



# Real-time RT-PCR as a tool to study differential gene expression in the potato-late blight interaction

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## 1. Introduction

Late blight, caused by the fungus-like oomycete *Phytophthora infestans*, is one of the most severe potato diseases worldwide. The present work describes a gene expression analysis strategy to study this potato-late blight interaction.

To detect differentially expressed genes, a fluorescent cDNA-AFLP technique was applied on four potato genotype pools differing in their resistance level to late blight and sampled at four different time points. Different expression profiles have been obtained and a total of 262 transcript-derived fragments have been isolated, cloned and sequenced.

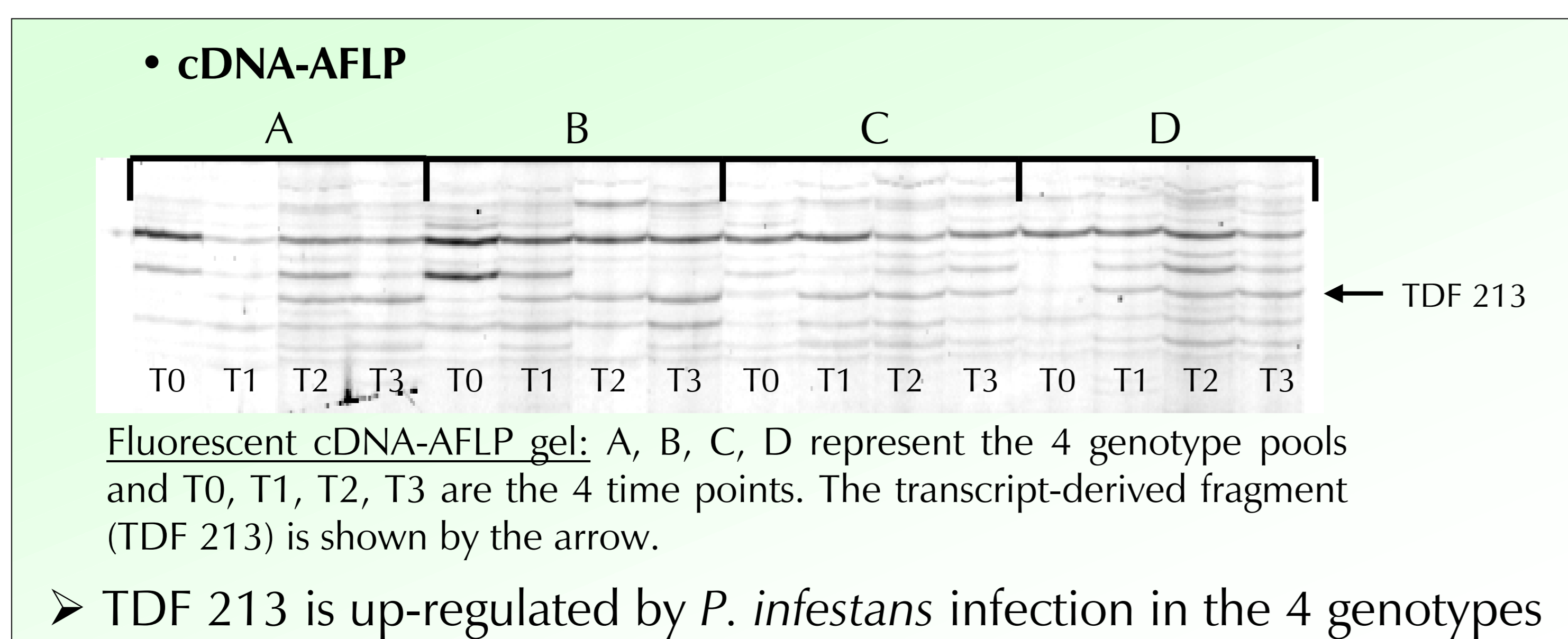
Depending on their homology with known putative functions, we focused on some transcripts. Real-time RT-PCR confirmed the results obtained by cDNA-AFLP and allowed an accurate quantification of the selected transcripts in the different samples. Here, we present the expression analysis of one of the selected transcripts (TDF 213).

## 2. Material

4 different potato genotype pools A, B, C, D sampled at 4 time points after *P. infestans* infection in field conditions (0, 1, 2, 3 weeks) constitute the 16 samples.

## 3. Results

### a) TDF 213 characterisation



### • Cloning and sequencing

➤ TDF 213 shares 91% homology with a wild tomato (*Lycopersicon peruvianum*) gene coding for a heat-shock protein (sequence compared to internet databases by using BLAST programs)

### b) Differential gene expression analysis by real-time RT-PCR

#### • Experiment

Experiment	Reverse Transcription	Chemistry	Assay optimisation	Reference Gene
5' Nuclease Assay	Multi™ Scribe RT	TaqMan® MGB Probe	Applied Biosystems (assay by design)	Potato rRNA 18s
SYBR® Green	Multi™ Scribe RT	SYBR® Green	Home	Potato rRNA 18s

#### • PCR efficiency calculation

PCR efficiencies were calculated by making a standard curve on a reverse transcribed total RNA sample.

	5' Nuclease Assay	SYBR®Green
E <sub>TDF 213 (target)</sub>	1.951	1.8623
E <sub>rRNA 18s (reference)</sub>	2.02	1.8823

➤ The 5' Nuclease assay allows to reach higher PCR efficiencies than the SYBR Green assay.

#### • Real-time RT-PCR data analysis

All experiments were performed on duplicates.

Data analysis:

- Mean Normalized Expression (MNE) was calculated by using the Q-Gene software (Muller *et al.*, 2002)
- Comparative CT method ( $2^{-\Delta\Delta CT}$ ) as described in the ABI Prism SDS User Bulletin #2

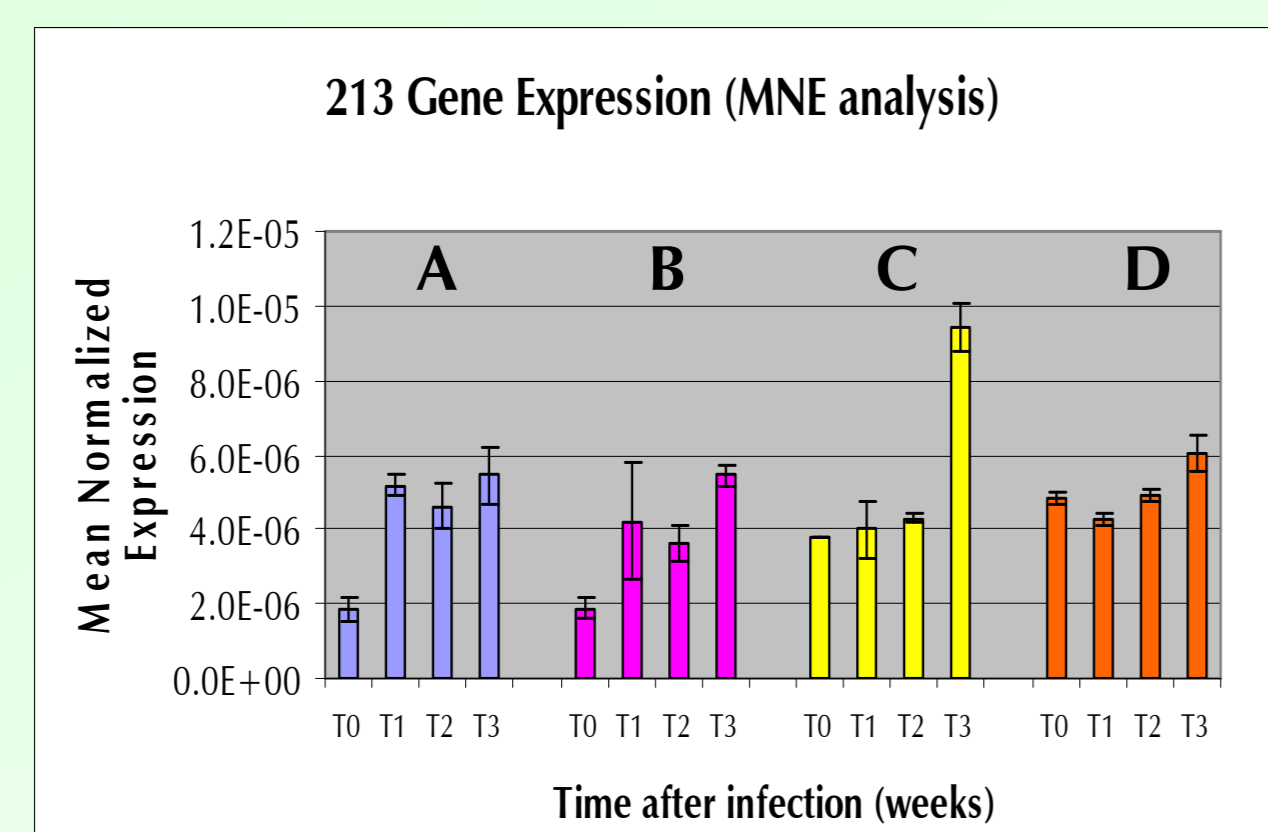


Figure 1: TDF 213 expression: 5' Nuclease Assay

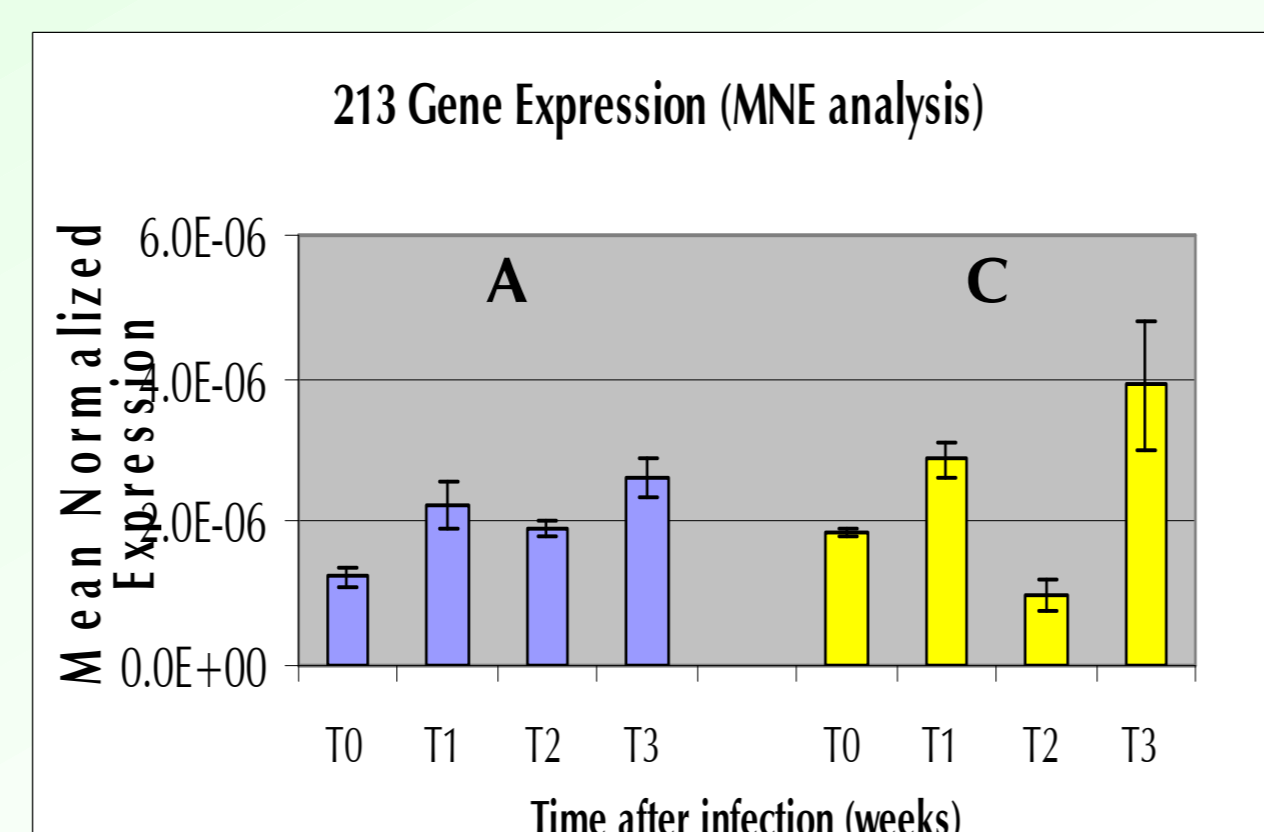


Figure 2: TDF 213 expression: SYBR Green Assay

➤ Both experiments (5' Nuclease Assay and SYBR® Green) confirm the cDNA-AFLP profile: TDF 213 expression increases upon pathogen infection.

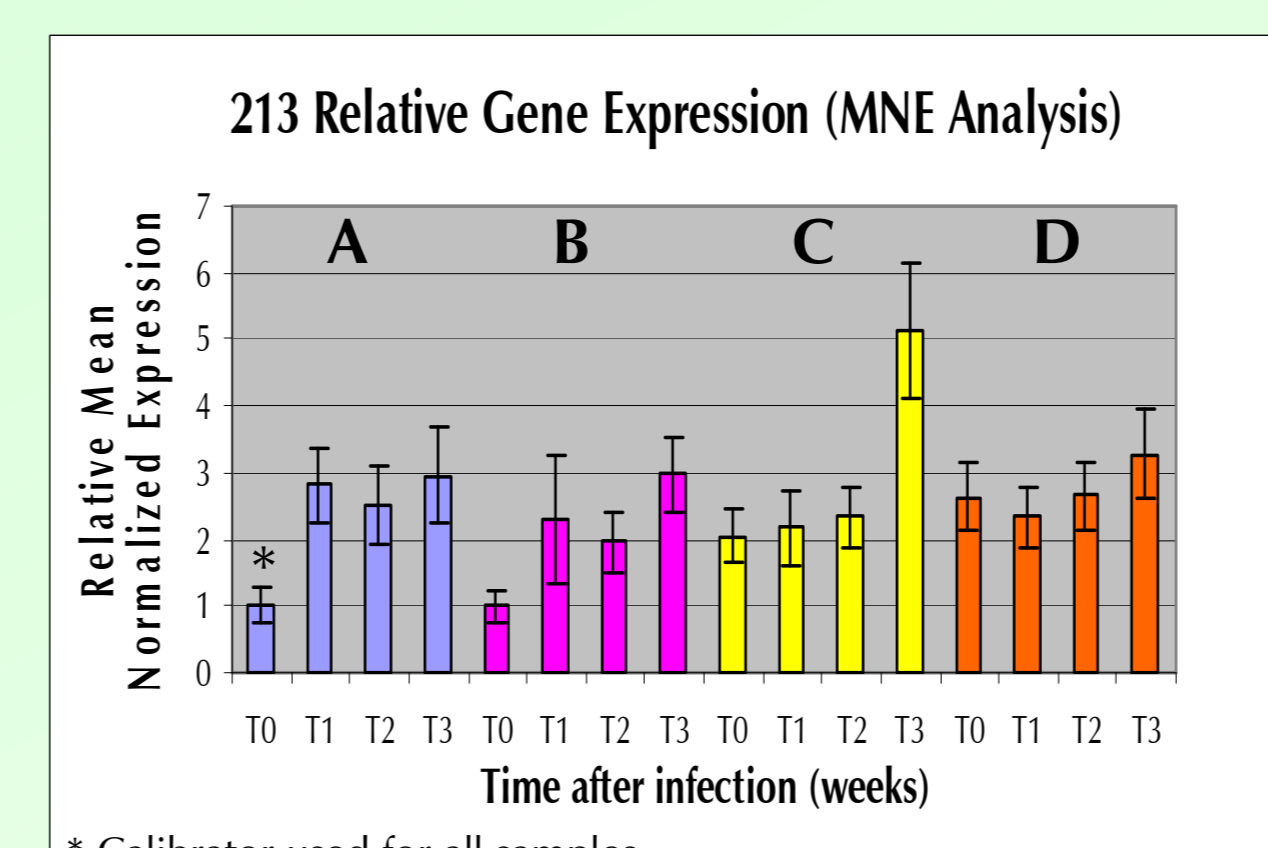


Figure 5: TDF 213 relative expression by the MNE analysis (5' Nuclease Assay data). Here, the MNE of the 16 samples is reported to sample T0 of genotype A (\*) considered as unique calibrator

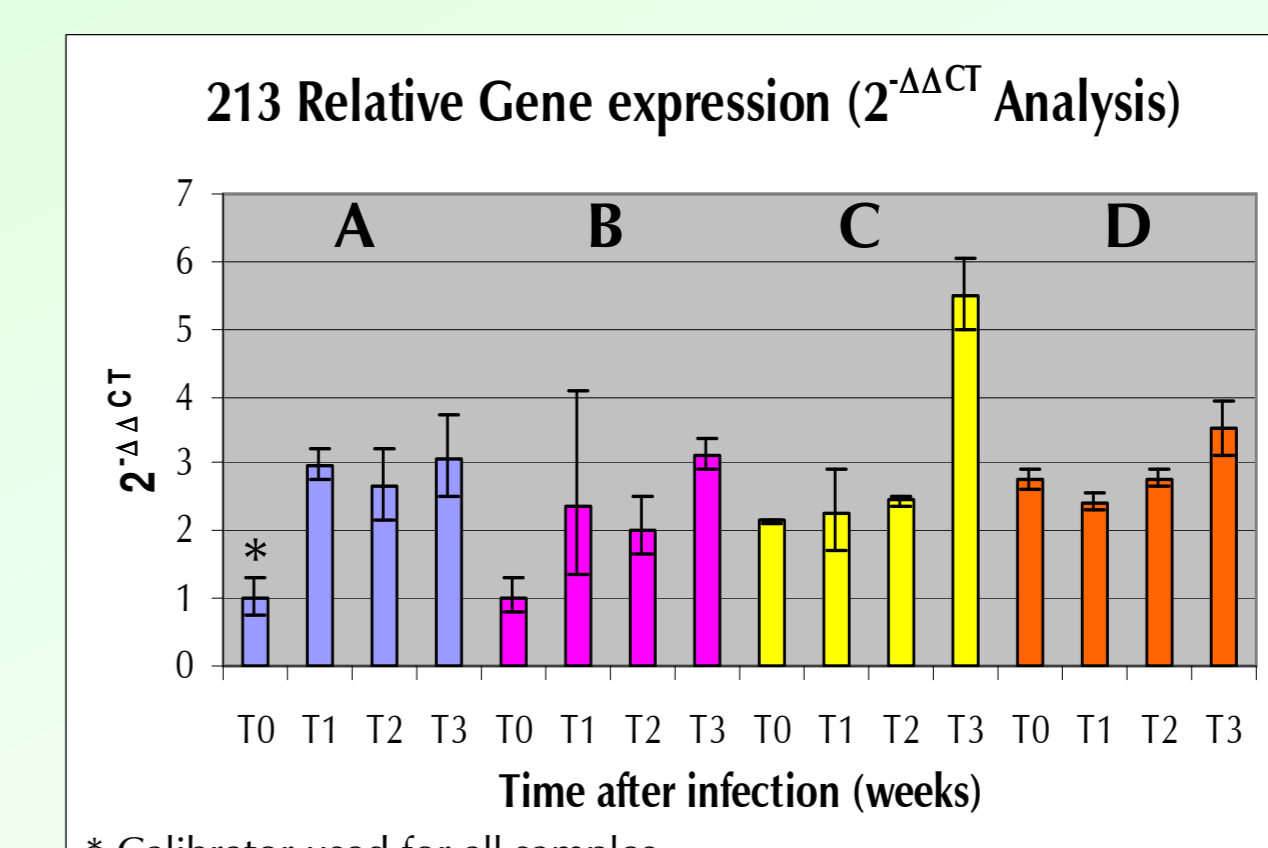


Figure 6: TDF 213 relative expression by the  $2^{-\Delta\Delta CT}$  method analysis (5' Nuclease Assay data). Here, sample T0 of genotype A (\*) is used as calibrator for the 16 samples

➤ The genotype D presents a higher constitutive TDF 213 expression level. Indeed at T0, the gene is expressed about 2.5 fold more in genotype D than in genotype A. Both analysis methods show similar results.

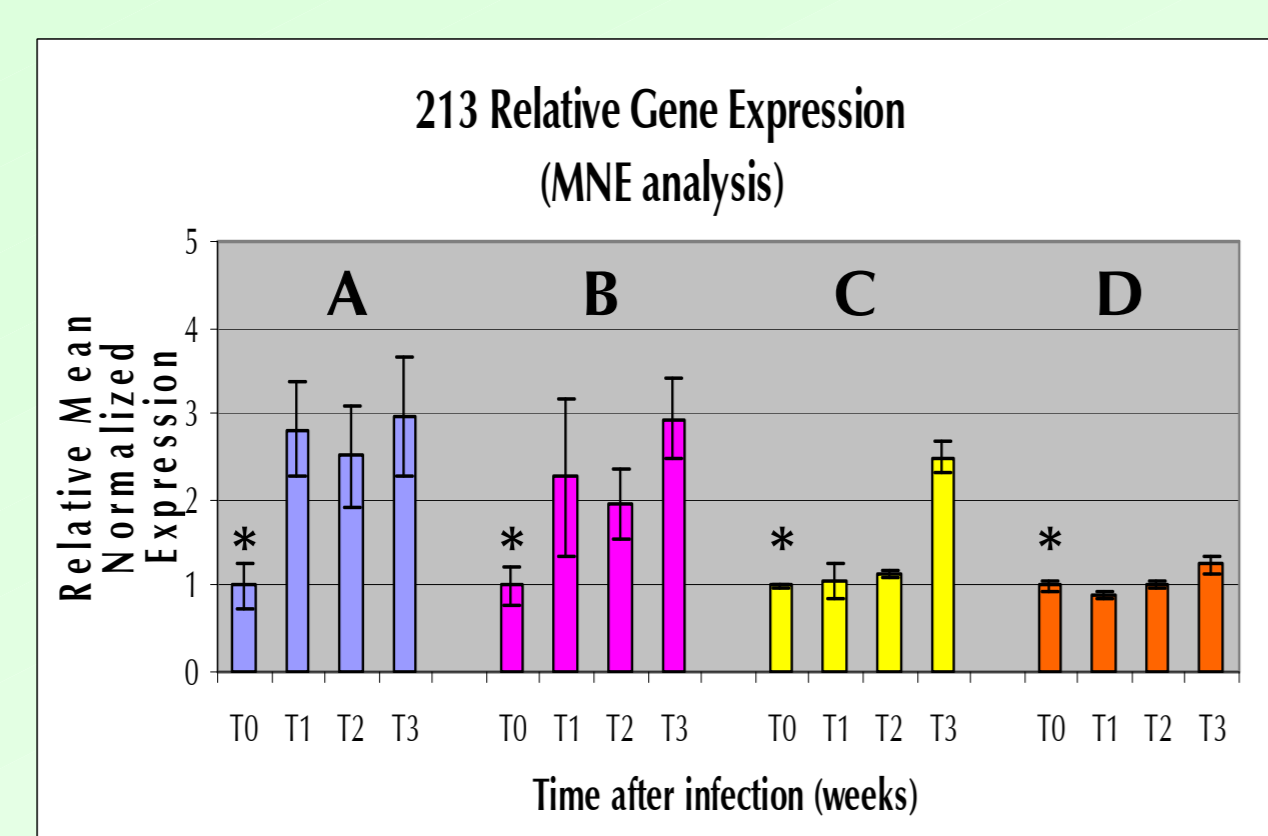


Figure 3: TDF 213 relative expression by the MNE analysis (5' Nuclease Assay data). In each genotype pool, the MNE of samples T0, T1, T2, T3 is reported to sample T0 used as a calibrator

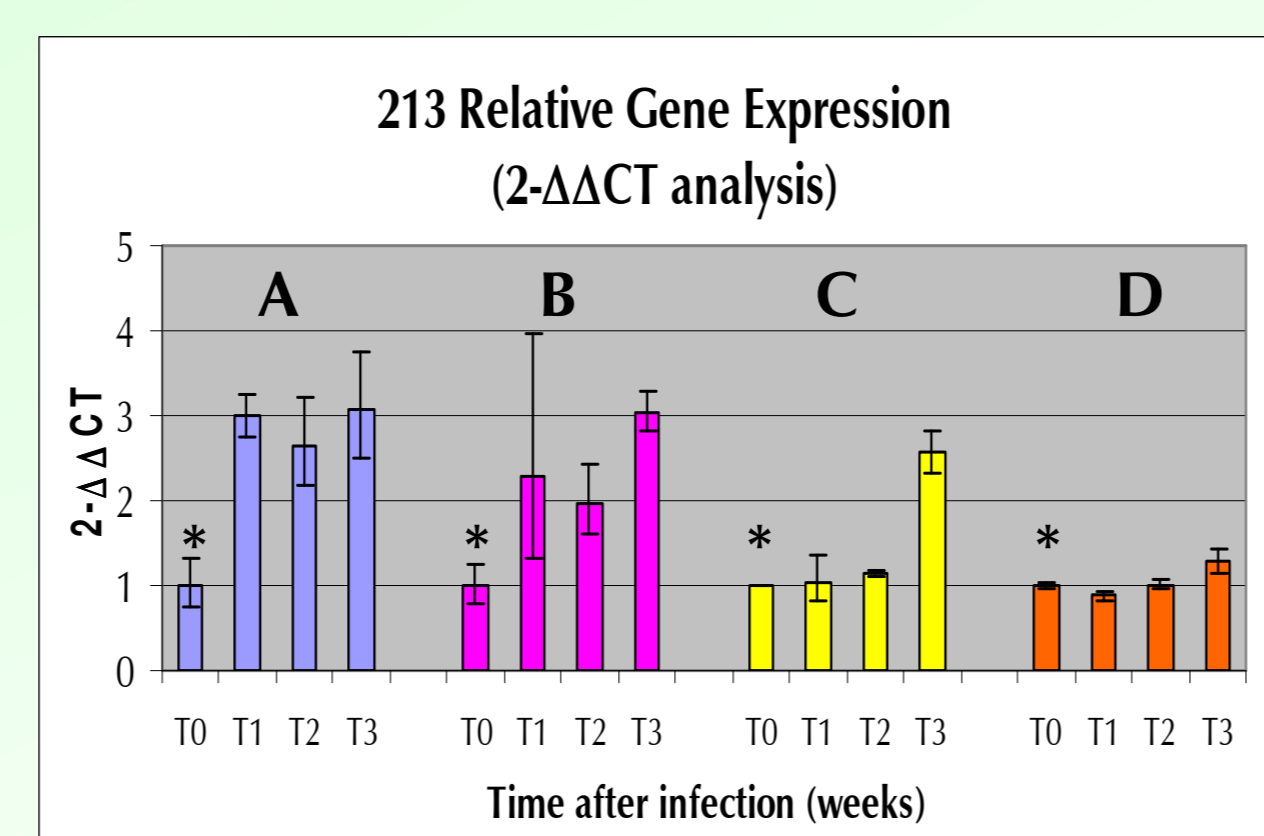


Figure 4: TDF 213 relative expression by the  $2^{-\Delta\Delta CT}$  method analysis (5' Nuclease Assay data). In each genotype pool, the calibrator used is sample T0

➤ The up-regulation of gene expression is more important in genotype A than in genotype D.  
 ➤ Both analysis methods show similar results. This is due to E reference and E target which are very close to each other in this case (respectively 2.02 and 1.951).

## 4. Conclusion

- Real-time RT-PCR experiments confirm the up-regulation of TDF 213 expression by *P. infestans* observed by cDNA-AFLP.
- In this case, the Mean Normalized Expression analysis method and the comparative CT method give very similar results.
- Depending on the calibrator used in the relative gene expression analysis, different aspects are pointed up (differential gene expression intra or inter genotype pools).

#### References:

- Muller *et al.* 2002. Processing of gene expression data generated by quantitative real-time RT-PCR. Biotechniques. 32(6):1372-1378.
- ABI Prism 7700 Sequence Detection System User Bulletin#2

The authors want to thank the International Potato Centre for providing with the potato cDNA samples