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Heritability of polyphenols, anthocyanins and antioxidant capacity of Cameroonian cocoa (*Theobroma cacao* L.) beans

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This study investigates the heritability of polyphenolic, anthocyanin and antioxidant capacity of beans derived from four cocoa clones and their offsprings. These compounds were analyzed at 280 nm (polyphenols) and 520 nm (anthocyanins) by reversed-phase high-performance liquid chromatography (RP-HPLC) method using a photodiode array detector (PDA). The antioxidant capacity of methanolic extracts prepared from cocoa beans was measured by the DPPH and ABTS assays. Samples displayed catechin and epicatechin as the two main polyphenols. Epicatechin, which represents 1 to 5% of defatted cocoa seed powder, was hundred-fold higher than catechin while cyanidin-3-arabinoside was three-fold higher than cyanidin-3-galactoside. The two main anthocyanins found in our samples represent about 0.05% of defatted cocoa seed powder. All these compounds were genotype-dependent. Unidentified substances called A, B and C were also found in cocoa seeds. Substance A is discussed as a derivative of caffeic acid and an ester-bound compound. Substances B and C are oligomers of proanthocyanidins. Antioxidant capacity of cocoa beans obtained by 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay was higher than those obtained by DPPH assay. According to correlation tests, flavanols contributed better in the antioxidant capacity than anthocyanin. No maternal effect was detected in the transmission of polyphenol compounds suggesting a nuclear heritability.

Key words: *Theobroma cacao*, cocoa beans, polyphenolic compounds, anthocyanins, heritability.

INTRODUCTION

Cocoa beans are the fruit from the plant *Theobroma cacao* L., a plant tree originated in the rain forests of America whose culture has extended to equatorial areas of Africa and Asia. Cocoa beans are important in the economy of several countries such as Ivory Coast, Ghana, Indonesia, Cameroon and Nigeria. Cameroon is the fifth largest producer of cocoa beans in the world and the fourth in Africa. Cameroonian cocoa germplasm is constituted of two main varieties: Forastero and Trinitario. Criollo, is considered to exhibit one of the best flavor qualities which is scarce in Cameroon. Cacao powders, chocolate, cocoa-related products are phenolic-rich foods derived from the fermented, roasted and milled seeds of *T. cacao* L. These products, consumed all over the world,

are popular because of the antioxidant and antiradical properties of some of its phenolic constituents (phenolic acids, procyanidins and flavonoids) (Wollgast and Anklam, 2000). Flavonoids in cocoa are mainly flavan-3-ols, either monomeric (catechin and epicatechin) or oligomeric procyanidins (ranging from dimers to decamers), with appreciable amounts of anthocyanins (especially cyanidin glycosides) and flavonols (quercetin glycosides) (Keli et al., 1996; Hertog et al., 1993).

Several methods are intensively studied to determine antioxidant capacities of samples. These methods differ in terms of their assay principle and experimental conditions. Most of them are based on the study of a reaction in which a free radical is generated and how this reaction is inhibited by the addition of the sample of interest. Stratil et al. (2006) determined antioxidant activities of several species of commonly consumed vegetables by the Trolox equivalent antioxidant capacity (TEAC), using diphenyl-p-picrylhydrazyl radical (DPPH), and ferric reducing antioxidant power (FRAP) methods. The phenolic content values of test substances and total antioxidant activity of the sets of samples correlate very well for all the methods used (Paixao et al., 2007). The antioxidant properties of simple polyphenols have been largely studied by means of *in vitro* DPPH scavenging tests (Lee et al., 2003; Othman et al., 2007) and many studies have confirmed the protective action of cocoa procyanidins and flavanols using *in vitro* cellular models (Kenny et al., 2004; Zhu et al., 2005). Moreover, other benign properties related to the bioactivity of phenolics from cocoa were largely studied. They can increase the antioxidant capacity of solutions and slow the oxidation of LDL. They may also induce endothelium-dependent vessel relaxation (Karim et al., 2000). Cocoa procyanidins can reduce the production of inflammatory cytokines, while increasing the production of anti-inflammatory cytokines (Mao et al., 2000a; Mao et al., 2000b). Schramm et al. (2001) reported that cocoa procyanidins can increase the synthesis of the antithrombotic lipid prostacyclin, while reducing the production of the proinflammatory cysteinyl leukotrienes. Cocoa polyphenol oligomers have been reported to protect against peroxynitrate-dependent oxidation and nitration reactions (Arteel and Sies, 1999). Finally, cocoa was shown to decrease the expression of the activated conformation of glycoprotein IIb/IIIa and CD62P (Pselectin) on epinephrine-activated platelets (Rein et al., 2000).

Cocoa beans used in the confectionery industry come from a wide range of geographical areas, and may have different chemical and organoleptic properties. The chocolate producer must therefore select and combine these beans in various proportions in order to meet certain quality standards and economic specification. This task can be avoided if a screening of some cocoa clones in terms of their polyphenolic components can be done. So, some clone with high potential of bioactive compounds can be exhibited and exploited at industrial levels. As it is true that there are at least two parental clones in cocoa plantation, pollination will augment the number of unidentified hybrids. It will be very interesting then, to know if the content of polyphenol of cocoa hybrid can be predicted on the basis of those of its parental clones.

The aim of this study was therefore (i) to use high performance liquid chromatography (HPLC) to determine polyphenolic contents in cocoa beans from two Trinitario and two Forastero cocoa clones and their offsprings, (ii) and to determine and compare the antioxidant capacity of these samples applying two commonly used spectrophotometric methods (DPPH and ABTS assay methods). In addition, the correlation between specific polyphenols and the antioxidant activity in samples were investigated. The heritability related to these traits was also undertaken.

MATERIALS AND METHODS

Cocoa plant materials

Four Clones available in gene banks of the Cameroon Cocoa Development Corporation (SODECAO) at Mengang Station (South Cameroon) were used to create ten progenies: One local Trinitario (SNK16), one Trinitario introduced from Trinidad (ICS40), and two Forestero (Sca12 and T79/501). Crossings were realized in Mengang Station of SODECAO in May, June and July 2012 using hand-pollination techniques (Cilas, 1991) (Table 1).

Post-harvest treatment of cocoa

Two thousand ripe cocoa pods from different parental cocoa clones and hybrids were harvested from the experimental plots of the SODECAO at Mengang Station in the South Region of Cameroon. The ripe pods were split and beans obtained were fermented using the traditional heap method. The fermentation was done by heaping the extracted cocoa beans on the fermenting platform covered with banana leaves. The heaped beans were again covered with

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Abbreviations: ABTS, 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid); **Cya-ara,** cyanidin 3-o-α-L-arabinoside; **Cya-gal,** cyanidin 3-o-β-D-galactoside; **DPPH,** 2,2-diphenyl-1-picrylhydrazyl; **GAE,** gallic acid equivalents; **HPLC,** high performance liquid chromatography; **PDA,** photodiode array detector; **SPSS,** statistical package for the social sciences; **TEAC,** trolox equivalent antioxidant capacity.

Table 1. General description of cocoa crossings evaluated in this study.

Family	Crossings	Families	Back-crossings
F40	(♀) ICS40 × (♂) SCa12	F12	(♀) SCa12 × (♂) ICS40
F45	(♀) ICS40 × (♂) T79/501	F50	(♀) T79/501 × (♂) ICS40
F20	(♀) ICS40 × (♂) SNK16	F25	(♀) SNK16 × (♂) ICS40
F61	(♀) SCa12 × (♂) T79/501	F15	(♀) T79/501 × (♂) SCa12
F79	(♀) SNK16 × (♂) T79/501	F16	(♀) T79/501 × (♂) SNK16

banana leaves and fermented for six days with consecutive openings and turnings after every two days. The fermented cocoa beans were then sun dried on the bamboo mat for twelve days.

Reagents and standards

Epicatechin and quercetin were obtained from Sigma. Protocatechic acid and catechin were obtained from Aldrich and Fluka respectively. 3-α-L-arabinosyl cyanidinin and 3-β-D-galactosyl cyanidin were purchased from polyphenols AS. All solvents used were of analytical grade and purchased from Merck (Darmstadt, Germany). Water was purified in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Extraction and analysis of total phenol content (TPC) and total flavonoids (TF)

Two grams of dry cocoa beans were milled in 10 mL of n-hexane for fat removal. Most of the residual seed fat was extracted by flushing the powder with 25 mL n-hexane in a Buchner funnel. The phenolic compounds were extracted by agitating 0.5 g of the fat-free sample on ice three times with 50 mL 60% aqueous acetone with constant shaking. After centrifugation at room temperature at 5000 rpm for 15 min, the three supernatants were combined in a flask containing 2 mL of glacial acetic acid. The acetone was removed by rotary evaporation under partial vacuum at 40 ±1°C. The aqueous phase obtained was adjusted to 100 mL with Milli-Q Plus water in a volumetric flask. Total contents of polyphenolic compounds were analyzed from this aqueous phase. TPC of cocoa extracts was determined spectrophotometrically according to a modified method of Lachman et al. (1998). Briefly, to a 0.5 mL aliquot of samples, 2.5 mL Folin-Ciocalteu's reagent, 30 mL distilled water and 7.5 mL of 20% Na_2CO_3 were added and filled up to 50 mL with distilled water. After 2 h the absorbance of blue coloration was measured at 765 nm against a blank sample. To determine the content of total flavonoids, these compounds were precipitated using formaldehyde, which reacts with C-6 or C-8 on 5, 7-dihydroxy flavonoids to form methyl derivates that further react with other flavonoid compounds also at positions C-6 and C-8. The condensed products of these reactions were removed by filtration and the remaining non-flavonoid phenols were determined as previously described. Total flavonoid content was calculated as the difference between total phenol and non-flavonoid content. Gallic acid was used as the standard and the results were expressed as mg gallic acid equivalents (GAE) per gram of defatted cocoa product (Kramling and Singleton, 1969). All measurements were performed in triplicate.

Purification of polyphenols

To clean the sample, 30 mL of the previous aqueous phase were mixed five times with 30 mL ethyl acetate. After 1 min of shaking, the aqueous phases were discarded and the organic phases combined, dried by the addition of 20 g anhydrous Na_2SO_4 and filtered after 5 min in the dark with Whatman paper No. 2. The salt residue was discarded and the clear organic phase was dried at 40°C under vacuum. The dried extract of polyphenolic compounds was dissolved in 5 mL of pure methanol (Lichrosolv, Merck) and filtered with Millipore paper (0.45 mm). The pure polyphenol extracts were stored at - 20°C until HPLC analyzed.

Purification of anthocyanins

Purification of anthocyanins was conducted from the 100 mL aqueous phase using a Sep-Paks Vac C18 6cc column (Waters). The column was first eluted with a mixture of pure methanol (10 mL): 2% acetic acid (10 mL). A 20 mL aliquot of the aqueous phase sample was loaded onto the column and washed with 2.5 mL of 2% acetic acid. Anthocyanins were then eluted twice from the column with 5 mL pure methanol analytical grade (Lichrosolv, Merck). The eluted fractions were combined and dried by rotary evaporation. The residues were re-suspended in 2 mL of a mixture of pure methanol and acetic acid 2%.

Analysis of polyphenolic and anthocyanin compounds by reverse-phase HPLC

Chromatographic analyses were carried out on Waters HPLC system equipped with an A2-200 automatic injector, Knauer HPLC pump 64, Knauer HPLC program 50 solvent controller, Waters 996 Photodiode Array Detector (PDA) and analyzed with Millenium TM 3.2 software (Millipore Corporation, Milford, MA, USA). Separation of polyphenols was performed on a LicroCart 250-4 octadecylsilyl (ODS) C18, 5 mm particle [RP-18 (5 mm)] column (Merck) at 26°C. The guard column consisted of a LicroCart 4-4Lichrospher 100 RP-18 (5 mm) (Merck). The binary mobile phase (Table 2) consisted of 2% acetic acid in water (A) and acetonitrile-water-concentrated acetic acid mixture (4:9:1 v/v/v) (B). Twenty microlitres of sample was injected into the column. The separation of polyphenols was monitored using a PDA detector at 280 nm and anthocyanins were recorded at 520 nm. Identification of each peak was confirmed by comparison of retention time and coelution with authentic standards of Protocatechic acid, catechinhydrate, epicatechin, cyanidin-3-galactoside and cyanindin-3-arabinoside.

ABTS radical scavenging assay

The Trolox equivalent antioxidant capacity (TEAC) of cocoa extracts was also estimated by the ABTS radical cation decolorization assay (Re et al., 1999). Stock solutions of ABTS (7 mM) and potassium peroxodisulfate (140 mM) in water were prepared, and mixed together to a final concentration of 2.45 mM

Table 2. Binary gradient used for the separation of polyphenolic and anthocyanin compounds in cocoa beans.

Times (min)	Flow rate (ml/min)	Solvent A (%)	Solvent B (%)
0	1.2	90	10
8	1.2	90	10
38	1.1	77	23
50	1.0	60	40
70	1.0	10	90
73	1.0	10	90
78	1.2	90	10
93	1.2	90	10

Table 3. Rate of success of hand-pollination.

Groups	Family	Crossing	Number of test	Number of success	Percentage of success (%)
1	F40	(♀) ICS40 × (♂) SCa12	1000	360	36
	F12	(♀) SCa12 × (♂) ICS40	1000	460	46
2	F45	(♀) ICS40 × (♂) T79/501	1000	110	11
	F50	(♀) T79/501 × (♂) ICS40	1000	220	22
3	F20	(♀) ICS40 × (♂) SNK16	1000	20	2
	F25	(♀) SNK16 × (♂) ICS40	1000	40	4
4	F61	(♀) SCa12 × (♂) T79/501	1000	360	36
	F15	(♀) T79/501 × (♂) SCa12	1000	410	41
5	F79	(♀) SNK16 × (♂) T79/501	1000	320	32
	F16	(♀) T79/501 × (♂) SNK16	1000	410	41

potassium peroxodisulfate. The mixture was left to react overnight (12 to 16 h) in the dark, at room temperature. On the day of analysis, the ABTS radical solution was diluted with methanol to an absorbance of 0.70 (± 0.02) at 734 nm. All measurements were performed as follows: 100 µL of cocoa extract were added to 2.0 mL of the ABTS radical solution, and the absorbance readings were taken after exactly 6 min against the appropriate reagent blank of 100 µL of methanol instead of the sample. The results, obtained from triplicate analyses, were expressed as Trolox equivalents, and derived from a calibration curve determined for this standard (100 to 1000 µM).

DPPH radical scavenging assay

Antioxidant capacity of the cocoa extracts was determined using the DPPH radical scavenging assay described by Brand-Williams et al. (1995), with some modifications. Briefly, 100 µL of methanol cocoa extract was added to 1.9 mL of 0.094 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol. The free radical scavenging capacity using the free DPPH radical was evaluated by measuring the decrease of absorbance at 517 nm every 2 min until the reaction reached its ‘‘plateau” state. Antioxidant capacity was expressed as mmol/L Trolox equivalents, using the calibration curve of Trolox (0 to 1000 µM), a water soluble vitamin E analogue.

Estimation of the heritability

For the different parameters measured, heritability was estimated according to Falconer (1974). This estimation considers the regression slope between means of parents and progeny.

Statistical analyses

Values are given as means of three measurements. Where appropriate, the data were tested by one-way ANOVA using the software SPSS 18.0 for windows, followed by Tukey post hoc test. Correlations between antioxidant capacities using DPPH and ABTS essays and content of polyphenolic compounds were also performed using the same software package.

RESULTS AND DISCUSSION

Hand-pollination

Hand-pollination test was less successful in F5 and F9 families with 11 and 18%, respectively. These results were better in F15 (41%) and F61 (36%) families (Table 3).

Table 4. Total polyphenols, total flavonoids, catechin, epicatechin, Cyanidin-3-galactoside and Cyanidin-3- arabinoside contents in defatted cocoa powder (DCP) determined by HPLC analysis.

Group	Clone/family	PPT (mg/g)	Flavonoid (mg/g)	Flavanol (µg/g)		Cyanidin (µg/g)	
				Catechin	Epicatechin	Cyanidin-3-galactoside	Cyanidin-3-arabinoside
1	ICS40	112.85^{a}	60.15^{a}	245.58^{ef}	30001.8^{d}	25.6^{b}	163.9^{c}
	Sca12	138.4^{cd}	66.41^{b}	234.3^{de}	28940.4^{c}	84.4^{cd}	252.2^{ef}
	F40	147.14^{d}	121.81^{d}	260.1^{fg}	31210.1^{de}	76.4^{c}	196.2^{d}
	F12	141.68^{d}	105.89^{c}	238.4^{de}	28750.1^{c}	73.6^{c}	242.5^{e}
2	ICS40	112.85^{a}	60.15^{a}	245.58^{ef}	30001.8^{d}	25.6^{b}	163.9^{c}
	T79/501	114.95^{a}	70.16^{a}	159.7b	36513.8^{ef}	319.1^{k}	789.1^{k}
	F45	168.53^{f}	129.53^{e}	253.3^{efg}	30722.1^{d}	251.9^{i}	529.5^{i}
	F50	117.65^{ab}	75.59^{a}	79.9^{ab}	14485.1^{b}	0,00	90.2^{b}
3	ICS40	112.85^{a}	60.15^{a}	245.58^{ef}	30001.8^{d}	25.6^{b}	163.9^{c}
	SNK16	130.18^{c}	84.51^{b}	754.5^{k}	45513.7^{g}	166^{g}	446.2^{h}
	F20	147.51^{d}	115.21^{cd}	241.6^{ef}	31203.2^{de}	98.8^{d}	275.3^{ef}
	F25	136.14^{cd}	98.14^{cd}	195.7^{c}	28613.9^{c}	118.2^{e}	260.7^{ef}
4	Sca12	138.4^{cd}	66.41^{b}	234.3^{de}	28940.4^{c}	84.4^{cd}	252.2^{ef}
	T79/501	114.95^{a}	70.16^{a}	159.7^{b}	36515.8^{ef}	319.1^{j}	789.1^{k}
	F61	191.24^{g}	165.12^{f}	212.3^{cd}	28956.2^{c}	142^{f}	365.4^{g}
	F15	145^{d}	110.16^{cd}	226.1^{cd}	4289.8^{a}	7.2^{a}	42.46^{a}
5	SNK16	130.18^{c}	84.51^{b}	754.5^{k}	45513.7^{g}	166^{g}	446.2^{h}
	T79/501	114.95^{a}	70.16^{a}	159.7^{b}	36515.8^{ef}	319.1^{j}	789.1^{k}
	F79	150.82^{d}	105.71^{cd}	554.5^{hi}	36342.1^{ef}	219.5^{h}	606.3^{j}
	F16	153.12^{de}	88.77^{c}	622.3^{ij}	41690.4^{g}	265.6^{i}	629.0^{j}
	Means	142.51^{d}	98.85^{c}	305.59^{fg}	29802.3^{ef}	132.02^{ef}	349.2^{g}
	Sums	1995.21	1198.23	4278.28	417232.7	1848.7	4888.96

*Values with the same letter in the same column are not significant different ($P < 0.05$).

Total phenol content and total flavonoid content of cocoa extracts

Total polyphenols, total flavonoids, catechin, epicatechin, cyanidin-3-galactoside and cyanidin-3-arabinoside contents in defatted cocoa extracts determined by HPLC analysis are presented in Table 4. TPC varied from 112.8 mg GAE/g in ICS40 beans to 191.24 mg GAE/g in F61 beans, while TFC ranged from 60.15 mg GAE/g in defatted ICS40 beans to 165.12 mg GAE/g in defatted F61 beans. According to multiple comparison tests, none hybrids displayed TPC values similar to those of their parent. For TFC, only F50 hybrid presented the same value compared to that of its parent. In a study on non-alkaloidal cocoa powder extract, obtained by using 70% methanol as the extraction solvent, Bonvehi and Coll (1997) estimated TPC of 58 mg GAE/g of cocoa powder was obtained. These results are different to those obtained in our study (142.51 mg GAE/g of defatted cocoa powder), but it is known that TPC varies depending on the cocoa bean variety, geographical origin, ripeness degree (harvest season) and post-harvest conditions, such as fermentation, drying, roasting, processing and storage (Wollgast and Anklam, 2000). In the evaluation of major chemical components in beans of some selected international cocoa clones in Ghana, Dadzie et al. (2014) confirmed the existence of significant correlations among the major chemical components in cocoa beans and the influence of genotype on the chemical component concentration. In 2-day-old fermented-like cocoa beans, the average total content ranged from 101.3 to 143.6 mg g^{-1} DM (Niemenak et al., 2006). Those values are close to our results and this may be because, the authors values were obtained in 6-day-old fermented-like beans. TFC in our samples represented about 70% of TPC and the assertion that flavonoids are the main polyphenol compounds found in cocoa is confirmed. Each sample of defatted cocoa seed polyphenols exhibits seven significant peaks at λ = 280 nm (Figure 1). Two of these peaks

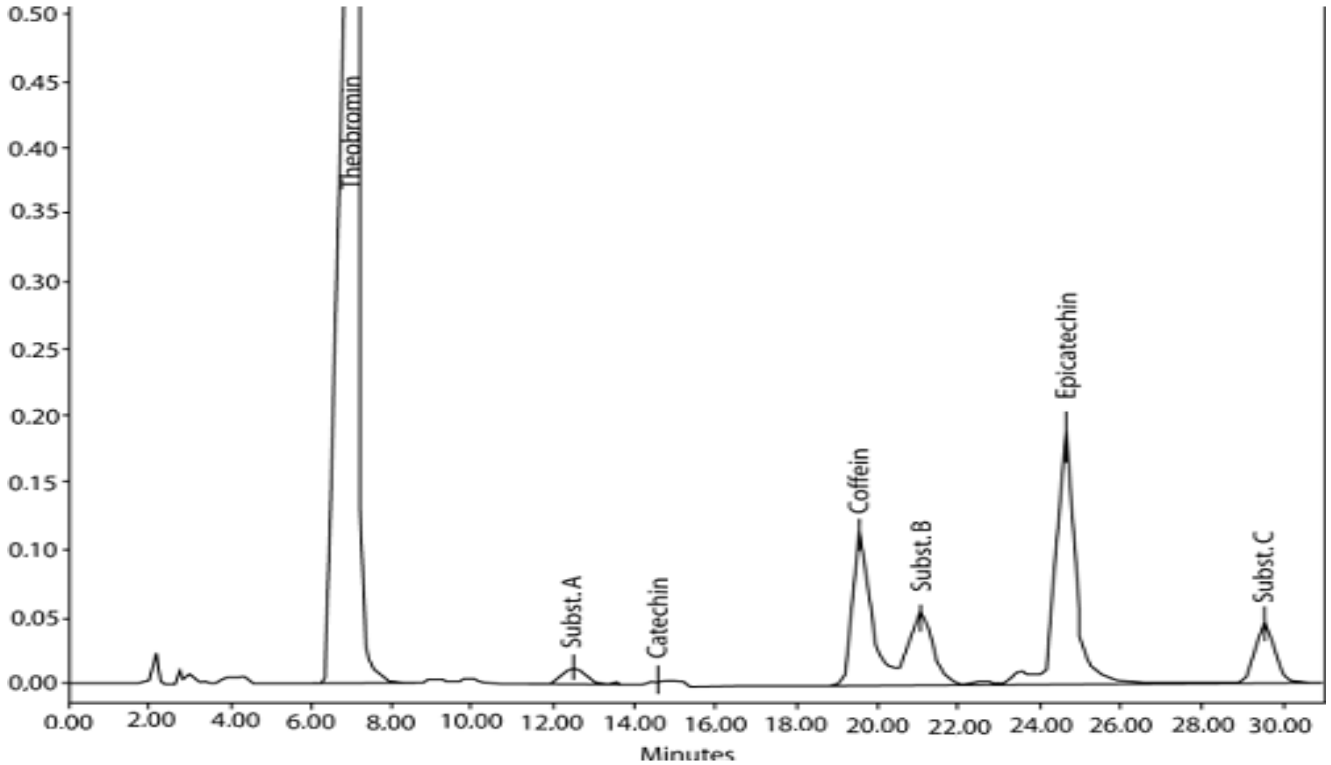

Figure 1. Representative chromatogram of a sample of defatted cocoa at λ = 280 nm. (AU: Absorption Units).

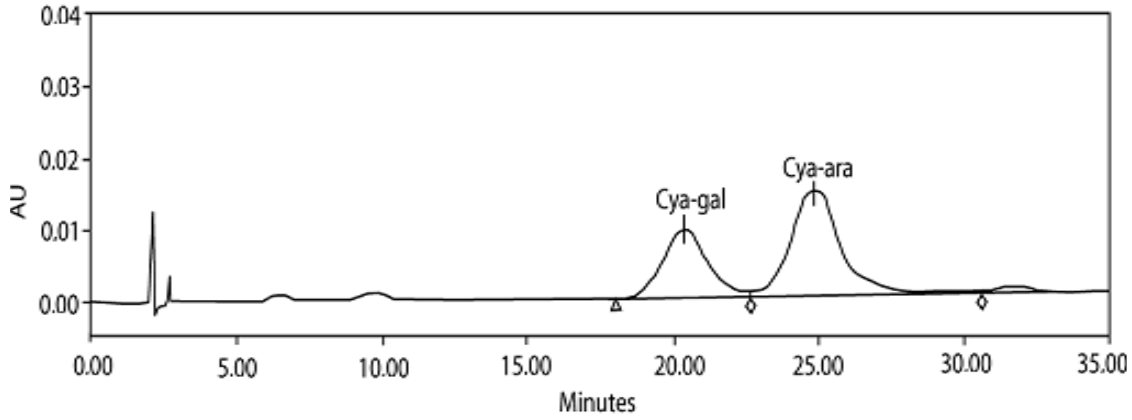

Figure 2. Representative chromatogram of a sample of defatted cocoa at λ = 530 nm. Cya-galcyanidin 3-o-β-D-galactoside, Cya-ara: cyanidin 3-o-α-L-arabinoside.

correspond to the two purine alkaloids; caffeine and theobromine, which are commonly found in cocoa seeds. HPLC chromatograms revealed three unidentified compounds, namely substances A (RT = 12.41 min), B (RT = 20.99 min), C (RT = 29.56 min). Protocatechic acid and quercetin were not detected. Two peaks were present at λ = 520 nm in defatted cocoa seed samples that contained anthocyanins (Figure 2). These two peaks were identified as cyanidin 3-galactoside and cyanidin 3-arabinoside.

Epicatechin content by the sum of means (29802.7 µg/g DCP on average) was hundred-fold higher than catechin content (305.6 µg/g DCP on average). Likewise the quantity of cyanidin-3-arabinoside by the sum of means (349.2 µg/g DCP on average), was about three-fold higher than Cyanidin-3-galactoside (132.02 µg/g

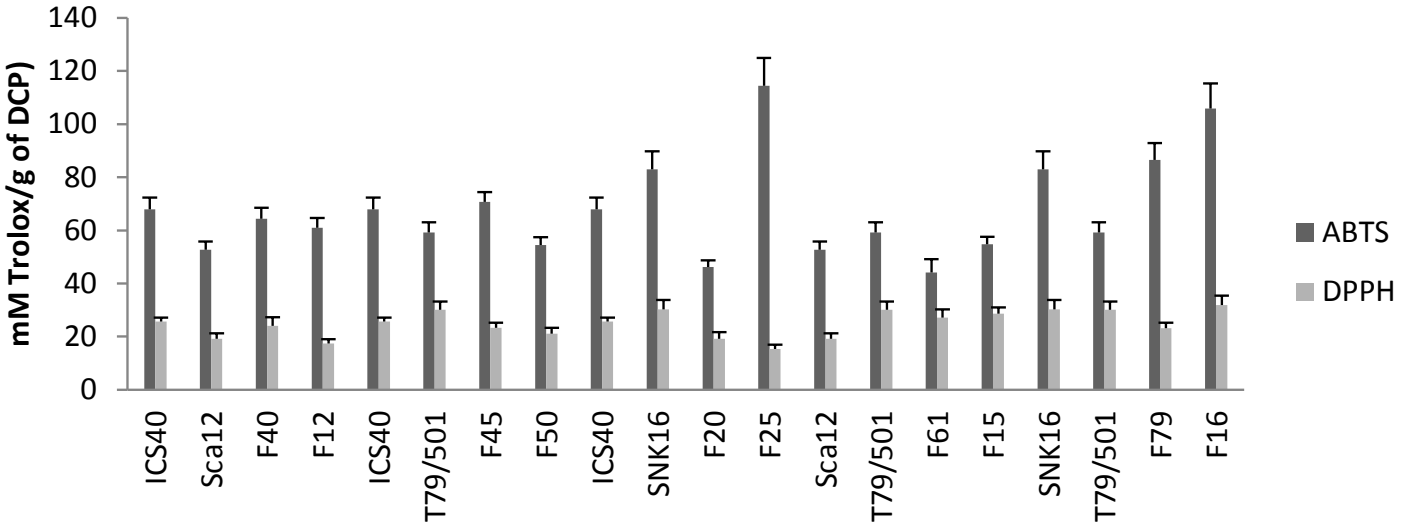

Figure 3. Antioxidant capacity of cocoa extracts determined by ABTS and DPPH assays. DCP: Defatted cocoa powder (The results are expressed as averages of three independent measurements. Values with the same letter are not significant ($P < 0.05$) different).

DCP on average). Among the four cocoa clones, SNK16 was found to be highest in catechin and epicatechin content, while T79/501 possessed the greatest quantity of cyanidin-3-galactoside and cyanidin-3-arabinoside.

Among hybrids, individuals of F16 family displayed the highest content in flavanols (catechin and epicatechin) and cyanidins. Except F40, F12 and F16 hybrids which displayed flavanol values similar to those of at least one of their parent, all other hybrids presented identical values to those of their parent in term of flavanols and cyanidins (Table 4). For Niemenak et al. (2006), it was well established that the predominant polyphenols identified in dried defatted cocoa bean was epicatechin followed by catechin.

In the same way, Wu et al. (2004) and Gu et al. (2004) also stated that cocoa beans are a concentrated source of antioxidants and flavonoids, with the flavan-3-ols and their derivatives being present in high concentrations. Among unidentified compounds, substance A, due to the PDA results will be a derivative of caffeic acid and an ester-bound compound. Substance B and C will be oligomers of proanthocyanidins that have been detected in cocoa seed (Adamson et al., 1999). As for the cyanidin 3-O-β-D-galactoside, a variation between 0 and 319.19 $\mu g.g^{-1}$ defatted cocoa was found, whereas the content of cyanidin 3-O-α-L-arabinoside ranged from 42.46 to 789.13 $\mu g.g^{-1}$ in defatted cocoa. These values were a little lower than those obtained by Elwers et al. (2009) in cocoa seeds and this can be due to variation in the methods used by the authors for the extraction of anthocyanins.

Antioxidant capacity of cocoa extracts

A standardized method for the determination of antioxidant properties of certain foods and beverages has not yet been established, so using more than one method for evaluating antioxidant capacity is highly recommended. In this study, two different antioxidant assays (ABTS and DPPH) for the evaluation of antioxidant capacity of various cocoa beans were applied and the obtained results are shown in Figure 3. Comparing the results of the applied radical scavenging assays on cocoa extracts, it can be noticed that methanol cocoa extracts exhibited high antioxidant potential, but the efficiency of radical scavenging differs markedly with regard to each cocoa clone.

Methanol extracts of SNK16 and T79/501 cocoa clones were the most efficient DPPH radical scavengers (30.2 and 30.1 mmol/L Trolox, respectively). F12 and F25 cocoa hybrid exhibited the least DPPH radical scavenging capacity in methanol extracts with 17.4 and 15.4 mmol/L Trolox, respectively. In the case of ABTS radical scavenging assay, methanol cocoa extract of F25 hybrid showed the best ABTS radical scavenging properties (114.42 mmol/L Trolox), better than F79 (86.5 mmol/L Trolox). F61 hybrids showed the lowest ABTS scavenging efficiency (44.1 mmol/L Trolox).

The antioxidant capacity of cocoa extracts obtained by ABTS assay was higher than that obtained by DPPH assay (Figure 3). Considering the fact that DPPH radical reacts only with lipophilic antioxidants, while ABTS radical reacts with both hydrophilic and lipophilic anti-oxidants, the difference between the results of these two radical scavenging assays becomes more obvious (Prior et al., 2005). Similar methodological differences were previously observed by Kim et al. (2002) and Arnao (2000), who also claim that these differences may be due to absorbance interruption at 517 nm by other com-pounds in the DPPH assay.

Table 5. Correlations between antioxidant capacities (ABTS, DPPH) and polyphenols and anthocyanins.

Compound	ABTS	DPPH	Mean
Catechin	0.66	0.62	0.64
Epicatechin	0.54	0.59	0.57
Cyanidin-3-Galactoside	0.38	0.42	0.40
Cyanidin-3-Arabinoside	0.54	0.44	0.39

Several studies showed a correlation between antioxidant activity and phenolic content (Nagai et al., 2003; Velioglu et al., 1998). Our findings report that cocoa beans exhibited the highest antioxidant activity. However, low correlation between ABTS, DPPH essays and TPC of cocoa beans were noticed (data not shown).

Our result is in agreement with those of Belščak et al. (2009) who stated that lower correlation coefficients between TPC and DPPH in both methanol and water extracts indicate that only a small content of the phenolic antioxidants in cocoa products account for the activity by scavenging free DPPH radicals. Arlorio et al. (2005) and Othman et al. (2007) suggested that high scavenging ability of cocoa extract compounds on DPPH and ABTS radicals could be attributed to other methanol-soluble compounds like methylxanthines, minor flavonoids and pigments.

Quite big correlation coefficients between flavanol and DPPH on one hand, flavanol and ABTS on the other hand in methanol extracts were found. These coefficients were about r = 0.64 for catechin and r = 0.57 for epicatechin (Table 5). For cyanidins, these coefficients were weaker than those of flavanol with r = 0.40 for cyanidin-3-galactoside and r = 0.39 for cyanidin-3-arabinoside (Table 5). These results indicated that flavanol contribute better than cyanidins in cocoa antioxidant capacity. These results also assumed that high scavenging ability on DPPH and ABTS radicals could not be exclusively due to flavanols and cyanidins in cocoa extracts. Arlorio et al. (2008), studying the impact of cocoa polyphenols in the antioxidant capacities, found a poor correlation between the contents of a specific polyphenolic fraction of cocoa (clovamide) and the antioxidant properties of cocoa. In Contrast, Othman et al. (2010) found that both ethanolic (r = 0.92) and water (r = 0.90) extracts of cocoa beans showed a significant positive and high correlation between epicatechin and ABTS value. These authors stated that epicatechin content in cocoa beans could be responsible for the antioxidant capacity.

Heritability

Heritability of biochemical compound contents was estimated using the regression between the average contents in flavanols and cyanidins of parents and those of their progenies (Figures 4 and 5). The calculated heritability (h^2) for catechin was 0.51 for the first crossings and 0.61 for reciprocal crossings. Concerning epicatechin, the heritability values obtained were 0.45 and 0.50, respectively, for the first crossings and their reciprocals. Cyanidin contents obtained in different crossings permitted estimation of the heritability of these characters. Heritability of the accumulated cyanidine-3-galactoside was weak for the two different crossings (h^2 = 0.42 for the first crossing and h^2 = 0.55 for reciprocal crossing).

For cyanidine-3-arabinoside, the estimation of the heritability was very close with 0.57 and 0.44. There was a weak relationship between flavanol contents of parents and those of progenies in the first crosses than reciprocal crossings (data not shown). These results were also observed for cyaniding-3-galactoside. Regardless of the studied character, the absence of a significant difference between the heritability values from reciprocal crossing portrays the absence of maternal heritability. This observation suggests that the heritability of biochemical compounds studied is nuclear rather than cytoplasmic. The relationship between phenolic compounds, amino acids, carbohydrates and resistance to *Phytophthora megakarya* in *T. cacao* detected no maternal effect in the transmission of these characters (Djocgoue et al., 2011; Ondobo et al., 2013).

Conclusion

Our results indicate that cocoa beans displayed high contents of polyphenolic compounds. Epicatechin content was hundred-fold higher than catechin content, likewise the quantity of cyanidin-3-arabinoside was about three-fold higher than Cyanidin-3-galactoside. However, those values were clone-dependant. According to multiple comparison tests, none of the offsprings displayed polyphenolic compounds values similar to those of their parent. Cocoa bean extracts gathered antioxidant capacity and those obtained by ABTS assay was higher than those obtained by DPPH assay. Flavanols contributed better in the antioxidant capacity than cyanidin and no maternal effect was detected in the transmission of polyphenol compounds.

Conflict of interests

The authors did not declare any conflict of interest.

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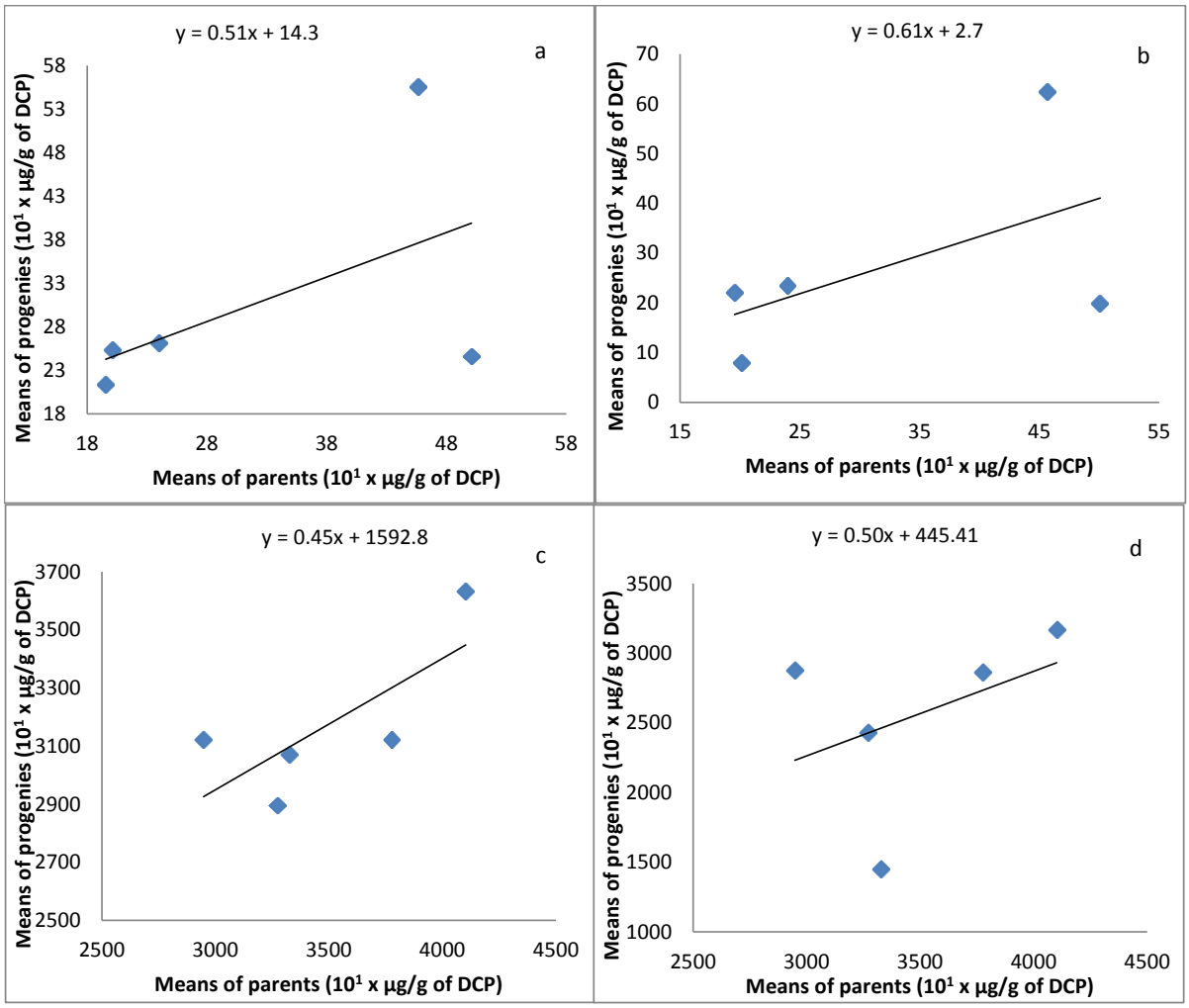

Figure 4. Evaluation of heritability (h^2) using the regression slope between parental clones and their progenies for catechin contents [crossing **(a)** and back-crossing **(b)**] and for epicatechin contents [crossing **(c)** and back-crossing **(d)**].

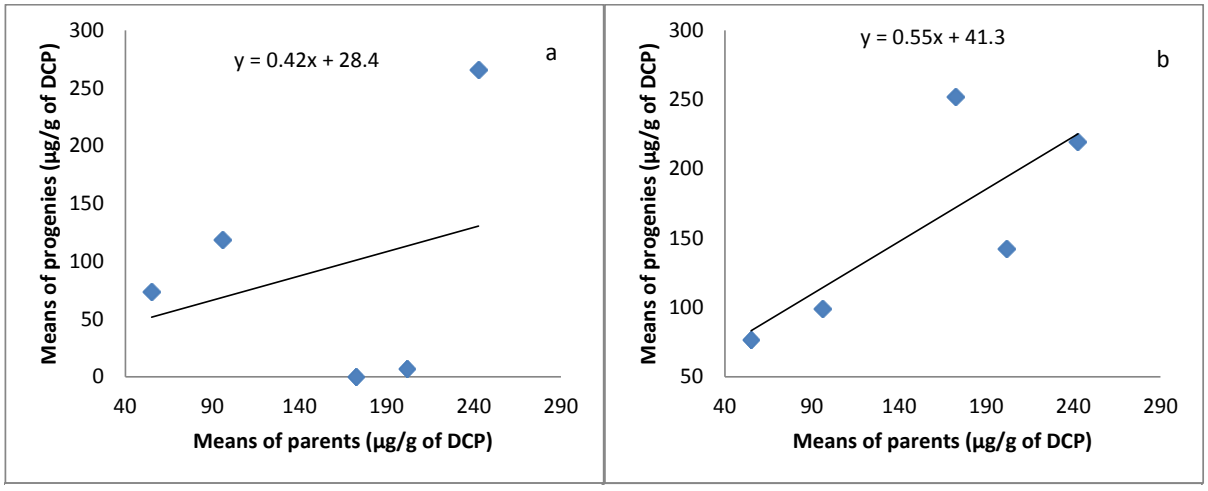

Figure 5. Evaluation of heritability (h^2) using the regression slope between parental clones and their progenies for Cyanidine-3-Galactoside contents [crossing (a) and back-crossing(b)] and for Cyanidine-3-Arabinoside contents [crossing (c) and back-crossing(d)].

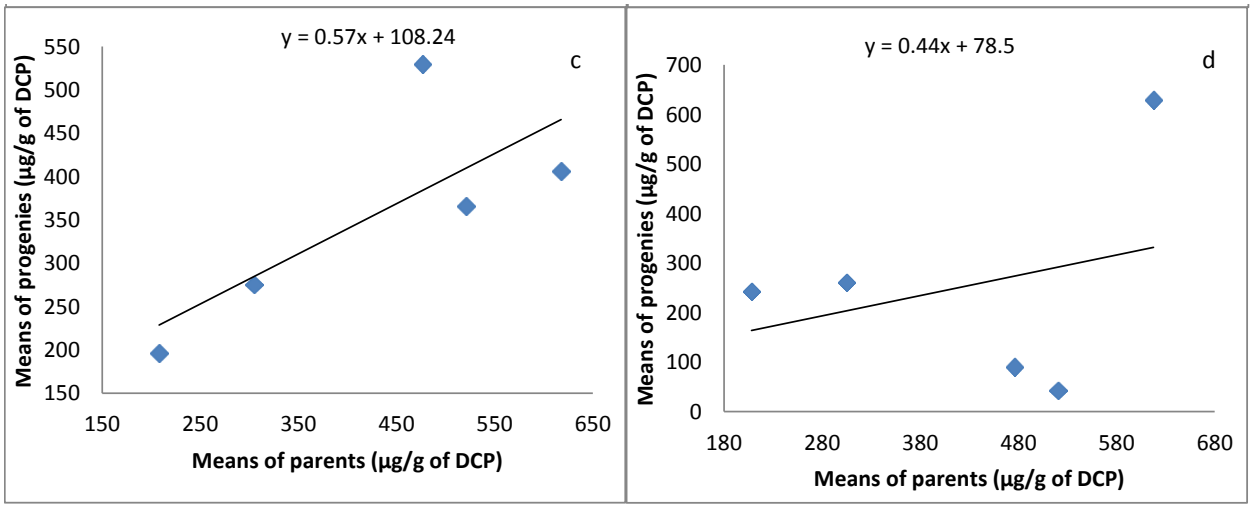

Figure 5. Contd.

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