Extracellular compound from culture filtrate of *Fusarium oxysporum* f. sp. *cubense* could be useful in agroecological approaches for banana production

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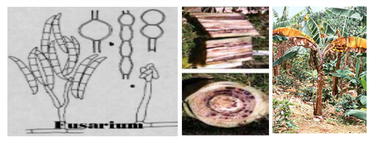
INTRODUCTION

Bananas and plantains (*Musa* spp.) are among the most important crops in the world providing staple food for hundreds of millions of people.

The production has been devastated by fungal infestations caused by ***Fusarium oxysporum* f. sp. *cubense*** (*Foc*).

Panama disease



The aim of this investigation was the isolation of extracellular microbial metabolites involved on a differential plant tissue response for both cellular and genetic level.

MATERIAL AND METHODS

Plant material. Leaves of two banana cultivars used in the experiments were sampled from field-grown plants at Experimental Station of Centro de Bioplantas, Cuba: 'Gros Michel' (susceptible) and 'Grande Naine' (resistant).

Culture methods. Pathogenic isolate of *Fusarium oxysporum* f.sp. cubense belonging to VCGs 01210 (race 1) was gently provided by Prof. Luis Pérez-Vicente (Instituto Nacional de Sanidad Vegetal, La Habana, Cuba). For production of culture filtrates (CF), a 1-cm diameter disk from the active mycelial growing of Foc on PDA plates during 7-days was transferred to 250-ml Erlenmeyer flask, each containing 100 ml of Czapek Dox (Oxoid). The cultures were incubated at 25 °C in the dark and the CF from five Erlenmeyer flasks were harvested daily and pooled. The aqueous phase and concentrated filtrate were then used in different experiments.

Bioassay. A leaf puncture bioassay on detached banana leaves was performed to evaluate the toxicity of CF and its fractions (Companioni *et al*., 2003)

Instrumental conditions. Liquid chromatography was made under the following conditions. The column used was a 150mm x 4.6 mm i.d., 5 µm, Restek Ultra C-18, with a 10 mm x 4.0 mm i.d. Restek guard column of the same material, Equipment: HPLC Finnigan Surveyor System. Two solvent mixtures were used as mobile phases. Mass spectrometry analyses were made on a Mass Spectrometer Thermo Finnigan LCQ Advantage Max (Ion Trap type) and a Thermo Finnigan Surveyor PDA detector (UV Vis 190 – 600 nm).

Elicitation and experimental design. The effectiveness of the *F. oxysporum* f. sp. cubense elicitor was determined by investigating the induction of response genes widely associated to plant defence. A leaf puncture bioassay on detached banana leaves, above mentioned was developed but using 5 µL of aqueous fraction corresponding to a total protein equivalent to 10 mg·mL^{-1}) as inductor,

RNA extraction. Total RNA was extracted from detached banana leaves using a modified CTAB method according to Li *et al*. (2013).

Quantitative RT-PCR. Expression levels of six selected defense-associated genes [NPR1, PR1, ACO, LOX, AOS and AOC (previously identified in banana)] were tested bthy quantitative RT-PCR (qRT-PCR) using a LightCycler® 480, 384-well PCR plates and the LightCycler® 480 SYBR Green I Master kit (Roche Diagnosis, Germany) following the manufacturer's instructions. Primers used for qPCR were as previously described [NPR1 (Zhao *et al*.; 2009), PR1 (Van den Berg *et al*.; 2007), ACO (Mbéguié-A-Mbéguié *et al*.; 2008), LOX, AOS and AOC (Zhao *et al*.; 2013)]. Expression data were normalized making use of the standard curve for the specific target gene and the endogenous control gene, β-actin (Van den Berg *et al*., 2004). The relative fold differences of each gene expression between induced and mock-treated samples at each time points were determined using the $2^{-\Delta\Delta Ct}$ formula (Livak and Schmittgen, 2001). The significance of differences for all treatments and between the two cultivars was analyzed by One-way ANOVA and the Tukey Highest Square Difference (HSD) test at $P < 0.05$.

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RESULTS

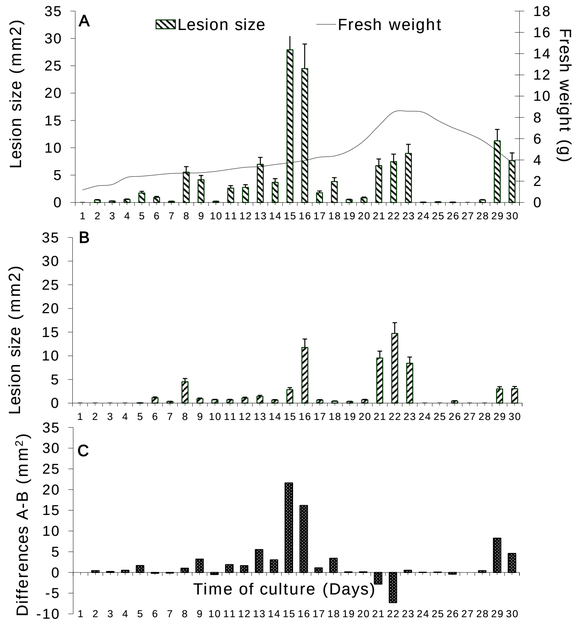

Figure 1. Effect of time after inoculation of the medium with *Fusarium oxysporum* f. sp. *cubense* race 1 on fungal culture fresh mass and phytotoxicity measured as leaf lesion size after application of the culture filtrate onto Gross Michel (A) and Grande Naine (B) leaves. (C) Specificity of culture filtrates. Each value represents the means of three replicates, and vertical bars indicate the standard error.

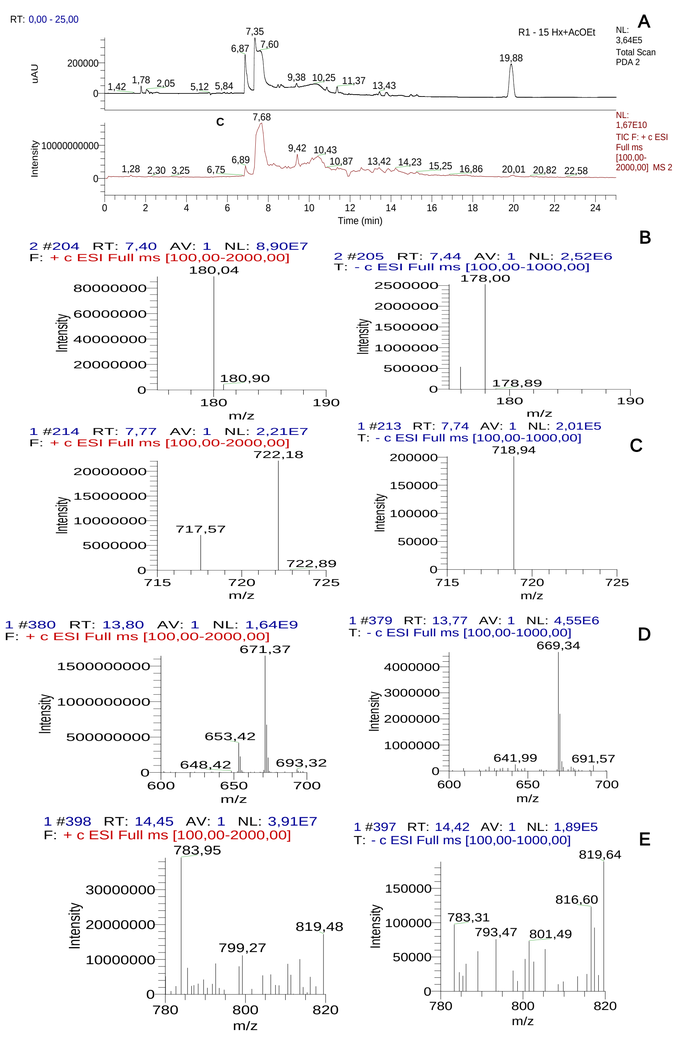

Figure 2. HPLC profile of Foc1.E fraction (Panel A). Total ion current chromatogram generated through ESI-MS analysis in positive ion (left) and negative ion (right) mode from the peaks eluted at 7.4 min, fusarico ac (Panel B), 7.7 min, fumonisin B1(Panel C), 13.8 min, enniatin (Panel D) and 14.4 min, beauvericin (Panel E).

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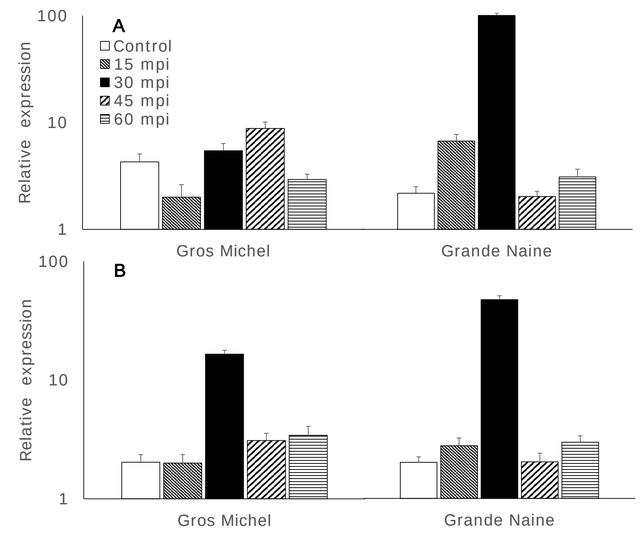

Figure 3. Fold changes in relative transcript abundance of the NPR1 (A) and PR1 (B) genes in susceptible (Gros Michel) and Resistant (Grande Naine) plants treated with an elicitor fraction from culture filtrate of *Fusarium oxysporum* f. sp. *cubense* race 1 or water (Control at 0 mpi).

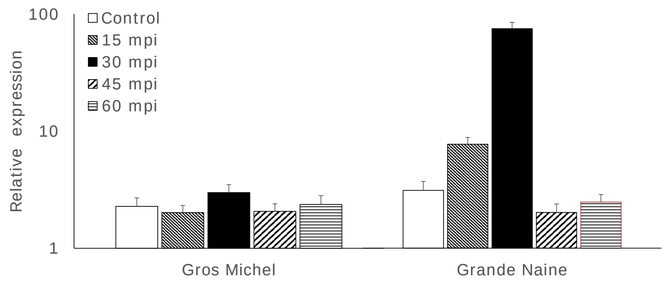

Figure 4. Fold changes in relative transcript abundance of the ACO gene in susceptible (Gros Michel) and Resistant (Grande Naine) plants treated with an elicitor fraction from culture filtrate of *Fusarium oxysporum* f. sp. *cubense* race 1 or water (Control at 0 mpi).

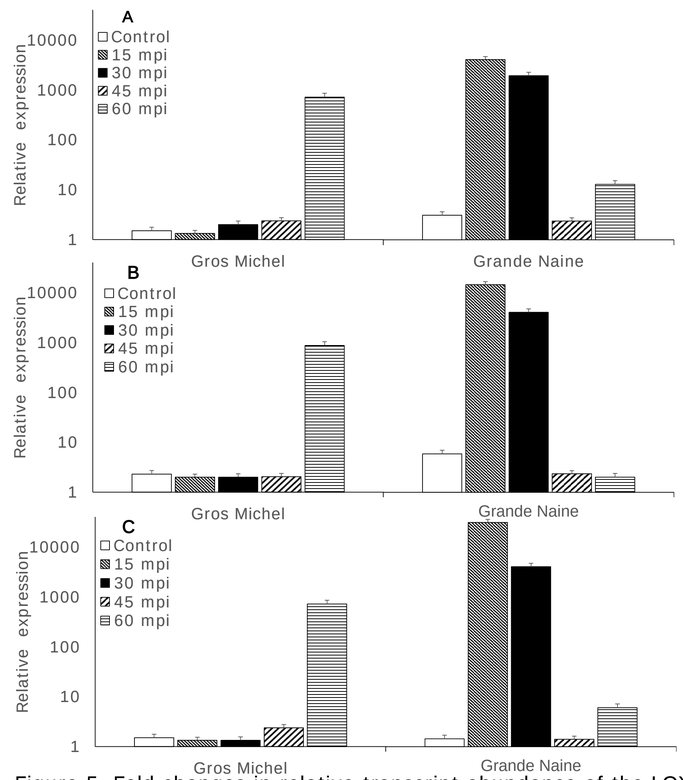

Figure 5. Fold changes in relative transcript abundance of the LOX (A), AOS (B) and AOC (C) genes in susceptible (Gros Michel) and Resistant (Grande Naine) plants treated with an elicitor fraction from culture filtrate of *Fusarium oxysporum* f. sp. *cubense* race 1 or water (Control at 0 mpi).

The gene expression (oxidative stress, jasmonate pathway and defense, PR1 and PR3) in response to inoculation of the aqueous extract of Foc R1 culture filtrate as elicitor was faster and stronger in the tolerant variety Grande naine than in the susceptible Gros Michel.

CONCLUSION

The main toxins isolated from a 15-days old specific culture filtrate of *Foc* race 1 are non-specific whereas extracellular proteins from the aqueous phase provide an important source for further investigations as elicitor for molecular *Foc* - Banana interactions and as a valuable tool for plant protection in organic farmer.

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