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Real-time PCR as a promising tool to monitor the epidemiology of *Venturia pirina*

M. van Driel, K. Kots and M. Wenneker[1]

Abstract

*Current control practices for pear scab (*Venturia pirina*) rely on prevention through orchard sanitation and extensive fungicide applications. Molecular quantification through a qPCR offers a sensitive, high-throughput approach to efficiently collect reliable data on the inoculum dynamics. A SYBR qPCR assay targeting the translation elongation factor 1α (EF1-α) gene of* V. pirina *was developed. The assay was validated based on specificity and sensitivity. The specificity was validated against 43* V. pirina *isolates and 13 non-target fungal species. A qPCR offers a prospective tool which can be employed in various aspects of pathogenic studies.*

Keywords: pear scab, SYBR qPCR, *Venturia pirina*

Introduction

Pear scab (*Venturia pirina*, syn. *Venturia pyrina*) hampers the European pear cultivation, as growers suffer economic losses due to scab damage (Perchepied *et al*., 2015). Current control methods to manage *V. pirina* include fungicide applications, cultivating tolerant cultivars, and sanitary measures. Preventative fungicide sprays are most effective when applied simultaneously with favourable weather conditions and the dispersal of the ascospores and conidia. Moisture and temperature data combined with quantification of the inoculum release are therefore key factors in developing efficient fungicide spraying schemes (Sokolova *et al*., 2014). Utilizing quantitative polymerase chain reaction (qPCR) enables the study of disease dynamics, specifically the high-throughput and sensitive assessment of the spore distribution. This short communication summarizes the efforts which contributed to developing and validating a SYBR-based qPCR protocol.

Material and Methods

A primer pair based on the translation elongation factor 1α (EF-1α) region was developed using SnapGene Viewer (version 7.0). Primers were developed in silico and pre-validated for specificity through multiple sequence alignment. Optimal reaction conditions were determined. Calibration curves using the EF1-α primer set were established employing mycelium DNA from a WUR isolate. The DNA were serially diluted in five- and tenfold, spanning concentrations from 10ng to 12.8fg and 10ng to 0.1fg, respectively. Standard curves are used to determine the sensitivity of a qPCR assay. The specificity of the EF1-α-based SYBR qPCR assay was validated using 10ng DNA of 39 *V. pirina* isolates and 13 non-target fungal species, including *Venturia inaequalis*.

Results and Discussion

Sensitivity Validation

Amplification efficiencies of 107.7 and 104.6 percent were achieved for the EF1-α primer set (figure 1). A reliable correlation was achieved between the Ct-value and the amount of DNA present (R2>0.99). The quantification range extended to 640fg, which was measured at an average Ct-value of 34. The detection limit extended to 128fg DNA (data not shown). Other

[1] Wageningen University and Research, Field crops, NL-6668 LA Randwijk

research efforts aimed at quantifying the primary inoculum have successfully quantified genomic DNA in the range of 4-40fg (Köhl *et al*., 2018; Torfs *et al*., 2019; Prencipe *et al*., 2020). Therefore, renewed efforts is directed at developing a TaqMan qPCR assay.

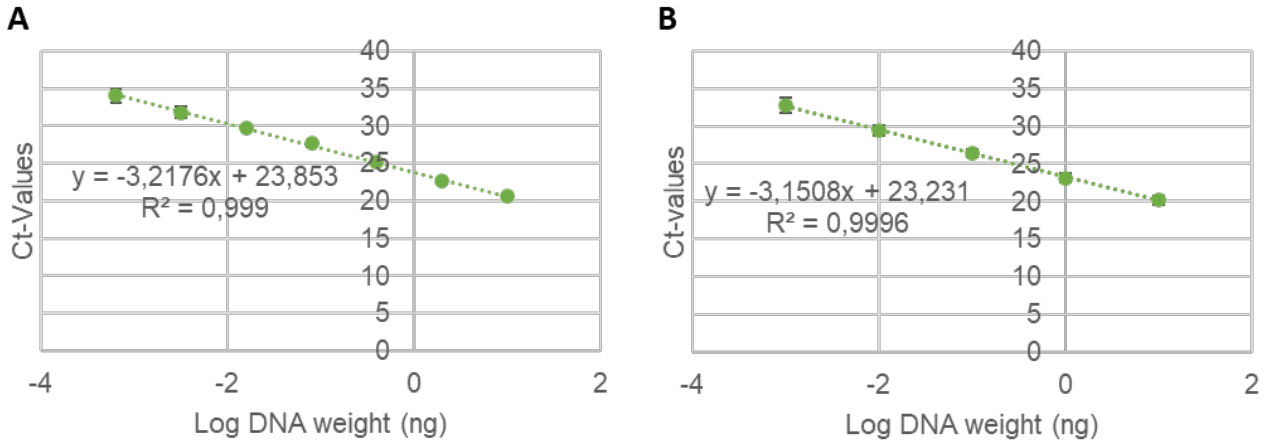

Figure 1: A standard curve obtained with a five- (**A**) and tenfold (**B**) dilution series of genomic DNA using the EF1-α primer set, showing the correlation between DNA weight (10ng to 640fg and 1pg, respectively) and the Ct-values. The data points represent three and five independent repetitions respectively, each consisting of three technical replicates. Standard deviation is included as error bars. However, the values are below 0, which means that they are not always visible in the graph.

Specificity Validation

A positive SYBR qPCR result was found for all 39 *V. pirina* isolates (data not shown). No Ct value lower than 34 (Ct-value corresponding to 640fg *V. pirina* DNA) was obtained for any of the 13 non-target fungal species, including *V. inaequalis*, *Botrytis cinerea*, *Neonectria ditissima* and *Stemphydium vesicarium* (data not shown). This shows that the SYBR qPCR assay is specific to *V. pirina*.

Acknowledgements

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