



E.P.S.O

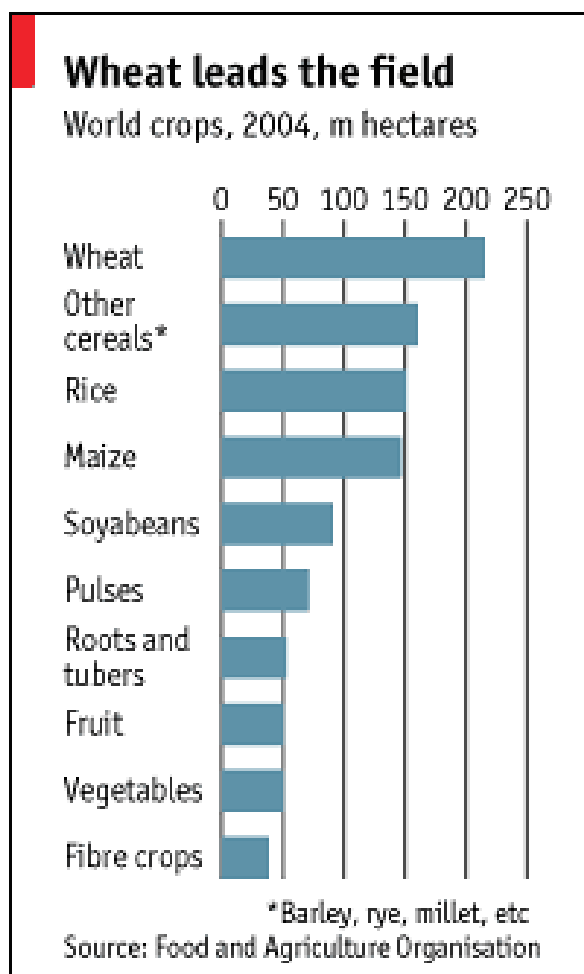
Genetic improvement of wheat quality for animal feeding



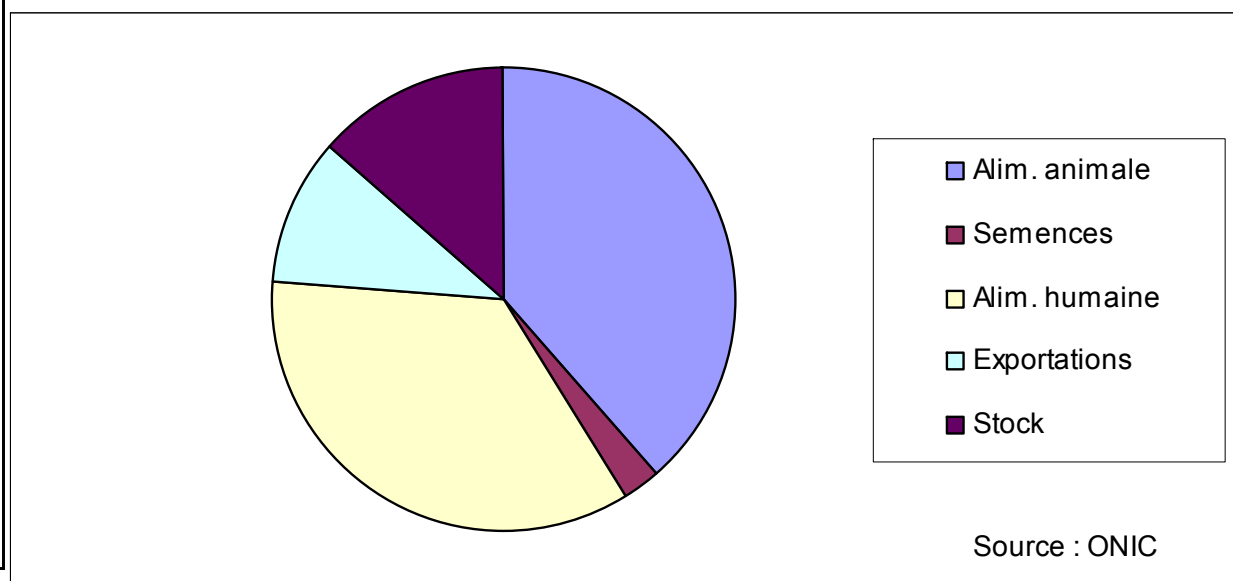
NUTRITION - FOOD
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ENVIRONMENT

INRA

World cereal production and uses



- Wheat is the most widely grown worldwide
- Its use for animal feeding is about 10% worldwide, but 30% in Europe



Animal feeding requirements

- Cereals are primarily a source of ENERGY (starch), mostly for **monogastics** (unable to use cellulose). Animal production (profitability) is correlated to
 - Low cost = yield, low inputs
 - High digestibility: problem of viscosity for poultry
- Protein content may be of interest (depending of the price/availability of other sources): if not conflicting with yield
- Protein quality (composition): lysin and methionin
- Mineral availability: phytase activity



Yield and protein content

How to deal with the
(genetic) negative correlation

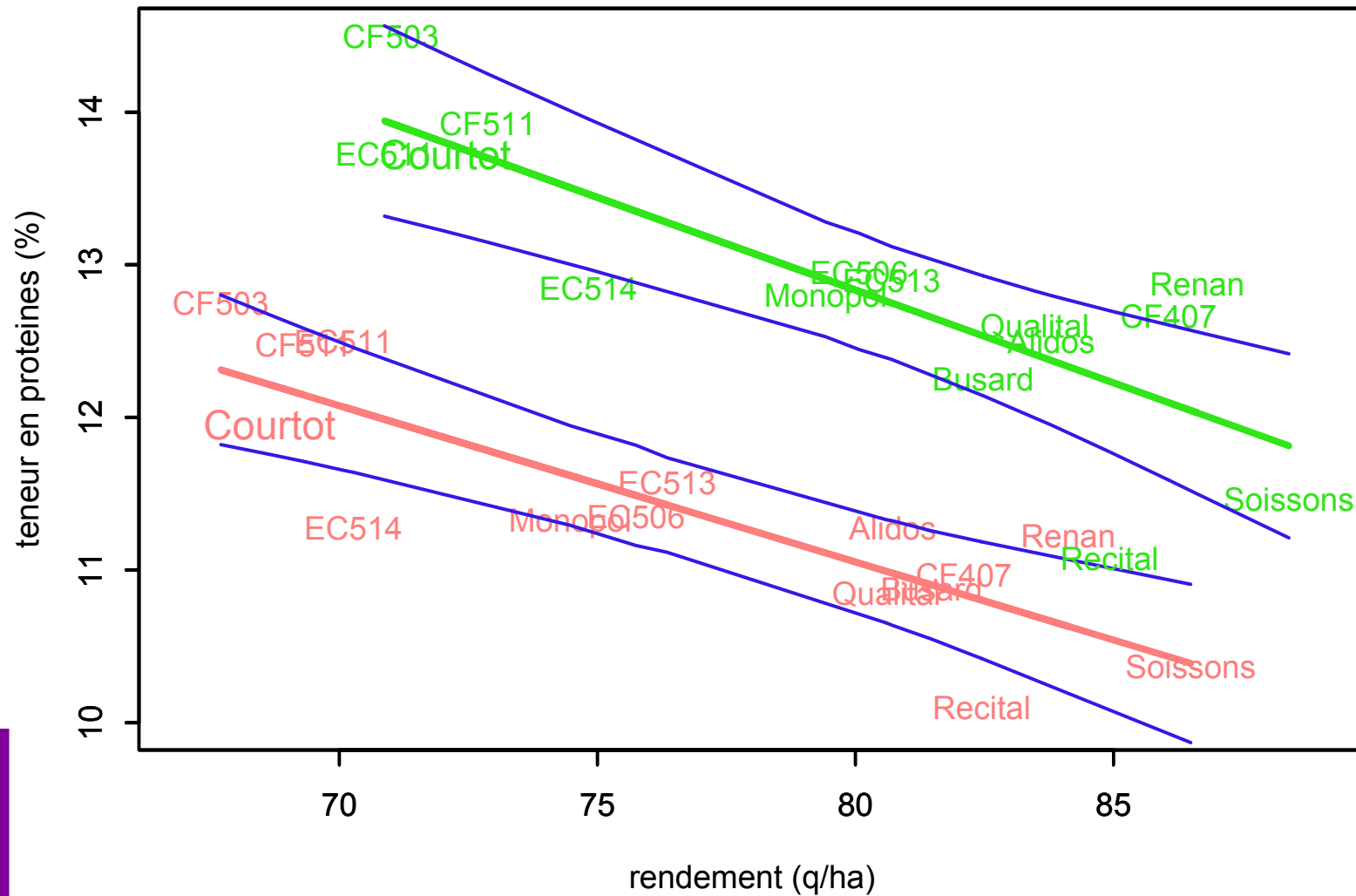
RENDEMENT-TENEUR EN PROTEINES SUR VALEURS MOYENNES

essais avec bles ameliorants

FERTILISATION N+: correlation = -0.83

FERTILISATION N: correlation = -0.85

Comparaison des 2 regressions : $F(2,25) = 50$ ($P < 0.001$)



COMBINED INDEX SELECTION ON YIELD AND PROTEIN

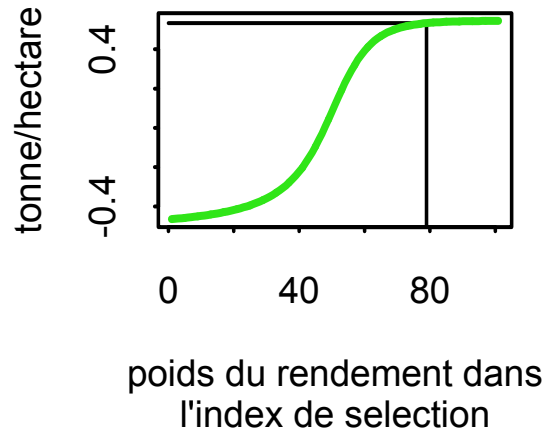
Up to now, protein % has rarely been used as selection criterion.

Use of quantitative genetics tools to predict the expected response to index selection with various economic weights W given to Yield vs Protein

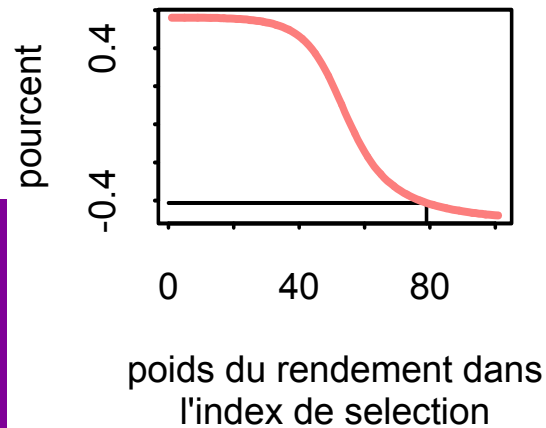
- Genetic material: breeding lines of INRA programme. Genetic correlation -0.75
- Selection intensity = 20% (best lines crossed).
- Expected response to selection estimated from G and P variance-covariance matrices. $\text{Gain} = i * P^{-1} * G * W$

**With current payment for extra-protein % (bread-wheat: 3€/T)
Optimum economic weight leads to improve only yield (0.4T/cycle)
Correlative response of protein content is -0.4% per cycle**

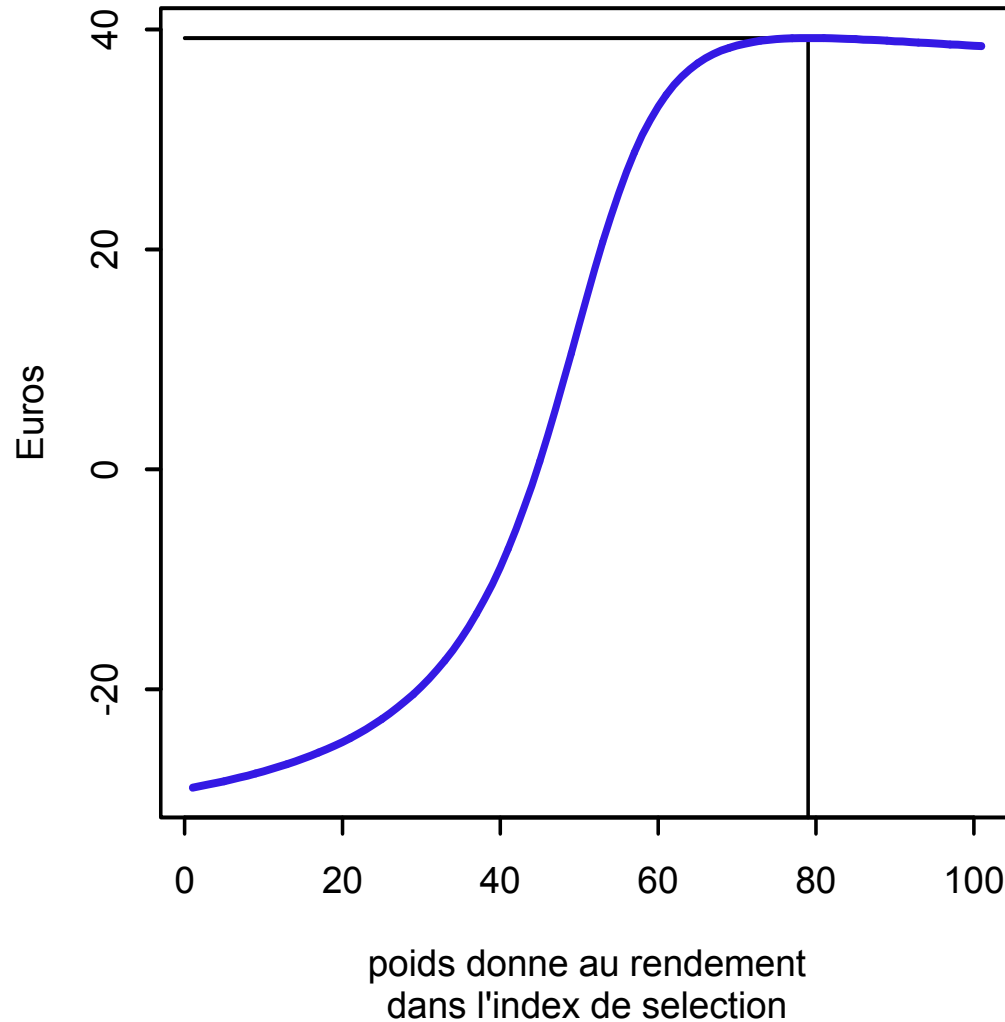
gain attendu pour le rendement



gain attendu pour la teneur en proteines

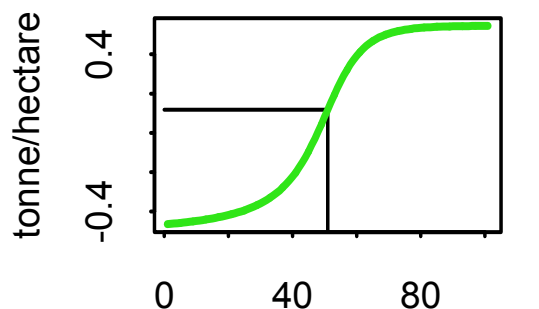


gain attendu pour le revenu par hectare (optimum = 78)



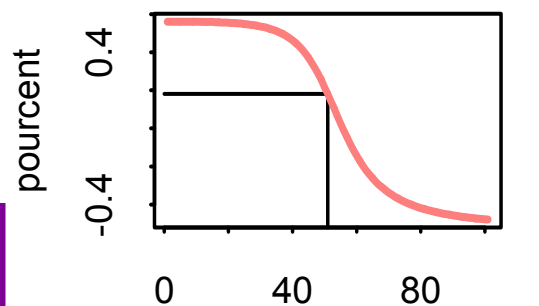
**To achieve a balanced response on both yield and protein content,
 Extra payment for protein should be 12€/T/%
 Yet the expected response would be limited (0.25T/ha and 0.3% prot)**

gain attendu pour le rendement



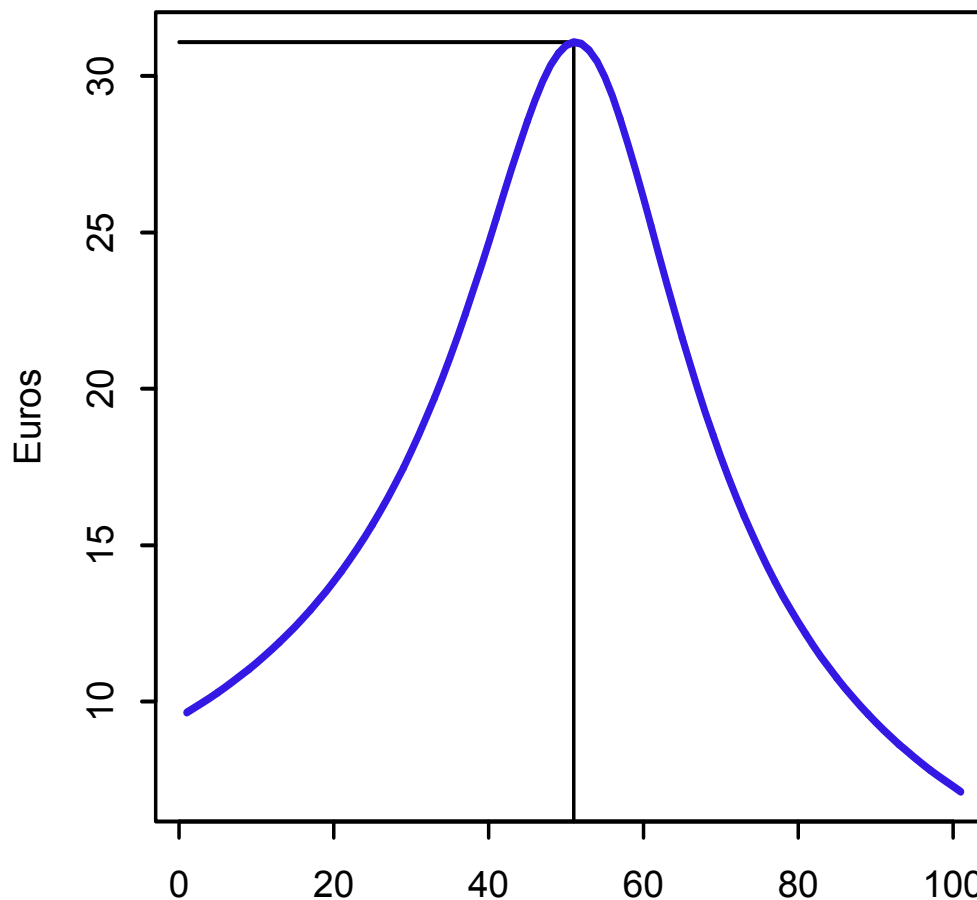
poids du rendement dans l'index de selection

gain attendu pour la teneur en proteines



poids du rendement dans l'index de selection

gain attendu pour le revenu par hectare (optimum = 50)

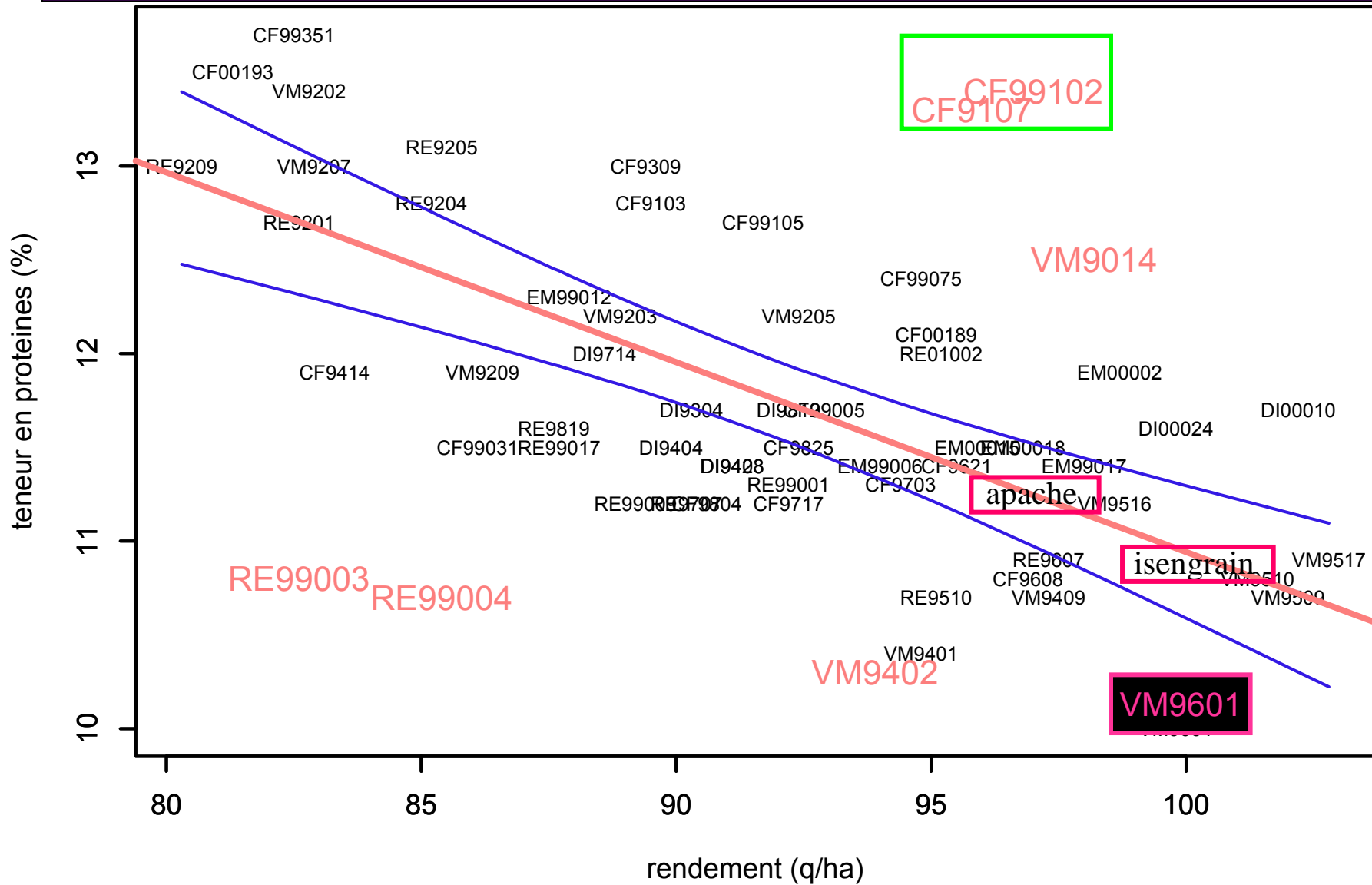


poids donne au rendement dans l'index de selection



How to conciliate yield and protein content?

Identification of breeding lines or cultivars with positive grain protein deviation (GPD)

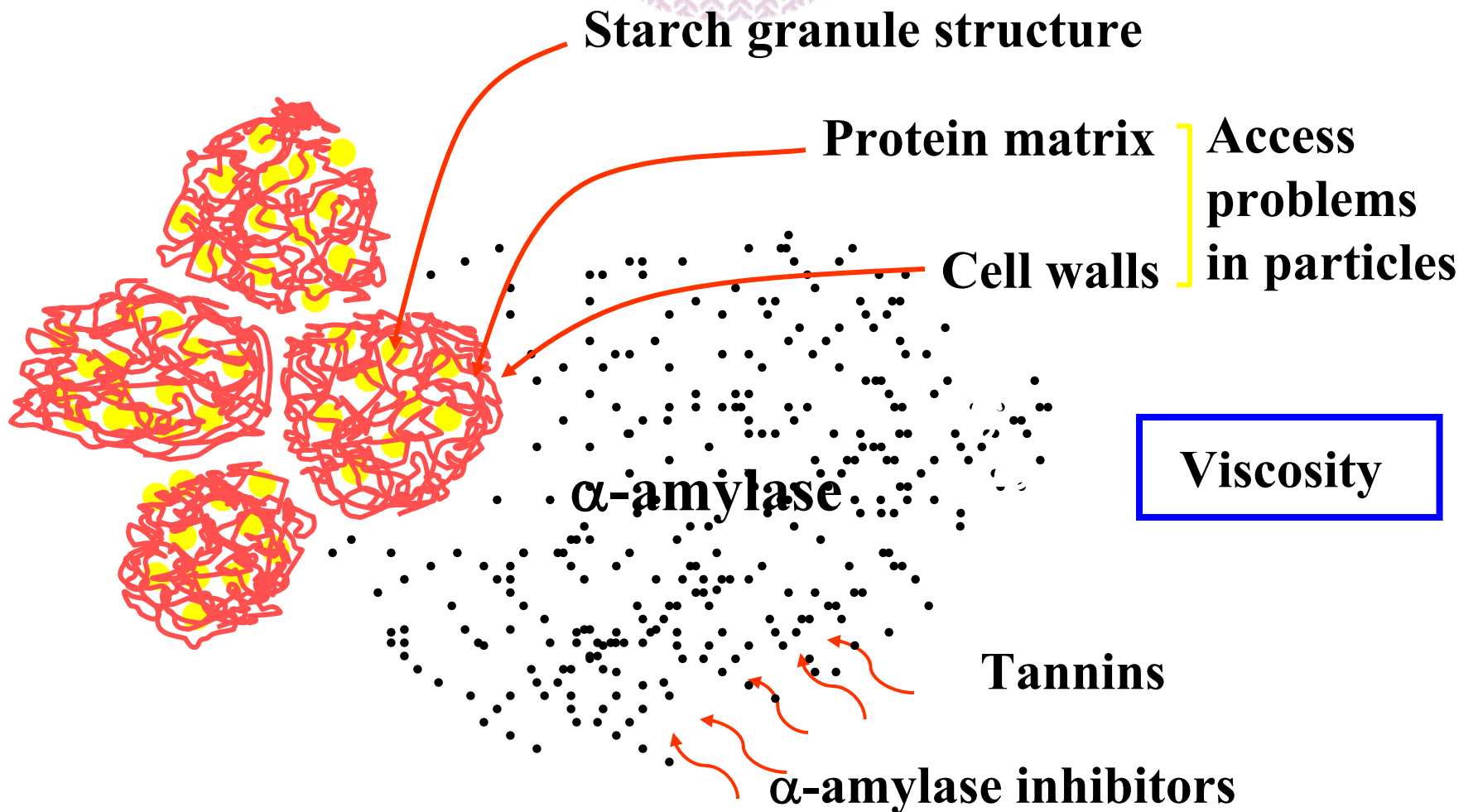




DIGESTIBILITY

Problems with pentosan
viscosity in poultry
Genetics of cell walls
composition

Factors affecting starch digestion



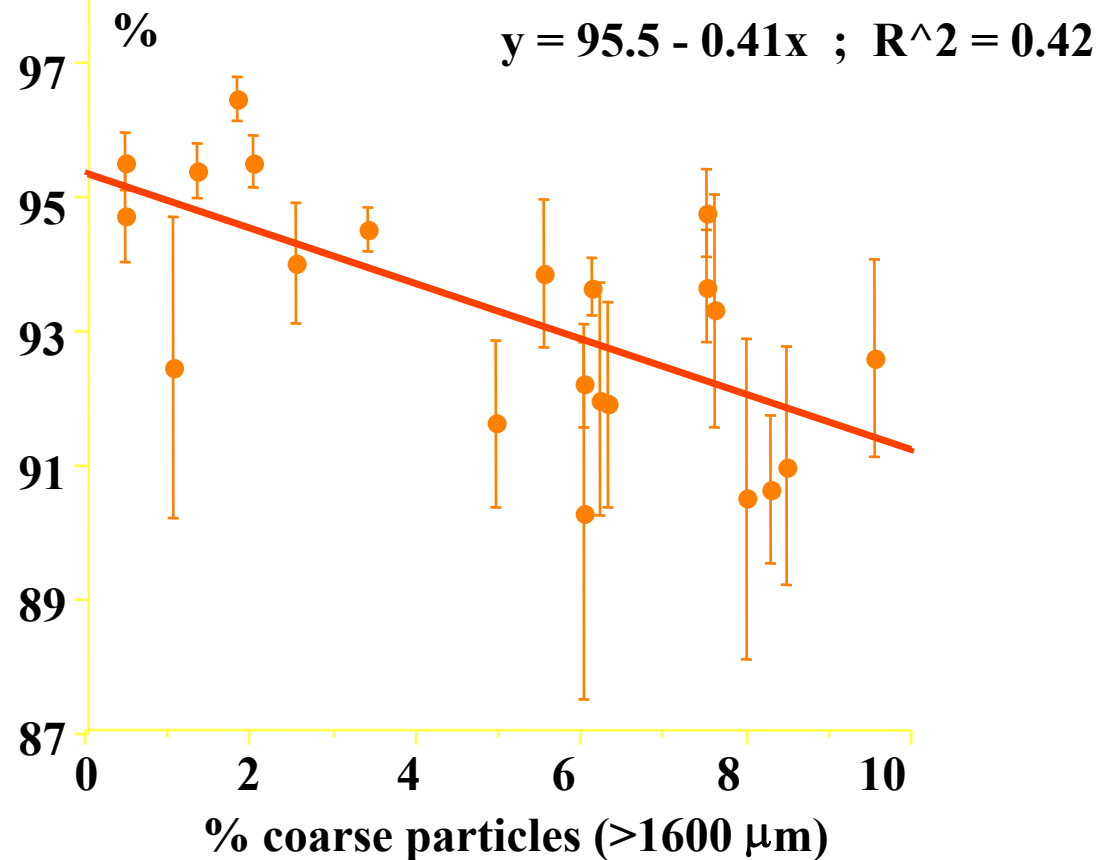
Effect of kernel hardness

Carré et al 2005, British poultry Sci 46:66-74

- Particle size ranges from $\sim 500 \mu\text{m}$ (soft varieties) to $\sim 900 \mu\text{m}$ (hard wheats).
- As expected, starch digestibility is negatively correlated to grain hardness ($r=-0.56$)
- Consequently, Apparent Metabolisable Energy (AME_N) is also negatively correlated
- However, grain hardness is strongly correlated to pellet durability ($r=0.84$), which is often considered as a desired trait to reduce food spillage

Relationship between wheat starch digestibility and particle size of wheat flours before pelleting

Starch digestibility



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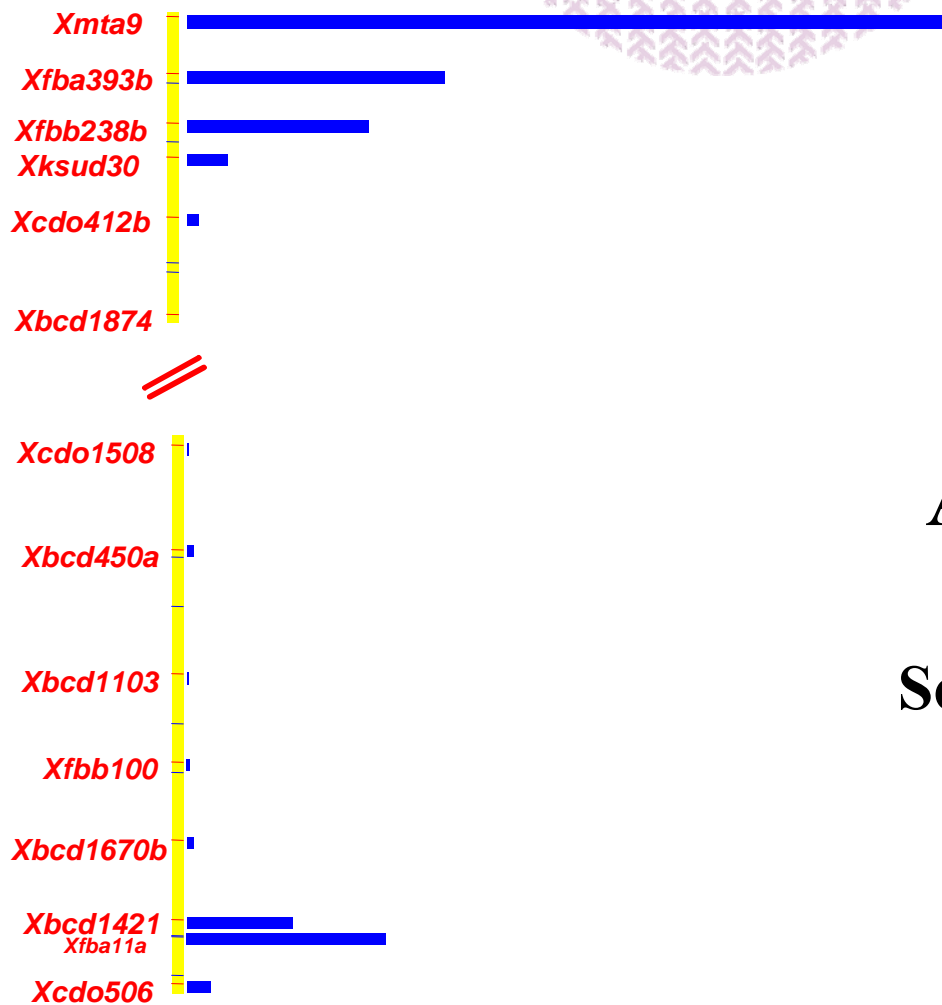
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After Carré et al. 2002

5 DS



**A major gene for
kernel hardness
Sourdille et al 1996 TAG**

5 DL



r2 VALUE (%)

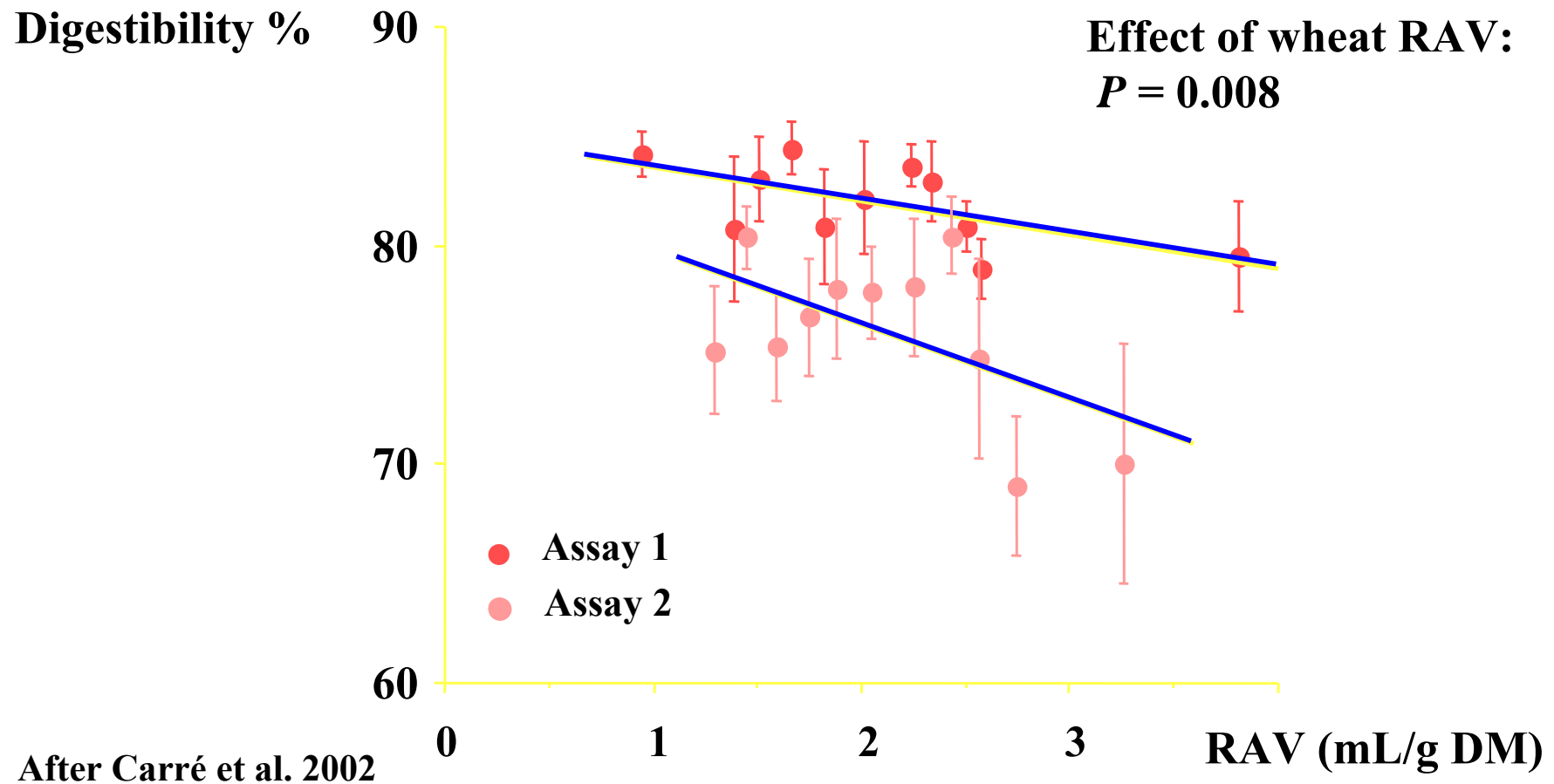
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Effect of viscosity (RAV) of wheats (55% in diets) on starch digestibility in 3 w. broiler chickens.



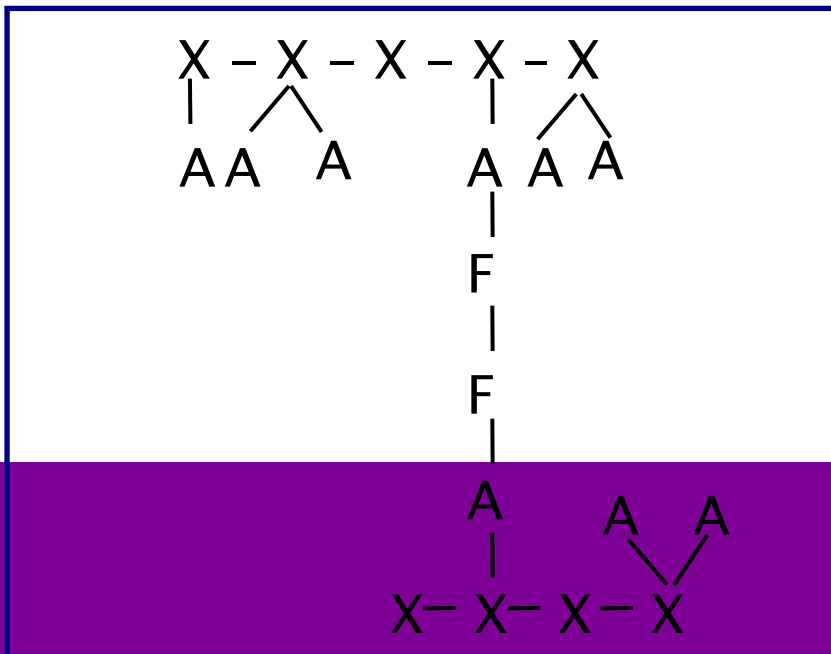
Problems with viscosity in birds diet

- A high dietary viscosity reduces the digestibility of the various components
- Causes inflammation of the intestinal mucosa, and induces over-consumption of water in birds (Carré et al, 1994).
- This over-consumption leads to more aqueous excreta which exacerbate both sanitary and environmental pollution problems (Carré et al, 1995).

Arabinoxylans and β -D-glucans are the major components of wheat endosperm cell walls and have impacts on processing and nutrition

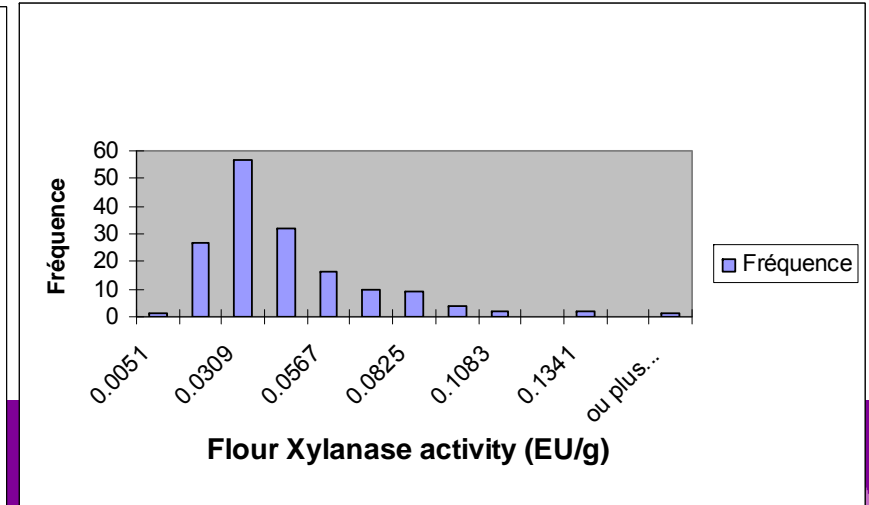
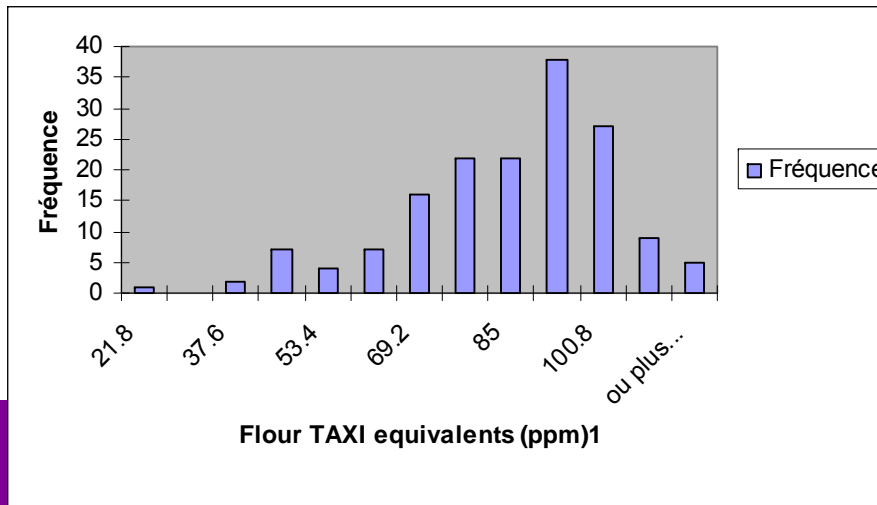
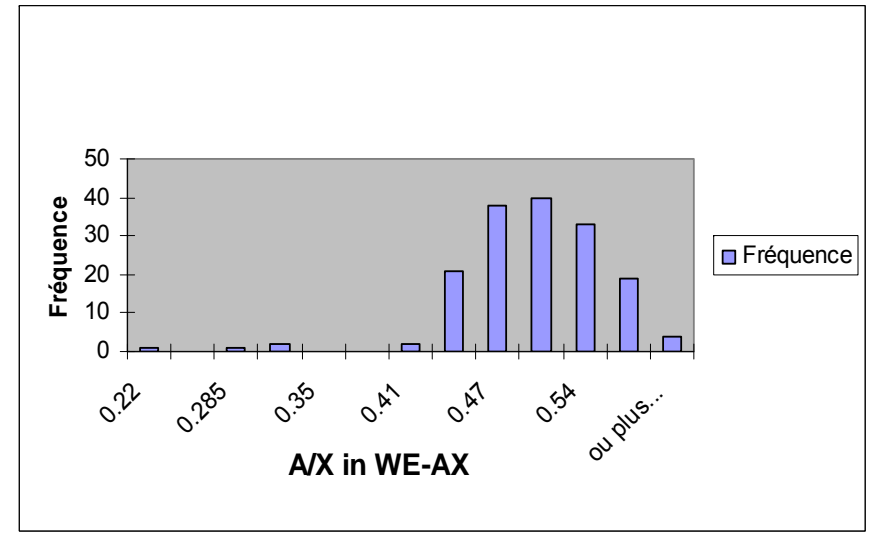
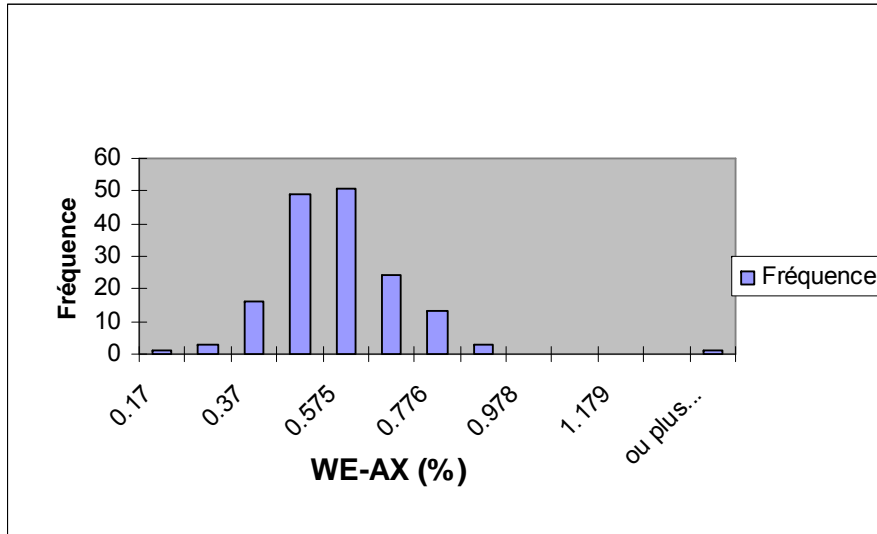
	% cell wall
arabinoxylan	70
(1 \rightarrow 3)(1 \rightarrow 4) β -glucan	20
glucomannan	2-7
cellulose	2-4
ferulic acid	✓

- ### Cell wall fibre
- are polymers of M_r 10^4 – 10^6
 - occur in water-soluble and water insoluble (ferulic acid cross-linked) forms
 - have high affinity for water
 - **form viscous solutions**
 - **affect intestinal absorption of lipids**
 - **have other effects on colon bacteria and composition**

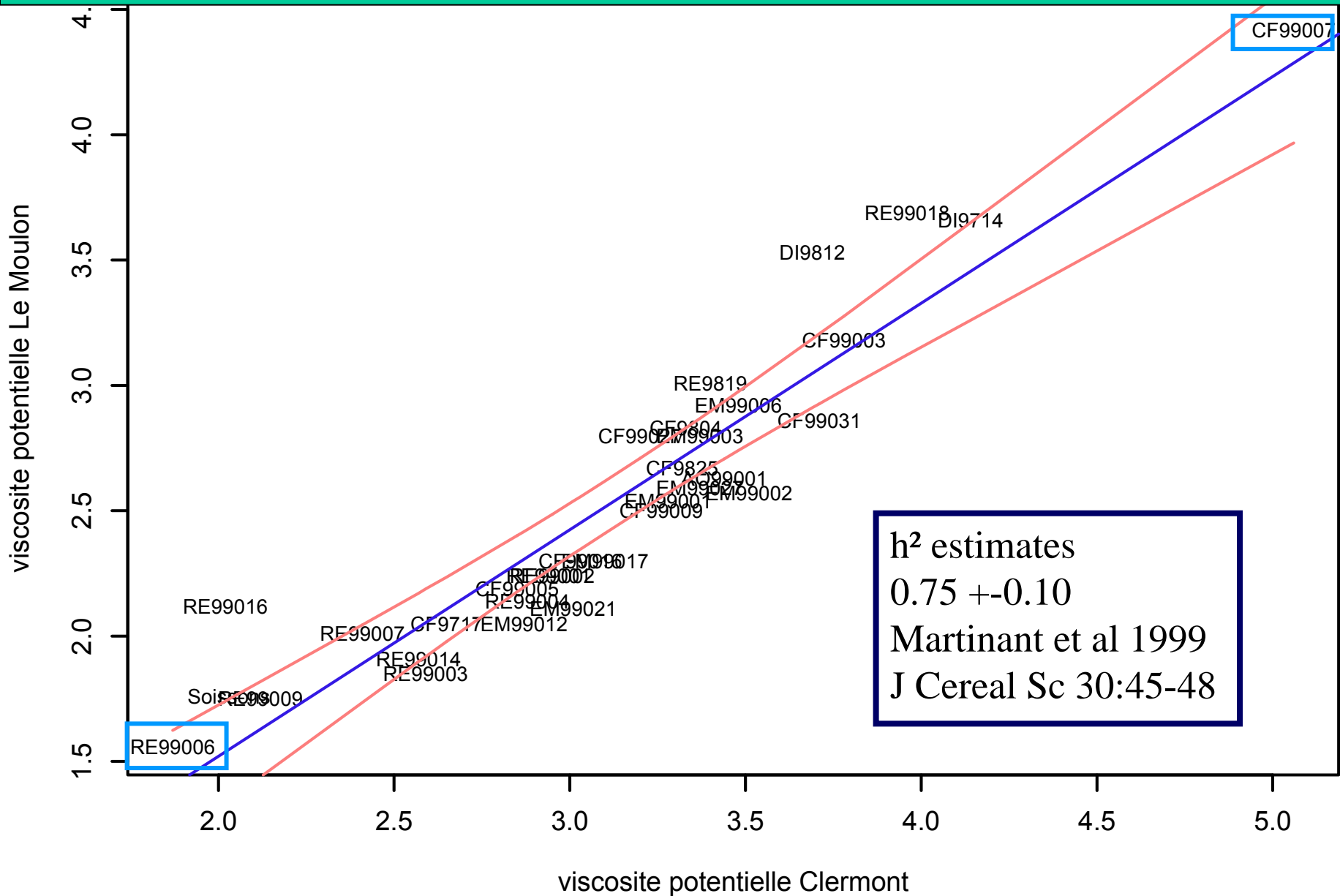




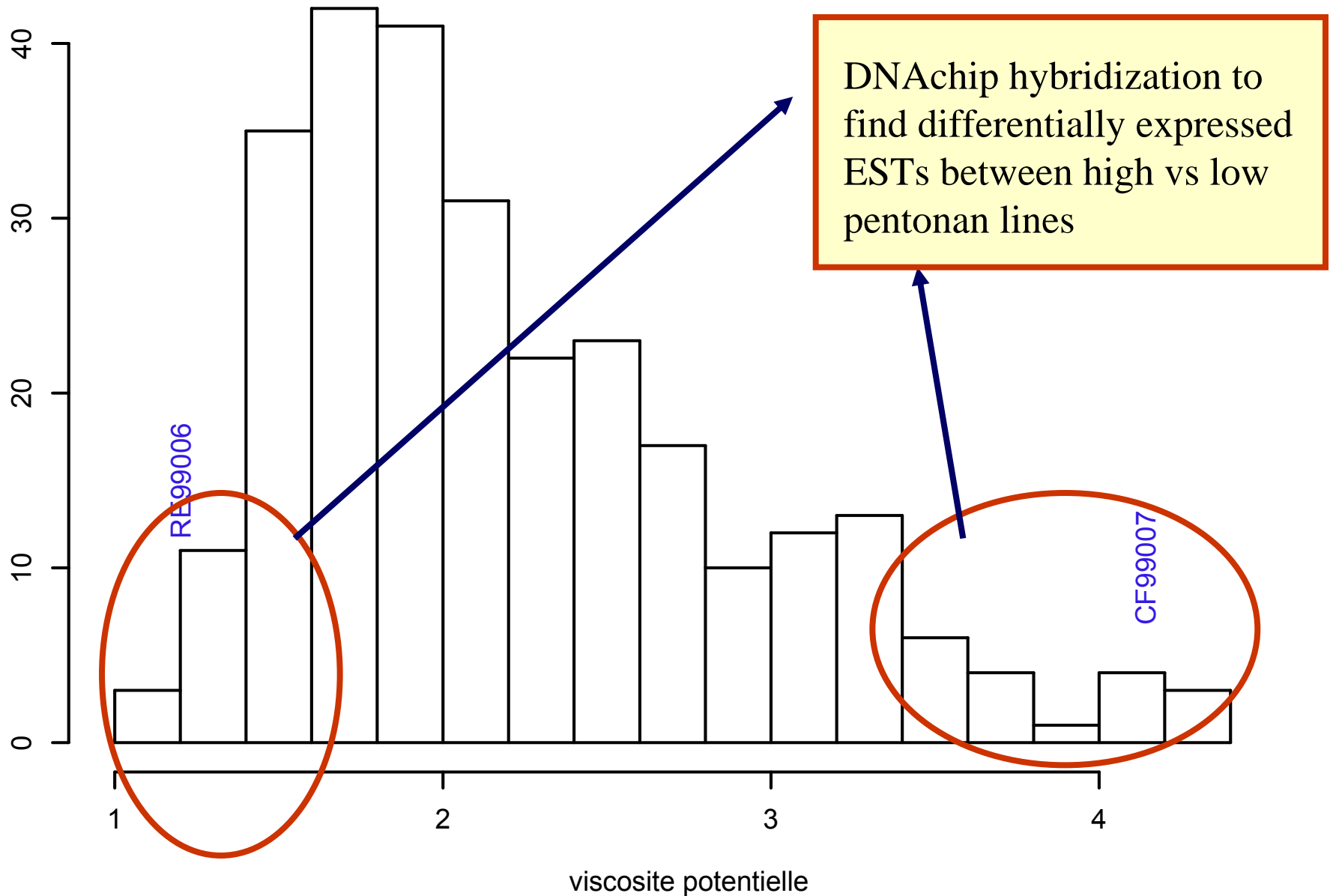
Range of variation in bread wheat core-collection



Choice of two contrasted breeding lines to develop doubled haploids
 NB r^2 between years = 0.87, means high h^2

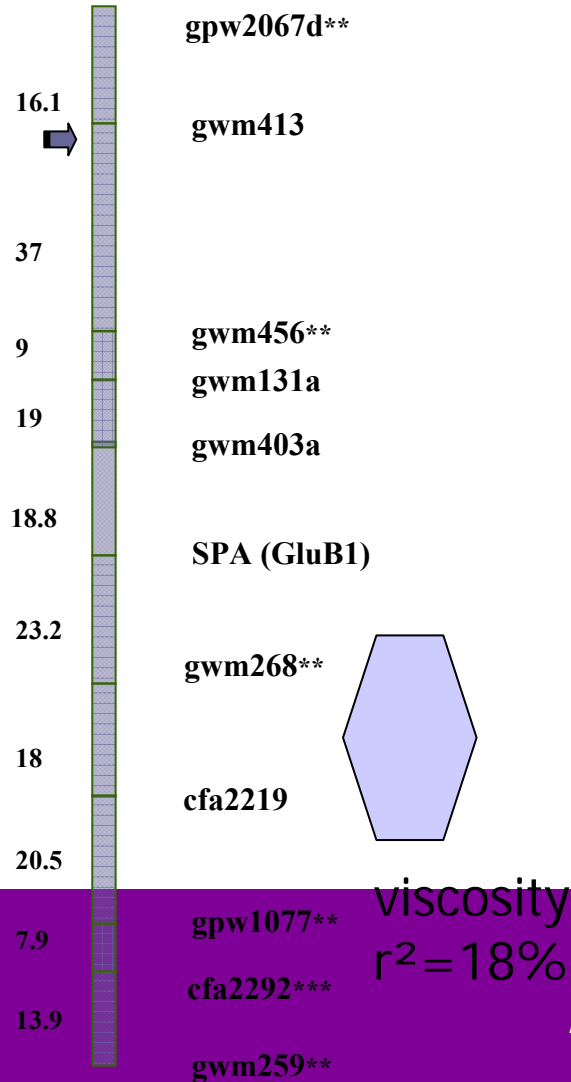


Distribution of potential viscosity in R6C7 DH population (harvest 2004)

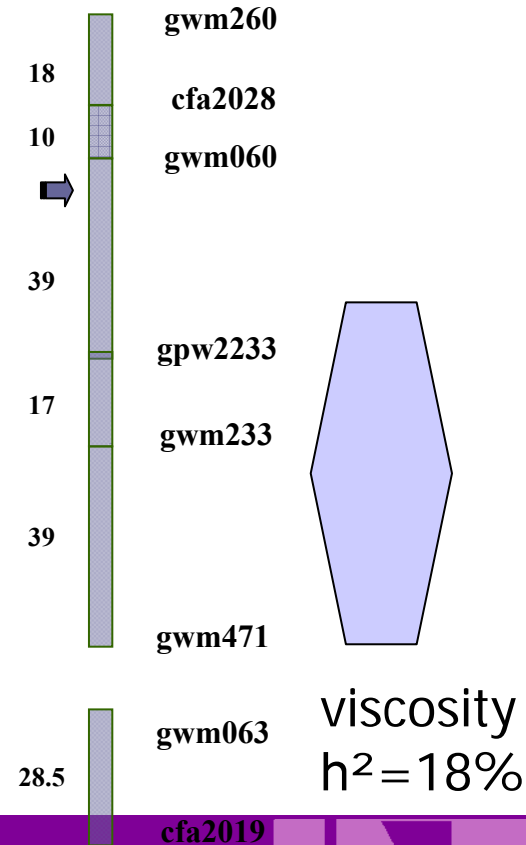


QTL analyses

1B



7A



► Search for candidate genes of endosperm cell wall composition

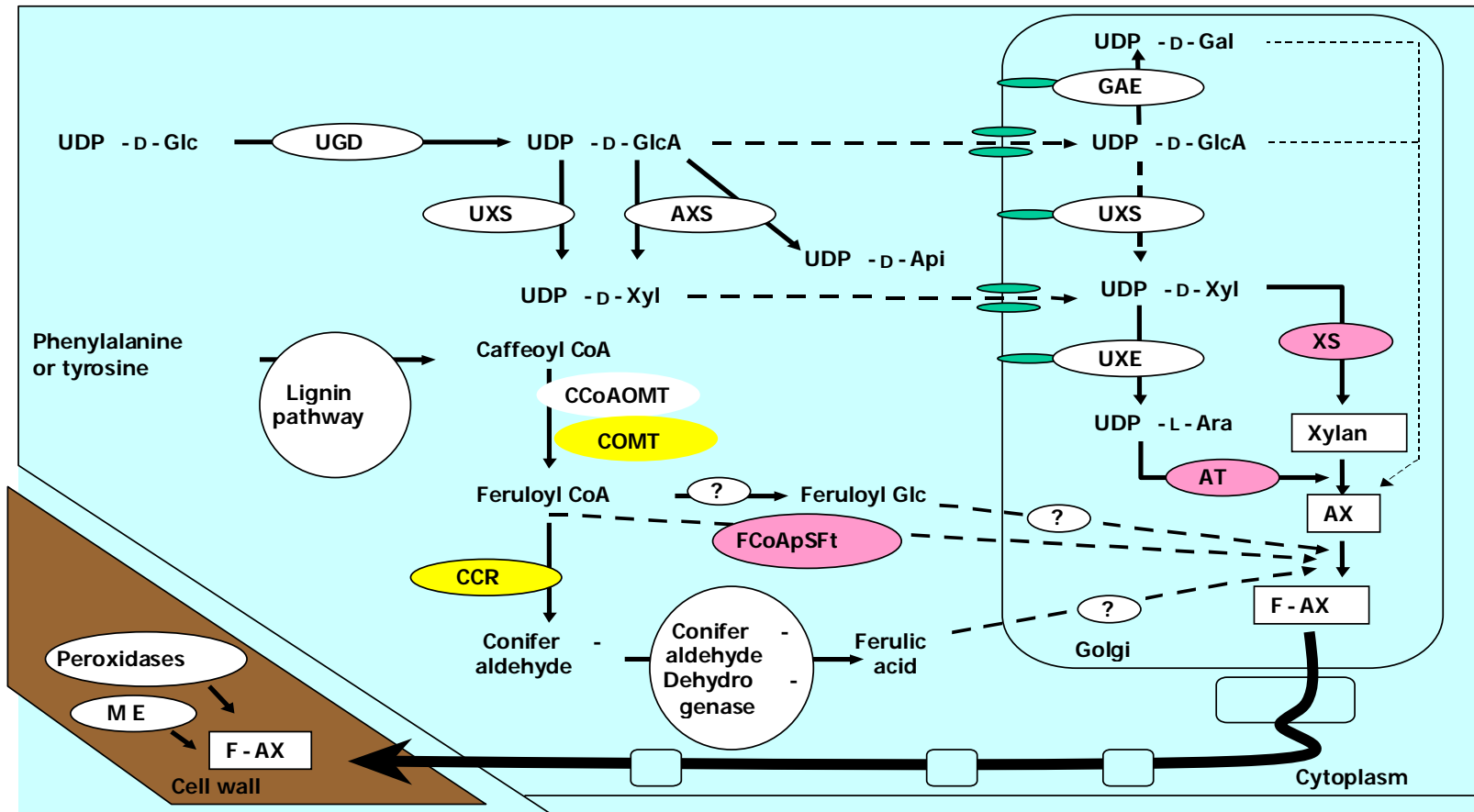
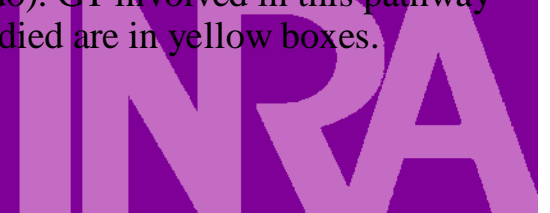


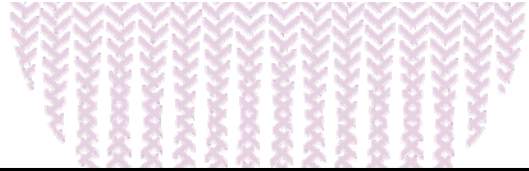
Figure 1. An overview of the pathway of AX synthesis in plant (from P.E. Sado). GT involved in this pathway are in pink boxes. Enzymes involved in early step of the AX synthesis and studied are in yellow boxes. Epimerases studied in the year 1 are not represented.

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Identification of candidate genes involved in arabinoxylans (AX) biosynthesis



Find matching EST contigs (eventually assignation NSF)



Design of specific primers



Assignation in deletion bin of CS



Polymorphism between parents of populations



Genes mapping and/or Difference in gene expression

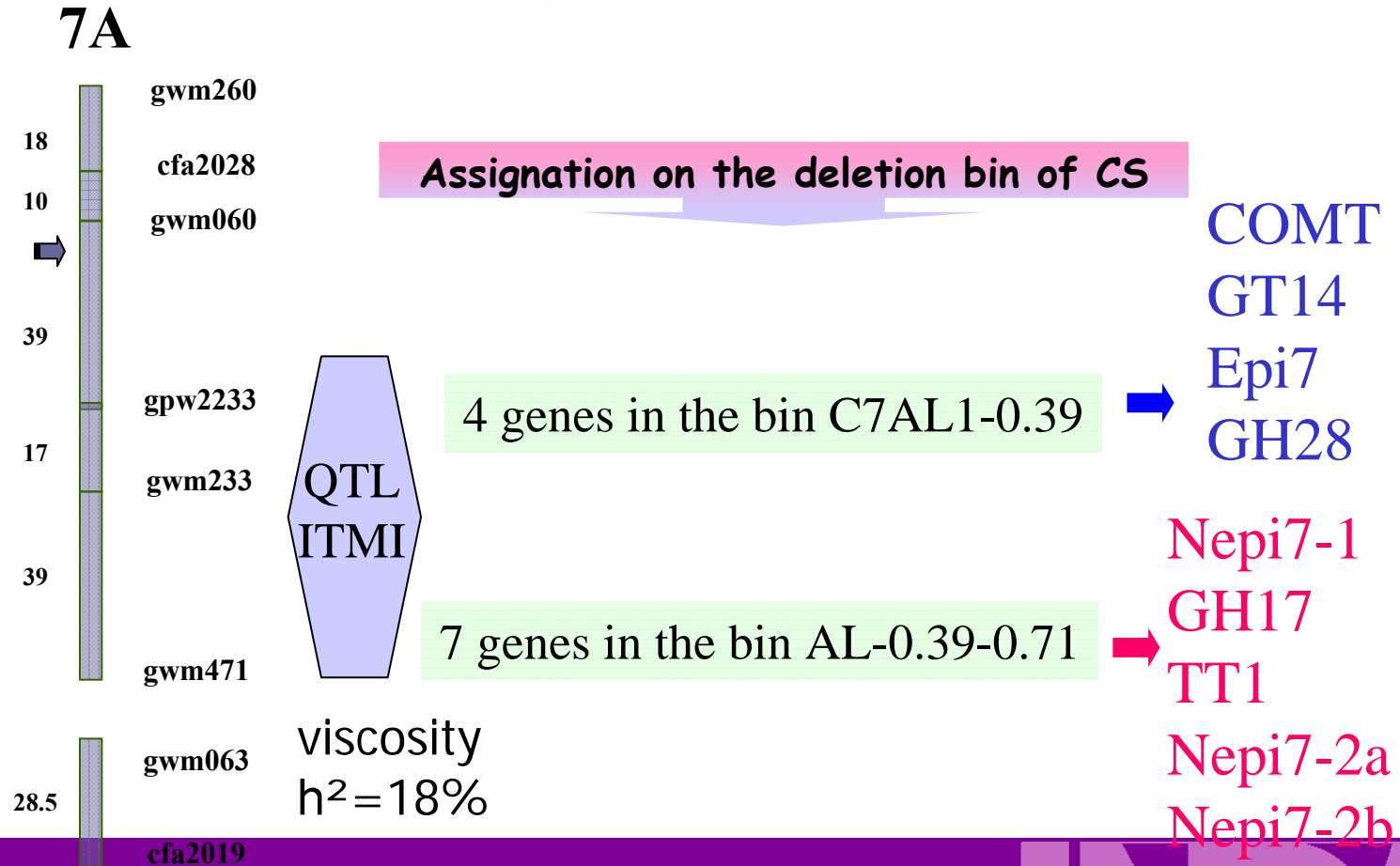


Allelic variant in core collection



Association SNP polymorphism / trait variation for validation

Candidate genes on chromosome 7A



Genome and allele specific markers

- ▶ Design of specific allele primers for genotyping HG core-collection

BD028EPI1_2R	AGCAAGTCGCTGTCGGGAGGCGGCCCGCGCTGACGGT	Allele 1 primer	CCAAGGATGGAACAGGGGTATGT
BD021EPI1_2R	AGCAAGTCGCTGTCGGGAGGCGGCCCGCGCTCACGGT		CCAAGGATGGAACAGGGGTATGT
BT026EPI1_2R	AGCAAGTCGCTGTCGGGAGGCGGCCCGCGCTGACGGT		GGGACCGACTACAACACCAAGGATGGAACAGGGGTATGT
BD052EPI1_2R	AGCAAGTCGCTGTCGGGAGGCGGCCCGCGCTGACGGT		GGGACCGACTACAACACCAAGGATGGAACAGGGGTATGT
BT037EPI1_2R	AGCAAGTCGCTGTCGGGAGGCGGCCCGCGCTGACGGT		CCAAGGATGGAACAGGGGTATGT
BD050EPI1_2R	AGCAAGTCGCTGTCGGGAGGCGGCCCGCGCTGACGGT	Allele 2 primer	CCAAGGATGGAACAGGGGTATGT
BT009EPI1_2R	AGCAAGTCGCTGTCGGGAGGCGGCCCGCGCTGACGGT		GGGACCGACTACAACACCAAGGATGGAACAGGGGTATGT
BD047EPI1_2R	AGCAAGTCGCTGTCGGGAGGCGGCCCGCGCTGACGGT		GGGACCGACTACAACACCAAGGATGGAACAGGGGTATGT
CONSENSUS	AGCAAGTCGCTGTCGGGAGGCGGCCCGCGCTCACGGT		GGGACCGACTACAACACCAAGGATGGAACAGGGGTATGT

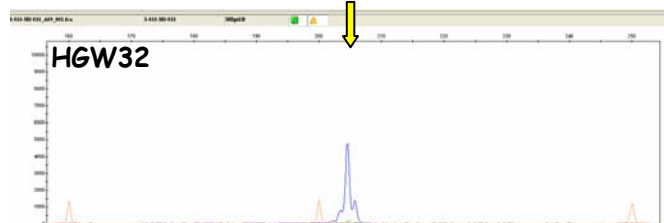
▲ SNP1

▲ SNP2

← SNP used to genotype HG collection

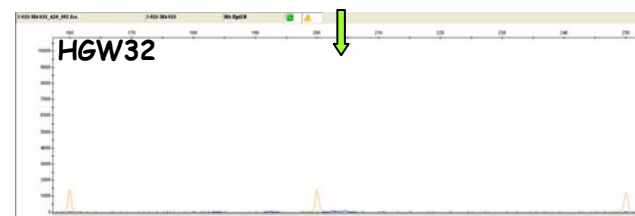
- ▶ PCR on HG core-collection

With allele 1 primer



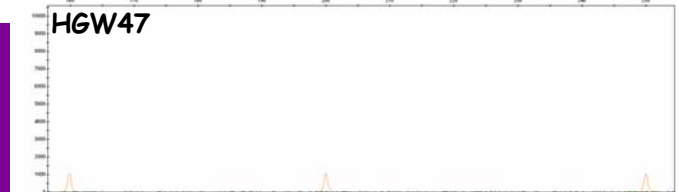
Amplification = Allele 1

With allele 2 primer



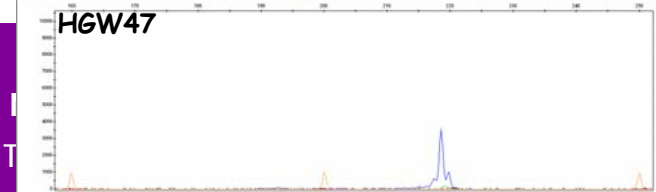
No amplification = Allele 1

HGW47



No amplification = Allele 2

HGW47



Amplification = Allele 2



post GENOMICS

(Perfect) Marker Assisted
Selection

Inducing new variation



Bioavailability of minerals

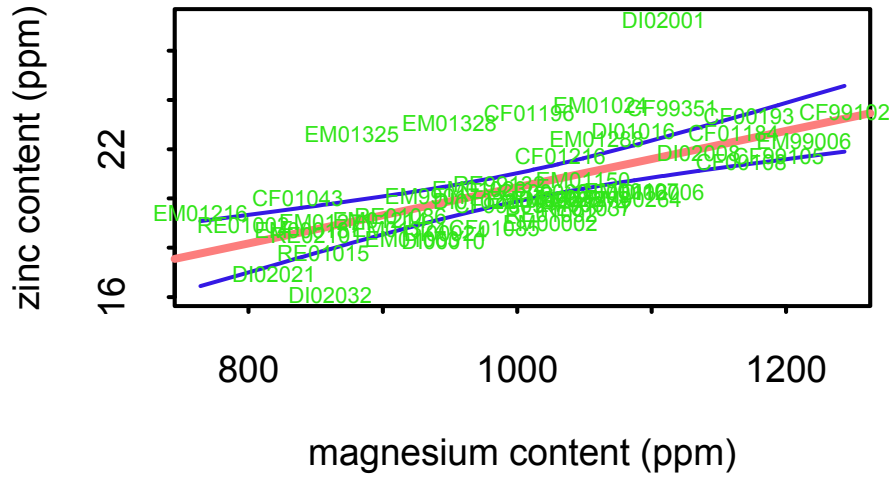
Phytic acid and phytasic
activity

Genotype and environment main effects on mineral content (ANOVA: F values)

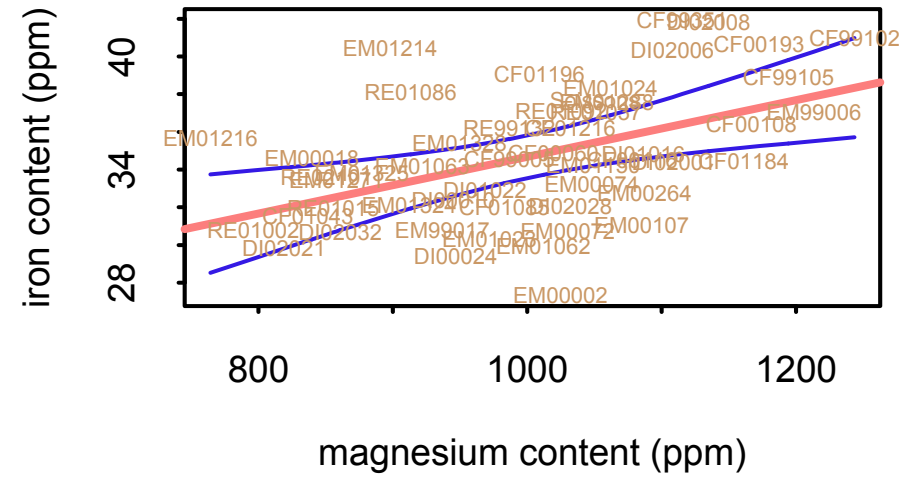
		Df	Yield	Magnesium	Zinc	Iron
ASG-2	Year	2	5.9 **	5.5 *	1.7 NS	6.6 **
	Génotype	10	7.7 ****	13.7 ****	3.1 *	2.1 NS
	Error	20				
IS	Location	2	708 ****	71.8 ****	95.4 ****	28.1 ****
	Génotype	50	3.2 ****	4.8 ****	2.8 ****	1.4 NS
	Error	100				

Relationship between the 3 minerals contents

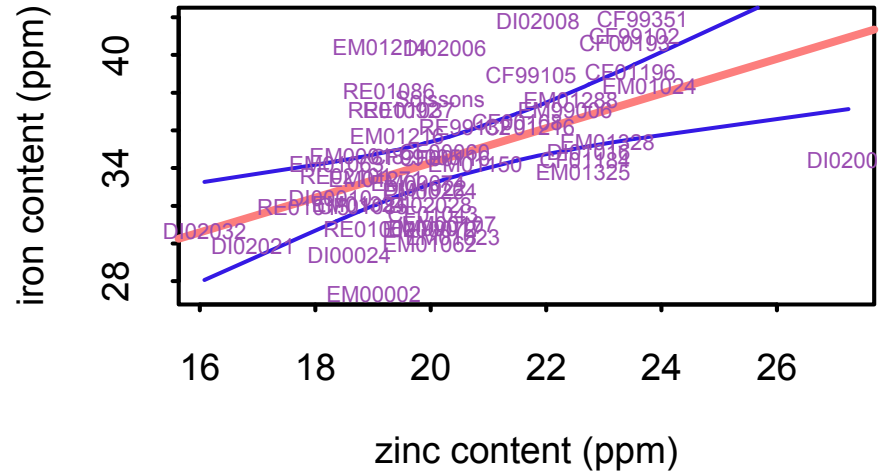
n = 51 correlation = 0.64 ****
 $Zn = 9.03 + 0.011 * Mg$



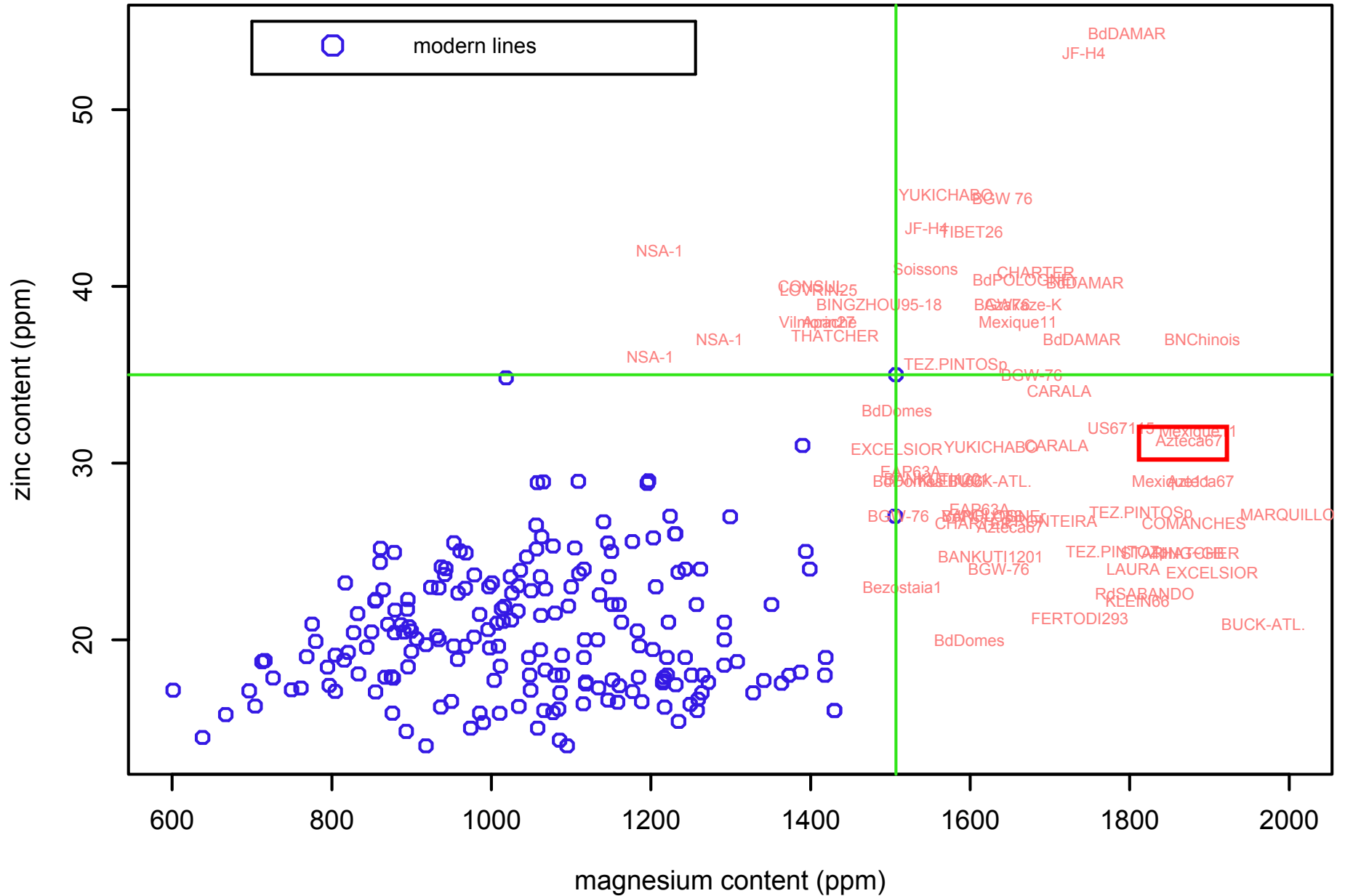
n = 51 correlation = 0.49 ***
 $Fe = 19.629 + 0.015 * Mg$



n = 51 correlation = 0.53 ****
 $Fe = 15.858 + 0.921 * Zn$



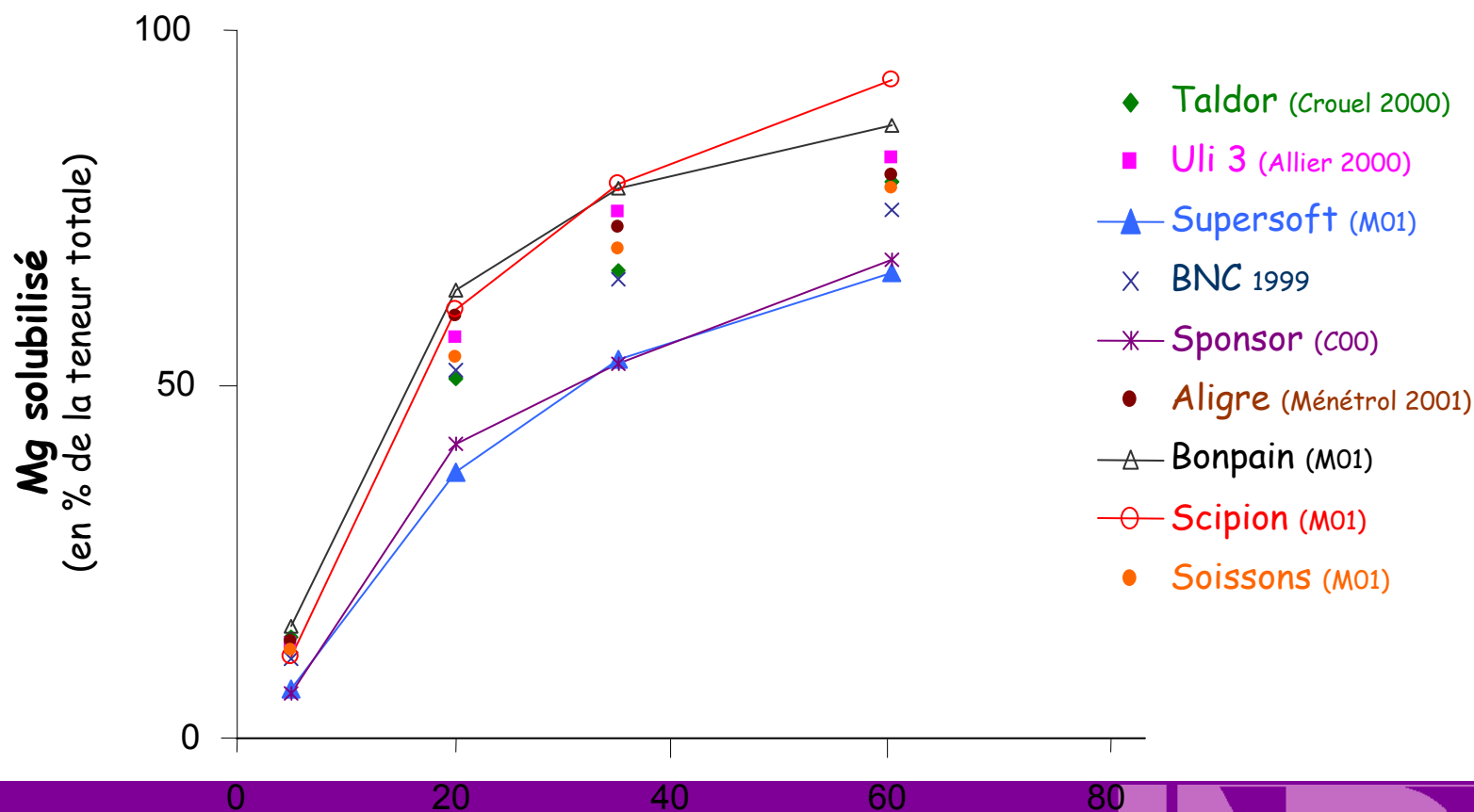
Range of variation of modern lines vs collections



CONCLUSIONS

- The genetic variability appears to be high for Mg, Zn and Fe, even in modern cultivars
 - experimental cross CF99102 (high-MG) x EM01216 (low-Mg)
- Heritability is high for MG, moderate for Zn and low for Fe, fortunately all these cations are positively correlated (also with toxic ones?)
- Genetic resources with high mineral content are often exotic, unadapted lines or old landraces.
 - Advanced backcross population developed from Apache (recurrent) x Azteca67

Bio-accessibility of Mg in bran: genetic variability



From F. Lenhardt, J. Abecassis

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Problems with Phosphorus in animal feeds

- **A total of 50-70% of grain phosphorus is in the form of phytic acid phosphorus (Reddy et al, 1982).**
- **This phytic acid phosphorus cannot be used by monogastric animals (Sauveur, 1989; Pointillart, 1994).**
- **In consequence, the phytic acid phosphorus is not available and therefore contributes to the pollution of surface water.**
- **Wheat, however, contains plant phytase whose activity varies depending on the variety (Sauveur, 1989; Barrier-Guillot et al, 1996a and b).**
- **This phytase is activated during digestion and liberates a substantial amount of the grain phosphorus (Frapin and Nys, 1993).**
- **A high phytase activity is, therefore, to be sought after in wheat**

Phytase activity in Triticale

Cultivar	n	Phytasic activity, UP / kg ^a
DI34-2	4	1012 ± 102 a
Aubrac	6	1320 ± 87 b
Trimaran	6	1424 ± 125 b
Capitale	4	1815 ± 126 c
Calao	6	2146 ± 145 d



A low phytic acid mutant in maize (lpa241)

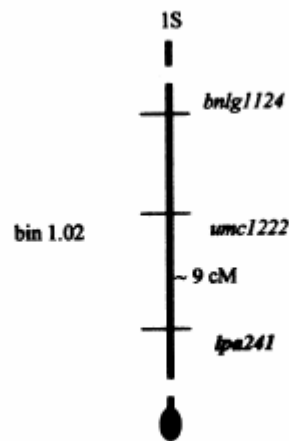
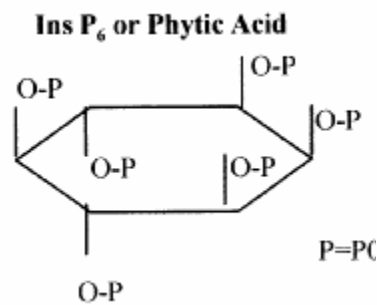


Fig. 4 Mapping of the *lpa241* locus. Approximate distance of *lpa241* from umc 1222 is shown on chromosome 1S

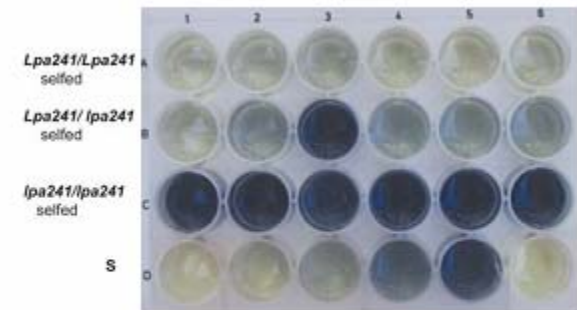
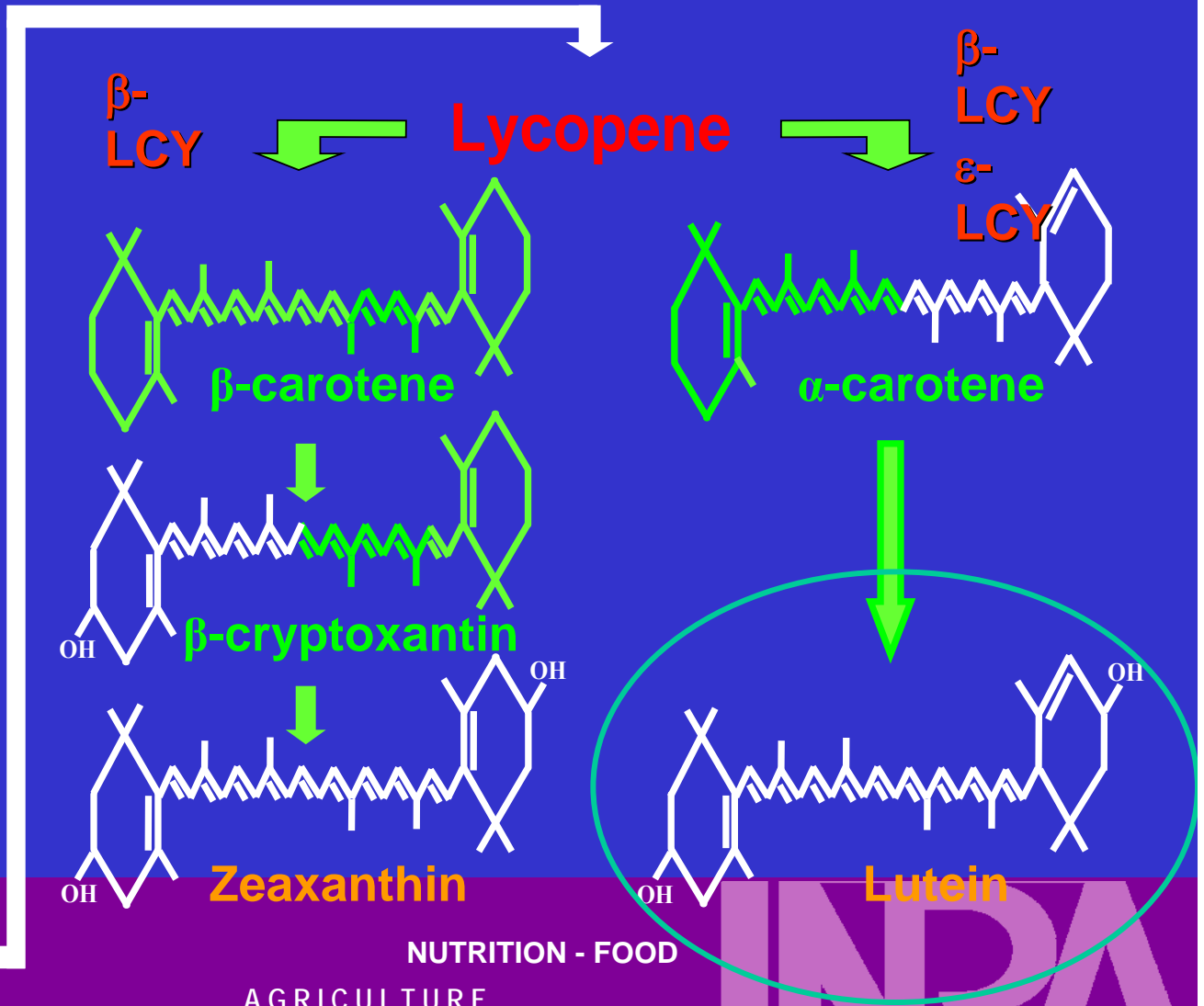
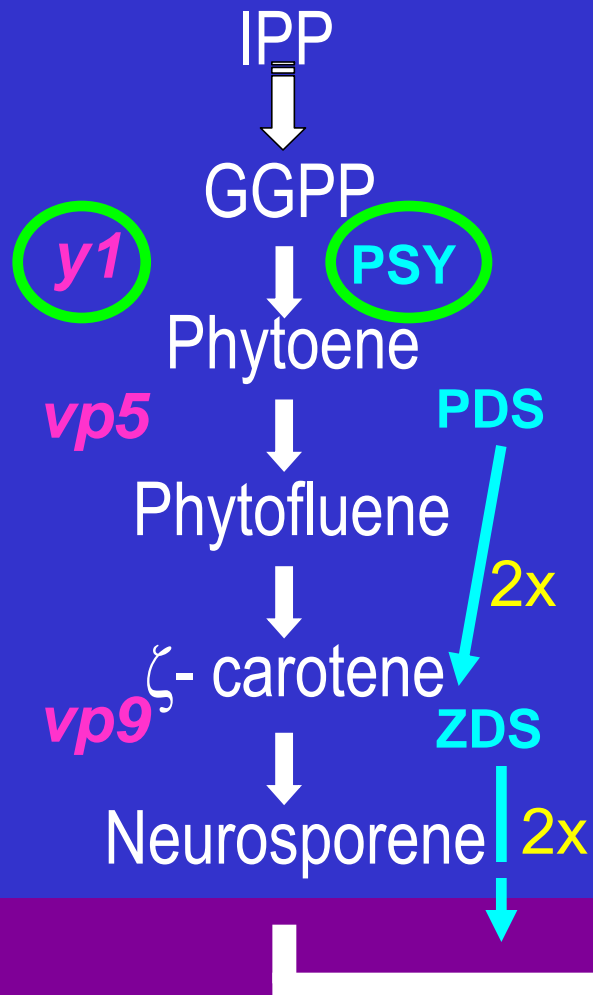


Fig. 2 Assay for free phosphate (HIP phenotype) in *lpa241* families. Single seeds from a given ear were crushed, extracted and assayed for free P using a microtitre plate-based colorimetric molybdenum staining assay. To allow for direct comparison, 100 mg of flour were extracted in 10 vol of HCl 0.4 N, and an equal aliquot vol was tested. The standards (S) contained 0, 0.20, 0.50, 1.50 and 3.0 µg of P

Genotype	Seed dry weight ^a mg	Total P ^b mg g ⁻¹	Pi free ^b mg g ⁻¹	Phytic acid P ^b mg g ⁻¹
+/+	184 ± 11	4.5 ± 0.42	0.3 ± 0.15	3.7 ± 0.21
<i>lpa241</i> /+	189 ± 5	4.4 ± 0.34	0.7 ± 0.18	3.3 ± 0.28
<i>lpa241</i> / <i>lpa241</i>	179 ± 6	4.6 ± 0.47	3.3 ± 0.40	0.4 ± 0.32

Carotenoid Pathway



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Contributions from

- G Charmet, FX Oury UMR1095 Plant Genetics and breeding Clermont-Ferrand F
- B Carré et al, Poultry Research Unit, Nouzilly F
- L Saulnier et al, UMR Biopolymers Nantes F