Detection of *Acidovorax citrulli* in seed extracts of melon and watermelon with Taqman PCR

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Introduction

Molecular assays previously developed for *A. citrulli* (Acit) vary in several aspects such as extraction procedures, DNA purification methods and the use of different Acit Taqman PCRs. Objective of this study is improvement, fixation and then validation of a reliable and high throughput method for detection of Acit.

DNA extraction

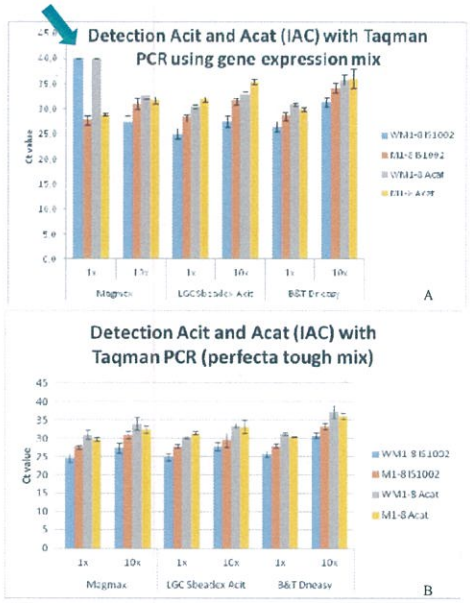

Fig. 1A-B. Detection of Acit in 8 melon (M) and 8 watermelon (WM) sub samples using 3 DNA extraction kits. The IS1002 Taqman (target) and the *A. cattleya* (Acat) Taqman (internal amplification control (IAC)) were performed with the AB gene expression mix (A) or the Perfecta tough mix (B). To monitor PCR inhibition undiluted (1x) and 10x diluted DNA were tested.

A. citrulli specific Taqman's

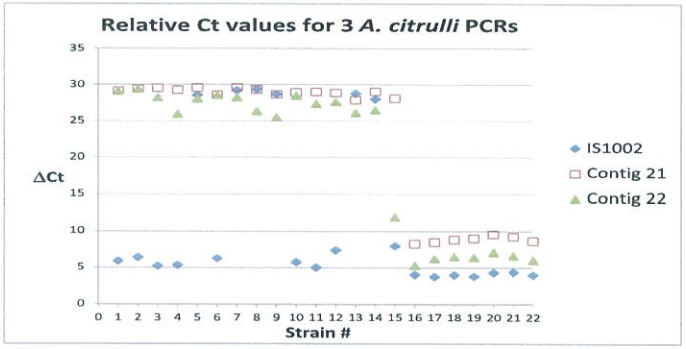

Fig. 2. Relative Ct values (universal bacterial Taqman - Acit specific Taqman PCRs (IS1002, Contig21 or Contig22) for Acit (strains 16-22) and related non Acit (strains 1-15). Identity of the strains based on AFLP analysis and pathogenicity assays on watermelon.

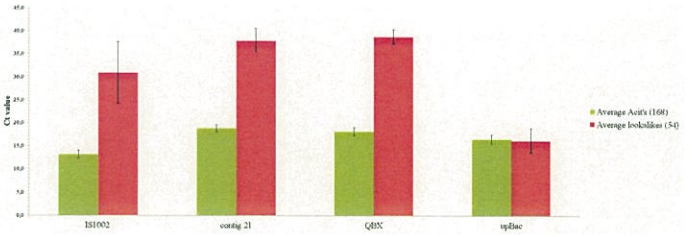

Fig. 3. Average Ct of 3 Acit Taqman PCRs (IS1002, Contig21 and QBX (Hazera) and a universal bacterial Taqman based on 168 Acit strains and 53 related Acidovorax strains (based on AFLP).

Table 1. Primers and probes for muliplex detection of *A. citrulli*

Target	Developed by	Sequence
Acit	Syngenta*	GAG TCT CAC GAG GTT GTT
Acit	IS1002	GAC CCT ACG AAA GCT CAG
Acit		6FAM TGC AGC CCT TCA TTG ACG G-BHQ1
Acit	Monsanto	GAA AGT GGT TGT TCT GGT GAT CAA
Acit	Contig22**	TTC GGA GGA CTC GGG ATT T
Acit		VIC ATG GTC TGC GAG CCA G MGB NFQ
Acat	Syngenta/	TGTAGCGATCCTTCACAAG
Acat	Naktuinbouw**	TGTCGATAGATGCTCACAAT
Acat		TEXAS RED CTT GCT CTG CTT CTC TAT CAC G-BHQ2

* EPPO poster, York, 2009

** Sequences were derived from ISHI AFLP study

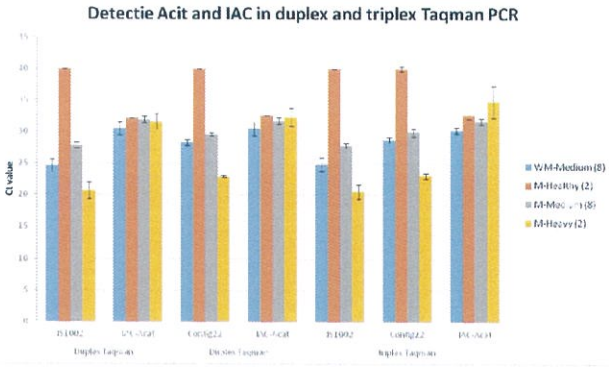

Fig. 4. Detection of *A. citrulli*/IAC in 20 seed sub samples (melon and watermelon) in duplex and triplex Taqman formats using the LGC Sbeadex customised *A. citrulli* kit and Perfecta qPCR toughmix.

Discussion /Conclusions

- The DNA extraction method is highly critical for a reliable assay. Especially for watermelon PCR inhibition (see arrow Fig.1A) in the undiluted DNA disappeared with the high throughput LGC Sbeadex *A. citrulli* kit in combination with more intensive washing. Results with this kit were comparable with the time consuming Qiagen Dneasy BT kit (Fig.1A)
- The Quanta Perfecta qPCR Toughmix decreased the inhibition of the Taqman PCR's compared to the AB gene expression mix (Fig. 1B).
- Several Taqmans with different targets are available for detection of Acit (Fig. 2-3). Taqman IS1002 (multicopy target) was selected for the high sensitivity. Taqman Contig22 was selected for the high specificity and slightly better sensitivity than Contig21 (Fig.2).
- Inhibition of the PCR's can be quantified with an internal amplification control (IAC) based on *A. cattleya* Taqman (Table 1).
- A triplex Taqman PCR with the IS1002 /Contig22/IAC Taqman gave similar results in comparison with two duplex Taqmans (Fig. 4).
- An improved method with the LGC Sbeadex *A. citrulli* kit and the Perfecta qPCR toughmix is currently under validation.
- The Taqman PCR does not discriminate between viable and non viable cells of *A. citrulli*.