Species:	*Pyrenophora teres*
Product Class(es):	Qo-Inhibitors
Method type described:	Molecular genetic detection of F129L mutation conferring QoI resistance in *Pyrenophora teres* in barley leaves.
Date of protocol:	2016-1
Version	1
comments	Pyrosequencing method used since several years

Method:

1. Sampling and DNA extraction:

   Discs of 2 mm diameter are punched out from leaves with typical symptoms. Samples with 15 discs each from a different leaf are collected and lyophilized. DNA is isolated using the MagAttract 96 DNA Plant Core Kit (Qiagen, Hilden , Germany) according to the instructions in the handbook (August 2003). Only at the disruption step RLT lysis buffer is added before the samples are shaken and thus they are not cooled in liquid nitrogen.

2. PCR and Pyrosequencing:

   A 167 bp PCR fragment containing the F129L mutation is amplified with the forward primer 5'-TCCTAACTTAAAAGGTTACACAAGGCTT-3' and the reverse primer 5'-Biotin-AACCATTTTGGGCTATGTTGGTA-3'. PCR run under the following conditions: Initial denaturation at 95°C for 2 min, 45 cycles at 95°C for 15 sec, 60°C for 30 sec and 72°C for 20 sec and final elongation at 72°C for 5 min (GoTaq® Hot Start Polymerase, Promega, Madison, WI).
   20 µl PCR products are prepared for pyrosequencing reactions using the PyroMark Q96 Vacuum Workstation (Qiagen) and Streptavidin Sepharose High Performance Beads (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) as described in the instructions of the workstation and in the PyroMark Q96 ID User Manual. The single-stranded DNA templates are transferred to 40 µl annealing buffer (Qiagen) containing the sequencing primer (0.4 µM) 5'-CGGAACTTAGACAGCC-3'. The sequencing run is set up

by PyroMark-Software v.1.0 (Qiagen). After incubation of samples at 80°C for 2 min. and equilibration to room temperature the sequencing reaction is performed with PyroMark Gold Q96 reagents on a PyroMark Q96 ID machine (both from Qiagen). Dispensation order of the nucleotides is GCTAGCATGTAC.

3. Analysis:

   Allele frequencies are calculated with PyroMark-Software.

BASF method adapted to our equipment.

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Field efficacy of pyraclostrobin against populations of Pyrenophora teres containing the F129L mutation in the cytochrome b gene

Feldwirkung von Pyraclostrobin gegen Populationen von Pyrenophora teres mit F129L Mutation im Cytochrom-b-Gen

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