Species:	*Zymoseptoria tritici*
Product Class(es):	Qo-Inhibitors
Method type described:	Molecular genetic detection of G143A mutation conferring QoI resistance in *Zymoseptoria tritici* in wheat leaves samples.
Date of protocol:	2016-1
Version	1
comments	Q-PCR method used since several years

Method:

1. Sampling and DNA extraction:

   Infected leaves are tested for the presence of the G143A substitution. 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. Q-PCR:

   The forward primers are allele-specific (amplification refractory mutation system = ARMS): For G143 (wild type allele), the primer is 5'-ACCTTATGG TCAAATGTCTTTATGATG-3' and for A143 (mutated allele) 5'-ACCTTATGG TCAAATGTCTTTATGATC-3'. The corresponding reverse primer is 5'-AGC AAAGAATCTGTTCAATGTTGC-3'. Singleplex SYBR Green Q-PCR assays are carried out in 384 well plates on the ABI 7900 HT SDS cycler (Applied Biosystems). The reaction volume is 10 µl comprising 2 µl template plus 8 µl Mastermix [FastStart Universal SYBR Green Master (ROX), Roche, at 1x and primers at a final concentration of 300 nM]. The cycling conditions are 10 min at 95°C once followed by 40 cycles of 15 sec at 95°C, 30 sec at 60°C and 30 sec at 71°C.

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