

*Relevance of the genetic  
background of the seed to the  
application of Real Time PCR  
strategy for GMO quantification in  
maize seed samples*



# Overview

- *GM seed testing*
  - ✓ *Application of the GMO legislation in the seed testing*
  - ✓ *Measurement units*
  - ✓ *Approaches for quantification of seed samples*
- *Real Time PCR and quantification of seed samples – importance of the genetic composition of the seed*
  - ✓ *Theoretical approach – creating models*
  - ✓ *Practical approach – proving the models*
- *Some important consequences*
  - ✓ *Compliance of the thresholds*
  - ✓ *Implementation of the traceability system*

## *GM seed testing*

- Many transgenic traits were introduced in the number of crop species
- In Europe only few are authorized
- Europe imports seeds from countries where the GMOs are cultivated
- The regulations ensure the consumer to choose between conventional and non-conventional products

## *GM seed testing*

Threshold regulations for food labelling (EC/49/2000;  
EC/50/2000) – (EC/2001/18)

New regulations on labeling and traceability, replacing &  
amending old legislation (in force since 20th April 2004)

EC/1829/2003 on GMO food and feed

EC/1830/2003 on traceability and labeling of GMO food and  
feed derived products

Comission Recommendation 2004/787/EC (4 October 2004)

# *GM seed testing*

## *Threshold regulations for mandatory labeling:*

### *Food/feed*

- 0.9% - authorized GMOs
- Zero tolerance – non-authorized GMOs
- 0.5% - non-authorized with positive evaluation

### *Separate threshold for seeds are proposed:*

- 0.3% for cross-pollinators (canola)
- 0.5% for self-pollinators and exceptions from cross-pollinators (corn, sugar beet, tomato, potato, cotton and chicory)
- 0.7% for exceptions from self-pollinators (soya)

# *GM seed testing*

## *Measurement units*

- **Seeds for sowing:** *number of transgenic seeds:total number of seeds*
- **Harvested products:** *number of transgenic grains/beets/fruits:total number grains/beets/fruits*
- **Milled, raw, pure or mixed products:** *number of transgenic particles: total number of particles*
- **Isolated proteins from seeds, grains or derived products:** *number of transgenic proteins:total number of proteins*
- **Isolated DNA from seeds, grains or derived products:** *number of transgenic haploid genomes:total number of haploid genomes*



# *GM seed testing*

## *Approaches for GMO quantification in seed samples*

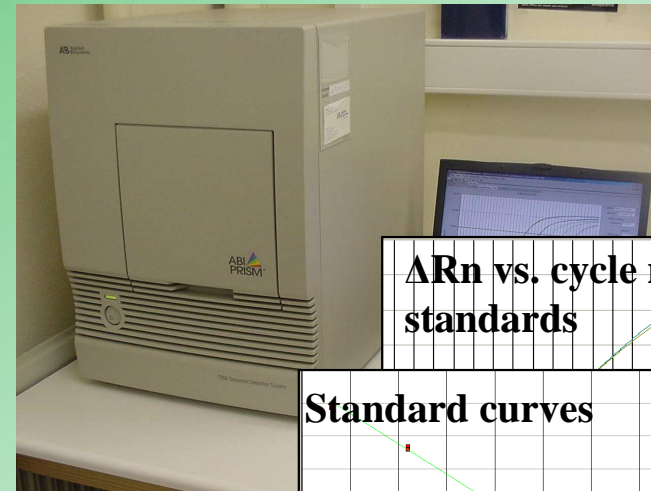
- Sequential testing plan
- Individual seed analysis
- Real Time PCR

# Real Time PCR: experimental setup

**DNA extraction**

**Real Time PCR run**

**Unknown sample**



$\Delta Rn$  vs. cycle number standards

Standard curves

$\Delta Rn$  vs. cycle number – endogene and transgene

**Homogenized sample (3000 seeds)**

Well	Sample Name	Detector	Task	Ct	StdDev Ct	Qty	Mean Qty	StdDev Qty
A1	NTC	SG 1	NTC	Undet.	Undet.			
A2	NTC	SG 1	NTC	36.21	Undet.			
A3	4.4	SG 1	Standard	21.34	0.118	4000.00		
A4	4.4	SG 1	Standard	21.17	0.118	4000.00		
A5	4	SG 1	Standard	22.73	0.049	1000.00		
A6	4	SG 1	Standard	22.66	0.048	1000.00		
A7	3	SG 1	Standard	25.84	0.017	1000.00		
A8	3	SG 1	Standard	25.81	0.017	1000.00		
A9	2	SG 1	Standard	29.23	0.008	100.00		
A10	2	SG 1	Standard	29.24	0.008	100.00		
A11	1	SG 1	Standard	33.07	0.194	10.00		
A12	1	SG 1	Standard	32.80	0.194	10.00		
B1	NTC	SG 2	NTC	Undet.	Undet.			
B2	NTC	SG 2	NTC	Undet.	Undet.			
B3	4.4	SG 2	Standard	21.15	0.058	4000.00		
B4	4.4	SG 2	Standard	21.07	0.058	4000.00		
B5	4	SG 2	Standard	22.40	0.032	1000.00		
B6	4	SG 2	Standard	22.35	0.032	1000.00		
B7	3	SG 2	Standard	25.66	0.104	1000.00		
B8	3	SG 2	Standard	25.80	0.104	1000.00		



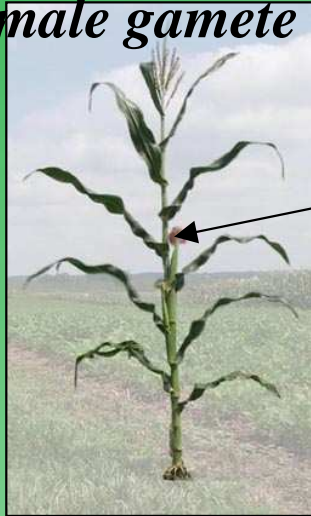
**Output**  
**Number of Transgenic genomes/total genome number**



# Real Time PCR quantification of seed samples

## Composition of the maize kernel

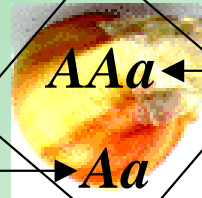
*Female gamete (A)*



*Male gamete (a)*



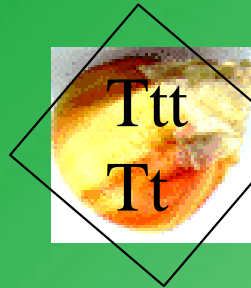
*Embryo  
(10%)*



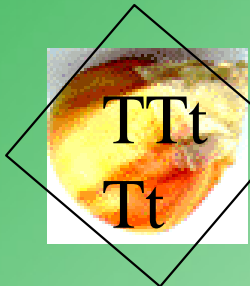
*Endosperm  
(85%)*

ILVO

# Experimental data – creating models



F1 seed:  
Transgene allele of  
paternal origin



F1 seed:  
Transgene allele of  
maternal origin



Real Time PCR  
quantification

0.35

0.65

Ratio transgenic  
genomes/  
total genomes in 1  
kernel



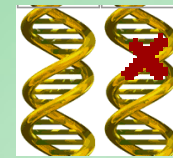
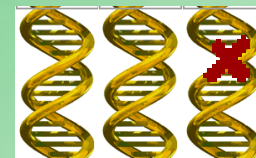
# Experimental data – creating models

Number of seeds



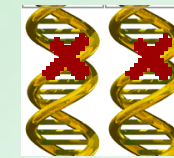
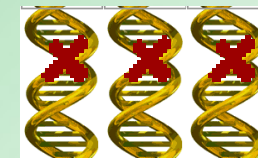
0.5 %

Number of genomes



Minimum

0.17%



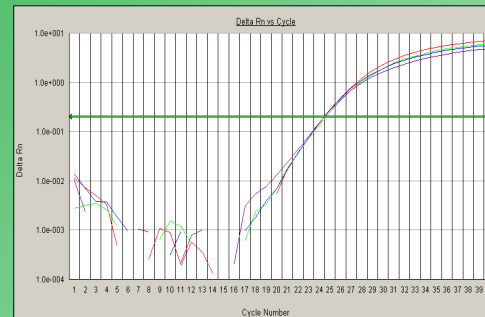
Maximum

0.5%

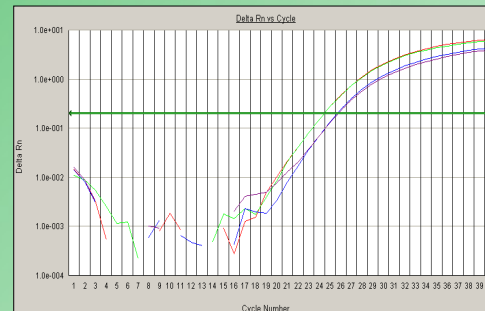
## *Experimental data – creating models*

- Seed/seed percentage and genome/genome percentage are not equivalent
- The genome/genome percentage is different in the heterozygotes depending on the transgenic parent

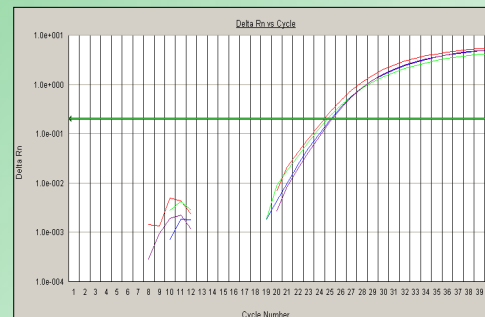
# Experimental data - proving the models



**Homozygous:**  
 **$0.96 \pm 0.05$**



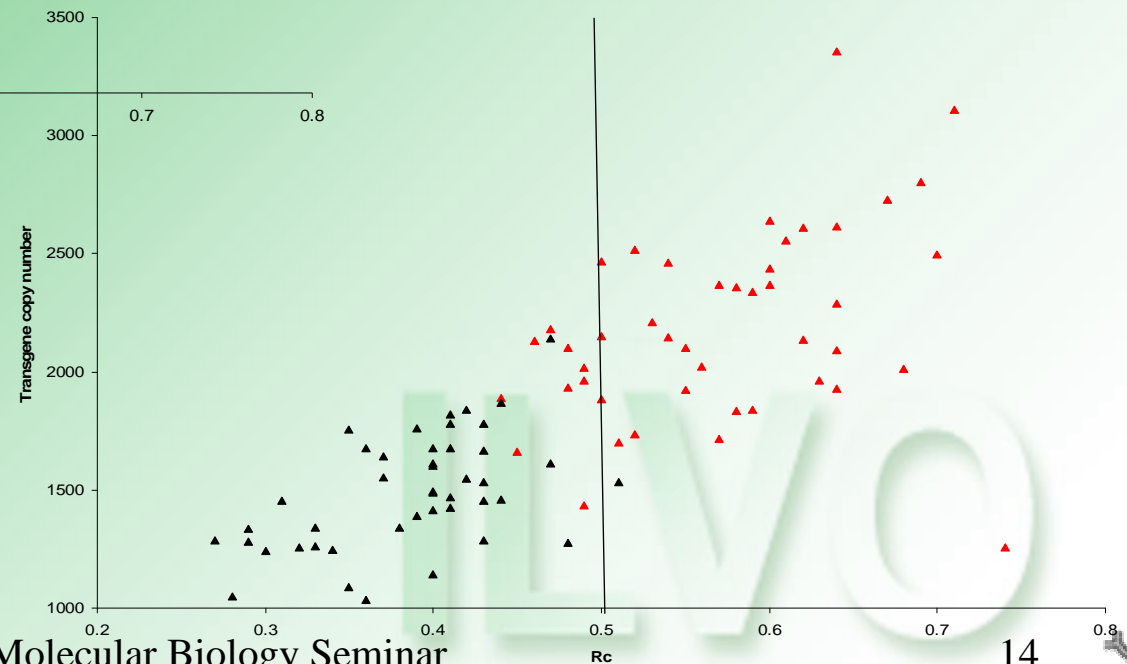
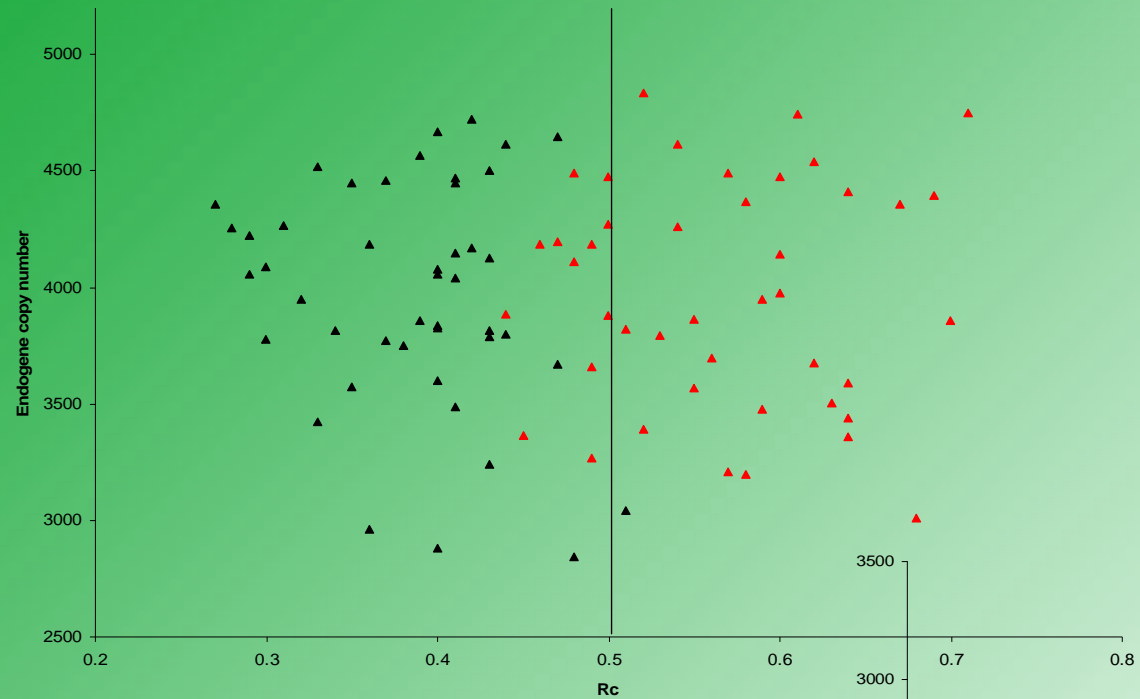
**Heterozygous paternal:**  
 **$0.39 \pm 0.03$**



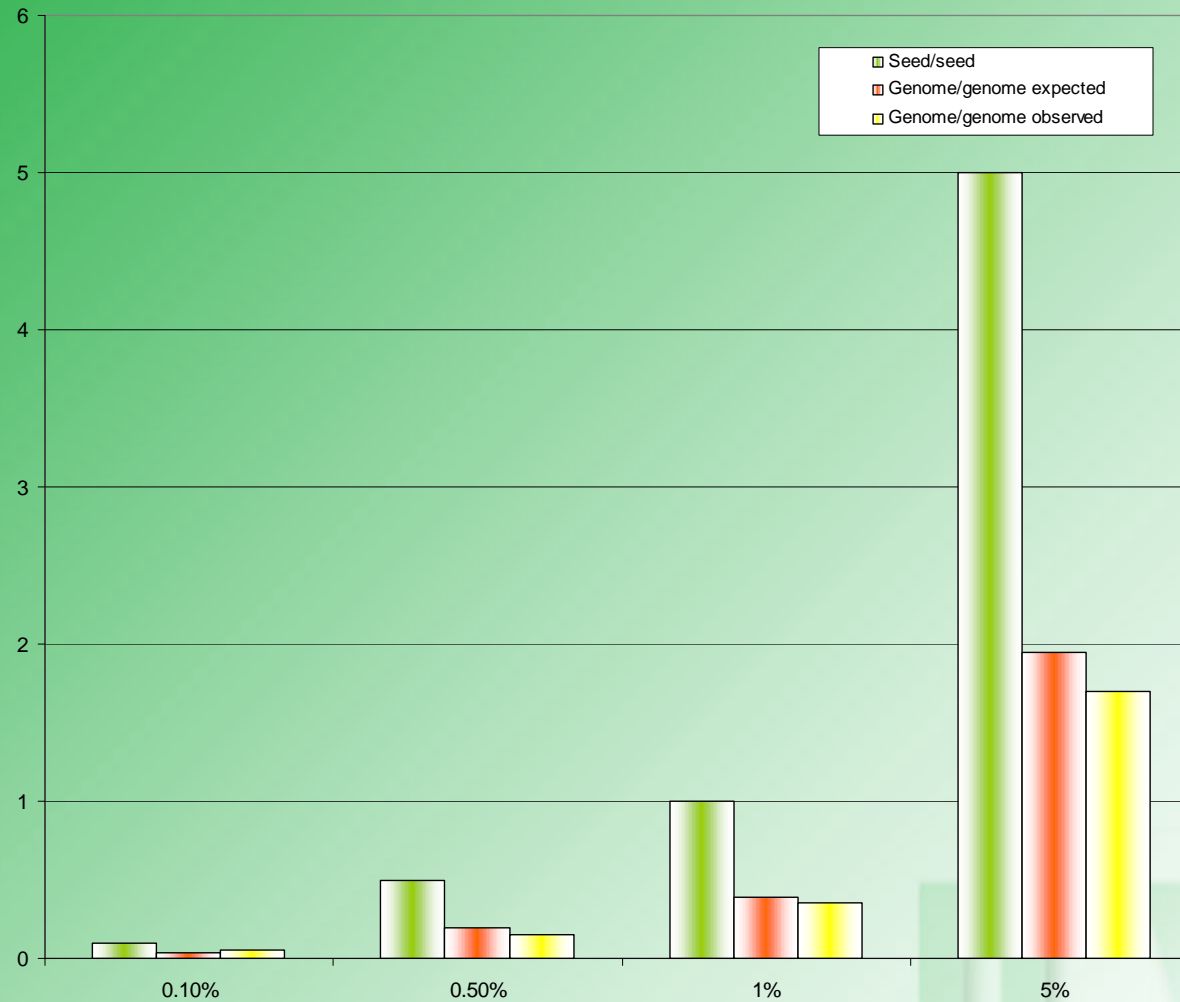
**Heterozygous maternal:**  
 **$0.57 \pm 0.05$**



# Experimental data - proving the models



# Experimental data - proving the models



## *Experimental data - proving the models*

- Conversion from number of seeds to number of genome is not possible
- One and the same measured genome number can be different number of seeds

# Consequences

**Seed thresholds:**  
• 0.5% for corn

*Number GMO seeds/  
total number of seeds*

**Threshold for food/feed:**  
• 0.9%

*Ingredient*

*Commission recommendation 2004/787/EC*

*Number GM genomes/total number genomes*

**Seed thresholds** -----> **Threshold for food/feed**

## *Consequences*

### *Compliance of the seed thresholds with the threshold for food/feed*

- What value of the threshold for seeds should be calculated?
- What is the relation between the 0.5% (in haploid genome) for maize seeds and the 0.9% (in haploid genomes) in the derived product?

## *Consequences*

### *Implementation of the traceability system*

- A seed sample with  $<0.5\%$  in haploid genomes can contain different number of GM seeds (15-51 for sample of 3000 seeds)
- Different number GM seeds is different number GM plants on the field
- *How do the transgenic genomes of homo- and heterozygotes seeds contribute to the contamination on the field, and then to the GM content of the harvested product?*



## *Conclusions*

- The use of the haploid genomes as a measurement unit facilitates the implementation of the traceability system for GM plants and GM derived products
- The haploid genomes as a measurement unit for GMO quantification in seeds should be implemented in the context of the seed testing

# Thank you for your attention!