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Detection of *Ralstonia solanacearum* phylotype II, race 2 causing Moko disease and validation of resistance observed in the hybrid plantain FHIA-21

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KEYWORDS

Bacterial wilt, *Musa*, *Ralstonia solanacearum*, Moko disease, comparative genome analyses, diagnostics

ABSTRACT

Vascular wilt of banana and plantains, also known as Moko disease, is caused by *Ralstonia solanacearum* (Rs) phylotype II, and is the main bacterial disease affecting these crops in the Americas. Upon comparative sequence analysis of 45 Rs genomes we developed an improved PCR protocol based on the nucleotide sequence of the hypothetical protein DUF3313. Next we tested the detection protocol with two Rs inoculation methods to validate field resistance reported in the hybrid plantain genotype FHIA-21, previously identified as susceptible to Moko in greenhouse experiments that wound the roots of the plants prior to inoculation. By using an inoculation protocol that did not wound the roots, we confirmed resistance in FHIA-21 to Moko disease (no Rs was detected by PCR in these plants). In contrast, the field-susceptible genotype Dominico

Hartón developed severe symptoms of Moko, independently of the inoculation method used. FHIA-21 showed an area under the disease progress curve (AUDPC) close to zero, while Dominico Hartón plants showed AUDPC values ranging from 65.79 to 88.42. The availability and analysis of genomic data provides significant improvements in diagnostics that together with improved inoculation methods and tolerant genotypes to Moko disease, will be of great use in Musa breeding programs.

INTRODUCTION

Vascular wilt of banana and plantains, also known as Moko disease, caused by *Ralstonia solanacearum* (Rs) race 2 (Smith) (Hayward, 1991) results in significant yield losses and high eradication costs (Ploetz et al., 2015). In the Americas, an inadequate use of infected planting material has contributed to the dissemination of Moko disease to Ecuador, Guyana, Honduras, Mexico, Trinidad, Venezuela and Colombia where in many cases up to 100% of the cultivated area has been affected including the destruction of the fruit bunch, thus preventing the plant from completing its growth cycle (Alvarez et al., 2015). Colombia is the fourth largest plantain exporter in the Americas, after Dominican Republic, Guatemala and Ecuador (FAOSTAT 2016); the crop generated in 2013 in Colombia 164,246 direct jobs and 2’594,350 transient and permanent jobs corresponding to an estimated 8% of total employment in the agricultural sector (Minagricultura, 2014). Nevertheless, early detection tools, seed certification programs and resistant plantain varieties are largely missing in this region (Cellier et al., 2015).

Rs is as a complex species, i.e., a group of related isolates, whose individual components might represent more than one species (Fegan and Prior, 2005; Prior et al., 2016). The current classification of Rs strains divides the complex into four phylotypes: phylotype I (isolates from

Asia), phylotype II (the Americas), phylotype III (Africa), and phylotype IV (Indonesian archipelago) (Fegan and Prior 2005; 2006). Phylotype II shows further diversity and is subdivided into IIA and IIB, each of which can be further resolved, based on sequence polymorphisms of the endoglucanase (*egl*) gene, into subgroups called sequevars (Fegan and Prior 2005; Santiago et al., 2017). Classification of isolates according to pathogen-host specificity identify two races whithin phylotype II: race 3 isolates that infect potato and race 2 isolates that infect banana and plantains (Buddenhagen et al., 1962; Fegan and Prior, 2005). Further genetic variation among Rs isolates from plantain, bananas, heliconias, and soil samples in Colombia and elsewhere, has been reported, adding extra complexity to the diagnostics of Rs isolates (Gomez et al., 2006).

Several genomic studies and sequence-based methods for the detection of Rs are reported (Cellier et al., 2015; Kubota et al., 2008; Ha et al., 2012; Horita et al., 2004; Fegan and Prior, 2005; Thammakijjawat et al., 2006; Smith and De Boer, 2009; Paret et al., 2010; Albuquerque et al., 2014; Lenarčič et al., 2014; Huang et al., 2017; Santiago et al., 2017) and a good amount of Rs sequence information is increasingly available in public databases. To improve specific diagnostics of Rs phylotype II, race 2 isolates, we retrieved forty-four Rs genome sequences from GenBank (NCBI) for comparative genome analyses. Twenty-nine corresponded to phylotype II, nine to phylotype I, five to phylotype III, and one to phylotype IV (**Table 1**). Using the GET_HOMOLOGUES software (Contreras-Moreira and Vinuesa, 2013) we first built a pan-genome employing the orthoMCL cluster algorithm (Li et al., 2003). Next, through the script parse_pangenome_matrix.pl (Contreras-Moreira and Vinuesa, 2013) we identified two unique genes out of eleven, that were present in most genomes of the phylotype II (**Table 1**). To corroborate the functional architecture of these genes we used the software SMART (Schultz et al., 1998), identifying as the best candidate

the RSPO-c01611 gene of Rs strain Po82, encoding a hypothetical lipoprotein containing a DUF3313-domain with the UniProtKB code D8NIF9 (this gene is also known as the RCFBP_11537 gene in Rs strain CFBP2957). The second gene identified encoded an uncharacterized protein with the UniProtKB code D8NI64 (also known as gene RCFBP_11543 of Rs strain CFBP2957) without known domains detected. We proceeded to align 24 sequences of the gene D8NIF9 (DUF3313-c01611) corresponding to phylotype II isolates from plantain, banana, heliconia, Anthurium, cucumber, tomato, pothos and potato isolates (**Table 1**) using the software MAFFT v.7 (Katoh 2013, 2016). Employing this alignment and using the software AlleleID v.7 (Primer Biosoft, Palo Alto, CA) were design primers for PCR (DUFF-F: 5'-AATCGTCCCGTTACCTGA-3' and DUFF-R: 5'-GTGGCGGTTGCCTTACAGGT-3') that amplify a 1200 bp region of the hypothetical protein DUF3313. The identity of all PCR products obtained from isolates infecting banana and plantain were validated by sequencing (**Supplementary Table 1**). BLAST analysis, did not show significant homology to any other soil-borne bacteria sequences and phylogenetic analysis grouped them with those of other Rs isolates of phylotype IIB, sequevar 4, pathogenic to banana and plantain (**Figure 1B**). For diagnostics, we designed primers BIOS1_F1: 5'-AATCGTCCCGTTACCTGA-3' and BIOS1_R1: 5'-GGTGAGCGTCAACTTCAC-3'. The specificity of these primers was tested on crude plant extracts obtained from infected plants and with purified bacterial DNA of Rs strains belonging to different phylotypes and hosts (**Figure 1C** and **Supplementary table 1**). Consistent results were obtained using either a standard Taq polymerase-based PCR (GoTaq, PROMEGA) or a quick PCR protocol using Phusion polymerase (Phusion®, New England Biolabs). The latter allowed us to complete a PCR reaction in ~25 minutes. PCR assays were performed in 25 µl reaction volume, with 0.5 µM of each primer and 20ng of bacterial DNA, following supplier instructions. PCR

conditions were as follows: 98°C for 30s followed by 30 cycles of 98°C for 8s, 55°C for 10s and 72°C for 8s. A final extension step of 5 min at 72°C was included before 1.2% agarose gel electrophoresis (**Figure 1C**). Detection of Rs was specific for isolates of phylotype II, as confirmed by using a multiplex PCR test described by Fegan and Prior (2005), using battery of DNA samples corresponding to different phylotypes (**Figure 1C**).

Plant inoculations were carried out in and insect-proof screenhouse at CIAT (Department of Valle del Cauca, Colombia). This region (Latitude 3.500136, Longitude -76.357031, at 980 m.a.s.l.) has an average annual precipitation of 1100 mm and relative humidity of 78%. The average temperature at the time of the assays was 24.5°C. FHIA-21 plants were provided originally from the Honduran Foundation for Agricultural Research (FHIA) and plants of the Moko-susceptible genotype Dominico Hartón were provided by certified plantain nurseries from Valle del Cauca, Colombia. Plants were grown in plastic pots containing 5 kg of a steam-sterilized 2:1 oxisol:sand mixture, for two months before inoculation. Rs infects its hosts via roots, and usually in the laboratory tests, roots are wounded to facilitate infection (Valencia-Valencia et al., 2014). However, the host-Rs interaction in the field may differ when proper agronomic practices, that maintain roots undamaged, are carried out. Indeed, previous field reports described the low incidence of Moko disease in FHIA-21 fields contaminated with Rs (Alvarez et al., 2015), even when this cultivar had been characterized as susceptible to Moko in a previous work (Valencia-Valencia et al., 2014). It is interesting that FHIA-21 was selected by the Honduran Foundation for Agricultural Research in screenings for resistance to Black Sigatoka Disease (BSD) and Fusarium Wilt (Tirado and Zapata, 2003). Field resistance of FHIA-21 to BSD was later confirmed also in Colombia (Cuellar et al., 2011). Taking into account field observations and recent reports on the

use of alternative Rs inoculation protocols that do not necessarily damage the roots of the plants (Singh et al., 2018), an evaluation of FHIA-21 under greenhouse conditions was carried out in order to validate its response Moko. For this, we tested two inoculation methods, one that did not wound the roots ('No Wound') and the one usually practiced with plantain and bananas, where the roots are damaged using a knife prior to inoculation ('Wounded') (**Figure 2**). Inoculated plants were incubated at >95% RH, for two days; then the conditions of high humidity were limited to 2 hours in the morning and 2 hours in the afternoon. Rs strains previously characterized as causing different degrees of severity (Gomez et al., 2006), were used in inoculation tests following "Wounded" and "No Wound" protocols (**Figure 2**). A split plot design was used that included three plants per observation and four repetitions per treatment. Symptoms severity was evaluated starting three days after inoculation and every two days based on a scale proposed by He et al. (1983), where 0 = Absence of symptoms; 1 = One wilted leaf; 2 = Two to three wilted leaves; 3 = Four or more wilted leaves; and 4 = Dead plant. Data were analyzed with SAS® Statistical Analysis System Version 9.4, and the area under the disease progress curve (AUDPC) was determined according to the formula:

$$\text{AUDPC} = \sum_{i=1}^{N_t-1} \frac{(y_i + y_{i+1})}{2}(t_{i+1} - t_i)$$

Where $\boldsymbol{y_i}$**=** Disease severity in the ***i***-th evaluation. $\boldsymbol{t_i}$ = Time in days of the ***i***-th evaluation. **N:** Number of evaluations (Mohapatra et al., 2008). Additionally, logistic models were carried out using the package "nlstools" of r project (Baty et al, 2015).

Bacterial suspensions were prepared from individual Rs colonies grown for 24 hours on nutrient agar (24 g/L). The bacterial suspensions were prepared in TE buffer (Tris 10 Mm pH 7.6 and EDTA

1 Mm, pH: 7.6) diluted to Absorbance$_{600}$ = 0.1, which corresponds to a concentration of 1x10^8 Colony Forming Units per milliliter (CFU/mL). Forty mL of the suspension was applied homogenously to the soil around each plant. Samples for PCR analysis were taken one week after inoculation and then every second day for 1.5 months.

Significant differences in disease severity were observed in FHIA-21 versus Dominico Hartón plantain with all Rs strains under evaluation (**Figure 2-3** and **Supplementary Figure 1**). FHIA-21 showed highly significant differences ($p<0001$) between inoculation methods, where the 'No Wound' inoculation was statistically similar to the control (zero value for incidence, severity, and AUDPC); and all asymptomatic FHIA-21 plants were negative to Rs in PCR tests. This result highlights the strong barrier of the FHIA-21 root system, which could be related to specific root exudates, structural resistance, or resistance to prevent penetration and colonization of the Rs complex into the vascular system, as reported in other similar systems (Tran et al., 2016; Singh et al., 2018). In our study, FHIA-21 showed a significant lower percentage of severely infected plants in the "Wounded" treatment with all Rs isolates tested (**Figure 3** and **Supplementary Figure 1**). Only in one case FHIA-21 inoculated with isolate CIAT-034 under a "No Wound" treatment was positive for Moko, but this corresponded to one single affected plant (**Supplementary Figure 1**). This could be due to some sort of unintended physical damage caused to the root at the time of inoculation, since the rest of strains evaluated did not cause any symptoms of Moko disease in FHIA-21, not even the most pathogenic ones, under a "No Wound" treatment (**Figure 3** and **Supplementary Figure 1**). On the other hand Dominico Hartón plants showed severe symptoms of Moko independently of the treatment and the Rs strain used and the "Wounded" treatment accelerated the development of symptoms by around 15 days as compared to the "No Wound" treatment.

Our results are in agreement with the field observations described by Alvarez et al. (2015), where incidence and severity of Moko disease in FHIA-21 did not exceed 5% in presence of a high Rs inoculum pressure. In contrast, the high susceptibility to Moko in Dominico Hartón plants (**Figure 3**), regardless of the inoculation method, was confirmed and were in agreement with the findings of Valencia-Valencia (2014).

Plants have strong and generic mechanisms that allows them to counter the attack of plant pathogens (Jones and Dangl, 2006; Garcion et al., 2007; Tran et al., 2016). In this regard, is good to remark that the FHIA-21 hybrid was obtained through plantain breeding (crossing AVP-67 (AAB) x SH-3142 (AA)), in search for options to manage BSD, a disease of great economic importance for plantain and banana all over the world. FHIA-21 also shows tolerance to races 1 and 2 of *Fusarium oxysporum* f. sp. *cubense* (Ploetz, 2015). To unravel the resistance mechanism observed in FHIA-21 will require further studies and we are confident that the improved detection and inoculation methods described here will greatly contribute to this aim.

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REFERENCES

Ailloud F, Lowe TM, Robene I, Cruveiller S, Allen C, Prior P (2016) In planta comparative transcriptomics of host-adapted strains of *Ralstonia solanacearum*. PeerJ 4:e1549

Ailloud F, Lowe T, Cellier G, Roche D, Allen C, Prior P (2015) Comparative genomic analysis of Ralstonia solanacearum reveals candidate genes for host specificity. BMC Genomics 16:270

Albuquerque GM, Santos LA, Felix KC, Rollemberg CL, Silva AM, Souza EB, Mariano RL (2014) Moko disease-causing strains of *Ralstonia solanacearum* from Brazil extend known diversity in paraphyletic phylotype II. Phytopathology 104(11):1175-1182

Alvarez E, Pantoja A, Gañan L, Ceballos, G (2015) Current status of Moko disease and Black Sigatoka in Latin America and the Caribbean, and options for managing them. Centro Internacional de Agricultura Tropical (CIAT). Cali, Colombia. Food and Agriculture Organization of the United Nations FAO. 50 p. (CIAT publication No. 404) E-ISBN: 978-958-694-147-1

Baty F, Ritz C, Charles S, Brutsche M, Flandrois J-P, Delignette-Muller M-L (2015). A Toolbox for Nonlinear Regression in R: The Package nlstools. *Journal of Statistical Software*, 66(5), 1-21.

Bocsanczy AM, Huguet-Tapia JC, Norman DJ (2014) Whole-genome sequence of *Ralstonia solanacearum* P673, a strain capable of infecting tomato plants at low temperatures. Genome Announcements 2(1):e00106-14

Bocsanczy AM, Huguet-Tapia JC, Norman DJ (2017) Comparative Genomics of *Ralstonia solanacearum* Identifies Candidate Genes Associated with Cool Virulence. Frontiers in Plant Science 8:1565

Cao Y, Tian B, Liu Y, Cai L, Wang H, Lu N, Wang M, Shang S, Luo Z, Shi J (2013) Genome sequencing of *Ralstonia solanacearum* FQY_4, isolated from a bacterial wilt nursery used for breeding crop resistance. Genome Announcements 1(3): e00125-13

Clarke CR, Studholme DJ, Hayes B, Runde B, Weisberg A, Cai R, Wroblewski T, Daunay MC, Wicker E, Castillo JA, Vinatzer BA (2015) Genome-Enabled Phylogeographic Investigation of the

Quarantine Pathogen *Ralstonia solanacearum* Race 3 Biovar 2 and Screening for Sources of Resistance Against Its Core Effectors. Phytopathology 105(5):597-607

Contreras-Moreira B, Vinuesa P (2013) GET_HOMOLOGUES, a Versatile Software Package for Scalable and Robust Microbial Pangenome Analysis. Applied and Environmental Microbiology 79:7696-7701

Cuellar A, Alvarez E, Castaño J (2011) Evaluación de Resistencia de Genotipos de Plátano y Banano a Sigatoka Negra (*Mycosphaerella fijiensis* Morelet.) Revista Facultad Nacional de Agronomia - Medellín 64:5853-5865.

Minagricultura. 2014. Ministerio de Agricultura y desarrollo rural, cadena de plátano. Available at: https://sioc.minagricultura.gov.co/Platano/Documentos/004%20-%20Documentos%20Competitividad%20Cadena/D.C.%202014%20Octubre%20-%20Indicadores%20platano.pdf. Accessed on February 11, 2019.

Fegan M, Prior P (2006) Diverse members of the *Ralstonia solanacearum* species complex cause bacterial wilts of banana. Australasian Plant Pathology 35:93–101

Fegan M, Prior P (2005) How complex is the *Ralstonia solanacearum* species complex. In Allen C, Prior P, Hayward AC, eds. Bacterial Wilt Disease and the *Ralstonia solanacearum* Species Complex. St. Paul, MN: APS press. pp. 449-461.

Gabriel DW, Allen C, Schell M, Denny TP, Greenberg JT, Duan YP, Flores-Cruz Z, Huang Q, Clifford JM, Presting G, González ET, Reddy J, Elphinstone J, Swanson J, Yao J, Mulholland V, Liu L, Farmerie W, Patnaikuni M, Balogh B, Norman D, Alvarez A, Castillo JA, Jones J, Saddler G, Walunas T, Zhukov A, Mikhailova N (2006) Identification of open reading frames unique to a

select agent: *Ralstonia solanacearum* race 3 biovar 2. Molecular Plant-Microbe Interactions 19(1):69-79

Garcion C, Lamotte O, Métraux JP (2007) Mechanisms of defence to pathogens: biochemistry and physiology. Chapter 6. In: Walter D, Newton A, Lyon G. Induced resistance for plant defence: a sustainable approach to crop protection. pp. 109-123

Gomez E, Alvarez E, Llano G (2006) Variabilidad genética de *Ralstonia solanacearum* raza 2, agente causante del Moko de plátano en Colombia. Fitopatología Colombiana 30(1):1-7

Guarischi-Sousa R, Puigvert M, Coll NS, Siri MI, Pianzzola MJ, Valls M, Setubal JC (2016) Complete genome sequence of the potato pathogen *Ralstonia solanacearum* UY031. Standards in Genomic Sciences 11:7

Guidot A, Elbaz M, Carrere S, Siri MI, Pianzzola MJ, Prior P, Boucher C (2009) Specific genes from the potato brown rot strains of *Ralstonia solanacearum* and their potential use for strain detection. Phytopathology 99(9):1105-1112

Ha Y, Kim JS, Denny T P, Schell MA (2012) A rapid, sensitive assay for *Ralstonia solanacearum* race 3 biovar 2 in plant and soil samples using magnetic beads and real-time PCR. Plant Disease 96:258-264

Hayward AC (1991) Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Annual Review of Phytopathology 29:65-87

He LY, Sequeira L, Kelman A (1983) Characteristics of strains of *Pseudomonas solanacearum* from China. Plant Disease 67 (12):1357-1361

Horita M, Yano K, Tsuchiya K (2004) PCR-based specific detection of *Ralstonia solanacearum* race 4 strains. Journal of General Plant Pathology 70:278–283

Huang W, Zhang H, Xu J, Wang S, Kong X, Ding W, Xu J, Feng J (2017) Loop-Mediated Isothermal Amplification Method for the Rapid Detection of *Ralstonia solanacearum* Phylotype I Mulberry Strains in China. Frontiers in Plant Science 8:76

Jones JDG, Dangl JL (2006) The plant immune system. Nature 444:323–329

Kang KM, Lee MH, Shim JK, Seo ST, Shrestha R, Cho MS, Hahn JH, Park DS (2007) PCR-based Specific Detection of Ralstonia solanacearum by Amplification of Cytochrome c1 Signal Peptide Sequences. Journal of Microbiology Biotechnology 17(11):1765-1771

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30(4):772-780

Kubota R, Vine BG, Alvarez AM, Jenkins DM (2008) Detection of *Ralstonia solanacearum* by Loop-Mediated Isothermal Amplification. Phytopathology 98:1045-1051

Lenarčič R, Morisset D, Pirc M, Llop P, Ravnikar M (2014) Loop-Mediated Isothermal Amplification of Specific Endoglucanase Gene Sequence for Detection of the Bacterial Wilt Pathogen *Ralstonia solanacearum*. PLoS ONE 9(4):e96027

Li L, Stoeckert CJ, Jr., Roos DS (2003) OrthoMCL: identification of ortholog groups for eukaryotic genomes. Genome Research 13:2178-2189

Mohapatra NK, Mukherjee AK, Suriya12AV, Nayak P (2008) Disease progress curves in the rice blast pathosystem compared with the logistic and Gompertz models. Journal of Agricultural and Biological Science 3:28-37

Paret ML, Kubota R, Jenkins DM, Alvarez AM (2010) Survival of *Ralstonia solanacearum* race 4 in drainage water and soil and detection with immunodiagnostic and DNA-based assays. HortTechnology 20:539–548.

Ploetz RC (2015) Management of Fusarium wilt of banana: A review with special reference to tropical race 4. Crop Protection 73:7-15

Ploetz RC, Kema GH, Ma LJ (2015) Impact of diseases on export and smallholder production of banana. Annual Review of Phytopathology 53:269-288.

Prior P, Ailloud F, Dalsing BL, Remenant B, Sanchez B, Allen C (2016) Genomic and proteomic evidence supporting the division of the plant pathogen *Ralstonia solanacearum* into three species. BMC Genomics 17:90-100

Ramesh R, Gaitonde S, Achari G, Asolkar T, Singh NP, Carrere S, Genin S, Peeters N (2014) Genome Sequencing of *Ralstonia solanacearum* Biovar 3, Phylotype I, Strains Rs-09-161 and Rs-10-244, Isolated from Eggplant and Chili in India. Genome Announcements 2(3):e00323-14

Remenant B, Coupat-Goutaland B, Guidot A, Cellier G, Wicker E, Allen C., Fegan M, Pruvost O, Elbaz M, Calteau A, Salvignol G, Mornico D, Mangenot S, Barbe V, Médigue C, Prior P (2010) Genomes of three tomato pathogens within the *Ralstonia solanacearum* species complex reveal significant evolutionary divergence. BMC Genomics 11:379-394

Salanoubat M, Genin S, Artiguenave F, Gouzy J, Mangenot S, Arlat M, Billault A, Brottier P, Camus JC, Cattolico L, Chandler M, Choisne N, Claudel-Renard C, Cunnac S, Demange N, Gaspin C, Lavie M, Moisan A, Robert C, Saurin W, Schiex T, Siguier P, Thébault P, Whalen M, Wincker P, Levy M, Weissenbach J, Boucher C (2002) Genome sequence of the plant pathogen *Ralstonia solanacearum*. Nature 415:497-502

Salgon S, Jourda C, Sauvage C, Daunay MC, Reynaud B, Wicker E, Dintinger J (2017) Eggplant resistance to the *Ralstonia solanacearum* species complex involves both broad-spectrum and strain-specific quantitative trait loci. Frontiers in Plant Science 8:828

Santiago TR, Lopes CA, Caetano G, Mizubuti ES (2017) Phylotype and sequevar variability of *Ralstonia solanacearum* in Brazil, an ancient centre of diversity of the pathogen. Plant Pathology 66:383-392

Schultz J, Milpetz F, Bork P, Ponting CP (1998) SMART, a simple modular architecture research tool: identification of signaling domains. Proceedings of the National Academy of Sciences, USA 95:5857-5864

Shan W, Yang X, Ma W, Yang Y, Guo X, Guo J, Zheng H, Li G, Xie B (2013) Draft Genome Sequence of *Ralstonia solanacearum* Race 4 Biovar 4 Strain SD54. Genome Announcements 1(6):e00890-13

Singh N, Phukan T, Sharma PL, Kabyashree K, Barman A, Kumar R, Ray SK (2018) An Innovative Root Inoculation Method to Study *Ralstonia solanacearum* Pathogenicity in Tomato Seedlings. Phytopathology 108:436-442

Smith DS, De Boer SH (2009) Implementation of an artificial reaction control in a TaqMan method for PCR detection of *Ralstonia solanacearu*m race 3 biovar 2. European Journal of Plant Pathology 124:405–412

Swanson JK, Yao J, Tans-Kersten J, Allen C (2005) Behavior of Ralstonia solanacearum Race 3 Biovar 2 During Latent and Active Infection of Geranium. Phytopathology 95:136-143

Thammakijjawat P, Thaveechai N, Kositratana W, Chunwongse J, Frederick RD, Schaad NW (2006) Detection of *Ralstonia solanacearum* in ginger rhizomes by real-time PCR. Canadian Journal of Plant Pathology 28:391–400

Tirado M, Zapata J (2003) Resistance of FHIA hybrids to *Mycosphaerella* spp. InfoMusa 12:25-28.

Tran TM, MacIntyre A, Hawes M, Allen C (2016) Escaping underground nets: extracellular DNases degrade plant extracellular traps and contribute to virulence of the plant pathogenic bacterium *Ralstonia solanacearum*. PLoS Pathogens 12:e1005686.

Valencia-Valencia L, Alvarez-Cabrera E, Castaño-Zapata J (2014) Resistencia de treinta y cuatro genotipos de plátano (Musa AAB) y banano (Musa AAA) a cinco cepas de *Ralstonia solanacearum* Raza 2 (Smith). Revista Agronomía 22(2):21-34

Weibel J, Tran TM, Bocsanczy AM, Daughtrey M, Norman DJ, Mejia L, Allen C (2016) A *Ralstonia solanacearum* strain from Guatemala infects diverse flower crops, including new asymptomatic hosts vinca and sutera, and causes symptoms in geranium, mandevilla vine, and new host African Daisy (Osteospermum ecklonis). Plant Health Progress 17(2):114-121.

Wicker E, Grassart L, Coranson-Beaudu R, Mian D, Guilbaud C, Fegan M, Prior P (2007) *Ralstonia solanacearum* strains from Martinique (French West Indies) exhibiting a new pathogenic potential. Applied and Environmental Microbiology 73(21):6790-6801

Xu J, Zheng HJ, Liu L, Pan ZC, Prior P, Tang B, Xu JS, Zhang H, Tian Q, Zhang LQ, Feng J (2011) Complete genome sequence of the plant pathogen *Ralstonia solanacearum* strain Po82. Journal of Bacteriology 193(16):4261-4262.

Yuan KX, Cullis J, Levesque CA, Tambong J, Chen W, Lewis CT., De Boer SH, Li XS (2015) Draft genome sequences of *Ralstonia solanacearum* race 3 biovar 2 strains with different temperature adaptations. Genome Announcements 3(4):e00815-15

Zou C, Wang K, Meng J, Yuan G, Lin W, Peng H, Li Q (2016) Draft Genome sequence of *Ralstonia solanacearum* Strain Rs-T02, which represents the most prevalent phylotype in Guangxi, China. Genome Announcements 4(2):e00241-16

FIGURES AND LEGENDS

Figure 1. A. Venn diagram showing the number of genes for each phylotype that were analyzed. From the 11 genes (in red) identified as exclusive for phylotype II, only 2 were detected in more than 80% of the analyzed genomes (Table 1). B. Maximum likelihood RAxML phylogeny of *R. solanacearum* (Rs) phylotype II, race 2 isolates, reconstructed from the nucleotide sequence of the hypothetical protein DUFF3313 domain. Values on branches indicates bootstrap support for each sub-group. Names marked as Colombia correspond to the group of isolates (100% sequence identity) characterized in this work (Supplementary Table 1). C. PCR amplification products from different Rs strains and related bacteria isolates. Upper gel show the results of a previously described multiplex PCR (Fegan & Prior, 2005) designed to identify the phylotypes of Rs strains. Lower gel show the results of the same set of samples using the BIOS1_F/BIOS1_R primers designed in this study. M. Molecular Marker Hyper Ladder II 100 bp; 1. Reaction blank (Nuclease-free water); 2. Genomic DNA of a not infected Dominico Hartón plant; 3. *Xanthomonas axonopodis* pv. manihotis from cassava; 4. *Burkholderia glumae* from rice; 5. Rs isolate G175 from eggplant, phylotype I; 6. Rs isolate G216 from tobacco, phylotype I; 7. Rs isolate G218 from Capsicum, phylotype I; 8. Rs isolate G217 from Heliconia, phylotype III; 9. Rs isolate V18 from banana; 10. Rs isolate V26 from plantain; 11. Rs isolate V31 from banana; 12. Rs isolate 066 from plantain; 13. Rs isolate 070 from plantain; 14. Rs isolate 072 from plantain; 15. Rs isolate 078 from plantain; 16. Rs isolate 088 from plantain; 17. Genomic DNA of a not infected Dominico Hartón plant; 18-20 Reaction blank (Nuclease-free water).

Figure 2. Reaction of plants of DH and FHIA-21 genotypes inoculated with a pathogenic isolate of *R. solanacearum* (Rs, CIAT-078) using two different inoculation methods named "Wounded" and

"No Wound". FHIA-21 "No wound" treated plants (**B**) show no symptoms of the disease and are negative in PCR tests to Rs, as compared to the "Wounded" and symptomatic treated plants, where some of the FHIA-21 plants develop severe symptoms (**A**). In contrast, all DH plants showed severe symptoms of Moko, independently of the treatment (**E** and **F**) Plants shown in **C**,**D** and in **G**, **H,** correspond to FHIA-21 and DH buffer inoculation controls, respectively.

Figure 3. Raw and processed data of disease severity of Rs strain CIAT-078 on four repetitions (R1-R4) assessed on 21 occasions (intervals of 1–3 days). Each assessment evaluated the severity of symptoms in 3 plants. **A** and **B** for genotype FHIA-21, using a "Wounded" and a "No Wound" treatment of the roots before inoculation, respectively. **C** and **D** show the same order of trratments for genotype Dominico Hartón. Solid, thicker lines show area under the logistic curve. And each dot correspond to an observation per plant. Observe that in the case of DH, all dots reach severity level 4 by the end of the experiment, while for FHIA-21, most of the dots remain in the 0 severity, according to the scale described in the main text. **r**: growth rate; **r_se**: standard error of the growth rate; **r_p**: p value of the growth rate; **auc_l**: area under the curve of the fitted logistic equation from time 0 to time t; **auc_e**: area under the curve of the measurements.

Table 1. List of isolates whose genome sequences were used in this work.

Supplementary Table 1. List of Rs isolates from Colombia tested in this work.

Supplementary Figure 1. Raw and processed data of disease severity for other Rs strains evaluated in this study.

Table 1

Sample_ID	Species	Phylotype	Seqvar	Host	Geographic origin	Reference
GMI1000	*R. pseudosolanacearum*	I		Tomato	Guyana	Salanoubat et al. 2002
FQY_4	*R. pseudosolanacearum*	I		Soil	China	Cao et al. 2013
P781	*R. pseudosolanacearum*	I	14	Mandevilla	United States	Bocsanczy et al. 2014
UW757	*R. pseudosolanacearum*	I	14	*Osteospermum*	Guatemala	Weibel et al. 2016
KACC10709	*R. pseudosolanacearum*	I			Korea	Jung et al. 2007
Rs-T02	*R. pseudosolanacearum*	I	14	Tomato	China	Zou et al. 2016
CFBP3858	*R. solanacearum*	IIA	1	Potato	Netherlands	CIRAD
Grenada 91	*R. solanacearum*	IIA	6	Banana	Grenada	Ailloud et al. 2015
IBSBF1900	*R. solanacearum*	IIA	6	Banana	Brazil	Wicker et al. 2007
UW181	*R. solanacearum*	IIA	6	Plantain	Venezuela	CIRAD
K60-1	*R. solanacearum*	IIA	7	Tomato	United States	Remenant et al., 2010
B50	*R. solanacearum*	IIA	24	Banana	Peru	Ailloud et al. 2015
CFBP2957	*R. solanacearum*	IIA	36	Tomato	French West Indies	Remenant et al., 2010
P597	*R. solanacearum*	IIA	38	Tomato	United States	Bocsanczy et al. 2017
CIP120	*R. solanacearum*	IIA	38	Potato	Peru	Bocsanczy et al. 2017
IPO1609	*R. solanacearum*	IIB	1	Potato	Nederland	Guidot et al. 2009
UY031	*R. solanacearum*	IIB	1	Potato	Uruguay	Guarischi-Sousa et al. 2016
RS2	*R. solanacearum*	IIB	1	Potato	N/D	Clarke et al., 2015
UW491	*R. solanacearum*	IIB	1	Potato	Colombia	cBio Corp
UW551	*R. solanacearum*	IIB	1	Geranium	Kenya	Swanson et al. 2005, Gabriel et al., 2006
MolK2	*R. solanacearum*	IIB	3	Banana	Philippines	Guidot et al. 2009
CFBP1416	*R. solanacearum*	IIB	3	Plantain	Costa Rica	Ailloud et al. 2015
CIP417	*R. solanacearum*	IIB	3	Banana	Philippines	Ailloud et al. 2015
Po82	*R. solanacearum*	IIB	4	Potato	Mexico	Xu et al. 2011
UW163	*R. solanacearum*	IIB	4	Plantain	Peru	Ailloud et al. 2015
UW163_1	*R. solanacearum*	IIB	4	Plantain	Peru	Ailloud et al. 2016
P673	*R. solanacearum*	IIB	4	Pothos	United States	Bocsanczy et al. 2014
UW179	*R. solanacearum*	IIB	4	Banana	Colombia	Ailloud et al. 2015
CFBP7014	*R. solanacearum*	IIB	4	Anthurium	Trinidad	CIRAD
IBSBF1503	*R. solanacearum*	IIB	4-NPB	Cucumber	Brazil	Ailloud et al. 2015
IBSBF1503_1	*R. solanacearum*	IIB	4-NPB	Cucumber	Brazil	Ailloud et al. 2016
CFBP6783	*R. solanacearum*	IIB	4-NPB	Heliconia	Martinique	Ailloud et al. 2015, Bocsanczy et al. 2017
CFBP6783_1	*R. solanacearum*	IIB	4-NPB	Heliconia	Martinique	Bocsanczy et al. 2017
23-10BR	*R. solanacearum*	IIB	27	Potato	Brazil	Clarke et al., 2015
POPS2	*R. solanacearum*	IIB	1	Potato	China	Clarke et al., 2015
CMR15	*R. pseudosolanacearum*	III		Tomato	Cameroon	Remenant et al., 2010
CFIA906	*R. pseudosolanacearum*	III				Yuan et al. 2015
NCPPB909	*R. pseudosolanacearum*	III	1		Egypt	Yuan et al. 2015
NCPPB 282	*R. pseudosolanacearum*	III	2		Colombia	Clarke et al. 2015
CFBP3059	*R. pseudosolanacearum*	III	23	*Solanum melongena*	Burkina Faso	Salgon et al. 2017
PSI07	*R. syzygii*	IV		Tomato	Indonesia	Remenant et al., 2010
SD54	*R. pseudosolanacearum*	I		Ginger	China	Shan et al. 2013
Rs-10-244	*R. pseudosolanacearum*	I		Eggplant	India	Ramesh et al. 2014
Rs-09-161	*R. pseudosolanacearum*	I		Chili	India	Ramesh et al. 2014

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Figure 1

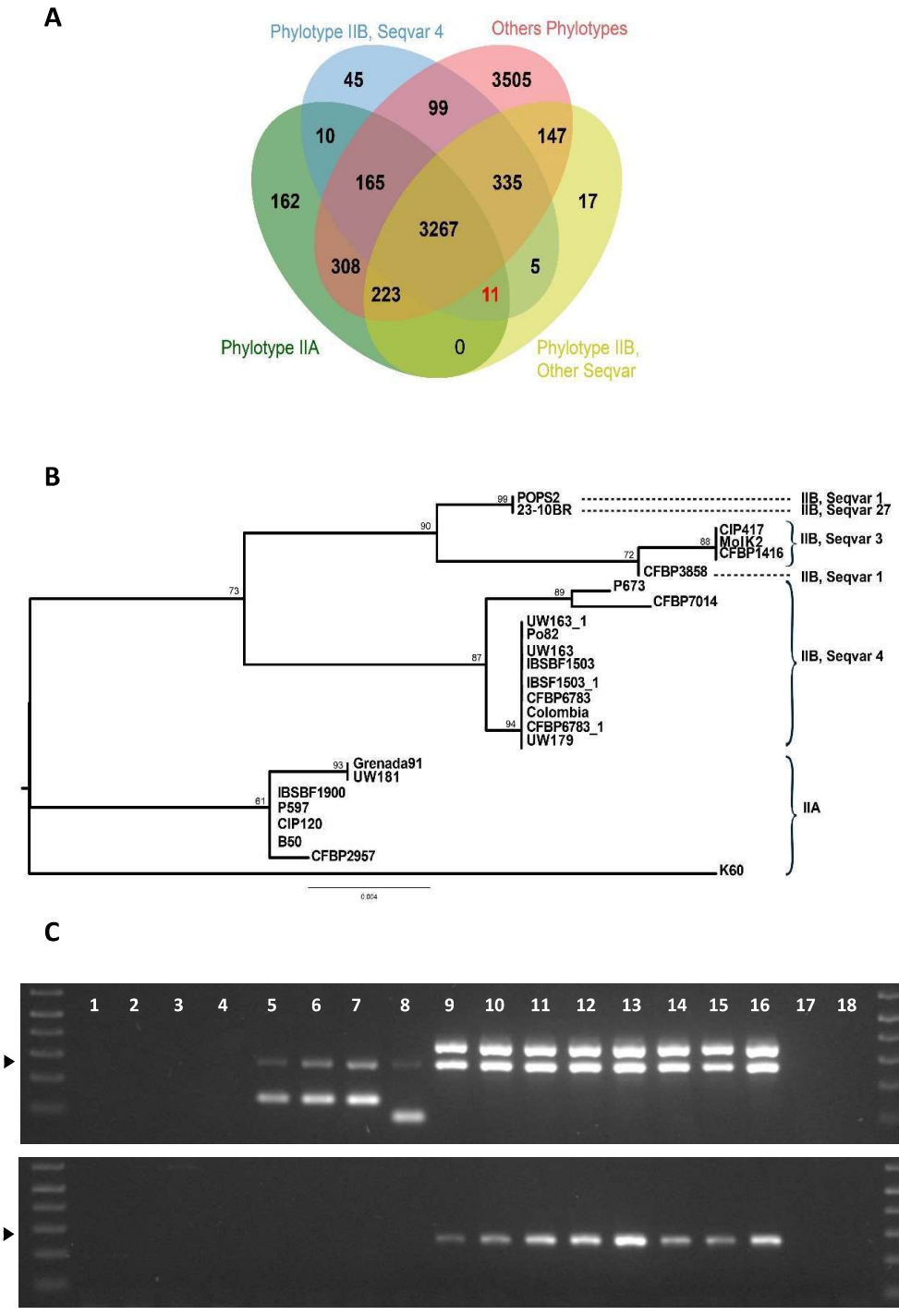

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Figure 2

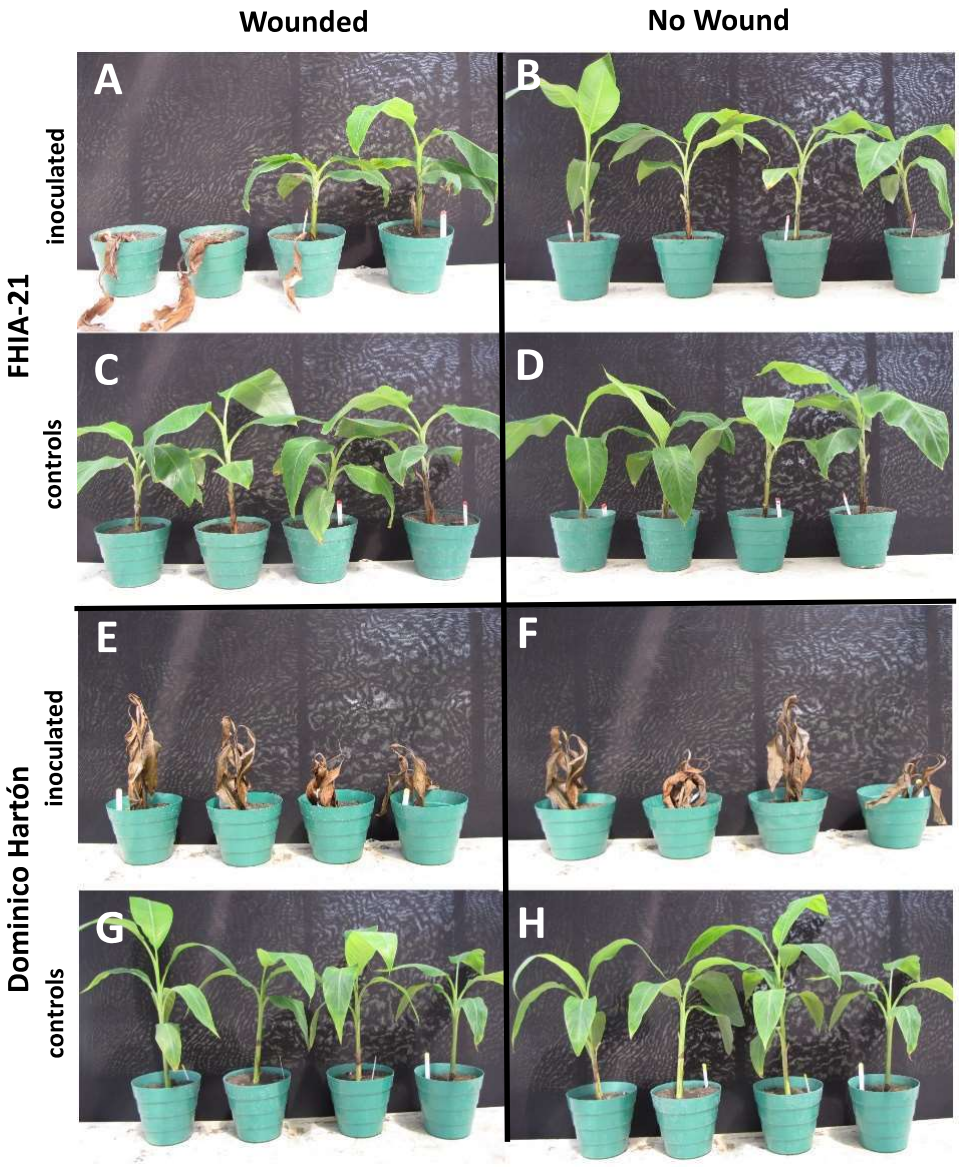

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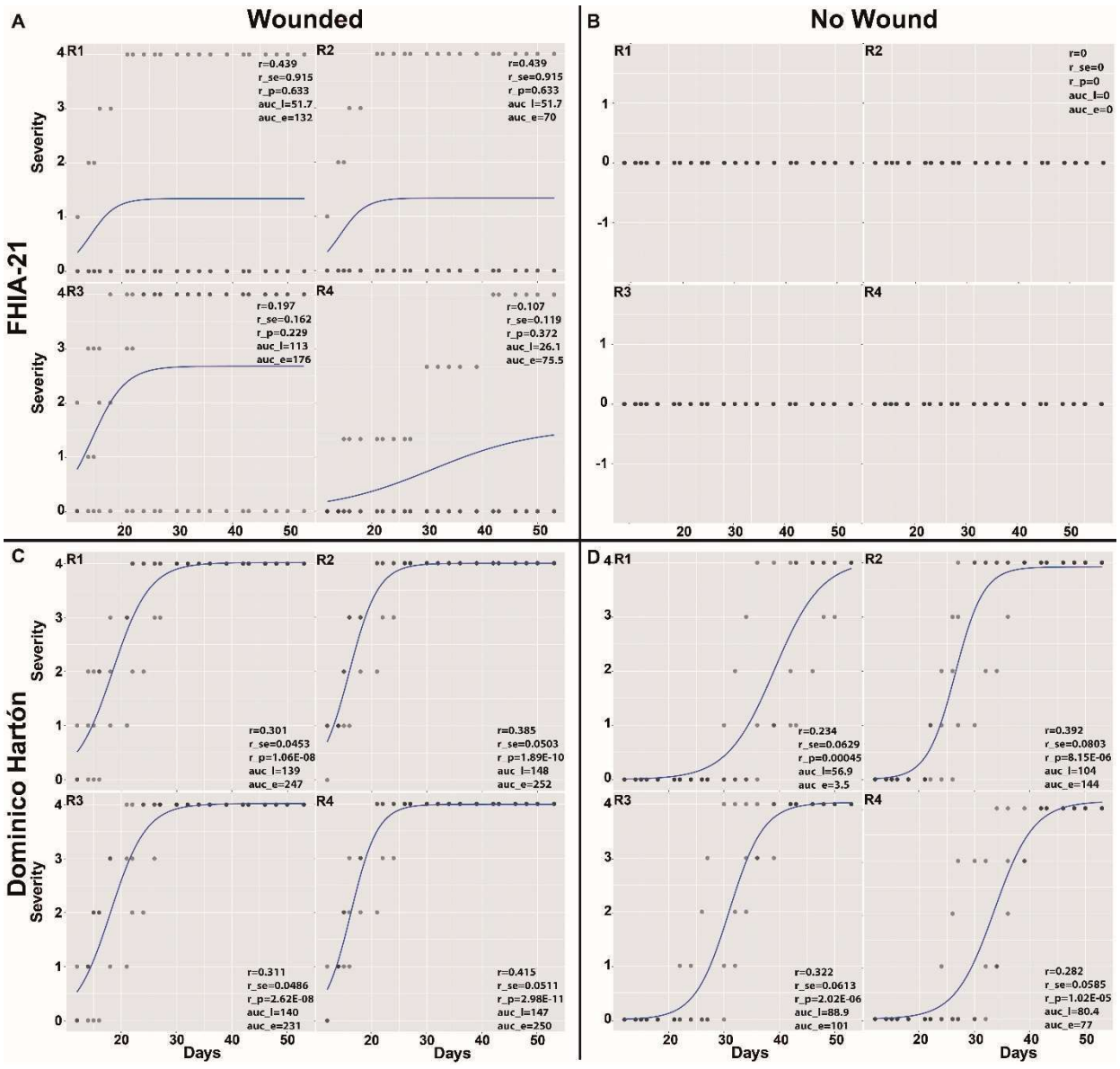
Figure 3



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