Vacciplant and Calliete cotreatment synergically induces phenolic compounds production in plantain *(Musa* x *paradisiaca* L.) and trigger the natural defense activation for protection to black leaf streak disease

ABSTRACT

Aims: To evaluate the synergistic action of two elicitors or stimulators of natural plant defense, vacciplant® and calliete®, on the synthesis of defense metabolites and their impact on tolerance to *Mycosphaerella fijiensis*, causal agent of the black leaf streak disease (BLSD) in banana.

Study design: Original article

Place and duration of study: The study was conducted in Abidjan, Lagunes region, southern Côte d'Ivoire in 2019.

Methodology: The ability of plants co-treated with vacciplant®+calliete® to product phenolic compounds was compared to vacciplant® and the control. Subsequently, the treated plants were inoculated with the pathogenic filtrate of *Mycosphaerella fijiensis* in order to induce the black leaf streak disease. Afterwards, the content of phenolic compounds was determined and then their analysis by ultra-high performance liquid chromatography was performed (U-HPLC). Finally, foliar pigments were quantified to have an indication of the sanitary state of elicited and infected plants.

Results: plants treated with vacciplant® and inoculated, as well as plants co-treated with vacciplant®+calliete® and inoculated, accumulated more phenolic compounds than the control plants. However, the phenol content of the co-treated plants (191.10 mg/g FL) was higher than that of the plants treated with vacciplant® alone (142 mg/g FL). In addition, qualitative analysis by U-HPLC revealed that treated plants synthesized de novo two compounds including *trans*-resveratrol, a stilbene with significant antifungal activity, in

plants co-treated with vacciplant®+calliete®. In addition, it allowed the plants to acquire a higher leaf greening state than those treated with vacciplant® alone, indicating a more intense photosynthetic activity and thus a good sanitary state

Conclusion: Vacciplant®+calliete® has a synergistic effect on the activation of the plantain's defense reactions and thus on the good tolerance to *M. fijiensis*, agent of plantain BLSD.

Keywords : black leaf streak disease, elicitor, *Mycosphaerella fijiensis*, natural defense, polyphenol, plantain, tolerance

1. INTRODUCTION

The plantain is a food crop that is grown in the countries of the humid tropics for its fruit called plantain. Worldwide, plantain is the fourth most important crop after rice, wheat and maize [1]. Sub-Saharan Africa produces almost 70% of the world's plantain production. Uganda, Cameroon, Ghana, Côte d'Ivoire, Rwanda and the Democratic Republic of Congo are the main producers of plantain [2]. In Côte d'Ivoire, plantain is the second most consumed food after rice [3]. Moreover, it is the 4th most important food crop after rice, cassava and yams [4], with 1,600,000 tonnes produced each year by 2,500,000 active farmers, 80% of whom are women [5]. It thus contributes to the diversification of farmers' incomes and the fight against poverty. However, bananas are subject to numerous parasitic constraints, among which fungal diseases contribute significantly to the drop in yields [6]. Indeed, the fungus *Mycosphaerella fijiensis*, the causal agent of black leaf streak disease (BLSD), is one of the main limiting factors in banana cultivation. The economic consequences of MRN are serious, as losses can represent 50% of the crop [7]. Moreover, the incidence of parasitism is such that the chemical control recommended for intensive cropping systems is inappropriate for plantain because it is a small-scale crop. Indeed, large quantities of pesticides are required to control BLSD, up to 50 fungicide applications per year [7]. Thus, the ecological costs to the environment of using fungicides to control BLSD are high, as are the resulting economic

costs. Moreover, Stover and Simmonds [8] evaluated the costs of pesticides at 27% of production. Consequently, it is necessary to look for effective and accessible alternatives to chemical control for sustainable ecoproduction in plantain. One of these alternatives is to give plants the ability to defend themselves or to increase their defenses instead of fighting the pest directly by using elicitors or natural plant defenses stimulators [9; 10]. Moreover, the elicitors enable the production of defense metabolites to be activated without the plant being attacked by pathogens; and among these metabolites, phenolic compounds occupy a prominent place [9 ; 11]. The plant is ready to retaliate in the event of a subsequent attack and simultaneously resist a wide spectrum of pathogens [12]. The aim of this study was to evaluate the synergistic action of two elicitors or stimulators of natural plant defense, vacciplant® and calliete®, on the synthesis of defense metabolites and their impact on tolerance to *Mycosphaerella fijiensis*, responsible of the black leaf streak disease in banana.

2. MATERIAL AND METHODS

2.1. Plant material

The plant material consists of vivoplants of plantain cv. French 2. This cultivar is susceptible to black leaf streak disease (BLSD) caused by *Mycosphaerella fijiensis* [13]. The plants were provided by the Centre National de Recherche Agronomique (CNRA).

2.2. Fungal material

The fungal material used is a virulent strain of M. fijiensis ST-W-D98.B1C1. It was isolated from the leaves of a plantain tree from the CNRA Wanita experimental plantation in Bimbresso (Côte d'Ivoire).

2.3. Production and maintenance of plantain vivoplants

Plantain vivoplants were produced in a germander from shoots taken from dehulled stumps. After 14 days, they were transferred under shade at room temperature and 80-90% relative humidity. The vivoplants were then grown in perforated black polyethylene bags containing

forest floor previously autoclaved at 121°C for 30 min under a pressure of 1 bar, as a substrate [14]. The vivoplants were maintained under shade for three months.

2.4. Preparation of elicitation solutions

Vacciplant® (45 g/L laminarin) and calliete® 80WP (alliete) were prepared respectively at 3 and 2%. A mixture of the both solutions (v/v) was also prepared for co-treatment and all the eliciting solutions were supplemented with 20% Tamoil® (SAE 5W-40 oil) at 5 mL/L. Distilled water was used as a control.

2.5. Preparation of the *M. fijiensis* filtrate

For the preparation of *M. fijiensis* toxic filtrate, 10 mL of inoculum (2.10^5 spores/mL) was added to 100 mL of liquid culture medium contained in Erlenmeyer flasks for the production of toxic metabolites according to Pinkerton and Strobel's method modified by Amari [15; 16]. The erlenmeyer flasks were placed in an enclosure for 28 days. The culture broths were separated from the mycelium by filtration through a sieve 80 µm in diameter. The extract obtained constituted the culture filtrate of *M. fijiensis*. Preparations without inoculation of fungal spores were used as control.

2.6. Treatments for banana leaves

The treatment of banana leaves was done according to Belhadj's technique [17]. After three months of cultivation of banana plants, 40 plants of the cultivar French 2 including control-plants (10 plants per treatment) were treated. The solutions were sprayed on the leaves using a hand sprayer and then the plants were incubated in a controlled environment for 48 h and 72 h respectively for the plants co-treated with vacciplant®+calliete® and treated with vacciplant®.

2.6. Foliar spray inoculation of banana with Mycosphaerella fijiensis filtrate

The French 2 banana plants were separated into three distinct groups (untreated, untreated then infected and treated then infected). After the incubation period, the leaves of the banana plants were slightly punctured with a sterile needle. Then, 25 mL of the fungal inoculum was sprayed on the upper and lower surfaces of each banana leaf. Three treatments and controls were carried out for each banana cultivar as (T0) plants not treated by elicitor and not inoculated (PNTNI), (T1) plants not treated with elicitor and inoculated (PNTI), (T2) plants treated by vacciplant® and inoculated (PTVI) and, (T3) plants treated with vacciplant®+calliete® and inoculated PT(V+Ct)I. The inoculated banana plants were placed under shade at room temperature. The plants were watered according to the humidity of the substrate. The leaves of the control plants were sprayed with sterile distilled water instead of elicitors. Two weeks after fungal inoculation, the second leaf after the cigar leaf of each banana cultivar was harvested. The leaves were then freeze-dried and stored at -20°C.

2.7. Quatitative determination of phenolic compounds by spectrophotometer

2.7.1. Extraction and phenolic compounds essay

The extraction of total phenolic compounds was carried out according to the method of Kouakou [19]. Briefly, 10 mL of methanol was added to 50 mg of freeze-dried leaf and placed overnight at 4°C. The mixture was then centrifuged at 2000 rpm for 10 min. Supernatant was filtered through a Millipore membrane (0.45 µm) and the filtrate was used to determination of phenols content. Briefly, 0.5 mL of Folin-Ciocalteur reagent and 0.9 mL of water were added to 0.1 mL of phenolic extract. After stirring at room temperature, 1.5 mL of a 17% (w/v) sodium carbonate solution were added. After 20 min of incubation at 25 °C in the dark, the mixture was monitored at 765 nm. Phenol content, expressed in milligrams of gallic acid equivalents per gram of extract, was determined using a calibration line with gallic acid ($y = 0,021x + 0,053$; $R^2 = 0,999$; where y is absorbance and x is the concentration of gallic acid).

2.8. Qualitative analysis of phenolic compounds by ultra-high performance liquid chromatography

2.8.1. Extraction and purification of phenolic compounds

Extraction and purification of phenolic compounds was carried as above [18]. Approximately 100 mg of leaf lyophilisate from each cultivar was placed in a haemolysis tube and 20 mL pure methanol was added. The mixture was then placed at 4°C for 15 h. After sonication for 5 min in ultrasound, the mixture was centrifuged at 5,000 rpm for 10 min. Then, 4 mL of supernatant was evaporated using a Speed Vac mini-concentrator. The dry residue obtained was dissolved in 1 mL of 30% methanol and then placed on a C18 grafted silica mini-column in the Supelco Visiprep™ refill system [19]. The resulting eluate was evaporated using Speed Vac ; residue was taken up in 1 mL of 50% methanol and filtered through a Millipore membrane at 0.45 µm. The filtrate was used as polyphenols extract.

2.8.2. Analytical conditions in ultra-high performance liquid chromatography

The analyses were performed using the Ultra High Performance Liquid Chromatography [20]. An aliquot of the methanolic solution was injected into Ultra High Performance Liquid Chromatography (UHPLC). The analysis of the samples was performed using Agilent LC system (1100-1200 series). The UHPLC was coupled to a nuclear magnetic resonance spectrometer (Bruker Avance III) with an operating frequency of 600 MHz for one proton. Separation of phenolic compounds was performed on a reverse phase C18 silica column (Zorbax Eclipse XDB-C18, 150 x 4.6 mm, 5 µm, Agilent). Distilled water filtered through the millipore membrane (0.45 µm) was acidified with 0.1% trifluoroacetic acid (TFA) to be solvent A and acetonitrile (HPLC grade) was acidified by TFA to be solvent B. The elution gradient profile was performed as follows : 2% B (0-1 min), 50% B (1-5 min), 40% B (5-10 min), 20% B (10-15 min), 10% B (15-16 min) and 2% B (16-20 min).

2.8.3. Separation and identification of phenolic compounds

The separation of phenolic compounds was carried out under a pressure of 550 to 1000 bar. The hydromethanolic extract was diluted with filtered distilled water (50/50, v/v). Approximately 10 µL of the purified phenolic compound extract was directly injected into U-HPLC with a flow rate of 1.3 mL/min and detection was performed at 284 nm. A reference library was previously made with commercially available phenolic compounds identified by ^{1}H-NMR [21 ; 22]. The retention time and/or the NMR spectra of the separated compounds were compared with those of the reference library for their identification.

2.8.4. Determination of leaf pigments

The extraction of leaf pigments was performed on the freeze-dried leaves. Thus, 50 mg of freeze-dried leaves were placed in a test tube and 10 mL of acetone was added and then tightly closed with a cap to avoid evaporation. The mixture was then placed on a rotary shaker for 10 min for 2 h. After 5 min sonication with ultrasound, the debris was removed by centrifugation (5000 rpm for 15 min). The absorbance of the crude extract was measured at 470, 647 and 663 nm with a spectrophotometer. Pigment contents are calculated in mg/g of freeze-dried leaves according to the following equations [22] : chlorophyll a (chla) = 12.25 OD663-2.79 OD647,

chlorophyll b (chl b) = 21.50 OD647-5.10 OD663, total chlorophylls (Chl t) = 7.15 OD663+ 18.71 OD647. The indicator of functional pigment equipment and green state of the leaves of elicited banana plants was calculated according to the method of Lichtenthaler [23]. Thus, the indicator was determined by chl a/chl b. In addition, the ratio of total chlorophylls to carotenoids (chl t/car), which indicates the greenness of the plant leaves, was determined.

2.9. Statistical analysis

The data obtained were analysed using Statistica 7.1 software. An analysis of variance with two classification criteria was performed to determine significant differences. In case of

significant differences, the means were classified into homogeneous groups using the Newman-Keuls test at 5%. All experiments were triplicate.

3. RESULTS AND DISCUSSION

Figure 1 shows the evolution of phenolic compounds in leaves of the banana cultivar French 2 at different treatment stages. The treatment stage (PNTNI) is the phenolic control stage. Indeed, the phenolic content in the control leaves (PNTNI) was 30.5 mg /mg FL. When the leaves were inoculated without elicitation (PNTI), this content decreased from 30.5 to 9.1 mg /mg FL, i.e. a decrease of 70.16% compared to the control. When the leaves were treated and inoculated, an increase in content was observed compared to the control. Thus, in the leaves treated respectively with vacciplant® and the vacciplant®+calliete® mixture and then inoculated, PTVI and PT(V+Ct)I, 142 and 191.10 mg /mg FL were obtained, corresponding to respective increases of 407 and 526.56% compared to the control. These results determine that without elicitation, the plant is susceptible to the establishment of infection. In addition, vacciplant® and vacciplant®+calliete® accumulated phenolic compounds auguring an acquisition of resistance to *M. fijiensis* [24]. Indeed, the parietal polysaccharides would be elicitors of defensins of phenolic nature.

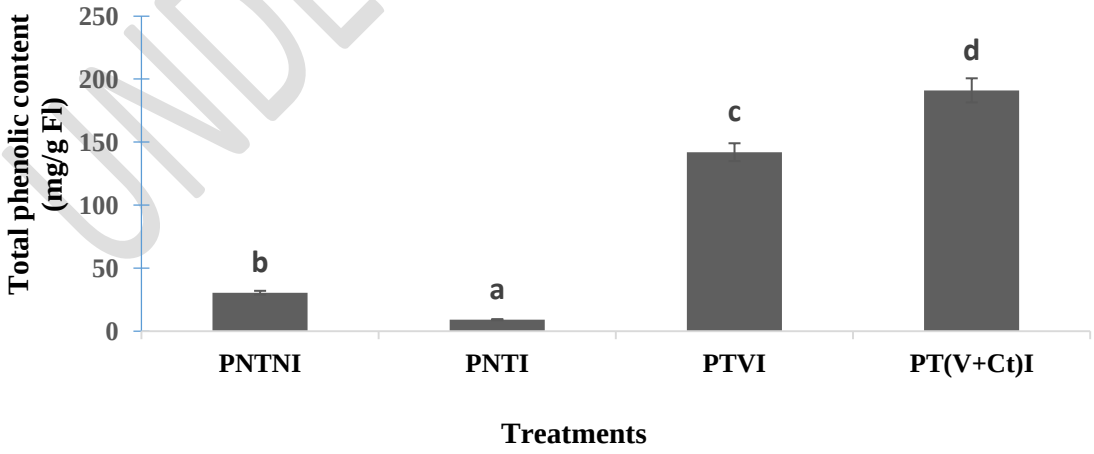

Figure 1. Phenolic compound content of elicited and inoculated leaves of the plantain cultivar French

PNTNI, Untreated and Inoculated Plants; PNTI, Untreated and Inoculated Plants; PTVI, Vacciplant Treated and Inoculated Plants; PT(V+Ct)I, Plants Treated with Vacciplant®+Calliette® and Inoculated. Histograms topped with the same letter are not significantly different at 5% (Newman-Keuls test); values represent the mean of triplicate. Bars are standard errors.

It is worth mentioning that calliette accumulated around 92.40 mg/g FL of phenolic compounds (data not shown) which is very low compared to the other both elicitors. In fact, these elicitors rich in parietal polysaccharides influence the activation of phenolic biosynthetic pathways. Indeed, according to several studies, polysaccharides would hydrolyze into reducing sugars that stimulate the accumulation of phenolic phytoalexins and thus activate defense reactions [25; 26]. In addition, phenol production from co-treated plants remains better and is even better when treated and inoculated with *Mycosphaerella fijiensis*. These results seem to indicate that vacciplant® and its co-treatment with calliete® would act as an early salicylic acid to mobilise phenolics in the leaves after elicitation [27]. As a result, a hypersensitivity reaction (HR) would be set up as reported by Beckers and Spoel [28]. HR relies on the rapid recognition of the pathogen by the plant and would aim to confine the toxic agent to its site of entry. Thus, in many interactions, it appears that the important contribution of the hypersensitive response to host resistance is directly related to the speed with which it is deployed [29]. The accumulation of phenolic compounds suggests that plants treated with these elicitors are able to develop a systemic immunity phenomenon, i.e. a systemic acquired resistance (SAR) against the black leaf stripe disease. The plantain has consequently been "vaccinated" and is ready to fight back in case of a subsequent attack by pathogens such as *M. fijiensis* [30]. In addition, it has long been recognised that plant responses to pathogen attack are characterised by a rapid accumulation of phenolic compounds at the site of infection. In addition, application of elicitor confers enhanced pathogen resistance in many plants was reported [31; 32]. Vacciplant® and co-treatment would relay the distress signal caused by the presence of elicitors to the nucleus of the

attacked cell to induce defence responses specific to the pathogenic attack and this accumulation of phenolic compounds may therefore be an important signal in the acquisition of pathogen tolerance as mentioned in several studies [33; 34]. Other studies on the involvement of phenolic compounds in plant resistance to fungi have already been demonstrated [35; 36]. Similarly, their involvement in plant tolerance to certain diseases has been proven by recent studies [33 ; 37]. Moreover, phenolic compounds accumulate in tissues adjacent to necrotic areas, suggesting that these compounds may be defensive [38].

The analysis of the phenolic compounds identified (Figure 2) in plantain leaves reveals that eight constitutive compounds were identified in the non-elicited plant (gallic acid, protocatechic acid, ferulic acid, p-coumaric acid, chlorogenic acid, vanillin, 3-p-coumaroylquinic acid and 3-hydroxybenzoic acid). However, when inoculated, there is no induction of new compounds, only four compounds are identified (gallic acid, protocatechic acid, p-coumaric acid and vanillin). In addition to these constitutive phenolic compounds. Elicited and inoculated-plant induced six new compounds (tryptophan, vanilic acid, caffeic acid, rutin, salicylic acid and *trans*-resveratrol). This plurality of phenolic metabolite biosynthesis has already been reported in cotton [27; 39].

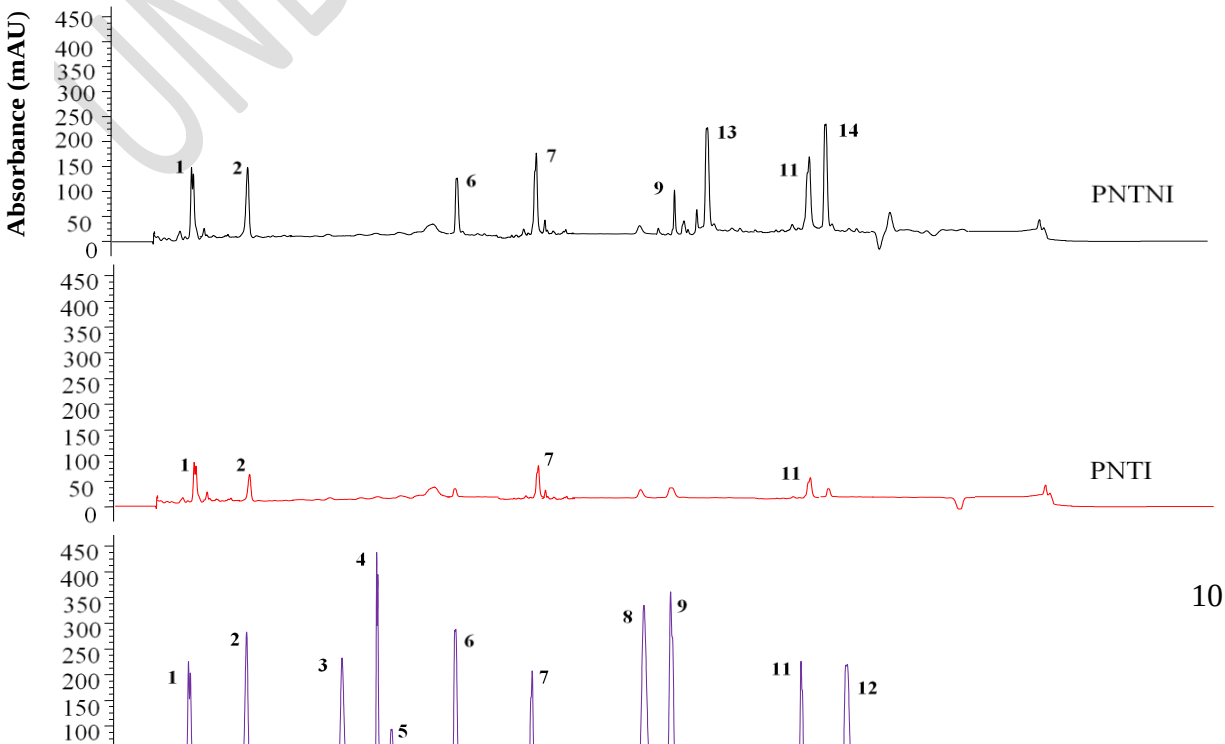

Figure 2. Chromatographic profiles of phenolic compounds extracted from the leaves of plantain cultivar French 2 at 284 nm

(1) gallic acid (1.486 min); (2) protocatechic acid (2.703 min); (3) tryptophan (3.143 min); (4) vanillic acid (5.067 min); (5) caffeine acid (5.203 min); (6) ferulic acid (6.554 min); (7) p-coumaric acid (7.905 min); (8) rutin (10.011 min); (9) chlorogenic acid (10.405 min); (10) trans-resveratrol (12.077 min); (11) vanillin (12.851 min); (12) salicylic acid (13.920 min); (13) 3-p-coumaroylquinic acid (11.082 min); and (14) 3-O-caffeoylquinic acid (13.378 min). PNTNI: untreated and non-injected plant; PNTI, untreated and inoculated plant, PTVI, Vacciplant-treated and inoculated plant; PT(V+Ct)I, Vacciplant-treated®+calliete® and inoculated plant.

Vacciplant® and vacciplant®+calliete® are elicitors that allow a good biosynthesis of phenolic compounds. Co-treatment induced trans-resveratrol, a stilbene with antifungal action reported in grapevine and cotton [40-41]. They are therefore key molecules in the induction and accumulation of phytoalexins. So, metabolism of phenolic compounds orientation in banana would be elicitor-dependent [9; 42]. In addition, phenolic compounds were reported to have beneficial actions on plant protection against pathogens [43; 44]. Thus, vacciplant® and vacciplant®+calliete® induce systemic acquired resistance (SAR) and potentiate phytoalexins (phenolic nature) accumulation [50]. Phytoalexins are plant antibiotics synthesised during the

hypersensitivity reaction or during HR [35; 36]. They accumulate and are thus thought to actively participate in plant defence [45; 46]. Their mode of action is linked to their antifungal power, their participation in the reinforcement of plant cell walls and their capacity to modulate and induce host defence reactions [25; 47]. In banana, Methyl jasmonate was showed to strongly stimulate the accumulation of phenolic phytoalexins, which can induce BLSD resistance [48]. Similarly, the involvement of phenolic compounds in the tolerance of plants to parasitic diseases has been reported [11; 37].

Table 1 shows the results on indicators of functional pigment equipment and leaf greenery. The Chla/Chlb ratio, which gives the indication of the functional pigment equipment of the leaves, remains lower than that of the control (PNTNI) and untreated and inoculated (PNTI). The same observation was observed in the Chlt/Car ratio, which gives an indication of the state of greenery of the leaves where the value of the PNTI remains below all values but also below 1.00 (reference value of the state of greenery). Thus, the controls (PNTNI) accumulate at the level of the indicator of the functional pigment equipment (Chla/Chlb) a value of 3.02 while that of the green indicator (Chlt/Car) is 3.7. When the untreated plants are inoculated (PNTI), their Chla/Chlb ratio is equal to 1.43, i.e. a decrease of 50.17%, while that of Chlt/Car is 0.63, i.e. a decrease of 77.42% compared with the control (PNTNI). When plants were treated with vacciplant® and vacciplant®+ calliete® followed by inoculation (PTVI and PT(V+Ct)I), the Chla/Chlb ratio was equal to that of the control for PTVI (3.02) and 3.31 for PT(V+Ct)I, an increase of 9.60% compared to the control. However, the green indicator (Chlt/Car) remained statistically identical to that of the control for PTVI, i.e. 3.72. On the other hand, in PT(V+Ct)I, this Chlt/Car ratio increased significantly to reach 5.56, i.e. a 50% increase.

Table 1. Indicators of functional pigment equipment and leaf green status in French 2 plantain elicited and inoculated with *Mycosphaerella fijensis*

Treatment	Functional pigment equipment indicator	Indicator of leaf greening status
PNTNI	3.02 ± 0.03^{b}	$3.70 \pm 0,04^{b}$
PNTI	0.97 ± 0.05^{c}	1.74 ± 0.09^{c}
PTVI	3.02 ± 0.01^{b}	3.72 ± 0.04^{b}
PT(V+Ct)I	3.31 ± 0.05^{a}	5.56 ± 0.01^{a}

Chl a, chlorophyll a; Chl b, chlorophyll b; Chl t, total chlorophyll; Car, carotenoids; PNTNI: Untreated and non-inoculated plant; PNTI, Untreated and inoculated plant, PTVI, Plant treated with vacciplant® and inoculated; PT(V+Ct)I, Plant treated with vacciplant®-calliete® combination and inoculated; within a column, means followed by the same letter are not significantly different (Newman-Keuls test at 5%).

The ratio of chlorophyll a to chlorophyll b was always greater than 1.00 except in unelicensed and infected plants (Chl a/Chl b = 0.97). Infection of the plants with the pathogen caused a 67.88% reduction in the functional pigment equipment of the leaves compared to the control. Analysis of this result suggests a degradation of chlorophyll a under the action of the pathogen in contrast to chlorophyll b. Indeed, the active site of chlorophyll biosynthetic enzymes such as magnesium chelatase and chlorophyllase would be located on chlorophyll a [49]. Thus, a bioconversion of chlorophyll a to chlorophyll b would be plausible as reported by several authors [50-53]. The elicitors used in this study (vacciplant® and the vacciplant®+calliete® co-treatment) would therefore have the role of protecting these enzymatic sites and also chlorophyll a with a view to ensuring important photosynthetic activity to maintain the plant in a good physiological state and thus a good health status

Moreover, Gamon *et al.* [54] reported that the photosynthetic activity of plants is significantly related to the ratio of chlorophyll pigments (total chlorophylls) and carotenoids (Chl t/Car). This ratio is an indicator of the greenness of the leaves [23]. For the elicited plants, the ratio is higher than 3.70 (PNTI), showing a positive effect of the treatment on the green state of the leaves. Moreover, the vacciplant®+calliete® co-treatment, which gives a value of 5.56, shows that this treatment is more favourable for the green state, which augurs a good photosynthesis, contrary to the vacciplant® treatment (3.72), which gives a similar result to the untreated and uninfected plant (3.70). In fact, in the non-elicited plants, the inoculation of the Mycosphaerella fijiensis filtrate caused a yellowing of the leaves (data not shown) and thus a very reduced green state (Chl t/Car=1.74). Thus, M. fijiensis filtrate appears to cause damage to the plant's photosynthetic apparatus, including chlorophyll a, the major photosynthetic pigment [55]. Consequently, the leaves appear to begin a state of yellowing that would lead to their senescence. The results obtained in these experiments show that chlorophyll a is the key pigment to evaluate the green state of leaves. It seems to provide a good appreciation of the photosynthetic activity of leaves and would better express the physiological state of the plants [56; 57]. Therefore, chl a/chl b and chl t/car could be regarded as indicators of plantain protection against black leaf streak disease (BLSD). Thus, vacciplant®+calliete® have a synergistic action on the protection of plantain against BLSD.

CONCLUSION

In the context of agriculture that is more respectful of the environment and human health, this study has been carried out in order to find new strategies for the protection of plants by exploiting their natural defense capacities. This research was conducted on French 2 plantain cultivar. To this end, vaccipant® and calliete® were applied as co-spray to the leaves and the plants were subsequently infected in vivo with virulent inoculum of *Mycosphaerella fijiensis*. The action of this co-treatment was compared to the action alone of vacciplant®, the results

of which were reported to be better for caliette®. Vaccciplant®-treated and inoculated plants and co-treated and inoculated plants synthesized more total phenolic compounds than control plants. However, phenolic compounds produced by co-treated plants (191.10 mg/g FL) were greater than those treated with vacciplant® alone (142 mg/g FL). Also, U-HPLC analysis revealed the *de novo* synthesis of four new compounds (phytoalexins) compared to the control (PNTNI) of eight compounds (constituent phenols or phytoanticipins). This study shows that the vacciplant®+ calliete® has a synergistic effect on the activation of the natural defenses in plantains against black line disease. Moreover, co-treatment has made it possible to induce *trans*-resveratrol, a stilbene with proven antifungal action. In addition, the level of synthesis of the constituent and induced phenolic compounds is greater than in the vaccine® alone. In addition, vacciplant®+ calliete® allowed the plants to acquire a higher leaf greenery than those treated with vacciplant® alone. These results show that vacciplant®+ calliete® co-treatment is synergistic and protects plantain against *M. fiji*ensis-induced black leaf streak disease.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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