



Original Research Article

Improvement of Cassava resistance to *Colletotrichum gloeosporioides* by Salicylic acid, Phosphorous acid and Fungicide Sumi 8

GOGBEU Seu Jonathan^{1*}, SEKOU Diabaté², KOUAKOU Kouassi Joseph³,
DOGBO Dénezon Odette³ and BEKRO Yves-Alain⁴

¹Université Jean Lorougnon Guédé, Laboratoire de Physiologie et Pathologie Végétales, BP 150 Daloa, Côte d'Ivoire

²Centre National de Recherche Agronomique, Laboratoire Central de Biotechnologies, 01 BP 1740 Abidjan 17

³Université Nangui Abrogoua, UFR des Sciences de la Nature, Pôle de Recherche Production Végétale, Unité de Recherche Agrophysiologie, 02 BP 801 Abidjan 02, Côte d'Ivoire

⁴Université Nangui Abrogoua, UFR des Sciences Fondamentales et Appliquées, 02 BP 801 Abidjan 02, Côte d'Ivoire

*Corresponding author

ABSTRACT

Keywords

Cassava,
Colletotrichum gloeosporioides,
phenolic
compounds,
natural defense,
Salicylic acid,
Phosphorous
acid, Sumi 8

Cassava is a major staple food in Côte d'Ivoire. It's attacked by several diseases of viral, bacterial, fungal and insect pests. This work was carried out to improve the plant's natural defense against pathogens by its stimulation of defense mechanisms. Four cassava cultivars were selected: *yacé*, *bonoua2*, *TMS30572* and *188/00158*. Among these, interest has been focused on phenolic compounds. Like this, salicylic acid (SA) and phosphorous acid (PA) as well as the fungicide Sumi 8 were used for elicitation of cassava plants. Results indicated *yacé* and *bonoua2* are more susceptible to *Colletotrichum gloeosporioides*. After the various treatments, all cultivars have amplified their defense mechanism. Sumi 8 seem to delay the spread of the pathogen speed. By against, accumulation of phenolic compounds was more stimulated by SA and PA. Phenolic compounds accumulation has been correlated with the resistance of plants to *C. gloeosporioides*. In these plants, especially those directly germinated in elicitation medium, symptoms of anthracnose and stem rot were reduced. This mode of induction of plant resistance was more beneficial for environment. Salicylic acid, phosphorous acid and Sumi 8 could be used as elicitors of stimulating natural defense in cassava.

Introduction

Cassava is a plant grown for its tuberous roots and leaves. It's used in the diet of more than 200 million people (FAO, 2008). Plant has quickly integrated into the traditional

African agriculture in tropical countries and it's now one of the staple crops. In Côte d'Ivoire, cassava is grown throughout the territory. With an annual production

estimated at over 2 million tonne, it's the second food crop after yam (FAO, 2010). Given its nutritional importance and its many derivatives, cassava has been subject of several research programs to improve local varieties or introduced from the International Institute of Tropical Agriculture (IITA, Nigeria) by the National Centre of Agricultural Research (CNRA, Côte d'Ivoire). However, among the cultivars popularized, many are prone to diseases caused by pathogenic microorganisms. Application of fungicides currently is the only means of protection of plants against diseases. This method has reduced the attack of plants by pathogens and increase crop yields. However, systematic misuse of these pesticides cause many long-term problems such as the induction of resistance in pathogenic fungi, environmental pollution (Buhot, 2003; Thakore, 2006), which could negatively influence health of consumers.

In this context, development of new strategies reliable and respectful of the environment becomes an issue in the fight against many diseases of plants grown economic interest. Among them, induction of plant resistance by means of natural defense stimulators plant needs to be taken into account. Indeed, it's an approach which, by its biological foundations, should be explored in the context of an efficient and environmentally friendly agriculture. Many studies have shown that the elicitor application on a plant, its active preventive defense reactions and leads to increased resistance of the latter to pathogens (Koussevitzky *et al.*, 2004; Ogawa *et al.*, 2005). Thus, these studies have shown that plant reacts when it's attacked. Defense responses can be implemented lignification of tissues attacked by the aggressor (Lange *et al.*, 1995), production of phytoalexins (Bacher *et al.*, 2001) and secondary

metabolites, especially phenolic compounds (Zawistowski *et al.*, 1991; Chériot, 2007). Plant phenolics may be devised in two classes: preformed phenolics that are synthesized during the normal development of plant tissues and induced phenolics that are synthesized by plants in response to physical injury, infection or when stressed by suitable elicitors such as heavy metal-salts, UV-irradiation, temperature, microorganism, (Lattanzio *et al.*, 2006; Dogbo *et al.*, 2012). Other studies have shown that similar reactions could be induced in the plant when treated with compounds of biotic or abiotic origin (Benhamou and Belanger, 1998; Nürnberger, 1999) commonly known elicitors. Thus, the treatment of various plants by phosphate salts, phosphorous acid or salicylic acid induced in them a protection against pathogens (Ogawa *et al.*, 2005; Ojha and Chatterjee, 2012). Treatment of leaves and roots cassava by salicylic acid induced accumulation of phenolic compounds in leaves (Dogbo *et al.*, 2008; Gogbeu *et al.*, 2012).

Different studies showed that, after attack by pathogens, there are wide often increases in phenolic synthesis in plants (Lattanzio *et al.*, 2006). Based on these results, we can consider that phenolic compounds can be a parameter for measuring plant defense. Indeed, the work done on this subject revealed that they reinforce pectocellulosiques walls and limit the invasion of the pathogen (Franke *et al.*, 2002).

According Zawistowski *et al.* (1991), some oxidized phenolic compounds become toxic to pathogenic microorganisms while others have antimicrobial activity. Apart from the recent work on cassava (Dogbo *et al.*, 2007; 2008; 2012; Gogbeu *et al.*, 2012), information on the role of phenolic

compounds in the natural defense of this plant has not been mentioned. This work was carried out to improve cassava's natural defense against *C.gloeosporoides* by its stimulation of defense mechanisms.

Materials and Methods

Plants (6 weeks of age) from four cassava cultivars were used for experimentation. These are *yacé*, *bonoua2*, *I88/00158* and *TMS30572*. *Yacé* and *bonoua2* are commonly grown in Côte d'Ivoire. *I88/00158* has been improved of CNRA and popularized as *bocou2*. *TMS30572* is a cultivar introduced from the IITA. The cuttings that were used to obtain plants were provided by the collection of the CNRA.

Cassava plants were obtained from hydroponic (Gogbeu *et al.*, 2012). Cuttings sterilized with alcohol 70% (v/v) were placed in two germination medium: nutrient medium containing phosphorous (P_2O_3) and dolomite ($CaMg(CO_3)_2$) at a 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 the mode of contamination: 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. gloeosporioides*] 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 the M_0 medium then transferred to the M_{SA} , M_{PA} or M_S medium (3 plants /

medium / contamination time) infected with *C. gloeosporioides*].

Pathogen was isolated from the 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 the dark, the 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.

Contamination of the plants was performed according to the method of Makambila (1983). The stems of the selected plants were pricked in the part not yet lignified [2/3 upper stem] using a thin heated to red needle. On the 3rd day after injection, the fungi (mycelial and conidia) were given in capsule form (1 mm²) collected by scraping on culture medium. After the various treatments, the humidity of the room was maintained by daily watering.

For measuring the speed propagation of *C. gloeosporioides* on cassava plants, two methods were used. The first was to evaluate the necrotic area (NA) appeared on the stem after the inoculation of the pathogen. The second method relates to estimating the distance (d) traveled by the fungi within the stem from the site of inoculation. The necrotic area was measured at 5th and 12th days from the date of inoculation of the pathogen. To determine the necrotic area, leaves 4 cm² of tracing paper (2 cm x 2 cm) of known weight were placed on the stem, and the limit of necrotic area was determined. The corresponding necrotic area was removed from 4 cm² and the remainder was weighed again. Weight difference is

proportional to the necrotic area according to the following formula:

$$NA = S - \left[\frac{S \cdot M'}{M} \right]$$

S : initial surface (4 cm²)

NA: necrotic area

M: initial weight of 4 cm² leaf

M': weight without necrotic leaf area

Distance traveled by the fungi within the stem was determined at 12th day after infection of the fungi (Day). To do this, the contaminated stems were cracked in length and the distance traveled by the fungi within the stem was measured using a ruler (Figure 1).

Samples (3rd leaf from the apex) used in the extraction of phenolic compounds were harvested at 5th and 12th Days. The extraction was performed according to the method of Dogbo *et al.* (2008) adapted to our biological material. 0.5 g of material was crushed in 5 ml of ethanol 80% (v/v) containing 15 mM ascorbic acid. The homogenate was centrifuged at 15000 g for 30 min at 4 °C. Supernatant was recovered and the pellet was taken up in 1 ml of extraction solvent and then ground, and centrifuged as before. Supernatants were adjusted to 10 ml with the extraction solvent to form the ethanolic extract. Phenols contained in the ethanolic extract were measured according to the method of Swain and Hillis (1959). 1 ml of the ethanolic extract was added to 0.5 ml of 0.5 N Folin-Ciocalteu reagents. After 30 min of incubation at 30 °C, this mixture was homogenized, and then 1.5 ml of sodium carbonate 17% (w/v) was added. Control tube wherein the ethanolic extract was

replaced by the extraction solvent is considered as 0 mg.L⁻¹ of the range. The reaction mixtures were stirred, and then incubated for 45 min at 28 °C. The absorbance was measured in a spectrophotometer at 725 nm. Amount of phenolic compounds contained in the ethanolic extract was determined using a calibration line produced with different concentrations of gallic acid to 100 mg.l⁻¹. Phenol content was expressed in milligram gallic acid equivalent per gram of fresh weight (mg AG/FW). A subsample of leaves was used fresh for the analysis, performing triplicate assays for each extraction.

SPSS version 11.5 software was used to compare the data. The analysis of variance (ANOVA) with one or two classification criteria was made at 5% level. When $p \leq 0.05$, difference is said to be significant. Homogeneous group's individuals are then determined by Duncan method.

Result and Discussion

Plants used to evaluate the necrotic area and migration rate of *C. gloeosporioides* were subjected to three different treatments: plants from the nutrient medium appointed control plants (PC); plants from the nutrient medium and transferred in the elicitation medium called elicited plants (PE) and plants from the medium elicitation appointed treated plants (PT). For PC, destruction of the cells by the heated needle caused necrosis in all cultivars. In *bonoua2* and *yacé*, necrosis was slightly higher compared to that of *TMS30572* and *I88/00158* (Table 1). However, statistical test performed showed no significant difference between the means of the necrotic area ($F = 0.64$; $P > 0.05$). When these plants were infected by the fungi, the first symptoms were observed at 5th Day. Necrotic area has changed rapidly in *bonoua2*, average in *yacé* and *I88/00158* and

slowly in *TMS30572* ($F = 4.18$, $P < 0.05$). According to Duncan test, difference of values from necrotic area observed is related to the fact that the average obtained in *yacé* and *bonoua2* be high compared to those of *TMS30572* and *I88/00158*. At 12th Day, the necrotic area increased from 6.38 mm² (5th Day) to 6.58 mm² (12th Day) for *bonoua2* and 4.67 mm² to 5 mm² for *yacé* during the same period. In *TMS30572* and *I88/00158*, this value did not exceed 4 mm² (Table 1). Statistical analysis performed on the values recorded at 12th Day also indicated a significant difference between cultivars ($F = 4.33$, $P < 0.05$). With Duncan, average value of the necrotic area in *TMS30572* would be small compared to those of other cultivars. In PE contaminated with fungi, various treatments have not led to a significant increase of the pathogen except *I88/00158* treated with Sumi 8. It was observed that necrotic area initially measured at 5th Day was reduced by the formation of new layer around the circumference for the plants treated by PA and Sumi 8 (Table 1). In the lot of PT, necrosis due to injury has not changed during experiment. When they have been contaminated, necrotic area measured at 5th Day was virtually unchanged after this period. Comparison between the different treatments (SA, PA and Sumi 8) in the same cultivar did not make a difference between elicitors. The average length of migration of the fungi within the stem contaminated PC showed a difference [$p < 0.05$ ($F = 6.83$)]. It was 3.63, 3.30 and 3.03 cm respectively for *yacé*, *bonoua2* and *I88/00158*. In *TMS30572*, this value was 1.4 cm (Table 2). In contaminated PE, growth of the pathogen within the stem was delayed in all cultivars except *TMS30572* [PA (1.17 cm) and Sumi 8 (1.50 cm)] and *I88/00158* [SA (3 cm) and PA (2.53 cm)] (Table 2). In general, contaminated PT prevented the progression of *C. gloeosporioides* outside *I88/00158* where the pathogen migration speed was

important for plants from the elicitation medium PA ($F = 14$, 29; $P < 0.001$) (Table 2). These results indicate that in response to injury and/or fungi, PC, PE and PT reacted differently. Necrotic surface due to the injury has not changed during the experiment for all cultivars regardless of the treatment. For against, in the presence of *C. gloeosporioides*, symptoms of disease were observed in PC of all cultivars. Similarly, the distance traveled by the pathogen within the stem, has to see that these plants were infected. These observations suggest that all cultivars are susceptible to anthracnose. In terms of the necrotic area and the migration rate of the pathogen within the stem, we can say that *yacé* and *bonoua2* are more susceptible compared to *I88/00158* and *TMS30572*. After the elicitation plants (PE), in *yacé* and *bonoua2*, pathogen growth was slower. Around the initial necrotic surface, the formation of new cell layers commonly called papillae was observed. So, we can say SA, PA and Sumi 8 helped improve the defense of cassava anthracnose. These phenomena were more pronounced with the results recorded in PT. In the latter, the speed of progression of necrosis on the stem and of the pathogen within the stem has been significantly reduced; this would correspond of the locking process to fungi. These physical responses may include the elaboration of cell wall thickenings and appositions, such as papillae, as well as the occlusion of plant vessels (Chérif *et al.*, 2007). However, the formation of papillae at the site inoculation of the pathogen was reported by Thordal-Christensen (2003) as a means of resistance of *Arabidopsis sp.* Indeed, in the site inoculation of the pathogen, the activation of enzymes, particularly peroxidases, strengthened lignification of cell walls. This helped to limit the spread of the fungi (Montes *et al.*, 2004).

Figure.1 Method for measuring the speed of propagation of the pathogen within the stem



A



B

Figure 1

A: Plant newly infected; **B:** symptom 12th days after infection by the pathogen
p: site of inoculation of the pathogen; *d*: Distance traveled by the pathogen

Table.1 Evolution of the necrotic area (mm²) on the stems of cassava plants inoculated with *Colletotrichum gloeosporioides*, 5th and 12th days after infection

Mode of contamination	Plants	Elicitors	Cassava cultivars							
			<i>yacé</i>		<i>Bonoua2</i>		<i>TMS30572</i>		<i>I88/00158</i>	
			5 th	12 th	5 th	12 th	5 th	12 th	5 th	12 th
Uncontaminated	PC	-	2,58 ± 0,32	2,58 ± 0,30	2,62 ± 0,44	2,64 ± 0,44	2,39 ± 0,07	2,41 ± 0,08	2,37 ± 0,07	2,38 ± 0,07
	PT	SA	2,30 ± 0,06	2,31 ± 0,05	2,37 ± 0,54	2,38 ± 0,53	2,45 ± 0,49	2,45 ± 0,49	2,37 ± 0,18	2,37 ± 0,17
	PT	PA	2,52 ± 0,43	2,52 ± 0,43	2,42 ± 0,63	2,42 ± 0,63	2,35 ± 0,81	2,35 ± 0,81	2,35 ± 0,45	2,35 ± 0,45
	PT	Sumi 8	2,51 ± 0,42	2,51 ± 0,42	2,41 ± 0,61	2,41 ± 0,61	2,33 ± 0,81	2,33 ± 0,81	2,29 ± 0,38	2,29 ± 0,38
Contaminated	PC	-	4,67 ± 0,30	4,97 ± 0,30	6,38 ± 2,73	6,58 ± 2,73	2,38 ± 0,84	2,51 ± 0,78	3,53 ± 0,35	3,83 ± 0,35
	PE	SA	2,60 ± 0,57	2,59 ± 0,57	2,79 ± 1,57	2,79 ± 1,57	2,25 ± 0,38	2,25 ± 0,38	2,45 ± 0,28	2,45 ± 0,28
	PE	PA	2,60 ± 0,57	2,38 ± 0,57	3,44 ± 2,68	3,22 ± 2,28	2,21 ± 0,22	1,99 ± 0,22	2,12 ± 0,43	1,90 ± 0,43
	PE	Sumi 8	2,60 ± 0,57	2,40 ± 0,57	3,44 ± 2,68	3,24 ± 2,68	1,90 ± 0,33	1,17 ± 0,34	2,12 ± 0,43	2,20 ± 0,15
	PT	SA	2,46 ± 0,80	2,47 ± 0,79	2,16 ± 0,14	2,19 ± 0,10	2,50 ± 0,62	2,50 ± 0,62	2,20 ± 0,19	2,02 ± 0,04
	PT	PA	2,41 ± 0,72	2,41 ± 0,72	1,99 ± 0,13	2,01 ± 0,16	2,16 ± 0,04	2,16 ± 0,04	2,30 ± 0,04	2,31 ± 0,20
	PT	Sumi 8	2,33 ± 0,60	2,33 ± 0,60	1,75 ± 0,35	1,78 ± 0,31	2,16 ± 0,04	2,16 ± 0,04	2,15 ± 0,23	2,17 ± 0,22

Each value is the average of 3 replicates ± standard deviation.

PC: control plant; **PE:** elicited plant; **PT:** treated plant; **SA:** salicylic acid, **PA:** phosphorous acid

Table.2 Effects of salicylic acid, phosphorous acid and fungicide Sumi 8 on the migration of *Colletotrichum gloeosporioides* (cm) inside the cassava stem, 12th days after contamination

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

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.3 Phenolic compounds accumulated (%) elicited in the leaves of cassava plants, treated and inoculated with *Colletotrichum gloeosporioides*, 0, 5th and 12th days after infection.

Cassava cultivars		Culture medium										
		Uncontaminated				Contaminated						
		PC	PT			PC	PE			PT		
		M ₀	M _{SA}	M _{PA}	M _S	M ₀	M _{0/S} _A	M _{0/P} _A	M _{0/S}	M _{SA}	M _{PA}	M _S
<i>yacé</i>	0	100	150	163	166	100	100	100	100	150	163	166
	5 th	100	150	163	166	109	110	126	156	110	86	151
	12 th	100	150	163	166	68	113	66	113	104	97	97
<i>bonoua2</i>	0	100	122	161	163	100	100	100	100	122	161	163
	5 th	100	122	161	163	113	133	136	136	125	167	166
	12 th	100	122	161	163	104	101	118	135	114	184	128
<i>I88/00.</i>	0	100	147	117	162	100	100	100	100	147	117	162
	5 th	100	147	117	162	116	157	170	98	128	134	175
	12 th	100	147	117	162	120	184	162	112	235	128	145
<i>TMS30</i>	0	100	135	137	141	100	100	100	100	135	137	141
	5 th	100	135	137	141	107	89	104	105	127	97	97
	12 th	100	135	137	141	102	109	125	121	135	109	91

PC: control plant; **PE:** elicited plant; **PT:** treated plant; **SA:** salicylic acid, **PA:** phosphorous acid, **TMS30:** TMS30572, **I88/00:** I88/00158. **M₀:** nutrient medium; **M_{SA}, M_{PA} and M_S:** nutrient medium respectively containing salicylic acid, phosphorous acid and the Sumi 8; **M_{0/SA}, M_{0/PA} and M_{0/S}:** germination in the nutrient medium and then transfer in the nutrient medium supplemented with salicylic acid, phosphorous acid or the Sumi 8. **100%** = 1.69 (*yacé*) 1.45 (*bonoua2*); 1.71 (*TMS30572*) and 1.05 (*I88/00158*) mg AG/FW.

These results have also highlighted that the growth of the fungi inside the stems is directly related to the necrotic area on the stems. *C. gloeosporioides* can progress only when tissues are damaged (Perfect *et al.*, 1999; Thomma *et al.*, 2001; Ton *et al.*, 2002).

Evolution of the phenolic compounds content in the leaves of cassava plants varied depending on the type of treatment. In

control plants (PC) uncontaminated, amount of phenolic compounds remained constant during the experiment. It was 1.69 (*yacé*), 1.45 (*bonoua2*), 1.71 (*TMS30572*) and 1.05 (*I88/00158*) mg AG/FW. By against in contaminated PC, elicited plants (PE) or treated (PT), amount of phenolic compounds has been an increase in all cultivars. This increase was greater for PT (Table 3). In *yacé*, after contamination of PC, amount of phenolic compounds increased slightly 5th

Day fell below its initial value in the 12th Day. By cons in PE, this value remained high compared to PC, except the data recorded at 12th Day for plants elicited at phosphorous acid (PA) and contaminated by the fungi. For uncontaminated PT, Sumi 8 allowed to record a slight increase from the Salicylic acid (SA) and PA.

In contaminated PT, the values recorded 5th and 12th Day remained low compared to day 0 (Table 3). In *bonoua2*, the values recorded 5th and 12th Day were high for all PE and PT compared to Pt (Table 3). Maximum rate of accumulation of phenolic compounds was recorded, however, with PA and Sumi 8 (Table 3). Values recorded for *I88/00158* have evolved almost the same as those obtained in *yacé* and *bonoua2*. Indeed, for each test performed, these values remained high at 5th and 12th Day compared to PC.

The highest accumulation of phenolic compounds was recorded with SA at 12th Day in contaminated PT (Table 3). Per cent accumulation of phenols in *TMS30572* was not significant compared to *yacé* and *bonoua2*. However, in this cultivar, germination of cuttings (PT) in different elicitation medium allowed plants synthesize a significant amount of phenolic compounds (Table 3). From this study, it's clear wounding and pathogen attacks affect the synthesis and accumulation of phenolic compounds. Similar results were reported by De Ascensao and Dubrey (2003) in banana.

Phenolics are often produced and accumulated in the subepidermal layers of plant tissues exposed to pathogen attack (Clé *et al.*, 2008). In addition, the mode of induction of cassava's defense showed that the germination of cuttings directly into the elicitation medium appear more suitable. Indeed, the treated plants (PT) were completely prevented the progression of the

pathogen. The duration of the plants in the elicitation medium would be a determining factor in the development of defense cassava. At the same time, amount of phenolic compounds synthesized was much higher in these plants (PT). That is why, phenolic compounds have been proposed for some time to serve as useful alternatives to the chemical control of pathogens of agricultural crops (Langcake *et al.*, 1981).

In resistant plants, phenolic compounds based defense responses are characterized by the early and rapid accumulation of phenolics at the infection site resulting in the effective isolation of the pathogen. At *I88/00158*, after stress (elicitation and / or contamination by the pathogen), phenolic compounds synthesis was twice as large. This synthesis was further amplified with salicylic acid and phosphorous acid. Even so, the growth of the pathogen was significantly delayed in all treated cultivars selected elicitors Sumi 8.

These are the cause of the synthesis of others defense-related compounds in cassava other than phenolic compounds. Such as phosphate salts stimulate the production of active forms of oxygen that lead to cell death at the site of infection (Oreber *et al.*, 2002).

This study showed that the response of cassava plants varied among cultivars and treatments submitted to the plants. Elicited in plants or those having germinated directly in the elicitation medium, amount of accumulated phenolic compounds was higher, especially in the presence of Sumi 8. This increase in the content of phenolic compounds has been correlated with the inhibition of the onset of symptoms of anthracnose. These elicitors could be used as a means of inducing natural defense in cassava.

References

- Bacher, T., Bacher, M., Hofer, O., Greger, H. 2001. Stress induced carbazole phytoalexins in glycosmis species. *Phytochemistry*, 58(1):129-135.
- Benhamou, N., Bélanger, R.R. 1998. Benzothiadiazole-mediated induced resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato. *Plant Physiol.*, 118(4): 1203-1212.
- Buhot, N. 2003. Rôle des élicites et des protéines de transfert de lipides dans l'induction de la résistance des plantes à leurs agents pathogène. Thesis, *Bourgogne University*, 278p.
- Cheriot, S. 2007. Rôle des produits de la réaction de Maillard dans l'inhibition de l'oxydation enzymatique des phénols et des lipides. Thesis, *Institute of Science and Industries Life and Environment*, AgroParis Tech., 241p
- Clé, C., Hill, L.M., Niggeweg, R., Martin, C.R., Guisez, Y., Prinsen, E., Jansen, M.A.K. 2008. Modulation of chlorogenic acid biosynthesis in *Solanum lycopersicum*; consequences for phenolic accumulation and UV-tolerance. *Phytochemistry*, 69:2149–2156
- De Ascensao, A.F.R.D.C., Dubrey, I.A. 2003. Soluble and wall-bound phenolic polymers in *Musa acuminata* roots exposed to elicitors from *Fusarium oxysporum* f.sp. cubens. *Phytochemistry*, 63: 679-686.
- Dogbo, D.O., Békro-Mamyrbekova, J.A., Békro, Y.-A., Gogbeu, S.J., Traoré, A., Sié, R.S. 2007. Influence de l'acide salicylique sur l'activité des polyphénoloxydases et l'accumulation des composés phénoliques chez le manioc (*Manihot esculenta* Crantz). *Afr. Sci.*, 3(2) : 243-258.
- Dogbo, D.O., Békro-Mamyrbekova, J.A., Békro, Y.-A., Sié, R.S., Gogbeu, S.J., Traoré, A. 2008. Influence de l'acide salicylique sur la synthèse de la phénylalanine ammonia-lyase, des polyphénoloxydases et l'accumulation des composés phénoliques chez le manioc (*Manihot esculenta* Crantz). *Sci. Nat.*, 5(1):1-13.
- Dogbo, D.O., Gogbeu, S.J., N'Zué, B., Yao, K.A., Zohouri, C.P., Békro-Mamyrbekova, J.A., Békro, Y.-A. 2012. Comparative activities of phenylalanine ammonia-lyase and tyrosine ammonia-lyase and phenolic compounds accumulated in cassava elicited cell. *Afr. Crop Sci. J.*, 20(2): 85-94.
- FAO (2008). <http://www.fao.org/newroom/fr/new/2008/10000899/index.html>.
- FAO (2010). Statistical databases. Rome (Italy).<http://www.fao.org>
- Franke, R., Hermm, M.R., Denault, J.W., Ruegger, M.O., Humphreys, J.M., Chapple, C. 2002. Changes in secondary metabolism and deposition of an unusual lignin in the ref8 mutant of Arabidopsis. *Plant J.*, 30: 47-59.
- Gogbeu, S.J., Dogbo, D.O., Zohouri, G.P., N'zue, B., Bekro, Y.-A., Békro-Mamyrbekova, J.A. 2012. Induction of polyphenol oxidases activities and phenolic compounds accumulation in cells and plants elicited of cassava (*Manihot esculenta* Crantz). *J. Sci. Res. Rev.*, 1(1), 7 - 14
- Koussevitzky, S., Ne'eman, E., Harel, E. 2004. Import of polyphenol oxidase by chloroplasts is enhanced by methyl jasmonate. *Planta* 219(3):412-419.

- Langcake, P., Irvine, J.A., Jeger, M.J. 1981. Alternative chemical agents for controlling plant disease. Philosophical transactions of the royal society of London B. *Biol. Sci.*, 295, 83-101.
- Lange, M.B., Lapierre, C., Sandermann, H.J. 1995. Elicitor-induced spruce stress lignin : structural similarity to early development lignins. *Plant Physiol.*, 108:1277-1287.
- Lattanzio, V., Veronica, M.T. Lattanzio, V.M.T., Angela Cardinali, A. 2006. Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. *Phytochemistry*, 23-67
- Makambila, 1983. Epidémiologie de l'anthracnose du manioc. In : Plantes-racines tropicales : culture et emplois en Afrique, actes du second symposium triennal de la société internationale pour les plantes-racines tropicales, Douala, Cameroun, 75-80.
- Montes, M.J., López-Braña, I., Delibes, A. 2004. Root enzyme activities associated with resistance of *Heterodera avenae* conferred by gene *Cre7* in a wheat / *Aegilops triuncialis* introgression line. *J. Plant Physiol.*, 161:493-495.
- Nürnbergger, T. 1999. Signal perception in plant pathogen defense. *Cell. Mol. Life Sci.*, 55: 167-182.
- Ogawa, D., Nakajima, N., Sano, T., Tomaoki, M., Aono, M., Kubo, A., Kanna, M., Ioki, M., Kamada, H., Saji, H. 2005. Salicylic acid accumulation under O₃ exposure is regulated by ethylene in tobacco plants. *Plant Cell Physiol.*, 46: 1062-1072.
- Ojha, S., Chatterjee, N.C. 2012. Induction of resistance in tomato plants against *Fusarium oxysporium* f. sp. *Lycopersici* mediated through salicylic acid and trichoderma *harzianum*. *J. Plant Prot. Res.*, 52(2): 220-225.
- Oreber, M., Siegrist, J., Buchenauer, H. 2002. Mechanisms of phosphate-induced resistance in cucumber. *Eur. J. Plant Physiol* 108: 345-353.
- Perfect, S.E., Hughes, H.B., O'Connell, R.J., Green, J.R. 1999. *Colletotrichum*: A model genus for studies on pathology and fungal-plant interactions. *Fungal Genet. Biol.*, 27:189-198.
- Swain, T., Hillis, W.E. 1959. The phenolic constituents of *Prunus domestica* I. The quantitative analysis of phenolic constituents. *J. Sci. Food Agri.*, 10: 63-68.
- Thakore, Y. 2006. The biopesticide market for global agricultural use. *Indus. Biotech.*, 2(3): 294-208.
- Thomma BP, Penninckx IA, Broekaert WF and Cammue BP (2001). The complexity of disease signaling in *Arabidopsis*. *Curr. Opin. Immunol.*, 13(11):951-959.
- Thordal-Christensen H (2003). Fresh insights into processes of nonhost resistance. *Curr. Opin. Plant Biol.*, 6(4): 351-357.
- Ton J, van Pelt J, van Loon L and Pieterse C (2002). Differential effectiveness of salicylate-dependent and jasmonate/ethylene-dependent induced resistance in *Arabidopsis*. *Mol. Plant Microbe Interact.*, 15(1):27-34.
- Zawistowski, J., Biliaderis, C.G., Eskin, N.A.M. 1991. Polyphenol oxidase. In *Oxidative enzymes in foods*. Robinson DS, Eskin NAM, Eds, Elsevier Appl. Sci., London 6: 217-273.