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HETEROSIS AND HERITABILITY ESTIMATES OF PURINE ALKALOIDS AND POLYPHENOLS IN COCOA

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ABSTRACT

Cocoa (*Theobroma cacao* L.) is an important allogamous tropical tree crop, whose centre of diversity is considered to be in Central America. Dry cocoa beans from five cocoa clones, and their intercrossed hybrids were analysed based on the variation of alkaloids and polyphenolic compounds contents, in order to gain insights on the heterosis and broad-sense heritability. Polyphenols and alkaloids were analysed at 280 nm by HPLC, using a Photodiode Array Detector (PDA); while anthocyanins were separated with the SEP-PAK Vac 6cc 1000 mg (waters) column and measured at 520 nm with a PDA. Dry cocoa beans displayed high content of purine alkaloids (2.1 and 8.8 mg g^{-1} for caffein and theobromine, respectively), and polyphenols (25 and 2978 µg g^{-1} for catechin and epicatechin, respectively). Among the five cocoa clones, SNK16 was the highest in purine alkaloid (caffein and theobromin) and flavanol (catechin and epicatechin); while T79/467 possessed the greatest quantity of cyanidin-3-galactoside and cyanidin-3-arabinoside. From all the parameters studied, anthocyanins (Cyanidin-3-galactoside and cyanidin-3-arabinoside) exhibited the highest level of heterosis. Parental genotypes SNK16 and T79/467 showed good aptitudes for the combination of characters because their reciprocal hybrids F5 and F9, distinguished themselves by better levels of mid-parent heterosis values. Besides, the heritability value in strict sense of this Cyanidin-3-galactoside was very high. Absence of significant difference between genotypes, coming from reciprocal crossbreeding for Cyanidin-3-galactoside, suggests that this character in cocoa would be nuclear contrary to purine alkaloids and flavan-3-ols, where their transmission to offsprings can be stated as cytoplasmic.

Key Words: Caffein, catechin, *Theobroma cacao*

RÉSUMÉ

Le cacaoyer (*Theobroma cacao* L.) est une importante plante tropicale allogame originaire d'Amérique Centrale. Les teneurs en alcaloïdes et en polyphénols ont été analysées sur des fèves de cinq clones de cacao et de leurs descendants issus des croisements réciproques afin de déterminer l'hétérosis et l'héritabilité de ces métabolites. Les alcaloïdes et les polyphénols ont été analysés par HPLC à 280nm utilisant un détecteur à barrettes de photodiode (PDA) alors que les anthocyanines l'ont été sur une colonne SEP-PAK Vac 6cc 1000 mg à 520nm utilisant le PDA. Ces fèves ont des teneurs élevées en alcaloïdes puriques (2,1 et 8,8 mg.g^{-1} de caféine et de théobromine respectivement) et en polyphénols (25 µg.g^{-1} de catéchine et 2978 µg.g^{-1} d'epicatéchine). Des cinq clones utilisés, SNK16 s'est distingué par des teneurs les plus élevées en caféine, théobromine, catéchine et épicatéchine. De tous les paramètres analysés, les anthocyanines ont montré un niveau d'hétérosis élevé. Les génotypes SNK16 et T79/467 ont présenté une meilleure aptitude à la combinaison et les hybrides issus de leur

croisement réciproque (F5 et F9) ont présenté une meilleure hétérosis par rapport au meilleur parent. L'utilisation de ces deux clones dans un champ semencier serait très importante pour des industries pharmaceutiques car leur croisement génère des hybrides à haut potentiel en alcaloïdes et en polyphénols. De plus, l'absence d'une différence significative de cyanidine-3-galactoside entre hybrides réciproques suggère que l'héritabilité de ce caractère serait nucléaire contrairement aux alcaloïdes puriques et aux flavan-3-ols dont l'héritabilité serait de nature cytoplasmique.

Mots Clés: Caffein, catechin, *Theobroma cacao*

INTRODUCTION

Cocoa (*Theobroma cacao* L.) is an important allogamous tropical tree crop, whose centre of diversity is considered to be in Central America. Three major cultivated types can be distinguished as Criollos, Forasteros and Trinitarios. Criollo is considered to exhibit one of the best flavour qualities; while Trinitario is a hybrid between Criollos and Forasteros; or an intermediate type.

Theobroma cacao beans constitute an important ingredient in different kinds of foods such as cakes, biscuits, child-foods, ice-creams and sweets. It constitutes an inexpensive fat source and is the principal raw material of chocolate (Tafuri *et al*., 2004).

Cocoa bean is also known to contain the two purine bases, theobromine and caffeine. The relative proportions of these bases have been determined in the mature bean and delipidated dry cocoa powder (Niemenak *et al*., 2006; Effa *et al*., 2015).

These compounds exert an excitatory effect on the central nervous system, promote the psychical and physical activities of organisms, stimulate heart and kidney functioning, and inhibit the bronchial muscle tone (Ashihara and Crozier, 2001). Therefore, they are widely used in modern pharmaceutical practice (Temple, 2009).

The major polyphenolic compounds contained in cocoa seeds are catechins (3.0-6.0%), leucocyanidins (2.5%) and tannins (2.0-3.5%) (Afoakwa *et al*., 2012). Flavanols, the most abundant flavonoids in cocoa, comprise the monomeric flavanols, (+)-catechin and (-)-epicatechin, and their oligomeric and polymeric forms (procyanidins). (-)-Epicatechin has been reported as the major monomeric flavanol in cocoa, representing 35% of the total phenolic content (Wollgast *et al*., 2000). These compounds are associated with a decrease in low-density lipoprotein (LDL) oxidation, oxidative stress, platelet activation, platelet function, and an increase in high-density lipoprotein (HDL) concentration, antioxidant status, and NO bioactivity, together with an improvement in endothelial function (Andres-Lacueva *et al*., 2008).

Intercrossing different varieties of plants frequently produces hybrid offsprings with superior vigour and increased yields, in a poorly understood phenomenon known as heterosis. Two types of heterosis have been considered (Hochholdinger and Hoecker, 2007). The first type refers to the phenomenon in which the hybrid F_1 offsprings exhibit phenotypic characteristics that are superior to the mean of the two parents (mid-parent heterosis). The second, the better of the two parents (best parent heterosis) indicates that a hybrid trait performs significantly better than the better of the two homozygous parental inbred lines.

Heterosis has been extensively exploited in plant breeding, particularly in maize (Sultan *et al*., 2014; Hochholdinger and Hoecker, 2007; Fernandez-Silva *et al*., 2009), fertility, resistance to disease and to insect pest, or to climatic rigours (Rhode *et al*., 2004; Korn *et al*., 2008). Despite the fact that purine alkaloids and flavanols are signature component in cocoa beans, little information is available on heterosis of these traits in cocoa.

Another important genetic factor used by breeders is heritability. This term denotes the proportion of genetically controlled variability and is a very important biometrical tool for guiding plant breeder for the adoption of appropriate breeding procedures. Springer and Robert (2007) partitioned it as very high (> 90%), high (70-90%) medium (50-70%) and low (<50%). Heritability, however, indicates only the effectiveness with which selection of genotype

can be based on phenotypic performance, but fail to indicate the genetic progress. Heritability estimates along with genetic gains are more effective and reliable in predicting improvement through selection. Estimates of genetic advance predict the extent of improvement that can be achieved for improving the different characters.

Heritability of biochemical metabolites in the *Theobroma cacao/Phythophthora megakarya* interaction has been studied by many authors. Some of these authors stated that 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). As for heterosis, little information is available on heritability of purine alkaloids and flavanols in cocoa.

The objective of this study was to estimate heterosis and broad-sense heritability of cocoa purine alkaloids, flavanols and cyanidins and to determine the relationships of traits in the F_1 generation of height crosses under the same environmental condition in Cameroon.

MATERIALS AND METHODS

Plant materials. Five cocoa clones: two local Trinitario (SNK16 and SNK413), one Trinitario introduced from Trinidad (ICS40), and two Forestero (Sca12 and T79/467) available in gene banks of the Cameroon Cocoa Development Corporation (SODECAO) at Mengang Station (South Cameroon), were used to create eight progenies. Crossings were realised in Mengang Station of SODECAO in May, June and July 2012, using hand-pollination techniques (Cilas, 1991) (Table 1).

Post-harvest treatment. One thousand ripe cocoa pods from different parental cocoa clones and hybrids were harvested from the experimental plots of the SODECAO at Mengang Station. The ripe pods were split and beans obtained were fermented using the traditional heap method (Afoakwa *et al*., 2012). 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 banana leaves and fermented for six days, with consecutive opening and turnings after every two days. The fermented cocoa beans were then sun-dried on a bamboo mat for twelve days.

Reagents and standards. Protocatechiuc acid and catechin were obtained from Aldrich and Fluka (Hamburg, Germany). Epicatechin was obtained from Sigma (Hamburg, Germany); while caffeine and theobromine were from Merck (Darmstadt, Germany). Water was purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA). All solvents used were of analytical grade, purchased from Merck (Darmstadt, Germany).

Extraction of polyphenols. Two grammes 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 500 mg of the fat-free sample on ice three times with 50 ml of 60% aqueous acetone with constant shaking. Each round of shaking was followed by

TABLE 1. General description of cocoa crossings evaluated for heterosis and heritability at Mengang SODECAO Station in South Cameroon

Family	Crossings	Family	Back-crossings
F5	(♀) SNK16 × (♂) T79/467	F9	(♀) T79/467 × (♂) SNK16
F70	(♀) T79/467 × (♂) SNK413	F30	(♀) SNK413 × (♂) T79/467
F10	(♀) SCa12 × (♂) T79/467	F80	(♀) T79/467 × (♂) SCa12
F90	(♀) T79/467 × (♂) ICS40	F95	(♀) ICS40 × (♂) T79/467

centrifugation at 5000 rpm, for 15 min, at room temperature. The three supernatants were combined in a flask containing 2 ml of glacial acetic acid. Acetone was removed by rotary evaporation under a partial vacuum at 40 °C. The aqueous phase obtained was adjusted to 100 ml with Milli-Q Plus water in a volumetric flask. The total content in polyphenolic compounds was analysed from this aqueous phase.

For qualitative determination of polyphenols, 50 mg of dry cocoa beans was blended in 3 ml pure MeOH. The homogenate was agitated on ice for 15 min and centrifuged at 5000 rpm for 10 min. For each sample, extraction was done three times and the extracts were combined and concentrated by rotary evaporation. The dried polyphenolic compounds were resuspended in 1.5 ml pure methanol (Lichrosolv, Merck), and filtered with Millipore paper (0.45 µm). The pure polyphenolic extracts were stored at -20 °C until analysed by HPLC.

Purification of anthocyanins was conducted from the 100 ml aqueous phase, using a Sep-PaksVac 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 2% acetic acid.

Analysis of polyphenolic, anthocyanin compounds and purine alkaloids. Total contents of polyphenolic compounds were determined by the Folin–Ciocalteu procedure (Singleton and Rossi, 1965). Chromatographic analyses were carried out on Waters HPLC system equipped with an A2-200 automatic injector and Waters 996 Photodiode Array Detector (PDA). Separation of polyphenols was performed on a LicroCart 250-4 octadecylsilyl (ODS) C18, 5 µm particle [RP-18 (5 ìm)] column (Merck) at 26 °C. The guard column consisted of a LicroCard 4-4 Lichrospher 100 RP-18 (5 µm) (Merck). The binary mobile phase 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 were injected into the column and polyphenol was detected at 280 nm; while anthocyanins were recorded at 520 nm, using a PDA detector. The identification of each peak was confirmed by a comparison of retention and coelution time with authentic standards of protocatechiuc acid, catechinhydrate, epicatechin, caffeine, theobromine, cyanidin-3-galactoside and cyanindin-3-arabinoside.

Statistical analyses. Data were subjected to analysis of variance and multiple range test (LSD) using the Statistical Package for the Social Sciences (SPSS) 18.0 Software for Windows. Principal component analysis (PCA) was performed to establish associations among variables, using the SPAD 5.5 Statistical Software Package. Cluster analyses with polyphenols and alkaloids, using the unweighted pairwise group methods with arithmetical average (UPGMA) on the basis of Nei's (1978) genetic distance, were performed with the assistance of SPAD 5.5.

Heterosis -The methods for calculating heterosis were as follows:

Mid-parent heterosis (MPH) = [F1 -MP] x 100 / µ

Best-parent heterosis (BPH) = [F1 -BP] x 100/ µ

Where: F1= hybrid value; MP = the mid-parent value of both parents; BP = value of the better parent and µ = average value of all parents and F1 combinations in the factorial mating design.

To investigate possible heterosis of purine alkaloids and flavanol in cocoa beans, we performed reciprocal crosses between the five parental clones studied. Theoretically, if a heterozygous dominance effect is greater than the mean parental homozygous effects for a cross, a positive middle-parent (MP) heterosis would be expected; otherwise, a negative MP heterosis would be expected (Zhu, 1993; McCarty *et al*., 2007; Wu *et al*., 2010).

Heritability-Broad-sense heritability (h^2_{bs}) estimates of each cross based were calculated for traits related to secondary metabolites (purine alkaloids and polyphenols), using the following relationships:

$$Vp = V_G + V_E$$

$$h^2_{bs} = V_G / V_G + V_E$$

$$h^2_{bs} = [V_{F1} - (V_{P1} + V_{P2})/2] / V_{F1}$$

Where: V_{F1} = variance of any cross; V_{P1} = variance of female parent and V_{P2} = variance of male parent. Estimation of environmental variance (V_E) for any cross was calculated as $(V_{P1} + V_{P2})/2$. In each cross, variances between F1 and parents were obtained from the analysis of variance following a completely randomised design, assigning replication as classes.

Genetic correlations (G) among biochemical traits were calculated using the equation described by Zeng *et al*. (2007):

$$G = \sigma_{gxy} / (\sigma^2_{gx} \sigma^2_{gy})^{1/2}, \sigma^2_{gx} = 1/r(V_x - V_{ex})$$

$$\sigma^2_{gy} = 1/r\,(V_y - V_{ey}), \sigma_{gxy} = 1/r(Cov_{xy} - Cov_{exy})$$

Where: σ_{gxy} is the genetic component of covariance between variable x and y; σ^2_{gx} and σ^2_{gy} are genetic components of variance for x and y, respectively; Vx and Vy are variances of x and y, respectively; Vex and Vey are errors for x and y, respectively; r is the number of replicates.

RESULTS

Hand-pollination test was better in F30 and F80 families, with the percentage of success of 39 and 23, respectively (Table 2). These results were less successful in F95 (13%) and F5 (11%) families.

Caffein, theobromin, catechin, epicatechin, cyanidin-3-galactoside and cyanidin-3-arabinoside contents in defatted cocoa powder were determined by HPLC analysis (Table 3). Cocoa beans were rich in purine alkaloid (2.1 and 8.8 $mg.g^{-1}$ for caffein and theobromine, respectively) and theobromin was four-folds higher than caffein content (2.14 mg g^{-1} DCP on average).

Among the five cocoa clones, SNK16 was highest in purine alkaloid (caffein and theobromin) and flavanol (catechin and epicatechin) contents; while T79/467 possessed the greatest quantity of cyanidin-3-galactoside and cyanidin-3-arabinoside (Table 3). According to multiple comparison tests, hybrids for groups 1 and 4 displayed caffein values similar to those of their parents. For theobromin, none of the parents presented comparable value compared to that of their offsprings.

Epicatechin was the most abundant monomeric flavanol in cocoa powder (Table 3).

TABLE 2. Rate of success of hand-pollination of cocoa in a heterosis and heritability study at Mengang SODECAO Station in South Cameroon

Groups	Families	Crossings	Number of test	Number of successes	Percentage of success
	F5	(♀) SNK16 × (♂) T79/467	500	55	11
1	F9	(♀) T79/467 × (♂) SNK16	500	90	18
	F70	(♀) T79/467 × (♂) SNK413	500	80	16
2	F30	(♀) SNK413 × (♂) T79/467	500	195	39
	F10	(♀) SCa12 × (♂) T79/467	500	105	21
3	F80	(♀) T79/467 × (♂) SCa12	500	115	23
	F90	(♀) T79/467 × (♂) ICS40	500	75	15
4	F95	(♀) ICS40 × (♂) T79/467	500	65	13

TABLE 3. Caffein, theobromin, catechin, epicatechin, cyanidin-3-galactoside and cyanidin-3- arabinoside contents in defatted cocoa powder (DCP) determined by HPLC analysis

Group	Clone	Purine alkaloids (mg g^{-1})		Flavan-3-ol (µg g^{-1})		Cyanidins (µg g^{-1})	
		Caffein	Theobromin	Catechin	Epicatechin	Cyanidin-3-galactoside	Cyanidin-3-arabinoside
	SNK16	3.27f	13.42d	75.57g	4551.7g	166.05d	446.24g
1	T79/467	2.46d	10.09c	24.81d	3423.6e	244.90g	599.20h
	F5	2.97e	12.20c	47.27f	4136.9f	226.25f	575.20h
	F9	3.11e	12.76c	29.18e	4328.1f	278.57h	774.50i
	T79/467	2.46 d	10.09c	24.81d	3423.6d	244.90g	599.20h
2	SNK413	1.01a	4.13a	11.49a	1403.2a	26.31a	78.25a
	F70	1.64 b	6.74b	12.39a	2285.9c	163.12d	421.65f
	F30	1.68 b	6.89b	14.70b	2335.9c	191.12e	418.05f
	Sca12	2.08c	8.53b	23.37d	2894.4d	84.4b	252.29c
3	T79/467	2.46 d	10.09c	24.81d	3423.6e	244.90g	599.20h
	F10	1.69 b	6.93b	20.08c	2350.3c	146.55d	343.94e
	F80	1.47 b	6.04b	13.41a	2050.3b	116.55c	305.05d
	ICS40	2.16 c	8.85b	24.58d	3001.8d	25.68a	163.95b
4	T79/467	2.46d	10.09c	24.81d	3423.6e	244.90g	599.20h
	F90	2.10 c	8.60b	11.93a	2916.8d	201.35e	456.8g
	F95	2.18 c	8.95b	15.26b	3036.8c	227.35f	496.8g
	Means	2.14	8.79	24.93	2978.20	161.40	410.43
	Sums	27.88	114.21	324.09	38716.58	2098.24	5335.57

Means with the same letter are not significantly different at the 0.05 probability level as calculated by the Tukey's test within the respective trial

Flavan-3-ol cocoa beans content displayed that epicatechin with an average of 29.8 mg g^{-1} DCP was 100-folds higher than catechin (0.29 mg g^{-1} DCP). For catechin and in group 2, F30 and F70 hybrids showed values not significantly different to their SNK413 parent. In the same way, catechin content of T79/467 and SNK413 clones were similar to their F10 offspring. Group 4 is special for epicatechin since F90 and F95 hybrids displayed the same value with ICS40 parental clone.

Cyanidin-3-arabinoside was about three-folds higher than cyanidin-3-galactoside, and the two main anthocyanins found in our samples represented about 6% of defatted cocoa seed powder. As for the cyanidin 3-O-β-D-galactoside, a variation between 25.68 and 278.57 µg g^{-1} defatted cocoa was found, whereas the content of cyanidin 3-O-α-L-arabinoside ranged from 78.25 to 774.5 µg g^{-1}.

Heterosis analysis. Table 4 summarises the results of Mid-parent heterosis (MPH) and Best-parent heterosis (BPH) of hybrids derived from the crossing of five cocoa clones.

F5 and F9 hybrids distinguished themselves from other hybrids by the fact that they show positive MPH to five of six traits, except for catechin where they displayed a negative MPH. Contrary to other offsprings, F10 and its reciprocal F80 showed a negative MPH for all traits. Negative mid-parent heterosis was detected for four of the six traits except Cyanidin-3-Galactoside and Cyanidin-3-Arabinoside. For BPH, all hybrids presented a negative BPH except F9 individual which presented a low positive BPH

TABLE 4. Mid-parent heterosis (MPH) and Best-parent heterosis (BPH) of hybrids derived from crossing of five cocoa clones in a study on heterosis and heritability of cocoa crossings at Mengang SODECAO Station in South Cameroon

Hybrids	Caffein		Theobromin		Catechin		Epicatechin		Cya.-3-Gal.		Cya.-3-Ara.	
	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH
F5	3.7	-9.2	3.8	-9.1	-5.8	-37.4	3.7	-9.1	10.1	-7.6	10.0	-4.0
F9	8.8	-4.7	8.5	-4.9	-41.9	-61.4	8.7	-4.9	35.6	0.1	48.2	0.3
F70	-5.5	-33.3	-5.2	-33.2	-31.7	-50.1	-5.3	-33.2	20.3	-33.4	24.5	-29.6
F30	-3.2	-31.7	-3.1	-31.7	-19.0	-40.7	-3.2	-31.8	40.7	-0.2	24.5	-0.3
F10	-25.6	-31.3	-25.6	-31.3	-16.6	-19.1	-25.6	-31.4	-11.0	-40.2	-19.2	-42.6
F80	-35.2	-40.2	-35.1	-40.1	-44.3	-45.9	-35.3	-40.3	-29.2	-52.4	-28.3	-49.1
F90	-9.1	-16.7	-9.2	-14.8	-51.7	-51.5	-9.2	-14.8	48.8	-17.8	19.7	-23.8
F95	-5.6	-11.4	-5.5	-11.3	-38.2	-38.5	-5.5	-11.3	68.0	-7.2	30.2	-17.1

MPH = Mid-parent heterosis, BPH = Best-parent heterosis

for cyanidin compounds (Table 4). In terms of Best-parent heterosis, no positive BPH was observed for all the six traits (Table 5).

Heritability. Broad-sense heritability for traits related to purine alkaloids and polyphenols were evaluated in the F1 population (Table 6).

The heritability estimates for theobromin were low to moderate, ranging from 0 to 0.80, with an average of 0.27 (Table 6). For purine alkaloids, the heritability estimate of F90 hybrid was zero. This value was near to zero for F5 (theobromin), F30 (catechin) and for F95 individual (theobromin and catechin).

Analysis of variation in the sample was visualised in the Principal Component Analysis (Fig. 1). The two first Principal Components generated from all the data represented 94.24% of the total variability. Caffein, epicatechin, theobromin and cyaniding-3-arabinoside were the dominating features in the first Principal Component (74.16% of the total variability); while catechin and cyaniding-3-galactoside were the features with the highest weight in the second principal component (20.08% of the total variability).

Additionally, correlations among these traits at genotypic and phenotypic levels are given in Table 7. All traits showed significant genotypic and phenotypic correlations, except Cyanidin-3-Galactoside and catechin.

Dendrogram generated by the unweight pair group (UPGMA) permitted a better visualisation of the distribution (Fig. 2). Considering the distance of D 1.0, the cluster generated four groups. The first group with three individuals (SNK16, F5 hybrid and his reciprocal F9), was dominated by high amounts of purine alkaloids and flavan-3-ol. The second group consisted of all parental clones, except SNK16, and distinguished itself by low amounts of cyaniding-3-galactoside and cyaniding-3-arabinoside. The third group included T79/467 parental clone and hybrids, obtained with its cross with ICS40 counterpart (F90 and F95) and was characterised by average amounts of all metabolites. Group four, more polymorphic, contained four hybrids belonging to two different families were characterised by low amounts of purine alkaloids.

TABLE 5. The heterosis over mid-parent or better parent of six secondary compounds of cocoa hybrids raised at Mengang SODECAO Station in South Cameroon

Property/ subsistance	MPH				BPH			
	Average	Range	+N	-N	Average	Range	+N	-N
Caffein	-8,97	-35.2 to 8.8	2	6	-22,31	-40.2 to -4.7	0	8
Theobromin	-8,92	-35.1 to 8.5	2	6	-22,06	-40.1 to -4.9	0	8
Catechin	-31,16	-51.7 to -5.8	0	8	-43,08	-61.4 to -19.1	0	8
Epicatechin	-8,95	-35.3 to 8.7	2	6	-22,07	-40.3 to -4.7	0	8
Cya.-3-Gal.	22,95	-29.2 to 68.0	6	2	-19,83	-52.4 to 0.1	1	7
Cya.-3-Ara.	13,69	-28.3 to 48.2	6	2	-20,77	-49.1 to 0.3	1	7

MPH = Mid-parent heterosis based on population mean; BPH = Best-parent heterosis based on population mean; +N = number of combinations with positive heterosis; -N = number of combinations with negative heterosis

TABLE 6. Broad-sense heritability estimates for some secondary metabolites in four crossings and reciprocal of cocoa raised at Mengang SODECAO Station in South Cameroon

Hybrids	Caffein	Theobromin	Catechin	Epicatechin	Cya-3-Gal	Cya-3-Ara
F5	0.86	0.07	0.72	0.92	0.99	0.54
F9	0.11	0.15	0.19	0.60	0.71	0.68
F70	0.86	0.80	0.43	0.34	0.88	0.49
F30	0.21	0.62	0.01	0.48	0.73	0.49
F10	0.90	0.18	0.66	0.69	0.97	0.35
F80	0.36	0.35	0.66	0.09	0.98	0.30
F90	0.00	0.00	0.96	0.50	0.82	0.51
F95	0.94	0.01	0.08	0.58	0.80	0.56
Mean	0.53	0.27	0.46	0.52	0.86	0.49

TABLE 7. Genotypic (G) and phenotypic (P) correlation coefficients among six biochemical components of cocoa raised at Mengang SODECAO Station in South Cameroon

Subsistance		Caffeine	Theobromin	Catechin	Epicatechin	Cya-3-gal.	Cya-3-ara.
Theobromin	G	0.93**					
	P	0.79**					
Catechin	G	0.80**	0.74**				
	P	0.80**	0.72**				
Epicatechin	G	0.93**	0.86**	0.70**			
	P	0.99**	0.79**	0.79**			
Cya-3-gal.	G	0.59**	0.45**	0.19	0.67**		
	P	0.57**	0.42**	0.21	0.59**		
Cya-3-ara.	G	0.71**	0.58**	0.29*	0.79**	0.97**	
	P	0.70**	0.48**	0.32*	0.72**	0.96**	1

Significant at * 0.05 and ** 0.01 probability level, respectively

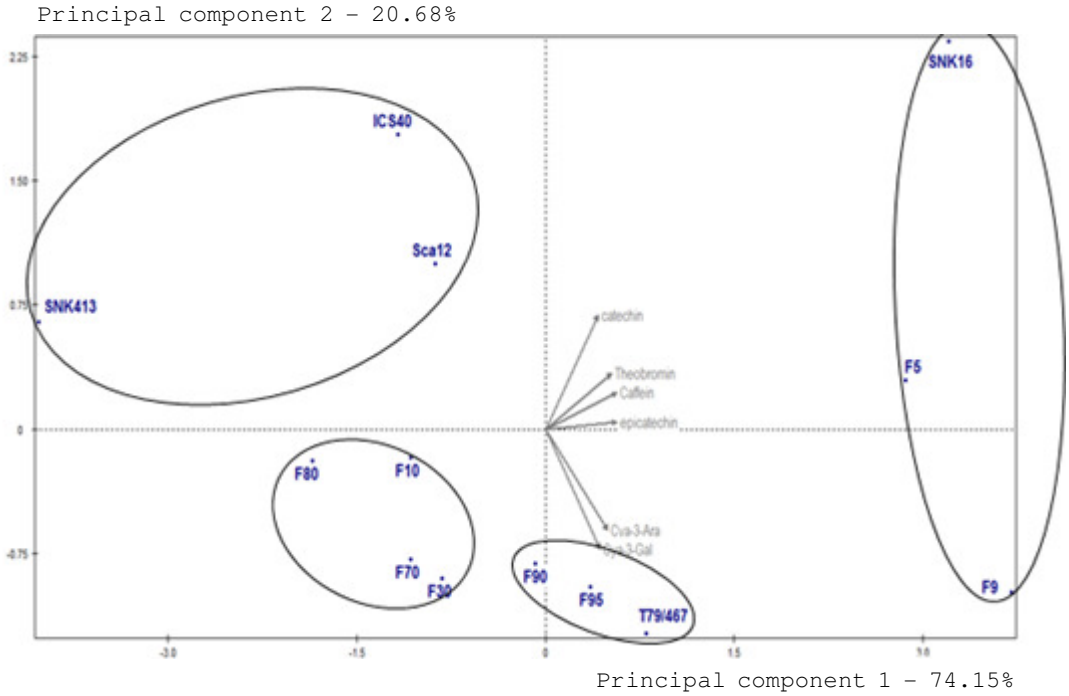

Figure 1. Loading plot of the first two principal components for caffein, theobromin, catechin, epicatechin, cyanidin-3-galactoside and cyanidin-3- arabinoside contents in defatted cocoa powder (DCP) determined by HPLC analysis.

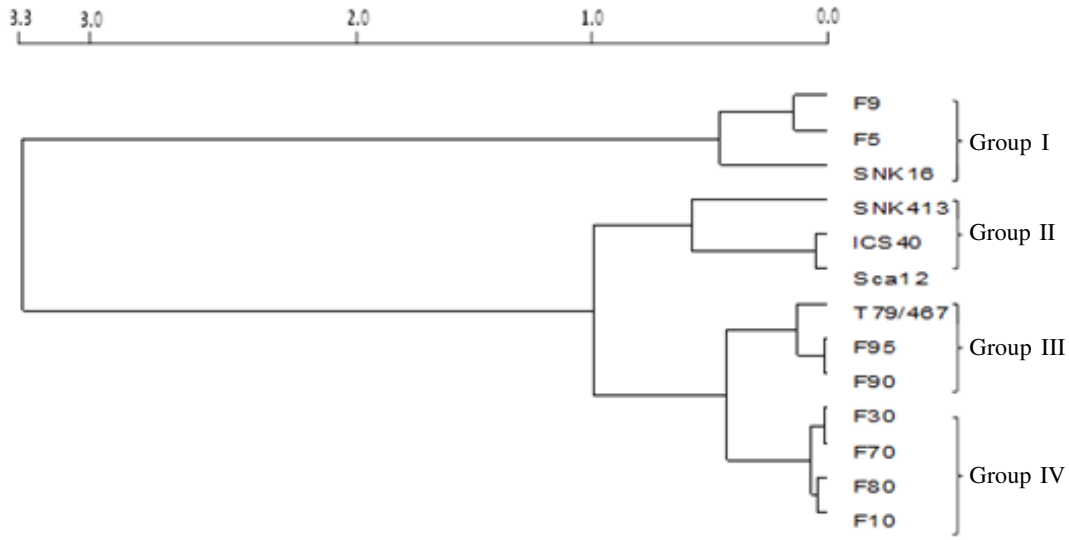

Figure 2. Dendrogram of cocoa clones and their offspring's generated by unweight pair group method (UPGAM) based on hierarchical cluster analysis using data on purine alkaloids, flavanols and cyanidins.

DISCUSSION

Analysis of purine alkaloids in cocoa beans in our study showed that theobromin was four-folds higher than caffein content. This result is in agreement with those obtained by many authors (Pura, 2001; Effa *et al*., 2015), who confirmed that theobromin is the dominant purine alkaloid present in cocoa beans. As expected, this study revealed that epicatechin was the most abundant monomeric flavanol in cocoa beans. Andres-Lacueva *et al*. (2008) also found that natural cocoa presented content of total flavonoids, equivalent to 2.65 mg g^{-1} (-)-epicatechin, representing the highest proportion. Recently, Tomas-Barberan *et al.* (2007) reported (+)-catechin values of 6.46 and 2.02 mg g^{-1} and (-)-epicatechin values of 25.65 and 3.30 mg g^{-1} for a polyphenol-rich and a conventional cocoa powder, respectively. Besides flavonoids, anthocyanin components were also detected in samples and the two main anthocyanins found (cyanidin-3-arabinoside and cyanidin-3-galactoside) represented about 6% of defatted cocoa seed powder. These values were similar to those obtained by Effa *et al.* (2015) in defatted cocoa powder and lower than those obtained by Elwers *et al*. (2009) in cocoa seeds. For the last authors, the difference could be due to variation in the methods used for the extraction of anthocyanins.

There was variation in the relative level of heterosis for different traits between different hybrids. This can be explained by the fact that heterosis is not the result of single locus action; nor does it simply reflect the overall extent of heterozygosity between parents. In fact, according to Springer and Robert (2007), the variation in levels of heterosis among traits leads to difficulties in accurately quantifying the amount of overall heterosis.

F90 hybrid and its reciprocal F95 displayed high value of MPH for cyanidin-3-galactoside. Furthermore, the parental genotypes (T79/467 and ICS40) showed good aptitudes for combination and their introduction into the biclonal planting field will be important for providing hybrids with high cyanidin attributes.

Hybrids derived from SNK16 and T79/467 clones (F5 and F9) exhibited low MPH and BPH for alkaloids and flavan-3-ols; while the genetic distance between their parents was low (~ D 0.5). In fact, as the genetic distance between parental inbreds increases, there is generally an increase in heterosis; yet when the parental distance exceeds a threshold, heterosis decreases. Thus, there appears to be a relationship between genetic diversity and heterosis; however, the correlation is not strong enough to be used as an accurate predictive tool (Melchinger, 1999).

The heritability estimates for some traits were high, especially for cyanidin-3-galactoside and cyanidin-3-arabinoside where the value of the heritability was superior or equal to 0.3 for all hybrids. According to Nair *et al.* (2012), a high value of heritability in broad sense indicates that, though the character is least influenced by the environmental effects, the selection for improvement of such character may be useful, because broad sense heritability is based on total genetic variance which includes both fixable (additive) and non fixable (dominance and epistatic) variances. The absence of significant difference between genotypes coming from reciprocal crossbreeding for cyanidins suggests that transmission of these metabolites in cocoa may be nuclear. In contrast, a significant difference was observed between values of the Broad-sense heritability of caffein contents in all reciprocal crossings, portraying presence of maternal heritability.

Examining a two-dimensional score plot in the space defined by PC1 and PC2, showed that the distribution of samples followed a pattern. SNK16 parental clone and its offsprings contained large amounts of caffeine, theobromine, catechin and epicatechin, thus PC1 clearly separates them from the other samples. Corresponding estimates of genotypic correlation coefficients were approximately equal to phenotypic correlation coefficients. The existence of positive correlations among components measured suggests that these traits can be simultaneously improved.

CONCLUSION

Dry cocoa beans display high contents of purine alkaloids and polyphenols. Theobromin content in cocoa beans is four-folds higher than caffeine content. Anthocyanins (Cyanidin-3-galactoside

and cyanidin-3-arabinoside) present the highest level of heterosis. Parental genotypes SNK16 and T79/467 show good aptitudes for the combination of the characters since their reciprocal hybrids F5 and F9 distinguished themselves by better level of mid-parent heterosis value. These two clones could be interesting for drug industries, and their introduction into the biclonal planting field will be important. Furthermore to the Principal component analysis, corresponding estimates of genotypic correlation coefficients are approximately equal to phenotypic correlation coefficients. Besides, the heritability value in the strict sense of this Cyanidin-3-galactoside is very high. The absence of significant difference between genotypes coming from reciprocal crossbreeding for Cyanidin-3-galactoside suggests that this character in cocoa would be nuclear contrary to purine alkaloids and flavan-3-ols where their transmission to offsprings can be stated as cytoplasmic.

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