Methodology of molecular diagnostic of *Rhizoctonia* spp. in plant and soil samples

European Root Health Forum 2015

M.Sc. Gesine Thomsen
Institute of Phytopathology, CAU Kiel

Introduction

Rhizoctonia spp. belong to a large, complex group of organisms with many subspecies. Isolates differ in pathogenicity, morphology, cultural and physiological characteristics. Damages caused by these pathogens include seedling damping-off and root rots as well as leaf blights. *Rhizoctonia* spp. are soil-borne, ubiquitous, necrotrophic pathogens with a wide host range and saprophytic behavior. The saprophytic behavior in combination with the localization in soil creates a further barrier for disease control. The pathogen cannot be eliminated but can be suppressed to a level that doesn't cause economic losses.

Qualitative diagnostic

For the molecular diagnostic of *Rhizoctonia* spp. specific primers are needed. The design is very difficult because the DNA sequences of the subgroups are highly conserved. The chosen ITS 1 and ITS 2 regions of sequenced isolates of each anastomosis group were clustered and examined for suitable regions for primer design (Fig. 1).

```
AG 1-1 A:   CCTGTGCACT-TGTG-AGACAG  -------CAATAG---TTGGTGG--------------
AG 1-1 B:   CCTGTGCACT-TGTG-AGACAG  TCAAGGTCCTTTGGGGTTGGGGGGCAGAGCTTTATTGC
AG 3:       CCTGTGAACT-TGTG-AGACAG  ---TTGGGGAATT---TATTTGTTATTTTTTGTAATAA
AG 10:      CCTGTGAACC-TGTG-AGACAG  ---ATGGGGAATT---TATTTGTTGTTTTTTGTAATAA
AG 2-2 IIIB: CCTGTGAACC-TGTG-AGGCAG  ---AGGCATGGATGGGAGAACTTTCATTT-------AC
AG 2-2 IV:  CCTGTGAACC-TGTG-AGGCAG  ---AGACATGGATGGGAAAACTTTCATTT-------AC
```

Highly conserved regions

Possible regions for primer design

Selected primers were digitally cross-tested with all stored sequence data of the National Center for Biotechnology Information (NCBI) and checked for the ability of the production of dimers, hairpins and loops.
Subsequently the primers were tested with a gradient of concentrations of the DNA of the target organism with a gradient of temperature for the detection of the annealing temperature in qualitative PCR. After the detection of an appropriate annealing temperature the primers were tested in a comprehensive cross test with DNA of many different soil and seed-borne diseases. Regarding to *R. cerealis* with the anastomosis groups AG 1-1 A, AG 1-1B, AG 2-1, AG 2-2 IIIB, AG 2-2 IV, AG 3, AG 4, AG 5, AG 6, AG 8, AG 9, AG 10 within the teleomorph *Thanatephorus cucumeris*, *R. zeae* and *R. oryzae* within *Waitea circinata, Fusarium culmorum, F. graminearum, Bipolaris sorokiniana, Microdochium nivale, Gaeumannomyces graminis, Tilletia caries and Ustilago tritici.* Additionally the primers were cross tested with corn and wheat DNA.

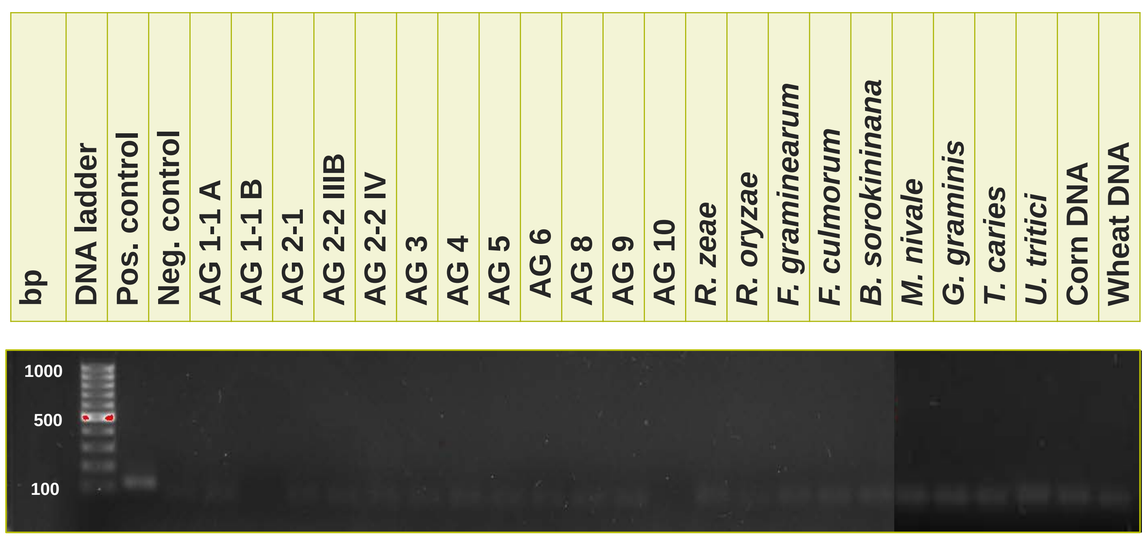

Fig. 2: Cross test for the detection of *R. cerealis*

Quantitative diagnostic

Qualitative PCR analysis only tells whether a pathogen has been detected. There is no indication of the present amount of the pathogen and an associated yield relevance. Quantitative real-time PCR is based on the measurement of the stepwise increase of the fluorescence intensity of an DNA-intercalating reference product during a PCR (SYBR-Green) (Fig. 3). With this method the measurement of the total amount of pathogen DNA in soils (ng/g) and the pathogen DNA in relation to plant DNA (‰) in plant samples is possible. For the classification of the measured values a standard series of samples with defined DNA content is necessary. With this information it is possible to deduce the measured values of samples with unknown DNA content.

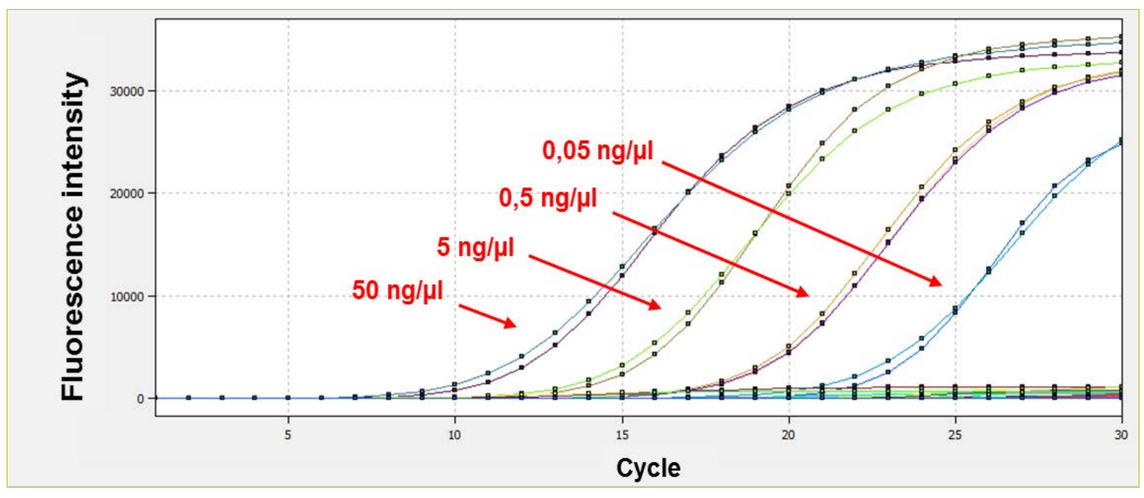

Fig. 3: Standard series of DNA dilutions of AG 2-2 IIIB

After qPCR the verification of the melting curve of samples with unknown pathogen content compared to the positive control is important. The product formed by the specific primers disintegrates during the progressive melting at the same temperature. This results in the release of SYBR-Green and sudden decrease of fluorescence (Fig. 4). Melting curves that are outside the range of the melting curve of the positive control indicate that the product does not match to the target product. It's possible to use these primers for pathogen detection in plant and soil material.

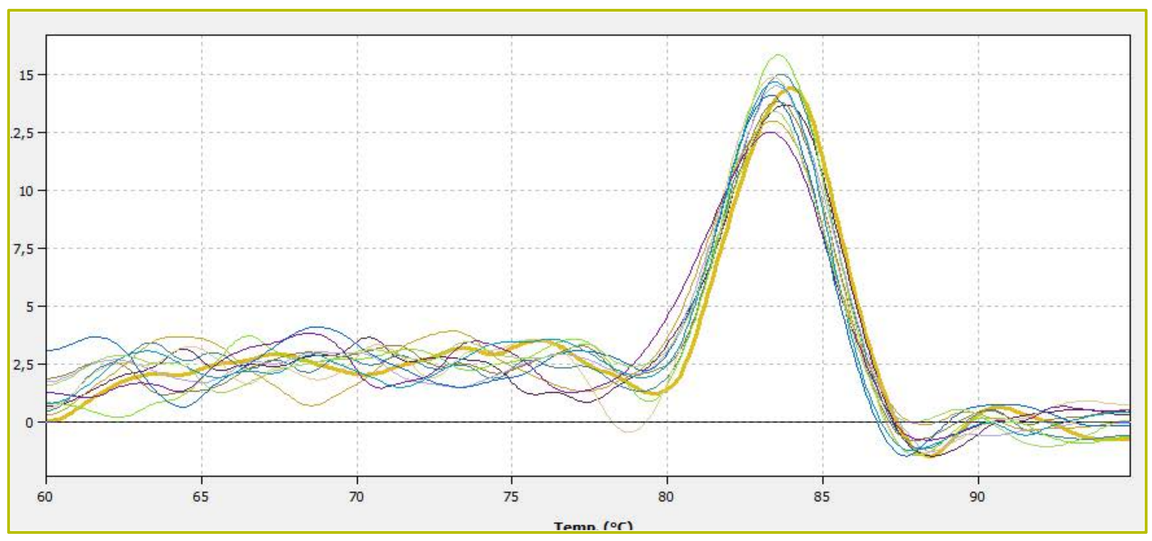

Fig. 4: Melting curve of the dilution series of *R. cerealis*