Gene Expression Plant – Virus Study Monitoring Potato Virus Y (PVY) with Real-Time PCR (qPCR) Results Using the PIPETMAX® 268

Application Note FB0213

Keywords

Real-time PCR (qPCR), PIPETMAX 268, Reproducibility, RNA, cDNA, Plants, Virus

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Introduction

Gene expression studies are often used to gain insight into complex interactions between organisms. Real-time PCR (qPCR) is frequently used in gene expression studies as it fits perfectly with its wide dynamic range, sensitivity, and ease of automation possibilities.

Plant – virus interactions are an example of very complex interactions that are best understood by monitoring gene expression of host plants after infection with a virus. In this case, we monitored the response of the potato plant cultivar Désirée (*Solanum tuberosum L.* cv. Désirée) to infection with Potato Virus Y (PVY) at the level of gene expression. Potato is the world's most widely grown tuber crop and the fourth largest food crop in terms of fresh produce after rice, wheat, and corn. Désirée is a tolerant cultivar, which means that PVY viruses multiply in the plant, but the plant tolerates PVY infection expression with very mild or no symptoms of infection. PVY, a member of the *Potyviridae* family, is an important potato pathogen worldwide.

In this application the PIPETMAX 268 was used for automated sample preparation in advance of qPCR which was performed on the sampled leaves from both virus-inoculated and mock-inoculated potatoes. The discussion describes the results for the gene relative expression of the Chlorophyll a/b binding gene (CAB) and PVY RNA relative gene expression levels, linear regression values from serial dilutions, cytochrome oxidase (COX) gene amplification curves, and statistical estimation of pipetting (%CV).

Materials

- RNeasy Plant Mini Kit - Qiagen, Hilden, Germany
- DNase I - Invitrogen, Carlsbad, CA
- High Capacity cDNA Reverse Transcription Kit - Applied Biosystems, Carlsbad, CA
- Fast Real-Time PCR System - Applied Biosystems 7900HT with 384 block

Sample Preparation

1. Two plants were inoculated with PVY and two were mock-inoculated (same procedure as inoculation with virus, except that virus was not present). Leaves from virus- and mock-inoculated plants were sampled at 4 days and 7 days post inoculation (dpi).
2. Reaction mixtures and sample dilutions were prepared by hand, master mixes and samples were loaded onto a 384-well plate and the sample preparation was carried out by the PIPETMAX, the plate was processed in the qPCR cycler, and then the data was analyzed (Figure 1).
3. Total RNA was extracted using RNeasy Plant Mini Kit, followed by DNase-treatment and then reverse transcribed using the High Capacity cDNA Reverse Transcription Kit. Relative concentration of PVY RNA and expression of CAB were followed using qPCR.
4. The standard curve method was used for relative gene expression quantification, and the transcript accumulation of each gene was normalized to COX and Elongation factor-1 (EF1). TaqMan chemistry was used in all genes.

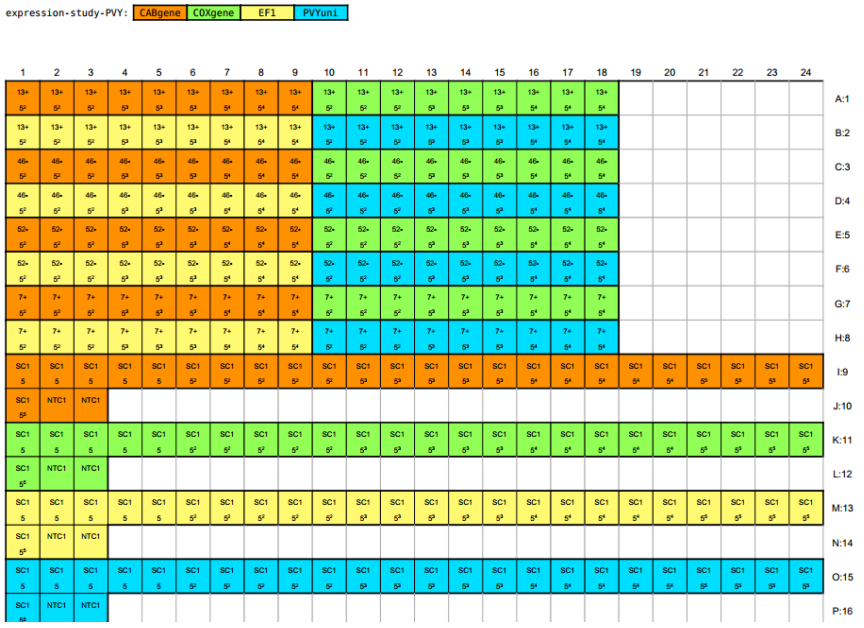
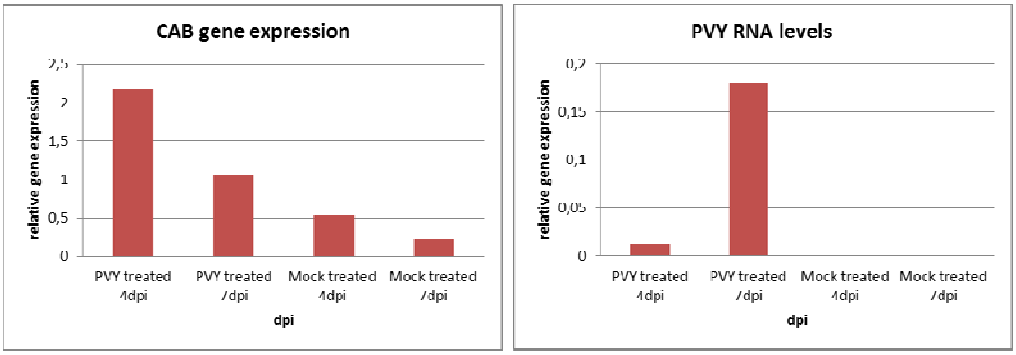


Figure 1: Samples and master mixes were loaded onto a 384-well qPCR plate for processing using PIPETMAX.

Materials & Methods (cont.)

Sample Analysis with qPCR

Samples were analyzed in 10 µl reactions on a 384-well plate on the Fast Real-Time PCR System (Applied Biosystems). Each sample was analyzed in three replicates and three dilutions (25-, 125-, 625-fold). A standard curve was used for quantification purposes for each gene (5 dilution points ranging from 5- to 3125-fold dilution were done in 5-fold steps (Figure 2), each step in 5 replicates; in PVY only 4 dilution points were used). Two no-template-controls (NTCs) were used for each gene.



(a) (b)

Figure 2: (a) Expression of CAB gene normalized to COX and EF1 in infected compared to non-infected (mock-inoculated plants) (b) levels of PVY RNA in samples.

Results

The CAB gene is involved in plant photosynthesis, a major primary metabolic pathway in plants. The gene expression of CAB was greater in PVY infected plants than in healthy (mock-inoculated) plant leaves (Figure 2a). This showed the higher metabolic rate in infected plants as they were struggling to overcome the viral infection; however, the plants started losing the battle with the virus over the course of several days as the CAB gene expression started dropping (CAB gene expression level at 4 dpi was higher than at 7 dpi).

By monitoring the presence of PVY viral RNA in the plants we were able to confirm the presence of the PVY virus in infected plants and showed that PVY accumulated in time: PVY level in 7 dpi plants was higher than in 4 dpi plants, indicating that the virus was spreading and multiplying throughout the plant (Figure 2b). No PVY virus was detected in mock-inoculated plants.

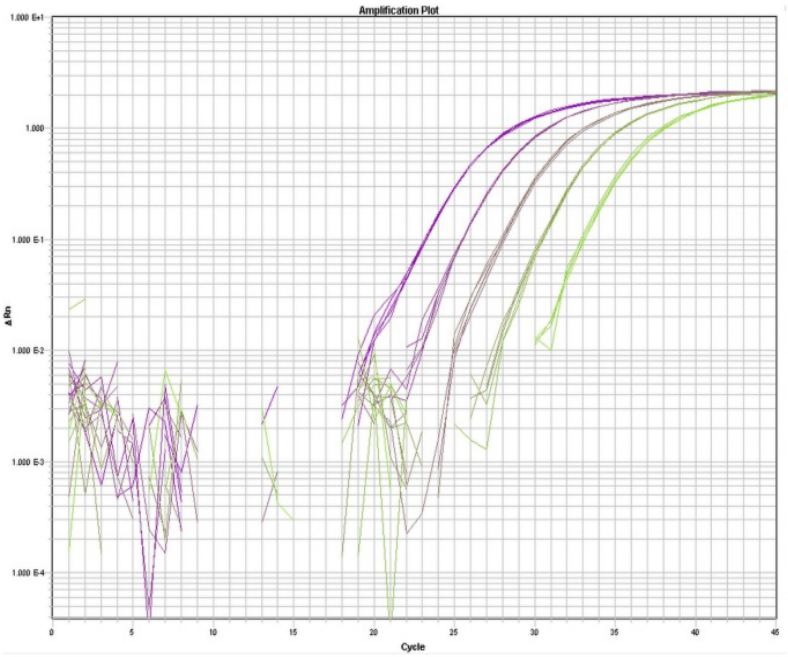


Figure 3: COX gene amplification curves of standard 5-fold dilution curve. Amplification curves from the left to right are of the following dilutions: 5x, 25x, 125x, 625x, and 3125x.

PIPETMAX Performance

Performance of PIPETMAX was assessed on three levels: results of negative controls (NTCs), estimations of serial dilutions, and statistical estimation of pipetting.

Negative Controls

All negative controls (NTCs, no template controls) in the experiment were negative (no signal was detected). This means that no cross-contamination occurred during the qPCR plate pipetting.

Serial Dilution Estimation

Serial dilutions showed how well the pipetting was executed. One of the advantages of serial dilutions was that one can apply linear regression, which showed the trend in the data. This also allowed R square (Pearson's coefficient of correlation) calculations, which indicated how individual measurements were close to the trend line (linear regression line in this case). The optimal value for R-square is 1.0. PIPETMAX reached values that were always above 0.99 (Figure 4).

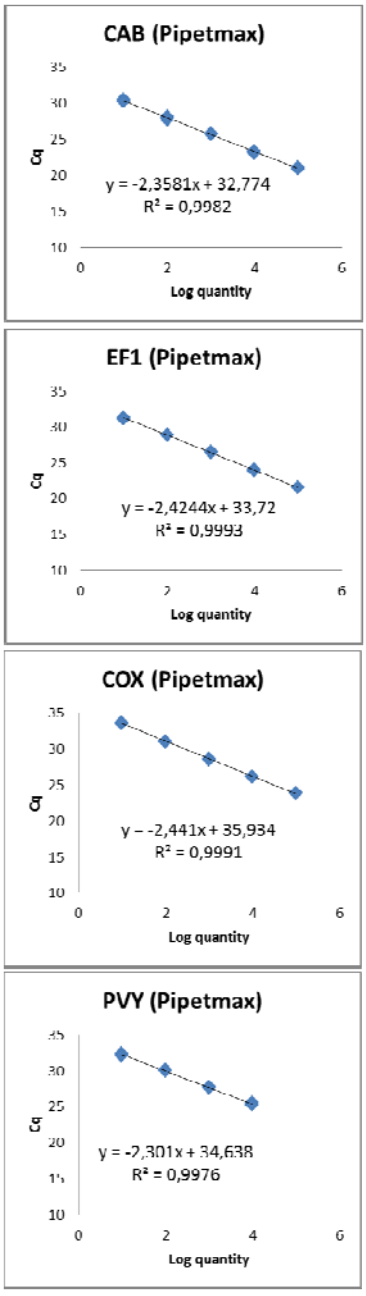


Figure 4: Linear regression parameters for serial dilutions performed by PIPETMAX.

Statistical Estimation of Pipetting

Coefficient of variation (CV) is one of the statistical parameters with which we estimate the dispersion of results. In qPCR, CV is usually estimated at the level of copy numbers (final results), and not Cq values.

In the table below, minimal and maximal CVs for CAB, EF1 and COX genes (across all samples in the experiment) were shown calculated at the level of relative copy numbers of genes. CVs measured at the level of copy number take into account a wide range of sources of technical variability—not just pipetting (variability that may have been introduced during the PCR amplification and data acquisition in qPCR cycler).

Gene	CV	CV - PIPETMAX
CAB	MIN	1.9
	MAX	10.2
	<5%	40% of samples had CV <5,0%
	<15%	100% of samples had CV <15,0%
COX	MIN	1.3
	MAX	15.6
	<5%	67% of samples had CV <5,0%
	<15%	93% of samples had CV <15,0%
EF1	MIN	1.8
	MAX	7.9
	<5%	50% of samples had CV <5,0%
	<15%	100% of samples had CV <15,0%

Summary

In this work, the expression of the potato gene, CAB, is followed at 4 days and 7 days post viral inoculation. The qPCR sample preparation method using the PIPETMAX does not introduce contamination, is accurate, and eliminates inherent variability.

References

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