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Effect of Menadione Sodium Bisulfite, an Inducer of Plant Defenses, on the Dynamic of Banana Phytoalexin Accumulation during Pathogenesis

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Using an authentic sample of 2-hydroxy-9-(p-hydroxyphenyl)-phenalen-1-one, a banana phenalenone-type phytoalexin, we studied its dynamic of accumulation during pathogenesis of banana plants (*Musa acuminata* (AAA), Grand Nain) inoculated with *Fusarium oxysporum* f.sp. cubense (FOC), Race 4, the causal agent of Panama disease. The results obtained demonstrate that banana plants treated prior inoculation with menadione sodium bisulfite (MSB), an inducer of plant defenses, are capable of changing the dynamic of accumulation (higher amount and speed of biosynthesis) of this banana phytoalexin, biosynthesized by the banana plant during pathogenesis.

KEYWORDS: Menadione sodium bisulfite; plant defense inducer; banana phytoalexins; *Fusarium oxysporum*; Panama disease

INTRODUCTION

Phytoalexins are antimicrobial, low molecular weight, secondary metabolite formed de novo as a result of physical, chemical, or biological stress, which resists or suppresses the activity of invaders, and its rate of production/accumulation depends either on host genotypes or both host and pathogen genotypes (*1*). Phytoalexin has been established as one of the major factors in plant defense resistance; however, its real contribution to resistance is still unclear.

Recently, several banana phytoalexins have been isolated from rhizomes of Musa acuminata, Grand Nain (Cavendish Subgroup), infected with *Fusarium oxysporum* f.sp. cubense (FOC) Race 4, the causal agent of the vascular wilt disease named Panama disease or elicited with kanamycin or from rhizomes infected with *Mycosphaerella fijiensis*, the causal agent of Black Sigatoka (*2*, *4*, *6*−*9*).

Fusarium wilt affects locally important cultivars in regions in which bananas are an important source of foods. Effective fungicides do not exist for this fatal disease. The highly susceptible cultivar, Gros Michel, used by the export trades until about 1960, was replaced by clones of Cavendish Subgroup. Although Cavendish cultivars are resistant to Race 1 and Race 2, the Race 4 of the pathogen (FOC-4) damages these cultivars in subtropical banana-growing regions. The continued use of the Cavendish cultivars is now threatened in these regions, and producers in the tropics, mindful of disastrous epidemics that occurred in Gros Michel, are concerned that Race 4, or a similar pathogen, might develop in their areas (*3*).

We synthesized several phenalenone-type phytoalexins according to Luis (*7*). One of these banana phytolaexins, the 2-hydroxy-9-(p-hydroxyphenyl)-phenalen-1-one (**Figure 1**), was found by us at highest concentration in a previous study.

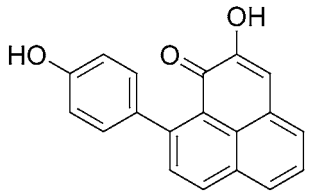

Figure 1. Structure of banana phytoalexin 2-hydroxy-9-(p-hydroxyphenyl)-phenalen-1-one used in this study.

A water soluble addition compound of vitamin K_3, menadione sodium bisulfite (MSB), first studied as a plant growth regulator (*10*), has recently been shown to induce resistance against Panama disease (*11*−*12*).

To investigate whether MSB banana plant treatments prior inoculation with FOC, Race 4, induce any effect on the dynamic of phytoalexin accumulation during pathogenesis, a comparative study with banana plants was carried out by inoculating MSB-treated and untreated banana (*Musa acuminata* (AAA), Grand Nain) plants with FOC, Race 4, and subsequently determining the phytoalexin accumulation at 0 (uninoculated) and 24, 48, 72, and 96 h after inoculation during pathogenesis.

MATERIALS AND METHODS

Growth and Maintenance of Plants. A total of 80 banana plants (30 cms high) of the cultivar Grand Nain (Subgroup Cavendish) from tissue culture were submitted to different treatments under identical experimental conditions. The plants (1 plant/pot) were distributed in blocks at random in a glass greenhouse. The maximum and minimum temperatures in the greenhouse during the experiment were 35 and 12 °C, respectively, while relative humidity oscillated between 40 and 95%.

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Pretreatment of Banana Plants and Pathogen Inoculation. Three foliar pretreatments were carried out in the form of aqueous solutions (deionized water) at a 60 μg/mL (70 mL/plant) dose of MSB and with an application frequency of 4 days between treatments. To each of the aqueous solutions of the treatments with MSB was added 40% polyglycolic alkylphenyl ether as adjuvant. Minimal medium (MM), as described Puhalla (*5*), was used to isolate FOC from diseased plants. Spores of FOC, Race 4 (VCG-0120) maintained at −80 °C in glycerol were cultured on PDA and incubated at 28 °C under white fluorescent light. At 24 h after the final pretreatment, inoculation was performed with FOC, Race 4 (VCG-0120), in the respective treatments. The plants corresponding to the treatments without MSB received the same pretreatments, the difference being that the aqueous solution was made up solely of deionized water and adjuvant. The initial step in the plant inoculation was to wash the root system. Subsequently, the root points (0.5 cm) were cut from all the plants in order to facilitate spore penetration. The root points of all 80 banana plants were cut at the same time. The plants were then submerged for 24 h in dilute solutions (deionized water) containing spores (10^5 spores/mL) of FOC, Race 4. After 24 h, the roots were washed with water and the plants transferred to a hydroponic system. The composition of the nutrient solution was (μM): 500Ca $(N0_3)_2 \times 4H_2O$; $200KNO_3$; $150KH_2PO_4$; $100K_2SO_4$; 200NaCl; 200KCl; $200MgSO_4 \times 7H_2O$; 40Fe ^{III}−EDTA; $14H_3BO_3$; $3ZnSO_4 \times 7H_2O$; $0.7CuSO_4 \times 5H_2O$; $3MnSO_4 \times H_2O$; $0.8Na_2\ MoO_4 \times 2H_2O$. The pH of the nutrient solution was adjusted to pH 5.5−5.8. The plants corresponding to each treatment were simultaneously removed from the hydroponic system at 0 (uninoculated) and at 24, 48, 72, 96 h after inoculation. All the roots of each plant were cut to the point of insertion with the rhizome.

Phytoalexin Extraction and Cuantification. Fresh weight of cut roots from each treatment at 0, 24, 48, 72, and 96 h after inoculation was left to macerate in bidistilled EtOH (150 mL) for 5 days at room temperature. The ethanolic extracts were then filtered and concentrated in a rotavapor to a fourth of their volume and repeatedly extracted with $CHCl_3$ (3 × 30 mL). The $CHCl_3$ extracts were taken to dryness. Taking as a reference the Rf in TLC (silica gel and *n*-hex/EtOAc (70:30) as solid and mobile phase, respectively, were employed), an authentic sample of banana phenalenone-type phytoalexin used as pattern and previously synthesized by us according to Luis et al. (*7*), the phytoalexin 2-hydroxy-9-(p-hydroxyphenyl)-phenalen-1-one (**Figure 1**) was identified. After being dissolved in $CHCl_3$ (1 mL), the pattern phytoalexin was injected (1 μL) with split ratio (20.0) into a GC (Hewlett-Packard Series 6890 Plus, 30 m × 0.32-mm HP-5 capillary column packed with cross-linked 5% phenyl methyl siloxane, automatic injector, FID detector, and N_2 as carrier gas at 15 mL min^{-1}, operating conditions: initial temp 100 °C for 1 min, rate 8 °C/min, final temp 280 °C for 1 min) and identified by its retention time and used as external standard. Each of the samples corresponding to the different treatments was taken to dryness and dissolved in $CHCl_3$ (1 mL) to be injected (1 μL) into the GC to quantify this phytoalexin in each of the extracts obtained from the corresponding treatments.

RESULTS AND DISCUSSION

Figure 2 shows that banana plant treated with MSB prior inoculation are capable of changing the dynamic of accumulation of the 2-hydroxy-9-(p-hydroxyphenyl)-phenalen-1-one, one of the banana phenalenone-type phytoalexins described in the literature (*7*).

MSB, a novel plant defense activator, has recently been shown to induce resistance against Panama disease, caused by FOC, Race 4 (*11*−*12*). MSB is a water soluble addition compound of vitamin K_3 and has been studied for the first time as a plant growth regulator (*10*). MSB induces an increase of free indoleacetic acid (IAA) level in the plant (*10*).

Beckman (*13*−*14*) added IAA to the list of host factors in his time−space model of host−parasite interactions in an infected vascular element and the surrounding contact parenchyma cells, because a reasonable and timely role in defense process has been established for this hormone. Thus, we have

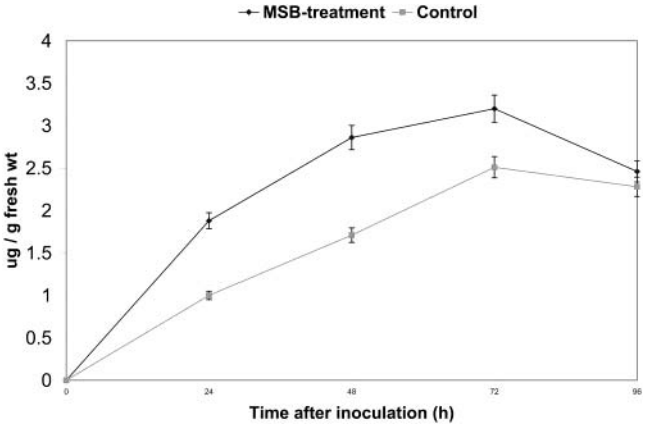

Figure 2. Dynamic of accumulation of 2-hydroxy-9-(p-hydroxyphenyl)-phenalen-1-one during pathogenesis. Phytoalexin of banana plants corresponding to each treatment were extracted as described in Materials and Methods. Statistical analysis was performed by the t-test to compare pairs of means using Statgraphics-Plus software, version 4.4 for Windows. Statistical analysis of phytoalexin concentration was done starting at 24 h time. Significant differences within each time period were found at $P = 0.05$, except at 96 h time. Mean value based on 8 replicates.

accomplished induced resistance against FOC by exogenous application of IAA (*11*). We suggest that an overexpression of free endogenous IAA level could act positively on other defense factors of the host, such as the phytoalexin synthesis, which could be involved in a reinforcement of resistance of banana plants to fusarial wilt, which is not observed in untreated plants (control).

We found a higher amount and speed of biosynthesis of the phytoalexin studied within each time period studied following the development of pathogenesis. The results obtained in this work suggest that pretreatments of banana plant with MSB bring about a positive influence on phytoalexin accumulation during pathogenesis, and this could be one of the defense mechanisms involved on resistance response. This work constitutes the first study of the dynamic of banana phytoalexin accumulation during pathogenesis in banana plants inoculated with FOC, Race 4.

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