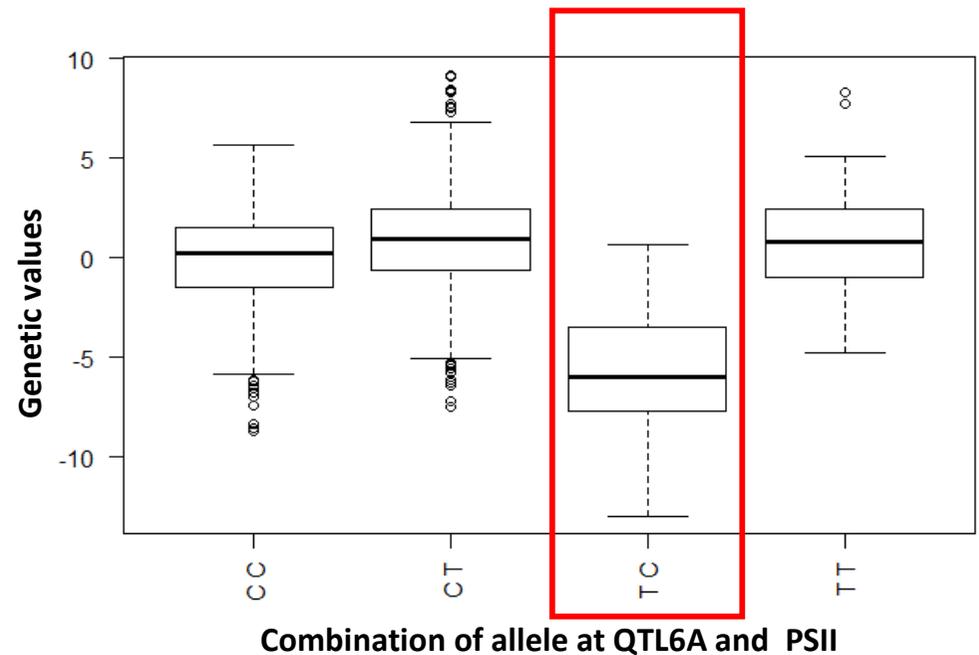
$$\text{NUE} = \text{ENV} + \text{Quality} + \text{Precocity} + \text{SNP}_i \times \text{SNP}_j + K$$

The effect of this region is significantly conditioned by others



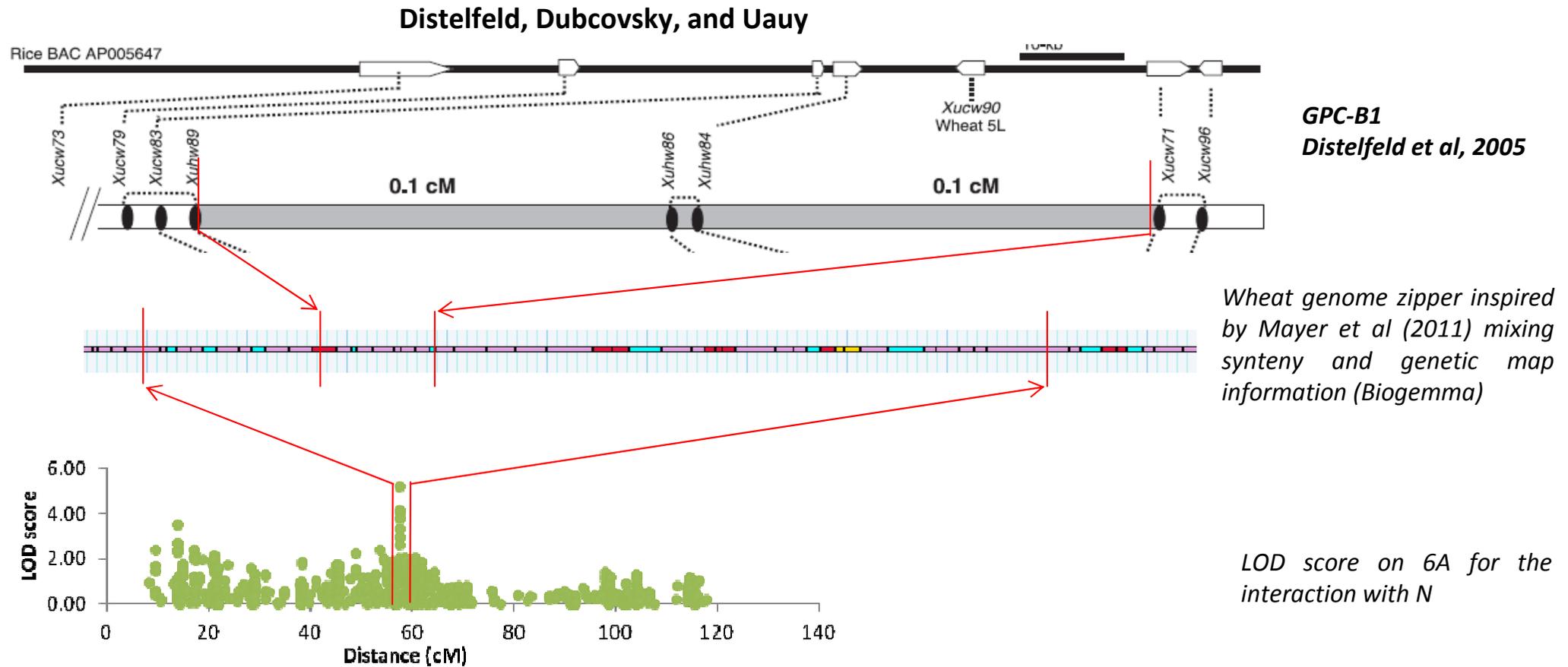
Interaction network based on significant epistatic interactions for NUE between SNP having a significant effect on NUE

20% of NUE genetic values can be explained by the interaction of QTL6A / Protein PSII



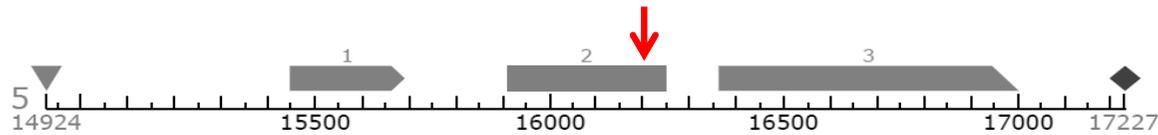
Boxplot of NUE genetic values as a function of alleles at QTL6A/PSII

A possible candidate gene

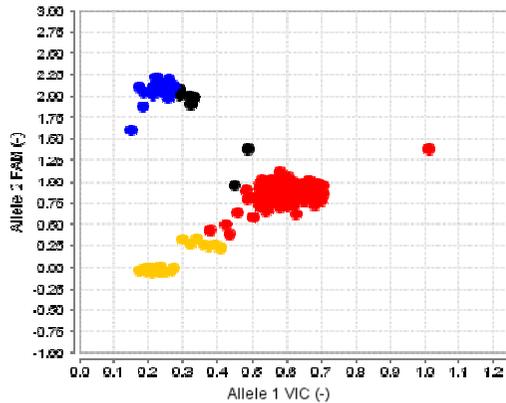


Looking for natural variants

Uauy et al. 2006 *TtNAM-A1* = IWGSC 6A seq



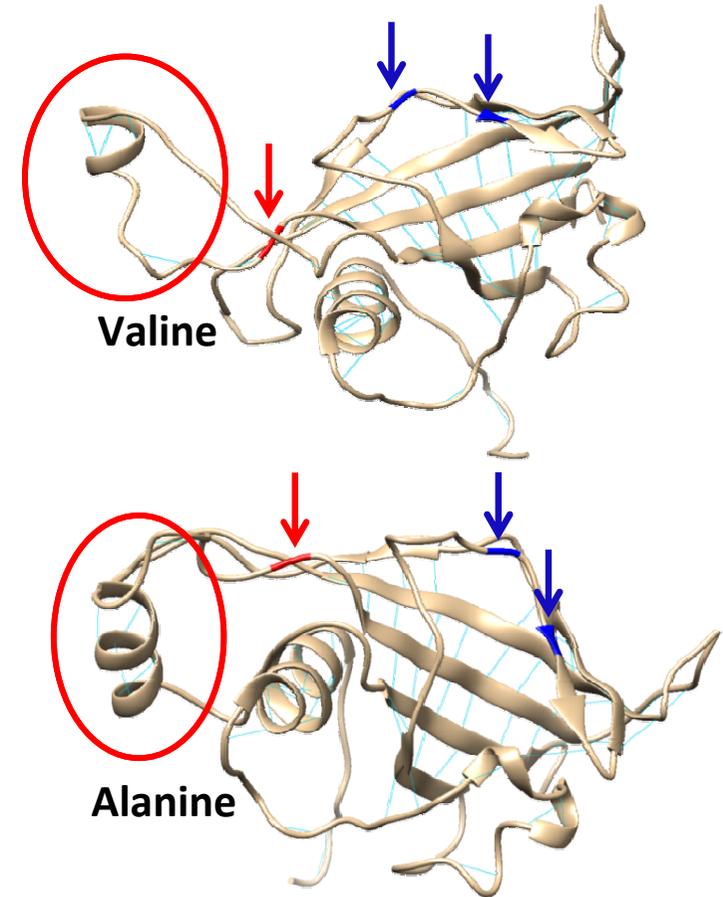
Genomic sequence of *TaNAM-A1*



Genotyping results of *TaNAM-A1*

GPC-A1 mapped in QTL6A confidence interval

→ polymorphism site
→ binding DNA aa



*Theoretical 3D structure of natural variant of *TaNAM-A1* in European elite bread wheat (Chimera)*

How to maintain / accelerate this improvement?

- ✓ Increase genetic diversity

- 2 new association panels (4600 INRA collection)
- 10 advanced backcross populations



- ✓ Identify linked molecular markers

- ✓ Develop phenotyping tools

Development of high-throughput techniques

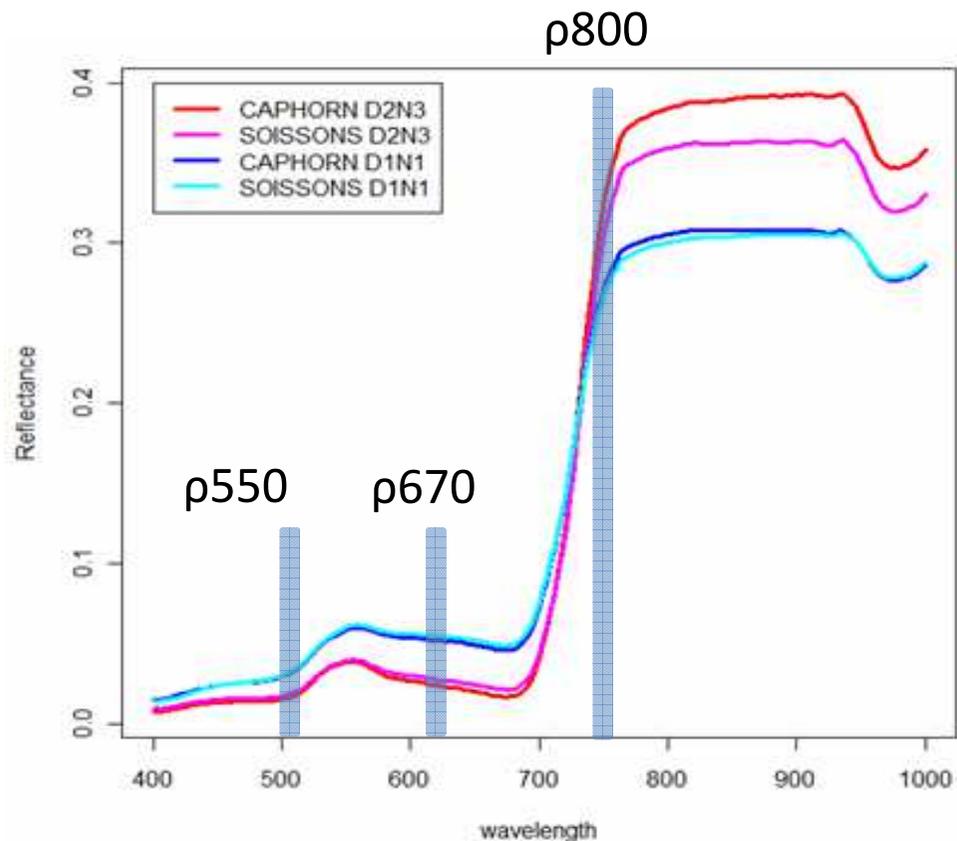
- ✓ NUE is difficult to phenotype
 - time-consuming (sampling, milling ...)
 - not repeatable (biomass, N content)

- ✓ May explain that we detected no additive genetic diversity in the elite panel

- ✓ The key trait is total plant N
 - biomass and N content are difficult to estimate
 - probably possible to approximate them estimating Leaf Area (LAI) and Chlorophyll Content (CC)

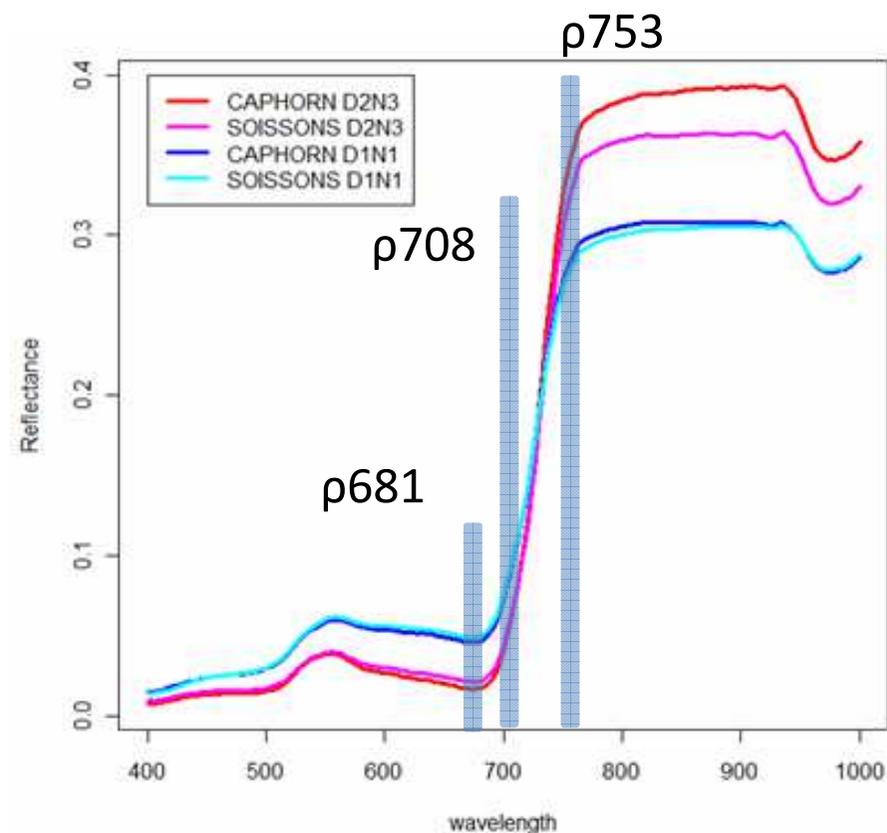
Based on indices using reflectance values

LAI: MCARI2 - Modified Chlorophyll
Absorption Ratio Index 2



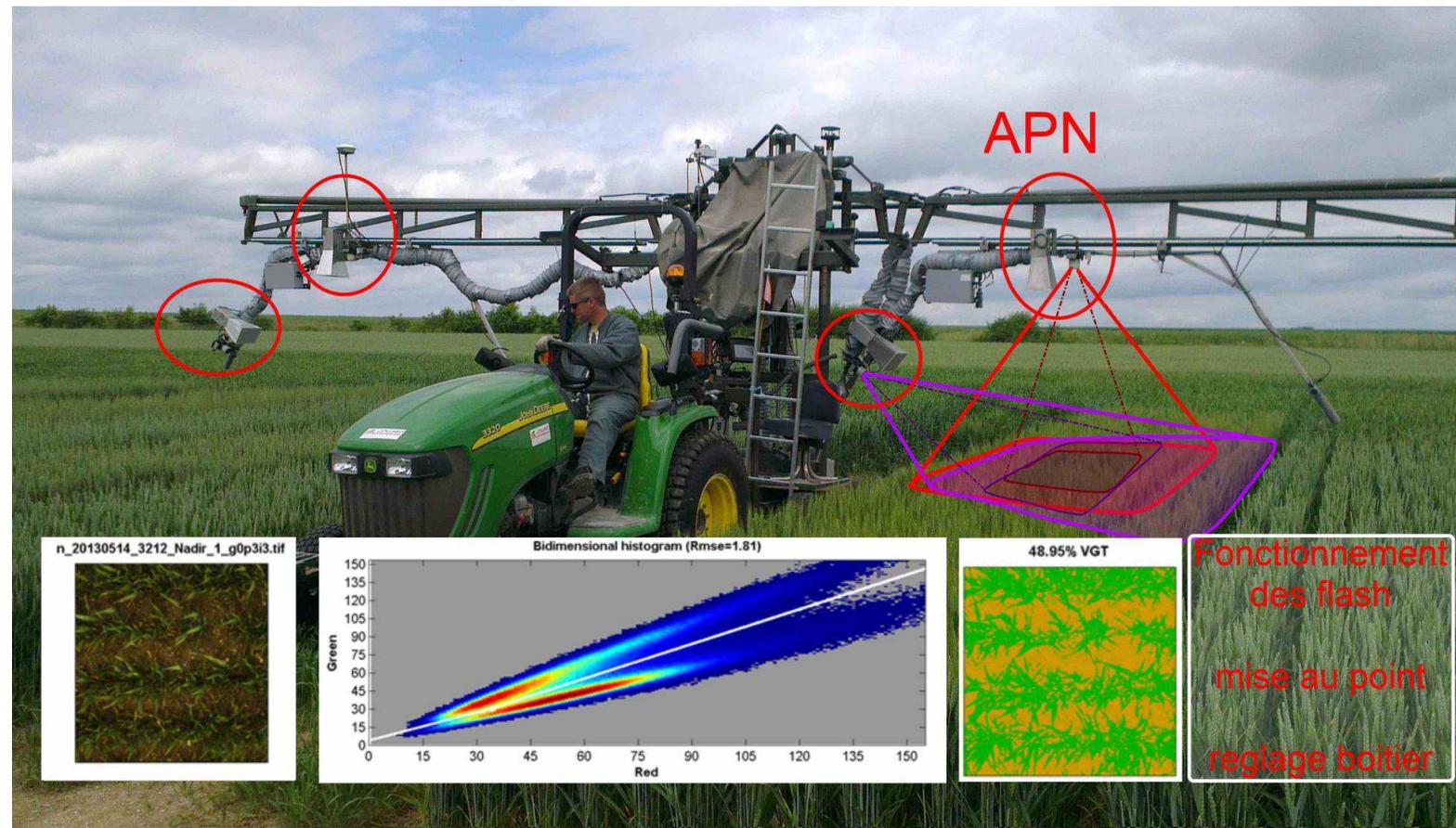
Haboudane et al., 2004

Chlorophyll: MTCI - MERIS
Terrestrial Chlorophyll Index



Dash and Curran, 2004

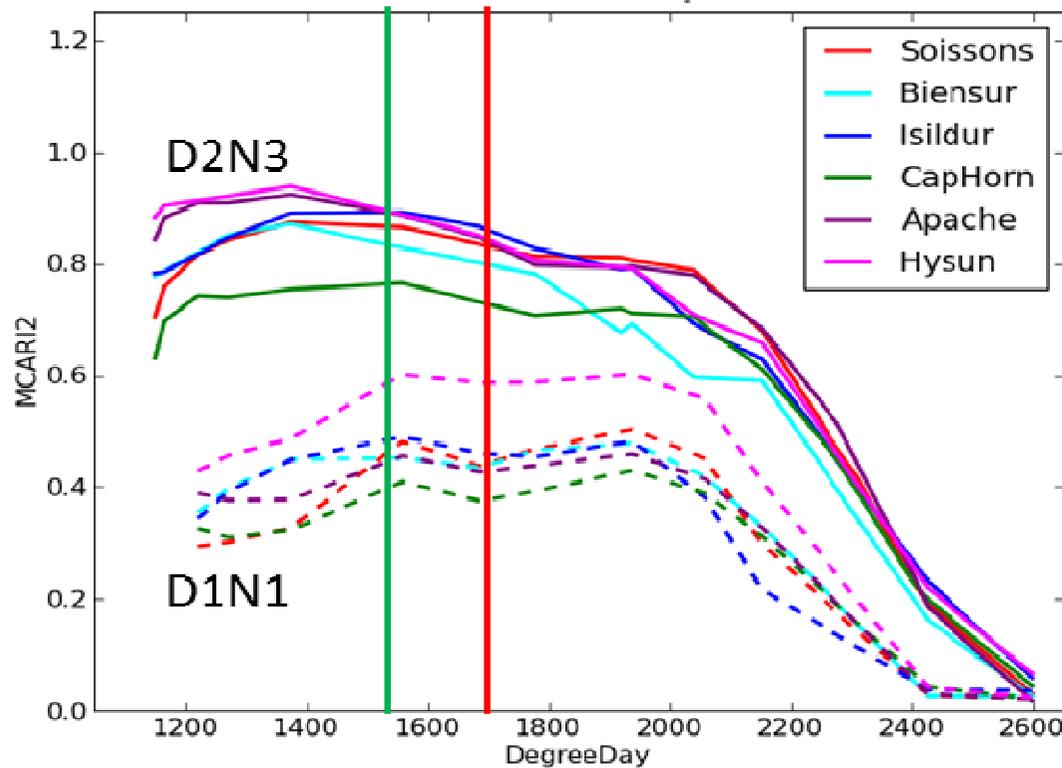
Developpement of a « PhenoMobile »



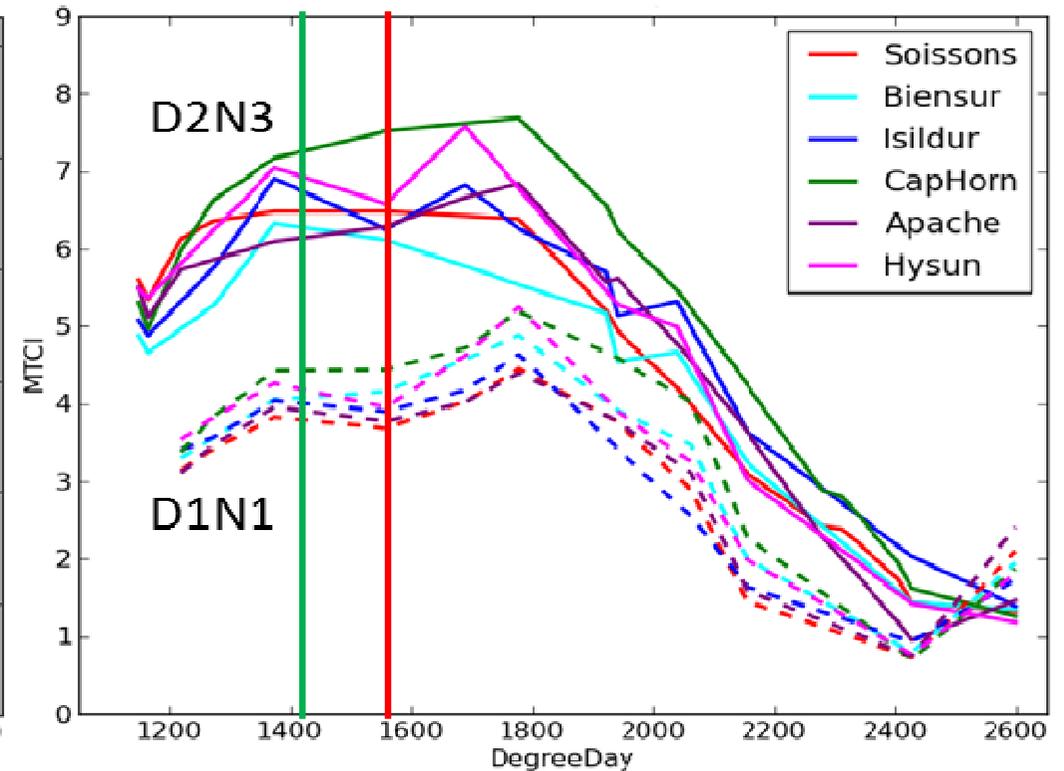
F. Baret, B. de Solan, P. Burger

Example of indices dynamics

Heading Flowering

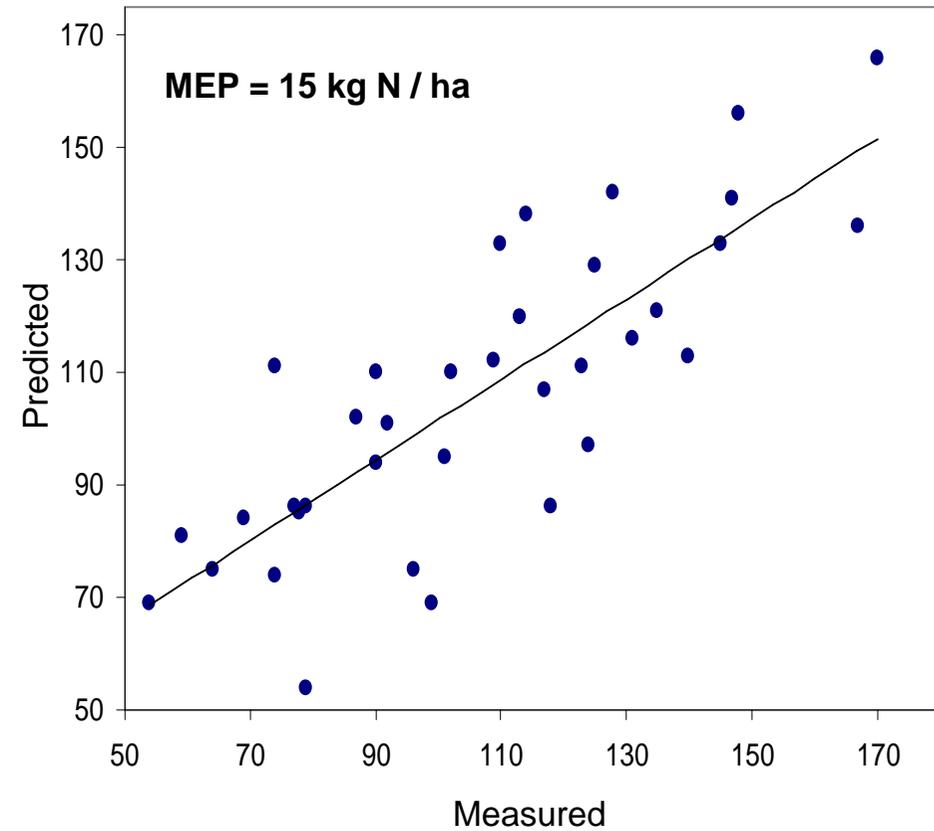


MCARI2 : LAI related



MTCI : Chlorophyll related

Estimation of absorbed N



Baret, Burger, et al

CONCLUSIONS

Estimation of genetic progress for NUE

- +0.33% / year
- significant G x N level interaction
- no significant genetic additive effect for N uptake

Association genetics in progress

- associated regions identified
- SNP x N level and SNP x SNP interactions detected

Phenotyping methods are currently developed

- LAI & Chlorophyll content dynamics

An second association panel was assembled (Breedwheat project)

- 220 elite lines, 100 common to the first panel
- genotyped (TaBW420k Affymetrix array, E. Paux et al)
- phenotyped
 - classical phenotyping (2012-2014)
 - high-throughput phenotyping (2012-2013)

Acknowledgments

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IA Breedwheat - J. Le Gouis (INRA GDEC) et al.



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Thank you for your attention

