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Pseudomonas chlororaphis CP07 strain reduces disease severity caused by *Phytophthora palmivora* in genotypes of *Theobroma cacao*

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Abstract Black pod rot is the most important disease affecting *Theobroma cacao* in Cuba. Plant growth promoting bacteria (PGPB) are considered an alternative for controlling plant disease in the context of the integrated management. The present work was aimed to evaluate *Pseudomonas chlororaphis* strain CP07, isolated from *T. cacao* rhizosphere, as potential PGPB against *P. palmivora* in cacao plants cultivated under controlled conditions. Three genotypes of Cuban traditional cacao of the group Trinitario and the genotype UF 677 were used. The effect of CP07 was evaluated by using detached leaf tests and *in planta* assays with plants obtained in vitro from both seeds and micrografting. Inoculation of *P. palmivora* in control plants showed that genotypes EICB-371, EICB-384 and UF 677 were highly susceptible to the disease (71.4 to 99% disease severity) while EICB-385 was significantly less susceptible (39 to 54.5%). In EICB-371 and EICB-385 genotypes, disease symptoms were significantly reduced in presence of CP07 compared to the control plants in all assays. In contrast, for EICB-384 and UF 677 genotypes, there was not reduction of disease severity in plants pre-treated with CP07. Results show the protective effect of treating cacao plants with CP07 against *P. palmivora*. Plant genotype significantly influenced the protective ability of bacteria affecting the CP07-mediated disease control. Results suggest that depending on cacao genotype, CP07 has a potential for inducing plant defense against *P. palmivora* which can be used in the control of black pod rot.

Keywords *Pseudomonas chlororaphis* · *Theobroma cacao* · *Phytophthora palmivora* · Biocontrol

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Introduction

In Cuba, cacao (*Theobroma cacao* L.) is a traditional crop, mostly grown in agro-forestry systems where traditional Cuban cacao and commercial genotypes are cultivated (Márquez Rivero and Aguirre Gómez 2008). Cuban traditional cacao (originally introduced between

the eighteenth and nineteenth centuries from Haiti and Dominican Republic) has been genetically identified as Trinitario (Bidot Martínez et al. 2015) and were adapted to the local environment. Thereby, Cuban ancient cacao is a valuable local genetic resource that can be used in the genetic improvement of cacao in Cuba. A core collection of these genotypes has been created for studying its genetic and agronomics characteristics and for the ex situ conservation (Bidot Martínez et al. 2017).

The most devastating plant disease in Cuban cacao production is black pod rot, causing from 9 to 17% of harvest losses (Hubeaux 2010). A recent study established *Phytophthora palmivora* (Butl.) as the predominant species affecting cacao fields in Cuba (Fernández Maura et al. in press). This pathogen has been found in 60% of farms in the main producing areas (Martínez de la Parte and Pérez Vicente 2015). Black pod disease mainly affects fruits but also roots, stems and leaves causing the blackening and necrosis of those organs limiting the growth and yield of diseased trees. The disease also affects the propagation of *T. cacao* plants, reducing survival and growth in the local nurseries (Márquez Rivero et al. 2006).

The control of black pod rot has been attempted through the use of resistant varieties, fungicide applications, phytosanitary measures and biological control (Acebo-Guerrero et al. 2012). Chemical methods are widely used, i.e. copper-based products and systemic fungicides are commonly applied for the prevention of the disease dissemination and once the symptoms appear, as a control measure. However, these products are costly for farmers and negatively impact the environment and human health (Hebbar 2007).

In the context of an integrated management, the use of biopesticides may be an alternative to reduce the use of chemical pesticides (Melnick et al. 2011; Dalal and Kulkarni 2013). Microorganisms with antagonistic effects could efficiently control pathogens infection in plants and thus reduce the incidence of the disease (Rezuanul Islam et al. 2012; Ali et al. 2014). Among these microorganisms, Plant growth promoting bacteria (PGPB) have been used to stimulate plant growth and to control plant pathogens. PGPB enhance plant growth by increasing the availability of nutrients to the plant, producing growth-stimulating products (phytohormones, enzymes and volatile compounds), or providing protection against phytopathogens (Tarkka et al. 2008; Almeida Câmara Leite et al. 2013). PGPB can: 1) antagonize plant pathogens directly e.g. by competing for resources, space, nutrients or production of antimicrobial compounds, or 2) are responsible for inducing certain mechanisms, such as induced systemic resistance (ISR), that helps the plant defend itself (Beattie 2006). From PGPB, plant-associated pseudomonads produce several compounds involved in plant growth stimulation. For instance, indole-3-acetic acid (IAA), cytokinin and the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase produced by *Pseudomonas* can modulate plant growth and thus contribute to overcome abiotic and biotic stresses (Santoyo et al. 2019). These bacteria have showed a great potential as biocontrol agents due to their antagonistic effect against a wide range of plant pathogens including bacteria, viruses, fungi and oomycetes (Meena 2014). Diverse antagonistic secondary metabolites are produced for disease suppression. As examples, the extracellular pigment pyoverdin is an efficient siderophore and pyochelin is an effective metal chelating agent possessing strong antimicrobial activity (Beneduzi et al. 2012). The antibiotics produced by pseudomonads include the phenazine, an electron transport inhibitor, phloroglucinols, which cause membrane damage, pyrrolnitrin, acting as a fungicide, cyclic lipopeptides that have surfactant properties against fungi, and hydrogen cyanide (HCN), which is a potent inhibitor of metalloenzymes. Lytic enzymes such as chitinases, glucanases, cellulases and lipases are also produced by pseudomonads to attack phytopathogens (Tarkka et al. 2008; Santoyo et al. 2019). In addition, *Pseudomonas* exhibit high motility and biofilm formation which are two important traits for successful root colonization that is necessary to exert their beneficial activity in host plants (Podile and Kishore 2007). *Pseudomonas* strains can also protect plants through induction of systemic resistance in which defensive mechanisms against pathogens are activated stimulating the synthesis of biochemical compounds involved in plant defense response (Bakker et al. 2007; Pieterse et al. 2014).

In *T. cacao*, some reports describe the antagonistic effect of bacterial isolates against *Phytophthora spp.* These antagonists include: (i) *Bacillus cereus* BT8 strain, a bacterial endophyte isolated from tomato plants with antagonistic effect against *Phytophthora capsici* in infected detached leaves (Melnick et al. 2008), (ii) unidentified bacteria from the yam rhizosphere that reduce the appearance of lesions on detached fruits infected with *P. palmivora* (Koranteng and Awuah 2011), (iii) *Bacillus* spp. endophytic isolates that inhibit *P. capsici*

growth in vitro (Melnick et al. 2011), and (iv) fluorescent *Pseudomonas spp.* isolated from cacao fruits that antagonize *P. palmivora* in vitro and in detached fruits (Akrofi et al. 2017). Detached leaf assay has been used in both screening for resistance to black pod disease and in vivo testing of microbial antagonism due to its correlation with the response of fruits to *Phytophthora spp.* infection (Nyassé et al. 1995; Tahi et al. 2006, 2007).

Pseudomonas chlororaphis CP07 was isolated from the rhizosphere of traditional cacao plants in the region of Baracoa (Cuba) and it displayed antagonistic effect against *P. palmivora*, both in vitro and in vivo, showing a reduction of symptom severity in detached cacao leaves (Acebo-Guerrero et al. 2015). These results suggest that CP07 strain has a potential as a biocontrol agent for black pod rot in cacao. The present study was aimed to investigate the capability of *P. chlororaphis* CP07 to reduce symptom severity in plants of different *T. cacao* genotypes infected with *P. palmivora* under controlled conditions.

Materials and methods

Plant material, seedlings preparation and culture conditions

The plant material was taken from the collection of the Instituto de Investigaciones Agroforestales UCTB Baracoa (IIAB), Guantánamo, Cuba. The hybrid line UF 677, a United Fruit Company selection (International Cocoa Germplasm Database [ICGD] 2015) and genotypes EICB-371, EICB-384 and EICB-385 of traditional Cuban cacao identified as Trinitario (Bidot Martínez et al. 2015) were used.

For obtaining seedlings, mature fruits were externally washed with tap water and soap. Seed disinfection was performed by immersion in $Ca(ClO)_2$ 2% solution (*w*/*v* in water) for 20 min followed by three rinses with sterile water in a Biological Safety Cabinet. After seeds disinfection the mucilage was removed with scalpel and seeds were sown on Murashige and Skoog medium (MS) (Murashige and Skoog 1962), supplemented with sucrose (3%), adjusted to pH 5.7 and solidified with Plant Agar (Duchefa Biochimie, Belgium) (6 g L^{-1}). The seedlings were maintained in a growth room at 25 ± 1 °C, 16 h/8 h (light/darkness photoperiod), and 23 μmol m^{-2} s^{-1} of photosynthetic photon flux (PPF). Micrografted plants were obtained using the protocol described by Miguelez-Sierra et al. (2017) for the in vitro micrografting of *T. cacao* using side graft with axillary buds. Genotypes EICB-371, EICB-384 and EICB-385 were grafted onto UF 677 rootstocks.

Microbial cultures and inocula preparation

Pseudomonas chlororaphis CP07 was grown in plates of King B (KB) Agar (Merck, Germany) at 28 °C for 24 h. Plates were flooded twice with 5 mL of sterile distilled water and scraped off to obtain a cell suspension that was adjusted to an OD_{600} of 0.6 (10^8 cfu mL^{-1}) with sterile water.

Phytophthora palmivora Mab 1 (Acebo-Guerrero et al. 2015) was grown in plates of unclarified V8 Agar for seven days at 24 °C in the dark. Suspensions of zoospores were prepared by the 'wet plate' method (Pistininzi et al. 2014) and the concentration of zoospores was adjusted to 10^5 mL^{-1}. A 1:1 mix of the zoospore suspension of *P. palmivora* Mab 1 and a solution of molten sterile agarose (2% *w*/*v*) maintained at 37 °C was used as inoculum.

Detached leaf assay

A detached leaf assay was performed to assess both the susceptibility to *Phytophthora palmivora* of the selected cacao genotypes and the effect of CP07 in reducing disease severity in infected leaves. Four-week-old seedlings of genotypes EICB-371, EICB-384, EICB-385 and UF 677 obtained as described in 2.1 were taken out of the containers and their roots were rinsed with water. Plantlets were planted in sterile pots (Ø8 cm) filled with 48 g of sterile perlite. Potted plants were individually placed into a transparent plastic container (140 mm height) with sterile perlite at the bottom and 150 mL of MS liquid medium (without sucrose) were poured into the vessels. A second plastic vessel was used to cover the plants and the union between vessels was sealed with Parafilm® to keep a high humidity inside. Plants were maintained in the culture conditions described in 2.1.

After 4 weeks, plants were either inoculated with CP07 or watered with sterile distilled water. Inoculum was prepared as described in 2.2. The pots were unsealed and 5 mL of inoculum were applied close to the roots. The containers were resealed and placed in the same conditions as before. After 10 days two-month-old leaves were collected and submitted to a 5 min

disinfection with $Ca(ClO)_2$ 2% (*w*/*v*, in water) followed by three rinses with sterile water under aseptic conditions. Disinfected leaves were placed in plates (12.5 × 12.5 × 1.5 cm) (Greiner Bio-One GmbH) with filter paper and 10 mL of sterile water. Immediately after they were inoculated with the agarose-zoospore suspension of *P. palmivora* Mab1 (prepared as described in 2.2). The inoculum was applied by placing 10 μL in six points of the abaxial side of leaves. The plates were maintained in the dark at 24 °C for 7 days. After this period, symptoms severity was evaluated according to the scale described by Nyassé et al. (1995) (0: no symptoms, 1: penetration points, 2: network of points, 3: web-like patch, 4: mottled patch, 5: true necrosis). Disease severity was calculated according to Yang et al. (2009) by the following equation: Disease severity = (Σ(number of infected leaves corresponding to the value of the scale × scale value)/(Total of plants × the highest scale value)) × 100.

Control treatments were set up as leaves (from plants inoculated and non-inoculated with CP07) with 10 μL of water and leaves inoculated with Mab1. The experiment was repeated three times with seven leaves per treatment.

In planta assay

The experiment was performed to evaluate the effect of CP07 strain in inducing disease reduction in plants using both four-week-old plants from in vitro germinated seeds and eight-week-old micrografted plants. In both experiments, plants were potted in sterile perlite and placed inside sealed plastics containers as described above.

After four weeks, plastic vessels were unsealed and roots were inoculated with CP07. The containers were resealed and placed in the same conditions as before. After 10 days, two fully developed leaves (approximately two months old) were inoculated on the abaxial surface with 100 μL of the agarose-zoospores suspension (prepared as described in 2.2) with a sterilized paintbrush (Widmer 2009). Plants were placed in a growth chamber at 25 °C, 16 h/8 h (light/darkness photoperiod), and 23 μmol m^{-2} s^{-1} of photosynthetic photon flux (PPF). After seven days, the severity of symptoms was evaluated. The treatments consisted of water-inoculated plants (5 mL of water in roots), plants inoculated with *P. clororaphis* CP07, plants infected with *P. palmivora* Mab1 and plants inoculated with CP07 and infected with Mab1. The experience was repeated three times. Seven plants were used as replicates per treatment.

Data processing

The assays were performed in a completely randomized design. Kruskall Wallis' ANOVA and Mann-Whitney U test were used for data analysis at $p < 0.05$. All experiments were repeated three times with similar results. Data of one experiment representative of the three replicates were used for figures and standard error is presented as vertical bars in the figures. The program Statistica 8.0 was used for data processing.

Results

The response to *P. palmivora* depends on the cacao genotype

Response to *P. palmivora* infection was evaluated in different cacao genotypes on detached leaves, seedlings or micrografted plants (Figs. 1, 4 and 5). Disease severity after 7 days of pathogen inoculation in detached leaves was significantly higher in genotypes EICB-371, EICB-384 and UF 677 than genotype EICB-385. These three genotypes were highly susceptible, with a disease severity ranging from 71.4 to 99% compared to EICB-385 (39 to 54.5%). EICB-384 was the most susceptible with a disease severity from 81.4 to 99%, followed by EICB-371 with 81.4 to 96.1%, and UF 677 with 71.4 to 74.5%.

P. chlororaphis CP07 reduces symptom severity in some cacao genotypes

Leaves of control plants treated with CP07 or treated with water did not present disease symptoms. Disease and symptom severity in detached *T. cacao* leaves after 7 days of pathogen inoculation in leaves from plants pre-treated or not with CP07 are shown in Figs. 1 and 2. In genotype EICB-371 the disease severity was 81.4% in the leaves of control plants (not treated with CP07 and infected with Mab1) but it significantly decreased to 19.9% in the leaves of the plants pre-treated with CP07. In EICB-384, disease severity was 98.5% in leaves of non-treated plants and no significant reduction was observed in presence of CP07 (92.3%). In genotype

EICB-385 with a significant reduction in susceptibility (39%) the symptoms were reduced to 29.4%. UF 677 presented a disease severity of 78% in non-treated leaves and there were not differences with CP07 (70.9%).

The effect of *P. chlororaphis* CP07 in reducing symptom severity against *P. palmivora* in *T. cacao* was evaluated *in planta* by using seedlings and micrografted plants (Figs. 3, 4 and 5). The conditions established for the *in planta* assay allowed growth and maintenance of plants throughout the experiment. After four weeks, plants from germinated seeds and micrografted plants developed numerous leaves in the systems with sterile perlite and nutrient medium (Fig. 3 a-c). In seedlings, the genotype EICB-371 showed a significant reduction of disease severity (from 77.1% to 22.8%) in plants pretreated with CP07. In EICB-384, severity was not significantly reduced in treated plants (75.7%) respect to non-treated plants (81.4%). Genotype EICB-385 showed a lower disease severity (51.4%) in control plants (not treated with CP07 and infected with Mab1) and the application of CP07 reduced the severity (35.7%). As for the UF 677 genotype inoculated with Mab1 the disease severity was 71.4% and the treatment with CP07 didn't reduce the disease severity significantly (65.7%).

The effect of CP07 in reducing disease severity in micrografted plants was also assessed. The genotypes EICB-371, EICB-384 and EICB-385 were grafted onto UF 677 genotype (rootstock). In micrografted plants, genotype EICB-371 had a significant reduction of severity from 96.1% to 22.3% in presence of CP07. In EICB-384 the disease severity with Mab 1 was 99% and there was not a significant reduction with CP07 (94.2%). Genotype EICB-385 had a disease severity of 54.7% with Mab 1 and it was reduced to 44.7% in treatment with CP07.

Discussion

The genotypes of Cuban cacao used in the study have been identified as Trinitario (Bidot Martínez et al. 2015). Results showed differences in susceptibility of these genotypes to *P. palmivora* probably due to the hybrid origin of this type of cacao that has been described as a highly heterozygotic group by Motamayor et al. (2003). Ancient cacao of the eastern region of Cuba is a hybrid where the predominant genetic groups are Criollo, Amelonado and Marañón although these groups are present in different proportions for each genotype (Bidot Martínez et al. 2015). These differences in the genetic composition between the genotypes can cause the differential response to *P. palmivora* inoculation.

In our experience, the inoculation of the root system with *P. chlororaphis* CP07 reduced significantly the disease severity in cacao plants whose leaves were infected with *P. palmivora*. PGPB antagonize a wide range of plant pathogens using both direct antagonism and indirect mechanisms, such as the induction of systemic resistance (Schmidt et al. 2014). The nonpathogenic interaction of rhizobacteria with plant roots has been reported to induce an increased level of resistance against pathogens that will be expressed upon subsequent infection by a pathogen (Meena 2014). In the ISR response, PGPB and the challenging pathogen are spatially separated and no contact between the two is established, thus the protective effect is plant-mediated (Tarkka et al. 2008). Hsu and Micallef (2017) found that root inoculation of spinach and three tomato cultivars with the root-colonizing *Pseudomonas* S4 strain limited the growth of *Salmonella enterica* serovar Newport populations inoculated on leaves. Impairment of *S. enterica* Newport leaf populations was also observed on spinach when plant roots were inoculated with *Pseudomonas* strain S2. Systemic defenses induced by rhizobacteria, as well as defenses induced by previous pathogen attacks may increase plant resistance to pathogen infection and in consequence, a more effective defense response is developed in plants (Pieterse et al. 2014).

In *P. chlororaphis* CP07, genes of secondary metabolites involved in ISR have been detected (Acebo-Guerrero et al. 2015), which could explain a possible effect of the strain in inducing plant defense against *P. palmivora*. For instance, genes of siderophores pyoverdine, pyochelin and achromobactin were detected and pyoverdine production was quantified. Also, genes for the production of the volatile compound 2,3-butanediol and its precursor acetoin were located in CP07 genome. All these bacterial metabolites are considered as elicitors of ISR in plants and could confer a protective effect against a broad spectrum of diseases (Beattie 2006; Meena 2014).

Plant responses of the three traditional Cuban genotypes pre-treated with *P. chlororaphis* CP07 were similar after challenge inoculation with *P. palmivora* on detached leaf assays and in plants obtained from seeds

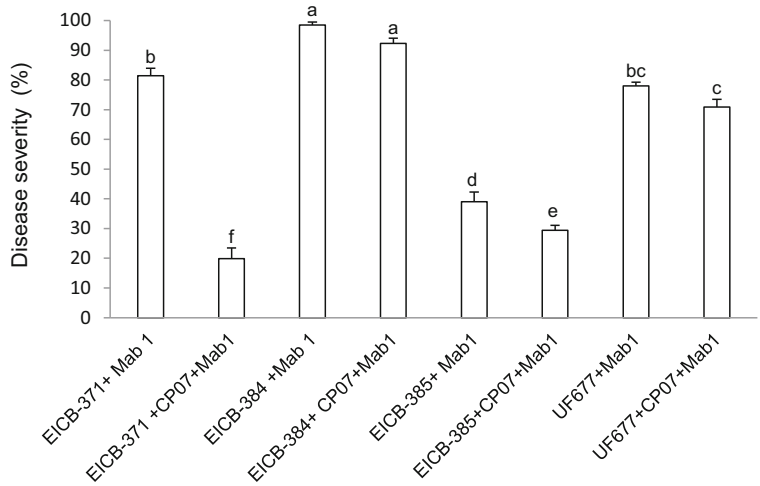

Fig. 1 Disease severity evaluated in detached leaves assay on different cacao genotypes. In vitro cacao plants are pre-treated or not with *P. chlororaphis* CP07 strain. Detached leaves were then inoculated with *P. palmivora* Mab1 strain. Disease severity was evaluated seven days after inoculation with the pathogen. Non common letters indicate significant differences using Mann-Whitney U test ($p < 0.05$). Vertical bars indicate standard error of means

but also on plants grafted onto UF 677 rootstocks. In all assays, the bacterial strain was inoculated in roots and the pathogen in leaves, thus, it is probably the observed responses are systemically induced. It has been demonstrated that after roots colonization, rhizobacteria send signals to the root system that can sensitize distal plant parts for enhanced pathogen defense. The elicitation of such systemic resistance is modulated by hormonal signaling pathways in the plant. The *Pseudomonas*-mediated ISR is dependent on jasmonic acid (JA) and ethylene (ET) signaling pathways although some strains have been reported to trigger a salicylic acid (SA)-dependent systemic resistance which resembles the systemic acquired resistance (SAR) elicited by pathogens (Weller et al. 2012; Pieterse et al. 2014; Agisha et al. 2017). The transcriptional regulator NPR1 which is an important regulator of SA biosynthesis also stimulates the regulation of defense genes in the ISR (Pieterse et al. 2014). After elicitation, a set of defense responses are triggered including phytoalexins and phenolic compounds accumulation, synthesis of resistance proteins, increase of defense enzymes activity and cells lignification (Podile and Kishore 2007; Tan et al. 2013). Further studies are needed to understand the metabolic pathway induced by CP07 on *T. cacao*.Our results show a significant reduction of disease severity caused by *P. palmivora* in some but not all genotypes of *T. cacao* pre-treated with *P. chlororaphis* CP07 suggesting that genotype influences the protective effect of the bacterial strain. For instance, EICB-371 had a high susceptibility to the infection of Mab 1 but, the treatment with CP07 resulted in a significant reduction of disease symptoms.

Fig. 2 Phenotype of disease severity induced on leaves of different genotypes of cacao infected with *P. palmivora* Mab1 strain (seven days post-infection). In vitro cacao plants are pre-treated or not with *P. chlororaphis* CP07 strain

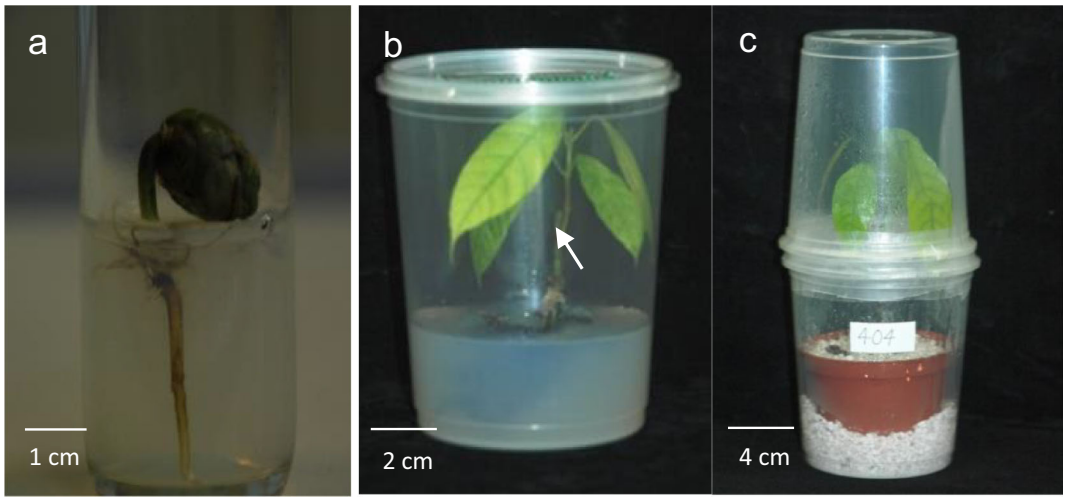

Fig. 3 Plants of *T. cacao* grown under controlled conditions for the *in planta* assay. **a** In vitro germination of seed, **b** eight-week-old micrografted plant and **c** plant growing in the system with sterile perlite. Arrow indicates the graft union

In EICB-385, the severity of symptoms in plants inoculated with Mab 1 was significantly less than the other genotypes and there was a slight but significant reduction with CP07. In contrast, EICB-384 and UF 677 genotypes didn't present disease reduction in presence of CP07.

In micrografted plants, CP07 colonized root system of the rootstock UF 677 and was able to protect leaves tissues of the scion of genotypes that have a beneficial interaction with the bacterial strain. In this case, the rootstock didn't seem to influence the protective effects of CP07, since the results were very similar to those obtained when in vitro germinated plants were used. It could mean that CP07 establishes specific interactions with each cacao genotype for inducing defense response against the pathogen.

The genotype effects on induced defense against pathogens could explain the response of cacao genotypes treated with *P. chlororaphis* CP07. Several studies provide evidences that induced resistance in plants is a host response greatly influenced by genotype and environment (Walters et al. 2011; Walters et al. 2013). Bruce (2014) states that a genotype with particular inducible defense traits will be activated by defense inducers but a genotype not carrying inducible traits cannot be activated. This author reviewed the natural variation between

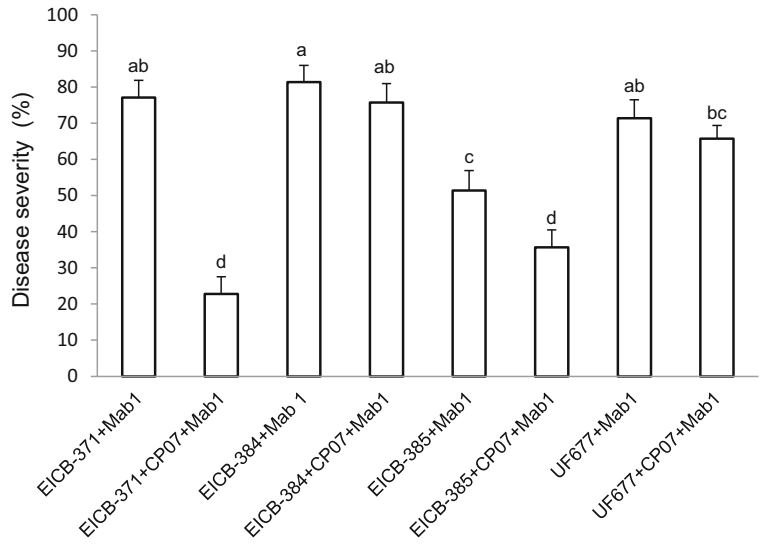

Fig. 4 Disease severity evaluated *in planta* on different cacao genotypes. In vitro cacao plants are pre-treated or not with *P. chlororaphis* CP07 strain. Leaves of seedlings were then inoculated with *P. palmivora* Mab1 strain. Disease severity was evaluated seven days after inoculation with the pathogen. Non common letters indicate significant differences using Mann-Whitney U test ($p < 0.05$). Vertical bars indicate standard error of means

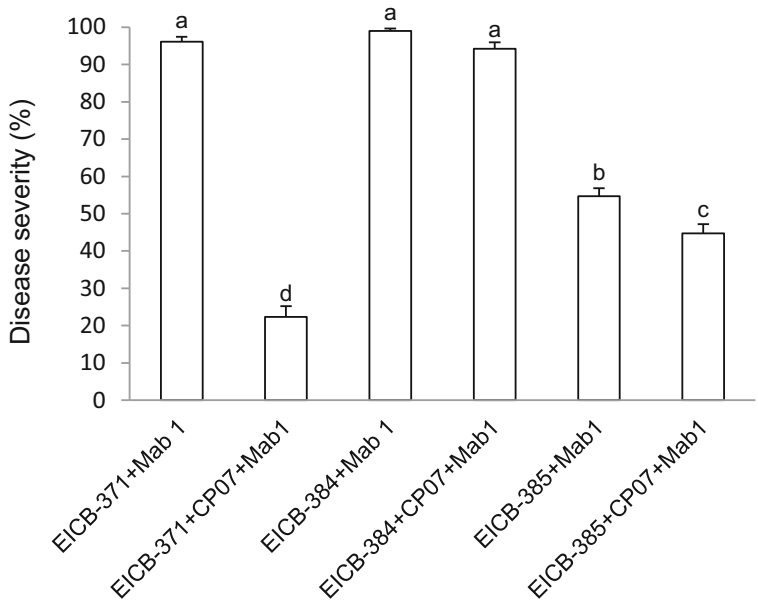

Fig. 5 Disease severity evaluated *in planta* on micrografted plants of different cacao genotypes. Micrografted plants consisting in genotypes of traditional Cuban cacao (EICB-371, EICB-384 and EICB-385) grafted onto UF 677 rootstocks. In vitro cacao plants are pre-treated or not with *P. chlororaphis* CP07 strain. Leaves were then inoculated with *P. palmivora* Mab1 strain. Disease severity was evaluated seven days after inoculation with the pathogen. Non common letters indicate significant differences using Mann-Whitney U test ($p < 0.05$). Vertical bars indicate standard error of means

different plant accessions in the activation of diverse defense biomolecules and pathways upon invaders attack (herbivores and microbial pathogens). For instance, transcriptional responses, induced camalexin accumulation and SA-dependent defenses, phenylalanine ammonia-lyase, polyphenol oxidase and peroxidase activity, constitutive and induced level of JA based defenses and methyl jasmonate-induced volatiles are differentially activated. Furthermore, level of resistance to attackers may influence responses to defense inducers. Resistant cultivars have greater constitutive level of defense molecules than susceptible cultivars which could mean there is less probability for further induction. However, resistant varieties sometimes have higher levels of both constitutive and induced defenses. Walters et al. (2013) report there was no relationship between the resistance rating of barley (*Hordeum vulgare*) varieties to the fungus *Rhynchosporium commune* and its ability to express induced resistance. In seven barley varieties which differed in their genetic resistance to *R. secalis*, inoculation with a combination of defense elicitors (acibenzolar-S-methyl, β-aminobutyric acid, and cis-jasmone) reduced disease in all except one and the magnitude of the reduction differed among cultivars (Walters et al. 2011).

Environmental factors also affect the elicitation of defense in plants and generate a genotype-environment interaction with many variables that often give rise to inconsistent and no reproducible induced defense. Field experimentation has provided information about the effect of previous biotic and abiotic stress in the magnitude of response to further elicitation and evidences suggest that not all host-pathogen interactions respond in the same way to subsequent induction (Walters et al. 2013). The information about this phenomenon is limited and depends on more investigation under field conditions to facilitate an effective use of defense elicitors.

Taking into account that defense induction in plants is highly affected by genotype, the future perspective is the use of particular plant defense activators in selected crop cultivars with the best genetic potential for induced defense in the control of target pathogens (Bruce 2014).

A thorough study of molecular and ecological interactions cacao genotype-CP07 could help to elucidate the pathways through which the bacterial strain can induce resistance to the pathogen. It is known ISR depends on the specific interactions established between the colonizing rhizobacteria and the host (Beneduzi et al. 2012). For instance, *Pseudomonas putida* Kt2440 induces a defense dependent on JA in the maize hybrid cultivar Delprim, which depends on the metabolite benzoxazinoid, present in root exudates (Neal and Ton 2013). In *Arabidopsis thaliana*, *Pseudomonas simiae* WCS417 produces volatile organic compounds (VOCs) that trigger the expression of the root-specific transcription factor MYB72, involved in the elicitation of the ISR response against pathogen infection (Zamioudis et al. 2015). Many other strains of *Pseudomonas* spp. are widely reported as inducers of

systemic resistance (Beattie 2006; Choudhary and Johri 2009). Two *Pseudomonas fluorescens* strains, UMAF6031 and UMAF6033, applied to melon seedlings, provided protection against powdery mildew and also against angular leaf spot (*Pseudomonas syringae* pv. *lachrymans*), with disease reductions of up to 60% (García-Gutiérrez et al. 2012). *P. fluorescens* PF-08, when applied individually or in combination with *Trichoderma harzianum*, enhanced significantly the accumulation of defense-related biomolecules and exhibited biocontrol potential against *Rhizoctonia solani*, the causal agent of sheath blight, in rice (*Oryza sativa* L.) (Singh et al. 2016).

In *T. cacao*, infection of *Ceratobasidium theobromae* (P.H.B. Talbot & Keane), the causal agent of vascular streak dieback, was significantly reduced by pre-treatment of leaves with *Bacillus* sp. and *Pseudomonas* sp. as elicitors of induced systemic resistance (Vanhove et al. 2016).

In the present study, *P. chlororaphis* CP07, isolated from the rhizosphere of traditional Cuban cacao, reduced symptoms of *P. palmivora* infection in this type of cacao which has been seen as a valuable genetic resource for improving cacao production in the country. The use of CP07 potentialities in obtaining cacao plants with enhanced defense responses against *P. palmivora* can contribute to the management of black pod rot by using a biocontrol agent already present in the agroecosystem. These approaches are of particular importance in the frame of the sustainable cacao production.

P. chlororaphis CP07 reduced *P. palmivora* infection in cacao plants under controlled conditions, which evidences the bacterial strain has a potential in inducing plant defenses against the pathogen. Further experiments must be conducted to investigate the protective effect of CP07 in cacao plants potted in substrate containing soil considering the influence of the microbial community of the soil in the expression of a particular biocontrol agent. Also, evidences of the biological mechanisms used by CP07 to induce plant defenses must be obtained to explain the effect in reducing disease severity in cacao plants. For that purpose, biochemical and molecular markers of systemic defense in plants are suitable.

Conclusions

The response to *P. palmivora* inoculation depended on the cacao genotype and significant differences in susceptibility of Cuban cacao genotypes were found. Under controlled conditions, pre-inoculation of *P. chlororaphis* CP07 in the rhizosphere of *T. cacao* plants significantly reduced disease severity caused by *P. palmivora*. Reduction of disease severity seems to be genetically-dependent. The genotypes EICB-371 and EICB-385 of traditional Cuban cacao had a beneficial interaction with CP07 that allowed a significant reduction of disease severity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animals rights No human and/or animal participants were involved in this research.

Informed consent All authors consent to this submission.

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