Journal of Scientific Research and Reviews Vol. 1(1), pp. 7 - 14, March 2012
Available online at http://www.wudpeckerresearchjournals.org/JSRR
2012 Wudpecker Research Journals

Full Length Research Paper

Induction of polyphenol oxidases activities and phenolic compounds accumulation in cells and plants elicited of cassava (*Manihot esculenta* Crantz)

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Accepted 9 March 2012

The aim of this study was to evaluate the activities of immobilize and cytoplasmic polyphenol oxidases, and quantify phenols in cells and seedlings of cassava cultivars *yacé* and *TMS4(2)1425* elicited by salicylic acid, phosphorous acid and Sumi 8 (diniconazole). Cells were elicited in cell suspension and seedlings by root uptake of products. With salicylic acid and phosphorous acid, polyphenol oxidases activities were multiplied by 1.6 to 3 in *yacé* and 1.2 to 4 in *TMS4(2)1425* according to the organs. They were 3 and 4 times higher in cells of *TMS4(2)1425* treated respectively with phosphorous acid and salicylic acid, and 3 times in leaves of *yacé* and *TMS4(2)1425* elicited by acid phosphorous. Amount of phenols increased from 25 to 110%. The highest accumulations were recorded in cells treated with salicylic acid (67.8% [*TMS4(2)1425*]; 110% (*yacé*)) and leaves treated with Sumi 8 (32% [*TMS4(2)1425*]; 68.9% (*yacé*)). With phosphorous acid, the increase of these compounds was greater than 50% in cells. It was 21% [*TMS4(2)1425*] and 38% (*yacé*) in leaves. Each elicitor would have a specific action in cassava cultivars.

Key words: Cassava, elicitation, phenols, phosphorous acid, polyphenol oxidases, salicylic acid, sumi 8.

INTRODUCTION

Plants respond to attack by pathogens by synthesizing a variety of organic molecules that can act indirectly (structural) or directly (biochemical) on the attackers. Work carried out by Ferreira et al., (2007) on cucumber (*Cucumis sativus*) and arabidopsis (*Arabidopsis thaliana*) reported biochemical and physiological changes. These changes were accompanied by structural changes including thickening of the walls of cortical cells, deposition of callose, lignin and phenolic compounds at sites of penetration of pathogen. Similarly, in rice (*Oryza sp*) and tea (*Camellia sinensis*), Nandakumar et al. (2001) and Saravanakumar et al. (2007) noted that the reaction of these plants to *Pseudomonas fluorescens* PF1 has been associated with increased production of salicylic acid. Similar reactions can be induced in plants by treating some of biotic and abiotic compounds commonly referred to as elicitors (Hilall, 2004; Iriti et al., 2004; Sels et al., 2008; Soylu et al., 2002; Van Hulten et al., 2006). Thus, treatment of various plants by phosphate salts, phosphorous acid or salicylic acid induced a stimulation of systematic protection of plants against pathogens (Ogawa et al., 2005; Reuveni and Reuveni, 1998). According to Ogawa et al. (2005), salicylic acid is involved in plant defence by acting as a signal molecular interaction in plant / pathogen. Indeed,

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after treatment of cassava leaves (*Manihot esculenta*) with salicylic acid, Dogbo et al. (2008) observed an increase of polyphenol oxidases (PPO) activities and amount of phenolic compounds in these organs. These authors suggested that PPO and phenols could be markers of resistance in this plant. The present study aims to compare the level of synthesis of phenolic compounds and polyphenol oxidases activities in cassava cultivars: *yacé* (local cultivar) and *TMS4(2)1425* (Improved cultivar) elicited by salicylic acid, phosphorous acid and Sumi 8 (diniconazole).

MATERIALS AND METHODS

Plant materiel

Plant materiel consist of cells and seedlings of two cultivars of cassava (*Manihot esculenta* Crantz): *yacé* (local cultivar) and *TMS4(2)1425* (improved cultivar) provided of National Centre of Agricultural Research (CNRA, Côte d'Ivoire).

Preparation of cell suspension and plants

Cell suspension was initiated from friable callus obtained by in vitro culture of immature petioles of leaves of cassava cultivars *yacé* and *TMS4(2)1425*. These petioles were disinfected by soaking successively in alcohol (70%) for 1 min, sodium hypochlorite (3.6%) for 7 to 8 min and rinsed with sterile distilled water were cut into pieces 0.5cm long. Extracts were placed on Murashige and Skoog (1962) medium enriched with vitamins B_5 of Gamborg et al. (1968), containing glucose (3%) and agar (0.8%). This medium was supplemented with growth regulators: 2,4-dichlorophenoxyacetic acid (0.2 mg/L), 6-benzylaminopurine (0.5 mg/L) and picloram (0.2 mg/L). Friable callus obtained after six weeks of culture, were separated and cells of same size were harvested after sorting through a sieve (diameter 2mm). For the cell suspension, a sample of 1.5g was transferred to Erlenmeyer flasks containing 20mL of liquid medium and placed in continuous agitation on an orbital shaker (Gerhardt) at 110rpm.

Cassava plants were obtained from hydroponic cuttings 20cm long previously sterilized with alcohol (70%) for 5 to 6min. Pots (diameter 7.2cm x height 12cm) were closed by a plug of polystyrene (thickness 2cm) at its center a hole was made for the passage of the cutting. To facilitate oxygenation of roots, the pots were laterally drilled four holes (diameter 0.5cm) equidistant from each other, 10 cm from the bottom. These pots received 200 mL of nutrient solution containing phosphorus (P_2O_3) and dolomite ($CaMg(CO_3)_2$) at a dose of 80 mg/L each. Germination and plant growth were conducted in a greenhouse illuminated by natural light (Dogbo et al., 2008). The media were renewed every 15 days.

Elicitation of cells and seedlings

Cell suspension was elicited after 5 days of acclimatization in culture medium using the technique of Gómez-Vásquez et al. (2004). Cells of each cultivar were divided into four groups: cells treated with phosphorous acid (0.1 mM), cells treated with salicylic acid (0.1 mM), cells treated with Sumi 8 (0.5 mM) and control cells. Each group consists of 51 recipients. These recipients were placed on the shaker and cells were harvested by filtration on a canvas (diameter 30 microns) from 0 to 12h, at 24, 48, 72 and 96h. Four groups of seedlings aged six weeks were established for each cultivar. Each package contains 24 selected seedlings. They were transferred in pots of nutrient solution containing salicylic acid (1mM; SIGMA), phosphorous acid (1mM; SIGMA) or Simu 8 (0.5mM; Syngeta Society). The control has not received elicitors. Seedlings were then placed in a greenhouse under the same conditions. Enzymes activities and amount of phenolic compounds were evaluated at 0, 4, 6, 12, 24, 48, 72 and 96h after treatment on the third leaf from the apex (Dogbo et al., 2008). Harvested leaves were immediately placed in ice. Only the limb was used for the different extractions.

Estimation of polyphenol oxidases activities

Polyphenol oxidases (PPO) activities were determined as per Gogbeu et al., (2011). Cytoplasmic polyphenol oxidases (PPOc) and immobilize (related to the cell walls) polyphenol oxidases (PPOl) were extracted separately. To extract PPOc, 1g of limbs or cells was ground in 10mL of Tris-HCl buffer (0.2M) pH 8 [cultivar *yacé* (yPPOc)] and sodium phosphate (0.2M) - citric acid (0.1 M) pH 4.5 [cultivar *TMS4(2)1425* (t_4PPOc)]. After centrifugation at 5000g for 30 min at 4 °C, the supernatant was recovered and the pellet was taken up in 5mL of extraction buffer and then ground and centrifuged as before. Supernatants were collected and treated with Dowex anion 3% (w/v) for 30 min at 4°C with agitation (orbital shaker at 110rpm). Supernatant from this second centrifugation represented the cytoplasmic enzyme extract partially purified. PPOl were extracted with citrate buffer (0.1 M) - sodium phosphate (0.2 M) pH 4.5 [cultivar *TMS4(2)1425*] and sodium phosphate (0.2 M) pH 7 (cultivar *yacé*). After exhausting the pellet by extraction, it was taken up in 5mL of extraction buffer supplemented with triton x-100 1% (v/v). The set was crushed, centrifuged and the supernatant was treated with Dowex. Supernatant collected after the second centrifugation constituted the partially purified extract of PPOl.

The enzyme was assayed in the reaction medium composed of 50 µL of partially purified enzyme extract, 5 mM $CuCl_2$ and 50 mM dopamine (yPPOc), 100 mM pyrogallol (yPPOl) or 125 mM catechol (t_4PPO). The reaction mixture was adjusted to 3 mL with the extraction buffer. After 5 min of incubation at 35 (yPPOc, and yPPOl t_4PPOl) or 40 °C (t_4PPOc), tubes were cooled in a bath regulated at 4 °C and PPO activities were determined in a spectrophotometer (Milton Roy) at 420nm (yPPOl and t_4PPO) or 470nm (yPPOc) against a control. Enzyme activity was expressed in absorbance per minute per milligram of protein (ΔDO /min/ mg prot.). Maximum stimulation (Sm) of polyphenol oxidases activities was expressed as the difference between high enzyme activity (EAh) and that of the control (EAc) (Sm = EAh - EAc).

Quantification of total phenolics

Phenolics were quantified following the procedure of Swain and Hillis (1959). 0.5g sample was crushed in 10mL of 80% ethanol containing 15mM ascorbic acid and centrifuged as described above. One mL of the ethanolic extract was added to 0.5 mL of 0.5 N Folin-Ciocalteu reagents and the solution was kept at 30 °C. At 30 min, 1.5mL of 17% (w/v) sodium carbonate was added and the reaction mixture was incubated for 45 min at 28°C. The absorbance of the developed blue colour was measured in a spectrophotometer at 725nm. Phenolics content were calculed according to a standard curve obtained from a Folin-Ciocalteu reaction with gallic acid and expressed in milligram gallic acid equivalent per gram of fresh weight (mg GA/FW)

Determination of protein

Protein concentrations of the extracts were determined using the dye-binding method of Bradford (1976), with bovine albumin as the

Table 1: Effect of elicitors salicylic acid, phosphorous acid and Sumi 8 on polyphenol oxidases activities (ΔDO /min/ mg prot.) in cell suspension of cultivar yacé[a]

Time (h post-elicitation)	Elicitors					
	Salicylic acid		Phosphorous acid		Sumi 8	
	PPOc	PPOl	PPOc	PPOl	PPOc	PPOl
0	4845.82 ±292	4845.82 ±292	4845.82 ±292	4845.82 ±292	4845.82 ±292	4845.82 ±292
1	4960.05 ±39	4960.05 ±39	5492.92 ±303	5492.92 ±303	4137.46 ±375	4137.46 ±375
2	4988.91 ±439	4988.91 ±439	7434.36 ±102	6192.05 ±395	4644.14 ±288	4179.73 ±259
3	4313.47 ±43	5176.16 ±52	9081.98 ±802	6650.95 ±638	4884.59 ±235	4396.13 ±212
4	4535.30 ±155	5442.36 ±186	9951.06 ±927	7295.60 ±842	4614.60 ±226	4939.66 ±119
5	6789.03 ±459	5431.22 ±367	8607.04 ±508	6885.64 ±406	5334.66 ±49	4801.20 ±44
6	7355.28 ±114	6298.01 ±463	6492.03 ±186	7572.98 ±679	5095.45 ±122	5095.45 ±122
7	7371.29 ±122	6411.26 ±147	6965.04 ±193	7298.37 ±770	5485.43 ±68	4936.88 ±61
8	7911.53 ±152	7185.20 ±224	4927.47 ±541	6405.71 ±704	5683.37 ±408	5115.03 ±367
9	7489.34 ±136	8238.27 ±1499	5679.46 ±951	7383.29 ±123	5564.48 ±520	5008.03 ±468
10	5110.28 ±836	7665.42 ±125	7126.37 ±588	8523.41 ±119	5956.56 ±301	4968.22 ±496
11	5807.85 ±209	6969.42 ±251	6516.21 ±705	8471.07 ±917	5599.27 ±416	5039.35 ±375
12	3543.77 ±248	4252.52 ±297	7401.24 ±342	8900.72 ±151	6336.91 ±160	5276.08 ±698
24	4652.64 ±294	3998.41 ±1172	4842.19 ±75	6294.85 ±97	6274.56 ±304	5647.10 ±274
48	4065.28 ±284	2845.70 ±199	4889.57 ±90	4889.57 ±90	5383.26 ±470	5406.67 ±121
72	4052.00 ±104	2836.40 ±72	2992.01 ±402	3889.61 ±563	4911.54 ±355	5418.34 ±106
96	3820.99 ±496	2674.69 ±347	2690.8 ±650	3498.07 ±845	4448.51 ±167	5338.21 ±201

[a] activity values are averages of three independent determinations ± standard deviation

standard, measuring optical density at 595 nm.

Statistical analysis of data

Data collected were subjected to analysis of variance (ANOVA) with one or two criteria for classification using the software SPSS 11.5. The difference between means at 95% confidence level calculated using the Least Significant Difference (LSD) test.

RESULTS

PPO activities in cells elicited

Cytoplasmic and immobilize PPO activities have evolved similarly in both cultivars (Tables 1 and 2). In cells of cultivar *yacé*, enzymes were strongly stimulated in the presence of salicylic acid (SA) and phosphorous acid (PA). The activation was maintained at a high level between 5-11 h with SA and between 1 to 24 h in the presence of PA. With Sumi 8, stimulation was delayed (12 to 24h) , Table1. Maximum stimulation (Sm) of PPO activities calculated as elicitors showed a significant difference ($p = 0.014$). The LSD test indicated that the effects of SA and Sumi 8 are similar. In cultivar *TMS4(2)1425*, activation was maintained for a long time (1 to 7h and after 12h) with SA and of short duration with PA (1-2 h, 12 - 24 h). With Sumi 8, activation took place from 8 to 10 h (Table 2). At the time of Sm ($p = 0.024$), homogeneous groups were on the one hand, salicylic acid-Sumi 8 and on the other hand, salicylic acid-

Table 2: Effect of elicitors salicylic acid, phosphorous acid and Sumi 8 on polyphenol oxidases activities (ΔDO /min/ mg prot.) in cell suspension of cultivar *TMS4(2)1425*[a].

Time (h post-elicitation)	Elicitors					
	Salicylic acid		Phosphorous acid		Sumi 8	
	PPOc	PPOl	PPOc	PPOl	PPOc	PPOl
0	1334.52 ±441	1334.52 ±441	1362.97 ±150	1362.97 ±150	1352.60 ±46	1352.60 ±80
1	2433.79 ±734	2190.41 ±660	2429.27 ±125	1457.56 ±75	1345.82 ±107	1370.40 ±118
2	2989,33 ±1489	2690,40 ±1340	2887,02 ±90	1732,21 ±54	1378,38 ±144	1337.53 ±255
3	2973.98 ±809	2973.98 ±809	1843.57 ±451	1738.03 ±542	1491.20 ±149	1342.08 ±134
4	3081.60 ±248	3081.60 ±112	1538.20 ±75	1384.38 ±67	1408.54 ±150	1267.68 ±135
5	3518.40 ±880	3166.56 ±792	1778.22 ±16	1600.40 ±14	1411.55 ±67	1270.40 ±60
6	3190.82 ±113	2871.74 ±102	1698.48 ±40	1528.64 ±36	1398.48 ±87	1258.64 ±78
7	3434.55 ±101	2747.64 ±2879	1828.48 ±22	1828.48 ±22	1541.81 ±79	1287.07 ±145
8	2284.75 ±161	1827.80 ±129	1894.46 ±136	1894.46 ±136	1884.46 ±121	1319.12 ±85
9	1618.08 ±109	1294.46 ±872	1854.83 ±173	2027.70 ±233	1854.83 ±173	1285.77 ±247
10	2185.60 ±195	1092.80 ±97	1985.52 ±100	2188.49 ±400	1984.19 ±102	1388.93 ±71
11	1618.08 ±109	1132.66 ±763	1727.60 ±13	2245.88 ±17	1770.09 ±73	1478.95 ±247
12	1307.83 ±101	906.14 ±772	2028.13 ±253	2410.21 ±234	1745.64 ±107	1571.07 ±96
24	2412.35 ±691	835.50 ±80	1663.02 ±65	2161.93 ±84	1858.52 ±140	1672.67 ±126
48	2191.43 ±1025	1095.71 ±512	1589.11 ±76	2065.84 ±100	1795.09 ±159	1615.58 ±148
72	2225.84 ±280	1112.92 ±140	1279.16 ±62	1662.90 ±81	1638.51 ±14	1638.51 ±14
96	1941.13 ±1807	970.56 ±903	1293.54 ±29	1681.60 ±38	1482.17 ±54	1630.39 ±60

[a] activity values are averages of three independent determinations ± standard deviation.

phosphorous acid.

Phenolic compounds accumulated in cells elicited

The results shown in Table 3 represent the rate of phenols (%) in cell suspensions treated or not with different reaction times. In cultivar *yacé*, phenolic compounds were accumulated in cells treated for 1 to 9h (salicylic acid (SA)), 1 to 10h (phosphorous acid (PA)) and 2 to 8h (Sumi 8). On the other side, in cultivar *TMS4(2)1425*, SA induced accumulation of phenols for 10 to 12h of contact. Treatment with PA and Sumi 8 stimulated the synthesis and accumulation of phenols during the experiment except in cells treated for 9, 11 and 12h by PA. However, the maximum levels of phenols were recorded with SA [210.36% (cultivar *yacé*) 167.85% (cultivar *TMS4(2)1425*] and PA [150.54% (cultivar *yacé*), 156.68 % (cultivar *TMS4(2)1425*]. The content of phenolic compounds of the cells was calculated by taking as 100%, the amount of phenols at 0 h after treatment. Each value used to calculate the percentage was the mean of 3 repetitions. **yacé*, 100% = 0.71mg GA/FW; ***TMS4(2)1425*, 100% = 0.37mg GA/FW

PPO activities in leaves of plants elicited by root absorption

In plants of cultivars *yacé* and *TMS4(2)1425* elicited, the

Table 3: Effect of elicitors salicylic acid, phosphorous acid and Sumi 8 on phenolic content (%) in cell suspension of cassava

Time (h post-elicitation)	Elicitors					
	Salicylic acid		Phosphorous acid		Sumi 8	
	yacé	*TMS4(2)1425*	*yacé*	*TMS4(2)1425*	*yacé*	*TMS4(2)1425*
0	100*	100**	100*	100**	100*	100**
1	104.14	100.14	122.88	103.00	96.94	122.11
2	126.49	93.05	134.14	105.99	118.29	114.69
3	139.46	74.39	129.10	156.68	112.34	127.62
4	210.36	62.94	150.54	156.40	115.32	114.44
5	195.86	70.44	139.55	117.44	107.03	124.96
6	176.31	39.65	112.43	107.36	106.40	115.27
7	155.05	78.47	106.94	107.08	111.98	123.76
8	154.59	65.40	98.56	101.91	127.93	126.16
9	121.89	76.84	125.41	99.18	82.07	120.97
10	58.65	139.51	125.14	111.99	73.78	127.11
11	31.35	167.85	92.79	85.56	77,93	145.68
12	29.82	154.22	91.71	87.19	69.64	136.37
24	43.78	88.96	74.59	107.08	71.26	126.67
48	27.39	97.28	74.32	109.54	64.59	116.34
72	40.99	77.38	80.45	105.72	62.88	122.49
96	38.47	70.98	78.83	120.98	62.07	111.65

activities of cytoplasmic polyphenol oxidases (PPOc) and immobilize polyphenol oxidases (PPOl) have evolved in a similar way (Tables 4 and 5). In cultivar *yacé* (Table 4), with salicylic acid (SA) and phosphorous acid (PA), maximum PPO activities measured in leaves were recorded at 72 h of treatment. They were stimulated twice as compared to controls. When treatment was done with Sumi 8, enzymes activities were inhibited. In cultivar *TMS4(2)1425* (Table 5), Sumi 8 inhibited the activity of enzymes, while salicylic acid induced a low stimulation at 48 h of treatment. However, with PA, this activity has tripled to 96 h of treatment. The test performed confirms the hypothesis that the elicitors act differently ($p = 0$) of enzyme activity in a given cultivar and polyphenol oxidases activities also varies between cultivars ($p = 0$).

Phenolic compounds from the leaves of plants elicited by root absorption

The analysis of Table 6 shows that in leaves of cultivars *yacé* and *TMS4(2)1425*, root absorption elicitors induced the synthesis and accumulation of phenolic compounds during the experiment. No significant differences occurred between cultivars ($p = 0.468$) and the effects of elicitors ($p = 0.183$) on the synthesis of phenols. However, maximum values of phenols were obtained from the cultivar *yacé*. They were 45.38, 38.78 and 68.98% respectively for 24 (salicylic acid), 72 (phosphorous acid) and 96 h (Sumi 8) treatment. In cultivar *TMS4(2)1425*, these amounts have varied from 21 to 32%. The content of phenolic compounds of the leaves was calculated by taking as 100%, the amount of phenols at 0 h after treatment. Each value used to calculate the percentage was the mean of 3 repetitions. **yacé*, 100% = 1.76mg GA /FW ; ***TMS4(2)1425*, 100% = 1.01mg GA/ FW.

DISCUSSION

Cells and leaves of cassava plants responded to elicitation by the increased synthesis of phenolic compounds and activation of both cytoplasmic and immobilize polyphenol oxidases (PPO) in the presence of salicylic acid (SA) and phosphorous acid (PA). Activation of enzymes was more pronounced in cells of cultivar *TMS4(2)1425* (3 to 4 times) and leaves of cultivar *yacé* (2.5 to 3 times). These results corroborate those obtained by Dogbo et al. (2008) on cassava and Shah (2003), Loake et al. (2007) and Vlot et al. (2009) from other plants that used salicylic acid. The early activation of enzymes in isolated cells (1 to 4 h) could be explained by a rapid entry elicitors in cells suspended in the nutrient medium by diffusion. On the other hand, the increase in reaction time found in leaves after elicitation by root absorption come from among others, the period of absorption and transport of the raw sap elicitors. The diversion of these substances by the leaves to high metabolism can also delay the entry of elicitor in the target cells of leaf selected.

Stimulation of PPO activities was concomitant accumulation of phenolic compounds. This synthesis was early and prolonged in both cultivars. Our results are consistent with those of Dogbo et al. (2008) who obtained an increase in the amount of phenols in elicited and not

Table 4: Effect of elicitors salicylic acid, phosphorous acid and Sumi 8 on polyphenol oxidases activities (ΔDO /min/ mg prot.) in leaves of cultivar *yacé*[a].

Time (h post-elicitation)	Elicitors					
	Salicylic acid		Phosphorous acid		Sumi 8	
	PPOc	PPOl	PPOc	PPOl	PPOc	PPOl
0	3921,05 ±375	5097,36 ±488	3921,05 ±375	5097,36 ±488	3921,05 ±375	5097,36 ±488
4	4071,05 ±385	5247,36 ±497	4031,05 ±370	5207,36 ±497	3811,05 ±361	4637,23 ±176
6	4086,05 ±386	5262,36 ±500	4046,05 ±371	5222,36 ±501	3796,05 ±359	4622,23 ±175
12	4921,05 ±365	6097,36 ±470	4921,05 ±373	6097,36 ±486	2921,05 ±385	3747,23 ±126
24	5650,57 ±315	7345,74 ±410	8725,55 ±542	11343,21 ±705	2853,63 ±376	2568,27 ±338
48	8944,21 ±600	11627,48 ±780	9747,13 ±931	12671,28 ±127	1057,20 ±48	951,48 ±43
72	9686,22 ±470	12592,08 ±611	11928,10 ±20	15506,53 ±0	930,20 ±25	1209,26 ±33
96	9305,80 ±697	12097,55 ±328	10340,26 ±723	13442,34 ±237	2268,55 ±486	2949,12 ±632

[a] activity values are averages of three independent determinations ± standard deviation

Table 5: Effect of elicitors salicylic acid, phosphorous acid and Sumi 8 on polyphenol oxidases activities (ΔDO /min/ mg prot.) in leaves of cultivar *TMS4(2)1425*[a].

Time (h post-elicitation)	Elicitors					
	Salicylic acid		Phosphorous acid		Sumi 8	
	PPOc	PPOl	PPOc	PPOl	PPOc	PPOl
0	3298.01 ±324	3957.62 ±389	3298.01 ±324	3957.62 ±389	3298.01 ±324	3957.62 ±389
4	3448.01 ±333	4107.62 398±	3408.01 ±317	4067.62 ±398	3188.01 ±324	3847.62 ±380
6	3463.01 ±334	4122.62 ±401	3423.01 ±316	4082.62 ±402	3173.01 ±322	3832.62 ±379
12	4298.01 ±315	4957.62 ±380	4298.01 ±309	4957.62 ±387	2298.01 ±333	2957.62 ±398
24	3699.30 ±388	4439.16 ±465	3699.30 ±388	4439.16 ±465	3332.23 ±249	3998.68 ±299
48	4625.83 ±68	5551.00 ±81	2063.21 ±59	2475.85 ±70	2063.21 ±59	2475.85 ±70
72	3711.33 ±228	4453.60 ±273	4080.04 ±88	4896.04 ±105	1413.37 ±489	1696.04 ±587
96	3825.33 ±85	4590.40 ±102	9671.37 ±109	11605.65 ±131	1671.37 ±109	2005.65 ±131

[a] activity values are averages of three independent determinations ± standard deviation

elicited leaves of the same plant. In seedlings, the establishment of systemic reaction is due to the transport of elicitor by the sap in leaves untreated.

Works on PA as elicitors of plant defence reactions are rare except those of Koller (1992) which showed its effect in plant resistance. However in agriculture, the use of PA as salts of phosphate and phosphite (fosetyl-Al) showed an effect on the stimulation of plant defence. Indeed, the works of Afek and Sztejnberg (1989) have indicated that fosetyl-Al was a systemic activation of defence response in plants. This induction is linked to stimulation of phenylpropanoid pathway leading to the accumulation of

Table 6: Effect of elicitors salicylic acid, phosphorous acid and Sumi 8 on phenolic content (%) in leaves of cassava

Time (h post-elicitation)	Elicitors					
	Salicylic acid		Phosphorous acid		Sumi 8	
	yacé	*TMS4(2)1425*	*yacé*	*TMS4(2)1425*	*yacé*	*TMS4(2)1425*
0*	100*	100**	100*	100**	100*	100**
4	111.31	106.84	110.29	106.22	111.31	106.84
6	125.34	115.32	124.31	114.69	125.34	115.32
12	110.29	106.22	109.35	105.65	138.43	123.23
24	145.38	110.41	115.89	107.66	166.12	106.70
48	103.22	125.94	117.07	121.16	161.32	132.06
72	114.15	105.40	138.73	116.99	165.61	116.68
96	113.08	117.05	120.75	108.52	168.98	114.70

antimicrobial phytoalexins potential (Dixon et al., 2002; Iriti and Faoro, 2009). The use of phosphate salts allowed Mitchell and Walters (2004) to induce both the activation of phenylpropanoid pathway and systemic resistance in barley (*Hordeum sp).* In addition to these actions, phosphate salts stimulate the production of active forms of oxygen that lead to cell death at the point of infection (Oreber et al., 2002).

Sumi 8 is a systemic fungicide (Ashour, 2009) which inhibited PPO activities in seedlings but their activation in cells was early in cultivar y*acé* (2h) and late in cultivar *TMS4(2)1425* (8h). However, the amount of phenolic compounds has been an increase in all organs of both cultivars. Its fungicidal action could be enhanced by phenol induced. Indeed, the structures of phenols such as lignin limit the expansion of fungi in plant. Others, their deleterious effect on spore germination, mycelial growth and production of hydrolytic enzymes of fungal pathogens reinforce the defences of plants (Dixon et al., 2002). This action is reinforced by the quinones produced by PPO (Shahidi and Naczk, 1995). The content of phenols initially low constitutive in cultivar *TMS4(2)1425*, has been an increase in all organs elicited. The initial high level of phenolic compounds in *yacé* or de novo synthesis after elicitation [cultivars *yacé* and *TMS4(2)1425*] is beneficial for these cultivars.

Conclusion

We can conclude that all elicitors used (salicylic acid, phosphorous acid, Sumi 8) induced the synthesis of phenolic compounds involved in plant resistance. These elicitors have certainly turned the biosynthetic pathway of phenylpropanoids in cassava.

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