Species:	*Zymoseptoria tritici*
Product Class:	QoI fungicides
Method type described:	Molecular biological detection of mutations conferring QoI resistance in *Zymoseptoria tritici* 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. The main cause for QoI 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 Septoria leaf spot samples can be determined quickly using the pyrosequencing method.

Method

1. Sampling and DNA extraction:

Around twenty randomly sampled leaves represent one sample. From each leaf per sample, typical Septoria leaf spot lesions (Ø approx. 1.5mm) are cut out with a cork-borer and pooled in a 50 ml Falcon-tube. For DNA extraction the plant material is mixed with Lysis buffer (RLT Buffer) and homogenized via stirring with satellite spheres of different sizes at 4500rpm for 3x20 minutes with 60'' break between the single homogenization steps. After mechanical disruption and centrifugation at 11.000rpm for 10 minutes, 200µl of the supernatant are used for DNA purification according to the BioSprint DNA Plant Kit procedure (Qiagen).

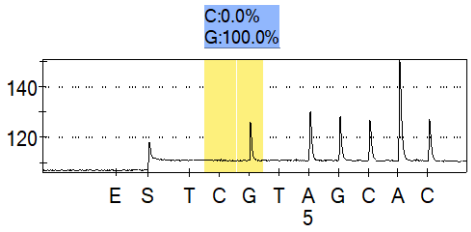
2. PCR and Pyrosequencing:

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), 0.5µl of primer SEPTTR-G143A-F1: GATGATGGCAACCGCATTCTTAG (10µM), 0.5µl of primer SEPTTR-G143A-R1B: ACTATGTCTTGTCCAACTCAAGG (10µM), and 6µl H_2O dest.

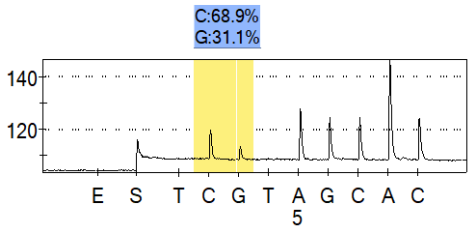
The PCR conditions are as follows: 15’ at 95°C followed by 94°C for 30’’, 59°C for 30’’ and 72°C for 1’ with 39 cycles, and final elongation at 72°C for 10’. For the detection of the G143A mutation the PCR product is analysed by pyrosequencing using the following specific sequencing primer (SEPTTR-G143A-S1: TGGTCAAATGTCTTTATGAG), 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

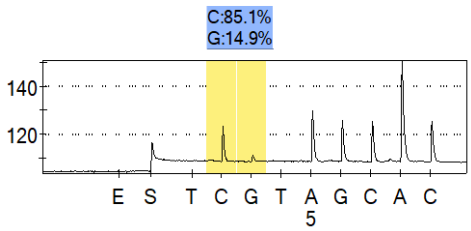
0% G143A



68.9% G143A



85.1% G143A



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