Species:	*Venturia inaequalis*
Product Class(es):	QoI fungicides
Method type described:	Molecular genetic detection of mutations conferring QoI resistance in *Venturia inaequalis* via Pyrosequencing
Date of protocol:	2016-02
Version	1
comments	Proven for detection of the mutation G143A in cytochrome b

Introduction

QoI fungicides inhibit one single target enzyme within the fungal mitochondrial respiration chain. From all resistance mechanisms to QoI fungicides that have been described up to now, the target mutation G143A is at the moment by far of the greatest importance on the practical level. The cause for this resistance is a single nucleotide polymorphism (SNP) in the fungal cytochrome b gene leading to an amino acid substitution of glycine with alanine at position 143 of the cytochrome b protein.
The level of resistance (percentage of mutation G143A) in scab samples can be determined quickly using the pyrosequencing method.

Method

1. Sampling and DNA extraction:

Around 20 randomly sampled leaves (or fruits) represent one sample. From each leaf (or apple) per sample, typical scab lesions (Ø approx. 6mm) are cut out with a cork-borer and pooled in a 50ml Falcon-tube. For DNA extraction the plant material is homogenized in liquid nitrogen by stirring with precooled satellite spheres (Vortex). After mechanical disruption spheres are removed and the homogenized cells are incubated in 1-2 ml Lysis buffer (Muller buffer) for 10 min at 95°C in a water bath. The suspension is then spun down and the supernatant, diluted to 1:200, serves as template for the PCR in order to copy the gene sequence of interest.

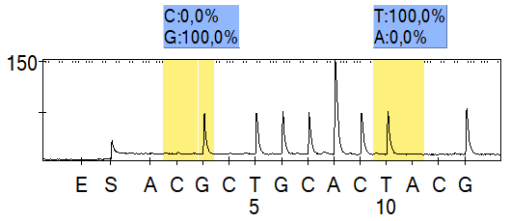
2. PCR and Pyrosequencing:

2.5µl of purified DNA are used for amplification of the cytochrome b gene fragment in a hot start PCR containing: 12.5 µl HotStarTaq Mastermix (Qiagen), 0.5 µl of primer VENTIN-XX-G143A-F1: GTAAAAGAGCAACGAGTAGACGG (10µM), 0.5 µl of primer VENTIN-XX-G143A-R1B: Bio_CGACTATATCTTGTCCTATTCACG (10µM), and 6 µl H_2O dest.

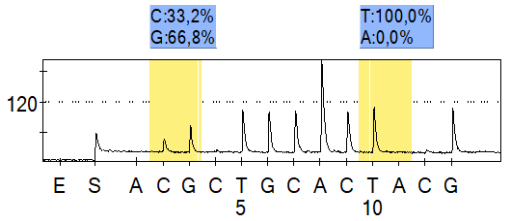
The PCR conditions are as follows: 15' at 95°C followed by 94°C for 30'', 57°C for 30'' and 72°C for 1' with 39 cycles, and final elongation at 72°C for 10'. For the detection of the G143A mutation the PCR product is analysed by pyrosequencing using the following specific sequencing primer (VENTIN-G143A-S1: CAAATGAGCCTATGGG) according to the manufactures´ instructions (Qiagen). Specific software calculates the allele frequency at the position of the mutation, thus indicating the percentage of G143 mutated fragments within the pooled DNA samples.

3. Examples

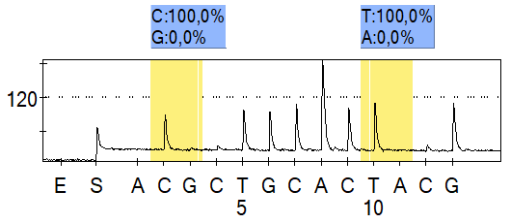
0% G143A



33.2% G143A



100% G143A



authors	Friederike Manger-Jacob, Thekla Taufferner, Dr. Andreas Mehl Bayer AG, 40789 Monheim, Germany andreas.mehl@bayer.com, friederike.manger-jacob@bayer.com