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Increasing Growth and Accumulation of Metabolites for Radical Scavenging Potential in *Manihot Esculenta Crantz* Calli Cultures by Abiotic Elicitors

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Abstract

Manihot esculenta Crantz (cassava) of Euphorbiaceae family is one of the well-known crops that possesses a broad range of therapeutic characteristics due to the rich content of vitamins, minerals and active secondary metabolites. The present work was planned to scrutinize the probable effect of different elicitor concentrations on the stimulation of callus growth and metabolite production such as total carbohydrates, total phenol and total flavonoids along with the antioxidant activity assessment. In vitro, calli cultures were formerly produced from stem segments of cassava using MS medium containing 5 mg/l 2,4dichlorophenoxyacetic acid (2, 4-D) and 0.2 mg/l benzylaminopurine (BAP). Salicylic acid was more efficient for callus production compared to methyl jasmonate and glutathione. Calli cultures of cassava recorded the maximum carbohydrate content with glutathione at concentrations of 4 and 2 mM (38.69 and 35.06 mg glu g-1dw), respectively. Similarly, the highest total phenol content (28.74 and 28.35 mg GAE g-1dw) was obtained using glutathione at concentrations of 4 and 2 mM, respectively. Whereas the highest content of flavonoids (1.73 $mgQEg^{-1}dw$), was achieved with 2 mM glutathione. The lowest carbohydrate, phenols and flavonoid contents were detected in the control calli cultures (11.62 mg glu $g^{-1}dw$; 0.44 $mgGAEg^{-1}dw$ and 0.16 $mgQEg^{-1}dw$ respectively). The highest free radical scavenging activity of calli cultures against DPPH free radicals has resulted in MeJ20 of 96.73%, while the lowest was in the control (free elicitor) medium of 80.21%. Although varied concentrations of elicitor were used in the present experiments, it can be concluded that the calli treated with elicitors had a higher metabolite content compared to the control. The obtained results indicated that with the appropriate type and concentration of elicitor, an improved metabolite content of *Manihot esculenta* Crantz can be achieved and hypothesized as a rich source of biological components to be useful in nutritional supplements and pharmaceuticals.

Keywords: *Manihot esculenta* Crantz, callus cultures, carbohydrates, phenols, flavonoids, polyphenols, DPPH scavenging activity

Introduction

Cassava (*Manihot esculenta* Crantz) is a perennial crop as the third most important material of calories in several tropics, consumed by about 800 million citizens as food and feed daily in Africa and many parts of the world [1]. The industrial prospective of cassava is becoming attractive to most investors [2, 3]. The tendency of plant tissue cultures for much important compound production has been delivered almost since the start of *in vitro* technology [4]. Among many strategies, elicitor applications were adopted for that purpose due to their enhancement of biomass and secondary metabolite accumulation via different enzymatic pathways [5]. Salicylic acid, glutathione and Methyl jasmonate were considered abiotic elicitors with remarkable effects on secondary metabolites accumulation [6]. Salicylic acid (SA) is involved in many abiotic stress mechanisms [7], in controlling programmed cell death [8]. Also, SA is necessary during the plant life cycle [9] and to mediate the hydroxyl radical formation that is needed in root developmental processes [10-11]. Methyl jasmonate is a well-known signal transduction elicitor which involved in plant defense mechanisms [12-13]. Glutathione is one of the most studied tripeptide antioxidants for efficient defense against pathogens. Furthermore, glutathione is known to play a principal role in signal transduction and redox regulation [14, 15]. Both phenols and flavonoids are valuable antioxidant

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structures, responsible for free radical's deactivation founded on their capability for hydrogen atoms donation to free radicals. As well they have ideal structural properties for scavenging free radicals [16]. Many scientific researches indicate a linear relationship between total phenols and flavonoid content with antioxidant activity [17, 18]. The DPPH assay is generally used to confirm the antioxidant capacity of isolated pure constituents and fractions as hydrogen atoms donors [19].

Material and Methods

Establishment of calli cultures and culture conditions

Stem segments initiated calli of *in vitro* growing cassava plantlets. Briefly, the stem explants were inoculated on Murashige and Skoog (MS) medium [20] supplemented with the optimized combinations of 2,4dichlorophenoxyacetic acid (2.4-D) combined with 0.2 mg/l benzylaminopurine (BAP) beside 3% sucrose plus 0.8% agar as reported before [21]. Medium was controlled to pH 5.8 before autoclaving for 20 minutes. The cultures were incubated after inoculation in the growth room at 25+2°C for 16 hrs photoperiod in light intensity equivalent 45 µmolms^{-1}usingtubes of cool white fluorescent. The formed calli were re-cultured on the same medium every 30 days.

Growth measurement

After 10 days in culture, the calli were weighed under sterile conditions and the average of fresh weight (g) difference was considered for growth measurements**.** The growth rate (gfw/day) was calculated according to Szoke et al. [22] as follows:

Growth rate = Ge - G s / 10

Where: Ge: Mass of callus (g) at the end of every week and Gs: The starting mass (g) of callus

Elicitation

Salicylic acid (50,100 and 150 mg/l) and glutathione (2,4 and 6 mM/l) were prepared by dissolving in H_2O, and then filter sterilized before added to the autoclaved and cooled down medium. Methyl jasmonate filtered sterilized solution was prepared by ethanol at a concentration of 95% before resolving to final concentrations of 10,20,30mg/l.The prepared solutions above elicitors were added to the callusing media after autoclaving. The effects of cassava culture growth and biochemical metabolites as exposed to those various abiotic elicitors' types and concentrations were studied.

Extraction of cassava phytochemical constituents

A Soxhlet extractor procedure was used for extraction as described before [23]. Appropriate dry samples of *in vitro* callus cultures were put in soxhlet apparatus with a method of maceration in 100 ml methanol at concentration of 85% at room temperature for 24 hrs. After collecting the extracts were filtered by a 0.45-µm filter, and then subjected to centrifugation at 14 000 rpm several times, before exposure to vacuum evaporator for full dryness using rotary evaporator device (BUCHI, RotaVapor, R-300). The residues were resolved in the least methanol volume at concentration of 85% before putting in the refrigerator at 4°C till the next use. The percentage yield of the extract was calculated using the formula:

Yield percentage (%) = weight of extract / weight of calli X 100

Determination of total carbohydrates content

According to Dubois et al. [24], about 0.5 g of dried calli were mixed with 10 ml of H_2SO_4 67% with agitation, then the reaction was preserved for 1h at room temperature, and the volume was completed to 100 ml of distilled water before filtration with filter paper.1 ml of filtrate was taken, then added 1 ml phenol 5% and 5 ml H_2SO_4 98% with slow agitation. The previous mixture was put in water bath at 30˚C for 10:15 min before reading by spectrophotometer UV-240 at 490 nm, the concentration of carbohydrates was calculated using the standard curve of glucose.

Determination of total phenols content

Total phenols were determined by the method of Folin–Ciocalteu micro- according to Slinkard and Singleton [25]. About30µl of extract solution was combined with about 2 ml of distilled water plus 100 µl of Folin–Ciocalteu's reagent before adding 300 µl Na_2CO_3 at concentration of 200 g/l. The mixture was put at 40 °C in a water bath for 25 min before measuring its absorbance at 760 nm. Calibration curve of gallic acid standard was used. Considering A solution of no extract was used as a control for analysis. Phenols content attributed to Gallic Acid Equivalent (GAE) was calculated using this equation:

$A = 0.98C + 9.925\ X10^{-3}$ (R2 = 0.9996)

Where A is absorbance of gallic acid and C is the concentration (mg GAE g^{-1} DW).

Determination of total flavonoids content

Total flavonoids were assessed using the method described by Ordonez et al. [26]. About500µl $AlCl_3$methanolic solution at concentration of 20 g l−1was combined with500µlof extract solution. The absorbance of the mixture was read at 420 nm after 1 h of incubation at room temperature. The appearance of yellow color indicated to flavonoid structures presence. Considering A solution of no extract was used as control for analysis. Calibration curve of quercetin standard was used. Flavonoids content attributed to Quercetin Equivalent (QE) was calculated using this equation:

Y = 0.0255X (R2 = 0.9812)

Where X is the absorbance and Y is the concentration (mg QE g^{-1} DW).

Determination of total antioxidant activity

Total antioxidant activity was evaluated by the described method of Brand- Williams et al. [27] using 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH·) to determine the free radical scavenging activity of the extracts. A bout1ml of DPPH· solution at concentration of 0.1 mM was prepared in methanol, and then added to 3ml of the extract solution, before the mixture was shaken vigorously for 30 min at room temperature. Control was prepared with the same procedure, and methanol is instead of the sample. Absorbances of samples were read in a spectrophotometer (JASCOV-730, serial No. A112261798, Japan) at 517 nm. The percentage of antioxidant activity was calculated using this formula:

RSC (%) = [A DPPH – As / A DPPH] ×100

Where: As is the mixture solution absorbance of extract and ADPPH is the DPPH solution absorbance. All determinations were performed in triplicates.

Statistical Analysis

All analyses were performed using three independent replicates, fresh weight and growth rate values were expressed as mean values ± SE according to Snedecor and Cochran [28]. All phytoconstituents (total carbohydrates, total phenols, total flavonoids, polyphenols and DPPH values) assessments were statistically analyzed using analysis of variance (ANOVA) by one-way to test the differentiation among all treatments. Program of MATATC software and least-significant-difference (LSD) test at $p<0.05$ ($LSD_{0.05}$) applying Duncan's Multiple Range Test (DMRT) was used according to Gomez and Gomez [29]. $LSD_{0.05}$ test was used to compose the significant differences between means of treatment [30].

Results and Discussion

Clusters of calli cultures derived from stem explants were re-cultured onto the optimal callusing media (MS + 5mg/l 2,4-D + 0.2mg/l BAP) supplemented with three elicitors (salicylic acid, methyl jasmonate and glutathione) at different concentrations to optimize callus productivity. The data were recorded at different time intervals (10, 20and 30 days) as illustrated in Table 1.

Table 1 Growth dynamics of cassava calli cultures

Treatments	10 days		20 days		30 days	
	FW	GR	FW	GR	FW	GR
Control	6.0±0.32	0.3±0.32	8.51±0.32	0.251±0.003	10.21±0.13	0.17±0.02
S50	9.83±0.52	0.68±0.05	12.62±0.73	0.28±0.05	19.69±1.2	0.71±0.04
S100	7.78±0.62	0.48±0.06	9.25±0.43	0.15±0.078	12.77±0.49	0.35±0.04
S150	5.32±0.35	0.23±0.08	7.79±0.49	0.25±0.05	9.62±0.53	0.18±0.026
G2	7.82±0.71	0.48±0.03	10.98±0.78	0.32±0.01	12.84±0.39	0.19±0.02
G4	7.96±0.39	0.49±0.08	11.27±0.63	0.33±0.066	13.3±0.57	0.203±0.089
G6	9.25±0.51	0.63±0.05	11.92±0.83	0.27±0.034	14.96±0.77	0.303±0.06
MeJ10	9.0±0.53	0.60±0.053	9.79±0.76	0.16±0.07	11.74±0.67	0.19±0.06
MeJ20	9.79±0.54	0.68±0.05	11.81±0.33	0.20±0.03	15.16±0.83	0.33±0.052
MeJ30	6.77±0.67	0.37±0.03	8.77±0.69	0.19±0.01	9.67±0.47	0.09±0.06

Where: Salicylic acid (S50-S100-S150;50,100,150mg/l); Glutathione (G2-G4-G6; 2,4,6 mM/l); Methyl jasmonate (MeJ10-MeJ20-MeJ30;10,20,30mg/l). Mean ± SE

Calli fresh weight and growth rate in the control treatment were (6; 8.51; 10.21g) and (0.3; 0.251; 0.17 g) after 10, 20 and 30 days of cultivation, respectively. On the other hand, the effect of different elicitors on callus cultures of cassava was relatively varied as shown in Table (1).

After 30 days of culture, the maximum calli fresh weight (19.69 g), was found with salicylic acid (50 mg/l) followed by(15.16 g) with methyl jasmonate (20 mg/l) and(14.96 g) with glutathione (6 mM/l), while salicylic acid (150 mg/l) and methyl jasmonate (30 mg/l)recorded the least value of calli fresh weights (9.62 and 9.67 g respectively).

Regarding growth rates of elicited cultures at the end of the experiment, the highest value of growth rate was achieved with S50(0.71), followed by S100 (0.35), then MeJ20(0.33), while MeJ30 treatment recorded the least value of growth rate (0.09) as shown in Table 1.

It could be concluded that salicylic acid was more efficient for callus production than methyl jasmonate and glutathione. As a result, the change in callus accumulation induced by a range of elicitors was dependent on both their concentration and the cultivation period as appeared in Figure 1.

Recently, many strategies using plant tissue culture technique have been intensively investigated to optimize plant metabolite production [31-33]. In vitro calli cultures were scrutinized to identify the occurrence of secondary metabolites in plant tissues during elicitation studies [34-35]. The plant cell and tissue culture system is a powerful biotechnology tool that can be applied as a platform for secondary metabolite production. The active metabolites extracted from the in vitro cultures are much easier than that of the complex organized tissues of a plant [4].

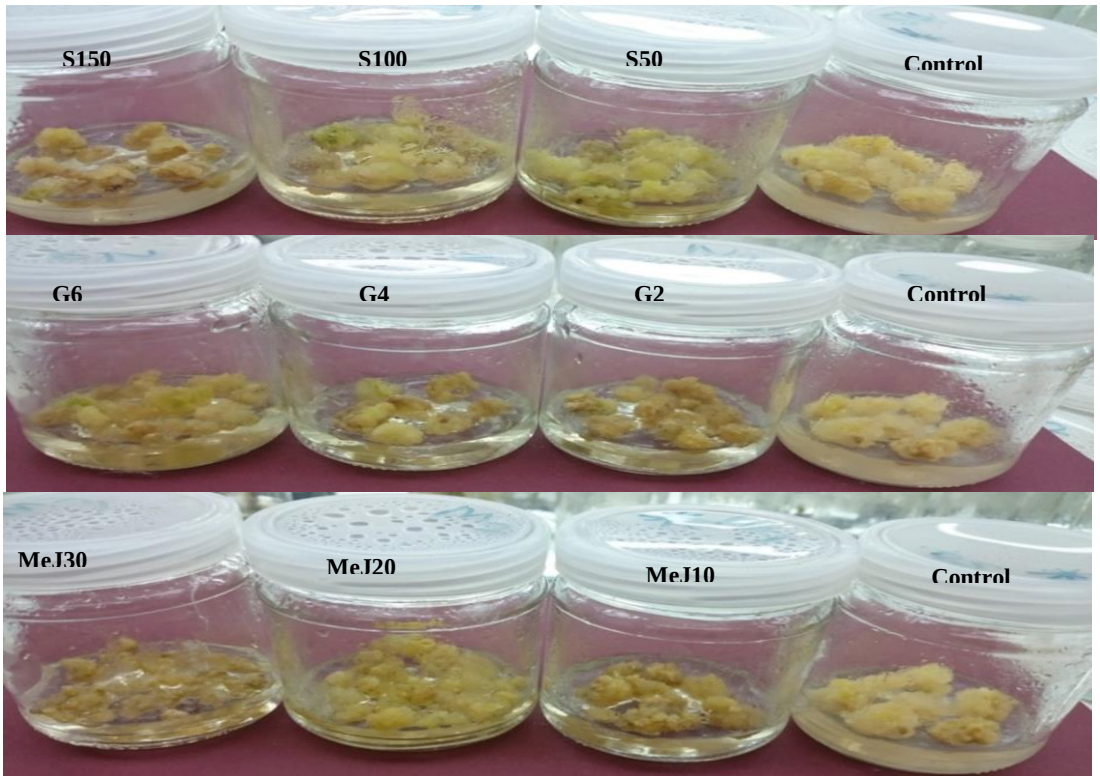

Figure 1. Development of cassava callus as affected by different elicitors.Where: Salicylic acid (S50-S100-S150;50,100,150mg/l); Glutathione (G2-G4-G6; 2,4,6 mM/l); Methyl jasmonate (MeJ10-MeJ20-MeJ30;10,20,30mg/l).

Data tabulated in Table 2 revealed that the minimum extraction yield was detected with control treatment (7.696 %) whilst the highest value of extraction yield (75.976 %), was recorded with medium addendum with salicylic acid (S100) followed by (G2) medium (48.776%) and (MeJ10) medium (45.296%) (Table2). Elevated concentration of glutathione and methyl jasmonate applications turned down the extraction yield percentage. While in the case of salicylic acid, the extraction yield started to increase from S50 (25.11%) to S100 recording the highest amount yield, then decreased at S150 recording 40.826% (Table 2). Compared with control, all elicitors recorded better extraction yields than that of control cultures.

Table 2 Extraction yield% of cassava dried calli

Treatments	Extraction yield% (w/w)
Control	7.696 ± 0.358^{i}
S50	25.11 ± 0.133^{g}
S100	75.976 ± 0.554^{a}
S150	40.826 ± 0.453^{e}
G2	48.776 ± 0.517^{b}
G4	43.686 ± 0.364^{d}
G6	30.493 ± 0.708^{f}
MeJ10	45.296 ± 0.547^{c}
MeJ20	24.183 ± 0.490^{g}
MeJ30	14.13 ± 0.280^{h}
LSD	1.139

Where: Salicylic acid (S50-S100-S150;50,100,150mg/l); Glutathione (G2-G4-G6; 2,4,6 mM/l); Methyl jasmonate (MeJ10-MeJ20-MeJ30;10,20,30mg/l).Means (±SE) the values with the same superscript letters in the same column are not significant whilst, values with different superscript letters in the same column are significantly different, LSD test at the 5 % probability level ($P<0.05$).

The extraction process of plant products which comprise a combination of active ingredients with several polarities is an important step in the detection and characterization of these compounds [36]. Many extraction techniques can be used, depending on the nature of the target compounds [37].

Due to a lack of information regarding *in vitro* produced metabolite in cassava callus cultures, we investigate the culture growth and contents of total carbohydrates, phenols and flavonoids in stem derived callus under elicitation with Salicylic acid, glutathione and Methyl jasmonate (Table 3).

The maximum value of total carbohydrates was recorded in the treatment of G4 (38.69 mg glu g^{-1}dw), followed by G2 (35.06 mg glu g^{-1}dw), S100 (22.48 mg glu g^{-1}dw), S150 (21.48mg glu g^{-1}dw), and then MeJ20 (21.28mg glu g^{-1}dw). Whereas control

treatment recorded the minimum carbohydrate content (11.62 mg glu g^{-1}dw) as shown in Table 3. It could be concluded that glutathione is the most efficient elicitor to raise total carbohydrate content in cassava calli cultures.

Table 3 Total carbohydrates, total phenols and total flavonoid contents of cassava calli cultures enhanced by different abiotic elicitors.

Treatments	Total carbohydrates (mg glug^{-1}dw)	Total phenols (mg GAE g^{-1}dw)	Total flavonoids (mg QE g^{-1}dw)	DPPH inhibition (%)
Control	11.62±0.619^{g}	0.44±0.054^{h}	0.16±0.040^{c}	80.21±0.622^{f}
S50	14.49±0.562^{f}	3.14±0.549^{g}	0.64±0.069^{b}	84.16±0.876^{e}
S100	22.48±1.167^{c}	21.47±0.289^{b}	1.09±0.129^{b}	88.21±0.530^{d}
S150	21.48±0.653^{c}	21.25±0.520^{b}	1.26±0.294^{ab}	84.05±1.174^{e}
G2	35.06±0.593^{b}	28.35±0.385^{a}	1.73±0.262^{a}	96.31±0.709^{a}
G4	38.69±0.665^{a}	28.74±0.733^{a}	1.63±0.222^{a}	96.60±0.607^{a}
G6	19.41±0.404^{d}	17.91±0.353^{d}	0.75±0.062^{b}	95.29±0.877^{a}
MeJ10	16.59±0.471^{e}	8.72±0.476^{f}	1.42±0.157^{ab}	90.19±0.718^{c}
MeJ20	21.28±0.997^{c}	19.91±0.566^{c}	1.49±0.191^{ab}	96.73±0.644^{a}
MeJ30	16.61±0.720^{e}	15.15±0.287^{e}	1.34±0.351^{ab}	92.72±0.427^{b}
LSD	1.756	1.118	0.498	1.821

Where: Salicylic acid (S50-S100-S150;50,100,150mg/l); Glutathione (G2-G4-G6; 2,4,6 mM/l); Methyl jasmonate (MeJ10-MeJ20-MeJ30;10,20,30mg/l); GAE: gallic acid equivalents; QE: quercetin equivalents. Results expressed as the means (±SE) the values with the same superscript letters in the same column are not significantly whilst, values with different superscript letters in the same column are significantly different, LSD test at the 5 % probability level ($P<0.05$).

Glutathione is a tripeptide (L--glutamyl-L-cysteinyl-glycine), and has diverse purposes in living cells [38-41]. Glutathione carries the active thiol group in shape of a cysteine residue; glutathione is antioxidant either directly by combining with either reactive oxygen or nitrogen species or by acting as a cofactor for various enzymes [42–45].

Bartoli et al., [46]; revealed that the boosting effect of glutathione might be due to its important roles in various biological processes [1]. In contrast to our results, total carbohydrate increment has been confirmed for *A. aucheri*and*O. Cartilaginous* cultures were treated with salicylic acid [47-48] and *B. oleracea* with methyl jasmonate [49].

The phenolic contents of the cassava callus extracts were calculated in terms of gallic acid spectrophotometrically at 750 nm (Table 3). Regarding phenolic content of cassava callus cultures, a comparable trend was remarked for the different elicitor treatments (Table 3). Generally, elicitor's application seems to significantly enhance total phenols accumulation compared to the control value (0.44 mg GAE g $^{-1}$dw).

For salicylic acid application, total phenols content was noticeably increased from S50 (3.14mgGAEg^{-1}dw) to S100 (21.47mgGAEg^{-1}dw) before faintly decreasing at S150 (21.25mgGAEg^{-1}dw). Similar behavior was observed with methyl jasmonate (8.72; 19.91; 15.15 mgGAEg^{-1}dw, respectively).

Table 3 revealed that the highest values of total phenols accumulation in cassava callus cultures were obtainedwithG4 and G2 of glutathione treatments (28.74 and 28.35mgGAEg^{-1}dw respectively), while control treatment recorded the minimum phenolic content (0.44mgGAEg^{1}dw). Phenolic molecules are significant plant ingredients with antioxidant activity due to their redox properties [50]. Groups of hydroxyls in plant extracts are accountable to facilitate the scavenging of free radicals. Both solvents and procedures of extraction are responsible for resolving the plants endogenous substances [51]. Furthermore, plant constituents are polar or non-polar naturally. Phenols compounds are commonly soluble in polar solvents owing to the existence of OH group; for that reason, methanol was chosen as the ideal solvent for extraction [52].

The total flavonoid contents were calculated in terms of catechin at 510 nm spectrophotometrically in cassava callus culture extracts. Data in Table 3 revealed that the application of salicylic acid increased the total flavonoid content recorded 0.64; 1.09 and 1.26 mgQEg^{-1}dw at S50, S100 and S150 respectively.

Total flavonoids content was slightly decreased with glutathione from G2 (1.73 mgQEg^{-1}dw) to G4 (1.63 mgQEg^{-1}dw), and then decreased significantly (0.75 mgQEg^{-1}dw) at G6 (Table 3). On the other hand, elicitation with methyl jasmonate gave total flavonoids values that fluctuated from 1.42 and 1.49 mgQEg^{-1}dw at MeJ10 and MeJ20 respectively and gradually decreased to record 1.34 mgQEg^{-1}dw atMeJ30 (Table 3). As in total carbohydrate and phenols content, the flavonoid content recorded the least value (0.16mgQEg^{-1}dw) with the control treatment. We can suggest that tested elicitor types and concentrations play a significant role in flavonoid accumulations in cassava callus cultures.

Flavonoids are one of the most studied secondary metabolites due to their antioxidant activity which depends on the position and number of free hydroxyl groups [53-54].

Phenolic and flavonoid compounds are necessary antioxidant modules which are responsible for radical scavenging founded on their capability for hydrogen atoms donation to free radicals [55]. Many reports indicate a direct relation between phenols and flavonoid content along with their antioxidant activity [56].

To evaluate cassava callus extracts regarding their antioxidant activity, DPPH methodology was applied to demonstrate the effect of elicitor type and concentration (Table 3). The DPPH is acknowledged as a simple, sensitive and rapidly performed technique to evaluate the radical scavenging potency of plant extracts [57-59]. The highest capacity of antioxidants in the callus cultures to scavenge DPPH

free radicals has achieved in MeJ20, G4 and G2 with 96.73%, 96.60 % and 96.31 % respectively, while the lowest was in control (free elicitor) medium of 80.21%.

From previously reported results, we confirmed that the crud extracts of cassava stem calli are considered a main source of indispensable phytoconstituents with a major role in therapeutic applications [21]. Likewise, the aqueous and methanolic extracts of cassava stem calli contained Phenolic acid, flavonoids and cinnamic acid derivatives such as gallic acid, sinapic acid, gentisic acid, syringic acid, caffeic acid, vanilic acid, protochuic acid, ferulic acid, coumarine, catachine, kaempferol and rutin which possess more potent antioxidant capacity [60]. In addition, the existence of phenolic compounds in cassava callus extracts implies their antioxidant characteristics which are responsible to protect the cell from oxidative damage [21].

The comprehensible correlation between antioxidant capacity and total phenols and total flavonoids is reflecting their importance in the antioxidant properties of *Manihot esculenta Crantz* calli. This agrees with numerous scientific reports on the relationship between the antioxidant capacity and total phenols and total flavonoids in many plant species [61-62].

Not only that but also there is a correlation between total carbohydrates and the sum of phenols and flavonoids (polyphenols) in the content in all studied samples of cassava callus cultures, where all abiotic elicitors increased the total carbohydrates amounts in a similar harmony with the increment of the polyphenols content more than the control as shown in Fig. 2.

Furthermore, it is observed that the highest amounts of both carbohydrates and polyphenols (38.69 and 30.37 mgglu/g DW, respectively) were verified using 4 mg/l glutathione, whilst 50 mg/l of salicylic acid is the least efficient elicitor (14.49 and 3.78 mgglu/g DW, respectively) compared to the control which recorded the lowest values (11.62 and 0.6 mgglu/g DW). Each abiotic elicitor affected the total carbohydrate accumulation as the same behavior on the polyphenols content at all its different concentrations as illustrated in Fig. 2. As well, it has appeared the presence of an incremental relationship of both carbohydrates and polyphenols dependent on increasing concentration of each elicitor till definite concentration before decreased slightly or significantly with the highest concentration of each elicitor (Fig. 2).

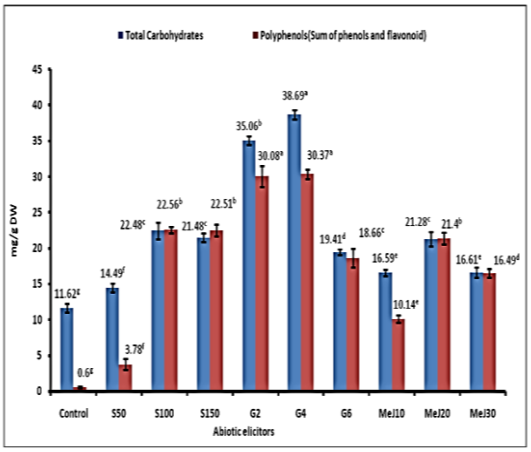

Figure 2. Total carbohydrates and sum of phenols and flavonoids content of cassava calli cultures enhanced by different abiotic elicitors, where: Salicylic acid (S50-S100-S150;50,100,150mg/l); Glutathione (G2-G4-G6; 2,4,6 mM/l); Methyl jasmonate (MeJ10-MeJ20-MeJ30;10,20,30mg/l).

Based on the previous investigations, it could be concluded this insight which recommends presenting a positive relationship between the total carbohydrates and the biosynthesis of phenols and flavonoids (polyphenols) in the in vitro cell cultures of *Manihot esculenta Crantz*. This insight is in line with Ibrahim et al. [63] who exhibited a higher effect of soluble sugar concentration in *Labisiapumila benthon* in the biosynthesis of its content of phenols and flavonoids structures, as well as agrees with Khatri et al. [64] who affirmed a significant positive relationship between phenols content and reducing sugar content in Nepalese Plants. Based on our results in Fig. 2 no doubt that all elicitors which enhanced the total carbohydrates content in cassava callus cultures, dependency increased their polyphenols accumulation as a result of the influenced role of soluble carbohydrates on the biosynthesis of phenols and flavonoids structures. This obtained scientific investigation has been confirmed by Cao et al. [65] who found a strong positive correlation between soluble carbohydrates (SC) and total phenols (TP)in all aquatic macrophytes, indicating to TP synthesis was dependent mostly on the SC availability. Upon all previous investigations, this research summarized the most potent capacity of all abiotic elicitors under study (salicylic acid, glutathione, methyl jasmonate) to enhance the bioactive metabolites accumulation (total carbohydrates, total phenols and total flavonoids) in cassava callus cultures with increment their extracts potentials as antioxidant activity. These findings match with Naik and Al-Khayri [66] who cleared that Salicylic acid, glutathione and methyl jasmonate were considered abiotic elicitors with wide effects on secondary metabolites accumulation. It

was reported that elicitation of *Salaciachinens* callus cultures with jasmonic acid enhanced the accumulation of total phenolic and flavonoid, content along with maximum antioxidant potential [67]. Furthermore, a mixture of salicylic acid and methyl jasmonate, in *Lonicera japonica* cultures improved the chlorogenic acids production with elevated antioxidant activity compared to *in vitro* control culture [68]. Consequently, the prospective cassava plants should be sponsored for health-boosting purposes.

Conclusion

Cassava stem callus cultures were scrutinized of their content accumulation of total carbohydrates, phenolics and flavonoids, using elicitation. The comprehensible correlation between antioxidant capacity and total phenols and flavonoids is reflecting their importance in the antioxidant properties of cassava callus cultures. So far to the authors' knowledge, the effect of different elicitors such as salicylic acid, glutathione and methyl jasmonate in cassava callus cultures were not reported before. These research findings could provide new insights for enhancing the production of potentially active plant metabolites in cassava, which are used as an alternative natural therapy for the safest progress in pharmacological purposes. Upon *Manihot esculenta Crantz*is a good starting point for the search for plant-based medicines for the promotion of the health of individuals to be a boon in the pharmaceutical industry.

Disclosure of conflict of interest: The authors declare that there is no competing interest.

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