Validation of a Nested PCR assay for detection of *Xanthomonas axonopodis* pv. *dieffenbachiae* in anthurium tissues in a european multicenter collaborative trial

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Xanthomonas axonopodis pv. *dieffenbachiae* (*Xad*) is the etiological agent of anthurium bacterial blight. *Xad* is a quarantine organism in several countries and particularly in Europe where this pathogen is listed in the A2 list of the European and Mediterranean Plant Protection Organization (EPPO).
A collaborative trial involving 15 European laboratories was performed to evaluate a Nested PCR assay (1) (N-PCR) for the detection of *Xad* from anthurium samples. This collaborative study was conducted according to the ISO 16140:2003 standard “Protocol for validation of alternative methods”(3). The ISO 16140 validation protocol comprises two phases: a comparative study of methods and a collaborative trial.
The comparative study was carried out in the organizing laboratory (CIRAD/LNPV Reunion Island). Methods such as the EPPO reference method for detection of *Xad*, the N-PCR assay, immunofluorescence (IF) and double antibody sandwich ELISA (DAS-ELISA) were compared. It was shown noticeably differences in performance between methods. The reference and N-PCR methods were the most efficient techniques, both combining a very good inclusivity, exclusivity, a relative accuracy above 95% and a low detection threshold (approximately 10^3 CFU.mL-1).
The collaborative trial consisted in determining the variability of the results obtained by several different competent laboratories using identical samples and in comparing these results to those obtained with the comparative study. Four blinded samples (8 replicates/sample) were analysed by the 15 laboratories. The four samples were constituted of an anthurium extract artificially contaminated with variable concentrations of the pathotype strain (0, 10^4 or 10^5 CFU.mL^{-1}), or with a non-target strain (10^7CFU.mL^{-1}).

The N-PCR assay was performed in comparison to a reference method (isolation of the bacteria on non-selective and semi-selective media followed by a serological identification by indirect ELISA) and to other methods, DAS-ELISA and IF, all included in the EPPO standard (2). The relative accuracy, relative specificity, relative sensitivity, accordance and concordance values obtained for the N-PCR assay were 96.0%, 95.0%, 97.5%, 94.0% and 93.0% respectively. The results obtained with the N-PCR assay were significantly different from the theoretically expected results ($p<0.05$),but it became non-significant when one laboratory (laboratory N) was excluded from the analysis ($p=0.15$). The results obtained with DAS-ELISA and IF were significantly different from the theorically expected results ($p<0.001$ and $p<0.05$ respectively). A significant variation between laboratories was shown for one of the four samples for the N-PCR assay ($p<0.05$). However, it became not significant when one laboratory (laboratory N) was excluded ($p=1.00$). Variations between laboratories were significant for one of the four evaluated samples for DAS-ELISA ($p<0.001$) and for the four evaluated samples for IF ($p<0.001$ to $p<0.05$). The analysis of the data suggested that these significant variations for DAS-ELISA and IF were linked to the characteristics of these methods and to the expertise of the laboratories. The N-PCR has been included into the EPPO standard as an alternative method for the detection of *Xanthomonas axonopodis* pv. *dieffenbachiae* in anthurium plants (2). Because of its high specificity, the addition of the N-PCR technique in the EPPO standard now makes the pathogenicity tests to anthurium optional, substantially reducing the amount of time for result delivery.

References:

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