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Physiological mechanism of resistance antibiosis to anthracnose of different Manihot varieties

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ABSTRACT

Cassava is one of the main food crops in Africa, particularly in Côte d'Ivoire. However, the cultivated varieties are prone to attack by diseases. The present work focused on the role of phenolic and in particular flavonoid resistance markers in the *Manihot esculenta-Colletotrichum gloeosporioides* pathosystem. The aim was to elucidate the involvement of flavonoid antibiosis in the natural defense of three cultivars of *M. esculenta* when confronted with attacks by *C. gloeosporioides*. The quantitative dosage approach for total phenolics and flavonoids as well as the identification of flavonoid antibiosis have been carried out. The results revealed that the cultivars 9620A, TMS30572 and YACE of *M. esculenta* have, after the *C. gloeosporioides* inoculation tests, reacted early 2 days after inoculation (JAI) and accumulated relatively high levels of antibiosis phenolic and flavonoid 9JAI. The three cultivars accumulated constitutive flavonoid antibiosis and 3 neosynthesized antibiosis from 7JAI to 9JAI. The accumulation of flavonoid antibacterials neosynthesized in the stems and in the leaves testify to the expression of a systemic resistance of the cassava plants. The cultivars 9620A and TMS30572 are more tolerant than cultivar YACE. This study approach has made it possible to discriminate between cultivars and can therefore be used as a complementary selection tool to traditional selection tests.

Keywords: *Manihot esculenta*, anthracnose, flavonoïd antibisis, glyphosate.

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INTRODUCTION

In Côte d'Ivoire, *Manihot esculenta* Crantz, known as cassava, is a main food crop. It is cultivated for its tuberous roots and leaves all over the country (Brou, 2014; Akpingny et al., 2017), with an estimated annual production of around 5.367 million tonnes (Faostat, 2019), this plant is the second most important food crop after yam and before plantain (N'Zué et al., 2015; Konan et al., 2017). Cassava crop is an important source of income for the people who grow it (Ekou, 2003; Manso, 2005). Thus, in order to support the dynamics of the crop, several research programmes have focused on the improvement of locally produced varieties, or introduced from the International Institute of Tropical Agriculture (ITTA) via the National Centre for Agronomic Research (CNRA). However, despite the efforts made, the improved cultivars remain prone to many diseases (Onzo et al., 2012: Djinadou et al., 2018). Work carried out by Xinzhang et al. (2012) on *Camelia oleifera* and *Lupinus angustifolius* showed that in plants that accumulated high levels of antibiosis and Gogbe et al. (2016) chez des plants de palmiers infectés par Fusarium oxysporum fse, the pathogen *Colletotrichum gloesporioides* remained confined and the plants were found to be resistant. Therefore, the present work on the natural defense of *Manihot esculenta* in interaction with *Colletotrichum gloeosporioides* was undertaken with the aim of developing approaches for the selection of resistant / tolerant plants. Specifically, depending on the cultivars and the time of infection, it involved, on the one hand, evaluating the contents of total phenolic and total flavonoid antibiosis and, on the other hand, identifying flavonoid antibiosis.

MATERIALS AND METHODS

Plant Material

The plant material used consists of 3 cultivars of *Manihot esculenta* of which one is traditional (YACE) and the other two improved 9620A (A), and TMS30572 (S). All these cultivars were supplied by the National Centre for Agronomic Research (CNRA).

Fungal material

The fungal agent used (*Colletotrichum gloeosporioides* f.sp. manihotis) is a necrotrophic fungus (Perfect et al., 1999). It was isolated from *Manihot esculenta* plants affected by anthracnose, on an experimental plot of the Centre National de Recherche Agronomique (CNRA). *Colletotrichum gloeosporioides* belongs to the order Melanconiales and the class Coelomycetes (Hawksworth et al., 1983). The conidia (Figure 25) are unicellular, elliptical or sickle-shaped, hyaline, without appendages and are produced apically on conidiophores in a sporiferous salmon- to orange-coloured jelly (Von Arx, 1981). Conidia and ascospores are embedded in a hydrophilic mucilaginous matrix of polysaccharides and glycoproteins. These matrices are water-soluble, with spores released and dispersed by the action of water (Louis & Cooke, 1985; McRae & Stevens, 1990).

Methodology

Expérimental device

Three blocks of plots (A, B and C) have been set up at the CNRA experimental site at Adiopodoumé in southern Côte d'Ivoire. Each block of plots consists of three (3) plots. Each plot consisted of three (3) rows of twenty (20) cassava plants arranged in totally randomized Fisher blocks.

Inoculation

The inoculation of the plants was done according to the methods of Terry et al. (1983), Ambang et al. (2007) and Brou (2014). It was carried out in 3 stages. The initial phase was the wounding of the stems, then the introduction of the inoculum in the form of a pellet at the wound site and finally the covering of the

inoculated stem portion. This inoculation was carried out on 4-month-old plants. For this purpose, the plants were notched with a heated needle (Figure 1) on the portions of the lignifying stems located between the 5th and 20th row of leaves from the apex of the plant (Figure 1). Three wound points were made on each plant (Ambang et al., 2007) and these were protected by sterilized transparent plastic wrapping the wounded stem portion to prevent possible infection. Thus, 50 wounded plants per cultivar on each plot were obtained. On the 4th day after the injury (Terry et al., 1983), 25 plants out of the 50 injured were selected to be inoculated by *C. gloeosporioides*. For this purpose, a 1 cm2 pellet of the 8-day old inoculum of *C. gloeosporioides* (consisting of 1.24 × 106 conidia counted on Malassez cells) was taken from petri dishes with a punch. This pellet was deposited at the site of the stem wound. After deposition of the inoculum, the inoculated stem portion was again covered with a new transparent plastic (Figure 32) which had been previously sterilized. Sterilization was performed by immersing the plastic in NaOCl (10%) for 3 min followed by abundant rinsing with sterilized distilled water. The positive control was represented by unharmed, uninoculated plants. The negative control was the wounded, uninoculated plants.

Sampling and packaging of samples

One day after inoculation, the leaves and stems of the 3 cultivars studied from Blocks A, B and C were sampled. The harvested organs were cleaned under running water and then dried for one day in a well-ventilated room and then in an oven for 7 days at 45 °C. The dried organs were pulverized with an electric mill (RETSCH, type SM 100) to obtain fine powders (Brou et al., 2012). These powders were used for hydro-methanol extraction (crude extracts) and selective extraction (flavonoid extracts).

Quantification of phenolic and flavonoid antibiosis

Extractions were carried out with the harvests of each block corresponding to the 1-15, 30, 45th DAI (Day After Inoculation) and the date of harvest of the tuberous roots (180 DAI). Thus, 324 extracts of which 162 for the leaves (54 uninjured, uninoculated extracts, 54 wounded/uninoculated extracts and 54 wounded, inoculated extracts) and 162 for the stems (54 uninjured, uninoculated extracts, 54 wounded, inoculated extracts and 54 wounded, inoculated extracts) were obtained. Each cultivar is presented by 18 extracts from the leaves and 18 extracts from the stems of unwounded and uninoculated plants (negative controls) (AFt, SFt, YFt, ATt, STt and YTt), by 18 extracts from leaves and 18 extracts from stems of injured/uninoculated plants (negative controls) (AFT, SFT, YFT, ATT, STT and YTT) and by 18 extracts from leaves and 18 extracts from stems of injured/inoculated plants (AFI, SFI, YFI, ATI, STI and YTI). A spectrophotometric assay of all these extracts (controls and inoculated) was carried out according to the protocol of Singleton and Rossi (1965), Brou et al. (2012), Dogbo et al. (2012) and Brou (2014).

Identification of flavonoid antibiosis by Thin Layer Chromatography (TLC)

TLC screening of the inoculated and positive controls extracts was carried out in order to identify the flavonoid antibiosis profile of cassava cultivars (Ekoumou, 2003; Dohou et al., 2003; Benkiki, 2006; Brou et al., 2010; Brou, 2014). The results of the extracts from uninjured-uninoculated plants (controls) obtained were compared with those of the extracts from injured-inoculated plants in order to show the role of phenolic antibiosis, in particular flavonoids, in the defense against anthracnose in Manihot esculenta.

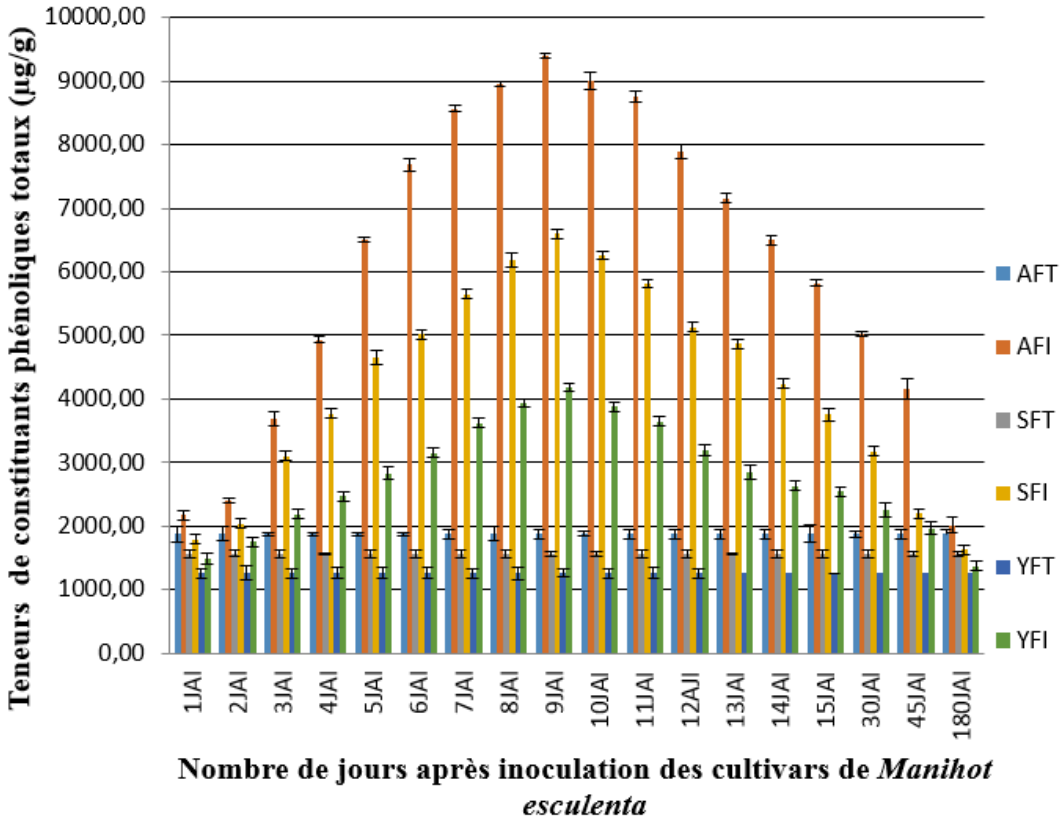

Figure 1: Total phenolic antibiosis levels in leaves (**F**) of inoculated (**I**) and uninoculated (**T**) *Manihot esculenta* cultivars (**A, S** and **Y**) as a function of number of days.
DAI: day after inoculation; **A**: 9620A; S: TMS30572; **Y**: YACE; **C**: Controls.

RESULTS

Evaluation of total phenolic antibiosis content in the mechanism of resistance of three *M. esculenta* cultivars against *C. gloeosporioides*

The amounts of total phenolic antibiosis contained in the leaves and stems increased steadily and significantly after inoculation of the different cultivars with the inoculum of *C. gloeosporioides* (Figures 1 and 2; Tables 1 to 4). This was not the case in uninoculated plants (Figures 1 and 2; Tables 1 to 4). This increase began on the 2nd day of inoculation, and reached its optimum stimulation on the 9th day after inoculation (DAI). From the 10th DAI, the total phenolic antibiosis content decreased steadily to reach a value similar to that of the 1st DAI (Figures 1 and 2; Tables 1 to 4). The stimulation weir, determined from a significant weir value of total phenolic antibiosis according to Duncan's test ($F = 34.62$; $P < 0.05$), was the date from which the *M. esculenta* cultivar reacted significantly to aggression by *C. gloeosporioides*. Thus, these thresholds obtained after 2 DAI were 2405.23 ±46.45 µg/g MS EAG, 2039.58 ±45.45 µg/g MS EAG and 1749.52 ±27.32 µg/g MS EAG at leaf level for cultivars A, S and Y respectively (Figure 1; Tables 1 and 2). For stems, these weirs were 1057.62 ± 16.78 µg/g MS EAG, 735.74 ±16.43 µg/g MS EAG and 513.64 ±15.95 µg/g MS EAG for cultivars A,

S and Y, respectively (Figure 2; Tables 3 and 4). As for the optimum stimulation or maximum accumulation stage, it was the date from which the cassava cultivar accumulated the highest amount of total phenolic antibiosis in its response to aggression by *C. gloeosporioides*. Thus, the optimum value for the leaves of cultivars A, S and Y was 9012.31±32.45, 6265.33 ± 17.25 and 3875.26±35.33 µg/g MS, respectively. Stems contained 5905.31±16.78, 4626.01±14.63 and 3779.45±13.74 µg/g MS for cultivars A, S and Y, respectively. These maximum values were recorded on the 9th DAI. At this stage of maximum accumulation, the leaves of cultivar A accumulated 5 times its phenolic antibiosis content and 8.51 times at the stem level. On the other hand, cultivar S accumulated 4.22 times its leaf and 9.25 times its stem accumulation, while the reference cultivar Y accumulated 3.32 and 7.22 times its constitutive phenolic content for leaves and stems, respectively. The proportions of accumulation of the improved cultivars (A and S) were higher than that of the reference cultivar Y. The values recorded in each period were highest for cultivar A, medium for S and low for Y. These values also varied with the organs. They were higher in the leaves than in the stems (Figures 1 and 2; Tables 1 to 4).

Evaluation of the total flavonoidics antibiosis content in the mechanism of resistance of three *M. esculenta* cultivars against *C. gloeosporioides*.

The results of the evaluation of the content of flavonoid antibiosis in the leaves and stems of plants inoculated with the inoculum of *C. gloeosporioides* (in µg/g quercetol equivalent) are presented in figures 3 and 4, and in tables V to VIII. It can be seen that the amounts of total flavonoid antibiosis increased steadily from the 2nd DAI to reach their maximum values by the 9th DAI for all plants of cultivars inoculated with the inoculum of *Colletotrichum gloeosporioides*. From the 10th DAI onwards, the flavonoid antibiosis contained in the organs declined steadily until the 180th DAI. The histograms also showed a bell-shaped curve for each of the 3 cultivars, and showed a significant stimulation threshold and stimulation optimum ($F = 27.53$; $p< 0.05$) following the mean comparison test. The stimulation threshold was found after 2 DIAs for all 3 cultivars studied. It was 787.65±2.85; 452.46±4.65 and 304.87±1.65 µg/g DM for leaves, and 117.63±2.45; 87.45±3.15 and 61.96±2.65 µg/g DM for stems of cultivars **9620A**, **TMS30572** and **YACE** respectively. The optimum stimulation (maximum accumulation stage) was determined according to the date from which the cassava cultivar accumulated the highest amount of total flavonoidics antibiosis in its response to aggression by *C. gloeosporioides*. Thus, this optimum value was 4303.28±4.65; 3273.45±2.37 and 1986.24±4.36 µg/g DM for the leaves of 3 cultivars 9620A, TMS30572 and YACE (Figure 3; Tables 5 and 6).

Identification of flavonoidics antibiosis by Thin Layer Chromatography (TLC)

The TLCs of extracts from leaves and stems of plants inoculated with the inoculum of *C. gloeosporioides* are shown in Figures 5 B to E and 6 B to E. Comparison of the chromatographic profiles of extracts from inoculated plants with those wounded and uninoculated (Figures 5 A and 6 A), showed that after treatment with the fungus, (at DAI 1; 7 and 9) neosynthesized flavonoid antibiosis, fluorescent blue (Rf 0.90), blue-green (Rf 0.85) and yellow-orange (Rf 0.84 and 0.88), appeared. In addition, these comparisons showed that the yellow-orange flavonoid antibiosis (Rf 0.84 and 0.88) present in the 7th DAI disappeared in the 9th DAI and at harvest. Two of the main flavonoidics antibiosis (orange of Rf 0.73 and green of Rf 0.80) were identified in both inoculated and control plants.

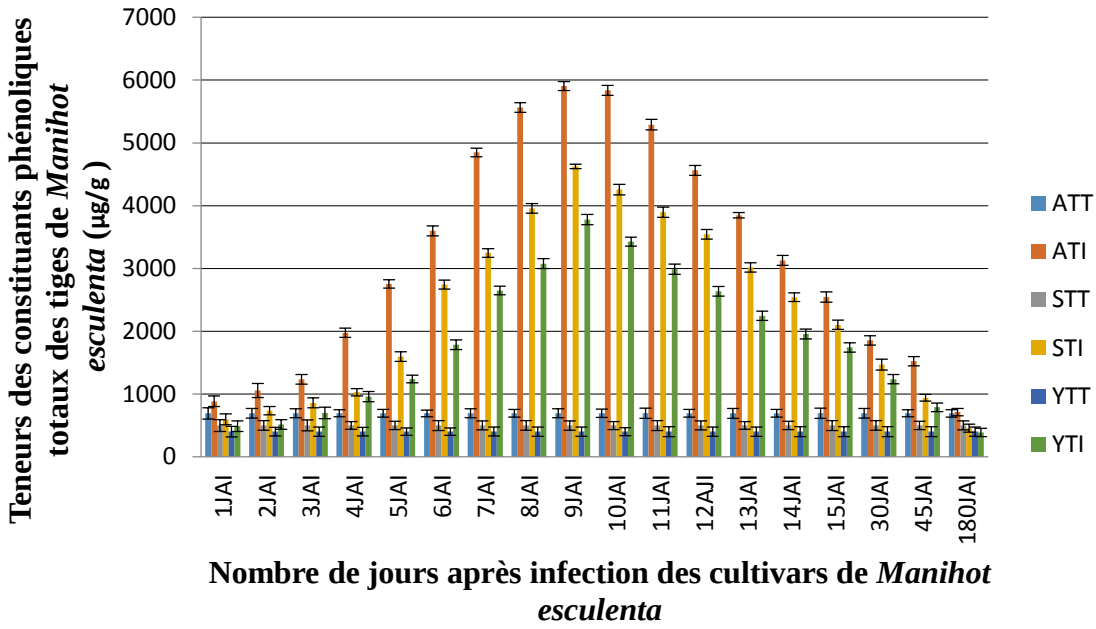

Figure 2: Total phenolic antibiosis levels in stems (**T**) of inoculated (**I**) and uninoculated (**T**) *Manihot esculenta* cultivars (**A, S** and **Y**) as a function of number of days.
DAI: day after inoculation; **A:** 9620A; **S:** TMS30572; **Y**: YACE; **C:** Controls.

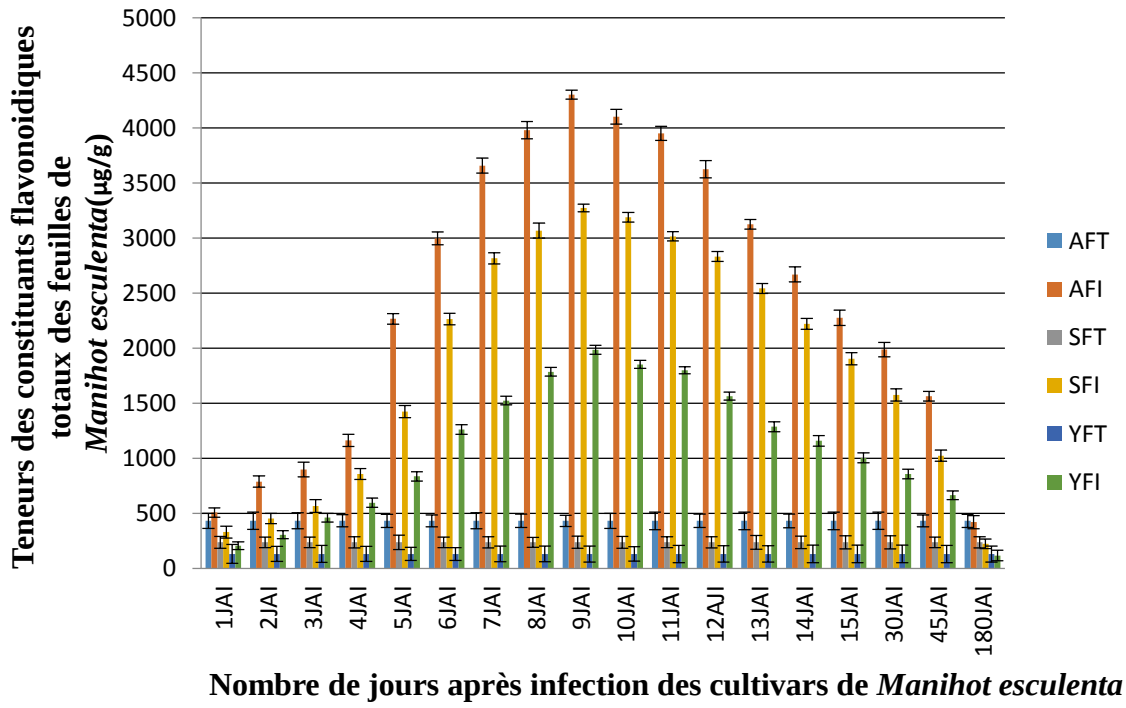

Figure 3: Total flavonoid antibiosis levels in leaf extracts (**F**) of inoculated (**I**) and uninoculated (**T**) *Manihot esculenta* cultivars (**A, S** and **Y**) as a function of the number of days.
DAI: day after inoculation; **A:** 9620A; **S:** TMS30572; **Y:** YACE; **T**: Controls.

Table 1: Amounts of total phenolic constituents from leaves of uninjured *Manihot esculenta* cultivars from 5th to 10th month after planting.

Cultivars of *M. esculenta*	Quantity in µg/g of phenolic constituents of cultivars in control plots from the 1st day of the 5th month to the 10th month after planting													
	1DAP	2DAP	3DAP	4DAP	5DAP	6DAP	7DAP	8DAP	9DAP	10DAP	15DAP	30DAP	45DAP	180DAP
Y	1262,76± 19,38	1262, 89 ±23,17	1260,97 ±26,47	1262,79 ±16,12	1263,01± 15,36	1262, 79 ±19,83	1262,45 ±36,04	1262,78± 25,36	1261,98± 16,25	1263,06 ±25,21	1262,76± 17,02	1263,01 ±15,71	1262,77 ±15,43	1262,49 ±34,17
S	1563,62± 25,72	1563,75± 16,32	1563,25 ±18,49	1562,95± 19,86	1563,67 ±26,47	1564,01± 35,16	1563,81 ±26,47	1563,29 ±35,39	1562,74 ±17,83	1563,85± 25,76	1562,75± 34,05	1563,25± 34,75	1561,93 ±25,12	1563,59 ±21,74
A	1873,06± 24,78	1872, 73 ±25,23	1871,99± 11,36	1873,13 ±15,09	1872,99 ±18,71	1873,74 ±24,05	1872,95± 18,16	1873,57± 14,37	1873,17 ±15,63	1874,04 ±25,44	1873,11 ±16,25	1872,78± 14,36	1873,31 ±16,65	1873,07 ±15,41

DAP: Day of the 5th month to the 10th month After Planting; Y: YACE; S: TMS30572; A: 9620A.

Table 2: Amounts of total phenolic antibiosis from leaves of cultivars of *M. esculenta* after injury.

Cultivars of *M. esculenta*	Amount in µg/g of total phenolic antibiotics according to the number of days (D) after (A) injury (B) of the cultivars													
	1DAB	2DAB	3DAB	4DAB	5DAB	6DAB	7DAB	8DAB	9DAB	10DAB	15DAB	30DAB	45DAB	180DAB
Y	1271,23± 11,84	1321,43 ±13,25	1385,04 ±15,05	1402,09 ±17,01	1361,46 ±16,23	1352,83 ±16,89	1349,75 ±15,25	1350,81 ±14,33	1353,84 ±13,21	1344,76 ±15,72	1338,03± 16,22	1322,05± 14,71	1317,51 ±16,02	1261,81 ±15,42
S	1564,52± 16,01	1597,64± 15,07	1654,42 ±17,43	1701,01± 14,37	1695,42 ±12,07	1685,72 ±18,01	1681,48 ±17,05	1680,25 ±16,73	1675,27 ±13,17	1670,01 ±14,81	1661,43± 17,21	1631,72± 12,86	1614,12 ±17,63	1562,89 ±14,46
A	1887,81 ±15,71	1898,93 ±16,07	1920,98 ±15,14	1984,77 ±16,21	1971,04± 14,23	1965,41 ±14,12	1963,95 ±16,73	1960,78 ±13,05	1957,51 ±16,34	1881,01 ±14,01	1878,15 ±15,02	1875,68± 13,74	1873,21 ±14,15	1873,97 ±17,12

DAB: Days (D) after (A) injury (B); Y: YACE; S: TMS30572; A: 9620A.

Table 3: Amounts of total phenolic antibiosis from stems of uninjured *M. esculenta* cultivars from 5th to 10th month after planting.

Cultivars of *M. esculenta*	Quantity in µg/g of total phenolic antibiosis of cultivars in control plots from the 1st day of the 5th month to the 10th month after planting													
	1DAP	**2DAP**	**3DAP**	**4DAP**	**5DAP**	**6DAP**	**7DAP**	**8DAP**	**9DAP**	**10DAP**	**15DAP**	**30DAP**	**45DAP**	**180DAP**
Y	401,01 ±11,31	400,85 ±18,23	401,15 ±10,01	402,05 ±21,41	400, 98 ±16,53	401,21 ±12,43	402,02 ±22,02	401,27 ±13,07	401,12 ±10,15	401,35 ±12,57	401,26 ±10,23	400,95 ±11,65	402,05 ±22,05	401,22 ±13,04
S	499,65 ±11,64	498,82 ±12,41	499,84 ±10,06	500,03 ±13,34	499,93 ±18,36	499,78 ±14,68	499,89 ±13,36	500,08 ±22,02	498,89 ±16,74	499,83 ±17,63	499,84 ±15,85	499,63 ±19,06	500,03 ±13,58	499,81 ±17,04
A	693,75 ±10,22	692,95 ±9,75	693,53 ±10,45	693,56 ±17,06	694,06 ±23,85	693,18 ±11,27	693,64 ±12,54	692,95 ±19,74	693,45 ±13,25	693,47 ±11,38	694,07 ±17,89	692,91 ±24,45	694,13 ±13,11	693,74 ±12,41

DAP: Day of the 5th month to the 10th month After Planting; Y: YACE; S: TMS30572; A: 9620A

Table 4: Amounts of total phenolic antibiosis from stems of cultivars of *M. esculenta* after injury.

Cultivars of *M. esculenta*	Amount in µg/g of total phenolic antibiosis according to the number of days (D) after (A) injury (B) of the cultivars													
	1DAB	**2DAB**	**3DAB**	**4DAB**	**5DAB**	**6DAB**	**7DAB**	**8DAB**	**9DAB**	**10DAB**	**15DAB**	**30DAB**	**45DAB**	**180DAB**
Y	407,33 ±9,76	422,63 ±11,07	458,14 ±11,21	461,23 ±13,44	459,99 ±14,01	460,01 ±10,09	455,71 ±13,46	455,07 ±11,82	453,89 ±13,19	454,01 ±14,03	448,12 ±14,05	439,07 ±13,74	432,33 ±11,63	404,29 ±15,44
S	511,33 ±10,68	544,52 ±13,23	568,11 ±12,75	573,23 ±11,63	570,12 ±14,08	568,44 ±13,77	562,76 ±12,01	560,21 ±14,39	551,68 ±16,13	548,32 ±14,89	540,71 ±13,71	531,43 ±11,59	520,03 ±14,29	502,69 ±17,13
A	719,73 ±13,43	778,49 ±12,11	806,17 ±13,77	862,06 ±14,13	852,18 ±13,66	849,92 ±12,48	833,05 ±10,01	831,69 ±13,67	825,23 ±12,91	792,44 ±13,19	779,87 ±12,13	751,05 ±14,62	731,58 ±10,25	703,74 ±13,59

DAB: days (D) after (A) injury (B); Y: YACE; S: TMS30572; A: 9620A.

Table 5: Quantities of total flavonoid antibiosis of leaves (5th to 10th month after planting) of uninjured *M. esculenta* plants.

Cultivars of *M. esculenta*	Amount in µg/g of flavonoid constituents of cultivars in control plots from the 5th month to the 10th month after planting													
	1DAP	2DAP	3DAP	4DAP	5DAP	6DAP	7DAP	8DAP	9DAP	10DAP	15DAP	30DAP	45DAP	180DAP
Y	130,69 ±15,31	130,68 ±14,25	130,98 ±16,45	131,11 ±14,12	130,49 ±15,26	130,41 ±14,24	129,95 ±16,25	130,67 ±15,21	128,97 ±14,59	130,41 ±15,02	131,11 ±13,42	130,65 ±12,39	131,21 ±14,28	130,67 ±16,43
S	236,04 ±15,42	236,35 ±16,18	236,14 ±17,07	237,48 ±16,46	236,67 ±15,41	235,95 ±18,36	236,03 ±16,18	237,01 ±17,34	235,97 ±15,56	236,81 ±18,55	236,75 ±17,45	237,45 ±18,65	235,94 ±17,42	236,49 ±18,92
A	430,85 ±23,26	430,82 ±24,15	430,74 ±23,32	429,99 ±24,65	431,05 ±22,21	430,27 ±24,59	431,25 ±24,25	430,81 ±25,67	430,28 ±23,63	428,92 ±22,54	430,26 ±26,25	430,87 ±24,36	431,13 ±20,65	430,21 ±15,41

DAP: DAP: Day of the 5th month to the 10th month After Planting; Y: YACE; S: TMS30572; A: 9620A.

Table 6: Amounts of total flavonoid antibiosis from leaves of *M. esculenta* cultivars after injury.

Cultivars of *M. esculenta*	Amount in µg/g of flavonoid antibiosis according to the number of days (D) after (A) injury (B) of the cultivars													
	1DAB	2DAB	3DAB	4DAB	5DAB	6DAB	7DAB	8DAB	9DAB	10DAB	15DAB	30DAB	45DAB	180DAB
Y	138,94 ±6,98	157,76 ±9,36	176,84 ±10,13	182,41 ±14,35	181,66 ±13,04	181,49 ±10,52	179,93 ±13,43	164,38 ±11,74	163,94 ±10,66	164,06 ±9,02	161,63 ±11,02	151,51 ±12,31	142,73 ±15,01	133,12 ±11,01
S	243,52 ±7,12	262,73 ±5,73	284,37 ±8,12	291,12 ±13,01	289,44 ±10,71	288,85 ±14,01	285,29 ±14,12	281,72 ±13,36	269,74 ±12,11	265,49 ±11,42	263,84 ±10,24	253,62 ±11,31	250,13 ±10,12	238,75 ±14,83
A	436,18 ±7,39	443,88 ±10,01	458,87 ±9,47	467,96 ±12,78	465,02 ±10,14	463,41 ±9,05	459,95 ±9,74	452,87 ±12,63	451,63 ±11,12	450,71 ±10,03	448,33 ±11,01	445,02 ±13,66	440,71 ±12,25	435,24 ±13,62

DAB: days (D) after (A) injury (B); Y: YACE; S: TMS30572; A: 9620A.

Table 7: Quantities of total flavonoid constituents from stems of uninjured *Manihot esculenta* cultivars from 5th to 10th month after planting.

Cultivars of *M. esculenta*	Amount in µg/g of flavonoid constituents of cultivars in control plots from the 5th month to the 10th month after planting													
	1DAP	2DAP	3DAP	4DAP	5DAP	6DAP	7DAP	8DAP	9DAP	10DAP	15DAP	30DAP	45DAP	180DAP
Y	31,46 ±7,35	31,62 ±9,23	30,89 ±8,36	31,46 ±7,12	32,07 ±9,26	31,61 ±8,24	32,04 ±6,23	31,47 ±8,21	30,85 ±9,59	31,66 ±8,28	31,36 ±9,45	32,16 ±8,39	31,25 ±7,28	31,45 ±6,42
S	44,65 ±10,54	45,02 ±9,46	44,14 ±10,26	44,31 ±9,42	43,92 ±10,63	44,48 ±10,38	43,96 ±9,16	44,68 ±7,34	44,65 ±7,54	45,03 ±9,15	44,64 ±13,41	44,66 ±10,63	45,03 ±12,18	44,64 ±10,91
A	71,16 ±6,26	71,52 ±7,15	71,38 ±8,32	71,63 ±8,65	70,96 ±7,61	70,88 ±9,58	72,09 ±9,24	71,15 ±9,65	71,33 ±7,63	70,99 ±10,64	71,17 ±7,53	71,11 ±8,46	72,03 ±10,25	71,14 ±10,35

DAP: DAP: Day of the 5th month to the 10th month After Planting; Y: YACE; S: TMS30572; A: 9620A.

Table 8: Quantities of total flavonoid antibiosis of the stems of cultivars of *M. esculenta* after injury.

Cultivars of *M. esculenta*	Amount in µg/g of flavonoid antibiosis according to the number of days (D) after (A) injury (B) of the cultivars													
	1DAB	2DAB	3DAB	4DAB	5DAB	6 DAB	7 DAB	8 DAB	9 DAB	10 DAB	15 DAB	30 DAB	45 DAB	180 DAB
Y	35,51 ±10,63	332,39 ±12,23	41,75 ±11,61	42,33 ±8,53	41,79 ±10,11	41,55 ±10,13	41,35 ±13,12	41,17 ±11,27	41,05 ±10,52	40,83 ±8,17	40,65 ±9,05	39,74 ±10,25	39,05 ±10,15	33,17 ±10,65
S	49,85±11, 59	50,59 ±10,11	52,31 ±11,41	53,39 ±7,18	53,02 ±9,36	52,78 ±11,56	52,69 ±10,44	52,48 ±13,41	52,44 ±12,36	52,39 ±10,23	52,04 ±10,15	51,96 ±13,06	51,03 ±11,38	47,41 ±11,32
A	74,09 ±10,89	76,18 ±8,41	78,04 ±10,45	79,47 ±10,36	79,36 ±13,59	78,88 ±12,25	78,19 ±10,14	78,01 ±11,14	77,03 ±12,65	77,02 ±13,48	76,97 ±10,09	76,81 ±10,31	76,13 ±12,21	72,49 ±10,72

DAB : days (D) after (A) injury (B) ; Y : YACE ; S : TMS30572 ; A : 9620A.

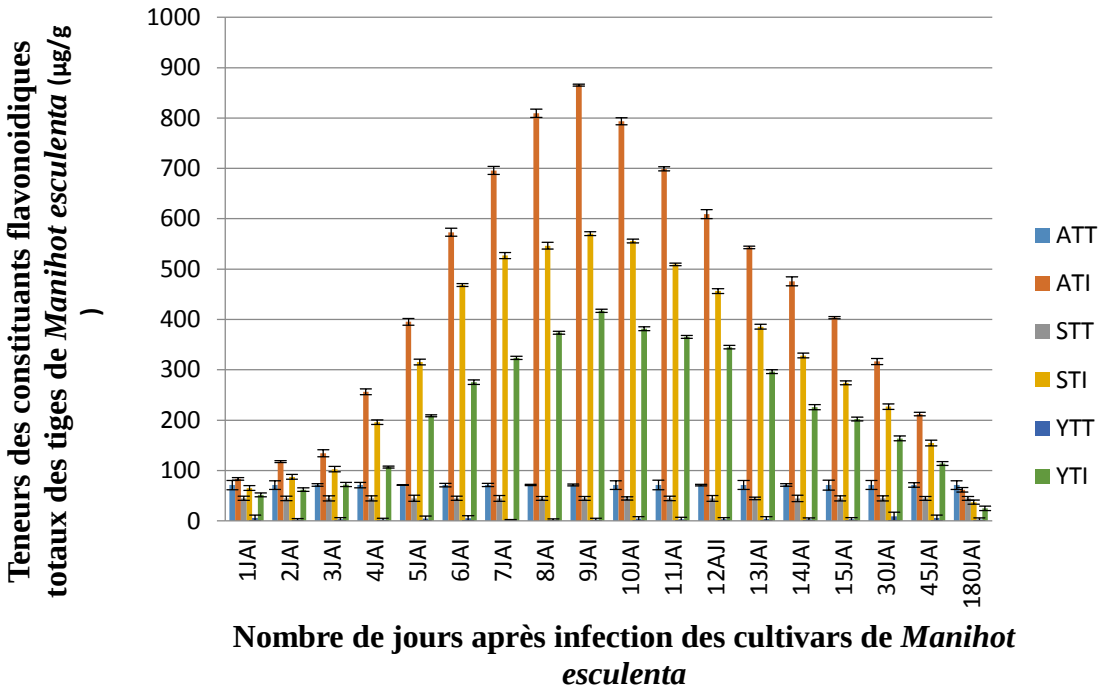

Figure 4: Total flavonoid antibiosis levels in stem (**T**) extracts of inoculated (**I**) and uninoculated (**T**) *Manihot esculenta* cultivars (**A**, **S** and **Y**) as a function of the number of days.
DAI: day after inoculation; **A**: 9620A; **S**: TMS30572; **Y**: YACE; **T**: Controls.

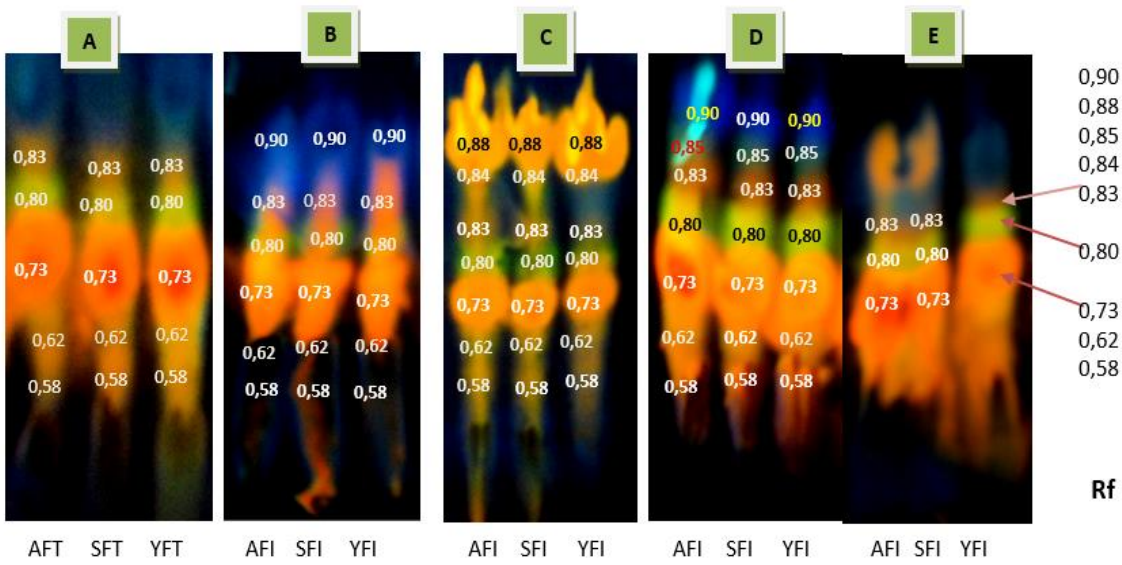

Figure 5: Comparative Thin Layer Chromatography of flavonoid antibiosis of leaves (F) of cultivars A, S and Y revealed by Neu reagent and viewed under UV light at 366 nm.
A: antibiosis of leaves of control (T) plants (F); **B**: antibiosis of leaves of plants inoculated at 1 Day After Inoculation (DAI); **C**: antibiosis of leaves of plants inoculated at 7 DAI; **D**: antibiosis of leaves of plants inoculated at the stage of maximum flavonoid accumulation (9 DAI); **E**: antibiosis at harvest stage at 180 DAI.

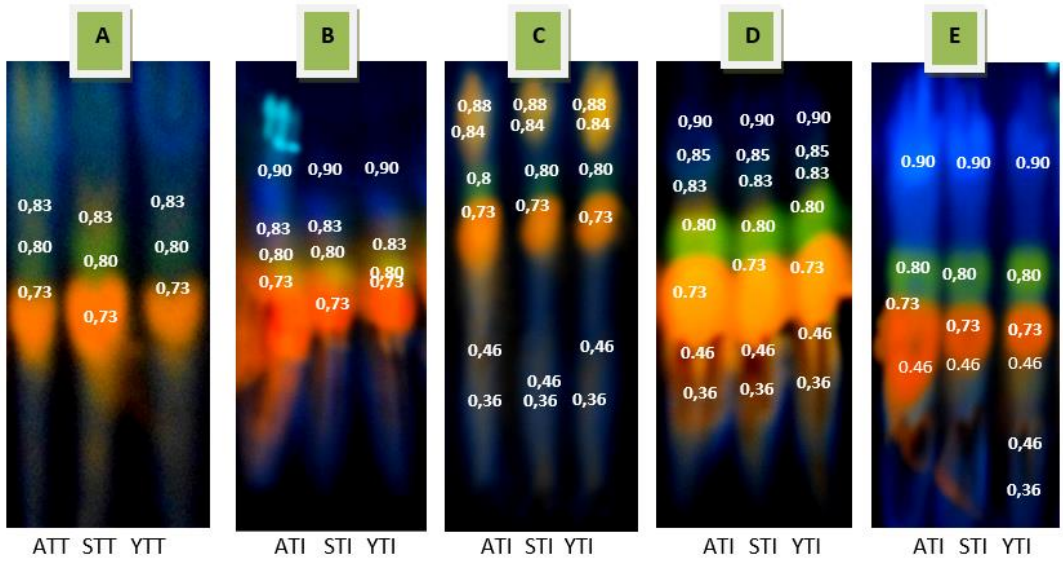

Figure 6: Comparative Thin Layer Chromatography of flavonoid antibiosis of stems (T) of cultivars A, S and Y revealed by Neu reagent and visualized under UV light at 366 nm.
A: antibiosis of stems (T) of control plants (T); **B**: antibiosis of stems (T) of plants inoculated at 1 Day After Inoculation (DAI); **C**: antibiosis of stems (T) of plants inoculated at 7 DAI; **D**: antibiosis of stems (T) of plants inoculated at the stage of maximum flavonoid accumulation (9 DAI); **E**: antibiosis at harvest stage at 180 DAI.

DISCUSSION

The comparative study of phenolic antibiosis and particularly flavonoid antibiosis in healthy plants inoculated with *C. gloeosporioides* showed that the presence of this pathogen induced an increase in the synthesis of phenolic antibiosis. This increase is general in all infected plants but with a variable amplitude depending on cultivars and organs. Stimulation of the synthesis of these constituents, initiated after inoculation of the pathogen, increased steadily with the contact time of the plant with the pathogen to reach an optimum at the 9th day after inoculation (9DAI). Beyond this period, the levels of phenolic and flavonoid antibiosis decrease and reach their minimum values. Stimulation was early in all cultivars. It started in the 2nd DAI and reached its maximum value in the 9th DAI. At this date, the leaves of cultivar **A** accumulated 5 times their phenol content and 8.51 times this content on the stems. On the other hand, the leaves of cultivar **S** accumulated 4.22 times the basic phenol content and the stems concentrated 9.25 times. The reference cultivar **Y** accumulated 3.32 and 7.22 times the phenol content for the leaves and stems respectively. Phenol accumulations in **A** and **S** are higher than those of the reference cultivar **Y**. Flavonoid antibiosis accumulation was also high. It was 9.99 to 15.2 times higher depending on cultivar and organs. This concomitant increase in phenolic and flavonoid antibiosis would result from the fact that most phenolic antibiosis whose flavonoids are derived from a common precursor which is p-coumaric acid. The latter results from the activity of phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL). Activation of these enzymes has been observed during elicitation of *Manihot esculenta* cells using salicylic acid (Dogbo et al., 2012) and infection of *Camellia oleifera* by *Colletotrichum gloeosporioides* (Xinzhang et al., 2012).

The synthesis of phenolic and flavonoidic antibiosis was early in all cultivars and organs. This very rapid accumulation of these constituents could be explained by their involvement in plant defense mechanisms (Broeckling et al., 2005*;* Suzuki et al., 2005; Naoumkina et al., 2007; Farag et al., 2009;

Mancilla et al., 2009). Concerning flavonoids in particular, Chen et al. (2007), Crawford et al. (2004) and Calatayud et al. (2011) found that resistance of cultivars of *Carmellia oleifera* and *Lupinus angustifolius* correlated with rapid and intense flavonoid production. In the latter species, the amounts of flavonoid antibiosis accumulated in the leaves after 7 days of contamination by *Colletotrichum lupinus* had been 50 times higher than in the control (Muth et al., 2009; Wojakwoska et al., 2013).

The synthesis of secondary metabolites, particularly phenolic and flavonoid antibiosis, increased with the time of contact with the pathogen. Indeed, according to Masella et al. (2005), Farag et al (2008) and Schmitt and Dirsch (2009) an accumulation of these compounds in order to assume physiological defense functions that are recognized to them is stimulated after the aggression of the plant by a pathogen. The increase in the observed accumulation time of flavonoids in both leaves and stems after fungal infection is thought to be related, on the one hand, to the contact time *Manihot esculenta-Colletotrichum gloeosporioides* and, on the other hand, to the transduction of the elicitor signal to initiate the synthesis of defense substances (Lozovaya et al., 2004; Grotewold, 2006; Farag et al., 2008; Schliemann et al., 2006; Bednarek and Osbourn, 2009; Jasin'ski et al., 2009.

Conclusion

The study of the defense reaction of *M. esculenta* against *C. gloeosporioides* has shown that inoculation of cassava cultivars with this pathogen induced an early reaction of antibiosis of these 2 DAI. In addition, it has induced a maximal and generalized accumulation of phenolic antibiosis and flavonoid antibiosis 9 DAI. The generalized accumulation of these antibiosis was stronger in cultivar A. But all cultivars showed the same stimulation threshold and the same maximum accumulation times. However, during the expression of this systemic defense, the inoculated cultivars have, over time, accumulated various types of flavonoid antibiosis. This flavonoid antibiosis has played the role of phenolic resistance markers in the defense reaction of cultivars of *M. esculenta* against *C. gloeosporioides*.

This study approach, through the parameters addressed, made it possible to discriminate the level of resistance of the cultivars and can therefore be used as a complementary tool to classics tests for cultivar selection.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

BKG carried out the work, collected the data and wrote the manuscript. MJLA and DS contributed to the evaluation of flavonoïd antibiosis. ZGP participated in the protocol of *C. gloeosporioides* inoculum production. MBJA and DDO supervised the work. KKN and BYA put their respective laboratories at our disposal to carry out the work. All these co-authors have read and validated the results of the work.

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