Genetic Transformation of *Phytophthora ramorum* With the Jellyfish GFP Gene[1]

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Abstract

The important quarantine organism *Phytophthora ramorum* has been dramatically increasing its host range in the past years and most of the studies concerning *P. ramorum* focus on these issues. Very little is known about the latency period. For sampling and analyzing potentially infected plant material, detailed information on the latency of the disease is of high importance. In order to provide such a tool for the infection process follow-up, we established a reliable method for the stable genetic transformation of *P. ramorum* isolates. The transferred genes were the marker gene nptII for resistance to geniticin and the target gene GFP. The first and most important step in this protocol was to develop a stable system to produce protoplasts from *P. ramorum* tissue. The next step was the transformation of protoplasts with plasmids containing marker gene and target gene, following an improved polyethylene glycol (PEG)-mediated protocol of protoplasts transformation. After transformation, protoplasts were cultivated on a selective medium and allowed to regenerate mycelium. The selected transformants were checked for integration and expression of transferred genes by PCR amplification and the use of anti-GFP antibodies.
About forty-three different transformed isolates were produced and then tested for GFP fluorescence, GFP expression and GFP gene integration. They were further tested for their infection potential on Rhododendron plants.

Introduction

The oomycete *Phytophthora ramorum* Werres, De Cock & Man in't Veld sp. nov. is a ubiquitous plant pathogen with a wide host range. One of the major diseases caused by *P. ramorum* is sudden oak death (SOD), a serious threat to native American oaks and European relatives where it has been discovered in numerous ornamental plant nurseries.

We are interested in the molecular, cellular and physiological processes underlying infection pathways and tissue colonization. Using visible genetic markers has been proved to be an useful tool for the analysis of plant-pathogen interactions.

Here, we analyse the suitability of the Green Fluorescent Protein (GFP) as a reporter gene in *P.ramorum*. A $CaCl_2$ and polyethylene-glycol-based DNA transformation

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protocol was developed and transformants expressing GFP were evaluated for usefulness in future studies.

Material and Methods

Transformation

The transformation vector used in the present study was p34GFN (Si-Ammour and others 2003) which contained the selectable marker nptII and the visible marker gfp expressed under the control of the ham34 promoter and terminator sequences. Four isolates of *P. ramorum* (BBA 9/95, BBA 26/02, BBA MSOD03-002 and PR01) were used as recipients for transformation according to the protocol of Judelson and others (1991) with some modifications.

Immunocytochemistry

The GFP expression was verified by immunocytochemical detection with Alexa Flour 488-labeled anti-GFP antibodies (Molecular Probes).

Microscopical analysis

For microscopical observation the Leica TCS SP2 confocal laser scanning microscope (CLSM) was used. GFP and anti-GFP antibody excitation was done with a 488 nm Argon Krypton laser at power levels from 10 to 50 percent. GFP expression of transgenic *P. ramorum* structures was measured by generating emission spectra profiles (lambda scan) from 500 to 580nm. Images and lambda scans were analysed with the Leica LCS software.

Results

About forty-three different transformed isolates were produced and then tested for GFP gene integration and GFP expression. Integration of GFP genes was approved with Real Time PCR and sequencing. GFP expression could be observed in all *P. ramorum* propagules obtainable from in vitro cultures like: germinating cysts, hyphae, zoosporangia and chlamydospores (Fig. 1).

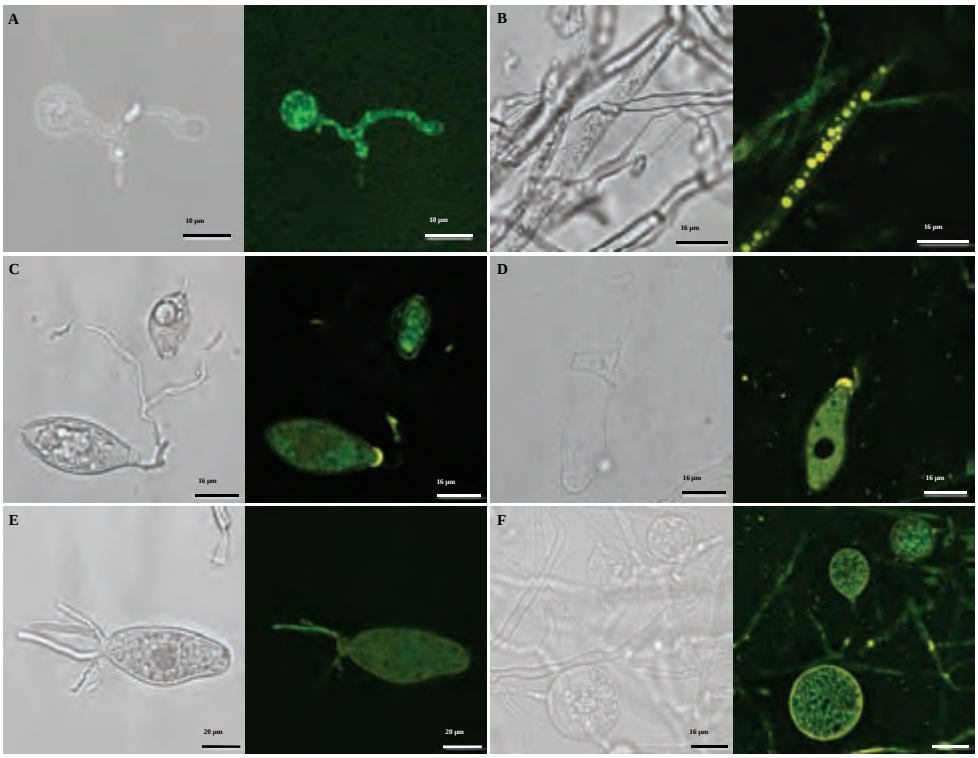

Figure 1-CLSM micrographs indicating GFP expression of transgenic *P. ramorum* strains BBA26/02_4 (**A** and **C**) and BBA9/95_6G (**B, D, E** and **F**). **A:** germinating cyst, **B**: hyphae grown in vitro, **C, D** and **E**: zoosporangia releasing zoospores and germinating respectively, **F**: chlamydospores grown in vitro. **Each left image:** transmission images. **Each right image:** CLSM signals with green signal indicating GFP expression in protoplasm and yellow signals showing natural *P. ramorum* autofluorescence in wall and papilla of zoosporangia and wall of chlamydospores.

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