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PCR-RFLP assay for distinguishing four *Frankliniella* species
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Introduction: Thrips (Thysanoptera) are tiny, slender insects with fringed wings. Until now more than 200 species were reported in the area of Poland and, among them seven species from the genus *Frankliniella*. Pest of this genus are polyphagous and feed on a number of ornamental and vegetable hosts as well as on many weed species. The most economically important *Frankliniella* species in fauna of Poland are *Frankliniella occidentalis* Pergande and *Frankliniella intonsa* Trybom. Proper pest identification is essential to successful their control. Because of similarity between *Frankliniella* species, especially in larval stadium, and similar host range morphological identification can cause some difficulties. For this reason molecular biology methods could be very useful.
Objectives: The aim of the study was to develop the fast and effective method to distinguish *Frankliniella* species occurring in Europe: *F. occidentalis*, *F. intonsa*, *F. pallida,* and *F. tenuicornis*.
Materials and methods: Material of the study constituted 15 populations of *F. occidentalis*, 6 populations of *F. intonsa*, 1 population of *F. pallida*, 1 population of *F. tenuicornis* and 3 *Thrips* species as negative control (*T. palmi*, *T. tabaci,* and *T. major*). First, the region of 18S-ITS1-5,8S-ITS2-28S rDNA was PCR amplified and sequenced. Received sequences were used to design universal primers, giving PCR products for all analyzed *Frankliniella* but not for *Thrips* species. Received PCR product was then cut by appropriate restriction enzymes to receive bands pattern distinctive for each species.
Results: PCR-RFLP reactions gave positive results for all tested *Frankliniella* populations. Samples containing *Thrips* species as well as no template control did not give any product.
Conclusions: Described protocol proved to be species-specific and sensitive.

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Hermaphrodite and female Papaya Distinction by HR-MAS NMR
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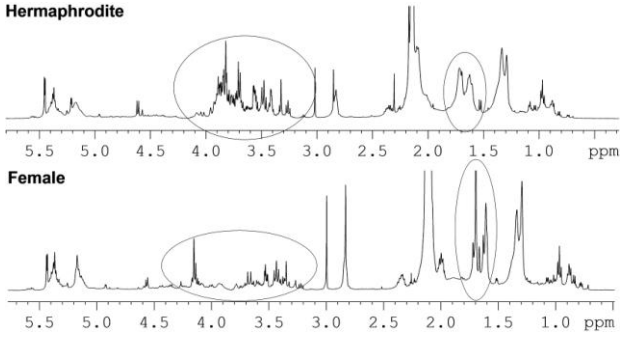
Introduction: Papaya (*Carica papaya* L.) from commercial seed usually produce hermaphroditic and female plants in a ration of 2:1. Males and females plants are usually removed. Flower morphology is the only way to identify visually the sex of the plant.
Objective: In this way this study correlate the sexual expression with chemical profile of hermaphrodite and female leaves through nuclear magnetic resonance spectroscopy (NMR).
Materials and methods: ^{1}H HR-MAS NMR measurements were performed at 28 °C on a Bruker Avance III 500 spectrometer equipped with a 4 mm HRMAS probe. Noesypr1d and 5 KHz were used as pulse sequence and rotating speed. Powdered leaves (15 mg) and 38 µL of acetone-d6 were added to a 50 µL spherical zirconium rotor. Triplicate experiments were applied for each leaf sample.
Results: The hermaphrodite and female ^{1}H NMR spectra (Figure 1) are very similar. However, expansions of the 3.1-4.3 and 0.7-1.9 ppm regions clearly indicates different chemical profiles according sex, particularly with respect to the carbohydrates and fatty acids compounds, whose signals are typically observed in this region of the ^{1}H spectrum. These compounds are observed in all plants however, the hermaphrodite leaves produce in higher concentration.

Figure 1. ^{1}H HR-MAS NMR spectra for papaya plants.



Conclusions: In this study, we demonstrate that it is possible to distinguish hermaphrodite and female papaya plants by ^{1}H HR-MAS NMR. The methodology employing demonstrated to be a powerful tool to evaluate chemical profile differences of papaya plants. This study also emphasises the remarkable advantage in using the HR-MAS NMR technique for plant analyses on the basis that the measurement is highly simplified since it does not require any pretreatment of the sample apart from the addition of a small amount of deuterum solvent necessary to produce homogeneous dough and a field frequency lock.

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Digital PCR for Detection and Quantification of Fire Blight and Potato Brown Rot

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Digital PCR (dPCR) is currently the most straightforward absolute quantification of the target nucleic acid copy numbers. Here we present the first assessment of digital PCR (ddPCR) format of qPCR assays for detection and absolute quantification of two quarantine bacteria, *Erwinia amylovora* (1) and *Ralstonia solanacearum* (2).

The evaluation combined the determination of the droplet based dPCR (Biorad) performance parameters of on a defined set of samples with known health status, including: (i) NTCs; (ii) negative plant material; (iii) serial dilutions of target DNA; and (iv) artificially prepared samples with target concentrations relevant to routine testing (in this case, log 3 cells/mL plant extract; Fig. 1) and (v) direct quantification of bacteria prior to DNA extraction.

In the dPCR format, the performance of the *E. amylovora* assay was comparable to its qPCR format, despite the reduced number of replicates (one in dPCR against three in qPCR), which makes the dPCR the first choice for characterisation of in-house reference materials, and for any application where quantification is also required. In contrast, for the *R. solanacearum* assay, where previous data and the data from this study indicate that its design might not be optimal, the dPCR format significantly improved both its analytical and diagnostic sensitivity. In general, the proprietary QuantaSoft analysis required data of high quality, while manual threshold selection was more suitable for non-optimal assays. The R script developed enabled automatic data analysis under different settings, and calculating additional parameters related to the occurrence of “rain” and to the quality of the separation of the negative and positive droplets. The data analysis and interpretation of the dPCR is considerably simpler than in qPCR.

Both dPCR assays accurately determined bacterial concentrations before and after DNA extraction (3). This is of particular importance in the field of plant health where no reference materials are commercially available.

References:

1 Pirc et al., 2009. Plant Pathol 58:872-881.
2 Weller et al., 2000. Appl Environ Microbiol 66:2853-2858.
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Figure 1: Heat maps of dPCR amplification of ***E. amylovora*** (a-d) and ***R. solanacearum*** (e-h). Samples are numbered sequentially.