American Journal of Agriculture and Forestry
2015; 3(3): 109-115
Published online May 12, 2015 (http://www.sciencepublishinggroup.com/j/ajaf)
doi: 10.11648/j.ajaf.20150303.18
ISSN: 2330-8583 (Print); ISSN: 2330-8591 (Online)

SciencePG
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Salicylic Acid, Phosphorous Acid and Fungicide Sumi 8 Effects on Polyphenol Oxidases Activities and Cassava Resistance to Anthracnose

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To cite this article:

Seu Jonathan Gogbeu, Koffi Mathurin Okoma, Koua Serge Beranger N'Goran, Dénézon Odette Dogbo. Salicylic Acid, Phosphorous Acid and Fungicide Sumi 8 Effects on Polyphenol Oxidases Activities and Cassava Resistance to Anthracnose. *American Journal of Agriculture and Forestry.* Vol. 3, No. 3, 2015, pp. 109-115. doi: 10.11648/j.ajaf.20150303.18

Abstract: In Côte d'Ivoire, cassava contributes enormously to improve food security of population by increasing national production and financial resources of vulnerable households. But, plant is attacked by several diseases including anthracnose. This study was done to improve its resistance to anthracnose by stimulating its natural defense following treatment plants with salicylic acid, phosphorous acid and fungicide Sumi 8 as elicitors. Polyphenol oxidases were chosen as resistance marker. Results showed that in the three cultivars (*yacé*, *TMS30572* and *I88/00158*), *yacé* was more susceptible to anthracnose ($p<0.05$; $F = 6.83$). After treatments, cassava resistance against anthracnose has been improved. Polyphenol oxidases activities were more stimulated in presence of elicitor's phosphorous acid and salicylic acid. Native-PAGE of polyphenol oxidases revealed 11 isoenzymes including 7 new isoenzymes detected in elicited plants, treated plants contaminated or uncontaminated by *Colletotrichum gloeosporioïdes*, pathogen of anthracnose. Recent isoenzymes were specific for each cultivar. Their appearance was correlated with plant resistance to *C. gloeosporioïdes*. In these plants, in particular those germinated directly in elicitation medium, anthracnose symptoms were lessened. These elicitors were thus induced and/or stimulated cassava defense especially polyphenol oxidases activities.

Keywords: Anthracnose, Cassava, Elicitors, Polyphenol Oxidases

1. Introduction

Polyphenol oxidases [PPO (EC. 1.14.18.1 and EC. 1.10.3.2)] are plant enzymes using molecular oxygen to oxidize phenolic compounds to quinones [1]. These reactions commonly contribute to tissue browning of fruits and vegetables, and consequently to the deterioration of consumer products quality. However, according to Mayer [2], Constabel and Barbehenn [3], role of PPO plants is associated with the defense against pathogenic microorganisms. In fact, role attributed to PPO is related to modification of endogenous phenolic compounds by these enzymes, in particular quinones that are toxic to pathogenic microorganisms [4] [5]. In tomato (*Solanum lycopersicum* L) plants infected by *Fusarium oxysporium*, PPO activities increased both in susceptible and resistant plants [6]. Other studies have indicated that induction of PPO activities could be caused by elicitors [7] [8]. Thus, in cassava processing plants by salicylic acid and phosphorous acid, similar responses were evoked [9] [10]. Therefore, Flurkey and Inlow [11] show that induction of PPO genes in response to plant hormones clearly suggests that these enzymes are released during plant resistance. Based on these results, PPO can be mentioned as marker for plant defenses. In order to establish a link between production of PPO under effect of

elicitor's salicylic acid, phosphorous acid or fungicide Sumi 8 and resistance of cassava to anthracnose, this study was conducted. Indeed, cassava in Côte d'Ivoire contributes enormously to improve food security of populations by increasing national production and financial resources of vulnerable households. With production estimated at over 2 million tonne, cassava is the second food after yams [12]. But despite these production efforts, cassava is prone to many diseases including anthracnose [13]. However, in developing countries such as Côte d'Ivoire, application of fungicides is only means of protection of plants against diseases. But, the misuse of these products leads to the induction of long-term pathogenic microorganisms resistance and environmental damage through the toxic residues accumulation [14] [15], which could act negatively on the health of consumers. Natural defense induction by elicitor application then appears as a means to fight against pathogenic microorganisms and especially environment preservation. Or in cassava, work on PPO activities induction and plant resistance to pathogens has not been discussed in the literature.

2. Materials and Methods

2.1. Plant and Experimental

Yacé, *TMS30572* and *I88/00158* plants aged of six week were used for experimentation. *Yacé* is a traditional cultivar commonly grown in Côte d'Ivoire. By cons, *TMS30572* cultivar was introduced by International Institute of Tropical Agriculture (IITA, Nigeria). As for *I88/00158* cultivar, it has been improved by National Centre of Agricultural Research (CNRA, Côte d'Ivoire) and now popularized as *Bocou2*.

Cassava plants were obtained from hydroponic according to Gogbeu *et al* [10] method. Cuttings sterilized with alcohol 70% (v/v) were placed in two germination medium: nutrient medium containing phosphorus (P_2O_3) and dolomite ($CaMg(CO_3)_2$) at dose of 80 mg L^{-1} each, namely M_0 medium, and M_0 medium supplemented with 1 mM of salicylic acid (SA, SIGMA), 1 mM of phosphorous acid (PA, SIGMA) or 0.5 mM of fungicide Sumi 8 (Syngeta Society) qualified M_{SA}, M_{PA} and M_S medium respectively. For each cultivar, plants were divided into two blocks according to contamination mode: block uncontaminated plants [plants from M_0 medium (3 plants) and plants from M_{SA}, M_{PA} and M_S medium (3 plants / medium) that have not been contaminated with *C. gloeosporioïdes*] and block contaminated plants [plants from M_0 medium (3 plants / contamination time), plants from M_{SA}, M_{PA} and M_S medium (3 plants / medium /contamination time) and plants from M_0 medium then transferred to M_{SA}, M_{PA} or M_S medium (3 plants /medium/contamination time) infected with *C. gloeosporioïdes*].

2.2. Estimation of Pathogen Propagation Speed

Pathogen was isolated from stems of cassava diseased plants. These stems were disinfected with alcohol 70% (v/v) and quickly flamed under a laminar flow hood. Samples of 0.5 cm collected around necrotic area were placed in Petri dishes containing PDA medium previously prepared. After 3 days of incubation at 28 °C in dark, fruiting bodies were collected using a sterile needle and transplanted into new PDA medium. After 5 to 6 subculture, pure cultures of fungi were obtained and stored at 4 °C in refrigerator. Plant contamination was performed according to Makambila [16] method. Stems of selected plants were pricked in part not yet lignified [2/3 upper stem] using a thin heated to red needle. On the 3rd day after injection, fungi (mycelial and conidia) were given in capsule form (1 mm^2) collected by scraping on culture medium. After various treatments, room humidity was maintained by daily watering. Distance traveled by fungi within stem was determined at 12th Day after plant contamination. To do this, contaminated stems were cracked in length and distance traveled by pathogen within stem was measured using a ruler [17].

2.3. Extraction and Assay of Polyphenol Oxidases Activities

Polyphenol oxidases were extracted using Gogbeu *et al* [18] method with some modification. Extraction buffer of PPO varied according to cultivar. There are 0.2 M sodium phosphate buffer pH 5 [PPO extracted from *I88/00158* (*i*PPO)] and pH 6 [PPO extracted from *TMS30572* (t_{30}PPO)], and 0.2 M Tris-HCl pH 7.5 [PPO extracted from *yacé* (*y*PPO)]. To do this, one g of limbs was ground in 10 mL of extraction buffer supplemented with Triton X-100 (100/1, v/v) and the whole was centrifuged at 15000 x g for 30 min at 4 °C. Supernatant was recovered and pellet was taken up in 5 mL of extraction buffer and then ground and centrifuged as before. Combined supernatants formed extracted PPO.

Enzyme activity was assayed in 3 mL of reaction mixture, consisting of 50 µL of enzymatic extract and 100 mM of dopamine [*y*PPO and *i*PPO] or pyrocatechol [t_{30}PPO]. After 5 min incubation at 30 °C (t_{30}PPO), 35 °C (*y*PPO) and 40 °C (*i*PPO), reaction mixtures were cooled in a controlled bath regulated at 4 °C. PPO activities were determined by measuring absorbance (spectrophotometer, Milton Roy) at 420 nm (*y*PPO, t_{30}PPO) or 470 nm (*i*PPO) against a control containing no substrate. PPO activities were expressed in absorbance per minute per milligram of protein (ΔDO/min/mg prot.). Maximum stimulation of PPO activities was expressed as difference between high enzyme activity and PPO activities extracted from plants from M_0 medium.

2.4. Native-PAGE of Polyphenol Oxidases

Polyacrylamide gel electrophoresis was performed according to Laemmli [19] for separating PPO isoenzymes of cassava leaves in nondenaturing conditions. It was performed with a discontinuous buffer system using 4% stacking gel and 10% resolving gel. Resolving gel was prepared by mixing 33.3 mL of acrylamide / bis-acrylamide (30% T, 2.6% C) to 40.2 mL of distilled water and 25 mL of 1.5 M Tris-HCl buffer pH 8.8. After 15 min, 500 µL of 10% (w/v) ammonium persulphate and 50 µL of TEMED were added. Whole was mixed and poured between two glass plates, separated on both sides by spacers which assembly is placed

on a support. Gel is covered with 1 mL of n-butanol. After 90 min of polymerization, n-butanol is rinsed thoroughly and 4% stacking gel is poured above resolving gel. This gel was prepared by mixing 1.3 mL of acrylamide / bis-acrylamide (30% T, 2.67% C) to 6.1 mL of distilled water, 2.5 mL of 0.5 M Tris-HCl buffer pH 6.8, 50 µL of 10% (w/v) ammonium persulphate and 10 µL of TEMED. Enzyme samples (32 µL enzyme extract and 8 µL of 0.1% bromophenol blue) were applied to well spaces in stacking gel. Migration was performed at 18 °C. It was carried out first at constant current of 9.8 mA and then increased to 12 mA and it was stopped when bromophenol blue reached the bottom of resolving gel. Revelation of PPO was to put essentially highlight functional proteins by Wu and Duan [20] method. Thus, after migration, gel removed from plates was immersed for 120 min into solution containing 0.2 M sodium phosphate buffer pH 5 and 100 mM dopamine for *i*PPO; 0.2 M sodium phosphate buffer pH 6 and 100 mM pyrocatechol for t_{30}PPO or 0.2 M Tris-HCl pH 7.5 and 100 M dopamine for *y*PPO. Isoenzyme bands were photographed. Calculated frontal reports were classified isoenzymes.

2.5. Amount of Protein

Amount of protein was determined using dye-binding method of Bradford [21], with bovine albumin as the standard, measuring optical density at 595 nm.

2.6. Statistical Analysis of Data

SPSS version 11.5 software was used to compare data. Analysis of variance (ANOVA) with one and two classification criteria was made. Difference between means at 95% confidence level calculated using Duncan test.

3. Results and Discussion

3.1. Colletotrichum Gloeosporioides Propagation Speed Within Cassava Stems

Analysis of table 1 shows that average length of fungi propagation in control plants (PC) varied among cultivars ($p < 0.05$; $F = 7.13$). It was 3.63; 3.03 and 1.40 cm respectively for *yacé*, *I88/00158* and *TMS30572*. When these plants were elicited and contaminated (PE), pathogen growth within stem was delayed in *yacé*. By cons in *TMS30572* and *I88/00158*, speed was slightly important [PA (1.17 cm) and Sumi 8 (1.50 cm) for *TMS30572*; SA (3 cm) and PA (2.53 cm) for *I88/00158*]. In contrast, plants (PT) obtained from cuttings directly into elicitation medium prevented pathogen growth. Indeed, apart from *I88/00158* where *C. gloeosporioides* progression speed was important for plants from elicitation PA ($F = 14.29$, $P < 0.001$) medium, first symptoms observed at 5th Day could not develop (Table 1). These results suggest that all cultivars are susceptible to anthracnose. In terms of progression speed of pathogen within stem, *yacé* would be more susceptible than *I88/00158* and *TMS30572*. But after elicitation of *yacé* plants, pathogen growth was slower. So, we can say SA, PA and Sumi 8 helped improve cassava defense system against anthracnose. These phenomena were more pronounced with results recorded in PT. Indeed, in the latter, pathogen propagation speed was significantly reduced; this would correspond to fungi arrest mechanism in migration process. Latter was confined at inoculation site. This result could be explained by the fact that these plants had already set up their defense system after different treatments. Similar results were obtained by several authors [22] [23]. They advocated pretreatment method with elicitors in plants as a means to fight against plant diseases. In bean (*Vigna mungo*), pretreatment of plant with SA 24 hours before virus (urdbean leaf crinke virus) inoculation, has helped reduce disease symptoms while contaminated plants at same time to SA treatment developed significant symptoms [23]. Our results indicate that in cassava, a long period of pretreatment may be recommended to reduce anthracnose impact.

3.2. Native-PAGE of Polyphenol Oxidases

In order to identify all functional PPO isoenzymes in cassava leaves, native-PAGE was performed. This showed existence of 4 isoenzymes constituent rated PPO_1, PPO_2, PPO_3 and PPO_4 in studied cultivars. When plants were stressed (elicitation and / or inoculation with pathogen), 7 new forms were induced: PPO_5, PPO_6, PPO_7, PPO_8, PPO_9, PPO_{10} and PPO_{11}. Indeed, in *yacé*, 4 distinct bands representing different forms of PPO were revealed (Figure 1). Among these isoenzymes, PPO_1 and PPO_3 were constantly present whatever treatment undergone by plants. In contrast, in PC contaminated, at 12th Day (line 3) and plants elicited with salicylic acid (SA) (lines 4 and 5) and phosphorous acid (PA) (lines 6 and 7), PPO_7 has been detected. In *yacé* plant treated, PPO_7 was found in all plants. After contamination, PPO_6 was demonstrated in all plants except those having germinated in fungicide Sumi 8 (lines 16 to 18). In *TMS30572*, 5 isoenzymes rated PPO_1, PPO_3, PPO_5, PPO_9 and PPO_{11} were found (Figure 2). Isoenzymes PPO_1 and PPO_3 are present in all plants. By against, PPO_9 and PPO_{11} were identified only in plants treated with SA (lines 10 to 12) and PA (lines 13 to 15) (Figure 2). PPO_5 form is specifically appeared at 12th Day in contaminated PC (line 3), plants elicited with SA and contaminated (lines 4 and 5), plants treated with SA and contaminated (lines 11 and 12) and plants treated with PA and contaminated at 5th Day (Figure 2). Four isoenzymes namely PPO_2, PPO_4, PPO_8 and PPO_{10} were observed in *I88/00158* (Figure 3). PPO_2 and PPO_4 are present in all plants regardless of treatment received. Isoenzymes PPO_8 and PPO_{10} are found in most elicited and treated plants. However, some differences were observed. PPO_8 was absent in plants elicited with Sumi 8 and contaminated (lines 8 and 9) as well as in plants treated with Sumi 8 and contaminated at 12th Day (line 18). This form was also absent in PC (line1) and contaminated witnesses during 5 days (line 2). As for isoenzyme PPO_{10}, it was detected in all treated plants except plants treated with Sumi 8 and contaminated (lines 17 and 18) and those treated with PA and contaminated at 5th Day (line 14). In elicited plants, PPO_{10} was identified by the presence of AP (lines 6 and 7) and SA after 5 days of infection (line 4)

(Figure 3). Examples of PPO isoenzymes induction were mentioned by Niranjan-Raj *et al*. [24], Karthiekeyan *et al*. [23] and, Wu and Duan [20] in bean (*V. mungo*) and millet (*Glycine max*). These authors noted the timeliness and amplification of PPO activities in resistant varieties compared to susceptible varieties. Thus, in millet, five isoenzymes were identified in resistant varieties whereas in susceptible varieties, they are four [24]. Fifth PPO has been induced during *Sclerospora graminicola* inoculation. In *V. mungo*, new forms were induced after treatment plants with SA and benzothiadiazole [23].

3.3. Polyphenol Oxidases Activities

Polyphenol oxidases (PPO) activities measured in cassava plant leaves subjected to different treatments was expressed in percentage, taking as 100%, enzyme activity obtained with plants from uncontaminated medium ($M_{0;}$ 0 Day) (Table 1). In response to *C. gloeosporioides*, plants from M_0, M_{AS}, M_{AP} and M_S medium have reacted differently. Indeed, in PC (M_0 uncontaminated), PPO activities remained constant for all cultivars during experiment. When these PC were contaminated, PPO activities increased rapidly at 5th Day before declining towards the end of experiment. Percentage of PPO stimulation was higher among *TMS30572* (39%), average for *yacé* (21%) and lower in *I88/00158* (15%) (Table 1). When plants were elicited and contaminated by fungi (M_{0SA}, M_{0PA} and M_{0S}) in the presence of PA, maximum stimulation of enzyme was 60 and 68% respectively in y*acé* and *TMS30572*. At *I88/00158*, it was 39%. In the presence of SA, value has hovered around 50% for *yacé* (52%) and *TMS30572* (49%). For *yacé* and *TMS30572*, PPO activities increased rapidly before falling at 12th Day for SA and PA (Table 1). In uncontaminated treated plants group (M_{SA}, M_{PA} and M_S uncontaminated), PPO activities were well above that of non-infected PC (M_0 uncontaminated) exception of plants resulting from Sumi 8 medium (Ms) (Table 1). In this environment, Sumi 8 negatively influenced enzyme activity evolution in *yacé*. When treated plants were infected (M_{SA}, M_{PA} and M_S contaminated), PPO activities varied among cultivars and elicitors (Table 1). Maximum stimulation of PPO exceeded 50% in all cultivars treated with SA. Greatest stimulation of enzyme was among *I88/00158*. It was 112%. In the presence of PA, significant stimulation was achieved in *TMS30572* (83%), followed *yacé* (64%). At *I88/00158*, value was 10%. For treated plants with Sumi 8, maximum stimulation of PPO did not exceed 15% (Table 1). These results show that increase of PPO isoenzymes was responsible for amplitude of their activity. In many plant tissues, increasing PPO genes in response to hormones related to defense such as salicylic acid and jasmonic acid is implicitly linked to the resistance of these pathogens [2] [11]. In cassava, induced isoenzymes identified were specific to cultivars. In contrast, intensity of reaction was more enhanced with treatment and / or contamination with *C. gloeosporioides*. Signal produced by pathogen or elicitor SA, PA or Sumi 8 was essential to trigger the synthesis and accumulation of defense gene products in treatment plants [25] [26] [27]. Beneficial role of Sumi 8 in improving cassava defense can therefore lead to the synthesis and accumulation of phenolic compounds although PPO activities were low in the presence of the latter [10]. According to these authors, metabolic pathways leading to phenol synthesis would be induced in cassava as suggested by Rodriguez *et al*.[28] work. On cassava tubers, they indicated accumulation of three hydroxycoumarins which scopoletin. It had an antimicrobial function.

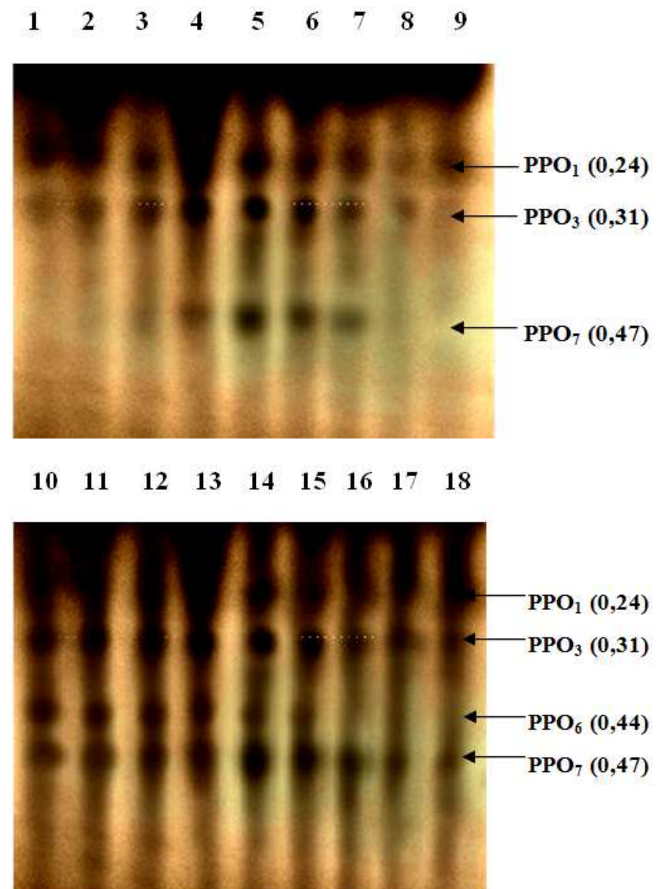

Figure 1*. Native-PAGE of polyphenol oxidases extracted of cassava yacé leaves treated with salicylic acid (SA), phosphorous acid (PA) and fungicide Sumi 8 and contaminated by Colletotrichum gloeosporioides (C.gl.).*

A : 1- control plant; 2- contaminated plant by C.gl at 5th Day; 3-contamined plant by C.gl at 12th Day; 4-elicited plant with SA and contaminated by C.gl, at 5th Day; 5- elicited plant with SA and contaminated by C.gl, at 12th Day; 6- elicited plant with PA and contaminated by C.gl, at 5th Day; 7- elicited plant with PA and contaminated by C.gl, at 12th Day; 8- elicited plant with Sumi 8 and contaminated by C.gl, at 5th Day; 9- elicited plant with Sumi 8 and contaminated by C.gl, at 12th Day.

B : 10- treated plant with SA; 11- treated plant with SA and contaminated by C.gl at 5th Day; 12-treated plant with SA and contaminated by C.gl at 12th Day; 13-treated plant with PA; 14-treated plant with PA and contaminated by C.gl, at 5th Day; 15-treated plant with PA and contaminated by C.gl, at 12th Day; 16-treated plant with Sumi 8; 17-treated plant with Sumi 8 and contaminated by C.gl, at 5th Day; 18-treated plant with Sumi 8 and contaminated by C.gl at 12th Day.

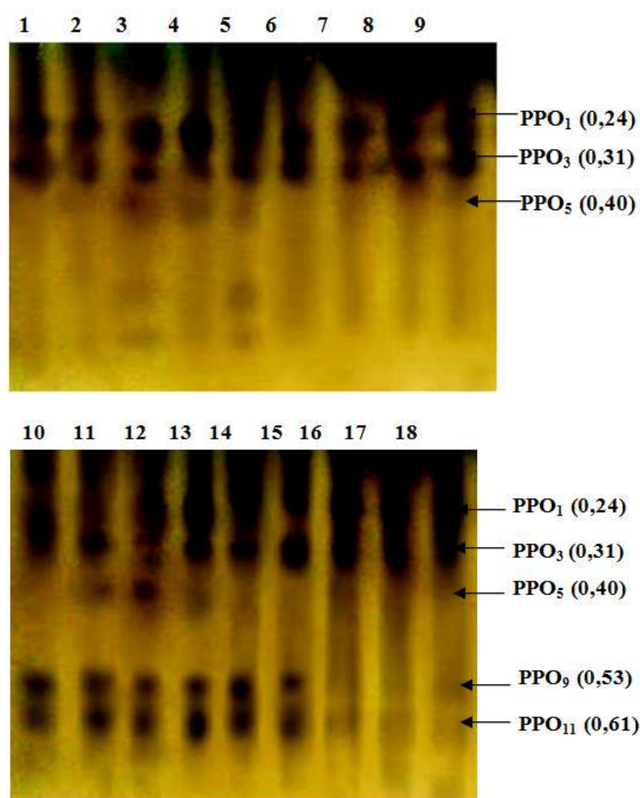

***Figure 2**. Native-PAGE of polyphenol oxidases extracted of cassava TMS30572 leaves treated with salicylic acid (SA), phosphorous acid (PA) and fungicide Sumi 8 and contaminated by Colletotrichum gloeosporioides (C.gl.).*

A : 1- control plant; 2- contaminated plant by C.gl at 5th Day; 3-contamined plant by C.gl at 12th Day; 4-elicited plant with SA and contaminated by C.gl, at 5th Day; 5- elicited plant with SA and contaminated by C.gl, at 12th Day; 6-elicited plant with PA and contaminated by C.gl, at 5th Day; 7- elicited plant with PA and contaminated by C.gl, at 12th Day; 8- elicited plant with Sumi 8 and contaminated by C.gl, at 5th Day; 9- elicited plant with Sumi 8 and contaminated by C.gl, at 12th Day.
B : 10- treated plant with SA; 11- treated plant with SA and contaminated by C.gl at 5th Day; 12-treated plant with SA and contaminated by C.gl at 12th Day; 13-treated plant with PA; 14-treated plant with PA and contaminated by C.gl, at 5th Day; 15-treated plant with PA and contaminated by C.gl, at 12th Day; 16-treated plant with Sumi 8; 17-treated plant with Sumi 8 and contaminated by C.gl, at 5th Day; 18-treated plant with Sumi 8 and contaminated by C.gl at 12th Day.

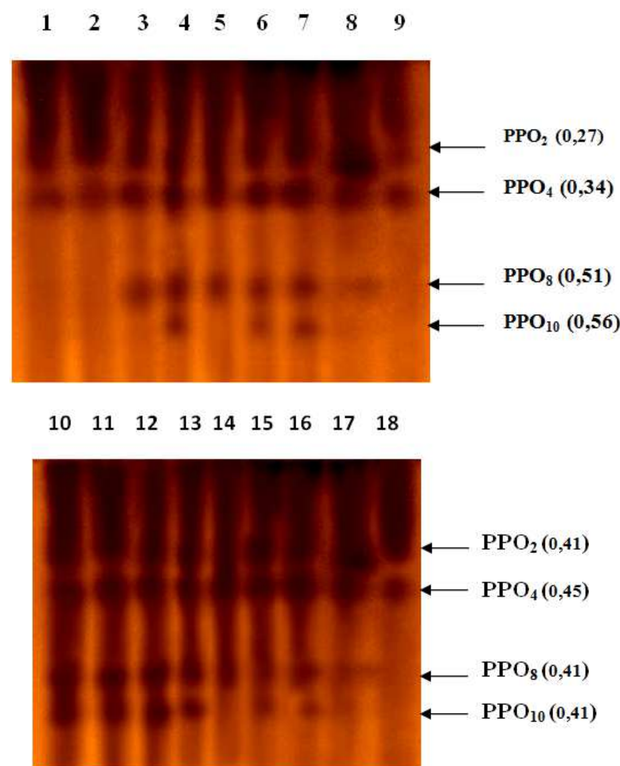

***Figure 3**. Native-PAGE of polyphenol oxidases extracted of cassava I88/00158 leaves treated with salicylic acid (SA), phosphorous acid (PA) and fungicide Sumi 8 and contaminated by Colletotrichum gloeosporioides (C.gl.).*

A : 1- control plant; 2- contaminated plant by C.gl at 5th Day; 3-contamined plant by C.gl at 12th Day; 4-elicited plant with SA and contaminated by C.gl, at 5th Day; 5- elicited plant with SA and contaminated by C.gl, at 12th Day; 6-elicited plant with PA and contaminated by C.gl, at 5th Day; 7- elicited plant with PA and contaminated by C.gl, at 12th Day; 8- elicited plant with Sumi 8 and contaminated by C.gl, at 5th Day; 9- elicited plant with Sumi 8 and contaminated by C.gl, at 12th Day.
B : 10- treated plant with SA; 11- treated plant with SA and contaminated by C.gl at 5th Day; 12-treated plant with SA and contaminated by C.gl at 12th Day; 13-treated plant with PA; 14-treated plant with PA and contaminated by C.gl, at 5th Day; 15-treated plant with PA and contaminated by C.gl, at 12th Day; 16-treated plant with Sumi 8; 17-treated plant with Sumi 8 and contaminated by C.gl, at 5th Day; 18-treated plant with Sumi 8 and contaminated by C.gl at 12th Day.

***Table 1**. Effects of salicylic acid, phosphorous acid and fungicide Sumi 8 on Colletotrichum gloeosporioïdes (cm) propagation inside cassava stem at 12th Day after plant contamination.*

Cassava cultivars	Elicitors						
		Salicylic acid		Phosphorous acid		Sumi 8	
	PC	PE	PT	PE	PT	PE	PT
yacé	3,63a1	1,17a2	0,10a3	1,40a2	0,10a3	1,90a2	0,10a3
	±0,85	±0,35	±0	±0,75	±0	±0,87	±0
bonoua2	3,30a1	1,03a2	0,13a3	1,10a2	0,10a3	1,70a2	0,10a3
	±0,75	±0,11	±0,05	±0,30	±0	±0,78	±0
TMS30572	1,40b1	0,77a2	0,17a3	1,17a12	0,10a3	1,50a1	0,10a3
	±0,62	±0,15	±0,05	±0,05	±0	±0,10	±0
I88/00158	3,03a1	3,00b1	0,20a2	2,53b1	0,87b3	0,93a3	0,10a2
	±0,20	±0,45	±0,10	±0,51	±0,80	±0,15	±0

PC: control plant; PE: elicited plant; PT: treated plant; each value is the average of 3 replicates ± standard deviation.
For each column, means followed a single alphabetical letter are not statistically different for a threshold of 5% according to the test Dancun.
For each line, means followed by the same figure not statistically different for a threshold of 5% according to the test Dancun.

Table 2*. Activities of polyphenol oxidases (%.) extracted of cassava leaves after treatment with salicylic acid (SA), phosphorous acid (PA) or fungicide Sumi 8 and/or inoculated by Colletotrichum gloeosporioides at 0, 5th and 12th Days after plant contamination.*

Cassava cultivars		**Culture medium**										
		Uncontaminated				**Contaminated**						
		PC	**PT**			**PC**	**PE**			**PT**		
		M0	**MSA**	**MPA**	**MS**	**M0**	**M0SA**	**M0PA**	**M0S**	**MSA**	**MPA**	**MS**
	0j	100	108	121	85	100	100	100	100	108	121	85
yacé	5j	100	108	121	85	121	152	161	84	161	164	75
	12j	100	108	121	85	113	129	150	105	139	134	91
	0j	100	115	104	101	100	100	100	100	115	104	101
I88/	5j	100	115	104	101	115	138	128	132	212	107	107
	12j	100	115	104	101	107	113	139	127	141	110	105
	0j	100	106	115	101	100	100	100	100	106	115	101
TMS30	5j	100	106	115	101	139	149	168	109	174	183	115
	12j	100	106	115	101	130	140	153	105	116	164	109

PC: control plant; PE: elicited plant; PT: treated plant; SA: salicylic acid, PA: phosphorous acid, *TMS30*: TMS30572, *I88/*: I88/00158. M_0: nutrient medium; M_{SA}, M_{PA} and M_S: nutrient medium respectively containing SA, PA and Sumi 8; M_{0SA}, M_{0PA} and M_{0S}: germination in the nutrient medium and then transfer in the nutrient medium supplemented with SA, PA or Sumi 8. 100% = *1247,61 (yacé); 1130,83(TMS30572) and 930,83 (I88/00158) ΔDO min^{-1}mg^{-1}prot.*

4. Conclusion

This work aims to investigate alternatives to use of pesticides as the only means of struggle against cassava enemies. Results of this study have demonstrated that several PPO isoenzymes were induced after different treatments with elicitors SA, PA and Sumi 8. SA and PA involved more amplification of PPO activities. By cons, fungicide Sumi 8 inhibits the activity of the enzyme. But it also improves the strength of cassava anthracnose.

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